Figure S1

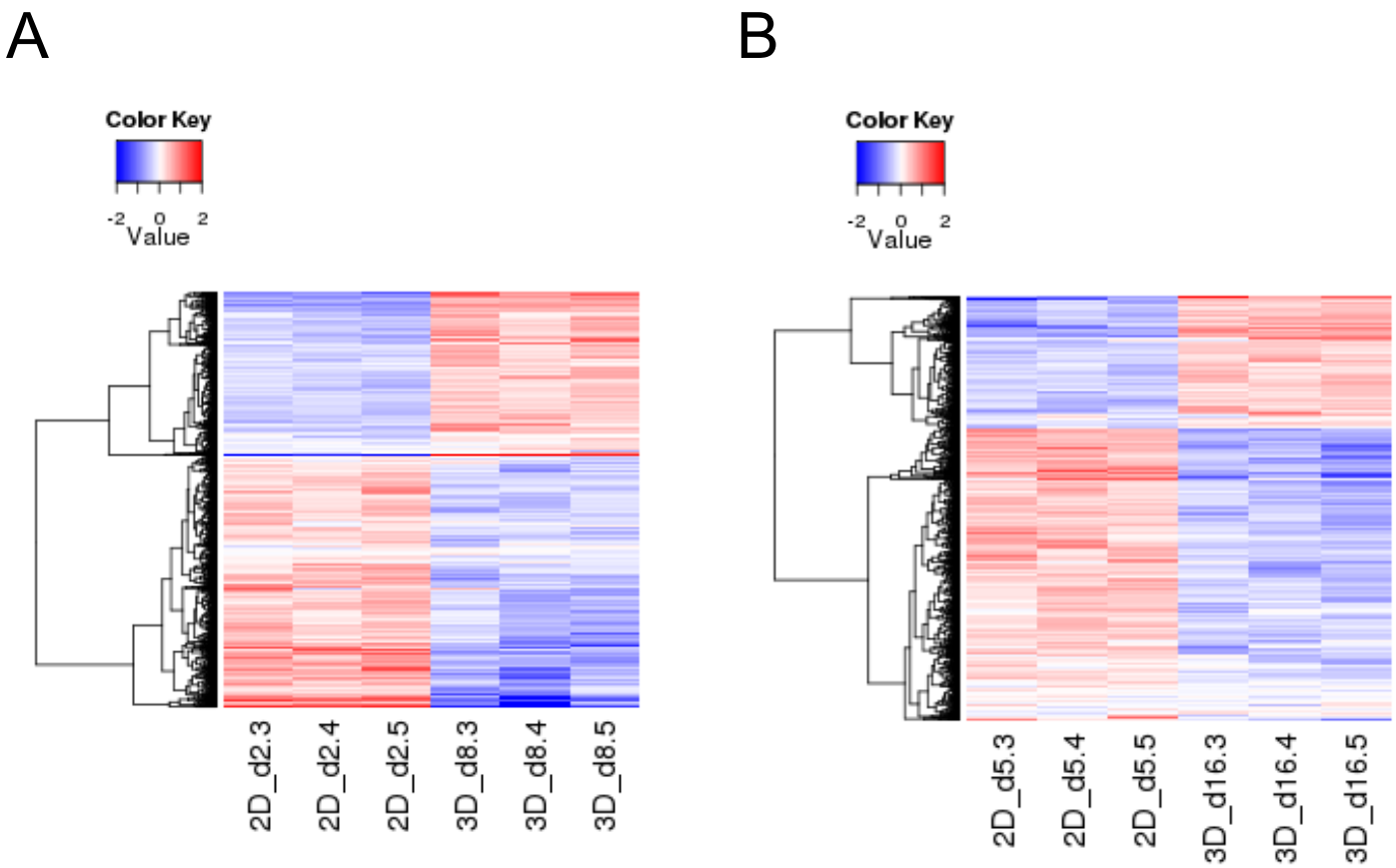


Figure S1: Genes differentially expressed between cells actively growing cells in 2D and 3D (A), and genes differentially expressed in proliferation-arrested cells in 2D and 3D (B).

