

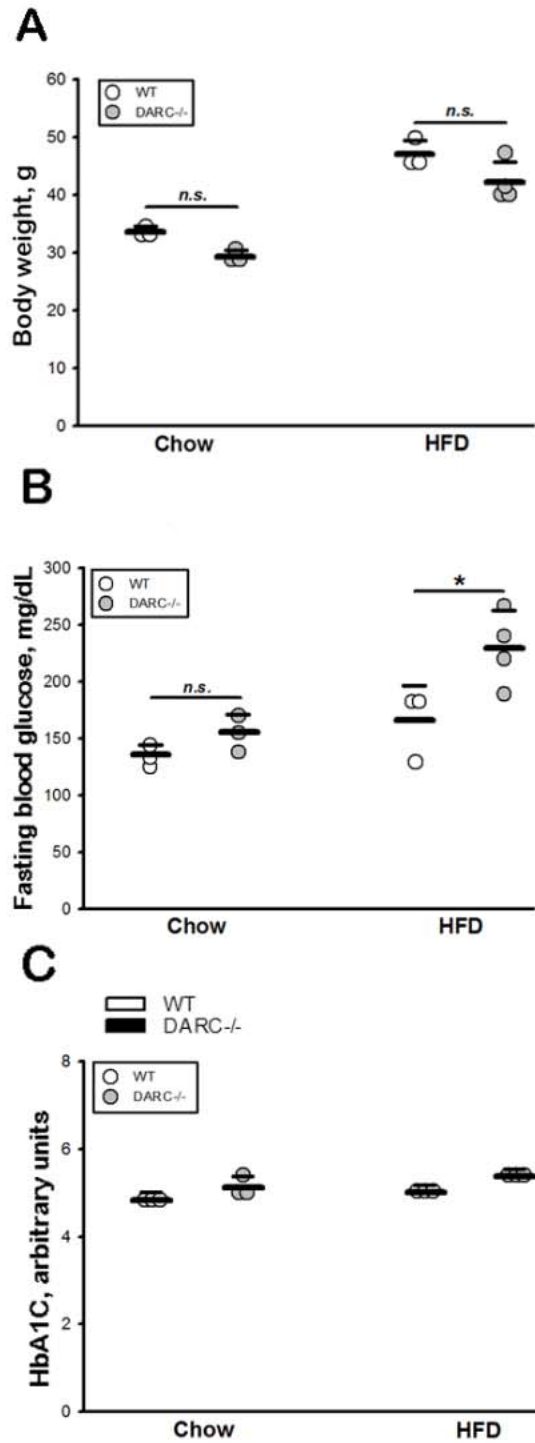
## **SUPPLEMENTAL MATERIAL**

### **Supplementary Materials and Methods**

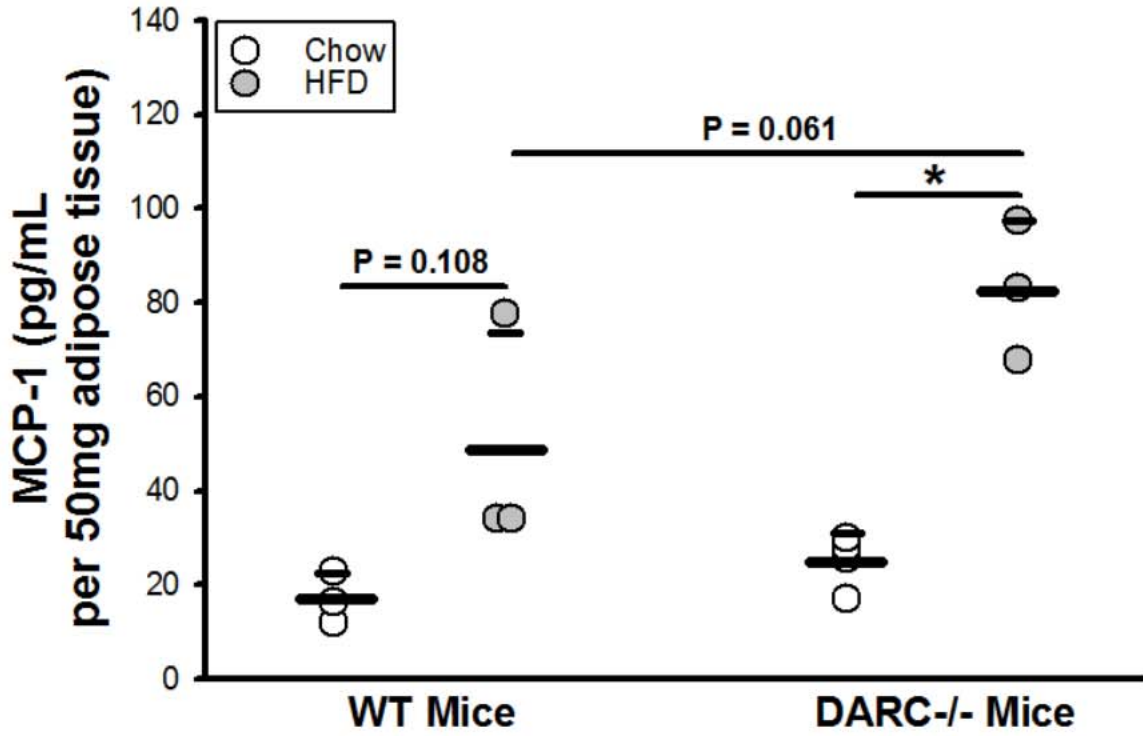
#### **Animals and collection of whole blood**

