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## Neutrophilic superoxide production can assess pharmacological and pharmacogenetic $\beta$ - adrenoreceptor effects

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## Short title

Neutrophilic ROS production to assess *ADRB2* pharmacogenetics.

## Abbreviations

*ADRB2*,  $\beta_2$ -adrenoceptor (gene);  $\beta_2$ AR,  $\beta_2$ -adrenoceptor (protein); LABA, long-acting  $\beta_2$ AR agonist; SABA, short-acting  $\beta_2$ AR agonist; fMLP, N-formyl-L-methioninyl-L-leucyl-L-phenylalanine; ISO, (*R*)-isoproterenol; SAL, (*R*)-salbutamol; FEN, (*R,R'*)-fenoterol; FORM, (*R,R'*)-formoterol; SNP, single nucleotide polymorphism; DF, degrees of freedom; ROS, reactive oxygen species

## Authorship contributions

M.T.R. and M.W. contributed equally to this work.

Participated in research design: M.T.R, M.W., M.K., Ro.S.

Conducted experiments: M.T.R., M.W., C.H., S.K.,

Performed data analysis: M.T.R, M.W., Ra.S.

Wrote or contributed to the writing of the manuscript: M.T.R., M.W., C.H., Ro.S.

## Conflict of interest statement

None to specify

## ABSTRACT

Asthma can be controlled well in most patients by inhaled  $\beta_2$ AR agonists and steroids. Poor response to  $\beta_2$ AR agonists is difficult to predict, especially in young children and by lung function testing, which may be affected by multiple influences. As an alternative approach, we analyzed *ex vivo* neutrophilic superoxide inhibition in response to  $\beta_2$ AR stimulation. In 60 healthy volunteers, this assay was unaffected by sex, age, smoking, atopy, or asthma status. Furthermore, we assessed effects of genetic variants in  $\beta_2$ AR by sequencing the *ADRB2* gene in our cohort and relating genotypes to  $\beta_2$ AR mediated neutrophilic superoxide inhibition. Gly16Arg genotypes correlated with minor decrease in overall adrenoresponse in this small study population. Taken together, *ex vivo* testing of the  $\beta_2$ AR response in human neutrophils represents a robust tool with good signal-to-noise ratio at physiological  $\beta_2$ AR agonist concentrations, and this assay may be useful to complement future pharmacogenetic studies in asthma. **(150/150 words)**

## KEY WORDS (5/5 words; alphabetical order)

*ADRB2*, asthma,  $\beta_2$ AR agonist, neutrophils, SNP

## INTRODUCTION

Asthma affects more than 300 million people worldwide (1). Although asthma can be controlled in the majority of patients with inhaled  $\beta_2$ AR agonists and steroids, treatment remains difficult in patients with severe asthma and long-term use of  $\beta_2$ AR agonists (2). Poorly controlled asthma is determined by environmental, socio-economic and genetic factors and represents an enormous economic burden (3). Early identification of patients who do not respond well to  $\beta_2$ AR agonists is of high clinical relevance but lung function tests are not feasible in all (especially young) children and lung function test results may be error prone due to multiple influences such as compliance.

An alternative approach to explore effects of  $\beta_2$ AR agonists is to test patient cells *ex vivo* for cellular adrenoresponses. Primary neutrophils represent a rather monomorphic, highly abundant, and easily accessible cell population for *ex vivo* testing of the  $\beta_2$ AR effects (4–6). Neutrophils contribute to a plethora of inflammatory processes including asthma, and one of the most important defense mechanisms in neutrophils is the production of reactive oxygen species (ROS) (7). ROS production is activated via the formyl-peptide receptor (6), inhibited via the  $\beta_2$ AR (5) and easily monitored

through the change of cytochrome c absorbance (8). Therefore, we assessed the pharmacological response towards four  $\beta_2$ AR agonists in neutrophils isolated from 60 healthy volunteers. Isoproterenol (ISO), a full  $\beta$ -adrenoceptor agonist, salbutamol (SAL) and fenoterol (FEN), both short acting  $\beta_2$ AR agonists (SABA), and formoterol (FORM), a long acting  $\beta_2$ AR agonist (LABA) commonly used in asthma or chronic obstructive pulmonary disease (COPD) treatment (9), were studied with respect to their effect on neutrophilic ROS production.

To test if subtle changes in  $\beta_2$ AR agonist response can be detected by this method, we investigated effects of *ADRB2* gene variants in all donors. So far, forty-nine different single nucleotide polymorphisms (SNPs) were identified in *ADRB2*, the gene encoding for the  $\beta_2$ AR, and several were proposed to influence  $\beta_2$ AR treatment response in patients (10). However, the influence of specific *ADRB2* SNPs on cellular adrenergic responses remain unclear and large, multi-center clinical trials thus far failed to elucidate the role of *ADRB2* variants to an extent that allowed for direct clinical implications (11).

## MATERIALS AND METHODS

### Study population

The study was approved by the local ethics committee. Sixty (male and female adults) participants gave their informed consent to collect pseudonomized data on *ex vivo* pharmacological responses in neutrophils, *ADRB2* genotyping and data obtained from a questionnaire (Supplemental Figures S1 and S2).

### Neutrophil isolation

Human neutrophils were isolated from venous blood (1.6 mg EDTA/ml blood). The isolation procedure was carried out at room temperature using 7 ml blood as described (8). In brief, the blood sample was diluted with PBS and layered on top of a separating solution with a density of 1.077 g/ml. After 30 minutes of centrifugation (RT, 400  $\times$  g), the monocytic cell layer was removed and the residual cell pellet was subjected to erythrocyte lysis by incubation with distilled water. The final preparation consisted of >98% viable neutrophils. Neutrophils were suspended in PBS to achieve a final density of  $10^6$  cells/ml and kept on ice until further use.

### Assessment of neutrophilic ROS production

10<sup>5</sup> neutrophils in 200 µl final volume per well were seeded into flat-bottom 96-well plates (Nunc Delta Surface, Nunc, Roskilde, Denmark). The reaction mix contained 1 mM CaCl<sub>2</sub>, 100 µM ferricytochrome c, and 0.3 µg/ml cytochalasin b (all final concentrations) in PBS. After pre-incubation with β<sub>2</sub>AR agonists (final concentrations between 1 nM and 10 µM, 3 min at 37 °C), ROS production was initiated by addition of fMLP (1 µM) and recorded as change of absorbance at 550 nm at 37 °C (A<sub>550</sub>; reduction of ferricytochrome c) using a Synergy 4 microplate reader (BioTek Instruments, Winooski, VT). Cells were analyzed in triplicates, and the difference between A<sub>550</sub> at time point of fMLP-addition and mean A<sub>550</sub> after 25 to 30 min was calculated. Full kinetic data and additional measurement at the isosbestic wavelength 556 nm served as internal controls (12).

### Genetic analysis

Genetic analysis of the complete exonic region of *ADRB2* (-237 bp from 5' of the ATG start codon to +1632 bp after the ATG start codon) is described in the Supplemental methods section. No *ADRB2* gene variants were preselected or excluded from the analyses. Primer sequences are given in Supplemental Table S1.

### Statistical analysis

Statistical methods are described in the Supplemental Methods section.

## RESULTS

fMLP activated neutrophilic ROS production was significantly inhibited by all four β<sub>2</sub>AR agonists (Figure 1A). FORM was the most potent and SAL the least potent inhibitor of fMLP-induced ROS production (Figure 1B and Supplemental Table S2). Although considerable inter-individual variability of the inhibitory potency of the four β<sub>2</sub>AR agonists occurred, donor-specific pharmacological responses for all four ligands were stable (Supplemental Figure S3).

The study cohort (n = 60 subjects, Supplemental Table S3) consisted of 27 male and 33 female subjects with Caucasian background. Six (10%) of the study participants were active smokers. Statistical analyses yielded no significant influence of sex, age, smoking, or atopy and asthma on

fMLP-stimulated ROS production or its inhibition by 100 nM ISO (Figure 1C and Supplemental Figure S4).

Polymorphic SNPs within the coding region of the ADRB2 gene identified in the cohort by sequencing were distributed according to Hardy-Weinberg equilibrium, and minor allele frequencies were in accordance with those of the HAPMAP-CEPH population (Supplemental Table S4) (13). In one subject, a previously unknown heterozygous variant (848C>G) causing an amino acid exchange (Thr283Ser) in transmembrane domain 6 (TM6) of the  $\beta_2$ AR was observed (Supplemental Figure S5). *In silico* analyses predicted a neutral effect of this gene variant with regard to protein structure and biological function (confidence of 63%; Supplemental Table S5).

When we analyzed  $\beta_2$ AR agonistic ROS inhibition in genotype-specific subgroups of our cohort (Supplemental Tables S6 and S7), a significant correlation between standardized pIC<sub>50</sub> values combining pharmacoresponse towards all four ligands and variant Gly16Arg was observed (Figure 2A). However, no significant influence of Gln27Glu on pharmacological responses was observed (Supplemental Figure S6 and S7) and when effects of individual ligands were tested separately, no statistically significant further correlations were observed (Figure 2B).

## DISCUSSION

The current study shows that  $\beta_2$ AR agonistic ROS inhibition in neutrophils can be used to assess pharmacological responses robustly and even to analyze the influence of pharmacogenetic variants in *ADRB2*. We observed effective inhibition of ROS production in all our 60 healthy donors with clinically relevant  $\beta_2$ AR agonist concentrations (16). In concordance with previous studies, FORM was the most potent and SAL the least potent inhibitor of ROS production (4). The highly significant correlation of standardized pIC<sub>50</sub> values for all agonists demonstrated similar pharmacological responses towards treatment with different  $\beta_2$ AR agonists in each individual proband. This suggested ligand-independent factors predisposing for strong or poor cellular  $\beta_2$ AR pharmacoresponse.

No confounding effects by sex, age, smoking or atopy and asthma were found in our study, supporting the robustness of our strategy and corroborating previous studies demonstrating no effect of age on ROS-production (17). We could not confirm the previously reported sexual dimorphism of neutrophil function that was described in a smaller study cohort (n=30) (5). In contrast, we did find a small but significant effect of smoking on the basal ROS production in

unstimulated neutrophils, which is in concordance with previous reports on increased neutrophil activation in smokers, an important factor in smoking-related airway disease (18). Of note, neutrophils are also important mediators of airway inflammation in asthma (14). However, the slightly increased ROS production at baseline in smokers' neutrophils did not affect fMLP-induced ROS production or impede the inhibitory effect of  $\beta_2$ AR in our pharmacological analysis.

We observed a significant correlation of the *ADRB2* variant Gly16Arg with a minor decrease in overall neutrophilic adrenoresponsiveness. This finding is consistent with preceding *in vitro* studies describing a  $\beta_2$ AR hypo-functionality for this variant (19). For Gln27Glu (11), a second variant described to possibly influence *ADRB2* function, we did not detect an allele-specific effect on neutrophilic ROS production, which is in agreement with *ex vivo* studies on lymphocytes (20). However, we cannot exclude subtle pharmacogenetic effects of the sequenced variants, especially of those with low frequencies in our cohort. Taken the moderate inter-individual variance of pharmacological responses and a probable overlap of evaluated subpopulations into account, the relatively small number of analyzed subjects combined with our extensive testing approach is a limitation of our study. Moreover, we cannot exclude that regulatory variants outside the sequenced exonic *ADRB2* region in *cis* and *trans* may also impact the cellular adrenoresponse.

