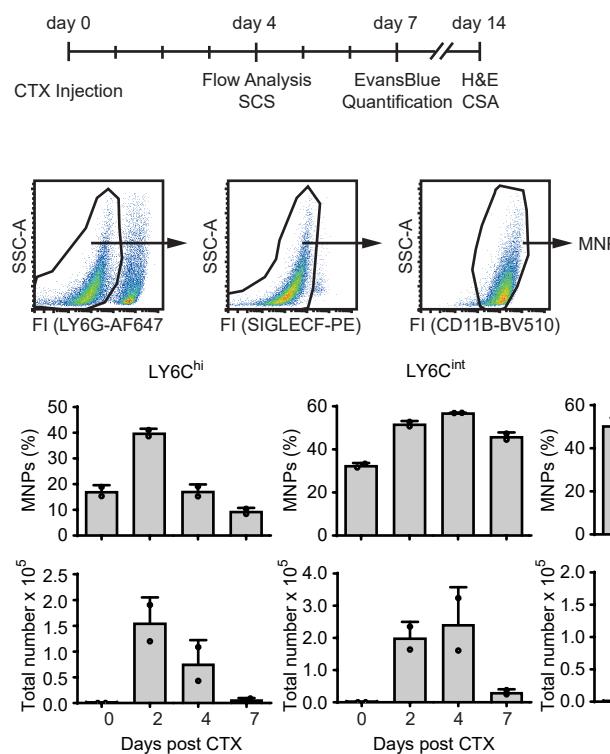


Supplemental information

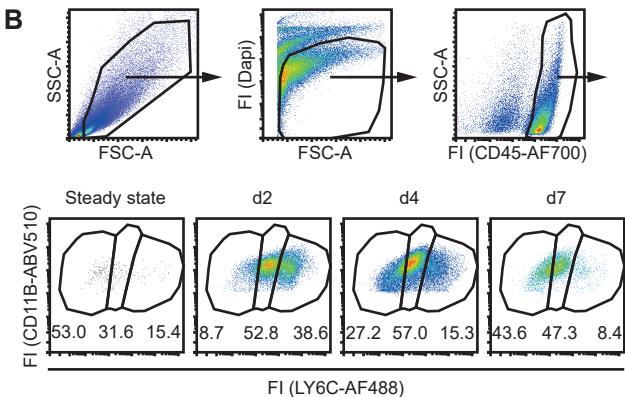
**IL-33-induced metabolic reprogramming
controls the differentiation of alternatively activated
macrophages and the resolution of inflammation**

Maria Faas, Natacha Ipseiz, Jochen Ackermann, Stephan Culemann, Anika Grüneboom, Fenja Schröder, Tobias Rothe, Carina Scholtysek, Martin Eberhardt, Martin Böttcher, Philipp Kirchner, Cornelia Stoll, Arif Ekici, Maximilian Fuchs, Meik Kunz, Benno Weigmann, Stefan Wirtz, Roland Lang, Joerg Hofmann, Julio Vera, David Voehringer, Alessandro Michelucci, Dimitrios Mougiakakos, Stefan Uderhardt, Georg Schett, and Gerhard Krönke

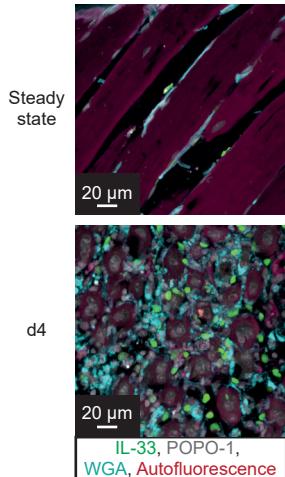
A Timeline Muscle Injury



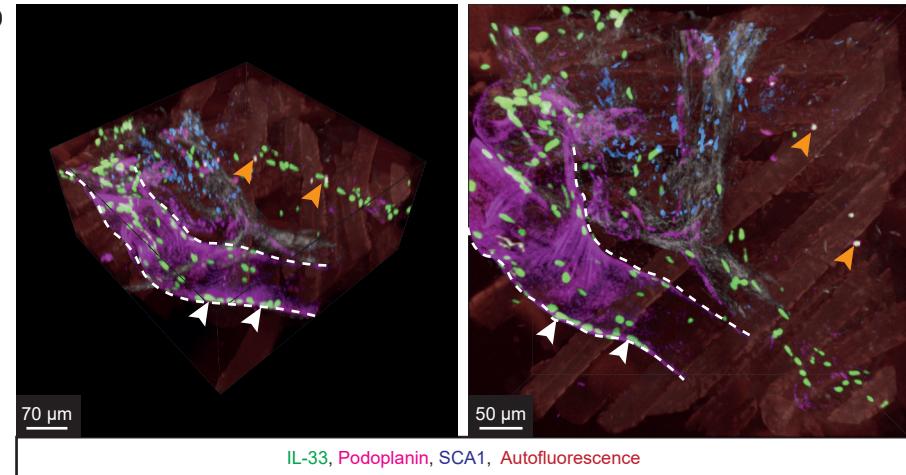
B



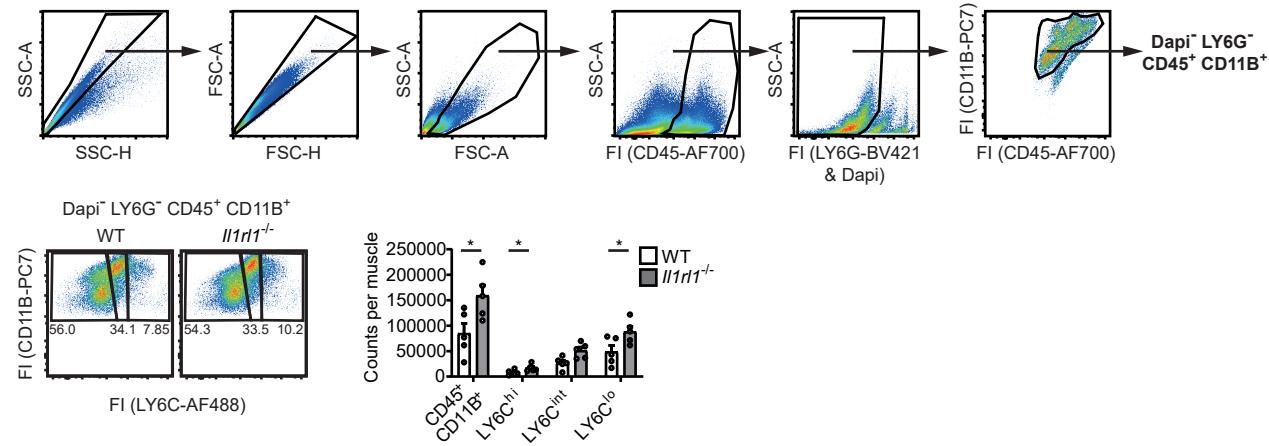
C



D



E



Supplementary Figure 1: IL-33 promotes the resolution of injury-induced inflammation and the differentiation of AAMs. Related to Figure 1.

