

Table S1. Genes that encode proteins with a high degree of sequence similarity (>50%) to EB-SUN (CG18190) are identified via Paralog Explorer.

Paralogs of EB-SUN (CG18190)					
	Microarray & RNA-Seq From FlyAtlas2	Alignment Length	Similarity	Common GO Slim	Tissue Expression Correlation
EB1		284	51%	GO:0043234 (proteincomplex); GO:0005622 (intracellular); GO:0005856 (cytoskeleton); GO:0043226 (organelle); GO:0008092 (cytoskeletal protein binding); GO:0005623(cell)	0.25037
CG32371	Very High expression in Testes	180	59%	GO:0008092 (cytoskeletal protein binding)	0.00695
CG2955	Very High expression in Testes	137	56%	GO:0008092 (cytoskeletal protein binding)	0.05561

Table S2. Probes specific to EB-SUN CDS for smi-FISH.

	EB-SUN Probes (5' to 3')	Seq with Flap-X (5' CCTCCTAAGTTTCGAGCTGGACTCAGTG 3')
1	catattctccgcattaacgc	catattctccgcattaacgcCCTCCTAAGTTTCGAGCTGGACTCAGTG
2	atgttgttcacccactgaag	atgttgttcacccactgaagCCTCCTAAGTTTCGAGCTGGACTCAGTG
3	agatcatctccatcatctgg	agatcatctccatcatctggCCTCCTAAGTTTCGAGCTGGACTCAGTG
4	tagtttggcgggtcatcttga	tagtttggcgggtcatcttgaCCTCCTAAGTTTCGAGCTGGACTCAGTG
5	ttgaaggcttcctggaagag	ttgaaggcttcctggaagagCCTCCTAAGTTTCGAGCTGGACTCAGTG
6	cgttttgtccagcttcaagc	cgttttgtccagcttcaagcCCTCCTAAGTTTCGAGCTGGACTCAGTG
7	aaagttgtcctggaagcgac	aaagttgtcctggaagcgacCCTCCTAAGTTTCGAGCTGGACTCAGTG
8	ggagcctgcgagtcgaagaa	ggagcctgcgagtcgaagaaCCTCCTAAGTTTCGAGCTGGACTCAGTG
9	cgacttaatgttctccaagc	cgacttaatgttctccaagcCCTCCTAAGTTTCGAGCTGGACTCAGTG
10	ctttatgggctttgccaacg	ctttatgggctttgccaacgCCTCCTAAGTTTCGAGCTGGACTCAGTG
11	gatcgctccttggcgaattg	gatcgctccttggcgaattgCCTCCTAAGTTTCGAGCTGGACTCAGTG
12	catcaatcagctctttgagc	catcaatcagctctttgagcCCTCCTAAGTTTCGAGCTGGACTCAGTG
13	gttgtagatctgattgctgg	gttgtagatctgattgctggCCTCCTAAGTTTCGAGCTGGACTCAGTG
14	ttgaccaaatacctcgaccag	ttgaccaaatacctcgaccagCCTCCTAAGTTTCGAGCTGGACTCAGTG
15	gtttttagagcaccgcttg	gtttttagagcaccgcttgCCTCCTAAGTTTCGAGCTGGACTCAGTG
16	ataatccgtcattgggatcc	ataatccgtcattgggatccCCTCCTAAGTTTCGAGCTGGACTCAGTG

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hsMAPRE3 ---MAVNVYSTSVTSENNLSRHDMLAWVNDLHLNYTKIEQLCSGAAYCQFMDMLFPGCVH 57
CG18190 MTDLKLTVALTSVNAENMSRHDMLQWVNNMVHGFKKIEELCSGAAYCQMEMIFPNCIN 60
      : . * *** . : * : * * * * * * * * * * * * * * * * * * * * * * * * * * * *
hsMAPRE3 LRKVKFQAKLEHEYIHNFKVLQAAFKKMGVDKIIPVEKLVKGFQDNFEFIQWFKKFFDA 117
CG18190 LKRVKMTAKLEHEYLHNLRLFOEAFNRLKLDKTVPIDRLIKGRFQDNFEFLQWFKKFFDS 120
      * : * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
hsMAPRE3 NYDGKDYNP LLARQQDVAPPNPVPQRTSPTGPKNMQTSGRLSNVAPPCILRKNPPSAR 177
CG18190 QAPGLENIKSLANAPLAKPIKPRQFAKERSPVNAV----- 155
      : * : * * . * . . . . * * . . :
hsMAPRE3 NGGHETDAQILELNQQLVDLKLTVDGLEKERDFYFSKLRDIELICQEHESEN--SPVISG 235
CG18190 -----DDALKELIDEMKNLSLKREDIMEASNQIYNKLRLEDLVNDMINNNQLVELCKR 209
      * : * * : : : * . . : : : : * * * * * * * * * * * * * * * * * * * *
hsMAPRE3 IIGILYATEEGFAPPEDEIEE-----HQQEDQDEY 266
CG18190 IQAVLYKTIDGEINEEPVEVNEDNGDEGAEHADPNDGLY 248
      * . : * * * : * * * * : * * * : : . * : .

