

Supplementary Information for
PFKP deubiquitination and stabilization by USP5 activates aerobic
glycolysis to promote triple-negative breast cancer progression

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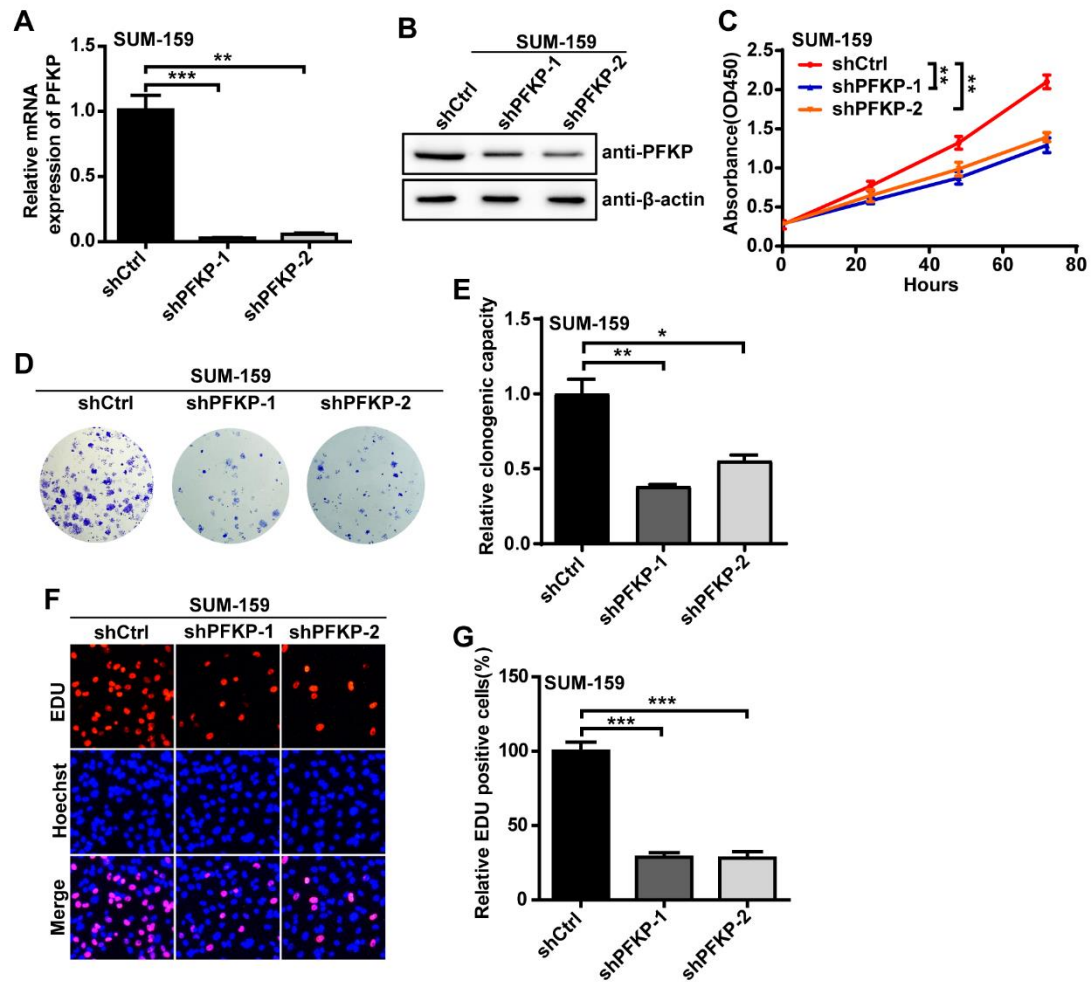
