**Generation of *Unc13BNull* and Ctrl (from Böhme et al., 2016)**

*Unc13* genomic transgenes were generated by Red/ET Recombineering. For this purpose, the Unc13 P[acman] BAC CH321-60O10 clone containing the *Drosophila unc13* gene was obtained from the BACPAC Resources Center, CA, USA used as a template for all cloning strategies. Based on the P[acman] technology and using Red/ET Recombineering in *E.coli* for modifying large DNA vector constructs (*GeneBridges Protocol*: Counter-Selection BAC Modification Kit, Version 3.2, January 2012), a genomic rescue construct encompassing the whole *Drosophila Unc13* locus including putative promotor regions was generated giving raise to ‘Ctrl*’*. Based on this wild type construct, an Unc13 isoform B specific deletion construct (*Unc13BNull*) was generated by removing the first 1000 bp of the *Unc13B* DNA sequence, causing a shift of the reading frame, which interrupts the translation of Unc13B by generating an early stop-codon. The cloning strategy was performed according to the Counter-Selection BAC Modification Kit by Red®/ET® Recombination (Gene Bridges Protocol, No. K002, Version 3.2, January 2012). For the Generation of transgenic flies both constructs were send for DNA micro-injection in embryos to Rainbow Transgenic Flies, Inc, CA, USA(service type: B/D2, injected fly strain: Strain 24862; y[1] M{vas-int.Dm}vZH-2A w[\*]; PBac{y[+]-attP-9A}VK00005) on LB agar.

To generate *Unc13BNull* the following sequence was deleted (first 1000 amino acids of the Unc13B exclusive exon):

ATGATGAACACATCTCAGCTGCAAGTGACCGGGGATACGGAAAAAAAAAGTCAGTTGCTTAAAAAGGAACTCAAAATAAACACCCAGGAAAAGCTTATCTTTGCAGAAAATGCCCTCAAAAGCCAGATTAAAATAAAGGAGCAGTTACGACTTCAGCAACAATCAACGATTTATGCGTCGTCACTATTGAGCTCTTCTGCTGCCGGATCAGTAAGAGCTCCGCTCTTAAGCCAGGGGCACCTTAATTCTATCCAACACAACATGGATTTTGACCTTGCCAAAGCACAAATACCGGAAATGCAACCACCGATGAGTAAGTCGCCCAATGGATTGGATTTCTCTTACTTAAGTTATCCAAGCATAAATACTAATGAAAGTATGATATCGATTAAAAGTGAACAACAGTTGTGTCAGAGCTACAATTCGGAGCAACACTCTGACTACATTATCTCCGATTATATGGATAAAATTGCAACAAGGATCAGTTTACTCGAGACAGAGCTGAAGTTTGCTTGGCGTGCATTGGATTTGCTTTCTACGGAGTACGGAAAGATTTGGATTCGTTTGGAAAAGCTTGAAAACATATCAATAGAACAACAGTCTGTCGTTGGCAACCTTGTGGATCTTATCGGCGCATCAAAAAAAGAACTTCAAAAAGTGGATATCGAAAGAATGAAAGTACCCTTATATCAAGATGAAGATCAATTGCTACCGTTGGAAATGGAAGACACATTAGATATAGATATCCAATCATCTAATCGTGACTTTGATAAGAATCTTACTTTTGAAAATCATGAAAAAACTTTTGTAACAAAACATACGCAAGCTACTAAATCGGAAGACCTTATGAATTCCGCTTACGCAATAGATTCCCATCCAAATTTTGAAAACATTGATTTCAATGGCAAAAACTTAGACATTGGGATAATAAAATTTGGTTTTGAAAAAGGCTACGAGCCAAAACAAAAAGGTAATCAATCGGATTTTGAAGCATACAAGGA

**Integration site of both constructs:**

Stock used for integration: #24862

https://bdsc.indiana.edu/Home/Search?presearch=24862

PBac{y[+]-attP-9A}VK00005

**Associated Genes:** [Eip75B](http://flybase.org/reports/FBgn0000568.html) (insert in)
**Comments:** 3rd chromosome attP docking site for phiC31 integrase-mediated transformation.
**Map:** Chr 3, 75A10, 3L:17952108..17952108.