

Supplementary figures and tables for:

HtsRC-mediated accumulation of F-actin regulates ring canal size during *Drosophila melanogaster* oogenesis

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Supporting Figure 2

Figure S1: Ring canals connecting nurse cells to oocytes retain more F-actin than nurse cell ring canals.

Figure S2: HtsRC persists on actin cables in *cheerio* (Filamin) mutants.

Supporting Figure 6

Figure S3: HtsRC/F-actin aggregates formation is consistent across various transgenes and can be regulated by Kelch.

Figure S4: Ectopic HtsRC expression drives F-actin aggregate formation independent of Arp2/3 complex activity.

Supporting Figure 7

Figure S5: HtsRC/F-actin aggregates rarely form at the apical surface in *cheerio* mutants.

Supporting Discussion section “HtsRC evolution and fecundity”

Figure S6: Predicted HtsRC homologs are present in 28 fly species.

Supporting Figures 3 and 5

Supplementary Table 1: Means, standard deviations and sample number for data plotted on violin plots in Figure 3 and Figure 5.

Reagent table

Figure S1: Ring canals connecting nurse cells to oocytes retain more F-actin than nurse cell ring canals.

Representative ring canals from several stages labeled with Phalloidin stain (magenta) and Filamin antibody (green). (left) The ring canals connecting pairs of nurse cells in the *hts* mutant never form a robust F-actin cytoskeleton although Filamin localizes normally. (right) The ring canals connecting nurse cells to the oocyte recruit an actin cytoskeleton at later stages (9 and 10) which partially colocalizes with the Filamin stain marking the inner rim. Arrows point to Filamin stain at the inner or outer rim to allow comparison of Filamin and F-actin channels.

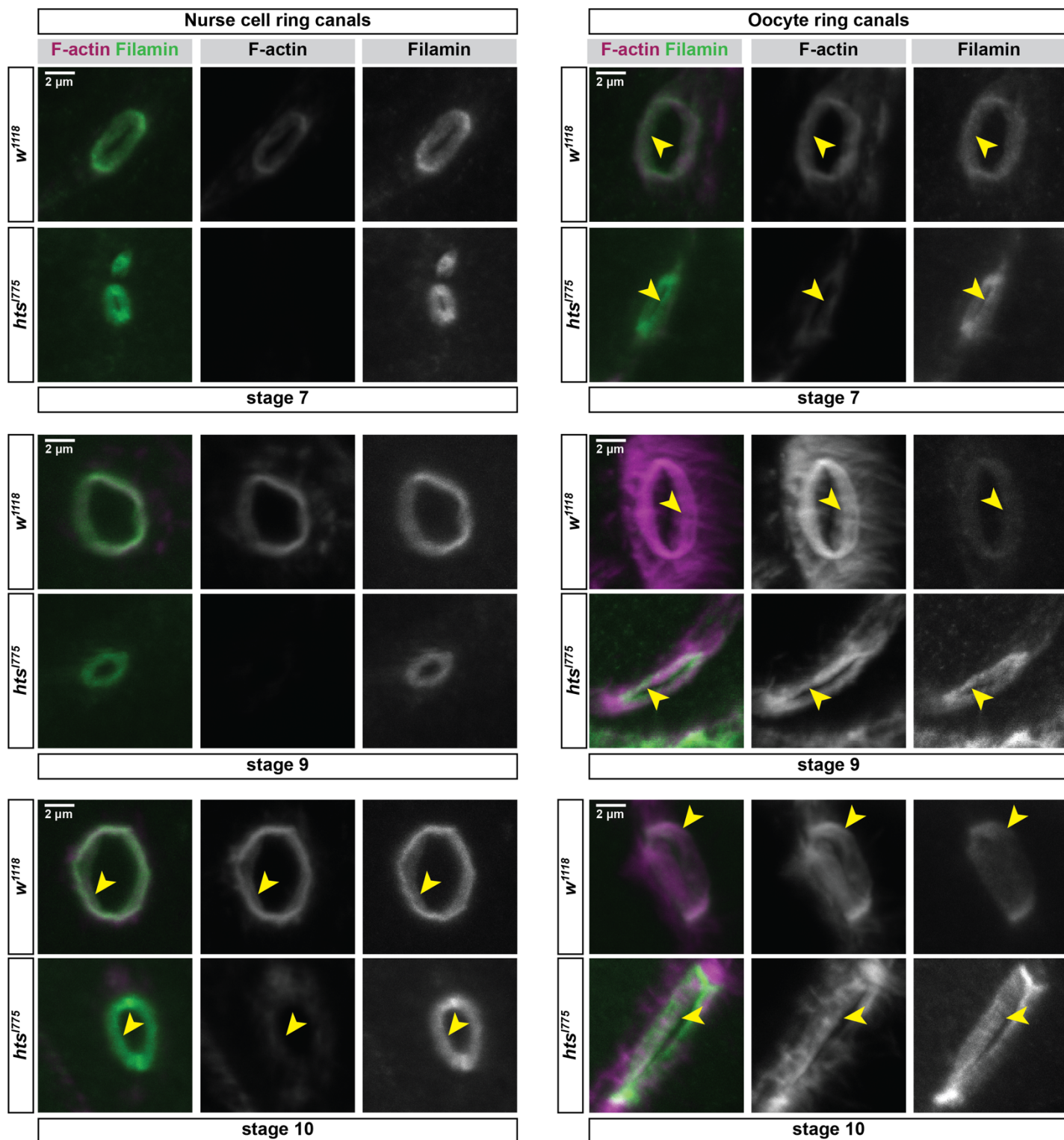


Figure S2: HtsRC persists on actin cables in *cheerio* (Filamin) mutants.

(A) Diagram of the *cheerio* (Filamin) gene and its protein products. Two independent transcription start sites promote the transcription of a full length Filamin (Filamin240) and a short Filamin (Filamin90). *cher*¹ is an allele with no known lesion that does not express detectable Filamin protein in the germline or somatic follicle cells. *cher*^{M113} and *cher*^{M919} were a gift from the David Ish-Horowicz lab. *cher*^{M113} causes a nonsense mutation in the actin binding domain, while *cher*^{M919} contains a point mutation which converts an AG to TG and eliminates a splice acceptor. *cher*^{M107480-GFSTF.0} (used in Figure 2) is an intronic insertion allele from the Mimic collection which has been swapped for a GFP containing cassette. GFP protein is inserted in the hinge of Filamin240 and roughly 70% of Filamin protein in ovary lysates contains GFP (results not shown). (B) HtsRC (green) and F-actin (magenta) stain of *w*¹¹¹⁸ control compared to three *cheerio* mutants. HtsRC localizes to both actin cables and ring canals in wild type and to cables in *cher* mutants. Antibody to the C-terminus of Filamin (not included in the merge) labels ring canals but does not stain actin cables.

