SUPPLEMENTARY INFORMATION

for

A novel strategy for the comprehensive analysis of the biomolecular composition of isolated plasmamembranes

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Supplementary Materials and Methods

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SUPPLEMENTARY MATERIALS AND METHODS

LC-MS and database analysis for the non-differential set-ups. The MS was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the six most abundant ion peaks per MS spectrum. Full scan MS spectra were acquired at a target value of 1E6 with a resolution of 60,000. The six most intense ions were then isolated for fragmentation in the linear ion trap. In the LTQ, MS/MS scans were recorded in centroid mode at a target value of 5,000 ion counts. Peptides were fragmented after filling the ion trap with a maximum ion time of 10 ms and a maximum of 1E4 ion counts. From the MS/MS data in each LC-run, Mascot generic files (mgf) were created using the Mascot Distiller software (version 2.2.1.0, Matrix Science). A peak list was only generated when the MS/MS spectrum contained more than 10 peaks, no de-isotoping was performed and the relative S/N limit was set at 2. The resulting mgf files were searched against the SwissProt database restricted to *Mus musculus* taxonomy. The following MASCOT parameters were set: the protease setting was trypsin (cleavage between Lys or Arg and Pro was accepted) with a maximum of one allowed missed cleavage and S-carbamidomethylated Cys was set as fixed modification, whereas acetylation of a protein's N-terminus, deamidation of Asn and Gln and pyroglutamate were considered as variable modifications, and tolerances for the precursor ion mass and fragment ions masses were set to ± 10 ppm and 0.5 Da respectively. Finally, peptide hits of which the MASCOT ion score of the MS/MS spectrum exceeded MASCOT's identity threshold score set at 99% confidence and which were ranked one were withheld and considered identified.

For the identification of N-glycosylated sites we withheld deamidation of asparagines (converted into aspartic acid by enzymatic cleavage of the glycan chain) in the consensus site N-X-S/T/C where X can be any amino acid except proline.

Postmetabolic labeling. PMs were prepared as described in the Methods section, and membrane proteins dissolved in 200 µl of 50mM triethylammoniumbicarbonate (pH 8.0), denatured for 10 min at 95°C prior to digestion with endoproteinase Lys-C (Roche Diagnostics GmbH, Mannheim, Germany) instead of trypsin. The protease was added in a 1/200 (w/w) ratio and digestion proceeded overnight at 37°C. Next, the peptide mixture from WT proteins was treated with 8 mg of $^{12}C_3$-propionyl-N-hydroxysuccinimide while those of PSENdKO and hPSEN1$_{rescue}$ were labeled with $^{13}C_3$ propionyl-N-hydroxysuccinimide ester (reagents were prepared as previously described (Pochlauer and Hendel 1998; Ghesquiere, Colaert et al. 2009; Ghesquiere, Jonckheere et al. 2011)). Propionylation of primary amino groups occurred at 30°C for 120 min. Excess reagents were quenched by adding 60 µl of 1 M glycine and incubation for 15 min at 25°C followed by the removal of unwanted O-propionylation by heating the sample for 60 min at 95°C. After acidification with 20 µl of 50% acetic acid, the samples were vacuum dried and re-dissolved in 50 µl of 2% acetonitrile. Light and heavy labeled peptides were mixed in a 1/1 (w/w) ratio (WT versus PSENdKO and WT versus hPSEN1$_{rescue}$), and separated using reversed phase-HPLC as described in "LC-MS and database analysis for the non-differential set-ups" section. In total 60 fractions were collected eluting between 20 and 80 min which were pooled into 15 fractions for further analysis on the Orbitrap XL mass spectrometer. Comparison of the UV-absorbance at 214 nm during the RP-HPLC separation confirmed that equal amounts of peptides were used in both analyses. Following identification, peptides were quantified with Mascot Distiller software in which those

with status 'False' were discarded. No manual validation of peptide ratios was performed. In each analysis the abundance of the light labeled (always WT peptides) was divided by the abundance of the heavy labeled (PSENdKO or $hPSEN1_{rescue}$) peptides

