Figure S1

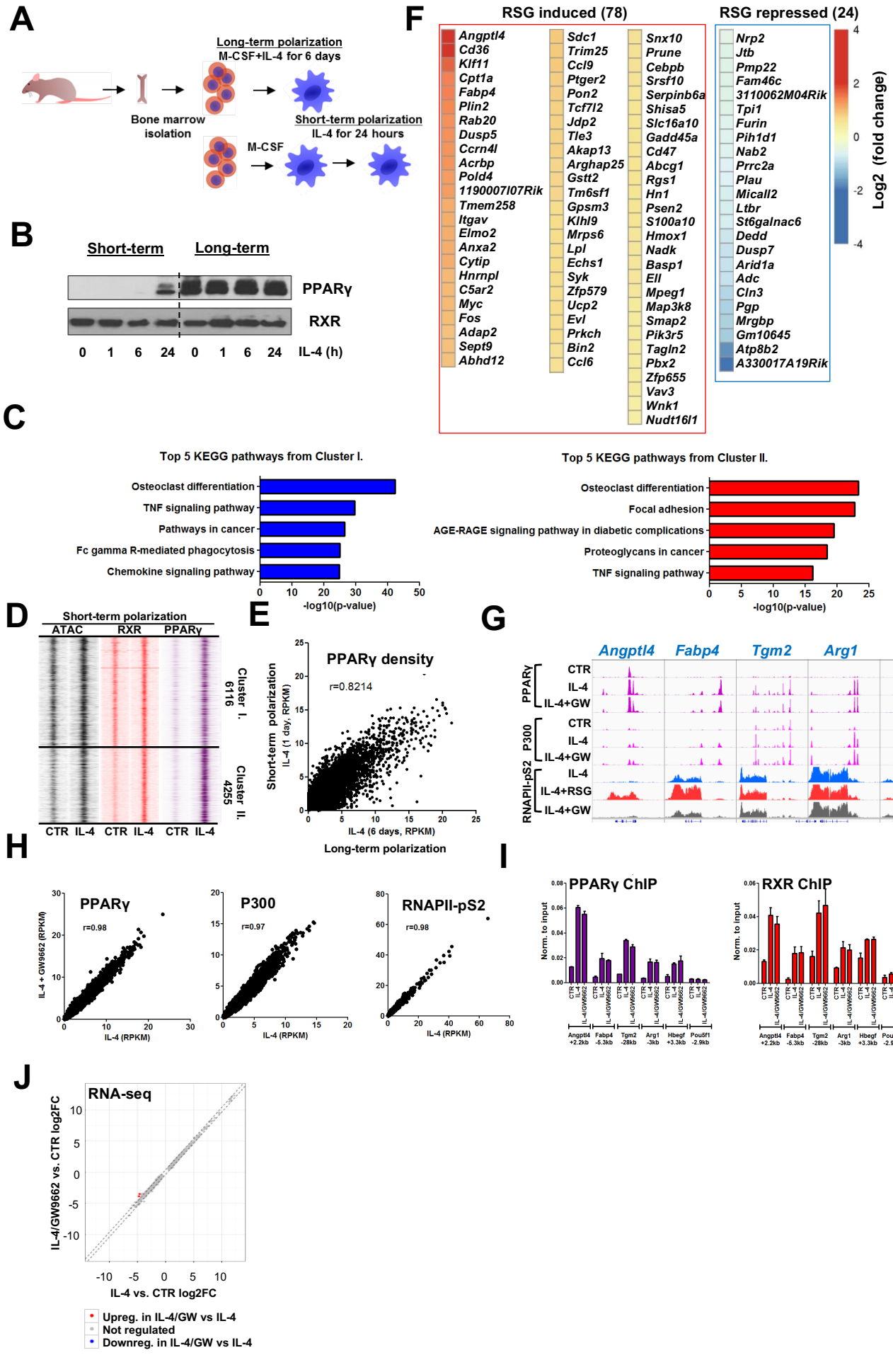
A
Long-term polarization
M-CSF+IL-4 for 6 days
Bone marrow isolation
Short-term polarization
IL-4 for 24 hours
M-CSF
B
Short-term
Long-term
PPARγ
RXR
0 1 6 24 0 1 6 24 IL-4 (h)
C
Top 5 KEGG pathways from Cluster I.
Osteoclast differentiation
TNF signaling pathway
Pathways in cancer
Fc gamma R-mediated phagocytosis
Chemokine signaling pathway
-log10(p-value)
Top 5 KEGG pathways from Cluster II.
Osteoclast differentiation
Focal adhesion
AGE-RAGE signaling pathway in diabetic complications
Proteoglycans in cancer
TNF signaling pathway
-log10(p-value)
D
Short-term polarization
ATAC
RXR
PPARγ
Cluster I. 6116
Cluster II. 4255
CTR IL-4 CTR IL-4 CTR IL-4
E
PPARγ density
r=0.8214
Short-term polarization
IL-4 (1 day, RPKM)
IL-4 (6 days, RPKM)
Long-term polarization
F
RSG induced (78)
Angptl4
Cd36
Klf11
Cpt1a
Fabp4
Plin2
Rab20
Dusp5
Ccrn4l
Acrbp
Pold4
1190007I07Rik
Tmem258
Itgav
Elmo2
Anxa2
Cytip
Hnrnpl
C5ar2
Myc
Fos
Adap2
Sept9
Abhd12
Sdc1
Trim25
Ccl9
Ptger2
Pon2
Tcf7l2
Jdp2
Tle3
Akap13
Arghap25
Gstt2
Tm6sf1
Gpsm3
Klhl9
Mrps6
Lpl
Echs1
Syk
Zfp579
Ucp2
Evl
Prkch
Bin2
Ccl6
Snx10
Prune
Cebpb
Srsf10
Serpinb6a
Shisa5
Slc16a10
Gadd45a
Cd47
Abcg1
Rgs1
Hn1
Psen2
S100a10
Hmox1
Nadk
Basp1
Ell
Mpeg1
Map3k8
Smap2
Pik3r5
Tagln2
Pbx2
Zfp655
Vav3
Wnk1
Nudt16l1
RSG repressed (24)
Nrp2
Jtb
Pmp22
Fam46c
3110062M04Rik
Tpi1
Furin
Pih1d1
Nab2
Prrc2a
Plau
Micall2
Ltbr
St6galnac6
Dedd
Dusp7
Arid1a
Adc
Cln3
Pgp
Mrgbp
Gm10645
Atp8b2
A330017A19Rik
Log2 (fold change)
G
Angptl4
Fabp4
Tgm2
Arg1
Hbegf
PPARγ
CTR
IL-4
IL-4+GW
P300
CTR
IL-4
IL-4+GW
RNAPII-pS2
IL-4
IL-4+RSG
IL-4+GW
H
PPARγ
r=0.98
IL-4 + GW9662 (RPKM)
IL-4 (RPKM)
P300
r=0.97
IL-4 (RPKM)
RNAPII-pS2
r=0.98
IL-4 (RPKM)
I
PPARγ ChIP
Norm. to input
Angptl4 +2.2kb
Fabp4 -5.3kb
Tgm2 -28kb
Arg1 -3kb
Hbegf +3.3kb
Pou5f1 -2.9kb
RXR ChIP
Norm. to input
Angptl4 +2.2kb
Fabp4 -5.3kb
Tgm2 -28kb
Arg1 -3kb
Hbegf +3.3kb
Pou5f1 -2.9kb
J
RNA-seq
IL-4/GW9662 vs. CTR log2FC
IL-4 vs. CTR log2FC
Upreg. in IL-4/GW vs IL-4
Not regulated
Downreg. in IL-4/GW vs IL-4

