SUPPLEMENTAL MATERIALS

Supplemental Figure 1

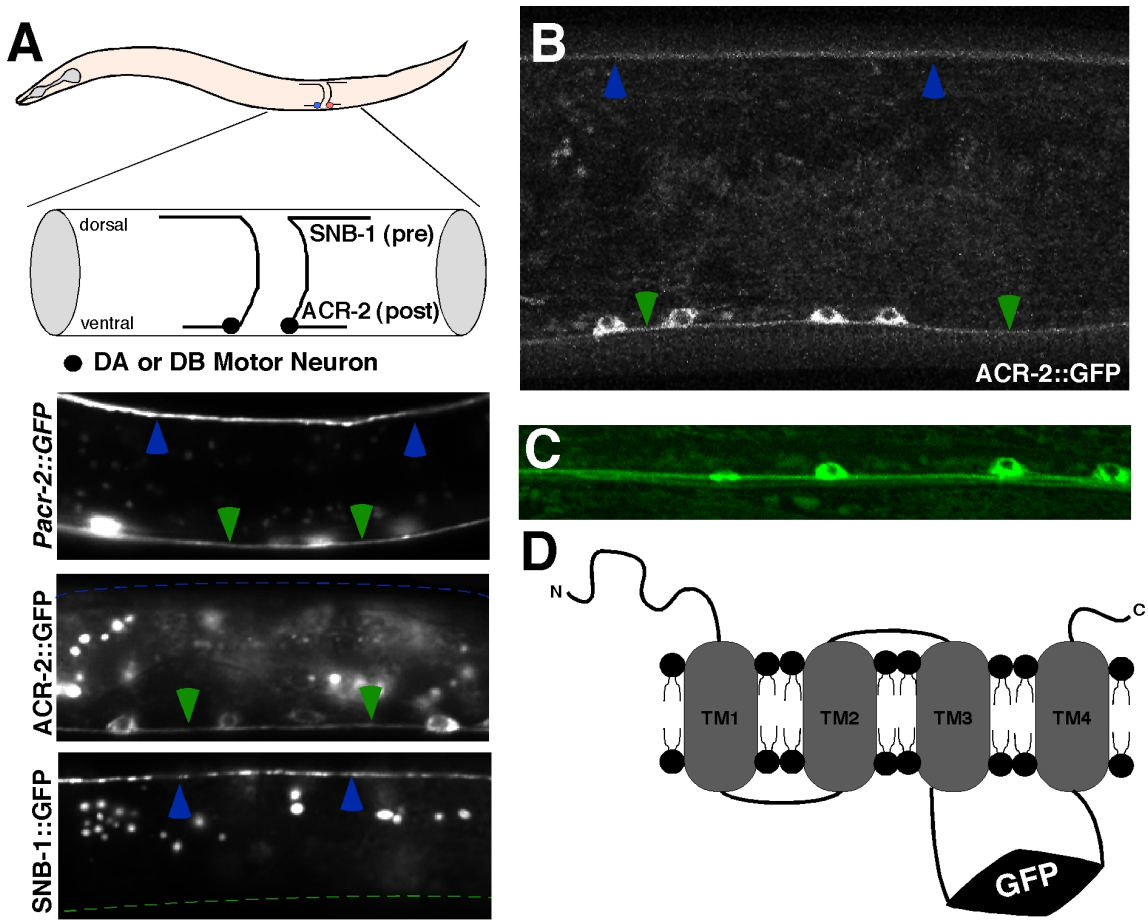

Figure S1. Expression of the non-alpha nAChR subunit ACR-2.

(**A**) Schematic depicting morphology of cholinergic DA and DB motor neurons in an L1 animal. Wide-field epifluorescent projection images of the ventral (green) and dorsal (blue) nerve cords in L1 animals expressing integrated arrays containing either the transcriptional reporter *Pacr-2::GFP* (*ufIs49*, top), full-length *Pacr-2::*ACR-2::GFP (*ufIs42*, middle) or a presynaptic marker (*Punc-4::SNB-1::GFP*, lower). *Pacr-2::GFP* fluorescence is present in both ventral and dorsal nerve cords. Expression of the synaptic vesicle marker synaptobrevin (SNB-1::GFP) is limited to axons of the dorsal nerve cord. ACR-2::GFP fluorescence is limited to dendrites in the ventral nerve cord. (**B**) Projection

of a confocal stack showing the dorsal and ventral nerve cords of an adult animal expressing full-length ACR-2::GFP. (**C**) Confocal image of ventral nerve cord showing diffuse ACR-2::GFP fluorescence. (**D**) Schematic of ACR-2 membrane topology and site of GFP insertion.

