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Supplemental Information

The *Drosophila* Small Conductance Calcium-Activated Potassium Channel Negatively Regulates Nociception

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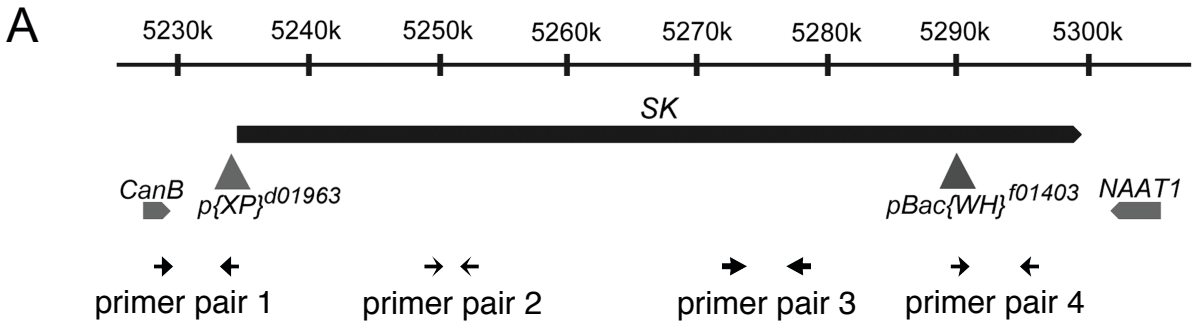

