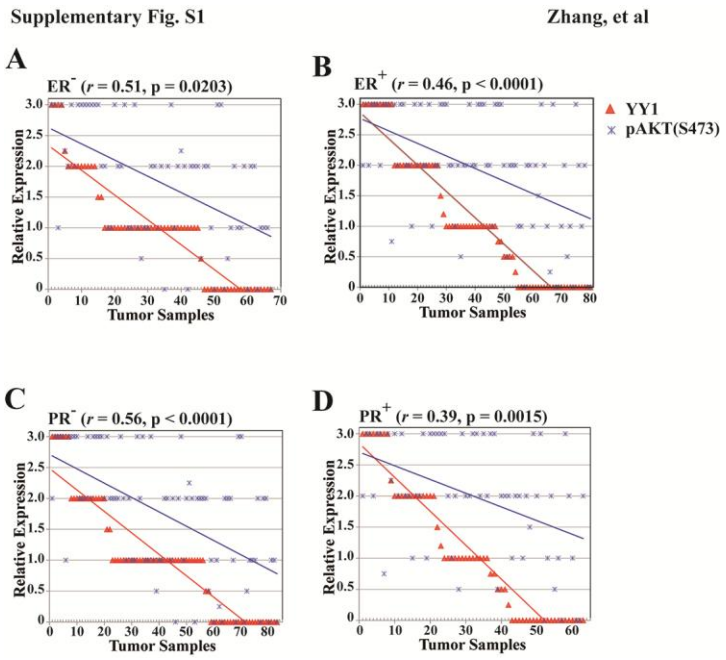
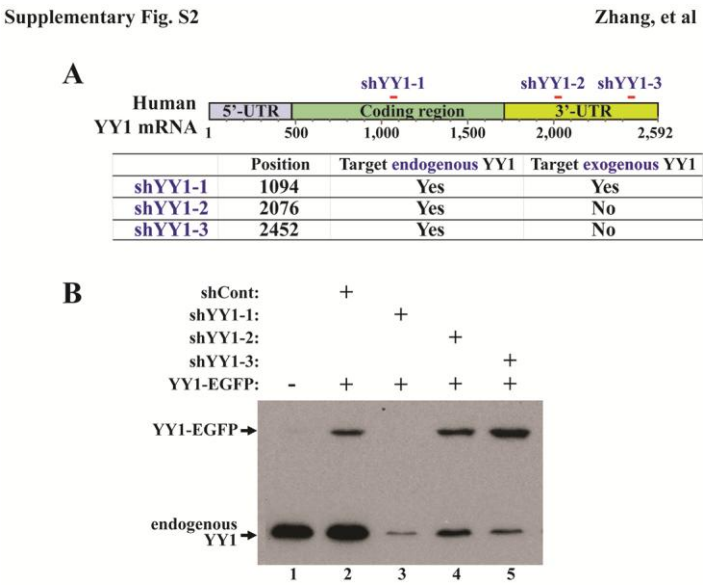


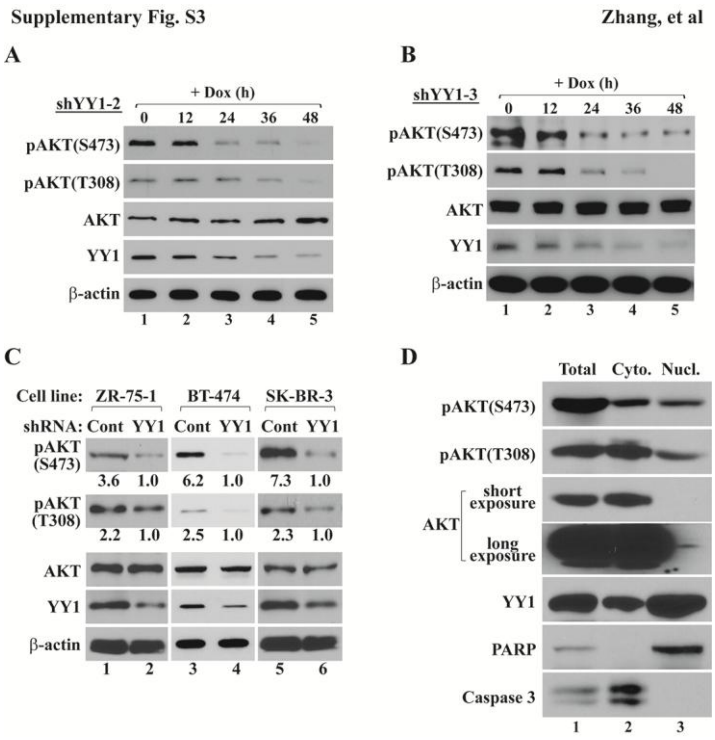
Supplementary Figure S1. Correlations between YY1 levels and AKT phosphorylation in breast cancer tissues with different estrogen receptor (ER) and progesterone receptor (PR) statuses.

