

Figure S1. **Toll-6 regulates activity of known FoxO kinases.** (A and B) Representative confocal projections of L3 nerves of indicated genotypes labeled with anti-pAkt. Bar, 10 μ m. (C) Quantification of pAkt intensity in nerves normalized to wild type. Wild type: 1.0 ± 0.05 arbitrary units ($n = 15$ nerves); Toll-6^{EX13}: 1.8 ± 0.1 arbitrary units ($n = 16$ nerves); and Toll-791-1: 1.2 ± 0.2 arbitrary units ($n = 11$ nerves). (D) Immunoblot of pAkt expression in the CNS of wild-type and Toll-6 mutants. (E and F) Representative confocal projections of L3 NMJ4 labeled with HRP and pJNK, and corresponding lookup tables applied to pJNK projections. Bar, 5 μ m. (G) Quantification of pJNK intensity at NMJ4 relative to wild type. Wild type (OregonR): 1.0 ± 0.04 arbitrary units ($n = 34$); Toll-6^{EX13}: 0.6 ± 0.04 arbitrary units ($n = 22$); and Toll-791-1: 1.1 ± 0.1 arbitrary units ($n = 13$). Anterior is up. Error bars are mean \pm SEM. n.s., not significantly different. ***, $P < 0.001$.

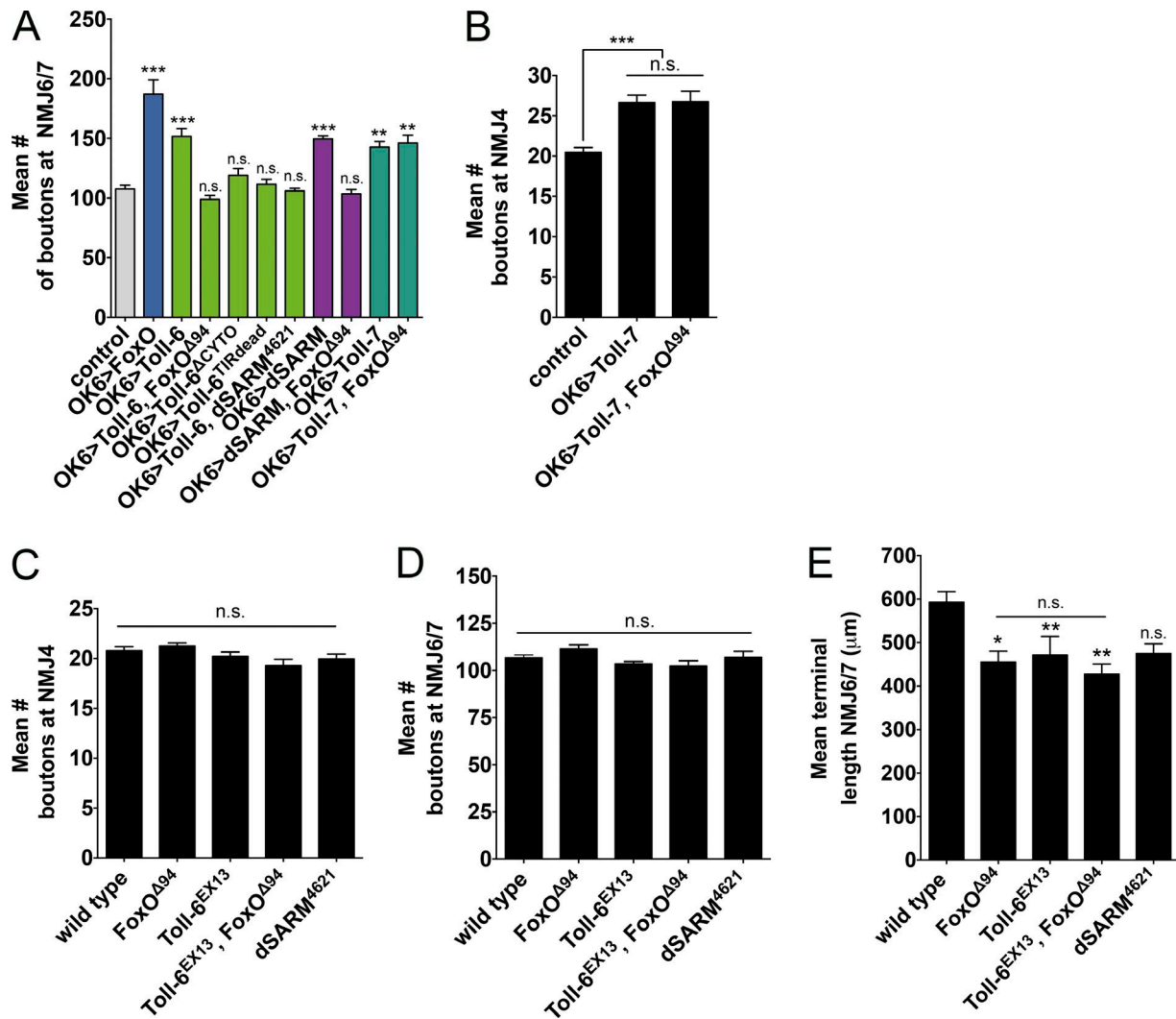


Figure S2. *Toll-6*, *dSARM*, and *foxO* overexpression and LOF NMJ phenotypes. (A) Quantification of bouton numbers at NMJ 6/7 (segment A2). Control: 107.5 ± 3.2 (*n* = 15); OK6>*foxO*: 187.1 ± 11.9 (*n* = 7); OK6>*Toll-6*: 151.6 ± 6.6 (*n* = 15); OK6>*Toll-6*, *foxO*^{Δ94}: 98.9 ± 3.4 (*n* = 14); OK6>*Toll-6*^{CYTO}: 119.1 ± 5.6 (*n* = 9); OK6>*Toll-6*^{IRdead}: 111.6 ± 4.0 (*n* = 13); OK6>*Toll-6*, *dSARM*⁴⁶²¹: 105.9 ± 2.3 (*n* = 17); OK6>*dSARM*: 149.5 ± 2.5 (*n* = 11); OK6>*dSARM*, *foxO*^{Δ94}: 103.5 ± 3.6 (*n* = 13); OK6>*Toll-7*: 142.7 ± 4.9 (*n* = 15); and OK6>*Toll-7*, *foxO*^{Δ94}: 146.2 ± 6.5 (*n* = 9). (B) Quantification of bouton numbers at NMJ4 in segments A2–A4. Control: 20.5 ± 0.6 (*n* = 15); OK6>*Toll-7*: 26.6 ± 0.9 (*n* = 19); and OK6>*Toll-7*, *foxO*^{Δ94}: 26.8 ± 1.3 (*n* = 28). (C) Quantification of bouton numbers at NMJ4 in segments A2–A4. Wild type (OregonR): 20.8 ± 0.4 (*n* = 42); *foxO*^{Δ94}: 21.3 ± 0.3 (*n* = 70); *Toll-6*^{EX13}: 20.2 ± 0.4 (*n* = 27); *Toll-6*^{EX13}, *foxO*^{Δ94}: 19.3 ± 0.6 (*n* = 10); and *dSARM*⁴⁶²¹: 20.0 ± 0.5 (*n* = 20). (D) Quantification of bouton numbers at NMJ 6/7 (segment A2). Wild type: 106.7 ± 1.6 (*n* = 23); *foxO*^{Δ94}: 111.4 ± 2.1 (*n* = 25); *Toll-6*^{EX13}: 103.5 ± 1.2 (*n* = 35); *Toll-6*^{EX13}, *foxO*^{Δ94}: 102.3 ± 2.8 (*n* = 16); and *dSARM*⁴⁶²¹: 106.9 ± 3.2 (*n* = 19). (E) Quantification of terminal length of NMJ6/7 (segment A2). Wild type: 592.6 ± 24.4 μm (*n* = 18); *foxO*^{Δ94}: 455.6 ± 24.6 μm (*n* = 15); *Toll-6*^{EX13}: 471.7 ± 42.5 μm (*n* = 18); *Toll-6*^{EX13}, *foxO*^{Δ94}: 427.6 ± 23.2 μm (*n* = 16); and *dSARM*⁴⁶²¹: 475.2 ± 22.2 μm (*n* = 16). Control is *OK6Gal4/+*. Error bars are mean ± SEM. n.s., not significantly different. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