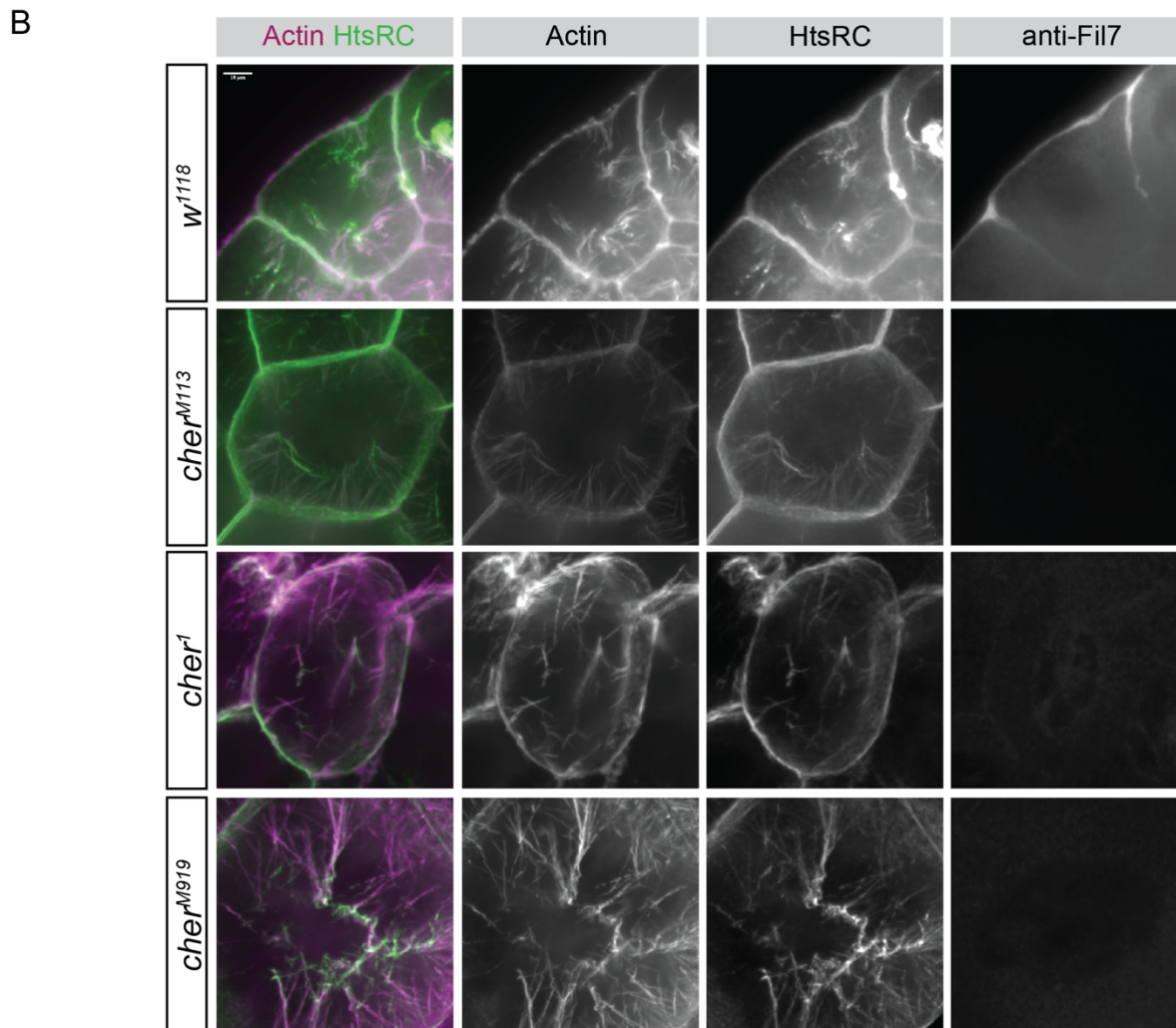
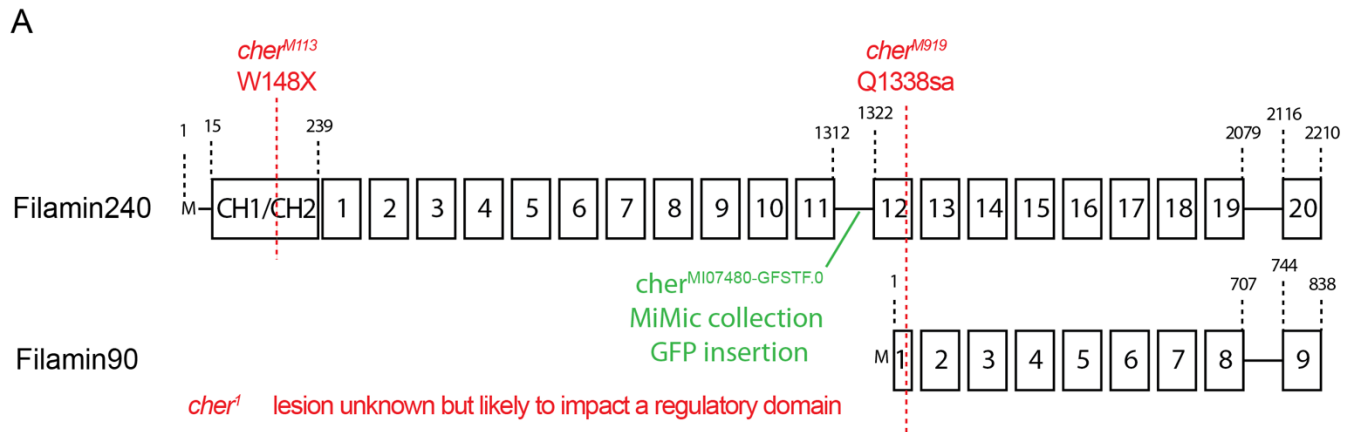


Figure S3: HtsRC/F-actin aggregates formation is consistent across various transgenes and can be regulated by Kelch. (A-E) View of somatic follicle cells from the basal end in *w¹¹¹⁸* control (A) compared with four different *ovhts* transgenes. (F-J) View of somatic follicle cells from the side with the basal end oriented downward and the apical end/germline oriented upward. (K-M) *UASh-ovhts::GFP-htsN4* transgene (same as C, H) driven in somatic follicle cells under the control of the hindsight-Gal4 driver. An mCherry control (K), mCherry::Kelch (L) or unregulated Kelch (M) is coexpressed with the same driver. (N-Q) Quantification of aggregate/foci features for aggregates in controls or the two Kelch overexpression conditions: number of aggregates per egg chamber region (N), average aggregate size (O), total summed aggregate area per egg chamber region (P), and total summed aggregate fluorescence (Q). Individual data points represent measurements from individual egg chambers. Significance was determined using one-way ANOVA, Tukey's multiple comparison test. significance thresholds as follows: $p < 0.05$ (*); $p < 0.0001$ (****).

