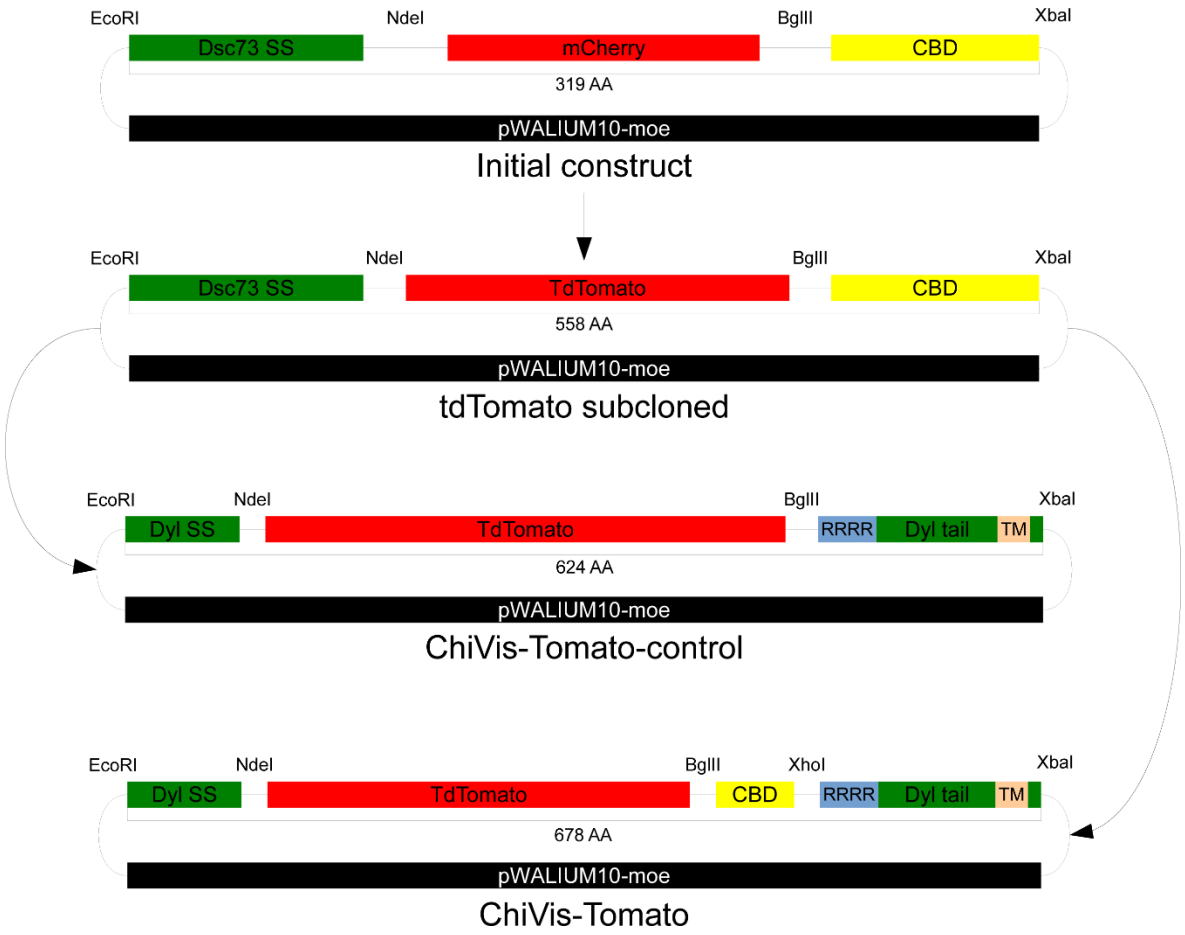

Fig. S1. The construction of UAS-ChtVis-Tomato. The steps taken to construct UAS-ChiVis-Tomato are shown. More details are provided in the Methods.

