SUPPLEMENTAL MATERIAL

IKKβ promotes metabolic adaptation to glutamine deprivation via phosphorylation and inhibition of PFKFB3

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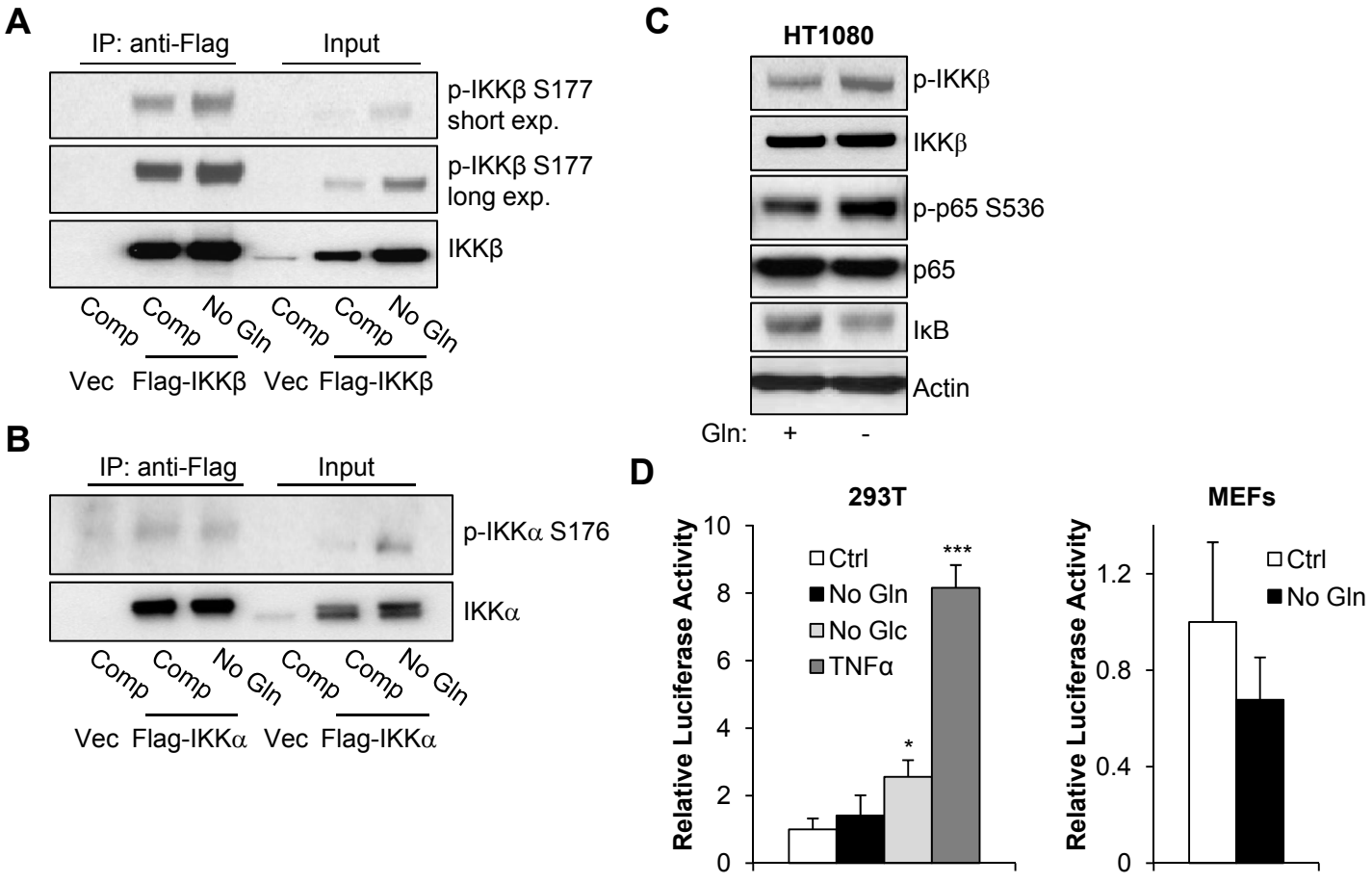
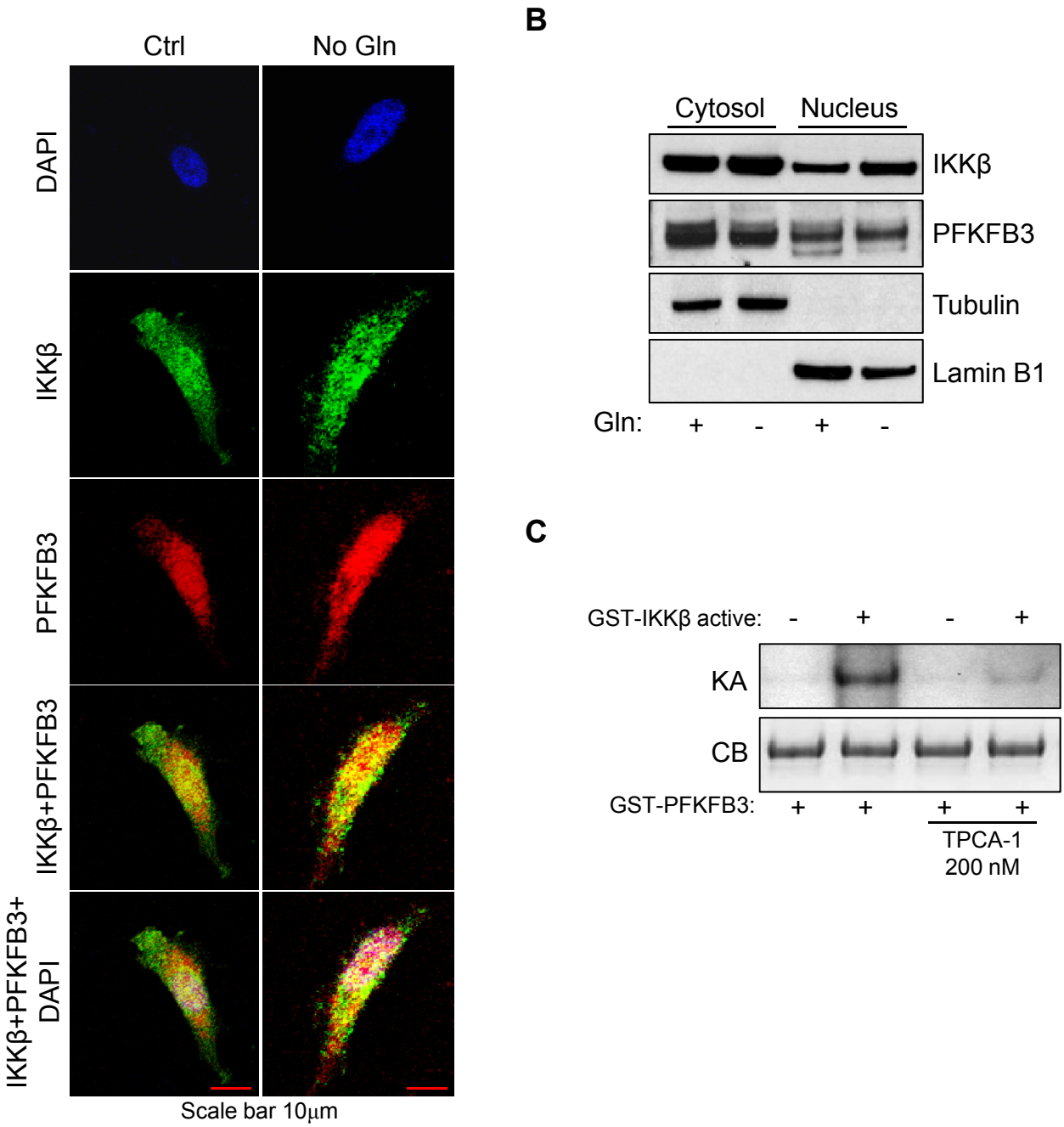
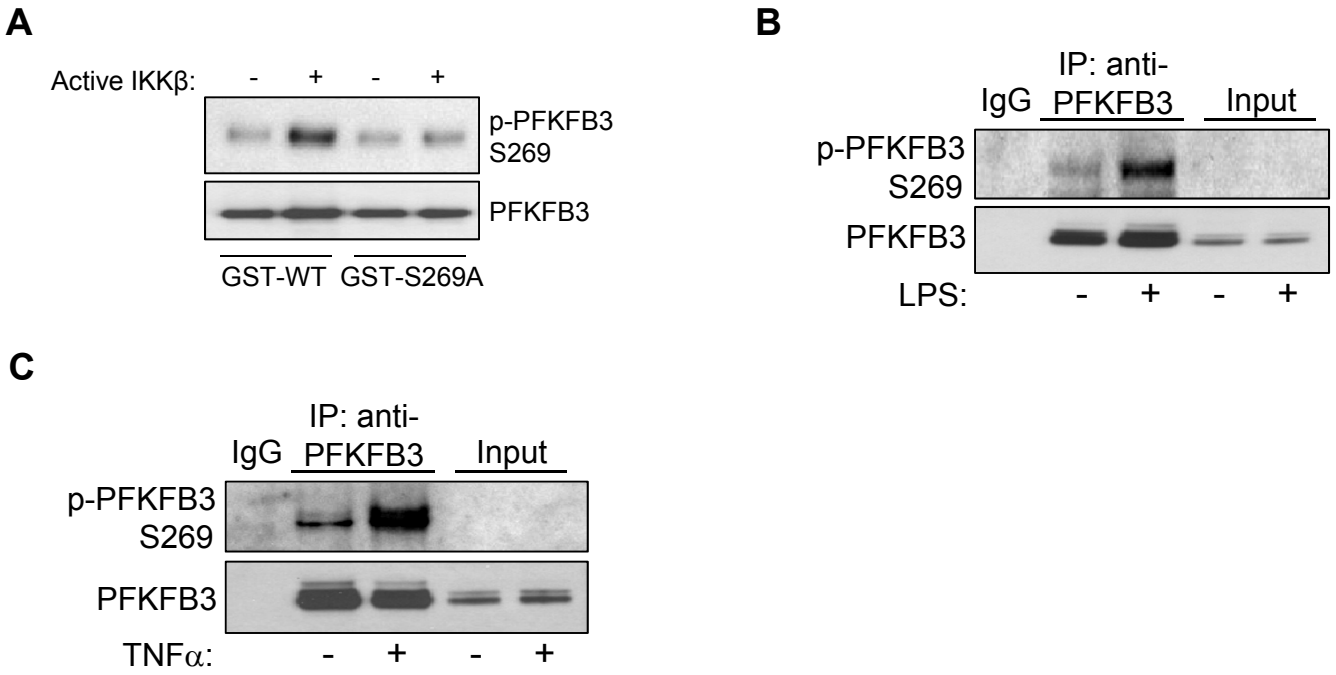


