

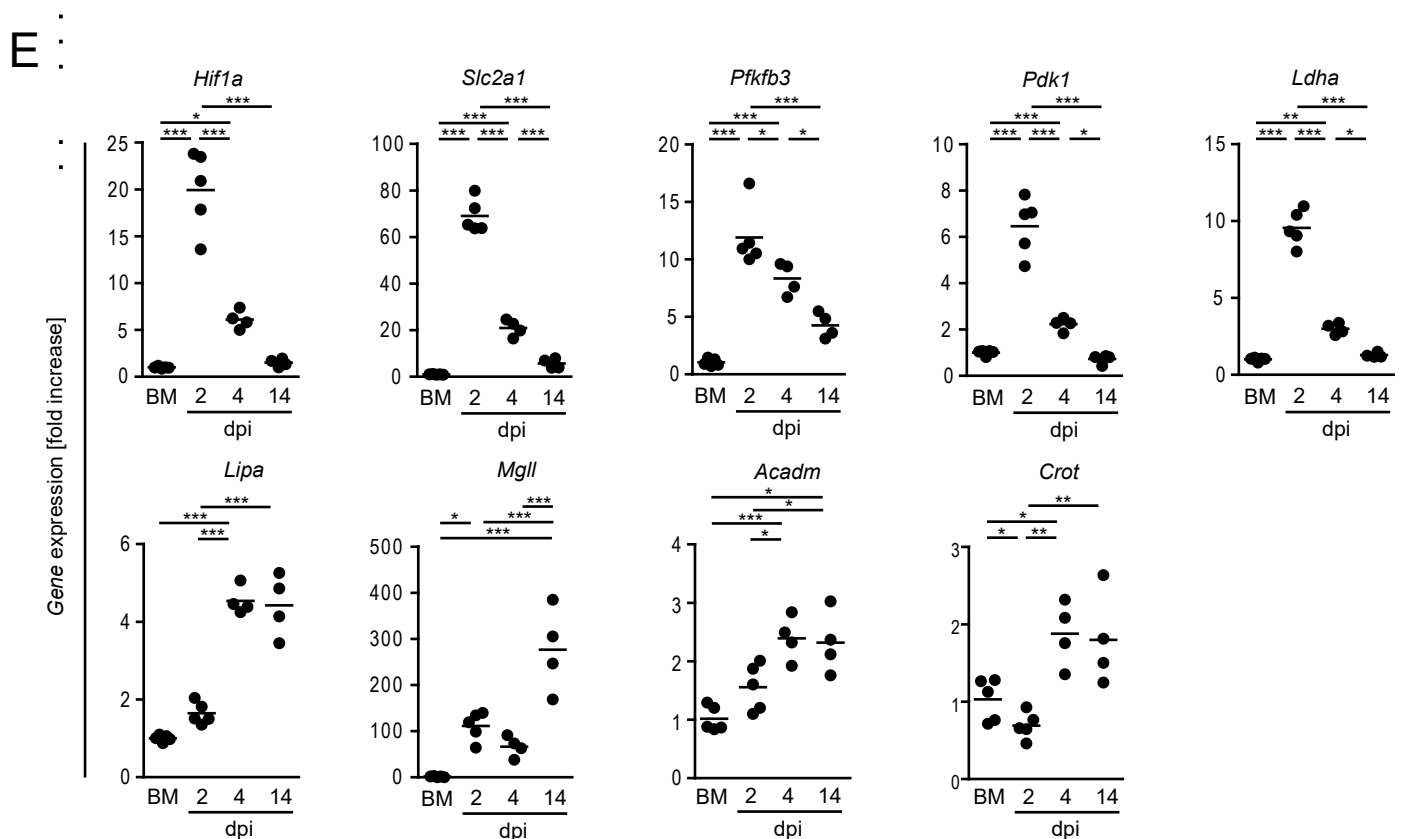
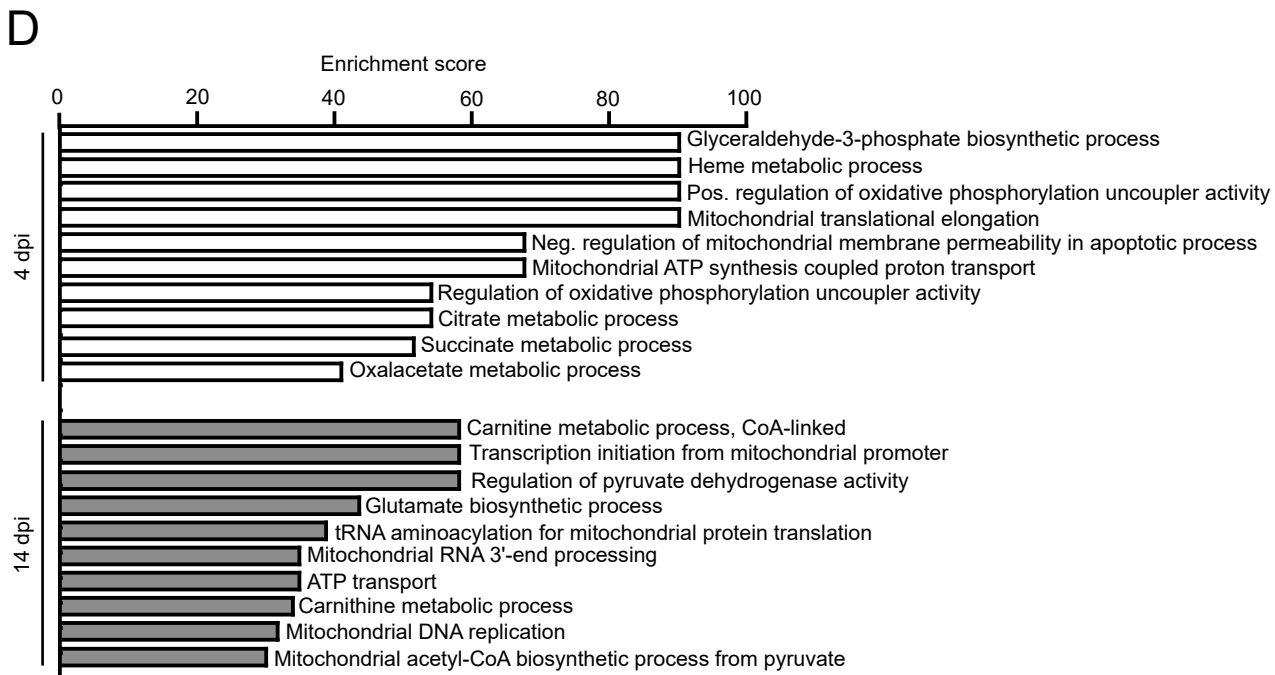
