SUPPLEMENTARY INFORMATION

Targeted suppression of microRNA-33 in lesional macrophages using pH low-insertion peptides (pHLIP) improves atherosclerotic plaque regression

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SUPPLEMENTAL FIGURE LEGENDS

Supp Figure 1. pH low inducible peptides (pHLIP) promotes delivery of miR-33 inhibitors to the atherosclerotic plaques. a-b, Representative images (**a**) and quantification (**b**) of tissues from low density lipoprotein receptor knockout (*Ldlr*$^{-/-}$) mice injected with fluorescently labeled targeted (A750-Var3) or non-inserting control (A750-5K-Var3) constructs. *Ldlr*$^{-/-}$ mice were fed a western diet (WD) for 3 months to induce atherosclerosis and tissues were harvested 4-24 hours post injection of constructs. **c**, Representative images demonstrating uptake of A546-Var3 into liver and kidney with A546-5K-Var3 as control.

Supp Figure 2. Targeted silencing of miR-33 does not result in differences in circulating lipids, lipoprotein profiles and body weight. a, Measurement of plasma total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides from Low density lipoprotein receptor knockout (*Ldlr*$^{-/-}$) mice injected anti-miR-33 peptide nucleic acid delivery vector (anti-miR33^{pHLIP}) or scrambled control (Src^{pHLIP}) (*n*= 6-13 each group). **b**, Lipoprotein profiles by FPLC from *Ldlr*$^{-/-}$ mice injected anti-miR33^{pHLIP} or Src^{pHLIP}. **c**, Body weight of *Ldlr*$^{-/-}$ mice injected anti-miR33^{pHLIP} or Src^{pHLIP} (*n*= 14-18 per group). Quantification represents the mean ± s.e.m. Data were analyzed by one-way ANOVA with Bonferroni correction for multiple comparisons.

Supp Figure 3. Selective targeting of miR-33 by pHLIP peptides shows no difference in circulating leukocytes composition and hepatic function. a, Quantification of peripheral blood counts by hemocytometer from *Ldlr*$^{-/-}$ mice injected anti-miR-33 peptide nucleic acid delivery vector (anti-miR33^{pHLIP}) or scrambled control (Src^{pHLIP}) (*n*= 7-9 each group). **b**, Quantification of serum hepatotoxicity markers, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), from *Ldlr*$^{-/-}$ mice injected anti-miR33^{pHLIP} or Src^{pHLIP} (*n*= 7-9 each group). Quantification represents the mean ± s.e.m. Data were analyzed by an unpaired two-sided Student's t-test.

