## Supplemental Figures

### Figure S1:

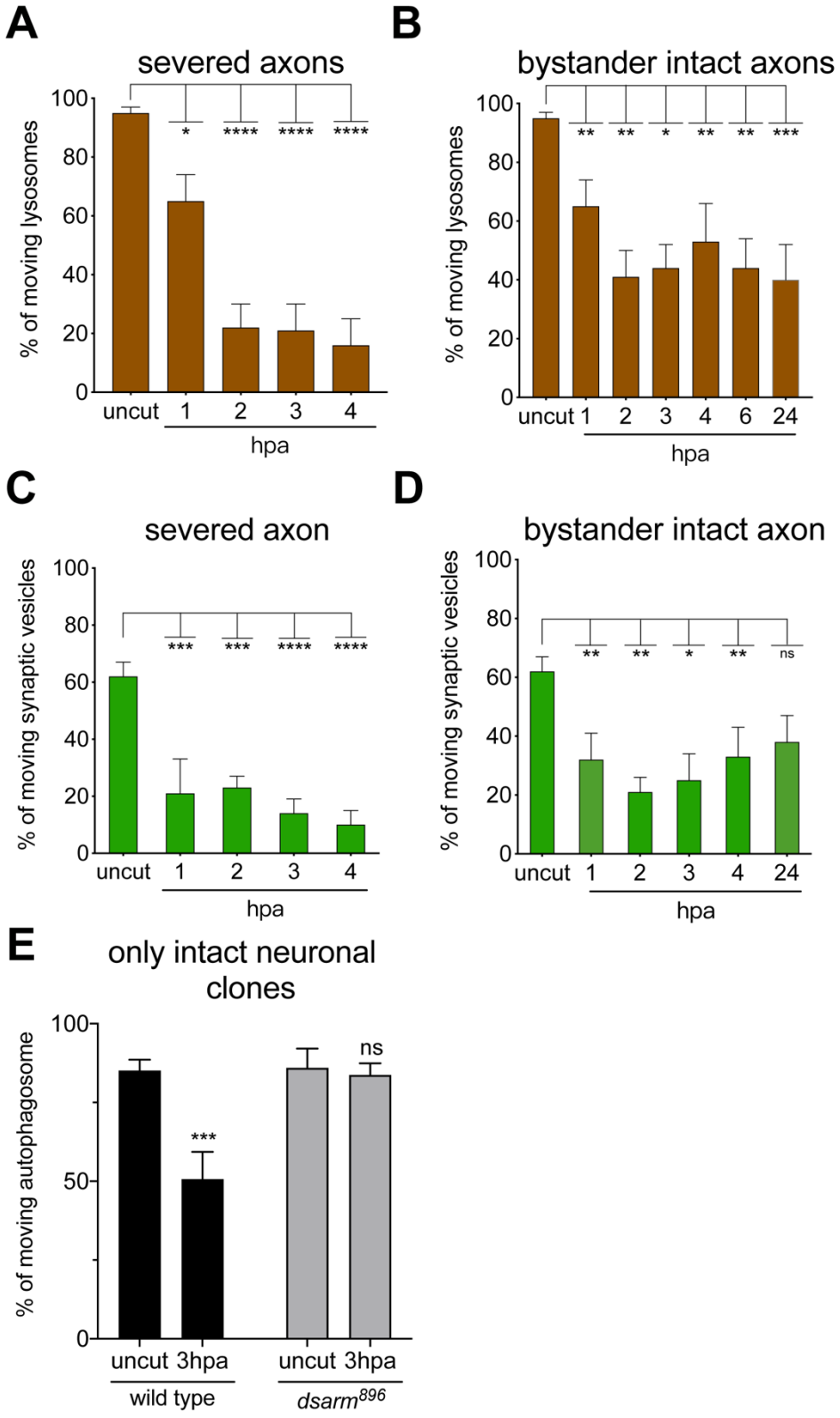

**Figure. S1: Nerve injury blocks trafficking of lysosomes and synaptic vesicles in both severed and intact axons. (Related to Figure 1)**

(A, B) Injury reduced the percentage of moving lysosomes in both severed axons and proximal intact axons. hpa, hours post axotomy. (For all, Ordinary one-way ANOVA with Sidak multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n = 10 axons of each, Error bar = S.E.M.).

