Supporting Information

Trib1 deficiency modulates function and polarization of bone marrow derived macrophages

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List of supplemental material:

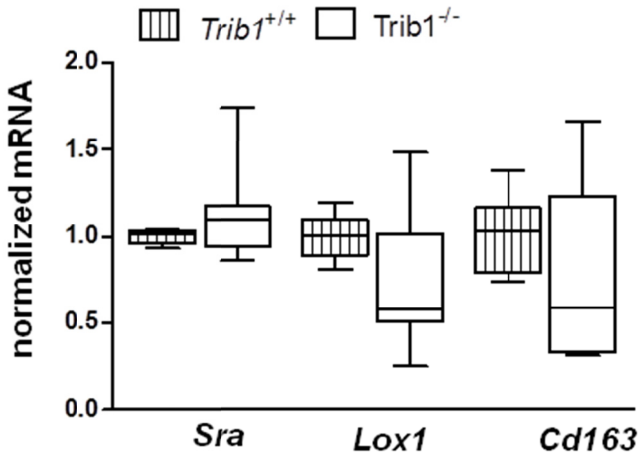

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Figure S1: Gene expression of *Sra*, *Lox1* and *Cd163* is comparable in WT and *Trib1*$^{-/-}$ BMDMs. BMDMs from WT and *Trib1*$^{-/-}$ mice were analyzed for mRNA expression of the scavenger receptors *Sra*, *Lox1* and *Cd163* by qPCR using the ΔCt method and *Actb* as a housekeeping gene. Values are expressed as changes compared to control group.

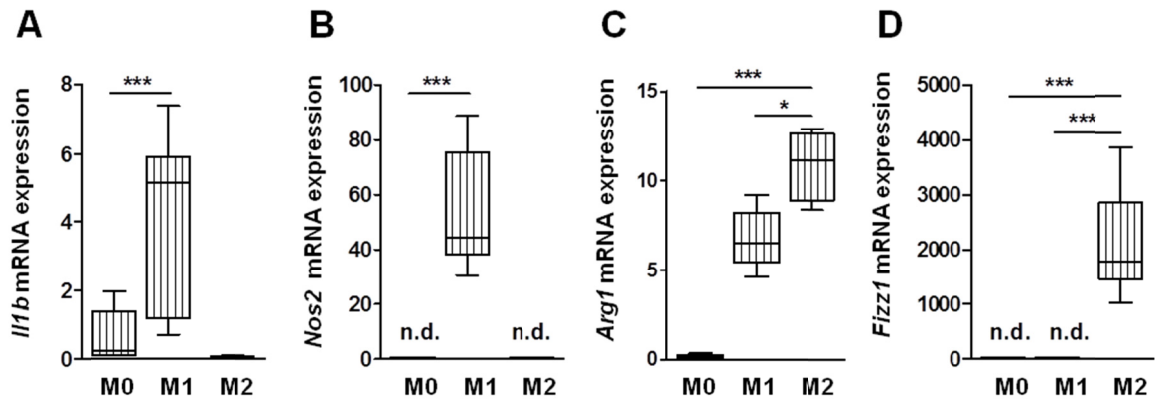

Figure S2: M1 and M2 marker gene expression of BMDMs in response to LPS/IFNγ or IL-4 treatment.

To validate the M1 or M2 polarization of macrophages, BMDMs from WT mice were treated with LPS (100ng/ml)/ IFNγ (20 ng/ml) for M1 or IL-4 (20 ng/ml) for M2 activation for 48h. RNA was isolated and M1 (*Il1b, Nos2)* and M2 *(Arg1, Fizz1)* marker gene expression was measured by qPCR using the ΔCt method and *Actb* as housekeeping gene; values are expressed as arbitrary units ($2^{-\Delta Ct}$ x 1000). (***p<0.001)

