Anti-IL-5 mepolizumab minimally influences residual blood eosinophils in severe asthma

Glenn Van Hulst, MSc*[,1], Joseph Jorssen, MSc*[,1], Nathalie Jacobs, PhD*, Monique Henket[†], Renaud Louis, MD, PhD[†], Florence Schleich, MD, PhD[†], Fabrice Bureau, DVM, PhD*[, ‡,1], Christophe J. Desmet, PhD*[,†,1]

* Laboratory of Cellular and Molecular Immunology, GIGA Institute and Faculty of Veterinary Medicine, Liege University, Liege, Belgium

[†] Laboratory of Pneumology, GIGA Institute, Faculty of Medicine and University Hospital Center, Liege University, Belgium

[‡] Walloon Excellence in Life Sciences and Biotechnology (Welbio), Wavres, Belgium

[1]Equal contributors

Corresponding author: Christophe J. Desmet, PhD, Laboratory of Cellular and Molecular Immunology, GIGA Institute, University of Liege, B34, 1 Avenue de l'Hopital, B4000 Liege, Belgium. Phone: +3243663708, Fax: +3243664198, email: christophe.desmet@uliege.be

Take home message: Asthma patients receiving anti-IL5 therapies retain residual blood eosinophils, of which potential alterations remain unknown. This study shows that these residual eosinophils harbour largely unaltered quiescent and activated gene expression programs

Abstract

Neutralizing antibodies against the cytokine interleukin (IL)-5 have become widely used for the control of severe eosinophilic asthma. Remarkably, patients receiving neutralizing anti-IL5 biological therapies retain a very stable population of residual blood eosinophils. Whether these residual eosinophils are endowed with particular biological activity has not yet been studied but is of importance in predicting potential long-term effects of IL5 neutralization in patients. To tackle the effect of IL5 depletion on residual eosinophils, we used a comparative RNA-sequencing approach and compared the gene expression program of eosinophils arising in IL5-depleted or IL5-replete human or murine hosts, at steady-state *in vivo* and following *in vitro* stimulation with the eosinophil-activating alarmin IL33. We compared blood eosinophils from patients with severe allergic eosinophilic asthma treated with anti-IL5 mepolizumab therapy to those of healthy controls and matched asthma patients receiving anti-IgE omalizumab therapy. We made similar comparisons on bone marrow eosinophils from mice genetically deficient or not for IL5. We report that restriction of IL5 availability did not elicit any detectable transcriptional response in steady-state residual eosinophils in mepolizumab-treated patients or IL5-deficient mice, and influenced only a handful of genes in their response to IL33. Together, these results support the notion that treatment with IL5 neutralizing antibodies spares a pool of circulating residual eosinophils largely resembling those of healthy individuals.

Keywords

Eosinophil, interleukin-5, mepolizumab, interleukin-33, severe eosinophilic asthma, transgenic mice

Abbreviations

BM: bone marrow, GSEAR: ranked gene set enrichement analysis, IL: Interleukin, NES: normalized enrichment score, FWERp: family-wise error rate p-value, PCA: principal component analysis, SOCS3: suppressor of cytokine signaling-3

Introduction

Eosinophils, evolutionarily conserved granulocytes characterized by their elevated content in acidophilic granule proteins (1), have become a cellular target of biological therapies in the precision treatment of so-called human eosinophilic diseases (2), especially so far of severe eosinophilic asthma (3–5). Indeed, in eosinophilic asthma, eosinophilic airway inflammation is associated with disease severity and there is a positive correlation between blood and tissue eosinophilia and the rate of exacerbations and risk of irreversible airway obstruction (6, 7).

Eosinophilia heavily depends on the bioavailability of a particular cytokine called Interleukin-5 (IL5) (8), as demonstrated initially in mouse models of asthma (9, 10). Eosinophilia results from increased production of eosinophils from bone marrow progenitors, increased eosinophil transit through the bloodstream and eosinophil extravasation in target tissues (11). The unique dependency of eosinophilia on IL5 instigated the introduction of neutralizing anti-IL5 monoclonal antibody-based biological treatments, namely mepolizumab and reslizumab. These biological therapies alleviate eosinophilia and consequently reduce disease exacerbations in severe eosinophilic asthma (3, 4, 12–14).

Remarkably, a very stable and interindividually consistent population of residual eosinophils persists in the blood of patients receiving anti-IL5 biological treatment, which amounts to about half the blood count of eosinophils in the general population (4). Whether these residual eosinophils are endowed with particular biological activity has not yet been studied but is of importance in predicting potential long-term effects of IL5 neutralization in patients. Indeed, because of its radical effect on eosinophil amplification, IL5 is still widely believed to act as a maturation factor for eosinophils, favouring progenitor engagement and progression along the eosinophil lineage (8, 15). In this line of thought, an early report examining the effect of mepolizumab on eosinophil development concluded that IL5 neutralization induces a maturational arrest of eosinophils in human bone marrow (16).

Therefore, even though the role of IL5 in eosinophil maturation has been less thoroughly studied, it is of high clinical relevance in the context of anti-IL5 biological therapies. Withdrawing IL5 during eosinophil development might, in addition to reducing their numbers, alter their biological activities as well. This could thereby have unforeseen long-term consequences given the various potential immune and homeostatic roles experimentally assigned to eosinophils (17, 18) and their putative heterogeneity (19, 20).

Here, through a comparative transcriptomic approach in mice and human, we studied whether residual eosinophils developing in conditions of IL5 restriction *in vivo* display alterations in their gene expression program.

Material and methods

Human subject characteristics and study design

We recruited 26 patients from the university asthma clinic of Liege (Centre Hospitalier Universtaire de Liege, Liege, Belgium) between February 2019 and May 2020. Ten healthy volunteers were enrolled by advertisement among the hospital and staff and were non-smoker, non-asthmatic, non-atopic. Asthma patient characteristics are given in Table 1 and Table 2. Asthma was diagnosed following the GINA guidelines (http://ginasthma.org/). Severe asthma was defined according to ATS criteria (21). All patients had a history of at least one serious exacerbation requiring hospitalization and two or more exacerbations requiring systemic corticosteroid treatments. In addition, patients presented with airflow limitation withhold <80 per cent FEV1, blood eosinophil counts >300/mm^3, poor symptoms control defined as ACQ consistently ≥1.5, ACT <20 or not controlled by National Asthma Education and Prevention Program (NAEPP) or Global Initiative for Asthma (GINA) guidelines. Patients receiving methylprednisolone up to 4 weeks prior to blood sampling were excluded from the study. Mepolizumab was administered as 100 mg subcutaneous dose every 4 weeks. Dosage and frequency of omalizumab administration was determined by the patient's age, pre-treatment serum total immunoglobulin E (IgE) level (IU/mL), and body weight.

The study was approved by the local ethics committee (IACUC, University of Liège) and written informed consent was obtained from all study participants. This research was undertaken in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and followed the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals of the International Committee of Medical Journal Editors.

Mice

C57BL/6J and *Il5*$^{-/-}$ (C57BL/6-*Il5*^{tm1Kopf}/J) mice were purchased from The Jackson Laboratory. The 2 strains were interbred and heterozygous *Il5*$^{+/-}$ progeny was further bred for generating littermates of the genotypes of interest. All mice were housed and bred in institutional specific pathogen-free facilities. Age- and sex-matched (females or males) mice

were used at 8 to 16 weeks of age. All animal experiments were approved by the Animal Ethics Committee of the University of Liege and complied with the ARRIVE guidelines, the EU Directive 2010/63/EU and the declaration of Helsinki for use and care of animals.

Isolation of eosinophils from human blood for cell sorting

Human blood polymorphonuclear cells were isolated by double-layer density centrifugation and blood eosinophils were purified using EasySep™ Human Eosinophil Isolation kit (Stemcell Technologies) following the manufacturer's instructions. Pre-sort cell viability was 95% or superior as assessed by trypan blue exclusion. Isolated cells were stained with CCR3, CD3, CD19, Siglec-8 (Miltenyi Biotec), and CD16 (BD Biosciences). Human blood eosinophils (CCR3+SiglecF+) were sorted (purity≥95%) into TRIzol® (ThermoFisher) and stored at -80°C for downstream RNA applications.

Ex vivo activation of eosinophils

Mouse bone marrow eosinophils were stimulated for 4 hours at 37°C and 5% CO_2 in culture medium and 100ng/mL purified IL33 (BioLegend), and 10ng/mL purified IL5 (Peprotech). Human blood eosinophils were stimulated for 6 hours at 37°C and 5% CO_2 in culture medium and 100ng/mL purified IL33 (PeproTech). Stimulated mouse bone marrow- and human blood eosinophils were resuspended in TRIzol® (ThermoFisher) and stored at -80°C for downstream RNA extraction.

RNA isolation

Human blood- and mouse bone marrow eosinophil RNA was isolated using phenol-chloroform phase separation RNA extraction procedure. Isolated RNA was treated with DNase (Zymo Research) for 15 minutes at room temperature. Treated RNA was purified with the RNA Clean & Concentrator-5 kit (Zymo Research). Purified RNA integrity and quantity was assessed using the RNA 6000 Pico kit (Agilent) for the presence of 18s and 28s rRNA peaks. All human samples had RNA integrity number (RIN) >7.9.

RNA sequencing & data processing

Full length cDNA was prepared using SMART-Seq v4 Ultra Low Input RNA kit (Takara Bio) following the manufacturer's instructions. Purified cDNA integrity and quantity was assessed using the High Sensitivity DNA kit (Agilent). cDNA libraries were prepared for sequencing

using Nextera XT DNA library preparation kit (Illumina) using the manufacturer's instructions and samples were sequenced on a NovaSeq™ 6000 sequencing system (Illumina). If samples were sequenced in different batches, groups were kept equal within every batch.

Differential gene expression analyses

Sequenced reads were aligned to the mouse genome (UCSC mm10) or the human genome (HG19) with RNA-seq Alignment (v2.0.2) using STAR aligner (version 2.6.1a) on BaseSpace (https://basespace.illumina.com). Differential gene expression was calculated using DESeq2 (1.26.0) in R (3.6.3 and 4.0.3) (22). If samples were sequenced in different batches, sample batch was taken into account in the DESeq2 design. GSEAR analyses on differentially expressed genes were performed on preranked list of significantly differentially expressed genes with baseMean>50 ordered according to their log2 fold change. Online GSEAR v7.2.1 (https://genepattern.broadinstitute.org/gp/pages/index.jsf) was used with the "h.all.v7.2.symbols" (Hallmarks) gene sets and default parameters, except for a "classic" scoring scheme and minimal gene set size of 20.

Statistical analyses

All statistical analyses were performed in R (3.5.0). All mouse experiments followed a randomized design. Sample sizes were determined by power analysis. Respect of tests assumptions and model fit were evaluated using diagnostic plots. Raw data were transformed when needed and back-transformed for graphical presentation. A p-value lower than 0.05 was considered significant.

Data deposition

RNA-sequencing data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession numbers E-MTAB-10188, E-MTAB-10189 and E-MTAB-10190

Additional details and methods are available in the Supplementary Material online

Results

Because much like in patients receiving mepolizumab, mice deficient for the *Il5* gene (*Il5*$^{-/-}$ mice) retain residual eosinophils (10), we first tested whether residual eosinophils in *Il5*$^{-/-}$ mice displayed alterations in their development or potential biological activities. We reasoned that such alterations should be reflected to some extent in the mature eosinophil gene expression program. In line with previous reports (10), *Il5*$^{-/-}$ mice raised in specific pathogen-free conditions displayed reduced numbers of eosinophils in their blood, lung, spleen and bone marrow (BM) compared with wild-type *Il5*$^{+/+}$ and heterozygous *Il5*$^{+/-}$ littermates (Figure 1A-D). We sorted BM eosinophils from *Il5*$^{+/+}$ and *Il5*$^{-/-}$ mice to very high purity (Figure 2A), retrieved high quality RNA and performed high-throughput RNA-sequencing of their poly-adenylated RNAs. In this analysis, samples from *Il5*$^{+/+}$ and *Il5*$^{-/-}$ mice did not segregate according to their genotype (Figure 2B). Further, analysis for differential gene expression returned no gene significantly regulated (adjusted $p<0.05$) according to mouse genotype (Figure 2C, D and Supplementary Figure 1). Hence, the gene expression program of steady-state mature BM eosinophils in mice is largely unperturbed by the total absence of IL5 during their development.

One may argue that BM eosinophils in the steady-state are quiescent cells with minimal levels of gene transcription, as reflected by their low content in RNA. Alterations in gene expression of residual *Il5*$^{-/-}$ eosinophils could consequently only become apparent following their activation. To address this possibility, we stimulated BM eosinophils from *Il5*$^{-/-}$ and *Il5*$^{+/+}$ mice *ex vivo* with both IL5 and the alarmin IL33, two very potent activating signals of eosinophils (23–25) and compared their transcriptome (Figure 3A). We observed that stimulation elicited a potent transcriptional response in eosinophils from both *Il5*$^{-/-}$ and *Il5*$^{+/+}$ mice as evidenced by the separation in a principal component analysis (PCA) of unstimulated and stimulated samples along the first principal component that captured 98% of variance in gene expression (Figure 3B). Compared with their unstimulated counterparts, 2660 genes were differentially expressed (adjusted $p<0.05$, $|\log2(\text{fold change})| > 1$) in stimulated eosinophils from both *Il5*$^{-/-}$ and *Il5*$^{+/+}$ mice (Figure 3C). Ranked gene set enrichment analyses (GSEAR) identified "Hallmark_TNFA_signaling_via_NFKB" as the most significantly upregulated hallmark process (Figure 3D, E), most likely reflecting the fact that IL33 activated the NF-κB pathway through its ST2 receptor (25). Notably, PCA suggested that the response of eosinophils from *Il5*$^{-/-}$ and *Il5*$^{+/+}$ mice to stimulation was highly similar. This was

confirmed by the fact that only one gene, Suppressor of cytokine signalling 3 (*Socs3*), was differentially expressed (padj<0.05) in the response to stimulation of *Il5*$^{-/-}$ versus *Il5*$^{+/+}$ eosinophils (Figure 3F).

These observations suggested that deprivation of IL5 has limited consequences on the development of eosinophils in mice. We next tested whether these observations would translate to human eosinophils. Toward this aim, we recruited 10 severe asthmatic patients with eosinophilic allergic asthma who received mepolizumab for at least 6 months, as well as 10 severe asthmatic patients with allergic asthma who received anti-IgE omalizumab for at least 6 months and 10 healthy patients. Mepoluzimab- and omalizumab-treated patients were matched for maintenance non-biological treatments in order to allow identifying potential treatment-related effects compared with healthy patients (Table 1 and Supplementary Table 1). As expected, blood of mepolizumab-treated patients contained only residual eosinophils (Figure 4A), which were around the typical 50 eosinophils/µl average (4). We sorted blood eosinophils of the 30 subjects to high purity and retrieved high quality RNA (Figure 4B). We subsequently compared polyadenylated RNA expression by RNA-sequencing. Sample clustering and PCA analyses indicated that eosinophil gene expression profiles failed to aggregate in function of the patient groups (Figure 4C, D). Pairwise differential gene expression analyses retrieved no differentially expressed genes (adjusted p<0.05, |log2(fold change)| >1) between subject groups, including in mepolizumab-treated versus healthy control patients (Figure 4E). Together, these results indicate that gene expression profiles of residual blood eosinophils from severe asthmatic patients receiving mepolizumab did not differ detectably from that of eosinophils from healthy patients or omalizumab-treated patients.

Like in our experiments with murine eosinophils, we also compared the response to activation of human eosinophils that developed in IL5-depleted versus IL5-replete conditions. To this end, we collected blood eosinophils from an additional 3 mepolizumab-treated and 3 omalizumab-treated severe asthmatic patients (Table 2). Half of the patient's sample was immediately processed for RNA-sequencing, while the other half of eosinophils were stimulated for 6h with IL33 before processing. We stimulated human eosinophils with IL33 alone to stay closer to the in vivo environment encountered by eosinophils in mepolizumab-treated patients, in whom IL5 is neutralized. Individual patients' RNA samples were subsequently sequenced and submitted to differential gene expression analysis using a paired-design (Figure 5A). Like in murine eosinophils, culture in the presence of IL33 had a very marked impact on human eosinophil gene expression. The first principal component in a PCA

captured 56% of variance in gene expression and separated IL33-cultured samples from their unstimulated counterparts in each patient, whereas PC2 did not separate patient samples based on treatment and captured only 23% of the variance. This suggested that IL33 stimulation, but not the patients' biological treatment, had a predominant effect on the eosinophil transcriptome (Figure 5B). Further substantiating this notion, the gene expression changes induced by culture in the presence of IL33 correlated highly between eosinophils from mepolizumab- and omalizumab-treated patients, as 1015 genes were significantly coregulated (adjusted $p<0.05$, Figure 5C and Supplementary Table 2). GSEAR analysis for Hallmark gene sets returned "Hallmark_TNFA_signaling_via_NFKB" as the most significantly upregulated hallmark process (Figure 5D), consistent with our results in murine eosinophils. In contrast, only 14 genes were differentially regulated in the response to IL33 between eosinophils from mepoluzimab- and omalizumab-treated patients (Figure 5E, F). Finally, based on differences in expression of these 14 genes, samples clustered first according to IL33 treatment, and only second according to the fact that samples came from mepolizumab- or omalizumab-treated patients (Figure 5F). In other words, only the magnitude of changes in gene expression induced by IL33 differed depending on the patients' treatment. Of potential interest still, one gene, *SOCS3*, was more robustly induced by IL33 in IL5-depleted eosinophils in both mice and human.

