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Supplemental information

CASCADE: high-throughput characterization of regulatory complex binding altered by non-coding variants

David Bray, Heather Hook, Rose Zhao, Jessica L. Keenan, Ashley Penvose, Yemi Osayame, Nima Mohaghegh, Xiaoting Chen, Sreeja Parameswaran, Leah C. Kottyan, Matthew T. Weirauch, and Trevor Siggers

Table S6. Antibodies used for experiments, Related to Figures 2, 3, 4, and 5. The antibodies listed were used for Western Blots, PBM experiments or both.

Antibody	Catalog Number	Application
Primary Antibodies		
P300	ab14984	PBM Experiment/Western Blot
SMARCA4	sc17796	PBM Experiment/Western Blot
GCN5	sc-365321x	PBM Experiment/Western Blot
RBBP5	a300-109A	PBM Experiment/Western Blot
TBLX1R1	sc-100908	PBM Experiment
HDAC1	ab7028	PBM Experiment
MED1	a300-793a	PBM Experiment
BRD4	a3910-985A50	PBM Experiment
P65	sc-372X	PBM Experiment
P65	sc-8008	Western Blot
IRF8	sc-6058X	PBM Experiment
IRF3	D83B9	PBM Experiment
IRF2	sc-374327	PBM Experiment
PU.1	sc-352X	PBM Experiment
Secondary Antibodies		
Donkey anti-goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	A11055	PBM Experiment
Goat anti-mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	A11029	PBM Experiment
Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	A11034	PBM Experiment
Goat anti-mouse IgG (H+L) Highly	A32728	PBM Experiment

Cross-Adsorbed Secondary Antibody, Alexa Fluor 647		
Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	A32733	PBM Experiment
HRP conjugated Goat anti-mouse	G-21234	Western Blot
HRP conjugated Goat anti-rabbit	G-21040	Western Blot

Figure S1. Model-based inference of transcription factors associated with CXCL10 promoter cofactor recruitment motifs, Related to Figure 2. (**a**) TF motifs matched to p300 recruitment preferences in LPS-stimulated macrophages (Replicate 1). (**b**) TF motifs matched to p300 recruitment preferences in LPS-stimulated macrophages (Replicate 2). (**c**) TF motif

matched to RBBP5 recruitment preferences in LPS-stimulated macrophages (**d**) TF motif matched to RBBP5 recruitment preferences in untreated macrophages. All COF recruitment preference tracks were converted to probability-based models (see Methods) prior to comparison. Similarity comparisons to known TF binding models was performed using TOMTOM and the full HOCOMOCOv11 motif database (771 total motifs – see Methods). COF: transcriptional cofactor, TF: transcription factor, ISRE: Interferon Stimulated Response Element, NF-kB: Nuclear Factor kappa light chain enhancer of activated B cells, LPS: Lipopolysaccharide, UT: Untreated.

Figure S2. Additional CASCADE-based analyses of transcription factor binding to the CXCL10 promoter segment, Related to Figure 2. Nucleotide binding preferences of IRF8 to the CXCL10 promoter segment in paired LPS-stimulated (track 11 - continued from Figure 2) and untreated (track 12) macrophages. Binding preferences of IRF3 (track 13) and p65 (track 14) in untreated macrophages. ISRE: Interferon Stimulated Response Element, NF-kB: Nuclear Factor kappa light chain enhancer of activated B cells, LPS: Lipopolysaccharide, UT: Untreated.

