Supplemental Materials

Decoding Functional High-Density Lipoprotein Particle Surfaceome Interactions

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Supplementary Tables:

Table S1. List of identified peptides in auto-CSC experiments with EA.hy926 cells, HAECs, HEPG2 cells, THP1 cells, activated THP1 cells, and foam cells. Identifications are filtered for FDR < 0.01 and NxS/T motifs.

Table S2. Matrix of all identified proteins in auto-CSC experiments with EA.hy926 cells, HAECs, HEPG2 cells, THP1 cells, activated THP1 cells, and foam cells.

Table S3. List of ranked and scaled protein abundances and the peptide counts (used for quantification) captured in auto-CSC experiments with EA.hy926 cells, HAECs, HEPG2 cells, THP1 cells, activated THP1 cells, and foam cells.

Table S4. Safequant output of the auto-CSC experiments quantitatively comparing VEGF-A treated vs untreated HAEC surfaceomes.

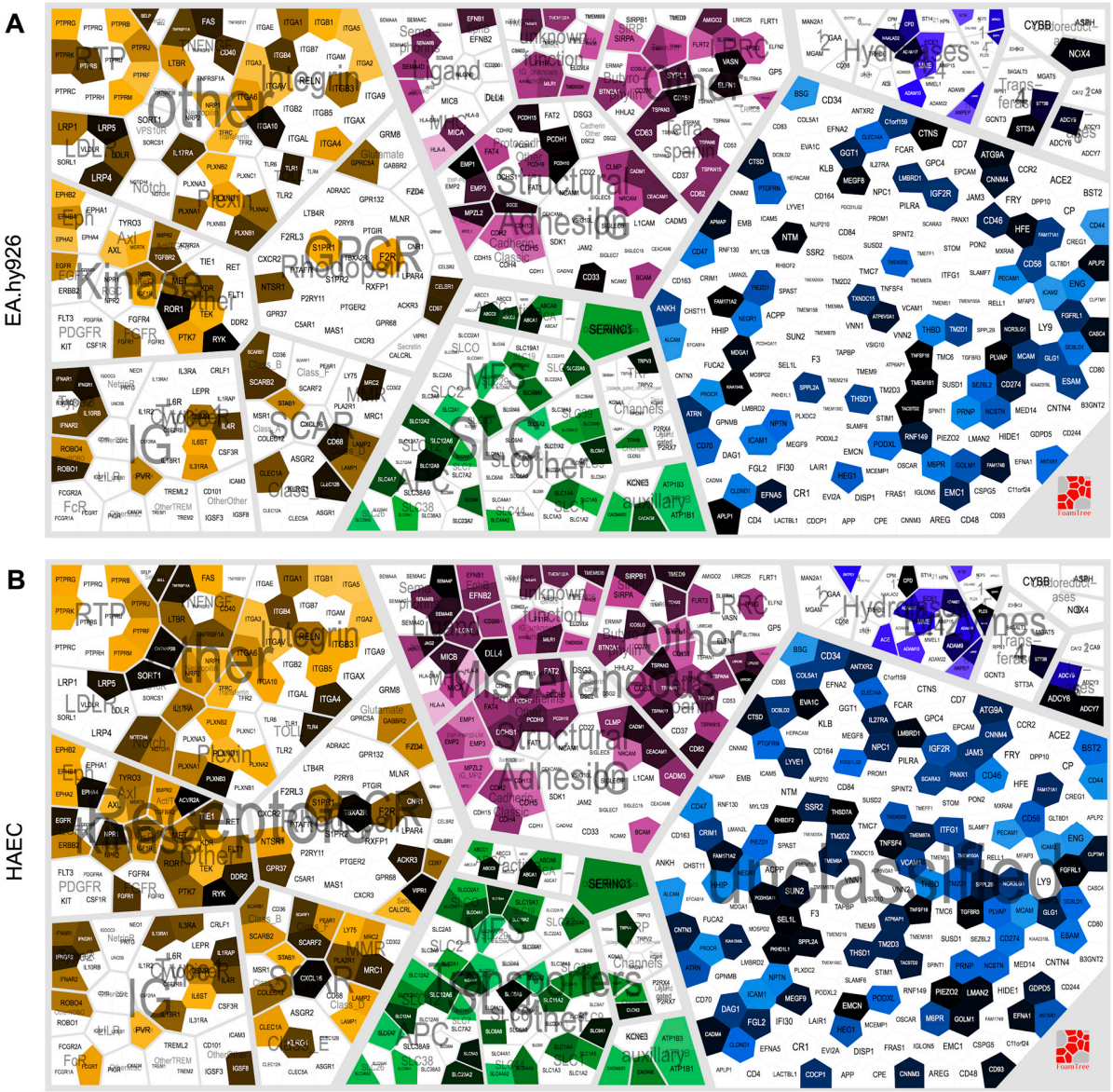
Table S5. Statistics from the Gene Ontology analysis of genes regulated by VEGF-A in HAECs.