Taken together, our work demonstrates that *ex vivo* testing of the  $\beta_2$ AR response in human neutrophils represents a robust and informative tool with good signal-to-noise ratio at physiological  $\beta_2$ AR agonist concentrations. This assay may be useful to complement future pharmacogenetic studies in asthma. Further analyses employing this test to assess *ex vivo* pharmacoresponses in neutrophils from asthma patients will shed light on its actual clinical relevance. On the long run, improved knowledge of *ADRB2* pharmacogenomics could contribute to the development of more precise treatment strategies in asthma.

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## Supplemental Materials and Methods

**Reagents** (*R*)-Isoproterenol hydrochloride (ISO), ferricytochrome c, cytochalasin B and fMLP were purchased from Sigma-Aldrich (St. Louis, MO). Enantiopure (*R,R'*)-fenoterol (FEN) fumarate was kindly provided by Dr. I. Wainer, SRI International (Menlo Park, CA). (*R*)-Salbutamol (SAL) and (*R,R'*)-formoterol (FORM) were provided by Boehringer Ingelheim (Ingelheim a. R., Germany). Ligand stocks (10 mM) were prepared in 1 mM HCl, aliquoted and stored at -20°C until use. Dilutions were prepared just before use in 1× PBS. Dulbecco's PBS (PBS, 10×) was purchased from PAA Laboratories (Pasching, Austria); Biocoll separating solution from Biochrom (Berlin, Germany).

**Genotyping** Genomic DNA was extracted from whole blood by means of standard salt-precipitation (1). The complete sequence of *ADRB2* including the 3'- and 5'- untranslated regions (-237 bp from 5' of the ATG start codon to +1632 bp after the ATG start codon) was determined after fragment PCR using Sanger sequencing (Supplemental Table S1).

**Sequence analysis** Chromatograms were analyzed using Vector NTI (Life Technologies, Carlsbad, CA) and ContigExpress (ContigExpress, New York, NY) software packages. Evaluation of the effects of single nucleotide variation on biological function of the  $\beta_2$ AR product was performed using PredictSNP 1.0 available online under <http://loschmidt.chemi.muni.cz/predictsnp> (2).

**Statistical analysis** Data were analyzed using the R-software package (3). Statistical differences between treatment with fMLP, basal activation of ROS-production and  $\beta_2$ AR-agonist-mediated inhibition of fMLP-activated ROS-production were evaluated using a paired two-tailed t-test with Bonferroni's correction for multiple testing. Statistical differences between independent subgroups at single concentrations of  $\beta_2$ AR-agonist treatment were evaluated by means of a one-way analysis of variance, which in most cases ( $k=2$ ) corresponded to an unpaired two-tailed t-test. Boxplots in Supplementary Figure 7 were used to analyze the distribution of the individual  $\Delta A_{550}$  for all conditions and subsettings tested. On this basis, we assumed normally distributed values.

For concentration-response analyses, the  $\Delta A_{550}$ -values were normalized to the values of full stimulation by 1  $\mu$ M fMLP and the maximal inhibition by 100 nM (*R*)-ISO. Concentration-response data of the ROS-assay were fitted to a log-logistic model to yield pIC<sub>50</sub> values as measure for overall  $\beta_2$ -AR responsiveness of each donor (logarithmic concentration of agonist to induce half maximal inhibition). Ambiguous fits were excluded from further analysis ( $n=4/240$  or 1.7% of all measurements). pIC<sub>50</sub> values were standardized: The centering of pIC<sub>50</sub> values was achieved by subtracting the mean calculated for each ligand group (ISO, FEN, SAL, FORM). Next, this value was scaled by division with the standard deviation of the same ligand group. This transformation

sets the overall mean of responsiveness to a value of zero to yield a standard deviation of 1. Standardization of responsiveness allowed for a combined analysis including the values of all four ligands in each donor.

Multivariate linear regression was used to assess the association of standardized responsiveness with the presence of minor alleles (according to the minor allele frequencies stated by the regarding NCBI SNP database entry; this corresponds to a dominant model for an effect by the minor allele) of the SNPs identified in the study population and additional categorical data (ligand, age quantile, sex, life-time prevalence for asthma or atopic diseases in general, smoking). A minimal multivariate linear model was constructed for the ligand attribute and each independent variable. After this pre-evaluation only variables with  $p < 0.25$  were used for building the extended multivariate linear model (4). Variables with  $p > 0.25$  were excluded from the model. The extended model was narrowed using stepwise backwards selection based on the Akaike information criterion (stepAIC) (5). The final model allowed the determination of significant explanatory variables and their estimated influence on the pharmacological response. Based on the standardization, estimates that were given by the regression model illustrated the estimated differences as multiple of the standard deviation of the original  $pIC_{50}$ . The analysis of variances applied to the final model yielded two-sided p-values, which were used for hypothesis generation without further correction for multiple testing. Values were considered significant when  $p < 0.05$ . If not stated otherwise, the error is given as standard deviation.

## Supplemental Tables

**Table S1. Amplification and sequencing primers used for direct sequencing of *ADRB2* within its genomic proximity on chromosome 5.**

|    | amplification primers (orientation) | genomic location<br>(length) | sequencing primers (orientation)                                   |
|----|-------------------------------------|------------------------------|--------------------------------------------------------------------|
| A. | ACATAACGGGCAGAACGCAC (Fwd)          | 148826595..1488278           | CTGACACAATCCACACCATCAG                                             |
|    | GAAGGCAATCCTGAAATCTGGG (Rev)        | 40<br>(1245 bp)              | (Rev)<br>GCAGTGGTGCCCTTTGG (Fwd)<br>CTATGCCAATGAGACCTGCTG<br>(Fwd) |
| B. | GCCTCTTCCATCGTGTCCTTCTAC<br>(Fwd)   | 148827435..1488284           | TCACCCTCTGCTGGCTGC (Fwd)                                           |
|    | CTCAAGTCCAAGGGTGTAGGAAGG<br>(Rev)   | 64<br>(1029 bp)              | AGGCAGAAGGATGCCCTTCC (Rev)                                         |

Fwd - forward; Rev - reverse, genomic location is given as the distance in base pairs (bp) from the beginning of the short chromosome arm (bp from pter).

**Table S2. Potencies for  $\beta_2$ AR -mediated inhibition of fMLP-induced ROS production in neutrophils**

|             | <b>pIC<sub>50</sub> <math>\pm</math> SD (<i>IC<sub>50</sub> in nM</i>)</b> | <b>pIC<sub>50</sub> (IQR)</b> | <b>n</b> |
|-------------|----------------------------------------------------------------------------|-------------------------------|----------|
|             | <b>mean</b>                                                                | <b>median</b>                 |          |
| <b>ISO</b>  | 8.5 $\pm$ 0.28 (3.16)                                                      | 8.47 (8.31–8.7)               | 60       |
| <b>FEN</b>  | 8.43 $\pm$ 0.31 (3.72)                                                     | 8.42 (8.19–8.62)              | 59       |
| <b>SAL</b>  | 7.28 $\pm$ 0.52 (52.48)                                                    | 7.37 (7.11–7.6)               | 58       |
| <b>FORM</b> | 9.99 $\pm$ 0.35 (0.10)                                                     | 9.94 (9.75–10.15)             | 59       |

Mean and median concentrations of  $\beta_2$ AR agonists for half-maximal inhibition (pIC<sub>50</sub>) of neutrophilic fMLP-induced ROS-production were calculated. Standard deviations and the 25th–75th percentiles (interquartile range = IQR) illustrate the scattering of this parameter within the population.

**Table S3. Cohort characteristics**

|                                                             |                  |
|-------------------------------------------------------------|------------------|
| <b>n of donors</b>                                          | 60               |
| <b>male (female)</b>                                        | 27 (33)          |
| <b>median age (range) / yrs</b>                             | 30 (22–56)       |
| <b>asthma-related questionnaire</b>                         |                  |
| <b>smoking</b>                                              | 6                |
| <b>had or has atopic disease</b>                            | 31               |
| <b>had or has asthma</b>                                    | 9                |
| <b>granulocytes count per ml whole blood / median (IQR)</b> |                  |
| <b>neutrophils</b>                                          | 3050 (2500–3800) |
| <b>eosinophils</b>                                          | 200 (100–300)    |
| <b>basophils</b>                                            | 0 (0–25)         |

Donors were recruited from the employees of Hannover Medical School, Hannover, Germany. Additional information was acquired with an asthma-related questionnaire (Supplemental Figure 2) and a whole blood count for each donor.

**Table S4. Genotype distribution within study cohort and predescribed SNP effects on gene expression**

| variation (substitution) |            | rs ID     | minor allele frequency |             | Described effects on gene expression                                                                                             |                               |
|--------------------------|------------|-----------|------------------------|-------------|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| nucleotide               | amino-acid |           | this study             | HAPMAP-CEPH | effect                                                                                                                           | citations [PMID]              |
| -47C>T                   | -          | rs1042711 | 0.36                   | -           | may alter $\beta_2$ -adrenoceptor expression                                                                                     | 17643132, 10193762            |
| -20C>T                   | -          | rs1801704 | 0.36                   | 0.48        | may alter $\beta_2$ -adrenoceptor expression                                                                                     | 17643132, 10193762            |
| 46G>A                    | Gly16Arg   | rs1042713 | 0.31                   | 0.36        | enhances agonist-stimulated receptor down-regulation; increased down-regulation in response to prolonged isoproterenol treatment | 7598936, 7915137;<br>22938397 |
| 79C>G                    | Gln27Glu   | rs1042714 | 0.36                   | 0.47        | no effect on receptor expression; Gln 27 may enhance down-regulation in presence of Arg16                                        | 7598936, 7915137;<br>22938397 |
| 252G>A                   | Leu84=     | rs1042717 | 0.18                   | 0.16        |                                                                                                                                  |                               |
| 523C>A                   | Arg175=    | rs1042718 | 0.2                    | 0.15        |                                                                                                                                  |                               |
| 848C>G                   | Thr283Ser* | -         | 0.01                   | -           | novel                                                                                                                            |                               |
| 1053G>C                  | Gly351=    | rs1042719 | 0.28                   | 0.30        |                                                                                                                                  |                               |
| 1239G>A                  | Leu413=    | rs1042720 | 0.32                   | 0.32        |                                                                                                                                  |                               |
| C>G (poly-C)             | -          | -         | 0.31                   | -           |                                                                                                                                  |                               |

Listed are all nucleotide variations, synonymous (e.g. Leu84=) and non-synonymous (e.g. Gly16Arg) amino-acid substitutions and their minor allele frequencies detected by complete sequencing of the ADRB2 exon including flanking up and downstream regions (See Genotype Analysis in Supplemental Materials and Methods). Important to say, the known SNP 491C>T (Thr164Ile, rs1800888, minor allele frequency (HAPMAP-CEPH) = 0.01) was not polymorph within our cohort. We also checked the diversity of the poly-C-repeat region. If available, rs IDs refer to Single Nucleotide Polymorphism Database (dbSNP) entries (6). Frequencies of the HAPMAP-CEPH cohort are given as reference (7). Minor allele frequencies found in the study population were not significantly different to HAPMAP-CEPH in a Chi<sup>2</sup>-test.

