Supplementary Information

Nanoparticle-Delivered miR-486-5p Inhibits H2O2-Induced Injury in Cultured Endothelial and Kidney Tubular Epithelial Cells

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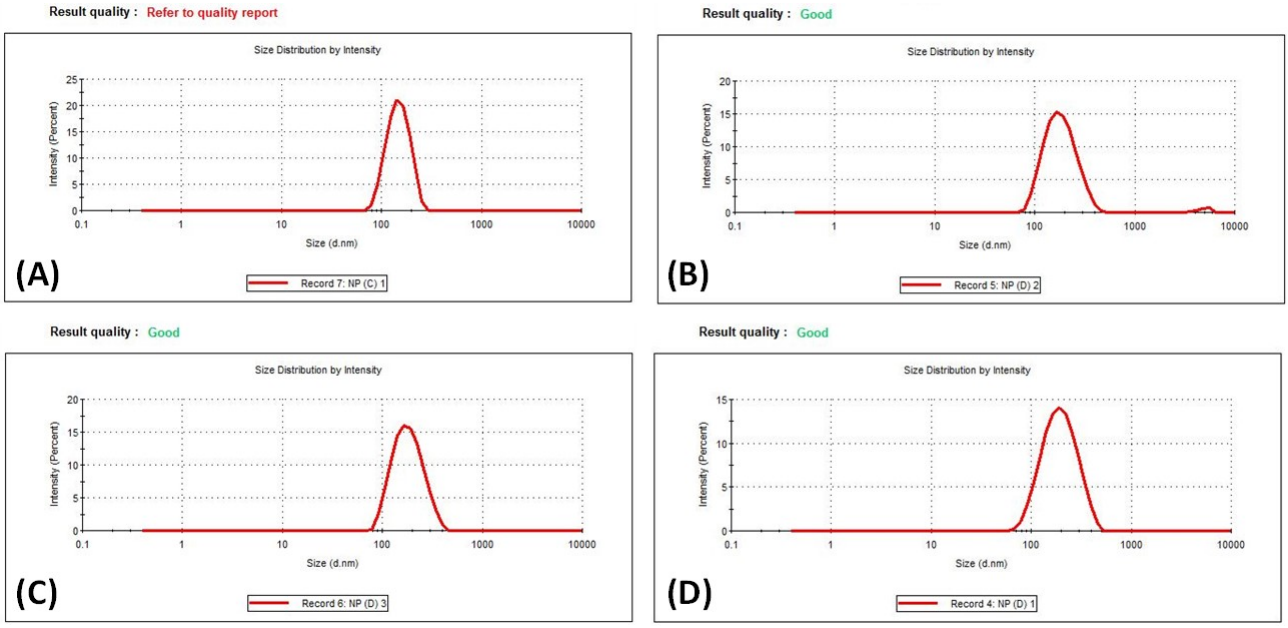

Fig.S1 | Dynamic light scattering (DLS) data measured by Zetasizer. Measurement of NP size distribution on intensity-size graphs for (A) polymeric (PNP), (B) hybrid (HNP), (C) poloxamer-based 1 (Pl-NP1), and (D) poloxamer-based 2 nanoparticles (Pl-NP2).