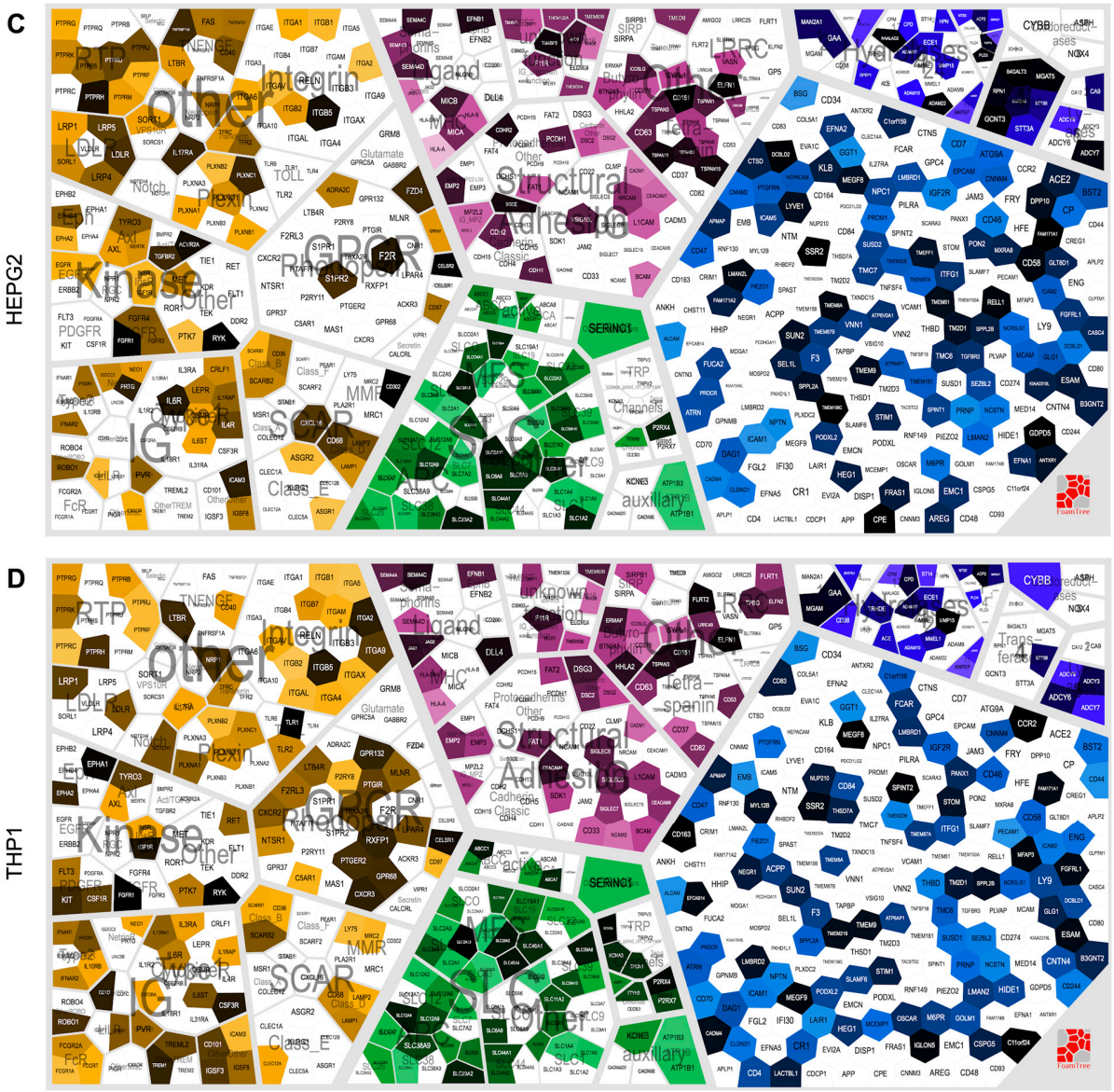
Table S6. Gene Ontology protein annotation of the analysis of VEGF-A-sensitive proteins on HAECs.