(C, D) Injury reduced the percentage of moving synaptic vesicles in both severed axons and proximal intact axons.

(E) Axon transport quantification in the wings with intact clones only. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, ***$p < 0.001$, n = 7 to 14 axons each, Error bar = S.E.M.).

**Figure S2:**

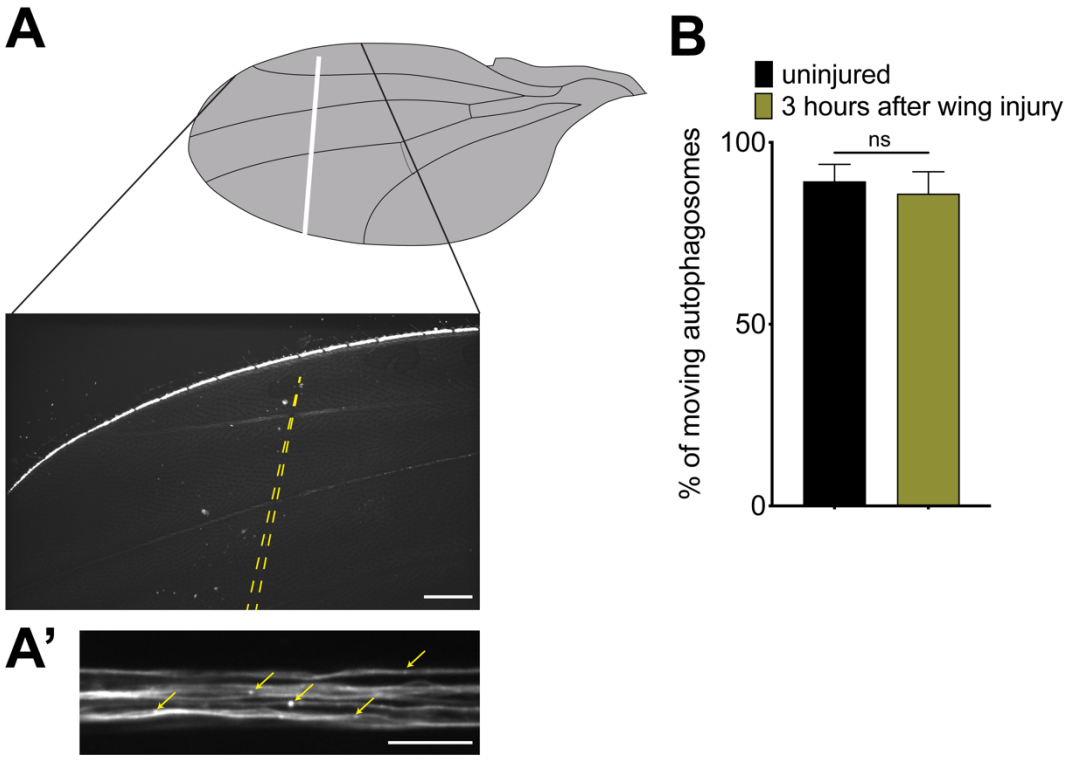
A
A'
B
uninjured
3 hours after wing injury
ns
% of moving autophagosomes
100
50
0

**Figure. S2: Wing injury, instead of nerve injury, is not sufficient to induce axon transport suppression. (Related to Figure 1)**

(A) Schematics and representative image indicate wing injury site. Yellow dash lines outline the the injury site. Scale bar = 100 μm. (A') Higher magnification image of the GFP-labeled axons in the wing margin. Autophagosomes were labeled with mCherry, and indicated by yellow arrows. Scale bar = 10 μm.

(B) Axon transport quantification in the wings before and after injury. Unpaired two-tailed t-test. (ns = not significant, n = 8 axons each, Error bar = S.E.M.).