Supplemental Figure 2

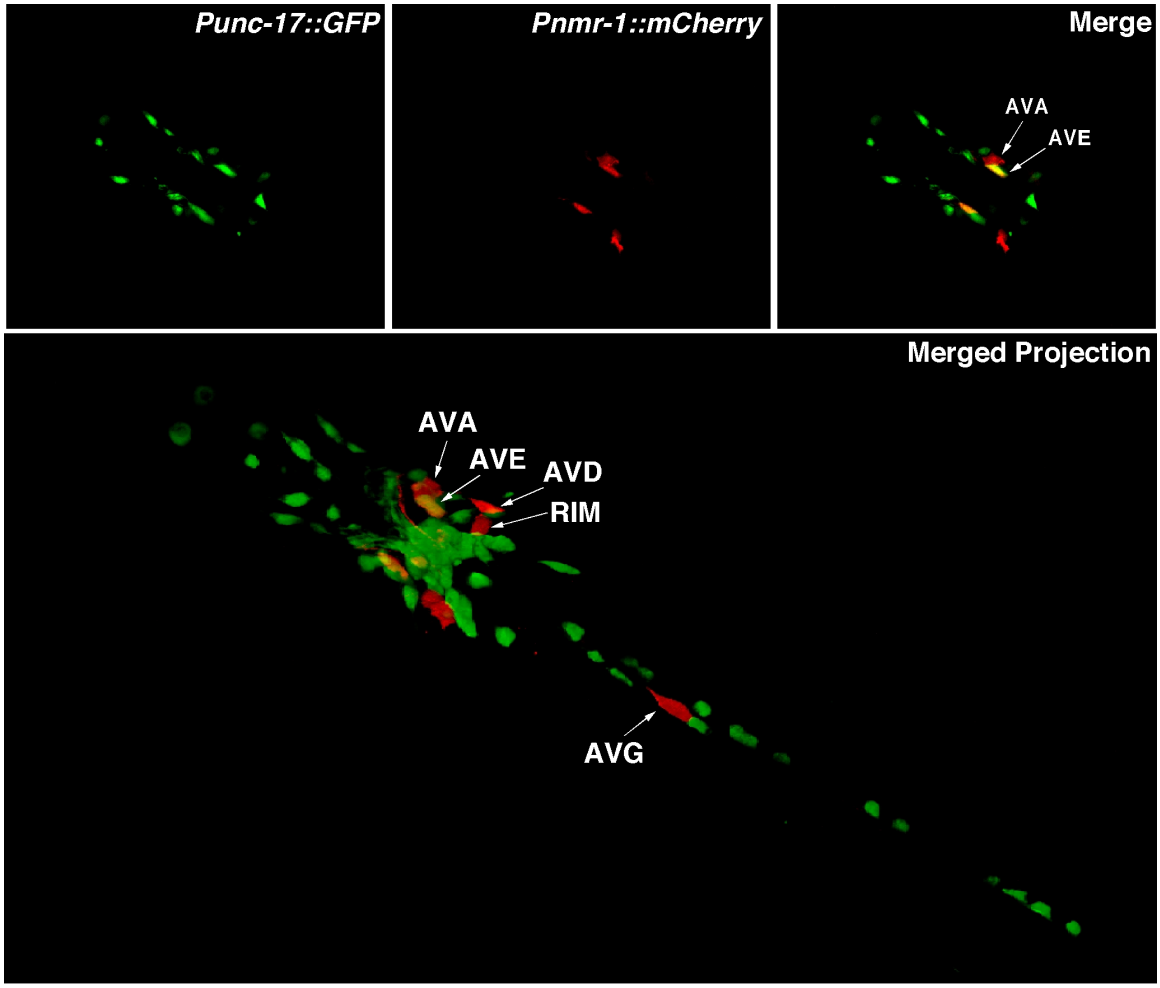

Figure S2. *C. elegans* command interneurons do not express *Punc-17::GFP*. (**A-C**) Single confocal images of an animal co-expressing an integrated array (*vsIs48*) containing the cholinergic neuron marker *Punc-17::GFP* together with an

extrachromosomal array (*ufEx158*) containing *Pnmr-1::mCherry*. (**D**) A confocal projection showing the relative positions of neuronal cell bodies expressing *Punc-17::GFP* and *Pnmr-1::mCherry* in the head of the worm. Neurons expressing mCherry are indicated. Only a single AVA neuron expresses mCherry due to mosaic expression of the array.

Supplemental Figure 3

Figure S3. Sequence features of ACR-2.

(A) Amino acid sequence of ACR-2. Predicted signal sequence (dashed box), transmembrane domains (underline), cys-loop (gray), gain-of-function mutation (box),

and site of GFP insertion (asterisks) are indicated. **(B)** Alignment of the second transmembrane domain of ACR-2 and various mammalian non-alpha subunits. The site of the 9' leucine to serine gain-of-function mutation is indicated (box).