LC-MS and database analysis for the differential experiments: WT vs PSENdKO and WT vs hPSEN1$_{rescue}$ LC-MS/MS analysis of the isolated peptides was performed as described in the previous section, and the generated mgf files were searched against the SwissProt database restricted to *Mus musculus* taxonomy. The following MASCOT parameters were set: the protease setting was endoproteinase Lys-C (cleavage C-terminal to Lys) with a maximum of one allowed missed cleavage, no fixed modifications, whereas acetylation of a protein's N-terminus, oxidation of methionine, deamidation of Asn and Gln and pyroglutamate were considered as variable modifications, and tolerances for the precursor ion mass and fragment ions masses were set to ± 10ppm and 0.5 Da, respectively. Determination of the light ($^{12}C_3$-propionyl) and heavy ($^{13}C_3$-propionyl) labeled peptides for further quantification was established using the quantitation option in Mascot. Finally, peptide hits of which the MASCOT ion score of the MS/MS spectrum exceeded MASCOT's identity threshold score set at 99% confidence and which were ranked one were withheld and considered identified. Quantification was performed using Mascot distiller software (version 2.2.1.0, Matrix Science).

PM Glycoproteomics: PM proteins were prepared and trypsin digested as described above. Following digestion the sample was acidified with acetic acid (5% final concentration) and methionines were converted into their sulfoxides using 0.5% (w/v) H_2O_2 (Sigma-Aldrich) for 30 min at 30°C. Next, the pH was adjusted to 7.8 with 200 µl of 1M TEAB. 100 µl of 50% catalase-agarose slurry (Sigma-Aldrich) was washed twice with 500 µl of 50 mM TEAB on a spin-cup paper filter (Thermo Scientific), the reaction mixture was then added to the catalase-agarose to reduce hydrogen peroxide during 5 min at 30°C. Peptides were obtained by centrifugation at 300xg for 10 min at 4°C. For alkylation of cysteines, 20 mM iodoacetamide (Sigma-Aldrich) and 10 mM triscarboxyethylphosphine (Pierce) was added for 15 min at 37°C, the mixture was acidified to 5% (f.c.) acetic acid, dried and re-dissolved in 100 µl of 1% acetic acid and separated by RP-HPLC. The COFRADIC (COmbined FRActional DIagonal Chromatography) isolation of N-glycosylated sequences was carried out as described before(Ghesquiere, Van Damme et al. 2006). Briefly, peptides were separated onto RP-HPLC and collected into primary fractions, fractions separated by 15 min were pooled, dried and re-dissolved in 50 mM ammonium bicarbonate pH 7.8 to which 0.8 U of PNGase F (Sigma-Aldrich) were added. N-deglycosylation was carried out for 30 min at 37°C and acidified with acetic acid (1% final concentration). These treated fractions were then submitted to identical RP-HPLC separations and peptides showing an altered chromatographic behavior were collected for LC-MS/MS analyses. The collected fractions were re-dissolved in 15 µl of 2% acetonitrile.

Reference:

Ghesquiere, B., N. Colaert, et al. (2009). "In vitro and in vivo protein-bound tyrosine nitration characterized by diagonal chromatography." Mol Cell Proteomics **8**(12): 2642-52.

