

Supplement

IGF2BP2 Deficiency in Macrophages Impairs Migration, Reprograms Metabolism, and Limits Tumor Progression

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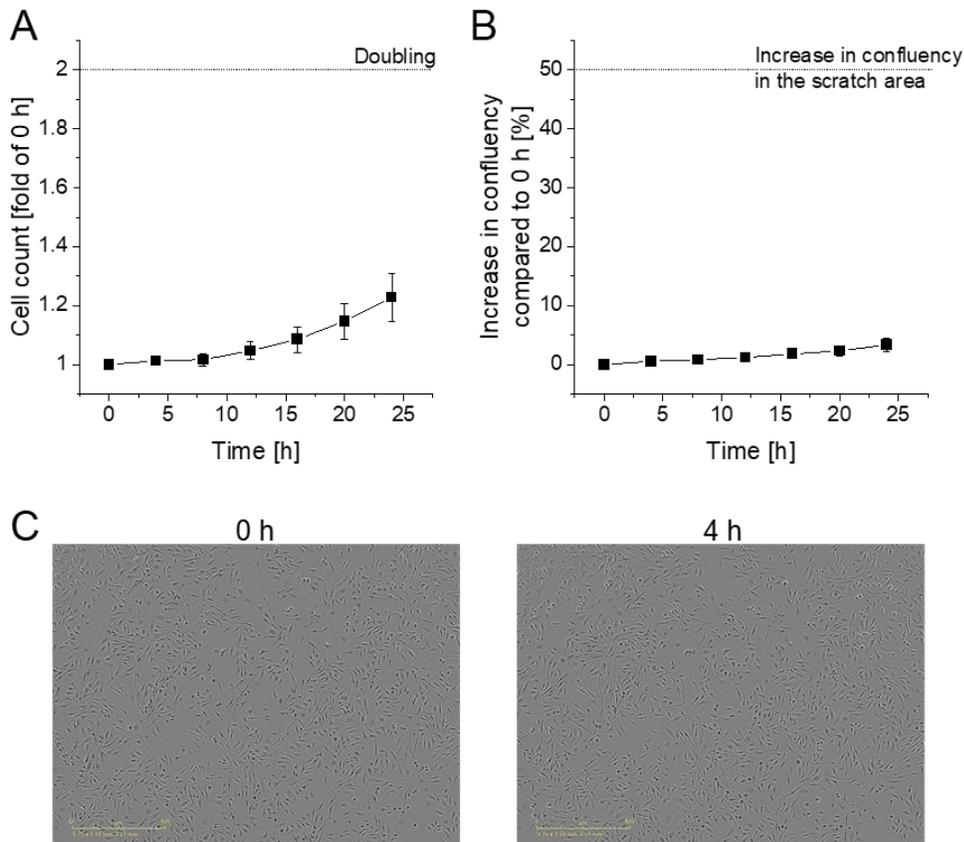
17 Supplemental Tables

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19 Table 1: Primer Sequences

