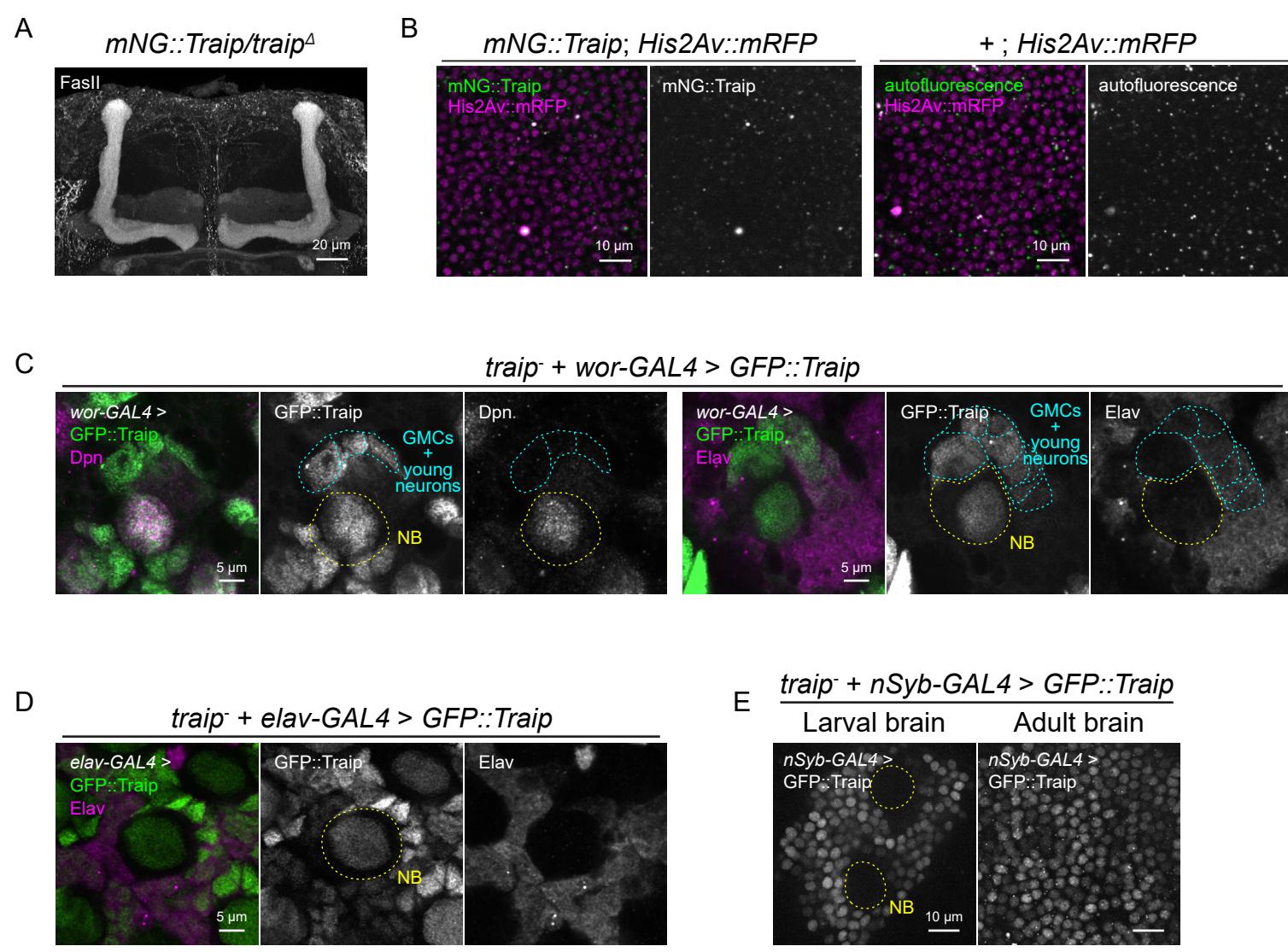


**Fig. S1. *Traip* is required for proper MB structure, supporting figures**

- (A) Confocal slices through control and *traip*<sup>-</sup> adult brains stained for N-Cadherin (CadN, magenta) and Neuroglian (Nrg, green), showing no obvious differences in neuropil regions or axon tracts, excluding the MBs.
- (B) Control and *traip*<sup>-</sup> full MB volumes (white) segmented from *OK107-GAL4 > mCD8::GFP* fluorescence (green).
- (C) Full MB volume measurements. N ≥ 18 MBs.
- (D)  $\alpha'/\alpha$  lobe cross-section measurements. N ≥ 18 MBs.
- (E)  $\alpha$  lobe cross-section measurements of controls and various combinations of *Traip* mutant alleles. Homozygous null animal MBs are similarly reduced, whereas hypomorphic animals have wild-type MB size. N ≥ 18 MBs/genotype.
- (F) Hypomorphic *traip*<sup>Z1447</sup> MBs have wild-type morphology.
- (G, H)  $\alpha$  lobe cross-section measurements show that *traip*<sup>-</sup> + *ubi-GFP::Traip* (G) and *traip*<sup>-</sup> + *Tub-GAL4 > GFP::Traip* (H) rescues have wild-type MB size. N ≥ 10 MBs.
- (I) Simple linear regression between KC number (X axis) and volumes (Y axis) of *OK107-GAL4 > mCD8::GFP*-positive full MBs ( $R^2 = 0.83$ , yellow squares) or FasII-positive  $\alpha/\beta$  lobes ( $R^2 = 0.84$ , blue circles).
- (J) Simple linear regression between KC number (X axis) and the cross sectional areas (Y axis) of *OK107-GAL4 > mCD8::GFP*-positive  $\alpha'/\alpha$  lobes ( $R^2 = 0.87$ , yellow squares) or FasII-positive  $\alpha$  lobes ( $R^2 = 0.87$ , blue circles).