Figure S2

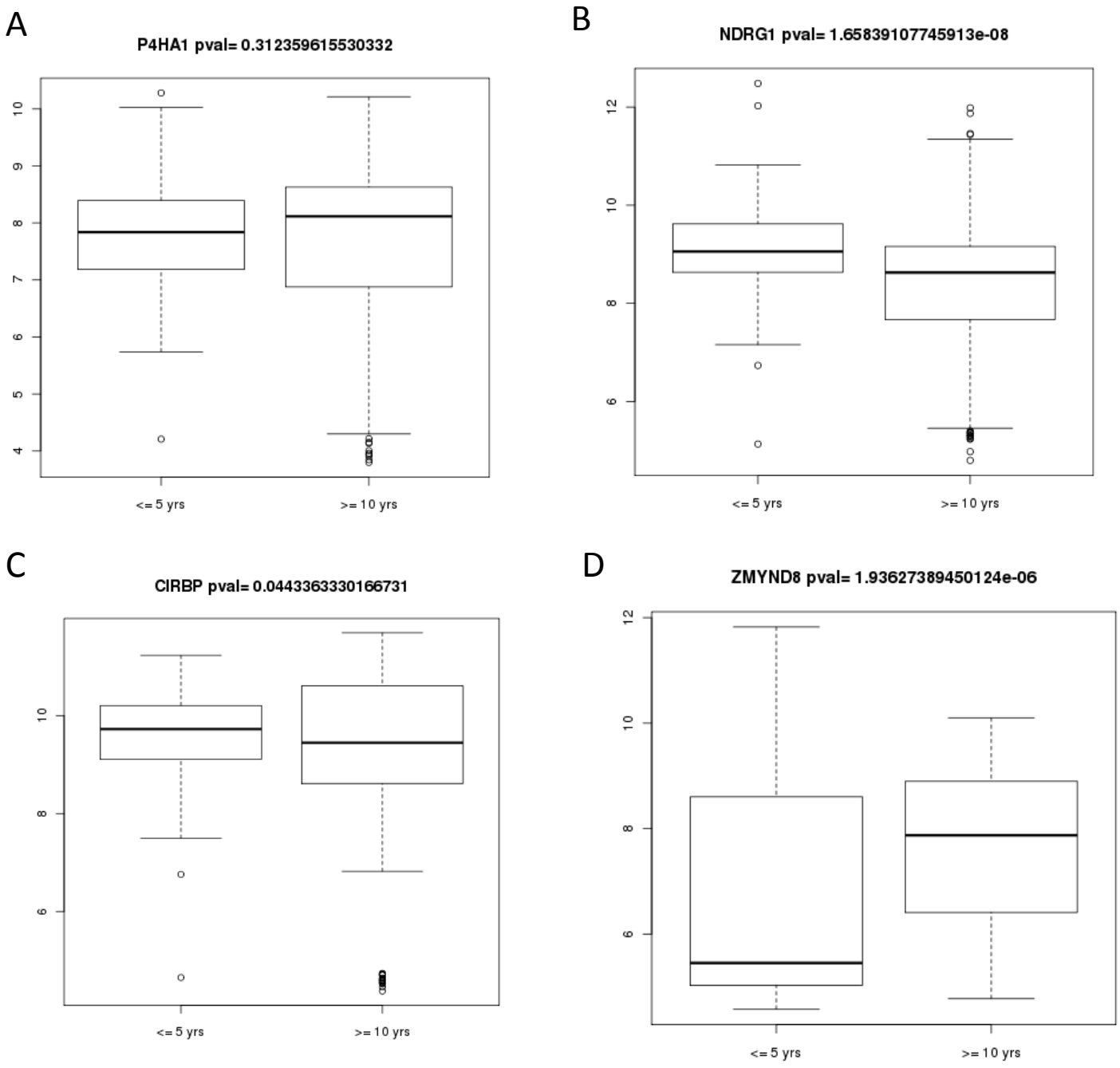
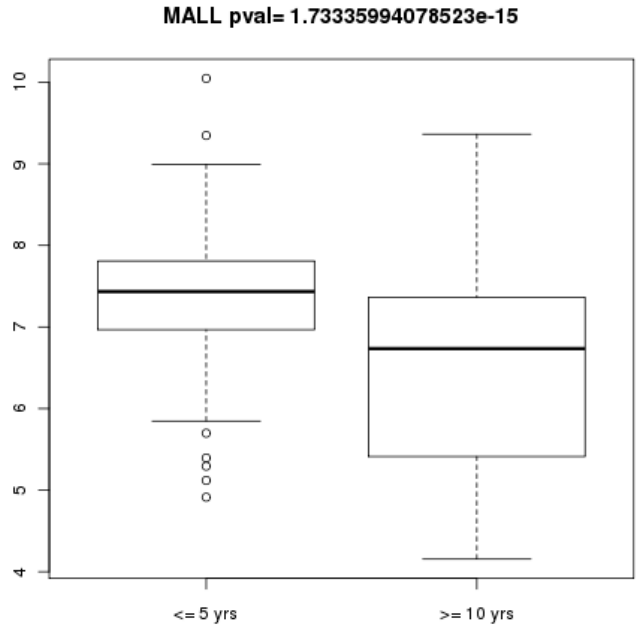


Figure S2: Box plots of gene expression levels in breast cancer patients for genes upregulated in proliferation-arrested 3D cultures. p-values are listed on top.

