Supplemental Data

Supplemental Experimental Procedures

The primer sequences used to PCR amplify each of the HER-2 cDNA constructs are listed below:

Truncated HER-2 (amino acids 1-1016)
5'-GGCCGAATTCATGGAGCTGGCGGCCTT -3'
5'-GGTTCACTCGAGTCAGTCCCCCATGTCATCGTCGTCCTCC-3'

25 kDa HER-2 (amino acids 1017-1125)
5'-GGCCGAATTCCTGGTGGATGCTGAGGAGTATC-3'
5'-GGCCCTCGAGTCAATCAGTCTCAGAGGGCAGGGGTACT-3'

22 kDa HER-2 (amino acids 1026-1254)
5'-GGCCGAATTCGGCTACGTTGCCCCCCTGACCTGCAG-3'
5'-GAGAGCCCTCGAGTCACACTGGCACGTCCAGACCCAG-3'

47 kDa HER-2 (amino acids 1017-1254)
5'-GGCCGAATTCCTGGTGGATGCTGAGGAGTATC-3'
5'-GAGAGCCCTCGAGTCACACTGGCACGTCCAGACCCAG-3'

The *EcoR*I sites in wild-type HER-2 cDNA were mutated without altering the coding sequence using the QuikChange site-directed mutagenesis kit (Stratagene) and the following primers:

***EcoR*I Site Ablation by Mutagenesis of Nucleotide 1450**
5'-TCCGGGGACGAATACTGCACAATGGCGCC-3'
5'-GGCGCCATTGTGCAGTATTCGTCCCCGGA-3'

***EcoR*I Site Ablation by Mutagenesis of Nucleotide 3072**
5'-GGGAGTTGGTGTCTGAGTTCTCCCGCATG-3'
5'-CATGCGGGCGAACTCAGACACCAACTCCC-3'

The candidate caspase cleavage sites in HER-2 were mutated using the QuikChange site-directed mutagenesis kit (Stratagene) and the following primers:

D1016E
5'-TGACATGGGGGAACTGGTGGATGCTGAGGAGTATC-3'
5'-GATACTCCTCAGCATCCACCAGTTCCCCCATGTCA-3'

D1019E
5'-GACCTGGTGGAGGCTGAGGAGTATCTGGTA CCC-3'
5'-ATACTCCTCAGCCTCCACCAGGTGCCCCATGTC-3'

D1087E
5'-GATGTATTTGAGGGTGACCTGGGAATGGG-3'

5’-CCCCCATTCCCAGGTCACCCTCAAATACATC-3’

D1125E
5’-CCCTCTGAGACTGAGGGCTACGTTGCCCCCCTGACC-3’
5’-GGGCAACGTAGCCCTCAGTCTCAGAGGG-3’

D1016E/ D1019E (for 4X HER-2)
5’-TGACATGGGGGAACTGGTGGAGGGCTGAGGAGTATCTGGTAC-3’
5’-GTACCAGATACTCCTCAGCCTCCACCAGTTCCCCCATGTCA-3’

The conserved L and D residues in the HER-2 BH3 domain were mutated in the 25 and 47 kDa HER-2 constructs using the QuikChange site-directed mutagenesis kit (Stratagene) and the following primers:

L1120E
5’-CCACAGTACCCGAGCCCTCTGAG-3’
5’-CTCAGAGGGCTCGGGTACTGTG-3’

D1125E (25 kDa HER-2 construct)
5’-CCCTCTGAGACTGAGTGACTCGAGCCGG-3’
5’-CCGGCTCGAGTCACTCAGTCTCAGAGGG-3’

D1125E (47 kDa HER-2 construct)
5’-CCCTCTGAGACTGAGGGCTACGTTGCCCCCCTGACC-3’
5’-GGGCAACGTAGCCCTCAGTCTCAGAG

Supplemental Figure Legends

Figure S1. HER-2 is proteolyzed by caspases in HER-2-overexpressing MDA-MB-453 breast cancer cells. HER-2-overexpressing MDA-MB-453 human breast cancer cells were treated with 2 μg/mL TRAIL and 1 μg/mL cycloheximide for 0-16 h with or without pretreatment with 100 nM epoxomicin and/or 50 μM zVAD-fmk for 1 h. HER-2 was detected by immunoblotting using a HER-2 mAb that recognizes the carboxyl-terminus.

Figure S2. The 47 and 25 kDa HER-2 products induce apoptosis in MDA-MB-468 breast cancer cells. MDA-MB-468 human breast cancer cells were co-transfected with cDNAs encoding FLAG-tagged HER-2 cleavage products (47 or 25 kDa) or FLAG vector, and GFP-positive cells were scored for apoptotic nuclei 24 h later (mean ± SEM, n = 3).

