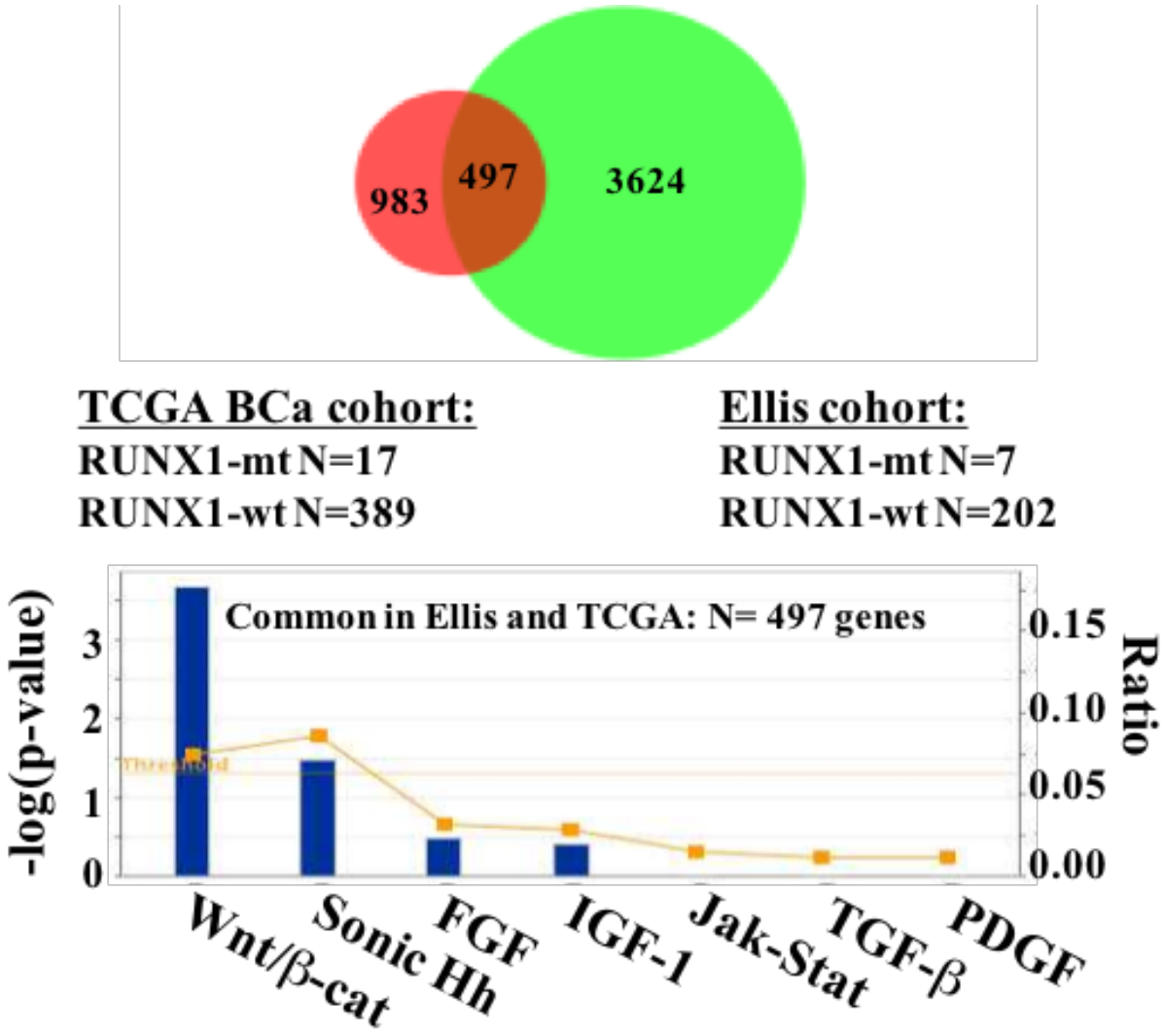
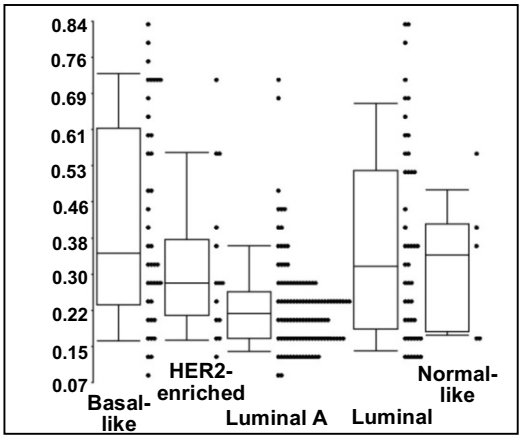