(**A**) and (**B**) Correlations between YY1 and pAKT(S473) levels in samples from 67 ER− (**A**) and 80 ER+ (**B**) breast cancer patients. (**C**) and (**D**) Correlations between YY1 and pAKT(S473) levels in samples from 83 PR− (**C**) and 63 PR+ (**D**) breast cancer patients.



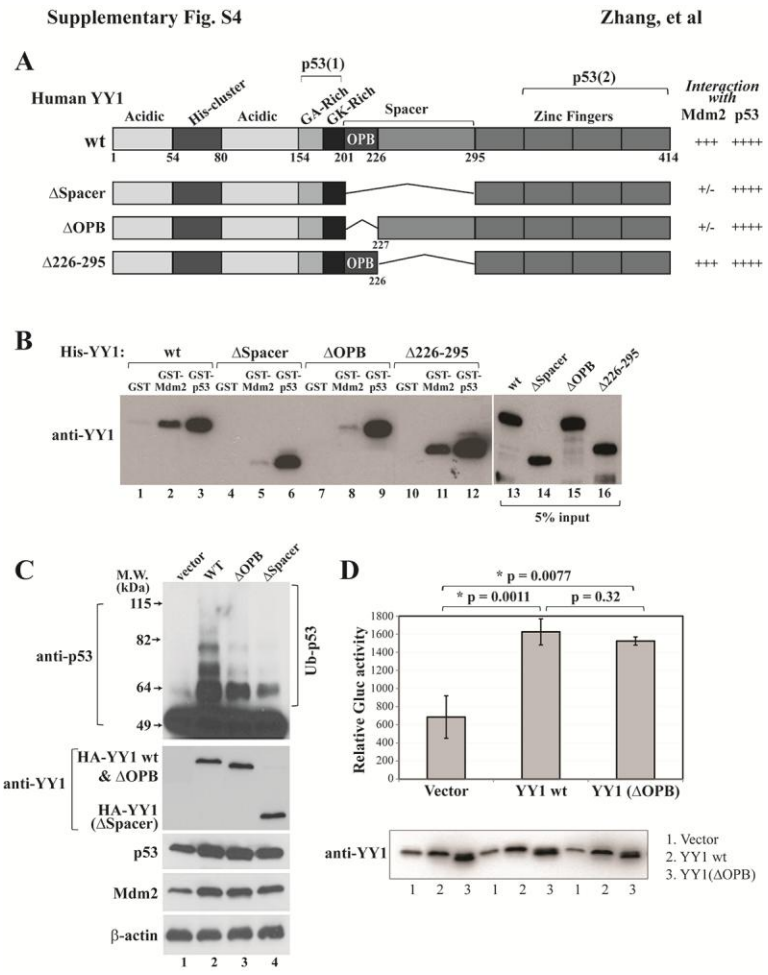
Supplementary Figure S2. Validation of YY1 shRNAs for their specificity to endogenous and/or ectopic YY1.

(**A**) The human YY1 mRNA and the positions of three shYY1s. With the first nucleotide designated as 1, the target site of shYY1-1 is in the coding region of nucleotide 1094 and the target sites of shYY1-2 and -3 are in the 3'-UTR of nucleotide 2076 and 2452, respectively. Their activity in targeting endogenous YY1 and exogenous YY1 (with the coding region expressed as an EGFP fusion protein) is indicated in the table. (**B**) Effects of YY1 shRNAs on endogenous YY1 and transfected YY1-EGFP in MDA-MB-231 cells.