Supplemental Figure S1, related to main Figure 1. IKKβ is activated in response to glutamine starvation. *(A)* HeLa cells expressing Flag-IKKβ were cultured in complete medium (24h) or glutamine-free medium (24h) followed by IP with anti-Flag conjugated agarose; immunoblotting was performed with indicated antibodies (10% input). *(B)* 293T cells ectopically expressing Flag-IKKα were cultured in complete medium (24h) or glutamine-free medium (24h) followed by IP with anti-Flag conjugated agarose; immunoblotting was performed with indicated antibodies (10% input). *(C)* HT1080 cells were cultured in complete or glutamine-free medium for 48h, then cells were lysed and immunoblotting was performed with indicated antibodies. *(D)* 293T and Wildtype MEFs were transfected with pbIIx and Renilla reporter constructs. 48h post-trasfection, cells were cultured in the indicated conditions followed by luciferase substrate and detection. $^{*}p < 0.05$, $^{***}p < 0.005$, Student's t test.



Supplemental Figure S2, related to main Figure 2. IKKβ co-localizes with and phosphorylates PFKFB3.

(A) HT1080 cells were cultured in complete medium or glutamine-free medium (24h) on chamber slides before staining with indicated antibodies and imaged using a Zeiss LSM 700 confocal microscope. Data presented are representative images. *(B)* HT1080 cells were cultured in complete or glutamine-free medium for 24h, then cells were lysed in cytoplasmic and nuclear fractionation buffers followed by immunoblotting with indicated antibodies. *(C)* ^{32}P-ATP *in vitro* kinase assay (KA) was performed using recombinant active GST-IKKβ (0.2μg) with recombinant GST-PFKFB3 (2μg) in the presence or absence of IKKβ inhibitor TPCA-2 (200 nM) followed by SDS-PAGE separation and autoradiograph exposure. Commassie blue (CB) staining confirmed loading in absence of KA band.



Supplemental Figure S3, related to main Figure 3. IKKβ phosphorylates PFKFB3 at Ser269. *(A)* Cold kinase assay using recombinant active GST-IKKβ, recombinant GST-PFKFB3 (GST-WT), or recombinant S269A GST-PFKFB3 (GST-S269A) was performed followed by immunoblotting with indicated antibodies. *(B)* HT1080 cells were treated with 50 ng/mL LPS for 16h, followed by immunoprecipitation with anti-PFKFB3 antibody; immunoblotting was performed with indicated antibodies (5% input). *(C)* MCF-7 cells were treated with 50 ng/mL TNFα for 15 minutes, followed by immunoprecipitation with anti-PFKFB3 antibody; immunoblotting was performed with indicated antibodies (5% input).

