

Nasal allergen challenge and environmental exposure chamber challenge: A randomized trial comparing clinical and biological responses to cat allergen

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Background: The direct-instillation nasal allergen challenge (NAC) and the environmental exposure chamber (EEC) are 2 methods of conducting controlled allergen provocations. The clinical and biological comparability of these methods has not been thoroughly investigated.

Objective: We sought to compare clinical and immunologic responses to cat allergen in NAC versus EEC.

Methods: Twenty-four participants were randomized to receive either NAC followed by a 2-day challenge in an EEC or a 2-day challenge in an EEC followed by NAC. Challenges were separated by 28-day washout periods. We measured total nasal symptom scores, peak nasal inspiratory flow, nasal (0-8 hours) and serum cytokines, serum antibodies, peripheral blood antigen-specific T lymphocytes, and gene expression in nasal scrapings. The primary outcome was the total nasal symptom score area under the curve for the first 3 hours after allergen exposure in NAC or after initiation of exposure in EEC.

Results: Both challenges increased IL-5 and IL-13 in nasal fluids and serum and resulted in altered nasal cell expression of gene modules related to mucosal biology and transcriptional regulation. Changes in gene modules, more so than cytokine

measurements, showed significant associations with total nasal symptom score and peak nasal inspiratory flow. Overall, EEC exposure generated larger responses and more early terminations compared with NAC. Although the 2 challenges did not correlate in symptom magnitude or temporality, striking correlations were observed in cytokine levels.

Conclusions: Although clinical outcomes of NAC and EEC were temporally different and nonequivalent in magnitude, immunologic responses were similar. Selection of a particular allergen challenge method should depend on considerations of study objectives and cost. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: Nasal allergen challenge, environmental exposure chamber, Fel d1, cat allergy, total nasal symptom score, peak nasal inspiratory flow, cat dander, epithelium

The pathophysiology of allergic rhinitis and the response to therapies can be studied using experimental allergen provocations (challenges). Two types of challenges are commonly used: the

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Abbreviations used

AUC: Area under the curve
 EEC: Environmental exposure chamber
 NAC: Nasal allergen challenge
 PNIF: Peak nasal inspiratory flow
 TNSS: Total nasal symptom score
 TOST: Two one-sided test

nasal allergen challenge (NAC) and the environmental exposure chamber (EEC). In the NAC, a known amount of allergen is applied directly in the nasal cavity, whereas during an EEC exposure, the study participant inhales aerosolized allergen delivered into a temperature/pressure/humidity-controlled room. The NAC is performed readily in the clinic and requires minimal equipment but may not be fully reflective of allergen exposure in everyday life.¹ The EEC represents a more naturalistic method of exposure, but requires a specialized chamber or facility to conduct the challenge and just as in everyday life, the exact amount of inhaled allergen is not easy to calculate.² Direct comparisons of NAC and EEC have occurred in only 1 study; however, immunologic responses were not assessed.³

Various clinical and physiologic outcomes can be measured in the course of these challenges including standardized symptom scores, such as the total nasal symptom score (TNSS), and objective measures of nasal obstruction such as the peak nasal inspiratory flow (PNIF). The NAC has also been extensively used to conduct mechanistic research and has unveiled many aspects of the pathophysiology of allergic rhinitis including the acute release of mast cell mediators upon allergen exposure, the nasal late-phase reaction, the local influx of inflammatory cells, the production of type 2 and other cytokines and chemokines, and the development of nasal hyperresponsiveness.⁴⁻¹¹ In contrast, with the exception of recent studies by Ahuja et al,^{12,13} the EEC model has not been used in this type of research.

The goal of our study was to characterize clinical and mechanistic aspects of allergic responses during NAC and EEC using cat allergen and to identify similarities and differences between the 2 methodologies. For the mechanistic objectives, we focused on outcomes that have not been previously examined in either model or have been examined only in 1 of the 2 models: these included measurements of inflammatory cytokines in nasal secretions and in serum, examination of allergen-induced changes in allergen-specific peripheral blood T cells, and changes in the transcriptomic profiles of nasal cells obtained by nasal brushing.

METHODS**Study participants**

This was a randomized open-label cross-over study conducted at Inflammax Research in Ontario, Canada (DBA Cliantha Research). Study participants were aged 18 to 65 years, with at least a 2-year history of moderate to severe allergic rhinitis with symptoms induced by cat exposure. After signing the informed consent form, participants were screened for eligibility. Participants were eligible if they had a positive skin prick test result to standardized cat extract. A wheal diameter greater than or equal to 5 mm larger than that achieved with the negative control was used to increase the probability that participants with moderate to severe cat allergy were enrolled. Exclusion criteria included a history of anaphylaxis to cat allergen, moderate to severe

asthma, chronic obstructive pulmonary disease, chronic tobacco use, recurrent or chronic sinusitis, and a prebronchodilator FEV₁ less than 80% of their predicted value. Individuals with other perennial or seasonal allergies that could not be avoided were also excluded. No participant owned a cat, and all participants were deemed to have low daily exposure to cat allergen. This study was approved by the Institutional Review Board Services protocol number 00000776. The study was conducted under Clinical Trial Application (CTA) number 179612 by Health Canada and was registered as NCT02163122 in [ClinTrials.gov](https://www.clinicaltrials.gov).

Study design

Participants were randomized into 2 groups using an opaque envelop system.¹⁴ Group A was assigned to undergo an NAC with measurement of nasal responses from 0 to 8 hours followed by a 2-day EEC with a 28-day washout period between the 2 challenges. Group B was assigned to undergo a 2-day EEC followed by an NAC with a 28-day washout period. An analysis of previous cat allergen challenge data demonstrated that EEC induced a priming effect and potentiated the clinical response on the last day of challenge. However, little increase in clinical response was observed after day 2. Therefore, EEC was performed over 2 days in our study.

Challenges

Participants underwent both an NAC and an EEC challenge. In the NAC, a single dose of cat allergen (Greer Laboratories, Lenoir, NC), which was 0.87 µg total Fel d1 and selected on the basis of a smaller dose-finding study (for additional details, see this article's [Online Repository](https://www.jacionline.org) at www.jacionline.org), was administered as a nasal spray with the use of a Bi-dose nasal applicator device (Aptar Pharma, Crystal Lake, Ill). TNSSs were captured on electronic tablets at 10, 30, 60, and 90 minutes and every 30 minutes thereafter for 8 hours total. PNIF was measured at 10, 30, and 60 minutes and every 60 minutes thereafter for 8 hours total. TNSS and PNIF recordings were made at the same time points during the NAC and EEC phases ([Fig 1](#)). During the EEC phase, participants stayed in a mobile temperature- and humidity-controlled room or EEC for 3 hours, with an exposure of 10 to 500 ng/m³ Fel d1 allergen and an estimated amount of 37.7 ng of Fel d1 inhaled on each day of the EEC (for additional details, see this article's [Online Repository](#)).

Serum cytokine measurements

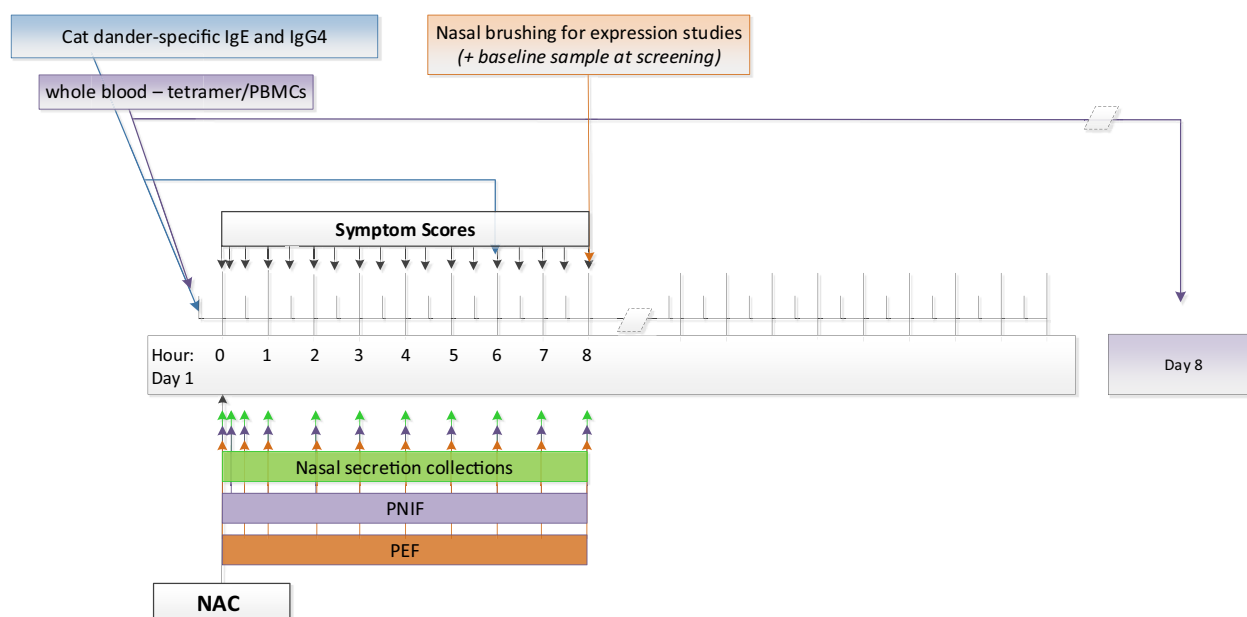
Blood was collected prechallenge and 6 hours postchallenge for NAC and pre-day 1, pre-day 2, and 6 hours postchallenge on day 2 of the EEC ([Fig 1](#)). Sera were frozen at -80°C until batched assays were performed. An ultrasensitive single-molecule digital immunoassay (Simoa HD-1 Analyzer, Quanterix, Billerica, Mass)¹⁵ was used to measure concentrations of IL-2, IL-4, IL-5, IL-10, and IL-13 in sera. The limits of detection for IL-2, IL-4, IL-5, IL-10, and IL-13 were 11.0, 4.6, 14.0, 3.8, and 5.5 fg/mL, respectively.

Nasal fluid collection and nasal cytokine measurements

Nasal secretions were collected as previously described¹⁶ and at time points indicated in [Fig 1](#). Briefly, a single autoclave-sterilized sponge was inserted into each nostril of each participant for 2 minutes before removal. Each sponge was then placed in a cryovial with an indwelling cellulose acetate filter. The sample was eluted by adding 50 µL of Milliplex Buffer (Millipore, Burlington, Mass) per sponge and by centrifugation. The supernatants from both sponges (1 from each nostril) were combined, transferred to 0.5-mL cryovials, and stored at -80°C until cytokine assays were performed.

After thawing of nasal fluid supernatants, cytokines were measured in batches. Measurements of IL-4, IL-5, IL-6, IL-9, IL-13, and eotaxin 1 were performed using a human cytokine/chemokine magnetic bead panel 96-well plate assay (Milliplex Map Kit, Millipore) and a Luminex xMAP Magpix

Nasal Allergen Challenge Visit Details



Environmental Exposure Chamber Visit Details

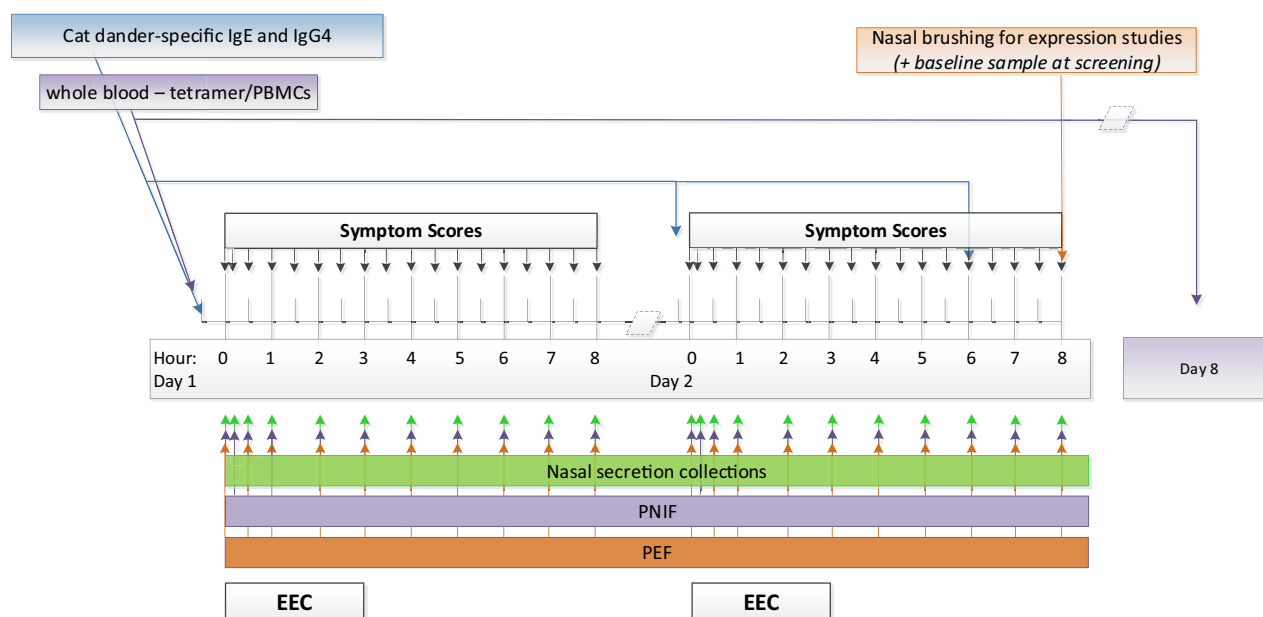


FIG 1. Timing of clinical assessments and mechanistic collections for NAC (top) and EEC (bottom). PEF, Peak expiratory flow.

platform (Millipore), according to the manufacturer's instructions. Twenty-five-microliter samples were analyzed in duplicate with standards and controls. The mean of the duplicate results was calculated and results below the lower limit of assay detection were given a value of 0.

Nasal brushing and RNA extraction

Nasal brushing was performed using a 3-mm cytology brush (Medical Packing Corporation, Camarillo, Calif) at study baseline (no allergen challenge), 8 hours after NAC, and 8 hours after the second day of the EEC

challenge. Brushes were immediately placed in cryovials containing RLT buffer (Qiagen, Camarillo, Calif) and frozen at -80°C until RNA extractions were performed.

RNA sequencing

Whole-genome transcriptional profiling was performed on nasal brushing RNA samples. Methods for RNA quality control, library preparation, and RNA sequencing have been described previously¹⁷ and are presented in detail in this article's [Online Repository](#). Differential gene expression was

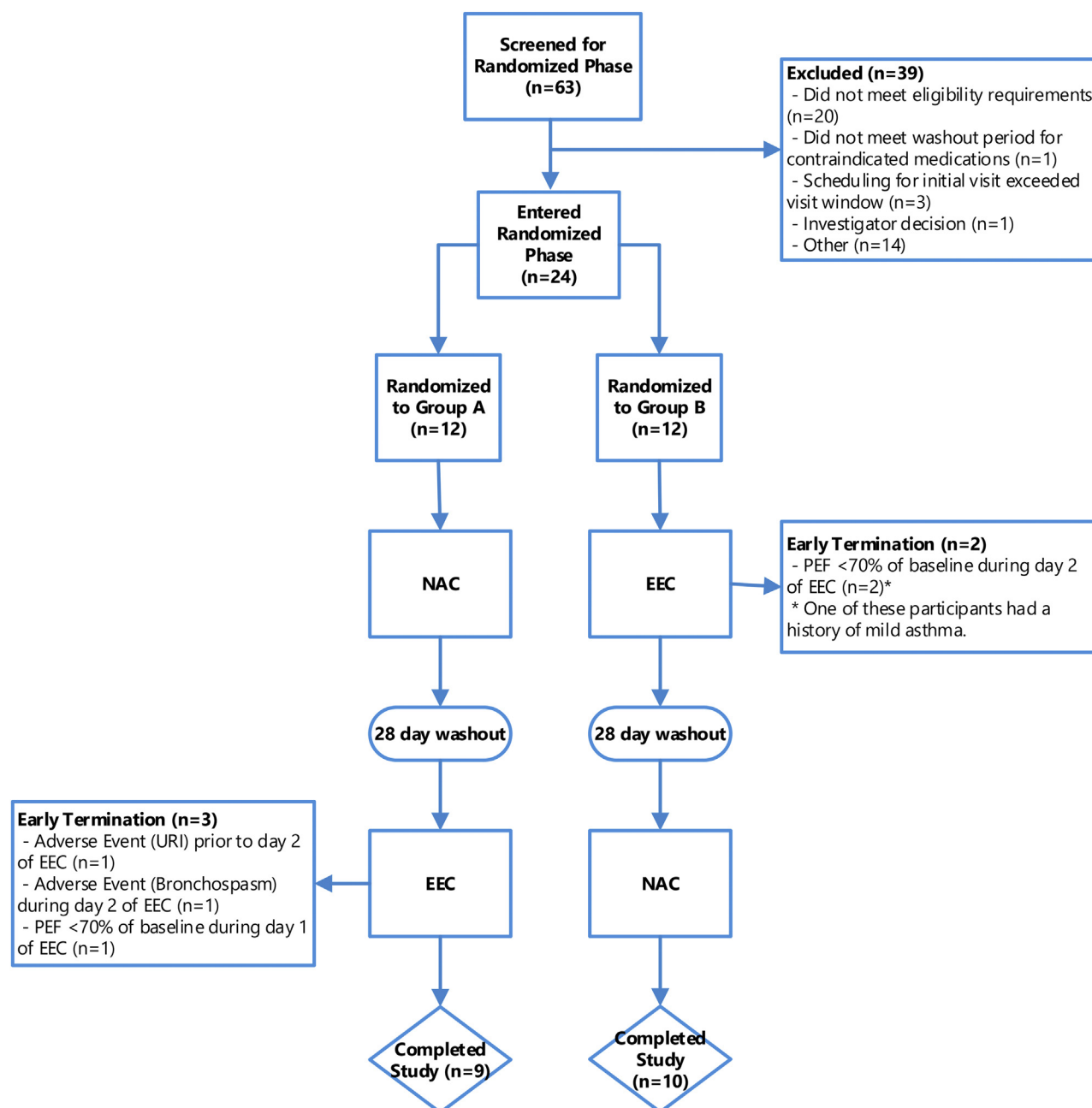


FIG 2. Consort diagram showing disposition of all participants screened and enrolled in the study. Group A underwent an NAC first followed by a 2-day EEC challenge after a 28-day washout period. Group B underwent the same challenges but started with the EEC challenge. Reasons for early withdrawal from the study and phase of study are also shown. *PEF*, Peak expiratory flow; *URI*, upper respiratory infection.

performed using a weighted linear model (limma in R) appropriate for RNA-sequencing data and empirical Bayes method, and modular analysis using weighted gene correlation network analysis as previously described.¹⁸ Full details are given in this article's [Online Respository](http://www.jacionline.org) at www.jacionline.org.

Statistical methods used for analysis of clinical and cytokine data

The study's sample size provided adequate statistical power to detect a correlation coefficient of 0.55 between NAC and EEC for the primary end point of TNSS area under the curve (AUC) 0 to 3 hours.

The AUC for the TNSS, adjusted for baseline, was computed from hour 0 to hour 3 of the NAC and from hour 0 to hour 3 on the second day of the EEC challenges. The trapezoidal rule was used to estimate the AUC of the TNSS; hour 0 was used as the base of the trapezoids in AUC calculations. The intraclass correlation coefficient was used to assess the equivalence of the 2 challenges in measuring TNSS AUC. To test the equivalence of the mean TNSS AUC between the EEC and the NAC, we computed a two one-sided test (TOST)¹⁹ using an equivalence margin of $\pm 20\%$. The null hypothesis of TOST is that the challenges are not equivalent, so that rejecting the null leads to a conclusion of equivalence of the challenges.

For cytokine data, paired *t* tests were performed to compare prechallenge to postchallenge cytokine levels. Pearson correlation coefficients (*r*) and *P* values

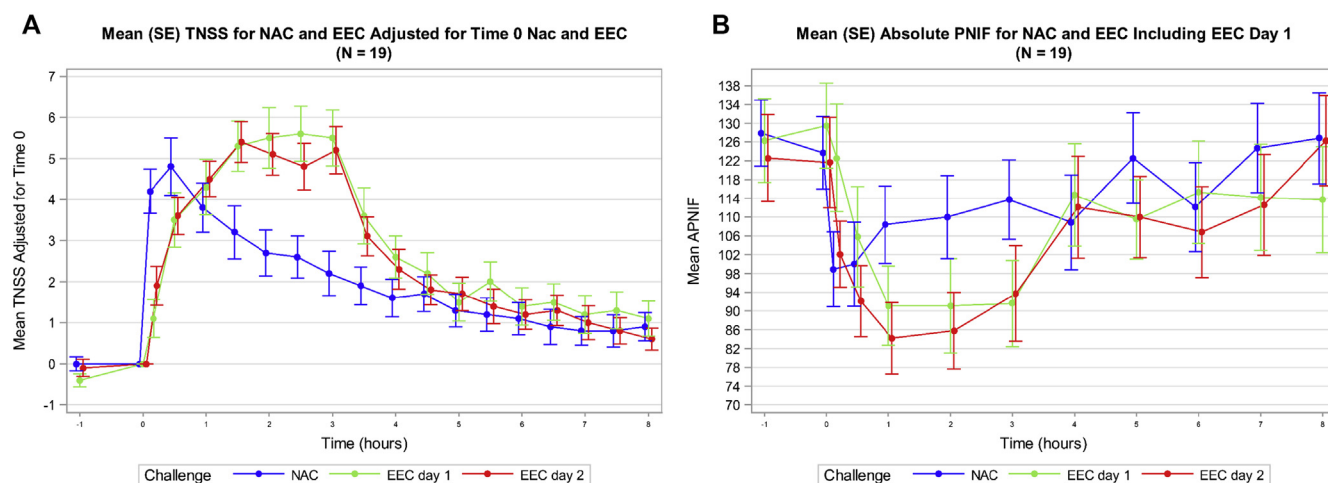


FIG 3. Clinical responses to NAC and EEC. **A**, Mean (SE) TNSSs shown over time for NAC, day 1 of EEC, and day 2 of EEC for all participants. TNSSs with NAC peaked earlier than EEC but have an overall reduced magnitude of response. **B**, Mean (SE) PNIF shown over time for NAC, day 1 of EEC, and day 2 of EEC for all participants. Similar to TNSSs, PNIF peaked earlier during NAC but EEC induced a greater magnitude of nasal air flow obstruction. *APNIF*, Absolute PNIF.

were calculated to determine whether levels of cytokines elicited by allergen were associated between the 2 challenges. The threshold for significance was $P < .05$ (2-sided). Other than analyses with RNA-sequencing data, mechanistic analyses were exploratory as described in the protocol and, therefore, were not adjusted for multiple comparisons.

All analyses were performed with SAS version 9.4 (SAS Institute Inc, Cary, NC) and R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

For details on MHC class II tetramer analyses, antibody measurements, RT-PCR, along with additional details on other assays and statistics, please see this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Data sets for these analyses are accessible through TrialShare, a public Web site managed by the Immune Tolerance Network (https://www.itntrialshare.org/CATEEC_primary.url).

RESULTS

Participant disposition and safety

The 2 groups were similar in regard to age, sex, race, and reactivity to cat dander (see [Table E2](http://www.jacionline.org) in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org). [Fig 2](http://www.jacionline.org) shows the randomization and participant disposition. Of the 24 enrolled participants, 5 stopped the study early (3 were initially randomized to NAC and 2 were initially randomized to EEC). All early terminations occurred during the EEC phase. Participants stopped the study because of bronchospasm (1), upper respiratory tract infection (1), and asymptomatic reductions in peak expiratory flow from baseline during the EEC phase (3). All adverse events, including those that did not result in early withdrawal, are presented in [Table E3](http://www.jacionline.org) in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Symptom scores

TNSSs collected over 8 hours after the NAC and during and after the EEC showed distinct patterns, with symptoms peaking earlier in the NAC compared with either day 1 or day 2 of the EEC ([Fig 3, A](http://www.jacionline.org)). Also, the 0- to 3-hour AUC of the TNSS was significantly greater with the EEC day 2 compared with the NAC (difference of least squares mean 3.4 with 95% CI 0.1-6.7). After

adjusting for baseline TNSSs, the interclass correlation coefficient of the TNSS 0- to 3-hour AUC between the 2 challenges (NAC vs EEC day 2) was 0.304 (95% CI, -0.174 to 0.666). In general, the correlation was low between the 2 challenges for most AUC intervals or between peak symptom values and no specific interval could be identified where a strong correlation was observed (see [Fig E1](http://www.jacionline.org) in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org). The TOST of equivalence of the 0- to 3-hour TNSS AUC had a P value of .756, suggesting that the challenges were not equivalent in terms of this outcome. Notably, we found no difference in the 0- to 3-hour AUC between day 1 and day 2 of the EEC challenges.

