Figures legends

Figure S1. Enhanced sensitivity of mutant ERBB2-expressing breast epithelial cells to ER stress.

Pbabe and NeuT cells were treated with thapsigargin for the indicated times (A) or incubated in the presence of different concentrations of tunicamycin for 48 hours (B). Apoptosis was determined as described under Materials and Methods. Error bars represent S.D. from three independent experiments. **P*<0.05, ***P*<0.01. (C) MDA-MB231 breast cancer cell line was infected with pbabe or NeuT retrovirus for 2 days and selected in the presence of puromycin. One week after infection, pbabe and NeuT cells were fixed and stained for β-galactosidase (β-gal) activity. Representative phase contrast microscopy images are shown.

Figure S2. Role of UPR branches in ER stress-induced apoptosis.

NeuT cells were transfected either with a siRNA oligonucleotide targeting Ire1α (A) or ATF6 (B) for 48 h and then treated with thapsigargin. After 30 h, apoptosis was measured as previously described. (C) ATF4 mRNA expression in NeuT cells treated with thapsigargin for the indicated times was assessed by RT-qPCR. Results are representative of two independent experiments.

Figure S3. Noxa and Bim-independent apoptosis upon ER stress in NeuT cells.

(A) pbabe and NeuT cells were treated with or without thapsigargin (TG, 100 nM) for 15 hours. The expression of Bcl-2 family genes was analyzed by RT-MLPA as described under Materials and Methods. Results of a representative experiment are shown. (B) NeuT cells were transfected either with a scrambled oligonucleotide or a

siRNA oligonucleotide targeting BIM (upper panel) or Noxa (lower panel) for 48 h. Cell were then treated with 100 nM thapsigargin for 30 h and apoptosis was measured as described in Methods. Results show the average and range of 2 independent experiments. BIM and Noxa levels were assessed by western blotting.

Figure S4. Role of CHOP in the regulation of TRAIL-R2 expression by thapsigargin in NeuT cells.

NeuT cells were transfected either with a siRNA oligonucleotide targeting CHOP or a scrambled oligonucleotide for 48h and then treated with 100 nM thapsigargin for the indicates times. CHOP (upper panel) and TRAIL-R2 (middle panel) mRNA levels were assessed by RT-qPCR as described in Methods. TRAIL-R2 protein levels (lower panel) were determined by western blot. Results are representative of 2 independent experiments.

Figure S5. Tunicamycin induces a TRAIL-R2 and caspase-8-dependent apoptotic pathway in NeuT cells.

NeuT cells were incubated in the presence or absence of Tunicamycin (1μg/ml) for the indicated times. (A) TRAIL-R2 (left panel) and CHOP (right panel) mRNA levels were assessed by RT-qPCR as described in Methods. Levels of TRAIL-R2 protein were assessed by western blotting and GAPDH was used as a protein loading control (B). (C) NeuT cells were transfected either with a siRNA oligonucleotide targeting TRAIL-R2, Caspase-8 or a scrambled oligonucleotide for 48 hours as described in Methods. Then cells were incubated in the presence or absence of Tunicamycin (1μg/ml) for 48 h and apoptosis was measured as described in Methods. Results show the average of two different experiments.

Figure S6. TRAIL-independent induction of apoptosis upon ER stress.

(A) NeuT cells were treated with thapsigargin (100 nM, left panel) or TRAIL (500 ng/ml, right panel) for 30 h in the presence or absence of TRAIL-R2/Fc. Apoptosis was determined as described under Materials and Methods. Results show the average of two different experiments. (B) NeuT cells were transfected with either a siRNA oligonucleotide targeting TRAIL or a scrambled oligonucleotide for 48 h as described in Methods. Then cells were incubated in the presence or absence of Thapsigargin (100 nM) for 30 h and apoptosis was determined (left panel). Results show the average of two different experiments. TRAIL knockdown was assessed by RT-qPCR (right panel) as described in Methods.