Supplementary information

Deep proteomics profiling of the cell secretome by DeepSec

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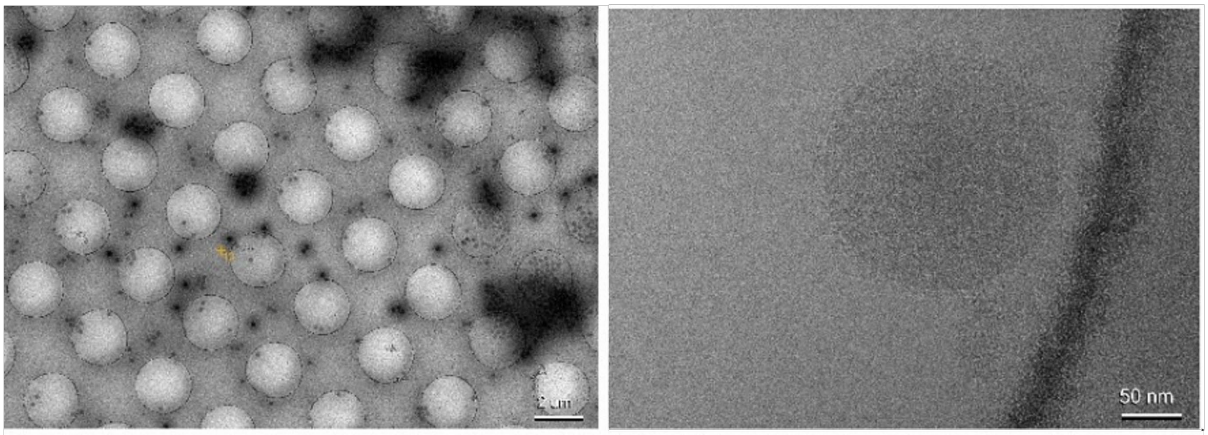
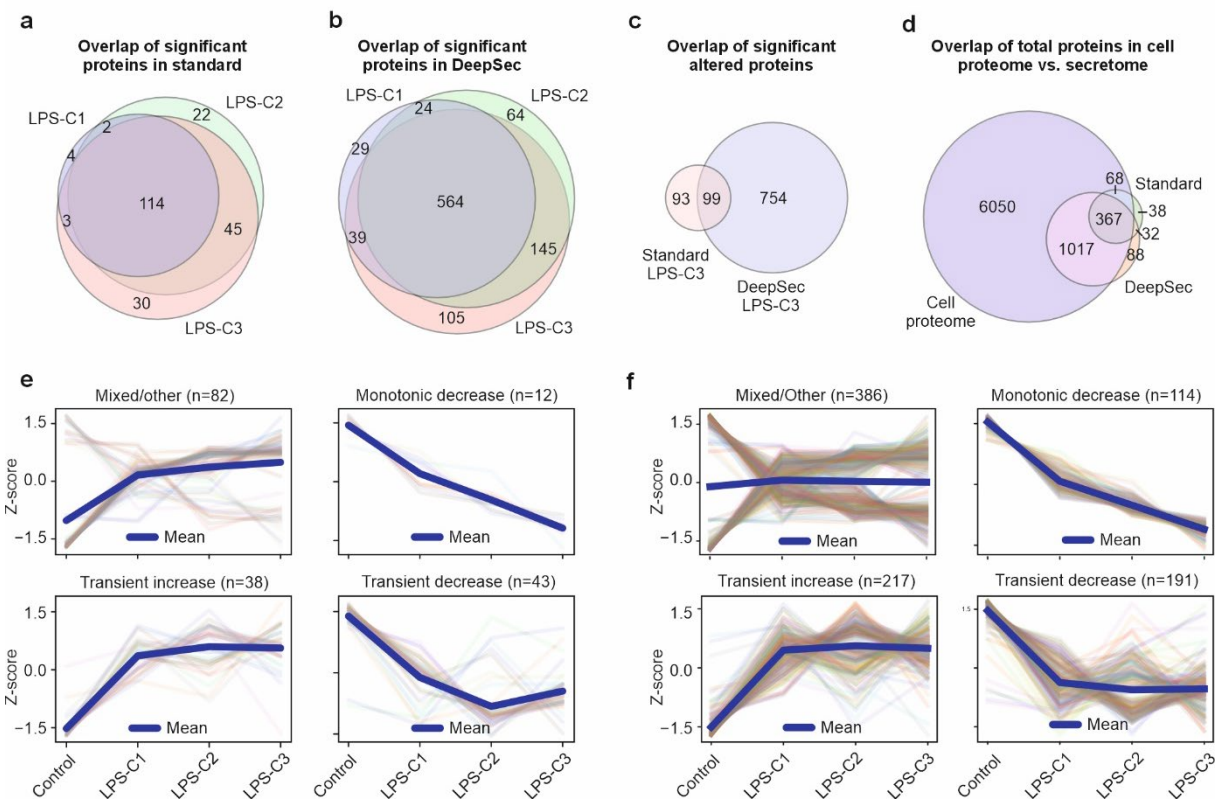