Table S7. Protein content of rHDL as identified by COMET. Peptide and protein identifications were filtered with an FDR of 1%. Common contaminants and keratins were removed from the protein list.

Table S8. Safequant output of the HATRIC-LRC experiment with APOA1, lipidated APOA1, and HDL as ligands on EA.hy926 cells.

Supplementary Figures:




C
HEPG2
D
THP1

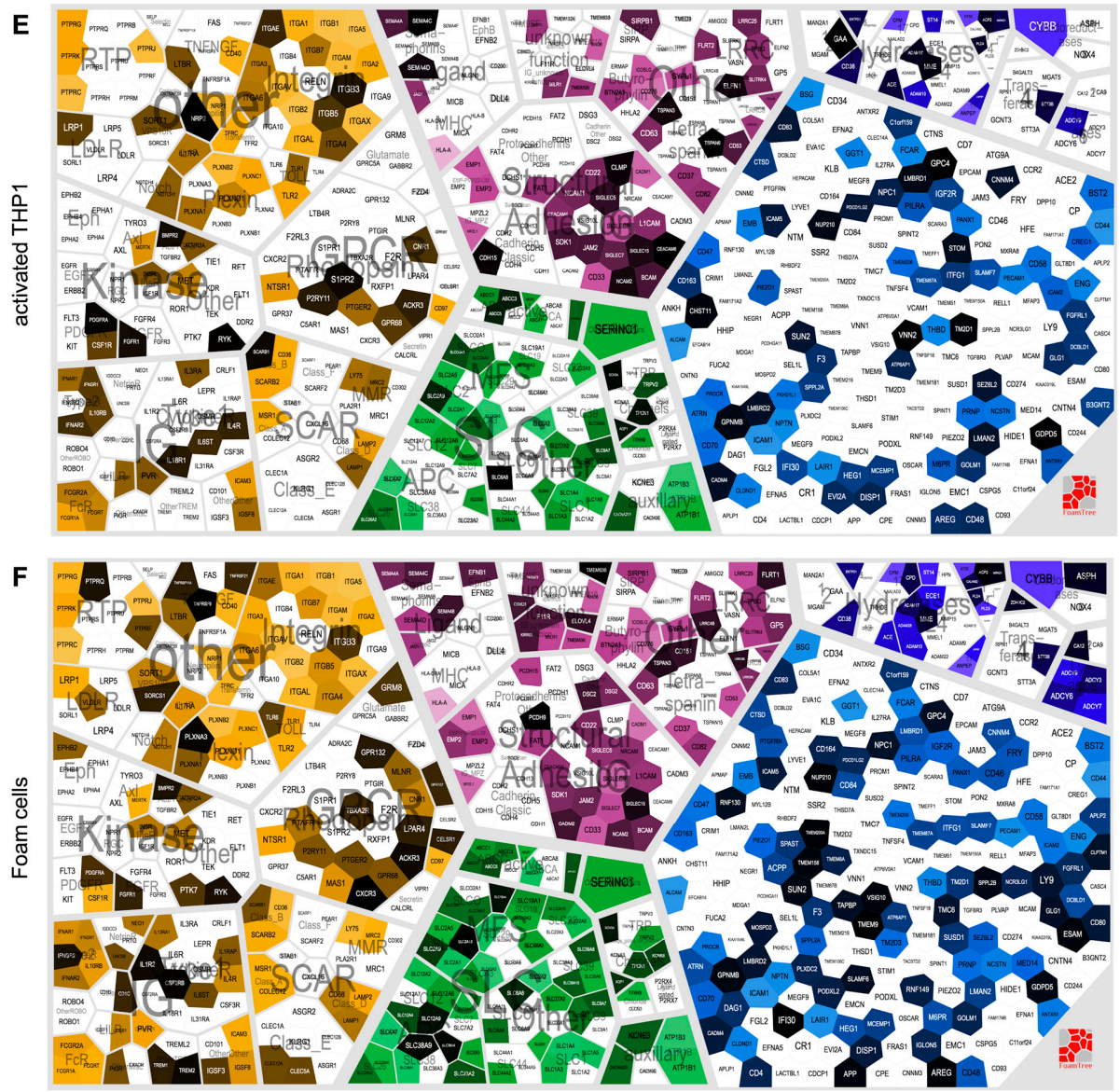


Figure S1. Voronoi treemaps of the quantitative surfaceome of different cell lines: (**A**) EA.hy926 