**Table S5. *In silico* analysis of effect by non-synonymous amino acid exchange on biological function**

| annotation                    |       | algorithm and principle                 |                                                    |                        |                                |                        |                    |                     |
|-------------------------------|-------|-----------------------------------------|----------------------------------------------------|------------------------|--------------------------------|------------------------|--------------------|---------------------|
| protein                       | SNP   | PredictSNP 1.0                          | MAPP                                               | PhD-SNP                | PolyPhen v1                    | PolyPhen v2            | SIFT               | SNAP                |
|                               |       | combined evaluation of the other scores | physico-chemical properties and sequence alignment | support vector machine | experts set of empirical rules | naïve Bayes classifier | sequence alignment | neural network      |
| score result and confidence   |       |                                         |                                                    |                        |                                |                        |                    |                     |
| ADRB2_HUMAN (UniProt# P07550) | T283S | neutral<br>63 %                         | deleterious<br>46 %                                | neutral<br>68 %        | neutral<br>67 %                | neutral<br>69 %        | neutral<br>53 %    | deleterious<br>62 % |
|                               | G16R  | deleterious<br>52 %                     | deleterious<br>46 %                                | deleterious<br>68 %    | deleterious<br>59 %            | neutral<br>75 %        | neutral<br>77 %    | deleterious<br>62 % |
|                               | E27Q  | neutral<br>83 %                         | neutral<br>80 %                                    | neutral<br>58 %        | neutral<br>67 %                | neutral<br>68 %        | neutral<br>77 %    | neutral<br>71 %     |

Result of web-based PredictSNP1.0 analysis tool for the combined scoring of effects by non-synonymous amino-acid exchanges (available under <http://loschmidt.chemi.muni.cz/predictsnp/> (date of query: 2014-08-18). This tool combines the algorithms MAPP (Multivariate Analysis of Protein Polymorphism), PhD-SNP (Predictor of human Deleterious Single Nucleotide Polymorphisms), PolyPhen-1 and -2 (Polymorphism Phenotyping v1 and v2), SIFT (sorts intolerant from tolerant) and SNAP (Screening for non acceptable polymorphisms) into a single score (PredictSNP 1.0).(2)

Table S6. Summary of data obtained per donor in a questionnaire (sex, prevalence, smoking, age) and by pharmacological analysis (A<sub>550</sub>, IC<sub>50</sub>) (1/2)

| ID | sex | life-time prevalence for |        | smoking | age       | A <sub>550</sub> ~ROS production |             |             | IC <sub>50,ISO</sub> |             |             | pIC <sub>50,ISO</sub> |                       | IC <sub>50,FEN</sub> |       | pIC <sub>50,FEN</sub> |      | IC <sub>50,SAL</sub> |                           | pIC <sub>50,SAL</sub> |       | IC <sub>50,FORM</sub> |       | pIC <sub>50,FORM</sub> |  |
|----|-----|--------------------------|--------|---------|-----------|----------------------------------|-------------|-------------|----------------------|-------------|-------------|-----------------------|-----------------------|----------------------|-------|-----------------------|------|----------------------|---------------------------|-----------------------|-------|-----------------------|-------|------------------------|--|
|    |     | asthma                   | atopic |         |           | group limits                     | basal       | max         | ISO 100 nM           | ISO 10 nM   | FEN 10 nM   | SAL 10 nM             | FORM 10 nM            | standardized         |       | standardized          |      | standardized         |                           | standardized          |       | standardized          |       |                        |  |
| 1  | f   | no                       | no     | no      | 22–25     | 0.007±0.003                      | 0.235±0.052 | 0.092±0.020 | 0.160±0.048          | 0.156±0.038 | 0.205±0.047 | 0.105±0.026           | 11.11<br>(12.48–9.74) | 7.95                 | -2.22 | 10.09<br>(11.34–8.84) | 8.00 | -1.56                | 174.00<br>(224.90–123.11) | 6.76                  | -1.30 | 0.23<br>(0.28–0.17)   | 9.64  | -0.92                  |  |
| 2  | m   | no                       | no     | yes     | 31.4–43.2 | 0.047±0.050                      | 0.275±0.055 | 0.139±0.091 | 0.190±0.095          | 0.194±0.085 | 0.230±0.069 | 0.163±0.107           | 9.50<br>(12.84–6.16)  | 8.02                 | -1.98 | 6.93<br>(10.64–3.23)  | 8.16 | -1.05                | 112.52<br>(161.72–63.33)  | 6.95                  | -0.89 | 0.24<br>(0.35–0.13)   | 9.62  | -0.97                  |  |
| 3  | f   | no                       | yes    | no      | 27.6–31.4 | 0.006±0.004                      | 0.217±0.048 | 0.089±0.050 | 0.139±0.058          | 0.148±0.053 | 0.185±0.052 | 0.121±0.058           | 8.25<br>(9.58–6.92)   | 8.08                 | -1.75 | 10.81<br>(13.42–8.20) | 7.97 | -1.65                | 1651.21<br>(NA–NA)        | 5.78                  | -3.45 | 0.64<br>(NA–NA)       | 9.19  | -2.08                  |  |
| 4  | m   | no                       | yes    | no      | 22–25     | 0.003±0.002                      | 0.210±0.017 | 0.049±0.022 | 0.110±0.054          | 0.113±0.031 | 0.173±0.039 | 0.054±NA              | 8.02<br>(9.53–6.51)   | 8.10                 | -1.71 | 8.55<br>(9.36–7.74)   | 8.07 | -1.33                | 104.97<br>(136.88–73.06)  | 6.98                  | -0.82 | 0.19<br>(0.23–0.16)   | 9.71  | -0.73                  |  |
| 5  | m   | no                       | no     | no      | 31.4–43.2 | 0.019±0.026                      | 0.285±0.025 | 0.105±0.051 | 0.165±0.036          | 0.175±0.036 | 0.252±0.017 | 0.122±0.056           | 7.94<br>(8.70–7.19)   | 8.10                 | -1.69 | 8.94<br>(9.93–7.95)   | 8.05 | -1.39                | 144.25<br>(187.71–100.80) | 6.84                  | -1.12 | 0.25<br>(0.31–0.19)   | 9.60  | -1.02                  |  |
| 6  | m   | yes                      | yes    | no      | 43.2–56   | 0.010±0.011                      | 0.224±0.012 | 0.091±0.021 | 0.138±0.025          | 0.147±0.027 | 0.182±0.029 | 0.126±0.025           | 5.92<br>(7.20–4.64)   | 8.23                 | -1.23 | 8.55<br>(12.00–5.10)  | 8.07 | -1.33                | 1168.87<br>(NA–NA)        | 5.93                  | -3.12 | 0.87<br>(1.07–0.68)   | 9.06  | -2.43                  |  |
| 7  | f   | no                       | no     | no      | 25–27.6   | 0.005±0.005                      | 0.236±0.058 | 0.083±0.040 | 0.134±0.052          | 0.142±0.043 | 0.188±0.047 | 0.118±0.065           | 5.59<br>(6.45–4.73)   | 8.25                 | -1.14 | 6.93<br>(8.42–5.43)   | 8.16 | -1.05                | 263.60<br>(NA–NA)         | 6.58                  | -1.70 | 0.35<br>(0.50–0.20)   | 9.46  | -1.39                  |  |
| 8  | f   | no                       | yes    | no      | 31.4–43.2 | 0.004±0.003                      | 0.217±0.049 | 0.054±0.029 | 0.104±0.038          | 0.116±0.042 | 0.171±0.049 | 0.071±0.048           | 5.34<br>(6.03–4.66)   | 8.27                 | -1.07 | 7.34<br>(8.21–6.48)   | 8.13 | -1.13                | 90.24<br>(114.94–65.54)   | 7.04                  | -0.68 | 0.20<br>(0.26–0.15)   | 9.69  | -0.78                  |  |
| 9  | m   | no                       | no     | no      | 27.6–31.4 | 0.007±0.003                      | 0.276±0.051 | 0.072±0.014 | 0.135±0.028          | 0.153±0.042 | 0.207±0.020 | 0.107±0.037           | 5.16<br>(7.48–2.83)   | 8.29                 | -1.02 | 7.68<br>(10.88–4.48)  | 8.11 | -1.19                | 166.88<br>(NA–NA)         | 6.78                  | -1.26 | 0.20<br>(0.27–0.12)   | 9.70  | -0.75                  |  |
| 10 | f   | no                       | no     | no      | 22–25     | 0.006±0.003                      | 0.179±0.023 | 0.046±0.003 | 0.085±0.010          | 0.094±0.011 | 0.149±0.011 | 0.057±0.015           | 5.13<br>(6.07–4.19)   | 8.29                 | -1.01 | 5.66<br>(7.33–3.99)   | 8.25 | -0.78                | 87.89<br>(120.17–55.61)   | 7.06                  | -0.65 | 0.19<br>(0.25–0.13)   | 9.72  | -0.71                  |  |