Supplementary Fig. 1. Annotations of genes differentially expressed in breast cancer tumors with *RUNX1* mutations in two independent cohorts suggest deregulation of Wnt/β-catenin signaling.
Genes differentially expressed in ER^+ tumors with wild-type versus mutant *RUNX1* in the breast cancer cohorts of both TCGA (https://tcga-data.nci.nih.gov/tcga/) and Ellis et al. (PMID: 22722193), listed in Supplemental Data 3, were interrogated by IPA™ for annotations related to major developmental signaling pathways. Line graph represent fold enrichment and bars represent statistical significance calculated by Fisher's exact test as implemented in the IPA™ software.



Supplementary Fig. 2. *RUNX1* methylation across breast cancer subtypes
RUNX1 methylation status in the five major breast cancer subtypes was assessed based on data for probe cg01265860 retrieved from the breast invasive carcinoma cohort of TCGA (https://tcga-data.nci.nih.gov/tcga/). Methylation levels differ significantly between subtypes (p=1.96e-08 by ANOVA).
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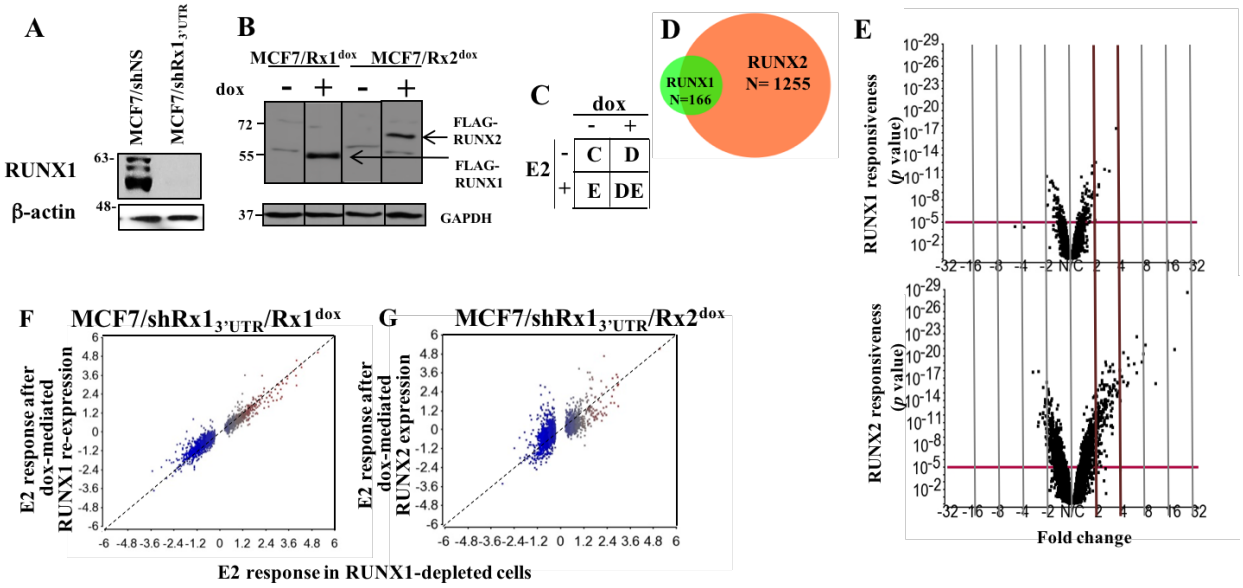
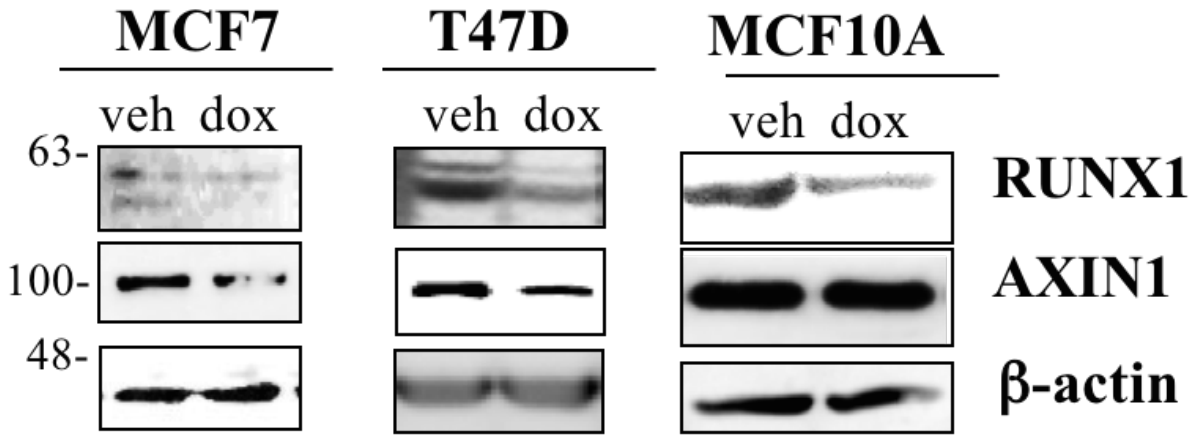
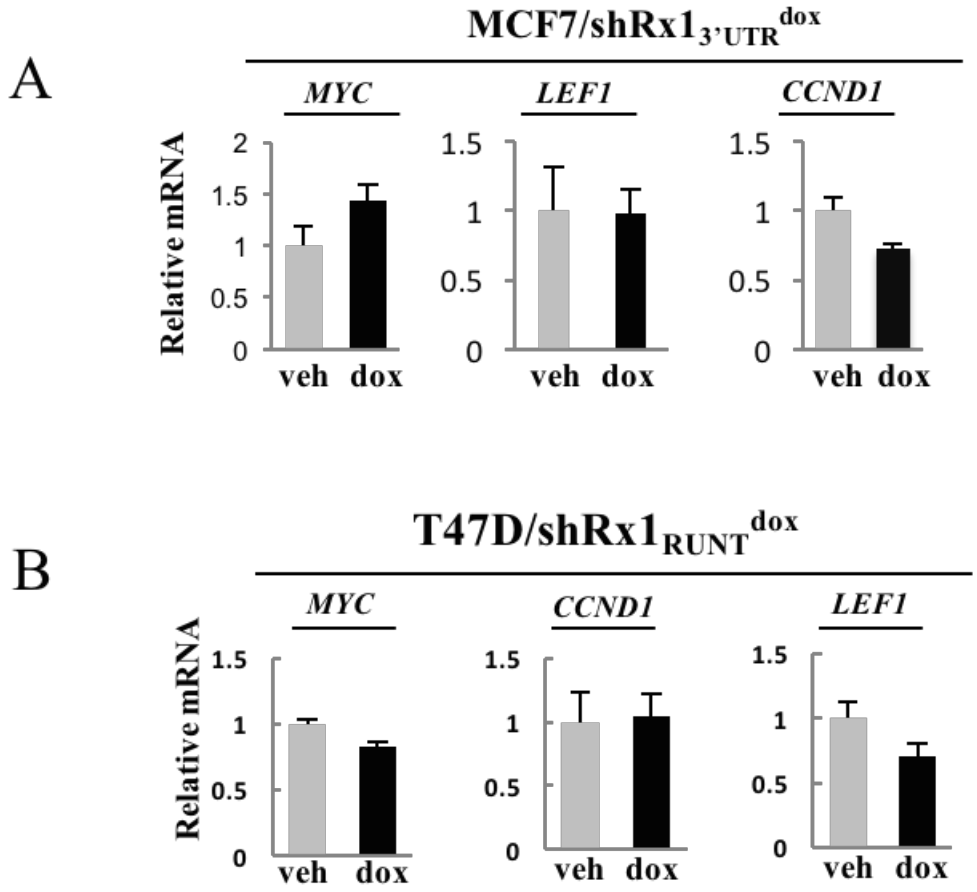