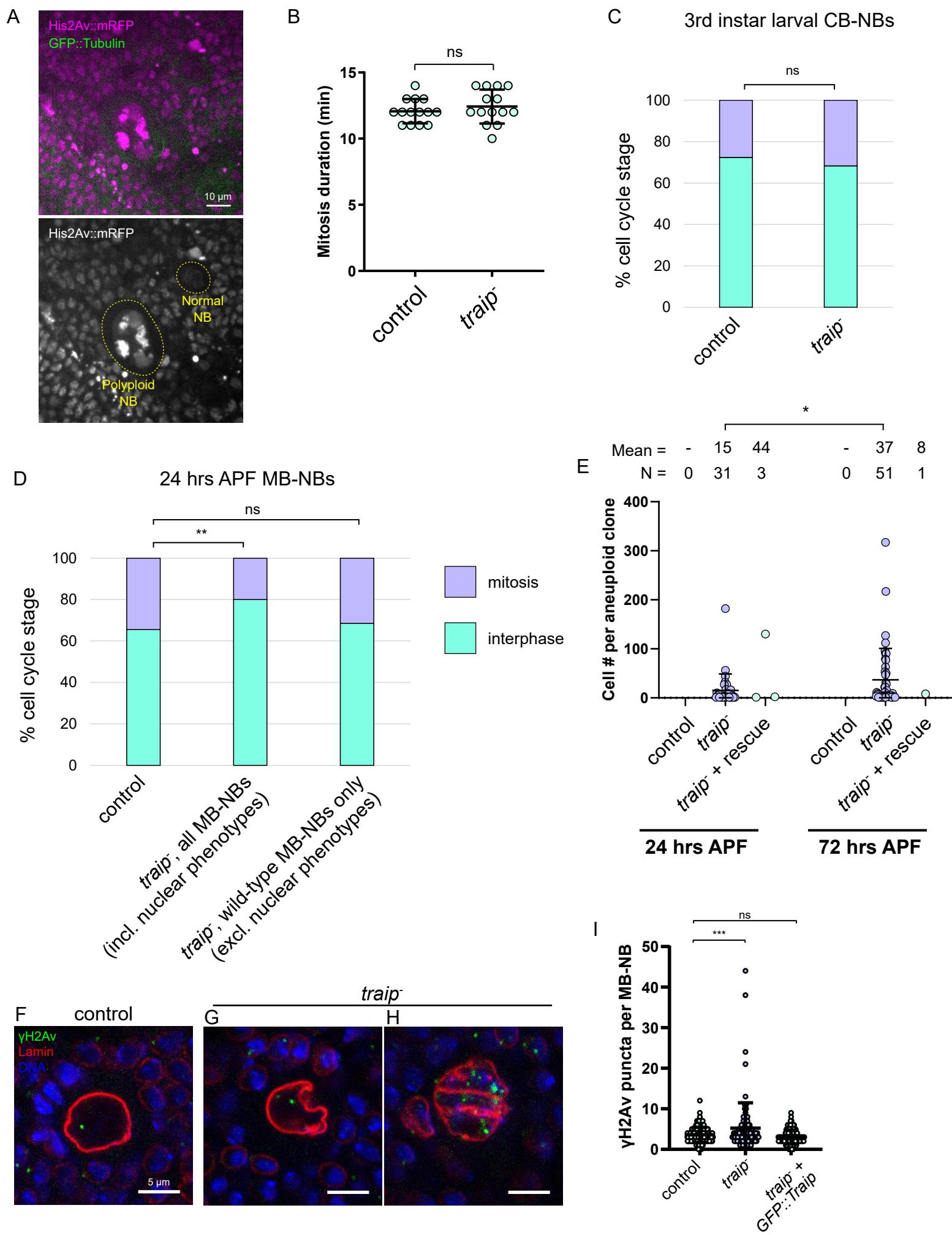
Ordinary one-way ANOVA was used for significance. ns = not significant, \*\*\*\* p < 0.0001. Scale bars = 50 μm (A, B), 20 μm (F).



**Fig. S2. *Traip* is required in neuroblasts, supporting figures**

- (A) *mNG::Traip<sup>CRISPR</sup>* encodes a functional protein that fully rescues *traip<sup>A</sup>* MB size.
- (B) Adult brains from either *mNG::Traip<sup>CRISPR</sup>* (left panels) or + (untagged *Traip*, right panels) with His2Av::mRFP (magenta). *mNG::Traip* (green, gray; left panels) does not have fluorescent signal above autofluorescence background of + (green, gray; right panels).
- (C) CB-NBs from *traip<sup>-</sup>* + *wor-GAL4 > GFP::Traip* 3<sup>rd</sup> instar larval brains, stained either Dpn (magenta; left panels) or Elav (magenta; right panels). *wor-GAL4 > GFP::Traip* (green) is expressed in NBs (Dpn-positive, yellow highlighting) and persists into daughter GMCs and neurons (Elav-positive, cyan highlighting).
- (D) *traip<sup>-</sup>* + *elav-GAL4 > GFP::Traip* has significant GFP::Traip expression in 3<sup>rd</sup> instar larval CB-NBs.
- (E) *traip<sup>-</sup>* + *nSyb-GAL4 > GFP::Traip* has GFP::Traip expression in larval and adult neurons, but no expression in larval NBs.

Scale bars = 10 μm (B, E), 5 μm (C, D).



**Fig. S3. *Traip* suppresses multinuclear phenotypes and mitotic DNA bridges, supporting figures**

(A) Live imaging of *traip*<sup>-</sup> 3<sup>rd</sup> instar larval CB-NBs expressing His2Av::mRFP (magenta) and GFP::Tubulin (green) showing likely polyploid NBs with enlarged nuclei and increased His2Av::mRFP fluorescence compared to a normal NB. Scale bars = 10  $\mu$ m.

(B) Total duration of mitosis for control and *traip*<sup>-</sup> 3<sup>rd</sup> instar larval CB-NBs, as measured from prophase onset to complete furrow constriction. N = 14 NBs.

(C) Mitotic index of fixed control and *traip*<sup>-</sup> 3<sup>rd</sup> instar larval CB-NBs.

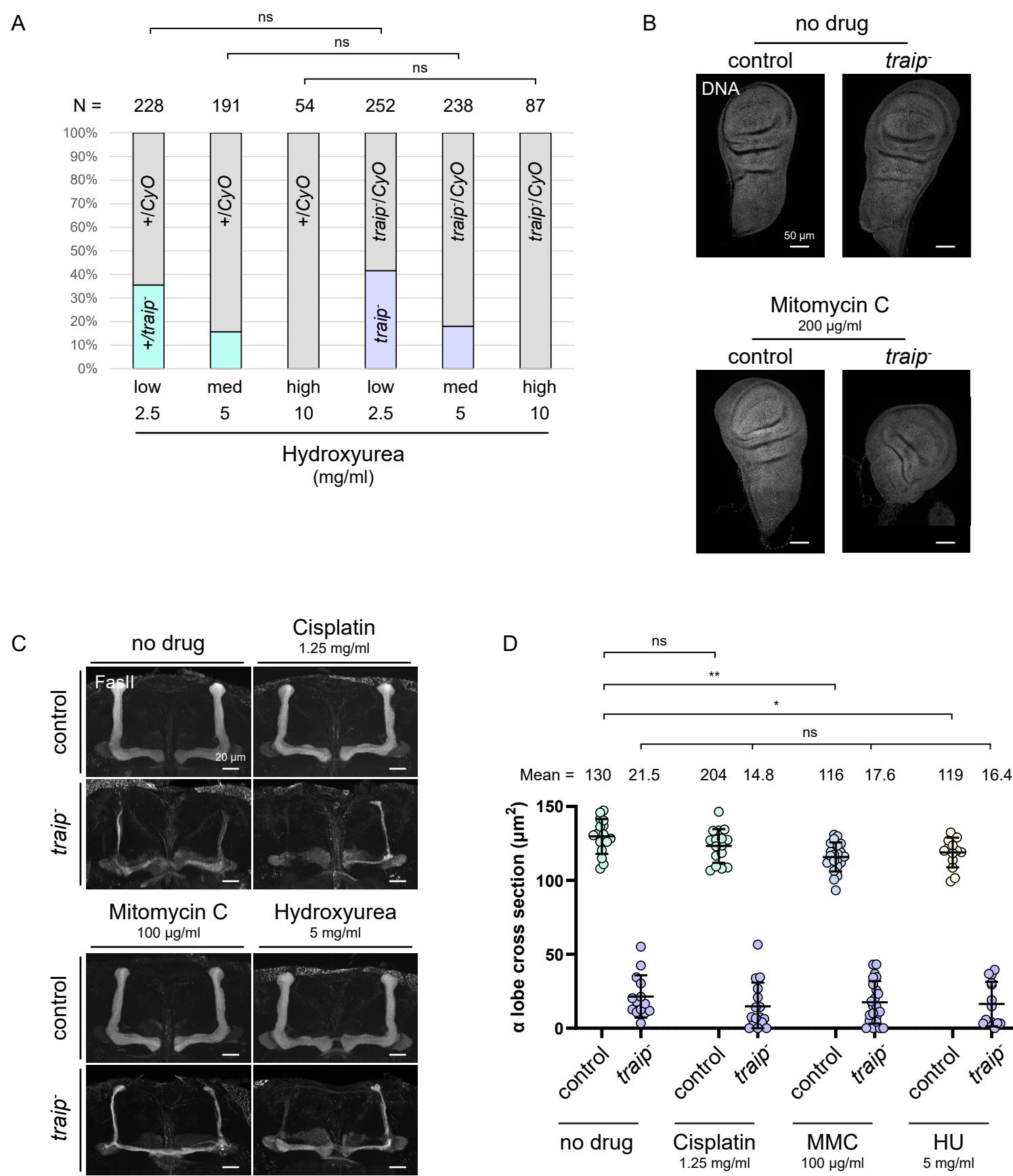
(D) Mitotic index of fixed control and *traip*<sup>-</sup> 24 hours APF MB-NBs, either including all *traip*<sup>-</sup> MB-NBs or only *traip*<sup>-</sup> MB-NBs without nuclear phenotypes (right column). \*\* p = 0.0025.

