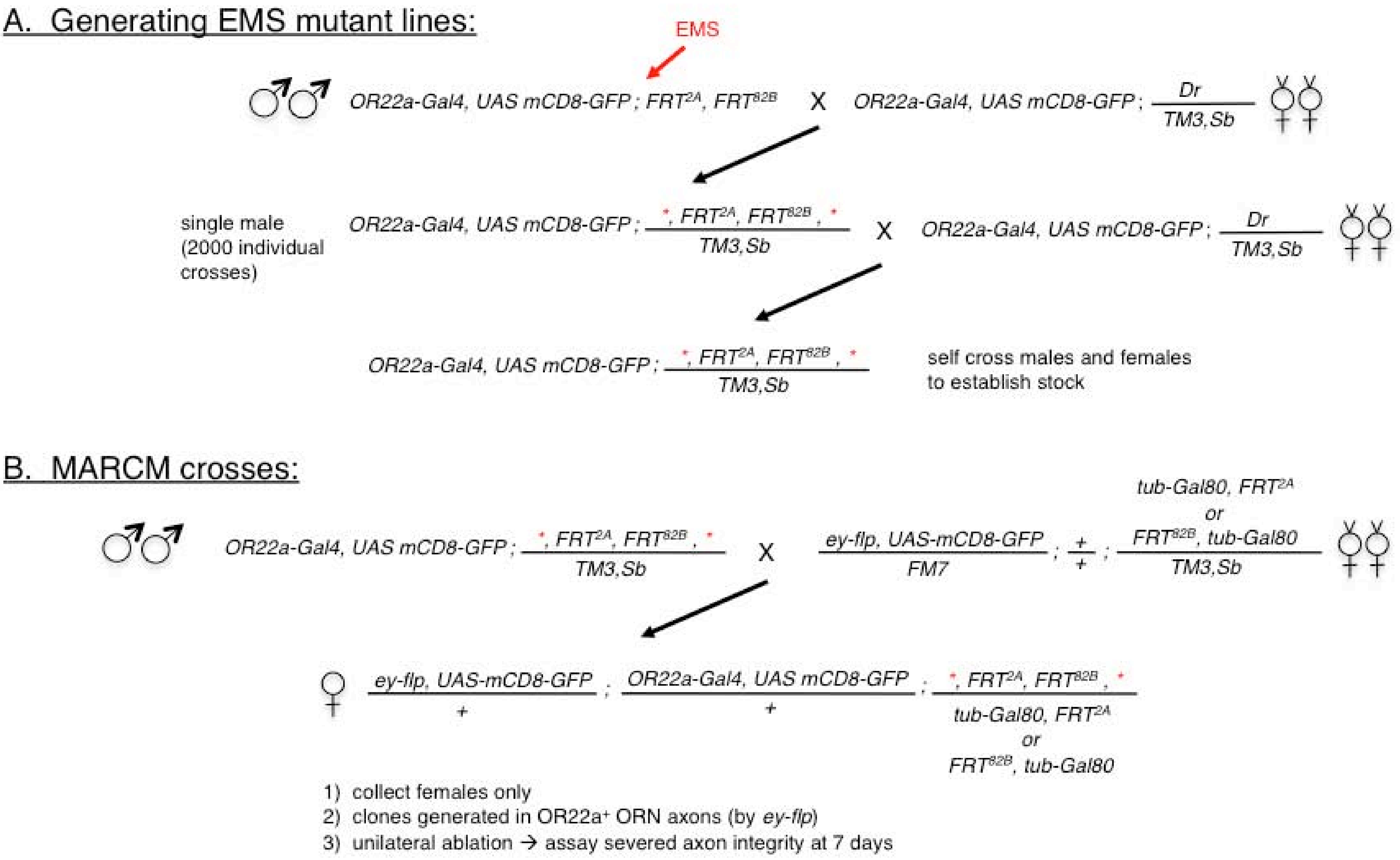


A. Generating EMS mutant lines:

EMS

♂♂ *OR22a-Gal4, UAS mCD8-GFP ;* FRT^{2A}, FRT^{82B} X *OR22a-Gal4, UAS mCD8-GFP ;* $\frac{Dr}{TM3,Sb}$ ♀♀

single male
(2000 individual
crosses)