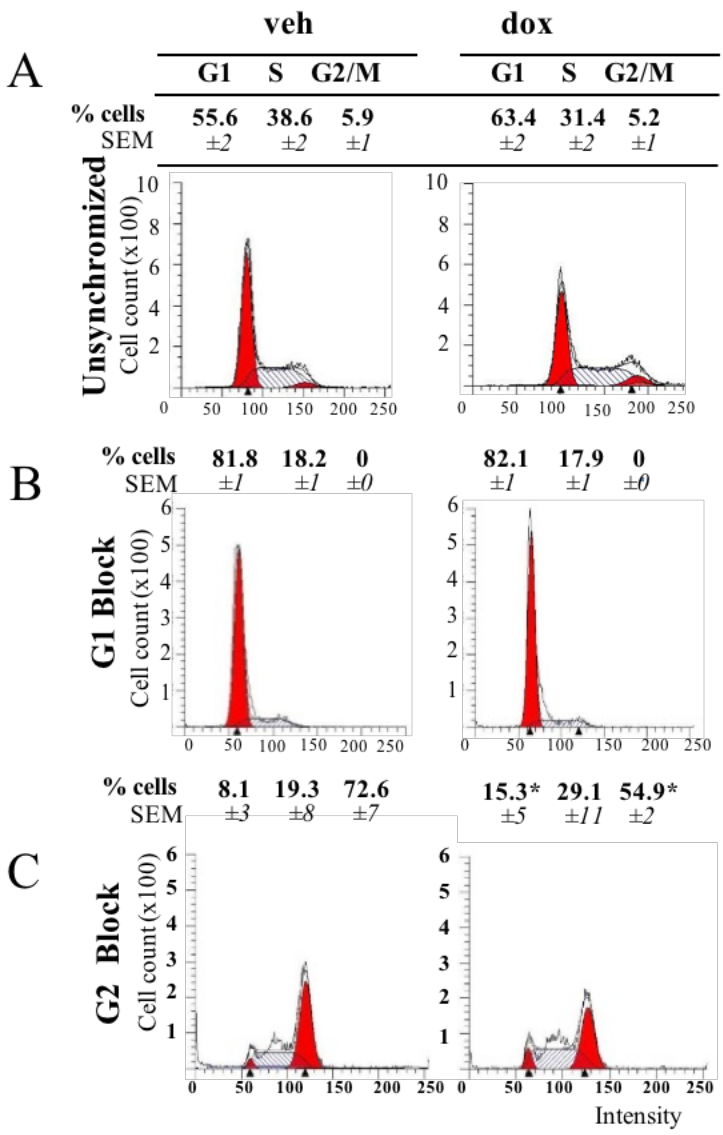
Supplementary Fig. 3. RUNX1 vs. RUNX2 transcriptome in MCF7 cells.
MCF7 cells were engineered to silence RUNX1 constitutively and express FLAG-RUNX1 or FLAG-RUNX2 in response to dox.
A-B. Western blot analysis demonstrating effective silencing of RUNX1 by constitutive expression of shRx1$_{3'UTR}$ versus a non-specific (*NS*) shRNA. Dox treatment for 48h induced expression of FLAG-RUNX1 and FLAG-RUNX2 in MCF7/shRx1$_{3'UTR}$/Rx1^{dox} and MCF7/shRx1$_{3'UTR}$/Rx2^{dox} cells, respectively. **C-E.** Cells were kept in CSS for 2 days and treated with dox and/or E2 for 48 h as indicated (C) and global changes in mRNA expression was profiled in triplicate using the HumanHT-12 v4 BeadChip (Illumina). Venn Diagram (D) and volcano plots (E) demonstrate far more robust response to RUNX2 as compared to RUNX1.
F-G. Scatter plots for the E2-responsive genes (fold-change >1.2-fold, FDR<0.05 in both the MCF7/shRx1$_{3'UTR}$/Rx1^{dox} and MCF7/shRx1$_{3'UTR}$/Rx2^{dox} cells) demonstrate strong attenuation of the global E2 response by RUNX2 (slope=0.6624, r=0.7223), but not by RUNX1 (slope=0.8865, r=0.9635).



Supplementary Fig. 4. MCF7, T47D (ER^+) and MCF10A (ER^-) cells were engineered to express $shRx1_{RUNT}$ upon doxycycline treatment. Cells were treated with 250 ng/ml dox for 4 days and whole cell extracts were subjected to western blot analysis of the indicated proteins.

