Supplementary Data

Escoter et al.,

Anti-inflammatory functions of the Glucocorticoid Receptor require DNA binding

Supplementary Figures (S1 – S5)

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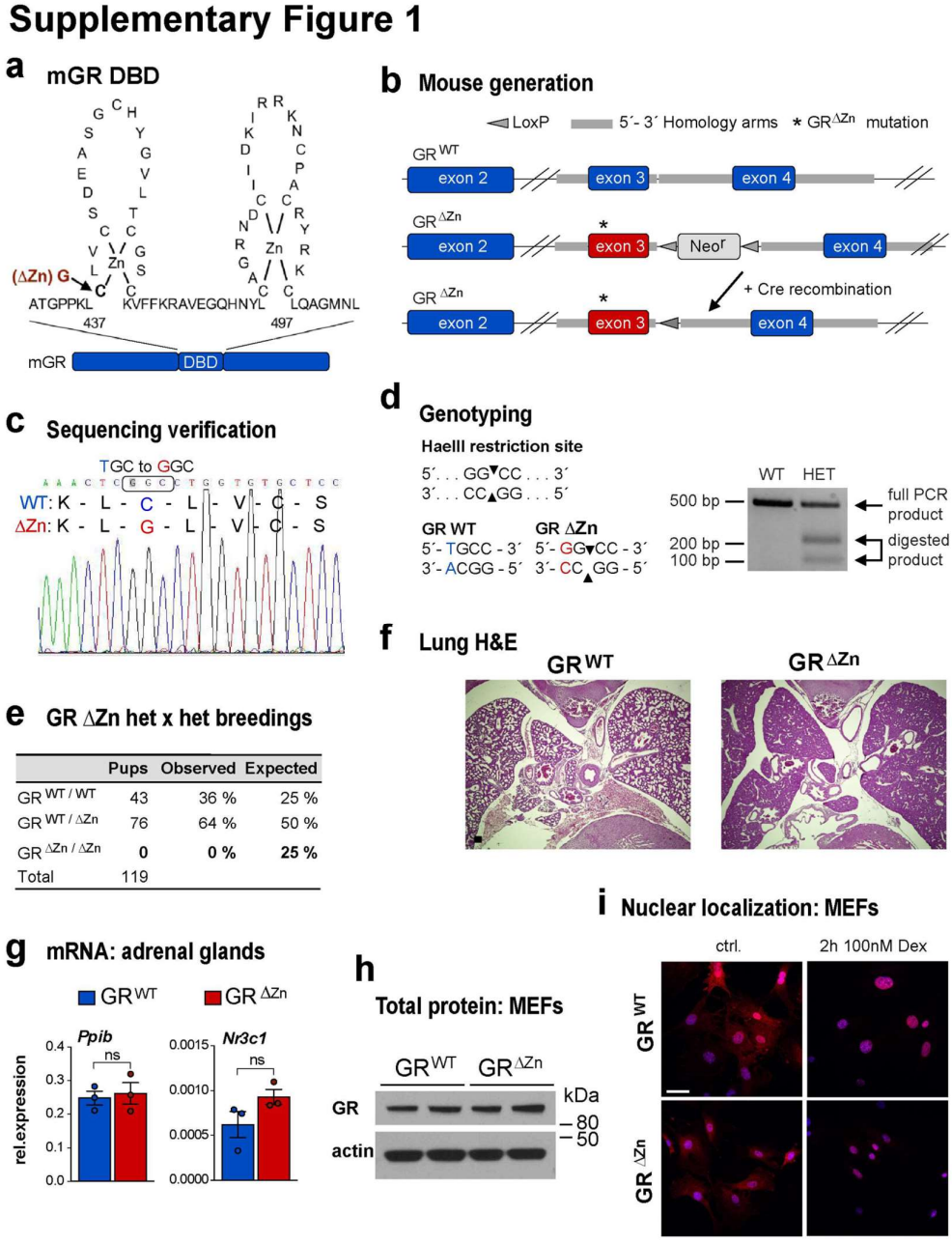
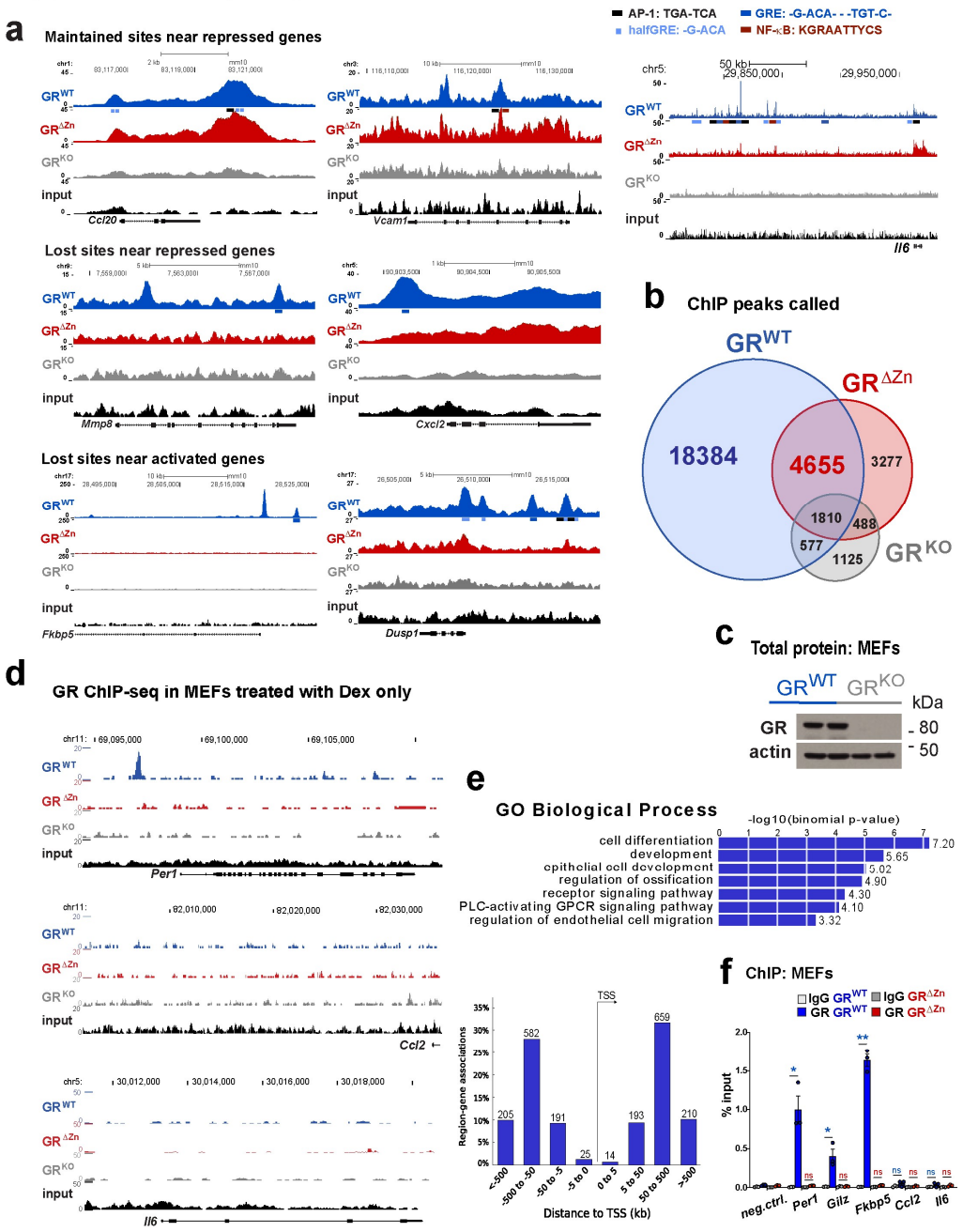


