Supplementary Materials for

Changes in rRNA Transcription Influence Proliferation and Cell Fate Within a Stem Cell Lineage

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Materials and Methods

Fly stocks

Fly stocks were maintained on standard cornmeal molasses agar at 22-25°C unless otherwise noted. The following stocks were used in this study (Bloomington *Drosophila* Stock Center # given in parentheses): *PBac{WH}f00130* (#18295), w^{1118} (#6326), *CyO, P{FRT(w$^+$)Hsp70-PBac\T}2/wg^{Sp-1}* (#8284), *P{ry[+t7.2]=neoFRT}42D P{w[+mC]=piM}45F* (#2120), *P{XP}d07339* (#19269), P{w[+mC]=His2Av-mRFP1}III.1 (#23650), P{y[+t7.7] v[+t1.8]=TRiP.HMS00029}attP2 (#33631), P{y[+t7.7] v[+t1.8]=TRiP.GL00641}attP40 (#38202), P{y[+t7.7] v[+t1.8]=TRiP.GL00340}attP2 (#35418), P{y[+t7.7] v[+t1.8]=TRiP.GL00556}attP2/TM3 (#36596), P{y[+t7.7] v[+t1.8]=TRiP.HMS00564}attP2 (#33694); *PBac{WH}f00102*, *PBac{RB}e00152*, *P{XP}d08197* (Exelixis collection at the Harvard Medical School); $bam^{\Delta 86}$, *y w hsFLP; [FRT]42D UbiGFP / CyO*, *P[daughterlessP-Gal4*] (*da-gal4*), *P[nanosP-Gal4]* (*nos-gal4*) (two lines, on the 2nd and 3rd chromosome respectively), and *hs-bam* (gift from D. Mckearin); the RpS2 protein trap line CB02294 (gift from A. Spradling).

The udd^1 allele was isolated as a background mutation from the Exelixis stock *PBac{WH}f00130*. Remobilization of the inserted piggyBac element were done by crossing those flies to a line carrying *T.ni* piggyBac Transposase under the control of *hsp70* promoter (Bloomington stock #8284), heat-shocking and outcrossing the progeny. Excision of the piggyBac transposon did not revert the recessive sterile phenotype. Meiotic recombination and deficiency lines were used to map the mutation causing the sterile phenotype.

The udd^{null} allele was generated by FLP/FRT mediated recombination in trans of the *P{XP}d08197* and *PBac{WH}f00102* element insertions (*30*). Three other deficiency lines *Exel(e00152-d08197)*, *Exel(d07339-f00102)* and *Exel(d07339-e00152)* were made similarly by FLP/FRT mediated recombination to map the disrupted gene by non-complementation of the sterile phenotype.

The phiC31 integrase transgenesis system (*31*) in conjunction with *Drosophila* Gateway™ expression system (*32, 33*) was used to generate transgenic flies *UASp-HA-Udd ORF* (96E landing site), *UASp-HA-Tif1A ORF* (51D landing site) and other UAS lines (Rainbow Transgenics). *da-gal4* and two *nos-gal4* lines were used to induce transgenic expression. Transgenic flies with *P[acman] Bac CH322-148I23, 138I13 and 11K08* (*34*) were also generated using phiC31 integrase transgenesis with 96E as the landing site (P[acman] Resources: http://www.pacmanfly.org/). In order to make the *Udd-GFP* transgenic line, a GFP tag was engineered into the *P[acman]* vector *CH322-148I23* replacing the *udd* stop codon with GFP. This *Udd-GFP* construct was used to transform flies carrying the 65B landing site. The *Udd-GFP* line rescued both the sterile phenotype of udd^1 homozygotes and the lethal phenotype of udd^{null} homozygotes.

The VALIUM 22 and VALIUM 20 vector (gift from N. Perrimon) were used to make UAS-RNAi lines for *TAF1B*, *RpI135* and *TAF1C-like*. The 21nt sequences CAGGACGATCCGACAGAAGAA (for *CG6241*, a.k.a. *Taf1B*), CGGAGTTTAAGCAGATACCTA (for *CG4033*, a.k.a. *RpI135*) and CAAATTCAATTTGTTAACTAA (for *CG10496*, a.k.a. *TAF1C-like*) were chosen using DSIR online tool (http://biodev.extra.cea.fr/DSIR/DSIR.html). The corresponding oligos

were designed, annealed, and ligated into VALIUM 22 vector and VALIUM 20 vector (*CG10496* only) following instructions from TRiP website (http://www.flyrnai.org/supplement/2ndGenProtocol.pdf). All these vectors carry attB sites, and after sequencing, the constructs were injected into embryos with attp40 as the landing site on the 2nd chromosome. Rainbow Transgenic Flies, Inc. performed all the microinjection work for this project.

For double RNAi experiments described in figs. S13 and S14, we used both newly generated RNAi lines (described above) and ones available from the Bloomington stock center. Depending on the chromosomal location of the RNAi construct insertion site, UAS-GFP controls on the 2nd or 3rd chromosome were used.

Generation of germ line clones and follicle clones marked by negative GFP

udd^{null} and *udd*^{1} were recombined onto a *FRT42D* chromosome by meiotic recombination. Germline clones and follicle clones for *udd*^{null} and *udd*^{1} were generated by FLP/FRT-mediated mitotic recombination (*35*). Adult *hs-FLP;FRT42D,ubiquitin-GFP*/*FRT42D,udd* females were heat-shocked at 37°C for 1 hour twice a day for 3 days. *hs-FLP;FRT42D,ubiquitin-GFP*/*FRT42D* flies were used as controls. Ovaries were dissected on days 4, 7, 14, 21 and 28 after clone induction.

For analysis in Fig.1D, the experiments were performed twice, and the numbers of germaria counted for each genotype at each time point are listed below:
A) For 4dph, *control*: n1=132, n2=121; *udd*^{1}: n1=94, n2=74; *udd*^{null}: n1=99, n2=122.
B) For 7dph, *control*: n1=120, n2=95; *udd*^{1}: n1=129, n2=121; *udd*^{null}: n1=115, n2=117.
C) For 14dph, *control*: n1=96, n2=80; *udd*^{1}: n1=115, n2=86; *udd*^{null}: n1=85, n2=126.
D) For 21dph, *control*: n1=96, n2=108; *udd*^{1}: n1=110, n2=104; *udd*^{null}: n1=114, n2=113.
E) For 28dph, *control*: n1=101, n2=117; *udd*^{1}: n1=115, n2=162; *udd*^{null}: n1=97, n2=145.

Immunofluorescent staining in ovaries and testes

Ovaries and testes were dissected in Grace's Medium. Tissue was fixed for 10 minutes with gentle rocking in 4% formaldehyde (EM grade) in PBS. After fixation, ovaries and testes were washed four times in PBT (PBS + 0.5% BSA + 0.3% Triton-X 100) at RT for 10 minutes and were incubated with primary antibodies overnight at 4°C. The samples were washed four times with PBT for 10 minutes, and incubated for five hours with secondary antibodies. Tissue was washed and mounted in VectaShield mounting medium with DAPI (Vector Laboratories). The images were taken with a Zeiss LSM 510 confocal microscope.

The following antibodies were used (dilutions noted in parentheses): rabbit anti-GFP (1:1000) (Molecular Probes), mouse anti-Hts (1B1) (1:20), rat anti-VASA(1:20), mouse anti-BamC A7 (1:10), mouse anti-Sxl (1:20) (Developmental Studies Hybridoma Bank, Iowa), goat anti-VASA (1:200) (Santa Cruz Biotechnology), rat anti-HA 3F10 (Roche), mouse anti-Fibrillarin 38F3 (1:800), rat anti-Brdu (1:50) (Abcam), mouse anti-Modulo monoclonal LA9 (1:200) (*36*) (gift from J. Pradel), rabbit anti-cleaved Caspase-3 (1:250) (Cell Signaling Technology), rabbit anti-phospho-Histone H3 (Ser 10)(1:250)(Upstate), mouse anti-beta-Galactosidase (IgG2a, 1:200) (Promega), guinea pig anti-Udd (1:800), guinea pig anti-A2bp1 (1:5000) (*37*); Cy3, Cy5, FITC (Jackson Laboratories) or Alexa 488 (Molecular Probes) fluorescence-conjugated secondary antibodies were used at a 1:200 dilution.

Generation of anti-Udd antibody

Full-length *Udd* ORF was cloned into the pDEST17 Gateway™ vector (Invitrogen) to produce 6×His-tagged Udd protein. The protein was expressed in BL21-AI™ *E. coli* (Invitrogen) and purified with Ni-NTA agarose (Invitrogen) under denaturing conditions. Polyclonal antisera were generated in two guinea pigs TX928 and TX927 (Covance). All the experiments described here were performed with antiserum from TX928.

S2 cell co-transfection and co-immunoprecipitation assays

The full-length ORFs of *Udd* (*CG18316*), *TAF1B* (*CG6241*), *RpI135* (*CG4033*) and *TAF1C-like* (*CG10496*) were generated by PCR from DGC cDNA clones GH26082, RE68448, SD02110 and LD41005 respectively. Note: RE68448 carried a point mutation that resulted in an amino acid change relative to the annotated sequence. This mutation was corrected by site-directed mutagenesis. The blunt-end PCR products with CACC at the 5' end were directionally cloned into pENTR™/D-TOPO® vector (Invitrogen), which were then fully sequenced and cloned into Drosophila Gateway™ vectors pAFW and pAHW using a LR reaction to generate N-terminal 3×FLAG tag and 3×HA tag destination expression vectors with *actin5C* promoters.

Transient transfections of S2 cells were performed using Effectene Transfection Reagent (Qiagen) following the manufacturer's instructions. For Co-IP studies, transfected cells were collected and lysed on ice in lysis buffer (50mM Tris pH8.0, 137mM NaCl, 1mM EDTA, 1% Triton X-100, 10% glycerol, 10mM NaF and protease inhibitors). Mouse anti-Flag M2 Agarose (Sigma) and rat anti-HA Affinity Matrix (Roche) were incubated with lysates overnight at 4°C. The beads were then quickly washed 4 to 5 times with lysis buffer and boiled in Laemmlli sample buffer with DTT.

Tandem affinity purification in S2 cells followed by Mass Spectrometry

The full-length ORF of *Udd* was cloned into Gateway™ vector pAFHW to generate 3×FLAG-3×HA-Udd (FH-Udd). Two 100mm plates of S2 cells were transiently transfected with either pAFHW (negative control) or pAFHW-Udd and lysed using the same lysis buffer as described above. After centrifugation, the supernatants were incubated with mouse anti-Flag M2 Agarose (Sigma) for 6 hours at 4°C, washed with lysis buffer, and eluted with 0.5mg/ml 3×FLAG peptide overnight at 4°C. The eluates were then incubated with rat anti-HA Affinity Matrix (Roche) for 10 hours at 4°C, washed with lysis buffer, and eluted with 1mg/ml HA peptide overnight at 4°C. The eluates were mixed with Laemmli sample buffer and boiled for western blot and silver staining. Silver staining was performed following the instructions in Invitrogen SilverQuest™ Staining Kit (Cat. No. LC6070). The protein bands from both control eluate and FH-Udd eluate were excised and sent to the UT Southwestern protein chemistry core for mass spectrometry analysis.

Immunofluorescent staining in S2 cells

The full-length *TAF1B (CG6241)* ORF and *TAF1C-like (CG10496)* ORF were cloned into the pHGW and pHWG Gateway™ vectors which carry a *hsp70* promoter and a GFP tag at the N- or C-terminus respectively. Transient transfections of S2 cells were

performed using the same method described above. Transfected S2 cells were resuspended, placed on Gold Seal® micro slides (Cat. No.3032) and allowed to settle for 30 minutes. Fresh fix buffer (4% formaldehyde in PBS) was used to flood the slide for 15 minutes, followed by one 5 minute wash in 1×PBS and two 5 minute washes with 1×PBS + 0.1% Triton-X 100. After the 3rd wash, the cells were pre-incubated with PBTA (1×PBS + 0.1% Triton-X 100 + 0.5%BSA) for 30 minutes. Primary antibodies were diluted in PBTA and the cells were incubated for 4 hours at room temperature. After two washes in 1×PBS and one wash with 1×PBS + 0.1% Triton-X 100, secondary antibodies were added to the cells and incubated for 1 hour at room temperature before final washes and mounting in VectaShield mounting medium with DAPI (Vector Laboratories). The images were taken with a Zeiss LSM 510 confocal microscope. The same antibody dilutions were used as mentioned in "Immunofluorescent staining in ovaries and testes".

