

Fig. S1. *Mab-zen* expression dynamics in the blastoderm of *Megaselia abdita*. *Mab-zen* in situ hybridizations of two embryos at consecutive blastoderm cellularization stages are shown in (A,B) lateral and (A',B') dorsal view with anterior to the left.

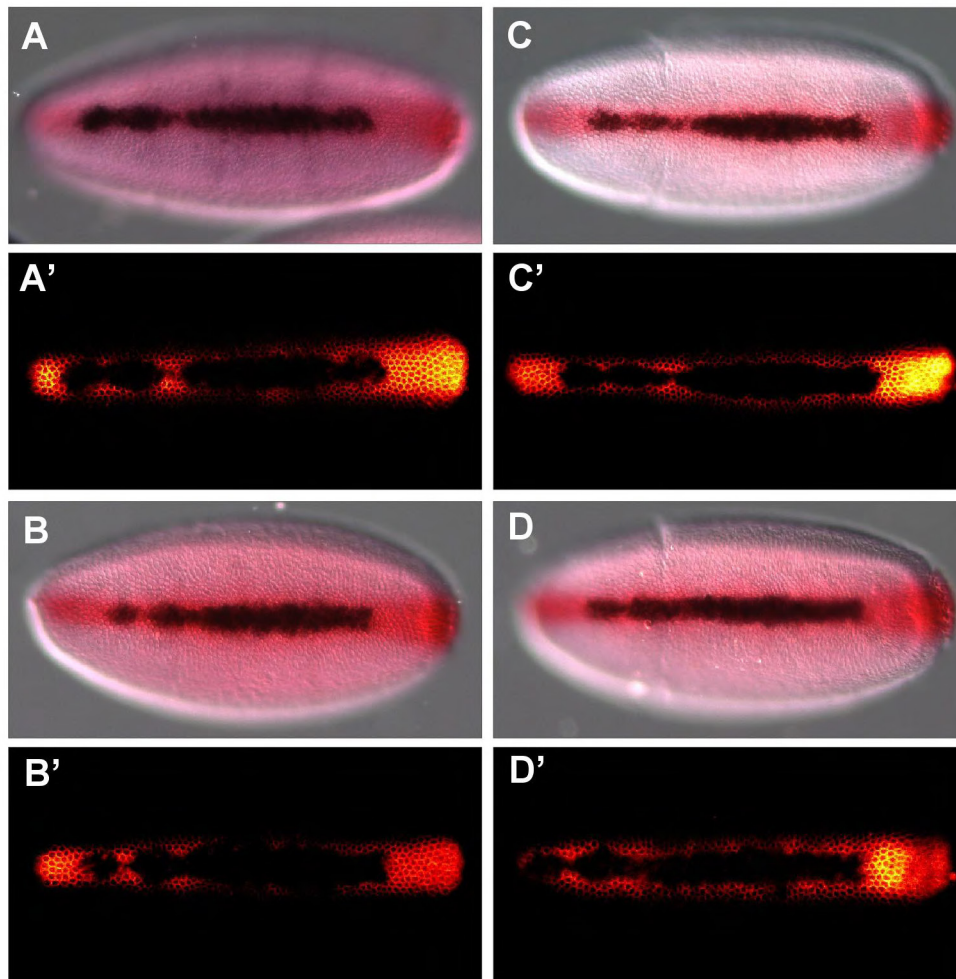


Fig. S2. pMad and *zen* transcript in *Drosophila melanogaster*. Double staining of pMad and *zen* transcript in *D. melanogaster* (A-B') at the cellular blastoderm stage and (C-D') during early gastrulation. All four embryos are shown in dorsal view with anterior to the left. *zen* transcript was detected using NBT/BCIP. pMad was detected using VectorRed and imaged in the Cy3 channel (A'-D'). NBT/BCIP quenches the pMad signal in the *zen* domain. Note that pMad is detected laterally adjacent to the *zen* domain, both shortly before and shortly after the onset of gastrulation.

