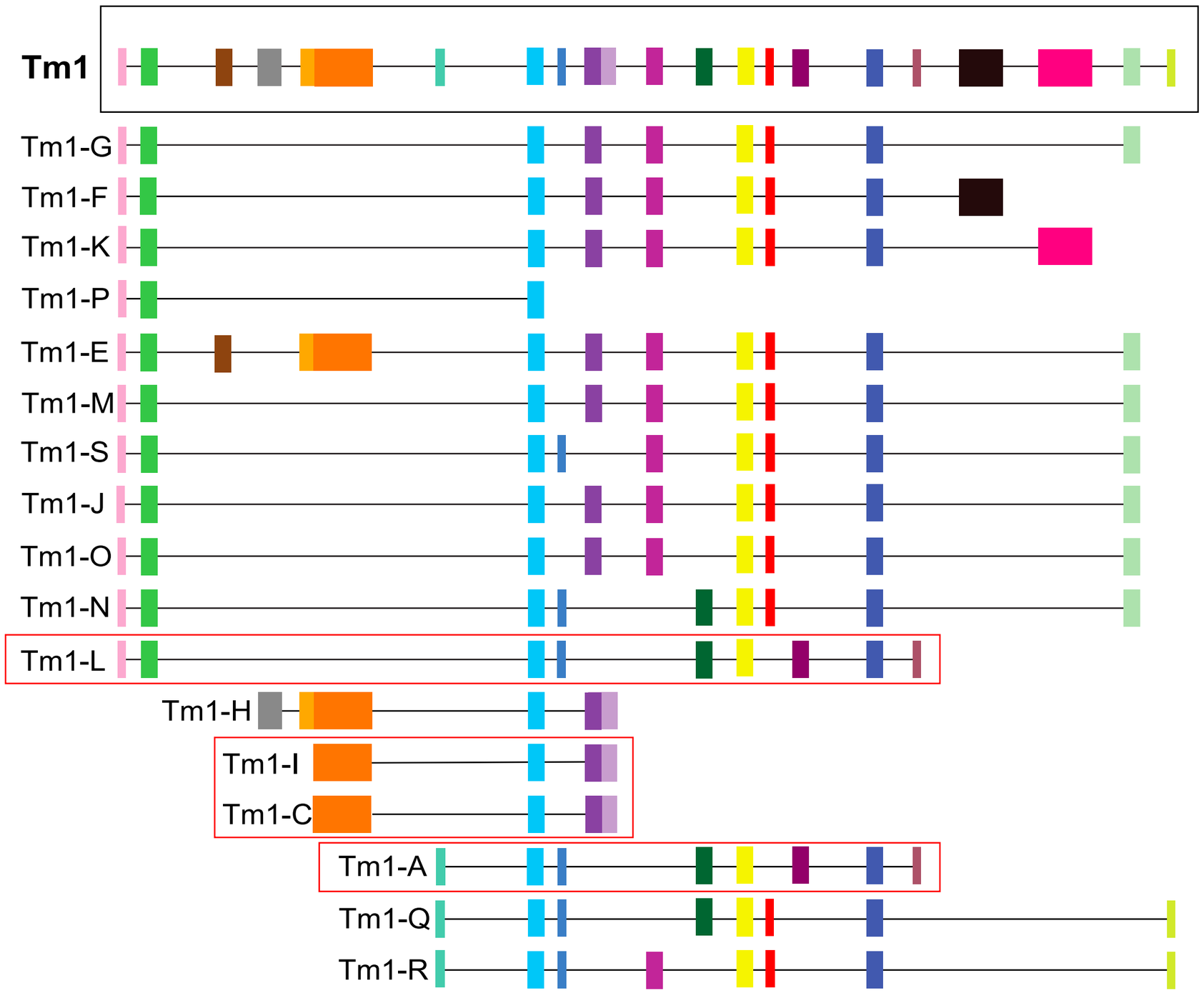
Figure S1

A



B

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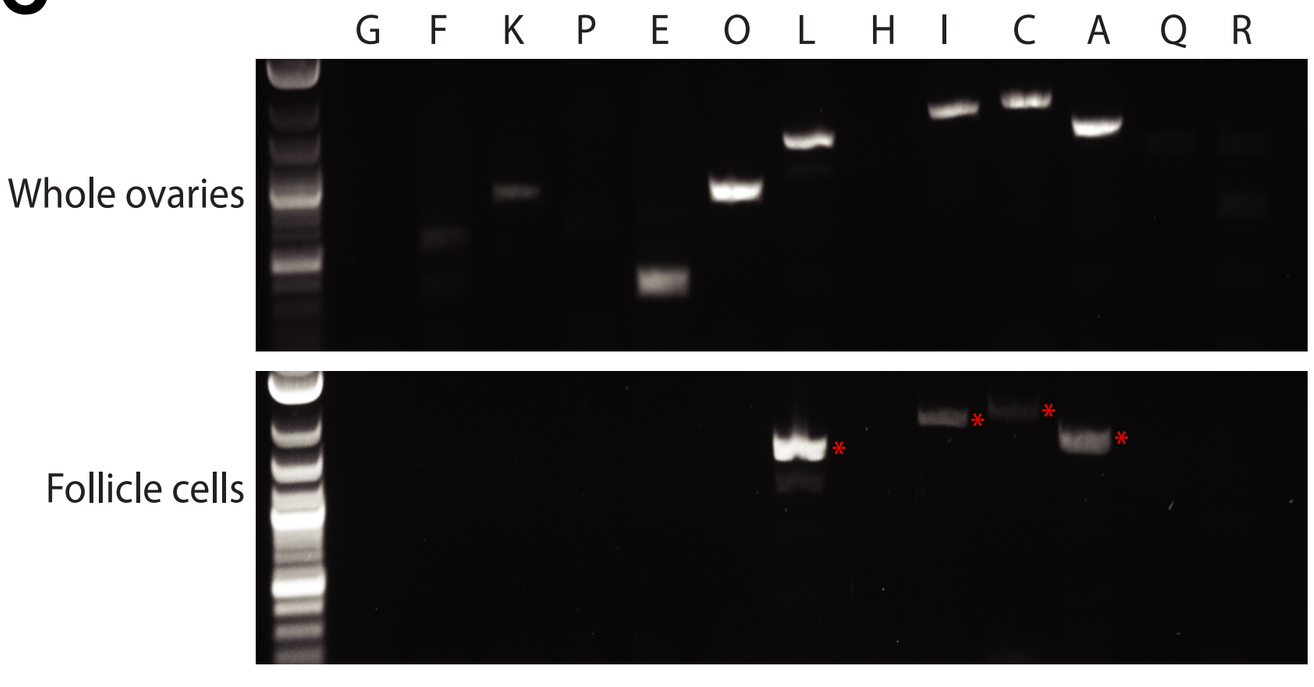
VTNSLGSNSPTPTDESSVQDEGASNPAAALIAEDAAPPAGTTTSKSKKKKREKGERSEKSDKSEKSDRKKKSSGKKERSKRSNPMEQSSDSLATDLSAGA
IDEGIALADDDDNQAAEWSKLRCTSEAAEIVAEREARRNKGRCADYPGLAFGRSIFSSDTMMKFNIIRNELHNIMNTQLKRAESEVAALNRRIQLLEEDL
ERSEERLGSATAKLSEASQAADESERARKILENRALADEERMDALENQLKEARFLAEEADKKYDEVQLKTNLSSIKLSNNNNNSNSNNIEISKSESCNAS
DIGGTNNNNASRTIASAAVGEETSTLSSTSHEHNNNPNNDT

