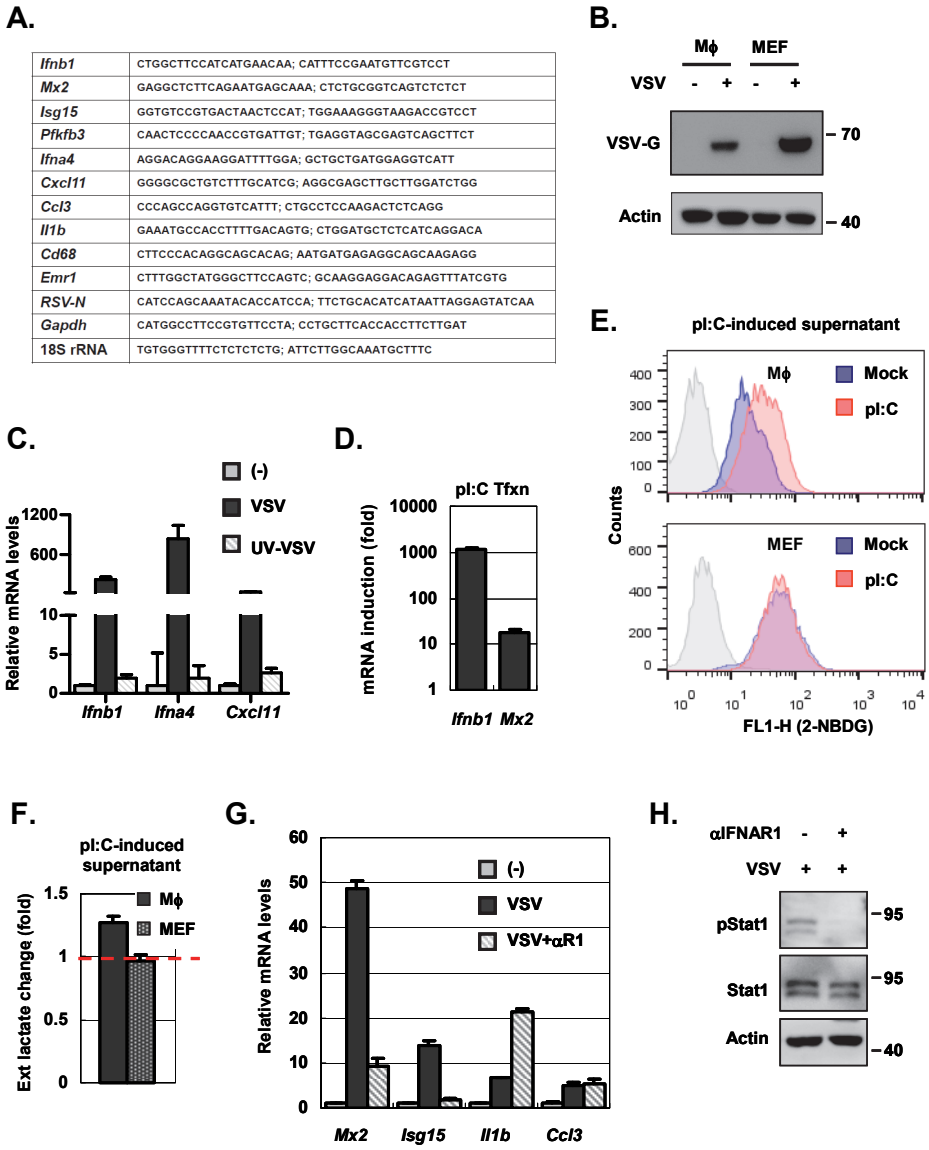
Supplemental Figures and Legends

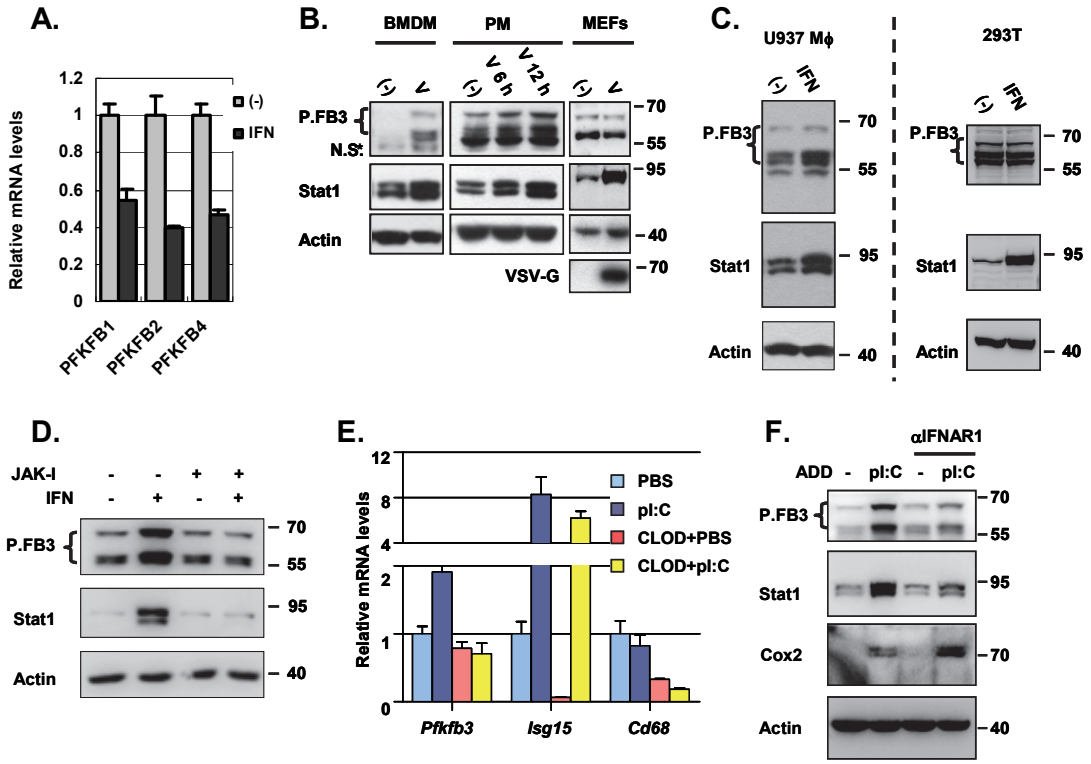
Supplemental Fig. 1



Supplemental Fig. 1: **VSV infection leads to IFN-dependent enhancement of glycolysis preferentially in macrophages. (A)** Sequences (5' to 3') for the major qPCR primers (in pairs) used in the present study. **(B)** PMs or MEFs were inoculated with VSV at MOI of 1. Cell lysates were harvested 8 h later and analyzed on WB. **(C)** PMs were inoculated with live VSV or UV-treated VSV at MOI of 1. Eight hours

following removing of the inoculum, total RNA was harvested for analysis of indicated markers. The mRNA levels of untreated cells were used for normalization. **(D)** PMs were mock-transfected or transfected with poly(I:C) (pI:C, 5 μg/ml) for 4 h and the cells were then fed with fresh medium. Six hours later, total RNA was harvested for analysis. The levels of induction of indicated mRNAs were normalized to the control levels and presented in log scale. **(E and F)** MEFs were transfected with poly(I:C) (3 μg/P100) for 4 h. The cells were fed with fresh medium and further incubated for 6 h. The conditional medium was harvested. PMs and MEFs were stimulated with the latter conditional medium for 8 h. In (E), 2-NBDG was then added to the culture medium for 15 min. The cells were subjected to flow cytometry to determine the level of 2-NBDG uptake. In (F), the relative levels of extracellular lactate over those in untreated cells were plotted. **(G)** PMs were infected with VSV (or mock-infected) in the presence of control IgG or a blocking antibody against IFNAR1. RNA samples were harvested 8 h after infection and subjected to qPCR analysis. **(H)** PMs were infected as in (G), but only for 4 h. The levels of pStat1 and Stat1 in the cell lysates were analyzed by WB. At this earlier time point, pStat1 but not Stat1 level was sensitive to receptor blockage. In this figure, data presented are representative of at least two independent experiments. Quantitative data were graphed using the means of triplicate or quadruplicate measurements (± STDEV).

