Liu et al., https://doi.org/10.1083/jcb.201611088

Figure S1. **Efficiency of Tango1 knockdown.** Confocal images of wild-type and *Cg>Tango1*^{i}, where Tango1 has been knocked down with two different constructs. Tango1 expression was visualized with anti-Tango1 antibody staining.

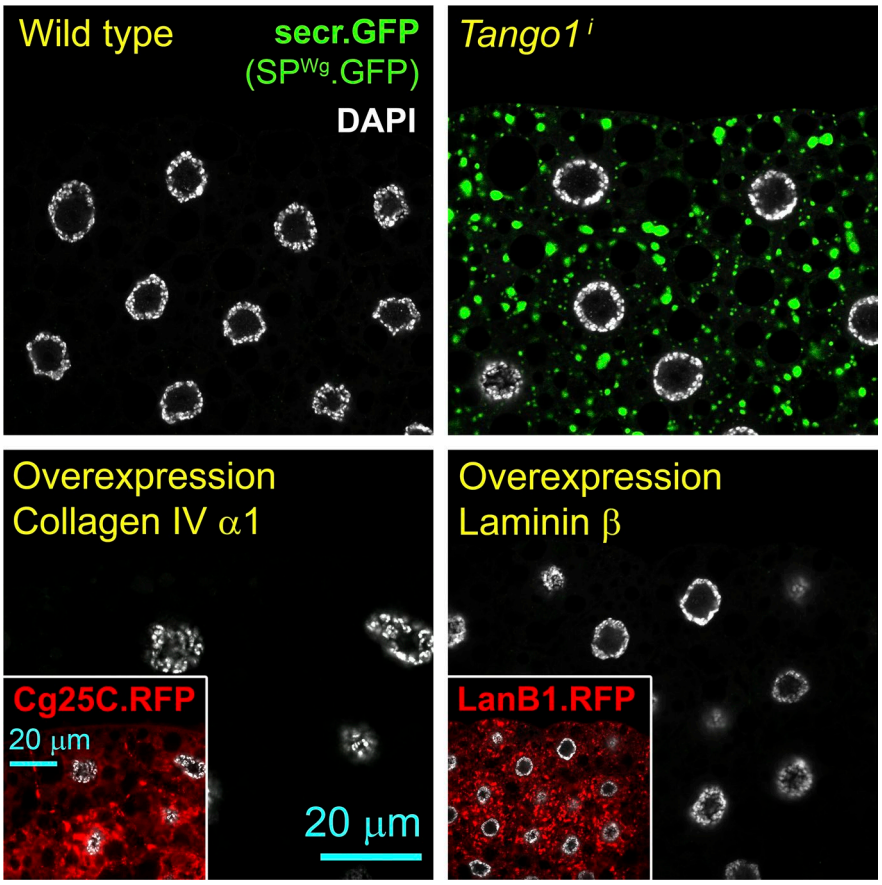

Figure S2. **Overexpression of Collagen IV or Laminin single chains does not impair general secretion.** Fat body cells expressing secreted GFP under control of BM-40-SPARC-GAL4 (*BM-40-SPARC>secr.GFP*, green). Images are of wild-type fat body, *Tango1*^{i} fat body, and fat body overexpressing Collagen IV α1 chain (Cg25C.RFP) and Laminin β chain (LanB1.RFP).