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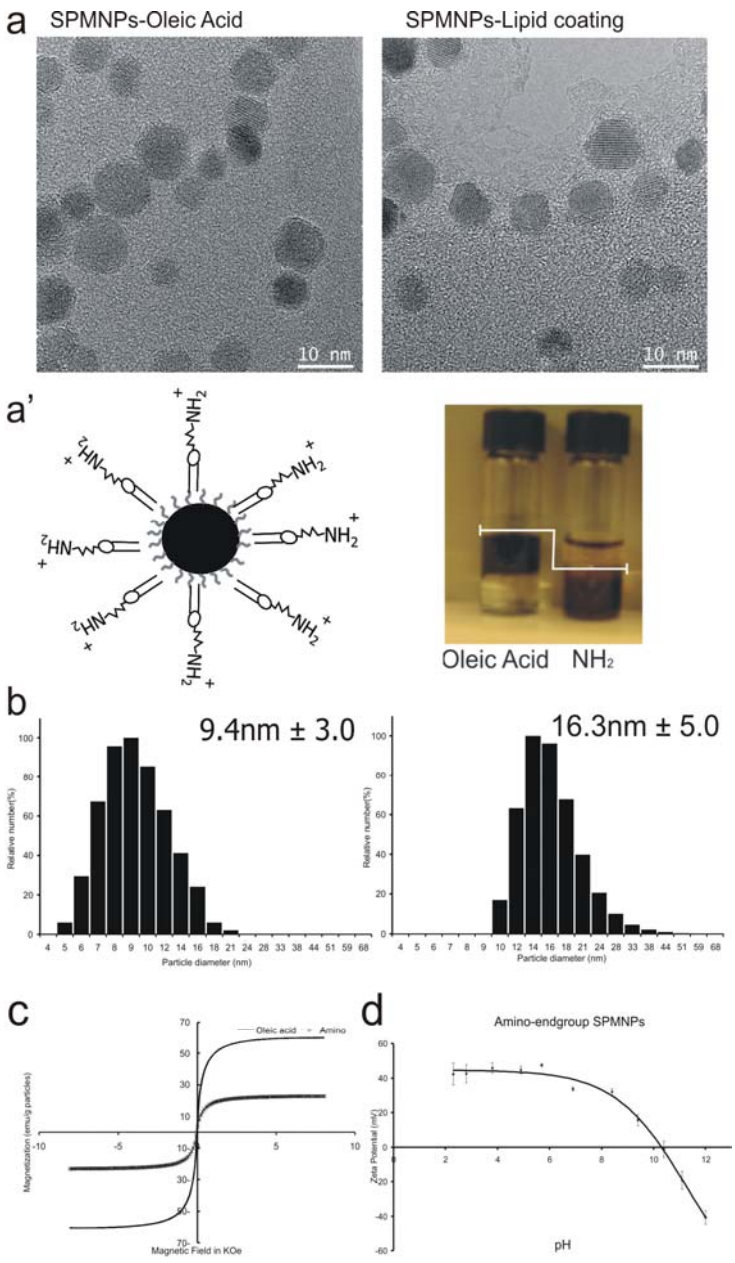
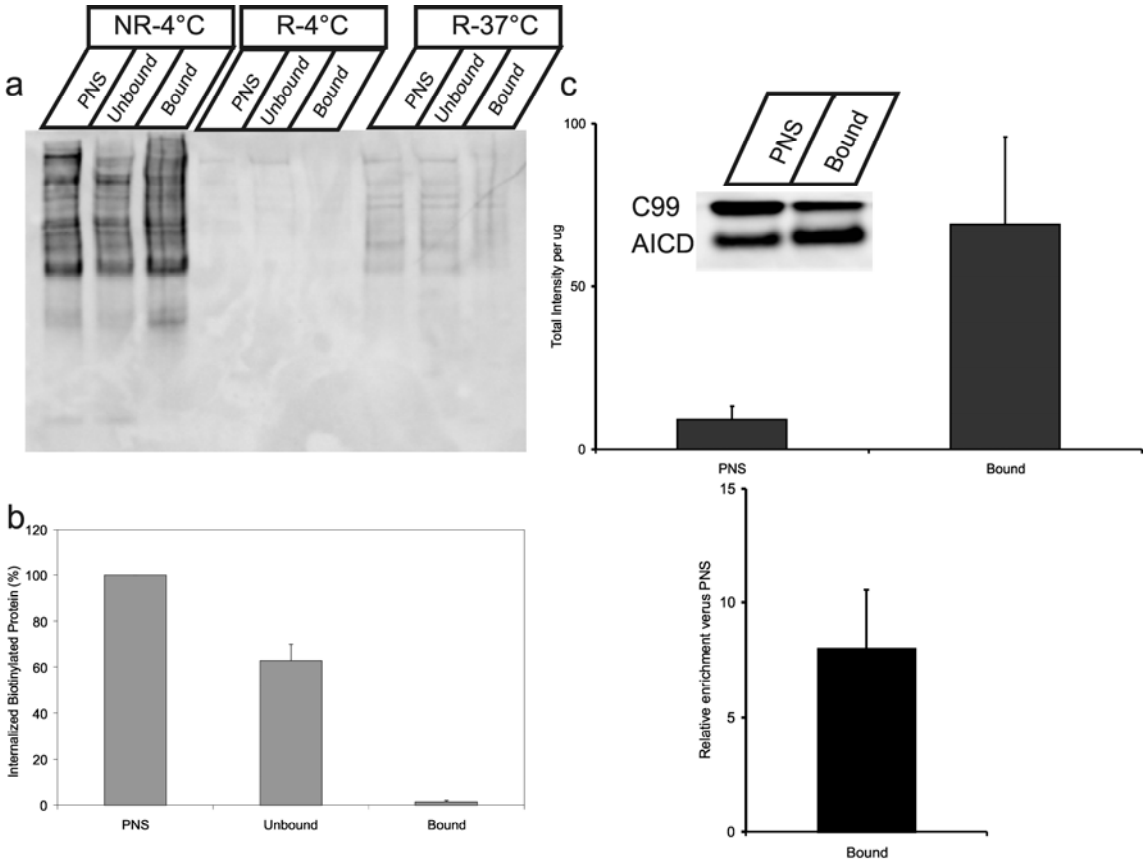
Ghesquiere, B., J. Van Damme, et al. (2006). "Proteome-wide characterization of N-glycosylation events by diagonal chromatography." J Proteome Res **5**(9): 2438-47.