Nasal obstruction

Similar to TNSS, maximum reduction in PNIF with NAC (20% after 10 minutes postchallenge) occurred earlier compared with either day 1 (30% at hour 1) or day 2 of the EEC (31% at hour 1) ([Fig 3, B](http://www.jacionline.org)). However, the 0- to 3-hour AUC of the change in PNIF over baseline in the EEC was not significantly different compared with the NAC (EEC least squares mean = -97.1; NAC least squares mean = -57.4, $P = .087$). The TOST of equivalence of the 0- to 3-hour AUC of the change in PNIF over baseline had a P value of .856, suggesting that the 2 challenges were not equivalent in terms of PNIF. As with TNSS, no significant differences between day 1 and day 2 in the PNIF 0- to 3-hour AUC were detected in the EEC.

Effect of challenge sequence on clinical outcomes

We tested whether the order of the challenges could affect the clinical results of the NAC or the EEC by comparing challenge-specific TNSS AUC for 0 to 3 hours between the 2 treatment groups (ie, Group A NAC vs Group B NAC and Group A EEC vs Group B EEC). No significant differences between challenge-induced increases in TNSSs and reductions in PNIF were detected between Group A and Group B, indicating that the order of the challenges did not influence the clinical outcomes.

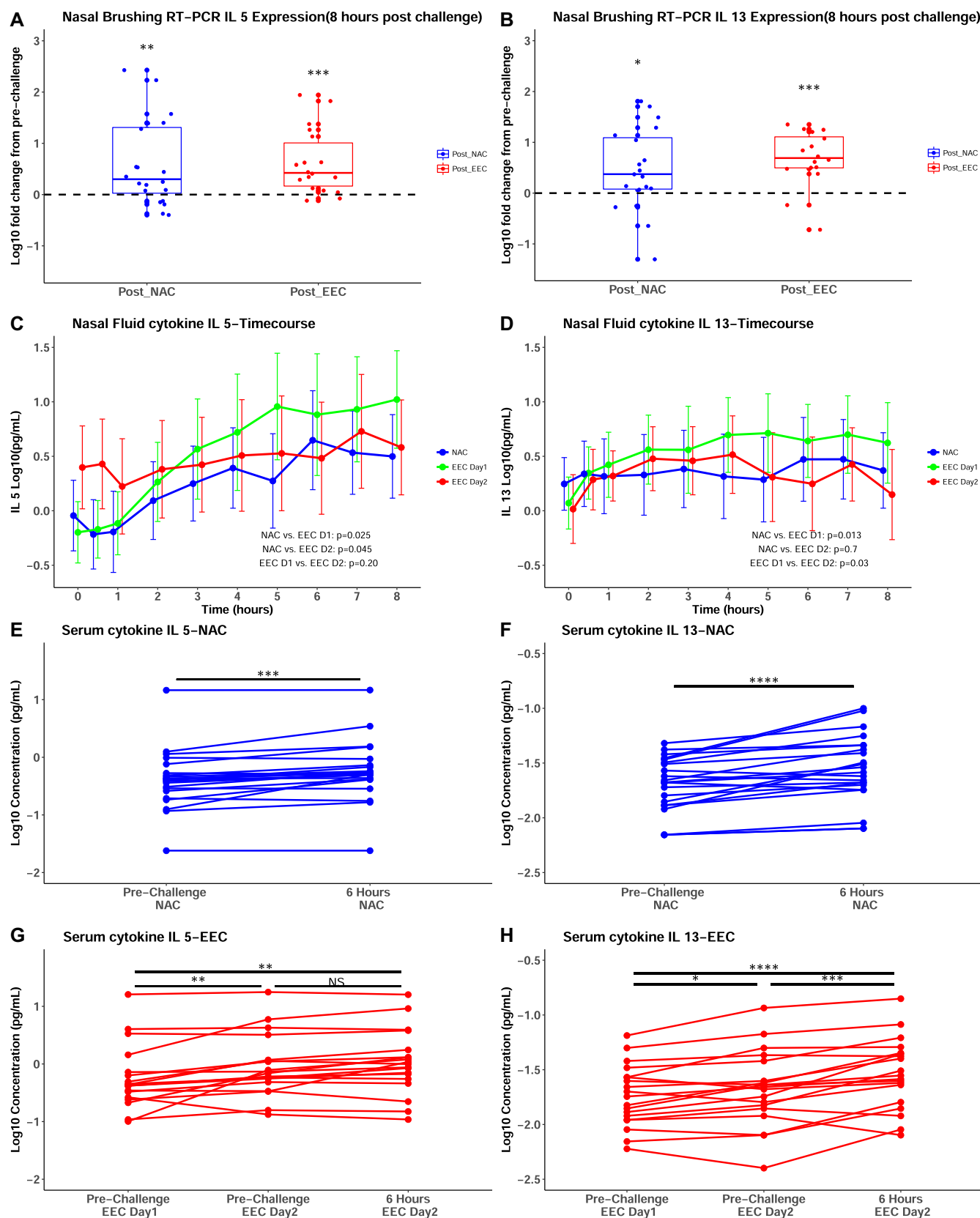


FIG 4. Increases in local and systemic type 2 immune responses after NAC and EEC. Log₁₀ fold increases in IL-5 (A) and IL-13 (B) gene expression from nasal brush samples collected at baseline and 8 hours after NAC (blue) or baseline and 8 hours after the second day of EEC (red). Levels of IL-5 (C) and IL-13 (D) in nasal fluid collected prechallenge and up to 8 hours after allergen challenge. Serum IL-5 and IL-13 concentrations prechallenge and 6 hours after NAC (E and F) or prechallenge day 1, prechallenge day 2, and 6 hours after EEC day 2 (G and H). NS, Not significant. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

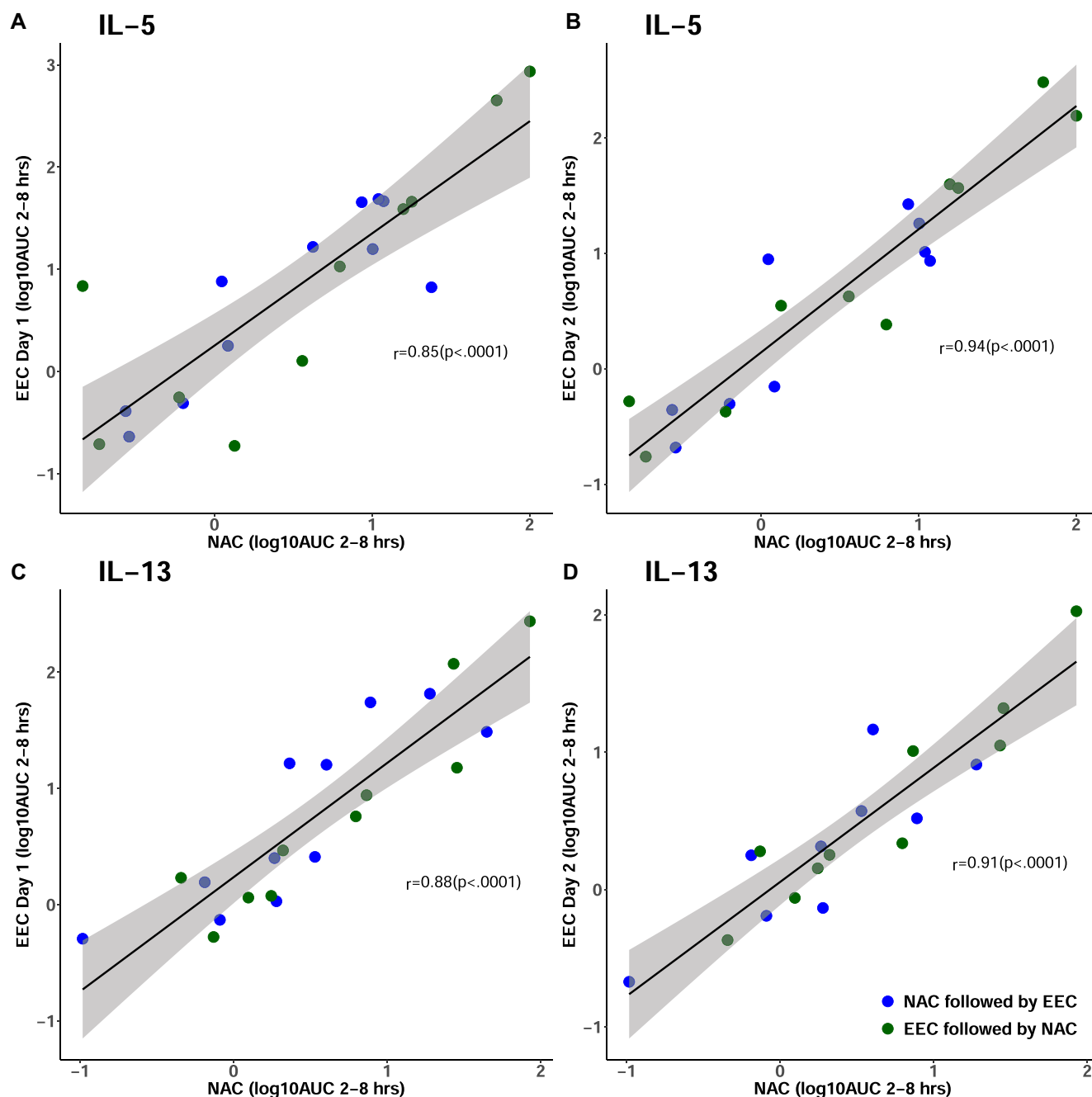


FIG 5. Relationship between nasal fluid cytokines elicited by NAC and EEC. Pearson correlations of log₁₀ 2- to 8-hour AUC of NAC vs log₁₀ 2- to 8-hour AUC of EEC day 1 for IL-5 (A) and IL-13 (C) and log₁₀ 2- to 8-hour AUC of NAC vs log₁₀ 2- to 8-hour AUC of EEC day 2 for IL-5 (B) and IL-13 (D). Participants colored by order of challenge received first (blue = NAC followed by EEC and green = EEC followed by NAC).

Local and systemic inflammatory and immunologic responses

Using RNA extracted from nasal brush samples, we performed RT-PCR to assess the expression levels of several type 1, type 2, and regulatory cytokine/chemokine genes (see Table E1 in this article's Online Repository at www.jacionline.org) before and 8 hours after allergen challenge (NAC and EEC day 2). Both challenges led to significant increases in the transcription of the type 2 cytokines IL-5 (for NAC: $P = .006$ and for EEC:

$P = .0006$; Fig 4, A) and IL-13 (for NAC: $P = .023$ and for EEC: $P = .0002$; Fig 4, B). There were no significant differences in the expression levels of IL-5 or IL-13 when NAC was compared with EEC. Overall, we observed increased expression of several type 2 cytokine genes, with small changes or decreased expression of type 1 and regulatory cytokines or alarmins (see Fig E2 in this article's Online Repository at www.jacionline.org).

In nasal fluids, we found significantly elevated levels of type 2 cytokines after both NAC and EEC over baseline (for IL-5: after

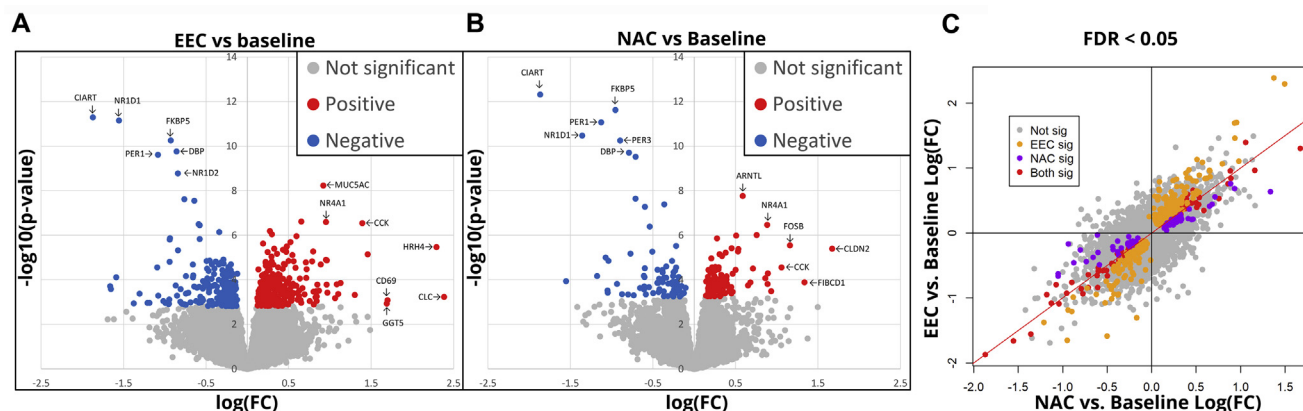


FIG 6. Similarities in gene expression after NAC and EEC. Volcano plots of nasal RNA-sequencing data showing genes with significant increases (red) or decreases (blue) in expression after (A) EEC or (B) NAC. C, Correlation in gene expression changes between NAC and EEC ($FDR < 0.05$). FC, Fold change; FDR, false discovery rate.

NAC, $P < .0001$; after EEC day 1, $P < .0001$; after EEC day 2, $P < .0001$, Fig 4, C; for IL-13: after NAC, $P < .0001$; after EEC day 1, $P < .0001$; after EEC day 2, $P < .0001$, Fig 4, D; for other cytokines/chemokines, see Fig E3, A-D, in this article's Online Repository at www.jacionline.org. The magnitude of IL-5 increase in nasal fluids was greater after EEC compared with NAC (NAC vs EEC day 1: $P = .025$ and NAC vs EEC day 2: $P = .045$). The increase in IL-13 after EEC was greater than NAC only on day 1 ($P = .013$).

NAC and EEC showed remarkable correlations in the allergen exposure-induced changes in IL-5 and IL-13 when expressed as 2- to 8-hour AUCs (Fig 5, A-D). After the first EEC exposure, nasal fluid IL-5 and IL-9, but not other cytokines, seemed to remain elevated for at least 24 hours because the EEC day 2 prechallenge levels were higher than the prechallenge levels on EEC day 1 (for IL-5, $P = .001$; for IL-9, $P = .0071$; see Fig 4, C, and Fig E3, C).

To determine whether a systemic response was also elicited by allergen challenge, we used an ultrasensitive single-molecule digital immunoassay and detected significant increases in circulating IL-5 and IL-13 6 hours after both challenges (for IL-5: after NAC, $P < .001$; after EEC day 2, $P < .01$; for IL-13: after NAC, $P < .0001$; after EEC day 2, $P < .0001$, Fig 4, E-H). No prechallenge to postchallenge changes in serum IL-2, IL-4, or IL-10 could be detected for either challenge. The changes from baseline in the serum levels of IL-5 and IL-13 induced by NAC and EEC were significantly correlated (for IL-5, $r = 0.71$, $P = .0007$; for IL-13, $r = 0.55$, $P = .014$; see Fig E4, A and B, in this article's Online Repository at www.jacionline.org) much like the nasal fluid cytokine data, albeit to a somewhat lesser extent.

Local and systemic type 2 cytokine responses did not correlate consistently with challenge-induced TNSS or PNIF in either challenge (see Table E4 in this article's Online Repository at www.jacionline.org). For NAC, there was a modest inverse correlation between type 2 cytokines (IL-5 and IL-13 in nasal fluid and/or serum) and delta PNIF ($r = -0.34$ to -0.52 and $P = .01$ to $.12$).

In the context of the systemic effects of NAC, we tested peripheral blood for changes in the number of cat allergen-specific CD4 T cells using MHC class II tetramers for Fel d1, at prechallenge and 7 days after the NAC and EEC day 1

exposure. Of the 9 participants who could be analyzed by tetramers, 5 had virtually no tetramer-positive CD4 cells at baseline and after both types of allergen challenge. However, in 4 participants with detectable tetramer-positive cells at baseline, we observed increases in cat allergen-specific CRTH2+ CD4 cells after both NAC and EEC (see Fig E5 in this article's Online Repository at www.jacionline.org). The magnitude of the increase appeared greater after EEC, but the difference did not reach significance, possibly due to the low number of cases.

Previous studies have demonstrated increases in allergen-specific antibody levels after allergen challenge.²⁰ Therefore, we measured levels of cat dander-specific IgE and IgG₄ in the serum before and 28 days after challenge. On average, the levels of IgE to cat dander increased after both NAC and EEC, but only the increase in IgE after EEC reached statistical significance ($P = .007$; see Fig E6, A, in this article's Online Repository at www.jacionline.org). We also noted a significant increase in IgG₄ after EEC ($P = .04$). Decreases in cat dander IgG₄ to IgE ratios were also observed after both challenges, but only the decrease after NAC was significant ($P = .037$, Fig E6, B).

Effects of nasal challenges on nasal transcriptomics

Using nasal brush samples obtained at baseline and 8 hours after each challenge, we investigated changes in global gene expression attributable to each challenge procedure with an unbiased RNA-sequencing approach. The expression of 462 genes was significantly changed after EEC (Fig 6, A; see Table E5, A and B, in this article's Online Repository at www.jacionline.org) and of 171 genes after NAC (Fig 6, B; see Table E5, A and B) (false discovery rate < 0.05). There was a high degree of overlap, with 102 genes from both lists showing significant changes (hypergeometric $P < .001$). Overall, differentially expressed genes showed a similar magnitude of change with each challenge and the changes were highly correlated (Fig 6, C; see Table E5, A and B).

To determine the discrete biological pathways represented in these differentially expressed genes, we conducted a modular

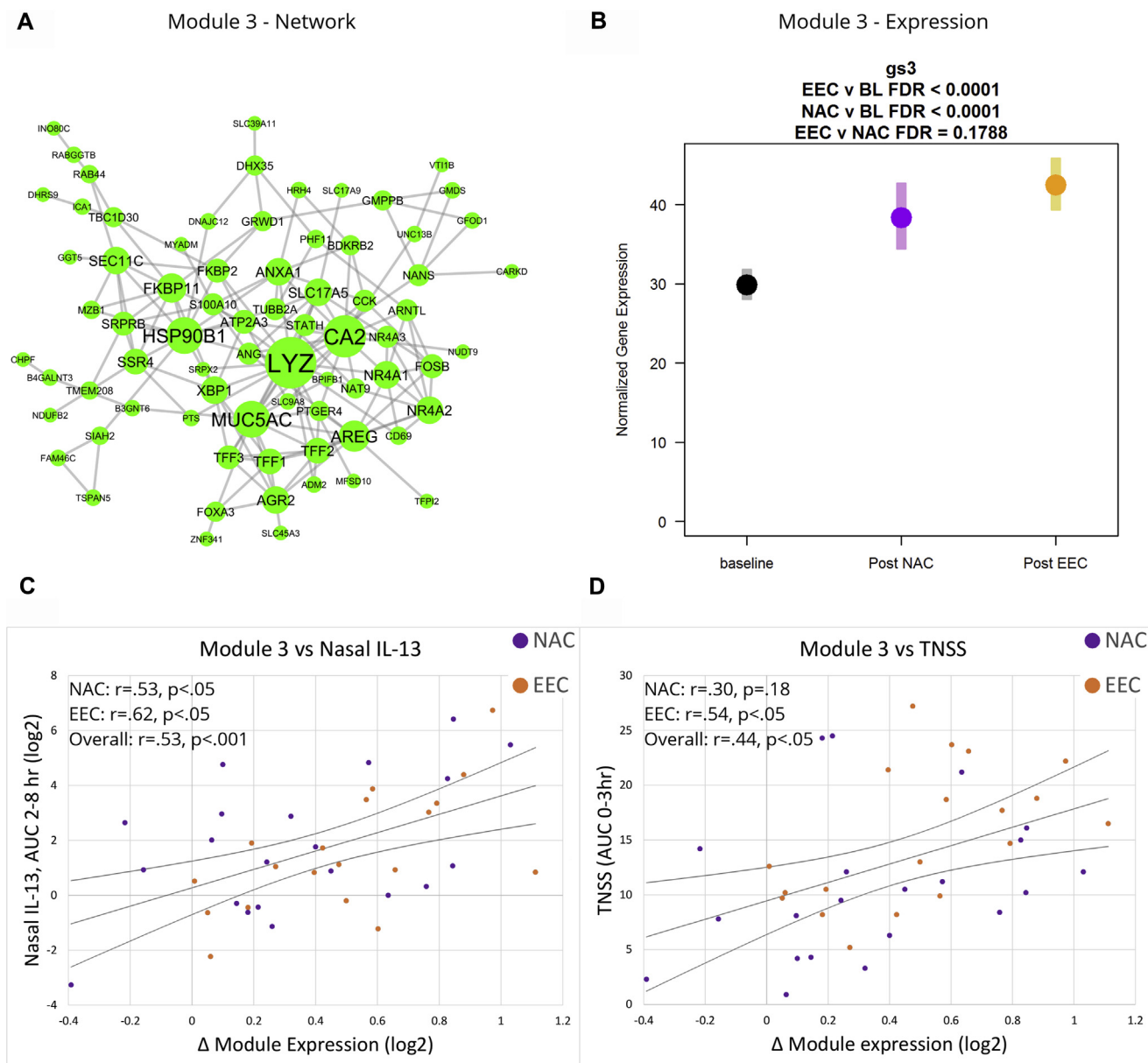


FIG 7. Gene network and module 3 expression. **A**, Gene-gene interaction network for module 3. **B**, Normalized gene expression of module 3 at baseline, post-NAC, and post-EEC. Correlation of module 3 expression with **(C)** nasal IL-13 levels and **(D)** TNSSs. BL, Baseline; FDR, false discovery rate.

network analysis of the 531 total differentially expressed genes (102 genes common to EEC and NAC and 429 genes unique to either EEC or NAC). The genes clustered into 8 different coexpression modules representing multiple molecular pathways. Each of these modules was significantly differentially expressed after both EEC and NAC compared with baseline (false discovery rate < 0.001). Generally, the observed change was greater with EEC than with NAC, though this difference was not significant for any module after multiple testing correction (lowest false discovery rate = 0.18). A complete list of modules, their enriched biological pathways, and their constituent genes is provided in Table E6 in this article's Online Repository at www.jacionline.org.

Of these modules, 2 (designated M3 and M4) were of particular interest because they also showed significant associations with both type 2 cytokine protein measurements and the clinical outcomes TNSS and PNIF. Specifically, the expression of M3, which contains 96 genes related to mucin and lysozyme production, prostaglandin synthesis, and mucosal healing (Fig 7, A), was increased after NAC (baseline to post-NAC expression change = 1.27; $P < .001$) and EEC (baseline to post-EEC expression change = 1.41; $P < .001$) (Fig 7, B), and the magnitude of change showed significant positive correlations with IL-5 and IL-13 levels in both nasal fluid and serum, as well as with TNSS, and significant inverse correlation with delta PNIF (Fig 7, C and D; see Table E7 in this article's Online Repository at www.jacionline.org).

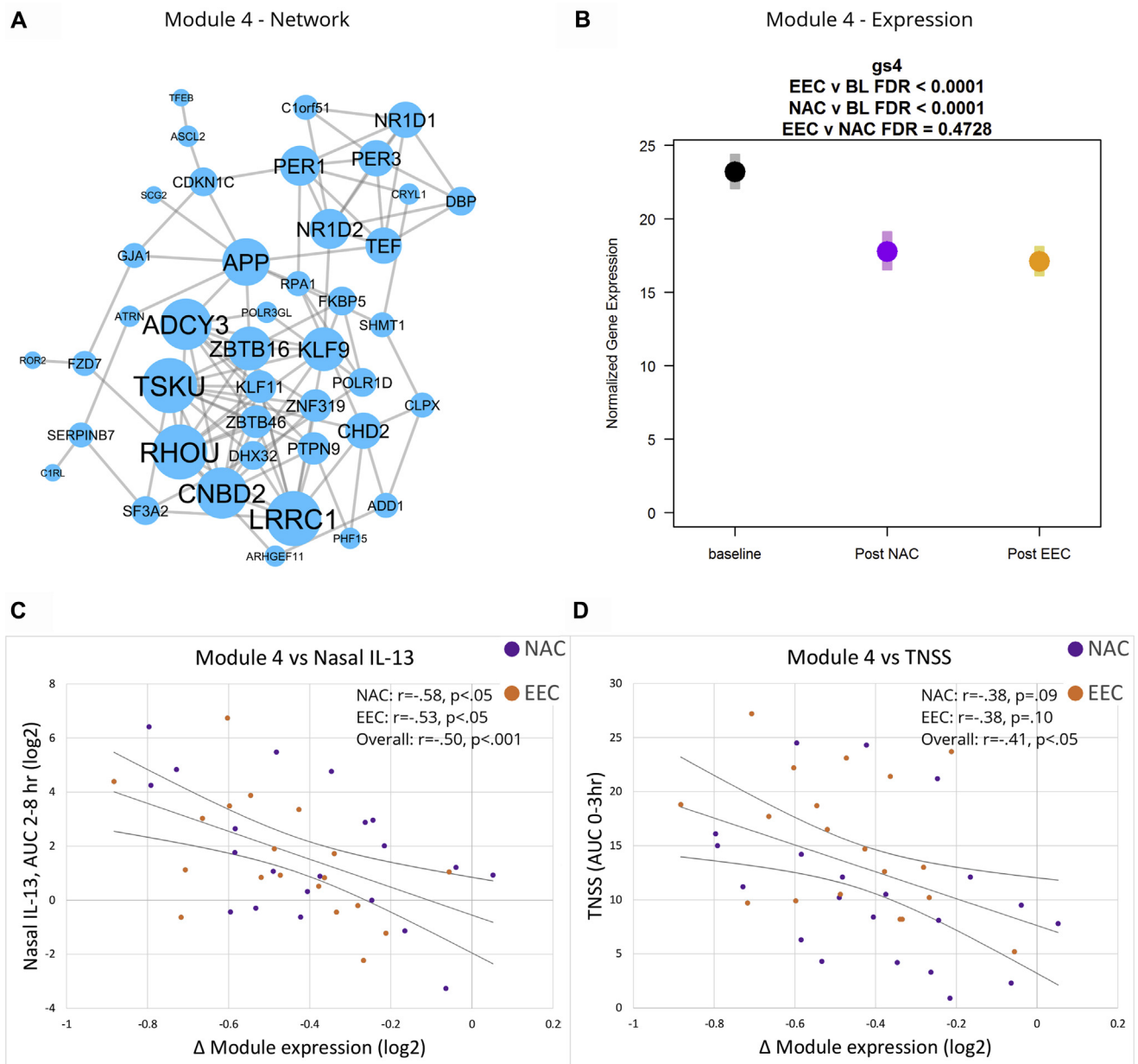


FIG 8. Gene network and module 4 expression. **A**, Gene-gene interaction network for module 4. **B**, Normalized gene expression of module 3 at baseline, post-NAC, and post-EEC. Correlation of module 3 expression with **(C)** nasal IL-13 levels and **(D)** TNSSs. BL, Baseline; FDR, false discovery rate.

