Supplementary information

Supplementary figures and legends (the legends are either in the same page as the figure or the following one)

Fig. S1

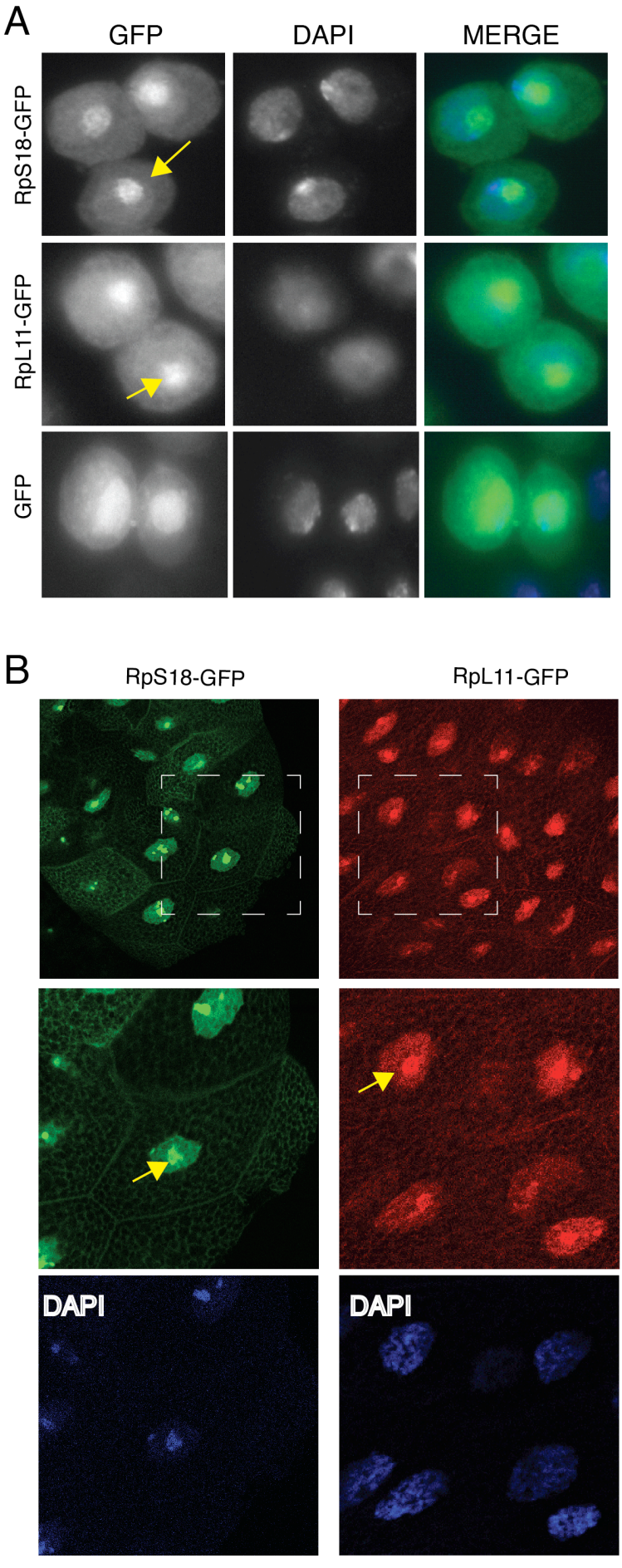

A
GFP
DAPI
MERGE
RpS18-GFP
RpL11-GFP
GFP
B
RpS18-GFP
RpL11-GFP
DAPI
DAPI

Figure S1. GFP tagged S18 and L11 concentrate in the nucleus and nucleolus.

(A) Fluorescence imaging of cell expressing GFP tagged version of the proteins indicated (left). Middle panels show DAPI staining, right panels Merge images. Arrow indicates the nucleolus. Bottom raw shows cells similarly expressing untagged GFP, which concentrates in the nucleus.

(B) Top row, confocal fluorescence images of salivary gland cells expressing either S18-GFP (left) or L11-RFP (right). Corresponding pictures below show close up of cells and DAPI staining; the arrow indicates the nucleolus.

Fig.S2

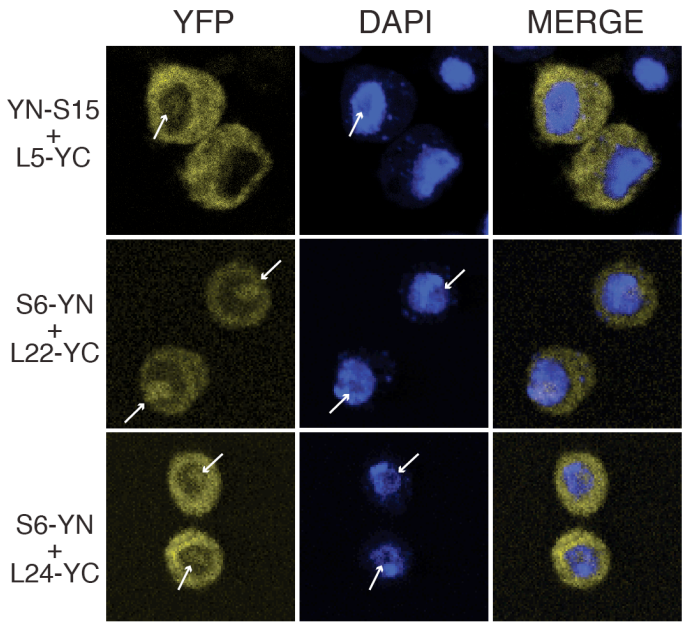


Figure S2. All RP pairs adjacent on the 80S produce also BiFC signal in the nucleolus.