Supplementary Figure S3. Effects of YY1 knockdown by different shRNAs and in various breast cancer cell lines, and the expression of YY1 and phosphorylated AKT in fractionated portions of MDA-MB-231 cells.

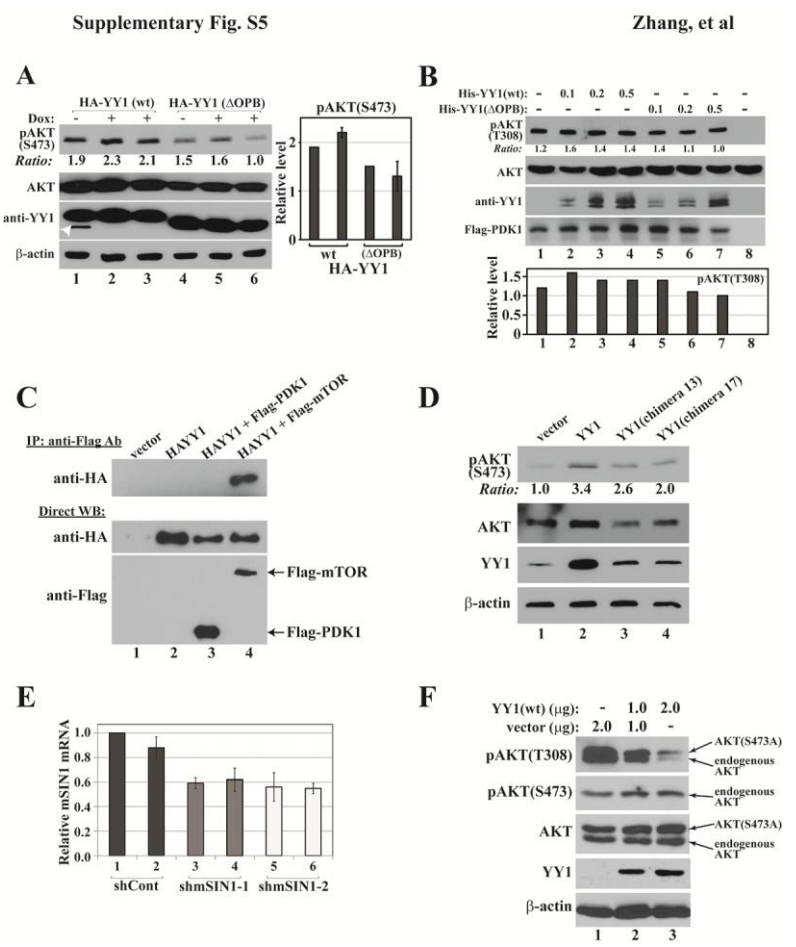
(**A**) and (**B**) Effects of YY1 knockdown by shYY1-2 (**A**) and shYY1-3 (**B**) on pAKT(S473) and pAKT(T308) in MDA-MB-231 cells. (**C**) Effects of YY1 knockdown by shYY1-1 on pAKT(S473) and pAKT(T308) in breast cancer ZR-75-1, BT-474 and SK-BR-3 cells. (**D**) Expression of pAKT(S473), pAKT(T308), total AKT and YY1 in cytoplasm (cyto.) and nucleus (nucl.) of fractionated MDA-MB-231 cells. To fractionate the cells, we used lysis Buffer A (20 mM HEPES, pH 8.0, 20% glycerol, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, pH 8.0, 1 mM DTT, 0.1% NP-40 and a proteinase inhibitor cocktail) to treat the cells for 10 min on ice, followed by centrifugation at 400 g for 5 min at 4℃. The supernatants were collected as the cytoplasmic fractions. The pellets were washed 3 times with Buffer A and then lysed in Buffer B (20 mM HEPES, pH 8.0, 20% glycerol, 500 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, pH 8.0, 1 mM DTT, 0.1% NP-40 and a proteinase inhibitor cocktail) for 30 min on ice, followed by centrifugation at 15,000 g at 4℃ for 15 min. The supernatants were collected as the nuclear fractions.



Supplementary Figure S4. The OPB domain (G201-S226) of YY1 is essential to its interaction with Mdm2 but dispensable in YY1-mediated Cdc6 expression.