C



D

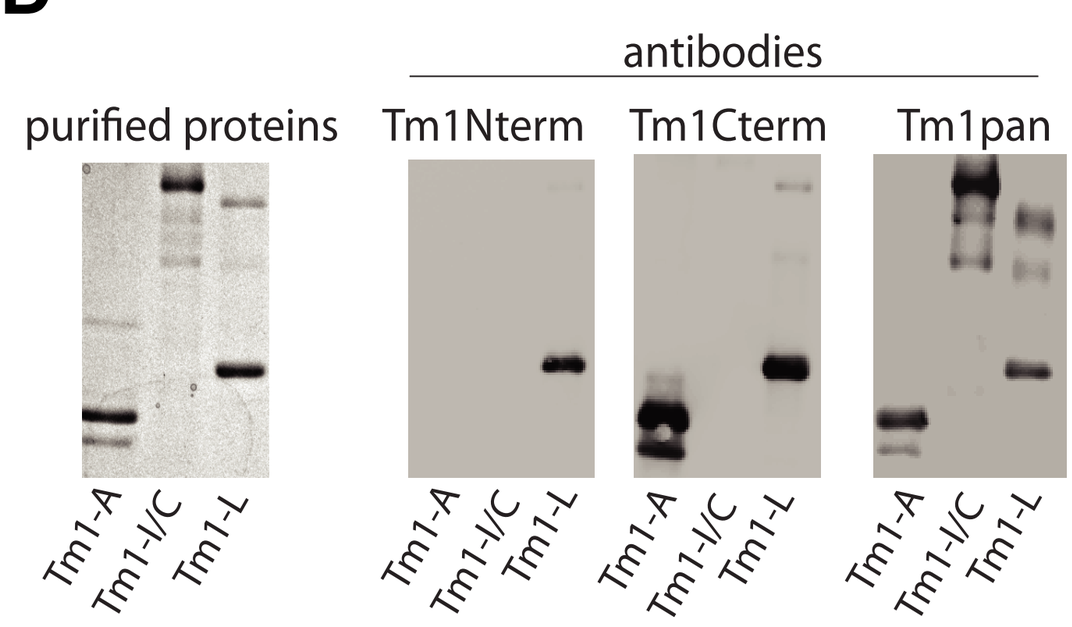


Figure S1. Genomic Organization and Isoform Detection by rt-PCR, Related to Figure 1

(A) Exon composition of the Tm1 gene adapted from www.flybase.org. Tm1-G/J/M/O encode the same protein but differ in the 5' and 3'untranslated regions (UTRs). Tm1-I/C encode the same protein but differ in the 3' UTRs. Four isoforms- Tm1-A, Tm1-I/C, and Tm1-L detected in follicle cells are outlined in red. (B) Amino acid sequence of Tm1-I/C. The amino acids coded by the common exon and shared between all Tm1 isoforms are in red. (C) rt-PCR results from mRNA extracted from whole ovaries or specifically from follicle cells using mRNA tagging (see Experimental Procedures for details.) Primers were designed to amplify the indicated isoform specifically, except for band O where the primers detected Tm1-M/O/J/N/S. While most isoforms were detected in whole ovary mRNA, only Tm1-A, Tm1-I/C and Tm1-L were detected from the follicle cell specific mRNA. (D) Tm1-A, -I/C and –L proteins were expressed in and purified from bacteria (left panel). Right panels show Western blots of the purified proteins using three antibodies: Tm1Nterm(L) recognizes isoform L but not A or I/C. Therefore this antibody recognizes an N-terminal epitope and should bind any isoform that shares the two N-terminal exons with L; Tm1Cterm(A/L) was raised against the full length –A isoform and reacts with Tm1-A and Tm1-L but not Tm1-I/C. Therefore this antibody should recognize any isoform that shares C-terminal exons with A and L; and Tm1pan(I/A/L) reacts with all isoforms.