YFP signal visualized in cells co-transfected with UAS-regulated plasmids indicated p-Act-GAL4. YFP signals are shown on the left, DAPI staining in the middle and the merged images on the right. All micrographs are confocal images taken with a 60X oil immersion objective. The nucleoli are indicated by arrows.

Fig. S3

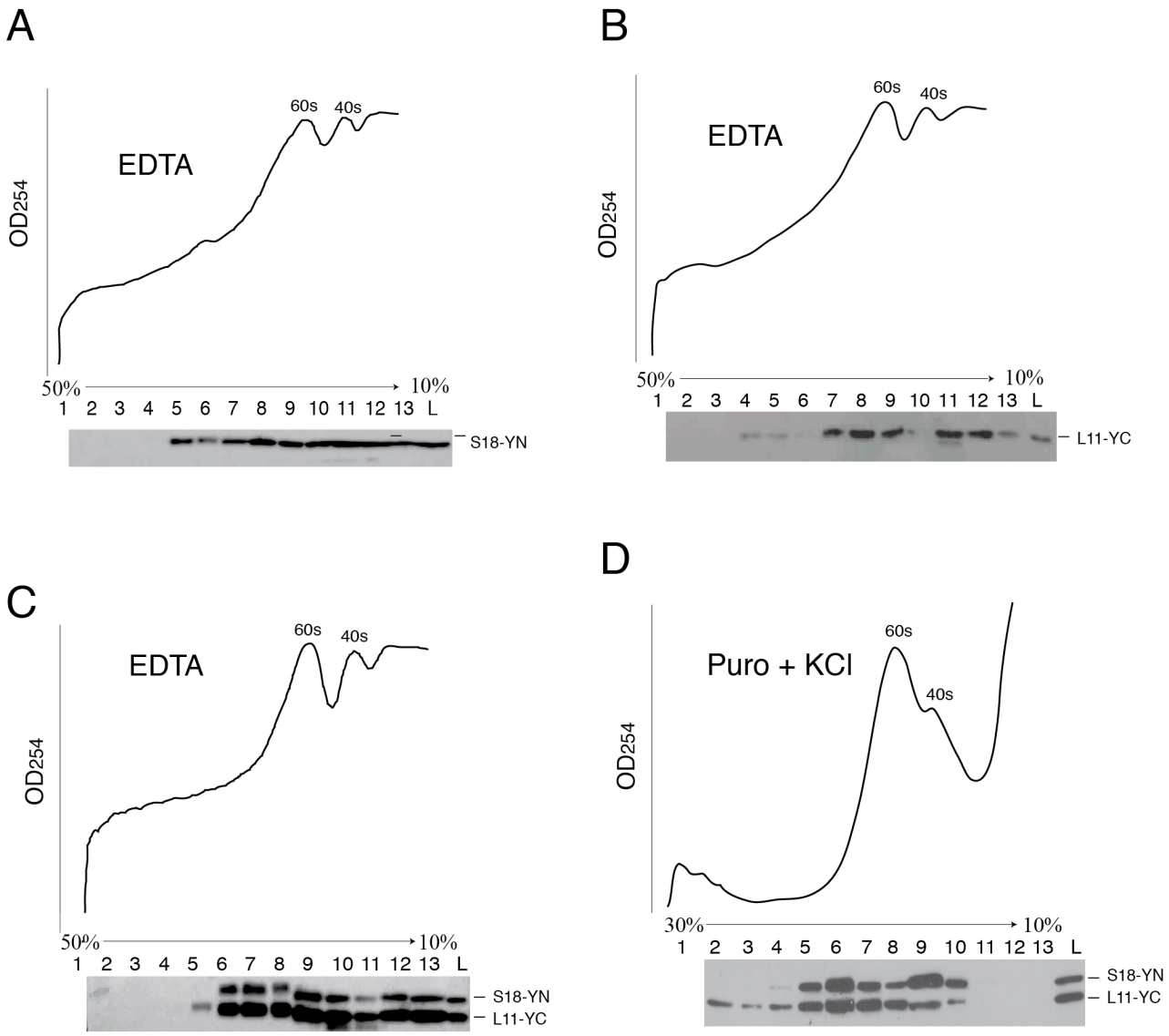

A
EDTA
60s
40s
OD254
50%
10%
1 2 3 4 5 6 7 8 9 10 11 12 13 L
S18-YN
B
EDTA
60s
40s
OD254
50%
10%
1 2 3 4 5 6 7 8 9 10 11 12 13 L
L11-YC
C
EDTA
60s
40s
OD254
50%
10%
1 2 3 4 5 6 7 8 9 10 11 12 13 L
S18-YN
L11-YC
D
Puro + KCl
60s
40s
OD254
30%
10%
1 2 3 4 5 6 7 8 9 10 11 12 13 L
S18-YN
L11-YC

Figure S3. The S18-YN and L11-YC constructs are incorporated into ribosomes.

(A-B) Polysomes profiling of extracts treated with EDTA, from cells expressing either S18-YN (A) or L11-YC (B). Western blotting of the different fractions with a polyclonal GFP antibody recognizing both YN and YC are shown below each profile (see Material and Methods). Positions of polysomes, monosomes (80S) and 60S and 40S subunits are indicated.

(C) Polysomes profiling as above of cells expressing both S18-YN and L11-YC.