| 11 | m   | yes                      | yes    | no      | 25–27.6   | 0.002±0.003                      | 0.203±0.052 | 0.078±0.052 | 0.114±0.079          | 0.118±0.080 | 0.156±0.069 | 0.100±0.071           | 5.11<br>(7.36–2.85)   | 8.29                 | -1.00 | 6.46<br>(8.77–4.16)   | 8.19 | -0.96                | 74.07<br>(130.65–17.48)   | 7.13                  | -0.49 | 0.17<br>(0.25–0.09)   | 9.76  | -0.60                  |  |
| 12 | f   | no                       | yes    | no      | 25–27.6   | 0.002±0.002                      | 0.220±0.026 | 0.044±0.004 | 0.089±0.007          | 0.098±0.010 | 0.161±0.030 | 0.072±NA              | 5.01<br>(5.78–4.23)   | 8.30                 | -0.97 | 6.39<br>(7.44–5.34)   | 8.19 | -0.94                | 64.82<br>(88.46–41.18)    | 7.19                  | -0.36 | 0.21<br>(0.25–0.17)   | 9.69  | -0.80                  |  |
| 13 | f   | no                       | yes    | no      | 25–27.6   | 0.004±0.000                      | 0.199±0.089 | 0.054±0.045 | 0.104±0.078          | 0.112±0.079 | 0.162±0.090 | 0.040±0.011           | 4.99<br>(6.40–3.58)   | 8.30                 | -0.97 | 7.07<br>(9.02–5.12)   | 8.15 | -1.08                | 62.47<br>(91.70–33.25)    | 7.20                  | -0.32 | 0.14<br>(0.21–0.07)   | 9.85  | -0.38                  |  |
| 14 | f   | no                       | no     | no      | 43.2–56   | 0.002±0.002                      | 0.167±0.034 | 0.029±0.007 | 0.062±0.015          | 0.071±0.020 | 0.125±0.024 | 0.031±NA              | 4.44<br>(5.08–3.80)   | 8.35                 | -0.78 | 5.34<br>(5.89–4.79)   | 8.27 | -0.70                | 87.88<br>(104.01–71.75)   | 7.06                  | -0.65 | 0.15<br>(0.20–0.10)   | 9.83  | -0.43                  |  |
| 15 | f   | no                       | no     | no      | 22–25     | 0.064±0.087                      | 0.292±0.029 | 0.072±0.033 | 0.127±0.020          | 0.141±0.017 | 0.212±0.030 | 0.089±0.043           | 3.94<br>(4.57–3.32)   | 8.40                 | -0.59 | 4.44<br>(5.27–3.62)   | 8.35 | -0.45                | 45.85<br>(62.95–28.75)    | 7.34                  | -0.03 | 0.16<br>(0.18–0.13)   | 9.80  | -0.49                  |  |
| 16 | m   | no                       | yes    | no      | 31.4–43.2 | 0.007±0.003                      | 0.239±0.039 | 0.043±0.001 | 0.091±0.016          | 0.102±0.023 | 0.175±0.031 | 0.062±0.009           | 3.81<br>(4.54–3.09)   | 8.42                 | -0.54 | 4.14<br>(4.94–3.34)   | 8.38 | -0.35                | 50.99<br>(67.24–34.74)    | 7.29                  | -0.13 | 0.13<br>(0.15–0.10)   | 9.89  | -0.26                  |  |
| 17 | m   | no                       | yes    | no      | 43.2–56   | 0.007±0.002                      | 0.192±0.028 | 0.039±0.005 | 0.079±0.018          | 0.076±0.025 | 0.128±0.029 | 0.055±NA              | 3.80<br>(4.72–2.88)   | 8.42                 | -0.54 | 3.00<br>(4.01–1.98)   | 8.52 | 0.08                 | 43.99<br>(55.62–32.37)    | 7.36                  | 0.01  | 0.23<br>(0.36–0.10)   | 9.64  | -0.91                  |  |
| 18 | m   | yes                      | yes    | no      | 27.6–31.4 | 0.005±0.009                      | 0.160±0.048 | 0.038±0.023 | 0.063±0.031          | 0.073±0.035 | 0.126±0.047 | 0.042±0.025           | 3.79<br>(4.22–3.35)   | 8.42                 | -0.53 | 4.97<br>(5.48–4.46)   | 8.30 | -0.60                | 49.19<br>(56.71–41.67)    | 7.31                  | -0.10 | 0.10<br>(0.11–0.09)   | 9.99  | -0.00                  |  |
| 19 | m   | no                       | no     | no      | 27.6–31.4 | 0.004±0.004                      | 0.216±0.045 | 0.051±0.027 | 0.089±0.028          | 0.099±0.047 | 0.157±0.045 | 0.064±0.033           | 3.77<br>(4.66–2.88)   | 8.42                 | -0.52 | 5.97<br>(6.89–5.05)   | 8.22 | -0.85                | 120.41<br>(165.34–75.48)  | 6.92                  | -0.95 | 0.10<br>(0.11–0.09)   | 9.98  | -0.03                  |  |
| 20 | f   | no                       | no     | no      | 31.4–43.2 | 0.002±0.001                      | 0.220±0.041 | 0.035±0.010 | 0.075±0.024          | 0.081±0.024 | 0.145±0.042 | 0.064±NA              | 3.64<br>(4.31–2.98)   | 8.44                 | -0.47 | 3.64<br>(4.04–3.24)   | 8.44 | -0.18                | 50.58<br>(57.79–43.37)    | 7.30                  | -0.12 | 0.12<br>(0.14–0.10)   | 9.92  | -0.18                  |  |
| 21 | f   | no                       | no     | yes     | 31.4–43.2 | 0.004±0.001                      | 0.216±0.027 | 0.036±0.009 | 0.074±0.030          | 0.079±0.020 | 0.165±0.029 | 0.047±0.016           | 3.47<br>(4.02–2.92)   | 8.46                 | -0.39 | 4.04<br>(4.65–3.43)   | 8.39 | -0.32                | 54.48<br>(64.56–44.41)    | 7.26                  | -0.19 | -                     | -     | -                      |  |
| 22 | m   | no                       | yes    | no      | 22–25     | 0.001±0.000                      | 0.167±0.029 | 0.041±0.016 | 0.073±0.026          | 0.080±0.024 | 0.119±0.033 | 0.036±NA              | 3.44<br>(4.35–2.54)   | 8.46                 | -0.38 | 4.14<br>(5.15–3.13)   | 8.38 | -0.35                | 65.97<br>(98.75–33.18)    | 7.18                  | -0.38 | 0.16<br>(0.20–0.11)   | 9.81  | -0.48                  |  |
| 23 | m   | no                       | yes    | no      | 43.2–56   | 0.008±0.012                      | 0.108±0.091 | 0.033±0.024 | 0.055±0.045          | 0.057±0.052 | 0.079±0.076 | 0.041±0.026           | 3.33<br>(4.74–1.93)   | 8.48                 | -0.33 | -                     | -    | -                    | -                         | -                     | -     | 0.12<br>(0.18–0.06)   | 9.92  | -0.18                  |  |
| 24 | f   | no                       | yes    | no      | 27.6–31.4 | 0.005±0.003                      | 0.197±0.009 | 0.042±0.011 | 0.067±0.004          | 0.075±0.003 | 0.149±0.002 | 0.054±0.019           | 3.27<br>(3.60–2.94)   | 8.49                 | -0.30 | 3.62<br>(3.97–3.27)   | 8.44 | -0.17                | 42.39<br>(47.79–37.00)    | 7.37                  | 0.05  | 0.09<br>(0.11–0.07)   | 10.05 | 0.15                   |  |
| 25 | f   | yes                      | yes    | no      | 22–25     | 0.009±0.009                      | 0.189±0.007 | 0.031±0.021 | 0.062±0.045          | 0.076±0.045 | 0.136±0.043 | 0.041±0.027           | 3.25<br>(4.20–2.31)   | 8.49                 | -0.29 | 5.13<br>(6.20–4.07)   | 8.29 | -0.64                | 49.97<br>(74.87–25.07)    | 7.30                  | -0.11 | 0.13<br>(0.17–0.10)   | 9.87  | -0.31                  |  |
| 26 | m   | no                       | yes    | no      | 27.6–31.4 | 0.002±0.000                      | 0.198±0.002 | 0.030±0.010 | 0.063±0.000          | 0.069±0.004 | 0.116±0.005 | 0.047±NA              | 3.25<br>(4.12–2.38)   | 8.49                 | -0.29 | 3.90<br>(4.74–3.06)   | 8.41 | -0.27                | 36.73<br>(49.51–23.95)    | 7.43                  | 0.18  | 0.17<br>(0.20–0.14)   | 9.78  | -0.56                  |  |
| 27 | m   | no                       | no     | no      | 27.6–31.4 | 0.004±0.002                      | 0.211±0.050 | 0.034±0.000 | 0.067±0.006          | 0.078±0.011 | 0.135±0.003 | 0.049±0.003           | 3.24<br>(4.11–2.37)   | 8.49                 | -0.29 | 4.65<br>(5.90–3.40)   | 8.33 | -0.51                | 41.59<br>(63.36–19.82)    | 7.38                  | 0.06  | 0.17<br>(0.21–0.13)   | 9.77  | -0.59                  |  |
| 28 | m   | no                       | no     | yes     | 27.6–31.4 | 0.003±0.004                      | 0.198±0.052 | 0.046±0.019 | 0.084±0.039          | 0.101±0.048 | 0.149±0.057 | 0.064±0.013           | 3.09<br>(3.75–2.42)   | 8.51                 | -0.21 | 5.04<br>(6.22–3.87)   | 8.30 | -0.62                | 50.21<br>(73.44–26.99)    | 7.30                  | -0.12 | 0.16<br>(0.22–0.10)   | 9.81  | -0.49                  |  |
| 29 | f   | no                       | yes    | no      | 43.2–56   | 0.002±0.003                      | 0.211±0.022 | 0.035±0.007 | 0.068±0.010          | 0.086±0.016 | 0.157±0.023 | 0.037±NA              | 3.08<br>(3.46–2.69)   | 8.51                 | -0.20 | 4.75<br>(5.23–4.26)   | 8.32 | -0.54                | 58.41<br>(69.41–47.42)    | 7.23                  | -0.26 | 0.10<br>(0.11–0.09)   | 10.00 | 0.02                   |  |
| 30 | f   | no                       | yes    | yes     | 22–25     | 0.047±0.063                      | 0.186±0.009 | 0.035±0.002 | 0.065±0.006          | 0.075±0.012 | 0.149±0.032 | 0.045±0.002           | 3.04<br>(3.47–2.61)   | 8.52                 | -0.19 | 3.56<br>(4.20–2.93)   | 8.45 | -0.15                | 105.36<br>(159.09–51.64)  | 6.98                  | -0.82 | 0.16<br>(0.18–0.14)   | 9.80  | -0.51                  |  |