(**A**) The domain structure of wt YY1 and the three mutants. The two p53 binding sites are indicated on the top. The domain mapping results in **B** are summarized in the right panel. "+/-" means very weak interaction. (**B**) *In vitro* protein binding studies of His-YY1 wt and its mutants ΔSpacer, ΔOPB and Δ226-295 with GST-Mdm2 and GST-p53. The data indicate that the binding site of Mdm2 is among the residues 201-226 of YY1 (compare lanes 2, 5, 8 and 11); the spacer region (201-295) is not responsible for YY1 binding to p53 (compare lanes 3, 6, 9 and 12). (**C**) Effects of YY1 wt, ΔOPB and ΔSpacer on *in vitro* p53 ubiquitination. pCMV/p53 and pcDNA3/Hisx6-ubiquitin were co-transfected with pcDNA3, pcDNA3/YY1 (wt), pcDNA3/YY1

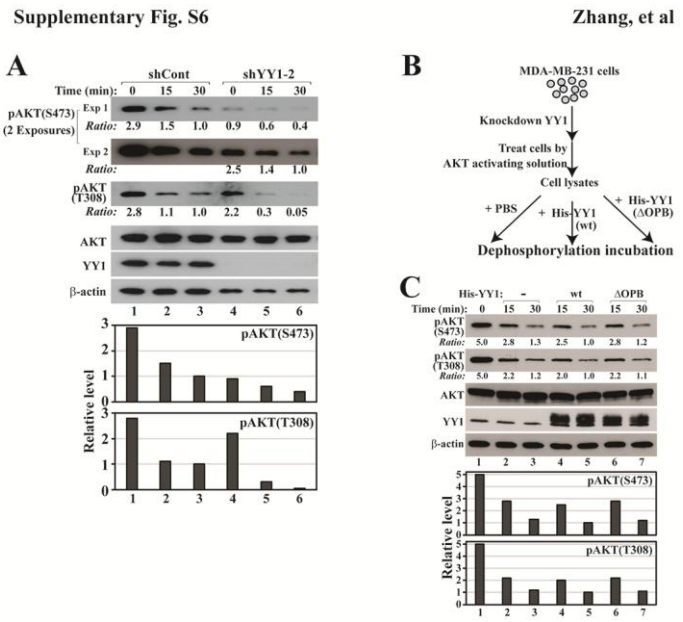
(ΔOPB) or pcDNA3/YY1 (ΔSpacer) into 293T cells. Cell lysates re-suspended in 6M guanidinium-HCl were incubated with Ni-NTA Agarose (Qiagen) for 3 h at 4 °C and then washed sequentially by 6M guanidinium-HCl and 8M Urea. The samples were analysed by Western blots using a p53 antibody (Do-1, Santa Cruz). The data indicate that deletion of the OPB domain or the Spacer domain abolished the activity of YY1 in promoting p53 ubiquitination, as we previously reported (Sui, *et al*. Cell. 2004; 117:859-872). (**D**) Reporter assays of the Cdc6-promoter activity affected by ectopic YY1 wt and YY1(ΔOPB). The experiments were carried out following our previously described procedure (Deng, *et al*, Mol Cell Biol. 2007; 27:3780-3792). Briefly, pcDNA3 vector, pcDNA3/YY1 (wt) and pcDNA3/YY1 (ΔOPB) were individually co-transfected with a reporter vector of the Cdc6 promoter-driven Gaussia luciferase (Gluc) and CMV-secreted alkaline phosphatase (SEAP) into MCF-7 cells in triplicates. The Gluc activity detected by its substrate Coelenterazine was presented after normalized by the SEAP activity detected by p-nitrophenyl phosphate (PNPP). The data indicate that deletion of the OPB domain did not significantly affect the transcriptional activity of YY1. Representative Western blots of these samples using an YY1 antibody (H-10) are shown in the lower panel. The experiments were repeated three times with similar results.



Supplementary Figure S5. Effect of YY1 wt and mutants on AKT phosphorylation.

