Figure S1. Efficiency of cell transfection. (A and B) The efficiency of transfection was detected by western blot when the MDA-MB-231 cells were transfected with (A) DN-Akt, CA-Akt, and (B) $RSK2^{S19A}$ and $RSK2^{S19E}$. (C and D) Reverse transcription-quantitative PCR was used to detect the efficiency of (C) siRNA-Akt and (D) siRNA-RSK2. $^{****}P<0.0001$. RSK2, ribosomal S6 kinase 2; $RSK2^{S19A}$, RSK2 Ser19Ala; $RSK2^{S19E}$, RSK2 Ser19Glu; p-, phosphorylated; CA, constitutively activated; DN, dominant negative, NC, negative control; t, total; si, small interfering.

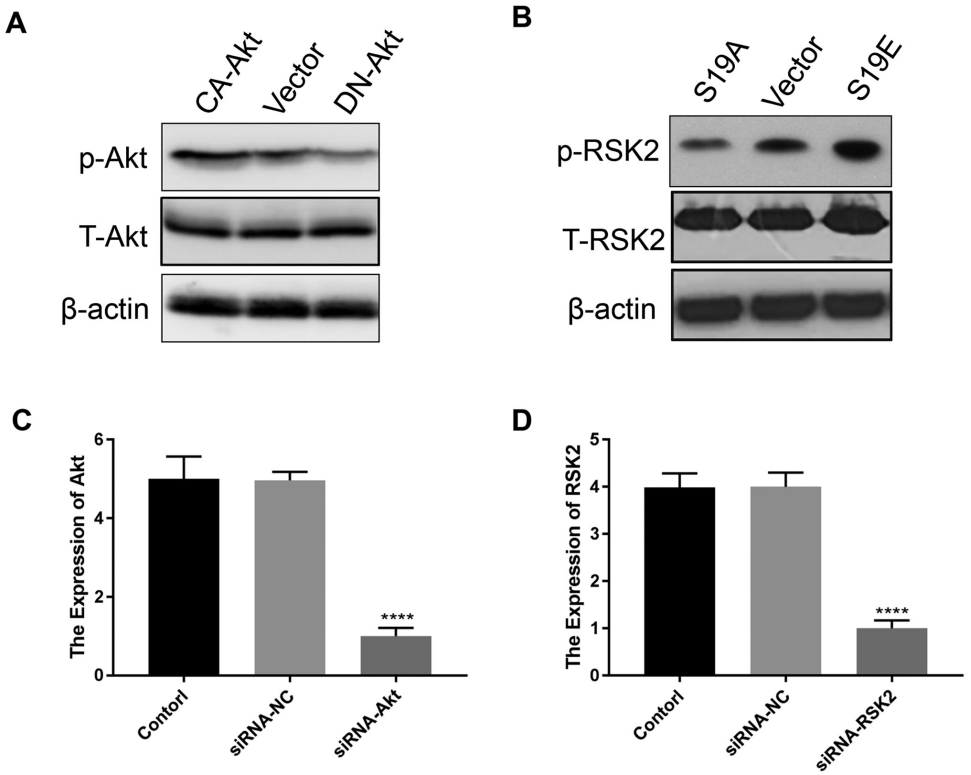

Figure S2. H2A.X S16ph regulator is not affected by Akt. MDA-MB-231 cells were treated with IGF for 1 h. Cell lysates were analyzed by western blotting with the indicated antibodies. RSK2, ribosomal S6 kinase 2; H2A.X, histone H2AX; H2A.X S16ph, H2A.X phosphorylated at the Ser16 site; p-, phosphorylated; IGF, insulin-like growth factor; t, total.

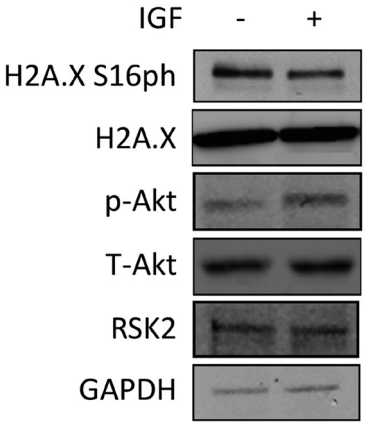

Figure S3. Specificity of the p-RSK2 antibody generated in-house. (A and B) The cells lysates of (A) MDA-MB-231 and (B) Dominant negative-Akt stable transfected MDA-MB-231 cells were analyzed by western blotting with a p-RSK2 antibody (1:1,000). (C and D) The peptide competition assay was performed to determine the specificity of the anti-RSK2-S19ph antibody. The anti-RSK2-S19ph antibody was pre-incubated with (C) phospho-RSK2 or (D) non-phospho-RSK2 peptides. Total protein of MDA-MB-231 cells was measured by western blot using anti-RSK2-S19ph antibody and anti-RSK2 antibody. RSK2, ribosomal S6 kinase 2; p-, phosphorylated.

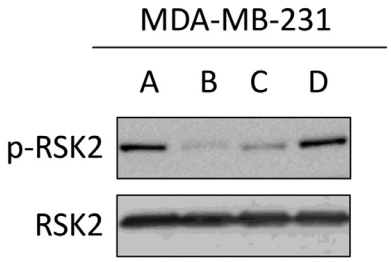

Figure S4. P-RSK2 is associated with Akt activity. RSK2 immunoprecipitates were subjected to western blot analysis to examine the Ser19 phosphorylation of RSK2. Western blotting was performed to detect the association between p-RSK2 and p-Akt. RSK2, ribosomal S6 kinase 2; p-, phosphorylated; WB, western blotting; t, total.

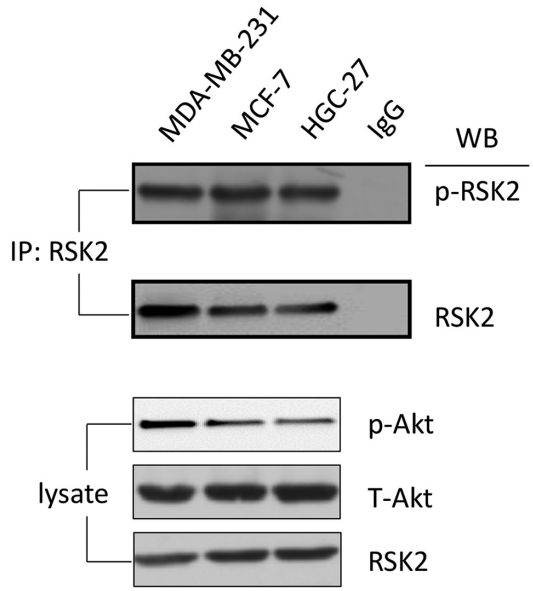

Figure S5. Transcriptional level analysis of Akt pathway target genes. (A) The transcription of several genes in MDA-MB-231 cells was confirmed by reverse transcription-quantitative PCR. The values are presented as the mean ± standard deviation percentage relative to GAPDH expression. (B) As detected by ChIP, increased levels of H2A.X S16ph are present on the differentially expressed genes. ERK1 was used as a negative control. The values are presented as the mean ± standard deviation of 3 independent experiments. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001 vs. RSK2 WT. RSK2, ribosomal S6 kinase 2; S19A, Ser19Ala; WT, wild type; FOXN2, Forkhead box N2; CDKN2A, Cyclin-dependent Kinase Inhibitor 2 A; PUMA, p53 upregulated modulator of apoptosis; Dram1, DNA damage-regulated autophagy modulator protein 1; ING3, inhibitors of growth family member 3; PSAT-1, phosphoserine aminotransferase 1; RTK6, Receptor tyrosine kinase 6.