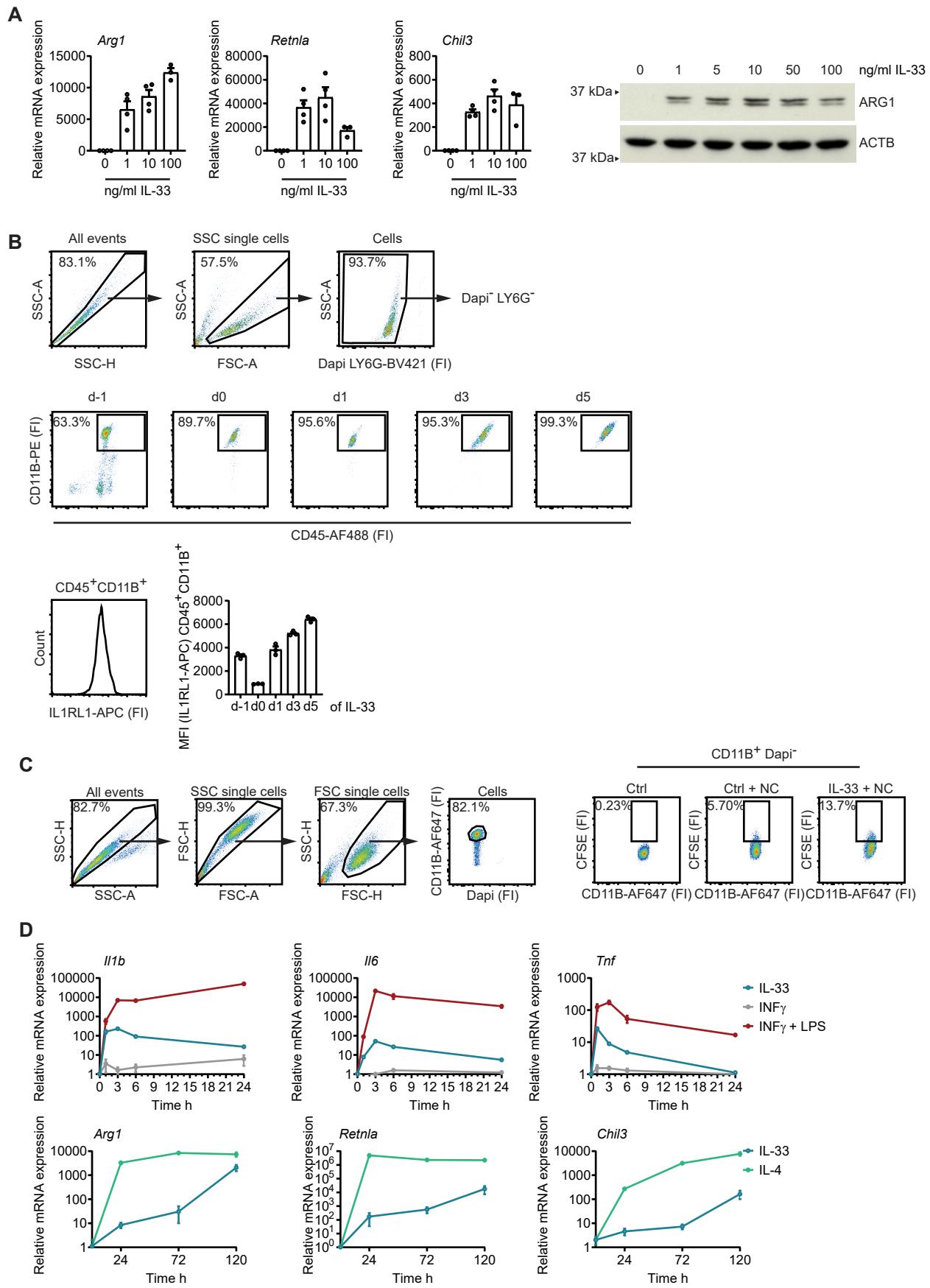
(A) Overview of CTX-induced muscle injury and subsequent analyses.

(B) Flow cytometry of injured muscles at indicated days post CTX Injection. Bar graphs show the percentage as well as the absolute numbers of Dapi⁻, LY6G⁻, SIGLECF⁻, CD45⁺, CD11B⁺ cells, referred to as mononuclear phagocytes (MNP), distinguished by the intensity of LY6C signal.

(C-D) Immunofluorescence microscopy of muscle. IL-33 depicted in green, podoplanin in magenta, SCA1 in blue, wheat germ agglutinin (WGA) in cyan and autofluorescence in red. Muscle taken from steady state (C upper panel and D) as well as 4 d post CTX Injection (C lower panel). White dashed lines indicate a podoplanin⁺ vessel structure; White arrows indicate exemplary podoplanin⁺ and IL-33⁺ cells; orange arrows indicate other IL-33⁺ cells. See also Movie S1.

(E) Flow cytometry of injured muscles at day 4 post CTX injection gated on single mononuclear phagocytes (Dapi⁻, LY6G⁻, CD45⁺, CD11B⁺). Graph shows absolute counts per muscle. WT (n=5) and *Il1rl1*^{-/-} (n=5).

Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001.



Supplementary Figure 2: IL-33 triggers the IL-4-independent differentiation of AAMs. Related to Figure 1.

