SUPPORTING INFORMATION

A Mechanistic Study of Tumor-Targeted Corrole Toxicity

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Figure S1. **Mechanism of HerGa-mediated cell death.** Schematic shows intracellular interactions mediating HerGa cytotoxicity. Based on the studies presented here and supported by our previous studies[3, 12], the schematic shows that HerGa undergoes HerPBK10-mediated uptake (a), followed by early endosome escape (b), thus enabling superoxide (O_2-) generation (c), which in turn mediates actin and microtubule disruption (d), and collapse of mitochondrial membrane potential (e).

Figure S2. Evaluation of apoptotic markers in treated MDA-MB-435 cells. **A**, Assessing the earliest and latest apoptotic markers. Cells were treated in triplicate with S2Ga or HerGa at 0.5 uM final corrole concentration. Parallel treatments included HerPBK10 alone (at equivalent protein concentration to HerGa) or 2 ug/mL anisomysin (an apoptosis-inducing control). At 24h after treatment, cells were monitored for specific apoptotic markers: (**Upper panel**) surface phosphatidylserine (PS), or (**Lower panel**) DNA laddering (TUNEL), as described (Procedures follow below). Bars represent marker elevation normalized by values obtained from mock-treated cells, or experimental/control ± 1 SD. *, $P<0.05$ compared to untreated as determined by 2-tailed unpaired t-test. **B,** Evaluating caspase activation. Cytosolic fractions from cells treated with 1 uM HerGa or S2Ga, HerPBK10 alone (at equivalent protein concentration to 1 uM HerGa), or PBS (Mock) for 24h were isolated as described (see following Methods) and immunoblotted for the activated forms of caspase 9 and 3 using antibodies from Abcam (Cambridge, MA, USA). **C**, Evaluating mitochondrial cytochrome c release. Micrographs show fluorescence confocal images of cells after treatment with HerGa (10 or 1 uM), S2Ga (1 uM), HerPBK10

alone (at equivalent protein concentration to 1 uM HerGa), or PBS (Mock). Red, mitochondria; Green, cytochrome c. n, nucleus. Bar, ~8 microns. These findings represent at least three independent experiments. ***Procedures.*** MDA-MB-435 cells receiving the indicated treatments were assayed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) or annexin V binding at 24h after treatment. Specifically, treated cells were washed with PBS and fixed in suspension in 1% paraformaldehyde. To process the cells for TUNEL, cells were washed with PBS and incubated with terminal deoxyuridine 5'-triphosphate TdT, BrdUTP, followed by washing and incubation with Alexa Fluor 488 –conjugated anti-BrdU mouse monoclonal antibody for 30 min using a TUNEL assay kit (Molecular Probes Invitrogen, Carlsbad, CA, USA). To process the cells for annexin V binding, the cells were incubated with propidium iodide in binding buffer (50 mM HEPES, pH 7.4; 700 mM NaCl, 12.5 mM CaCl2), washed, and incubated for 30 min with Alexa Fluor 488–labeled annexin V using a surface phosphatidylserine labeling kit (Molecular Probes Invitrogen, Carlsbad, CA, USA). For both assays described earlier, cells were assessed by flow cytometry at 10,000 total events for each sample.

To assess for activated caspases, MDA-MB-435 cells growing in T75 flasks were treated with HerGa or S2Ga (each at 1 uM corrole concentration), equivalent dose of HerPBK10, or PBS in a 5 mL total volume of complete media for 4h with rocking at 37°C/5% CO2, followed by supplementation with 5 mL complete media, and cell harvest 24h later for isolation of cytosolic fractions using a commercial extraction kit (Abcam, Cambridge, MA, USA), following the manufacturer's procedures. Aliquots from each fraction (40 ug each) were electrophoresed and immunoblotted using indicated antibodies from Abcam (Cambridge, MA, USA). HRP-conjugated secondary antibody blotting and chemiluminescence detection were performed following standard established procedures.

Cytochrome c release was assessed in treated cells growing on coverslips at 24h after indicated treatments. The media was replaced with the prewarmed (37°C) staining solution containing MitoTracker® probe (50nM final), and incubated at 37°C for 30 minutes, followed by fixation and permeabilization before cytochrome c staining using an Alexa fluor 488 cytochrome c apoptosis detection

kit (Invitrogen), following the manufacturer's protocol. Immunostained cells were imaged using a Leica confocal microscope to assess cytochrome c and mitochondria localization (cytochrome c - ex: 488nm, em:510-560nm, mitochondria – ex: 535nm, em: 560-590nm).