Figure S3. The HER-2 BH3-like domain is dispensable for mitochondrial localization. Confocal images of MDA-MB-231 cells transfected with cDNAs encoding FLAG-tagged 25 or 47 kDa HER-2 (WT or 2XE BH3 mutant) cleavage products. Transfected MDA-MB-231 cells were immunostained with FLAG mAb (green). Mitochondria were labeled with Mitotracker Deep Red 633 (red), and nuclei were stained with DAPI (blue). Colocalization (yellow) is shown in the merged image. Bar, 10 μm.

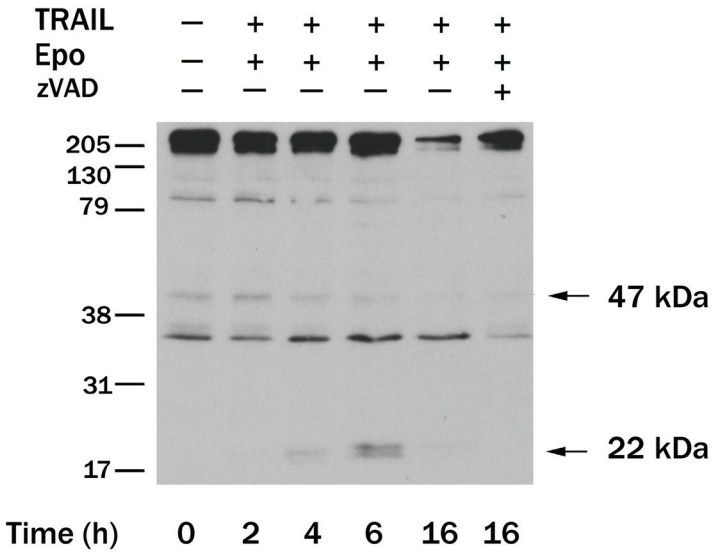

Figure S1. *Strohecker et al.*

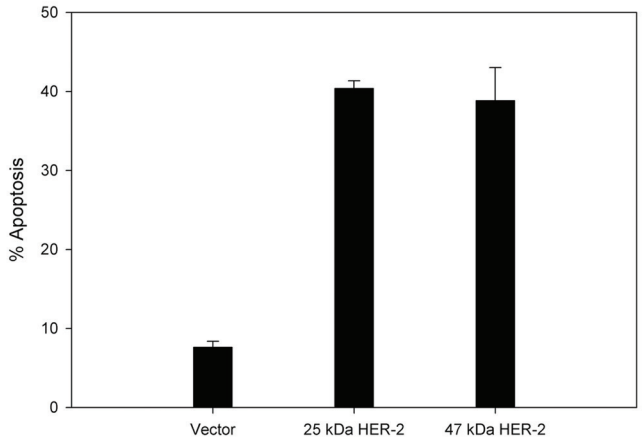

Figure S2. *Strohecker et al.*

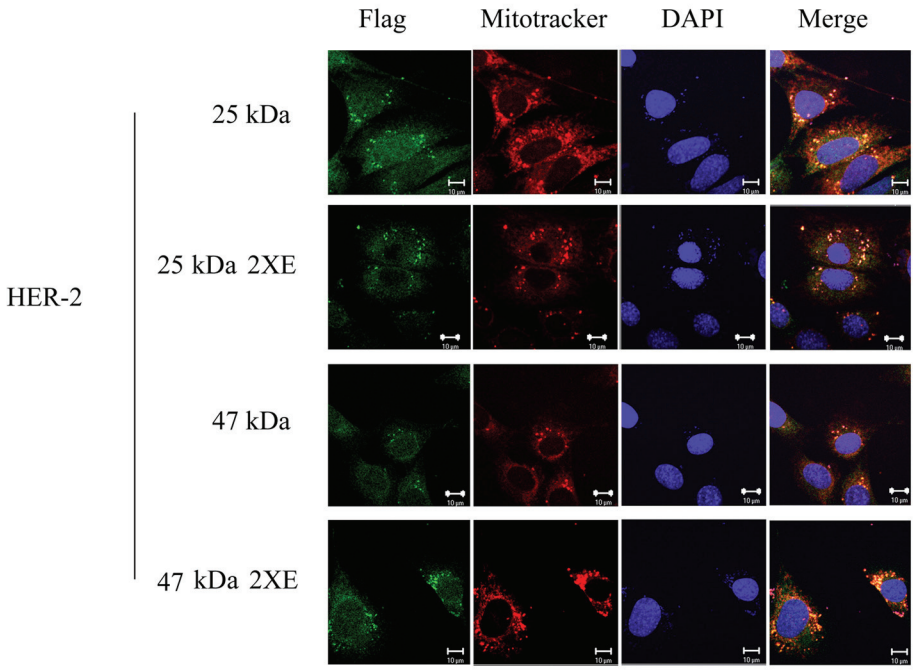

Figure S3. *Strohecker et al.*