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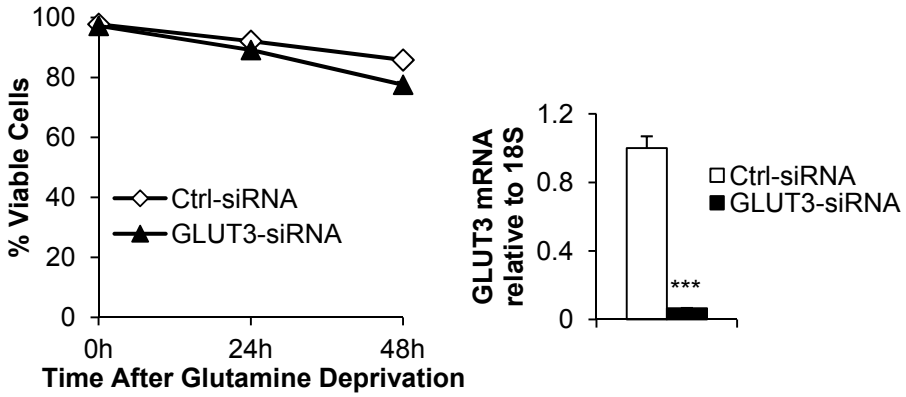
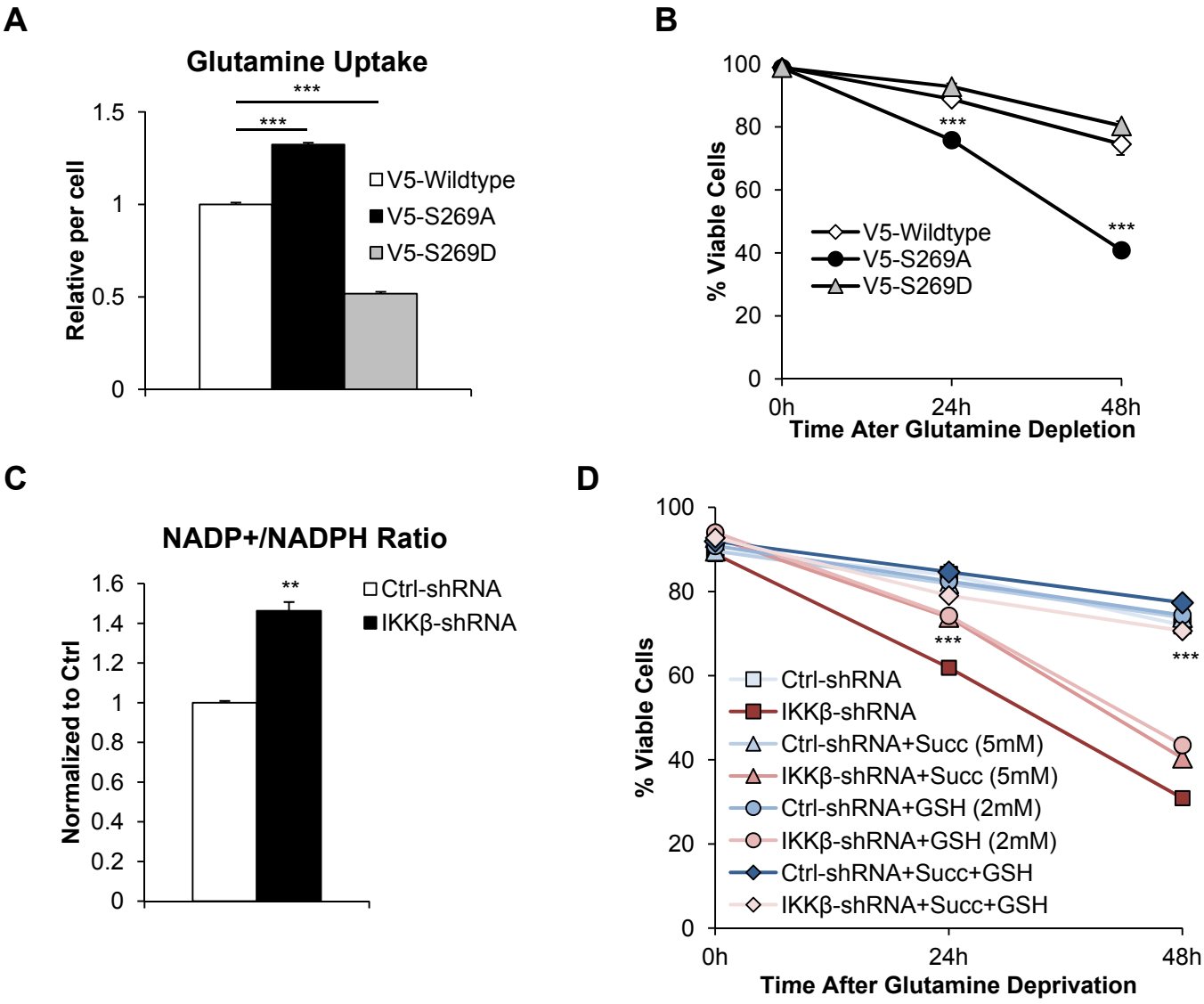