Six week old C57BL/6 WT or DARC<sup>-/-</sup> mice (strain #29873, MMRRC-UNC, Chapel Hill, NC) were fed either 10% fat chow diet (CD) or 60% HFD (Research Diets, Inc., D12492) for  $\geq 12$  weeks. The animals were anesthetized using isoflurane, and whole blood collected in EDTA tubes by cardiac puncture unless indicated otherwise. All animal experiments were approved by the University of Cincinnati and Georgia Regents University Institutional Animal Care and Use Committees.

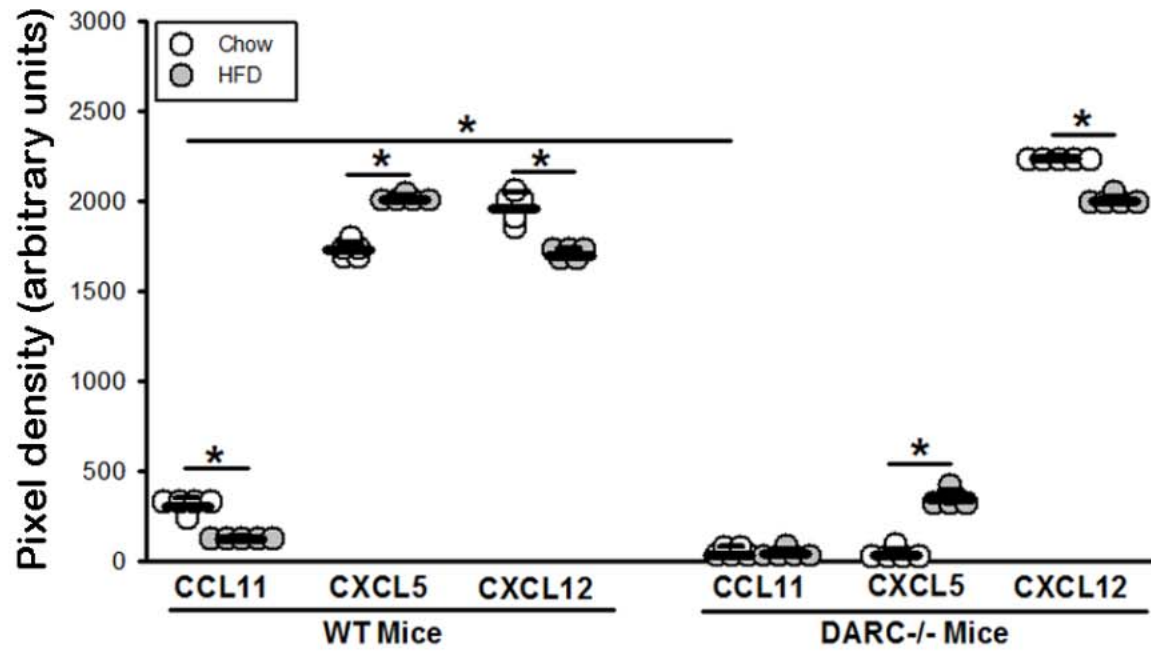