Figure S2

E



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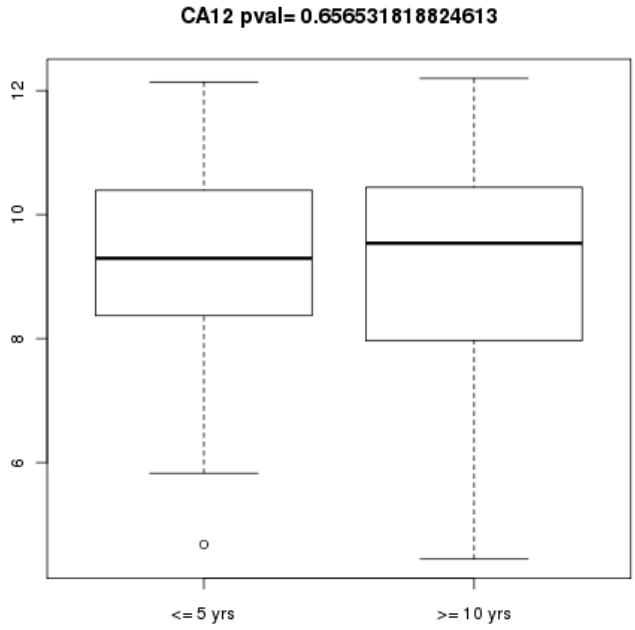

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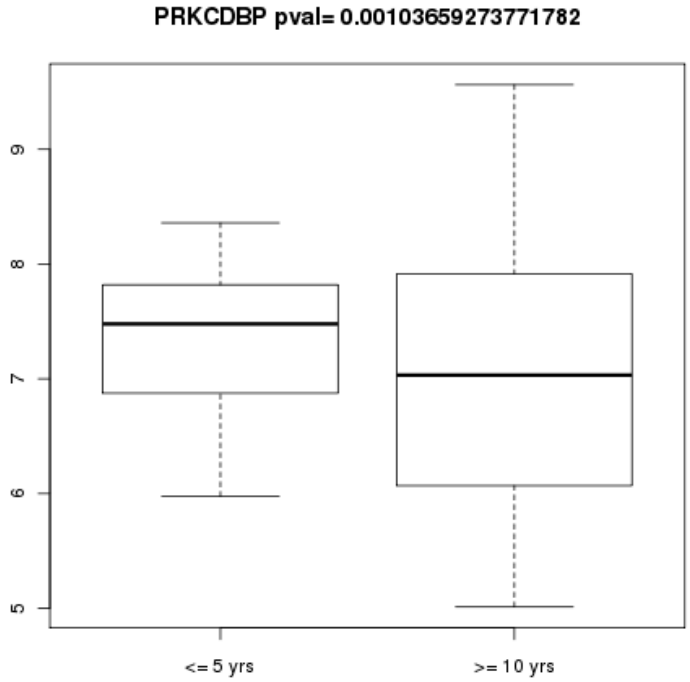

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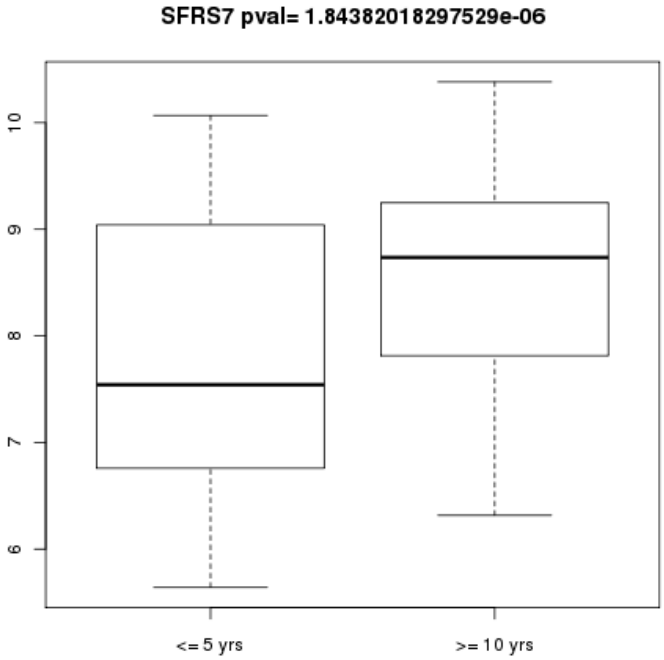
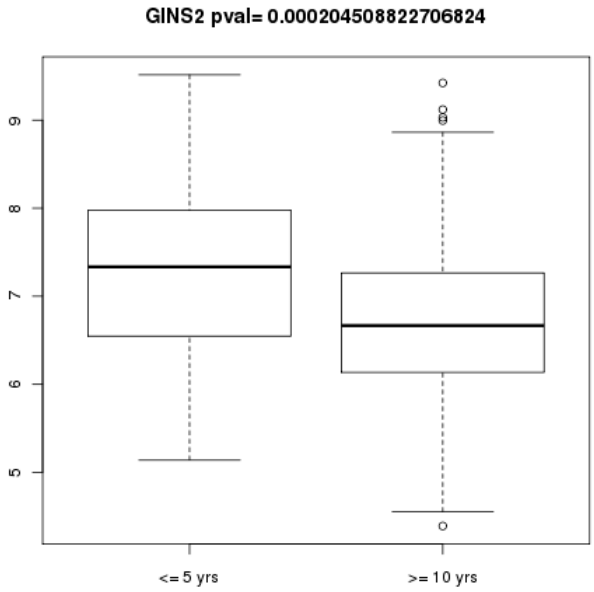