**Figure S3:**

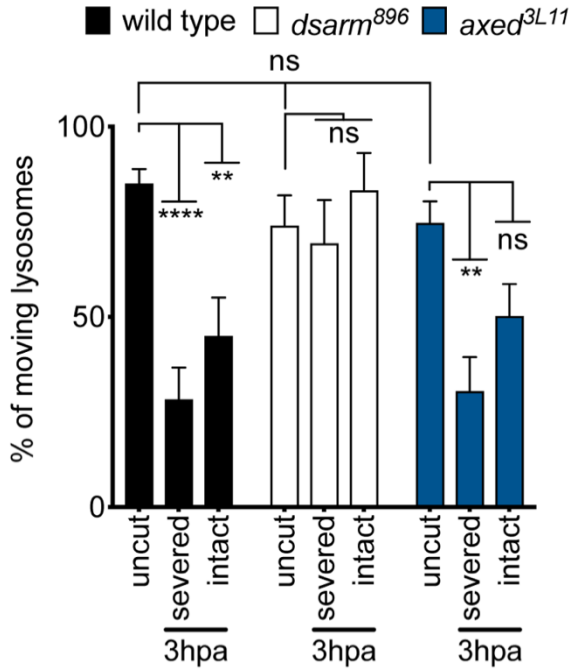
wild type
dsarm896
axed3L11
ns
ns
**
****
**
ns
100
50
0
% of moving lysosomes
uncut
severed
intact
uncut
severed
intact
uncut
severed
intact
3hpa
3hpa
3hpa

**Figure S3: dSarm, but Axed, is required for the blockade of vesicle trafficking in both severed and intact axons after nerve injury. (Related to Figure 2-4)**

Lysosome trafficking in axons was suppressed in control and *axed* mutant animals 3hpa, but not in *dsarm* (*dsarm*$^{896}$) null mutants. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, ***p < 0.001, ****p < 0.0001, n = 10 axons of each, Error bar = S.E.M.).

**Figure S4:**

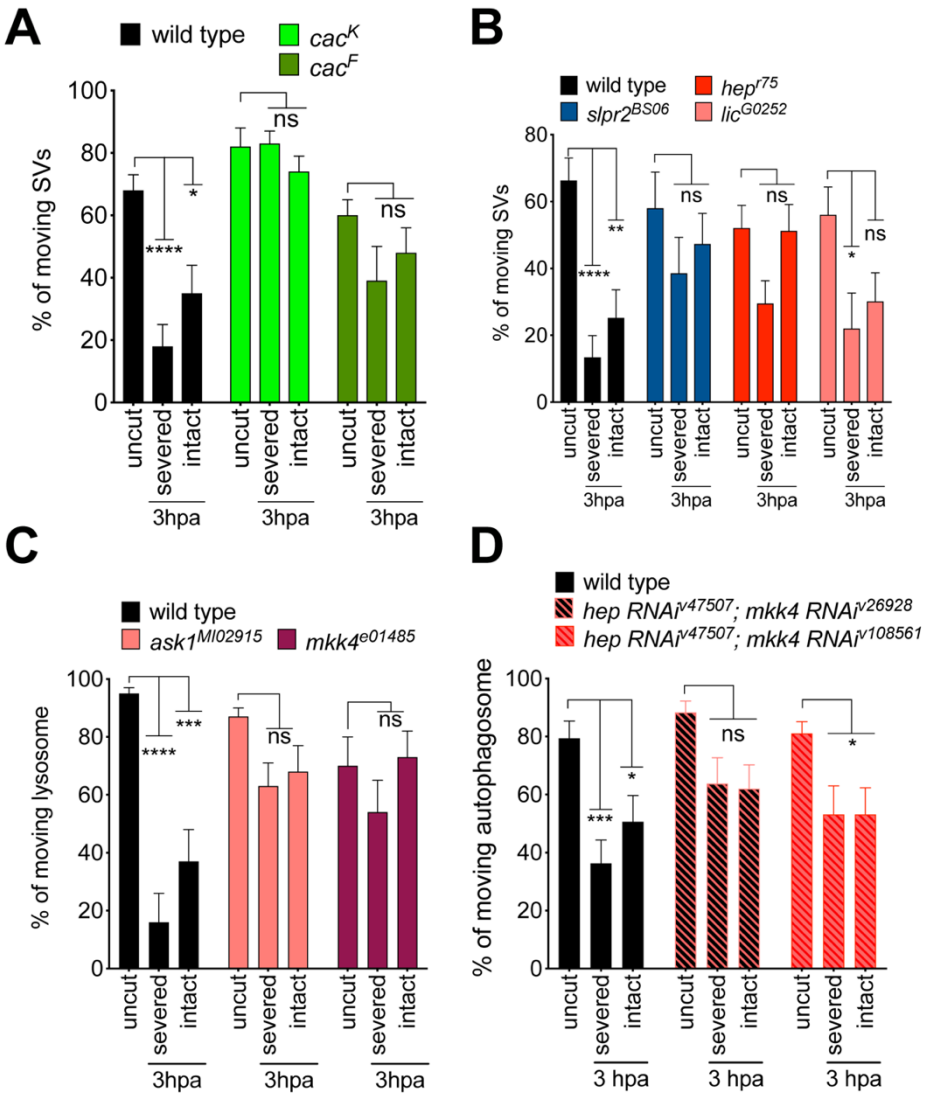
A
wild type
cac^K
cac^F
% of moving SVs
100
80
60
40
20
0
****
*
ns
ns
uncut
severed
intact
3hpa
B
wild type
slpr2^BS06
hep^r75
lic^G0252
% of moving SVs
80
60
40
20
0
****
**
ns
ns
*
ns
C
wild type
ask1^MI02915
mkk4^e01485
% of moving lysosome
100
80
60
40
20
0
****
***
ns
ns
D
wild type
hep RNAi^v47507; mkk4 RNAi^v26928
hep RNAi^v47507; mkk4 RNAi^v108561
% of moving autophagosome
100
80
60
40
20
0
***
*
ns
*
3 hpa

