

Supplementary Information

Supplementary Figure 1S. *Deletion of PS1 and PS2 in skin of epidermal- PS cDKO mice*

PCR analysis of PS1 and PS2 genes in control (PS1 f/f; PS2+/+) and epidermal PS cDKO (PS1 f/f; PS2-/-; K14-Cre-ER^{T2}) mice. Genomic DNA from skin and brain of 8 week-old littermate control and epidermal PS cDKO mice was purified and amplified by using specific primers for genes of interest (see Materials and Methods). PCR fragments corresponding to Cre recombinase (700 bp), intact floxed PS1 (262 bp), PS1 null (372 bp), PS2 (540 bp) and PS2 -/- (326 bp) alleles are displayed. Undeleted floxed PS1 was still present in total skin (dermis + epidermis) of PS1f/f; PS2-/-; K14-Cre-ER^{T2} due to absence of Cre-recombinase expression in some layers of the skin epidermis (Li et al., 2000).

Supplementary Figure 2S. *Loss of PS affects trafficking in late endosomal compartments but not internalization of EGFR*

(a) Cells were treated with EGF (150 ng/ml) for the indicated times or treated with EGF for 5 min and chased for 25 min (last lane). Cells were incubated with sulfo-NHS-LC-biotin (0.25 mg/ml) for 30 min at 4°C, washed and lysates (200 µg) were incubated with 25 µl of UltraLink immobilized NeutrAvidin protein beads. Analysis of immunoprecipitates by Western blotting shows similar internalization rate in control and PS-/- fibroblasts whereas EGFR recycling (last lane) is impaired in PS-/- fibroblasts. Mean values ± s.d. of three independent experiments are shown.

(b) Cell surface EGFR was labeled by incubating PS1/PS2 and PS-/- fibroblasts with EGF-Alexa488 (150 ng/ml) at 4°C for 15 min (time 0). EGFR internalization was analyzed by incubating cells for 15-180 min at 37°C. Images were obtained using a Leica TCS SP5 AOBS confocal microscope and analyzed with MetaMorph 6.1 software. The number of vesicles and

area of vesicles (arbitrary units) per cell were analyzed (n=10-30 cells per condition). Data represent mean \pm s.e.m. * $P < 0.05$

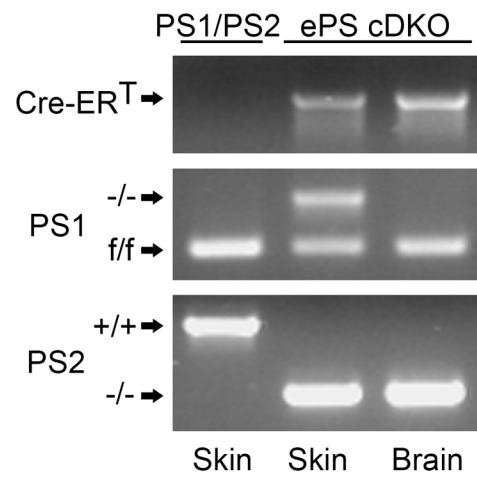
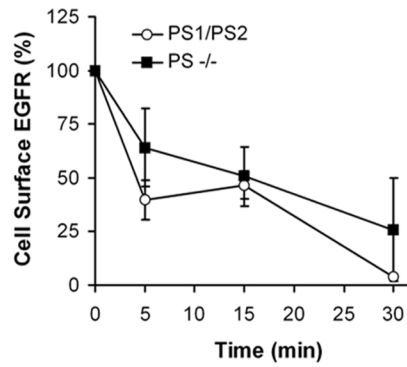
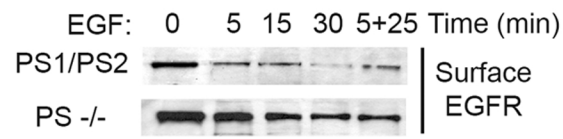


Figure S1

a



b

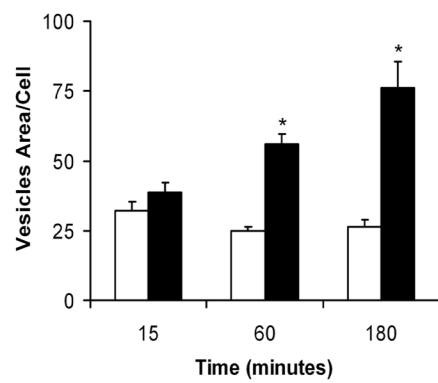
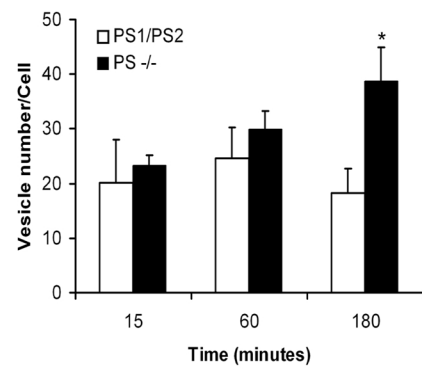
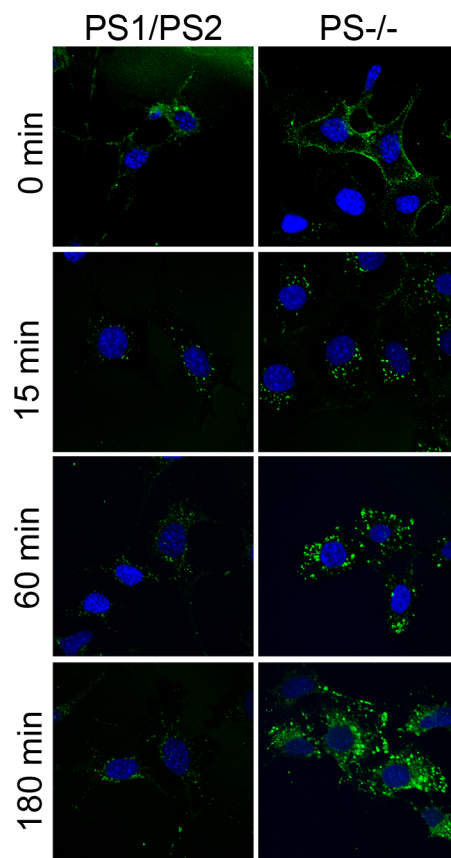


Figure S2