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Supplementary Data

The N-Terminal SH3 Domain of Grb2 is Required for Endosomal Localization of AβPP

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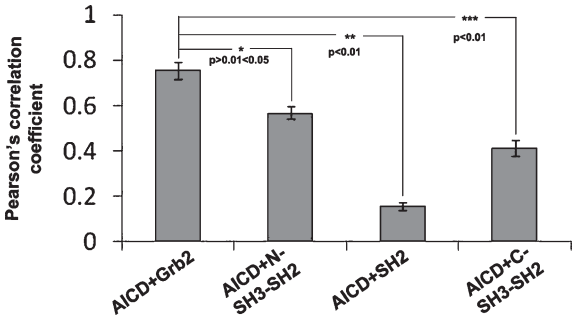
Supplementary Table 1

Mutation	Primer sequence
Grb2_G173D	Forward: 5′CCAGGAGGATGACGAGCTGGGCTTC 3′
	Reverse: 5′GAAGCCCAGCTCGTCATCCTCCTGG 3′
Grb2_P49L	Forward: 5′GACGGCTTCATTCTCAAGAACTACATAG 3′
	Reverse: 5′CTATGTAGTTCTTGAGAATGAAGCCGTC 3′



Supplementary Figure 1. The degree of co-localization of double transfected cells was calculated in terms of 'Pearson's correlation coefficient'. For each experiment ($n = 5$) individual images were analyzed and mean Pearson's correlation coefficients were calculated. The error bar indicates standard error.

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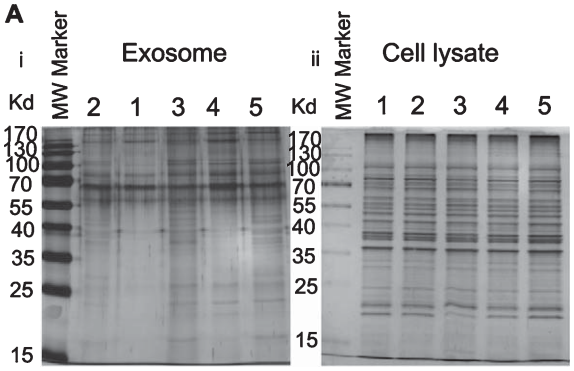
A