Sup. Figure 2

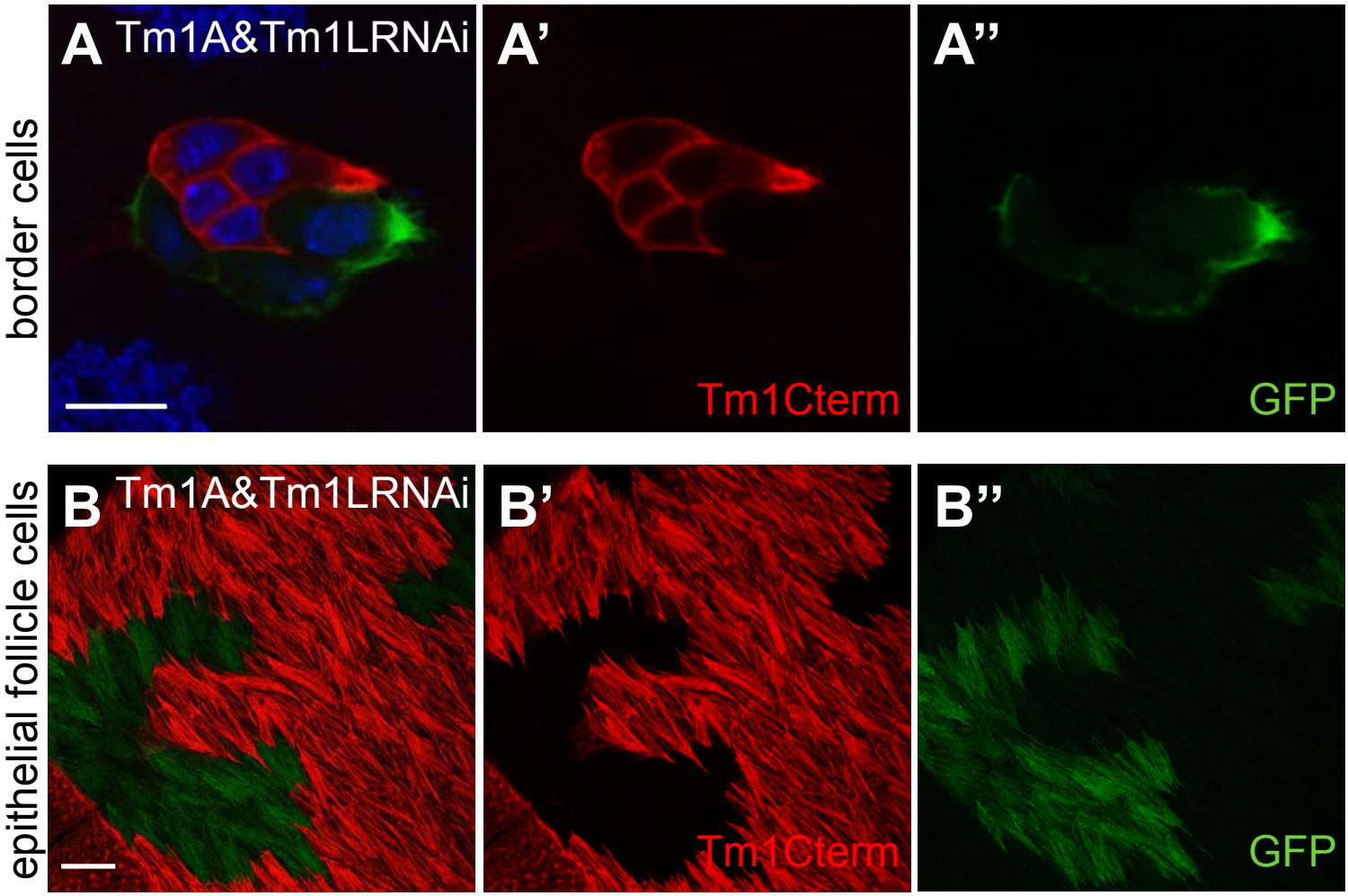

A Tm1A&Tm1LRNAi
A'
Tm1Cterm
A''
GFP
border cells
B Tm1A&Tm1LRNAi
B'
Tm1Cterm
B''
GFP
epithelial follicle cells

Figure S2. Effective Knockdown of Tm1-A and Tm1-L Isoforms, Related to Figure 2. (A-B) Co-expression of Tm1-A and Tm1-L RNAi lines in a subset (GFP+, green) of border cells (A) and epithelial follicle cells (B) results in effective knockdown of both Tm1-A and Tm1-L isoforms detected by Tm1Cterm (A/L) antibody (red). Scale bars, 10μm.