(D) Polysomes analysis of cells expressing both S18-YN and L11-YC after treatment of the extract with puromycin and KCl (the sample was fractionated on a 10-30% gradient).

Fig. S4

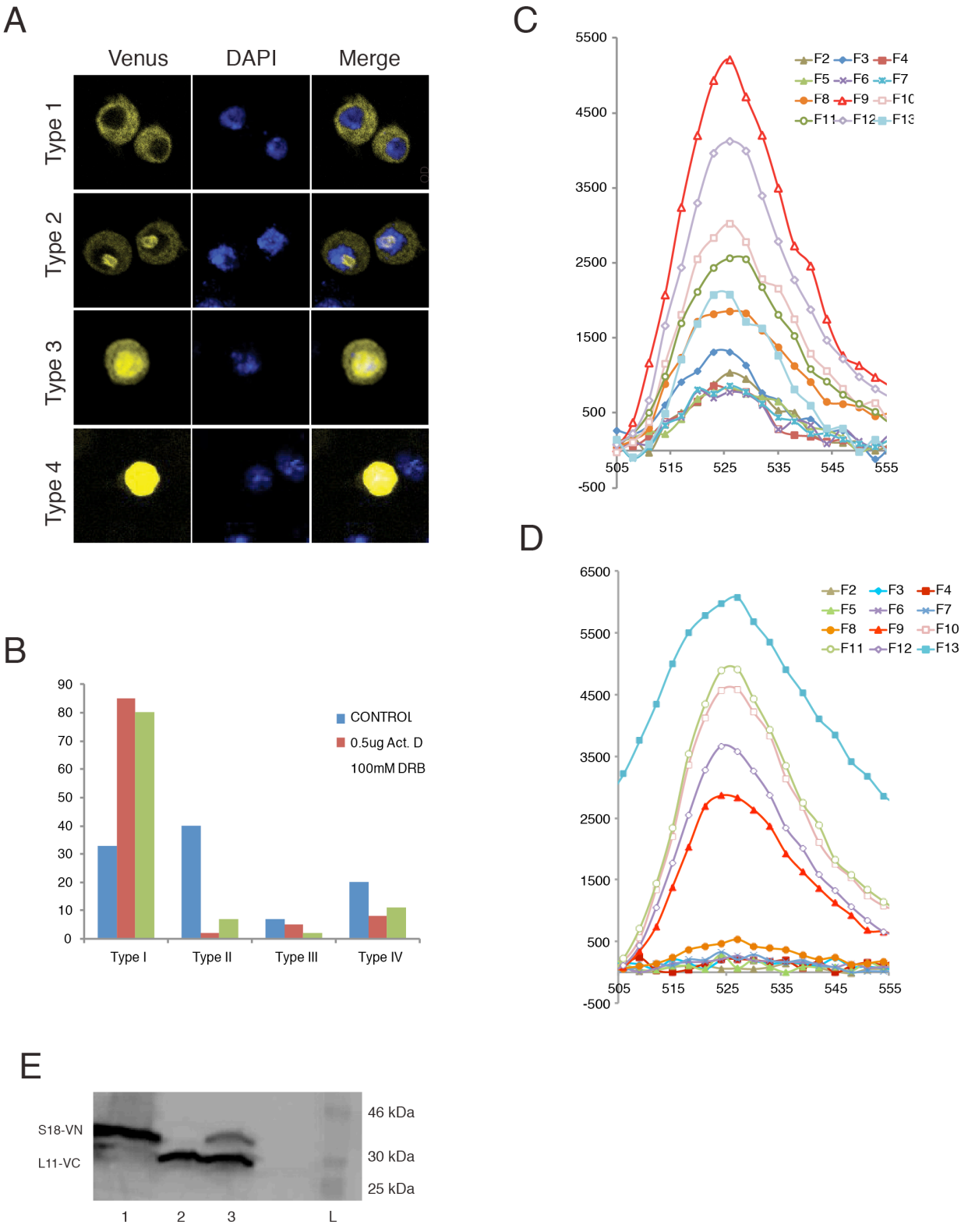

A
Venus
DAPI
Merge
Type 1
Type 2
Type 3
Type 4
B
90
80
70
60
50
40
30
20
10
0
CONTROL
0.5ug Act. D
100mM DRB
Type I
Type II
Type III
Type IV
C
5500
4500
3500
2500
1500
500
-500
505
515
525
535
545
555
F2 F3 F4
F5 F6 F7
F8 F9 F10
F11 F12 F13
D
6500
5500
4500
3500
2500
1500
500
-500
505
515
525
535
545
555
F2 F3 F4
F5 F6 F7
F8 F9 F10
F11 F12 F13
E
S18-VN
L11-VC
46 kDa
30 kDa
25 kDa
1
2
3
L

Figure S4. Visualization of S18 and L11 interaction using Venus-based BiFC.

(A) Venus signal visualized in cells co-transfected with plasmid expressing S18-VN and L11-VC and p-Act-GAL4. Venus signals are shown on the left, DAPI staining in the middle and the merged images on the right. Cells were classified in four types based on the subcellular pattern of the signal. Type 1 and 2 appear morphologically normal and show a normal DAPI stain while Type 3 and 4 show a shrunk morphology and punctated DAPI. Frequencies of the different types are shown in below in panel C.

(B) Percentage of cells showing Type 1-4 patterns in transfected cells treated for 4 hours prior fixation with actinomycin D (0.5 μg/ml) or DRB (100 μM). Values are based on scoring 100 clearly fluorescent cells in a set of several micrographs taken from three parallel equal transfections.