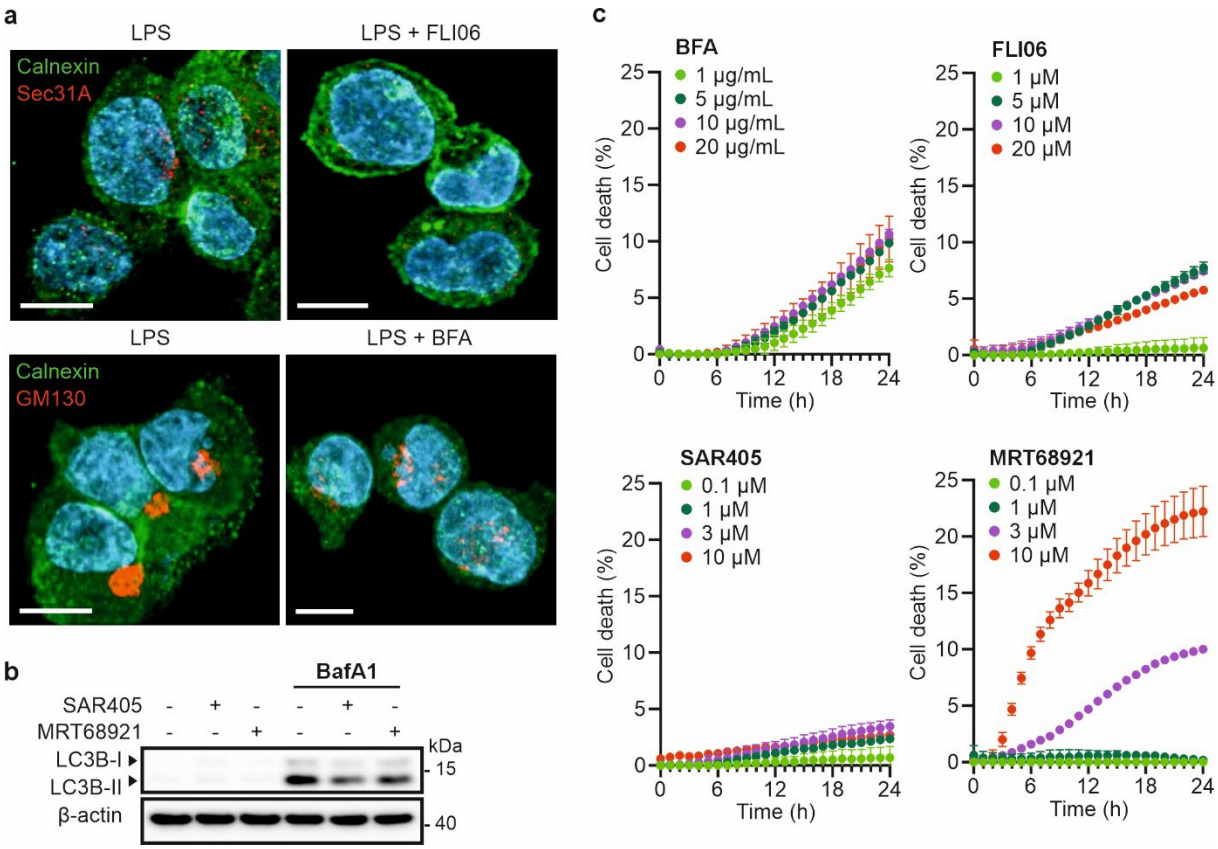


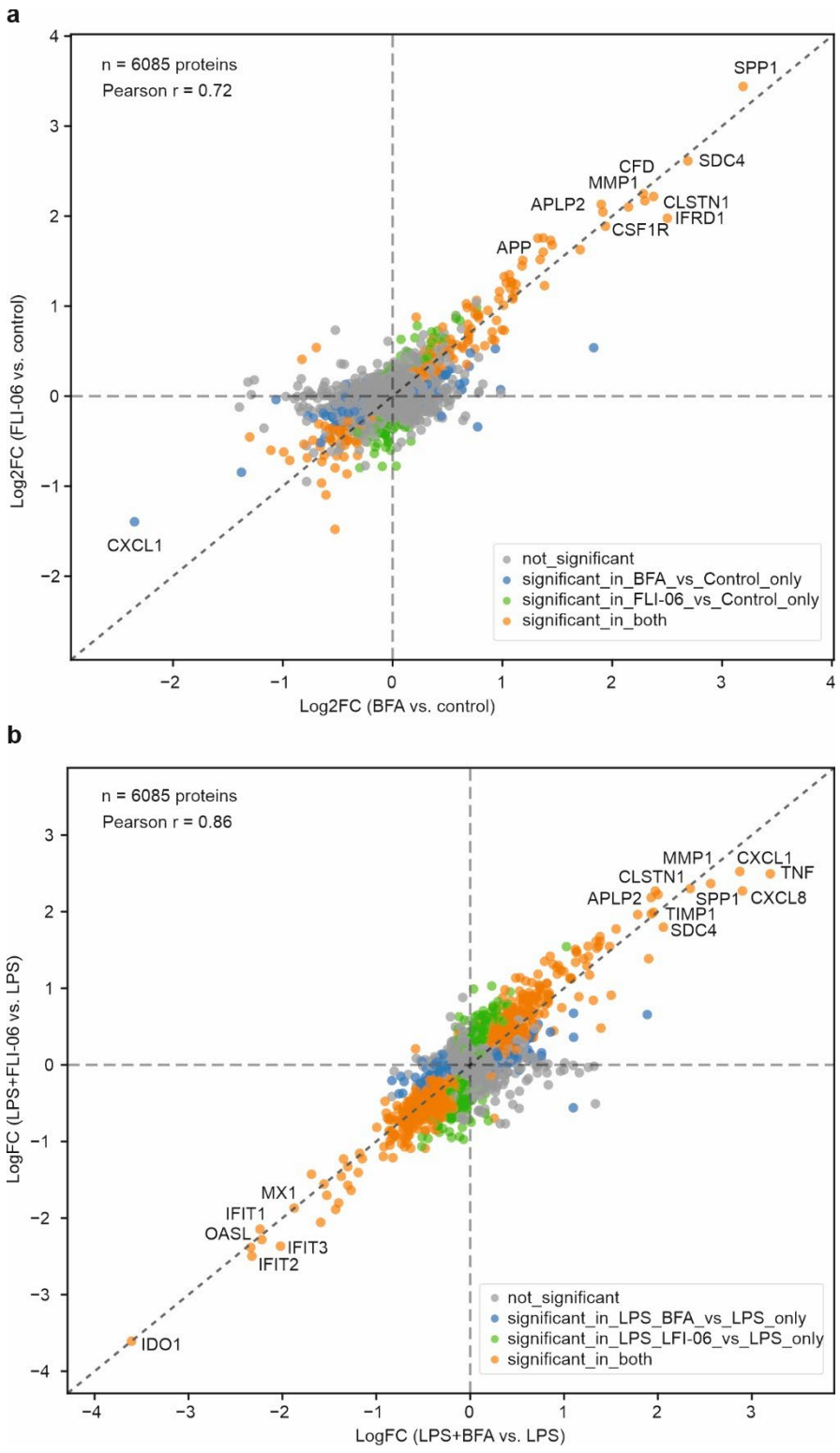
Supplementary Fig. 1. Representative cryo-TEM images of protein-corona-coated polystyrene nanoparticles, showing no detectable extracellular vesicles or large protein aggregates under the conditions examined. These images establish a solid foundation for reliable, robust, and contamination-free proteomics analysis using nanoparticle-based technologies.