Gene	Accession number	Primer forward sequence	Primer reverse sequence
<i>Acss2</i>	NM_019811.3	TATGTGACCGGAGATGGCTGC	TCGTGTTCCACAAGTGCCGA
<i>Aldh2</i>	NM_001308450	GGACCTACCTAGCGGCCTTG	TGGTACTTGTTCAGCCCAGCC
<i>Aldoc</i>	NM_001303423	CATCTGCCAGCAGAATGGGAT	GCTTGAGCAGAGTCCCTTCG
<i>Bpgm1</i>	NM_007563.5	GTCTGCAGCACGGCGTTAC	CAAGAACAGCAGTATATCTGCAAG G
<i>Cxcl1</i>	NM_008176.3	AACCGAAGTCATAGCCACACT	CCGTTACTTGGGGACACCTT
<i>Fgf2</i>	NM_008006.2	GCTCTACTGCAAGAACGGCG	TAACACACTTAGAAGCCAGCAGC
<i>Gapdh</i>	NM_001289726. 2	TCGGTGTGAACGGATTTGGC	TGAAGGGGTTCGTTGATGGCA
<i>Gpi1</i>	NM_008155.4	CCTGTCTACGAACACGGCCA	GCTGACCACAGCGAATAGCG
<i>Hif1a</i>	NM_001313919	TCAAGCAGCAGGAATTGGAACAT	TCATCCATTGACTGCCCCAGC
<i>Hk2 (genomic DNA)</i>	NC_000072.7	GCCAGCCTCTCCTG ATTTTAGTGT	GGGAACACAAAAGACCTCTTCTGG
<i>Il1β</i>	NM_008361.3	CCAAAAGATGAAGGGCTGCTT	GGAAGGTCCACGGGAAAGAC
<i>Il6</i>	NM_031168.2	AAGAAATGATGGATGCTACCAA ACT G	GTA CTCCAGAAGACCAGAGGAAAT T
<i>Igf2bp2</i>	NM_183029.2	TTGGATGGGCTGTTGGCTGA	GTGACGTTGACAACGGCAGTT
<i>Mito 16S</i>	AP014886.1	CCGCAAGGGAAAGATGAAAGAC	TCGTTTGGTTTCGGGGTTTC
<i>Mito Nd1</i>	AP014886.1	CTAGCAGAAACAAACCGGGC	CCGGCTGCGTATTCTACGTT
<i>Mmp2</i>	NM_008610.2	CATCGCTGCACCATCGC	GCATGGTCTCGATGGTGTTT
<i>Mmp9</i>	NM_013599.3	GCCGACTTTTGTGGTCTTCC	TACAAGTATGCCTCTGCCAGC
<i>Mrc1</i>	NM_008625.2	TTCAGCTATTGGACGCGAGG	GAATCTGACACCCAGCGGAA
<i>Nos2</i>	NM_010927.3	CTTCCTGGACATTACGACCC	TACTCTGAGGGCTGACACAA
<i>Pgk1</i>	NM_008828.3	CAAGCTGGACGTGAAGGGGAA	AACAGCAGCCTTGATCCTTGGT
<i>Ppia</i>	NM_008907.1	GCGTCTCCTTCGAGCTGTTT	CACCCTGGCACATGAATCCT
<i>RNA18S</i>	NR_003278.3	AGGTCTGTGATGCCCTTAGA	GAATGGGGTTCAACGGGTTA
<i>Tgfb</i>	NM_011577.1	ACCCTGCCCTATATTTGGA	CGGGTTGTGTTGGTTGTAGAG
<i>Tnf</i>	NM_013693.2	CCATTCTGAGTTCTGCAAAGG	AGGTAGGAAGGCCTGAGATCTTAT C
<i>Vegfa</i>	NM_001025250. 3	CCACCATGCCAAGTGGTCCC	ACCAGGGTCTCAATCGGACG

