Supplemental Materials

Transplanted perivascular adipose tissue accelerates injury-induced neointimal hyperplasia: role of MCP-1

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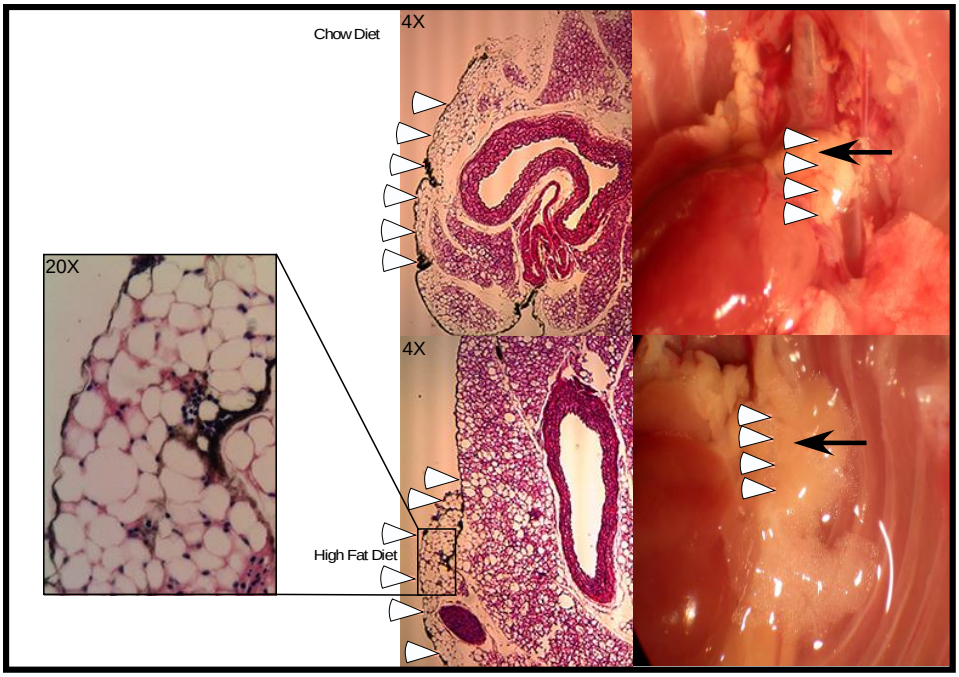
Supplemental

Table.

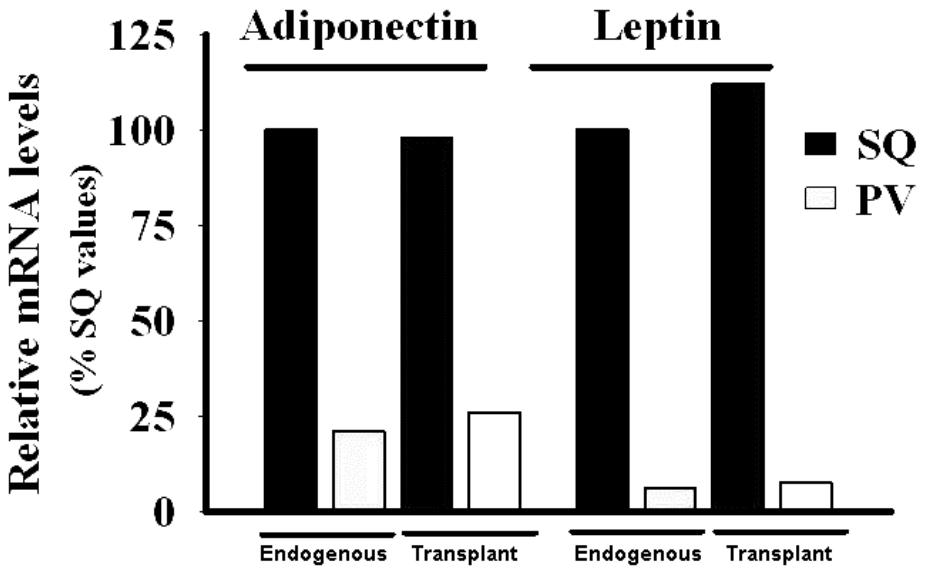
Primers sequences used in this study.

