

Table S1. **Detailed description of R9AP-syntaxin 3 chimeric molecules**

Construct	Cartoon	Residues from R9AP	Residues from Stx3	Figure
Myc-R9AP		1–251	—	Fig. 6 A
Myc-Stx 3		—	1–286	Fig. 6 B
Myc-R9AP/Stx 3 _(TM)		1–226	257–286	Fig. 6 C
Myc-Stx 3/R9AP _(SH)		129–218	1–161 and 259–286	Fig. 6 D
Myc-Stx 3/R9AP _(TM)		265–285	1–264	Fig. 6 E
Myc-R9AP/Stx 3 _(SH)		1–127 and 231–251	162–263	Fig. 6 F

Table S2. **Predicted length of transmembrane domains used in this study**

Construct Name	Sequence	Domain length	Figure
GFP-R9AP TM	GFP_DGQL IIVSLLCGTALVAITLYSIL	17	Figs. 3 D and 9 A
GFP-R9AP TM shorter (-2)	GFP_DGQL IIVLLCGTALVAITLYSIL	15	Fig. 9 B
GFP-R9AP TM shortest (-4)	GFP_DGQL IIVLLCGTALAILYSIL	13	Fig. 9 C
GFP-mGluR1 tmd 1	GFP_IIAIAF SCLGILVTLFVTLFVLYRDTPVV	24	Fig. 7
GFP-Cyt b5 TM	GFP_SWWTN WVIPAISALAVALMYRLYMAED	14	Fig. 8 A
GFP-Cyt b5 TM longer (+4)	GFP_SWWTN WVIPAILSVALLAVALMYRLYMAED	18	Fig. 8 B
GFP-BclXL TM	GFP_RKGQERFNRFWFL TGMTVAGVVLGS LFSRK	13	Fig. 8 C
GFP-BclXL TM K233S	GFP_RKGQERFNRFWFL TGMTVAGVVLGS LFSRS	13	Fig. 8 D
GFP-BclXL TM K233S, longer (+4)	GFP_RKGQERFNRFWFL TGMTVALLLGVVLLGS LFSRS	19	Fig. 8 E
GFP-Stx 3 TM	GFP_DARRKK IMILICCVILAIIVIASTI GGIFA	18	Figs. 4 D and 10

The residues shown in bold are predicted to form each respective transmembrane domain as calculated using the DAS server (Cserzo, M., E. Wallin, I. Simon, G. von Heijne, and A. Elofsson. 1997. *Protein Eng.* 10:673–676). The position of the K233S point mutation in BclXL is underlined.