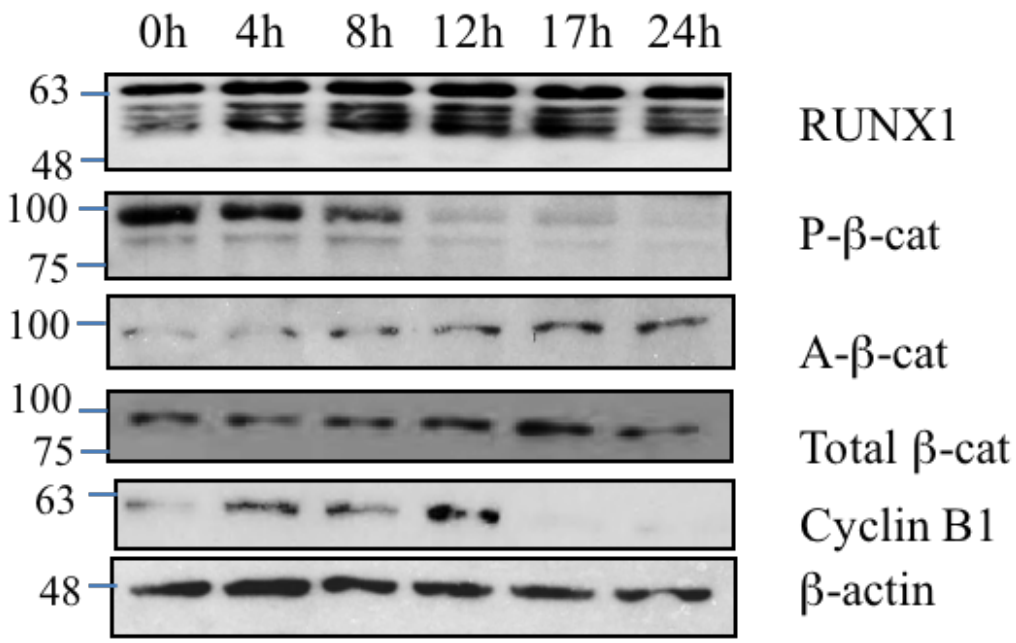


Supplementary Fig. 5. MCF7/shRx1$_{3'UTR}^{dox}$ (A) and T47D/shRx1$_{RUNT}^{dox}$ (B) cells were treated with 250 ng/ml dox for 48h and total RNA was subjected to RT-qPCR analysis of the indicated mRNAs. Data was corrected for 18S RNA and represent mean±SEM from triplicate experiments.

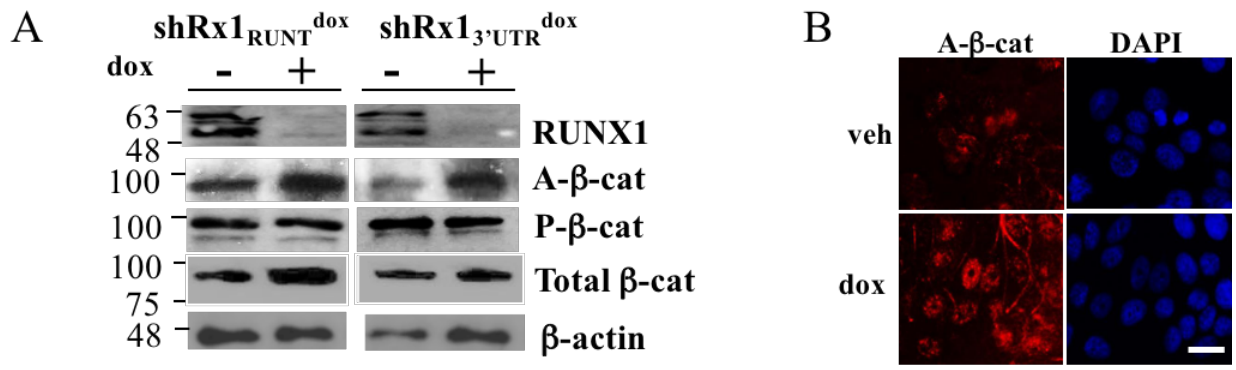


Supplementary Fig. 6: Compromised G2/M arrest, but not G1/S arrest in MCF7/shRx1$_{RUNT}^{dox}$ cultures.