cells, (**B**) HAECs, (**C**) HEPG2 cells, (**D**) THP1 cells, (**E**) activated THP1 cells, and (**F**) foam cells. The background proteome comprises all identified proteins across the different cell types. Color scale corresponds to the cell type-specific ranked and scaled abundance levels of the proteins.

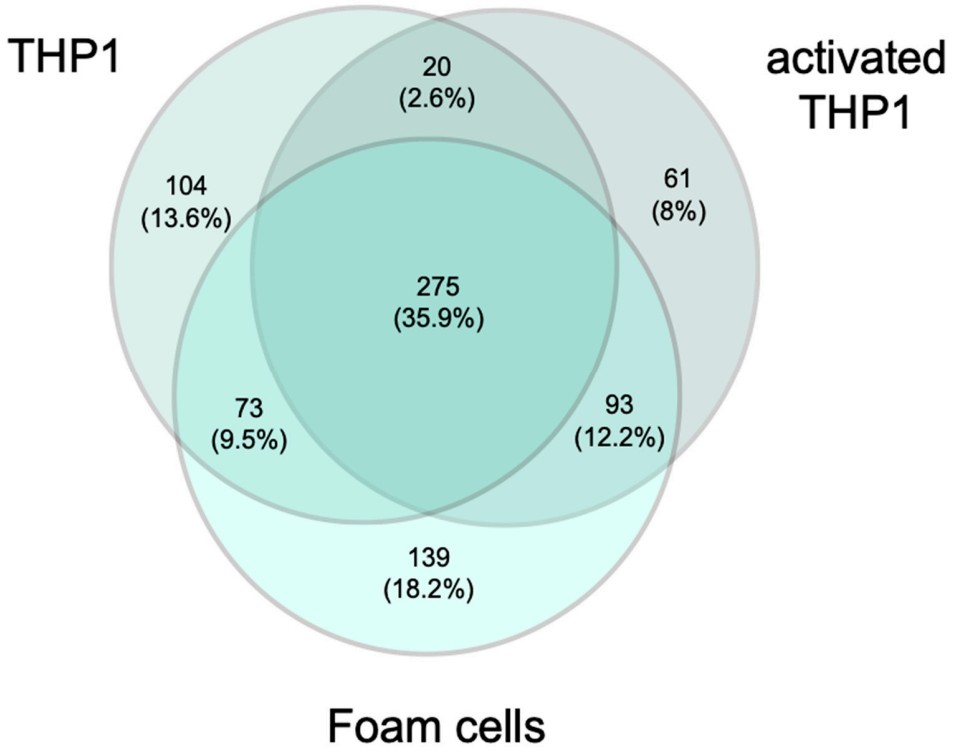


Figure S2. Venn diagram of overlap of identified proteins in the auto-CSC experiments with THP1 cells, activated THP1 cells, and foam cells.