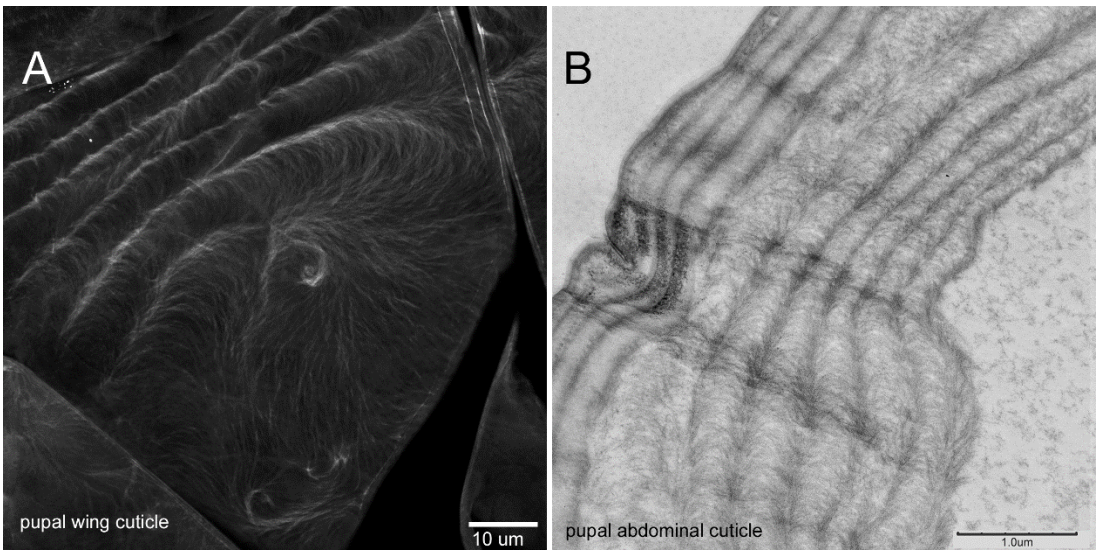

Fig. S2. A. A confocal image of pupal wing cuticle labelled by the ChtVis-Tomato. This is an en face view. The image is a maximum projection of a small number of optical sections. B. A transmission electron microscope image of pupal abdominal cuticle. This is a z section through the cuticle. Outside is to the left and the space between the pupal cuticle and the developing adult abdomen is to the right. Note the very different scales between A and B.