Discussion

In this work, we show that depletion of IL5, through genetic deficiency in mice or through the administration of anti-IL5 neutralizing antibodies in human, results in only minimal perturbations in the gene expression program of residual eosinophils in the steady-state or following acute activation. As such, our study supports the notion that anti-IL5 biological therapies leave residual circulating eosinophils largely unaltered, albeit in reduced numbers.

This conclusion is based on congruent observations of the role of IL5 in two distant organisms. First, we studied syngeneic mouse strains differing only for their genetic proficiency or deficiency at producing IL5. Second, we compared severe allergic asthmatic patients receiving anti-IL5 or anti-IgE biological treatments. In both cases, depletion of IL5 had no detectable effect on the gene expression program of steady-state residual eosinophils. In addition, eosinophils in both organisms responded almost uniformly to acute cytokine stimulation. Indeed, only the response of a handful of genes differed between eosinophils that developed in IL5-depleted versus IL5-replete conditions, namely one gene in murine eosinophils and 14 genes in human eosinophils. Remarkably, the sole differentially expressed

gene in stimulated murine eosinophils experiencing IL5 restriction, namely Socs3, was also more robustly induced in stimulated eosinophils from mepolizumab-treated patients. Altogether, these results suggest that IL5 only plays a minimal role in priming the eosinophil gene expression program per se, but that this role, minimal as it is, is conserved between human and mice.

SOCS3 encodes a negative regulator of signalling by different cytokines and growth factors, including IL12, a key regulator of auxiliary T cell polarization (26). SOCS3 is a suspected driver of asthma risk in genetic association studies (27), of which expression correlates with asthma severity (28). This is most likely explained by the fact that SOCS3 is a marker of auxiliary type 2 T cells and facilitates their polarization in airway allergy (28). Yet, the role of SOCS3 in eosinophils themselves remains to be established. Determining whether increased stimulation-induced expression of SOCS3 in eosinophils in IL5-depleted conditions has biological consequences would hence be worthwhile pursuing.

The absence of a major impact of the absence of IL5 on residual eosinophils may seem at odds with its previous proposal as an eosinophil maturation factor. However, the uniform reduction in maturing eosinophil progenitors that was observed in the bone marrow of mepolizumab-treated patients (16) may be more consistent with a reduction in eosinophil amplification, rather than with an impairment of eosinophil maturation. This notion is also consistent with the observation that IL5 is dispensable for mouse eosinophil maturation after differentiation is initiated (29). From a fundamental standpoint, our results are hence mainly in line with the notion that the major effect of IL5 on eosinophilopoiesis is in promoting eosinophil expansion rather than in influencing their differentiation per se.

In this study, we relied solely on gene expression profiling for determining the effect of IL5 on residual eosinophil function. We do not exclude that IL5 has activities not directly related to gene expression control. Yet, we argue that major changes in differentiation or activity in any cell are reflected, at least indirectly and to some extent, in its gene expression program. We could however not detect any gene expression signature of IL5 depletion in steady-state eosinophils, and only very limited changes in gene expression following eosinophil activation. Our current findings are thereby also consistent with a report that mepolizumab does not alter the expression of activation markers on eosinophils in the bronchoalveolar lavage fluid or their release of eosinophil peroxidase in the lung mucosa of treated patients (30). Our analysis in human arguably comprised a limited number of patients (10 per group), and might thereby not have captured genes with elevated interindividual variability and low differences between groups. Nevertheless, as discussed above, our

analyses in syngeneic mice, which differ only by the expression of IL5, showed striking similarities with our analyses in human eosinophils.

Altogether, our results indicate that the restriction of IL5 bioavailability has no detectable impact on the gene expression program of residual quiescent steady-state eosinophils, and only minimally influences their response to activation. From a clinical perspective, our work supports the notion that treatment with IL5 neutralizing antibodies spares a pool of circulating residual eosinophils largely resembling those of healthy individuals.

Acknowledgements

This work was supported by the Fonds Wetenschappelijk Onderzoek - Vlaanderen (FWO) and the Fonds de la Recherche Scientifique (FRS) – FNRS (Belgium) under EOS Project No. 30565447 (U-HEAD), by a Research Project grant (T.0052.18 REGEOS) of the FRS-FNRS, by the Leon Fredericq Foundation (Liege University), and by Liege University. JJ is a doctoral Research Fellow, and CJD is a Research Associate of the FRS-FNRS. Funding sources were not involved in the research. The authors thank the Flow Cytometry platform, the Mouse Facility, and the Genomics platform of the Interdisciplinary Group for Applied Genoproteomics of the University of Liege. We also thank R.Fares, C.François and I.Sbai for excellent technical and secretarial assistance.

Conflict of Interest

J. Jorssen reports PhD student scholarship from Fonds de la Recherche scientifique (FRS)-FNRS (Belgium). N. Jacobs reports consulting fees and lecture fees from GSK, outside the submitted work. L. Renaud reports grants from GSK, AZ, Novartis, Chiesi and Teva; royalties from patent AU2016328384, CA2997506, EP 3337393, US2020345266; consulting fees and lecture payments from GSK, AZ, Novartis, Sanofi and Chiesi, outside the submitted work. F. Schleich reports grants from GSK, Astrazeneca, Teva, Chiesi and Novartis; consulting fees from GSK, Astrazeneca, Amgen, Chiesi and Novartis; lecture payments from GSK, Astrazeneca, Teva, Chiesi and Novartis, outside the submitted work. C. Desmet reports salary from Fonds de la Reherche Scientifique – FNRS (Belgium), during the submitted work; consulting fees in Advisory Board on eosinophil research from AstraZeneca; lecture fees for presentation at several scientific symposia in Europe on our research on eosinophils from GSK, outside the submitted work. All other authors have nothing to disclose.

References

1. Rothenberg, M. E., and S. P. Hogan. 2006. The eosinophil. *Annu Rev Immunol* 24: 147–174.

2. Roufosse, F. 2018. Targeting the Interleukin-5 Pathway for Treatment of Eosinophilic Conditions Other than Asthma. *Front Med* 5: 49.

3. Haldar, P., C. E. Brightling, B. Hargadon, S. Gupta, W. Monteiro, A. Sousa, R. P. Marshall, P. Bradding, R. H. Green, A. J. Wardlaw, and I. D. Pavord. 2009. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360: 973–984.

4. Pavord, I. D., S. Korn, P. Howarth, E. R. Bleecker, R. Buhl, O. N. Keene, H. Ortega, and P. Chanez. 2012. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 380: 651–659.

5. FitzGerald, J. M., E. R. Bleecker, P. Nair, S. Korn, K. Ohta, M. Lommatzsch, G. T. Ferguson, W. W. Busse, P. Barker, S. Sproule, G. Gilmartin, V. Werkström, M. Aurivillius, and M. Goldman. 2016. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 388: 2128–2141.

6. Graff, S., S. Demarche, M. Henket, V. Paulus, R. Louis, and F. Schleich. 2019. Increase in blood eosinophils during follow-up is associated with lung function decline in adult asthma. *Respir. Med.* 152: 60–66.

7. Hammad, H., and B. N. Lambrecht. 2021. The basic immunology of asthma. *Cell* .

8. Dougan, M., G. Dranoff, and S. K. Dougan. 2019. GM-CSF, IL-3, and IL-5 Family of Cytokines: Regulators of Inflammation. *Immunity* 50: 796–811.

9. Foster, P. S., S. P. Hogan, A. J. Ramsay, K. I. Matthaei, and I. G. Young. 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183: 195–201.

10. Kopf, M., F. Brombacher, P. D. Hodgkin, A. J. Ramsay, E. A. Milbourne, W. J. Dai, K. S. Ovington, C. A. Behm, G. Köhler, I. G. Young, and K. I. Matthaei. 1996. IL-5-Deficient Mice Have a Developmental Defect in CD5+ B-1 Cells and Lack Eosinophilia but Have Normal Antibody and Cytotoxic T Cell Responses. *Immunity* 4: 15–24.

11. Klion, A. D., S. J. Ackerman, and B. S. Bochner. 2020. Contributions of Eosinophils to Human Health and Disease. *Annu. Rev. Pathol. Mech. Dis.* 15: 179–209.

12. Pavord, I. D., A. Menzies-Gow, R. Buhl, P. Chanez, M. Dransfield, N. Lugogo, O. N. Keene, E. S. Bradford, and S. W. Yancey. 2020. Clinical Development of Mepolizumab for

the Treatment of Severe Eosinophilic Asthma: On the Path to Personalized Medicine. *J Allergy Clin Immunol Pr.* .
13. Castro, M., J. Zangrilli, M. E. Wechsler, E. D. Bateman, G. G. Brusselle, P. Bardin, K. Murphy, J. F. Maspero, C. O'Brien, and S. Korn. 2015. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 3: 355–366.
14. Schleich, F., S. Graff, H. Nekoee, C. Moermans, M. Henket, C. Sanchez, V. Paulus, F. Guissard, A.-F. Donneau, and R. Louis. 2020. Real-world experience with mepolizumab: Does it deliver what it has promised? *Clin. Exp. Allergy* 50: 687–695.
15. Mack, E. A., and W. S. Pear. 2020. Transcription factor and cytokine regulation of eosinophil lineage commitment. *Curr Opin Hematol* 27.
16. Menzies-Gow, A., P. Flood-Page, R. Sehmi, J. Burman, Q. Hamid, D. S. Robinson, A. B. Kay, and J. Denburg. 2003. Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 111: 714–719.
17. Weller, P. F., and L. A. Spencer. 2017. Functions of tissue-resident eosinophils. *Nat Rev Immunol* 17: 746.
18. Marichal, T., C. Mesnil, and F. Bureau. 2017. Homeostatic Eosinophils: Characteristics and Functions. *Front Med* 4: 101.
19. Van Hulst, G., H. M. Batugedara, J. Jorssen, R. Louis, F. Bureau, and C. J. Desmet. 2020. Eosinophil diversity in asthma. *Biochem. Pharmacol.* 179: 113963.
20. Mesnil, C., S. Raulier, G. Paulissen, X. Xiao, M. A. Birrell, D. Pirottin, T. Janss, P. Starkl, E. Ramery, M. Henket, F. N. Schleich, M. Radermecker, K. Thielemans, L. Gillet, M. Thiry, M. G. Belvisi, R. Louis, C. Desmet, T. Marichal, and F. Bureau. 2016. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest* 126: 3279–3295.
21. Wenzel, S., J. Fahy, C. Irvin, S. Peters, S. Spector, S. Szefler, T. Casale, M. Cloutier, J. Elias, M. Liu, and V. Taggert. 2000. Proceedings of the ATS Workshop on Refractory Asthma. *Am J Respir Crit Care Med* 162: 2341–2351.
22. Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15: 550.
23. Cherry, W. B., J. Yoon, K. R. Bartemes, K. Iijima, and H. Kita. 2008. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol* 121: 1484–1490.

24. Angulo, E. L., E. M. McKernan, P. S. Fichtinger, and S. K. Mathur. 2019. Comparison of IL-33 and IL-5 family mediated activation of human eosinophils. *PLoS One* 14: e0217807.
25. Bouffi, C., M. Rochman, C. B. Zust, E. M. Stucke, A. Kartashov, P. C. Fulkerson, A. Barski, and M. E. Rothenberg. 2013. IL-33 Markedly Activates Murine Eosinophils by an NF-κB–Dependent Mechanism Differentially Dependent upon an IL-4–Driven Autoinflammatory Loop. *J Immunol* 191: 4317 LP – 4325.
26. Rottenberg, M., and B. Carow. 2014. SOCS3, a Major Regulator of Infection and Inflammation. *Front Immunol* 5: 58.
27. Hao, K., Y. Bossé, D. C. Nickle, P. D. Paré, D. S. Postma, M. Laviolette, A. Sandford, T. L. Hackett, D. Daley, J. C. Hogg, W. M. Elliott, C. Couture, M. Lamontagne, C.-A. Brandsma, M. van den Berge, G. Koppelman, A. S. Reicin, D. W. Nicholson, V. Malkov, J. M. Derry, C. Suver, J. A. Tsou, A. Kulkarni, C. Zhang, R. Vessey, G. J. Opiteck, S. P. Curtis, W. Timens, and D. D. Sin. 2012. Lung eQTLs to Help Reveal the Molecular Underpinnings of Asthma. *PLOS Genet.* 8: e1003029.
28. Seki, Y., H. Inoue, N. Nagata, K. Hayashi, S. Fukuyama, K. Matsumoto, O. Komine, S. Hamano, K. Himeno, K. Inagaki-Ohara, N. Cacalano, A. O'Garra, T. Oshida, H. Saito, J. A. Johnston, A. Yoshimura, and M. Kubo. 2003. SOCS-3 regulates onset and maintenance of TH2-mediated allergic responses. *Nat. Med.* 9: 1047–1054.
29. Fulkerson, P. C., K. L. Schollaert, C. Bouffi, and M. E. Rothenberg. 2014. IL-5 Triggers a Cooperative Cytokine Network That Promotes Eosinophil Precursor Maturation. *J Immunol* 193: 4043 LP – 4052.
30. Kelly, E. A., S. Esnault, L. Y. Liu, M. D. Evans, M. W. Johansson, S. Mathur, D. F. Mosher, L. C. Denlinger, and N. N. Jarjour. 2017. Mepolizumab Attenuates Airway Eosinophil Numbers, but Not Their Functional Phenotype, in Asthma. *Am J Respir Crit Care Med* 196: 1385–1395.

Tables

Table 1: Demographic and functional characteristics of asthma patients in Figure 4

	Mepolizumab (n=10)	**Omalizumab (n=10)**	**p-value**
Gender (M/F)	2/8	2/8	1
Age (years)	56.1 ± 9.45	39.5 ± 9.6	0.0011
BMI	27.2 ± 2.5	26.2 ± 5.5	0.59
FeNO (ppb)	49.11 ± 35.0	18.7 ± 12.2	0.019
FEV1 (% predicted)	71.3 ± 13.3	71.2 ± 20.2	0.99
FEV1 postBD (% predicted)	79.7 ± 13.2	79.3 ± 17.8	0.96
FVC (% predicted)	84.5 ± 17.5	82.3 ± 9.7	0.73
FEV1/FVC	68.6 ± 6.6	73.3 ± 13.4	0.33
FEV1/FVC postBD	74.1 ± 9.5	76.2 ± 12.6	0.68
RV (% predicted)	133 ± 31.7	94.9 ± 49.9	0.056
TLC (% predicted)	102.5 ± 19.0	90.5 ± 19.2	0.18
DLCO (% predicted)	83.9 ± 9.7	78.3 ± 12.3	0.32
KCO (% predicted)	96 ± 18.3	92.8 ± 13.9	0.71
ICS (yes/no)	10/0	10/0	1
OCS (yes/no)	0/10	0/10	1
LABA (yes/no)	10/0	10/0	1
LAMA (yes/no)	1/9	1/9	1
LTRA (yes/no)	0/10	4/6	0.00016

Results are expressed as mean ± SD. Comparisons between patient groups were performed using unpaired t tests for continuous variables. Chi-square test was applied for categorical variables. BMI: body mass index; FeNO: exhaled nitric oxide. FEV1: forced expiratory volume in 1 s; postBD: post-bronchodilator treatment; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; DLCO: diffusing capacity of the lung for carbon monoxide; KCO: gas transfer coefficient; ICS: inhaled corticosteroids; OCS: oral corticosteroids; LABA: long-acting beta 2 agonist, LAMA: long-acting muscarinic antagonist. SABA: short acting beta agonist; LTRA: leukotriene receptor antagonist.

Table 2. Demographic and functional characteristics of asthma patients in Figure 5.

	Mepolizumab (n=3)	Omalizumab (n=3)	p-value
Gender (M/F)	0/3	1/2	1
Age (years)	34 ± 13.9	52.3 ± 27.5	0.36
BMI	23.9 ± 1.2	26.3 ± 1.5	0.092
FeNO (ppb)	35.3 ± 37.1	30.3 ± 9.5	0.83
FEV1 (% predicted)	85 ± 11.5	70.7 ± 2.1	0.10
FEV1 postBD (% predicted)	88 ± 11.5	79.3 ± 14.5	0.45
FVC (% predicted)	87.3 ± 17.6	84 ± 14.6	0.81
FEV1/FVC	83 ± 12.5	69.3 ± 14.8	0.29
FEV1/FVC postBD	80.7 ± 7.1	75.3 ± 18.2	0.66
RV (% predicted)	103.0 ± 23.5	116.7 ±14.6	0.52
TLC (% predicted)	96.0 ± 6.2	82 ± 18.2	0.072
DLCO (% predicted)	79.3 ± 12.1	75.3 ± 24.1	0.73
KCO (% predicted)	85.3 ± 3.2	100 ± 7.8	0.39
ICS (yes/no)	3/0	3/0	1
OCS (yes/no)	0/3	0/3	1
LABA (yes/no)	3/0	3/0	1
LAMA (yes/no)	0/3	0/3	1
LTRA (yes/no)	2/1	0/3	0.1258

Results are expressed as mean ± SD. Comparisons between patient groups were performed using unpaired t tests for continuous variables. Chi-square test was applied for categorical variables. BMI: body mass index; FeNO: exhaled nitric oxide. FEV1: forced expiratory volume in 1 s; postBD: post-bronchodilator treatment; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; DLCO: diffusing capacity of the lung for carbon monoxide; KCO: gas transfer coefficient; ICS: inhaled corticosteroids; OCS: oral corticosteroids; LABA: long acting beta 2 agonist, LAMA: long acting muscarinic antagonist. SABA: short acting beta agonist; LTRA: leukotriene receptor antagonist.

