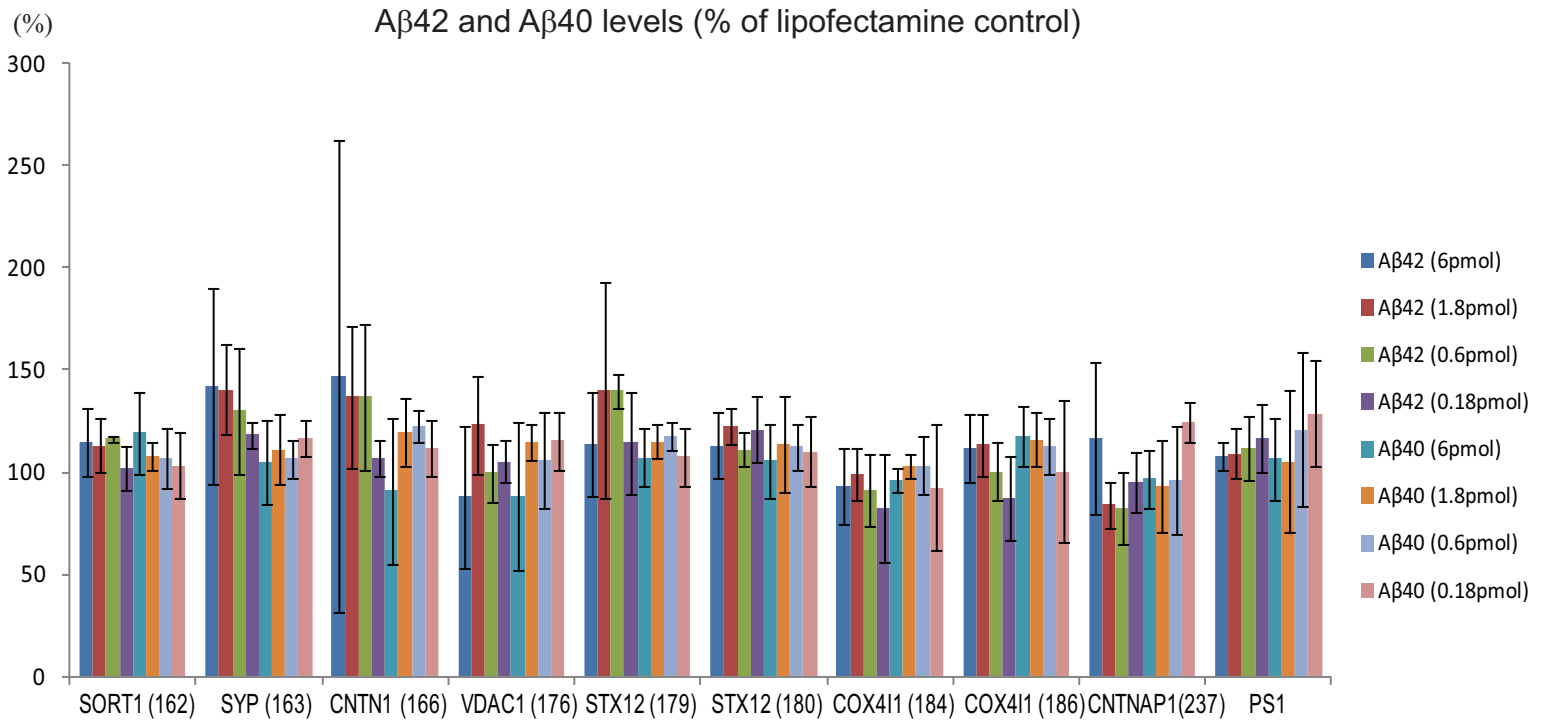
Supplementary Figure 1. Silencing of GSAPs in BE(2)-C cells did not change Aβ levels. Different amounts of siRNA (6/1.8/0.6/0.18 pmol) were transfected into neuroblastoma BE(2)-C cells, and the levels of Aβ42 and Aβ40 were measured by sandwich ELISA. The Aβ levels are expressed as % of Aβ from cells treated with Lipofectamine (LF) only, and all values were adjusted for cell viability. The results are presented as mean ± SD (error bars) of three independent experiments.

Supplementary Figure 2. The siRNAs directed to the GSAPs resulted in an efficient knock-down of the corresponding protein. HEK-293 APP695 cells were treated with siRNA as described in Experimental Procedures. After harvesting the media for Aβ analysis, cells were lysed and samples from three independent experiments were loaded on an SDS-PAGE gel and western blotting was performed. The following antibodies were used: SORT1 (BD), SYP (Stressgen), CNTN1 (R&D systems), VDAC1 (Santa Cruz), STX12 (R&D systems), COX4I1 (Molecular Probes), CNTNAP1 (AbCam) and PS1-CTF (MilliPore).