(E) KC number per aneuploid clone in control, *traip*<sup>-</sup>, and *traip*<sup>-</sup> + GFP::*Traip* rescue expressing *OK107-GAL4* > NLS::mCherry + mCD8::GFP at 24 and 72 hours APF pupal brains. The average number of KCs per clone in *traip*<sup>-</sup> increases from 15 at 24 hours APF to 37 at 72 hours APF. \* p = 0.0287.

(F-H) Control and *traip*<sup>-</sup> 24 hours APF MB-NBs stained for  $\gamma$ H2Av (green), Lamin (red), and DAPI (blue). Most control (F) and *traip*<sup>-</sup> (G) MB-NBs have few  $\gamma$ H2Av puncta. Rare *traip*<sup>-</sup> MB-NBs have extremely elevated  $\gamma$ H2Av puncta (H).

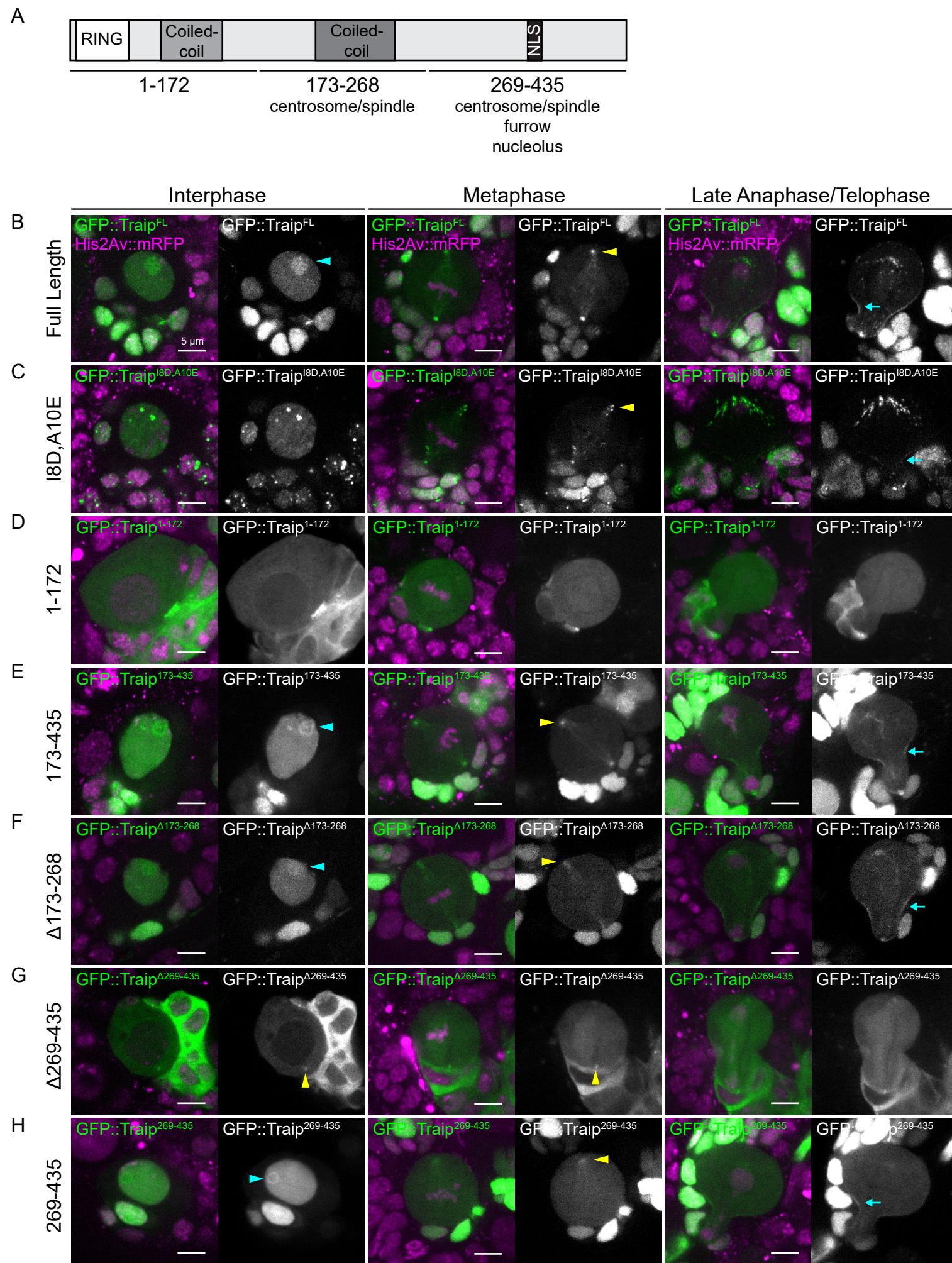
(I)  $\gamma$ H2Av puncta counts per MB-NB for control, *traip*<sup>-</sup>, and *traip*<sup>-</sup> + GFP::*Traip*.  $\gamma$ H2Av puncta in *traip*<sup>-</sup> were significantly higher than controls, primarily due to the small number of *traip*<sup>-</sup> MB-NBs with extremely elevated puncta. Ordinary one-way ANOVA was used for significance. \*\*\* p = 0.0004. N  $\geq$  100 MB-NBs.

T-test (B), chi-squared test (C, D) Two-tailed Mann-Whitney test (E), and ordinary one-way ANOVA (I) were used for significance. ns = not significant.



