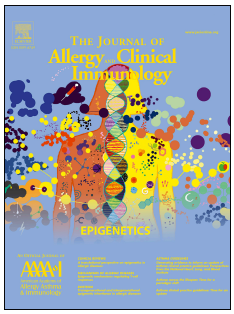
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Oral JAK/SYK-inhibition (ASN002) suppresses inflammation and improves epidermal barrier markers in atopic dermatitis

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Title: Oral JAK/SYK-inhibition (ASN002) suppresses inflammation and improves epidermal barrier markers in atopic dermatitis

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Conflicts of interest:

LD, NR, SG, DJZ are employees of Asana BioSciences.

RB is an investigator, consultant, advisory board member, speaker for and/or receives honoraria from Abbvie, Aquinox Pharma, AntibioTx, Asana BioSciences, Astellas, Boehringer-Ingelheim, Brickell Biotech, Eli-Lilly, Dermavant, Dermira, Dignity Sciences, Galderma, Glenmark, GSK-Stiefel, Hoffman LaRoche Posay, Kiniksa, Leo Pharma, Neokera, Pfizer, Regeneron, Ralexar, Sanofi, Sienna, and Vitae. RB is also an employee and shareholder of Innovaderm Research.

EGY is an employee of Mount Sinai and has received research funds (grants paid to the institution) from: Abbvie, Celgene, Eli Lilly, Janssen, Medimmune/Astra Zeneca, Novartis, Pfizer, Regeneron, Vitae, Glenmark, Galderma, Asana, Innovaderm, Dermira, UCB. EGY is also a consultant for Sanofi Aventis, Regeneron, Stiefel/GlaxoSmithKline, MedImmune, Celgene, Anacor, AnaptysBio, Dermira, Galderma, Glenmark, Novartis, Pfizer, Vitae, Leo Pharma, Abbvie, Eli Lilly, Kyowa, Mitsubishi Tanabe, Asana Biosciences, and Promius. The rest of the authors declare they have no conflicts of interest.

Target Journal: *Journal of Allergy and Clinical Immunology*

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- 7 supplementary figures, 13 supplementary tables.

Key messages:

- The oral JAK/SYK inhibitor ASN002 induces rapid and sustained improvements in cellular infiltrates, atopic dermatitis (AD) molecular pathways, and epidermal barrier abnormalities in moderate-to-severe AD patients.
- This is the first paper to dissect the molecular and cellular pathway modulated by JAK/SYK inhibition, further supporting the use of this new class of oral therapeutics in moderate-to-severe AD and beyond.

Capsule Summary (33/35 words):

The JAK/SYK inhibitor ASN002 induced robust inhibition of cellular infiltrates, inflammatory pathways, with concurrent improvement in the epidermal barrier pathology and parallel rapid improvements in clinical disease measures in moderate-to-severe atopic dermatitis patients.

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Abstract: 228/250 words

Background: Moderate-to-severe atopic dermatitis (AD) has been associated with significant disease burden and systemic abnormalities, and often requires systemic treatments. Currently, safe and effective oral systemic treatments for moderate-to-severe AD are not yet available. ASN002 is an oral inhibitor of JAK/SYK signaling pathways, targeting several cytokine axes (Th2/Th22/Th17/Th1) and epidermal differentiation.

Objective: To evaluate the effect of ASN002 on cellular and molecular biomarker profile of patients with moderate-to-severe AD, and to correlate changes in biomarkers to improvements in clinical severity measures and pruritus.

Methods: 36 patients with moderate-to-severe AD were randomized to dose escalation of ASN002 20mg, 40mg, 80mg, and placebo groups. Skin biopsies were performed at baseline, day15, and day29. Gene expression studies were conducted using microarray and qRT-PCR, and cellular infiltrates and protein expression were studied by immunohistochemistry.

Results: ASN002 reversed the lesional skin transcriptome towards a non-lesional phenotype. It also rapidly and significantly suppressed key inflammatory pathways implicated in AD pathogenesis, including Th2 (IL4R, IL-13, CCL13/MCP-4, CCL17/TARC, CCL18/PARC, CCL22/MDC, CCL26/Eotaxin-3), Th17/Th22 (LCN, PI3/elafin, CCL20, S100A7/S100A8/S100A9, IL36G/IL36RN), Th1 (IFN-γ, CXCL9/ CXCL11, MX1) axes and barrier-related measures (FLG,

CLDN23). Significant improvements in AD gene-signatures were observed predominantly in the 40mg and 80mg groups. Smaller and largely non-significant molecular changes were seen in the 20mg group or placebo.

Conclusion: The JAK/SYK inhibitor ASN002 significantly suppressed key AD inflammatory pathways, corresponding to clinical response. ASN002 may be an effective novel therapeutic agent for moderate-to-severe AD.

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Key words: Atopic dermatitis, ASN002, JAK, SYK, JAK inhibitors, SYK inhibitors, tyrosine kinase, Th2, Th22, Th17, barrier.

Abbreviations:

AD	Atopic Dermatitis
BSA	Body Surface Area
DC	Dendritic cells
DEG	Differentially expressed genes
FCH	Fold change
FDR	False Discovery rate
K16	Keratin 16
FLG	Filaggrin
RT-PCR	Real-time PCR
MADAD	Meta-analysis derived AD transcriptome
EASI	Eczema Area and Severity Index
EASI-50	≥ 50% reduction in EASI score
EASI-75	≥ 75% reduction in EASI score
H&E	Hematoxylin and eosin
IHC	Immunohistochemistry
IL	Interleukin

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Introduction:

Atopic dermatitis (AD) affects up to 10% of adults and 25% of children worldwide, with up to 1/3 of patients suffering from moderate-to-severe disease.[1-5] AD is a heterogeneous disease primarily involving Th2, and Th22 activation, with variable Th1 and Th17-skewing depending on subtype.[6-15] While all subtypes show Th2/Th22 activation, some subtypes, including Asian, pediatric, and intrinsic AD show increased Th17 activation.[8,10-12] Despite its high prevalence and impact on quality of life, systemic treatment options for moderate-to-severe AD are limited, presenting a large unmet need for safer and more efficacious treatments.[16,17] The only FDA-approved oral therapeutic for AD is glucocorticoids, which harbor many side-effects and is inappropriate for long term use.[18] Other immune-suppressants used off-label for AD such as cyclosporine A, are inadequate for continuous use.[19-22] Dupilumab, a novel Th2-specific monoclonal antibody targeting IL-4Rα, recently approved by the FDA, has shown efficacy in moderate-to-severe AD, with ~30% of patients approaching disease resolution (EASI-90).[5,23-25]

Oral Janus Kinase (JAK) inhibitors targeting JAK1 or JAK1/JAK2 signaling pathways have recently shown efficacy in early clinical trials for moderate-to-severe AD patients.[26-30] Depending on their specificity, JAK inhibitors target cytokine axes involved in the pathogenesis of AD, specifically Th2 (IL-4, IL-5, IL-13, TSLP), Th22 (IL-22), and Th1 pathways (IFN-γ, IL-12, IL-23).[26-28,31,32] Spleen tyrosine Kinase (SYK), a non-receptor tyrosine kinase, regulates B-cell and dendritic-cell differentiation, Th17/IL-17-signaling, and inhibits keratinocyte

terminal differentiation.[33-41] In mice, SYK inhibition was shown to inhibit Th17 pathway signaling and improve terminal epidermal differentiation.[41-43] Thus, dual JAK-SYK inhibition may broaden the range of cytokines targeted to successfully resolve AD across the different disease subtypes.[9,10,12,21,44-48]

ASN002 is the first oral dual JAK/SYK inhibitor tested in AD patients. A phase 1B clinical trial has been recently performed in moderate-to-severe AD patients over 4 weeks (ClinicalTrials.gov: NCT03139981),[49] evaluating 3 daily doses (20mg, 40mg, 80mg) compared to placebo. 80%-100% of patients in the higher doses of ASN002 achieved EASI-50 response by day29.[49] The present study evaluates the effects of the three ASN002 doses, as compared to placebo, on the molecular and cellular profiles of lesional and non-lesional AD skin, after 15 and 29 days of treatment. Overall, both higher doses of ASN002 significantly and rapidly reduced inflammatory cellular infiltrates, suppressed major inflammatory pathways, and improved the epidermal barrier abnormalities implicated in AD, and treatment response biomarkers were correlated with clinical responses.

Material and Methods:

Study Design and Patients

This mechanistic study is part of a randomized, double-blinded, placebo-controlled, phase 1B clinical trial (ClinicalTrials.gov: NCT03139981) conducted at 10 centers across the United States and Canada. 36 patients were enrolled and randomized 1:1:1:1 to dose escalation groups of oral 20mg, 40mg, 80mg and

placebo group for 28 days.[49] Each patient was evaluated at baseline (D1), day 15 (D15), and day 29 (D29) for safety, efficacy and skin biopsies were taken for evaluating biomarkers (See Online Repository/OR for details). Additional study design and clinical trial outcome data has been recently reported.[49]

In Vitro Biochemical assays

ASN002, tamatinib and tofacitinib were evaluated for inhibiting JAK1, JAK2, JAK3, and TYK2 tyrosine kinase activity using an *in-vitro*, time-resolved fluorescence resonance energy transfer (TR-FRET) assay. The IC_{50} of ASN002, tamatinib, and tofacitinib were determined using an *in-vitro* radiometric assay. IL17-mediated CCL20 release was evaluated using neonatal human epidermal keratinocytes (NHEKs) treated with ASN002 (1μm), tamatinib (1μm), tofacitinib (1μm), an anti IL-17 antibody, and IL-17-DMSO. See methods in Online Repository/OR for details.

Immunohistochemistry/IHC

IHC was performed on frozen OCT-embedded cryostat tissues using purified mouse anti-human monoclonal antibodies, as previously described.[50] Antibodies and dilutions can be found in the OR (Table E1). Epidermal thickness and inflammatory cells were quantified per millimeter by computer-assisted analysis software (image J 1.42; National institute of Health, Bethesda, MD), as described.[10,51-54]

Quantitative real-time PCR and gene-array analysis

RNA was profiled using affymetrix Human U133 Plus 2.0 gene arrays (Affymetrix, Santa Clara, Calif) available through the Gene Expression Omnibus (GSE133385), and real time PCR by EZ-PCR Core reagents (Life technologies, Grand Island, NY) as previously described and detailed in the OR.[10,52,54-56] Taqman Low Density Array/TLDA cards were used for qRT-PCR to analyze mRNA expression of inflammatory markers. Primers and probes used are listed in Table E2 and were generated by Primer Express Algorithm (Applied Biosystems, Foster City, CA).[10] See the OR for details.

Statistical Analyses

All analyses were performed using statistical language R (www.R-Project.org) and Bioconductor. Standard R packages such as ArrayQualityMetrics and GC Robust Multi-array Average (GCRMA) were used for quality control and normalization. [11,57,58] Gene expression was modeled by linear mixed-effect models with time, treatment and tissue as a fixed interaction term, and a random effect for each patient, using R's Limma linear modeling framework. P-values were adjusted for multiplicity using Benjamin-Hochberg procedure that controls the false discovery rate (FDR).[59] Spearman correlations between clinical scores and biomarkers were considered significant if p-value <0.05. A non-parametric multivariate previously published μ stat approach, [60] was used to integrate skin, blood, and clinical changes. See OR for details.

Results

Study Population, Safety, and Clinical Efficacy

36 patients were randomized to receive placebo, 20mg, 40mg, or 80mg ASN002 (n=9 per group) in an ascending-dose study design.[49] There were no significant differences between study arms in baseline demographic data (age, gender, clinical severity measures) (Table E3).[49] Lesional and non-lesional biopsies were collected at baseline, and lesional biopsies at day15 and day29. The number of biopsies available for analyses at each time-point is listed in Table E4.

ASN002 shows higher affinity for SYK (and TYK2) pathways and greater inhibition of IL-17-induced CCL20 compared to selected JAK Inhibitors

To determine the affinity of ASN002 to JAK1, JAK2, JAK3, TYK2, and SYK as compared to other JAK and/SYK inhibitors we used an in-vitro radiometric assay,[61] that determined the IC_{50} of ASN002, tamatinib, tofacitinib, and also compared with reported upadicitinib[62] and baricitinib[63] data. ASN has much higher affinity for SYK and TYK2 compared to tofacitinib, and higher SYK and TYK2 affinity compared to Tamatinib (Table E5). ASN002 also shows decreasing affinity to JAK2>JAK3>JAK1.

Based upon the SYK activity of ASN002 we hypothesized that IL-17 will be more suppressed with ASN002 compared to the pan-JAK tofacitinib. We thus compared between inhibition of IL-17-mediated CCL20 release in human

keratinocytes by similar concentrations (1μM) of ASN002, tamatinib, tofacitinib and an IL-17 neutralizing antibody. ASN002 showed the highest inhibition of CCL20 release, which was similar to that of the IL-17 antagonist (Fig E1). See details in OR methods and results.

ASN002 improves epidermal barrier abnormalities

Epidermal hyperplasia was evaluated at baseline, day15/D15, and day29/D29 using epidermal thickness, along with protein and gene expression of the proliferation marker keratin 16/K16, as quantified by fold changes compared to baseline and to placebo (Fig 1). ASN002 improved epidermal hyperplasia as evident by decreased epidermal thickness on H&E staining, and a rapid reversal of K16 staining already evident at D15 in 7/14 of the patients on 40mg (3/8) or 80mg (4/6) doses, with 10/15 (4/7 on 40mg and 6/8 on 80mg) of these patients achieving more resolution of K16 staining at D29 (Figs 1A-B, E2A). Only minimal improvements were seen in the 20mg and placebo arms at D29 (Fig 1A-B), where only 4/15 (1/7 on 20mg and 3/8 on placebo) patients had improvement in K16 staining by D29. The 40mg and 80mg doses also induced significant reductions in K16 mRNA expression at D15 and D29 as compared to baseline ($p<0.05$ for both doses), and improvements in epidermal thickness ($p<0.05$ for 80mg at D29), whereas minimal improvements and even exacerbations were seen in the 20mg and placebo groups (Fig 1D-E).

We also evaluated protein expression of the epidermal differentiation marker, filaggrin (FLG), by immunohistochemistry. At baseline, lesional skin

253 showed faint, interrupted FLG staining, as compared to continuous and more
254 intense staining in non-lesional skin (Fig 1C). Both 40mg and 80mg ASN002
255 doses induced more robust FLG staining already by D15, and further at D29 (Fig
256 1C and Fig E2B), as compared to the discontinuous lesional staining in placebo
257 arm at both time-points.
258
259 **ASN002 suppresses inflammatory cell infiltrates in lesional skin**
260 We assessed cellular infiltrates using immunohistochemical staining for T-
261 cells ($CD3^+$) and myeloid dendritic cells/DC ($CD11c^+$) (Figs 1F-G and E2C-D).
262 ASN002 significantly reduced $CD3^+$ T cell infiltrates in lesional skin at D29 as
263 compared to baseline, predominantly in 80mg group, and approaching
264 significance in the 40mg dose ($p<0.05$ for D29 80mg, Fig 1F), with no significant
265 difference compared to placebo. Progressive reductions in $CD11c^+$ DCs were
266 seen in both 40mg and 80mg arms at D29 as compared to baseline ($p<0.01$ for
267 both at D29; Fig 1G), with 80mg approaching significance versus placebo (Figs
268 1G, E2D). No significant decreases in T-cell or DC infiltrates were seen in the
269 20mg or placebo arms.
270
271 **ASN002 improves the AD Transcriptome**
272 Affymetrix U133Plus 2.0 gene-arrays were used to define the AD skin
273 phenotype or transcriptome (defined as differentially expressed genes [DEGs]
274 between lesional and non-lesional skin), using criteria of fold changes/(FCHs)≥2,
275 and false discovery rate/(FDR)<0.05 (Table E6), with DEGs depicted in two

heatmaps, representing changes in the established robust meta-analysis derived atopic dermatitis (MADAD) transcriptome (Fig 2),[20,51,64] and in this study's transcriptome (Fig E3). Progressive improvements in genomic profiles were seen with both transcriptomes in the higher doses, with red-to-blue or blue-to-red transitions in skin lesions resembling non-lesional skin by D29, as compared to minimal changes in the 20mg and placebo groups (Fig 2A, Fig E3A). Significant gene expression alterations in the MADAD transcriptome towards non-lesional skin profile were evident at D15 in the 40mg and 80mg groups, and in the 40mg group at D29 (Fig 2, Table E7); smaller transcriptomic improvements were seen with placebo and 20mg groups, corresponding to modest clinical responses in these groups.[49] The mean MADAD transcriptomic percent improvements in the 40mg group of 49.9% at D15 and 79.29% at D29 were significantly higher than percent improvements of 8.28% at D15 and 21.81% at D29 obtained in placebo (p<0.001 at both time points) (Fig 2B). Significant genomic improvements compared to placebo of 45.77% (p<0.001) and 22.91% (p<0.01) were also seen at D15 in the 80mg and 20mg group, respectively. Similar genomic changes were seen in this study's transcriptome, with significant mean transcriptomic improvements compared to placebo of 44.08% at D15 and 48.26% at D29 in the 80mg group (p<0.001), and 60.05% at D15 and 69.25% at D29 in the 40mg group (p<0.001; Fig E3B).

ASN002 significantly suppressed Th2, Th17/Th22, and Th1 cytokine pathways

We investigated the effect of ASN002 on inflammatory pathways based on a previously defined immune gene-subset, [20,64-67] as depicted in a heatmap showing DEGs at D15 and D29 compared to baseline (p<0.05) (Fig 3). We found significant decreases in various inflammatory markers in the higher ASN002-treated dose groups as compared to baseline, but not compared to placebo. There was significant, progressive suppression of Th2 (IL4R, CCL13/MCP-4, CCL17/TARC, CCL18/PARC, CCL22/MDC, TNFSF4/OX40L, FCER1A), Th17/Th22 (IL36RN, IL36G, LCN2, S100A7/S100A8/S100A9), and Th1 (OASL, MX1, IL12RB2)-related genes in the 40mg group at both D15 and D29 compared to baseline (p<0.05, Fig 3). Similarly, there were significant reduction in genes related to Th2 (CCL18, CCL22, CCL13, FCER1A), Th17 (LCN2, IL36G, PI3/elafin), and Th1 (OASL, MX1) markers at D29 in the 80mg group as compared to baseline (p<0.05, Fig 3). There was also significant down-regulation of genes related to T-cell/T-cell activation-(IL15, CCR7, ITK, CD28, CD5), dendritic cells-(CD1b, CD83), and general inflammation-(MMP12, TNFSF10/TRAIL), mostly in the 40mg group (p<0.05, Fig 3). Markers of general inflammation (MMP12), and dendritic cells (CD1B, CD83, CD86) were also significantly down regulated in the 80mg group at D29 (p<0.05, Fig 3). Negative regulator genes-(IL34, IL37) were significantly up-regulated in the 40mg group at D29, with only IL-34 up-regulated in the 80mg group (p<0.05, Fig 3). There were minimal modulations of genes in the 20mg and placebo group. ASN002 80mg also significantly up regulates several barrier-related markers, including filaggrin 2 (FLG2) and scielin (SCEL; p<0.05), with FLG showing similar trends (p=0.06).

322 Additional barrier measures, including the tight junction marker claudin 23
323 (CLDN23), as well as CDH11, were significantly increased by ASN002 40mg
324 ($p<0.05$; Table E8).

325 We confirmed modulation of selected key AD immune markers using qRT-
326 PCR, as shown by individual graphs (Fig 4, Fig E4) and in summary heat maps
327 for all patients (Fig 5). [20,55,57,66] Corresponding to microarray data, ASN002
328 decreased mRNA expression of markers related to general inflammation-
329 (MMP12), T-cell/ NK-cell activation (IL-15, IL-15RA, IL-2RA), innate immunity
330 (CXCL8/IL-8, IL17-C), Th1 (IFN-γ, STAT1, CXCL9, CXCL10, IL-12RB1, CCL5),
331 Th2 (IL-13, IL-31, IL-10, IL-4R, CCL13/MCP-4, CCL17/TARC, CCL18/PARC,
332 CCL22/MDC, CCL26/Eotaxin-3), Th9 (IL-9) and Th17/Th22 (IL-22, IL-32,
333 S100A7, S100A8, S100A9, S100A12, IL-19, PI3/elafin, CCL20, IL-23p19) axis
334 (Figs 4-5, Fig E4). mRNA expression of markers related to T-cell/NK-cell
335 activation-(IL-15, IL-15RA, IL-2RA), Th1-(IL-12RB1, STAT1, CXCL9, CXCL10),
336 were significantly decreased compared to baseline and also compared placebo
337 at D29, predominantly for the 40mg ($p<0.05$, Fig 4, Fig E4). IL-13 expression was
338 significantly down regulated in both 40mg and 80mg groups as compared to
339 baseline ($p<0.05$ for both) and also compared to placebo for the 40mg group
340 ($p<0.05$) (Fig 4). Expression of the Th2-induced chemokines (CCL13, CCL18,
341 CCL22, CCL26) was reduced in both the 40mg and 80mg groups, achieving
342 higher significance compared to baseline and placebo for the 40mg dose (Fig 4).
343 Additional Th1 (IFN-γ), Th2 (IL4R), and Th17/Th22 axis genes (IL-22, IL-19, IL-
344 23A/IL-23p19, PI3/elafin, S100A7/S100A8/S100A9/S100A12, IL-32) were

345 significantly down-regulated in the 40mg group as compared to baseline at D29
346 (p<0.05; Figs 4-5, Fig E4). IL-9/Th9 was down-regulated in both higher doses at
347 D29, approaching significance in the 40mg (Fig 5, Fig E4). FOXP3, the T-
348 regulatory marker showed significant decrease at D29 in the 40mg group (Fig 5).
349 Epidermal cytokine and/or innate immune markers (CXCL8/IL-8, IL-17C) showed
350 small reductions with ASN002, approaching significance for IL-17C in 40mg at
351 D29 and CXCL8/IL-8 in 80mg at D29 (Fig 5 and Fig E4). A summary of RT-PCR
352 and immunohistochemistry data by patient (ordered by EASI50 response) is
353 shown as a heatmap (Figure E5).
354
355 **Biomarkers correlated with clinical and histologic improvements**
356 To evaluate the relationship between clinical responses to ASN002 and
357 changes in expressions of molecular and cellular markers evaluated in lesional
358 skin, percent EASI and percent BSA improvements were correlated with changes
359 in inflammatory and epidermal hyperplasia (K16) markers at D15 and D29 (Table
360 1). Spearman Correlation coefficients were used, with significance determined by
361 p-value<0.05. A heatmap depicting correlations between skin biomarkers with
362 clinical scores and with each other at D15 and D29 are shown in Fig E6, with
363 correlations between clinical severity and biomarkers shown in Table E9 (D15)
364 and Table E10 (D29).
365 Already at D15, percent EASI improvement significantly correlated with
366 improvements in lesional expressions of markers of general inflammation
367 (MMP12), Th1 (IFN-γ), Th2 (IL-13, IL-5, CCL13, CCL17, CCL22, CCL26), and

Th17/22 (IL-22, CCL20) (Table 1A, $p<0.05$). At D29, percent EASI improvements significantly correlated with additional markers including those related to innate immunity (IL-6), T-cell/NK-cell activation (IL-15), Th1 (CXCL10), and Th17/22 (S100A7, S100A9, S100A8) axes (Table 1B, $p<0.05$). The epidermal hyperplasia marker (KRT16) also correlated with percent EASI improvement at D29 (p=0.05, Table 1B).

Improvement in BSA showed similar trends, showing strong significant correlations with changes in general inflammation (MMP12), Th2 (IL-13, CCL22, CCL17), and Th17/Th22 (IL-22, CCL20) markers, as early as D15 (Table 1C, $p<0.05$) with additional markers showing correlations at D29 (Table 1D, $p<0.05$).

We also evaluated for correlations between reversal of the epidermal hyperplasia measured using a histologic score/histoscore (obtained by averaging the Z scores for changes in epidermal thickness and K16 expression)[52,54] and changes in biomarkers and clinical scores at D15 and D29 (Table 2). A summary of the correlations with the histoscore is found in Table E11 for D15, and Table E12 for D29. As expected, we found significant correlations between the histoscore improvement and changes in epidermal hyperplasia (S100s, thickness, KRT16), general inflammation (MMP12), T-cell/NK-cell activation (IL2RA, IL-15, IL-15RA), epidermal cytokine/innate immunity (IL-6, IL17C, CXCL8/IL-8, IL-1B), Th1 (IL12RB1, CXCL10, STAT1), Th2 (IL-13, IL-4R, CCL17/CCL18/CCL22), Th17/22 (PI3/elafin, IL-22, IL-23p19, IL-19), and T-regulatory cells (FOXP3) by D15, which were sustained until D29 (Table 2, $p<0.05$).

An integrated skin and serum biomarker approach improves correlations with clinical improvement

We also employed a multivariate biomarker analysis, as recently validated.[68] Unlike the above skin biomarkers, which showed significant correlations in the univariate spearman correlations, the recently reported serum biomarkers did not show individual correlations with improvement in clinical disease severity. [49] However, the non-parametric multivariate approach using μ statistics, [60,68] yielded a significant integrated biomarker correlation (*r*=0.54, p=0.008) between clinical improvement and an integrated biomarker μ score that included several serum biomarkers, such as the Th2 chemokine CCL13, and the Th17 biomarker CCL20 (Fig E7). While skin univariate analyses yielded significant correlations, an integrated μ score of lesional skin biomarkers showed a higher and more significant correlation (*r*= 0.66; p=0.0006). The biomarkers included in the μ score correlation included the innate cytokine IL-6, the Th1/IFN-related chemokine CXCL10, the Th2-related chemokine CCL13, and the general inflammatory marker MMP12, reflecting various pathways modulated by JAK/SYK inhibition. Upon integrating the skin and serum biomarkers, we obtained an increased correlation with clinical improvement (*r*= 0.76; p=0.00003), with an integrated biomarker profile that includes innate immunity (IL-6), general inflammation (MMP12), Th2 (CCL13) and Th1/T-cell activation (IL-15RA) markers, representing some of the axes and biomarkers modulated by ASN002 (Fig E7). The combined score includes primarily skin biomarkers with a single serum biomarker.

ASN002 induced significant improvements in AD signature gene pathways

We evaluated the effect of ASN002 on previously defined AD-related gene signatures of up- or down-regulated genes. These gene pathways include those related to T-cells, dendritic cells, epidermal differentiation complex (EDC-CE), untreated keratinocytes and cytokine-treated keratinocytes, and T-cell cytokine responses. [20,55,57,64,69-72] Pathway improvements were quantified by percent change from baseline in each treatment group compared to placebo. ASN002 induced strong significant improvements compared to placebo in most evaluated pathways in both higher doses by D29 (Fig 6). Specifically, pathways related to immune genes, genes up-regulated in T-cells, dendritic-cells, untreated keratinocytes, and keratinocyte treated by IL-1, IL-4, IFN-γ, and TNFα, showed significant improvements from baseline in the 40mg and some in the 80mg group, as compared to placebo by D29 (p<0.05; Fig 6). Gene-sets related to IL-17-treated keratinocytes and Th22/IL-22 pathway showed significant improvements of greater than 100% in the 40mg group versus placebo (p<0.05; Fig 6). The 20mg and placebo groups showed only modest, non-significant improvements (Fig 6). Epidermal differentiation complex genes (EDC-CE) showed large, but non-significant improvements, in the higher doses (Fig 6).

Discussion

This is the first study evaluating the molecular effects of a targeted kinase inhibitor in moderate-to-severe AD patients, with appropriate correlations to clinical responses, and a multivariate biomarker approach integrating skin and

serum. ASN002, an oral JAK-SYK inhibitor, was recently shown to have rapid onset of clinical efficacy in patients with moderate-to-severe AD, with greater than 80% of patients achieving EASI-50 in the higher (40 and 80mg) doses, and rapid significant reduction in pruritus NRS, with limited clinical efficacy in the 20mg and placebo groups.[49] The current study evaluated the effects of ASN002 on cellular infiltrates, inflammatory pathways, and skin barrier abnormalities implicated in AD pathogenesis.

In both higher doses, ASN002 suppressed $CD3^+$ T-cell and $CD11c^+$ DC infiltrates and improved the three major components of the barrier defects in AD, including epidermal hyperplasia, S100As, and epidermal differentiation measures such as FLG, FLG2, and CLDN23 expression.[10,20,65,72-77] The improvements in epidermal barrier were paralleled by significant reductions in Th2 (IL-13, IL-31), Th22/Th17 (IL-22, S100) pathway cytokines,[33,52,72] which are key to the pathogenesis of AD that has been linked with down regulation of filaggrin,[74] and other epidermal barrier differentiation measures such as tight junctions.[33,52,72,74,76-80]

The AD genomic signature (MADAD transcriptome),[64] showed significant improvements in both the 40mg and 80mg groups as early as day15, with further changes towards the non-lesional phenotype by day29, with ~80% improvement from baseline in the 40mg group. No significant changes were seen in placebo at both day15 and day29, and the 20mg showed significant, but modest changes from baseline at day15, but not at day29. Robust suppression of key inflammatory AD pathways, were seen, predominantly in the 40mg group, and

particularly at day29, with little changes in placebo or 20mg groups. These included inhibition of mRNA expressions of T-cell/DC-(IL-15, IL-15RA, IL-2RA, CCR7, CD1b, CD83), general inflammation (MMP12), Th2-(IL4R, IL-10, IL-13, CCL13, CCL17, CCL18, CCL22, CCL26, FCER1A), Th17/Th22-(LCN, PI3/elafin, CCL20, IL12RB2, S100As, IL36G, IL36RN), and Th1-(IFN-γ, CXCL9, CXCL11, MX1, OASL), and barrier (FLG, CLDN 23) related products. In parallel with the cellular and molecular improvements we also observed significant improvements in gene signatures of cytokine-treated keratinocyte and AD-associated pathways. [11,20,52,66,67] These significant molecular improvements are likely due to the successful inhibition of JAK-STAT as well as SYK signaling pathways, as JAK-family kinases are involved in Th2 (IL-4, IL-5, IL-13, TSLP), Th22 (IL-22), Th1 pathways (IFN-γ, IL-12), [26-32] Th17 (IL-17), [81] and T-cell/NK/Th1 activation (IL-15).[82] JAK signaling has also been shown to have a direct effect on neuronal itch. [81,82] Our data showed significant and robust early modulation of the itch cytokine IL-31, which signals through JAK1/JAK2, with 40mg ASN002. SYK signaling further regulates Th17, B-cell and DC signaling, [34-39] and inhibits keratinocyte terminal differentiation.[34-39,83-85] The rapid inhibition of various axes and amelioration of the barrier pathology may be induced by the broadened spectrum of cytokines targeted with JAK/SYK inhibition. [31,35-38,86,87] Previous studies have shown that concomitant JAK/SYK inhibition has a synergistic therapeutic effect on inflammatory diseases. [33,87] Thus, concomitant SYK inhibition may increase the clinical efficacy of JAK inhibition in AD.

Our study also correlated clinical responses to changes in molecular and histological tissue responses. We found significant correlations between improvements in clinical scores (EASI and BSA) with reductions in inflammatory markers, epidermal hyperplasia, and the composite histological improvement score (histoScore) combining epidermal thickness and k16 mRNA expression, as early as day15.[54] Markers significantly correlated with clinical improvements are among those crucial to the AD pathogenesis and barrier abnormalities, including Th2 (IL-5, IL-13), Th17/Th22 (IL-22, CCL20), and Th1 cytokines and/or their associated chemokine (CCL13/CCL17/CCL22, CCL26, CXCL10, S100As, the epidermal proliferation/hyperplasia marker K16, etc). Among skin biomarkers significantly associated with EASI and BSA improvements was also the pro-inflammatory cytokine IL-6, which has been associated with atherosclerosis and induction of C-reactive protein.[88,89] Even higher correlations were obtained between changes in tissue biomarkers and the histoscore. Particularly high correlations were obtained between improvements in histoScore at both day15 and day29 particularly with IL-22/IL-17-induced S100As, as well as other IL-22/IL-17 associated markers including PI3/elafin, IL-23p19, and CXCL1. High correlations were also found between improvements in histoscore and suppressions of Th2 associated measures such as IL-4R, CCL17, CCL22, and CCL18. While individual serum biomarkers did not correlate with clinical disease improvements,[49] upon integrating changes in skin and serum biomarkers, the correlations with clinical improvement improved to 0.76 (compared to ≤0.64 with individual skin biomarkers) with a profile that includes primarily skin biomarkers

505 (IL-6, Th2/CCL13, Th1/T-cell activation/IL-15RA, and MMP12), that reflect the
506 cytokine modulation by ASN002. Whereas skin biomarkers are generally more
507 sensitive for assessing molecular improvement,[49] combining skin and blood
508 measures provides the most robust biomarker approach.

509 These significant correlations may help define a set of tissue disease
510 response biomarkers that can potentially serve to monitor treatment response in
511 future studies with JAK and/or SYK inhibitors. Many of these biomarkers have
512 been previously identified as key to AD pathogenesis and were shown to mirror
513 treatment responses in AD patients treated with broad (cyclosporine A,[90] NB-
514 UVB,[67] topical steroids,[91] and calcineurin antagonists),[92]and specific agents
515 targeting Th2 (dupilumab[93] GBR830[94]) Th22 (fezakinumab)[52] and Th17/IL-23
516 (ustekinumab)[95] axis. While ASN002 potently inhibited Th2
517 (CCL17/CCL18/CCL22/CCL26), Th22/Th17 and hyperplasia-related genes
518 (S100As, K16) similar to dupilumab, it also suppressed Th1-related products
519 (CXCL9/CXCL10, STAT1, IL-12RB1), which were not modulated by
520 dupilumab.[54,66] Of note ASN002 40mg dose showed more robust and significant
521 inhibition of the mRNA of the itch cytokine IL-31 at both week 2 and 4 compared
522 with dupilumab at either 4 or 16 weeks.[93] While overall Th2 inhibition with
523 ASN002 at 4 weeks is similar to dupilumab at 4 weeks, higher Th17/Th22
524 inhibition is seen after 4 weeks of ASN002 treatment compared to dupilumab
525 treatment for both 4 and 16 weeks, likely due to the indirect inhibition of the
526 Th17/Th22 pathways with dupilumab, whereas ASN002 exerts robust Th17/Th22
527 inhibition via SYK/TYK2 signaling. Since SYK and TYK2 are involved in IL-

23/Th17/Th22 signaling, we believe that these data explain the particularly robust and early inhibition of Th17/Th22 pathway in our data as well as the effects on hyperplasia. This is also supported by the pathway analyses we provide that show the highest improvements in the Th17 and Th22 pathways (over 100% improvement). Of note, the inhibition of Th17/Th22 pathways with ASN002 in our AD cohort is much higher than that seen in psoriasis, a Th17-centered disease, at weeks 1 and 2 with the pan JAK inhibitor, tofacitinib, which took much longer to improve psoriasis clinically and molecularly, likely due to the lack of direct modulation of the Th17 pathway.[96,97] A comparison of treatment response biomarkers across different therapeutics is listed in Table E13. As illustrated in the table, ASN002 at 4 weeks shows overall larger responses in Th17/Th22 markers as well as some Th2/Th1 markers compared to other broad or specific systemic treatments, including NB-UVB,[67] ustekinumab/anti-IL-12/23p40,[98] Fezakizumab/anti IL-22,[52] GBR830/anti OX40 at later weeks,[94] as well as compared to a mild-to-moderate topical steroid at 4 weeks (Table E13).[91] Percent improvement in the AD transcriptome with ASN002 40mg at 4 weeks was larger than that seen with dupilumab at 4 weeks, and the magnitude of Th17/Th22 modulation at week 4 is even larger than with dupilumab at week 16. Overall ASN002 showed significant cellular and molecular suppression of key-AD inflammatory pathway such including those related to Th2, Th22/Th17 and also Th1 pathways, and improvements in epidermal barrier measures. While AD is primarily Th2/Th22-driven, it is a heterogeneous disease with variable contributions of Th1 and Th17 immune pathways in distinct disease subtypes. [6-

[8,13-15,66,99] For example, AD in both pediatric and Asian adults populations have strong Th2 and Th17 polarization, while AD in European American adults has mainly Th2/Th22 activation. [10,12,100] The broader range of cytokines targeted by JAK/SYK inhibition may make this class of therapeutics applicable across all AD subtypes, as to AD patients with concomitant allergic contact dermatitis (ACD), which often involves Th1/Th17 activation.[101,102] Furthermore, these data can inform other disease indications. The robust suppression of hallmark Th17 genes (LCN, PI3/elafin, CCL20, S100As, IL36G, IL36RN, IL-19) may have implications for possible treatment of psoriasis,[103] acne vulgaris,[104-106] hidradenitis suppurativa,[107] ichthyoses,[108] and other Th17-driven diseases. [10-12,109,110] The robust modulation of Th17 is also emphasized by the higher inhibition of the IL-17-induced release of CCL20 in keratinocytes by ASN002, as compared to tofacitinib, a pan JAK inhibitor, that indeed did not show robust Th17/Th22 modulation at week 2 in psoriasis patients.[96]

Our study had several limitations, including a small sample size, short study duration of only 4 weeks, and non-significant differences in baseline EASI between the groups (the 20 and 80mg were more severe ~29, whereas 40mg and placebo were ~21), perhaps accounting for the greater clinical and biomarker effects seen in the 40mg vs the 80mg group, and the lack of efficacy in the 20mg group. Future larger and longer studies in diverse ethnic and age groups are needed to further assess the clinical efficacy and associated molecular response to ASN002 in moderate-to-severe patients across the AD spectrum.

In summary, ASN002 couples clinical responses with robust tissue improvements in cellular infiltrates, inflammatory pathways and the associated barrier pathology of AD as early as day15, with sustained, progressive changes until day29.

ACCEPTED MANUSCRIPT

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926 **Figure Legends**

927 **Figure 1. Histology and barrier changes.** Representative histological images in
928 placebo and 40mg treatment group of hematoxylin and eosin (H&E) (**A**), keratin
929 16 (K16) (**B**), and filaggrin (FLG) (**C**). Number in lower right corner of image for
930 K16 staining (B) shows number of patients with K16 staining post-treatment. Fold
931 change (FCH) in epidermal thickness (ET) at day 15 and day 29 vs baseline in
932 lesional skin (**D**). Fold change (FCH) of K16 mRNA expression in lesional and
933 nonlesional skin at D15 and D29 vs baseline (**E**). Fold change (FCH) of $CD3^+$ T-
934 cells in lesional and nonlesional skin at D15 and D29 vs baseline (**F**). Fold
935 change (FCH) of $CD11c^+$ dendritic cells in lesional and nonlesional skin at D15
936 and D29 vs baseline (**G**). +p<0.1; *p<0.05; **p<0.01; ***p<0.001. *Black stars*:
937 significance of comparison between placebo and ASN002 groups, *red stars*:
938 significance of comparison vs baseline.
939
940 **Figure 2**. **MADAD Transcriptomic Changes. A)** Heatmap of genomic changes
941 in meta-analysis-derived atopic dermatitis (MADAD) transcriptome in treatment
942 groups of 80mg, 40mg, and 20mg, compared to placebo group. Differentially
943 expressed genes (DEGs) defined by criteria of fold change (FCH)>2 and false
944 discovery rate (FDR) < 0.05. Upregulated (red) and downregulated (blue) genes
945 at baseline in lesional and nonlesional skin and after treatment with respective
946 doses (80mg, 40mg, 20mg) in lesional skin at day 15 and day 29. **B)** Overall
947 percent improvements in MADAD transcriptome at day 15 and day 29 with
948 respective treatment doses of 80mg, 40mg, and 20mg, compared to placebo.

949 ***(p<0.001), **(p<0.01) compared to placebo group on respective treatment day.
950 Light blue bar on the boxplots represent the mean ± SEM.
951
952 **Figure 3**. **Immune Gene-Subset.** Heat map of mean gene expressions of a
953 curated list of AD immune genes based on previous profiling studies. Table
954 shows gene symbols with fold changes at day 15 (D15) and day 29 (D29) vs day
955 1 (D1) for each treatment groups of 80mg, 40 mg, and 20mg. +p<0.1; *p<0.05;
956 **p<0.01;
957
958 **Figure 4. Fold changes of inflammatory markers measured by qRT-PCR.**
959 Fold changes (FCH) of inflammatory markers in lesional skin at day 15 (D15) and
960 day 29 (D29) in the 80 mg treatment group, the 40 mg group, and the 20 mg
961 group, as compared to baseline and placebo group. *Black stars:* significance of
962 comparison between placebo and ASN002. *Red stars:* significance of
963 comparison vs baseline. +p<0.1; *p<0.05; **p<0.01; ***p<0.001.
964
965 **Figure 5**. **Heatmap of genes measured by qRT-PCR**. Heat map of mean gene
966 expressions measured by qRT-PCR in lesional skin at day 1 (D1), day 15 (D15),
967 and day 29 (D29) and in nonlesional skin at day 1 (D1). Table shows gene
968 symbols with fold changes at day 15 (D15) and day 29 (D29) vs day 1 (D1) for
969 each treatment groups of 80 mg, 40 mg, and 20mg. +p<0.1; *p<0.05; **p<0.01;
970

971 **Figure 6**. **Gene pathway analysis**. Percent improvements from baseline
972 dysregulation of selected AD-related gene signature pathways in lesional skin at
973 day 29 in respective treatment groups. Numbers at bottom of graph indicate
974 numbers of genes in each gene set. +p<0.1; *p<0.05; ***p<0.001. *Black stars:*
975 significance of comparison between placebo and ASN002.
976
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Tables

Table 1. Biomarker correlations with clinical improvements

A) Biomarker Correlation with EASI %Change at Day 15		
Markers	**Correlation**	**P-Value**
% EASI Improvement	1	0
MMP12	0.647	0.006
CCL20	0.650	0.007
IL-13	0.638	0.007
IL-22	0.627	0.008
CCL17	0.615	0.010
CCL26	0.603	0.012
IFN-γ	0.574	0.018
CCL22	0.551	0.024
IL-5	0.532	0.030
CCL13	0.527	0.032

B) Biomarker Correlation with EASI %Change at Day 29		
Markers	**Correlation**	**P-Value**
% EASI Improvement	1	0
IL-31	0.651	0.0008
MMP12	0.493	0.017
IL-5	0.479	0.021
IL-6	0.468	0.024
IL-15	0.467	0.025
CXCL10	0.448	0.032
S100A7	0.447	0.033
IL-22	0.443	0.034
S100A9	0.439	0.036
CCL13	0.436	0.037
S100A8	0.433	0.039
IL12B/IL-12/23p40	0.429	0.041
KRT16	0.411	0.052

C) Biomarker Correlation with BSA %Change at Day 15		
Markers	Correlation	P-Value
% BSA Improvement	1	0
CCL22	0.668	0.003
CCL17	0.629	0.007
IL-22	0.593	0.012

IL-13	0.545	0.024
MMP12	0.509	0.037
CCL20	0.487	0.047

6

D) Biomarker Correlation with BSA %Change at Day 29		
Markers	Correlation	P-Value
% BSA Improvement	1	0
IL-31	0.624	0.001
IL12B/IL-12p40	0.472	0.023
IL-9	0.466	0.024
IL-22	0.446	0.033
IL-13	0.425	0.043
MMP12	0.424	0.044
IL-19	0.421	0.045
IL-6	0.415	0.049

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8 Table 2. Biomarker correlations with histologic score (Histoscore)

A) Biomarker Correlation with HistoScore at Day 15		
Markers	**Correlation**	**P-Value**
histoSCORE	1	0
KRT16	0.956	$<10^{-10}$
S100A9	0.929	$<10^{-10}$
S100A8	0.907	$<10^{-10}$
S100A12	0.890	$<10^{-10}$
S100A7	0.885	$<10^{-10}$
PI3/elafin	0.880	$<10^{-10}$
IL-17C	0.828	$<10^{-4}$
IL23A/IL-23p19	0.826	$<10^{-4}$
CXCL1	0.821	$<10^{-4}$
CXCL8	0.750	0.0008
Thickness	0.738	0.0010
MMP12	0.730	0.0013
JAK3	0.725	0.0014
IL-4R	0.716	0.0017
Il-31	0.706	0.0021
IL1B	0.699	0.0024
CCL17	0.657	0.0052
CCL22	0.615	0.0101
IL-19	0.613	0.0104
IL-12RB1	0.608	0.0112
CXCL10	0.603	0.0120
CCL18	0.600	0.0124
IL-2RA	0.571	0.0185
FOXP3	0.556	0.0223
IL-13	0.554	0.0230
STAT1	0.534	0.0292
IL-32	0.524	0.0327
CCL20	0.522	0.0337
IL-15	0.522	0.0337
IL-15RA	0.505	0.0408
IL-22	0.500	0.0430
CCL26	0.498	0.0441
IL-9	0.493	0.0465
CCL5	0.488	0.0490
IL-6	0.488	0.0490

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B) Biomarker Correlation with HistoScore at Day 29		
Markers	**Correlation**	**P-Value**
HistoSCORE	1	0
KRT16	0.941	$<10^{-5}$
S100A9	0.927	$<10^{-5}$
S100A8	0.902	$<10^{-5}$
S100A12	0.881	$<10^{-5}$
CXCL8/IL-8	0.877	$<10^{-5}$
JAK3	0.869	$<10^{-5}$
Thickness	0.867	$<10^{-5}$
S100A7	0.852	$<10^{-5}$
IL-4R	0.848	$<10^{-6}$
CXCL1	0.817	$<10^{-5}$
CCL18	0.808	$<10^{-5}$
IL-2RA	0.802	$<10^{-5}$
PI3/elafin	0.801	$<10^{-5}$
IL-32	0.796	$<10^{-4}$
MMP12	0.782	$<10^{-4}$
CCL22	0.778	$<10^{-4}$
IL-1B	0.754	$<10^{-4}$
FOXP3	0.731	0.0002
CCL17	0.722	0.0002
IL-15	0.711	0.0003
IL-19	0.696	0.0005
IL23A/IL-23p19	0.682	0.0007
CXCL10	0.642	0.0016
IL-22	0.615	0.0029
IL-10	0.609	0.0032
IL-12RB1	0.608	0.0032
IL12B/IL-12p40	0.574	0.0060
PDE4B	0.566	0.0069
CXCL9	0.553	0.0086
CCL13	0.546	0.0096
IL-15RA	0.543	0.0101
STAT1	0.525	0.0133
IL-6	0.521	0.0140
CCL5	0.518	0.0147
IL-13	0.513	0.0158

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IL-17C	0.506	0.0174
IL-2	0.451	0.0364

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Figure 1

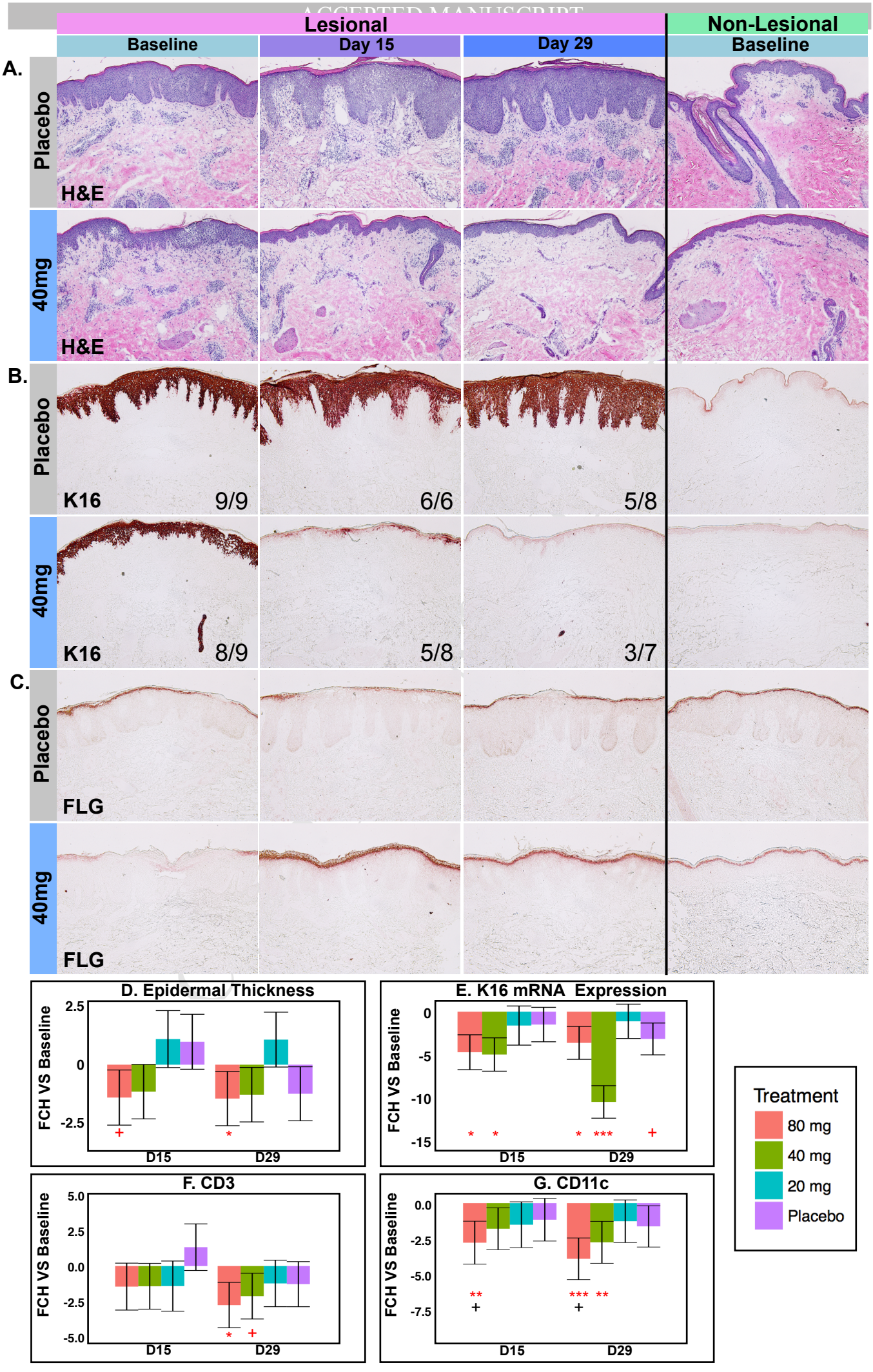
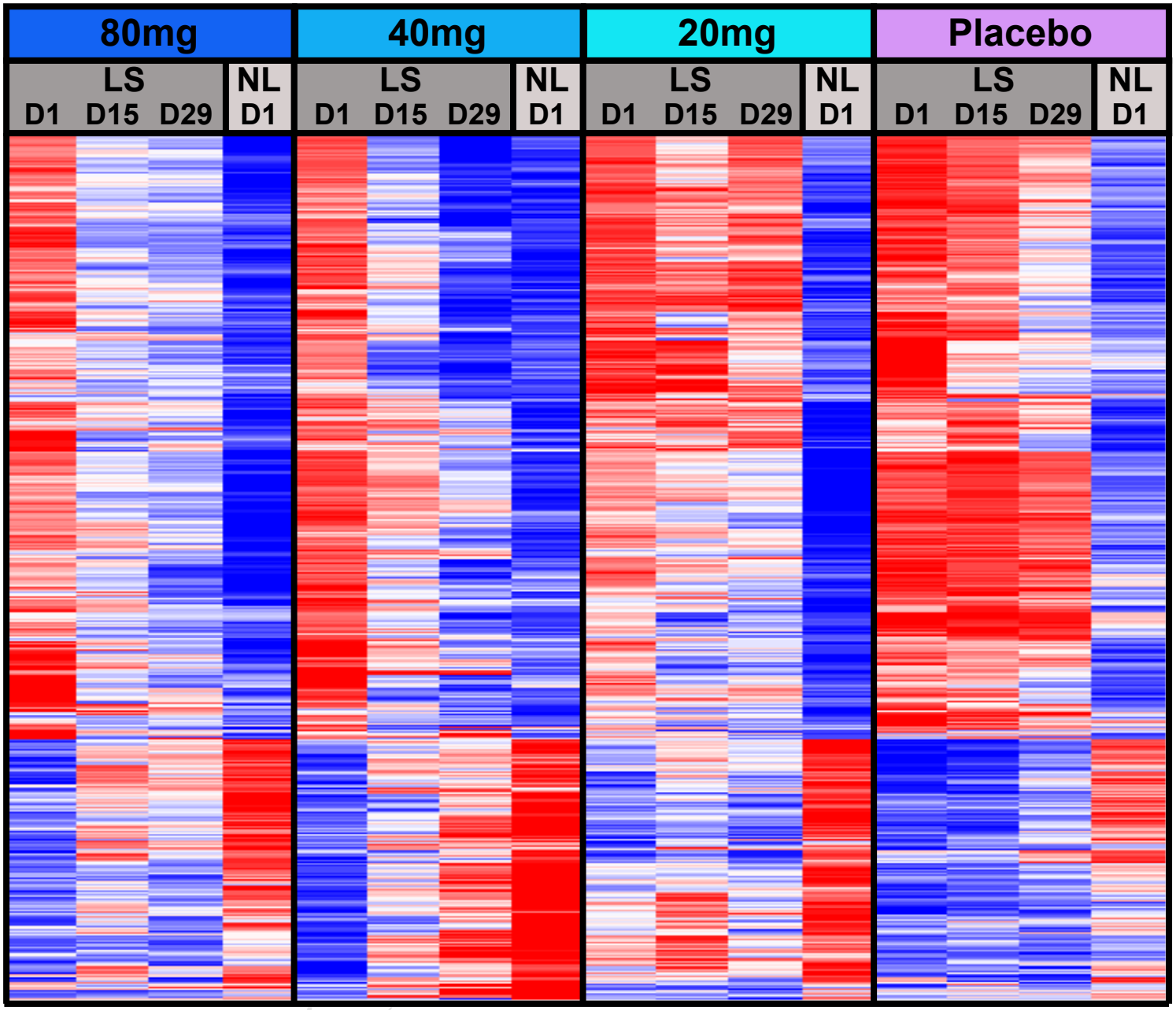
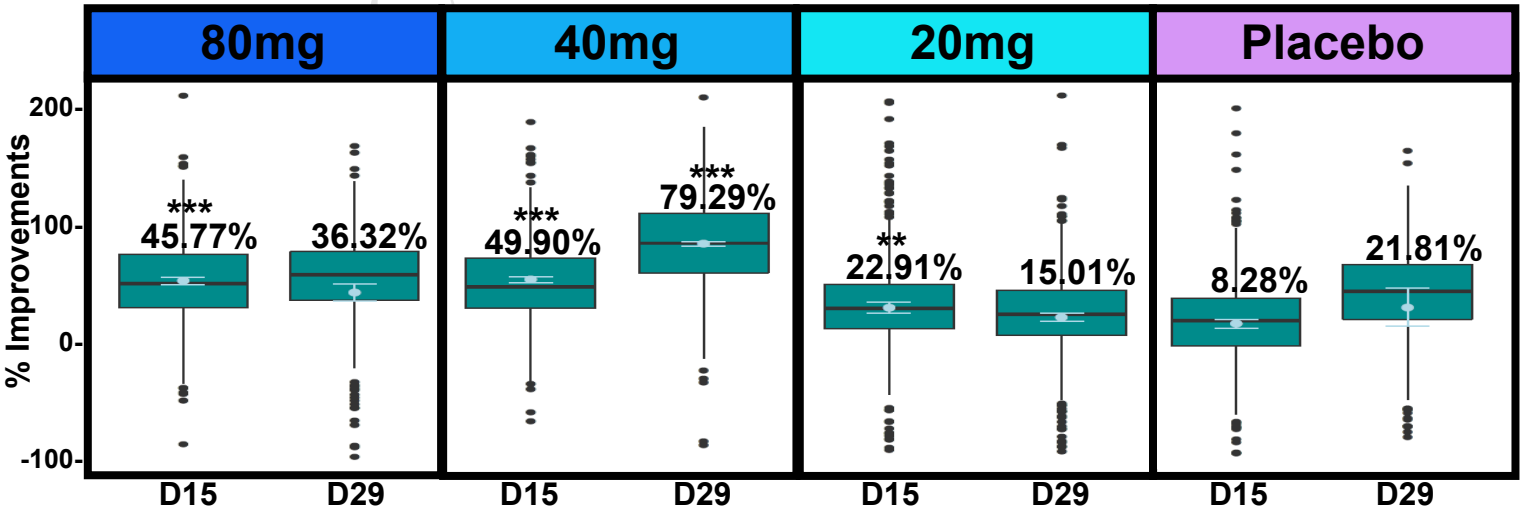

Lesional
Non-Lesional
Baseline
Day 15
Day 29
Baseline
A.
Placebo
H&E
40mg
H&E
B.
Placebo
K16
9/9
6/6
5/8
40mg
K16
8/9
5/8
3/7
C.
Placebo
FLG
40mg
FLG
D. Epidermal Thickness
FCH VS Baseline
2.5
0
-2.5
D15
D29
E. K16 mRNA Expression
FCH VS Baseline
0
-5
-10
-15
D15
D29
F. CD3
FCH VS Baseline
5.0
2.5
0.0
-2.5
-5.0
D15
D29
G. CD11c
FCH VS Baseline
0.0
-2.5
-5.0
-7.5
D15
D29
Treatment
80 mg
40 mg
20 mg
Placebo

Figure 2

A. MADAD Transcriptome

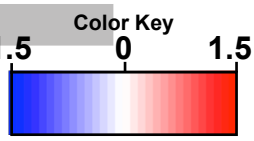


B.Transcriptomic Improvements



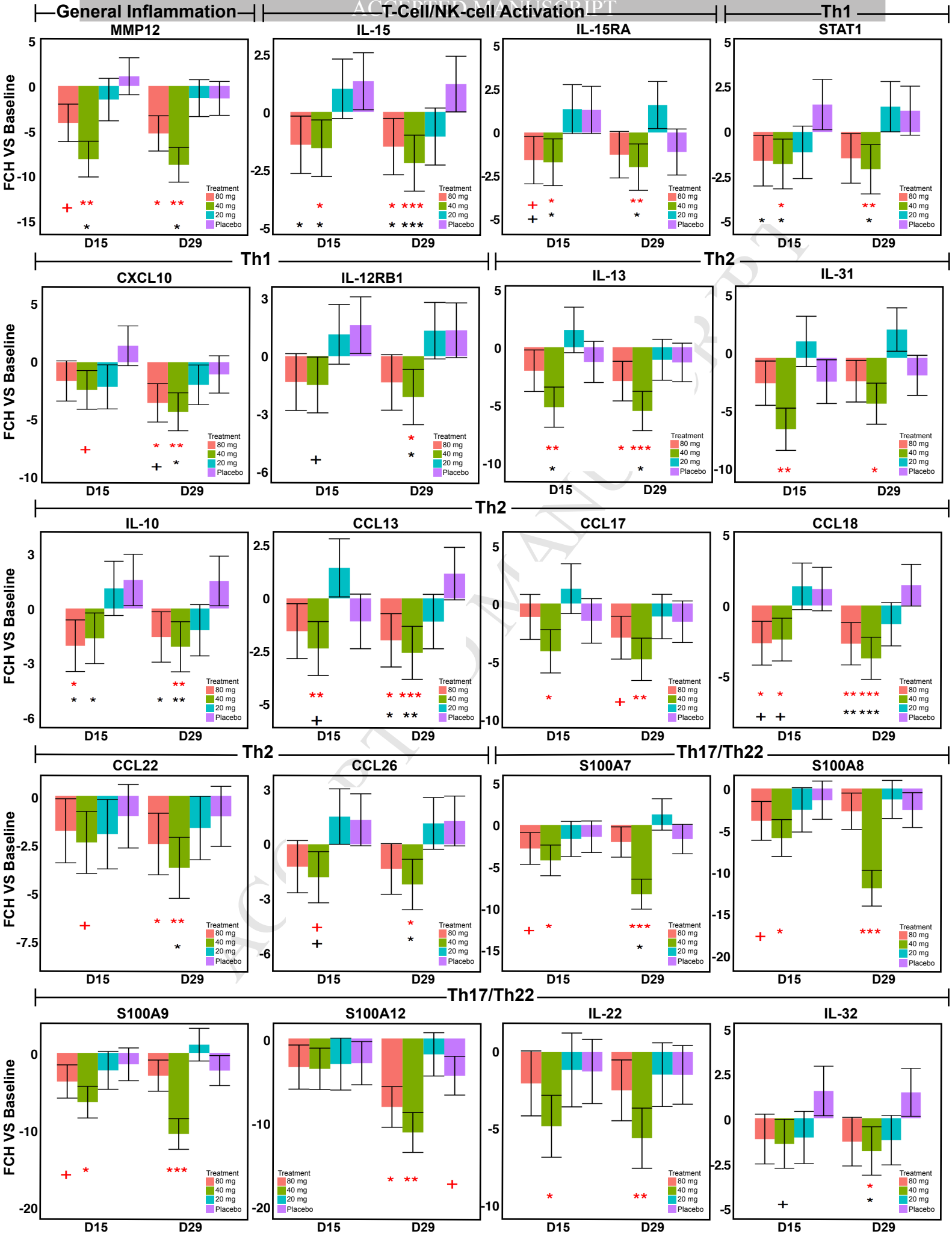
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Figure 3