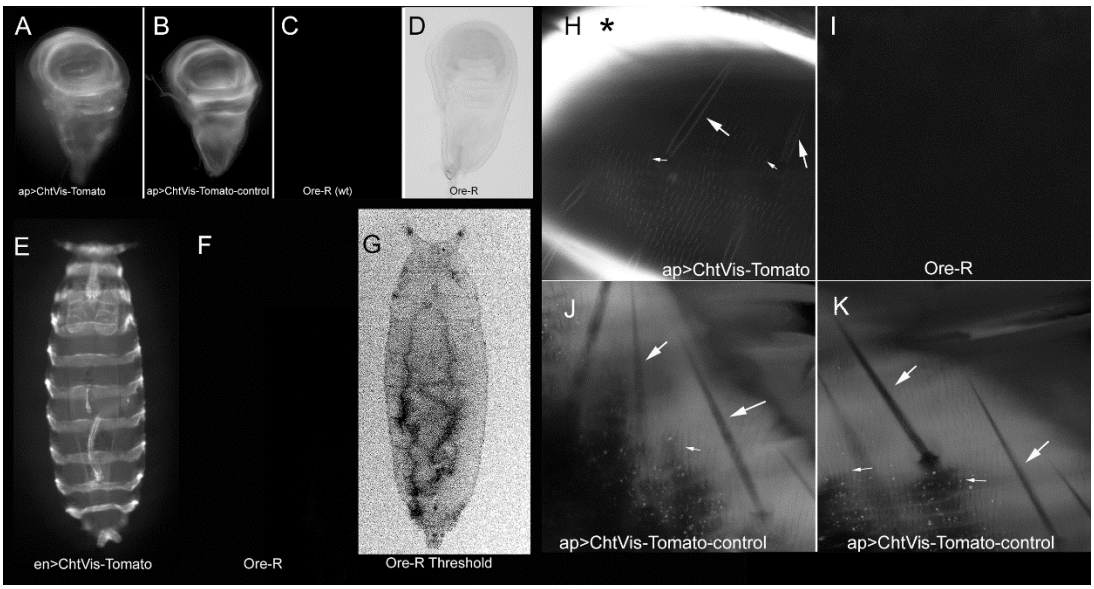

Fig. S3. Autofluorescence is negligible in this system. A-D are living third instar wing discs. A-C show td-Tomato fluorescence. All three images were taken with the same microscope settings. Note no fluorescence is obvious in the Ore-R wing disc. Note also that *ap>ChtVis-Tomato* and *ap>ChtVis-Tomato-control* show equivalent levels of fluorescence. E is an en-Gal4/UAS-ChtVis-Tomato white prepupae and F is an Oregon-R white prepupae . Both E and F were taken with the same microscope settings. Note the enormous difference between these two. G is the image shown in F, where we did an inverse thresholding in ImageJ to show the very faint image of the pupae. H-K are confocal micrographs of living pupae and all were taken at with the same microscope settings. Each is a maximal projection of 2-5 optical sections. H is a 53 hr *ap>ChtVis-Tomato* pupae. The large arrow points to a bristle and the small arrow to a hair. The asterisk is on a section of pupal cuticle that is too bright to see any detail. I is a 53 hr Ore-R pupae. Comparing this image to H establishes that autofluorescence was negligible in these experiments. J is a 53 hr *ap>ChtVis-Tomato-control* pupae and K a 62 hr *ap>ChtVis-Tomato-control* pupae. In both notice how the bristles (large arrows) and hairs (small arrows) can be seen as dark structures due to their exclusion of secreted reporter.