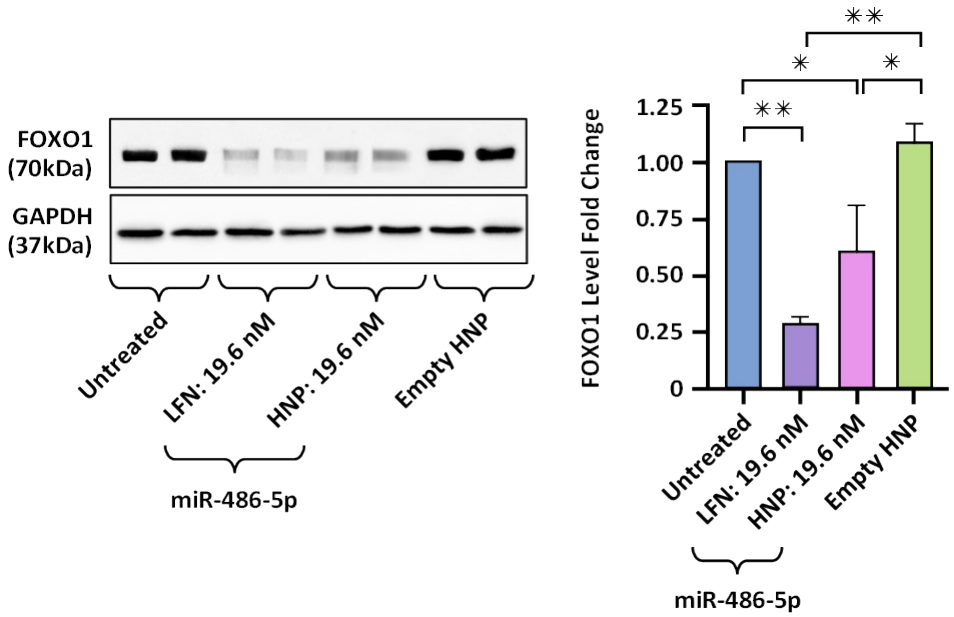

Fig.S2 | **Functional assessment of hybrid nanoparticle (HNP)-encapsulated miR-486-5p on HK2 cells.** Mean levels of FOXO1 protein relative to negative control (untreated cells) and the housekeeping GAPDH protein (loading control), measured by western blotting in HK2 cells (n=3) 72 h post-treatment with HNPs that were either empty or encapsulating 19.6 nM of miR-486-5p. Each pair of bands represents technical duplicates. Lipofectamine (LFN)-borne miR-486-5p acted as positive controls. ↑P<0.05. ↑↑P<0.01. Statistical analysis performed by one- or two-way ANOVA. HNP hybrid nanoparticle. Error bars represent standard error. FOXO1 forkhead box O1. GAPDH glyceraldehyde-3-phosphate dehydrogenase.