Immune Genes	80mg D15 Vs D1 LS	80mg D29 Vs D1 LS	40mg D15 Vs D1 LS	40mg D29 Vs D1 LS	20mg D15 Vs D1 LS	20mg D29 Vs D1 LS	Placebo D15 Vs D1 LS	Placebo D29 Vs D1 LS
IL36RN	-1.09	-1.22	-1.39*	-1.84***	-1.14	-1.00	-1.14	-1.35+
IL36G	-1.55	-2.06*	-2.45**	-4.02***	-1.51	-1.27	-1.39	-2.07*
LCN2	-2.24	-3.15*	-3.06+	-4.27*	-1.56	-1.05	-1.37	-1.91
OASL	-13.60***	-16.60***	-8.97**	-22.70***	-1.75	-1.53	-1.06	-4.45*
IL12RB2	-1.56*	-1.44+	-1.58*	-1.73*	-1.03	1.06	-1.10	-1.39
PI3	-3.19	-3.82*	-2.38	-2.57	-1.90	1.02	-2.43	-2.12
MX1	-3.29***	-4.29***	-2.64**	-3.57***	-1.79	-1.45	1.07	-1.45
S100A12	-1.27	-4.11+	1.16	-2.80	-4.45	-2.79	-2.04	-9.19**
CCL19	-1.59	-1.24	-1.89	-3.25*	1.09	-1.04	1.14	-1.07
IL4R	-1.68	-1.50	-1.15	-2.29*	-1.26	1.12	1.18	-1.14
TNFSF10	1.06	1.02	-1.09	-1.58*	-1.12	-1.11	-1.18	-1.06
CCL18	-1.42	-2.33**	-1.57	-2.13*	-1.21	-1.28	-1.23	-1.01
CCL22	-1.90	-3.57*	-2.70	-4.11*	-2.91	-2.30	-1.78	-1.49
CCL13	-1.27	-2.01*	-1.67+	-2.30**	1.04	-1.32	-1.13	-1.02
MMP12	-4.66	-8.74*	-14.50**	-39.50***	-2.27	-1.52	-1.20	-2.21
CD1B	-1.76	-5.86*	-3.96+	-17.20***	-3.54	-3.74+	-2.26	-1.77
S100A7	-1.31	-1.32	-1.57+	-2.28**	-1.11	1.04	-1.05	-1.05
S100A8	-1.63	-1.63	-2.08*	-3.47***	-1.29	-1.01	-1.10	-1.20
S100A9	-3.88	-4.03	-7.37*	-17.50**	-2.34	-1.09	-1.49	-1.81
CCL17	2.02	-1.57	-2.84	-4.83*	-1.11	-1.58	1.31	-1.85
ITK	-1.47	-1.67	-1.71	-3.40*	-1.60	-2.20	-1.01	-1.35
CD28	-1.22	-1.68	-1.88	-5.84**	-1.09	-1.36	1.05	-1.27
IL7R	-1.58	-1.65	-1.17	-2.19*	-1.25	-1.24	1.08	-1.24
CD2	-1.10	-1.66	-1.31	-3.26*	-1.01	-1.23	1.45	-1.17
LCK	-1.19	-1.50	-1.39	-2.80*	-1.05	-1.37	1.16	-1.00
CD3G	-1.08	-1.53	-1.36	-2.86*	1.18	-1.29	1.34	-1.12
CD3E	-1.07	-1.25	-1.60	-2.35*	1.62	-1.39	1.40	1.03
CCR2	-1.46	-1.35	-1.29	-2.36*	-1.29	-1.33	1.17	1.10
CD27	-1.23	-1.33	-1.58	-1.92*	-1.02	1.07	1.14	1.00
CD5	-1.24	-1.49	-1.78+	-2.16*	1.57	-1.07	1.14	1.17
CCR7	-1.02	-1.45+	-1.56+	-1.70*	1.04	-1.40	1.30	-1.16
IL6R	-1.28	-1.31	-1.52*	-1.67*	1.11	1.24	1.27	1.06
CCR1	-1.78	-1.90+	-2.60*	-1.97+	-1.13	-1.06	1.08	1.39
XCL2	-1.85	-2.66*	-1.91	-3.56**	-1.51	-1.70	-1.50	-1.23
XCL1	-1.86	-2.05+	-2.18+	-3.42**	-1.39	-1.42	-1.52	-1.17
ITGAM	-1.41	-1.75+	-1.56	-1.93*	-1.03	-1.28	1.06	1.06
CD86	-1.95+	-2.76**	-1.96+	-2.02+	-1.01	-1.55	1.12	-1.11
CD83	-1.52	-2.27**	-1.35	-2.19*	-1.67	-1.94*	-1.10	-1.50
CTLA4	-1.03	-3.49*	-2.03	-13.00***	-2.28	-2.37	-1.12	-1.57
IL15	-1.28	-1.67+	-1.68+	-2.41**	-1.57	-1.63	1.02	1.11
CX3CR1	-1.21	-1.19	1.16	-1.10	-1.25	-2.00*	1.32	1.37
IFNGR1	1.02	1.07	-1.15	1.13	-1.60*	-1.24	-1.45+	-1.11
STAT1	-1.60+	-1.61+	-2.00*	-1.22	-1.81+	-1.47	-1.66+	-1.51
CXCL16	-1.37+	-1.50*	-1.24	-1.02	-1.47+	-1.30	-1.08	-1.14
TNFSF4	1.12	-1.83+	-2.38*	-3.20**	-1.19	-1.71	-1.08	-1.19
CXCL11	1.01	-2.38	-4.69**	-3.15+	-1.29	-1.35	-1.04	-1.55
FCER1A	-1.21	-1.45*	-1.26	-1.55*	-1.20	-1.57*	-1.15	1.11
CCR6	-1.00	-1.17	-1.18	-1.49	-1.86	-2.49*	-1.15	1.05
CCL20	-1.05	-1.32	-3.75+	-2.89	-6.34*	-3.41	-1.15	-1.62
PDE4B	-1.45	-1.73*	-1.23	1.22	-1.29	-1.90*	-1.48	-1.48
CXCL3	-2.56	-1.70	1.29	2.44	-2.70	-4.19*	1.05	-2.11
IL6	-6.89*	-1.85	-1.01	1.61	-1.77	-2.81	1.03	-2.30
IL1RN	-2.31**	-2.12**	1.08	1.01	-1.39	1.01	1.30	-1.42
IL37	3.80+	4.20*	4.54*	8.19**	2.23	1.65	-1.01	3.03+
IL34	1.24	1.61	2.19+	2.84*	1.85	1.49	1.00	1.32
CXCL14	1.95	2.64*	1.50	1.18	2.15	2.09+	1.44	1.76
CCL14	1.56+	1.31	1.60*	1.59*	1.46	1.21	1.18	1.45+
PDE5A	1.12	1.16	1.29	1.92**	-1.29	-1.40	-1.09	1.14
CCL28	1.22	1.12	1.18	2.19*	-1.00	-1.47	-1.03	-1.03
PDE9A	1.41	-1.00	3.69*	3.34*	1.29	-1.00	1.95	1.22
IL16	1.63+	1.45	1.81*	1.03	1.42	1.27	1.15	1.28
PDE1A	1.75	1.45	2.74**	1.31	1.36	1.08	1.58	1.50

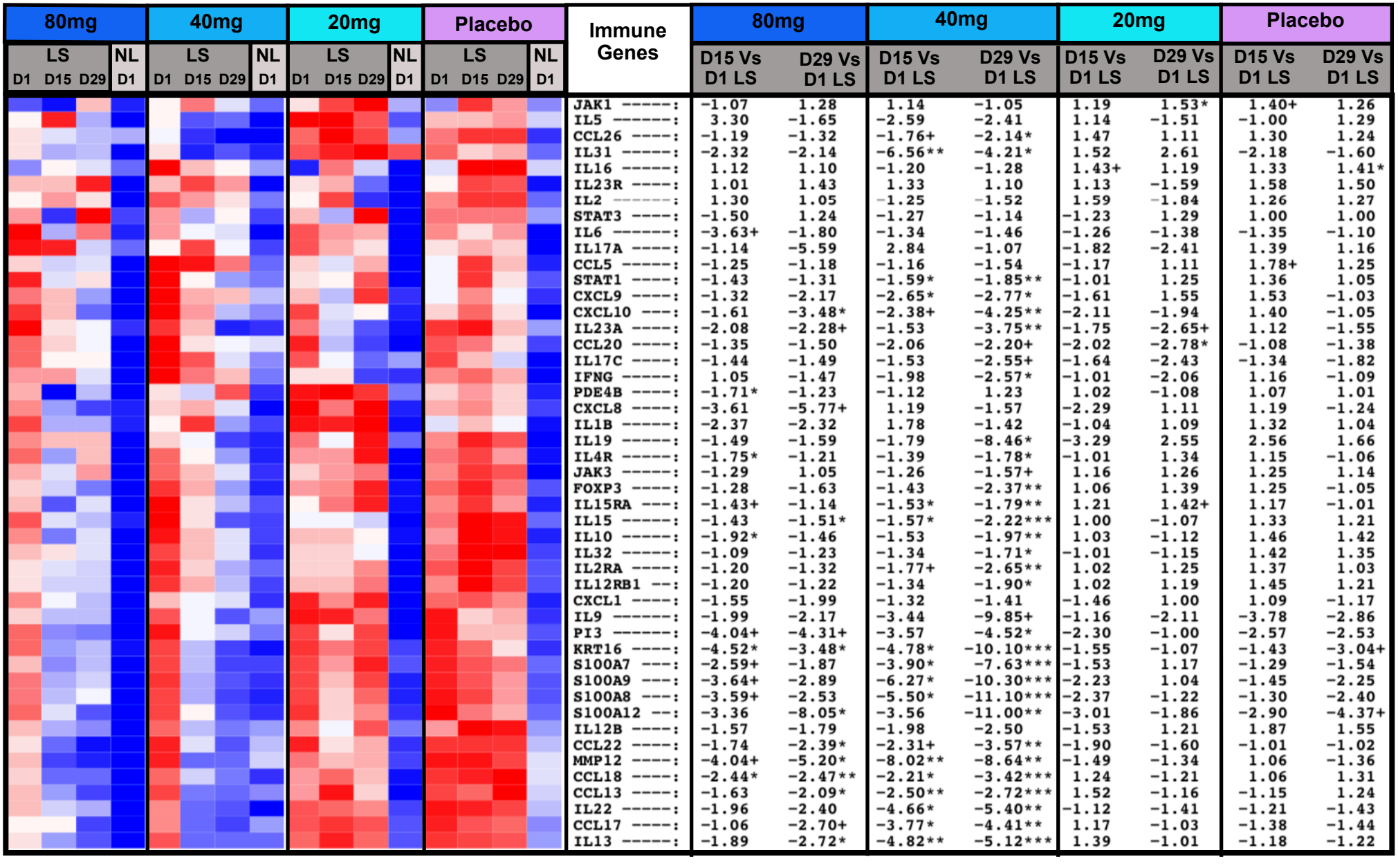
Figure 4.



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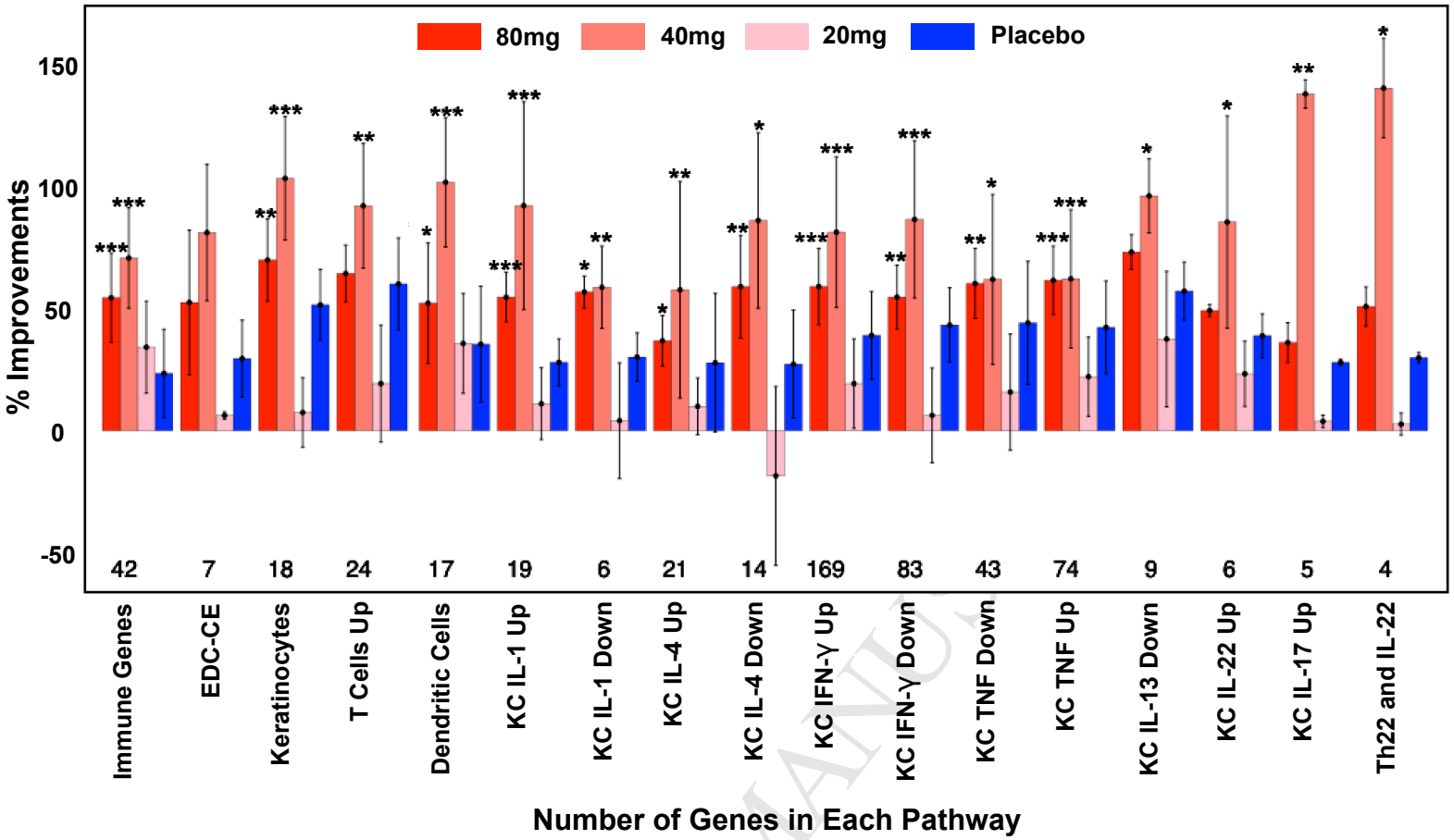
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Figure 5 - Heatmap of genes measured by RT-PCR



Immune Genes	80mg D15 Vs D1 LS	80mg D29 Vs D1 LS	40mg D15 Vs D1 LS	40mg D29 Vs D1 LS	20mg D15 Vs D1 LS	20mg D29 Vs D1 LS	Placebo D15 Vs D1 LS	Placebo D29 Vs D1 LS
JAK1 -----:	-1.07	1.28	1.14	-1.05	1.19	1.53*	1.40+	1.26
IL5 ------:	3.30	-1.65	-2.59	-2.41	1.14	-1.51	-1.00	1.29
CCL26 ----:	-1.19	-1.32	-1.76+	-2.14*	1.47	1.11	1.30	1.24
IL31 -----:	-2.32	-2.14	-6.56**	-4.21*	1.52	2.61	-2.18	-1.60
IL16 -----:	1.12	1.10	-1.20	-1.28	1.43+	1.19	1.33	1.41*
IL23R ----:	1.01	1.43	1.33	1.10	1.13	-1.59	1.58	1.50
IL2 ------:	1.30	1.05	-1.25	-1.52	1.59	-1.84	1.26	1.27
STAT3 ----:	-1.50	1.24	-1.27	-1.14	-1.23	1.29	1.00	1.00
IL6 ------:	-3.63+	-1.80	-1.34	-1.46	-1.26	-1.38	-1.35	-1.10
IL17A ----:	-1.14	-5.59	2.84	-1.07	-1.82	-2.41	1.39	1.16
CCL5 -----:	-1.25	-1.18	-1.16	-1.54	-1.17	1.11	1.78+	1.25
STAT1 ----:	-1.43	-1.31	-1.59*	-1.85**	-1.01	1.25	1.36	1.05
CXCL9 ----:	-1.32	-2.17	-2.65*	-2.77*	-1.61	1.55	1.53	-1.03
CXCL10 ---:	-1.61	-3.48*	-2.38+	-4.25**	-2.11	-1.94	1.40	-1.05
IL23A ----:	-2.08	-2.28+	-1.53	-3.75**	-1.75	-2.65+	1.12	-1.55
CCL20 ----:	-1.35	-1.50	-2.06	-2.20+	-2.02	-2.78*	-1.08	-1.38
IL17C ----:	-1.44	-1.49	-1.53	-2.55+	-1.64	-2.43	-1.34	-1.82
IFNG -----:	1.05	-1.47	-1.98	-2.57*	-1.01	-2.06	1.16	-1.09
PDE4B ----:	-1.71*	-1.23	-1.12	1.23	1.02	-1.08	1.07	1.01
CXCL8 ----:	-3.61	-5.77+	1.19	-1.57	-2.29	1.11	1.19	-1.24
IL1B -----:	-2.37	-2.32	1.78	-1.42	-1.04	1.09	1.32	1.04
IL19 -----:	-1.49	-1.59	-1.79	-8.46*	-3.29	2.55	2.56	1.66
IL4R -----:	-1.75*	-1.21	-1.39	-1.78*	-1.01	1.34	1.15	-1.06
JAK3 -----:	-1.29	1.05	-1.26	-1.57+	1.16	1.26	1.25	1.14
FOXP3 ----:	-1.28	-1.63	-1.43	-2.37**	1.06	1.39	1.25	-1.05
IL15RA ---:	-1.43+	-1.14	-1.53*	-1.79**	1.21	1.42+	1.17	-1.01
IL15 -----:	-1.43	-1.51*	-1.57*	-2.22***	1.00	-1.07	1.33	1.21
IL10 -----:	-1.92*	-1.46	-1.53	-1.97**	1.03	-1.12	1.46	1.42
IL32 -----:	-1.09	-1.23	-1.34	-1.71*	-1.01	-1.15	1.42	1.35
IL2RA ----:	-1.20	-1.32	-1.77+	-2.65**	1.02	1.25	1.37	1.03
IL12RB1 --:	-1.20	-1.22	-1.34	-1.90*	1.02	1.19	1.45	1.21
CXCL1 ----:	-1.55	-1.99	-1.32	-1.41	-1.46	1.00	1.09	-1.17
IL9 ------:	-1.99	-2.17	-3.44	-9.85+	-1.16	-2.11	-3.78	-2.86
PI3 ------:	-4.04+	-4.31+	-3.57	-4.52*	-2.30	-1.00	-2.57	-2.53
KRT16 ----:	-4.52*	-3.48*	-4.78*	-10.10***	-1.55	-1.07	-1.43	-3.04+
S100A7 ---:	-2.59+	-1.87	-3.90*	-7.63***	-1.53	1.17	-1.29	-1.54
S100A9 ---:	-3.64+	-2.89	-6.27*	-10.30***	-2.23	1.04	-1.45	-2.25
S100A8 ---:	-3.59+	-2.53	-5.50*	-11.10***	-2.37	-1.22	-1.30	-2.40
S100A12 --:	-3.36	-8.05*	-3.56	-11.00**	-3.01	-1.86	-2.90	-4.37+
IL12B ----:	-1.57	-1.79	-1.98	-2.50	-1.53	1.21	1.87	1.55
CCL22 ----:	-1.74	-2.39*	-2.31+	-3.57**	-1.90	-1.60	-1.01	-1.02
MMP12 ----:	-4.04+	-5.20*	-8.02**	-8.64**	-1.49	-1.34	1.06	-1.36
CCL18 ----:	-2.44*	-2.47**	-2.21*	-3.42***	1.24	-1.21	1.06	1.31
CCL13 ----:	-1.63	-2.09*	-2.50**	-2.72***	1.52	-1.16	-1.15	1.24
IL22 -----:	-1.96	-2.40	-4.66*	-5.40**	-1.12	-1.41	-1.21	-1.43
CCL17 ----:	-1.06	-2.70+	-3.77*	-4.41**	1.17	-1.03	-1.38	-1.44
IL13 -----:	-1.89	-2.72*	-4.82**	-5.12***	1.39	-1.01	-1.18	-1.22

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Online Repository Tables

Table E1. Immunohistochemistry antibodies

Antigen	Manufacturer	Clone	Isotype	Dilution
CD3	BD Biosciences	SK7	IgG1	1:100
CD11c	BD Biosciences	B-ly6	IgG1	1:100
K16	Santa Cruz	LL025	IgG1	1:100
Filaggrin	Abnova	FLG01	IgG1	1:100

Table E2. qRT-PCR Primers and Probes

Gene	Assay ID
MMP12	Hs00159178_m1
CCL13	Hs00234646_m1
CCL17	Hs00171074_m1
CCL18	Hs00268113_m1
CCL20	Hs01011368_m1
CCL22	Hs01574247_m1
CCL26	Hs00171146_m1
CCL5	Hs00174575_m1
CXCL1	Hs00236937_m1
CXCL10	Hs00171042_m1
CXCL9	Hs00171065_m1
PI3	Hs00160066_m1
FOXP3	Hs01085834_m1
18S	Hs99999901_s1
RPLP0	Hs99999902_m1
IFN-γ	Hs00989291_m1
IL-10	Hs00961622_m1
IL-12p40	Hs01011518_m1
IL-12RB1	Hs01106578_m1
IL-13	Hs00174379_m1
IL-15	Hs01003716_m1
IL-15RA	Hs00542604_m1
IL-16	Hs00913644_m1
IL-17A	Hs00174383_m1
IL-19	Hs00604657_m1
IL-1B	Hs01555410_m1
IL-2	Hs00174114_m1
IL-22	Hs01574154_m1
IL-23p19	Hs00372324_m1
IL-23R	Hs00332759_m1
IL-2RA	Hs00907777_m1
IL-32	Hs00992441_m1
IL-17C	Hs00171163_m1

IL-4R	Hs00965056_m1
IL-5	Hs00174200_m1
IL-6	Hs00174131_m1
CXCL8	Hs00174103_m1
IL-9	Hs00174125_m1
JAK1	Hs01026983_m1
JAK3	Hs00354555_m1
PDE4B	Hs00277080_m1
K16	Hs00373910_g1
S100A12	Hs00942835_g1
S100A7	Hs00161488_m1
S100A8	Hs00374264_g1
S100A9	Hs00610058_m1
STAT1	Hs01013996_m1
STAT3	Hs00374280_m1

Table E3. Baseline demographics in patients treated with ASN002

Characteristics	Placebo (n=9)	20mg (n=9)	40mg (n=9)	80mg (n=9)	P-Values
Age (y), mean (SD)	29.9(9.3)	38.2(14.4)	42.4(13.9)	33.1(10.4)	0.16
Gender					
Male, no. (%) Female, no. (%)	3(33.3%) 6 (66.7%)	5(56.6%) 4(44.4%)	5(56.6%) 4(44.4%)	5(56.6%) 4(44.4%)	0.83
SEVERITY					
EASI, mean (SD)	21.6(6.2)	29.0(13.5)	21.8(6.2)	28.2(11.7)	0.23
IGA severity, n (%) 3 = moderate 4 = severe	8 (88.9%) 1(11.1%)	5(55.6%) 4(44.4%)	8(88.9%) 1(11.1%)	4(44.4%) 5(55.6%)	0.12
BSA (%)	25.1(10.5)	44.1(20.2)	31.4(18.0)	36.9(25.9)	0.22
Pruritus NRS, mean (SD)	5.9(2.6)	5.4(2.3)	6.1(2.5)	6.0(2.6)	0.87

BSA = body surface area; EASI = Eczema Area and Severity Index; IGA = Investigator's Global Assessment; NRS = Numeric Rating Scale; SD = standard deviation.

Table E4. Number of Patients in Biopsy Cohort

Group	Placebo	20mg	40mg	80mg	Total
Non-lesional Baseline	**9**	**7**	**9**	**8**	**33**
Lesional Baseline	**9**	**7**	**9**	**8**	**33**
Lesional Day 15	**6**	**4**	**8**	**6**	**24**
Lesional Day 29	**8**	**7**	**7**	**8**	**30**

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Table E5. ASN002 Inhibition of tyrosine kinase activity with TR-FRET assay.

Kinase	Inhibition of kinase activity, IC_{50} (nM), Mean				
	ASN002	**Tofacitinib**	**Tamatinib**	**Upadacitinib***	**Baricitinib***
JAK1	46	21	11	43	5.9
JAK2	4	7	4	200	5.7
JAK3	11	4	12	2,300	>400
TYK2	8	3,600	500	4,700	53
SYK	5	>10,000	14	--	--

IC_{50} values determined in biochemical kinase assays using purified partial or full-length enzymes.
*Literature values for upadacitinib (Kremer et al., 2016) and baricitinib (Akahane et al., 2017). -- Not Recorded

Table E9. Biomarker correlations with clinical improvements at day 15

A) Biomarker Correlation with EASI improvements at Day 15		
Markers	**Correlation**	**P-Value**
% EASI improvements	1	0
MMP12	0.647058824	0.006147347
CCL20	0.639705882	0.006916832
IL-13	0.637254902	0.007189792
IL-22	0.62745098	0.008369235
CCL17	0.615196078	0.010056159
CCL26	0.602941176	0.012004687
IFN-y	0.573529412	0.017924249
CCL22	0.551470588	0.023724262
IL-5	0.531862745	0.030039073
CCL13	0.526960784	0.03180662
CXCL10	0.480392157	0.052952303
STAT1	0.475490196	0.055686656
CCL18	0.453431373	0.069345624
IL-2	0.401960784	0.110871343
CXCL9	0.399509804	0.113219607
IL-19	0.384803922	0.128071321
IL-2RA	0.379901961	0.133318329
S100A9	0.37745098	0.135998519
IL-32	0.375	0.138716814
S100A8	0.367647059	0.14710262
FOXP3	0.345588235	0.174388365
S100A7	0.345588235	0.174388365
IL-12RB1	0.340686275	0.180895503

IL-9	0.335784314	0.187567031
IL-15	0.335784314	0.187567031
JAK3	0.289215686	0.259348601
IL-15RA	0.284313725	0.267804269
CXCL8/IL-8	0.269607843	0.294209854
PI3/elafin	0.262254902	0.307996656
S100A12	0.25245098	0.326982813
KRT16	0.25245098	0.326982813
CCL5	0.227941176	0.377437625
IL-4R	0.220588235	0.393394453
IL-1B	0.220588235	0.393394453
STAT3	0.218137255	0.398796167
IL-17C	0.213235294	0.409722689
IL12B/IL-12p40	0.193627451	0.455039932
CXCL1	0.181372549	0.484633929
PDE4B	0.114040487	0.662972783
IL23A/IL-23p19	0.105392157	0.687298291
IL-10	0.083333333	0.751205676
JAK1	0.073529412	0.780154518
IL23R	-0.017156863	0.951004026
IL-16	-0.031862745	0.905925224
IL-6	-0.107843137	0.680314781
IL-17A	-0.147149016	0.573039516

B) Biomarker Correlation with BSA improvements at Day 15		
Markers	**Correlation**	**P-Value**
% BSA improvement	1	0
CCL22	0.668323429	0.003361035
CCL17	0.628865219	0.006846667
IL-22	0.593106217	0.01209071
IL-13	0.545016523	0.023667335
MMP12	0.509257521	0.036803495
CCL20	0.487062278	0.047377046
IL-2	0.47103238	0.056329338
CCL26	0.462400897	0.061642611
CCL18	0.455002482	0.066484819
CXCL8/IL-8	0.455002482	0.066484819
IL12B/IL-12p40	0.452536344	0.068159525
IL12RB1	0.448837137	0.070729494
CCL5	0.447604068	0.071601747

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S100A8	0.436506446	0.079810326
IL-32	0.430341101	0.084655856
FOXP3	0.422942687	0.090747441
S100A12	0.421709618	0.091792578
IL-15	0.413078134	0.09935207
S100A9	0.409378927	0.102724189
PI3/elafin	0.406912789	0.105017049
IL-1B	0.406912789	0.105017049
CCL13	0.394582098	0.117028362
JAK3	0.394582098	0.117028362
IL-9	0.381018339	0.131320984
S100A7	0.374852993	0.138201745
IFN-y	0.364988441	0.149721388
CXCL1	0.362522303	0.152700694
CXCL10	0.361289234	0.154205386
IL-15RA	0.358823096	0.157244961
IL-2RA	0.348958543	0.169808943
STAT1	0.330462507	0.195143642
IL-5	0.329229438	0.196916133
KRT16	0.302101919	0.23859297
CXCL9	0.292237367	0.2550306
IL-17C	0.2749744	0.285450616
IL23A/IL-23p19	0.247846881	0.337487819
IL-19	0.226884707	0.381184155
PDE4B	0.214068691	0.409356232
IL-4R	0.198524118	0.444962931
IL-6	0.127006113	0.627146098
IL-17A	0.103024413	0.693968751
STAT3	0.09124711	0.727620489
IL-16	0.050555831	0.847200299
IL-10	0.01356376	0.958794065
IL-23R	-0.029593657	0.910230719
JAK1	-0.163998185	0.529376103

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Table E10. Biomarker correlations with clinical improvements at day 29

A) Biomarker Correlation with EASI improvements at Day 29		
Markers	**Correlation**	**P-Value**
% EASI Improvement	1	0
MMP12	0.492710665	0.01691137
IL-5	0.479069806	0.02073067
IL-6	0.468495196	0.024146747
IL-15	0.466518423	0.024832771
CXCL10	0.448233273	0.031947519
S100A7	0.446750693	0.032588688
IL-22	0.44329134	0.034124452
S100A9	0.439337794	0.035949134
CCL13	0.436372635	0.037367514
S100A8	0.433407476	0.038829626
IL12B/IL-12p40	0.42945393	0.040848679
KRT16	0.410674586	0.051588214
IL-10	0.405238461	0.055072848
IL-13	0.401284915	0.0577192
IL-4R	0.387447504	0.067757921
IL-1B	0.382505571	0.071648117
CCL17	0.380128522	0.073578567
IFN-γ	0.356807523	0.094663624
STAT1	0.353842363	0.097635874
PI3/elafin	0.348900431	0.10274227
IL-2RA	0.341981725	0.11021808
CCL18	0.333086247	0.120404868
IL-19	0.328638508	0.125746707
IL-15RA	0.301457879	0.162146838
CCL26	0.278230797	0.198618235
FOXP3	0.269829512	0.213078323
S100A12	0.268841125	0.214824571
JAK3	0.244131463	0.261602015
IL-9	0.226834699	0.297949401
CXCL9	0.212503095	0.330317282
CXCL1	0.211020515	0.333781533
CCL22	0.187793433	0.390852271
IL23A/IL-23p19	0.177415375	0.418020649
CCL20	0.156165065	0.476737472
JAK1	0.148257973	0.499602541

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CCL5	0.144304427	0.511233673
IL12RB1	0.141833461	0.518569023
IL-32	0.140845075	0.5215172
IL-2	0.120088958	0.58520978
IL-17C	0.094885103	0.666722745
CXCL8/IL-8	0.085001238	0.699779987
STAT3	0.046454165	0.833296573
PDE4B	-0.023721276	0.914444864
IL-16	-0.092414137	0.674933869
IL-23R	-0.158141838	0.471105475
IL-17A	-0.207486504	0.342126363

B) Biomarker Correlation with BSA improvements at Day 29		
Markers	**Correlation**	**P-Value**
% BSA Improvement	1	0
IL12B/IL-12p40	0.47242183	0.02282916
IL-9	0.46648564	0.024844279
IL-22	0.446698338	0.032611515
IL-13	0.425426989	0.042988972
MMP12	0.423942941	0.043799494
IL-19	0.420974846	0.045456348
IL-6	0.414543973	0.049213803
CCL13	0.405145004	0.055134303
CCL17	0.39831786	0.059768703
IL-4R	0.374969369	0.077903662
STAT1	0.370022544	0.082228176
S100A7	0.367054448	0.084907975
IL-15	0.349245877	0.102379062
KRT16	0.346772464	0.105000608
S100A8	0.341825639	0.110391201
PI3/elafin	0.329458575	0.124749157
S100A9	0.318080876	0.139107429
CCL26	0.288399924	0.182023589
CXCL10	0.280485003	0.194854344
CCL18	0.27603286	0.202335284
IL-2RA	0.273064765	0.207428885
CXCL1	0.260697701	0.229575765
IL-15RA	0.255256193	0.239795952
IL-1B	0.241405082	0.267133583
S100A12	0.228543336	0.29422637

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CCL22	0.214692225	0.325241674
FOXP3	0.196883653	0.367894983
IL-5	0.190429107	0.384114472
IL23A/IL23p19	0.184516589	0.399321143
IL-10	0.177096351	0.418871773
JAK3	0.168192065	0.443005457
IL-32	0.143952621	0.512274977
CCL5	0.124660001	0.570899829
IFN-γ	0.121691906	0.580174093
CXCL9	0.086074763	0.696162601
CXCL8	0.080138572	0.716243538
IL-16	0.072718334	0.741601333
JAK1	0.069255556	0.753526357
IL-12RB1	0.054909762	0.803484594
IL-17C	0.028691588	0.896603738
CCL20	0.009893651	0.964264204
IL-2	-0.008409603	0.969621961
STAT3	-0.016819207	0.939284713
PDE4B	-0.029680953	0.893057877
IL-17A	-0.074247359	0.736353781
IL-23R	-0.254266828	0.241685572

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Table E11. Biomarker correlations with histologic score (Histoscore) at Day 15

Markers	Correlation	p-value
histoSCORE	1	0
KRT16	0.955882353	$<10^{-10}$
S100A9	0.928921569	$<10^{-10}$
S100A8	0.906862745	$<10^{-10}$
S100A12	0.889705882	$<10^{-10}$
S100A7	0.884803922	$<10^{-10}$
PI3/elafin	0.879901961	$<10^{-10}$
IL-17C	0.828431373	$<10^{-4}$
IL23A/IL-23p19	0.825980392	$<10^{-4}$
CXCL1	0.821078431	$<10^{-4}$
CXCL8/IL-8	0.75	0.000791476
Thickness	0.737745098	0.001066177
MMP12	0.730392157	0.001262828
JAK3	0.725490196	0.00140874
IL-4R	0.715686275	0.001739743
IL-1B	0.698529412	0.002463109
CCL17	0.656862745	0.005229559
CCL22	0.615196078	0.010056159
IL-19	0.612745098	0.010424039
IL-12RB1	0.607843137	0.011192078
CXCL10	0.602941176	0.012004687
CCL18	0.600490196	0.01242829
IL-2RA	0.571078431	0.018506509
FOXP3	0.556372549	0.022322675
IL-13	0.553921569	0.023015045
STAT1	0.534313725	0.029184665
IL-32	0.524509804	0.032720363
CCL20	0.522058824	0.033654488
IL-15	0.522058824	0.033654488
IL-15RA	0.504901961	0.040789821
IL-22	0.5	0.04302948
CCL26	0.49754902	0.044184367
IL-9	0.492647059	0.046565823
CCL5	0.487745098	0.049044953
IL-6	0.487745098	0.049044953
IFN-γ	0.43872549	0.079762573
CXCL9	0.43872549	0.079762573

CCL13	0.414215686	0.099658467
PDE4B	0.409564761	0.102552869
STAT3	0.409313725	0.104039021
IL-10	0.401960784	0.110871343
% BSA improvement	0.374852993	0.138201745
IL12B/IL-12p40	0.321078431	0.208580093
IL-2	0.318627451	0.212229312
% EASI improvement	0.306372549	0.23111252
IL-17A	0.296750516	0.247424926
IL-5	0.279411765	0.276432988
IL-23R	0.232843137	0.367008293
JAK1	-0.125	0.632167169
IL-16	-0.272058824	0.289700718

Table E12. Biomarker correlations with histologic score (Histoscore) at Day 29

Markers	Correlation	p-value
HistoSCORE	1	0
KRT16	0.941276115	$<10^{-5}$
S100A9	0.926595144	$<10^{-5}$
S100A8	0.901750423	$<10^{-5}$
S100A12	0.881422925	$<10^{-5}$
CXCL8/IL-8	0.876905703	$<10^{-5}$
JAK3	0.869000565	$<10^{-5}$
Thickness	0.866741954	$<10^{-5}$
S100A7	0.852060982	$<10^{-5}$
IL-4R	0.847543761	$<10^{-6}$
CXCL1	0.817052513	$<10^{-5}$
CCL18	0.808018069	$<10^{-5}$
IL-2RA	0.802371542	$<10^{-5}$
PI3/elafin	0.801242236	$<10^{-5}$
IL-32	0.795595709	$<10^{-4}$
MMP12	0.782044043	$<10^{-4}$
CCL22	0.777526821	$<10^{-4}$
IL-1B	0.753811406	$<10^{-4}$
FOXP3	0.731225296	0.00016787
CCL17	0.722190853	0.000221309
IL-15	0.710897798	0.000306961
IL-19	0.696216827	0.000457495
IL23A/IL-23p19	0.681535855	0.000664708

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CXCL10	0.642010164	0.00164165
IL-22	0.614906832	0.002848577
IL-10	0.609260305	0.003176244
IL-12RB1	0.608130999	0.003245425
IL12B/IL-12p40	0.574251835	0.00599385
PDE4B	0.566346697	0.006857686
CXCL9	0.552795031	0.008579094
CCL13	0.546019198	0.009565839
IL-15RA	0.542631282	0.010093403
STAT1	0.524562394	0.013330532
IL-6	0.521174478	0.014023552
CCL5	0.517786561	0.01474594
IL-13	0.513269339	0.015756418
IL-17C	0.506493506	0.017378081
IL-2	0.451157538	0.036396885
IL-17A	0.406635592	0.060374804
% BSA Improvement	0.361017007	0.098801315
CCL20	0.34839074	0.112506246
IFN-γ	0.33822699	0.123935335
STAT3	0.332580463	0.130641863
IL-9	0.299830604	0.174831965
CCL26	0.281761717	0.203272246
% EASI Improvement	0.248517378	0.264757082
IL-5	0.227067728	0.309521381
IL-23R	0.212874082	0.339917719
JAK1	0.172219085	0.441700268
IL-16	0.104460757	0.642617455

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Table E13. Treatment response biomarkers across different therapeutics

Pathway	Marker	ASN002		Cyclosporine A[1]		Narrow Band-UVB[2]	Dupilumab * 200mg[3]		Fezakinumab † IL-22 High[4]		GBR830 ‡ Two doses[5]		Ustekinumab[6] §		Triamcinolone acetonide cream 0.025%[7]	
		Wk2	Wk4	Wk2	Wk12	Wk12	Wk4	Wk16	Wk4	Wk12	Wk4	Wk10	Wk4	Wk16	Wk4	Wk16
General inflammation	MMP12	-8.02*	-8.6**	-4.8**	-11.7**	-9.6**	-6.5**	-20.1**	-1.9	-10.7***	-1.4	-3.8***	-2.8*	-3.2*	-2.2	-4.9
Th1	CXCL9	-2.65*	-2.8*	-5.0**	-5.7**				-1.5	-4.6***			-1.6	-1.8		-4.4**
	CXCL10	-2.4	-4.3*	-4.4**	-5.0**	-3.1	1	-1.1	-2.3	-8.1***	-1.6	-2.9***	-1.5	-2.2	-1.2	-4.6**
	IFNγ	-2.0	-2.6*	-1.8	-2.4		-1.1	1.0			-1.6	-2.1**				
	MX1	-2.6**	-3.6***	-1.9**	-2.2**	-0.5			-1.7*	-2.1***			-1.7*	-1.7*		
	STAT1	-1.6*	-1.9**	-1.6**	-1.9**		-1.6*	-2.2***	-1.1	-1.2				-1.4		-1.8*
Th2	IL-13	-4.8**	-5.1***	-6.6**	-18.6**	-4.0**	-1.31	-2.3**	-1.01	-1.6	1.1	-1.9*			-1.1	-2.4
	CCL26	-1.8	-2.1*	-1	-1.5		-5.3**	-6.7**	1.3	-1.2	-1.3	-1.4*				
	CCL22	-2.3	-3.6**	-2.3**	-2.3**	-2.0**	-1.6*	-2.4**	-1.6	-2.9***			-2.0*	-1.5		
	CCL18	-2.2*	-3.4***	-3.3**	-6.1**	-6.3**	-6.6**	-9.9**	-1.7*	-3.0***	-1.34	-1.6***			-1.3	-4.6
	CCL17	-3.8*	-4.4**	-5.1**	-4.4**	-2.9**	-2.6*	-2.9**	-1.5	-3.3***	-1.6	-3.0***	-3.1*	-3.4**	-1.5	-3.2
	CCL13	-2.5**	-2.7***	-2.5*	-2.0*		-2.9	-4.0**	-1.7*	-2.3***						
	IL-31	-6.6**	-4.2*	1.3	1.0		-1.3	-1.4*			-1.3	-2.3*				
Th17	IL-17A	2.8	-1.1	-1.5	-1.9*	-1.1	-1.3	-1.9**	1.0	1.1	-1.3	-1.2				
	IL-19	-1.8	-8.5*	-7.3**	-13.3**											
	PI3	-3.6	-4.5*	-5.3**	-8.4**	-3.5	-3.9**	-6.8**	-3.0***	-5.4			-2.1*	-2.3*		-3.6**
	IL23p19	-1.5	-3.8**	1.1	-1.1		-1.2	-1.1			-1.1	-1.3*				
	IL23p40	-2.0	-2.5		-4.3**	-1.4*	-1.1	-1.6	-1	-1.5*	-1.3	-1.6*			-1.1	-2.6
Th22	IL-22	-4.7*	-5.4**	-4.7**	-10.7**	-2.8*	-2.2*		-1.5	-4.43**	-1.3	-2.6***	-2.2*	-1.3	-1.4	-4.3**
Th17/Th22	S100A7	-3.9*	-7.6***	-3.8**	-7.8**	-3	-2.3*	-2.1*	-3.8***	-2.6***					-2.0	-7.3**
	S100A8	-5.5*	-11.1***	-3.6**	-6.8**	-1.8	-3.6*	-7.7*	-1.5*	-3.3***			-1.5	-1.3	-2.9	-10.9**
	S100A9	-6.2*	-10.3***	-5.4**	-9.5**	-4.6	-3.3***	-10.0***	-5.3*	-22.1***	-1.8***	-3.5***	-2.5	-2.2	-2.6	-9.4**
	S100A12	-3.5	-11.0**	-6.7**	-22.2**		-3.6**	-8.9*	-3.4**	-6.0***	-2.1***	-4.9***	-2.7*	-3.6**	-2.2	-10.5**
Hyperplassia	K16	-4.8*	-10.1***	-4.0**	-6.1**	-2.8*	-4.2**	-6.1*	-2.3***	-2.85	-1.5**	-3.3***	-2.2**	-2.5**	-2.5	-5.4**
	Thickness	-1.5	-1.5*	-1.3***	-1.8***	-2.5***	-1.3	-1.5***	-1.2	-1.2	-1.2*	-1.3*	-1.2	-1.2	1.0	-1.1
Epidermal Differentiation	FLG	1.2	1.3	1.7*	1.1		1.5*	1.7*	1.1	1.2						
	LOR	1.4	1.5	1.8	1.3	1.1	1.8**	2.1**	1.2	1.4*						
	CLDN23	1.6	2.3*	1.4	1.9**		1.7**	2.4***	1.3	1.6**			1.7*	1.7*		

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* Dupilumab is a monoclonal antibody targeting IL-4 and IL-13

† Fezakinumab is a monoclonal antibody targeting IL-22

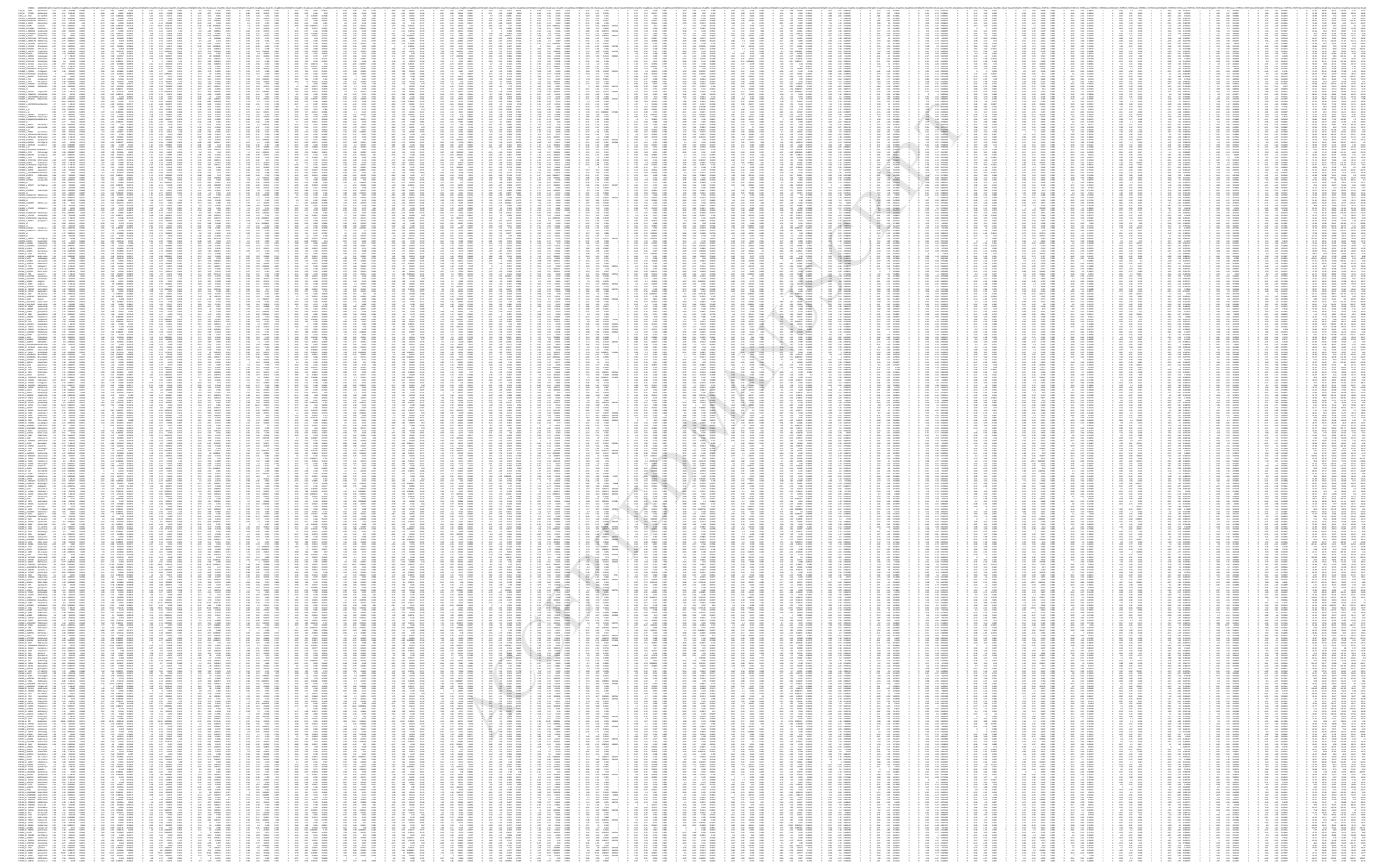
‡ GBR830 is a monoclonal antibody targeting OX40

§ Ustekinumab is a monoclonal antibody targets IL-12-23 p40

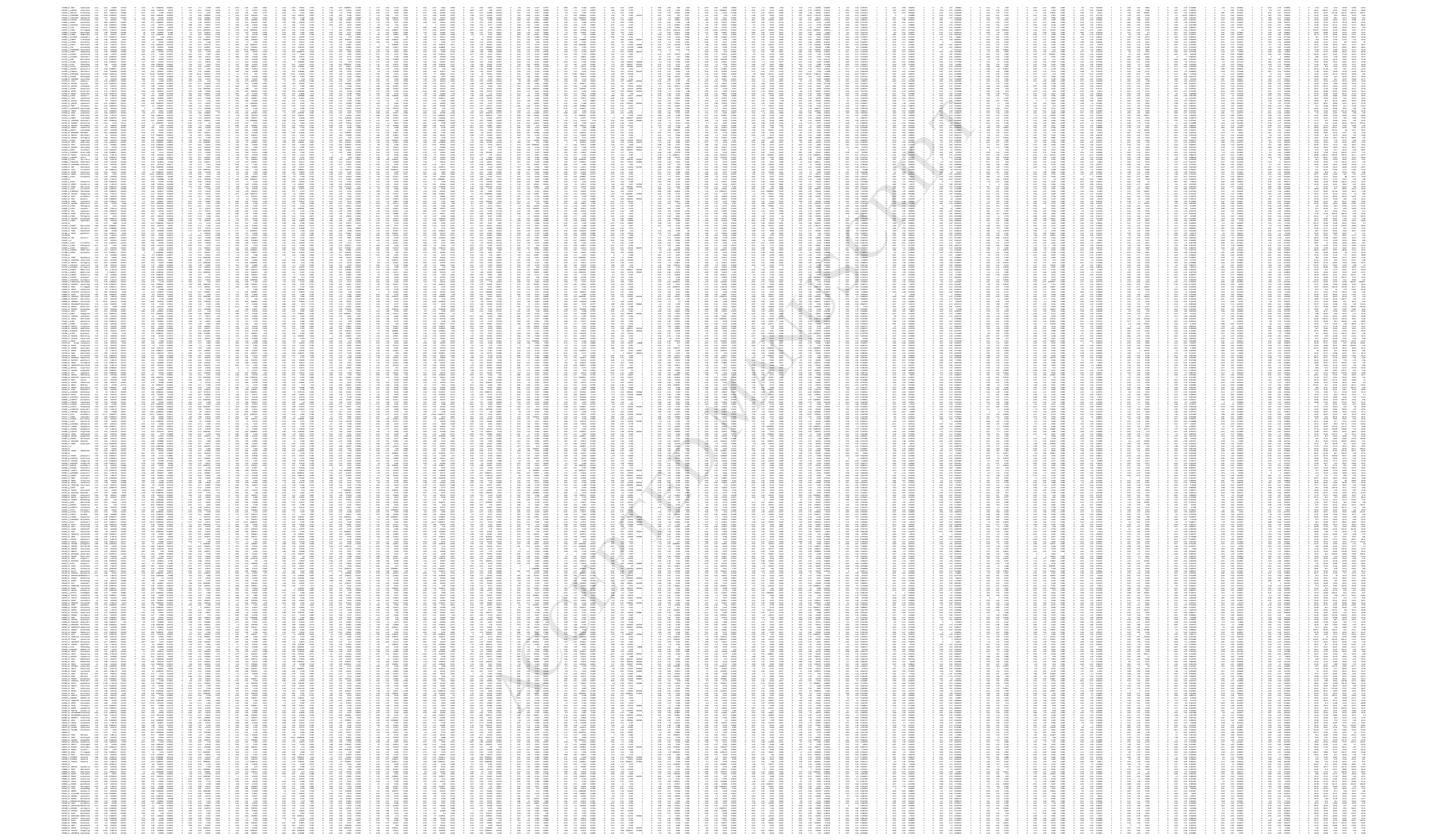
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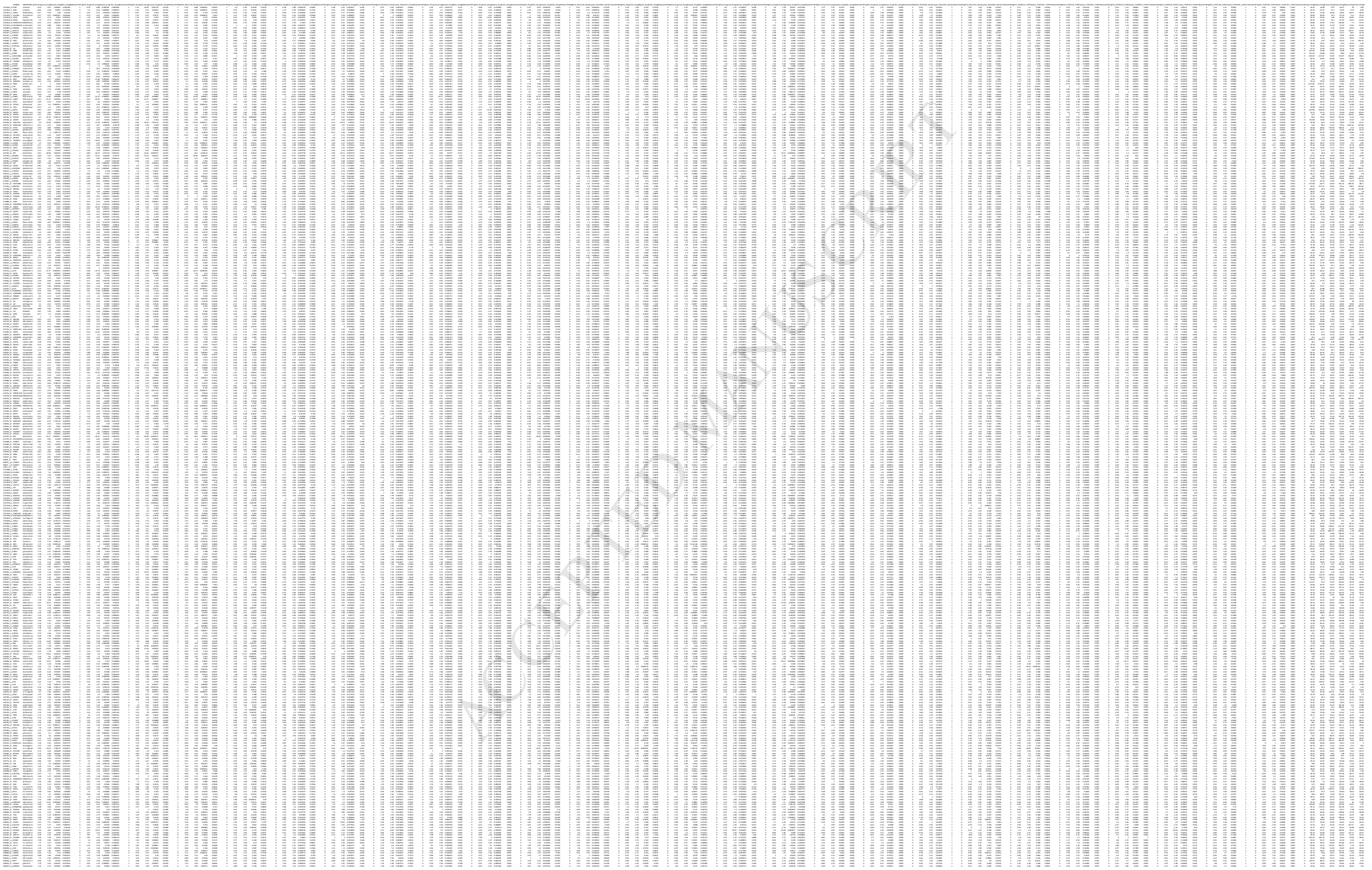
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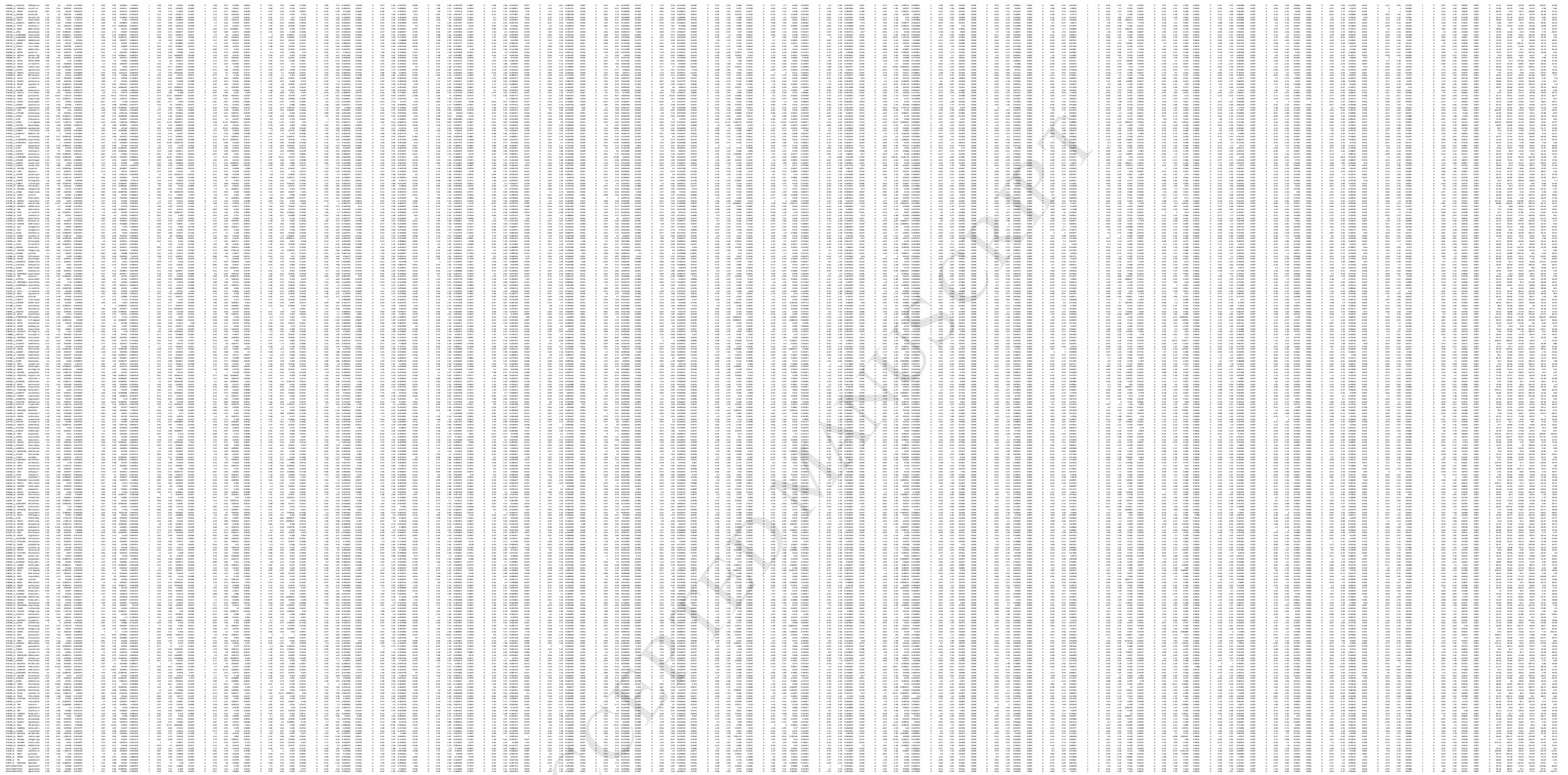


