Legends for Supplementary Figures

Supplementary Figure 1. Relative distribution of mitochondrial and cytosolic proteins. Mitochondrial fractions were prepared from pooled mice livers (n≥5 per group) freshly obtained from different groups by differential centrifugation followed by two separate washing steps, as described.[22,23] Relative purities of the mitochondria fractions with potential contamination of the cytosolic fractions were determined by using a specific marker protein for each fraction. Mitochondrial proteins and cytosolic proteins (20 μg/lane) were separated by 12% SDS-PAGE and subjected to Coomassie blue staining (left panels) and immunoblot analysis (right panels) by using the specific antibody against: (A) α-ATP synthase (from InVitrogen Co.) and (B) peroxiredoxin-II (catalog No. LF-PA0007 from LabFrontier Co.) as specific marker proteins for mitochondrial and cytosolic proteins, respectively. Lanes #1, 2, and 3 represent the mitochondrial and cytosolic proteins from mouse liver tissues of: sham control, IR without MnTMPyP, and IR with MnTMPyP, respectively. Mitochondrial peroxiredoxin-III could have been recognized by the anti-peroxiredoxin-II antibody (B, right panel). This figure represents a typical result from two separate experiments.

Supplementary Figure 2. Schematic diagram to identify oxidatively-modified Cys residues of mitochondrial proteins. Oxidized and/or *S*-nitrosylated Cys residues of mitochondrial (Mito) proteins in sham control or I/R injured liver tissues in the absence and presence of MnTMTyP were labeled with *N*-ethylmaleimide. The *N*-ethylmaleimide-labeled proteins were then treated with either 15 mM DTT or Asc for 30 min to reduce the oxidatively-modified Cys residues before they were incubated with biotin-*N*-maleimide (biotin-NM), as detailed.[23,29,30] The biotin-NM labeled proteins in different groups were then purified with streptavidin-agarose beads before they were subjected to immunoblot analysis or 2-DE analysis.

Supplementary Figure 3. The levels of serum transaminases following I/R injury in the absence and presence of MnTMPyP. (A) Serum ALT levels following I/R injury (reperfusion for 10-h or 24-h) without or with MnTMPyP are shown. *significantly

different from the sham controls at $p< 0.001$; **significantly different from the sham controls at $p< 0.01$; #significantly different from the I/R injury samples (reperfusion for 10-h without MnTMPyP) at $p< 0.005$; ##significantly different from the I/R injury samples (reperfusion for 24-h without MnTMPyP) at $p< 0.05$. (B) Serum AST levels following I/R injury (reperfusion for 10-h or 24-h) without or with MnTMPyP are shown. *significantly different from the sham controls at $p< 0.005$; **significantly different from the sham controls at $p< 0.05$; #significantly different from the I/R injury samples (reperfusion for 10-h without MnTMPyP) at $p< 0.01$; ##significantly different from the I/R injury samples (reperfusion for 24-h without MnTMPyP) at $p< 0.05$.

Supplementary Figures 4 and 5. Typical hematoxilin and eosin stained liver slides for different groups (reperfusion for 10-h) in the absence or presence or MnTMTyP. Each liver slice was fixed in 10% neutral formalin and then paraffin-embedded. All sections of formalin-fixed liver tissues (5 μm slices) were stained with H&E. Magnification: 100x (Supplementary Fig. 4) and 400x (Supplementary Fig. 5). Large areas of necrotic damage are clearly visible in the absence of MnTMPyP. However, this peroxynitrite scavenger significantly reduced the severity of the necrotic damage observed after 10-h reperfusion. Similar histology patterns were also observed in 4 – 5 liver sections/group.

Supplementary Figures 6 and 7. Typical hematoxilin and eosin stained liver slides for different groups (reperfusion for 24-h) in the absence or presence or MnTMTyP. Each liver slice was fixed in 10% neutral formalin and then paraffin-embedded. All sections of formalin-fixed liver tissues (5 μm slices) were stained with H&E. Magnification: 100x (Supplementary Fig. 6) and 400x (Supplementary Fig. 7). More prominent necrotic damage are clearly visible in the absence of MnTMPyP. Again, this scavenger significantly reduced the severity of the necrotic damage observed after 24-h reperfusion. Similar histology patterns were also observed in 4 – 5 liver sections/group.

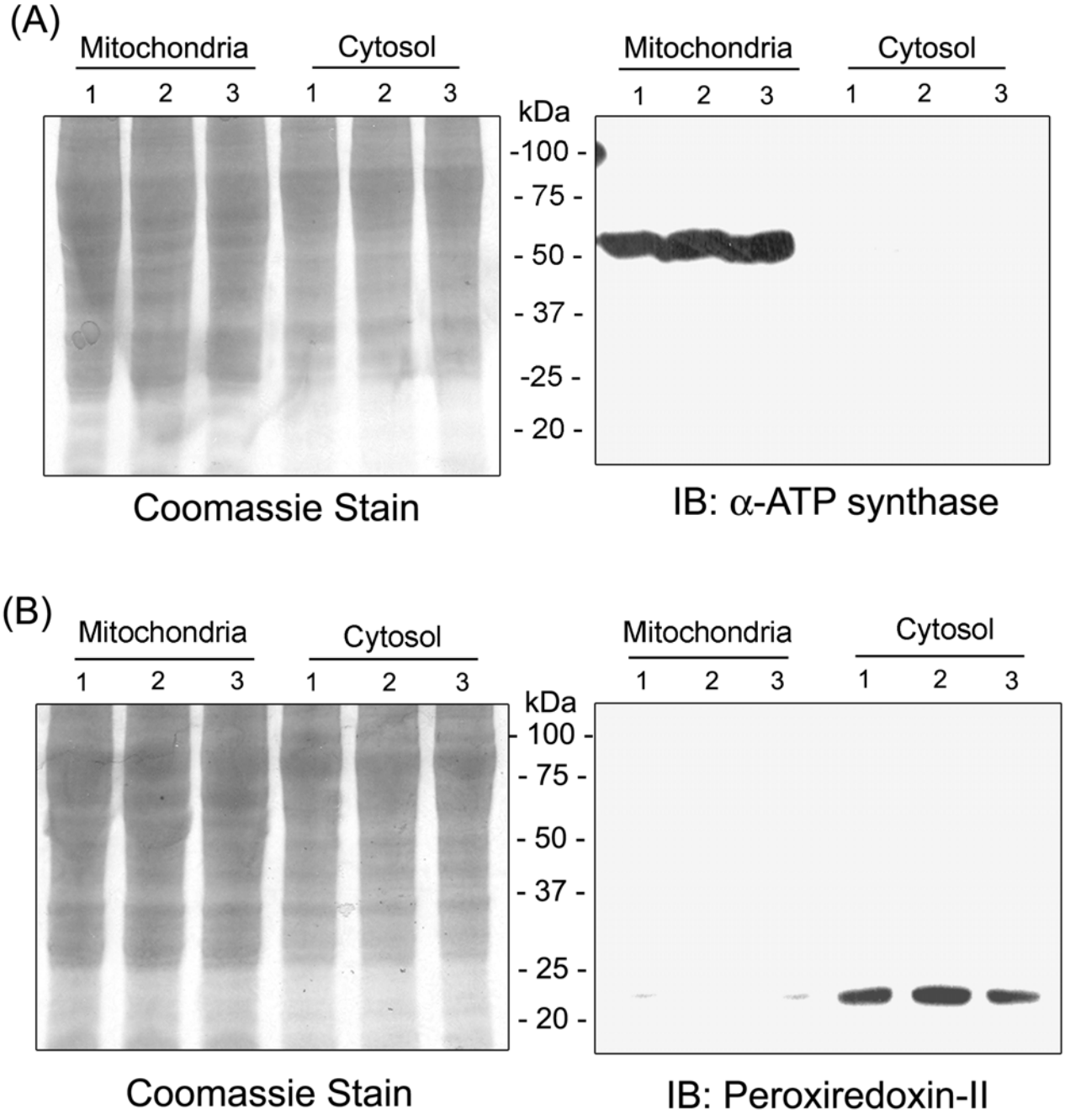
Supplementary Figures 8 and 9. Comparison of oxidized and *S*-nitrosylated mitochondrial proteins by 2-DE in hepatic I/R injury (reperfusion for 10-h or 24-h as