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Figure S1. Protein alignment of EB-SUN with human microtubule-associated protein family member 3 (MAPRE3). Underlined text in bold indicates the CH domain (14-116 in MAPRE3 and 17-119 in EB-SUN), and white texts highlighted in purple represent the EB homology domain (194-264 in MAPRE3 and 151-223 in EB-SUN). Asterisk (*), residues that are identical in two sequences in the alignment: colon (:), conserved substitutions. period (.), semi-conserved substitutions (as defined in Clustal Omega).

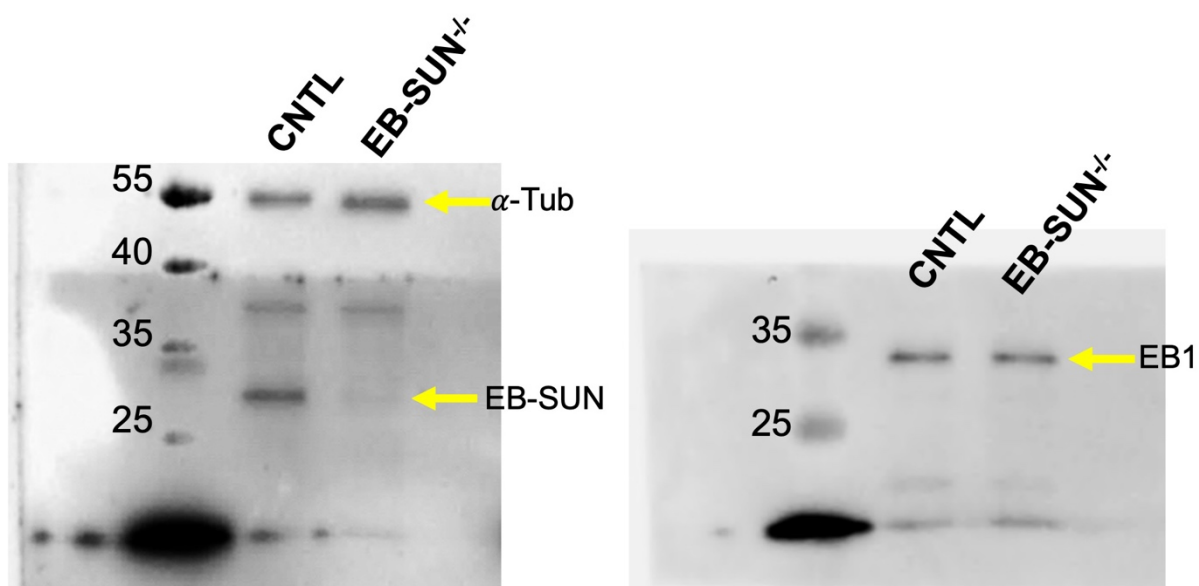


Figure S2. Immunoblot with ovary extracts of CNTL (W^{1118}) and homozygous EB-SUN KO. The same PVDF membrane was stripped then probed for EB1.

