

Figure S3. Compound 15 causes Rad53 activation in vivo. Cells were treated with increasing amounts of either compound 13 or 15 and fixed with 10% TCA. Cell extracts were fractionated on SDS-PAGE, and Rad53 protein detected by immunoblot analysis. The positions of Rad53 and the activated, phosphorylated form of Rad53 (Rad53-P) are indicated. The immunoblots were re-probed to detect tubulin (tub), as a loading control. Compound 15 activated Rad53 at concentrations of $45 \,\mu\text{M}$ and $67.5 \,\mu\text{M}$ (0.8 EC50 and 1.6 EC50). By contrast, compound 13 did not induce detectable Rad53 phosphorylation, even at 112.5 $\,\mu\text{M}$ (12.5 EC50).