20 **Supplemental Figures**

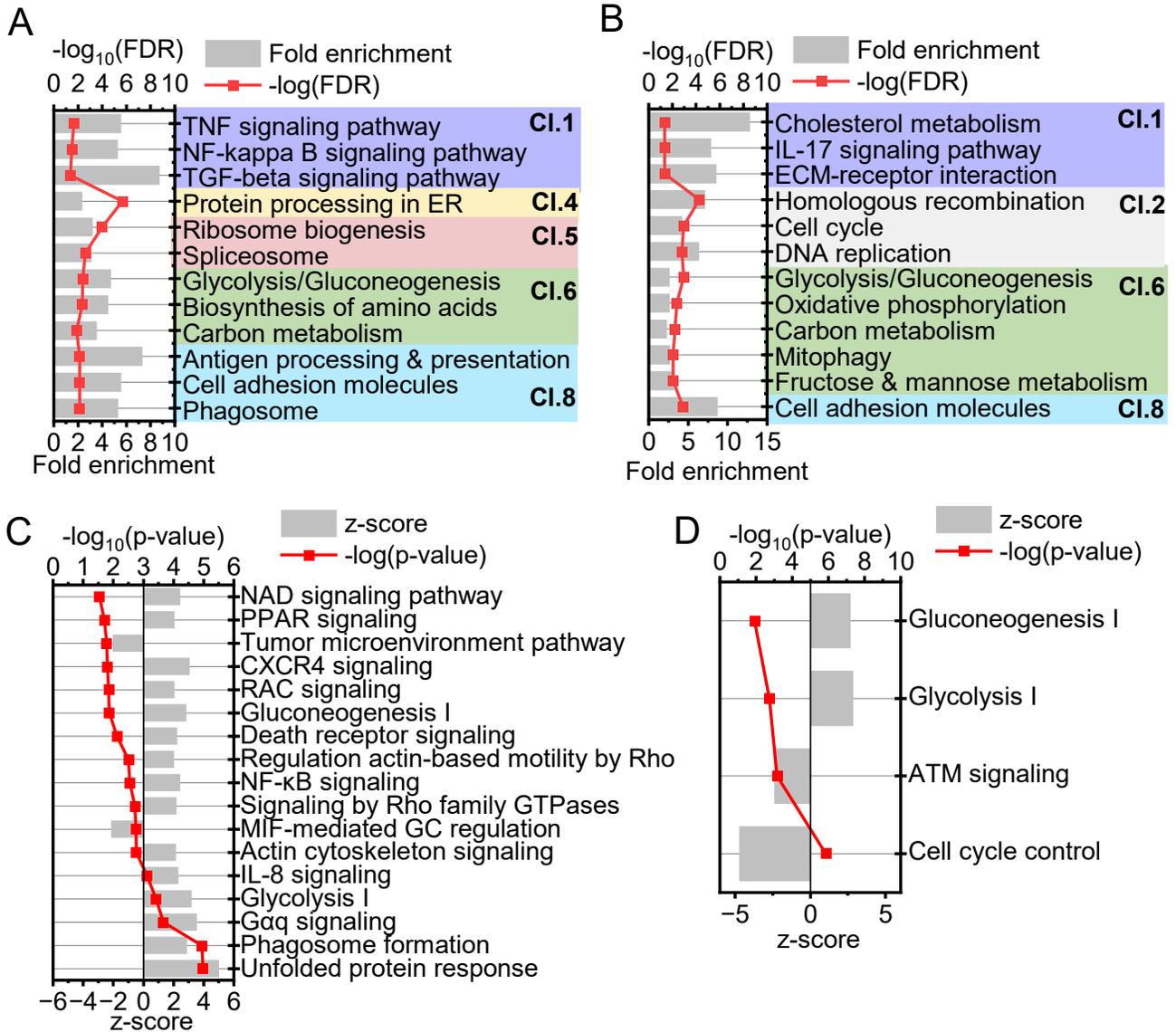


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22 **Supplemental Figure 1.** Proliferation of bone marrow-derived macrophages under culture conditions used for
23 migration assays (n=8). (A) Cell counts over 24 h, normalized to baseline at 0 h. BMMs showed only a 1.2-fold
24 increase after 24 h. (B) Confluency increase measured by IncuCyte live-cell imaging. Confluency increased by
25 3.3 % after 24 h, corresponding to ~0.13 % per hour. (C) Representative phase-contrast images of BMM cultures
26 at 0 h and 4 h, illustrating negligible changes in cell density over the assay period. Scale bar: 400 μ m.

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Supplemental Figure 2. Pathway Analysis in WT and IGF2BP2 KO Macrophages.

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(A, B) Selected KEGG pathways enriched in clusters (CI) identified as shown in Figure 1C and 1D. Pathway enrichment significance is shown by false discovery rate (FDR) values (fold change > 2, FDR < 0.05). (C, D)

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Ingenuity Pathway Analysis (IPA) of differentially expressed genes (DEGs; p < 0.05) in untreated (A, C) and IL-4-treated (8 h, 20 ng/ml) (B, D) BMMs (n = 3 mice per group) from WT and IGF2BP2 MΦ-KO mice n = 3

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mice per group) (Supplementary file S5). Pathways are ranked by activation z-scores (z-score > 2, p-