Supplementary Figure 1. Generation and phenotypic analysis of GR$^{\Delta Zn}$ mice.

a, First and second zinc finger of the Glucocorticoid Receptor DNA-Binding Domain (DBD). In GR ΔZn, Cysteine 437 is replaced by Glycine (red). **b,** GR ΔZn mouse generation by classical gene targeting in ES cells. The point mutation was introduced to exon 3 by homologous recombination and verified by sequencing (**c**). **d**, The C437G mutation created a new HaeIII restriction site. Mouse genotyping was done by PCR amplification of exon 3 followed by HaeIII digestion. **e**, Number of wild type, heterozygous and homozygous mutant pups born from het x het crosses. **f**, H&E staining of E18.5 lungs of wild type and homozygous mutants. Scale bar, 100 µm. **g**, Gene expression by qRT-qPCR (normalized to *Rpl38)* in E18.5 adrenal glands; peptidylprolyl isomerase B (*Ppib*, housekeeping) and *Nr3c1*. n = 3, ns = not significant. **h**, Western blot in MEFs showing total GR protein levels in wild type and mutant MEFs. Representative blot from (n = 3). **i**, Immunofluorescent detection of GR (red) in wildtype and mutant MEFs treated with 100nm Dex for 2 h (blue = DAPI). Representative images from n = 3, scale bar = 50 µm.

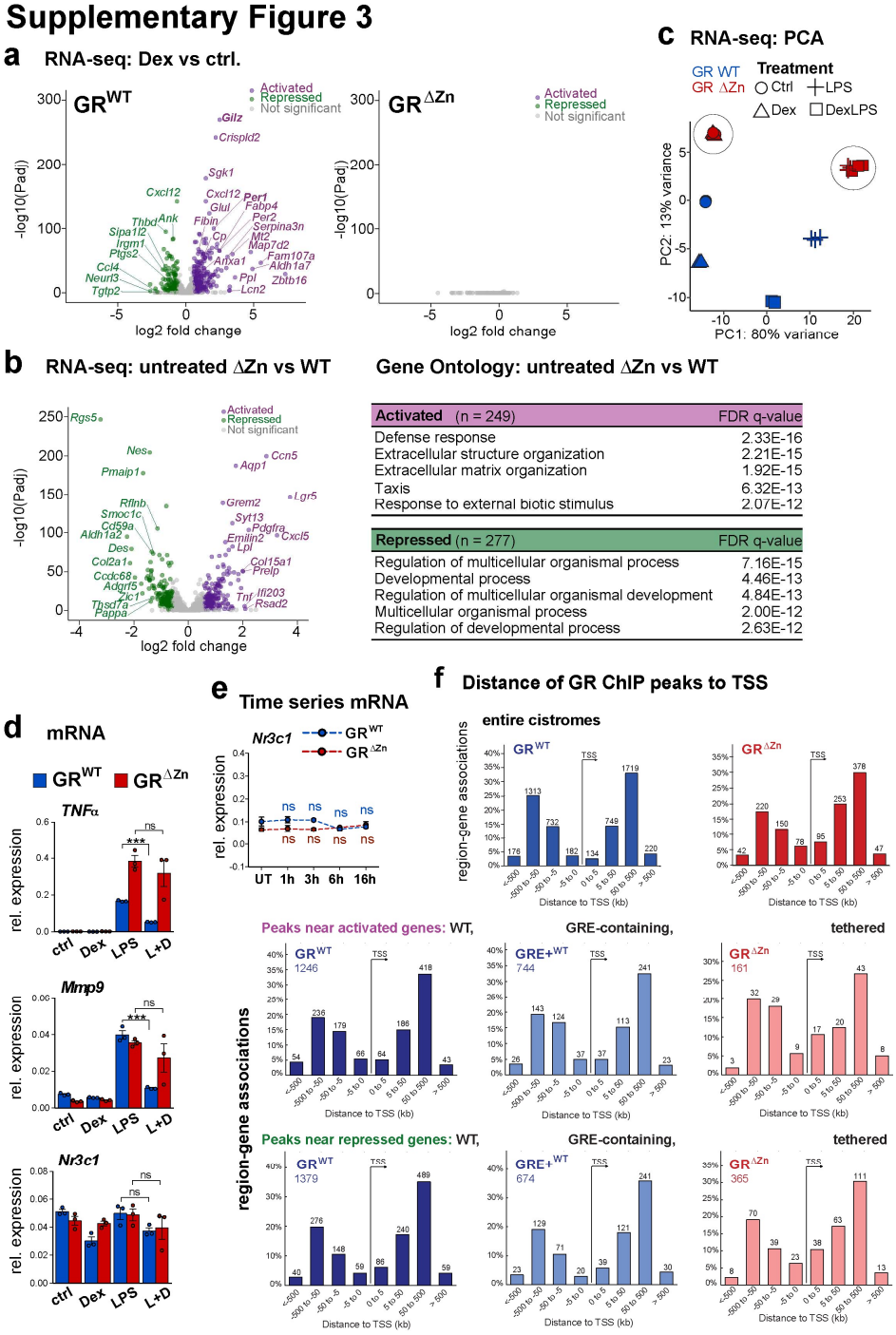
Supplementary Figure 2



Supplementary Figure 2. GR ChIP-Seq data in MEFs.