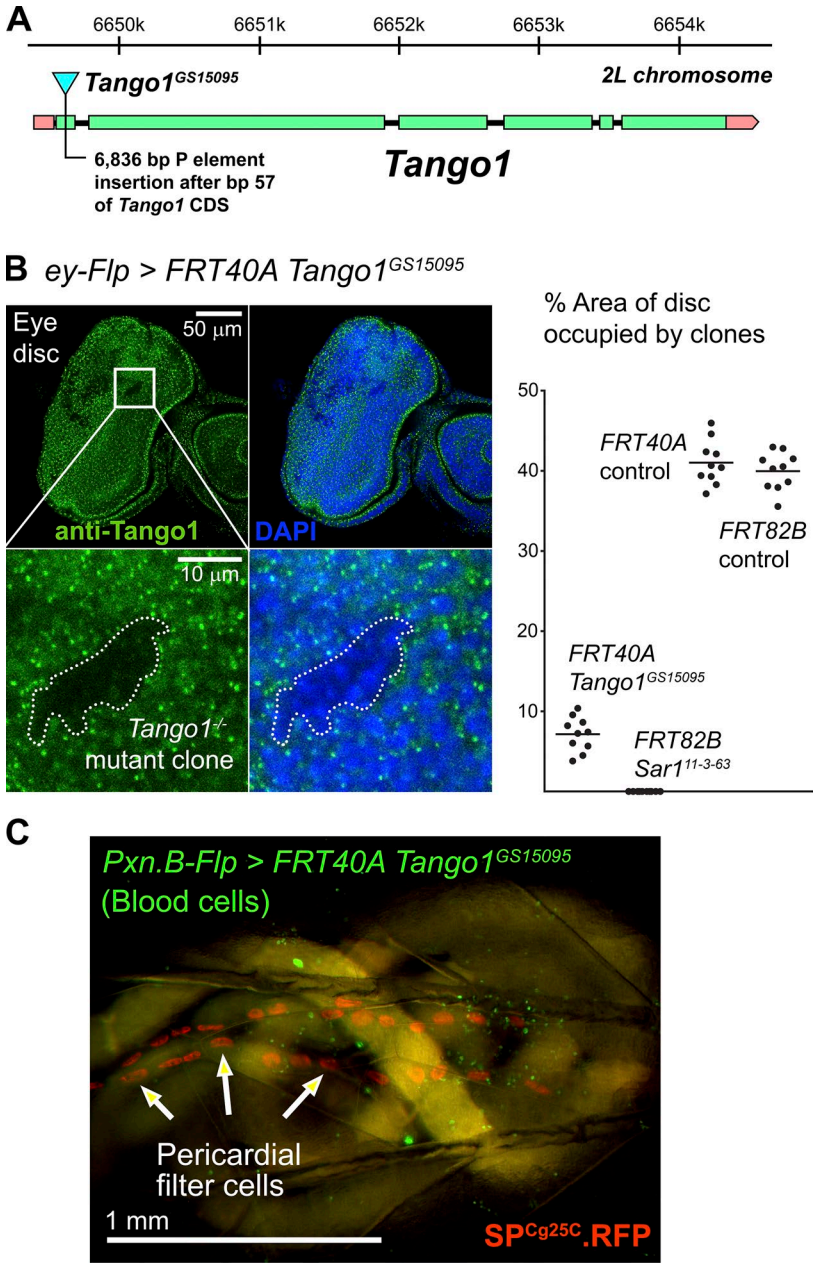

Figure S3. **Analysis of *Tango1* mutant mosaics.** (A) Graphic representation of the genomic organization of the *Tango1* gene and *Tango1*^{GS15095} mutation caused by a transposon insertion into the first coding exon of the gene. (B) Confocal image of an eye disc showing clones of *FRT40A Tango1*^{GS15095} homozygous mutant cells (absence of Tango1 antibody staining). Unlike *FRT82B Sar1*$^{11\text{-}3\text{-}63}$ clones, *FRT40A Tango1*^{GS15095} clones can be recovered but show reduced viability, as indicated by the reduced area they occupy in discs when compared with wild-type *FRT40A* control clones. Area occupied by clones in 10 discs per genotype (n = 10) is represented in the graph. Horizontal lines indicate mean area. (C) Detail of a larva containing *FRT40A Tango1*^{GS15095} homozygous mutant hemocytes (blood cells) expressing cytoplasmic GFP as a marker and secreted RFP (*act>SP*^{Cg25C}*.RFP*). RFP accumulation in pericardial filter cells shows that *FRT40A Tango1*^{GS15095} mutant cells are capable of secretion.