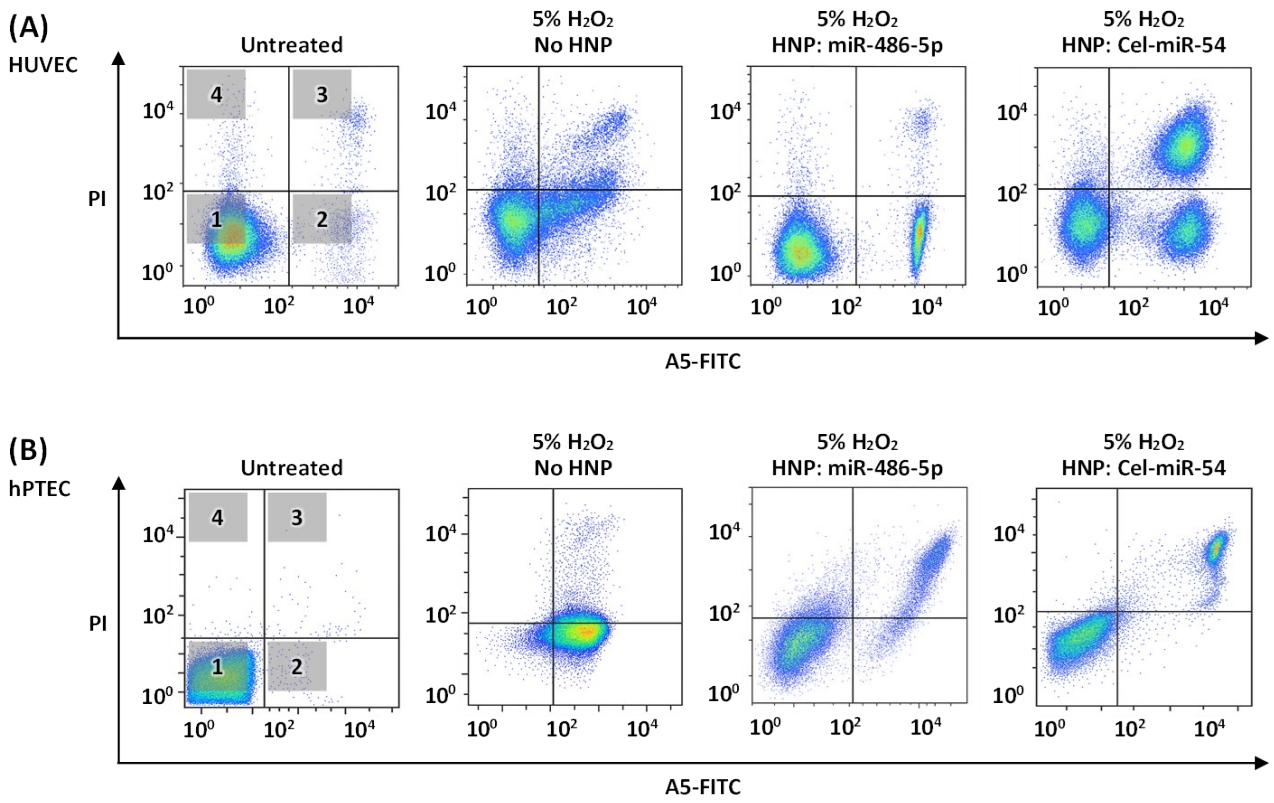

Fig.S3 | Apoptosis assessment in cells treated with miR-486-5p-loaded hybrid nanoparticles (HNPs). Flow cytometry plots depicting apoptotic stages of **(A)** HUVECs (n=1,2) and **(B)** hPTECs (n=1) at 72 h post-treatment with HNPs that were either empty or encapsulating 19.6 nM of miR-486-5p or Cel-miR-54. All cells were exposed to 5% hydrogen peroxide (H_2O_2) for 2 h prior to staining. Cells were labeled with propidium iodide (PI) and annexin5 (A5-FITC) to assess apoptosis. In each plot, quadrant gating denotes: Q1 – viable (unstained) cells; Q2 – early apoptotic ($A5^+/PI^-$) cells; Q3 – late apoptotic ($A5^+/PI^-$) cells; and Q4 – necrotic ($A5^-/PI^+$) cells. HNP hybrid nanoparticle. FITC fluorescein isothiocyanate.

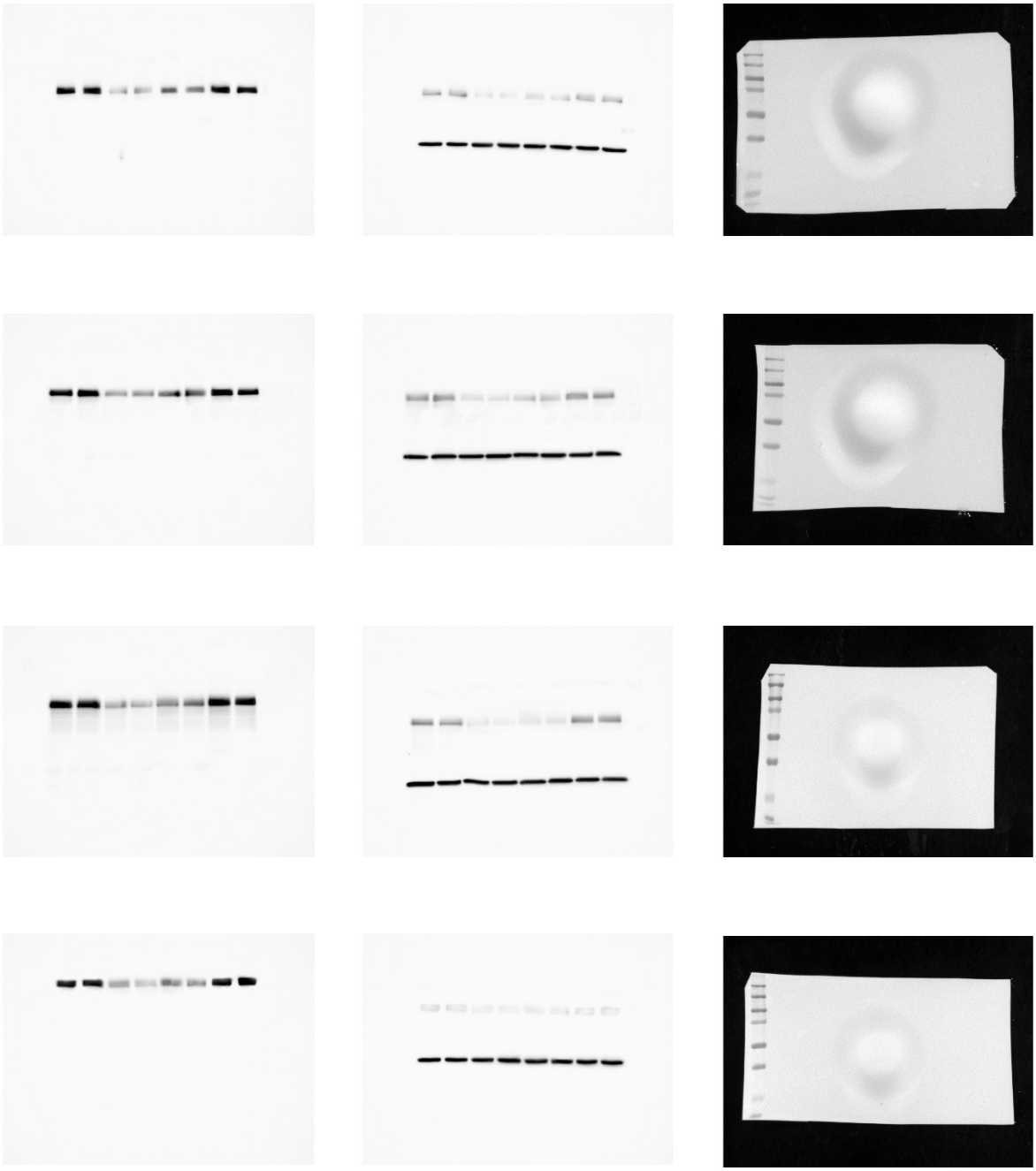

Fig.S4 | **Unedited western blot images of all replicates for Fig.5A.** Four replicates of HUVECs that were untreated or treated with lipofectamine plus miR-486-5p, miR-486-5p-loaded HNPs, and empty HNPs. The left, middle, and right panels respectively represent FOXO1 bands, GAPDH bands, and the ladders.

