Supplemental Data (7 figures with legends)

microRNA-687 induced by HIF-1 targets PTEN in renal ischemia-reperfusion injury

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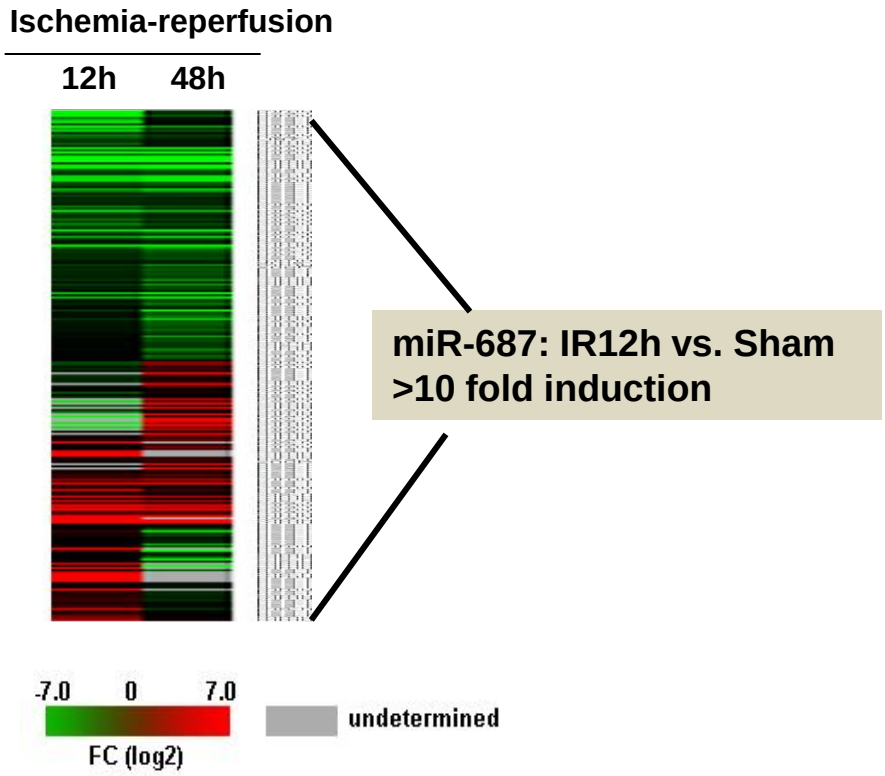
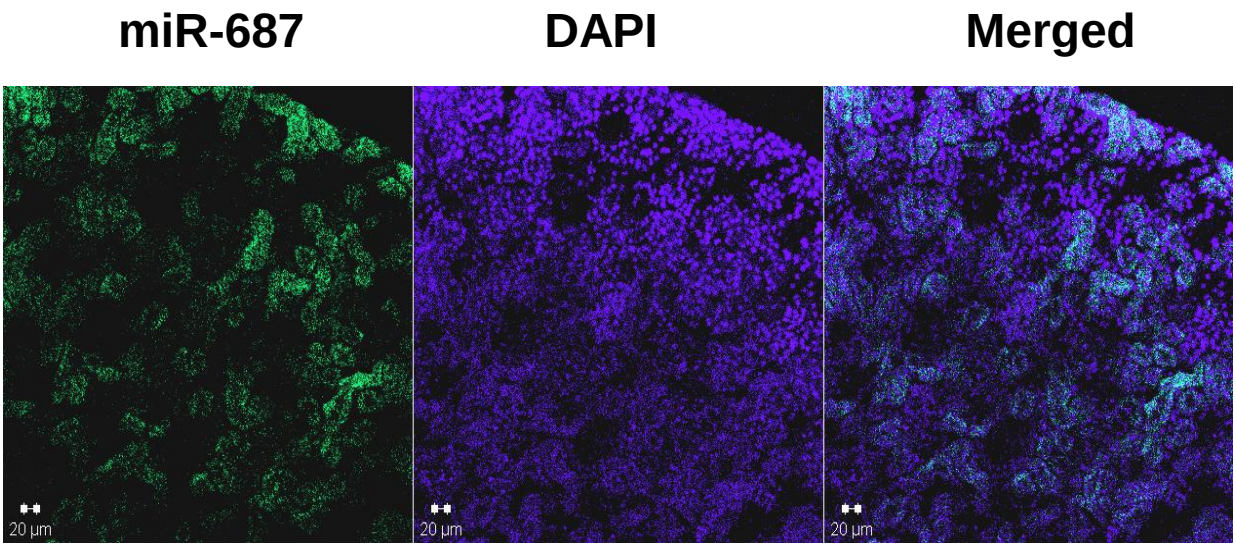
Supplemental Figure 1