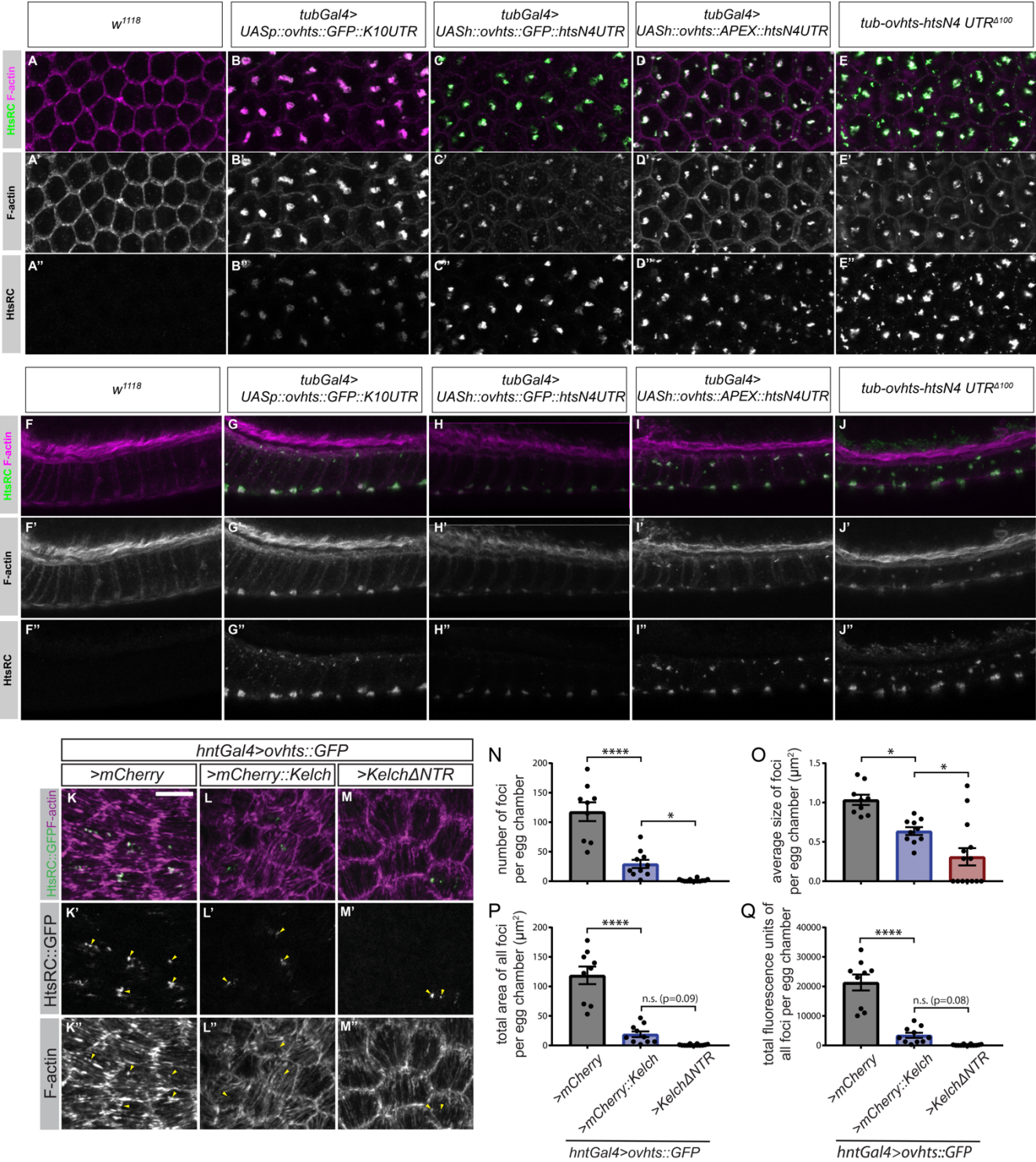


Figure S4: Ectopic HtsRC expression drives F-actin aggregate formation independent of Arp2/3 complex activity.

Heatshocks were performed on flies heterozygous for *FRT40A Tubulin-Gal80* and either a wildtype control *FRT40A* chromosome or an *FRT40A* chromosome containing a mutant allele. Clones expressing two copies of the control allele or two copies of the mutant allele were marked by the expression of *CD8::GFP* (blue) and *HtsRC::V5::APEX* (green) driven by *tubulin-Gal4* expression. Neighboring cells (no GFP) contained either one or two copies of *tubulin-Gal80* and did not express GFP or HtsRC protein. Samples were also stained with Phalloidin. (A) Control clone (B) *ArpC1^{Q29sd}* mutant clone (C) *ArpC4^{SH1036}* mutant clone. Quantifications were performed on HtsRC aggregates from regions taken from GFP positive clones (D-F). Regions were defined by a 24.3 micron by 24.3 micron square in the xy plane, but had a variable number of z-slices depending on the thickness of the somatic epithelium. Mean density (fluorescent intensity divided by volume) intensities for both HtsRC (D) and F-actin (E) channels were calculated for aggregates within each region. Total volume occupied by aggregates (F) and total HtsRC intensity were also determined for each region. Significance was determined for each experiment using a one-way ANOVA and Tukey's multiple comparison tests. P-value summaries for the multiple comparison tests are displayed on graphs, with $p < 0.05$ indicated by an *. ANOVA P values are as follows: (D) 0.0515, (E) 0.808, (F) 0.7287, and (G) 0.1512.

