Aubergine and piRNAs repress the proto-oncogene Cbl for germline stem cell self-renewal
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### Appendix Figure S1: Aub candidate target mRNAs

(A) List of candidate target mRNAs of Aub in GSCs. Increase of protein levels in *aub<sup>hn2</sup>* mutant versus control (*aub<sup>hn2/+</sup>*) GSCs was determined using the clonal analysis, as shown in (B-C’’). +: increased protein levels; -: no increase. Ref. 1 (Wang & Lin, 2004); Ref. 2 (Gilboa & Lehmann, 2004); Ref. 3 (Harris et al, 2011); Ref.4 (Neumuller et al, 2008); Ref. 5 (Xia et al, 2010); Ref. 6 (Hanyu-Nakamura et al, 2008); Ref. 7 (Xin et al, 2013); Ref. 8 (Davies et al, 2013); Ref. 9 (Sanada et al, 2009).

(B-C’’') Immunostaining of *aub<sup>hn2</sup>* mosaic germaria 7 days after clone induction, with anti-GFP (green) and anti-Fused (Fu, B, B’’) or anti-Nanos (Nos, C, C’’) (red). DAPI (blue) was used to visualize DNA. White arrowheads indicate control (*aub<sup>hn2/+</sup>*) GSCs; yellow arrowheads indicate *aub<sup>hn2</sup>* mutant GSCs.
Appendix Figure S2: GFP-Aub iCLIP in GSCs in nanos, mei-P26 and fused mRNAs

(A-C) Significant GFP-Aub crosslinks in cultured GSCs (Ma et al, 2017), in nanos (A), mei-P26 (B) and fused (C) mRNAs. Thin boxes are 5'- and 3'UTRs, lines are introns, and thick boxes are exons. Significant crosslink clusters are indicated in red.
Appendix Figure S3: Cbl expression pattern in the germarium

(A-A'') Immunostaining of germaria expressing GFP-Par1 to label spectrosomes with anti-GFP (green) and anti-Cbl 10F1 (red). DAPI (blue) was used to visualize DNA. White arrowheads indicate GSCs; yellow arrowheads indicate cystoblasts.

(B) Quantification of Cbl protein levels in GSCs and cystoblasts using fluorescence intensity of immunostaining with 10F1 antibody. Fluorescence intensity was measured in arbitrary units using ImageJ. The number of scored cells (n) is indicated. Intensity in GSCs was set to 1. The error bar indicates standard deviation. ***p-value <0.001 using the two-tailed Student’s t test.

(C, D) Immunostaining with anti-Vasa (green) and anti-Hts (red) of wild-type germaria (C) and of germaria overexpressing Cbl with Hsp83-CblS (D). DNA (blue) was revealed with DAPI. The white arrow indicates the lower number of germ cells. Scale bar: 10 μm in A, C, D.
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