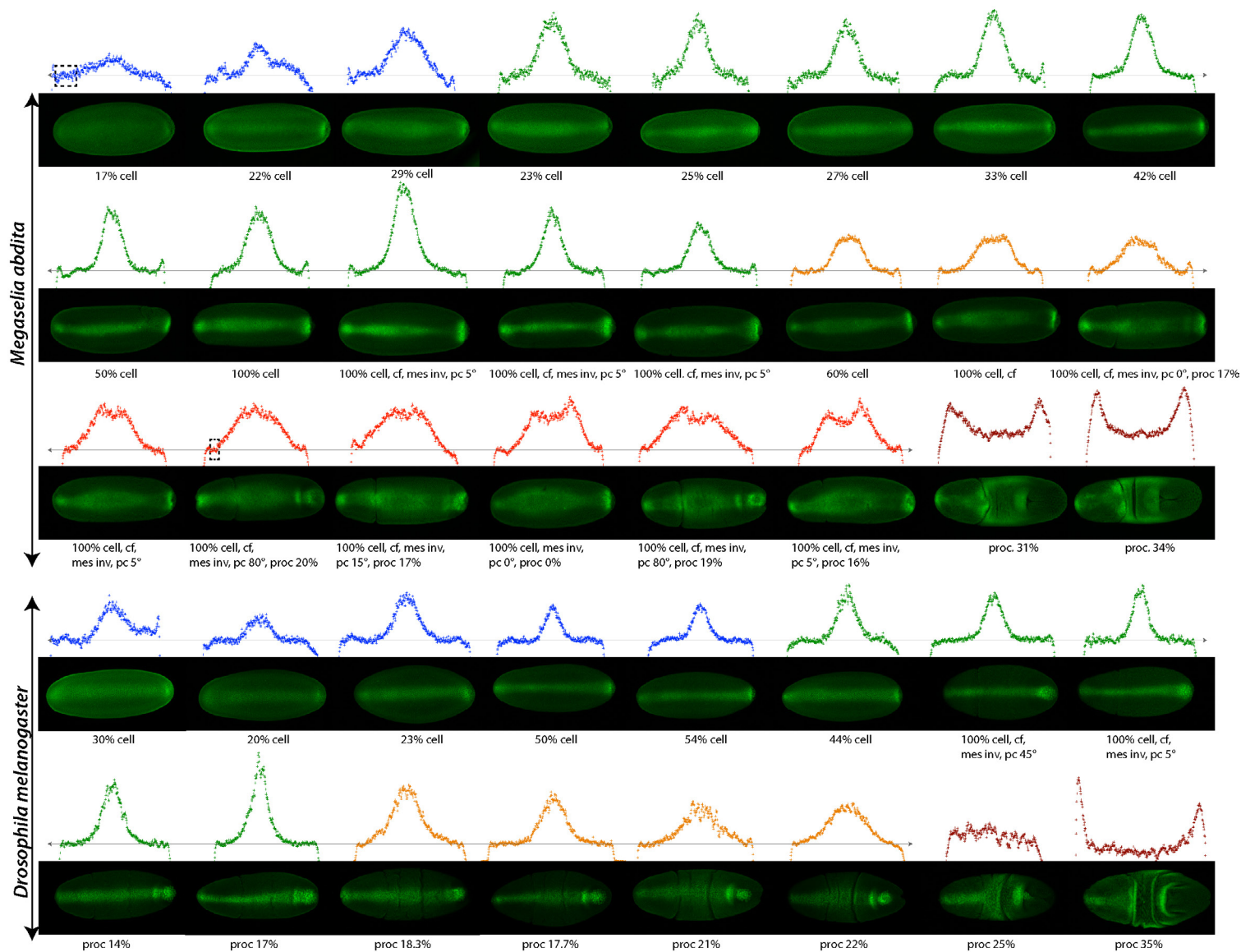


Fig. S3. pMad profiles of *M. abdita* and *D. melanogaster* embryos. Cross-sectional pMad profiles (at 50% egg length) of individual embryos are shown above dorsal projections of their confocal images. The embryos are shown in dorsal view with anterior to the left and grouped according to the shape of their pMad profiles as indicated by the color of the profile. Background intensity was calculated as the average intensity in the windows highlighted (dashed rectangles). All values in a profile were adjusted to these background values, which were treated as zero (gray line), except for profiles shown in dark brown (late gastrulation stages). Note the dorsocentral depression of pMad level in early gastrula stages of *M. abdita* (arrows), which is not conserved in *D. melanogaster*. Advanced gastrulation stages of both species exhibited an apparent reduction of the pMad level in the serosa of *M. abdita* or the amnioserosa of *D. melanogaster*, respectively. Abbreviations below images indicate morphological features of the respective embryos: cell, blastoderm cellularization; cf, cephalic furrow; mes inv, mesoderm invagination; pc, pole cell angle relative to length axis; proc, position of the proctodeum (see Materials and methods).