OR22a-Gal4, UAS mCD8-GFP ; $\frac{*,\ FRT^{2A},\ FRT^{82B},\ *}{TM3,Sb}$ X *OR22a-Gal4, UAS mCD8-GFP ;* $\frac{Dr}{TM3,Sb}$ ♀♀

OR22a-Gal4, UAS mCD8-GFP ; $\frac{*,\ FRT^{2A},\ FRT^{82B},\ *}{TM3,Sb}$

self cross males and females
to establish stock

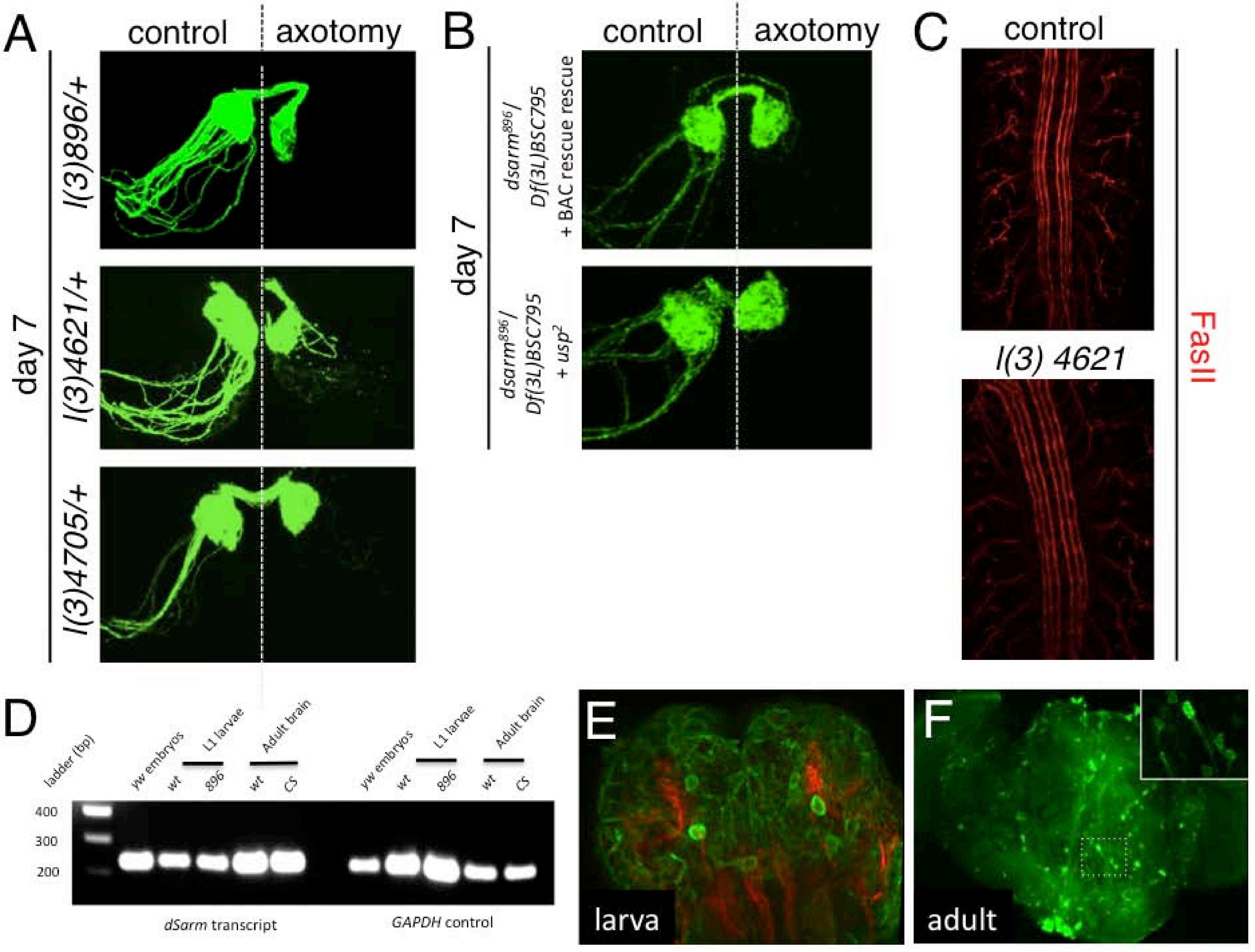
B. MARCM crosses:

