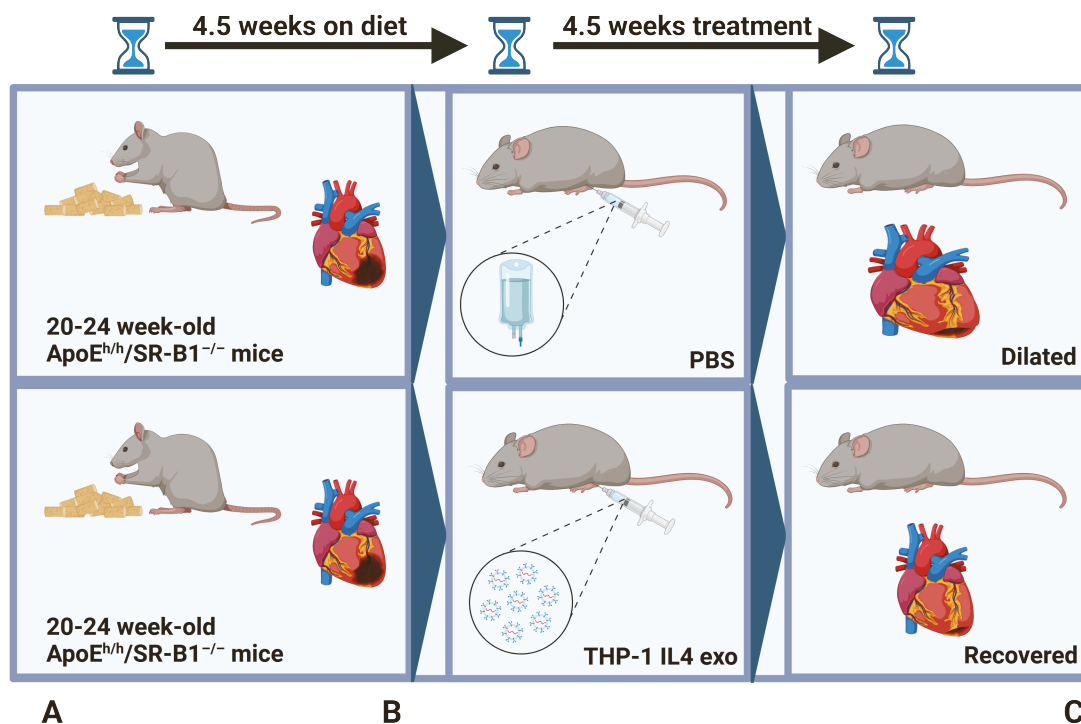


Supplemental Information

**M2 macrophage exosomes reverse heart failure
post-myocardial infarction by suppressing
type 1 interferon signaling in myeloid cells**

