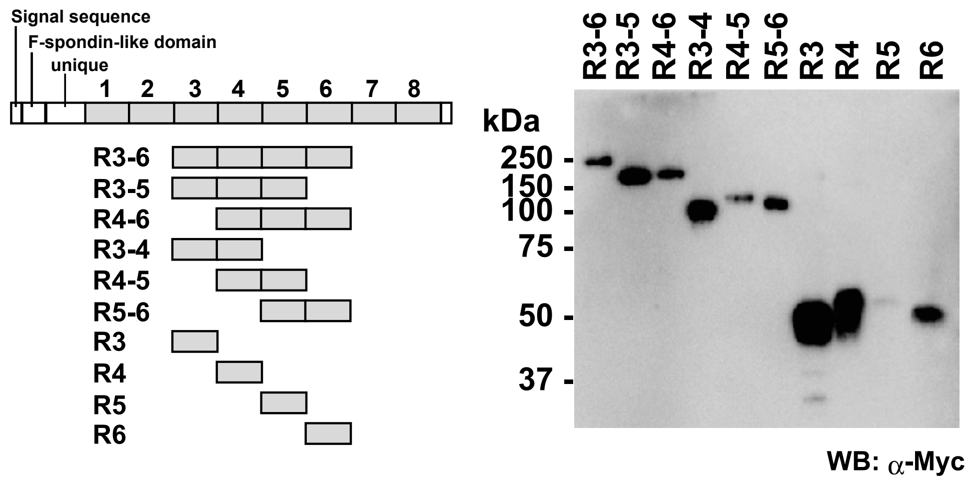
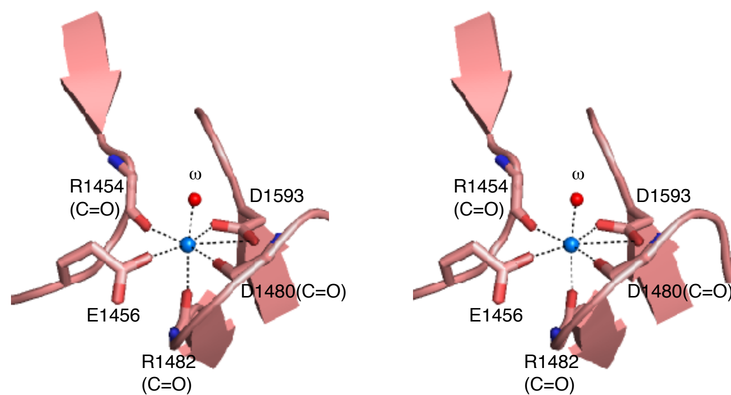
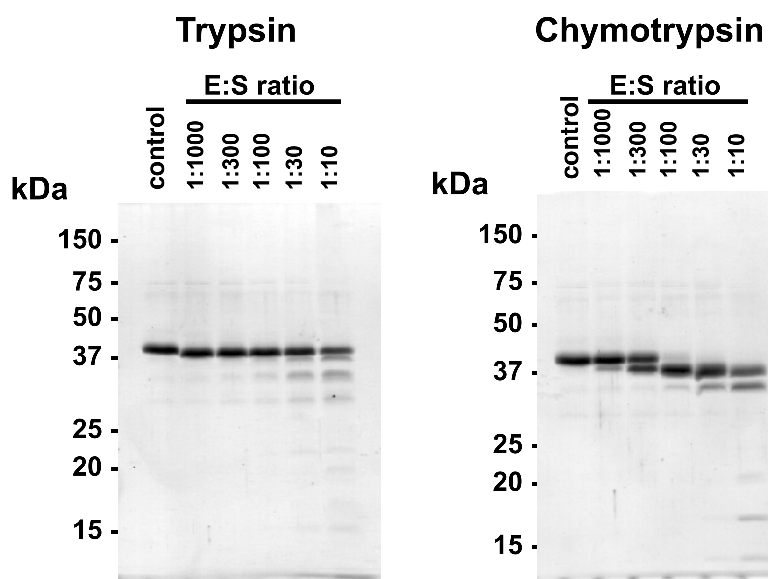


Supplementary Figure 1

Expression of reelin fragments. (Left) Domain organization of reelin protein and the design of its fragments are shown. (Right) The culture supernatant from 293T cells transiently transfected with various reelin fragments was precipitated with Ni-NTA beads, while bound proteins were separated by SDS-PAGE and subjected to Western blotting using anti-Myc antibody.