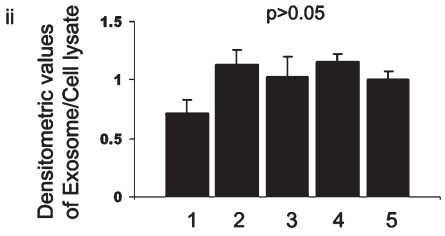


B

i

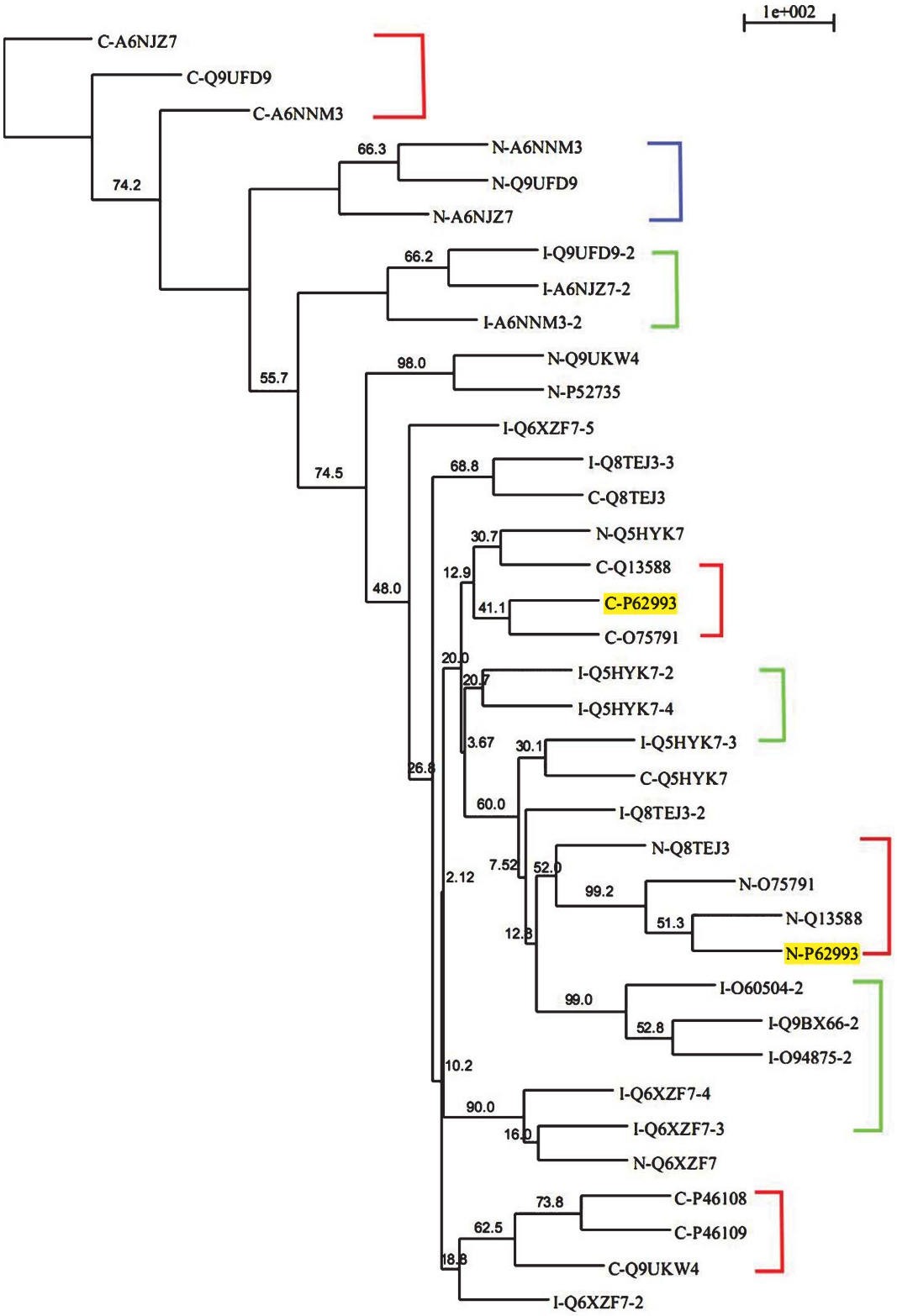
Densitometric values of Exosome/Cell lysate	Dsred (1)	Grb2 -Dsred (2)	NSH3 -SH2 (3)	SH2 (4)	CSH3 -SH2 (5)
I	0.48	1.4	1.2	1.18	.87
II	0.93	1.03	.68	1.25	1.14
III	0.73	0.97	1.25	1.01	0.97
Average	0.71	1.13	1.03	1.15	1
SD	0.22	0.23	0.310	0.126	0.135
SE	0.12	0.13	0.17	0.07	0.07

ii



Supplementary Figure 2. A) 3×10^6 cells were cultured in each plate and were transfected with either empty vector DsRed (control) or Grb2-DsRed or DsRed fusion constructs of different domains of Grb2 (N-SH3-SH2-DsRed, SH2-DsRed, C-SH3-SH2-DsRed). After 48 h of transfection, the medium was collected to prepare exosomes and proteins were prepared from the cells. Exosome pellet was dissolved in 75 μl HEPES/NaOH buffer and the cells were lysed in 100 μl lysis buffer. [i] 20 μl exosome and [ii] 5μl cell lysate was loaded in 12% SDS-PAGE and the proteins were visualized by silver staining. B) The amount of protein in each lane was measured by the PDQuest software of VersaDoc (Bio-Rad). [i] The densitometric values obtained from the software were used to calculate exosome/cell lysate ratio. Ratios obtained from three independent experiments were taken to calculate mean ratio, standard deviation (SD), and standard error (SE). [ii] The result was plotted graphically and the error bar represents SE. *p* value was calculated for each transfection (Grb2 and its different domains) with respect to empty vector control and using paired *t*-test.

Supplementary Figure 3. A dendrogram of a subset of human SH3 domains generated using the PHYLIP package. The bootstrap values other than 100% are shown in nodal points with the scaling value on the top. To define the position of SH3 domains whether they belong to N-terminal, Intermediate, or C-terminal positions, and each protein's "UniProt" id is preceded by "N", "I", or "C". Clustering of N-terminal, Intermediate, and C-terminal domains are shown with blue, green, and red braces. The N-terminal and C-terminal domains of Grb2 (UniProt id: P62993) are highlighted in yellow.



Supplementary Figure 3.