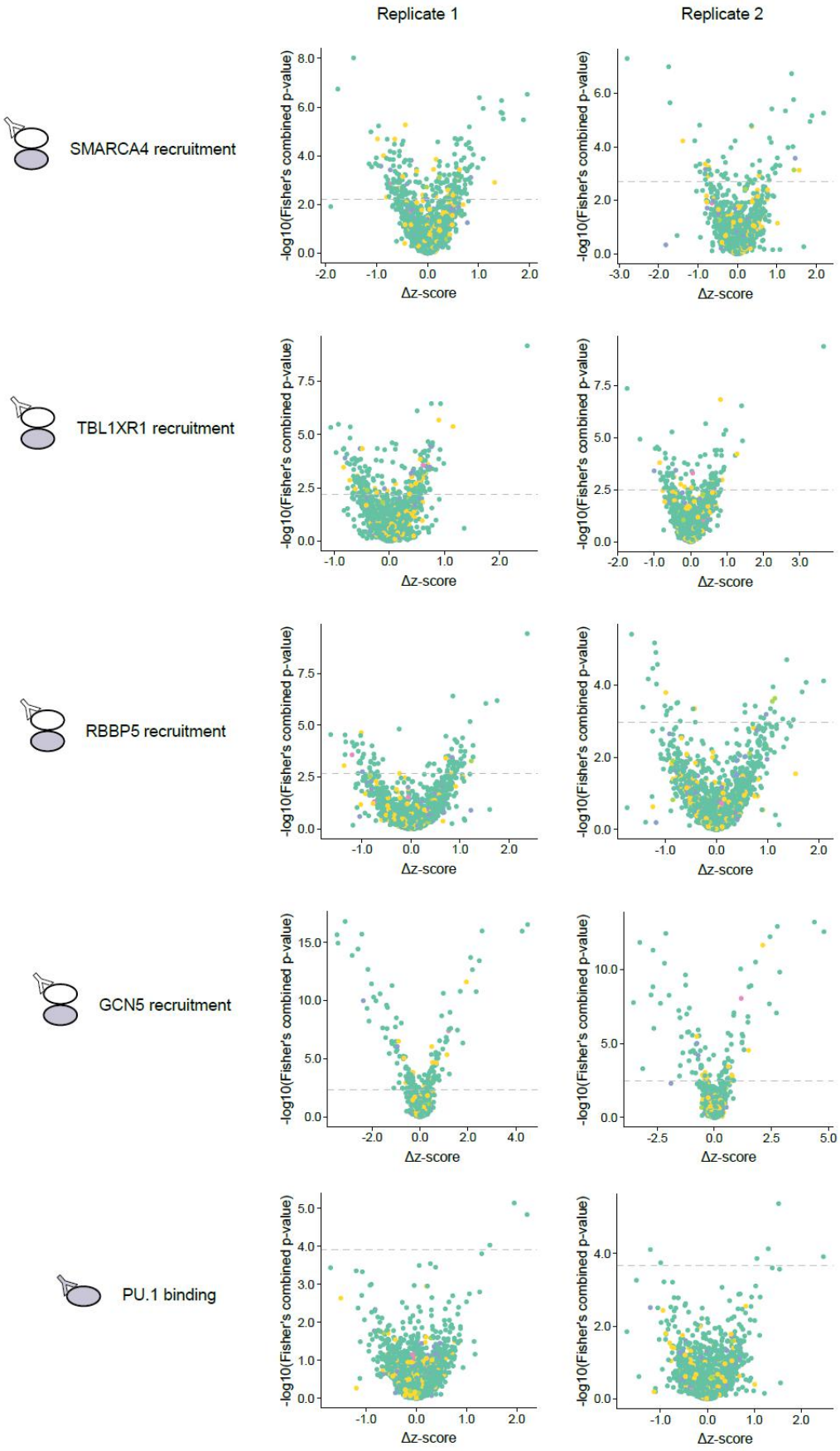
Replicate 1
Replicate 2
SMARCA4 recruitment
TBL1XR1 recruitment
RBBP5 recruitment
GCN5 recruitment
PU.1 binding
-log10(Fisher's combined p-value)
Δz-score

Figure S3. Statistical significance and direction-of-effect for changes in cofactor recruitment and transcription factor binding across reference and SNP probe pairs screened, Related to Figure 3. Rows represent volcano plots obtained for different COFs (SMARCA4, TBL1XR1, RBBP5, GCN5) and TF PU.1. Left column shows the volcano plots obtained in a first replicate and the right column shows the volcano plots obtained in a technical replicate experiment. Statistical significance threshold for each experiment ($q < 0.05$, see Methods) is shown as a grey dashed line. COF: transcriptional cofactor, TF: transcription factor.

