Fernandes et al., http://www.jcb.org/cgi/content/full/jcb.201406026/DC1

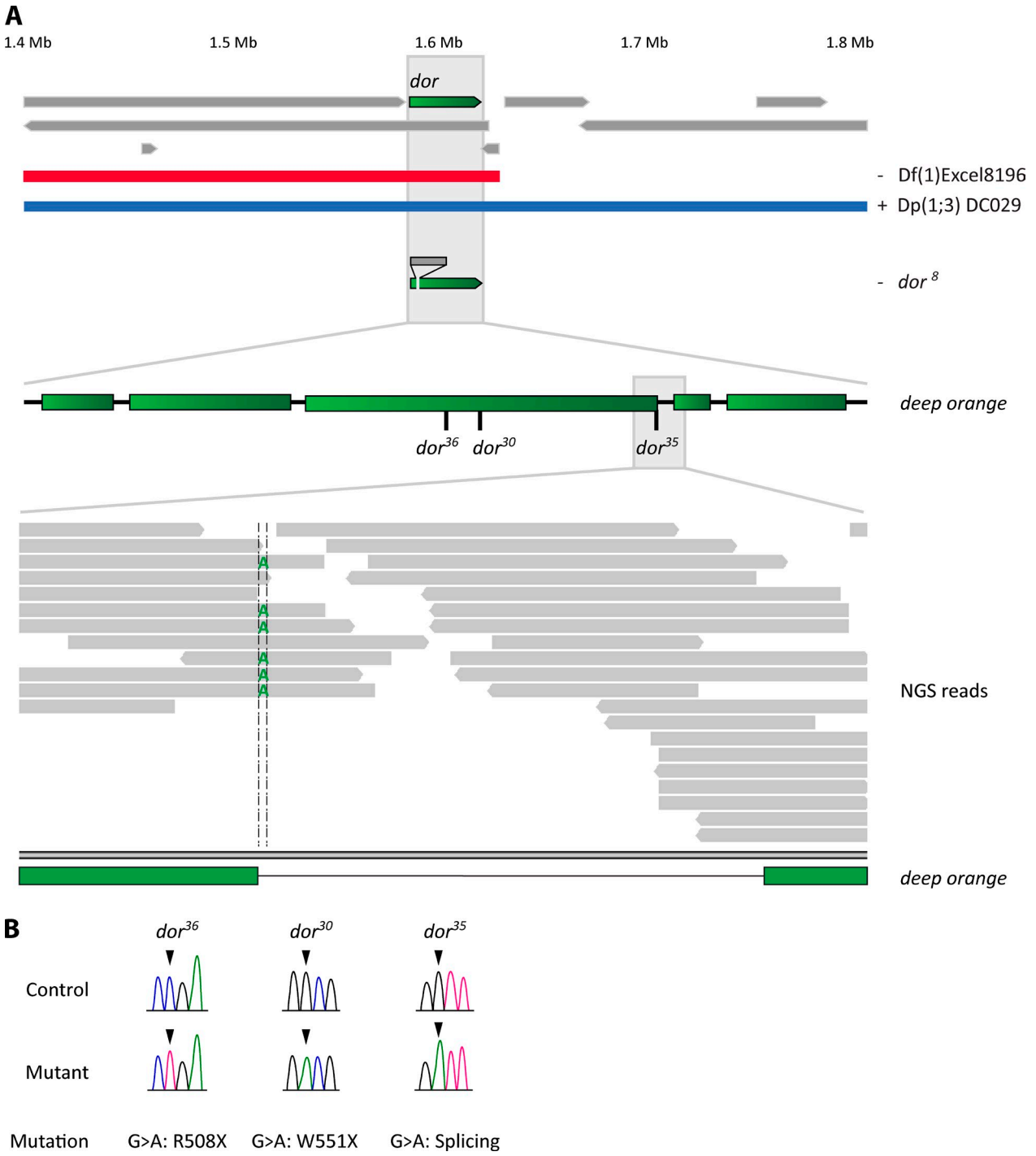

Figure S1. **Identification of *dor* EMS-induced lesions.** (A) Schematic representation of the genomic region around the *dor* locus (green) and complementation mapping with deficiency and duplications. We isolated a duplication Dp(1;3)DC029 (blue) that rescues the lethality associated with dor^{36}, dor^{35}, and dor^{30} and a deficiency Df(1)Exel8196 (red) and lethal allele dor^{8} that fails to complement the alleles. Highlight of the whole genome sequencing (next generation sequencing [NGS]) reads of $dor^{35/+}$ identifying a splice donor mutation in *CG3093/dor*. (B) G to A mutations identified by Sanger sequence in all *dor* alleles. Arrowheads indicate the identified EMS-induced mutation. R and W refer to the amino acids Arginine and Tryptophan, respectively, whereas X indicates a stop codon.