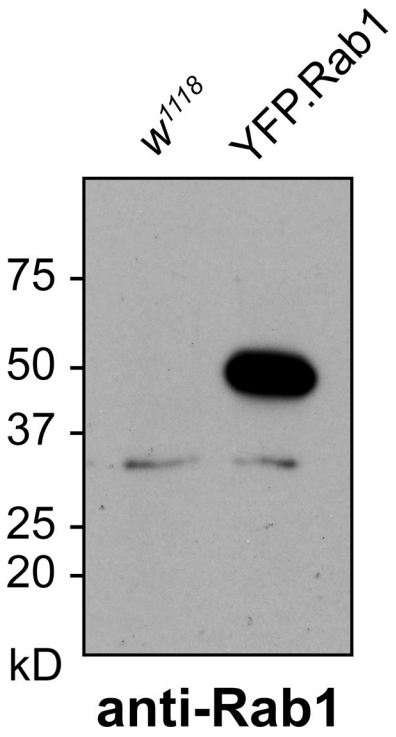

Figure S4. **Anti-Rab1 antibody.** Fat body lysates from wild-type and *Cg>YFP.Rab1* larvae were Western blotted with rabbit anti-Rab1 antibody generated in this study. YFP.Rab1 expression results in a strong band of higher molecular weight than endogenous, untagged Rab1.

Table S1. **Predicted molecular mass of secreted proteins in this study**

Proteins	Molecular mass
	kD
Vkg (Col IV α2)	193.8
Cg25C (Col IV α1)	174.3
Col IV trimer α1 + α2 + α1	542.4
Rfabg (ApoB)	372.7
Trol (Perlecan)	316.8–496.9
Ndg (Nidogen)	149.1
Fat-spondin	84.9
Fer1HCH (Ferritin)	13.6–27.9
Hedgehog	52.1
Sgs3	32.2
VSVG	57.5
GFP/RFP/YFP	26.9

Table S2. **Rescue of Tango1 knockdown by cytoplasmic Tango1**

w ; Cg-GAL4 x w ; UAS-Tango1.RNAi^{NIG11098R} / TM6B		*w ; Cg-GAL4 ; UAS-GFP.Tango1^{CYT} x w ; UAS-Tango1.RNAi^{NIG11098R} / TM6B*	
w ; Cg-GAL4 / + ; Tango1. RNAi^{NIG11098R}/ +	*w ; Cg-GAL4 / + ; TM6B / +*	*w ; Cg-GAL4 / + ; Tango1.RNAi^{NIG11098R} / GFP.Tango1^{CYT}*	*w ; Cg-GAL4 / + ; TM6B / GFP. Tango1^{CYT}*
0	78	101	87

Number of adults and their genotype in the progeny of indicated crosses.