a, Representative GR ChIP-Seq tracks from wild type (n = 3), homozygous mutant (n = 4) and knockout (n = 2) MEFs at *Ccl20, Vcam1, Mmp8, Cxcl2, Fkbp5, Dusp1* and *Il6* loci. Cells were treated with 100 ng/µl LPS for 3 h and 1 µM Dex overnight. **b**, Venn diagram showing the numbers of reproducible peaks called in GR wild type (n = 3), homozygous $GR^{\Delta Zn}$ mutant (n = 4) and knockout (n = 2) MEFs. The 18,384 and the 4,655 peaks were used for further analyses. **c**, GR western blot in wild type and GR knockout MEFs (n = 2), actin as loading control. **d**, Representative GR ChIP-Seq tracks from wild type (n = 2), homozygous mutant (n = 2) and knockout (n = 2) MEFs at *Per1, Ccl2* and *Il6* loci. Cells were treated with 1 µM Dex overnight. **e**, Genomic distribution of wild type GR ChIP-Seq peaks, by distance to the transcription start site (TSS), in MEFs treated with Dex only (top). GO functional annotation of the Dex-induced wild type GR ChIP peaks, based on linear proximity to the nearest gene (GREAT analysis) (bottom). **f**, GR ChIP-qPCR for selected loci in MEFs treated with 1 µM Dex overnight (n=3), shown as % input together with a negative site. Values are mean ± SEM, ns = not significant, *p<0.05, **p<0.01.

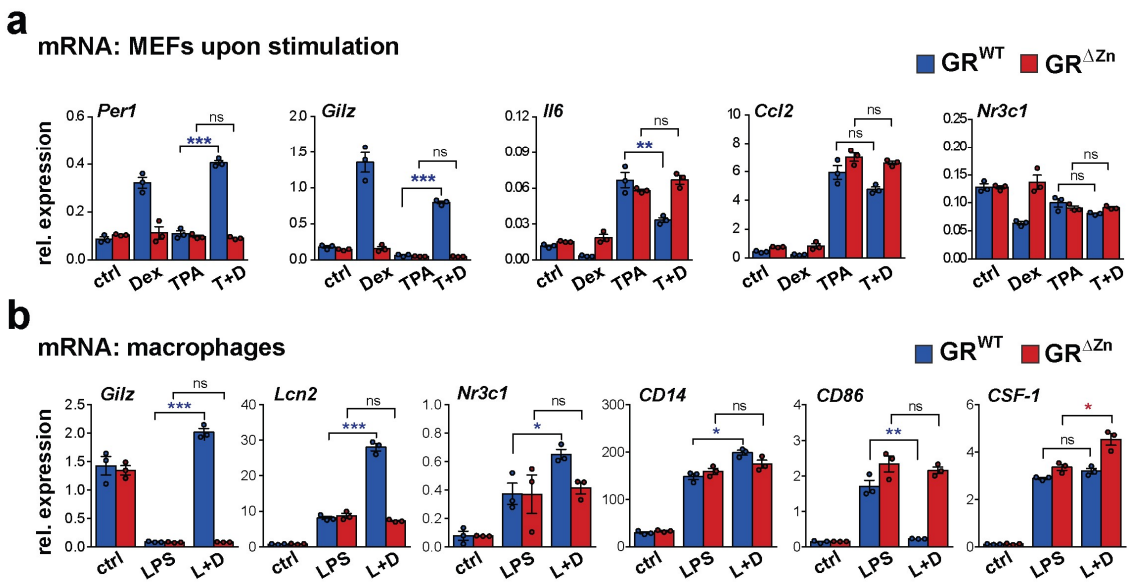
Supplementary Figure 3



Supplementary Figure 3. RNA expression in wild type and GR$^{\Delta Zn}$ MEFs.