Figure S1 (Related to Figure 1). Characterization of the PPARγ:RXR heterodimer cistrome in alternatively polarized macrophages.

A; Scheme of macrophage polarization systems used in the study. Bone marrow-derived cells were either differentiated in the presence of M-CSF or M-CSF+IL-4 (5ng/ul) for 6 days. Short-term polarization was performed on M-CSF differentiated cells for 24 hours. **B;** Western blot analyses of total protein from the two polarization models. Polarization of M-CSF-differentiated cells were performed in the presence of IL-4 (20ng/ul) and samples were collected at the indicated time points. Long-term polarization was performed as described above. On the 6^{th} day of differentiation cells were exposed to IL-4 (20ng/ul) and samples were collected at the indicated time points. **C;** KEGG pathway analysis of the genes annotated to the PPARγ:RXR heterodimer-bound genomic regions from Cluster I. and II. Top five functional terms are shown. The closest genes that were located +/- 100kb from the heterodimer-bound loci were annotated yielding 1578 genes for Cluster I. and 1119 genes for Cluster II., respectively. **D;** Read distribution plot of ATAC-seq, PPARγ and RXR ChIP-seq in non-polarized (CTR) and IL-4 polarized (IL-4, 24h – short-term polarization) macrophages in a 1.5 kb window around the summit of the RXR peaks determined from the long-term polarization system shown in Fig. 1A. Cluster I. represents constitutive RXR-bound genomic regions, while cluster II. shows *de novo* PPARγ:RXR sites. **D;** Correlation plot of the PPARγ cistromes obtained from the two polarization settings. RPKM (Reads Per Kilobase per Million reads) values are presented. Pearson correlation coefficient is indicated. **E;** Heat maps depicting RSG-induced and repressed genes, regulated in the two replicates and showing at least 1.3 fold difference. Fold changes are relative to IL-4+vehicle-treated cells. **F;** Genome browser view of