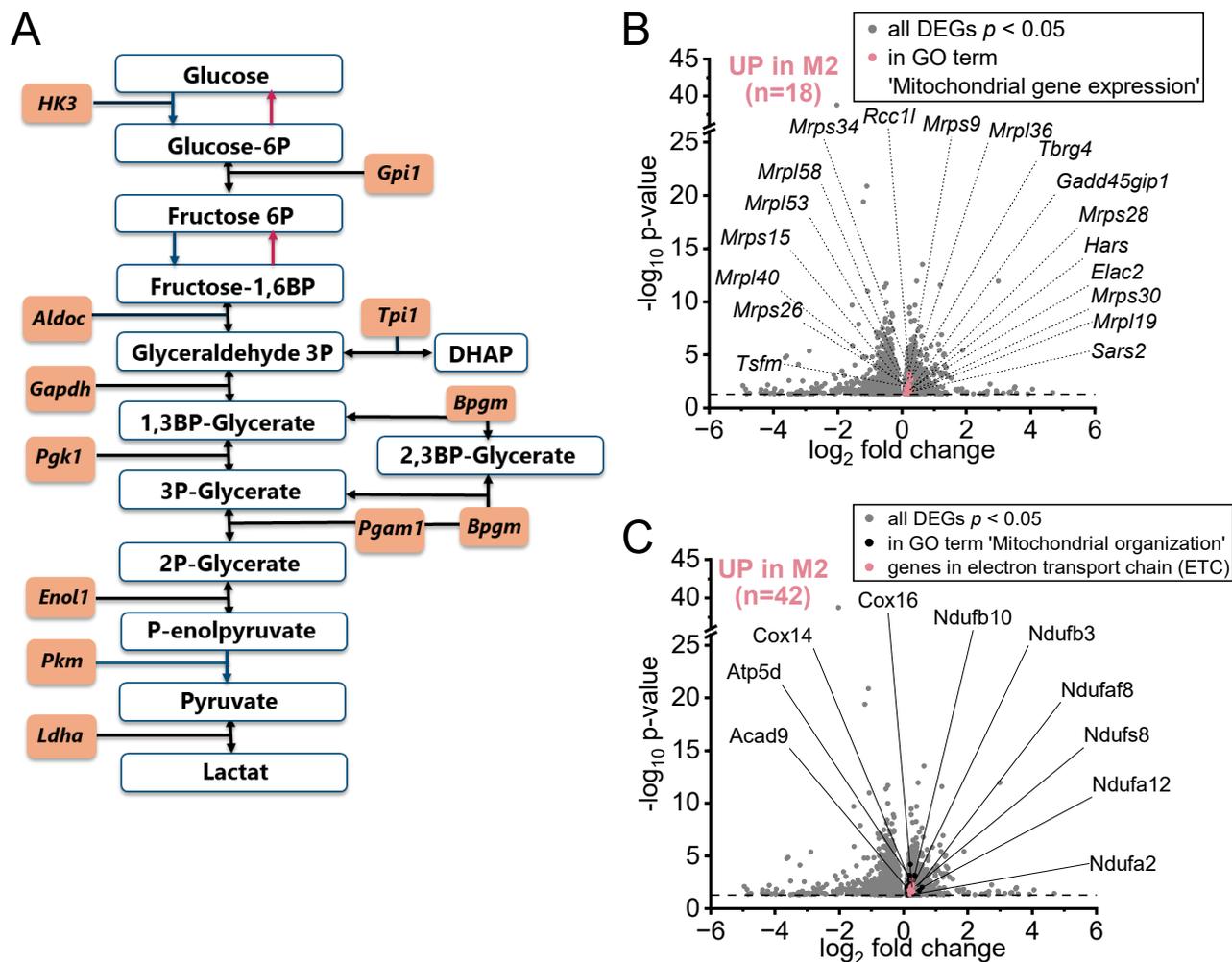
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value < 0.05), indicating predicted pathway activation states.

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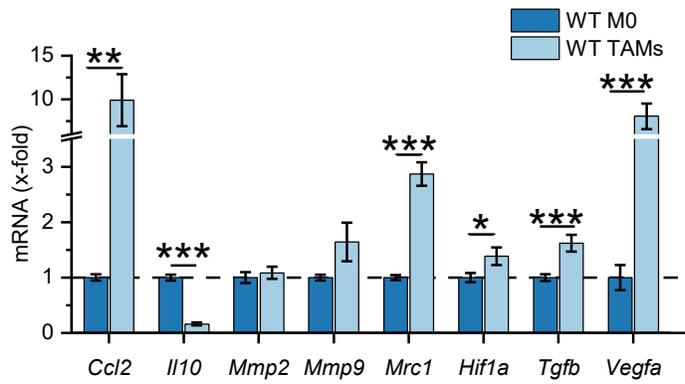
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39 **Supplemental Figure 3. Alterations in Glycolytic and Mitochondrial Pathways in IGF2BP2 KO**
 40 **Macrophages.** (A) Schematic representation of the glycolytic pathway, highlighting genes (purple) that are
 41 significantly upregulated ($p < 0.05$) in IGF2BP2 KO M2-polarized macrophages in KEGG pathway
 42 “Glycolysis” (B, C) Volcano plots of DEGs (shown in gray) of KO M2 vs. WT M2 macrophages, with
 43 significantly upregulated genes in (B) the GO term "Mitochondrial gene expression" (GO:0140053)
 44 highlighted in purple, and (C) the GO term "Mitochondrial gene expression" (GO:0140053) shown in black,
 45 with a subset of genes from the electron transport chain (ETC) highlighted in purple (n = 3 mice per group).