Martin Ng, Alex S. Gao, Tuan Anh Phu, Ngan K. Vu, and Robert L. Raffai

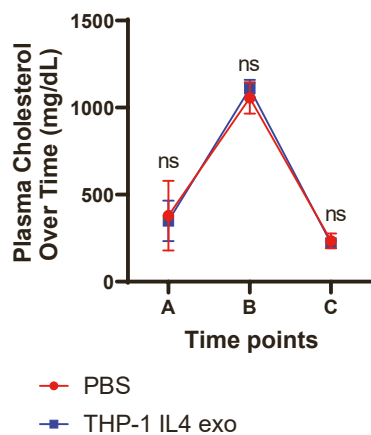
A



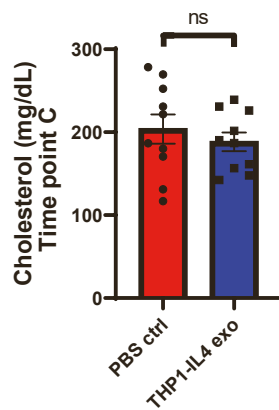
Time points

plpC + triweekly exo injections

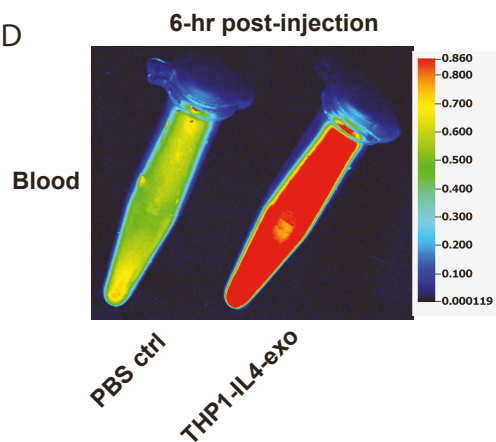
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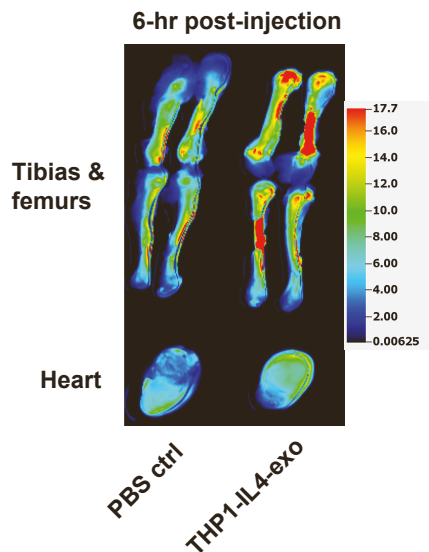
C



D



E



F

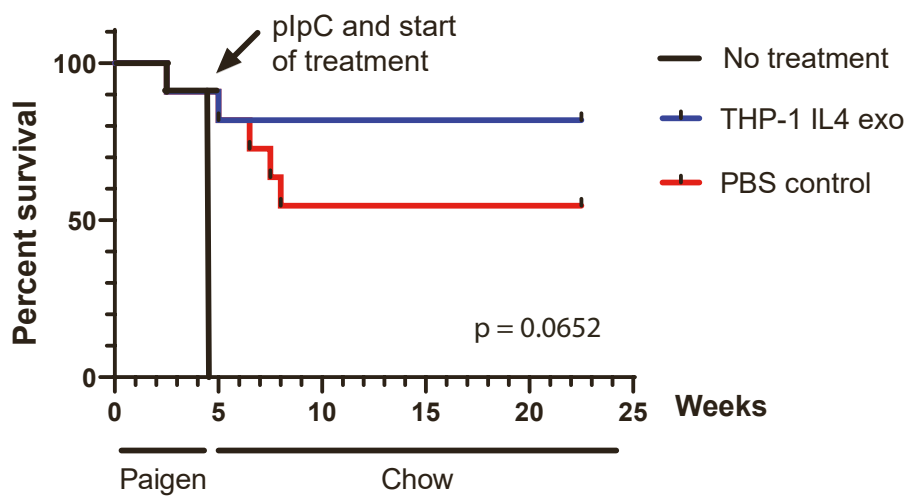


Figure S1. Study design utilizing ApoE^{h/h}/SR-B1^{-/-}Mx1-Cre⁺ mice

(A) Schematic depicting the time course involving diet modification, plasma lipid lowering, and exosome treatments. Time point A corresponds to the period before starting Paigen's atherogenic diet. Time point B occurs when mice have been on the diet for 4.5 weeks. Time point C occurs when mice have experienced tri-weekly treatments of PBS vs THP1-IL4-exo for 4.5 weeks.

(B) Plasma cholesterol levels in both treatment groups at Time point A, B, and C; n = 5 per group.

(C) Plasma cholesterol levels at Time point C; data pooled from two independent experiments; n = 10 per group.

(D and E) Images of DiR fluorescence in the blood (D), tibias, femurs, and hearts (E) 6 hr post-injection from ApoE^{h/h}/SR-B1^{-/-}Mx1-Cre⁺ mice that had been fed a Paigen's diet for 4.5 weeks, induced with plpC, returned to a chow diet, and injected IP with PBS or 10¹⁰ particles of DiR-labeled THP1-IL4-exo.

(F) Kaplan-Meier survival curve of ApoE^{h/h}/SR-B1^{-/-}Mx1-Cre⁺ mice fed Paigen diet followed by a plpC injection and chow diet, treated with PBS vs THP1-IL4-exo for up to 23 weeks. p < 0.0652 as determined by a log-rank test; n = 10 per group.

