mastermind regulates niche ageing independently of the Notch pathway in the Drosophila ovary.

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Supplementary Material and Methods

Supplementary References

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Figure S1 (related to Figure 1).
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**SUPPLEMENTARY MATERIALS AND METHODS**

**Genotypes**

Figure 1

(B, C) y w

(D) bab1>tau::GFP; w; bab1-Gal4, UAS-tau::GFP/TM2

Figure 2

(A) w; bab1-Gal4, UAS-tau::GFP/TM2

(B) mam enhancer>tau::GFP: w; mam(GMR28A08)-Gal4/UAS-tau::GFP

(C, D) Control: w; UAS-mam/++; TM6B/

bab1>mam: w; UAS-mam/++; bab1-Gal4, tub-G80ts/+ 

(E) hh>tau::GFP: w; tub-G80ts/+; hh-Gal4/UAS-tau::GFP

(F) hh>mam: w; UAS-mam/tub-G80ts; hh-Gal4/+ 

(G) ptc>tau::GFP: w; tub-G80ts/+; ptc-Gal4/UAS-tau::GFP

(H) ptc>mam: w; UAS-mam/tub-G80ts; ptc-Gal4/+ 

(F, H) Control: w; UAS-mam/tub-G80ts; TM6B/+ 

Figure 3

(A) y w; DE-cadherin::GFP; MKRS/TM6B

(C) Control: w; UAS-mam/DE-cadherin::GFP; TM6B/+ 

bab1>mam: w; UAS-mam/ DE-cadherin::GFP; bab1-Gal4, tub-G80ts/+ 

Figure 4

(A) y w; DE-cadherin::GFP; MKRS/TM6B

(B) w; Resille::GFP

(E) y w

(G) Control: w; UAS-mam/++; TM6B/+ 

bab1>mam: w; UAS-mam/++; bab1-Gal4, tub-G80ts/+ 

Figure 5
(A, E, F) y w
(C, G) Control: w; UAS-mam/+; TM6B/+ 

bab1>mam: w; UAS-mam/+; bab1-Gal4, tub-G80ts/+ 

Figure 6

(A) Control: w; UAS-mam/UAS-SOD1; TM2/TM6B 
bab1>mam+GFP: w; UAS-mam/+; UAS-tau::GFP/bab1-Gal4, tub-G80ts 
bab1>mam+SOD1: w; UAS-mam/UAS-SOD1; bab1-Gal4, tub-G80ts/TM2 

(B-D) Control: w; UAS-Dicer/+; UAS-(DI RNAi or N\textsuperscript{intra})/TM2 
bab1>DI RNAi: w; UAS-Dicer/+; UAS-DI RNAi/bab1-Gal4, tub-G80ts 
bab1>N\textsuperscript{intra}: w; UAS-N\textsuperscript{intra}/+; bab1-Gal4, tub-G80ts/TM2 
bab1>mam: w; UAS-mam/+; bab1-Gal4, tub-G80ts/+ 

Suppl. Figure 1 
w;; bab1-Gal4, UAS-tau::GFP/TM2 

Suppl. Figure 2 

(A, B) Control: w; UAS-dicer/+; bab1-Gal4, tub-G80ts/TM2 
bab1>mam RNAi: w; UAS-dicer/+; bab1-Gal4, tub-G80ts/UAS-mam RNAi 
(C) y w/w; TM6B/+ 
y w/w; bab1-Gal4, tub-G80ts/+ 

(D) Control: w; tj-Gal4/+; tub-G80ts/TM2 
bab1>mam\textsuperscript{short}: w;; bab1-Gal4, tub-G80ts/UAS-mam\textsuperscript{short} 

(E) Control: w; UAS-mam\textsuperscript{short}/TM6B 
tj>mam\textsuperscript{long}: w; UAS-mam\textsuperscript{long}/tj-Gal4; tub-G80ts/TM2 

(F) w; tub-G80ts/+; TM6B/+ 
w; tub-G80ts/+; hh-Gal4/+ 
w; tub-G80ts/+; ptc-Gal4/+ 

