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Supplemental information

The insulin and IGF signaling pathway sustains breast cancer stem cells by IRS2/PI3K-mediated regulation of MYC

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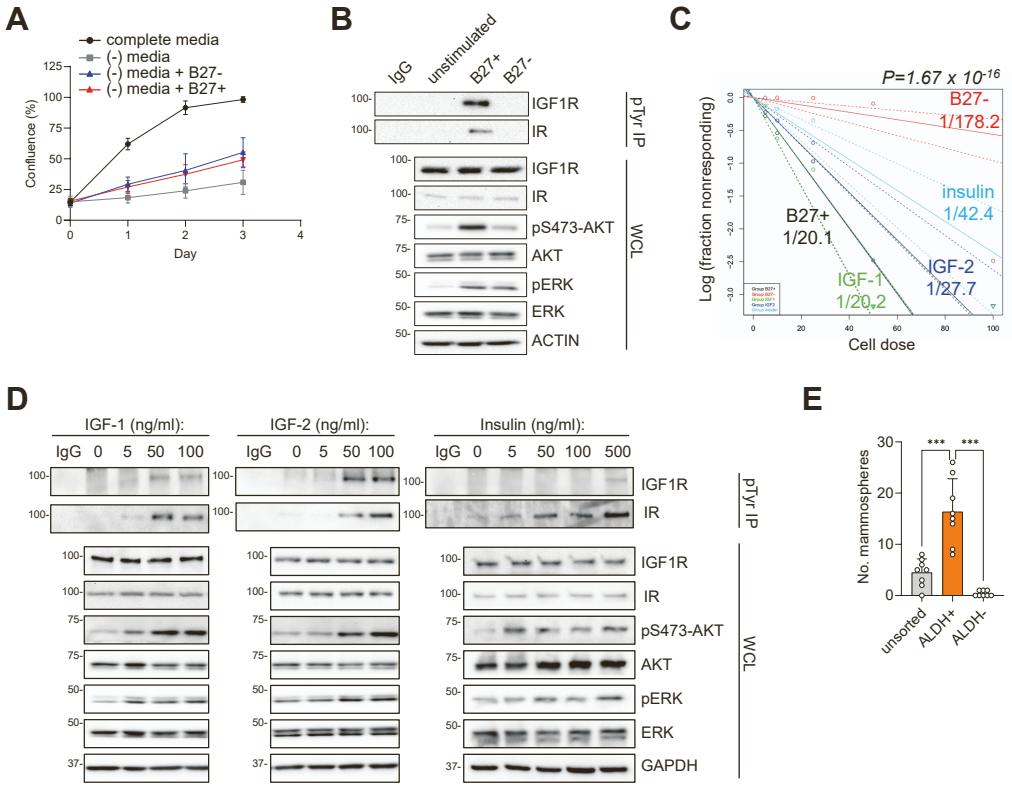

Figure S1. Analysis of signaling in response to IGF-1, IGF-2, and insulin, Related to Figure 1

