

C-terminally phosphorylated p27 activates self-renewal driver genes to program cancer stem cell expansion, mammary hyperplasia and cancer

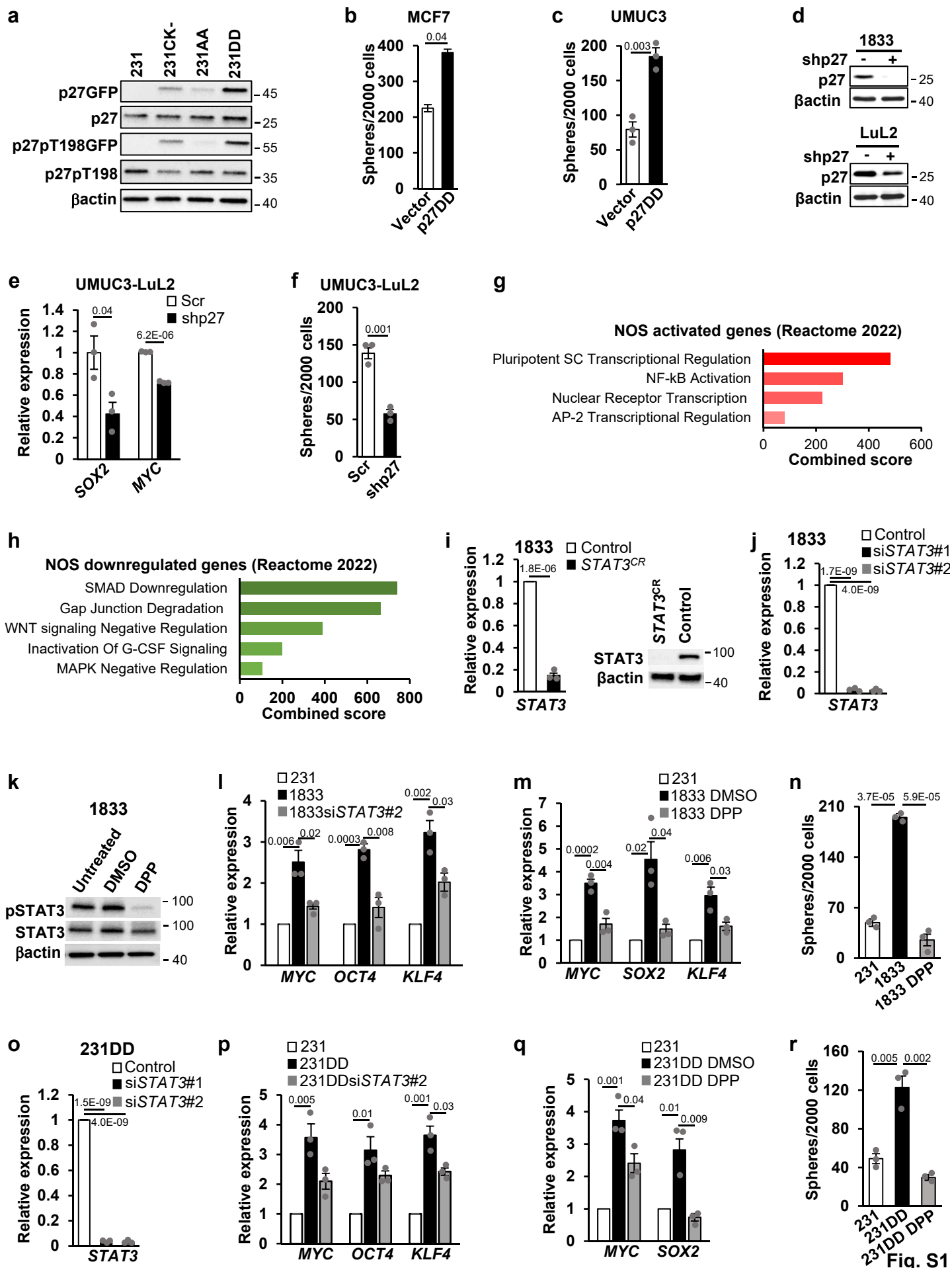


Fig. S1

Fig. S1 | p27pTpT drives stem cell properties via STAT3.

