Genomic and drug target evaluation of 90 cardiovascular proteins in 30,931 individuals

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Abstract

Circulating proteins are vital in human health and disease and are frequently used as biomarkers for clinical decision-making or as targets for pharmacological intervention. Here we map and replicate protein quantitative trait loci (pQTL) for 90 cardiovascular proteins in over 30,000 individuals, resulting in 451 pQTLs for 85 proteins. For each protein we further perform pathway mapping to obtain trans-pQTL gene and regulatory designations. We substantiate these regulatory findings with orthogonal evidence for trans-pQTLs using mouse knock-down experiments (ABCA1, TRIB1) and clinical trial results (CCR2, CCR5), with consistent regulation. Finally we evaluate known drug targets, and suggest new target candidates or repositioning opportunities using Mendelian randomization. This identifies 11 proteins with causal evidence of involvement in human disease that have not previously been targeted, including (gene symbols) EGF, IL16, PAPPA, SPON1, F3, ADM, CASP8, CHI3L1, CXCL16, GDF15, and MMP12. Taken together these findings demonstrate the utility of large-scale mapping the genetics of the proteome, and provide a resource for future precision studies of circulating proteins in human health.

Main

Proteins circulating in blood are derived from multiple organs and cell types, and consist of both actively secreted and passively leaked proteins. Plasma proteins are frequently used as biomarkers to diagnose and predict disease and have been of key importance for clinical practice and drug development for many decades.

Circulating proteins are attractive as potential drug targets as they can often be directly perturbed using conventional small molecules or biologics such as monoclonal antibodies[1]. However, a prerequisite for successful drug development is efficacy, which is predicated on the drug target playing a causal role in disease. One approach to clarifying causation is through Mendelian randomization (MR), which has successfully predicted the outcome of randomized controlled trials (RCT) for pharmacological targets such as PCSK9, LpPLA2 and NPC1L1, and is increasingly becoming a standard tool for triaging new drug targets[2].

Recent technological developments of targeted proteomic methods have enabled hundreds to thousands of circulating proteins to be measured simultaneously in large studies[3-6]. This has paved the way for studies of genetic regulation of circulating proteins using genome-wide association studies (GWAS) for detection of protein quantitative trait loci (pQTL), some of which are referenced here [3,4,7-9].

Here, we present a genome-wide meta-analysis of 90 cardiovascular-related proteins, many of which are established prognostic biomarkers or drug targets, measured using the Olink Proximity Extension Assay CVD-I panel [10] in 30,931 subjects across 14 studies. The identified pQTLs were combined with other sources of information to suggest new target candidates underpinned by insights into *cis*- and

trans- regulation of protein levels and to evaluate past and present efforts to therapeutically modify the proteins analysed in the present investigation. We also show that protein-centric polygenic risk scores (PRS) can predict a substantial fraction of inter-individual variability in circulating protein levels, explaining a proportion of disease susceptibility attributable to specific biological pathways.

These are the first results to emerge from the SCALLOP consortium, a collaborative framework for pQTL mapping and biomarker analysis of proteins on the Olink platform (www.scallop-consortium.com).

Results

Genome-wide meta-analysis of 90 proteins reveals 467 independent genetic loci associated with plasma levels of 85 proteins

Ninety proteins in up to 21,758 participants from 13 cohorts passed quality control (QC) criteria and were available for GWAS meta-analysis [Supplementary Table 1]. We found a total of 401 pQTLs that were significant at a discovery *P*-value threshold conventional for GWAS ($P<5x10^{-8}$) [Figure 1] [Supplementary Table 2]. Conditioning each of these primary pQTLs using the GCTA-COJO software, we identified an additional 144 proximal pQTLs that independently surpassed conventional genome-wide significance ($P<5x10^{-8}$), termed as secondary pQTLs. We attempted to replicate the primary and secondary pQTLs in two independent studies (9,173 participants) whereupon the discovery and replication datasets were meta-analysed, leading to 315 primary pQTLs and 136 secondary pQTLs surpassing a Bonferroni corrected *P*-value ($P<5.6x10^{-10}$). The discovery *P*-values were used for pQTLs absent in the replication dataset ($n_{snp}=25$) [Supplementary Table 2].

Some proteins such as SCF, RAGE, PAPPA, CTSL1 and MPO showed association with more than nine primary pQTLs, but most proteins (22 of 85) were associated with 2 primary pQTLs. We also observed that some proteins were associated with multiple conditionally significant (secondary) pQTLs such as CCL-4 with 4 secondary signals, implicating complex genetic regulation of circulating CCL-4 at the *CCL4* locus.

Analysis of *trans*-pQTLs suggests common mechanisms by which genetic variants affect plasma protein levels

A "best guess" causal gene for each of the CVD-I trans-pQTLs was assigned by a hierarchical approach based on analysis of protein-protein interactions (PPI), literature mining, genomic distance to gene and manual review of literature around the gene as well as the genomic context of the association signal. In total, 326 primary trans-pQTLs were assigned to unique genes and 30 trans-pQTLs were assigned more than one gene, with *ABO, ST3GAL4, JMJD1C, SH2B3, ZFPM2* showing association with the levels of five or more CVD-I proteins [Extended Figure 2A and 2B] [Supplementary Table 2]. Extending this analysis to pQTLs from literature expanded the list of genes with five or more protein associations to include also *KLKB1, GCKR, FUT2, TRIB1, SORT1 and F12* [Supplementary Table 4].

Gene ontology (GO) analysis of genes assigned to all significant trans-pQTLs showed functional enrichment for chemokine binding, glycosaminoglycan binding, receptor binding and G-protein coupled chemoattractant activity [Figure 2C]. A broader classification of genes assigned to both cis- and trans-pQTLs [Figure 2A, 2B] [Supplementary Table 2] using a wider set of tools (Online Methods) suggested that transcriptional regulation, post-translational modifications, such as glycation and sialylation, cell-signalling events, protease activity and receptor binding are potential common mechanisms by which trans-pQTLs influence circulating protein levels. The default gene calls and paths for the CVD-I *trans*-pQTLs based on PPI and literature mining can be visualised using the SCALLOP CVD-I network tool [Extended Figure 2C] whereas details on the classification of genes are available in the Online Methods, [Supplementary Information 1] and [Supplementary Table 3].

Evidence of mRNA expression mediating associations with a third of cis pQTLs

We investigated the overlap of the CVD-I *cis*- and *trans*-pQTLs with expression quantitative trait loci (eQTL) by a combination of approaches and eQTL studies, including direct genetic lookups and co-localisation using PrediXcan [11] and SMR / HEIDI [12]. For direct lookups, three studies were used: LifeLines-DEEP (whole blood), eQTLGen meta-analysis (whole blood and PBMCs) and GTEx (48 tissue types). Of 545 pQTLs from [Supplementary table 2], eQTL data were available for 434 SNP-transcript

pairs, including 168 *cis*-pQTLs and 266 *trans*-pQTLs. Of these, 72 (43%) of *cis*-pQTLs had at least one corresponding eQTL (FDR<0.05) in any of the eQTL datasets investigated, implicating 42 of the 75 proteins with a *cis*-pQTL. At a more stringent eQTL p-value of $P<5x10^{-8}$, the percentage with a corresponding eQTL was 26 %, similar to some previous reports [13-15] [Supplementary Table 5].

Co-localisation analysis of CVD-I cis-pQTLs and mRNA levels was performed in selected tissues from the GTEx project by first imputing mRNA expression of the CVD-I protein-encoding transcripts using the PrediXcan[11] algorithm in one of the SCALLOP CVD-I cohorts (IMPROVE), and then testing imputed mRNA levels for association with CVD-I plasma protein levels using linear regression. Twenty-six of the 90 CVD-I proteins were associated with their corresponding mRNA transcript (FDR<0.05) in at least one of the 20 GTEx tissues investigated [Extended Figure 3]. All 26 proteins were among the 42 proteins found to also be an eQTL by direct lookups. Proteins CCL4, CD40, CHI3L1, CSTB and IL-6RA all associated with their corresponding transcript across five or more tissues whereas proteins ST2 and RAGE showed significant association exclusively in lung, and CTSD exclusively in skeletal muscle.

To further investigate if the CVD-I protein pQTLs overlap with eQTLs, we used the SMR/HEIDI methods[12], using data from the Consortium for the Architecture of Gene Expression (CAGE) study. SMR/HEIDI tests the hypothesis that there is a single variant affecting protein and gene expression (pleiotropy or causality), with the alternative hypothesis being that protein and gene expression are affected by two distinct variants. In total, 125 associations between 96 genes and 54 proteins were identified at an experiment-wise SMR test significance level ($P_{SMR}<0.05/8558$) and a stringent HEIDI test threshold ($P_{HEIDI} > 0.01$) [Supplementary Table 6], of which 23.2 % were in *cis*-pQTL regions, such as IL-8 and U-PAR. The 96 genes were located in 74 loci, suggesting that pleiotropic associations between protein and mRNA expression were present for 18.4 % of significant and suggestive primary loci using SMR / HEIDI.

A minor proportion of *cis*-acting pQTLs are in high linkage-disequilibrium with non-synonymous coding variants

"Pseudo-pQTLs" caused by epitope effects, i.e. differential assay recognition depending on presence of protein-altering variants, is a theoretical possibility for *cis*-pQTLs and likely dependent on the method of protein quantification [4,16]. To evaluate the potential for pseudo-pQTLs among the CVD-I pQTLs, we investigated presence of protein-altering variants for sentinel variants or variants in high linkage disequilibrium with a sentinel variant. Of the 90 proteins, 85 had at least one pQTL, including 12 with only *cis*-pQTLs, 10 with only *trans*-pQTLs and 63 with both *cis*- and *trans*-pQTLs. Of the 170 primary or secondary *cis*-pQTLs for 75 proteins, 20 *cis*-pQTLs for 18 proteins had a sentinel variant in high linkage disequilibrium (LD; R^2>0.9) with a protein-altering variant, which suggests potential to affect assay performance [Supplementary Table 2].

Orthogonal evidence supports causal gene to protein relationships for a subset of the CVD-I *trans*-pQTLs

Of the 326 *trans*-pQTLs identified, eight were assigned to gene products targeted by compounds or antibodies that have been in clinical development [Supplementary Table 7]. Assuming that *trans*-pQTLs represent causal relationships between gene variants and proteins, we hypothesized that the downstream CVD-I proteins associated with CVD-I *trans*-pQTL genes would be modulated on therapeutic modification of the gene product. Support for this hypothesis was obtained by previous work showing that circulating FABP4 is upregulated upon treatment with glitazones (PPARG inhibitors)[17]; that circulating IL-6 is increased after treatment with tociluzumab[18] (IL6R inhibitor) and that circulating TNF-R2 is decreased upon infliximab (TNFA inhibitor) treatment in patients with Crohn's disease[19], which supports CVD-I *trans*-pQTLs for these proteins. Along these lines, we present novel evidence from a clinical trial supporting our observations that a CCR5 variant is a trans-pQTL for plasma CCL-4 and a variant in CCR2 is a trans-pQTL for plasma MCP-1 [Supplementary table 2]. CCR5 and CCR2 are targeted in combination by the small-molecule dual-inhibitor PF-04634817 [20]. To test whether dual inhibition of CCR5 and CCR2 resulted in a change of circulating CCL-4 and MCP-1 respectively, we measured these proteins in 350 type 2 diabetes patients in a randomized, double-

blind, placebo-controlled phase-II trial evaluating the efficacy of PF-04634817 in diabetic nephropathy (NCT01712061). In addition, we also measured known or suspected ligands of CCR5 and CCR2, including CCL-3, CCL-5 (RANTES) and CCL-8, and 5 additional proteins that were present on the Olink CVD-I panel, and for which assays were readily available. Compared to placebo, we observed a 9.25-fold increase in circulating MCP-1 levels ($p < 0.0001$) and a 2.11-fold increase in circulating CCL4 levels ($p < 0.0001$) at week 12 [Figure 3A]. An alternative ligand for CCR-2; CCL-8 did not change following exposure to PF-04634817, and neither did other CCR-5 ligands, such as CCL-5 (RANTES) and CCL-3. Moreover, EN-RAGE, FGF-23, KIM-1, myoglobin and TNFR-2 were unchanged following PF-04634817 exposure [Extended Figure 4]. We conclude that CVD-I *trans*-pQTLs at *CCR5* and *CCR2* were concordant with the effects of PF-04634817 in human.

Two of the genes implicated by CVD-I *trans*-pQTLs, *ABCA1* and *TRIB1* for circulating SCF levels, were also investigated in the mouse. Mice with liver-specific or whole-body knockdown of *ABCA1*[21] and *TRIB1*[22] respectively showed decreased plasma levels of SCF compared to matched wild-type controls [Figure 3B], concordant with the human CVD-I *trans*-pQTLs.

Mendelian randomization analysis revealed 25 CVD-1 proteins causal for complex traits with strong evidence

To identify potential causal disease pathways indexed by proteins, we conducted an MR analysis of 85 proteins across 38 outcomes. 25 proteins showed strong evidence of causality for at least one disease or phenotype and an additional 24 proteins showed intermediate evidence of causality. [Figure 4A] [Extended Figure 7] [Supplementary Figure 1]. Using open-source information (clinicaltrials.gov) (www.ebi.ac.uk/chembl/) (www.drugbank.ca/) (www.opentargets.org) and Clarivate Integrity (integrity.clarivate.com), we identified records on past or present clinical drug development programs for 14 of the 25 proteins, all of which have been in phase 2 trials or later [Supplementary Table 7]. Of the 14 proteins, seven proteins were targeted for an indication different from the phenotype implicated by our MR analysis. Eleven of the 25 proteins have never been

targeted in clinical trials, but may provide new promising target candidates for indications closely related to the traits in the MR analysis.

Several published MR findings were confirmed, including that *IL6RA* variants associated with higher circulating levels of interleukin-6 (IL-6) and soluble IL6-RA were associated with lower risk of coronary heart disease (CHD), rheumatoid arthritis (RA) and atrial fibrillation but higher risks of atopy, such as asthma and eczema[23]. We also replicated previous findings suggesting a causal contribution of IL-1ra to rheumatoid arthritis (RA) but an inverse causal relationship with cholesterol levels [24], and a protective role of genetically higher MMP-12 against stroke [4,25].

Some novel MR observations included higher levels of CD40 protein and increased risk of RA, higher MMP-12 and increased risk of eczema, and higher TRAIL-R2 proteins levels and prostate cancer. Further, Dkk-1 has been targeted by a humanised monoclonal antibody (DKN-01) in clinical trials for advanced cancer (NCT01457417, NCT02375880), and was in our study causally linked to higher risk of bone fractures and lower risk of estimated bone mineral density (eBMD). In addition, strong evidence for protective roles of PLGF in CHD, CASP-8 in breast cancer and ST2 in asthma was observed. RAGE was causally linked to several traits, including lower body mass index (BMI) and a corresponding lower risk of type 2 diabetes (T2D), higher total cholesterol and triglycerides and higher risk of prostate cancer and schizophrenia. A small molecule brain penetrant RAGE inhibitor was tested in a phase 2 trial of Alzheimer's disease (NCT00566397), but was stopped early for futility. We saw no strong signal for Alzheimer's disease (or vascular disease) in our MR analysis. Our findings identify potential target-mediated effects across multiple other complex phenotypes that might manifest in beneficial and/or harmful effects on patients receiving RAGE-modifying therapies.

We also collated observational evidence for 23 of the 50 protein-trait pairs identified as causal in the MR analysis [supplementary table 10]. The direction of effect inferred from observational studies was concordant with the effect direction from MR estimates for 12 pairs.

Heritability analysis and polygenic risk scores (PRS) demonstrates large differences in genetic architecture

We calculated SNP-heritability contributed by the major reported loci (major loci h_{SNP}^2, any pQTL included in [supplementary table 2]), as well as additional genome-wide SNP-heritability (polygenic h_{SNP}^2) for each protein included in the entire SCALLOP CVD-I meta-analysis. We observed a large range of different genetic architectures: Differences in magnitude of the genetic component (h_{SNP}^2) ranged from 0.01 (EGF) to 0.46 (IL-6RA). Differences in the contribution from non-genome-wide significant SNPs ranged from essentially monogenic (e.g. IL-6RA) to others showing considerable locus heterogeneity with genetic contributions originating entirely from a polygenic background with no single dominating locus (e.g. PDGF-B and Galanin) [Figure 5].

In addition, we calculated the out of sample variance explained in the independent Malmo Diet and Cancer (MDC) study (N=4,678) both for genome-wide significant loci (major loci $V.E._{PRS}$), as well as additional variance explained by adding PRS (polygenic $V.E._{PRS}$) [Figure 5]. The protein PRS' applied in the MDC study for 11 proteins exceeded 10 % of variance explained ($V.E._{PRS}$) and the PRS' for another 14 proteins exceeded 5 % of variance explained, suggesting that the genetic contribution to inter-individual variability of CVD-I protein levels is considerable.