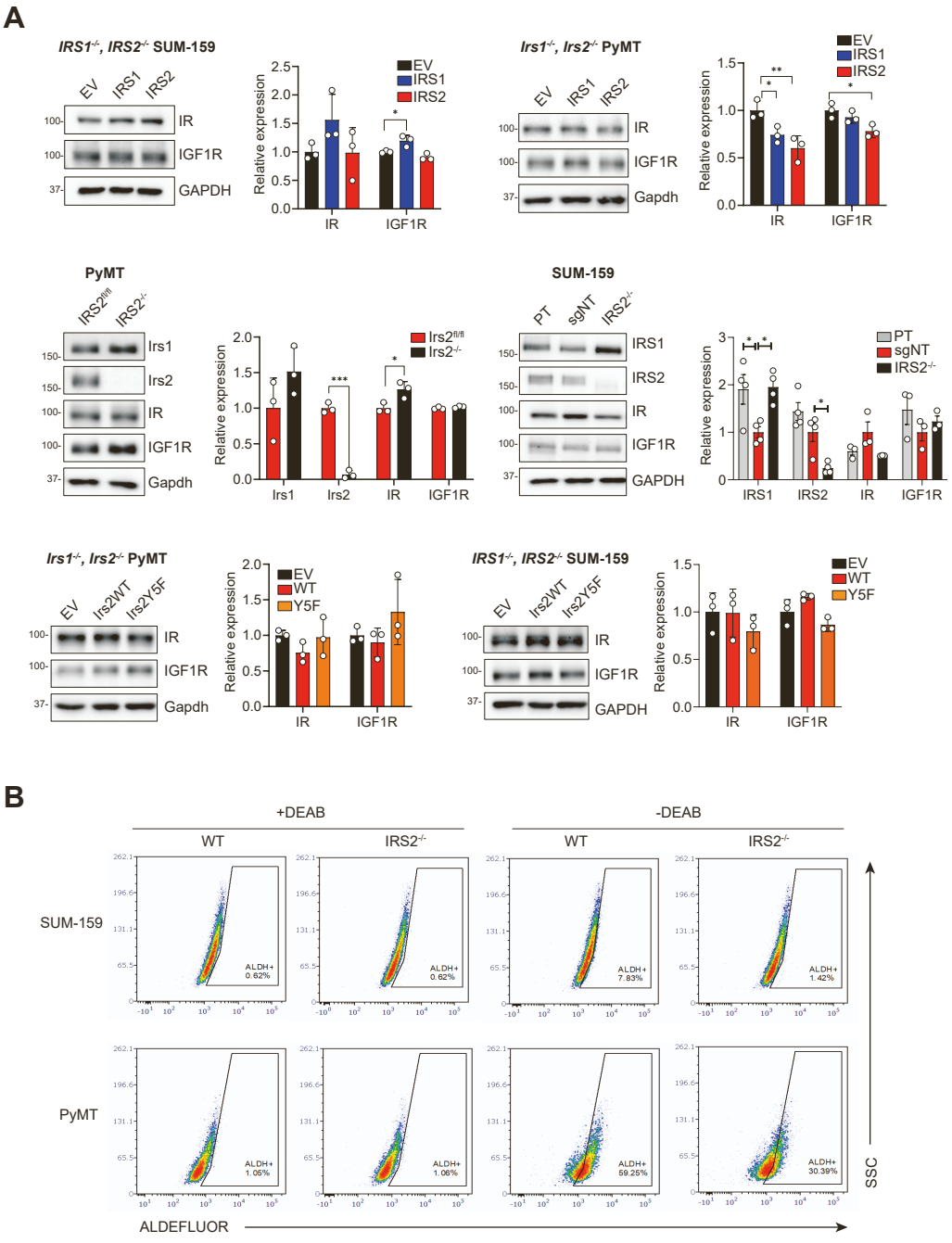

Figure S2. IRS2 regulates breast cancer stemness. Related to Figure 2