PPARγ, P300 and RNAPII-S2 enrichments in the presence of the indicated compounds (RSG-rosiglitazone, PPARγ agonist; GW-GW9662, PPARγ antagonist). **G;** Correlation plot of PPARγ, P300 and RNAPII-S2 RPKM (Reads Per Kilobase per Million reads) values from IL-4 and IL-4+GW9662-treated macrophages. Pearson correlation coefficient is indicated. **H;** ChIP-qPCR measurements for PPARγ and RXR in the presence of IL-4 and IL-4+GW9662 on the indicated enhancers. *Pou5f1* -2.9kb region was used as a negative control. Each bar represents mean +/- SD of duplicate determinations. **I;** Scatter plot depicting IL-4-regulated genes (based on CPM-Counts Per Million values) in the presence of GW9662. Genes not regulated by GW9662 are depicted in grey. Changes were considered significant using edgeR GLM (General Linear Model, $p<0.05$) and 1.3-fold change using two replicates for each conditions.

Figure S2

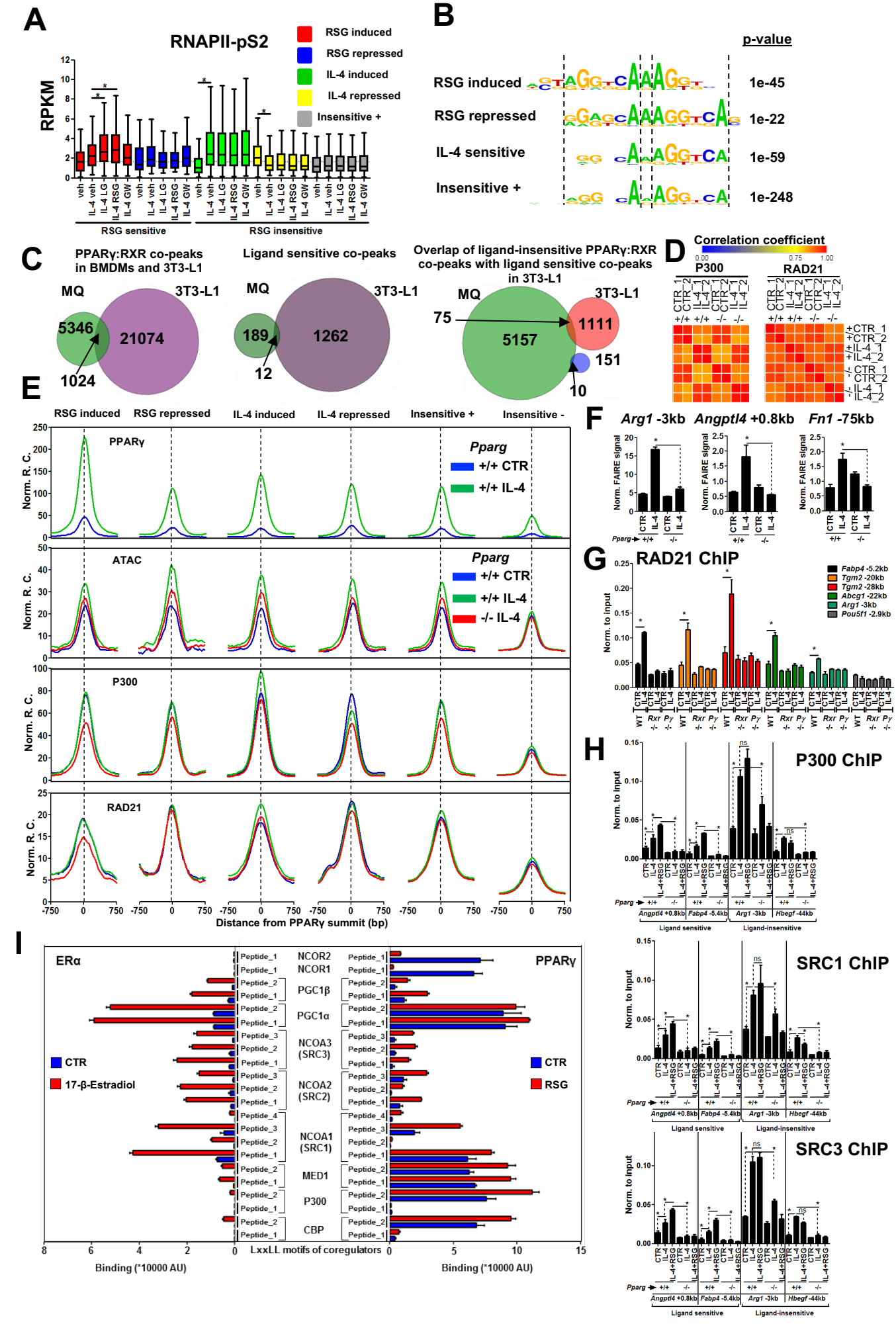
A
RNAPII-pS2
RPKM
RSG induced
RSG repressed
IL-4 induced
IL-4 repressed
Insensitive +
RSG sensitive
RSG insensitive
B
p-value
RSG induced
1e-45
RSG repressed
1e-22
IL-4 sensitive
1e-59
Insensitive +
1e-248
C
PPARγ:RXR co-peaks in BMDMs and 3T3-L1
MQ
3T3-L1
5346
21074
1024
Ligand sensitive co-peaks
189
1262
12
Overlap of ligand-insensitive PPARγ:RXR co-peaks with ligand sensitive co-peaks in 3T3-L1
75
1111
5157
151
10
D
Correlation coefficient
P300
RAD21
E
RSG induced
RSG repressed
IL-4 induced
IL-4 repressed
Insensitive +
Insensitive -
PPARγ
ATAC
P300
RAD21
Norm. R. C.
Pparg
+/+ CTR
+/+ IL-4
-/- IL-4
Distance from PPARγ summit (bp)
F
Arg1 -3kb
Angptl4 +0.8kb
Fn1 -75kb
Norm. FAIRE signal
G
RAD21 ChIP
Norm. to input
Fabp4 -5.2kb
Tgm2 -20kb
Tgm2 -28kb
Abcg1 -22kb
Arg1 -3kb
Pou5f1 -2.9kb
H
P300 ChIP
Ligand sensitive
Ligand-insensitive
I
ERα
PPARγ
CTR
17-β-Estradiol
RSG
NCOR2
NCOR1
PGC1β
PGC1α
NCOA3 (SRC3)
NCOA2 (SRC2)
NCOA1 (SRC1)
MED1
P300
CBP
LxxLL motifs of coregulators
Binding (*10000 AU)
SRC1 ChIP
SRC3 ChIP

