# Glucocorticoid Activation of Anti-Inflammatory Macrophages Protects Against Insulin Resistance

## Supplementary Information

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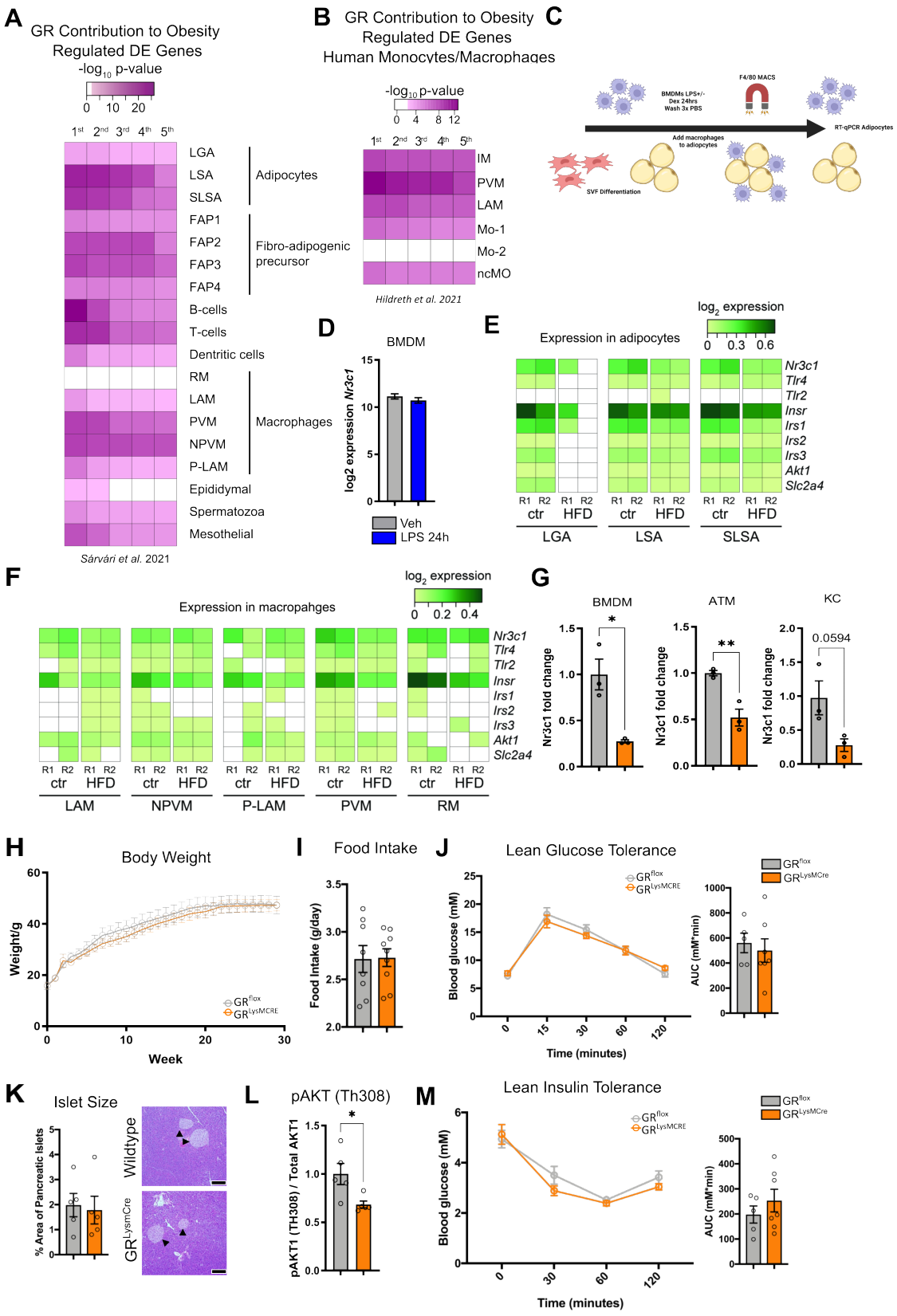
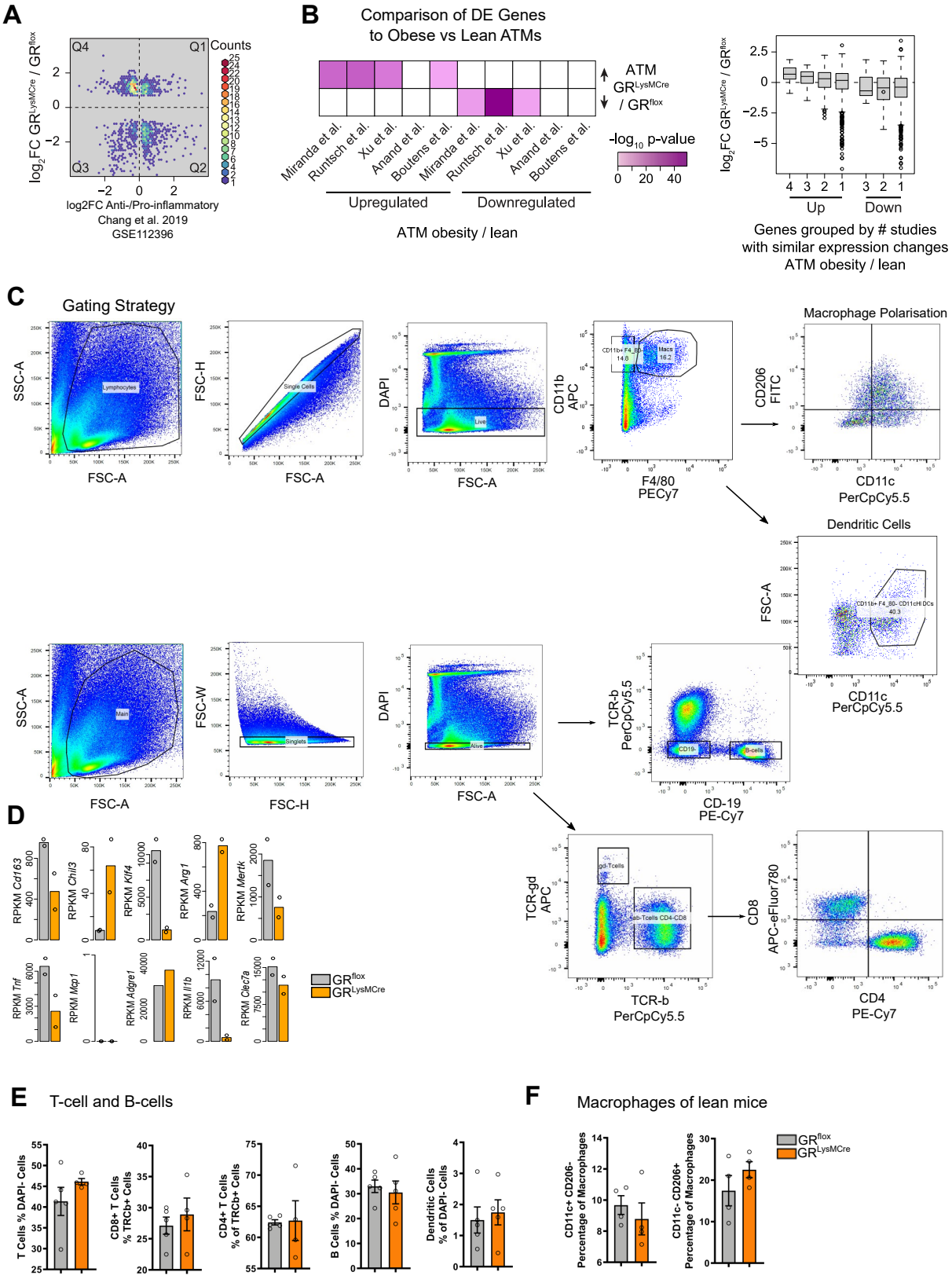