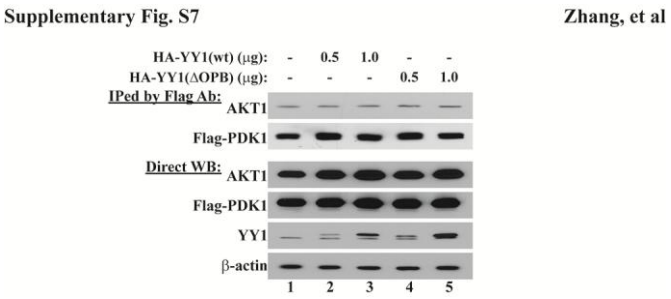
(**A**) Effects of YY1 wt and YY1(ΔOPB) on AKT phosphorylation. MDA-MB-231 cells with Dox-inducible shYY1-3 were infected by lentiviruses generated from pSL5-HA-YY1 wt or YY1(ΔOPB), and cultured in the absence or presence of Dox, as indicated. (**B**) *In vitro* phosphorylation assay to determine the effects of His-YY1 wt and ΔOPB on PDK1-mediated AKT phosphorylation at T308. Flag-PDK1 expression plasmid was transfected into HeLa cells. Two days post transfection, Flag-PDK1 was immunoprecipitated (IP) by anti-Flag antibody-conjugated agarose and used in *in vitro* assays for AKT(T308) phosphorylation with purified recombinant GST-AKT and different amounts of recombinant YY1(wt) and YY1(ΔOPB). The data indicate that YY1 has no effect on PDK1-mediated AKT(T308) phosphorylation. (**C**) Co-IP studies to test the interaction of YY1-mTOR and YY1-PDK1. pcDNA3/HA-YY1 was co-transfected with Flag-PDK1 or Flag-mTOR expression vector, with indicated empty vector co-

transfection as controls. Two days post transfection, the cell lysates were IPed by anti-Flag antibody-conjugated agarose followed by Western blots using a HA antibody. Direct Western blot analyses were carried out using HA and Flag antibodies to detect HA-YY1, Flag-PDK1 and Flag-mTOR, respectively. (**D**) Effects of ectopically expressed YY1 wt, zinc finger chimera 13 and chimera 17 on pAKT(S473). 184B5 cells were individually infected by lentiviruses carrying pSL2 empty vector, pSL2/YY1(wt), pSL2/YY1(chimera 13) and pSL2/YY1(chimera 17). Three days post infection, cell lysates were analysed by indicated antibodies. (**E**) Real-time PCR to determine the silencing of shmSIN1-1 and shmSIN1-2. 184B5 cells were individually infected by lentiviruses expressing a control shRNA, shmSIN1-1 and shmSIN1-2. Two days post infection, the cells were selected by 1.5 mg/ml puromycin in the culture medium. Three days post the selection, one portion of each shRNA-treated cells was collected for Real-Tome PCR analyses to quantify mSIN1 mRNA levels. Primers for this quantitation are AGT ACT TTG GCC CTG GTT GA and GAC ATT GGG CTC GCT CTG CTT CT. Another portion of each shRNA-treated cells was infected by lentivirus of pSL2 vector or pSL2/YY1. After 2 days, the cell lysates were tested by Western blot analyses shown in Figure 4C. (**F**) Effects of YY1 on T308 phosphorylation of AKT(S473A) mutant. Different amounts of empty vector or YY1 expressing plasmid as indicated were co-transfected with AKT(S473A) into MCF-7 cells. Two days post transfection, cell lysates were collected for Western blot analyses to detect pAKT(T308), pAKT(S473), total AKT, YY1 and β-actin expression. Reduced pAKT(T308) signal for both endogenous AKT and AKT(S473A) was detected compared to the control, likely due to the competition of YY1 in binding to AKT PH domain against PIP3. On the other hand, ectopically expressed AKT(S473A) could also compete with endogenous AKT in binding to the OPB domain of YY1, and consequently attenuate YY1's stimulative effects on pAKT(S473), which in turn reduced pAKT(S308).