Figure S2

I



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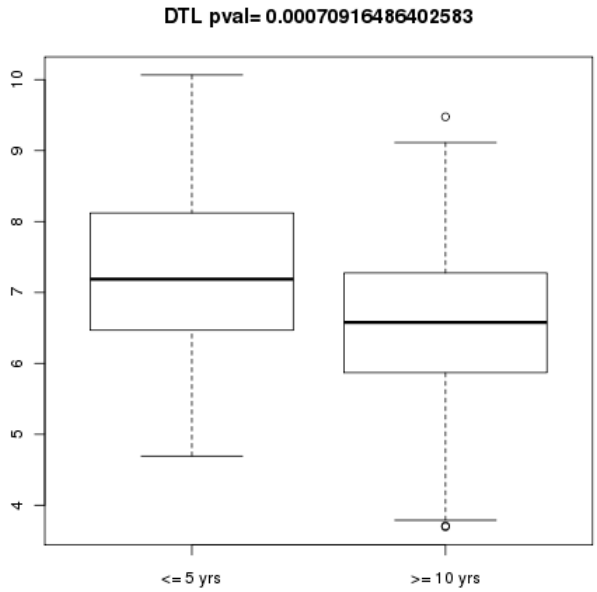

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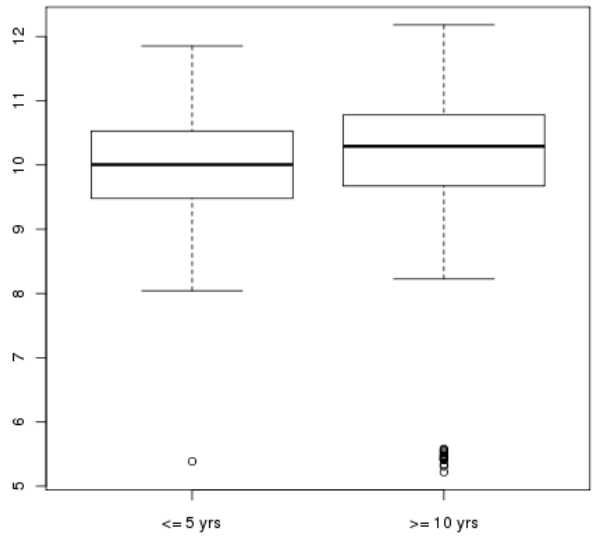


