

Supplementary Information

Exosome-based *Ldlr*-gene therapy for familial hypercholesterolemia in a mouse model

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Table S1: Sequences of PCR primers

Name		Sequence
qPCR		
<i>Ldlr</i>	Forward	5'- TGA CTCAGACGAACAAGGCTG-3'
	Reverse	5'- CTA ACTAAACACCAGACAGAGGC-3'
Mouse <i>Gapdh</i>	Forward	5'-AGGTCGGTGTGAACGGATTTG-3'
	Reverse	5'-TG TAGACCATGTAGTTGAGGTCA-3'
<i>Col1a1</i>	Forward	5'-GCTCCTCTTAGGGGCCACT-3'
	Reverse	5'-ATTGGGGACCCTTAGGCCAT-3'
<i>Tnfa</i>	Forward	5'-GACGTGGA ACTGGCAGAAGAG-3'
	Reverse	5'-TTGGTGGTTTGTGAGTGTGAG-3'
<i>Mcp1</i>	Forward	5'-CCTGCTGTT CACAGTTGCC-3'
	Reverse	5'-ATTGGGATCATCTTGCTGGT-3'
Human GAPDH	Forward	5'-GGAGCGAGATCCCTCCAAAAT-3'
	Reverse	5'-GGCTGTTGTCATACTTCTCATGG-3'
Nested PCR		
External primer of <i>Ldlr</i>	Forward	5'- CTCCCAGGATGACTTCCGAT -3'
	Reverse	5'- CGCAGTGCTCCTCATCTGAC -3'
Internal primer of <i>Ldlr</i>	Forward	5'- CGACGGGGATGTCGACTGTGTTGA -3'
	Reverse	5'- TCGGCCCTGGCAGTTCTGTG -3'
Cloning		
<i>Ldlr</i>	Forward	5'-GGTTAATTAACAATGAGCACCGCGGATCTGA-3'
	Reverse	5'-GGTTCTGAATCATGCCACATCGTCCTCCAGGC-3'

Supplementary Figure Legends

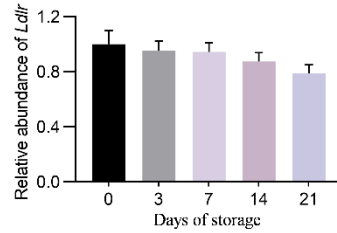


Figure S1: Stability of the *Ldlr* mRNA in the exosomes. qPCR analysis of the *Ldlr* mRNA in the exosomes. About 10^9 exosomes were preserved for indicated periods, followed by RNA isolation. Relative abundance was analyzed by qPCR and normalized to the day 0 sample.

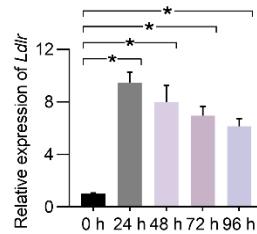


Figure S2: Functional duration of the delivered *Ldlr* mRNA in recipient cells. qPCR analysis of the *Ldlr* mRNA in recipient cells following incubation with exosomes. About 10^6 cells were incubated with 40 μ g exosomes and harvested at different times post-incubation. Data are expressed as mean $\pm$ SEM. *, $p < 0.05$ by one-way ANOVA.

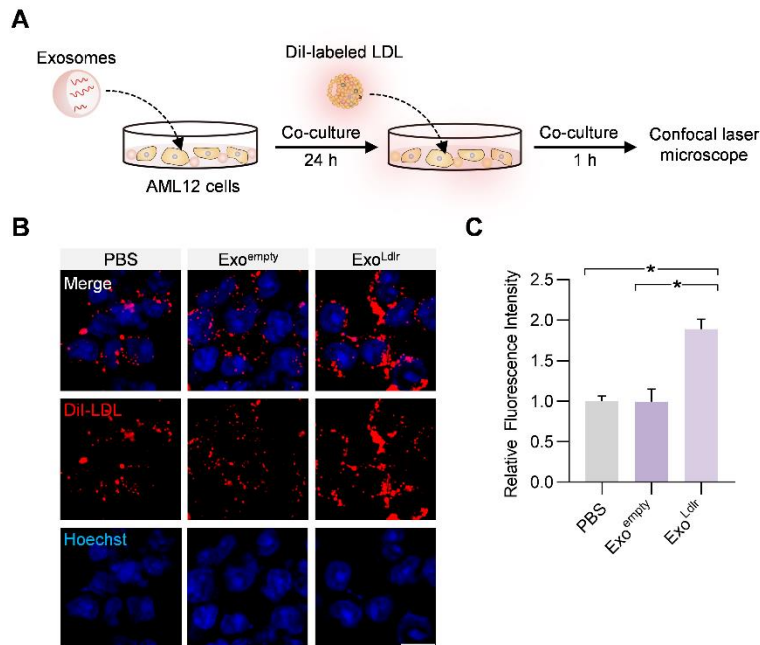


Figure S3: Exo^{Ldlr} promote LDL uptake in AML12 cells. A. Schematic representation of the