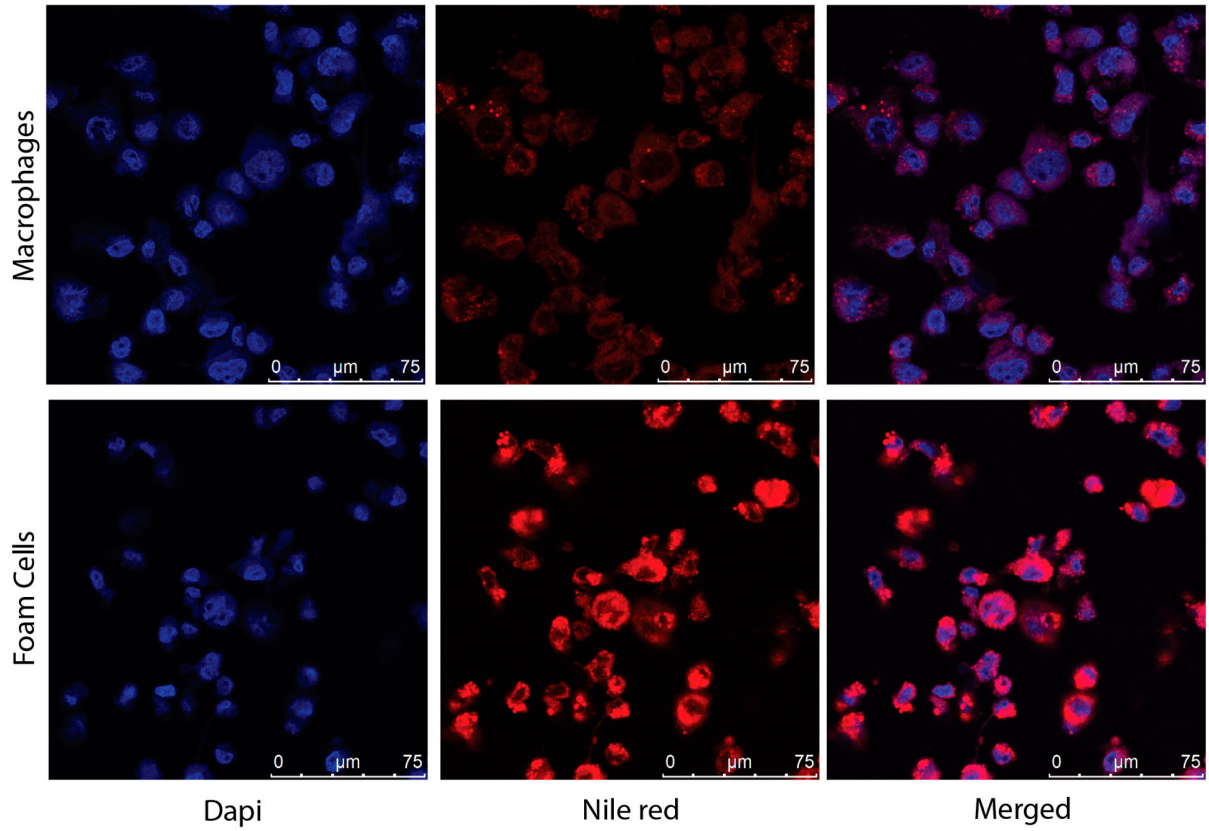


Figure S3. Microscopy images of the lipid content within THP1 cells, activated THP1 cells, and foam cells. Lipids were stained with Nile red.

