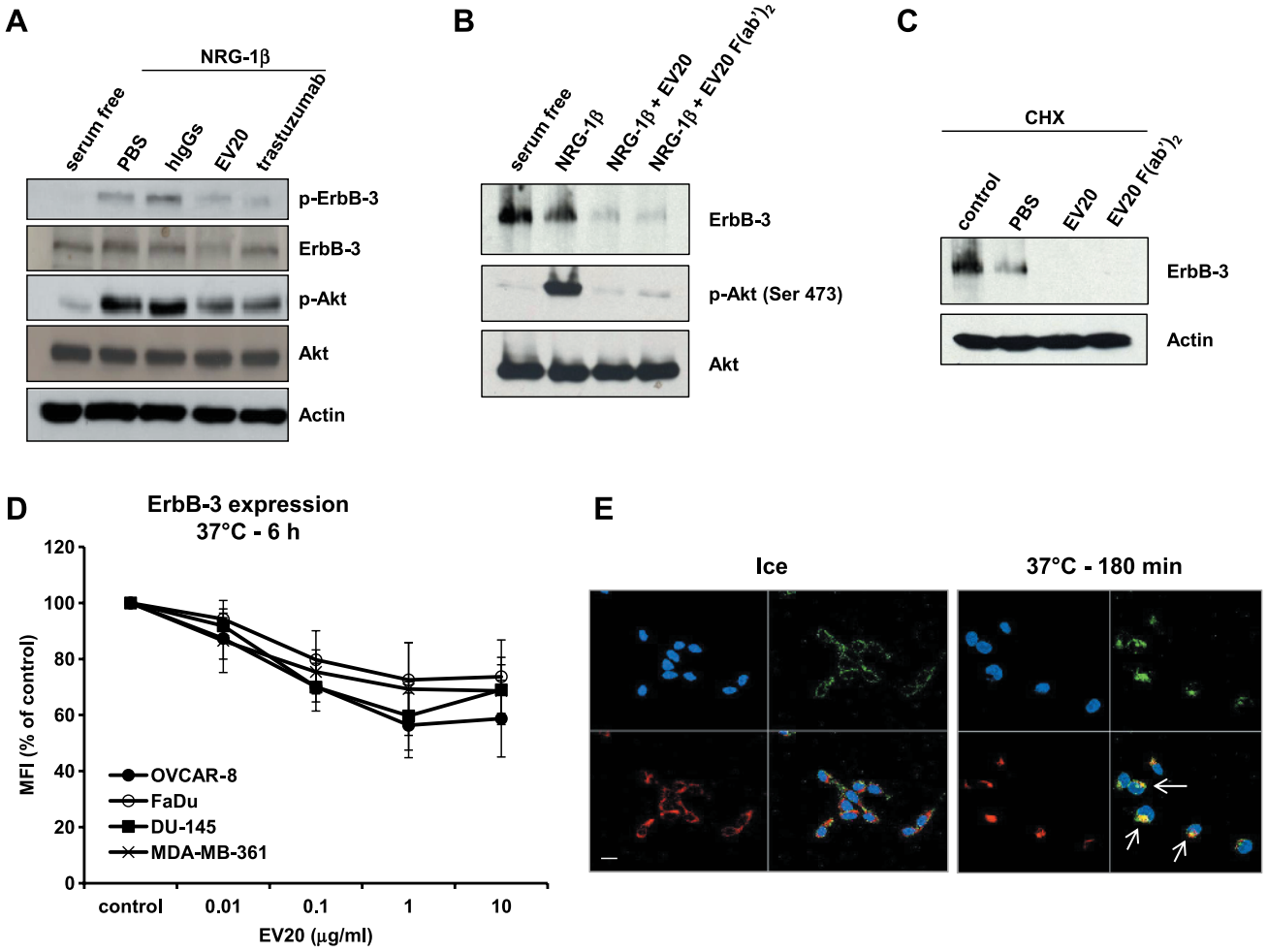

Figure W1. (A) After 24 hours of serum deprivation, OVCAR-8 cells were treated for 2 hours as indicated before ligand stimulation (5 minutes). hIgGs and trastuzumab were used as negative and positive control antibodies, respectively. (B) Serum-starved (24-hour) IR-8 cells were incubated with the FL EV20 or F(ab')2 truncated form for 2 hours before NRG-1β stimulation. Cell lysates were immunoblotted as indicated. (C) IR-8 cells were incubated for 3 hours with CHX in the presence or absence of FL EV20 or F(ab')2 truncated form. Cell lysates were immunoblotted as indicated. (D) Cells were exposed to increasing doses of EV20 for 6 hours and then analyzed for surface ErbB-3 expression by FACS. Plotted results are an average ± SD of three independent experiments. (E) IR-8 cells were treated with 10 μg/ml EV20, kept on ice for 30 minutes, and then returned at 37°C for 180 minutes. After harvesting, cells were fixed, permeabilized, and then incubated with the rabbit antibody C-17 recognizing the C-terminal residue of ErbB-3. Secondary goat anti-human and anti-rabbit antibodies were used to visualize EV20 (green) and ErbB-3 (red). Cell nuclei are shown in blue. Bar, 10 μm.