(C) Venus emission spectra, 505 nm to 555 nm, of the fractions collected from the gradient shown in Fig. 5A (emetine treated sample); fractions, F2 (heaviest) to F13 (lightest), are in different colors. Emission values were normalized by subtraction of the background reading of a similarly prepared control extract from untransfected cells. Venus emission is at 528 nm.

(D) Venus emission spectra of fractions as above, but from the gradient shown Fig. 5B (puromycin-treated sample).

(E) Western blotting of S2 cells expressing S18-VN (lane 1), L11-VC (lane 2) or both (lane 3). Lane 4 shows three of the protein ladder bands with molecular weights.

Fig. S5

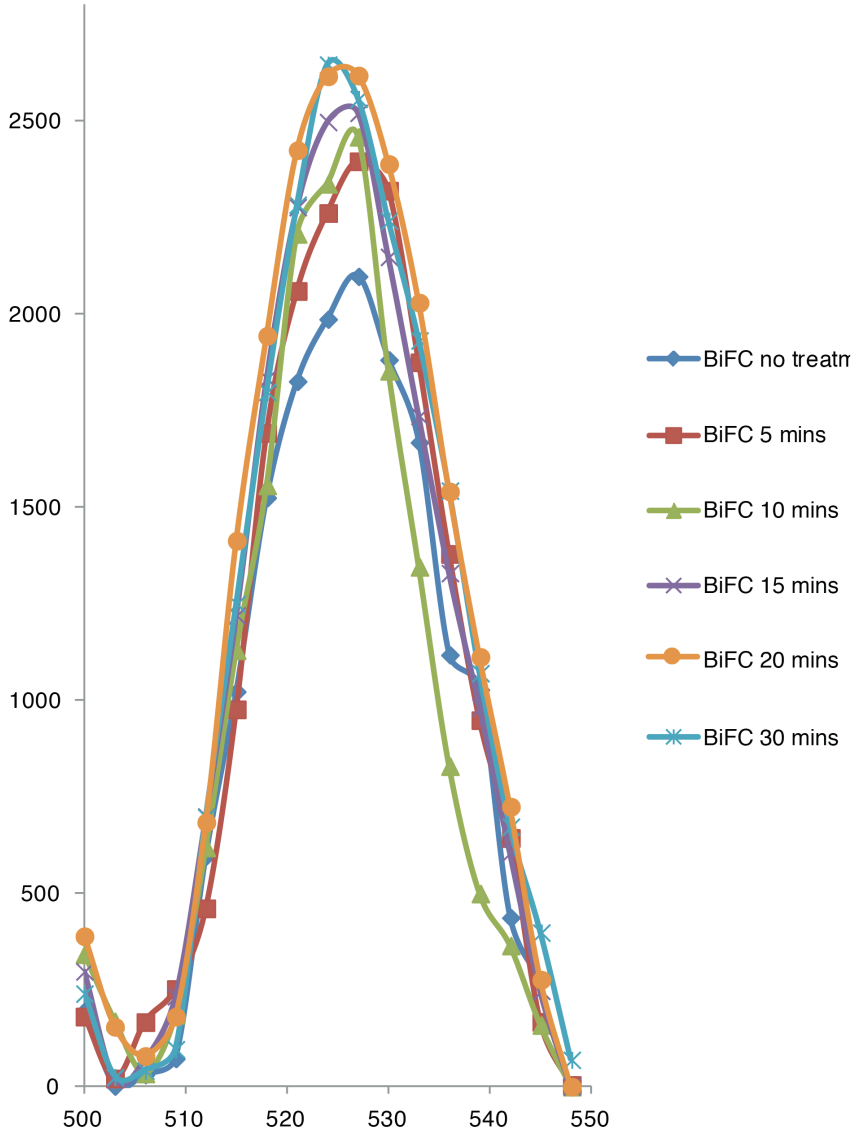


Figure S5. Emetine treatment swiftly increases BiFC signal.

Fluorescence emission spectra, 500nm to 550nm, in extracts of transfected cells pre-incubated prior to lysis with or without the indicated drug. The three experiments were performed with the same batch of transfected cells, which was split into aliquots. Emetine concentration was 50 μg/ml and incubation times are indicated. The fluorescence of each sample was normalized as in Fig. 3 E.

Fig. S6

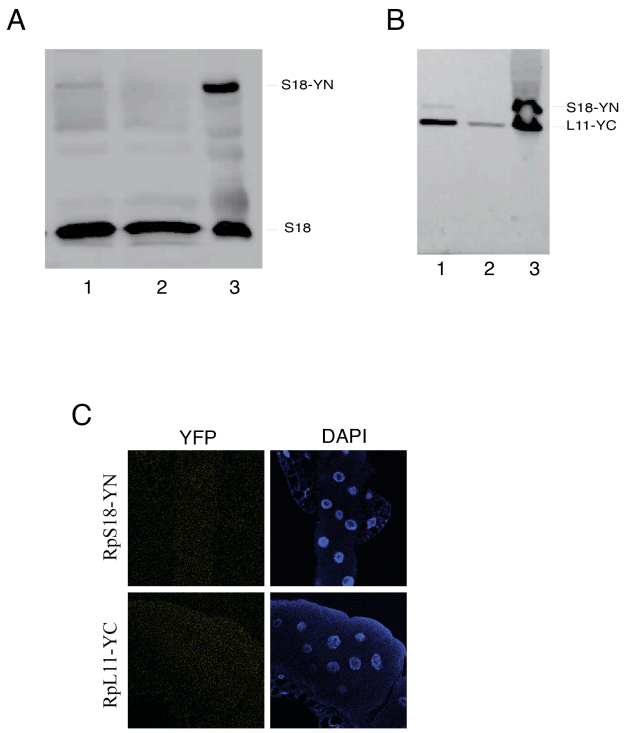


Figure S6. The YN and YC fragments alone do not produce YFP fluorescence.