Supplemental Figure 4

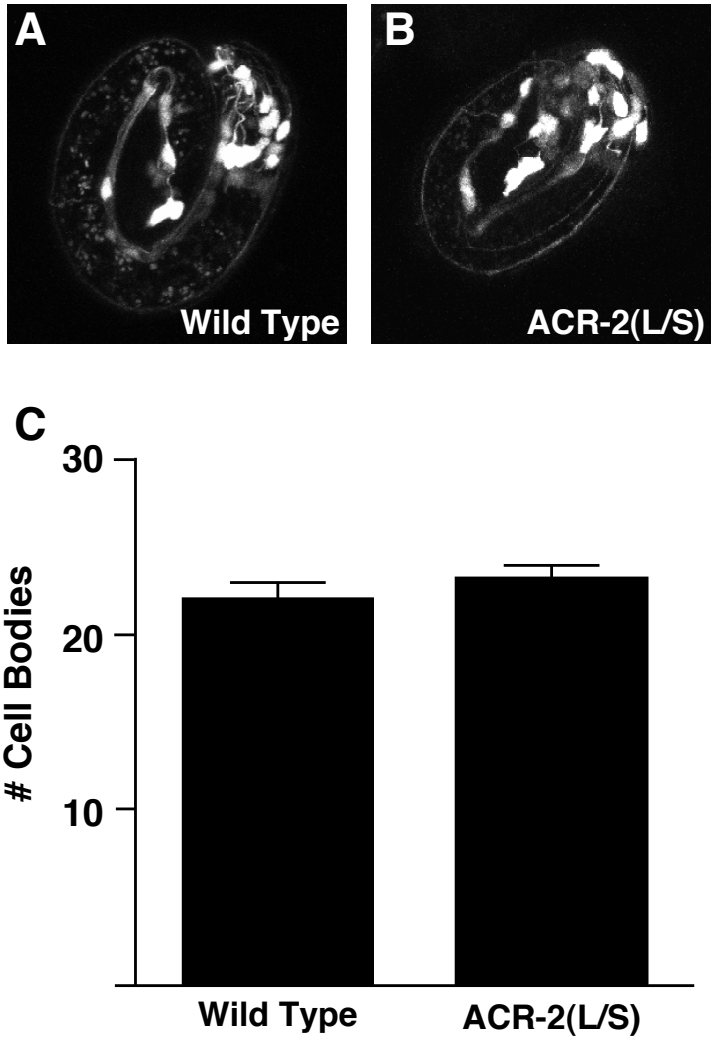

Figure S4. Wild type and ACR-2(L/S) animals have the same number of *acr-2* expressing cells at the 3-fold embryo stage.

(A and B) Confocal images of wild type (A) and ACR-2(L/S) (B) 3-fold embryos expressing an integrated array containing *Pacr-2::GFP (ufIs49)*. Images represent Z-projections of 23 slices (0.5 µm/slice). **(C)** Quantification of the average number of *Pacr-2::GFP* labeled cell bodies present in wild type and ACR-2(L/S) 3-fold embryos. Data represents the mean ± SEM of at least ten animals per genotype.

Supplemental Figure 5

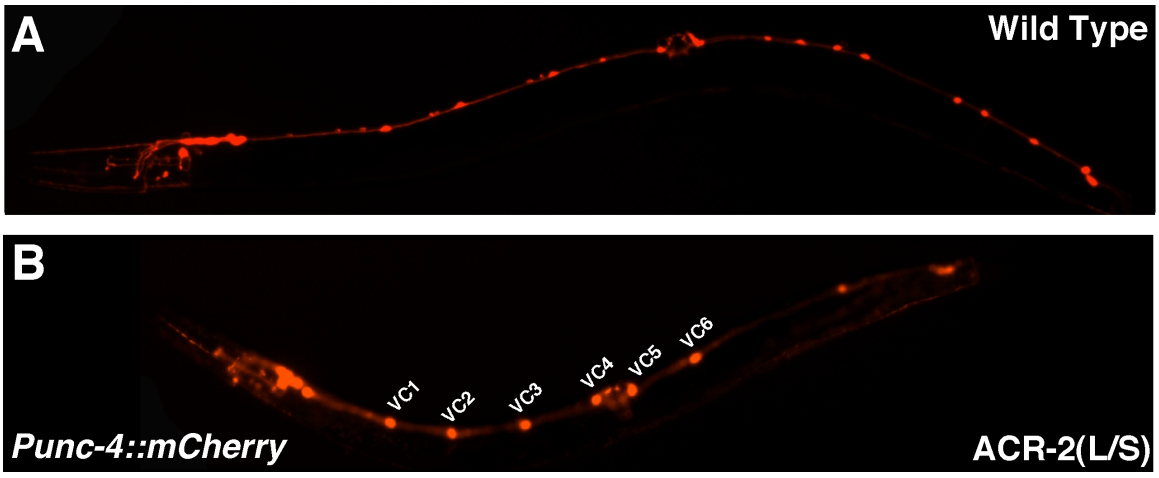

Figure S5. The VC neurons remain present in transgenic ACR-2(L/S) animals. Confocal images of wild type (A) and ACR-2(L/S) (B) animals expressing an integrated array (*ufIs26*) that contains *Punc-4::mCherry*.