**Fig. S4. *Traip* is required for inter-strand crosslink repair, supporting figures**

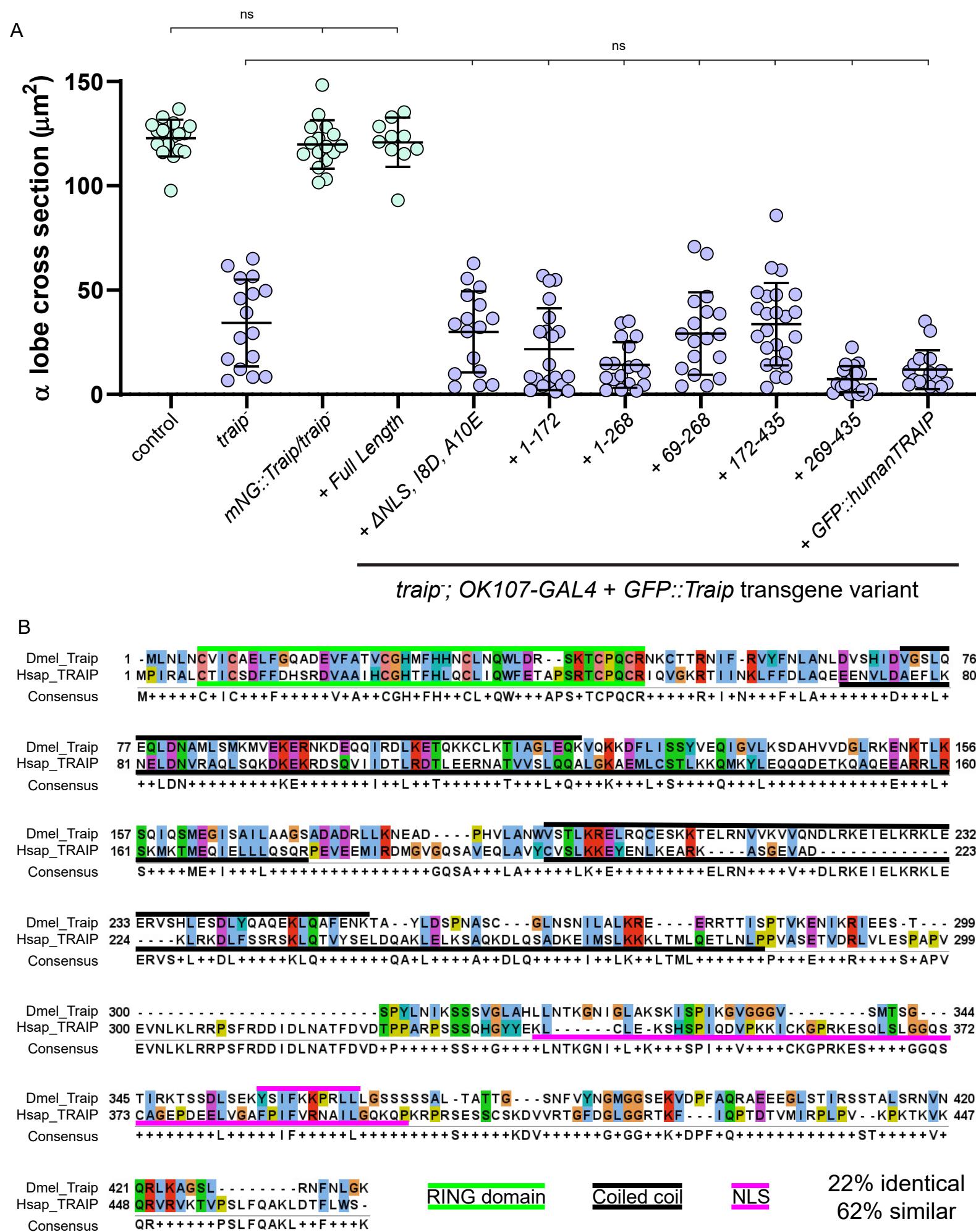
- (A) Hydroxyurea treatment survival assay results. Both control and *traip*<sup>-</sup> crosses produced offspring that were increasingly sensitive to higher doses of hydroxyurea. Chi-squared tests were used for significance.
- (B) Wing discs from control and *traip*<sup>-</sup> 3<sup>rd</sup> instar larvae treated with either no drug or high doses of Mitomycin C and stained for DAPI (gray). *traip*<sup>-</sup> discs treated with Mitomycin C are severely reduced in size compared to controls.
- (C) MBs from control and *traip*<sup>-</sup> treated with no drug or medium doses of Cisplatin, Mitomycin C, or Hydroxyurea, stained with FasII. Scale = 20  $\mu$ m.
- (D)  $\alpha$  lobe cross-section measurements of control and *traip*<sup>-</sup> treated with no drug or medium doses of Cisplatin, Mitomycin C (MMC), or Hydroxyurea (HU). Ordinary one-way ANOVA was used for significance. ns = not significant, \*\* p = 0.0013, \* p = 0.0357. N  $\geq$  14 MBs.



**Fig. S5. Traip localization depends on distinct domains**

(A) Schematic of major Traip protein features, transgene fragment breakpoints, and localization domains.

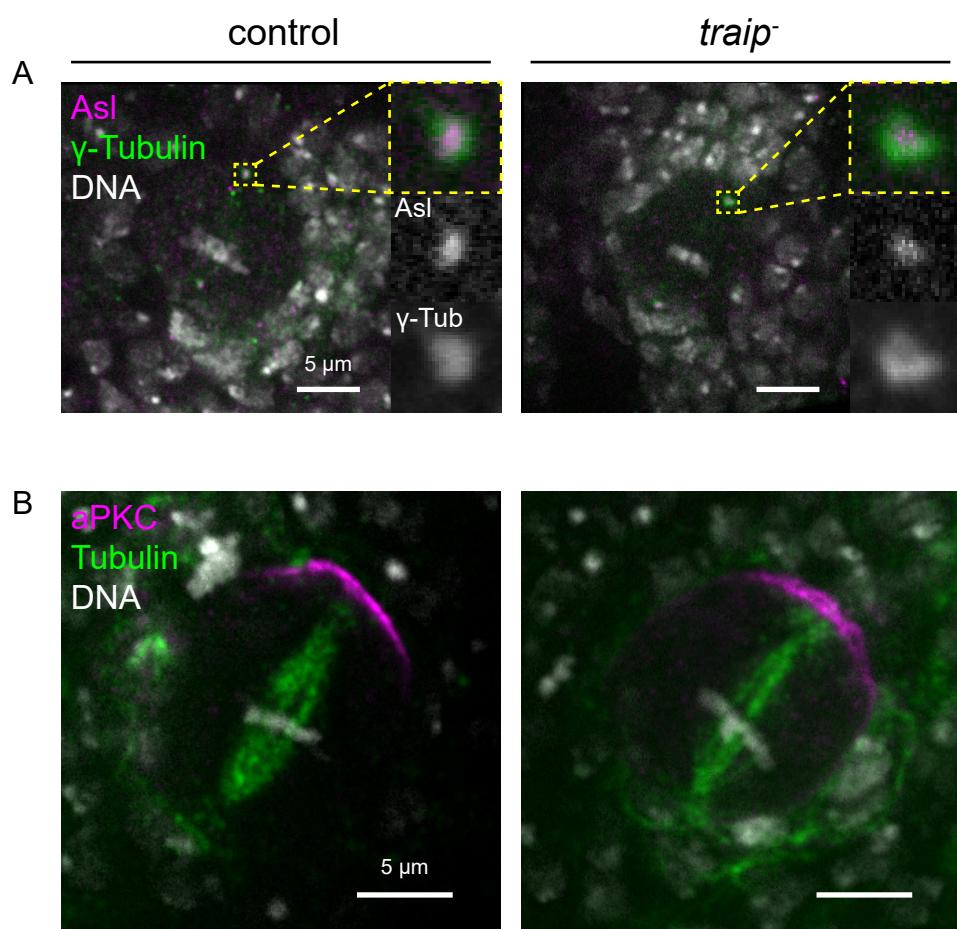
(B-H) Localization of GFP::Traip variant transgenes (green) expressed via *wor-GAL4* with His2Av::mRFP (magenta) in CB-NBs during interphase, metaphase, and late anaphase/telophase. Yellow arrowhead denotes centrosome localization, cyan arrow denotes cytokinetic furrow localization, and cyan arrowhead denotes nucleolar localization. GFP::Traip variants include: (B) Full Length, reproduced from Figure 7D; (C) RING domain mutant I8D, A10E; (D) RING domain and first coiled coil 1-172; (E) second coiled coil and C-terminal domain 173-435; (F) a deletion of the second coiled coil Δ173-268; (G) deletion of the C-terminal domain Δ269-435; and (H) the C-terminal domain alone 269-435. Scale bar = 5 μm.



**Fig. S6. Transgene rescues attempted**

(A) α lobe cross-section measurements of control, *traip*<sup>-</sup>, and *traip*<sup>-</sup> + GFP::Traip variant transgenes expressed via *OK107-GAL4*. Transgenes included *mNG::Traip<sup>CRISPR</sup>*, Full Length, ΔNLS + I8D,A10E, 1-172, 1-268, 69-268, 172-435, 269-435, and humanTRAIP. Ordinary one-way ANOVA was used for significance. ns = not significant. N ≥ 10 MBs.

