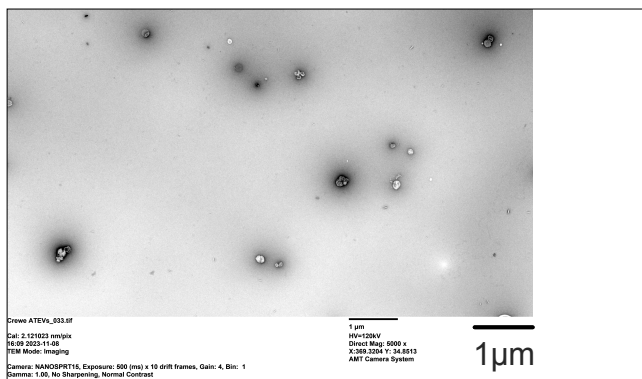


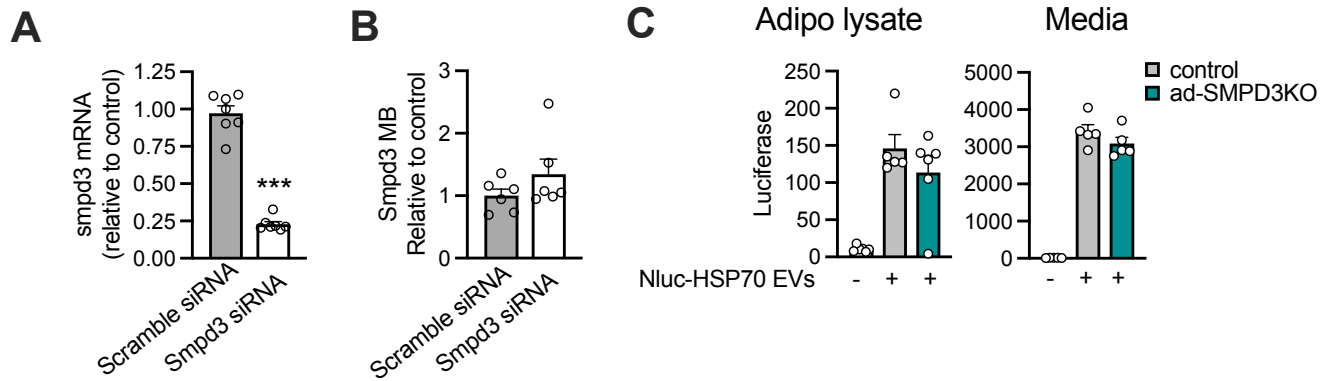
SUPPLEMENTARY INFORMATION

Adipocytes signal to recruit specific mRNAs from surrounding cells to restore
expression deficits

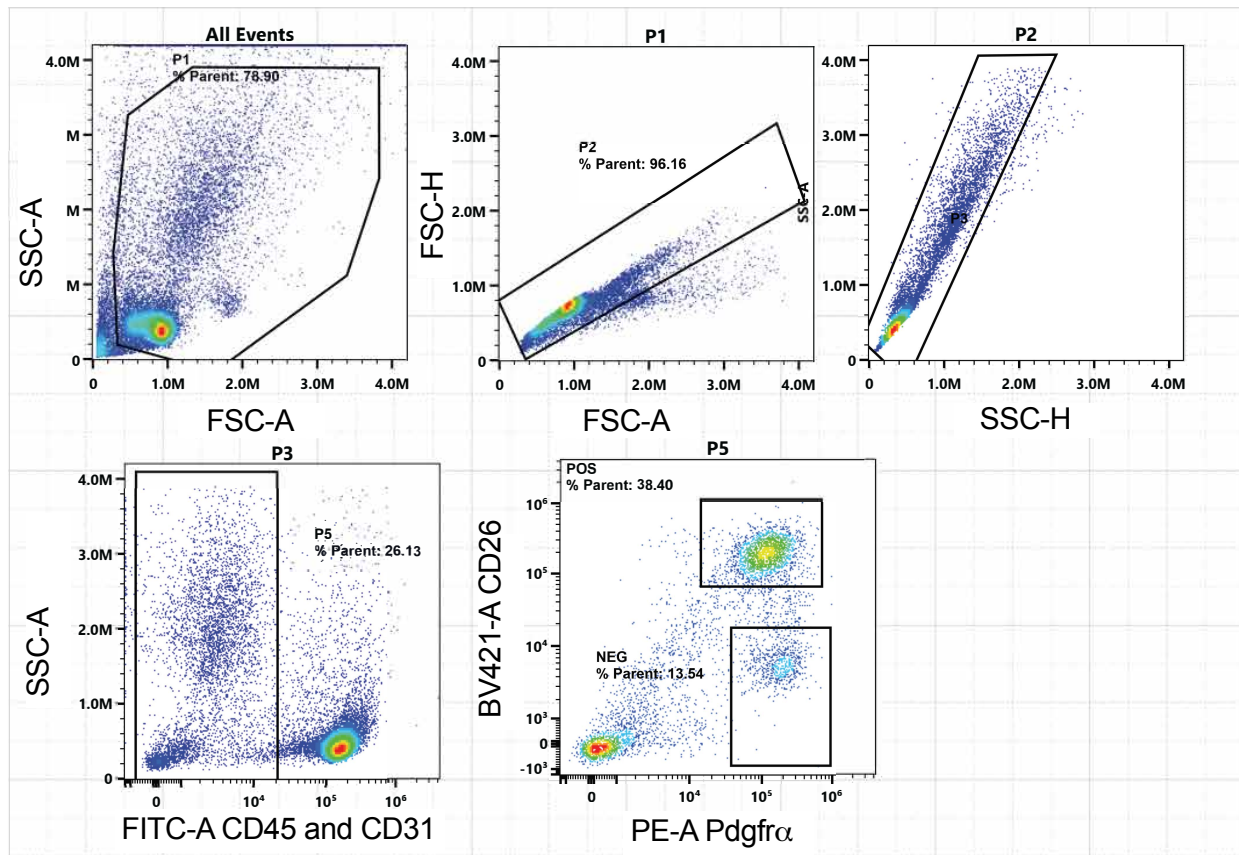
Clair Crewe, Christy M. Gliniak, Toshiharu Onodera, Shihwei Chen, Jan-Bernd Funcke,
May-Yun Wang, Snigdha Tiash, Yun-Ling Pai, Marjori Russo, Saket Awadhesbhai Patel,
Ze Yu, Yi-Cian Zheng, Alex Larkin, Chao Xing, Laurent Gautron¹, Chun-Kan Chen,
Chen Liu, Philipp E. Scherer