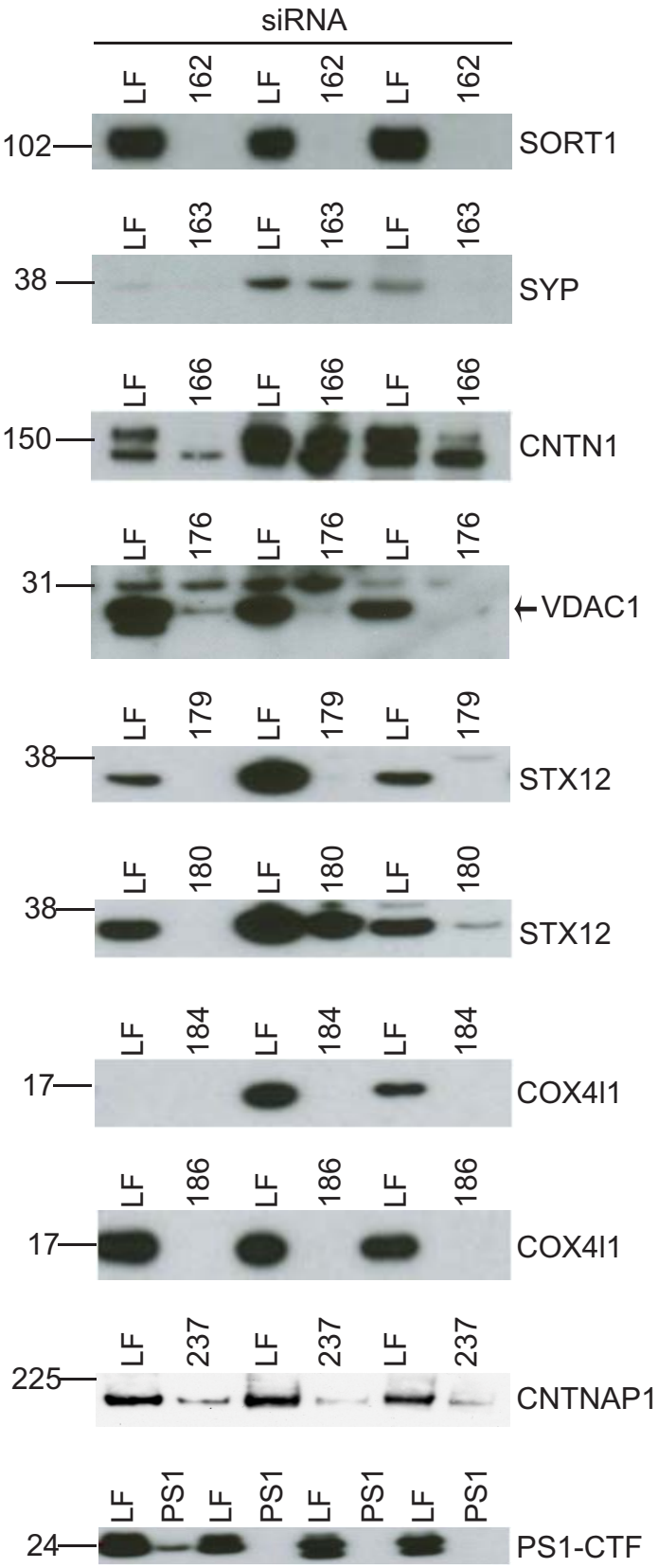
Supplementary Figure 3. The siRNAs directed to the GSAPs did not significantly alter the expression levels of γ-secretase complex components and APP-CTF. HEK-293 APP695 cells were treated with siRNA as described in Experimental Procedures. After harvesting the media for Aβ analysis, cell lysates from three independent experiments were analyzed by SDS-PAGE and Western blotting. Representative data is shown here. Note that siRNA treated samples are in duplicates. The following antibodies were used: nicastrin (Chemicon), PS1-NTF (Calbiochem), Aph-1aL (Nordic Biosite), Pen-2 (UD1, a kind gift from Dr. Jan Näslund, Karolinska Institutet) and APP-CTF (C1/6.1, a generous gift from Dr. Paul M. Mathews, The Nathan S. Kline Institute).