Figure S1. Hwang et al.

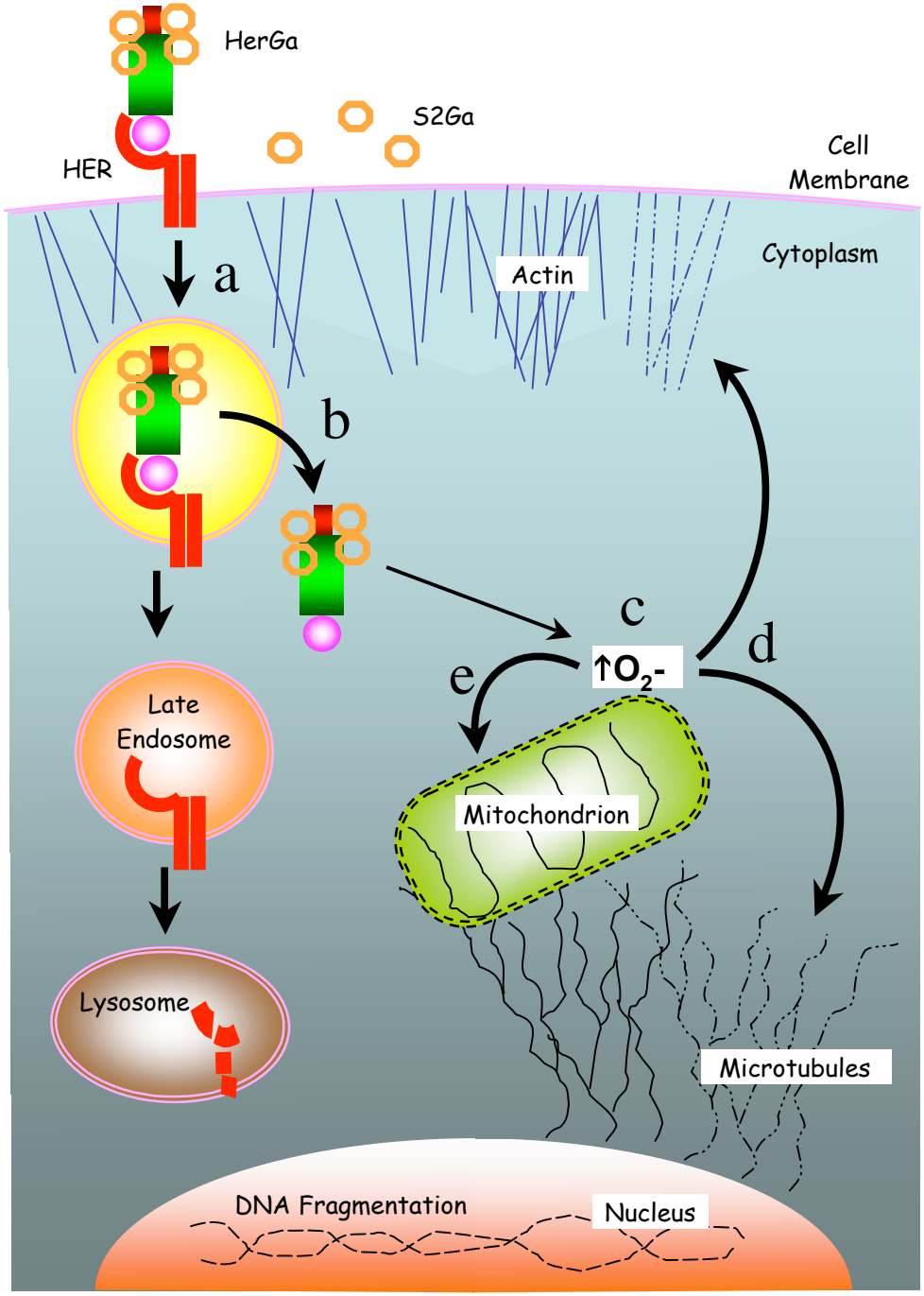

Figure S2. Hwang et al.