L

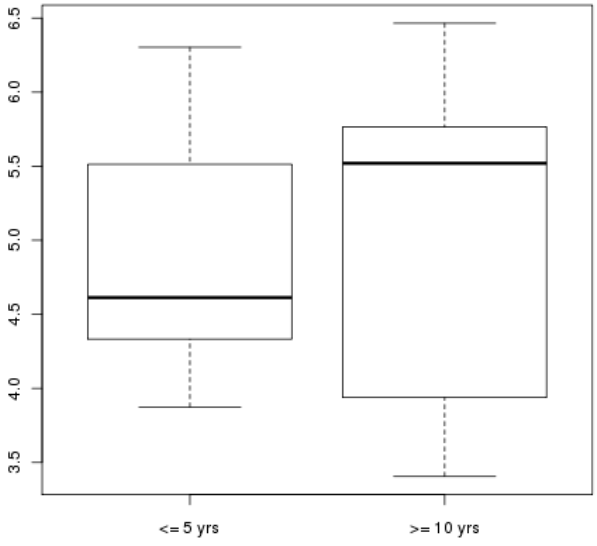


Figure S3

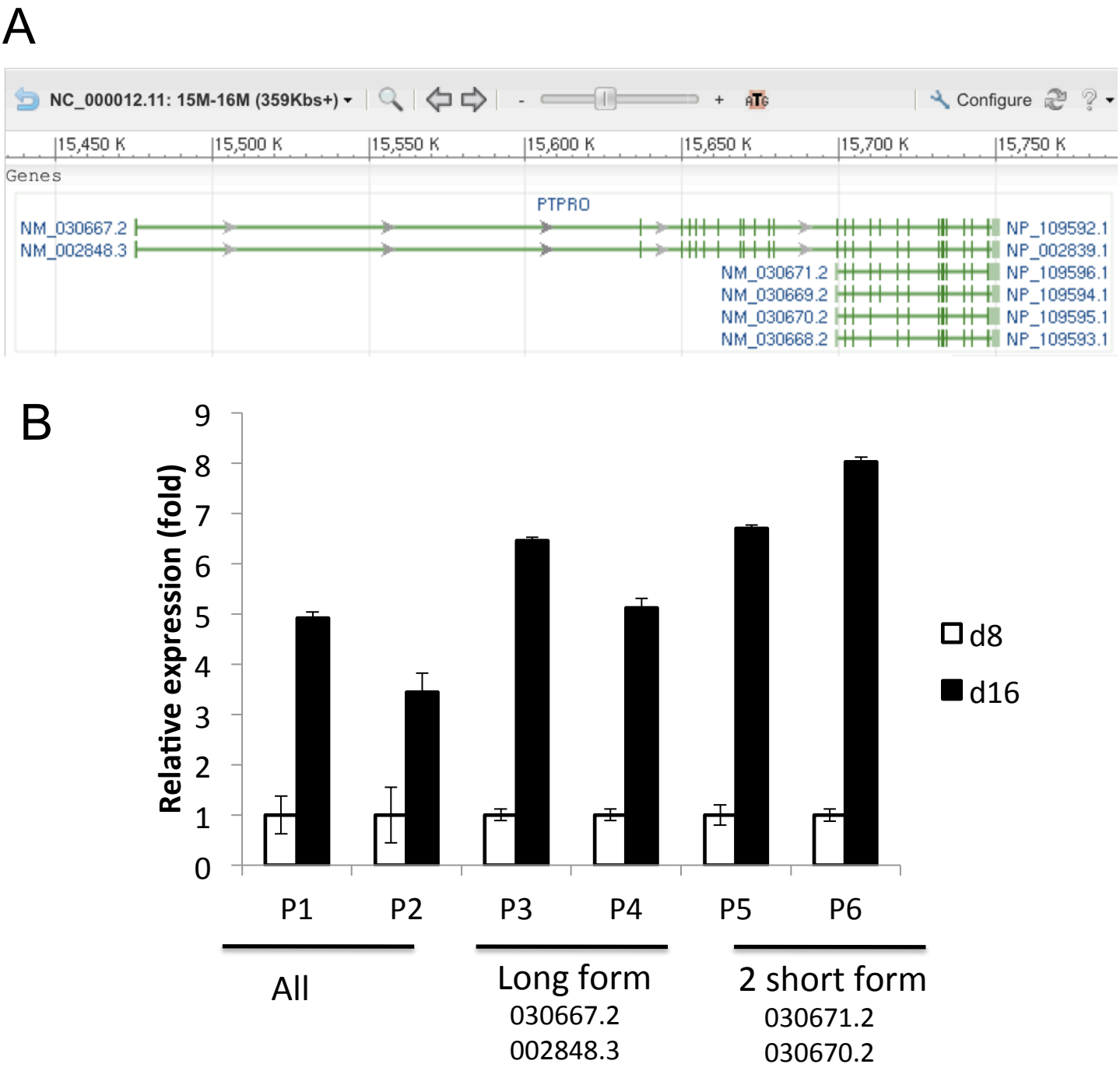
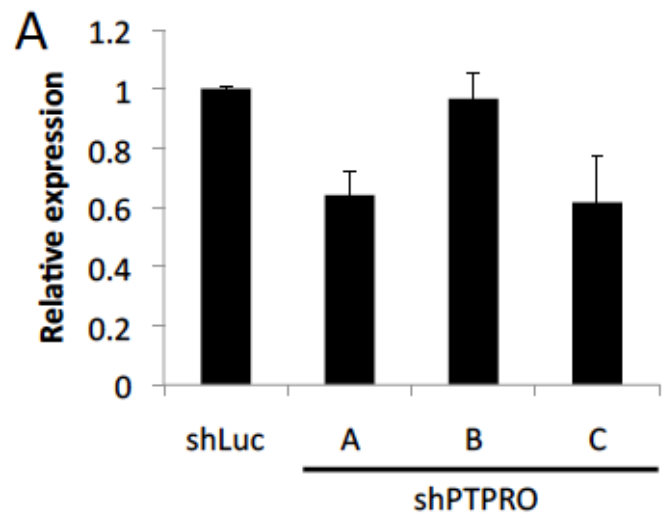