(A) Western blot of salivary gland extracts (equivalent to ~ 15 pairs each) with antibody detecting S18 showing both endogenous S18 (bottom) and S18-YN fusion (top). Lanes 1 and 2 are from two different strains both expressing S18-YN and L11-YC but at different level. The intensity of the S18-YN band is about ~ 5% of S18 in lane 1 and too weak to quantify in lane 2. Lane 3 was loaded with a whole protein extract of S2 cells transfected with plasmids expressing the same constructs.

(B) Western blot of Salivary glands or S2 cells expressing S18-YN and L11-YC with GFP antibody detecting both S18-YN and L11 YC. In Lanes 1 and 2 are extract of salivary glands (equivalent to 8 pairs each), and in lane 3 extracts of S2 cells.

(C) Confocal imaging of glands expressing either S18-YN or L11-YC alone. No YFP fluorescence could be detected.

Fig. S7

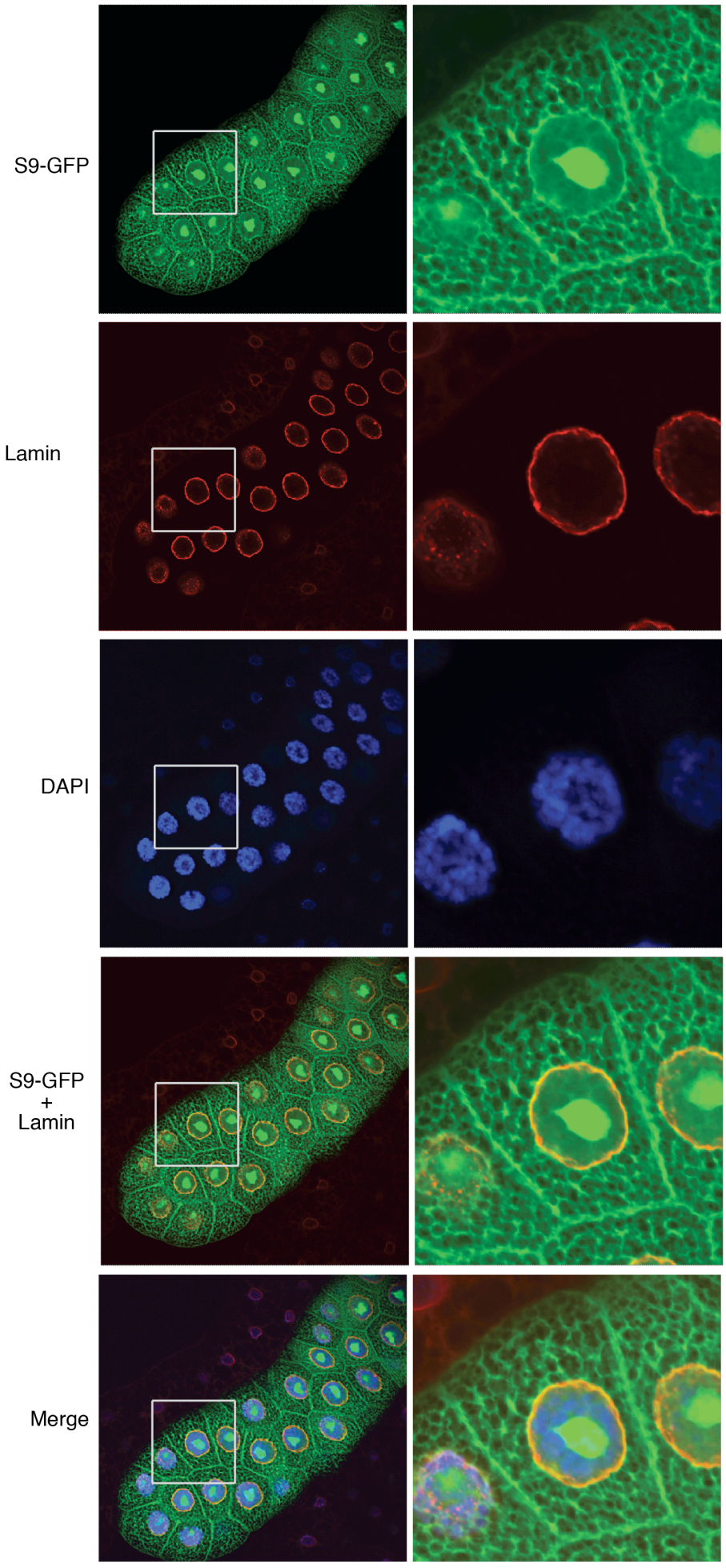


Figure S7. Nuclear lamina immunostaining shows no evidence of nuclear envelope invaginations.

Indirect immunostaining of salivary glands expressing S9-GFP with Drosophila lamin Dm0 antibody (Material and Methods). Confocal micrographs show S9-GFP in green, lamina in red (Alexa Fluor® 647 fluorescence), and DAPI in blue. Merged images are shown in the two bottom panels.