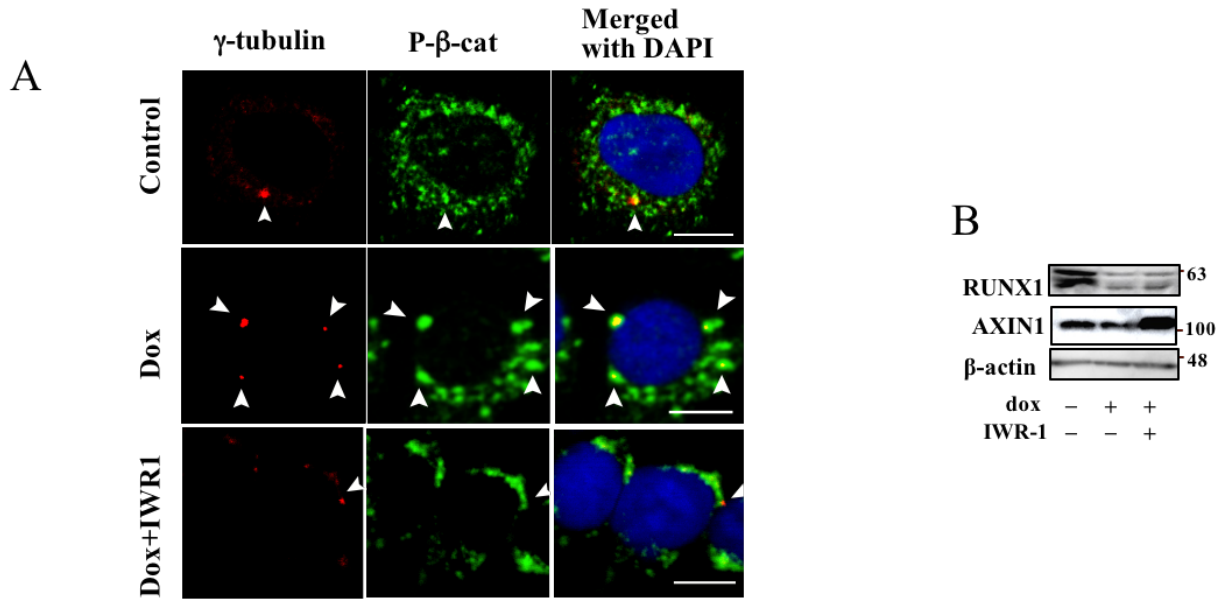
MCF7/shRx1$_{RUNT}^{dox}$ cells were treated with dox and synchronized in order to investigate effects of RUNX1 silencing on P-b-cat as described in Figure 6H. Cells were subjected to a double thymidine block to induce a G1 block (B), which was followed by nocodazole treatment to induce a G2 block (C). The synchronized (B, C) and control cells (A) were subjected to propidium iodide staining and flow cytometry analysis to determine the percentage of cells in G1, S and G2/M. Data are Mean ± SEM from three independent experiments, with a representative cell cycle profile shown for each condition. *p<0.05 by t-test.



