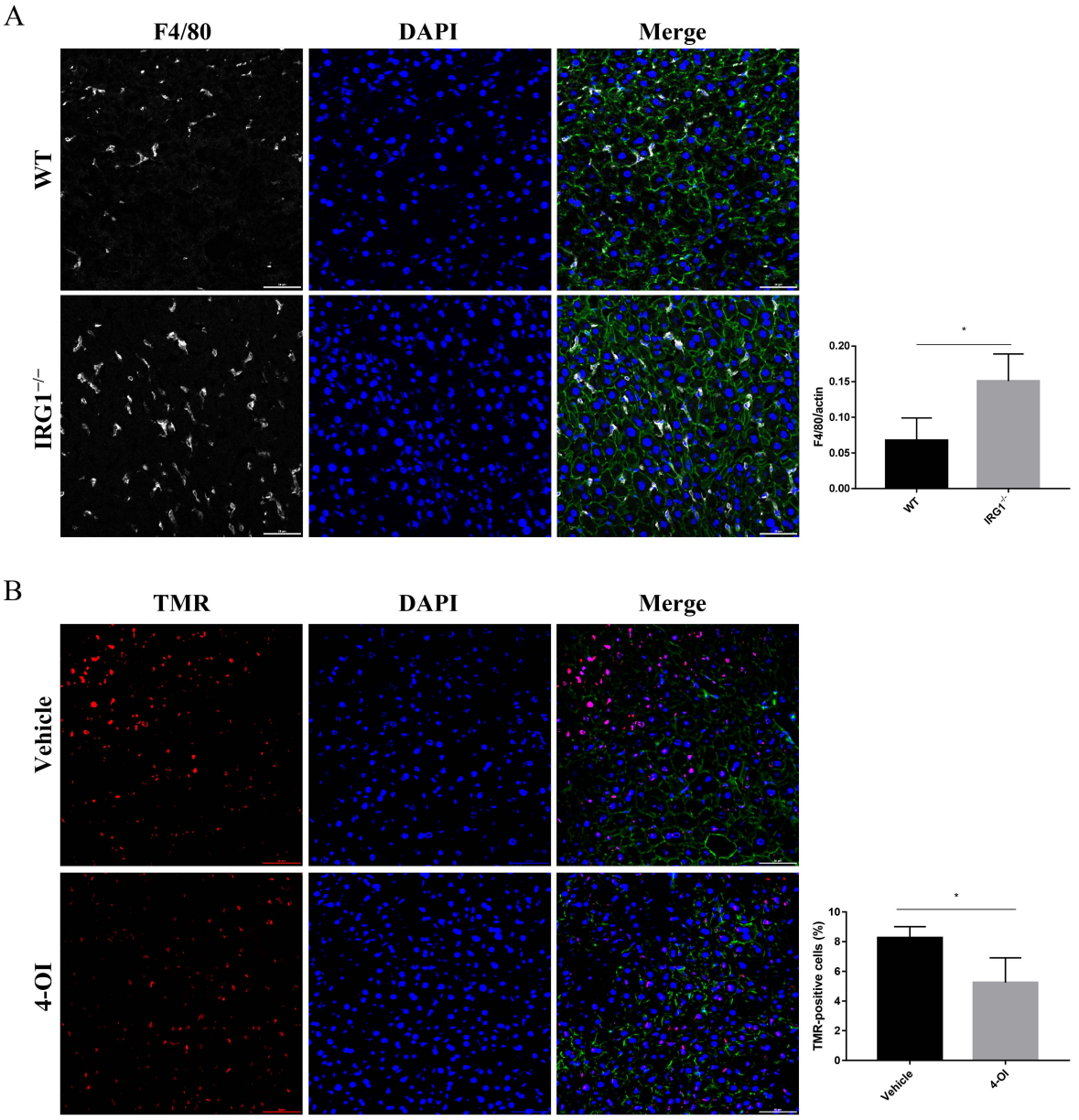
Supporting FIG. 1. (A) Confocal microscopy images of livers from WT (C57BL/6NJ) and IRG1$^{-/-}$ mice after 1-hour ischemia and 6-hour reperfusion; liver tissues were stained with F4/80 (white), DAPI (blue), and β-actin (green); and images were taken using confocal microscopy (scale bar = 50 μm). Area of F4/80 (μm^2)/β-actin (μm^2) was quantified and represented in bar graph; n = 4 for each group. (B) Confocal images (scale bar = 50 μm) of livers from WT mice after 1-hour ischemia and 6-hour reperfusion; 25 mg/kg body weight of 4-octyl itaconate (4-OI) or vehicle control was injected intraperitoneally 2 hours before hepatic I/R and at the time of reperfusion. Liver tissues were stained with TMR (red), DAPI (blue), and β-actin (green). Percentage of TMR-positive cells was quantified and represented in bar graph; n = 3 per group. Images are representative of data from multiple mice per experimental group. Data are presented as means ± SD. *$P < 0.05$.

Supporting FIG. 2. (A) Western blot for cleaved caspase-3 and caspase-3 in whole cell lysates from WT (C57BL/6NJ) and IRG1$^{-/-}$ hepatocytes treated with or without TNF-α (30ng/ml) for 12 hours. Densitometry of cl-caspase3 bands relative to β-actin loading control was quantified and presented in bar graph. (B-D) Primary hepatocytes isolated from WT (B6J) mice were pretreated with 4-OI or vehicle control 1 hour prior to normoxia or 10-hour hypoxia and 10-hour reoxygenation (H/R). Quantitation of band density in Western blot for Nrf2 and its downstream mediators (HO-1 and NQO1) in whole cell lysates and (E) quantitation of band density in Western blot for nuclear Nrf2 (Nu-Nrf2) in total nuclear protein from hepatocytes after normoxia or H/R. (F,G) Primary hepatocytes isolated from WT (C57BL/6J) and Nrf2$^{-/-}$ mice were pretreated with 4-OI (125 μM) or vehicle control 1 hour prior to normoxia or H/R (10 hour/10 hour). Quantitation of band density in Western blot for Nrf2 and HO-1 in whole cell lysates. (H,I) Quantitation of band density in Western blot for Nrf2 and HO-1 in whole cell lysates from primary human hepatocytes pretreated with 4-OI (125 μM) or vehicle control 1 hour before normoxia or 10-hour hypoxia and 10-hour reoxygenation (H/R). Quantitation of band density was performed across at least three separate blots and presented in bar graph. Images are representative of data. Data are presented as means ± SD. *$P < 0.05$, **$P < 0.01$.

Supporting Figure1



Supporting Figure2