Supplemental Figure S4, related to main Figure 5. GLUT3 is not involved in IKKβ-driven low glutamine cell survival. *(A)* HT1080 cells were transiently transfected with 20nM Control (Ctrl) siRNA or GLUT3 siRNA. 48h post-transfection, cells were cultured in glutamine-free medium and viability was assessed by PI exclusion (left panel). Data presented are mean +/- SEM of three independent experiments performed in duplicate. 48h post-transfection, cells were lysed and qPCR was performed (right panel). $^{***}p < 0.005$, Student's t test.



Supplemental Figure S5, related to main Figure 6. Enhanced aerobic glycolysis in IKKβ-deficient cells leads to increased glutamine dependence.
(A,B) HT1080 cells depleted of endogenous PFKFB3 and ectopically expressing Wildtype, S269A, or S269D PFKFB3 were cultured in complete medium for 48h followed by glutamine uptake measurement using the Nova Bioprofile 100 analyzer. Metabolite levels per cell were normalized to Wildtype (A). Or cells were cultured in complete or glutamine-free medium and viability was assessed by propidium iodide exclusion at the indicated time points (B) Data presented are mean +/- SEM of three independent experiments performed in duplicate. *(C)* HT1080 cells transduced with Control (Ctrl) or IKKβ shRNA were cultured in complete medium 24h followed by NADPH measurement. *(D)* HT1080 cells transduced with Control (Ctrl) or IKKβ shRNA were cultured in the indicated conditions for 24h and 48h, and viability was assessed by propidium iodide exclusion at the indicated time points. Data presented are mean +/- SEM of three independent experiments performed in duplicate. $^{**}p < 0.01$, $^{***}p < 0.005$, Student's t test.

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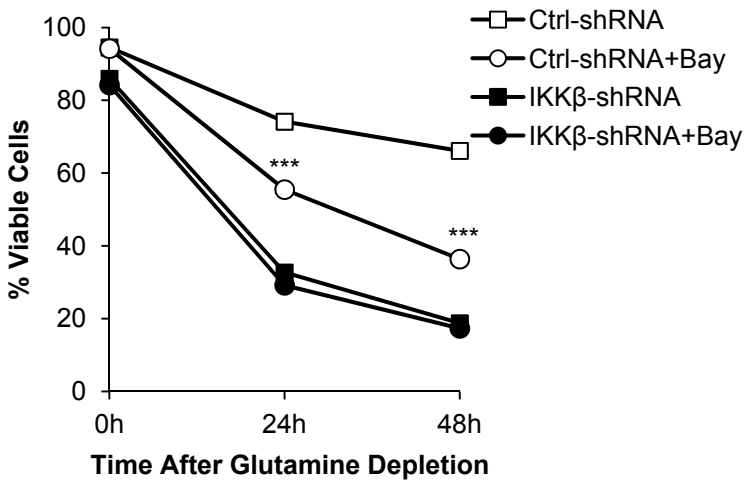

Supplemental Figure S6, related to main Figure 7. Bay 11-7082 sensitizes Control but not IKKβ-deficient cells to glutamine depletion.

(A) HT1080 cells transduced with either Control (Ctrl) or IKKβ shRNA were cultured in glutamine-free medium with or without Bay 11-7082 (2.5μM) and viability was assessed by propidium iodide exclusion at the indicated time points. Data presented are mean +/- SEM of three independent experiments. $^{***}p < 0.005$, Student's t test.