(B) Amino acid alignment of *Drosophila* Traip and human TRAIP proteins. Known domains for Traip and TRAIP are highlighted above and below the sequences, respectively, including RING domain, coiled coils, and NLS. Traip and TRAIP are 22% identical and 62% similar in amino acid sequence.



**Fig. S7. *Traip* is not required for proper centrosome or spindle formation**

(A) Control and *traip*<sup>-</sup> 24 hours APF metaphase MB-NBs stained for  $\gamma$ -Tubulin (green), Asl (magenta), and DAPI (grey). There were no obvious centrosome defects in *traip*<sup>-</sup>.

(B) Control and *traip*<sup>-</sup> 24 hours APF metaphase MB-NBs stained for Tubulin (green), aPKC (magenta), and DAPI (grey). There were no obvious polarity defects in *traip*<sup>-</sup>.

**Table S1. Details for Reagents and Strains**

Reagent or Resource	Source	Identifier
<b>Antibodies</b>		
mouse anti-FasII	Developmental Studies Hybridoma Bank	1D4
rat anti- CadN	Developmental Studies Hybridoma Bank	DN-Ex #8
mouse anti-Nrg	Developmental Studies Hybridoma Bank	BP104
mouse anti-Elav	Developmental Studies Hybridoma Bank	7E8A 10
mouse anti-Lamin	Developmental Studies Hybridoma Bank	ADL84.12
mouse anti- $\gamma$ H2Av	Developmental Studies Hybridoma Bank	UNC93-5.2.1
mouse anti-Tubulin	Developmental Studies Hybridoma Bank	E7
rat anti-Cadherin	Developmental Studies Hybridoma Bank	DCAD2
mouse anti-GFP	ThermoFisher	A11122
mouse anti-Phospho-tyrosine	Millipore	4G10
rabbit anti-Phospho-histone H3	Millipore	H3S10P
rabbit anti-aPKC	Santa Cruz	C20
mouse anti- $\gamma$ -Tubulin	Sigma	GTU-88

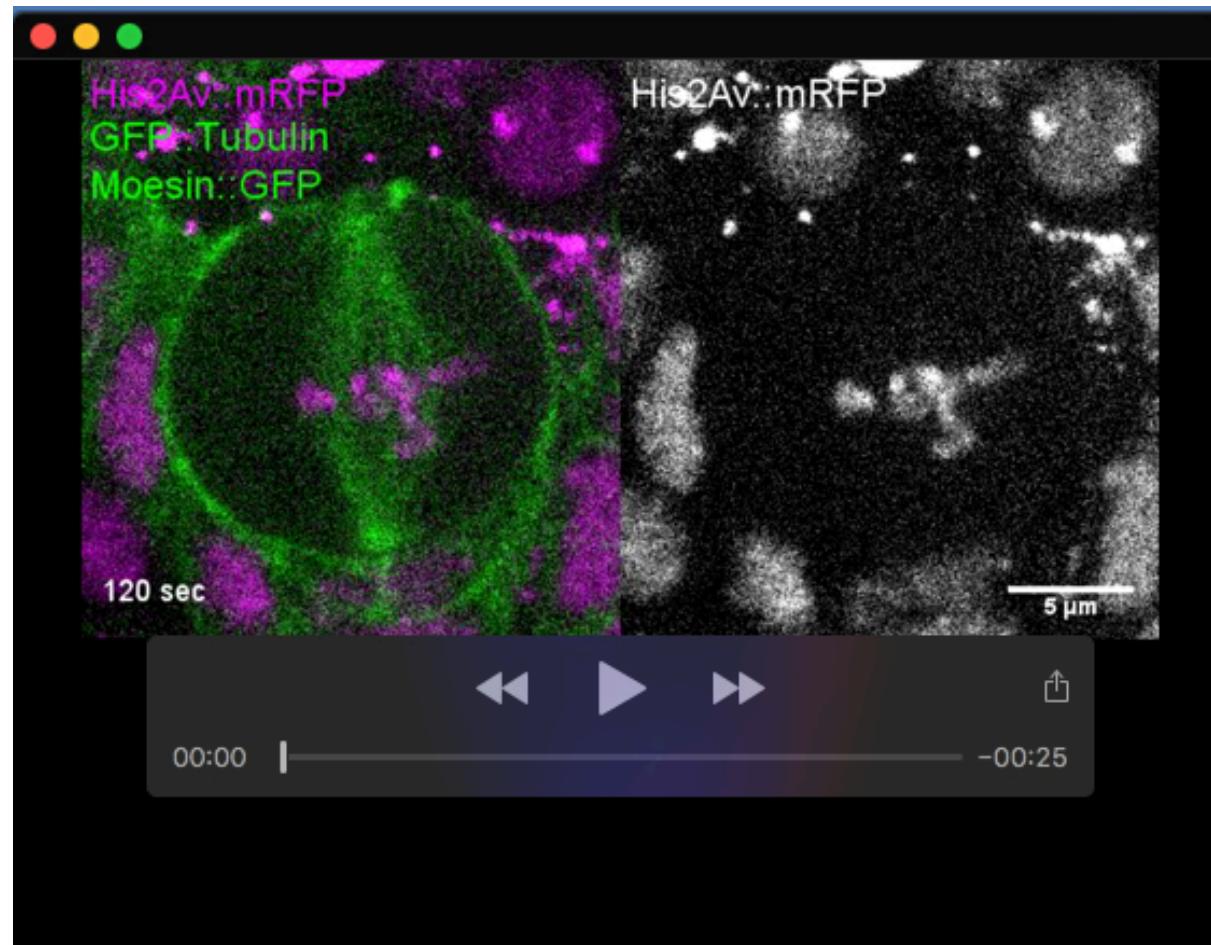
rabbit anti-Dcp-1	Cell Signaling Tech	9578
guinea pig anti-Asl	Rusan Lab	Klebba et al., 2013
guinea pig anti-Dpn	Skeath lab	N/A
Alexa Fluor 488/568/647 conjugated secondary antibodies	Thermo Fisher Scientific	Variable host and target species
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
Alexa Fluor 568 Phalloidin	Molecular Probes/Fisher Scientific	Cat # A12380
Phalloidin–Atto 647N	Sigma Aldrich	Cat # 65906
Cisplatin	Sigma Aldrich/Calbiochem	Cat # 232120
Mitomycin C	Sigma Aldrich/Calbiochem	Cat # 475820
Hydroxyurea	Sigma Aldrich/Calbiochem	Cat # 400046
<b>Drosophila Strains</b>		
yw	Peifer Lab (UNC-Chapel Hill)	N/A
<i>nopo</i> <sup>Exc142</sup>	Merkle et al., 2009	N/A
<i>nopo</i> <sup>Z1447</sup>	Bloomington Drosophila Stock Center	BDSC: 57334
<i>Df(2R)Exel7153</i>	Bloomington Drosophila Stock Center	BDSC: 7893
<i>Df(3L)H99</i>	Bloomington Drosophila Stock Center	BDSC: 1576

<i>mCherry</i> RNAi	Bloomington Drosophila Stock Center	BDSC: 35787
<i>Drice</i> RNAi	Bloomington Drosophila Stock Center	BDSC: 32403
<i>Tub-GAL4</i>	Bloomington Drosophila Stock Center	BDSC: 5138
<i>OK107-GAL4</i>	Bloomington Drosophila Stock Center	BDSC: 854
<i>wor-GAL4</i>	Bloomington Drosophila Stock Center	BDSC: 56554
<i>elav-GAL4</i>	Bloomington Drosophila Stock Center	BDSC: 8760
<i>nSyb-GAL4</i>	Bloomington Drosophila Stock Center	BDSC: 51635
<i>UAS-mCD8::GFP</i>	Bloomington Drosophila Stock Center	BDSC: 32186
<i>UAS-NLS::mCherry</i>	Giniger Lab (NINDS-NIH)	N/A
<i>ubi-GFP::Tubulin</i>	Dr. Tomer Avidor-Reiss, University of Toledo	N/A
<i>ubi-moesin::GFP</i>	Kiehart Lab (Duke)	N/A
<i>His2Av::mRFP</i>	Bloomington Drosophila Stock Center	BDSC: 23650
<i>UAS-mCherry::Tubulin</i>	Rusan Lab	N/A