**Supplementary Figure 1. Related to Figure 1**

(**A**) Data from *Sárvári et al.*[1] were analysed, and genes induced by obesity were determined in each cell type and subjected to LISA analysis to estimate the prediction score of GR. fibro-adipogenic progenitors (FAPs), perivascular-like macrophages (PVM), non-perivascular-like

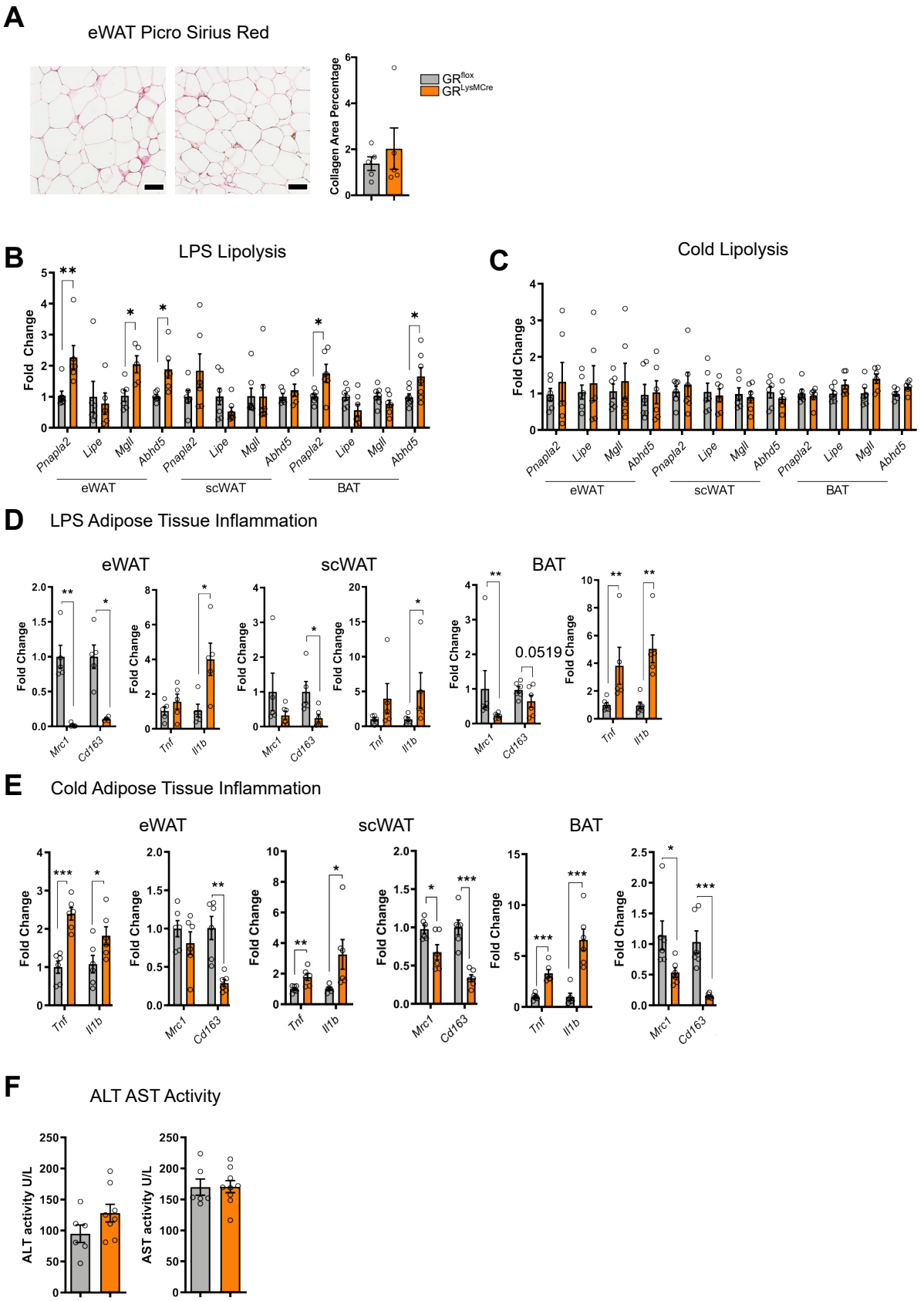
macrophages (NPVM), lipid-associated macrophages (LAM), proliferative LAM (P-LAM), collagen-expressing macrophages (CEM), regulatory macrophages (RM), lipogenic adipocytes (LGA), lipid-scavenging adipocytes (LSA), stressed lipid-scavenging adipocytes (SLSA). (**B**) Human monocyte and macrophage obesity regulated genes from *Hildreth et al.* [2] were analysed by LISA for GR enrichment. (**C**) Schematic representation of adipocyte-macrophage co-cultures. Created with Biorender.com (**D**) Gene expression of *Nr3c1* in BMDMs after LPS treatment. (N=2) (**E**) *Nr3c1* and *Tlr4, Tlr2, Insr, Irs1, Irs2, Irs3, Akt* and *Slc2a4* gene expression in adipocytes during chow and HFD. (**F**) *Nr3c1* and *Tlr4, Tlr2, Insr, Irs1, Irs2, Irs3, Akt* and *Slc2a4* gene expression in adipose tissue macrophages during chow and HFD (**G**) *Nr3c1* (GR) deletion was investigated via qPCR from indicated macrophage populations (BMDM = bone marrow derived macrophages, ATM = Adipose tissue resident macrophages, KC = Kupffer cells). (N=3) (**H**) $GR^{flox}$ and $GR^{LysMCre}$ mice were subjected to 60% HFD for 29 weeks. Weight was monitored. (**I**) Food intake of mice on 60% HFD (($GR^{flox}$ N=8, $GR^{LysMCre}$ N=9)). (**J**) Lean $GR^{flox}$ and $GR^{LysMCre}$ mice were fasted overnight, given 2 mg/kg glucose, and blood glucose levels were traced for 120 mins ($GR^{flox}$ N=5, $GR^{LysMCre}$ N=7). (**K**) Pancreas was harvested from obese $GR^{flox}$ and $GR^{LysMCre}$ mice, fixed and stained using H&E. Pancreatic islet size was quantified relative to total area of the image (N=5). (**L**) Baseline AKT phosphorylation was measured by ELISA from muscle of lean and obese $GR^{flox}$ and $GR^{LysMCre}$ mice. (N=5) (**M**) Lean $GR^{flox}$ and $GR^{LysMCre}$ mice were fasted overnight, and given 0.5 μ i.U./g insulin, blood glucose levels were traced for 120 mins ($GR^{flox}$ N=5, $GR^{LysMCre}$ N=7). Original images at 10x magnification. Black arrows indicate islets. Data show mean ±SEM. Statistical analysis by student's t-test or 2-way ANOVA with a Bonferroni post-hoc test or two-tailed student's T-test *$p < 0.05$. Wilcoxon rank-sum test for A and B. Scale bar: 200μm in **K**.