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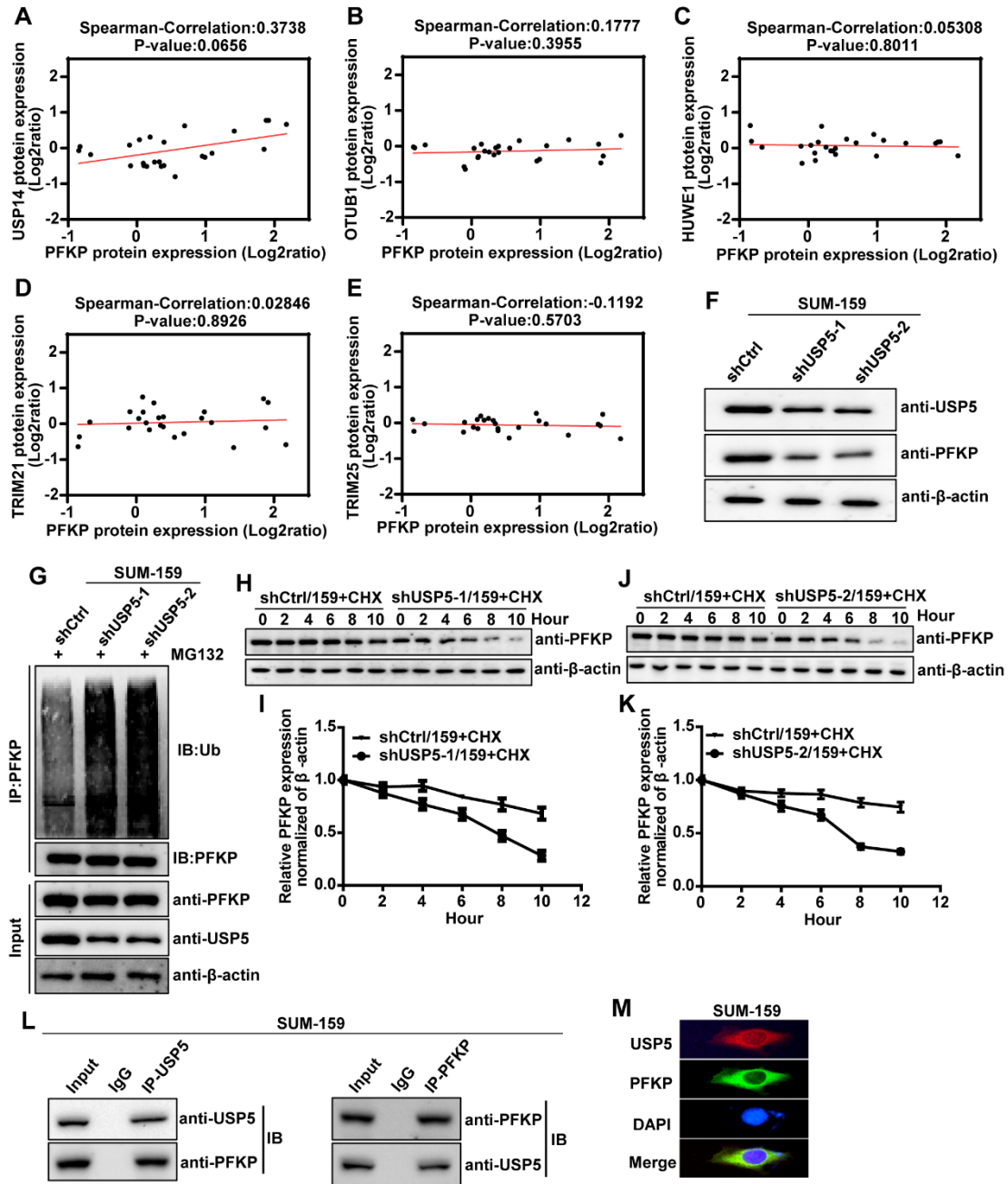
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This file includes: Supplementary Fig. S1-S4 and Supplementary Tables S1.