TNFα (F)	TCTCATGCACCACCATCAAGG-3
TNFα (R)	ACCACTCCCCGCAGAACTCA
MCP-1 (F)	GGCTCAAGCCAGATGCAGTTAC
MCP-1 (R)	GCCTACTCATTGGGATCATCTT
MIP-1α (F)	ACTGACCTGGAACTGAATGCCTGA
MIP-1α (R)	ATGTGGCTACTTGGCAGCAAACAG
RPLPO (F)	AGCTGAAGCAAAGGAAGAGTCGGA
RPLPO (R)	ACTTGGTTGCTTTGGCGGGATTAG

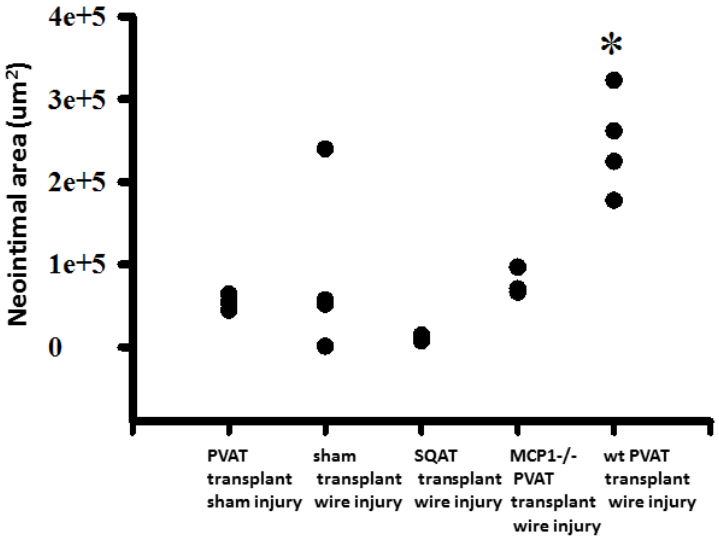
Supplemental Figures.



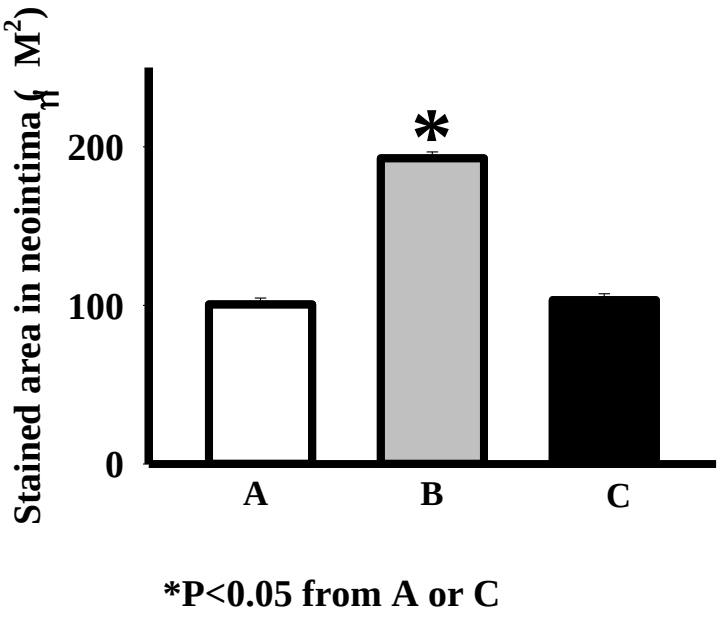
Supplemental Figure I. Representative anatomy and histology of thoracic aortic PVAT. C57Bl/6J mice were fed a chow diet (upper panels) or high fat diet (lower panels) for 4 weeks. Mice were euthanized, and thoracotomy was performed to expose PVAT at the lesser curvature of the thoracic aorta (black arrows) using a dissecting microscope. A strip of white adipose tissue was visually identified at the inferior margin of the lesser curvature (white arrows, right panels) and marked with a black tissue marking pen. Aortic arch with PVAT was harvested en bloc and processed for H&E staining (middle panels). White arrows in middle panels correspond to the regions marked in the right panels, identifying the site where PVAT was harvested for transplantation. Left panel shows 20x magnification of the adipose tissue histology contained within the insert (note the black marking).