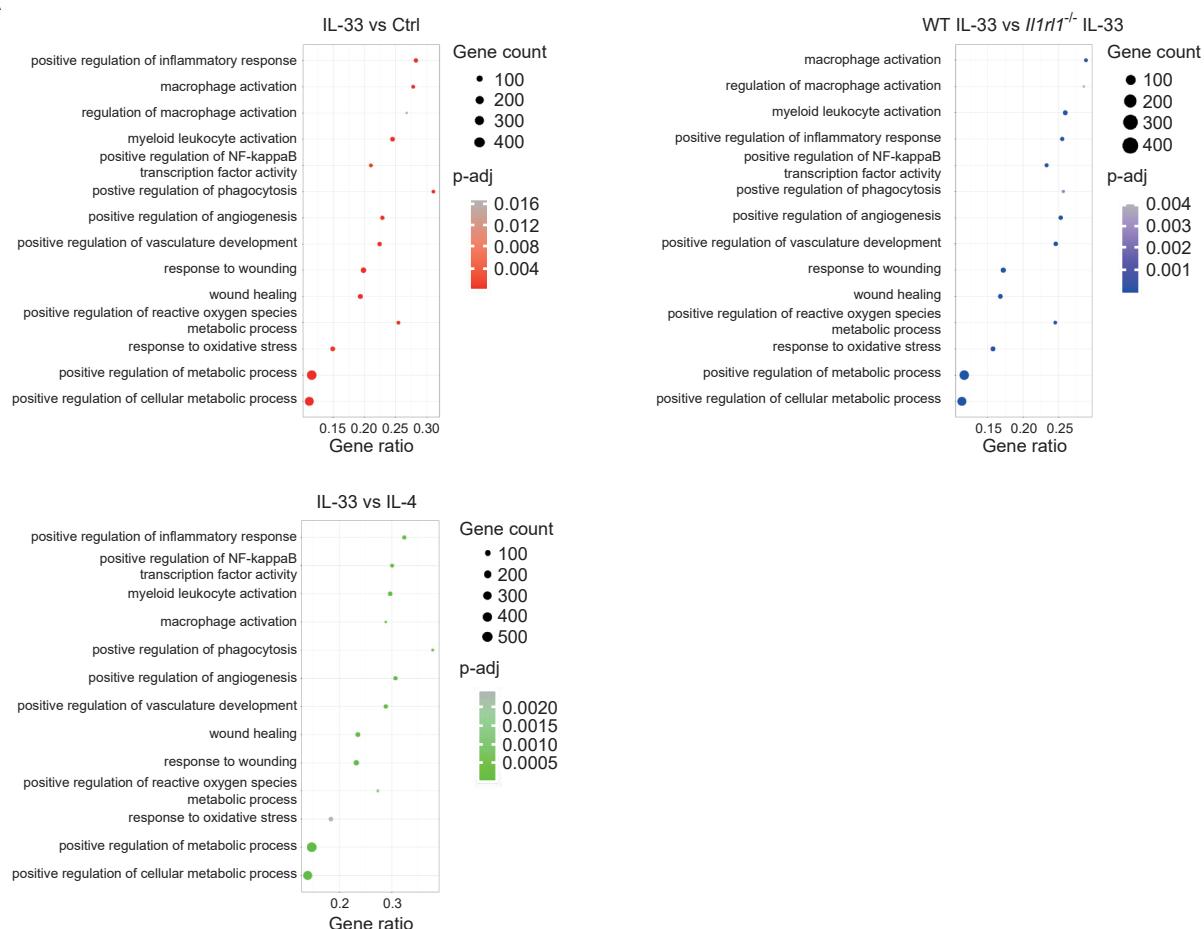
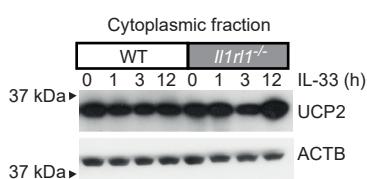
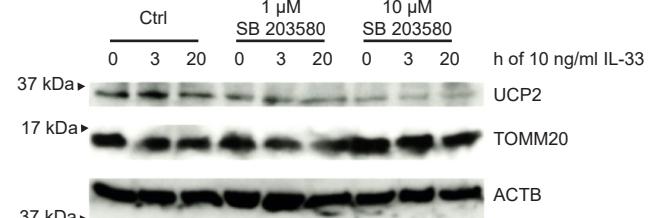
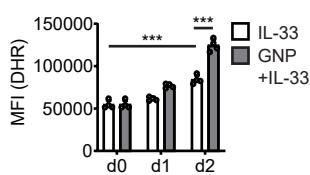
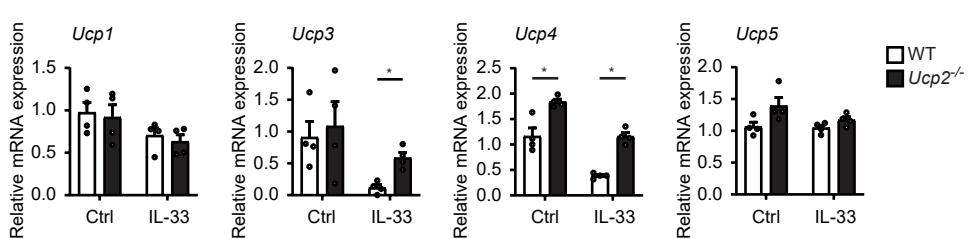
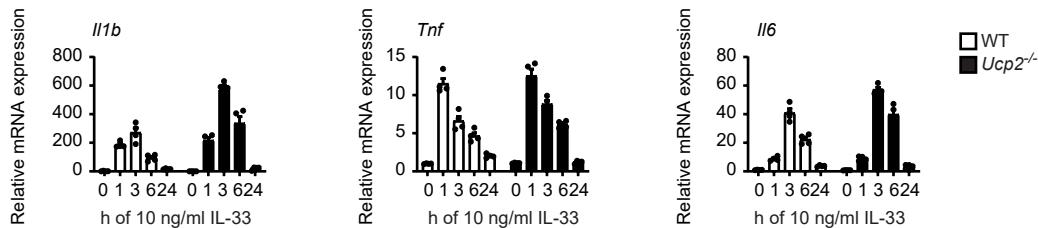
(A) Quantitative real-time PCR-based measurement of mRNA expression of indicated genes and western blot-based determination of ARG1 expression in BMDMs upon stimulation with vehicle (Ctrl) or IL-33 at the indicated concentrations for 5 days.

(B) Flow cytometry determining IL1RL1 expression on BMDMs treated with 10 ng/ml IL-33 for the indicated time points with d-1 indicating the day of isolation and d0 indicating the day of IL-33 stimulation. Histogramm shows IL1RL1 expression on day 5.

(C) Gating strategy to measure the uptake of necrotic cells (NC) in BMDM treated with vehicle (Ctrl) or IL-33 (10 ng/ml for 5d).