Pochlauer, P. and W. Hendel (1998). "One-pot formation of succinimidyl esters by the system chlorophosphate/hydroxysuccinimide/base." Tetrahedron **54**(14): 3489-3494.

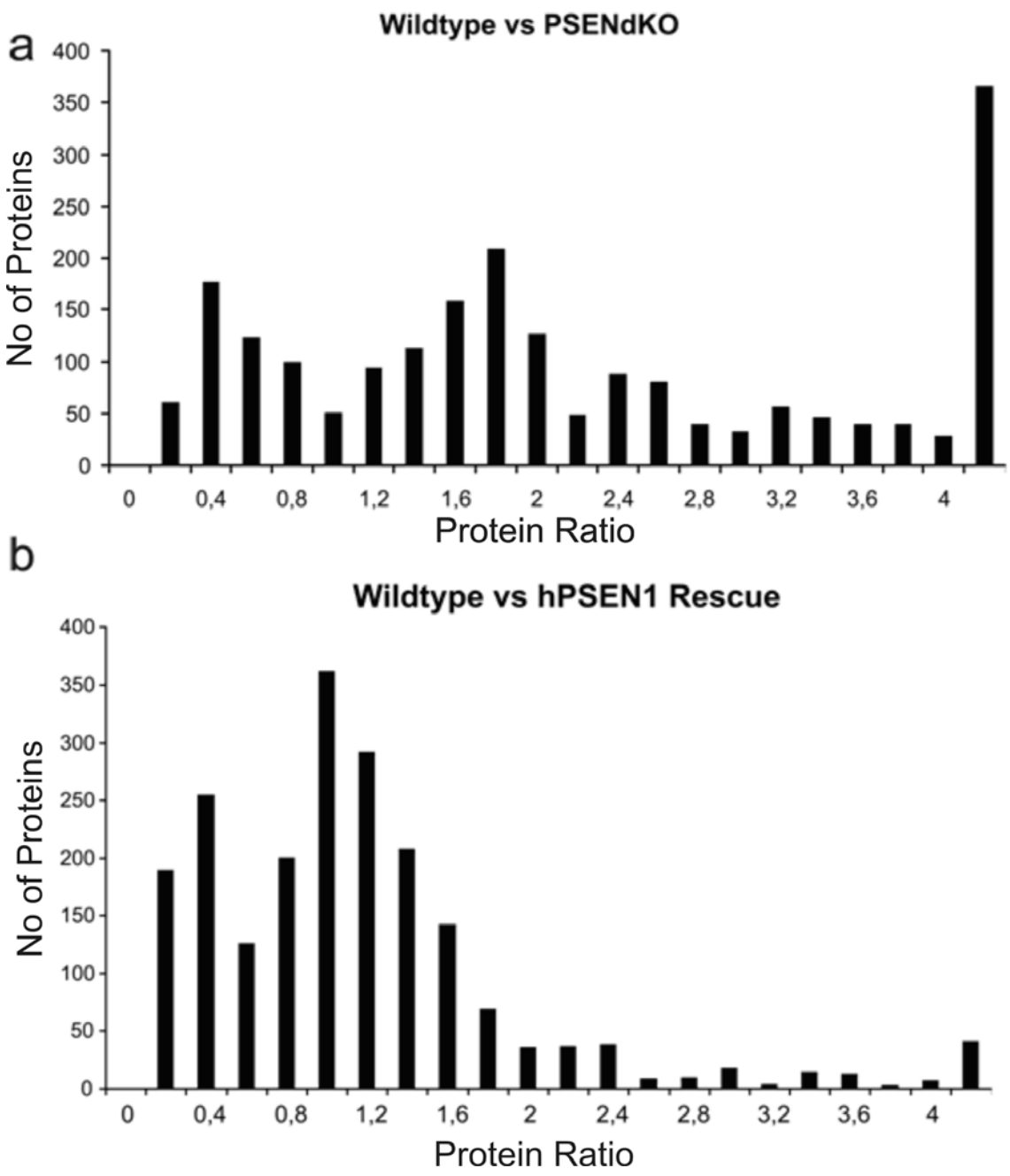


Supplementary Figure 1: SPMNP Synthesis and Characterization. (a) TEM of Fe_3O_4 nanoparticles coated with oleic acid (left) and nanoparticles coated with NH_2 end-group phospholipids (right); a') left: pictorial representation of nanoparticles coated with NH_2 end-group phospholipids; right: nanoparticles remain dispersed in the water phase of a water-organic solvent mixture