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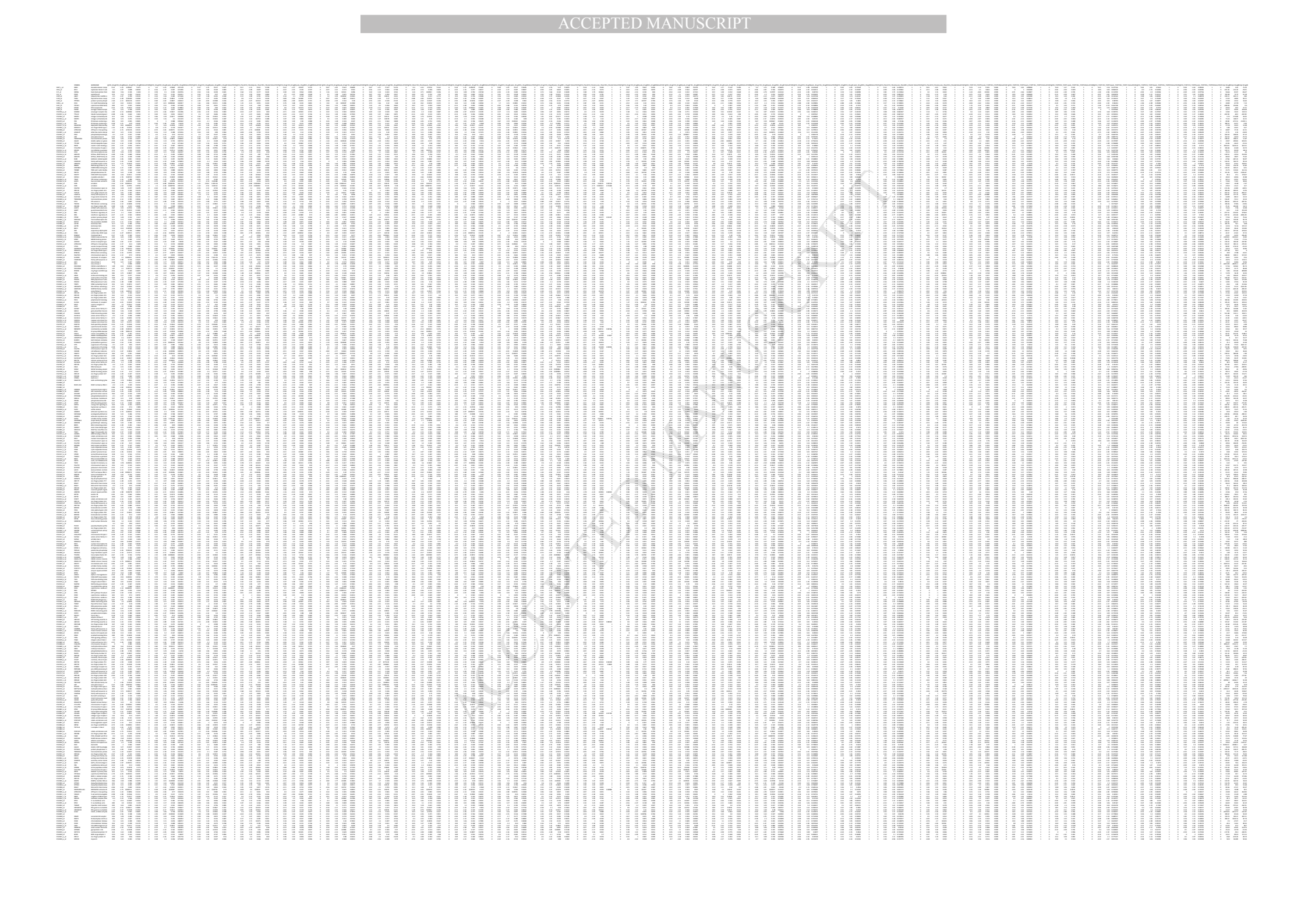
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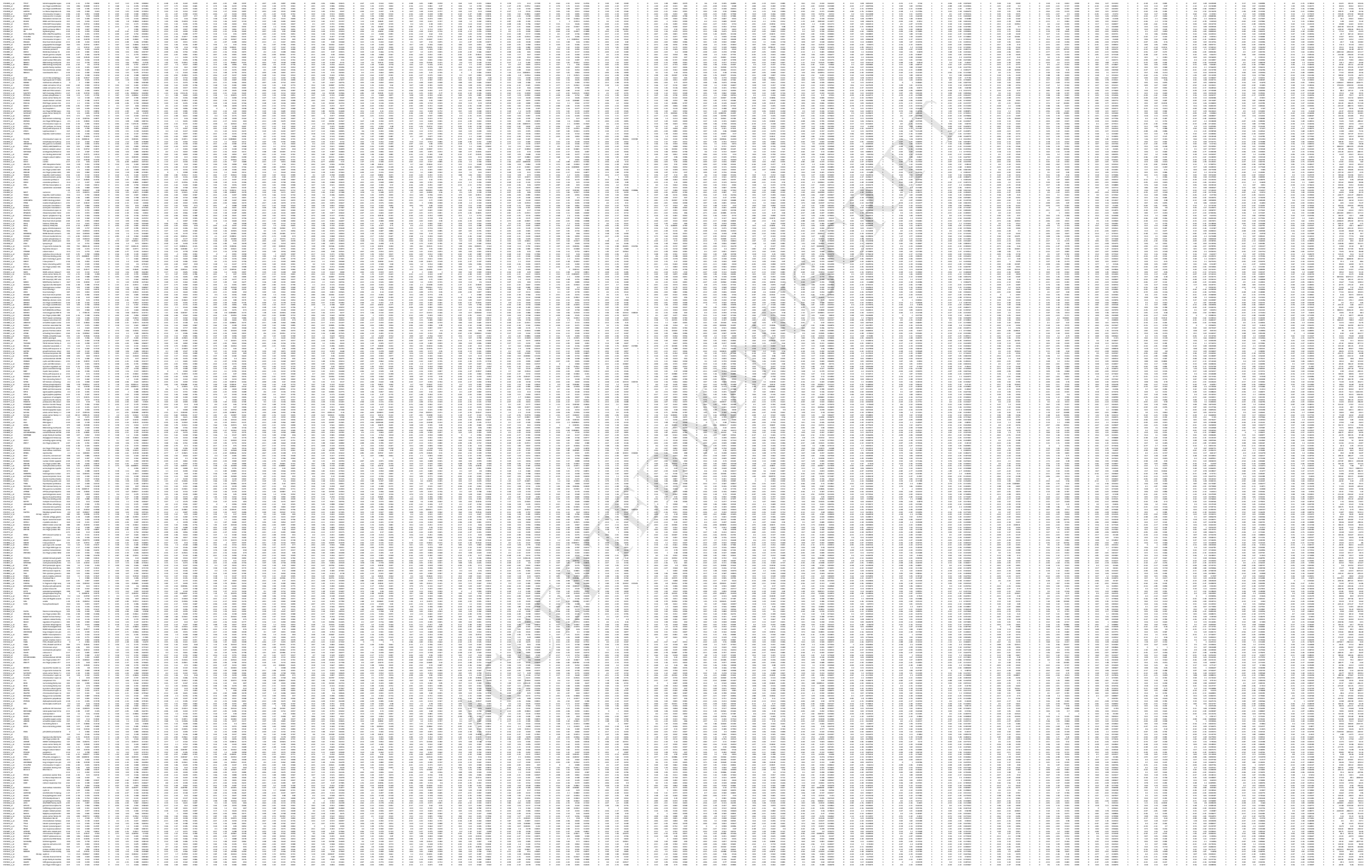