a & b, Volcano plots showing transcripts with significant fold changes in wild type MEFs (left) and GR$^{\Delta Zn}$ (right). Dex compared to vehicle for **a**, and GR$^{\Delta Zn}$ compared to wild type in untreated MEFs for **b**, n = 3. Functional annotation of differentially regulated genes from **b**. **c**, Principal Component Analysis (PCA) of all RNA-Seq samples, (n = 3). **d**, qRT-PCR for GR target genes in GR wild type and ΔZn MEFs treated with Dex and/or LPS, n = 3, normalized to *U36b4*. Values represent mean ± SEM, ns = not significant, ***p<0.001. **e**, qRT-PCR for *Nr3c1* in MEFs treated with Dex for 0-16 h. Student´s t-test compared to untreated (UT, 0h) condition, ns = not significant. **f**, Genomic distribution of GR ChIP-Seq peaks, by distance to the transcription start site (TSS), in MEFs treated with Dex and LPS (see Figure 2). All wild type, GRE-containing wild type and GR$^{\Delta Zn}$ maintained peaks associated with nearby mRNA expression changes (nearest target genes) are shown below.

Supplementary Figure 4



Supplementary Figure 4. MEF and macrophage gene expression in response to inflammatory stimuli.

a, qRT-PCR for GR target genes in GR wild type and ΔZn MEFs treated with vehicle, Dex, TPA and TPA+Dex (T+D), respectively. n = 3, normalized to *U36b4*. Values represent mean ± SEM, ns = not significant, **p<0.01, ***p<0.001. **b**, qRT-PCR for GR target genes (normalized to *U36b4*) and macrophage differentiation markers in fetal liver derived macrophages treated with vehicle, LPS for 6 h or LPS for 6 h + Dex overnight (L+D), n = 3 biological replicates. Values represent mean ± SEM. ns = not significant, *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 5

a Peptide counts

	GR WT1	GR WT2	GR WT3	GR ΔZn1	GR ΔZn2	GR ΔZn3
ARID1A	9	11	12	4	5	4
ARID5B	5	5	5	4	2	5
p52 / NFKB2	7	6	7	5	7	5
SMARCA5	14	14	13	14	15	11
SMARCAD1	16	15	12	17	17	16
SMARCB1	7	8	8	7	9	9
SMARCC1	13	10	11	7	9	8
SMARCC2	11	12	12	1	6	7
SMARCD2	10	9	11	8	8	5
SMARCD3	8	10	10	4	4	5
SMARCE1	6	6	6	4	4	5

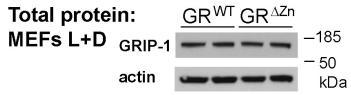
b Peptides functional annotation

Process description	FDR q-value
Protein-DNA complex subunit organization	1.63E-1
Chromosome organization	2.16E-1
DNA metabolic process	1.45E-1
Chromatin organization	2.23E-1
Nucleosome organization	2.32E-1

