Supplementary Information

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Anti-LRP/LR specific antibodies and shRNAs impede amyloid beta shedding in Alzheimer's disease

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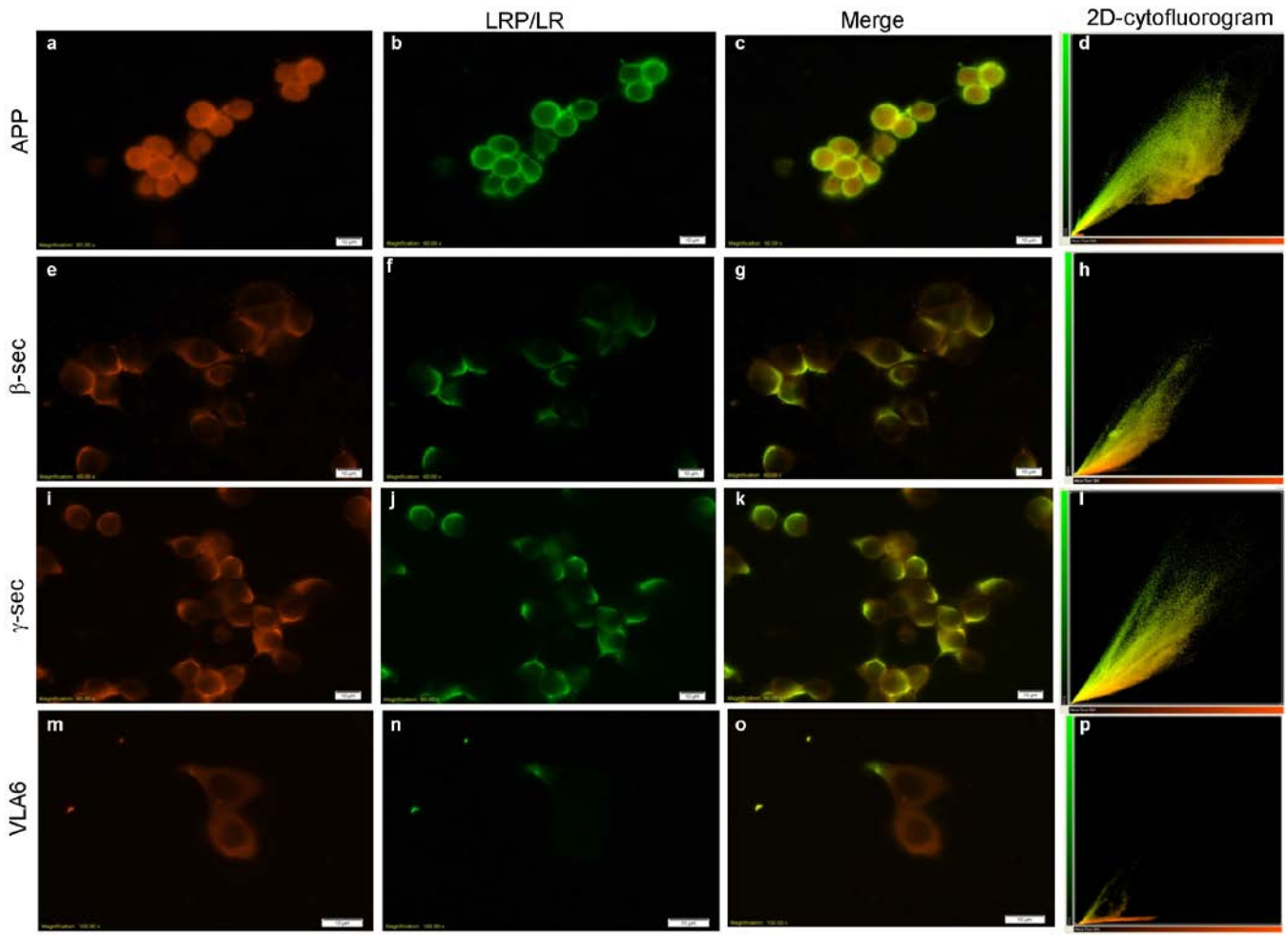