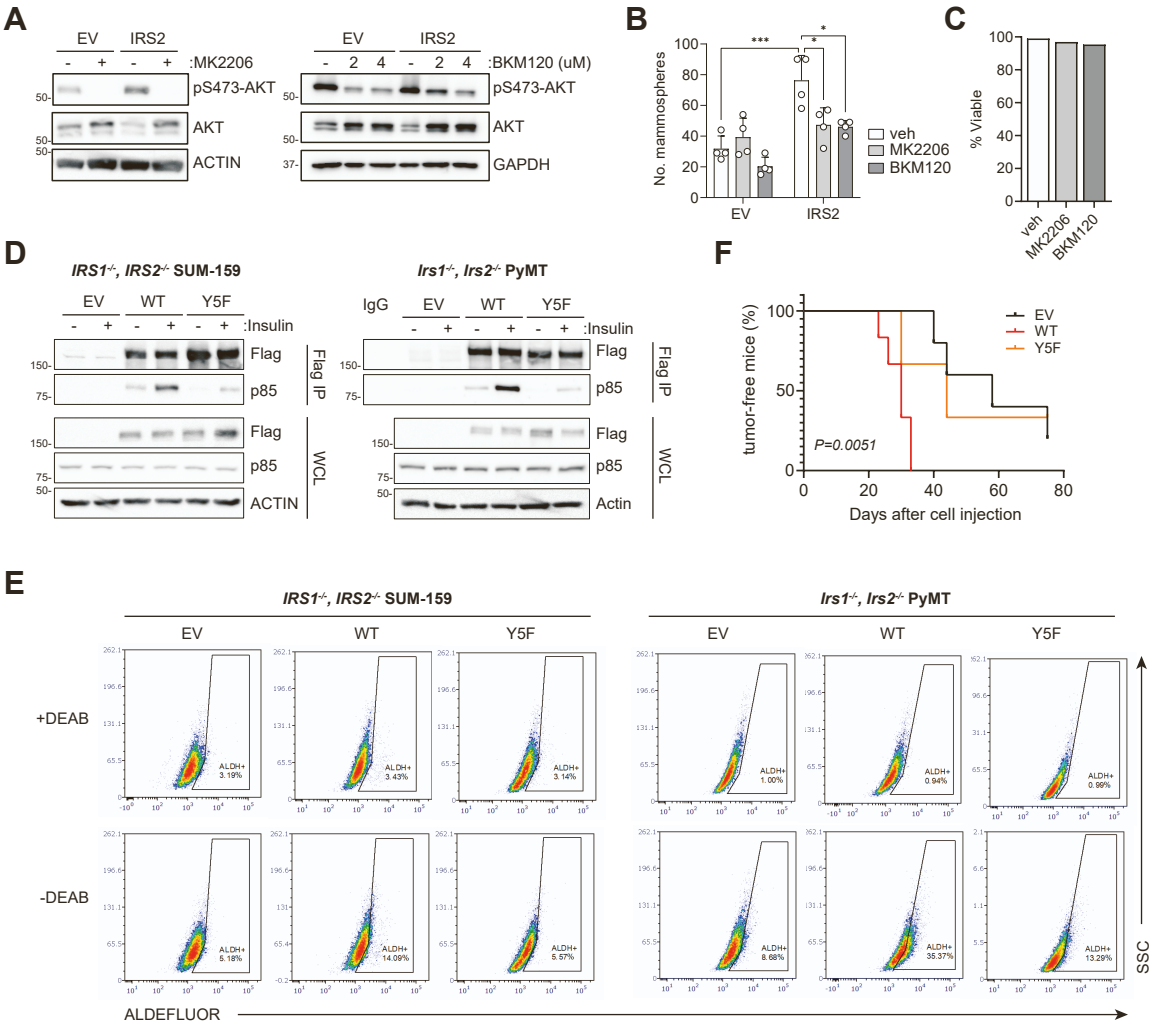

Figure S3. IRS2 activation of PI3K is required for self-renewal of breast CSCs. Related to Figure 2