Figure S2 (Related to Figure 2). Characterization of the ligand-sensitive, -insensitive PPARγ cistrome in alternatively activated macophages.

A; Box plot depicting RNAPII-pS2 enrichments (RPKM) in the presence of the indicated compounds (LG-LG268, RXR agonist; RSG, PPARγ agonist; GW-GW9662, PPARγ antagonist) determined by ChIP-seq on PPARγ:RXR-bound loci. "veh" stands for solvent control. Changes were considered significant at $p<0.05$ using two tailed paired t test using two replicates for each conditions. **B;** *De novo* motif enrichments for DR1 at the indicated enhancer groups. **C;** Venn diagram representation of the overlapping PPARγ:RXR-bound genomic regions between macrophages and 3T3-L1 adipocytes under the indicated analytical circumstances. **D;** Heat map representation of the Pearson correlation coefficients between the replicates of the P300 and RAD21 ChIP-seq experiments. **E;** Histograms depicting read enrichments for ATAC-seq, PPARγ, P300 and RAD21 ChIP-seq around RXR summits on the different enhancer categories from wild-type +/+ and *Pparg* -/- cells. One representative example of the two replicates is shown. **F;** FAIRE-qPCR analysis of chromatin accessibility on the indicated loci in wild-type +/+ and *Pparg* -/- macrophages in the presence or absence of IL-4. Mean +/- SD of duplicate determinations are shown and changes were considered significant using two tailed, unpaired t test at $p<0.05$. **G;** ChIP-qPCR measurements for RAD21 in the presence of IL-4 in *Pparg* -/- (*Pγ* -/-) and *Rxra/b* -/- (*Rxr* -/-) macrophages on the indicated enhancers. *Pou5f1* -2.9kb region was used as a negative control. **H;** ChIP-qPCR analysis for P300, SRC1 and SRC2 in wild-type +/+ and *Pparg* -/- macrophages in the absence (CTR) and presence of IL-4 or IL-4+RSG on the indicated gene loci. ChIP-qPCR on panel G and H represents mean +/- SD of at least two replicates for all the qPCR-based measurements, and changes were considered significant at $p<0.05$ using two tailed unpaired t test. **I;** Bar graph representation of MARCoNI assay results for the indicated coregulator peptides in the presence of apo- (unliganded) and holo-PPARγ and ERα receptor ligand binding domains. Mean +/- SEM of four replicate measurements are shown.