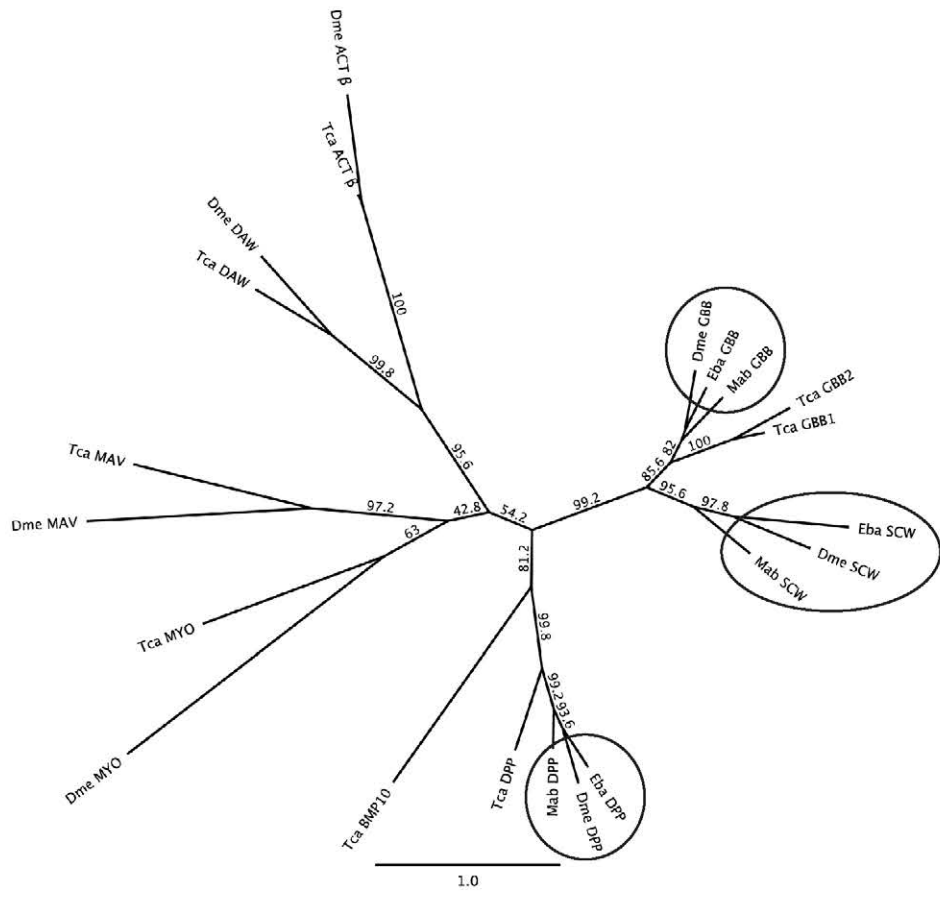


Figure S4A

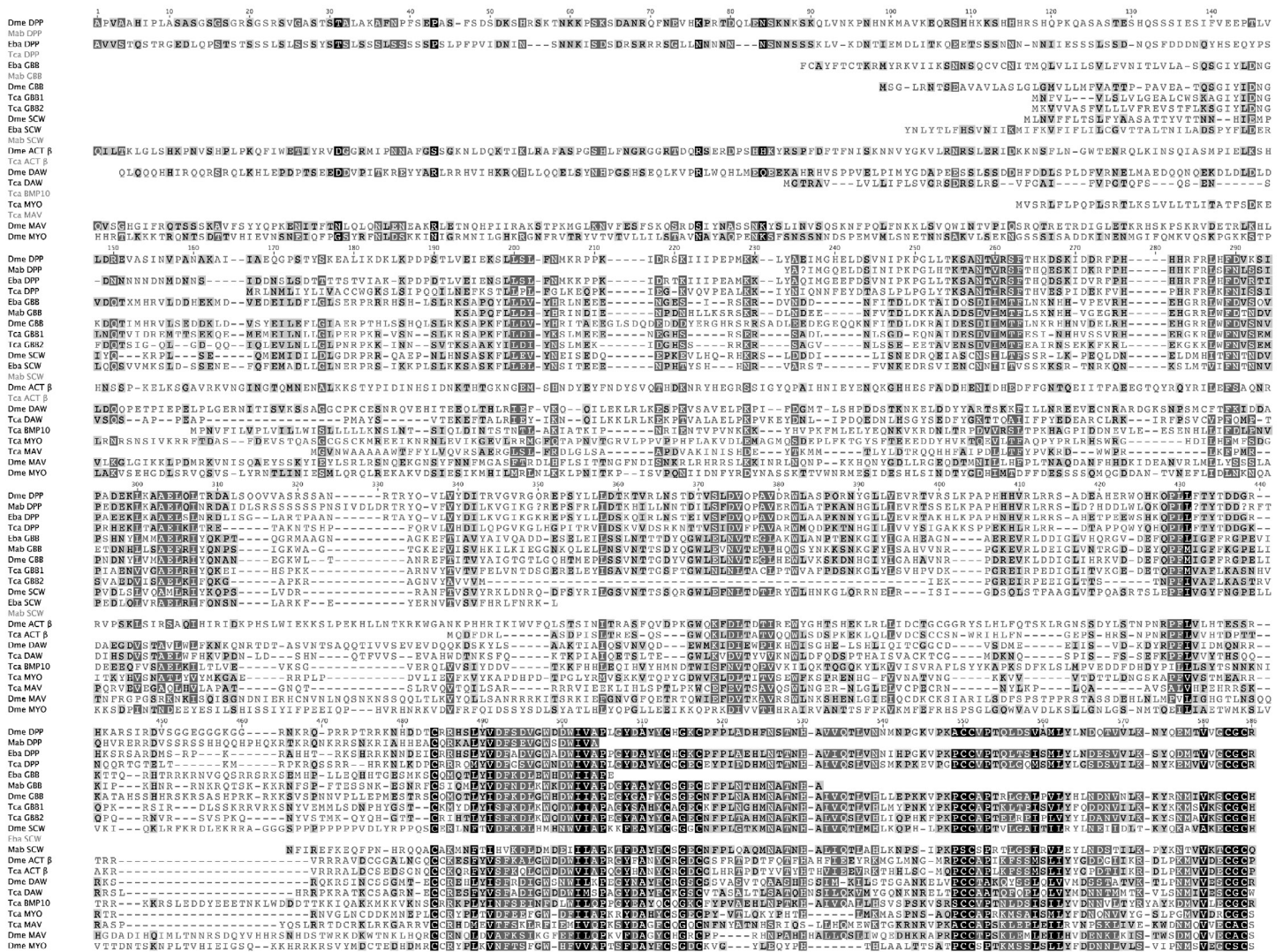


Figure S4B

Fig. S4. Phylogenetic analysis of BMP ligands. (A) Maximum likelihood tree of BMP ligand homologs of *Drosophila melanogaster* (*Dme*), *Episyrphus balteatus* (*Eba*), *Megaselia abdita* (*Mab*) and *Tribolium castaneum* (*Tca*). Homologs of Gbb, Scw and Dpp are circled. The gene tree was generated using the maximum likelihood method in PHYML with the Jones-Taylor-Thornton substitution model and 500 bootstrap iterations. The scale indicates one amino acid change per position. (B) ClustalW alignment of predicted protein sequences as used for tree building. Gap penalty was set to 3.0 and the extended gap penalty to 1.8. In the alignment, positions that were represented in less than 20% of the sequences and not conserved have been deleted manually. Highlighting indicates similarity to the consensus sequence using Blosum62 similarity matrix and threshold of 1; black, 100%; dark gray, 80% to <100%; light gray, 60% to <80%; unmarked, <60%.