[jacionline.org](http://www.jacionline.org)). Key hub genes in this network include mucin 5AC (*MUC5AC*) and lysozyme (*LYZ*), important secretory products of goblet and serous cells that likely reflect increased epithelial mucus production and hypersecretion in response to allergen. The network also includes several receptor genes involved in allergic inflammation, vasodilation, and tissue edema including *HRH4*, *BDKRB2*, and *PTGER4*, and the 3 trefoil factors (*TFF1*, *TFF2*, and *TFF3*), which are secretory proteins involved in mucosal healing.

M4, which contains genes related to the negative regulation of transcription (Fig 8, A), was decreased after NAC (baseline to post-NAC expression change = 0.76; $P < .001$) and EEC (baseline to post-EEC expression change = 0.74; $P < .001$) (Fig 8, B), and showed significant inverse correlations with IL-5 and IL-13

levels, as well as with TNSS, and significant positive correlation with delta PNIF (Fig 8, C and D; see Table E8 in this article's Online Repository at www.jacionline.org). The module is a network of 61 genes composed predominantly of nuclear proteins including at least 11 genes involved in negatively regulating gene expression.

A third biologically interesting module was M1, which contains genes related to cilia production. The expression of this module was significantly increased with allergen exposure (baseline to post-NAC expression change = 1.17, $P < .001$, and baseline to post-EEC expression change = 1.27, $P < .001$; see Fig E7 in this article's Online Repository at www.jacionline.org), but was not significantly associated with other mechanistic or clinical variables.

DISCUSSION

EEC and NAC are routinely used in allergic rhinitis research, but many outcomes have never been directly compared. Using a cross-over design, we examined clinical and immunologic outcomes aiming at assessing how interchangeable these methodologies are and at identifying differences, if present.

In designing this study, we also took the opportunity to investigate the suitability of some techniques for which relatively little experience is available in the context of NAC. This includes measurement of serum cytokines using ultrasensitive assays, assessment of allergen-induced changes in allergen-specific CD4⁺ T lymphocytes in peripheral blood, and nasal mucosal transcriptomics. Notably, transcriptomics post-EEC allergen exposure have been assessed in the recent work of Ahuja et al¹² and our study offered a chance at replicating some of their findings.

Our study shows that the 2 types of allergen challenges, NAC and EEC, do not correlate with each other with respect to subjective clinical symptoms or the objective measure of nasal obstruction (PNIF) elicited by allergen exposure. Thus, the 2 methodologies cannot be used interchangeably when clinical outcomes are considered. Another study that compared clinical outcomes between NAC and EEC observed good agreement between both challenge modalities with respect to peak and mean symptom scores.³ It is unclear whether differences in the type/amount of allergen used in challenges, or simply the small number of participants used in experiments, may explain the lack of agreement with our data. Importantly, in our study, the effects of the 2 challenges on immunologic outcomes and on nasal mucosal transcriptomics show striking similarities and are highly correlated.

Contrasts between the 2 types of challenges in terms of the symptom and PNIF responses to allergen exposure are illustrated in Fig 3, A and B, and show both kinetic and magnitude differences. We were hoping that the differences in kinetics, which were expected, would be mitigated by expressing these outcomes as AUCs over the first 3 hours after allergen exposure in the NAC or the 3 hours of continuous allergen exposure in the EEC. However, the lack of correlation or agreement between the 2 challenges, even when AUC outcomes are considered, suggests that additional qualitative differences are present. These differences probably reflect the fact that, in NAC, a large amount of allergen is delivered within a few seconds to the nasal mucosa, resulting in a sudden, high local allergen concentration. In contrast, with EEC, allergen is inhaled at approximately the same rate for 3 hours, presumably resulting in different distribution along the nasal airways and probably different local concentrations. Variable distribution, concentration, and the timing of allergen exposure may translate into differential patterns of mast cell activation, as well as target organ stimulation. As an example, exposure of a wider mucosal surface will result in activation of a larger number of sensory nerve endings by mast cell products such as histamine and, given that neural responses mediate many rhinitis symptoms,²¹ result in qualitatively and quantitatively different symptoms within the same individual. Additional factors influencing the symptomatic response may include the size of the particles within which allergen is delivered (droplets of a solution primarily confined to the nose in NAC vs aerosolized dry particles capable of reaching the lower airways in EEC) and, perhaps, the total dose of allergen delivered by each challenge.

Although the dose of allergen delivered by the NAC is known, the dose delivered by the EEC is difficult to calculate. Based on approximations and assumptions of homogeneous concentration of allergen particles in the EEC air, continuous nasal breathing, normal respiratory rates, and average tidal volumes, we have calculated the average total dose of Fel d 1 inhaled through the nose in the EEC to be approximately 38 ng/d. Notwithstanding a degree of erroneous assumptions behind this calculation, the amount of allergen delivered by the EEC appears to be substantially lower than that delivered by the NAC, which was 872 ng (0.1 mL per nostril of a 4.36 µg/mL Fel d 1 solution). It is, therefore, surprising to observe the EEC generating significantly greater changes over baseline in the TNSS 0- to 3-hour AUC, compared with the NAC, and a similar trend with PNIF, albeit not reaching statistical significance. Also, differences in some immunologic outcomes and even in the magnitude of transcriptomic responses trended in the same direction. As discussed above, it is likely that the differences between the 2 challenges may reflect differential allergen distribution, local concentration, and mast cell activation and that the total dose of allergen delivered is of lesser importance.

The observed differences in the magnitude of the clinical outcomes (TNSS and PNIF) between the 2 challenge methods might have relevance to their use in clinical trials. To examine the implication of the larger clinical signal in the EEC, we conducted sample size calculations for a hypothetical clinical trial with the objective to detect a 30% difference in 0- to 3-hour AUC TNSS between an active agent and placebo with 80% statistical power. The number of study participants needed was lower for EEC day 1 (N = 102) and EEC day 2 (N = 62) compared with NAC (N = 168). Because there were no statistically significant differences in the cat allergen-induced symptoms and the reduction in PNIF between EEC day 1 and 2, an observation that may only apply to perennial allergens, the smaller number of participants needed with EEC day 2 outcomes was largely due to reduced SD. This analysis highlights the fact that either NAC or EEC can be used to test a potential therapeutic, but choosing the best challenge method for a study ultimately depends on available resources and specific study objectives. For example, the EEC, besides mimicking natural allergen exposure more closely than the NAC, shows an advantage for sample size, but this should be balanced by other factors such as time, cost, and organizational complexity, where the NAC approach may be more advantageous. It is also important to note that all 5 participants who were either removed or dropped out of the study were in the EEC phase and the reasons for withdrawal were related to lower or upper respiratory tract symptoms or reduction in lung function.

The differences in clinical outcomes between NAC and EEC might be expected to yield different immunologic responses, but our data suggest that although the magnitude of immunologic responses differs slightly, both challenges elicit fundamentally the same local and systemic type 2 responses. This was supported by various methodologies ranging from local expression (RT-PCR) and production (protein assays) of cytokines/chemokines, to changes in systemic cytokine levels, changes in antigen-specific T cells, and to the overall patterns of gene expression in nasal brushings assessed by RNA sequencing.

Measurements of nasal cytokine mRNA through RT-PCR showed a clear type 2 immune response including IL-5, IL-13, CCL26, and CCL2 (Fig E2). Not surprisingly, type 1 cytokine

signal was unchanged or downregulated. This was consistent after both types of allergen challenges. Cytokine protein measurements confirmed the same response pattern and, except for the occasional elevation of a non-type 2 cytokine (such as IL-6), both challenges were concordant. Furthermore, for IL-5 and IL-13, strong correlations between the 2 challenges were observed in the allergen-induced changes in nasal, as well as in serum, cytokines (Figs 5 and E4). It is important to note that the use of an ultrasensitive assay methodology for the first time in an allergen challenge setting allowed us to detect changes in serum type 2 cytokines, which could be a useful biomarker to monitor the response to inhaled allergens. Interestingly, in the nasal RT-PCR measurements, we failed to detect an increase in thymic stromal lymphopoietin and IL-33 expression after challenge, epithelial products also strongly associated with type 2 immune responses. Increases in alarmin expression have been observed after bronchial allergen challenge in at least 1 other human study.²² Location, or timing of sampling, or different allergens used may explain the inconsistency in outcomes.

Nasal brushing transcriptomics yielded biologically insightful results that were associated with clinical responses. Specifically, we identified 2 relevant networks of genes that showed opposite change patterns in response to the allergen challenges and were significantly associated with both clinical measurements (ie, TNSS and PNIF) and local and systemic type 2 cytokine measurements. We saw upregulation of a gene module (M3) characteristic of multiple aspects of allergic inflammation including mucin and lysozyme production, upregulation of histamine, bradykinin, and prostaglandin receptors, and mucosal healing. The direct association of this gene network to clinical symptoms and changes in nasal patency is consistent with these biologic pathways having a direct role in the severity of the allergic response. Furthermore, this association directly links the clinical response with local and systemic type 2 inflammation, specifically IL-5 and IL-13 production. As an inverse finding, we observed a decrease in a gene module (M4) that is related to negative regulation of transcription. This suggests that, in addition to induction of type 2 immune response genes, induction of the allergic immune response requires a disinhibition of transcriptional regulators of epithelial cell function and offers an opportunity for more precise mechanistic outcomes and potential biomarkers that can be tested in future allergen challenge and interventional studies. Because the prechallenge nasal RNA was collected at a different time of day than the postchallenge sampling, we cannot rule out diurnal variation playing a role in the gene expression differences we observed. However, given that the M3 results are biologically relevant and directly linked to physiologic changes, it is unlikely that diurnal variation was a major contributor to our observations.

Because the analysis was based on nasal brushings, we assumed that most cells were nasal epithelial cells. Therefore, it was expected that many of the differentially expressed genes represent epithelial functions, such as the upregulation of ciliary genes (M1), and barrier secretory molecules such as *MUC5AC* and *LYZ* (M3). However, we also observed changes in the expression of many genes typically associated with leukocytes rather than epithelial cells. In particular, in M3, several genes including *NR4A1*, *NR4A2*, *NR4A3*, *GFOD1*, *FOSB*, and *CD69* are known to be expressed by mast cells, eosinophils, and/or basophils, suggesting that part of the observed response derives from

intraepithelial inflammatory cells relevant to the allergic response. All these cell types are known to undergo transepithelial migration during symptomatic allergic rhinitis, whereas T cells tend to compartmentalize in tissues. It may be that a higher proportion of such cells in the epithelium plays a direct role linking the observed epithelial response with type 2 cytokine levels and the magnitude of clinical response.

Our findings are consistent with and expand upon previous work. Ahuja et al¹² previously found that expression levels of 10 individual genes positively associate with symptom scores collected during EEC with house-dust mite allergen.¹² Two of the genes they described, CD69 and CLC, are components that we found coexpressed within a larger module (M3) that represents the multifaceted epithelial inflammatory response. Furthermore, our work demonstrates the link between this immune response at the epithelium and local and systemic type 2 inflammation.

Several limitations ought to be mentioned: first, the 2 challenges differed with respect to the total dose of allergen delivered to the nose; to match the 2 challenges would require technology not practical to implement. Most importantly, our intention was to compare the 2 challenge methodologies in the manner they are regularly applied in research protocols. Second, our study was only conducted with cat allergen and one should be cautious in extrapolating the results to other allergens given potentially different direct effects on the epithelium and on the innate immune system. Third, the participants/staff were not blinded to the allergen challenge that they received/administered, something that would have required 2 additional sham challenges; the consequences of this limitation are unknown. Fourth, the study was not powered to detect all immunologic differences between the 2 types of challenges. It is, therefore, possible, that, with higher numbers of participants, more differences between the NAC and the EEC outcomes would have been detected. However, the strong similarities that were observed between the 2 challenges in immunologic outcomes and nasal transcriptomics offer substantial certainty to our conclusions.

In summary, our study demonstrates that although the different methodologies of allergen challenge yield different clinical responses, immunologic responses are very similar. In addition, molecular analysis of local tissue responses correlates well with clinical symptoms and local and systemic immunologic measurements. Furthermore, the data presented in this article can be used to guide the choice of allergen challenge methodology in future trials and *in vivo* mechanistic studies of respiratory allergy.

We acknowledge other Cat EEC vs NAC study contributors: Rebecca Parkin, Jorge Pardo, Judith Evind, Kristina Harris, and Peter Sayre. We also thank the patients for their participation in the study.

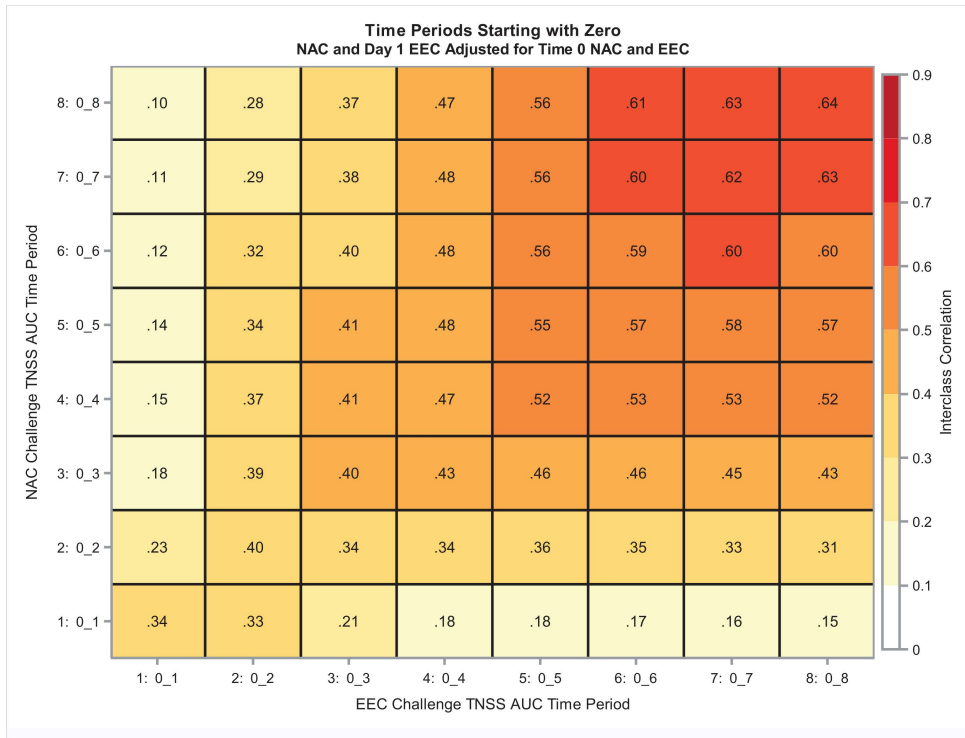
Key messages

- NAC and EEC did not correlate in regard to symptom magnitude or temporality.
- Immunologic responses correlated well between challenges, indicating similar biological pathways are elicited by both allergen challenge methodologies.
- Gene expression changes of local tissues correlated well with clinical and immunologic responses.