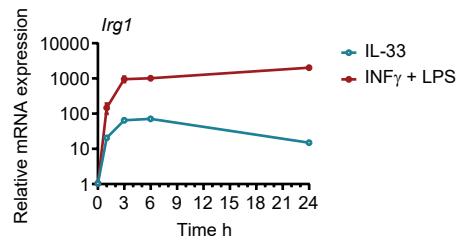
(D) Relative mRNA expression of markers for classical activation (*Il1b*, *Il6*, *Tnf*) or markers for alternative activation in macrophages (*Arg1*, *Retnla*, *Chil3*) BMDMs upon stimulation with IL-33 (10 ng/ml), INF γ (10 ng/ml), INF γ + LPS (10 ng/ml) or IL-4 (20 ng/ml) for the indicated time points.

Data are presented as mean + SEM

A**B****C****D****E****F**

**Supplementary Figure 3: IL-33 induced mitochondrial rewiring promotes AAM differentiation.
Related to Figure 3.**

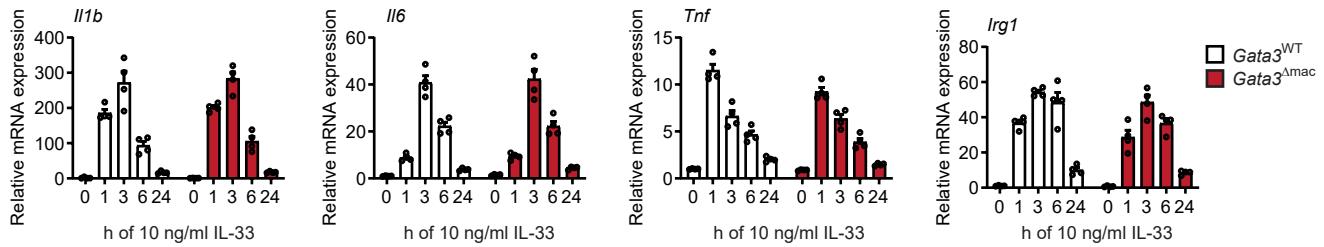
- (A) Pathway analysis of bulk mRNA sequencing data comparing vehicle (Ctrl) treated, IL-33 (10 ng/ml for 36 h) and IL-4 (20 ng/ml for 36 h) treated BMDM from WT or *Il1rl1*^{-/-}.
- (B) Western Blot of UCP2 protein from cytoplasmic fractions of WT or *Il1rl1*^{-/-} BMDM treated with IL-33 (10 ng/ml) for the indicated time points. Samples shown are the corresponding cytoplasmic fractions to the mitochondrial fractions shown in Fig. 3 G.
- (C) Western Blot of UCP2 protein from mitochondrial fractions BMDM treated with IL 33 (10 ng/ml) and/or SB203580 (1 μ M and 10 μ M) for the indicated time points.
- (D) Flow cytometry data showing the mean fluorescence intensity (MFI) of DHR in BMDM treated with GNP (100 μ M) and/or IL-33 (10 ng/ml) for the indicated time points.
- (E-F) Quantitative real-time PCR-based measurement of mRNA expression of indicated genes in BMDMs isolated from WT or *Ucp2*^{-/-} mice treated with vehicle (Ctrl) or IL-33 (10 ng/ml for 5d).
- Data are presented as mean + SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 4: IL-33-mediated mitochondrial uncoupling results in the induction of the transcription factor GATA3. Related to Figure 4.

Relative mRNA expression of *Irg1* in BMDMs upon stimulation with IL-33 (10 ng/ml) or INF γ (10 ng/ml) + LPS (10 ng/ml) for the indicated time points.

Data are presented as mean +/- SEM.

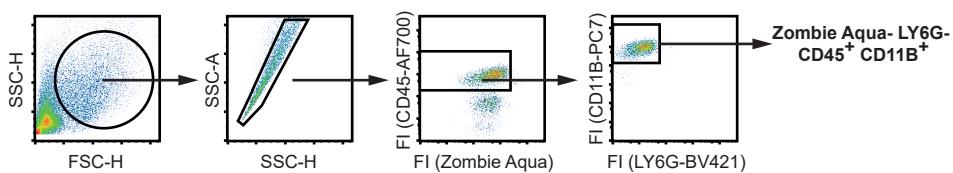


Supplementary Figure 5: GATA3 controls the IL-33-induced polarization of AAMs. Related to Figure 5.

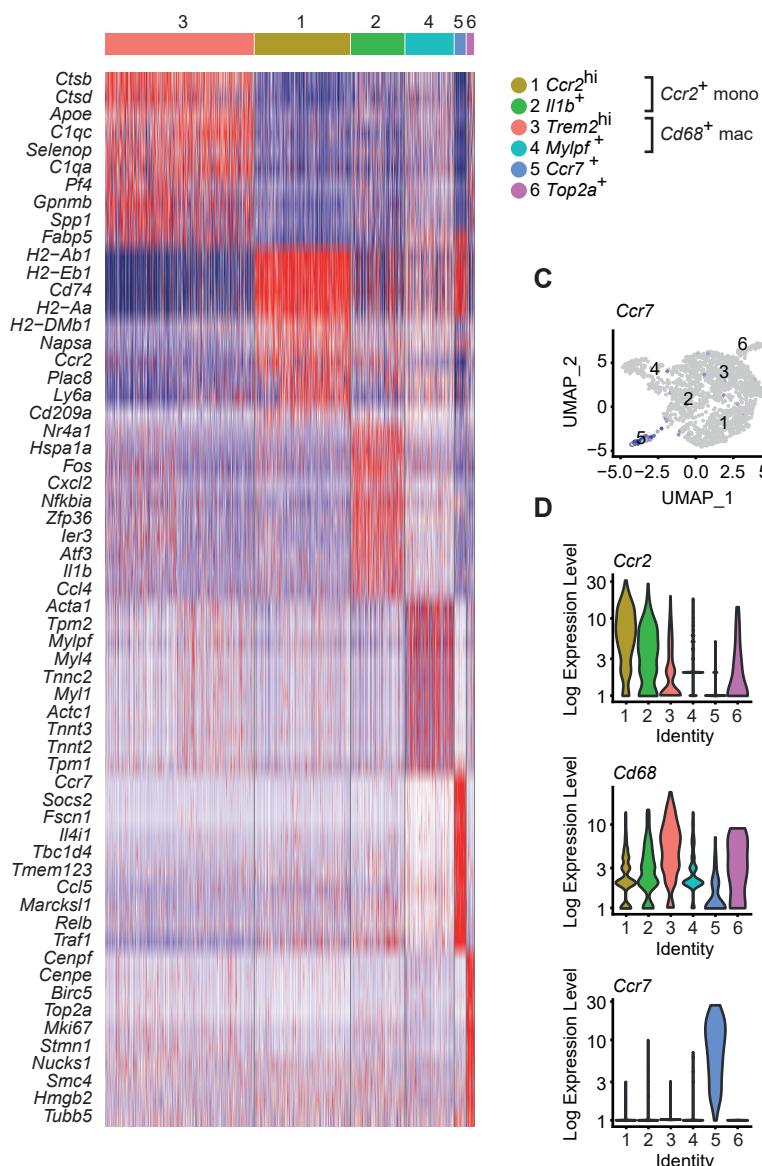
Relative mRNA expression of indicated genes in BMDMs isolated from *Gata3^{WT}* and *Gata3^{Δmac}* mice upon stimulation with IL-33 (10 ng/ml) for the indicated time points. Data was generated from mRNA isolated from the same experiment as data shown in supplementary figure 3F.