A polygenic risk score for circulating ST2 levels shows a dose-response relationship with asthma

Since circulating ST2 showed strong evidence of causation in asthma and inflammatory bowel disease (IBD) and the polygenic $V.E._{PRS}$ model for ST2 explained nearly 20 % of its variance, we attempted to quantify the effect of the ST2 polygenic $V.E._{PRS}$ on circulating ST2 levels in the MDC study, and risk of asthma and IBD in 337,484 unrelated White British subjects in the UK Biobank. The range of circulating ST2 across 11 categories of the ST2 PRS in MDC was nearly 1.2 standard deviations [Figure 6A]. Corroborating the Mendelian randomization analysis, the ST2 PRS showed a strong negative dose-response relationship with risk of asthma ($p=1.2x10^{-8}$) and a positive trend for risk of IBD ($p=0.13$) [Figure 6B and 6C]. Overlaying the linear trends for ST2 levels, asthma and IBD using meta-

regression, an increase in the PRS equivalent to a 1 standard deviation higher circulating ST2, corresponded to a 8.6 % (95%CI 3.8%, 13.2%; P=0.004) reduction in the relative risk of asthma and a 4.3 % (95%CI -3.8%, 13.0%; P=0.263) increase in the relative risk of IBD [extended Figure 8].

Reverse Mendelian randomization identifies widespread causal relationships, where complex phenotypes affects CVD-I proteins

To investigate whether genetic susceptibility (liability) to complex disease and phenotypes causally alter circulating levels of CVD-I proteins, we also performed MR using 38 complex phenotypes (including continuous risk factors, such as adiposity and clinical outcomes, such as T2D) as exposure and CVD-I protein levels as outcomes. All CVD-I proteins were causally altered by at least one complex phenotype. BMI and estimated glomerular filtration rate (eGFR) causally affected 32 and 29 of the 85 tested proteins respectively [Figure 7A] [Extended Figure 7] [Supplementary Figure 2]. BMI seemed to causally affect protein levels in both positive and negative directions, whereas only REN (renin) was causally decreased with genetically higher eGFR. In an effort to elucidate whether these estimates were recapitulated in simple observational analyses, we compared effect estimates from linear regression analyses of associations of BMI and eGFR with each respective CVD-I protein in one of the participating study cohorts (IMPROVE). The correlation between the observational and MR estimates were high for BMI (R=0.78), and more modest for eGFR (R=0.50) [Figure 7B-C].

Discussion

Using a meta-analysis approach including >30,000 individuals, we identified and replicated 315 primary and 136 secondary pQTLs for 85 circulating proteins to yield new insights for translational studies and drug development. Our study demonstrates that pQTLs can be harnessed to enhance evaluation of therapeutic hypotheses for protein targets, and to support those hypotheses with basic insights into potential protein regulatory pathways and biomarker strategies. However, we also observed large differences between proteins in relation to genetic architecture, suggesting that the relative strength to apply these strategies is likely protein-dependent.

Our pQTL-based framework was developed to address several key challenges associated with drug development, including a) mapping of protein regulatory pathways, b) identification of new target candidates c) repositioning of drugs, d) target-associated safety and e) matching of target mechanisms to patients by protein biomarkers or genetic PRS' [Figure 8].

The mapping of *trans*-pQTLs, which typically have smaller effects on protein levels [Extended Figure 9], was aided by the large SCALLOP discovery sample size, yielding on average 4 independent pQTLs per protein. A causal gene was assigned for each *trans*-pQTL to generate hypotheses that can be further tested using *in vitro* or *in vivo* perturbation experiments. The robustness of causal gene assignments for a few selected *trans*-pQTLs was demonstrated using samples from a randomised controlled trial testing a dual small-molecular inhibitor of the protein products of assigned genes (*CCR5*, *CCR2*) and transgenic mice with liver-specific knockdown of assigned genes (*ABCA1*, *TRIB1*). Although further studies will be needed for orthogonal validation of most of the genes assigned from the CVD-I *trans*-pQTLs, several of the implicated genes have previously been identified as regulators of some of the CVD-I proteins including *CASP1*[26], *NLRC4*[26] and *GSDMD*[27] for IL-18, *FLT1*[28] for PLGF, *ADAM17* [29] for TNFR1 and *SLC34A1*[30] for FGF-23 [Supplementary Table 2].

Further, we attempted to estimate the proportion of pQTLs that were likely to be driven by effects on mRNA expression, using multiple eQTL approaches and datasets. The lowest estimate was obtained with SMR/HEIDI, suggesting that 18.4 % of pQTLs were also eQTLs whereas direct look-up and co-localisation analysis using PrediXcan yielded estimates between 26 % - 29 %. We conclude that the majority of pQTLs identified for the CVD-I proteins were not explained by eQTLs.

Clinical-stage targeting with any drug modality was reported for 35 of the 90 proteins on the Olink CVD-I panel [Supplementary Table 7]. Our MR analysis identified 11 proteins with causal evidence of involvement in human disease that have not previously been targeted. Among those, four proteins were causal for a disease phenotype and did not show strong evidence of inverse causality with another phenotype (increasing specificity for intended indication), including CHI3L1 and SPON1 for

atrial fibrillation and PAPPA for type-2 diabetes. Strong causal evidence was also identified for proteins targeted in phase-2 or later development. The MR evidence was concordant with drug indications for several protein targets but for some also suggested alternative indications or that monitoring of target-associated safety might be warranted. Monoclonal antibodies that block the CD40 ligand binding to CD40 – a critical element in T cell activation – have been shown to have positive clinical effects in patients with autoimmune diseases; but increased risk of thromboembolism precluded further clinical development[31]. These observations from clinical trials are in line with our findings that genetically *lower* levels of CD40 are associated with *lower* risk of RA, but *higher* risk of stroke. There are ongoing efforts to modify CD40L antibodies to retain efficacy while avoiding thromboembolism [31]. However, our results suggest that decreasing circulating CD40 levels may have target-mediated beneficial effects on RA risk, while increasing the risk of ischemic stroke, i.e. that the increased risk of thromboembolism (manifest as stroke) is an on-target adverse effect. TRAIL-R2 is a key receptor for TRAIL, which has been shown to selectively drive tumour cells into apoptosis. Therefore, considerable effort to agonise TRAIL-R2 for treating cancers has been made in the past years[32]. We demonstrated that increased circulating TRAIL-R2 is protective against prostate cancer, which may suggest that this cancer type should be investigated in clinical trials evaluating the efficacy of TRAIL-R2 agonists.

Biomarkers can be broadly classified as generic biomarkers for disease risk or prognosis, or as biomarkers reflecting the activity of specific disease processes or biology. Biomarkers that enable matching of target mechanisms to patient subgroups with greater than average benefit from treatment are enablers of precision medicine. We showed that CCR2/CCR5 small-molecule inhibition modulated circulating levels of CCL-4 and MCP-1, which may suggest that *trans*-pQTLs can guide selection of exploratory biomarkers to monitor the efficacy of target mechanisms. We also identified multiple complex traits causally affecting circulating protein levels. For example, eGFR and BMI causally influenced over 1/3 of the CVD-I proteins, suggesting that future biomarker studies should consider these traits as potential confounders. Moreover, the causal phenotype-to-protein

associations may represent pathway-related causality to the complex phenotype of interest; or alternatively, 'reverse causality' which might pose an opportunity to evaluate implicated proteins as surrogate biomarkers for efficacy in interventional trials [33]. We found that higher BMI causally lowered RAGE, while higher circulating levels of RAGE were causally linked to a lower risk of T2D. Thus, developing a hypothetical therapeutic to increase RAGE might represent a mechanism by which it is possible to off-set the risk of T2D arising from the global increases in obesity.

Protein-centric PRS' may allow stratification of individuals with genetic propensity for high circulating protein levels. Only 10 % of the protein-centric PRS' explained 10 % or more of the protein variance in the independent replication cohort, including ST2, a prognostic biomarker for heart failure[34]. ST2 showed evidence of inverse causality in asthma and positive causality in IBD. By constructing a genome-wide polygenic risk score for ST2 levels from the MDC study, applying it to the UK Biobank and comparing asthma and IBD prevalence across eleven quantiles of the ST2 PRS, estimated the magnitude of ST2 increase required to decrease the risk of asthma to similar levels as individuals in the highest ST2 PRS category. Such use of PRS for proteins may be expanded to other disease endpoints and may be of use in precision medicine, to guide which patients may obtain most benefit from drugs that pharmacologically alter individual proteins.

In conclusion, our findings provide a comprehensive toolbox for evaluation and exploitation of therapeutic hypothesis and precision medicine approaches in complex disease. Such approaches provide an excellent opportunity to rejuvenate the drug development pipeline for new treatments.

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Author Contributions

La.F, SG, QW, DHH, ÅKH, DVZ, EF, EMD, EI, AM contributed to meta-analysis. La.F, ÅKH, DVZ, YW, JRG, YC, AC, FM, EF, Lu.F, TQ, RW, HJW, JY, AM contributed to functional analysis. La.F, SG, QW, GDS, TP, TQ, JY, LW, ASB, MVH, EI, AM contributed to Mendelian randomization. JP, NE, SEB, TB, ADB, St.E, AK, MAK, SHC, JD, Sö.E, CF, Lu.F, PF, VG, Ch.H, AH, ÅJ, PKJ, LL, CML, SL, EMD, MM, APM, RM, MWN, OP, BP, EP, JS, PS, UV, HJW, AZ, JÄ, JF, GS, TE, Ca.H, UG, ML, Ag.S, JFW, LW, ASB, EI, AM contributed to cohort level analysis. BS, LM, AM contributed to mouse experiments. KP, JDG, JL, WZ, AQ, AM contributed to clinical trials. La.F, ÅKH, An.S, JRG, FM, EF, AI, TW, AM contributed to other downstream analysis. La.F, SG, ÅKH, GE, CF, OM, KM, PMN, JN, MOM, MS, AM contributed to replication analysis. La.F, SG, QW, MVH, EI, AM contributed to writing. La.F, SG, QW, ASB, MVH, EI, AM contributed to project planning. All authors gave final approval to publish.

Competing Interests Statement

The other authors declare no competing interests

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Figure and table legends

Figure 1. Chromosomal location of all associations discovered. Cis-pQTLs are shown in red (bold) and trans-pQTLs in blue if they are significant at a conventional GWAS significance threshold of $P<5x10^{-8}$. The gene annotations refer to the gene closest to the pQTL. A version of this figure with only loci selected according to the criteria for Mendelian randomization is available as [Extended figure 1].

Figure 2. Classification of cis- and trans-pQTL genes. A. The gene ontology label of all cis-pQTL genes, i.e. the protein-encoding genes. **B**. The gene-ontology label of all best-guess trans-pQTL genes. **C**. Gene set enrichment analysis of genes assigned to all significant trans-pQTLs, showing the top-gene sets from the Gene Ontology set Molecular Function.

Figure 3. Clinical trial in humans and knock down experiment in mice corresponds to trans pQTL effects. A) In humans treated with a small molecule dual-inhibitor of CCR5 and CCR2 (PF-04634817) the induction of MCP-1 and CCL4, mirrors the observed CVD-I trans-pQTLs. Box plots elements are according to standards for box-and-whisker diagrams. **B)** In mice, knockdown of ABCA1 or TRIB1 resulted in decreased circulating SCF levels mirroring CVD-I trans-pQTLs for SCF. Shown in the plot are SCF levels of individual mice represented by circles (wild-type in blue and transgenic mice in red) and the median level per group. P-value is calculated using a two-sided T-test.

Figure 4. Main findings of Mendelian randomization analysis. A. Heatmap of Mendelian randomization analyses of 38 complex traits. ICD-10 chapter of indication and clinical trial stage indicated for each target **B.** Forest plot showing CVD-I proteins with strong evidence of causality in the Mendelian randomization analysis. Drug development abbreviations: PC: pre-clinical, Ph1: Phase 1, Ph2: Phase 2, Ph3: Phase 3, post-MA: post-marketing authorisation. ICD-10 chapters of disease: A-B: infectious and parasitic; C-D: neoplasms; D: blood and immune; E: endocrine, nutritional and metabolic; F: mental and behavioural; G: nervous system; H: eye, adnexa, ear and mastoid; I: circulatory system; J: respiratory system; K: digestive system; L: skin and subcutaneous tissue; M: musculoskeletal and connective tissue; N: genitourinary; O: pregnancy, childbirth, puerperium; P: perinatal; Q: congenital, deformations and chromosomal; R: clinical and lab findings; S-T: injury, poisoning; U: provisional assignment (new diseases unknown aetiology); V-Y: external causes; Z: health status & health services

Figure 5. **SNP heritability and variance explained by genetics. A**. SNP-Heritability in the SCALLOP consortium discovery cohorts stratified by contributions major loci (light red) and polygenic effects (dark red). In the independent MDC cohort, additional variability explained by adding major loci (light blue) and polygenic risk scores (dark blue). Significance was reported according to the LDSC algorithm (blue) or a linear regression model (red). **B**. Differences in how protein levels are affected by

polygenic (non-genome-wide significant) loci vs major loci, shown for both the SCALLOP consortium discovery cohorts as h_{SNP}^2 and for the MDC cohort as variability explained.

Figure 6. Mendelian randomization using polygenic risk scores. A. Association of a polygenic risk score (PRS) with ST2 levels in the independent MDC cohort. **B**. Association of the ST2 PRS with asthma in the UK-biobank. **B**. Association of the ST2 PRS with inflammatory bowel disease (IBD) in the UK-biobank. The ST2 PRS was divided into 11 quantiles, with the middle group (quantile number 6) as the reference category. Effect estimates are presented as quantile-specific mean differences (ST2) and odds ratios (asthma and IBD) relative to the reference category.

Figure 7. Mendelian randomization with proteins as outcome. A. Heatmap showing the causal estimates of 38 complex traits on CVD-I protein levels. **B.** Correlation between beta-values for association between body mass index and circulating levels of CVD-I proteins in the IMPROVE cohort, and causal estimates from the Mendelian randomization analysis of body mass index genetic liability on same CVD-I proteins. **C.** Same as B but for estimated glomerular filtration rate.

Figure 8. Protein-trait relationships that support target validation, repositioning, target-mediated safety and new candidates for drug development. For more information, see data presented in [Supplementary Table 7].

URLs

www.scallop-consortium.com

www.ebi.ac.uk/gwas/

www.proteinatlas.org

www.uniprot.org

http://www.pantherdb.org

david.ncifcrf.gov

clinicaltrials.gov

www.ebi.ac.uk/chembl

www.drugbank.ca

www.opentargets.org

neic.no/tryggve/

Data availability

The full summary statistics of the Olink CVD-I protein GWAS have been deposited at the SCALLOP-CVD-I online resource, allowing access to interactive SCALLOP-CVD-I tools and unrestricted download access for secondary analyses. Additionally, a full copy has been deposited at https://doi.org/10.5281/zenodo.2615265 for long-term retention, as well as with GWAS catalog. A copy of the polygenic scores have been deposited at the PGS catalog.

Online Methods

Selection of proteins

Proteins for the Olink PEA CVD-I panel were selected by mining the literature for protein biomarkers associated with cardiovascular risk or prognosis in human observational studies and in animal models and by bringing in protein biomarker suggestions from leading cardiovascular disease researchers [10]. The list of proteins curated from these sources was then pruned down based on availability of high-quality antibodies and relative abundance of the proteins in human plasma.

Intra- and inter-plate coefficients of variation (CV) of the CVD-I panel are available from Olink Proteomics AB (https://www.olink.com/resources-support/document-download-center/). In addition, we calculated the inter-plate coefficient of variation using data from a pooled plasma sample in one of the participating cohorts -the IMPROVE study. The mean inter-plate CV was averaged across proteins was 16.6 %, (range 11 % -26 %) [Supplementary Table 1].

Cohorts and data collection

Summary statistics from GWAS of Olink CVD-I proteins were obtained from 13 cohorts of European ancestry. The details of all study cohorts are shown in [Supplementary Table 9]. Together the cohorts included a total of 21,758 individuals; although the average per-protein sample size was 17,747, since not all proteins passed quality control (QC) in all cohorts. Each cohort provided data imputed to 1000 Genomes Project phase 3 reference or later or to the Haplotype Reference Consortium (HRC) reference, which resulted in the testing of 21.4M SNPs. Because imputation schemes varied by cohort, this resulted in an average of 20.3M SNPs under investigation for each protein.

Each cohort applied quality control measures for call rate filters, sex mismatch, population outliers, heterozygosity and cryptic relatedness as documented in [Supplementary Table 8]. Prior to running the genetic analyses, NPX values of proteins (on the $\log_2$ scale) were rank-based inverse normal transformed and/or standardised to unit variance, thus avoiding potential Olink batch-differences

668 between cohorts. Genetic analyses were conducted using additive model regressions, with
669 adjustment for population structure and study-specific parameters [Supplementary Table 8]. Forest
670 plots of cohort-specific effects are available for all significant and suggestive pQTLs using the online
671 tool. Each contributing cohort uploaded the resulting summary statistics in a standardized format
672 using a secure computational cluster provided by Neic Tryggve (https://neic.no/tryggve/). All meta-
673 analysis was performed in duplicate at two different research centres using completely separate
674 bioinformatic pipelines (L.F. and S.G.).