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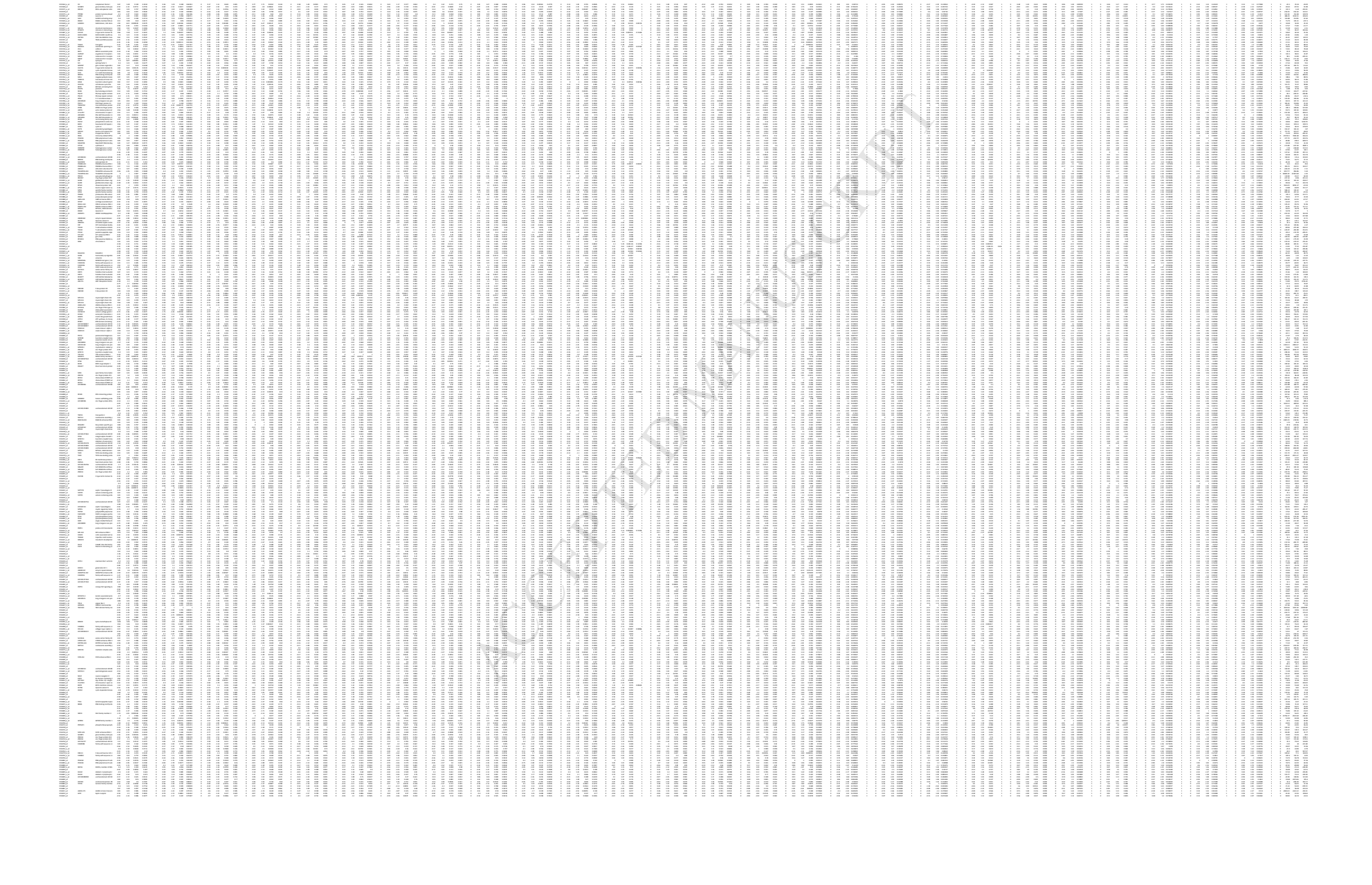
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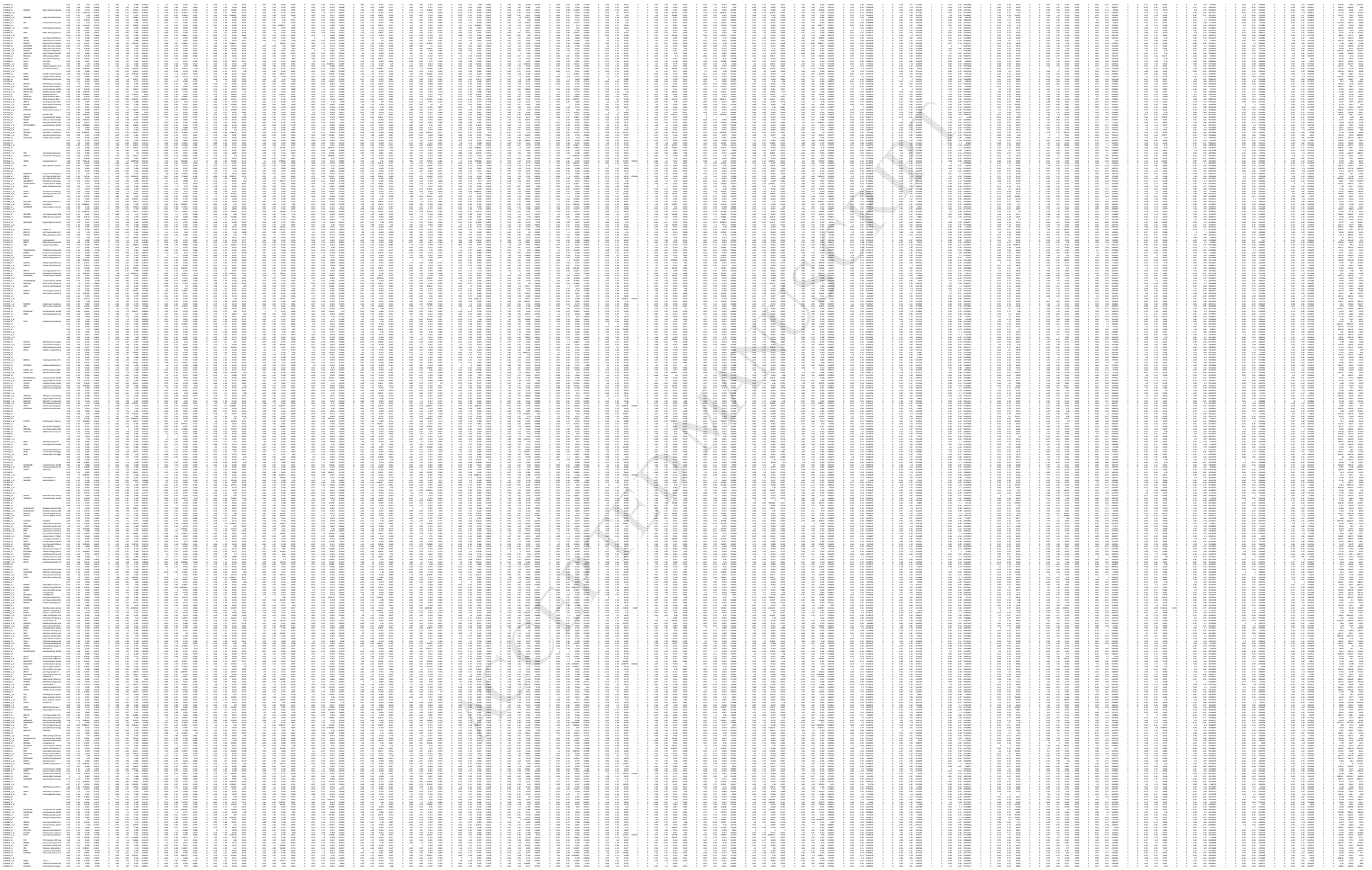
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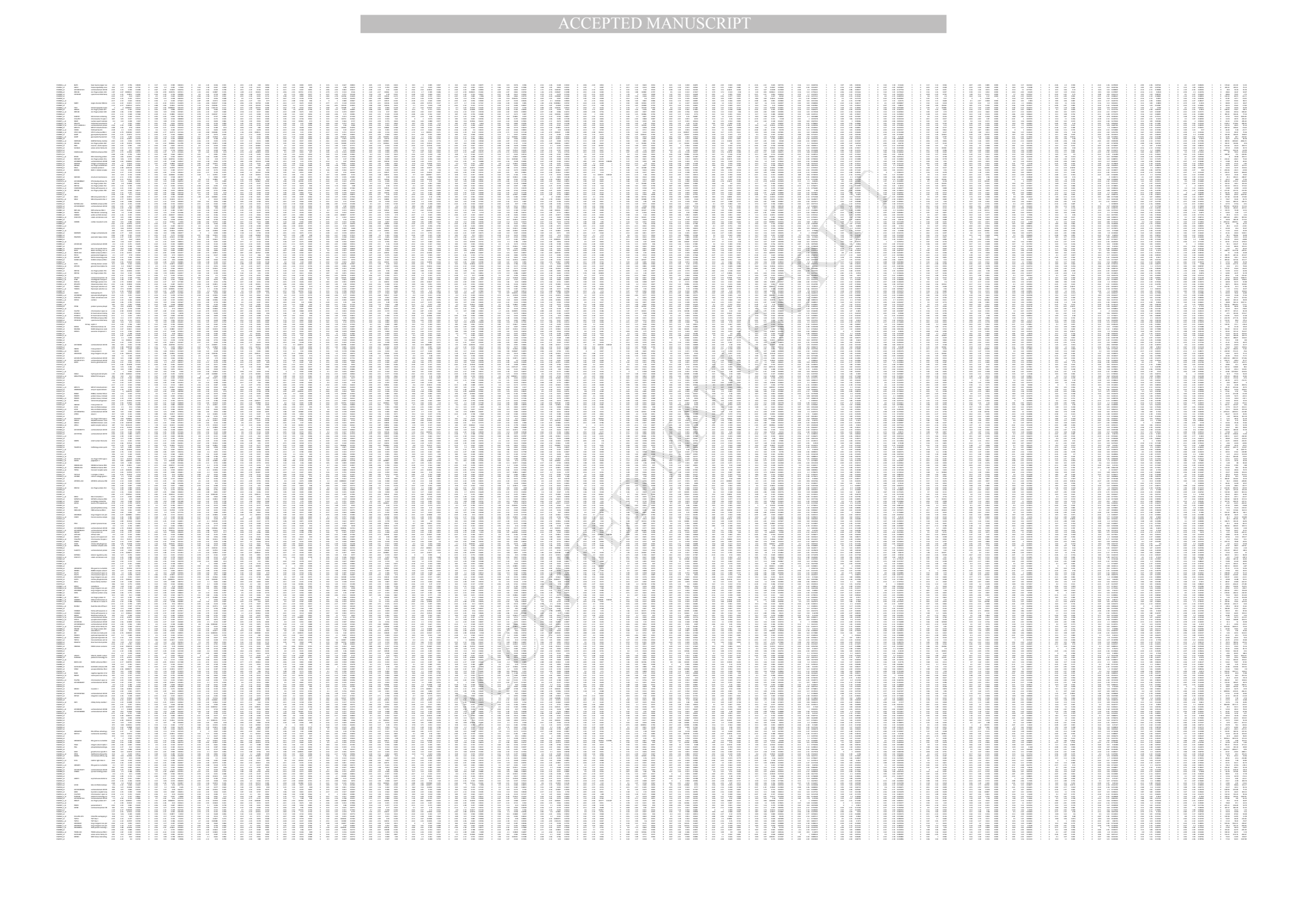
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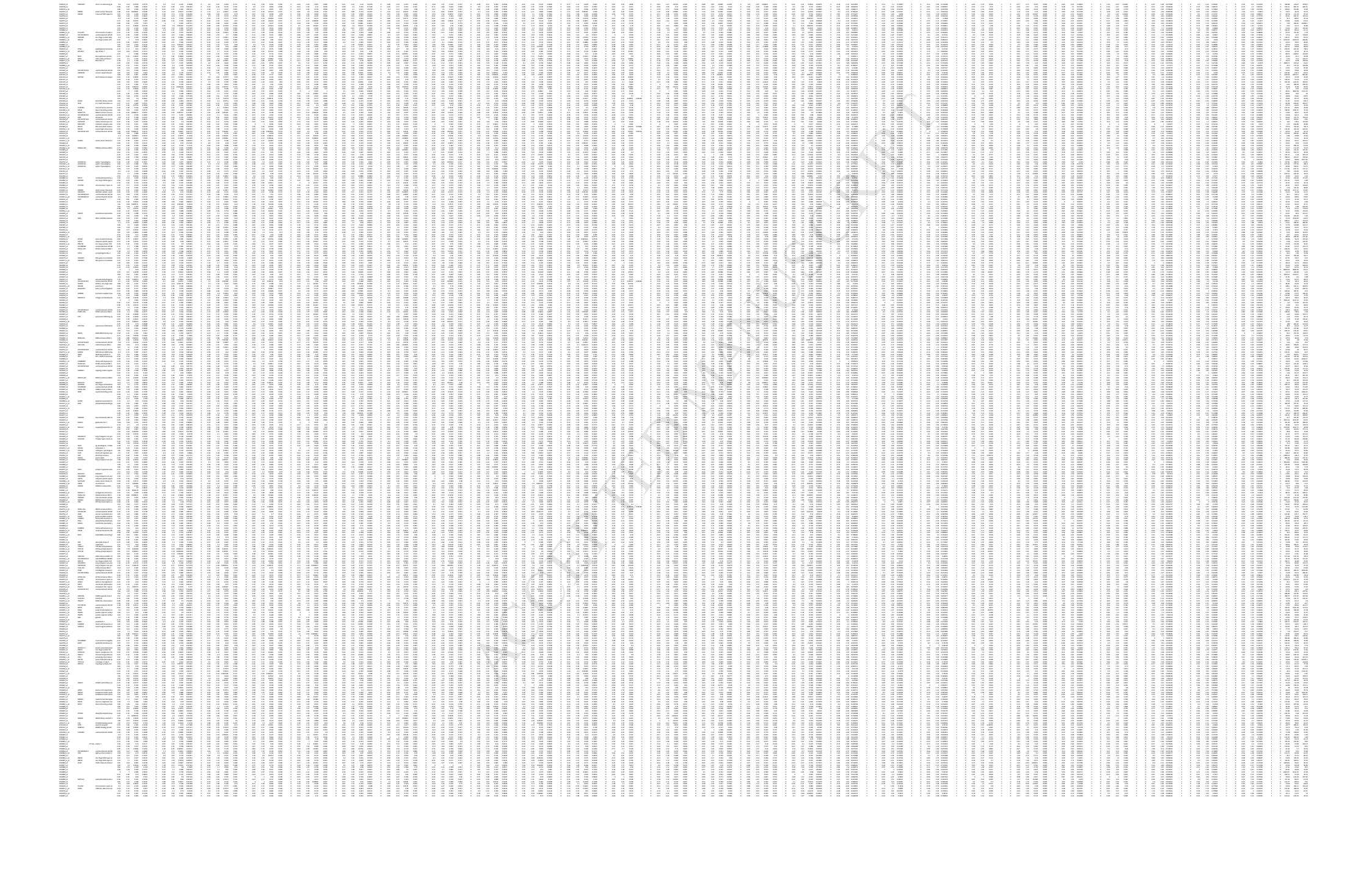
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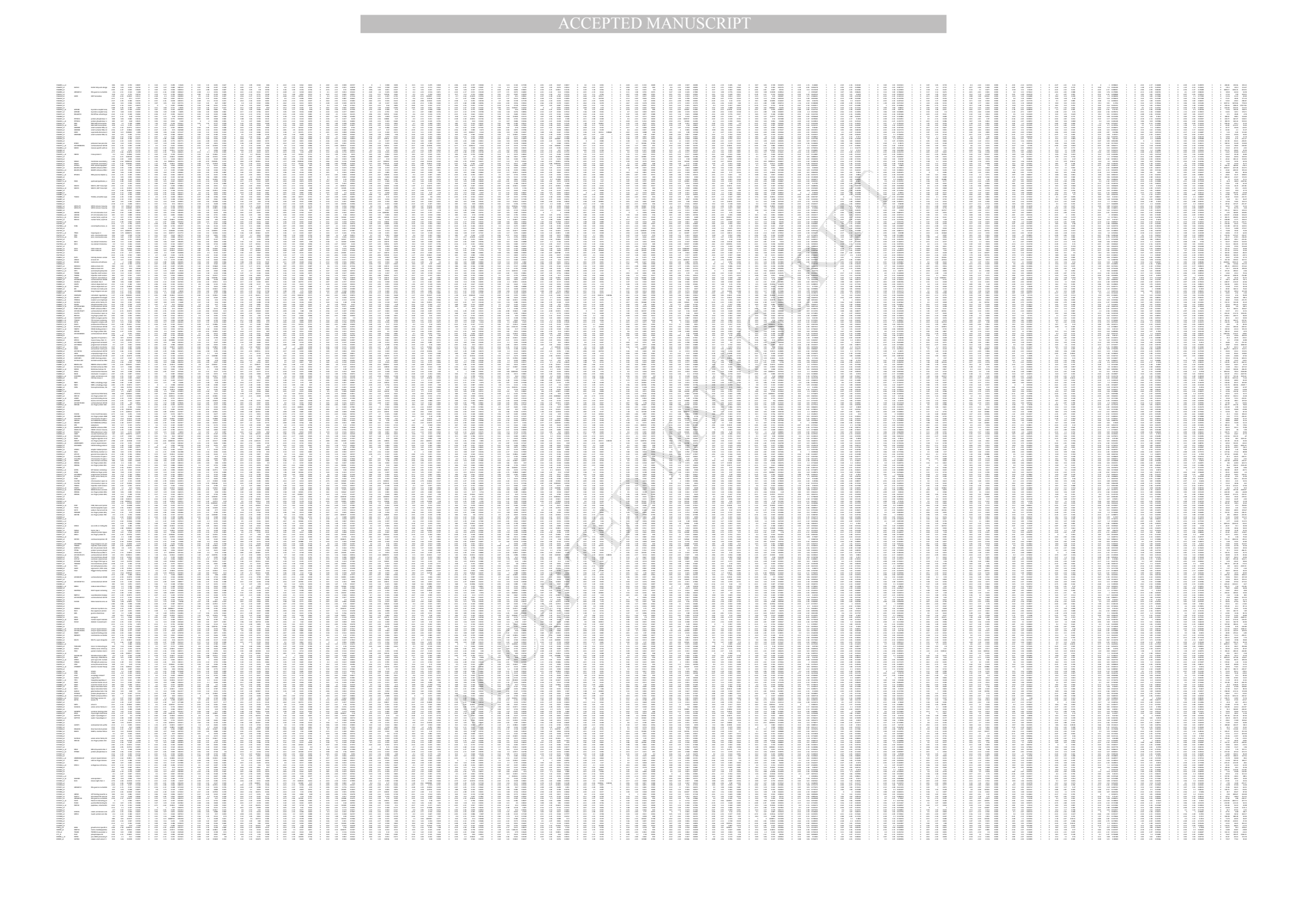
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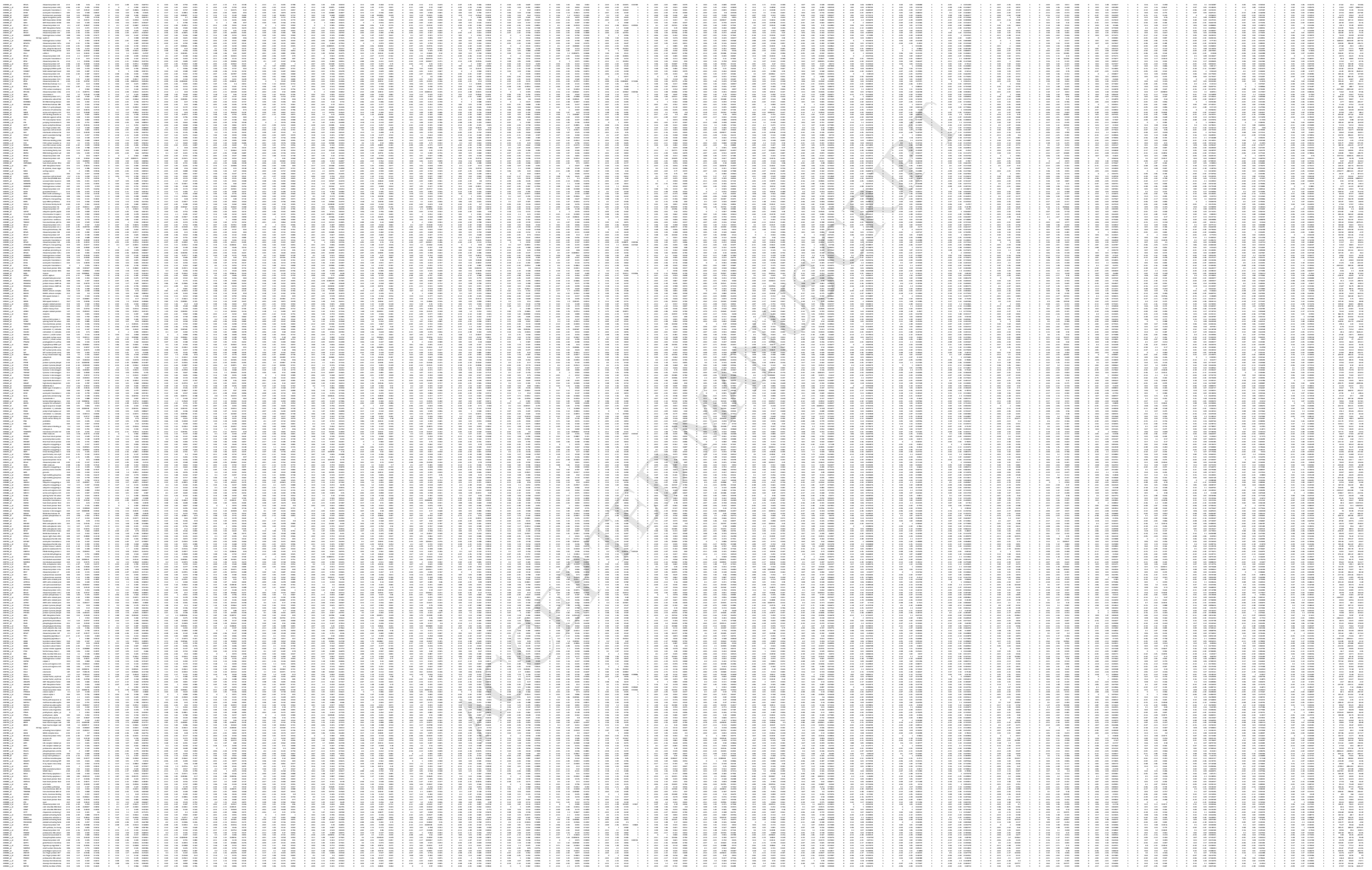
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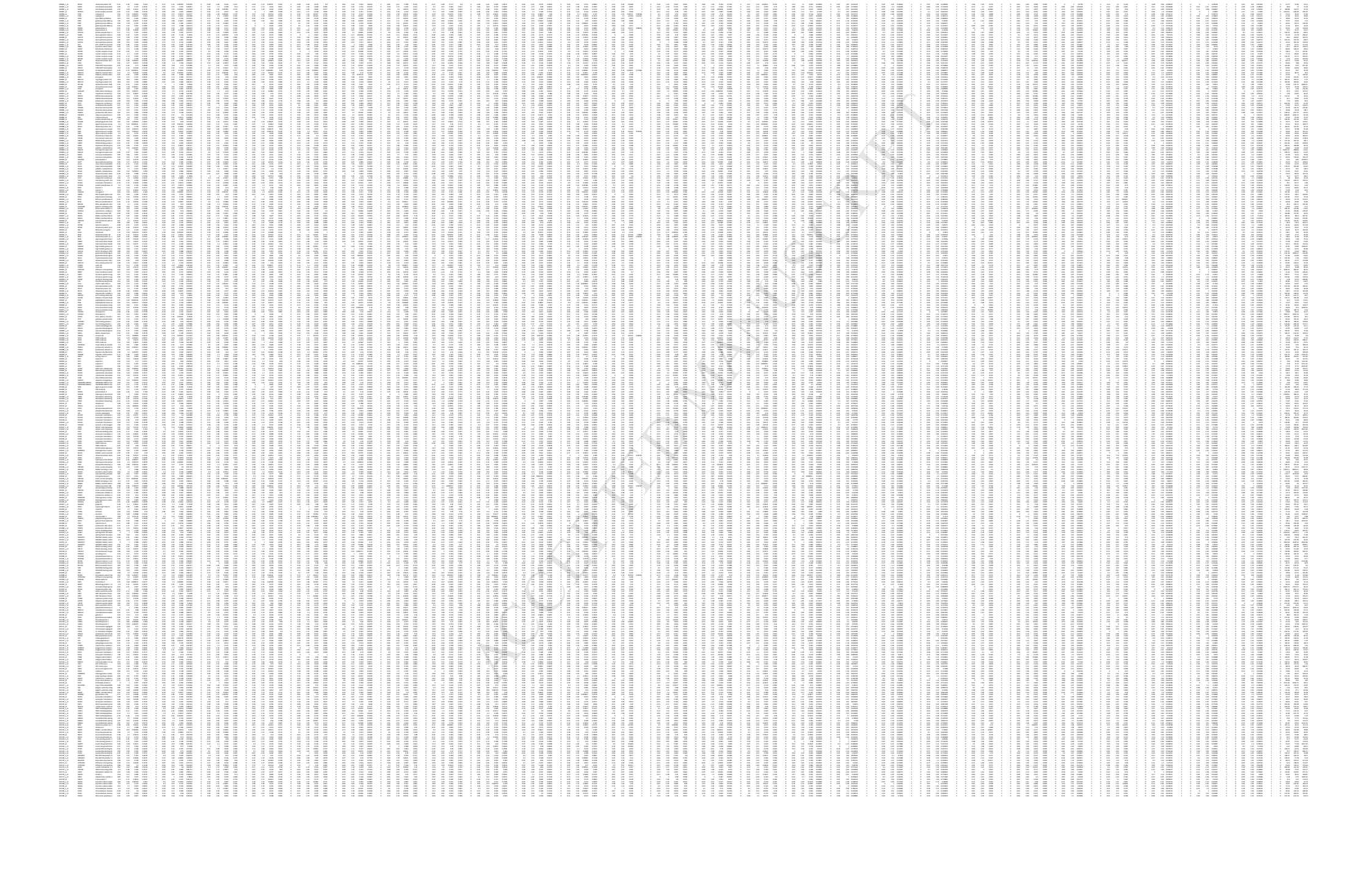
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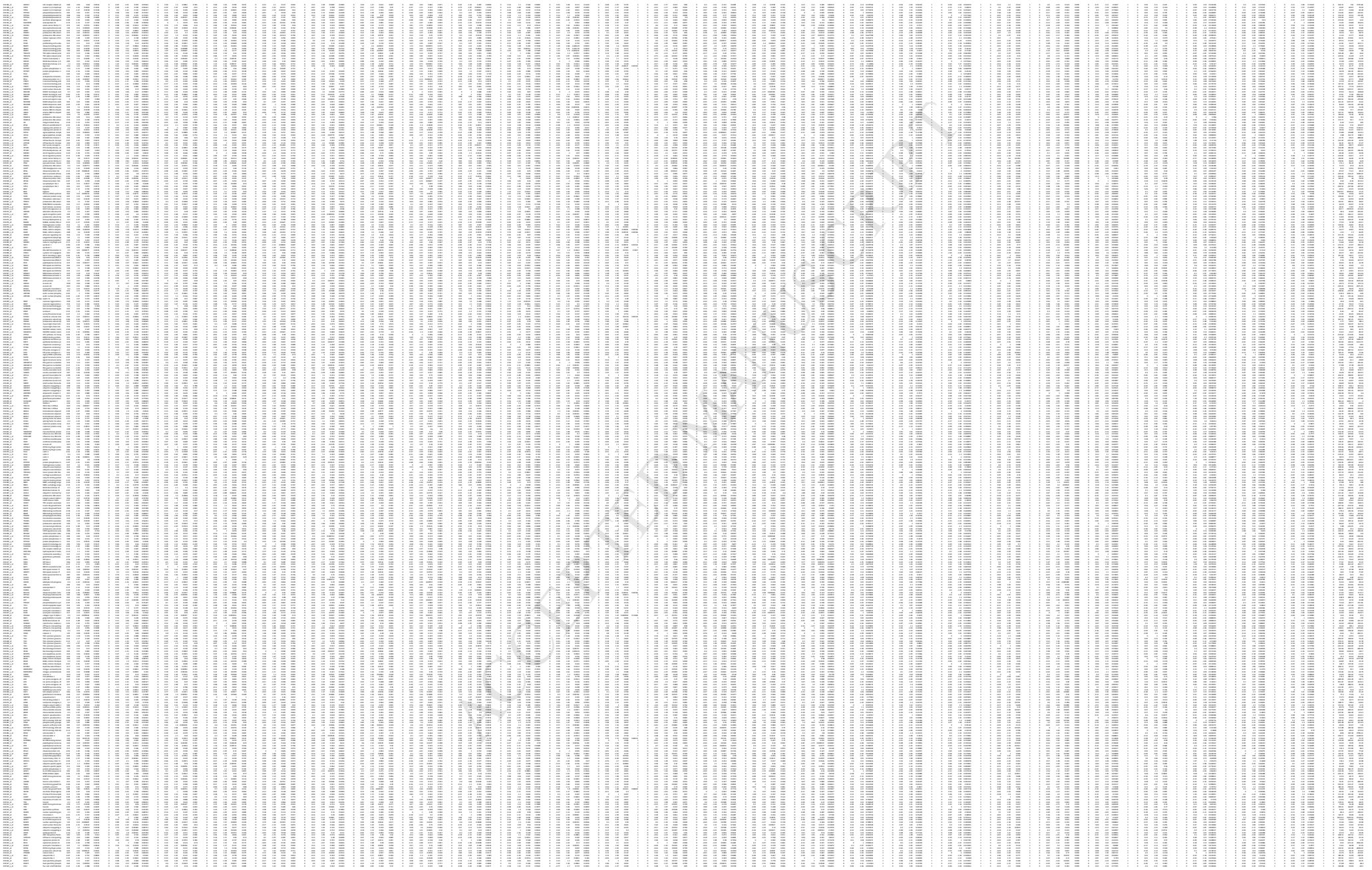
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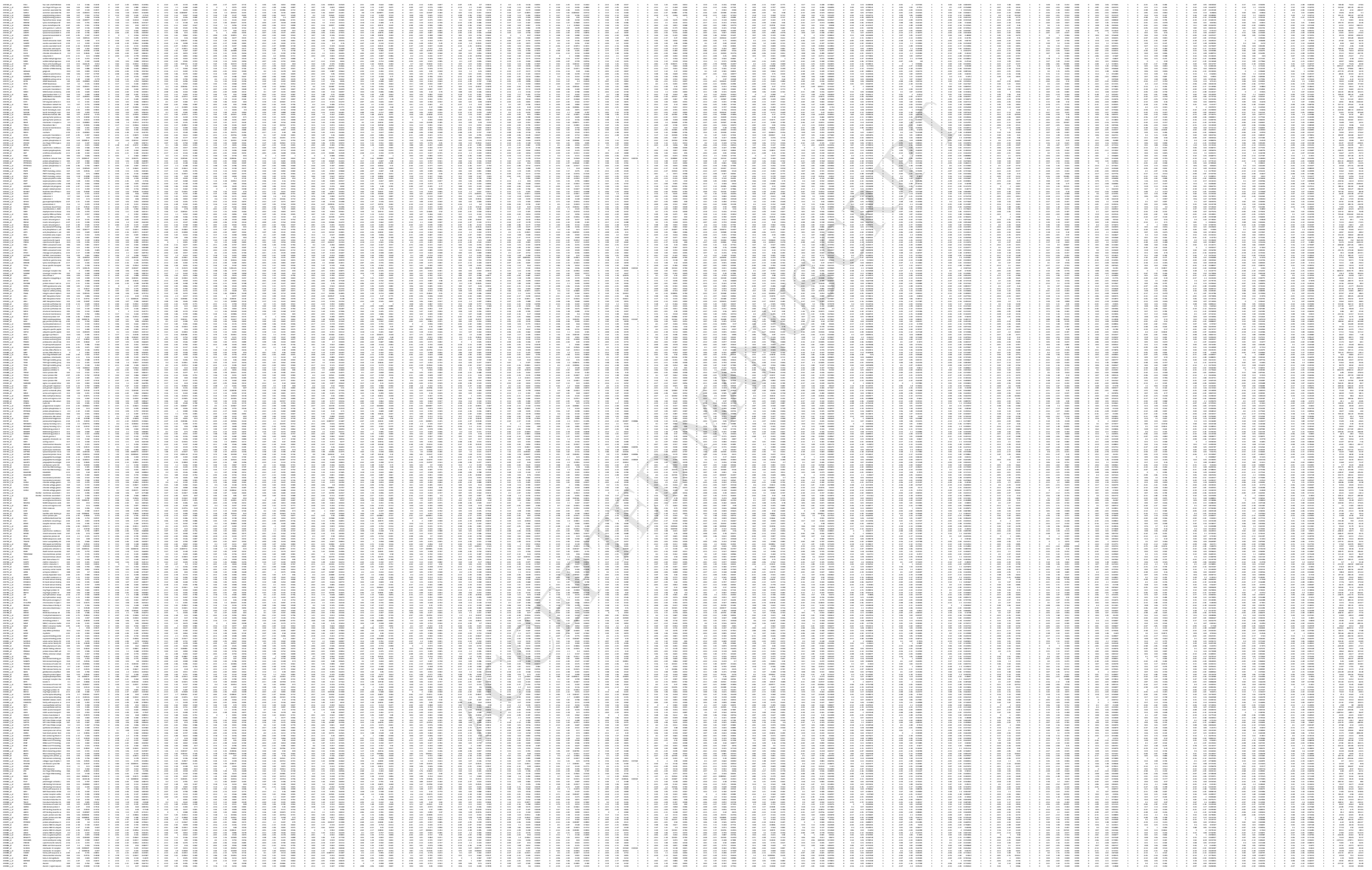
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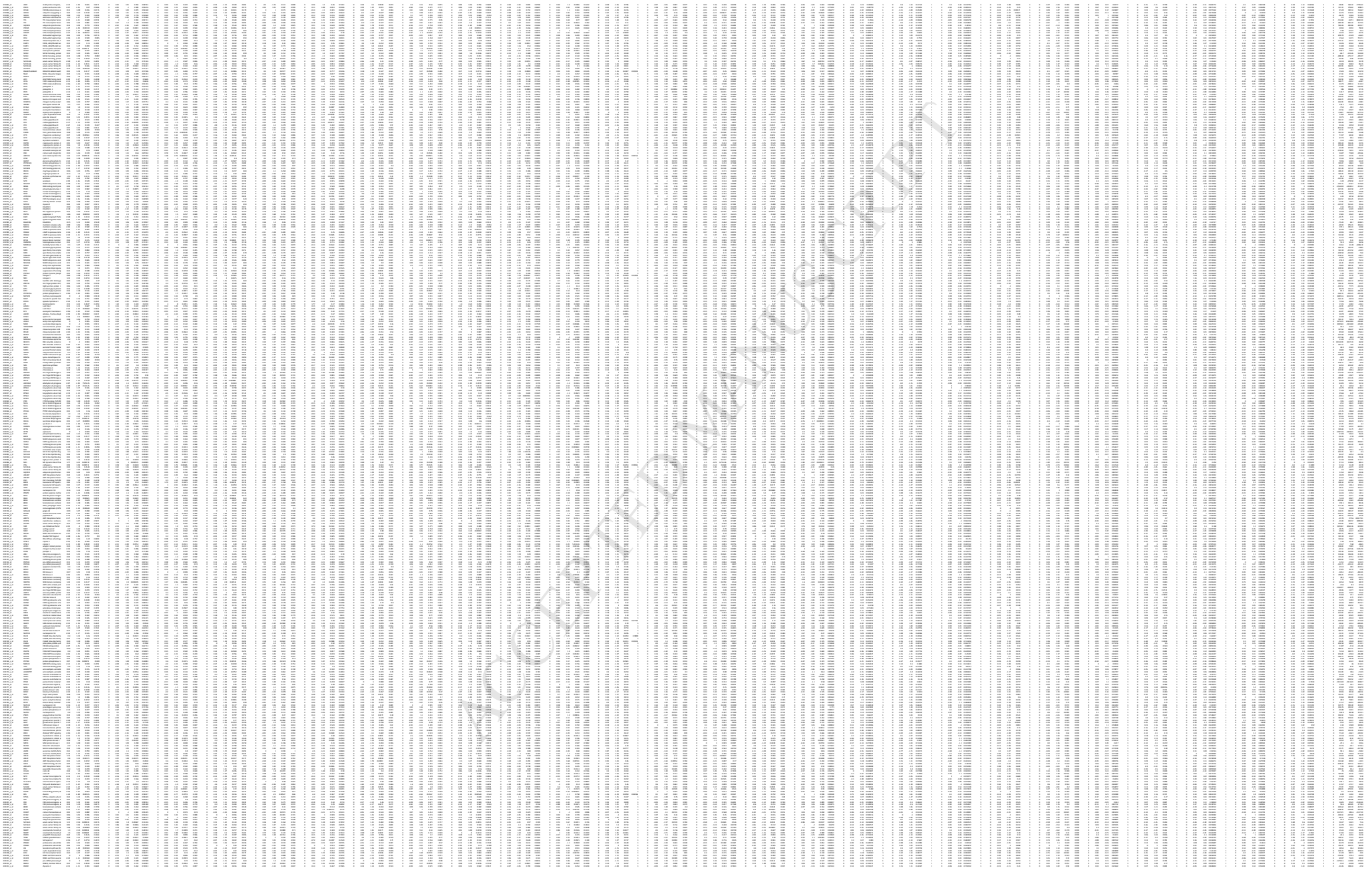
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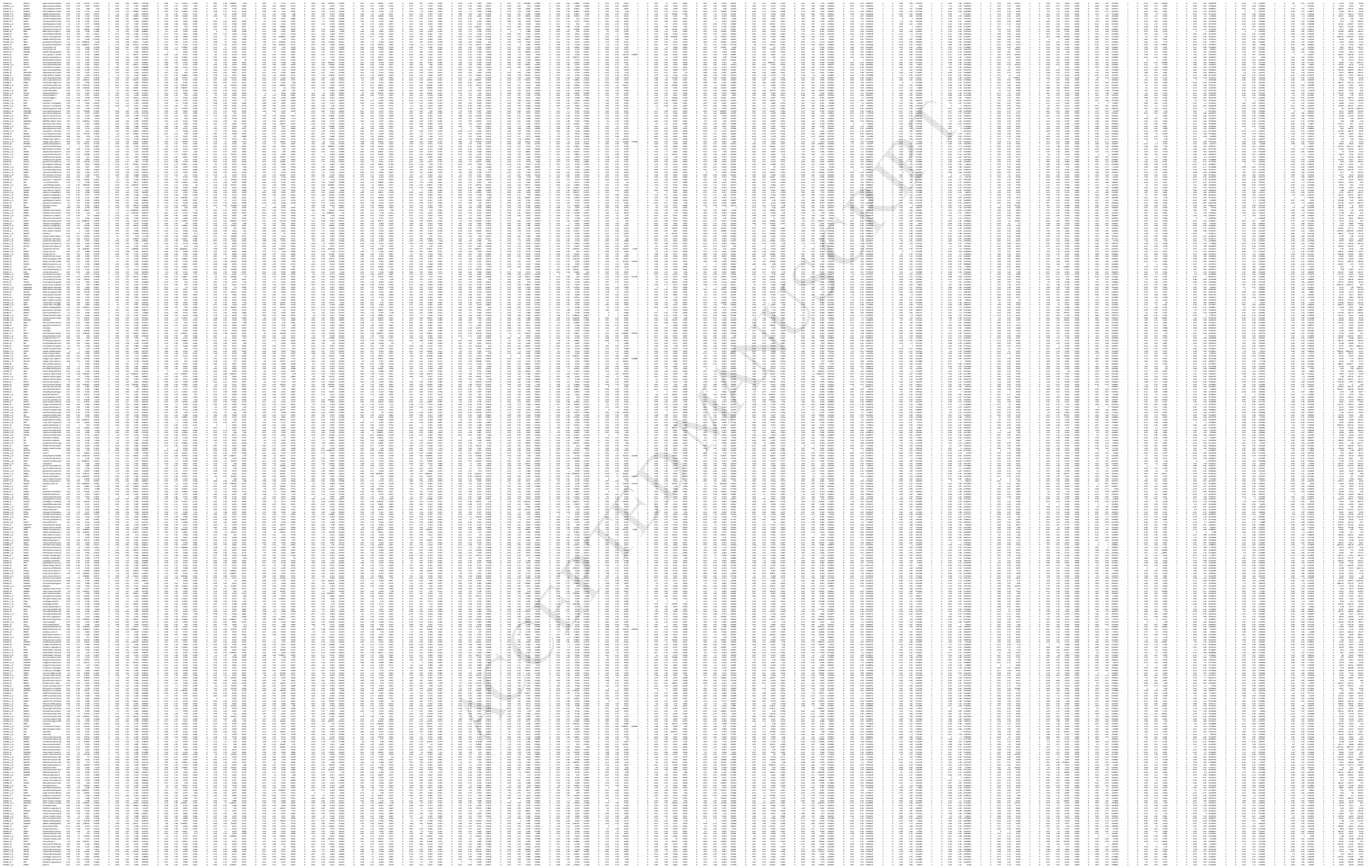
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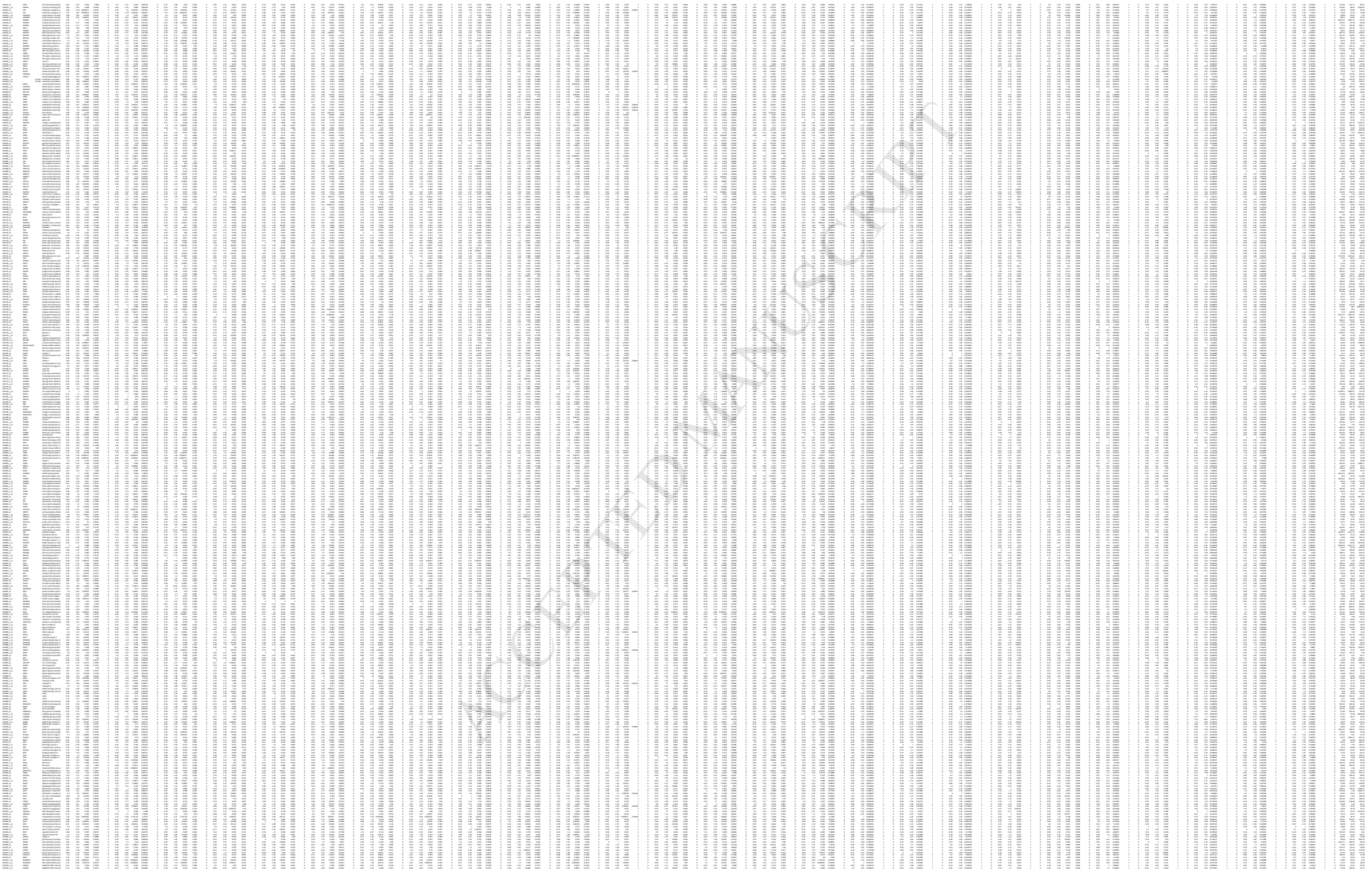
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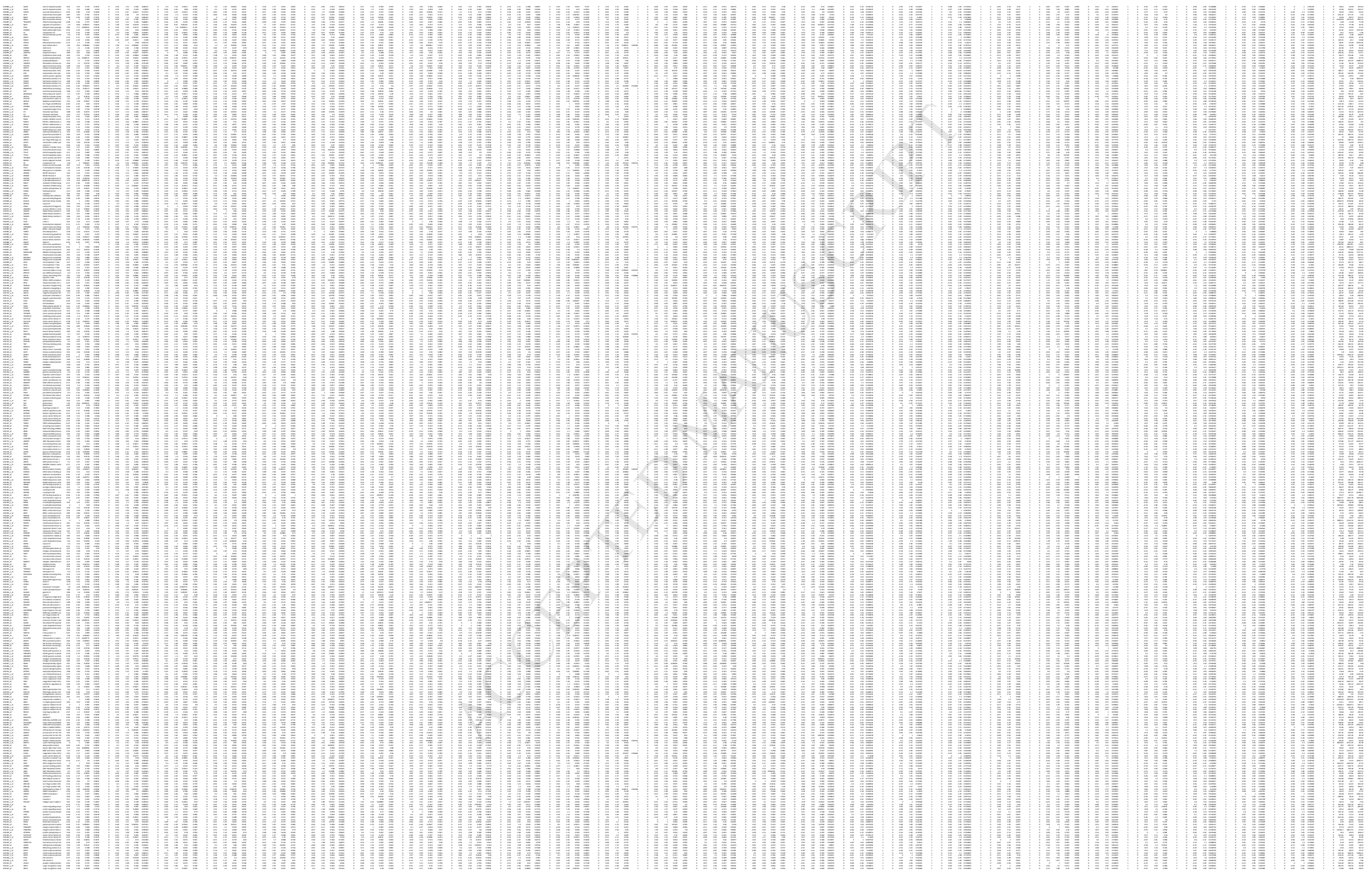
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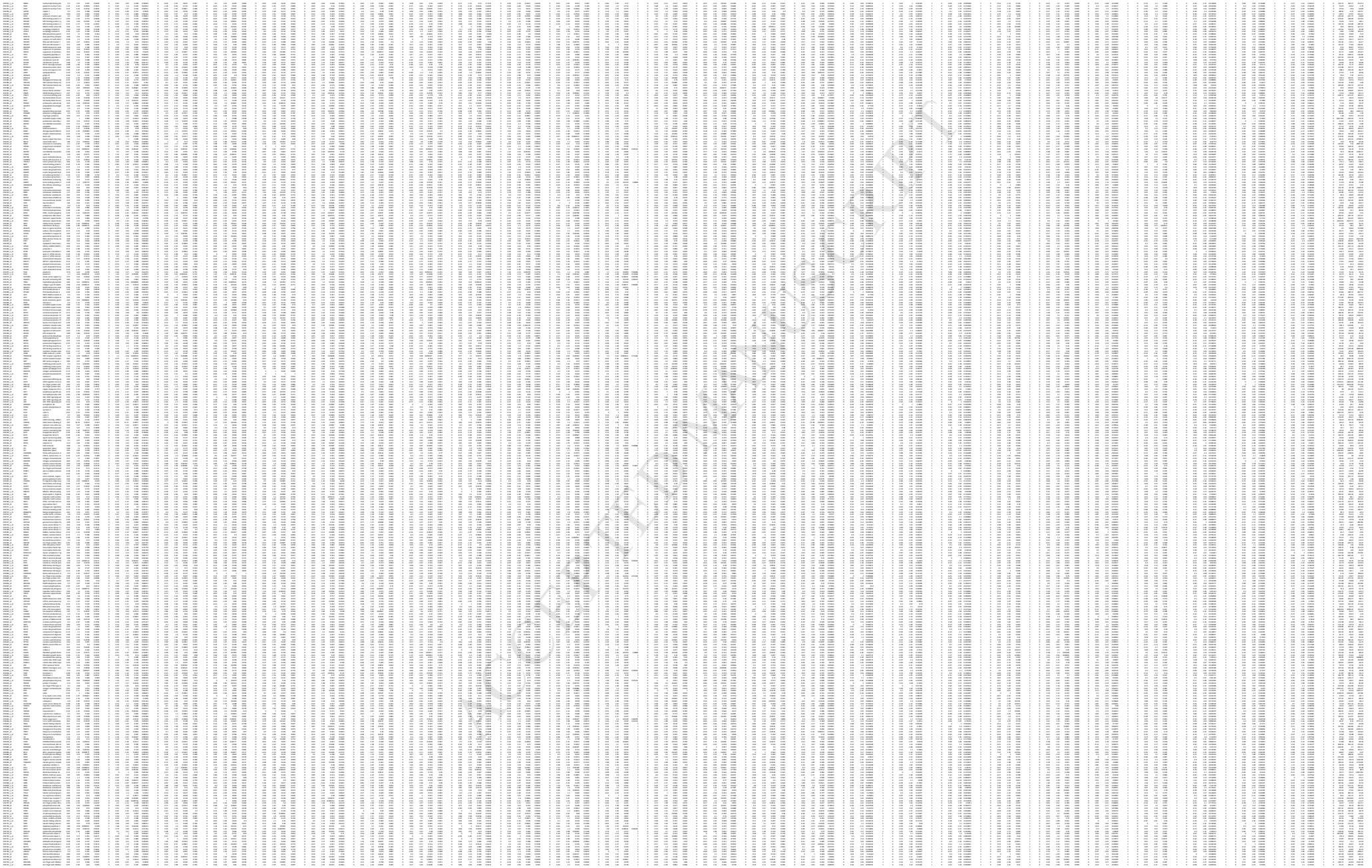
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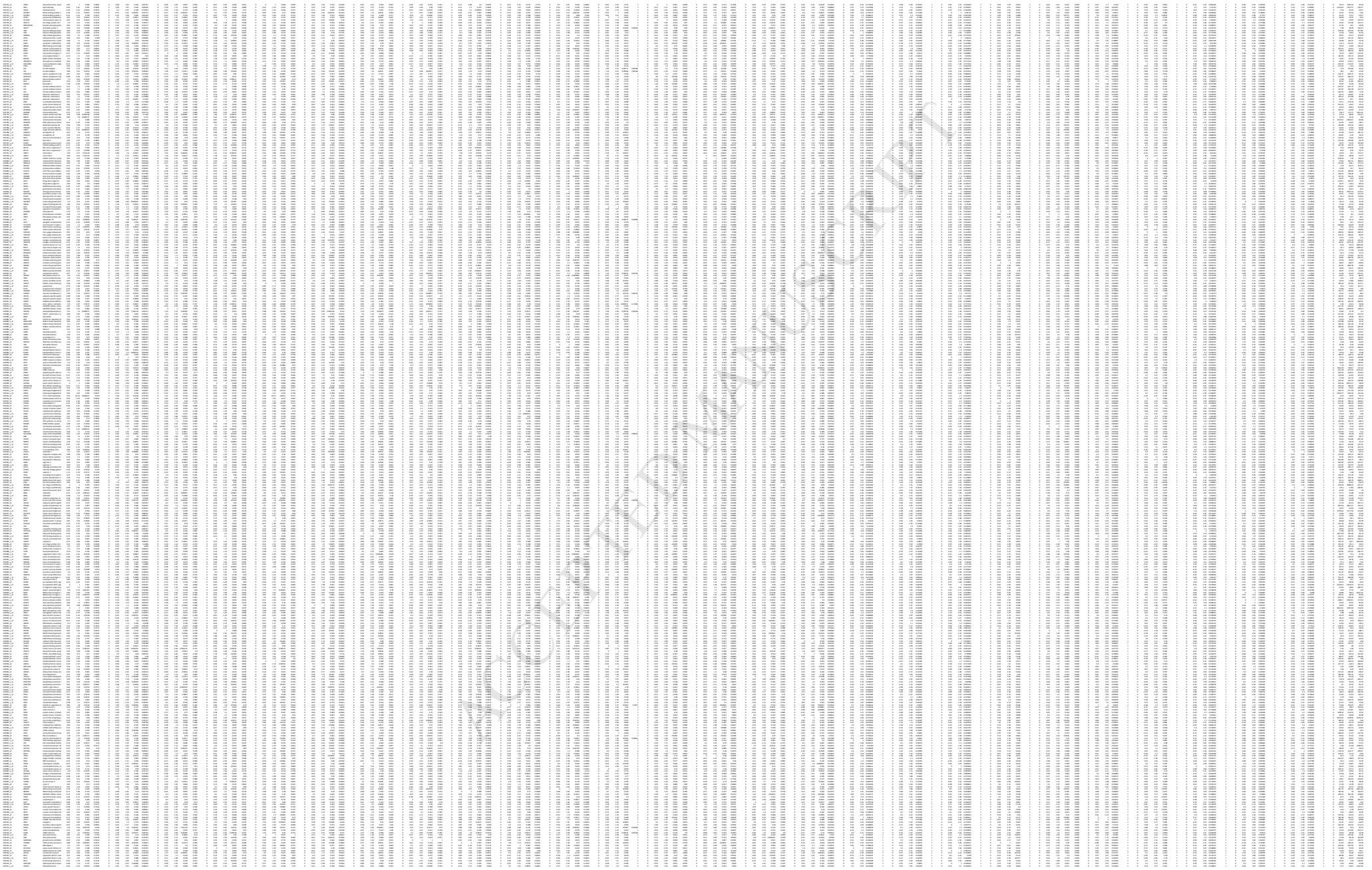
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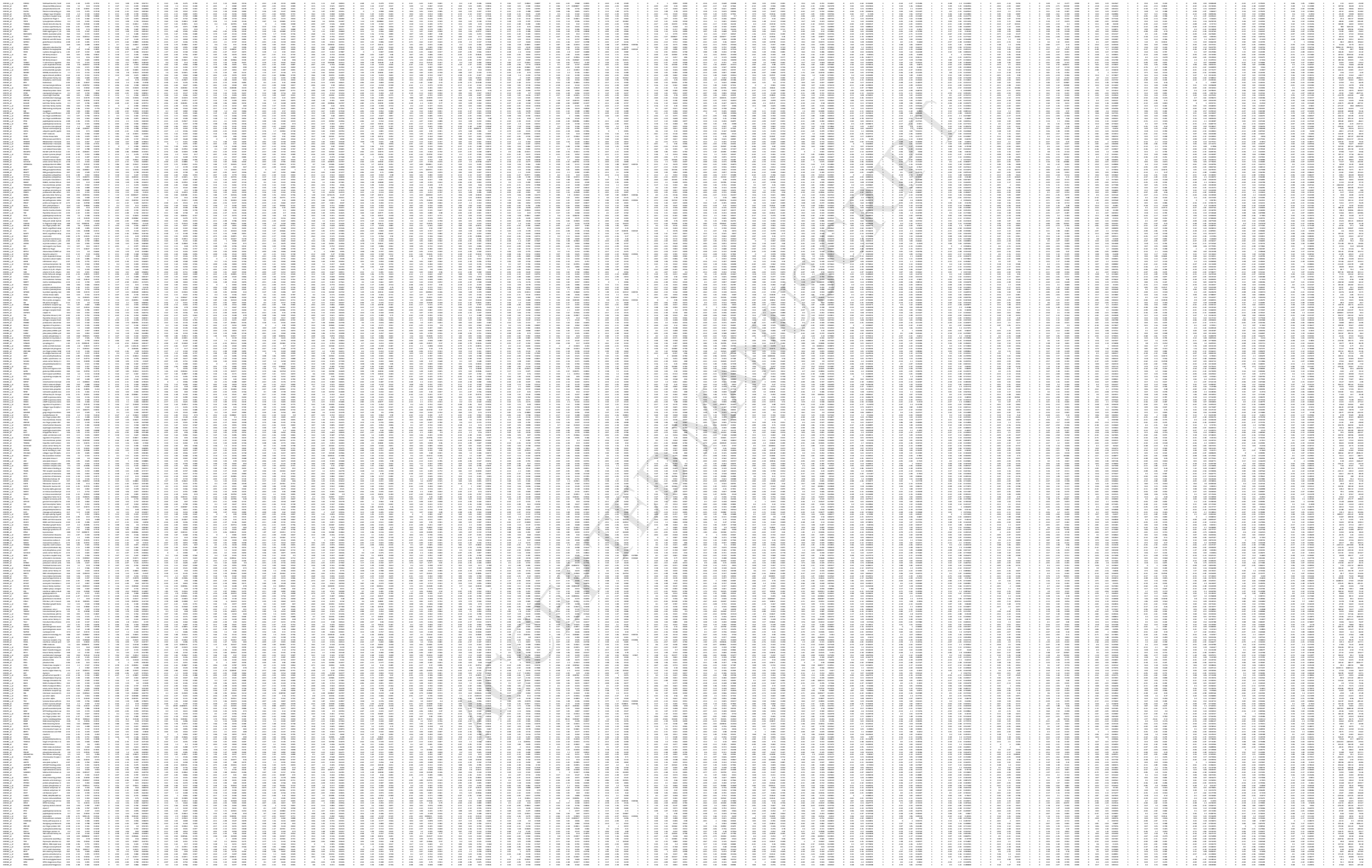
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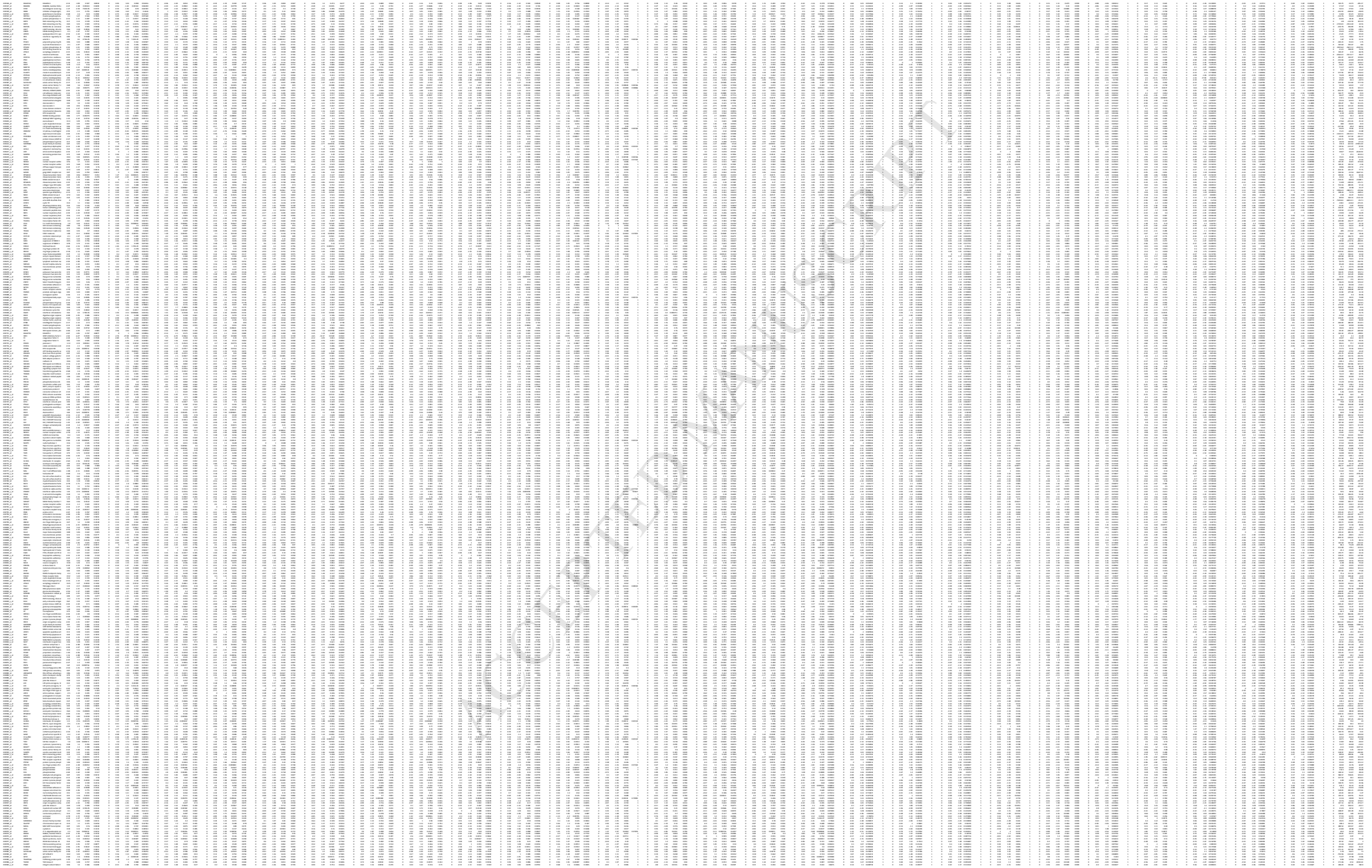
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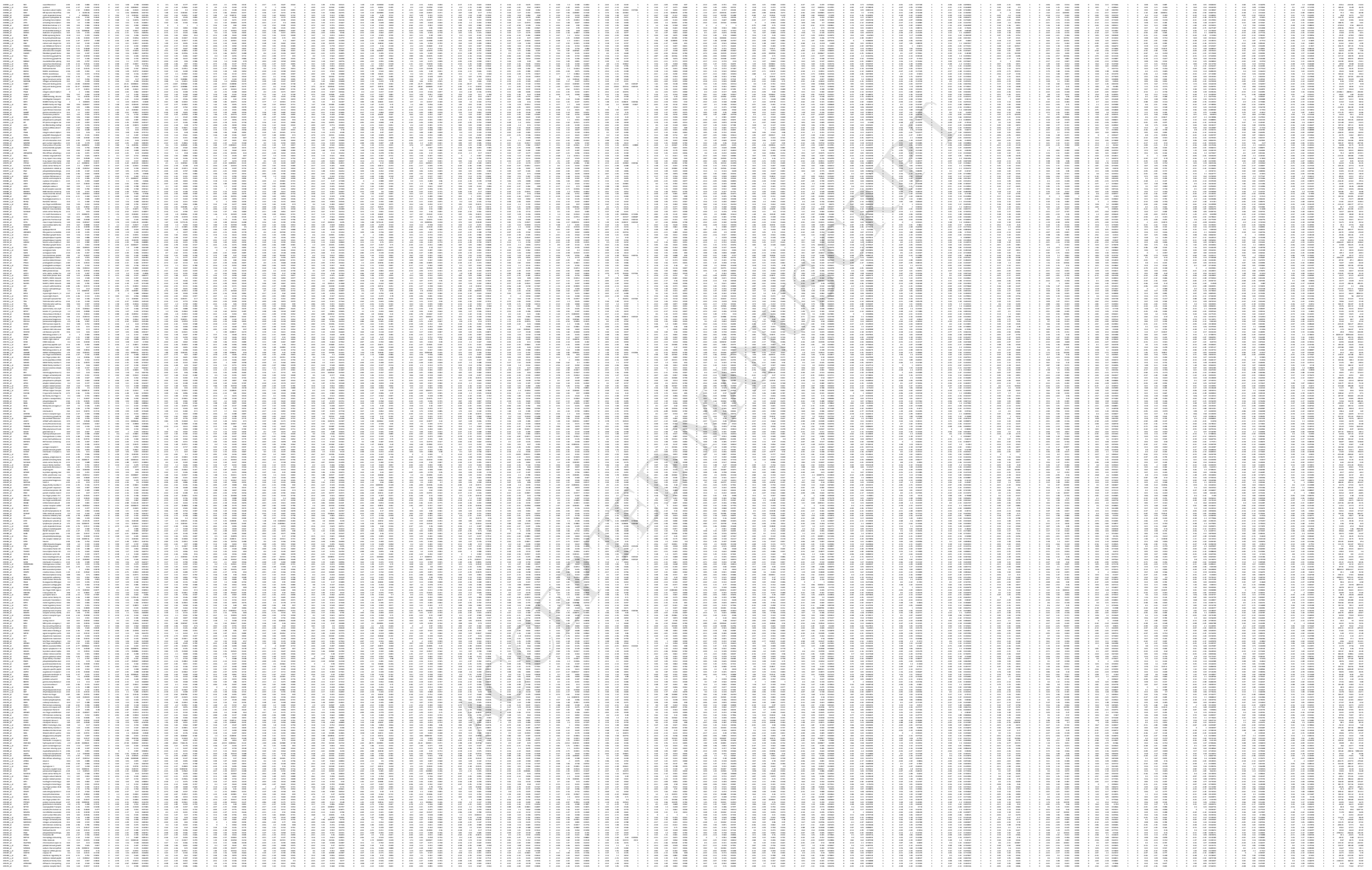
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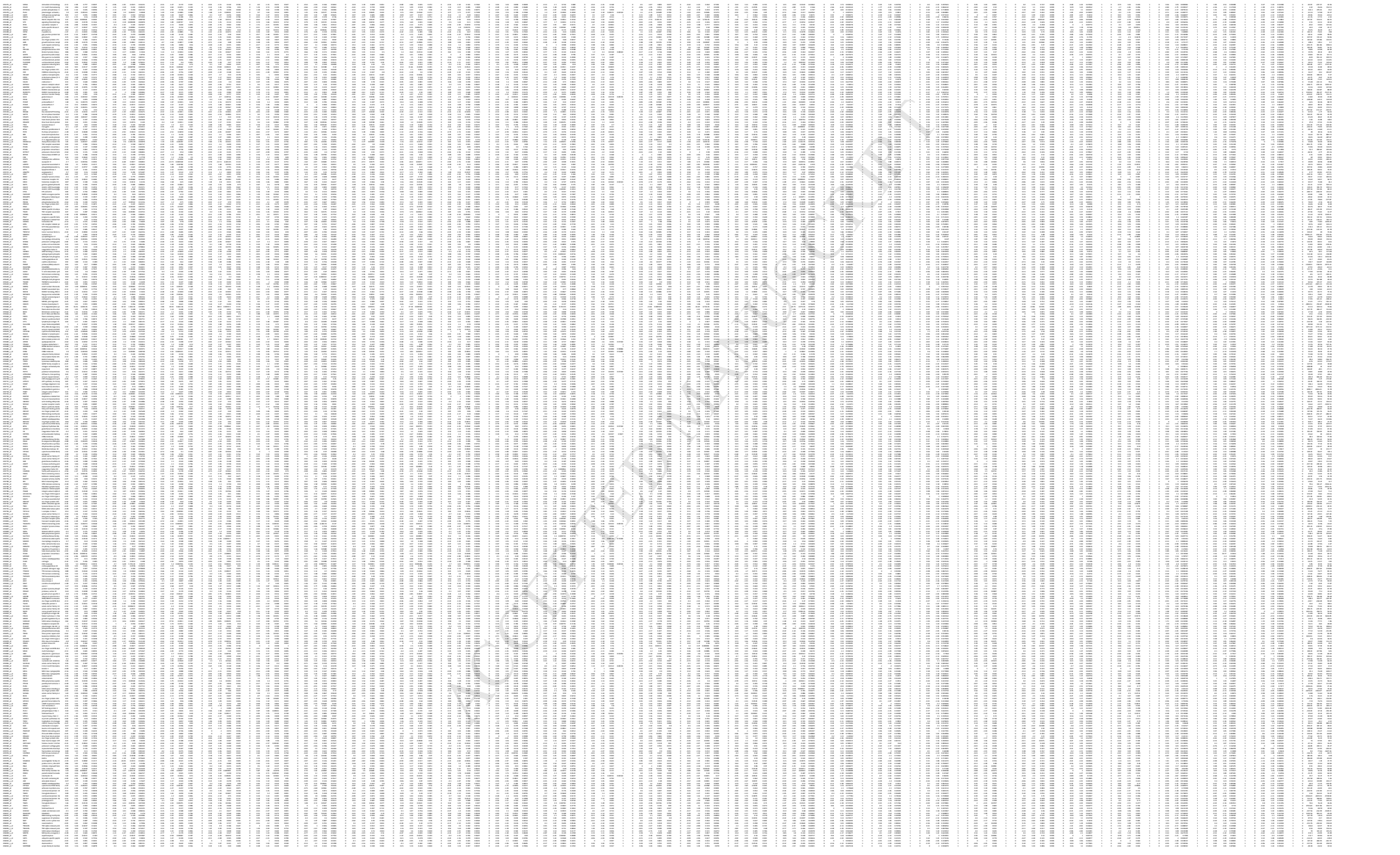
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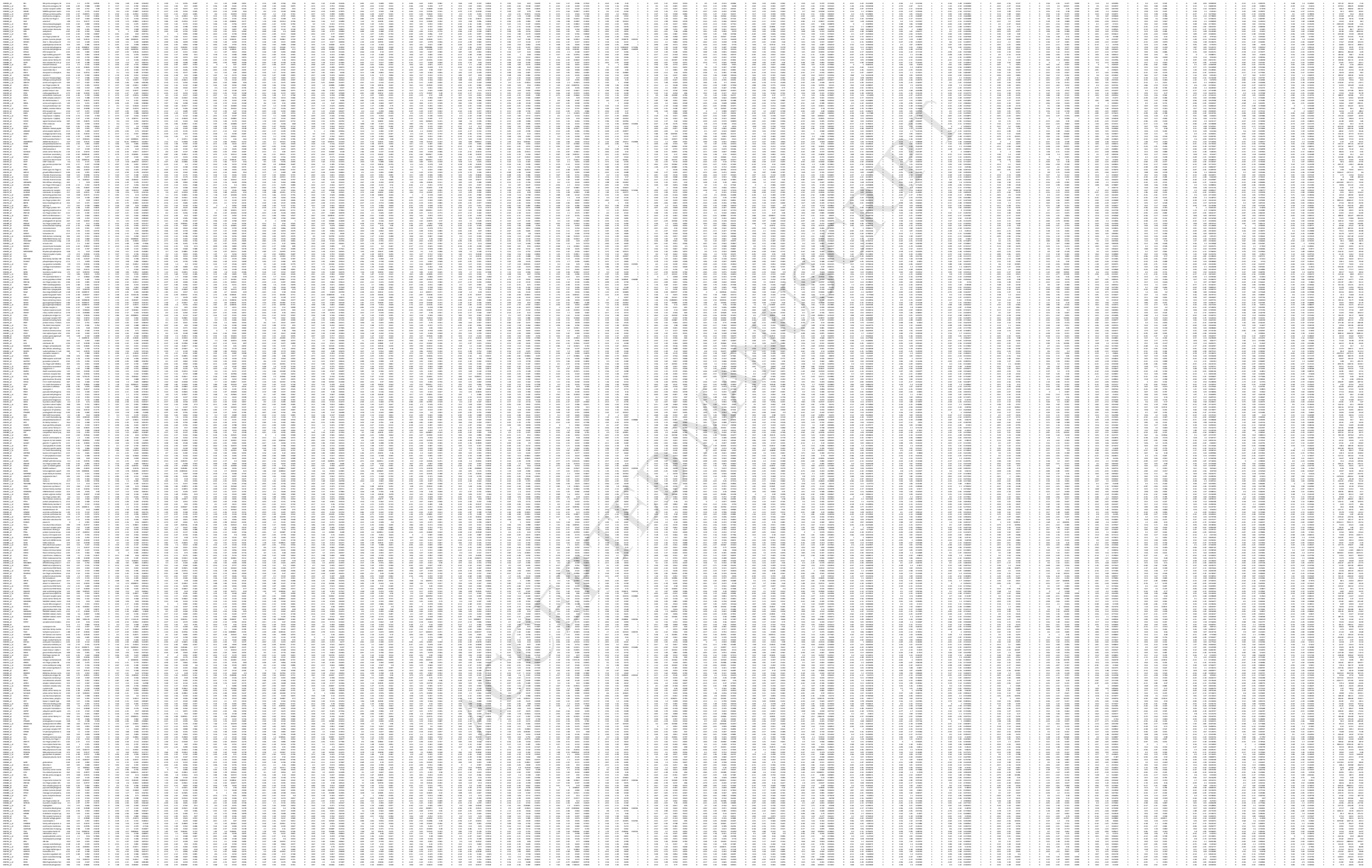
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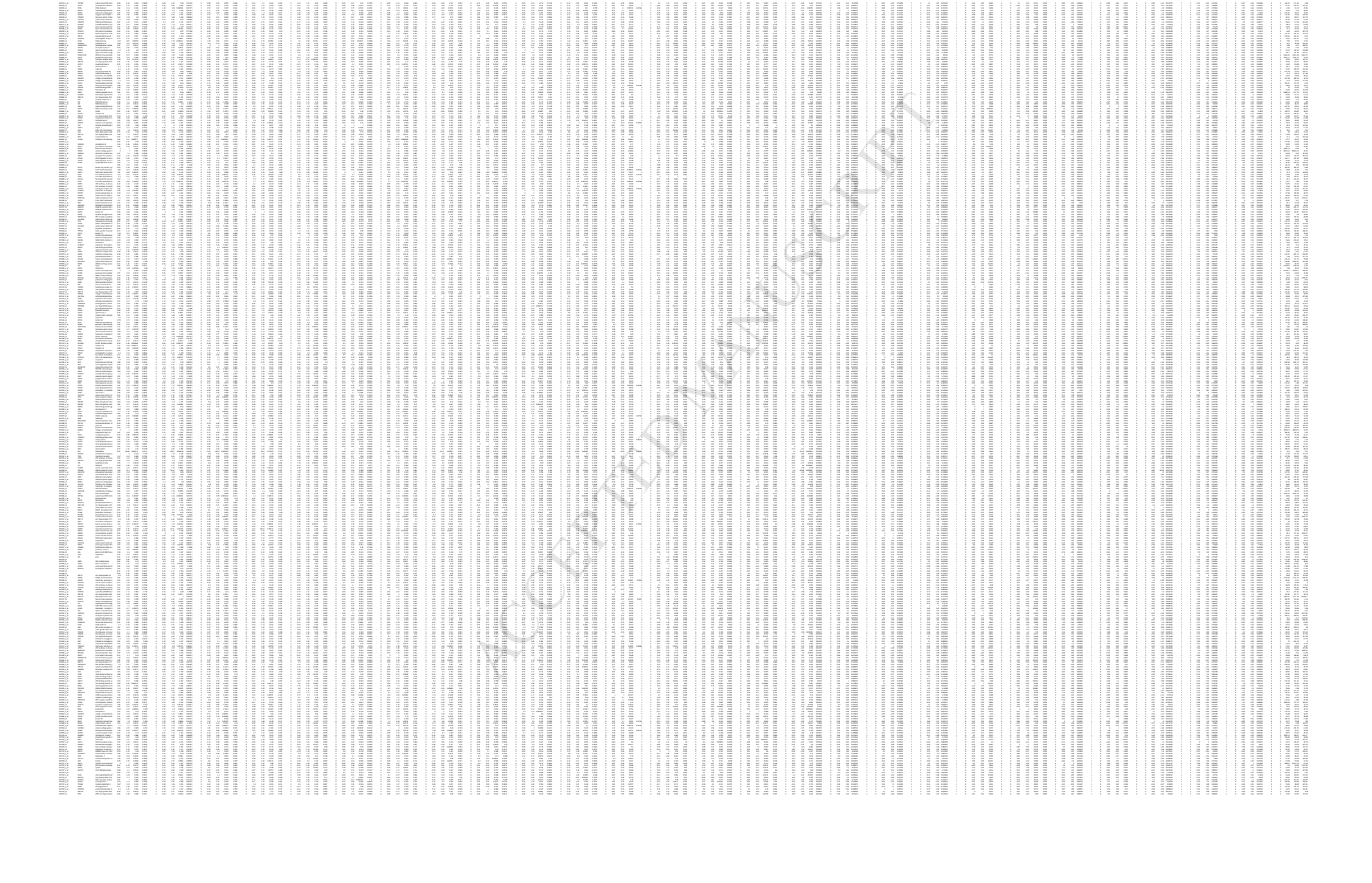
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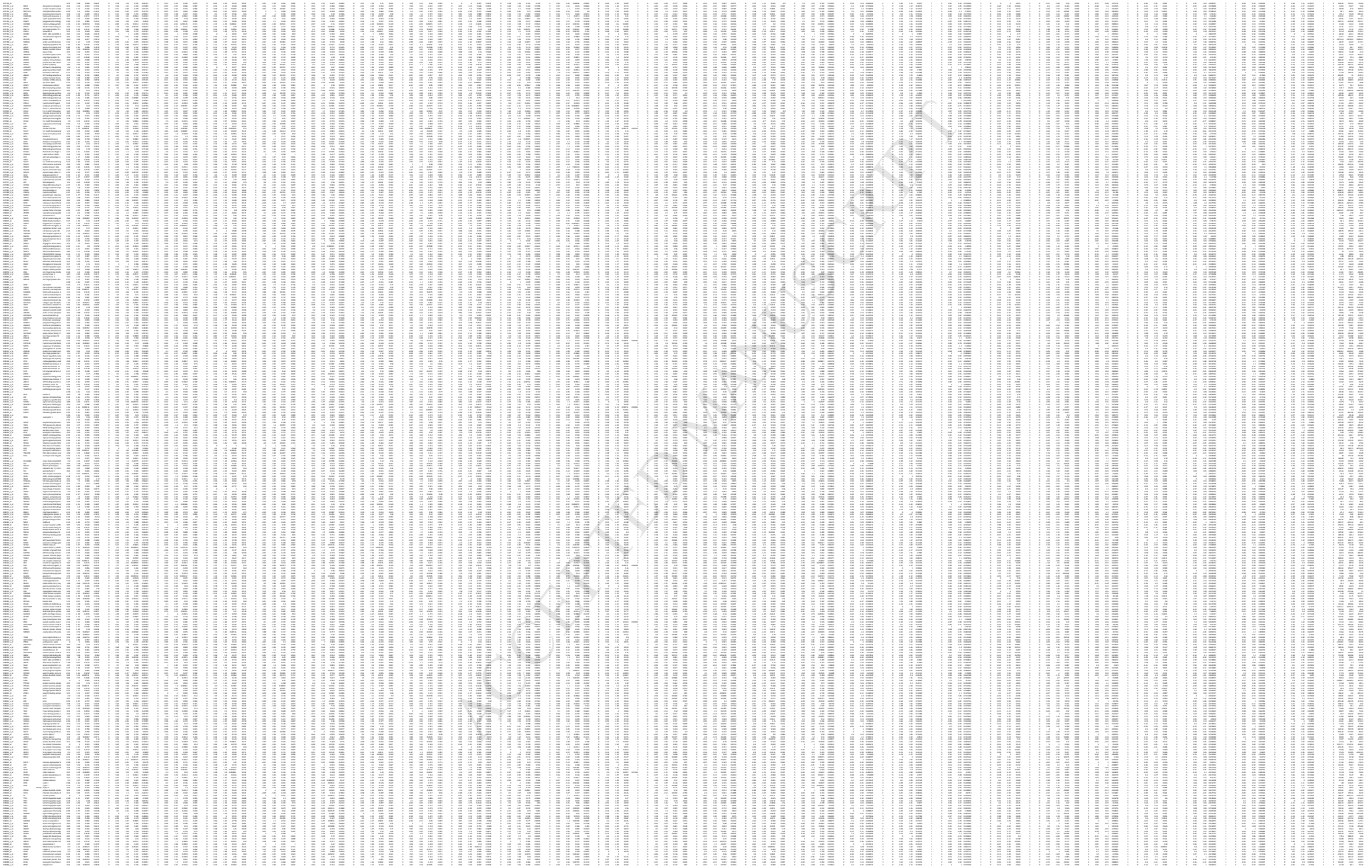