Color traces back donor-specific data in the figures of the publication (Fig. 1, Supplementary Figure 3, 4 and 6), donor IDs were assigned post-hoc using the order of IC<sub>50,ISO</sub>, A<sub>550</sub> = absorbance measured at 550 nM was proportional to ROS production, IC<sub>50</sub> values and its transformations were calculated from the concentration-response data of neutrophilic ROS-assays. Errors are given as standard deviations or confidence intervals. Failing error calculation is denoted with NA and was only accepted for IC<sub>50</sub> values if plotted curves allowed a tolerable fit based on R<sup>2</sup>.

**Table S6 (continued). Summary of data obtained per donor (2/2)**

| ID | sex | life-time prevalence for |        | smoking | age       | A <sub>550</sub> ~ROS production |             |             |             |             |             | IC <sub>50,ISO</sub> | pIC <sub>50,ISO</sub> | IC <sub>50,FEN</sub> | pIC <sub>50,FEN</sub> | IC <sub>50,SAL</sub> | pIC <sub>50,SAL</sub> | IC <sub>50,FORM</sub> | pIC <sub>50,FORM</sub> |           |            |                     |              |              |
|----|-----|--------------------------|--------|---------|-----------|----------------------------------|-------------|-------------|-------------|-------------|-------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|-----------------------|------------------------|-----------|------------|---------------------|--------------|--------------|
|    |     | asthma                   | atopic |         |           | group                            | basal       | max         | ISO 100 nM  | ISO 10 nM   | FEN 10 nM   |                      |                       |                      |                       |                      |                       |                       |                        | SAL 10 nM | FORM 10 nM | standardized        | standardized | standardized |
| 31 | f   | no                       | yes    | no      | 31.4–43.2 | 0.003±0.001                      | 0.217±0.003 | 0.035±0.008 | 0.062±0.003 | 0.086±0.014 | 0.181±0.000 | 0.054±NA             | 2.86<br>(3.13–2.59)   | 8.54                 | -0.09                 | 4.90<br>(5.32–4.48)  | 8.31                  | -0.58                 | 65.45<br>(70.68–60.21) | 7.18      | -0.37      | 0.14<br>(0.16–0.12) | 9.85         | -0.37        |
| 32 | f   | no                       | yes    | no      | 22–25     | 0.004±0.004                      | 0.197±0.022 | 0.023±0.007 | 0.047±0.004 | 0.056±0.005 | 0.148±0.009 | 0.033±0.012          | 2.74<br>(2.95–2.53)   | 8.56                 | -0.02                 | 2.73<br>(2.96–2.49)  | 8.56                  | 0.21                  | 42.77<br>(47.80–37.74) | 7.37      | 0.04       | 0.08<br>(0.09–0.07) | 10.11        | 0.31         |
| 33 | m   | no                       | no     | no      | 43.2–56   | 0.005±0.003                      | 0.137±0.109 | 0.030±0.010 | 0.047±0.031 | 0.061±0.050 | 0.098±0.089 | 0.029±0.008          | 2.49<br>(2.97–2.01)   | 8.60                 | 0.13                  | 3.27<br>(3.97–2.57)  | 8.49                  | -0.03                 | 35.32<br>(50.04–20.60) | 7.45      | 0.22       | 0.11<br>(0.14–0.09) | 9.94         | -0.13        |
| 34 | m   | yes                      | yes    | no      | 31.4–43.2 | 0.004±0.002                      | 0.130±0.068 | 0.026±0.013 | 0.043±0.029 | 0.051±0.037 | 0.097±0.069 | 0.035±0.018          | 2.33<br>(2.97–1.69)   | 8.63                 | 0.23                  | 2.86<br>(3.69–2.04)  | 8.54                  | 0.14                  | 36.06<br>(49.12–23.00) | 7.44      | 0.20       | 0.12<br>(0.15–0.09) | 9.93         | -0.16        |
| 35 | m   | no                       | yes    | no      | 43.2–56   | 0.005±0.006                      | 0.128±0.049 | 0.020±0.005 | 0.036±0.015 | 0.036±0.008 | 0.071±0.027 | 0.030±0.013          | 2.32<br>(2.75–1.89)   | 8.63                 | 0.24                  | 2.29<br>(2.69–1.89)  | 8.64                  | 0.44                  | 18.19<br>(21.80–14.57) | 7.74      | 0.85       | 0.08<br>(0.09–0.07) | 10.08        | 0.23         |
| 36 | f   | no                       | no     | no      | 27.6–31.4 | 0.004±0.002                      | 0.241±0.076 | 0.052±0.036 | 0.087±0.051 | 0.115±0.046 | 0.181±0.063 | 0.096±0.047          | 2.30<br>(3.18–1.43)   | 8.64                 | 0.25                  | 6.52<br>(7.91–5.14)  | 8.19                  | -0.97                 | 74.63<br>(97.44–51.82) | 7.13      | -0.49      | 0.24<br>(0.32–0.16) | 9.62         | -0.96        |
| 37 | f   | no                       | no     | no      | 27.6–31.4 | 0.004±0.000                      | 0.164±0.025 | 0.024±0.008 | 0.050±0.038 | 0.055±0.041 | 0.103±0.036 | 0.030±0.014          | 2.22<br>(3.16–1.28)   | 8.65                 | 0.31                  | 2.30<br>(3.28–1.33)  | 8.64                  | 0.44                  | 21.01<br>(30.62–11.40) | 7.68      | 0.72       | 0.09<br>(0.12–0.06) | 10.07        | 0.19         |
| 38 | f   | no                       | yes    | no      | 27.6–31.4 | 0.005±0.002                      | 0.171±0.095 | 0.036±0.035 | 0.053±0.048 | 0.066±0.059 | 0.126±0.098 | 0.022±0.005          | 2.02<br>(2.40–1.65)   | 8.69                 | 0.45                  | 2.96<br>(3.49–2.43)  | 8.53                  | 0.10                  | 35.17<br>(44.80–25.53) | 7.45      | 0.22       | 0.08<br>(0.09–0.07) | 10.11        | 0.31         |
| 39 | f   | no                       | no     | no      | 25–27.6   | 0.004±0.002                      | 0.219±0.029 | 0.037±0.000 | 0.055±0.001 | 0.070±0.001 | 0.150±0.013 | 0.053±0.001          | 1.99<br>(2.34–1.63)   | 8.70                 | 0.48                  | 3.04<br>(3.53–2.55)  | 8.52                  | 0.06                  | 36.58<br>(42.09–31.06) | 7.44      | 0.19       | 0.13<br>(0.15–0.11) | 9.89         | -0.28        |
| 40 | f   | no                       | no     | no      | 43.2–56   | 0.002±0.002                      | 0.129±0.033 | 0.015±0.004 | 0.032±0.014 | 0.035±0.016 | 0.075±0.033 | 0.023±0.003          | 1.92<br>(2.36–1.47)   | 8.72                 | 0.54                  | 1.43<br>(1.86–0.99)  | 8.85                  | 1.08                  | 15.73<br>(22.03–9.43)  | 7.80      | 0.99       | 0.03<br>(0.04–0.03) | 10.49        | 1.29         |
| 41 | f   | yes                      | yes    | no      | 22–25     | 0.003±0.003                      | 0.089±0.042 | 0.019±0.002 | 0.028±0.003 | 0.028±0.003 | 0.053±0.023 | 0.026±0.000          | 1.89<br>(2.31–1.47)   | 8.72                 | 0.56                  | 1.24<br>(1.51–0.96)  | 8.91                  | 1.28                  | 13.48<br>(18.72–8.25)  | 7.87      | 1.14       | 0.06<br>(0.09–0.03) | 10.21        | 0.56         |
| 42 | m   | no                       | no     | no      | 25–27.6   | 0.006±0.003                      | 0.170±0.092 | 0.040±0.030 | 0.064±0.061 | 0.076±0.080 | 0.123±0.101 | 0.030±0.013          | 1.89<br>(2.42–1.36)   | 8.72                 | 0.56                  | 1.86<br>(2.45–1.26)  | 8.73                  | 0.73                  | 18.55<br>(31.23–5.87)  | 7.73      | 0.84       | 0.08<br>(0.12–0.04) | 10.09        | 0.25         |
| 43 | f   | no                       | no     | no      | 43.2–56   | 0.003±0.000                      | 0.082±0.013 | 0.017±0.001 | 0.023±0.001 | 0.033±0.011 | 0.053±0.012 | 0.022±0.000          | 1.87<br>(2.12–1.62)   | 8.73                 | 0.58                  | 2.33<br>(2.90–1.76)  | 8.63                  | 0.42                  | 18.72<br>(21.53–15.91) | 7.73      | 0.83       | 0.07<br>(0.08–0.06) | 10.17        | 0.45         |
| 44 | m   | no                       | no     | no      | 25–27.6   | 0.003±0.001                      | 0.206±0.044 | 0.024±0.005 | 0.049±0.012 | 0.065±0.024 | 0.133±0.029 | 0.033±NA             | 1.86<br>(2.24–1.48)   | 8.73                 | 0.59                  | 2.79<br>(3.38–2.21)  | 8.55                  | 0.18                  | 29.87<br>(38.35–21.39) | 7.52      | 0.38       | 0.11<br>(0.12–0.09) | 9.97         | -0.05        |
| 45 | m   | no                       | yes    | no      | 27.6–31.4 | 0.002±0.002                      | 0.184±0.060 | 0.031±0.014 | 0.050±0.024 | 0.059±0.026 | 0.113±0.082 | 0.027±NA             | 1.85<br>(2.15–1.55)   | 8.73                 | 0.59                  | 2.36<br>(2.72–1.99)  | 8.63                  | 0.41                  | 34.79<br>(57.39–12.20) | 7.46      | 0.24       | 0.11<br>(0.17–0.06) | 9.94         | -0.13        |
| 46 | m   | no                       | no     | no      | 31.4–43.2 | 0.005±0.004                      | 0.122±0.051 | 0.039±0.038 | 0.053±0.056 | 0.056±0.055 | 0.089±0.064 | 0.047±0.044          | 1.77<br>(2.50–1.04)   | 8.75                 | 0.66                  | 1.97<br>(2.68–1.27)  | 8.70                  | 0.64                  | 24.16<br>(31.59–16.73) | 7.62      | 0.58       | 0.03<br>(0.05–0.02) | 10.48        | 1.27         |
| 47 | f   | no                       | yes    | no      | 25–27.6   | 0.021±0.033                      | 0.214±0.065 | 0.058±0.058 | 0.076±0.065 | 0.081±0.062 | 0.149±0.069 | 0.096±0.067          | 1.73<br>(1.97–1.49)   | 8.76                 | 0.70                  | 1.86<br>(2.07–1.64)  | 8.73                  | 0.73                  | -                      | -         | -          | 0.05<br>(0.06–0.05) | 10.27        | 0.71         |
| 48 | m   | yes                      | yes    | no      | 31.4–43.2 | 0.004±0.000                      | 0.175±0.022 | 0.022±0.002 | 0.035±0.010 | 0.048±0.022 | 0.107±0.024 | 0.031±0.003          | 1.53<br>(1.70–1.36)   | 8.82                 | 0.90                  | 1.85<br>(2.20–1.51)  | 8.73                  | 0.73                  | 17.79<br>(19.68–15.91) | 7.75      | 0.88       | 0.07<br>(0.08–0.07) | 10.13        | 0.35         |
| 49 | m   | yes                      | yes    | no      | 31.4–43.2 | 0.006±0.002                      | 0.174±0.040 | 0.029±0.015 | 0.040±0.024 | 0.046±0.028 | 0.117±0.056 | 0.039±0.027          | 1.41<br>(1.73–1.09)   | 8.85                 | 1.03                  | 1.54<br>(1.86–1.22)  | 8.81                  | 0.98                  | 19.37<br>(24.33–14.41) | 7.71      | 0.79       | 0.09<br>(0.11–0.07) | 10.04        | 0.13         |
| 50 | m   | no                       | yes    | no      | 22–25     | 0.012±0.020                      | 0.166±0.067 | 0.045±0.027 | 0.066±0.047 | 0.077±0.065 | 0.117±0.075 | 0.028±NA             | 1.37<br>(1.75–0.99)   | 8.86                 | 1.07                  | 2.29<br>(3.15–1.43)  | 8.64                  | 0.44                  | 34.45<br>(58.31–10.60) | 7.46      | 0.24       | 0.01<br>(0.01–0.00) | 11.26        | 3.30         |
| 51 | m   | no                       | yes    | no      | 43.2–56   | 0.000±0.003                      | 0.151±0.030 | 0.020±0.008 | 0.031±0.008 | 0.040±0.013 | 0.086±0.022 | 0.033±NA             | 1.30<br>(1.50–1.11)   | 8.89                 | 1.15                  | 1.69<br>(1.97–1.41)  | 8.77                  | 0.86                  | 21.11<br>(24.26–17.97) | 7.68      | 0.71       | 0.10<br>(0.11–0.09) | 9.99         | -0.01        |
| 52 | f   | no                       | yes    | no      | 25–27.6   | 0.024±0.034                      | 0.174±0.035 | 0.055±0.047 | 0.070±0.043 | 0.075±0.037 | 0.107±0.017 | 0.060±0.042          | 1.21<br>(1.63–0.78)   | 8.92                 | 1.26                  | 1.31<br>(1.79–0.83)  | 8.88                  | 1.20                  | 9.98<br>(17.21–2.75)   | 8.00      | 1.43       | 0.06<br>(0.10–0.03) | 10.19        | 0.52         |
| 53 | f   | no                       | no     | no      | 43.2–56   | 0.002±0.001                      | 0.179±0.084 | 0.023±0.011 | 0.036±0.018 | 0.062±0.049 | 0.123±0.086 | 0.018±0.000          | 1.20<br>(1.49–0.91)   | 8.92                 | 1.27                  | 2.66<br>(3.33–1.98)  | 8.58                  | 0.24                  | 25.88<br>(35.90–15.86) | 7.59      | 0.52       | 0.05<br>(0.06–0.04) | 10.28        | 0.74         |
| 54 | f   | no                       | no     | no      | 22–25     | 0.001±0.001                      | 0.178±0.085 | 0.015±0.009 | 0.023±0.016 | 0.029±0.018 | 0.100±0.054 | -                    | 1.17<br>(1.33–1.02)   | 8.93                 | 1.31                  | 1.17<br>(1.33–1.02)  | 8.93                  | 1.35                  | 16.02<br>(17.00–15.04) | 7.80      | 0.98       | 0.02<br>(0.03–0.01) | 10.72        | 1.89         |
| 55 | f   | no                       | no     | yes     | 31.4–43.2 | 0.004±0.001                      | 0.206±0.085 | 0.039±0.029 | 0.046±0.033 | 0.062±0.051 | 0.154±0.108 | 0.043±0.030          | 1.12<br>(1.23–1.02)   | 8.95                 | 1.38                  | 1.54<br>(1.82–1.25)  | 8.81                  | 0.98                  | 25.45<br>(32.27–18.64) | 7.59      | 0.53       | 0.07<br>(0.08–0.06) | 10.17        | 0.45         |
| 56 | f   | yes                      | yes    | no      | 22–25     | 0.002±0.001                      | 0.155±0.028 | 0.016±0.004 | 0.025±0.007 | 0.033±0.010 | 0.093±0.025 | -                    | 1.02<br>(1.14–0.90)   | 8.99                 | 1.53                  | 1.39<br>(1.53–1.24)  | 8.86                  | 1.12                  | 25.25<br>(27.87–22.64) | 7.60      | 0.54       | 0.04<br>(0.05–0.04) | 10.35        | 0.94         |
| 57 | m   | no                       | no     | no      | 43.2–56   | 0.004±0.004                      | 0.133±0.086 | 0.020±0.004 | 0.023±0.005 | 0.019±0.008 | 0.063±0.033 | 0.027±0.010          | 0.97<br>(1.12–0.82)   | 9.01                 | 1.61                  | 0.76<br>(0.92–0.59)  | 9.12                  | 1.94                  | 8.37<br>(13.19–3.56)   | 8.08      | 1.60       | 0.02<br>(0.03–0.02) | 10.60        | 1.59         |
| 58 | f   | no                       | no     | no      | 22–25     | 0.014±0.017                      | 0.108±0.055 | 0.026±0.019 | 0.031±0.016 | 0.034±0.011 | 0.072±0.028 | 0.028±0.016          | 0.96<br>(1.12–0.80)   | 9.02                 | 1.63                  | 0.31<br>(0.48–0.14)  | 9.51                  | 3.15                  | 7.93<br>(12.81–3.05)   | 8.10      | 1.65       | 0.01<br>(0.02–0.00) | 10.84        | 2.21         |
| 59 | f   | no                       | no     | yes     | 25–27.6   | 0.002±0.001                      | 0.142±0.030 | 0.020±0.004 | 0.030±0.001 | 0.033±0.003 | 0.067±0.007 | -                    | 0.84<br>(1.10–0.59)   | 9.07                 | 1.83                  | 0.67<br>(0.90–0.45)  | 9.17                  | 2.09                  | 12.30<br>(15.42–9.17)  | 7.91      | 1.23       | 0.05<br>(0.07–0.04) | 10.27        | 0.71         |
| 60 | f   | no                       | no     | no      | 22–25     | 0.004±0.000                      | 0.130±0.022 | 0.015±0.004 | 0.019±0.006 | 0.021±0.006 | 0.055±0.032 | 0.016±0.007          | 0.82<br>(0.91–0.72)   | 9.09                 | 1.88                  | 0.91<br>(0.99–0.82)  | 9.04                  | 1.69                  | 5.26<br>(7.27–3.25)    | 8.28      | 2.04       | 0.01<br>(0.02–0.00) | 10.96        | 2.53         |