675 Data cleaning and meta-analysis

676 A per-protein filtering threshold of >80% samples above the Olink detection limit was applied to each
677 cohort, leaving data on 90 of the 92 proteins to be analysed. The remaining files had an average of
678 3% missing samples (per cohort statistics available in [Supplementary Table 8]). Minor allele
679 frequencies were compared with those reported in 1000 Genomes EUR. A per-SNP filter was applied
680 based on imputation quality level (at default setting for respective imputation algorithm) and minor
681 allele count (at least 10 alleles per cohort). This resulted in the omission of 10% of the SNPs. Finally,
682 meta-analysis was performed using METAL (2011-03-25) [35], applying the inverse-variance weighted
683 approach (i.e. the STDERR option). Throughout the manuscript, P-values from this test are reported
684 as-is, with multiple testing burden handled through appropriate thresholds. *Cis*-pQTLs were defined
685 as a signal within 1 Mb of the gene encoding the protein and all other signals were defined as *trans*-
686 pQTLs. See [extended figure 5] for flow chart overview of meta analysis.

687 Replication analyses

688 We sought to replicate the findings in the Malmö Diet and Cancer (MDC) population-based cohort
689 with 4,678 individuals, and in the Swedish Mammography Cohort Clinical (SMCC, part of the Swedish
690 national research infrastructure SIMPLER described at www.simpler4health.se) population-based
691 study of 4,495 women. In MDC, genotypes were imputed to the Haplotype Reference Consortium
692 reference (HRC Unlimited v1.0.1) and data were analysed using linear regression in EPACTS 3.3.0

(linear Wald test). The genotypes in SMCC were measured using Illumina's Global Screening Array and were imputed up to HRC v1.1 and 1000G phase3 (v5), and linear regressions of rank-based inverse-normal transformed protein values adjusting for age, storage time, and PC1-15 were performed using PLINK v2 (4 Mar 2019).

Conditional and joint association analysis

To identify secondary signals at the 401 loci reported in [Supplementary table 2], we performed analyses conditioning on the primary signal using conditional-joint analysis in GCTA (version 1.26.0) [36,37]. The Stanley cohort was chosen as an ancestrally well-matched LD-reference cohort. Meta-analysis summary data were processed with filtering for MAF (0.01) and r^2 (<0.001) to ensure that secondary association signals identified were not driven by LD with the primary signal. See [Extended figure 6] for a flow chart of signal selection criteria.

Cross-reference of pQTLs with other complex traits

For each pQTL association, we searched PubMed and the EBI GWAS catalogue (URL: https://www.ebi.ac.uk/gwas/ : November 2018) for published SNPs with any complex trait within 10kb or having an LD of r^2 >= 0.85.

Comparison between eQTLs and pQTL

To identify eQTL that corresponded to each pQTL, we used three independent eQTL studies: LifeLines-DEEP [38], GTEx[39] and eQTLGen[40]. Each SNP-protein pQTL pair was first converted to SNP-gene pairs using Olink platform protein identification and the gene annotation of Ensembl v91. Then, the significance of eQTLs for these SNP-gene pairs was assessed in three eQTL datasets, using two different cut-offs: a stringent genome-wide significance threshold ($P<5x10^{-8}$) and a nominal significance of $P<0.05$.

In the eQTL dataset of LifeLines-DEEP, individual-level whole blood RNA-seq, protein and genotype data were available. This allowed for a direct comparison of the concordance of blood eQTLs and

pQTLs. To do so, we re-tested eQTL associations for all pQTL pairs, using a previously published pipeline [41]. The resulting eQTLs were considered genome-wide significant if it passed the permutation-based FDR <0.05 level, or to be nominally significant if the *P*-value was < 0.05.

In the eQTL datasets of GTEx v7 and eQTL-Gen, we did not have access to individual level data. Thus, the comparisons were conducted using publicly available eQTL results. In these datasets, we considered an eQTL genome-wide significant if it was within the reported genome-wide significant list, and nominally significant if it had a nominal *P*-value < 0.05. Altogether, if one pQTL pair had at least one significant eQTL effect in any dataset irrespective of allelic direction it was considered an overlapping pQTL-eQTL pair.

Expression SMR analysis

We performed an SMR and HEIDI (heterogeneity in dependent instruments) analysis[12] to identify the expression levels of genes that were associated with protein abundance through pleiotropy using pQTL summary statistics from this study and cis-eQTL summary data from published studies[42,43].

The eQTL summary data used in the SMR analysis were from the Consortium for the Architecture of Gene Expression (CAGE), comprising 38,624 normalized gene expression probes and ~8 million SNPs from 2,765 blood samples. The eQTL effects were in standard deviation (SD) units of expression levels. We excluded the gene probes in the major histocompatibility complex (MHC) region and included only the gene probes with at least one cis-eQTL at $P<5\times10^{-8}$ (a basic assumption of SMR), resulting in 9,538 gene expression probes.

The SMR test uses a SNP instrument (i.e., the top associated eQTL) to detect association between two phenotypes (i.e., gene and protein in this case). The HEIDI test utilises LD between the SNP instrument and other SNPs in the cis-region to distinguish whether the association identified by the SMR test is driven by a set of shared genetic variants between two traits (pleiotropic or causal model) or distinct sets of variants in LD (linkage model)[12]. Only the associations that surpassed the genome-

wide significance level of the SMR test ($P_{SMR} < 0.05 / m$ with m being the number of SMR tests) and were not rejected by the HEIDI test ($P_{HEIDI} > 0.01$) were reported as significant.

PrediXcan and transcript-wide association of CVD-I protein levels

Imputation of gene expression was performed in the IMPROVE study. After standard quality control, genotypes were pre-phased using Eagle2, and then subsequently imputed by minimac4 using the 1000 Genomes reference. A filter on RSQ 0.8 and minor allele frequency 0.01 was set on the imputed genotypes prior to prediction with PrediXcan, which used 44 tissue models based on GTEx v7.

Using protein data collected on the CVD-I chip in the same individuals, the associations between protein levels in plasma and the predicted expression of their respective coding gene across 20 tissues (from the PrediXcan model) were modelled by a linear model in R. False discovery rate were estimated based on Q-values (using the R package qvalue). In total, 64 genes in one to 18 tissues were tested for associations between protein levels and predicted expression. Heatmaps were constructed (using the pheatmap package in R) for any gene with a significant association (FDR<0.05) in at least one tissue.

Systems Biology

Two sets of network analysis were performed, one using the protein-protein interaction (PPI) data from the inBio Map™ (InWeb_InBioMap) and one using significant associations from text-mining (TM). These two networks each had 13,033 and 14,635 nodes, respectively; and 147,882 and 193,777 edges, respectively. In both setups, the shortest path between any of the cis-gene intermediaries to the protein was identified; altogether 12,436 pairs were compared. Of the 372 trans-pQTL associations reported in [Supplementary Table 2], 335 associations had both cis-gene intermediaries and plasma protein in the network allowing their analysis. The likelihood of a path arising by chance was calculated by permutation sampling, using 1,000,000 random networks were generated with a conserved degree distribution. A new algorithm was developed for *de novo* random network

765 generation, which generated random networks with a nearly conserved degree distribution in a
766 feasible time-frame. Further details are available in [Supplementary Information 1].

767 Assignment of cis-intermediary genes

768 To assign the most plausible causal gene for each of the CVD-I trans-pQTLs we applied a hierarchical
769 approach based on analysis of InWeb_InBioMap PPI, TM, and genomic distance between gene and
770 lead variant at each locus. Results were then manually reviewed by literature, gene expression
771 analysis (proteinatlas.org) and published pQTLs which led to the re-assignment of 52 genes. The
772 algorithmic gene assignment was overruled or complemented for instances when the assigned gene
773 was different from the gene assigned by multiple prior studies [Supplementary table 4]. Gene
774 Ontology analysis of most plausible genes was performed using the DAVID bioinformatics tools and
775 the GO MF gene set definition, with default settings. The Panther pathway tool, Uniprot and the
776 Human Protein Atlas were used to classify the genes according to basic functional class (see URLs).

777 Human in-vivo validation of trans-pQTLs

778 PF-04634817 is a competitive dual inhibitor of CCR2 and CCR5 receptors. In the recent B1261007
779 study, (ClinicalTrials.gov Identifier: NCT01712061), samples were collected from subjects with
780 diabetic nephropathy and treated with PF-04634817 for 12 weeks. CCL-2 (MCP-1) was measured in
781 serum by ELISA at Eurofins (The Netherlands). CCL4 (MIP-1b) and CCL-8 were measured in plasma
782 using Luminex assays (Bio-Rad, Berkeley, CA). CCL5 (RANTES), was measured in plasma as part of a
783 multi-analyte panel at Myriad Rules Based Medicine (Austin, TX).

784 Mouse in-vivo validation of trans-pQTLs

785 Plasma from transgenic- and matched control mice were randomised on a PCR plate. The samples
786 included five mice with targeted deletion of hepatocyte ABCA1[21] together with five matched control
787 mice, three mice with whole-body TRIB1[22] knockdown and three controls and four mice with liver-
788 specific knockdown of TRIB1 and four matched controls. Protein levels of stem cell factor (SCF) was
789 measured using the Olink PEA Mouse exploratory panel according to the manufacturer's instruction

790 (Olink Proteomics, Uppsala, Sweden). The plasma levels of SCF were normalised against average
791 protein concentrations using information on an additional 91 proteins. TRIB1 whole-body and liver-
792 specific mice were analysed jointly as were the respective wild-type controls. The median plasma
793 levels of SCF were compared using the Mann-Whitney U test for unpaired samples.

794 Mendelian randomization

795 To study the causal effects of the protein on selected disease outcomes, we performed two-sample
796 Mendelian randomization analyses. We used between-study heterogeneity to guide the instrumental
797 variable selection. In the presence of between-study heterogeneity (*P-het*<$9x10^{-5}$), variants had to
798 surpass a Bonferroni-corrected p-value threshold in discovery (*P*<$5.6x10^{-10}$) and show nominal
799 significance (P<0.05) in the replication studies (9,173 individuals), with directionally concordant beta
800 coefficients. In the absence of between-study heterogeneity we included variants showing
801 conventional genome-wide significance (*P*<$5x10^{-8}$) in a meta-analysis of the discovery and replication
802 datasets. From these, we created two sets of instrumental variables (IVs) for each of the 85 proteins
803 with variants reaching multiple testing-corrected significance in our discovery GWAS: (a) *cis* IVs
804 including one or more independent variants (LD r^2=0.001 within ±1Mb of the transcript boundaries of
805 the gene encoding the protein); and (b) *pan* IVs including all independent (LD r^2=0) variants
806 associated with the protein, i.e. combining *cis* and *trans* pQTLs. The per-allelic beta coefficients from
807 the main GWAS analyses were used as weights in the IVs. For the outcomes, we obtained the
808 relevant SNP-to-trait summary statistics from publicly-available GWAS as outcomes [Supplementary
809 Table 9]. When lead variants from our main GWAS were not available in these summary statistics, we
810 replaced them with proxies (LD r^2>0.85). For each individual SNP-protein and SNP-outcome
811 association, we generated an instrumental variable Wald ratio estimate, with standard errors
812 obtained using the delta method. When the instrument included more than one SNP, summary IV
813 estimates were generated by combining individual SNP Wald estimates by inverse-variance weighted
814 fixed-effect meta-analysis. We report associations with a Benjamini-Hochberg false discovery rate

(FDR) ≤ 5%, applied separately to summary estimates from *cis*-pQTL and *pan*-pQTL IVs, using pooled estimates for all 38 diseases. We graded the evidence of causality using a framework outlined in [Extended Figure 7], using the following categories: strong (*cis*-IV estimate FDR≤ 5%); intermediate (pan-IV estimate FDR≤ 5% with: (i) no heterogeneity between cis-IV estimate and pan-IV estimate; *and* (ii) no evidence of the MR estimate being unduly influenced by a *trans*-pQTL in leave-one-out analysis); or weak (pan-IV estimate FDR≤ 5% but: no *cis*-pQTL IV available; heterogeneity between *cis*- and all- IVs; or evidence of undue influence by a trans-pQTL). Heterogeneity between *pan*-IV and *cis*-IV estimates were calculated using Cochran's Q tests, with $P<0.05$ denoting evidence against the null hypothesis, and applying a Bonferroni adjustment for multiple testing. Mendelian randomization was conducted in duplicate by two separate analysts and analyses were performed in Stata (StataCorp, Texas, USA) version 13.3 using the *mrivests*, *metan* and *multproc* commands and R. Of the 2437 IV estimates derived using *cis*-pQTL instruments across the 85 proteins and 38 outcome traits, the IV estimates of 50 protein-to-disease associations met the FDR≤5% (corresponding to an uncorrected $P\leq 1.1\times10^{-3}$). Of the 3044 IV estimates composed using all pQTL instruments, 281 IV estimates met FDR≤ 5% (corresponding to $P\leq 4.7\times10^{-3}$; [Figure 4A]).

Heritability analyses

We estimated the total SNP-heritability (h_{SNP}^2) for the plasma level of each protein from the summary statistics of each individual GWAS by summing the contributions from two independent partitions of the SNPs: primary major loci and polygenic background. We defined the variance explained by primary major loci (major loci h_{SNP}^2) as the sum of the estimated variance explained ($2*\beta^2*f*(1-f)$), where f is the minor allele frequency, and owing to the fact that the phenotypic variance has been standardized across lead SNPs indexing all primary genome-wide significant loci. We used LDSC regression[44] to estimate the contribution of the polygenic background (polygenic h_{SNP}^2) for each protein, which we define as the contribution of all loci not indexed by a genome-wide significant lead SNP. LDSC regression is known to perform poorly when large effect, major genes are present, as it

was derived under the assumption of a simple polygenic genetic architecture[44]. To account for this and avoid double counting the variance explained by major loci through LD surrogates, prior to estimating the LDSC regression polygenic h_{SNP}^2, we censored all SNPs within 10 Mb of genome-wide significant lead SNPs for all primary loci.

Polygenic risk score calculation

Polygenic risk scores were derived using LDpred algorithm[45], which adjusts the effect of each SNP allele for those of other SNP alleles in linkage disequilibrium (LD) with it, and also takes into account the likelihood of a given allele to have a true effect according to a user-defined parameter, which we used as all 7 default LDpred-settings, with values from 1 through $1x10^{-5}$. The algorithm was directed to use HapMap3 SNPs that had a minor allele frequency >0.05, Hardy-Weinberg equilibrium P>1e-05 and imputation score >0.95. Variance explained in the independent MDC-study was tested according to a step-wise model, first including non-genetic covariates, then additional variability explained by adding SNPs from genome-wide significant SNPs (major loci $V.E._{PRS}$), and then additional variability explained by adding the 7 LDpred-derived scores as additional covariates (polygenic $V.E._{PRS}$).

ST2 polygenic risk score for asthma and inflammatory bowel disease in the UK biobank

Prior to analysis subjects who were not White British (based on self-reported ancestry in combination with genetic PCA) in the maximum unrelated subset were filtered out. All bi-allelic SNPs with MAF >= 1% and MaCH rsq >= 0.8 were kept. The Z-score transformed LDpred PRS (wt2) for ST2 was calculated as described for MDC in 337,484 White British UK Biobank participants. Association with asthma and IBD were tested using logistic regression adjusting for age, sex, PC1-10, genotype batch using either the continuous PRS or the PRS quantile-bins as predictors. The UK Biobank protocol has been described previously[46] and is available online (https://www.ukbiobank.ac.uk). The genotype quality control (QC), phasing, and imputation was performed centrally and has been previously described [47]. Outcomes (defined based on self-reported data at baseline and/or the

inpatient and death registry [including primary and secondary causes as well as prevalent and incident disease]) Asthma: Self-reported touchscreen (6152), self-reported nurse interview (20002), or ICD-10 "J45". Conflicting self-reported results set to missing unless "J45" was reported. Inflammatory bowel disease: nurse interview (20002) or ICD-10 K50-K52.

Meta-regression analysis for ST2 PRS, asthma and IBD

We estimated the per-quantile and per-SD associations of the weighted PRS for ST2 (MDC study) on risks of asthma and IBD (UK Biobank) by taking the quantile associations with ST2, asthma and IBD and conducting meta-regression analyses whereby the dependent variable was the quantile-specific logOR and corresponding SE of asthma or IBD and the independent variable was the quantile specific beta coeffient for ST2. This was conducted using the "metareg" package in STATA SE v13.1 (Statacorp, USA). Plots from the metaregression are presented in [Extended Figure 8].

Observational evidence

Observational evidence for the CVD-I proteins showing strong evidence of causality in Mendelian randomization was collated from literature or by de-novo analysis in the IMPROVE cohort [supplementary table 10]. To identify evidence from literature, we searched for the protein name or aliases in combination with the implicated trait trait/disease in PubMed. For clinical outcome traits, only those reported as “significant” by the paper were included, and the table provides the directional information provided. For quantitative outcome traits, standardised betas and p-values are reported.