Fig.S8

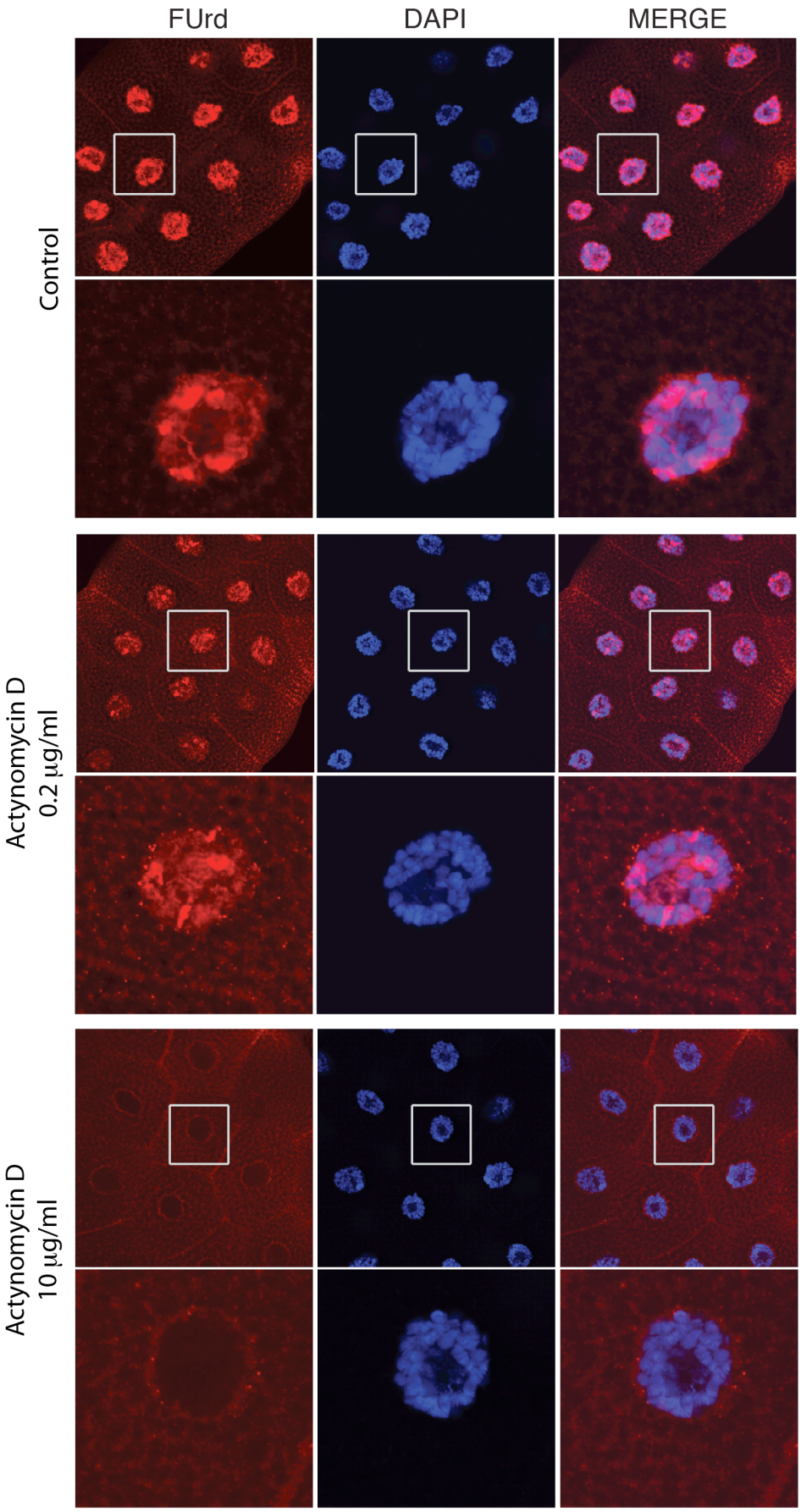

FUrd
DAPI
MERGE
Control
Actynomycin D
0.2 µg/ml
Actynomycin D
10 µg/ml

Figure S8. Actinomycin rapidly inhibits transcription in salivary glands.

Confocal images of salivary glands briefly (15 minutes) incubated with 5-Fluorouridine (FUrd) to label newly synthesized transcripts. FUrd incorporation (in red) is apparent at the chromosomes in the untreated gland, slightly reduced in presence of low concentration of actinomycin (middle panels) and absent at high concentration (bottom panels). DAPI and merged images of the same sections are shown on the right. High magnification zoom shown close ups of the nuclei indicated by the square.