Fig S1| Co-localisation of LRP/LR with the AD relevant proteins APP, β- and γ-secretase. Cell surface receptors on N2a (mouse neuroblastoma) cells were indirectly immunolabelled to allow for detection using the Olympus IX71 Immunofluorescence Microscope and Analysis Get It Research Software . (a), APP (detected by anti-APP (rabbit polyclonal IgG) (Abcam), (e), β-secretase (detected using anti-BACE (M-83) (rabbit polyclonal IgG) (Santa Cruz Biotechnology)), (i), γ-secretase (detected by anti-PEN-2 (FL-101) (rabbit polyclonal IgG) (Santa Cruz Biotechnology)) and (m), VLA6 (detected by anti-very late antigen-6 (VLA6) CD49-f (rabbit monoclonal IgG) (Immunotech) were all indirectly labelled with Alexaflour 633, while an anti-human FITC conjugated antibody (Cell Lab) was used to label LRP/LR (b, f, j, n). The merges between LRP/LR and relevant proteins are shown (c, g, k, o) and the corresponding 2D-cytofluorograms (acquired using CellSens Software) have been included to confirm the degree of co-localisation (d, h, l, p).

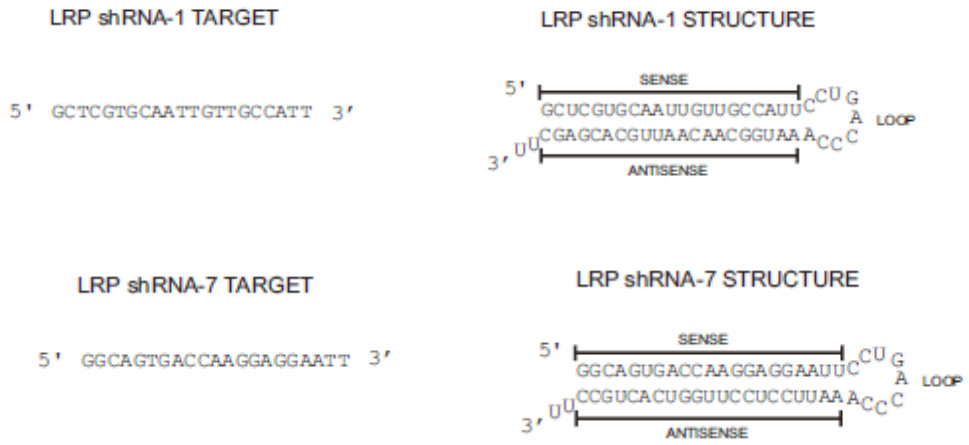


Figure S2| LRP/LR target sequences and structure of shRNA1 and shRNA7. shRNAs 1 and 7 were designed to target the LRP/LR sequences shown above. The hairpin structure of the shRNAs is also shown.