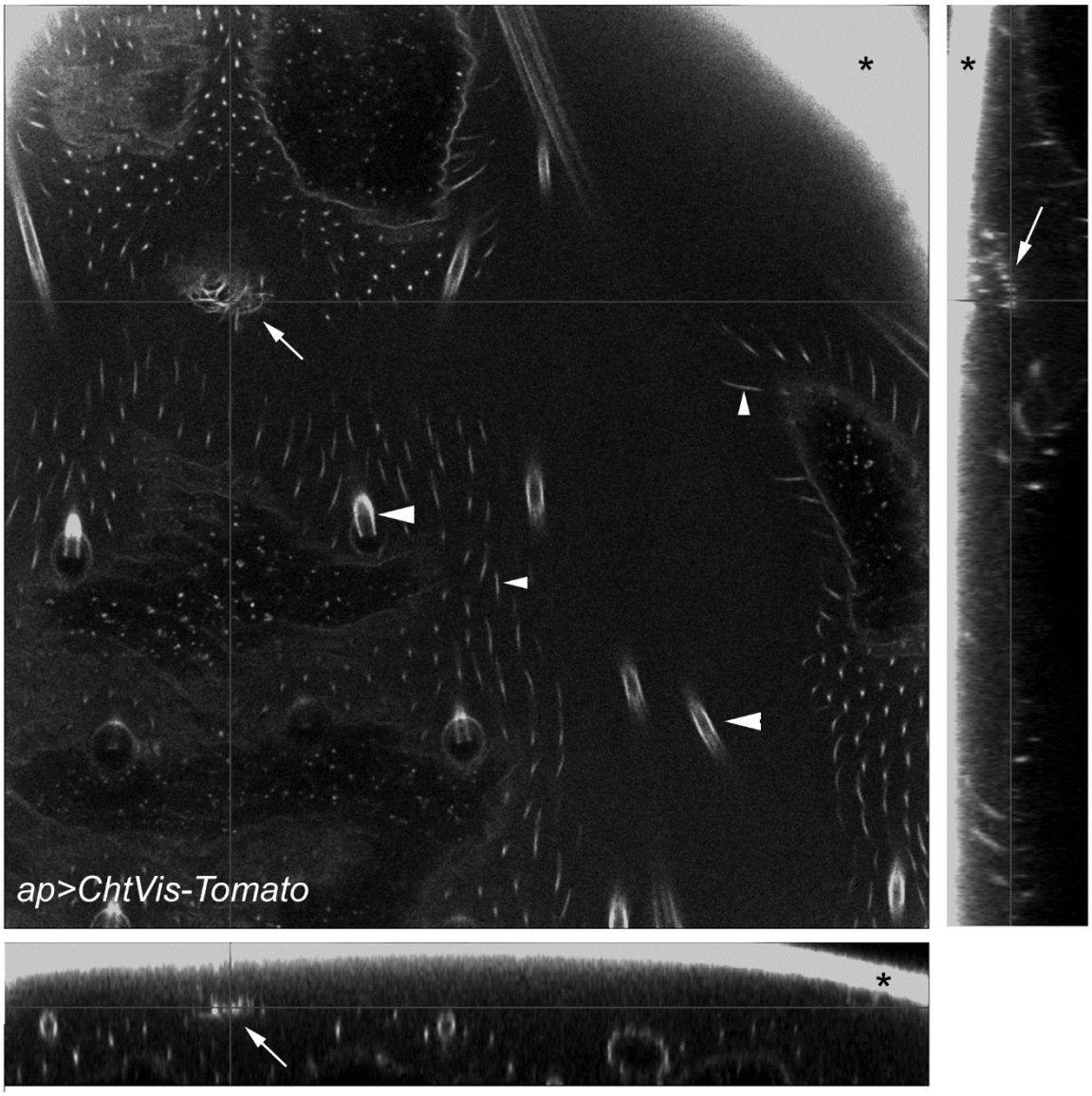

Fig. S4. Orthogonal views of a stack of images of an *ap-Gal4/UAS-ChtVis-Tomato* live notum. One optical section is shown en face. The position of the orthogonal views are indicated by the faint lines. These were drawn over a region of chitin fibers (arrow). Hairs (small arrowheads) and bristles (large arrowheads) are also visible. The pupal wing cuticle is visible in this image (asterisks). Note how the fibrous chitin is below the pupal cuticle but above the surface of the epithelial cells.