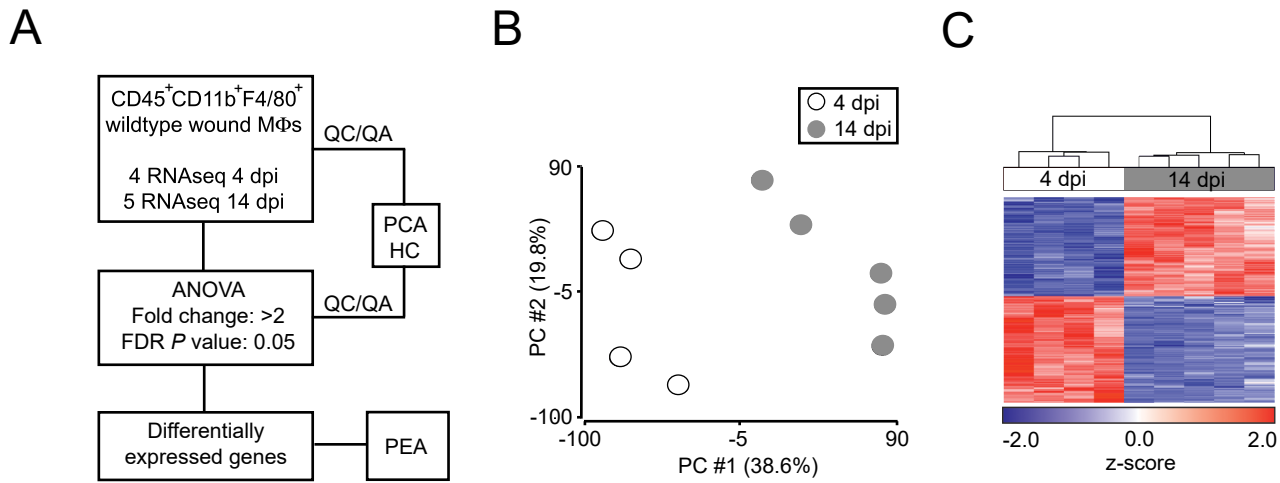
Supplemental information