Western blots

To detect proteins by western blot, ovaries were dissected in Grace's medium, physically disrupted and extracted with Laemmlli sample buffer (Bio-Rad) with DTT using a pestle followed by boiling at 95°C for 10 minutes. For blotting, the following primary antibodies were used: guinea pig anti-Udd TX928 (1:5000), mouse anti-Actin (1:100) (Developmental Studies Hybridoma Bank, Iowa), goat anti-VASA (1:1000) (Santa Cruz Biotechnology), rat anti-HA 3F10 (1:5000) (Roche), rabbit anti-H2B (1:1000) (Upstate), rabbit anti-Mad (1:3000) (gift from L. Raftery), rabbit anti-Medea (1:3000) (gift from L. Raftery (*21*)). HRP-conjugated anti-guinea pig, anti-mouse, anti-goat and anti-rat secondary antibodies (Jackson Laboratories) were used at a 1:2000 dilution. In order to detect S2 cell co-immunoprecipitation results, the following antibodies were used: HRP-conjugated mouse anti-Flag M2 (1:10,000) (Sigma), rat anti-HA 3F10 (1:5000) (Roche), mouse anti-Fibrillarin (1:5000) (Abcam) and guinea pig anti-Udd (1:8000). For protein level semi-quantification of western blots in Fig. 4 and fig. S16C, the experiments were repeated 3 times, and Image J software (Gel Analysis function) and Graphpad software were used to generate the graph. The values were normalized to those of $bam^{\Delta 86}$ mutant extracts.

RT-PCR and Northern blot

Total RNA was isolated from ovaries and testes of newly eclosed flies using TRIzol (Invitrogen). The RNA concentration was measured with a Nanodrop 2000c spectrometer.

Two-step RT-PCR was performed for all the experiments. The reverse transcription step was performed with Invitrogen SuperScript® III First-Strand Synthesis SuperMix (Cat. No. 18080-400) using a primer mixture of both oligo$(dT)_{20}$ and random hexamers.

The 2nd step, for non-real time PCR (fig. S3C), Roche Taq polymerase was used with the following primer sequences for *udd* and *RpL32* mRNA detection:

udd-F: 5'-ATGAAAACAAAAGATGAGAAGCCATCG-3'
udd-R: 5'-CTAGGATAGAATAGCATTTAATGAATCGTC-3'
RpL32-F: 5'-CACCAGTCGGATCGATATGC-3'
RpL32-R: 5'-CACCAGGAACTTCTTGAATCC-3'

The 2nd step, for real-time PCR (qPCR), Power SYBR® Green PCR Master Mix from Applied Biosystems (Part No. 4367659) was used and reactions were executed on a Bio-Rad CFX96 Real-Time PCR Detection System. Standard curves were created for each primer set. The qPCR results for all genes were normalized to the reference gene αTublin84B. RNA samples without RT reaction (No-RT) were also included for each RT sample. The following primer sets were used for detection of each gene, all amplifying 50-150 bp fragments:

udd-F	5'-CTCCTCCGGCCAGTAACGAG-3'
udd-R	5'-ATCCCGGGCCAGTCGTAGTT-3'
TAF1B-F	5'-GCACTGCCACCTCGGCTACT-3'
TAF1B-R	5'-TGGCCTCATAGCGCGGATAC-3'
TIF-IA-F	5'-TGCGGTGGGATACATGGCTA-3'
TIF-IA-R	5'-TGCTAAGCGGCAAAAATCGTG-3'
αTubulin84B-F	5'-TGGGCCCGTCTGGACCACAA-3'
αTubulin84B-R	5'-TCGCCGTCACCGGAGTCCAT-3'

Northern blots were performed using Ambion NorthernMax® Kit (Cat. No. AM1940, for steps including formaldehyde gel electrophoresis, membrane transfer and hybridization steps), Roche DIG Wash and Block Buffer Set (Cat. No. 11585762001, for protocol and buffer recipes used in Washing/ Blocking/ DIG Detection steps following Hybridization.), Roche Anti-Digoxigenin-AP antibody (Cat. No. 11093274910) and CDP-star reagent from the New England BioLabs Phototope®-Star Detection Kit (discontinued) with the following probes which were DIG-labeled with Roche DIG Oligonucleotide 3'-End Labeling Kit, 2nd generation (Cat. No. 03 353 575 910):
Pre-rRNA and rRNA processing probe (*14*):
5'-CACCATTTTACTGGCATATATCAATTCCTTCAATAAATG-3'
Mature 5S rRNA probe:
5'-ACGAGAACCGATGTATTCAGCGTGGTATGGTC-3'

In situ run-on transcription assay

Ovaries were dissected in Grace's Medium, washed once with ice-cold PBS, and permeabilized with digitonin (200ng/µl; Sigma) in permeabilization (PB) buffer (22mM NaCl, 100mM CH_3COOK, 2mM $MgCl_2$, 8mM KCl, 11mM K_2HPO_4, add 1mM dithiothreitol and protease inhibitors (Roche Cat. No. 04 693 159 001) freshly before use) for 5 minutes on ice. Then the ovaries were washed once with PB buffer and incubated on ice for 10 minutes with PB buffer supplemented with α-amanitin (250ng/µl; Sigma) to inhibit RNA polymerases II and III. Subsequently, transcription mix was added to give final concentrations of 2mM ATP, 0.5mM CTP, 0.5mM GTP, and 2mM 5-bromouridine 5'triphophate (BrUTP; Sigma). The run-on transcription was carried out at 25°C for 20 minutes and was terminated by rinsing the ovaries with ice-cold PBS. For control experiments, (A) actinomycin D (0.72ng/µl; Sigma) was added to the transcription reaction mixture to inhibit global transcription. The fixation and staining procedures were the same as mentioned above for the immunofluorescent staining. Rat Anti-BrdU antibody (Abcam Cat. No. ab6326) was used at 1:50 dilution. Note: the preparation steps

for BrUTP incorporation make nucleolar Udd staining look less compact compared to the regular immunofluorescent staining protocols.

Chromatin Immunoprecipitation and Real-Time PCR

For ChIP experiments presented in Fig. 2G, 200 pairs of ovaries from 3- to 6- day-old *da-gal4/UASp-HA-Udd* flies were used per immunoprecipitation reaction, and *da-gal4* flies were used as negative control. For ChIP experiments in fig. S9B, 400 pairs of ovaries from 3- to 6- day-old udd^{null}/udd^{null}*;da-gal4/UASp-HA-Udd* flies were used per immunoprecipitation reaction, and the same extracts were used in negative control without adding anti-HA antibody.

Chromatin from each 200 pairs of ovaries was cross-linked for 10 minutes at room temperature in a 1.5ml tube with 1ml 1% formaldehyde in 1×PBS; Cross-linking was stopped by adding 100µl of 1.25M Glycine solution to each tube. After three quick washes with cold 1×PBS, 400µl ChIP sonication buffer (1% Triton X-100, 0.1% Deoxycholate, 50mM Tris 8.1, 150mM NaCl, 5mM EDTA, and a protease inhibitor cocktail tablet (Roche; Cat. No. 04 693 159 001) was added to each tube. Ovaries were disrupted with a pestle and kept on ice for 10-20 minutes. The cell lysate was sonicated on wet ice for 4 minutes using 10 second pulses followed by 10 second "cooling-off" period and centrifuged at maximum speed for 10 mins at 4°C. The volume of the supernatant was brought up to 1ml with ChIP sonication buffer. After 1 hour preabsorption with 40µl Protein G Agarose (Millipore Cat. No. 16-201) at 4°C, 30µl of the supernatant (3%) were kept as Input and the rest incubated overnight with 3µl anti-HA antibody (Abcam rabbit polyclonal ChIP grade anti-HA ab9110) at 4°C. The next day 40µl Protein G Agarose were added and incubated for 5 hours at 4°C with rotation. The beads were then washed for 5 minutes at 4°C with 1ml of the following buffers: 2 washes with ChIP Sonication Buffer; 3 washes with High Salt Wash Buffer (1% Triton X-100, 0.1% Deoxycholate, 50mM Tris-Cl 8.1, 500mM NaCl, 5mM EDTA); 2 washes with LiCl Immune Complex Wash Buffer (0.25M LiCl, 0.5% IGEPAL CA630, 0.5% deoxycholate, 1mM EDTA, 10mM Tris, pH 8.1); 1 wash with TE buffer (10mM Tris-Cl pH8.1, 1mM EDTA). Elution Step: Each ChIP sample was incubated with 250µl Elution Buffer (1%SDS, 0.1M $NaHCO_3$) at room temperature for 20 minutes; after repeating once and the supernatants were combined. 500µl of elution buffer was added to the Input samples. 20µl 5M NaCl was added to each sample, mixed and incubated overnight at 65°C. The next day, 10µl RNaseA was added to each sample and incubated for 30 minutes at 37°C. Then 10µl 0.5M EDTA, 20µl 1M Tris-HCl pH6.5 and 1µl Proteinase K were added to each sample and incubated at 45°C for 2 hours. With addition of 5 times volume of Qiagen PB binding buffer, the samples were passed through a column from Qiagen PCR Purification Kit, washed once with Qiagen PE buffer and eluted the DNA with 30µl nuclease-free water. Real-Time PCR was used for quantification of precipitated DNA using the Standard Curve method. Biorad iTaq™ Fast SYBR® Green supermix with ROX (172-5100) was used as the PCR reaction buffer.

Primers used to amplify rDNA sequence fragments (around 350bp each) in the 5' non-transcribed spacer (NTS), external transcribed spacer (ETS) and the 5' end of 18s rRNA were described by Guerrero et al (*38*) as follows:

1-F 5'-GGTTGCCAAACAGCTCGTCATC
1-R 5'-CGAGGTGTTTGGCTACTCTTG
2-F 5'-GAGTAGCCAAACACCTCGTC
2-R 5'-GAGAGGTCGGCAACCAC
3-F 5'-GCTGTTCTACGACAGAGGGTTC
3-R 5'-CAATATGAGAGGTCGGCAACCAC
4-F 5'-GGTAGGCAGTGGTTGCCG
4-R 5'-GGAGCCAAGTCCCGTGTTC
5-F 5'-ATTACCTGCCTGTAAAGTTGG
5-R 5'-CCGAGCGCACATGATAATTCTTCC
6-F 5'-TTCTGGTTGATCCTGCCAGTAG
6-R 5'-CGTGTGTACTTAGACATGCATGGC

The primer sets 1-6 amplified rDNA regions labeled by bars from left to right in Fig. 2 and fig. S9B. Primers 5S control-F AAGTTGTGGACGAGGCCAAC and 5S control-R CGGTTCTCGTCCGATCACCGA were used to amplify a fragment of the 5s rDNA which served as a negative control.

Live imaging

Ovaries from newly eclosed *udd*^{null}; *Udd-GFP, His2Av-mRFP* flies were dissected in Scheider's Drosophila medium (Gibco®) supplemented with 10% Fetal Bovine Serum (Hyclone), 1:10,000 Penicillin-Streptomycin and 200 ug/ml human insulin (Sigma). The muscle sheath was removed and separated ovarioles were placed on a drop of medium on a microscope slide, and a coverslip was placed on top. A single germarium was imaged every 3-4 min for 12-14 hours using a Resonance Scanning Confocal Microscope Leica SP5. 4D data sets were processed using Image J (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012) and Adobe Photoshop, CS4.

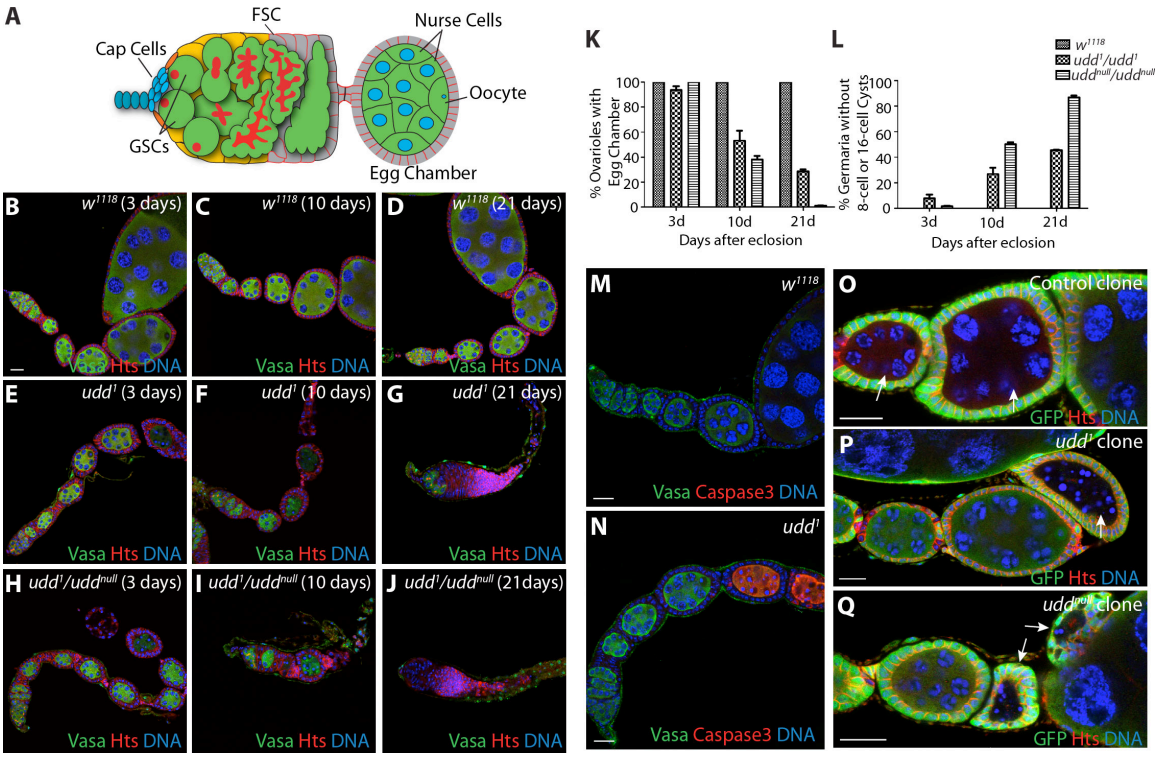

Fig. S1 *udd* mutant ovaries exhibit a germ cell loss phenotype that worsens with age