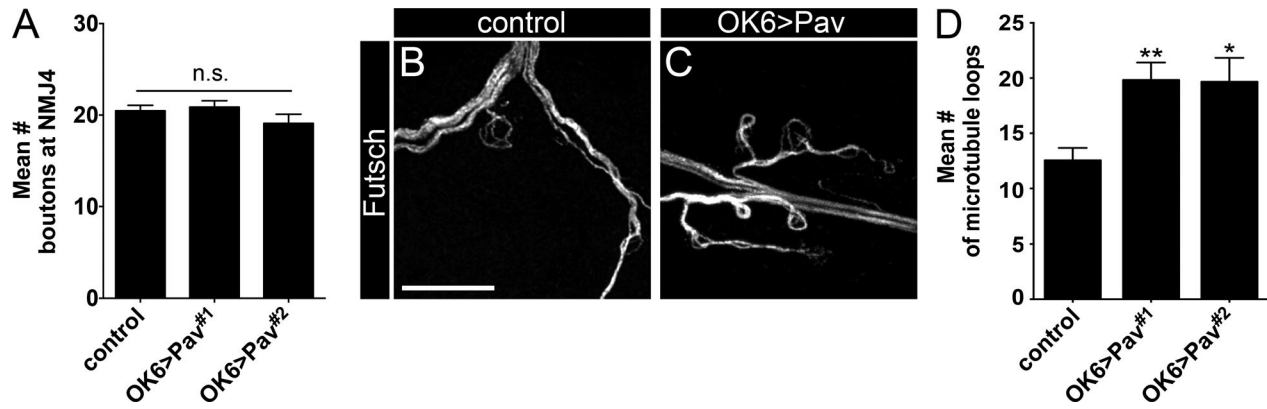


Figure S3. **Pav-KLP overexpression elevates MT stability at the NMJ.** (A) Quantification of bouton numbers at NMJ4 in segments A2–A4. Control: 20.5 ± 0.6 ($n = 15$); OK6>Pav#1: 20.9 ± 0.7 ($n = 8$); and OK6>Pav#2: 19.1 ± 1.0 ($n = 11$). (B and C) Representative confocal projections of Futsch-positive MT loops at NMJ6/7 of indicated genotypes. Bar, 10 μm . (D) Quantification of Futsch-positive MT loops at NMJ6/7 of segment A2. Control (OK6Gal4/+): 12.6 ± 1.2 ($n = 9$); OK6>Pav#1: 19.8 ± 1.6 ($n = 6$); and OK6>Pav#2: 19.7 ± 2.2 ($n = 6$). Error bars are mean \pm SEM. n.s., not significantly different. *, $P < 0.05$; **, $P < 0.01$.