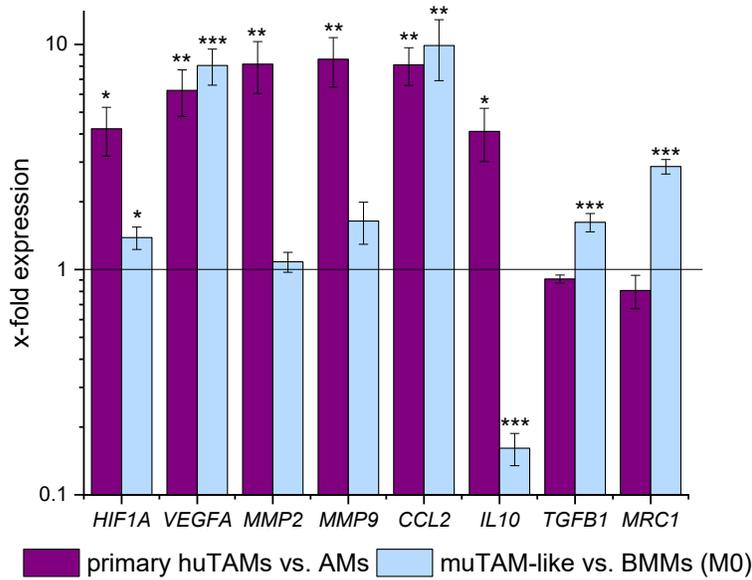
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48 **Supplemental Figure 4. Elevated TAM Marker Expression in LLC1 TAM-like Macrophages.** BMMs
 49 were cultured in standard medium (M0) or TCM from LLC1 cells (TAM-like) for 8 hours. mRNA expression
 50 was measured by qPCR and normalized to *Ppia*. Expression of TAM-like macrophage markers is shown as a
 51 fold change of WT M0 (n = 6 mice per group, duplicates). Data are presented as mean ± SEM. Statistical
 52 significance was determined by Student's t-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