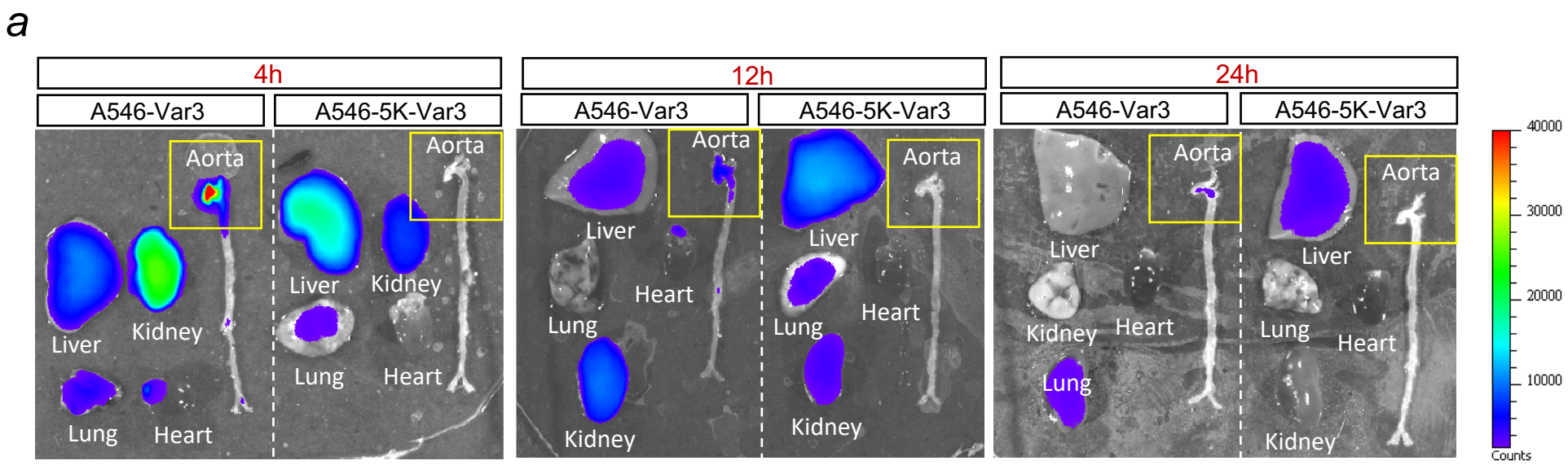
Supp Figure 4. Targeted silencing of miR-33 does not influence macrophage and smooth muscle cell content in atherosclerotic lesions. Low density lipoprotein receptor knockout (*Ldlr*$^{-/-}$) mice were placed on a western diet (WD) for 3 months, and then switched to a chow diet and received anti-miR-33 peptide nucleic acid delivery vector (anti-miR33^{pHLIP}) or scrambled control (Src^{pHLIP}) at a dose of 1 mg/kg body weight every week for a total of 5 injections. Representative immunofluorescence staining of macrophage (CD68 positive) and smooth muscle cell (α-smooth muscle actin) in cross sections of the aortic root from *Ldlr*$^{-/-}$ mice injected with anti-miR33^{pHLIP} or Src^{pHLIP} vectors. Quantification of macrophage and smooth muscle cell content are shown in the right panel. Data were analyzed by one-way ANOVA with Bonferroni correction for multiple comparisons (*n*= 6-13 per group). Scale bar: 100 μm.

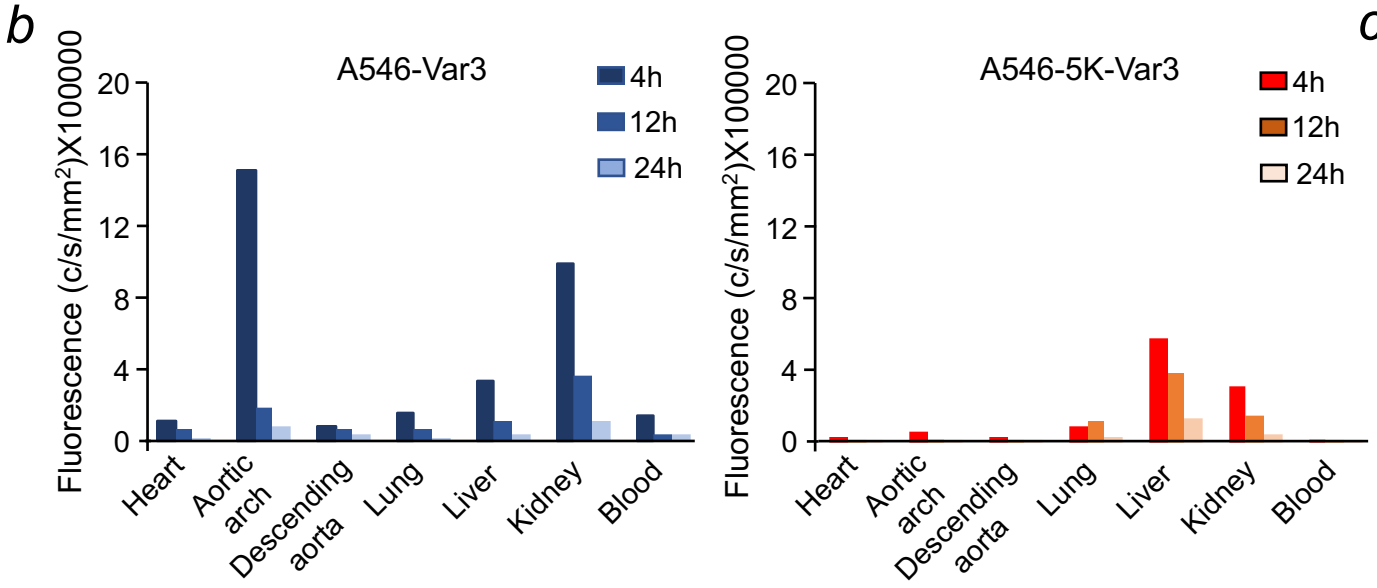
Supp Figure 5. pHLIP-mediated miR-33 silencing does not influence hepatic ABCA1 and ABCG1 expression. Representative Western blot analysis of ABCA1 and ABCG1 in the liver from mice injected with anti-miR-33^{pHLIP} or Src^{pHLIP}. Quantification are shown in the bottom panels. Data were analyzed by an unpaired two-sided Student's t-test (*n*= 5 per group). Quantification represents the mean ± s.e.m.