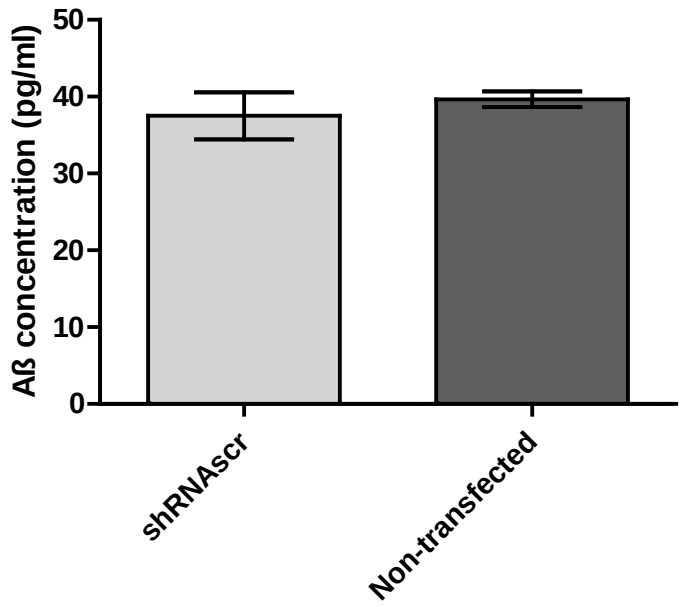
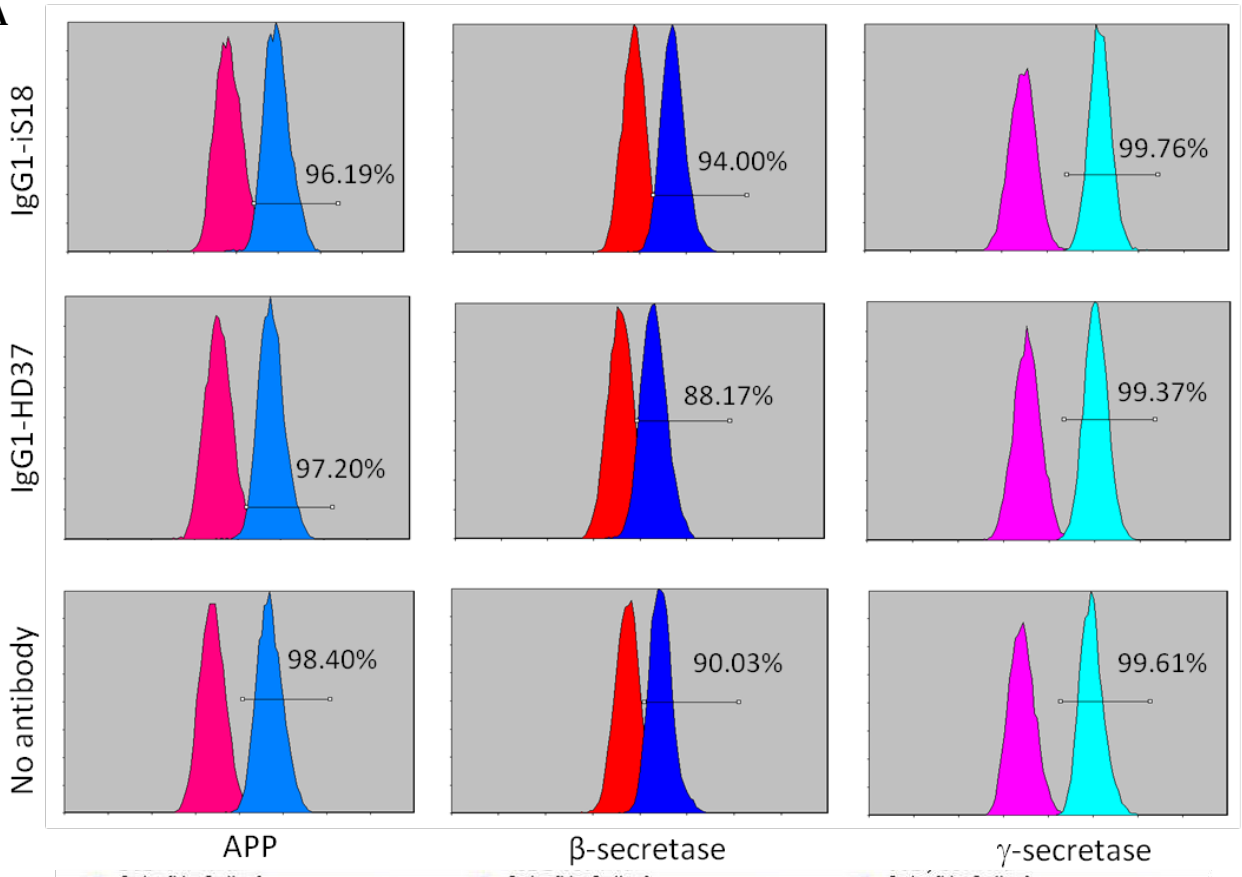


Figure S3| Aβ concentration of the cell culture medium of shRNAscr-transfected and mock-transfected HEK293 cells. HEK293 cells were either transfected with the scrambled control (shRNAscr) or mock-transfected with no plasmid. 72 hours post transfection, the Aβ concentration of the cell culture medium was analysed using an Aβ ELISA. Data shown (Mean±s.d); n=3; Student's *t*-test; $p>0.05$.

