Supplementary Information

**Title:** miR-146a is essential for lipopolysaccharide (LPS)-induced cross-tolerance against kidney ischemia/reperfusion injury in mice.

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Supplementary S1: (a) Time course of miR-146a and miR-21 levels between kidney I/R injury and sham controls groups. miR-146a and miR-21 was examined and normalized to U6 by TaqMan-based real-time PCR, fold changes were calculated against the mean value of Sham group at each time point. (n=3 mice per each time point.*P<0.05,**P < 0.01,#P<0.001,vs. Sham group, respectively). (b) Proinflammatory cytokine mRNA profile in mice at 24h after kidney I/R surgery with LNA-anti-miR-146a treatment. LNA-anti-miR-146a and LNA anti-scrambled oligonucleotide were administered 24h before I/R surgery. (c) Representative periodic acid–Schiff (PAS)-stained renal sections from mice treated with either I/R+Scrambled control and I/R+anti-miR-146a procedures (original magnification ×200). (d) Semiquantitative analysis of tubular damage in I/R+Scrambled control and I/R+anti-miR-146a mouse kidney at 24h after reperfusion. ("P<0.001 vs. I/R+ Scrambled control).
Supplementary S2 (a) The expression of phosphorylated p65 (p-p65) (nuclear extracts), and IkBα, BeL-xL, T-P65 (cytosolic extracts) in the 6h groups was confirmed by western blot analysis. Co-detection of Histone H3 (nuclear) and β-actin (cytoplasm) were performed to assess equal loading. (b) Western blots from three experiments were quantified by densitometry analysis. The ratios of phosphor-protein to total protein for p65 were calculated. The fold changes relative to LPS+Sham protein are shown. (n=3, #P<0.001, **P<0.01, vs. LPS+I/R+Scrambled control group).(c) The expression of phosphorylated p65 (p-p65) (nuclear extracts), and IkBα, BeL-xL, T-P65 (cytosolic extracts) in the 48h groups were confirmed by western blot analysis.(d) The Western blots from three experiments were quantified by densitometry analysis. The ratios of phosphor-protein to total protein for p65 were calculated. The fold changes relative to LPS+Sham protein are shown. (n=3, **P<0.01, *P<0.05 vs. LPS+I/R+Scrambled control group)
Supplementary S3: (a) Representative kidney sections immunostained for TLR4 (original magnification ×200 and ×400, Bar=50um, the black arrow indicates positive area). TLR4 was expressed by tubular cells and displayed a similar marked elevation in mice receiving anti-miR-146a or anti-scrambled oligonucleotides at 24h after I/R injury. (b) TLR4 expression was scored in mouse kidney. (n=6mice per group, *P<0.05, vs. LPS+I/R+Scrambled control).