Figure legends

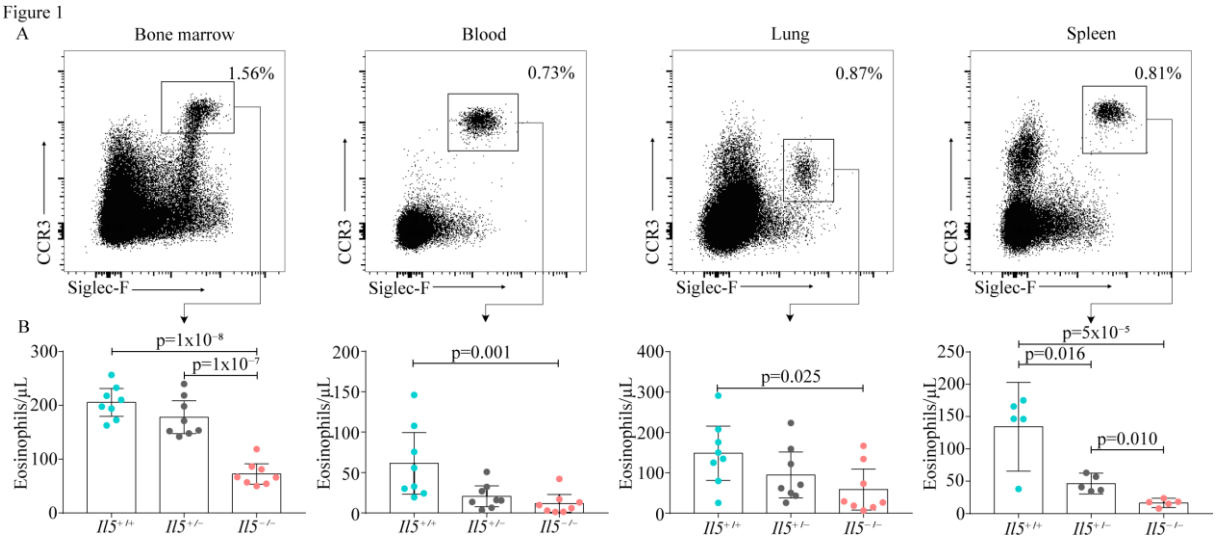


Figure 1. Il5-deficient mice retain residual eosinophils. A. Representative plots of flow cytometric gating strategy with percentage of eosinophils in indicated organs. **B.** Quantification of eosinophils in specified organs of *Il5*$^{+/+}$, *Il5*$^{+/-}$ and *Il5*$^{-/-}$ mice as in A. Data was pooled from 2 to 3 independent experiments, presented as means +/- 95% confidence interval and analysed by one-way ANOVA followed by Tukey honestly significant difference tests. Only significant differences of interest are indicated for clarity of presentation.

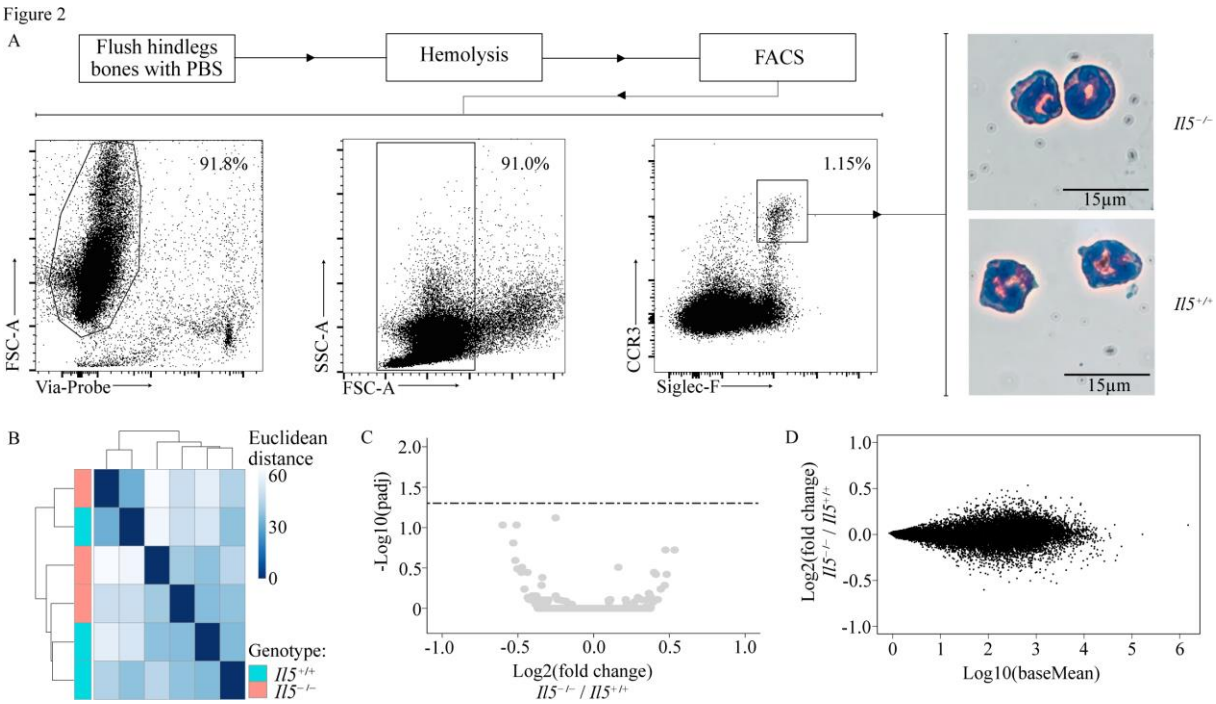


Figure 2. Genetic deficiency in Il5 has no detectable impact on mouse residual eosinophils. A. Isolation strategy of *Il5*$^{+/+}$ and *Il5*$^{-/-}$ mouse bone marrow eosinophils (left) and representative post-sort light microscopy picture (right). **B.** Sample clustering, volcano plot and MA plot based on RNA-sequencing of biological triplicates in A.

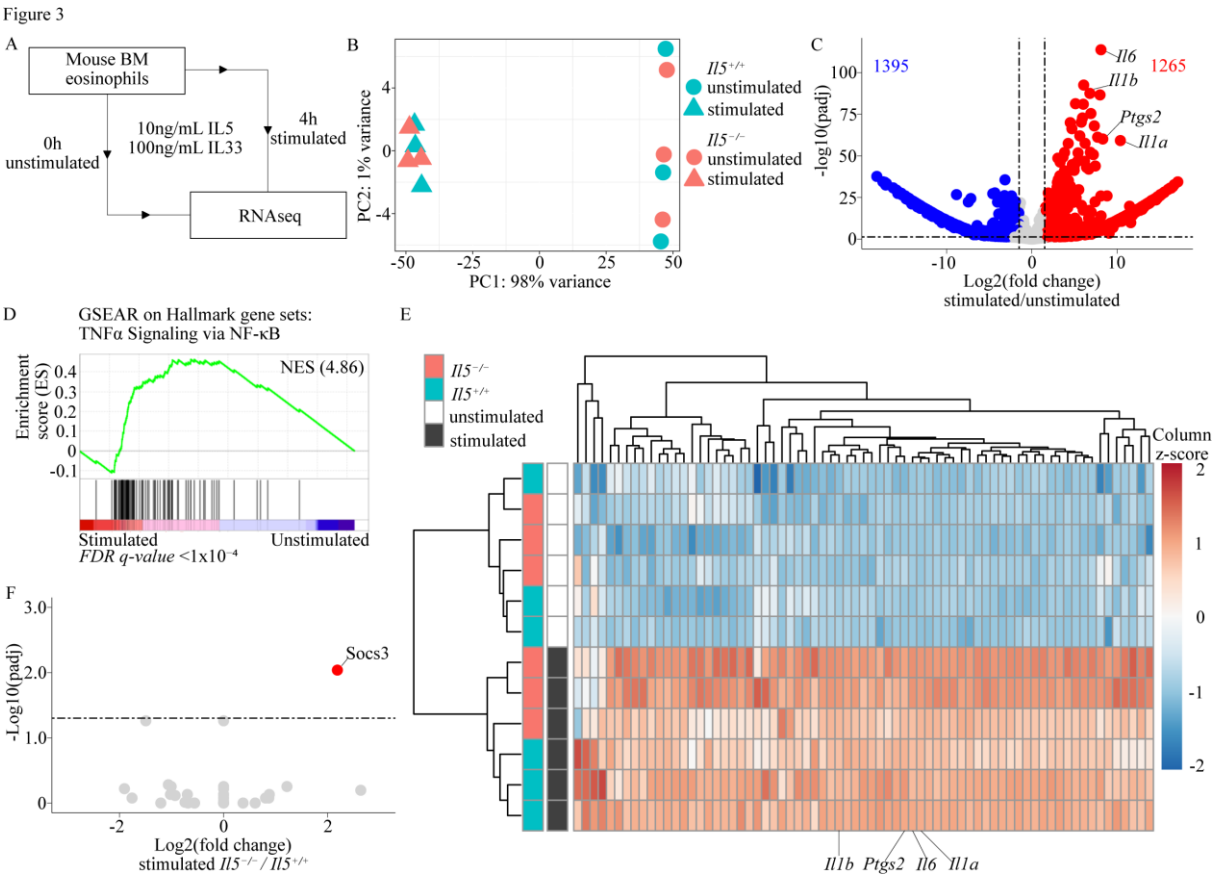


Figure 3. Response to stimulation of *Il5*$^{+/+}$ and *Il5*$^{-/-}$ bone marrow eosinophils. A. Experimental outline. **B.** Principal component analysis. **C.** Changes in gene expression of *Il5*$^{+/+}$ and *Il5*$^{-/-}$ eosinophils in response to stimulation. **D.** Ranked gene set enrichment analysis plot for the indicated Molecular Signature database (MisgDB) hallmark gene set. **E.** Heatmap of changes in expression of genes in D. **F.** Changes in gene expression of *Il5*$^{-/-}$ versus *Il5*$^{+/+}$ eosinophils in response to stimulation.

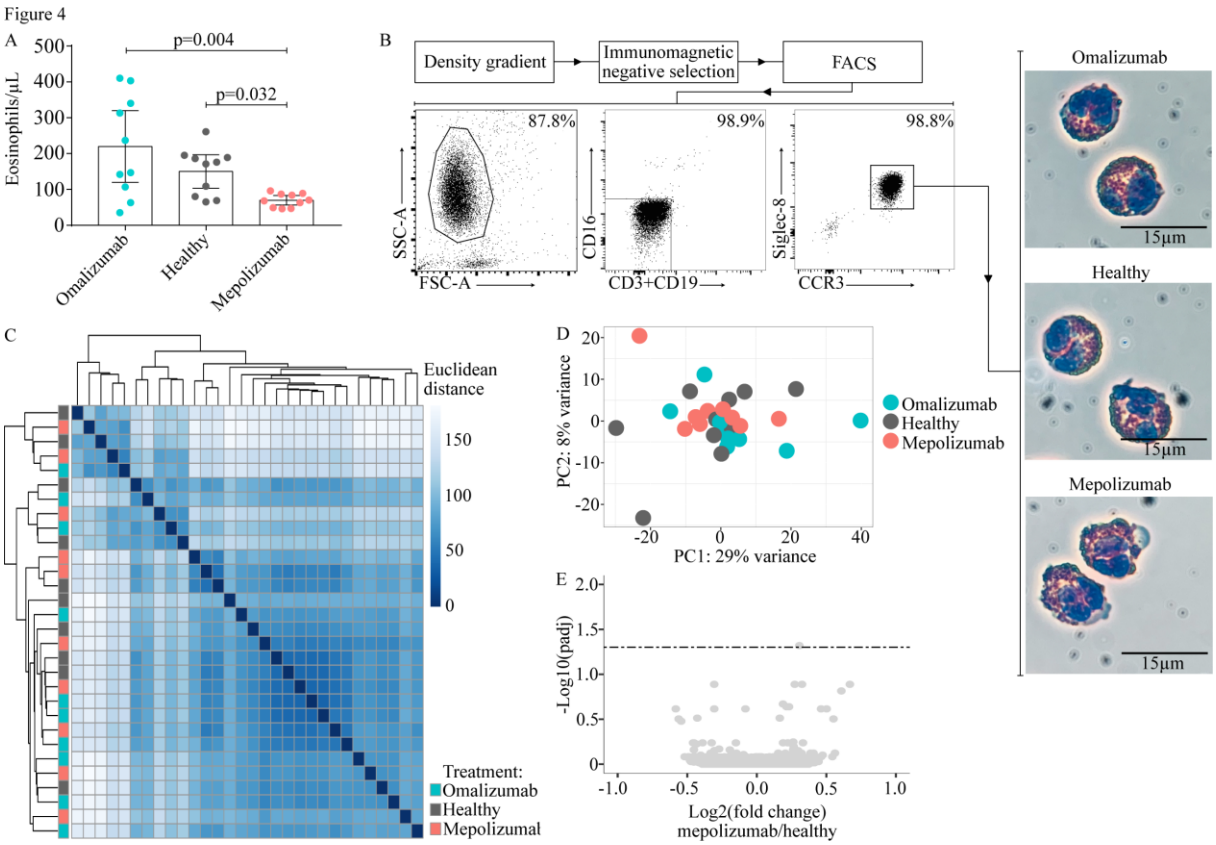


Figure 4. Transcriptomic impact of mepolizumab on human blood eosinophils. A. Eosinophil blood counts in healthy controls, and in severe allergic asthmatic patients receiving mepolizumab or omalizumab treatment (one-way ANOVA followed by Tukey honestly significant difference tests). **B.** Blood eosinophil sorting strategy and representative post-sort light microscopy pictures of eosinophils from the 3 groups of donors in A. **C.** Sample clustering by RNA-sequencing. **D.** Principal component analysis. **E.** Differentially expressed genes in eosinophils from mepolizumab-treated versus healthy control donors.

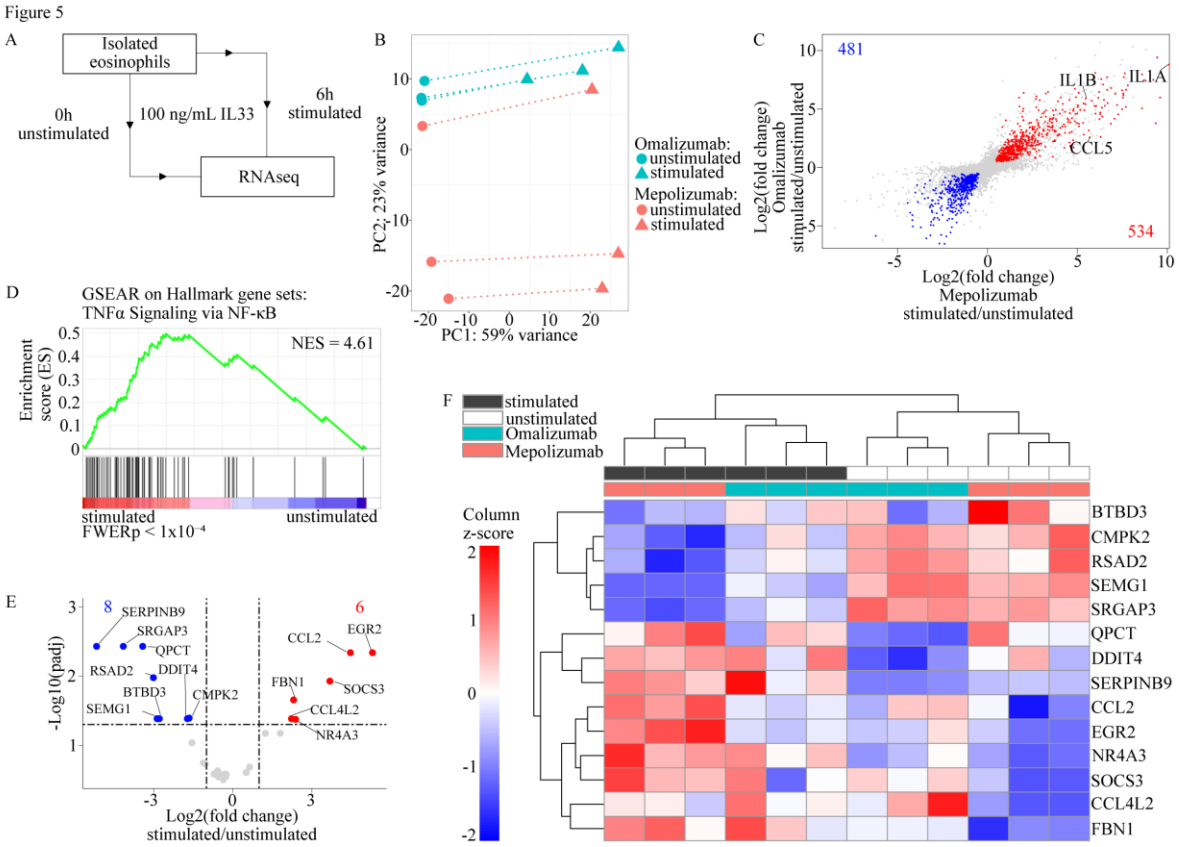


Figure 5. Transcriptomic response to stimulation of blood eosinophils from mepolizumab- or omalizumab-treated patients. A. Experimental outline. **B.** Principal component analysis. **C.** Correlation of changes in gene expression in response to IL33 in eosinophils from both patient groups. **D.** Ranked gene set enrichment analysis plot for the indicated Molecular Signature database (MisgDB) hallmark gene set. **E-F.** Differentially expressed genes in response to stimulation of eosinophils from mepolizumab- versus omalizumab-treated patients presented as a volcano plot (E) or a heatmap (F).