for several weeks. (b) Dynamic Light Scattering (DLS) graphs of Fe_3O_4 nanoparticles coated with oleic acid (left) and nanoparticles coated with NH_2 end-group lipids (right). (c) Magnetic properties measurement by alternating gradient field magnetometer

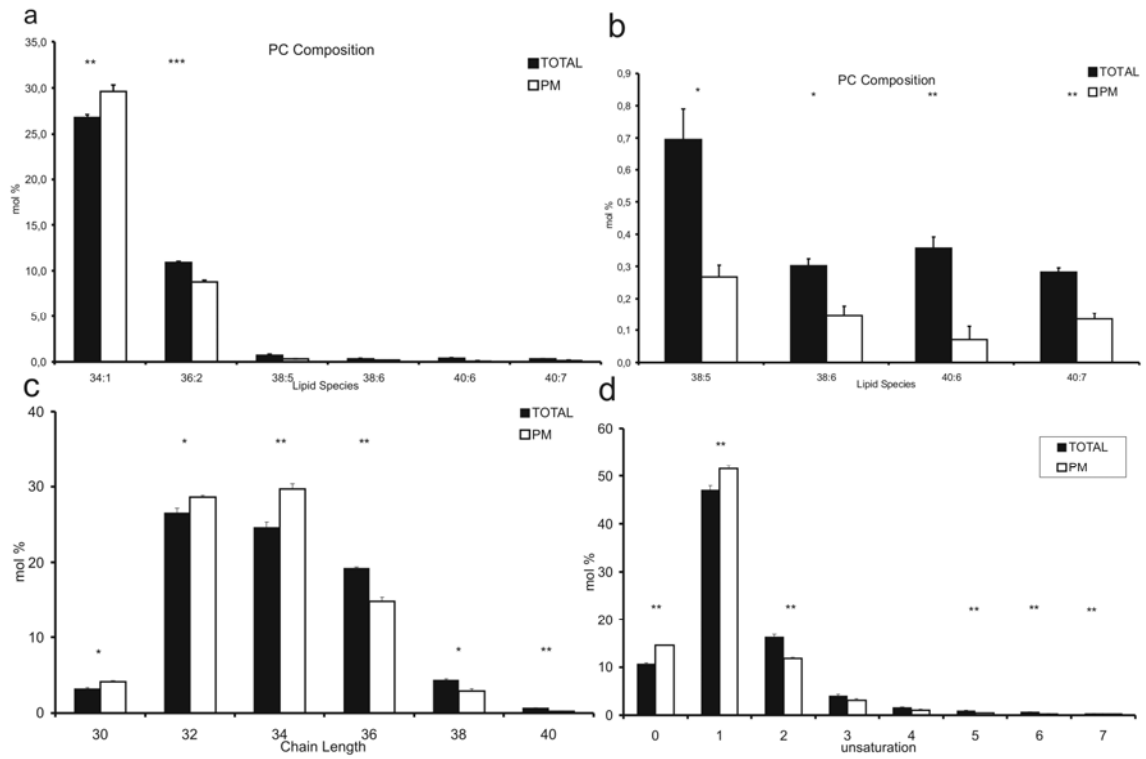
(AGFM) – Oleic Acid coated (Dark line) and Lipid coated Fe_3O_4 nanoparticles (dotted line) where X-axis is the magnetic field (KOe) and Y-axis is the magnetization (emu/g particles) (d) Zeta Potential measurement on NH_2 end-group lipid coated Fe_3O_4 nanoparticles for a pH range 2-11.