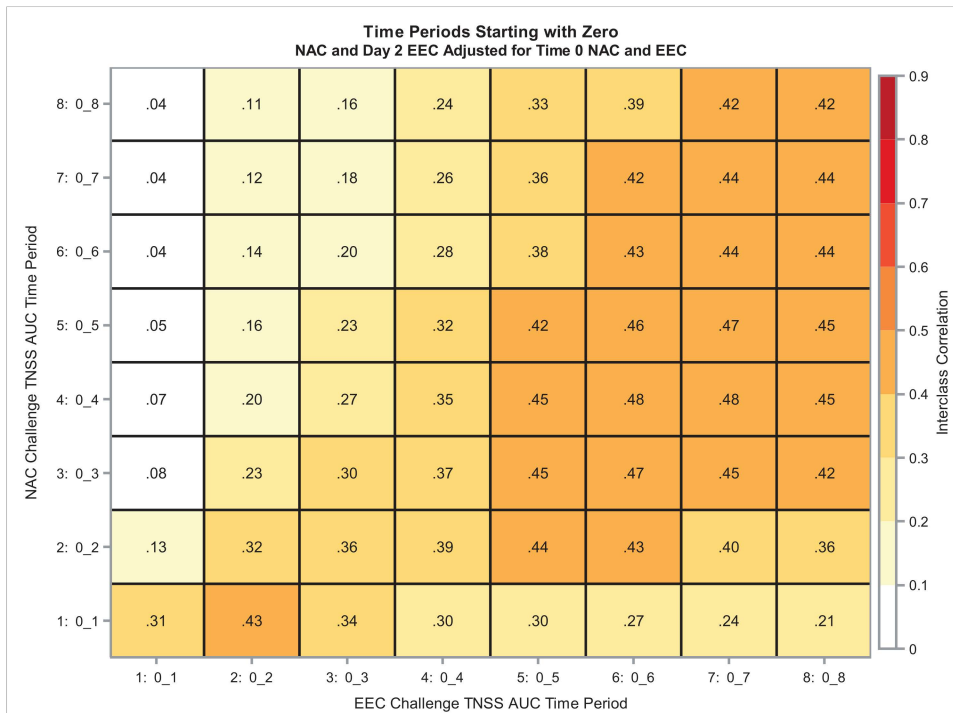
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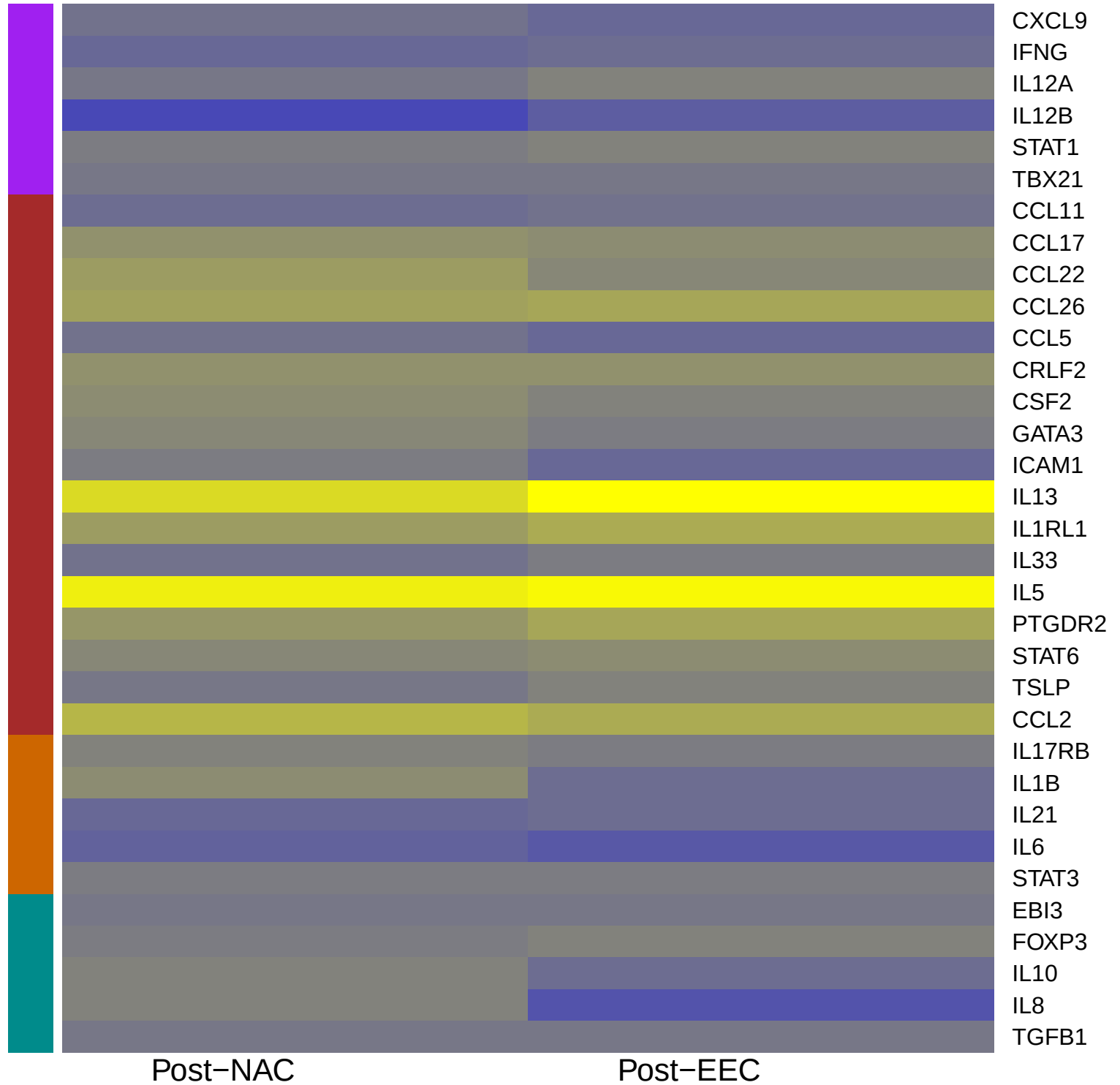
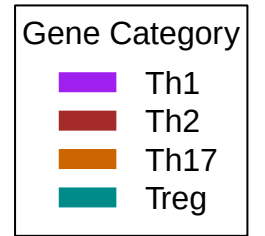
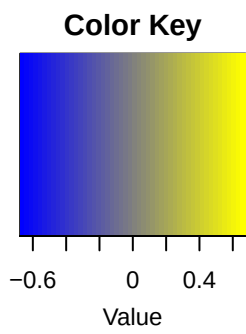
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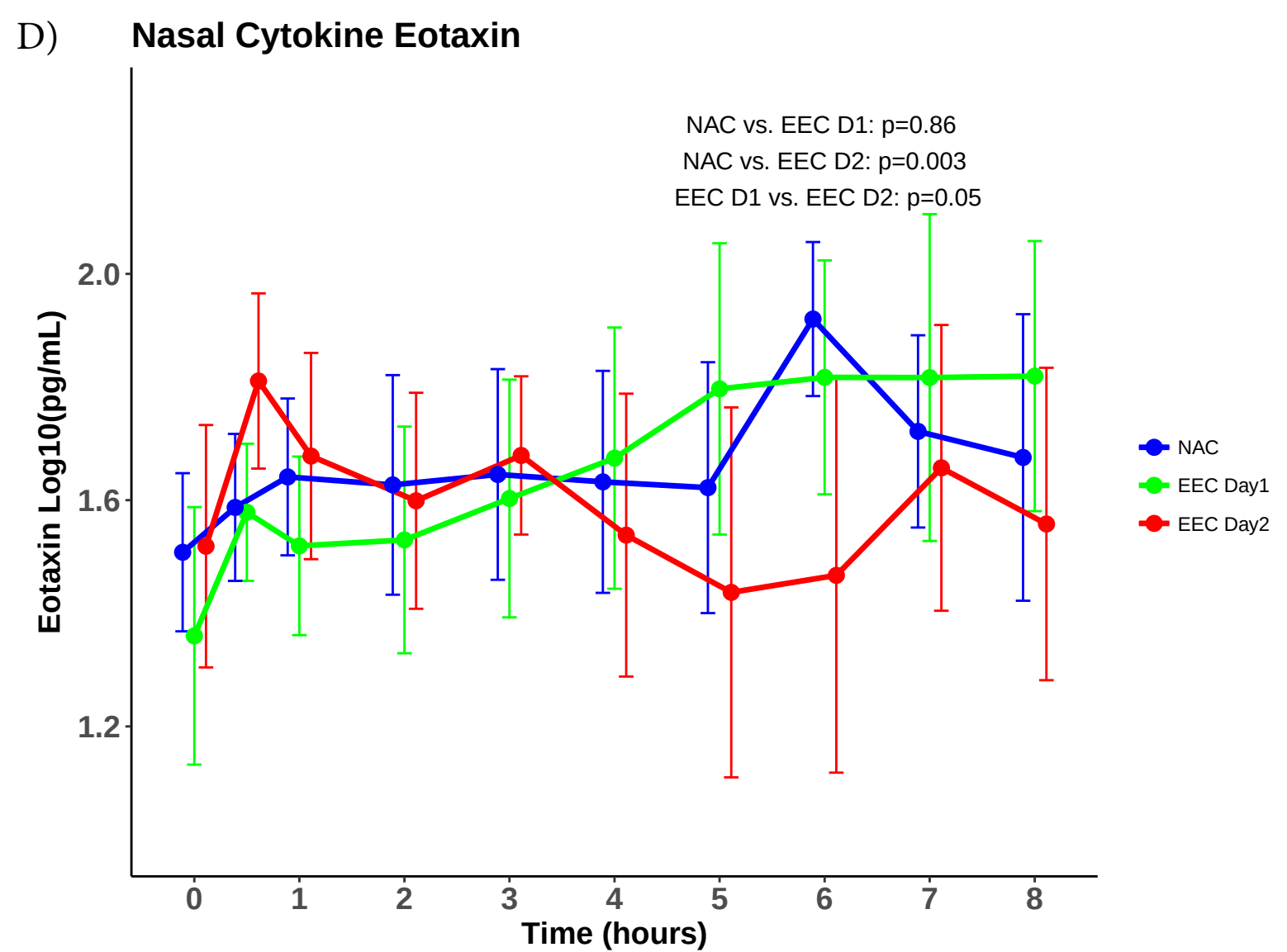
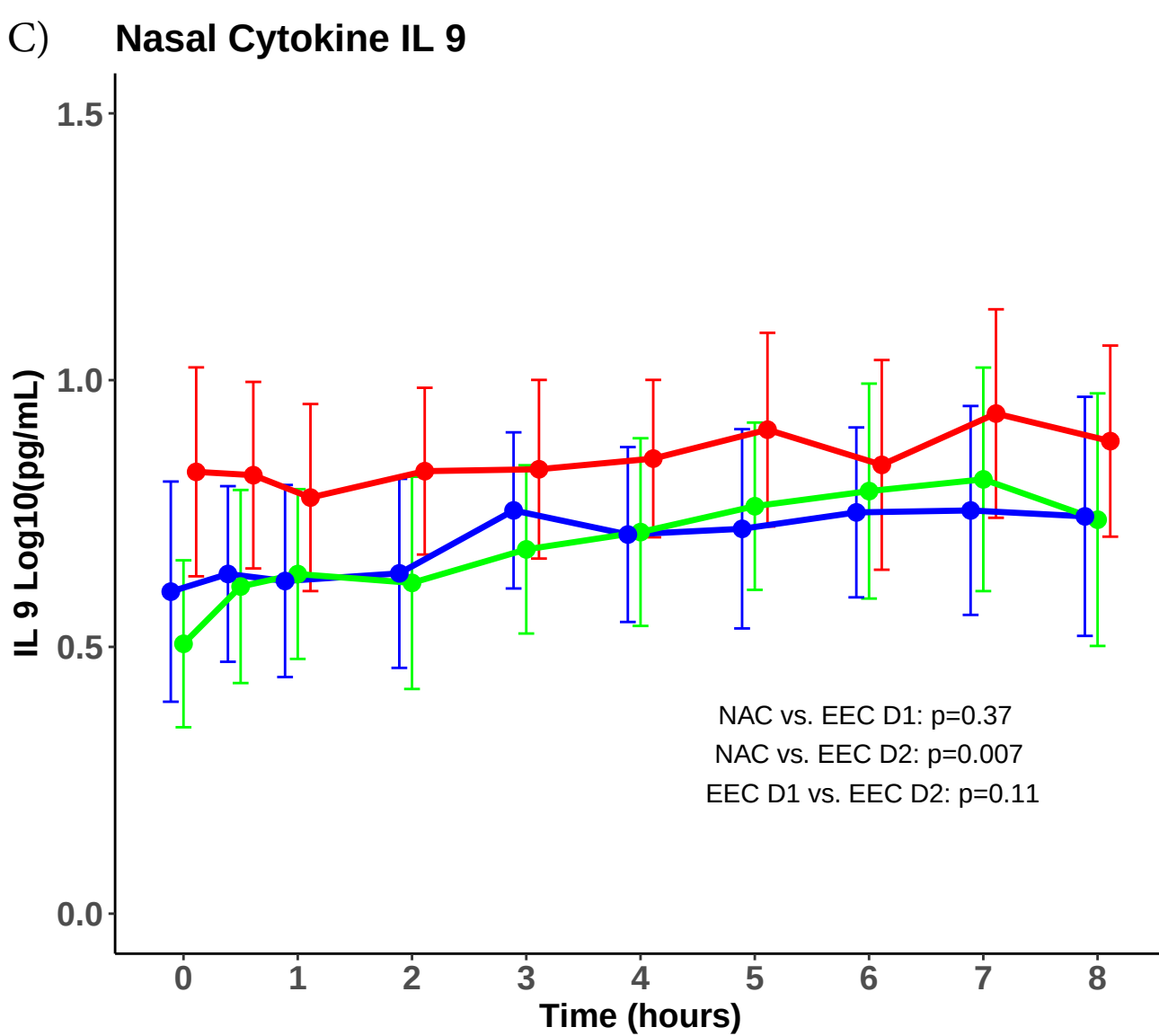
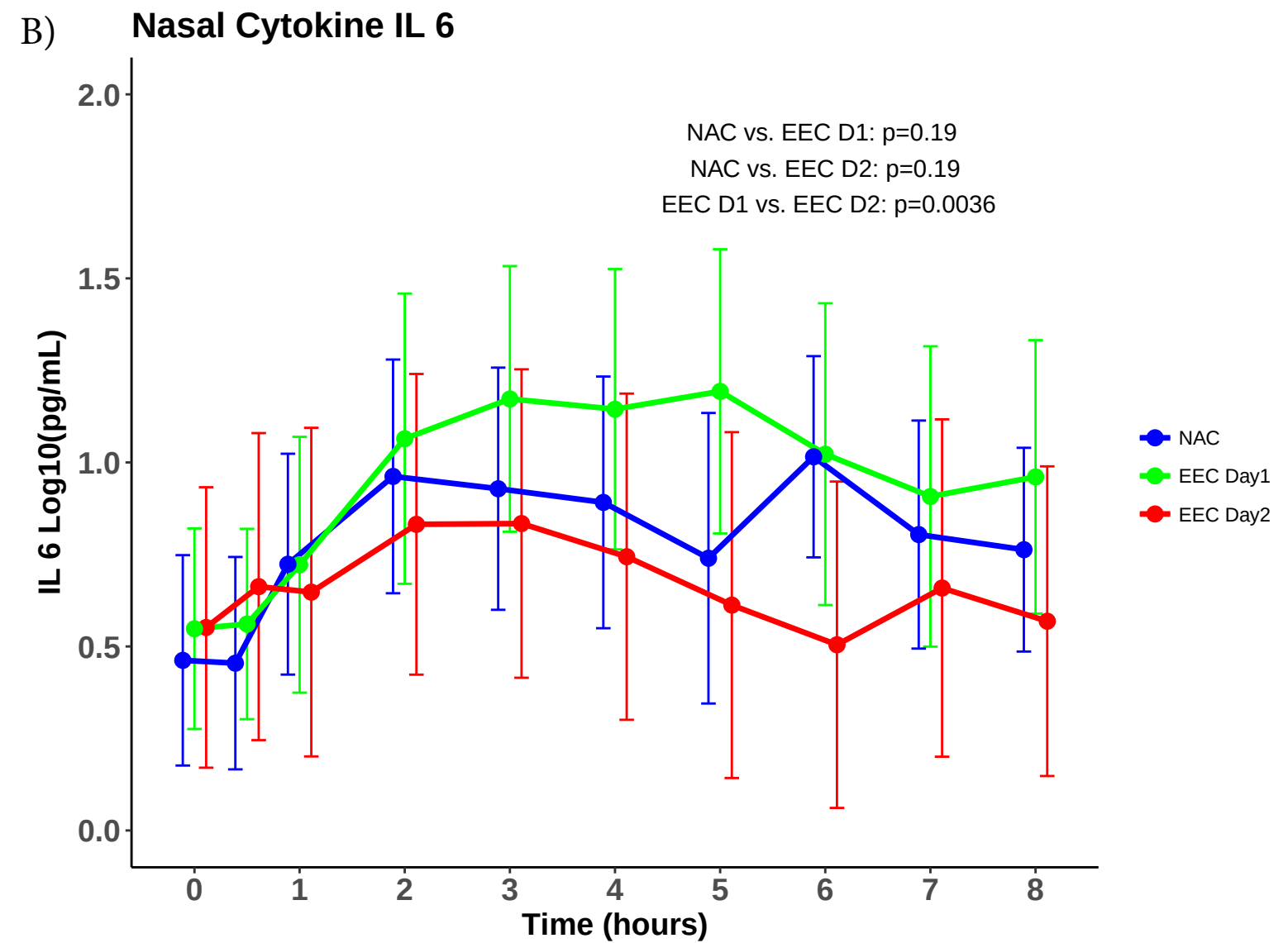
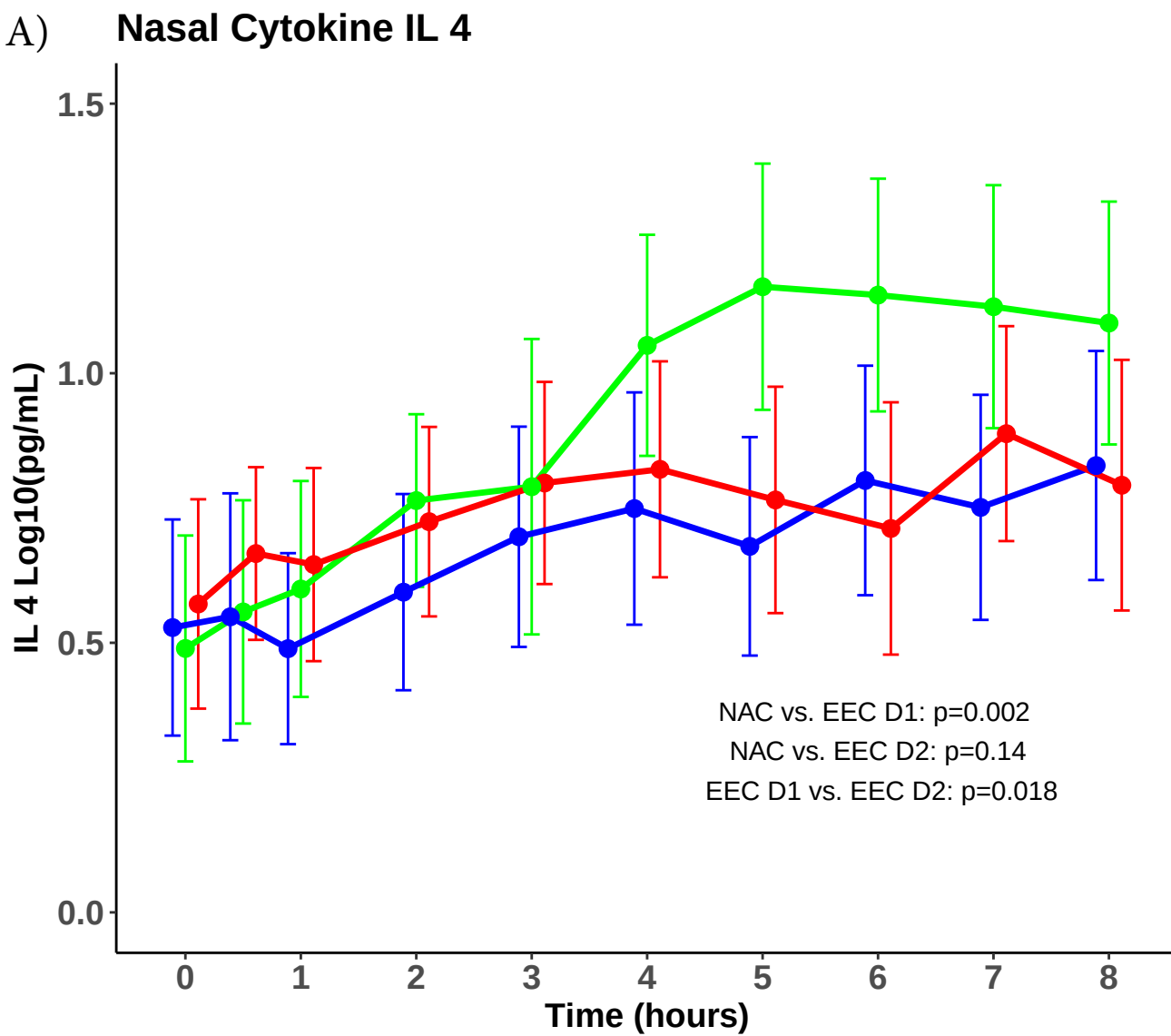


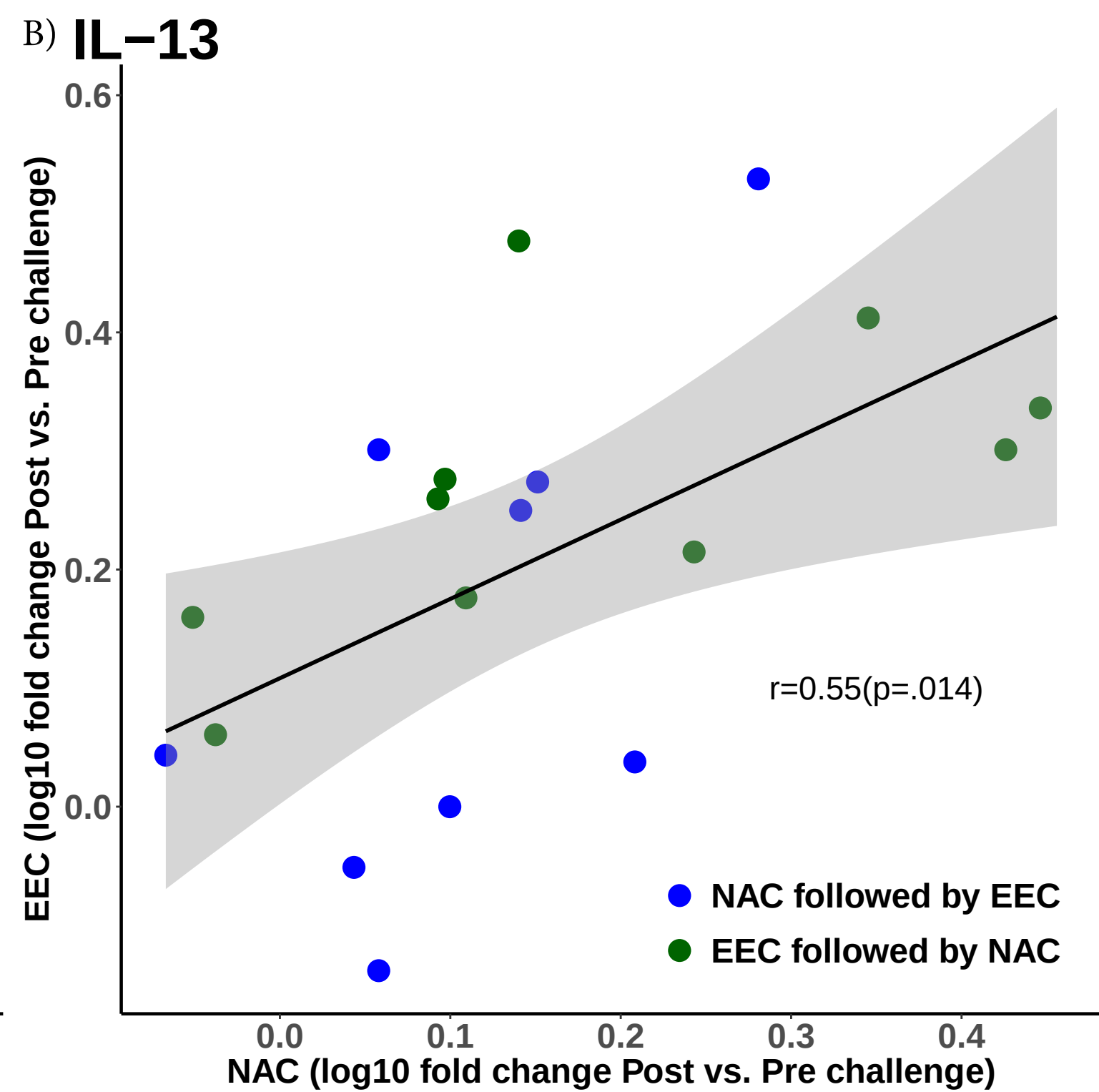
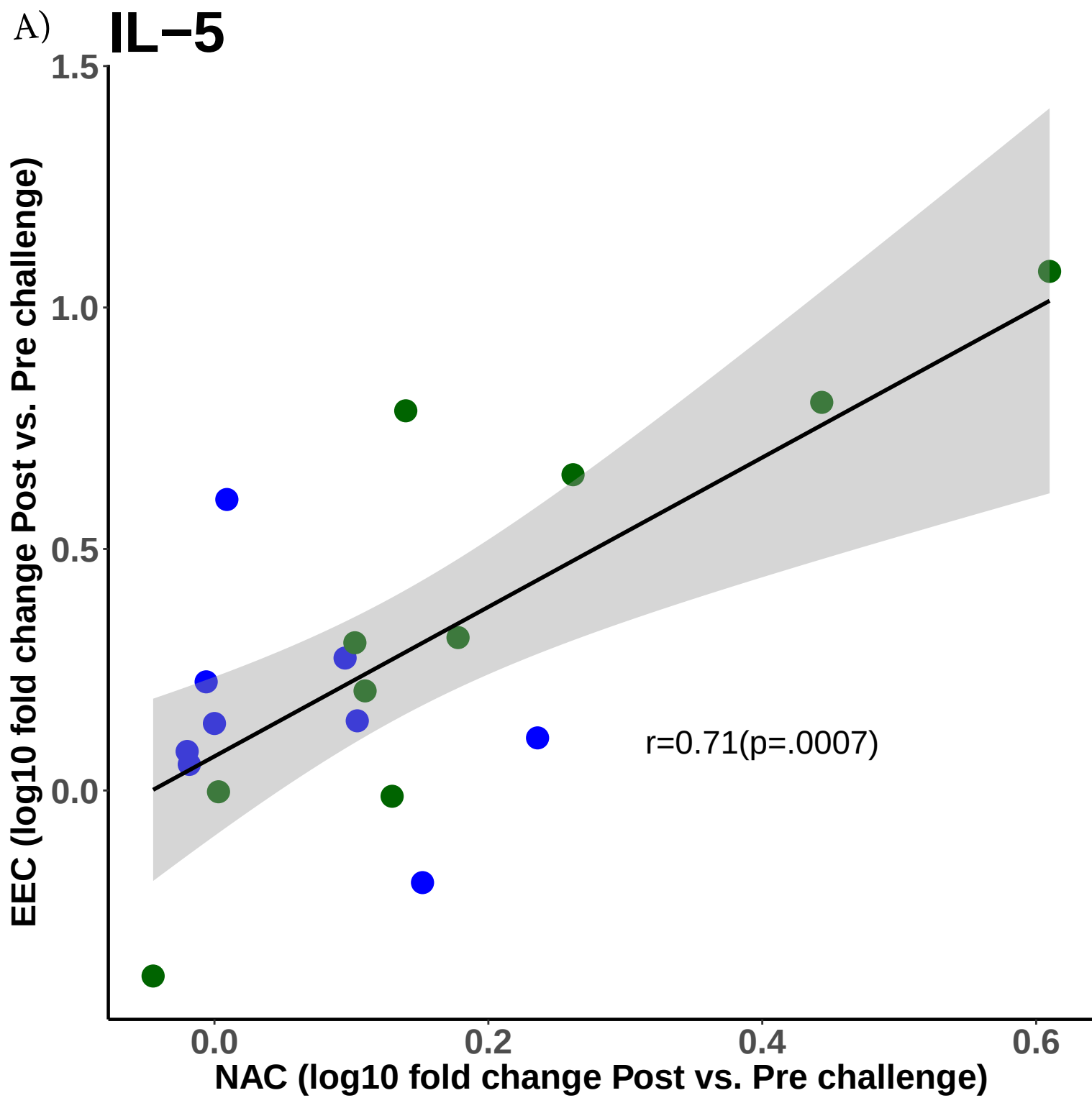
B



Nasal Brushing RT-PCR Expression (log10 fold change from pre-challenge)

