Supporting Figures S1-S3 for:

Title: Enhancer turnover is associated with a divergent transcriptional response to glucocorticoid in mouse and human macrophages.

Running Title: The genomic response of macrophages to glucocorticoids

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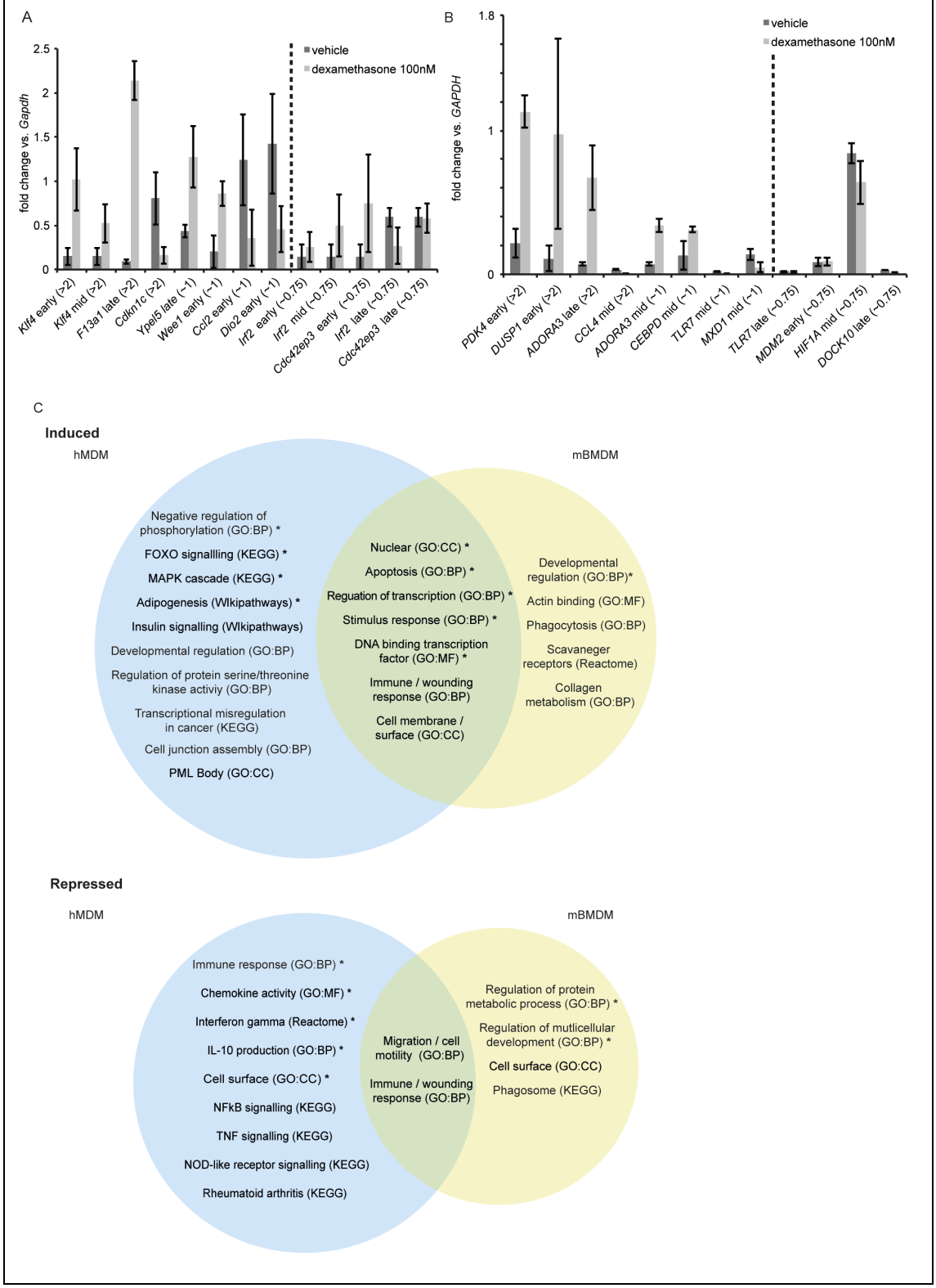
A
fold change vs. Gapdh
vehicle
dexamethasone 100nM
Klf4 early (>2)
Klf4 mid (>2)
F13a1 late (>2)
Cdkn1c (>2)
Ypel5 late (~1)
Wee1 early (~1)
Ccl2 early (~1)
Dio2 early (~1)
Irf2 early (~0.75)
Irf2 mid (~0.75)
Cdc42ep3 early (~0.75)
Irf2 late (~0.75)
Cdc42ep3 late (~0.75)
B
fold change vs. GAPDH
vehicle
dexamethasone 100nM
PDK4 early (>2)
DUSP1 early (>2)
ADORA3 late (>2)
CCL4 mid (>2)
ADORA3 mid (~1)
CEBPD mid (~1)
TLR7 mid (~1)
MXD1 mid (~1)
TLR7 late (~0.75)
MDM2 early (~0.75)
HIF1A mid (~0.75)
DOCK10 late (~0.75)
C
Induced
hMDM
mBMDM
Negative regulation of phosphorylation (GO:BP) *
FOXO signallling (KEGG) *
MAPK cascade (KEGG) *
Adipogenesis (Wlkipathways) *
Insulin signalling (Wlkipathways)
Developmental regulation (GO:BP)
Regulation of protein serine/threonine kinase activiy (GO:BP)
Transcriptional misregulation in cancer (KEGG)
Cell junction assembly (GO:BP)
PML Body (GO:CC)
Nuclear (GO:CC) *
Apoptosis (GO:BP) *
Reguation of transcription (GO:BP) *
Stimulus response (GO:BP) *
DNA binding transcription factor (GO:MF) *
Immune / wounding response (GO:BP)
Cell membrane / surface (GO:CC)
Developmental regulation (GO:BP)*
Actin binding (GO:MF)
Phagocytosis (GO:BP)
Scavaneger receptors (Reactome)
Collagen metabolism (GO:BP)
Repressed
hMDM
mBMDM
Immune response (GO:BP) *
Chemokine activity (GO:MF) *
Interferon gamma (Reactome) *
IL-10 production (GO:BP) *
Cell surface (GO:CC) *
NFkB signalling (KEGG)
TNF signalling (KEGG)
NOD-like receptor signalling (KEGG)
Rheumatoid arthritis (KEGG)
Migration / cell motility (GO:BP)
Immune / wounding response (GO:BP)
Regulation of protein metabolic process (GO:BP) *
Regulation of mutlicellular development (GO:BP) *
Cell surface (GO:CC)
Phagosome (KEGG)