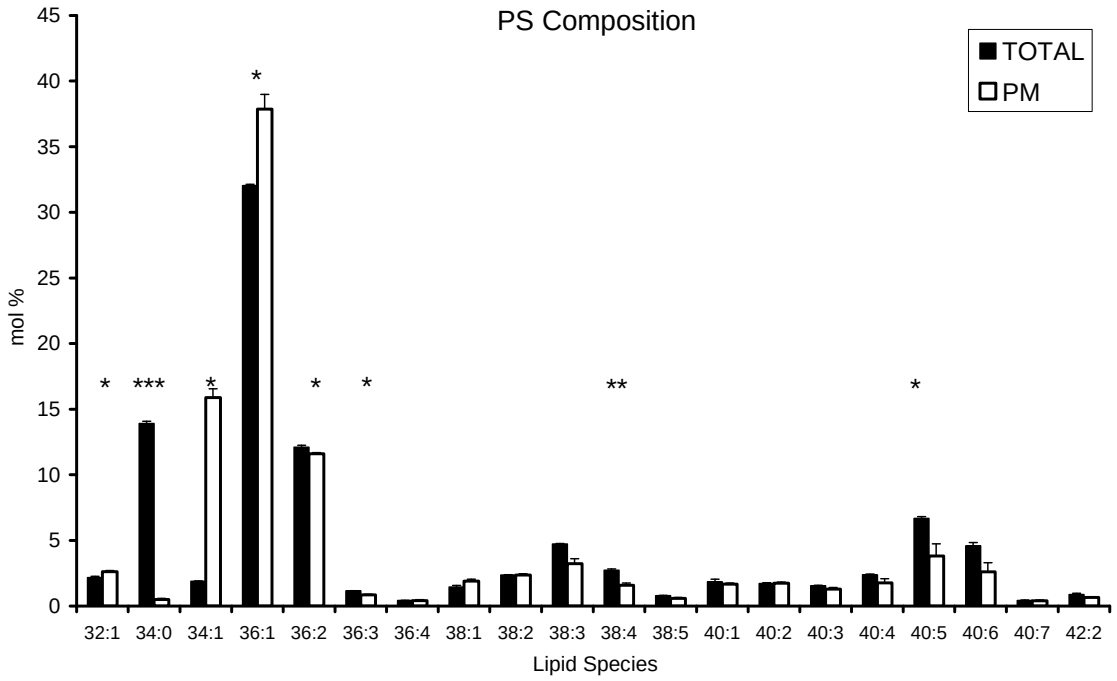
Supplementary Figure 2: Quality Control and enzymatic activity on magnetically isolated PM fractions (a) Biotin internalization. WT MEFs were incubated with NHS-SS-biotin and allowed to internalize bound biotin (20min at 37°C). After removing remaining surface bound biotin using 100 mM 2-sodium-2-mercaptoethanesulfonate, PM were isolated using SPMNPs and analyzed by western blotting using neutravidin-HRP to detect biotin. Equal amounts (5,5µg) of PNS, unbound (UB) and bound PM (B) fractions were resolved in precast 4-12% SDS-PAGE gels. Comparison between non-reduced and Sodium 2-mercaptoethanesulfonate-treated MEFs (following 20min at 4°C, or 37°C, respectively) revealed that less than 1.25% of biotin compared to PNS was recovered in the PM fraction, as quantified in (b). (c) Quantification of in vitro AICD production by γ-secretase in PNS and PM fractions (top) as well as the relative PM enrichment of γ-secretase activity versus PNS. Data are in mean ± SEM (n=3).