**Supplementary Figure 2. Related to Figure 2.**

(**A**) Scatterplot of genes differentially expressed between $GR^{LysMCre}$ and $GR^{flox}$ ATMs of obese mice from analysis shown in Fig. 2A compared to differentially expressed genes between anti- and pro-inflammatory ATMs from eWAT of obese mice (GSE112396)[3] . (**B**) Differentially

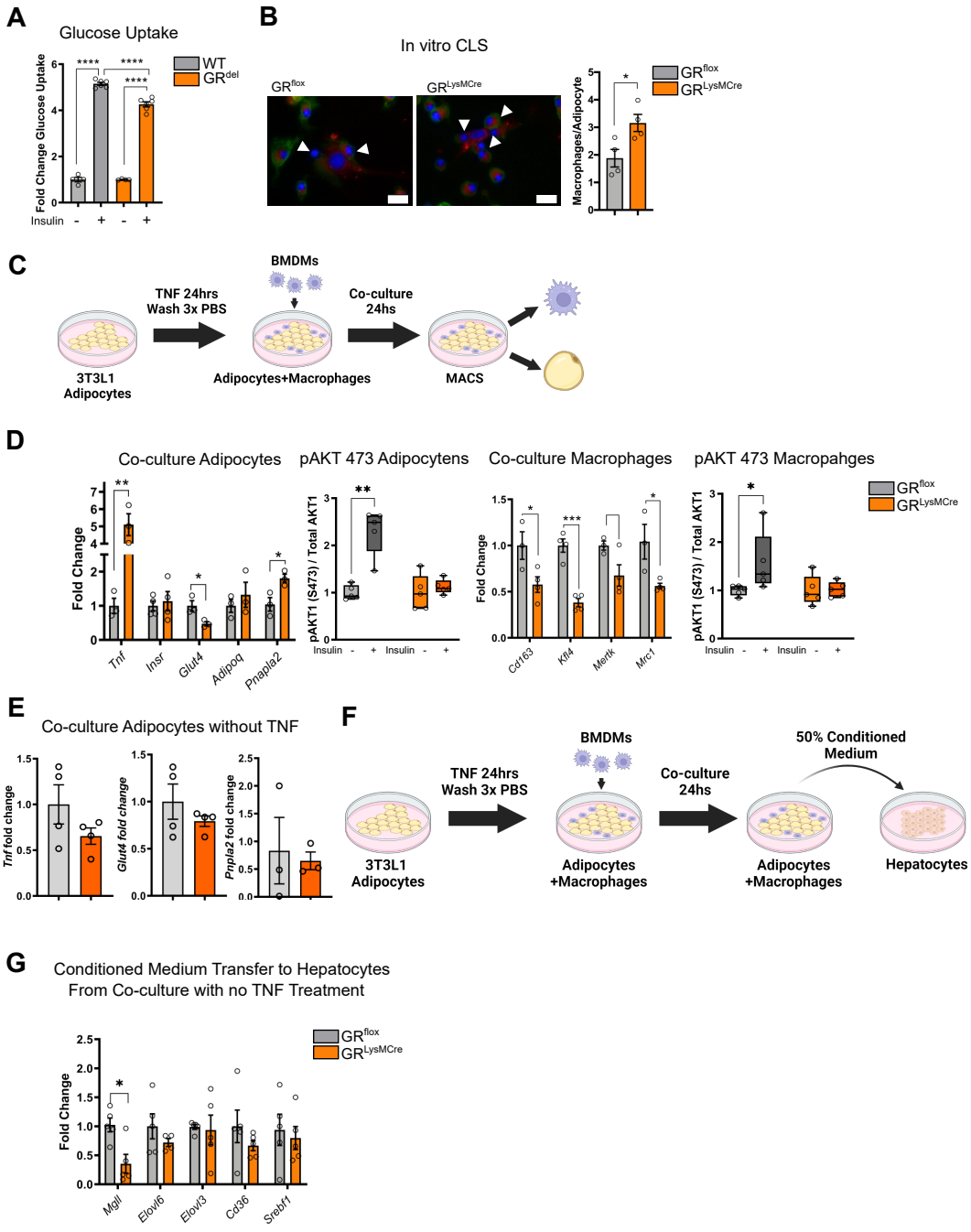
expressed genes between $GR^{LysMCre}$ and $GR^{flox}$ ATMs were compared to various published datasets of ATMs between lean and obese mice. Boxplots show median, IQR, 1.5xIQR and outlier values. (N=2) (**C**) FACS gating strategy of SVF from obese $GR^{flox}$ and $GR^{LysMCre}$ mice related to Fig.3. (**D**) Selected macrophage genes from RNASeq of RNA preparations of ATMs of obese $GR^{LysMCre}$ and $GR^{flox}$ mice (**E**) SVF T cell relative number, polarisation, B cell relative number and dendritic cell relative number quantified by FACS ($GR^{flox}$ N=5, $GR^{LysMCre}$ N=4). (**F**) SVF from lean $GR^{flox}$ and $GR^{LysMCre}$ mice was analysed for macrophage polarisation by FACS (N=4). Data show mean +/- SEM. Statistical analysis by two-tailed student's t-test. $p < 0.001$***.