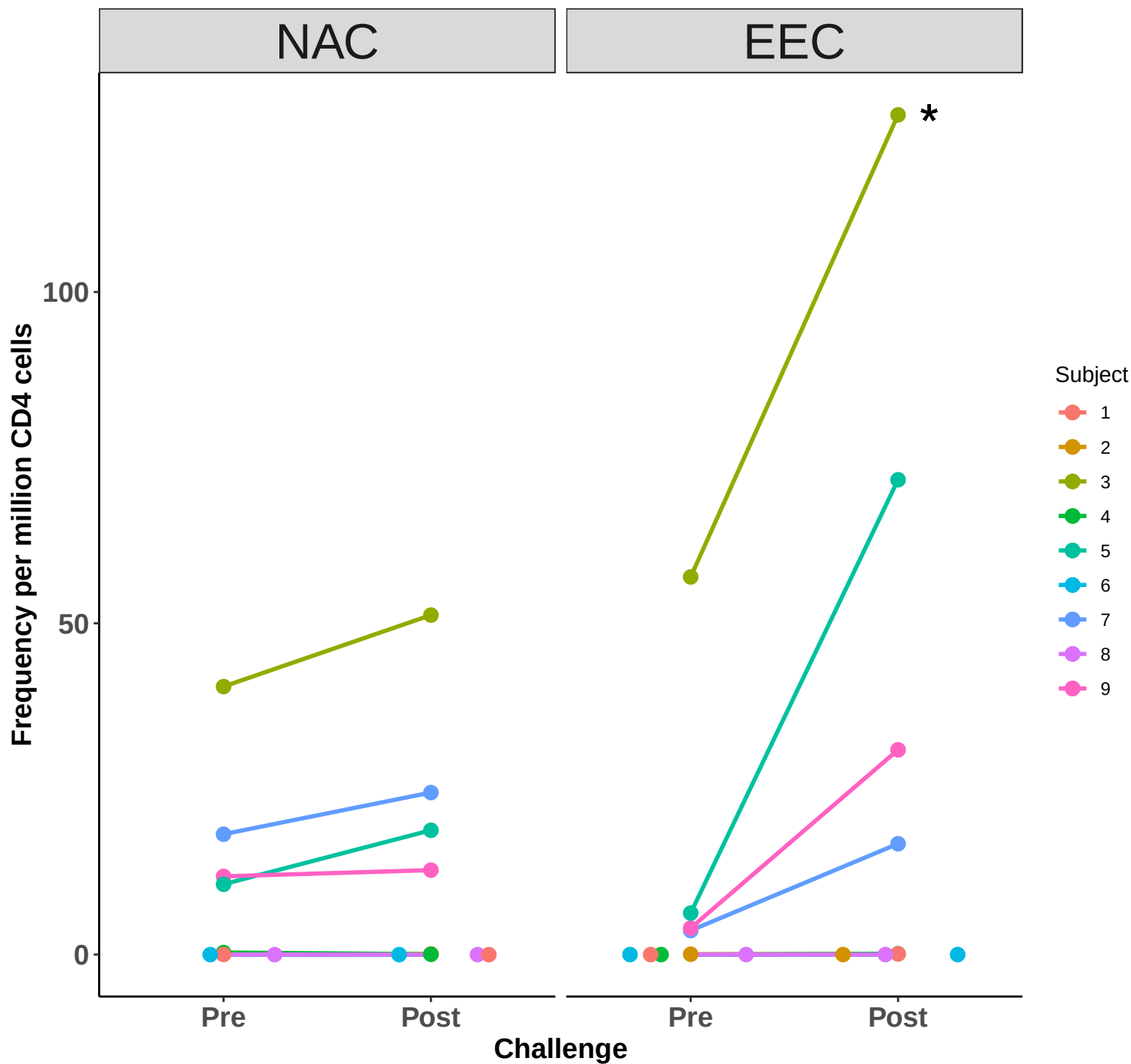


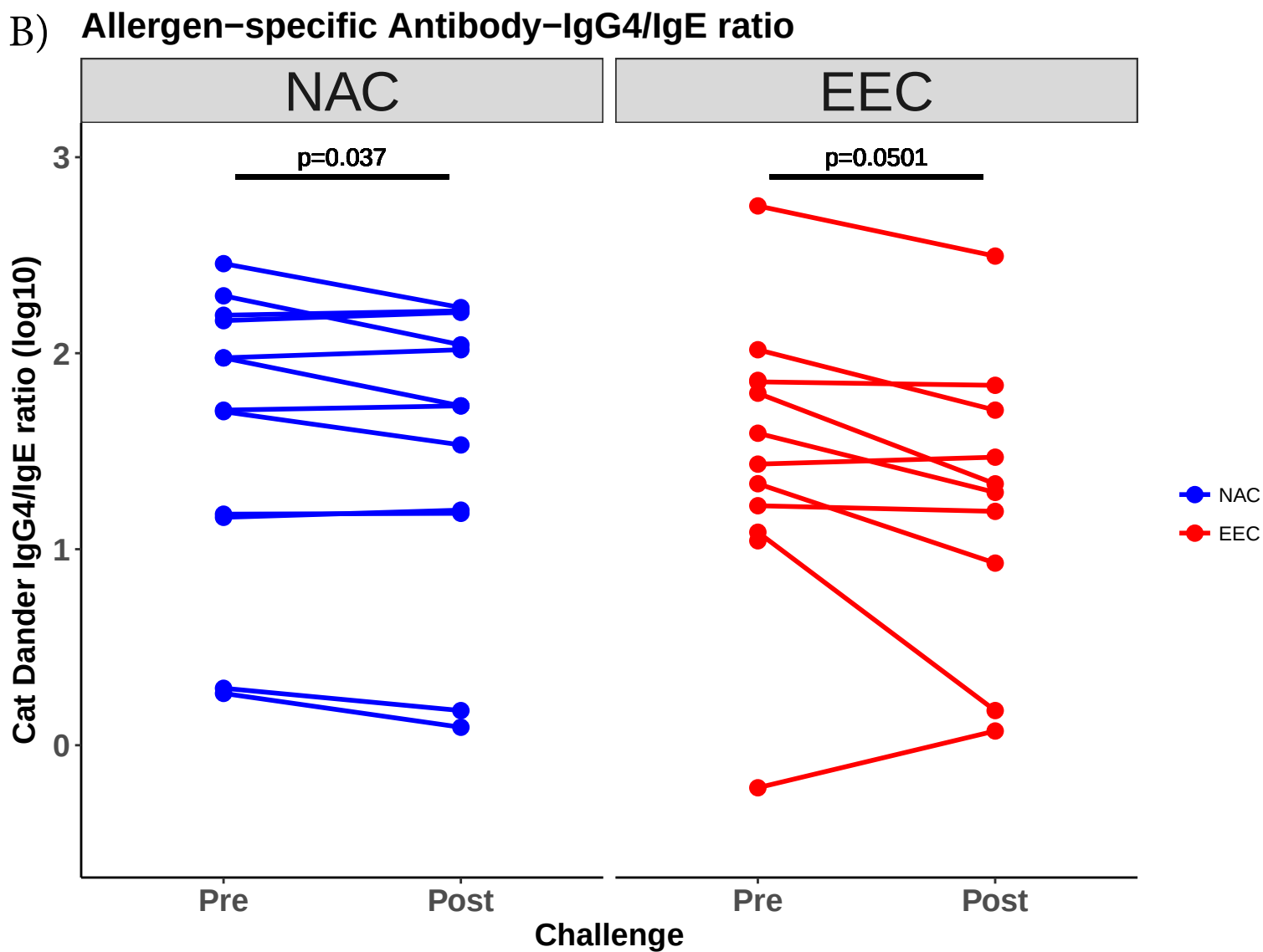
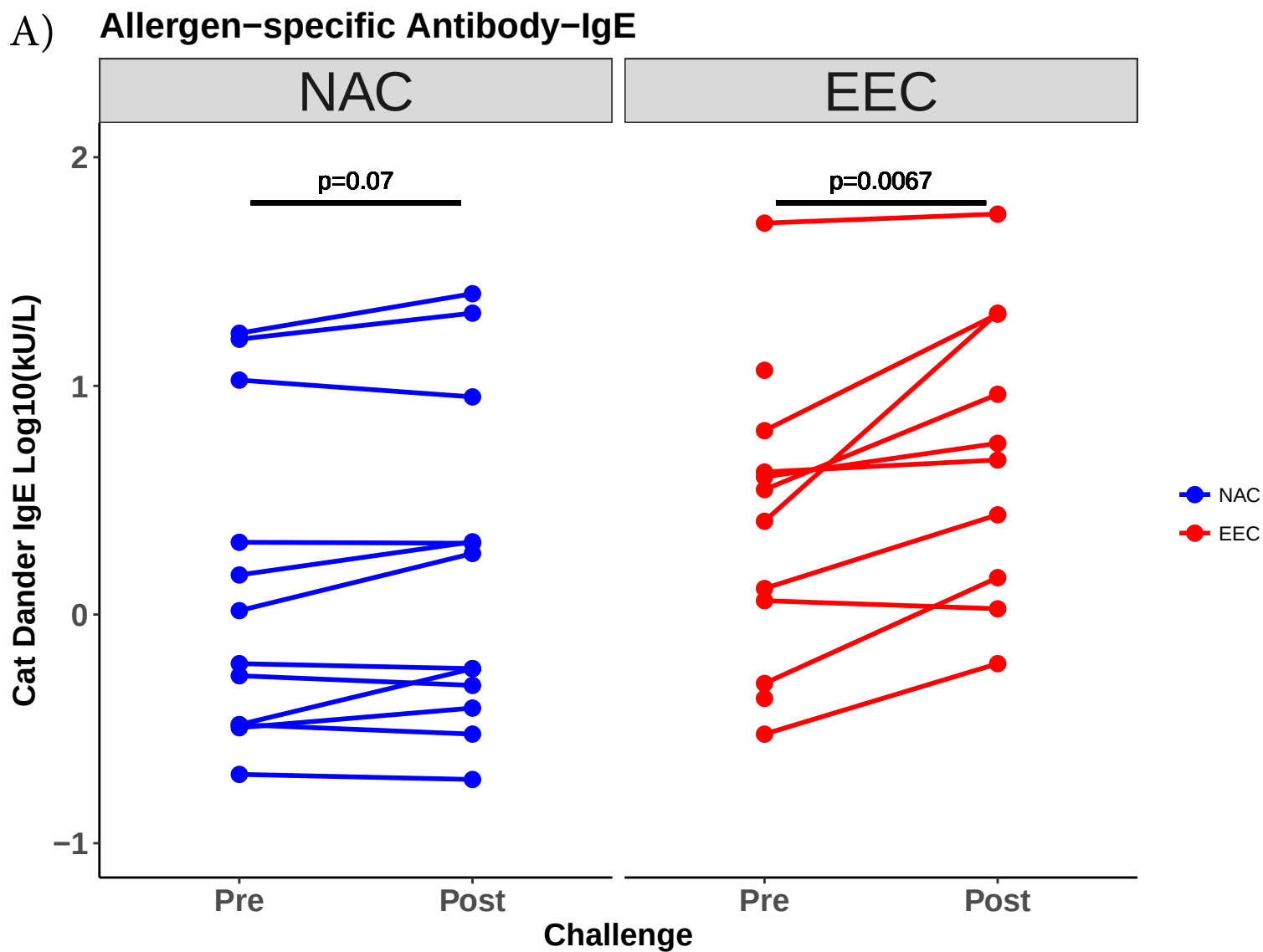




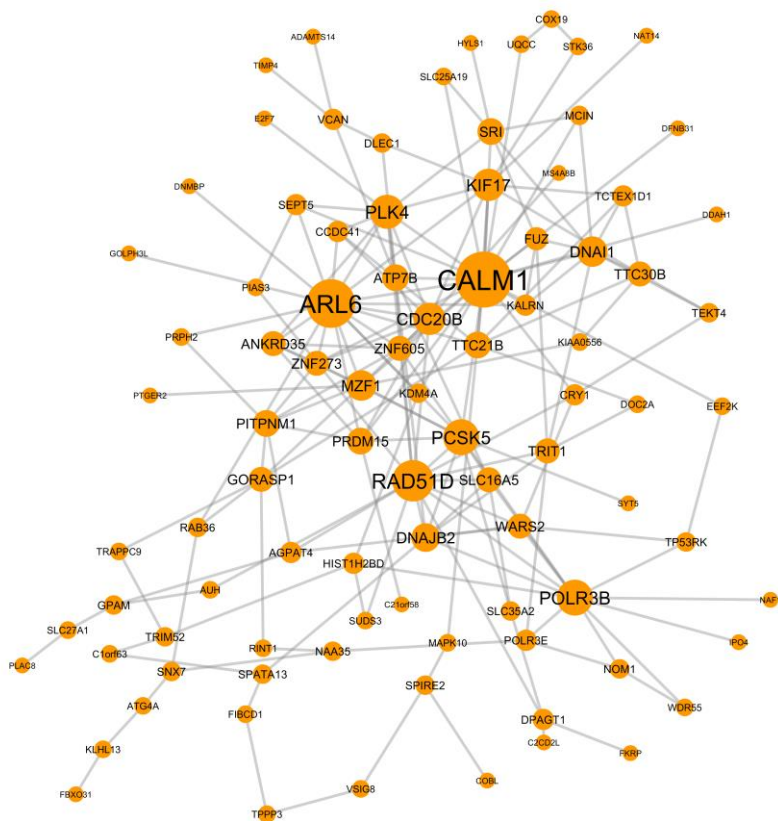
Allergen-specific Cellular Data

Th2 cells (CD4+ Trm+ CD45RA- CRTH2+) per million CD4 cells

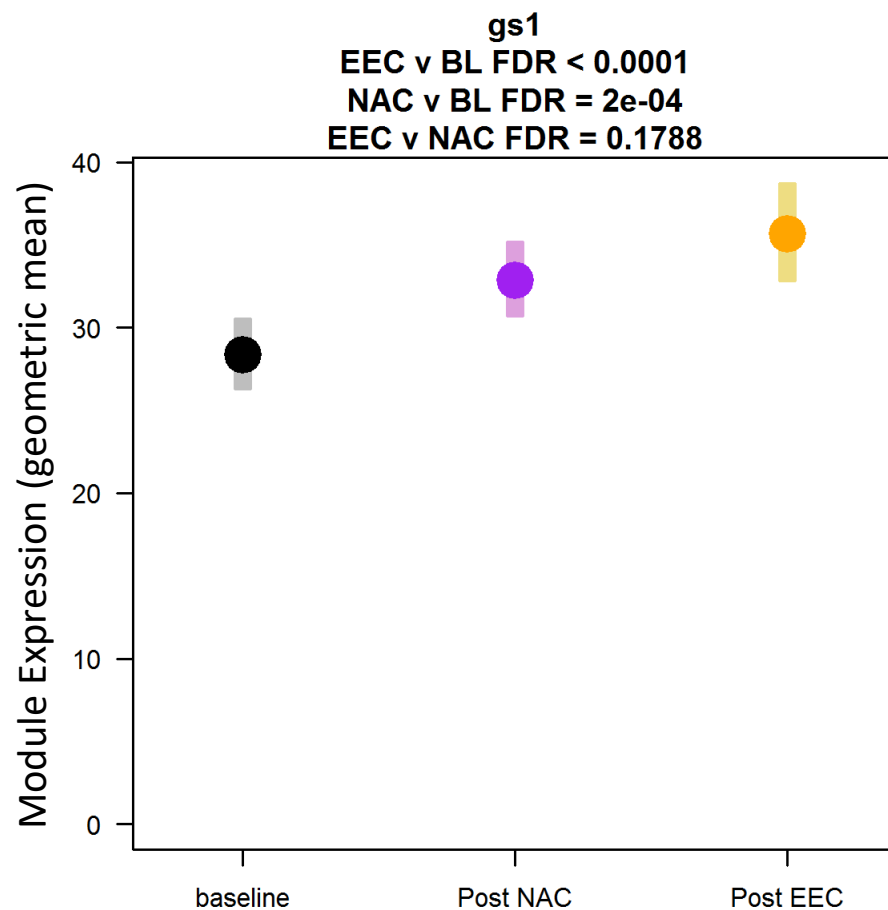




A) Module 1 - network



B) Module 1 - expression



Online Repository Material

Nasal Allergen Challenge and Environmental Exposure Chamber Challenge: A Randomized Trial Comparing Clinical and Biological Responses to Cat Allergen

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E-Methods:

Amount of Allergen Used in Challenges

For NAC, 6 participants with cat allergy (history of at least 2 years of cat-induced rhinoconjunctivitis, and SPT $\geq$ 5 mm to cat allergen) underwent a single-visit, stepwise dose-escalating nasal allergen challenge, to identify the most appropriate dose of allergen to use in the randomized phase. Considerations for choosing the most appropriate dose included tolerability and obtaining a range of TNSS. Cat allergen (Greer Laboratories, NC, USA) at a concentration reported by the manufacturer of 14.4 mcg/mL of Fel d1 was used for challenges. Four 3.3-fold dilutions in normal saline were made for the following concentrations: 4.4 mcg/mL, 1.3 mcg/mL, 0.4 mcg/mL, and 0.1 mcg/mL. Disposable Bi-dose nasal applicator devices (Aptar Pharma, Crystal Lake, IL, USA), were used to deliver one 100 μ L spray of allergen to each nostril. The challenge began at 0.1 mcg/mL, with increasing concentrations administered at 10-min intervals, with response (TNSS and PNIF) recorded immediately before the next dose, continuing to the 14.4 mcg/mL undiluted concentration. The 4.4 mcg/mL concentration was selected for NAC challenges in the randomized phase based on this pilot experiment.

For EEC, the amount of cat allergen inhaled during EEC was estimated using the following formula:

Total volume of air inhaled per study participant = 0.0005 m^3 (tidal volume) $\times$ 14 (respiratory rate per minute) $\times$ 360 (total minutes in the EEC over 2 days) = 1.26 m^3 .

Total dose of allergen = 1.26 m^3 of inhaled air $\times$ 37.7 ng of Fel d 1 per m^3 (based on previous EEC work from Inflamax*) = 75.4 ng Fel d 1 entered each participant's airways (total dose)

* In past Inflamax studies, airborne samplers were placed within the EEC and average airborne cat allergen (Fel d1) was measured by ELISA (Indoor Biotechnologies, VA, USA) to estimate levels of cat allergen per m^3 . The amount of Fel d 1 as a function of particle size was not known and, therefore, particle size could not be considered when estimating the amount of Fel d1 deposited to the nasal cavities.

Tetramer assays

To determine frequencies of Fel d 1-specific CD4 T cells before and 7 days after allergen challenges were initiated, MHC class II tetramers and flow cytometry were utilized. Tetramers for 8 HLA types were used for experiments and included tetramers for HLA-DRB1:0101, HLA-DRB1:0301, HLA-DRB1:0401, HLA-DRB1:0701, HLA-DRB1:1101, HLA-DRB1:1301, HLA-DRB1:1401, and HLA-DRB5:0101. For *ex vivo* tetramer staining, PBMCs from subjects with appropriate HLA genotypes were isolated from fresh whole blood, and CD4 cells were enriched by negative selection (Stem Cell Technologies). CD4 cells were first treated with dasatinib at a final concentration of 50 nM in order to improve tetramer staining¹ and then cells were stained with PE-labeled pooled tetramers (10 μ g/mL final concentration) for 100 minutes at 37°C. Tetramer-positive cells were enriched using the EasySep PE Positive Selection Kit (Stem Cell Technologies, USA) and then stained with anti-CD3 BV421 (BD Biosciences, USA), anti-CD4 FITC (Biolegend, USA), anti-CD14 BV510 (Biolegend, USA), anti-CD19 BV510 (Biolegend,

USA), anti-CD45RA PerCP-Cy5.5 (BD Biosciences, USA), and anti-CRTH2 AF647 (BD Biosciences, USA) for 30 minutes at room temperature. Frequency of Fel d 1-specific Th2 cells was expressed per million CD4+ T cells (number of CD4-positive, tetramer-positive, CD45RA-negative, CRTh2-positive cells X 1 million / the number of CD4+ T cells stained for enrichment).

Serum Antibody Measurements

Levels of cat dander-specific IgE and IgG4 were measured using an ImmunoCAP FEIA system (Viracor-IBT Laboratories, Inc., MO, USA).

Nasal Brushing RNA Extraction

Samples were thawed, vortexed, and quick-spun, and the supernatant transferred to fresh tubes. Seventy percent EtOH was used to wash the brushes and original tubes, which was then transferred to the fresh tubes. The samples were then spun through a Qias shredder column (Qiagen) and extracted using RNeasy mini kits (Qiagen) with 25 ul elution volumes following the manufacturer's protocol.

RT-PCR

RNA was quantitated using a QIAbit (Qiagen). RNA was diluted and cDNA synthesised using the Superscript VILO cDNA synthesis kit (Invitrogen) according to the manufacturer's instructions. Ten microliters TaqMan Gene Expression Master Mix (Thermo Fisher), 1 ul TaqMan Gene Expression Assay (Thermo Fisher) (primer details listed in Table E1) and 9 ul cDNA template with RNA free water was mixed to make 20 ul reactions. Samples underwent 40 cycles of amplification on Applied Biosystems 7900HT Real-Time PCR System in an ABI PRISM™ 384-Well Clear Optical Reaction Plate (Applied Biosystems) and analysed using Thermo Fisher's online Cloud platform using housekeeping genes EEF2, GAPDH and RPLP0. <https://www.thermofisher.com/uk/en/home/digital-science.html>.

RNA-Sequencing

RNA quality was assessed using RNA electrophoresis (Agilent) and NanoDrop 1000 (NanoDrop Products, Thermo Fisher Scientific). Sequencing libraries were constructed from total RNA using TruSeq RNA Sample Preparation Kits v2 (Illumina) and clustered onto a flowcell using a cBOT amplification system with a HiSeq SR v4 Cluster Kit (Illumina). Single-read sequencing was carried out on a HiSeq2500 sequencer (Illumina), using a HiSeq SBS v4 Kit to generate 58-base reads, with a target of approximately 10 million reads per sample. Samples were sequenced in 2 batches using repeated samples across batches to allow assessment for batch effects. Processing batch did not show a batch effect on gene expression variability, so the replicate with the highest percent aligned was included for analysis and the other replicate removed. The final dataset included 64 nasal samples and 14,949 genes.

Bcl files were deconvoluted and converted to fastq format using Casava from Illumina. Fastq files were aligned to the Ensembl version of the human genome (GRCh38) (GenBank assembly accession id GCA_000001405.15), using TopHat (version 1.4.1).² The single-paired flag was set to "single," while all other TopHat parameters were set to defaults. HTSeq-count³ was used to generate gene counts with mode as "Intersection (nonempty)" and minimum alignment quality set to 0 and otherwise set to default parameters. For quality control, samples were kept that had counts >1 million, percent of reads aligned >80% and median CV coverage <1. Genes were filtered to include those that had a trimmed mean of M values (TMM) normalization count of at least 1 in at least 10% of libraries and were classified as protein coding using BioMart.^{4, 5} Each of the 2 sample batches was independently normalized within its batch using TMM normalization and counts were transformed to log2 counts per million along with observations level weights using voomWithQualityWeights from the limma R package.⁶

Statistics for clinical and mechanistic data

Least squares (LS) mean TNSS AUC was estimated for both challenges as well as the difference between the 2 challenges. Estimates of the LS means and ICC for the TNSS AUC were derived from a repeated measures model assuming a compound symmetry covariance structure among the two challenges within a participant. The model adjusted for challenge (NAC or EEC), challenge sequence (NAC then EEC or EEC then NAC) and challenge period (first or second).

For comparisons of nasal fluid cytokine levels, the 2-8 hour AUC was calculated for each cytokine for NAC, EEC day 1, and EEC day 2 challenges. Pearson correlation coefficients (r) and p-values were calculated using log10 2-8 hour AUC data for NAC, EEC Day 1, and EEC Day 2.

A t-test was performed on nasal RT-PCR data to determine whether there was a significant increase in gene expression from study baseline (no allergen challenge) to 8 hours post NAC or 8 hours post EEC challenge.

Statistical method used for the analysis of RNA-Sequencing data

Differences in gene expression were assessed comparing RNA-sequencing of the post-NAC, post-EEC, and baseline nasal brush samples. Differences were tested using a weighted linear model (limma in R) appropriate for RNA-seq data and empirical Bayes method.⁴ The primary model included terms to account for percent aligned, which contributed variability to the data, and sequencing batch. Because multiple samples came from each participant, a random effect was included for participant. Multiple testing correction was performed using the Benjamini-Hochberg method and genes with false discovery rates (FDR) < 0.05 were considered significant.

Genes that showed significant differential expression among the 3 timepoints after multiple testing correction (FDR<0.05) were coassociated into modules using weighted gene correlation network analysis (WGCNA).⁷ Parameters used were: power=7, which was the lowest power for which the scale-free topology fit index reached 0.90; networkType="signed" and TOMType="signed" to cluster positively correlated genes, minModuleSize=15 and

maxBlockSize=600 to control the size of modules, and deepSplit=4 to maximize sensitivity of module detection. Module level expression values were calculated using the geometric mean of the genes assigned to a module.

Differential expression of modules among the 3 timepoints was analyzed using the same weighted linear model that was used for differential gene expression. The changes in module expression from the baseline sample to post-challenge samples were calculated and associated to cytokine measurements and TNSS by Pearson correlation.

Pathway analysis

The biological function of modules was investigated using Database for Annotation Visualization and Integrated Discovery version 6.8.^{8, 9} This database uses a modified Fisher exact test to identify specific biological/functional categories that are overrepresented in gene sets in comparison with a reference set (the default human genome was used as the reference set). Each module was submitted and all available enrichment categories downloaded and ranked according to FDR. Modules were considered to have significant enrichment if one or more of the DAVID default categories showed enrichment with an FDR<0.05.

STRING version 10.5,^{10, 11} which is a database of known and predicted protein-protein interactions was used to determine interaction networks of each module. All STRING interaction sources were used and a minimum interaction score of 0.15 was required for a gene to be included within a network. An interaction network was significant if its protein-protein interaction enrichment p-value was <0.05 meaning it has more interactions than expected from a random set of proteins of the same size drawn from the human genome. Cytoscape version 3.5.1¹² was used to draw interaction networks according to a prefuse force directed layout using the combined interaction score exported from STRING. The size of each gene was made proportional to the number of interactions in the network as a percentage of the maximum number of interaction of any node in that network. Unconnected nodes were excluded.

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E-Tables:

Table E1:
Genes and primers used for RT-PCR work.

Table E2:
Baseline demographic and clinical characteristics of participants randomized into the study by group.

Table E3:
List of adverse events that occurred during the CAT EEC study and whether AE resulted in termination from the study.

Table E4:
Relationship of cytokine expression and protein levels with clinical response to allergen challenge.

Table E5:
Differentially Expressed Gene List. Genes listed by ensembl id and gene symbol that were upregulated (A) or downregulated (B) following NAC or EEC, along with the fold changes in expression from baseline and associated p-values.

Table E6:
Module gene lists. Complete list of modules and their constituent genes.

Table E7:
Module 3 correlations with clinical and cytokine variables.

Table E8:
Module 4 correlations with clinical and cytokine variables.

E-Figures:

Figure E1:

Correlation of NAC TNSS scores with TNSS scores of EEC Day 1 (A) or EEC day 2 (B) at various time points after allergen challenge.

Figure E2:

Transcriptional analysis of RNA extracted from nasal brushes at study baseline and after NAC and EEC. Expression levels are displayed in a color scale from high (yellow) to low (blue) with genes grouped by category (purple = Th1, red = Th2, orange = Th17, and green = Treg).

Figure E3:

Levels of IL-4 (A), IL-6 (B), IL-9 (C), and eotaxin (D) in nasal fluid collected pre-challenge and up to 8 hours after allergen challenge.

Figure E4:

Relationship between serum cytokines elicited by NAC and ECC. Pearson correlations of log₁₀ fold changes pre to 6 hours post NAC vs. log₁₀ fold changes pre to 6 hours post EEC Day 2 for IL-5 (A) and IL-13 (B). Participants colored by order of challenge received first (blue = NAC followed by EEC and green = EEC followed by NAC).

Figure E5:

Analysis of allergen-specific CD4 responses. Cat allergen-specific CD4 responses were measured before and 7 days after NAC or Day 1 EEC in HLA compatible CAT EEC study participants. Frequencies of allergen-specific Th2 cells for 9 participants with measurable tetramer positive cells determined by flow cytometry. * = participant who did not complete EEC day 2.

Figure E6:

Cat dander-specific antibody responses. Levels of IgE to cat dander were measured in sera pre and 28 days after NAC or EEC (A). IgG4 to IgE ratios were calculated using cat dander-specific IgG4 and IgE levels measured in sera pre and 28 days after NAC or EEC (B).

311 Figure E7:

312 Gene network and module 1 expression. Gene-gene interaction network (A). Normalized gene
313 expression of module 1 at baseline, post NAC, and post EEC (B).