Supplemental Material and methods

Quantitation of mouse and human eosinophils

Mouse lungs were flushed with 10mL of PBS to remove blood from the lung. Lungs were macerated and mixed with 3mL of digestion buffer consisting of HBSS with Ca and Mg (ThermoFisher, 24020117), 5% FBS, 1mg/mL collagenase A (Roche, 10103578001), and 0.05mg/mL DNase I (Roche, 4716728001) and incubated at 37°C for 1h with shaking. Mouse lung cell suspension was passed through a cell strainer (70μm) and washed twice in ice-cold PBS prior to the staining with conjugated surface markers. Mouse spleen was homogenised and passed through a cell strainer (70μM). The recovered spleen cell suspension was lysed with lysis buffer, consisting of of UltraPure distilled water (Invitrogen, 15657708) supplemented with 150mM NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA, at room temperature for 5 minutes and washed with ice-cold PBS twice prior to the staining with conjugated surface markers. Mouse blood was collected from the eye sinus with a capillary under terminal anesthesia. The recovered mouse blood was lysed with lysis buffer at room temperature for 5 minutes and washed with ice-cold PBS twice prior to the staining with conjugated surface markers. All recovered lung, spleen, and blood single-cell suspensions were stained with anti-CCR3-APC (BioLegend, J073E5), anti-Siglec-F-PE (BD Biosciences, E50-2440), anti-CD3-FITC (BD Biosciences, 55306), anti-CD19-APC-eFluor 780 (eBioscience, 47-0193-80), anti-Ly-6G-PE-Cy7 (BD Biosciences, 560601), anti-CD45-V500 (BD Biosciences, 562129), anti-CD11b-BV421 (BD Biosciences, 562605), and green nucleic acid stain (BD Biosciences, 565799). Staining reactions were performed at 4°C following a 5-minute incubation with Fc receptor antibodies to reduce non-specific binding (BD Biosciences, 553141). Mouse eosinophils were defined as $CCR3^+$Siglec-F^+ cells (Figure E1). Absolute mouse eosinophil counts were quantitated using CountBright absolute counting beads for flow cytometry (ThermoFisher, C36950). Human whole blood eosinophils were quantitated using an automated hematology analyzer (Cell-Dyn, Abbott Laboratories).

Isolation of eosinophils from mouse bone marrow

Single-cell mouse bone marrow suspensions were recovered from hind leg bones (pelvis, tibia, femur). Bones were flushed with 10mL of ice-cold PBS containing 10mM EDTA and passed through a cell strainer (70μM). The recovered single-cell suspension was lysed in lysis buffer at room temperature for 5 minutes and washed with ice-cold PBS twice. Mouse bone marrow cells were stained with anti-CCR3-APC (BioLegend, J073E5), anti-Siglec-F-

PE (BD, E50-2440), and green nucleic acid stain (BD Biosciences, 565799). Absolute eosinophil counts were quantitated using CountBright absolute counting beads for flow cytometry (ThermoFisher, C36950). Bone marrow eosinophils ($CCR3^+$ $Siglec\text{-}F^+$ cells) were sorted into TRIzol® (ThermoFisher, 15596026) using a BD FACSAria III (BD Biosciences) at a purity of 90 per cent or higher and stored at -80°C for downstream RNA applications.

Isolation of eosinophils from human blood

From every donor patient, we sampled 30mL of venous blood in BD Vacutainer EDTA tubes (BD biosciences, 367525). Polymorphonuclear cells were isolated by double-layer density gradient centrifugation. Recovered cells were purified by negative selection immunomagnetic purification (Stemcell™ Technologies, 17956) following the manufacturer's instructions. Briefly, cells were washed twice with ice-cold PBS supplemented with 2% heat inactivated fetal bovine serum (FBS, Sigma-Aldrich) and 1mM EDTA. Cell suspension was sedimented at 300xg for 5 minutes and adjusted to $5x10^7$ cells per mL. For every mL of sample, 50µL of antibody cocktail was added, and cells were incubated at room temperature for 5 minutes. An equal volume of RapidSpheres™ was added and samples were immediately incubated in an EasySep™ magnet at room temperature for 3 minutes. Pre-sort eosinophil viability was 95 per cent or superior as assessed by trypan blue exclusion. Human blood eosinophils were stained with anti-CCR3-FITC (REA574, Miltenyi Biotec), anti-CD3-PE (REA613, Miltenyi Biotec), anti-CD19-PE (REA675, Milenyi Biotec), anti-Siglec-8-APC (REA1045, Milentyi Biotec), and anti-CD16-APC-H7 (3G8, BD Biosciences). Staining reactions were performed at 4°C following a 5-minute incubation with Fc receptor antibodies to reduce non-specific binding (BD Biosciences, 564219). Eosinophils were sorted into TRIzol® (ThermoFisher, 15596026) using a BD FACSAria III (BD Biosciences) at a purity of 95% or higher and stored at -80°C for downstream RNA applications.

Bright-field microscopy

Sorted human blood- and mouse bone marrow eosinophils were sedimented at 300xg for 5 minutes and resuspended in Freeflex Geloplasma 3% (Fresenius Kabi, RVG 20107), loaded into a cytofunnel and spun at 800xg for 4 minutes. Slides were left to dry overnight and cells and stained using Hemacolor Rapid staining kit (Sigma-Aldrich, 1116610001). Bright-field cell images were taken at 1500x total magnification using an oil immersion objective.

Ex vivo activation of eosinophils

Culture medium consisted of sterile filtered IMDM supplemented with 20% heat inactivated fetal bovine serum (Sigma-Aldrich), 1% non-essential amino acids (Sigma-Aldrich), 1mM sodium pyruvate (Sigma-Aldrich), 2mM l-glutamine (Sigma-Aldrich), 25mM HEPES (Sigma-Aldrich), 50μM β-mercaptoethanol (Sigma-Aldrich), 100U/mL penicillin (Millipore). Human blood eosinophils (purity≥95%) were cultured for 6 hours at 37°C and 5% CO_2 in culture medium with additional 100ng/mL purified IL33 (PeproTech, 200-33). Stimulated human blood eosinophils were sedimented at 300xg for 5 minutes, resuspended in TRIzol® (ThermoFisher) and stored at -80°C for downstream RNA extraction. Mouse bone marrow eosinophils (purity≥90%) were cultured for 4 hours at 37°C and 5% CO_2 in culture medium with additional 100ng/mL purified IL33 (BioLegend, 580504), and 10ng/mL purified IL5 (Peprotech, 215-15). Stimulated mouse bone marrow eosinophils were sedimented at 300xg for 5 minutes, resuspended in TRIzol® (ThermoFisher) and stored at -80°C for downstream RNA extraction.

RNA extraction

Human blood and mouse bone marrow eosinophils were sorted as described above directly into TRIzol (ThermoFisher). For every mL of TRIzol, 200μL of chloroform was added, and the samples were vigorously mixed and incubated for 2 minutes at room temperature. Samples were centrifuged at 10,000xg for 15 minutes at 4°C to separate the phases. The RNA-containing upper aqueous phase was transferred to a new microcentrifuge tube containing 475μL of isopropanol and 2μL of glycoblue (ThermoFisher, AM9515). Samples were centrifuged at 10,000xg for 15 minutes and supernatant was discarded. One volume of 75% ethanol was added and samples were centrifuged at 10,000xg for 1 minute to precipitate the RNA and discard the supernatant. RNA pellet was resuspended in 40μL of DNase/RNase-free water for a 15-minute DNase treatment (Zymo Research, E1010). DNase treatment was followed by column-based RNA purification with the RNA Clean & concentrator-5 kit (Zymo Research, R1016). Briefly, 100μL of RNA binding buffer was added to every 50μL sample and mixed thoroughly. One volume of 100% ethanol was added and the sample was transferred into a Zymo-Spin™ IC column in a collection tube. Columns were centrifuged at 10,000xg for 30 seconds and flow-through was discarded. The column was washed once with RNA prep buffer and twice with RNA wash buffer, following the manufacturer's instructions. RNA was eluted in 10μL of DNase/RNase-free water and stored

at -80°C. Purified RNA integrity and quantity was assessed using the RNA 6000 Pico kit (Agilent) for the presence of 18s and 28s rRNA peaks. All human samples had RNA integrity number (RIN) >7.9.

RNA sequencing & data processing

Full length cDNA was prepared from isolated RNA using SMART-Seq v4 Ultra Low Input RNA kit (Takara Bio, 634889) following the manufacturer's instructions, with 17 cycles of cDNA amplification. Final cDNA quality was assessed using Agilent High Sensitivity DNA kit (Agilent, 5067-4626). cDNA libraries were prepared for sequencing using Nextera XT DNA library preparation kit (Illumina, FC-131-1024) using the manufacturer's instructions and samples were sequenced on a NovaSeq™ 6000 sequencing system (Illumina). If samples were sequenced in different batches, groups were kept equal within every batch.

Differential gene expression analyses

Sequenced reads were aligned to the mouse genome (UCSC mm10) or the human genome (HG19) with RNA-seq Alignment (v2.0.2) using STAR aligner (version 2.6.1a) on BaseSpace (https://basespace.illumina.com). Uniquely mapped reads were used to calculate gene expression. Differential gene expression was calculated using DESeq2 (1.26.0) in R (3.6.3 and 4.0.3) (19). If samples were sequenced in different batches, sample batch was taken into account in the DESeq2 design. GSEAR analyses on differentially expressed genes were performed on preranked list of significantly differentially expressed genes with baseMean>50 ordered according to their log2 fold change. Online GSEAR v7.2.1 (https://genepattern.broadinstitute.org/gp/pages/index.jsf) was used with the "h.all.v7.2.symbols" (Hallmarks) gene sets and default parameters, except for a "classic" scoring scheme and minimal gene set size of 20.

Statistical analyses

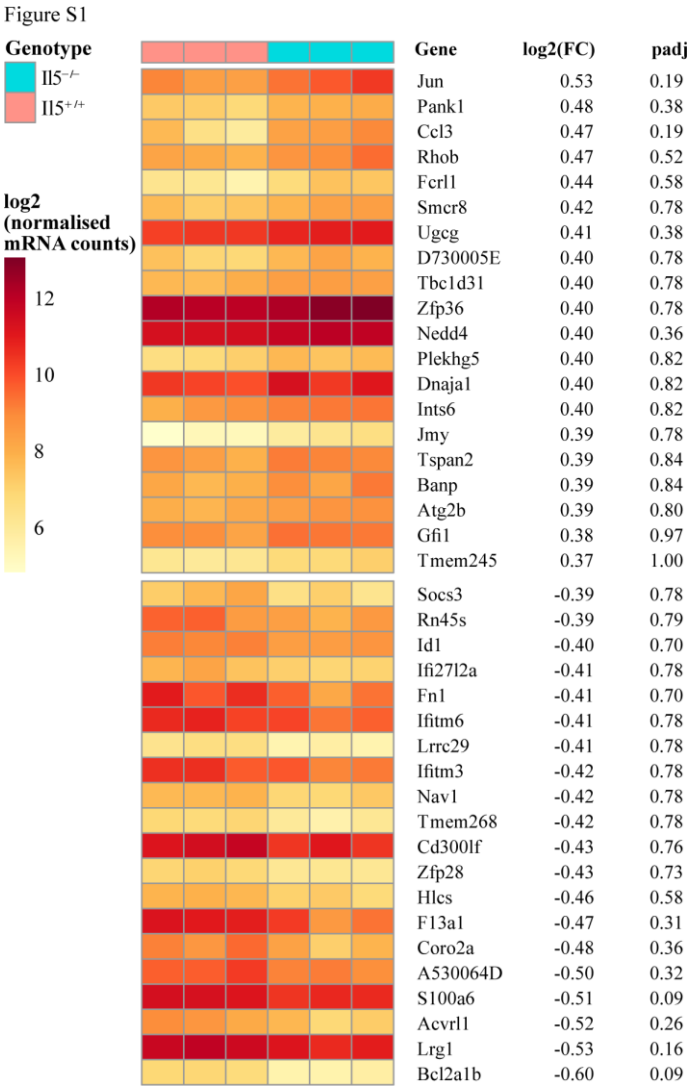
All statistical analyses were performed in R (3.5.0). Patient demographic and functional characteristics were expressed as mean ± SD. Comparisons between patient groups were performed using unpaired t tests. Chi-square test was applied for categorical variables. All mouse experiments followed a randomized design. Sample sizes were determined by power analysis. Respect of tests assumptions and model fit were evaluated using diagnostic plots. Raw data were transformed when needed and back-transformed for graphical presentation.

Error bars in all graphs represent mean +/- 95% confidence interval. In Figure 1A, data was pooled from 2 to 3 independent experiments and was analyzed by one-way ANOVA followed by Tukey honestly significant difference tests. In Figure 3A, data was analyzed by one-way ANOVA followed by Tukey honestly significant difference tests. A p-value lower than 0.05 was considered significant. For clarity of presentation, only results of intergroup comparisons of interest are displayed in figures.

Data deposition

RNA-sequencing data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession numbers E-MTAB-10188, E-MTAB-10189 and E-MTAB-10190

Supplementary figure legend



Supplementary Figure 1. Heatmap of mRNA expression, log2(fold change) and adjusted p-value of the top- and bottom- 20 genes in the comparison of bone marrow eosinophils from *Il5*$^{-/-}$ versus *Il5*$^{+/+}$ mice, ordered by fold change.

Supplementary Table 1. Response to biological treatment of patients in Table 1

	Mepolizumab	**Omalizumab**
Blood eosinophils (cells/μl)		
Pre-treatment	598.6 ± 694.6	278.0 ± 188.6
On treatment	65.7 ± 31.0 (**p=0.02**)	200.0 ± 185.1 (p=0.43)
FeNO (ppb)		
Pre-treatment	52.8 ± 28.8	35.6 ± 36.6
On treatment	49.1 ± 35.0 (p=0.60)	22.9 ± 12.1 (p=0.47)
FEV1 (% predicted)		
Pre-treatment	77.8 ± 17.8	69.0 ±23.2
On treatment	71.3 ± 13.3 (p=0.087)	69.0 ± 22.2 (p=1)
FEV1 postBD (% predicted)		
Pre-treatment	81.9 ± 19.2)	74.4 ± 22.7
On treatment	80.8 ± 13.5 (p=0.78)	79.5 ± 20.2 (p=0.45)
Exacerbations/year		
Pre-treatment	2.60 ± 1.51	2.22 ± 1.79
On treatment	0.13 ± 0.28 (**p=7.1x10^{-4}**)	0.38 ± 0.55 (**p=0.015**)
ACQ		
Pre-treatment	2.50 ± 1.76	3.41 ±0.89
On treatment	1.57 ± 0.68 (p=0.21)	2.91 ±1.22 (p=0.24)

Results are expressed as mean ± SD and p-values are given for comparisons of variables pre- and post-establishment of the treatment in each patients group (paired t-tests). ACQ: asthma control questionnaire; FeNO: exhaled nitric oxide; FEV1: forced expiratory volume in 1 s; postBD: post-bronchodilator treatment.