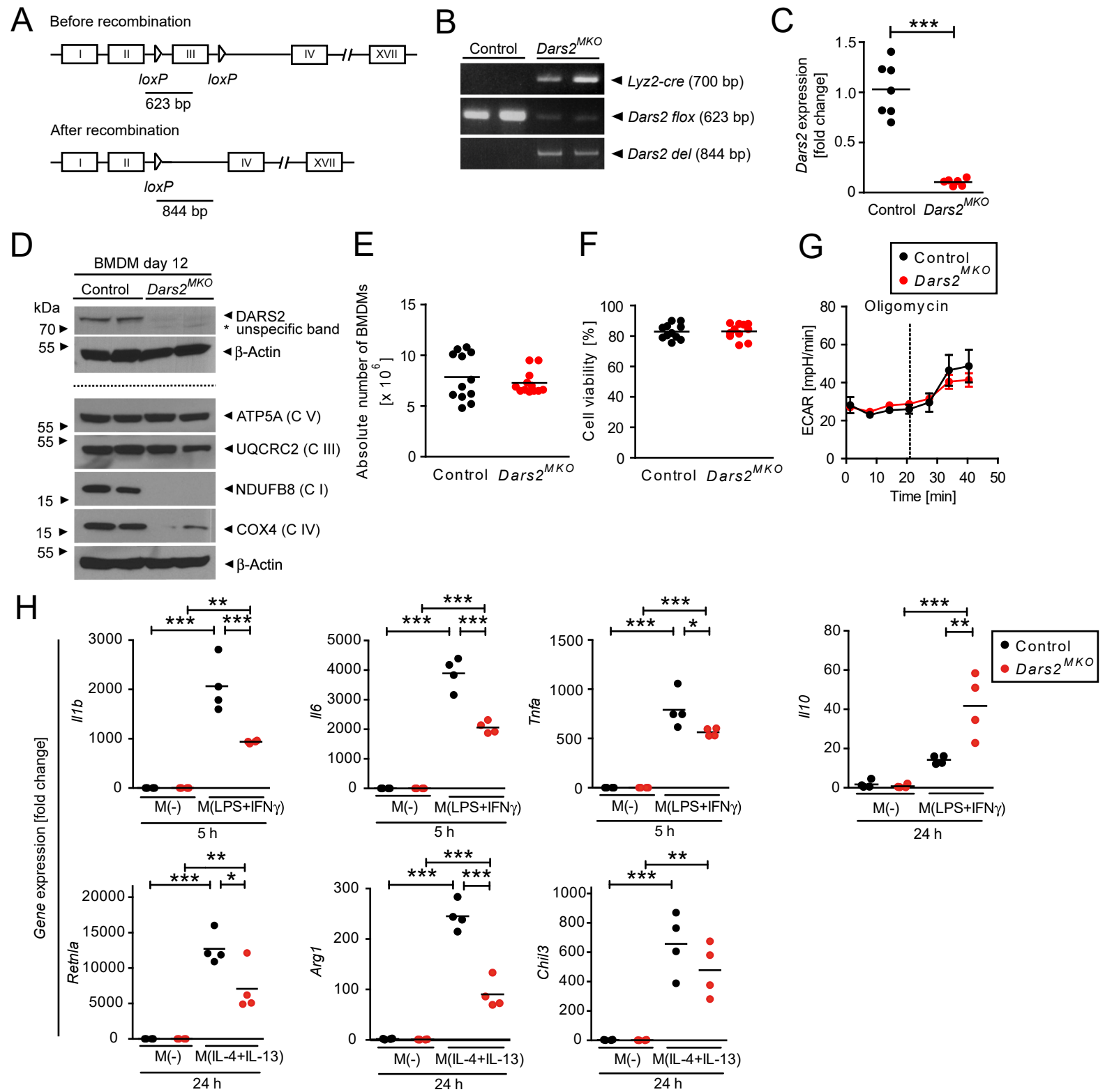
Mitochondrial metabolism coordinates

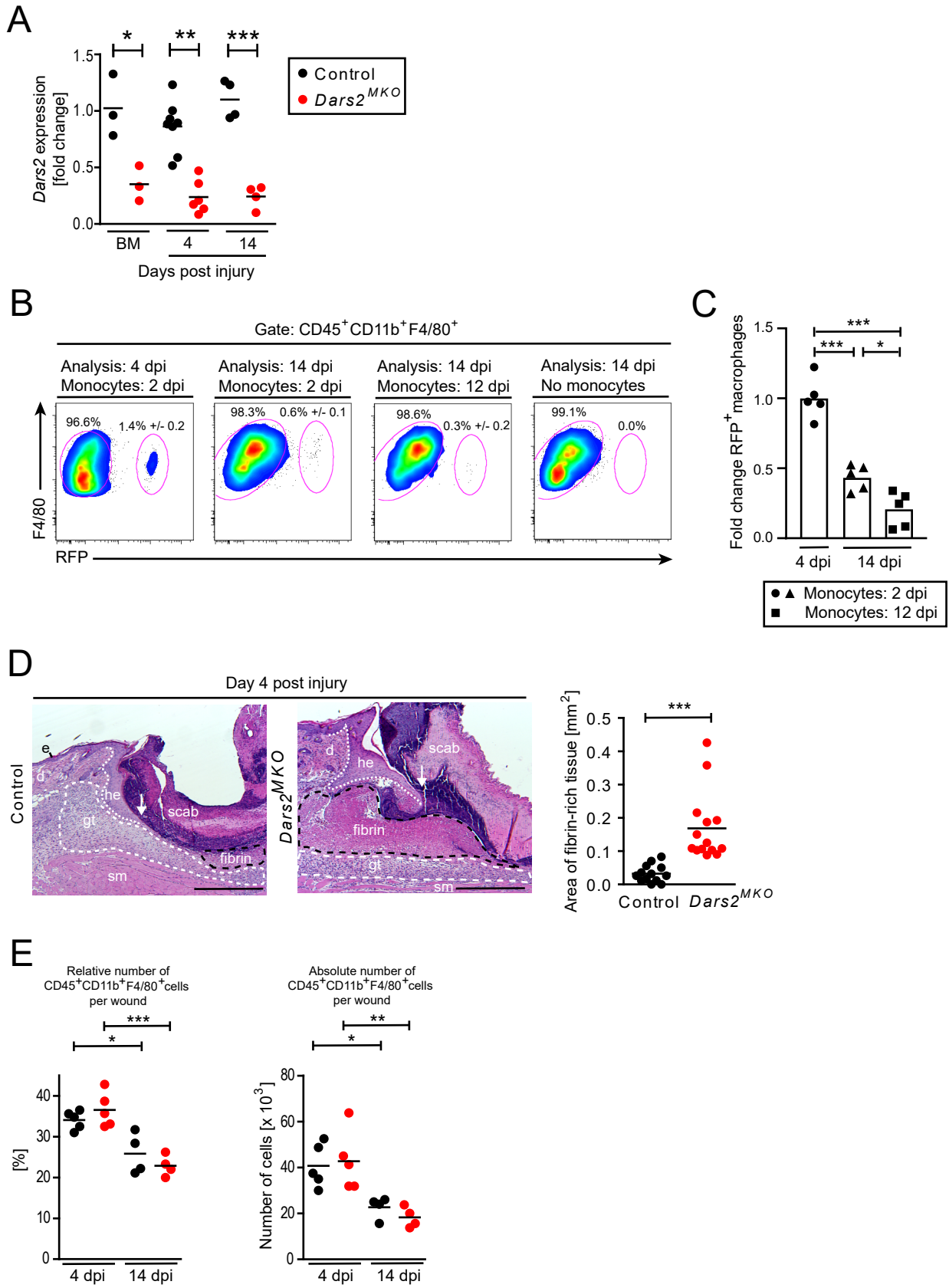
stage-specific repair processes

in macrophages during wound healing

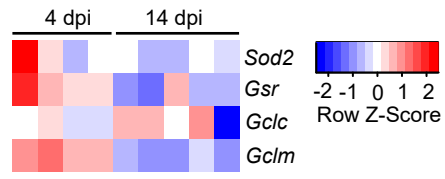
Sebastian Willenborg, David E. Sanin, Alexander Jais, Xiaolei Ding, Thomas Ulas, Julian Nüchel, Milica Popović, Thomas MacVicar, Thomas Langer, Joachim L. Schultze, Alexander Gerbault, Axel Roers, Edward J. Pearce, Jens C. Brüning, Aleksandra Trifunovic, and Sabine A. Eming