experimental procedure. AML12 cells were treated with indicated exosomes followed by incubation with 5 µg/ml DiI-labeled LDL 24 h later. LDL uptake was visualized by confocal microscopy at 1 h post-incubation. B. Fluorescence images of DiI-labeled LDL taken up by cells with different treatments. Nuclei were counterstained with Hoechst. PBS served as negative control. Scale bar=10 µm. C. Quantification of the fluorescence signal intensity. Data are expressed as mean ± SEM of three independent experiments. At least 100 cells were counted in each group per replicate. *, $p < 0.05$ by one-way ANOVA.

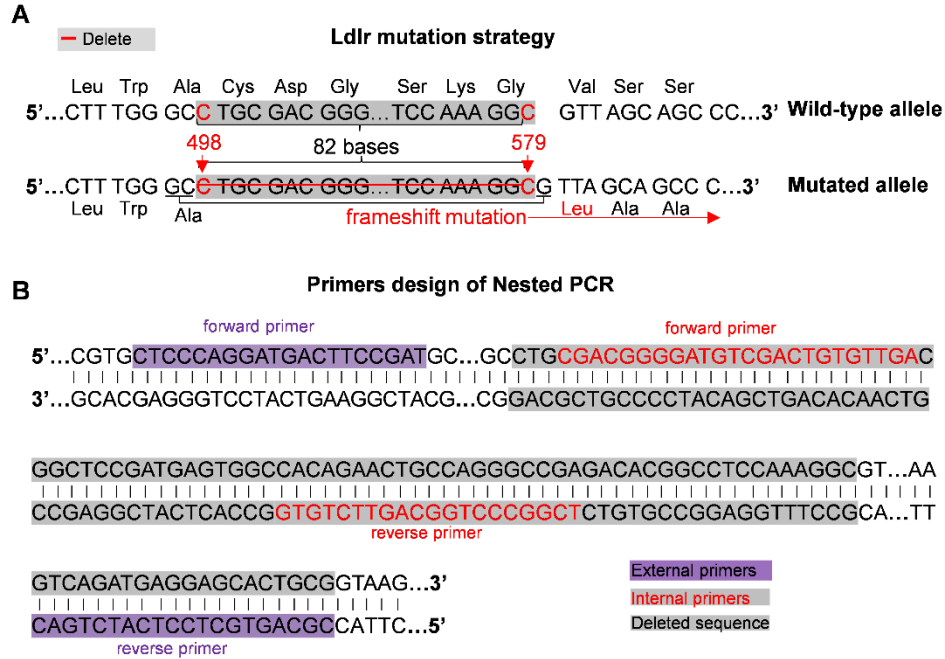


Figure S4: Illustration of *Ldlr* gene deletion strategy and primer design. A. *Ldlr* gene deletion strategy. In the knockout mice, an 82 bp sequence was deleted, resulting in a frameshift. B. Schematic representation of the location of the primers for nested PCR and qPCR.

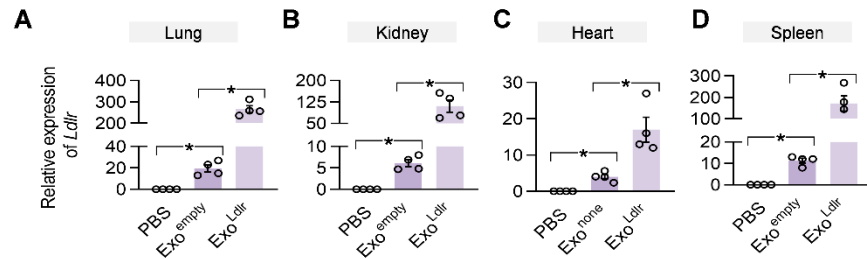


Figure S5: RNA expression of *Ldlr* in different organs of mice receiving exosome therapy. (A-D) qPCR analysis of wildtype of *Ldlr* mRNA in the lung (A), kidney (B), heart (C), and spleen (D). *Ldlr*^{-/-} mice were fed with a high-fat diet for 8 weeks, followed by injection of indicated exosomes. *Ldlr* expression at mRNA and protein levels was examined 3 days after injection. Data are

expressed as mean $\pm$ SEM. *, $p < 0.05$ by one-way ANOVA.