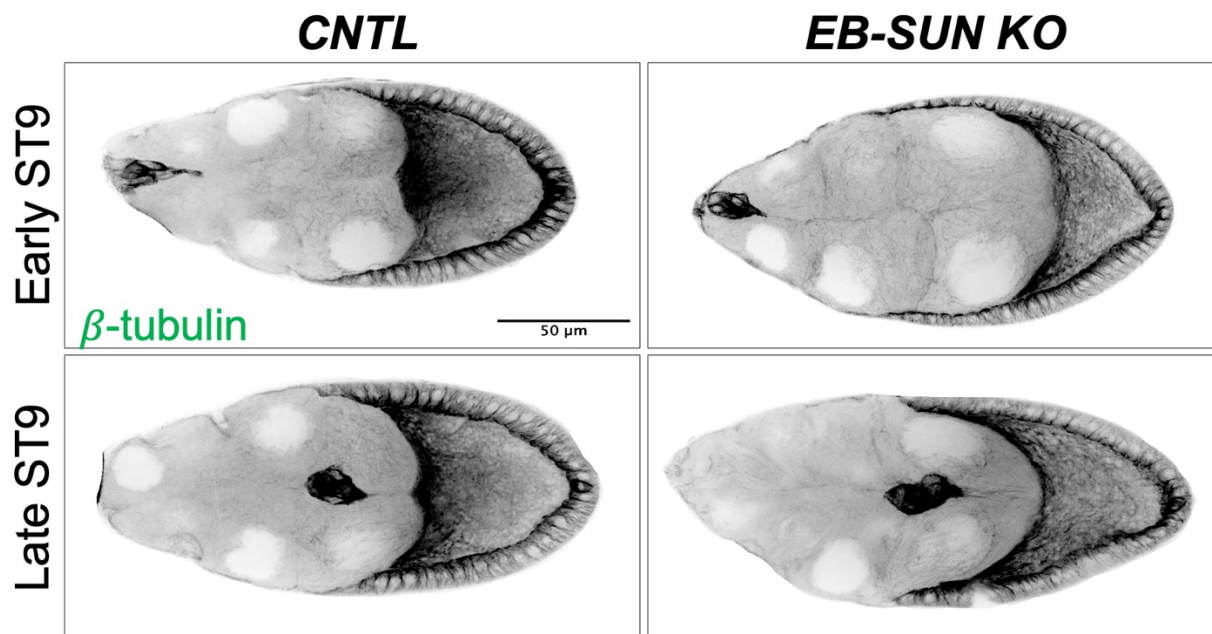


Figure S3. Microtubule staining of egg chambers at ST9. No significant difference in microtubule staining was observed between control and EB-SUN KO egg chambers.

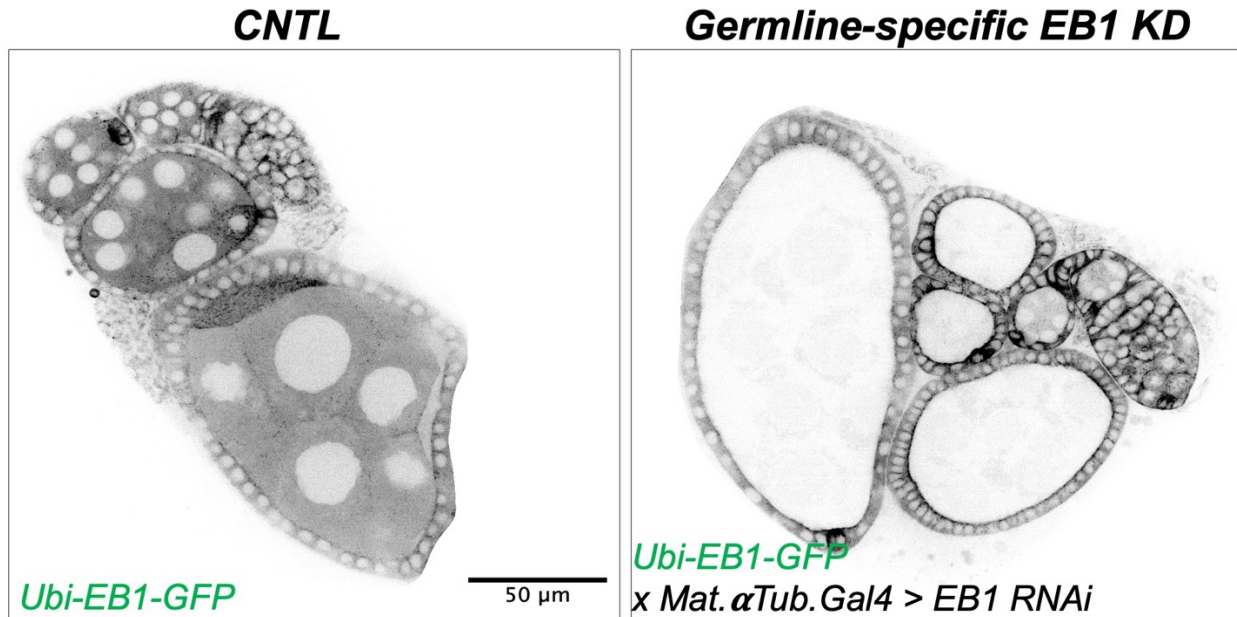


Figure S4. Germline-specific KD of EB1. To test EB1 RNAi efficiency, a fly line expressing GFP-tagged EB1 under a ubiquitin promoter (*ubi-EB1-GFP*) was crossed with an *EB1 RNAi* line driven by a germline-specific Gal4 (*Mat. α Tub-Gal4^{V37}*). As the germline-specific *Mat. α Tub-Gal4^{V37}* turns on between ST2 and ST3 after cell divisions, EB1 comets are completely abolished in germline cells (nurse cells and oocytes) within egg chambers past ST3, without affecting follicle cells. In control, *ubi-EB1-GFP* was crossed with *Mat. α Tub-Gal4^{V37}* alone. (See also Supplemental Movie S5)