Suppl. Figure 3
(A) y w
(B) Control: w; UAS-mam/+; TM6B/+ 
    bab1>mam: w; UAS-mam/+; bab1-Gal4, tub-G80ts/+ 

Suppl. Figure 4
(A, B) Control: w; UAS-mam/+; TM6B/+ 
    bab1>mam: w; UAS-mam/+; bab1-Gal4, tub-G80ts/+ 
(C, D) w; UAS-mam/+; UAS-DE-cadherin/TM6B 
    w; UAS-nls::GFP/+; UAS-DE-cadherin/TM6B 
    bab1> GFP+DE-cad: w; UAS-nls::GFP/+; UAS-DE-cadherin/bab1-Gal4, tub-G80ts 
    bab1>mam+GFP: w; UAS-mam/UAS-nls::GFP; bab1-Gal4, tub-G80ts/TM2 
    bab1>mam+DE-cad: w; UAS-mam/+; UAS-DE-cadherin/bab1-Gal4, tub-G80ts 

Suppl. Figure 5
(A) Control: w; UAS-mam/DE-cadherin::GFP; TM6B/+ 
    bab1>mam: w; UAS-mam/ DE-cadherin::GFP; bab1-Gal4, tub-G80ts/+ 
(C) y w 
(E) Control: w; UAS-mam/+; TM6B/+ 
    bab1>mam: w; UAS-mam/+; bab1-Gal4, tub-G80ts/+ 

Suppl. Figure 6
(A) y w 
(C) Control: w; UAS-mam/+; TM6B/+ 
    bab1>mam: w; UAS-mam/+; bab1-Gal4, tub-G80ts/+ 
(E) bab1>hh::GFP: w; UAS-hh::GFP/Sp; bab1-Gal4, tub-Gal80ts/+ 
    Control: w; UAS-hh::GFP/UAS-mam; +/TM6B 
    bab1>mam+GFP: w; UAS-nls::GFP/UAS-mam; bab1-Gal4, tub-Gal80ts/+ 
    bab1>mam+hh: w; UAS-hh::GFP/UAS-mam; bab1-Gal4, tub-Gal80ts/+
Suppl. Figure 7

(A, B) Control: w; UAS-mam/UAS-SOD1; TM2/TM6B
bab1>mam+SOD1: w; UAS-mam/UAS-SOD1; bab1-Gal4, tub-G80ts/TM2

(C) Control: w; UAS-SOD1/CyO; TM6B/TM2
bab1>SOD1: w; UAS-SOD1/CyO; bab1-Gal4, tub-G80ts/TM6B

Fly stocks

Flies were grown on standard medium at 18, 25 or 29°C, depending on the experiment. To overexpress GFP in niche cells, we used bab1-Gal4 [1], mam-Gal4 (BDSC 49444; it expresses Gal4 under the control of DNA sequences in or near mam; GMR28A08), hh-Gal4 [2] or ptc-Gal4 (BDSC 45900) in combination with UAS-tau::GFP [3] or UAS-nls::GFP (BDSC 4775). The DE-cadherin::GFP line (BDSC 60584) is a genetically modified version of the endogenous shotgun gene, engineered to express a GFP-tagged E-cadherin under its native regulatory sequences [4]. As a membrane marker for live imaging we used Resille::GFP [5]. For overexpression experiments we used the following lines: UAS-hh::GFP [6], UAS-mam (long isoform; BDSC 27743), UAS-mamShort [7], UAS-Nintra (BDSC 52008), UAS-SOD1 [8] (BDSC 33605) or UAS-DE-cadherin [9] in combination with tub-Gal80ts (BDSC 7017) and bab1-Gal4 [1] or traffic-jam-Gal4 [10]. To knock-down mam or Delta levels we utilised UAS-mam RNAi (BDSC 28046) and UAS-Delta RNAi (BDSC 34938) in combination with UAS-Dicer (BDSC 24650), bab1-Gal4 and tub-Gal80ts. Where necessary, flies of the appropriate genotype were shifted from 18°C to 29°C for 1 or 2 weeks upon eclosion and prior to dissection.