Color traces back donor-specific data in the figures of the publication (Fig. 1, Supplementary Figure 3, 4 and 6), donor IDs were assigned post-hoc using the order of IC<sub>50,ISO</sub>, A<sub>550</sub> = absorbance measured at 550 nM was proportional to ROS production, IC<sub>50</sub> values and its transformations were calculated from the concentration-response data of neutrophilic ROS-assays. Errors are given as standard deviations or confidence intervals. Failing error calculation is denoted with NA and was only accepted for IC<sub>50</sub> values if plotted curves allowed a tolerable fit based on R<sup>2</sup>.

**Table S7. Summary of genetic data obtained per donor (1/2)**

| ID | sex | genotype as the single nucleotide alleles |                      |                     |                    |                    |                     |                     |                     |             |                      |                      | poly-C-repeat<br>C>G |
|----|-----|-------------------------------------------|----------------------|---------------------|--------------------|--------------------|---------------------|---------------------|---------------------|-------------|----------------------|----------------------|----------------------|
|    |     | rs1042711<br>-47C>T                       | rs34399297<br>-23G>A | rs1801704<br>-20C>T | rs1042713<br>46G>A | rs1042714<br>79C>G | rs1042717<br>252G>A | rs1800888<br>491C>T | rs1042718<br>523C>A | -<br>848C>G | rs1042719<br>1053G>C | rs1042720<br>1239G>A |                      |
| 1  | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GA                   | CG                   |
| 2  | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 3  | f   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 4  | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 5  | m   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 6  | m   | TT                                        | GG                   | TT                  | GG                 | CC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 7  | f   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CA                  | CC          | CC                   | AA                   | GG                   |
| 8  | f   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | CC                   | AA                   | GG                   |
| 9  | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 10 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 11 | m   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | GG                   | GA                   | CC                   |
| 12 | f   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 13 | f   | TT                                        | GG                   | TT                  | GG                 | CC                 | AA                  | CC                  | AA                  | CC          | CC                   | AA                   | GG                   |
| 14 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 15 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 16 | m   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CC                  | CC          | GC                   | GA                   | CC                   |
| 17 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 18 | m   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 19 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GA                   | CG                   |
| 20 | f   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 21 | f   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CA                  | CC          | CC                   | AA                   | CG                   |
| 22 | m   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 23 | m   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 24 | f   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 25 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 26 | m   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CC                  | CC          | GC                   | AA                   | CG                   |
| 27 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 28 | m   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 29 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 30 | f   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CA                  | CC          | CC                   | AA                   | GG                   |

Color traces back donor-specific data in the figures of the publication (Fig. 1, Supplementary Figure 3, 4 and 6), donor IDs were assigned post-hoc using the order of IC<sub>50,ISO</sub>, *ADRB2* genotypes were determined as described in Materials and Methods and known and new polymorphic sites were analyzed for each donor. If available, rs IDs refer to Single Nucleotide Polymorphism Database (dbSNP) entries (6). Analysis of linkage disequilibrium inbetween analyzed SNPs is provided in Supplemental Fig. S8.

**Table S7 (continued). Summary of genetic data obtained per donor (2/2)**

| ID | sex | genotype as the single nucleotide alleles |                      |                     |                    |                    |                     |                     |                     |             |                      |                      | poly-C-repeat<br>C>G |
|----|-----|-------------------------------------------|----------------------|---------------------|--------------------|--------------------|---------------------|---------------------|---------------------|-------------|----------------------|----------------------|----------------------|
|    |     | rs1042711<br>-47C>T                       | rs34399297<br>-23G>A | rs1801704<br>-20C>T | rs1042713<br>46G>A | rs1042714<br>79C>G | rs1042717<br>252G>A | rs1800888<br>491C>T | rs1042718<br>523C>A | -<br>848C>G | rs1042719<br>1053G>C | rs1042720<br>1239G>A |                      |
| 31 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 32 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 33 | m   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 34 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CG          | GC                   | GA                   | CG                   |
| 35 | m   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 36 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 37 | f   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 38 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 39 | f   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 40 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 41 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GG                   | CG                   |
| 42 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GA                   | CG                   |
| 43 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 44 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 45 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 46 | m   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 47 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 48 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 49 | m   | TT                                        | GG                   | TT                  | GG                 | CC                 | AA                  | CC                  | CA                  | CC          | CC                   | AA                   | GG                   |
| 50 | m   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 51 | m   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 52 | f   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 53 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |
| 54 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | CC                   | AA                   | GG                   |
| 55 | f   | CT                                        | GG                   | CT                  | GG                 | GC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 56 | f   | CT                                        | GG                   | CT                  | GA                 | GC                 | GG                  | CC                  | CC                  | CC          | GC                   | GA                   | CG                   |
| 57 | m   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GA                   | CG                   |
| 58 | f   | TT                                        | GG                   | TT                  | AA                 | CC                 | GG                  | CC                  | CC                  | CC          | GG                   | GA                   | CG                   |
| 59 | f   | TT                                        | GG                   | TT                  | GA                 | CC                 | GA                  | CC                  | CA                  | CC          | GC                   | GA                   | CG                   |
| 60 | f   | CC                                        | GG                   | CC                  | GG                 | GG                 | GG                  | CC                  | CC                  | CC          | GG                   | GG                   | CC                   |

Color traces back donor-specific data in the figures of the publication (Fig. 1, Supplementary Figure 3, 4 and 6), donor IDs were assigned post-hoc using the order of IC<sub>50,ISO</sub>, *ADRB2* genotypes were determined as described in Materials and Methods and known and new polymorphic sites were analyzed for each donor. If available, rs IDs refer to Single Nucleotide Polymorphism Database (dbSNP) entries (6). Analysis of linkage disequilibrium inbetween analyzed SNPs is provided in Supplemental Fig. S8.

## Supplemental Figures

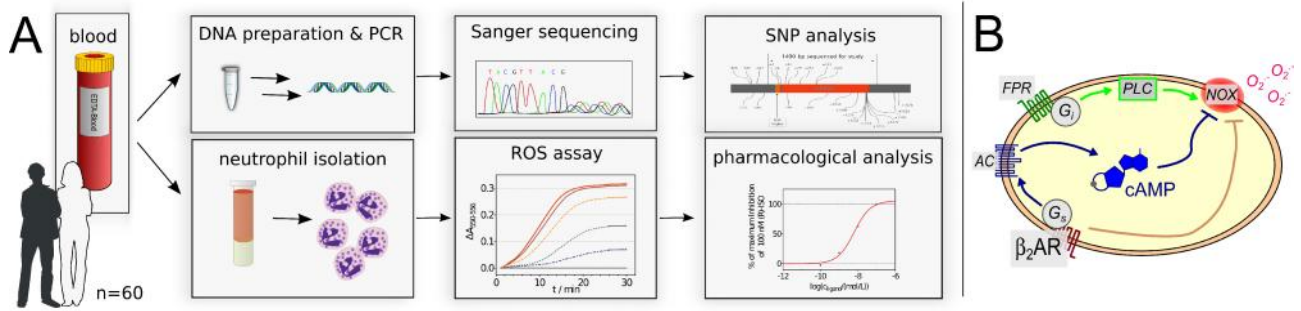


**Fig. S1. Study concept.** **A**, Neutrophils from 60 donors were isolated and  $\beta_2AR$ -mediated inhibition of the fMLP-induced ROS production was examined. Moreover, DNA of each donor was isolated for genotyping of the *ADRB2* region. **B**, In neutrophils, fMLP induces ROS production (reactive oxygen species like superoxide,  $O_2^{\cdot-}$ ) via  $G_i$ -protein- and phospholipase-c(PLC)-mediated activation of NADPH-oxidase (NOX).  $\beta_2AR$ -Activation inhibits this process by cAMP-dependent and -independent mechanisms. ROS production was monitored photometrically by cytochrome c reduction.