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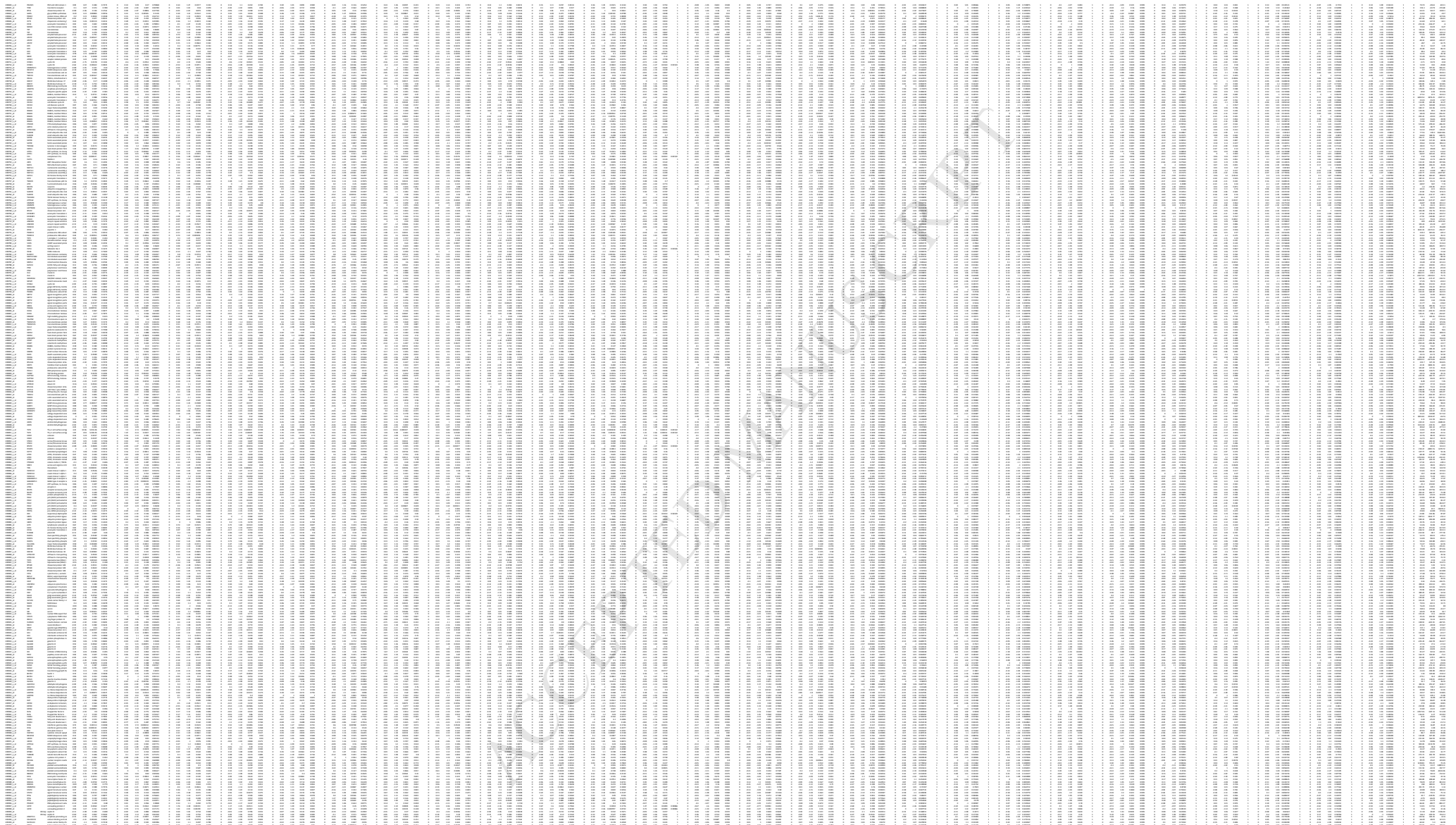
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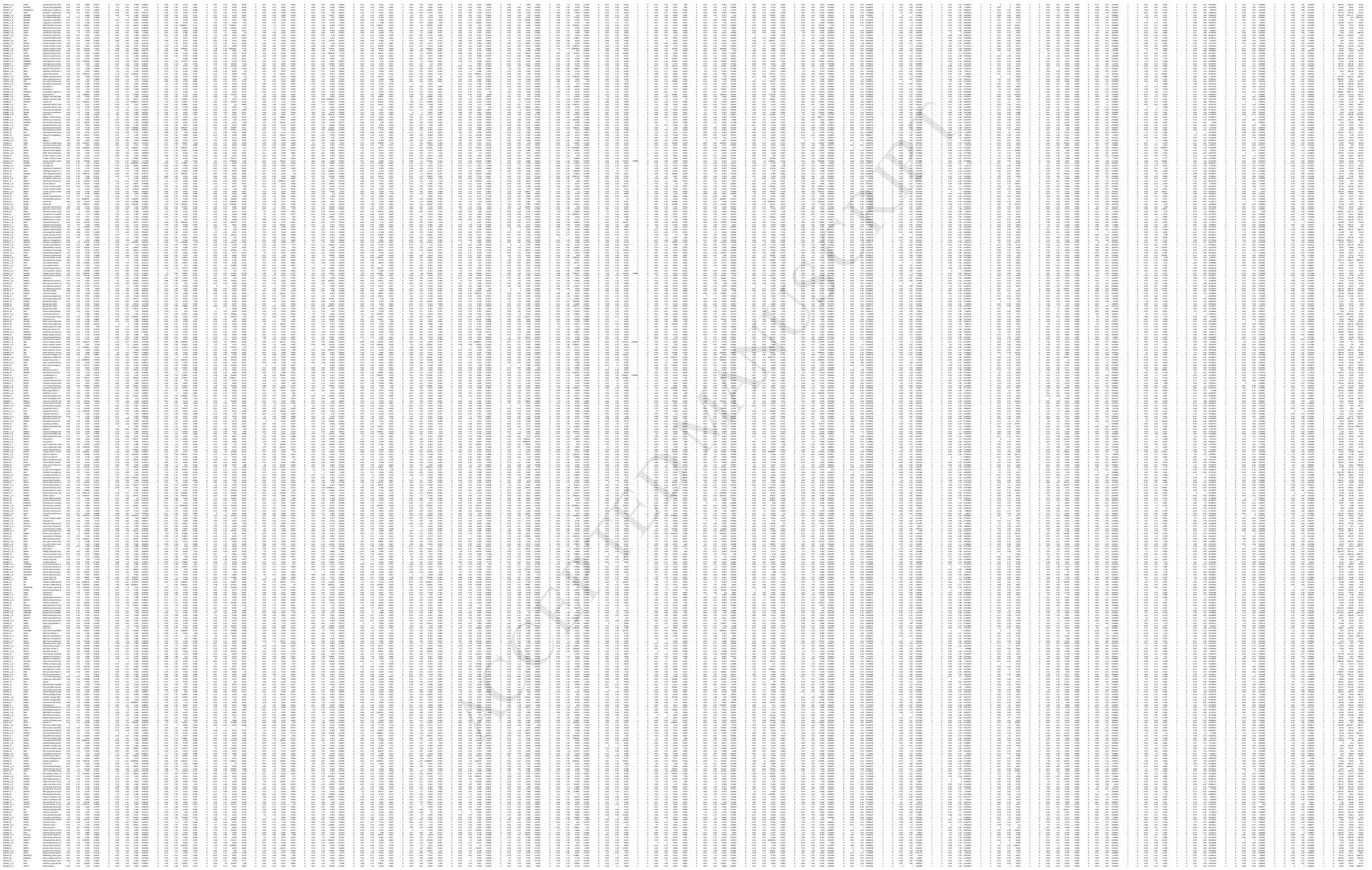
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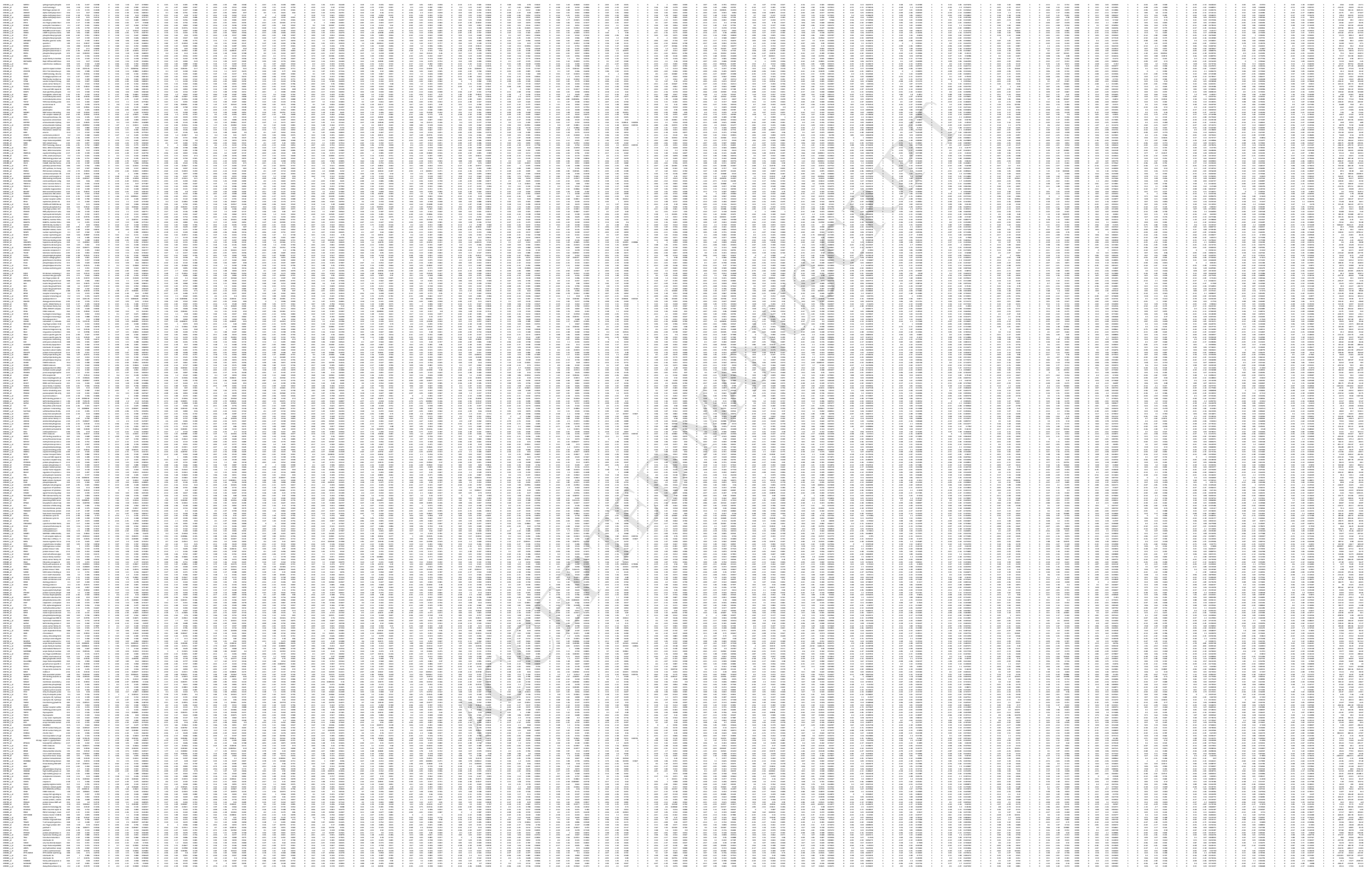
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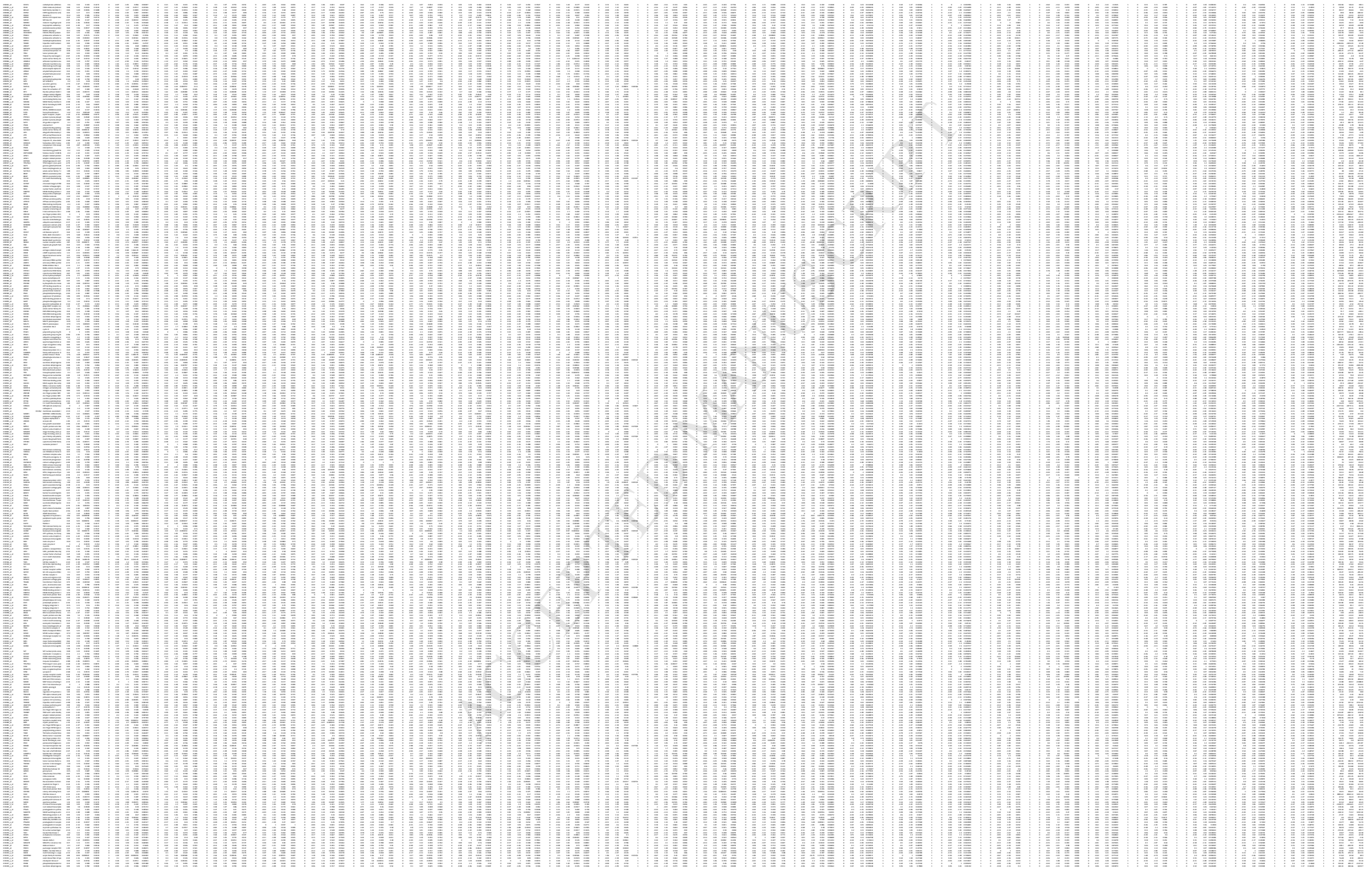
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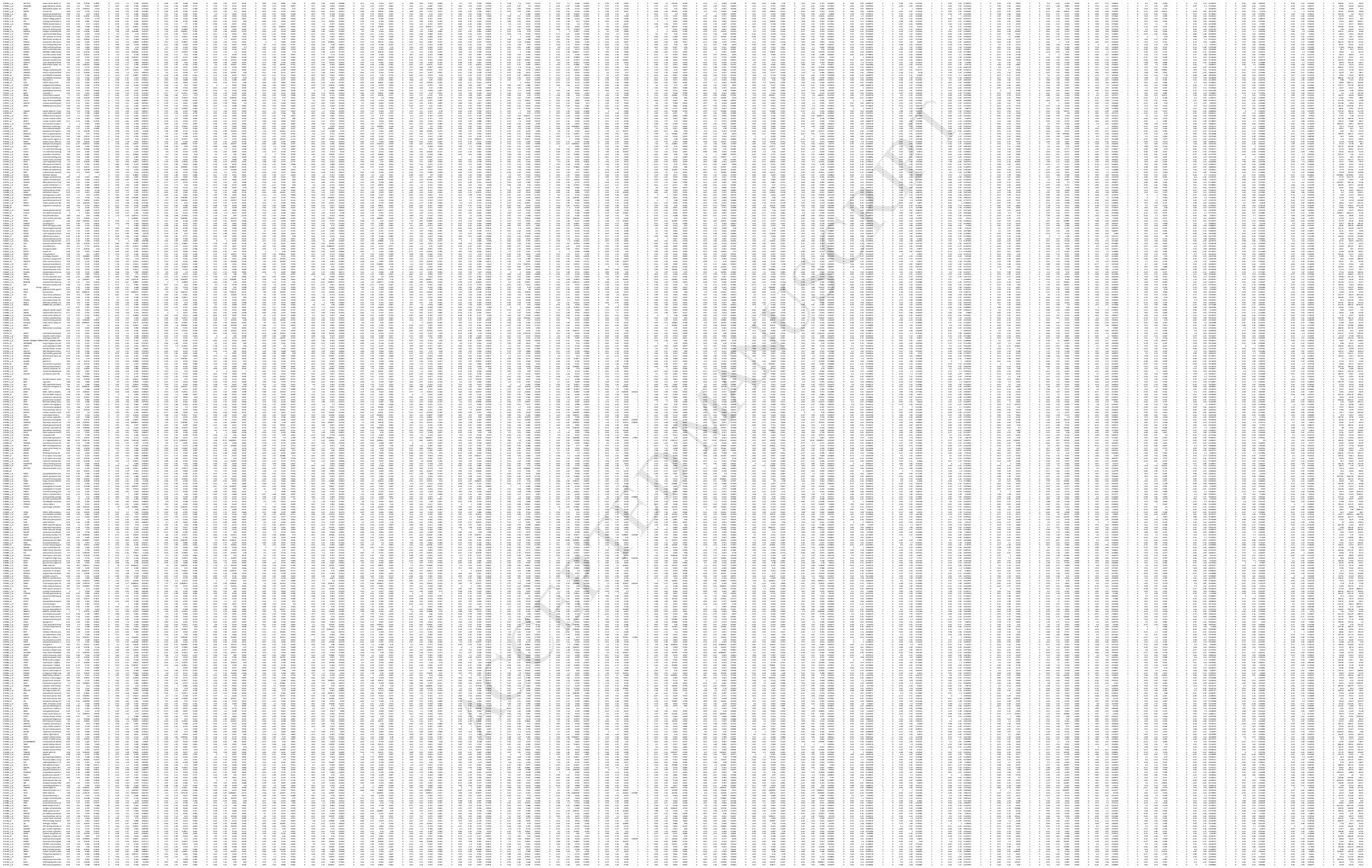
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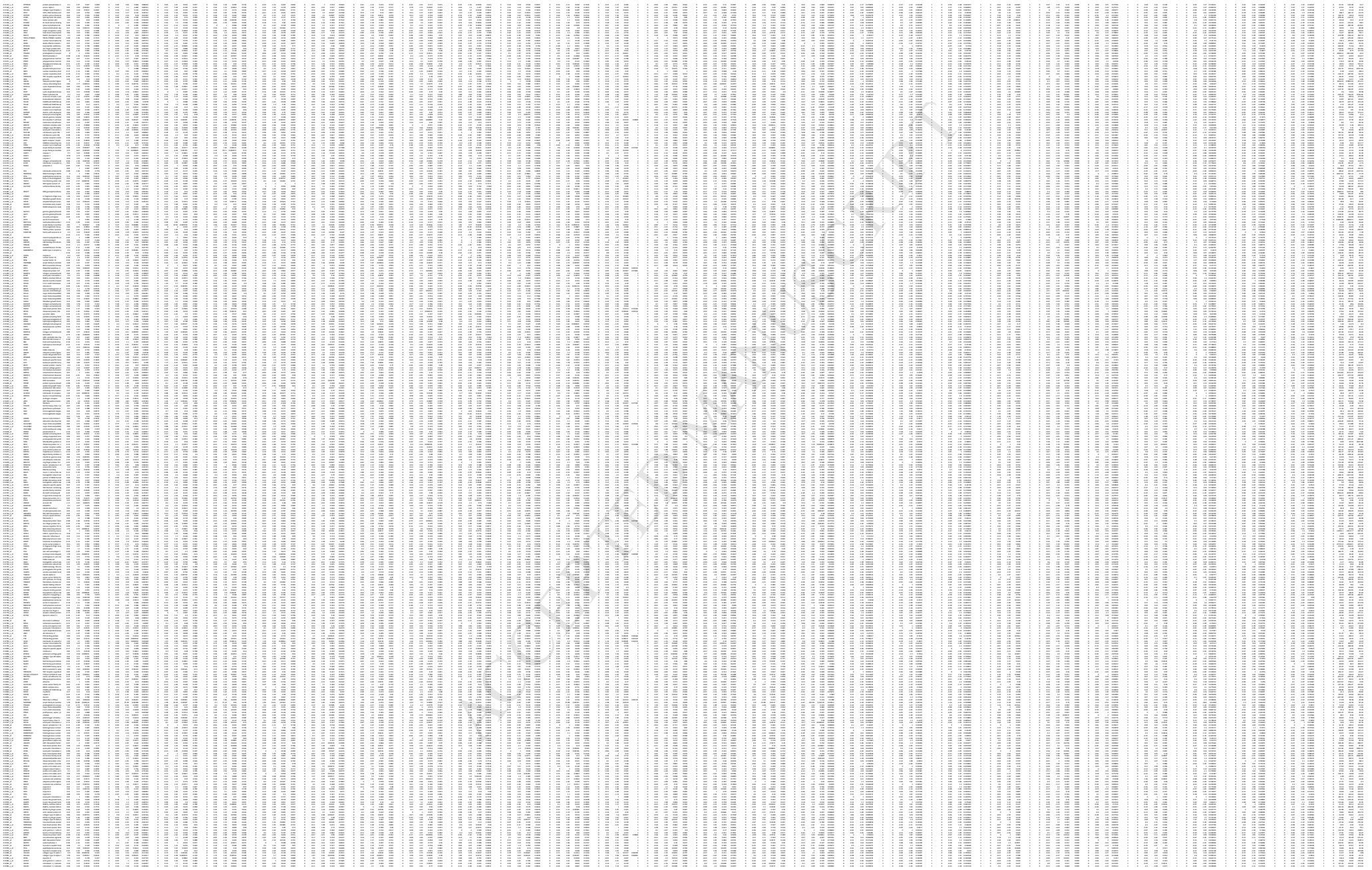
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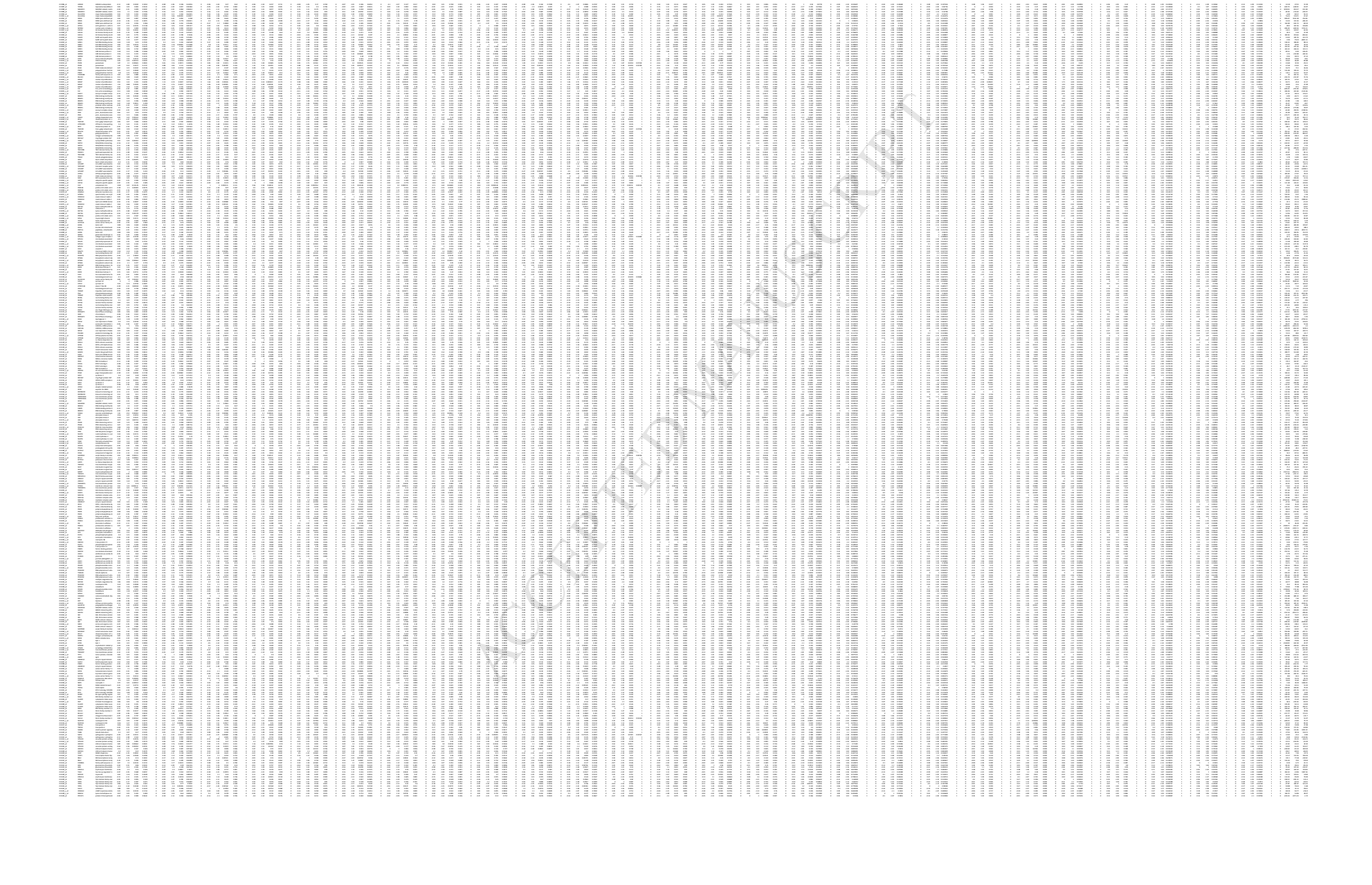
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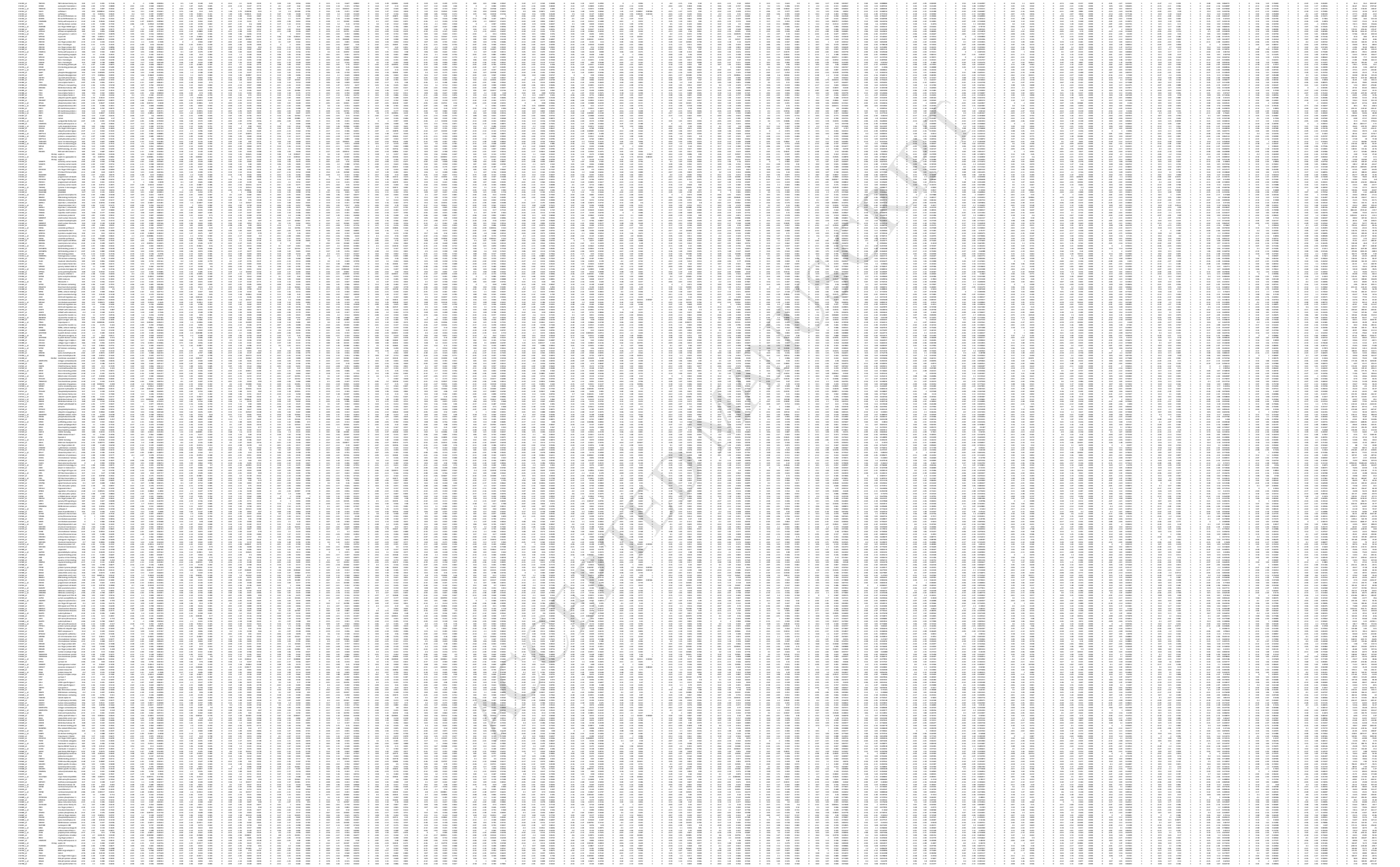
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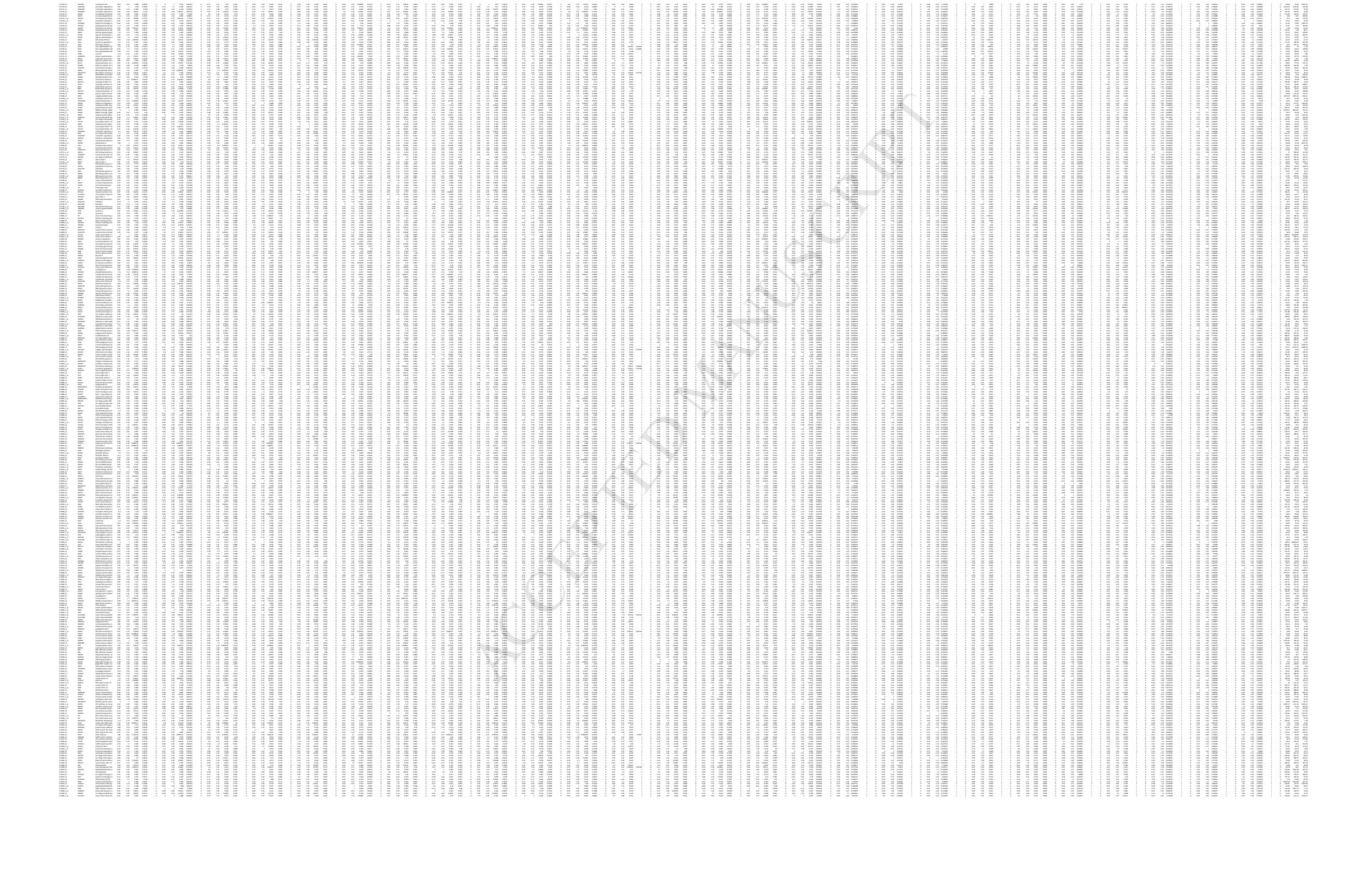
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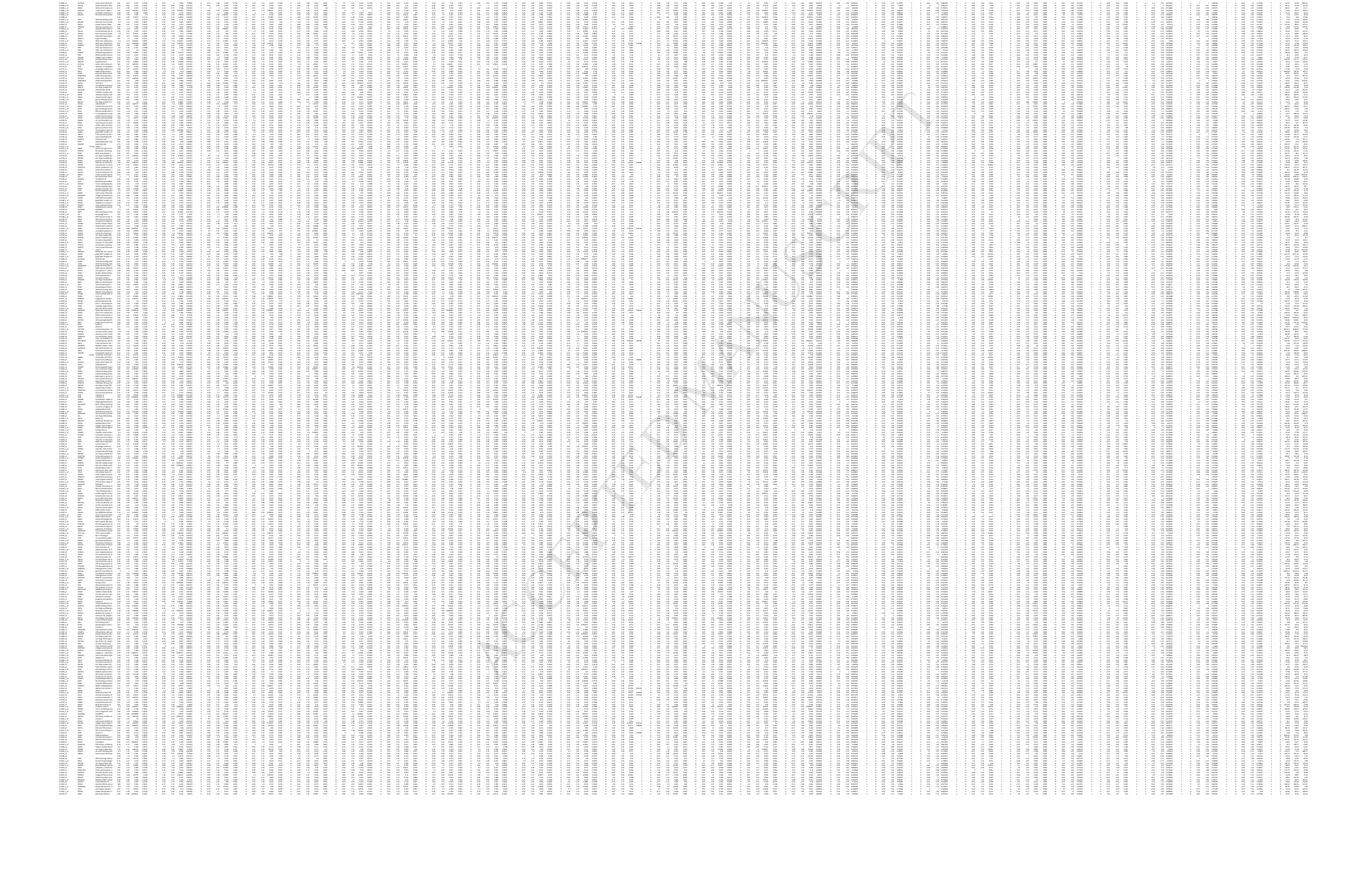
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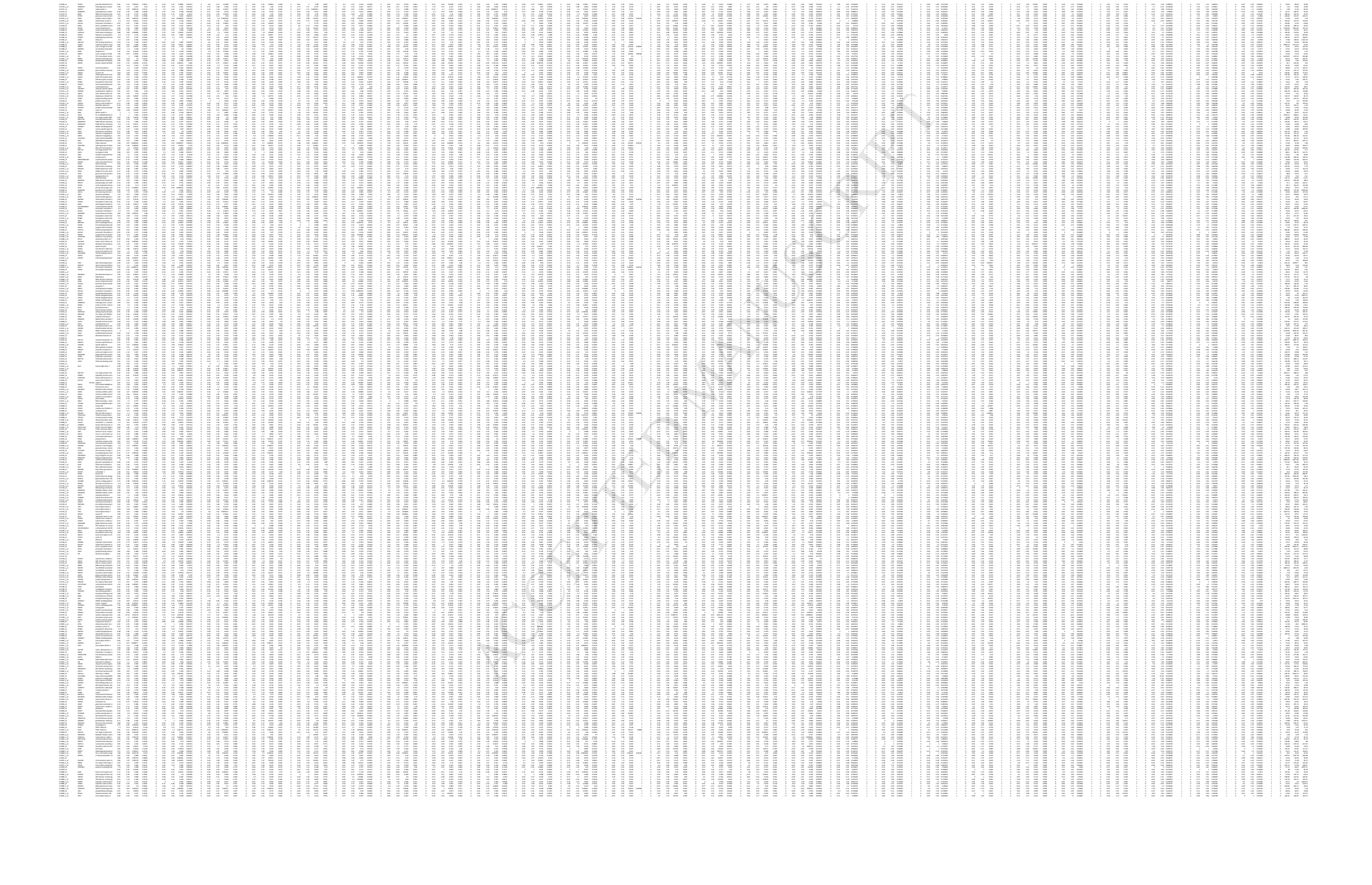
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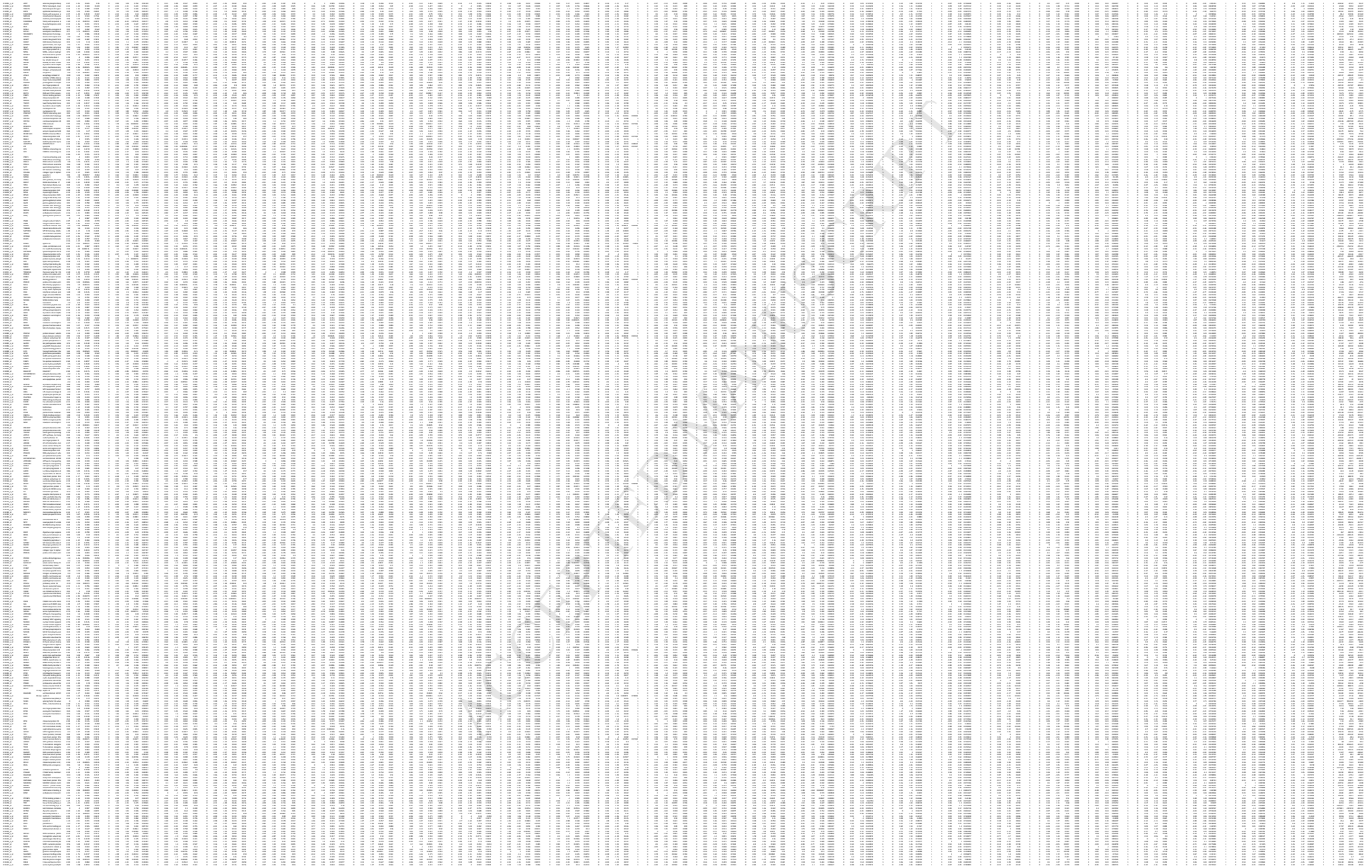
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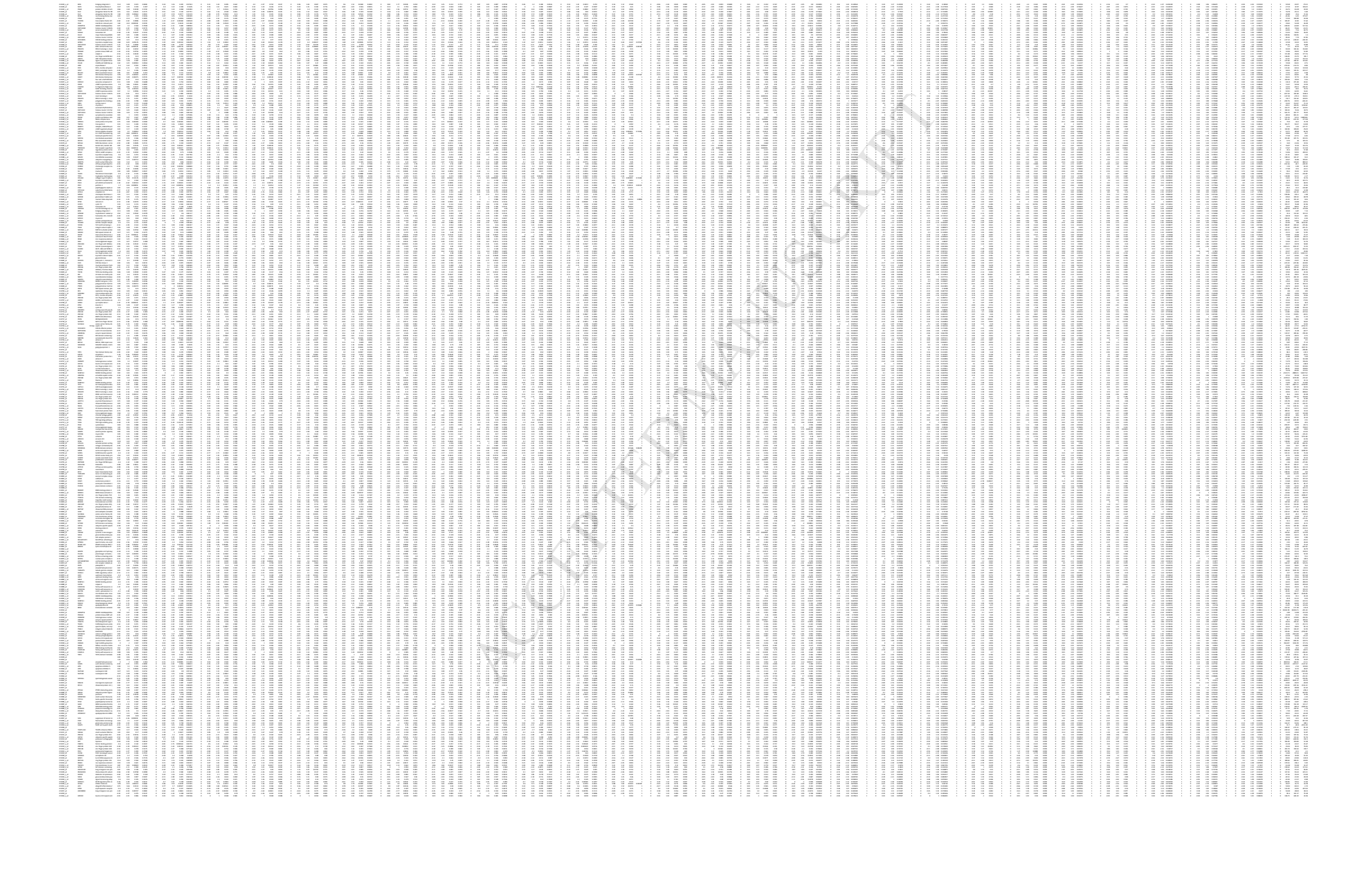
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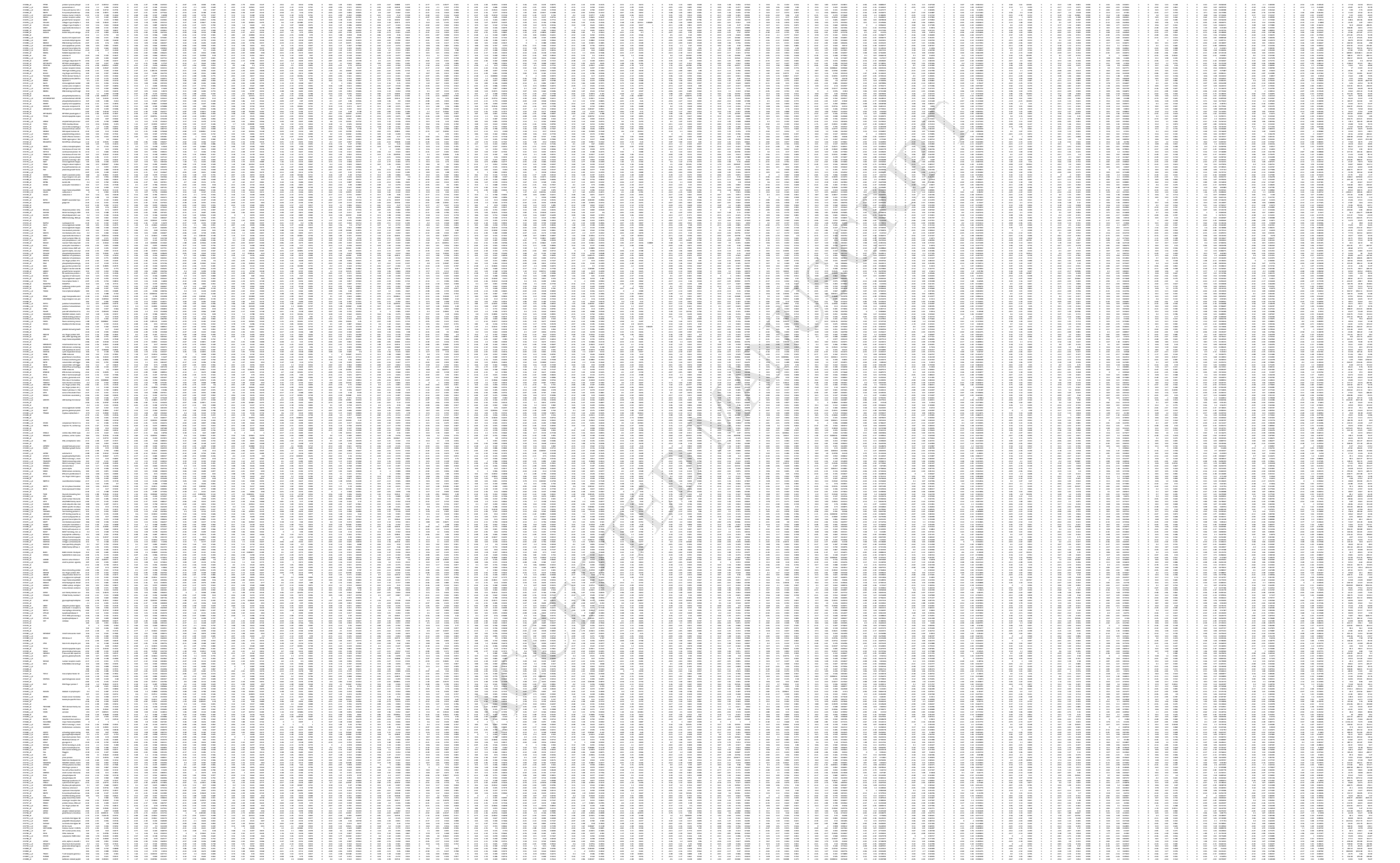
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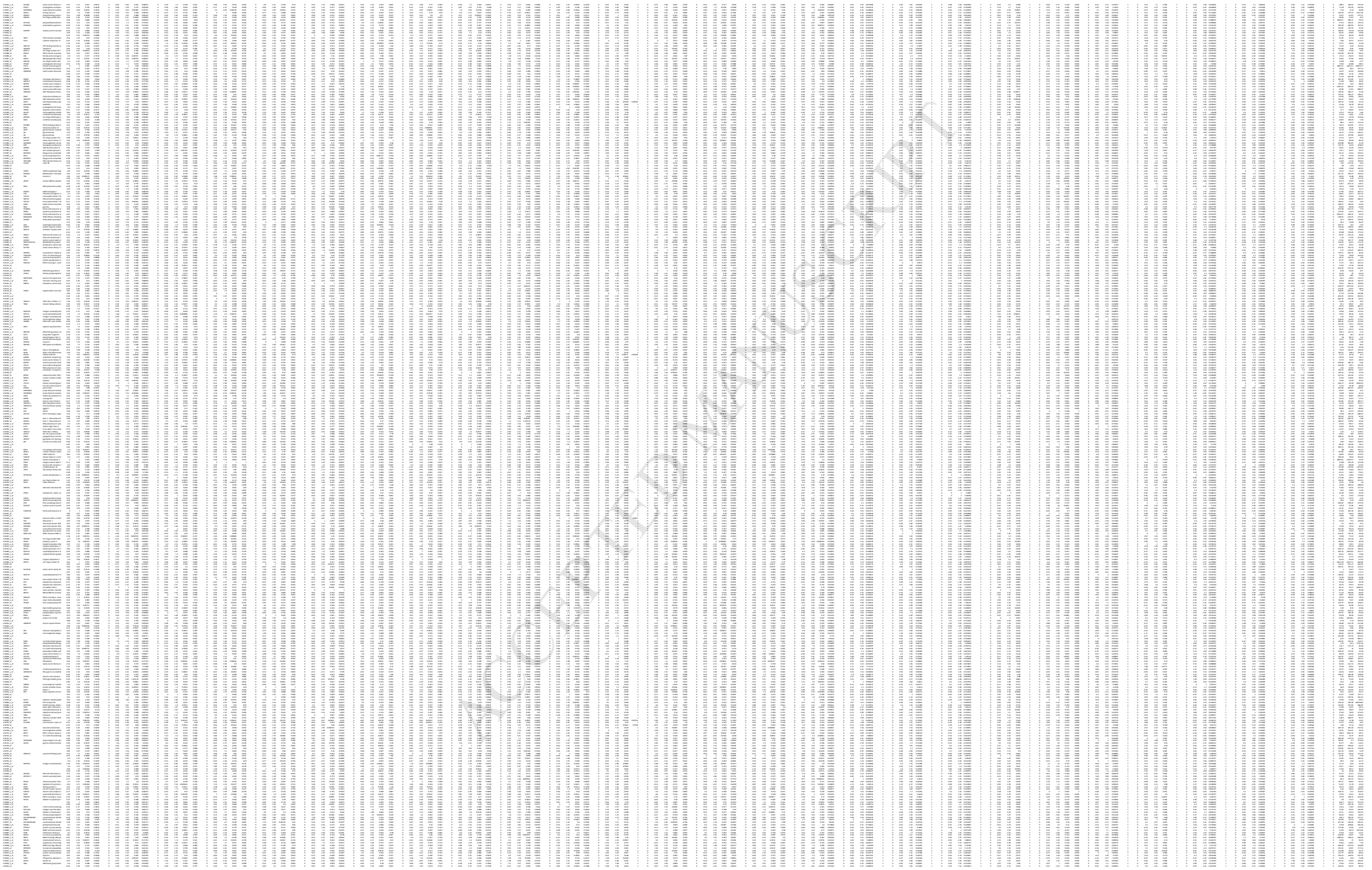
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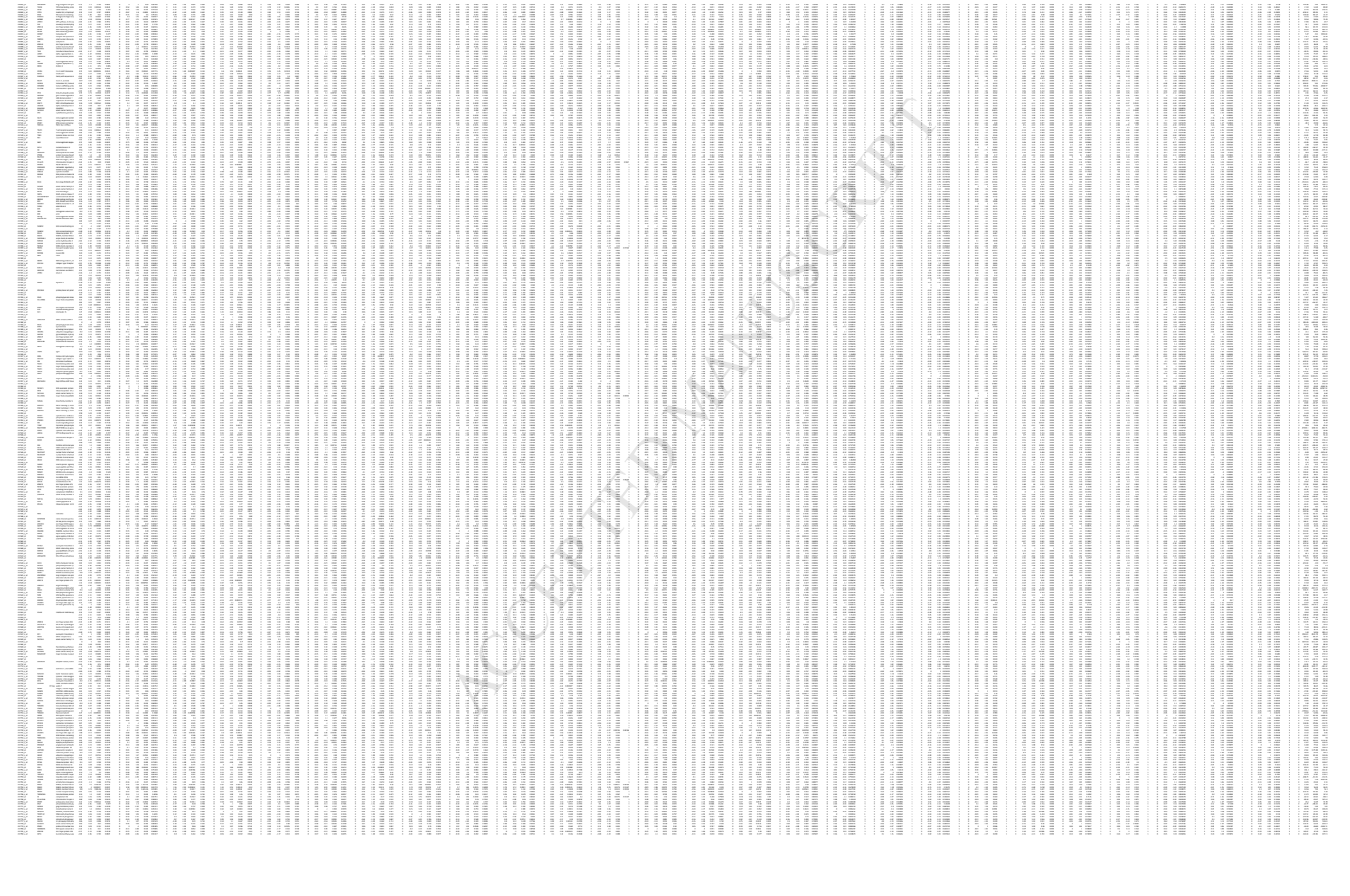
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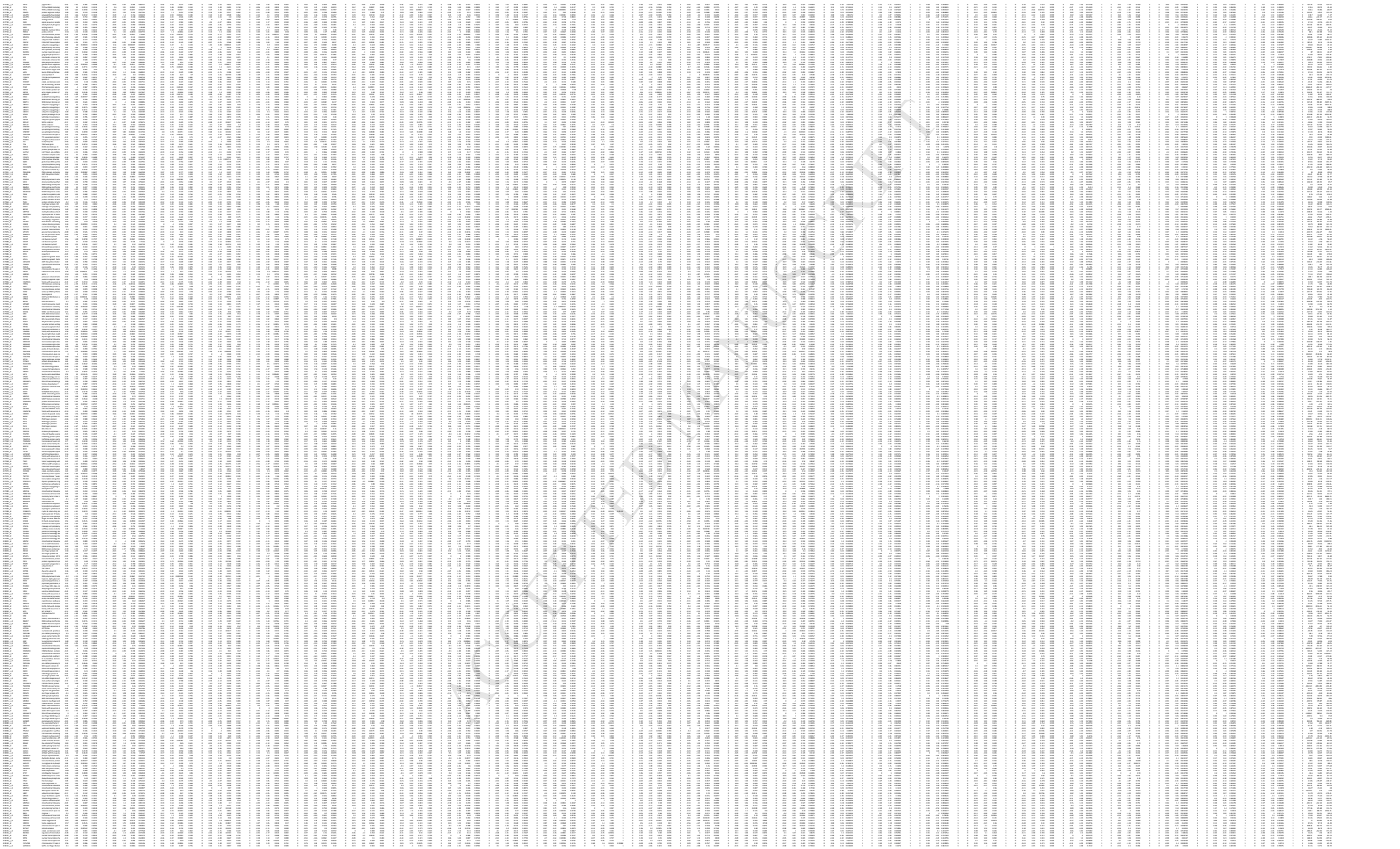
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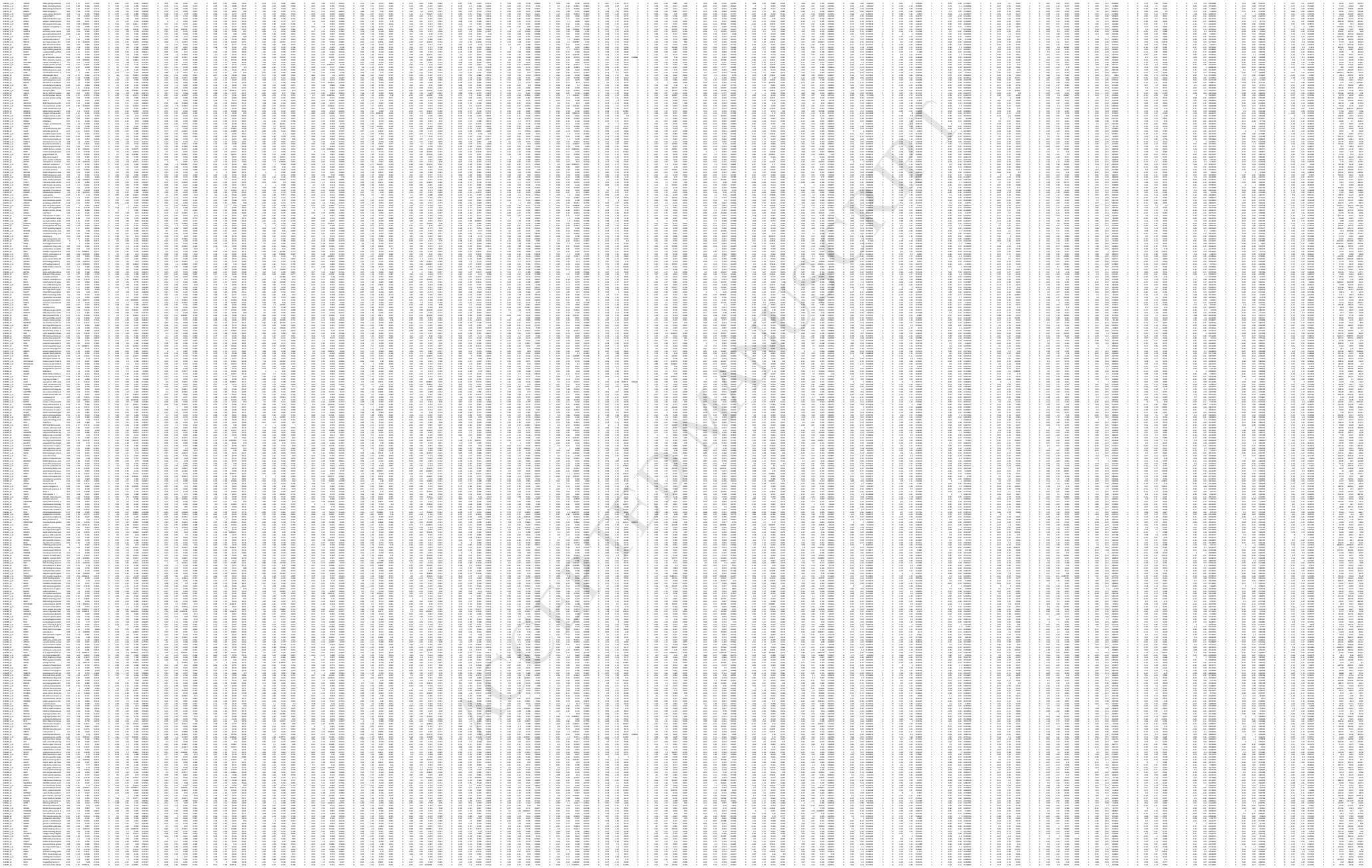
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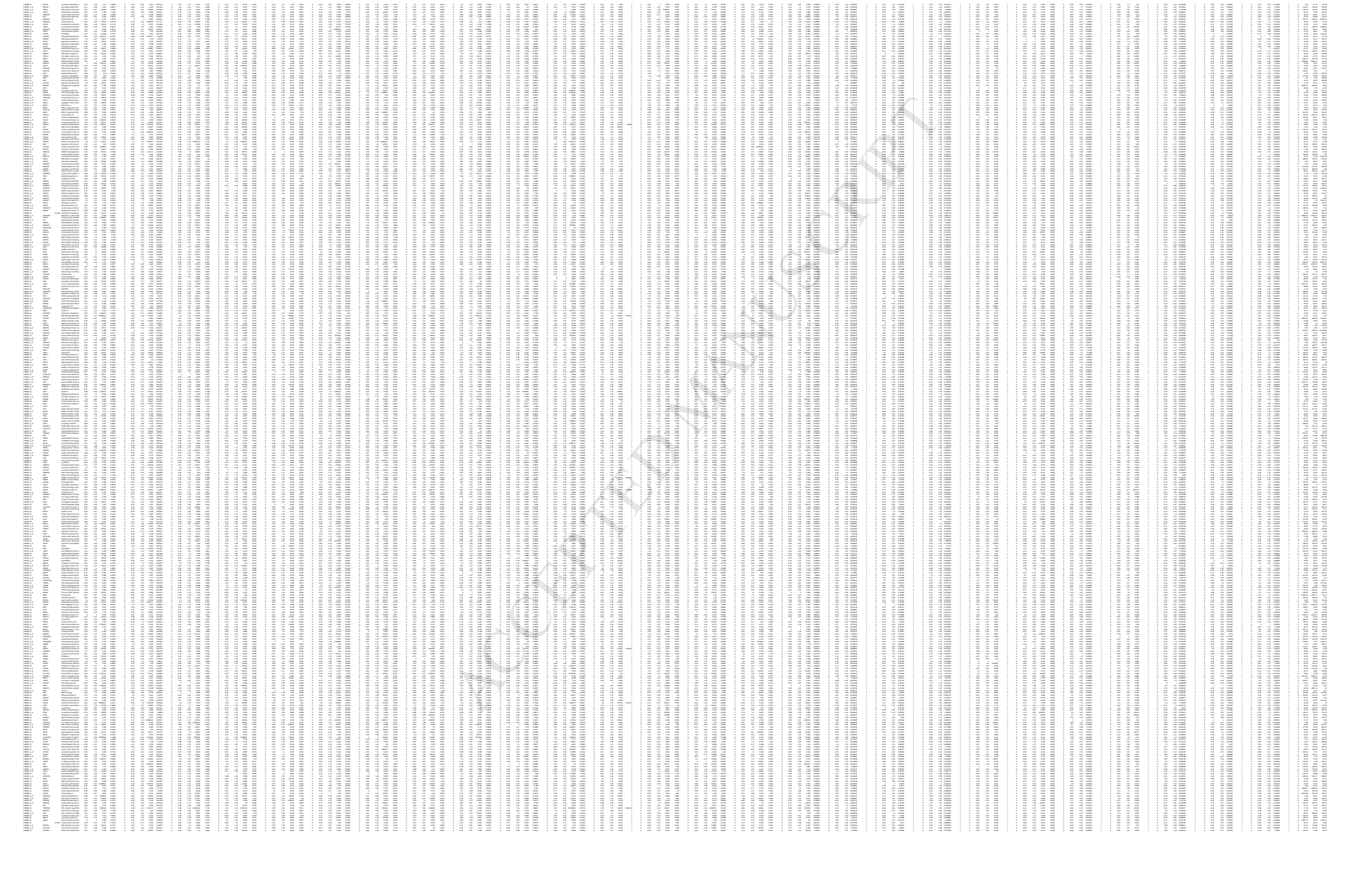
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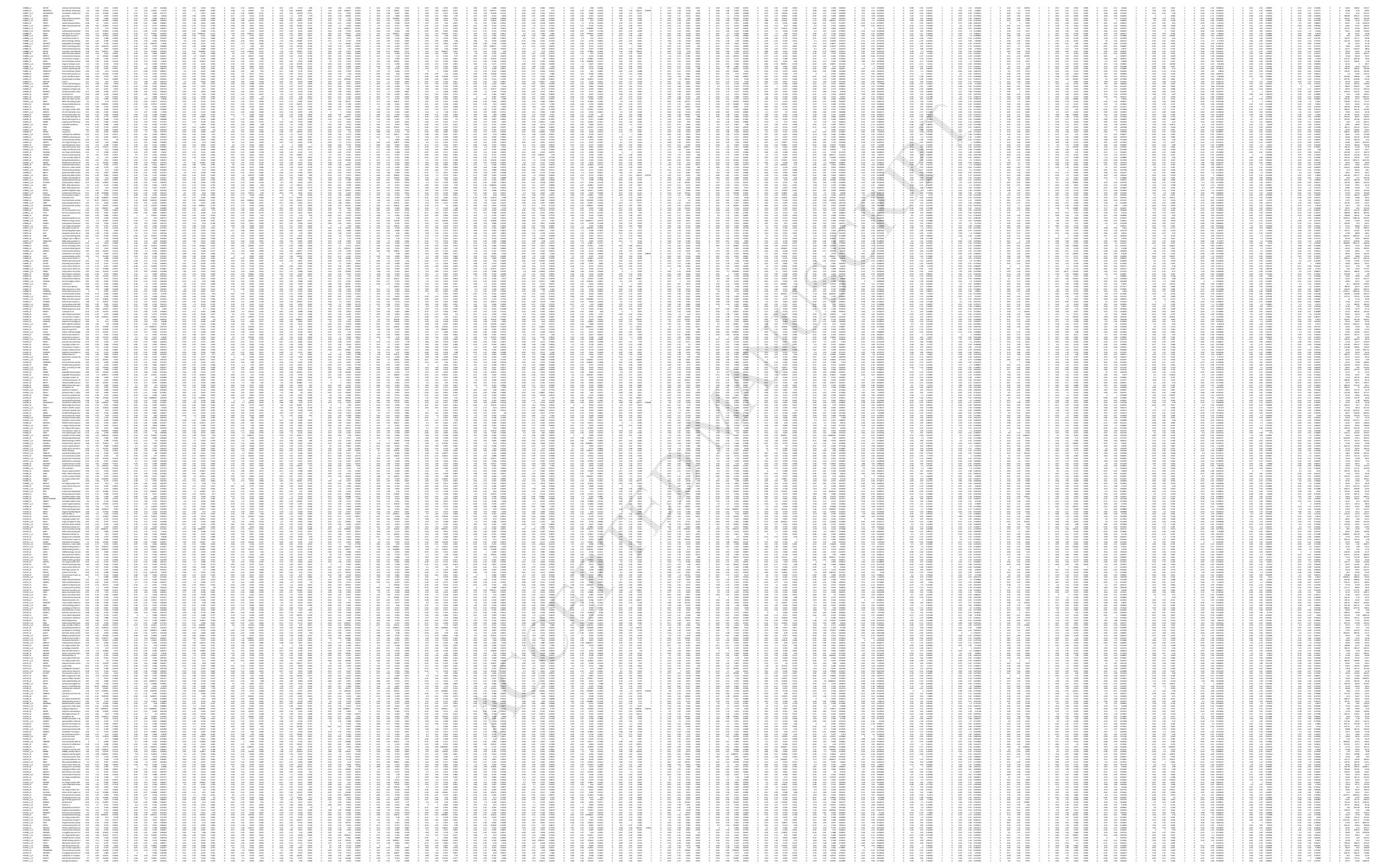
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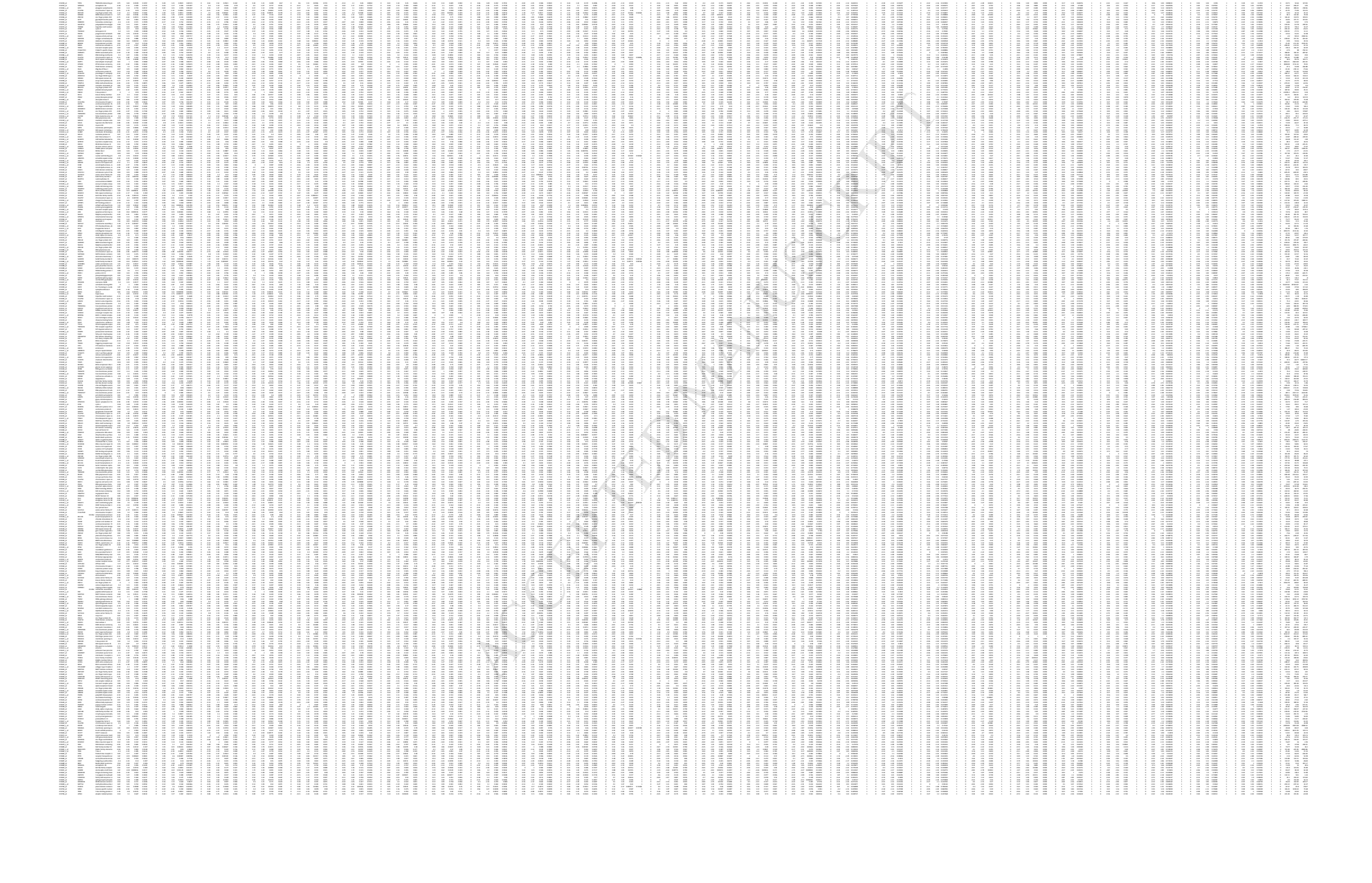
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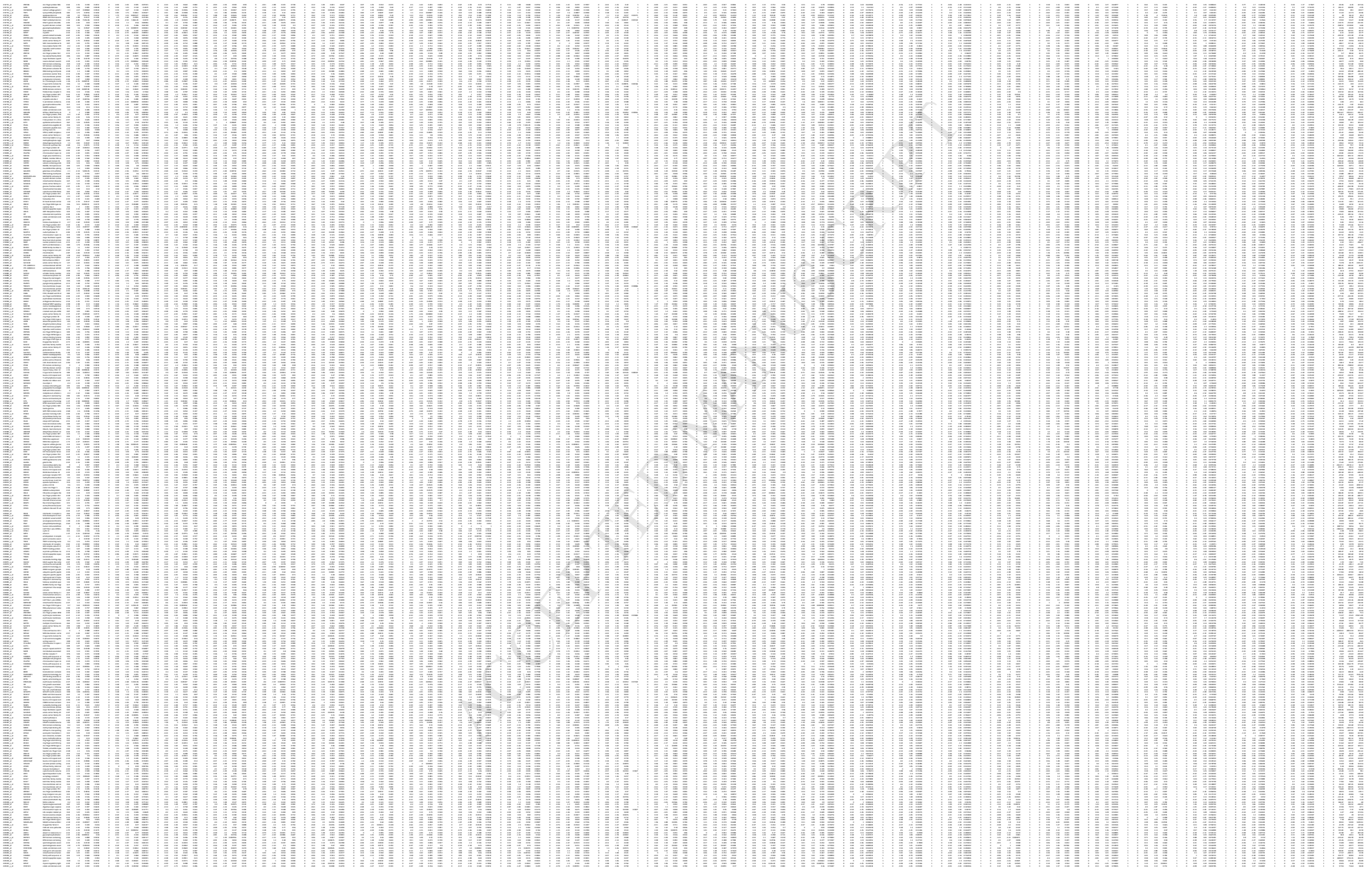
