Molecules and Cells





Supplementary Fig. S1. Effect of PKM1 or PKM2 knockdown on cell growth. (A, B) The effect of siRNA against PKM1 (A) or PKM2 (B) was analyzed by Western blotting. Cells were transfected with each siRNA against PKM1 or PKM2 and incubated for 48 h. (C) The effect of knocking down PKM1 or PKM2 on MDA-MB-231 and HCC1937 TNBC cell growth was accessed by MTT assay. Cells were transfected and incubated for 72 h and cell growth was measured at an absorbance of 570 nm. (D) The effect of knocking down PKM1 or PKM2 on HCC1937 TNBC cell growth was accessed by soft agar assay. Colonies were counted using a microscope and the Image-Pro PLUS (v.6) computer software program. For C and D, data are shown as means \pm S.D. of triplicate values from 3 independent experiments and the asterisk (*) indicates a significant difference (p < 0.05). (E) The effect of knocking down PKM1 or PKM2 on the expression of phosphorylated p65 and total p65. Cells were transfected for 72 h and cells were analyzed by Western blotting. Band density was measured using the Image J (NIH) software program and similar results were obtained from 3 independent experiments.

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Supplementary Fig. S2. The transcriptional level of PKM1 and PKM2 in shPKM cells and shControl cells was analyzed by RT-PCR. β -Actin was used to verify equal protein loading. Band density was measured using the Image J (NIH) software program.



Supplementary Fig. S3. Effect of 2-DG on TNBC cell growth. Effect of 2-DG on MCF10A normal breast cell (A) or HCC1937 TNBC cell (B) growth was accessed by MTT assay. Cells were seeded and treated with 2-DG for 48 h. Cell growth was measured at an absorbance of 570 nm. (C) Effect of PKM knockdown on anchorage-independent cell growth was determined by soft agar assay. Cells were seeded and incubated for 3 weeks. Colonies were counted using a microscope and the Image-Pro PLUS (v.6) computer software program. All data are shown as means \pm S.D. of triplicate values from 3 independent experiments and the asterisk (*) indicates a significant difference (p < 0.05).

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Supplementary Fig. S4. Effect of PKM overexpression on MCF10A normal breast cell growth. (A) The effect of stable expression of PKM1, PKM2, or both PKM1 and PKM2 was analyzed by Western blotting. Cells were transfected for 48 h and treated with G418 (200 μ g/ml) for 1 week. (B-D) The effect of stable expression of PKM1, PKM2, or both PKM1 and PKM2 on MCF10A cell growth was accessed by MTT assay (B), soft agar assay, (C) or foci formation assay (D). (E, F) The effect of PKM overexpression on the activity of NF- κ B was analyzed by luciferase assay (E) and Western blotting (F). For B, C and E, all data are shown as means ± S.D. of triplicate values from 3 independent experiments. For D and F, similar results were obtained from 3 independent experiments.