Immunohistochemistry

Most antibody stainings were performed as previously reported [11] with minor modifications. Ovaries were fixed with 1xPBS + 0.1% Tween-20 + 4% Formaldehyde (FA), washed with 1xPBS + 0.1% Triton X-100 (PBS-T), permeabilized for 1 hour with 1xPBS + 1% Triton X-100 and blocked for 1 hour with 1xPBS + 0.1% Tween-20 + 10% BSA + 1% NP40. Anti-DE-cadherin staining was performed following [12]. In brief, ovaries were dissected in Schneider’s insect medium (Biowest L0207-500), fixed in 1xPBS + 0.1% Tween-20 + 4% FA for 15 min, washed with PBS-T and incubated in 0.5% goat serum diluted with PBS-T for 1 hour.
Primary anti-DE-cadherin and its secondary antibodies were incubated at 4°C overnight, then washed with PBS-T. Primary antibodies were used at the following concentrations: mouse anti-Hts (1B1) (Developmental Studies Hybridoma Bank [DSHB], University of Iowa), 1:100; rabbit anti-Vasa (a gift from R. Lehmann), 1:3000; goat anti-GFP, FITC (Abcam, ab6662), 1:500; alpaca anti-GFP-Booster_Ato488 (ChromoTek, gba 488-100), 1:200; mouse anti–Lamin C (LC28.26-s) (DSHB), 1:30; rat anti-DE-cadherin (DCAD2) (DSHB), 1:50; guinea pig anti-Hh (raised against a His-tagged fragment of the Hh protein (aa. 82-257) produced in E. coli; polyclonal antibodies were affinity purified), 1:100; rabbit anti-phospho-Mad (Abcam, 52903), 1:1000; rabbit anti-cleaved Dcp-1 (Cell Signaling Technology, 9578), 1:100; guinea pig anti-Nuf [13], 1:500; mouse anti–Ptc (Apa-1) (DSHB), 1:100; mouse anti-Rab11 (BD Biosciences), 1:100. Secondary antibodies (FITC from Abcam and Cy2-, Cy3- and Cy5-conjugated from Jackson ImmunoResearch) were used at 1:100. DNA staining was performed using the DNA dye Hoechst (Sigma B2883 10mg/ml in H₂O) at 1:1000 in PBT.

**Imaging and Image Analysis**

Images were captured with Leica SPE or SP5 confocal microscopes controlled by the Leica LAS AF software and using the 63x oil immersion objective. In most experiments Z-sections were taken every 0.7 μm. Images were analysed utilising ImageJ and processed with Adobe Photoshop and Adobe Illustrator. To analyse GSC numbers, image series were acquired in the SPE confocal microscope at 400 or 600 Hz, zooming in the germarium. To analyse fluorescence intensity, z-series of control and experimental samples were acquired in the SP5 confocal microscope at 400 Hz with exactly the same settings. Average fluorescence intensity per pixel was quantified with the Image J “Region of Interest manager” analysis tool. In general, to quantify fluorescent intensity we created a MAX projection of the CpC cluster and drew approx. 15 small squares (of ~0.1-0.5 microns² each) per germarium that contained regions of bright signal. The average pixel intensity was calculated and plotted. Average background levels were measured in each germarium with at least 5 boxes in GSC nuclei or cytoplasm. To quantify Ptc levels, we obtained the MAX projection image from 5-6 z-sections and scored overall fluorescent signal of areas of similar size (approx. 1200 microns² each) in the anterior half of the germarium. To quantify DE-cadherin::GFP we measured the
fluorescent signal in both cap cell-cap cell and cap cell-GSC interfaces. To quantify pMad levels in GSCs, we obtained MAX projections for individual GSCs and scored fluorescence intensity in a minimum of 5 boxes of similar areas (~1-2 micron\(^2\) each) per projection. Average background levels were measured in each germarium with at least 5 boxes in the nucleus or cytoplasm of older cysts (which do not express pMad).