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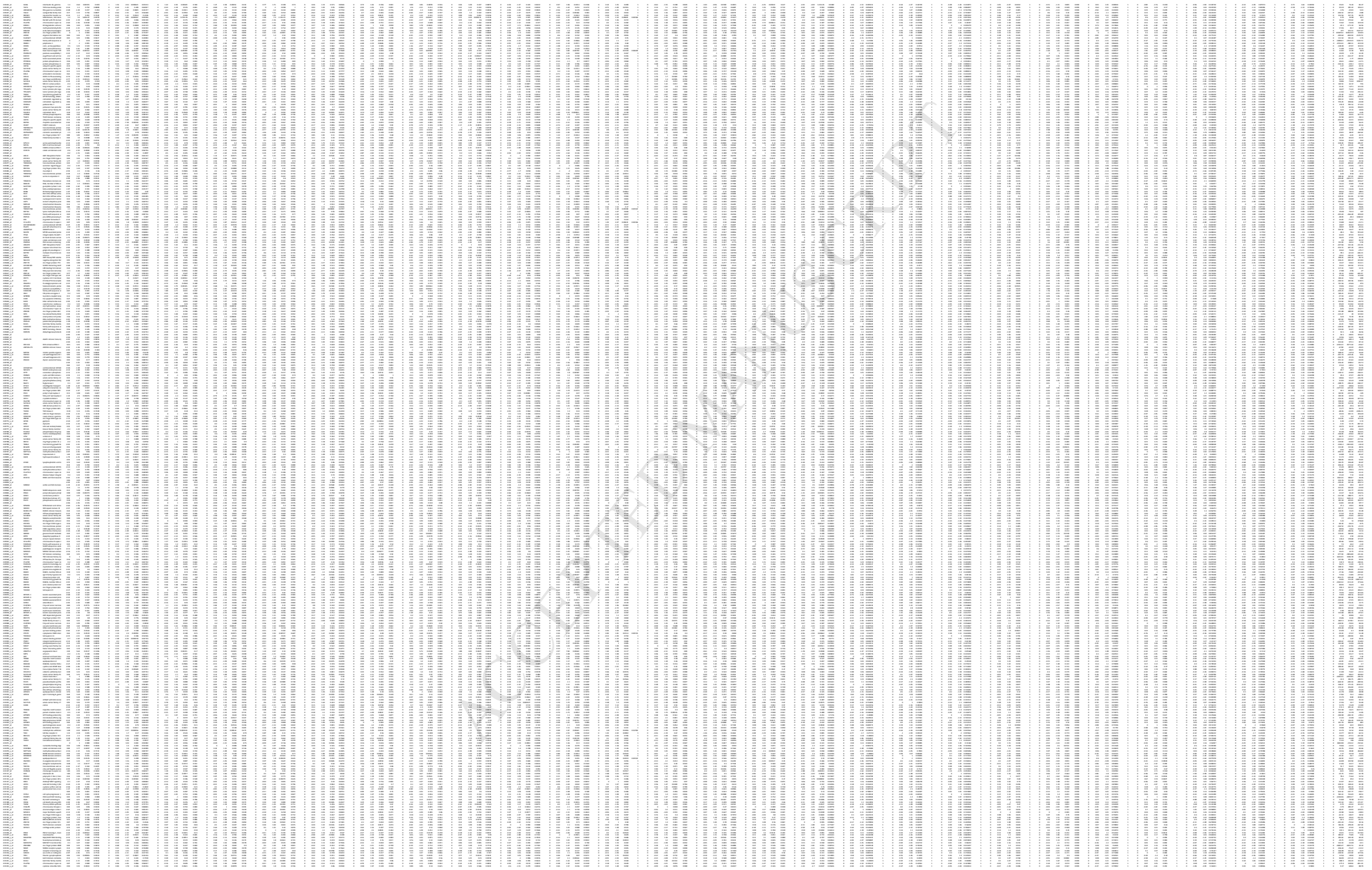
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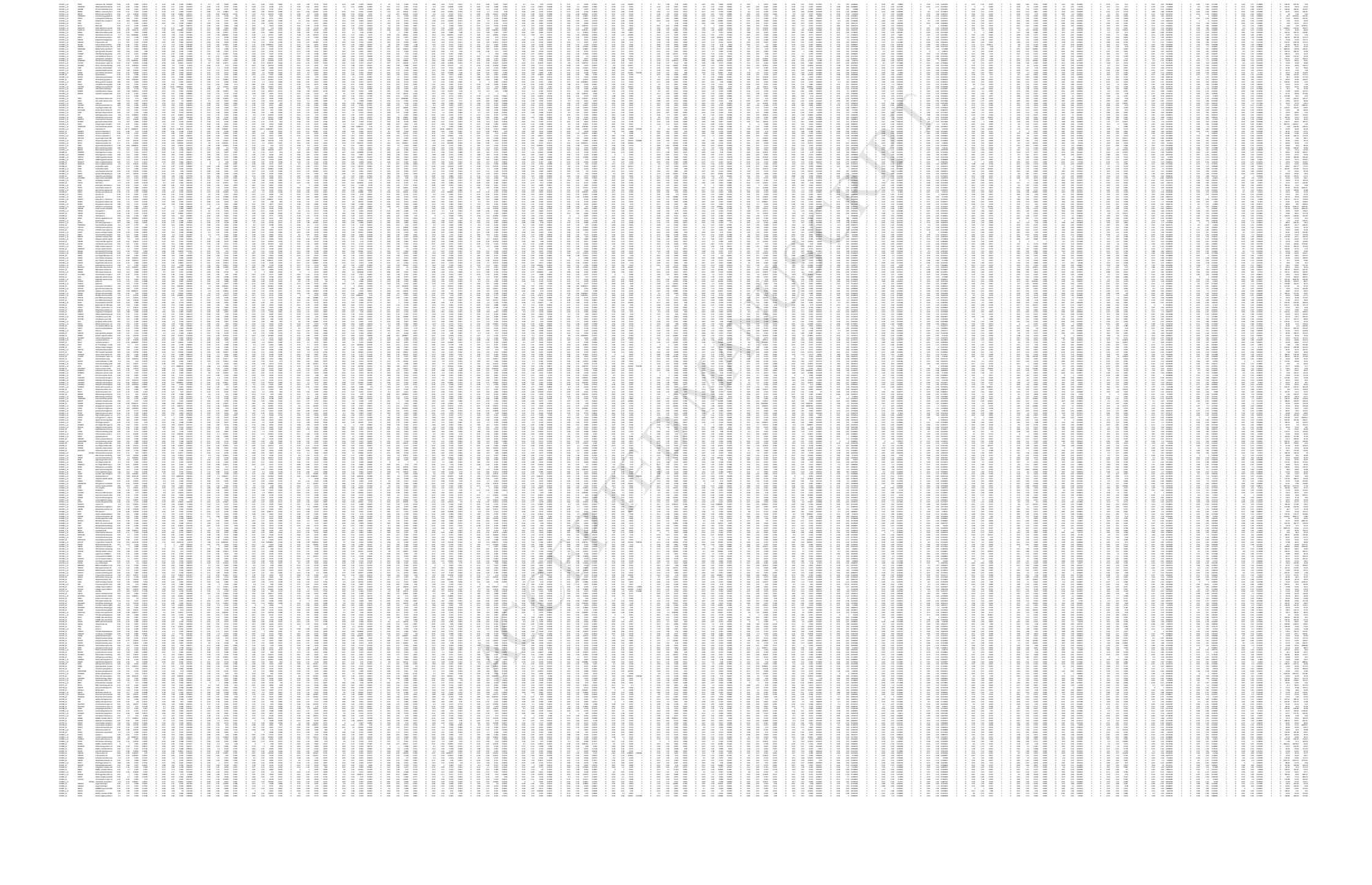
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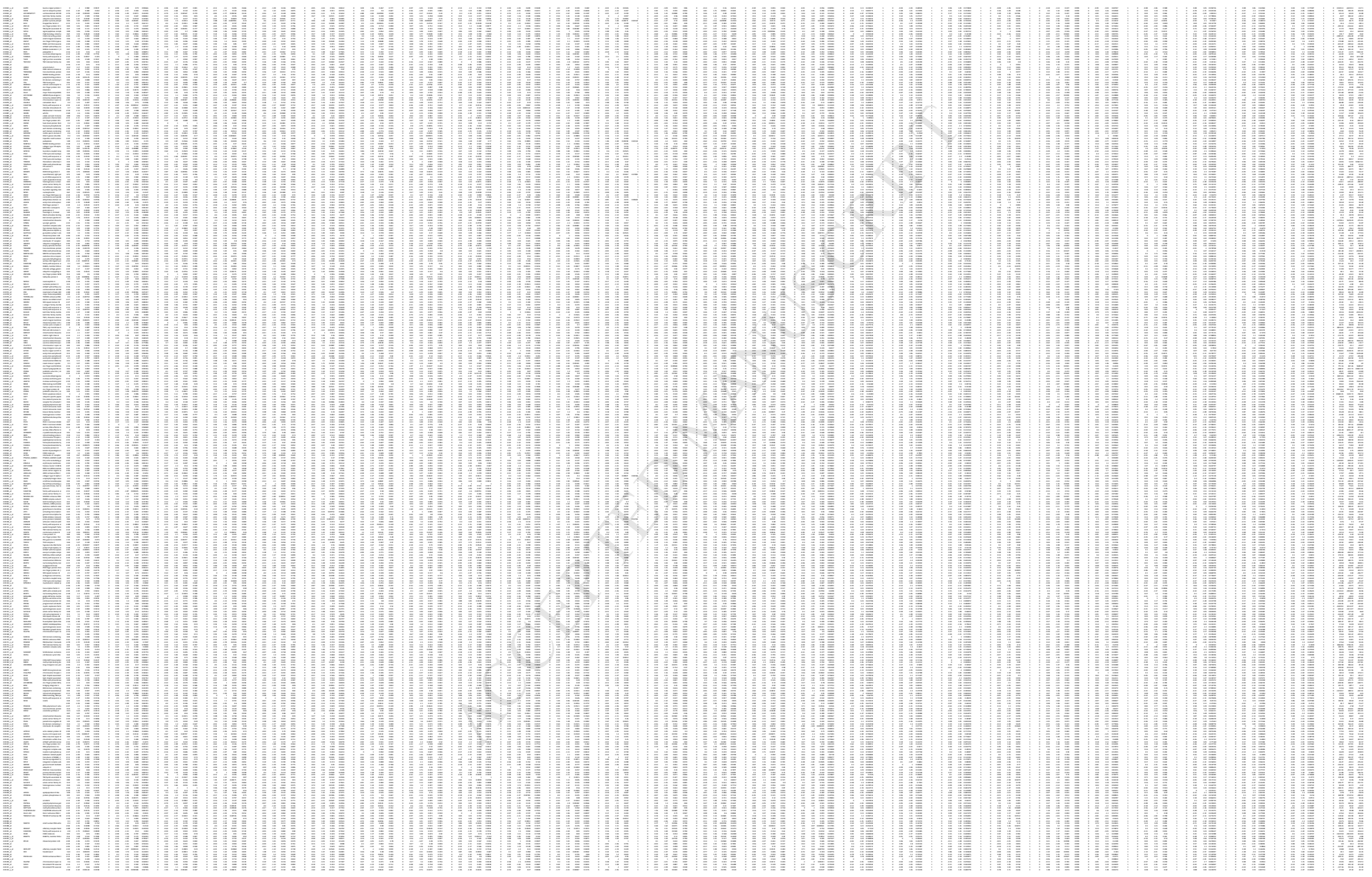
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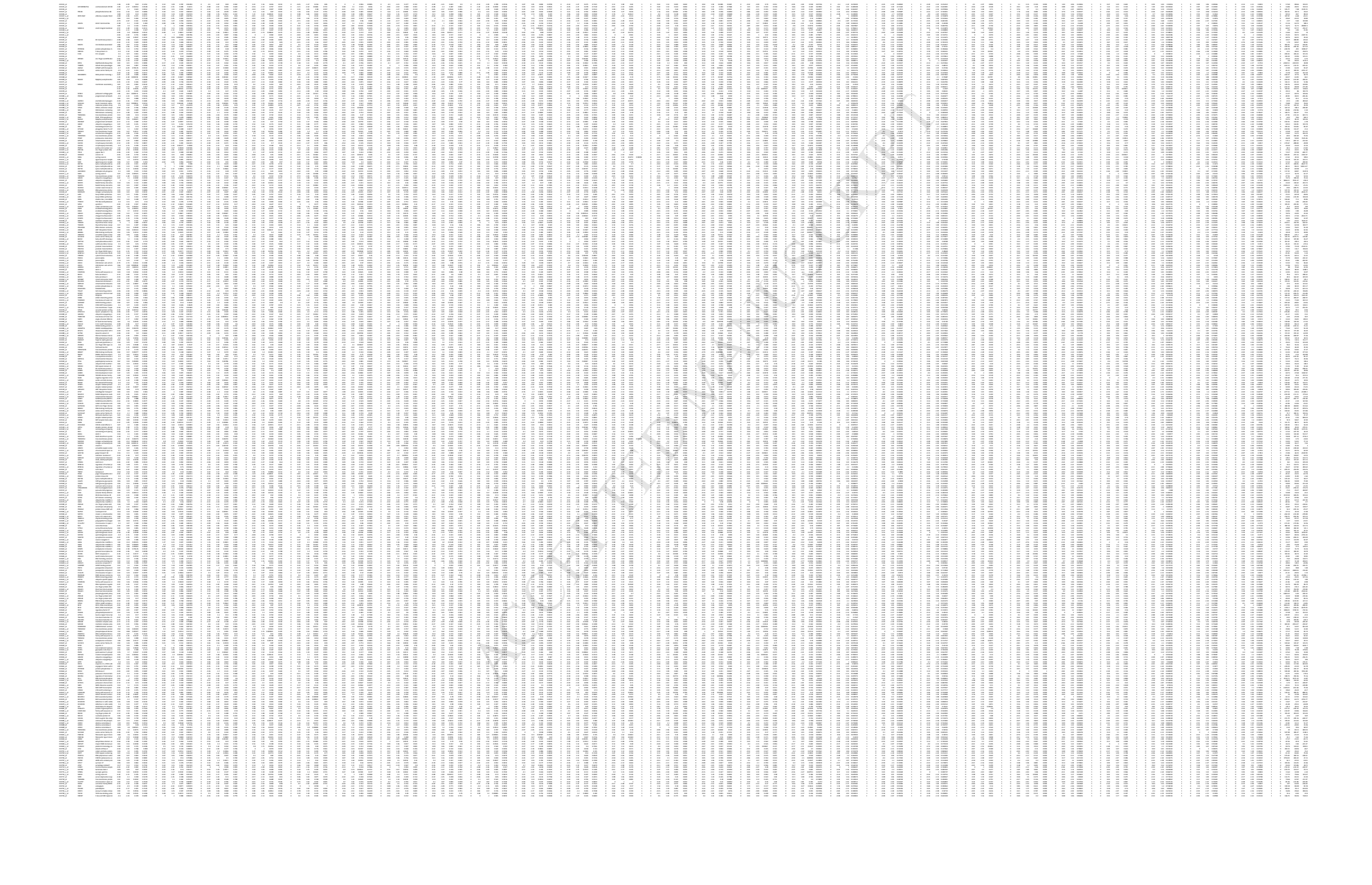
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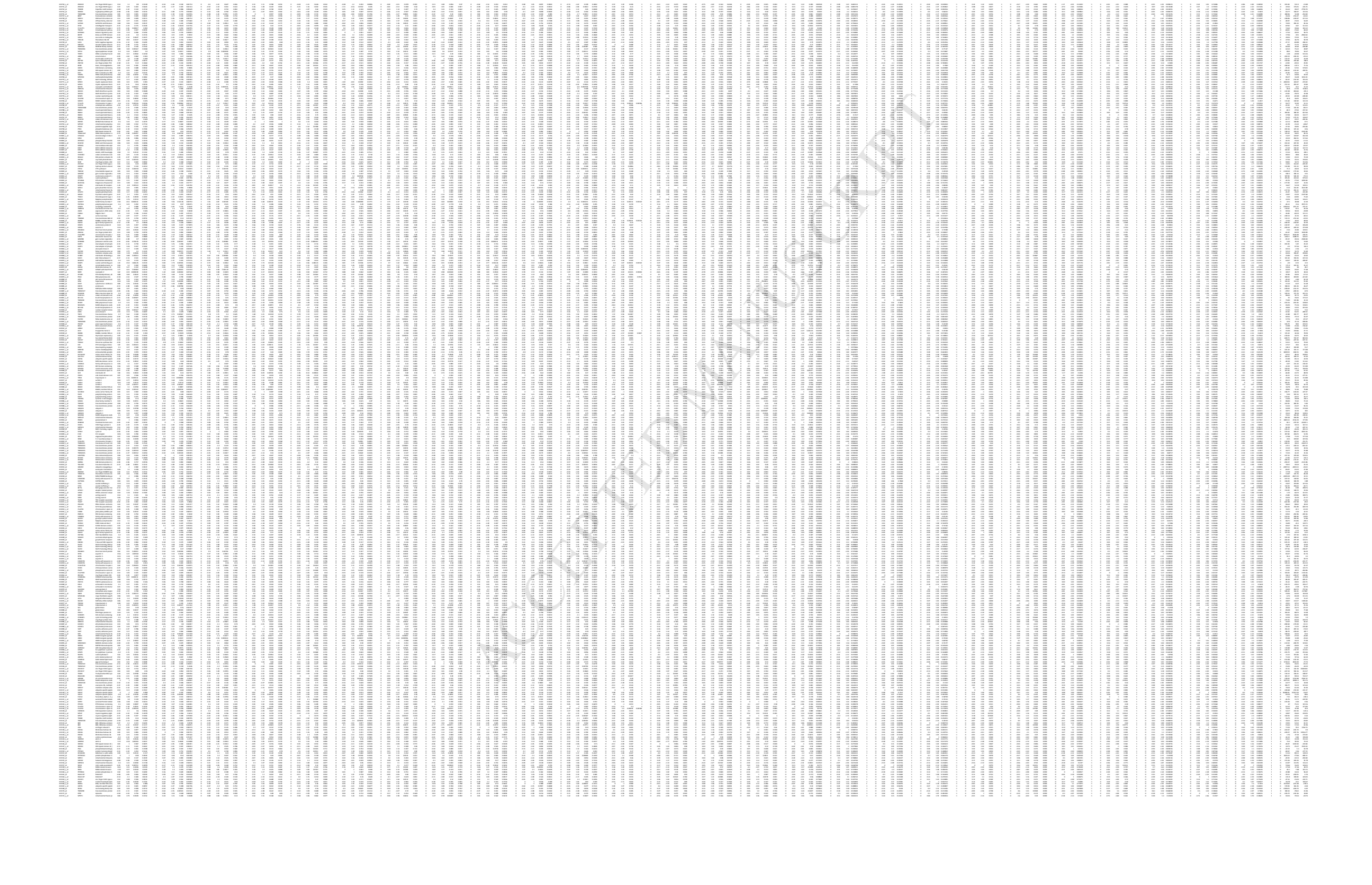
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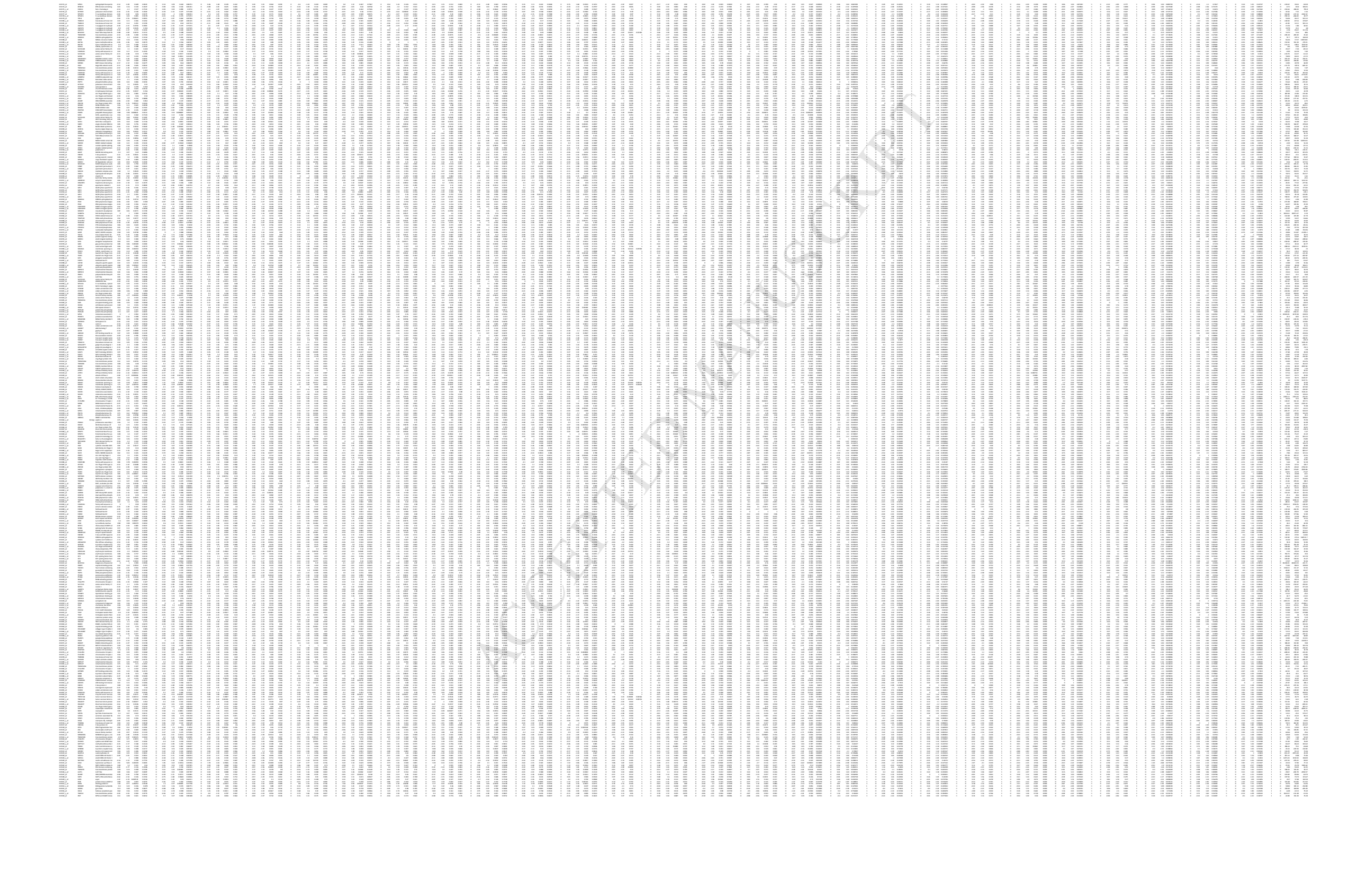
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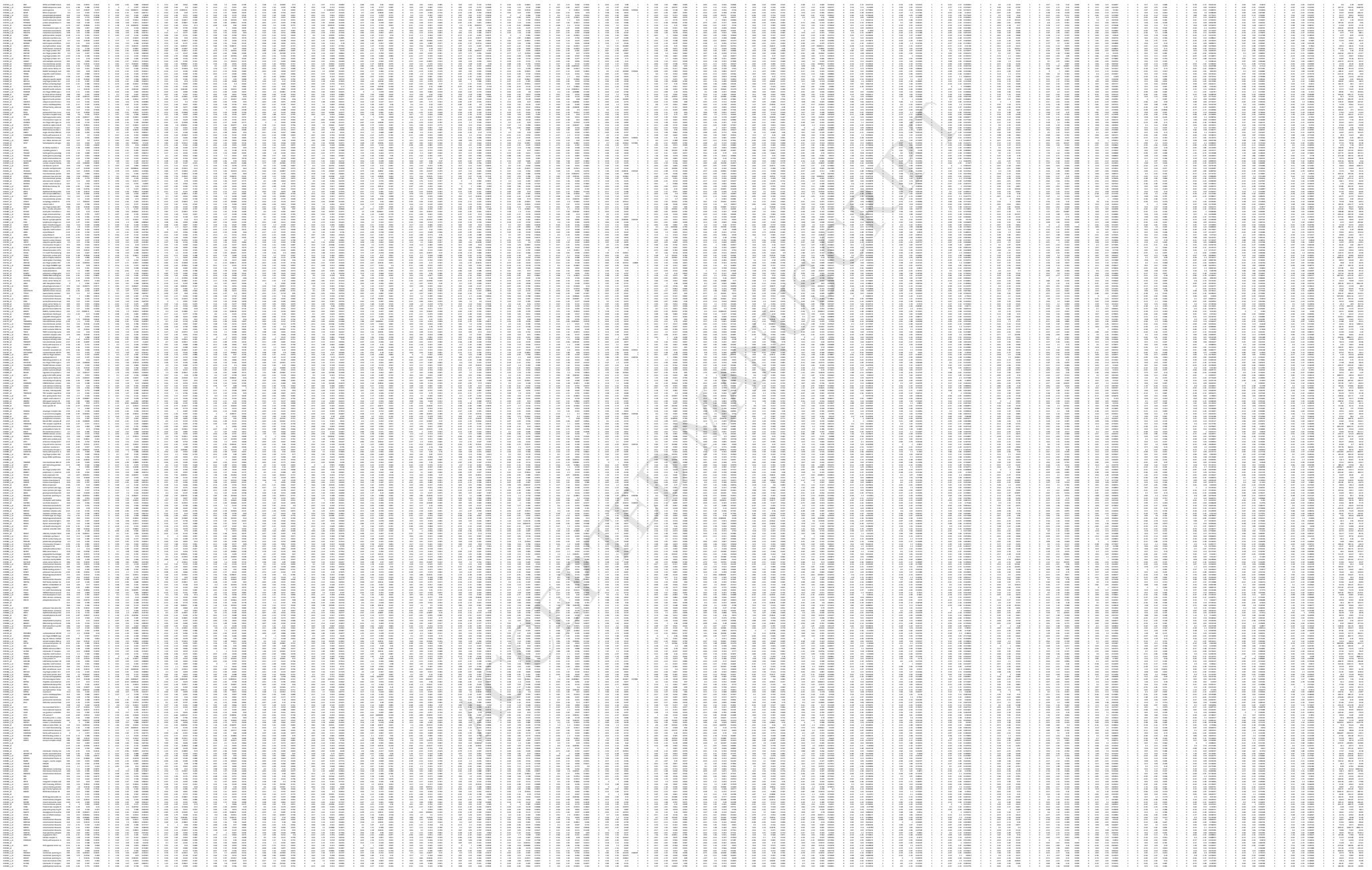
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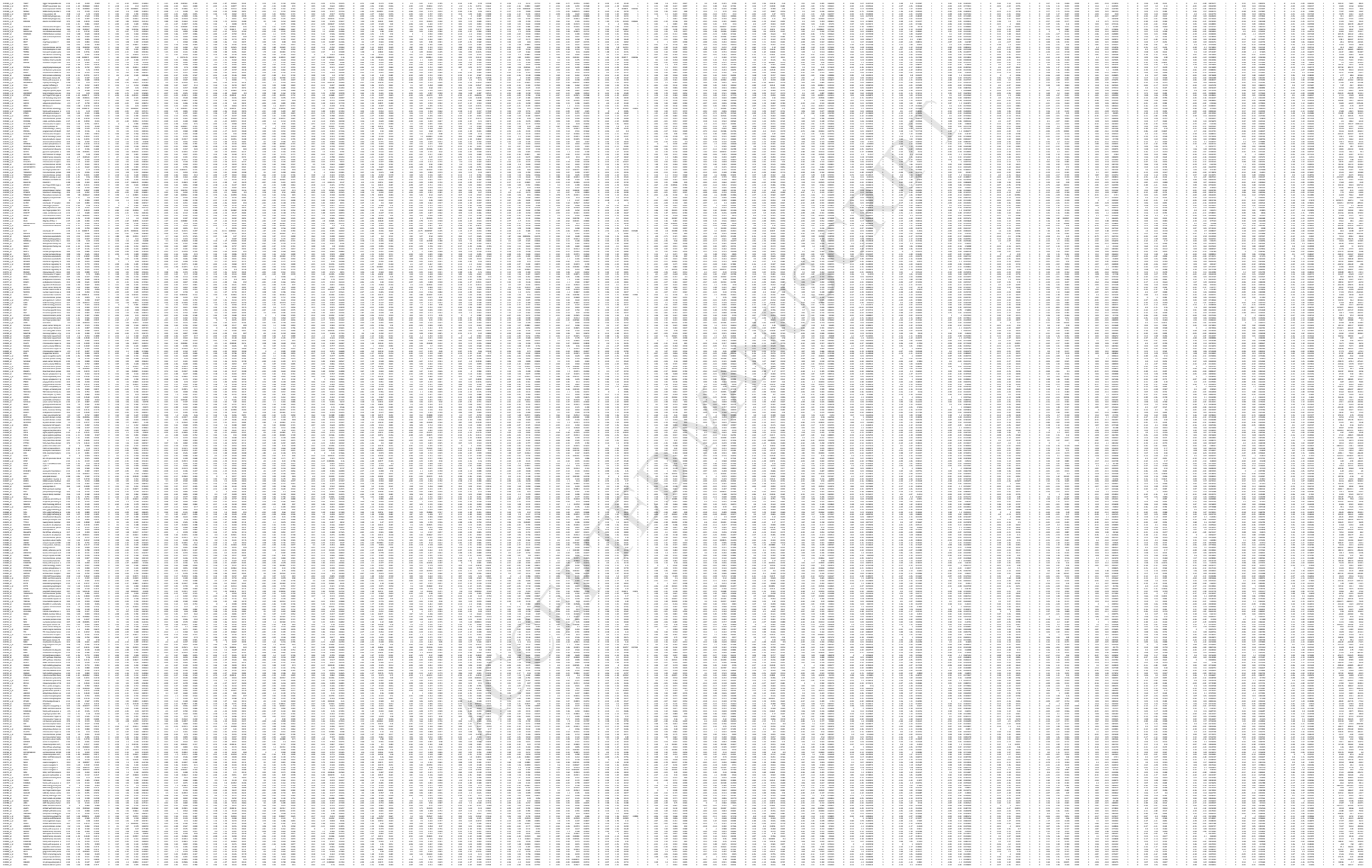
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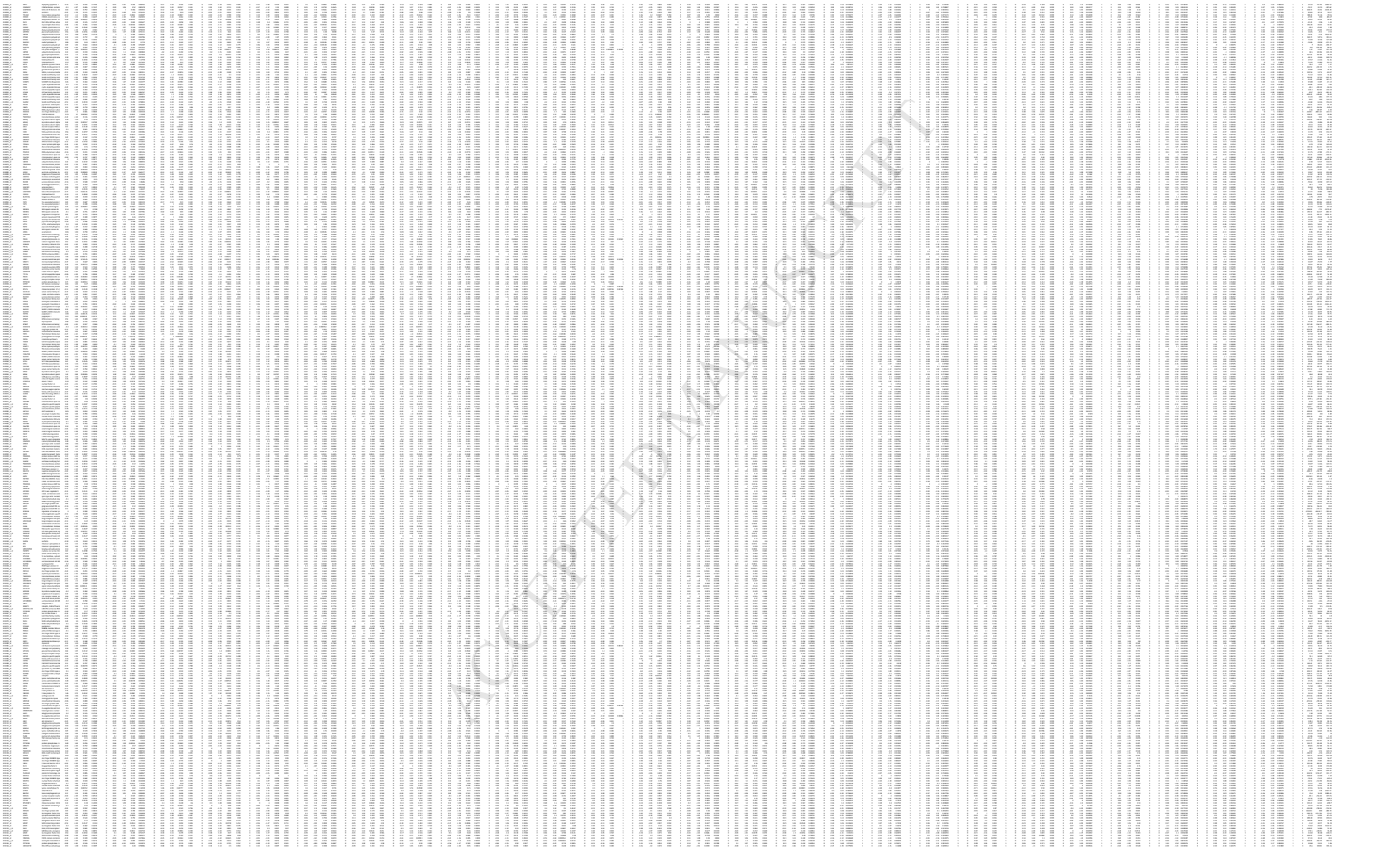
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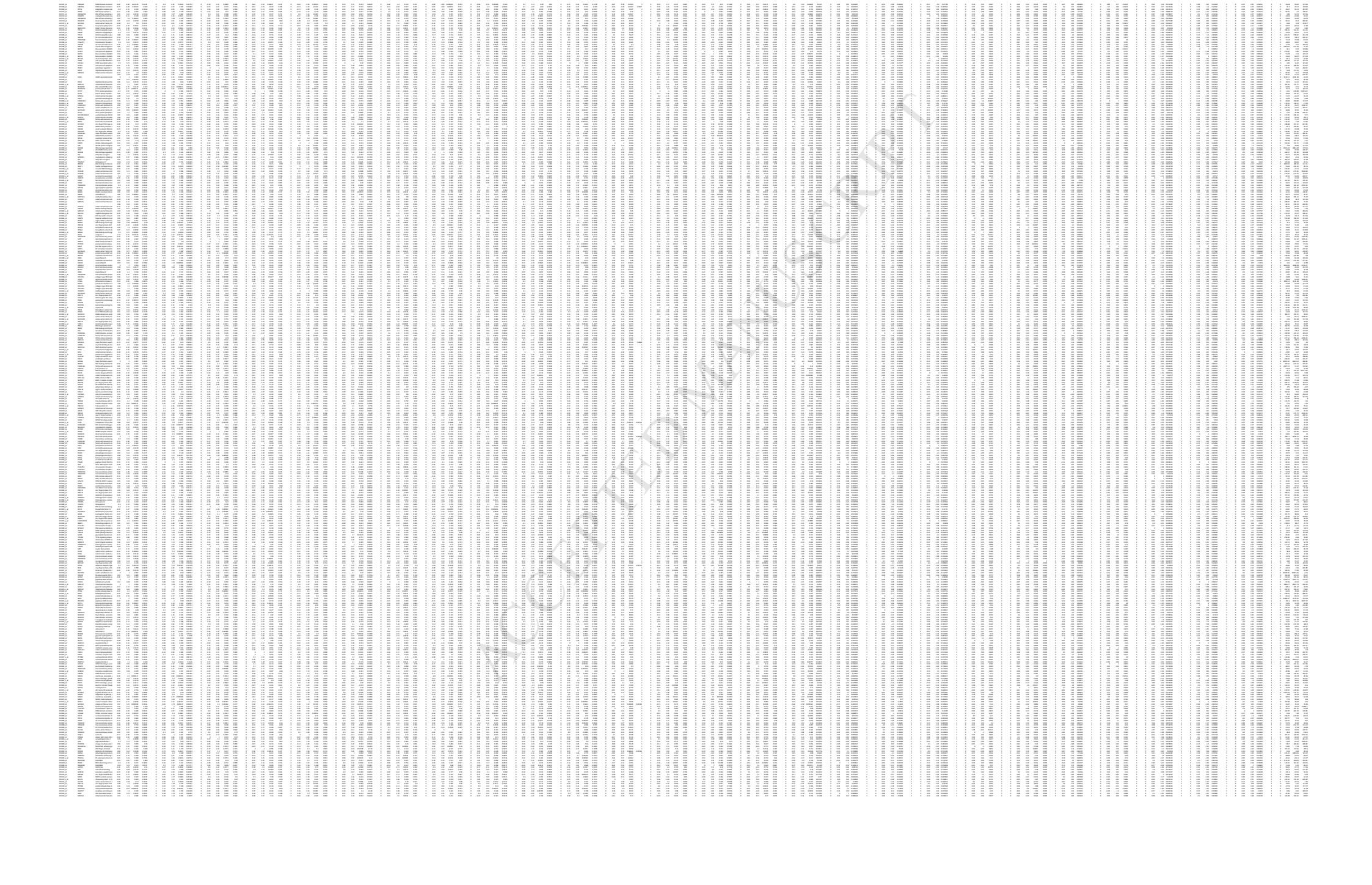
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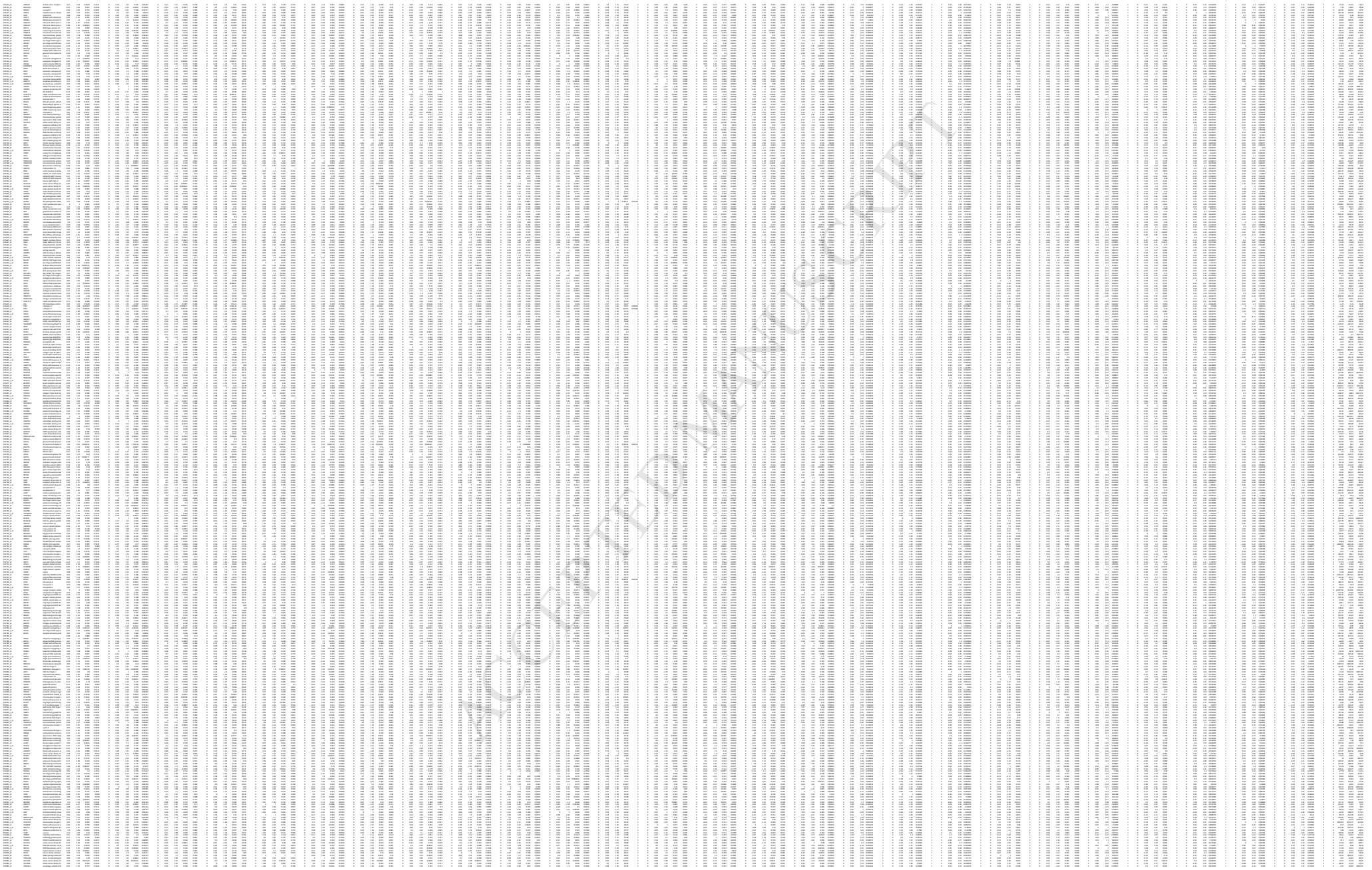
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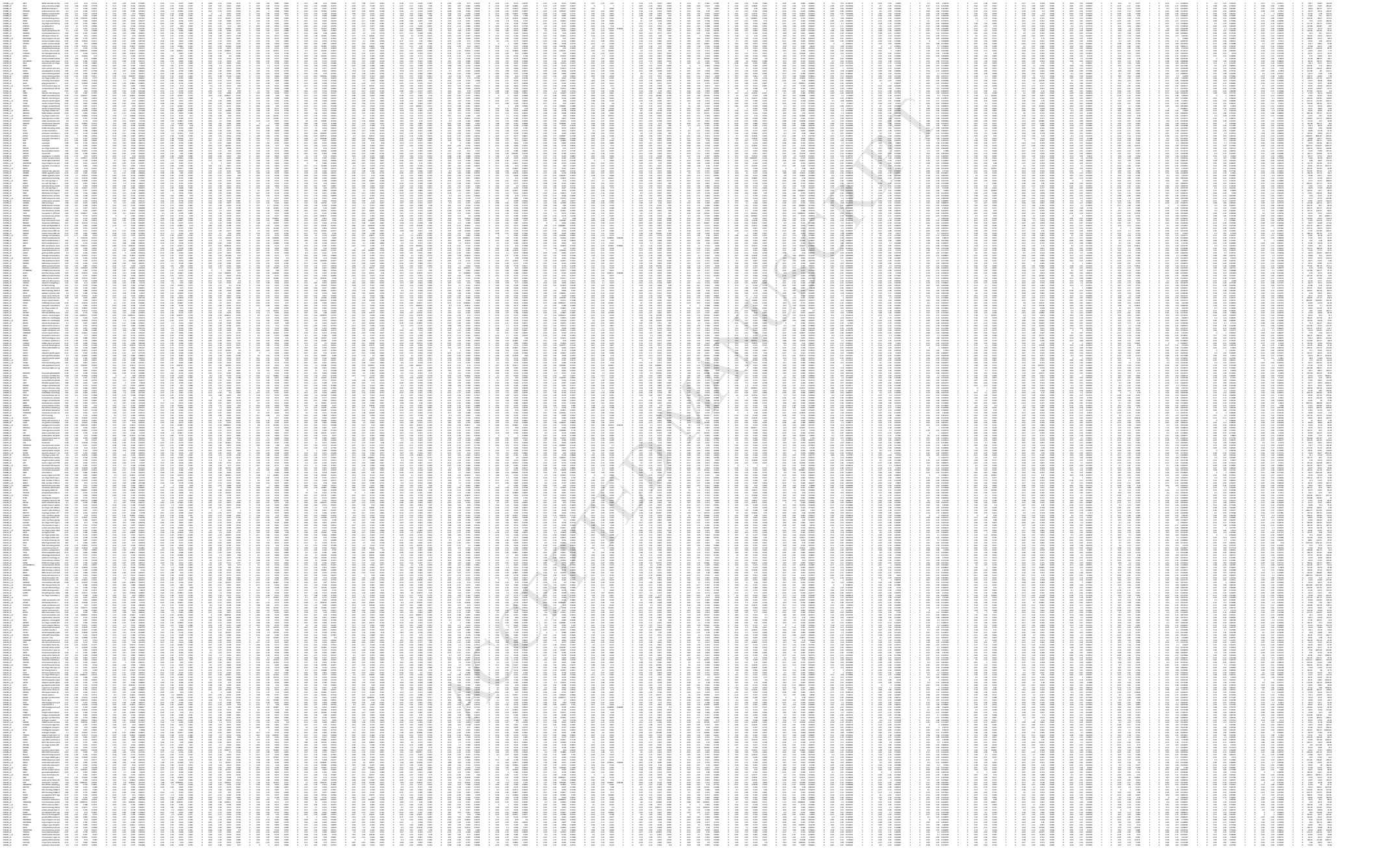
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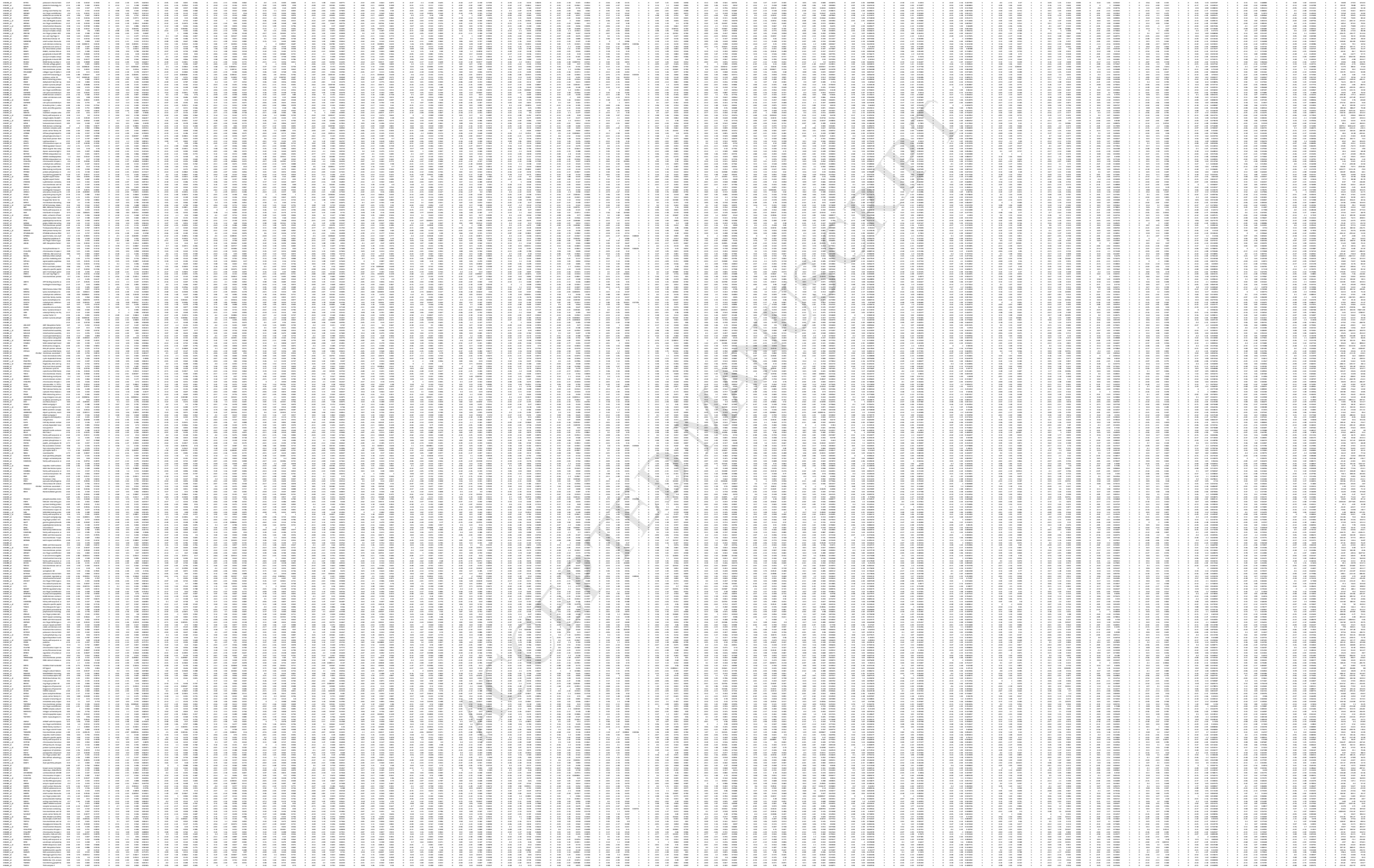
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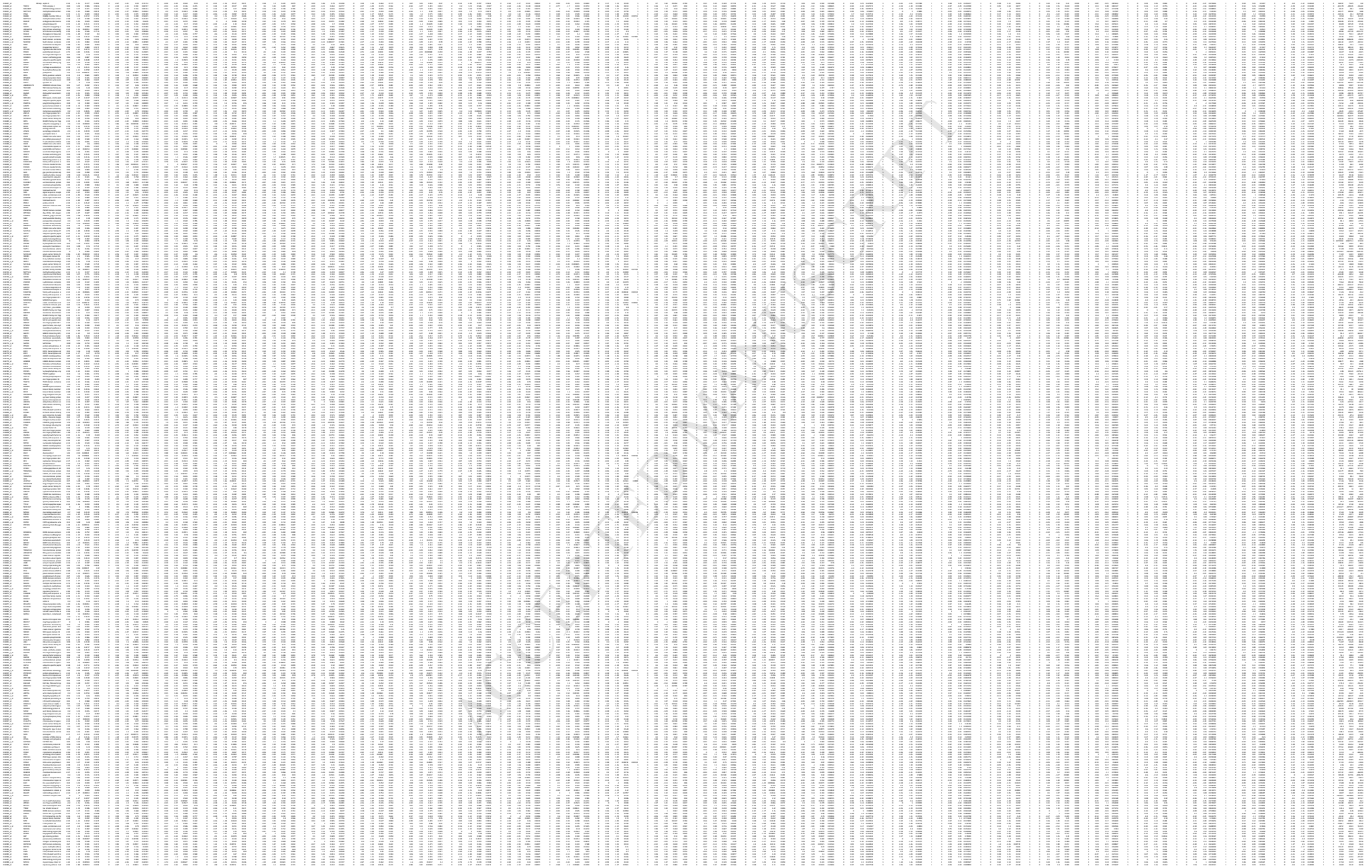
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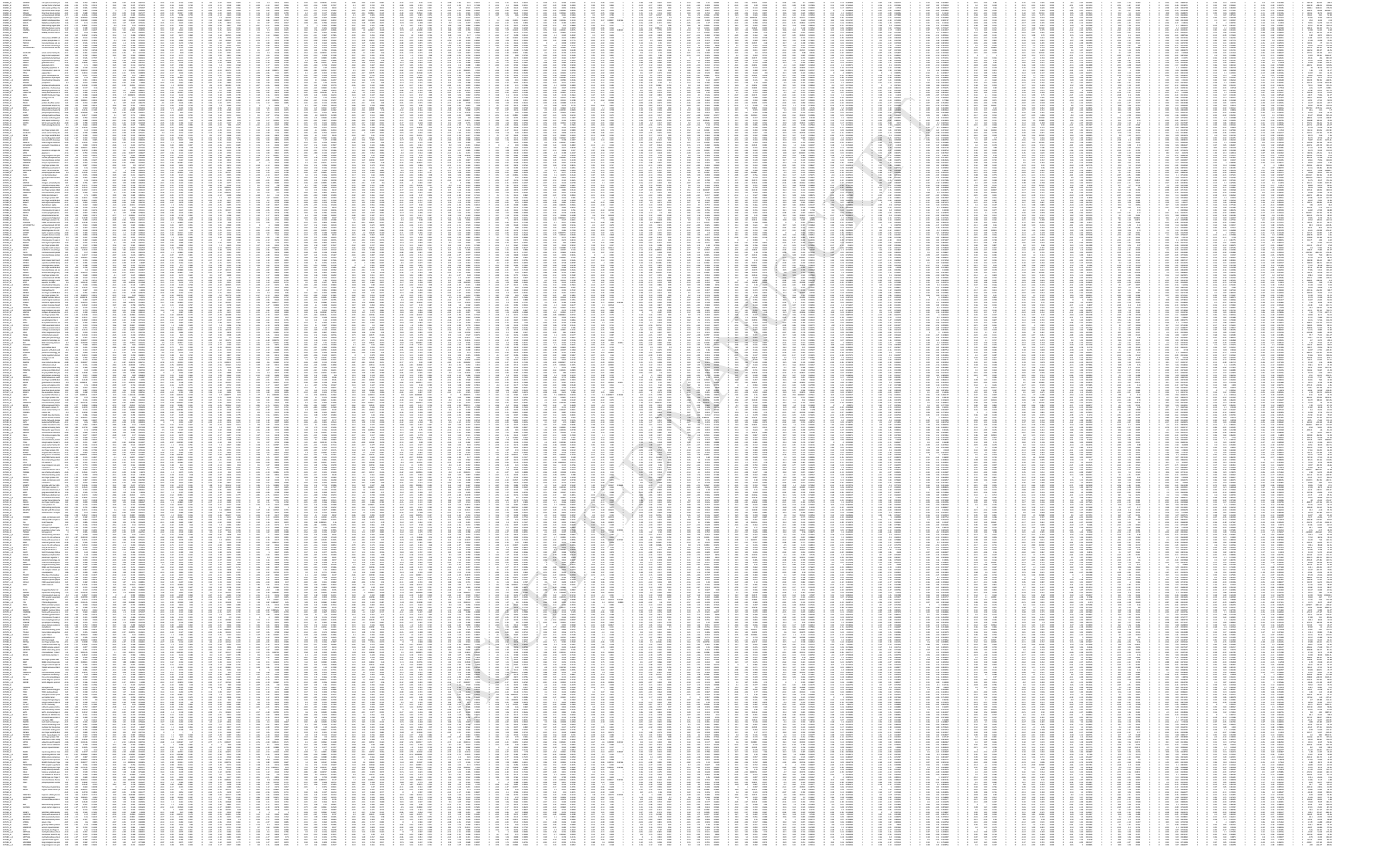
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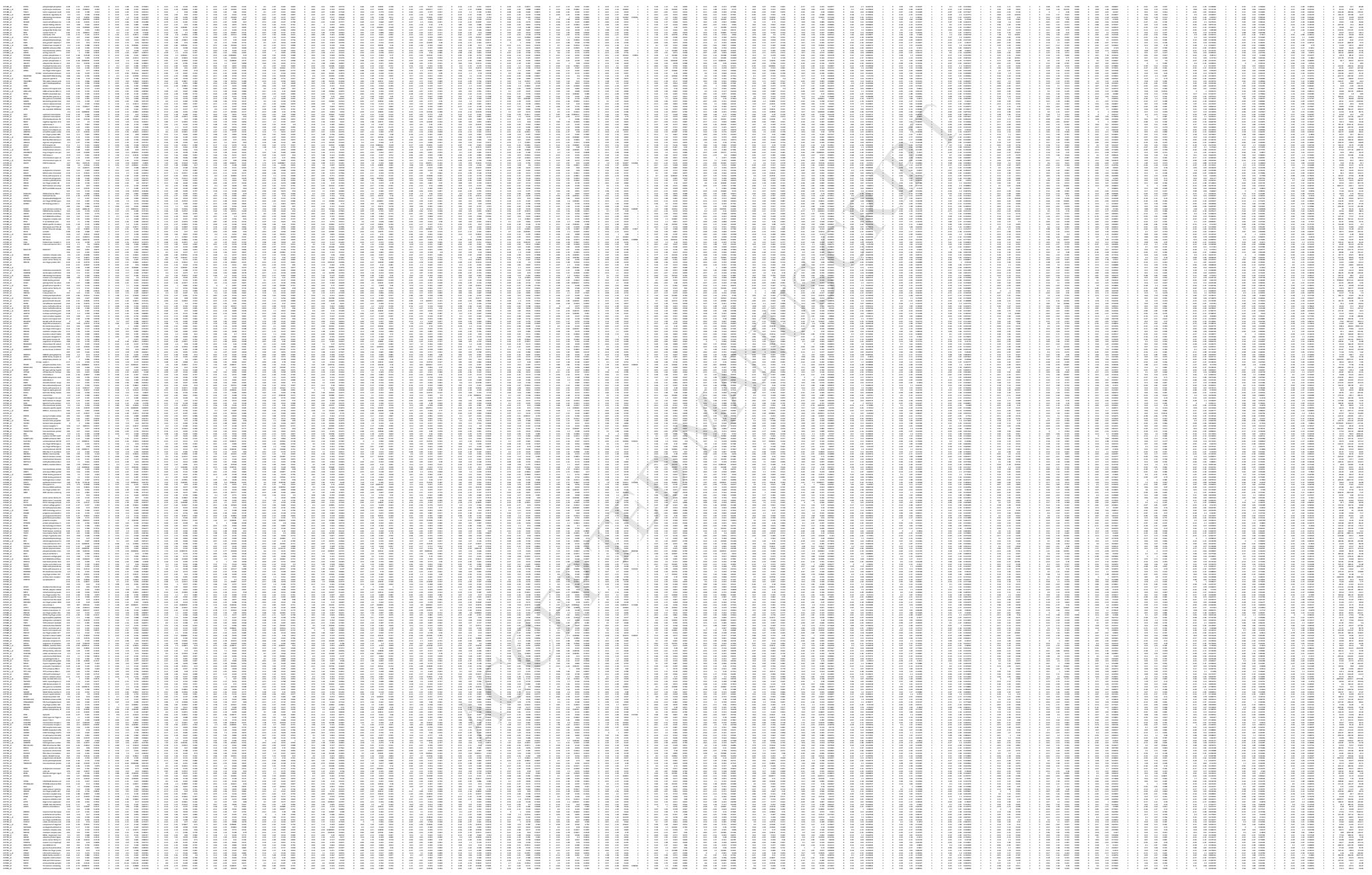
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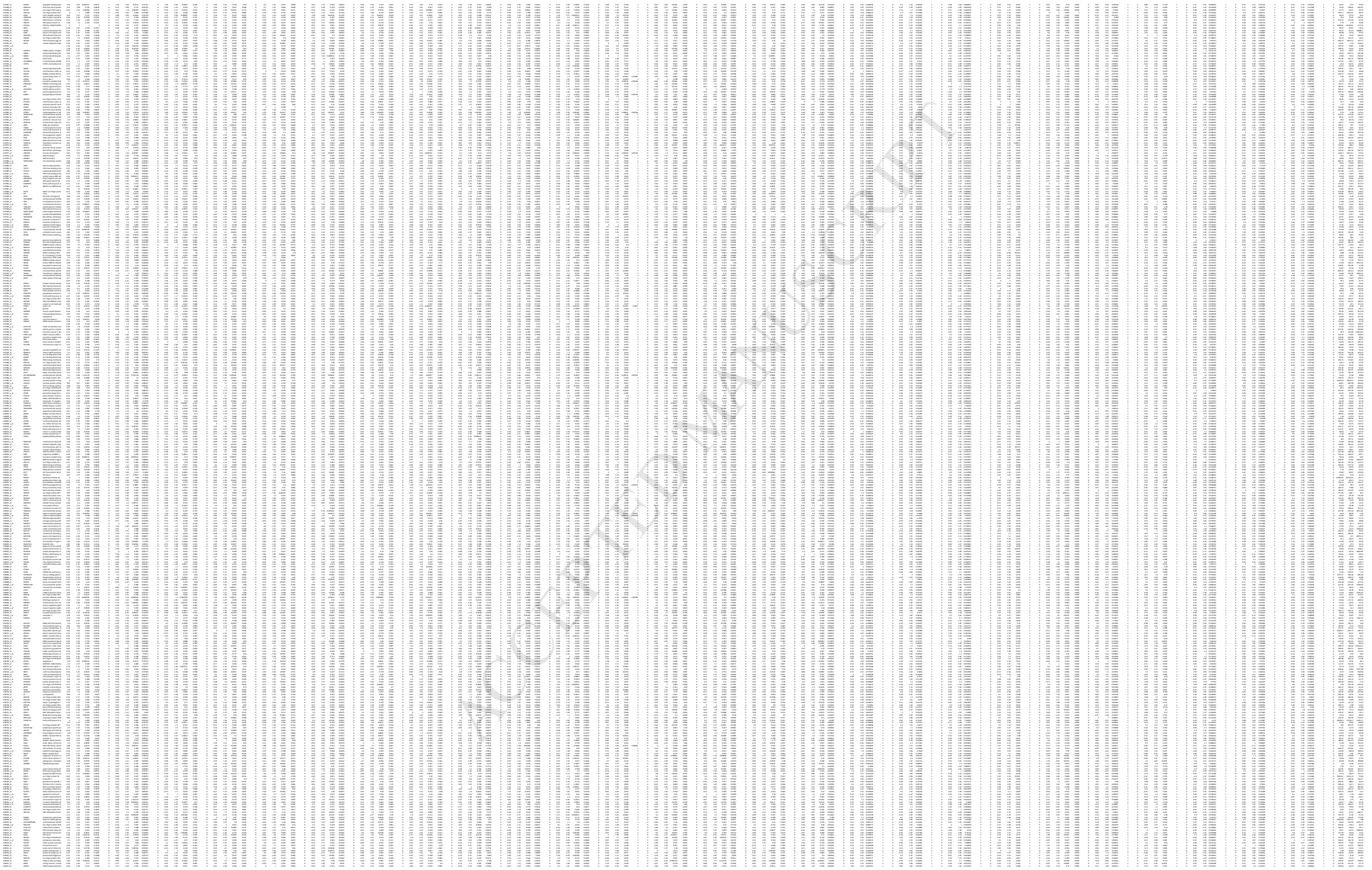
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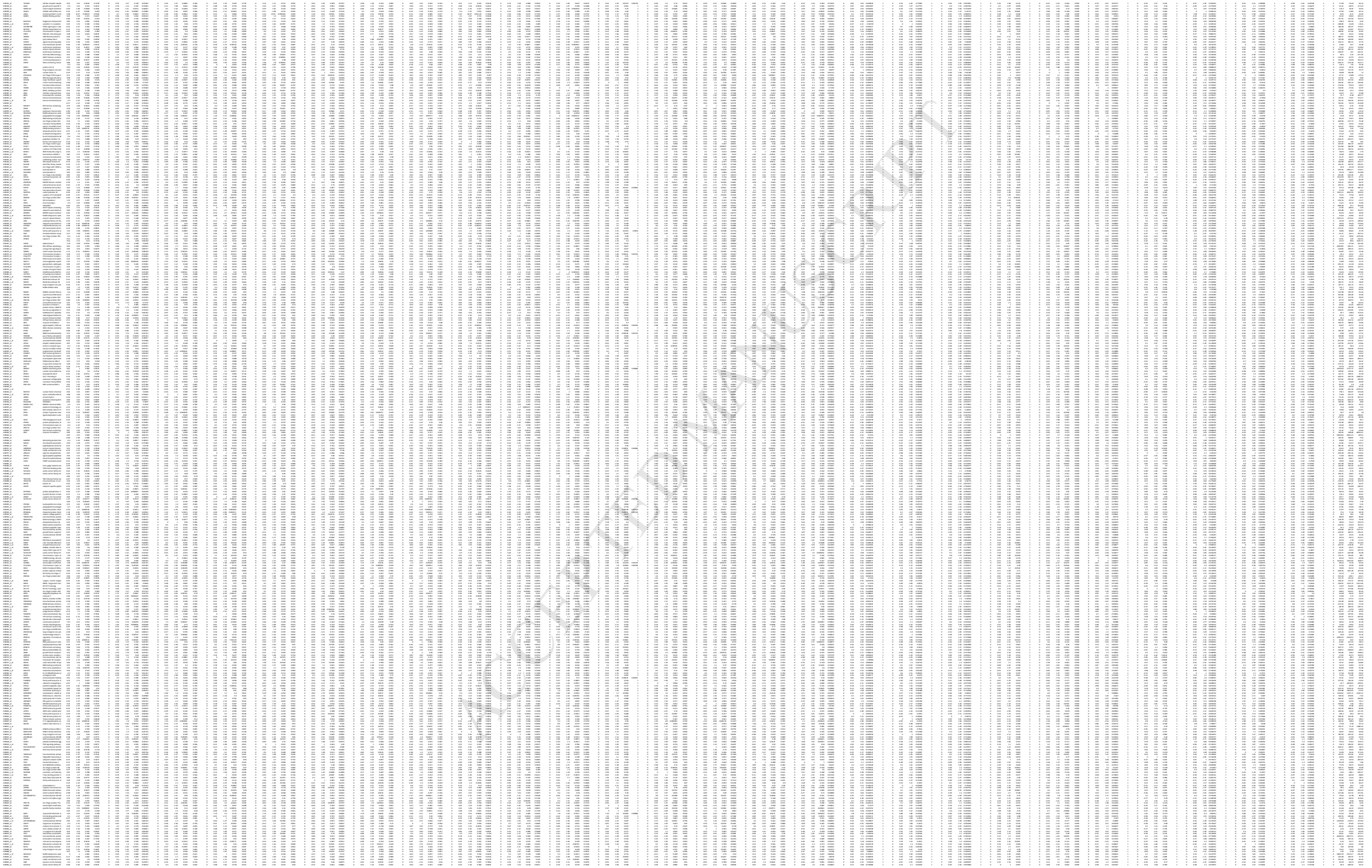
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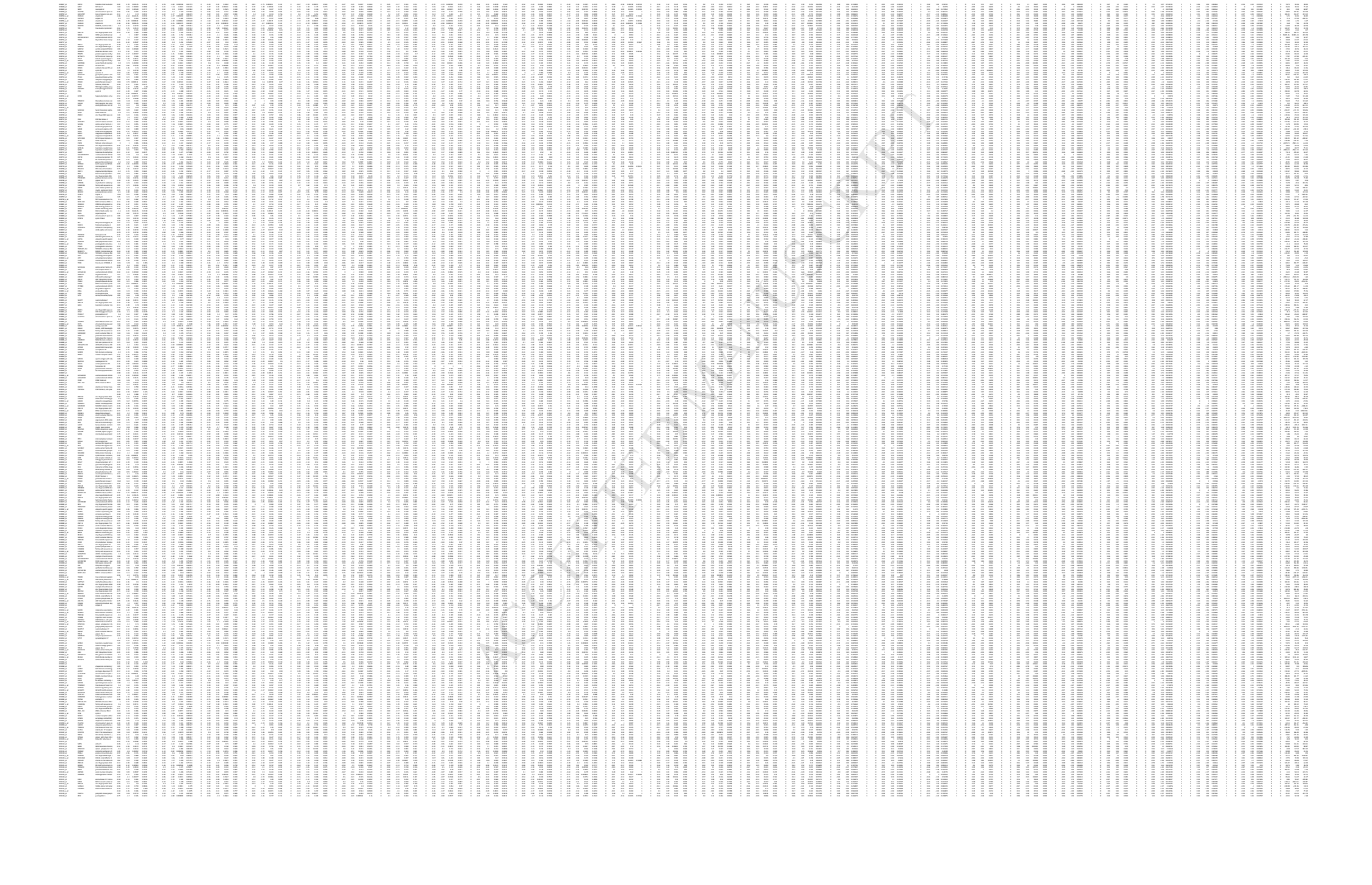
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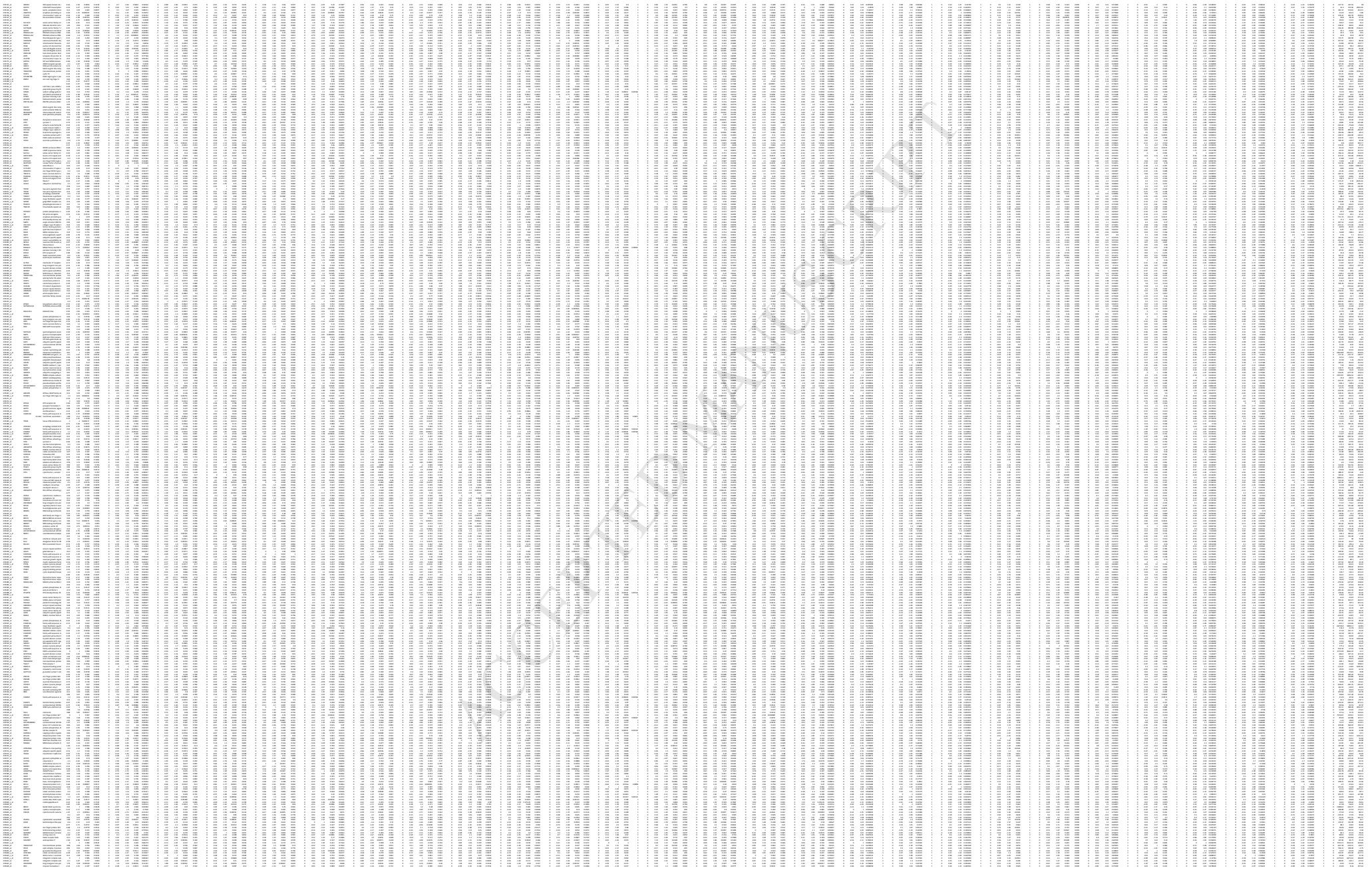
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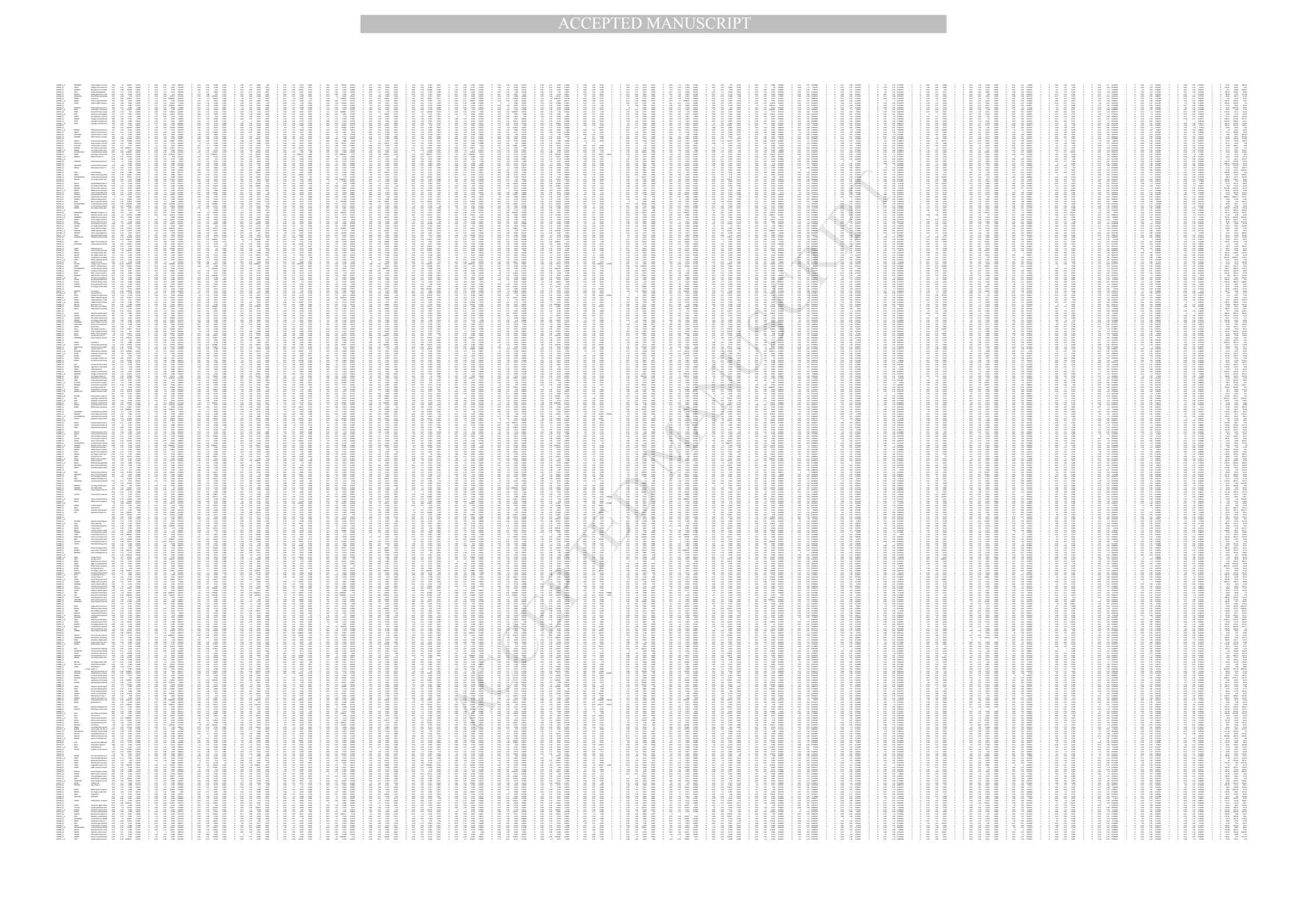
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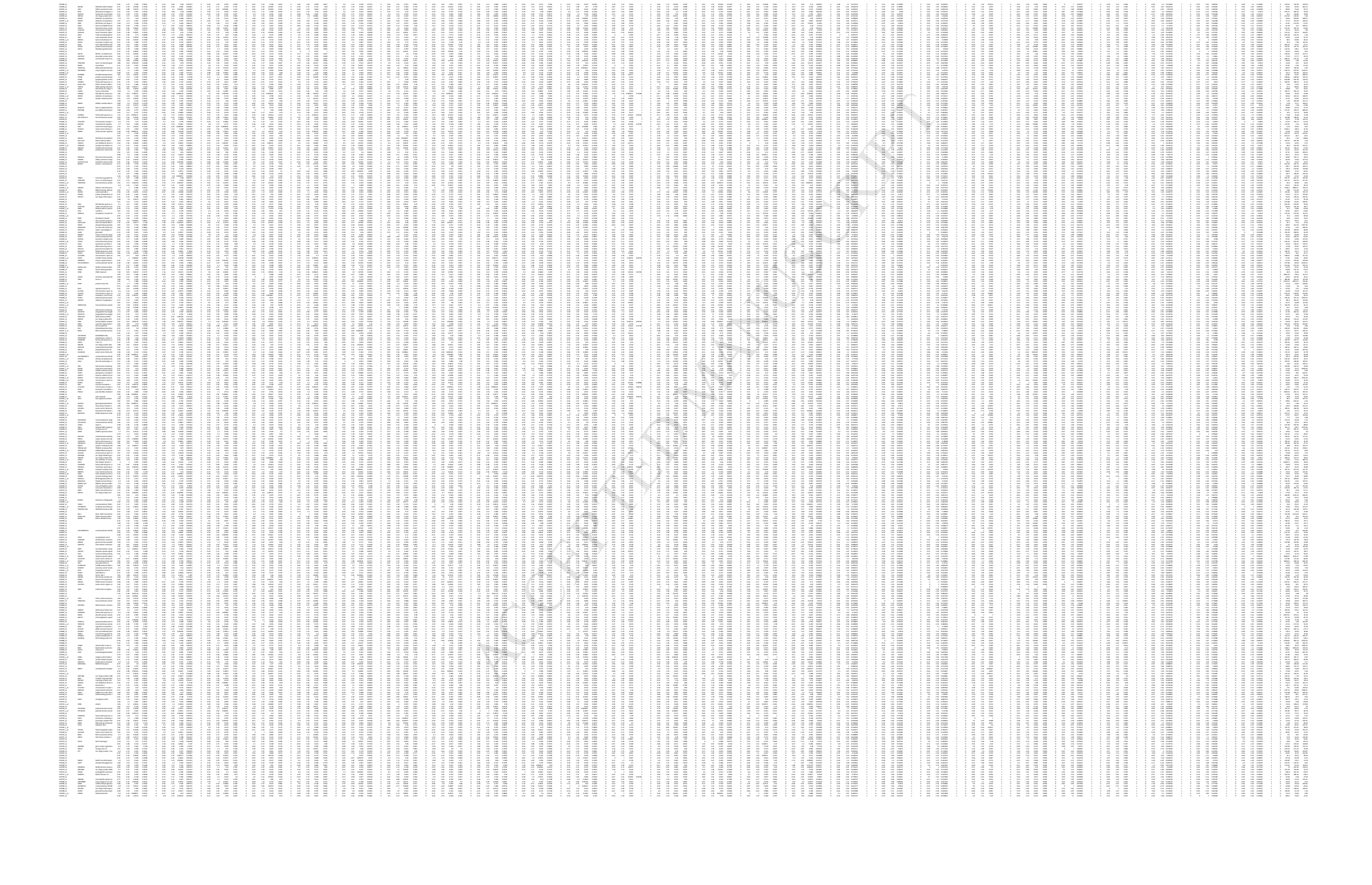