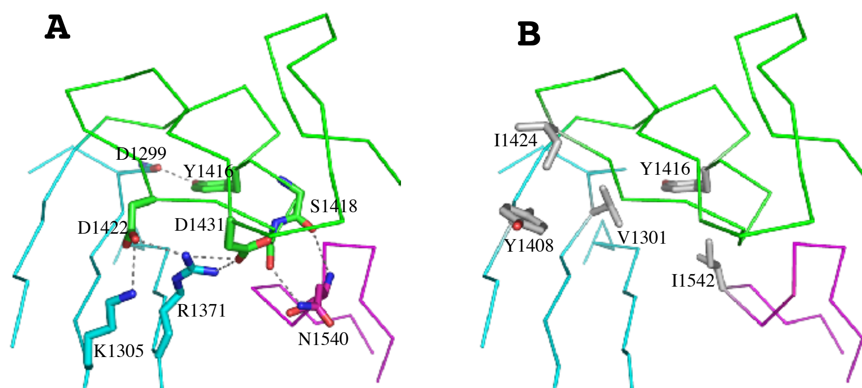


Supplementary Figure 2
Stereo view of the Ca^{2+} binding site in the subrepeat B. Hepta-coordination geometry typical for a protein-bound $Ca^{2+}$is shown. Side chains and mainchain carbonyls that participate in the primary coordination shell, together with one water molecule (ω), are shown.

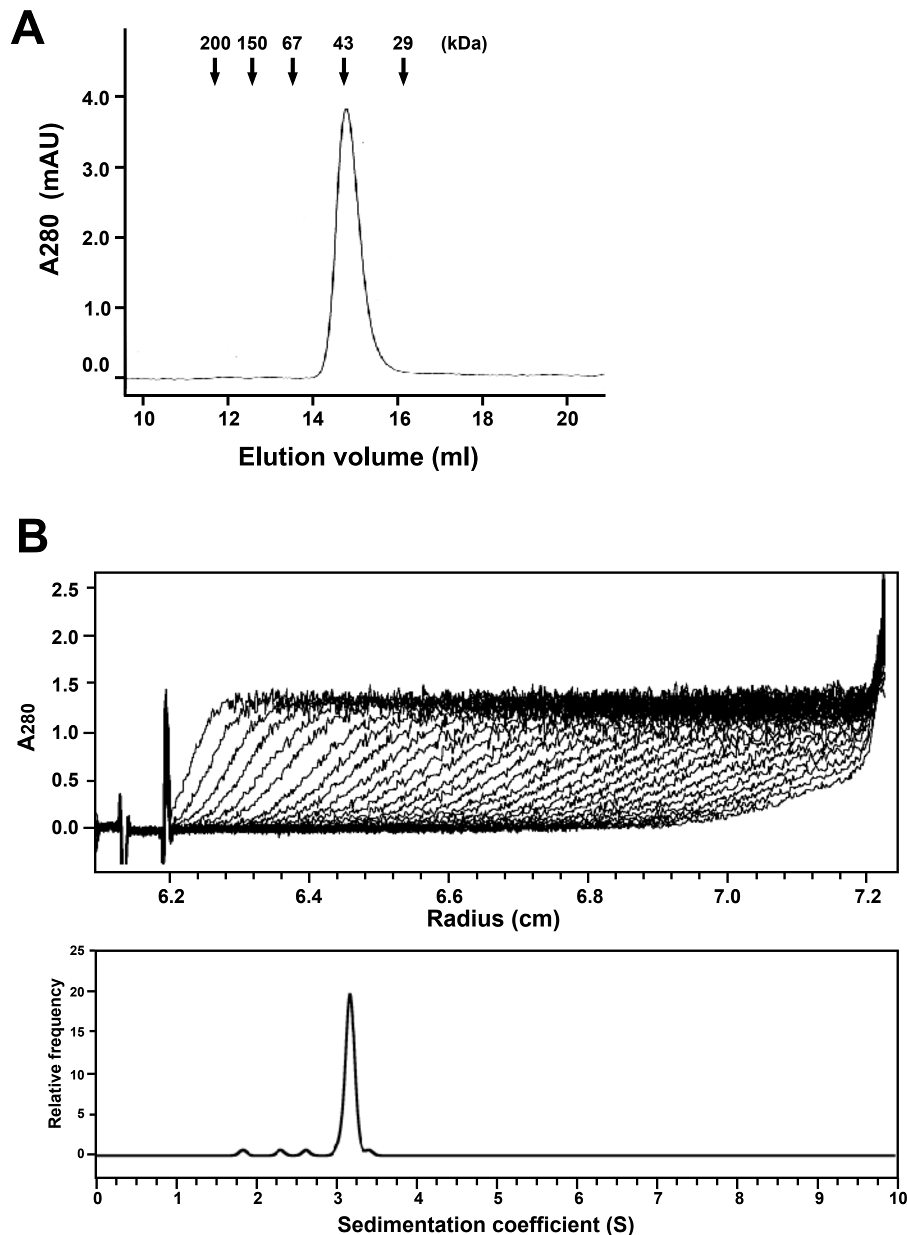


Supplementary Figure 3

Extended protease treatment of R3 fragment. Untagged and purified R3 fragments (500 μg/ml) were incubated with increasing concentrations of trypsin (left) or chymotrypsin (right) for 16h at 25∝C, and analyzed on a reducing SDS-PAGE. Even at a enzyme/substrate ratio (w/w) of 1/10, most of the R3 fragment remained largely intact after trypsin treatment. Chymostrypsin treatment resulted in a reduction of apparent molecular weight by ~2 kDa, most likely corresponding to a cleavage at Tyr-1241 in the long A-B loop, as well as a partial production of a smaller fragment (35 kDa) when the enzyme concentration was high. This can be regarded as a lack of efficient cleavage, considering the relatively broad substrate specificity of chymotrypsin. These results strongly argue against the notion that the N-terminal part of the subrepeat A is unstructured.