<i>M{RFP[3xP3.PB] GFP[E.3xP3]=vas-Cas9}ZH-2A</i>	BestGene/Bloomington Drosophila Stock Center	BDSC: 51323
<b>Recombinant DNA</b>		
pPWG	Drosophila Genomics Resource Center	DGRC: 1078
pUC57 simple	GenScript	Cat # SD1176
pUC6-chiRNA	Addgene	Plasmid # 45946
pUC-attP-3xP3-TR	Rusan Lab	N/A
pOT2-nopo	BDGP Drosophila Gold Collection	GH03577
<i>FancD2</i> cDNA	Twist Bioscience	synthesized oligo
<i>hsTRAIP</i> cDNA?	Dharmacon, Inc	MHS6278-202825899
pENTR	Thermo Fisher Scientific	Cat # K2400-20
pPGW	Drosophila Genomics Resource Center	DGRC: 1077
pUGW	Drosophila Genomics Resource Center	DGRC: 1283
pUNW	Rusan Lab	N/A
pPT20TRW	Rusan Lab	N/A
<b>Software and Algorithms</b>		
MetaMorph for CSU-10 and CSU-22 systems	Molecular Devices	
Nikon Elements	Nikon	

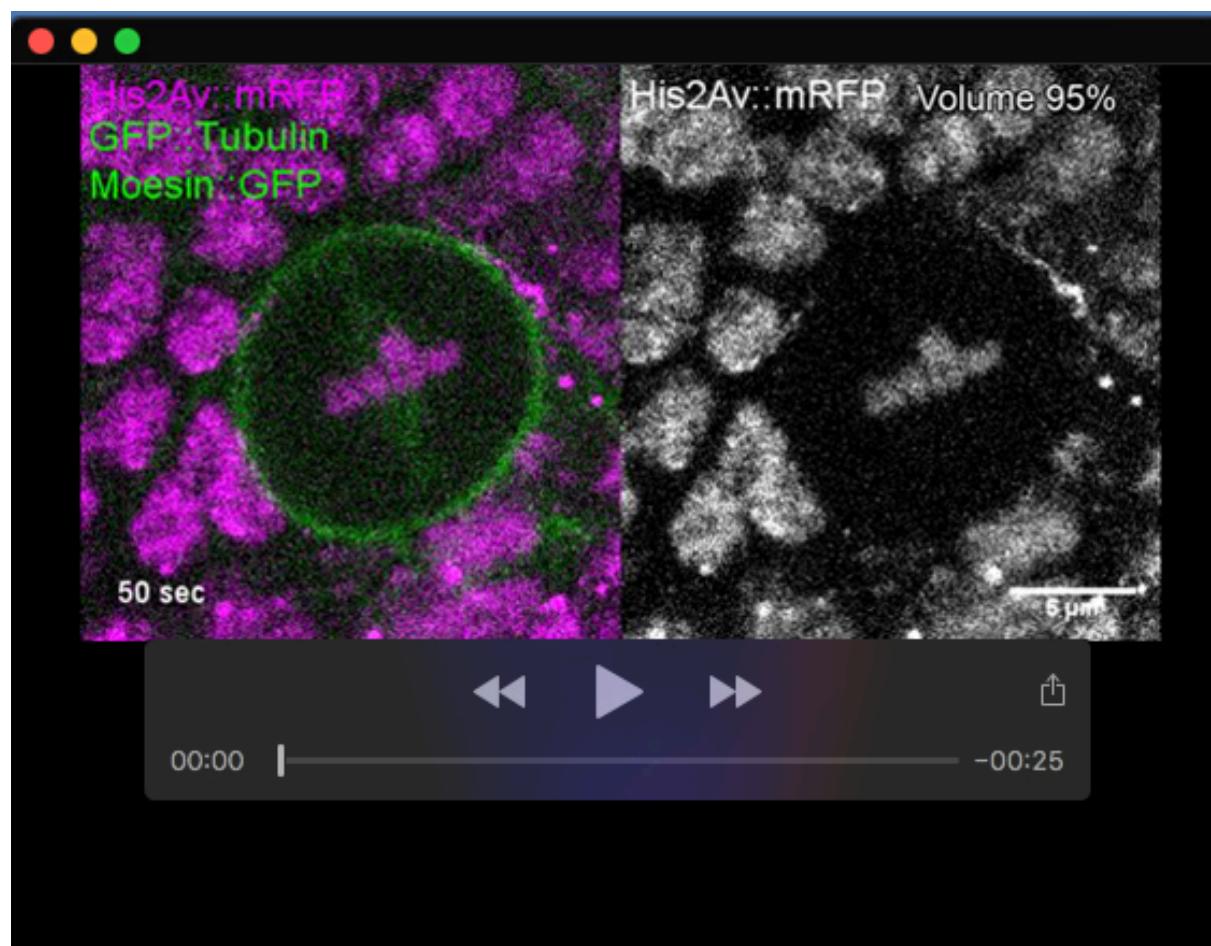
Zen Black	Zeiss	
Skyscan	Bruker	
NRecon, Bruker MicroCT, v1.7.0.4	Bruker	
FIJI/ImageJ	NIH	<a href="http://fiji.sc/">http://fiji.sc/</a>
Dragonfly v3.6	Object Research Systems	<a href="http://www.theobjects.com/dragonfly/">http://www.theobjects.com/dragonfly/</a>
Aivia	SVision	<a href="https://www.aivia-software.com/">https://www.aivia-software.com/</a>
Excel	Microsoft	<a href="https://products.office.com/en-us/excel">https://products.office.com/en-us/excel</a>
Flycrispr design tool		<a href="http://flycrispr.molbio.wisc.edu/tools">http://flycrispr.molbio.wisc.edu/tools</a>
cNLS Mapper	Kosugi <i>et al.</i> , 2009	<a href="http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi/">http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi/</a>
Prism 9	GraphPad	<a href="http://www.graphpad.com/scientificsoftware/prism/">www.graphpad.com/scientificsoftware/prism/</a>
Photoshop/Illustrator	Adobe	<a href="http://www.adobe.com/uk/products/">www.adobe.com/uk/products/</a>

[Click here to download Table S1](#)



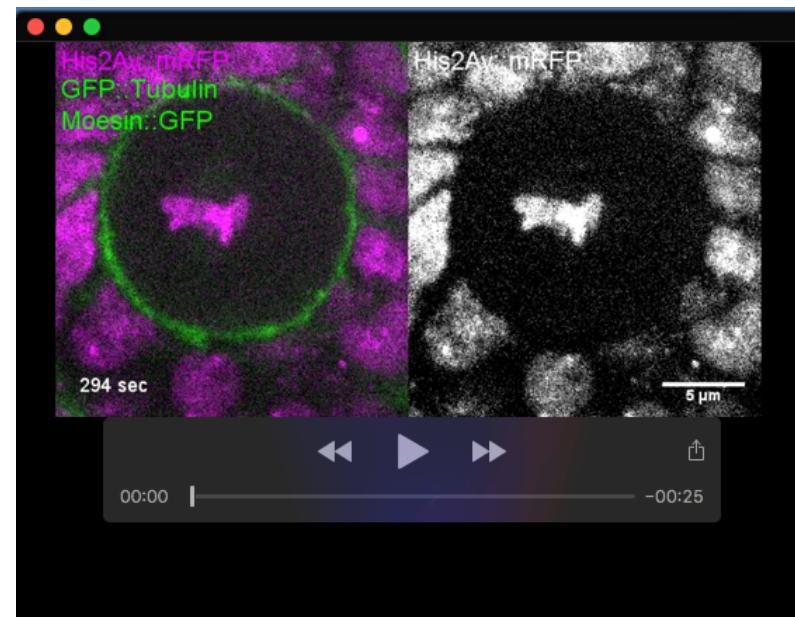
### Movie 1. Control NB mitosis

Control CB-NB expressing His2Av::mRFP (magenta, gray) and both GFP::Tubulin and Moesin::GFP (green), showing normal mitotic chromosome segregation.