Supplementary table 1. Differentially expressed genes following IL33 stimulation of blood eosinophils from mepolizumab- and omalizumab-treated patients

Gene	log2(FC) mepolizumab	padj mepolizumab	log2(FC) omalizumab	padj omalizumab
SLAMF7	9.52	7.58E-05	13.88	1.00E-07
IL1A	10.76	7.92E-11	11.58	4.12E-11
C3	11.37	9.22E-07	10.60	9.28E-07
EBI3	10.60	1.35E-04	10.95	9.34E-05
COL13A1	10.30	1.28E-06	11.22	1.15E-07
CD22	8.92	9.37E-17	11.11	1.50E-19
KCNN1	10.12	4.39E-04	9.76	1.01E-03
KCNH4	10.03	8.60E-05	9.50	6.84E-05
ARHGAP31	9.66	4.17E-06	9.73	6.18E-06
C15orf48	9.68	2.43E-63	9.68	2.99E-49
MIR3142HG	8.88	2.70E-09	9.84	6.46E-11
SEMA6B	7.04	2.64E-07	11.11	5.91E-10
MELTF	9.52	6.58E-11	8.22	6.27E-13
ENG	8.83	2.28E-12	8.25	2.60E-11
DNAAF1	9.30	1.22E-09	7.30	4.16E-09
NANOS3	8.63	3.32E-08	7.56	5.50E-06
CXCR5	7.66	5.48E-09	8.45	2.15E-08
CRLF2	7.29	2.98E-13	8.62	3.49E-09
CCM2L	8.14	2.97E-07	7.33	8.63E-09
TNFAIP6	5.91	4.46E-06	9.45	4.10E-12
CD79B	6.36	2.60E-05	8.82	7.01E-08
SLC30A4	6.82	1.54E-14	8.14	2.46E-12
DNPH1	6.57	5.61E-11	8.23	8.44E-11
TRIP10	5.93	1.18E-05	8.69	3.26E-07
LOC644090	6.52	2.34E-05	7.92	1.67E-05
CDKN1A	8.32	1.14E-54	6.11	1.82E-32
LMNA	6.84	7.38E-07	7.29	2.17E-07
SERPINB9	3.96	1.37E-12	10.01	2.19E-18
DSE	7.09	1.13E-12	6.63	3.51E-11
TPSAB1	5.22	6.03E-06	8.37	2.81E-07
ELOVL7	6.59	1.45E-10	6.88	1.91E-07
LINC00926	5.54	7.73E-21	7.45	1.79E-24
HCAR3	5.57	9.86E-03	7.38	4.07E-04
ETV5	6.68	8.03E-05	6.18	9.58E-05
GRASP	6.28	5.59E-10	6.40	8.89E-08
BIRC3	7.29	2.68E-15	5.29	3.14E-08
IL1B	6.55	3.99E-10	5.95	2.49E-08
ZNF563	6.95	1.78E-13	5.45	2.64E-09
SLC2A6	6.29	2.88E-07	6.02	1.32E-06
DUSP2	5.46	2.28E-12	6.75	1.86E-12
SPATC1	4.37	4.32E-05	7.63	2.22E-08
IRAK2	4.77	1.13E-02	7.19	1.10E-04

LINC01094	5.53	2.13E-15	6.26	2.73E-19
STAT4	4.55	2.99E-07	6.79	6.20E-09
OLR1	4.53	8.42E-16	6.53	1.91E-30
CFAP58-DT	5.96	3.12E-05	5.10	6.65E-04
MFSD2A	6.04	2.97E-21	4.76	1.73E-14
PPM1N	6.06	1.08E-08	4.69	9.50E-07
TRAF4	6.29	9.02E-20	4.34	1.36E-10
FAM229B	5.76	1.30E-05	4.86	6.66E-04
NR4A1	6.84	2.40E-19	3.75	3.10E-07
NFKB2	5.42	4.01E-41	5.11	1.95E-36
TMEM52B	5.07	2.89E-07	5.42	3.52E-07
CXCL8	4.56	1.03E-04	5.90	3.27E-07
CCL3	6.54	1.67E-09	3.79	1.94E-03
ZC3H12A	5.01	3.94E-34	5.24	2.48E-37
FAM57A	7.23	1.10E-09	3.00	5.78E-03
SLC1A5	3.26	6.95E-04	6.75	1.22E-10
PLIN4	5.62	1.57E-05	4.34	1.11E-03
SLC7A11	5.82	3.10E-29	4.13	5.79E-13
PA2G4P4	6.33	3.04E-10	3.53	1.19E-04
COL15A1	5.82	1.42E-05	4.02	1.55E-04
LINC01678	4.06	2.84E-05	5.75	2.01E-06
HIVEP2	4.65	4.93E-07	5.13	4.44E-08
OPTN	4.22	4.90E-06	5.55	3.84E-08
UPB1	5.08	8.50E-11	4.63	1.25E-09
LOC100288175	6.46	6.75E-17	3.24	6.67E-05
HCAR2	4.32	2.67E-02	5.21	7.11E-03
ITGA1	5.33	5.46E-04	4.10	1.60E-02
POU2F2	5.08	1.93E-23	4.32	1.24E-17
SKIL	4.68	2.20E-86	4.61	5.90E-85
TIMP1	3.39	4.02E-09	5.83	1.38E-18
TGIF1	4.15	5.95E-13	5.02	3.51E-18
QPRT	4.37	5.65E-09	4.78	3.01E-11
PI3	3.88	1.63E-06	5.17	4.94E-05
RELB	3.90	2.35E-22	5.06	2.44E-36
TNFAIP3	4.28	9.77E-25	4.54	6.75E-28
MAP1LC3A	4.05	1.17E-12	4.77	3.23E-17
TRIM36	4.68	1.95E-43	4.09	1.84E-35
NR4A3	6.33	1.04E-16	2.37	4.09E-03
TNFSF4	3.23	2.87E-02	5.40	2.14E-04
NFIA	4.02	8.18E-16	4.61	2.40E-21
CHN2	4.18	9.72E-04	4.38	7.14E-03
TMEM176A	5.24	7.35E-11	3.32	2.13E-05
VILL	3.77	2.11E-03	4.75	7.23E-05
GRK5	4.61	1.35E-20	3.86	1.88E-14
INSIG1	4.66	1.18E-05	3.70	1.15E-03
PIM3	3.83	2.21E-13	4.42	2.81E-16

MAFG	4.27	6.85E-21	3.95	1.17E-17
TNFRSF18	2.74	8.97E-03	5.45	2.73E-05
LGALS3	4.36	5.39E-20	3.83	2.40E-15
PLIN5	3.96	1.89E-06	4.10	1.66E-06
SLC11A2	4.26	2.68E-18	3.79	1.65E-14
NFKBIZ	4.30	4.37E-17	3.71	1.16E-12
CD83	4.04	6.82E-34	3.92	1.82E-32
PLEKHO1	3.64	4.08E-08	4.31	8.11E-11
MAFF	5.32	5.68E-13	2.58	1.61E-03
IL10RA	4.44	3.26E-27	3.38	5.79E-16
MARCH3	4.22	3.26E-27	3.59	4.75E-20
BMF	3.99	7.11E-14	3.80	1.09E-12
DMXL2	4.10	3.61E-17	3.65	1.77E-13
SUCNR1	3.21	7.46E-08	4.51	1.65E-14
LRG1	3.04	3.04E-18	4.62	2.98E-41
ENO3	2.59	8.93E-03	5.03	6.29E-08
IL9R	4.13	1.62E-11	3.49	7.95E-09
CXCL16	4.41	5.00E-22	3.18	1.53E-11
RAB3C	3.95	3.64E-14	3.56	8.73E-12
CCL5	2.50	1.80E-04	4.78	1.00E-13
SLC43A3	3.56	7.85E-15	3.68	1.28E-15
PILRA	3.52	3.88E-22	3.71	1.79E-24
ABI3	3.88	8.34E-05	3.32	1.12E-03
RGL1	4.17	9.45E-08	2.97	2.40E-04
TFRC	3.64	1.24E-18	3.48	5.35E-17
CDC42EP3	2.98	1.62E-09	4.13	3.23E-17
CD82	3.17	1.35E-20	3.88	1.07E-30
LINC01146	4.14	1.01E-07	2.92	4.81E-04
FAH	3.44	8.89E-10	3.61	4.65E-10
TMEM205	3.30	3.13E-20	3.73	8.35E-25
LACC1	3.95	2.53E-15	3.05	3.13E-09
TRAF3	3.16	1.81E-19	3.83	3.07E-28
GRINA	3.33	8.15E-11	3.66	1.20E-12
CKAP2L	2.84	1.61E-02	4.12	3.87E-04
SRC	3.85	7.96E-07	3.10	5.05E-05
LOC100130357	2.77	6.83E-04	4.16	1.99E-07
MFSD12	3.83	4.29E-16	3.03	4.37E-10
NFE2L3	3.21	2.63E-07	3.62	3.89E-09
LINC01619	3.64	5.76E-07	3.17	5.58E-06
TK1	2.88	2.91E-02	3.89	1.10E-02
PHACTR1	4.28	3.98E-10	2.48	7.10E-04
ADM	3.64	1.80E-03	3.09	2.20E-02
SHISAL2A	2.67	3.20E-04	4.03	1.42E-07
NFKBID	3.37	2.74E-17	3.25	5.99E-16
LOC100130476	3.07	1.86E-07	3.54	2.42E-09
KLHL5	3.64	1.24E-24	2.94	8.58E-17

GNA15	3.26	2.69E-13	3.30	2.97E-13
LPXN	3.52	2.06E-23	3.02	3.23E-17
NFKBIE	3.26	7.21E-21	3.21	3.26E-20
SNX25	3.38	2.69E-06	3.05	1.32E-05
IER3	2.53	1.40E-07	3.89	5.54E-16
NFKBIA	2.91	3.95E-13	3.44	4.12E-18
LINC00877	3.63	3.08E-09	2.71	2.61E-05
THBS1	3.57	1.91E-04	2.76	8.37E-03
BTG3	3.35	9.72E-04	2.97	5.67E-03
GPR35	3.61	2.19E-17	2.68	1.01E-09
DUSP16	3.34	8.39E-04	2.93	7.84E-03
PPIF	3.52	1.49E-13	2.74	3.13E-08
GPR174	4.01	3.66E-09	2.21	3.69E-03
TRAF1	3.47	3.30E-05	2.72	4.40E-03
GK	3.39	8.10E-15	2.77	7.62E-10
BCL3	3.43	1.97E-17	2.72	5.64E-11
TNFAIP8L1	2.94	6.16E-18	3.14	1.90E-20
DOT1L	2.95	5.72E-08	3.12	1.27E-08
TIPARP	3.54	3.18E-21	2.52	8.11E-11
ARRDC2	3.23	8.92E-24	2.81	4.48E-18
DUSP10	2.89	5.37E-03	3.16	2.94E-03
PRKCH	2.79	1.91E-10	3.23	5.07E-14
MAPK6	4.28	2.45E-10	1.69	4.57E-02
NFKB1	2.90	3.91E-13	2.99	8.25E-14
ADAMDEC1	3.34	7.60E-04	2.51	1.94E-02
MGAT3	2.30	3.25E-02	3.48	5.37E-04
PRR5L	3.04	4.56E-21	2.74	3.23E-17
PLAU	2.88	6.95E-04	2.84	4.10E-02
GCH1	3.09	9.51E-06	2.63	2.68E-04
SGPP2	3.16	1.47E-05	2.50	1.76E-03
SNN	2.84	4.03E-11	2.78	1.41E-10
TBC1D8	2.48	3.24E-05	3.08	6.94E-08
CFP	2.60	5.35E-06	2.93	2.12E-07
HMG20B	3.04	4.17E-18	2.49	4.26E-12
ATP2C1	2.88	9.77E-19	2.64	9.90E-16
PIM2	3.16	3.96E-15	2.32	3.46E-08
SOD2	2.80	8.15E-11	2.67	1.07E-09
FMNL3	3.12	9.66E-09	2.27	7.94E-05
PDXK	2.49	7.40E-06	2.89	1.25E-07
RTN2	2.97	8.49E-05	2.42	2.04E-03
ZBTB25	2.53	3.57E-04	2.85	2.42E-05
SYNGR2	2.71	5.46E-10	2.65	1.90E-09
FCAR	2.41	8.73E-05	2.93	5.19E-06
IRAK3	2.22	1.09E-07	3.12	2.16E-14
LOC284454	2.63	3.05E-08	2.70	1.63E-08
LGALS1	3.14	3.54E-13	2.20	1.42E-06

FPGS	2.65	8.14E-06	2.68	8.58E-06
LINC00528	3.30	6.64E-07	2.01	7.58E-03
TMEM120A	2.84	5.82E-10	2.46	1.86E-07
ARL3	3.30	4.73E-05	1.99	2.68E-02
PTGER2	2.76	5.31E-10	2.52	3.04E-08
IL2RG	2.59	6.34E-17	2.62	3.76E-17
GSAP	2.70	3.78E-08	2.52	4.85E-07
IRF5	2.17	4.00E-06	3.03	2.47E-11
ABCC4	2.25	5.59E-04	2.90	7.77E-06
SLC1A4	2.31	4.57E-04	2.82	1.05E-05
HINT1	2.49	3.04E-11	2.62	3.32E-12
MDFIC	2.55	7.68E-08	2.54	1.19E-07
CCDC28B	2.37	1.53E-03	2.72	1.84E-04
PLAUR	2.79	1.56E-15	2.29	1.88E-10
DDIT4	1.26	2.37E-02	3.79	5.46E-16
SMCO4	2.10	4.49E-03	2.94	3.23E-05
VDR	2.85	4.64E-14	2.18	8.34E-09
AURKA	2.75	6.60E-04	2.26	9.95E-03
MVD	2.28	8.97E-03	2.68	1.77E-03
ATXN1	2.48	6.62E-10	2.43	1.97E-09
MRPL14	2.53	1.53E-06	2.36	1.13E-05
NR6A1	2.85	6.32E-06	2.01	3.07E-03
IFIH1	2.33	6.38E-09	2.48	5.12E-10
S1PR1	3.59	6.15E-15	1.20	3.95E-02
SERPINB8	2.49	5.36E-03	2.28	1.60E-02
PLK2	2.68	1.20E-05	2.10	6.32E-04
FKBP2	2.33	1.27E-07	2.42	2.29E-07
NBN	2.48	1.24E-10	2.27	8.11E-09
GPR160	1.97	3.03E-10	2.77	1.01E-19
IER5	2.72	1.78E-13	2.02	1.58E-07
IFNGR2	2.51	9.68E-11	2.19	3.88E-08
TNIP1	2.57	2.69E-10	2.13	4.04E-07
COLGALT2	1.84	5.68E-04	2.86	1.42E-08
PRKCI	2.07	3.97E-05	2.62	9.80E-08
SRXN1	3.04	2.60E-15	1.62	1.27E-04
SPAG1	2.35	6.55E-05	2.31	1.06E-04
DOCK10	2.01	6.05E-06	2.64	7.20E-10
CCDC57	2.58	6.76E-05	2.06	2.33E-03
DHCR7	1.72	4.45E-02	2.90	4.00E-04
SPN	1.63	4.46E-05	2.99	6.95E-16
OTUD5	2.36	2.01E-09	2.25	1.78E-08
BTNL8	2.25	1.88E-05	2.34	1.29E-07
ZNRF3	2.43	4.30E-07	2.15	1.27E-05
NECTIN1	1.78	3.24E-02	2.80	4.31E-04
SAMSN1	2.30	8.15E-11	2.29	1.44E-10
SLC39A1	1.40	2.97E-02	3.18	1.27E-08

MLLT6	2.55	1.33E-11	2.02	2.61E-07
ALG2	2.03	1.31E-06	2.54	5.87E-10
DUSP5	2.16	1.78E-02	2.40	8.24E-03
PIGV	2.10	2.28E-05	2.42	5.71E-07
PLAC8	2.03	9.35E-04	2.49	3.03E-05
VOPP1	2.75	1.01E-16	1.76	4.40E-07
C16orf87	2.30	1.31E-05	2.20	3.73E-05
SLC7A5	2.76	3.48E-05	1.72	2.84E-02
REL	2.55	1.86E-07	1.90	2.85E-04
NECAB3	2.14	3.13E-04	2.24	1.85E-04
FADS1	2.27	1.43E-07	2.10	1.30E-06
SQSTM1	2.33	4.54E-13	2.04	6.11E-10
GAS7	2.41	4.27E-11	1.96	1.57E-07
NSMF	1.99	9.02E-03	2.36	1.63E-03
ST3GAL2	2.39	6.97E-08	1.94	2.73E-05
CALHM6	2.27	1.32E-03	2.05	3.59E-03
CD44	2.37	1.78E-10	1.95	4.04E-07
SLC12A4	2.62	5.45E-05	1.68	2.02E-02
MCRIP2	1.62	4.16E-03	2.62	4.85E-07
IL10RB-DT	2.52	4.66E-05	1.70	1.41E-02
MCTP1	2.04	1.04E-04	2.16	4.04E-05
RNASEK	2.15	1.34E-04	2.03	4.23E-04
NAB1	2.36	3.58E-06	1.82	8.40E-04
IVNS1ABP	2.01	2.33E-08	2.12	4.07E-09
ZSCAN16	2.18	1.59E-04	1.96	1.22E-03
PRR7	2.45	1.04E-06	1.67	2.57E-03
RAB5A	2.13	6.84E-15	1.98	1.09E-12
LOC339192	1.71	2.31E-03	2.38	7.83E-06
MARCKS	1.48	2.78E-02	2.60	4.39E-03
LYSMD2	2.19	1.14E-12	1.88	1.94E-09
CD4	1.20	3.19E-02	2.83	1.78E-09
ZNF267	2.50	4.64E-12	1.53	9.92E-05
NCOR2	1.76	7.00E-05	2.26	1.54E-07
SEC62	1.94	1.43E-09	2.06	1.75E-10
E2F3	1.95	2.03E-05	2.05	7.83E-06
ZMIZ1	2.36	1.16E-08	1.63	2.78E-04
KLF10	1.85	1.42E-05	2.14	3.14E-07
LAT2	1.11	3.07E-02	2.86	3.58E-11
NFE2L2	2.15	2.03E-09	1.79	1.64E-06
NXT1	1.70	7.58E-05	2.24	8.89E-08
BCOR	2.44	1.38E-05	1.45	2.65E-02
ANPEP	2.01	5.45E-16	1.87	9.99E-14
PFKFB3	1.81	1.25E-04	2.08	9.86E-06
LYRM7	2.11	2.27E-03	1.76	1.94E-02
USF2	1.49	3.73E-04	2.37	1.88E-09
STARD10	2.15	1.78E-10	1.71	9.38E-07