4A


IgG1-iS18
IgG1-HD37
No antibody
96.19%
94.00%
99.76%
97.20%
88.17%
99.37%
98.40%
90.03%
99.61%
APP
β-secretase
γ-secretase

B

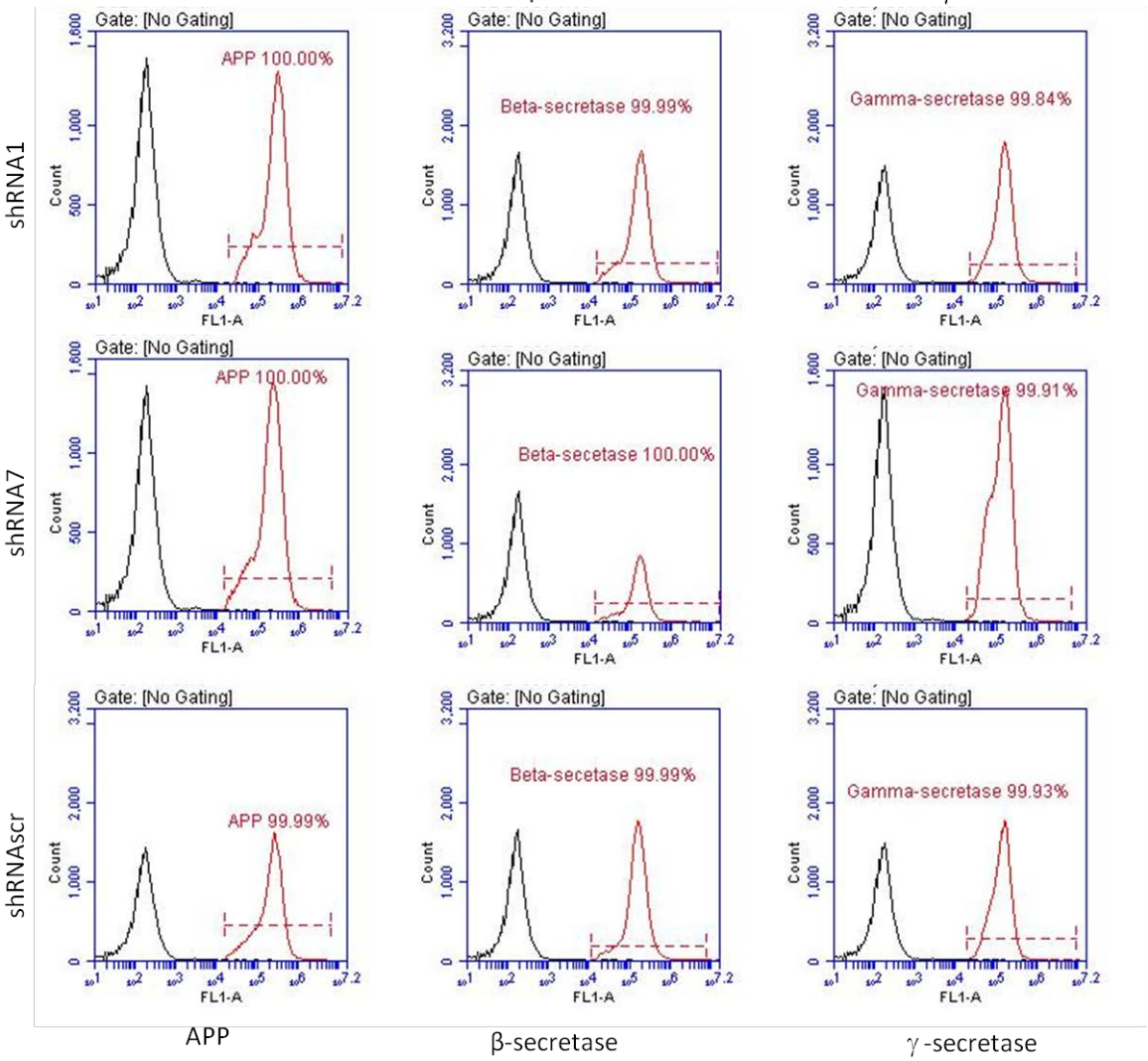

shRNA1
shRNA7
shRNAscr
Gate: [No Gating]
APP 100.00%
Beta-secretase 99.99%
Gamma-secretase 99.84%
APP 100.00%
Beta-secetase 100.00%
Gamma-secretase 99.91%
APP 99.99%
Beta-secetase 99.99%
Gamma-secretase 99.93%
Count
FL1-A
APP
β-secretase
γ-secretase

Figure S4| Flow cytometry histogram overlay plots for β-secretase, γ-secretase and APP after antibody or shRNA treatments. (A) HEK293 cells were incubated with either 50 μg/ml IgG1-iS18, IgG1-HD37 or no antibody for 18 hours after which APP, β- and γ-secretase cell surface levels were ascertained by flow cytometry (Coulter EPICS® XL-MCL). Images shown are averages of 3 independent experiments. (B) HEK293 cells were transfected with either shRNA1, shRNA7 or shRNAscr. 