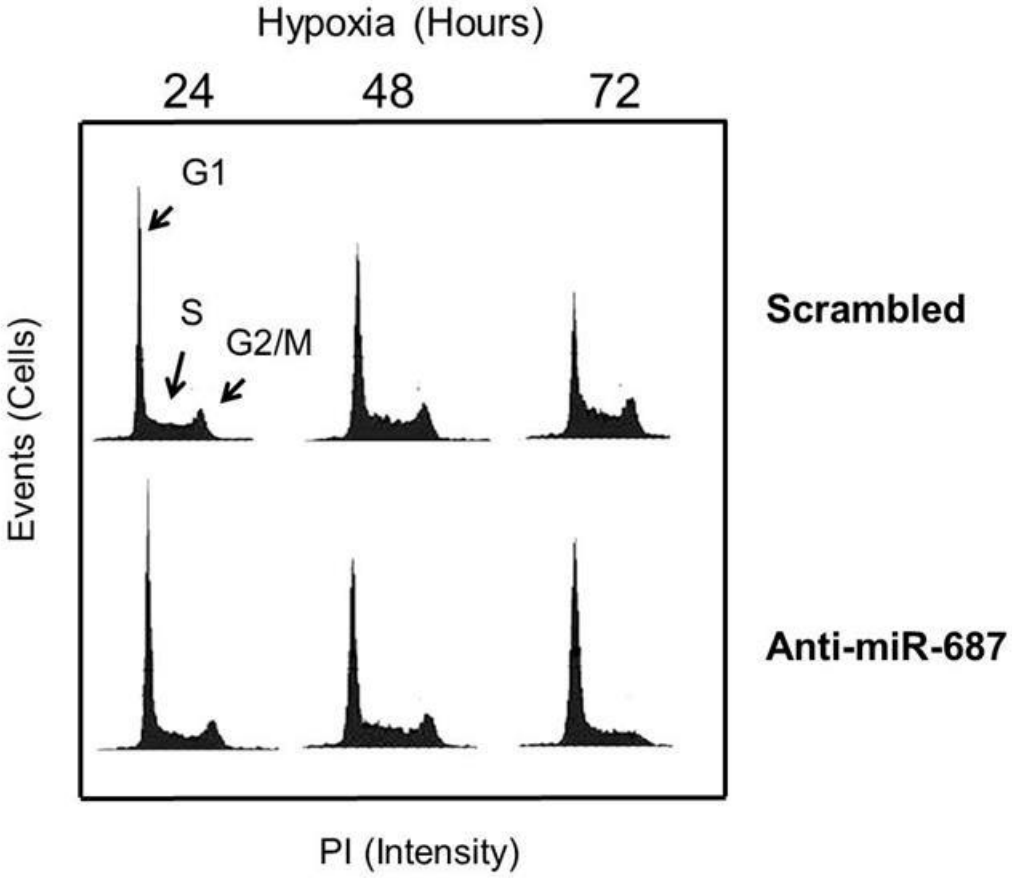
Heatmap of microarray analysis of microRNA expression during renal IRI. C57BL/6 mice were subjected to 30 minutes of ischemia followed by 12 or 48 hours of reperfusion. Sham-operated mice were used as control. Total RNA was extracted from renal cortical tissues for microRNA microarray analysis. ΔCt values of all miRNAs were used to generate the heat map.