Sup Figure 1

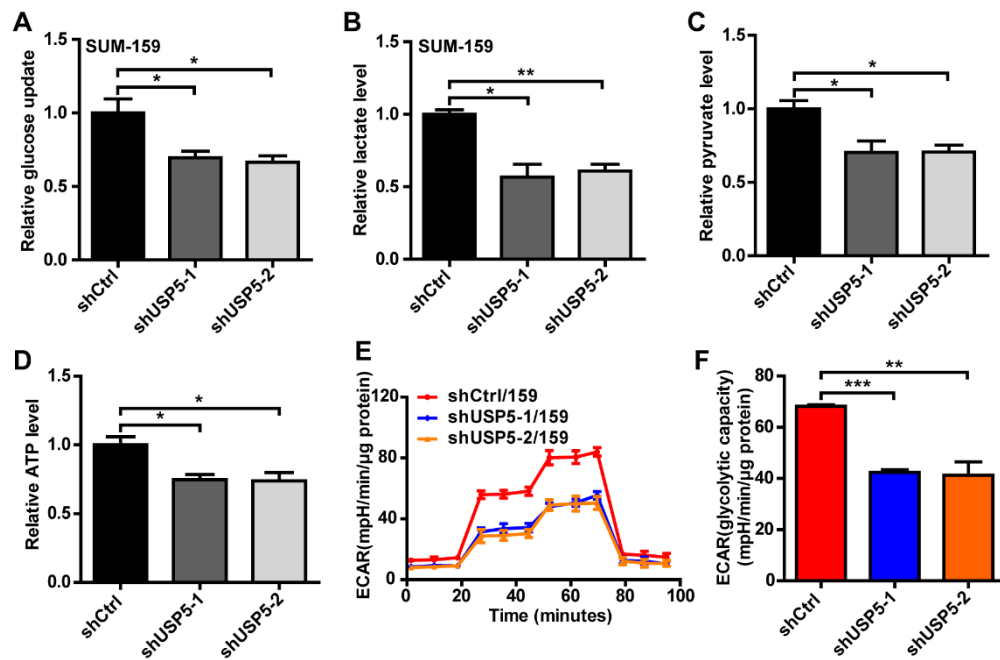


Supplementary Figure 1. (A, B) qRT-PCR (A) and western blot (B) analysis of PFKP expression with PFKP knockdown in SUM-159 cells. *** $P < 0.001$, ** $P < 0.01$. (C) CCK-8 assay determination of SUM-159 cell proliferation in response to PFKP knockdown. ** $P < 0.01$. (D, E) Clone formation assay determination of SUM-159 cell proliferation in response to PFKP knockdown. ** $P < 0.01$, * $P < 0.05$. (F, G) EDU incorporation assay determination of SUM-159 cell proliferation in response to PFKP knockdown. *** $P < 0.001$.