indicated) without or with MnTMPyP. Oxidized mitochondrial proteins (10 mg/sample) from sham-control (top) and I/R injured mice (middle and bottom panels) in the absence (left panels) or presence (right panels) of MnTMPyP, as indicated. All mitochondrial proteins from three different groups as indicated were processed concurrently and labeled with *N*-ethylmaleimide. The *N*-ethylmaleimide-labeled proteins were then treated with either 15 mM DTT (Supplementary Fig. 8) or Asc (Supplementary Fig. 9) for 30 min to reduce the oxidatively-modified Cys residues before they were incubated with biotin-*N*-maleimide (biotin-NM), as detailed.[23,29,30] The biotin-NM labeled proteins in different groups were then purified with streptavidin-agarose beads before they were subjected to 2-DE analysis. This figure represents a typical result from two separate experiments.

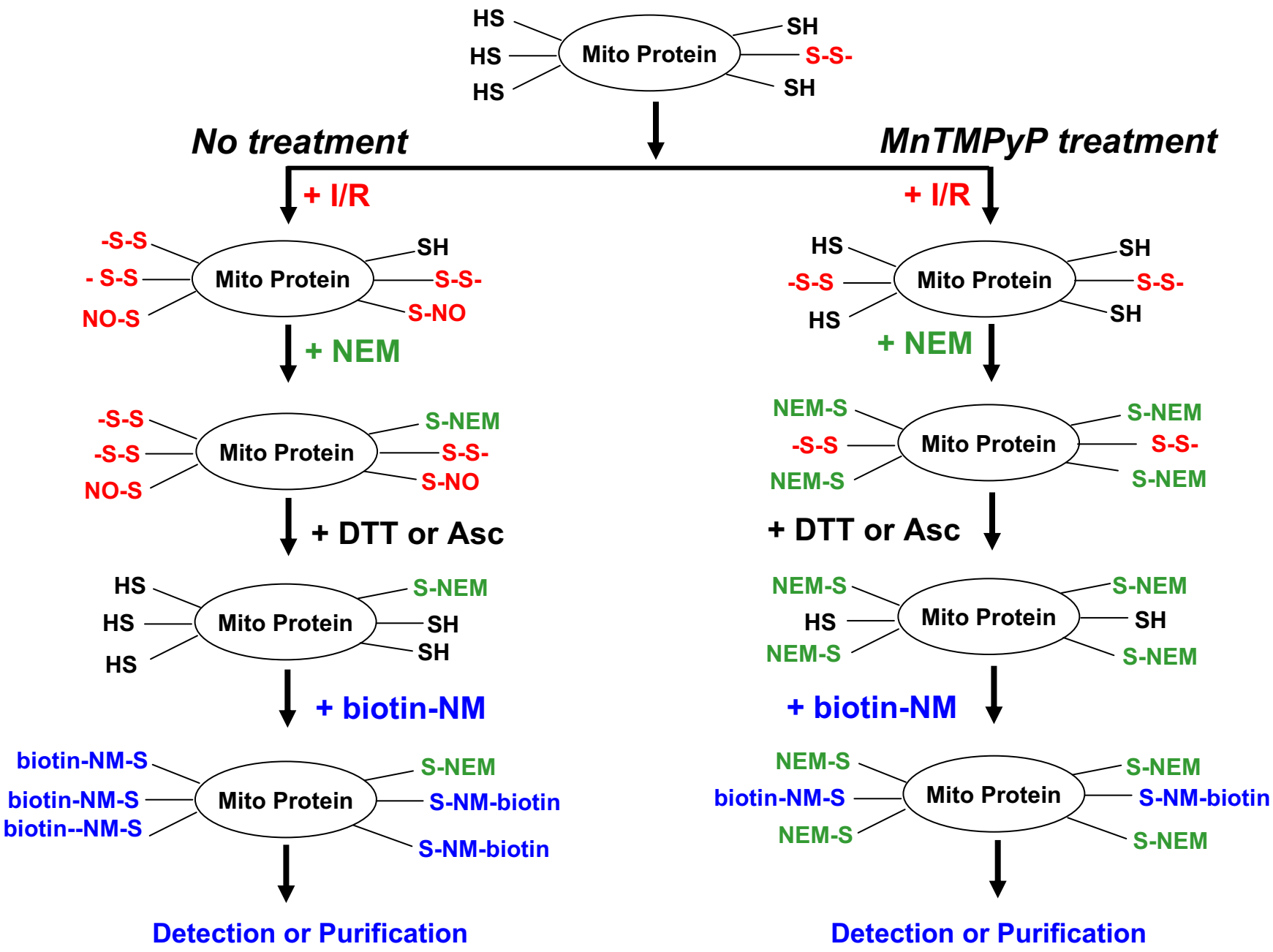
Supplementary Figure 10. Inactivation of mitochondrial ALDH2 activity and the level of lipid peroxidation in I/R injured mice (reperfusion for 10-h or 24-h) without or with MnTMPyP. (A) Catalytic activities of mitochondrial ALDH2 from the indicated liver samples were determined and presented. *significantly different from the sham controls at $p< 0.005$; #significantly different from the I/R injury samples (reperfusion for 10-h without MnTMPyP) at $p< 0.005$; ##significantly different from the I/R injury samples (reperfusion for 24-h without MnTMPyP) at $p< 0.001$. (B) Hepatic malondialdehyde (MDA) levels in differently treated mice were measured and presented. *significantly different from the sham controls at $p<0.01$; **significantly different from the sham controls at $p< 0.005$; #significantly different from the I/R subjected samples (reperfusion for 10-h without MnTMPyP) at $p< 0.05$; ##significantly different from the I/R subjected samples (reperfusion for 24-h without MnTMPyP) at $p< 0.05$.