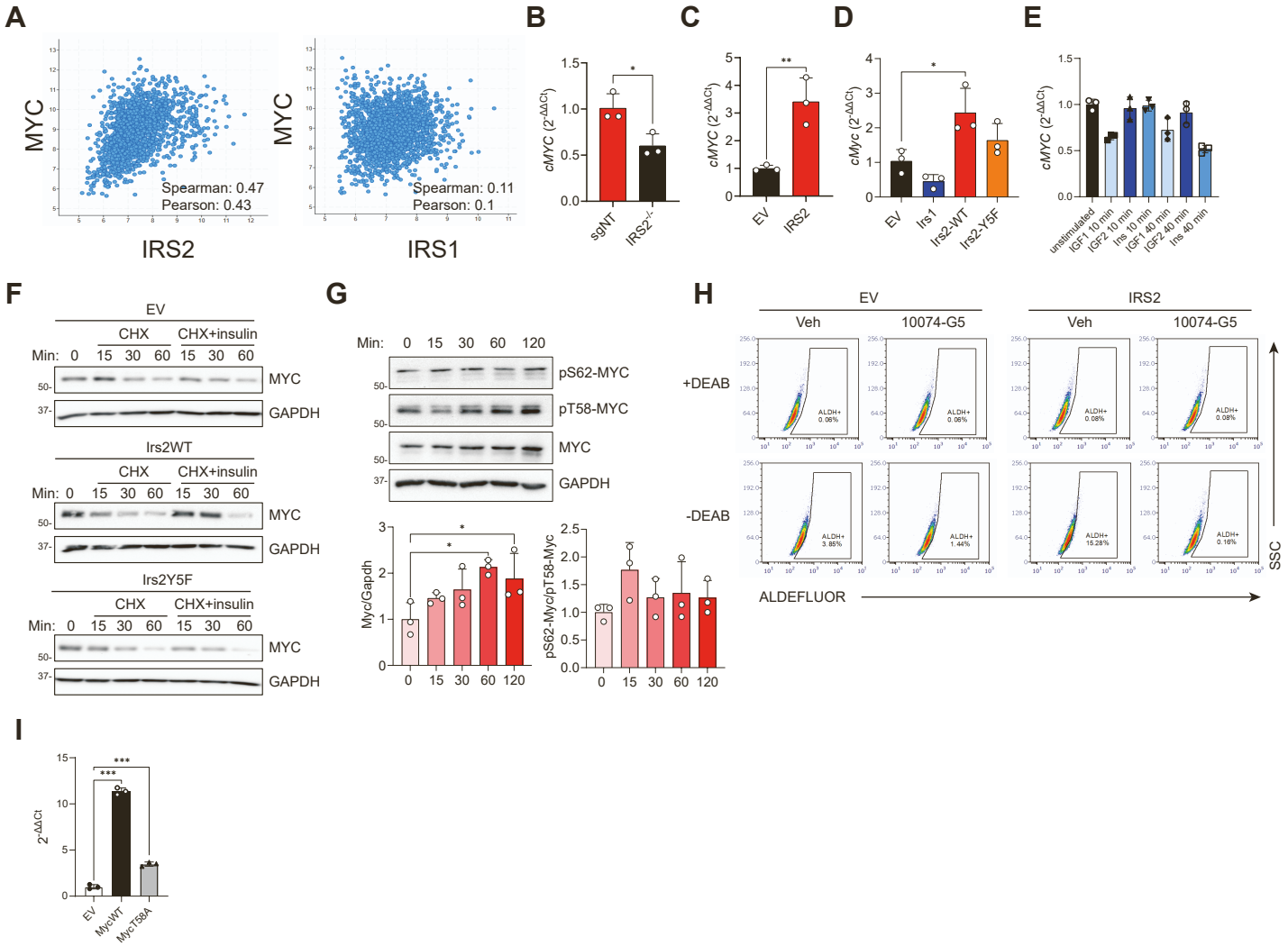

Figure S4. IRS2 regulates self-renewal through MYC. Related to Figures 3 and 4

Figure S1. Analysis of signaling in response to IGF-1, IGF-2, and insulin. Related to Figure 1 (A) SUM-159 cells were plated in 96-well plates with complete growth medium. Medium was replaced with the indicated media (Day 0) and growth rate was monitored by measuring cell confluency using a Celigo imaging cytometer. The data shown represent the mean ± S.D. of a representative experiment performed three times independently. (B) SUM-159 cells were stimulated with B27 supplement containing insulin (B27+) or without insulin (B27-) for 15 minutes, followed by immunoprecipitation and immunoblotting. (C) *In vitro* limiting dilution assays with *Irs2*$^{-/-}$ PyMT cells expressing mIrs2-WT were performed in B27+, B27- or B27- medium supplemented with individual ligands (50 ng/ml). Data are presented as a log-log plot, and the frequency of stem cells is calculated by extreme limiting dilution analysis. (D) SUM-159 cells were stimulated with IGF-1, IGF-2 or insulin in a dose-dependent manner for 10 minutes, followed by immunoprecipitation and immunoblotting. (E) ALDH^{+} or ALDH^{-} SUM-159 cells were sorted by flow cytometry and plated into low attachment plates to analyze mammosphere formation. The data shown are a representative assay. WCL, whole cell lysate. ***P<0.001.