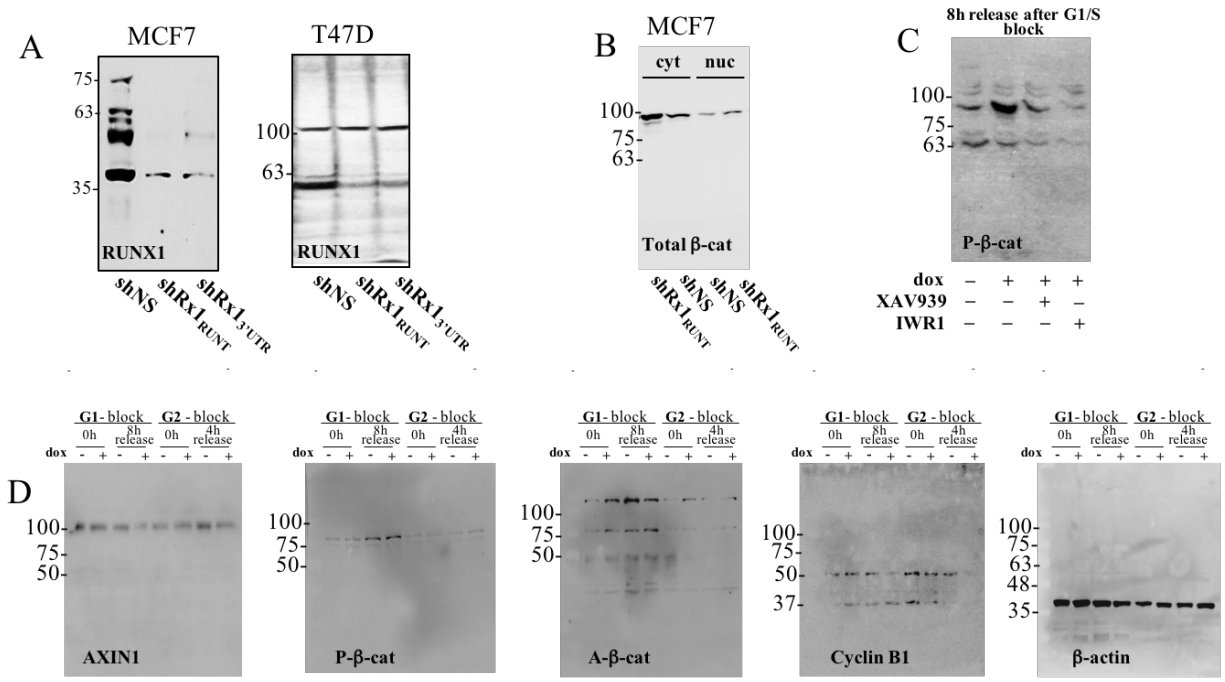
Supplementary Fig. 7. Cell cycle-dependent P-β-catenin expression in MCF7 cells
Western blot analysis of the indicated proteins at the indicated time points after release of MCF7 cells from a double thymidine block.



Supplementary Fig. 8. ***A*****,** MCF7 cells conditionally expressing either shRx1$_{RUNT}^{dox}$ (*left*) or shRx1$_{3'UTR}^{dox}$ (*right*) were treated with 250 ng/ml dox for 4 days followed by western analysis of the indicated proteins. ***B*****,** Immunofluorescence analysis of A-β-cat in MCF7/shRx1$_{RUNT}^{dox}$ cells treated with dox for 48h. Scale bar = 50 μm



Supplementary Fig. 9. **Immunofluorescence imaging of P-β-cat in MCF7 cells**
MCF7/shRx1$_{RUNT}^{dox}$ cells were treated with dox for 48 hours to silence *RUNX1* and the medium was supplemented with 5μM IWR1 for the last 36h. ***A***, Centrosomal P-β-cat was identified based on co-localization with γ-tubulin. Immunofluorescence microscopy was carried out essentially as described previously (Liang et al. 2008, PMID:18552835). Cells grown on coverslips were fixed in methanol at -20C° and permeabilized with 0.5% Triton X-100 in PBS for 5 min. Primary antibody for P-β-cat (Cell Signaling Technology #9561) was used at a 1:200 dilution and anti-γ-tubulin antibody (C7604, Sigma-Aldrich) was used at a 1:200 dilution. Confocal images were acquired using a Nikon Eclipse C1 laser-scanning microscope (Nikon, PA) fitted with a 60×Nikon objective (PL APO, 1.4NA) and a Nikon image software. Scale bar = 10 μm. ***B***, Western blot analysis of the cell lysates from parallel cultures.



Supplementary Fig. 10. Uncropped scans of key western blots. (A) RUNX1 knockdown in MCF7 and T47D cells expressing a non-specific short hairpins RNA (shNS) or shRNAs targeting the RUNT domain of RUNX1 (shRx1RUNT) or its 3'UTR (shRx13'UTR). (B). Increased total b-catenin levels in nuclear and cytoplasmic protein extracts from RUNX1-depleted versus control MCF7 cells. (C) AXIN stabilizers reverse effect of RUNX1 depletion on P-β-cat in late S-phase. (D). Effect of RUNX1 knockdown on expression of the indicated proteins in the indicated phases of the cell cycle.