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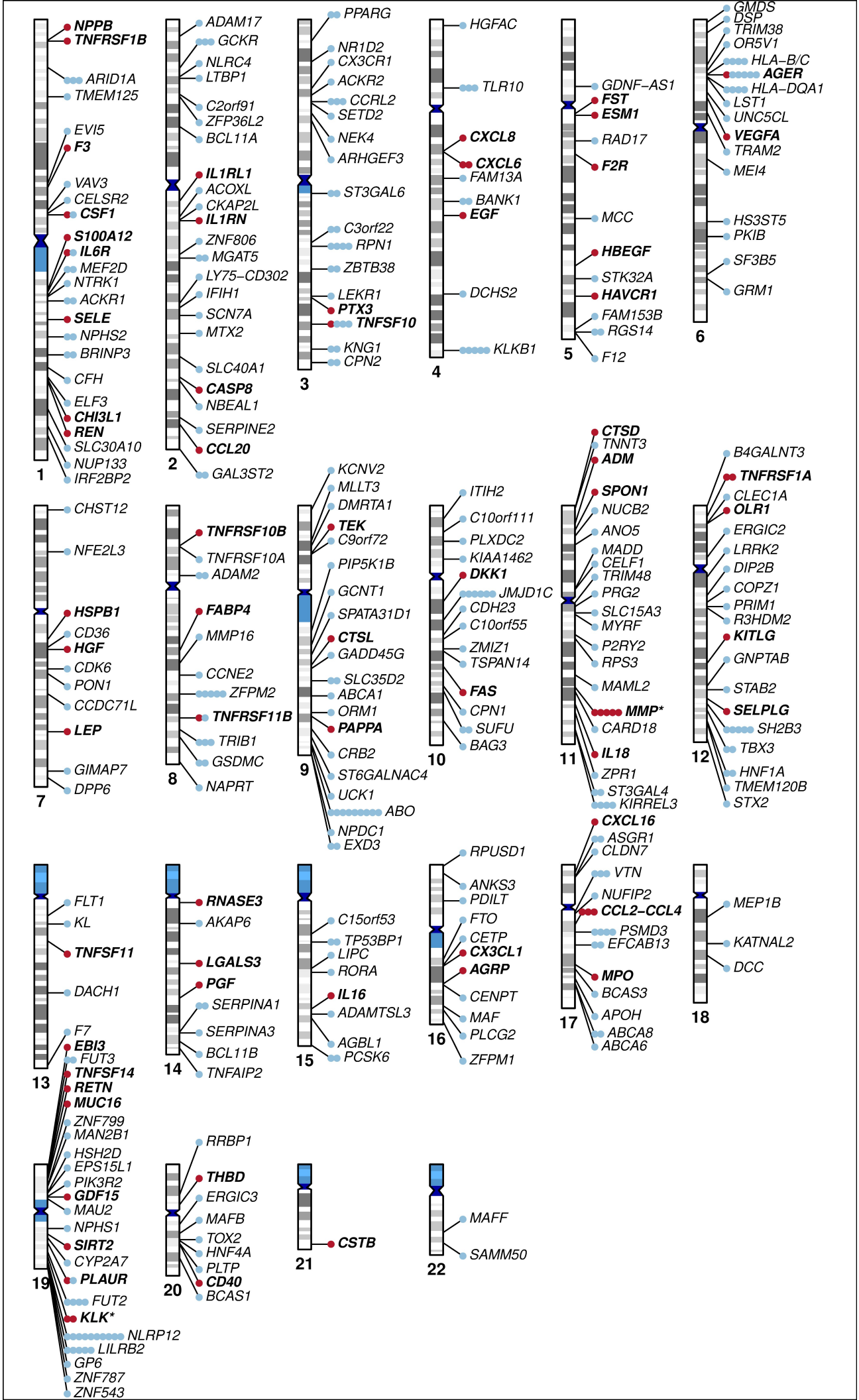
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KLKB1
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AGBL1
PCSK6
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ANKS3
PDILT
FTO
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CX3CL1
AGRP
CENPT
MAF
PLCG2
ZFPM1
16
CXCL16
ASGR1
CLDN7
VTN
NUFIP2
CCL2–CCL4
PSMD3
EFCAB13
MPO
BCAS3
APOH
ABCA8
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SAMM50
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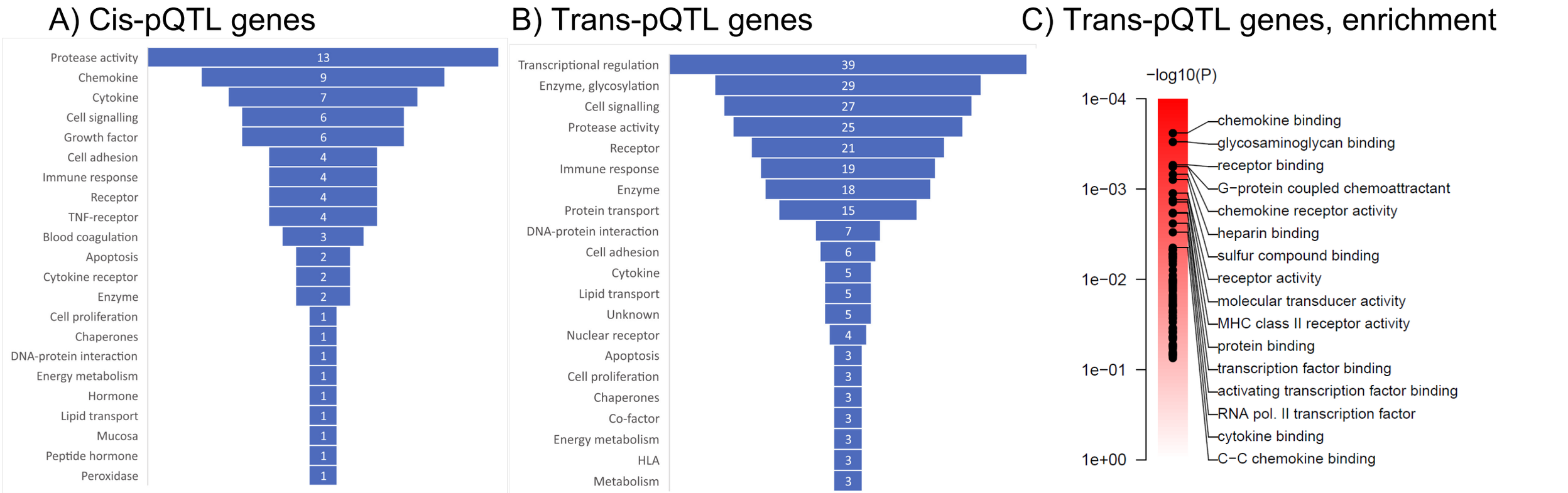

A) Cis-pQTL genes
Protease activity 13
Chemokine 9
Cytokine 7
Cell signalling 6
Growth factor 6
Cell adhesion 4
Immune response 4
Receptor 4
TNF-receptor 4
Blood coagulation 3
Apoptosis 2
Cytokine receptor 2
Enzyme 2
Cell proliferation 1
Chaperones 1
DNA-protein interaction 1
Energy metabolism 1
Hormone 1
Lipid transport 1
Mucosa 1
Peptide hormone 1
Peroxidase 1
B) Trans-pQTL genes
Transcriptional regulation 39
Enzyme, glycosylation 29
Cell signalling 27
Protease activity 25
Receptor 21
Immune response 19
Enzyme 18
Protein transport 15
DNA-protein interaction 7
Cell adhesion 6
Cytokine 5
Lipid transport 5
Unknown 5
Nuclear receptor 4
Apoptosis 3
Cell proliferation 3
Chaperones 3
Co-factor 3
Energy metabolism 3
HLA 3
Metabolism 3
C) Trans-pQTL genes, enrichment
−log10(P)
1e−04
1e−03
1e−02
1e−01
1e+00
chemokine binding
glycosaminoglycan binding
receptor binding
G−protein coupled chemoattractant
chemokine receptor activity
heparin binding
sulfur compound binding
receptor activity
molecular transducer activity
MHC class II receptor activity
protein binding
transcription factor binding
activating transcription factor binding
RNA pol. II transcription factor
cytokine binding
C−C chemokine binding

MCP-1

CCL4

Log2(fold-change)

4

2

0

-2

Placebo

PF-04634817

Placebo

PF-04634817

P=0.017

P=0.008

SCF protein level

0.25

0.20

0.15

0.10

WT

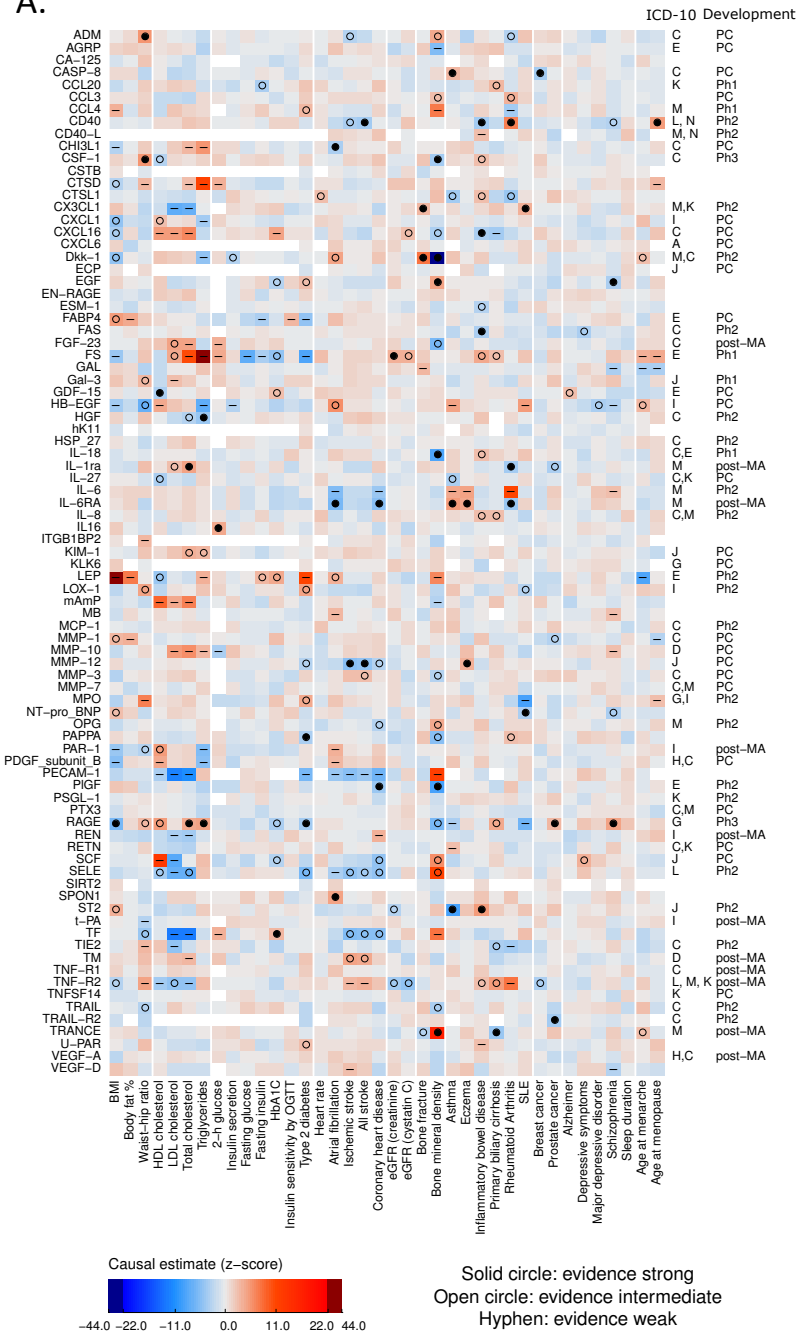
KO

ABCA1

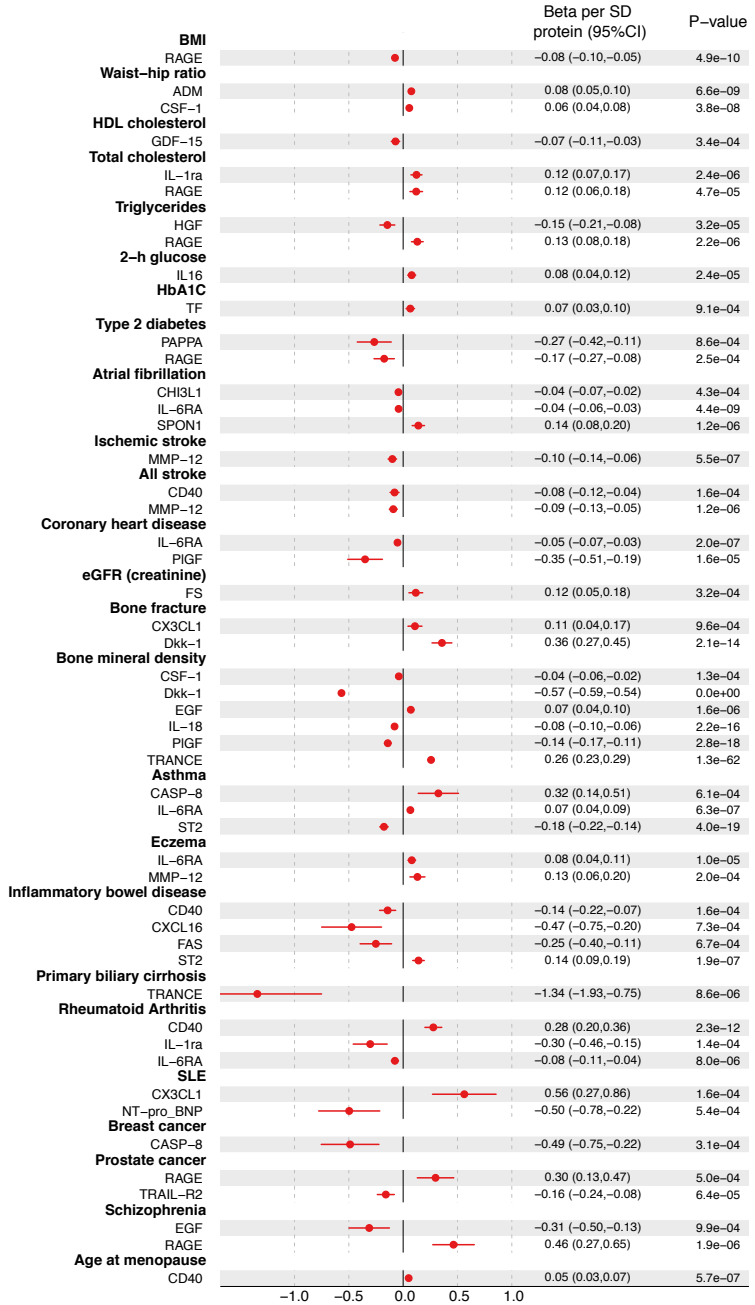
Trib1

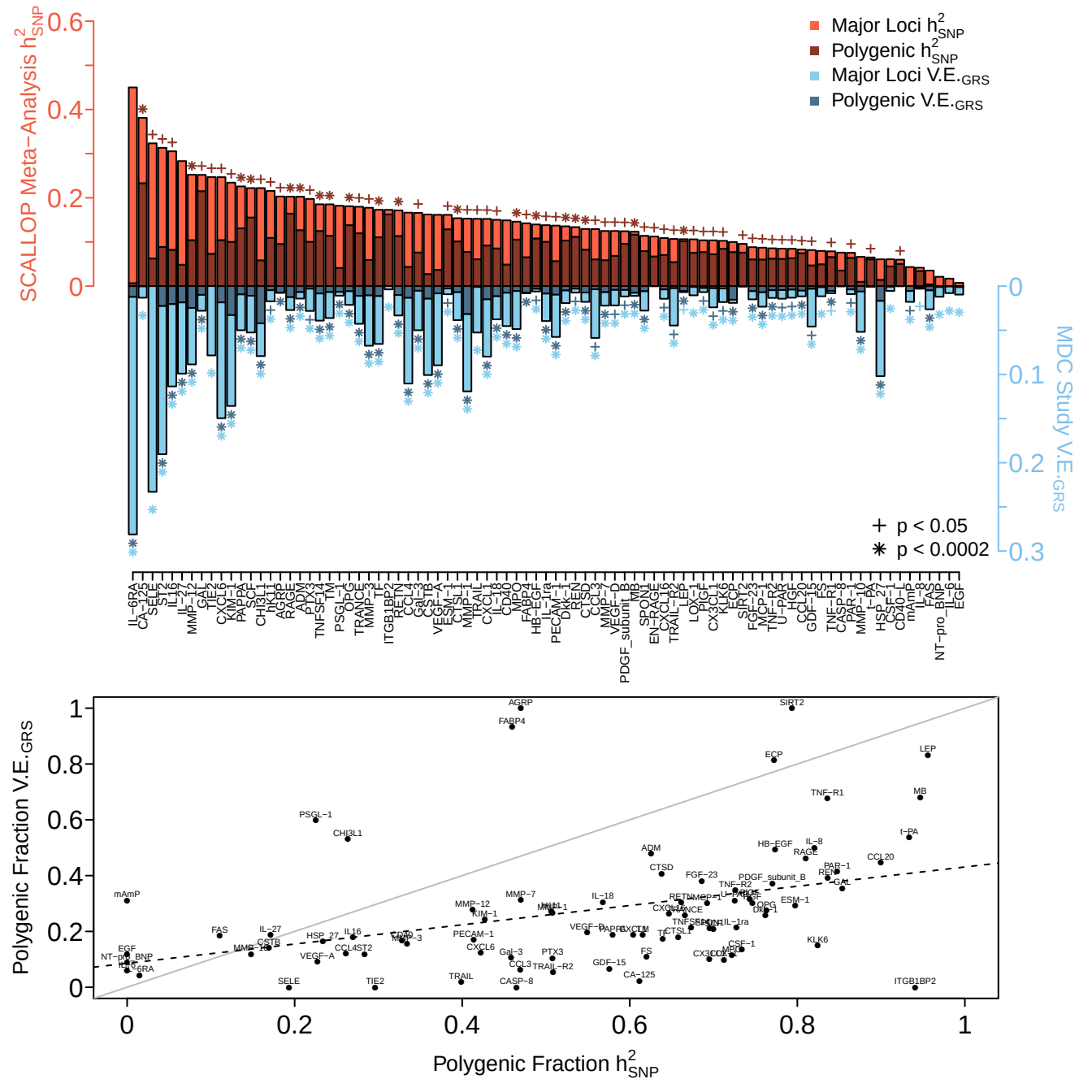
Mouse

A.