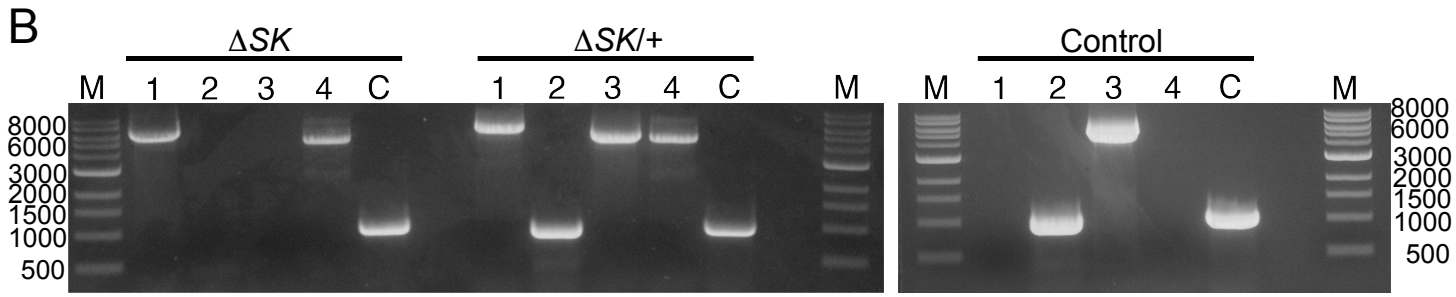

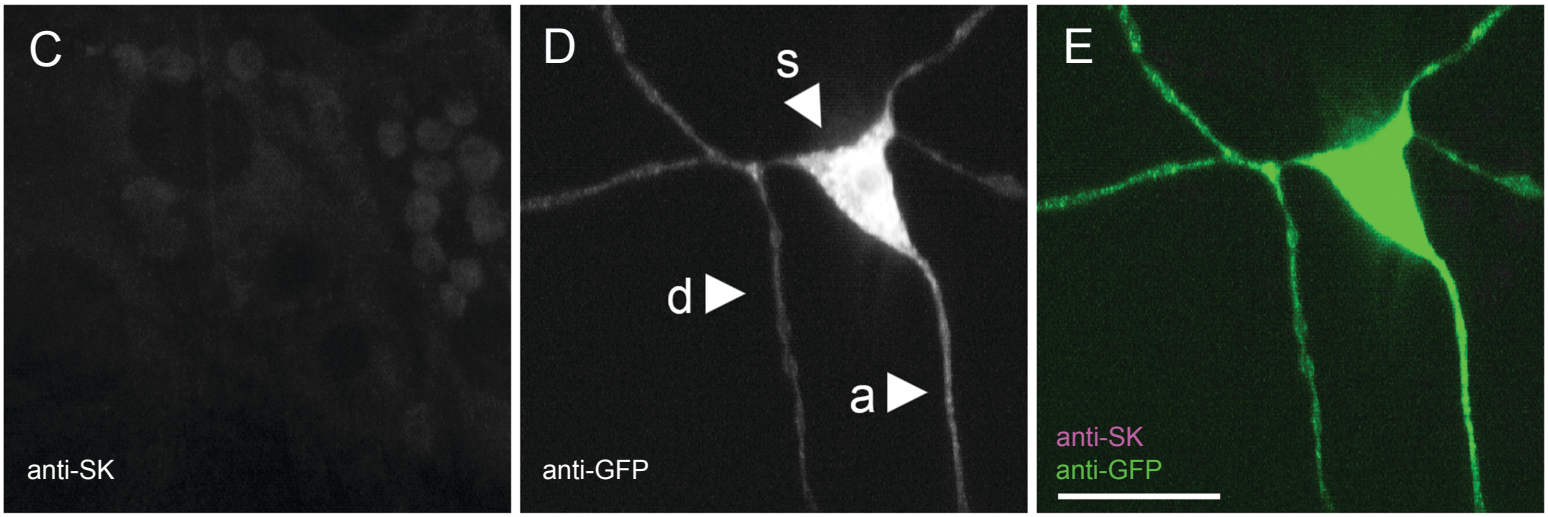

Figure S1. Confirmation of *SK* deletion by PCR and immunostainting. Related to Figure 2.
(A) Arrowheads indicate the *P{XP}*^{d01963} and *pBac{WH}SK*^{f01403} transposable elements used to generate the FLP-FRT mediated *SK* deletion (*ΔSK*). The arrows numbered 1 - 4 represent distinct primer pairs for PCR confirmation of the *SK* deletion (primer set 1: 7.6kb, primer set 2: 900bp, primer set 3: 5.3kb, primer set 4: 5.8kb). **(B)** Agarose gel electrophoresis of PCR amplified products using primer pairs 1 and 4 that flank the *SK* deletion, and *SK*-specific primer pairs 2 and 3. Lane M is the NEB 1 kb DNA ladder. The *ΔSK* transgenic animals show amplified products for primer set 1 and 4 and do not show a product for the *SK*-specific primer pairs set 2 and 3. Transgenic animals heterozygous for the *SK* deletion (*ΔSK*/+) show products for all primer pairs as they have one copy of the WT and mutant chromosome each. Control transgenic animals (isogenic *white*) do not show products for primer set 1 and 4; they do not contain the transposable elements in their genome. Control primers against *Caα 1D* (1kb) demonstrate integrity of the genomic DNA preps. All transgenic animals used for PCR analysis were virgin females (n>5). **(C-E)** Maximum intensity projection from z-stack confocal micrographs representative of the dorsal md neuron cluster (abdominal segment A4-A6) in larvae. Endogenous SK proteins are not at detectable levels in sensory neurons of *SK* null mutant larvae when immunostaining with anti-SK antibodies targeting the long isoform. SK protein expression (C and magenta in E) and class IV neuron (D and green in E) labeling. Scale bar is 10μm.