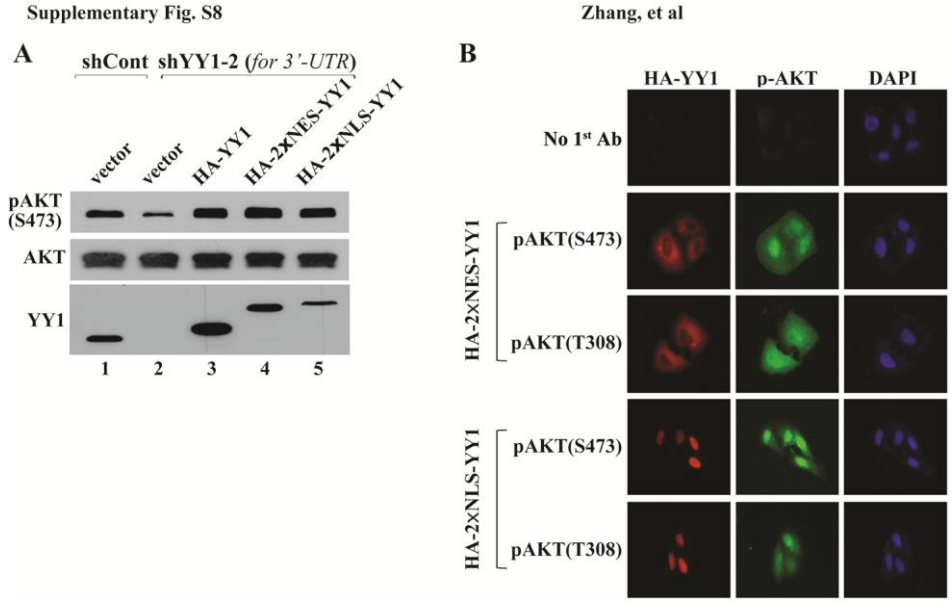
Supplementary Figure S6. Effect of YY1 wt and mutants on AKT dephosphorylation.

(**A**) Effects of YY1 knockdown on *in vitro* AKT dephosphorylation at S473 and T308. Cells were treated by an AKT phosphorylation solution (100 μM of both hydrogen peroxide and sodium orthovanadate) for 15 min to maximally activate AKT prior to collection. Cell lysates were then incubated at 30 ℃ for 15 and 30 min followed by Western blot analyses. In response to the AKT activating solution, the cells expressing shCont showed over 3-fold higher pAKT(S473) levels than the cells with silenced YY1, but this increase was not detected in the changes of pAKT(T308). This result reinforced the prediction above that YY1 directly regulates AKT phosphorylation at S473 but not T308. Interestingly, with YY1 knockdown, the dephosphorylation rate of pAKT(S473) was virtually unaffected, but pAKT(T308) dephosphorylation was accelerated. (**B**) and (**C**) Effects of His-YY1 wt and ΔOPB on AKT dephosphorylation at S473 and T308. The AKT activating solution was used to treat MDA-MB-231 cells with silenced endogenous YY1. Cell lysates were then supplied with PBS, purified His-YY1, or His-YY1(ΔOPB) prior to dephosphorylation incubation. In this experimental setting, dephosphorylation rates of phos-AKT at S473 or T308 were comparable among these three groups, suggesting that YY1 is not involved in AKT deactivation.



Supplementary Figure S7. YY1 does not affect PDK1-AKT interaction.

HA-YY1 wt and ΔOPB expressing plasmids were individually co-transfected with pcDNA3/Flag-PDK1 and HA-AKT. Two days post transfection, cell lysates were analysed by co-IP studies using Flag antibody-conjugated agarose beads and Western blots were carried out using the indicated antibodies.



Supplementary Figure S8. YY1 tagged by either NES or NLS promoted AKT phosphorylation at S473.

(**A**) MDA-MB-231 cells were infected by lentivirus expressing shCont or shYY1-2 and lentivirus carrying empty pSL2 vectors, pSL2/HA-YY1, pSL2/HA-2×NES-YY1 or pSL2/HA-2×NLS-YY1, as indicated. After 72 h, cell lysates were examined by Western blot analyses using YY1, pAKT(S473) and AKT antibodies. (**B**) Immunostaining by a HA antibody (for HA-YY1) to confirm the subcellular localization of HA-2×NES-YY1 and HA-2×NLS-YY1. To detect the signal of phosphorylated AKT, pAKT(S473) and pAKT(T308) antibodies were used as primary antibodies with DAPI to staining nuclei.