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Supplementary Data

Figure S1 ERα represses *de novo* synthesis of RelB. (a1) T47D cells were treated with 1 μM ICI 182,780 (ICI) or equivalent volume of vehicle ethanol (Eth) for 48 h and WCEs (25 μg) analyzed by immunoblotting for RelB, ERα, and β-actin. RelB levels normalized to β-actin in cells treated with ICI 182,780 are presented relative to cells treated with control ethanol. (a2) T47D cells were treated as above and mRNA (20 μg) subjected to Northern blot analysis using human *RELB* cDNA as probe. Ethidium bromide staining of the gel confirmed equal loading. (a3) T47D cells were treated as above, and nuclear extracts (5 μg) subjected to EMSA using oligonucleotides containing either NF-κB, AP-1 or Oct-1 elements, as probes. (a4) T47D cells were transiently transfected, in triplicate, with 0.5 μg NF-κB or AP-1 element driven constructs κB-Luc or TRE-CAT, respectively and 0.5 μg SV40-β-gal. Post-transfection (6 h), cells were treated with 1 μM ICI 182,780 or equivalent volume of vehicle ethanol for 40 h. Luciferase and CAT activities were determined and values normalized by β-gal activity presented as the mean ± S.D. from three separate experiments (with the control ethanol sample set to 1). (b) Stable Hs578T transfectants expressing either sense *RELB* (si-Con) or siRNA *RELB* (si-*RELB*) were prepared. (b1) RNA (20 μg) was subjected to Northern blot analysis using human *RELB* cDNA as probe. Ethidium bromide staining of the gel confirmed equal loading. (b2) Nuclear extracts (25 μg) were analyzed by immunoblotting for levels of RelB, p65 and Lamin B, as loading control. (c) WCEs were prepared from MDA-MB-231 cells transiently expressing sense *RELB* (si-Con) or siRNA *RELB* (si-*RELB*). Samples (50 μg) were analyzed by immunoblotting for E-cadherin, γ-catenin (γ-caten), Snail, fibronectin (FN), β-actin, and RelB.

Figure S2 RelB expression promotes mesenchymal phenotype via induction of Bcl-2. (a) Stable MCF-7 clones expressing either RELB or EV DNA were isolated: RELB(1), RELB(2), EV(1), and EV(2). Cells were transiently transfected, in triplicate, with 0.5 μg κB-Luc vector, and 0.5 μg SV40-β-gal. Post-transfection (48 h), luciferase and β-gal activities were determined and normalized values presented relative to EV transfection, which was set at 1 (mean ± S.D from three separate experiments). Inset: nuclear extracts (25 μg) from the cells, indicated below, were subjected to immunoblot analysis for RelB, p65, and Lamin B. (b) WCEs (25 μg) from MCF-7 RELB and control cells were analyzed by immunoblotting for cyclin D1, product of an NF-κB target gene, and for β-actin. (c) ZR-75 cells were transiently transfected, in triplicate, with 1.0 μg WT or CRE-Mutant P1 *BCL2* promoter-Luc vector, the indicated dose of p52 or RelB expression vectors, 0.5 μg SV40-β-gal and empty vector pcDNA3 (EV) to make a total of 3.5 μg DNA. Post-transfection (48 h), luciferase and β-gal activities were determined and normalized luciferase activities presented (mean ± S.D. from three separate experiments).

Figure S3 RelB reduces E-cadherin expression in MCF-7 cells. The levels of E-cadherin in stable MCF-7 RELB and control cells were determined by indirect immunofluorescence microscopy using mouse anti-E-cadherin (primary) and Alexa594 anti-mouse (secondary) antibodies. Nuclei were stained with DAPI. Fluorescence microscopy was performed using a Zeiss Axiovert 200M microscope. Individual and merged images are shown. Scale bar, 100 μm.

Figure S4 Bcl-2 reduces E-cadherin expression in MCF-7 cells. The levels of E-cadherin in MCF-7 stable cells with either EV or full length Bcl-2 DNA were determined by indirect

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immunofluorescence microscopy using mouse anti-E-cadherin (primary) and Alexa594 anti-mouse (secondary) antibodies, as in Figure S3. Scale bar, 100 μm.

