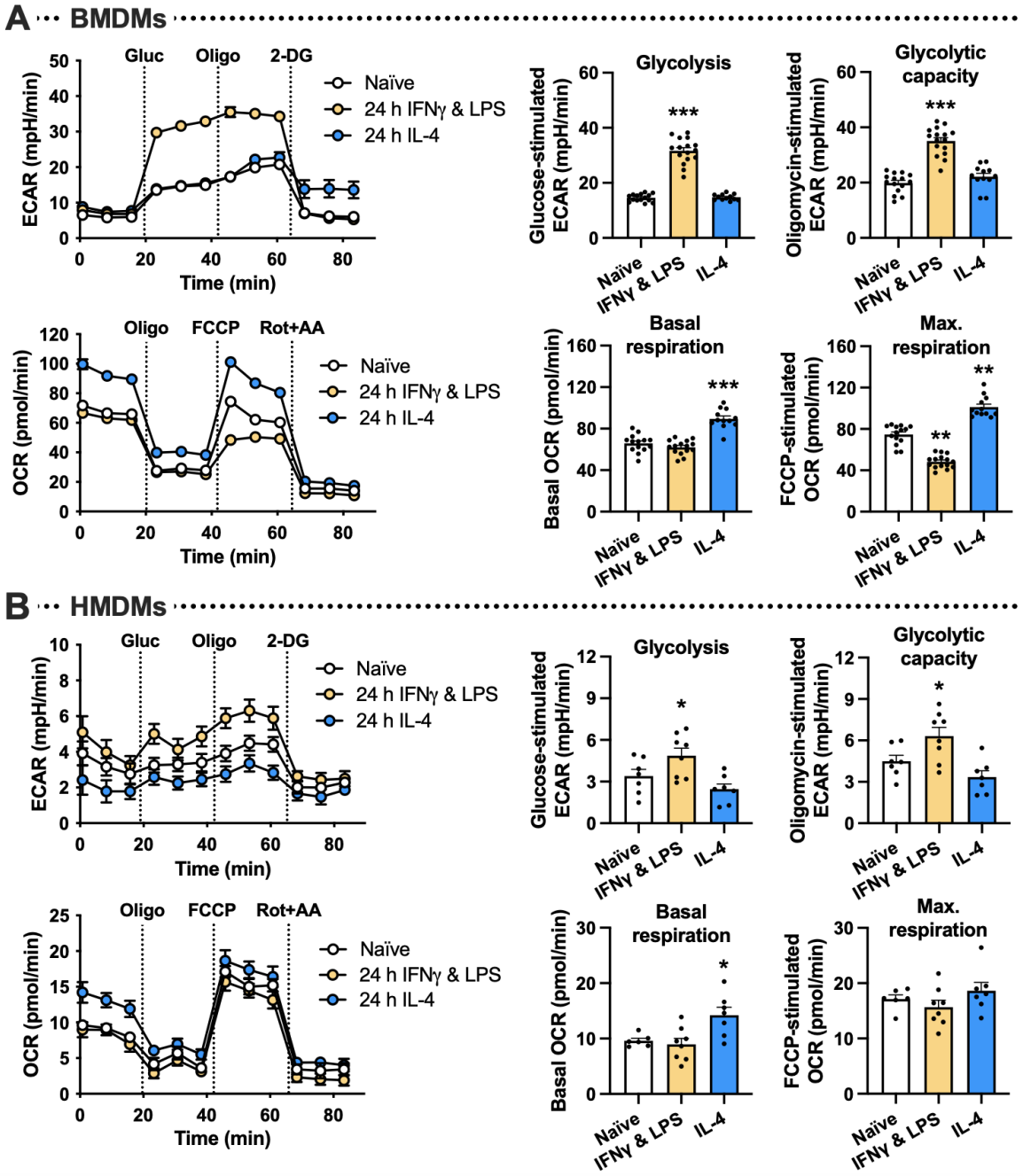
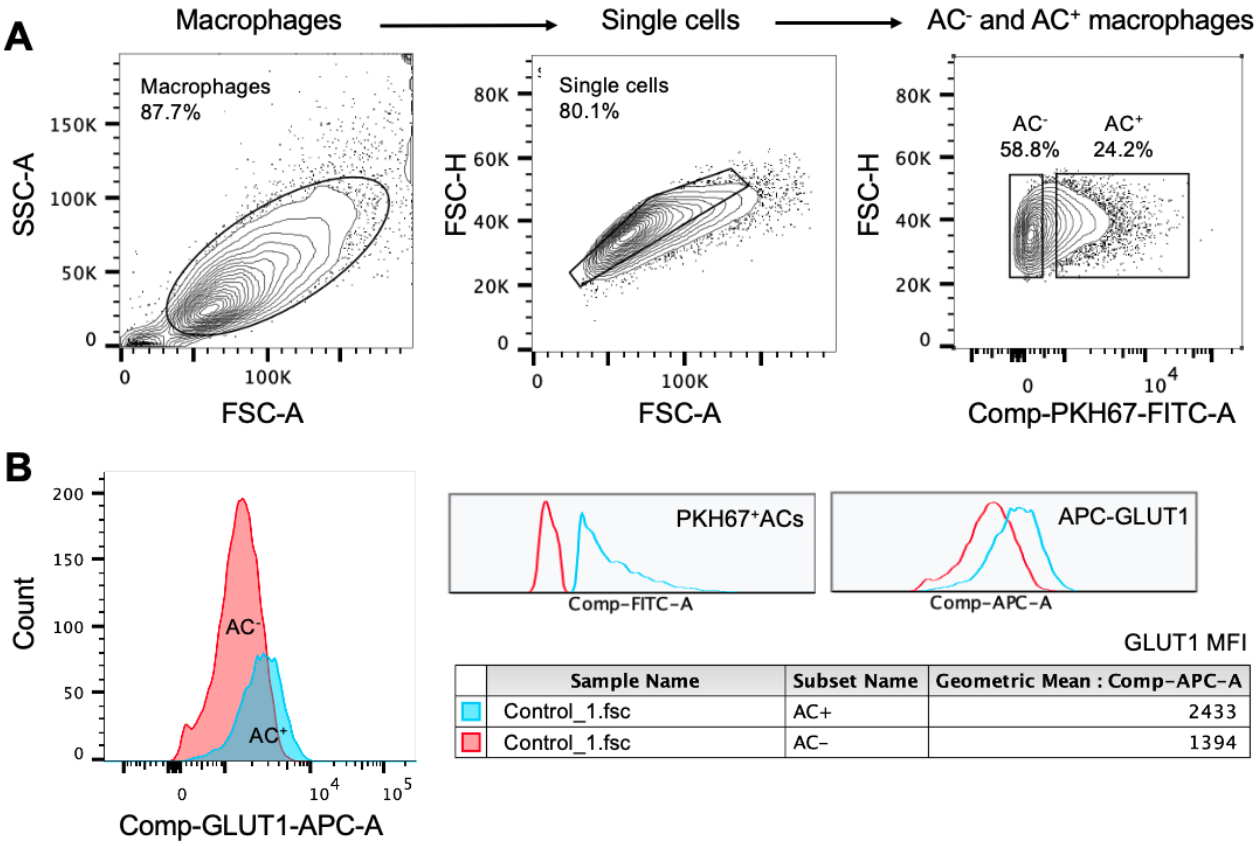
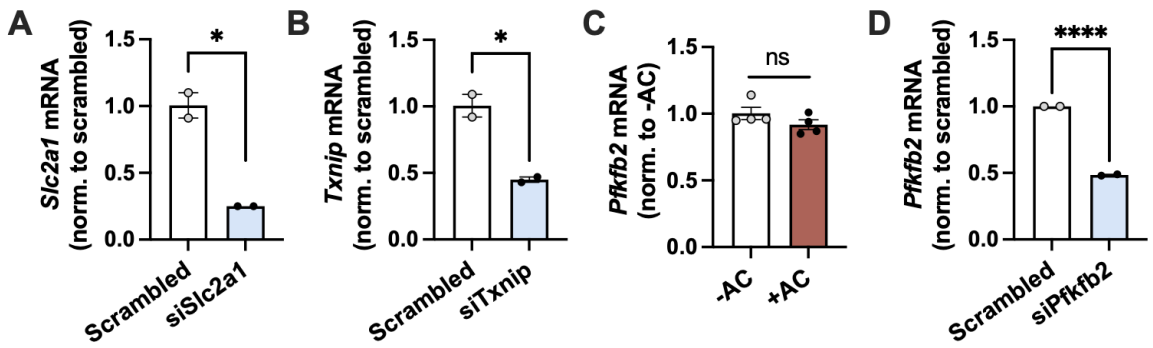


Suppl. Figure 1. Seahorse analysis of macrophages treated with either IFNγ and LPS or IL-4. Naïve BMDMs (**A**) and HMDMs (**B**) were polarized towards a pro-inflammatory phenotype with IFNγ and LPS or a pro-resolving phenotype with IL-4 for 24 h followed by Seahorse analysis. The extracellular acidification