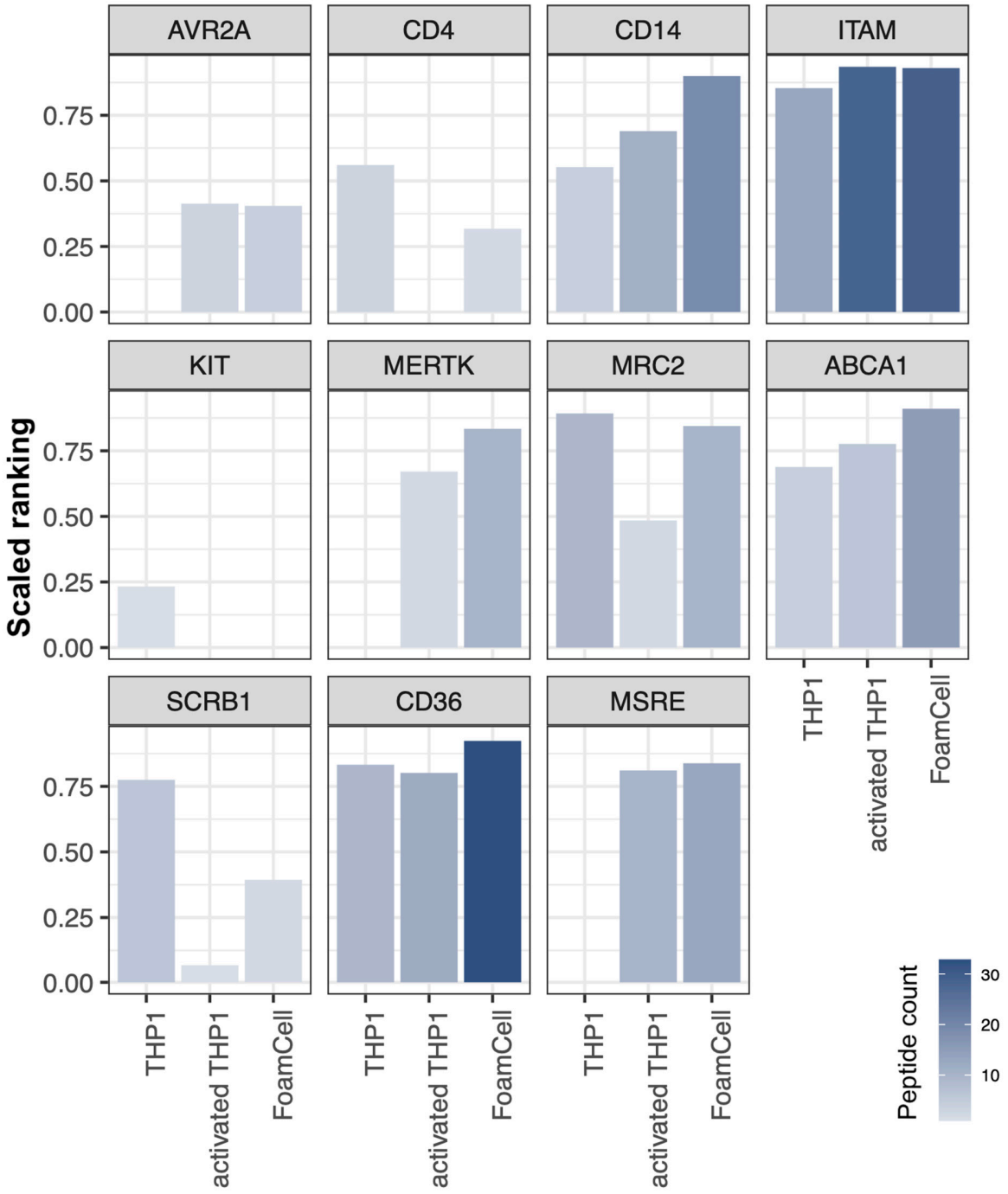


Figure S4. Bar charts of selected proteins and their scaled ranked abundances determined with auto-CSC on THP1 cells, activated THP1 cells, and foam cells. Included are proteins previously shown to have altered surface abundance upon THP1 differentiation [1]: AVR2A, CD4, CD14, ITAM, KIT, MERTK, and MRC2. Data for the APOA1/HDL receptors ABCA1 and SCRB1 as well as the oxLDL receptors CD36 and MSRE [2] are also shown.