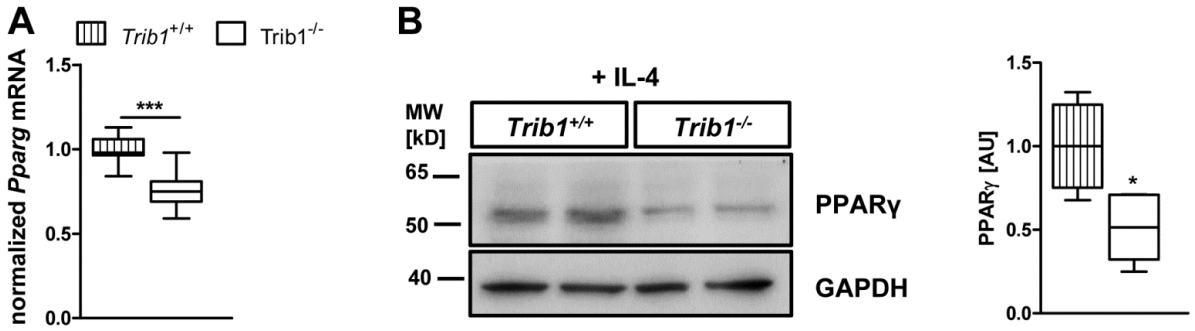

Figure S3: *Trib1* deficient BMDMs show lower levels of PPARγ mRNA and protein after IL-4 treatment.

For M2 polarization WT and *Trib1*$^{-/-}$ BMDMs were treated with IL-4 (20 ng/ml) for 48h. RNA was isolated and *Pparg* gene expression was measured by qPCR using the ΔCt method and *Actb* as housekeeping gene; values are expressed as changes compared to control group [A]. Whole cell lysates were prepared using RIPA lysis buffer and analyzed by immunoblotting using anti-PPARγ and anti-GAPDH antibodies [B]. Densitometric analysis was performed using the ImageJ software and is shown next to the blot; values are expressed as changes compared to control group (mean ± SD in arbitrary units (AU)). The striped column represents WT und the solid white column *Trib1*-deficient BMDMs. (***p<0.001)

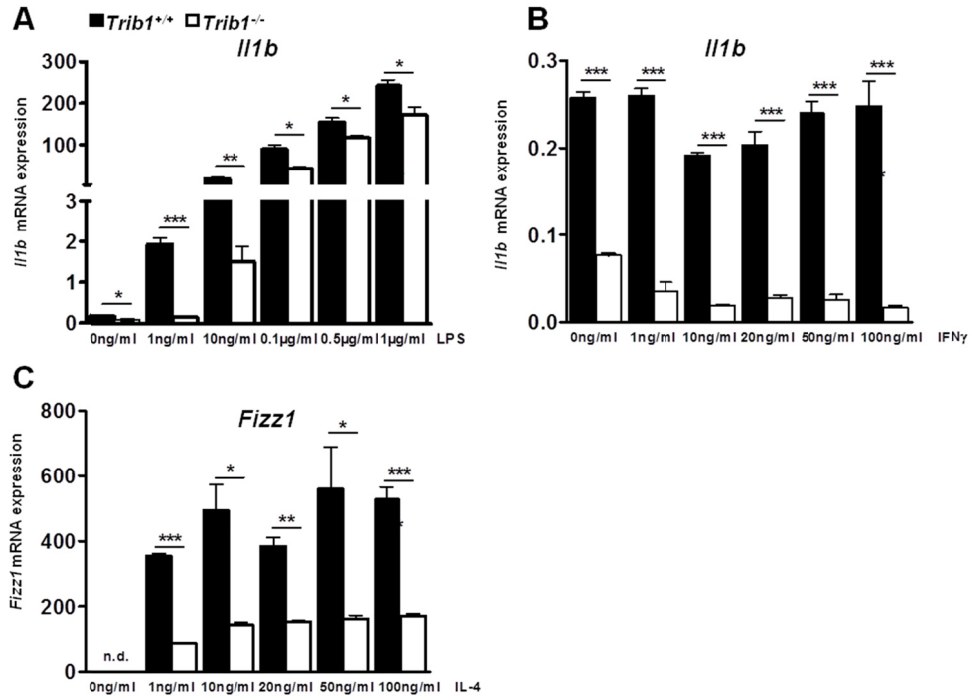

Figure S4: *Trib1*$^{-/-}$ BMDMs retain lower expression of *Il1b* and *Fizz1* in response to increasing concentration of LPS, IFNγ or IL-4.