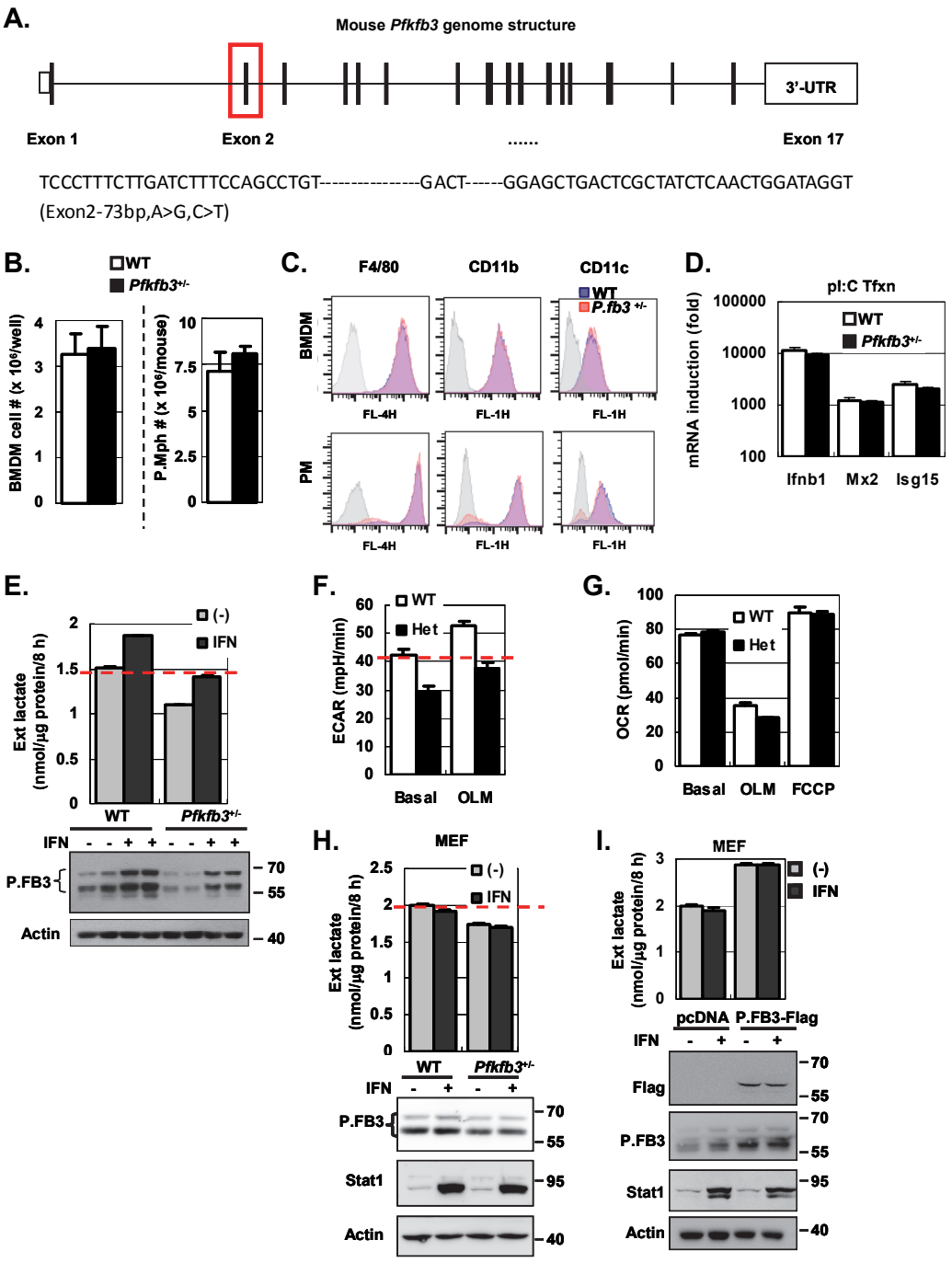
Supplemental Fig. 2



Supplemental Fig. 2: Type I IFN induces glycolytic regulator PFKFB3 in macrophages. (A) PMs were treated with 1000 IU/ml of mIFNβ for 8 h and RNA samples were analyzed for the levels of other *Pfkfb* family members. **(B)** BMDMs (MOI of 5), PMs (MOI of 1) and MEFs (MOI of 1) were treated with VSV (V) for 8 h, if not specifically indicated. The cell lysates were subjected to Western analysis with indicated antibodies. 'P.FB3' is the abbreviation for PFKFB3. Two major bands representing PFKFB3 (marked by a bracket) and non-specific signal (N.S.) are indicated. **(C)** U937 macrophages or the non-immune 293T cells were stimulated with 1000 IU/ml of hIFNα for 8 h as indicated. Cell lysates were analyzed on Western. **(D)** **(D)** PMs were pre-treated with Jak-inhibitor (5 μM) for 1 h and were then stimulated with IFN for 8 h. Cell lysates were analyzed on immunoblot. **(E)** Mice were

intranasally instilled with PBS or chlodronate liposome (CLOD). Two days later, the mice were injected i.p. with 50 μg poly(I:C). 12 h later, lungs were harvested from the mice and the RNA samples were subjected to qPCR analyses. **(F)** PMs were stimulated with poly(I:C) (50 μg/ml) for 8 h in the presence in the presence of control IgG or blocking antibody against IFNAR1. Cell lysates were subjected to Western analysis. In this figure, data presented are representative of at least two independent experiments. The qPCR analyses were graphed using the means of quadruplicate measurements (± STDEV).