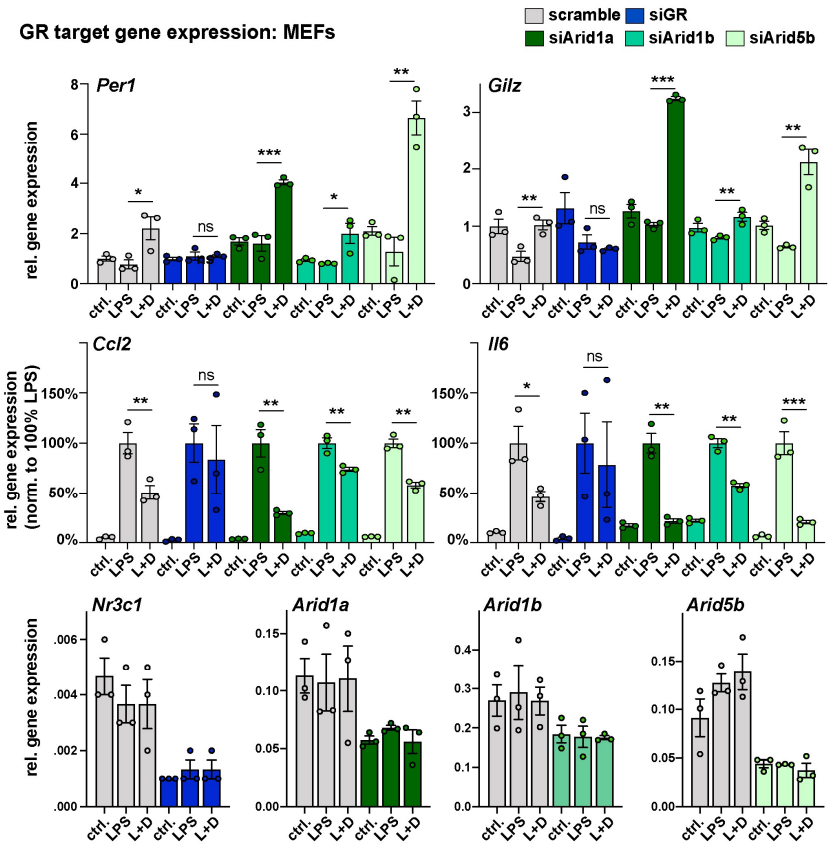
c RNA-seq read counts

	GR^{WT}	$GR^{\Delta Zn}$
Arid1a	2857	3058
Arid5b	2020	1754
Cbp / Crebbp	876	951
Cebpb	258	334
Nr3c1	1535	2282
Grip-1 / Ncoa2	861	769
p300	1702	1764
p52 / Nfkb2	3871	6950
p65 / Rela	2649	3844
Smarca4	5718	6058
Smarca5	4564	4934
Smarcad1	826	869
Smarcb1	1975	2007
Smarcc1	4701	4507
Smarcc2	2662	2660
Smarcd2	1500	1456
Smarcd3	371	187
Smarce1	2842	2857

d



e



Supplementary Figure 5. Co-regulator peptide counts, transcript levels and knockdown effects.

a, Mass spec peptide counts for selected co-regulators and transcription factors. **b,** Functional annotation of peptides enriched in wild type and mutant interactomes. **c**, Average DESeq normalised read counts of mRNA transcripts for the selected co-regulators in LPS and Dex treated MEFs, data from RNA-Seq shown in **Fig. 3**. **d**, Western blot showing total GRIP-1 protein levels in wild type and $GR^{\Delta Zn}$ MEFs treated with Dex overnight and LPS for 3 h. Representative blot from (n = 3). **e**, qRT-PCR for GR target genes in MEFs treated with vehicle, Dex and/or LPS, n = 3, normalized to *U36b4***.** Bars show *Per1*, *Gilz*, *Ccl2* and *Il6* expression after siRNA knockdown of *GR* itself, *Arid1a, Arid1b, Arid5b* (or non-targeting controls). Please note that Ccl2 and Il6 expression were normalized to LPS samples to account for effects on the TLR4 response. Knockdown efficiency is shown below. Values represent mean ± SEM, ns = not significant, *p<0.05, **p<0.01, ***p<0.001.