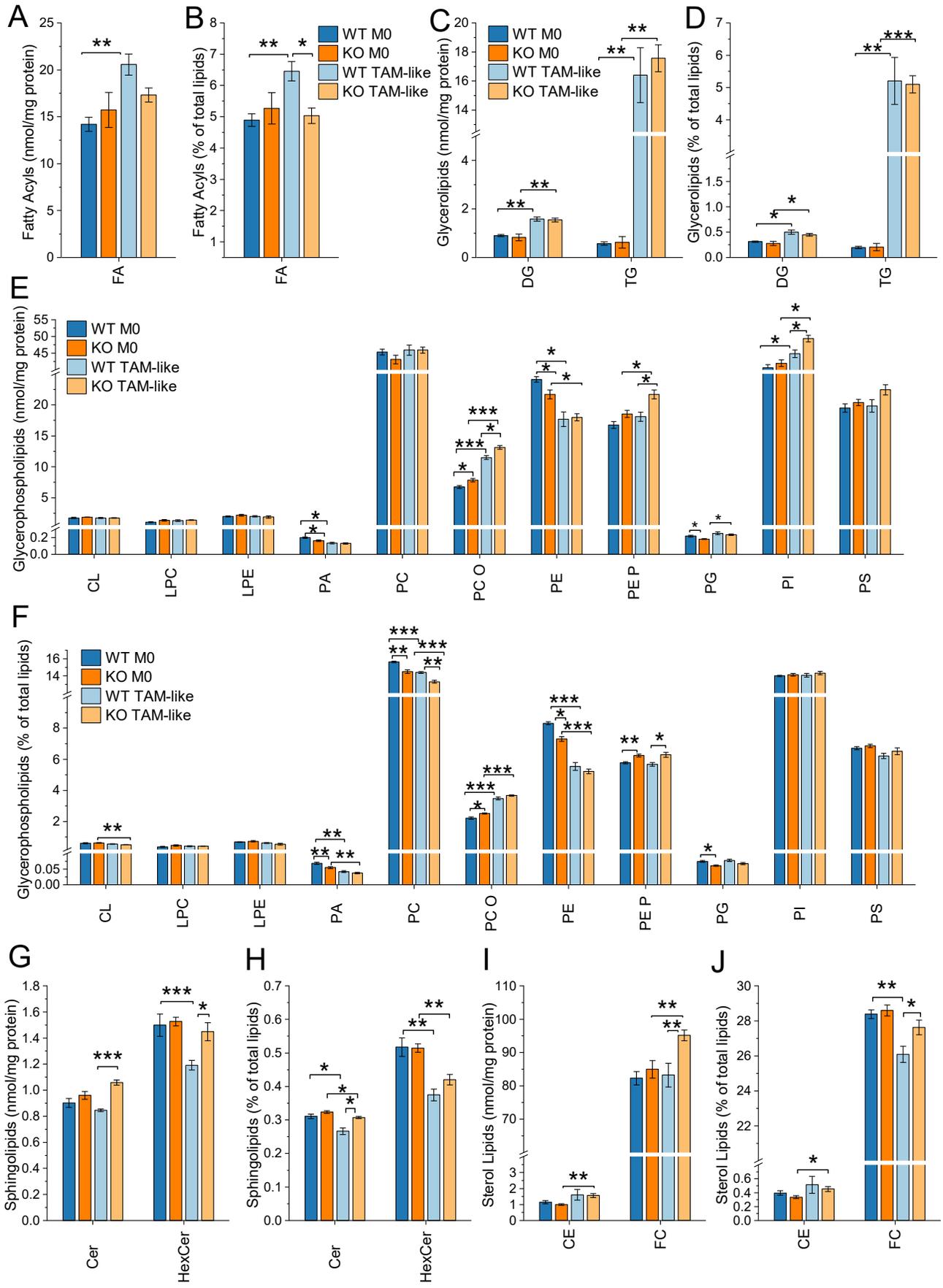
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55 **Supplemental Figure 5. Comparison of TAM-associated gene expression in murine and human TAM(-**
 56 **like) macrophages.** mRNA expression of selected TAM-associated genes in murine BMMs cultured in
 57 standard medium (M0) or tumor-conditioned medium from LLC1 cells (TAM-like; n = 6 mice per group,
 58 duplicates) and in human tumor-associated macrophages (TAMs) versus matched alveolar macrophages
 59 (AMs) from GEO dataset GSE162669 (n = 3 donors per group, triplicates). Expression values are shown as x-
 60 fold change relative to M0 (for murine data) or AMs (for human data). Data are presented as mean ± SEM.
 61 Statistical significance was determined by Student's t-test (*p < 0.05; **p < 0.01; ***p < 0.001).