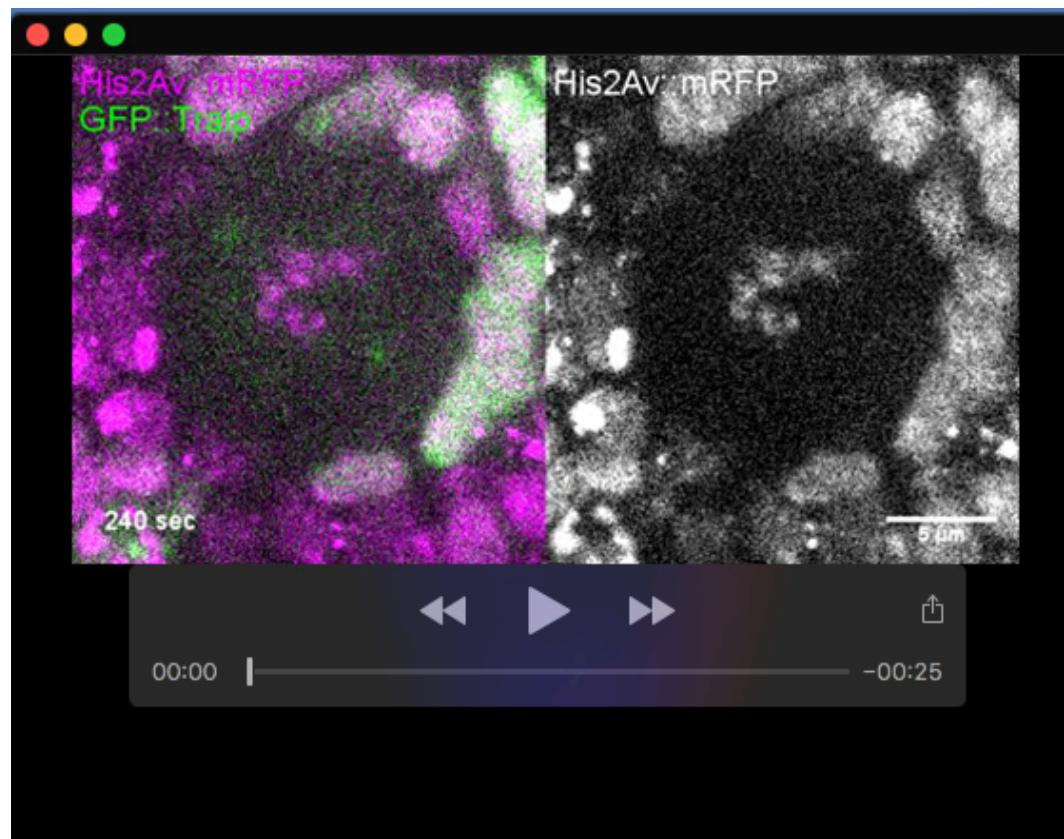
### Movie 2. *traip*- NB mitosis with a typical DNA bridge

*traip*<sup>-</sup> CB-NB expressing His2Av::mRFP (magenta, gray) and both GFP::Tubulin and Moesin::GFP (green). 26% (5/19) of *traip*<sup>-</sup> NBs had chromosome bridges during anaphase.



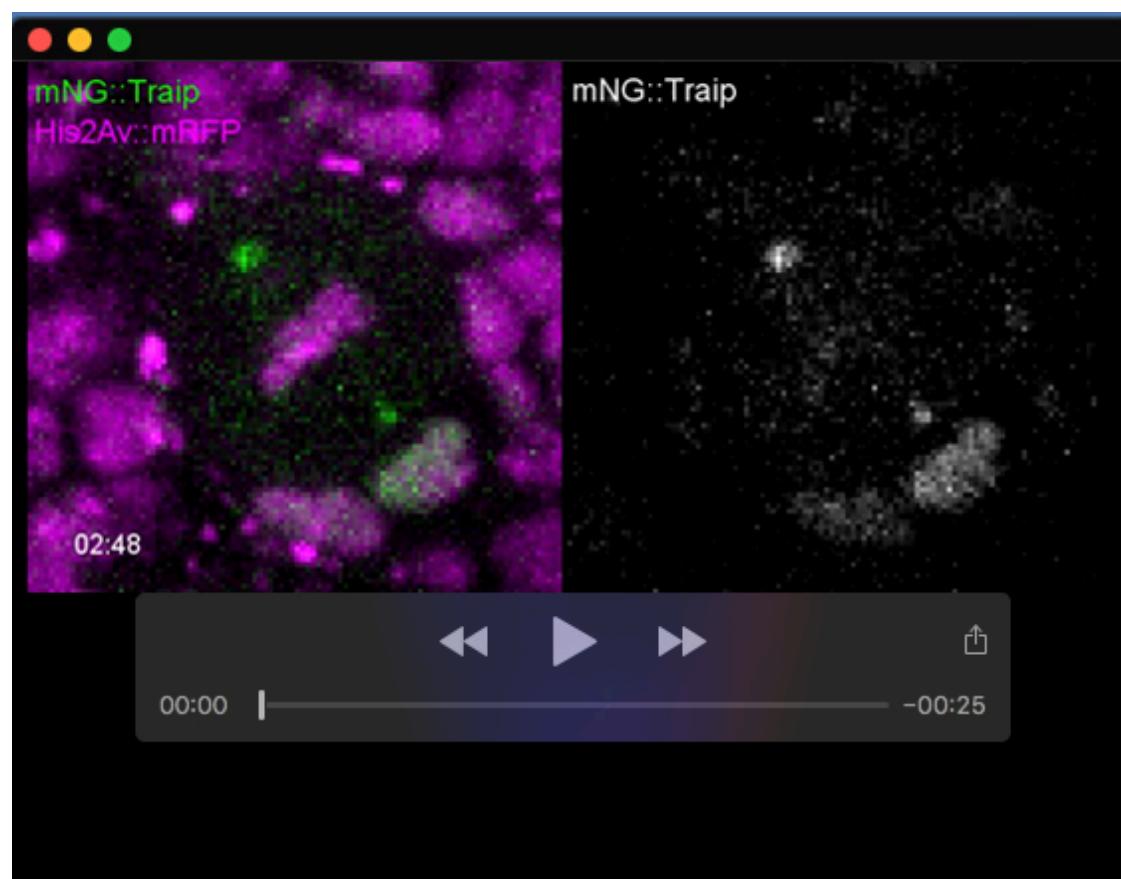
**Movie 3. *traip*<sup>-</sup> NB mitosis with a severe DNA bridge**

*traip*<sup>-</sup> CB-NB expressing His2Av::mRFP (magenta, gray) and both GFP::Tubulin and Moesin::GFP (green), showing a prominent chromosome bridge and cortical disruption.