a Western blots show p27 and p27pT198 with β -actin control in 231, 231p27CK-, 231p27CK-AA, and 231p27CK-DD

b-c Effect of p27CK-DD induction on sphere formation in MCF7 (**b**) and in UMUC3-LUL2 cells (**c**).

d Western blots confirm p27 depletion in 1833 (top) and LUL2 (bottom) cells.

e-f Effect of p27 depletion on embryonic stem cell transcription factors (ES-TF) expression (**e**) and sphere formation (**f**) in UMUC3-LUL2 cells

g-h Pathway analysis of p27-regulated NOS (Nanog, Oct4, and Sox2) target genes.

i *STAT3* CRISPR knockout confirmed in 1833 cells.

j *STAT3* siRNA knockdown confirmed in 1833 cells.

k 1833 cells treated with STAT3 inhibitor (5, 15-DPP) for 48 h.

l-m Effect of *STAT3* siRNA knockdown (**l**) and STAT3 inhibitor (**m**) on p27pTpT-upregulated ES-TFs expression in 1833 cells.

n Sphere assay in 1833 cells treated with STAT3 inhibitor.

o *STAT3* siRNA knockdown confirmed in 231DD cells.

p-q Effect of *STAT3* siRNA knockdown (**p**) and STAT3 inhibitor treatment (**q**) on p27pTpT-upregulated ES-TFs expression in 231p27CK-DD cells.

r Sphere assay in 231DD cells treated with STAT3 inhibitor.

All graphs show mean $\pm$ SEM from N = 3 biological replicate assays. p values were represented by paired one-tailed Student's T Test. Source data are provided as a Source Data file.