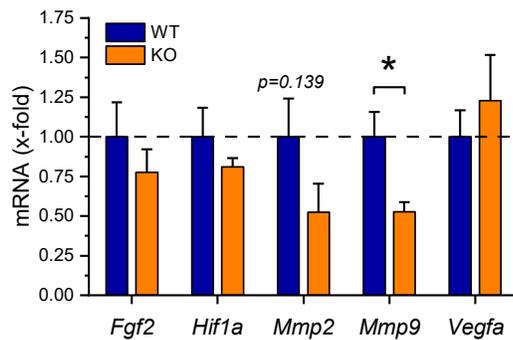
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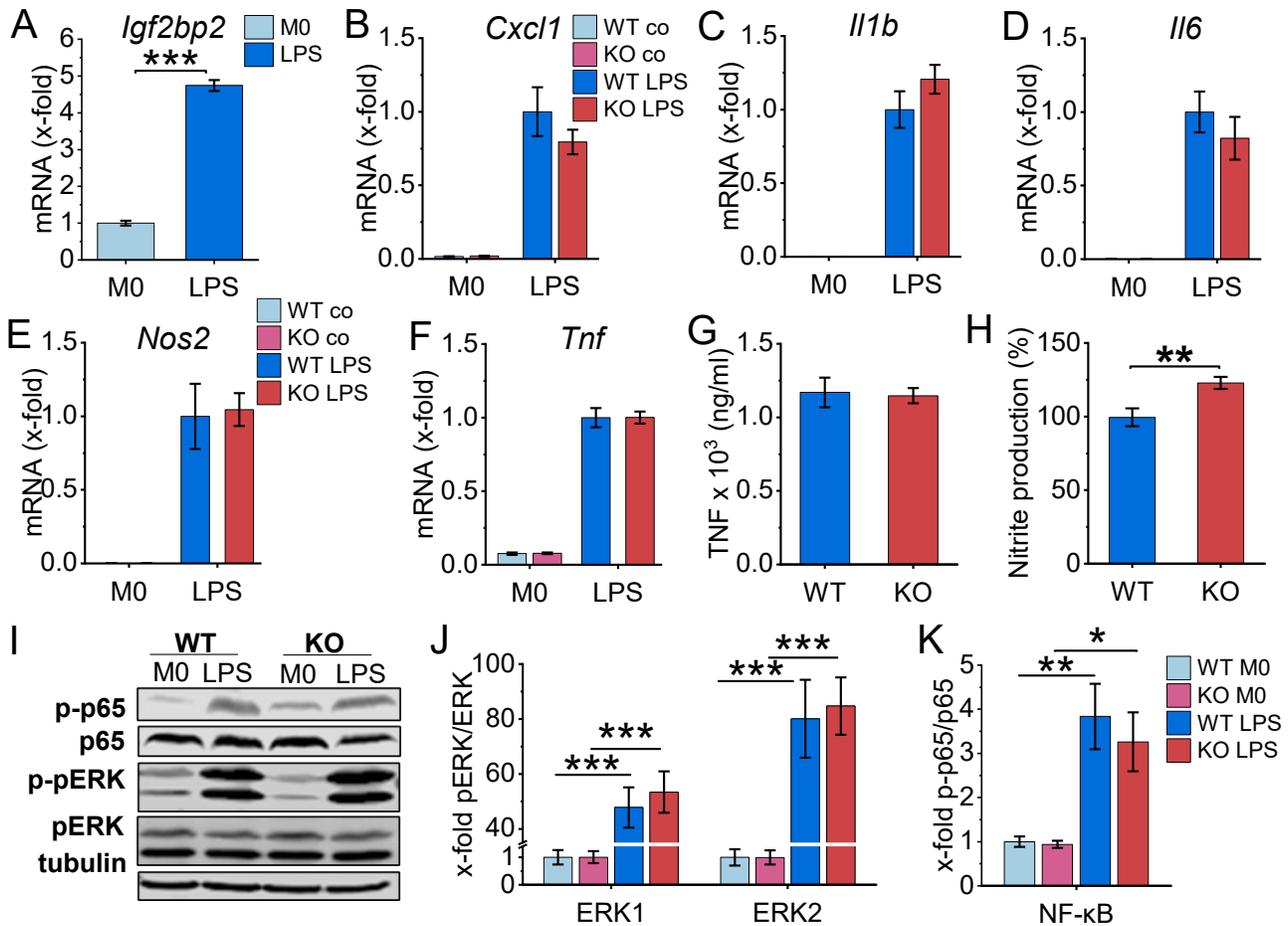
65 **Supplemental Figure 6. Lipid Classes in WT and IGF2BP2 KO Macrophages (M0 and TAM-like**
66 **macrophages).** BMMs were cultured either in standard medium (M0) or in TCM from LLC1 cells for
67 24 hours to induce TAM-like polarization. Lipid concentrations in WT and IGF2BP2 KO M0 and TAM-like
68 macrophages were analyzed by mass spectrometry. Data are displayed as (A, C, E, G, I) absolute
69 concentrations (nmol/mg protein) and (B, D, F, H, J) relative abundance, expressed as a percentage of total
70 detected lipids. Lipid categories: (A-B) Fatty acyls, (C-D) Glycerolipids, (E-F) Glycerophospholipids, (G-H)
71 Sphingolipids, (I-J) Sterol lipids. Data are presented as mean \pm SEM (n = 4 mice per group). Statistical
72 significance was determined by ANOVA with Bonferroni's post hoc test (* $p < 0.05$; ** $p < 0.01$;
73 *** $p < 0.001$).