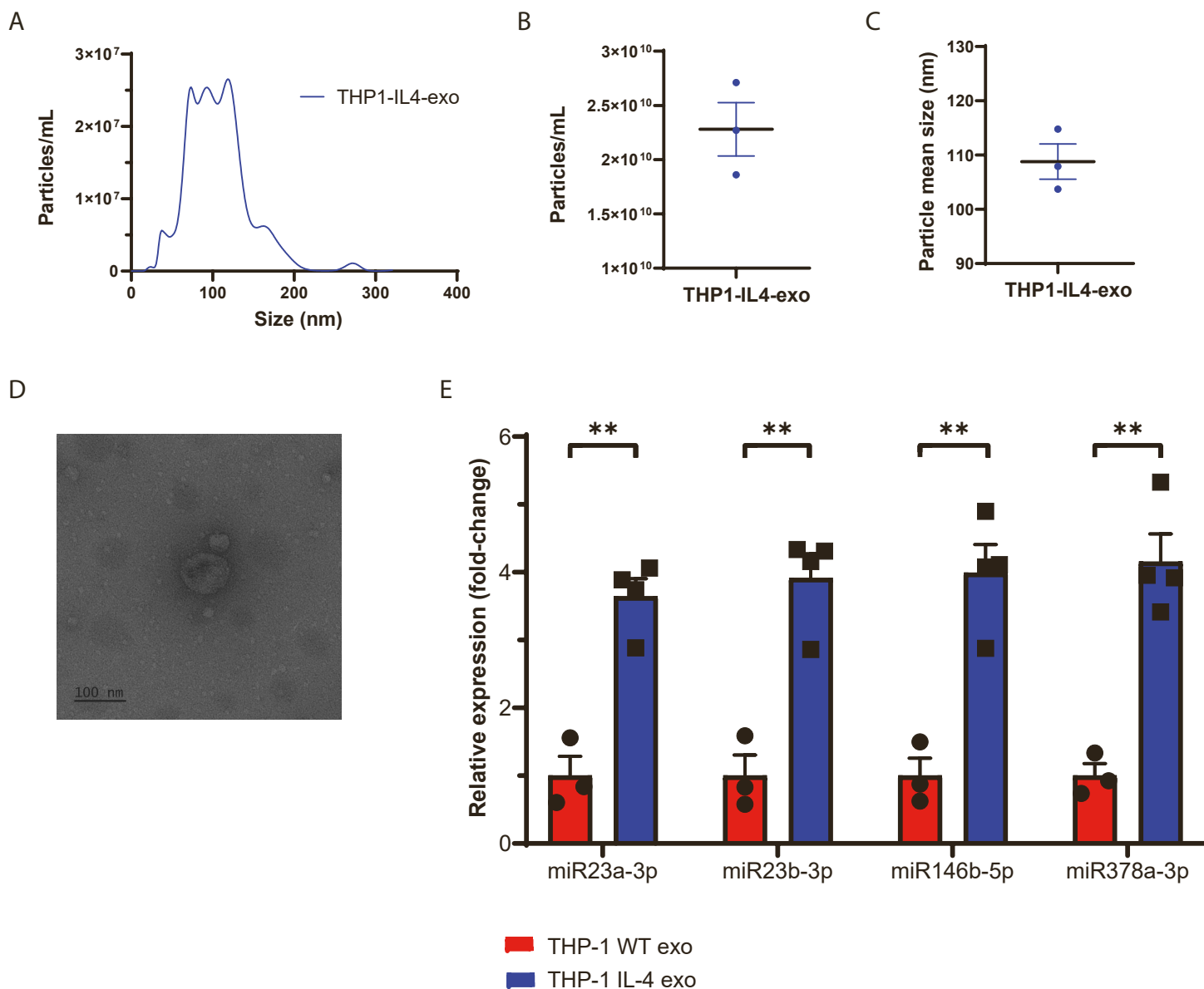


Figure S2. Biophysical parameters of THP1-IL4-exo

(A) Representative concentrations and size distributions of THP1-IL4-exo purified from THP-1 conditioned cell culture supernatant after a 24 hr culture period with IL4-supplemented exosome-free media determined using Nanoparticle Tracking Analysis (NTA).

(B and C) Average particle concentration per milliliter (B) and mean diameter in nanometers (C) determined using NTA; n = 3.

(D) Electron micrograph depicting a single purified THP1-IL4-exo in the center; scale bar: 100nm.

(E) RT-qPCR analysis of miR-23a-3p, -23b-3p, -146b-5p, and -378a-3p microRNA levels in THP-WT-exo vs THP1-IL4-exo. Results were normalized to U6 snRNA and UniSp6 (spike-in control). Separate preparations of exosomes were analyzed; n = 3-4 per group.

