Luo et al., http://www.jcb.org/cgi/content/full/jcb.201409142/DC1

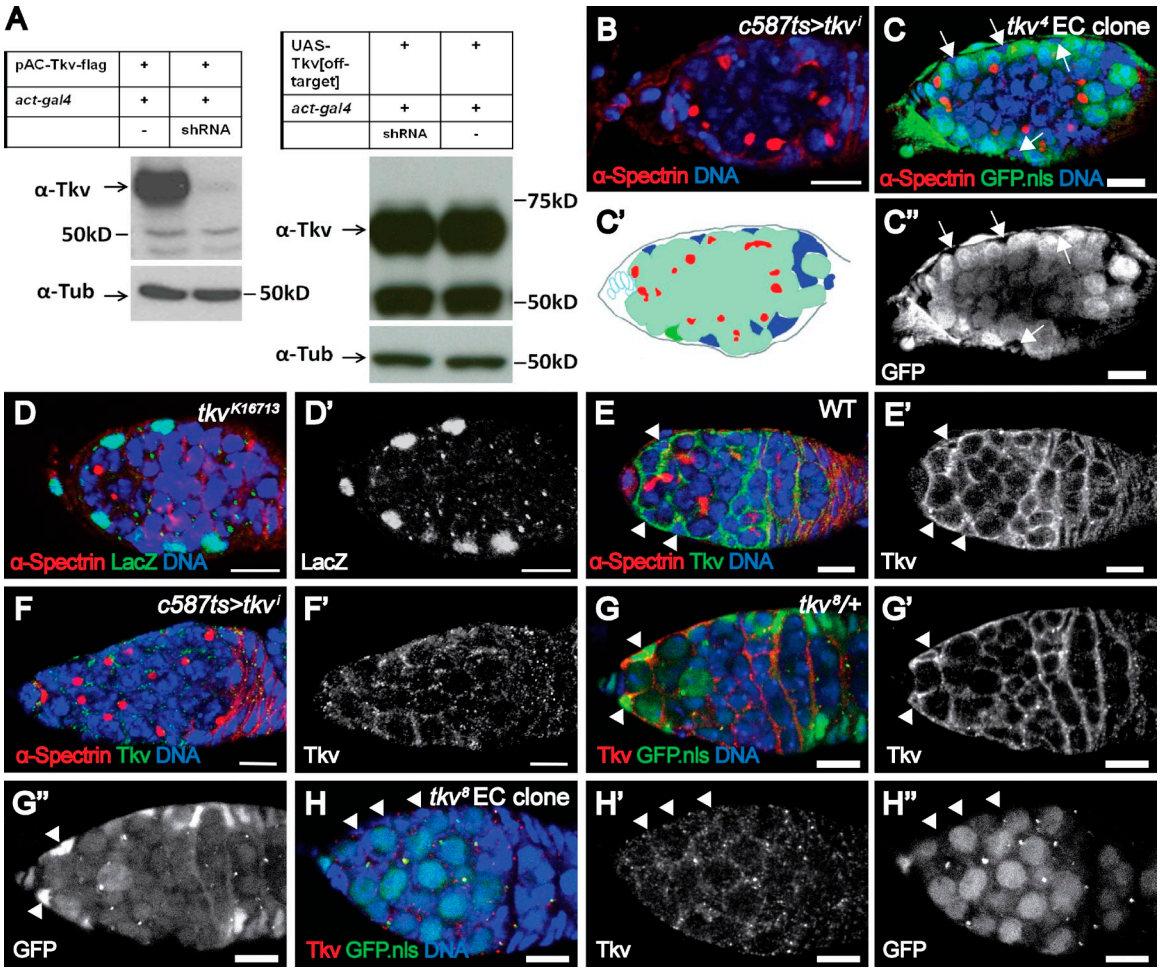


Figure S1. **Tkv functions in ECs.** (A) Western blotting results show that *tkv* shRNA (based on the construct used in BL40937) effectively knocked down the WT (left) but not the off-target (right) Tkv variant in S2 cells. (B) A tkv^{i} (v105834) germarium exhibits more spectrosome-containing cells. (C) A germarium carrying ECs mutant for tkv^{4} (arrows) exhibits more spectrosome-containing cells. (C′) A cartoon illustrating positions of mutant ECs (blue). (D) A Tkv^{K16713} reporter is expressed in ECs. (E and F) Tkv is strongly expressed in ECs of the WT germarium (E, arrowheads) but not of the tkv^{i} (v105834) germarium (F). (G and H) Tkv is strongly detected in ECs (two ECs marked by arrowheads) of the tkv^{8}/+ germarium, but strongly reduced in ECs (arrowheads) mutant for tkv^{8}. Bars, 10 µm.

