Tang et al., http://www.jcb.org/cgi/content/full/jcb.200911078/DC1

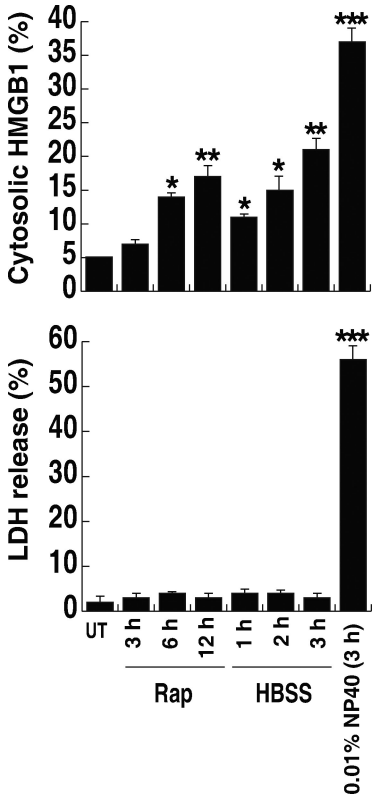

Figure S1. **Autophagic stimuli promote cytosolic HMGB1 translocation that is not dependent on plasma membrane disruption.** MEF cells were starved (HBSS), treated with 1 μM rapamycin (Rap) and 0.01% NP-40 at indicated times, and then immunostained with HMGB1-specific antibody and Hoechst 33342. Images of 1,000 cells were analyzed to obtain the mean cytosolic HMGB1 intensity per cell by imaging cytometric analysis. In parallel experiments, lactate dehydrogenase release was analyzed. *, $P < 0.05$; **, $P < 0.005$; and ***, $P < 0.005$ versus untreated (UT) group; $n = 3$. Data are means ± SEM.

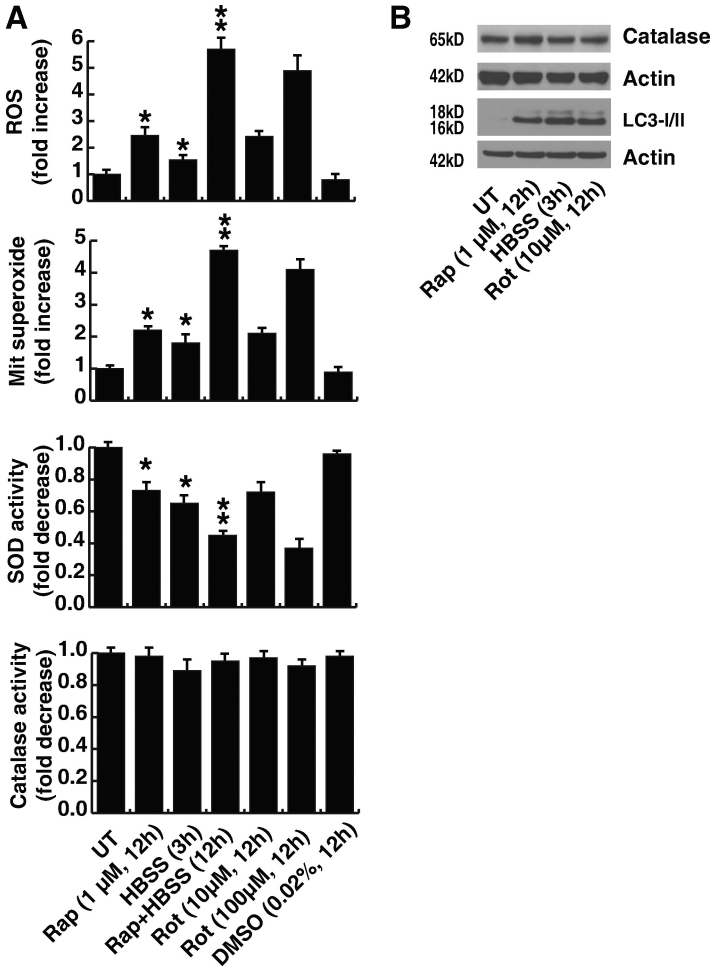

Figure S2. **Autophagic stimuli increase ROS and mitochondrial superoxide levels and decrease SOD activity, while having no effect on catalase activity levels.** (A) Effects of autophagy on ROS production and SOD/catalase activity. Panc2.03 cells were starved (HBSS) and then treated with rapamycin (Rap) or rotenone (Rot) at the indicated doses and time. ROS and mitochondrial superoxide production was assessed by measuring the fluorescent intensity of CM-H2DCFDA or MitoSox red on a fluorescent plate reader. The DMSO is used as a vehicle. In parallel experiments, SOD and catalase activity were assayed by colorimetric assay kit according to manufacturer's instructions. *, $P < 0.05$; and **, $P < 0.005$ versus untreated (UT) group; $n = 3$. (B) Effects of autophagy on expression of catalase and LC3. Panc2.03 cells were starved (HBSS), stimulated with rapamycin, or poisoned with Rot at the indicated doses and times, and the protein level of catalase and LC3-I/II were assessed by Western blotting. Actin was used as a loading control. Blots are representative of three independent experiments with similar results. Data are means ± SEM.