Figure S1. Additonal validation and analysis of microarray expression data. Combinations of genes and time points from our microarray data were selected to represent a range of fold change responses at several time points (early, <2h; mid, 4-10h; late, >10h) in (A) mBMDM and (B) hMDM treated with dexamethasone (see methods). Expression was measured using RT-qPCR to establish a threshold where all tested conditions were confirmed, represented by the vertical dashed line. Values in brackets represent the approximate log2 fold change for each gene at the given time point using our microarray data. Error bars represent the standard deviation of the mean for 3 biological replicates in mBMDM and 4 biological replicates in hMDM. (C) Functional annotation of gene expression data from mBMDM and hMDM. Filtered results from mining multiple public functional annotation databases are shown for induced and repressed gene sets. Terms enriched in mBMDM data in yellow and hMDM in blue. Duplicate terms have been removed. All terms shown are significant to -log p-value > 6.5. * = Enriched in genes regulated 0-2h. Abbreviations: KEGG = Kyoto Encyclopedia of Genes and Genomes, GO = Gene Ontology, BP = Biological Process, MF = Molecular Function, CC = Cellular Compartment.

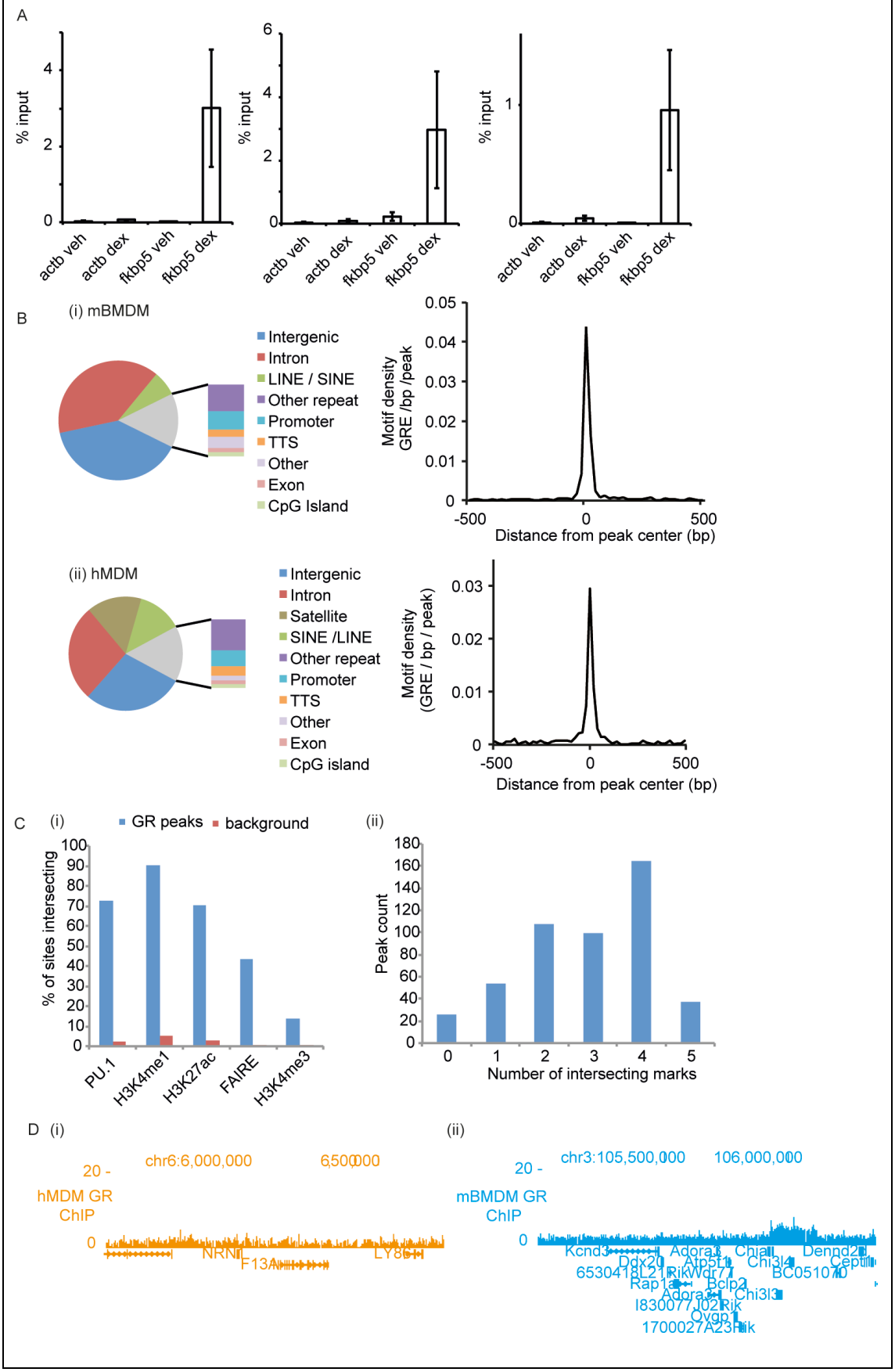
A
% input
actb veh
actb dex
fkbp5 veh
fkbp5 dex
B
(i) mBMDM
Intergenic
Intron
LINE / SINE
Other repeat
Promoter