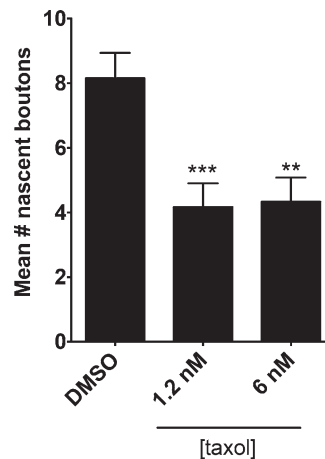


Figure S4. **Taxol-induced MT stability impairs nascent bouton formation.** Quantification of nascent boutons at wild-type NMJ6/7 segments A2 and A3 treated with taxol or 0.2% DMSO before high K^+ stimulation. DMSO: 8.2 ± 0.8 ($n = 26$); 1.2 nM taxol: 4.2 ± 0.7 ($n = 18$); and 6 nM taxol: 4.3 ± 0.7 ($n = 15$). Wild type is OregonR. Error bars are mean \pm SEM. n.s., not significantly different from DMSO treatment. **, $P < 0.01$; ***, $P < 0.001$.

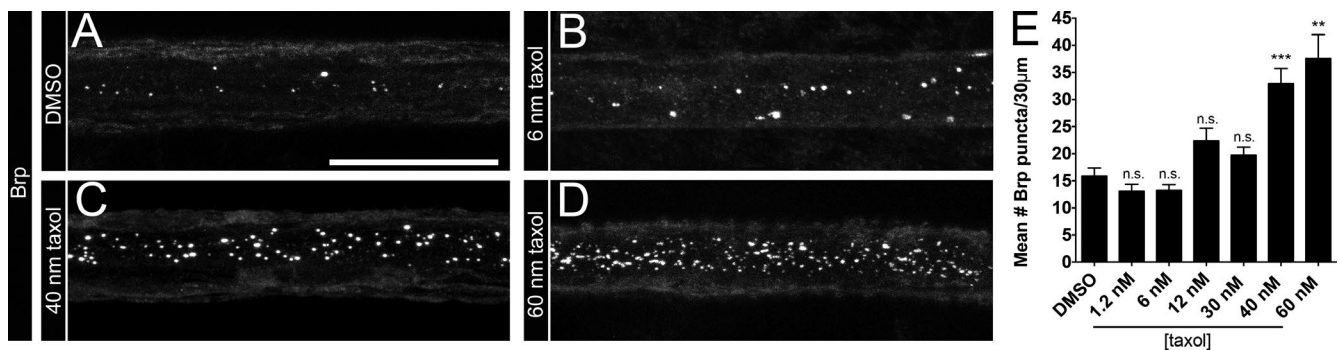


Figure S5. **Taxol-induced MT stability impairs axonal transport.** (A–D) Representative confocal projections of wild-type nerves labeled with Brp and treated with indicated concentrations of taxol or DMSO. Bar, 20 μm . (E) Quantification of Brp accumulations per 30 μm of nerve. 0.2% DMSO: 15.9 ± 1.5 ($n = 16$); 1.2 nM taxol: 13.1 ± 1.3 ($n = 46$); 6 nM taxol: 13.3 ± 1.1 ($n = 44$); 12 nM taxol: 22.4 ± 2.4 ($n = 47$); 30 nM taxol: 19.8 ± 1.5 ($n = 42$); 40 nM taxol: 32.9 ± 2.8 ($n = 27$); and 60 nM taxol: 37.6 ± 4.4 ($n = 16$). Wild type is OregonR. Error bars are mean \pm SEM. n.s., not significantly different. **, $P < 0.01$; ***, $P < 0.001$.