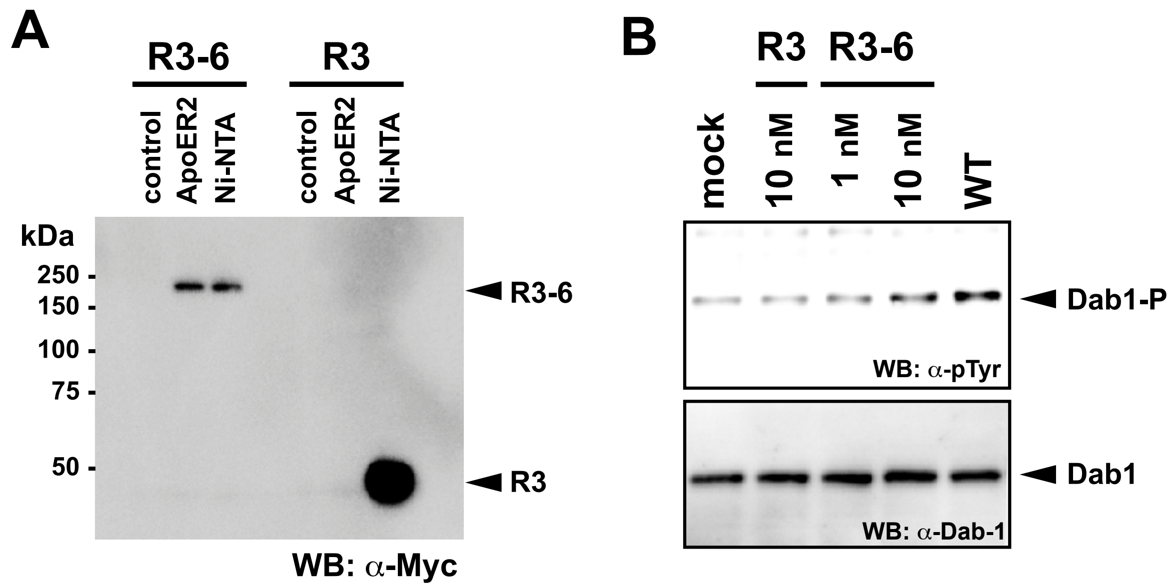
Supplementary Figure 4
Close-up view of EGF-subrepeat interfaces. Residues involved in hydrogen bonding (A) or in hydrophobic (B) interactions across the EGF-subrepeats interface are highlighted in the stick models.



Supplementary Figure 5

Hydrodynamic properties of the R3 fragment. (A) Analytical gel filtration. Purified and untagged R3 protein (10 μg) was analyzed on a Superdex 200 10/30 column equilibrated with 50 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.5, at a flow rate of 0.5 ml/min. Elution positions of standard globular proteins are shown on the top. β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), and carbonic anhydrase (29 kDa).
(B) Analytical ultracentrifugation. Sedimentation velocity analysis of purified R3 was performed at 4∝C using Beckman Optima XL-A ultracentrifuge as described in the Supplementary Methods. The upper panel shows the overlaid scans of absorbance versus radius distributions at different times, and the lower panel shows the sedimentation coefficient distributions (corrected to values at 20∝C) calculated from the data. The data indicate that R3 behaves as a homogeneous species with a sedimentation coefficient of 3.11 S. The maximal theoretical sedimentation coefficient (s_{max}) for a sphere with a molecular mass of 43,248 is 4.47 S, yielding a frictional ratio f/f_0 of 1.43. The frictional ratio for the hydrated protein (f/f_{hyd}) can be derived from the following equation, assuming a hydration factor (δ) of 0.3:

$$f/f_{hyd} = f/f_0(1+0.3)^{-\frac{1}{3}} = 1.32$$



Supplementary Figure 6

Biological activity of the reelin R3-6 fragment. (A) Interaction of reelin fragments with hGH-ApoER2 fusion protein was analyzed by a solid-phase binding assay (See Supplementary Methods). Culture supernatants from 293T cells transiently transfected with R3 or R3-6 were incubated with receptor(ApoER2)-immobilized or control beads. Ni-NTA agarose was used to estimate amounts of total recombinant fragments present in the medium. Proteins bound were probed with anti-Myc antibody. R3-6 fragment could be pulled-down with the receptor-immobilized beads but not with the control beads. The binding was efficient because the amount of proteins bound by the receptor beads was comparable to that by the Ni-NTA beads. In contrast, the R3 fragment was not bound by the receptor, even though the total production and secretion level was much higher than that of R3-6. (B) The R3-6 fragment was produced using CHO Lec 3.2.8.1 cells and purified to homogeneity. Mouse cortical neurons were incubated for 20 min with the purified reelin fragments at the indicated concentration or with culture supernatant from the cells transfected with full-length reelin (WT). Cell lysates were immunoprecipitated with anti-Dab1 antibody and subjected to Western blotting using anti-phosphotyrosine (top) or anti-Dab1 (bottom) antibodies.