Supplementary Figure 11. Inactivation of ATP synthase and complex I activities following hepatic I/R injury. (A) Mitochondrial ATP synthase activities from sham control and I/R injured mouse livers without or with MnTMPyP are presented. *significantly different from the sham controls at $p< 0.0005$; #significantly different from the I/R injury samples (reperfusion for 10-h without MnTMPyP) at $p< 0.0005$; ##significantly different from the I/R injury samples (reperfusion for 24-h without MnTMPyP) at $p< 0.0005$. (B) NADH-ubiquinone oxidoreductase (Complex I) activities in indicated groups were determined and presented. *significantly different from the

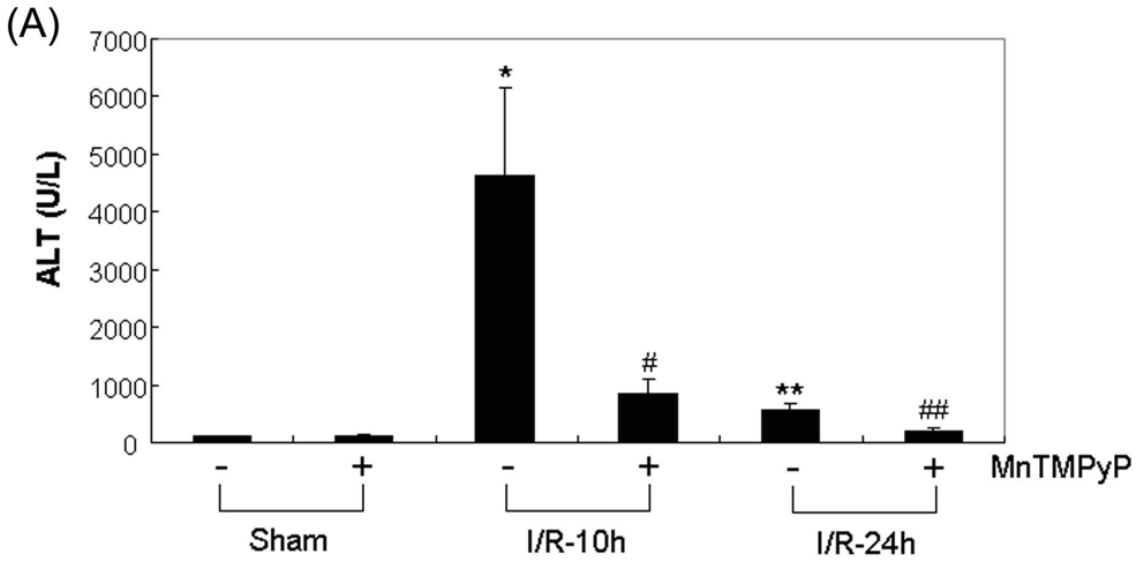
sham controls at $p< 0.005$; **significantly different from the sham controls at $p< 0.01$; # significantly different from the I/R subjected samples (reperfusion for 10-h without MnTMPyP) at $p< 0.005$; ##significantly different from the I/R subjected samples (reperfusion for 24-h without MnTMPyP) at $p< 0.05$.

