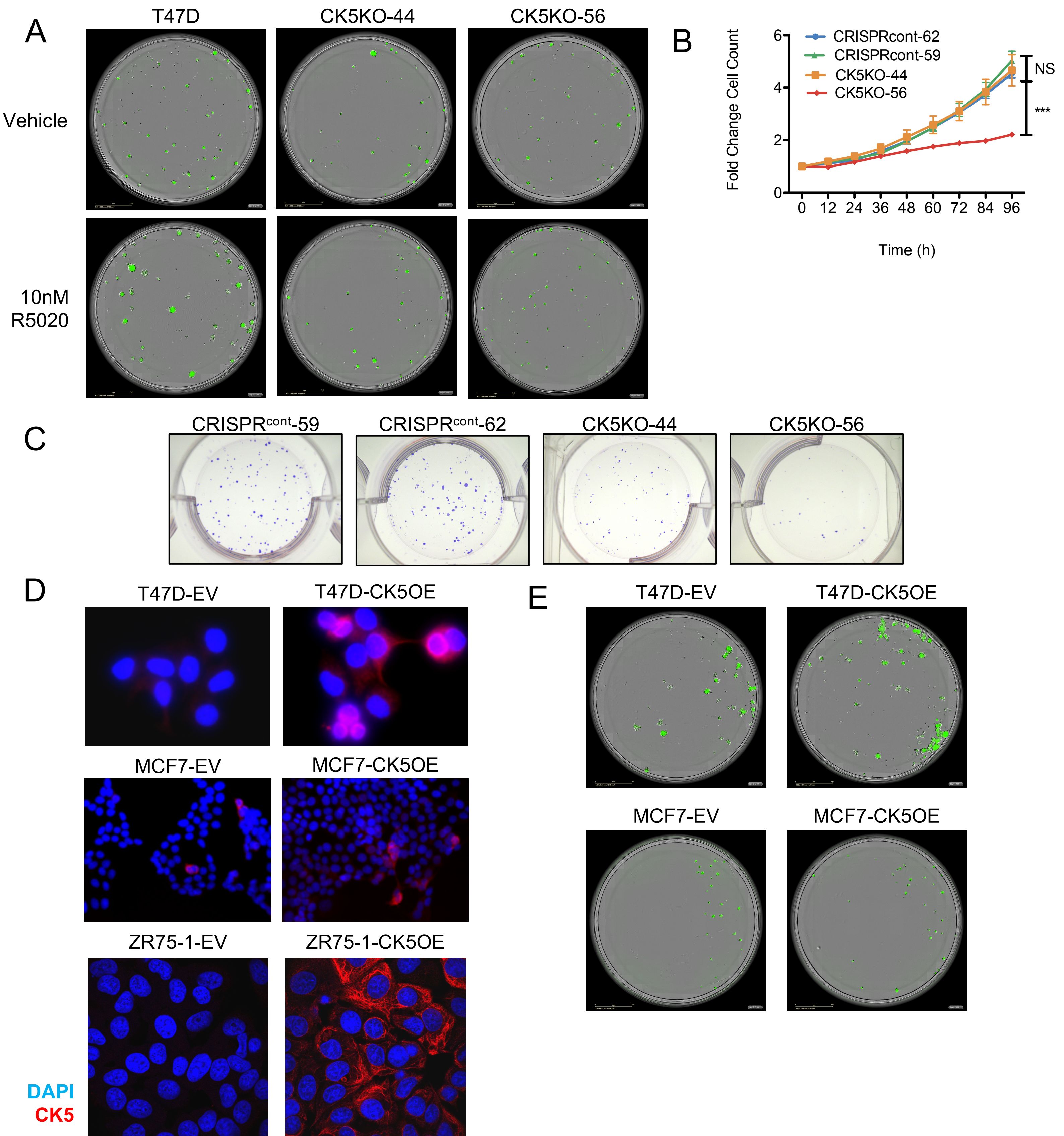
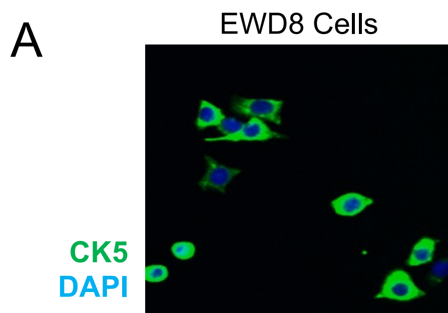