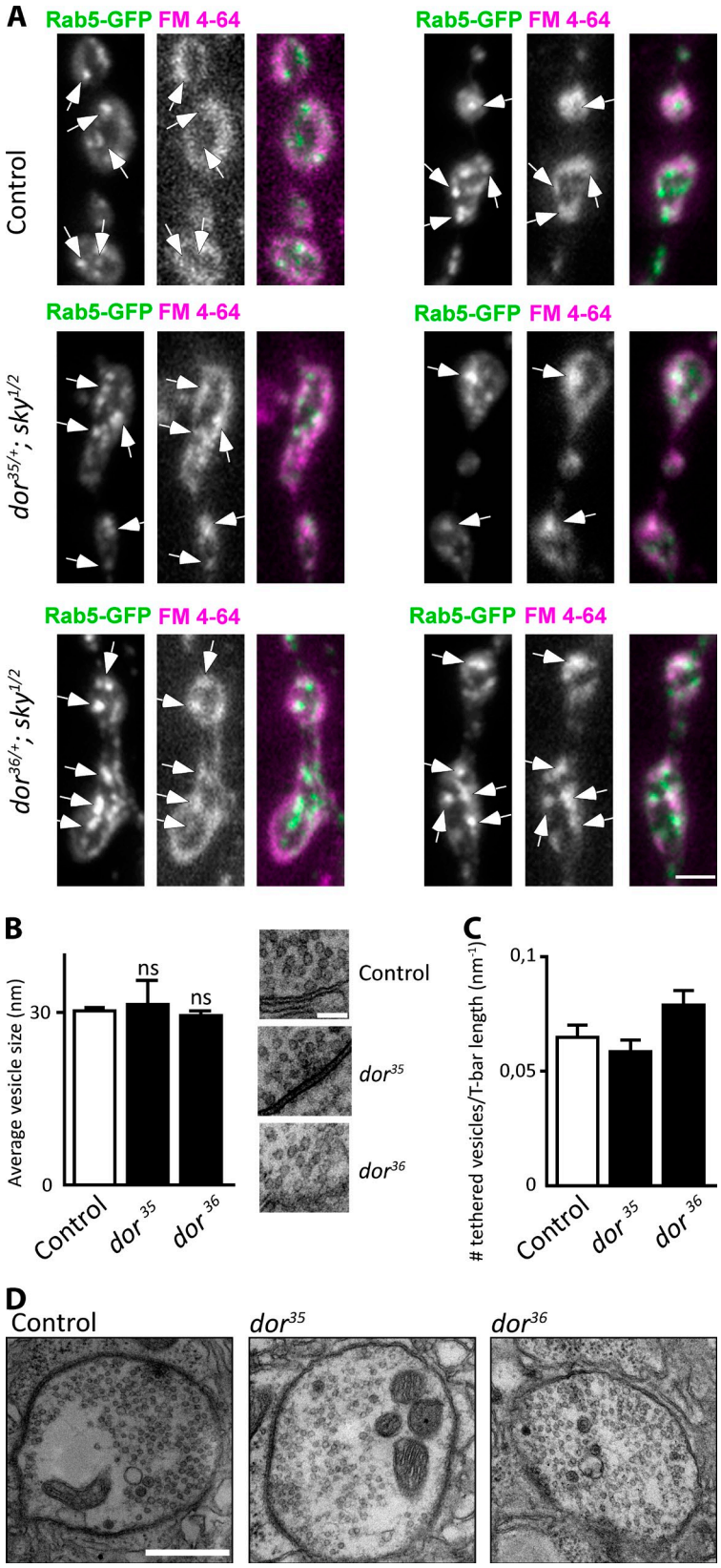

Figure S2. **Cellular and synaptic analyses of *dor* mutants.** (A) Two different examples (left and right) of imaging of control (*UAS-Rab5-GFP* and *vGlutGAL4*) and $dor^{35\text{ or }36/+}$; $sky^{1/2}$ mutants that express Rab5-GFP (*vGlutGAL4*) and were stimulated in the presence of FM 4–64 for 5 min in 90 mM KCl. Although the FM 4–64 in controls distributes in the typical doughnut-like pattern, in the mutants, the dye concentrates in blebs. Arrows show the location of Rab5-GFP–positive endosomes, many of which colocalize with the FM 4–64 accumulations in the double mutants. Bar, 2 µm. (B–D) TEM micrographs of synaptic vesicles (B) and bouton overviews (D) and quantification of the mean vesicle size (B) and tethered number of vesicles at T bars (C) in FRT19A controls and dor^{35} and dor^{36} mutants (n = 8–22). Bars: (A and D) 500 nm; (B) 100 nm. Error bars: SEM. ANOVA (Dunnett's test): ns, not significant.