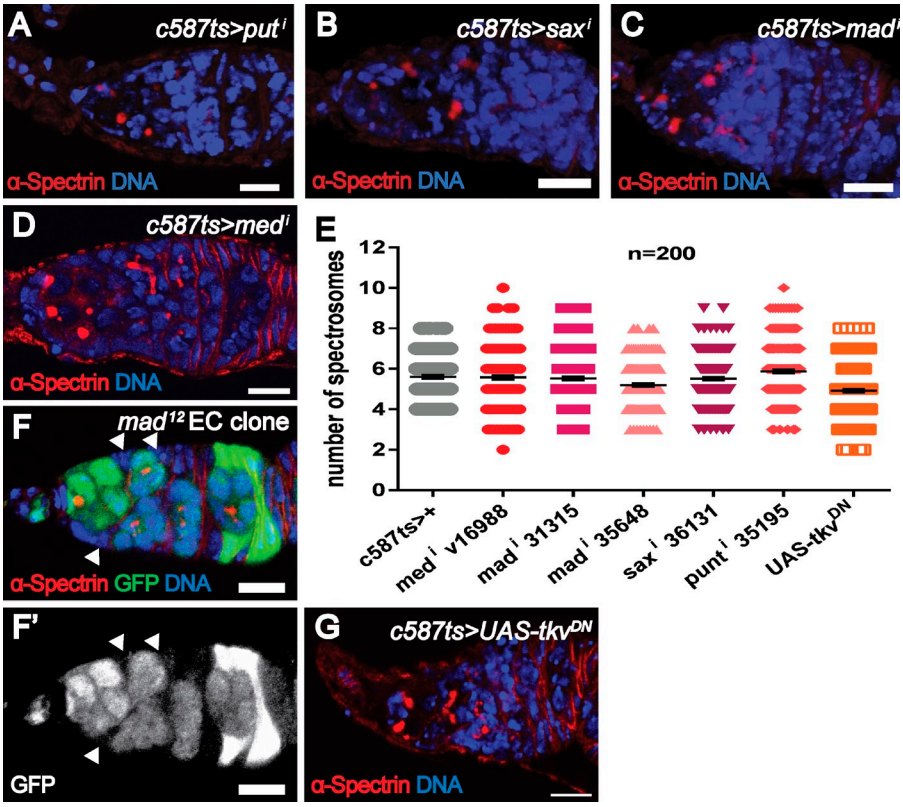


Figure S2. **Tkv functions independently of the canonical Dpp signaling pathway.** (A–D) Germaria with compromised canonical Dpp signaling components including Put (A, BL-35195]), Sax (B, BL-36131), Mad (C, Bl-31315), and Med (D, v16988) in ECs do not exhibit more spectrosome-containing cells. (E) Statistical data for spectrosome counts in these backgrounds. (F) A germarium containing ECs mutant for *mad*^{12} (arrowheads) does not exhibit more spectrosome-containing cells. Genotype: *c587.UAS-flp; FRT40A.ubi-GFP/FRT40A.mad*^{12}. (G) A germarium with ectopic Tkv^{DN} expression in the ECs does not exhibit more spectrosome-containing cells (see E for statistical data). Bars, 10 µm.