**Figure S4: Cacophony and the TIR-1-like-MAPK signaling pathway promote blockade of lysosome and SV trafficking after nerve injury. (Related to Figure 4)**

(A) Synaptic vesicle movement in the two independent alleles of *cac* (*cac*$^{K}$ and *cac*$^{F}$) in both severed axons and proximal intact axons in injured wings (3hpa). (For all, two-way ANOVA with Sidak multiple comparisons test. ns = not significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, n = 10 axons of each, Error bar = S.E.M.).

(B) Strong alleles of *mkk4* and *ask1*, (*mkk4*$^{e01485}$, and *ask*$^{MI02915}$) exhibited normal lysosome transport in both severed axons in injured wings 3hpa compared to uninjured wings.

(C) In severed axons, synaptic vesicle movement was not significantly changed in *slpr* or *hep* (*slpr*$^{BS06}$ and *hep*$^{r75}$) mutant axons (3hpa) compared to uninjured wings (uncut). An allele of *lic*, (*lic*$^{G0252}$) showed a mild reduction in trafficking after injury compared to uninjured wings.

(D) The axon clones with double RNAi knockdown of *mkk4* and *hep* (*hep RNAi*$^{v47507}$ and *mkk4 RNAi*$^{v26928}$) showed no significant suppression of axon transport after injury (middle group). Two alleles of mkk4 RNAi were tested (*mkk4 RNAi*$^{v26928}$ and *mkk4 RNAi*$^{v108561}$). To enhance the RNAi efficiency, *5xuas-Gal4* was included in all groups.