Table E1

Gene	Assay ID
<i>TGFB1</i>	<i>Hs00998133_m1</i>
<i>IL8</i>	<i>Hs00174103_m1</i>
<i>IL10</i>	<i>Hs00961622_m1</i>
<i>FOXP3</i>	<i>Hs01085834_m1</i>
<i>EBI3</i>	<i>Hs01057148_m1</i>
<i>STAT3</i>	<i>Hs01047580_m1</i>
<i>IL6</i>	<i>Hs00985639_m1</i>
<i>IL21</i>	<i>Hs00222327_m1</i>
<i>IL1B</i>	<i>Hs00174097_m1</i>
<i>IL17RB</i>	<i>Hs00218889_m1</i>
<i>CCL2</i>	<i>Hs00234140_m1</i>
<i>TSLP</i>	<i>Hs00263639_m1</i>
<i>STAT6</i>	<i>Hs00598625_m1</i>
<i>PTGDR2</i>	<i>Hs01867513_s1</i>
<i>IL5</i>	<i>Hs01548712_g1</i>
<i>IL33</i>	<i>Hs00174379_m1</i>
<i>IL1RL1</i>	<i>Hs00249384_m1</i>
<i>IL13</i>	<i>Hs00174379_m1</i>
<i>ICAM1</i>	<i>Hs00164932_m1</i>
<i>GATA3</i>	<i>Hs00231122_m1</i>
<i>CSF2</i>	<i>Hs00929873_m1</i>
<i>CRLF2</i>	<i>Hs00845692_m1</i>
<i>CCL5</i>	<i>Hs00982282_m1</i>
<i>CCL26</i>	<i>Hs00171146_m1</i>
<i>CCL22</i>	<i>Hs01574247_m1</i>
<i>CCL17</i>	<i>Hs00171074_m1</i>
<i>CCL11</i>	<i>Hs00237013_m1</i>
<i>TBX21</i>	<i>Hs00203436_m1</i>
<i>STAT1</i>	<i>Hs01013996_m1</i>
<i>IL12B</i>	<i>Hs01011518_m1</i>
<i>IL12A</i>	<i>Hs01073447_m1</i>
<i>IFNG</i>	<i>Hs00989291_m1</i>
<i>CXCL9</i>	<i>Hs00171065_m1</i>
<i>EEF2</i>	<i>Hs00157330_m1</i>
<i>GAPDH</i>	<i>Hs02758991_g1</i>
<i>RPLP0</i>	<i>Hs99999902_m1</i>

Table E2

Demographic and Baseline Characteristics
 Evaluable Sample

	GROUP A NAC Followed by EEC N=12	GROUP B EEC Followed by NAC N=12	Overall N=24
Age at Screening (years)			
N	12	12	24
Mean (SD)	38.3 (12.25)	41.7 (12.48)	40.0 (12.22)
Median (Q1, Q3)	39.0 (26.8, 47.1)	45.2 (30.9, 51.4)	41.3 (29.0, 50.2)
Min, Max	19, 58	24, 62	19, 62
Gender n (%)			
Male	2 (16.7%)	6 (50.0%)	8 (33.3%)
Female	10 (83.3%)	6 (50.0%)	16 (66.7%)
Ethnicity n (%)			
Hispanic or Latino	0	2 (16.7%)	2 (8.3%)
Not Hispanic or Latino	11 (91.7%)	10 (83.3%)	21 (87.5%)
Unknown	1 (8.3%)	0	1 (4.2%)
Race n (%)			
White/Caucasian	6 (50.0%)	4 (33.3%)	10 (41.7%)
Black or African American	6 (50.0%)	4 (33.3%)	10 (41.7%)
Asian	0	1 (8.3%)	1 (4.2%)
Mixed Race	0	2 (16.7%)	2 (8.3%)
Other	0	1 (8.3%)	1 (4.2%)
Cat Skin Prick Test at Screening (mm)			
N	12	12	24
Mean (SD)	6.5 (1.50)	6.7 (0.83)	6.6 (1.19)
Median (Q1, Q3)	6.0 (5.5, 7.3)	6.5 (6.0, 7.5)	6.5 (5.8, 7.5)
Min, Max	5, 10	6, 8	5, 10

Table E2 (cont.)

Demographic and Baseline Characteristics
 Evaluable Sample

	GROUP A NAC Followed by EEC N=12	GROUP B EEC Followed by NAC N=12	Overall N=24
Allergic Rhinitis due to Cat Exposure (years)			
N	12	12	24
Mean (SD)	18.8 (13.40)	20.3 (10.08)	19.5 (11.62)
Median (Q1, Q3)	15.0 (8.0, 31.5)	20.0 (12.5, 27.5)	20.0 (9.5, 30.0)
Min, Max	2, 40	5, 40	2, 40
Cat Allergen Levels in House Dust (ug/g)			
N	12	11	23
Mean (SD)	25.3 (86.94)	0.3 (0.70)	13.3 (62.79)
Median (Q1, Q3)	0.0 (0.0, 0.3)	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)
Min, Max	0, 301	0, 2	0, 301
Asthma Diagnosis at Screening n (%)			
Yes	2 (16.7%)	1 (8.3%)	3 (12.5%)
No	10 (83.3%)	11 (91.7%)	21 (87.5%)
Cat Dander IgE (KU/L)			
N	12	12	24
Mean (SD)	4.2 (6.42)	7.3 (14.29)	5.8 (10.95)
Median (Q1, Q3)	0.8 (0.3, 6.3)	3.0 (0.8, 5.3)	1.4 (0.5, 5.3)
Min, Max	0.2, 17	0.3, 51.5	0.2, 51.5

Table E3

Subject	AE	Group Allocation	Phase of Study	Reason for Termination?
1	Cough	A	Pre - NAC	No
	Nausea		Post-NAC	No
	Upper respiratory tract infection		NAC	No
	Upper respiratory tract infection		EEC	Yes
2	Headache	B	EEC	No
3	Bronchospasm	A	EEC	Yes
4	Headache	A	Post-NAC	No
5	Bronchospasm	B	EEC	No
6	Headache	A	Post-NAC	No

Table E4

Nasal RT-PCR Data		
Comparison	Pearson Correlation Coefficient (r)	p-value
Nasal IL-13 Expression (post NAC) vs. TNSS (NAC AUC 0-3 hr)	0.26	0.275
Nasal IL-5 Expression (post NAC) vs. TNSS (NAC AUC 0-3 hr)	0.08	0.737
Nasal IL-13 Expression (post NAC) vs. Delta PNIF (NAC AUC 0-3 hr)	-0.51	0.025
Nasal IL-5 Expression (post NAC) vs. Delta PNIF (NAC AUC 0-3 hr)	-0.43	0.056
Nasal IL-13 Expression (post EEC D2) vs. TNSS (EEC D2 AUC 0-3 hr)	0.48	0.057
Nasal IL-5 Expression (post EEC D2) vs. TNSS (EEC D2 AUC 0-3 hr)	0.20	0.437
Nasal IL-13 Expression (post EEC D2) vs. Delta PNIF (EEC D2 AUC 0-3 hr)	-0.09	0.742
Nasal IL-5 Expression (post EEC D2) vs. Delta PNIF (EEC D2 AUC 0-3 hr)	-0.06	0.821

Serum IL-5 and IL-13 Data		
Comparison	Pearson Correlation Coefficient (r)	p-value
Serum IL-13 (post NAC) vs. TNSS (NAC AUC 0-3 hr)	0.44	0.041
Serum IL-5 (post NAC) vs. TNSS (NAC AUC 0-3 hr)	0.05	0.824
Serum IL-13 (post NAC) vs. Delta PNIF (NAC AUC 0-3 hr)	-0.34	0.118
Serum IL-5 (post NAC) vs. Delta PNIF (NAC AUC 0-3 hr)	-0.52	0.012
Serum IL-13 (post EEC D2) vs. TNSS (EEC D2 AUC 0-3 hr)	0.38	0.113
Serum IL-5 (post EEC D2) vs. TNSS (EEC D2 AUC 0-3 hr)	0.42	0.071
Serum IL-13 (post EEC D2) vs. Delta PNIF (EEC D2 AUC 0-3 hr)	0.05	0.851
Serum IL-5 (post EEC D2) vs. Delta PNIF (EEC D2 AUC 0-3 hr)	-0.26	0.278

Nasal Fluid IL-5 and IL-13 Data		
Comparison	Pearson Correlation Coefficient (r)	p-value
Nasal Fluid IL-13 (NAC AUC) vs. TNSS (NAC AUC 0-3 hr)	0.01	0.971
Nasal Fluid IL-5 (NAC AUC) vs. TNSS (NAC AUC 0-3 hr)	-0.14	0.553
Nasal Fluid IL-13 (NAC AUC) vs. Delta PNIF (NAC AUC 0-3 hr)	-0.30	0.182
Nasal Fluid IL-5 (NAC AUC) vs. Delta PNIF (NAC AUC 0-3 hr)	-0.50	0.022
Nasal Fluid IL-13 (EEC D2 AUC) vs. TNSS (EEC D2 AUC 0-3 hr)	0.25	0.306
Nasal Fluid IL-5 (EEC D2 AUC) vs. TNSS (EEC D2 AUC 0-3 hr)	0.27	0.265
Nasal Fluid IL-13 (EEC D2 AUC) vs. Delta PNIF (EEC D2 AUC 0-3 hr)	0.05	0.854
Nasal Fluid IL-5 (EEC D2 AUC) vs. Delta PNIF (EEC D2 AUC 0-3 hr)	0.00	0.997

Table E5A - Upregulated genes

ensemblID	geneSymbol	EEC Fold Change	EEC P value	EEC FDR value	NAC Fold Change	NAC P value	NAC FDR value
ENSG00000215182	MUC5AC	1.89329911	5.87238E-09	1.25409E-05	1.307171333	0.003209153	0.11532121
ENSG00000080493	SLC4A4	1.573831228	2.41566E-07	0.000345524	1.382187508	5.37308E-05	0.015749433
ENSG00000123358	NR4A1	1.935676949	2.54248E-07	0.000345524	1.847362411	3.416E-07	0.000425548
ENSG00000187094	CCK	2.626757141	2.86773E-07	0.000357248	2.083199824	2.77946E-05	0.011375455
ENSG00000072778	ACADVL	1.208935931	6.47465E-07	0.000645264	1.089847286	0.009074053	0.165021927
ENSG00000109736	MFSD10	1.229280881	9.00204E-07	0.000791597	1.072995048	0.053225452	0.335724589
ENSG00000106541	AGR2	1.510809394	1.10216E-06	0.000915344	1.325642107	0.00018571	0.028197716
ENSG00000101452	DHX35	1.436962538	2.01919E-06	0.001372037	1.311852206	9.88177E-05	0.021723905
ENSG00000058600	POLR3E	1.141398365	2.37197E-06	0.001541677	1.085912669	0.001061998	0.069879511
ENSG00000167588	GPD1	1.394376493	3.12896E-06	0.001948948	1.28343062	0.000169905	0.027020266
ENSG00000102359	SRPX2	1.391226373	3.26595E-06	0.00195291	1.189832716	0.006223724	0.145983569
ENSG00000134489	HRH4	4.897428713	3.41745E-06	0.001964903	2.82332374	0.001694997	0.090494691
ENSG00000204991	SPIRE2	1.252871926	4.01723E-06	0.002224205	1.172301861	0.00035677	0.039742073
ENSG00000160145	KALRN	1.349240594	4.49971E-06	0.002402364	1.26091425	0.000122567	0.023941997
ENSG00000158292	GPR153	1.738078566	5.32215E-06	0.002637757	1.415720866	0.00164079	0.088549332
ENSG00000145780	FEM1C	1.386013268	5.56746E-06	0.002637757	1.369389834	3.84416E-06	0.002971896
ENSG00000173486	FKBP2	1.186138205	5.64641E-06	0.002637757	1.114500384	0.001400325	0.080513275
ENSG00000134285	FKBP11	1.282669662	6.78263E-06	0.003072529	1.069046873	0.17653015	0.53401217
ENSG00000108176	DNAJC12	2.749574407	7.20105E-06	0.003166131	1.913879156	0.002462171	0.103100816
ENSG00000117152	RGS4	1.459431153	7.73302E-06	0.003302883	1.449954457	4.17485E-06	0.002971896
ENSG00000167632	TRAPPC9	1.225046038	9.09086E-06	0.003774979	1.192141669	3.85268E-05	0.012652502
ENSG00000101194	SLC17A9	1.936356154	1.2662E-05	0.004901524	1.573311116	0.001231179	0.07451376
ENSG00000197520	FAM177B	1.949462652	1.3437E-05	0.004901524	1.569960754	0.001725392	0.090524481
ENSG00000162772	ATF3	1.41452406	1.42888E-05	0.004901524	1.297308473	0.000403922	0.041932131
ENSG00000107281	NPDC1	1.47544704	1.47292E-05	0.004901524	1.178471967	0.043573529	0.311070047
ENSG00000112619	PRPH2	1.723408066	1.47891E-05	0.004901524	1.063371595	0.600000353	0.84832686
ENSG00000043514	TRIT1	1.227170951	1.50268E-05	0.004901524	1.137050164	0.002879692	0.110949798
ENSG00000148396	SEC16A	1.165732421	1.57384E-05	0.004901524	1.086671803	0.008599733	0.162319962
ENSG00000170608	FOXA3	1.635336069	1.65222E-05	0.004906247	1.315158437	0.008220232	0.158788737
ENSG00000100395	L3MBTL2	1.16579184	1.74093E-05	0.004906247	1.061263004	0.060504914	0.352352148
ENSG00000140391	TSPAN3	1.184994225	1.89572E-05	0.004906247	1.100467461	0.006808599	0.152020438
ENSG00000116874	WARS2	1.214362725	1.9292E-05	0.004906247	1.184147608	7.97024E-05	0.019230201
ENSG00000214274	ANG	1.358764381	1.95969E-05	0.004906247	1.249985433	0.000760696	0.059275291
ENSG00000160182	TFF1	1.852712641	2.04893E-05	0.004906247	1.450950741	0.004372623	0.132007392
ENSG00000198722	UNC13B	1.244487087	2.07197E-05	0.004906247	1.189702182	0.00023555	0.031180885
ENSG00000183508	FAM46C	1.370687486	2.09241E-05	0.004906247	1.271236313	0.000419526	0.042463202
ENSG00000150787	PTS	1.233196478	2.10047E-05	0.004906247	1.172940249	0.0004204	0.042463202
ENSG00000117472	TSPAN1	1.168110576	2.33967E-05	0.005380891	1.046484169	0.157235994	0.510842518
ENSG00000102760	RGCC	1.294499954	2.74863E-05	0.006095008	1.303102013	6.06461E-06	0.003777492
ENSG00000166959	MS4A8	1.498782595	2.89847E-05	0.006279605	1.199106867	0.034591566	0.2853816
ENSG00000161677	JOSD2	1.345491349	3.23454E-05	0.006715714	1.19969392	0.005353078	0.138929115
ENSG00000205277	MUC12	1.644710165	3.35021E-05	0.006860589	1.265888721	0.036915009	0.292502336
ENSG00000160679	CHTOP	1.097762241	3.42459E-05	0.00691343	1.054827135	0.008439772	0.160312775
ENSG00000179178	TMEM125	1.206422844	3.46851E-05	0.00691343	1.120959228	0.005248858	0.138929115
ENSG00000139044	B4GALNT3	1.187860673	3.949E-05	0.00755189	1.122756224	0.002351544	0.10072559
ENSG00000168701	TMEM208	1.166034092	5.31774E-05	0.009665948	1.110184461	0.002437038	0.102913228
ENSG00000119899	SLC17A5	1.239004033	5.62654E-05	0.009678599	1.149432115	0.004061929	0.128437098
ENSG00000163482	STK36	1.296112114	5.63274E-05	0.009678599	1.082892525	0.169119498	0.524355754
ENSG00000145287	PLAC8	1.14332905	5.86022E-05	0.009843188	1.110851517	0.000606966	0.051848809
ENSG00000109065	NAT9	1.249645245	6.05482E-05	0.0099715	1.136496854	0.011272507	0.179053912
ENSG00000090382	LYZ	1.311276851	6.07001E-05	0.0099715	1.139438313	0.029793971	0.263700454
ENSG00000130304	SLC27A1	1.168133699	6.41057E-05	0.010193038	1.025748524	0.461276052	0.768827706
ENSG00000178980	SELENOW	1.15632936	6.47761E-05	0.010193038	1.06234416	0.061342281	0.355153276
ENSG00000124532	MRS2	1.130154779	6.5604E-05	0.010215779	1.127345546	3.63148E-05	0.012391954

ENSG00000148090	AUH	1.141274477	6.8827E-05	0.010329926	1.05242185	0.087548354	0.407451438
ENSG00000153234	NR4A2	1.30813029	7.12435E-05	0.010441365	1.274541257	0.000136002	0.023941997
ENSG00000112699	GMD5	1.26050047	7.42878E-05	0.010586412	1.194945487	0.000940655	0.066329514
ENSG00000047578	KIAA0556	1.213684574	7.43577E-05	0.010586412	1.145428119	0.002327284	0.100254247
ENSG00000182957	SPATA13	1.180043565	7.98121E-05	0.01091906	1.128343687	0.00178797	0.090912799
ENSG00000135046	ANXA1	1.228334914	8.03463E-05	0.01091906	1.226476229	3.37913E-05	0.012391954
ENSG00000188833	ENTPD8	1.640343716	8.30833E-05	0.011189303	1.217349875	0.095082403	0.417657788
ENSG00000131061	ZNF341	1.263904181	8.50336E-05	0.011199547	1.157352387	0.008172608	0.158581723
ENSG00000125999	BPIFB1	1.423060748	8.52814E-05	0.011199547	1.19928716	0.023896634	0.240235904
ENSG00000127954	STEAP4	1.320870172	8.67242E-05	0.011199547	1.271886698	0.000253311	0.0323654
ENSG00000158373	HIST1H2BD	1.554296009	8.76545E-05	0.011199547	1.28966279	0.016464714	0.207530357
ENSG00000158715	SLC45A3	1.289540505	8.87375E-05	0.011241842	1.072095934	0.230982221	0.594777651
ENSG00000152154	TMEM178A	1.349275	8.95629E-05	0.01124379	1.316281141	0.000148435	0.024996665
ENSG00000177548	RABEP2	1.190482387	9.15247E-05	0.011307459	1.193770927	2.63875E-05	0.011375455
ENSG00000166562	SEC11C	1.176298069	9.2787E-05	0.011369456	1.111510745	0.005309042	0.138929115
ENSG00000196497	IPO4	1.277217623	9.67674E-05	0.011760776	1.184989139	0.003512851	0.121553767
ENSG00000188735	TMEM120B	1.215380999	9.88286E-05	0.011877057	1.071662419	0.126166706	0.468690211
ENSG00000100219	XBP1	1.259108135	9.93131E-05	0.011877057	1.142392214	0.01222534	0.185917205
ENSG00000115339	GALNT3	1.247512286	0.00010933	0.012778763	1.193350411	0.000814112	0.061874213
ENSG00000173540	GMPPB	1.204177095	0.000109417	0.012778763	1.135939498	0.003661063	0.123507218
ENSG00000181788	SIAH2	1.147281266	0.000112227	0.013005288	1.146574557	4.82385E-05	0.014716679
ENSG00000172269	DPAGT1	1.138277915	0.000116783	0.013326612	1.077008007	0.015441364	0.203916031
ENSG00000012171	SEMA3B	1.394262826	0.000122859	0.013695711	1.161108083	0.061511981	0.355647922
ENSG00000008405	CRY1	1.220920002	0.000123955	0.013695711	1.211268541	9.35048E-05	0.020862729
ENSG00000103319	EEF2K	1.149887466	0.000124629	0.013695711	1.128012635	0.000370407	0.040714811
ENSG00000164855	TMEM184A	1.27603096	0.000128345	0.013695711	1.12301261	0.043181646	0.310508432
ENSG00000133872	SARAF	1.117187139	0.000129179	0.013695711	1.064157642	0.016904675	0.210660532
ENSG00000198488	B3GNT6	2.189291028	0.000139243	0.014455179	1.426879352	0.069374439	0.371182975
ENSG00000107537	PHYH	1.206259775	0.000140489	0.014483937	1.175536547	0.00043004	0.043145383
ENSG00000168785	TSPAN5	1.307300279	0.000149876	0.015170951	1.266341471	0.000384564	0.041288812
ENSG00000173588	CEP83	1.311374722	0.000150197	0.015170951	1.31287284	7.01205E-05	0.018390017
ENSG00000187492	CDHR4	1.42612463	0.000151378	0.015187579	1.131141147	0.141827519	0.490668729
ENSG00000117245	KIF17	1.467787629	0.000152792	0.015227224	1.117092479	0.243936841	0.60500594
ENSG00000170502	NUDT9	1.124300582	0.000155908	0.0154349	1.123420452	7.39283E-05	0.018972804
ENSG00000149927	DOC2A	2.119912331	0.000160177	0.015579529	1.487093626	0.040998731	0.302960962
ENSG00000073737	DHRS9	1.223765579	0.000163313	0.015579529	1.186816353	0.000544775	0.048561953
ENSG00000183718	TRIM52	1.197253831	0.00016429	0.015579529	1.103137575	0.026539407	0.25103604
ENSG00000126549	STATH	1.800839331	0.000164349	0.015579529	1.319346375	0.053690385	0.336668442
ENSG00000125740	FOSB	1.946447665	0.000164664	0.015579529	2.235622389	2.83676E-06	0.002494514
ENSG00000145990	GFOD1	1.215564065	0.000176071	0.016240065	1.063795047	0.191045938	0.550596824
ENSG00000103264	FBXO31	1.18448554	0.000177077	0.016240065	1.041737857	0.31498333	0.668657456
ENSG000000272398	CD24	1.272740225	0.000180238	0.016271624	1.239499035	0.000323522	0.037490953
ENSG00000072134	EPN2	1.155475814	0.000187026	0.01632187	1.098766215	0.00803494	0.158581723
ENSG00000186198	SLC51B	1.475836746	0.000187361	0.01632187	1.027253001	0.786209994	0.930264506
ENSG00000003096	KLHL13	1.510050651	0.000187591	0.01632187	1.416340139	0.000966808	0.067241536
ENSG00000125384	PTGER2	1.367671094	0.000187796	0.01632187	1.261638215	0.002962781	0.112986258
ENSG00000111707	SUDS3	1.1771003	0.000203514	0.016960357	1.129576206	0.002639991	0.105822197
ENSG00000176142	TMEM39A	1.209070953	0.000210779	0.017218249	1.227922698	2.72404E-05	0.011375455
ENSG00000198855	FICD	1.269540062	0.000213898	0.017378056	1.182809315	0.005194481	0.138929115
ENSG00000138134	STAMBPL1	1.312427548	0.000216663	0.017507515	1.422746064	1.03783E-06	0.001034302
ENSG00000137955	RABGGTB	1.131370215	0.000221398	0.017793992	1.076025727	0.016367995	0.207530357
ENSG00000102100	SLC35A2	1.124998368	0.000226165	0.017888429	1.03536142	0.222717291	0.585012451
ENSG00000180879	SSR4	1.167437853	0.000229368	0.017888429	1.061522004	0.112544937	0.447336949
ENSG00000141956	PRDM15	1.201659109	0.000230185	0.017888429	1.159314775	0.001526925	0.085171643
ENSG00000138316	ADAMTS14	1.588178211	0.000237929	0.018334011	1.059369074	0.632741705	0.865992533