Table S3. **Experimental genotypes**

Figure	Genotype
Fig. 1	**Imaging of ERES–Golgi units through SIM microscopy**
A–C	*w*^{1118}
D	*w Gmap*^{KM0132}*.GFP*
E	*w ; Cg-GAL4 / + ; UAS-RFP.Rab1.3.1 / +*
Fig. 2	**Tango1 knockdown impairs general secretion in fat body cells**
A	*w ; vkg*^{G454}*.GFP UAS-myr.RFP / + ; BM-40-SPARC-GAL4 / + w ; vkg*^{G454}*.GFP UAS-myr.RFP / + ; BM-40-SPARC-GAL4 / UAS-PH4αEFB. RNAi*$^{VDRC.v2464}$ *w ; vkg*^{G454}*.GFP UAS-myr.RFP / + ; BM-40-SPARC-GAL4 / UAS-Tango1.RNAi*^{VDRC21594} *w ; vkg*^{G454}*.GFP UAS-myr.RFP / + ; BM-40-SPARC-GAL4 / UAS-PH4αEFB.RNAi*$^{VDRC.v2464}$ *Tango1.RNAi*^{VDRC21594}
B	*w ; vkg*^{G454}*.GFP / +; BM-40-SPARC-GAL4 / + w ; vkg*^{G454}*.GFP / +; BM-40-SPARC-GAL4 / UAS-Tango1.RNAi*^{VDRC21594}
C	*w ; Cg-GAL4 / + ; UAS-Tango1.RNAi*^{VDRC21594} */ +*
D	*w ; Cg-GAL4 / + ; UAS-SP*^{Vkg}*.RFP / + w ; Cg-GAL4 / + ; UAS-SP*^{Cg25C}*.RFP / + w ; Cg-GAL4 / + ; UAS-SP*^{Vkg}*.RFP / UAS-Tango1.RNAi*^{VDRC21594} *w ; Cg-GAL4 / + ; UAS-SP*^{Cg25C}*.RFP / UAS-Tango1.RNAi*^{VDRC21594}
E	*w ; Cg-GAL4 / + ; Rfabg.sGFP*$^{fTRG.900}$ */ + w trol*$^{CPTI-002049}$*.YFP / w ; BM-40-SPARC-GAL4 UAS-myr.RFP/ + w*^{1118} *w ; fat-spondin*^{CPTI001685}*.YFP / + ; BM-40-SPARC-GAL4 UAS-myr.RFP / + w ; Cg-GAL4 UAS-myr.RFP / + ; Fer1HCH*^{G188}*.GFP/ + w ; UAS-secr-GFP / + ; BM-40-SPA RC-GAL4 UAS-myr.RFP / + w ; Cg-GAL4 / + ; Rfabg.sGFP*$^{fTRG.900}$ */ UAS-Tango1.RNAi*^{VDRC21594} *w trol*$^{CPTI-002049}$*.YFP / w ; BM-40-SPARC-GAL4 UAS-myr.RFP / UAS-Tango1.RNAi*^{VDRC21594} *w ; Cg-GAL4 / +; UAS-Tango1.RNAi*^{VDRC21594} */ + w ; fat-spondin*^{CPTI001685}*.YFP / +; BM-40-SPA RC-GAL4 UAS-myr.RFP / UAS-Tango1.RNAi*^{VDRC21594} *w ; Cg-GAL4 UAS-myr.RFP / + ; Fer1HCH*^{G188}*.GFP / UAS-Tango1.RNAi*^{VDRC21594} *w ; UAS-secr.GFP / + ; BM-40-SPARC-GAL4 UAS-myr.RFP / UAS-Tango1.RNAi*^{VDRC21594}
Fig. 3	**Tango1 is widely expressed and required for secretion in salivary glands and disc cells**
A	*w*^{1118}
B	*w ; Sgs3-GFP / + ; He-GAL4 UAS-myr.RFP / + w ; Sgs3-GFP / + ; He-GAL4 UAS-myr.RFP / UAS-Tango1.RNAi*^{VDRC21594}
C	*w ; UAS-hh.GFP / + ; hh-GAl4 / + w ; UAS-hh.GFP / + ; hh-GAL4 / UAS-Tango1.RNAi*^{VDRC21594}
D	*w ; UAS-secr.GFP / + ; rn-GAL4 / + w ; UAS-secr.GFP / + ; rn-GAL4 / UAS-Tango1.RNAi*^{VDRC21594}
Fig. 4	**Tango1 differentially affects secretion of Collagen IV**
A	*w ; vkg*^{G454}*.GFP / + ; UAS-SP*^{Vkg}*.RFP BM-40-SPARC-GAL4 / UAS-Tango1.RNAi*^{VDRC21594} *w ; vkg*^{G454}*.GFP / + ; UAS-SP*^{Vkg}*.RFP BM-40-SPA RC-GAL4 / UAS-Sec23.RNAi*^{VDRC24552GD} *w ; vkg*^{G454}*.GFP / + ; UAS-SP*^{Vkg}*.RFP BM-40-SPARC-GAL4 / UAS-Sar1.RNAi*^{VDRC34192GD}