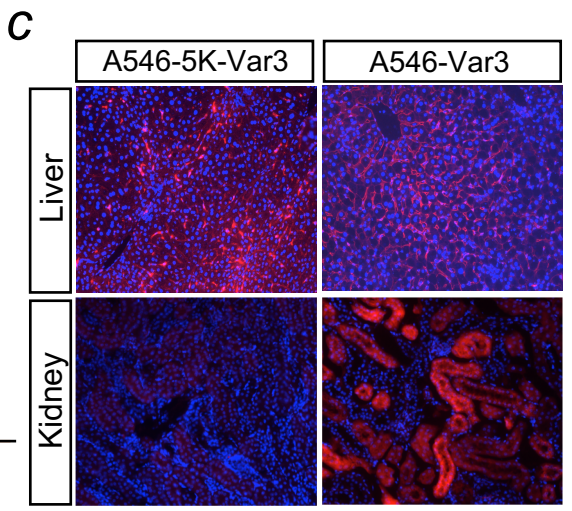
Supp Figure 6. Single cell RNAseq analysis of whole aortic cells treated with anti-miR-33 peptide nucleic acid delivery vector (anti-miR33^{pHLIP}) or scrambled control (Src^{pHLIP}). a, Heatmap of the 20 most upregulated genes in each cluster defined in **Fig. 4a** and selected enriched genes used for biological identification of each cluster. **b**, Proportions of cells extracted from the atherosclerotic aortas of anti-miR33^{pHLIP} and Src^{pHLIP}-treated mice.

Supp Figure 7. Gene expression features from indicated monocytes/macrophages clusters. a, Uniform manifold approximation and projection (UMAP) plots of *Cd14, Cd68, Adgre1*, and *Csf1r* in sorted cells from atherosclerotic plaques of mice treated with anti-miR-33 peptide nucleic acid delivery vector (anti-miR33^{pHLIP}) or scrambled control (Src^{pHLIP}) . **b**, UMAP plots of most highly upregulated genes for clusters of Trem2^{high} Mac, F10^{+} Mono, Inflammatory Mac and Stem-like Mac. c, UMAP plots of most highly upregulated genes for ECM^{high} Mac cluster.

