

Additional Information

Targeted Delivery of MerTK Protein via Cell Membrane Engineered Nanoparticle Enhances Efferocytosis and Attenuates Atherosclerosis in Diabetic ApoE^{-/-} Mice

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Table S1. Primers used in the study.

Gene	Forward	Reverse
<i>Il1β</i>	5'GCAACTGTTCTGAACTCAACT3'	5'ATCTTTTGGGGTCCGTCAACT3'
<i>Nos2</i>	5'GTTCTCAGCCCAACAATACAAGA3'	5'GTGGACGGGTCGATGTCAC3'
<i>Il6</i>	5'TTCCATCCAGTTGCCTTCT3'	5'CAGAATTGCCATTGCACAAC3'
<i>Tnfa</i>	5'CTGAACTTCGGGGTGATCGG3'	5'GGCTTGTCACCTCGAATTTTGAGA3'
<i>Il10</i>	5'GCTCTTACTGACTGGCATGAG3'	5'CGCAGCTCTAGGAGCATGTG3'
<i>Ym1</i>	5'CAGGTCTGGCAATTCTTCTGAA3'	5'GTCTTGCTCATGTGTGTAAGTGA3'
<i>Fizz1</i>	5'CCAATCCAGCTAACTATCCCTCC3'	5'ACCCAGTAGCAGTCATCCCA3'
<i>Ptgs2</i>	5'TGAGCAACTATTCCAAACCAGC3'	5'GCACGTAGTCTTCGATCACTATC3'
<i>Ptges</i>	5'GGATGCGCTGAAACGTGGA3'	5'CAGGAATGAGTACACGAAGCC3'
<i>Mertk</i>	5'CAGGGCCTTTACCAGGGAGA3'	5'TGTGTGCTGGATGTGATCTTC3'
<i>β-actin</i>	5'GGCTGTATCCCTCCATCG3'	5'CCAGTTGGTAACAATGCCATGT3'