Table S1. RNAi and LOF phenotypes of canonical Toll pathway members

| Genotype | Mean bouton number \pm SEM | | | | Mean number of Futsch loops \pm SEM | | | |
|--------------------------------------|-------------------------------|----|-----------------------------|----|---------------------------------------|----|----------------------------|----|
| | NMJ6/7 A2 | n | NMJ4 | n | NMJ6/7 A2 | n | NMJ4 | n |
| Wild type | 106.7 \pm 1.6 | 23 | 20.8 \pm 0.4 | 42 | 12.6 \pm 0.5 | 22 | 4.2 \pm 0.2 | 51 |
| MyD88 ^{CO3881} | 103 \pm 2.9 ^a | 10 | 21.5 \pm 0.9 ^a | 13 | 11.5 \pm 1.0 ^a | 6 | 3.3 \pm 0.4 ^a | 15 |
| Rel ^{E20} | 111.3 \pm 6.4 ^a | 8 | 19.4 \pm 1.0 ^a | 14 | 12.6 \pm 0.9 ^a | 8 | 2.9 \pm 0.3 ^a | 15 |
| Control | 106.8 \pm 5.2 | 12 | 21.1 \pm 0.5 | 29 | 11.9 \pm 0.9 | 11 | 3.6 \pm 0.5 | 18 |
| D42>dcr-2; FoxO RNAi | 105.3 \pm 1.7 ^a | 8 | 20.9 \pm 0.6 ^a | 25 | 17.8 \pm 1.3 ^b | 12 | 7.1 \pm 0.6 ^c | 19 |
| D42>dcr-2; Toll-6 RNAi ^{#1} | 103.0 \pm 3.7 ^a | 6 | 20.7 \pm 1.9 ^a | 10 | 21.2 \pm 2.6 ^d | 6 | 5.7 \pm 0.5 ^a | 10 |
| D42>dcr-2; Toll-6 RNAi ^{#2} | 107.5 \pm 4 ^a | 10 | 19.9 \pm 0.7 ^a | 14 | 17.8 \pm 0.9 ^d | 18 | 6.8 \pm 0.5 ^d | 11 |
| D42>dcr-2; dSARM RNAi ^{#1} | 98.9 \pm 5.4 ^a | 11 | 19.9 \pm 0.5 ^a | 31 | 18.6 \pm 1.0 ^d | 11 | 6.1 \pm 0.5 ^b | 12 |
| D42>dcr-2; dSARM RNAi ^{#2} | 104.3 \pm 4 ^a | 12 | 20.2 \pm 0.7 ^a | 35 | 19.2 \pm 1.8 ^d | 10 | 5.3 \pm 0.4 ^a | 18 |
| D42>dcr-2; dSARM RNAi ^{#3} | 111.3 \pm 2.8 ^a | 13 | 21.0 \pm 0.7 ^a | 31 | 18.8 \pm 1.1 ^c | 22 | 7.7 \pm 0.6 ^c | 18 |
| D42>dcr-2; dSARM RNAi ^{#4} | 105.3 \pm 3.3 ^a | 13 | 20.6 \pm 0.7 ^a | 19 | 16.8 \pm 1.3 ^a | 13 | 5.4 \pm 0.4 ^a | 19 |
| D42>dcr-2; dSARM RNAi ^{#5} | 82.9 \pm 3.6 ^a | 10 | 21.0 \pm 0.6 ^a | 7 | 11.5 \pm 1.2 ^a | 10 | 4.9 \pm 0.5 ^a | 7 |
| D42>dcr-2; cact RNAi ^{#1} | 82.6 \pm 6.4 ^a | 8 | 19.3 \pm 1.2 ^a | 8 | 12.9 \pm 2.0 ^a | 8 | 3.0 \pm 0.6 ^a | 8 |
| D42>dcr-2; cact RNAi ^{#2} | 90.6 \pm 7.3 ^a | 10 | 19.8 \pm 0.7 ^a | 12 | 9.4 \pm 0.8 ^a | 10 | 2.6 \pm 0.4 ^a | 12 |
| D42>dcr-2; cact RNAi ^{#3} | 107.8 \pm 6.5 ^a | 9 | 22.9 \pm 1.1 ^a | 11 | 9.3 \pm 0.7 ^a | 9 | 3.0 \pm 0.3 ^a | 11 |
| D42>dcr-2; dl RNAi ^{#1} | 100.9 \pm 5.6 ^a | 9 | 20.9 \pm 0.5 ^a | 14 | 13.4 \pm 1.6 ^a | 9 | 4.4 \pm 0.5 ^a | 14 |
| D42>dcr-2; dl RNAi ^{#2} | 111.9 \pm 6.2 ^a | 15 | 22.3 \pm 1.3 ^a | 14 | 14.4 \pm 1.2 ^a | 13 | 3.1 \pm 0.5 ^a | 14 |
| D42>dcr-2; dl RNAi ^{#3} | 109.5 \pm 11.6 ^a | 8 | 20.1 \pm 0.7 ^a | 9 | 13.4 \pm 1.9 ^a | 7 | 3.1 \pm 0.5 ^a | 9 |
| D42>dcr-2; dl RNAi ^{#4} | 100.3 \pm 5.3 ^a | 15 | 18.7 \pm 0.8 ^a | 17 | 10.2 \pm 0.9 ^a | 13 | 3.1 \pm 0.4 ^a | 17 |
| D42>dcr-2; Myd88 RNAi | 167.1 \pm 8.4 ^b | 10 | 22.4 \pm 1.1 ^a | 16 | 14.5 \pm 1.6 ^a | 10 | 3.6 \pm 0.5 ^a | 16 |
| D42>dcr-2; Pli RNAi | 104.6 \pm 6.3 ^a | 5 | 17.2 \pm 1 ^b | 14 | 14.5 \pm 1.7 ^a | 10 | 2.8 \pm 0.4 ^a | 14 |
| D42>dcr-2; wek RNAi | 140 \pm 5.5 ^a | 6 | 19.9 \pm 0.8 ^a | 27 | 16 \pm 1.8 ^a | 6 | 3.1 \pm 0.3 ^a | 27 |