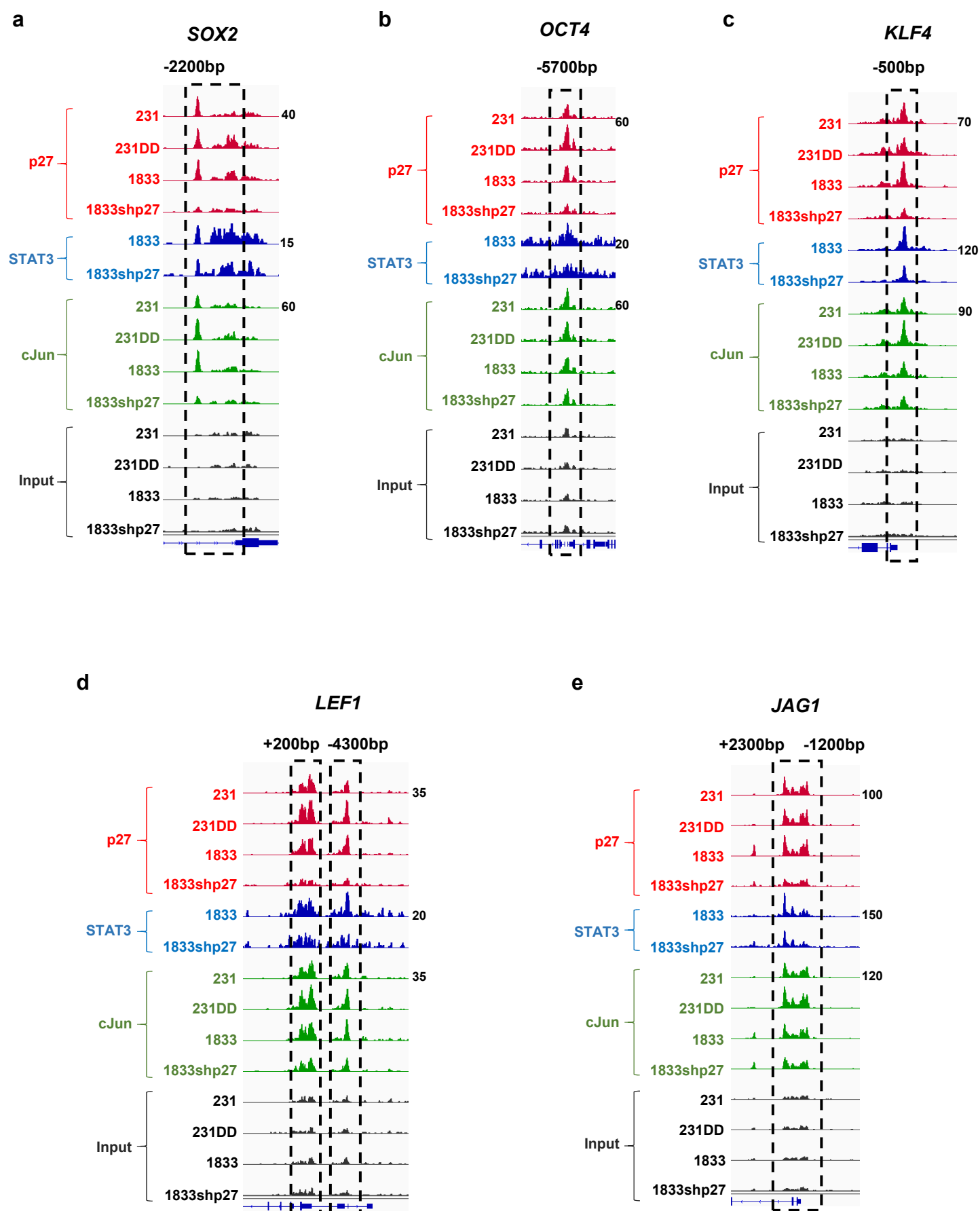


Fig. S2

Fig. S2 | p27-dependent STAT3, cJun enrichment at +/- 5 kb of TSS of the SC and CSC driver genes. a-e
p27, STAT3, and cJun co-occupied peaks at *SOX2* (**a**), *OCT4* (**b**), *KLF4* (**c**), *LEF1* (**d**), and *JAG1* (**e**) promoters