WT and *Trib1*$^{-/-}$ BMDMs were stimulated either with LPS in a concentration range from 1ng/ml to 1µg/ml [A], or IFNγ from 1ng/ml to 100ng/ml [B] or IL-4 from 1ng/ml to 100ng/ml [C] over 48h. RNA was isolated and *Il1b* and *Fizz1* gene expression was measured by qPCR using the ΔCt method and *Actb* as housekeeping gene; values are expressed as arbitrary units ($2^{-\Delta Ct}$ x1000). The striped column represents WT und the solid white column *Trib1*-deficient BMDMs. (*p<0.05, **p<0.01, ***p<0.001, n.d. = not detectable)

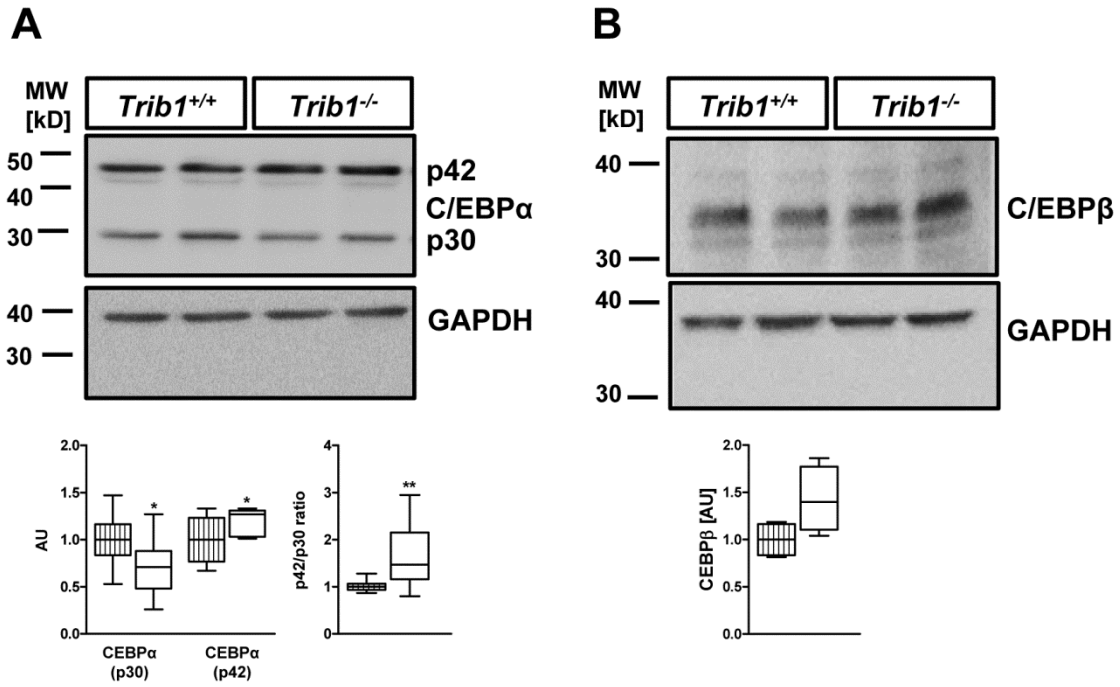

Figure S5: Protein abundance of CEBP/α is increased in *Trib1*$^{-/-}$ BMDMs.
Whole cell extracts from WT and *Trib1*$^{-/-}$ BMDMs were isolated using RIPA buffer and analyzed by immunoblotting. WT and *Trib1*$^{-/-}$ lysates were probed with antibodies against C/EBPα [A] and C/EBPβ [B] and GAPDH as loading control. Densitometric analysis was performed using the ImageJ software and is shown below each immuoblot; values are expressed as changes compared to control group (mean ± SD in arbitrary units (AU)). A representative blot from three independent experiments is presented for each protein. The striped column represents WT und the solid white column *Trib1*-deficient BMDMs. (*p<0.05, **p<0.01)