Figure S5 Full scans of original Western blots and EMSA gel. (a) Full scans of immunoblots of ERα and RelB in Figure 1a top panel. ns, nonspecific band. (b) Full scans of AP-1 and NF-κB EMSA in Figure 1e. (c) Full scan of E-cadherin immunoblot in Figure 4i.

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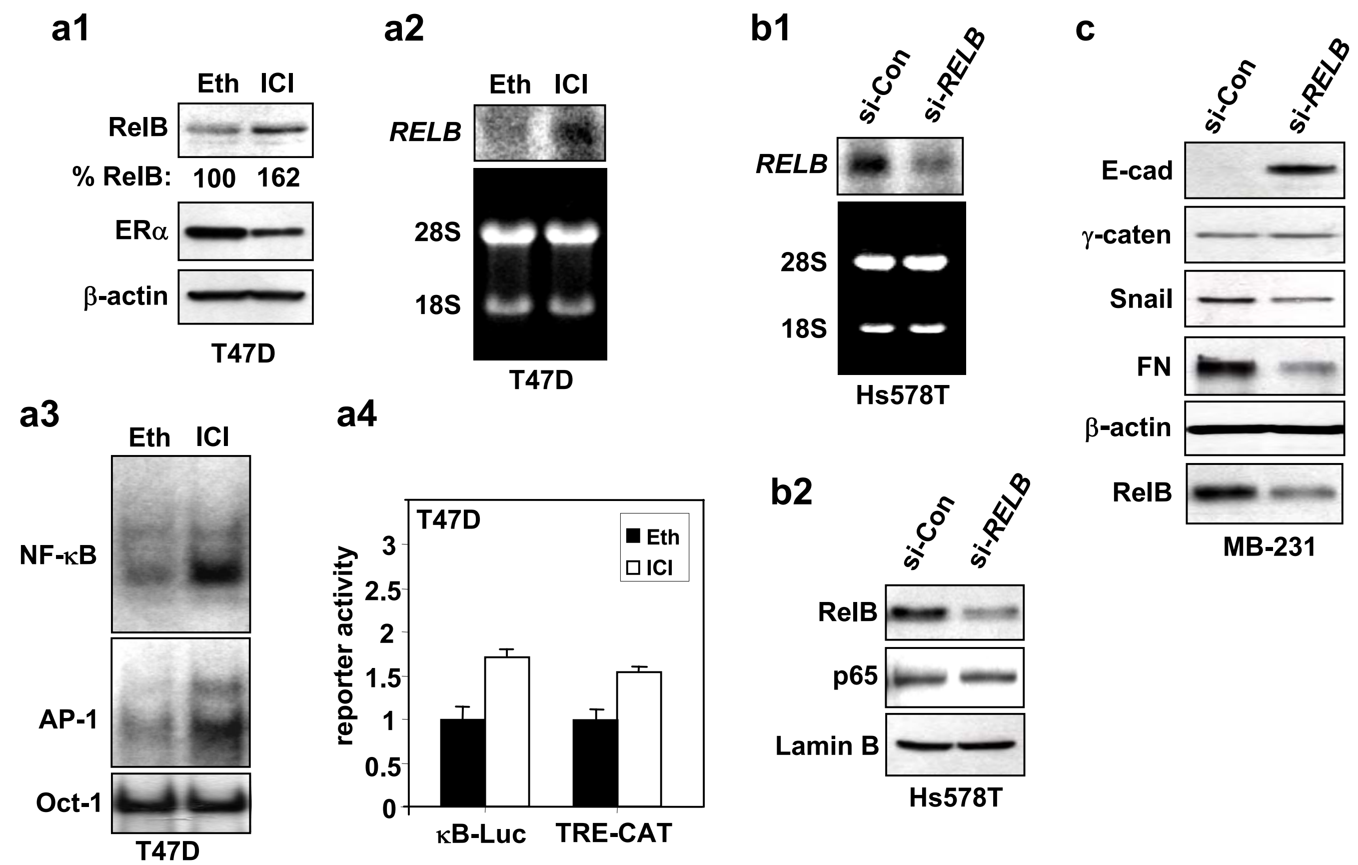