B	*w ; vkg*^{G454}*.GFP UAS-myr.RFP / + ; BM-40-SPARC-GAL4 / + w ; UAS-secr.GFP / + ; BM-40-SPARC-GAL4 UAS-Dcr2 / + w ; Ub-VSVG. GFP / + ; BM40-SPARC-GAL4 UAS-myr.RFP / + w ; vkg*^{G454}*.GFP UAS-myr.RFP / + ; BM-40-SPARC-GAL4 / UAS-Tango1.RNAi*^{VDRC21594} *w ; vkg*^{G454}*.GFP UAS-myr.RFP / +; BM-40-SPARC-GAL4 / UAS-Sec23. RNAi*^{VDRC24552GD} *w ; vkg*^{G454}*.GFP UAS-myr.RFP / +; BM-40-SPA RC-GAL4 / UAS-Sar1. RNAi*^{VDRC34192GD} *w ; UAS-secr.GFP / +; BM-40-SPARC-GAL4 UAS-Dcr2 / UAS-Tango1.RNAi*^{VDRC21594} *w ; UAS-secr. GFP / +; BM-40-SPARC-GAL4 UAS-Dcr2 / UAS-Sec23. RNAi*^{VDRC24552GD} *w ; UAS-secr.GFP / +; BM-40-SPARC-GAL4 UAS-Dcr2 / UAS-Sar1. RNAi*^{VDRC34192GD} *w ; Ub-VSVG.GFP / + ; BM-40-SPARC-GAL4 UAS-myr.RFP / UAS-Tango1.RNAi*^{VDRC21594} *w ; Ub-VSVG.GFP / + ; BM-40-SPARC-GAL4 UAS-myr.RFP / UAS-Sec23. RNAi*^{VDRC24552GD} *w ; Ub-VSVG.GFP / + ; BM-40-SPARC-GAL4 UAS-myr.RFP / UAS-Sar1. RNAi*^{VDRC34192GD}
Fig. 5	**Loss of Tango1 produces smaller ERES uncoupled from Golgi**
A	*w ; Cg-GAL4 / + ; Sec16.sGFP*$^{fTRG.1259}$ */ + w ; Cg-GAL4 / + ; Sec16.sGFP*$^{fTRG.1259}$ */ UAS-Tango1.RNAi*^{VDRC21594}
B	*w ; Cg-GAL4 / + ; Sec16.sGFP*$^{fTRG.1259}$ */ + w ; Cg-GAL4 / + ; Sec16.sGFP*$^{fTRG.1259}$ */ UAS-Tango1.RNAi*^{VDRC21594}
C	*y w ; Sec16.sGFP*$^{fTRG.1259}$ *(y) w ; Tango1*^{GS15095} *; Sec16.sGFP*$^{fTRG.1259}$
D	*w ; Cg-GAL4 UAS-Grasp65.GFP / + w ; Cg-GAL4 / + ; UAS-YFP.Rab1 / + w ; Cg-GAL4 UAS-Grasp65.GFP / + ; UAS-RFP.Rab1 / + w ; Cg-GAL4 UAS-Grasp65.GFP / + ; UAS-Tango1.RNAi*^{VDRC21594} */ + w ; Cg-GAL4 / +; UAS-YFP.Rab1 / UAS-Tango1.RNAi*^{VDRC21594} *w ; Cg-GAL4 UAS-Grasp65.GFP / + ; UAS-RFP.Rab1.3.1 / UAS-Tango1.RNAi*^{VDRC21594}
E	*w ; Cg-GAL4 / + ; Sec16.sGFP*$^{fTRG.1259}$ */ UAS-Tango1.RNAi*^{VDRC21594} (anti-GM130) *w ; Cg-GAL4 UAS-Grasp65.GFP / + ; UAS-Tango1. RNAi*^{VDRC21594} (anti-Sec16) *w ; Cg-GAL4 / + ; UAS-YFP.Rab1 / UAS-Tango1.RNAi*^{VDRC21594} (anti-GM130) *w ; Cg-GAL4 UAS-Grasp65.GFP / + ; UAS-RFP.Rab1.3.1 / UAS-Tango1.RNAi*^{VDRC21594} *w ; Cg-GAL4 / + ; UAS-YFP.Rab1 / UAS-Tango1.RNAi*^{VDRC21594} (anti-Sec16)
Fig. 6	**The cytoplasmic part of Tango1 directs ERES localization and can rescue Tango1 loss**
C	*w ; Cg-GAL4 / + ; UAS-GFP.Tango1*^{CYT}*.3.1 / +*
D	*w ; Cg-GAL4 / + ; UAS-Tango1.RNAi*^{NIG11098R} */ + w ; Cg-GAL4 / + ; UAS-GFP.Tango1*^{CYT} */ UAS-Tango1.RNAi*^{NIG11098R}
Fig. 7	**Tango1 overexpression increases EREs's size and number**
A	*w /* Y OR *y v sc ; ptc-GAL4 / UAS-Tango1.attP2*
B	*w ; Cg-GAL4 / + ; UAS-SP.GFP.Tango1.3.1 / + w ; Cg-GAL4 / + ; UAS-GFP.Tango1*^{CYT}*.3.1 / +* (30°C overexpression)
C	*w*^{1118} (anti-Tango1) *w ; Cg-GAL4 / + ; UAS-SP.GFP.Tango1.3.1 / +*
D	*w ; Cg-GAL4 / + ; UAS-Tango1.RNAi*^{NIG11098R} */ +* (anti-Sec16) *w*^{1118} (fat body, anti-Tango1) *w ; Cg-GAL4 / + ; UAS-SP.GFP.Tango1.3.1 / + w*^{1118} (salivary gland, anti-Tango1)
E	*w*^{1118} (anti-Gmap) *w ; Cg-GAL4 / + ; UAS-SP.GFP.Tango1.3.1 / +* (anti-Gmap)
Fig. 8	**Multiple interactions of Tango1 at the ERES–Golgi interface**
A	*w ; Cg-GAL4 ; UAS-Tango1.FLAG.3.1 UAS-Tango1.HA.3.1 w ; Cg-GAL4 ; UAS-Tango1.FLAG.3.1 w ; Cg-GAL4 ; UAS-Tango1.HA.3.1*
B	*w ; Cg-GAL4 ; UAS-Tango1.FLAG.3.1 w*^{1118}
C	*w ; Cg-GAL4 ; UAS-YFP.Rab1 w*^{1118}
D	*w ; Cg-GAL4 UAS-Grasp65.GFP w*^{1118}
E	*w ; Cg-GAL4 ; UAS-Tango1.FLAG.3.1 w*^{1118}
F	*w ; Cg-GAL4 ; UAS-YFP.Rab1 w*^{1118}
G	*w ; Cg-GAL4 UAS-Grasp65.GFP w*^{1118}
H	*y w ; Sar1*^{CA07674}*.GFP / TM3, Ser Sb w*^{1118}
Fig. S1	**Efficiency of Tango1 knockdown**