Supplementary Table 1. Genotyping PCR primers

	Forward	Reverse
GRZn	AATCATGCCAAGCATAACCC	AATGTCTATCATTAGTGGAC
NeoLoxP	CTATTCGGCTATGACTGGGC	CACCATGATATTCGGCAAGC

Supplementary Table 2. ChIP-qPCR primers

Gene	Forward	Reverse
Foxl2 (neg. ctrl)	GCTGGCAGAATAGCATCCG	TGATGAAGCACTCGTTGAGGC
MEFs		
Ccl2	GGAGAAAACGGGAAACCCCA	ATTGTGCAATCTGCTGTCGC
Fkbp5	CTCAGCAGCTGGGTAAGTGG	TGCAGGAGCGGTTGATCTG
Gilz	CCCGGGACTAGGGTACAGAA	GCCACAAGGGTGTGGTTTGA
Il6	GGAGCCCACCAAGAACGATAG	CAGAGAGGAACTTCATAGCGGT
Kfl9	CACAGCCCTTCTGACTCACC	CCGAGTATGGTTCTGCCTCG
Per1	GTAGGTCCCGCAAAGAGAACC	GACAGCGGTCCTGTACAAAAG
Macrophages		
Per1	TGGAACATCCTGTTCTCAGCG	AAGGAAGGCTGTGGCCAAC
Dusp1	ACAGACAGAATGGTGGTTTTTACTCC	CCCCTTGCTTTCAAATGTTACAC
Nos2	TGCCAAGAGATGCAGTTGAGG	GCTTGGGTTCGAGGCCTAC
Mmp13	TGCACCAAACACATCAAACTTTCTG	CTTAGTAACTAGGGCAAACCCCC
Il1β	GGGAAGAGGCTATTGCTACCC	ATGCCCATTTCCACCACGAT
Ccl2	GATCTGGCTGGAGAAAACGG	TCTGCTGTCGCAACACTCGT
Livers		
Acox2	GACGGCACATTGAGTTCC	ATGACTACGCAAGGCACAC
Adh5	ACTAGGTTTGGTTCCGTGGT	TCGCGCATCTAAAGCAATGA
Fah	CCAGTTCTCTCAACGTGCCT	AGCCTTAACCTGAGCCAACC
Gbe1	TACTTCCGAGCAGCGTTTGT	AGGTCGCTCTTCGATGTTGG
Hilpda	GACTCCCCGAGAACTCTGC	AGCCCCAAAGACAAACGGAC

Supplementary Table 3. qRT-PCR primers

Gene	Forward	Reverse
Arid1a	TGGGACTAACCCATACTCGCA	GAATCTGCTGTGCATAAGAGAGG
Arid1b	CTCCCCTGCGAGTATTCCAG	TGCCTGTCATAAAACCTCTTTCC
Arid5b	GTGATGAGTTCGCGCCAAATC	GCTGATAACTTTACCGTCACAGT
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
CD14	AGATTGGTCCAGCGCTTTCA	GAACTGCCCCAGATCTGCTT
CD86	GCAGCACGGACTTGAACAAC	TTGTAAATGGGCACGGCAGA
CSF-1	CAATGCTAACGCCACCGAGA	GTTGCAATCAGGCTTGGTCA
Cyp11a1	AACCTTTCCTGAGCCCTACG	TAGCCAACCATTGTCGCCAG
Cyp11b1	GCAGCCCTTTGAAGCCATAC	CGGCAACGTCACAAACACAA
Cyp11b2	TGGCATTGTGGCGGAACTAA	AGCCAGCTCAAAAAGGGTCA
Cyp17a1	TGGAGGCCACTATCCGAGAA	CACATGTGTGTCCTTCGGGA
Dusp1	GTTGTTGGATTGTCGCTCCTT	TTGGGCACGATATGCTCCAG
Gilz	ACCACCTGATGTACGCTGTG	TCTGCTCCTTTAGGACCTCCA
Il1β	TGCCACCTTTTGACAGTGATG	ATGTGCTGCTGCGAGATTTG
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Lcn2	GGCCAGTTCACTCTGGGAAA	TGGCGAACTGGTTGTAGTCC
Lpin1	GCCGACTGTCTCACTTTAG	CCTTGAGCTATGAGGAATGG
Mmp9	CCAGCCGACTTTTGTGGTCT	CTTCTCTCCCATCATCTGGGC
Mmp13	ACCTCCACAGTTGACAGGCT	AGGCACTCCACATCTTGGTTT
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Nr3c1	AGCTCCCCCTGGTAGAGAC	GGTGAAGACGCAGAAACCTTG
Per1	ACCAGGTCATTAAGTGTGTGC	CTCTCCCGGTCTTGCTTCA
Ppib	GGAGCGCAATATGAAGGTGC	TTATCGTTGGCCACGGAGG
Rpl38	AGCAGGTACCTTTACACCCTG	AGATCCTTCACTGCCAAACCC
TNFα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
U36b4	AGCGGTTTTGCTTTTTCATC	TATGGGATTCGGTCTCTTCG

Supplementary List 1. ChIP-Seq peak list

Supplementary List 2. RNA-Seq differentially expressed genes

Supplementary List 3. ChIP-Mass Spectrometry proteins list