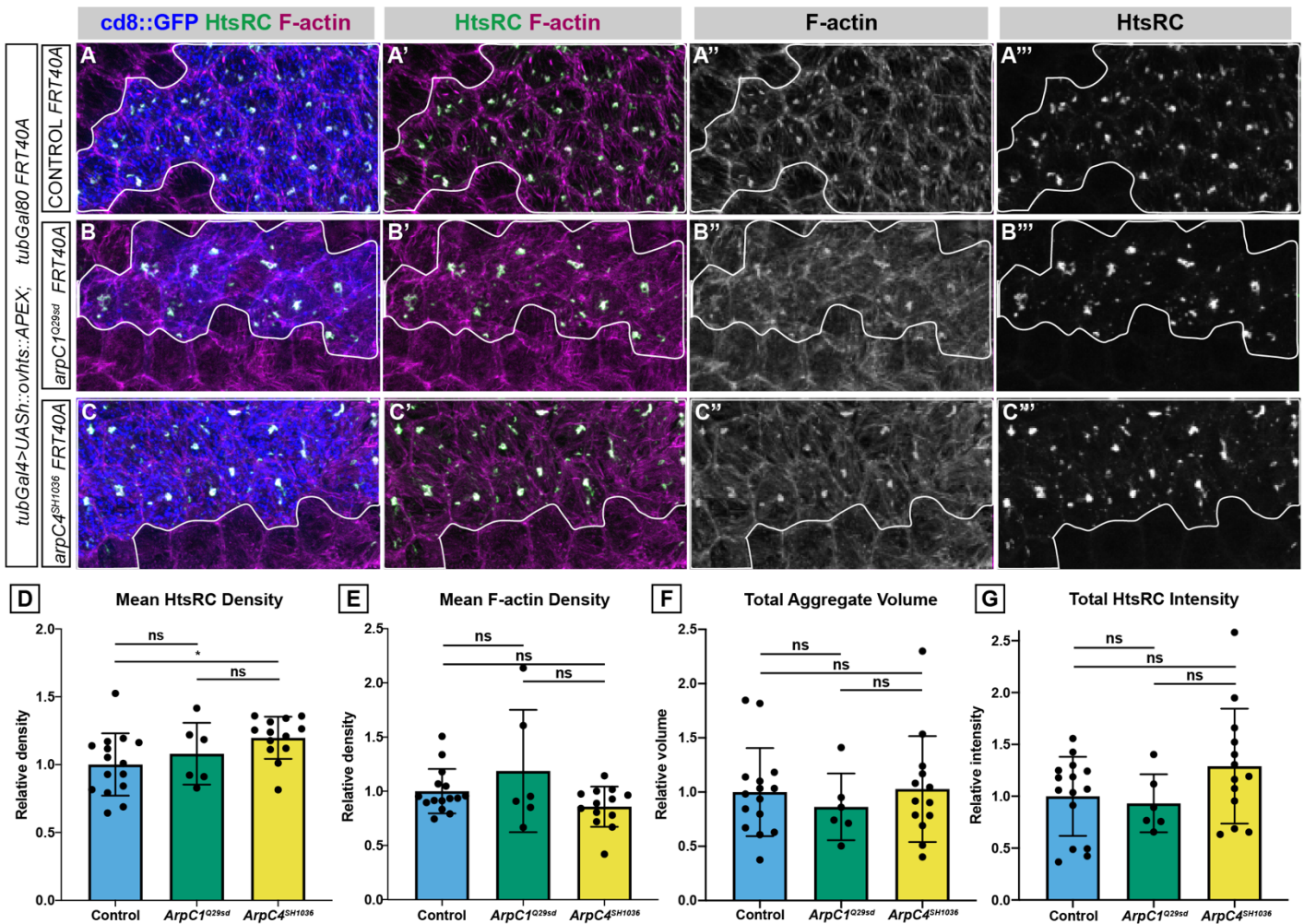


Figure S5: HtsRC/F-actin aggregates rarely form at the apical surface in *cheerio* mutants. Aggregate location within follicle cells was determined in Imaris for all aggregates within three regions of interest (regions p, q, and r; 24x24 micron squares) per egg chamber. Z-position at the center of mass (based on HtsRC intensity) for each aggregate was used as a proxy for the location of the entire aggregate or cluster of aggregates; aggregates in close proximity were reported by