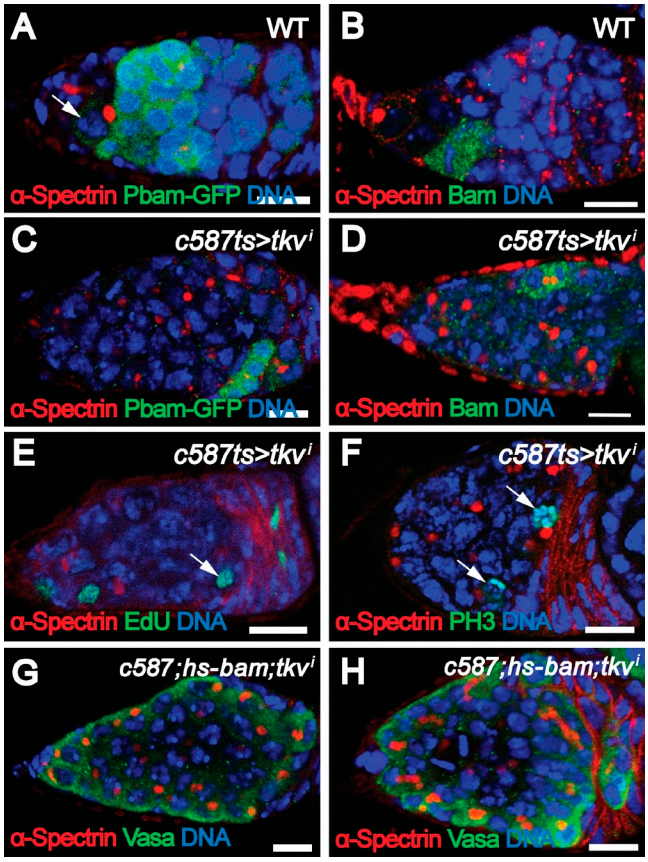
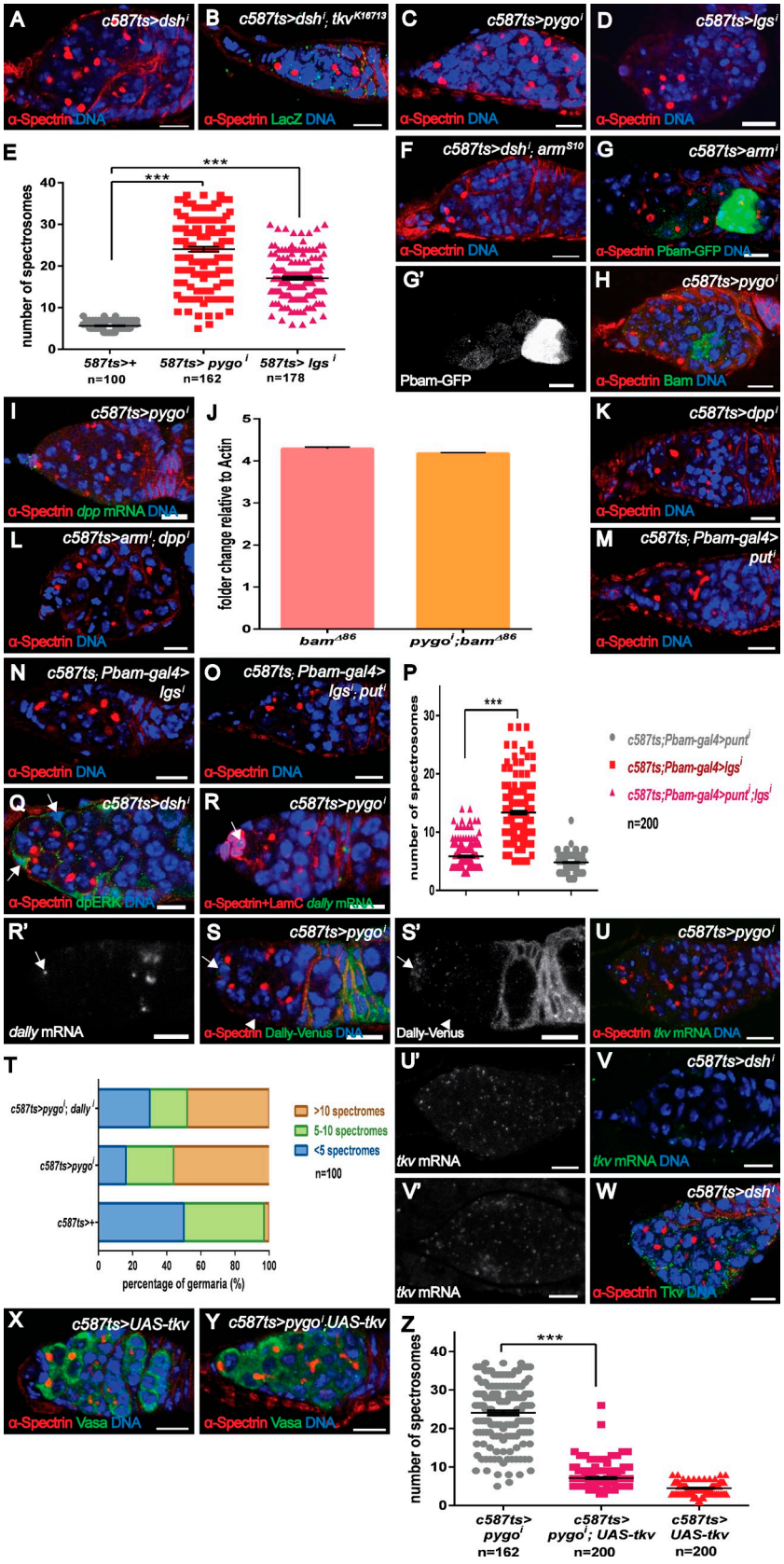