Supplemental Figure 2



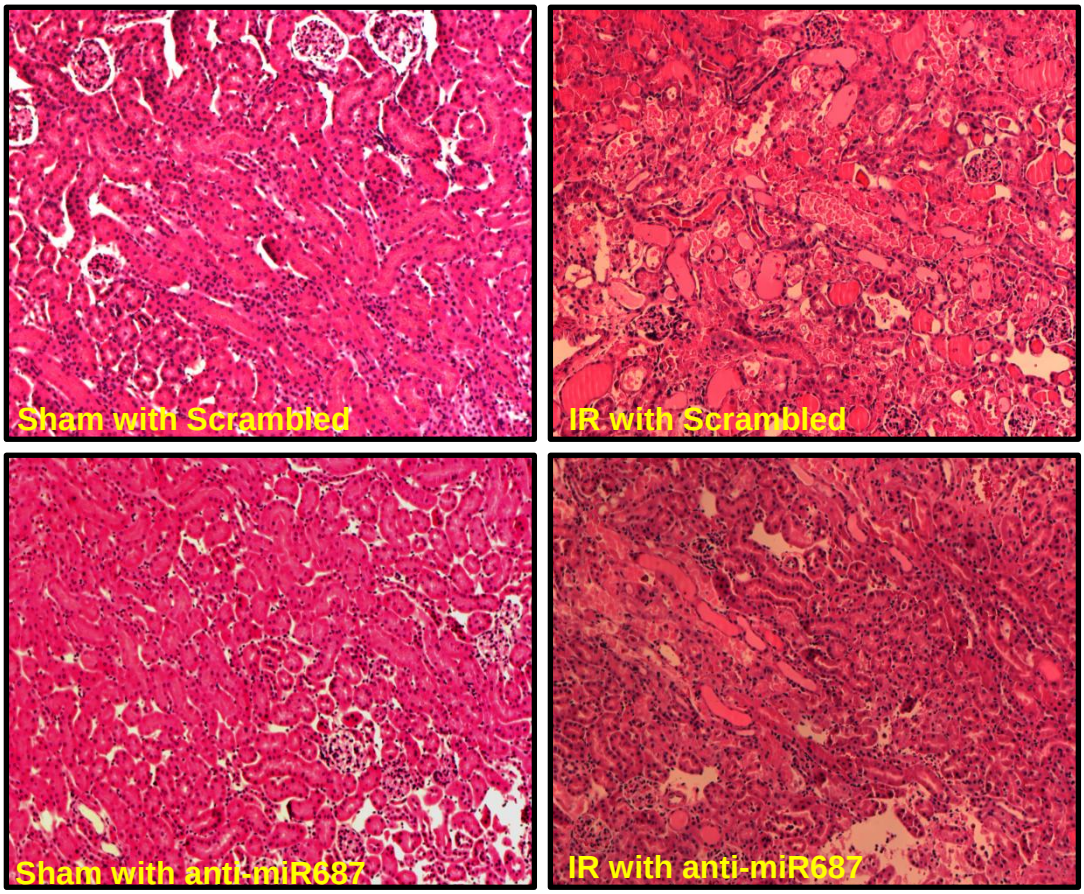
FISH analysis of miR-687 induction during renal IRI in kidney tissues. C57BL/6 mice were subjected to 30 minutes of renal ischemia followed by 12 hours of reperfusion. Cryosections of renal tissues were used for FISH analysis using a specific miR-687 probe with DAPI co-staining. Green-miR-687; Blue-nuclei. The results show induction of miR-687 in specific renal tubules in cortical tissues.