Figure S3

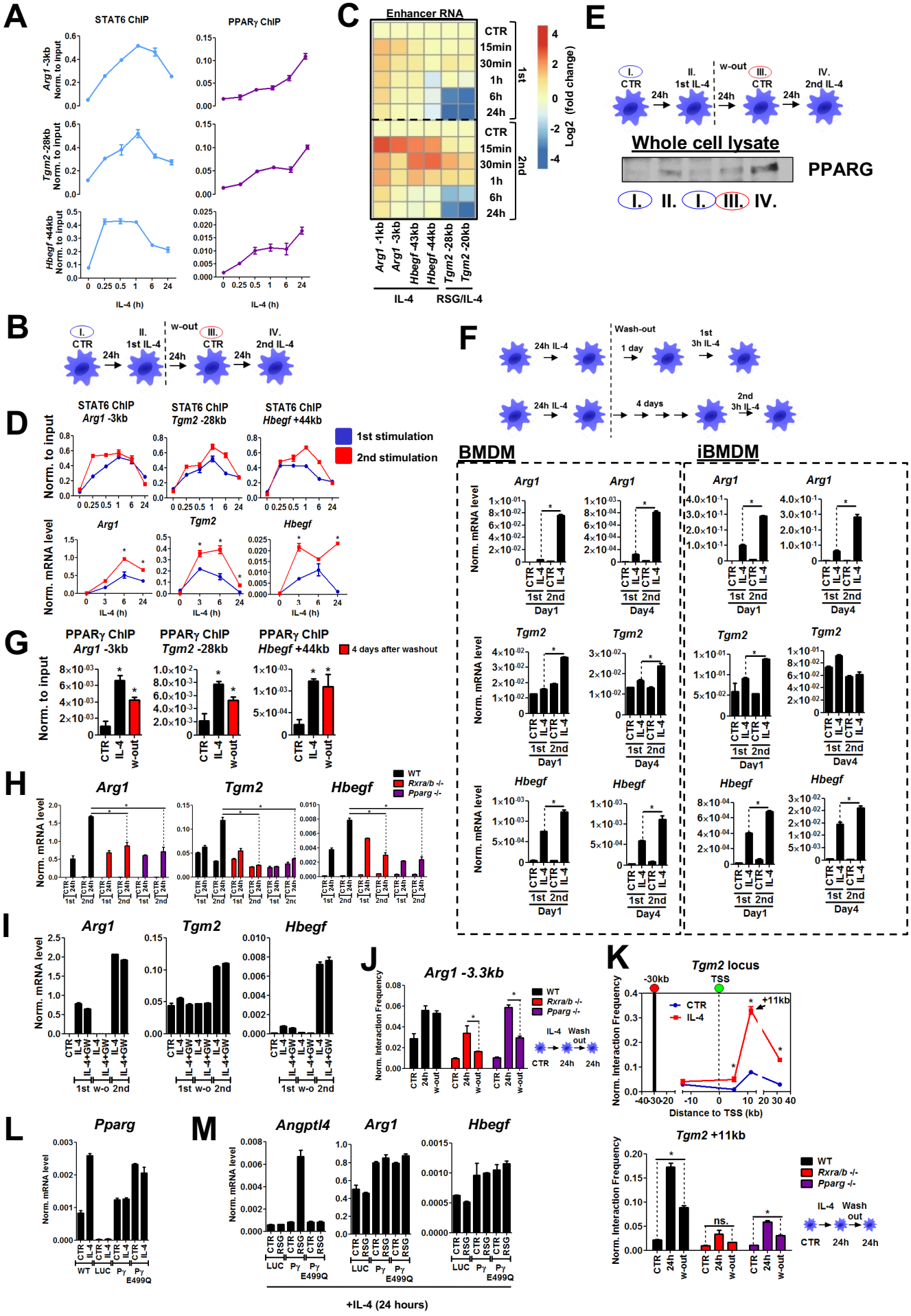
A
STAT6 ChIP
PPARγ ChIP
Arg1 -3kb Norm. to Input
Tgm2 -28kb Norm. to Input
Hbegf +44kb Norm. to Input
IL-4 (h)
B
I. CTR
II. 1st IL-4
w-out
III. CTR
IV. 2nd IL-4
24h
C
Enhancer RNA
CTR
15min
30min
1h
6h
24h
1st
2nd
Log2 (fold change)
Arg1 -1kb
Arg1 -3kb
Hbegf -43kb
Hbegf -44kb
Tgm2 -28kb
Tgm2 -20kb
IL-4
RSG/IL-4
D
STAT6 ChIP
Arg1 -3kb
Tgm2 -28kb
Hbegf +44kb
1st stimulation
2nd stimulation
Norm. to input
Norm. mRNA level
E
Whole cell lysate
PPARG
F
24h IL-4
Wash-out
1 day
1st 3h IL-4
4 days
2nd 3h IL-4
BMDM
iBMDM
Arg1
Tgm2
Hbegf
Norm. mRNA level
Day1
Day4
G
PPARγ ChIP
4 days after washout
Norm. to input
H
WT
Rxra/b -/-
Pparg -/-
I
1st w-o 2nd
J
Arg1 -3.3kb
Norm. Interaction Frequency
IL-4 Wash out
K
Tgm2 locus
-30kb
TSS
+11kb
CTR
IL-4
Distance to TSS (kb)
Tgm2 +11kb
ns.
L
Pparg
WT LUC Pγ Pγ E499Q
M
Angptl4
+IL-4 (24 hours)