Figure S3. **EC-expressed Tkv restricts germline proliferation.** (A) A control (P*bam-GFP*/+) germarium showing Pbam-GFP expression in the CB (arrow) and cyst. (B) A WT germarium showing Bam expression in the cyst. (C) A *tkv*^{i} (BL40937) germarium contains many cells with low/no Pbam-GFP expression. (D) A *tkv*^{i} (v3059) germarium exhibits ectopic spectrosome-containing cells that do not express Bam. (E and F) Some of the ectopic spectrosome-containing cells in *tkv*^{i} (v3059) germarium are positive for EdU (E, arrow) or anti-phosphorylated Histone 3 (pH3; F, arrows). (G and H) Ectopic Bam expression in *tkv*^{i} germarium (G) promotes the differentiation of spectrosome-containing cells (H). Bars, 10 μm.

Figure S4. **Wnt signaling modulates Tkv expression in ECs.** (A–E) Knockdown of Wnt signaling components, including Dsh (A and B, BL-31306), Pygo (C, NIG-11518R-1), and Lgs (D, Bl-37476), in the ECs leads to the formation of ectopic spectrosome-containing cells and the down-regulation of tkv^{K16713} reporter expression (compared with Fig. S1 D). (E) Statistical analyses of spectrosome-containing cell number in $pygo^{i}$ or lgs^{i} germaria. (F) The overexpression of Arm^{S10} in the ECs of a dsh^{i} (BL-31316) germarium suppresses the formation of ectopic spectrosome-containing cells. (G) A arm^{i} (v107344) germarium showing ectopic low levels of Pbam-GFP–expressing cells. (H) Bam is not detected in the ectopic spectrosome-containing cells in a $pygo^{i}$ (NIG-11518R-1) germarium. (I) A $pygo^{i}$ (BL-38208) germarium showing that *dpp* mRNA is detected only in the cap cells. (J) Quantitation of *dpp* mRNA level in $bam^{\Delta 86}$ and $pygo^{i};bam^{\Delta 86}$ germaria. (K and L) knockdown of Dpp in the ECs of arm^{i} (BL-35004) germarium does not suppress the formation of ectopic spectrosome-containing cells. (M–P) knockdown of Put in the germline in lgs^{i} (BL-37476) germarium suppresses the formation of ectopic spectrosome-containing cells. (Q) dpERK is detected in the ECs (arrows) of a dsh^{i} (BL-31306) germarium, similar to WT germarium (Fig. 3 A). (R–S) *dally* (shown by in situ hybridization and reporter expression) is normally expressed in a $pygo^{i}$ (NIG-11518R-1) germarium (arrows indicate cap cells). (T) Knocking down Dally in the ECs of a $pygo^{i}$ (NIG-11518R-1) germarium does not suppress the formation of ectopic spectrosome-containing cells. (U and V) *tkv* mRNA is down-regulated in $pygo^{i}$ (U, NIG-11518R-1) and dsh^{i} (V, BL-31316) germaria. (W) Tkv is reduced in the dsh^{i} (BL-31316) germarium. (X–Z) The expression of Tkv in ECs of the $pygo^{i}$ (NIG-11518R-1) germarium strongly suppresses the formation of ectopic spectrosome-containing cells. Error bars represent SEM. ***, $P < 0.001$. Bars, 10 µm.