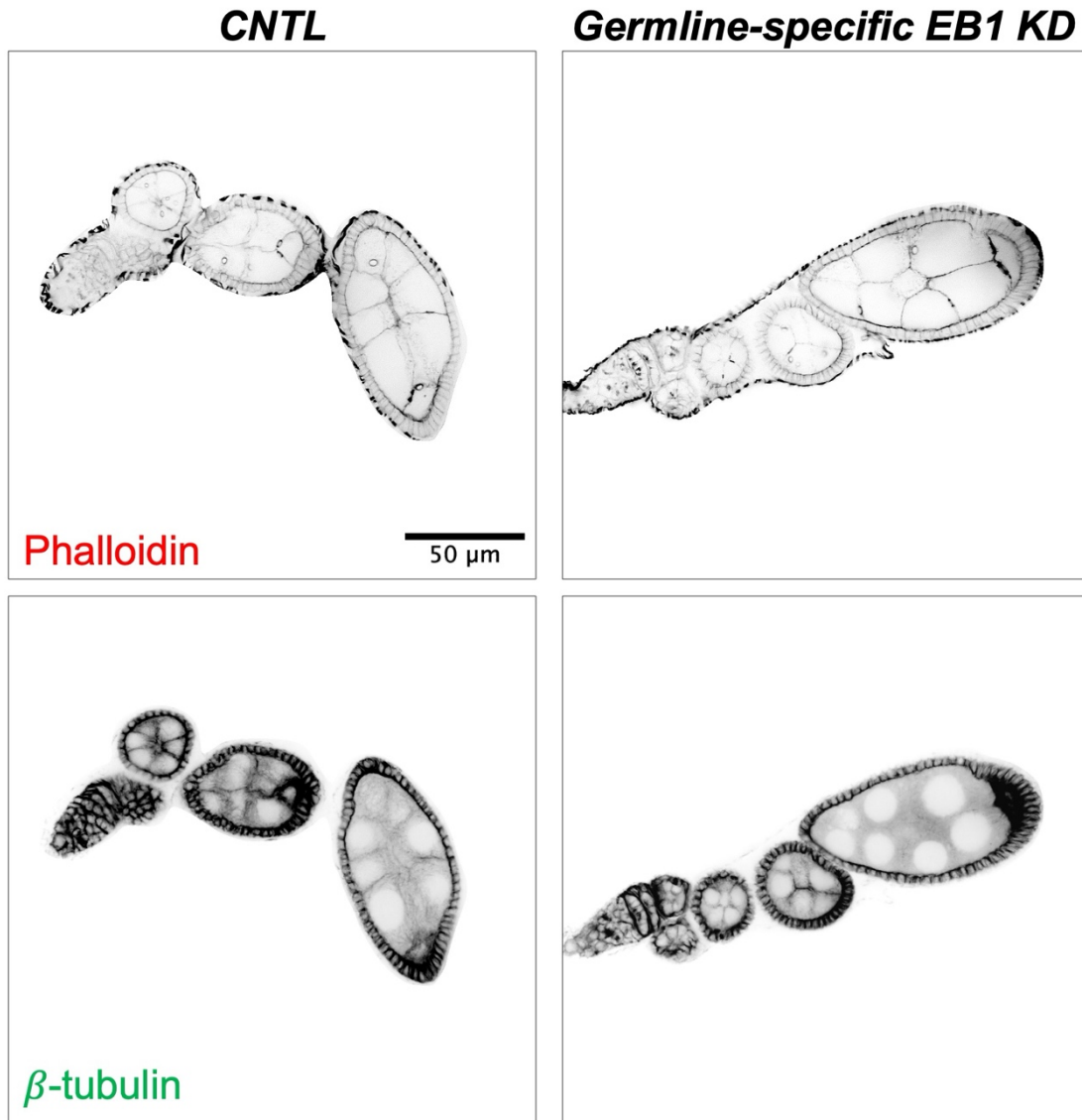


Figure S5. Germline-specific KD of EB1. EB1 was depleted by expressing EB1 RNAi by two Gal4 lines: *nos-Gal4^[vp16]* which activates RNAi in primordial germ cells and a postmitotic germline-specific Gal4 (*Mat. α Tub-Gal4^[V2H]*), which turns on between ST2 and ST3 to ensure continuous depletion of EB1 throughout oogenesis. Overall, EB1 depletion in early cell divisions and oogenesis appears to be largely unaffected. Scale Bar, 50 μ m