(A) Schematic of a *Drosophila* germarium. Each germarium houses two to three germline stem cells that are maintained by a cluster of cap cells. These stem cells carry round fusomes (red), which become branched as the stem cell daughters form multicellular cysts. Mature 16-cell cysts bud off of the germarium to form egg chambers. **(B-D)** w^{1118} control, **(E-G)** udd^{1} homozygous and **(H-J)** udd^{1}/udd^{null} mutant ovarioles dissected **(B,E,H)** 3 days, **(C,F,I)** 10 days or **(D,G,J)** 21 days after eclosion stained for Vasa (green), Hts (red) and DNA (blue). Vasa (green) is a germline specific RNA helicase. Hts (hu-li-tai-shao, red), in the germline, labels an ER-like structure called the fusome. In addition, Hts also labels the membranes of follicle cells surrounding germ cells. **(K)** Graph showing changes in the percentage of ovarioles that contain egg chambers over time. **(L)** Graph showing changes in the percentage of germaria that do not contain 8-cell and 16-cell cysts over time. **(M)** w^{1118} control and **(N)** udd^{1} homozygous ovarioles stained for Vasa (green), Cleaved (Activated) Caspase 3 (red) and DNA (blue). **(O)** Wild type control, **(P)** udd^{1} and **(Q)** udd^{null} clones in ovaries dissected 14 days after heat shock-mediated clone induction stained for GFP (green), Hts (red) and DNA (blue). The control germline clones formed late stage egg chambers (arrows in O) while the udd^{1} and udd^{null} clones exhibit egg chamber degeneration (arrows in P,Q). Scale bars represent 20 μm.

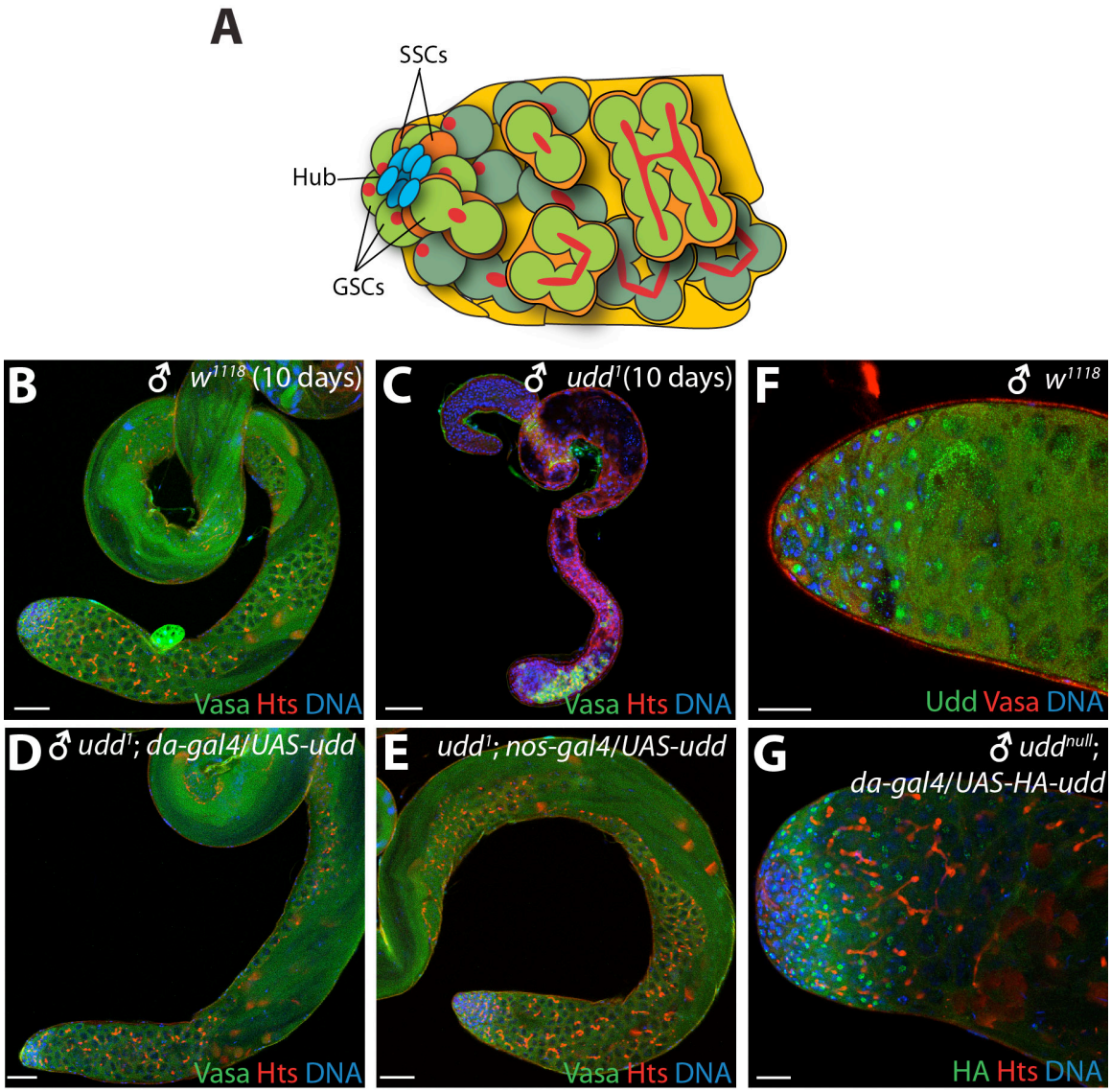

Fig. S2 Phenotypic analysis of *udd* mutant testes

(A) Schematic of the tip of a *Drosophila* testis. GSCs are located next the hub (blue). Similar to female germline, the cytoplasmic marker Vasa and the fusome marker Hts are used to label the male germline. **(B)** w^{1118} control, **(C)** udd^{1}, **(D)** udd^{1}/udd^{1}; *da-gal4/UAS-HA-udd* and **(E)** udd^{1}/udd^{1}; *nos-gal4/UASp-HA-udd* rescued flies stained for Vasa (green), Hts (red) and DNA (blue) 10 days after eclosion. Both ubiquitous and germline-specific expression of HA-Udd rescues the sterility of udd^{1} male homozygotes. **(F)** Udd is enriched in the nucleoli in the wild type testis (green), co-stained with Vasa (red) and DNA (blue). **(G)** A testis from rescued udd^{null}/udd^{null}; *da-gal4/UAS-HA-udd* male stained for HA (green), Hts (red) and DNA (blue). HA-tagged Udd is also nucleolar. Scale bars represent 50 µm (B-E) and 20 µm (F-G).

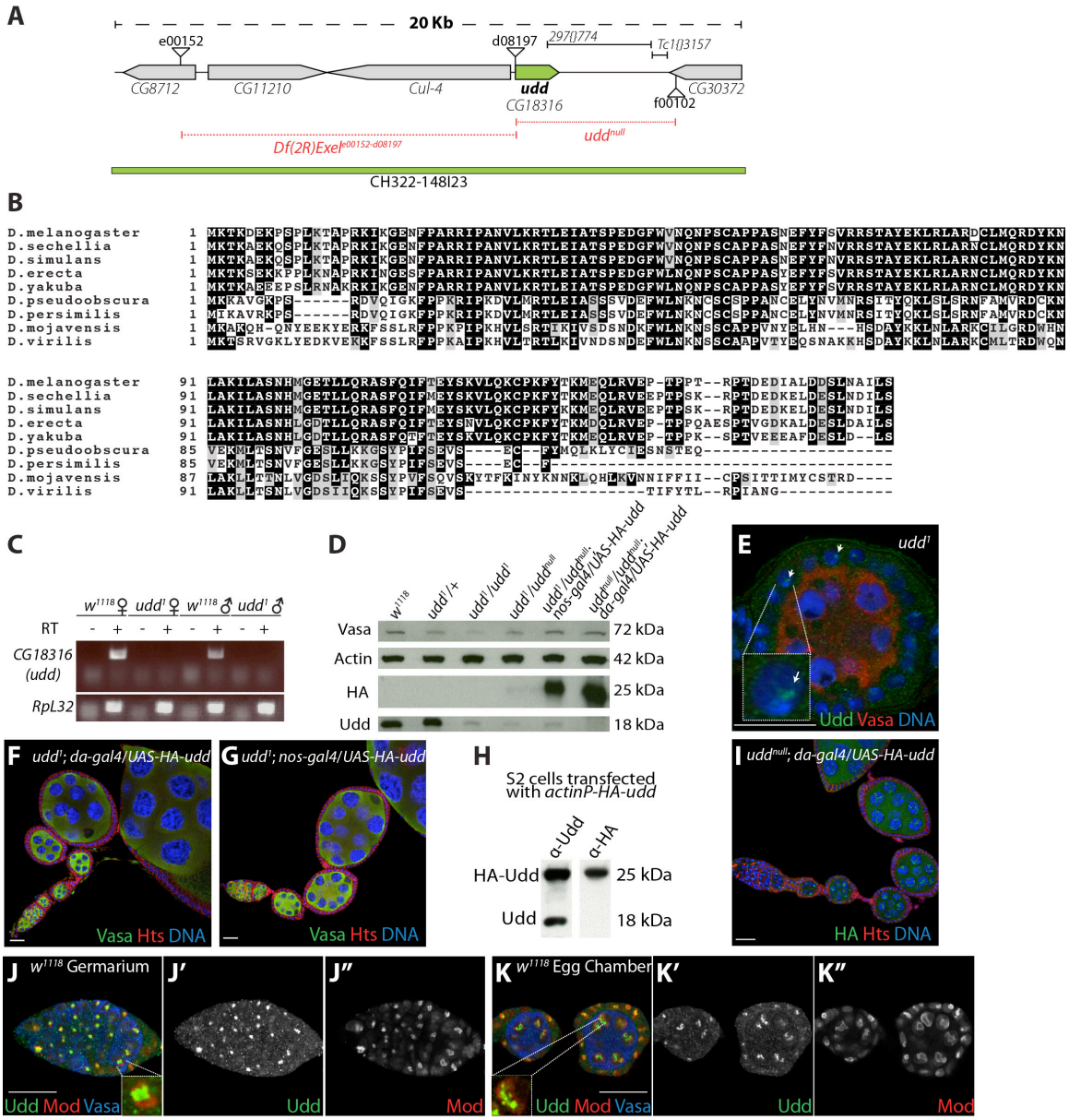

Fig. S3 *udd*^{1} disrupts *CG18316*

(A) Schematic of the region where *udd* maps. This 20 kb interval contains five ORFs. The CH322-148I23 genomic clone rescues the *udd*^{1} phenotype. Two molecularly defined deletions were made using FRT/FLP-mediated recombination: *Df(2R)Exel*$^{e00152\text{-}d08197}$ and *udd*^{null}. The *udd*^{1} allele complemented *Df(2R)Exel*$^{e00152\text{-}d08197}$ but did not complement *udd*^{null}. The *udd*^{null} homozygotes are embryonic lethal, a phenotype rescued by ubiquitous expression of HA-Udd (see panel I and fig. S2G). 297{}774 and Tcl{}3157 are natural transposons that lie immediately downstream of the *udd* gene, previously known as *CG18316*. **(B)** Sequence alignment of Udd orthologs from different *Drosophila* species. **(C)** The *udd*^{1} mutation results in reduced levels of *udd* mRNA. EtBr stained agarose gel showing the products of RT-PCR reactions using primers specific for *CG18316* (*udd)* or *RpL32*, which serves as a loading control. Control PCR samples without RT reactions were run in parallel. **(D)** Western blot analysis shows that Udd protein levels are greatly

reduced in udd^{1}/udd^{1} and udd^{1}/udd^{null} mutant ovaries compared to w^{1118} and $udd^{1}/+$ heterozygous ovaries. The polyclonal antibody against full-length Udd specifically recognizes this 18 kDa protein. Vasa (germline only) and Actin serve as loading controls, and the anti-HA blot shows the expression of HA-Udd in the sterility- and lethality-rescued flies. Ovarian lysates from newly eclosed flies were used. **(E)** udd^{1}/udd^{null} mutant egg chamber stained for Udd (green), Vasa (red) and DNA (blue). The arrows point to the remaining nucleolar Udd in somatic cells. The severe loss of Udd in the germline likely explains the specific sterile phenotype observed in udd^{1} mutants. **(F)** udd^{1}/udd^{1}; *da-gal4*/*UASp-HA-udd* rescued ovaries and **(G)** udd^{1}/udd^{1}; *nos-gal4*/*UASp-HA-udd* rescued ovaries stained for Vasa (green), Hts (red) and DNA (blue) 10 days after eclosion. **(H)** Western blot analysis showing that anti-Udd antiserum recognizes both endogenous Udd (18 kDa) and HA-tagged Udd (25 kDa), using transiently transfected S2 cells. **(I)** An ovariole from udd^{null}/udd^{null}; *da-gal4*/*UAS-HA-udd* rescued flies stained for HA (green), Hts (red) and DNA (blue). **(J-J")** w^{1118} germarium and **(K-K")** egg chamber stained for Udd (green), Modulo (red), Vasa (blue). Grayscale images show that Udd is enriched in the center of nucleoli, surrounded by Modulo. Scale bars represent 20 μm.