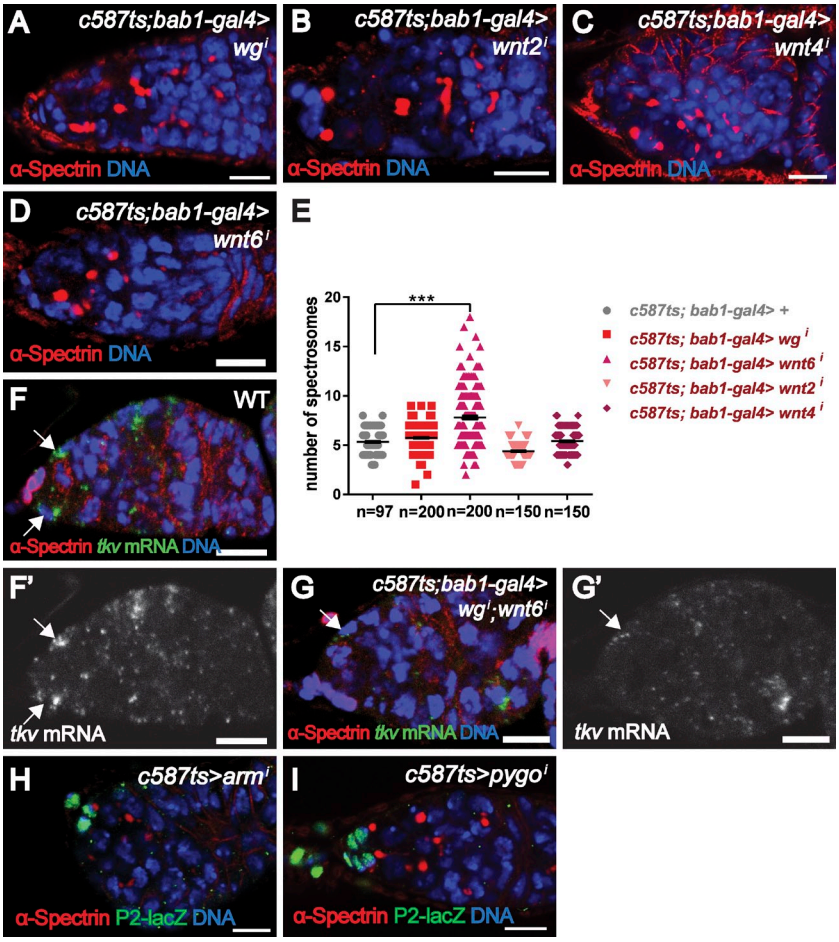


Figure S5. **The function of Wnts in the germarium.** Knockdown of Wg (A, v104579), Wnt2 (B, v104338), or Wnt4 (C, BL-29442) using *c587;bab1-gal4* does not result in the formation of ectopic spectrosome-containing cells. (D) Removing Wnt6 (BL-30493) from cap cells and ECs leads to a slight increase in the number of spectrosomes. (E) Statistical analyses of the number of spectrosomes in A–D. (F–G) *tkv* mRNA is down-regulated in the ECs (arrows) of a germarium with compromised Wg and Wnt6 functions in cap cells and ECs. (H and I) The P2-lacZ reporter is down-regulated in the ECs of *arm*^{i} (F, BL-35004) and *pygo*^{i} (G, BL-38208) germaria. ***, P < 0.001. Bars, 10 μm.

Table S1. **Primers used to generate *tkv* reporter lines**

Name	Location	Primers
P1	2L:5231kb..5237kb	5′-tggatgcgctcgggagaacaggag-3′ 5′-ctggagatgctgcttacagcagag-3′
P2	2L:5237kb..5245kb	5′-ggatgggattgtatggacggagtg-3′ 5′-atccgcagttaaggcatatcctgtg-3′