*Ex vivo* ovariole culture was performed following [14]. In short, ovarioles were isolated in Schneider medium supplemented with 10% Foetal Bovine Serum (Sigma, F3018), 0.6% (V/V) streptomycin/penicillin antibiotic mix (Invitrogen, 15140-122) and 0.20 mg ml\(^{-1}\) insulin (Sigma, 15500). Individual ovarioles were transferred to a 35 mm Poly-d-lysine Coated plate (Mattek, P35GC-1.5-10-C) containing supplemented Schneider medium.

**Fluorescence Recovery After Photobleaching (FRAP)**

Z-sections were taken every 0.3 μm. Pre-bleach z-stacks were collected with the 488 nm laser line at 20% power. The pre-bleach signal was considered 100% fluorescent intensity. One or several regions of interest per niche which corresponded to CpC-CpC or CpC-GSC surfaces were bleached with the 488 nm and 405 nm laser lines at 100% power for three 2.6 second-long scans (400 Hz). The post-bleach signal was arbitrarily assigned a 0% value. Three Z-stacks were then collected at 10-min. time intervals (400 Hz, 2 line average). Fluorescence intensity was quantified in single planes of the bleached regions in each time point.

**Isolation of niche cells by Fluorescence-Activated Cell Sorting (FACS)**

Preparation of single-cell suspensions were done optimizing a previous protocol [15]. In short, 120 ovary pairs were dissected in Schneider’s insect medium (Biowest L0207-500) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco 10500-064; S-FBS), washed twice with Cell Dissociation Solution Non-enzymatic 1x (Sigma C5914) and incubated with the digestion solution (670μl Trypsin 1X [SIGMA T4299] + 330μl Collagenase [SIGMA C9407] 10 mg/ml in PBS + 100μ DNAse I [Sigma AMPD1-1KT] + 100μl DNAse I buffer [Sigma AMPD1-1KT]) at 25°C for 15 min, rolling at 40 rpm in a dissecting well (NOTE: prior to treatment, ovaries were teased apart manually with the dissecting forceps). Ovaries were then mechanically disaggregated in a blue pipette tip and transferred to a FACS tube (BD
Falcon 352054). Trypsin and DNAse were inactivated with 1ml S-FBS and 100 μl Stop Solution, respectively. The supernatant was filtered through a 70μm nylon mesh and cells were collected by centrifugation at 420 x g for 5 minutes (4°C) and re-suspended in 1ml S-FBS. The resulting cell suspension was filtered again through a 70μm nylon mesh and propidium iodide was added at a final concentration of 1:500.

To isolate terminal filament and cap cells (but not escort cells), the suspended cells were separated according to cell size (forward and side scatter plots), negative propidium iodide label and GFP-signal intensity in a FACSARia cell sorter (BD) equipped with a 100 μm nozzle and at low pressure (20 psi). Cells were sorted directly into 0.5 ml lysis buffer and snap frozen in liquid nitrogen. Typically, we could sort ~1,000 TFCs and CpCs per experiment.

**Sample preparation, Microarray hybridization and data analysis**

RNA from three biological replicas per each of the three time-points (1-, 3- and 4-week old samples) was isolated using magnetic beads from ~400 cells. cDNA synthesis, library preparation and amplification (Pico Profiling) are described elsewhere [16]. After reverse-transcription, each cDNA sample was added to an amplification mix that was subdivided into five equivalent parts for PCR amplification (26 cycles). Amplified cDNA collections were purified with the PureLink PCR Purification Kit (ThermoFisher Scientific), resuspended in 40μl and their concentrations measured using a Nanodrop 1000 spectrophotometer. The nine cDNA collections were hybridized to GeneChip Drosophila Genome 2.0 Arrays from Affymetrix. Raw data (CEL files) generated with Affymetrix’s AGCC software were used as input for the DTT software. For the resulting datasets, RMA (Robust Microarray Average) normalization and linear modelling by limma (Linear Models for Microarray Analysis) was performed. The p-value for the False Discovery Rate (FDR) was ≤ 0.05.