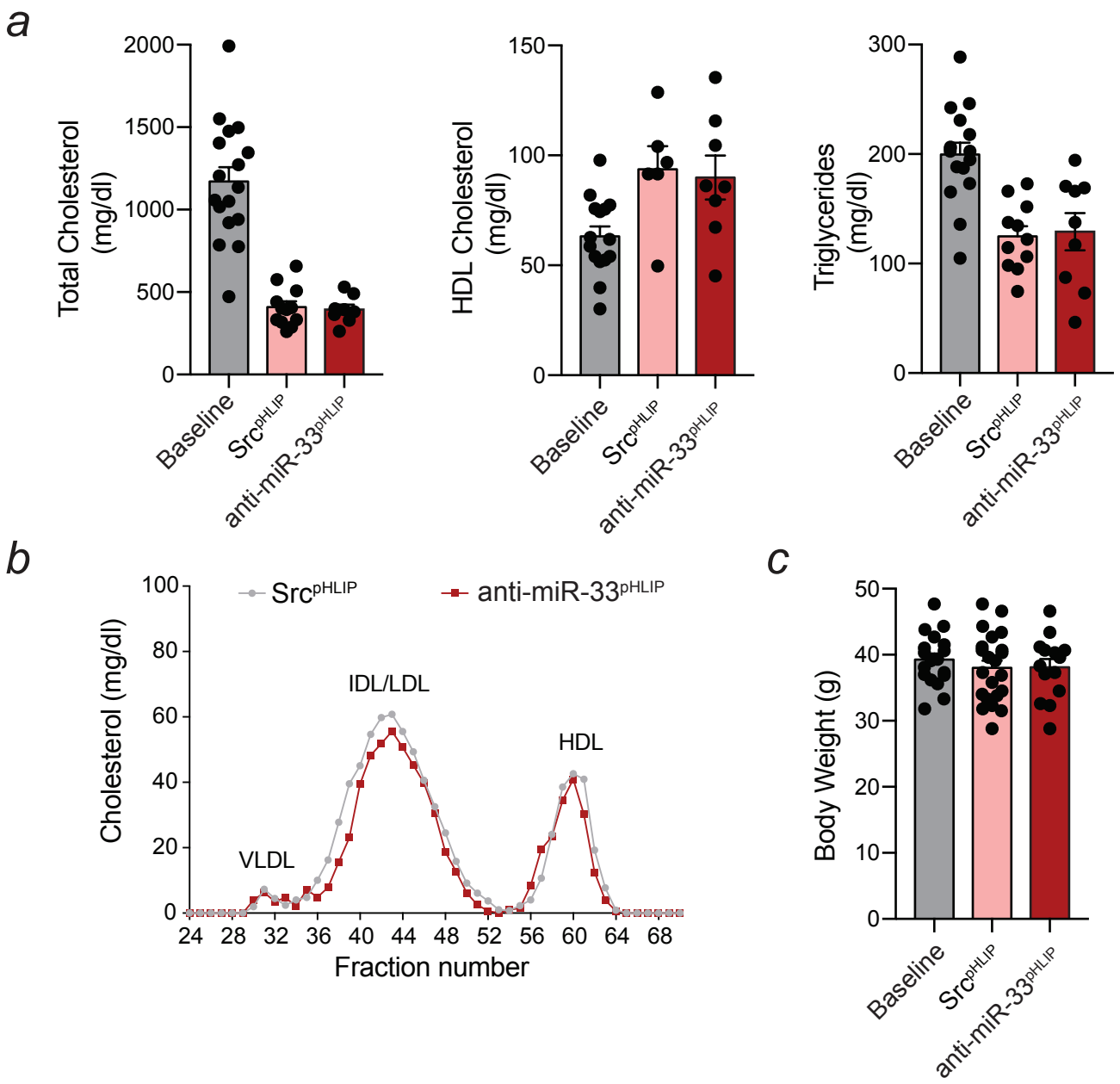
Supplementary Fig 1



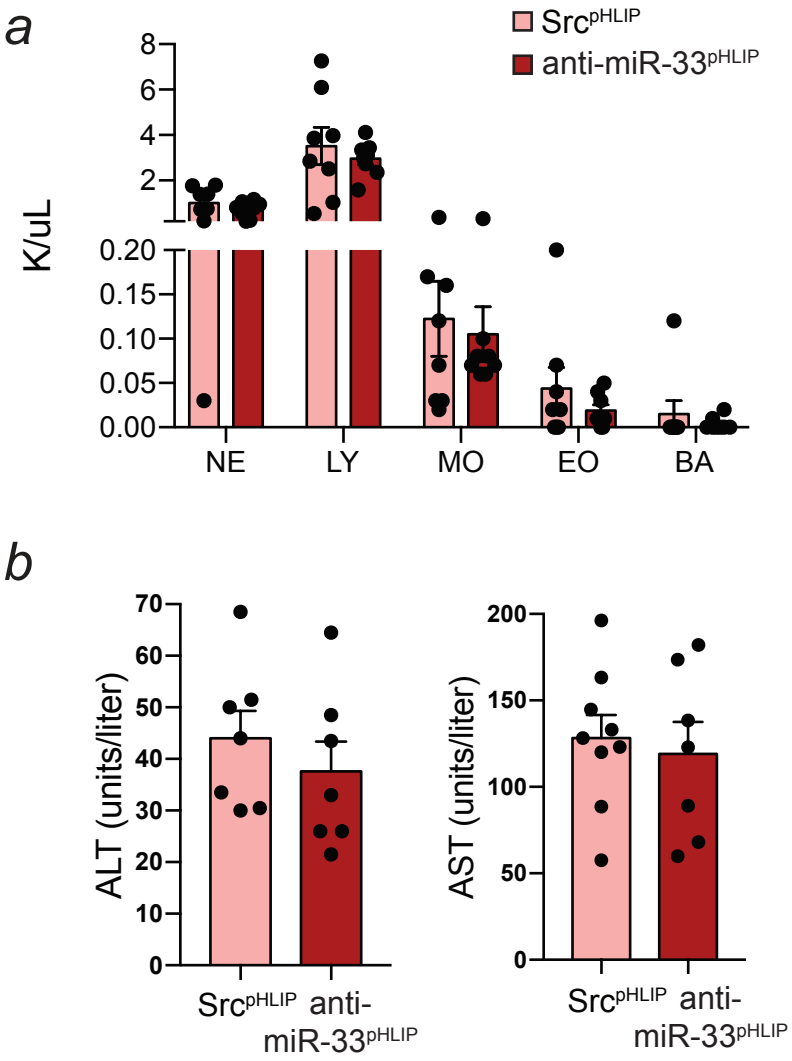




Supplementary Figure 2