Data are presented as mean + SEM.

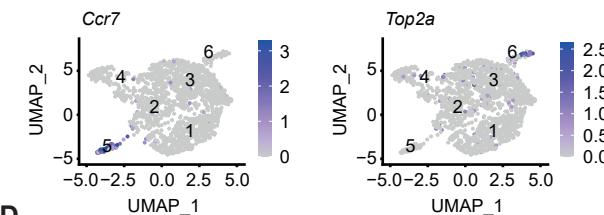
A Gating strategy single cell sequencing



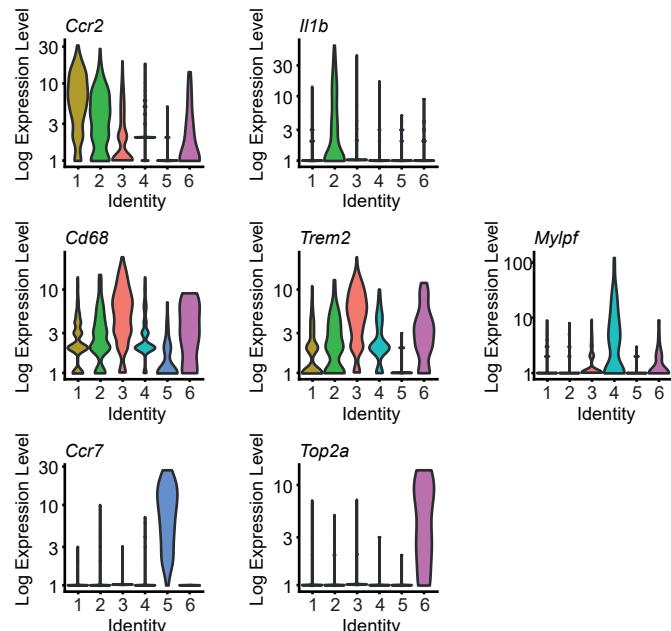
B Top 10 heatmap of cluster markers



C



D



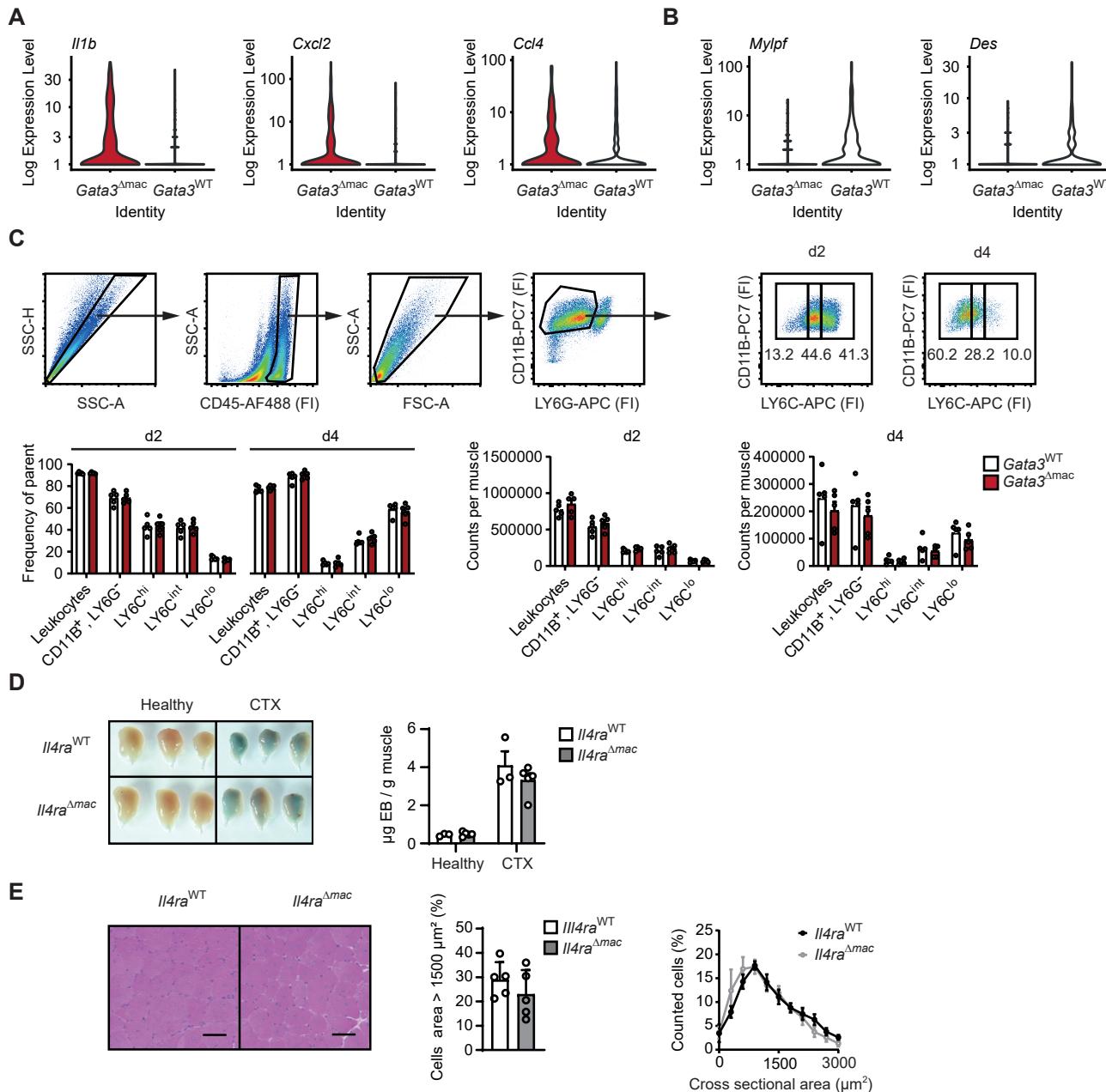
Supplementary Figure 6: Muscle injury results in the differentiation of pro-resolving macrophages. Related to Figure 6.

