

Supporting Information

Stat3 mediated alterations in lysosomal membrane protein composition

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Supplementary Table S10: Exclusive unique peptide counts of 39 proteins whose representation changed with OSM stimulation

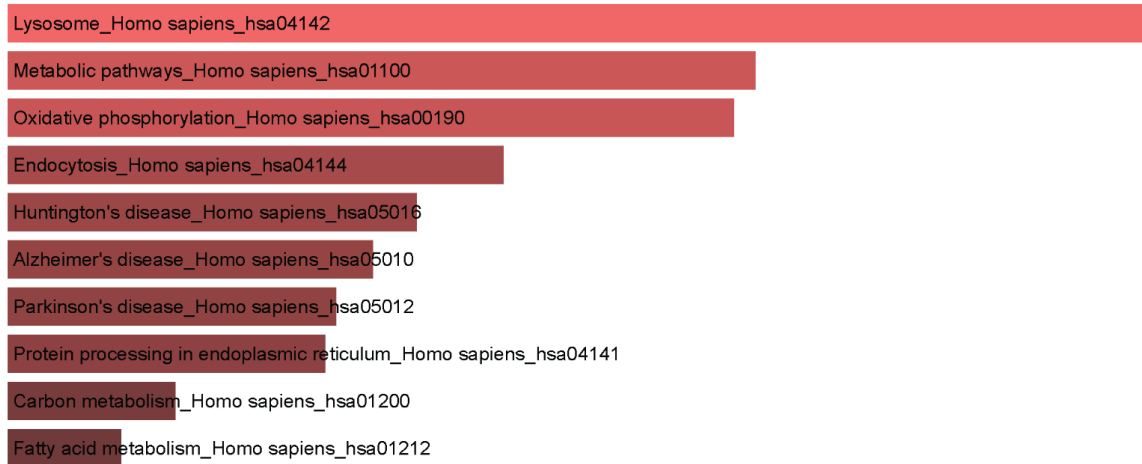
Supplementary Table S11: Microarray data from Stat3 knockout and control mammary glands at 24h involution showing fold downregulation of *Flot1*, *Flot2* and *CD63* mRNA in Stat3 knockout samples.

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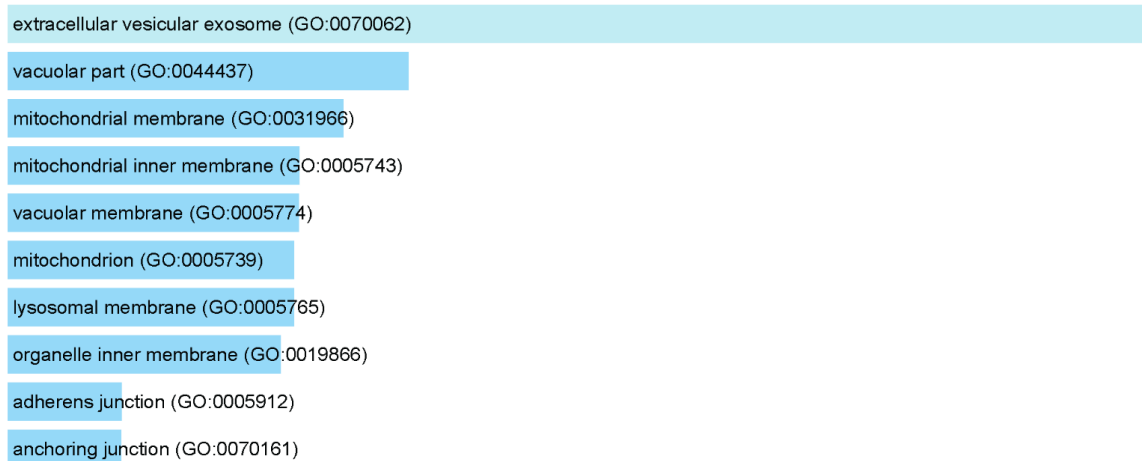
Supplementary Table S13: Representation of heavily glycosylated lysosomal membrane proteins with or without PNGase F enzyme treatment