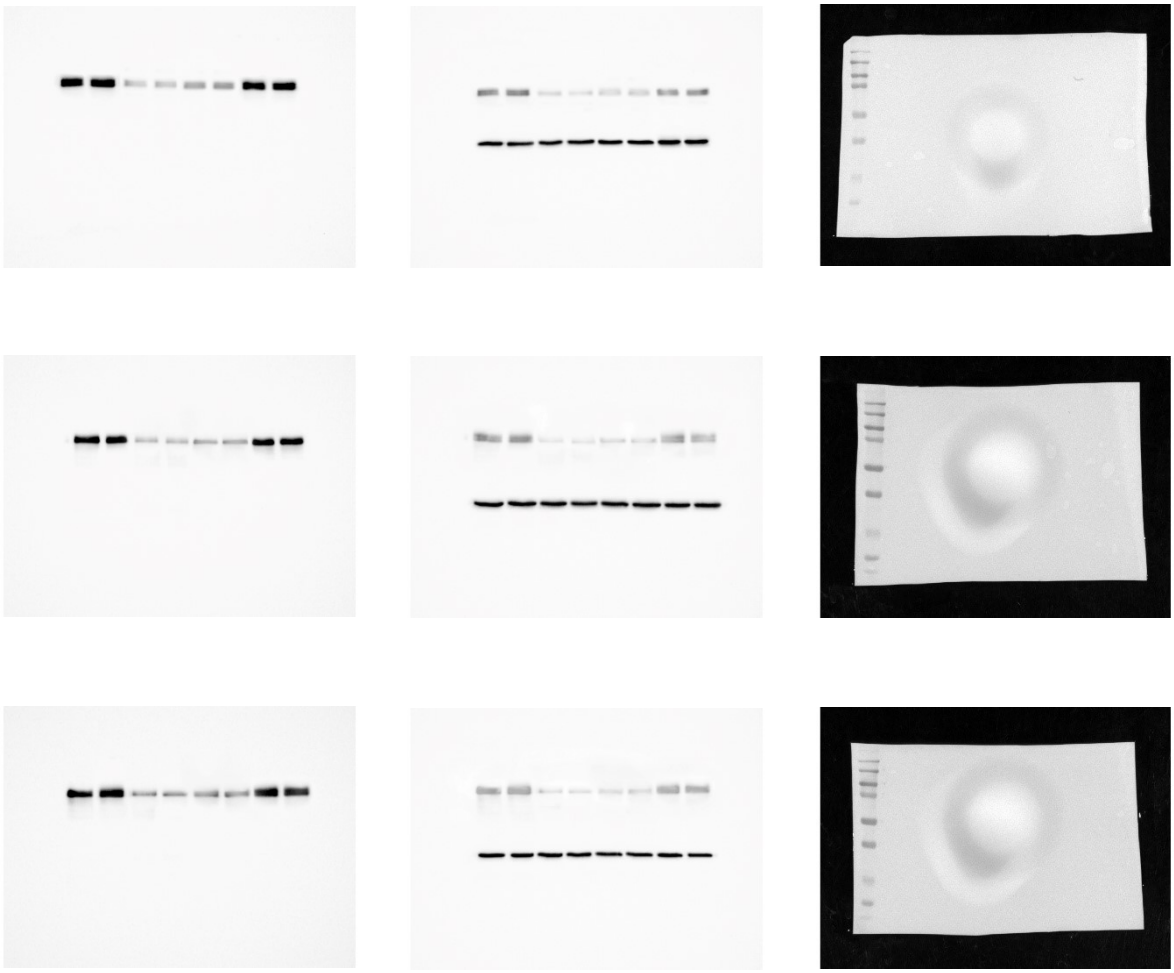

Fig.S5 | **Unedited western blot images of all replicates for Fig.5B.** Three replicates of hPTECs that were untreated or treated with lipofectamine plus miR-486-5p, miR-486-5p-loaded HNPs, and empty HNPs. The left, middle, and right panels respectively represent FOXO1 bands, GAPDH bands, and the ladders.

