

File S1.

Strains used in these studies

The reference strain for *unc-17(e245) IV* (outcrossed 6x) is RM908.

The reference strain for *sup-1(e995) III* (outcrossed 7x) is RM3670.

The reference strain for *sup-1(md44) III* (outcrossed 3x) is RM3277.

The reference strain for *sup-1(e995 e2636) III* (outcrossed 6x) is RM3571.

The *unc-17* strains used for the isolation of suppressor mutations were:

CB933: *unc-17(e245) IV*

PR1443: *unc-17(p300) dpy-13(e184) IV*

The strain used for DNA sequencing to identify the *sup-1* gene was RM921: *dpy-18(e364) sup-1(e995) III; unc-17(e245) IV*

The strains used to assess behavioral suppression and protein abundance (Figures 1 and 5) were:

N2: wild type

RM908: *unc-17(e245) IV*

RM3660: *sup-1(e995) III; unc-17(e245) IV*

RM3670: *sup-1(e995) III*

RM3643: *sup-1(e995 e2636) III; unc-17(e245) IV*

RM3571: *sup-1(e995 e2636) III*

The strains used to determine allele-specific suppression by *sup-1* (Tables 1 and S1) were:

CB2108: *dpy-18(e364) sup-1(e995) III*

CB933: *unc-17(e245) IV*

CB283: *unc-17(e283) IV*

CB284: *unc-17(e284) IV*

CB327: *unc-17(e327) IV*

CB334: *unc-17(e334) IV*

CB335: *unc-17(e335) IV*

CB356: *unc-17(e359) IV*

CB464: *unc-17(e464) IV*

CB795: *unc-17(e795) IV*

PR1170: *unc-17(p300) IV*

PR1158: *cha-1(b401)* IV

PR1154: *cha-1(p1154)* IV

CB81: *unc-18(e81)* X

The strain used to compare the expression patterns of *sup-1* and *unc-17* reporters (Figure 3) was:

RM3549: *pha-1(e2123)* III; *mdIs139* [FRM186 = *Punc-17::NLS-YFP*; pBX]; *mdEx1044* [FRM466 = *Psup-1::NLS-CFP::LacZ*; pBX; pBS]

The strains used to assess behavioral suppression of *unc-17(e245)* phenotypes by transgenic expression of SUP-1(G84E) were:

RM3483: *pha-1(e2123)* III; *unc-17(e245)* IV; *mdEx995* [FRM260 = *Psup-1::SUP-1(G84E)::GFP*; pBX; pBS]

RM2086: *pha-1(e2123)* III; *mdEx186* [pBX] (control: pBX rescue of *pha-1*)

RM2501: *pha-1(e2123)* III; *unc-17(e245)* IV; *mdEx186* [pBX] (control: pBX rescue of *unc-17*; *pha-1*)

The strains used to determine the subcellular localization of SUP-1, UNC-17, and cytochrome b5 fusion proteins (Figure 4) were:

RM3480: *pha-1(e2123)* III; *mdEx992* [FRM408 = *Psup-1::SUP-1(+)::CFP*; FRM403 = *Punc-17::YFP::UNC-17(+)*; pBX]

RM3623: *pha-1(e2123)* III; *mdEx1099* [FRM489 = *Punc-17b::cytochrome b5::YFP*; FRM491 = *Punc-17b::SUP-1(+)::CFP*; pBX; pBS]

The strains used to analyze overexpression of UNC-17(G347R) and YFP::UNC-17(G347R) (Figure 6) were:

RM3220: *pha-1(e2123)* III; *unc-17(e245)* IV; *mdEx791* [RM#349p = *Punc-17::NLS-GFP*; RM#883p = *Punc-17::UNC-17(G347R)*; pBX]

RM3674: *pha-1(e2123)* III; *unc-17(e245)* IV; *mdEx1112* [FRM419 = *Punc-17::YFP::UNC-17(G347R)*; pBX; pBS]

RM2086: *pha-1(e2123)* III; *mdEx186* [pBX] (control: pBX rescue of *pha-1*)

RM2501: *pha-1(e2123)* III; *unc-17(e245)* IV; *mdEx186* [pBX] (control: pBX rescue of *unc-17*; *pha-1*)

The *unc-17(md1447)* IV strain (Figures 6 and S3) is RM580.

The strains used for BiFC analysis of UNC-17 and SUP-1 proteins (Figure 7) were:

RM3478: *pha-1(e2123)* III; *mdEx990* [FRM394 = *Punc-17::NYFP::UNC-17(+)*; FRM401 = *Psup-1::SUP-1(+)::CCFP*; pBX; pBS]

RM3482: *pha-1(e2123)* III; *mdEx994* [FRM387 = *Punc-17::NYFP::UNC-17(G347R)*; FRM409 = *Psup-1::SUP-1(G84E)::CCFP*; pBX; pBS]

RM3689: *pha-1(e2123)* III; *mdEx1139* [FRM387 = *Punc-17::NYFP::UNC-17(G347R)*; FRM401 = *Psup-1::SUP-1(+)::CCFP*; pBX; pBS]

RM3465: *pha-1(e2123)* III; *mdEx978* [FRM394 = *Punc-17::NYFP::UNC-17(+)*; pBX; pBS]

RM3496: *pha-1(e2123)* III; *mdEx1001* [FRM401 = *Psup-1::SUP-1(+)::CCFP*; pBX; pBS]

The interaction of NYFP::UNC-17(G347R) with full-length SUP-1(G84E)::CCFP and the Δ C-terminus, the Δ N-terminus, and SNT-1::SUP-1 chimeric versions of SUP-1(G84E)::CCFP (Figure 8) were studied in strains RM3482, RM3634, RM3636, and RM3673, respectively:

RM3482: *pha-1(e2123) III; unc-17(e245) IV; mdEx994* [FRM387 = *Punc-17::NYFP::UNC-17(G347R)*; FRM409 = *Psup-1::SUP-1(G84E)::CCFP*; pBX; pBS]

RM3634: *pha-1(e2123) III; unc-17(e245) IV; mdEx1038* [FRM387 = *Punc-17::NYFP::UNC-17(G347R)*; FRM437 = *Psup-1::SUP-1(G84E) Δ 97-103::CCFP*; pBX; pBS]

RM3636: *pha-1(e2123) III; unc-17(e245) IV; mdEx1007* [FRM387 = *Punc-17::NYFP::UNC-17(G347R)*; FRM420 = *Psup-1::SUP-1(G84E) Δ 20-71::CCFP*; pBX; pBS]

RM3673: *pha-1(e2123) III; unc-17(e245) IV; mdEx1116* [FRM387 = *Punc-17::NYFP::UNC-17(G347R)*; FRM513 = *Psup-1::SNT-1(1-72)::SUP-1(73-103,G84E)::CCFP*; pBX; pBS]

RM2086: *pha-1(e2123) III; mdEx186* [pBX] (control: pBX rescue of *pha-1*)

RM2501: *pha-1(e2123) III; unc-17(e245) IV; mdEx186* [pBX] (control: pBX rescue of *unc-17; pha-1*)

The *unc-123* mutant strains were:

ER10: *unc-123(jd5) III*

ER50: *unc-123(jd5jd10) III*