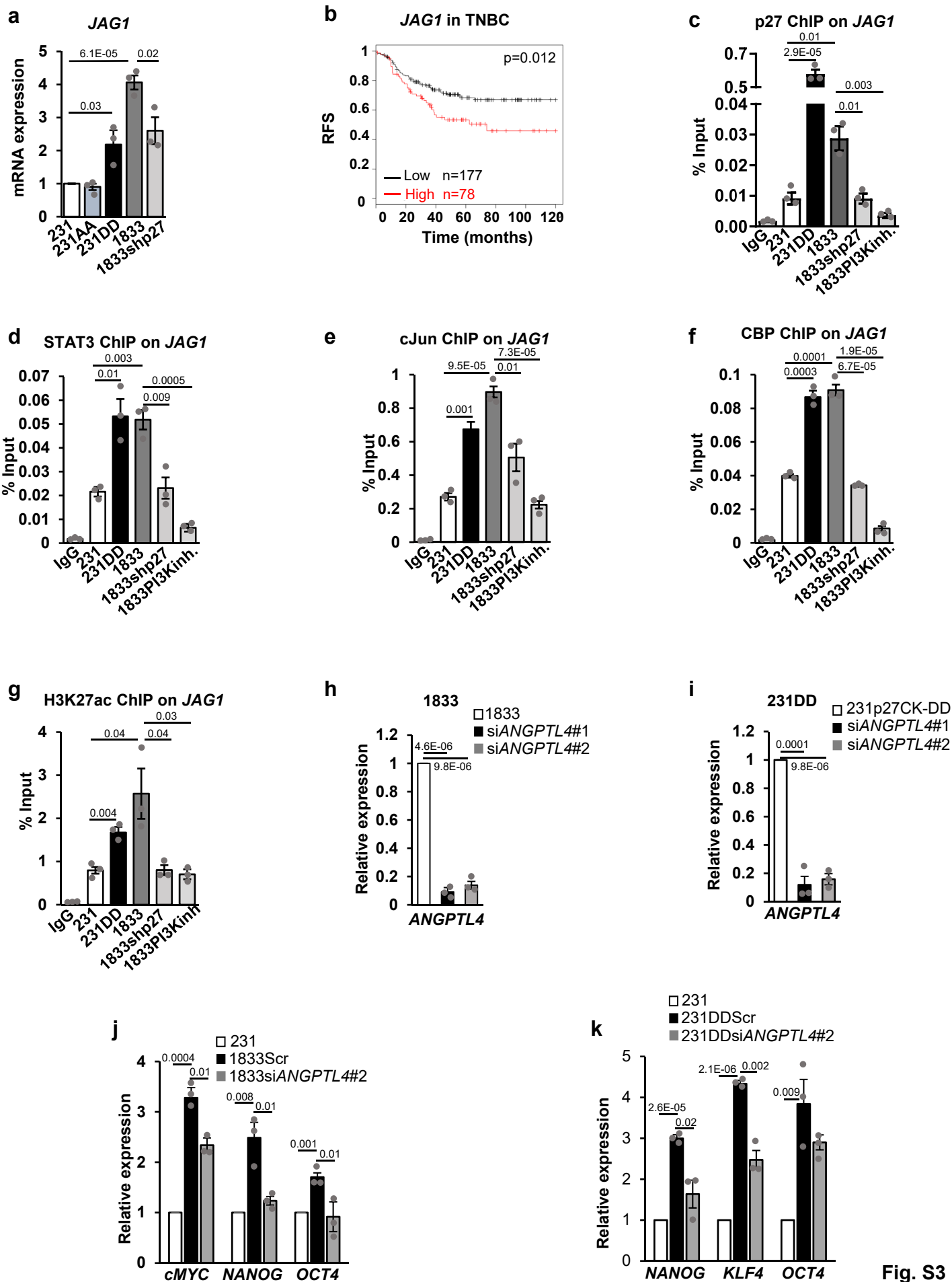


Fig. S3

Fig. S3 | p27/STAT3/cJun co-activate *JAG1*.

a *JAG1* mRNA expression.

b KM plot shows relapse-free Triple Negative Breast Cancer (TNBC) Relapse Free Survival (RFS) with *JAG1* differential expression.

c-g ChIPqPCR shows p27 (**c**) STAT3 (**d**), cJun (**e**), and CBP (**f**) binding and H3K27ac (**g**) at +2kb of the *JAG1* TSS, at a site where p27, STAT3 and cJun each bind on ChIPseq.

h-i *ANGPTL4* siRNA knockdown confirmed by qPCR in 1833 (**h**) and 231p27CK-DD cells (**i**).

j-k Effect of *ANGPTL4* depletion on p27-driven increase in embryonic stem cell transcription factors (ES-TFs) expression in 1833 (**j**) and in 231p27CK-DD (**k**).

All graphs show mean $\pm$ SEM from N = 3 biological replicate assays. p-values were represented by paired one-tailed Student's T Test. Source data are provided as a Source Data file.