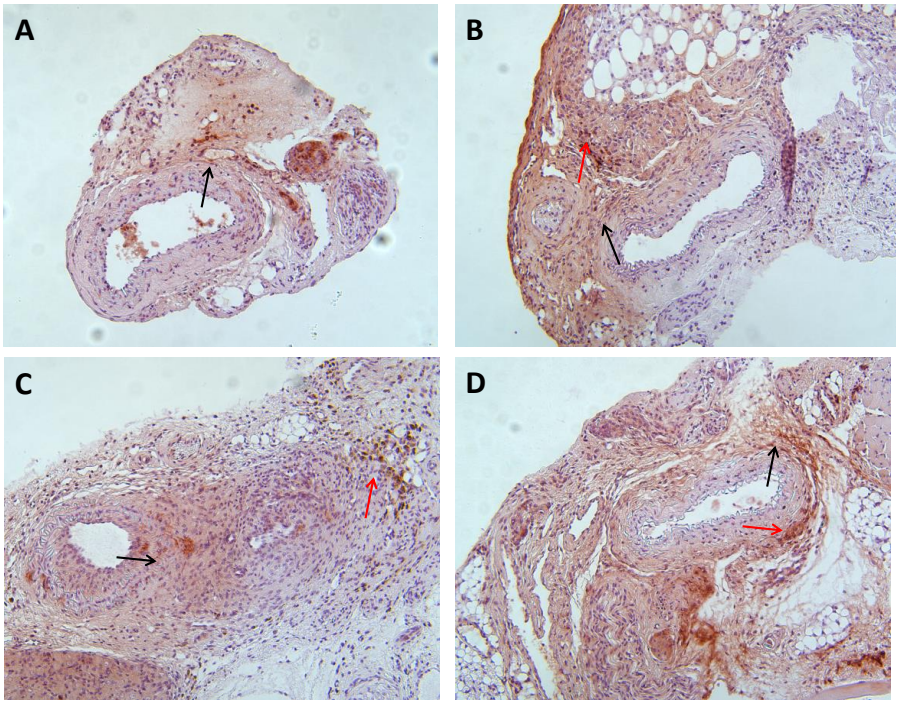
Supplemental Figure II. Comparison of expression of the adipocyte genes adiponectin and leptin in transplanted SQ or PV adipose tissue versus the corresponding endogenous adipose tissues from the same animals. Expression levels were normalized to the SQ data and expressed as % SQ values. Because of the small volume of transplanted adipose tissues, tissues were pooled from 3-4 animals and run in duplicate to obtain these results.



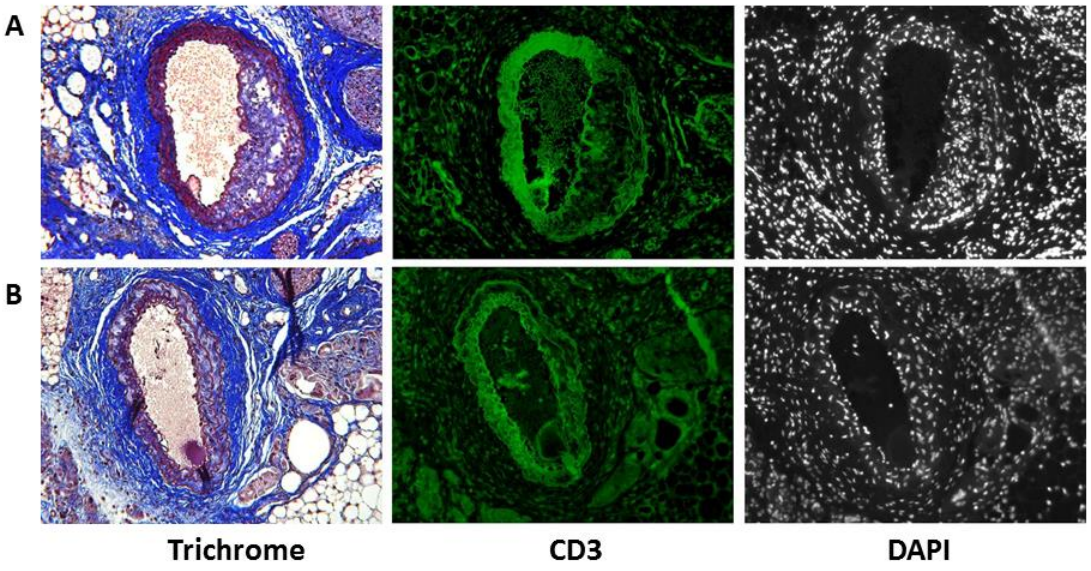
Supplemental Figure III. Scatter plot of individual neointimal area data points from experiments shown in Figure 2. Please see legend for Figure 2 for additional experimental details.