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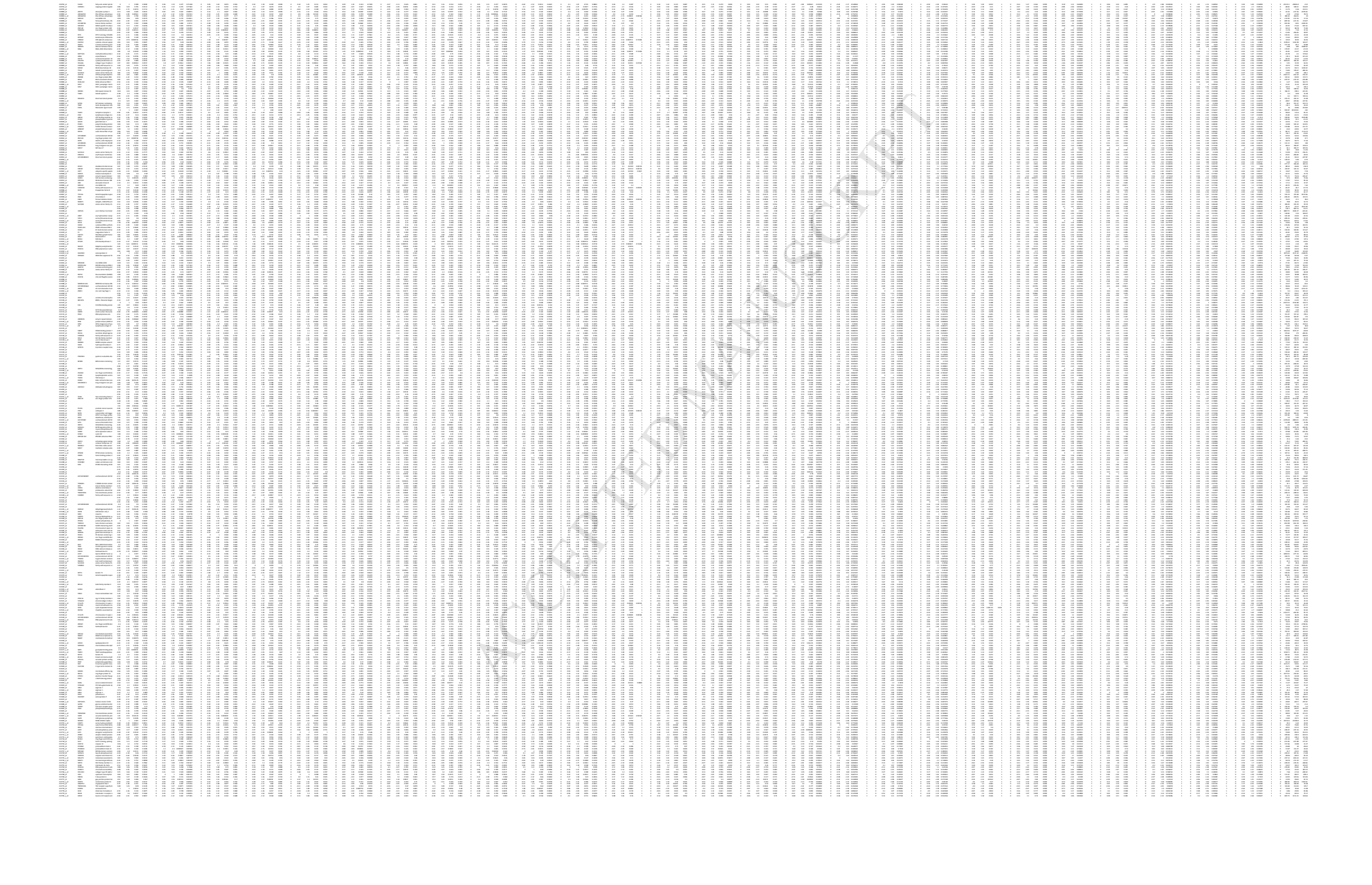
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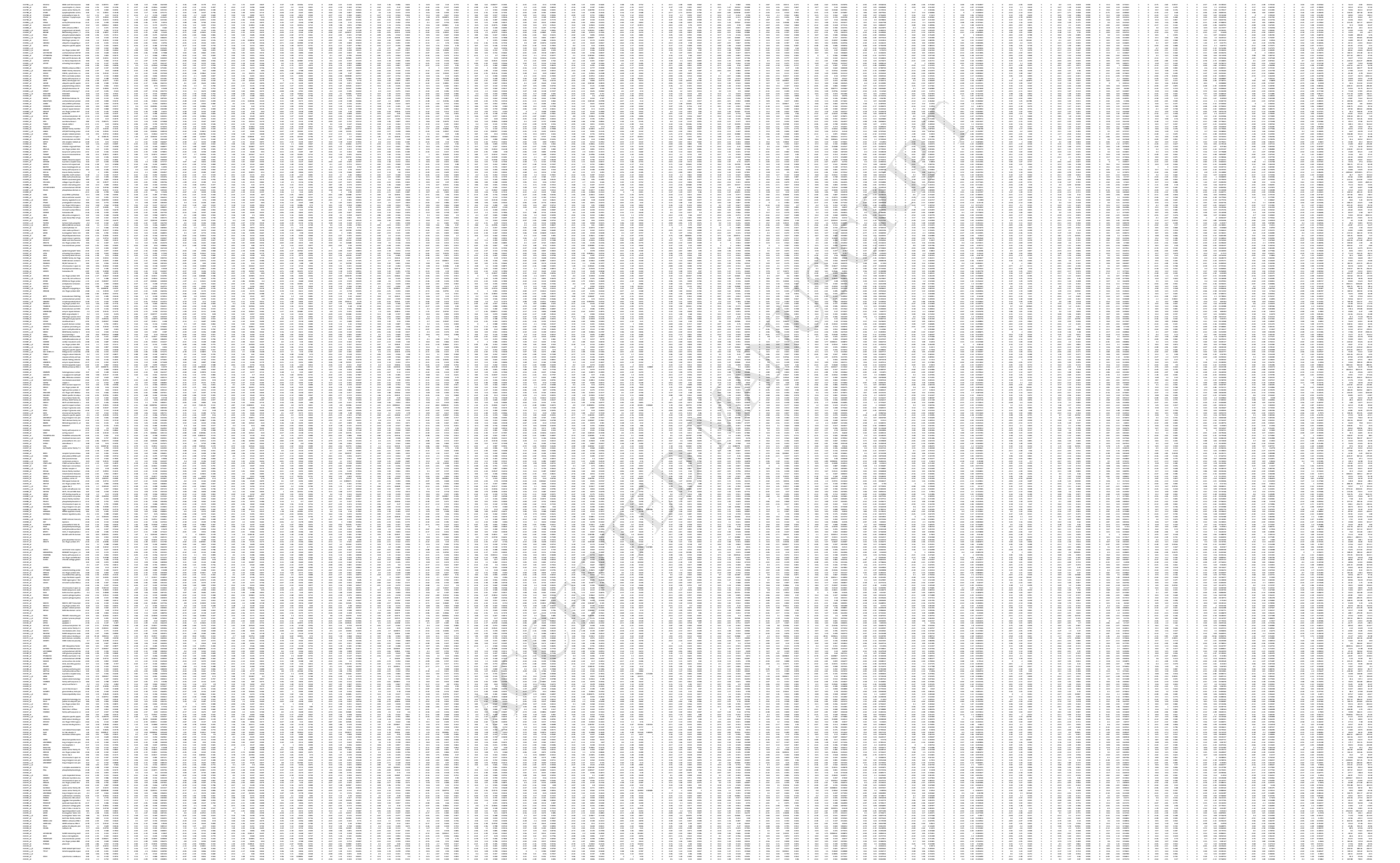
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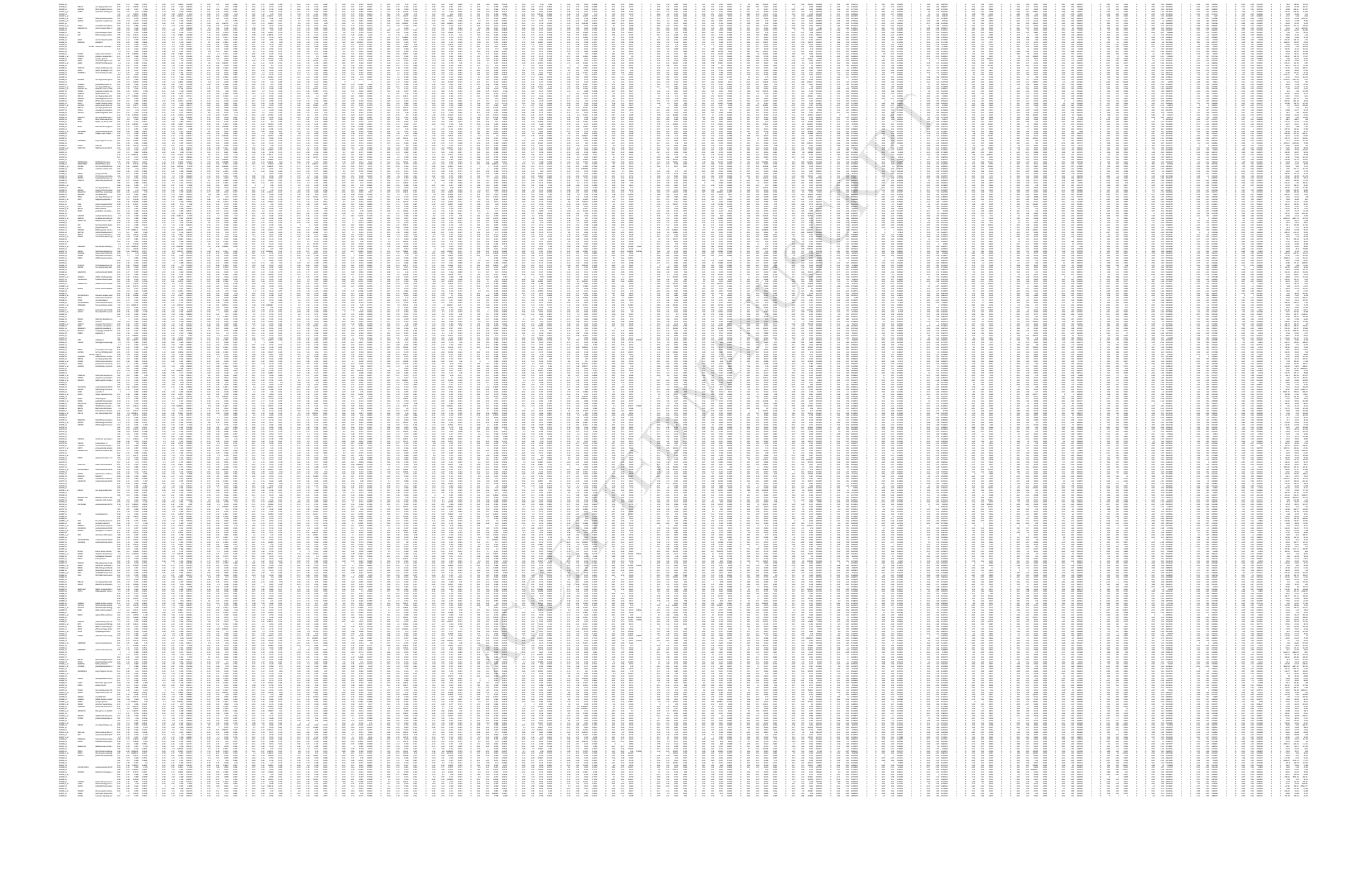
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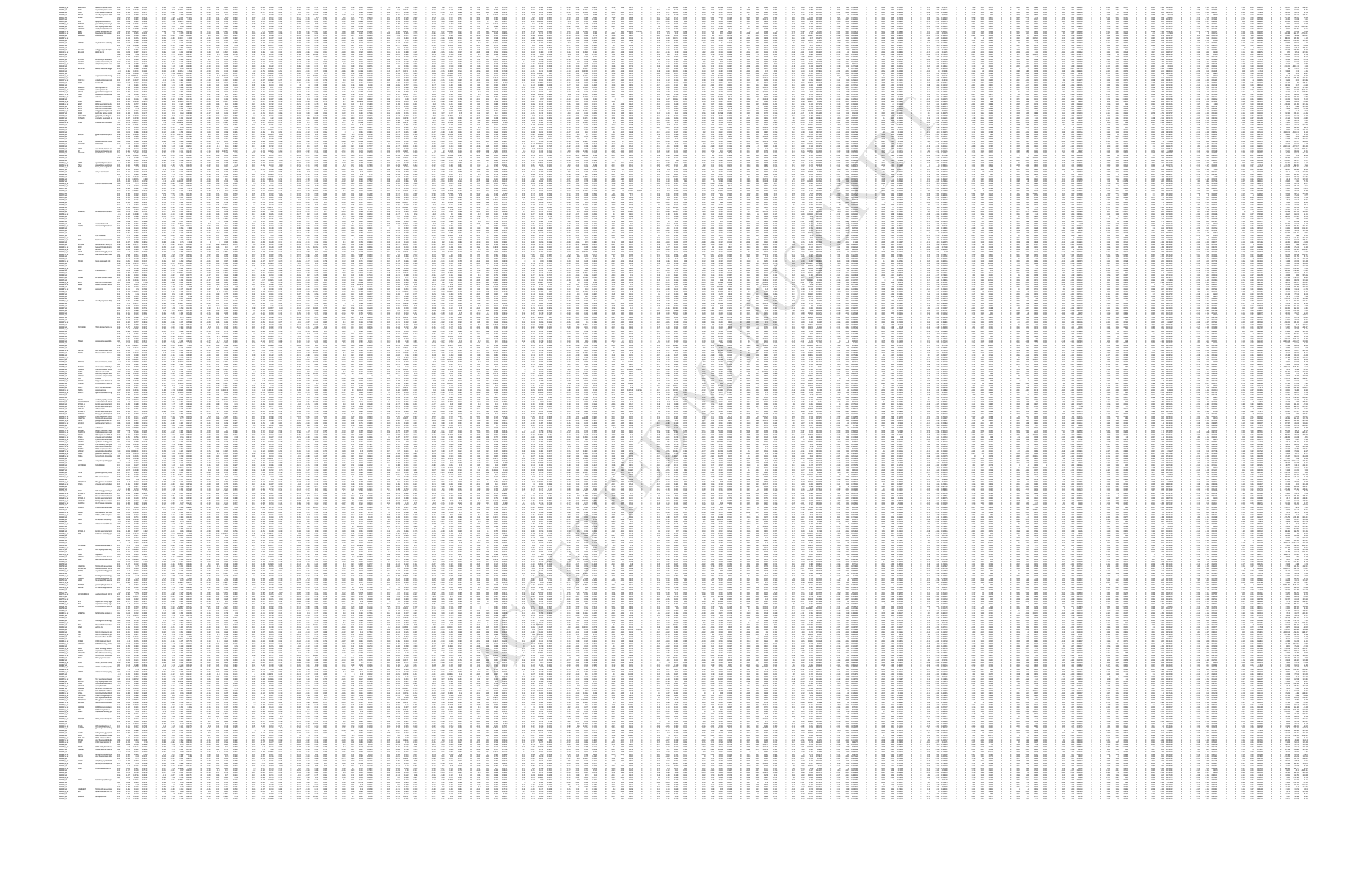
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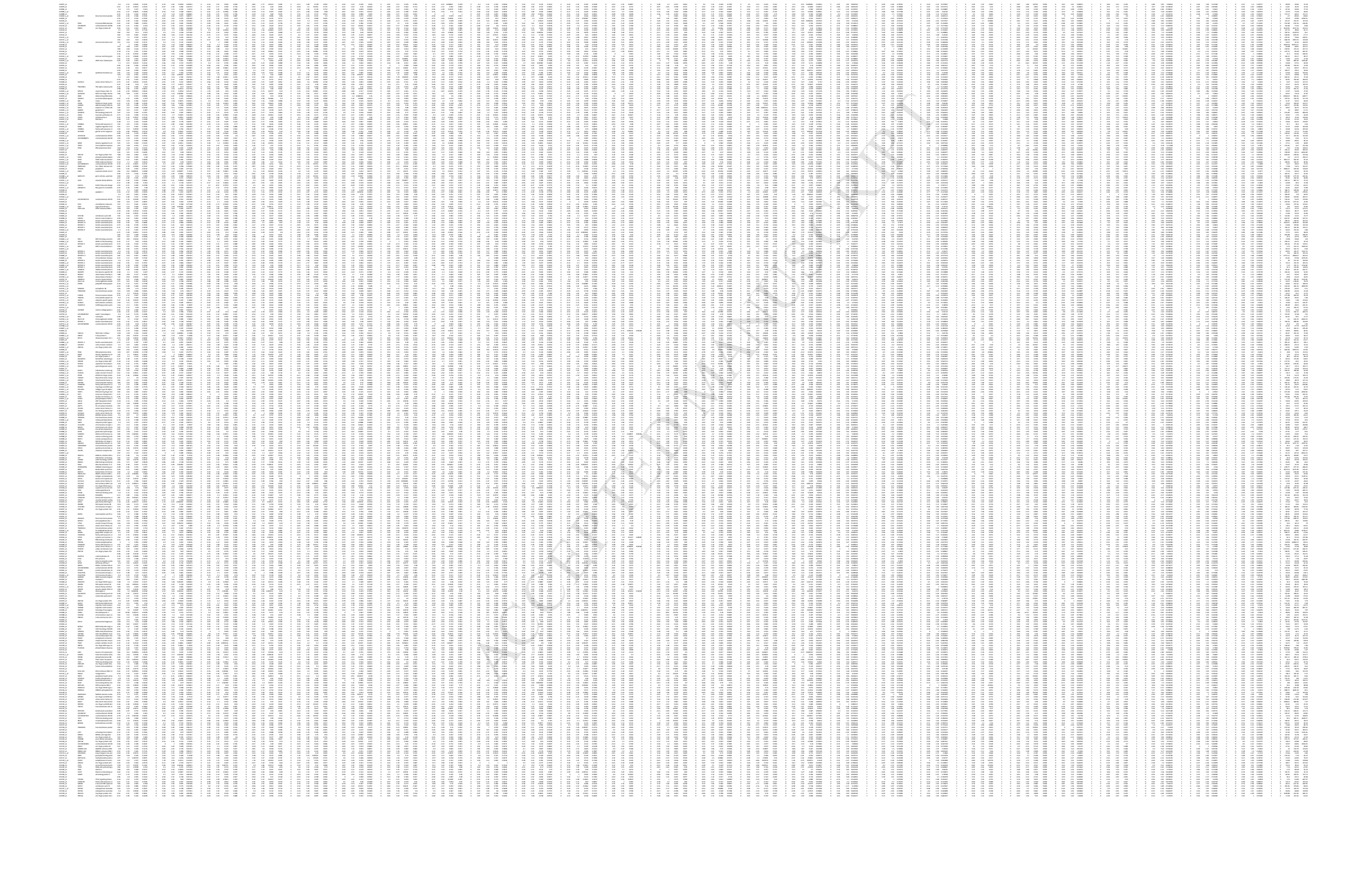
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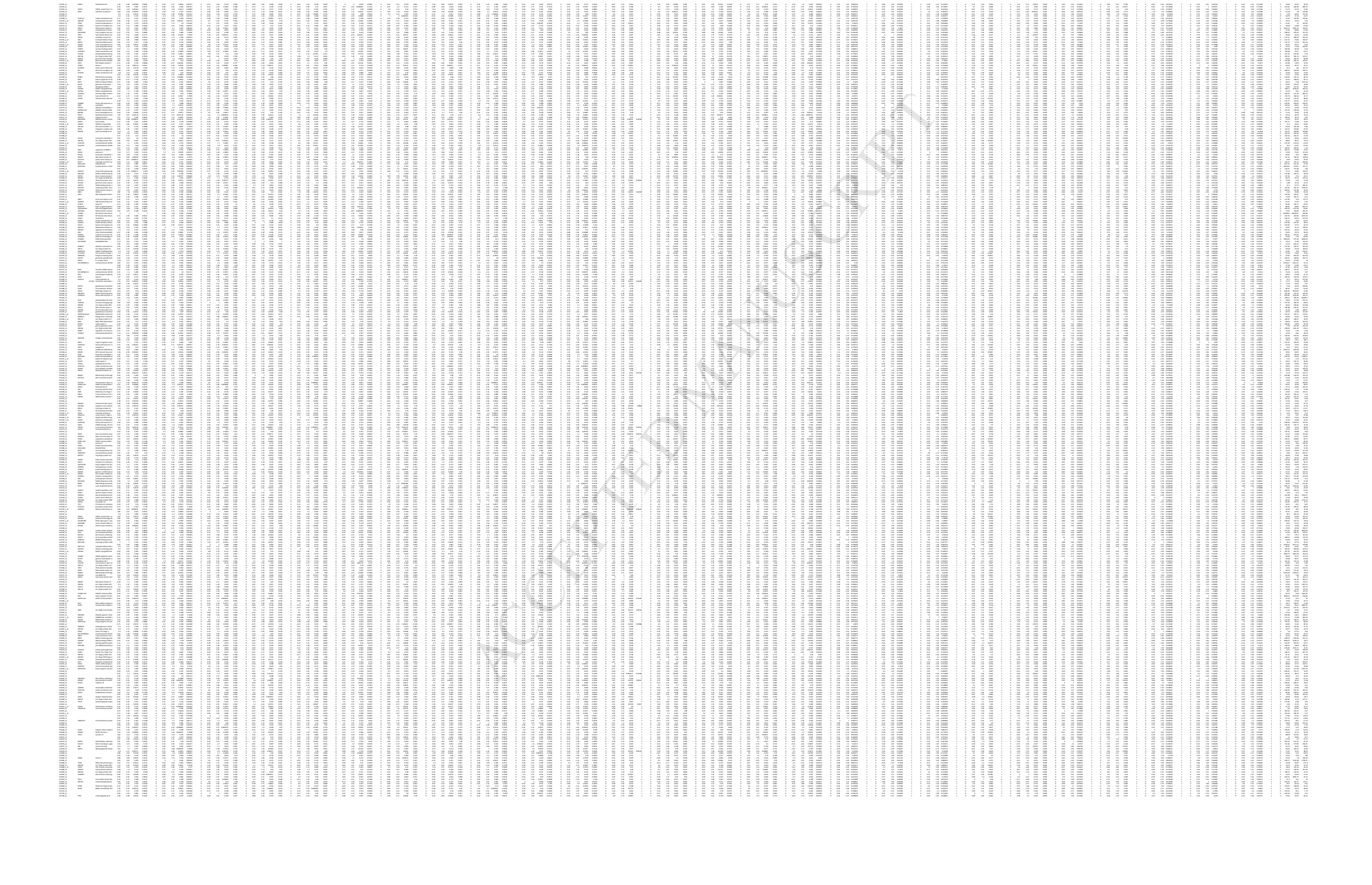
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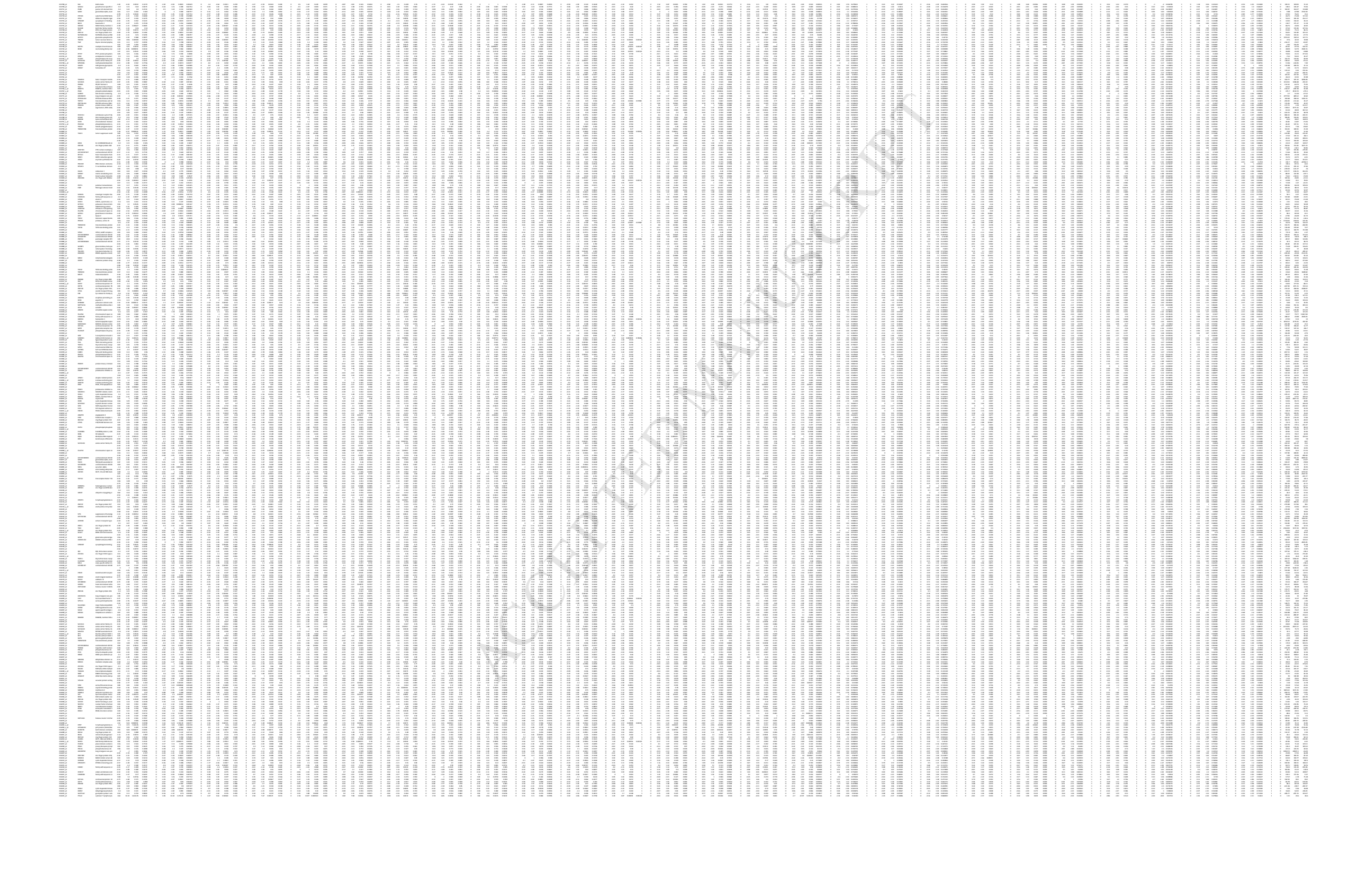
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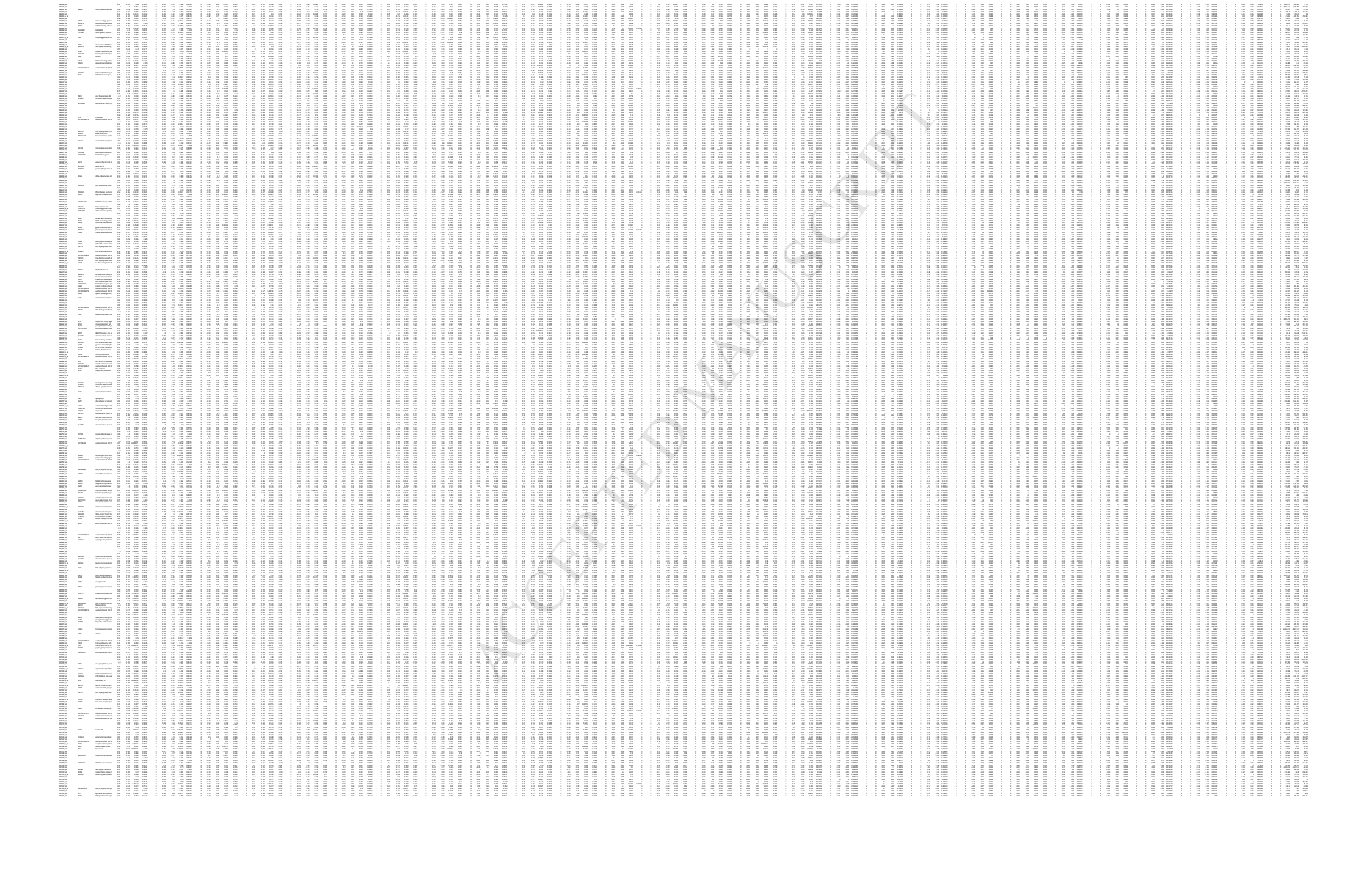
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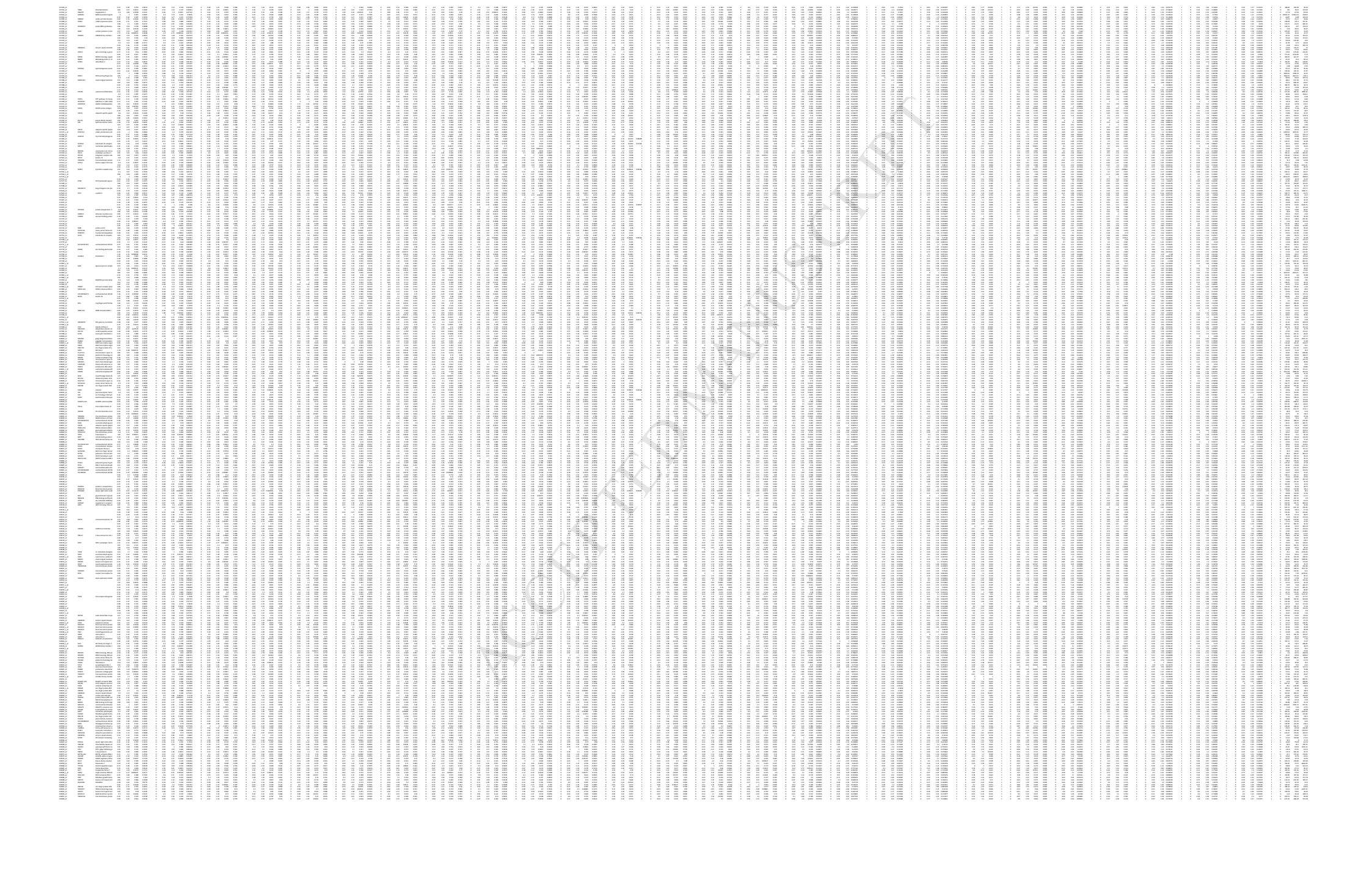
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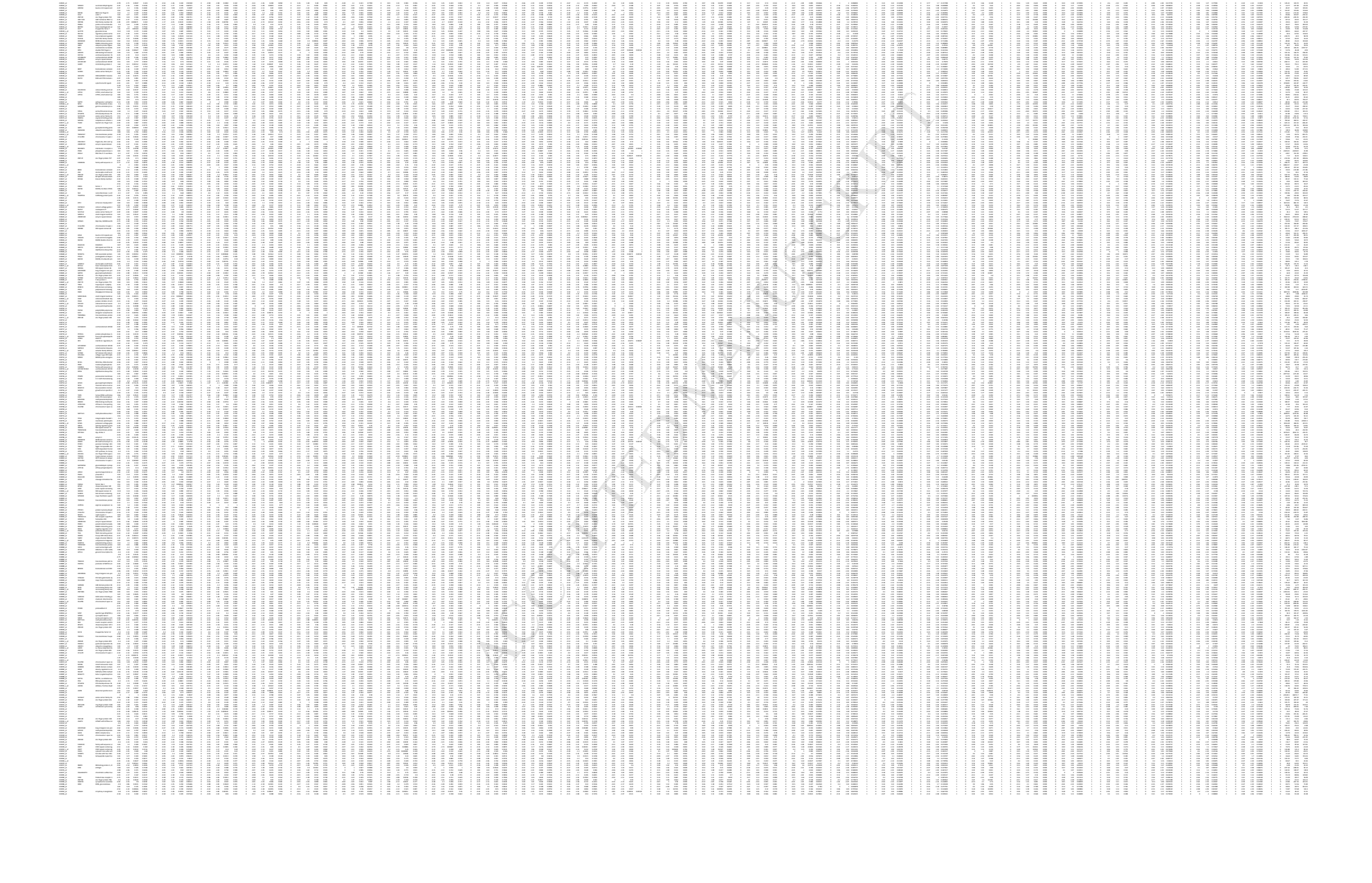
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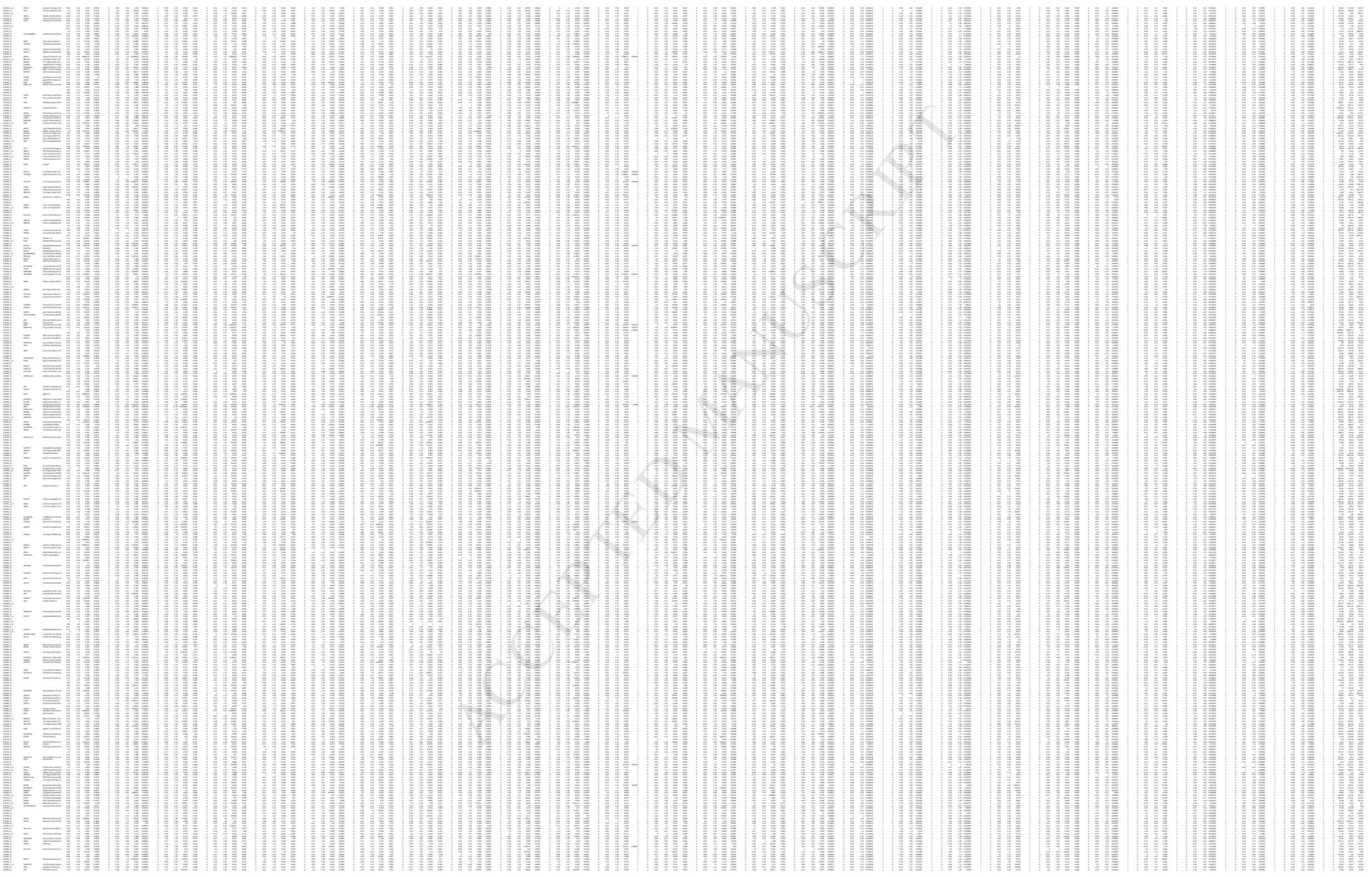
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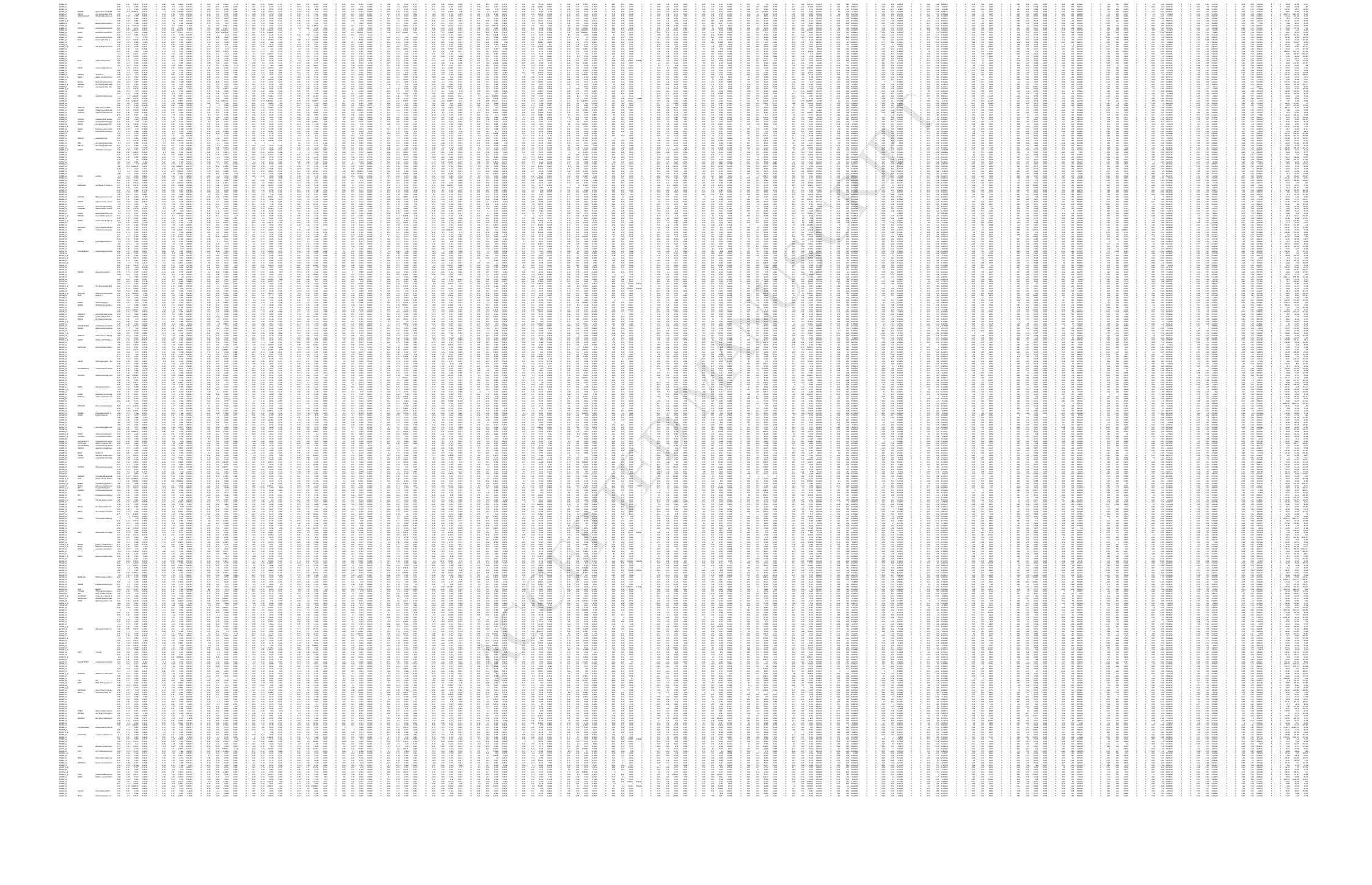
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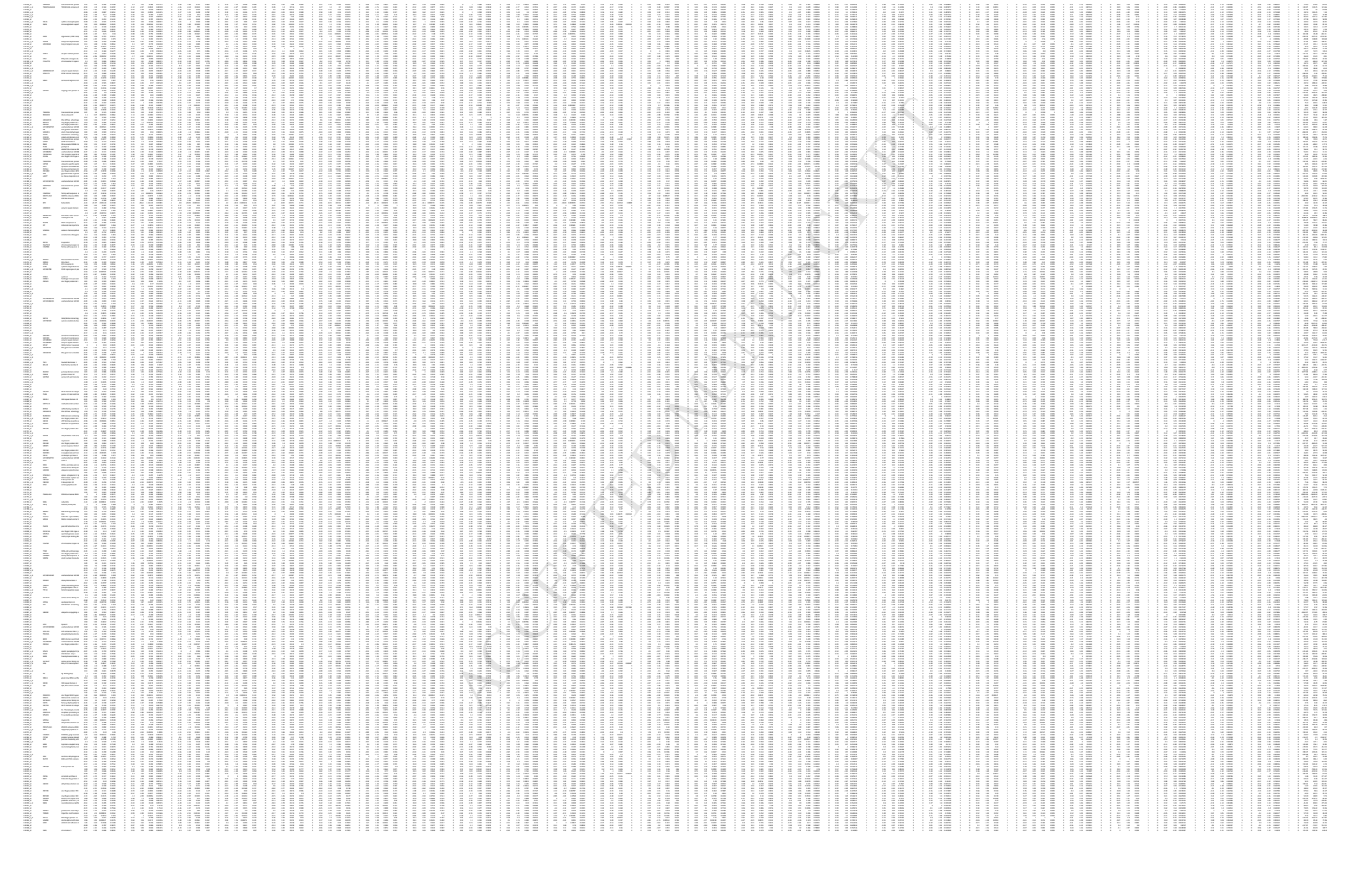
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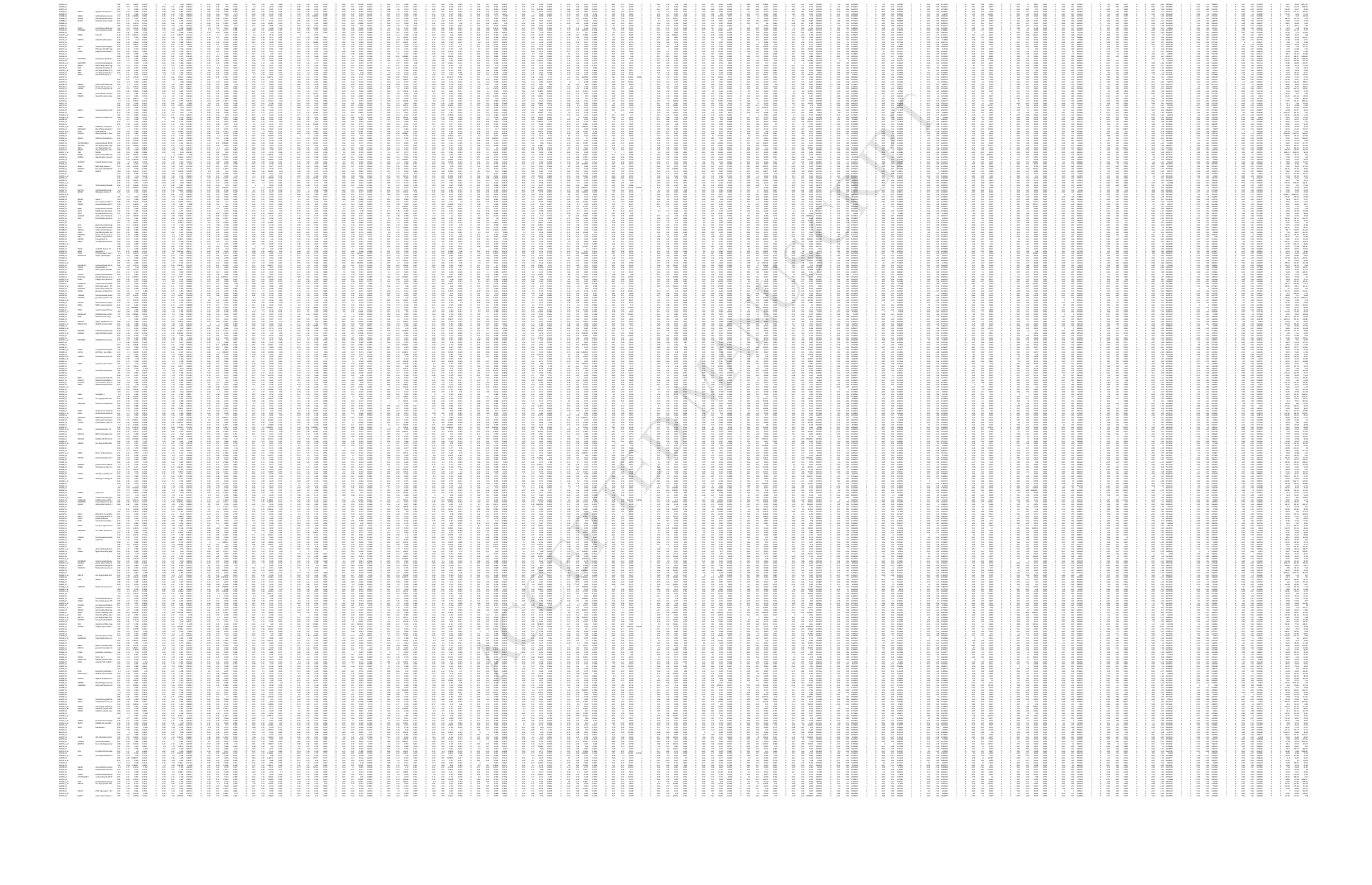
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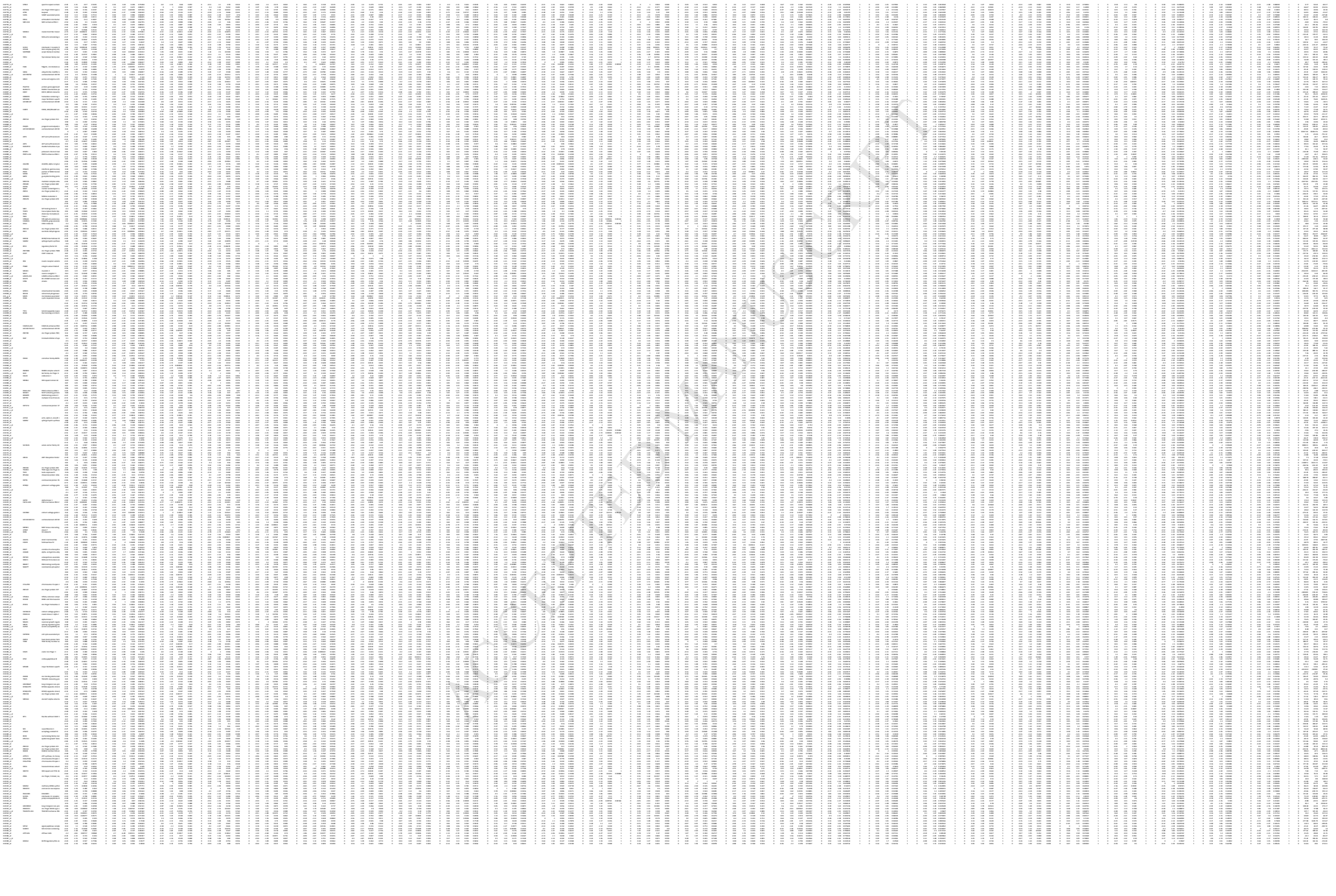
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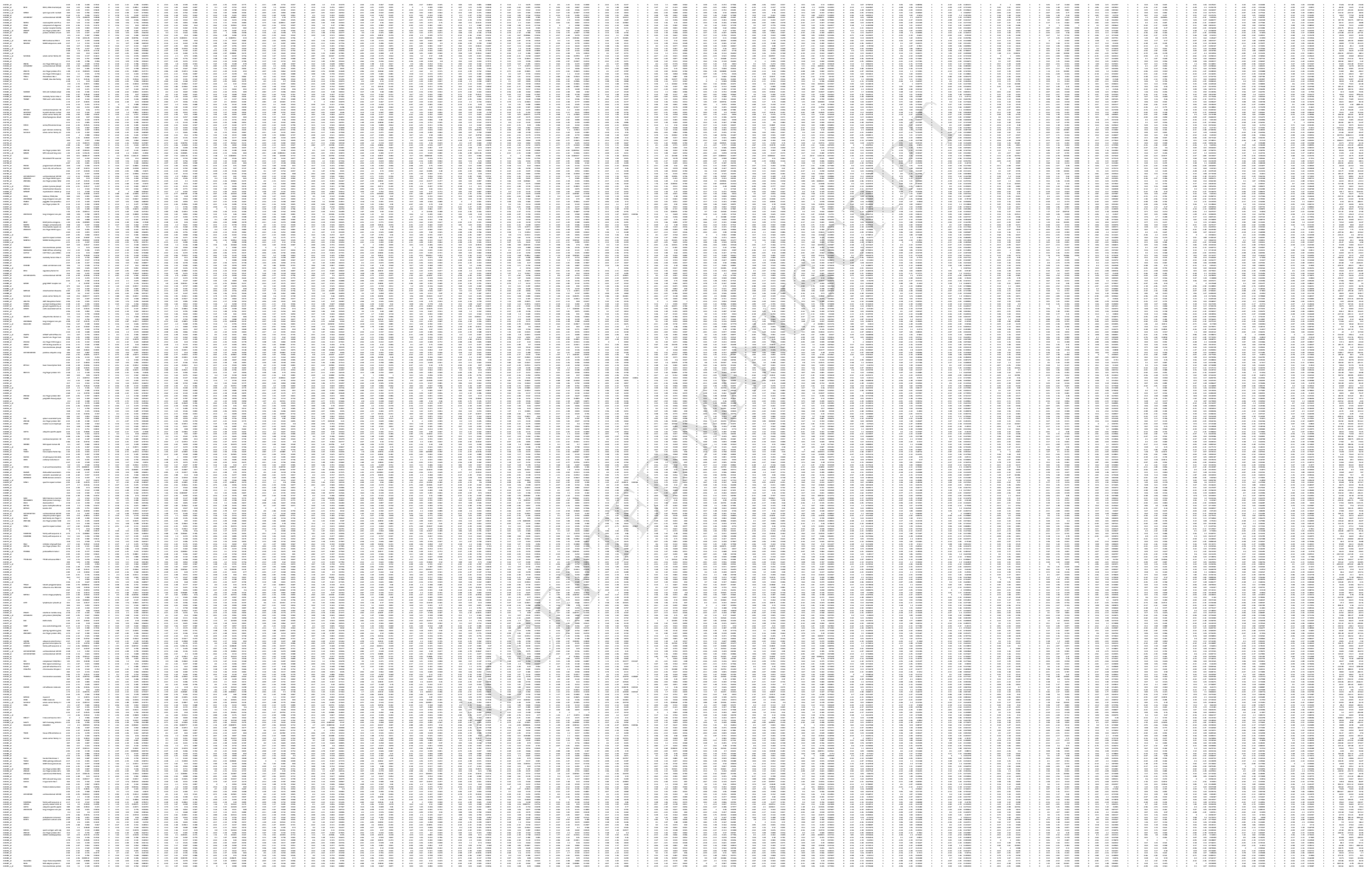
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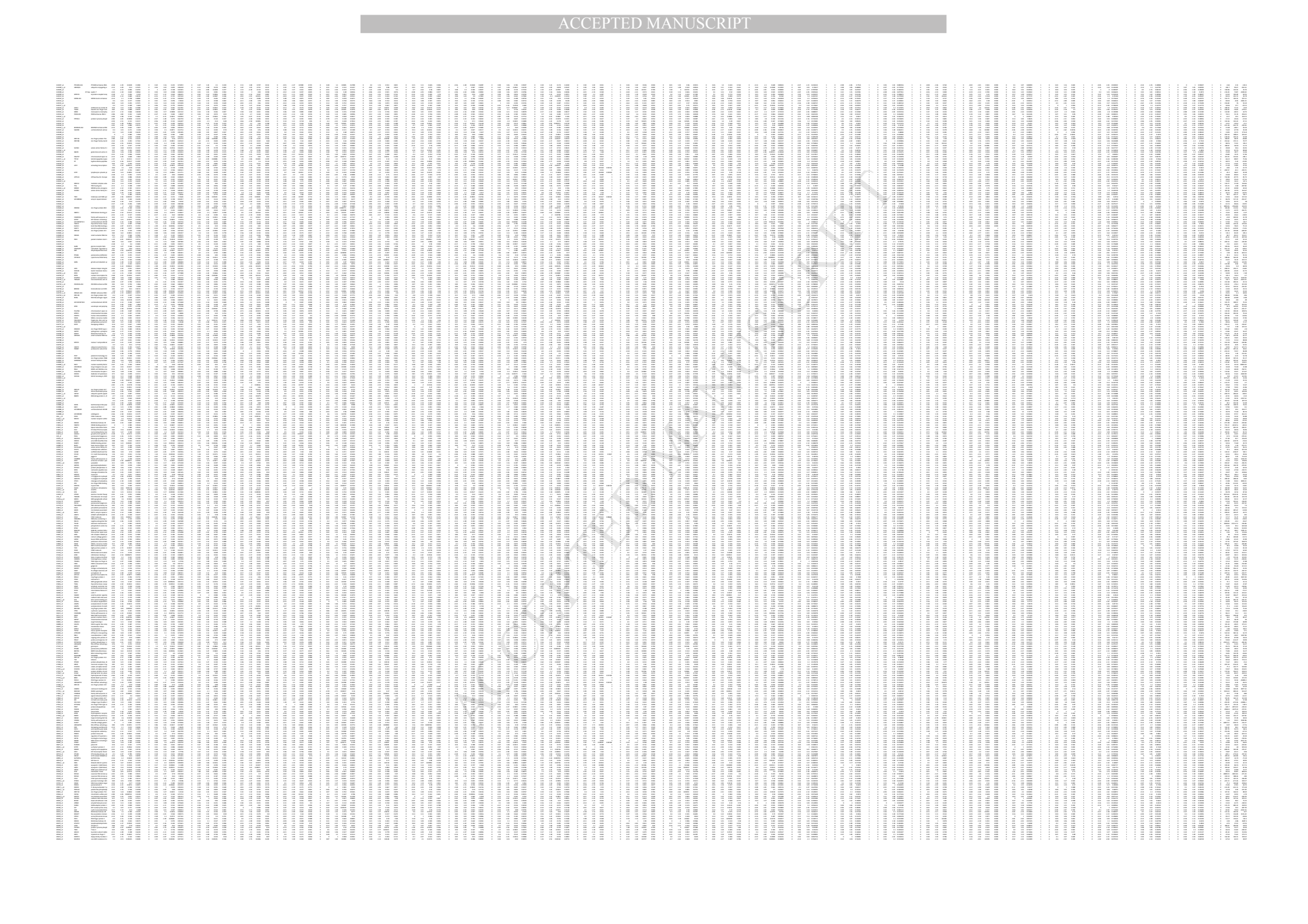
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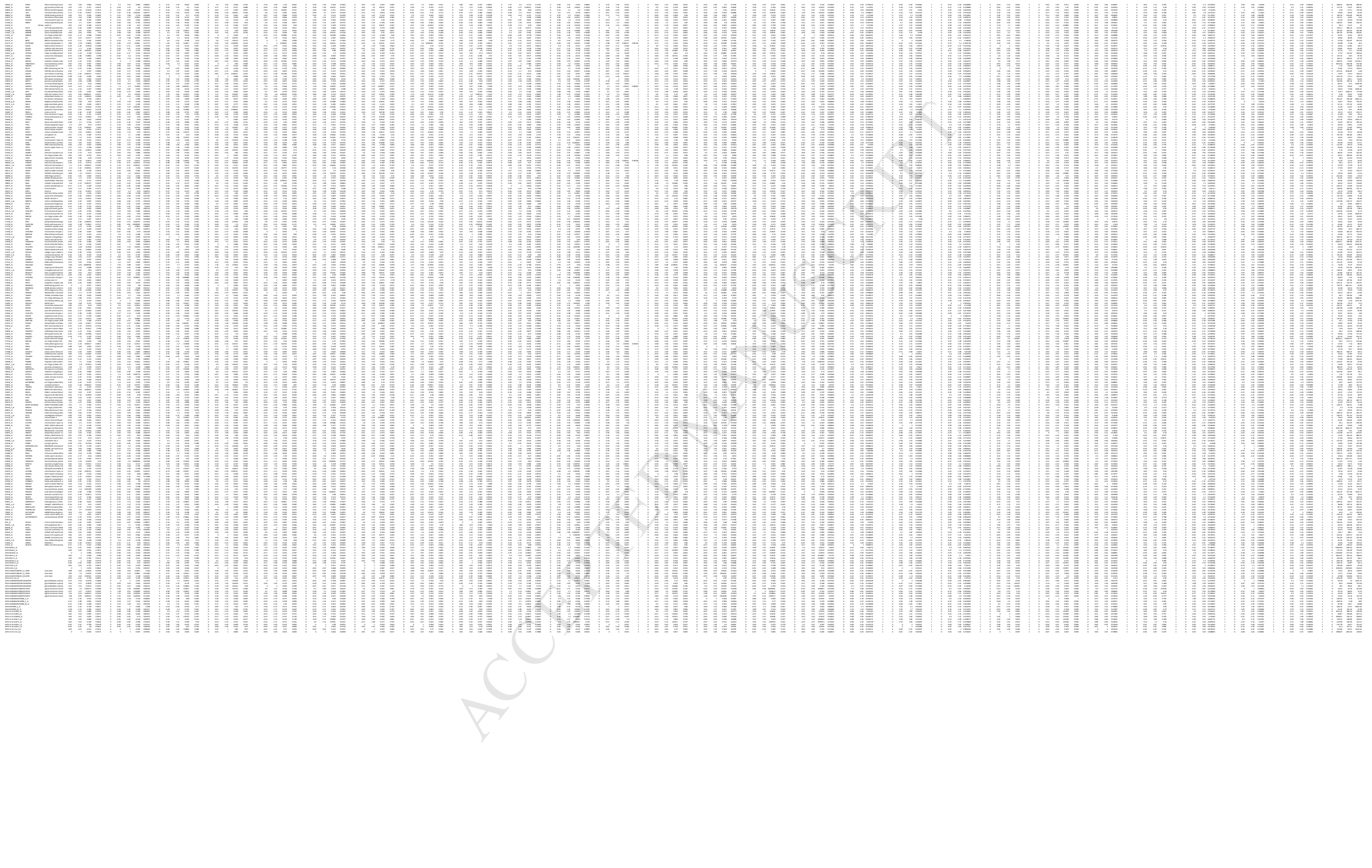
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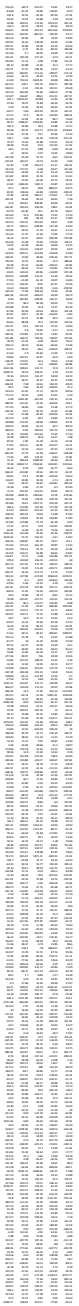
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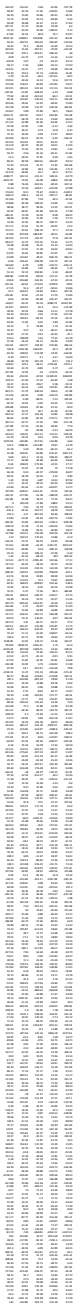
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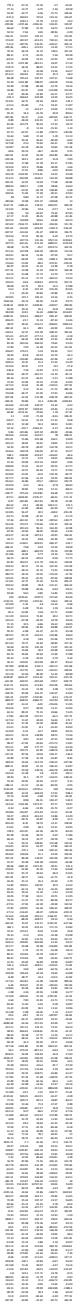
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Figure E1