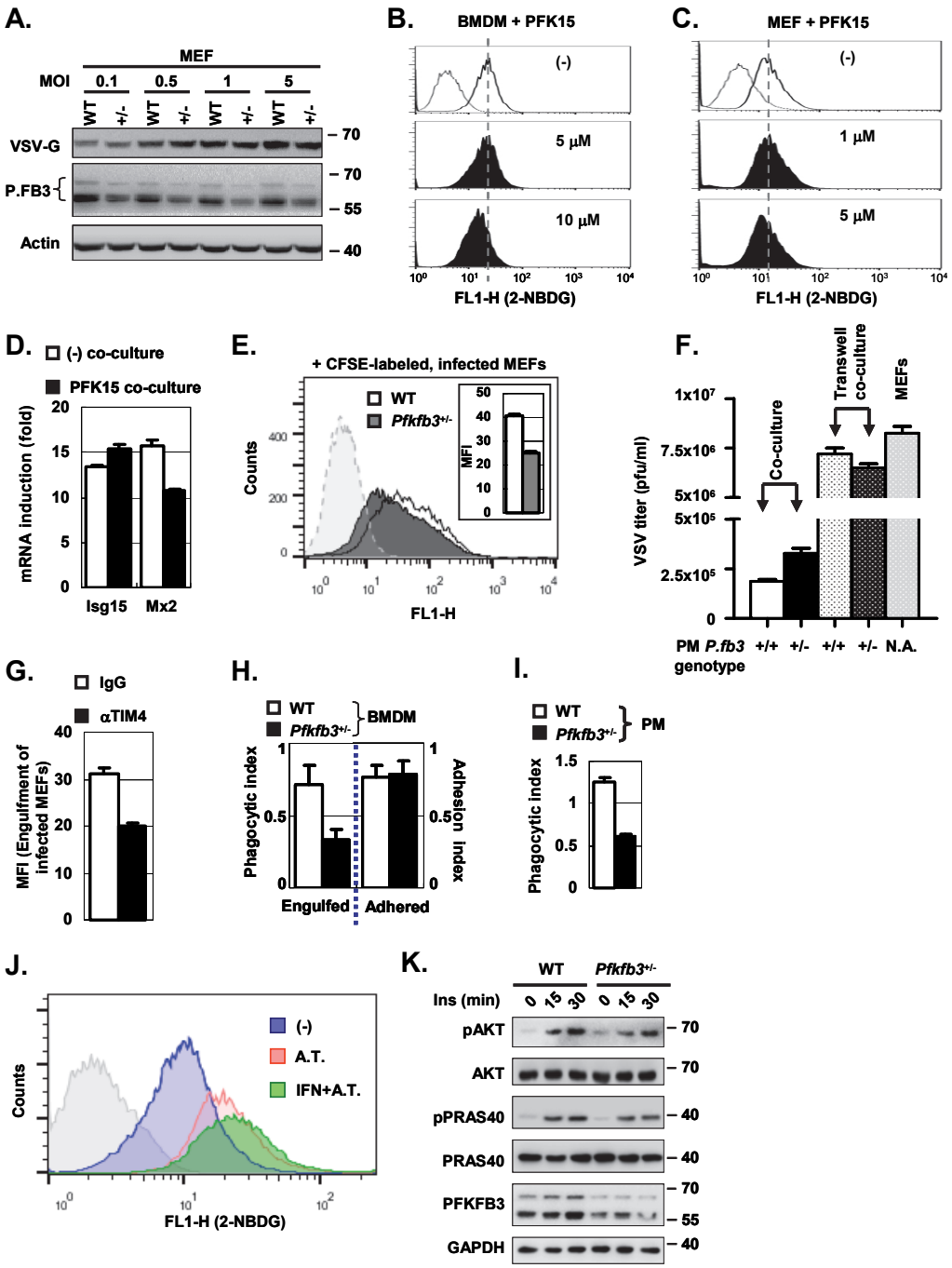
Supplemental Fig. 3



Supplementary Fig. 3: **PFKFB3 enhances glycolysis in macrophages. (A)** Genome structure of mouse *Pfkfb3* gene is depicted. The exon harboring the indel mutation is highlighted. **(B)** Left: Bone marrow cells were plated (triplicates) at a density of 2x 10^6 in a 60-mm dish in the presence of macrophage-differentiating L-medium. On day