ENSG00000170190	SLC16A5	1.237325519	0.000244115	0.018509693	1.140381313	0.013279531	0.192803393
ENSG00000099326	MZF1	1.252923428	0.000245161	0.018509693	1.08053738	0.167171689	0.522049211
ENSG00000129696	TTI2	1.177365153	0.000256582	0.018858989	1.184297478	6.71736E-05	0.018218015
ENSG00000213995	NAXD	1.110923866	0.000257045	0.018858989	1.078427838	0.004318522	0.131481834
ENSG00000137266	SLC22A23	1.179359247	0.000258374	0.018858989	1.071129848	0.089921675	0.409579258
ENSG00000203985	LDLRAD1	1.430341847	0.000262279	0.018941135	1.250115408	0.012052554	0.185554721
ENSG00000103489	XYLT1	1.351624596	0.000264857	0.018944243	1.444714677	5.35001E-06	0.003550796
ENSG00000177096	FAM109B	1.259863407	0.000279838	0.019698937	1.13945175	0.025156812	0.24435944
ENSG00000066135	KDM4A	1.137055016	0.000280679	0.019698937	1.103744826	0.002502512	0.103916827
ENSG00000136147	PHF11	1.134558473	0.000290023	0.020052705	1.065644463	0.045653513	0.317630035
ENSG00000197446	CYP2F1	1.37885052	0.000291085	0.020052705	1.415857614	3.59734E-05	0.012391954
ENSG00000003147	ICA1	1.169881302	0.000295328	0.020166309	1.063514625	0.118625925	0.45669301
ENSG00000184702	SEPT5	1.304112192	0.000295433	0.020166309	1.125127575	0.080341063	0.391466279
ENSG00000184465	WDR27	1.273888176	0.000299792	0.020342948	1.136337932	0.03972781	0.29916054
ENSG00000274897	PANO1	1.547921969	0.000302161	0.020346839	1.165738033	0.175806946	0.533605313
ENSG00000259120	SMIM6	1.824431493	0.000309697	0.020760786	1.578995802	0.003668469	0.123507218
ENSG00000234602	MCIDAS	1.373458656	0.000311853	0.020812026	1.365612214	0.000212672	0.030161596
ENSG00000137440	FGFBP1	1.787202253	0.000323517	0.021285282	1.856727737	5.14787E-05	0.01539109
ENSG00000081307	UBA5	1.099347557	0.000324032	0.021285282	1.076113043	0.002640423	0.105822197
ENSG00000077232	DNAJC10	1.193296439	0.000324826	0.021285282	1.129118775	0.006845782	0.152020438
ENSG00000143543	JTB	1.118021618	0.000326064	0.021285282	1.057633723	0.045736634	0.317630035
ENSG00000106078	COBL	1.190857683	0.000341244	0.022083358	1.121343627	0.010502599	0.175248717
ENSG00000103175	WFDC1	1.743218048	0.000343005	0.022101635	1.379956012	0.027490492	0.25454327
ENSG00000160181	TFF2	1.78572712	0.000361887	0.023118984	1.51547552	0.006049602	0.144465664
ENSG00000119508	NR4A3	1.958406533	0.000380145	0.024002333	1.338917086	0.105728201	0.43589856
ENSG00000275410	HNF1B	1.527896434	0.000384252	0.024002333	1.301464719	0.021037423	0.225896514
ENSG00000105072	C19orf44	1.354130394	0.000387464	0.024034037	1.164004797	0.058099389	0.347133397
ENSG00000008226	DLEC1	1.425982586	0.000392489	0.024093869	1.121264836	0.203405759	0.564035001
ENSG00000149300	C11orf52	1.469620143	0.000393016	0.024093869	1.05375868	0.607481315	0.852802513
ENSG00000153391	INO80C	1.154387933	0.000401691	0.024509688	1.117480858	0.003129571	0.11532121
ENSG00000104177	MYEF2	1.371770758	0.000408018	0.024760449	1.127528658	0.160283092	0.514288892
ENSG00000013503	POLR3B	1.176728184	0.000409113	0.024760449	1.08813759	0.045999527	0.318361056
ENSG00000143365	RORC	1.201936572	0.000419738	0.025074088	1.098295487	0.047975666	0.323068862
ENSG00000196659	TTC30B	1.343037836	0.000421257	0.025074088	1.210696779	0.014174429	0.197929232
ENSG00000145414	NAF1	1.182597058	0.000426413	0.025074088	1.140311789	0.003258072	0.115964087
ENSG00000156642	NPTN	1.100367314	0.00042692	0.025074088	1.076528296	0.003151884	0.11532121
ENSG00000157150	TIMP4	1.469415517	0.000427689	0.025074088	1.272859224	0.017885376	0.214268612
ENSG00000122735	DNAI1	1.325868279	0.000427714	0.025074088	1.139255516	0.072077839	0.376912595
ENSG00000260314	MRC1	2.145311282	0.000434644	0.025380828	1.976498559	0.000881603	0.064288245
ENSG00000099139	PCSK5	1.248619862	0.000438769	0.025423119	1.074830337	0.212839785	0.573907278
ENSG00000197818	SLC9A8	1.199489946	0.000451431	0.025772126	1.126728401	0.011983273	0.18505987
ENSG00000157470	FAM81A	1.326637901	0.000453794	0.025772126	1.208483461	0.012900438	0.190186044
ENSG00000105825	TFPI2	1.616684945	0.000455825	0.025772126	1.291343896	0.045136021	0.316512857
ENSG00000197747	S100A10	1.233863437	0.000457384	0.025772126	1.1130059	0.048260865	0.323334251
ENSG00000167595	PROSER3	1.19116025	0.000458183	0.025772126	1.140350994	0.005145471	0.138929115
ENSG00000160298	C21orf58	1.364853173	0.000458585	0.025772126	1.220548915	0.015121071	0.201645752
ENSG00000160051	IQCC	1.261937118	0.000463557	0.025953971	1.242745072	0.000547437	0.048561953
ENSG00000124786	SLC35B3	1.193470317	0.000474356	0.026361158	1.14075204	0.005021559	0.138528501
ENSG00000165376	CLDN2	2.46583126	0.000480645	0.02651351	3.187143504	4.00874E-06	0.002971896
ENSG00000067248	DHX29	1.124436563	0.000501866	0.027381022	1.124354374	0.000242408	0.03178728
ENSG00000176903	PNMA1	1.183187431	0.000514888	0.027989311	1.121057002	0.010049098	0.172607096
ENSG00000156535	CD109	1.542657155	0.000540414	0.028852324	1.451294987	0.001738625	0.090524481
ENSG00000140067	FAM181A	1.420429447	0.000549847	0.029251484	1.049255706	0.612655217	0.856582757
ENSG00000156958	GALK2	1.218953306	0.000556711	0.029511625	1.131049261	0.02015332	0.223470421
ENSG00000144834	TAGLN3	1.791250216	0.000562263	0.02970058	1.538100577	0.006986624	0.152249331

ENSG00000135924	DNAJB2	1.159260833	0.000577574	0.030212287	1.090419114	0.026774092	0.251533243
ENSG00000162694	EXTL2	1.225328736	0.000580034	0.030212287	1.124899509	0.033069149	0.279600687
ENSG00000181027	FKRP	1.209242226	0.00058698	0.030362522	1.164165557	0.003354754	0.118558421
ENSG00000105205	CLC	5.222877513	0.000593185	0.030577689	2.59300566	0.043246873	0.310517534
ENSG00000126217	MCF2L	1.215832328	0.000610731	0.03115981	1.065892143	0.218200102	0.579682481
ENSG00000243284	VSIG8	1.772894554	0.000617595	0.03129638	1.21585998	0.217358116	0.578601374
ENSG00000163060	TEKT4	1.368593324	0.000631021	0.031761398	1.225707246	0.017409601	0.211650759
ENSG00000255587	RAB44	1.452235038	0.000633576	0.031782998	1.169782361	0.124791132	0.466080115
ENSG00000179820	MYADM	1.276487455	0.000652559	0.032517035	1.209105899	0.00425969	0.131024895
ENSG00000100568	VTI1B	1.088145985	0.000667973	0.033067952	1.070420254	0.003142914	0.11532121
ENSG00000148444	COMMD3	1.145565602	0.000670251	0.033067952	1.0293654	0.427928493	0.750501014
ENSG00000104267	CA2	2.090184422	0.000675497	0.033147747	1.737378883	0.006621567	0.150892997
ENSG00000137267	TUBB2A	1.231584483	0.000676304	0.033147747	1.194447906	0.001838552	0.091849401
ENSG00000152760	TCTEX1D1	1.292353129	0.000703771	0.034047461	1.168968801	0.027342885	0.25435519
ENSG00000172915	NBEA	1.225594315	0.000712483	0.034219028	1.12189221	0.035749099	0.289184676
ENSG00000108389	MTMR4	1.099202283	0.000712671	0.034219028	1.045622685	0.079269256	0.389904184
ENSG00000120314	WDR55	1.128064892	0.000720302	0.0343586	1.07678374	0.023485484	0.239529623
ENSG00000153904	DDAH1	1.181638714	0.000721694	0.0343586	1.151493744	0.002211083	0.099348189
ENSG00000105447	GRWD1	1.138482993	0.00073261	0.034754598	1.094024617	0.011073041	0.178283392
ENSG00000151135	TMEM263	1.245138589	0.000734661	0.034754598	1.212878132	0.001623651	0.087941856
ENSG00000061918	GUCY1B3	1.440465717	0.000739447	0.034760992	1.690937738	9.78877E-07	0.001034302
ENSG00000145354	CISD2	1.142347538	0.000749171	0.035036207	1.106332575	0.006352334	0.147455029
ENSG00000129566	TEP1	1.201517074	0.000756085	0.0351461	1.064399934	0.209034756	0.570021994
ENSG00000101019	UQCC1	1.126165077	0.000757499	0.0351461	1.121385897	0.000546217	0.048561953
ENSG00000018510	AGPS	1.182133173	0.000759395	0.0351461	1.135234635	0.005896945	0.143118329
ENSG00000143457	GOLPH3L	1.133194361	0.000762474	0.035179713	1.104473661	0.003877798	0.126294554
ENSG00000253304	TMEM200B	1.524411607	0.000783976	0.035716881	1.383584628	0.006172356	0.145950308
ENSG00000165449	SLC16A9	1.305383733	0.000790889	0.035716881	1.202116548	0.011742983	0.182860268
ENSG00000100554	ATP6V1D	1.181015689	0.00079444	0.035716881	1.151934974	0.002213054	0.099348189
ENSG00000163644	PPM1K	1.238007181	0.000794751	0.035716881	1.13942872	0.027114416	0.253017108
ENSG00000198952	SMG5	1.14260799	0.000808126	0.036061721	1.158367355	0.000104678	0.022678773
ENSG00000095380	NANS	1.129732703	0.00081722	0.036359007	1.103162932	0.003638636	0.123507218
ENSG00000198039	ZNF273	1.285401563	0.000827609	0.03662175	1.084641948	0.249866933	0.610636061
ENSG00000105048	TNNT1	1.563654446	0.000828025	0.03662175	1.421583915	0.005505117	0.139484732
ENSG00000110848	CD69	3.246309364	0.000831594	0.036642311	1.940000093	0.052696007	0.333934976
ENSG00000109339	MAPK10	1.159682606	0.000834924	0.036642311	1.085566793	0.043228943	0.310517534
ENSG00000129990	SYT5	1.441562747	0.000838444	0.036648829	1.100704598	0.343254641	0.688859395
ENSG00000133794	ARNTL	1.268757166	0.000845609	0.036854247	1.503328606	1.73272E-08	3.23781E-05
ENSG00000101844	ATG4A	1.107991585	0.000852211	0.037034023	1.086297691	0.003841066	0.125730709
ENSG00000123607	TTC21B	1.36121045	0.000855173	0.037055002	1.323294995	0.001495737	0.083744458
ENSG00000186603	HPDL	1.448319626	0.000872472	0.037695347	1.381938989	0.001775559	0.090912799
ENSG00000070214	SLC44A1	1.149235344	0.000883924	0.038080078	1.102919943	0.01128994	0.179053912
ENSG00000114745	GORASP1	1.093487656	0.000897491	0.03855341	1.054893602	0.02995961	0.264577835
ENSG00000113966	ARL6	1.280854777	0.000900107	0.038555011	1.239434247	0.002457787	0.103100816
ENSG00000160180	TFF3	1.790040264	0.000905383	0.03858104	1.348257984	0.05925035	0.349559071
ENSG00000164287	CDC20B	1.689083663	0.000911606	0.03858104	1.81690474	8.5723E-05	0.019714973
ENSG00000204396	VWA7	1.27912958	0.000929524	0.039032192	1.130806577	0.06954936	0.371182975
ENSG00000146909	NOM1	1.125847224	0.000967673	0.040407098	1.066620371	0.052029849	0.33305578
ENSG00000125454	SLC25A19	1.201819154	0.000989314	0.041073305	1.091401744	0.091188551	0.411563244
ENSG00000123562	MORF4L2	1.110263008	0.00099187	0.041073305	1.093261852	0.002492143	0.103774495
ENSG00000074370	ATP2A3	1.202971549	0.000997942	0.041149491	1.137299222	0.012558935	0.18830845
ENSG00000116863	ADPRHL2	1.175596075	0.001000444	0.041149491	1.049058146	0.282998619	0.640312752
ENSG00000100228	RAB36	1.286353955	0.001005222	0.041170053	1.189720492	0.013548375	0.193865559
ENSG00000162929	KIAA1841	1.281773905	0.001029481	0.041605624	1.274958543	0.000758887	0.059275291
ENSG00000181143	MUC16	1.298517484	0.001048754	0.041661487	1.017521392	0.805148151	0.936200397

ENSG00000095397	WHRN	1.232414493	0.00105108	0.041661487	1.130333047	0.03892639	0.297862324
ENSG00000138640	FAM13A	1.2330977	0.001056238	0.041661487	1.173640169	0.00715628	0.153262272
ENSG00000131788	PIAS3	1.105130702	0.001084372	0.042339544	1.040589822	0.150453485	0.500696605
ENSG00000185379	RAD51D	1.172718906	0.001084851	0.042339544	1.184783255	0.000257075	0.032567953
ENSG00000143776	CDC42BPA	1.215089598	0.001084987	0.042339544	1.184552043	0.002293896	0.100254247
ENSG00000128165	ADM2	1.572293056	0.00108759	0.042339544	1.064519045	0.637529088	0.868296496
ENSG00000144867	SRPRB	1.172272154	0.001105675	0.04234005	1.09972955	0.033014029	0.279600687
ENSG00000123191	ATP7B	1.219299421	0.001110602	0.04234005	1.256554526	8.49147E-05	0.019714973
ENSG00000172716	SLFN11	1.381363495	0.001113152	0.04234005	1.318230114	0.003298628	0.11685115
ENSG00000172315	TP53RK	1.111609539	0.001117131	0.04234005	1.066591532	0.030665927	0.26808476
ENSG00000142207	URB1	1.164228391	0.00113172	0.042722426	1.114185964	0.012204808	0.185917205
ENSG00000075142	SRI	1.148239349	0.001140886	0.042754007	1.116558196	0.005057081	0.138626286
ENSG00000133195	SLC39A11	1.159729588	0.001141136	0.042754007	1.129133851	0.004187669	0.129969847
ENSG00000099998	GGT5	3.22704289	0.001174057	0.043443006	1.90843278	0.063541597	0.359266922
ENSG00000168672	FAM84B	1.120828441	0.001198768	0.043816521	1.117662883	0.000786915	0.060326112
ENSG00000240230	COX19	1.151235175	0.001199823	0.043816521	1.071375705	0.084909186	0.401296594
ENSG00000204385	SLC44A4	1.111479663	0.001201737	0.043816521	1.068015462	0.027112374	0.253017108
ENSG00000123989	CHPF	1.193699175	0.001205129	0.04383328	1.077520788	0.132127652	0.476936545
ENSG00000115368	WDR75	1.114956906	0.001211217	0.043947766	1.154631817	1.09941E-05	0.005667282
ENSG00000026652	AGPAT4	1.362296676	0.001233862	0.044661023	1.183282959	0.06120682	0.354918833
ENSG00000168398	BDKRB2	1.172035775	0.001251174	0.045068217	1.110218579	0.021123066	0.226195354
ENSG00000085831	TTC39A	1.1436548	0.001254156	0.045068217	1.133626469	0.001311529	0.077317159
ENSG00000171522	PTGER4	1.231356937	0.001267968	0.045130621	1.179206677	0.006137823	0.145873315
ENSG00000110697	PITPNM1	1.172014741	0.001278945	0.045320479	1.128910643	0.007638979	0.157383603
ENSG00000169314	C22orf15	1.409952072	0.001281063	0.045320479	1.26360451	0.018724412	0.218509947
ENSG00000170476	MZB1	1.746091409	0.001282398	0.045320479	1.1841732	0.300602276	0.656909055
ENSG00000148950	IMMP1L	1.215883762	0.001285462	0.045321629	1.026048688	0.6477248	0.872413802
ENSG00000038427	VCAN	1.339308763	0.001326452	0.046221742	1.210424998	0.026599964	0.251037162
ENSG00000172375	C2CD2L	1.160633072	0.001334158	0.046312055	1.121598645	0.007687722	0.157429933
ENSG00000090266	NDUFB2	1.120185482	0.001338338	0.046312055	1.090724078	0.007874072	0.158581723
ENSG00000198668	CALM1	1.105103717	0.001341515	0.046314798	1.094675635	0.001855653	0.092017579
ENSG00000159713	TPPP3	1.283486138	0.001386249	0.047530295	1.135036624	0.071874321	0.376912595
ENSG00000117616	RSRP1	1.261667125	0.001390925	0.047530295	1.131103614	0.065514921	0.363783187
ENSG00000154760	SLFN13	1.212919717	0.001398779	0.047631765	1.076125674	0.184192275	0.543513639
ENSG00000160055	TMEM234	1.212567318	0.001410397	0.047701425	1.14039523	0.019933399	0.222678439
ENSG00000213145	CRIP1	1.326336546	0.001424242	0.047814894	1.220187139	0.016622247	0.208904775
ENSG00000129083	COPB1	1.090132109	0.001435337	0.047814894	1.08516327	0.001277726	0.076267832
ENSG00000095203	EPB41L4B	1.206431279	0.001440894	0.047814894	1.181815902	0.002358291	0.100725969
ENSG00000166598	HSP90B1	1.12518461	0.001446649	0.047814894	1.069440701	0.046237011	0.318458796
ENSG00000198786	MT-ND5	1.342811252	0.001447418	0.047814894	1.127529031	0.144956024	0.494652885
ENSG00000010361	FUZ	1.299142778	0.001449761	0.047814894	1.200929665	0.016364579	0.207530357
ENSG00000273559	CWC25	1.162698454	0.001452135	0.047814894	1.190439543	0.000126252	0.023941997
ENSG00000175309	PHYKPL	1.188801097	0.001478819	0.048554495	0.981725389	0.714023257	0.900905947
ENSG00000090971	NAT14	1.294177181	0.001490836	0.048554495	1.160116119	0.046855222	0.320301974
ENSG00000221909	FAM200A	1.122313059	0.003850162	0.080333939	1.211821469	1.37236E-06	0.001282215
ENSG00000078687	TNRC6C	1.137536298	0.007273709	0.104625823	1.237993656	7.07973E-06	0.004070573
ENSG00000183864	TOB2	1.15934007	0.005660855	0.093313203	1.263885187	8.59904E-06	0.004760999
ENSG00000107554	DNMBP	1.107593516	0.005571963	0.093131803	1.16337204	2.75973E-05	0.011375455
ENSG00000112541	PDE10A	1.466701066	0.001572665	0.050342124	1.638061366	3.13852E-05	0.012030193
ENSG00000075213	SEMA3A	1.13889524	0.024408649	0.176272892	1.256091971	6.00707E-05	0.017269179
ENSG00000162627	SNX7	1.086029046	0.005348107	0.091803492	1.117097167	0.000107627	0.022984437
ENSG00000148925	BTBD10	1.029190774	0.354330629	0.632842123	1.125414731	0.000116744	0.023941997
ENSG00000164934	DCAF13	1.070032588	0.012440335	0.131707201	1.106417145	0.000119084	0.023941997
ENSG00000130720	FIBCD1	1.553116173	0.082653655	0.314169074	2.526053607	0.000130625	0.023941997
ENSG00000165891	E2F7	1.355887091	0.015978366	0.146749708	1.603308095	0.000131401	0.023941997

ENSG00000174796	THAP6	1.152220525	0.015281037	0.143851526	1.241397933	0.000148014	0.024996665
ENSG00000163877	SNIP1	1.101311382	0.01073092	0.123873758	1.148650392	0.000152183	0.025277619
ENSG00000109321	AREG	1.686714203	0.002232515	0.060241649	1.853282931	0.000156159	0.025374167
ENSG00000160588	MPZL3	1.178781739	0.002464044	0.062644556	1.214467067	0.000178486	0.028086161
ENSG00000142731	PLK4	1.326815986	0.022432089	0.168452868	1.571762754	0.000180751	0.028114143
ENSG00000135249	RINT1	1.111983535	0.002147033	0.05871254	1.131341173	0.000188697	0.028197716
ENSG00000168806	LCMT2	1.119270539	0.004387238	0.084355014	1.150983742	0.000201961	0.029311837
ENSG00000163659	TIPARP	1.166072406	0.05268896	0.253018714	1.331666979	0.000204904	0.029452924
ENSG00000005700	IBTK	1.129526077	0.005265695	0.091099576	1.167699825	0.000214673	0.030161596
ENSG00000112782	CLIC5	1.197393586	0.00530721	0.091402623	1.257386403	0.000220704	0.030467064
ENSG00000119927	GPAM	1.089841188	0.070563395	0.29099371	1.185980005	0.000227241	0.030882094
ENSG00000100483	VCPKMT	1.115240215	0.040947404	0.225045126	1.209032375	0.000246862	0.032089876
ENSG00000106415	GLCCI1	1.127195677	0.033532467	0.204299834	1.218235529	0.000313303	0.037468489
ENSG00000135040	NAA35	1.083978177	0.018515253	0.155759434	1.126422856	0.000318914	0.037490953
ENSG00000263429	LINC00675	1.289405353	0.003297063	0.074116981	1.3422317	0.00032053	0.037490953
ENSG00000205835	GMNC	1.602540461	0.010767291	0.124006346	1.909567563	0.00032867	0.037794491
ENSG00000198331	HYLS1	1.158767365	0.115615638	0.369524218	1.382209952	0.000346466	0.039237326
ENSG00000101343	CRNKL1	1.090318501	0.017246787	0.151304117	1.132956016	0.000358899	0.039742073
ENSG00000028839	TBPL1	1.112507052	0.009908114	0.119120811	1.152348264	0.000386111	0.041288812
ENSG00000181449	SOX2	1.154253195	0.001905716	0.056079807	1.167123384	0.000411457	0.042158015
ENSG00000143867	OSR1	1.156111277	0.112541519	0.364152201	1.367067551	0.000411738	0.042158015
ENSG00000198483	ANKRD35	1.21708515	0.003195667	0.072381851	1.250323542	0.000438137	0.043655545
ENSG00000074266	EED	1.088486205	0.050340263	0.248981738	1.156837254	0.000469245	0.045256404
ENSG00000196458	ZNF605	1.203244419	0.004076965	0.082027665	1.241494317	0.000481387	0.046129844
ENSG000000085511	MAP3K4	1.101196553	0.009137693	0.115438257	1.131569831	0.000497554	0.047311916
ENSG00000111490	TBC1D30	1.148423149	0.003597966	0.077613272	1.17107876	0.000503976	0.047370092
ENSG000000080608	PUM3	1.108996904	0.004899631	0.08797964	1.130208674	0.00050963	0.047370092
ENSG00000171723	GPHN	1.122368853	0.021196798	0.164530513	1.183949932	0.000511539	0.047370092
ENSG00000173085	COQ2	1.072973095	0.042928175	0.229272341	1.123826701	0.000513342	0.047370092
ENSG00000110066	KMT5B	1.050854107	0.092959694	0.33091575	1.104852906	0.000516628	0.047380828
ENSG00000104131	EIF3J	1.094939334	0.026384497	0.182771941	1.146816378	0.000539526	0.048561953

Table E5B - Downregulated genes

ensemblID	geneSymbol	EEC Fold Change	EEC P value	EEC FDR value	NAC Fold Change	NAC P value	NAC FDR value
ENSG00000159208	CIART	0.273172408	5.07848E-12	5.27767E-08	0.274148128	4.8768E-13	7.29032E-09
ENSG00000126368	NR1D1	0.340202432	7.06091E-12	5.27767E-08	0.390368032	3.34968E-11	1.25186E-07
ENSG00000096060	FKBP5	0.525498673	5.52644E-11	2.75382E-07	0.516102408	2.37737E-12	1.77696E-08
ENSG00000105516	DBP	0.551472796	1.73071E-10	6.4681E-07	0.57839896	1.97789E-10	4.92792E-07
ENSG00000179094	PER1	0.471981479	2.43465E-10	7.27912E-07	0.457960057	8.47334E-12	4.22227E-08
ENSG00000174738	NR1D2	0.558039449	1.67415E-09	4.17114E-06	0.610958045	2.27894E-08	3.78532E-05
ENSG00000049246	PER3	0.589072991	2.42012E-08	4.52231E-05	0.537104369	5.60392E-11	1.67546E-07
ENSG00000167074	TEF	0.640369993	2.84938E-08	4.73282E-05	0.611587073	3.00783E-10	6.42344E-07
ENSG00000182704	TSKU	0.664344597	3.19246E-07	0.000367108	0.661113849	5.31584E-08	7.22422E-05
ENSG00000117643	MAN1C1	0.669980038	3.5556E-07	0.000379662	0.688331895	4.07431E-07	0.000468514
ENSG00000136848	DAB2IP	0.789413763	7.17142E-07	0.000670035	0.777732791	4.12139E-08	6.16106E-05
ENSG00000168874	ATOH8	0.522025404	1.3453E-06	0.001058466	0.585508673	6.70076E-06	0.004006787
ENSG00000119138	KLF9	0.669836479	1.47896E-06	0.001105449	0.703697374	5.46313E-06	0.003550796
ENSG00000088882	CPXM1	0.521081261	1.56638E-06	0.001115041	0.745891697	0.009043771	0.164714
ENSG00000127423	AUNIP	0.558366566	4.74302E-06	0.002444948	0.657215705	0.000199463	0.029233022
ENSG00000043143	JADE2	0.818100784	1.23445E-05	0.004901524	0.830218417	1.42588E-05	0.006875955
ENSG00000156802	ATAD2	0.728845657	1.38613E-05	0.004901524	0.809436942	0.001169204	0.072350181
ENSG00000151474	FRMD4A	0.710009706	1.44422E-05	0.004901524	0.815041242	0.003852075	0.125730709
ENSG00000168209	DDIT4	0.766579789	1.51127E-05	0.004901524	0.836473088	0.001342973	0.078117156
ENSG00000166396	SERPINB7	0.520328887	1.56702E-05	0.004901524	0.772433815	0.039062055	0.297862324
ENSG00000120278	PLEKHG1	0.799946351	1.69813E-05	0.004906247	0.875269745	0.004202787	0.130077554
ENSG00000167183	PRR15L	0.800651798	1.84412E-05	0.004906247	0.833377131	0.000130801	0.023941997
ENSG00000107829	FBXW4	0.819006618	1.85451E-05	0.004906247	0.917410969	0.035608418	0.288405193
ENSG00000125257	ABCC4	0.742019888	2.03648E-05	0.004906247	0.801842797	0.000537663	0.048561953
ENSG00000064655	EYA2	0.871144164	2.03742E-05	0.004906247	0.907698748	0.001004564	0.06766258
ENSG00000182326	C1S	0.632553855	2.06511E-05	0.004906247	0.708271963	0.000397388	0.04183488
ENSG00000100100	PIK3IP1	0.787143381	2.07459E-05	0.004906247	0.838837376	0.000618453	0.052529821
ENSG00000146083	RNF44	0.852126697	2.68621E-05	0.006084267	0.87929817	0.000253188	0.0323654
ENSG00000109906	ZBTB16	0.468990826	2.7725E-05	0.006095008	0.486711269	1.4182E-05	0.006875955
ENSG00000184602	SNN	0.758506567	3.12167E-05	0.006636969	0.829722048	0.00171813	0.090524481
ENSG00000136048	DRAM1	0.712920493	3.15222E-05	0.006636969	0.745269005	9.00756E-05	0.020402125
ENSG00000067113	PLPP1	0.810524574	3.67325E-05	0.007210195	0.845116495	0.000321241	0.037490953
ENSG00000139178	C1RL	0.869851281	3.71386E-05	0.007210195	0.859236525	3.02664E-06	0.002513623
ENSG00000006210	CX3CL1	0.722057023	3.9909E-05	0.00755189	0.735096411	3.54841E-05	0.012391954
ENSG00000266412	NCOA4	0.835321267	4.12953E-05	0.007716543	0.894122144	0.004795796	0.137079062
ENSG00000100207	TCF20	0.88242092	5.35713E-05	0.009665948	0.904549451	0.000443885	0.043655545
ENSG00000087274	ADD1	0.918412872	5.36674E-05	0.009665948	0.930665008	0.000231166	0.031132476
ENSG00000121851	POLR3GL	0.851250523	5.43678E-05	0.009675533	0.917544459	0.01528574	0.203297623
ENSG00000119986	AVPI1	0.755884586	5.51834E-05	0.009678599	0.815483892	0.001160097	0.072299488
ENSG00000170779	CDCA4	0.813086314	5.83437E-05	0.009843188	0.865947845	0.00199754	0.094199468
ENSG00000148985	PGAP2	0.873643847	6.28469E-05	0.010193038	0.92065406	0.006499211	0.149242257
ENSG00000176387	HSD11B2	0.634926857	6.36449E-05	0.010193038	0.655198745	6.69884E-05	0.018218015
ENSG00000164331	ANKRA2	0.859347976	6.75723E-05	0.010329926	0.886684205	0.00056082	0.04910421
ENSG00000162337	LRP5	0.858227705	6.85573E-05	0.010329926	0.858949868	2.81552E-05	0.011375455
ENSG00000104147	OIP5	0.596960381	6.91011E-05	0.010329926	0.924257116	0.457857368	0.76749381
ENSG00000088812	ATRN	0.865185156	7.03556E-05	0.010413326	0.930003034	0.026325787	0.25103604
ENSG00000141579	ZNF750	0.839804123	7.3937E-05	0.010586412	0.907825573	0.014846853	0.200131293
ENSG00000113552	GNPDA1	0.833739187	7.5946E-05	0.010710536	0.896611505	0.00800124	0.158581723
ENSG00000178150	ZNF114	0.332342185	7.72385E-05	0.010791008	0.708065989	0.11593174	0.452733432
ENSG00000006468	ETV1	0.633065158	7.82327E-05	0.010828716	0.814350919	0.04350442	0.310751183
ENSG00000159403	C1R	0.714440464	8.61239E-05	0.011199547	0.746952155	0.000266249	0.033168007
ENSG00000100297	MCM5	0.82340847	8.72808E-05	0.011199547	0.861765847	0.001075873	0.069879511
ENSG00000172059	KLF11	0.740939688	9.02572E-05	0.01124379	0.774564766	0.000334437	0.038164116
ENSG00000112561	TFEB	0.786735051	0.000101786	0.012076139	0.80394825	0.00014882	0.024996665