Imaris as a single aggregate. The position of the nuclei was calculated using the center of mass for DAPI stain and used to normalize the relative aggregate position between samples. Although the x and y dimensions of the regions of interest were fixed, the z dimension varied with the depth of the follicle cell layer.

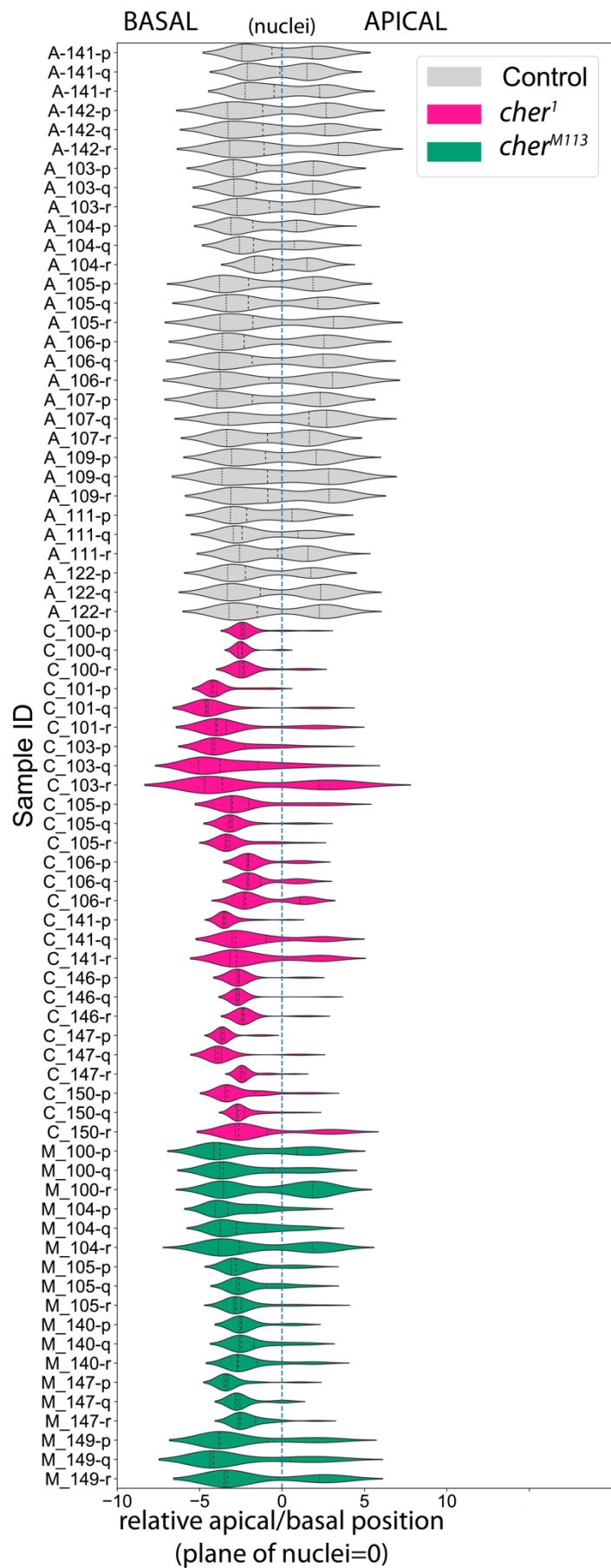
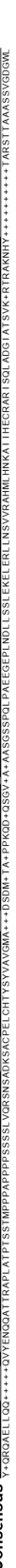
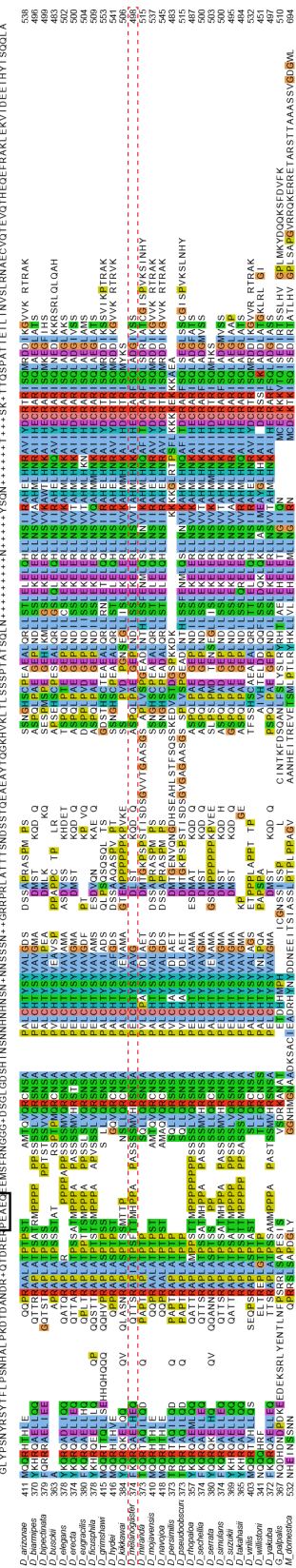
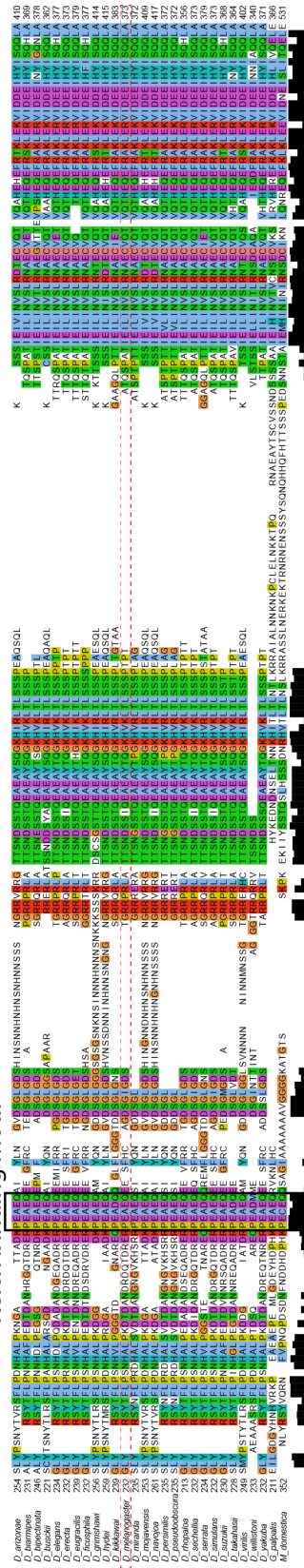
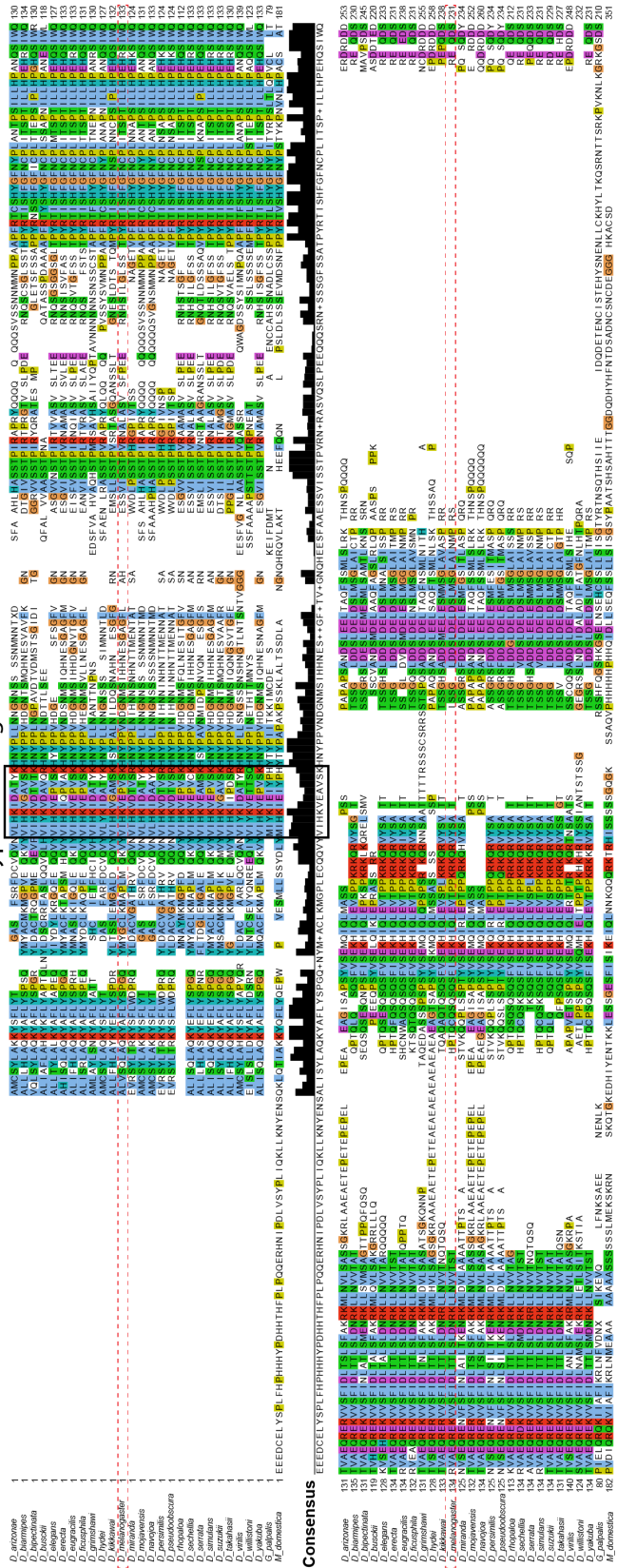