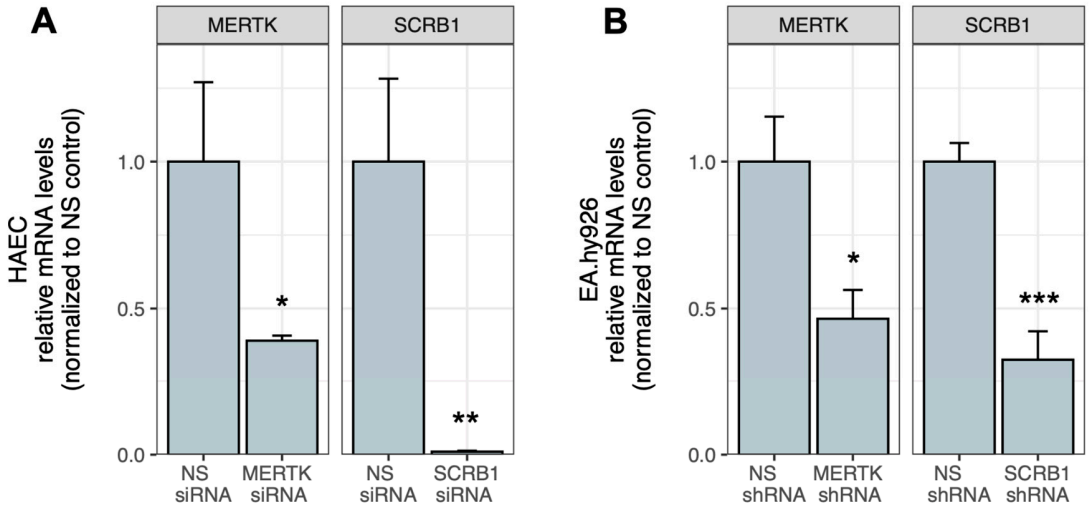


Figure S5. mRNA levels upon silencing of *MERTK* and *SCRB1* in EA.hy926 and HAECs. Significance of *MERTK* and *SCRB1* silencing using shRNA in EA.hy926 cells and siRNA in HAECs compared to control cells treated with non-silencing (NS) vector or control, respectively, was assessed with Student's *t*-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

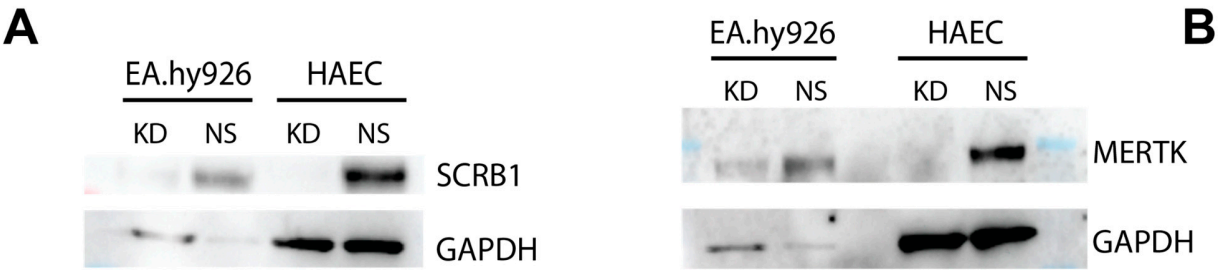


Figure S6. Western blot analysis of total protein abundance levels upon silencing of (**A**) *SCRB1* and (**B**) *MERTK* in EA.hy926 cells and HAEC with shRNA or siRNA, respectively (KD), and upon treatment with non-silencing controls (NS).