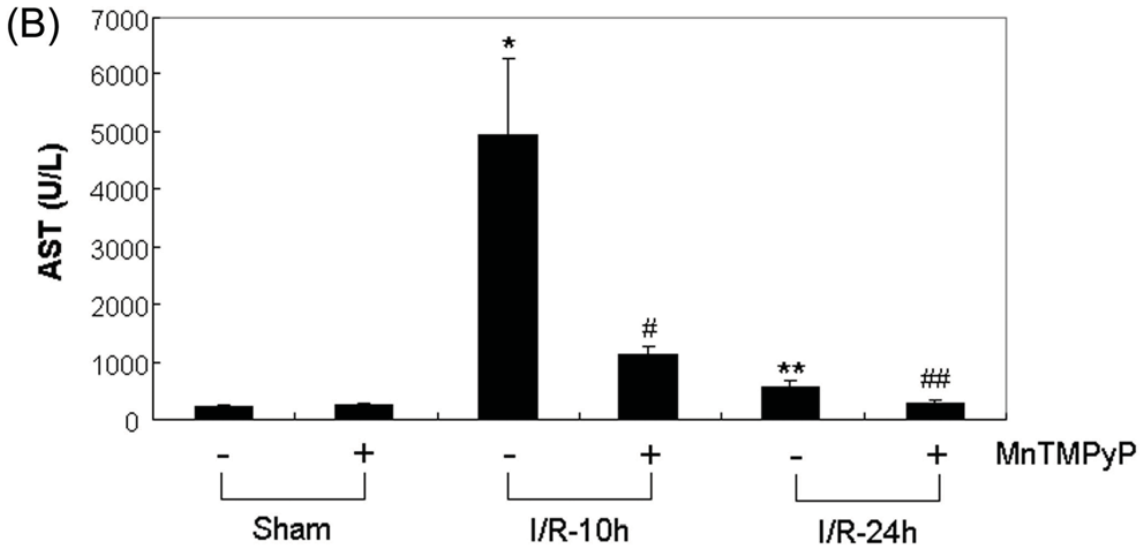


Moon *et al.*, Supplementary Figure 1

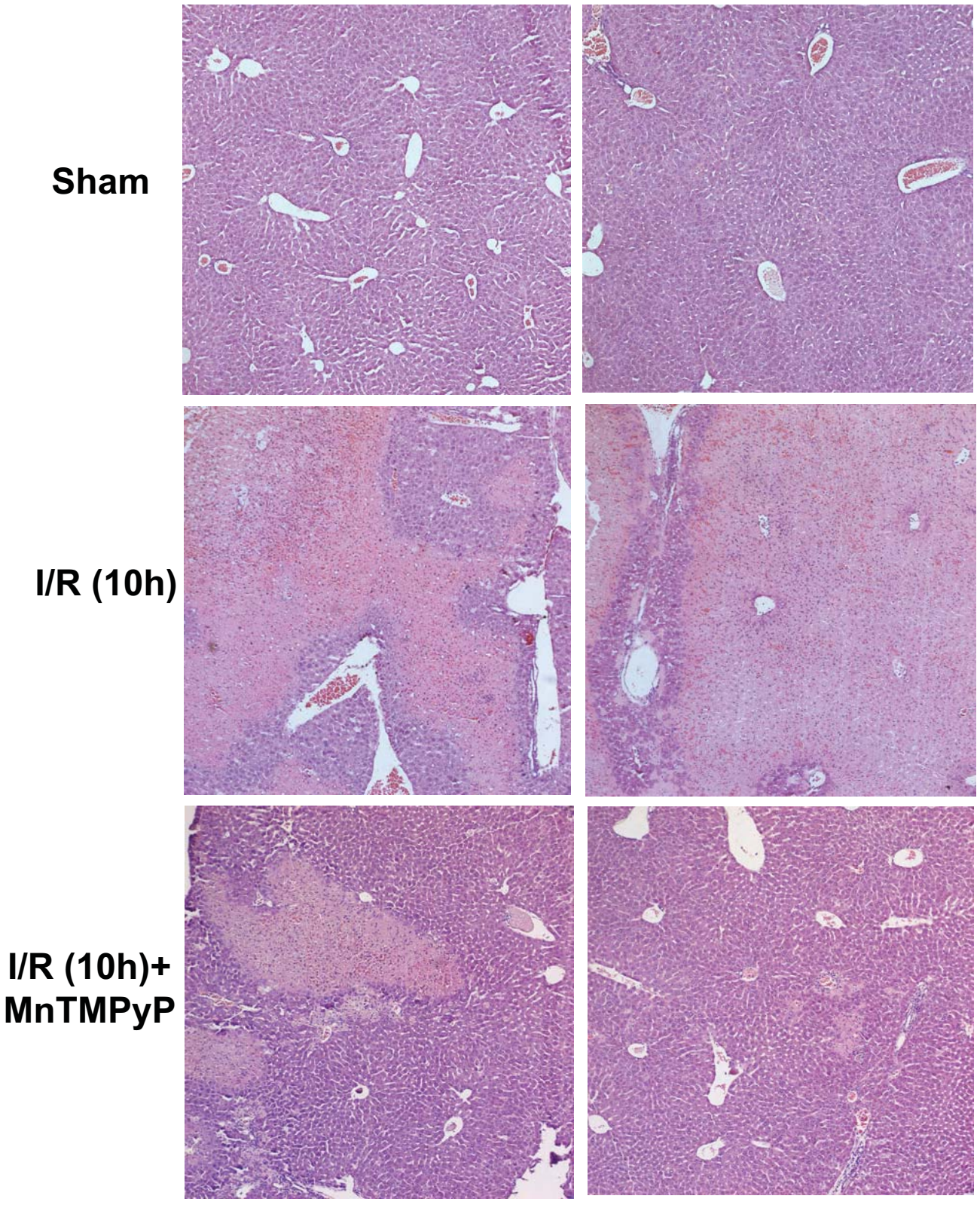


Moon *et al.*, Supplemental Fig. 2