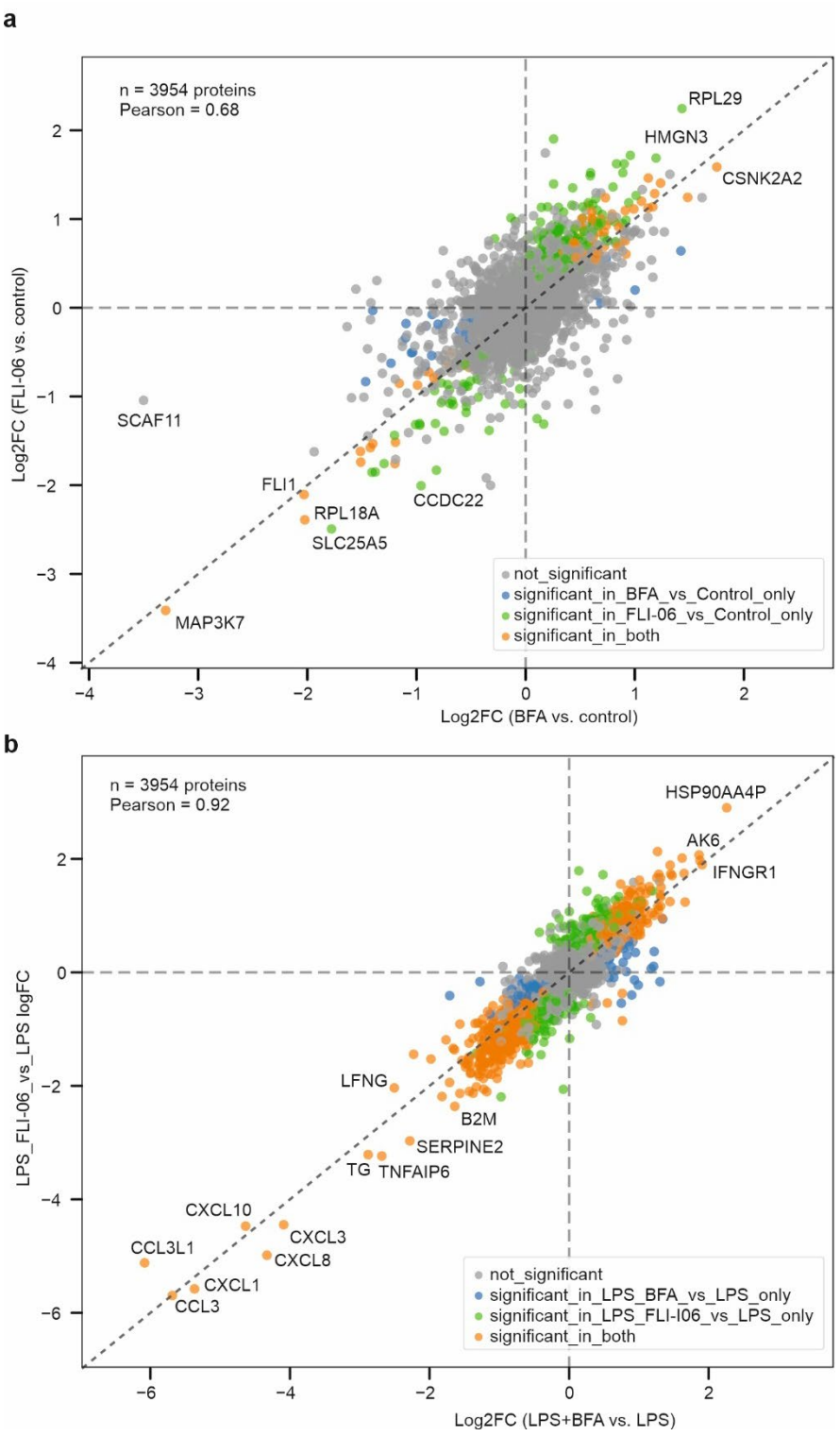
Supplementary Fig 2. Data overview, secretome coverage and assessment of quantitative accuracy. a-b, Overlap of the proteins quantified across increasing LPS concentrations using standard and DeepSec workflows. **c,** Overlap of significant detected proteins by both approaches (log2 fold change >0.5 and FDR <0.05). **d,** Overlap of total proteins in the cell proteome and in the secretomes. **e-f,** Clustering of dose-dependent patterns in standard (e) and DeepSec approaches (f). The secreted proteins' behavior was classified as monotonically increased as shown in Fig 2c-d, as well as transient upregulation, monotonically decreased, transiently decreased and mixed/other profiles.