**Supplementary Figure 3. Relate Figure 3**

**A**) Picro sirius red staining of eWAT of obese GR$^{flox}$ and GR$^{LysMCre}$ mice (N=5). (**B**) Lean GR$^{flox}$ and GR$^{LysMCre}$ mice were subjected to endotoxin shock for 24 hours. RT-qPCR of lipolytic

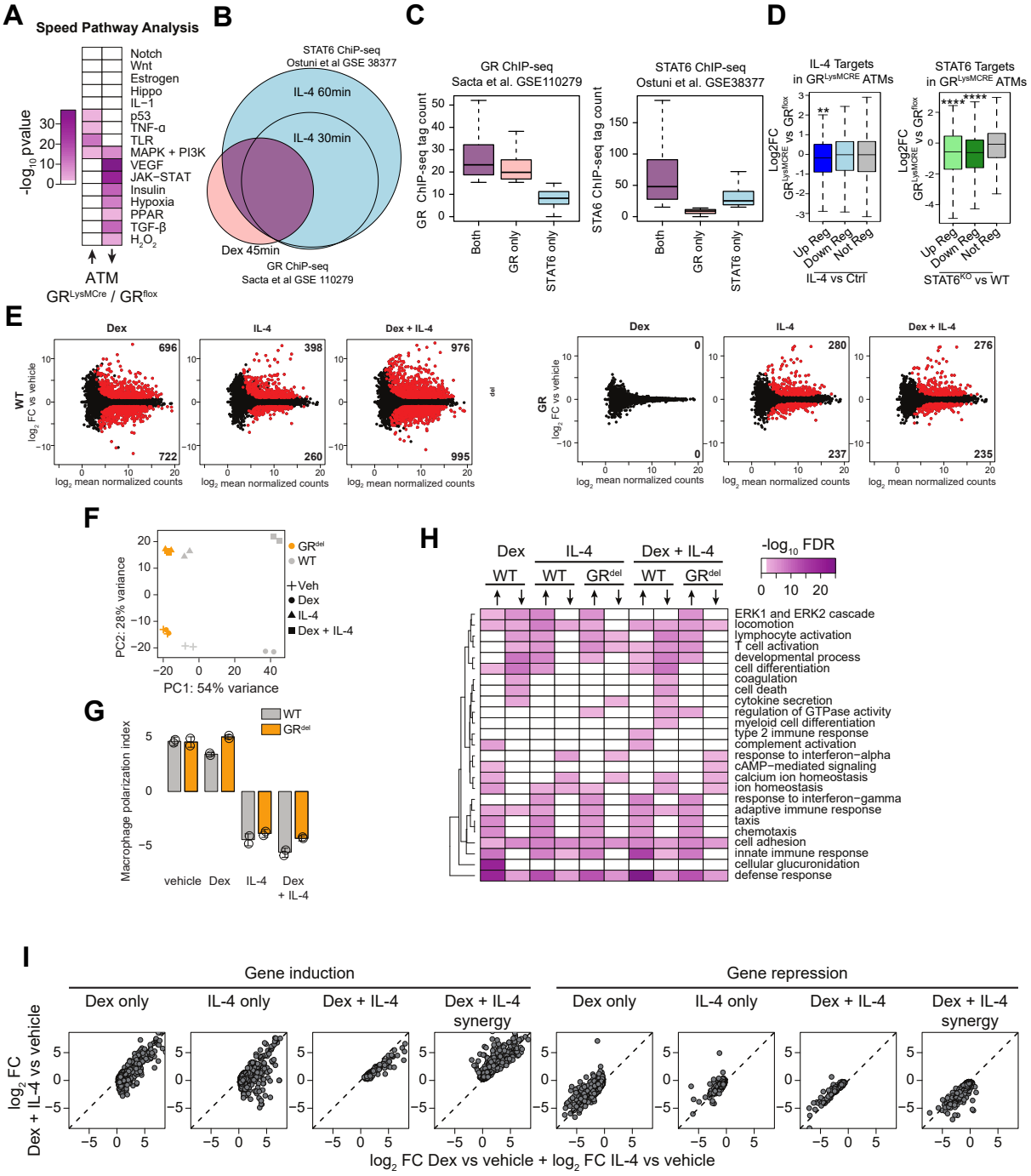
markers (eWat $GR^{flox}$: *Pnpla2* N=7, *Lipe* N=6, *Mgll* N=6, *Abhd5* N=6; $GR^{LysMCre}$: *Pnpla2* N=6, *Lipe* N=5, *Mgll* N=5, *Abhd5* N=6; scWat $GR^{flox}$: *Pnpla2* N=6, *Lipe* N=7, *Mgll* N=7, *Abhd5* N=5; $GR^{LysMCre}$: *Pnpla2* N=6, *Lipe* N=7, *Mgll* N=7, *Abhd5* N=7; BAT $GR^{flox}$: *Pnpla2* N=7, *Lipe* N=7, *Mgll* N=7, *Abhd5* N=7; $GR^{LysMCre}$: *Pnpla2* N=7, *Lipe* N=7, *Mgll* N=7, *Abhd5* N=7) and (**D**) inflammation markers (eWat $GR^{flox}$: *Mrc1* N=5, *Cd163* N=5, *Tnf* N=5, *Il1b* N=5; $GR^{LysMCre}$: *Mrc1* N=5, *Cd163* N=5, *Tnf* N=5, *Il1b* N=5; scWat $GR^{flox}$: *Mrc1* N=5, *Cd163* N=5, *Tnf* N=5, *Il1b* N=5; $GR^{LysMCre}$: *Mrc1* N=6, *Cd163* N=6, *Tnf* N=5, *Il1b* N=5; BAT $GR^{flox}$: *Mrc1* N=6, *Cd163* N=6, *Tnf* N=6, *Il1b* N=6; $GR^{LysMCre}$: *Mrc1* N=6, *Cd163* N=6, *Tnf* N=5, *Il1b* N=6). (**C**) Obese $GR^{flox}$ and $GR^{LysMCre}$ mice were subjected to 4°C cold stress for 12 hours. RT-qPCR of lipolytic genes (N=6) and (**E**) inflammatory markers (N=6). (**F**) Serum ALT and AST measurement of obese $GR^{flox}$ and $GR^{LysMCre}$ mice ($GR^{flox}$ N=6, $GR^{LysMCre}$ N=8). Data show mean +/- SEM. Statistical analysis by two-tailed Student's t-test. $p < 0.05$*, $< 0.01$**, $<0.001$***. Scale bar: 200µm in **A**.