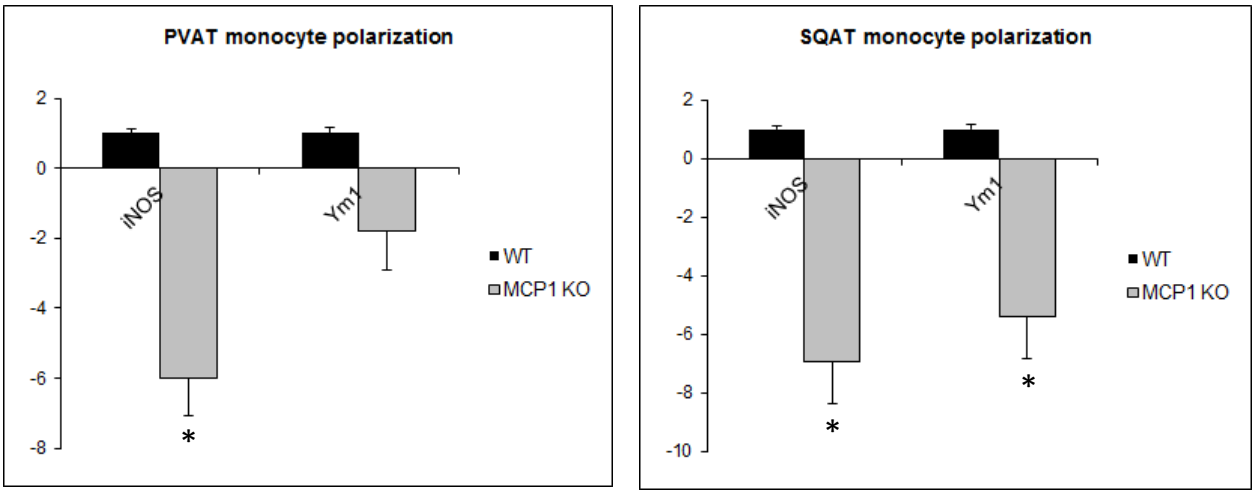
Supplemental Figure IV. Quantification of α-smooth muscle actin expression in the neointima. Images (40x) from each animal were analyzed using ImageJ software, and the total area (um2) of positively stained intima was calculated and expressed relative to sham PVAT transplantation + wire injury (column A). Column B represents wt PVAT transplantation + wire injury, and column C represents MCP-1-/- PVAT transplantation + wire injury.



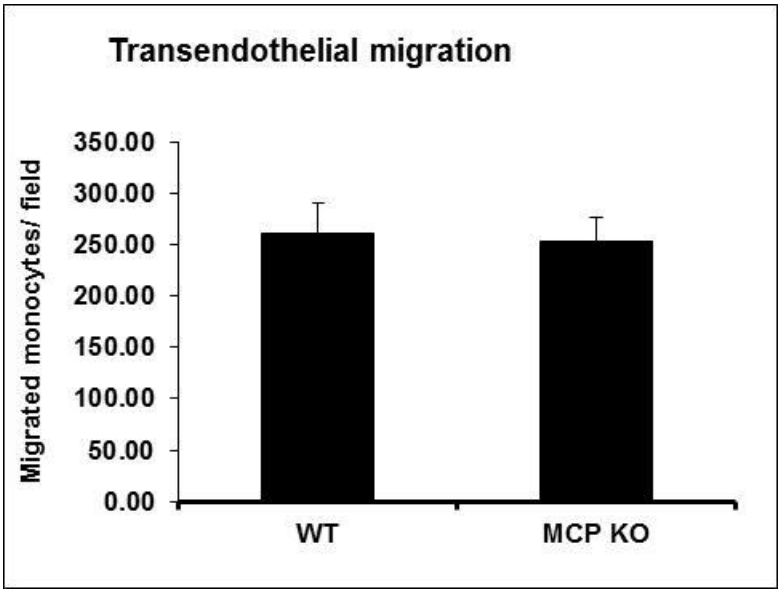
Supplemental Figure V. Representative staining for Mac3 (brown staining) in carotid artery sections (all photographed at 10x). Panel A: sham PVAT transplantation + wire injury, Panel B: wt PVAT transplantation + sham injury, Panel C: wt PVAT transplantation + wire injury, Panel D: MCP-1-/- PVAT transplantation + wire injury. Black arrows denote location of external elastic lamina, and red arrows in B-D show clustering of macrophages near transplanted PVAT.



Supplemental Figure VI. Representative immunostaining for T cells in carotid artery sections. Left panels show trichrome staining, middle panels CD3 staining, and right panels nuclear staining (DAPI). A: wt PVAT transplantation + wire injury, B: MCP-1-/- PVAT transplantation + wire injury. Images (all photographed at 20x) are representative of histology from 3 separate mice in each group.



Supplemental Figure VII. Expression (mRNA) of iNOS and Ym1 in transplanted PVAT. Mice underwent transplantation of PVAT or SQAT and two weeks later were subjected to wire injury. After 2 more weeks, tissues were harvested and RNA isolated to quantify mRNA expression of iNOS and Ym1 by real-time PCR. Data were normalized to wt values and expressed as fold change. *, $p<0.05$ compared to corresponding WT value, n=3-4.



Supplemental Figure VIII. Comparison of potency of conditioned medium from wt PVAT and MCP-1-/- PVAT to elicit monocyte chemotaxis. After feeding the mice a Western diet for 4 weeks, PVAT (10 mg) was harvested and incubated in vitro in culture medium for 4 hours. Media were collected and aliquots placed in 24-well plates fitted with inserts to elicit monocyte/macrophage transendothelial migration as described in *Materials and methods*. N=3, p=n.s.

The NIHMS has received the file 'ATVB_ATVB-2014-303983D_supp1.pdf' as supplementary data. The file will not appear in this PDF Receipt, but it will be linked to the web version of your manuscript.