Figure S3 (Related to Figure 3). Transcriptional memory to IL-4 is predominant and it requires PPARγ on a select set of genes.

A; ChIP-qPCR measurements carried out for STAT6 and PPARγ on the indicated IL-4 time course in wild type macrophages. Each data point represent mean +/- SD of duplicate determinations. **B;** Experimental setup to study transcriptional memory in macrophages. Cells were stimulated with IL-4 for 24 hours (24h, II. 1st IL-4) or left untreated (I. CTR). After stimulation, IL-4 containing media is removed followed by extensive washing steps (w-out) and 24 hours of incubation and collected (III. CTR) or the 2nd stimulation was performed (IV. 2nd IL-4). **C;** Heat map representation of enhancer RNA measurements by qPCR on the indicated loci. First (1st) and second (2nd) IL-4 simulation was performed for the indicated period of time in the same experimental system as described for panel B. Heat map represents log2 fold change from three independent measurements. Enhancers sensitive to IL-4 only or IL-4 and RSG are marked at the bottom. **D;** STAT6 ChIP-qPCR and gene expression (mRNA) measurements performed on the presented genes and gene loci in the presence of IL-4 for the indicated period of time. 2nd stimulation was performed after 24 hours of IL-4 pre-treatment followed by wash out, resting (24 hours) and restimulation for the indicated periods of time. Mean +/- SD of duplicate (ChIP) and triplicate (mRNA) measurements are shown and significant changes were determined at $p<0.05$ using two tailed unpaired t test. **E;** Western blot from IL-4 stimulated macrophages according to the experimental setup presented on panel B, for PPARγ. Blue (basal without IL-4 stimulation) and red circles (after first IL-4 stimulation and wash-out) show the two control states. **F;** Experimental setup used to study transcriptional memory after 4 days of IL-4 exposure in macrophages. Cells were stimulated with IL-4 for 24 hours (24h, 1st IL-4) or left untreated (CTR, 1st). After stimulation, IL-4 containing media were removed followed by extensive washing steps (wash-out). Cells were further incubated for 24 hours (1 day) or for 4 days in fresh media and collected (2nd CTR) or second stimulation was performed with IL-4 (2nd IL-4) (top). Gene expression measurements (mRNA) of the indicated genes was performed in primary macrophages bone marrow-derived macrophages (BMDM) and in immortalized BMDMs (iBMDM) using the setup presented above (bottom). Mean +/- SD of triplicate determinations are shown and considered significant at $p<0.05$ using two tailed unpaired t test. **G;** ChIP-qPCR measurements for PPARγ on the indicated enhancers after the first stimulation and 4 days after wash-out (red). Two tailed, unpaired t tests were performed and significant differences are indicated at $p<0.05$ (n=2). **H;** Gene expression (mRNA) analysis of *Arg1, Tgm2* and *Hbegf* upon first (1st) and second (2nd) IL-4 exposure from wild type (WT), *Pparg* and *Rxra/b* -/- cells. Each data point represents mean +/- SD of triplicate determinations and changes were considered significant at $p<0.05$ using two tailed unpaired t test. **I,** Gene expression analysis (mRNA) of *Arg1, Tgm2* and *Hbegf* upon first (1st) IL-4 exposure, after wash-out (w-o) and after second (2nd) IL-4 stimulation in the presence or absence of the PPARγ antagonist GW9662 (GW). Mean +/- SD of triplicate measurements are presented. **J,** 3C-qPCR carried out at the *Arg1* locus in *Rxra/b* -/- and *Pparg* -/- macrophages and interaction frequency is presented at the focal point of the interaction (+11kb). Experimental scheme is also shown. Each data point represents mean +/- SD of duplicate determinations. Differences were considered significant at $p<0.05$ using two tailed unpaired t test. **K;** 3C-qPCR performed on the *Tgm2* locus. Each data point represents mean +/- SD of duplicate determinations. Differences were considered significant at $p<0.05$ using two tailed unpaired t test. **L;** Gene expression (mRNA) measurements of *Pparg* in wild type (WT) and *Pparg* -/- macrophages expressing luciferase, wild type PPARγ (Pγ) and transactivation function mutant PPARγ (Pγ E499Q). Mean +/- SD of triplicate determinations are shown. **M;** Gene expression (mRNA) measurements of the indicated genes from the same model as on panel L. Cells were either treated with RSG or left untreated (CTR) to test ligand inducible gene transcription. Mean +/- SD of triplicate measurements are shown.