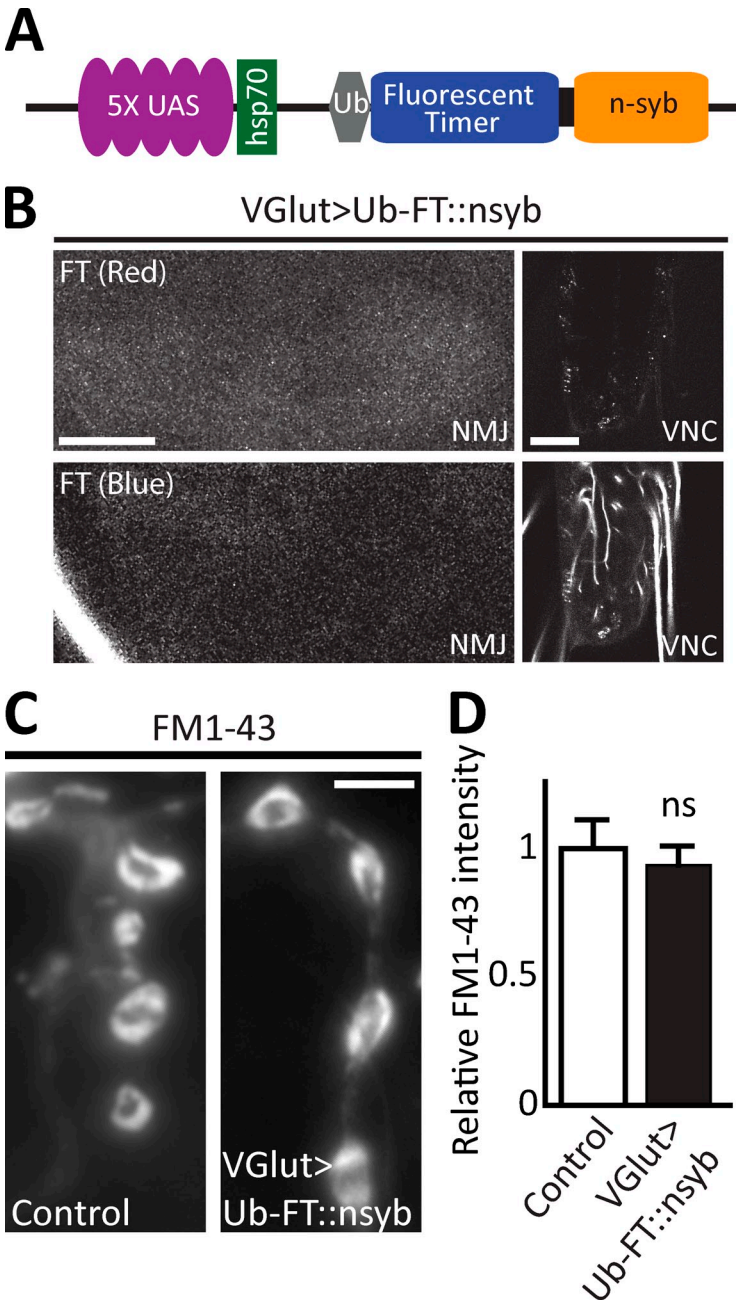

Figure S3. **Characterization of Ub-nSyb-FT.** (A) Schematic of the construct to express a chimeric FT fused to an ubiquitin (Ub) moiety and nSyb (Ub-FT::nSyb). (B) Images of animals expressing the Ub-FT::nSyb using *vGlutGAL4* showing labeling of the red and blue forms of the timer at the larval NMJ and the ventral nerve cord (VNC). Note the absence of the red and blue forms at boutons and the weak labeling at the VNC. (C and D) Images of FM 1–43 labeling at boutons (C) and quantification of labeling intensity (D) in controls and in animals expressing the Ub-FT::nSyb using *vGlut-GAL4* (n = 19). Bars: (B [NMJ] and C) 5 µm; (B, VNC) 50 µm. Error bars: SEM. *t* test.

Table S1. **List of primers used in this study**

Primer name	Primer sequence
#1-NotI-Ub_F	5′-GCTAGCGGCCGCCAAAATGCAGATCTTCGTGAAAACCC-3′
#2-Ub-Link-FT_R	5′-CCTCGCCCTTGCTCACGCGGGACTGCTGACGCAGGCGGAGCACCAAGTGC-3′
#3-Ub-link-FT_F	5′-CTTGGTGCTCCGCCTGCGTCAGCAGTCCCGCGTGAGCAAGGGCGAGGAGG-3′
#4-FT_GSlink_R	5′-TGAACCTGAACCAGAACCTGAACCAGATCCCTTGTACAGCTCGTCCATGC-3′