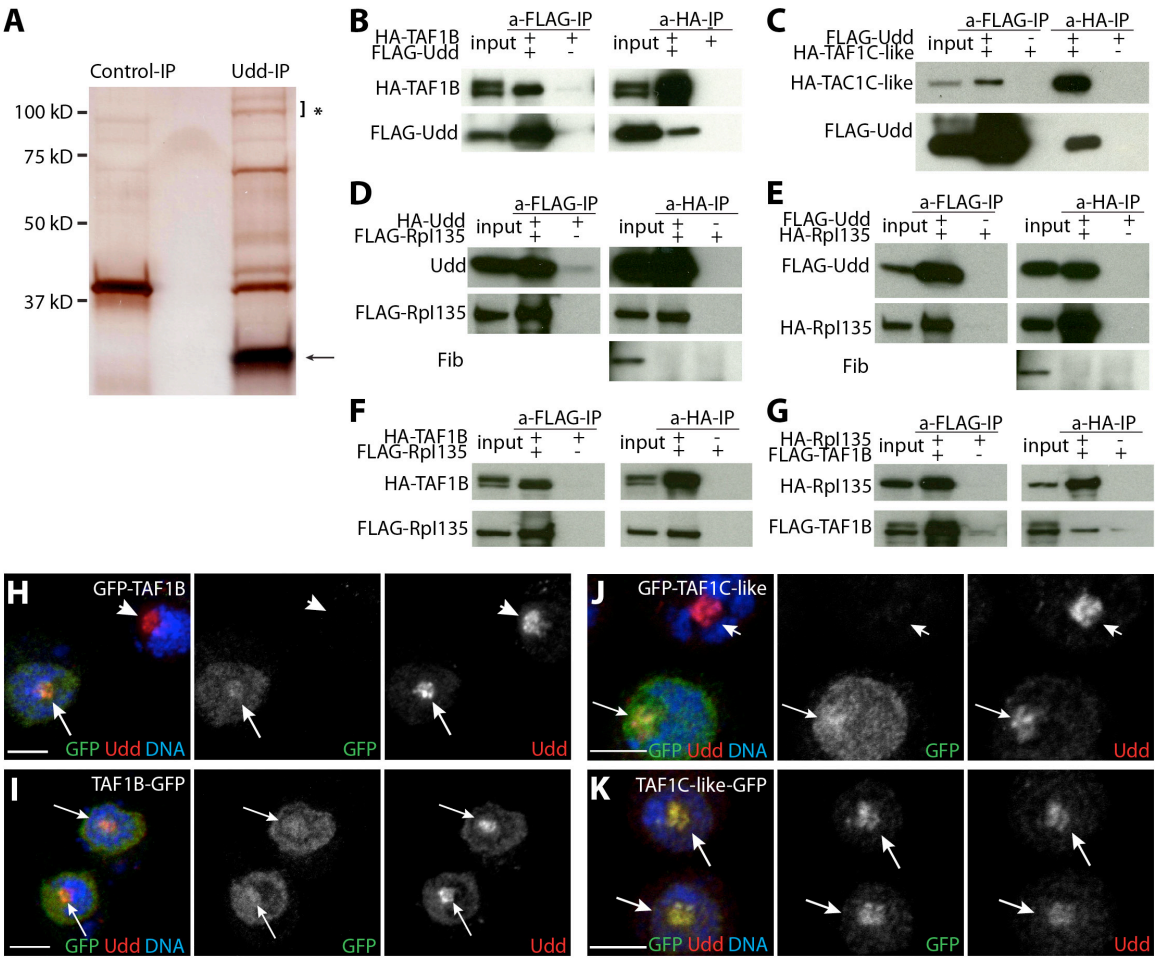

Fig. S4 Udd associates with *Drosophila* TAF1B and TAF1C-like

(A) Silver-stained SDS Polyacrylamide gel of tandem-affinity-purified and peptide-eluted 3×Flag-3×HA-Udd (Udd-IP). S2 cells transfected with empty vector were used as a negative control (Control-IP). Tandem mass spectrometry shows that two bands (bracket) contain TAF1B and TAF1C-like. **(B)** Western blots of co-immunoprecipitation (Co-IP) between FLAG-tagged Udd, HA-tagged TAF1B from transfected S2 cells. **(C)** Western blots of Co-IP between FLAG-tagged Udd and HA-tagged TAF1C-like from S2 cells (also see **Fig. 2B** for interaction tests between HA-tagged Udd and FLAG-tagged Taf1B and Taf1C-like.). **(D)** Western blots of Co-IP between HA-tagged Udd, FLAG-tagged RpI135 and endogenous Fib (negative control) from S2 cells. **(E)** Western blots of Co-IP between FLAG-tagged Udd and HA-tagged RpI135 from S2 cells. **(F)** Western blots of Co-IP between FLAG-tagged RpI135 and HA-tagged TAF1B from S2 cells. **(G)** Western blots of Co-IP between FLAG-tagged TAF1B and HA-tagged RpI135 from S2 cells. TAF1B tagged with GFP (green) at the **(H)** N-terminus (also see **Fig. 2C**) and **(I)** C-terminus co-localizes with Udd (red) in the nucleolus. TAF1C-like factor tagged with GFP (green) at the **(J)** N-terminus and **(K)** C-terminus (also see **Fig. 2D**) co-localizes with Udd (red) in the nucleolus. The *TAF1B* and *TAF1C-like* ORFs were introduced into the pHGW and pHWG gateway vectors, which both contain a hsp70 promoter and N- or

C-terminal GFP tags respectively. Transfected S2 cells at room temperature were examined for GFP expression. Arrows point out the co-localization of tagged TAF1B or tagged TAF1C-like with endogenous Udd in the nucleoli. Scale bars represent 5 μm (H,I).

A The Taf1B/RRN7 family

```
gi_82078563     5 E.[9].CGQ     CAAV.[1].WGVSDEGQFFC.[1].SCHNVIE 41  Taf1B_DANRE
gi_74726856     3 L.[9].CTQ     CAAV.[1].WGLTDEGKYYC.[1].SCHNVTE 39  Taf1B_HUMAN
gi_342187029    3 V.[9].CSQ     CAAV.[1].WGLTDEGKYYC.[1].SCHNVTD 39  Taf1B_MOUSE
gi_26399334     3 G.[7].CSV.[2].CKST     WYFKNAGQTFC     RRGHAQH 37  RRN7_SCHPO
gi_1008140      3 T.[6  CGT.[2].CPSR     LWRIIDGRRTC     QYGHVME 36  RRN7_SACCE
gi_74956228     1 M.[7].CNA     CGGY    RFSVNDGFKYC.[1].RCGALFE 34  Taf1B_CAEEL
gi_74869043     5 L.[9].CDV     CEGT    TFQEREGFYYC.[1].ECGTQKD 40  Taf1B_DROME
```

DANRE: Danio rerio; **SCHPO**: Schizosaccharomyces pombe; **SACCE**: Saccharomyces cerevisiae; **CAEEL**: Caenorhabditis elegans; **DROME**: Drosphila Melanogaster.

B *Drosophila* vs Human Taf1B alignment

```
1   MEEVLETMQLENMHCDVCEGTTFQ-EREGFYYCVECGTQKDQIRAVDITAEDNFDDTAAG  59 TAF1B_DROME
1   --MDLEEAEEFKERCTQCAAVSWGLTDEGKYYCTSCHNVTERYQEVTNT--DLIPNTQ--  54 TAF1B_HUMAN

60  RYTARTIRQKKDTEKEDEDDITSWEFY-----NYVLRGFLQELLNMGAKPELKLMTL-QV 113 TAF1B_DROME
55  ------IKALNRGLKKKNNTEKGWDWYVCEGFQYILYQQAEALKNLGVGPELKNDVLHNF 108 TAF1B_HUMAN

114 WAAYLDSMEVAFSKSNKTGLPKLNVRALPIDARIIYNHKTFKKGKKGKKSTLTGDPNDER 173 TAF1B_DROME
109 WKRYLQKSKQAYCKNPVY----------------------TTGR---KPTVLED----N 138 TAF1B_HUMAN

174 AKFRLWNRTKRNL---DASGYRSHGGASESEGEQSLHLQWSMRARKSLKRHMPLKHLDKH 230 TAF1B_DROME
139 LSHSDWASEPELLSDVSCPPFLESGAESQSD----IHT------R----KPFPVSKASQ- 183 TAF1B_HUMAN

231 SRDSKGSMSCHSLRPRVKQLHNFDRNIYCLNIIKLYVVLGIALNMVEDDIQLSDLLRFID 290 TAF1B_DROME
184 ----SETSVCSGSLDGVEYSQRKEKGIVKMTMPQTLAFCYLSLLWQREAITLSDLLRFVE 239 TAF1B_HUMAN

291 EEHLTKRCMLNYLPGNVAAKGKA--LLKDMELSKMKDKVTNKLLRVNIACMSRFINLSEY 348 TAF1B_DROME
240 EDHIPYINAFQHFPEQMKLYGRDRGIFGIESWPDYEDIYKKTVEVGTFLDLPRFPDITED 299 TAF1B_HUMAN

349 QKPNLHSLAERYILELALPPRLLKYVNSLLDLHPP------TFFNAMTVHPYPRYEARTM 402 TAF1B_DROME
300 CYLHPNILCMKYLMEVNLPDEMHSLTCHVVKMTGMGEVDFLTFDPIAKMAKTVKYDVQAV 359 TAF1B_HUMAN

403 AYILYAMKLLFGLDDLKERNISESAAKINEKLLEVGGDEAPLLFVFTEWMEFVEMRKVIV 462 TAF1B_DROME
360 AIIVVVLKLLFLLDDSFEWSLSNLAEKHNEKN-----KKDKPWFDFRKWYQIMKK----- 409 TAF1B_HUMAN

463 SHYNQSFARRFGVSTRTGCQVDDILAKEWKEKEQGETFGWM----------QGSAAMKRQ 512 TAF1B_DROME
410 -AFDEK-------------------KQKWEEAR--AKYLWKSEKPLYYSFVDKPVAYKKR 447 TAF1B_HUMAN

513 H--ENLTHIIETMLKDH--FGESSKESMEKEHIEFQPSLTPAHSYFNRILLQVSRSDGAK 568 TAF1B_DROME
448 EMVVNLQKQFSTLVESTATAGKKSPSSF---QFNWTEEDTDRTCFHGHSLQGVLKEKGQS 504 TAF1B_HUMAN

569 MKITIP-DHMKVDHSARNLDPFVLETTELSQYL-------SQHGLKLRVEELACQEDI-- 618 TAF1B_DROME
505 LLTKNSLYWLSTQKFCRCYCTHV-TTYEESNYSLSYQFILNLFSFLLRIKTSLLHEEVSL 563 TAF1B_HUMAN

619 QNVGIFRPL-TIIRGDGREYRANTEIKTETWISELKRKEKRPDFRFTQPTGTYGARYLKR 677 TAF1B_DROME
564 VEKKLFEKKYSVKRKKSRSKK-------------VRRH---------------------- 588 TAF1B_HUMAN
```

Fig. S5 *Drosophila* CG6241 is a homolog of human TAF1B

(A) The TAF1B/RRN7 family includes multiple members from yeast to human. **(B)** Sequence alignment of *Drosophila melanogaster* TAF1B (1-677aa, full-length 872aa) and Human TAF1B (1-588aa, full length 588aa). Uniprot online alignment tool was used. Magenta: Zinc finger. Blue: conserved Zinc-binding cysteine. Dark grey and * indicate identical amino acids.