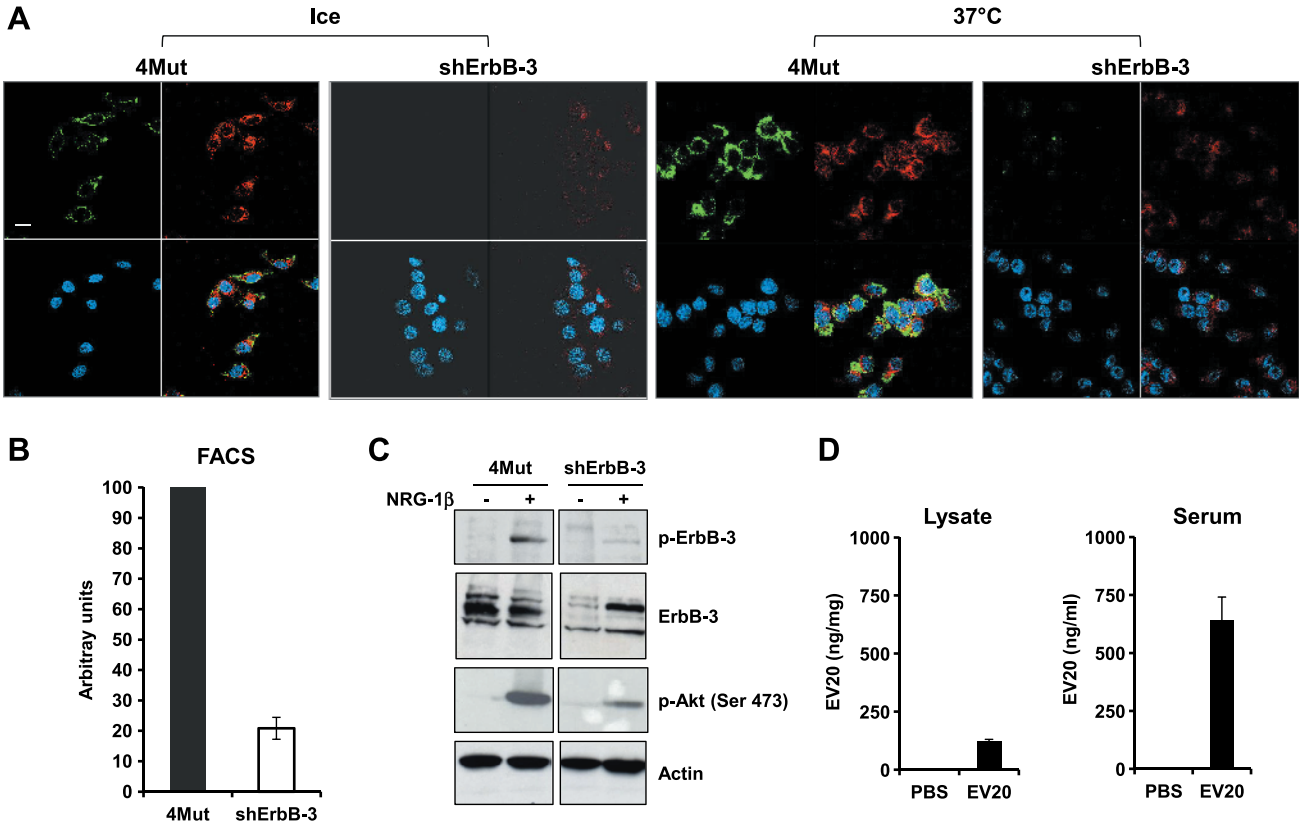

Figure W2. (A) Control 4Mut and shErbB-3 IR-8 cells were maintained on ice for 30 minutes in the presence of 10 μg/ml EV20 and returned at 37°C for 60 minutes. EV20 and ErbB-3 receptor were visualized in green and red, respectively. Cell nuclei are shown in blue. Bar, 10 μm. (B) Control and shErbB-3 IR-8 cells were analyzed by FACS for ErbB-3 expression. Plotted results are an average ± SD of three independent experiments. (C) Control and shErbB-3 IR-8 cells were serum starved for 24 hours and then stimulated with 10 ng/ml NRG-1β for 5 minutes. Cell lysates where then probed with the indicated antibodies. (D) ELISA for determination of EV20 concentration in serum (left) and tumor tissue (right) of mice 24 hours after antibody injection.