Figure S3

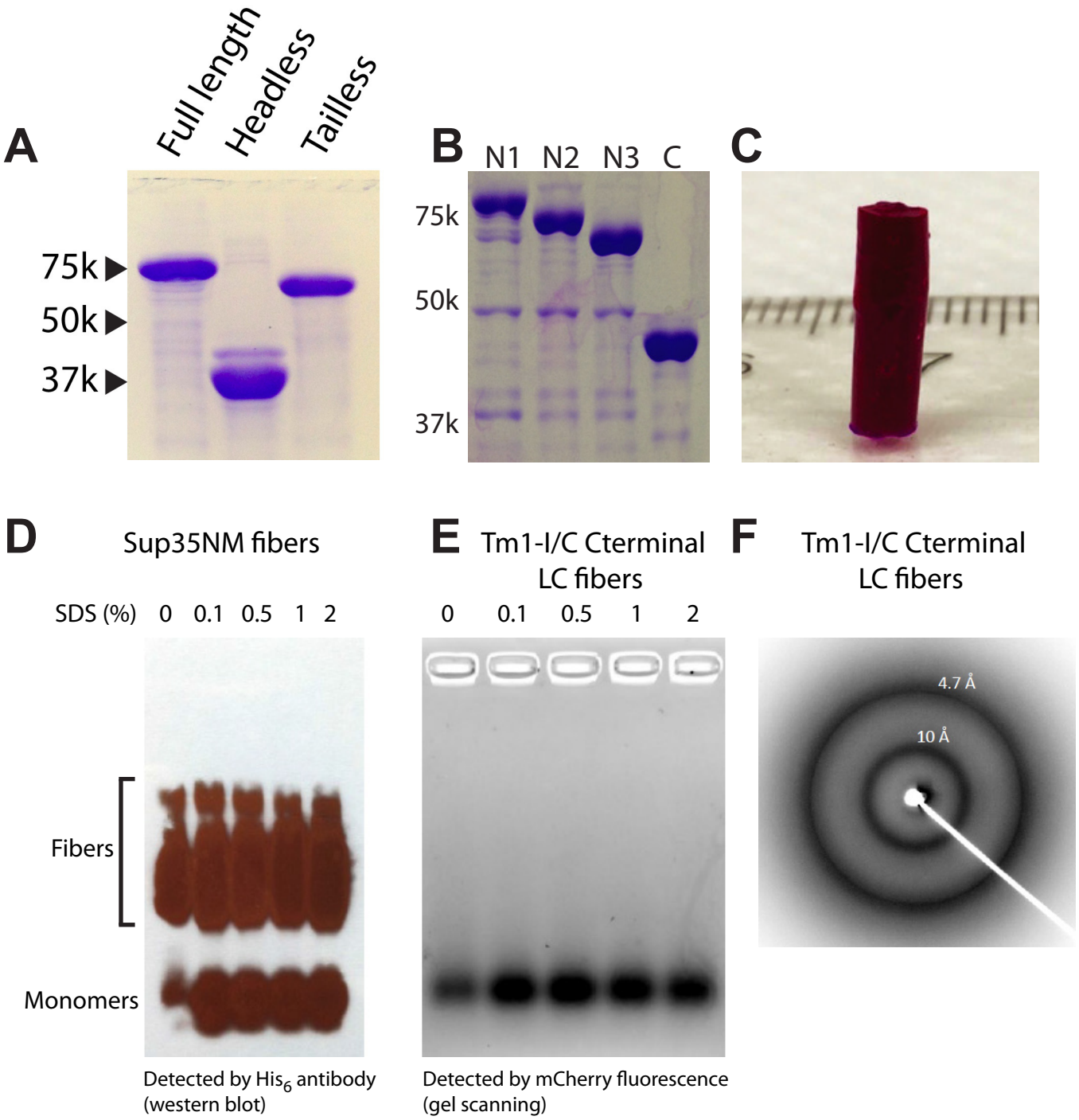
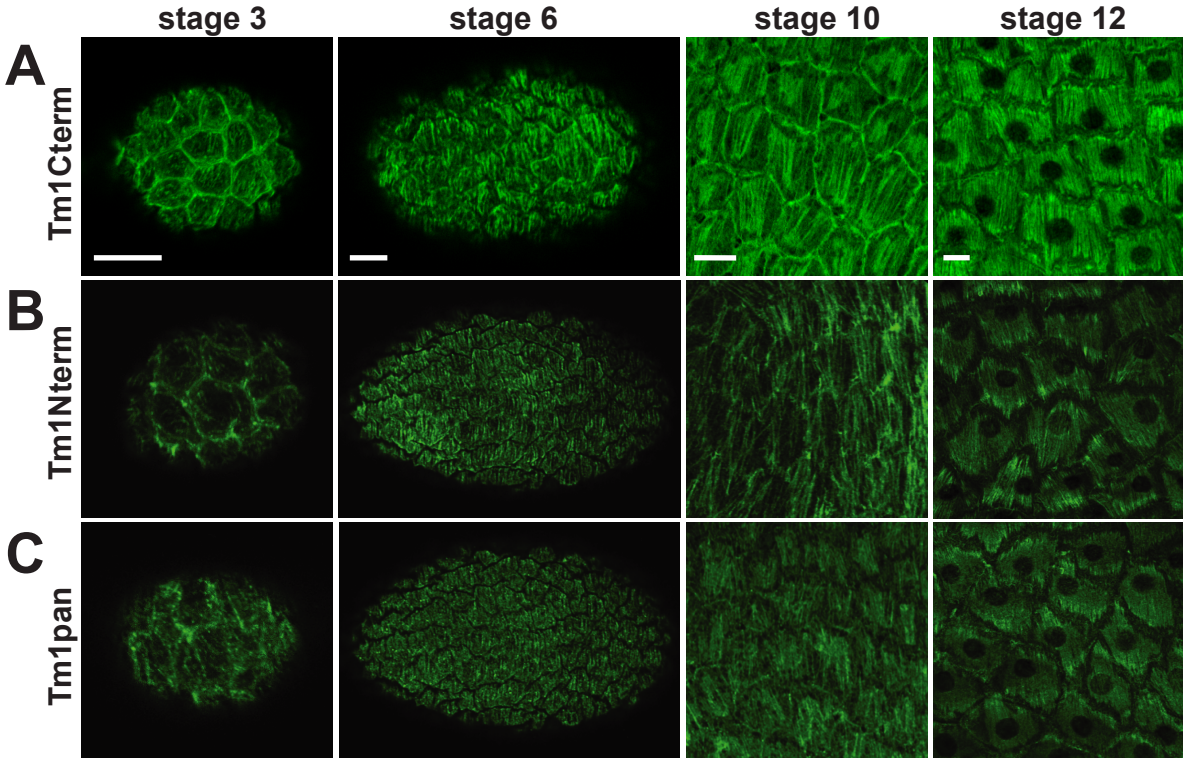