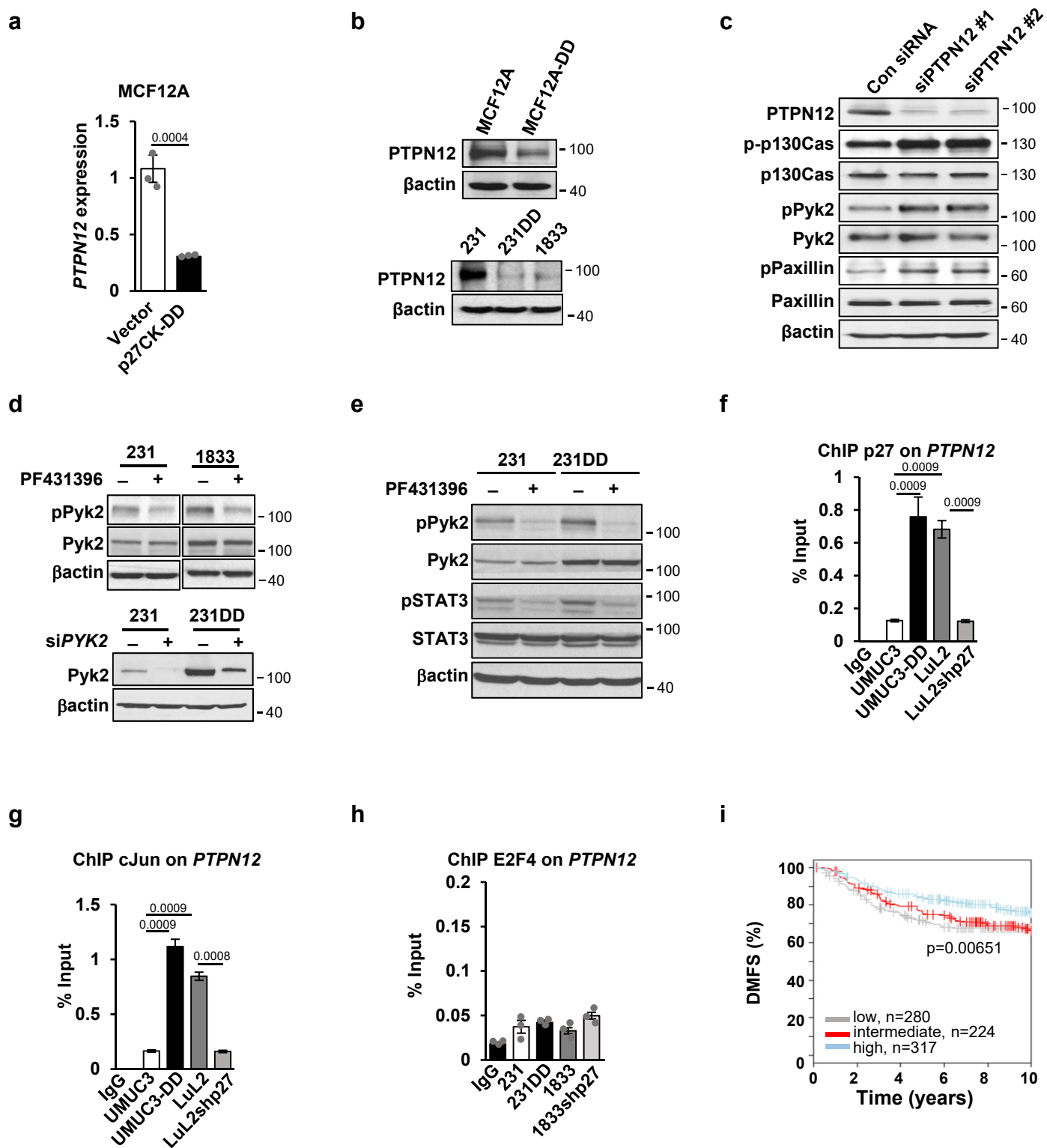


Fig. S4

Fig. S4 | p27/cJun co-repress *PTPN12*.

a *PTPN12* expression in p27CK-DD transduced MCF12A cells.

b Western blots show *PTPN12* level in MCF12A, MCF12Ap27CK-DD (top), 231, 231DD, and 1833 cells (bottom).

c Western blot depicts effect of *PTPN12* knockdown on P130Cas, Pyk2, Paxillin, and their phosphorylation level.

d Western blot confirmed effect of Pyk2 kinase inhibitor treatment on phospho-Pyk2 level (top) and confirmed *PYK2* siRNA knockdown (bottom).

e Effect of Pyk2 inhibitor treatment on phospho-STAT3 (Y705).

f-g ChIPqPCR analysis with anti-p27 (**f**) and anti-cJun antibodies (**g**) at the +2 kb site of *PTPN12* TSS in indicated cells.

h ChIPqPCR analysis for E2F4 bindings at the +2 kb site of *PTPN12* TSS in indicated cells.

All graphs show mean $\pm$ SEM from N = 3 biological replicate assays. p-values were represented by paired one-tailed Student's T Test.

i KM plot shows Distant metastasis-free survival (DMFS) with *PTPN12* differential expression.

Source data are provided as a Source Data file.