Figure S4

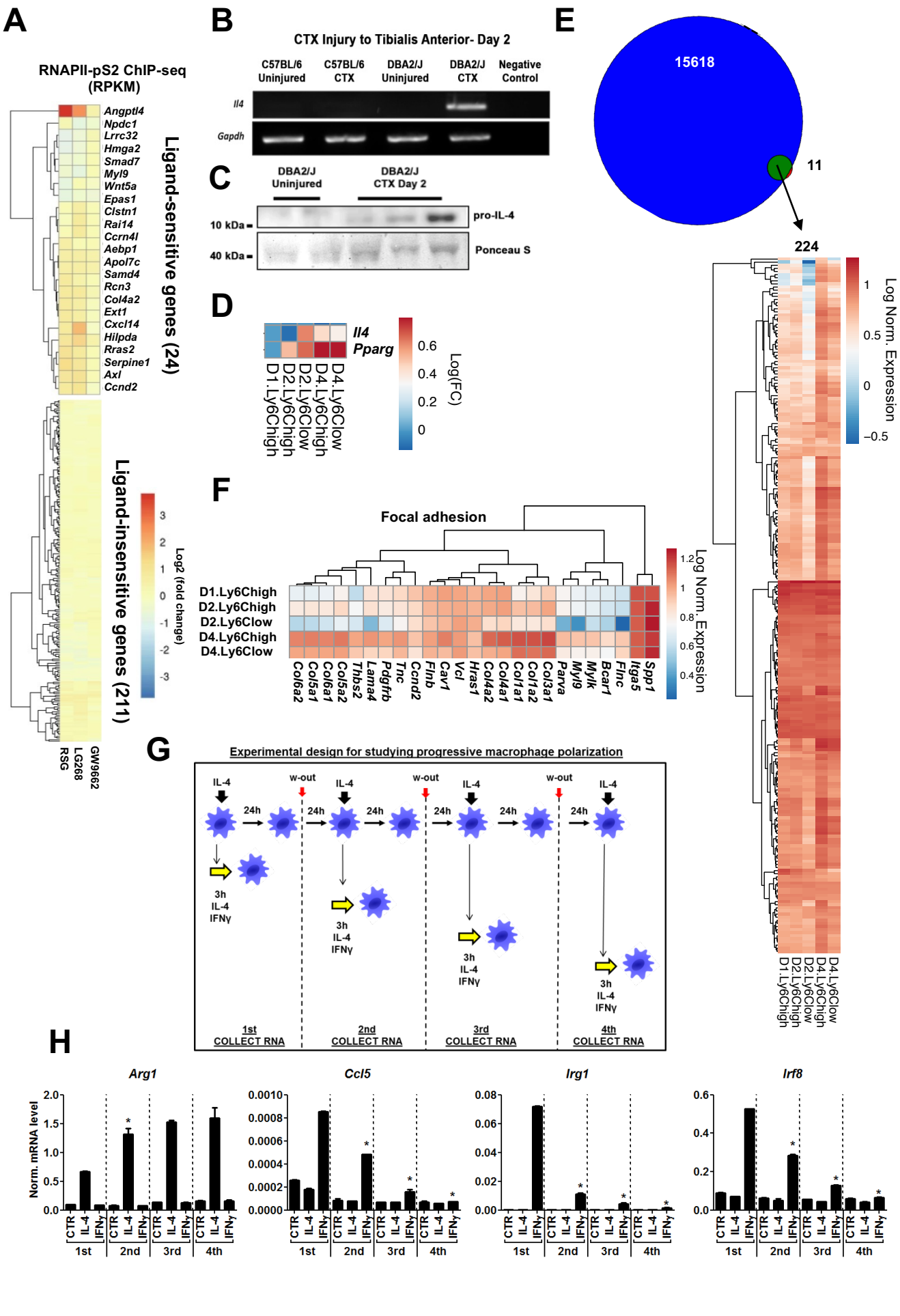
A
RNAPII-pS2 ChIP-seq (RPKM)
Angptl4
Npdc1
Lrrc32
Hmga2
Smad7
Myl9
Wnt5a
Epas1
Clstn1
Rai14
Ccrn4l
Aebp1
Apol7c
Samd4
Rcn3
Col4a2
Ext1
Cxcl14
Hilpda
Rras2
Serpine1
Axl
Ccnd2
Ligand-sensitive genes (24)
Ligand-insensitive genes (211)
Log2 (fold change)
RSG
LG268
GW9662
B
CTX Injury to Tibialis Anterior- Day 2
C57BL/6 Uninjured
C57BL/6 CTX
DBA2/J Uninjured
DBA2/J CTX
Negative Control
Il4
Gapdh
C
DBA2/J Uninjured
DBA2/J CTX Day 2
10 kDa
40 kDa
pro-IL-4
Ponceau S
D
Il4
Pparg
D1.Ly6Chigh
D2.Ly6Chigh
D2.Ly6Clow
D4.Ly6Chigh
D4.Ly6Clow
Log(FC)
E
15618
11
224
Log Norm. Expression
F
Focal adhesion
D1.Ly6Chigh
D2.Ly6Chigh
D2.Ly6Clow
D4.Ly6Chigh
D4.Ly6Clow
Col6a2
Col5a1
Col6a1
Col5a2
Thbs2
Lama4
Pdgfrb
Tnc
Ccnd2
Flnb
Cav1
Vcl
Hras1
Col4a2
Col4a1
Col1a1
Col1a2
Col3a1
Parva
Myl9
Mylk
Bcar1
Flnc
Itga5
Spp1
Log Norm. Expression
G
Experimental design for studying progressive macrophage polarization
IL-4
w-out
24h
3h
IL-4
IFNγ
1st COLLECT RNA
2nd COLLECT RNA
3rd COLLECT RNA
4th COLLECT RNA
H
Arg1
Ccl5
Irg1
Irf8
Norm. mRNA level
CTR
IL-4
IFNγ
1st
2nd
3rd
4th