(A) Gating strategy during FACS-based sort of mononuclear phagocytes prior to scRNAseq.

(B) Heatmap of the top 10 cluster markers in the identified clusters based on scRNAseq analysis of mononuclear phagocytes.

(C) UMAP visualization of the clustermarkers *Ccr7* and *Top2a*.

(D) Violin plot visualization of the cluster markers *Ccr2*, *I1b*, *Cd68*, *Trem2*, *Mylpf*, *Ccr7* and *Top2a*.



Supplementary Figure 7: GATA3 controls the differentiation of pro-resolving macrophages upon muscle injury. Related to Figure 7.

(A-B) Violin plot visualization of differentially expressed genes *II1b*, *Cxcl2* & *Cc14* in Cluster 1 & 2 (A) and *Mylpf* & *Des* in Cluster 3 & 4 (B).

(C) Flow cytometry of injured muscles day 2 and day 4 post CTX injection. Gated on single Dapi⁺CD45⁺CD11B⁺LY6G⁻ mononuclear phagocytes. Graphs show frequency of cells as well as absolute counts per muscle. *Gata3*^{WT} (n=5) and *Gata3*^{Δmac} (n=5) mice.

(D) Macroscopic pictures and quantification of Evans Blue accumulation in healthy and CTX injected muscles of *II4ra*^{WT} (n=3) *II4ra*^{Δmac} (n=4) mice on day 7 after injury.

(E) Histology, quantification and comparison of cross sectional areas from muscle sections of CTX injected *II4ra*^{WT} (n=5) and *II4ra*^{Δmac} (n=5) mice on day 14 after injury. Scale bar indicates 50 µm.

Data are presented as mean + SEM.

Supplementary Table 1: Top 50 DEG of bulk mRNA-Seq: *Gata3*^{Δmac} IL-33 vs WT IL-33. Related to Figure 5.

Gene	KoIL33.value	WtIL33.value	log2foldchange	pvalue	padjust	significance	gene_name
ENSMUSG00000019987	28,18572395	256,2393468	-3,184455364	4,13E-166	7,02E-162	TRUE	<i>Arg1</i>
ENSMUSG00000020334	35,8700529	3,722795792	3,268321507	3,05E-146	2,59E-142	TRUE	<i>Slc22a4</i>
ENSMUSG00000030117	61,92927071	13,25108873	2,224510594	3,11E-82	1,77E-78	TRUE	<i>Gdf3</i>
ENSMUSG00000038085	4,854883006	18,64067379	-1,940945456	3,68E-46	7,83E-43	TRUE	<i>Cnbd2</i>
ENSMUSG00000020388	7,019206263	23,82490298	-1,763090434	8,48E-42	1,44E-38	TRUE	<i>Pdlim4</i>
ENSMUSG00000025453	11,52867296	29,50779923	-1,355869793	2,11E-39	3,13E-36	TRUE	<i>Nnt</i>
ENSMUSG00000078566	109,4056215	300,1749391	-1,456116569	2,21E-39	3,13E-36	TRUE	<i>Bnip3</i>
ENSMUSG0000007682	2,293531838	0,438762612	2,386058486	3,11E-34	4,07E-31	TRUE	<i>Dio2</i>
ENSMUSG00000025150	92,98250155	249,5839192	-1,424493733	1,15E-33	1,39E-30	TRUE	<i>Cbr2</i>
ENSMUSG00000063458	8,566913697	1,393083277	2,620494092	5,31E-33	6,02E-30	TRUE	<i>1700112E06Rik</i>
ENSMUSG00000033207	13,63871071	5,121529565	1,413060674	6,30E-32	6,70E-29	TRUE	<i>Mamdc2</i>
ENSMUSG00000035448	1,623918233	5,065208045	-1,641142423	8,02E-32	8,02E-29	TRUE	<i>Ccr3</i>
ENSMUSG000000102418	1,247948877	4,592171429	-1,879617487	3,58E-26	3,38E-23	TRUE	<i>Sh2d1b1</i>
ENSMUSG00000006154	2,01118574	7,092829426	-1,818314771	4,52E-26	4,05E-23	TRUE	<i>Eps8l1</i>
ENSMUSG00000035352	12,24681893	40,1043899	-1,7111352929	1,81E-24	1,46E-21	TRUE	<i>Ccl12</i>
ENSMUSG00000055782	7,435101632	15,00573897	-1,013089932	5,77E-24	4,46E-21	TRUE	<i>Abcd2</i>
ENSMUSG00000021796	1,945109035	0,276789395	2,812990473	8,76E-24	6,48E-21	TRUE	<i>Bmpr1a</i>
ENSMUSG00000057897	1,751098911	5,384034886	-1,620427036	1,70E-23	1,20E-20	TRUE	<i>Camk2b</i>
ENSMUSG00000078636	17,42644779	36,41041773	-1,063072696	2,94E-21	2,00E-18	TRUE	<i>Gm7336</i>
ENSMUSG00000031762	22,10234377	52,80182562	-1,25638834	5,52E-21	3,61E-18	TRUE	<i>Mt2</i>
ENSMUSG00000107470	10,75528861	3,316213988	1,6974373	1,45E-20	9,11E-18	TRUE	<i>CAAA01093177.1</i>
ENSMUSG00000022797	24,12819791	48,9716407	-1,021226281	2,96E-20	1,80E-17	TRUE	<i>Tfrc</i>
ENSMUSG0000000318	185,9549149	426,0529443	-1,196079705	5,61E-20	3,29E-17	TRUE	<i>Clec10a</i>
ENSMUSG00000028238	140,1082251	79,65497123	0,814705387	1,06E-19	6,00E-17	TRUE	<i>Atp6v0d2</i>
ENSMUSG0000003420	64,56517434	128,5967098	-0,994025533	1,67E-19	9,16E-17	TRUE	<i>Fcgtr</i>
ENSMUSG00000035105	10,91792767	21,80570458	-0,998006483	2,51E-19	1,33E-16	TRUE	<i>Egln3</i>
ENSMUSG00000021876	77,52722238	161,7081794	-1,060617662	6,83E-19	3,52E-16	TRUE	<i>Rnase4</i>
ENSMUSG0000003541	41,98758642	80,54520099	-0,939835696	2,04E-18	1,02E-15	TRUE	<i>ler3</i>
ENSMUSG00000047945	56,61355528	111,9269268	-0,983337631	2,27E-18	1,10E-15	TRUE	<i>Marcks1</i>
ENSMUSG00000019478	3,070223	8,875086485	-1,531417565	2,78E-18	1,31E-15	TRUE	<i>Rab4a</i>
ENSMUSG00000029648	1,366480588	4,439570119	-1,699954799	4,79E-18	2,20E-15	TRUE	<i>Flt1</i>
ENSMUSG00000072812	21,49373647	11,53429996	0,897985906	9,98E-18	4,46E-15	TRUE	<i>Ahnak2</i>
ENSMUSG0000006611	81,41059172	152,2018523	-0,902697424	1,97E-17	8,59E-15	TRUE	<i>Hfe</i>
ENSMUSG00000017009	81,88377907	151,0950033	-0,883806279	2,17E-17	9,24E-15	TRUE	<i>Sdc4</i>
ENSMUSG00000026656	163,8620189	316,8460657	-0,951300507	2,29E-17	9,50E-15	TRUE	<i>Fcgr2b</i>
ENSMUSG0000002992	83,80184708	161,8411135	-0,949524118	2,97E-17	1,20E-14	TRUE	<i>Apoc2</i>
ENSMUSG00000062300	5,276031178	11,91824497	-1,175646692	1,02E-16	4,02E-14	TRUE	<i>Pvr12</i>
ENSMUSG00000055546	1,00957662	0,0410397	4,620585412	1,38E-16	5,32E-14	TRUE	<i>Timd4</i>
ENSMUSG00000040950	118,4775656	257,674771	-1,120937242	1,95E-16	7,37E-14	TRUE	<i>Mgl2</i>
ENSMUSG0000003617	8,644884109	20,15231262	-1,221026739	2,10E-16	7,75E-14	TRUE	<i>Cp</i>
ENSMUSG00000028793	88,78423853	155,2790714	-0,806487835	2,23E-16	8,08E-14	TRUE	<i>Rnf19b</i>
ENSMUSG00000026069	2,091331688	5,510735325	-1,397822778	2,38E-16	8,43E-14	TRUE	<i>Il1rl1</i>
ENSMUSG00000059089	41,93413672	82,56182556	-0,977349617	3,22E-16	1,12E-13	TRUE	<i>Fcgr4</i>
ENSMUSG0000003882	71,6436439	42,43242979	0,75567147	3,64E-16	1,24E-13	TRUE	<i>Il7r</i>