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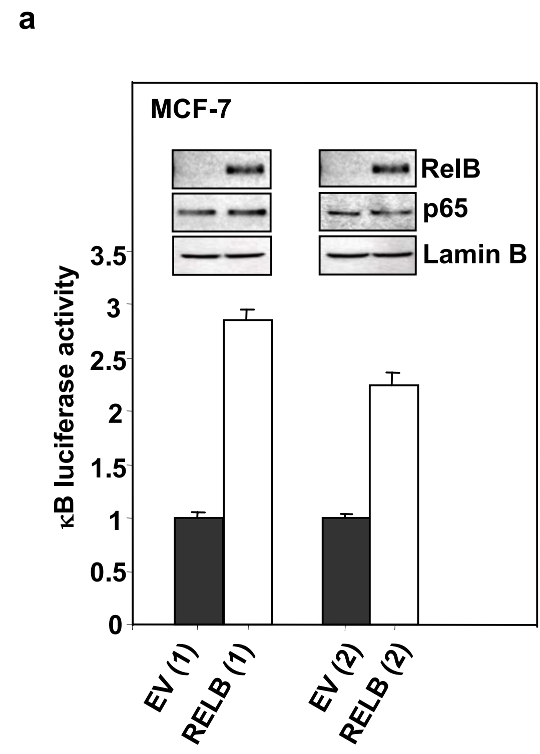