**Quantification of mRNA levels by qPCR**

RNA was isolated from ~20 ovaries with RNAeasy Micro (Qiagen 74004) and QIAshredder (Qiagen 79654) columns. To synthesize cDNA, 10 μl of mRNA were incubated for 5 min at 65°C with 1 μl (0.5 g) of Oligo(dT)$_{23}$ Anchored, (Sigma O4387) + 1 μl of 10mM dNTP mix and then on ice for 1 min. 4 μl of 5x First Strand buffer (Y02321) + 1 μl RNaseOUT Recombinant
Ribonuclease Inhibitor (40 units/μl) (100000840) + 2 μl 0.1 M DTT (Y00147) from Invitrogen were added and incubated for 1 min at 42°C. Next, 1 μl of Super Script II RT from Invitrogen (18064-014) was added and incubated for 50 min at 42°C, 15 min at 70°C and then on ice for 1 min. Finally, mix was incubated for 20 min at 37°C with 1 μl of RNase H from Invitrogen (18021-014). Primers used were (5’-3’): RpL32, F: ATGACCATCGCCGACTGG, R: GCTTAGCATATCGATCCGACTGG; β-Tub, F: GCAGTTCACCCTATATGTC, R: CCGACACCAGATCGTTCA; mam long isoforms, F: ACAATCGACCACACAACGC, R: TCGCCACTCAAATGCAGATGC; mam short isoform, F: AAATCTTCAGCCAAGCCAGC, R: TGCCGGTATTGGAGCCATGG. The relative mRNA amounts of mam long and short isoforms were determined by real-time quantitative PCR (CFX Connect, Bio-Rad) using the comparative cycle threshold (Ct) method [17] and the EVAGreen supermix kit (Bio-Rad 1864033). Ribosomal protein L32 (RpL32) and β-Tubulin (β-Tub) were used for normalisation. qPCR reactions contained 5 μl of SYBR Premix Ex Taq (Tli RNaseH Plus) kit from Takara (RR420A), 1 μl of template cDNA, 2 μl of F+R primers and 2 μl distilled water. The PCR conditions were: 95°C for 10 min (1x), 95°C for 15 sec and 67°C for 45 sec (40x). Ct values obtained resulted from three biological replicas and three technical replicas.

**Quantification of mRNA levels by droplet digital PCR**

Pico profiled samples from sorted niche cells (terminal filament cells and cap cells; see above) were used to quantify mRNA levels of mam long and short isoforms by droplet-digital PCR (ddPCR). Primers used were (5’-3’): RpL32, F: ATGACCATCGCCGACTGG, R: GCTTAGCATATCGATCCGACTGG; β-Tub, F: GCAGTTCACCCTATATGTC, R: CCGACACCAGATCGTTCA; mam long isoforms, F: ACAATCGACCACACAACGC, R: TCGCCACTCAAATGCAGATGC; mam short isoform, F: CCGACCAAGTCTACCAACGC, R: TGTCAGGGCCATTGTCAACA. RpL32 was used for normalisation. Each reaction contained 10 μl of Master Mix ddPCR EVAGreen (Bio-Rad), 150 nM of each primer and 2.5 ng of cDNA template (except in the case of the housekeeping control, where we used 0.5 ng). Samples were prepared in duplicate with 10% additional volumen. Droplet generation, PCR amplification and droplet analysis were done using the QX200 AutoDG ddPCR system (Bio-Rad). PCR conditions were: 95°C for 5 min (1x), 95°C for 1 min and 65.2°C for 2 min (44x). For
all steps a ramp rate of 2°C/s was used. Data were analysed with the Quantasoft software 1.7.4.0917 (Bio-Rad).