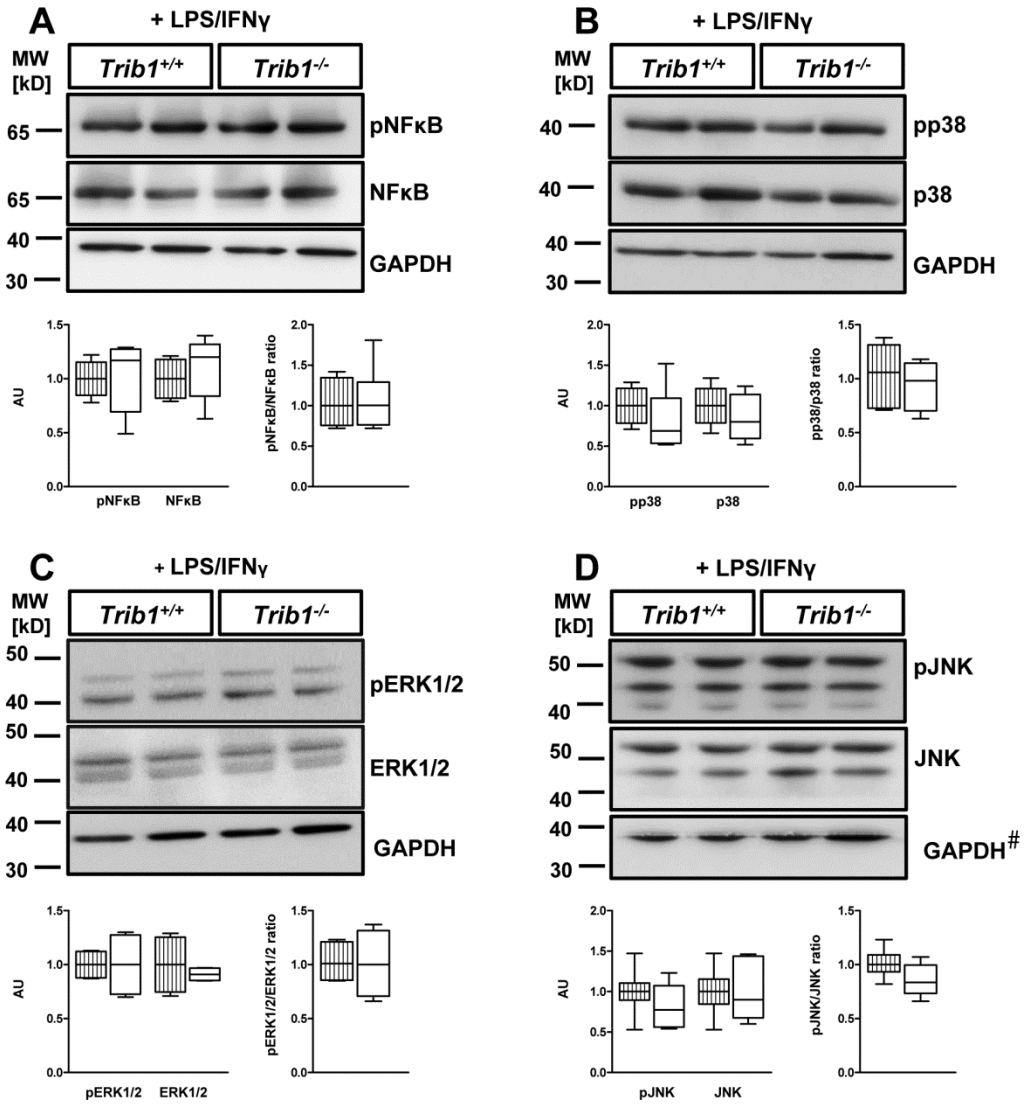

Figure S6: NFκB and MAPK signaling in response to LPS/ IFNγ treatment is not altered in *Trib1*$^{-/-}$ BMDMs.

BMDMs from WT and *Trib1*$^{-/-}$ mice were treated with LPS (100ng/ml)/ IFNγ (20 ng/ml) for 30 min to initiate M1 polarization. Total cell lysates were prepared and analyzed by immunoblotting. Abundance of total and phosphorylated protein was determined for NFκB [A], p38 [B], ERK1/2 [C] and JNK [D]. An antibody against GAPDH was used as loading control. # The GAPDH loading control in Figure S6D is identical with the GAPDH loading control for JAK1 in Figure 4D, as both western blots were created from the same membrane (upper part of the cut membrane was probed with JAK1).

