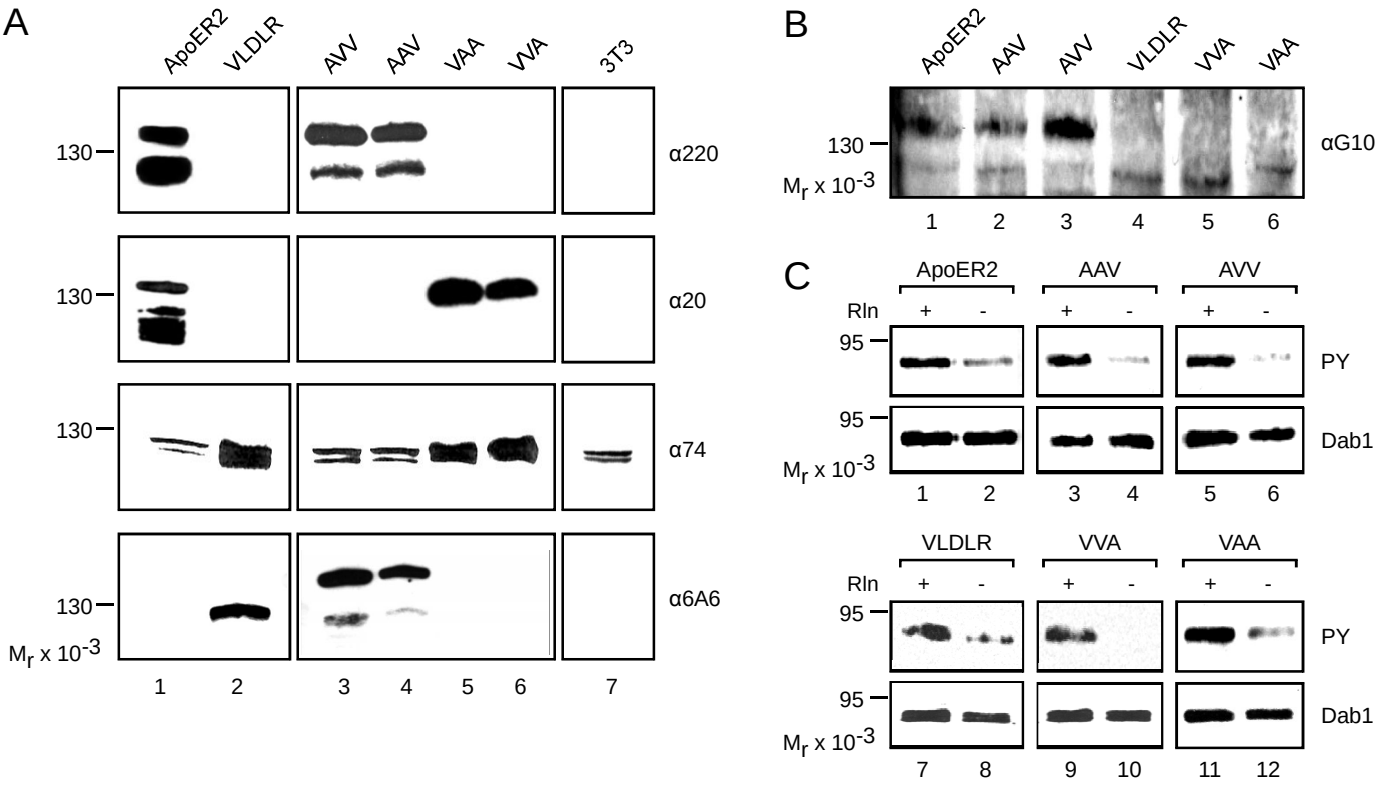
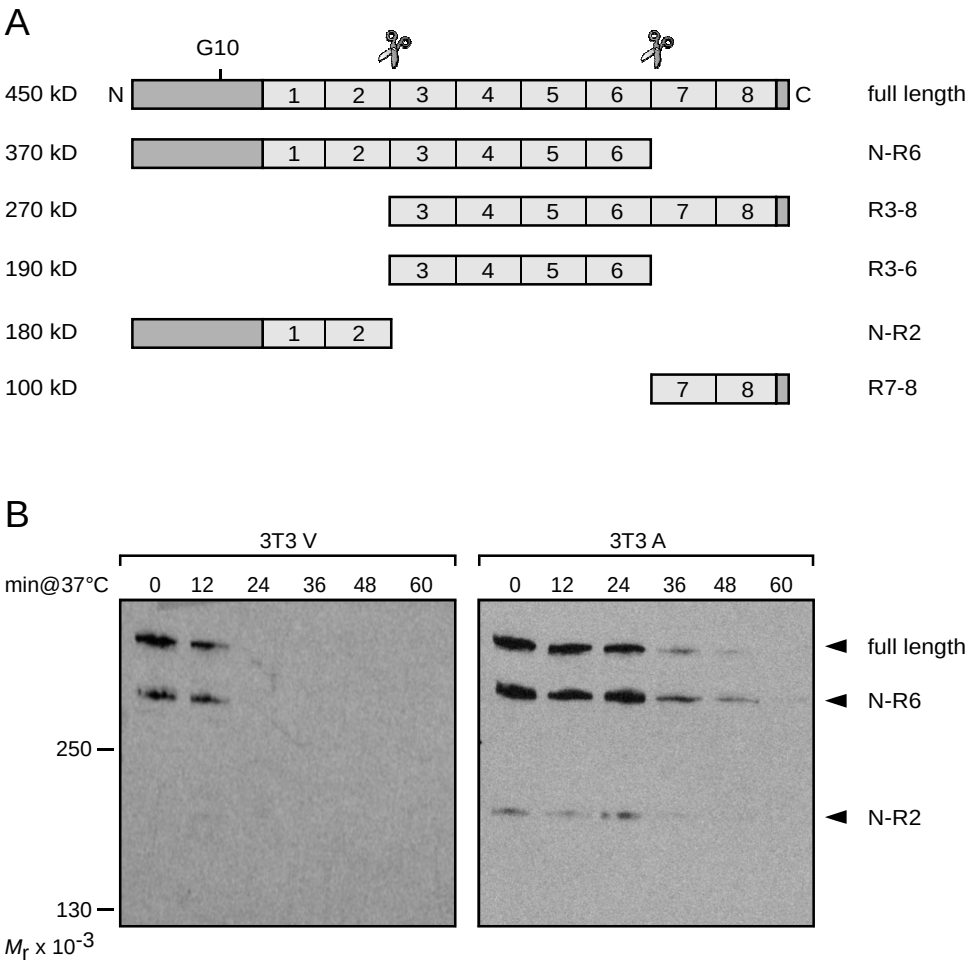
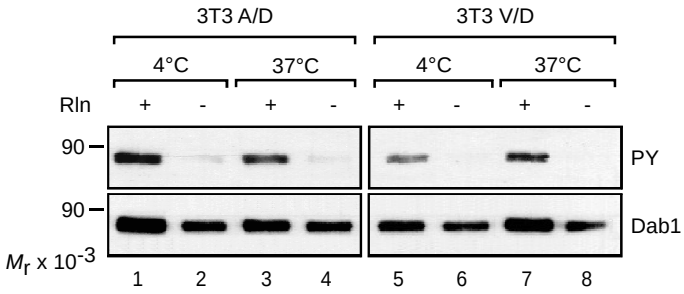
Supplemental figure 1