Quantification of the number of boutons at NMJ4 in A2–A4 and NMJ6/7 in A2 and Futsch-positive loops at NMJ6/7 in A2 of indicated genotypes. Wild type is OregonR; control is D42>dcr-2.

^aNot significantly different.

^bP < 0.05.

^cP < 0.001.

^dP < 0.01.

Table S2. RNAi phenotypes of mRNAs up-regulated in Toll-6 LOF mutants

| Genotype | Mean bouton numbers \pm SEM | | | |
|--------------------------------------|-------------------------------|----|-----------------------------|----|
| | NMJ6/7 A2 | n | NMJ4 | n |
| Control | 108.3 \pm 6.2 | 10 | 19.3 \pm 0.9 | 15 |
| D42>dcr-2; Klp61F RNAi | 113.6 \pm 2.6 ^a | 7 | 21.6 \pm 0.7 ^a | 11 |
| D42>dcr-2; Klp67A RNAi ^{#1} | 96.8 \pm 5.0 ^a | 8 | 16.9 \pm 0.9 ^a | 12 |
| D42>dcr-2; Klp67A RNAi ^{#2} | 106.6 \pm 7.5 ^a | 5 | 18.3 \pm 1.0 ^a | 10 |
| D42>dcr-2; Klp3A RNAi ^{#1} | 121.8 \pm 6.6 ^a | 10 | 22 \pm 0.7 ^a | 20 |
| D42>dcr-2; Klp3A RNAi ^{#2} | 104.8 \pm 4.6 ^a | 12 | 19.9 \pm 0.9 ^a | 16 |
| D42>dcr-2; Pav RNAi ^{#1} | 152.5 \pm 5.6 ^b | 13 | 28.5 \pm 0.9 ^c | 30 |
| D42>dcr-2; Pav RNAi ^{#2} | 159.2 \pm 8.2 ^b | 12 | 28.6 \pm 1.1 ^c | 21 |
| D42>dcr-2; Pav RNAi ^{#3} | 143.3 \pm 7.4 ^d | 9 | 27.9 \pm 1.3 ^c | 25 |

Quantification of the number of boutons at NMJ4 in A2–A4 and NMJ6/7 in A2 of indicated genotypes. Control is D42>dcr-2.

^aNot significantly different.

^bP < 0.01.

^cP < 0.001.

^dP < 0.05.