Supplemental Figure 1. A. Representative images of tumorsphere assay using T47D CRISPR^{cont} and CK5KO cells stably expressing ZsGreen analyzed in Figure 1B.**B.** Nuclear-GFP labeled CRISPRcont and CK5KO T47D cells were seeded in phenol red-free media at 1000 cells/well in a 96 well plate in replicates of 6. Proliferation was assessed by measuring nuclear-GFP count using Incucyte Zoom. Fold change in cell count normalized to 0h timepoint is shown. Two-way ANOVA with Bonferroni post-tests were used to determine statistical significance. ***P<0.001. **C.** Representative images of colony formation assays using T47D CRISPR^{cont} and CK5KO cells analyzed in Figure 1C. **D.** EV and CK5OE T47D, MCF7, and ZR75-1 cells without hormone treatment were analyzed by fluorescent immunocytochemistry for CK5 (red) and DAPI (blue). **E.** Representative images of tumorsphere assay using T47D (top) and MCF7 (bottom) EV and CK5OE cells analyzed in Figure 1E.

A



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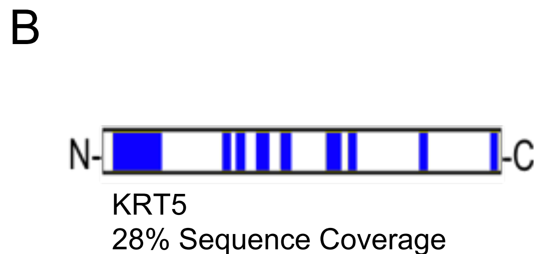
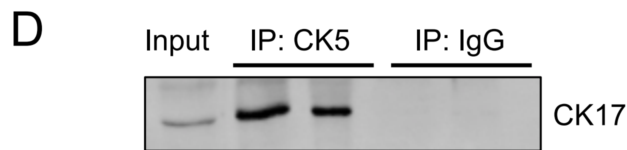


C

Common cytokeratin interacting proteins identified by IP-MS			
	Average Spectral Count		
	CK5	IgG	Fold Change
KRT5	40	23.5	1.7
KRT18	35.5	7	5.1
KRT8	30	14	2.1
KRT17	8.5	1	8.5
KRT19	6.5	0	6.5
ITA3	19	5.5	3.5
ITA6	30	13	2.3
PKP3	10	5.5	1.8
PLEC	8.5	1.5	5.7