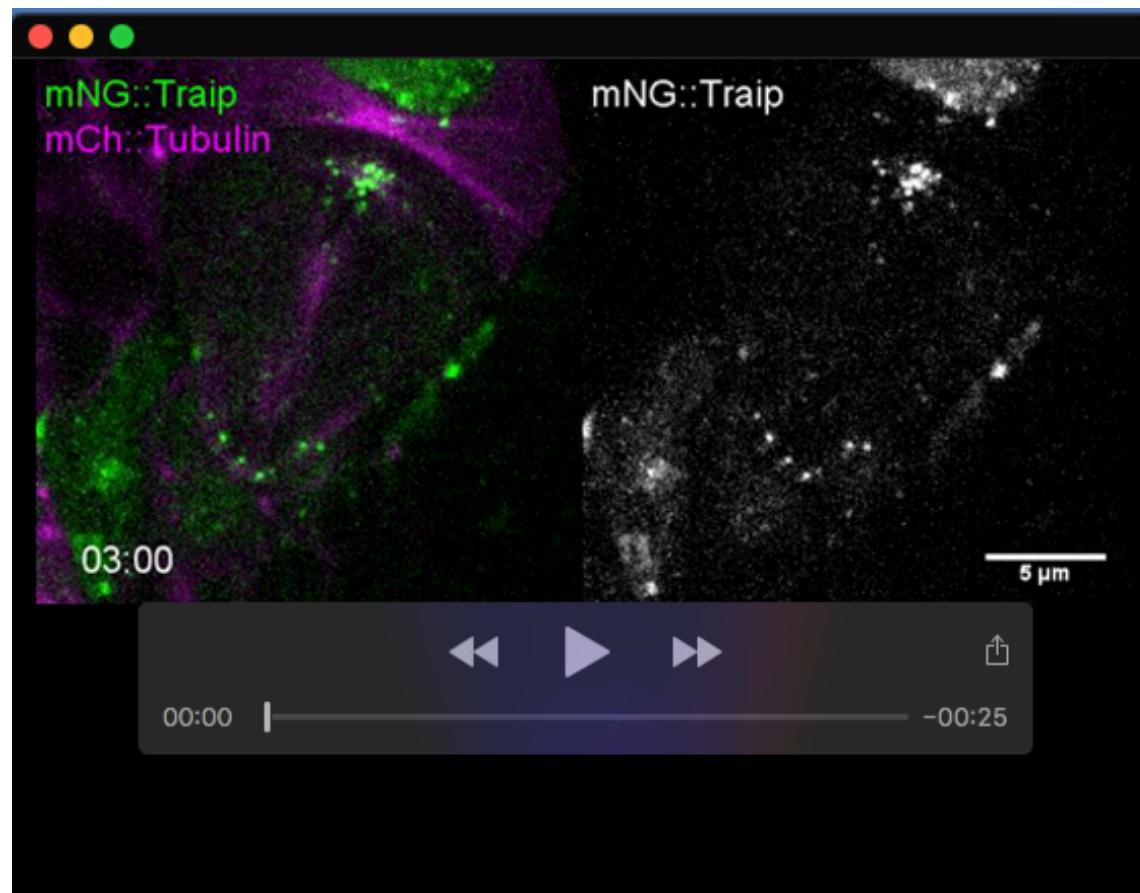
**Movie 4. GFP::Traip rescues *traip*<sup>-</sup> NB mitotic DNA bridges**

CB-NBs from *traip*<sup>-</sup> with *wor-GAL4* > GFP::Traip (green) and His2Av::mRFP (magenta, gray) have normal mitotic chromosome segregation.



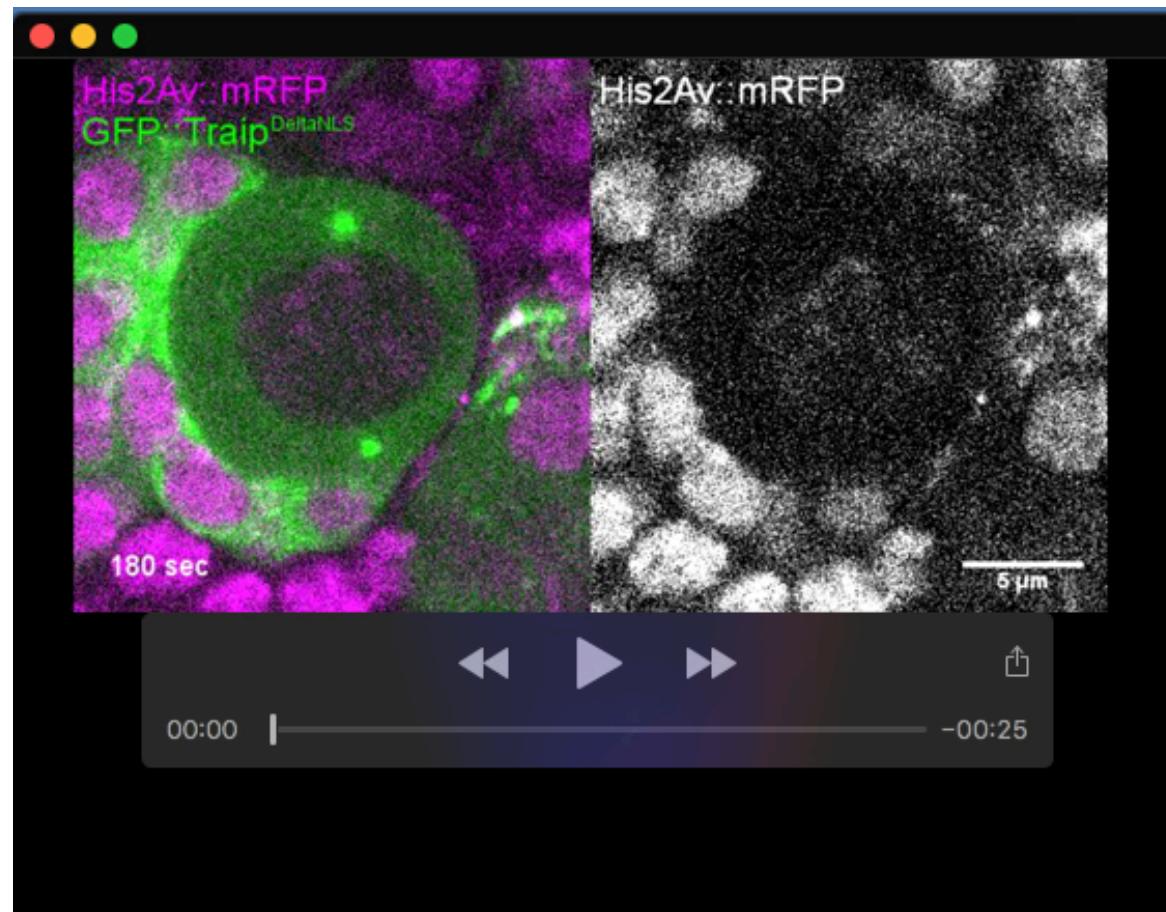
**Movie 5. mNG::Traip localization during NB mitosis, low magnification**

Mitotic CB-NB expressing mNG::Traip (green, gray) and His2Av::mRFP (magenta) at 40x magnification. At mitotic onset mNG::Traip is released from the nucleus and localizes to the centrosomes and spindle.



**Movie 6. mNG::Traip localization during NB mitosis, high magnification**

Mitotic CB-NB expressing mNG::Traip (green, gray) and mCherry::Tubulin (magenta) at 100x magnification. High magnification imaging shows mNG::Traip moving pole-wards along microtubules as puncta.



**Movie 7. GFP::Traip<sup>ΔNLS</sup> rescues *traip*<sup>-</sup> NB mitotic DNA bridges**

CB-NBs from *traip*<sup>-</sup> with *wor-GAL4 > GFP::Traip<sup>ΔNLS</sup>* (green) and His2Av::mRFP (magenta, gray) have normal mitotic chromosome segregation (22/23).