Figure S6 (on following page): Predicted HtsRC homologs are present in 28 fly species. Clustal Omega alignment of HtsRC proteins from 26 *Drosophila* species as well as *Musca domestica* and *Glossina palpalis*. Gaps are indicated by blank space. Residue conservation within this group is indicated by black boxes under the alignment. Conserved residues are colored according to the Clustal X color scheme with non-conserved residues left white: hydrophobic (blue), positive charge (red), negative charge (magenta), polar (green), cysteines (pink), glycines (orange), prolines (yellow), and aromatics (cyan). The site of Ovhts polyprotein cleavage and Kelch binding found in *D. melanogaster* are indicated by black outlines. *D. melanogaster* sequence is indicated by a red dashed line. Species in alignment (in order) are as follows: *D. arizonae*, *D. biarmipes*, *D. bipectinata*, *D. busckii*, *D. elegans*, *D. erecta*, *D. eugracilis*, *D. ficusphila*, *D. grimshawi*, *D. hydei*, *D. kikkawai*, *D. melanogaster*, *D. miranda*, *D. mojavensis*, *D. navojoa*, *D. persimilis*, *D. pseudoobscura*, *D. rhopaloa*, *D. sechellia*, *D. serrata*, *D. simulans*, *D. suzukii*, *D. takahashii*, *D. virilis*, *D. willistoni*, *D. yakuba*, *G. palpalis*, *M. domestica*.



Supplementary Table 1:

Means, standard deviations and sample number for data plotted on violin plots in Figure 3 and Figure 5.

Figure 3C and D (ring canal diameter)						
Genotype	RC type	Stage	Mean diameter	SD	N ring canals	N egg chambers
<i>w¹¹¹⁸</i>	nurse cell to nurse cell	2	1.72	0.43	22	2
		3	2.25	0.43	33	3
		4	2.95	0.52	55	5
		5	3.79	0.39	77	7
		6	4.31	0.40	44	4
		7	4.52	0.44	55	5
		8	5.13	0.68	34	4
		9	5.85	0.52	42	4
		10a	6.53	0.86	34	4
		10b	8.99	1.15	22	2
<i>hts^{I775fs}</i>	nurse cell to nurse cell	2	1.41	0.26	38	4
		3	1.89	0.39	22	2
		4	2.26	0.46	55	5
		5	2.30	0.66	44	4
		6	2.86	0.74	31	3
		7	2.93	0.85	33	3
		8	3.18	1.09	33	3
		9	4.13	1.09	44	4
		10a	4.60	1.44	22	2
		10b	5.22	2.35	33	3
<i>w¹¹¹⁸</i>	oocyte to nurse cell	2	2.22	0.59	8	2
		3	2.86	0.65	12	3
		4	3.39	0.65	19	5
		5	4.31	0.72	28	7
		6	4.84	0.52	16	4
		7	5.14	0.51	20	5
		8	6.01	0.95	12	3
		9	6.74	0.88	16	4
		10a	7.51	1.72	12	3
		10b	10.99	2.37	6	2
<i>hts^{I775fs}</i>	oocyte to nurse cell	2	2.00	0.53	16	4
		3	2.81	0.50	8	2
		4	3.41	0.67	20	5
		5	4.57	1.03	16	4
		6	5.01	0.99	12	3
		7	5.07	1.20	12	3
		8	5.92	1.55	12	3
		9	8.46	5.31	16	4
		10a	11.29	2.86	8	2
		10b	10.82	3.96	12	3

Figure 5G (ring canal diameter)					
Condition	Stage	Mean diameter	SD	N ring canals	N egg chambers
w¹¹¹⁸ control	2	1.74	0.57	25	2
	3	1.87	0.54	28	2
	5	3.55	0.78	73	5
	7	5.00	0.89	15	1
	9	8.18	1.16	75	5
	10A	11.30	2.39	58	4
	10B	13.83	1.83	30	2
HtsRC overexpression (<i>matGal4</i> > <i>ovhts::GFP</i>)	2	2.29	0.62	26	2
	3	3.14	0.67	29	2
	5	4.62	1.12	30	2
	7	7.50	1.60	39	3
	9	11.41	1.97	65	5
	10A	15.28	3.18	19	2
	10B	22.20	4.18	22	2

Reagent Table; Gerdes et al.