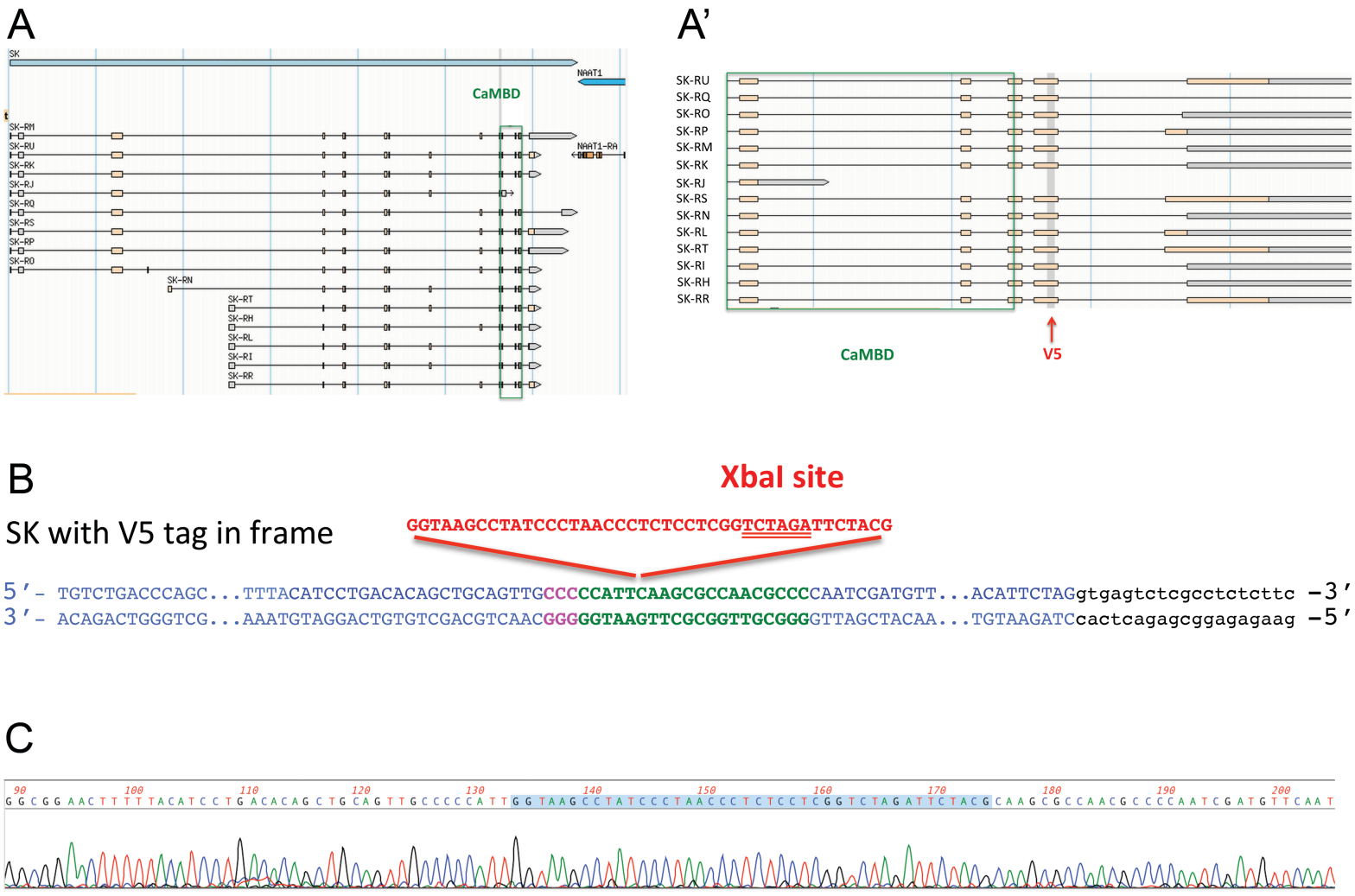

Figure S2. CRISPR engineered *V5* epitope at *SK* locus. Related to Figure 4.
(A) Image of *SK* gene structure and predicted/known *SK* transcripts taken from the Flybase Genome Browser version FB2017_04, released August 22, 2017 (Gramates et al., 2017). The exons encoding the calmodulin-binding domain (CaMBD) in 13 out of the 14 predicted *SK* transcripts are boxed in green. **(A')** Zoomed in view of the *SK* locus shows the in-frame *V5* epitope tag (red arrow) that was engineered downstream of the CaMBD using an ssODN template (shaded grey region) for CRISPR mediated homologous repair. Predicted transcripts are indicated and the *V5* tag was inserted within last common exon after the CaMBD. **(B)** Predicted sequence of the in frame *V5* tag (red sequence) contains an XbaI restriction site (double underlined red sequence) for PCR-RFLP analysis. Exon sequence in black, intron sequence in blue, gRNA sequence in green, and PAM site in magenta. **(C)** Sanger sequencing results show precise insertion of V5 tag. Sequences are from cloned cDNA (generated by reverse transcription and amplified via PCR) targeting *SK* transcripts that span the *V5* insertion and flank exon splice sites.