**Supplementary Figure 4. Related to Figure 3.**

Differentiated 3T3L1 adipocytes were co-cultured with either WT or $GR^{del}$ fetal liver derived macrophages (FLDMs) and insulin stimulated glucose uptake assessed (WT N=4, $GR^{del}$ N=5) (**B**) Number of $GR^{flox}$ and $GR^{LysMCre}$ BMDMs contacting TNF pre-treated 3T3L1 adipocytes determined by IF of CD11b (green) and LipidTox (red) (N=4). (**C**) Cartoon depicting co-cultures of TNF pre-treated 3T3L1 adipocytes and either $GR^{flox}$ or $GR^{LysMCre}$ BMDMs. Created with Biorender.com (**D**) 3T3L1s cocultured with either $GR^{flox}$ or $GR^{LysMCre}$ macrophages as in S4C were separated by MACS and gene expression or AKT phosphorylation quantified by RT-qPCR (Adipocytes: $GR^{flox}$: *Tnf* N=3, *Insr* N=4, *Glut4* N=3, *Adipoq* N=3, *Pnpla2* N=3; $GR^{LysMCre}$ *Tnf* N=3, *Insr* N=4, *Glut4* N=3, *Adipoq* N=3, *Pnpla2* N=3; pAKT Adipocytes N=5; Macropahges: $GR^{flox}$: *Cd163* N=3, *Klf4* N=4, *Mertk* N=3, *Mrc1* N=3; $GR^{LysMCre}$ *Cd163* N=4, *Klf4* N=4, *Mertk* N=4, *Mrc1* N=4; pAKT Macropahges N=5). (**E**) 3T3L1 adipocytes were co-cultured with BMDMs without TNF pretreatment ($GR^{flox}$: *Tnf* N=4, *Glut4* N=4, *Pnpla2* N=3; $GR^{LysMCre}$