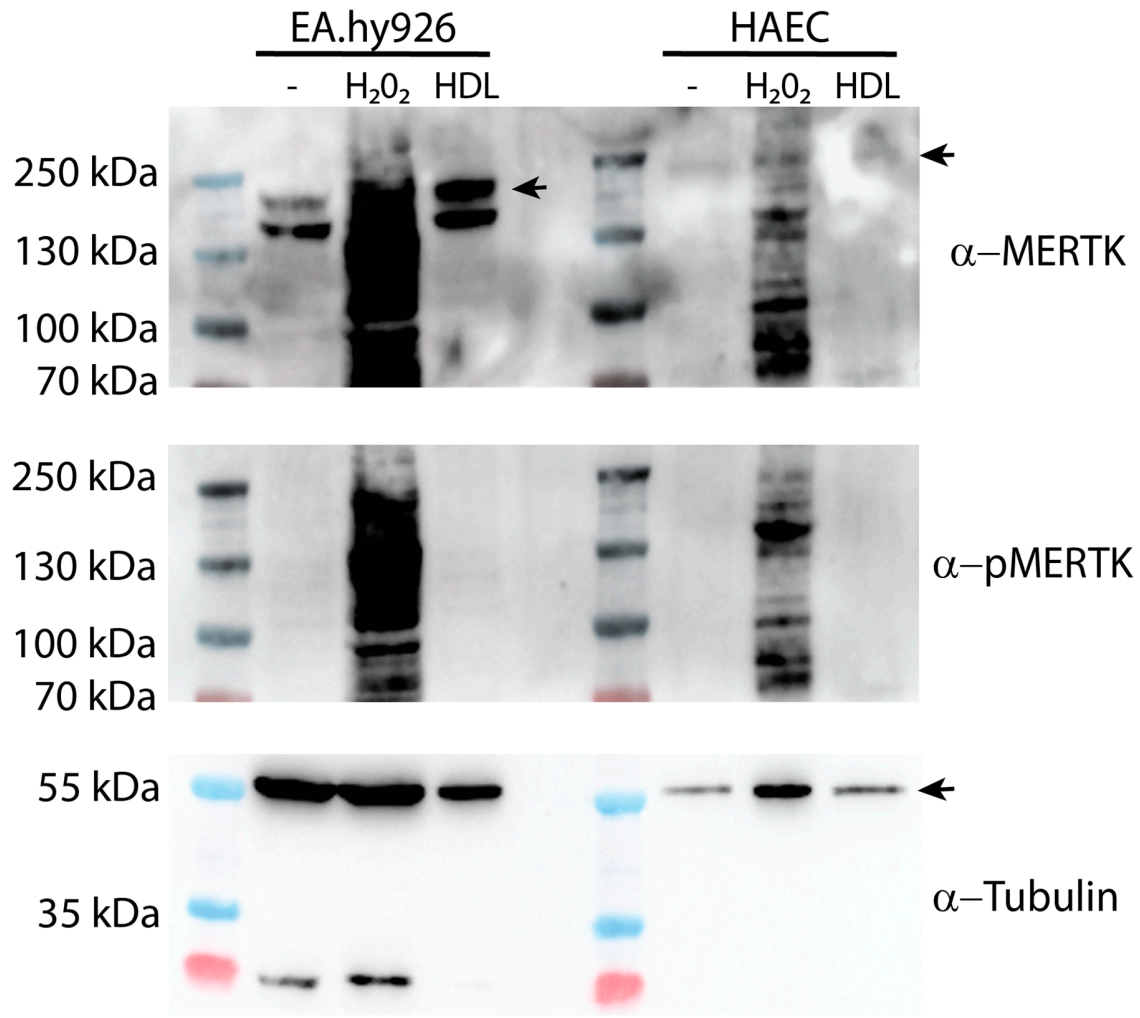


Figure S7. Effect of HDL treatment on phosphorylation of MERTK in EA.hy926 cells and HAECs as shown by Western blot. H_2O_2 treatment served as positive control [3]. Tubulin was used as loading control.

References:

1. Kalxdorf, M.; Gade, S.; Christian Eberl, H.; Bantscheff, M. Monitoring Cell-surfaceN-Glycoproteome Dynamics by Quantitative Proteomics Reveals Mechanistic Insights into Macrophage Differentiation. *Molecular & Cellular Proteomics* 2017, *16*, 770–785.
2. Das, R.; Ganapathy, S.; Mahabeleshwar, G.H.; Drumm, C.; Febbraio, M.; Jain, M.K.; Plow, E.F. Macrophage Gene Expression and Foam Cell Formation Are Regulated by Plasminogen. *Circulation* **2013**, *127*, 1209–1218, e1–e16.
3. Anwar, A.; Keating, A.K.; Joung, D.; Sather, S.; Kim, G.K.; Sawczyn, K.K.; Brandão, L.; Henson, P.M.; Graham, D.K. Mer Tyrosine Kinase (MerTK) Promotes Macrophage Survival Following Exposure to Oxidative Stress. *J. Leukoc. Biol.* **2009**, *86*, 73–79.