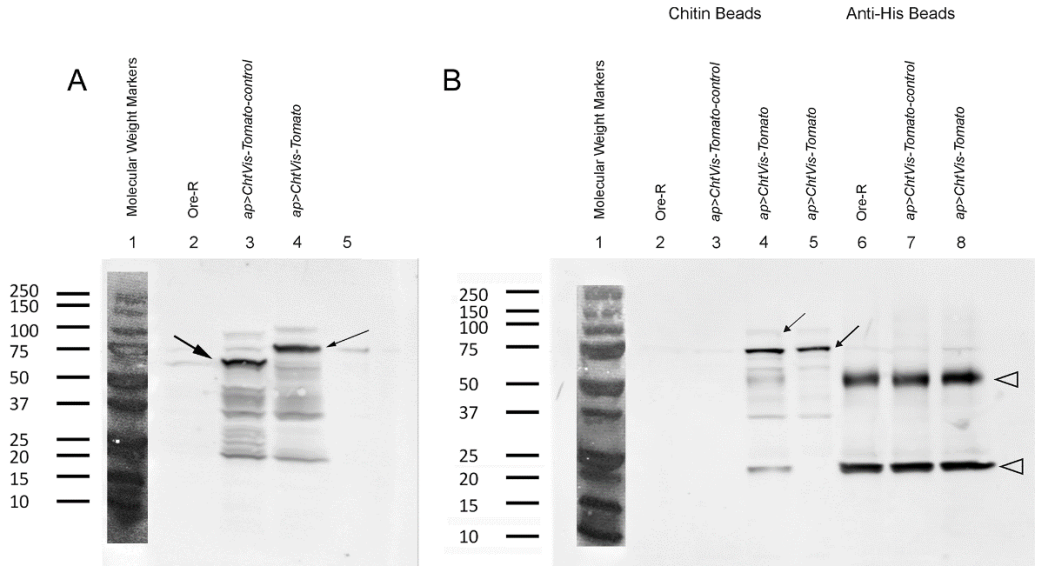

Fig. S5. Biochemical evidence that ChtVis-Tomato binds chitin. Both blots were probed with anti-Tomato monoclonal antibody. A. A control Western of wing disc samples homogenized in SDS sample buffer. Lane 1-molecular weight standard, lane 2 - Ore-R, lane 3 - *ap>ChtVis-tomato-control*, lane 4 - *ap>ChtVis-Tomato*, lane 5 - no sample. The thicker arrow points to the prominent band that corresponds to ChVis-Tomato-control (lane 3). As expected it is smaller than the ChtVis-Tomato protein (lane 4 - thin arrow). In both of these samples degradation products are seen. No protein found in Ore-R reacts with the antibody. The very weak band seen at the size of ChtVis-Tomato-control in the Ore-R sample is spillover from the neighboring lane. B. Wing disc samples were homogenized in bead binding buffer and then incubated with either Chitin magnetic beads or anti-His magnetic beads. Bound proteins were then released by incubation in SDS sample buffer. Lane 1 - molecular weight markers, lane 2 - Ore-R, lane 3 - *ap>ChtVis-Tomato-control*, lane 4 - *ap>ChtVis-Tomato*, lane 5 - replicate *ap>ChtVis-Tomato*, lane 6 - Ore-R, lane 7 - *ap>ChtVis-Tomato-control*, lane 8 - *ap>ChtVis-Tomato*. Note the *ap>ChtVis-Tomato* extract proteins released from the chitin beads. No proteins from the Ore-R or *ap>ChtVis-Tomato-control* extracts bound to the beads. The thicker arrow points to a prominent band around 75kd and the thinner arrow a faint band around 85kd. Uncleaved ChtVis-Tomato is predicted to be 76264kd, approximately the size of the prominent band and it may be that most of the reporter is uncleaved. However, the situation may be more complicated. It is possible that the 75kd band represents cleaved ChtVis-Tomato released from the membrane and the 85kd band the uncleaved protein. ChtVis-Tomato contains both potential N-linked and O-linked (mucin like) glycosylation sites and the protein could be glycosylated, which would lead to it migrating slower in the gel. This can explain the existence of the 85kd band and is consistent with the hypothesis that most of the protein is cleaved. We also observed a series of lower molecular weight bands in these samples that presumably represent degradation products. Similar bands were not seen in the Ore-R or *ap>ChtVis-Tomato-control* samples. In all of the samples eluted from the anti-His beads a prominent band was seen at around 50kd and one at ~25K (open arrowheads). The nature of these bands is uncertain but we suspect they represent anti-His6 antibody H and L chains released from the beads during the experiment. In all lanes on the gel there was a very faint band around the size of the strong ChtVis-Tomato band. We suspect this represents leakage during the application of the two *ap>ChtVis-Tomato* samples. The imaging of both blots was optimized for detecting the sample. The molecular weight sample images were quite faint so they were modified to facilitate detection of the bands.