Supplementary Table 1. Associations between RUNX1 and AXIN1 status, controlling for ER intensity (p-values based on the Pearson chi-square test, for the 2x2 table)

ER low		
RUNX1 Status	AXIN1 Status	
	Negative	Positive
Negative	2	7
Positive	7	6
Odds Ratio = 0.24 95% CI: (0.036, 1.66) p=0.15		

ER high		
RUNX1 Status	AXIN1 Status	
	Negative	Positive
Negative	2	1
Positive	0	6
Odds Ratio = 21.7 95% CI: (0.64, 730.0) p=0.033		

p-value for comparing the 2 odds ratio's (0.24 and 21.7) is p=0.006 – suggesting that the association between RUNX1 and AXIN1 is different, depending on the intensity of ER expression

Supplementary Table 2 Sequences of oligonucleotides used in this study

For dox-inducible pSLIK-RUNX1 vector	
F-Flag-mRx1	GACTACAAAGACGATGACGACAAGCGTATCCCCGTAGATGCCAG
R-mRx1_MfeI	CTCAGCCAATTGTCAGTAGGGCCGCCACACGGCCTCC
For dox-inducible pSLIK shRUNX1 targeting the RUNT domain	
T_h_Rx1_Runt	AGCGACCTCGAAGACATCGGCAGAAATAGTGAAGCCACAGATGTATTTCTGCCGATGTCTTCGAGG
B_h_Rx1_Runt	ggcaCCTCGAAGACATCGGCAGAAATACATCTGTGGCTTCACTATTTCTGCCGATGTCTTCGAGGT
For dox-inducible pSLIK shRUNX1 targeting the 3'UTR region	
T_h_Rx1_3UTR	AGCGCCGCTTGGTTATCCAGAAGTATTAGTGAAGCCACAGATGTAATACTTCTGGATAACCAAGCGA
B_h_Rx1_3UTR	ggcaTCGCTTGGTTATCCAGAAGTATTACATCTGTGGCTTCACTAATACTTCTGGATAACCAAGCGG

Primer sequences for RT-qPCR		
Gene	Forward primer	Reverse primer
GAPDH	F: 5' -AGCCACATCGCTCAGACAC- 3'	R: 5' -GCCCAATACGACCAAATCC- 3'
18S	F: 5' -GTAACCCGTTGAACCCCATT-3'	R: 5' -CCATCCAATCGGTAGTAGCG- 3'
AXIN1	F: 5' -CAAGCAGAGGTATGTGCAGGA- 3'	R: 5' -CACAACGATGCTGTCACACG- 3'
AXIN2	F: 5' -AAACGCAATGGGAAAGGCAC- 3'	R: 5' -TGTGCTTTGGGCACTATGGG- 3'
CCND1	F: 5' -CTCCTGTGCTGCGAAGTGG- 3'	R: 5' -CTTCTGTTCCTCGCAGACCTCC- 3'
LEF1	F: 5' -ATCACACCCGTCACACATCC	R: 5' -TGGGAAAACCTGGACATGGA
c-Myc	F: 5' -AGAGAAGCTGGCCTCCTACC- 3'	R: 5' -CGTCGAGGAGAGCAGAGAAT- 3'
SOX2	F: 5' -TCAGGAGTTGTCAAGGCAGAG- 3'	R: 5' -AGAGGCAAACTGGAATCAGGA- 3'
NANOG	F: 5' -ACCTCAGCTACAAACAGGTGA- 3'	R: 5' -CTTCTGCGTCACACCATTGC- 3'
ALDH1A3	F: 5' -TGGCACGAATCCAAGAGTGG- 3'	R: 5' -TTGTCCACGTCGGGCTTATC- 3'
CD44	F: 5' -TCCCTGGATCACCGACAGCACA- 3'	R: 5' -CCTCTTGGTTGCTGTCTCAGTTGCT- 3'
Axin1 (mouse)	F: 5' -TGTCCAGTGATGCTGACACG-3'	R: 5' -AAGTGCGAGGAATGTGAGGTA- 3'

Primer sequences for ChIP-qPCR		
Gene region	Forward primer	Reverse primer
AXIN1	F: 5' -CAGGTGATTTCTCGGTCACTC- 3'	R: 5' -AGGCCTCCTAGGCACAG- 3'
Negative	F: 5' -ATGGTTGCCACTGGGGATCT- 3'	R: 5' -TGCCAAAGCCTAGGGGAAGA- 3'