n=3-4/group; *p<0.05, **p<0.01.

Figure 4: Inhibition of PI3K disrupts Akt/HIF-1 signaling and recreates liver IRI in Keap1HKO OLTs. Groups of WT or Keap1HKO liver donor mice were pre-treated with Ly294002 or DMSO (-1 h). (A) sALT levels (IU/L): (☐) sham; (▩) WT+DMSO; (▨) WT+ Lly294002; (⬚) Keap1 HKO+DMSO; (■) Keap1 HKO+Ly294002. Mean±SD; n=4 mice/group; *p<0.05, **p<0.01. (B) Western analysis of p-Akt and HIF-1 in OLTs. β-actin served as an internal control. Representative of three experiments. (C) Representative H&E staining of OLTs (n=4) at 24h: Panel (a) WT+DMSO; (b) WT+Ly294002; (c) Keap1HKO+DMSO; (d) Keap1HKO+Ly294002 (magnification x100). Keap1-dependent Nrf2 activation promoted Trx1/Akt/HIF-1 signaling in mouse hepatocytes *in vitro*. (D) Western blot expression of Nrf2, Trx1 and p-Akt in primary H_2O_2-stressed hepatocyte (WT, Keap1HKO or Nrf2KO) cultures. Representative of three experiments. (E) Primary H_2O_2-stressed hepatocytes were pretreated with PI3K inhibitor (LY294002) or DMSO; p-Akt/HIF-1 expression was analyzed by Western blots. Representative of three experiments. (F) Quantitative RT-PCR-assisted detection of HIF-1 , HO-1, and Cyclin D1 in LY294002/DMSO-pretreated H_2O_2-stressed hepatocyte cultures. (G) LDH release (U/L) in LY294002/DMSO-treated hepatocytes. (F-G): (☐) WT cells; (▩) WT cells+DMSO; (▨) WT cells+Ly294002; (▨) Keap1 HKO cells; (⬚) Keap1 HKO cells+DMSO; (■) Keap1 HKO cells+Ly294002. Mean±SD; n=3-4/group; *p<0.005, **p<0.0005.

Supplementary Figure 1: Schematic representation of the regulatory mechanisms by which Keap1-Nrf2 signaling may affect liver IRI. See text for details.

Supplementary Figure 2: OLT histology and cell viability. (A) Representative H&E OLT staining (a-c; magnification x40) and TUNEL staining (d-f; magnification x100). (a/d): WT WT; (b/e): Keap1 HKO Keap1 HKO; (c/f): Nrf2 KO Nrf2 KO. (B) Apoptotic and necrotic cells in OLTs. Keap1 HKO reduced cell death in IR-stressed OLTs. Mean SD; n=4-6 mice/group. *p<0.0001. (▩) Apoptotic cells; (■) Necrotic cells; (▩) Total non-viable cells.

Supplementary Figure 3: Mouse hepatocyte (HP) cultures. (A) Representative (n=3) Western blot-assisted analysis of H_2O_2-stressed HP cultures. Keap1 silencing activated Nrf2-mediated Trx1 and promoted Akt/HIF-1 signaling. MTT assay in HP without (B) or with (C) HIF-1 inhibitor (YC-1). Mean±SD; n=3-4/group; *p<0.05, **p<0.005. (▩) WT cells+NS siRNA; (▩) WT cells+Keap1 siRNA; (■) WT cells+Nrf2 siRNA; (▨) Keap1 HKO cells+Nrf2 siRNA; (▨) WT cells+Keap1 siRNA; (▩) WT cells+Keap1 siRNA+YC-1.

Supplementary Table 1. Primers used in qRT-PCR studies.