Additional Figures and Figure legends

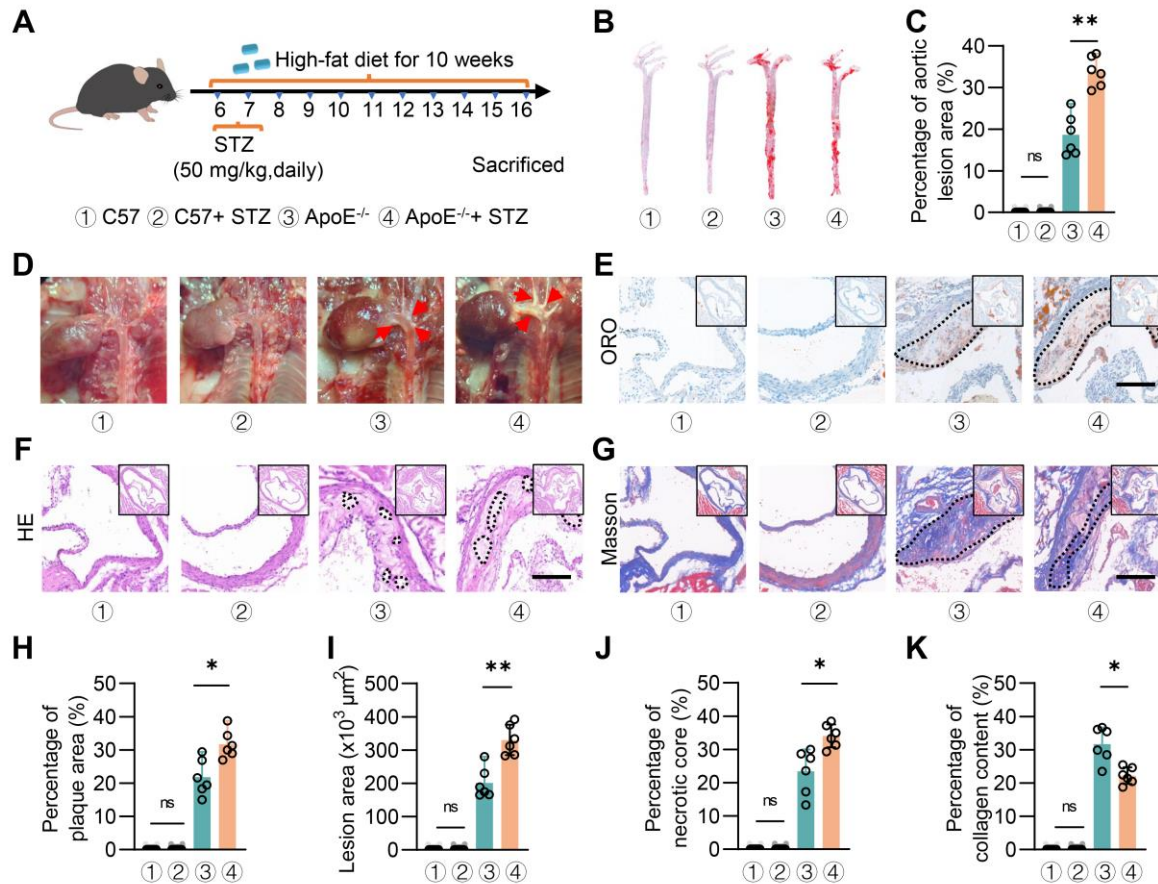


Figure S1. Diabetes exacerbates atherosclerotic lesions in ApoE^{-/-} mice.

A. Schematic diagram showing the experimental procedure. B. Representative images of Oil Red O staining of the atherogenic lesion areas. C. Percentage of the aortic lesion. D. Representative aortic arch view of the atherosclerotic lesions in mice treated as indicated. E-G. Representative images of the atherogenic lesion areas stained with H&E, Masson's trichrome, and Oil Red O. Scale bar = 200 μm. H-K. Quantitative analysis of the aortic lesion percentage, lesion area, necrotic core area, plaque collagen area relative to plaque area. Data are presented as mean ± SEM (n= 6 per group). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. *P < 0.05, **P < 0.01.

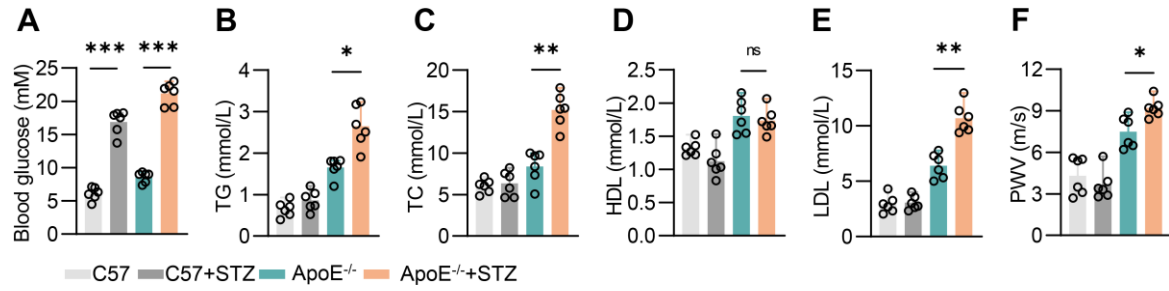


Figure S2. Effects of diabetes on serum levels of biochemical parameters and arterial stiffness.

A-E. Serum biochemistry measured in mice. Examination of the blood glucose, total triglyceride, total cholesterol, HDL, and LDL levels. F. The PWV of the C57, C57+STZ, ApoE^{-/-} and ApoE^{-/-}+STZ mice. Data are presented as mean $\pm$ SEM (n= 6 per group). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001. ns, no significance.

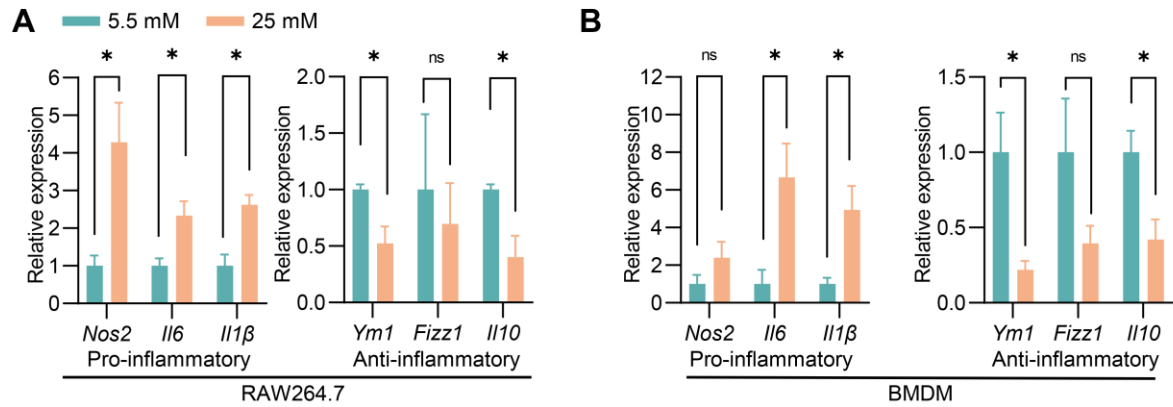


Figure S3. High glucose changes inflammatory gene expression.