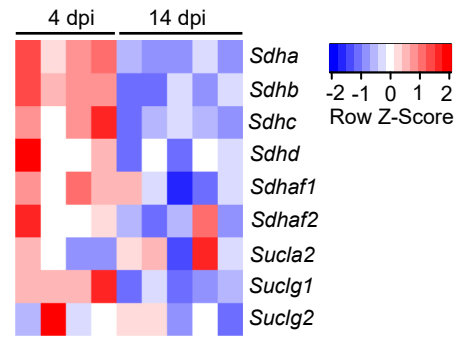




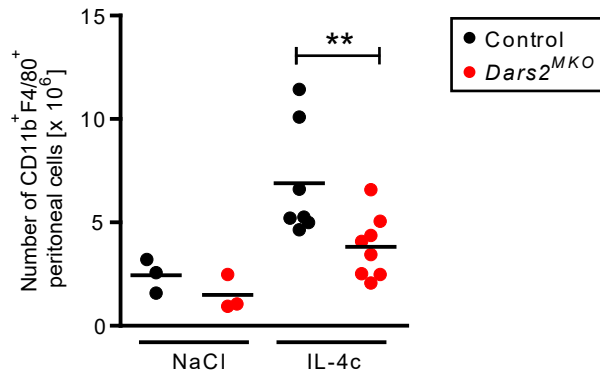
A



B



A



B

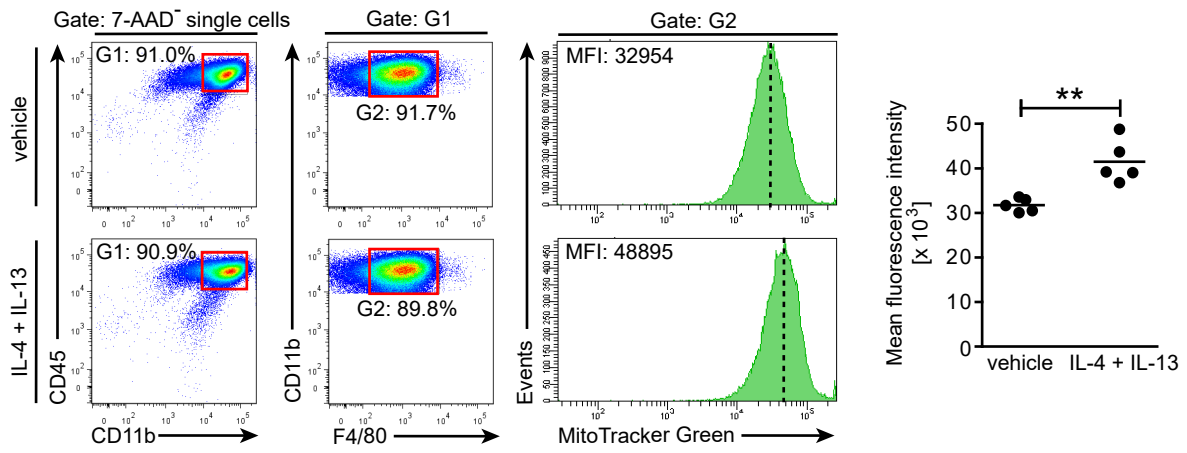


Figure S6. Related to Figure 7

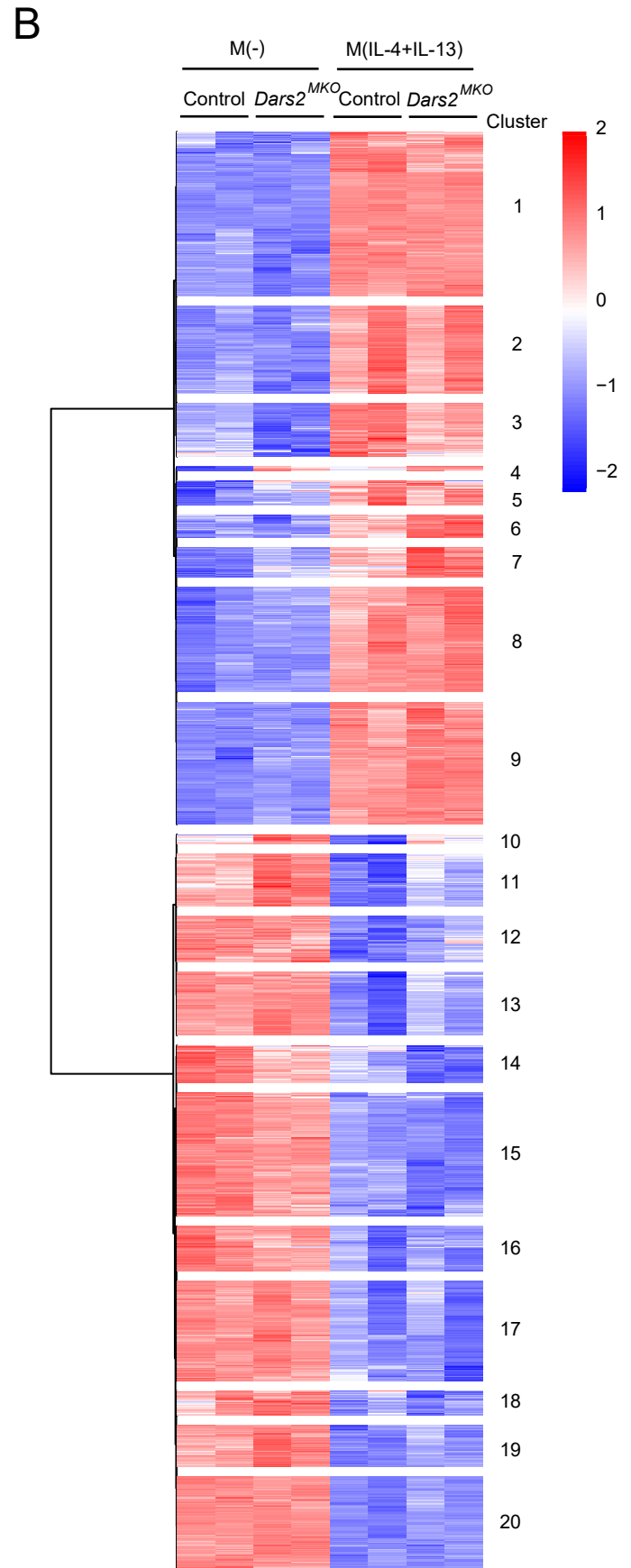
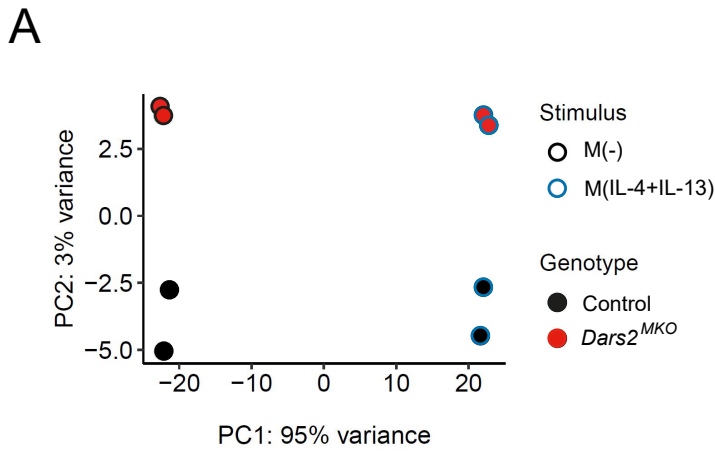


Figure S1. Related to Figure 1. Distinct metabolic transcriptional profiles in early and late phase wound MΦs

(A-D) Bulk RNA-seq analysis of sorted wound MΦs isolated from wild-type mice at 4 and 14 dpi. 1 technical replicate of $n = 4$ biological replicates at 4 dpi, 5 at 14 dpi.

(A) Schematic illustrating the workflow of the bulk RNA-seq analysis.

(B) PCA based on all identified genes.

(C) HC showing the z-transformed expression values of the 1,000 most variable genes.

(D) GOE analysis of genes encoding proteins with mitochondrial localization (based on MitoCarta 3.0 datasets) and being differentially expressed in early versus late phase wound MΦs.

(E) qRT-PCR in sorted wild-type wound MΦs at indicated time points normalized to wild-type blood monocytes. 1 technical replicate of $n = 5$ biological replicates per group for BM, 5 at 2 dpi, 4 at 4 dpi, 4 at 14 dpi. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by 1-way ANOVA with Tukey's Multiple Comparison Test. BM, blood monocytes.