## Supplementary Figures



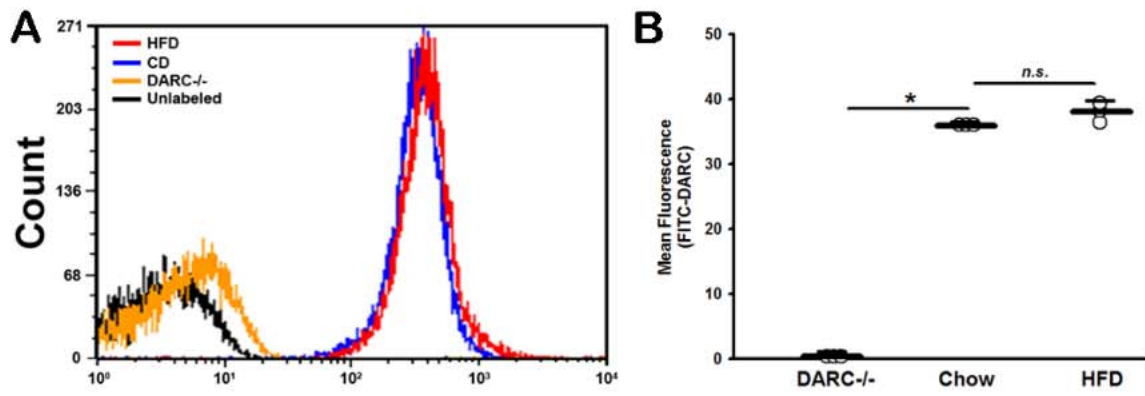
**Suppl. Fig 1:** Weight gain (A), fasting glucose (B), and Hb1AC levels (C) in WT and DARC<sup>-/-</sup> mice maintained on chow and HFD. Animals were fed an obesogenic, 60% fat diet for 12 weeks. n=3-4; \*, P<0.05.



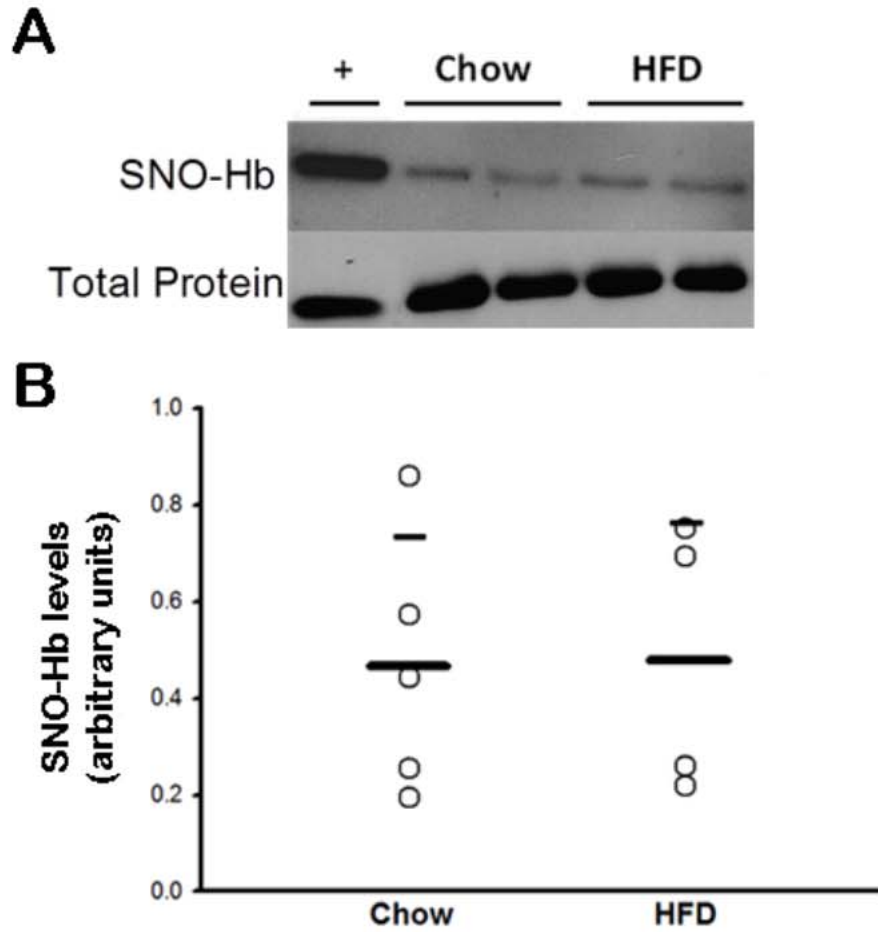
**Suppl. Fig 2:** Levels of MCP-1 protein secreted by subcutaneous adipose tissues are similar in WT and DARC-/- mice maintained on chow and HFD.  $n=3$ ; \*,  $P<0.05$ .



