

## **SUPPLEMENTARY METHODS**

### **5'-RACE and RT-PCR experiments**

5' RACE experiments were performed with GeneRacer™ Kit (Invitrogen) on OSS cell or ovary RNA extracts according to the manufacturer's instructions. A first PCR amplification followed by a nested PCR was carried out with the Universal and nested primers included in the kit and the following reverse oligonucleotides specific for the *Drosophila flam* sequence. Gel-isolated products were sequenced directly, or cloned first into the PCR- vector. Primers are listed in supplementary Table S5 online.

### **Plasmid construction**

Different parts of the *flam* promoter were inserted into pGL3-Enhancer Vector, upstream of the Firefly Luciferase. Site-specific deletions were obtained by oligonucleotide-directed mutagenesis. NC construct was obtained by adding 159 bp of GFP coding sequence upstream SFII promoter. The original promoter of control plasmid, pRL-null Vector, was changed to the Actin promoter.

### **Cell culture and transfection**

OSS cells were cultured as previously described [25] and transfected using Xfect Transfection Reagent (Clontech 631317). Assays were performed 48 hrs after transfection using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was detected by TD 20/20 luminometer (Turner Designs). The firefly Luciferase signals were normalized to the Actin Renilla Luciferase transfection control.

siRNAs are transfected using Xfect siRNA transfection reagent (Clontech 631331). Total RNAs are extracted 48 or 96 hours after transfection.

OSS cells were treated either with 15ug/ml of alpha amanitin (A2263, Sigma) for 4, 10 or 20 hours or with 0,001ug/ml of Actinomycin D (A1410, Sigma) or with 20ug/ml of ML-60218(CAS number 577784-91-9, Tebu-bio) during 4 hours.

### **Immunostaining**

Immunofluorescent staining was performed as described in [27] using the following primary antibodies: rat anti-Ci (1/100; 2A1, Hybridoma) and rabbit anti-Myc (1:100, Santa Cruz). Images were acquired on a Leica SP5 microscope.

### **Western Blot**

Proteins extracted from OSS cells were resolved by electrophoresis on a 4-12% Bis-Tris Gel (NuPAGE® Novex®) and then detected by western blotting using anti-Ci antibodies (1/500).

### **Chromatin immunoprecipitation assays**

ChIP from 80 ovaries was performed as described in[28]. Antibodies against Ci (2A1, Hybridoma) and RNA PolII Phospho-S5 (ab5131, Abcam), were added and samples were incubated overnight at 4°C in a rotating wheel. qPCR was performed using SYBR Green I Master on LightCycler® 480 on at least two biological-independent extractions and three technical replicates. Enrichment was calculated relative to Input and Rp49. Primers are listed in supplementary Table S5 online.

### **Genomatix analyses**

Potential transcription factor binding elements in the promoter region were searched for using the Genomatix MatInspector Program ([www.genomatix.de](http://www.genomatix.de)). Alignment of *flam* promoter

sequences from the *Drosophila melanogaster* subgroup species was performed using the Genomatix DiAlign Program ([www.genomatix.de](http://www.genomatix.de)).