rate (ECAR), a measure of glycolysis, was measured at baseline and after the addition of glucose ("glycolysis"), oligomycin ("glycolytic capacity"), and 2-DG. The oxygen consumption rate (OCR), a measure of oxidative phosphorylation, was measured at baseline ("basal respiration") and after the addition of oligomycin, FCCP ("maximal respiration), and rotenone plus antimycin A (*n* = 15-16 and *n* = 7-8 wells/group for BMDMs and HMDMs, respectively). All values are means ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to the naïve groups, determined by one ANOVA with Fisher's LSD post hoc analysis.

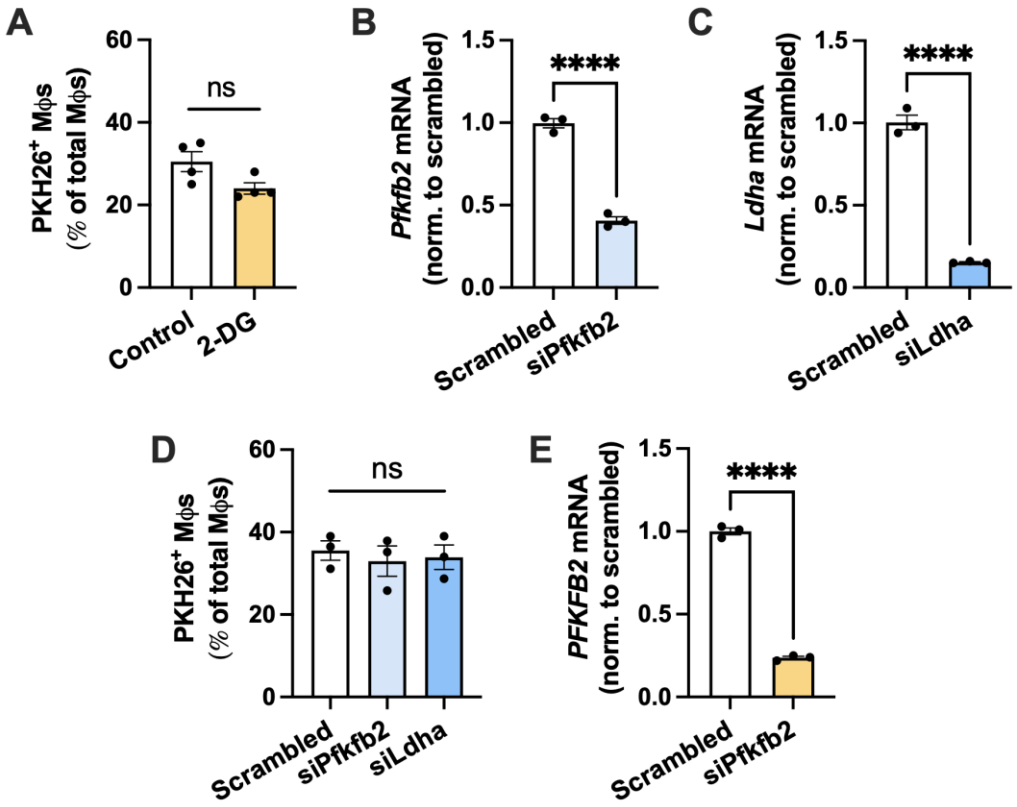


	Sample Name	Subset Name	Geometric Mean : Comp-APC-A
	Control_1.fsc	AC+	2433
	Control_1.fsc	AC-	1394

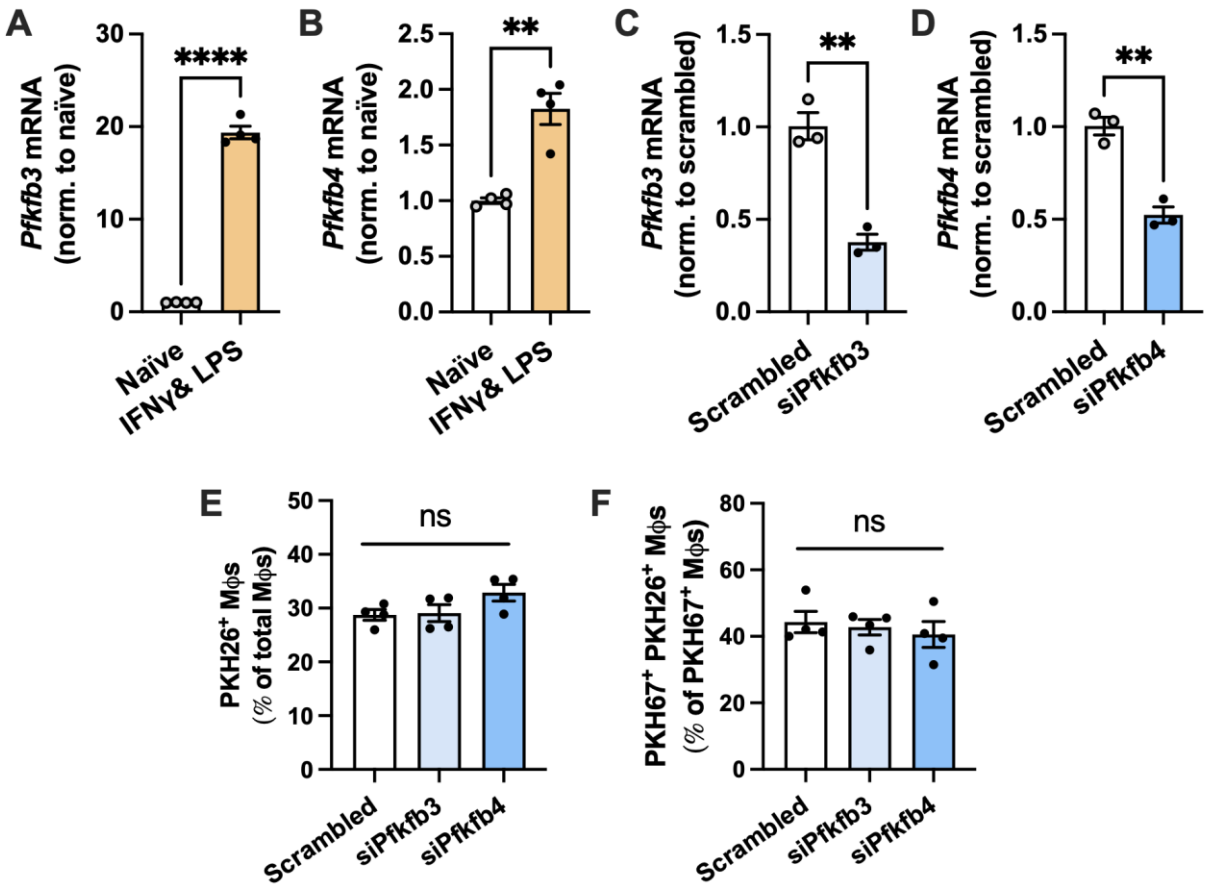
Suppl. Figure 2. Flow cytometry analysis of cell-surface GLUT1 expression in efferocytic macrophages. BMDMs were incubated with PKH67-labeled apoptotic cells (ACs) for 45 min, rinsed and harvested 1 h later for flow cytometric analysis of cell-surface GLUT1 using an APC-conjugated GLUT1 antibody. (**A**) Gating strategy for AC^- and AC^+ macrophages, with PKH67 detected in the FITC channel. (**B**) The mean fluorescent intensity (MFI) of cell-surface GLUT1 based on the flow cytometric data.



