SUPPLEMENTARY MATERIALS

Suppl. Fig. 1. Effects of ML385 and brusatol on the protective effects of bardoxolone methyl (BM) treatment on dRib-induced decreases in l-[14C]cystine uptake (A), intracellular GSH (B), and cell viability (C) in isolated rat renal tubular epithelial cells (RTECs). (A) Isolated RTECs were co-stimulated with 0.2 μM bardoxolone methyl (BM), 100 μM ML385, or 100 μM brusatol and 50 mM dRib for 4 h in the extracellular fluid buffer containing 1.7 μM l-[14C]cystine (0.1 μCi/mL) at 37℃. The radioactivity incorporated into the cells was determined by a liquid scintillation counter. (B and C) Isolated RTECs were co-stimulated with 0.2 μM bardoxolone methyl (BM), 100 μM ML385, or 100 μM brusatol and 50 mM dRib for 6 h in DMEM media containing 10% FBS. The intracellular GSH and cell viability were measured using a glutathione assay kit and LDH release assay, respectively. These experiments were performed thrice, in triplicate. Data are presented as the mean ± SD. \*\*p < 0.01 vs. control; ††p < 0.01 vs. 50 mM dRib-alone group; ‡‡p < 0.01 vs. 50 mM dRib plus 0.2 μM BM group, as determined by one way analysis of variance and Duncan's post hoc test.

Suppl. Fig. 2. Effects of ML385, brusatol, and/or bardoxolone methyl (BM) treatments on 2-deoxy-d-ribose (dRib)-induced increase in intracellular levels of lipid reactive oxygen species (ROS). NRK-52E cells were co-stimulated with 100 μM ML385, 100 μM brusatol, 0.2 μM BM, and/or 50 mM dRib for 6 h in DMEM media containing 10% FBS. Intracellular lipid ROS level was quantified by flow cytometry using the lipophilic fluorescent dye C11-BODIPY. Cells were incubated with 4 μM C11-BODIPY during the final 30 min. The histogram is representative of four independent experiments.

Suppl. Fig. 3. Effects of bardoxolone methyl (BM) treatment on 2-deoxy-d-ribose (dRib)-induced changes in SLC7A11 (A), ACSL4 (B), CHAC1 (C), and PTGS2 (D) mRNA expressions in isolated rat renal tubular epithelial cells (RTECs). Isolated RTECs were co-stimulated with 0.2 μM bardoxolone methyl (BM), 100 μM ML385, or 100 μM brusatol and 0, 10, 20, or 30 mM dRib for 6 h in DMEM media containing 10% FBS. The mRNA levels were analyzed by real-time quantitative reverse transcription‑quantitative polymerase chain reaction. Relative expression of target genes was calculated using the 2-ΔΔCT method. This experiment was performed thrice, in triplicate. Data are presented as the mean ± SD. \*p < 0.05 and \*\*p < 0.01, vs. 0 mM dRib group; †p < 0.05 and ††p < 0.01 vs. dRib alone, as determined by one-way analysis of variance and Duncan's post-hoc test.

Suppl. Fig. 4. Immunofluorescence confocal microscopy analysis of NRK-52E cells treated with 50 mM dRib alone or in combination with 0.2 μM BM for 6 h. Representative images from three independent experiments show Keap1 (green), DAPI (blue), and merged staining under control and experimental conditions. Scale bar = 20 μm.