### "Pharmakogenetische Effekte von Sequenzvarianten des $\beta 2$ -Adrenorezeptors."

Der Gesundheitszustand und die Medikation eines Probanden können bei der Auswertung der genetischen und funktionellen Analyse eine wichtige Rolle spielen.

**Wir bitten Sie daher die folgenden Fragen zu beantworten!**

[illegible]

**Fig. S2. Asthma-related questionnaire (two pages).** Each donor's data-set was complemented with the information of the attached questionnaire in German language. In brief, this included: 1) prevalence for atopic and auto-immune diseases during childhood, adulthood or both, 2) results of positive prick tests if available, 3) smoking status, 4) if relevant, medication at time of blood donation and the week before. Personal data (sex, age, address) were collected and stored separately.

☐ Ja      ☐ Nein☐ Nein☐ Ja[illegible][illegible]

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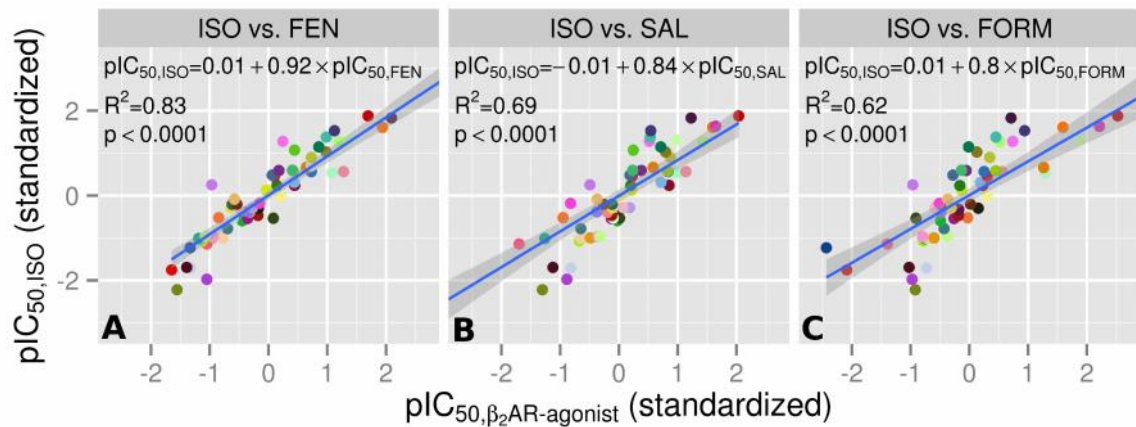


**Fig. S3. Stable subject-specific pharmacological responses towards ISO with individual pharmacological responses towards the three other  $\beta_2AR$  agonists.** Standardized responsiveness values ( $pIC_{50}$ ) of the ISO-induced inhibition of ROS production were plotted against respective standardized  $pIC_{50}$  values for, **A**, FEN, **B**, SAL, and, **C**, FORM in each donor. Negative values describe a lower responsiveness compared to the mean, positive values describe a higher responsiveness compared to the mean. Linear regression was applied and best-fit equations are given including  $R^2$  as measure of the goodness of fit. Linear regressions illustrate the correlation of all subject-specific pharmacological responses for the  $\beta_2AR$  agonists ( $p < 0.0001$ ). Specific comparison for each donor can be traced by color (see Supplemental Table S6/7).

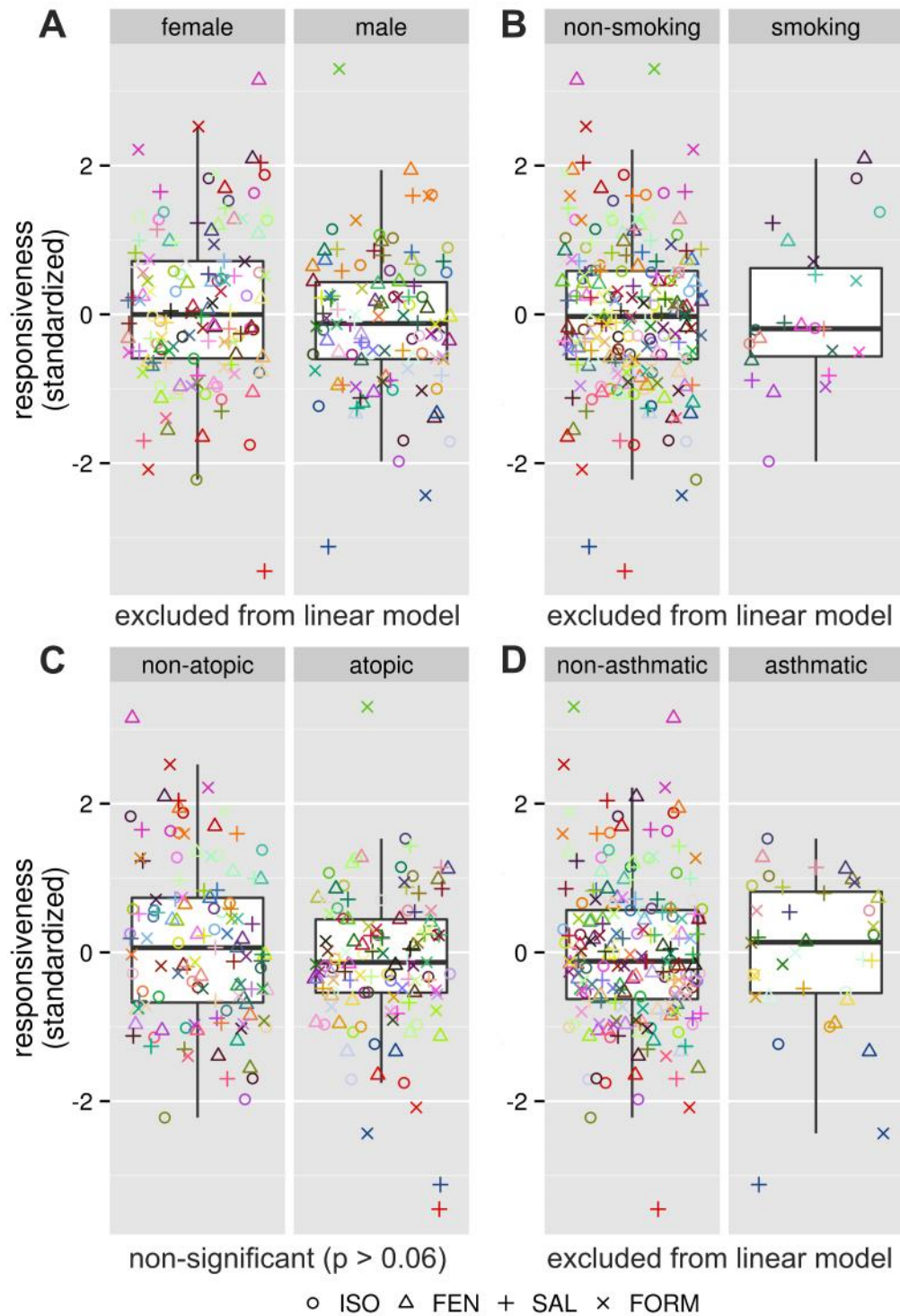


**Fig. S4. No influence of sex, smoking, or atopic disease status on standardized responsiveness.** Boxplot illustration (median and 25<sup>th</sup>-75<sup>th</sup> percentiles) and statistical analysis by multivariate linear regression on influence of **A**, sex, **B**, smoking, **C**, atopic disease and asthma on the overall pharmacological response to the four examined  $\beta_2$ AR-agonists (ISO (circle), FEN (triangle), SAL (plus), FORM (cross)). Factors may have been excluded from the final linear model based on the applied model-building strategy (see Statistical Analysis in Supplemental Methods). Donor-specific responsiveness can be traced by color (see Table S6/7).

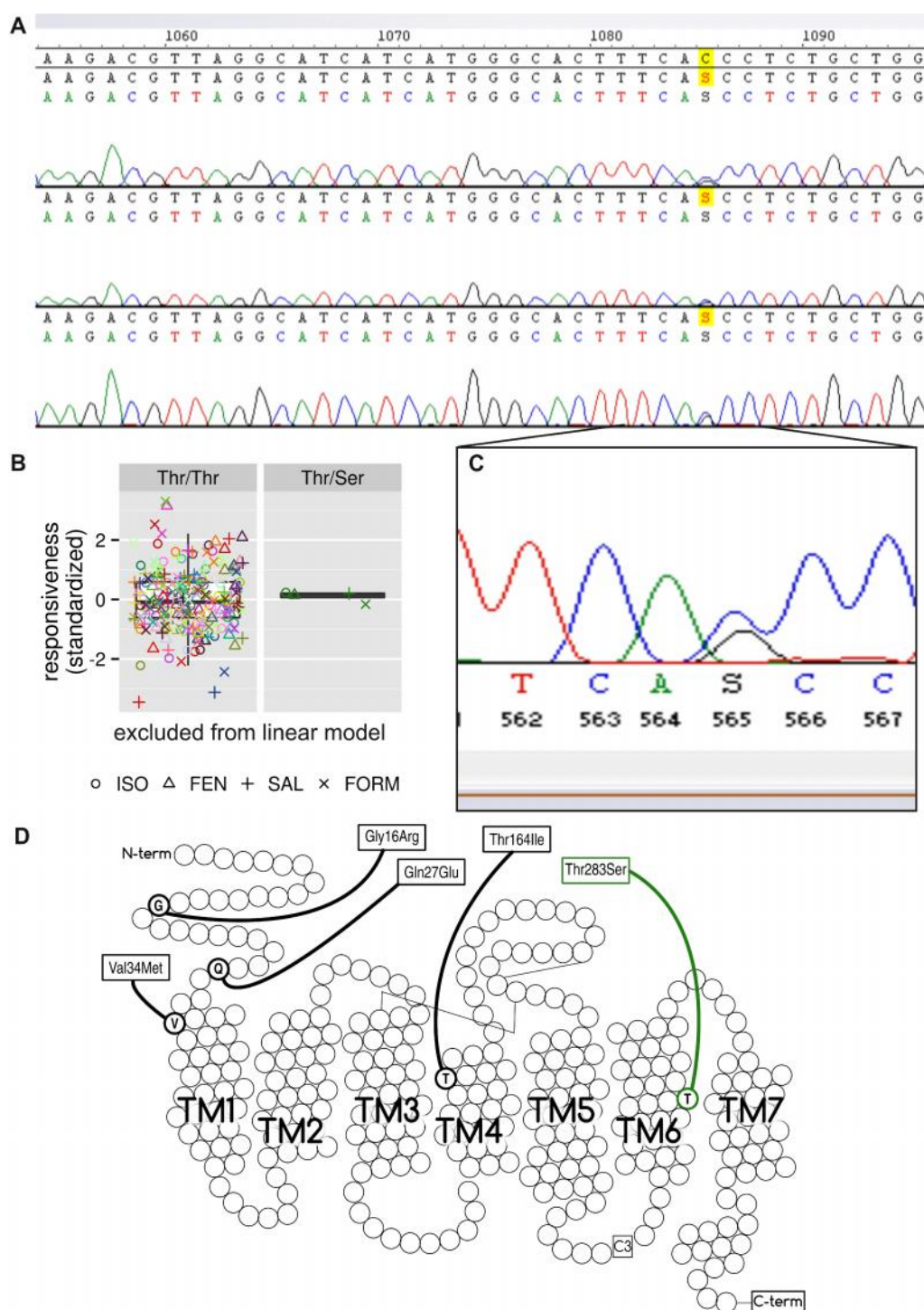


**Fig. S5. Newly-discovered variation in *ADRB2*.** In one donor Sanger sequencing identified a heterozygous presence of a novel single nucleotide variation (848C>G). **A**, Representative chromatograms from three independent runs from the same fragment, but two different sequencing primers (Table S1). **B**, the  $\beta_2$ AR-mediated pharmacological response did not differ from homozygous wild-type population and was excluded from the final linear model based on the applied model-building strategy (see Statistical Analysis in Supplemental Methods, specific comparison for each donor can be traced by color, see Supplemental Table S6/7). **C**, zoom-into region of interest on chromatogram (S denotes the heterozygous locus). **D**, the novel variation (Thr283Ser) causes a non-synonymous amino-acid exchange at position 283 in the transmembrane domain 6 (TM6) of  $\beta_2$ AR.