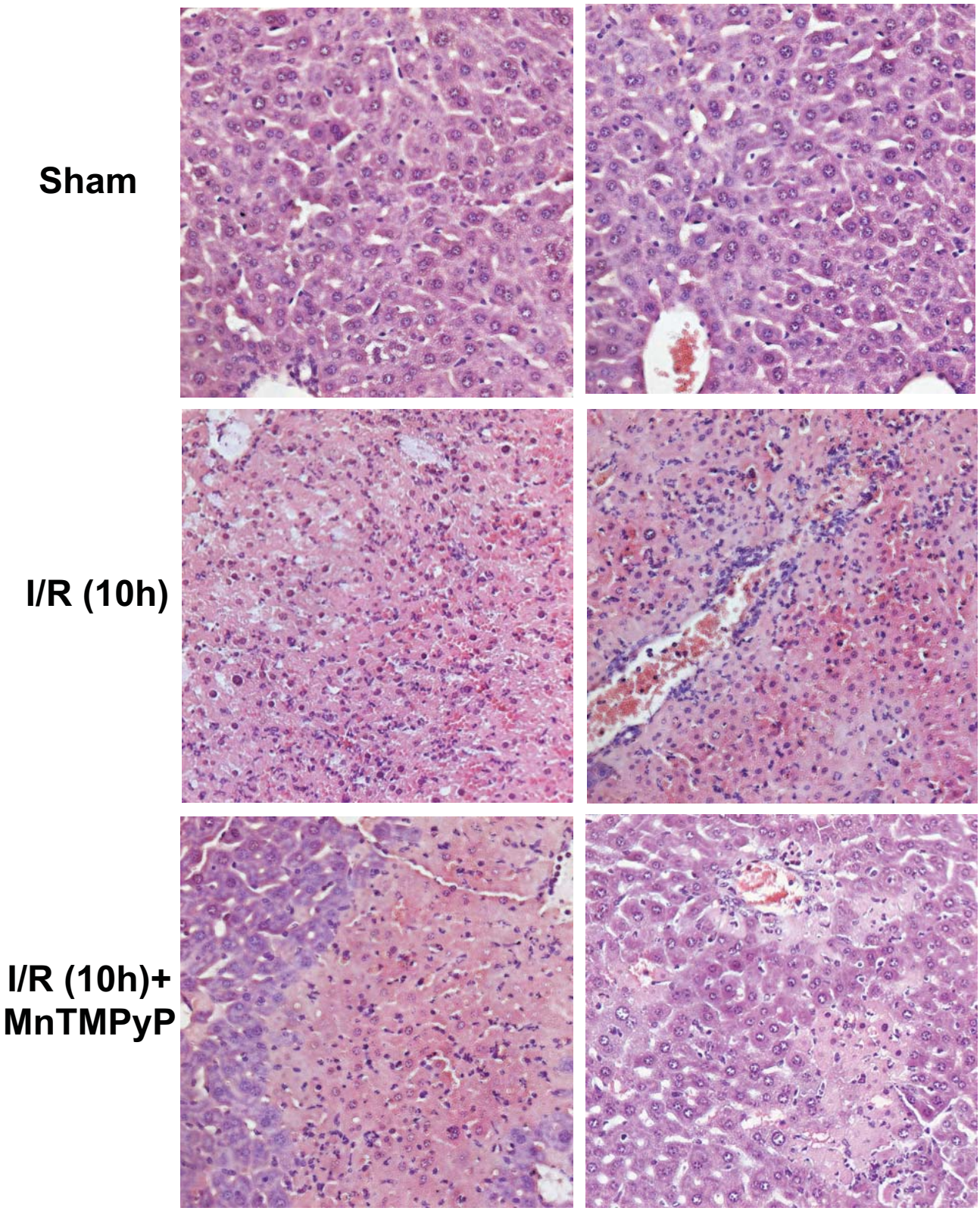




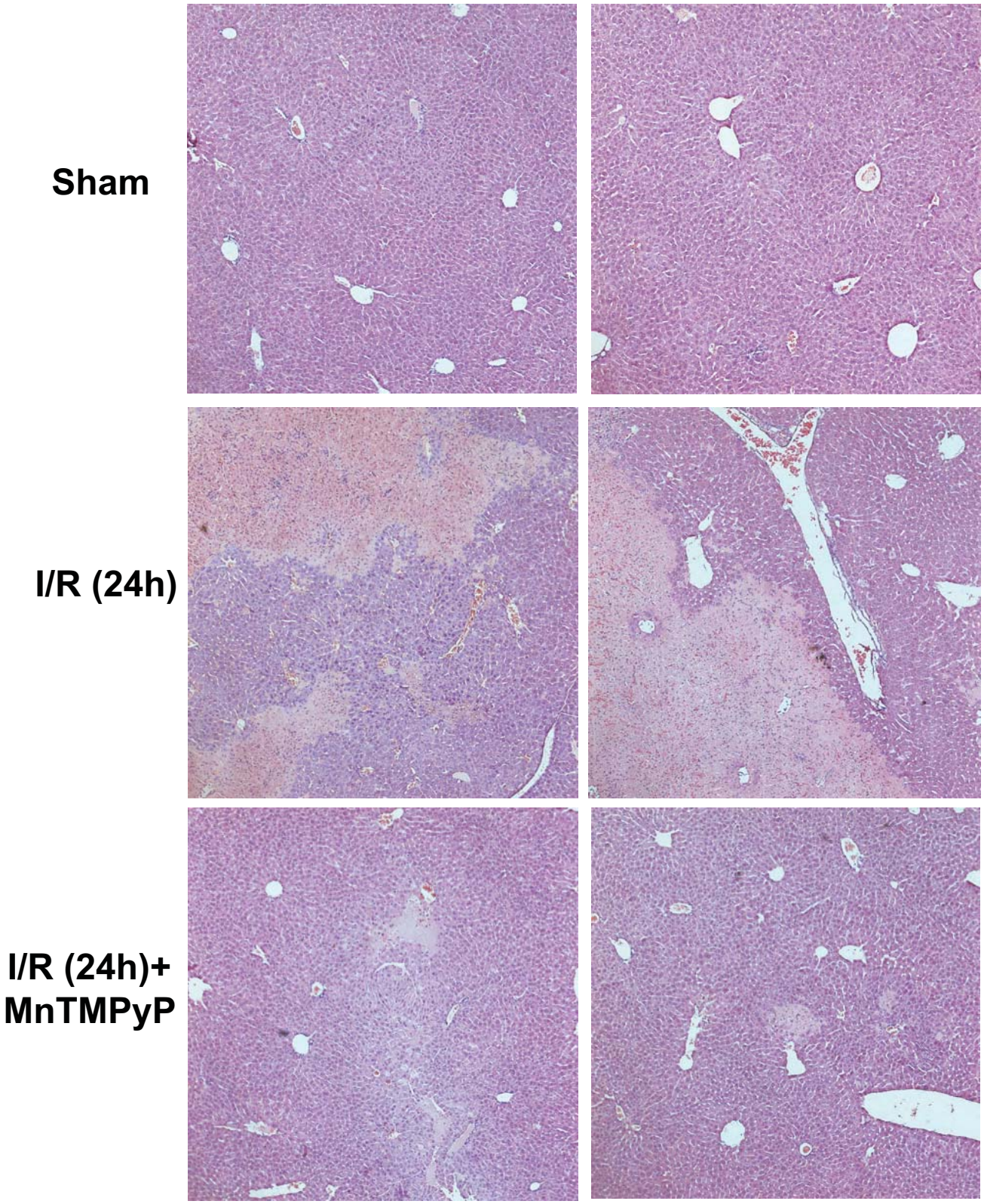
Moon *et al.*, Supplemental Figure 3