Supplementary Figure 4. The subcellular localization of overexpressed NotchΔE is similar to that of endogenous. HEK-293 cells or HEK-293 cells stably expressing NotchΔE were incubated in the presence or absence of γ-secretase inhibitor, L-685,458 (5 μM). Immunocytochemical analysis by confocal microscopy was performed using the C20 antibody (Santa Cruz), which recognizes all variants of Notch (red). In the absence of L685,458, only weak cytoplasmic staining was observed in the wt cells (A), while the HEK-293 NotchΔE cells showed intense nuclear staining (this nuclear staining indicates cleaved NotchΔE, NICD) (C). Blocking of γ-secretase activity with L-685,458 results in a perinuclear staining, which is similar for the two cell lines (B, D). Note that no nuclear staining (D) and no NICD band in western blot analysis (E) was observed in HEK/NotchΔE cells treated with L-685,458. Scale bars: 10 μm.

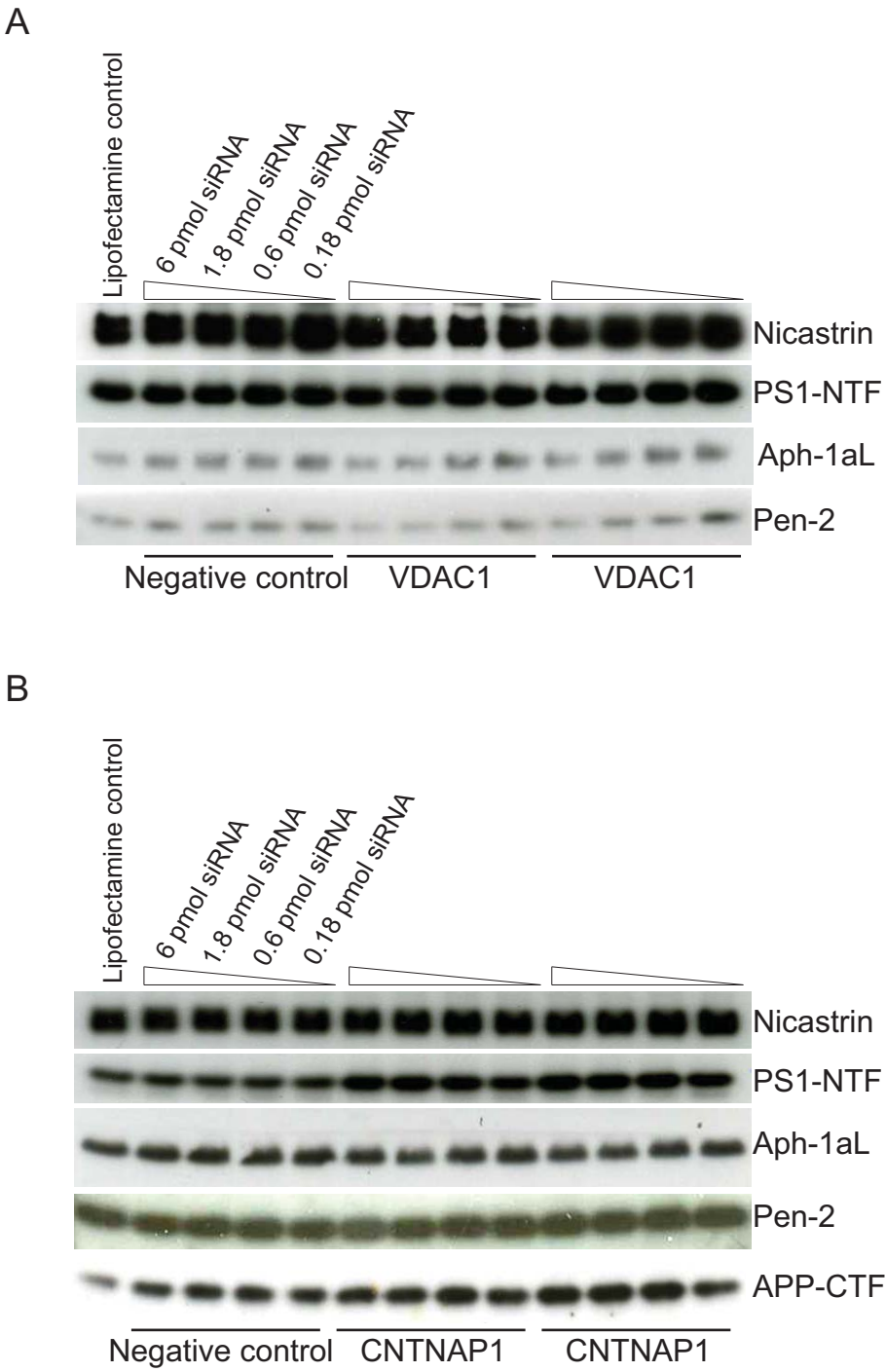
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4