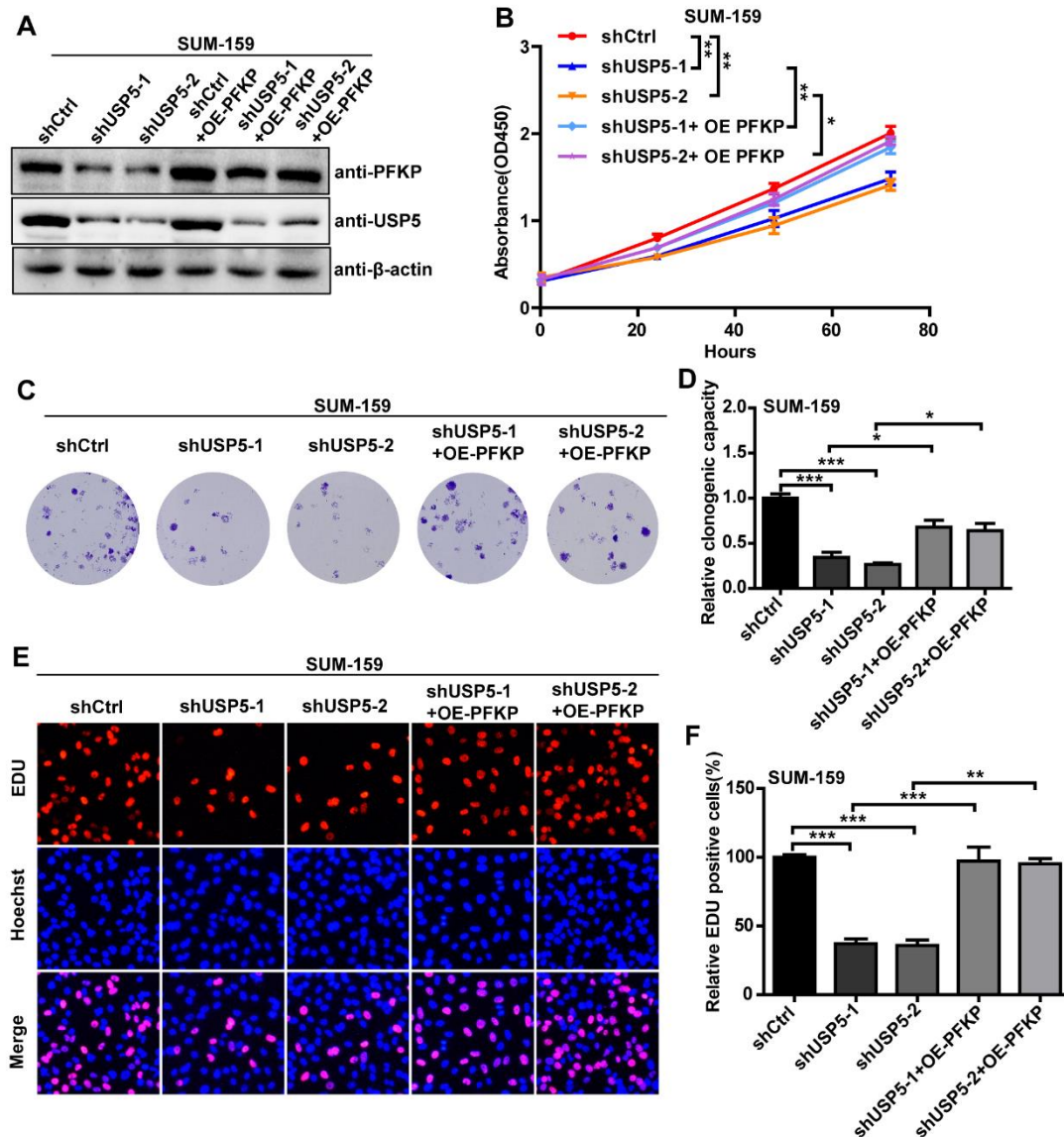


Supplementary Figure 2. (A-E) The correlation between USP14 (A), OTUB1 (B), HUWE1 (C), TRIM21 (D), TRIM25 (E) protein expression and PFKP protein expression in TNBC was analyzed by LinkedOmics platform. (F) The USP5 and PFKP protein expression in control and USP5 knockdown SUM-159 cells were analyzed by Western blot. (G) Co-IP analysis of PFKP protein ubiquitination in control and USP5 knockdown MDA-MB-231SUM-159 cells. (H-K) Control and USP5 knockdown SUM-159 cells were treated with CHX for the indicated time points and analyzed by Western blot (H, J), quantification of PFKP protein levels normalized to β-actin in time after addition of CHX (I, K). (L) The binding of USP5 to PFKP was detected by Co-IP assay in SUM-159 cells. (M) Immunofluorescence co-localization of USP5 and PFKP in SUM-159 cells.

Sup Figure 3



Supplementary Figure 3. (A-D) Glucose uptake (A), lactate level (B), pyruvate level (C), and ATP level (D) were significantly decreased after USP5 knockdown in SUM-159 cells. (E, F) ECAR values (E) and calculated glycolytic capacity (F) of control and USP5 knockdown SUM-159 cells.



Supplementary Figure 4. (A) The USP5 and PFKP protein expression in control and USP5 knockdown SUM-159 cells in the presence or absence of overexpressed PFKP were analyzed by Western blot. (B-F) SUM-159 cell proliferation following USP5 knockdown in the presence or absence of overexpressed PFKP was evaluated using CCK8 assay (B), Clone formation assay (C, D), and EDU incorporation assay (E, F). *** $P < 0.001$, ** $P < 0.01$.

Table S1. Primer used in this study.

Construction of PFKP expression vector	
PFKP forward	5'- ATGGACGCGGACGACTCCC -3'
PFKP reverse	5'- TCAGACACTCCAGGGCTGCACAT -3'
Construction of shRNA vector	
shPFKP-1	5'- GGGATGCTCAAGGTATGAA -3'
shPFKP-2	5'- GGCTGAAGGAGCAATTGAT -3'
shUSP5-1	5'- GCCTCTACATCTGTATGAA -3
shUSP5-2	5'- GCATCGACATGCTGAAGAT -3'
Quantitative RT-PCR	
PFKP forward	5'- GCATGGGTATCTACGTGGGG -3'
PFKP reverse	5'- CTCTGCGATGTTTGAGCCTC -3'
USP5 forward	5'- GCTGCTGTCAGTATTACCGAC -3'
USP5 reverse	5'- AAAGCCCAGAAACGTGTTCATA -3'
β -actin forward	5'- CATGTACGTTGCTATCCAGGC -3'
β -actin reverse	5'- CTCCTTAATGTCACGCACGAT -3'