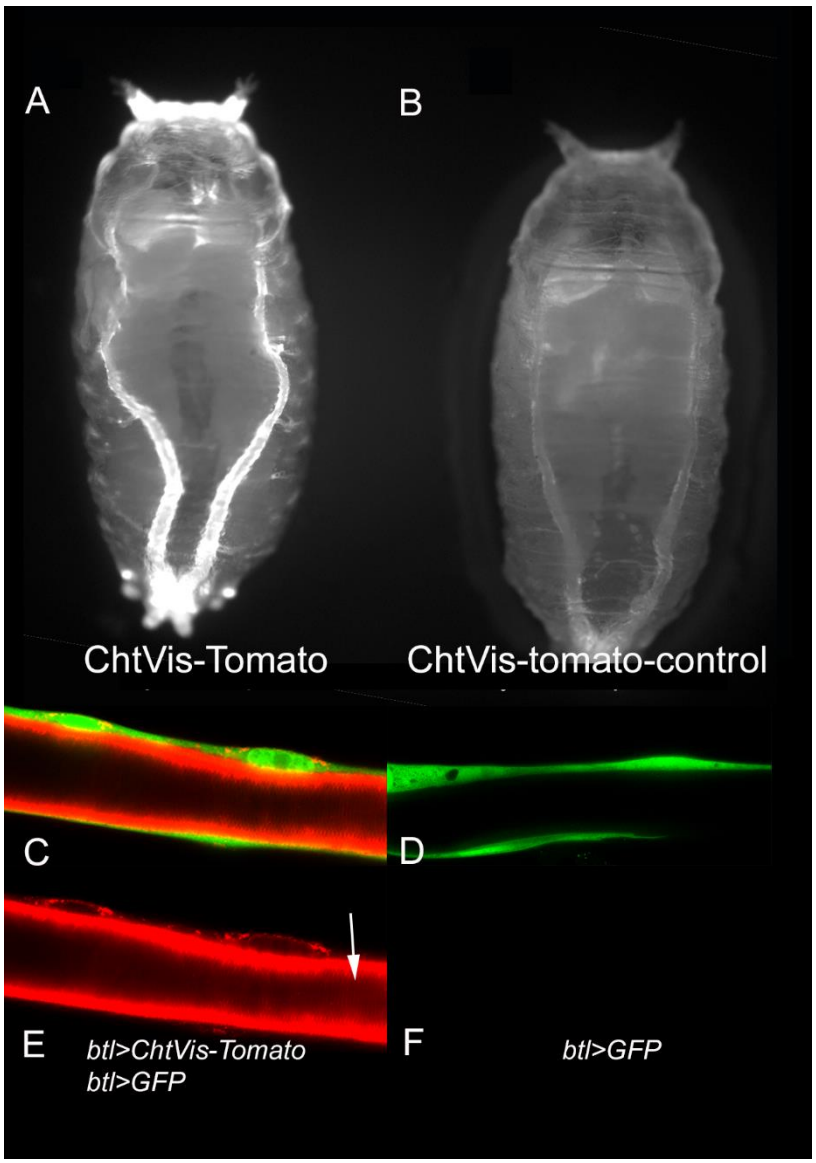

Fig. S6. The ChtVis-Tomato works to visualize chitin in trachea. A. A white prepupae showing brightly staining of the main tracheal branches by the ChtVis-Tomato. B. The bright staining of the tracheal branches is not seen when the ChtVis-Tomato-Control is used. Note A and B were from a single image of the two types of white prepupae placed next to one another on a microscope slide. C. A confocal image (single optical plane) of a *btl-Gal4 UAS-GFP/UAS-ChtVis-Tomato* trachea dissected out of a third instar larva and then imaged directly without staining or fixation. The arrow points to the distinctive taenidia that are typical of tracheal cuticle. D. A confocal image (single optical plane) of a *btl-Gal4 UAS-GFP* trachea dissected out of a third instar larva and then imaged directly without staining or fixation. This shows that fluorescence is negligible in this experiment.

ChtVis-Tomato construct

Restriction sites: EcoRI, NdeI, BglII, XbaI
CDS
dyl signal peptide and tail
tdTomato
Chitin-binding Domain
STOP

GAATTCAAAATGGGACACCTGACGCGACGCAGCAGCCTAATGGCCATAGTCCTGTGCCTGGT
ACTCAACACACAGCACCTGTCGGTGCATGGCGATGCCTCG**CA****TATG**GTGAGCAAGGGCGAG
GAGGTCATCAAAGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCATGAACGGCCACGA
GTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCT
GAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGT
ACGGCTCCAAGGCGTACGTGAAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTC
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CCCAGGACTCCTCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCACCAAC
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GCCTGTACCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGGCCCTGAAGCTGAAGGA
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TCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCACCAACTTCCCCCCCGA
CGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCC
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TACGTGGACACCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTA
CGAGCGCTCCGAGGGCCGCCACCACCTGTTCCTGTACGGCATGGACGAGCTGTACA**AG****ATC**
TATACGACAAATCCTGGTGTATCCGCTTGGCAGGTCAACACAGCTTATACTGCGGGACAATTG
GTCACATATAACGGCAAGACGTATAAATGTTTGCAGCCCCACACCTCCTTGGCAGGATGGGAA
CCATCCAACGTTCCTGCCTTGTGGCAGCTTCAAGGTGC**CTCGAG**AAGGCGCAGAGACACCAT
GGACATTGTGGTGAAGCCACAGAGGATCTACAAGAGGAACGCGCAGGAGATGACCGATGTG
AACACCAGCCGAATTATCCAGGTGGTGGCGCCCGGAGACGTTAACTTTGCGCTGAACAGCAA
TGCCAGCAACGAGACGGTGGTCATCCAGTCGGCCAGGTCCGCGGATGCGGAGACCATCTGC
ATGTCGGTGCCCAGCTTTGTGGGCGGATTGGTGATGCTGCTGCTCGTCCTGGCCGTCGCCTC
TCTAGTCGCCGCCTTCCTCTTCGTCCGCGTGCGTCACTTCGATCGCAAGGGAGCGGGCATGG
CCTATGTGAAC**TAA****TCTAGA**