Supplementary Figure 3



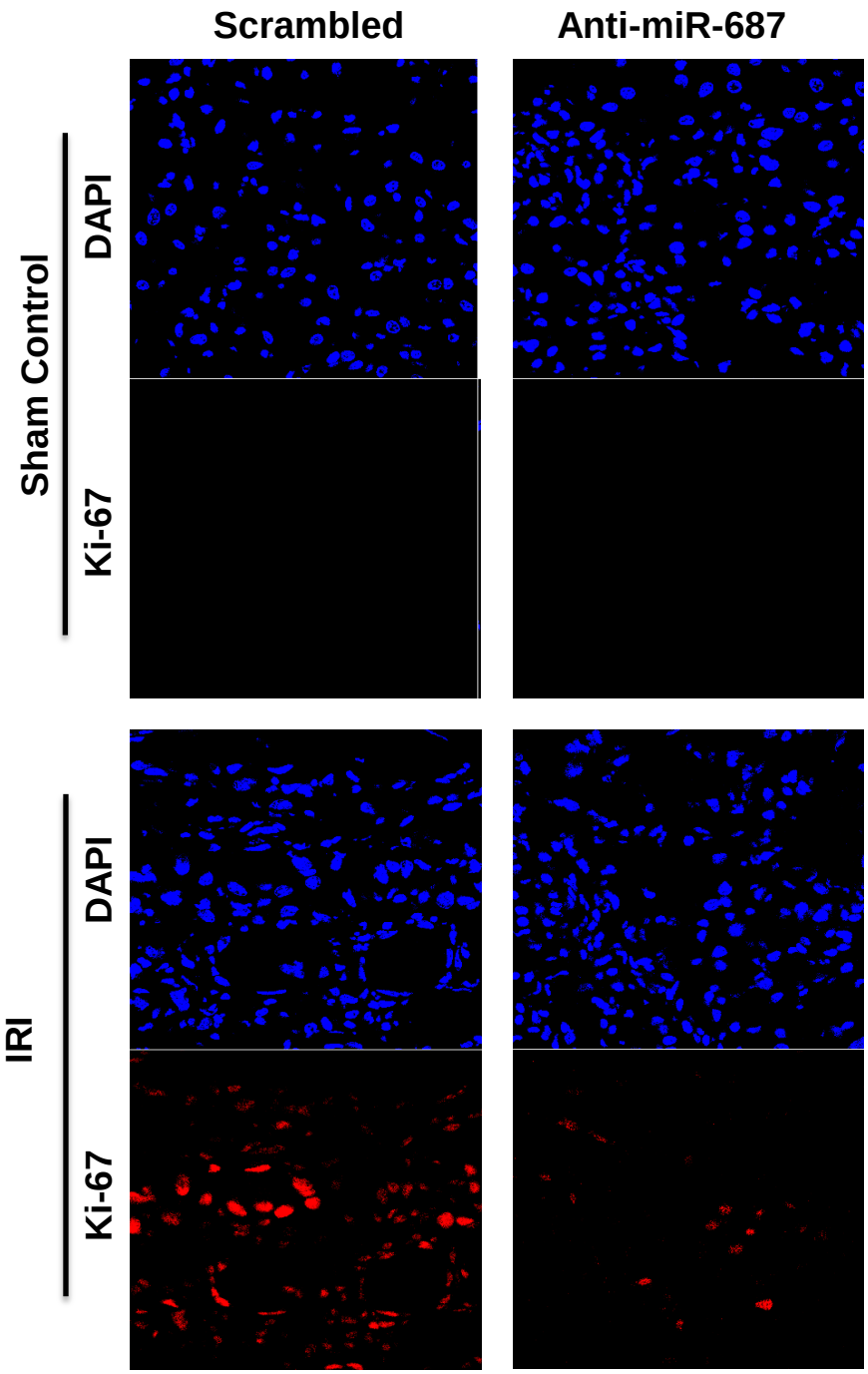
Representative FACS analysis of cell cycle. HEK cells were transfected with either scrambled LNA or anti-miR-687 LNA, and then subjected to hypoxia for 24-72 hours. For FACS analysis of cell cycle distribution of the cell population, the cells were stained with PI. The results show that hypoxia induced an increase in cells in S and G/M phase accompanied by a decrease in cells in G1 phase (Scrambled); anti-miR687 suppressed hypoxia-induced cell cycle changes.

Supplementary Figure 4



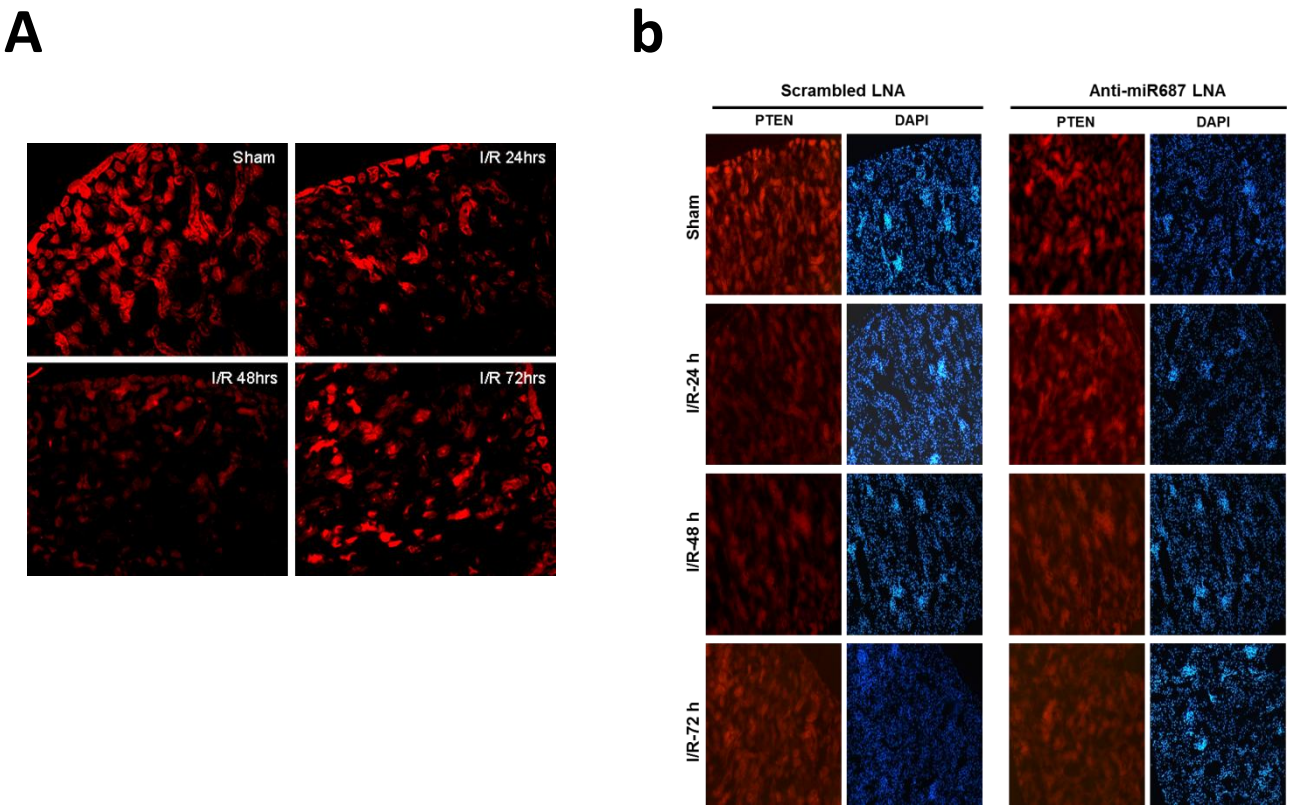
Representative histology showing the protective effect of anti-miR-687 in renal IRI. C57BL/6 mice were subjected to 30min renal ischemia and 48h reperfusion in the presence of scrambled LNA or anti-miR-687 LNA. Kidney tissues were collected for H&E staining for histological analysis.