Supplementary Figure S1

A



B

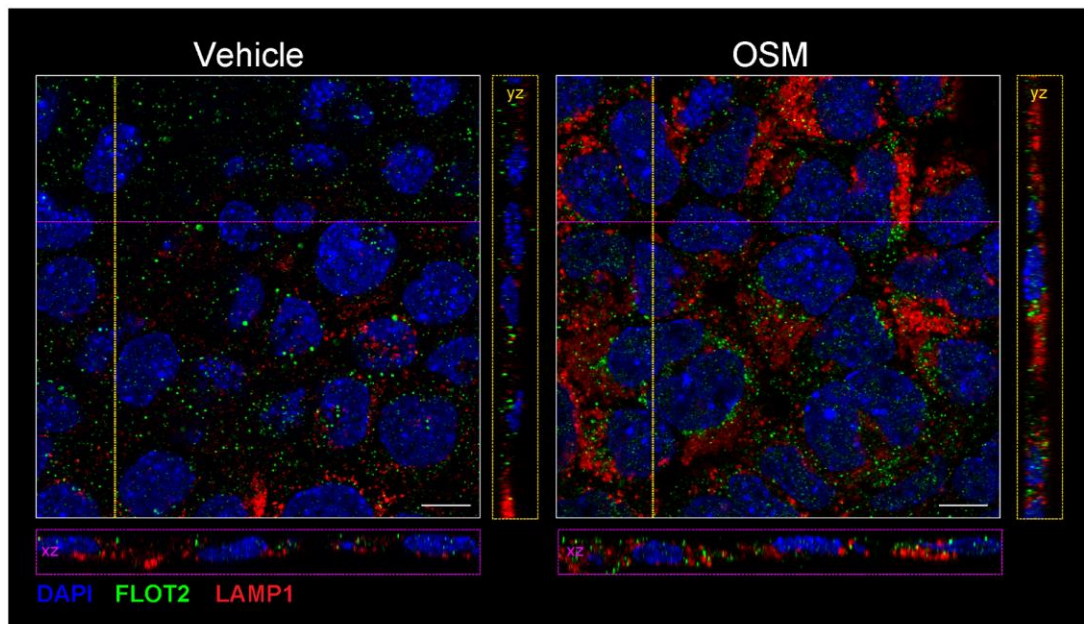


Supplementary Figure S1.

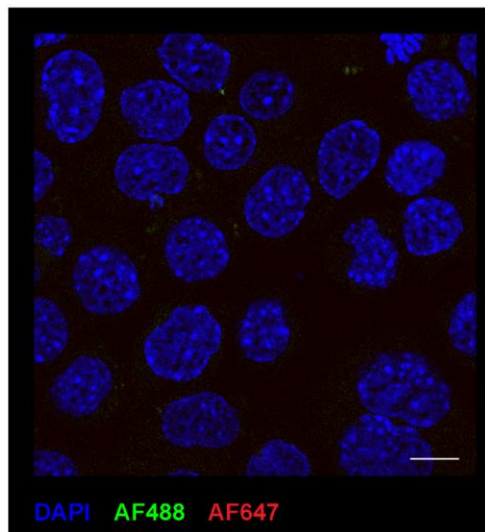
A, Significantly enriched KEGG (2016) pathways derived from mass-spectrometry analysis of EpH4 lysosomal preparations isolated using iron nanoparticles. **B**, Significantly enriched GO annotations according to the category Cellular Component. Associated with Supplementary Table S3.