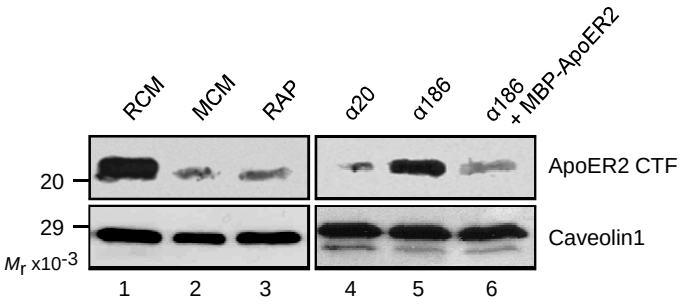
Supplemental figure 2



Supplemental figure 3



Supplemental figure 4



Supplemental Figure 1: Expression and functional characterization of chimeric receptors. **(A)** *wt* receptors (lanes 1 and 2) and chimeric receptors (lanes 3-6) were expressed in 3T3 fibroblasts, total cell extracts were separated by SDS-PAGE and the receptors analyzed by Western blotting using the indicated antibodies in combination with the appropriate HRP-coupled secondary antibodies. Mock transfected 3T3 fibroblasts (lane 7) were used as control. **(B)** Total cell extracts derived from cells expressing ApoER2 (lane 1), VLDLR (lane 4) or the respective chimeric receptors (lanes 2, 3, 5, 6) were separated by SDS-PAGE and the receptors analyzed by ligand blotting using Reelin as ligand. Bound Reelin was detected by incubating the blots with an antibody against Reelin (G10) in combination with an HRP-coupled goat-anti-mouse antibody. **(C)** Dab1 phosphorylation mediated by wt (lane 1, 2, 7, and 8) and chimeric receptors (lanes 3-6 and 9-12). 3T3 cells expressing one of the receptors and Dab1 were incubated with RCM or MCM for 30 minutes and Dab1 was immunoprecipitated from cell extracts and analyzed by Western blotting using antibodies against Dab1 and phosphorylated tyrosine in combination with an HRP-coupled goat-anti-mouse antibody.

Supplemental Figure 2: Full-length Reelin and Reelin fragments show similar endocytosis and degradation kinetics. **(A)** Reelin is cleaved at two sites, generating three fragments and two intermediates. Reelin antibody G10 recognizes N-R6 and N-R2 in addition to full length Reelin. **(B)** Western blot partially presented in Fig. 3A. 3T3 cells expressing ApoER2 or VLDLR were incubated with RCM at 4°C to allow binding of Reelin to the respective receptors. Cells were then shifted to 37°C for the indicated time periods to allow internalization and degradation of the ligand. Cell extracts were prepared and analyzed for cell-associated Reelin by Western blotting using Ab G10 in combination with an HRP-coupled goat-anti-mouse antibody. Black arrows indicate the respective Reelin fragments.

Suppemental Figure 3: Dab1 phosphorylation is independent of Reelin internalization. 3T3 cells expressing Dab1 and either ApoER2 (lanes 1-4) or VLDLR (lanes 5-8) were incubated with RCM or MCM for 30 minutes at 4°C or 37°C to inhibit or allow endocytosis, respectively. Dab1-phosphorylation was measured by immuno-precipitating Dab1 from total cell extracts and analyzing the precipitate by Western blotting using antibodies against Dab1 and phosphorylated tyrosine in combination with an HRP-coupled goat-anti-mouse antibody.

Supplemental Figure 4: Secretase-mediated cleavage of ApoER2 is induced by multivalent ligands. ApoER2-expresssing 3T3 fibroblasts were incubated for 5 h with RCM (lane 1), MCM (lane 2), 20 µg/ml recombinant RAP (lane 3), Ab 20 (lane 4, 1:100, targets ApoER2 intracellular domain), Ab 186 (lane 5, 1:100, targets ApoER2 ligand binding domain), or Ab 186 and recombinant ApoER2 N-terminal fragment (lane 6, MBP-ApoER2) and total cell extracts were analyzed by Western blotting for ApoER2-CTF (Ab 20 in combination with an HRP-coupled goat-anti-rabbit antibody). Caveolin1 was highlighted using a caveolin1 antibody and used as a loading control.