
Supplementary information

TFEB induces mitochondrial itaconate synthesis to suppress bacterial growth in macrophages

In the format provided by the
authors and unedited

Supplementary Information

| Motif | Transcription Factor | p-value |
|----------------|--------------------------|---------|
| TCGTAGCACT | Zfp691 | 1e-4 |
| CATTACCGTA | Homeobox protein Nkx-6.1 | 1e-4 |
| GTGACTGTGTAT | FOXH1 | 1e-4 |
| AAAGGCCACACGT | Mlxip | 1e-4 |
| CACTAAAGGCC | Homeobox protein Nkx-2.1 | 1e-4 |
| ACCAACTACTCC | Hmx1 | 1e-4 |
| ATCCACTCCTGA | ZNF354C | 1e-4 |
| GCCAGTTACCCCT | Spz1 | 1e-4 |
| GAGCAACATGAT | Homeobox protein Six1 | 1e-4 |
| GCTCAAGGTATC | RAR:RXR (NR) | 1e-4 |
| GCCGCTTGCCTA | at_AC_acceptor | 1e-4 |
| TCCTGGCTTGAT | SREBF2 | 1e-4 |
| TCCTTTCTCAAT | Irf3 | 1e-4 |
| CAGTTGGTCTTT | Mybl1 | 1e-4 |
| TCCTAGCTATCT | GATA | 1e-4 |
| GTTTGATCACCT | Cux1 | 1e-4 |
| TCTACAAGTGAT | Zbtb3 | 1e-4 |
| ACAAACCACAAT | RUNX | 1e-4 |
| CCTCCCCACCTCG | TBX15 | 1e-4 |
| CCCTTCACGTGT | PRDM1 | 1e-4 |
| ACCCGTCACCTC | Eomes-2 | 1e-4 |
| GTITGTCAATGTGC | TFEB | 1e-4 |
| TACCAAGCTTGCA | Tcf21 | 1e-4 |
| ATTTTCACATCG | Eomes-1 | 1e-4 |
| GTGAGTTACAAT | Homeobox protein Pknox1 | 1e-4 |
| GCACTATCTACT | Mnx1 | 1e-4 |
| CGGTCTAGCC | SMAD3 | 1e-4 |
| CACGTCCACT | HIF-1A | 1e-3 |
| TACTTGAAAC | Nkx-2.2 | 1e-3 |
| GTATAAAAGGC | TATA-Box | 1e-3 |
| CCTAAACTGC | BMYB (HTH) | 1e-3 |
| ATGTGCTTAC | NRL | 1e-3 |
| GACACAACTC | KLF10 | 1e-3 |

Supplementary Table 1. Potential *Irg1* regulators upon TFEB activation.

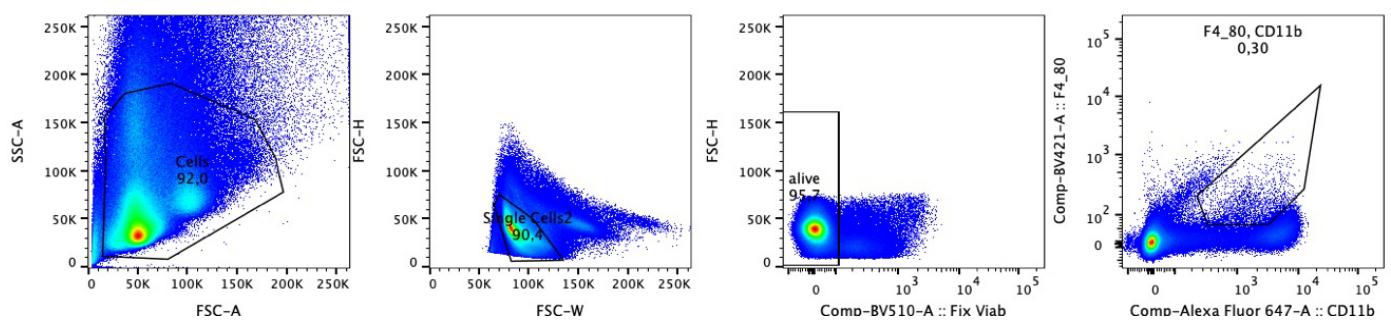
List of identified transcription factor binding motifs in significantly opened chromatin regions of the *Irg1* locus from ATAC-seq analysis (shown in Figure 4h) in TFEB-GFP- versus GFP-expressing BMDMs. Identified motifs are shown in column 1. Column 2 indicates the respective transcription factor. Column 3 represents the significance of enrichment of the binding motifs in the gained *Irg1* peaks calculated via the HOMER motif analysis software.

Supplementary Table 2
Oligonucleotide sequences

| Cloning primers | |
|-------------------------|--|
| Irg1-forw | TTCAAGCTTGCACCATGATGCTCAAGTCTGTACAGAG |
| Irg1-rev | GGCCCGCGGTACCTTGATATTGGTAAACCTGGGCAACG |
| BFP-forw | CCACCGGTGCCACCATGAGCGAGCTGATTAAGGAG |
| BFP-rev | TGTGCTGGCGGCCGCTCAATTAAGCTTGTGCCAG |
| RT-qPCR primers | |
| Irg1-forw | GCAACATGATGCTCAAGTCTG |
| Irg1-rev | TGCTCCTCCGAATGATACCA |
| Tfeb-forw | TGATCCCCAAGGCCAACATGAC |
| Tfeb-forw | TCCCTGGACTTTGCAGGTC |
| b2m fw | CTGCTACGTAACACAGTTCCACCC |
| b2m rev | CATGATGCTTGATCACATGTCTG |
| Actin fw | TCGTTGCCGGTCCACACCCG |
| Actin rev | CTTGCACATGCCGGAGCCGT |
| ChIP-qPCR primer | |
| -800bp_FW | GGCCACCATTCCCTCACAAAT |
| -800bp_RV | ACGAGTAAGTGAGCATGGGT |
| -200bp_FW | GTCTTCAAGAGTGGGATGCC |
| -200bp_RV | ATAATTACAGAGGGATGGCTG |
| negative-control_FW | TGCTCCACAGTGTCCATGTACA |
| negative-control_RV | AGCAATTCATGGGTGAGAGAAG |

Supplementary Figure

Flow cytometry gating strategy



Supplementary Figure 1: Flow cytometry gating strategy to identify splenic macrophages.

Relevant for Figure 4d, g and Extended Data Figure 4 c,h. From splenic cells suspensions (SSC-A to FSC-A), doublets (FSC-W to FCH-H) and dead cells (viable to FSC-H) were excluded. Macrophages were identified as double positive F4/80+ and CD11b+ population.