TTS
Other
Exon
CpG Island
Motif density GRE /bp /peak
Distance from peak center (bp)
(ii) hMDM
Intergenic
Intron
Satellite
SINE /LINE
Other repeat
Promoter
TTS
Other
Exon
CpG island
Motif density (GRE / bp / peak)
Distance from peak center (bp)
C
(i)
GR peaks
background
% of sites intersecting
PU.1
H3K4me1
H3K27ac
FAIRE
H3K4me3
(ii)
Peak count
Number of intersecting marks
D
(i)
chr6:6,000,000
6,500,000
hMDM GR ChIP
NRN1
F13A
LY86
(ii)
chr3:105,500,000
106,000,000
mBMDM GR ChIP
Kcnd3
Adora3
Chia
Dennd2c
Ddx20
Atp5f1
Chi3l4
Cept
6530418L21Rik
Wdr77
BC051076
Rap1a
Bclp2
Adora2b
Chi3l3
I830077J02Rik
Qvgp1
1700027A23Rik

Figure S2. Additional data supporting GR ChIP-seq data. (A) Replication of GR ChIP-qPCR in hMDM. ChIP-qPCR was performed for GR in hMDM, each panel shows an independent biological replicate. Signal from vehicle treated (veh) and 100nM dexamethasone treated (dex) samples is shown for a known GR binding site at +88kb in the *FKBP5* locus (*fkbp5*) and the promoter of *ACTB* (actb). Error bars represent 2xSEM for 3 technical replicates of each condition.

(B) GR binding occurs at sites that are enriched for the GRE and lie distant from gene promoters in both mBMDM and hMDM. The classification of genomic sites where GR is bound is shown from (i) mBMDM and (ii) hMDM, when treated with 100nM dexamethasone. GRE motif density (GRE/bp/peak) for the GR bound sites for each species is shown alongside.

