

**Supplemental material for Molecular Pharmacology, Ms # 82651**

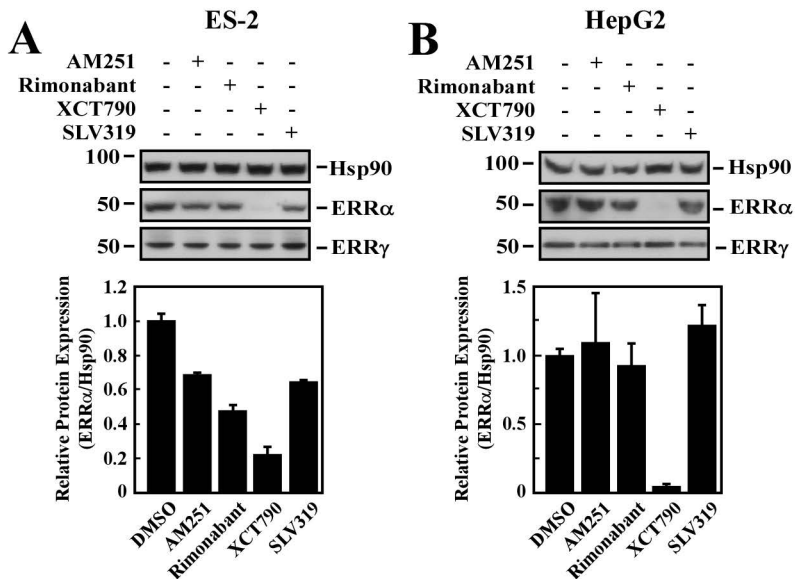
The biarylpyrazole compound AM251 alters mitochondrial physiology via proteolytic degradation of ERR $\alpha$ .

Susan M. Krzysik-Walker, Isabel Gonzalez-Mariscal, Morten Scheibye-Knudsen, Fred E. Indig, and Michel Bernier

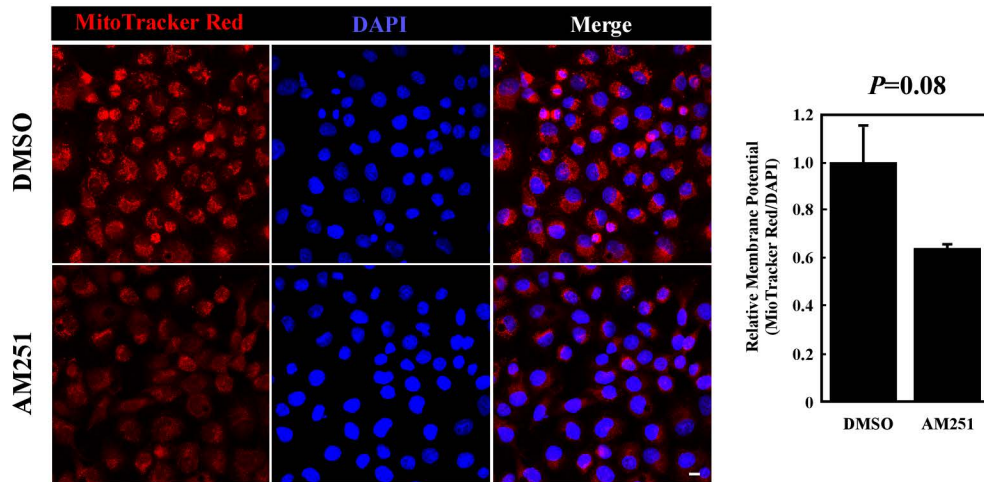
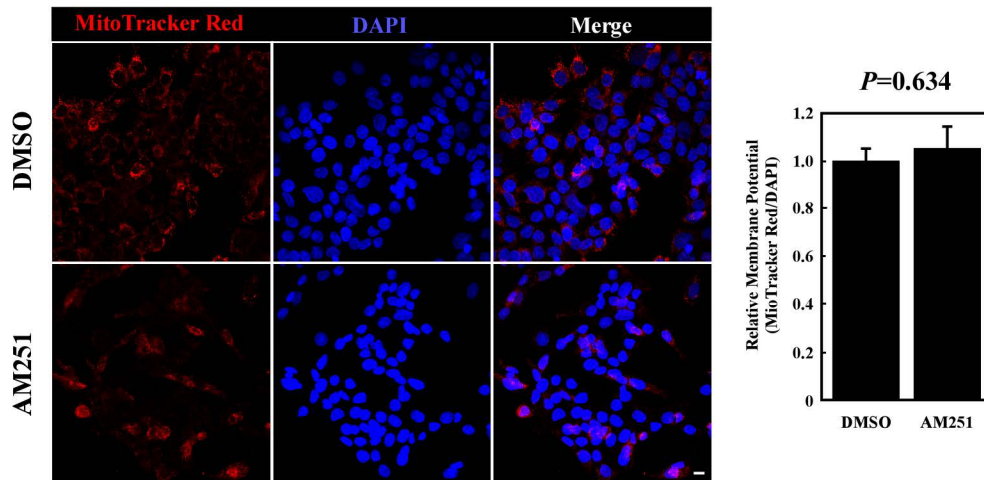
**Supplemental Figure 1.** Biarylpyrazole compounds destabilize ERR $\alpha$  in a cell type-specific manner.