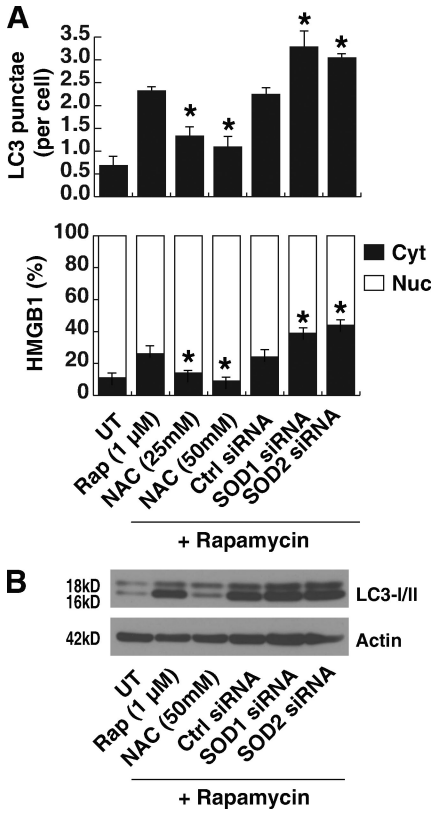

Figure S3. **Antioxidant or knockdown of SOD1 and SOD2 increases rapamycin-induced autophagy and HMGB1 translocation.** (A) Panc2.03 cells were pretreated with an antioxidant (NAC) at the indicated concentrations for 1 h or pretreated with SOD1 or SOD2 siRNA and then stimulated with 1 μM rapamycin (Rap) for 12 h. Cells were immunostained with HMGB1- or LC3-specific antibody and Hoechst 33342. Images of 1,000 cells were analyzed to obtain the mean nuclear (Nuc)/cytosolic (Cyt) HMGB1 intensity and LC3 punctae per cell by imaging cytometry. *, $P < 0.05$ versus rapamycin group; $n = 3$. UT, untreated. Ctrl, control. (B) Western blot analysis of LC3-I/II expression under the conditions indicated in A. Actin was used as a loading control. Data are means ± SEM.

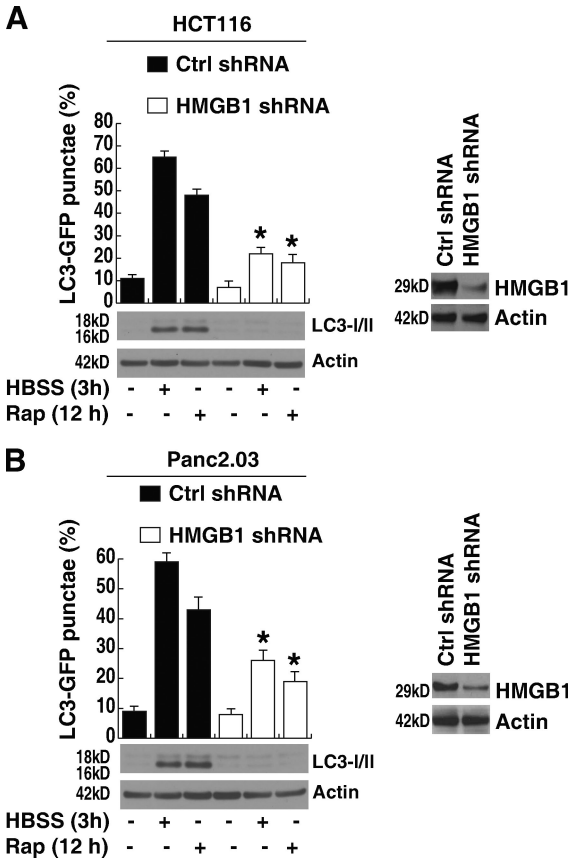

Figure S4. **Knockdown of HMGB1 limits autophagy.** HCT116 (A) and Panc2.03 (B) cells were cotransfected with control (Ctrl) shRNA, HMGB1 shRNA, and LC3-GFP for 48 h and then were treated with 1 μM rapamycin (Rap) or starvation (HBSS) for the indicated time. Autophagy was assessed by quantifying the percentage of cells with LC3-GFP punctae or LC3-I/II expression as indicated. *, $P < 0.05$ versus control group; $n = 3$. Blots are representative of three independent experiments with similar results. Data are means ± SEM.

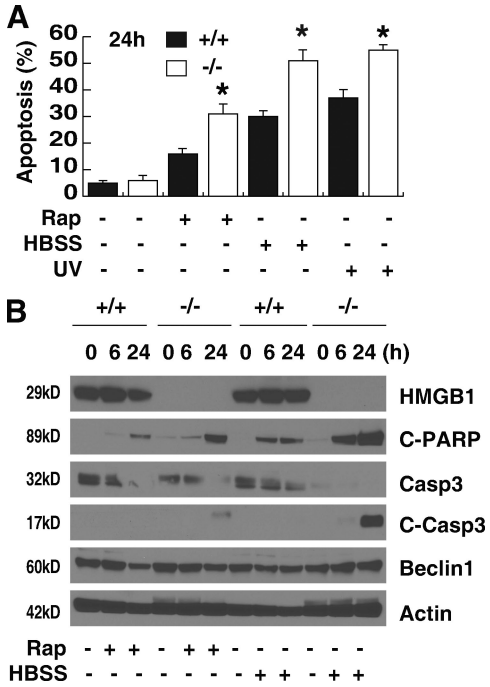

Figure S5. **HMGB1 depletion promotes apoptosis.** Hmgb1$^{-/-}$ and Hmgb1$^{+/+}$ MEFs were treated with 1 μM rapamycin (Rap), starvation (HBSS), or 5 min of UV light at 50 mJ/cm^2 for the indicated time and then were assayed for apoptosis by FACS (A) or by Western blot (B) as indicated. *, $P < 0.05$ versus HMGB1$^{+/+}$ group; $n = 3$. Blots are representative of three independent experiments with similar results. Data are means ± SEM.