Fig.S6 | **Unedited western blot images of Fig.5C.** One replicate of HUVECs that were untreated or treated with lipofectamine plus miR-486-5p; Cel-miR-54-loaded HNPs; and miR-486-5p-loaded PNPs, Pl-NP1s, and Pl-NP2s. The left, middle, and right panels respectively represent FOXO1 bands, GAPDH bands, and the ladders.

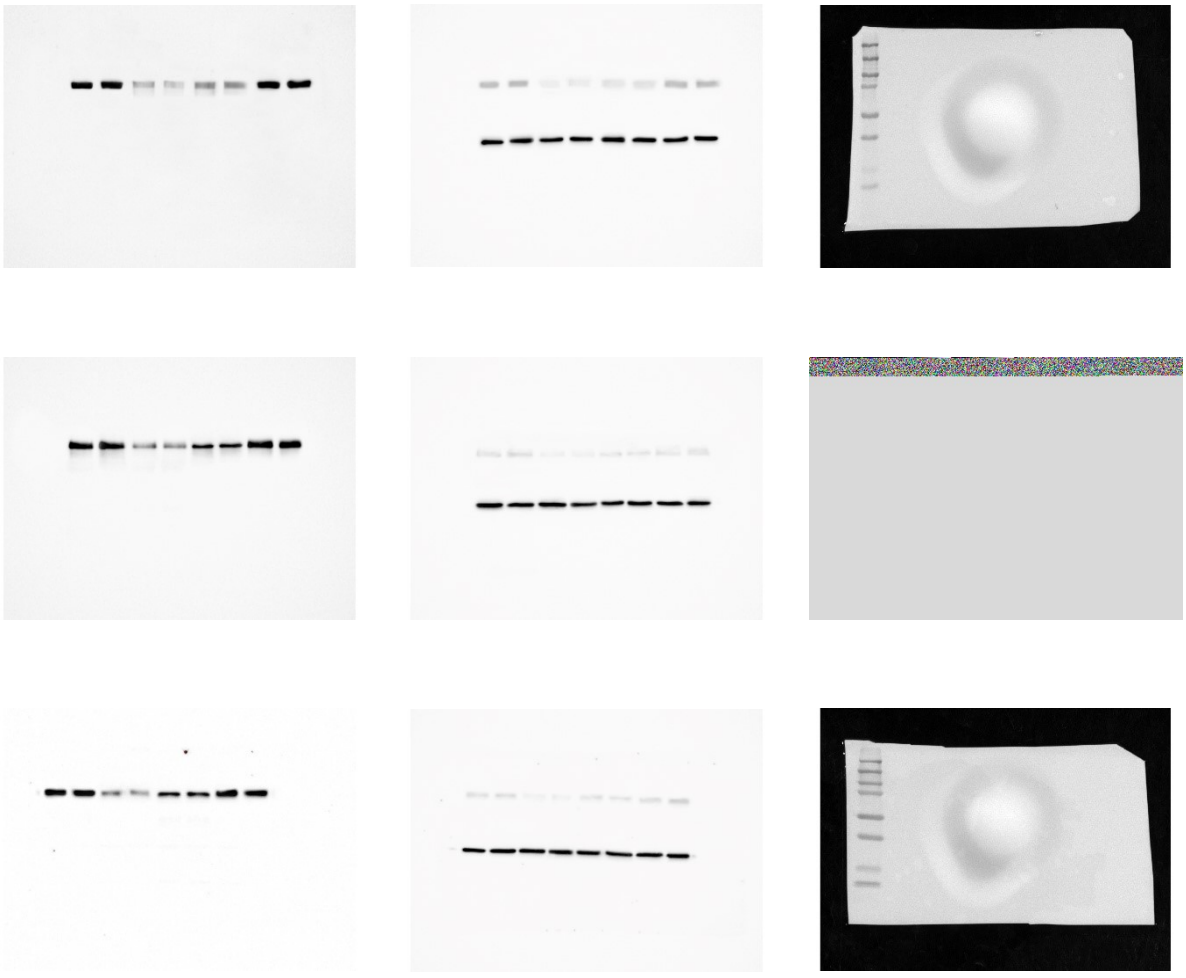

Fig.S7 | **Unedited western blot images of all replicates for supplemental Fig.2.** Three replicates of HK2 cells that were untreated or treated with lipofectamine plus miR-486-5p, miR-486-5p-loaded HNPs, and empty HNPs. The left, middle, and right panels respectively represent FOXO1 bands, GAPDH bands, and the ladders.