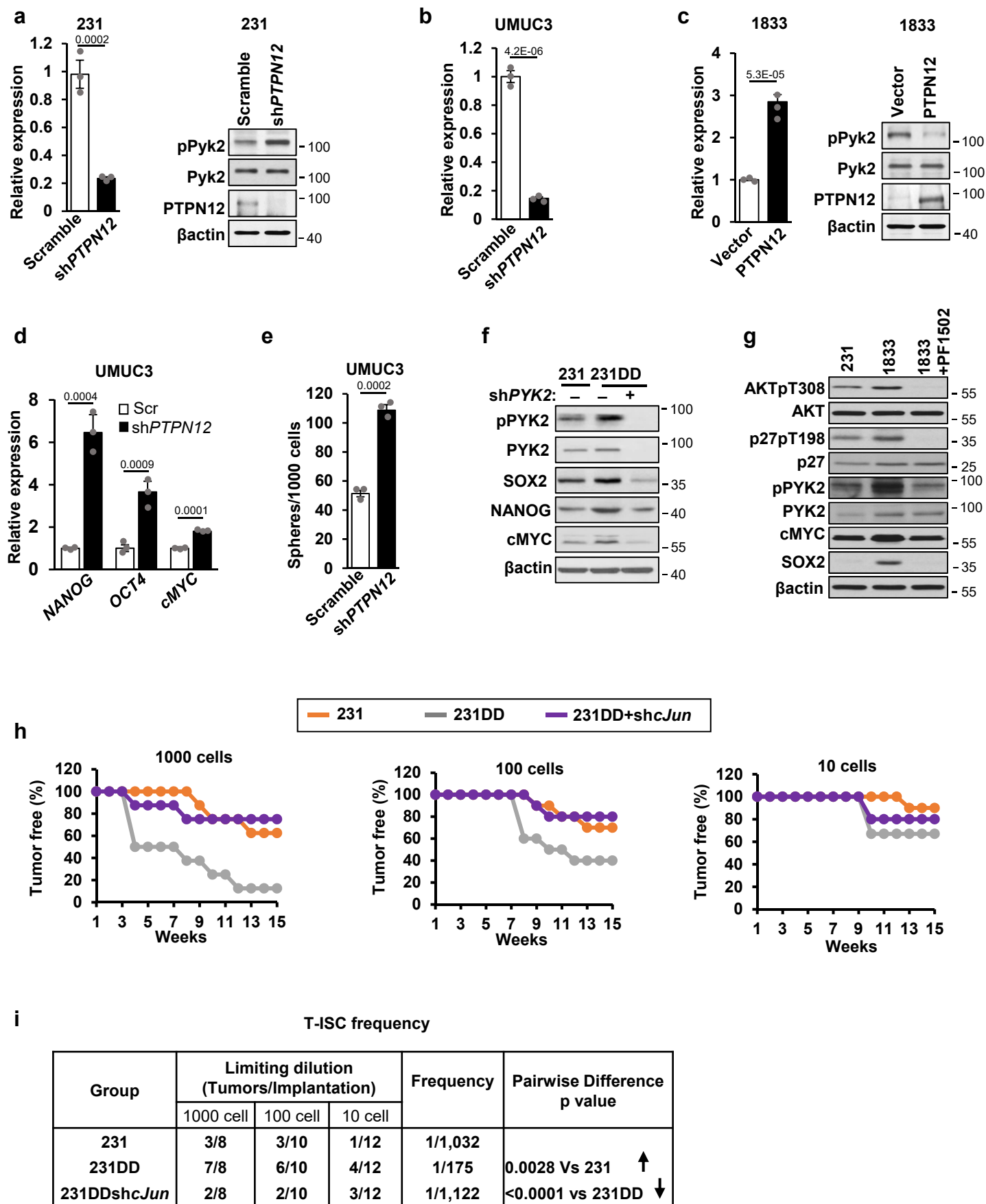


Fig. S5

Fig. S5 | p27pTpT increases T-ISC via cJun.

a-b qPCR confirmed *PTPN12* knockdown in 231 (**a**) and UMUC3 cells (**b**).

c qPCR and western blot confirmed *PTPN12* overexpression in 1833 cells.

d-e Effects of *PTPN12* knockdown on embryonic stem cell transcription factors (ES-TFs) expression (**d**) and sphere formation (**e**) in indicated cells. All graphs show mean $\pm$ SEM from N = 3 biological replicate assays. p-values were represented by paired one-tailed Student's T Test.

f Western blot shows effect of *PYK2* knockdown on p27pTpT-driven ES-TFs upregulation.

g Western blot depicts effect of PI3K inhibitor treatment on p27pTpT-driven ES-TFs upregulation.

h Effect of cJun knockdown on p27pTpT-driven increase of the Tumor Initiating Stem Cell (T-ISC) frequency. Graphs showing tumor free mice (%).

i T-ISC frequency was calculated by L-Calc Limiting Dilution Software (<http://www.stemcell.com/en/Products/All-Products/LCalc-Software.aspx>) from STEMCELL Technologies.