DEPDC7	1.57	1.37E-02	2.29	9.12E-05
SLC9A8	1.89	2.72E-06	1.95	1.42E-06
TGIF2	2.12	1.09E-07	1.70	3.83E-05
SMG9	2.08	4.19E-03	1.74	2.87E-02
TSPAN18	1.84	2.42E-04	1.97	7.70E-05
FHOD1	2.24	4.26E-05	1.55	1.10E-02
FPR1	1.42	4.10E-06	2.35	1.09E-15
GALC	2.37	5.08E-09	1.40	2.29E-03
SMAD3	1.95	1.96E-05	1.81	1.17E-04
SPATA9	1.76	3.70E-04	2.00	3.73E-05
NAMPT	1.89	5.78E-11	1.87	1.61E-10
PKM	1.82	1.62E-09	1.91	2.84E-10
RAPGEF1	2.03	2.59E-04	1.70	4.29E-03
METTL21A	2.07	2.50E-06	1.65	4.16E-04
VEGFB	1.97	4.84E-03	1.74	1.94E-02
NFAT5	2.12	1.04E-07	1.59	1.93E-04
SPPL2A	1.93	1.94E-10	1.77	9.03E-09
NECAP1	2.04	9.30E-06	1.65	8.03E-04
TNFRSF1B	1.85	7.88E-08	1.83	1.50E-07
RUNX3	1.92	3.65E-15	1.75	1.44E-12
GPR108	1.63	6.38E-09	2.03	2.28E-13
EVI2A	1.76	1.62E-05	1.90	4.69E-06
ISOC1	2.13	3.63E-04	1.53	2.24E-02
ANP32A	1.86	1.42E-07	1.80	4.65E-07
DLGAP1-AS1	1.70	1.42E-02	1.95	5.11E-03
TANK	1.95	2.21E-07	1.64	2.71E-05
CHMP4B	1.78	5.63E-03	1.79	7.52E-03
DDHD1	1.78	1.71E-06	1.79	1.46E-06
SMPD2	1.74	1.50E-06	1.83	3.75E-07
EZH2	1.49	2.61E-03	2.05	1.27E-05
NSMCE2	1.54	1.93E-05	1.98	1.63E-08
P2RY10	1.99	5.54E-08	1.52	9.24E-05
BID	1.97	1.49E-07	1.53	1.18E-04
MIR3064	1.45	4.39E-03	2.05	2.15E-05
CRTC2	1.63	1.54E-05	1.87	5.09E-07
FLVCR1	1.69	2.11E-06	1.79	4.65E-07
LIMK2	1.91	6.38E-09	1.55	5.92E-06
MARCKSL1	2.19	3.23E-13	1.26	1.37E-04
LOC100507507	1.42	1.03E-03	2.03	5.46E-07
INTS9	1.82	3.28E-04	1.62	2.19E-03
DHRSX	1.90	1.76E-06	1.51	3.28E-04
DNAJB4	2.16	2.92E-06	1.25	1.98E-02
CCDC61	1.52	9.23E-03	1.88	9.82E-04
C4orf46	1.51	1.69E-02	1.88	2.21E-03
TMEM123	1.97	2.22E-08	1.41	1.91E-04
KCNE3	1.82	1.46E-05	1.56	3.81E-04

TP53INP2	1.89	3.95E-03	1.49	4.01E-02
KIF3B	1.76	5.53E-03	1.63	1.47E-02
BMP2K	1.75	1.36E-05	1.63	7.77E-05
GRK3	1.52	1.40E-06	1.84	2.44E-09
RAB21	1.85	2.99E-07	1.51	6.97E-05
DYNLT3	1.67	1.68E-02	1.66	2.44E-02
HCST	1.60	4.97E-06	1.72	1.19E-06
DNMT3A	1.92	1.23E-04	1.40	8.64E-03
AGO2	2.10	7.15E-08	1.20	6.97E-03
HMGA1	1.69	6.36E-05	1.61	1.80E-04
PDE4B	1.89	2.92E-06	1.40	1.29E-03
LOC101927851	1.36	2.90E-02	1.93	1.06E-03
MAN2B1	1.47	8.38E-08	1.81	1.49E-11
ABCF1	1.82	3.73E-06	1.45	5.41E-04
GPX7	1.61	8.06E-03	1.65	8.49E-03
TFE3	1.67	1.48E-03	1.58	3.64E-03
UBE2E1	1.59	1.36E-02	1.65	1.35E-02
TPRA1	1.52	1.69E-03	1.71	3.47E-04
FCER1G	1.90	8.52E-06	1.33	4.70E-03
SESN2	1.84	2.25E-04	1.36	1.35E-02
GPR137B	1.87	2.88E-07	1.32	7.89E-04
SELENOH	1.29	5.31E-04	1.87	9.57E-08
MSL1	1.68	9.32E-05	1.47	1.12E-03
SERTAD1	1.54	1.08E-02	1.61	8.25E-03
LIMS1	1.78	2.82E-06	1.36	8.87E-04
UNC119	1.55	1.30E-04	1.60	9.39E-05
QKI	1.34	2.77E-04	1.80	3.04E-07
EIF5A	1.58	3.11E-07	1.53	1.28E-06
TMEM106A	1.45	1.16E-02	1.64	4.22E-03
PQLC1	1.75	2.62E-03	1.34	3.98E-02
GAPT	1.53	2.51E-09	1.55	2.03E-09
CLCF1	1.65	1.90E-04	1.43	1.95E-03
PHF23	1.74	9.33E-06	1.33	1.61E-03
SPG21	1.27	1.57E-03	1.79	2.52E-06
ZNF433-AS1	1.34	8.05E-03	1.73	4.27E-04
FTH1	1.80	1.56E-11	1.25	1.13E-05
LINC01303	1.24	7.50E-03	1.78	3.97E-05
RIT1	1.82	4.92E-07	1.20	3.00E-03
NSMAF	1.82	4.32E-05	1.20	1.86E-02
SAMD8	1.88	6.31E-05	1.14	3.96E-02
ZHX2	1.65	7.98E-05	1.36	2.21E-03
CARD19	1.79	3.09E-10	1.20	1.08E-04
NINJ1	1.50	1.09E-08	1.48	2.59E-08
ITM2A	1.70	4.59E-07	1.29	3.06E-04
NFKBIB	1.44	2.35E-03	1.55	1.07E-03
MIR5047	1.20	6.74E-04	1.78	9.07E-08

TRAF2	1.61	1.39E-04	1.36	2.58E-03
CDKN2C	1.51	4.73E-03	1.45	9.03E-03
ZBTB1	1.76	4.98E-06	1.19	5.30E-03
RHOBTB3	1.44	3.83E-06	1.49	1.91E-06
EIF1AX	1.06	2.24E-02	1.87	8.93E-06
CSTA	1.09	3.78E-02	1.84	3.06E-04
APBA3	1.34	1.61E-03	1.59	1.16E-04
TCP11L1	1.63	3.59E-05	1.30	2.14E-03
ATG7	1.28	1.12E-03	1.64	1.27E-05
LPCAT1	1.54	4.46E-04	1.37	3.04E-03
SLC20A1	1.64	1.10E-07	1.27	9.92E-05
IGFLR1	1.23	4.67E-04	1.66	7.79E-07
RRM2B	1.74	8.47E-10	1.14	2.16E-04
FLOT1	1.38	1.37E-04	1.49	3.85E-05
FNDC3B	1.76	4.93E-07	1.10	5.20E-03
CDK9	1.50	2.66E-07	1.36	5.24E-06
RCC2	1.06	1.06E-02	1.79	1.71E-06
SLC38A1	1.26	8.09E-04	1.58	1.51E-05
STAP1	1.11	1.35E-02	1.72	2.54E-05
POLD3	1.47	2.81E-04	1.36	1.05E-03
P2RY8	1.29	1.13E-05	1.53	1.05E-07
CD58	1.51	9.81E-06	1.31	2.28E-04
IRF2BP2	1.55	1.59E-08	1.26	1.09E-05
TFDP1	1.76	1.54E-08	1.05	2.91E-03
ASCL2	1.30	3.76E-02	1.50	1.54E-02
ZDHHC18	1.35	2.16E-04	1.43	9.18E-05
AK2	1.31	4.37E-06	1.46	2.08E-07
GSTP1	1.26	7.17E-04	1.51	4.29E-05
RNF144B	1.22	3.62E-02	1.54	7.02E-03
CREG1	1.60	1.27E-04	1.16	1.30E-02
STRN4	1.47	3.48E-04	1.23	4.88E-03
LMNB1	1.40	6.66E-04	1.29	2.66E-03
N4BP1	1.63	5.25E-05	1.06	2.14E-02
ARMCX3	1.06	1.83E-02	1.63	7.89E-05
RFFL	1.49	3.99E-03	1.18	4.04E-02
SMAD7	1.37	1.24E-02	1.28	2.72E-02
GPR65	1.65	1.21E-05	0.98	2.66E-02
PLPP5	1.27	1.80E-03	1.35	9.93E-04
ZFP36	1.58	1.64E-05	1.03	1.45E-02
DENND5A	1.41	5.36E-04	1.19	6.14E-03
RIF1	1.44	8.92E-04	1.16	1.37E-02
PADI2	1.03	1.17E-03	1.55	1.77E-07
WDR11	1.50	3.24E-05	1.07	7.14E-03
POR	1.42	1.68E-05	1.16	9.79E-04
SWAP70	1.38	2.18E-06	1.20	7.09E-05
HMBOX1	1.13	2.14E-02	1.42	3.08E-03

SLC3A2	1.45	3.23E-04	1.11	1.31E-02
DDIT3	1.35	3.85E-04	1.19	3.08E-03
HSDL1	0.95	4.89E-02	1.59	2.61E-04
PRKD3	1.31	1.16E-02	1.21	3.12E-02
LYRM1	1.46	1.29E-03	1.05	4.10E-02
RNF115	1.03	1.74E-02	1.45	3.96E-04
DPH3	1.34	1.63E-03	1.13	1.35E-02
DBF4	1.12	4.85E-02	1.34	1.71E-02
GPX1	1.04	5.23E-04	1.41	9.13E-07
SQOR	1.20	2.44E-03	1.25	1.81E-03
EPM2AIP1	1.13	2.02E-02	1.32	6.17E-03
RAB33A	0.92	4.25E-02	1.50	2.61E-04
YWHAE	1.29	1.70E-03	1.13	9.90E-03
NDFIP2	1.35	1.18E-05	1.06	1.15E-03
PRDX6	1.04	6.01E-03	1.37	1.76E-04
PTPN7	0.90	4.77E-02	1.51	2.12E-04
SLC36A4	1.52	2.81E-06	0.86	2.46E-02
ARHGAP18	1.09	3.74E-02	1.29	1.35E-02
ANP32E	1.33	3.64E-04	1.05	9.90E-03
CLEC2B	1.14	2.86E-02	1.23	2.17E-02
TRIM8	1.19	1.61E-02	1.18	2.32E-02
PIGQ	1.10	3.79E-02	1.25	1.86E-02
PCTP	1.02	8.83E-03	1.33	3.89E-04
MFNG	0.79	4.19E-03	1.53	4.58E-10
PPCDC	1.46	3.04E-05	0.86	4.00E-02
UBE2R2	1.27	5.55E-05	1.05	1.67E-03
CYSTM1	0.98	3.15E-02	1.32	3.00E-03
GSTO1	1.31	3.87E-05	0.99	4.77E-03
FBRS	1.13	3.69E-02	1.16	4.15E-02
ORAI2	0.94	1.89E-04	1.34	2.49E-08
RHBDD2	1.27	7.30E-05	1.01	3.25E-03
RIN3	1.30	5.00E-05	0.97	5.70E-03
SERP1	1.26	1.01E-05	1.00	1.06E-03
PTP4A2	1.15	3.00E-06	1.10	1.08E-05
MED19	1.19	1.17E-02	1.05	4.09E-02
ZBED1	1.11	7.32E-03	1.12	8.16E-03
TESK2	0.99	2.78E-02	1.24	4.59E-03
RBM17	1.21	2.48E-03	1.00	2.36E-02
SERPINA1	0.89	1.30E-02	1.32	7.41E-05
MDM1	1.05	3.77E-02	1.16	2.39E-02
DAGLB	0.86	3.26E-02	1.35	2.54E-04
SAE1	1.14	1.62E-02	1.07	3.35E-02
TM9SF4	0.89	4.39E-02	1.31	1.55E-03
RAP2C	1.23	1.55E-03	0.95	2.66E-02
CYBB	0.96	3.49E-02	1.22	5.58E-03
MARF1	1.08	3.85E-04	1.10	3.47E-04

RILPL2	0.99	9.48E-03	1.19	1.39E-03
PDLIM7	1.15	2.21E-03	1.02	1.04E-02
MAD2L1BP	1.22	3.97E-03	0.94	4.88E-02
SEC13	1.13	4.65E-04	1.02	2.34E-03
JUP	1.08	3.35E-02	1.06	4.92E-02
IL13RA1	0.83	3.91E-02	1.30	4.20E-04
R3HDM4	1.00	9.63E-04	1.13	1.69E-04
HCK	1.12	2.35E-03	1.01	9.92E-03
SMS	1.06	2.27E-03	1.05	3.29E-03
ADORA3	0.84	1.62E-02	1.27	8.81E-05
ETV6	1.12	3.01E-03	0.99	1.46E-02
IFNAR1	1.06	4.93E-03	1.03	8.59E-03
POLD4	0.80	1.14E-02	1.28	8.90E-06
NECAP2	1.08	3.37E-03	0.99	1.09E-02
HCP5	1.04	5.45E-03	1.03	7.53E-03
GRIPAP1	0.91	4.38E-02	1.14	9.54E-03
NPL	0.79	3.01E-02	1.27	1.33E-04
IL10RB	1.11	1.69E-03	0.95	1.31E-02
CDC42SE2	1.19	1.18E-04	0.85	1.39E-02
DVL3	0.92	3.94E-02	1.12	1.04E-02
ZEB2	1.03	3.16E-03	1.01	5.34E-03
TRAPPC5	0.86	4.47E-02	1.18	4.13E-03
HLA-DRA	1.05	5.23E-04	0.98	1.94E-03
MCMBP	0.73	1.72E-02	1.28	4.04E-06
RAP1A	0.85	7.84E-03	1.16	1.16E-04
BASP1	0.91	2.77E-02	1.07	8.84E-03
TYMP	0.93	1.42E-02	1.06	5.06E-03
FBXL15	0.92	3.85E-02	1.06	1.92E-02
ARL6IP5	0.91	4.19E-03	1.06	6.86E-04
RBM7	1.00	1.39E-02	0.94	2.94E-02
TRIM13	1.09	1.68E-03	0.86	2.55E-02
IMPDH1	0.75	4.97E-02	1.19	5.97E-04
ZNF641	1.02	1.15E-02	0.90	3.24E-02
UBXN1	0.86	1.97E-02	1.06	3.00E-03
ORAI1	0.84	2.60E-02	1.07	3.48E-03
ASCC1	0.96	3.08E-02	0.95	4.44E-02
SFT2D1	1.12	1.39E-04	0.79	1.81E-02
DEK	0.93	4.54E-03	0.96	4.17E-03
TMX2	0.92	2.70E-02	0.97	2.37E-02
RBX1	1.06	1.45E-04	0.83	6.40E-03
AZIN1	0.85	1.47E-02	1.03	2.52E-03
EHD1	0.82	3.79E-02	1.04	6.17E-03
SS18	0.85	1.96E-02	0.98	6.90E-03
APMAP	0.95	3.99E-03	0.88	1.14E-02
PCBP1	0.82	6.85E-03	0.98	9.79E-04
FAM129A	0.92	8.83E-03	0.87	2.08E-02

TGFBR2	0.98	4.26E-03	0.81	3.12E-02
UBXN7	0.85	2.62E-02	0.94	1.56E-02
ANAPC16	0.81	2.13E-02	0.97	5.23E-03
FNBP1	1.04	2.52E-04	0.72	2.67E-02
NDE1	0.82	4.94E-02	0.92	2.88E-02
NRDE2	0.97	3.64E-03	0.74	4.91E-02
KTN1	0.94	4.04E-03	0.76	3.42E-02
GALNT6	0.82	3.20E-02	0.88	2.36E-02
STK17A	0.68	4.90E-02	1.01	1.57E-03
KRAS	0.95	1.67E-03	0.74	2.59E-02
MAP3K2	0.88	1.10E-02	0.80	3.32E-02
DDAH2	0.95	3.25E-03	0.72	4.99E-02
CREB1	0.75	3.07E-02	0.91	7.89E-03
PLAGL1	0.83	2.04E-02	0.82	2.94E-02
TAPBP	0.79	4.06E-03	0.85	2.17E-03
AMPD3	0.78	1.58E-02	0.85	9.17E-03
SDCBP	0.94	9.88E-04	0.69	3.28E-02
UBE2A	0.92	3.97E-03	0.71	4.71E-02
ADAP1	0.76	3.00E-02	0.86	1.45E-02
SRGN	0.83	1.24E-02	0.77	3.12E-02
RPS27A	0.82	1.27E-02	0.77	2.84E-02
BAZ1A	0.78	4.33E-02	0.81	4.49E-02
TUBB4B	0.76	4.24E-02	0.83	2.96E-02
PNRC1	0.75	6.61E-03	0.84	2.61E-03
RAC1	0.67	3.71E-02	0.87	4.86E-03
KDELR2	0.77	1.88E-02	0.77	2.36E-02
SAT1	0.76	1.71E-02	0.76	2.17E-02
CD63	0.63	1.86E-02	0.88	6.19E-04
JMJD6	0.75	2.55E-02	0.73	4.19E-02
TYROBP	0.74	5.91E-03	0.70	1.25E-02
HLA-A	0.78	4.65E-03	0.62	4.62E-02
TMSB4X	0.61	2.53E-02	0.63	2.63E-02
VSIR	-0.67	2.07E-02	-0.62	4.60E-02
ALOX5	-0.70	1.98E-02	-0.64	4.87E-02
TBXAS1	-0.64	4.52E-02	-0.72	2.44E-02
MCL1	-0.72	1.75E-02	-0.66	4.65E-02
CEBPB	-0.79	1.67E-02	-0.73	4.04E-02
RBL2	-0.81	1.06E-02	-0.74	2.95E-02
ESYT1	-0.80	1.55E-02	-0.81	1.73E-02
FGD3	-0.99	8.62E-05	-0.66	2.40E-02
IFITM2	-0.90	1.68E-03	-0.75	1.57E-02
FOXO3	-0.83	2.00E-02	-0.83	2.55E-02
LYST	-0.86	1.25E-02	-0.80	2.96E-02
ZMYND11	-0.84	3.51E-02	-0.89	2.82E-02
PGAM1	-0.75	2.94E-02	-0.99	2.74E-03
SLMAP	-0.93	4.59E-03	-0.82	1.93E-02