♂♂ *OR22a-Gal4, UAS mCD8-GFP ;* $\frac{*,\ FRT^{2A},\ FRT^{82B},\ *}{TM3,Sb}$ X $\frac{ey\text{-}flp,\ UAS\text{-}mCD8\text{-}GFP}{FM7}$; $\frac{+}{+}$; $\frac{tub\text{-}Gal80,\ FRT^{2A}\ \text{or}\ FRT^{82B},\ tub\text{-}Gal80}{TM3,Sb}$ ♀♀

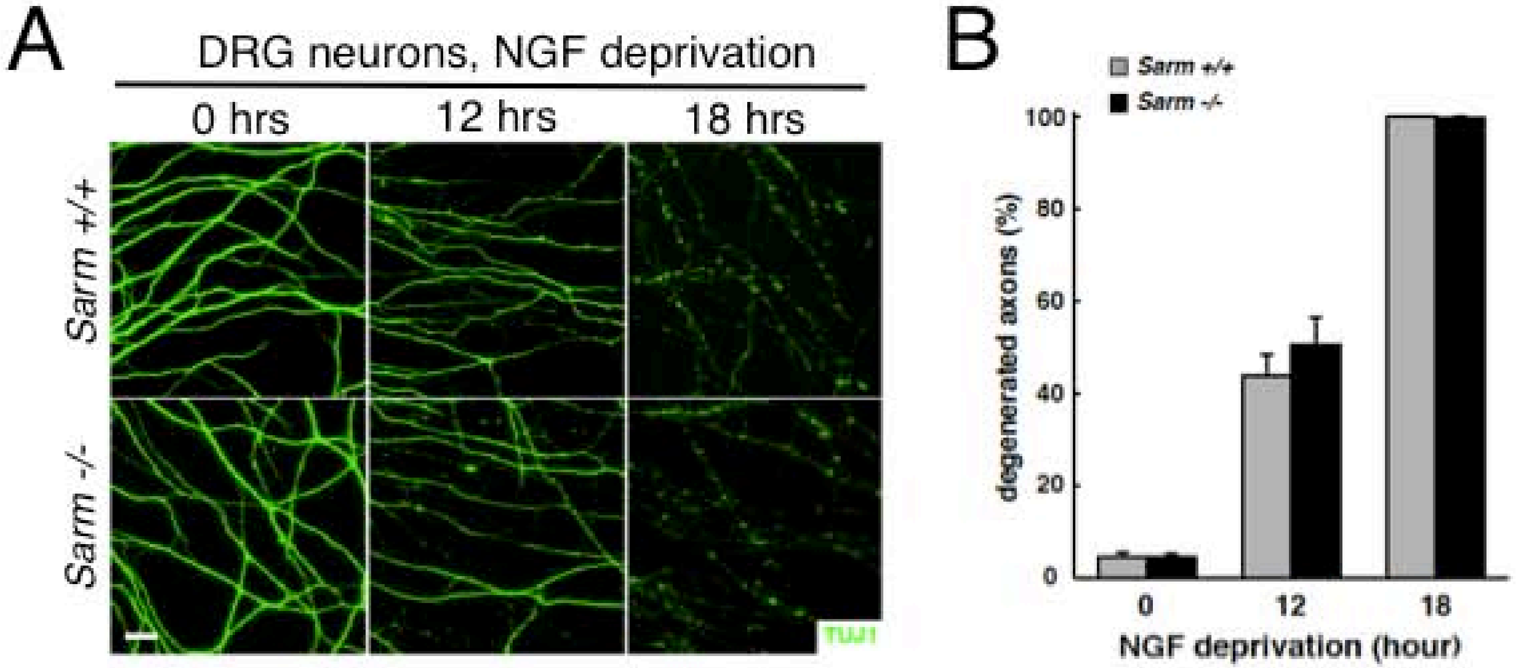
♀ $\frac{ey\text{-}flp,\ UAS\text{-}mCD8\text{-}GFP}{+}$; $\frac{OR22a\text{-}Gal4,\ UAS\ mCD8\text{-}GFP}{+}$; $\frac{*,\ FRT^{2A},\ FRT^{82B},\ *}{tub\text{-}Gal80,\ FRT^{2A}\ \text{or}\ FRT^{82B},\ tub\text{-}Gal80}$