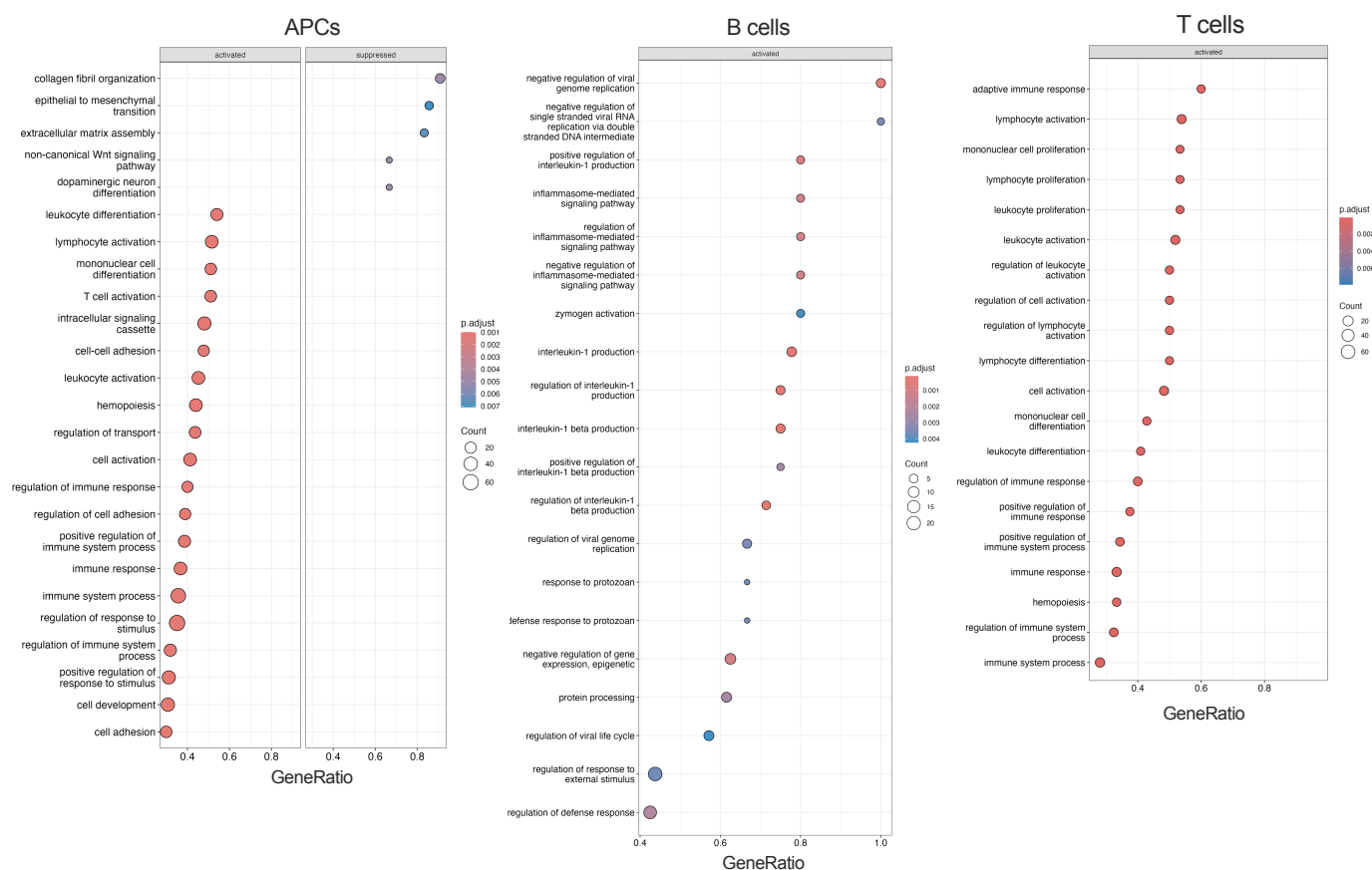
Supplementary Figure 1. ATsEV electron micrograph. ATsEVs were imaged at a 5000X magnification to demonstrate the presence of multiple EVs in a single field of view.