**Statistical Analysis**

Samples were collected from at least 5 different adult females grown in similar conditions. Graphs represent the mean values and standard error of each dataset. Fluorescence arbitrary units (AU) were normalised to controls. P-values were obtained using a two-tailed Student’s t-test to determine values that were significantly different (*: P≤0.05, **: P≤0.005, ***: P≤0.0005). In all graphs, bar comparisons without asterisks indicate non-significant differences.

**Supplemental References**

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**SUPPLEMENTARY TABLE 1 (related to Figure 1 and Figure S1)**

| Linear Fold Change | Increase/Decrease | p value | FDR  | Gene Symbol |
|-------------------|------------------|---------|------|-------------|
| **3 weeks vs 1 week** |                  |         |      |             |
| 8.615             | Decrease         | 0.045   |      | rdo         |
| 5.947             | Decrease         | 0.027   |      | CG34259     |
| 5.067             | Decrease         | 0.033   |      | CG13405     |
| 4.717             | Decrease         | 0.007   |      | CG10013     |
| 3.796             | Decrease         | 0.033   |      | hiw         |
| 3.105             | Decrease         | 0.013   |      | ru          |
| 2.718             | Decrease         | 0.009   |      | FucTD       |
| 2.673             | Decrease         | 0.033   |      | CG5080      |
| 3.588             | **Increase**     | 0.033   |      | mam         |
| **4 weeks vs 1 week** |                  |         |      |             |
| 7.835             | Decrease         | 0.022   |      | rdo         |
| 7.157             | Decrease         | 0.035   |      | CG5707      |
| 6.787             | Decrease         | 0.024   |      | CG12744     |
| 6.539             | Decrease         | 0.048   |      | Phf7        |
| 5.670             | Decrease         | 0.035   |      | yin         |
| 5.558             | Decrease         | 0.022   |      | CG10195     |
| 5.347             | Decrease         | 0.039   |      | Fili        |
| 4.638             | Decrease         | 0.047   |      | Rp55b       |
| 4.541             | Decrease         | 0.025   |      | CG14434     |
| 4.140             | Decrease         | 0.022   |      | CG13405     |
| 3.995             | Decrease         | 0.012   |      | CG10013     |
| 3.874             | Decrease         | 0.026   |      | CG34259     |
| 3.615             | Decrease         | 0.031   |      | CG30001     |
| 3.506             | Decrease         | 0.022   |      | Acer        |
| 3.375             | Decrease         | 0.022   |      | CG34200     |
| 3.297             | Decrease         | 0.012   |      | CG5080      |
| 2.884             | Decrease         | 0.035   |      | CG11858     |
| 2.851             | Decrease         | 0.012   |      | CG32803     |
| 2.679             | Decrease         | 0.020   |      | Cap-G       |
| 2.589             | Decrease         | 0.015   |      | ru          |
| 2.567             | Decrease         | 0.035   |      | Smu1        |
| 2.470             | Decrease         | 0.029   |      | CG9203      |
| 2.377             | Decrease         | 0.035   |      | CG6629      |
| 2.360             | Decrease         | 0.038   |      | RhoGEF4     |
| 2.326             | Decrease         | 0.047   |      | ko          |
| 2.279             | Decrease         | 0.012   |      | FucTD       |
| 2.202             | Decrease         | 0.040   |      | CG10903     |
| 2.120             | Decrease         | 0.035   |      | l(2)k14505  |
| 2.088             | Decrease         | 0.022   |      | nxf4        |
| Linear Fold Change | Change in Expression | Genes |
|--------------------|----------------------|-------|
| 2.051              | Decrease             | 0.035 | TppII |
| 2.049              | Decrease             | 0.047 | CG1136 |
| 2.034              | Decrease             | 0.035 | mRpL41 |
| 5.227              | Increase             | 0.020 | CG11893 |
| 4.541              | Increase             | 0.048 | CG8620 |
| 3.700              | Increase             | 0.019 | CG43111 |
| 3.545              | Increase             | 0.012 | Hsp22 |
| 3.474              | Increase             | 0.012 | GV1 |
| 3.323              | Increase             | 0.020 | l(2)41Ab |
| 3.254              | Increase             | 0.048 | TpnC25D |
| 3.206              | Increase             | 0.012 | cenG1A |
| 3.070              | Increase             | 0.018 | dm |
| 2.996              | Increase             | 0.016 | CG30421 |
| 2.958              | Increase             | 0.020 | CG30031 // CG4269 |
| 2.957              | Increase             | 0.026 | Pvf2 |
| 2.903              | Increase             | 0.014 | CG5953 / gag-int-pol |
| 2.873              | Increase             | 0.026 | Arc2 |
| 2.765              | Increase             | 0.020 | CG18547 |
| 2.718              | Increase             | 0.024 | CG34301 |
| 2.677              | Increase             | 0.035 | Rcd2 |
| 2.520              | Increase             | 0.035 | clumsy |
| 2.473              | Increase             | 0.047 | mam |
| 2.471              | Increase             | 0.047 | Gadd45 |
| 2.467              | Increase             | 0.022 | CG31710 |
| 2.442              | Increase             | 0.020 | CG40005 // cta |
| 2.435              | Increase             | 0.022 | Spn42Da |
| 2.434              | Increase             | 0.031 | Cpr92A |
| 2.406              | Increase             | 0.012 | MESK2 |
| 2.388              | Increase             | 0.039 | MRP |
| 2.386              | Increase             | 0.023 | CG15784 |
| 2.379              | Increase             | 0.022 | CG11447 |
| 2.044              | Increase             | 0.033 | Hsp70Bb // Hsp70Bc |
| 2.033              | Increase             | 0.035 | Hsp70Ba // Hsp70Bb // Hsp70Bbb // Hsp70Bc |