**Figure S5:**

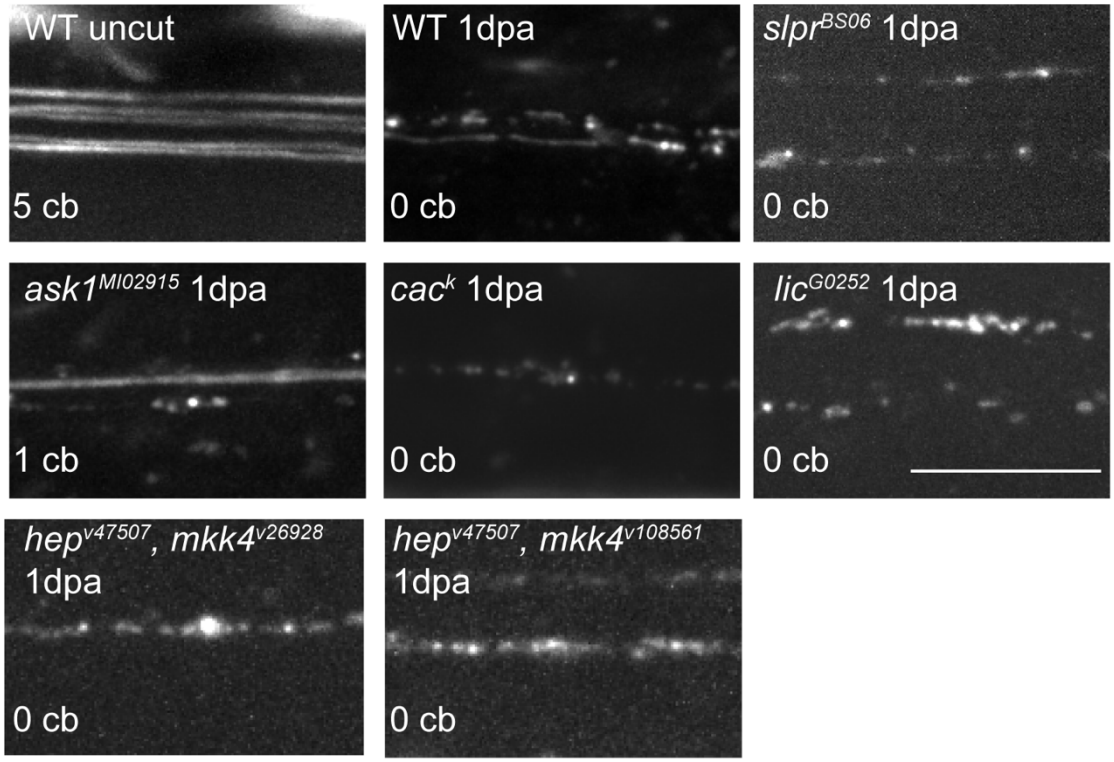
WT uncut
5 cb
WT 1dpa
0 cb
slpr^BS06 1dpa
0 cb
ask1^MI02915 1dpa
1 cb
cac^k 1dpa
0 cb
lic^G0252 1dpa
0 cb
hep^v47507, mkk4^v26928
1dpa
0 cb
hep^v47507, mkk4^v108561
1dpa
0 cb

**Figure S5: Loss of MAPK components does not suppress axon degeneration. (Related to Figure 4)**

Loss of function mutations in *slpr*, *ask1*, *cac*, and *lic*, and *mkk4*, *hep* double RNAi knockdown (*hep RNAi*$^{v47507}$, *mkk4 RNAi*$^{v26928}$ and *hep RNAi*$^{v47507}$*, mkk4 RNAi*$^{v108561}$) with *5xuas-Gal4*, are unable to suppress axon degeneration even at 1day post axotomy (dpa). The number of remaining cell bodies (cb), and therefore predicted axon number were indicated at lower left corner. Scale bar = 10 um.