B. MR with strong evidence




Major Loci h^2_{SNP}
Polygenic h^2_{SNP}
Major Loci V.E.$_{GRS}$
Polygenic V.E.$_{GRS}$
SCALLOP Meta−Analysis h^2_{SNP}
MDC Study V.E.$_{GRS}$
+ p < 0.05
* p < 0.0002
IL−6RA
CA−125
SELE
ST2
IL−6
IL−12
MMP−12
GAL
TIE2
CXCL6
KIM−1
PAPPA
SCF
CHI3L1
hK11
AGRP
RAGE
ADM
PTX3
TNFSF14
TM
PSGL−1
OPG
TRANCE
MMP−3
TF
ITGB1BP2
RETN
Gal−3
CSTB
VEGF−A
ESM−1
CTSL1
MMP−7
TRAIL
CXCL1
IL−18
CD40
MPO
FABP4
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IL−1ra
PECAM−1
DKK−1
REN
CTSD
CCL3
MMP−7
VEGF−D
PDGF_subunit_B
MB
SPON1
EN−RAGE
CXCL16
TRAIL−R2
LEP
PAI−1
CX3CL1
KLK6
ECP
SIRT2
FGF−23
MCP−2
TNF−R2
U−PAR
HGF
CCL20
GDF−15
FS
TNF−R1
CA−19−9
PAR−1
MMP−10
t−PA
HSP_27
CSF−1
CD40−L
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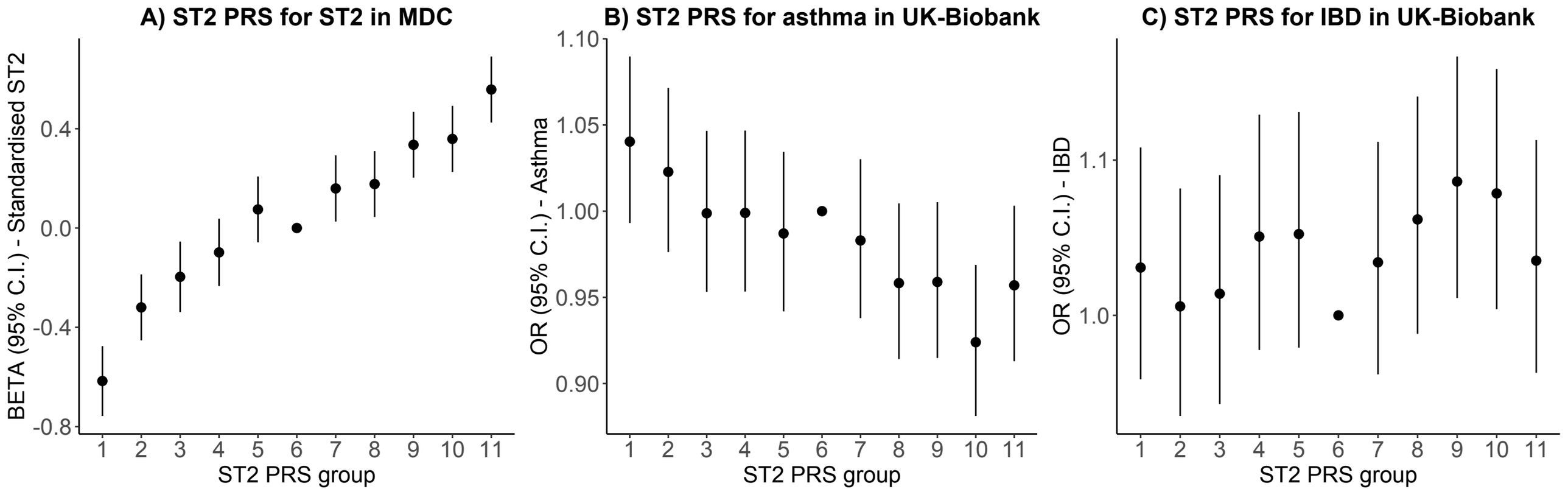
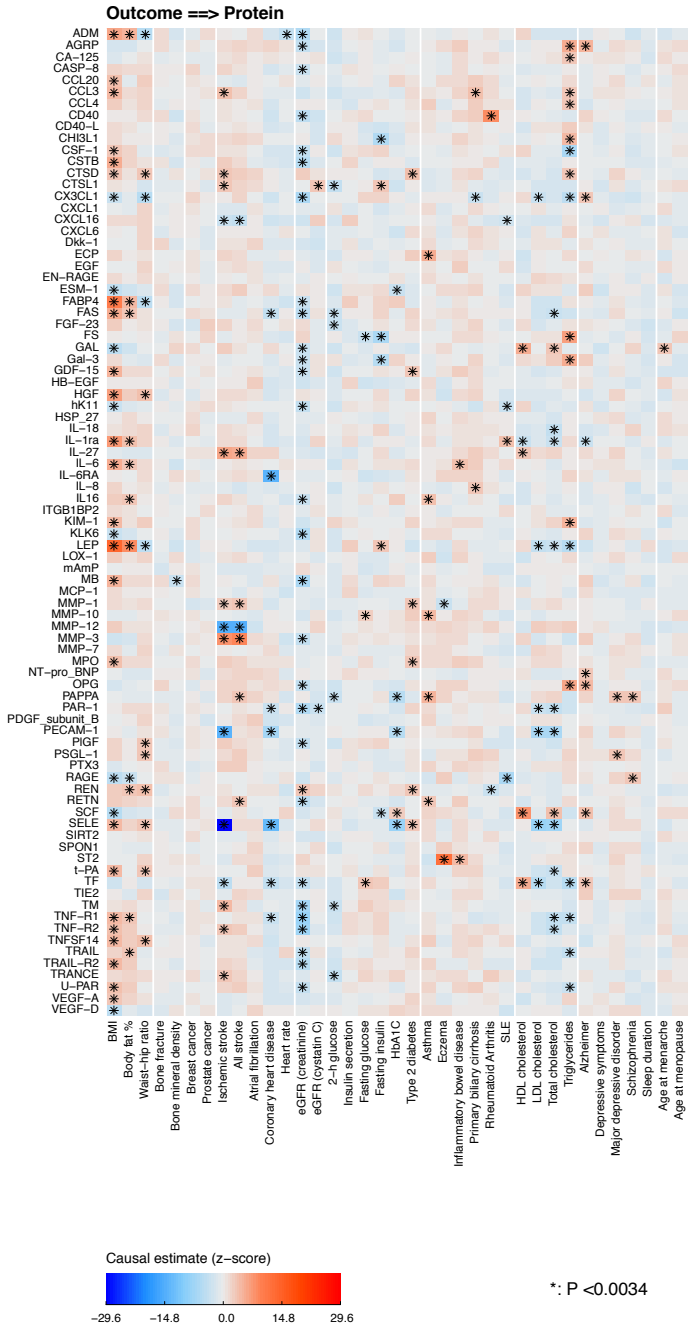
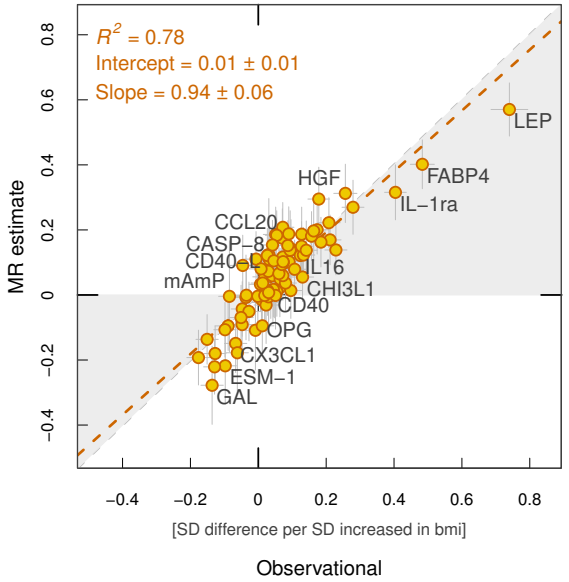
IL−8
FAS
NT−pro_BNP
IL−6
EGF
Polygenic Fraction V.E.$_{GRS}$
Polygenic Fraction h^2_{SNP}


A) ST2 PRS for ST2 in MDC
BETA (95% C.I.) - Standardised ST2
0.4
0.0
-0.4
-0.8
1 2 3 4 5 6 7 8 9 10 11
ST2 PRS group
B) ST2 PRS for asthma in UK-Biobank
OR (95% C.I.) - Asthma
1.10
1.05
1.00
0.95
0.90
1 2 3 4 5 6 7 8 9 10 11
ST2 PRS group
C) ST2 PRS for IBD in UK-Biobank
OR (95% C.I.) - IBD
1.1
1.0
1 2 3 4 5 6 7 8 9 10 11
ST2 PRS group

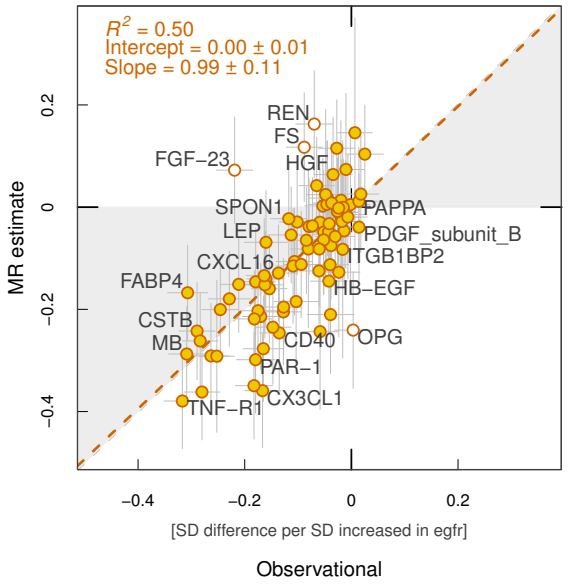
A. 37 traits to 85 proteins



B. BMI ==> proteins: MR vs Observational



C. eGFR ==> proteins: MR vs Observational



Target validation

CASP-8: breast cancer
CD40: IBD, RA
DKK1: eBMD
IL-1RA: RA
IL-6RA: RA, CHD
ST2: asthma
TRAIL-R2: prostate cancer
TRANCE: eBMD

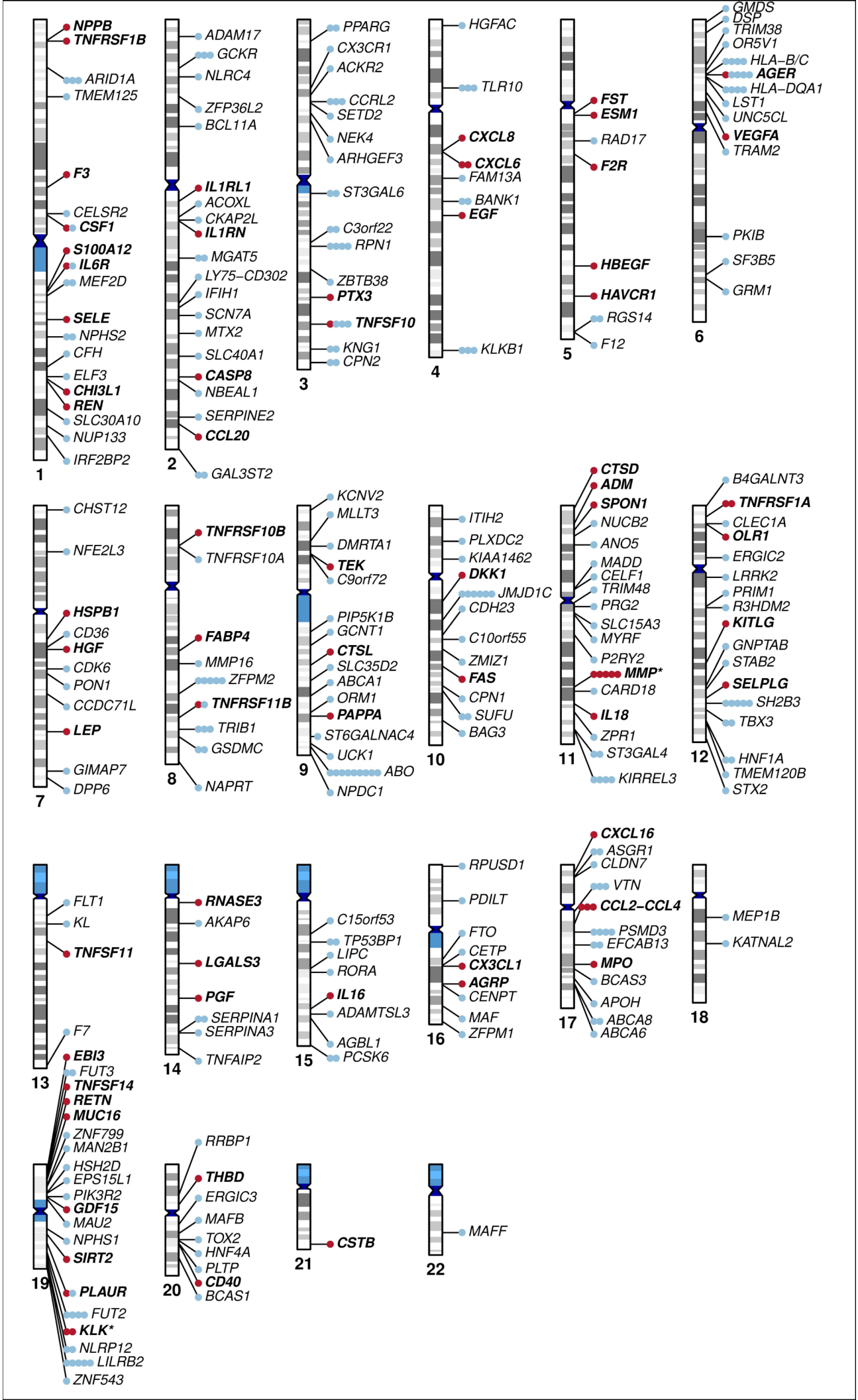
New target candidates

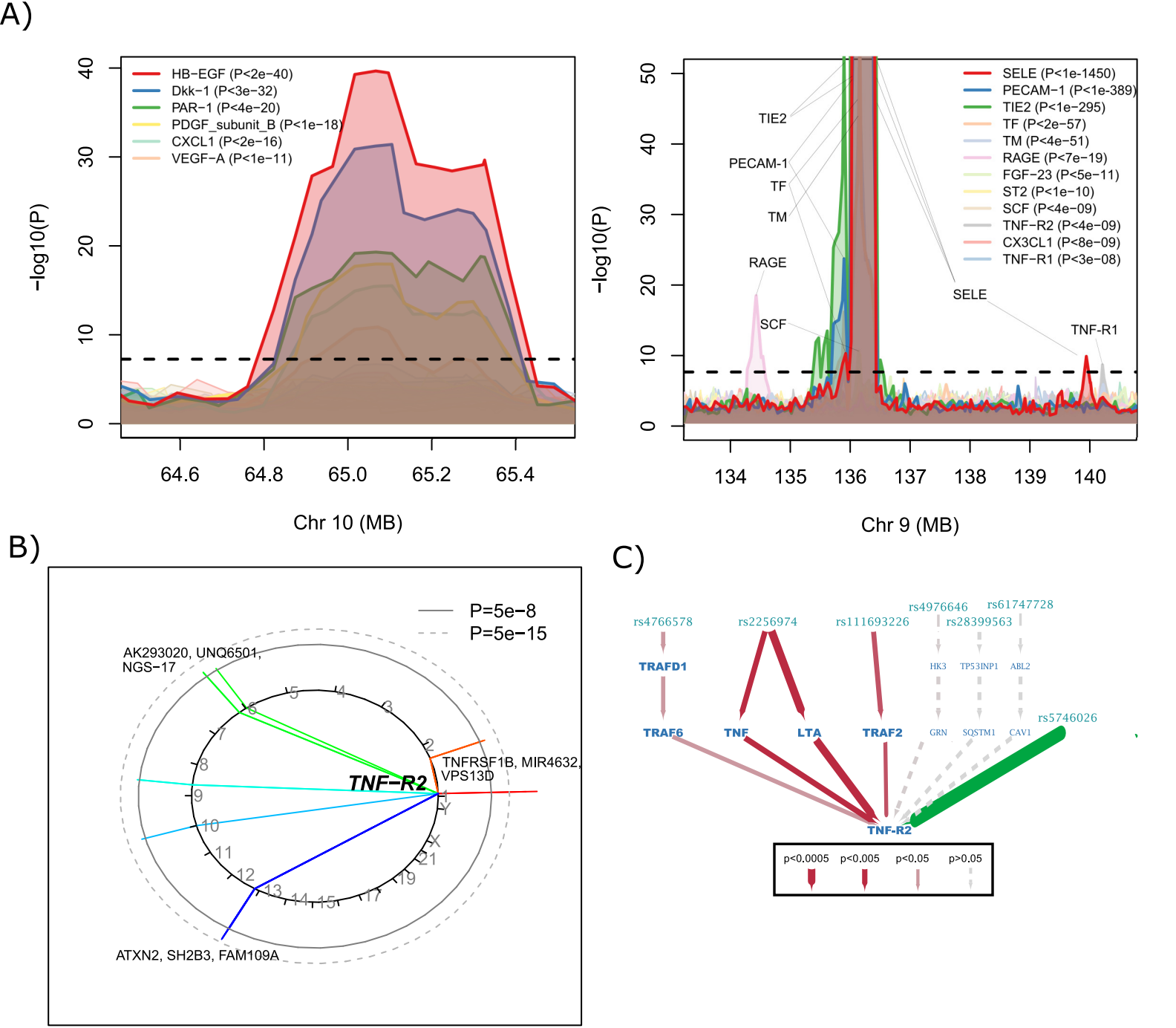
EGF: SCZ, eBMD
IL16: 2h glucose
PAPPA: T2D
SPON1: Afib
TF: HbA1c