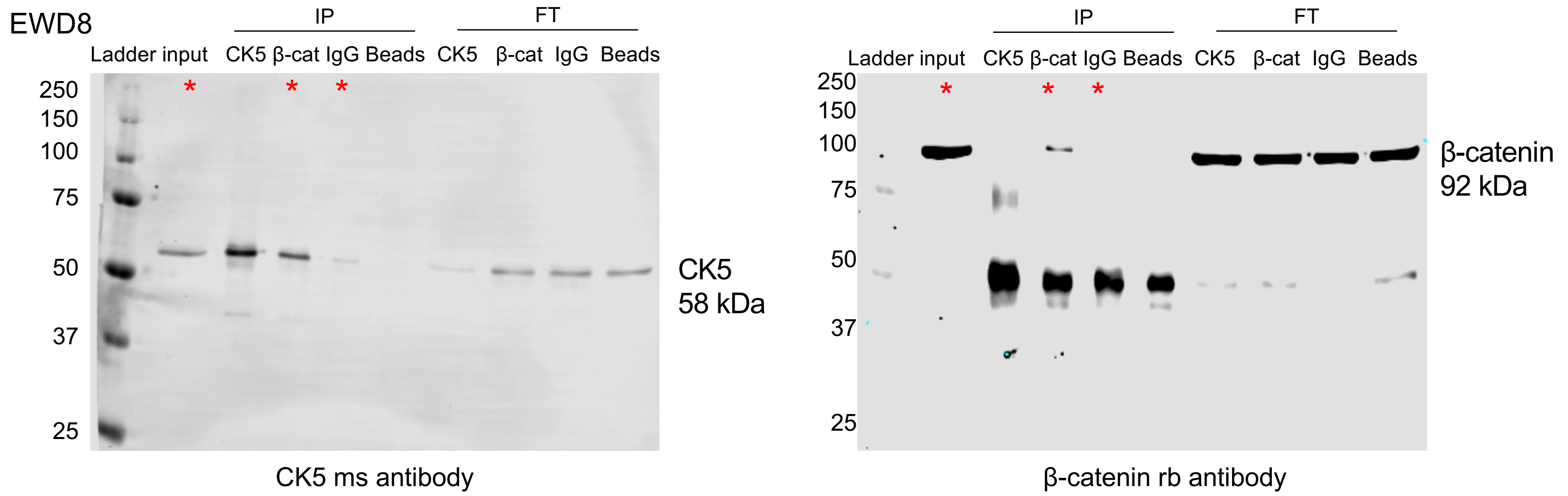
D

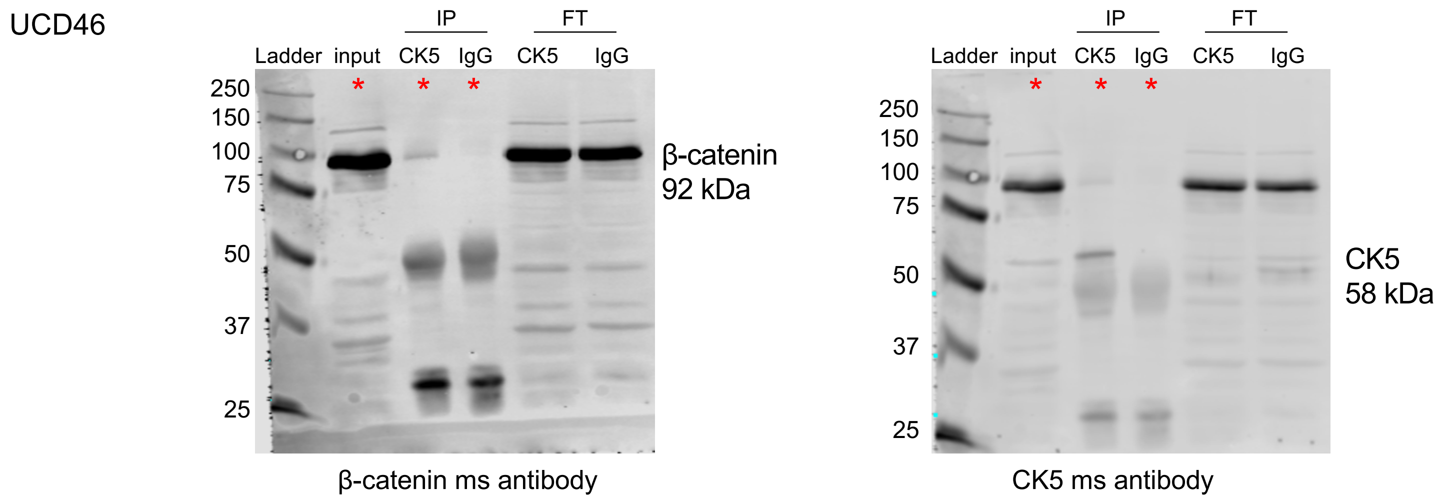


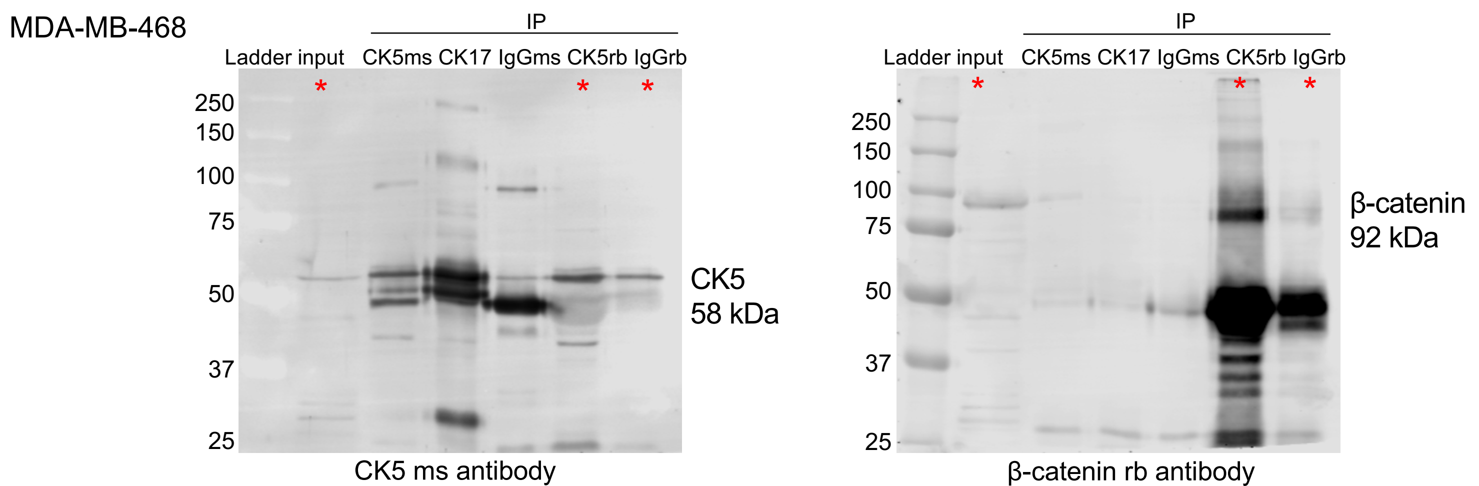
E

Significantly differentially regulated Wnt/β-catenin pathway genes in EWD8 cells compared to parental T47D cells		
Gene Symbol	Gene Name	Expression Fold Change
AKT3	AKT serine/threonine kinase 3	6.808
AXIN2	axin 2	5.018
CDH2	cadherin 2	6.566
CDH3	cadherin 3	8.066
CDKN2A	cyclin dependent kinase inhibitor 2A	5.071
DKK1	dickkopf WNT signaling pathway inhibitor 1	6.284
FZD8	frizzled class receptor 8	8.04
GJA1	gap junction protein alpha 1	128.321
KREMEN2	kringle containing transmembrane protein 2	4.895
MYC	v-myc avian myelocytomatosis viral oncogene homolog	2.735
PPP2R2B	protein phosphatase 2 regulatory subunit Bbeta	55.215
SOX15	SRY-box 15	3.131
TGFB2	transforming growth factor beta 2	5.071
TGFBR3	transforming growth factor beta receptor 3	5.783
TP53	tumor protein p53	2.286
WNT10A	Wnt family member 10A	3.055
WNT3A	Wnt family member 3A	3.877
WNT5A	Wnt family member 5A	35.448
WNT7A	Wnt family member 7A	10.281