Supplementary Figure S2

A



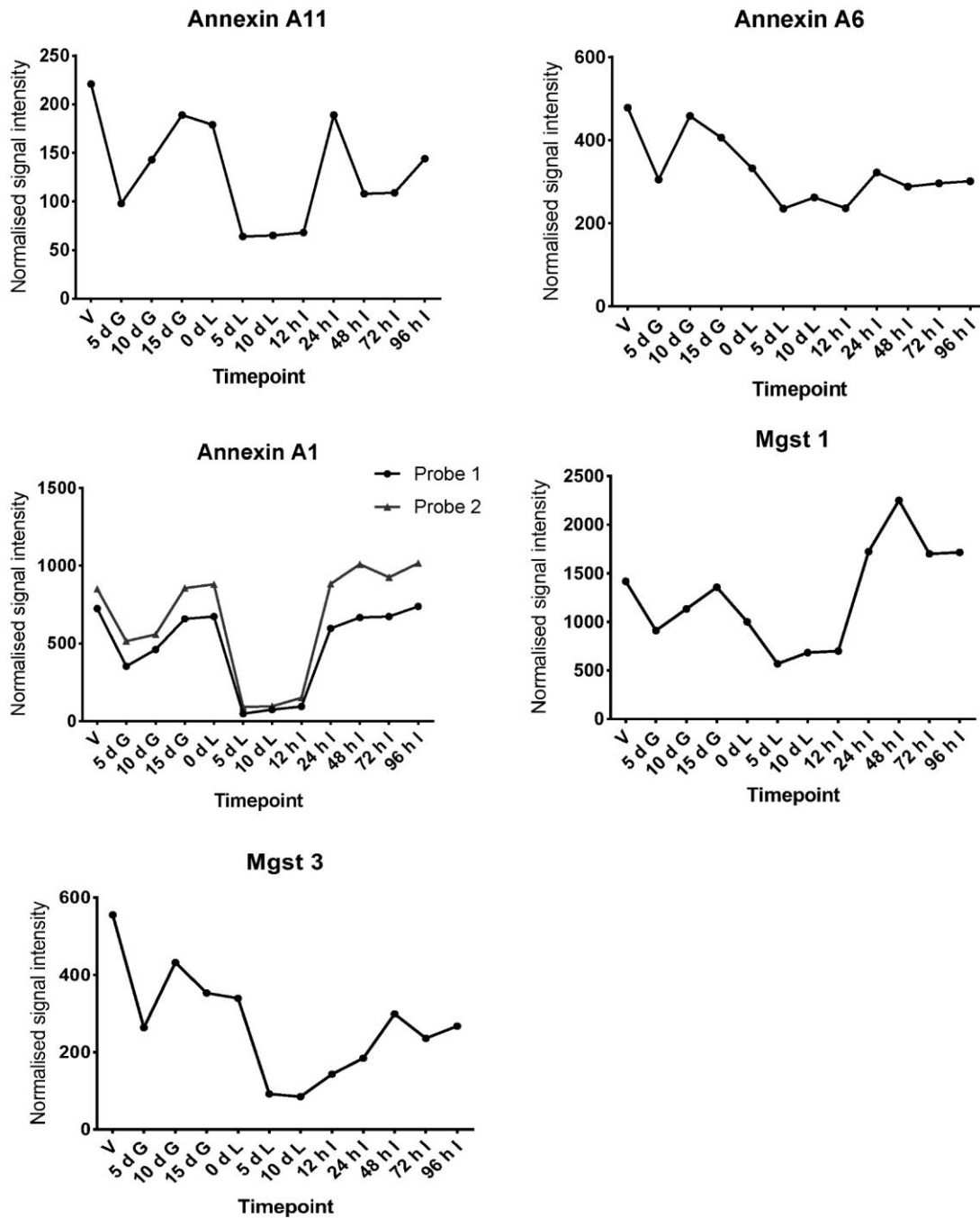
B



Supplementary Figure S2.