1) collect females only
2) clones generated in OR22a^{+} ORN axons (by *ey-flp*)
3) unilateral ablation → assay severed axon integrity at 7 days

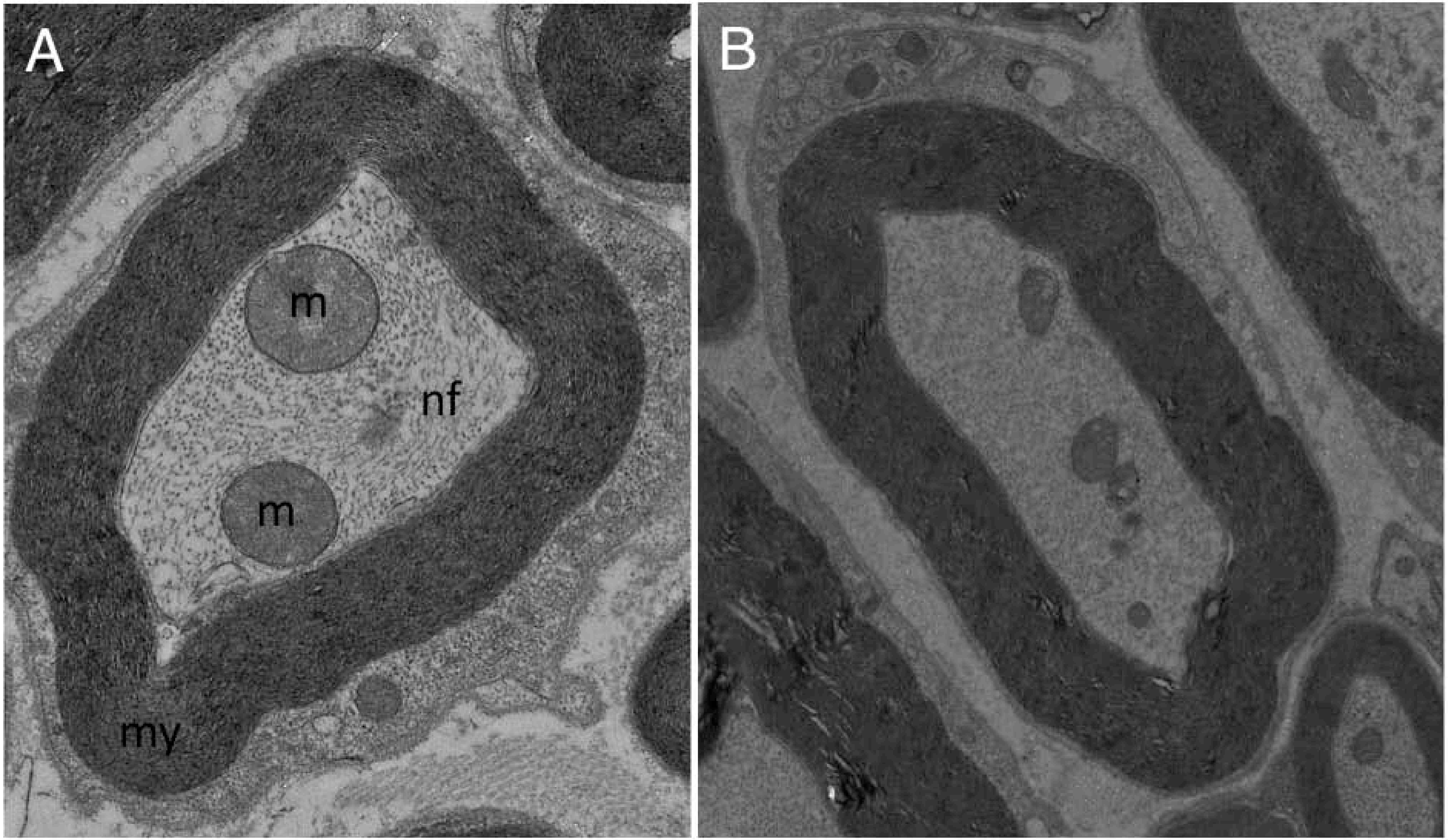