A
Full length
Headless
Tailless
75k
50k
37k
B
N1 N2 N3 C
75k
50k
37k
C
D
Sup35NM fibers
SDS (%) 0 0.1 0.5 1 2
Fibers
Monomers
Detected by His6 antibody
(western blot)
E
Tm1-I/C Cterminal
LC fibers
0 0.1 0.5 1 2
Detected by mCherry fluorescence
(gel scanning)
F
Tm1-I/C Cterminal
LC fibers
4.7 Å
10 Å

Figure S3. In Vitro Expression and Analysis of Tm1-I/C Protein Domains, Related to Figure 3

(A, B) Coomassie stained polyacrylamide gel showing the purified proteins diagrammed in Figure 3. (C) Hydrogel formed by the C-terminal low complexity domain of Tm1-I/C (D) Western blot showing the insolubility of yeast prion Sup35 fibers even in 2% SDS (E) Labile Tm1-I/C C-terminal domain fibers dissociate even in the lowest concentrations of SDS. (F) X-ray diffraction from m-Cherry-Tm1-I/C C-terminal domain hydrogel shows a typical pattern for cross-β fibers.

Figure S4


stage 3
stage 6
stage 10
stage 12
A
Tm1Cterm
B
Tm1Nterm
C
Tm1pan

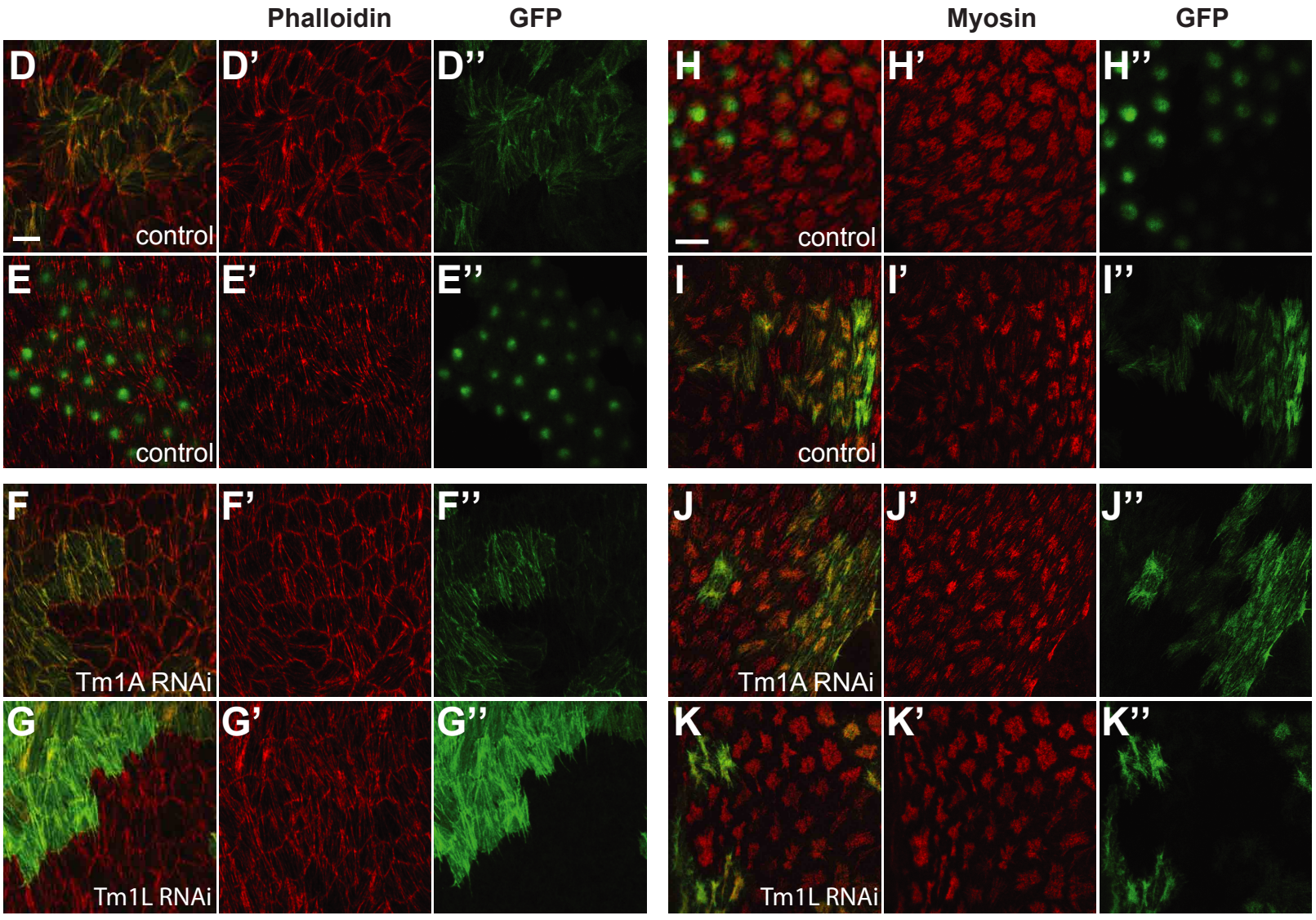

Phalloidin
GFP
Myosin
GFP
D
D'
D''
control
H
H'
H''
control
E
E'
E''
control
I
I'
I''
control
F
F'
F''
Tm1A RNAi
J
J'
J''
Tm1A RNAi
G
G'
G''
Tm1L RNAi
K
K'
K''
Tm1L RNAi

Figure S4. Canonical Tm1 Expression and Knockdown in Epithelial Follicle Cells, Related to Figure 4.