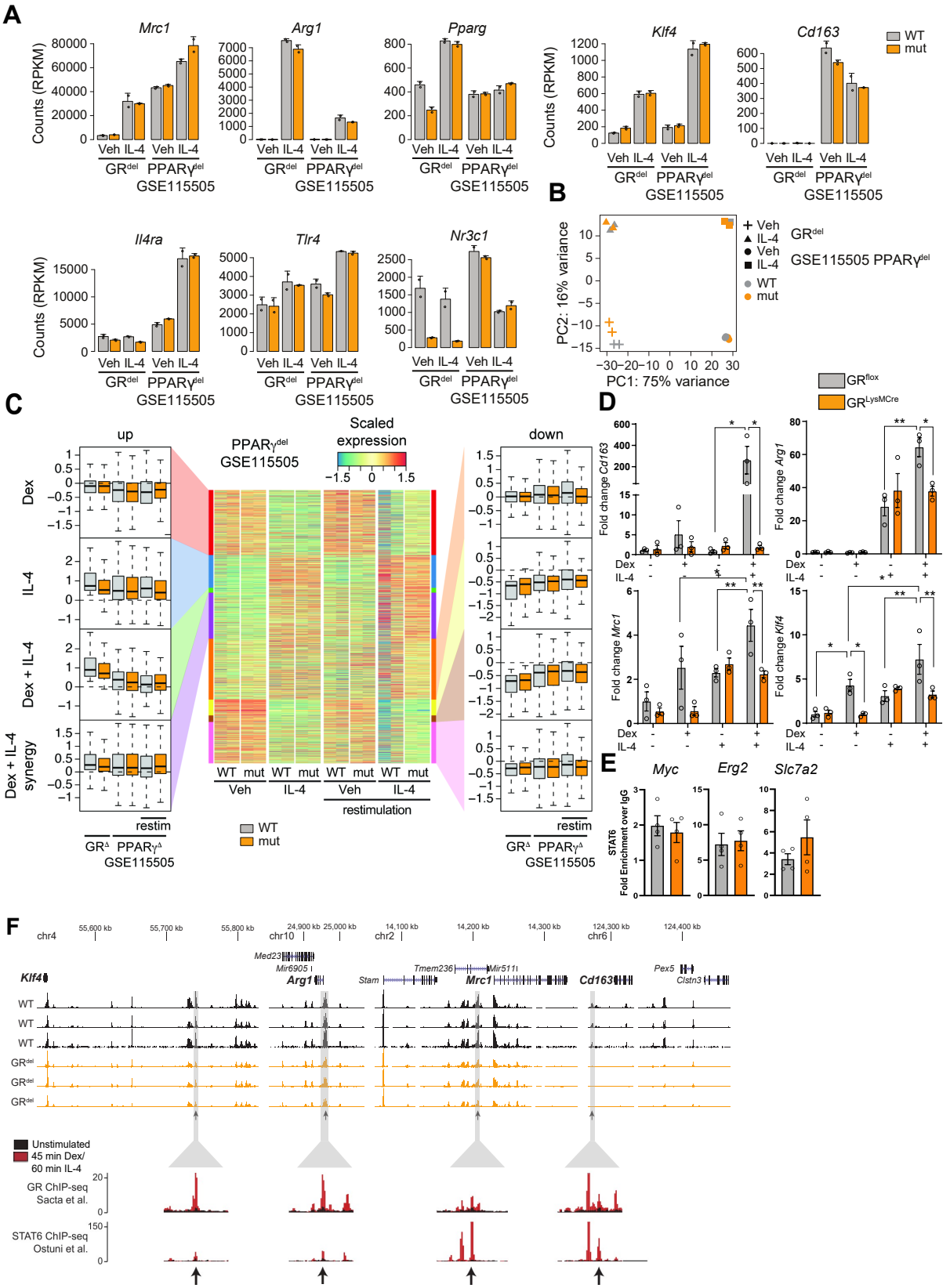
*Tnf* N=4, *Glut4* N=4, *Pnpla2* N=3). (**F**) Cartoon depiction of co-culture conditioned medium transfer to hepatocytes. Created with Biorender.com (**G**) Primary hepatocytes were treated with co-culture conditioned medium without TNF pre-treatment of adipocytes (N=5). Data show mean ±SEM. Boxplots show median, IQR and min/max values. Statistical analysis two-tailed students t-test (B, D, G), two-way ANOVA with a Sidak post-hoc test (A). $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** images at 40x original magnification. Scale bar: 25μm in **B**.




**Supplementary Figure 5. Related to Figure 5.**

(**A**) Speed pathway analysis of differentially expressed genes from $GR^{flox}$ and $GR^{LysMCRE}$ ATMs in 2A. Significance calculated using Hypergeometric test one-sided (**B**) Euler plot comparing binding loci of public ChIP-seq data for GR and STAT6. (**C**) Tag density for GR binding was determine for GR only bound, STAT6 only, GR+STAT6 bound or neither sites from the indicated studies. Tag density of STAT6 was compared for STAT6 only bound, GR only, STAT6+GR bound or neither sites from the indicated studies. (n= GR only: 2602; STAT6 only: 23665; Both: 8813) (**D**) The $log_2$FC between $GR^{LysMCre}$ and $GR^{flox}$ ATMs for IL-4 or $STAT6^{KO}$ up- or down-regulated was compared to non-IL-4 or $STAT6^{KO}$ regulated genes. Box plots show median, interquartile range and min/max values excluding outliers. P. value calculated using Wilcoxon rank sum test. (**E**) MA plots derived from RNA-seq of WT and $GR^{del}$ FLDMs treated

with dex, IL-4 or IL-4+dex compared to vehicle. (**F**) PCA of RNA-seq data derived from S5D. (**G**) Macrophage polarization index based on RNA-seq counts from WT and $GR^{del}$ FLDMs in S5D. (N=2) (**H**) Gene ontology analysis of genes up or down regulated by dex (only WT, no genes regulated in $GR^{del}$), IL-4 and dex+IL4 compared to vehicle control (GOseq benjamini-hochberg corrected padj < 0.01). (**I**) Scatter plots of quantification of dex+IL-4 synergism comparing $log_2$FC of dex+IL-4 to log2FC dex + log2FC IL-4. Exact p.value: D: IL4 targets: 0.0050, Stat6 targets: Up-regulated: 6.849e-10, Down-regulated: <2.2e-16




**Supplementary Figure 6 Related to Figure 5.**

(**A**) Gene expression from GR$^{del}$ and PPARγ$^{del}$ BMDMs with and without IL-4 treatment. (**B**) Principal component analysis for the GR$^{del}$ and PPARγ$^{del}$ BMDMs before and after stimulation

with IL-4. (**C**) Heat map showing gene expression of first and second stimulation with IL-4 in PPAR$\gamma^{del}$ BMDMs for the clusters identified in Figure 4B along with boxplots quantifying the induction and repression levels for GR and PPAR$\gamma$ deficiency. (**D**) qPCR validation of dex+IL-4 co-regulated genes (N=3). (**E**) ChIP PCR for STAT6 in GR$^{flox}$ and GR$^{LysMCre}$ BMDMs upon dex + IL-4 stimulation nearby genes of the IL-4 only activated group (N=4). (**F**) Design for the ChiP-qPCR Primers for their respective genes. Data show mean ±SEM. Boxplots show median, IQR and min/max values. Statistical analysis by two-way ANOVA (D), Mann-Whitney test, two-sided (E). $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

## Supplementary References

1. Sárvári, A. K. *et al.* Plasticity of Epididymal Adipose Tissue in Response to Diet-Induced Obesity at Single-Nucleus Resolution. *Cell Metabolism* **33**, 437-453.e5 (2021).