A HHpred (search for sequence and structure homology)

Input Protein Sequence	Proteome searched	Hit Ranking	Hit Name	Probability (%)	E-value	P-value	Score
dTaf1C-like	*Homo* sapiens	**1**	**Taf1C isoform 2**	98.9	**7.60E-09**	**1.20E-13**	**117.3**
		2	**Taf1C isoform 1**	98.9	**1.40E-08**	**2.10E-13**	**115.9**
		3	gi|56243590	98.1	3.70E-05	5.80E-10	96.4
		4	gi|8922301	97.8	0.0025	3.90E-08	74.5
		5	gi|2136115	97.7	0.0018	2.90E-08	76.2
hTaf1C isoform 1	*Drosophila* melanogaster	**1**	**CG10496**	100	**5.20E-70**	**1.10E-74**	**408.6**
		2	gi|24586100	98.6	4.70E-06	9.60E-11	57.9
		3	gi|20129115	98.5	1.20E-05	2.40E-10	55.8
		4	gi|19922838	98.3	5.10E-05	1.00E-09	52.3
hTaf1C isoform 2	*Drosophila* melanogaster	**1**	**CG10496**	100	**7.40E-48**	**1.50E-52**	**419.1**
		2	gi|17933648	99.8	3.20E-17	6.50E-22	177.5
		3	gi|28573273	99.7	6.50E-15	1.30E-19	162.9
		4	gi|20129115	99.5	7.80E-12	1.60E-16	131.5
hTaf1C isoform 6	*Drosophila* melanogaster	**1**	**CG10496**	100	**4.20E-49**	**8.60E-54**	**431.2**
		2	gi|17933648	99.5	3.90E-12	8.00E-17	138.4
		3	gi|28573273	99.4	1.70E-11	3.60E-16	136.1
		4	gi|20129115	99.2	1.40E-08	3.00E-13	107.1
		5	gi|45552461	99.2	4.70E-09	9.50E-14	120.8

B PHYRE2 predicted 3D Models

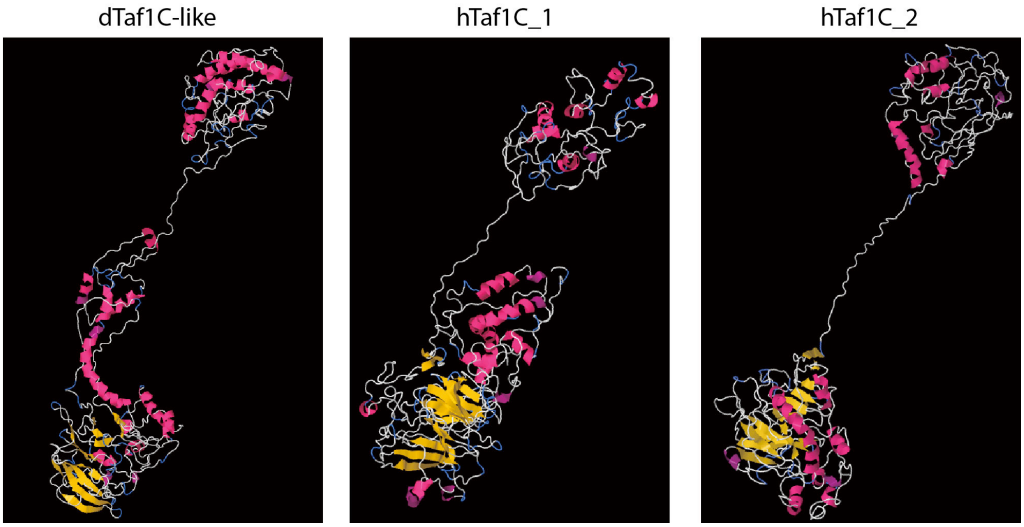

Fig. S6 The protein encoded by *CG10496*, TAF1C-like, resembles human TAF1C

(A) HHpred server (*39*), which detects protein homology according to both primary sequence and secondary structure, shows that the top hits for TAF1C-like in *Homo sapiens* proteome are isoforms of human TAF1C, and vice versa. The best hit for different human TAF1C isoforms in the *Drosophila melanogaster* proteome is the protein encoded by *CG10496*, which we refer to as TAF1C-like. **(B)** The 3D models for TAF1C-like, human TAF1C isoform 1 & 2, predicted by PHYRE2 protein fold recognition server (*40*), exhibits highly similar structures.

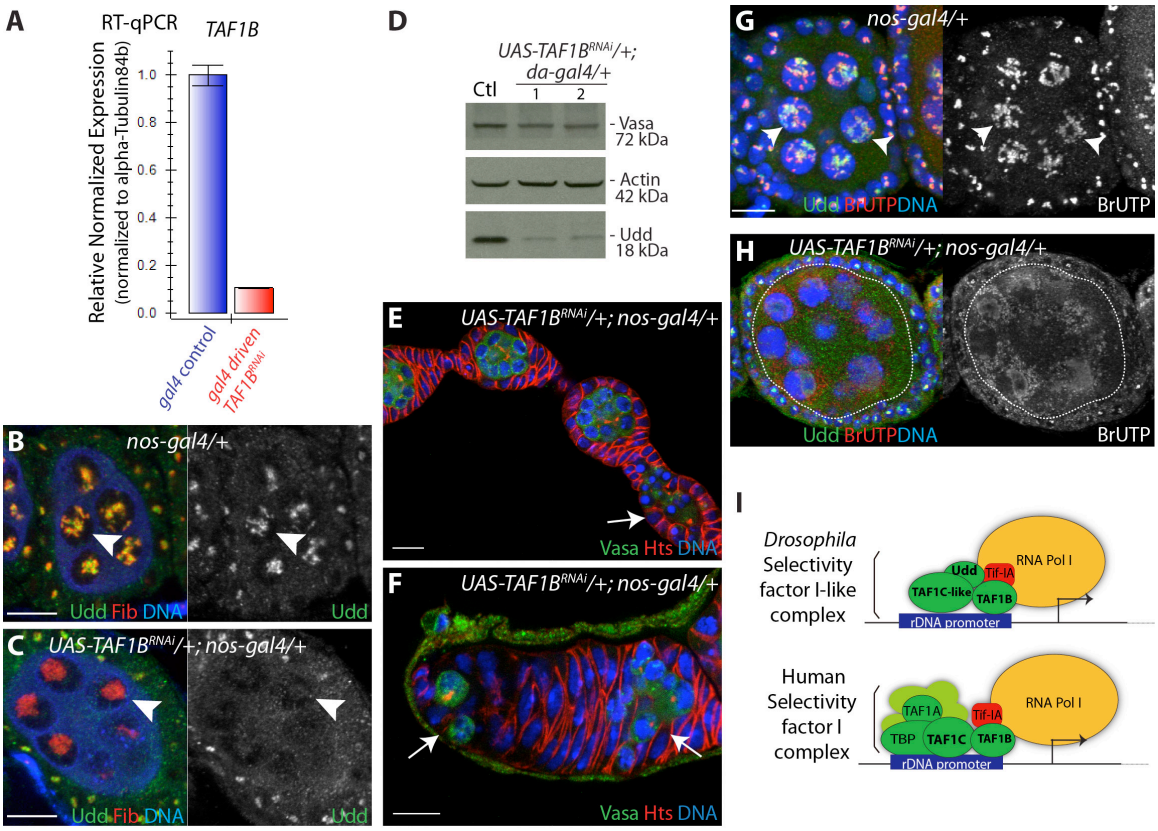

Fig. S7 Germline-specific knock-down of *TAF1B* exhibits germ-cell loss and reduced rRNA transcription

(A) RT-qPCR demonstrates that the mRNA level of *TAF1B* is greatly reduced when knocking down TAF1B ubiquitously using the UAS-*TAF1B*^{RNAi} line. Total RNA was isolated from ovarian lysates from *daughterless (da)-gal4/+* and *UAS-TAF1B*^{RNAi}*/+*; *da-gal4/+* flies kept at room temperature. The RNA levels of *TAF1B* were normalized to *α-Tubulin84B*. Note: Ubiquitous knock-down of *TAF1B* using *da-gal4* exhibits lethality at 29°C, and here crosses were kept at room temperature. **(B-C)** Knock-down of TAF1B greatly reduces the nucleolar expression of Udd, but not that of Fibrillarin (arrowheads point to nurse cell nucleoli). Egg chambers from **(B)** *nanos* (*nos)-gal4/+* control and **(C)** *UAS-TAF1B*^{RNAi}*/+*; *nos-gal4/+* ovaries stained for Udd (green), Fibrillarin (red) and Vasa (blue). Here TAF1B is only knocked down in the germline but not in the surrounding somatic cells. **(D)** Western blot of extracts from *da-gal4/+* (Ctl) and two independent *UAS-TAF1B*^{RNAi}*/+*; *da-gal4/+* lines demonstrates reduced expression of Udd protein. Actin and Vasa control for loading. **(E,F)** *UAS-TAF1B*^{RNAi}*/+*; *nos-gal4/+* flies kept at 29°C were examined. **(E)** Egg chambers and a **(F)** germarium stained for Vasa (green), Hts (red), DNA (blue). Arrows point to germ cell loss phenotype in the egg chamber and the germarium. Nascent rRNA labeled by BrUTP incorporation (red) and Udd (green) in egg chambers from **(G)** *nos-gal4/+* control and **(H)** *UAS-TAF1B*^{RNAi}*/+*; *nos-gal4/+* females (both raised at 29°C). **(G)** Arrowheads point to control nuclei and **(H)** the white dotted line marks RNAi knockdown nurse cells. Knock-down of *TAF1B* results in a dramatic reduction of rRNA transcription. **(I)** Model comparing the *Drosophila* SL1-like and human SL1 complexes. Scale bars represent 10 µm.

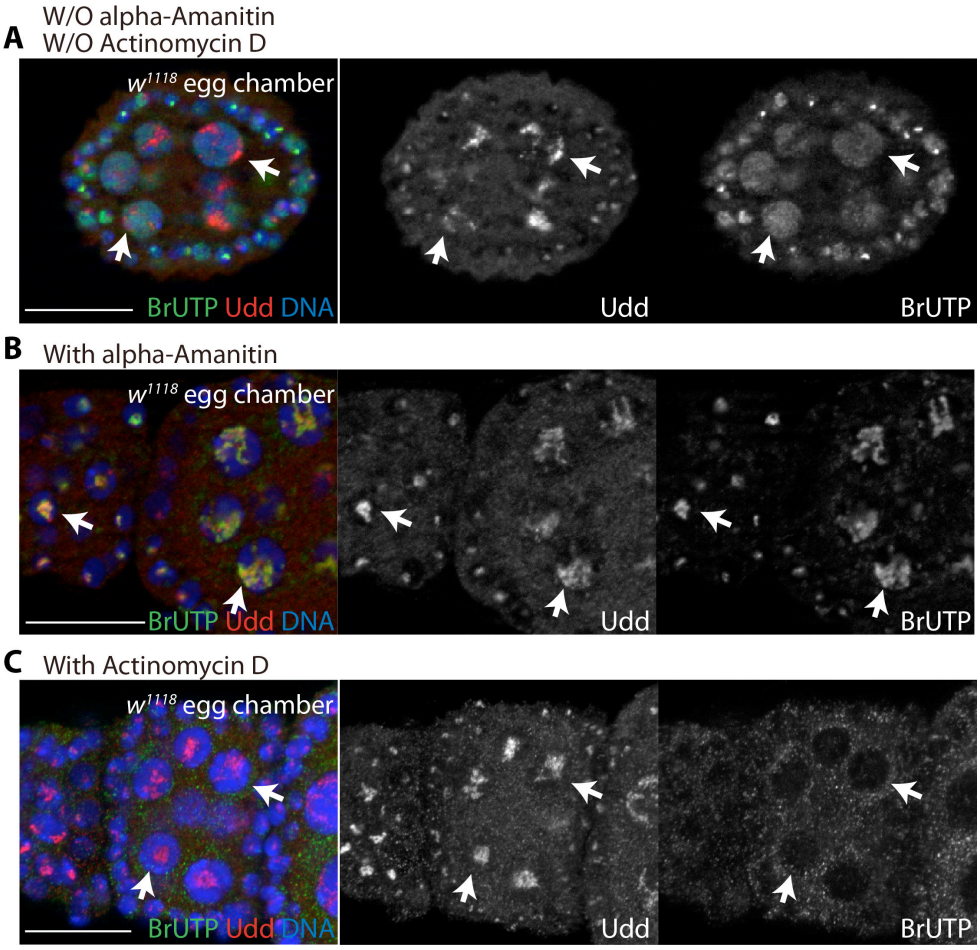

Fig. S8 Actinomycin D inhibits global transcription, while alpha-amanitin specifically inhibits transcription by RNA Pol II and Pol III in cultured w^{1118} ovaries.

(**A-C**) Egg chambers were stained for Udd (red), BrUTP (green) and DNA (blue). **(A)** Ovaries cultured for 20 minutes without alpha-Amanitin or Actinomycin D exhibited global nuclear BrUTP labeling, as expected, since without drug, Pol I, Pol II and Pol III RNAs all incorporate BrUTP. **(B)** Ovaries treated with alpha-Amanitin, displayed BrUTP incorporation into pre-rRNAs within nucleoli. **(C)** No BrUTP incorporation was observed in the nuclei or nucleoli of egg chambers treated with Actinomycin D. Arrows point to individual nuclei. Scale bars represent 20 µm.