IRF9	-0.95	2.94E-03	-0.81	2.04E-02
PTPN1	-0.83	3.94E-02	-0.94	2.16E-02
HP1BP3	-0.81	8.01E-03	-0.95	1.53E-03
GCA	-0.74	9.99E-03	-1.06	8.27E-05
LRMP	-0.97	2.46E-03	-0.84	1.48E-02
UIMC1	-0.93	5.98E-03	-0.89	1.27E-02
NHSL2	-0.90	4.08E-02	-0.93	4.24E-02
CAP1	-0.92	9.52E-04	-0.93	9.71E-04
NUP50	-0.87	4.06E-03	-1.00	9.11E-04
NFIL3	-0.85	9.95E-03	-1.02	1.63E-03
IPCEF1	-0.94	8.95E-03	-0.96	9.92E-03
RAB11FIP1	-0.85	4.06E-02	-1.07	8.26E-03
TLE4	-0.94	3.46E-04	-0.98	2.10E-04
FOXN2	-1.10	4.57E-04	-0.83	1.89E-02
LST1	-1.14	7.85E-05	-0.80	1.38E-02
DOK2	-1.14	2.27E-04	-0.80	2.18E-02
FCGRT	-1.09	2.89E-04	-0.85	1.06E-02
GPI	-0.96	9.24E-03	-0.98	9.75E-03
S100A4	-1.06	8.44E-03	-0.91	3.83E-02
TBC1D10C	-1.21	2.11E-05	-0.78	1.57E-02
ABCA5	-0.91	4.38E-02	-1.09	1.64E-02
MYB	-1.00	3.23E-02	-1.00	4.16E-02
NOTCH2NLB	-0.97	2.82E-02	-1.05	1.93E-02
CDK5RAP3	-1.12	3.97E-03	-0.91	3.51E-02
NNT	-0.99	1.07E-02	-1.04	8.59E-03
PPIP5K2	-0.97	4.84E-03	-1.07	2.07E-03
LAMTOR4	-1.21	2.94E-05	-0.83	1.12E-02
ATM	-1.07	1.86E-02	-0.97	4.91E-02
ANAPC5	-1.23	2.33E-04	-0.83	3.06E-02
ACAP1	-1.12	3.28E-03	-0.98	1.59E-02
GMCL1	-1.09	2.01E-02	-1.02	4.34E-02
AP1M1	-1.29	4.41E-05	-0.85	1.73E-02
COMMD9	-1.02	2.76E-02	-1.12	1.63E-02
ADI1	-1.13	1.94E-03	-1.01	8.26E-03
ATG16L2	-1.39	1.01E-05	-0.75	4.88E-02
STAT1	-0.91	1.60E-02	-1.24	5.34E-04
WARS	-1.02	2.06E-03	-1.13	5.97E-04
INPP1	-1.04	8.41E-04	-1.11	3.88E-04
CARD16	-0.95	3.61E-02	-1.21	6.36E-03
CYSLTR1	-1.40	5.03E-06	-0.76	3.98E-02
IFI6	-1.24	1.03E-03	-0.94	2.06E-02
TBCB	-1.07	1.02E-02	-1.12	8.47E-03
C11orf21	-1.10	5.64E-03	-1.11	6.25E-03
PARP12	-1.28	9.95E-05	-0.94	8.68E-03
GRK6	-1.02	4.70E-03	-1.21	6.19E-04
NME4	-1.16	7.08E-03	-1.07	1.89E-02

TMCC3	-0.83	2.17E-02	-1.41	1.33E-05
PADI4	-0.97	2.90E-03	-1.30	3.03E-05
FGL2	-1.34	9.96E-06	-0.93	5.87E-03
ANXA1	-0.77	4.10E-02	-1.50	5.36E-06
MLKL	-1.31	1.60E-03	-0.96	4.19E-02
ARMC10	-1.08	2.80E-02	-1.20	1.56E-02
PDK3	-0.96	3.70E-02	-1.32	2.80E-03
OAT	-1.27	2.23E-03	-1.02	2.66E-02
PGP	-1.29	7.25E-04	-1.00	1.60E-02
PARP4	-1.11	8.89E-04	-1.18	4.00E-04
CSF2RA	-0.98	2.75E-03	-1.33	2.09E-05
RAB31	-0.75	4.61E-02	-1.56	1.40E-06
CLINT1	-0.94	8.64E-03	-1.37	4.44E-05
TAGAP	-1.14	3.91E-04	-1.20	1.85E-04
GCNT1	-1.39	3.12E-05	-0.96	8.76E-03
IQGAP2	-0.83	4.28E-02	-1.52	2.86E-05
PMM1	-1.09	2.21E-02	-1.26	8.34E-03
ETS1	-1.22	3.60E-03	-1.14	9.51E-03
PYGL	-1.27	1.37E-04	-1.10	1.81E-03
CASP4	-1.04	3.82E-02	-1.32	9.03E-03
SPTLC2	-1.06	9.34E-03	-1.31	1.01E-03
ANTXR2	-1.16	2.21E-03	-1.22	1.40E-03
EIF4E3	-1.24	5.89E-04	-1.14	2.04E-03
TAPBPL	-1.29	1.72E-03	-1.09	1.35E-02
SCP2	-1.31	2.76E-03	-1.09	2.39E-02
PRKDC	-1.08	2.77E-02	-1.34	5.42E-03
PPP2R5A	-1.33	2.10E-04	-1.09	4.53E-03
PGLS	-1.33	3.64E-03	-1.11	2.55E-02
MZT2A	-1.24	1.98E-03	-1.20	3.80E-03
ELOVL5	-1.39	2.19E-06	-1.05	9.31E-04
TLR4	-1.17	5.96E-04	-1.27	1.83E-04
MYD88	-1.46	4.59E-07	-0.99	2.05E-03
ADGRG5	-1.26	1.44E-04	-1.20	4.16E-04
ACSS1	-1.38	1.80E-03	-1.07	2.93E-02
LBR	-0.97	7.79E-04	-1.49	3.62E-08
RNASET2	-1.43	1.79E-08	-1.03	1.34E-04
MGME1	-1.50	1.53E-06	-0.97	5.58E-03
ASL	-1.24	2.45E-03	-1.23	3.40E-03
PQLC3	-1.38	1.30E-04	-1.11	3.64E-03
KBTBD11	-1.19	3.57E-02	-1.30	2.62E-02
UNC93B1	-1.38	2.71E-05	-1.11	1.36E-03
TMSB10	-1.35	4.67E-06	-1.14	2.28E-04
DDX21	-1.73	3.27E-09	-0.78	2.91E-02
ARHGAP15	-1.42	1.73E-06	-1.08	6.37E-04
FCGR2C	-1.45	8.35E-04	-1.06	2.72E-02
TSNAX	-1.48	5.48E-05	-1.03	1.25E-02

HMOX2	-1.42	1.40E-05	-1.09	1.83E-03
RNF144A	-1.18	4.31E-02	-1.33	2.44E-02
CAMK1D	-1.35	8.84E-05	-1.16	1.37E-03
IFNAR2	-1.25	3.15E-03	-1.27	3.05E-03
ARMH1	-1.65	3.48E-08	-0.87	1.33E-02
PATJ	-1.46	1.11E-04	-1.06	1.10E-02
P2RY2	-1.21	6.21E-03	-1.32	2.57E-03
PSTPIP1	-1.31	1.13E-02	-1.22	2.73E-02
ZDHHC7	-1.61	9.58E-06	-0.93	2.93E-02
MBOAT7	-1.12	3.54E-04	-1.42	3.11E-06
ZC3HAV1	-1.19	1.43E-02	-1.36	5.08E-03
TUBA4A	-1.21	5.90E-05	-1.36	5.09E-06
CRBN	-1.56	6.68E-06	-1.00	1.05E-02
CAT	-1.47	6.43E-08	-1.12	1.04E-04
H1F0	-1.25	3.75E-03	-1.34	2.80E-03
PTEN	-1.53	6.64E-07	-1.06	1.72E-03
APBB1IP	-1.67	6.76E-08	-0.92	9.51E-03
PPP2R3B	-1.42	6.15E-05	-1.17	1.85E-03
IFI35	-1.45	2.97E-03	-1.15	3.35E-02
NADK	-1.43	1.13E-05	-1.18	6.14E-04
IKBIP	-1.31	3.02E-03	-1.30	4.31E-03
PGM2	-1.56	8.19E-07	-1.05	2.56E-03
HEATR5B	-1.47	3.12E-03	-1.15	3.69E-02
FAM126A	-1.70	3.62E-06	-0.92	3.49E-02
NLRP1	-1.25	3.36E-03	-1.38	1.26E-03
BIN2	-1.44	1.40E-05	-1.21	5.93E-04
C1RL	-1.34	2.60E-02	-1.31	3.76E-02
USP32	-1.38	2.35E-03	-1.27	7.61E-03
HPS3	-1.34	4.04E-03	-1.34	5.27E-03
TPST2	-1.30	6.18E-03	-1.39	3.96E-03
EAF2	-1.23	5.21E-03	-1.48	6.04E-04
RCSD1	-1.71	3.01E-07	-0.99	9.75E-03
MYH9	-1.23	3.34E-02	-1.48	9.91E-03
UCP2	-1.25	1.13E-02	-1.47	2.82E-03
C5orf56	-1.43	1.56E-02	-1.30	4.21E-02
KLHL9	-1.72	1.98E-07	-1.01	7.06E-03
RNF216P1	-1.60	5.90E-04	-1.14	2.80E-02
CTSA	-1.44	3.27E-06	-1.31	3.39E-05
GALNT10	-1.40	1.24E-02	-1.36	2.10E-02
TECR	-1.16	1.19E-02	-1.60	3.56E-04
ERP29	-1.68	5.48E-05	-1.08	2.48E-02
ARHGAP26	-1.55	5.46E-04	-1.22	1.32E-02
ZBTB20	-1.47	5.13E-03	-1.30	2.11E-02
UPP1	-1.32	1.79E-04	-1.47	1.86E-05
GADD45A	-1.18	1.40E-02	-1.63	3.70E-04
CELF2	-1.90	1.03E-08	-0.91	2.40E-02

MZT2B	-1.30	4.70E-03	-1.52	7.94E-04
EMC1	-1.57	7.21E-03	-1.25	4.91E-02
DOP1B	-1.44	9.37E-03	-1.39	1.63E-02
MACF1	-1.70	8.92E-05	-1.13	2.18E-02
SPECC1	-1.40	3.16E-03	-1.44	2.96E-03
RARA	-1.26	1.10E-02	-1.58	9.79E-04
SSBP2	-1.54	3.68E-04	-1.30	4.51E-03
MTRR	-1.44	1.88E-02	-1.42	2.59E-02
RALGPS1	-1.38	5.70E-03	-1.49	2.73E-03
KIAA0930	-1.42	1.63E-02	-1.45	1.77E-02
IL6R	-1.35	3.14E-02	-1.53	1.60E-02
DOCK8	-1.39	3.50E-03	-1.50	1.72E-03
FLI1	-1.82	1.38E-07	-1.08	5.85E-03
CYFIP2	-1.39	7.42E-06	-1.54	4.65E-07
RNASE2	-1.15	2.55E-03	-1.78	4.28E-07
PAPSS1	-1.59	3.70E-07	-1.34	4.21E-05
GVINP1	-1.81	1.99E-05	-1.14	1.86E-02
MAN2B2	-1.66	1.22E-05	-1.31	1.20E-03
MYADM	-1.61	2.32E-05	-1.35	7.78E-04
TSPAN32	-1.47	2.63E-02	-1.51	2.87E-02
ARHGAP1	-1.53	2.46E-04	-1.44	8.27E-04
VIM	-0.97	3.67E-02	-2.01	5.98E-07
WDR7	-1.47	3.73E-03	-1.53	2.70E-03
SVIL	-1.39	1.45E-03	-1.61	1.95E-04
PLD3	-1.80	1.31E-08	-1.20	4.95E-04
SLC40A1	-1.16	1.68E-02	-1.84	3.76E-05
ABHD2	-1.51	2.85E-03	-1.51	3.95E-03
P2RY13	-1.31	1.55E-02	-1.71	1.02E-03
NEK6	-1.24	4.78E-02	-1.79	2.41E-03
DHTKD1	-1.64	7.32E-04	-1.41	6.17E-03
CD300A	-1.67	2.83E-11	-1.40	5.94E-08
SIN3B	-1.65	4.03E-03	-1.42	2.18E-02
CD52	-1.78	4.67E-06	-1.29	2.38E-03
CAMKK2	-1.68	2.11E-06	-1.41	1.32E-04
ERLIN1	-1.09	3.35E-02	-1.99	1.82E-05
TSC22D3	-1.85	2.35E-06	-1.24	4.56E-03
ELAC2	-1.56	9.42E-03	-1.55	1.33E-02
CEBPE	-1.96	1.70E-05	-1.16	3.04E-02
PNPLA6	-1.51	1.69E-02	-1.62	1.14E-02
SP110	-2.01	7.18E-11	-1.12	1.24E-03
ELP1	-1.61	1.33E-02	-1.52	2.78E-02
SH3GLB2	-1.71	7.22E-04	-1.42	8.59E-03
CD55	-1.30	4.30E-05	-1.83	2.13E-09
STN1	-1.02	4.10E-02	-2.14	5.19E-07
STK17B	-1.74	5.45E-10	-1.41	1.37E-06
DYRK2	-1.64	2.19E-04	-1.52	9.30E-04

CCDC125	-2.00	4.69E-05	-1.18	4.38E-02
ANXA6	-1.41	2.87E-02	-1.79	4.28E-03
FECH	-1.40	3.68E-03	-1.81	8.56E-05
IFITM1	-1.67	4.39E-09	-1.54	1.18E-07
CHAF1A	-1.62	9.10E-03	-1.60	1.35E-02
ACOT11	-1.64	1.64E-02	-1.58	2.48E-02
SFXN1	-1.32	8.66E-03	-1.93	3.73E-05
MINDY1	-1.69	6.26E-08	-1.57	5.93E-07
CDCA7L	-1.87	3.83E-05	-1.39	4.56E-03
PRKCB	-1.67	3.39E-07	-1.61	1.18E-06
TCTA	-1.71	9.46E-04	-1.58	3.18E-03
GPR27	-2.09	3.53E-05	-1.22	3.75E-02
ACSL1	-1.96	6.61E-05	-1.35	1.58E-02
SKP2	-1.67	1.12E-04	-1.64	1.83E-04
DGLUCY	-1.89	6.93E-06	-1.42	1.59E-03
CASP3	-1.69	1.84E-09	-1.63	1.01E-08
REX1BD	-1.64	1.22E-03	-1.68	9.71E-04
APOBEC3C	-2.07	1.27E-07	-1.25	4.28E-03
ALAS1	-1.84	7.48E-06	-1.48	6.60E-04
NAA80	-1.97	8.97E-05	-1.37	1.47E-02
LGALS9	-1.97	4.69E-05	-1.38	1.00E-02
NOTCH2	-1.30	1.16E-02	-2.07	1.31E-05
CDKN2D	-1.81	6.84E-05	-1.56	1.12E-03
CTSS	-1.90	3.21E-10	-1.48	2.97E-06
CRIP1	-1.59	7.89E-04	-1.79	1.30E-04
LOC100294145	-1.72	4.66E-05	-1.66	9.23E-05
HDAC10	-1.40	3.34E-02	-1.98	1.45E-03
DTX3L	-1.69	1.31E-02	-1.70	1.56E-02
GNAQ	-1.40	6.72E-03	-1.99	4.46E-05
MAP3K4	-1.97	2.34E-03	-1.46	4.52E-02
VSTM1	-1.97	8.12E-07	-1.46	6.62E-04
DDX60L	-1.78	1.60E-05	-1.66	7.09E-05
C2CD5	-2.09	2.60E-07	-1.35	2.04E-03
ZMYND8	-2.14	2.18E-08	-1.30	2.16E-03
SEMA4D	-1.92	2.34E-05	-1.53	1.69E-03
FBP1	-2.21	3.48E-07	-1.24	1.40E-02
LINC00537	-2.35	1.89E-10	-1.12	8.93E-03
PSD4	-2.14	1.59E-04	-1.35	4.05E-02
ITGAM	-1.72	3.98E-10	-1.76	2.15E-10
ABCC5	-1.49	4.06E-03	-1.99	5.94E-05
SYTL1	-2.05	3.50E-12	-1.45	2.80E-06
LRRC25	-2.07	2.88E-08	-1.44	3.54E-04
DHRS1	-2.25	8.17E-07	-1.26	1.68E-02
ABTB2	-1.64	3.19E-02	-1.87	1.33E-02
SAMD9L	-1.80	5.86E-07	-1.72	2.03E-06
PIEZO1	-1.66	2.00E-02	-1.85	9.86E-03