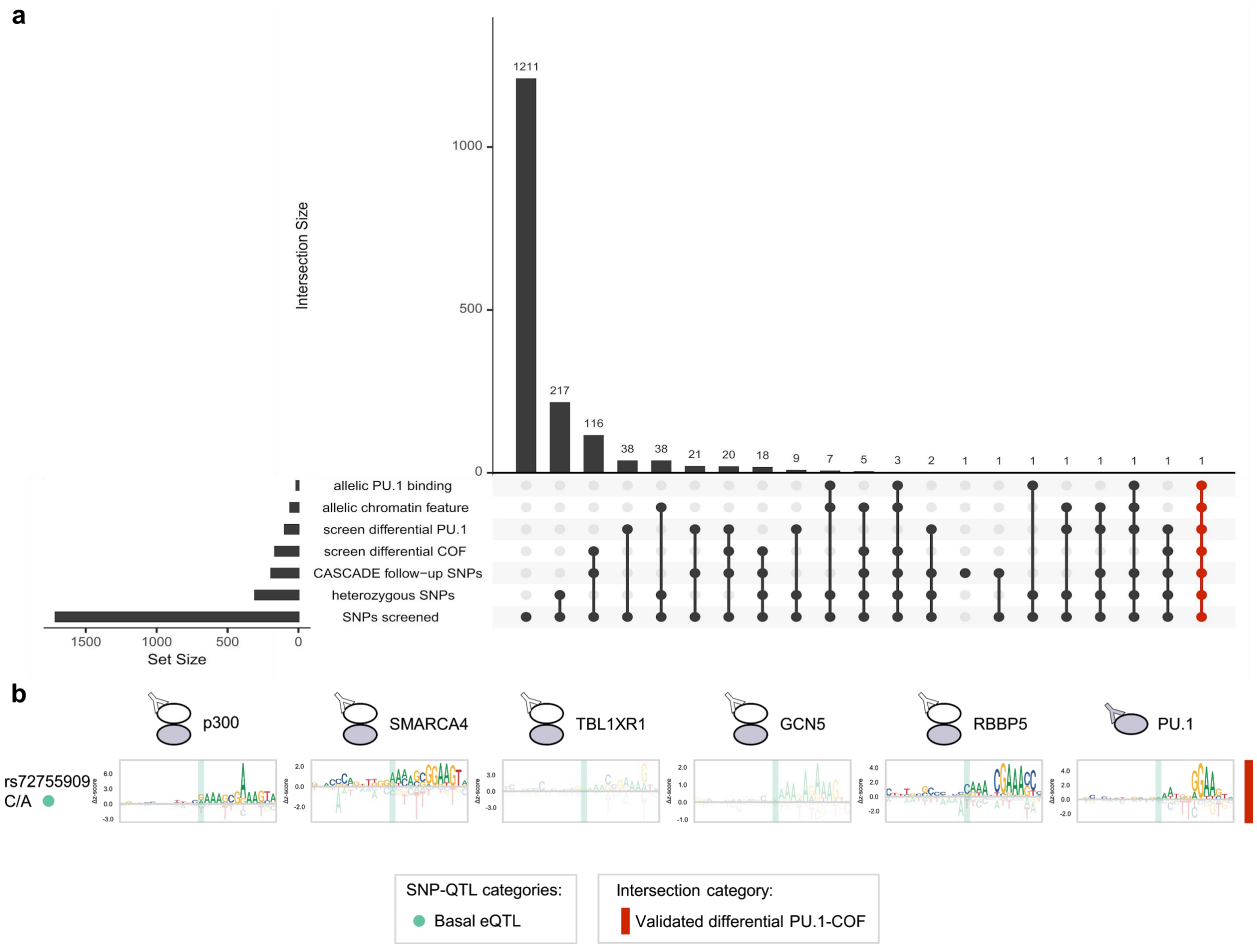

Figure S4. Comparison of differential cofactor recruitment screen with allelic imbalance data, Related to Figures 3 and 4. (**a**) UpSet plot demonstrating the size of each set intersection shown. Numbers at the top of each set intersection correspond to the number of SNP-QTLs found within that set intersection. Allelic PU.1 and chromatin feature sets were generated using publicly available ChIP-seq data (see Methods). Differential PU.1 binding and COF recruitment (sets labeled “screen differential PU.1” and “screen differential COF”) were obtained from an array-based COF recruitment and TF binding assay (see Methods). SNPs in the “CASCADE follow-up SNPs” correspond to the set characterized in detail using the CASCADE technique. Heterozygous SNPs in the THP-1 cell line were determined from genotyping array data (see Methods). The set intersection marked in red shows the single example of a SNP that perturbs PU.1 binding in both ChIP-seq and our assay (**b**) COF

recruitment logos for the rs72755909 profiled using CASCADE for p300, SMARCA4, TBL1XR1, GCN5, and RBBP5 recruitment as well as PU.1 binding. The red set intersection indicated is the same as in (**a**).