Figure S2. Related to Figure 3. Efficient *Dars2* gene deletion in BMDMs isolated from *Dars2^{MKO}* mice

(A) Scheme illustrating the *Dars2* gene construct and the 2 *loxP* sites flanking exon 3 along with the PCR fragment length before and after successful recombination.

(B) PCR analysis of genomic DNA isolated from BMDMs cultured in growth medium. 1 technical replicate of $n = 2$ biological replicates per genotype.

(C) qRT-PCR analysis of *Dars2* gene expression in BMDM cultured in growth medium. 1 technical replicate of $n = 7$ biological replicates (control), 6 (*Dars2^{MKO}*).

(D) Western blot analysis of DARS2 and subunits of MRC complexes in BMDM cultured in growth medium. Dashed line indicates the use of different gels. 1 technical replicate of $n = 2$ biological replicates per genotype.

(E) Quantification of absolute numbers of BMDMs cultured in growth medium. 1 technical replicate of $n = 12$ biological replicates (control), 11 (*Dars2^{MKO}*).

(F) Quantification of cell viability of BMDMs cultured in growth medium analyzed by trypan blue staining. 1 technical replicate of $n = 12$ biological replicates (control), 11 (*Dars2^{MKO}*).

(G) EFA of ECARs in BMDMs cultured for 24 h in growth medium. $n = 8$ technical replicates of 1 biological replicate per genotype. The mean value \pm SEM is represented.

(H) qRT-PCR analysis in BMDMs stimulated with LPS (50 ng/mL) and IFN γ (50 ng/mL) [M(LPS+IFN γ)] for 5 h or with IL-4 (50 ng/mL) and IL-13 (50 ng/mL) [M(IL-4+IL-13)] for 24 h or with vehicle [M(-)]. 1 technical replicate of $n = 4$ biological replicates per group.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's unpaired two-tailed t -test (C) or 1-way ANOVA with Tukey's Multiple Comparison Test (H).

Figure S3. Related to Figure 3. Wound healing response in *Dars2^{MKO}* mice

(A) qRT-PCR analysis of *Dars2* gene expression in sorted wound M Φ s normalized to blood monocytes. 1 technical replicate of $n = 3$ biological replicates per group for BM, 8 controls at 4 dpi, 6 *Dars2^{MKO}* at 4 dpi, 4 per group at 14 dpi. BM, blood monocytes.

(B and C) Flow cytometric analysis of RFP⁺ macrophages among wound M Φ s at 4 or 14 dpi. RFP⁺ monocytes were adoptively transferred either at 2 or at 12 dpi. Representative flow cytometry plots ($n = 5$ per group in total) (B) and quantification of the fold change of RFP⁺ cells within total M Φ s normalized to the group which received monocytes at 2 dpi and was analyzed at 4 dpi (C). 1 technical replicate of 5 biological replicates per group.

(D) Representative H&E-stained wound sections at 4 dpi ($n = 13$ control, 14 *Dars2^{MKO}* wounds in total) and quantification of fibrin-rich granulation tissue. d, dermis; e, epidermis; gt, granulation tissue; he, hyperproliferative epithelium; sm, skeletal muscle. Dotted line underlines the newly formed epithelium. White and black dashed lines indicate the gt and the

fibrin rich-area, respectively. Arrows indicate the epithelial tips. Scale bar, 500 μm . 1 technical replicate of 13 biological replicates (control), (14 *Dars2*^{MKO}).

(E) Relative and absolute numbers of M Φ s per wound at indicated time points post injury. One technical replicate of $n = 5$ biological replicates per group at 4 dpi, 4 at 14 dpi.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's unpaired two-tailed t -test (A, D) or 1-way ANOVA with Tukey's Multiple Comparison Test (B, E).

Figure S4. Related to Figure 4. High expression of genes involved in ROS detoxification and succinate metabolism in early phase wound M Φ s

(A, B) Heatmaps of the bulk RNA-Seq analysis in sorted wound M Φ s isolated from wild-type mice at 4 and 14 dpi showing the z-transformed expression values of indicated genes. 1 technical replicate of 4 biological replicates at 4 dpi, 5 at 14 dpi.

Figure S5. Related to Figure 6. Attenuated IL-4c-induced proliferation of tissue-resident M Φ s in *Dars2*^{MKO} mice

(A) Quantification of CD11b⁺F4/80⁺ peritoneal M Φ s in control and *Dars2*^{MKO} mice treated as indicated. 1 technical of $n = 3$ biological replicates per genotype in the NaCl group, 7 controls in the IL-4c group, 8 *Dars2*^{MKO} in the IL-4c group.

(B) Representative flow cytometry plots ($n = 5$ per group in total) of mitochondrial mass analysis by MTG fluorescent staining in CD11b⁺F4/80⁺ BMDMs isolated from control mice. 7-AAD⁻CD11b⁺F4/80⁺ single cells were gated (G2) and the mean fluorescence intensity (MFI) of MTG was analyzed. 1 technical replicate of 5 biological replicates per group.