A, Confocal fluorescence imaging of EpH4 cells treated with OSM for 24 h and immunostained for flotillin 2 and LAMP1. Main images show a single thin optical slice, orthogonal views show XZ (purple line and box) and YZ (orange line and box) planes. Scale bars: 10 μ m. Representative images of n=2 independent experiments are shown. **B**, No primary antibody controls. Scale bar: 10 μ m

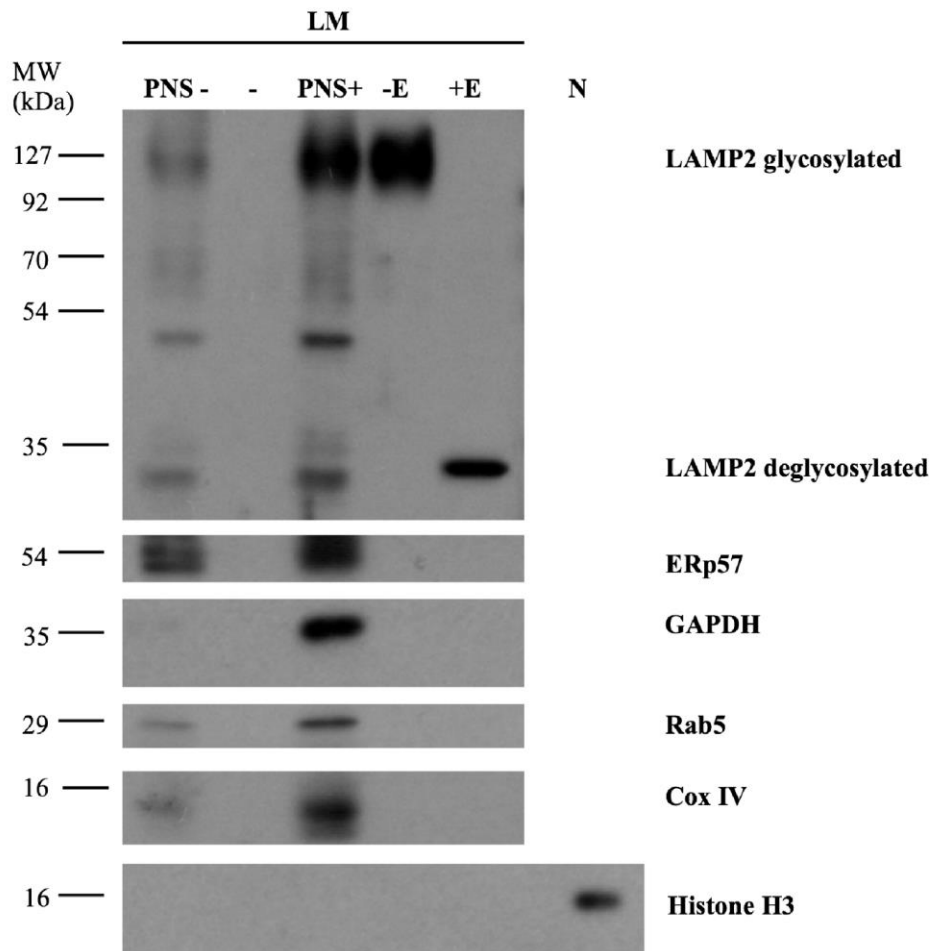
Supplementary Figure S3



Supplementary Figure S3.

Microarray analysis of twelve different timepoints during the mammary gland pregnancy cycle showing the involution related expression profiles of selected proteins that were more abundant in OSM treated EpH4 cell lysosomes. V, virgin; d G, days gestation; d L, days lactation; h I, hours involution.

Supplementary Figure S4



Supplementary Figure S4.

LAMP2 deglycosylation by PNGase F. EpH4 cell lysosomes were isolated using the magnetic nanoparticle separation protocol and fractionated by freezing in liquid nitrogen and thawing at 37°C five times. The lysosomal membrane fraction (LM) was either untreated (UT) or denatured at 95°C and incubated with (+ E) or without (- E) PNGase F for 30 min at 37°C. N, nuclear lysate. Organelle marker proteins: LAMP2, lysosomes; ERp57, endoplasmic reticulum; GAPDH, cytoplasm; Rab5, early endosomes; Cox IV, mitochondria; Histone H3, nucleus.