**Table S1.** List of genes whose expression is either up- or down-regulated during ageing in terminal filament and cap cells. Expression levels of 1-week old cells have been compared to cells isolated from 3- or 4-week old ovaries. *p* value for the FDR (False Discovery Rate) was set <0.05. Linear Fold Change was set >2.
Supplementary Figure Legends

Supplementary Figure S1 (related to Figure 1): Graphical representation of genes up- or down-regulated during ageing in niche support cells. Numerical values of identified differences in expression and the p values of False Discovery Rates can be found in Table S1.

Supplementary Figure S2 (related to Figure 2): Effect of mam mRNA reduction on GSC numbers and Hedgehog and DE-cadherin levels. Overexpression of mam short isoform in somatic cells of the ovary does not affect GSC numbers. Overexpression of mam long isoform in somatic cells of the ovary does not affect mam short isoform levels. Additional controls to discard the effect of different chromosomal backgrounds on GSC numbers. (A) A slight reduction in mam mRNA levels does not affect significantly GSC numbers whereas a stronger reduction decreases them. (B) Quantification of Hh and DE-cad levels in bab1>mam RNAi grown at 22°C and kept for 4 weeks at 22°C. (C) Graph showing that females carrying either the TM6B balancer chromosome or the recombinant chromosome with the bab1-Gal4, tub-Gal80ts constructs contain similar GSC numbers. (D) Graph showing that bab1>mam<sup>Short</sup> does not induce GSC loss. (E) Real time quantitative PCR data showing that overexpression of mam long isoform in somatic cells of the ovary (using the traffic-jam Gal4 driver) induces high levels of mam<sup>Long</sup> specifically, without affecting mam<sup>Short</sup> isoform expression. We used two different housekeeping genes to normalise our measurements, β-tubulin and RpL32. (F) Graph showing that females carrying the tub-Gal80<sup>ts</sup> construct and the TM6B balancer chromosome do not show differences in GSC numbers with females carrying the tub-Gal80<sup>ts</sup> construct and the hh- or ptc-Gal4 lines. Numbers in bars represent germaria analysed (n). Error bars are shown for the different samples.