Supplemental Figure 3



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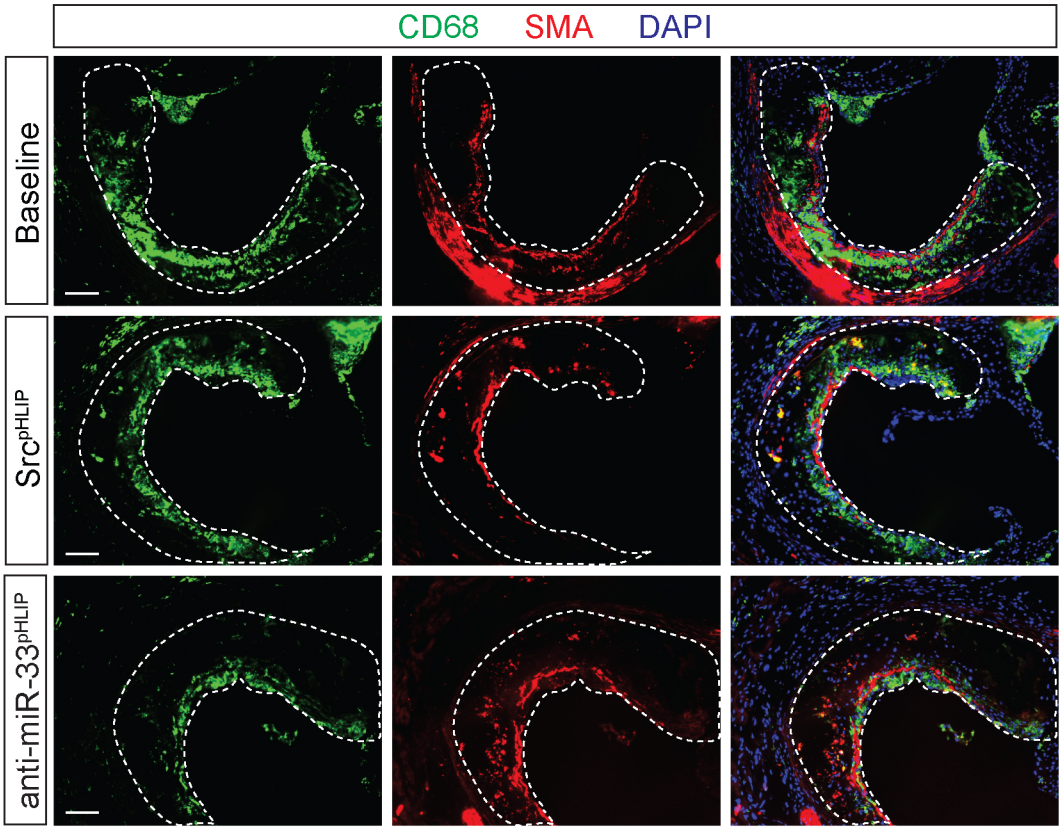