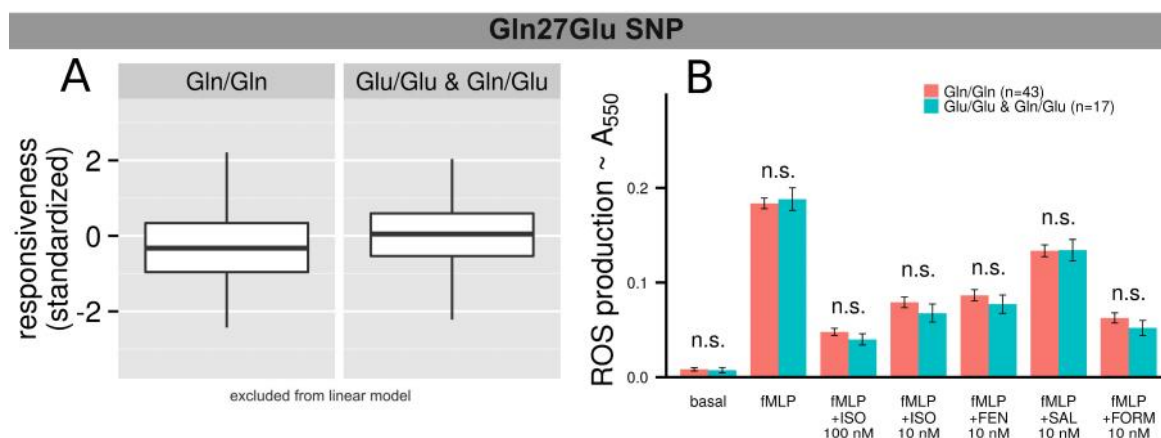


**Fig. S6. No Influence of the *ADRB2* variant Gln27Glu on the  $\beta_2$ AR mediated pharmacological response.** Analysis based on the presence of the SNP Gln27Glu (**A** and **B**). **A**,  $pIC_{50}$  values for the four  $\beta_2$ AR agonists were standardized as parameter for overall  $\beta_2$ AR responsiveness and multivariate linear regression analysis was applied. Gln27Glu was already excluded from the linear model during pre-evaluation (see Statistical Methods in Supplemental Materials and Methods). Box-plots show the median of the genotype-specific subgroups with 25<sup>th</sup>-75<sup>th</sup> percentiles. **B**, Pairwise-comparison of the genotype-specific subgroups was based on ROS production without (basal) and with fMLP-stimulation (1  $\mu$ M), and the inhibitory effect on fMLP-induced ROS-production at single concentrations of 100 nM ISO or 10 nM ISO, FEN, SAL, and, FORM. (Mean  $\pm$  SD, n.s., non-significant ( $p < 0.05$ ) in an unpaired two-tailed t-test).

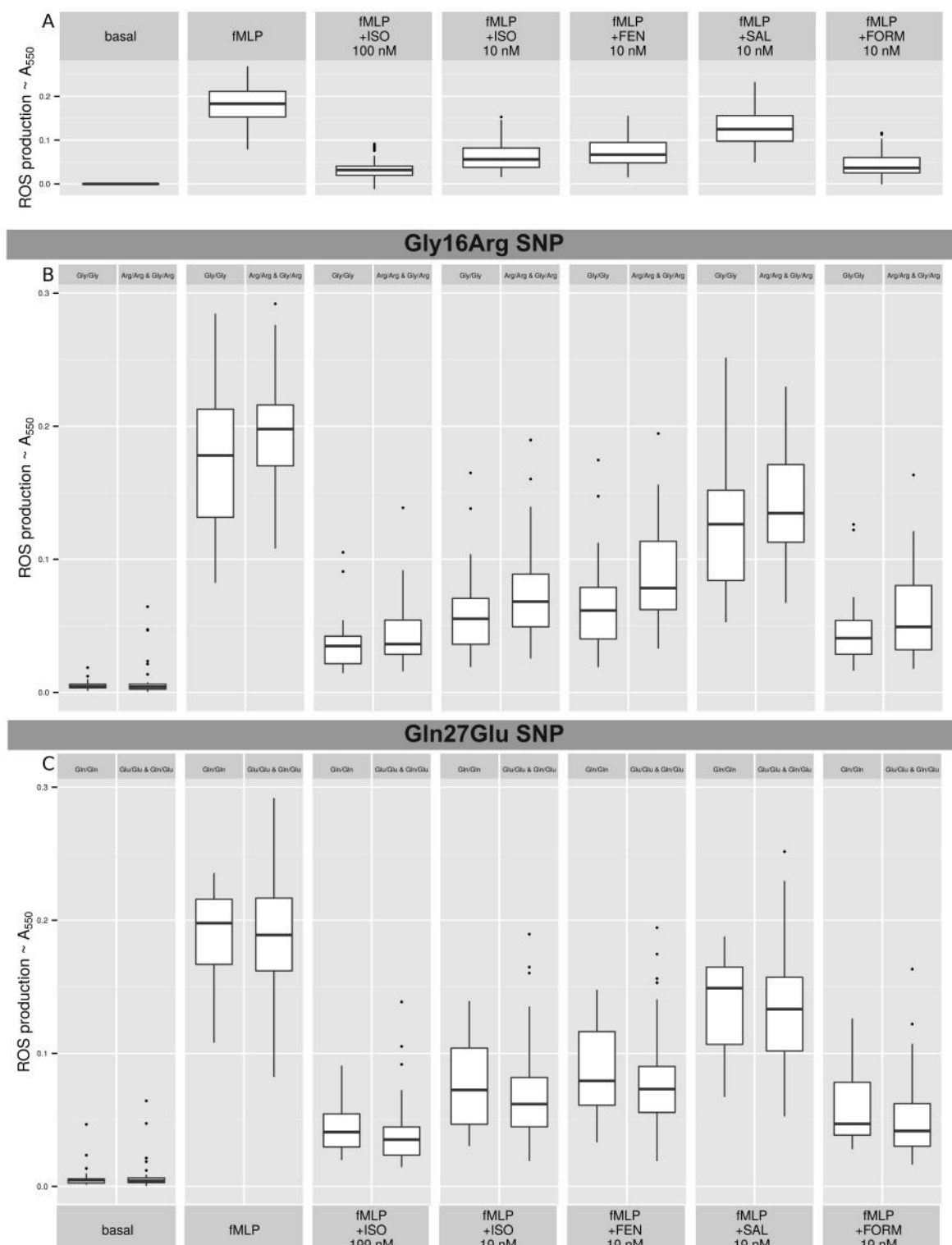


**Fig S7: Boxplot analysis of ROS production.** Visual evaluation of the individual  $A_{550}$  values depicting the ROS production under basal conditions, or after stimulation with 1  $\mu$ M fMLP, or with simultaneous  $\beta_2$ AR inhibition using 100 nM ISO, 10 nM ISO, 10 nM FEN, 10 nM SAL, or 10 nM FORM. Box plots display the median per ligand with the 25th-75th percentiles. **A** Plots are based on the individual differences to basal condition (see Fig 1A). **B** Values were subsetting according individual genotype of the Gly16Arg SNP (homozygous major Gly-allele (Gly/Gly) or presence of minor Arg-allele (Arg/Gly & Arg/Arg), ref. to Fig 1D). **C** Subsetting according genotype of the Gln27Glu SNP (homozygous Gln-allele or presence of minor Glu-allele, ref. to Fig S6).

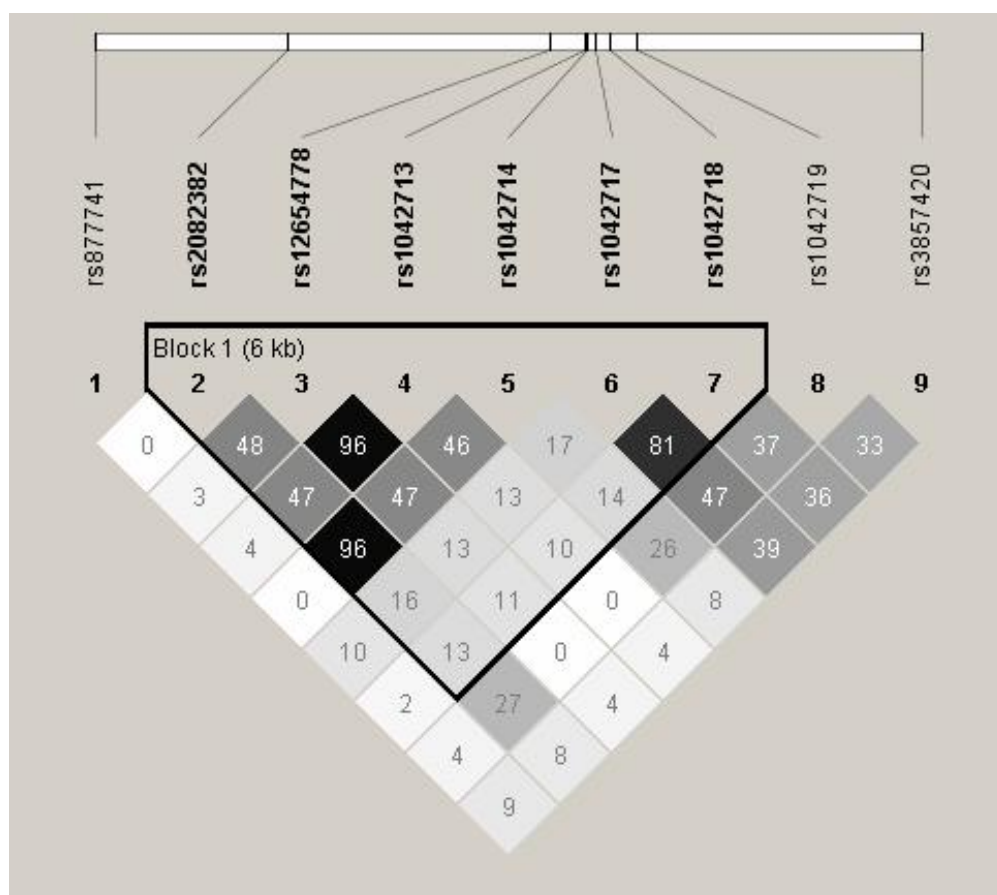


**Fig S8: Linkage disequilibrium (LD) between genotyped *ADRB2* polymorphisms.** Chromosomal positions are provided according to NCBI Genome Build 37.3. Only polymorphisms found in the study population are depicted.

## Supplemental References

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