Figure S3. Analysis of expression levels for different isoforms of PTPRO. A) A snapshot image of the genomic alignment for different isoforms of PTPRO from the NCBI webpage. B) qRT-PCR analysis of the relative expression of different isoforms of PTPRO in 3D day 8 and day16. Two primer pairs (P1 and P2) amplify the common regions for all 6 isoforms. Two primer pairs (P3 and P4) amplify the common regions for the two long isoforms NM_030667.2 and NM_002848. 3. Two primer pairs (P5 and P6) amplify the common regions for the two short isoforms NM_030671.2 and NM_030570.2. (mean±s.d., n=3)

Figure S4



C

P value (compare to shLuc)

	A	B	C
Day 4	<0.001	<0.001	<0.001
Day 8	<0.001	<0.001	<0.001
Day 12	<0.001	0.5	<0.001

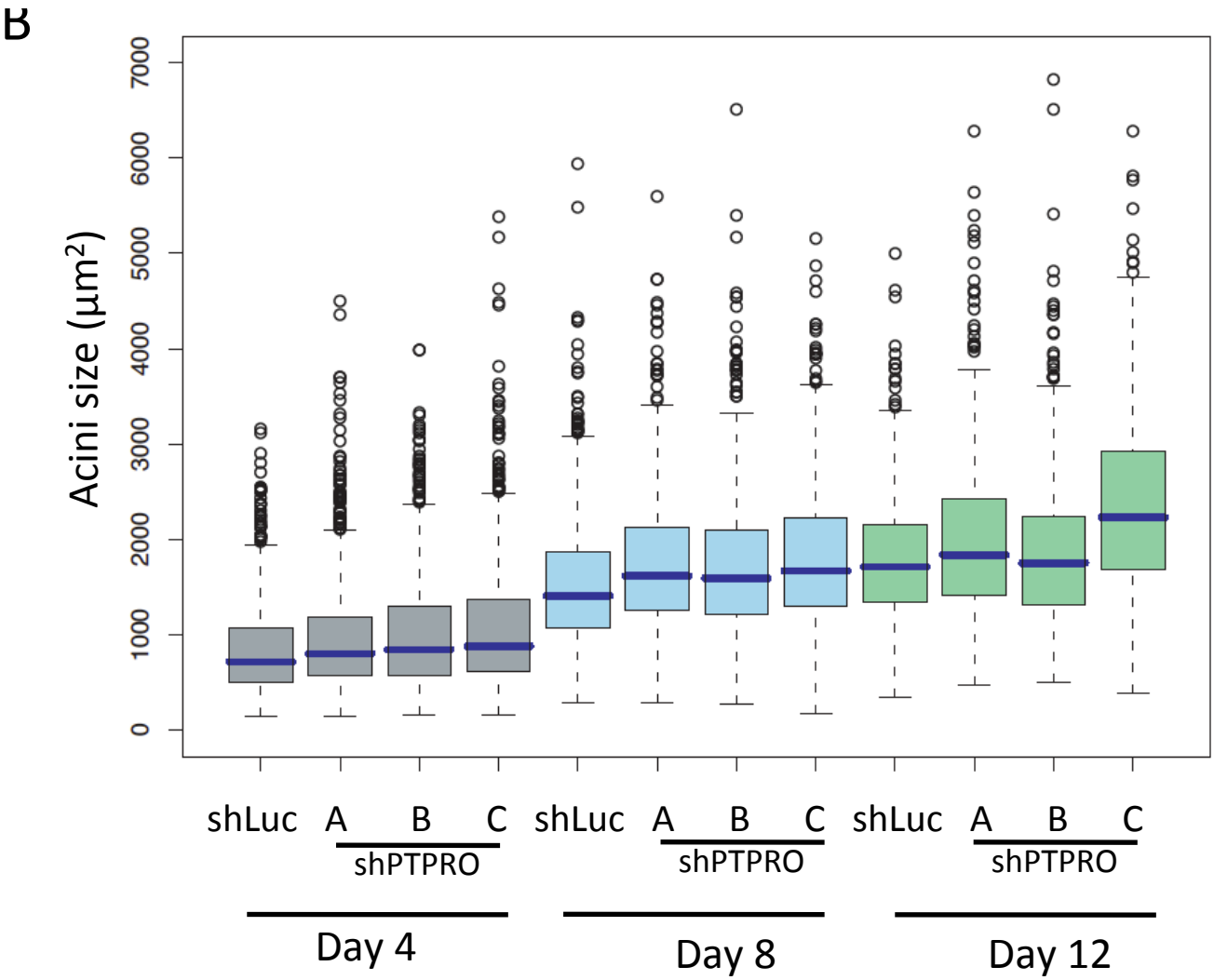


Figure S4. Loss of PTPRO induces proliferation. (A) Three independent short hairpin RNA (shRNA) were designed and tested for expression changes in expression of mRNA by quantitative