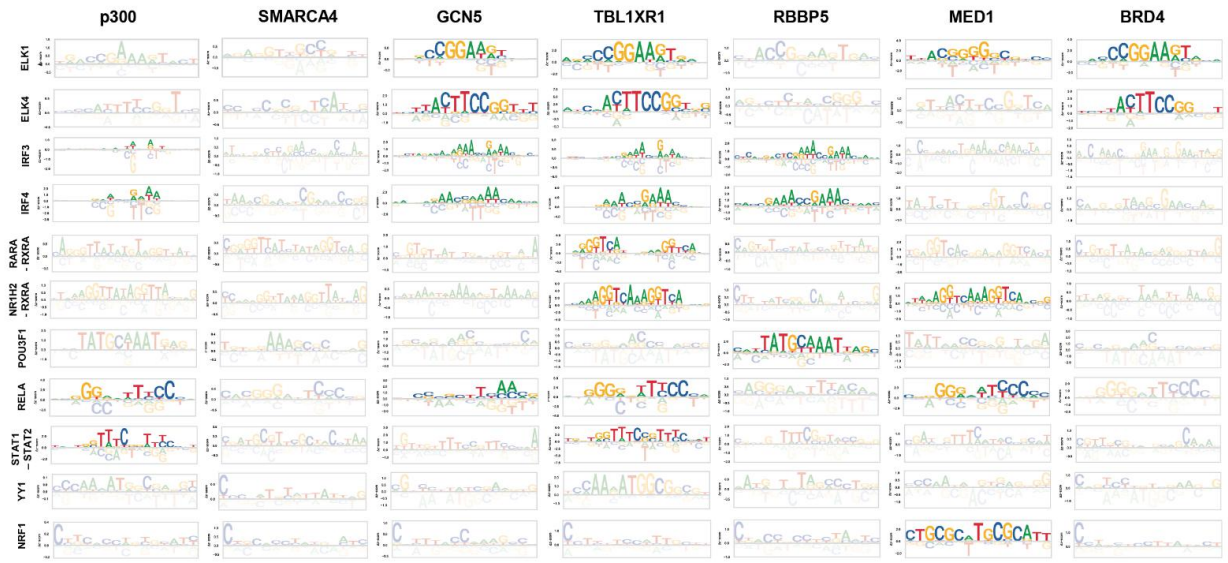

Figure S5. Profiling cofactor recruitment to a broad set of transcription factor consensus sites, Related to Figures 3, 4, and 5. COF recruitment motifs were determined by profiling COF recruitment to ~350 transcription factor (TF) consensus sites (and all single-nucleotide variants) (see Methods). COF recruitment motifs for p300, SMARCA4, GCN5, TBL1XR1, RBBP5, MED1, and BRD4 are shown for select TF binding sites to which binding was significant in at least one experiment. Motifs are only included if COF binding to the TF consensus sequence was of sufficient affinity that it met an imposed z-score threshold (z-score > 1.5, see Methods). Motifs shown were found to match the JASPAR motifs for the TFs indicated on the left hand side of each row..