Supplemental Figure 6

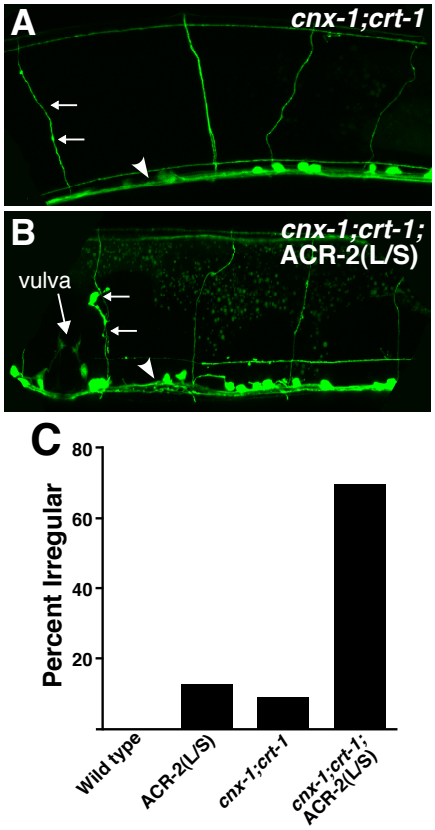

Figure S6. Motor neuron processes are irregular in *cnx-1;crt-1* double mutants expressing transgenic ACR-2(L/S).

(**A and B**) Confocal images of commissural processes and ventral nerve cord processes in *cnx-1;crt-1* double mutants (A) and *cnx-1;crt-1* double mutants expressing the ACR-2(L/S) transgene (B). Arrowheads show normally fasciculated ventral nerve cord in *cnx-1;crt-1* double mutants (A) and defasciculation in *cnx-1;crt-1;*ACR-2(L/S) animals (B). Arrows show normal commissural process in (A) and ectopic sprouting in (B). In each case, a region immediately posterior of the vulva was imaged and the ventral nerve cord is positioned at the bottom. Images show Z-projections of 34 confocal planes (A) or 33 confocal planes (B) (0.5 μm/slice). For all fluorescent imaging, animals are expressing an integrated *Punc-17::GFP* transgene (*vsIs48*). (**C**) Quantification of the percentage of

irregular commissural processes in wild type (15 commissures from 5 animals), transgenic ACR-2(L/S) (39 commissures from 10 animals), *cnx-1;crt-1* (75 commissures from 19 animals) and *cnx-1;crt-1*;ACR-2(L/S) (93 commissures from 17 animals). Since *Punc-17::*GFP also labels AS motor neurons that project commissural axons and do not express *acr-2*, the percentage of axonal defects in ACR-2(L/S) expressing neurons is likely higher.

Supplemental Table 1. Suppressors of ACR-2(L/S) induced paralysis isolated from a forward genetic screen.

Allele[†]	**% Paralyzed*** (levamisole)	**Gene**
Wild type	90±3	
e264	3±3	*unc-38*
uf62	0	*unc-38*
uf67	0	*unc-38*
uf153	0	*unc-38*
E306	0	*unc-50*
uf86	3±3	*unc-50*
e883	0	*unc-74*
uf124	0	*unc-74*
uf72	5±5	*unc-74*
ok1075	3±2	*unc-63*
uf79	5±5	*unc-63*
ok367	94±4	*acr-12*
uf65	95±5	*acr-12*
uf65	75±12	*acr-12*
uf65	85±5	*acr-12*
uf65	70±10	*acr-12*
uf65	100	*acr-12*
uf66	90±4	*acr-12*
uf66	98±3	*acr-12*
uf66	85±5	*acr-12*
uf77	95±5	*acr-12*
uf77	45±5	*acr-12*
uf77	93±5	*acr-12*
uf77	95±5	*acr-12*
uf77	75±5	*acr-12*
uf77	95±5	*acr-12*
uf94	78±6	*acr-12*
uf94	80±10	*acr-12*

uf95	100	*acr-12*
uf95	85±5	*acr-12*
uf95	100	*acr-12*
uf117	100	*acr-12*
uf117	85±5	*acr-12*
uf117	90±10	*acr-12*

Gray shading denotes available alleles used as reference

*denotes percentage of animals paralyzed after 120 minutes in the presence of 200 μM levamisole

[†]Alleles listed multiple times indicated duplicate isolates of the same allele. Forty-four additional levamisole-resistant mutants (<20% paralyzed) were isolated but not characterized further.