Fig. S9

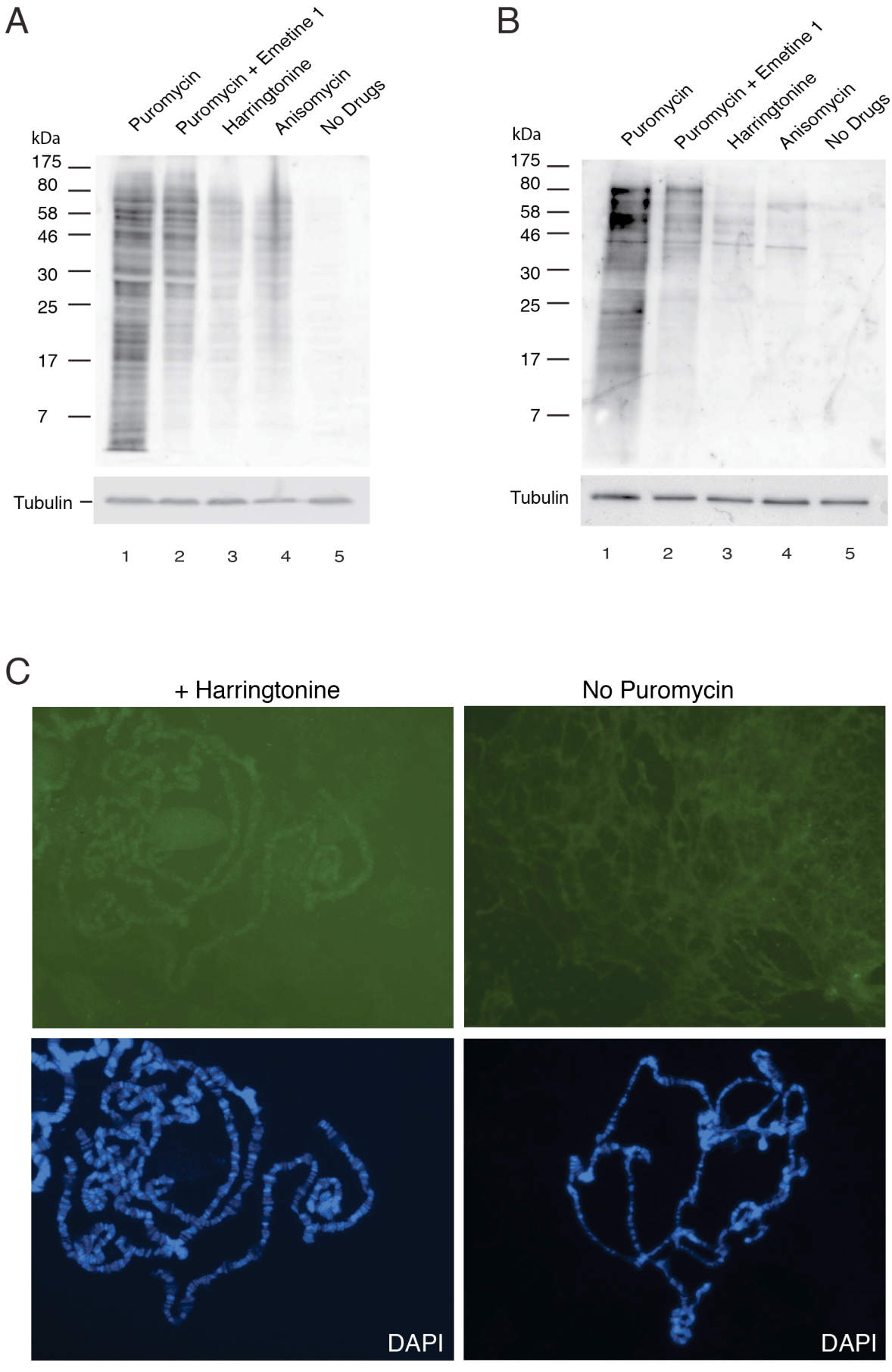

A
Puromycin
Puromycin + Emetine 1
Harringtonine
Anisomycin
No Drugs
kDa
175
80
58
46
30
25
17
7
Tubulin
1
2
3
4
5
B
Puromycin
Puromycin + Emetine 1
Harringtonine
Anisomycin
No Drugs
kDa
175
80
58
46
30
25
17
7
Tubulin
1
2
3
4
5
C
+ Harringtonine
No Puromycin
DAPI
DAPI

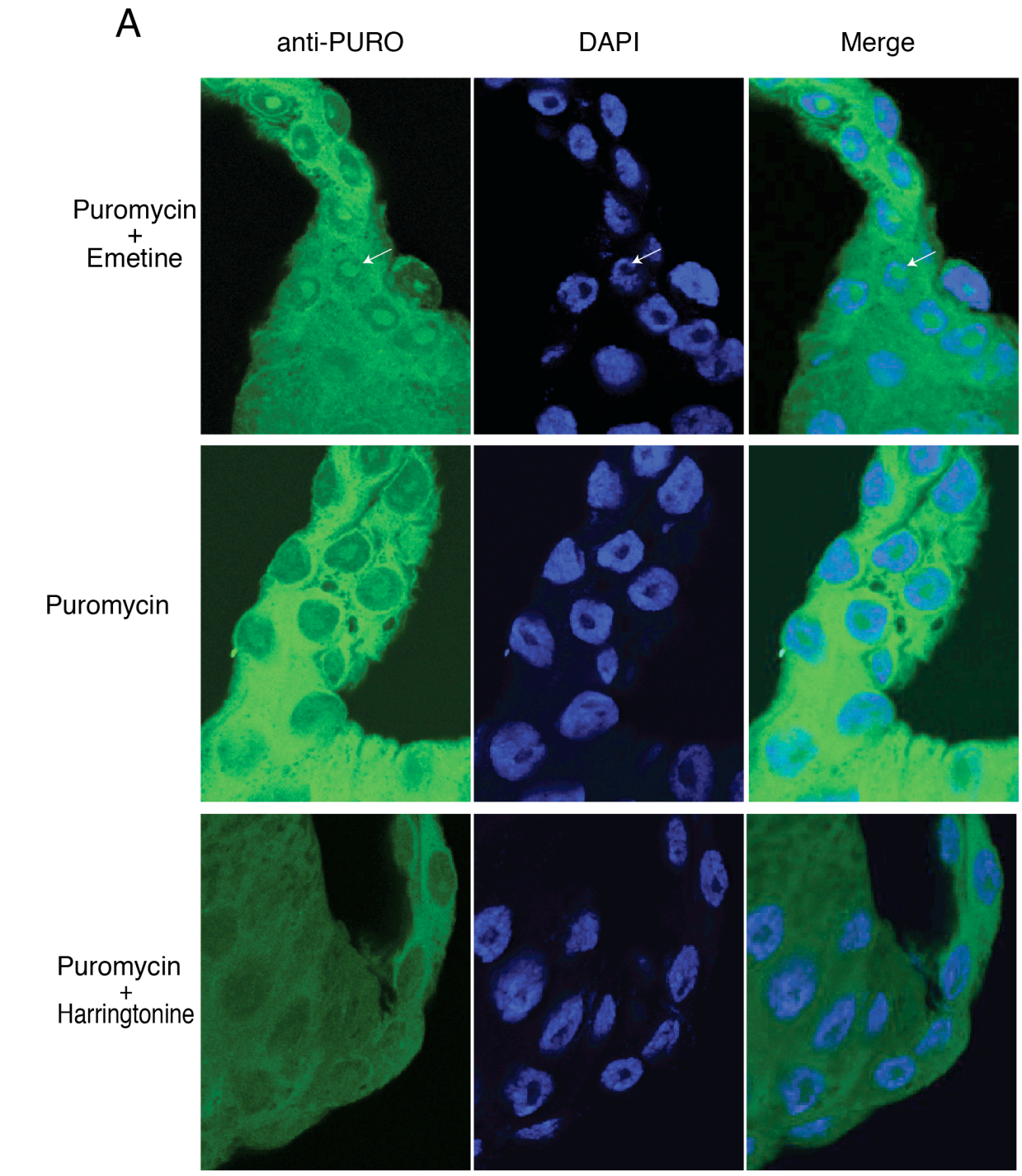
Figure S9. The puromycin antibody detects ribopuromycylated polyteptides.