**Supplemental Figure 2.** Cell type-specific effects of AM251 on mitochondrial membrane potential.

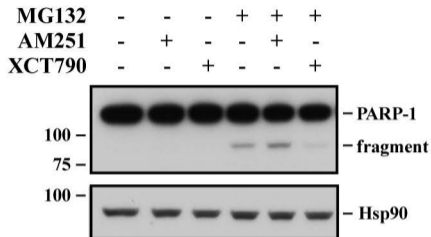
**Supplemental Figure 3.** Lack of cleavage of PARP-1 by AM251 or XCT790.



**Supplemental Fig. 1. Biarylpyrazole compounds destabilize ERR $\alpha$  in a cell type-specific manner.** Serum-depleted ES-2 (A) and HepG2 (B) cells were incubated with either vehicle (DMSO) or 5  $\mu$ M of AM251, rimonabant or SLV319. The ERR $\alpha$  inverse agonist XCT790 (2.5  $\mu$ M) was included as a positive control. Total cell extracts were prepared and analyzed by immunoblotting with antibodies against ERR $\alpha$ , ERR $\gamma$  and Hsp90, the latter serving as a loading control. The relative expression of ERR $\alpha$  was determined by densitometry and normalized to Hsp90. Bars represent means  $\pm$  range of two dishes from a single experiment. Comparable results were obtained in a second independent experiment. The migration of molecular-mass markers (values in kilodaltons) is shown on the left of immunoblots.

**A****B**

**Supplemental Fig. 2. Cell type-specific effects of AM251 on mitochondrial membrane potential.** Forty-eight hours after seeding PANC-1 (A) and HepG2 (B) cells into chamber slides, cells in serum-free medium were treated with vehicle (0.1% DMSO) or AM251 (5  $\mu$ M) for 16 h. The spent medium was replaced with serum-free medium containing 100 nM MitoTracker Red CMXRos for 10 min at 37  $^{\circ}$ C. Cells were rinsed in PBS, fixed, permeabilized and then mounted in Prolong Gold antifade medium containing 4',6-diamidino-2-phenylindole (DAPI, blue) to counterstain cell nuclei. Images were captured by confocal microscopy with Z-stack images processed for maximum projection using the Zeiss Zen software. Relative membrane potential was calculated as histogram density of MitoTracker Red CMXRos normalized to DAPI (Bars). Means  $\pm$  SD (n=3-4) are presented. Student's t-test was performed to determine significance. Scale bar, 10  $\mu$ m.



**Supplemental Fig. 3. Lack of cleavage of PARP-1 by AM251 or XCT790.** Control and MG132-treated PANC-1 cells were incubated with AM251 (5  $\mu$ M) or XCT790 (2.5  $\mu$ M) for 16 h. Total cell extracts were immunoblotted with antibodies against PARP-1 and Hsp90, which served as a loading control. Note the weak formation of the 89-kDa fragment of PARP-1 in MG132-treated cells.