**Figure S6:**

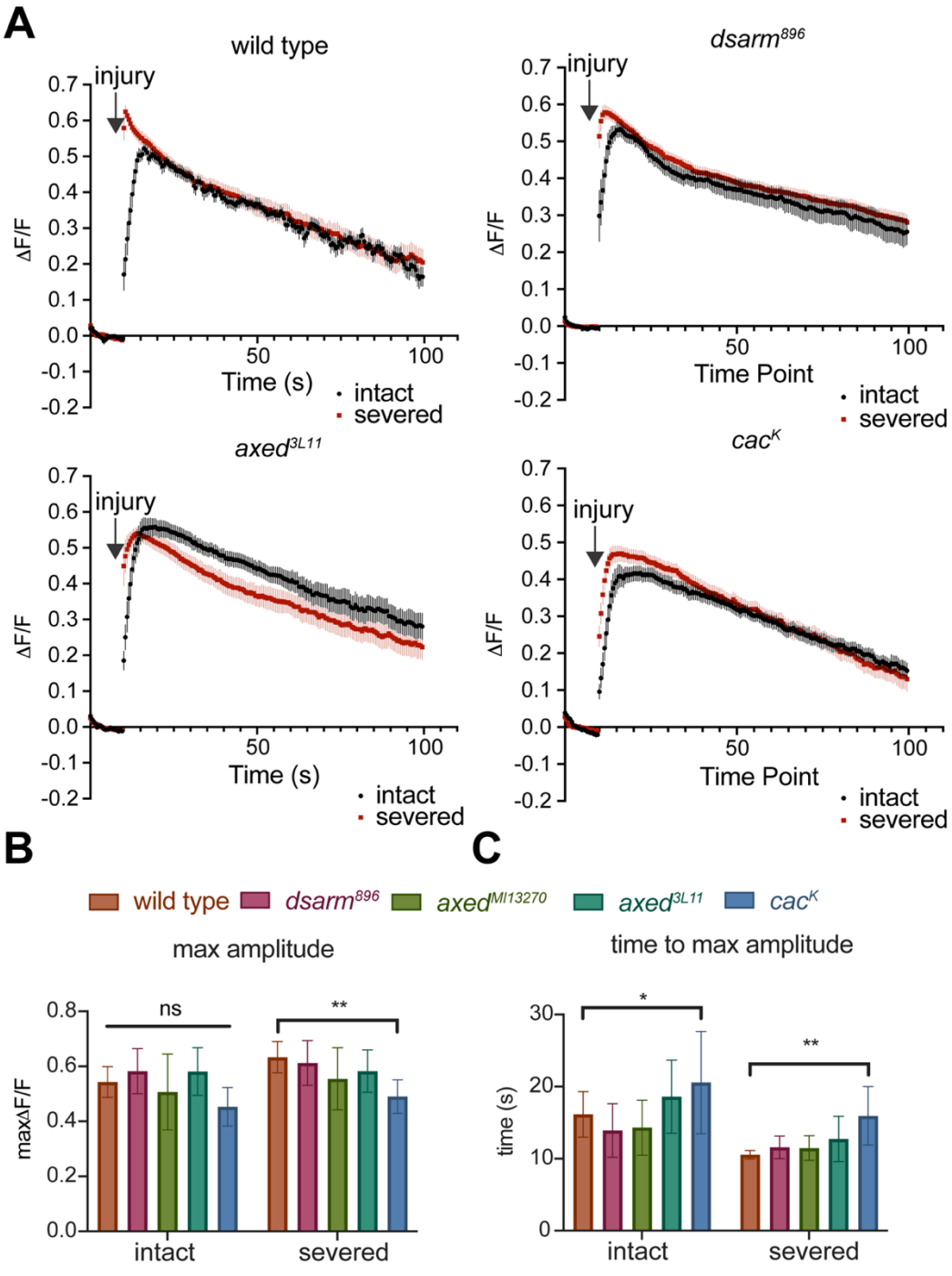
A
wild type
injury
ΔF/F
Time (s)
intact
severed
dsarm896
Time Point
axed3L11
cacK
B
wild type
dsarm896
axedMI13270
axed3L11
cacK
max amplitude
maxΔF/F
ns
**
intact
severed
C
time to max amplitude
time (s)
*

**Figure S6: Increase of calcium influx in severed and intact axons after axotomy is mildly reduced in *cacophony* mutants. (Related to Figure 4)**

(A) The GCamp6s signal intensity in different genotypes over time after injury is plotted. (Error bar = S.E.M., n = 9-15 wings)
(B, C) Maximum amplitude (B) and time to max amplitude (C) of GCamp6s intensity after injury in different genotypes. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, *$p < 0.05$, **$p < 0.01$, n = 9-15 wings. Error bar = S.E.M.).