** $P < 0.01$ by Student's unpaired two-tailed t -test (A, B).

Figure S6. Related to Figure 7. RNA-seq analysis in BMDM isolated from control and *Dars2*^{MKO} mice

(A, B) Bulk RNA-seq analysis in BMDMs isolated from control and *Dars2*^{MKO} mice. 1 technical replicate of $n = 2$ biological replicates per group.

(A) PCA based on all identified genes. (B) HC showing the z-transformed expression values of the 2,810 differentially expressed genes comparing M(IL-4 + IL-13) versus M(-) separately for control and *Dars2*-deficient BMDMs. P value < 0.01 and $\text{Log}_2\text{FC} > 0.5$ or < -0.5 .

Table S1: Oligonucleotides used for mouse genotyping and qRT-PCR. Related to Figure 3, Figure 4, Figure 6, Figure 7, Figure S1, Figure S2, Figure S3.

Oligonucleotides		
Genotyping		
Primer 1 <i>Dars2 flox</i> 5' ATGAATTCTAGGCCAGCCAC3'	Sigma-Aldrich	N/A
Primer 2 <i>Dars2 flox</i> 5' TGGCAATCTCTTAGGACTAAG3'	Sigma-Aldrich	N/A
Primer 1 <i>Lyz2-cre</i> 5' CTTGGGCTGCCAGAATTTCTC3'	Sigma-Aldrich	N/A
Primer 2 <i>Lyz2-cre</i> 5' TTACAGTCGGCCAGGCTGAC3'	Sigma-Aldrich	N/A
Primer 3 <i>Lyz2-cre</i> 5' CCCAGAAATGCCAGATTACG 3'	Sigma-Aldrich	N/A
Primer 1 <i>Il4ra flox</i> 5' CCCTTCCTGGCCCTGAATTT 3'	Sigma-Aldrich	N/A
Primer 2 <i>Il4ra flox</i> 5' GTTTCCTCCTACCGCTGATT 3'	Sigma-Aldrich	N/A
Primer 1 <i>Il4ra del</i> 5' GGCTGCTGACCTGGAATAACC 3'	Sigma-Aldrich	N/A
Primer 2 <i>Il4ra del</i> 5' CCTTTGAGAACTGCGGGCT 3'	Sigma-Aldrich	N/A
qRT-PCR		
<i>Atf4</i> forward 5' AACATCCAATCTGTCCCGG 3'	Sigma-Aldrich	Kaspar et al., 2021
<i>Atf4</i> reverse 5' GTTCTCCAGCGACAAGGC 3'	Sigma-Aldrich	Kaspar et al., 2021
<i>Arg1</i> forward 5' GCTTCGGAACCTCAACGGGAGGG 3'	Sigma-Aldrich	N/A
<i>Arg1</i> reverse 5' ACCAGAAAGGAACTGCTGGGATACA 3'	Sigma-Aldrich	N/A
<i>Acadm</i> forward 5' AACACAACACTCGAAAGCGG 3'	Sigma-Aldrich	PrimerBank ID: 158508465c1
<i>Acadm</i> reverse 5' TTCTGCTGTTCCGTCAACTCA 3'	Sigma-Aldrich	PrimerBank ID: 158508465c1
<i>Ccl22</i> forward 5' CTCTGCCATCACGTTTAGTGAA 3'	Sigma-Aldrich	PrimerBank ID: 154240695c1
<i>Ccl22</i> reverse 5' GACGGTTATCAAAACAACGCC 3'	Sigma-Aldrich	PrimerBank ID: 154240695c1
<i>Crot</i> forward 5' GGTGGCTCAATGTTGCCTAC 3'	Sigma-Aldrich	PrimerBank ID: 142387204c2
<i>Crot</i> reverse 5' GGTGGCTCAATGTTGCCTAC 3'	Sigma-Aldrich	PrimerBank ID: 142387204c2
<i>Ddit3</i> forward 5' TGCCTTTCACCTTGGAGACGG 3'	Sigma-Aldrich	Kaspar et al., 2021
<i>Ddit3</i> reverse 5' CGCAGGGTCAAGAGTAGTGAAGG 3'	Sigma-Aldrich	Kaspar et al., 2021
<i>Dars2</i> forward 5' AGTCCTAAGAGATTGCCACGG 3'	Sigma-Aldrich	PrimerBank ID: 141801674c3
<i>Dars2</i> reverse 5' GACGGGAGATTACTGTCCCAG 3'	Sigma-Aldrich	PrimerBank ID: 141801674c3
<i>Il6</i> forward 5' ACACATGTTCTCTGGGAAATC 3'	Sigma-Aldrich	N/A
<i>Il6</i> reverse 5' AAGTGCATCATCGTTGTTTCATACA 3'	Sigma-Aldrich	N/A
<i>Gclc</i> forward 5' GGACAAACCCCAACCATCC 3'	Sigma-Aldrich	PrimerBank ID: 324710985c2
<i>Gclc</i> reverse 5' GTTGAACCTCAGACATCGTTCCT 3'	Sigma-Aldrich	PrimerBank ID: 324710985c2