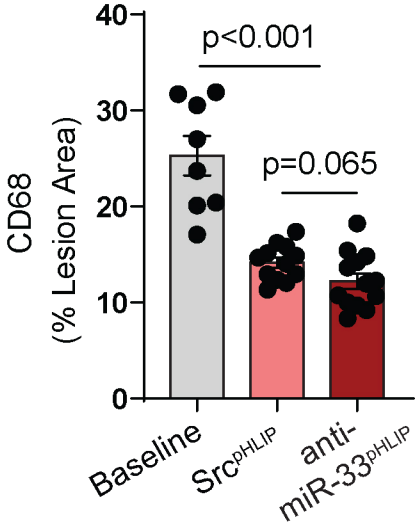

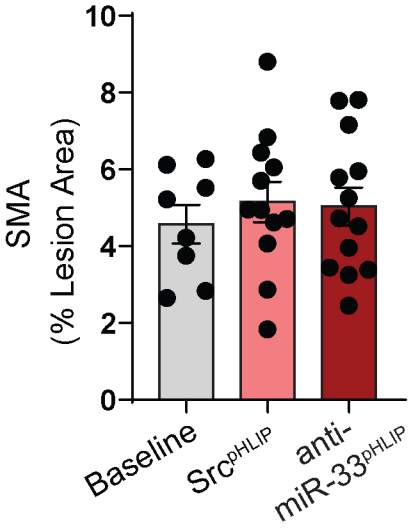

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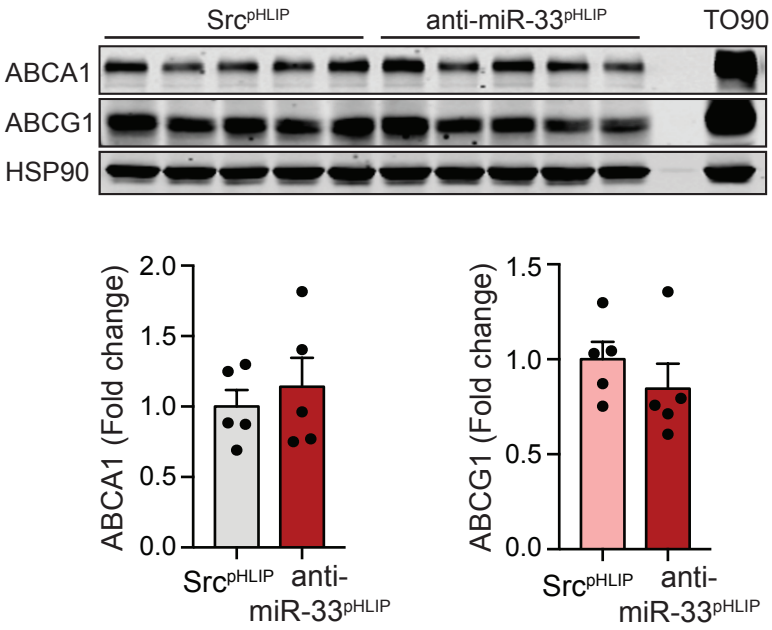