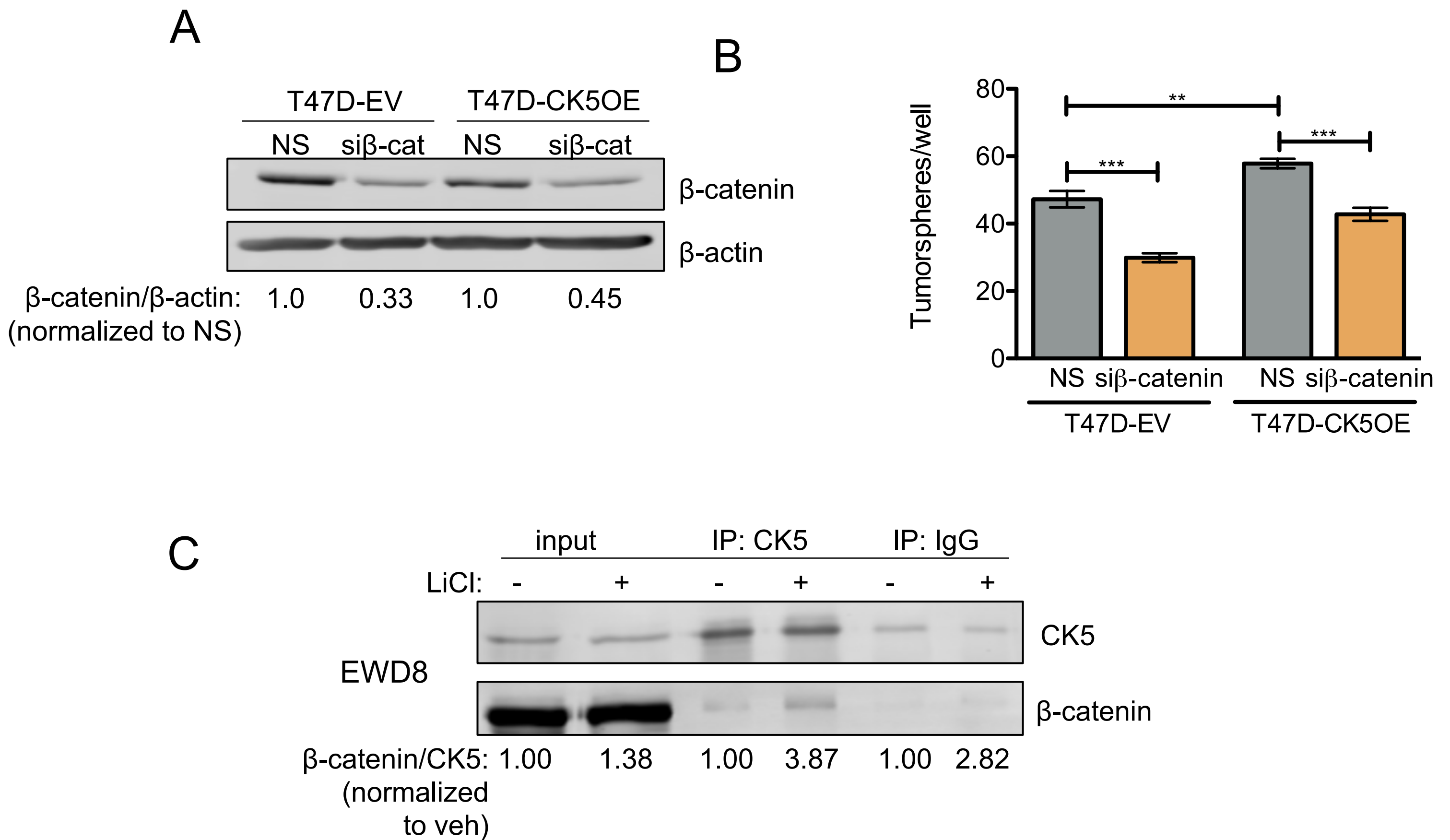
Supplemental Figure 2. A. ICC staining of CK5 (green) and DAPI (blue) shows abundant CK5 in EWD8 cells. **B.** Sequence coverage obtained for CK5 in EWD8 IP-MS experiment was generated by Scaffold. **C.** Table shows average spectral counts of keratins and desmosomal components pulled down in EWD8 IP-MS experiment. Fold change of CK5 spectral counts over IgG spectral counts are shown. **D.** Co-IP was performed in untreated EWD8 cells using a CK5 antibody and IgG negative control and was analyzed by immunoblot for CK17 expression to confirm the interaction between CK5 and CK17. **E.** Table shows Wnt/ β -catenin pathway genes that are differentially expressed in EWD8 cells compared to parental T47D cells.