<i>Gclm</i> forward 5' GGACAAACCCCAACCATCC 3'	Sigma-Aldrich	PrimerBank ID: 142373116c3
<i>Gclm</i> reverse 5' CCTGCTCTTCACGATGACCG 3'	Sigma-Aldrich	PrimerBank ID: 142373116c3
<i>Gsr</i> forward 5' GACACCTCTTCCTTCGACTACC 3'	Sigma-Aldrich	PrimerBank ID: 160298212c1
<i>Gsr</i> reverse 5' CACATCCAACATTACGCAAG 3'	Sigma-Aldrich	PrimerBank ID: 160298212c1
<i>Hif1a</i> forward 5' GGGGAGGACGATGAACATCAA 3'	Sigma-Aldrich	PrimerBank ID: 226061947c3
<i>Hif1a</i> reverse 5' GGGTGGTTTCTTGTACCCACA 3'	Sigma-Aldrich	PrimerBank ID: 226061947c3
<i>Il10</i> forward 5' AGCCGGGAAGACAATAACTG 3'	Sigma-Aldrich	N/A
<i>Il10</i> reverse 5' CATTTCGGATAAGGCTTGG 3'	Sigma-Aldrich	N/A
<i>Il1b</i> forward 5' CGACCCCAAAGATGAAGGGCTGC 3'	Sigma-Aldrich	N/A
<i>Il1b</i> reverse 5' GCTCTTGTTGATGTGCTGCTGCG 3'	Sigma-Aldrich	N/A
<i>Ldha</i> forward 5' CAAAGACTACTGTGTAAGTCCGA 3'	Sigma-Aldrich	PrimerBank ID: 257743038c1
<i>Ldha</i> reverse 5' TGGACTGTACTTGACAATGTTGG 3'	Sigma-Aldrich	PrimerBank ID: 257743038c1
<i>Lipa</i> forward 5' CTGGTGAGGAACACTCGGTC 3'	Sigma-Aldrich	PrimerBank ID: 162287342c2
<i>Lipa</i> reverse 5' AGCCGTGCTGAAGATACACAA 3'	Sigma-Aldrich	PrimerBank ID: 162287342c2
<i>Mgll</i> forward 5' CGGACTTCCAAGTTTTTGTGTCAGA 3'	Sigma-Aldrich	PrimerBank ID: 6754690a1
<i>Mgll</i> reverse 5' GCAGCCACTAGGATGGAGATG 3'	Sigma-Aldrich	PrimerBank ID: 6754690a1
<i>Mrc1</i> forward 5' TGCCGACATGCCAGGACGAAA 3'	Sigma-Aldrich	N/A
<i>Mrc1</i> reverse 5' GTGGGCTCTGGTGGGCGAGT 3'	Sigma-Aldrich	N/A
<i>Nos2</i> forward 5' CCACCTTGGTGAAGGGACTGAGCT 3'	Sigma-Aldrich	N/A
<i>Nos2</i> reverse 5' AGGGGCAAGCCATGTCTGAGACT 3'	Sigma-Aldrich	N/A
<i>Pdk1</i> forward 5' GGACTTCGGGTCAGTGAATGC 3'	Sigma-Aldrich	PrimerBank ID: 227908810c1
<i>Pdk1</i> reverse 5' TCCTGAGAAGATTGTCGGGGA 3'	Sigma-Aldrich	PrimerBank ID: 227908810c1
<i>Pfkfb3</i> forward 5' CAACTCCCAACCGTGATTGT 3'	Sigma-Aldrich	PrimerBank ID: 295293219c1
<i>Pfkfb3</i> reverse 5' TGAGGTAGCGAGTCAGCTTCT 3'	Sigma-Aldrich	PrimerBank ID: 295293219c1
<i>Retn1a</i> forward 5' TATGAACAGATGGGCCTCCT 3'	Sigma-Aldrich	N/A
<i>Retn1a</i> forward 5' GGCAGTTGCAAGTATCTCCAC 3'	Sigma-Aldrich	N/A
<i>Slc2a1</i> forward 5' GCTGTGCTTATGGGCTTCTC 3'	Sigma-Aldrich	N/A
<i>Slc2a1</i> reverse 5' CACATACATGGGCACAAAGC 3'	Sigma-Aldrich	N/A
<i>Sod2</i> forward 5' CAGACCTGCCTTACGACTATGG 3'	Sigma-Aldrich	PrimerBank ID: 31980762a1
<i>Sod2</i> reverse 5' CTCGGTGGCGTTGAGATTGTT 3'	Sigma-Aldrich	PrimerBank ID: 31980762a1

<i>Tnfa</i> forward 5' GACCCTCACACTCAGATCATCTTCT 3'	Sigma- Aldrich	N/A
<i>Tnfa</i> reverse 5' CCTCCACTTGGTGGTTTGCT 3'	Sigma- Aldrich	N/A
<i>Vegfa</i> forward 5' TGTACCTCCACCATGCCAAGT 3'	Sigma- Aldrich	N/A
<i>Vegfa</i> reverse 5' CGCTGGTAGACGTCCATGAA 3'	Sigma- Aldrich	N/A
<i>Chil3</i> reverse 5' TACCAGTTGGGCTAAGGACAGGCC 3'	Sigma- Aldrich	N/A
<i>Chil3</i> forward 5' ACTGAACGGGGCAGGTCCAAACT 3'	Sigma- Aldrich	N/A