(A-C) Stress fiber labeling with anti Tm1 antibodies. Tm1Cterm antibody (A), Tm1Nterm antibody (B) and Tm1pan antibody (C) as they develop during stages 3 to 12. (D-E) Control clones (GFP+, green) made using the MARCM (D) and Flpout (E) techniques (see Experimental Procedures for details) and stained with phalloidin (red) to label F-actin stress fibers. GFP+ and GFP- cells show similar patterns and intensity of phalloidin labeling. (F-G) Phalloidin staining of stress fibers in clones of cells (GFP+, green) expressing either Tm1-A RNAi (F) or Tm1-L RNAi (G). GFP+ (RNAiexpressing) and GFP- (wild type) cells show similar patterns and intensity of phalloidin labeling similar to control clones. (H-K) Myosin accumulation detected by sqh:sqhmcherry (red) in MARCM (H) and FLP-Out (Moesin-GFP positive, green) clones of cells expressing either no RNAi (H, I), or Tm1-A RNAi (J) or Tm1-L RNAi (K). In all cases, clones show similar myosin accumulation to the neighboring wild type (GFPnegative) cells. All scale bars 10μm.

Figure S5

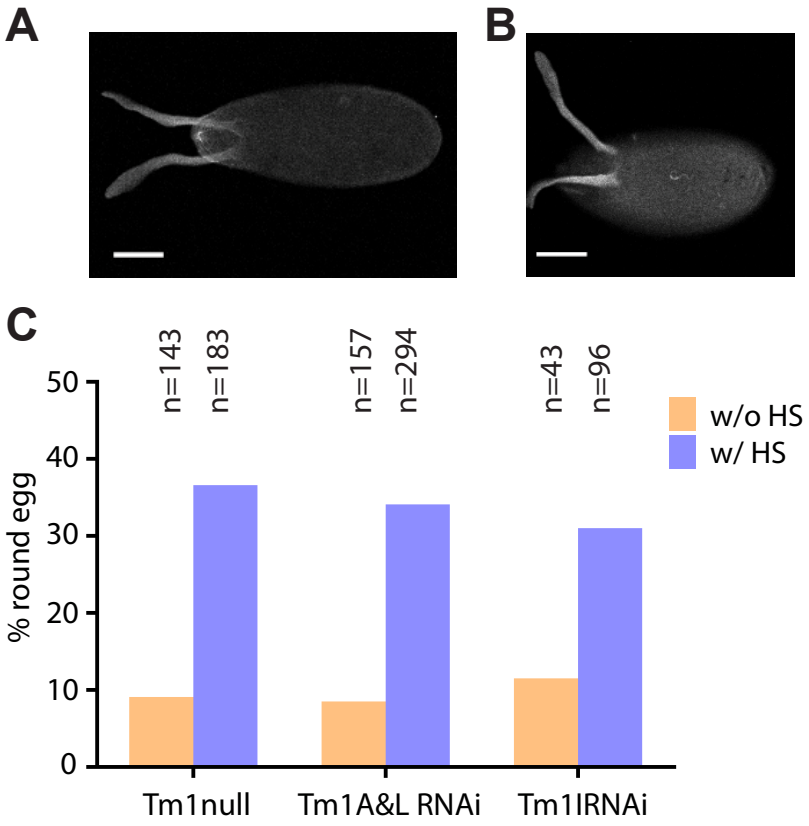

A
B
C
n=143
n=183
n=157
n=294
n=43
n=96
w/o HS
w/ HS
% round egg
50
40
30
20
10
0
Tm1null
Tm1A&L RNAi
Tm1IRNAi

Figure S5. Round Egg Phenotype of Eggs from Tm1 Mosaic Females, Related to Figure 5.