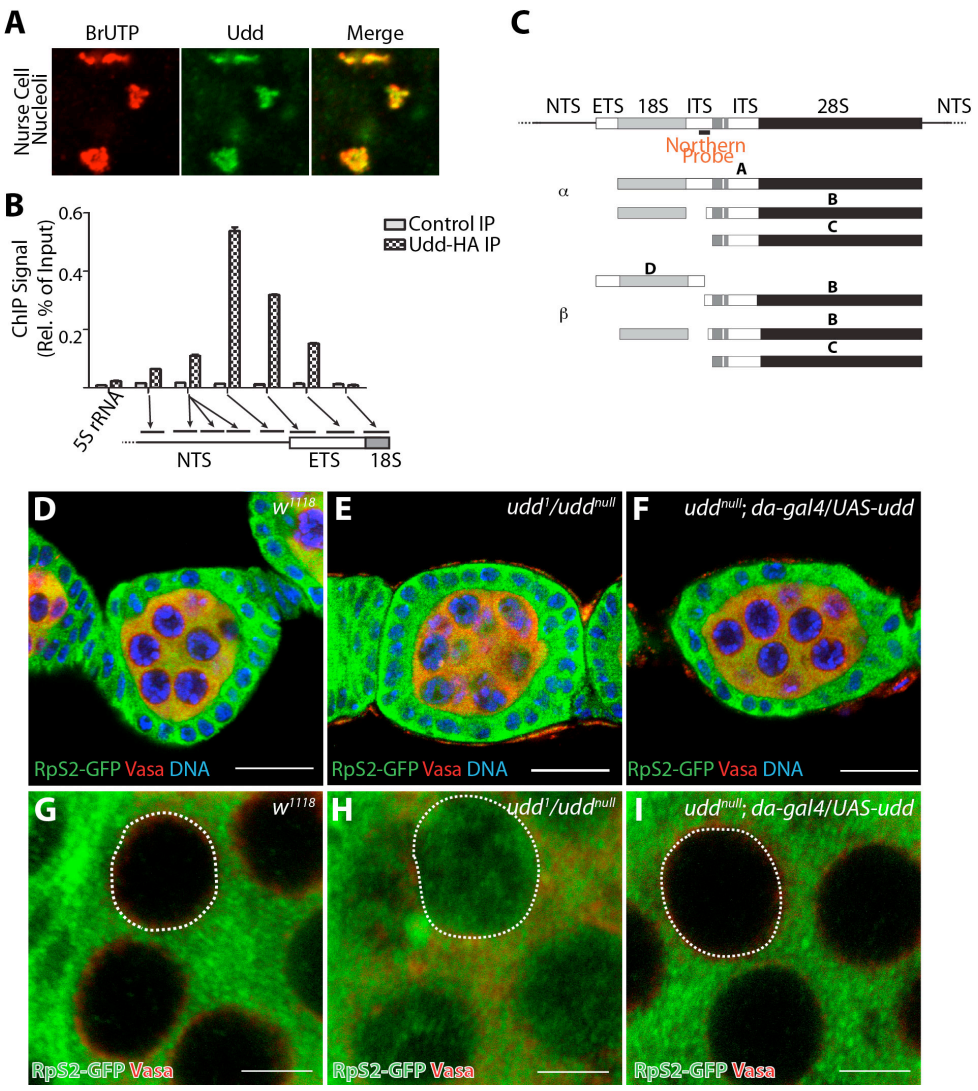

Fig. S9 Udd associates with nascent rRNA, the rRNA gene promoter region and regulates ribosome biogenesis

(A) Nurse cells from w^{1118} egg chambers, pulse-labeled for nascent rRNA transcripts using BrUTP, stained for BrUTP (red) and Udd (green). This staining shows significant co-localization between Udd and newly synthesized rRNAs. **(B)** ChIP-qPCR analysis from udd^{null}/udd^{null}; *da-gal4*/*UAS-HA-udd* reveals that a rescuing HA-tagged Udd transgene driven by *da-gal4* associates with specific sites within the rRNA promoter and external transcribed spacer (ETS), as indicated by the 5th and 6th arrows and bars. The same extracts were subjected to the ChIP protocol without anti-HA antibody as a negative control. Note: this experiment shows similar results to ChIP-qPCR results in **Fig. 2G**, using a different genetic background; in both experiments, 3- to 6- day-old flies were used. Error bars represent standard deviation. **(C)** Schematic of a single *Drosophila* rRNA gene showing the two different rRNA processing pathways, referred to as α and β, and the location of the probe used in the Northern blot analysis which is in the internal transcribed spacer (ITS). **(D-I)** A cytoplasmic RpS2-GFP protein trap reporter

accumulates in the nuclei of *udd* mutants and the rescuing HA-Udd transgene reverses the accumulation. **(D)** w^{1118}, **(E)** udd^{1}/udd^{null} and **(F)** udd^{null}/udd^{null}; *da-gal4*/*UAS-HA-udd* egg chambers which carry the RpS2-GFP protein trap (*CB02294*) (*41*) stained for GFP (green), Vasa (red) and DNA (blue). **(G)** w^{1118}, **(H)** udd^{1}/udd^{null} and **(I)** udd^{null}/udd^{null}; *da-gal4*/*UAS-HA-udd* nurse cells stained for RpS2-GFP (green) and Vasa (red). The dotted lines outline nurse cell nuclei. (D-F) Scale bars represent 20 μm. (G-I) Scale bars represent 5 μm.

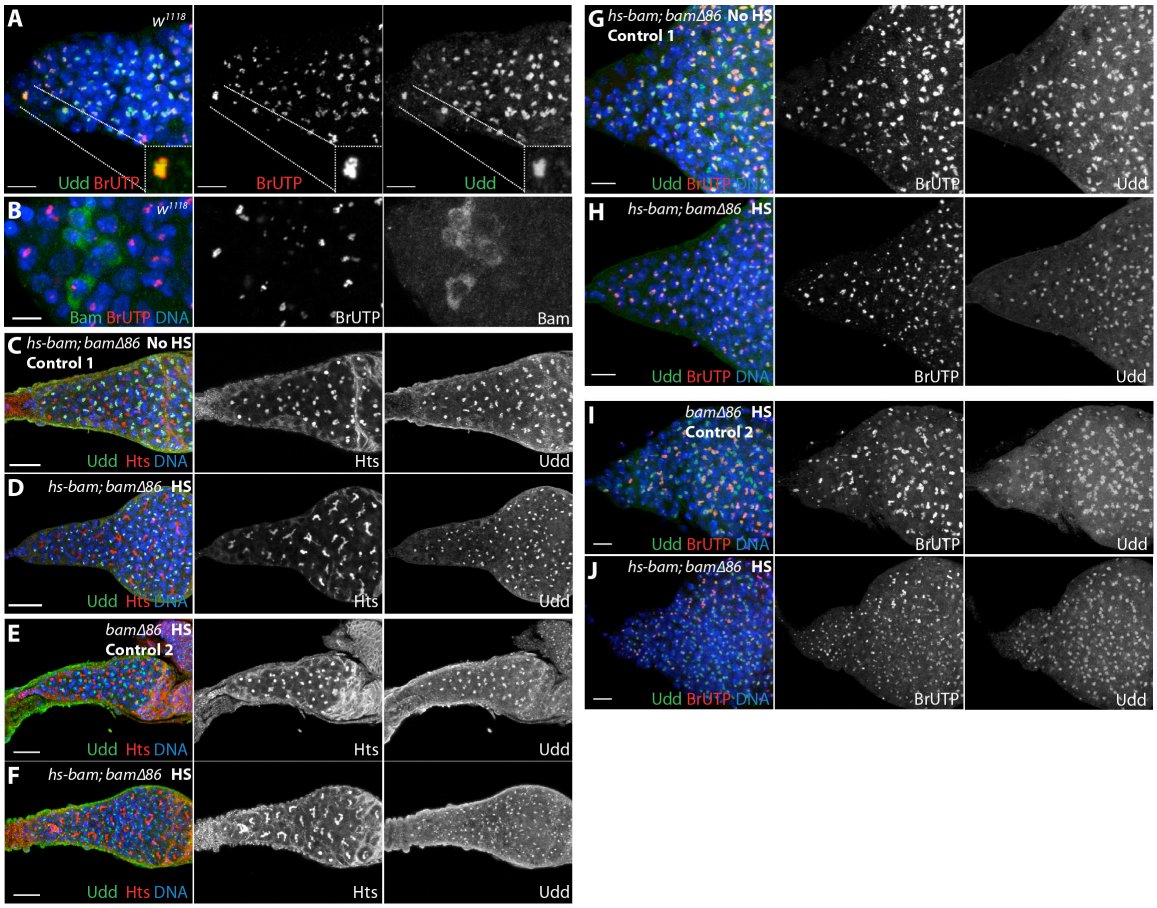

Fig. S10 Bam expression promotes multicellular cyst formation and correlates with low levels of rRNA transcription

(A) Separate grayscale channels for w^{1118} germarium in **Fig. 3A**, stained for Udd (green), BrUTP (red), DNA (blue). **(B)** Separate grayscale channels for w^{1118} germarium in **Fig. 3B**, stained for Bam (green), BrUTP (red), DNA (blue). Two different controls are shown in panels C and G and panels E and I. **(C,D,G,H)** Ovaries from *hs-bam*; $bam^{\Delta 86}$ females subjected to **(B,F)** no heat-shock or **(D,H)** two one-hour heat-shocks at 37°C on two consecutive days, were dissected 36 hours after the 1st heat shock. **(E,F,I,J)** Ovaries from $bam^{\Delta 86}$ **(E,I)** and *hs-bam*; $bam^{\Delta 86}$ **(F,J)** females were subjected to the same heat-shock procedure as **(D,H)**. **(C-F)** Ovaries were stained for Udd (green), Hts (red) and DNA (blue). **(G-J)** Ovaries were pulse labeled with BrUTP (red) in the presence of α-amanitin, and stained for Udd (green) and DNA (blue). **(G,H)** Separate grayscale channels for **Fig. 3D and 3E**. **(A-B, G-J)** Scale bars represent 10 μm. **(C-F)** Scale bars represent 20 μm.

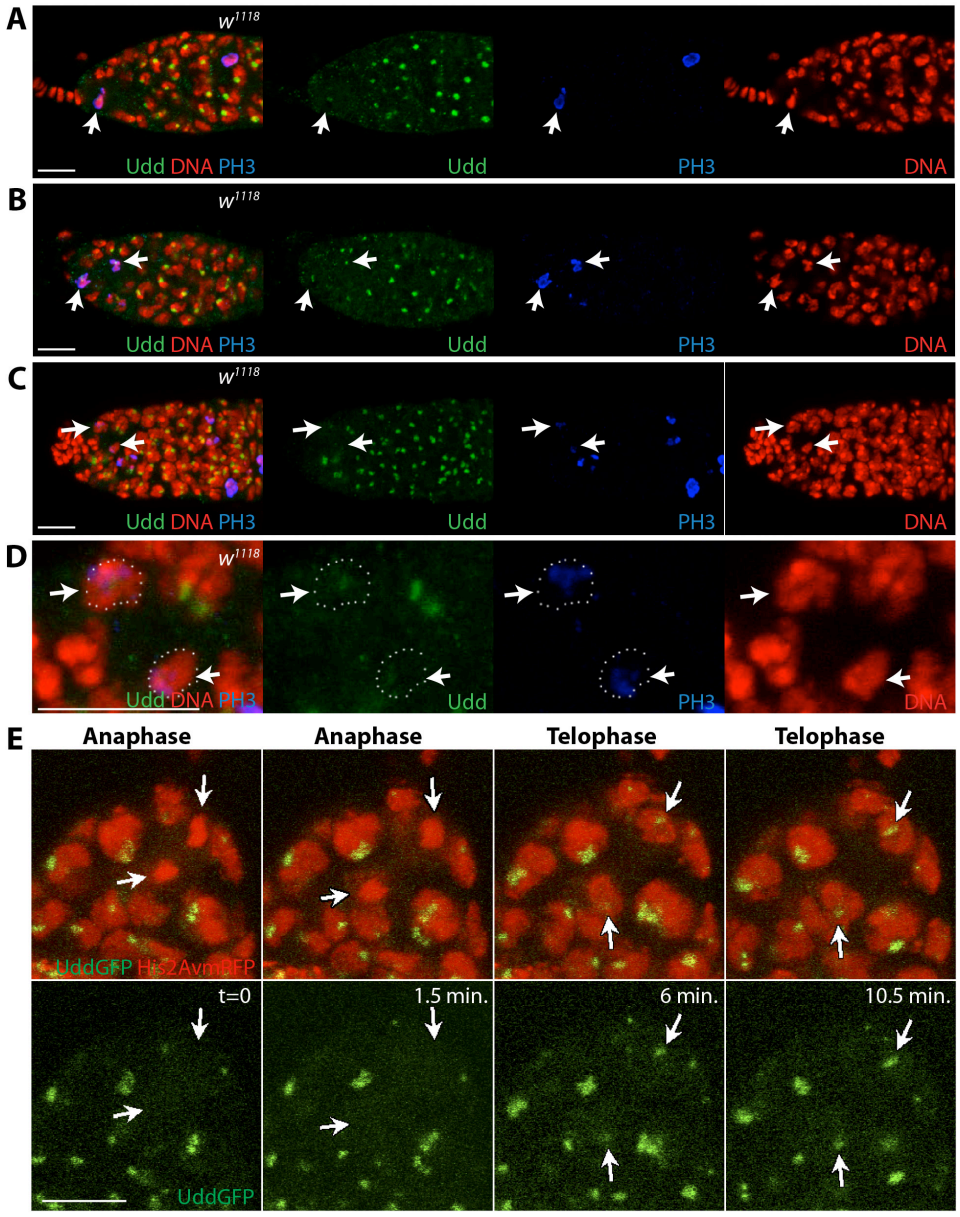

Fig. S11 Udd enriched in the anterior stem cell daughter immediately after GSC mitotic division.