*p < 0.05 and **p < 0.01 as determined using unpaired Student's t test. Data are represented as mean ± SEM.

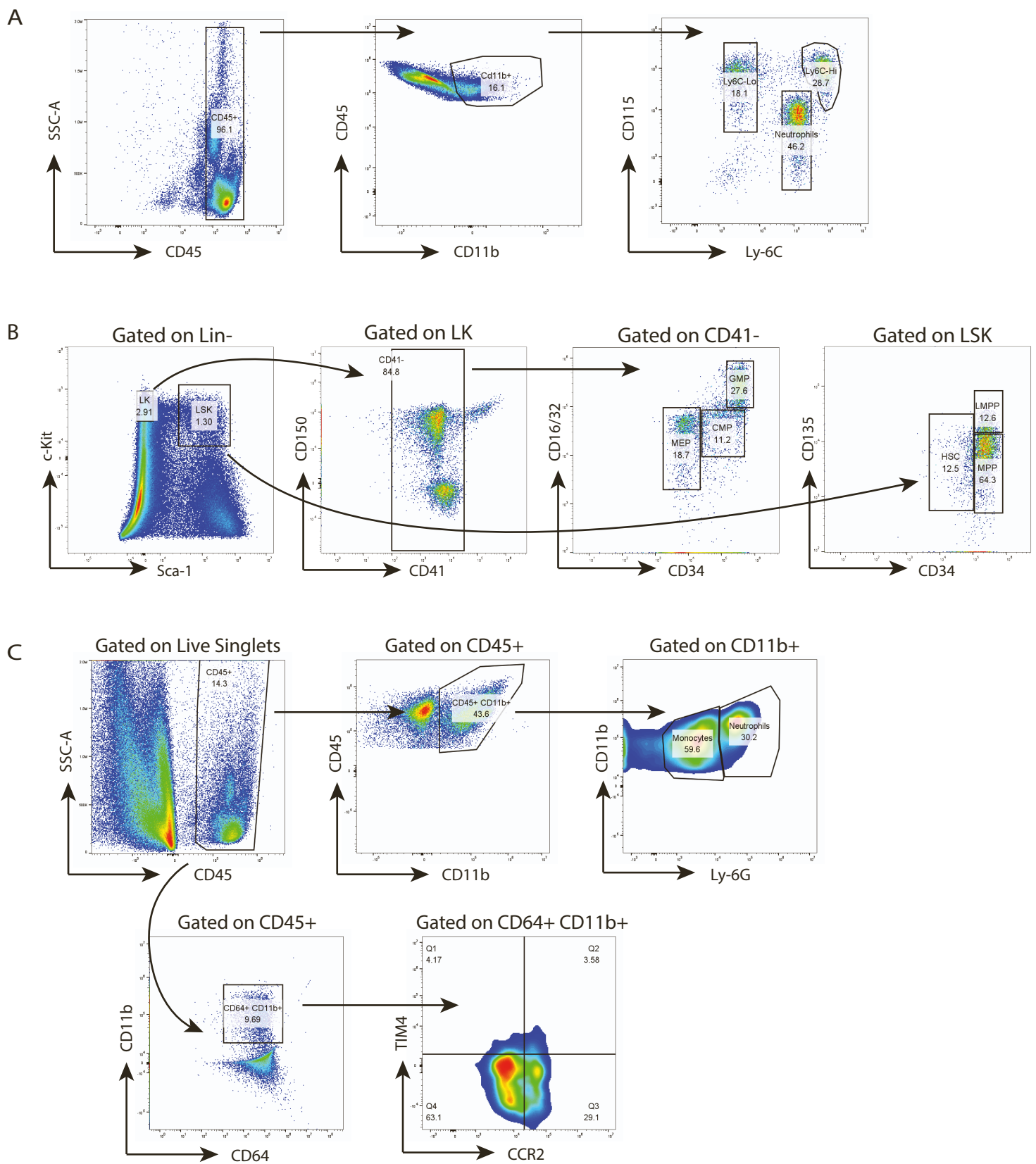


Figure S3. Flow cytometry gating strategies

(A) Representative flow cytometry plots of leukocyte subsets from peripheral blood.

(B) Representative flow cytometry plots of leukocyte progenitor subsets from bone marrow.

(C) Representative flow cytometry plots of leukocyte subsets from digested whole heart tissues.