Densitometric analysis was performed using the ImageJ software and is shown below each immuoblot; values are expressed as changes compared to control group (mean ± SD in arbitrary units (AU)). A representative blot from two to three independent experiments is presented for each protein. The striped column represents WT und the solid white column *Trib1*-deficient BMDMs.

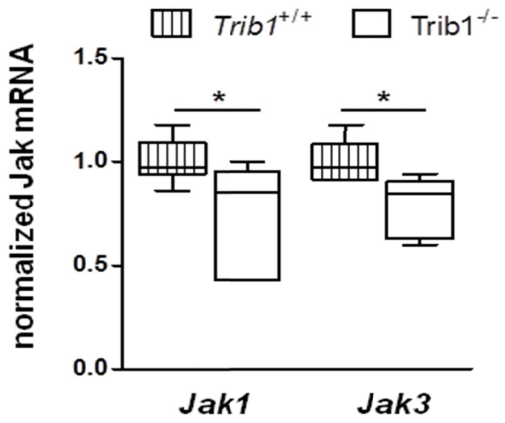

Figure S7: *Jak1* and *Jak3* gene expression is reduced in *Trib1*$^{-/-}$ BMDMs.
RNA from WT and *Trib1*$^{-/-}$ BMDMs was isolated and *Jak1* and *Jak3* gene expression was measured by qPCR using the ΔCt method and *Actb* as housekeeping gene; values are expressed as changes compared to control group. (*$p<0.05$)

Table S1: List of primers for quantitative RT-PCR

Gene name	Forward primer (5´- 3´)	Reverse primer (5´- 3`)
Il6	TCCAGTTGCCTTCTTGGGAC	GTGTAATTAAGCCTCCGACTTG
Il1b	TGCCACCTTTTGACAGTGATG	TGATGTGCTGCTGCGAGATT
Nos2	ACCTTGTTCAGCTACGCCTT	CATTCCCAAATGTGCTTGT
Arg1	ACAAGACAGGGCTCCTTTCAG	GGCTTATGGTTACCCTCCCG
Cd206	CAGGTGTGGGCTCAGGTAGT	TGTGGTGAGCTGAAAGGTGA
Fizz1	GGAACTTCTTGCCAATCCAG	GCCACAAGCACACCCAGTAG
Cd68	AGCTGCCTGACAAGGGACACT	AGGAGGACCAGGCCAATGAT
Cd36	TGGTGATGTTTGTTGCTTTTATGAT	GCTCATCCACTACTTATTTTCCATTCT
Srb1	CCCTCCCTCATCAAGCAGC	GAACTCCCTGTAGACATAGGGTC
Scarf1	GGTGACAGTTTCTCATCACGATCCA	AGGCTGGCTTCTGGTGACTC
Cxcl16	ACTGGCTTGAGGCAAATGTT	GGTTCCAGTTGCAGTCCAAA
Stab1	CTTCTCCAACCCCTGCTACG	CACAGCCACAGTCCTCCTG
Marco	CTGTCGCATGCTCGGTTAC	TGCAGTCCCACAAACTGTTC
Ccr2	TCATAAAGGAGCCATACCTGTAAA	GTATGCCGTGGATGAACTGA
Trib1	TAGCTGAGCGCGAGCATGTGT	CGTTTTCGGCTCCGCACATAGGA
Pparg1	CGCGGGCTGAGAAGTCACGTT	GGTGGGCCAGAATGGCATCT
Actb	GTGCGTGACATCAAAGAG	GCCACAGGATTCCATACC
Sra	CATGAACAAGAGGATGCTGACT	GGAAGGGATGCTGTCATTGAA
Lox1	ATGAAGCCTGCGAATGACGA	CTGGCGTAATTGTGTCCACTG
Cd163	GGTCATTCAGAGGCACACTG	CTGGCTGTCCTGTCAAGGCT
Jak1	ACGCTCCGAACCGAATCATC	GTGCCAGTTGGTAAAGTAGAACC
Jak3	ACACCTCTGATCCCTCAGC	GCGAATGATAAACAGGCAGGATG