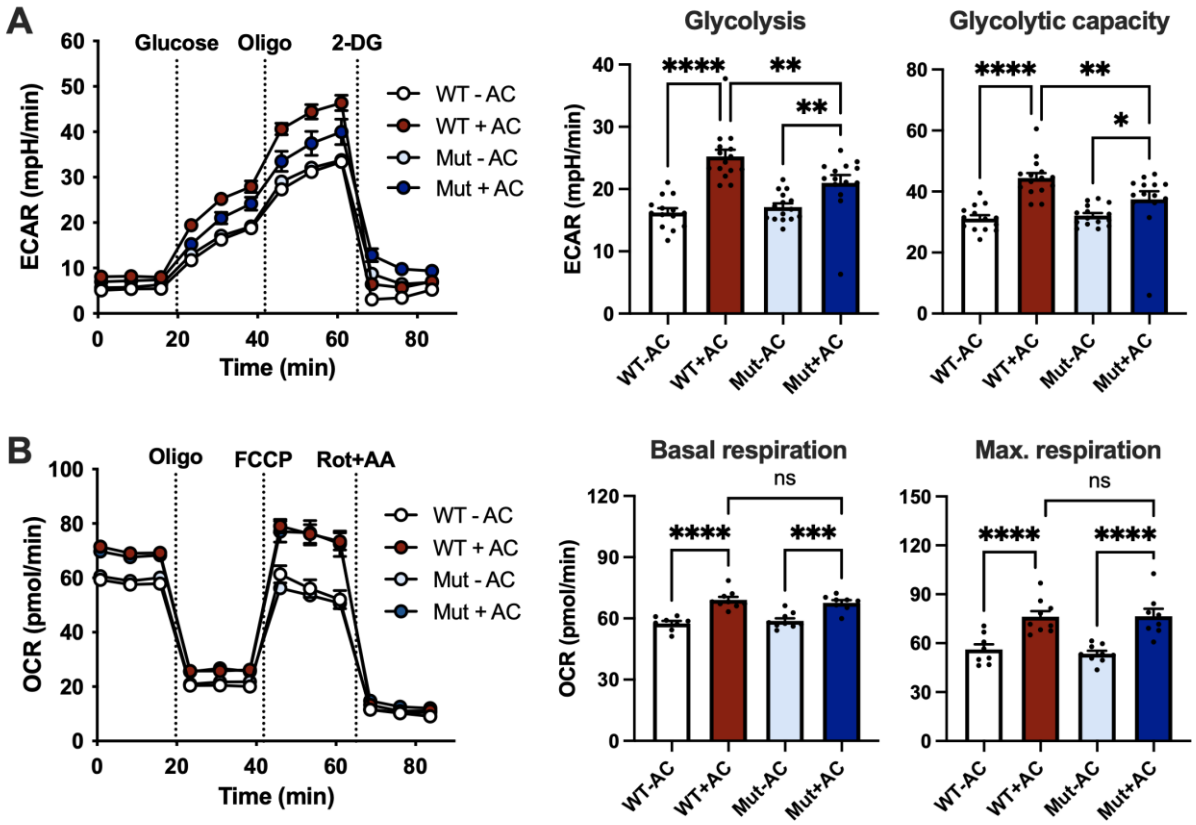
Suppl. Figure 3. RT-qPCR analysis to test siRNA silencing efficiency and efferocytosis-induced gene expression. (**A**) BMDMs were transfected with scrambled RNA or siSlc2a1, and *Slc2a1* expression was measured by RT-qPCR (n = 2 wells/group). (**B**) BMDMs were transfected with scrambled RNA or siTxnip, and *Txnip* expression was measured by RT-qPCR (n = 2 wells/group). (**C**) BMDMs were incubated in the absence or presence of ACs for 45 min, rinsed, and *Pfkfb2* expression was measured 1 h later (n = 4 wells/group). (**D**) BMDMs were transfected with scrambled RNA or siPfkfb2, and *Pfkfb2* expression was measured by RT-qPCR (n = 2 wells/group). All values are means ± SEM. *P < 0.05, ****P < 0.0001 as compared with the indicated control groups, determined by the two-tailed Student's t-test. ns, not significant (P > 0.05).