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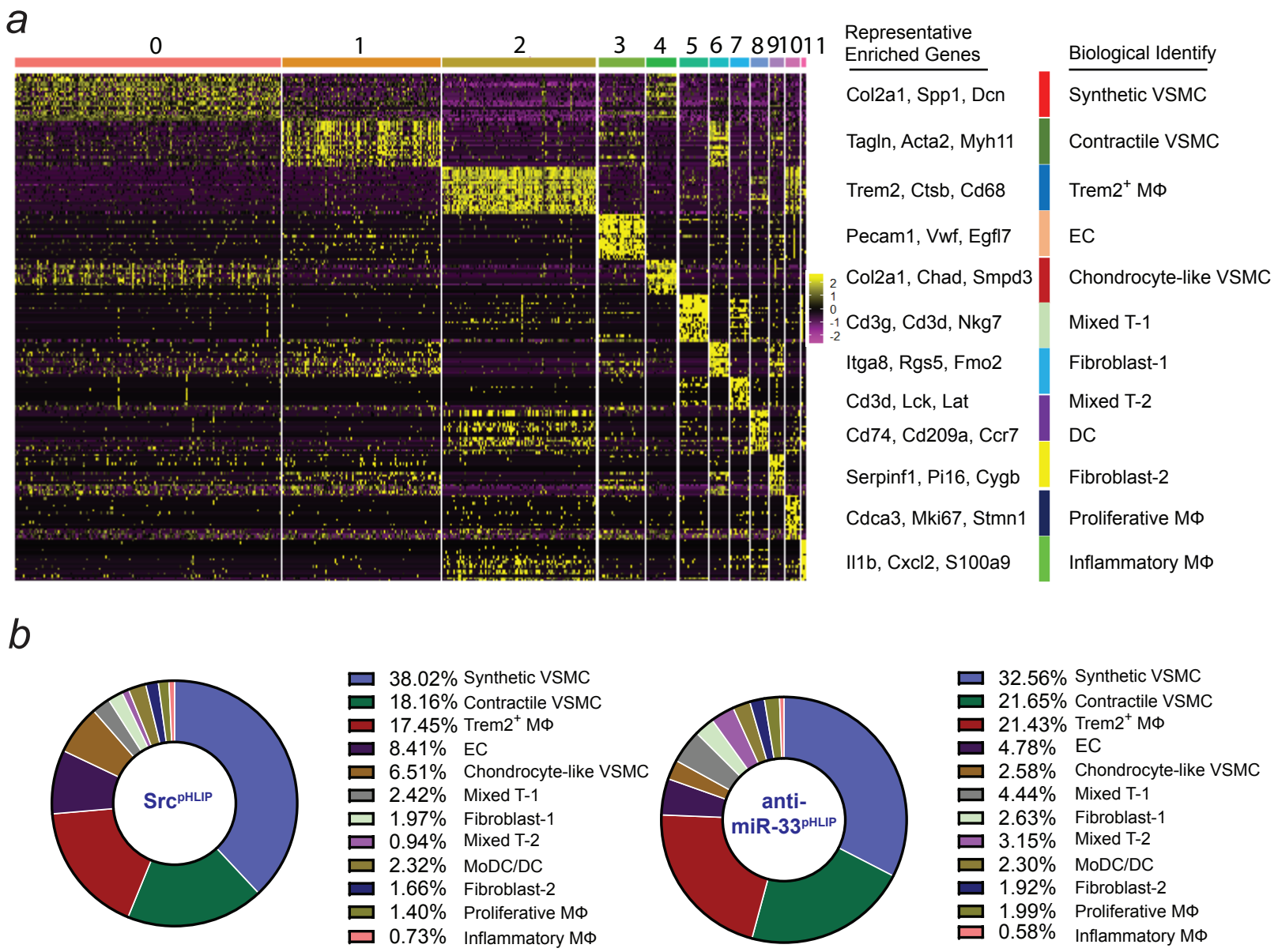

Supplemental Figure 7