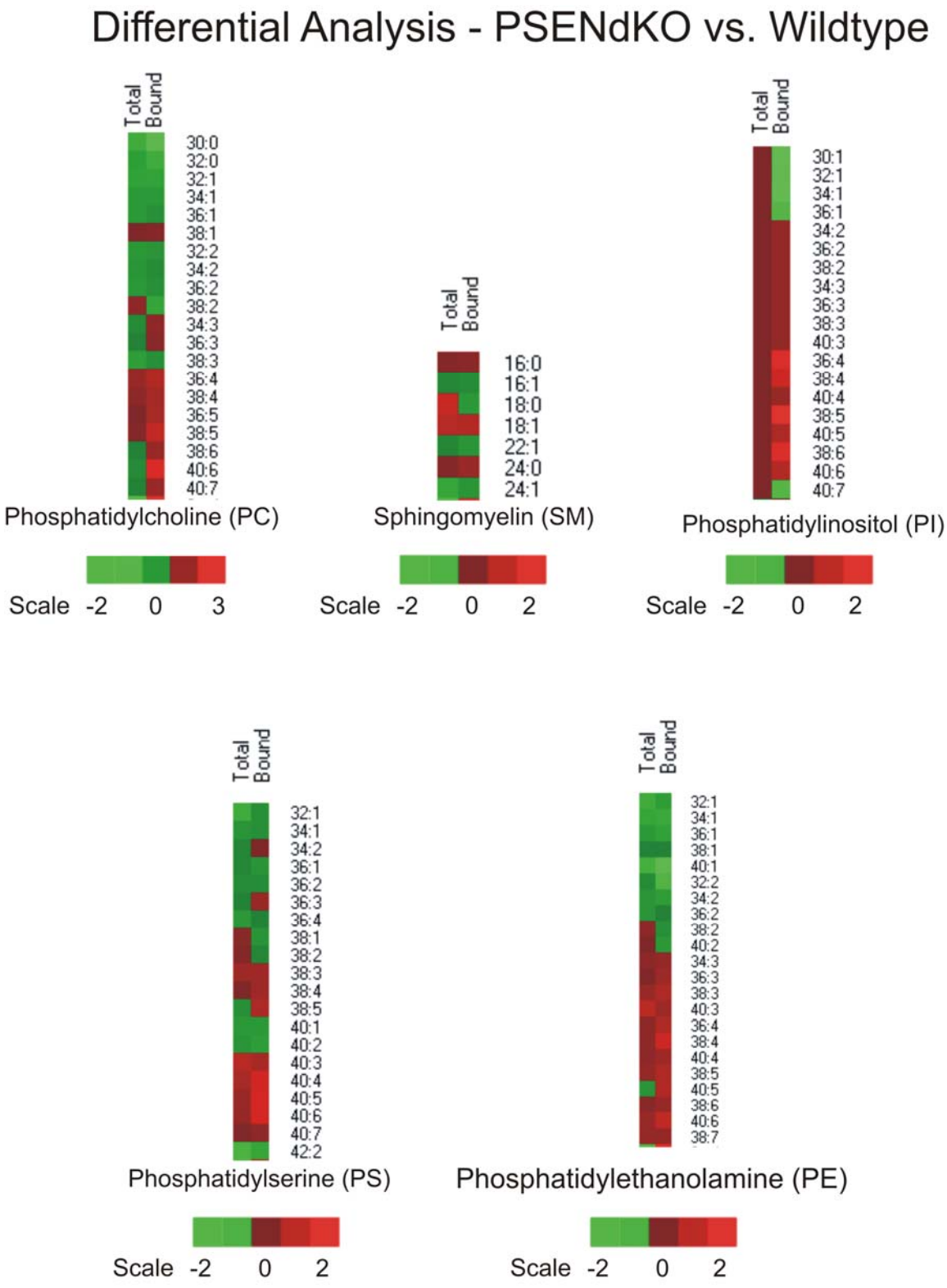
Supplementary Figure 3: Quantitative Proteomics using Shotgun approach on WT, PSENdKO and PSEN1$_{rescue}$ MEFs. Histogram of protein ratio's (with status 'TRUE' and belonging to predicted membrane proteins) between WT and PSENdKO PM proteomes (a) and between WT and PSEN1$_{rescue}$ PM proteomes (b). The more unimodal re-distribution of the protein ratio's towards 1/1 ratio's in WT versus PSEN1$_{rescue}$ (i.e. the WT and rescue sample are more similar - contain more 1/1 ratio's- than the WT and PSENdKO sample) show that the broad variation in protein ratio's observed between WT and PSENdKO is caused by PSEN deficiency.) (X-axis – Protein ratio; Y-axis- No of Proteins.)