**Figure S7:**

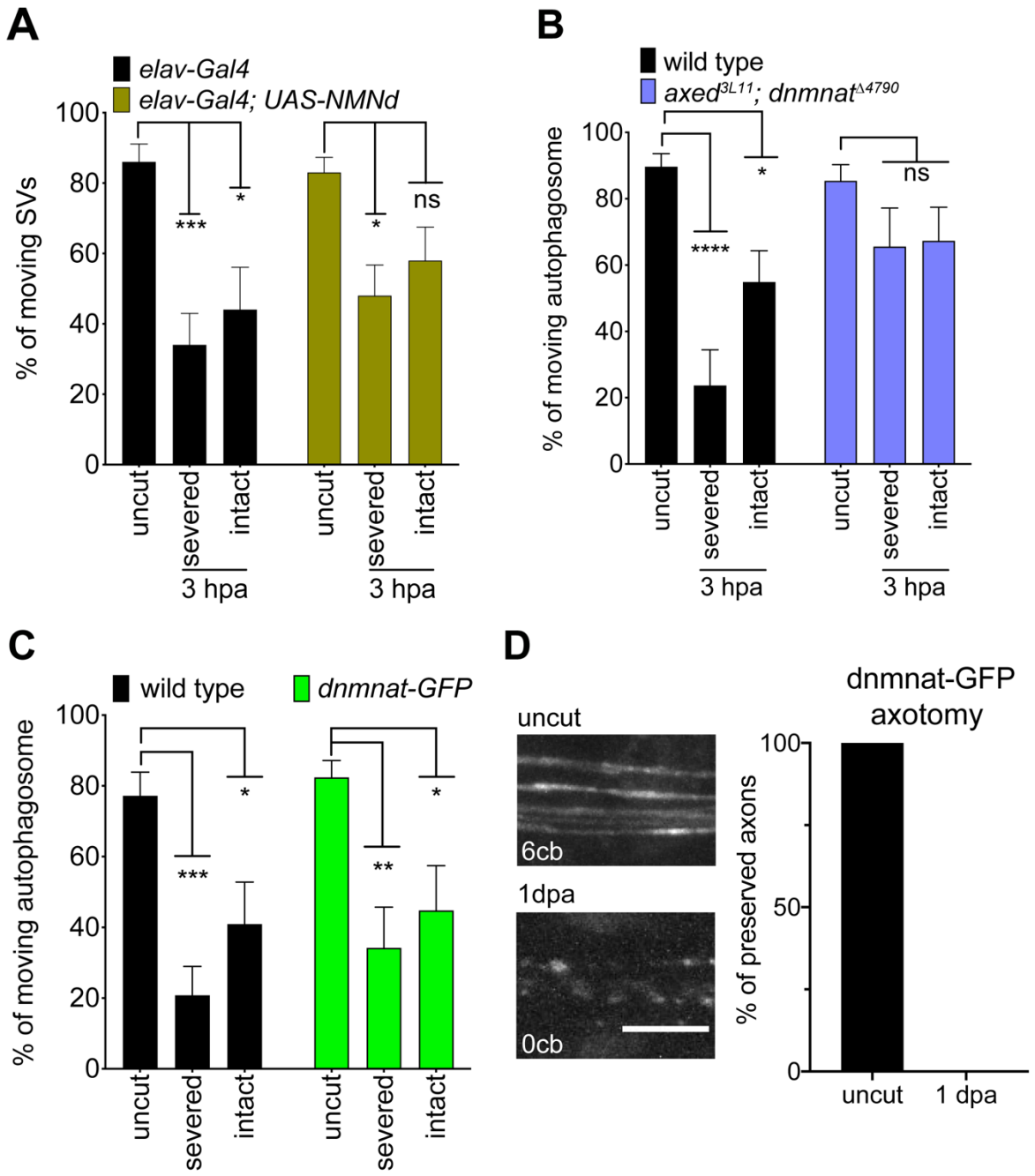
A
elav-Gal4
elav-Gal4; UAS-NMNd
% of moving SVs
100
80
60
40
20
0
***
*
*
ns
uncut
severed
intact
uncut
severed
intact
3 hpa
3 hpa
B
wild type
axed3L11; dnmnatΔ4790
% of moving autophagosome
100
80
60
40
20
0
****
*
ns
uncut
severed
intact
uncut
severed
intact
3 hpa
3 hpa
C
wild type
dnmnat-GFP
% of moving autophagosome
100
80
60
40
20
0
***
*
**
*
uncut
severed
intact
uncut
severed
intact
D
uncut
6cb
1dpa
0cb
dnmnat-GFP
axotomy
% of preserved axons
100
50
0
uncut
1 dpa

**Figure S7: NMN deamindase expression does not modify injury-induced suppression of axon transport and dNmnat levels to not drop in bystander neuron cell bodies. (Related to Figures 5.)**

(A) Severed and intact axons expressing NMN deamidase (NMNd) showed partial decrease of axon transport after axonal injury 3hpa compared to that in uninjured wings. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, **p < 0.01, *p < 0.05, n = 10~20 axons of each, Error bar = S.E.M.).

(B) The severed and intact *axed*$^{3L11}$, *dnmnat*$^{\Delta 4790}$ clones showed no reduction of axon transport after axonal injury 3hpa when comparing to uninjured wings. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, ****p < 0.0001, *p < 0.05, n = 10~20 axons of each, Error bar = S.E.M.).

(C) The axon clones with endogenously GFP labeled dNmnat (dNmnat-GFP) showed suppression of axon transport, which is similar to wild type axon clones. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, ***p < 0.001, **p < 0.01, *p < 0.05, n = 10~20 axons of each, Error bar = S.E.M.).