Supplementary Figure 1



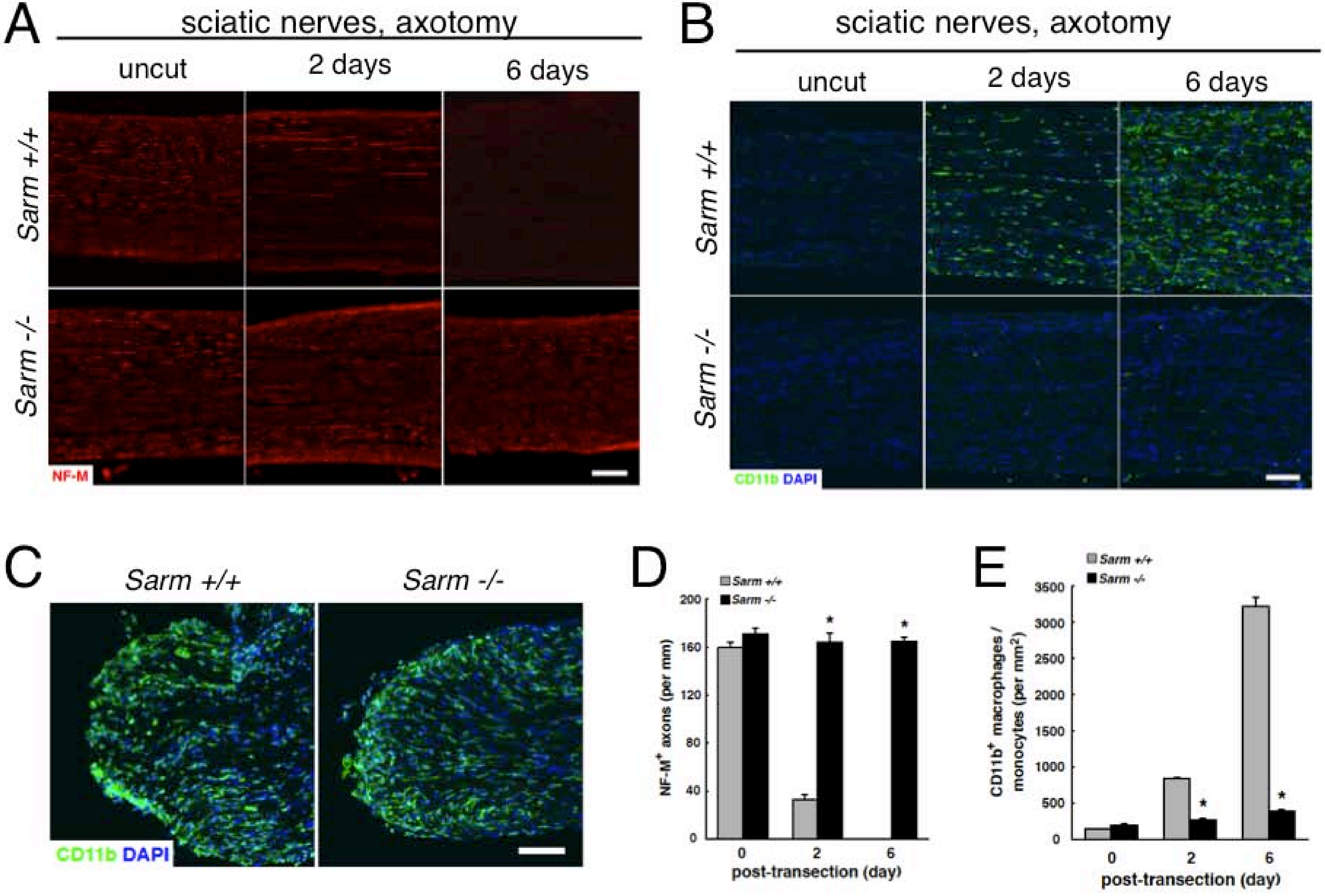
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Supplementary Table 1: Mutants tested that fail to suppress Wallerian Degeneration