Supplementary Figure 4: Molecular composition of the PC class in PMs and Total fraction of WT MEFs. (a, b) The abundance of each lipid species of PC is represented in mol% (absolute amount of lipid species/absolute amount of total identified lipid species). (c) Chain length of PC – The relative abundances of lipid chain length (both chains combined) with respect to total PC lipid composition. (d) Saturation of PC – The relative abundances of saturated and unsaturated lipids with respect to total PC lipid composition. Data are mean ± SEM (n=3). * $P \leq 0.05$; ** $P \leq 0.01$; and ***$P \leq 0.001$.



Supplementary Figure 5: Molecular composition of major PS species in PMs and Total fraction of WT MEFs. Data are mean ± SEM (n=3). * P ≤ 0.05, ** P ≤ 0.01 and *** P ≤ 0.001.



Supplementary Figure 6: Differential analysis of lipid species between the PSENdKO and WT lipid classes. Heatmap profiles of the lipid species levels for PI, PS, PE, SM and PC were generated with the average $\log_2$-values of the PSENdKO/WT ratio in total and PM.

Supplementary Table I: Lipid class composition (mol% ± SEM) for MEFs Wildtype and PSENdKO (n=3)

Lipid Classes	Wildtype		PSENdKO	
	TOTAL	PM	TOTAL	PM
Phosphatidylcholine (PC)	51.8 ± 0.5	67.2 ± 3.5	54.6 ± 3.5	68.6 ± 2.7
Sphingomyelin (SM)	3.9 ± 0.4	7.8 ± 3	2.8 ± 0.5	6.8 ± 0.5
Phosphatidylserine (PS)	11.5 ± 0.4	9.0 ± 0.7	11.3 ± 1.5	7.4 ± 1.2
Phosphatidylethanolamine (PE)	23.9 ± 0.9	11.6 ± 1.5	24.6 ± 0.9	10.9 ± 1
Phosphatidylinositol (PI)	8.7 ± 1.1	4.3 ± 1.8	8.5 ± 0.4	6.2 ± 0.8

Supplementary Table II: Average nmol of lipid/mg protein absolute level (mean ± SEM, n=3) for Wild-type (PNS & PM fraction) and PSENdKO (PNS & PM fraction) and normalization of PSENdKO with respect to Wild-type;

Lipid Classes	TOTAL			PM		
	Wildtype	PSENdKO	Ratio	Wildtype	PSENdKO	Ratio
Cholesterol	1129 ± 150	2353 ± 450	2.1	12138 ± 297	5577 ± 220	0.45
Total SM	89.3 ± 43	131.5 ± 69	1.5	529.2 ± 18	731.9 ± 2	1.5
SM d18:1/16:0	55.3 ± 27	87.4 ± 45	1.6	190 ± 9	301 ± 4.3	1.5
SM d18:1/18:0	0.92 ± 0.5	1.30 ± 0.6	1.5	62.2 ± 12	75 ± 5.6	1.2
SM d18:1/18:1	0.99 ± 0.4	4.4 ± 0.34	4.5	49.2 ± 12	100 ± 7	2
Total PC	1060.3 ± 402	2661.2 ± 1525	1.2	6165.9 ± 644	7295.8 ± 1443	1.2
Total PI	162 ± 41	219 ± 32	1.4	407.2 ± 112	666.4 ± 143	1.6