ENSG00000248485	PCP4L1	0.689223464	0.000114849	0.013206754	0.847851274	0.053847534	0.336947168
ENSG00000186350	RXRA	0.889109316	0.000121987	0.013695711	0.927517097	0.006819043	0.152020438
ENSG00000111275	ALDH2	0.825346489	0.000123933	0.013695711	0.915116082	0.048084386	0.323068862
ENSG00000162407	PLPP3	0.808556854	0.000125708	0.013695711	0.848781112	0.001280569	0.076267832
ENSG00000186184	POLR1D	0.903512489	0.00012648	0.013695711	0.932278893	0.003730672	0.123932922
ENSG00000157978	LDLRAP1	0.846529804	0.000127391	0.013695711	0.858902292	0.000182425	0.028114143
ENSG00000124145	SDC4	0.869951367	0.000132539	0.013952954	0.890101493	0.000582583	0.050592209
ENSG00000125817	CENPB	0.851336357	0.000135551	0.014170343	0.90536275	0.009295741	0.166167208
ENSG00000166289	PLEKHF1	0.717194952	0.000145797	0.014928197	0.733745417	0.000137736	0.023941997
ENSG00000085998	POMGNT1	0.812225462	0.000162959	0.015579529	0.891139653	0.01985054	0.222639868
ENSG00000138617	PARP16	0.852847126	0.00016316	0.015579529	0.918725588	0.024685452	0.242615146
ENSG00000145901	TNIP1	0.823466436	0.00016601	0.015608091	0.891560936	0.014077873	0.197376649
ENSG00000140332	TLE3	0.761750143	0.000167517	0.015651278	0.909682123	0.135480451	0.481420273
ENSG00000213190	MLLT11	0.719343375	0.000175226	0.016240065	0.772243086	0.001255519	0.075680484
ENSG00000132383	RPA1	0.904224423	0.000178808	0.016271624	0.921137608	0.000939279	0.066329514
ENSG00000101057	MYBL2	0.547840144	0.000181674	0.016271624	0.732010989	0.024697339	0.242615146
ENSG00000165475	CRYL1	0.878343265	0.000181775	0.016271624	0.944168834	0.063890691	0.360213964
ENSG00000160752	FDPS	0.857171257	0.000186755	0.01632187	0.900376427	0.005263908	0.138929115
ENSG00000119403	PHF19	0.747546116	0.000189073	0.01633787	0.882504899	0.067929211	0.36905596
ENSG00000101224	CDC25B	0.698378929	0.000190331	0.016352054	0.747908485	0.000939741	0.066329514
ENSG00000181333	HEPHL1	0.315764974	0.000194117	0.01658201	0.340837693	0.000115663	0.023941997
ENSG00000100711	ZFYVE21	0.873960187	0.000199198	0.016919418	0.893866097	0.000815387	0.061874213
ENSG00000134202	GSTM3	0.639918941	0.000201389	0.016960357	0.86881684	0.165236344	0.519754103
ENSG00000155367	PPM1J	0.774616589	0.000203828	0.016960357	0.872448759	0.026447568	0.25103604
ENSG00000117298	ECE1	0.84492307	0.000204219	0.016960357	0.883594364	0.002957295	0.112986258
ENSG00000187193	MT1X	0.667591281	0.000207338	0.017124262	0.684167983	0.000215887	0.030161596
ENSG00000082996	RNF13	0.871954043	0.000210647	0.017218249	0.911054734	0.00583504	0.142762716
ENSG00000150991	UBC	0.866585708	0.000222914	0.017820045	0.872554075	0.000189279	0.028197716
ENSG00000157212	PAXIP1	0.849565711	0.000227039	0.017888429	0.841091769	4.62626E-05	0.01465717
ENSG00000108602	ALDH3A1	0.655228035	0.000228922	0.017888429	0.861144307	0.15018355	0.500132298
ENSG00000123240	OPTN	0.836518917	0.00023095	0.017888429	0.904565144	0.022290524	0.232533874
ENSG00000169410	PTPN9	0.908334421	0.000239919	0.018392545	0.922250685	0.000854942	0.063584685
ENSG00000176974	SHMT1	0.82721496	0.000241477	0.018417569	0.894799986	0.016888275	0.210660532
ENSG00000175471	MCTP1	0.687721152	0.000249406	0.01873515	0.66043004	3.04794E-05	0.011990453
ENSG00000103429	BFAR	0.908517643	0.000250683	0.01873515	0.917148782	0.000395064	0.04183488
ENSG00000170365	SMAD1	0.872073588	0.000251908	0.01873515	0.938543662	0.058787923	0.348888026
ENSG00000133818	RRAS2	0.862983355	0.000258619	0.018858989	0.956025963	0.211253219	0.571898654
ENSG00000244094	SPRR2F	0.317917837	0.00026107	0.018941135	0.518820231	0.008303964	0.159197348
ENSG00000074219	TEAD2	0.570848152	0.000264188	0.018944243	0.720062769	0.016976913	0.210717916
ENSG00000140931	CMTM3	0.725360453	0.000275097	0.019582999	0.817689532	0.009765532	0.169918995
ENSG00000163719	MTMR14	0.890325712	0.000276584	0.01959555	0.913234221	0.001979039	0.093622301
ENSG00000220205	VAMP2	0.854147354	0.000287149	0.019965918	0.853664086	0.000123957	0.023941997
ENSG00000186318	BACE1	0.846433689	0.000287154	0.019965918	0.882645745	0.003205464	0.11532121
ENSG00000132694	ARHGEF11	0.8976719	0.000300742	0.020342948	0.926369495	0.005227915	0.138929115
ENSG00000198056	PRIM1	0.775421924	0.00032347	0.021285282	0.873578931	0.032077589	0.275118708
ENSG00000174080	CTSF	0.772652709	0.00033945	0.022062747	0.868939635	0.027960664	0.25706271
ENSG00000138031	ADCY3	0.815696327	0.000357092	0.022910585	0.849842116	0.001904013	0.092713658
ENSG00000183734	ASCL2	0.724543475	0.000365567	0.02325472	0.810523457	0.0093759	0.166461197
ENSG00000138080	EMILIN1	0.609178749	0.000383192	0.024002333	0.851596717	0.170656954	0.52674106
ENSG00000116574	RHOU	0.787753262	0.000383745	0.024002333	0.836585978	0.003805367	0.125025131
ENSG00000085117	CD82	0.87726832	0.000385348	0.024002333	0.923453761	0.017903109	0.214268612
ENSG00000249242	TMEM150C	0.813935159	0.000393264	0.024093869	0.867773556	0.007526366	0.156483522
ENSG00000155760	FZD7	0.596967484	0.000420958	0.025074088	0.76635141	0.034570811	0.2853816
ENSG00000262655	SPON1	0.404304512	0.000422348	0.025074088	0.891644864	0.573477295	0.83427734
ENSG00000142192	APP	0.834454101	0.000437446	0.025423119	0.832689775	0.000161028	0.025883989

ENSG00000137310	TCF19	0.809614267	0.000441098	0.025459348	0.866987983	0.009255113	0.166167208
ENSG00000196139	AKR1C3	0.812180421	0.000455932	0.025772126	0.827555474	0.000623339	0.052645705
ENSG00000067066	SP100	0.857264648	0.000468375	0.026125913	0.899769748	0.008801614	0.163854705
ENSG00000168003	SLC3A2	0.814676671	0.000479385	0.02651351	0.830440219	0.000693914	0.056376724
ENSG00000013563	DNASE1L1	0.882311457	0.000500302	0.027381022	0.905449276	0.002787771	0.109095273
ENSG00000084112	SSH1	0.883318268	0.000500895	0.027381022	0.910453447	0.004278875	0.131344779
ENSG00000148798	INA	0.472934379	0.000520822	0.028197425	0.696763538	0.049494294	0.32622186
ENSG00000183722	LHFPL6	0.553940846	0.000522489	0.028197425	0.606055564	0.001421946	0.08144317
ENSG00000126351	THRA	0.829038942	0.000527891	0.028386463	0.839589607	0.000561698	0.04910421
ENSG00000174151	CYB561D1	0.9058241	0.000536121	0.02872571	0.915391427	0.000860841	0.063642103
ENSG00000188368	PRR19	0.674080067	0.00057837	0.030212287	0.953927169	0.623731772	0.862142317
ENSG00000184445	KNTC1	0.829036308	0.000579794	0.030212287	0.900924204	0.033054899	0.279600687
ENSG00000089351	GRAMD1A	0.793292259	0.000586608	0.030362522	0.869516057	0.022046319	0.230953345
ENSG00000108219	TSPAN14	0.849766059	0.000598172	0.030728766	0.955530118	0.285464569	0.642585429
ENSG00000177084	POLE	0.828778487	0.000605281	0.030987509	0.847493993	0.001171232	0.072350181
ENSG00000182095	TNRC18	0.887009573	0.000616857	0.03129638	0.910215212	0.003666798	0.123507218
ENSG00000135446	CDK4	0.860594506	0.000623824	0.031505213	0.924844442	0.047958586	0.323068862
ENSG00000119242	CCDC92	0.890685312	0.000637802	0.031887956	0.921182846	0.008785207	0.163837083
ENSG00000089327	FXD5	0.776233066	0.000669175	0.033067952	0.840424037	0.010807919	0.176307144
ENSG00000161011	SQSTM1	0.821564789	0.00069125	0.033677042	0.91915956	0.109746074	0.442056546
ENSG00000162817	C1orf115	0.830539206	0.000691608	0.033677042	0.93762452	0.185702932	0.545076208
ENSG00000148337	CIZ1	0.896023353	0.000701178	0.03403217	0.921822918	0.006442441	0.148853236
ENSG00000131153	GINS2	0.685062615	0.000714184	0.034219028	0.77382815	0.010810519	0.176307144
ENSG00000204175	GPRIN2	0.678723008	0.000737461	0.034760992	0.79615502	0.024147031	0.241185682
ENSG00000138867	GUCD1	0.909775576	0.000749989	0.035036207	0.955182134	0.071185528	0.374965629
ENSG00000172159	FRMD3	0.518624625	0.000771729	0.03549718	0.649623212	0.011306228	0.179053912
ENSG00000162512	SDC3	0.800924954	0.000782421	0.035716881	0.765923778	3.64737E-05	0.012391954
ENSG00000136827	TOR1A	0.897828162	0.000788672	0.035716881	0.915968592	0.003190957	0.11532121
ENSG00000168610	STAT3	0.917270843	0.000789516	0.035716881	0.934000401	0.004118394	0.12906892
ENSG00000113916	BCL6	0.856794314	0.00079562	0.035716881	0.857223884	0.000386677	0.041288812
ENSG00000166188	ZNF319	0.870258666	0.000804637	0.036013545	0.886198997	0.001741739	0.090524481
ENSG00000168309	FAM107A	0.689155159	0.000835844	0.036642311	0.758345843	0.007090221	0.15306928
ENSG00000185668	POU3F1	0.650062976	0.000912905	0.03858104	0.77578658	0.030928628	0.269907802
ENSG00000111206	FOXN1	0.740902456	0.000913492	0.03858104	0.844639512	0.038243572	0.296322829
ENSG00000130558	OLFM1	0.493985855	0.000913619	0.03858104	0.732638211	0.094488632	0.416217676
ENSG00000135631	RAB11FIP5	0.85550877	0.000923307	0.038880341	0.883057645	0.00408105	0.128437098
ENSG00000152804	HHEX	0.727362638	0.000932378	0.03904236	0.906224485	0.24294322	0.604567146
ENSG00000050165	DKK3	0.569607417	0.00097763	0.040709153	0.745077301	0.051508198	0.33189485
ENSG00000109084	TMEM97	0.848301435	0.001001968	0.041149491	0.923422745	0.079390348	0.389904184
ENSG00000108055	SMC3	0.892384863	0.001009132	0.041217252	0.966165647	0.268404893	0.629602468
ENSG00000155254	MARVELD1	0.678906198	0.001017282	0.041436937	0.742431986	0.005885954	0.143118329
ENSG00000168062	BATF2	0.739518062	0.001030929	0.041605624	0.813878613	0.013273332	0.192803393
ENSG00000165795	NDRG2	0.885011124	0.00103163	0.041605624	0.94582443	0.099627653	0.42592771
ENSG00000100385	IL2RB	0.620222193	0.001032556	0.041605624	0.808919084	0.098670731	0.423498586
ENSG00000151632	AKR1C2	0.670362685	0.001036432	0.041649524	0.773242377	0.021575069	0.229066556
ENSG00000166855	CLPX	0.906461004	0.00104642	0.041661487	0.925646797	0.005244084	0.138929115
ENSG00000145431	PDGFC	0.816933079	0.00104843	0.041661487	0.917951666	0.121809601	0.46103906
ENSG00000164463	CREBRF	0.803683712	0.001050184	0.041661487	0.902429293	0.090724187	0.410555197
ENSG00000116717	GADD45A	0.797297366	0.001053671	0.041661487	0.877951941	0.037865321	0.295278397
ENSG00000085840	ORC1	0.666717417	0.001077026	0.042339544	0.747523906	0.010205995	0.173177554
ENSG00000011465	DCN	0.432431682	0.001095876	0.04234005	0.537675932	0.007325835	0.153905374
ENSG00000115414	FN1	0.439284452	0.001097509	0.04234005	0.522377001	0.005702694	0.141822582
ENSG00000134954	ETS1	0.778506797	0.00110109	0.04234005	0.8574471	0.026912205	0.252548995
ENSG00000173575	CHD2	0.868238115	0.001110737	0.04234005	0.921031404	0.037490509	0.29413988
ENSG00000205336	ADGRG1	0.892922808	0.001115702	0.04234005	0.898204844	0.000980578	0.067241536

ENSG00000173080	RXFP4	0.502753539	0.001118542	0.04234005	0.543830882	0.001224814	0.07451376
ENSG00000171169	NAIF1	0.873492264	0.001118758	0.04234005	0.893331846	0.003438056	0.119803041
ENSG00000009709	PAX7	0.829801247	0.001138508	0.042754007	0.790939562	2.51566E-05	0.011375455
ENSG00000129757	CDKN1C	0.781808451	0.001148078	0.04290654	0.829700896	0.00675486	0.151847227
ENSG00000076248	UNG	0.846828045	0.001157581	0.04309723	0.869147103	0.003202749	0.11532121
ENSG00000268089	GABRQ	0.385016444	0.001158946	0.04309723	0.432667852	0.001649855	0.088718278
ENSG00000149646	CNBD2	0.804922303	0.00117075	0.043428139	0.775909716	8.10424E-05	0.019230201
ENSG00000087495	PHACTR3	0.576163076	0.001181856	0.043623626	0.475464121	1.00275E-05	0.005353598
ENSG00000171951	SCG2	0.517685772	0.001188541	0.043762305	0.442469412	6.8246E-05	0.018218015
ENSG00000144730	IL17RD	0.769572323	0.00119845	0.043816521	0.777364679	0.000902245	0.0650882
ENSG00000152661	GJA1	0.516331176	0.001239021	0.044739423	0.768849286	0.134547418	0.480725119
ENSG00000089876	DHX32	0.888495392	0.001257264	0.045071549	0.924575151	0.019804457	0.222639868
ENSG00000174371	EXO1	0.588483714	0.001263302	0.045101748	0.759699617	0.05878253	0.348888026
ENSG00000125386	FAM193A	0.901039033	0.00126414	0.045101748	0.93483689	0.023570255	0.239529623
ENSG00000140577	CRTC3	0.857204888	0.001297268	0.045482684	0.897508866	0.013860968	0.196405314
ENSG00000187595	ZNF385C	0.750283633	0.001297874	0.045482684	0.784478131	0.003784318	0.124882485
ENSG00000184708	EIF4ENIF1	0.900199756	0.001299158	0.045482684	0.940879232	0.041105494	0.30359982
ENSG00000104897	SF3A2	0.877533802	0.001306308	0.045626169	0.920209201	0.026272204	0.25103604
ENSG00000205795	CYS1	0.596299345	0.001337323	0.046312055	0.701984866	0.014894742	0.200346136
ENSG00000100139	MICALL1	0.843424852	0.001346689	0.046386295	0.861284059	0.002521667	0.103990689
ENSG00000157837	SPPL3	0.88712571	0.001389802	0.047530295	0.88663455	0.000656125	0.054477605
ENSG00000100292	HMOX1	0.746731509	0.00139262	0.047530295	0.993201255	0.930410201	0.9798638
ENSG00000166508	MCM7	0.859787971	0.001403333	0.047678249	0.884355058	0.005046016	0.138626286
ENSG00000170604	IRF2BP1	0.860515195	0.001409447	0.047701425	0.935167136	0.113470698	0.449136211
ENSG00000149573	MPZL2	0.848705007	0.001417393	0.047814894	0.886245792	0.010903842	0.17657288
ENSG00000070540	WIP1	0.80767729	0.001425572	0.047814894	0.822738711	0.001843255	0.091849401
ENSG00000117748	RPA2	0.89312528	0.001427251	0.047814894	0.93333799	0.032976535	0.279530077
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Table E6

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ENSG00000119242	6	CCDC92
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ENSG00000205795	6	CYS1
ENSG00000011465	6	DCN
ENSG00000050165	6	DKK3
ENSG00000013563	6	DNASE1L1
ENSG00000006468	6	ETV1
ENSG00000168309	6	FAM107A
ENSG00000115414	6	FN1
ENSG00000183722	6	LHFPL6
ENSG00000117643	6	MAN1C1
ENSG00000155254	6	MARVELD1
ENSG00000187193	6	MT1X

ENSG00000141905	6	NFIC
ENSG00000009709	6	PAX7
ENSG00000157212	6	PAXIP1
ENSG00000145431	6	PDGFC
ENSG00000162407	6	PLPP3
ENSG00000185668	6	POU3F1
ENSG00000198056	6	PRIM1
ENSG00000262655	6	SPON1
ENSG00000074219	6	TEAD2
ENSG00000126351	6	THRA
ENSG00000249242	6	TMEM150C
ENSG00000273820	6	USP27X
ENSG00000018510	7	AGPS
ENSG00000100554	7	ATP6V1D
ENSG00000197446	7	CYP2F1
ENSG00000164934	7	DCAF13
ENSG00000077232	7	DNAJC10
ENSG00000072134	7	EPN2
ENSG00000221909	7	FAM200A
ENSG00000156958	7	GALK2
ENSG00000115339	7	GALNT3
ENSG00000106415	7	GLCCI1
ENSG00000162929	7	KIAA1841
ENSG00000110066	7	KMT5B
ENSG00000123562	7	MORF4L2
ENSG00000156642	7	NPTN
ENSG00000112541	7	PDE10A
ENSG00000080608	7	PUM3
ENSG00000124786	7	SLC35B3
ENSG00000070214	7	SLC44A1
ENSG00000080493	7	SLC4A4
ENSG00000181449	7	SOX2
ENSG00000138134	7	STAMBPL1
ENSG00000028839	7	TBPL1
ENSG00000174796	7	THAP6
ENSG00000151135	7	TMEM263
ENSG00000078687	7	TNRC6C
ENSG00000081307	7	UBA5
ENSG00000156802	8	ATAD2
ENSG00000164463	8	CREBRF
ENSG00000134954	8	ETS1
ENSG00000125386	8	FAM193A
ENSG00000151474	8	FRMD4A
ENSG00000152804	8	HHEX
ENSG00000163565	8	IFI16
ENSG00000144730	8	IL17RD
ENSG00000100385	8	IL2RB

ENSG00000128908	8	INO80
ENSG00000162337	8	LRP5
ENSG00000149573	8	MPZL2
ENSG00000248485	8	PCP4L1
ENSG00000119403	8	PHF19
ENSG00000120278	8	PLEKHG1
ENSG00000082996	8	RNF13
ENSG00000133818	8	RRAS2
ENSG00000163082	8	SGPP2
ENSG00000170365	8	SMAD1
ENSG00000108055	8	SMC3
ENSG00000084112	8	SSH1
ENSG00000168610	8	STAT3
ENSG00000100207	8	TCF20
ENSG00000141579	8	ZNF750

Table E7

Variable	Overall		EEC only		NAC only	
	R	P-value	R	P-value	R	P-value
IL-13 nasal postchallenge avg AUC 2-8hr (log2)	0.53	<0.001	0.62	<0.05	0.53	<0.05
IL-13 serum postchallenge 6hr (log2)	0.54	<0.001	0.54	<0.05	0.58	<0.05
IL-5 nasal postchallenge avg AUC 2-8hr (log2)	0.48	<0.05	0.63	<0.05	0.37	0.10
IL-5 serum postchallenge 6hr (log2)	0.46	<0.05	0.52	<0.05	0.37	0.09
TNSS AUC 0-3hr	0.44	<0.05	0.54	<0.05	0.30	0.18
Delta PNIF AUC 0-3hr	-0.35	<0.05	-0.17	0.48	-0.44	<0.05

Table E8

Variable	Overall		EEC only		NAC only	
	R	P-value	R	P-value	R	P-value
IL-13 nasal postchallenge avg AUC 2-8hr (log2)	-0.50	<0.001	-0.53	<0.05	-0.58	<0.05
IL-13 serum postchallenge 6hr (log2)	-0.25	0.20	-0.06	0.81	-0.38	0.06
IL-5 nasal postchallenge avg AUC 2-8hr (log2)	-0.5	<0.001	0.54	<0.05	0.48	<0.05
IL-5 serum postchallenge 6hr (log2)	-0.37	<0.05	-0.21	0.39	-0.46	<0.05
TNSS AUC 0-3hr	-0.41	<0.05	-0.38	0.10	-0.38	0.09
Delta PNIF AUC 0-3hr	0.48	<0.05	0.29	0.22	0.59	<0.05