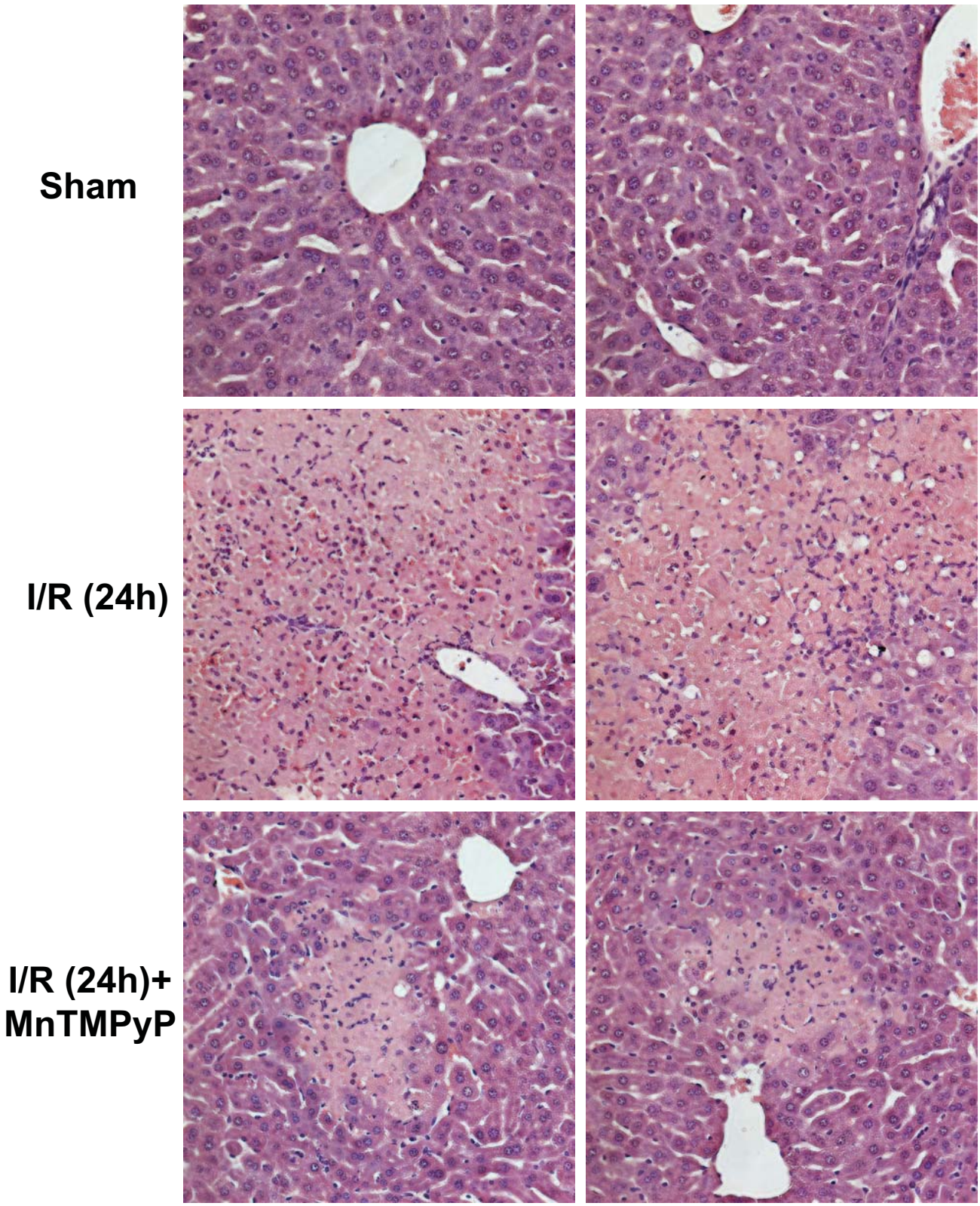
Moon *et al.*, Supplemental Figure 4.



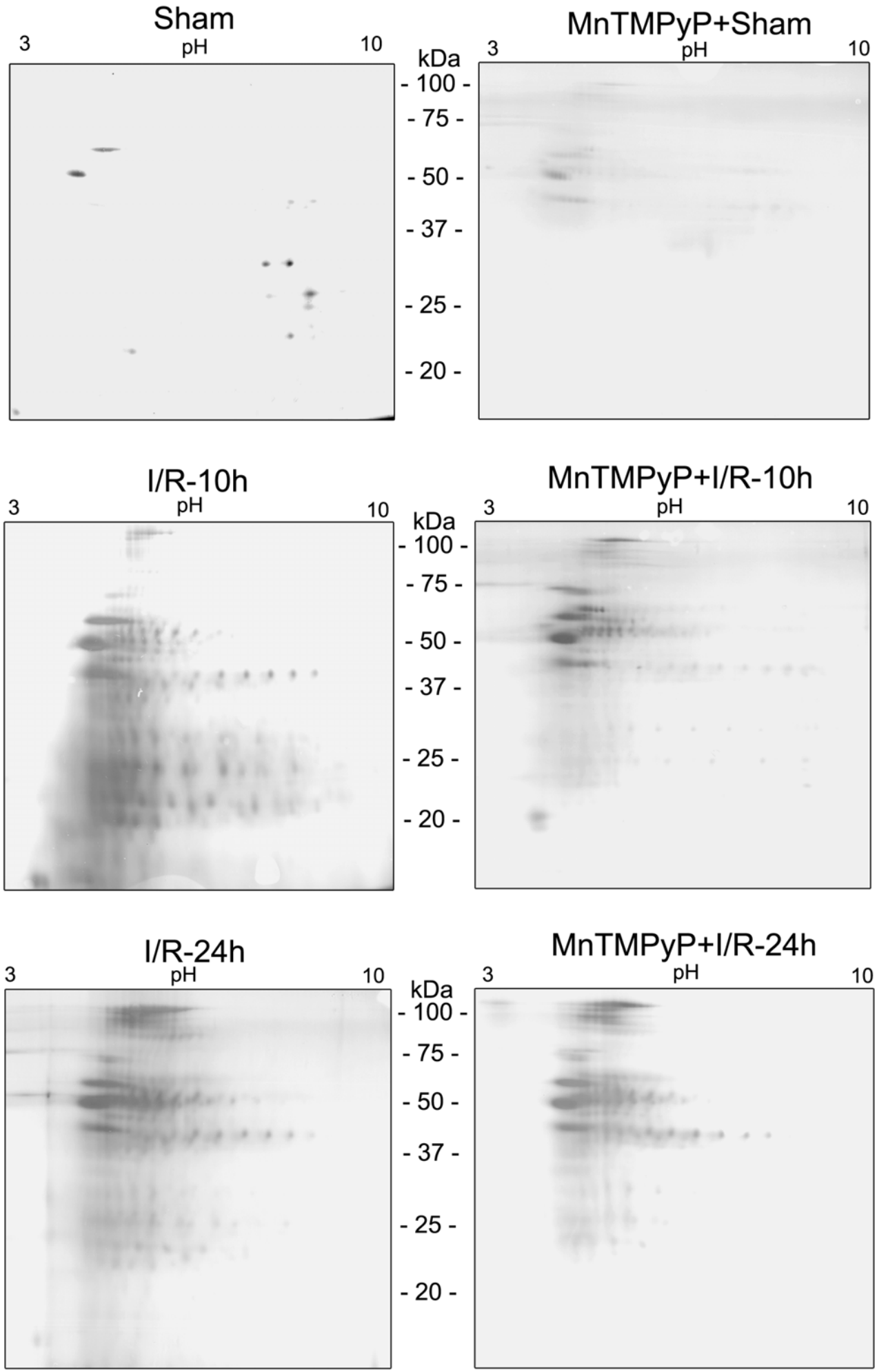
Moon *et al.*, Supplemental Figure 5.