Supplementary Figure 2. SMPD3 Knockdown by siRNA. **A-B**, *in vitro* differentiated adipocytes were reverse-transfected with either scramble siRNA or siRNA against SMPD3. **A**, qPCR confirmation of SMPD3 knockdown ($n=7$, $P<0.0001$). **B**, molecular beacon (MB) transfer from stromal vascular fraction cells (SVF) to scramble, or knockdown adipocytes ($n=6$). **C**, SVF cells were microporated with a plasmid to express a Nano luciferase-HSP70 fusion protein to track EV production (media) and transfer to adipocytes (adipo-lysate). Adipo lysate: - $n=6$; control + $n=5$; ad-SMPD3KO + $n=6$. Media: - $n=5$; control + $n=5$; ad-SMPD3KO + $n=5$. Data are presented as mean $\pm$ s.e.m. Statistics determined by 2-tailed Student's *t* test.



Supplementary Figure 3. Gating strategy for FACS sorting of preadipocyte populations. Hematopoietic cells (CD45) and endothelial cells (CD31) were excluded and preadipocytes (PDGF α^+) and preadipocyte sub populations (DPP4; CD26 $^{-/+}$) were sorted.



Supplementary Figure 4. scRNAseq Gene ontology analysis. Gene set enrichment analysis (GSEA) was performed on ranked gene lists derived from single-cell RNA sequencing (scRNA-seq) data. Differential gene expression for each cell population (APCs, B cells, and T cells) was conducted using the Seurat package (v4.4.0), and genes were ranked based on adjusted *p*-value and log2 fold change. Enrichment significance was assessed using normalized enrichment scores (NES) and adjusted *p*-values.

Target (<i>mus</i>)	Forward Primer Sequence	Reverse Primer Sequence
Smpd3 exon 3	CTATGAGAAGCGCCAGAGGG	CAGACTGCAGTGGAGACCAG
Smpd3 exon 2-3 (mature mRNA)	AGCGCCCTCCTGTGCG	ACACCGGAGTCTGCTGAAAA
Smpd3 intron 2 - exon 3 (pre- mRNA)	CCAAGACAGCCAGTTAGGGA	ACAGACAGTCATGGTTCGCT
Smpd1	TGGGACTCCTTTGGATGGG	CGGCGCTATGGCACTGAAT
Smpd2	TGGGACATCCCCTACCTGAG	TAGGTGAGCGATAGCCTTTGC
Smpd5	TCATGGGAGTTACCCAGCTC	CGACATTCATCCCCTTGTCT
Sptlc1	AGGGTTCTATGGCACATTTGATG	TGGCTTCTTCGGTCTTCATAAAC
Sptlc2	CAAAGAGCTTCGGTGCTTCAG	GAATGTGTGCGCAGGTAGTCTATC
Cers2	AGAGTGGGCTCTCTGGACG	CCAGGGTTTATCCACAGTGAC
Cers3	CCTGGCTGCTATTAGTCTGATG	CTGCTTCCATCCAGCATAGG
Cers4	CTGTGGTACTGTTGTTGCATGAC	GCGCGTGTAGAAGAAGACTAAG
Cers5	TGGCCAATTATGCCAGACGTGAG	GGTAGGGCCCAATAATCTCCCAGC
Cers6	GCATTCAACGCTGGTTTCGAC	TTCAAGAACCGGACTCCGTAG
Cre	GCGGTCTGGCAGTAAAACTATC	GTGAAACAGCATTGCTGTCACTT
Gapdh	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT
B2M	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC

Supplementary Table 1. Primer sequences