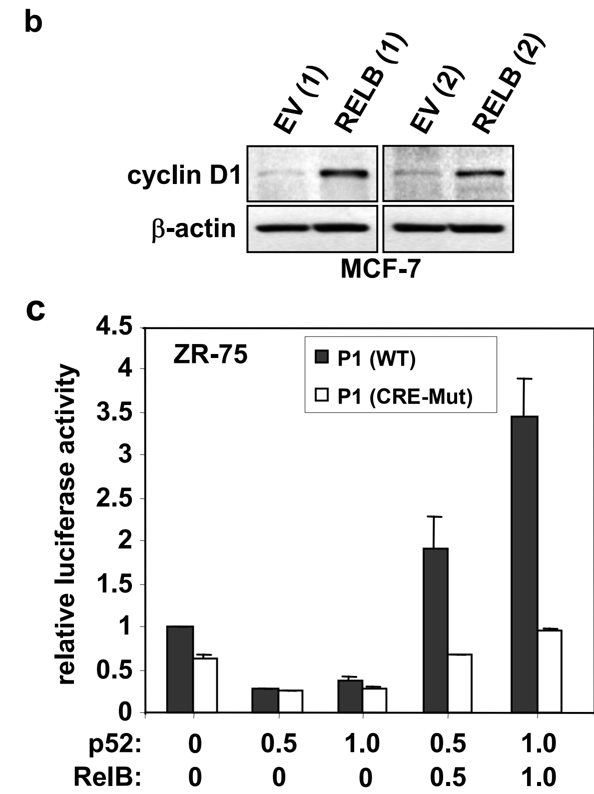

Fig S2. Wang et al.,

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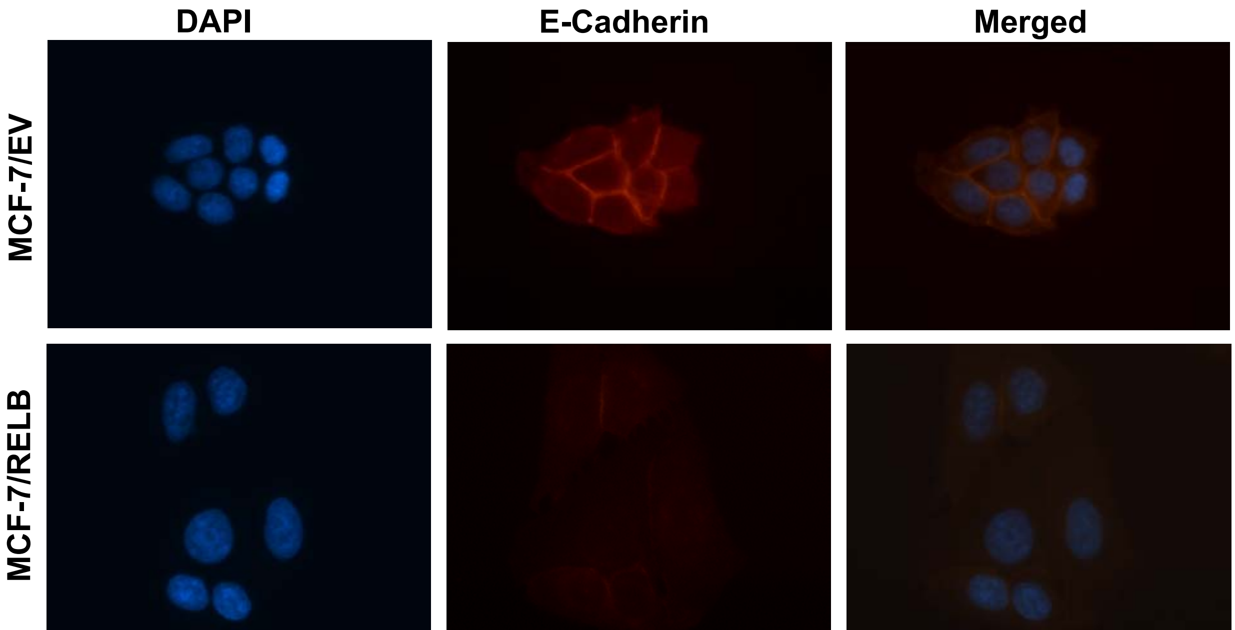

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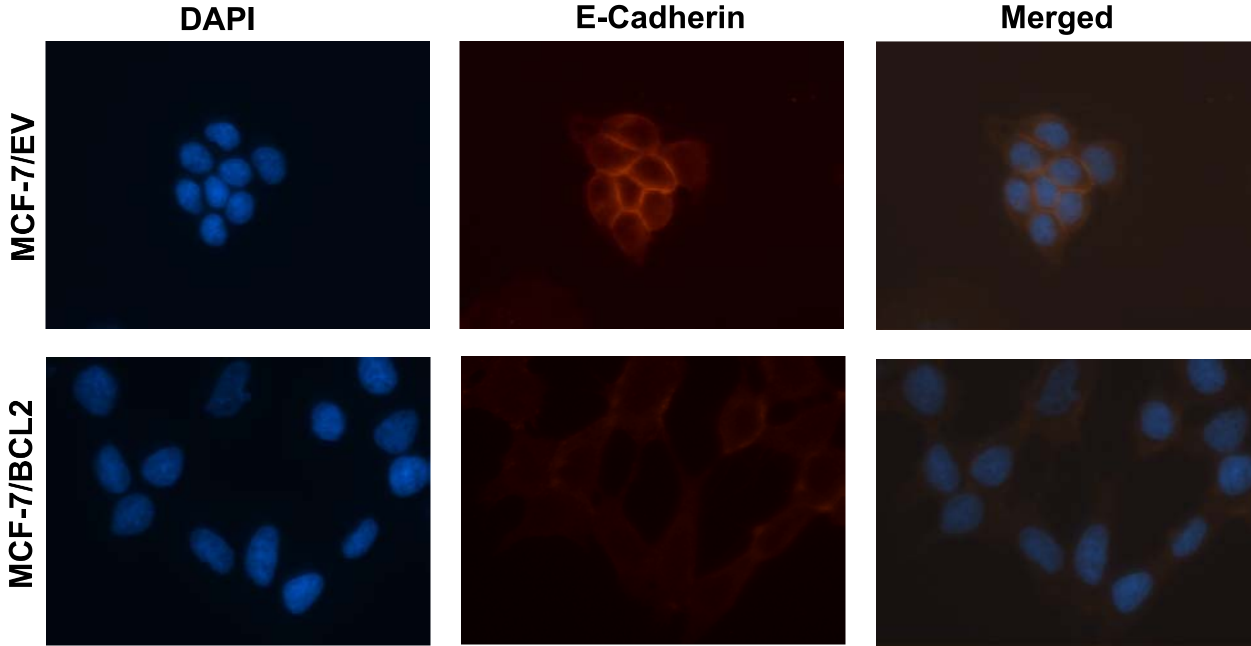