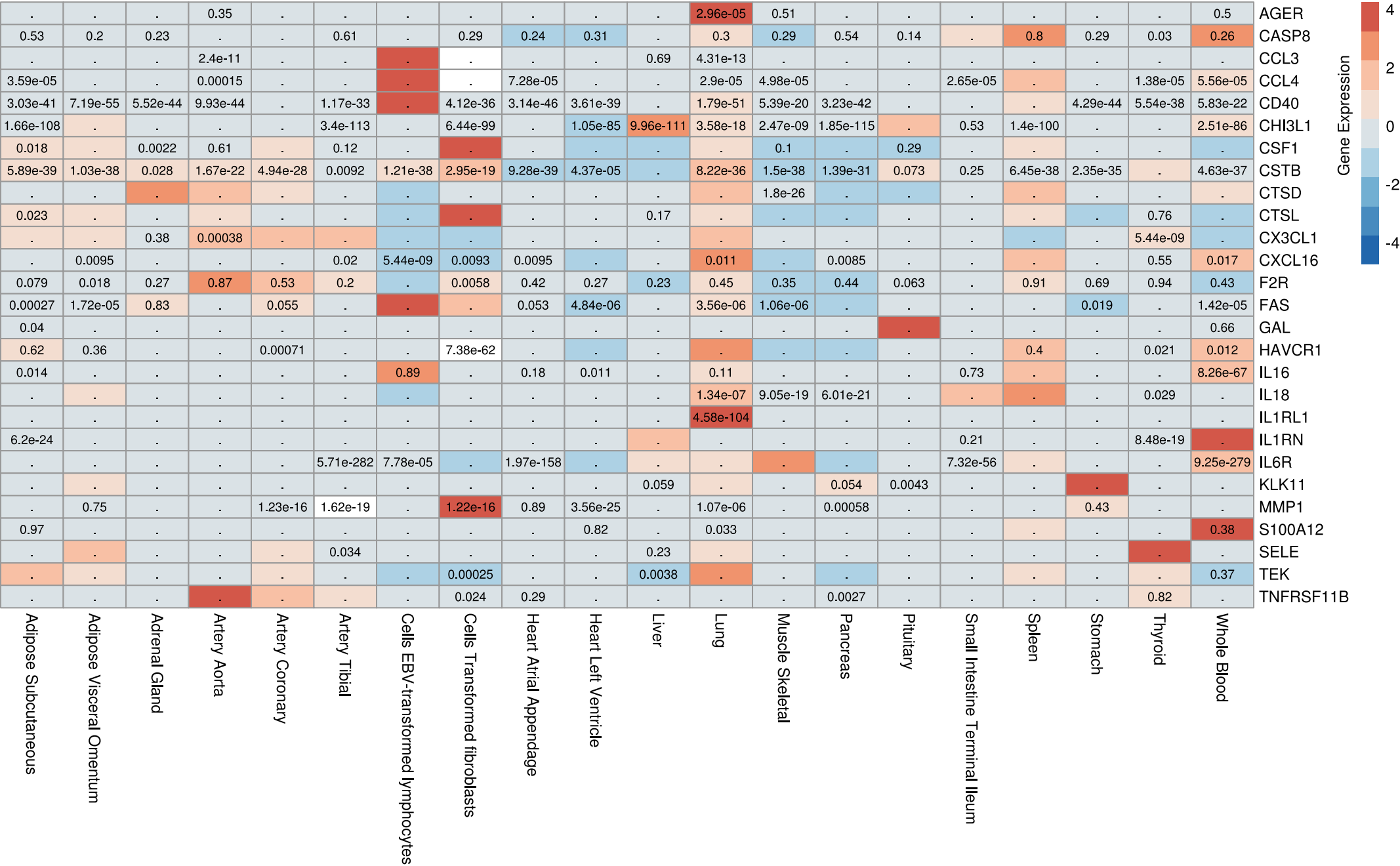
Repositioning & target-mediated safety

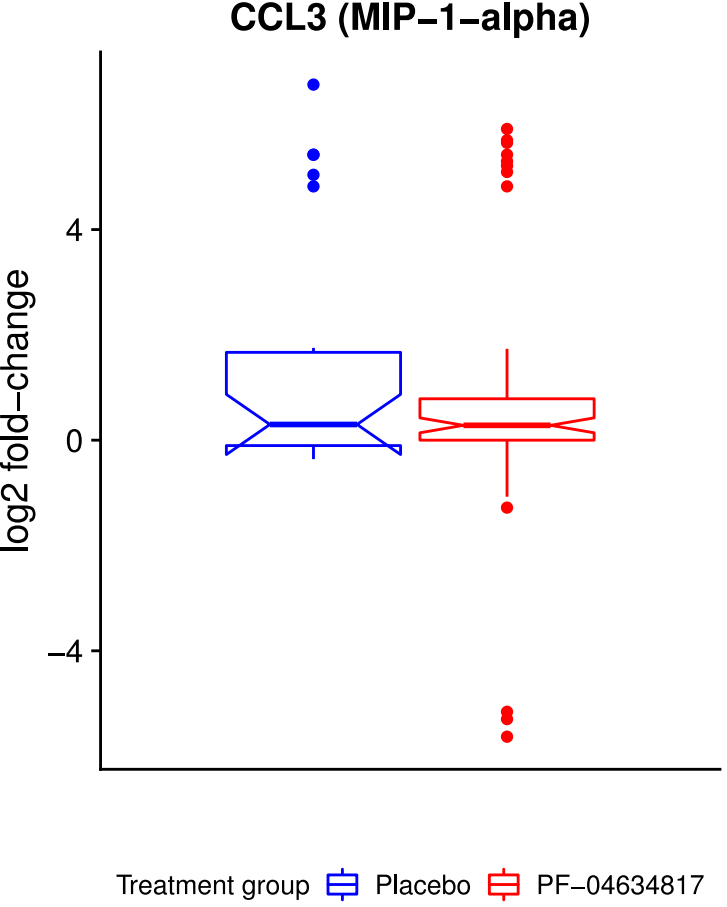
*(latter denoted by *)*

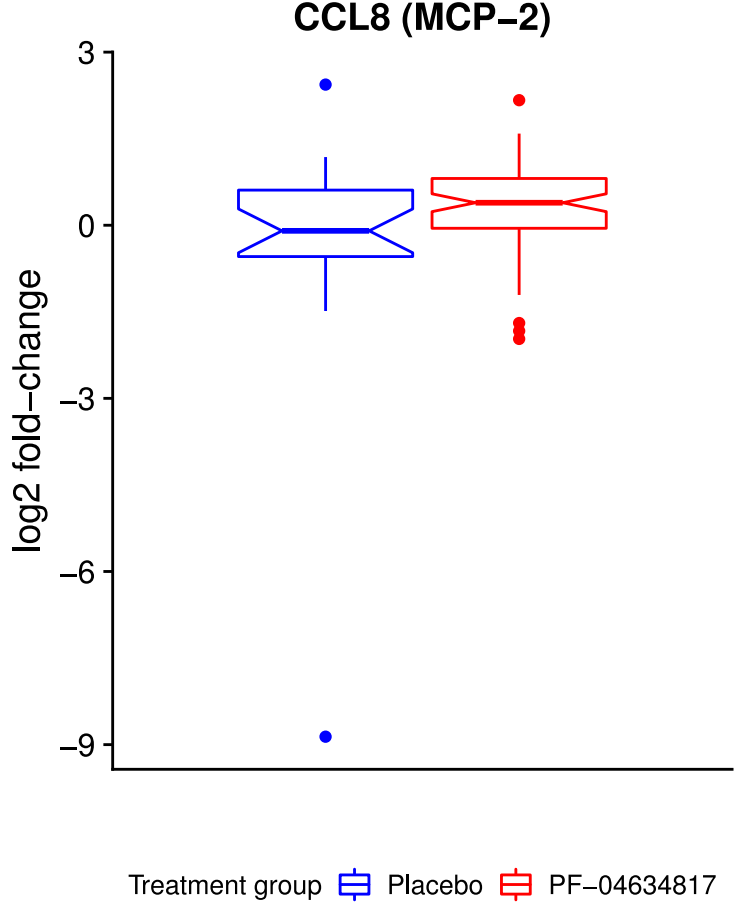
ADM: WHR
CASP-8: asthma*
CD40: stroke*
CHI3L1: AFib
CSF: WHR, eBMD
CX3CL1: fracture, SLE
CXCL16: IBD
FAS: IBD
GDF-15: HDL-C
HGF: TG
IL-1RA: total cholesterol*
IL-6RA: asthma, eczema*
IL-6RA: AFib
IL18: eBMD
MMP-12: eczema
PIGF: CHD, eBMD
RAGE: Lipids, BMI, T2D, prostate cancer, SCZ
ST2: IBD*

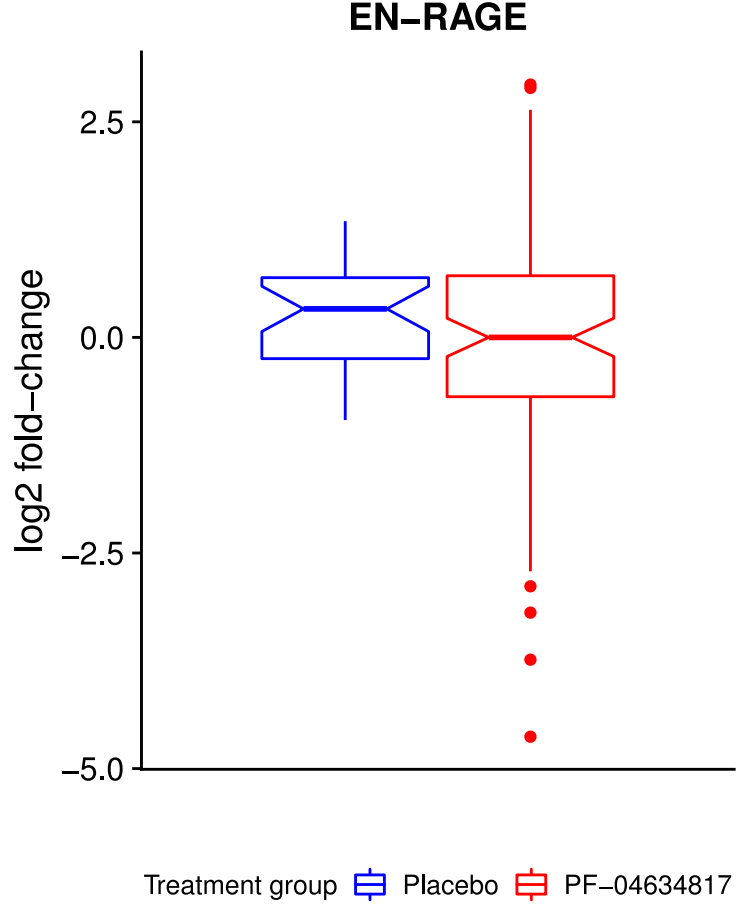

NPPB
TNFRSF1B
ARID1A
TMEM125
F3
CELSR2
CSF1
S100A12
IL6R
MEF2D
SELE
NPHS2
CFH
ELF3
CHI3L1
REN
SLC30A10
NUP133
IRF2BP2
1
ADAM17
GCKR
NLRC4
ZFP36L2
BCL11A
IL1RL1
ACOXL
CKAP2L
IL1RN
MGAT5
LY75–CD302
IFIH1
SCN7A
MTX2
SLC40A1
CASP8
NBEAL1
SERPINE2
CCL20
GAL3ST2
2
PPARG
CX3CR1
ACKR2
CCRL2
SETD2
NEK4
ARHGEF3
ST3GAL6
C3orf22
RPN1
ZBTB38
PTX3
TNFSF10
KNG1
CPN2
3
HGFAC
TLR10
CXCL8
CXCL6
FAM13A
BANK1
EGF
KLKB1
4
FST
ESM1
RAD17
F2R
HBEGF
HAVCR1
RGS14
F12
5
GMDS
DSP
TRIM38
OR5V1
HLA–B/C
AGER
HLA–DQA1
LST1
UNC5CL
VEGFA
TRAM2
PKIB
SF3B5
GRM1
6
CHST12
NFE2L3
HSPB1
CD36
HGF
CDK6
PON1
CCDC71L
LEP
GIMAP7
DPP6
7
TNFRSF10B
TNFRSF10A
FABP4
MMP16
ZFPM2
TNFRSF11B
TRIB1
GSDMC
NAPRT
8
KCNV2
MLLT3
DMRTA1
TEK
C9orf72
PIP5K1B
GCNT1
CTSL
SLC35D2
ABCA1
ORM1
PAPPA
ST6GALNAC4
UCK1
ABO
NPDC1
9
ITIH2
PLXDC2
KIAA1462
DKK1
JMJD1C
CDH23
C10orf55
ZMIZ1
FAS
CPN1
SUFU
BAG3
10
CTSD
ADM
SPON1
NUCB2
ANO5
MADD
CELF1
TRIM48
PRG2
SLC15A3
MYRF
P2RY2
MMP*
CARD18
IL18
ZPR1
ST3GAL4
KIRREL3
11
B4GALNT3
TNFRSF1A
CLEC1A
OLR1
ERGIC2
LRRK2
PRIM1
R3HDM2
KITLG
GNPTAB
STAB2
SELPLG
SH2B3
TBX3
HNF1A
TMEM120B
STX2
12
FLT1
KL
TNFSF11
F7
EBI3
FUT3
TNFSF14
RETN
MUC16
ZNF799
MAN2B1
HSH2D
EPS15L1
PIK3R2
GDF15
MAU2
NPHS1
SIRT2
PLAUR
FUT2
KLK*
NLRP12
LILRB2
ZNF543
13
RNASE3
AKAP6
LGALS3
PGF
SERPINA1
SERPINA3
TNFAIP2
14
C15orf53
TP53BP1
LIPC
RORA
IL16
ADAMTSL3
AGBL1
PCSK6
15
RPUSD1
PDILT
FTO
CETP
CX3CL1
AGRP
CENPT
MAF
ZFPM1
16
CXCL16
ASGR1
CLDN7
VTN
CCL2–CCL4
PSMD3
EFCAB13
MPO
BCAS3
APOH
ABCA8
ABCA6
17
MEP1B
KATNAL2
18
RRBP1
THBD
ERGIC3
MAFB
TOX2
HNF4A
PLTP
CD40
BCAS1
20
CSTB
21
MAFF
22