Source data are provided as a Source Data file.

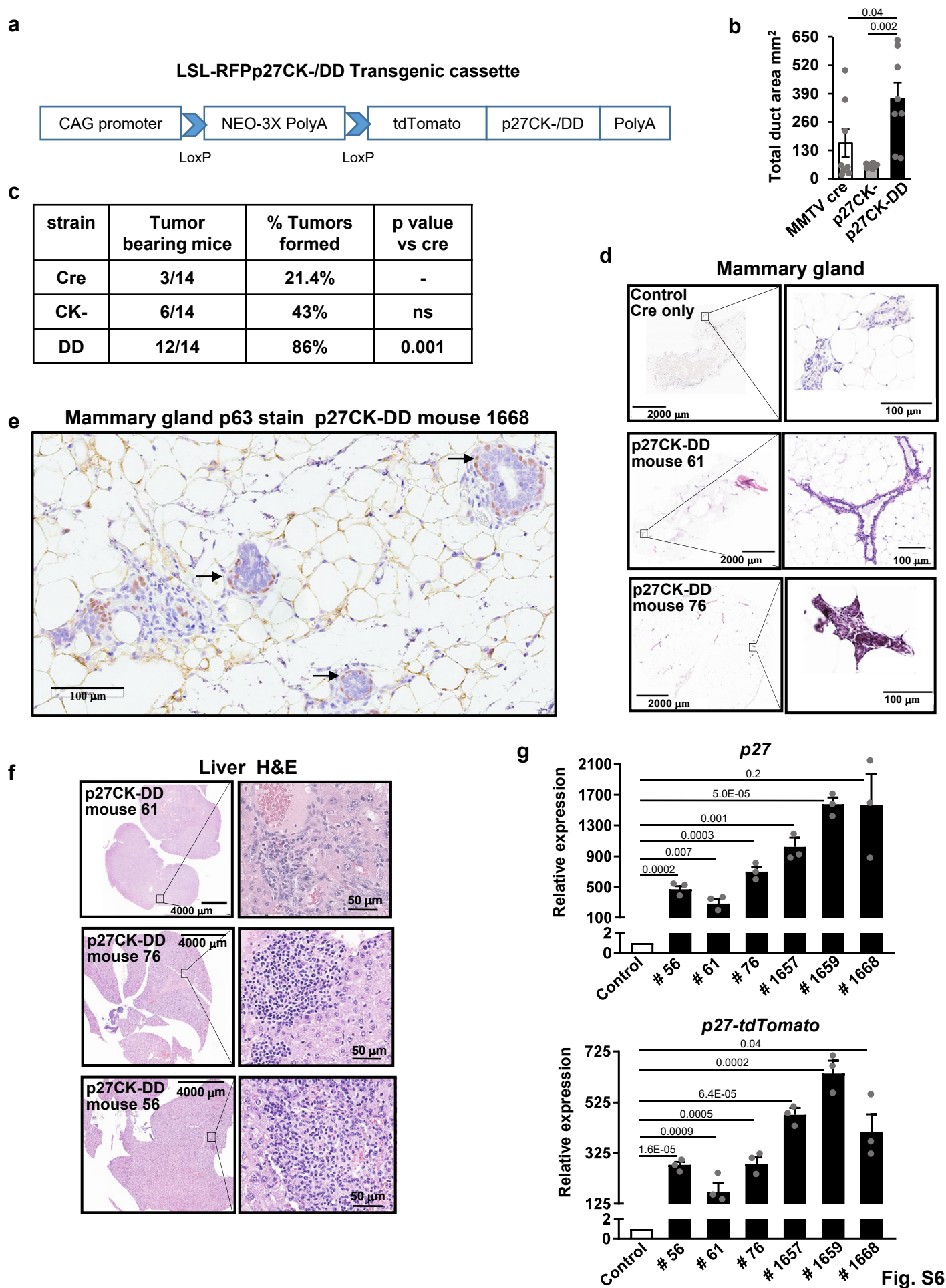


Fig. S6

Fig S6 | p27CK-DD increases mammary duct area, microinvasive cancers that metastasize to liver.