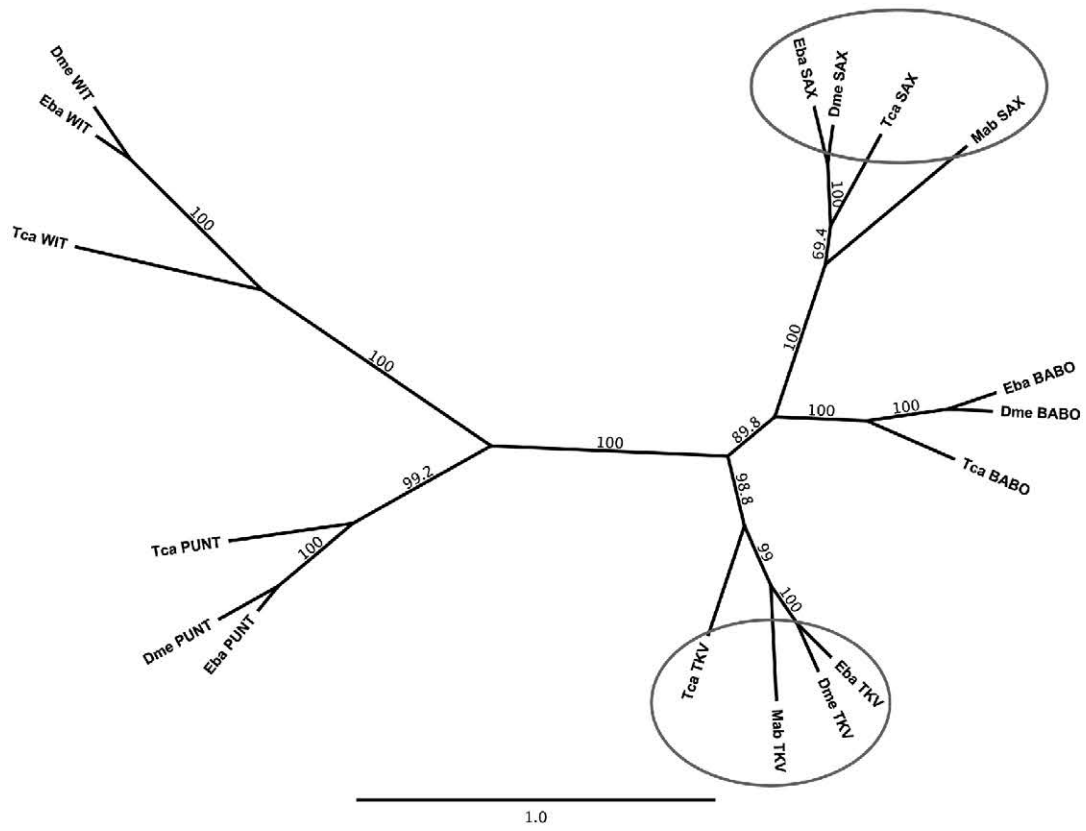


Figure S5A



Fig. S5. Phylogenetic tree of Sax and Tkv homologs. (A) Maximum likelihood tree of BMP ligand homologs of *Drosophila melanogaster* (Dme), *Episyrphus balteatus* (Eba), *Megaselia abdita* (Mab) and *Tribolium castaneum* (Tca). Homologs of Sax and Tkv are circled. The gene tree was generated using the maximum likelihood method in PHYML with the Jones-Taylor-Thornton substitution model and 500 bootstrap iterations. **(B)** ClustalW alignment of predicted protein sequences as used for tree building. Gap penalty was set to 3.0 and the extended gap penalty to 1.8. Highlighting indicates similarity to the consensus sequence using Blosum62 similarity matrix and threshold of 1; black, 100%; dark gray, 80% to <100%; light gray, 60% to <80%; unmarked, <60%.

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1         10        20        30        40        50        60        70
Dme SOG  L I V C L A G V T E G R R H A P L M F E E S T G R R S N R P A V T E C Q F G K V I R E L G S T W Y A D L G P P F G V M Y C I K C E C V A I P K K R R I
Eba SOG  M L V E D F E - A R R P S R Q G - A E C Q F G K T L H E L G S T W W A D L G P P F G V M Y C I K C E C V A M P K K R R I
Mab SOG
Aga SOG  V T E C Q F G K T I R E L H T T W F A D L G P P F G V M Y C I M C E C V P F Q K K R R V
Cri SOG
Tca SOG  L T W G L F C V L V T L A T A R N K A P L I D D G T R T - R N K A A E C V F G K Q V R E L G S Q W I P D L G V P I G V L Y C M K C E C V P I Q K K R R I