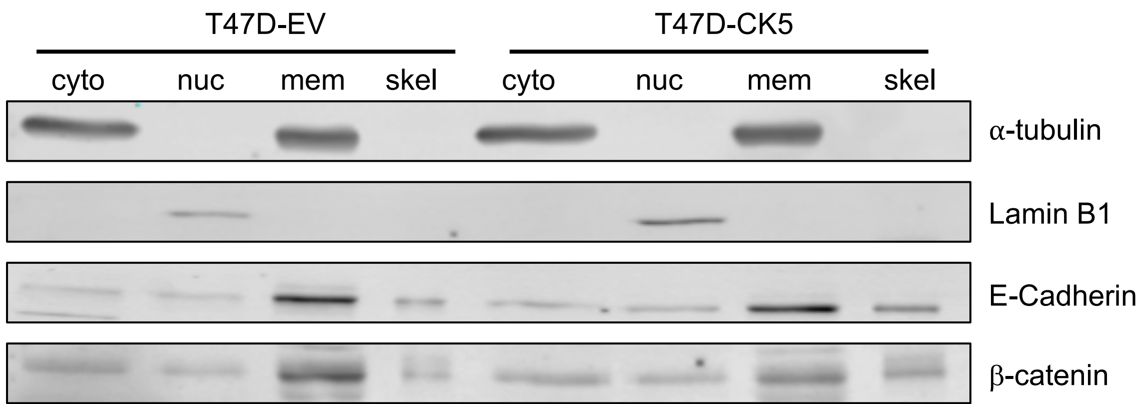


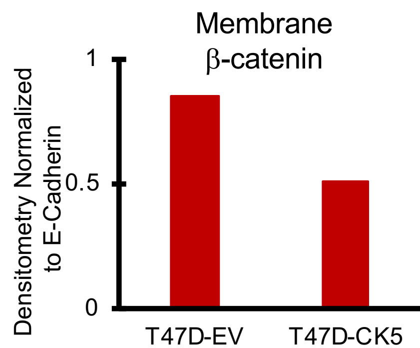


Supplemental Figure 3. Full blots are shown for co-IP experiments from Figure 2E for EWD8 (**A**), UCD46 (**B**), and MDA-MB-468 (**C**). Red asterisks indicate the lanes used in Figure 2E.



Supplemental Figure 4. A. β-catenin knockdown was performed in T47D-EV and CK5OE cells using 10nM siRNA for 48h. Knockdown was validated by immunoblot for β-catenin by comparing to nonsilencing siRNA transfected control (NS). Experiment was repeated twice. **B.** β-catenin knockdown was performed as described. 48h after transfection, cells were trypsinized, counted and re-plated into a tumorsphere formation assay. Tumorsphere assay was repeated 3 times, error bars represent SEM, ANOVA/Tukey was used to determine statistical significance. ***P*<0.01, ****P*<0.001. **C.** EWD8 cells were treated with 50mM LiCl for 24h, lysates were harvested, and a co-IP was performed with CK5 and IgG antibodies and analyzed by immunoblot for CK5 and β-catenin pull-down. Co-IP was repeated twice.





Supplemental Figure 5. Subcellular fractionation was performed in T47D-EV and T47D-CK5 cells. SDS-PAGE was performed with cytoplasmic (cyto), nuclear (nuc), membrane (mem), and cytoskeletal (skel) fractions and an immunoblot was performed with antibodies against alpha-tubulin (cytoplasmic control), Lamin B1 (nuclear control), E-cadherin (membrane control), and β-catenin. Membrane β-catenin was quantified using densitometry and normalizing to E-cadherin.

ER, PR, and CK5 status in cell line and PDX models				
	Model	**ER**	**PR**	**CK5**
Cell Lines	T47D	+	+	– (+ with P4[#])
	MCF7	+	+ with E2[&]	– (+ with P4[#])
	ZR75-1	+	+ with E2[&]	– (+ with P4[#])
	EWD8	–	–	+
	MDA-MB-468	–	–	+
PDX models	UCD46	+	–	high
	UCD15	+	–	low

[&]E2 refers to 17β-estradiol treatment
[#]P4 refers to progesterone or R5020 treatment

Supplemental Figure 6. Table outlines ER, PR, and CK5 expression in the cell lines and PDX models used in the study.