72 hours post transfection, the cell surface levels of APP, β- and γ-secretase were ascertained by flow cytometry (BD Accuri C6) using the methodology described above.

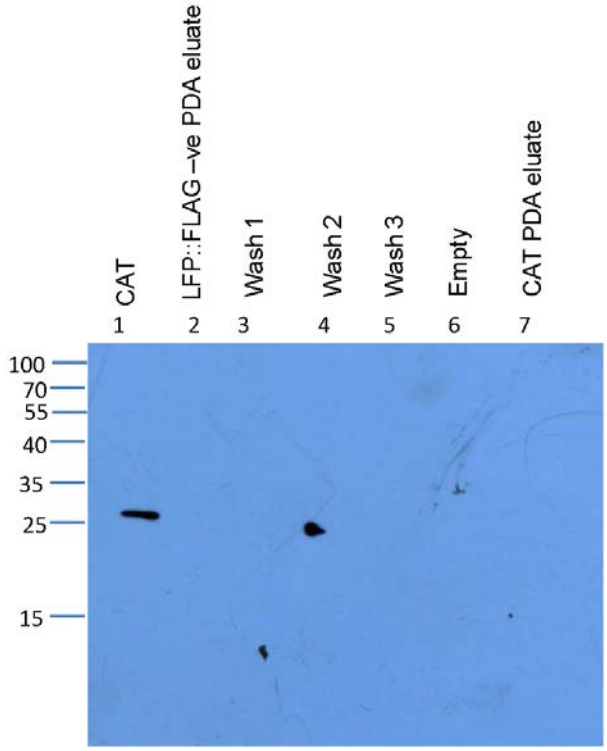


Figure S5| CAT fails to immunoprecipitate with LRP::FLAG. Cell lysates containing CAT (chloramphenicol acetyl transferase) were mixed with LRP::FLAG positive cell lysates and were subjected to a FLAG® Immunoprecipitation assay. CAT was only detected in the CAT cell lysate (positive control, lane 1) and possibly in residual amounts in the wash fractions of the assay (lane 4). It however is completely lacking in lane 7 which contains the eluate of the assay and hence all the proteins that would be interacting the LRP::FLAG protein.