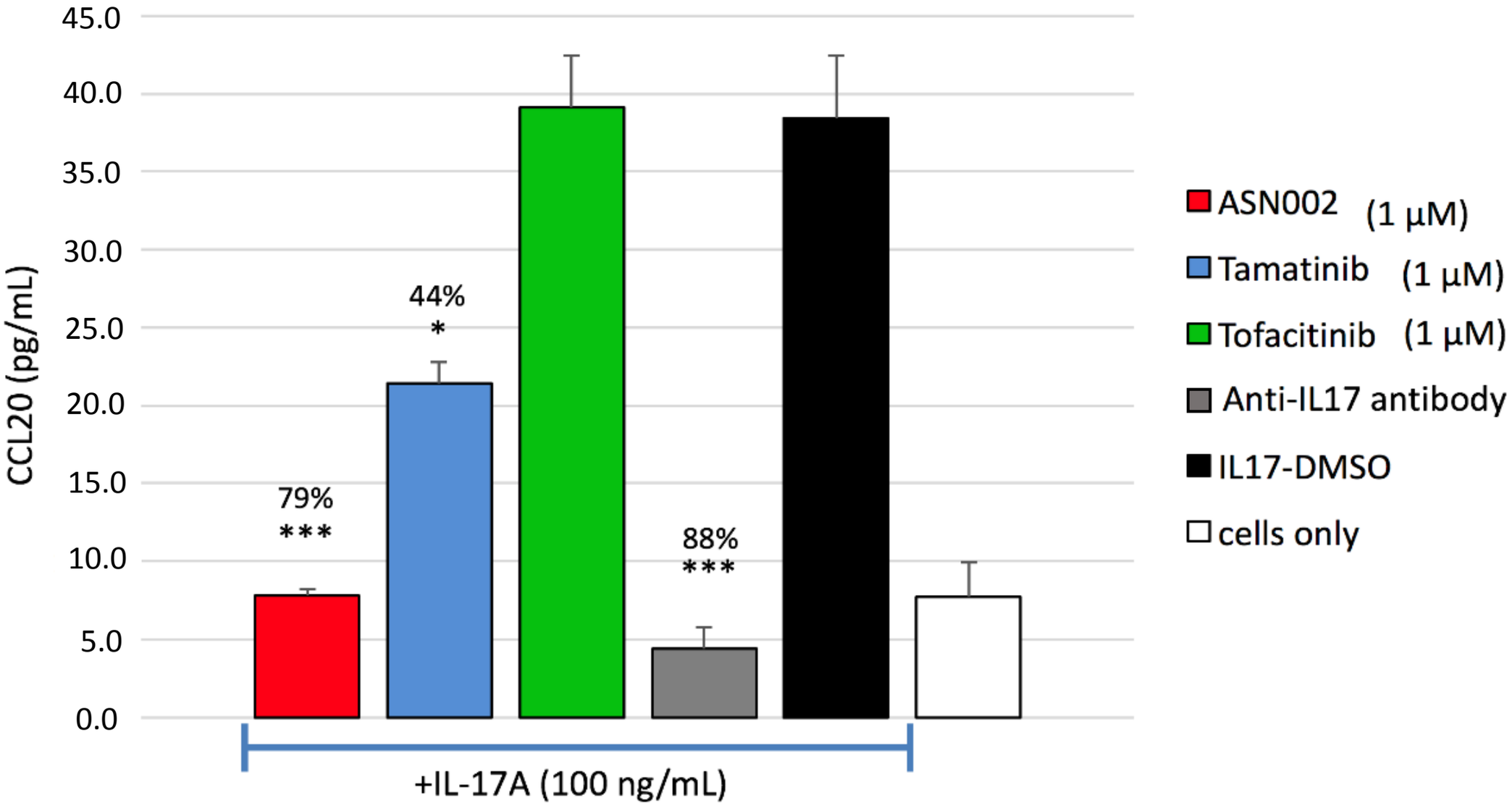

45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
CCL20 (pg/mL)
79%
***
44%
*
88%
***
+IL-17A (100 ng/mL)
ASN002 (1 µM)
Tamatinib (1 µM)
Tofacitinib (1 µM)
Anti-IL17 antibody
IL17-DMSO
cells only

Figure E2

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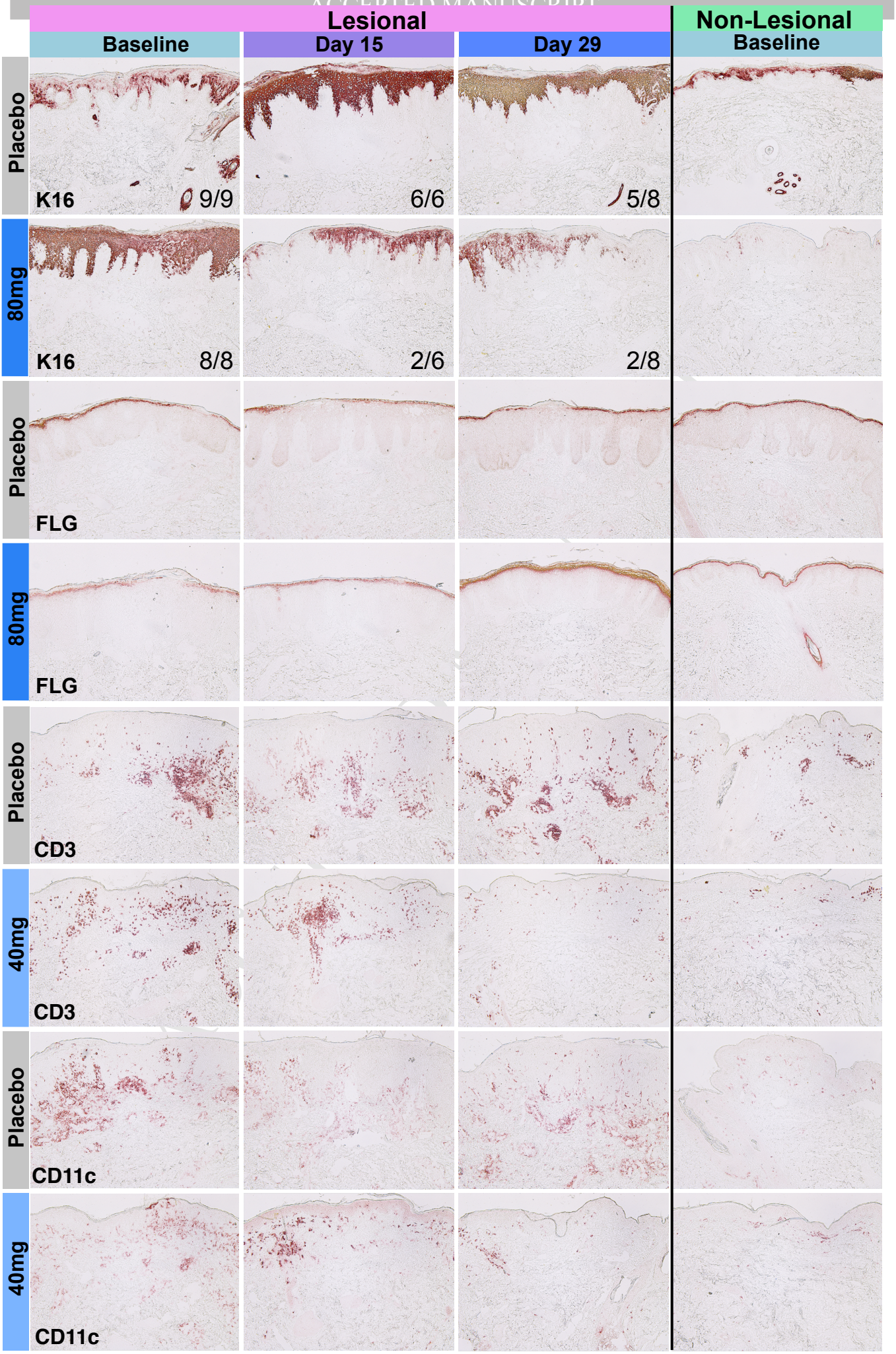
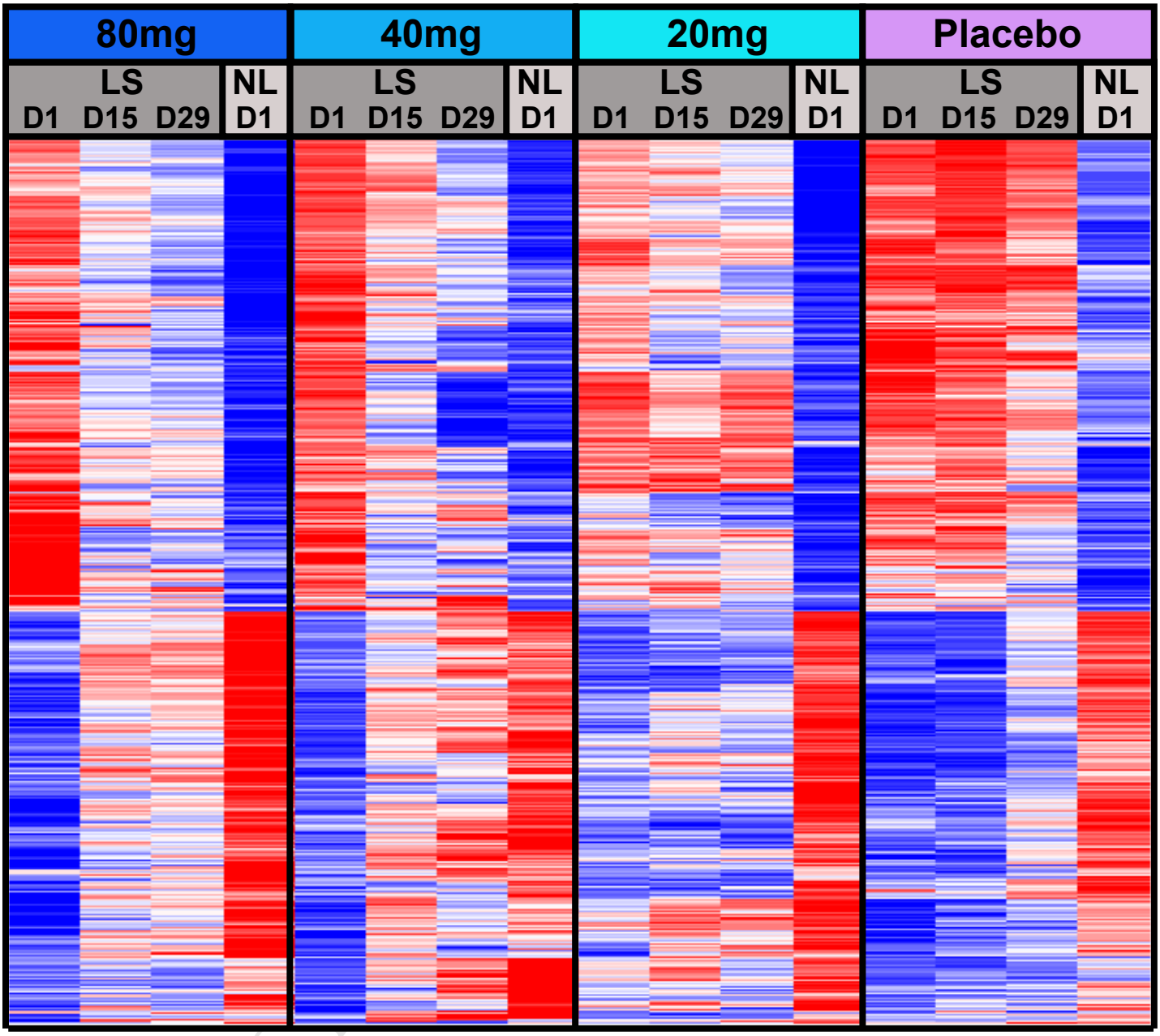


Figure E3

A. ASN002 Study Transcriptome



B.Transcriptomic Improvements

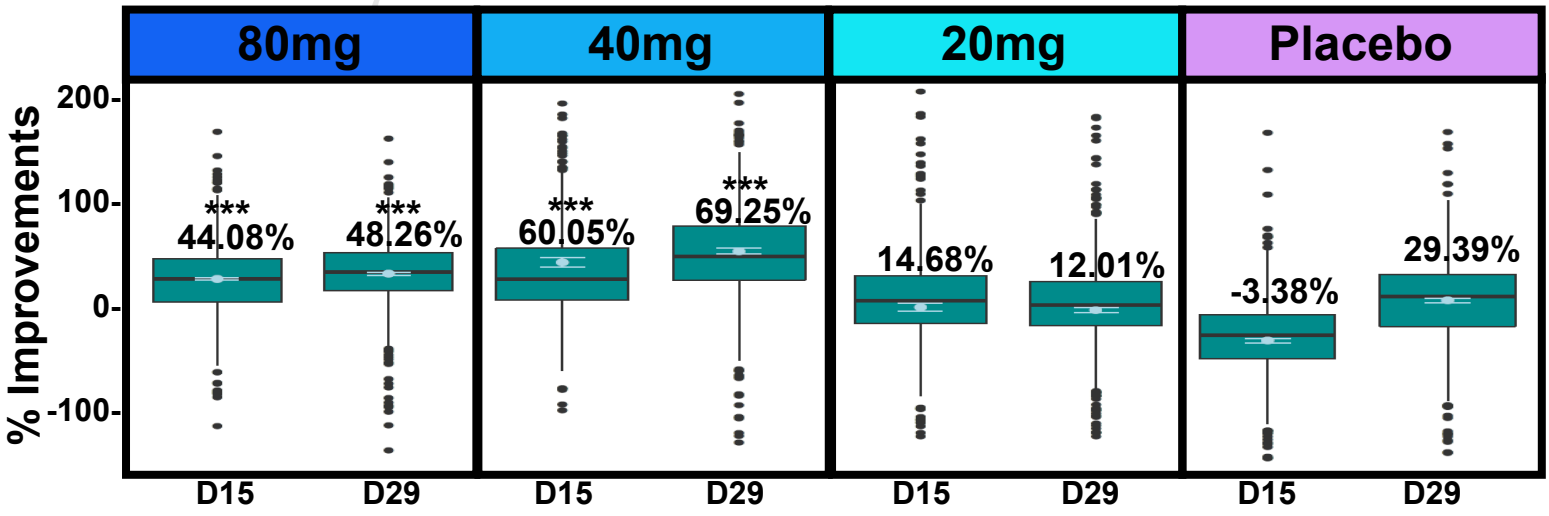
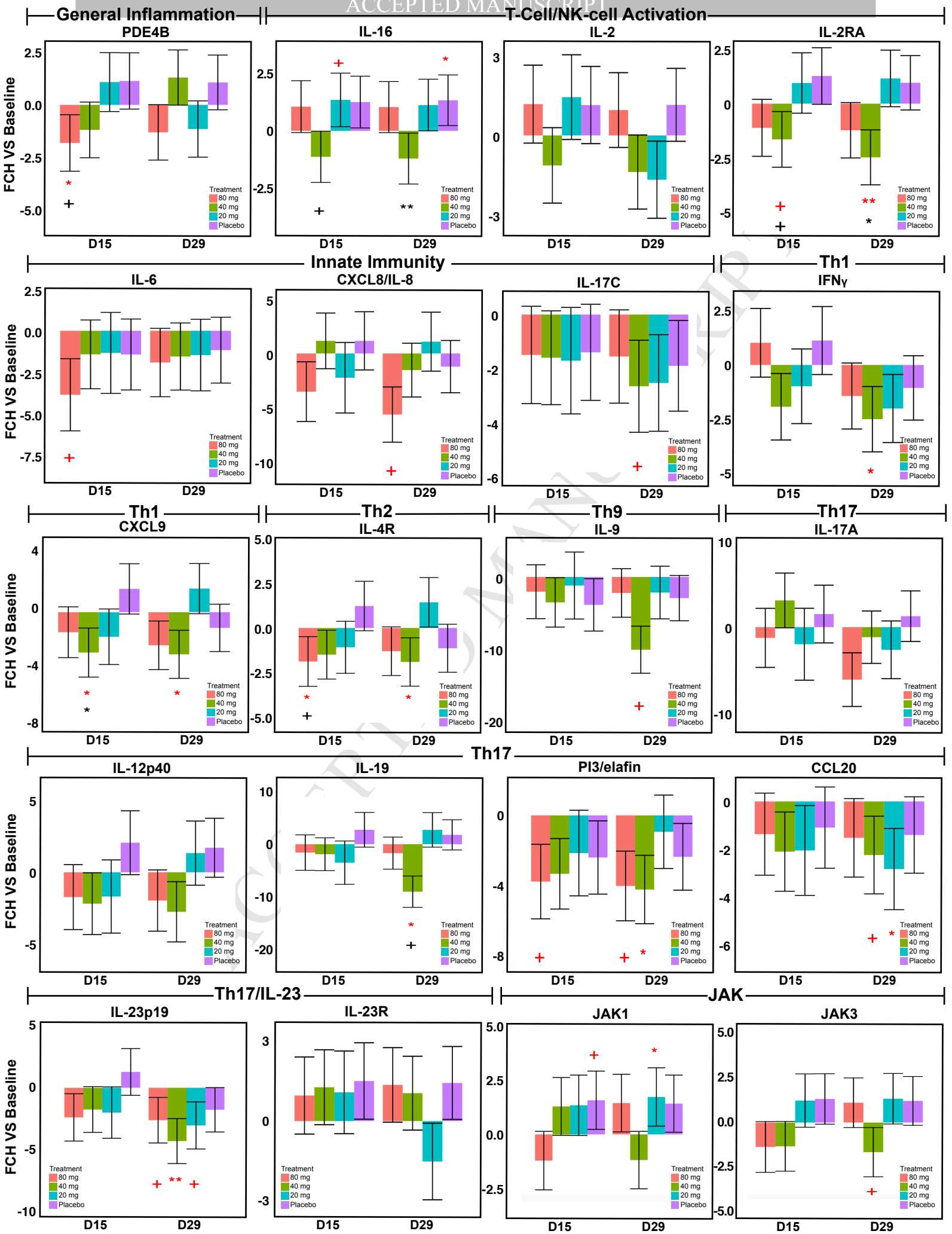


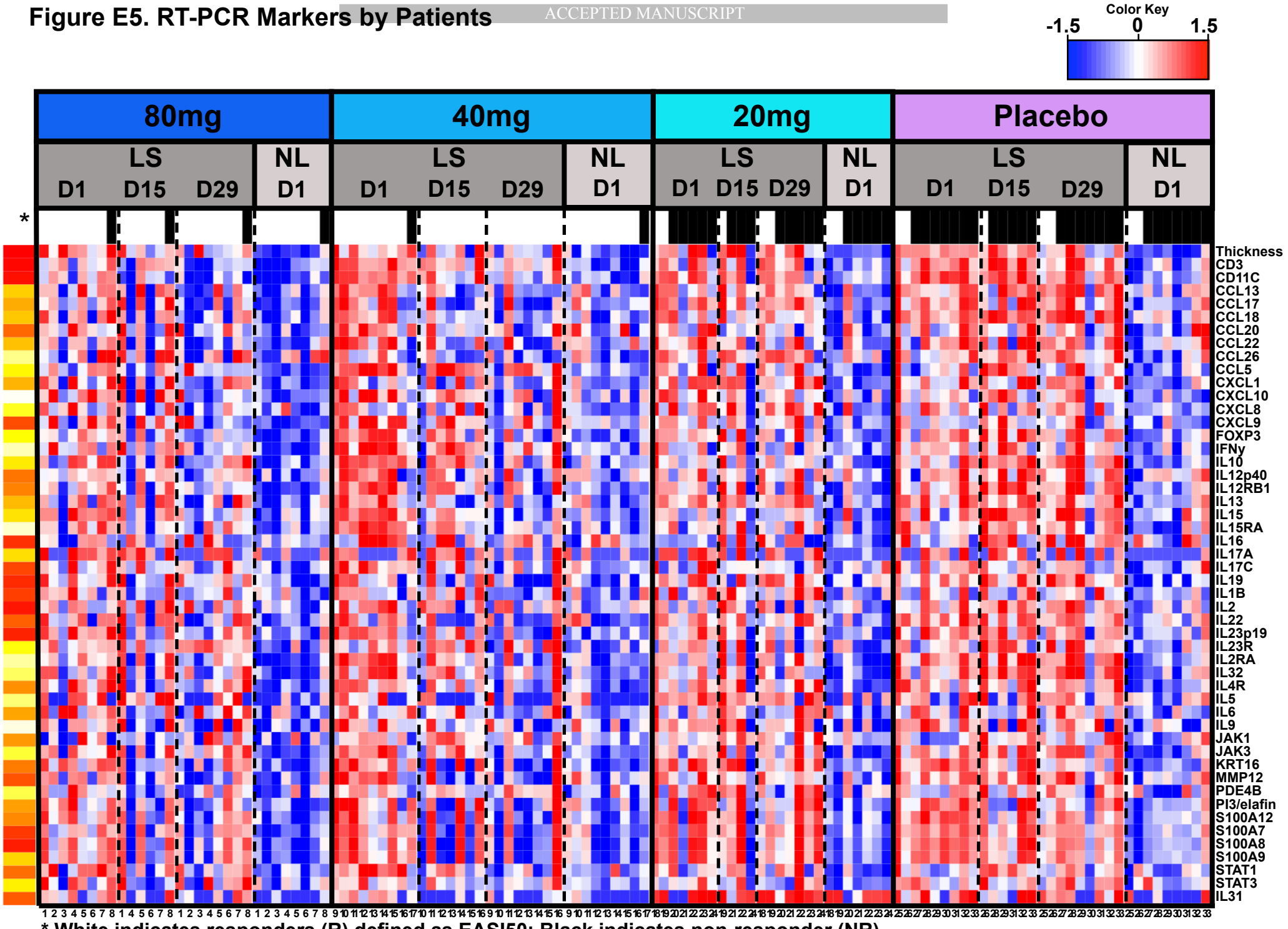
Figure E4.

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Figure E5. RT-PCR Markers by Patients

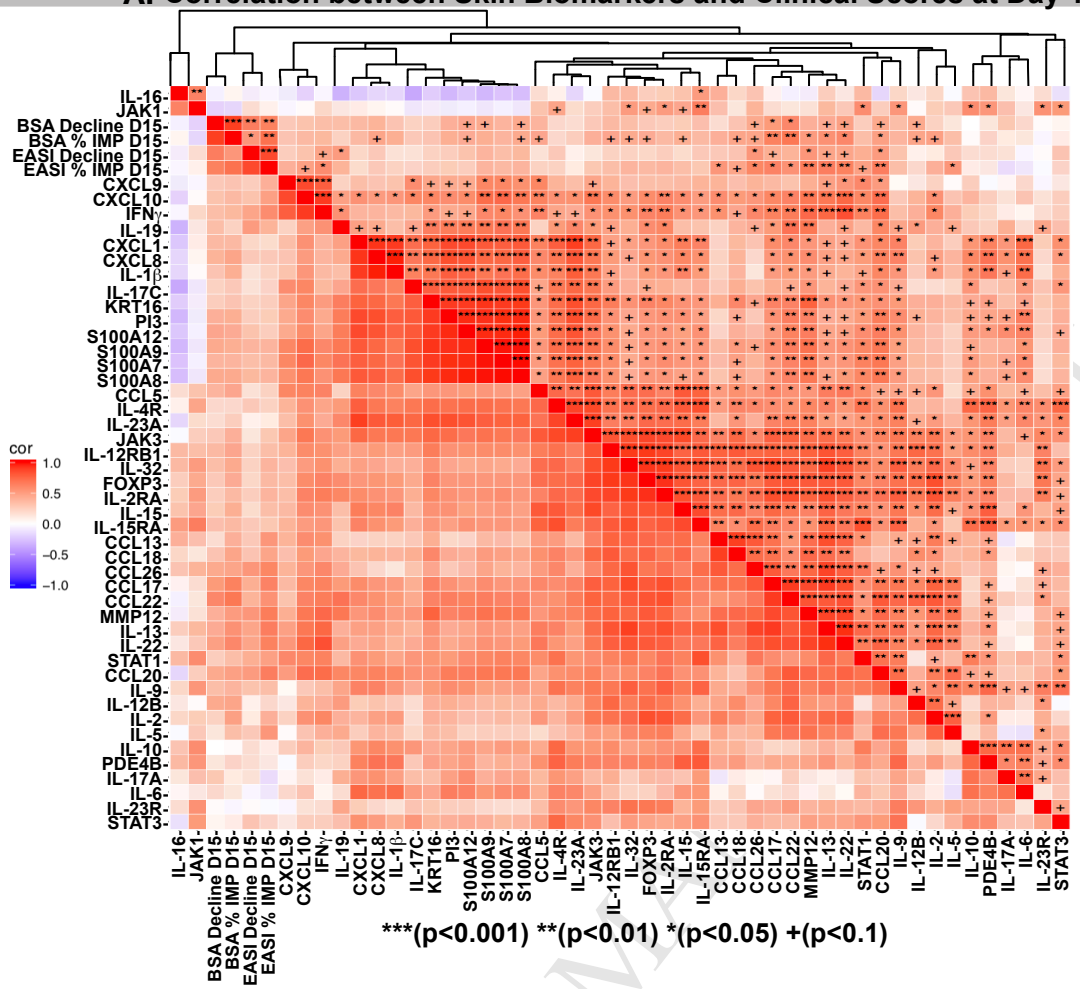


* White indicates responders (R) defined as EASI50; Black indicates non-responder (NR).

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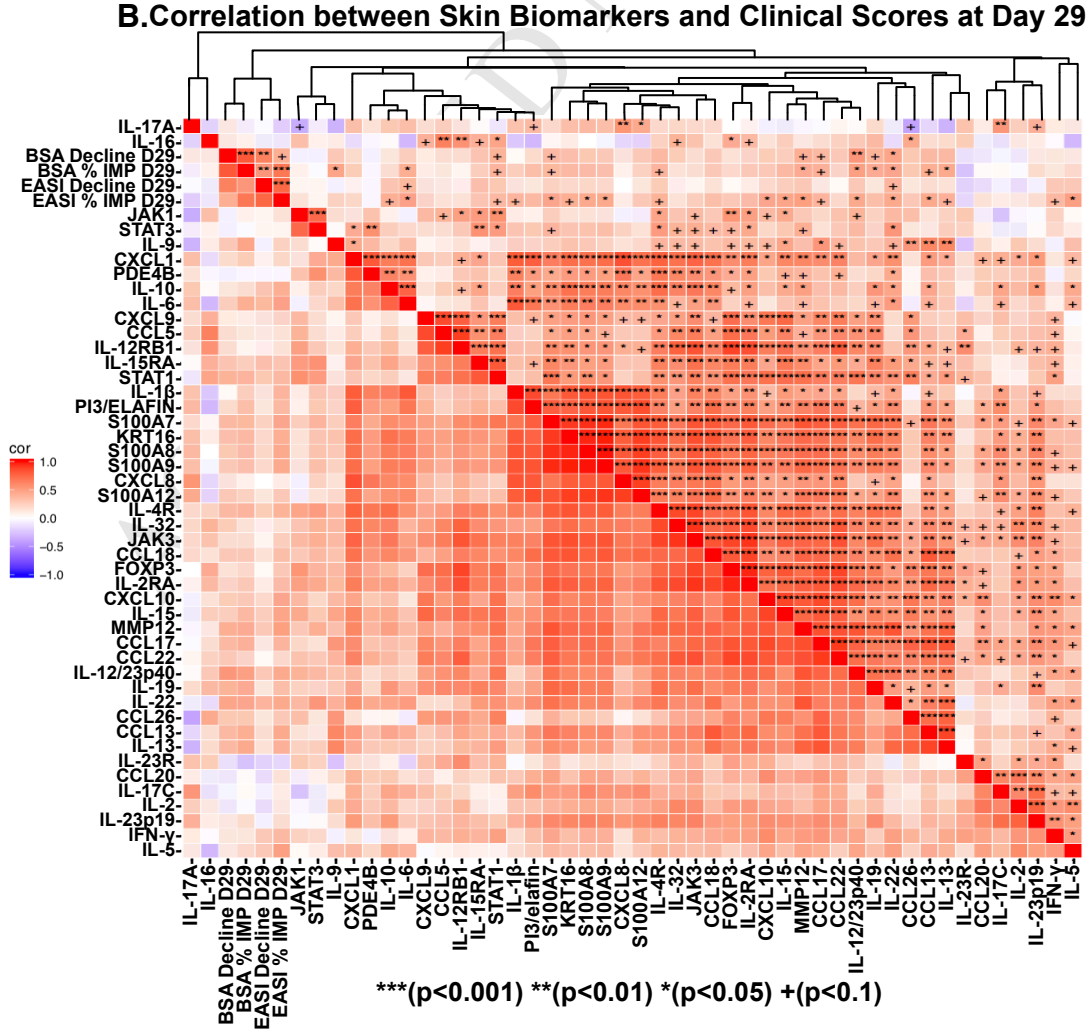
Figure E6

A. Correlation between Skin Biomarkers and Clinical Scores at Day 15



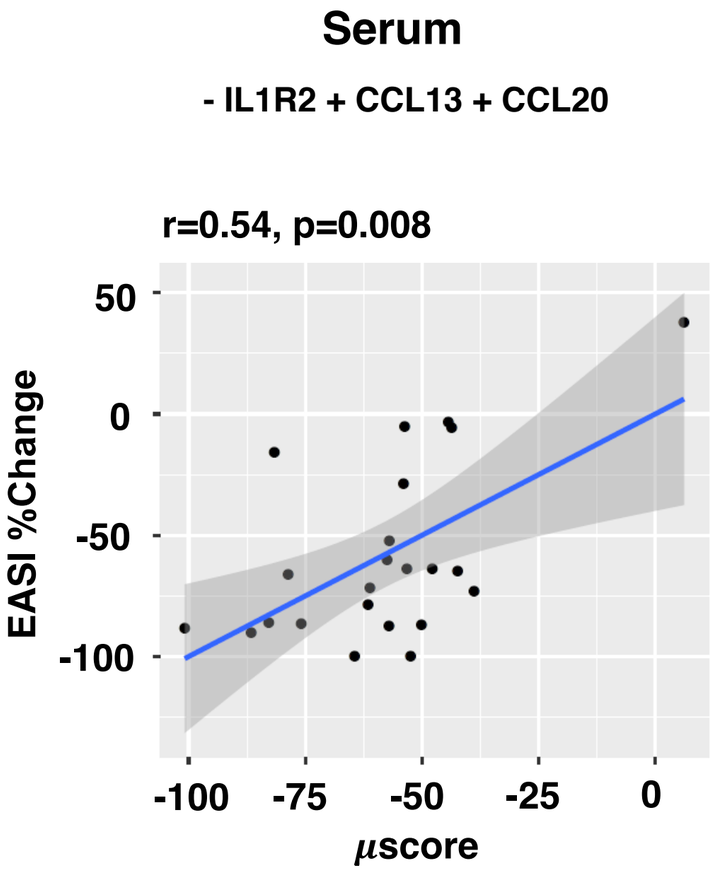
***(p<0.001) **(p<0.01) *(p<0.05) +(p<0.1)

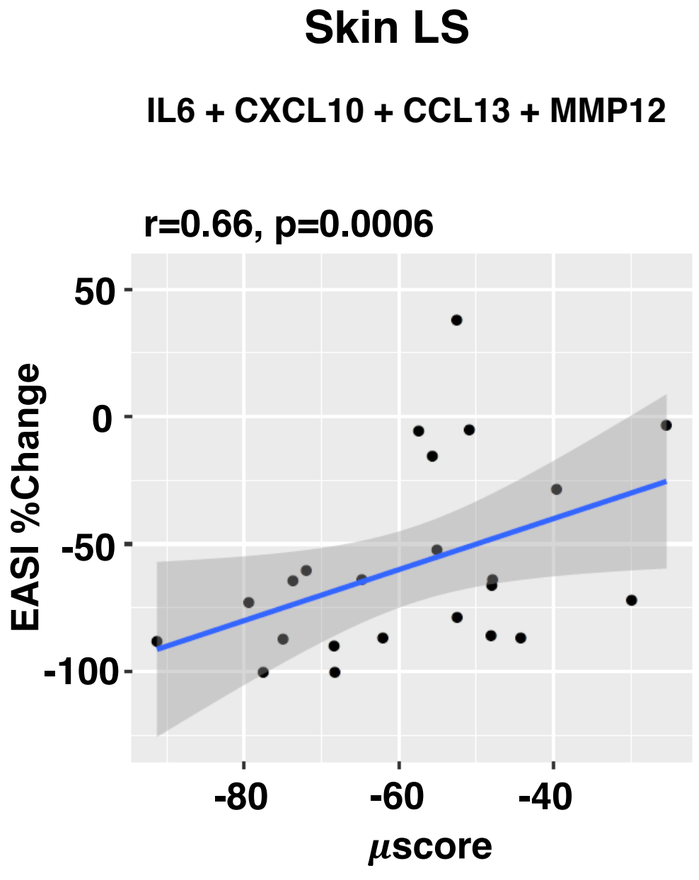
B.Correlation between Skin Biomarkers and Clinical Scores at Day 29

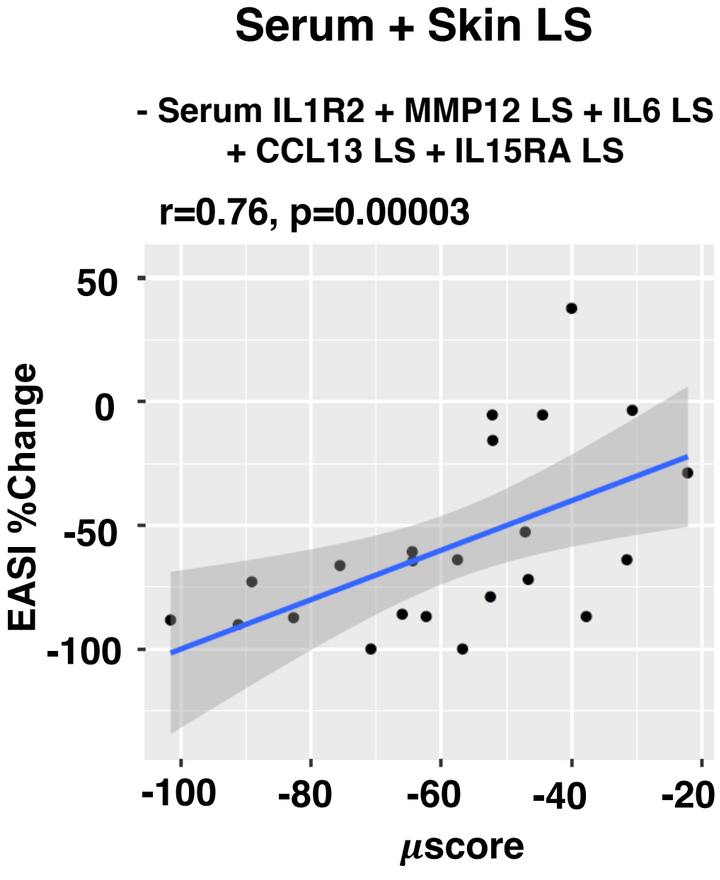


***(p<0.001) **(p<0.01) *(p<0.05) +(p<0.1)

Figure E7


Serum
- IL1R2 + CCL13 + CCL20
r=0.54, p=0.008
EASI %Change
50
0
-50
-100
-100
-75
-50
-25
0
μscore


Skin LS
IL6 + CXCL10 + CCL13 + MMP12
r=0.66, p=0.0006
EASI %Change
50
0
-50
-100
-80
-60
-40
μscore


Serum + Skin LS
- Serum IL1R2 + MMP12 LS + IL6 LS
+ CCL13 LS + IL15RA LS
r=0.76, p=0.00003
EASI %Change
50
0
-50
-100
-100
-80
-60
-40
-20
μscore

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Material & Methods:

Study Design and Patients

Inclusion criteria consisted of an age range of 18 to 75 years old, moderate-to-severe atopic dermatitis defined by an Eczema Area and Severity Index/EASI score of greater than 16, and an inadequate response to topical corticosteroids. Patients were also prohibited from using concomitant topical or systemic medications. Additional information about study design was previously published.[1]

Biopsy collection

Biopsy specimens (4.5 mm) were collected from lesional and nonlesional skin at baseline, and at 15 and 29 days of treatment (lesional only). Post-treatment specimens were taken from the same location as pretreatment biopsies but at least 1 cm away from prior scars, as described.[2-4] Researchers performing biomarker analysis were blinded to treatment allocation and clinical response.

Statistical Analyses

Microarray chips

Quality control of Affymetrix chips was done using the *arrayQualityMetrics* R package. Expression measures were obtained using GC Robust Multi-array Average (GCRMA) algorithm.[5] Batch effects corresponding to the hybridization date and chip lot number were detected by principal component analyses and

adjusted using ComBat algorithm from R's *sva* package.[6] Probe sets with expression >3 in at least 15 samples (10% of all samples) were used for further analyses. Gene expression changes were modeled using a mixed-effects model, with treatment, tissue type and time point as a fixed interaction term, and a random effect for each patient. Fold changes (FCHs) for the comparisons of interest were estimated, and hypothesis testing was conducted with contrasts under the general framework of linear models in the R *limma* package.[7] p-values were adjusted for multiple hypotheses using the Benjamini-Hochberg procedure, which controls the false discovery rate (FDR).

Correlations between biomarkers and clinical improvements

Correlation heat maps were generated using unsupervised hierarchical clustering of Spearman Correlation Coefficients based on Euclidean distance and McQuitty agglomeration.[5,8] Spearman correlations between clinical scores and biomarkers were considered significant if p-value <0.05. To correlate changes in multiple serum and skin biomarkers with clinical improvement, we used the nonparametric multivariate μ statistics approach.[9] This approach uses U-statistics for scoring multivariate ordinal data and then correlates them with the outcome in question.

RT-PCR and Immunohistochemistry

qRT-PCR expression values were normalized to house-keeping gene RPLP0. qRT-PCR values under the limit of detection (LOD) were estimated to 20% of the

minimal value over the LOD. Log2-scale qRT-PCR expression and Immunohistochemistry (IHC) counts were modeled by similar mixed-effects model, with treatment, tissue type and time point as a fixed interaction term, and a random effect for each patient. Standard packages (*nlme, lsmeans* and *gmodels*) of R's linear mixed-effects models framework were used.

Inhibition of JAK tyrosine kinase activity with TR-FRET assay ASN002, tamatinib and tofacitinib were evaluated for inhibiting JAK1, JAK2, JAK3, and TYK2 tyrosine kinase activity using an *in-vitro*, time-resolved fluorescence resonance energy transfer (TR-FRET) assay. The enzyme was incubated with substrate to be phosphorylated and ATP in the assay buffer in the presence/absence of an inhibitor compound. After 60-minute incubation, the reaction was terminated with 40mM EDTA and Eu-labeled anti-phospho-tyrosine antibody added to the reaction mix and incubated at room temperature for an additional 60 minutes. The reaction plate was read in Perkin Elmer Multi-label Counter Victor X5 (Excitation: 340 nm Emission: 615 & 665 nm). IC_{50} values were determined using purified or full-length enzymes.

Inhibition of SYK tyrosine kinase activity with radiometric assay

The IC_{50} of ASN002, tamatinib, and tofacitinib were determined using an *in-vitro* radiometric assay. This assay used the ability of a recombinant SYK to phosphorylate a substrate peptide in the presence of ATP. By using a

radiolabeled form of ATP, it was possible to quantify the phosphorylation status of the substrate.

IL17-mediated CCL20 release in human epidermal keratinocytes

Neonatal human epidermal keratinocytes (NHEKs) were cultured in keratinocyte growth media supplemented with growth factors and maintained in T75 flasks at 37˚C/5% CO_2. Cells were treated with IL-17 A (100 µg/mL stock in 4 mM HCl) at 0, 50, 100, 150, 250 ng/mL final for 0, 9, 12, 18, and 24 hours. CCL20 release was measured by in the culture media by ELISA. Cells were pretreated with tofacitinib, tamatinib, and ASN002 for 30 minutes followed by IL-17 A (100 µg/mL stock in 4 mM HCl) at 100 ng/mL final for 24 hours. BAY-61-3606 (10 µM) and neutralizing IL-17A antibody (Nab) AF-317-NA R & D Systems (5 µg/mL) were used as the positive reference control; goat IgG (5 µg/mL) was used as the negative reference control.

Results

IL17-mediated CCL20 release in human epidermal keratinocytes

Highly significant downregulation of CCL20 was achieved with ASN002 and anti-IL17 antibody ($p<0.001$) compared to IL-17+DMSO control by one-way ANOVA with Dunnett's multiple comparison test. While Tamatinib also achieved significance ($p<0.05$), Tofacitinib did not show any change in CCL20 compared to IL-17+DMSO (Fig. E1). These results confirm the strong modulation of Th17 pathway with ASN002, as an effect of TYK2/SYK affinity

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Supplementary Figure Legends

Figure E1: ASN002 strongly inhibits CCL20 release. Bar graph demonstrating inhibition of CCL20 release as compared to IL-17A antagonists between various JAK and SYK inhibitors. +p<0.1; *p<0.05; **p<0.01; ***p<0.001.

Figure E2. Histologic Representation of Cellular Infiltrates. Representative histological images in placebo and 80mg ASN002 groups of K16 expression, with number in lower right corner indicating number of patients with K16 staining post-treatment **(A),** filaggrin expression (**B**), in placebo and 40mg ASN002 groups of $CD3^+$ T-cells (**C**) and $CD11c^+$ dendritic cells (**D**).

Figure E3. **ASN002 Study Transcriptomic Changes**. **A)** Heatmap of mean genomic changes of study transcriptome in treatment groups of 80mg, 40mg, and 20mg, compared to placebo group. Number of differentially expressed genes (DEGs) defined by criteria of fold change (FCH)>2 and false discovery rate (FDR) < 0.05. Upregulated (red) and downregulated (blue) genes at baseline in lesional and nonlesional skin and after treatment with respective doses (80mg, 40mg, 20mg) in lesional skin at day 15 and day 29. **B)** Overall percent improvements in study transcriptome at day 15 and day 29 under respective treatment groups of 80mg, 40mg, and 20mg, compared to placebo. ***(P<0.001), **(P<0.01) compared to placebo group on respective treatment day. Light blue bar on the boxplots represent the mean ± SEM. □

Figure E4. Fold Changes (FCH) of AD immune markers measured by qRT-PCR. Fold changes (FCH) of inflammatory markers in lesional skin at day 15 (D15) and day 29 (D29) in respective treatment groups of ASN002 compared to placebo group and baseline (D1). *Black stars:* significance of comparison between placebo and ASN002 groups, *red stars:* significance of comparison vs baseline. +p<0.1; *p<0.05; **p<0.01; ***p<0.001.

Figure E5. RT-PCR markers by patient ordered by EASI50 responders. ID located on the bottom of the heatmap identifies patients at different time points.

Figure E6. Clinical scores and skin biomarkers correlations. Heat map of Spearman's correlations between skin biomarkers and clinical severity scores on day 15 **(A)**, and day 29 **(B),** ordered by unsupervised clustering (and shown by dendrogram). Significance determined by +p<0.1; *p<0.05; **p<0.01; ***p<0.001.

Figure E7. Integrating skin and blood biomarkers with clinical improvement by a non-parametric multivariate approach using μ statistics. Top significant correlations in serum, skin, and serum and skin combined at Day 29.

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