RT-qPCR Analysis of pro- and anti-inflammatory cytokine mRNA levels in RAW 264.7 (A) or BMDMs (B). Data are presented as mean $\pm$ SEM of three independent experiments. Statistical significance was determined by student's t test, *P < 0.05. ns, no significance.

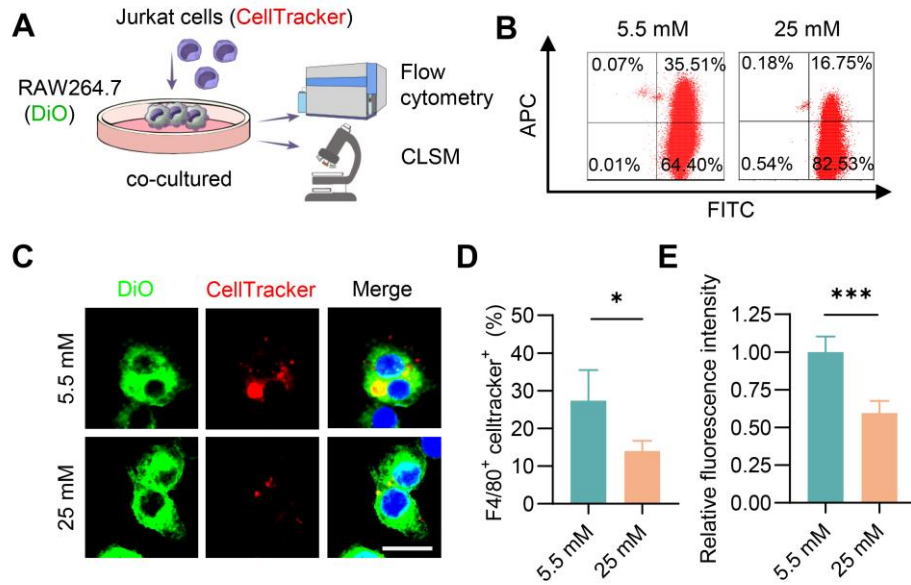


Figure S4. High glucose damages the efferocytosis capacity in RAW264.7 cells.

A. Schematic of efferocytosis assay in vitro. DiO labeled RAW264.7 cells were co-cultured with Celltracker labeled Jurkat cells, followed by immunofluorescence and flow cytometry analysis. B. Flow cytometry analysis of RAW264.7 uptaking apoptotic Jurkat cells. C. Representative confocal laser scanning microscopy (CLSM) showing the localization of apoptotic cells (Jurkat cells) endocytosed by RAW264.7 cells. Scale bar =10 μ m. D. Quantitative analysis of flow cytometry data. E. Quantitative analysis of fluorescence intensity. Data are presented as mean $\pm$ SEM of three independent experiments. Statistical significance was determined by student's t test, *P < 0.05, ***P < 0.001.

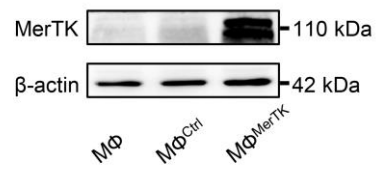


Figure S5. Increased MerTK expression through viral infection.

A. Western blot analysis of MerTK expression in RAW 264.7. Data are presented of three independent experiments.

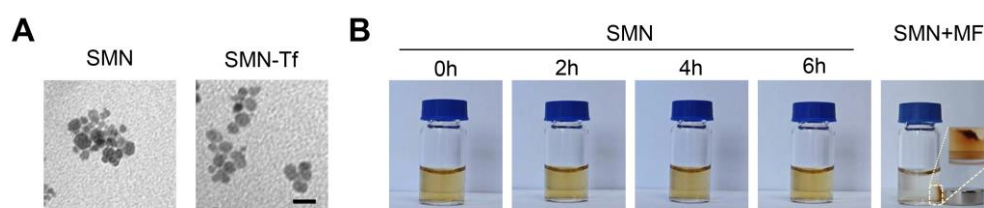


Figure S6. Characterization of the SMN-Tf.

A. Representative TEM images of SMN and SMN-Tf. B. MF induces aggregation of the SMN in vitro. Scale bar = 20 nm. Data are representative of three independent experiments.