#5-Gslink-nSyb_F	5′-GGATCTGGTTCAGGTTCTGGTTCAGGTTCAGCGGACGCTGCACCAGCTGG-3′
#6-KpnI-nSyb_R	5′-GCTAGGTACCTTACACGCCGCCGTGATCGCCAGCTCC-3′
#7-NotI-FT_F	5′-GCTAGCGGCCGCCAAAATGGTGAGCAAGGGCGAGGAGGATAAC-3′
#2a-Ub-link-MediumFT_R	5′-CCTCGCCCTTGCTTACGCGGGACTGCTGACGCAGGCGGAGCACCAAGTGC-3′
#3a-Ub-link-MediumFT_F	5′-CTTGGTGCTCCGCCTGCGTCAGCAGTCCCGCGTAAGCAAGGGCGAGGAGG-3′
#7a-NotI-MediumFT_F	5′-GCTAGCGGCCGCCAAAATGGTAAGCAAGGGCGAGGAGGATAAC-3′
Check-lig-FT's_F	5′-CTAGTCAAGGCACTATACATC-3′
Check-lig-FT's_R	5′-CGCCACAGTACCAGTATCC-3′
Seq_insFT_1_F	5′-GCTTGGATTTCACTGGAACTAG-3′
Seq_insFT_1_R	5′-GAATGCCCTCCTTATCCTG-3′
Seq_insFT_2_F	5′-CAAGACCATCACCTTGGAG-3′
Seq_insFT_2_R	5′-CGCAGCTTCACCTTGTAG-3′
Seq_insFT_3_F	5′-GCGTGATGAACTTCGAGGAC-3′
Seq_insFT_3_R	5′-TCCACGACCTCATCGACC-3′
Seq_insFT_4_F	5′-ACACAATCCGCAGCAGATC-3′
Seq_insFT_4_R	5′-TCATCAGTTCCATAGGTTGG-3′
Vps39-F	5′-AACTGGCTATTGAGCTGACG-3′
Vps39-R	5′-TATCTGGCGAATGGTTTGGG-3′
Rab7-F	5′-CGAAAGAGGTGGTGGTCAAC-3′
Rab7-R	5′-TAAAACGCCACTCCAAGCG-3′
RP49-F	5′-ATCGGTTACGGATCGAACAA-3′
RP49-R	5′-GACAATCTCCTTGCGCTTCT-3′

F, forward; R, reverse.