

E08-02-0160 Kaplan

Supplementary Table 1. Tagged SNARE genes

Listed are the SNARE genes tagged with either RFP or GFP (indicted) used in this study. All were expressed in all neurons under the SNB-1 promoter. SNARE homologue names were assigned based on highest BLAST identities to the mouse protein database. *C. elegans* cosmid/gene identifications are listed. Expected localization is based on homologous protein localization patterns. Abbreviations: IC – Intermediate Compartment, PM – Plasma Membrane, Endo – Endosomes, Lys – Lysosome and SV – Synaptic Vesicle.

Supplementary Table 2. Summary of *unc-18* point mutations tested.

The listed *unc-18* transgenes were expressed in *unc-18(md299)* mutants. Point mutations expected to disrupt either the closed-Syntaxin binding (C) or the N-terminal binding (N) modes of UNC-18 are indicated, based on the cited prior publications. Transgenic animals were assayed for locomotion, aldicarb resistance, and accumulation of endogenous UNC-64 in cell bodies. Locomotion: (Unc) indicates Uncoordinated movement, (WT) indicates wild-type movement on NGM plates. The percentage of animals paralyzed after 140 minutes on 1mM aldicarb are indicated as follows: less than 20% (Resistant), 80-100% paralyzed (+++), 60-80% paralyzed (++) , and 20-60% paralyzed (+). Anti-UNC-64 cell body accumulation: (+++) indicates the *unc-18* mutant phenotype of UNC-64 accumulation, (WT) indicates a wild type UNC-64 distribution with little cell body staining.

Supplementary Table 1. Tagged SNARE genes.

| SNARE homologue | <i>C. elegans</i> gene ID | Expected Localization | N-Terminal Tag |
|-------------------|---------------------------|-----------------------|----------------|
| Bet-1 | Y59E9AL.7 | IC | GFP |
| Sec-22 | F55A4.1 | IC | RFP |
| Membrin | B0272.2 | Golgi | RFP |
| Gos-28 | F08F8.8 | Golgi | GFP |
| Ykt-6 | B0361.10 | Golgi | RFP |
| Vti-1 | Y57G11C.4 | Golgi, Endo, SV | GFP |
| SNAP-23 | T14G12.2 | PM | GFP |
| SNAP-29 | K02D10.5 | PM | GFP |
| VAMP-3 | C30A5.5 | Endo, PM | GFP |
| VAMP-3 | T14D7.3 | Endo, PM | GFP |
| VAMP-3 | ZK795.4 | Endo, PM | GFP |
| VAMP-7 | Y69A2AR.6 | Endo, Lys | GFP |
| Endobrevin/VAMP-8 | B0513.9 | Endo | GFP |
| Snb-2 | F23H12.1 | SV, PM | GFP |

Supplemental Table 2. UNC-18 rescue experiments and point mutations tested.

| UNC-18 constructs expressed in <i>unc-18(md299)</i> | Stx Binding mode | Locomotion | Aldicarb Res. | UNC-64 transport defect | Reference |
|---|------------------|------------|---------------|-------------------------|--|
| - | NA | Unc | Resistant | +++ | |
| wild-type | NA | WT | +++ | WT | |
| W28S | C | WT | +++ | WT | (Kauppi <i>et al.</i> , 2002) |
| D34N | C | WT | +++ | WT | (Wu <i>et al.</i> , 1998; Ciufo <i>et al.</i> , 2005) |
| R39C | C | WT | +++ | WT | (Harrison <i>et al.</i> , 1994; Wu <i>et al.</i> , 1998; Ciufo <i>et al.</i> , 2005) |
| S42F | C | WT | +++ | WT | (Ehrhard <i>et al.</i> , 2000; Kauppi <i>et al.</i> , 2002) |
| E59K | C | WT | +++ | WT | (Kauppi <i>et al.</i> , 2002) |
| D112N | ND | WT | +++ | WT | (Ehrhard <i>et al.</i> , 2000) |
| L116K | N | WT | + | WT | (Peng and Gallwitz, 2004) |
| S311A, S313A | ND | WT | +++ | WT | (Fujita <i>et al.</i> , 1996) |
| S311D, S313D | ND | WT | +++ | WT | (Fujita <i>et al.</i> , 1996) |
| T574A | ND | WT | +++ | WT | (Shuang <i>et al.</i> , 1998) |
| T574D | ND | WT | ++ | WT | (Shuang <i>et al.</i> , 1998) |
| D34N, L116K | C+N | Unc | Resistant | +++ | |
| R39C, L116K | C+N | Unc | Resistant | +++ | |
| W28S, L116K | C+N | WT | ND | ND | |
| S42F, L116K | C+N | Unc | Resistant | +++ | |

References cited in E08-02-0160, Supplemental Table 2, McEwen and Kaplan:

Ehrhard, K. N., Jacoby, J. J., Fu, X. Y., Jahn, R., and Dohlman, H. G. (2000). Use of G-protein fusions to monitor integral membrane protein-protein interactions in yeast. *Nat. Biotechnol.* *18*, 1075--1079.

Fujita, Y., Sasaki, T., Fukui, K., Kotani, H., Kimura, T., Hata, Y., Sudhof, T. C., Scheller, R. H., and Takai, Y. (1996). Phosphorylation of Munc-18/n-Sec1/rbSec1 by protein kinase C: its implication in regulating the interaction of Munc-18/n-Sec1/rbSec1 with syntaxin. *J. Biol. Chem.* *271*, 7265--7268.

Kauppi, M., Wohlfahrt, G., and Olkkonen, V. M. (2002). Analysis of the Munc18b-syntaxin binding interface. Use of a mutant Munc18b to dissect the functions of syntaxins 2 and 3. *J. Biol. Chem.* *277*, 43973--43979.

Shuang, R., Zhang, L., Fletcher, A., Groblewski, G. E., Pevsner, J., and Stuenkel, E. L. (1998). Regulation of Munc-18/syntaxin 1A interaction by cyclin-dependent kinase 5 in nerve endings. *J. Biol. Chem.* *273*, 4957--4966.