GALT	-2.19	1.18E-04	-1.33	4.60E-02
ERAP2	-1.89	5.74E-06	-1.63	8.89E-05
CEP250	-1.51	1.44E-02	-2.01	6.34E-04
ANXA2	-1.59	1.58E-05	-1.93	9.07E-08
ALMS1	-1.52	3.89E-02	-2.01	3.30E-03
CPPED1	-2.12	6.15E-15	-1.43	5.04E-07
ST3GAL1	-2.31	1.26E-10	-1.27	1.72E-03
HPCAL1	-1.72	2.83E-03	-1.86	1.33E-03
PDIK1L	-1.87	1.27E-02	-1.71	3.26E-02
PRKX	-2.08	2.89E-05	-1.51	5.34E-03
RMND1	-1.68	2.79E-02	-1.91	1.14E-02
XKR8	-2.09	4.89E-14	-1.51	1.84E-07
ALS2	-1.71	5.30E-04	-1.90	9.19E-05
EPN2	-2.04	4.44E-04	-1.57	1.39E-02
DPY19L3	-1.42	3.14E-03	-2.21	6.41E-07
SIRPB2	-2.51	3.76E-10	-1.15	1.69E-02
HK3	-1.84	1.43E-07	-1.85	1.55E-07
RNF141	-1.69	5.03E-05	-2.02	7.60E-07
SNTB1	-2.22	1.80E-04	-1.49	2.66E-02
GNG2	-2.19	7.14E-08	-1.52	5.87E-04
HLA-DMA	-1.98	3.69E-04	-1.73	2.76E-03
SLC25A1	-2.12	1.10E-06	-1.62	4.28E-04
IL17RA	-2.11	9.17E-08	-1.63	9.92E-05
CEP85L	-2.41	7.15E-08	-1.33	9.51E-03
HHEX	-1.72	1.50E-03	-2.02	1.39E-04
DIXDC1	-2.18	1.18E-05	-1.58	3.08E-03
ALDH3B1	-2.21	1.09E-09	-1.56	5.05E-05
MPRIP	-2.32	3.32E-04	-1.48	4.49E-02
ADCY7	-1.86	4.65E-03	-1.94	3.57E-03
CEMIP2	-2.13	1.19E-04	-1.69	4.36E-03
CHRM3-AS2	-1.97	6.13E-03	-1.85	1.20E-02
MPP7	-2.21	4.37E-06	-1.61	1.87E-03
PRIMPOL	-2.23	2.02E-07	-1.60	4.18E-04
SIGIRR	-2.07	8.94E-04	-1.78	6.90E-03
COLGALT1	-1.59	7.88E-04	-2.27	3.51E-07
TTC38	-2.23	1.16E-06	-1.63	8.27E-04
LILRA2	-1.25	4.71E-02	-2.63	1.40E-06
NRROS	-2.00	3.55E-03	-1.90	7.94E-03
ISG15	-2.53	3.51E-13	-1.36	3.68E-04
SAMHD1	-2.12	1.02E-04	-1.78	1.01E-03
TMED8	-2.26	5.01E-05	-1.65	7.47E-03
ADGRE5	-2.15	3.37E-14	-1.76	1.68E-09
C3orf86	-2.22	5.60E-06	-1.70	1.03E-03
EXOC6	-2.07	4.19E-06	-1.85	6.91E-05
BACE2	-2.05	8.80E-10	-1.89	2.05E-08
WDR43	-2.17	4.14E-03	-1.79	3.24E-02

TUBA1A	-2.05	1.70E-10	-1.91	3.98E-09
ABHD10	-2.33	9.12E-06	-1.63	4.91E-03
TMEM273	-2.44	4.50E-12	-1.52	7.07E-05
DSC2	-1.98	1.75E-09	-1.99	1.47E-09
SP4	-1.53	1.16E-02	-2.44	1.16E-05
LTB4R	-2.17	3.61E-03	-1.82	2.55E-02
TMEM71	-2.21	6.52E-12	-1.82	3.97E-08
SIRPB1	-1.97	1.48E-11	-2.06	1.28E-12
CLEC12A	-2.08	2.51E-06	-1.98	1.12E-05
IFIT2	-2.36	1.88E-10	-1.73	8.25E-06
PIK3R6	-2.73	1.05E-11	-1.36	3.37E-03
MPHOSPH9	-1.88	4.39E-03	-2.21	7.32E-04
ACADSB	-2.19	1.82E-04	-1.90	2.04E-03
NAAA	-2.24	8.16E-06	-1.86	3.99E-04
PARP9	-2.39	1.43E-09	-1.72	3.03E-05
CELF2-AS1	-2.10	2.50E-02	-2.02	3.95E-02
RCL1	-2.11	2.03E-03	-2.02	2.94E-03
LINC01504	-2.71	6.68E-12	-1.43	9.49E-04
RNASE3	-1.73	8.59E-03	-2.42	9.62E-05
ENC1	-1.86	3.63E-02	-2.29	7.81E-03
AKR7A2	-2.20	5.69E-04	-1.96	3.41E-03
44450	-2.24	1.20E-09	-1.96	1.68E-07
PMP22	-1.84	2.50E-05	-2.36	1.32E-08
RHOB	-1.74	2.37E-02	-2.48	4.83E-04
LILRB2	-2.08	3.03E-08	-2.14	1.96E-08
PXN	-2.20	1.25E-06	-2.05	8.42E-06
CCDC88C	-2.03	2.47E-02	-2.23	1.53E-02
ZFP64	-2.37	1.03E-03	-1.89	1.32E-02
NOD1	-2.54	6.80E-06	-1.72	5.22E-03
IQSEC1	-2.01	2.64E-05	-2.27	1.40E-06
MIR223	-2.26	2.19E-09	-2.03	1.59E-07
SCPEP1	-2.44	4.82E-15	-1.85	1.06E-08
CISH	-2.61	5.91E-05	-1.69	1.96E-02
NUP210	-2.27	6.43E-04	-2.05	3.18E-03
CASP1	-2.27	7.61E-09	-2.05	3.04E-07
LZTR1	-2.69	5.45E-10	-1.64	4.91E-04
FOCAD	-1.96	2.02E-04	-2.39	5.58E-06
ROPN1L	-1.77	1.19E-03	-2.61	2.68E-07
DGKD	-2.09	9.62E-04	-2.29	2.85E-04
MGAM	-1.89	7.34E-03	-2.50	2.29E-04
SLC16A14	-2.46	2.43E-03	-1.94	2.72E-02
DEF8	-2.81	5.20E-17	-1.59	1.07E-05
TEC	-2.02	4.46E-07	-2.39	1.47E-09
SELPLG	-2.54	1.19E-10	-1.87	6.40E-06
F5	-2.49	1.00E-18	-1.92	3.14E-11
SYNE1	-2.53	4.78E-13	-1.90	2.08E-07

NFE2	-2.10	2.33E-04	-2.32	4.33E-05
TOB1	-2.77	8.15E-11	-1.67	3.54E-04
PACS2	-2.47	3.19E-03	-1.99	2.74E-02
NLRC5	-2.84	4.04E-11	-1.63	6.06E-04
ALDH3A2	-2.34	2.33E-05	-2.14	1.80E-04
CACNA1D	-2.16	8.50E-03	-2.32	4.79E-03
DUSP1	-2.29	6.29E-16	-2.21	9.10E-15
CR1	-2.05	1.23E-06	-2.48	2.29E-09
SLC39A10	-2.65	1.17E-03	-1.90	3.81E-02
SYNE2	-2.66	4.56E-21	-1.89	8.44E-11
LINC01410	-3.21	7.46E-13	-1.34	1.10E-02
SIRPD	-2.52	1.97E-06	-2.04	1.60E-04
HACD4	-2.49	1.98E-18	-2.07	1.02E-12
FBXL13	-2.23	5.58E-06	-2.34	1.70E-06
44257	-2.78	8.15E-11	-1.81	7.70E-05
IL18RAP	-1.80	1.86E-03	-2.80	3.05E-07
ARHGEF18	-2.19	1.60E-04	-2.44	2.02E-05
CNKSR2	-2.86	1.36E-04	-1.79	3.01E-02
PECAM1	-3.19	2.68E-15	-1.47	1.27E-03
CSGALNACT1	-2.07	6.38E-03	-2.63	3.34E-04
THEM4	-3.02	1.58E-05	-1.70	3.06E-02
KHDRBS3	-2.59	4.10E-03	-2.13	2.39E-02
MAD1L1	-2.53	4.60E-07	-2.21	2.09E-05
HEXDC	-2.63	1.85E-09	-2.12	2.54E-06
FAM124B	-2.14	8.12E-04	-2.64	9.45E-06
TRPV2	-2.85	8.22E-08	-1.95	6.57E-04
RCAN3	-2.32	2.48E-02	-2.49	1.64E-02
TFF3	-2.41	1.77E-04	-2.44	1.66E-04
TRIB2	-1.90	3.08E-02	-2.95	1.68E-04
PRMT7	-2.67	1.49E-03	-2.19	1.46E-02
CARS2	-2.98	2.01E-20	-1.89	1.84E-08
FAAH	-2.87	7.45E-15	-2.03	4.98E-08
SLC35G1	-2.67	2.83E-03	-2.24	1.90E-02
ACACB	-3.01	7.38E-07	-1.92	3.53E-03
LINC00299	-3.17	1.50E-06	-1.78	1.66E-02
DUSP6	-1.50	1.79E-02	-3.44	3.89E-10
APOL1	-3.09	4.12E-05	-1.88	1.73E-02
LRRC45	-2.58	6.88E-03	-2.40	1.78E-02
CXCR1	-2.72	2.43E-05	-2.26	1.27E-02
FCER1A	-2.77	6.88E-15	-2.23	1.73E-08
KIF21B	-2.43	1.21E-04	-2.58	4.44E-05
FBXW8	-2.52	1.75E-03	-2.52	3.05E-04
PLCB2	-3.05	6.49E-13	-2.03	7.07E-06
44256	-1.99	3.31E-02	-3.09	4.55E-04
TNFRSF10C	-2.92	9.30E-15	-2.18	2.31E-08
TSEN34	-2.38	1.55E-09	-2.75	1.85E-12

OXER1	-3.39	2.20E-10	-1.74	4.48E-03
IGFBP7	-2.38	1.54E-06	-2.76	1.73E-08
C20orf197	-3.07	2.65E-04	-2.08	2.87E-02
CYSLTR2	-3.45	5.92E-17	-1.71	1.32E-04
MYCT1	-3.41	1.81E-09	-1.83	3.12E-03
MCCC1	-2.42	1.73E-06	-2.85	8.78E-09
BANK1	-3.14	2.84E-08	-2.15	4.07E-04
THBS4	-2.91	1.95E-10	-2.38	2.32E-07
PLD4	-3.21	7.54E-12	-2.10	1.63E-05
GLIPR2	-2.68	2.03E-20	-2.64	1.08E-19
ITGA4	-2.58	2.25E-23	-2.74	1.31E-26
LOC643802	-2.92	2.98E-09	-2.41	2.66E-06
CLC	-2.73	3.33E-17	-2.68	2.40E-16
CCND3	-2.40	3.34E-08	-3.03	1.28E-12
IFIT3	-3.50	1.76E-21	-2.01	1.12E-07
PFKFB4	-3.21	1.26E-08	-2.33	9.64E-05
TRIM22	-3.33	4.29E-17	-2.21	5.42E-08
TMEM254	-2.66	4.81E-02	-2.94	3.57E-02
LPAR1	-2.71	3.32E-04	-2.88	1.29E-04
RRP12	-2.29	3.40E-03	-3.31	8.86E-06
UBA7	-3.34	5.08E-13	-2.26	3.18E-06
ADAMTS7P1	-3.03	1.33E-03	-2.60	7.03E-03
CMPK2	-3.96	2.97E-21	-1.68	1.58E-04
TRAM2	-3.26	6.31E-05	-2.40	4.55E-03
HERC5	-4.06	1.31E-10	-1.65	2.53E-02
NLRP12	-3.43	2.52E-14	-2.30	1.13E-06
SLAMF6	-3.52	1.98E-15	-2.22	1.18E-06
NUDT16P1	-2.76	8.22E-03	-3.06	2.60E-03
TXK	-2.46	1.67E-03	-3.39	1.03E-05
EPS8L2	-3.46	2.69E-04	-2.41	1.83E-02
EEF2K	-2.97	2.25E-10	-2.93	2.15E-10
MX1	-3.63	1.25E-10	-2.29	1.40E-04
PRDM5	-3.40	9.12E-09	-2.53	4.44E-05
CSRP1	-2.94	2.64E-05	-2.99	2.15E-05
LSS	-4.00	7.35E-08	-1.98	1.73E-02
IL5RA	-3.84	2.09E-20	-2.14	1.53E-06
ACPP	-3.59	1.66E-14	-2.42	7.64E-07
PDLIM1	-2.90	5.08E-18	-3.18	1.17E-22
CBFA2T3	-3.81	8.88E-08	-2.27	2.85E-03
CENPU	-1.93	4.39E-03	-4.18	8.11E-11
XAF1	-3.84	1.02E-15	-2.31	1.37E-06
ALDH6A1	-3.24	1.22E-11	-2.94	9.12E-10
RIPOR2	-3.18	1.66E-20	-3.02	2.04E-18
CIITA	-4.02	1.15E-07	-2.22	7.94E-03
CENPV	-4.25	7.58E-14	-2.09	3.77E-04
GSTM1	-4.08	6.77E-04	-2.32	4.20E-03

CLEC12B	-3.75	1.72E-08	-2.67	9.22E-05
TREML2	-2.86	1.29E-06	-3.56	5.31E-10
TRPM6	-3.99	5.45E-10	-2.44	3.55E-04
DHRS9	-2.56	3.77E-05	-3.87	7.12E-11
SLC7A8	-4.10	2.25E-09	-2.36	8.27E-04
LOC105370401	-3.63	1.36E-12	-2.85	2.66E-08
ALPL	-3.90	2.33E-04	-2.63	2.06E-02
TOX2	-4.07	4.32E-05	-2.53	2.44E-02
REC8	-3.52	2.01E-09	-3.31	8.11E-09
VCL	-3.46	2.63E-11	-3.40	8.93E-11
GSTM4	-4.02	1.83E-20	-3.00	4.41E-12
SELL	-3.87	7.85E-45	-3.15	8.18E-30
LILRA1	-3.82	8.02E-16	-3.29	3.20E-12
FLNB	-3.79	9.00E-04	-3.33	5.63E-03
FAM117B	-4.53	1.53E-05	-2.62	1.15E-02
HLA-DMB	-4.32	4.31E-10	-2.92	3.28E-05
PYROXD2	-4.40	3.43E-27	-2.93	5.52E-13
SPATC1L	-3.32	6.83E-04	-4.08	1.66E-05
IL34	-4.83	1.81E-07	-2.64	2.89E-03
HRH2	-2.89	4.80E-02	-4.59	6.14E-04
FKBP5	-4.60	4.50E-20	-2.95	2.92E-08
SEMA3C	-2.92	2.13E-02	-4.69	6.47E-04
EPX	-4.23	1.52E-03	-3.45	1.15E-02
MS4A6A	-3.89	4.05E-03	-3.85	2.79E-02
TMEM38A	-3.94	3.24E-05	-3.87	7.09E-05
KLHL13	-5.20	1.27E-07	-2.69	1.12E-02
MEIS2	-5.45	1.53E-11	-2.51	1.14E-03
CCDC170	-4.64	1.01E-08	-3.40	7.95E-06
SLC39A14	-3.43	1.94E-02	-4.69	3.05E-03
RSAD2	-5.77	1.01E-22	-2.36	4.44E-05
NOV	-4.67	2.42E-04	-3.48	1.24E-02
SMPD3	-5.03	1.51E-35	-3.16	1.47E-14
CNTNAP3	-3.23	8.41E-03	-5.02	1.87E-04
ANKRD55	-5.07	2.96E-22	-3.38	6.85E-11
IFIT1	-5.00	1.52E-09	-3.55	1.88E-06
CSF1R	-4.27	1.60E-03	-4.33	1.30E-03
MRVI1	-3.65	2.29E-04	-5.07	4.24E-06
ITGA6	-4.58	2.54E-29	-4.28	2.52E-26
LRRC17	-4.28	1.19E-06	-4.67	1.33E-08
FLT3	-4.33	8.90E-10	-4.68	8.45E-07
LOC100506585	-6.24	8.02E-12	-3.22	3.50E-04
ADAM19	-5.28	2.89E-15	-4.42	5.64E-11
CYP4F12	-5.63	1.26E-04	-4.12	1.03E-02
AGAP7P	-6.59	2.68E-08	-3.56	5.82E-07
DDX60	-7.34	1.21E-10	-2.85	1.16E-04
CYP7B1	-6.73	3.15E-10	-3.75	6.91E-08

MAP2K6	-6.03	5.49E-06	-4.79	3.90E-04
SRGAP3	-7.76	8.96E-21	-3.12	9.87E-08
SLC47A1	-7.52	1.42E-05	-3.49	4.83E-03
PCSK6	-8.12	1.15E-06	-3.74	1.87E-03
KLF2	-5.92	1.29E-69	-6.27	2.62E-82
SEMG1	-9.38	9.68E-11	-3.30	5.28E-06
CDK15	-10.10	2.26E-11	-5.53	2.05E-10

List of common significantly differentially expressed genes (adjusted p-value <0.05) in the response of blood eosinophils from mepolizumab- or –omalizumab-treated patients to ex vivo stimulation with recombinant human IL33 compared with freshly concomitantly isolated blood eosinophils from the same patients (n=3/group). Log2(FC): log2(fold change); padj: adjusted p-value