(D) dNmnat-GFP axon clones showed normal axon degeneration after axotomy. Scale bar = 5μm, dpa = days post axotomy, cb = cell body. (100% of preserved axons before injury, and 0% of preserved axons on 1dpa, n = 20 wings).

**Figure S8:**

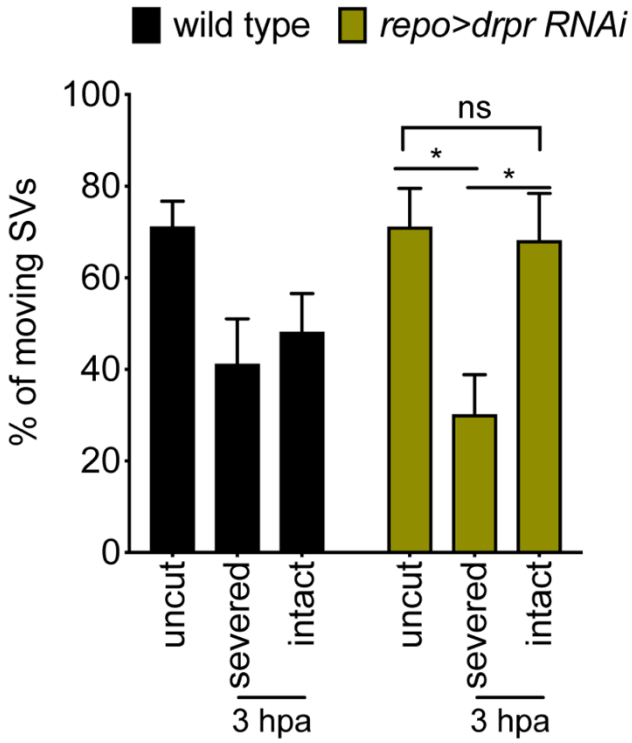
wild type
repo>drpr RNAi
% of moving SVs
100
80
60
40
20
0
ns
*
*
uncut
severed
intact
uncut
severed
intact
3 hpa
3 hpa

**Figure S8: Draper functions in glia to suppress axon transport in the intact neurons. (Related to Figures 7)**

Severed axons, but not intact axons, showed decrease of synaptic vesicle (SV) transport after axonal injury 3hpa in glial-specific draper knockdown flies (*repo-gal4*, *uas-drpr*$^{RNAi}$). Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, **p < 0.01, *p < 0.05, n = 10~20 axons of each, Error bar = S.E.M.).

**Figure S9:**

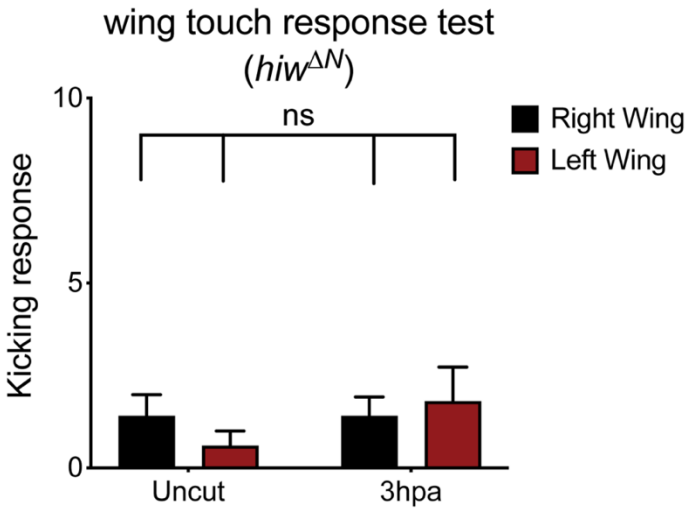
wing touch response test
(hiw^ΔN)
ns
Right Wing
Left Wing
Kicking response
10
5
0
Uncut
3hpa

**Figure S9. *hiw*$^{\Delta N}$ mutant showed defective kicking responses after gentle touch. (Related to Figure 6)**

Uncut: Both side of the wings are intact. 3hpa: Right wings are intact, but the left wings are injured. *hiw*$^{\Delta N}$ mutant showed defective kicking responses upon bristle stimulation even in the animals without any injury. Two-way ANOVA with Sidak multiple comparisons test. (ns = not significant, n = 13~14 animals of each, Error bar = S.E.M.).