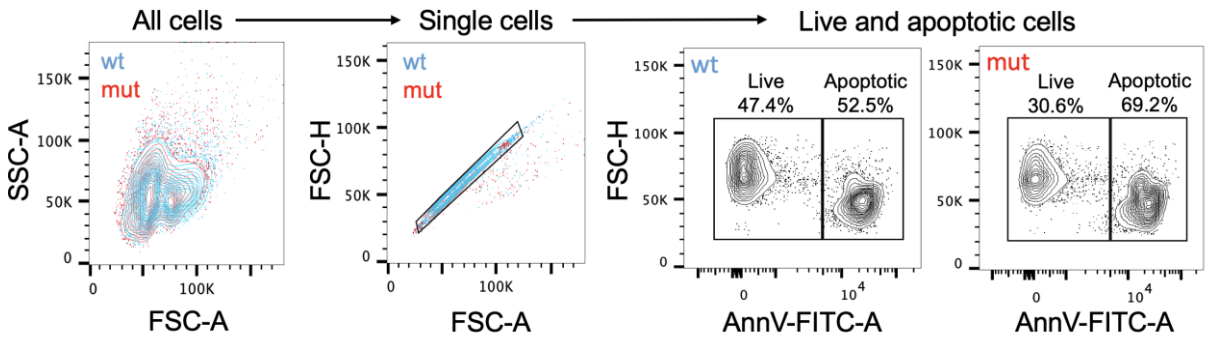
Suppl. Figure 4. Additional efferocytosis assays and validation of siRNA silencing efficiency. (**A**) BMDMs were pretreated without or with 10 mM 2-DG for 1 h, followed by incubation with PKH26-labeled apoptotic Jurkat cells (ACs) for 45 min. Unengulfed ACs were then rinsed away, and the number of PKH26$^+$ macrophages were quantified as a measure of single efferocytosis (n = 4 wells/group). (**B**) BMDMs were transfected with scrambled RNA or siPfkfb2, and *Pfkfb2* gene expression was measured by RT-qPCR (n = 3 wells/group). (**C**) BMDMs were transfected with scrambled RNA or siLdha, and *Ldha* gene expression was measured by RT-qPCR (n = 3 wells/group). (**D**) BMDMs were transfected with scrambled RNA, siPfkfb2 or siLdha, followed by a single efferocytosis assay as described for panel A (n = 3 wells/group). (**E**) HMDMs were transfected with scrambled RNA or siPfkfb2, and *PFKFB2* gene expression was measured by RT-qPCR (n = 3 wells/group). All values are means ± SEM. ****P < 0.0001 as compared with the indicated control groups, determined by the two-tailed Student's t-test (A-C & E) or two-way ANOVA with Fisher's LSD post hoc analysis in panel D. ns, not significant (P > 0.05).