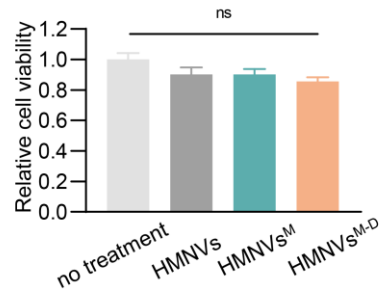


Figure S7. Cell viability in cells with indicated treatments.

Cell viability was measured using cell counting kit-8. Data are presented as mean $\pm$ SEM of three independent experiments. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. ns, not significant.

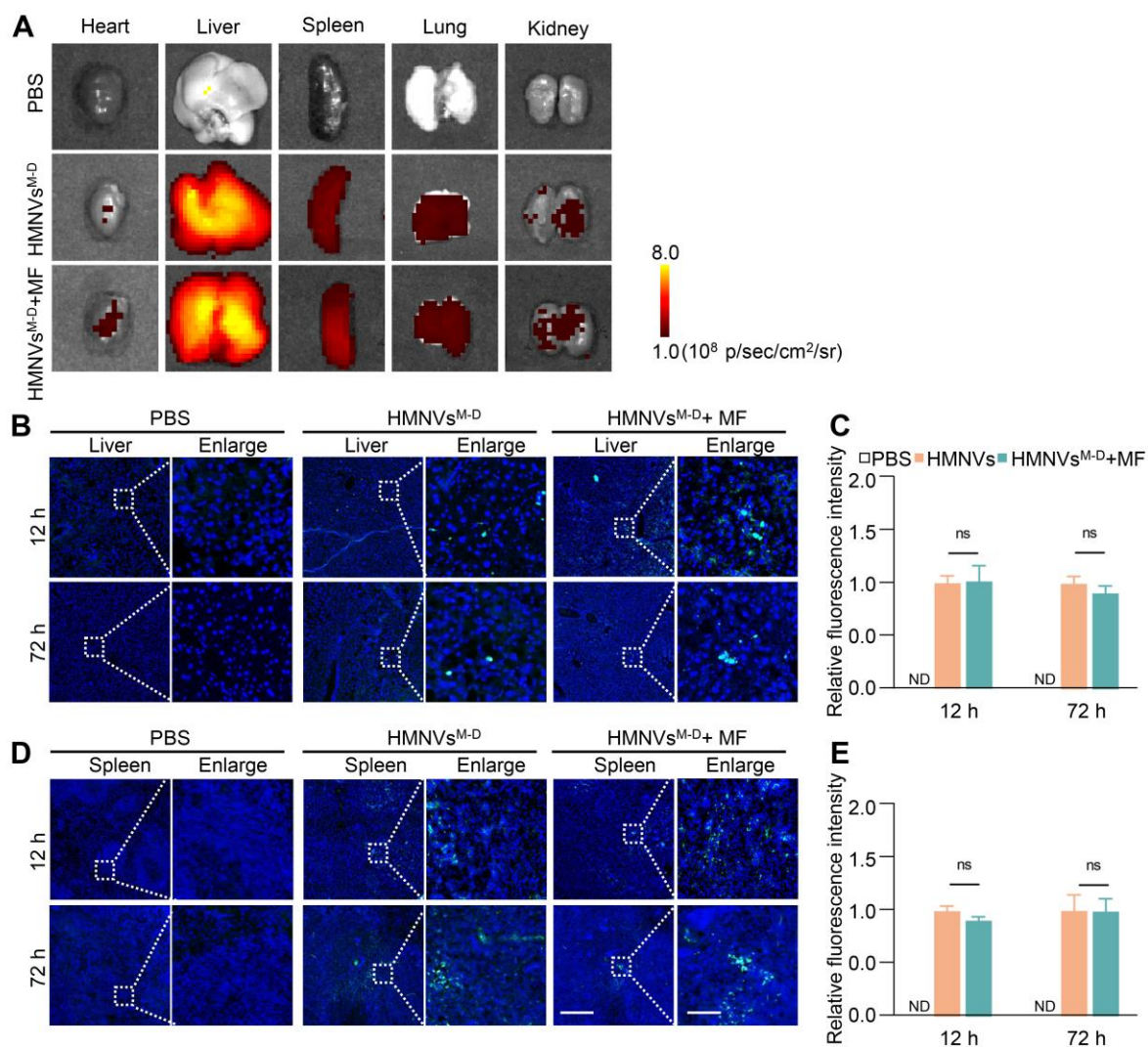


Figure S8. Biodistribution of HMNVs after systemic administration.