Supplementary Figure S3 (related to Figures 2 and 3): mam overexpression in niche cells does not induce GSC apoptosis. (A) Z-projection of germaria from young (1 week) and aged (4 weeks) flies stained with antibodies to Hts (red; to distinguish germline cells and cysts) and Dcp1 caspase (green; to label apoptotic cells). Dcp1 expression is indistinguishable in young (n=22) vs. aged germaria (n=20). (B) Z-projection of germaria from control and experimental flies overexpressing mam for 1 week stained with antibodies to Dcp1 caspase (to label
apoptotic cells) and with the DNA dye Hoechst (blue). Dcp1 expression is indistinguishable in control (n=6) vs. experimental germaria (n=10). Dashed lines outline Dcp1 positive cells or cell clusters, which never corresponded to GSCs. Scale bars 5 μm.

Supplementary Figure S4 (related to Figure 3): mam overexpression reduces DE-cadherin levels. (A) Z-projection of germaria from bab1>mam flies kept for two weeks at 29°C stained to visualise DNA and the endogenous DE-cadherin protein. (B) Quantification of DE-cadherin levels shows they decrease in experimental niches. At least 15 measurements per germarium were taken. (C) DE-cadherin overexpression for two weeks in niche cells does not increase GSC numbers and it cannot rescue the GSC loss produced by mam overexpression. Numbers in bars represent germaria analysed. Dashed lines outline CpC clusters. Scale bar 5 μm.

Supplementary Figure S5 (related to Figure 4): Effect of mam overexpression on DE-cadherin::GFP recycling and of ageing and mam over-expression on Nuf levels in CpCs. (A) Images showing DE-cadherin::GFP FRAP in 1-week old CpCs from control and bab1>mam niches. (B) Both conditions show similarly low fluorescence recoveries. (C) Z-projection of germaria from 1-week and 6-week old flies stained to visualise TFC and CpC nuclear membranes (Lamin-C; green) and the Nuf protein (red). (D) Nuf levels decrease with age in niche cells. (E) Z-projection of germaria from bab1>mam flies kept for two weeks at 29°C stained to visualise TFC and CpC nuclear membranes (Lamin-C; green) and the Nuf protein (red). (F) Nuf levels do not change in experimental CpCs. Numbers in bars represent germaria analysed. Data plotted in (D, F) were obtained from at least 15 measurements/germarium. Purple dashed boxes in (A) demarcate the photobleached regions; dashed lines in (C, E) outline CpC clusters. n= number of germaria analysed. Pre-bleach levels are considered 100% intensity and the post-bleach signal is arbitrarily considered 0%. Later time points are referenced to the post-bleach values.

Supplementary Figure S6 (related to Figure 5): dpp signalling in aged and mam-overexpressing niches. (A) Z-projections of germaria from 1-week and 6-week old flies stained to visualise pMad (red) and spectrosomes (Hts; green). (B) Quantification of pMad
levels in (A). (C, D) Similar experiments to (A, B) but comparing control and experimental flies overexpressing *mam* for 2 weeks. (E) Graph depicting the quantification of GSC numbers in control and experimental conditions in which *mam* and/or *hh::GFP* are overexpressed. *hh* overexpression does not induce extra GSCs. 2-week *hh* overexpression in niche cells cannot rescue the GSC loss produced by high *mam* levels. Dashed lines outline GSCs. Numbers in bars represent germlaria analysed. Scale bars 5 μm.

**Supplementary Figure S7 (related to Figure 6):** *SOD1* overexpression does not affect GSC numbers. (A) Z-projections of germlaria from control and experimental flies overexpressing *SOD1+mam* for 2 weeks stained to visualise Hh (red), DE-cad (green) and DNA (blue). (B) Quantification of Hh and DE-cad levels in (A). (C) Quantification of GSC numbers in control and *SOD1*-overexpressing niches. Numbers in bars represent germlaria analysed. Dashed lines outline CpC clusters. Scale bars 5 μm.
Fig. S1

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Fig. S2

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Fig. S3

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**Fig. S4**

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