a Schematics of transgenic p27CK- and p27CK-DD expression targeting vectors.

b Duct area was quantitated from whole mounts and graphed as mean % $\pm$ SEM. p-values were represented by one-way ANOVA with post hoc 2 by 2 comparisons using Tukey correction. N = 8 mice for MMTVCre controls, N = 8 mice for TGp27CK-, N = 8 mice for TGp27CK-DD

c Table shows tumor formation in MMTV cre, MMTV Cre X p27CK-, and MMTV Cre X p27CK-DD mice.

d Mammary gland hematoxylin and eosin (H&E) staining from MMTV Cre control mouse and the indicated p27CK-DD MG.

e p63 staining of breast myoepithelial cells is seen at the perimeter of normal ducts (upper right arrow) and is interrupted in the tumor area in the lower left. This is an expanded view of the mammary tumor from p27CK-DD mouse 1668 shown in Fig 7d.

f H&E staining of the indicated MMTV Cre X p27CK-DD mice livers showing representative micro metastases.

g qPCR shows transgenic *p27CK-DD* expression using TGp27-specific primers and primers spanning the tdTomato and p27 sequences in metastatic liver compared to control liver. N = 5 control mice and N=6 TGp27CK-DD mice. All graphs show mean $\pm$ SEM. Each TGp27CK-DD mice is compared by the average of 5 control mice. p-values were represented by paired one-tailed Student's T Test.

Source data are provided as a Source Data file.

Table S1. Primer sequence

Gene	Forward	Reverse
c-MYC (qPCR primer)	GTGGAAAAGAGGCAGGCTCC	CTTGACCCTCTTGGCAGCAG
SOX2 (qPCR primer)	ACCAGCTCGCAGACCTACAT	ACCAGCTCGCAGACCTACAT
OCT4 (qPCR primer)	TCAGCCAAACGATCTGC	TTCGCTTTCTCTTTCGGGCC
NANOG (qPCR primer)	CAGACCTGGTGCACCCAATC	CTTCCAAGGCAGCCTCCAAG
KLF4 (qPCR primer)	CCCACACTTGTGATTACGC	GGTAAGGTTTCTCACCTGTG
STAT3 (qPCR primer)	CAGTGACAGCTTCCCAATGG	ATGTGATTCTTTGCTGGCCG
ANGPTL4 (qPCR primer)	AGAAGCAGCACCTGCGAAT	CTCTTTCTTCGGGCAGGCTT
JAG1 (qPCR primer)	ACCTTCAACCTCAAGGCCAG	CGCCTCCACAAGCAACGTAT
PTPN12 (qPCR primer)	TGAAGATTCACCTCCTCCC	CCGCTGGATGATCACATTC
p27 (qPCR primer)	TAGCGGAGCAATGCGCAGGA	AACCGGCATTTGGGGAACCGT
p27 (PCR primer)	ACTACTACGTGGACACCAAGCTG	GGGGTTTGTGATTCTGAGCATC
GAPDH	ATCAAGTGGGGCGATGCTG	ACCCATGACGAACATGGGG
c-MYC (ChIPqPCR primer)	AAAGAACGGAGGGAGGGATC	GCGAGTTAGATAAAGCCCCG
JAG1 (ChIPqPCR primer)	GTGGACCCTGAGCCGAAG	TCAAAGTGTGCCTCAAGGAG
ANGPTL4 (ChIPqPCR primer)	GAGAATGAGCTGGCTTGGAG	AGCCTGCCAGGAAGAACTC
PTPN12 (ChIPqPCR primer)	TGGAGAAGTGGCCTTAGGGT	ATTGAGCCACTGCACTCCAA