Table S2. **Primers for ChIP experiment**

Name	Forward primer	Reverse primer
1	5′-tcctgtgtgtttctggttgtt-3′	5′-gcatcaacggcaatctatagt-3′
2	5′-tcttcttggctctctccctt-3′	5′-gcactgcgatgctaactcaa-3′
3	5′-actcctggcattaaaaacgca-3′	5′-tttaagttcgggaggcataga-3′
4	5′-tcggggtctgctctgcttt-3′	5′-tcagcagccacatccacaat-3′
5	5′-tacaatatactgtggcggcta-3′	5′-ctgtcaatttctcaagcgtag-3′
6	5′-aggttgattctagccatacata-3′	5′-tggatggaatagtgttcccc-3′
7	5′-gtgttgttgggtgtgtgtaaa-3′	5′-tgcaaaagtgtgcaagaacga-3′
8	5′-aggcgcgtcaaacataatttg-3′	5′-tccctcctttggcattaagtt-3′
9	5′-ggggatactaaccactatact-3′	5′-aacttatgatcataacactcgg-3′
10	5′-caaaaaaagggagagcgggt-3′	5′-tatttcaggcgcccgacaaa-3′
11	5′-aaaggggttaggcctcctg-3′	5′-agcgccaaccacttttttctg-3′
12	5′-gtgtaaattatgggaaaccgc-3′	5′-aggcactttcagctaacaaata-3′
13	5′-atgtgggtgcagccaaagaa-3′	5′-agccaatttgcacgtgctcc-3′
14	5′-tgacagtttaacagccgcca-3′	5′-tgggctgcacggtgttctt-3′
15	5′-gattgtaaagtcaaggcatgc-3′	5′-cagaccacacccaattgattt-3′
16	5′-gttgtgggttcgggttcata-3′	5′-agaaacgtgtgacttaccgaa-3′
17	5′-gttttgaaactgggtcgtcg-3′	5′-ctatttttgagtggccaatgc-3′
18	5′-gcatcggcgtgtgaacatta-3′	5′-cgcgtaggcagaatgtgtaa-3′
−1	5′-aacatgaagaagaaggattgtc-3′	5′-gataacctattcattgcaagct-3′
−2	5′-tgggtaagaactggtaagatc-3′	5′-agggctttaagcagatactatt-3′
−3	5′-tgacttttaccatcgcagcc-3′	5′-ctacttttttggctccaacctt-3′
−4	5′-cctggactttgccaccattt-3′	5′-cccaaagtcagtcgaaatgg-3′
N4	5′-tcaatcagacgtcagaggtaccg-3′	5′-ctgatggaagaaccgtgttgg-3′

Table S3. **Primers used for quantitative real-time PCR**

Name	Forward primer	Reverse primer
dpp	5′-ccgctcctgttcacctacac-3′	5′-ggtgtcgtcgtggttgttg-3′
actin 5c	5′-ggatctccaagcaggagtacg-3′	5′-tcctccagcagaatcaagacc-3′

Table S4. **Primers used for in situ experiments**

Name	Forward primer	Reverse primer
wnt2	5′-tacatccgtcatgctctgtgg-3′	5′-gacttcgctcgcagtagttgg-3′
wnt4	5′-aatgctggtcaggtggtgaac-3′	5′-cgtgcttcagattgtcattgc-3′
wnt5	5′-ttgcacaacatgaccaagacc-3′	5′-cgtgacggatgtgttgttctc-3′
wnt6	5′-caccaacatccttctcgatcc-3′	5′-cttcagccagcaggtcttcac-3′
wnt10	5′-ctccaagctcttcctcgactg-3′	5′-ttcttatgcatgtggctgagg-3′
wg	5′-ggctcgaaggaccttgtctatc-3′	5′-atgcaagcagttcaagcagtg-3′
tkv	5′-ctacaagcgacgcgagaagctgc-3′	5′-gagacccactgaatacatatcag-3′
dally	5′-cgtgtgtgccagtgtgtgt-3′	5′-taatacgactcactatagggcgccgcctgtgtatctatg-3′
dpp	5′-aggacgatctggatctagatcggt-3′	5′-actttggtcgttgagatagagcat-3′

Table S5. **Primers used for generating constructs of luciferase assay**

Name	Forward primer	Reverse primer
#1	5′-tttctgtttgccccgtaggt-3′	5′-caatccgcagttaaggcatat-3′
#2	5′-ccacccagcaatttgcaatc-3′	5′-gcactgttcccctcgaaatt-3′
#3	5′-tggatgggattgtatggacg-3′	5′-acgcaatgagcgcgacttga-3′

Table S6. **Primers used for antibody generation**

Name	Forward primer	Reverse primer
Tkv	5′-caccatggccgccgccgctttgagtggc-3′	5′-tcagttgcagaagtcctccttgtcgcagc-3′
α-Spectrin	5′-gtgaagatcctcgagacagtcgag-3′	5′-tgcaggtagtaggactcatc-3′