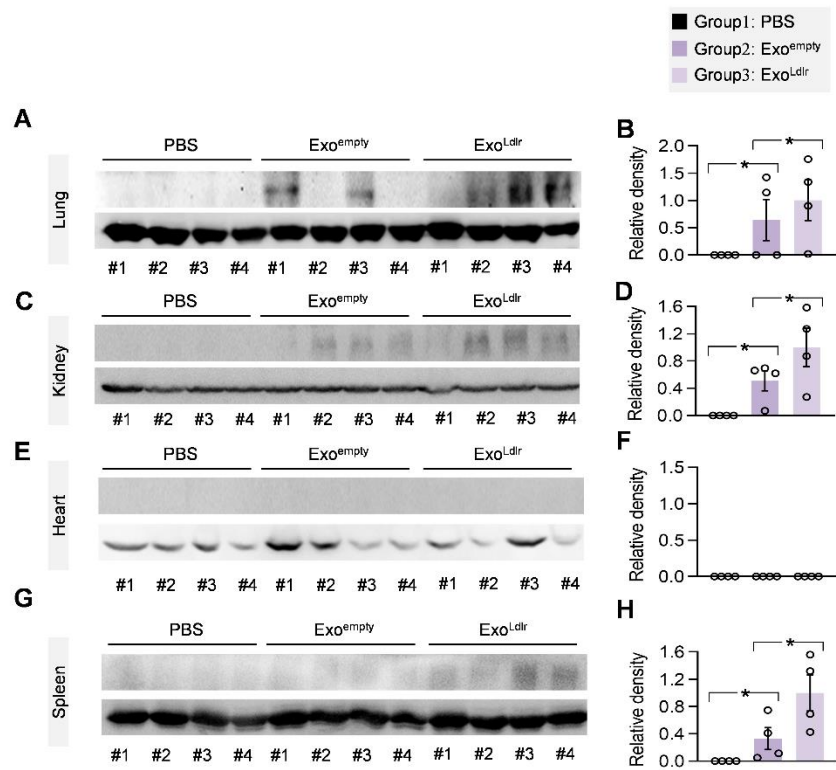


Figure S6: Protein expression of LDLR in various organs of mice receiving exosome therapy. (A-H) Western blot analysis of the LDLR expression in the lung (A), kidney (C), heart (E), and spleen (G) from mice treated as indicated. Quantification analysis of the Western blot bands by densitometry in B, D, F, and H. Data are expressed as mean $\pm$ SEM. *, $p < 0.05$ by one-way ANOVA.

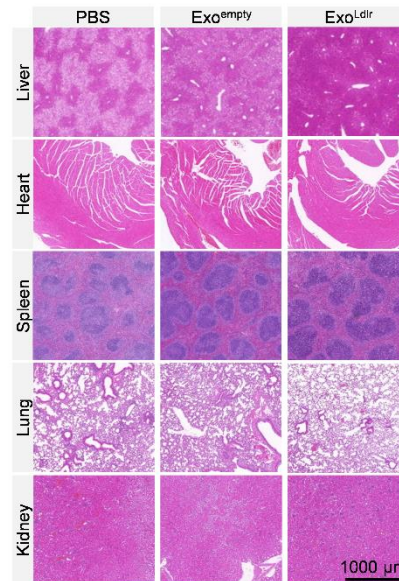


Figure S7 H&E staining of different organs from mice with indicated groups. No significant

toxicity was observed in the liver, heart, spleen, lung, and kidney from the HE staining results.

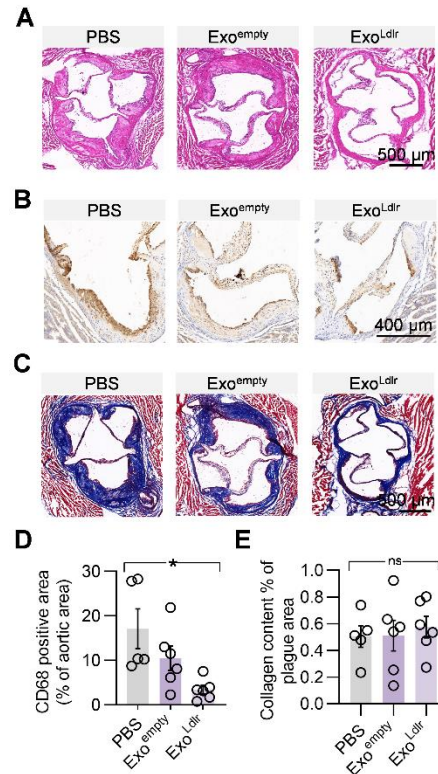


Figure S8: Exo^{Ldlr} reduces the inflammation of atherosclerotic plaques. A. H&E staining of aortic root sections from mice treated as indicated. Scale bar=500 μm. B. CD68 immunostaining in aortic root sections from mice treated as indicated. Scale bar=400 μm. C. Representative Masson's trichrome staining images of an aortic sinus. Scale bar=500 μm. D. Percentage of CD68-positive area in the plaque. Data are expressed as mean ± SEM. *, $p < 0.05$ by one-way ANOVA. E. Percentage of collagen content in the plaque. Data are expressed as mean ± SEM. ns, no significance.