Data type	Experimental species	Symbol/name used in publication	Source – public	Source – published	Source – unpublished	Identifiers	New reagent	Comments
Data type (mandatory). Duplicate rows as needed. Order is flexible, but row titles must be preserved.	Experimental species (mandatory, "NA" okay)	Symbol/name used in publication (mandatory)	Source – public (stock center, company, data repository) (one of D,E,F mandatory)	Source – published (PMID or this paper) (one of D,E,F mandatory)	Source – unpublished (description, incl. lab of origin) (one of D,E,F mandatory)	Identifiers (format as ID, source identifier) Separate multiple entries with semi-colon, space	New reagent (mandatory for new entities) Description, progenitor(s)	Comments (optional) Genotypes, purpose of reagent, additional information
antibody	NA	HtsRC antibody	Developmental Studies Hybridoma Bank			RRID:AB_528289		Mouse monoclonal anti-Hts (HtsRC 4B); 1:10 dilution
antibody	NA	Flamin antibody		PMID:10556087		RRID:AB_2569614		Rat polyclonal anti-Flamin (CFIL 7); 1:500 dilution
antibody	NA	Goat anti-mouse Alexa Fluor 633	Thermo Fisher Scientific			RRID:AB_2535719		Goat anti-mouse IgG Secondary Antibody, Alexa Fluor 633; 1:500 dilution
antibody	NA	Goat anti-mouse IgG Secondary Antibody, Alexa Fluor 647; 1:500	Thermo Fisher Scientific			RRID:AB_2535804		Goat anti-mouse IgG Secondary Antibody, Alexa Fluor 647; 1:500 dilution
antibody	NA	Goat anti-rat IgG Secondary Antibody, Alexa Fluor 488; 1:500	Thermo Fisher Scientific			RRID:AB_2534074		Goat anti-rat IgG Secondary Antibody, Alexa Fluor 488; 1:500 dilution
antibody	NA	TRITC-Phalloidin	Sigma-Aldrich			RRID:AB_2315148		1:200 dilution
other	NA	DAPI stain						1:50,000 dilution
genetic reagent (in whole organism)	Drosophila melanogaster	Wild type; w ¹¹¹⁸	Bloomington Drosophila Stock Center			RRID:BDSC_3605		
genetic reagent (in whole organism)	Drosophila melanogaster	matGal4	Bloomington Drosophila Stock Center			BDSC_7063; FB:FBi0016914		w ¹¹¹⁸ ; P[w ¹¹¹⁸ +mC]-mata4-GAL-VP16/V37
genetic reagent (in whole organism)	Drosophila melanogaster	hntGal4		PMID:12814552		FB:FBaI0130627		Gift from Mary Baylies lab
genetic reagent (in whole organism)	Drosophila melanogaster	tubGal4	Bloomington Drosophila Stock Center			BDSC_5138; FB:FBi0012687		
genetic reagent (in whole organism)	Drosophila melanogaster	htr ¹ LP1; tubulinGal80 FRT40A; UAS-mCD8::GFP tubulinGal4		this paper			Component stocks/insertions: RRID:BDSC_5; FB:FBi0002044; RRID:BDSC_5192; RRID:BDSC_30030;	
genetic reagent (in whole organism)	Drosophila melanogaster	cher1		PMID:9093858		FB:FBaI0060473		
genetic reagent (in whole organism)	Drosophila melanogaster	cherM113			David Ish-Horowitz lab		This work/Laboratory of D. Ish-Horowitz; mutations generated on FRT82 chromosome by EMS. Allele was generated as an extension of the work done in Hayashi et al 2014 but not published.	Gift of David Ish-Horowitz.
genetic reagent (in whole organism)	Drosophila melanogaster	cherM919			David Ish-Horowitz lab		This work/Laboratory of D. Ish-Horowitz; mutations generated on FRT82 chromosome by EMS. Allele was generated as an extension of the work done in Hayashi et al 2014 but not published.	Gift of David Ish-Horowitz.
genetic reagent (in whole organism)	Drosophila melanogaster	htr1755f		PMID:30559276		FB:FBaI0345602		
genetic reagent (in whole organism)	Drosophila melanogaster	htrR913s		PMID:30559276		FB:FBaI0345601		
genetic reagent (in whole organism)	Drosophila melanogaster	UASp-mCherry		PMID:22428051		FB:FBaI0284922		
genetic reagent (in whole organism)	Drosophila melanogaster	UASp-TAPmCherry-Kelch		PMID:26384358		FB:FBaI0283122		
genetic reagent (in whole organism)	Drosophila melanogaster	UASp-Kelch-deltaNTR		PMID:30559276		FB:FBaI0345599		
genetic reagent (in whole organism)	Drosophila melanogaster	tubulin-ovhts-hsN4UtrDelta100		PMID:24677508		FB:FBaI0301360		Tubulin direct drive construct missing region of 3'UTR required for Swallow dependent localization at at oocyte cortex
genetic reagent (in whole organism)	Drosophila melanogaster	UASH-Ovhts::GFP-hsN4Utr		PMID:17215303		FB:FBaI0212988		Contains UAS promoter with ovhts/hisN4 3'UTR sequence in place of typical 3'UTR
genetic reagent (in whole organism)	Drosophila melanogaster	UASH-Ovhts::GFP-K18Utr		this paper			ovhts::GFP PCR amplified from UASH-ovhts::GFP-hsN4Utr construct and inserted into pDONR201; Insert was then swapped into pPW-attB using Gateway recombination. Resulting plasmid was transformed into a strain containing the attP2 landing site on chromosome III (88A4).	
genetic reagent (in whole organism)	Drosophila melanogaster	UASH-Ovhts::V5::APEX-hsN4Utr		this paper			APEX was PCR amplified from pDNA3-mito-APEX using a primer containing V5 tag sequence. Resulting PCR product was restriction cloned into UASH-ovhts::GFP (hsN4Utr) in frame in the place of GFP. Plasmid was inserted using p-element insertion	
genetic reagent (in whole organism)	Drosophila melanogaster	htr Df. w ¹¹¹⁸ ; Df(2R)BSC135/CyO	Bloomington Drosophila Stock Center			BDSC_9423; FB:FBaI0044866		
genetic reagent (in whole organism)	Drosophila melanogaster	cher-M07480-GFSTF.0	Bloomington Drosophila Stock Center			BDSC_60261; FB:FBaI0314454		
genetic reagent (in whole organism)	Drosophila melanogaster	ArpC1-Q25SD FRT40A	Bloomington Drosophila Stock Center			BDSC_9137; FB:FBaI0135790		
genetic reagent (in whole organism)	Drosophila melanogaster	ArpC4-SH1036 FRT40A	Bloomington Drosophila Stock Center			BDSC_29488; FB:FBaI0321477		
genetic reagent (in whole organism)	Drosophila melanogaster	isogenized neoFRT40A		PMID:8404527		FB:FBi0002071		Gift of Tian Xu
gene (source not applicable)	Drosophila melanogaster	htr	NA	NA	NA	FB:FBgn0263391		
gene (source not applicable)	Drosophila melanogaster	cherio	NA	NA	NA	FB:FBgn0014141		
gene (source not applicable)	Drosophila melanogaster	kelch	NA	NA	NA	FB:FBgn0001301		
gene (source not applicable)	Drosophila melanogaster	ArpC1	NA	NA	NA	FB:FBgn0001961		
gene (source not applicable)	Drosophila melanogaster	ArpC4	NA	NA	NA	FB:FBgn0284255		
software, algorithm	NA	ImageJ/Fiji	NIH					https://fiji.sc/
software, algorithm	NA	Imaris 9.3	Bitplane					https://www.bitplane.com/
software, algorithm	NA	Prism 8	GraphPad					https://www.graphpad.com/scientificsoftware/prism/
software, algorithm	NA	Jalview		PMID:19151095				http://www.jalview.org/
software, algorithm	NA	Jupyter Notebook	Anaconda					http://www.anaconda.com/
software, algorithm	NA	NCBI Blast	NIH					https://blast.ncbi.nlm.nih.gov/
software, algorithm	NA	Vectorbase		PMID:25510499				https://www.vectorbase.org/blast
software, algorithm	NA	SPOT-Disorder-Single application		PMID:30395465				
software, algorithm	NA	MetaDisorder (GeneSilo)		PMID:22624656				http://genesilo.clinicaldisorder/
recombinant DNA reagent	NA	pUASH-ovhts-GFP		PMID:17215303				
recombinant DNA reagent	NA	pCDNA3-mito-APEX		PMID:23371551			Addgene Plasmid #42607	
recombinant DNA reagent	NA	pPW-attB (gateway vector)			Lab of Michael Buszczak, UT Southwestern			
recombinant DNA reagent	NA	pDONR201 (gateway vector)	Thermo Fisher Scientific					
gene (source not applicable)	D. arizonae	predicted HtsRC homolog				XM_018011272		
gene (source not applicable)	D. biarmipes	predicted HtsRC homolog				XM_017057880		
gene (source not applicable)	D. bipunctata	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. busckii	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. elegans	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. erecta	predicted HtsRC homolog				XM_018166883		
gene (source not applicable)	D. eugracilis	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. fuscipennis	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. grimshawi	predicted HtsRC homolog				XM_016872118		
gene (source not applicable)	D. hydei	predicted HtsRC homolog				XM_020511683		
gene (source not applicable)	D. ikkawai	predicted HtsRC homolog				XM_017885381		
gene (source not applicable)	D. melanogaster	HtsRC				XM_001688785		
gene (source not applicable)	D. miranda	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. mojavensis	predicted HtsRC homolog				XM_00209844.1		
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gene (source not applicable)	D. persimilis	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	D. pseudoobscura	predicted HtsRC homolog				XM_001688785		
gene (source not applicable)	D. rhopaloea	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. sechellia	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	D. serrata	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	D. simulans	predicted HtsRC homolog				XM_018166883		
gene (source not applicable)	D. suzukii	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. takahasi	predicted HtsRC homolog				XM_017050448.2		
gene (source not applicable)	D. willisii	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	D. willisii	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	D. yakuba	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	G. pallipes	predicted HtsRC homolog				XM_020589809		
gene (source not applicable)	M. domestica	predicted HtsRC homolog				XM_020589809		