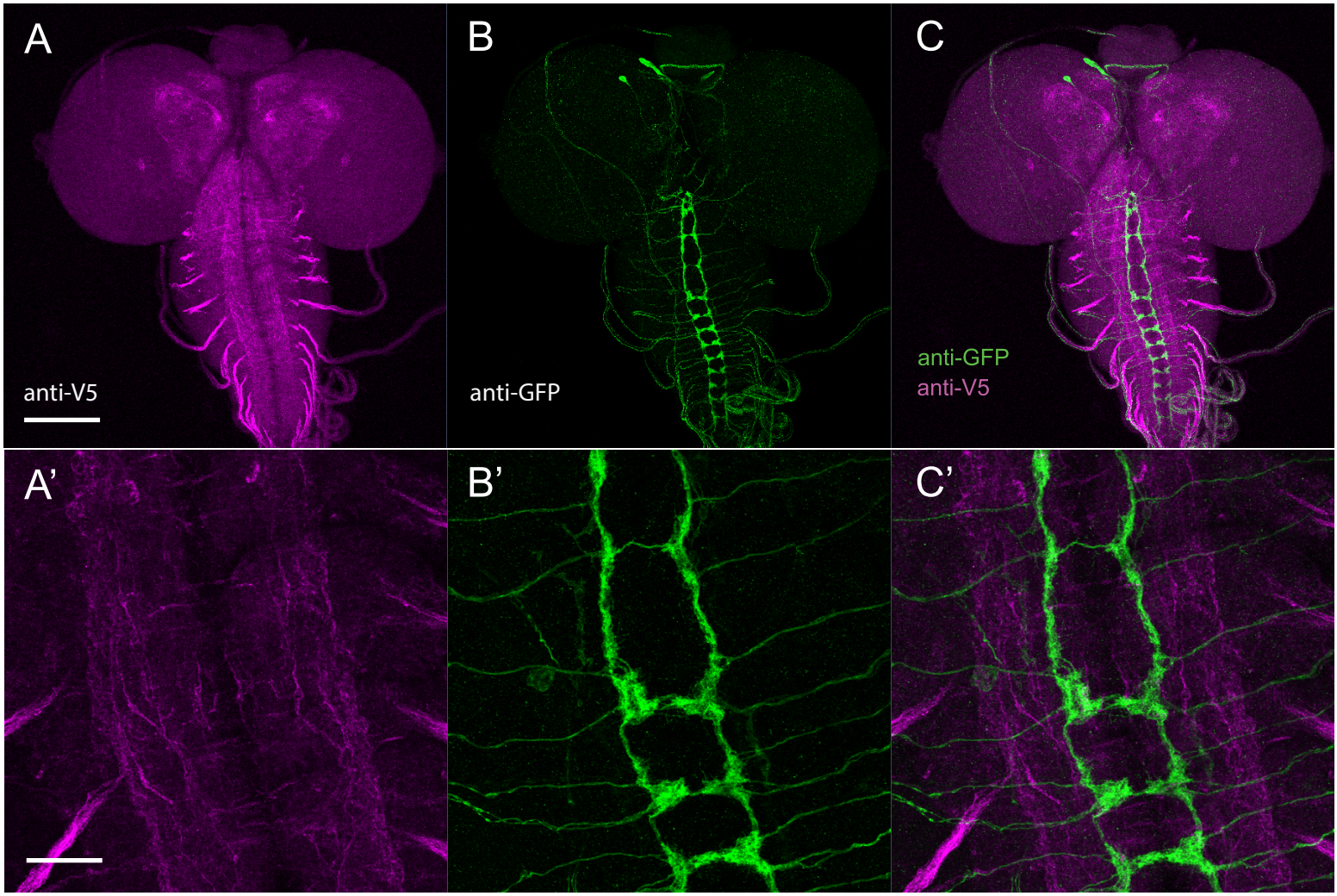

Figure S3. Immunostaining for SK::V5 proteins in larval brains. Related to Figure 4.
(A-C) Maximum intensity projection from z-stack images representative of larval brains. SK::V5 proteins do not localize to class IV axon terminals in the ventral nerve cord. SK::V5 protein expression (magenta in A,C) and Class IV projected terminals (green in B,C). Scale bars are 100μm. **(A'-C')** High magnification images of A, B, and C, respectively. Scale bars are 20μm.