2. Hildreth, A. D. *et al.* Single-cell sequencing of human white adipose tissue identifies new cell states in health and obesity. *Nat Immunol*. **22**:639-653 (2021).

3. Chang, J. C. et al. Adaptive adipose tissue stromal plasticity in response to cold stress and antibody-based metabolic therapy. Sci Rep **9**, 8833 (2019).

**Supplementary Table 1**

**RT-qPCR Primer**

| Name | Forward | Reverse | |
|---|---|---|---|
| Abhd5 | TGGTGTCCCACATCTACATCA | CAGCGTCCATATTCTGTTTCCA | |
| Adgre1 | CCTGGACGAATCCTGTGAAG | GGTGGGACCACAGAGAGTTG | |
| Adipoq | GGAGAGAAAGGAGATGCAGGT | CTTTCCTGCCAGGGGTTC | |
| Arg1 | CTCCAAGCCAAAGTCCTTAGAG | AGGAGCTGTCATTAGGGACATC | |
| Cd163 | GGCTAGACGAAGTCATCTGCA | CTTCGTTGGTCAGCCTCAGAGA | |
| Cd36 | TGGCAAAGAACAGCAGCAAA | CACAGTGTGGTCCTCGGG | |
| Chil3 | GCTCTCCAGAAGCAATCCTGA | GGAAGATCCCAGCTGTACGTT | |
| Clec7a | CTTCACCTTGGAGGCCCATT | CGCCAAAATGCTAGGGCAC | |
| Elovl3 | TTCTCACGCGGGTTAAAAATGG | GAGCAACAGATAGACGACCAC | |
| Elovl6 | TCAGCAAAGCACCCGAAC | AGCGACCATGTCTTTGTAGGAG | |
| Glut4 | CTGTCGCTGGTTTCTCCAAC | CAGGAGGACGGCAATAGAA | |
| Il1b | GGCTGTGGAGAAGCTGTGGCA | GGGTCCGACAGCACGAGGCT | |
| Insr | TCTTTCTTCAGGAAGCTACATCTG | TGTCCAAGGCATAAAAAGAATAGTT | |
| Klf4 | GCAGTCACAAGTCCCCTCTC | TGGTAAGGTTTCTCGCCTGT | |
| Lipe | CCAGCCTGAGGGCTTACTG | CTCCATTGACTGTGACATCTCG | |
| Mcp1 | AACGCCCCACTCACCTGCTG | TGGGGTCAGCACAGACCTCTC | |
| Mertk | GCTGGCATTTCATGGTGGAA | CATTGTCTGAGCGCTGCAC | |
| Mgll | ACCATGCTGTGATGCTCTCTG | CAAACGCCTCGGGGATAACC | |
| Mrc1 | CCACAGCATTGAGGAGTTTG | ACAGCTCATCATTTGGCTCA | |
| Nr3c1 | GGCCGCTCAGTGTTTTCTAA | GCAGAGTTTGGGAGGTGGT | |
| Pnpla2 | CAACGCCACTCACATCTACGG | GGACACCTCAATAATGTTGGCAC | |
| Rpl | CCTGCTGCTCTCAAGGTT | TGGCTGTCACTGCCTGGTACTT | |
| Srebf1 | TGACCCGGCTATTCCGTGA | CTGGGCTGAGCAATACAGTTC | |
| Tnf | AGGGGCCACCACGCTCTTCT | TGAGTGTGAGGGTCTGGGCCAT | |

**ChIP Primer**

| | | | |
|---|---|---|---|
| Arg1 | CAAAGGGTTCTGCAGGGGAA | TCAAGAGCCCTAAGTGCCTG | |
| Cd163 | CTCAGTTGGGGTGAGACTTCA | TGCAAGCTCTAGCCAGACAAC | |
| Erg2 | CAAATTGGCCATGTGACGGC | GCAGGTCTTCAATACCCGTGA | |
| Klf4 | GGTGACAAAGCAGGTGAAGC | TGTACAAAGCCGGTCTAGCG | |
| Mrc1 | TCCAGGTCCATCCATTTGGC | TGGAAAGAACCCAGATGCCC | |
| Myc | TTGGCAATTCACTCCTCCCC | GCGCTAGACGCGAGAATATG | |
| Slc7a2 | AGCTTGGAGTTAGACACCGC | CCTGGAGCGTTTGTCACGG | |