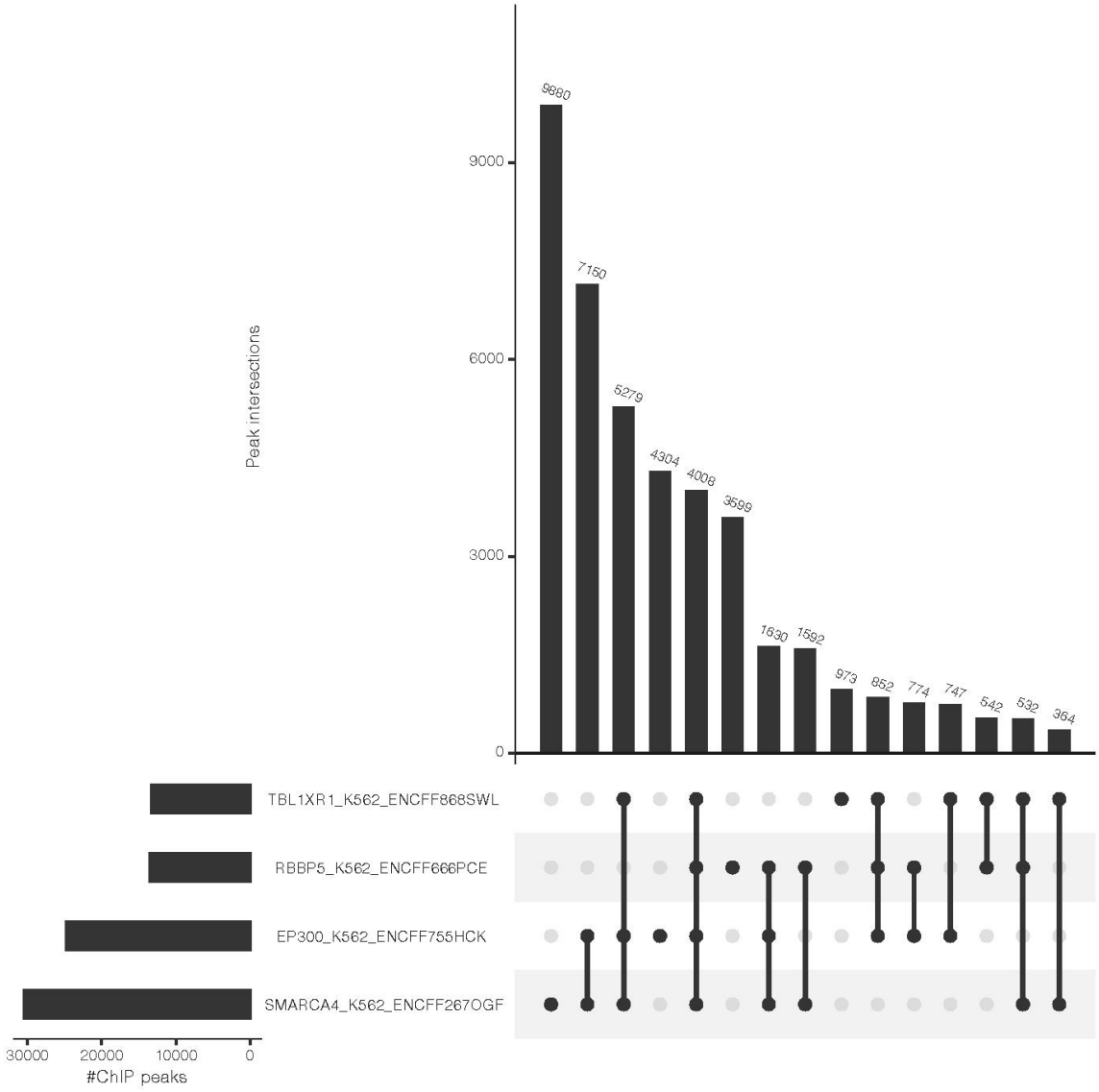

Figure S6. Overlap of ChIP-seq peaks for four cofactors in K562 cells, Related to Figures 3, 4, and 5. Shown is an UpSet plot (generated using R) quantifying the level of binding overlap for four of our target COFs in ENCODE ChIP-seq data sets from K562 cells. The ENCODE dataset identifiers are shown for each dataset. Overlap was counted if 1-bp from each ChIP-seq peak overlapped.

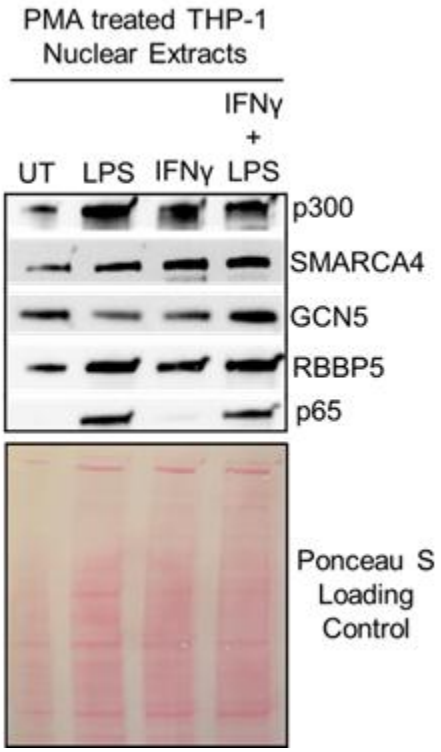

Figure S7. Western blot of PMA treated THP-1 nuclear extracts, Related to Figures 2, 3, 4, and 5. The protein expression levels of p300, SMARCA4, GCN5, RBBP5, and p65 of PMA treated THP-1 cells were evaluated by western blotting. 30ug of nuclear extract were loaded for all samples. PMA treated THP-1 cells were treated with LPS for 45 min to induce p65 expression. PMA-treated THP-1 cells were treated with IFNγ for 3 h to prime the immune response. PMA-treated THP-1 cells were treated with IFNγ for 1 h and LPS were treated with IFNγ for 2 h followed by LPS stimulation for 45 min. Ponceau S staining was used as a loading control. UT: untreated, LPS: Lipopolysaccharide, IFNγ: Interferon gamma, IFNγ + LPS: Interferon gamma and lipopolysaccharide.