Suppl. Figure 5. Pfkfb3 and Pfkfb4 are upregulated by IFNγ and LPS, and partial silencing of Pfkfb3 and Pfkfb4 does not affect efferocytosis. (**A-B**) Expression levels of *Pfkfb3 and Pfkfb4* were measured by RT-qPCR in naïve macrophages and macrophages polarized towards a pro-inflammatory phenotype with IFNγ and LPS (*n* = 4 wells/group). (**C**) BMDMs were transfected with scrambled RNA or siPfkfb3, and *Pfkfb3* expression was measured (*n* = 3 wells/group). (**D**) BMDMs were transfected with scrambled RNA or siPfkfb4, and *Pfkfb4* expression was measured (*n* = 3 wells/group). (**E**) BMDMs transfected with scrambled RNA, siPfkfb3, or siPfkfb4 were incubated with PKH26-labeled apoptotic Jurkat cells (ACs) for 45 min. Unengulfed ACs were removed by rinsing, and the number of PKH26$^+$ macrophages were quantified (*n* = 4 wells/group). (**F**) BMDMs transfected with scrambled RNA, siPfkfb3, or siPfkfb4 were first incubated with PKH67-labeled ACs for 45 min, rinsed, and 2 h later incubated with PKH26-labeled ACs for 45 min. The number of PKH67$^+$ PKH26$^+$ Mφs relative to PKH67$^+$ Mφs was quantified as a measure of continual efferocytosis (*n* = 4 wells/group). All values are means ± SEM. ***P* < 0.01, *****P* < 0.0001 as compared with the indicated control groups, determined by the two-tailed Student's t-test (A-D) or one ANOVA with Fisher's LSD post hoc analysis (E-F). ns, not significant (*P* > 0.05).



Suppl. Figure 6. Macrophages from PFKFB2 mutant mice show attenuated efferocytosis-induced glycolysis. BMDMs from PFKFB2 mutant mice and wild-type littermates were subjected to Seahorse analysis. (**A**) The extracellular acidification rate (ECAR), a measure of glycolysis, was measured at baseline and after the addition of glucose ("glycolysis"), oligomycin ("glycolytic capacity"), and 2-DG (*n* = 8-9 wells/group). (**B**) The oxygen consumption rate (OCR), a measure of oxidative phosphorylation, was measured at baseline ("basal respiration") and after the addition of oligomycin, FCCP ("maximal respiration), and rotenone plus antimycin A (*n* = 8-9 wells/group). All values are means ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 as compared with the indicated control groups, determined by two ANOVA with Fisher's LSD post hoc analysis. ns, not significant (*P* > 0.05).



Suppl. Figure 7. Flow cytometric analysis of apoptotic thymocytes from the dexamethasone-thymus experiment. All cells were isolated from the thymi of hematopoietic PFKFB2 mutant and wild-type mice of the dexamethasone-thymus experiment (see Fig. 5 of the main manuscript) and subjected to flow cytometric analysis. The cells were immunostained for the apoptotic cell marker annexin V (AnnV; FITC) and, using the depicted gating strategy, quantified for the percentages of $AnnV^-$ (live) and $AnnV^+$ (apoptotic) thymocytes.

Supplementary Table

Supplementary Table 1. Primer sequences used for quantitative RT-PCR analysis

Gene	Species	Primer sequence (5' → 3')	
		Forward	**Reverse**
Ldha	Mouse	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGGA
Pfkfb2	Mouse	ACATGCTCATGGGCTTCCTAT	GTTGAGGTAGCGTGTTAGTTTCT
Rplp0	Mouse	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
Slc2a1	Mouse	GCAGTTCGGCTATAACACTGG	GCGGTGGTTCCATGTTTGATTG
Slc16a1	Mouse	TGTTAGTCGGAGCCTTCATTTC	CACTGGTCGTTGCACTGAATA
Txnip	Mouse	TCTTTTGAGGTGGTCTTCAACG	GCTTTGACTCGGGTAACTTCACA