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An isoform-specific allele of the *sax-7* locus

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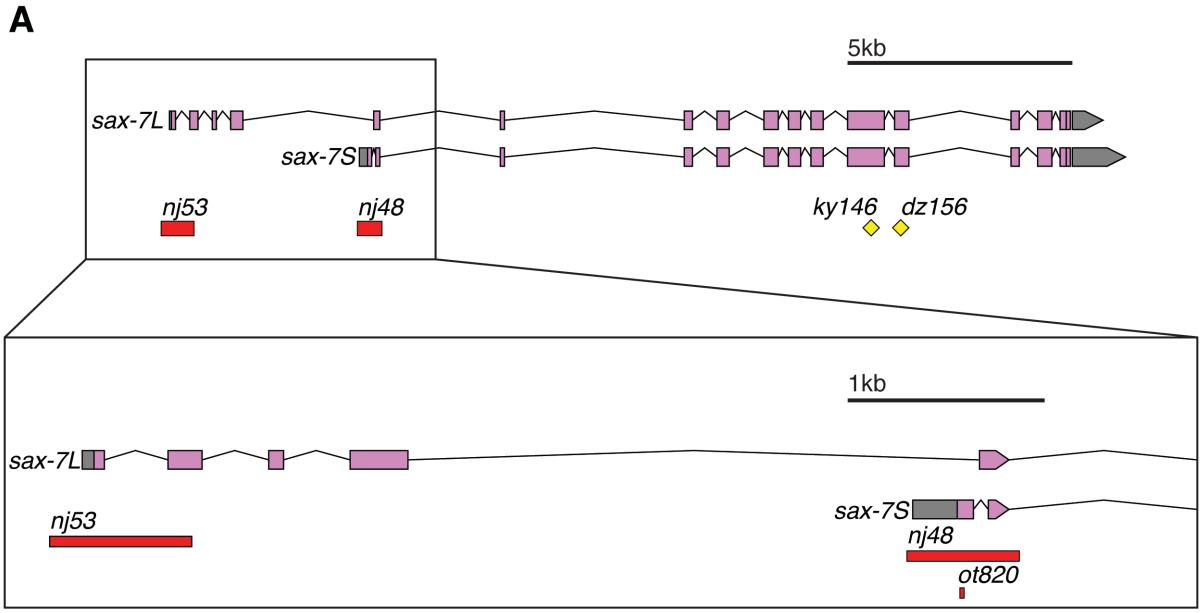

Figure 1 *ot820* is a novel isoform-specific allele of the *sax-7* locus. A. *sax-7* locus encodes two isoforms of an L1CAM homolog. Existing null alleles affect either the long isoform (*nj53*) or both isoforms (*nj48, ky146,* and *dz156*). *ot820* is a short deletion mutant that affects only the short isoform. **B.** sgRNA targets the beginning of the first exon of *sax-7S*, and *ot820* is a 8bp deletion 15bp from the start codon.

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Description

The immunoglobulin superfamily member *sax-7* produces a long and short isoform that appear to have distinct functions (Chen *et al.* 2001; Wang *et al.* 2005; Sasakura *et al.* 2005; Pocock *et al.* 2008). An isoform-specific allele for the long isoform, *sax-7L*, was previously reported (Sasakura *et al.* 2005), but an isoform-specific allele for the short isoform, *sax-7S*, has been lacking (Fig. 1A). We used CRISPR/Cas9 to generate such an allele, using the *unc-22* co-CRISPR method (Kim *et al.* 2014). The *ot820* allele was isolated using sgRNA targeted to the first exon of *sax-7S* (sgRNA sequece: 5' - TGGGGTTACGAGAGACGAT - 3'). Twitching progeny were screened by PCR and Sanger sequencing, and an 8bp deletion 15bp from the start codon was isolated (screening primers: 5' - GGTGCTTCTCTGGTGGTAGC - 3' and 5' - TGTTGGCAAACAAAATACACG - 3', Fig. 1B). While the *sax-7L* isoform is predicted to be entirely unaffected by this allele, the resultant frameshift is predicted to generate a 49 amino acid protein in which all but the first five amino acids of *sax-7S* are aberrant and has no predicted signal sequence, before terminating in a premature stop in the second exon (Fig. 1B). This allele therefore likely represents a null for the *sax-7S* isoform. After we generated this allele, a recent paper reported an additional *sax-7S*-specific allele, with somewhat similar, but not identical sequence properties (Chen et al., 2019). Consistent with previous evidence of distinct functions of *sax-7* isoforms, these authors showed differing axon fasciculation defects between these two alleles, which is further distinct from a total null allele. These new *sax-7S* alleles should help to reveal new insights into the role of L1CAM/SAX-7 isoforms in the nervous system.

Reagents

OH13830 *sax-7(ot820) IV; oyIs14 V*. Will be available at CGC.

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