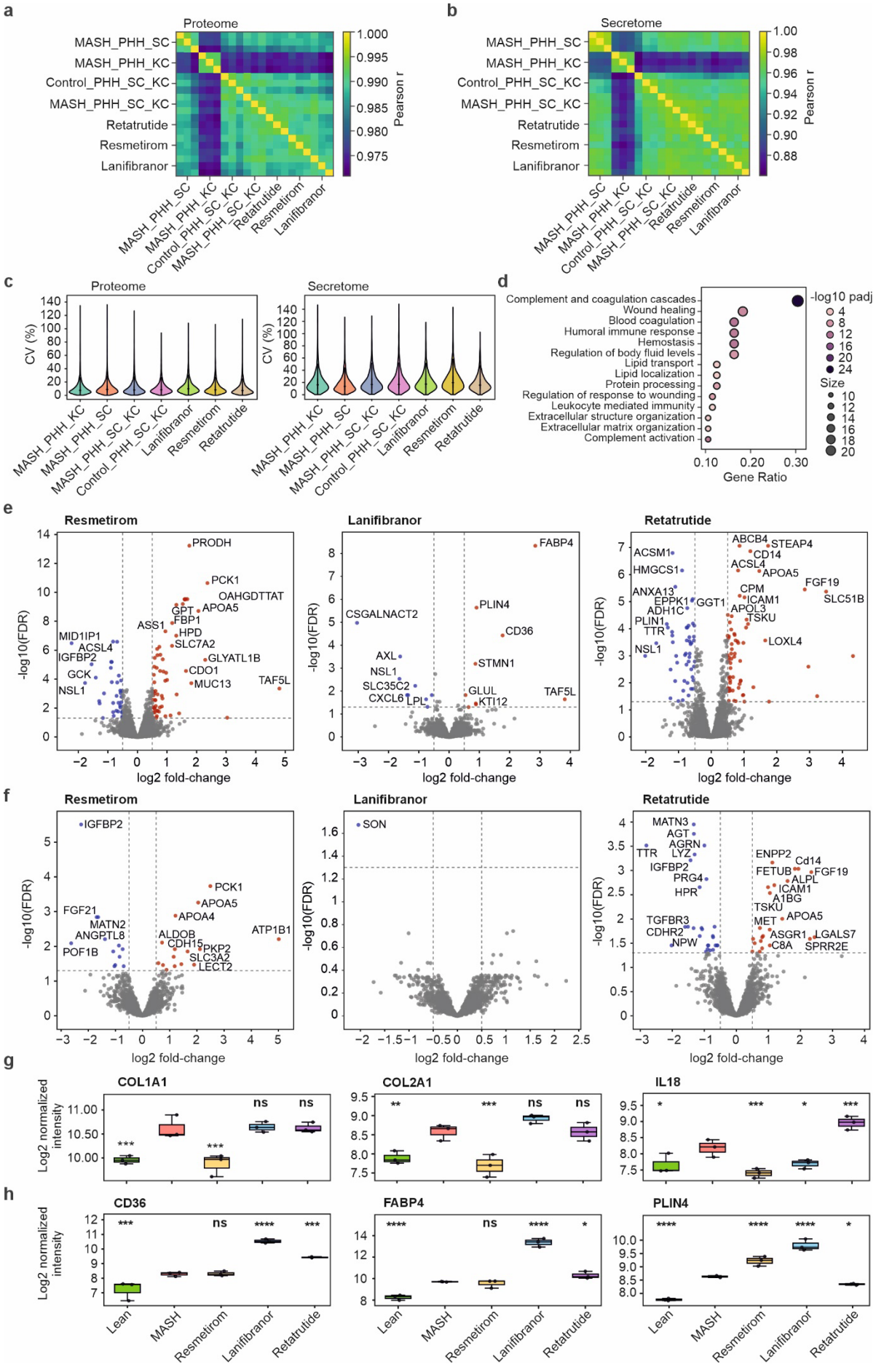
Supplementary Fig 3. Validation of ER-Golgi trafficking and autophagy inhibition. THP1 cells were stimulated with LPS and treated with the indicated inhibitors. **a,** Immunofluorescence of ER exit sites by Sec31a staining following FLI-06 treatment (10 µM, 6 h) (upper panels) and of Golgi disruption by GM130 staining upon BFA treatment (5 µg/mL, 6 h) (lower panels). Calnexin was used as an ER marker. Nuclei were stained with Hoechst. **b,** Western blot analysis of the autophagy marker LC3B-II following inhibition of early autophagy with SAR405 or MRT68921 (1 µM, 6 h each). Bafilomycin A1 (0.1 µM) was used to block autophagosome-lysosome fusion and inhibit autophagic flux, enabling assessment of autophagosome formation. **c,** Cell viability following treatment with increasing concentrations of inhibitors for 24 h, measured using the Incucyte live-cell imaging system.



Supplementary Figure 4. The correlation of the proteome response to LPS combined with BFA or combined with FLI-06, vs. LPS stimulation alone.



Supplementary Fig 5. The correlation of the THP1 cell secretomes in response to LPS combined with BFA or combined with FLI-06, vs. LPS stimulation alone.


a
Proteome
MASH_PHH_SC
MASH_PHH_KC
Control_PHH_SC_KC
MASH_PHH_SC_KC
Retatrutide
Resmetirom
Lanifibranor
Pearson r
b
Secretome
c
Proteome
Secretome
CV (%)