Reagent Table; Gerdes et al.

Data type	Experimental species	Symbol/name used in publication	Source – public	Source – published	Source – unpublished	Identifiers	New reagent	Comments
Data type (mandatory). Duplicates rows as needed. Order is flexible, but row titles must be preserved.	Experimental species (mandatory, "NA" okay)	Symbol/name used in publication (mandatory)	Source – public (stock center, company, data repository) (one of D,E,F mandatory)	Source – published (PMID or this paper) (one of D,E,F mandatory)	Source – unpublished (description, incl. lab of origin) (one of D,E,F mandatory)	Identifiers (format as ID, source identifier) Separate multiple entries with semi-colon, space	New reagent (mandatory for new entities) Description, progenitor(s)	Comments (optional) Genotypes, purpose of reagent, additional information
antibody	NA	HtsRC antibody	Developmental Studies Hybridoma Bank			RRID:AB_528289		Mouse monoclonal anti-Hts (HtsRC 4B); 1:10 dilution
antibody	NA	Flamin antibody		PMID:10556087		RRID:AB_2569614		Rat polyclonal anti-Flamin (CFIL 7); 1:500 dilution
antibody	NA	Goat anti-mouse Alexa Fluor 633	Thermo Fisher Scientific			RRID:AB_2535719		Goat anti-mouse IgG Secondary Antibody, Alexa Fluor 633; 1:500 dilution
antibody	NA	Goat anti-mouse IgG Secondary Antibody, Alexa Fluor 647; 1:500	Thermo Fisher Scientific			RRID:AB_2535804		Goat anti-mouse IgG Secondary Antibody, Alexa Fluor 647; 1:500 dilution
antibody	NA	Goat anti-rat IgG Secondary Antibody, Alexa Fluor 488; 1:500	Thermo Fisher Scientific			RRID:AB_2534074		Goat anti-rat IgG Secondary Antibody, Alexa Fluor 488; 1:500 dilution
antibody	NA	TRITC-Phalloidin	Sigma-Aldrich			RRID:AB_2315148		1:200 dilution
other	NA	DAPI stain						1:50,000 dilution
genetic reagent (in whole organism)	Drosophila melanogaster	Wild type; w ¹¹¹⁸	Bloomington Drosophila Stock Center			RRID:BDSC_3605		
genetic reagent (in whole organism)	Drosophila melanogaster	matGal4	Bloomington Drosophila Stock Center			BDSC_7063; FB:FBi0016914		w ¹ ; P[w ⁺ +mC]-mata ^{ph4} -GAL-VP16 V37
genetic reagent (in whole organism)	Drosophila melanogaster	hntGal4		PMID:12814552		FB:FBaI0130627		Gift from Mary Baylies lab
genetic reagent (in whole organism)	Drosophila melanogaster	tubGal4	Bloomington Drosophila Stock Center			BDSC_5138; FB:FBi0012687		
genetic reagent (in whole organism)	Drosophila melanogaster	htr ¹ P1; tubulinGal80 FRT40A; UAS-mCD8::GFP tubulinGal4		this paper			Component stocks/insertions: RRID:BDSC_5; FB:0002044; RRID:BDSC_5192; RRID:BDSC_30030;	
genetic reagent (in whole organism)	Drosophila melanogaster	cher1		PMID:9093858		FB:FBaI0060473		
genetic reagent (in whole organism)	Drosophila melanogaster	cherM113			David Ish-Horowitz lab		This work/Laboratory of D. Ish-Horowitz; mutations generated on FRT82 chromosome by EMS. Allele was generated as an extension of the work done in Hayashi et al 2014 but not published.	Gift of David Ish-Horowitz.
genetic reagent (in whole organism)	Drosophila melanogaster	cherM919			David Ish-Horowitz lab		This work/Laboratory of D. Ish-Horowitz; mutations generated on FRT82 chromosome by EMS. Allele was generated as an extension of the work done in Hayashi et al 2014 but not published.	Gift of David Ish-Horowitz.
genetic reagent (in whole organism)	Drosophila melanogaster	hst175fs		PMID:30559276		FB:FBaI0345602		
genetic reagent (in whole organism)	Drosophila melanogaster	htrR913s		PMID:30559276		FB:FBaI0345601		
genetic reagent (in whole organism)	Drosophila melanogaster	UASp-mCherry		PMID:22428051		FB:FBaI0284922		
genetic reagent (in whole organism)	Drosophila melanogaster	UASp-TAPmCherry-Kelch		PMID:26384358		FB:FBaI0283122		
genetic reagent (in whole organism)	Drosophila melanogaster	UASp-Kelch-deltaNTR		PMID:30559276		FB:FBaI0345599		
genetic reagent (in whole organism)	Drosophila melanogaster	tubulin-ovhts-hsN4UTRdelta100		PMID:24677508		FB:FBaI0301360		Tubulin direct drive construct missing region of 3'UTR required for Swallow dependent localization at at oocyte cortex
genetic reagent (in whole organism)	Drosophila melanogaster	UASH-Ovhts::GFP-hsN4UTR		PMID:17215303		FB:FBaI0212988		Contains UAS promoter with ovhts/hisN4 3'UTR sequence in place of typical 3'UTR
genetic reagent (in whole organism)	Drosophila melanogaster	UASH-Ovhts::GFP-K18UTR		this paper			ovhts::GFP PCR amplified from UASH-ovhts::GFP-hsN4UTR construct and inserted into pDONR201; Insert was then swapped into pPW-attB using Gateway recombination. Resulting plasmid was transformed into a strain containing the attP2 landing site on chromosome III (88A4).	
genetic reagent (in whole organism)	Drosophila melanogaster	UASH-Ovhts::V5::APEX-hsN4UTR		this paper			APEX was PCR amplified from pDNA3-mito-APEX using a primer containing V5 tag sequence. Resulting PCR product was restriction cloned into UASH-ovhts::GFP (hsN43'UTR) in frame in the place of GFP. Plasmid was inserted using p-element insertion	
genetic reagent (in whole organism)	Drosophila melanogaster	hts Df. w ¹¹¹⁸ ; Df(2R)BSC135/CyO	Bloomington Drosophila Stock Center			BDSC_9423; FB:FBaI0044866		
genetic reagent (in whole organism)	Drosophila melanogaster	cher-M07480-GFSTF.0	Bloomington Drosophila Stock Center			BDSC_60261; FB:FBaI0314454		
genetic reagent (in whole organism)	Drosophila melanogaster	ArpC1-Q25SD FRT40A	Bloomington Drosophila Stock Center			BDSC_9137; FB:FBaI0135790		
genetic reagent (in whole organism)	Drosophila melanogaster	ArpC4-SH1036 FRT40A	Bloomington Drosophila Stock Center			BDSC_29488; FB:FBaI0321477		
genetic reagent (in whole organism)	Drosophila melanogaster	isogenized neoFRT40A		PMID:8404527		FB:FBi0002071		Gift of Tian Xu
gene (source not applicable)	Drosophila melanogaster	hts	NA	NA	NA	FB:FBgn0263391		
gene (source not applicable)	Drosophila melanogaster	cheerio	NA	NA	NA	FB:FBgn0014141		
gene (source not applicable)	Drosophila melanogaster	kelch	NA	NA	NA	FB:FBgn0001301		
gene (source not applicable)	Drosophila melanogaster	ArpC1	NA	NA	NA	FB:FBgn0001961		
gene (source not applicable)	Drosophila melanogaster	ArpC4	NA	NA	NA	FB:FBgn0284255		
software, algorithm	NA	ImageJ/FIJI	NIH					https://fiji.sc/
software, algorithm	NA	Imaris 9.3	Bitplane					https://www.bitplane.com/
software, algorithm	NA	Prism 8	GraphPad					https://www.graphpad.com/scientificsoftware/prism/
software, algorithm	NA	Jalview		PMID:19151095				http://www.jalview.org/
software, algorithm	NA	Jupyter Notebook	Anaconda					http://www.anaconda.com/
software, algorithm	NA	NCBI Blast	NIH					https://blast.ncbi.nlm.nih.gov/
software, algorithm	NA	Vectorbase		PMID:25510499				https://www.vectorbase.org/blast
software, algorithm	NA	SPOT-Disorder-Single application		PMID:30395465				
software, algorithm	NA	MetaDisorder (GeneSilo)		PMID:22624656				http://genesilo.clinicaldisorder/
recombinant DNA reagent	NA	pUASH-ovhts-GFP		PMID:17215303				
recombinant DNA reagent	NA	pCDNA3-mito-APEX		PMID:23371551			Addgene Plasmid #42607	
recombinant DNA reagent	NA	pPW-attB (gateway vector)			Lab of Michael Buszczak, UT Southwestern			
recombinant DNA reagent	NA	pDONR201 (gateway vector)	Thermo Fisher Scientific					
gene (source not applicable)	D. arizonae	predicted HtsRC homolog				XM_018011272		
gene (source not applicable)	D. biarmipes	predicted HtsRC homolog				XM_017057880		
gene (source not applicable)	D. bipunctata	predicted HtsRC homolog				XM_017204448.2		
gene (source not applicable)	D. busckii	predicted HtsRC homolog				XM_017202483.2		
gene (source not applicable)	D. elegans	predicted HtsRC homolog				XM_017272506.1		
gene (source not applicable)	D. erecta	predicted HtsRC homolog				XM_018166883		
gene (source not applicable)	D. eugracilis	predicted HtsRC homolog				XM_017272803.2		
gene (source not applicable)	D. foushiana	predicted HtsRC homolog				XM_017284820.3		
gene (source not applicable)	D. grimshawi	predicted HtsRC homolog				XM_016872116		
gene (source not applicable)	D. hydei	predicted HtsRC homolog				XM_020511683		
gene (source not applicable)	D. ikkawai	predicted HtsRC homolog				XM_017388381		
gene (source not applicable)	D. melanogaster	HtsRC				XM_001688785		
gene (source not applicable)	D. miranda	predicted HtsRC homolog				XM_017221055.1		
gene (source not applicable)	D. mojavensis	predicted HtsRC homolog				XM_002709844.1		
gene (source not applicable)	D. novojoki	predicted HtsRC homolog				XM_020389860		
gene (source not applicable)	D. persimilis	predicted HtsRC homolog				XM_020315197		
gene (source not applicable)	D. pseudoobscura	predicted HtsRC homolog				XM_001688204		
gene (source not applicable)	D. rhopaloea	predicted HtsRC homolog				XM_017222787		
gene (source not applicable)	D. sechellia	predicted HtsRC homolog				XM_020344448.1		
gene (source not applicable)	D. serrata	predicted HtsRC homolog				XM_020344448.1		
gene (source not applicable)	D. simulans	predicted HtsRC homolog				XM_016815886.1		
gene (source not applicable)	D. suzukii	predicted HtsRC homolog				XM_017272841		
gene (source not applicable)	D. takahasi	predicted HtsRC homolog				XM_017145521		
gene (source not applicable)	D. willis	predicted HtsRC homolog				XM_020315197		
gene (source not applicable)	D. willistoni	predicted HtsRC homolog				XM_020173252		
gene (source not applicable)	D. yakuba	predicted HtsRC homolog				XM_016182287		
gene (source not applicable)	G. pallipes	predicted HtsRC homolog				GP0028402-RA; GP0028401-RA		
gene (source not applicable)	M. domestica	predicted HtsRC homolog				XP_012246254.1; XM_012246252.2		