Fig. S4, Wang et al.,

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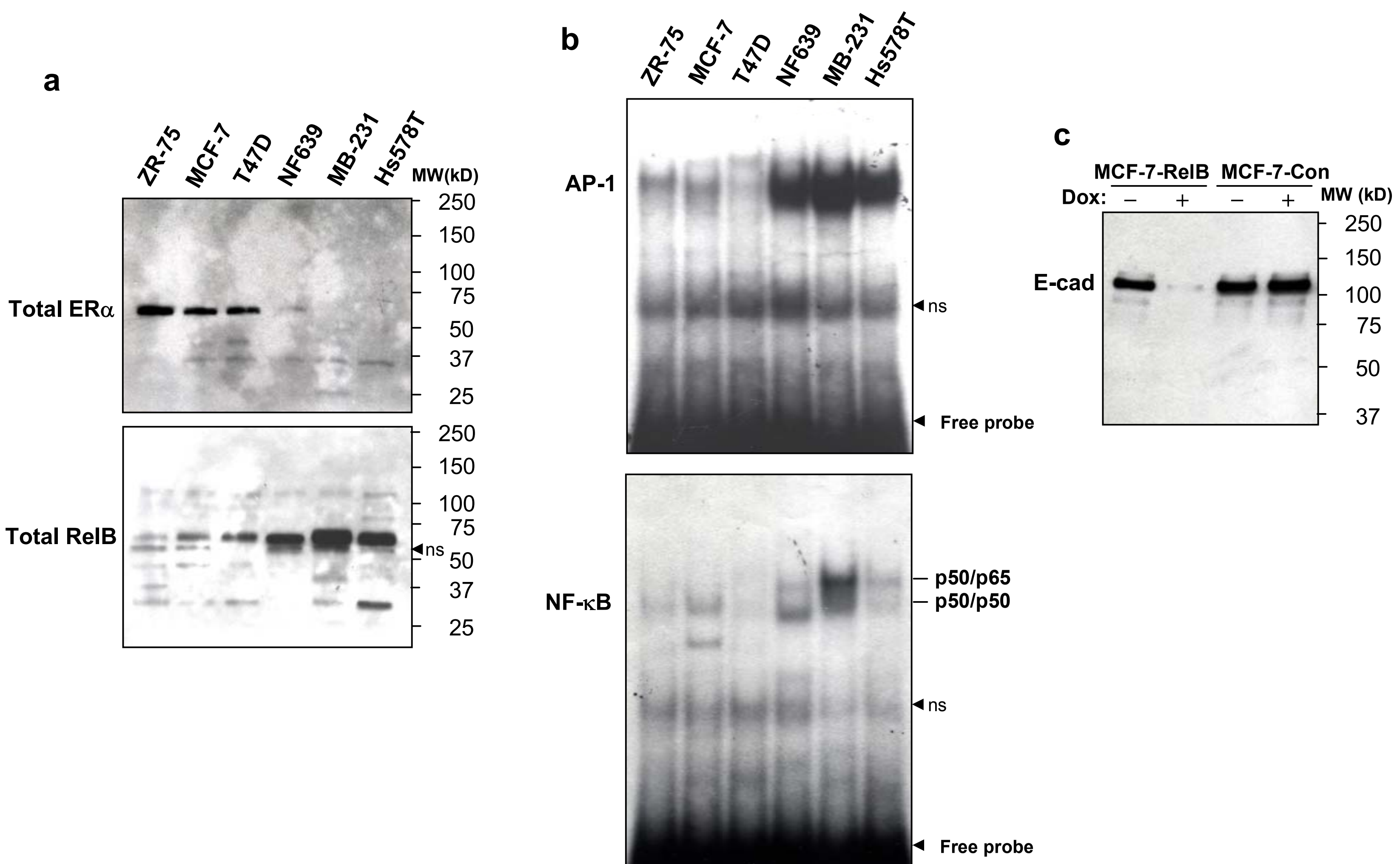

Fig. S5, Wang et al.