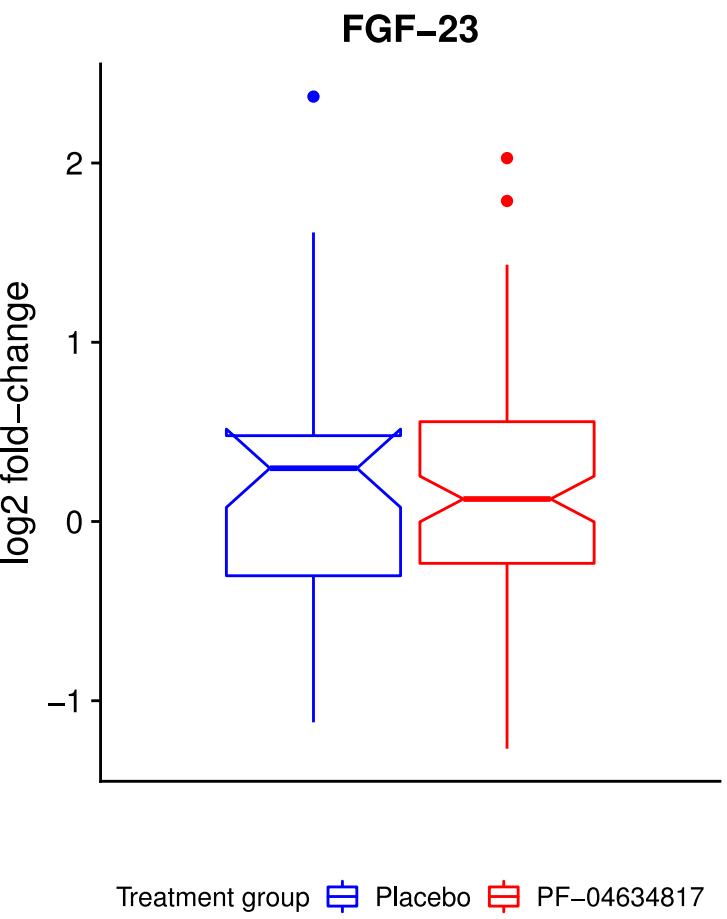

A)
HB−EGF (P<2e−40)
Dkk−1 (P<3e−32)
PAR−1 (P<4e−20)
PDGF_subunit_B (P<1e−18)
CXCL1 (P<2e−16)
VEGF−A (P<1e−11)
−log10(P)
Chr 10 (MB)
SELE (P<1e-1450)
PECAM−1 (P<1e-389)
TIE2 (P<1e−295)
TF (P<2e−57)
TM (P<4e−51)
RAGE (P<7e−19)
FGF−23 (P<5e−11)
ST2 (P<1e−10)
SCF (P<4e−09)
TNF−R2 (P<4e−09)
CX3CL1 (P<8e−09)
TNF−R1 (P<3e−08)
TIE2
PECAM−1
TF
TM
RAGE
SCF
SELE
TNF−R1
Chr 9 (MB)
B)
P=5e−8
P=5e−15
AK293020, UNQ6501, NGS−17
TNF−R2
TNFRSF1B, MIR4632, VPS13D
ATXN2, SH2B3, FAM109A
C)
rs4766578
rs2256974
rs111693226
rs4976646
rs61747728
rs28399563
TRAFD1
HK3
TP53INP1
ABL2
TRAF6
TNF
LTA
TRAF2
GRN
SQSTM1
CAV1
rs5746026
TNF-R2
p<0.0005
p<0.005
p<0.05
p>0.05

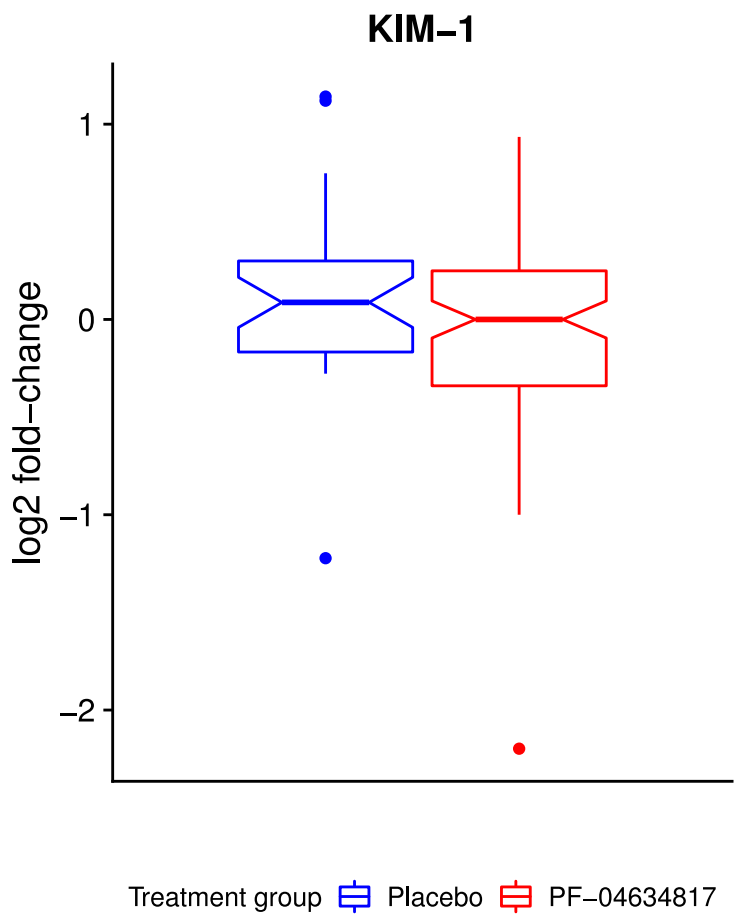

Gene Expression
4
2
0
-2
-4
AGER
CASP8
CCL3
CCL4
CD40
CHI3L1
CSF1
CSTB
CTSD
CTSL
CX3CL1
CXCL16
F2R
FAS
GAL
HAVCR1
IL16
IL18
IL1RL1
IL1RN
IL6R
KLK11
MMP1
S100A12
SELE
TEK
TNFRSF11B
Adipose Subcutaneous
Adipose Visceral Omentum
Adrenal Gland
Artery Aorta
Artery Coronary
Artery Tibial
Cells EBV-transformed lymphocytes
Cells Transformed fibroblasts
Heart Atrial Appendage
Heart Left Ventricle
Liver
Lung
Muscle Skeletal
Pancreas
Pituitary
Small Intestine Terminal Ileum
Spleen
Stomach
Thyroid
Whole Blood