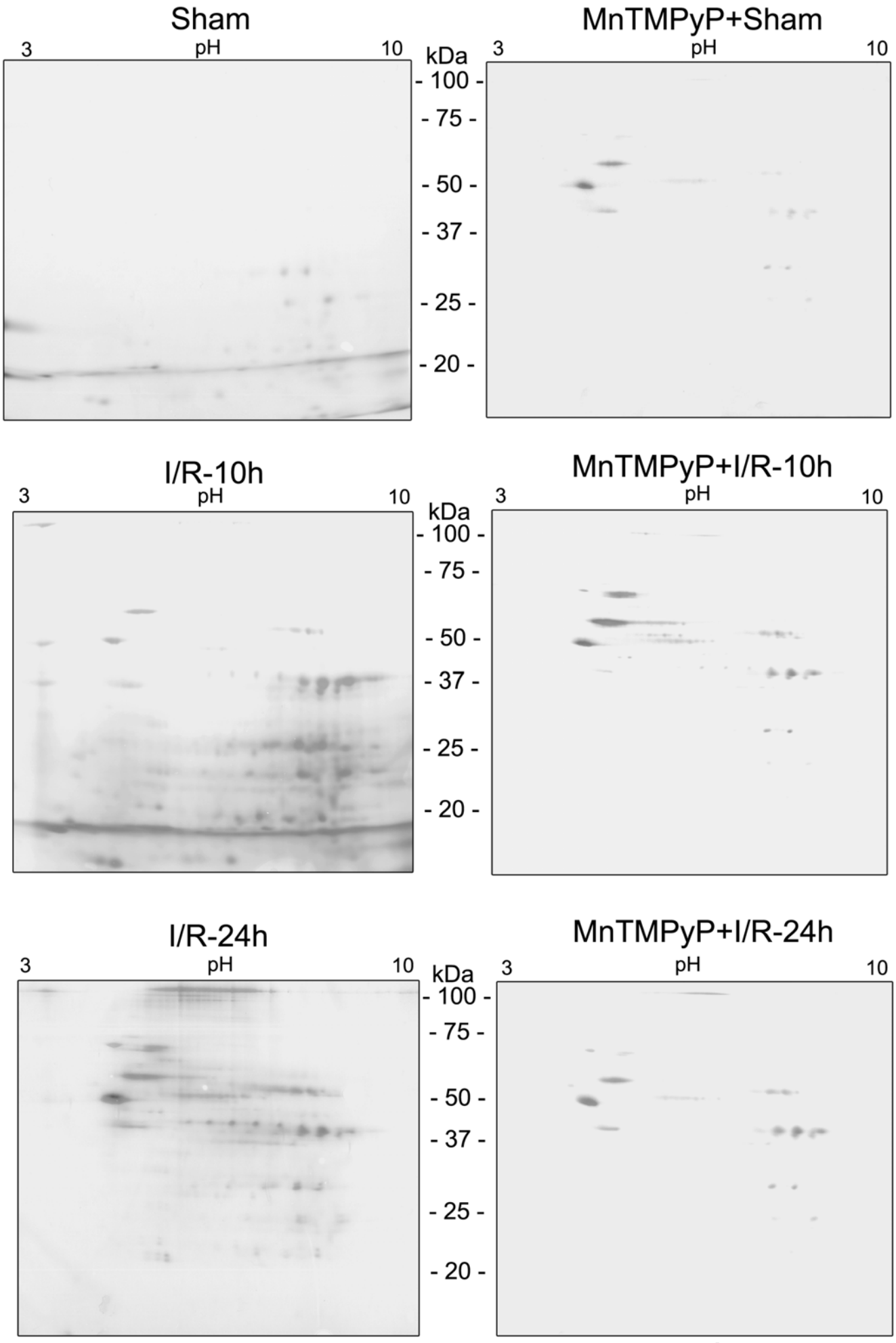
Moon *et al.*, Supplemental Figure 6.



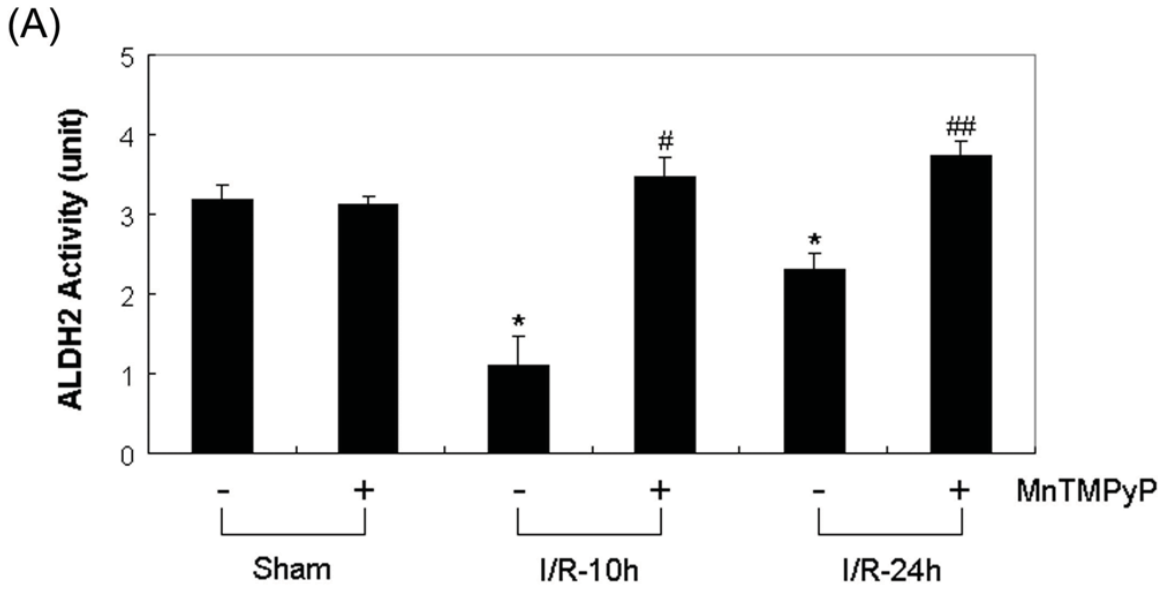
Moon *et al.*, Supplemental Figure 7.