Supplementary Table 2: Selection of DEG of cluster 1 & 2 single cell mRNA-Seq: Gata3^{Δmac} vs Gata3^{WT}. Related to Figure 7.

Gene	p-value	avg_log Fold Change	pct.1	pct.2	p-value_adj
<i>Bst2</i>	7,79E-55	-0,922105662	0,749	0,964	9,98E-51
<i>Rpsa</i>	1,22E-49	0,75841808	1	1	1,56E-45
<i>Irft</i>	7,68E-49	-0,943696673	0,697	0,974	9,83E-45
<i>Ly6a</i>	1,12E-39	-1,220394035	0,39	0,816	1,44E-35
<i>Isg15</i>	1,51E-36	-1,065079849	0,498	0,849	1,93E-32
<i>Oasl2</i>	1,89E-34	-0,668862127	0,414	0,839	2,42E-30
<i>Ifi27l2a</i>	7,44E-34	-0,838419641	0,948	0,974	9,53E-30
<i>H2-T23</i>	2,76E-32	-0,620067241	0,749	0,941	3,53E-28
<i>Ly6e</i>	4,10E-32	-0,529727654	0,944	0,997	5,25E-28
<i>Zbp1</i>	1,29E-31	-0,744740883	0,227	0,686	1,65E-27
<i>Ms4a4c</i>	5,55E-31	-0,765281371	0,757	0,964	7,11E-27
<i>Ifitm3</i>	3,42E-29	-0,836476795	0,88	0,992	4,38E-25
<i>Ifit3</i>	1,13E-28	-0,634556154	0,131	0,599	1,45E-24
<i>Cd52</i>	1,85E-27	-0,512613666	0,988	0,997	2,37E-23
<i>Rtp4</i>	8,09E-27	-0,584426091	0,219	0,645	1,04E-22
<i>Mndal</i>	1,03E-26	-0,589421296	0,697	0,936	1,32E-22
<i>Rnf213</i>	1,76E-25	-0,551400272	0,39	0,77	2,25E-21
<i>Gdpd3</i>	2,78E-25	0,490459174	0,426	0,087	3,56E-21
<i>Tap1</i>	3,51E-25	-0,534764172	0,526	0,849	4,49E-21
<i>Psme1</i>	3,64E-25	-0,485648624	0,745	0,918	4,66E-21
<i>Stat1</i>	4,72E-25	-0,620264632	0,586	0,855	6,04E-21
<i>Ifi209</i>	5,71E-25	-0,592905246	0,347	0,74	7,31E-21
<i>Ifi213</i>	3,02E-21	-0,526277509	0,215	0,587	3,87E-17
<i>Ifit2</i>	1,05E-20	-0,538789675	0,104	0,464	1,34E-16
<i>Ifi211</i>	5,01E-20	-0,594594002	0,167	0,526	6,41E-16
<i>Ifi203</i>	1,68E-18	-0,561467841	0,622	0,827	2,15E-14
<i>Ifi47</i>	3,76E-18	-0,523591657	0,131	0,464	4,81E-14
<i>Ifi204</i>	6,71E-17	-0,539090752	0,514	0,763	8,59E-13
<i>Irgm1</i>	3,89E-14	-0,345399493	0,175	0,467	4,98E-10
<i>Fcgr3</i>	4,72E-13	0,4269541	0,677	0,449	6,05E-09
<i>Fcgr1</i>	1,19E-12	-0,421277073	0,673	0,811	1,52E-08
<i>Ifit1</i>	1,82E-12	-0,328662218	0,116	0,372	2,33E-08
<i>Ifih1</i>	2,97E-11	-0,34846243	0,211	0,452	3,81E-07
<i>Ifit3b</i>	8,06E-11	-0,285327984	0,076	0,296	1,03E-06
<i>Ifi35</i>	1,70E-10	-0,309858637	0,167	0,395	2,18E-06
<i>Cxcl2</i>	1,10E-09	0,99093868	0,319	0,125	1,40E-05
<i>Cxcr4</i>	8,57E-09	0,504516501	0,498	0,311	0,00010973
<i>Il1b</i>	3,54E-08	0,841298864	0,414	0,23	0,000453888
<i>Axl</i>	7,25E-08	-0,293567623	0,295	0,508	0,000928418
<i>Fcgr4</i>	1,28E-07	-0,392603961	0,191	0,37	0,001644576
<i>Ccl3</i>	1,50E-06	0,377897695	0,418	0,245	0,019176719
<i>Hspa1a</i>	1,50E-06	0,534219858	0,514	0,339	0,019238627
<i>Jun</i>	1,93E-06	0,382034081	0,857	0,758	0,024715738
<i>Ccl5</i>	2,10E-06	-0,442490807	0,064	0,204	0,026945075
<i>Cxcl10</i>	1,01E-05	-0,598575473	0,131	0,283	0,129591767
<i>Tnf</i>	5,24E-05	0,269410362	0,327	0,189	0,670929582
<i>Ccl4</i>	0,000312829	0,265508646	0,39	0,255	1