ChtVis-Tomato-Control construct

Restriction sites: EcoRI, NdeI, BglII, XbaI
CDS
dyl signal peptide and tail
tdTomato
STOP

GAATTCAAAATGGGACACCTGACGCGACGCAGCAGCCTAATGGCCATAGTCCTGTGCCTG
GTACTCAACACACAGCACCTGTCGGTGCATGGCGATGCCTCG**CA****TATG**GTGAGCAAGGG
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CCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCG
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GCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGACCATCTACATGGCC
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CACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCTCCGAGGGCCGCCACCACCTG
TTCCTGGGGCATGGCACCGGCAGCACCGGCAGCGGCAGCTCCGGCACCGCCTCCTCCGA
GGACAACAACATGGCCGTCATCAAAGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTC
CATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCA
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CACCACCTGTTCCTGTACGGCATGGACGAGCTGTACA**AG****ATCT**CAAGAAGGCGCAGAGAC
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GACCATCTGCATGTCGGTGCCCAGCTTTGTGGGCGGATTGGTGATGCTGCTGCTCGTCCT
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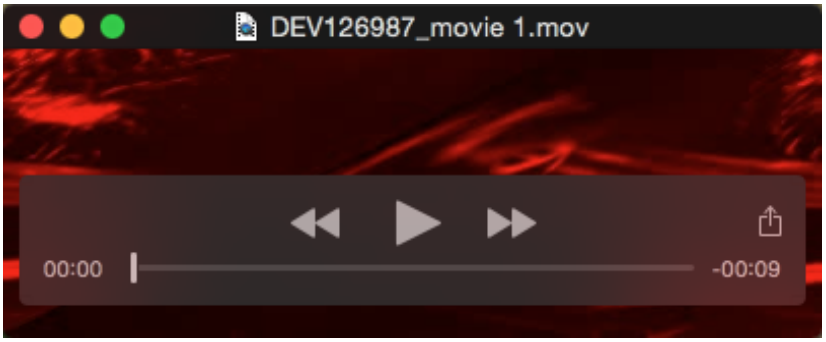
Fig. S7. Sequences of ChtVis-Tomato and ChtVis-Tomato-Control constructs.



Movie 1. FRAP shows evidence for two components of the ChtVis-Tomato. Shown is a movie of the FRAP experiment analyzed in Fig. 5.

Table S1. The sequence of the primers used in the construction of ChtVis-Tomato

Primer name	sequence, complementary (binding) bases, **restriction site**
dyl_SP_F	CAAT**GAATTC**AAAATGGGACACCTGACG
dyl_SP_R	AAGTG**CATATG**CGAGGCATCGCCATG
tdTomato_F	AGTAGC**CATATG**GTGAGCAAGGGCGAGGAG
tdTomato_R	TAGGTACC**AGATCT**TGTACAGCTCGTCCATGCCG
CBD_F	ATG**AGATCT**ATACGACAAATCCTGGTGTATCCGCTTG
CBD_R	ACATT**CTCGAG**GCACCTTGAAGCTGCCACAAGGCAGGAA
dyl_tail_F	CAGT**CTCGAG**AAGGCGCAGAGACACCAT
dyl_tail_R	GAT**TCTAGA**TTAGTTCACATAGGCCATGCCC
dyl_tail_control_F	ATG**AGATCT**CAAGAAGGCGCAGAGACACCATG