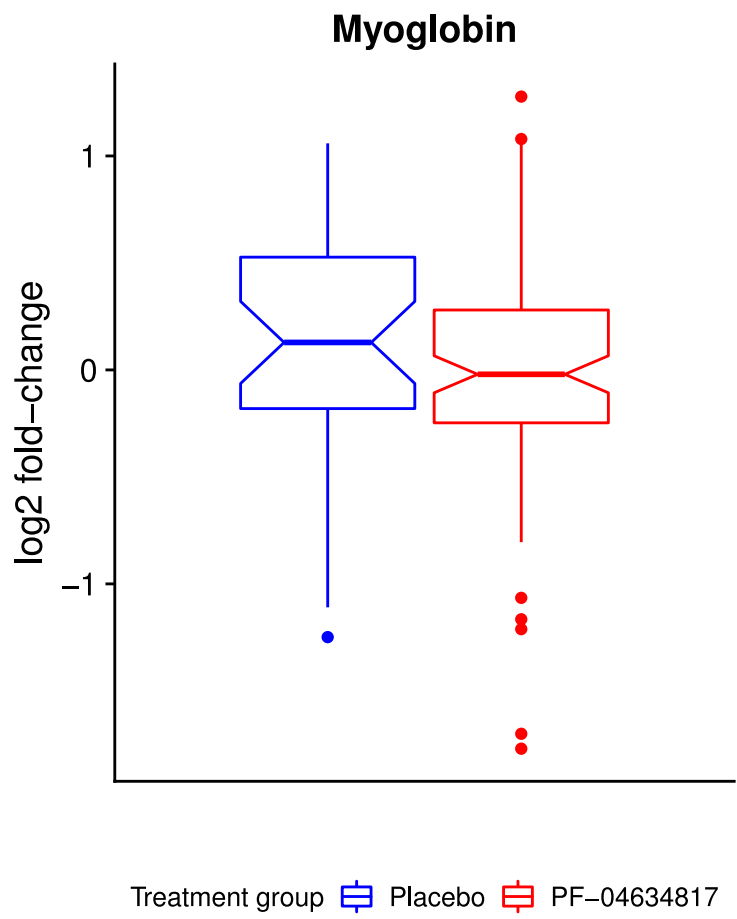

CCL3 (MIP−1−alpha)
log2 fold−change
4
0
−4
Treatment group
Placebo
PF−04634817

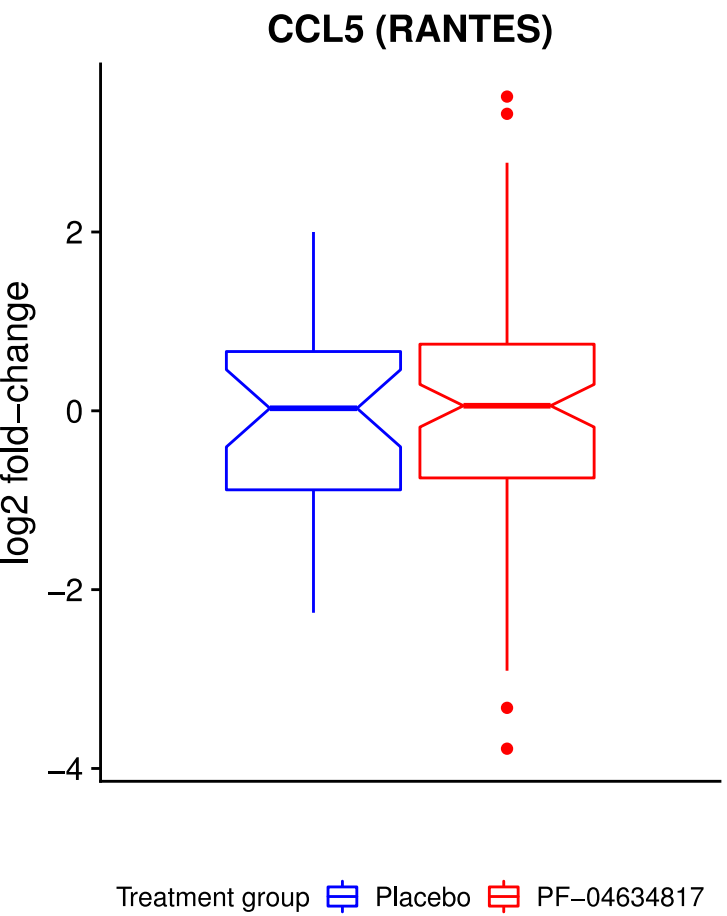

CCL8 (MCP−2)
log2 fold−change
3
0
−3
−6
−9
Treatment group
Placebo
PF−04634817

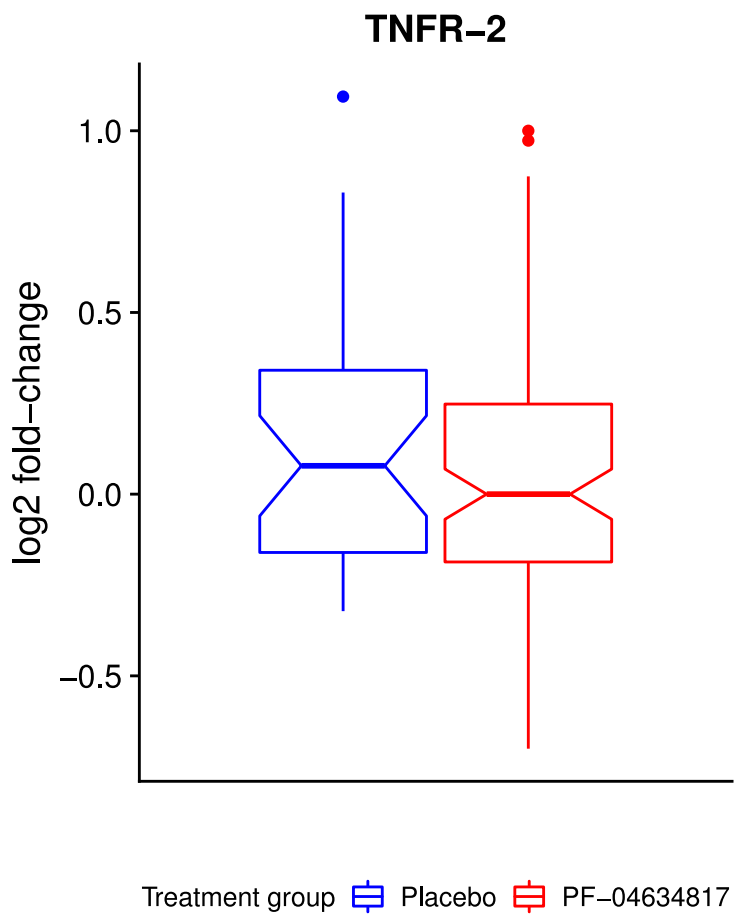

EN−RAGE
log2 fold−change
2.5
0.0
−2.5
−5.0
Treatment group
Placebo
PF−04634817


FGF−23
log2 fold−change
2
1
0
−1
Treatment group
Placebo
PF−04634817

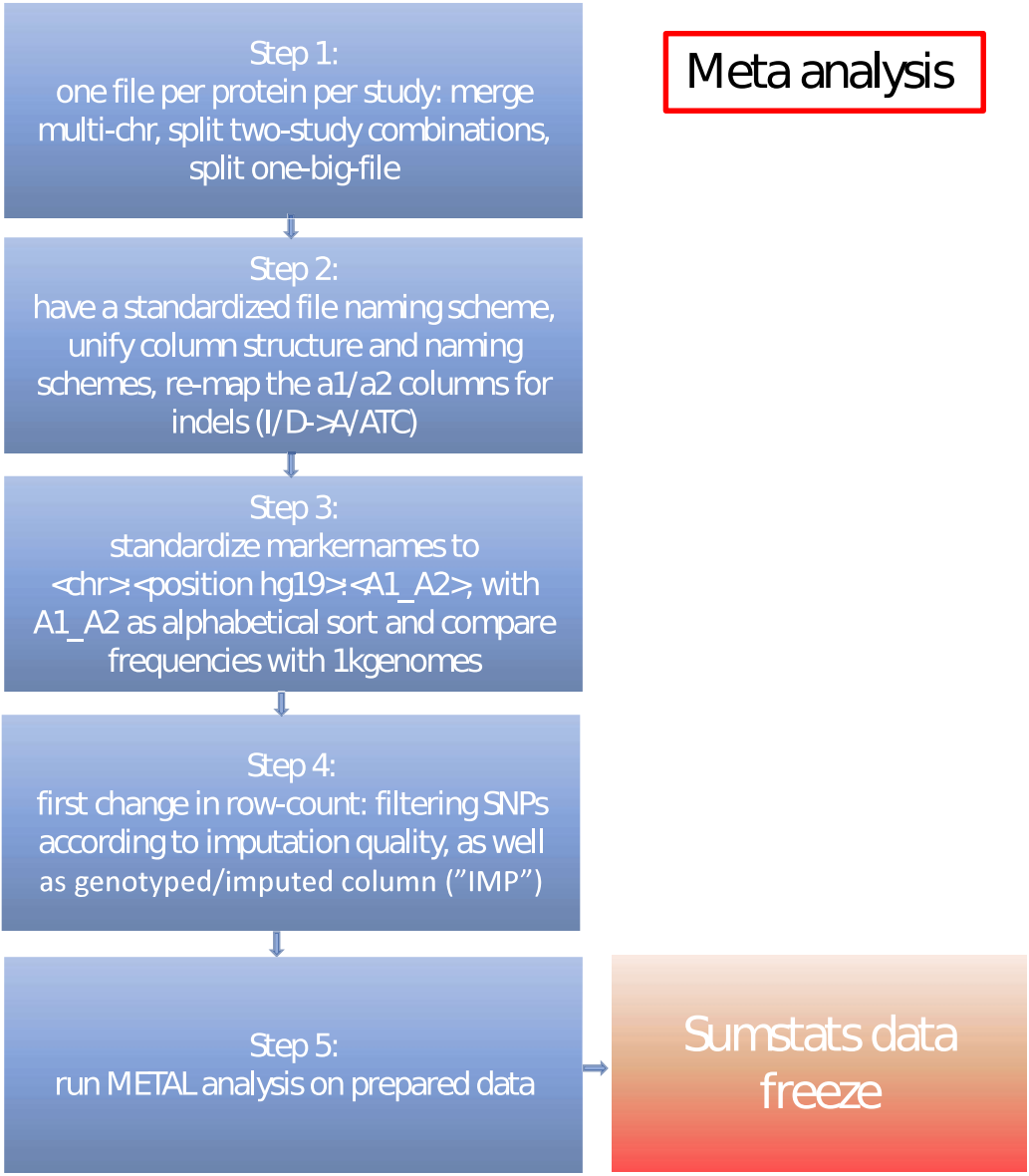

KIM−1
log2 fold−change
1
0
−1
−2
Treatment group
Placebo
PF−04634817

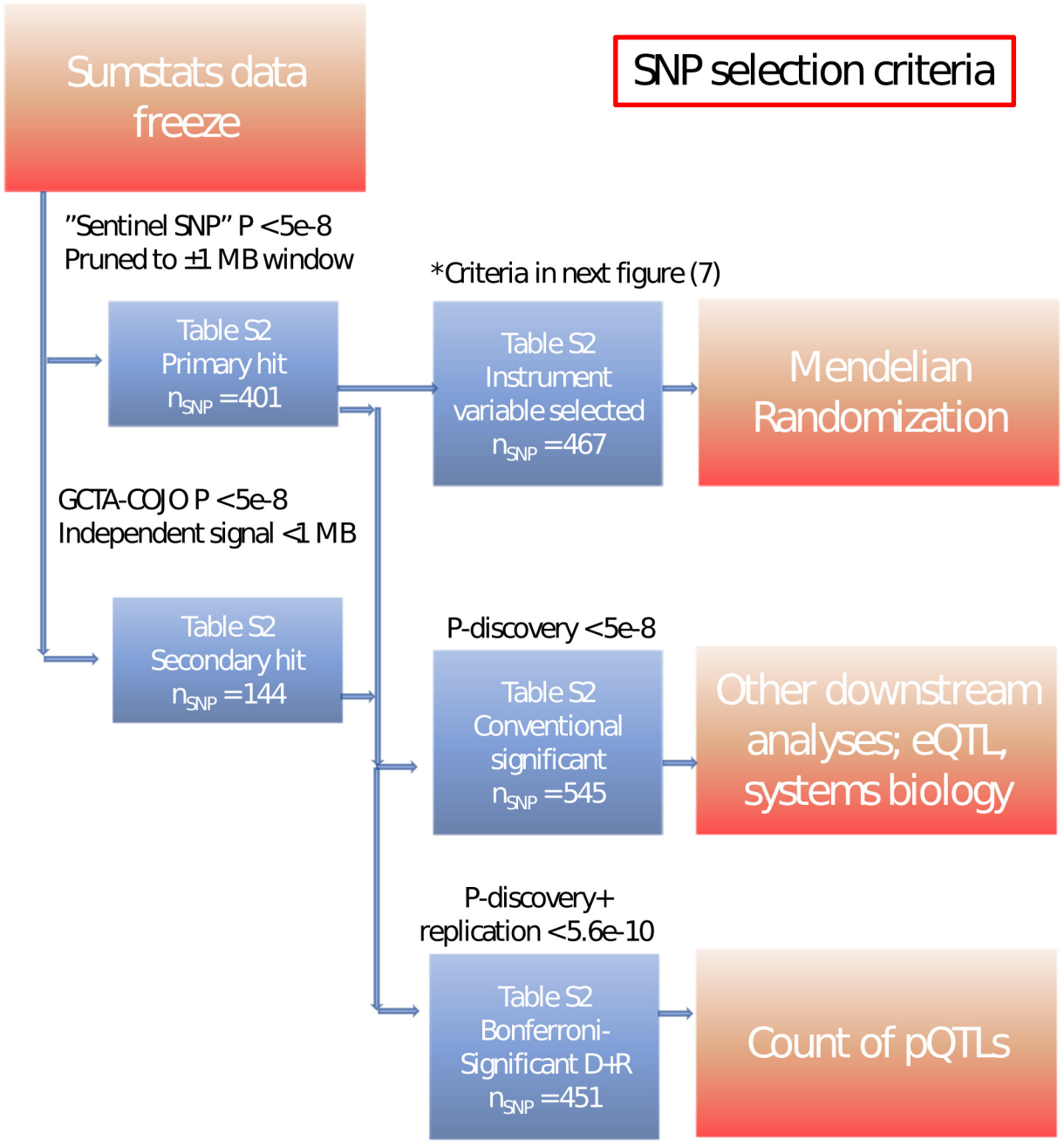

Myoglobin
log2 fold−change
1
0
−1
Treatment group
Placebo
PF−04634817


CCL5 (RANTES)
log2 fold−change
2
0
−2
−4
Treatment group
Placebo
PF−04634817

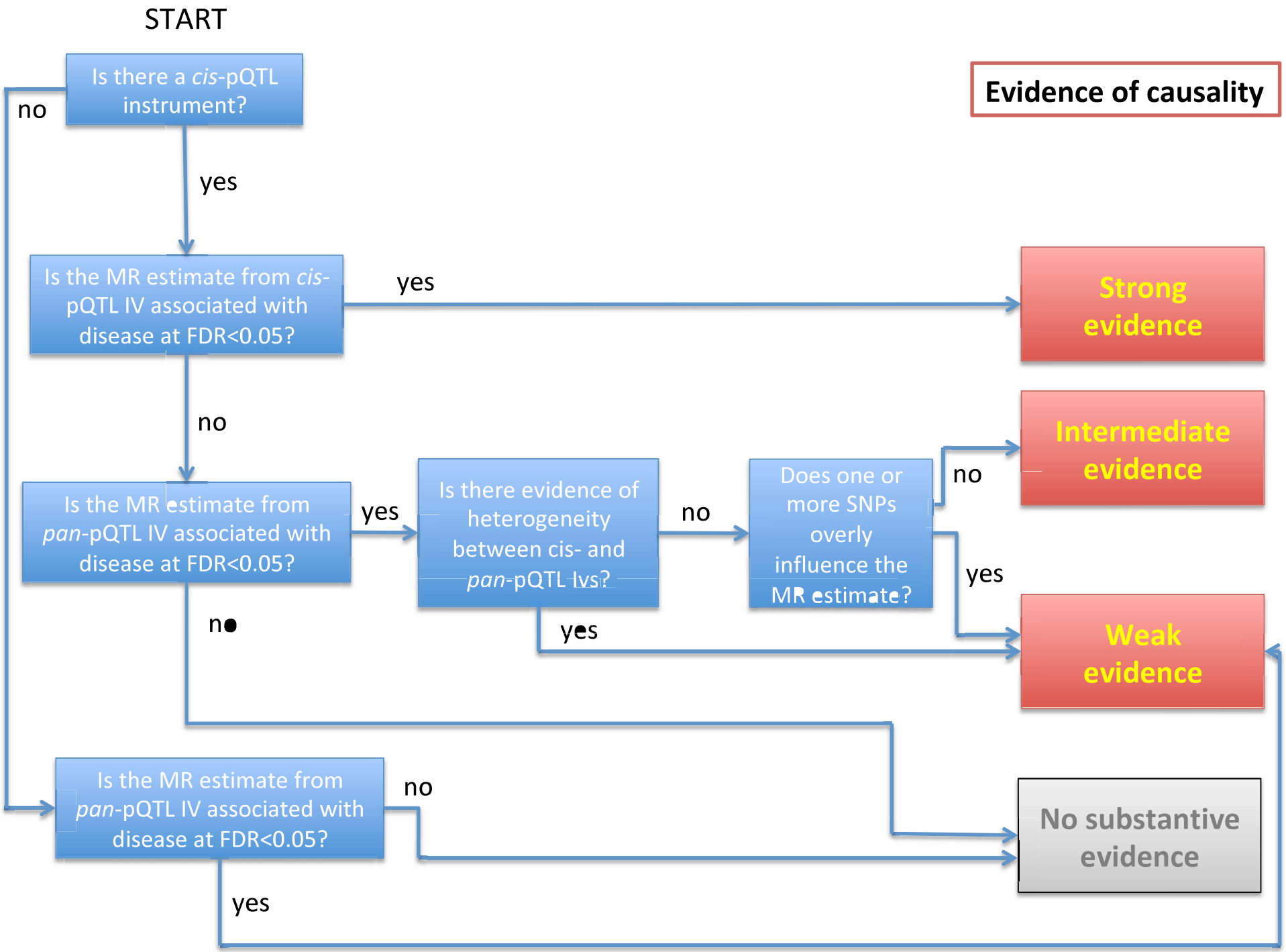

TNFR−2
log2 fold−change
1.0
0.5
0.0
−0.5
Treatment group
Placebo
PF−04634817

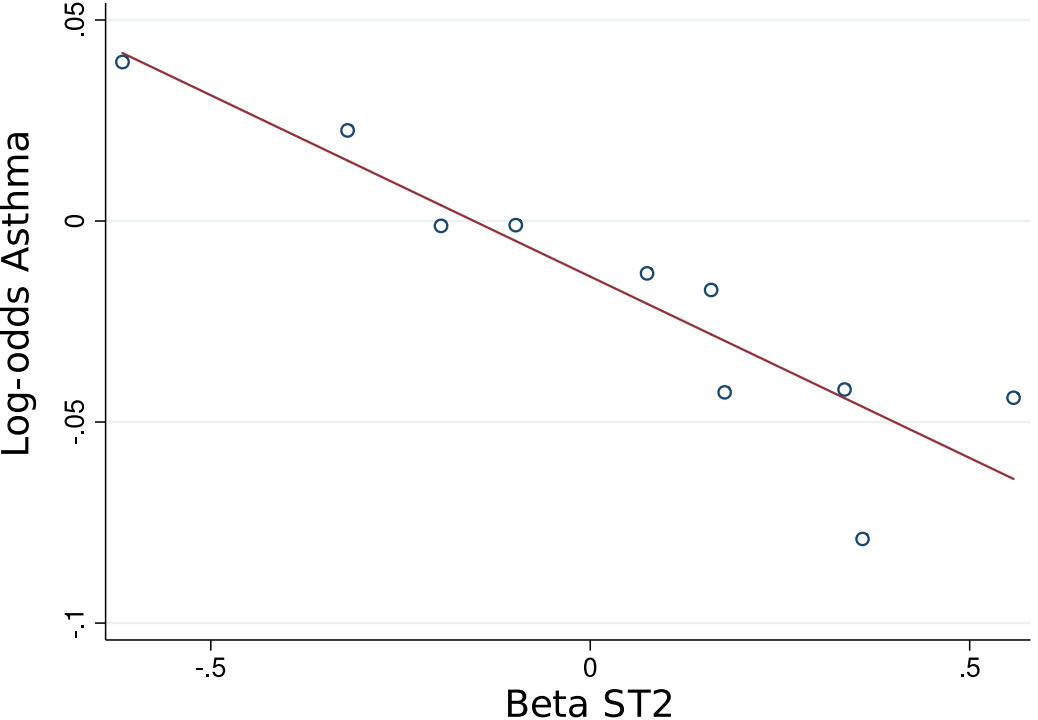
A)

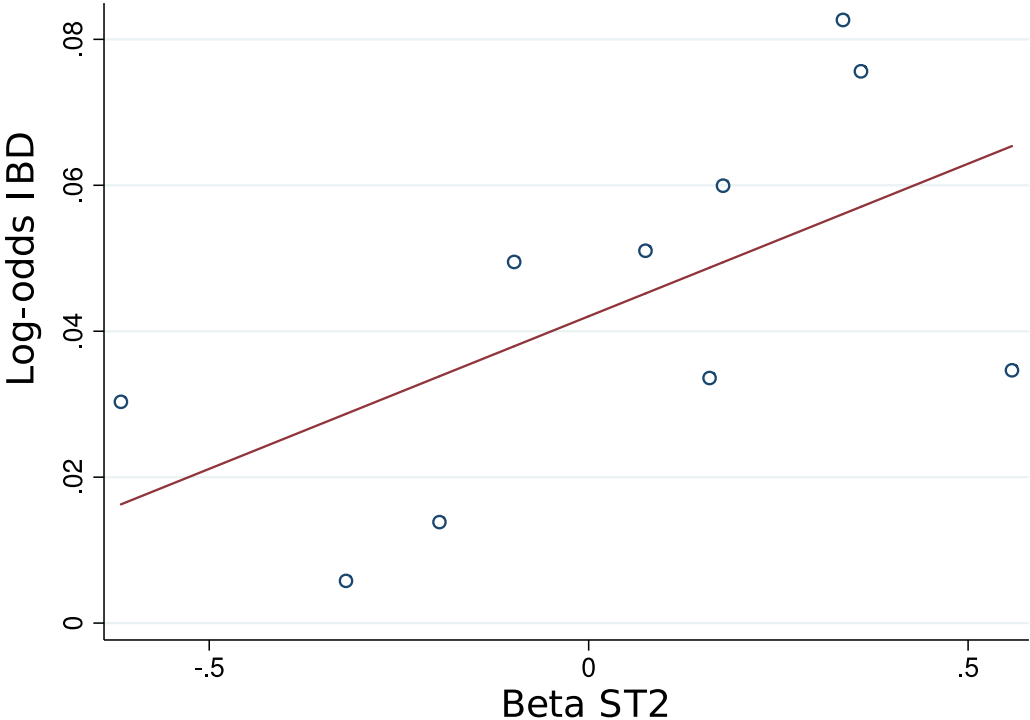

Step 1:
one file per protein per study: merge multi-chr, split two-study combinations, split one-big-file
Step 2:
have a standardized file naming scheme, unify column structure and naming schemes, re-map the a1/a2 columns for indels (I/D->A/ATC)
Step 3:
standardize markernames to <chr>:<position hg19>:<A1_A2>, with A1_A2 as alphabetical sort and compare frequencies with 1kgenomes
Step 4:
first change in row-count: filtering SNPs according to imputation quality, as well as genotyped/imputed column (”IMP”)
Step 5:
run METAL analysis on prepared data
Meta analysis
Sumstats data freeze

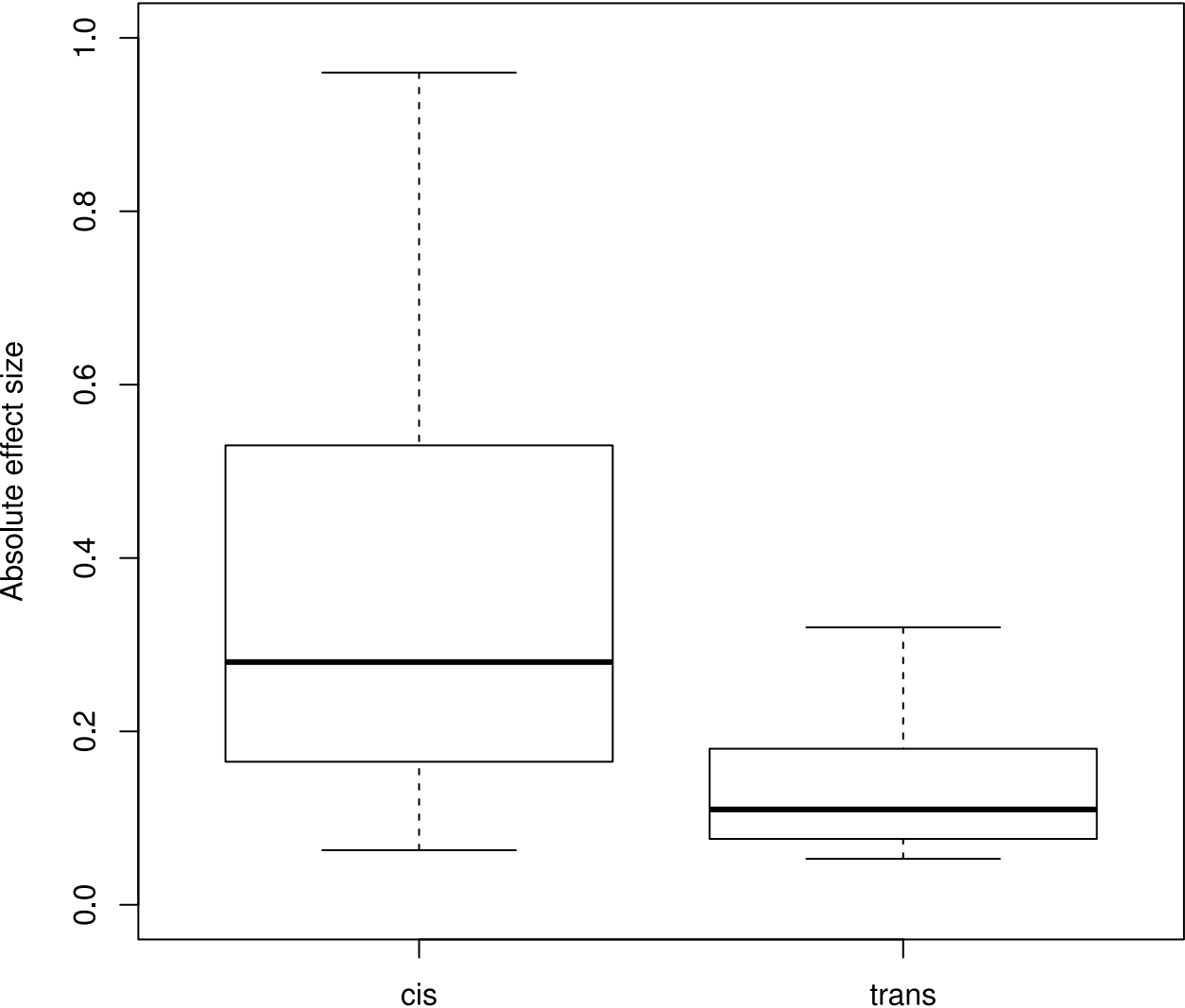

Sumstats data freeze
SNP selection criteria
"Sentinel SNP" P <5e-8
Pruned to ±1 MB window
*Criteria in next figure (7)
Table S2
Primary hit
n_{SNP} =401
Table S2
Instrument variable selected
n_{SNP} =467
Mendelian Randomization
GCTA-COJO P <5e-8
Independent signal <1 MB
Table S2
Secondary hit
n_{SNP} =144
P-discovery <5e-8
Table S2
Conventional significant
n_{SNP} =545
Other downstream analyses; eQTL, systems biology
P-discovery+ replication <5.6e-10
Table S2
Bonferroni-Significant D+R
n_{SNP} =451
Count of pQTLs

c)


START
Is there a *cis*-pQTL instrument?
no
yes
Is the MR estimate from *cis*-pQTL IV associated with disease at FDR<0.05?
yes
Strong evidence
no
Is the MR estimate from *pan*-pQTL IV associated with disease at FDR<0.05?
yes
Is there evidence of heterogeneity between cis- and *pan*-pQTL Ivs?
no
Does one or more SNPs overly influence the MR estimate?
no
Intermediate evidence
yes
Weak evidence
yes
no
Is the MR estimate from *pan*-pQTL IV associated with disease at FDR<0.05?
no
No substantive evidence
yes
Evidence of causality


Log-odds Asthma
.05
0
-.05
-.1
-.5
0
.5
Beta ST2


Log-odds IBD
.08
.06
.04
.02
0
-.5
0
.5
Beta ST2


Absolute effect size
0.0
0.2
0.4
0.6
0.8
1.0
cis
trans