reverse transcription PCR. (B) Stable cell lines expressing each of the shRNA were plated on 3D, phase images collected at indicated days and size measured as detailed in the methods section. (C) Statistical analysis of acini size for shRNA cells compared to the size of control RNAI (shLuc) cells.

Figure S5

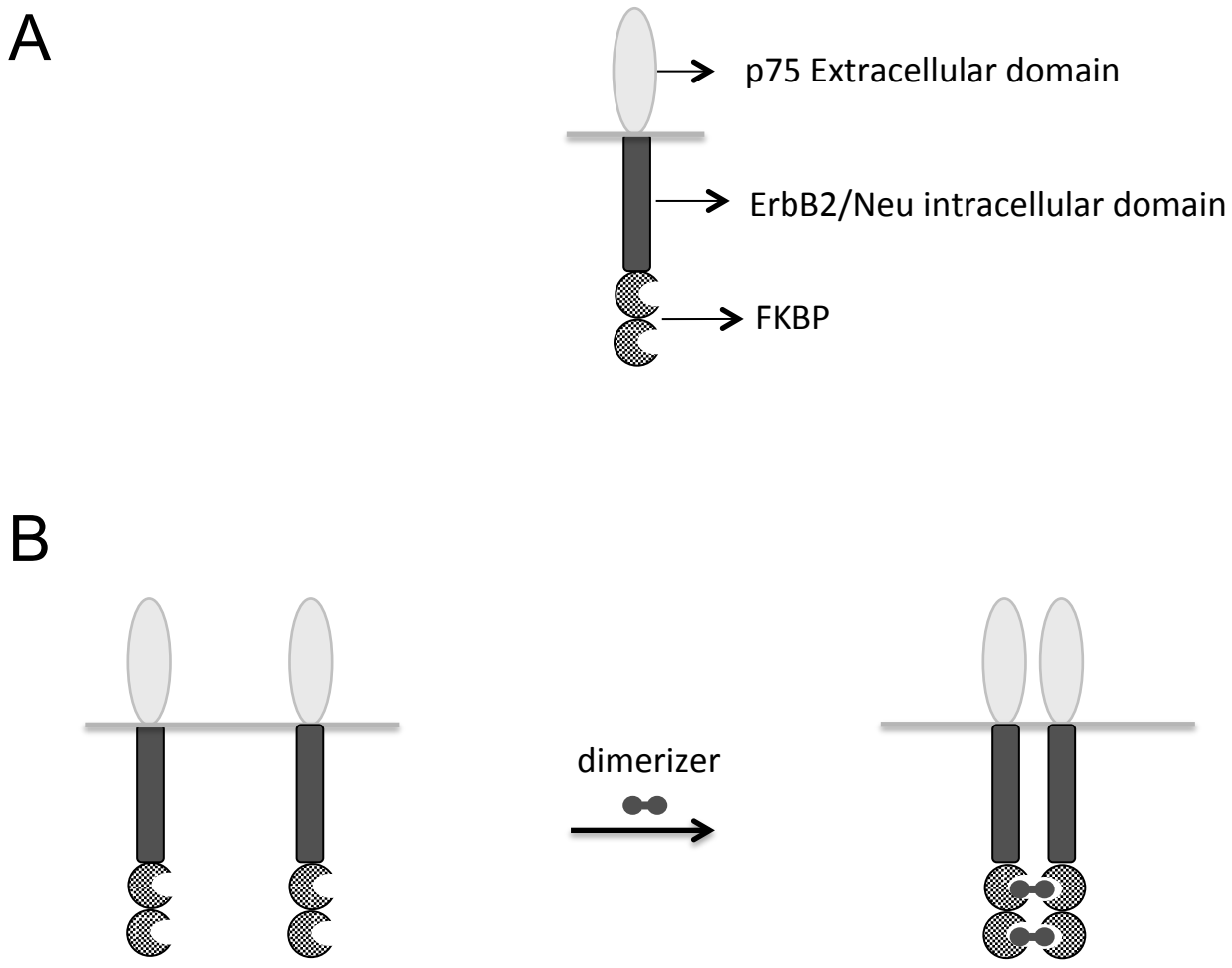


Figure S5. Controlled dimerization system. (A) Chimeric ErbB2 receptor – Intracellular domain of Neu (rat homolog of human HER2) was fused to extracellular and transmembrane domain of p75, low affinity nerve growth factor receptor and two copies of FK506 binding protein and a HA epitope tag. (B) Addition of a dimerizing ligand (dimerizer) to cells expressing the chimeric receptor will induce dimerization and activation of the chimera. We have previously demonstrated that the dimerizer does not have any detectable non-specific activity in cells that do not express the receptor chimera.