Table S3. Oligonucleotide sequences. Related to STAR Methods

OLIGONUCLEOTIDE	SOURCE	ADDITIONAL INFORMATION
5'-GCAGTCGTGTATTTGCTGTCG-3'	IDT (This study)	Primer targeting *CanB* sequence flanking *SK* deletion (primer pair 1, Figure S1)
5'-TACTATTCCTTTCACTCGCACTTATTG-3'	IDT (This study)	Primer targeting residual *P{XP}* transposable element sequence flanking *SK* deletion (primer pair 1, Figure S1)
5'-ATGTCAATTCAGAAGCTTAACGACAC-3'	IDT (This study)	Primer targeting *SK* locus (primer pair 2, Figure S1)
5'-TGCAGTTGCTGCTGATGCAGAT-3'	IDT (This study)	Primer targeting *SK* locus (primer pair 2, Figure S1)
5'-TTATTCATGATAGATAACTGCGCTGACG-3'	IDT (This study)	Primer targeting *SK* locus (primer pair 3, Figure S1)
5'-CACCATGCCGCAGGTGAGGGTGATA-3'	IDT (This study)	Primer targeting *SK* locus (primer pair 3, Figure S1)
5'-CCTCGATATACAGACCGATAAAAC-3'	IDT (This study)	Primer targeting residual *pBac{WH}* transposable element sequence flanking *SK* deletion (primer pair 4, Figure S1)
5'-ATGCTGGTGCCCTACGATCTATGT-3'	IDT (This study)	Primer targeting remaining *SK* sequence flanking *SK* deletion (primer pair 4, Figure S1)
5'-CAACCGGATGTGAAGTGCG-3'	IDT (This study)	Primer targeting *Caα1D* sequence (control primer pair, Figure S1)
5'-CTTGGCACTTCGCCTGAAGG-3'	IDT (This study)	Primer targeting *Caα1D* sequence (control primer pair, Figure S1)
5'-ATGTCAATTCAGAAGCTTAACGAC-3'	IDT (This study)	Primer targeting *SK-M* mRNA isoform sequence for cDNA cloning (open reading frame)
5'-TCAGCTAGAATGTGGAAACAGCAT-3'	IDT (This study)	Primer targeting *SK-M* mRNA isoform sequence for cDNA cloning (open reading frame)

OLIGONUCLEOTIDE	SOURCE	ADDITIONAL INFORMATION
5'- ATGTCGCCGGCCTTCTGC-3'	IDT (This study)	Primer targeting *SK-V* mRNA isoform sequence for cDNA cloning (open reading frame)
5'-TCAGCTAGAATGTGGAAACAGCAT-3'	IDT (This study)	Primer targeting *SK-V* mRNA isoform sequence for cDNA cloning (open reading frame)
5'-CACCATGTCAATTCAGAAGCTTAACGAC-3'	IDT (This study)	Primer targeting *SK-M* mRNA isoform sequence for *pENTR* subcloning
5'-TCAGCTAGAATGTGGAAACAGCATG-3'	IDT (This study)	Primer targeting *SK-M* mRNA isoform sequence for *pENTR* subcloning
5'-CACCATGTCGCCGGCCTTCT-3'	IDT (This study)	Primer targeting *SK-V* mRNA isoform sequence for *pENTR* subcloning
5'-TCAGCTAGAATGTGGAAACAGCATGGGC-3'	IDT (This study)	Primer targeting *SK-V* mRNA isoform sequence for *pENTR* subcloning
5'-CCATTCAAGCGCCAACGCCC-3'	IDT (This study)	gRNA cloned into *pU6-BbsI-chiRNA* vector for CRISPR gene editing (*SK::V5* allele generation)
5'-GAGCGGATCGAGCAGCGGCGGAACTTTTTACATCCTGACACAGCTGCAGTTGCCCCCATTGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTAGATTCTACGCAAGCGCCAACGCCCCAATCGATGTTCAATGCAGCGCCCATGCTGTTTCCACATTCTAGG-3'	IDT (This study)	ssODN donor repair template for CRISPR gene editing (*SK::V5* allele generation)
5'-GAGCGTTTAACCAACCTAGAG-3'	IDT (This study)	Primer targeting *SK* locus for XbaI PCR-RFLP analysis (*SK::V5* allele)
5'-GCAGTTAGTGTTCGTCCAAAG-3'	IDT (This study)	Primer targeting *SK* locus for XbaI PCR-RFLP analysis (*SK::V5* allele)