Supplementary Table 3: Selection of DEG of cluster 3 & 4 single cell mRNA-Seq: *Gata3*^{Δmac} vs *Gata3*^{WT}. Related to Figure 7.

Gene	p-value	avg_log Fold Change	pct.1	pct.2	p-value_adj
<i>Irft</i>	1,51E-59	-0,94634987	0,626	0,956	1,93E-55
<i>Bsf2</i>	3,78E-53	-0,747370464	0,783	0,974	4,84E-49
<i>lsg15</i>	3,56E-51	-0,722320006	0,38	0,875	4,56E-47
<i>Ifi27l2a</i>	3,89E-45	-0,787347612	0,869	0,982	4,99E-41
<i>Ifitm3</i>	2,44E-43	-0,709938323	0,734	0,986	3,13E-39
<i>Stat1</i>	5,61E-38	-0,71212172	0,511	0,855	7,18E-34
<i>Gdpd3</i>	2,04E-35	0,51300769	0,434	0,082	2,61E-31
<i>Zbp1</i>	2,93E-32	-0,500065186	0,291	0,687	3,75E-28
<i>Rnf213</i>	6,69E-32	-0,514377234	0,417	0,779	8,56E-28
<i>Cd52</i>	2,01E-31	-0,537662375	0,934	0,984	2,57E-27
<i>Mndal</i>	2,83E-30	-0,577262942	0,586	0,869	3,62E-26
<i>Ms4a4c</i>	1,64E-29	-0,572422457	0,514	0,845	2,10E-25
<i>Oasl2</i>	1,88E-28	-0,469775084	0,36	0,729	2,40E-24
<i>Parp14</i>	3,24E-28	-0,55145509	0,46	0,785	4,15E-24
<i>Rpsa</i>	2,57E-27	0,396356428	1	0,998	3,29E-23
<i>Ifit3</i>	9,07E-26	-0,549044796	0,171	0,522	1,16E-21
<i>Psme1</i>	2,76E-25	-0,411134172	0,706	0,904	3,54E-21
<i>Ifit2</i>	4,61E-25	-0,53293392	0,089	0,414	5,91E-21
<i>Ly6a</i>	5,61E-25	-0,664046473	0,277	0,618	7,19E-21
<i>Mylpf</i>	1,99E-13	-0,873205785	0,206	0,444	2,55E-09
<i>Myl1</i>	8,24E-11	-0,551379478	0,097	0,277	1,06E-06
<i>Tnnt3</i>	6,71E-10	-0,462079469	0,1	0,269	8,60E-06
<i>Cxcl2</i>	6,28E-09	0,864771488	0,203	0,072	8,04E-05
<i>Myl4</i>	8,16E-08	-0,449214483	0,1	0,239	0,001045451
<i>Tnncc2</i>	1,78E-07	-0,567631916	0,123	0,265	0,002280418
<i>Tnnt2</i>	2,06E-07	-0,379696815	0,089	0,221	0,002643482
<i>Il1b</i>	4,89E-07	0,406165008	0,214	0,094	0,006259744
<i>Des</i>	2,16E-06	-0,343728928	0,131	0,259	0,027656506

Supplementary Table 4: Primer sequences for qPCR. Related to STAR Methods.

Target	Forward 5'- 3'	Reverse 5'- 3'
<i>Actb</i>	TGTCCACCTTCCAGCAGATGT	AGCTCAGTAACAGTCCGCCTAGA
<i>Arg1</i>	TTT TAG GGT TAC GGC CGG TG	CCT CGA GGC TGT CCT TTT GA
<i>Chil3</i>	CCAGCAGAACGCTCCTCCAGAAG	CTCCCTTCTATTGGC CTGTCC
<i>Gata3</i>	CAATCTGACCGGGCAGGT	CAGAGACGGTTGCTTCCG
<i>Retnla</i>	AGGAACCTCTGCCAATCCAG	AGTCAACGAGTAAGCACAGG
<i>Tnfa</i>	GCTGAGCTCAAACCCCTGGTA	CGGACTCCGCAAAGTCTAAG
<i>Ucp1</i>	CGACTCAGTCCAAGAGTACTTCTCTTC	GCCGGCTGAGATCTGTTTC
<i>Ucp2</i>	TCCCCTGTTGATGTGGTCAA	CAGTGACCTGCGCTGTGGTA
<i>Ucp3</i>	CCTACGACATCATCAAGGAGAAGTT	TCCAAAGGCAGAGACAAAGTGA
<i>Ucp4</i>	GAATGCCTATGCCGAGGA	AGTAGGAAC TTGCTCGTCCGG
<i>Ucp5</i>	TCCCAACTGCTCAGCGTG	GGTGCTTCTGGTAATATCATAAACG
<i>Il1b</i>	ATGAAGGGCTGCTTCCAAAC	ATGTGCTGCTGCGAGATTG
<i>Il6</i>	TCC TTC CTA CCC CAA TTT CC	GCC ACT CCT TCT GTG ACT CC
<i>Irg1</i>	GCG AAC GCT GCC ACT CA	ATC CCA GGC TTG GAA GGT C