7, the fully differentiated macrophages were counted (±STDEV). Right: five days after i.p. injection of thioglycollate, the peritoneal macrophages were harvested (n=2). The numbers of PMs isolated from the WT and *Pfkfb3*$^{+/-}$ mice were counted (± range). **(C)** The WT and *Pfkfb3*$^{+/-}$ BMDMs (upper) and PMs (lower) were subjected to flow cytometry analysis using the indicated antibodies. **(D)** BMDMs were mock-transfected or transfected with poly(I:C) (pI:C, 5 μg/ml) for 4 h and the cells were then fed with fresh medium. Eight hours later, total RNA was harvested for analysis. The levels of induction of indicated mRNAs were normalized to the control levels in the WT cells and presented in log scale. **(E)** Thioglycollate-elicited PMs from the WT or *Pfkfb3*$^{+/-}$ mice were stimulated with IFN for 8 h. The culture supernatant was analyzed for the levels of extracellular lactate and the cell lysates were analyzed using indicated antibodies. **(F and G)** WT and *Pfkfb3*$^{+/-}$ PMs were treated as in (E). Cells were then subjected to ECAR (F) and OCR (G) analysis using Seahorse metabolic analyzer. Under given nutrient/stress conditions, the average levels of the latter parameter at several time points were averaged. **(H and I)** WT or *Pfkfb3*$^{+/-}$ primary MEFs (H) and MEFs transfected with empty plasmid or Flag-tagged PFKFB3 (I) were stimulated and analyzed as in (E). In this figure, data presented are representative of at least two independent experiments. Metabolic analyses were graphed using the means of triplicate or quadruplicate measurements (± STDEV).

Supplemental Fig. 4



Supplementary Fig. 4: **PFKFB3 on macrophage extrinsic antiviral activity and efferocytosis. (A)** WT and *Pfkfb3*$^{+/-}$ primary MEFs were infected with VSV at indicated MOIs. 14 h after infection, the lysates were harvested and analyzed using the indicated antibodies. **(B and C)** BMDMs (B) or MEFs (C) were treated with

indicated concentration of PFK15 for 3 h. Cells were then incubated with glucose analog, 2-NBDG, and subjected to flow cytometry analysis. A left-ward shift of the peak indicates a decreased capacity to uptake glucose. **(D)** WT MEFs were first infected with VSV at MOI of 1 for 14 h. Unattached and loosely attached cells were collected. Infected MEFs were then added in a ratio of 2:1 into BMDMs that had been pre-treated with DMSO or PFK15 for 3 h. RNA samples were harvested after 12 h and later analyzed (means of quadruplicate measurements, ±STDEV). **(E)** MEFs were labeled with CSFE and were then infected and collected as in (D). Infected MEFs were then added in a ratio of 10:1 into BMDMs. Four h later, the cells were washed extensively and stained with F4/80 antibody (FL4-H). To measure efferocytosis of infected MEFs, the CSFE (FL1-H) fluorescence of F4/80-positive cells was determined by flow cytometry. For quantitation, the MFI was determined and shown in the inset. (**F**) The infected MEFs were prepared as in (D) and added at a ratio of 1:2 to wells containing (or w/o) WT or *Pfkfb3*$^{+/-}$ PMs. In some groups, infected MEFs were segregated from the PMs by 3 μm pore-sized transwell membranes. The culture supernatants were harvested in 12 h for viral titer analysis (means of duplicate measurements, ± range). **(G)** BMDMs were pre-incubated with IgG or anti-TIM4 (100 μg/ml) and were then subjected to the flow cytometry-based efferocytosis assay as in (E). The MFI levels of CSFE fluorescence were shown. **(H)** The efferocytosis activities of WT and *Pfkfb3*$^{+/-}$ BMDMs were measured using apoptotic thymocytes (20:1). Four h following cargo addition, the average number of engulfed apoptotic cells per total macrophage in 10 different microscopic fields were determined

(±STDEV) and were shown on the left. To determine the capacity of BMDMs to initially bind the cargos, the BMDMs were initially added with a greater excess (ratio of 100:1) of apoptotic thymocyte. After a brief incubation of 15 min, the attached apoptotic thymocytes were quantitated similarly as above and the results were shown on the right. **(I)** WT and *Pfkfb3*$^{+/-}$ PMs were subjected to efferocytosis assay using apoptotic thymocytes. **(J)** Serum-starved PMs that had undergone pre-treatment w/ or w/o IFN (8 h) were added with A.T. (20:1). Cells were harvested in 1 h for assay of 2-NBDG uptake. **(K)** WT and *Pfkfb3*$^{+/-}$ PMs were added with 100 nM of insulin for indicated times. The cell lysates were harvested and analyzed in Western. Data presented in this figure are representative of two independent experiments.