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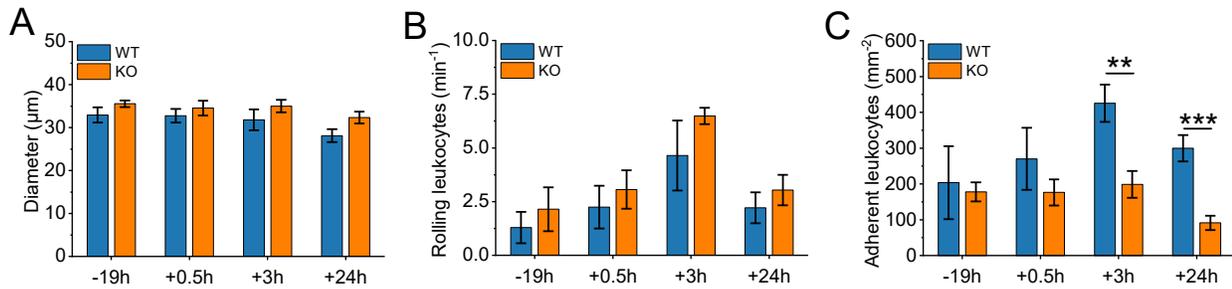
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76 **Supplemental Figure 7. Expression of Angiogenesis-related Genes in IGF2BP2 M Φ -KO Tumors.** RNA
77 was isolated from tumor tissue and mRNA expression levels of *Fgf2*, *Hif1a*, *Mmp2*, *Mmp9*, and *Vegfa* were
78 normalized to *Ppia* and expressed as fold change relative to WT tumor tissue. Data are presented as mean \pm
79 SEM (n = 8 WT, n = 6 KO). Statistical analysis was performed using Student's t-test (* $p < 0.05$; ** $p < 0.01$;
80 *** $p < 0.001$).



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 82 **Supplemental Figure 8. Regulation and Role of IGF2BP2 in LPS-activated BMMs (A-G)** BMMs were
 83 treated with 100 ng/ml LPS for 4 hours. (A, B) mRNA expression data were normalized to *Ppia* and expressed
 84 as x-fold of untreated WT control. A: *Igf2bp2* expression (n = 8 mice per group, duplicates). (B-F) Expression
 85 of *Cxcl1*, *Il1b*, *Il6*, *Nos2*, and *Tnf* (n = 5 mice per group, duplicates). (G) TNF secretion was measured by
 86 ELISA in LPS-treated BMMs (n = 8 mice per group, duplicates). (H) The relative nitrite production in
 87 LPS/IFN- γ (100 ng/ml; 25 ng/ml; 24 h) treated BMMs was measured by Griess assay, with data normalized to
 88 WT (set to 100%) (n = 8 mice per group, triplicates). (I) ERK and NF- κ B activation in WT and IGF2BP2 KO
 89 BMMs were assessed by Western blotting after 15 minutes of LPS (100 ng/ml) treatment.
 90 (J, K) Phosphorylated ERK (pERK) and phosphorylated p65 (pp65) signal intensities were quantified and
 91 normalized to their respective total (unphosphorylated) protein levels. Tubulin served as a loading control
 92 (n = 5 mice per group, duplicates). Data are presented as means \pm SEM. Statistical analysis was performed
 93 using ANOVA with Bonferroni's post hoc test (J,K) or Student's *t*-test (A, H) ($p < 0.05$; * $p < 0.01$;
 94 ** $p < 0.001$).

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Supplemental Figure 9. IGF2BP2 Modulates Leukocyte Adherence in Arterioles. Intravital fluorescence microscopy of arterioles was used to assess microcirculatory changes. Quantification includes (A) arteriolar diameter (μM), (B) rolling leukocytes, and (C) adherent leukocytes in dorsal skinfold chambers of WT and IGF2BP2 MΦ-KO mice (n = 8 mice per group). Mice were exposed to LPS treatment for 0.5 hours, and WT and IGF2BP2 MΦ-KO animals were analyzed simultaneously. Measurements were obtained using intravital fluorescence microscopy and computer-assisted image analysis. Data are presented as mean ± SEM, with statistical significance determined using Student's t-test for individual time points (*p < 0.05; **p < 0.01; ***p < 0.001).

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