Supplementary Figure 5



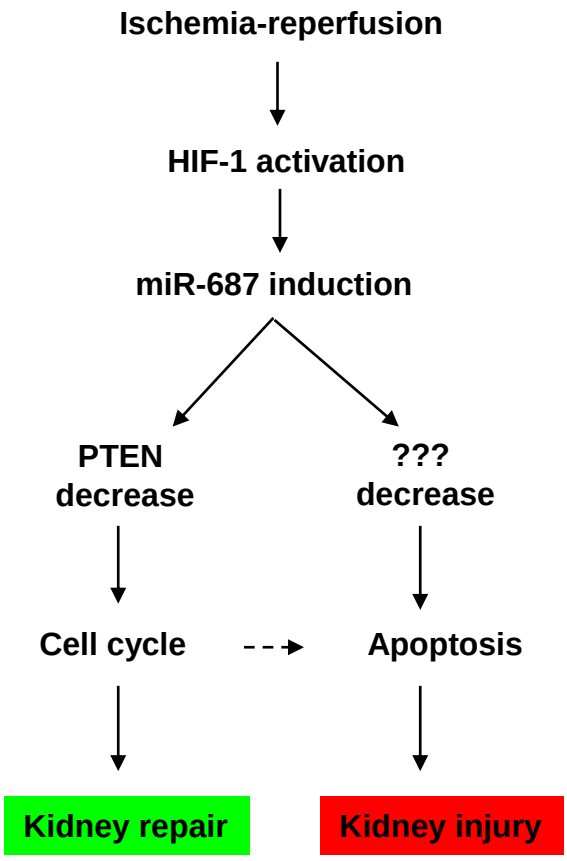
Representative ki67 staining showing the effect of anti-miR687 on cell cycle activation during renal IRI. C57BL/6 mice were subjected to renal IRI or sham operation in the presence of either scrambled or anti-miR-687 LNA. Renal tissues cryosections were processed for immunofluorescence analysis of Ki-67. The results show that renal IRI induced the number of Ki-67 positive proliferative cells, which was reduced in the presence ofanti-miR-687.

Supplemental Figure 6



PTEN decrease in renal IRI and the inhibition by anti-miR-687. **(a)** C57BL/6 mice were subjected to IRI and renal tissues were collected at indicated time-points. Cryosections of renal tissues were used for immunofluorescence analysis of PTEN expression. The results show a transient yet remarkable decrease of PTEN within 48 hours of reperfusion. **(b)** C57BL/6 mice were subjected to IRI in the presence of either scrambled or anti-miR-687 LNA. Renal tissues sections were collected at indicated time-points for immunofluorescence analysis of PTEN. The results show that anti-miR-687 prevented the decrease in PTEN observed during the early (24-48h) reperfusion period in the scrambled LNA group.

Supplemental Figure 7



Schematic diagram of HIF-1/miR-687/PTEN signaling axis in renal IRI. Ischemia-reperfusion leads to the activation of HIF-1 in kidney tubular cells, which transactivates the expression and induction of miR-687. miR-687 then targets and represses PTEN, contributing to cell cycle activation and cell proliferation for kidney repair. miR-687 may also lead to apoptosis by targeting other downstream genes. Dashed arrow: the activation of cell cycle may increase cellular sensitivity to apoptosis.