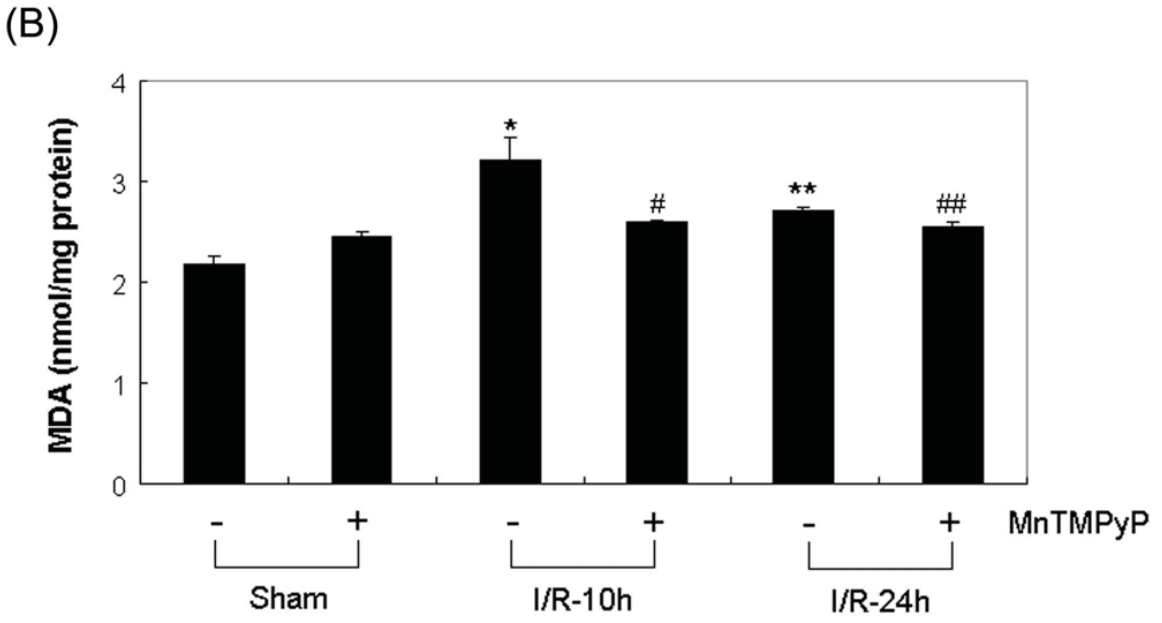


Moon *et al.*, Supplemantary Figure 8



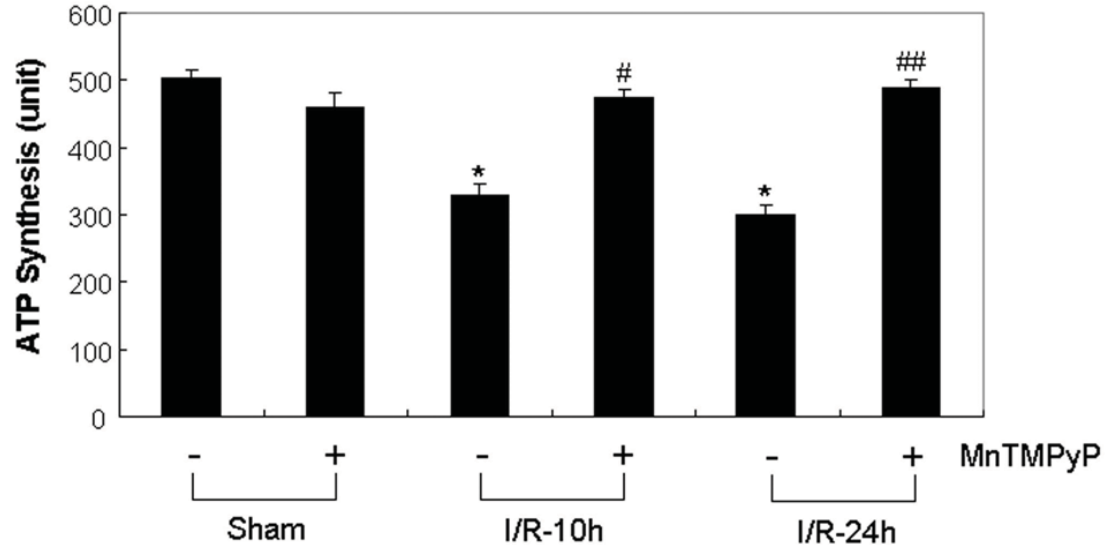
Moon *et al.*, Supplemantary Figure 9





Moon *et al.*, Supplemental Figure 10.

(A)



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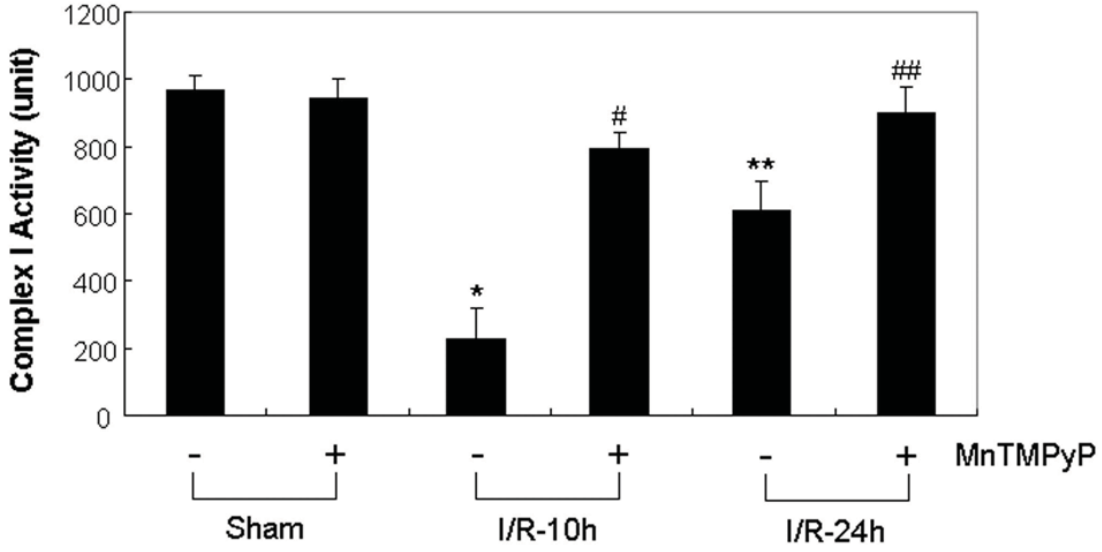

Moon *et al.*, Supplemental Figure 11.