Supplementary Figure 3 Molecular modeling and functional characterization of BADN as a dominant-negative mutant for RIM1 function.  (a) X-ray structure of the β subunit and AID (yellow and red) complex adapted from the previous paper by Opatowsky et al.  (b) Energetically minimized structure of BADN by molecular modeling.  BADN is designed to suppress AID binding without destruction of the β subunit 3D structure.  Inset indicates the amino acid sequence (370-393) of BADN compared with the sequence of rat βab and the AID sequence of Ca,2.1.  BADN is constructed on the basis of rat βab, and hexa-peptide (GELNGG (red)) containing the essential region (ELNG) for β subunit binding of AID with flanking glycine residues as spacers replacing the original tri-peptide (ENQ (blue)) to suppress AID binding.  (c) Domain structure of βab and BADN.  (d) Dose-dependent binding of recombinant-BADN to the GST-RIM11079-1463 is shown.  This result shows that BADN has affinity to RIM11079-1463 comparable with that of βab.  (e) Interaction of recombinant BADN and RIM1 in HEK293 cells.  The interaction is evaluated by IP with anti-FLAG antibody, followed by WB with anti-βab antibody.  Top: HEK293 cells co-transfected with βab and FLAG-RIM1.  FLAG-Ca,2.1(I-II linker) is used as a positive control.  Bottom: HEK293 cells co-transfected with BADN and FLAG-RIM1.  (f) Rapid disruption of the RIM1-βab interaction by BADN.  The dissociation is measured in the presence of excessive BADN protein (200 nM).  This result suggests that virtually complete disruption of the RIM1-βab interaction is attained within 6 h of incubation.