(A) Western blotting of S2 cells treated with the indicated translation inhibitor drugs (lanes 1-4) or untreated (lane 5). Incubation with puromycin (50 μg/ml), with or without emetine (100 μg/ml), was for 15 minutes in Shields and Sang M3 media (Sigma) at room temperature. Harringtonine (2 μM) or anisomycin (2 μg/ml) were added 15 minutes beforehand.

(B) Western blotting of dissected salivary glands (seven pairs) briefly cultured in M3 media for the time required for the labeling reaction as described above, except for anisomycin which was present at 20 μg/ml.

(C) Puromycin immunostaining with Alexa488-conjugated antibody of polytene chromosomes squashes from puromycin-labelled glands treated with emetine plus harringtonine and in control glands not labeled with puromycin (see Fig 7 and Material and Methods).

Fig S10


A
anti-PURO
DAPI
Merge
Puromycin
+
Emetine
Puromycin
Puromycin
+
Harringtonine

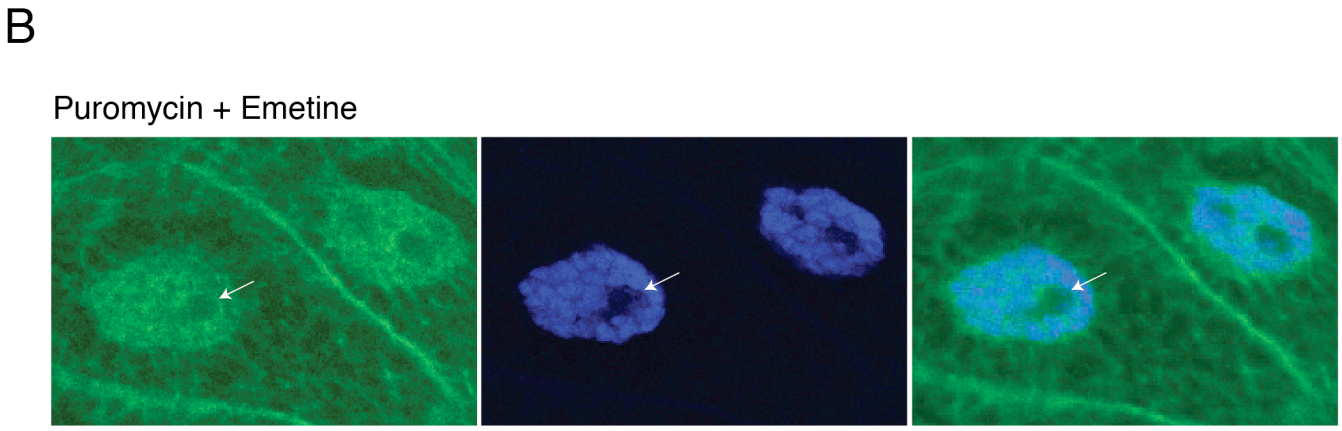

B
Puromycin + Emetine

Figure S10. Puromycin incorporation in whole salivary glands.

(A) Direct immunostaining of puromycin-labeled whole salivary glands using Alexa488-conjugated anti-puromycin antibody (in green). Labeling procedure consisted of 15 minutes incubation in media containing puromycin (50 µg/mL) and emetine (100 µg/mL), or puromycin only, or pretreated with harringtonine 15 minutes before adding puromycin and emetine (details in Material and Methods). DAPI and merged images are shown on the right.

(B) Puromycin immune fluorescence in larger salivary gland cells (locate distally on the lobe) as above. Note strong anti-puromycin fluorescence on the chromosome; the arrows point to the nucleoli.

Movie S1. BiFC signal in two cells co-transfected with S18-YN and L11-YC.

Z-stack of confocal images showing both YFP and DAPI. The cell on the right is an example of a Type 2 cell with apparent YFP signal in the nucleolus.