Gene	Mutant Allele	Allele Type
ASK1	DN	OE
Atg1	Δ3D	LOF
Atg1	KQ #5B DN	OE
Atg1	68 DN	OE
Atg1	EP3009	LOF
Atg1	KG03098	LOF
Atg1	EP3348	LOF
Atg2	EP3697	LOF
Atg6	00096	LOF
Atg7	d77	LOF
Atg7	d14	LOF
Atg18	KG03098	LOF
Bsk/Jnk	DN	OE
buffy	H37	LOF
cullin-3	mds1	LOF
cullin-3	UAS	OE
cyt-c d	bln1	LOF
damm	f02209	LOF
dark	CD4	hypomorph
dark	CD8	hypomorph
debcl	E36	LOF
debcl	W105	hypomorph
dIAP/thread	th1	LOF
dIAP/thread	FL	OE
dIAP2	G2326	OE
Dronc	DN	OE
Dronc	51	LOF
drp1	KG03815	LOF
Hsp22	EP(3)3247	OE
ik2	DN	OE
omi/htra2	FL	OE
omi/htra2	Δ07	LOF
p35	FL	OE
pmn	FL	OE
puckered	FL	OE
roc1b	dc3	LOF
SOD1	FL	OE
TOR	TED DN	OE

All mutants or misexpression constructs were crossed into a background where a subset of ORNs were labeled with GFP. Constructs tested for overexpression phenotypes were co-expressed with GFP. ORN axons were severed, and axon degeneration and clearance from the brain was assayed 7 days after axotomy. LOF = reported amorphic loss of function allele; OE = overexpression construct; DN = dominant negative; FL = full length gene.

Supplementary Table 2 – Production of MARCM clones and persistence of severed axons in mutant backgrounds

mosaic chromosome	**number of axons (uninjured)***	**number of axons 7 days after axotomy***
wild type	11.08 +/-1.52	0
l(3)896	4.71 +/-1.76	5.43 +/-2.12
l(3)4621	7.44 +/-0.66	6.13 +/-0.74
l(3)4705	4.94 +/-0.85	5.00 +/-1.61

*Represents number of individual axon fibers identified in z-stacks from confocal imaging of entire antennal lobe. n≥10 antennal lobes for all.

Supplementary Table 3 – Identification of *dsarm* through re-sequencing of mutant genomes

	One mutant line (# of genes)	Any two mutant lines (maximum # of genes)	All three mutant lines (# of genes)
Unique coding variants; genome wide	92 (*l(3)896*) 997 (*l(3)46210)* 272 (*l(3)4705)*	166	6
Unique coding variants; chromosome 3L	34 (*l(3)896*) 132 (*l(3)46210)* 46 (*l(3)4705)*	8	3
+ nonsynonymous or splice site and heterozygous changes	17 (*l(3)896*) 66 (*l(3)46210)* 23 (*l(3)4705)*	2	1