(A) Wild type, elongated egg (B) Round egg from a Tm1 mosaic female (C) Percentage of round eggs obtained from mosaic females for the indicated mutations or RNAi-expressing cells in the absence of heat shock induction of FLP recombinase expression (w/o hs) or following clone induction with heat shock (w/ hs).

Supplementary experimental procedures

Transgenic flies expressing RNAi hairpins

P[UAS-Tm1I/C-RNAi] was obtained from VDRC (v34119). To generate RNAi lines specific for Tm1-A and Tm1-L, the hairpin sequences (Tm1-A: AAGAAGATGCGCCAGACCAAA; Tm1-L: GCAAGCGATGAAAGTCGACAA) were selected and cloned into the VALIUM20 vector by TRiP facility (Harvard). The amplified plasmid was injected into y,v;nanos-integrase;attP40 (2nd chromosome) or y,v;nanos-integrase;attP2 (3rd chromosome) by Rainbow transgenics.

Generating knock out lines using ends out targeting and CRISPR

In order to generate Tm1null, we took advantage of ends out targeting method (Gong and Golic, 2003). The 135 basepair gene region that is common to all isoforms was targeted for deletion using left arm primers (Fwd: 5' GTTGGCCTTCTAGCTTTCTG 3'; Rev: 5' GACAAACGTTCGATGGACGA 3') and right arm primers (Fwd: 5' AGTACCACGTTACCATCTA 3'; Rev: 5' GGTCCCAGTTATGGATTCCA 3').
The left and right arms were amplified using above primers and w^{1118} genomic DNA as the template. The two ~3kb fragments were inserted into pW35 vector using Infusion cloning (Clontech) and was verified by sequencing. The cloned plasmid was amplified was injected into w1118 flies (Bestgene). The flies were crossed to hs-flp hs-ISceI /CyO and heat shocked to excise and linearize the common exon targeting insert. Successful deletion/replacement with w+ was confirmed by PCR. For generating CRISPR Tm1null line, two guide RNAs (Tm1-Start: ccatcgaacgtttgtcttatagG, Tm1-End: ctacgaattcatatagtctacgg) under U6 promoter were injected into Cas9 expressing flies (Rainbow Transgenics). Sucessful deletion was verified by PCR and antibody staining.

Generating transgenic lines

Tm1-I was amplified using specific primers and Drosophila EST LD11194 as a template. LD37158 was used for Tm1-A amplification. Since cDNA for Tm1-L did not exist, two PCR fragments amplified from RE08101 and LD37158 were combined to make full length Tm-L. The amplified sequences were cloned into HA-UASt vector for N terminal HA tag expression or EGFP-UASt vector for N terminal EGFP tagged protein. Some of the expression vectors (with C-terminal tags) were made by utilizing available Gateway vectors and its cloning system (Invitrogen). Cloned plasmid was verified by sequencing and sent to Bestgene for transgenic fly generation. Collection of UAS-EGFP-Tm1-A, UAS-EGFP-Tm1-I/C, UAS-EGFP-Tm1-L, UAS-Tm1-A-RFP, UAS-Tm1-I/C-EGFP, UAS-HA-Tm1-A, UAS-HA-Tm1-I/C, and UAS-HA-Tm1-L was made for this project.

Generating Tm1Cterm (A/L) and Tm1pan (I/A/L) antibodies

For polyclonal antibody generation, Tm1-I isoforms were amplified using specific primers and EST LD11194 as a template. LD37158 was used for Tm1-A amplification. Each amplified sequence was cloned into pET28-a vector (Clonetech). The plasmids were transformed into BL21 cells and the protein was induced using 0.5mM IPTG. The His tagged proteins were purified using Ni-NTA His Bind Purification kit (Novagen) and eluted with 100 mM and 250 mM imidazole. Total of 5mg of each protein was purified and concentrated using Amicon Ultra centrifugal filters (Millipore, USA) and sent out to Antibodes, Inc for antibody generation.