(A-D) Fixed germaria stained for endogenous Udd (green), PH3 (blue) and DNA (red), with arrows pointing out the dividing GSC. **(A)** Metaphase; **(B)** Anaphase; **(C)** Telophase; **(D)** Magnified image of telophase in (C). Note: Udd-GFP signal is weaker than immunofluorescent staining against endogenous Udd, and Udd-GFP disappears before metaphase and reappears in early telophase. Scale bars represent 10 μm. **(E)** Still images from a second GSC live cell imaging experiment with time stamps showing GFP-tagged Udd (green) and mRFP-tagged Histone H2Av (red). The corresponding GFP-Udd channel alone is also included below. Arrows point to a dividing GSC. Scale bars represent 10 μm.

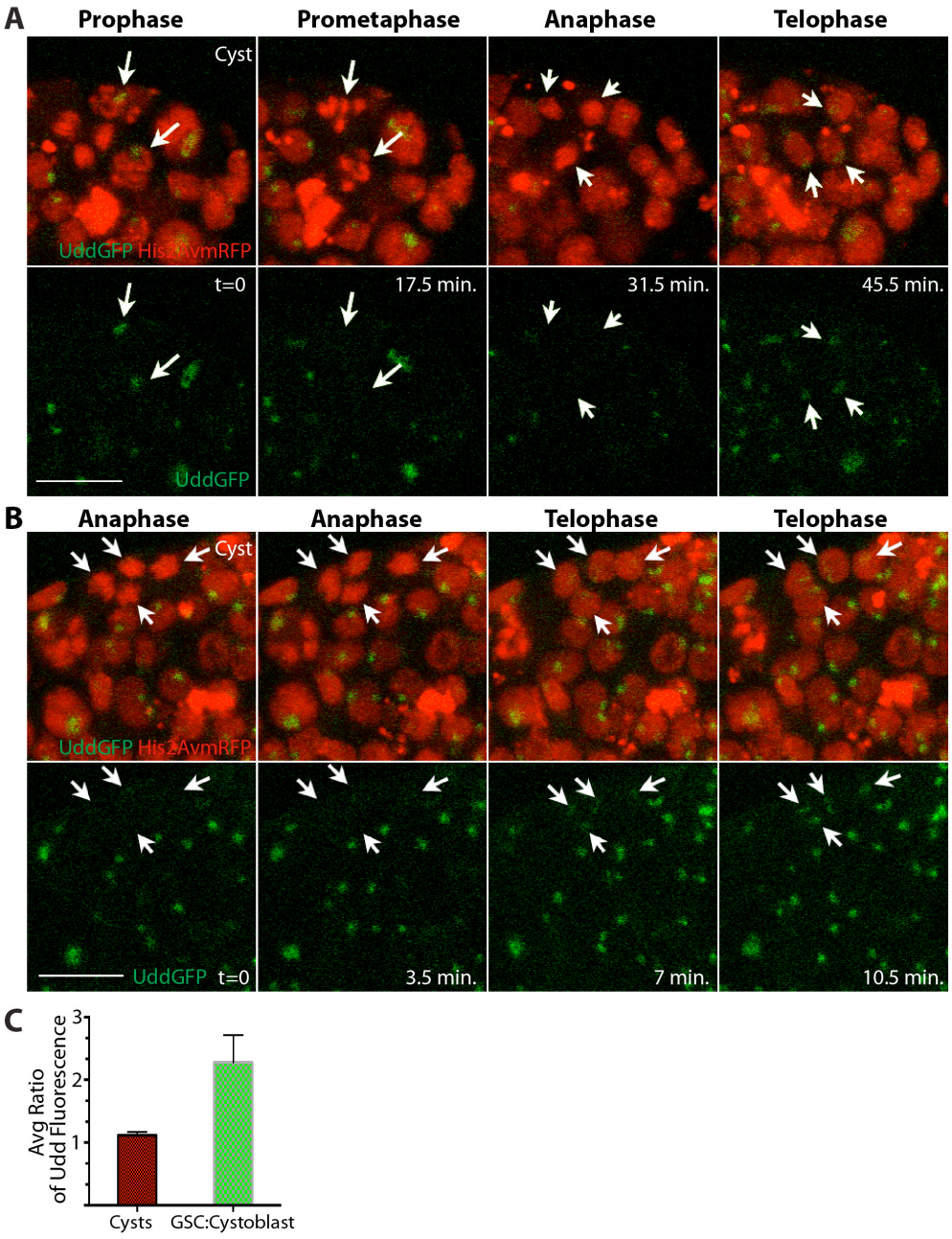

Fig. S12 Udd evenly distributes during cyst divisions

(A,B) Still images from two independent GSC live-cell imaging experiments showing GFP-tagged Udd (green) and mRFP-tagged Histone H2Av (red). Time stamps are indicated. Arrows point to dividing cysts. Scale bars represent 10 µm. **(C)** Quantification of the average ratio of Udd-GFP fluorescence between GSCs and cystoblasts and different cells within the same cyst from collected movies.

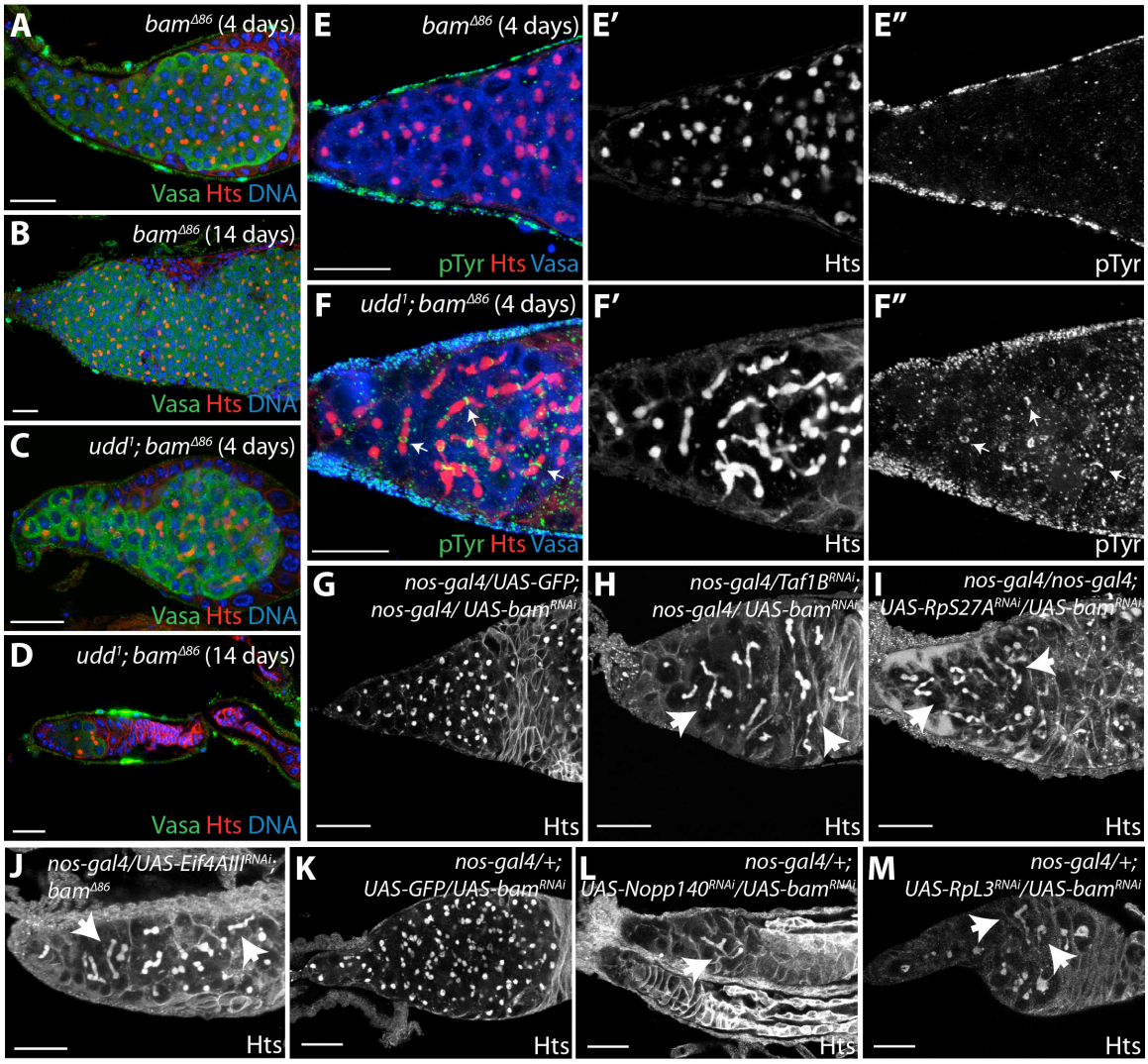

Fig. S13 Down-regulation of ribosome biogenesis promotes multicellular cyst formation in a *bam* loss-of-function background

(A-D) *bam*$^{\Delta 86}$ germaria grew larger over time while *udd*^{1} *bam*$^{\Delta 86}$ double mutant germaria exhibited germ-cell loss similar to *udd*^{1} single mutants. **(A, B)** *bam*$^{\Delta 86}$ and **(C, D)** *udd*^{1} *bam*$^{\Delta 86}$ germaria stained for Vasa (green), Hts (red) and DNA (blue). **(A, C)** Germaria from 4 day-old flies. **(B, D)** Germaria from 14 day-old flies. **(E-E'')** *bam*$^{\Delta 86}$ and **(F-F'')** *udd*^{1} *bam*$^{\Delta 86}$ double mutant germaria stained for Phosphotyrosine (pTyr) (green), Hts (red) and DNA (blue), corresponding to the image in **Fig. 4**. Arrows point to ring canals labeled by pTyr. **(E', F')** Hts alone. **(E'', F'')** pTyr alone. **(G-M)** Down-regulation of factors involved in ribosome biogenesis and protein translation in undifferentiated *bam* mutants also induces cyst formation. Germaria from 3-6 day-old flies. **(G)** *nos-gal4/UAS-GFP; nos-gal4/UAS-bam*^{RNAi}, **(H)** *nos-gal4/UAS-TAF1B*^{RNAi}*; nos-gal4/UAS-bam*^{RNAi}, **(I)** *nos-gal4/nos-gal4; UAS-RpS27A*^{RNAi}*/UAS-bam*^{RNAi} stained for Hts. **(J)** *nos-gal4/UAS-Eif4AIII; bam*$^{\Delta 86}$, compared to *bam*$^{\Delta 86}$ single mutants stained for Hts. **(K-M)** One copy of *nos-gal4* was used to drive **(K)** *UAS-GFP* and *UAS-bam*^{RNAi}, **(L)** *UAS-Nopp140*^{RNAi} and

UAS-bam^{RNAi}, **(M)** *UAS-RpL3*^{RNAi} and *UAS-bam*^{RNAi}. RpL3 is a component of the large ribosomal subunit, and Nopp140 is involved in rRNA processing and ribosome assembly. Arrows mark branched fusomes. Scale bars represent 20 μm.