Table S3. **Experimental genotypes** *(Continued)*

Figure	Genotype
	w^{1118} w ; Cg-GAL4 / +; UAS-Tango1.RNAi^{VDRC21594} / + w ; Cg-GAL4 / +; UAS-Tango1.RNAi^{NIG11098R} / +
Fig. S2	**Overexpression of Collagen IV or Laminin single chains does not impair general secretion**
	w ; UAS-secr.GFP/ + ; BM-40-SPARC-GAL4 UAS-myr.RFP / + w ; UAS-secr.GFP/ + ; BM-40-SPARC-GAL4 UAS-myr.RFP / UAS-Tango1. RNAi^{VDRC21594} w ; UAS-secr.GFP / UAS-Cg25C.RFP.2.1 ; BM-40-SPARC-GAL4 / + w ; UAS-secr.GFP/ + ; BM-40-SPARC-GAL4 / UAS-LanB1.RFP.3.1
Fig. S3	**Analysis of *Tango1* mutant mosaics**
B	*y w ey-Flp ; FRT40A Tango1^{GS15095} / FRT40A y w ey-Flp ; FRT40A Tango1^{GS15095} / FRT40A tub-GAL80 ; act-y^{+}-GAL4 UAS-GFP / + y w ey-Flp ; FRT40A / FRT40A tub-GAL80 ; act-y^{+}-GAL4 UAS-GFP / + y w ey-Flp ; act-y^{+}-GAL4 UAS-GFP / + ; FRT82B Sar1$^{11-3-63}$ / FRT82B tub-GAL80 y w ey-Flp ; act-y^{+}-GAL4 UAS-GFP / + ; FRT82B / FRT82B tub-GAL80*
C	*w Pxn.B-Flp.F12a ; FRT40A Tango1^{GS15095} / FRT40A tub-GAL80 ; act-y^{+}-GAL4 UAS-GFP / UAS-SP^{Cg25C}.RFP.3.1*
Fig. S4	**Anti-Rab1 antibody**
	w^{1118} w ; Cg-GAL4 ; UAS-YFP.Rab1