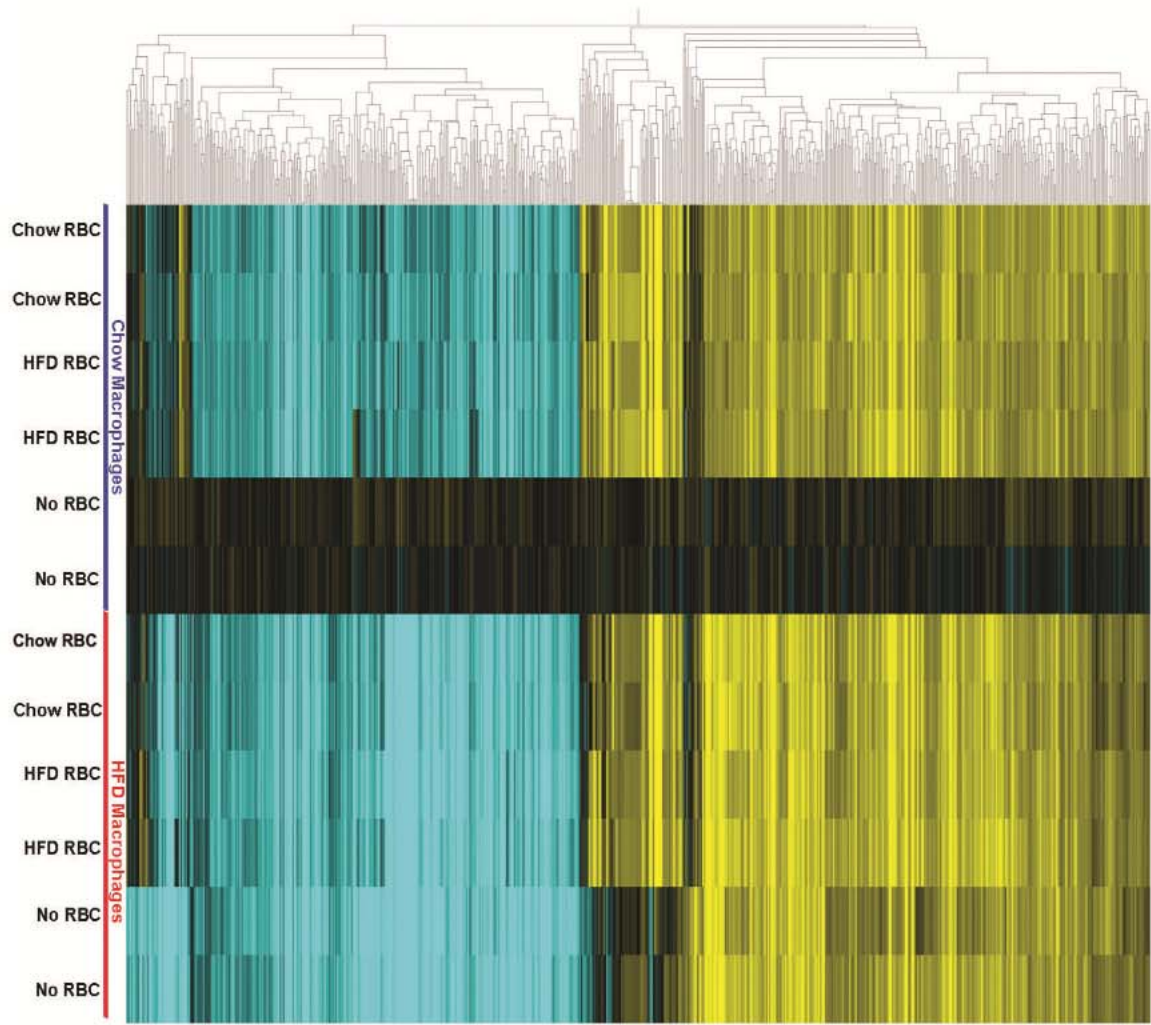
**Suppl. Fig 3:** Levels of CCL11, CXCL5, and CXCL12 on RBC membranes of WT and DARC<sup>-/-</sup> mice maintained on chow and HFD. n=5; \*, P<0.05.



**Suppl. Fig 4:** Levels of RBC-DARC are unaffected by HFD. **A**, representative flow cytometry experiment; **B**, calculated mean fluorescence in graph form; n=3, P<0.05.



**Suppl. Fig 5:** Levels of SNO-Hb as assessed by biotin switch assay are unaffected by HFD. **A**, representative western blot; **B**, calculated mean levels by analysis of relative densitometry in graph form;  $n=4-5$ , *n.s.* chow vs HFD.



**Suppl. Fig. 6:** Complete unsupervised clustering analysis, changes in gene expression (n=642) observed in macrophages isolated from WT mice maintained on CD or HFD, and exposed to either CD-RBC or HFD-RBC. Note that the bulk of the RBC response signature is recapitulated by HFD-exposed macrophages not exposed to RBC.