d
Complement and coagulation cascades
Wound healing
Blood coagulation
Humoral immune response
Hemostasis
Regulation of body fluid levels
Lipid transport
Lipid localization
Protein processing
Regulation of response to wounding
Leukocyte mediated immunity
Extracellular structure organization
Extracellular matrix organization
Complement activation
-log10 padj
Size
Gene Ratio
e
Resmetirom
Lanifibranor
Retatrutide
-log10(FDR)
log2 fold-change
f
Resmetirom
Lanifibranor
Retatrutide
-log10(FDR)
log2 fold-change
g
COL1A1
COL2A1
IL18
Log2 normalized intensity
h
CD36
FABP4
PLIN4
Log2 normalized intensity
Lean
MASH
Resmetirom
Lanifibranor
Retatrutide

Supplementary Fig 6. DeepSec resolves disease- and treatment-associated proteome and secretome changes in patient-derived 3D MASH liver spheroids. **a-b,** The reproducible measurement of intracellular and extracellular proteome in spheroids. **c,** The CV of proteome and secretome analysis on the spheroids. **d,** Pathways enriched for the top 105 upregulated proteins in the MASH model secretome vs. control. **e,** The MASH spheroids' proteome in response to different drugs. **f,** The MASH spheroids' secretome in response to different drugs. **g,** The effect of resmetirom on the expression of collagens and IL-18. **h,** The impact of lanifibranor on the expression of proteins involved in fatty acid and lipid handling.