A. Quantitative fluorescence analysis of the distribution of the HMNVs in different organs, including heart, liver, spleen, lung, and kidney B. Representative fluorescence images of the HMNVs in the liver (green). The nuclei were counter-stained with Hoechst (blue). C. Quantitative analysis of the fluorescence area. D. Representative fluorescence images of the HMNVs in the spleen (green). The nuclei were counter-stained with Hoechst (blue). Scale bar = 500 or 100 μ m. E. Quantitative analysis of the fluorescence area. Data are presented as mean $\pm$ SEM (n = 3 per group). Statistical significance was determined by student's t test, ns, no significance.

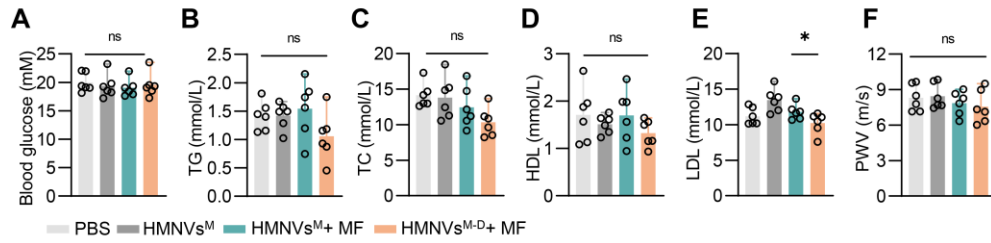


Figure S9. HMNVs attenuate atherosclerotic lesions in diabetic ApoE^{-/-} mice.

A-E. Serum biochemistry measured in mice treated as indicated. Examination of the blood glucose (A), total triglyceride (B), total cholesterol (C), HDL (D), and LDL (E) levels. F. The PWV of mice treated as indicated. Data are presented as mean $\pm$ SEM (n= 6 per group). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. *P < 0.05. ns, no significance.

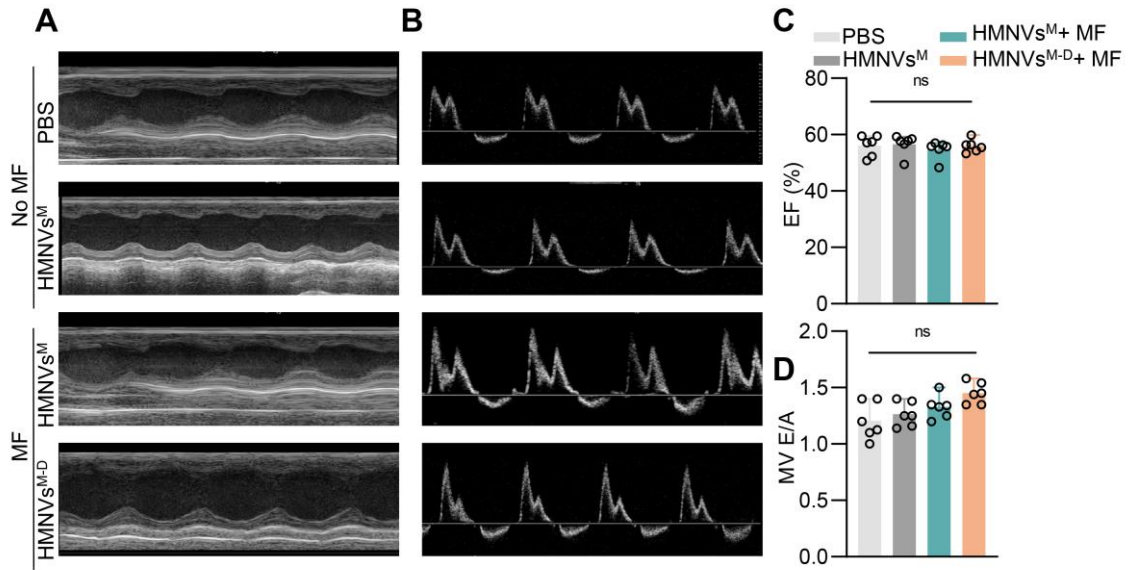


Figure S10. Cardiac function change in mice receiving HMNV treatments.

A. Representative images of M mode echocardiography of mice with indicated treatments. B. Representative images of mitral flow Doppler echocardiography. C. Quantification of systolic function parameter ejection-fraction (EF). D. Quantification of diastolic function parameter E/A. Data are presented as mean $\pm$ SEM (n= 6 per group). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. ns, no significance.