Figure S4 (Related to Figure 4). Newly infiltrating macrophages possess and progressively induce the gene signature of IL-4/PPARγ-dependent transcription memory during muscle regeneration.

A; Heat map depicting the RPKM values of RNAPII-S2 ChIP-seq results in the presence of the solvent control (CTR), RXR agonist (LG268), PPARγ agonist (RSG) and PPARγ antagonist (GW9662) on the gene set exhibiting PPARγ-mediated transcriptional memory. DiffBind analysis was performed to identify significantly ($p<0.05$) changing RNAPII-pS2 enrichments on the gene bodies. Genes significantly changing and exhibiting at least 1.5 fold difference compared to solvent control are considered ligand sensitive using two replicates. **B;** PCR analysis of the expression of *Il4* and *Gapdh* in DBA2J uninjured and cardiotoxin (CTX) injured whole muscle lysates. **C;** Western blot for pro-IL-4 in DBA2J uninjured and CTX injured whole muscle lysates. **D;** Heat map representation (Log_{10}(Fold Change)) of *Il4* and *Pparg* expression during the time course of regeneration in DBA2J mice derived sorted macrophages following the injury. **E;** Venn diagram representation of the overlap between the gene signature of macrophages derived from the regenerating tissue of DBA2J mice and the gene set observed on Figure 4B. Heat map depicts the overlapping section containing 224 genes (values represented as Log_{10}(normalized expression)). **F;** Heat map representation (Log_{10}(normalized expression)) of the genes related to the focal adhesion functional category (as determined on Figure 4F) from the DBA2J muscle infiltrating macrophages (D, day). **G;** Experimental model used to study progressive macrophage polarization. **H;** Gene expression measurements at the mRNA level for *Arg1*, *Ccl5*, *Irg1* and *Irf8* after the indicated number of IL-4 stimulations (see experimental setup on panel G) followed by either IL-4 or IFNγ exposure. Mean +/- SD of triplicate measurements are shown and differences were considered significant at $p<0.05$ using two tailed unpaired t test.

Supplemental table titles

Table S1: MARCoNI data set of coregulator peptides. Related to Figure S2.

Table S2: Primers used in this study. Related to Figures 1, 2, 3, 4 and all the supplemental figures.

Table S3: QC of sequencing data. Related to Figures 1, 2, 3, 4 and all the supplemental figures.