Figure S6

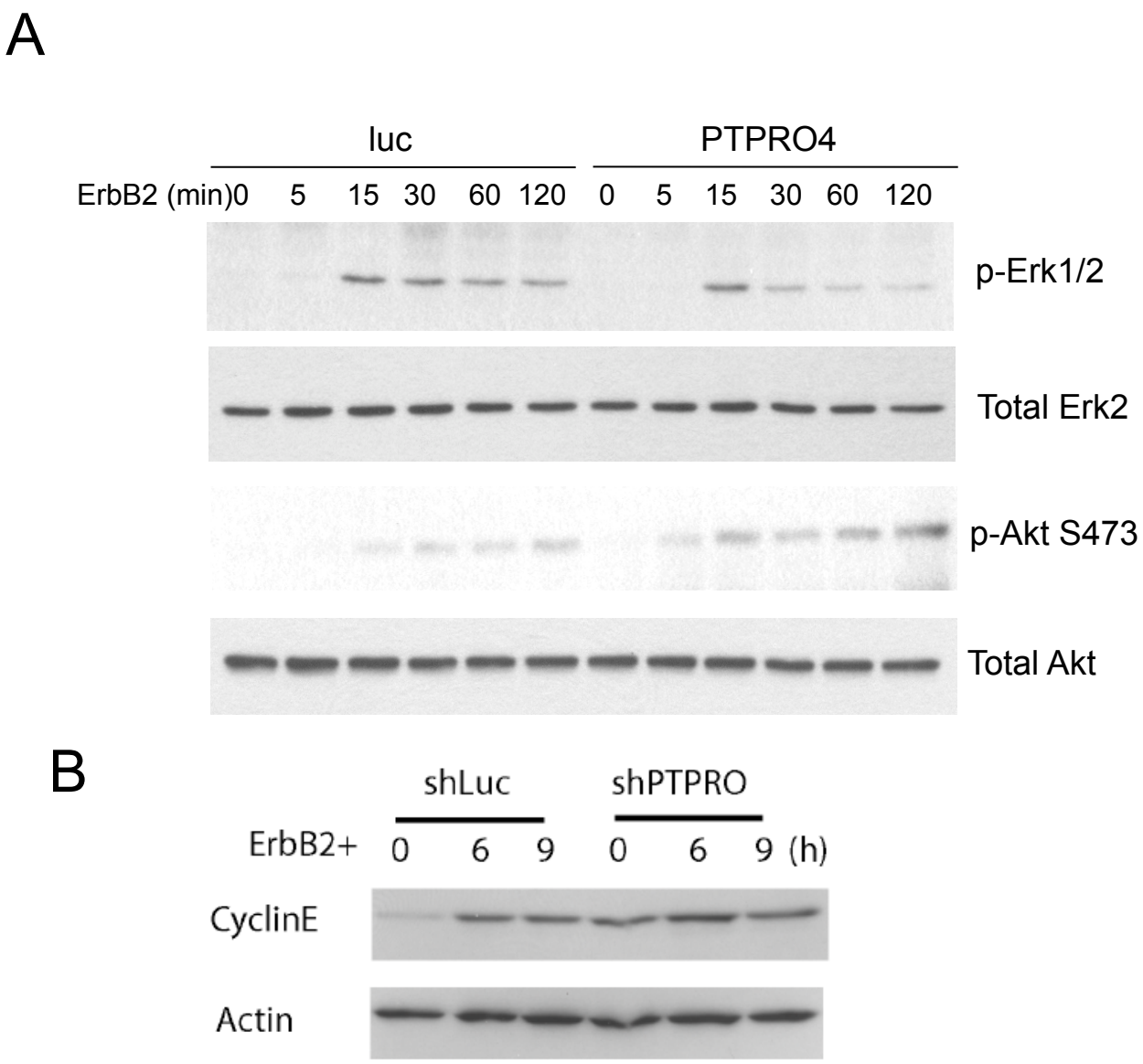


Figure S6: Loss of PTPRO and activation of downstream signaling. (A) Control or sh.PTPRO cells were stimulated with dimerizer for indicated lengths of time and lysates used for immunoblotting with phosphor-specific or total antibodies as indicated. (B) ErbB2 activation induced changes in expression of Cyclin E.