(C) GR bound sites in mBMDM display marks consistent with enhancers. Comparison was made with published data(57) from non-stimulated mBMDM. (i) Blue = counts of GR bound sites co incident with the previously reported ChIP peaks for PU.1, H3K4me1, H3K27ac, H3K4me3 and open chromatin measured by formaldehyde assisted identification of regulatory elements (FAIRE-seq). A 1000x permuted background for each is shown in red, p values are reported in the main text. (ii) Percentage of GR peaks overlapping 0-5 of the marks described in (i).

(D) Chip-seq data showing absence of GR binding in a wider area for (i) the human F13A1 locus which is mouse specific (Figure 4C) and (ii) mouse Adora3 which is human specific (Figure 4E).

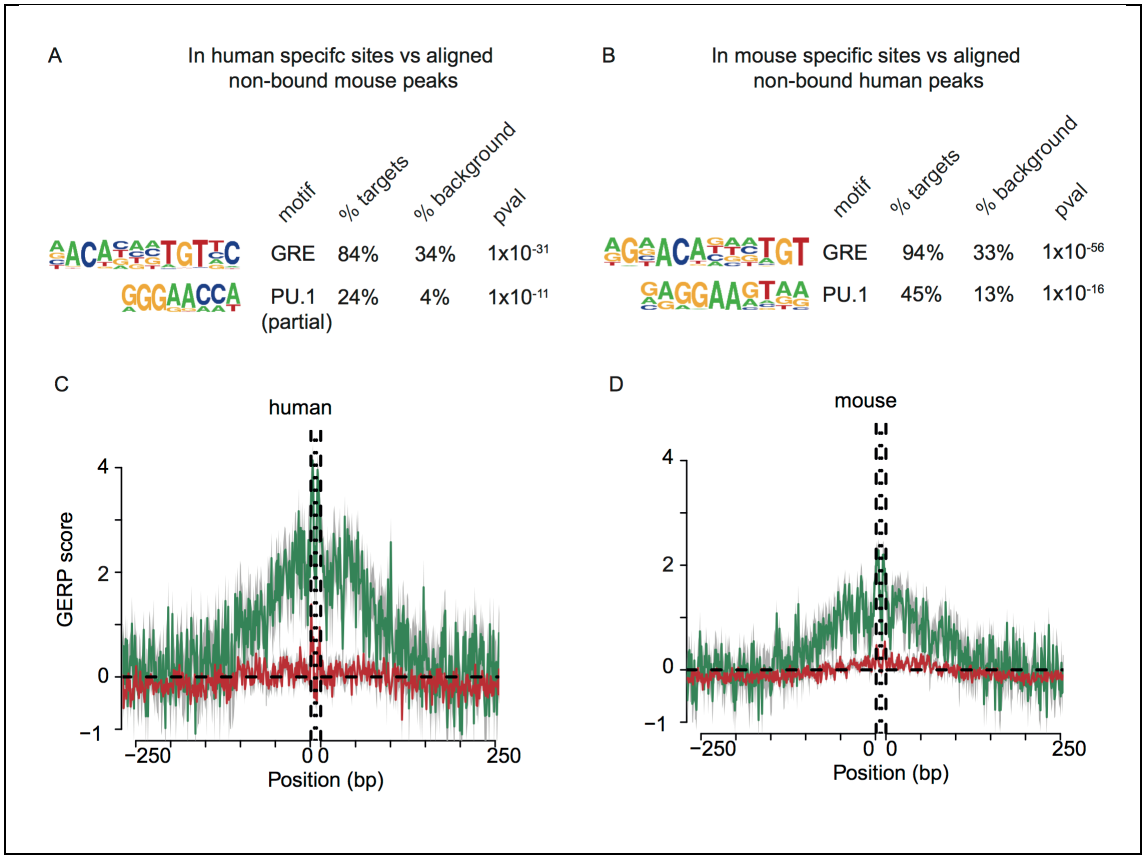

Figure S3. (A) Motifs enriched in the human specific peaks compared to the aligned mouse sites that are not bound by GR in hMDM. (B) motifs enriched in the mouse specific peaks compared to the aligned human sites that are not bound by GR in mBMDM. (C) Mean per base constraint scores calculated using GERP (72) across a 500bp region surrounding the the GRE in shared (green) and species-specific (red) peaks found in hMDM, where the grey bars represents the standard error of the mean. Vertical dashed lines delineate the GRE, as derived de novo from our human data. (D**)** Analogous to (C) for GR bound peaks and GRE motif found in mBMDM.