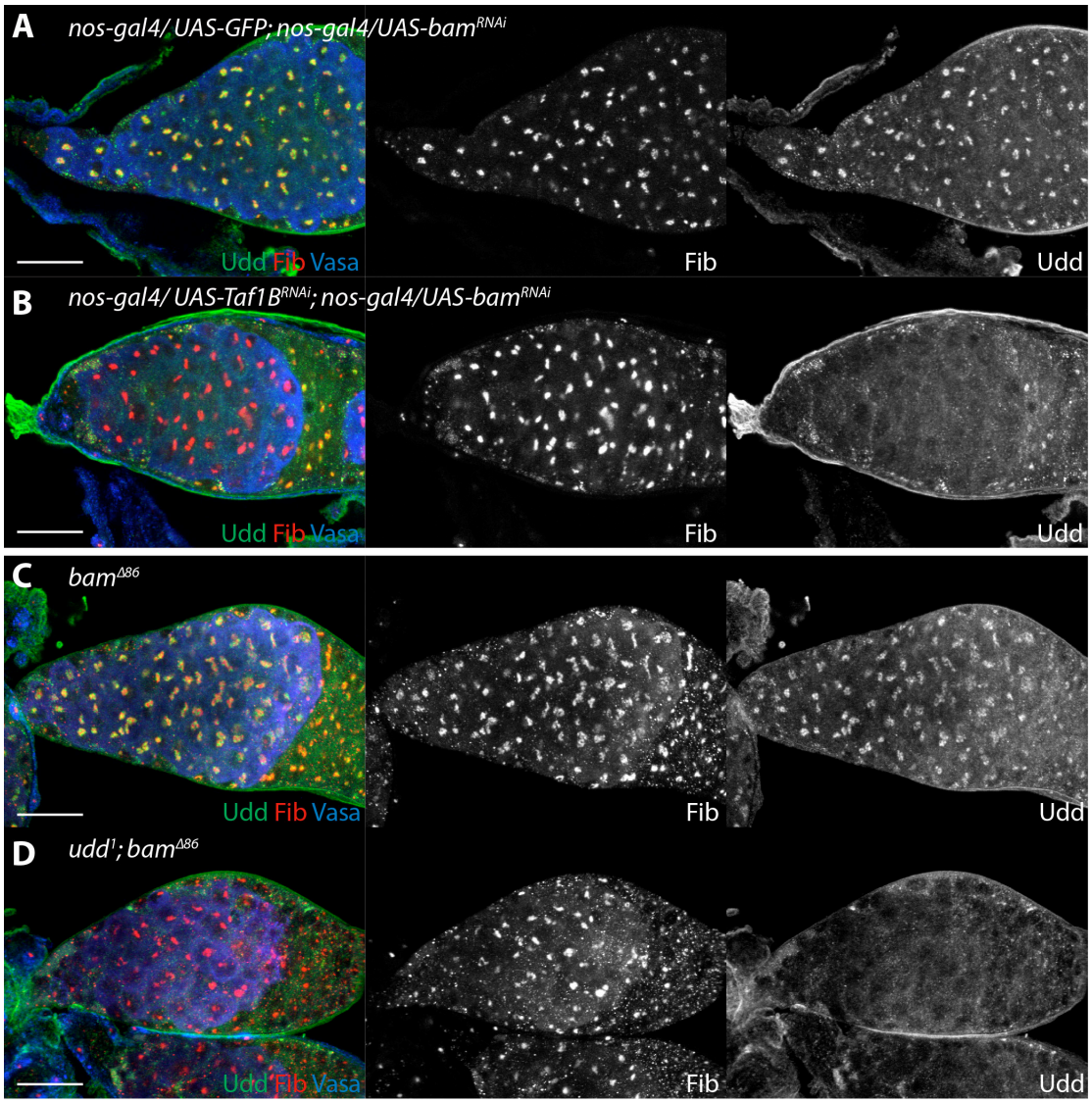

Fig. S14 Fibrillarin expression in *bam TAF1B* double RNAi knock-down or *udd bam* double mutants

Fibrillarin, an rRNA processing factor, still localizes to the nucleoli of germ cells that have reduced levels of Udd and TAF1B. (**A-D**) Germaria from 2-4 day old flies stained for Udd (green), Fibrillarin (red), Vasa (blue). **(A)** Control *nos-gal4/UAS-GFP;nos-gal4/UAS-bam*^{RNAi}; **(B)** *nos-gal4/UAS-TAF1B*^{RNAi}*; nos-gal4/UAS-bam*^{RNAi}; **(C)** control *bam*$^{\Delta 86}$; **(D)** *udd*^{1}; *bam*$^{\Delta 86}$. Scale bars represent 20 µm.

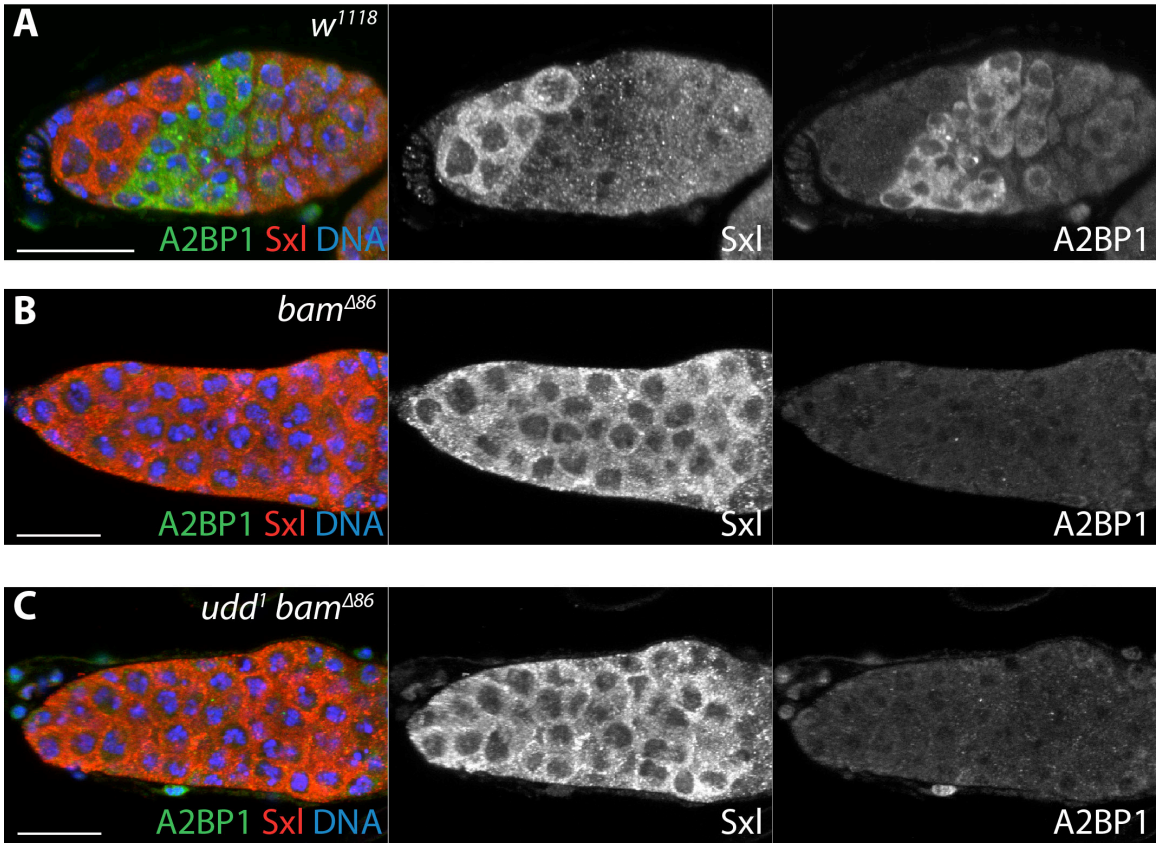

Fig. S15 Sxl and A2bp1 expression in *w*^{1118}, *bam* and *udd bam* double mutants

Sex-lethal (Sxl) is usually highly expressed in GSCs, CBs, 2-cell cysts; cytoplasmic A2bp1 is highly expressed in 4-, 8- and 16-cell cysts (*37*). Here, both double mutants and *bam* single mutants exhibit high levels of Sxl and low levels of A2bp1. Ovaries from *w*^{1118} **(A)**, *bam*$^{\Delta 86}$ **(B)** and *udd*^{1}; *bam*$^{\Delta 86}$ **(C)** stained for A2bp1 (green), Sxl (red), DNA (blue). Scale bars represent 20 µm.

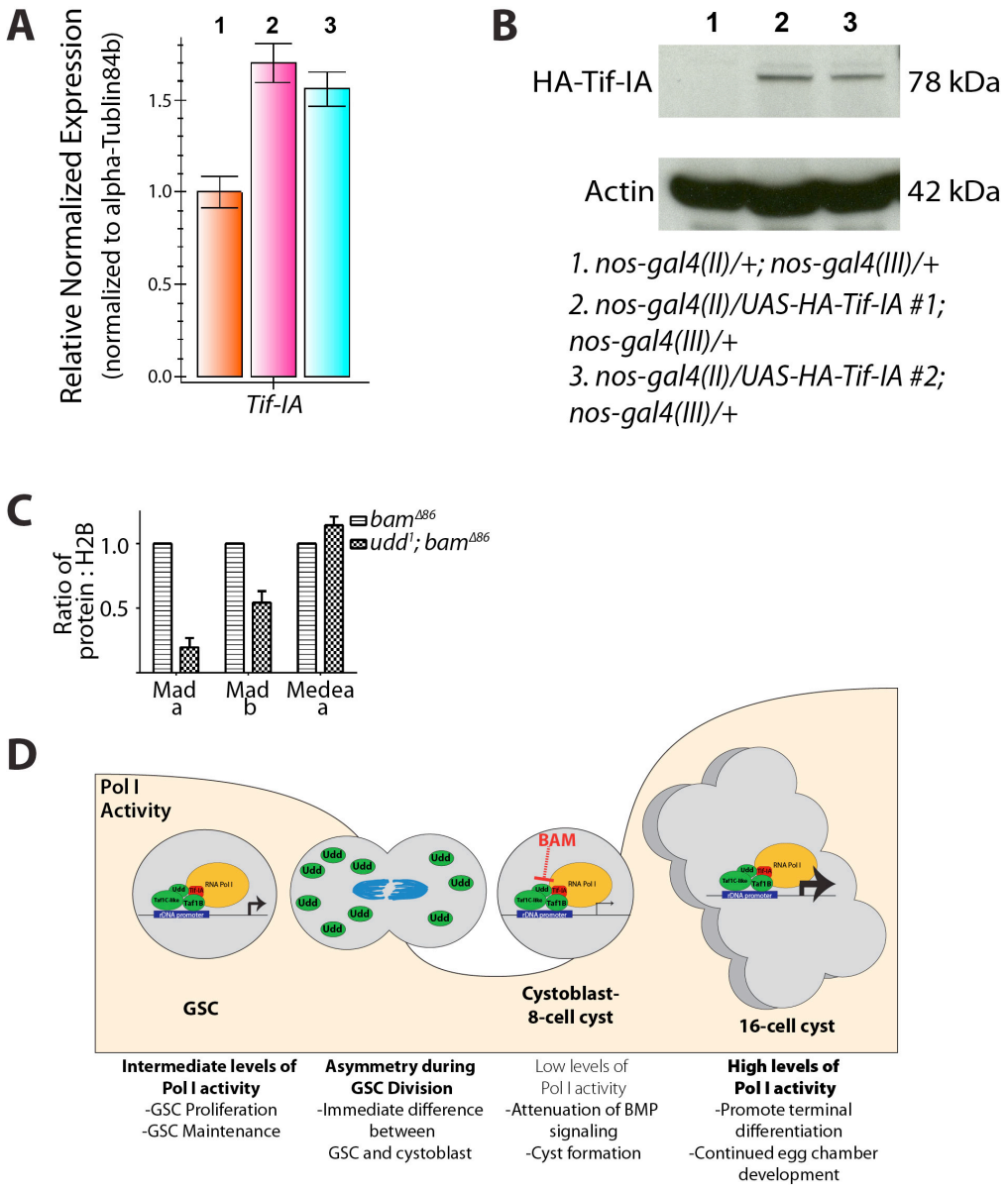

Fig. S16 Modulating rRNA synthesis influences cyst development and BMP signaling in the germ line.

(A) RT-qPCR demonstrates the ovarian mRNA level of *TIF-IA* is increased in flies carrying *TIF-IA* transgene expressed in the germline. The RNA level of *TIF-IA* was normalized to *α-Tublin84B*. **(B)** Western blot demonstrates transgenic protein expression of TIF-IA. Two copies of *nos-gal4* were used to drive the germline expression of two individual *UAS-HA-Tif1A* lines in **(A-B)**. Flies carrying two copies of *nos-gal4* alone were used as negative controls. Actin serves as a loading control. **(C)** Semi-quantification of three independent western blots corresponding to Fig 4I using Image J, and the ratio of Mad or Medea protein versus H2B in double mutants is normalized that in $bam^{\Delta 86}$ single mutants respectively. Error bars represent standard deviation. **(D)** Model describing how

the modulation of Pol I activity affects stem cell activity and the differentiation of stem cell daughters.

Movie Legends. In all movies Udd-GFP is in green and His2Av-mRFP is in red. Z-stacks were taken every 3.5 minutes for Movies S1, S3 and S7. A continuous scan was taken for Movie S5. Scale bars represent 10 μm.

Movie S1. Udd is enriched in the GSC following mitosis, relative to its differentiating daughter, the cystoblast. Arrow pointing to a GSC undergoing mitosis, starting from prophase. Udd is discretely localized during prophase and prometaphase. Udd diffuses during metaphase, anaphase and early telophase. Udd then reappears in late telophase and is enriched in the GSC relative to the cystoblast and remains high following mitosis.

Movie S2. Udd-GFP channel of Movie S1 alone.

Movie S3. Udd appears evenly distributed in multicellular cysts before and after mitosis. Arrows pointing to cysts undergoing mitosis starting from prophase. Udd diffuses during metaphase, anaphase and early telophase. Udd then reappears at equal levels in the newly formed daughters and remains equal following mitosis.

Movie S4. Udd-GFP channel of Movie S3 alone.

Movie S5. Udd is enriched in the GSC following mitosis, relative to the cystoblast. Arrows pointing to chromosomes in anaphase of a dividing GSC. Udd is diffused during anaphase and early telophase. Udd then reappears in late telophase and is enriched in the GSC relative to the cystoblast and remains high following mitosis.

Movie S6. Udd-GFP channel of Movie S5 alone.

Movie S7. A second independent example of how Udd appears evenly distributed in multicellular cysts before and after mitosis. Arrows pointing to cysts undergoing mitosis starting from prophase. Udd diffuses during metaphase, anaphase and early telophase. Udd then reappears at equal levels in the newly formed daughters and remains equal following mitosis.

Movie S8. Udd-GFP channel of Movie S7 alone.

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