Figure S2. IRS2 regulates breast cancer stemness. Related to Figure 2 (A) Expression of IR and IGF1R in cell lines used in this study. (B) Representative density plots of ALDEFLUOR assays of SUM-159 and PyMT cells (related to Figure 2F and 2G).

Figure S3. IRS2 activation of PI3K is required for self-renewal of breast CSCs. Related to Figure 2 (A) *IRS1*$^{-/-}$, *IRS2*$^{-/-}$ SUM-159 cells expressing EV or IRS2 were treated with MK2206 (1 uM) or BKM120 for 24 hours followed by immunoblotting. (B) *IRS1*$^{-/-}$, *IRS2*$^{-/-}$ SUM-159 cells expressing EV or IRS2 were treated with MK2206 (1 uM) or BKM120 (4 uM) for 24 hours. Cells were trypsinized and viable cells (C) were assayed for mammospheres. (D) *IRS1*$^{-/-}$, *IRS2*$^{-/-}$ SUM-159 cells and *Irs1*$^{-/-}$, *Irs2*$^{-/-}$ PyMT cells expressing EV, Irs2-WT (WT), or Irs2-Y5F (Y5F) were stimulated with insulin (100 ng/ml, 10 minutes), followed by immunoprecipitation and immunoblotting. (E) Representative density plots of ALDEFLUOR assays of *IRS1*$^{-/-}$, *IRS2*$^{-/-}$ SUM-

159 cells and *Irs1*$^{-/-}$, *Irs2*$^{-/-}$ PyMT cells expressing EV, Irs2-WT, or Irs2-Y5F (related to Figure 2K and 2L). (F) Percentage of tumor free mice injected with 10^5 *Irs1*$^{-/-}$, *Irs2*$^{-/-}$ PyMT cells expressing EV, Irs2-WT, or Irs2-Y5F cells. WCL, whole cell lysate. *p<0.05; **p<0.01.

Figure S4. IRS2 regulates self-renewal through MYC. Related to Figures 3 and 4 (A) Correlation between mRNA expression of *IRS2 and IRS1 with MYC* in cBioPortal. (B-E) Fold change of MYC mRNA expression in (B) *IRS2*$^{+/+}$ (sgNT) or *IRS2*$^{-/-}$ SUM159 cells; (C) *IRS2*$^{-/-}$ SUM-159 cells expressing EV or IRS2; (D) *Irs1*$^{-/-}$, *Irs2*$^{-/-}$ PyMT cells (EV) with restored expression of mIrs1, mIrs2 or mIrs2-Y5F; (E) IRS1-/-, IRS2-/- SUM-159 cells expressing IRS2 were serum starved and stimulated with insulin, IGF-1 or IGF-2 (50 ng/ml) for 10 or 40 minutes. (F) *IRS1*$^{-/-}$, *IRS2*$^{-/-}$ SUM-159 cells with restored Irs2-WT or Irs2-Y5F were treated with cycloheximide in the absence or presence of insulin for the indicated times. (G) Irs1$^{-/-}$, Irs2$^{-/-}$ PyMT cells expressing Irs1 were stimulated with IGF1 (50 ng/ml) for the time periods indicated. (H) Representative density plots of ALDEFLUOR assays of *IRS1*$^{-/-}$, *IRS2*$^{-/-}$ SUM-159 cells expressing EV or IRS2 after treatment with 10074-G5 (related to Figure 4A). (I) Fold change of exogenous *Myc* mRNA expression in *IRS2*$^{-/-}$ PyMT cells expressing EV, MycWT or MycT58A. The data shown represent the mean ± S.D. of three independent experiments. *p<0.05; **p<0.01; ***p<0.001.