80        90        100       110       120       130       140       150
Dme SOG  V A R V O C R N I K N E C P P A K C D D P I S L P G K C C K T C P G D R N D T D V A L D V - E V P - N E E E E R - - N M K H Y A A L L T G R T S Y F L K
Eba SOG  V A R V O C R N I K N E C P P A N C D D P I L L P G K C C K T C P G E S I D P E I A L D V - E M P V N N E E E R R R N M K H F A A L L T G R T S Y F L K
Mab SOG  Q C R N M K N E C P P A N C D D P I L L P G K C C K T C P G E N N T - E M I Q D Q D P T P F N I E E E E R N - M K H F A A L L T G K T S H F L R
Aga SOG  V G R V O C R N I K N E C P K P T C D D P I L L P G R C C K T C P G D A Q S P D V V Q D L - E Q A - T M F E E E E R - S K H F A A L L T G R T S L L K
Cri SOG  O C R N I K N E C P K P T C D E P I L L P G R C C K T C P G D S S S P D I L O D T - E V A - T I N E P E D - - M K H F G A L L T G R T S T M L K
Tca SOG  V A R V O C R S I K N E C P E P T C D E P V H R P G R C C K T C P G D L Y D S D I I O D I V E O N - V L D E E E K S T K H Y A A L L T E R S S L V L R

160       170       180       190       200       210       220
Dme SOG  G E E M K S M Y T T Y N E O N V V A T A R E I F E K K N L Y S F Y T S - - S R I G R E R A I O F V D D A G V I L E E H Q L E T T L A - G T L S V Y O N
Eba SOG  G E E M K A M Y S T H N E O N V V A T G R E I F E K K - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Mab SOG  R E D T - T V S S M H N E N N L I A T G R E N F Q K K N L Y S F Y V S A N K Q M G R P T T T O F V N D V G T I L E E H N L T T S T N - G P L S V Y E K
Aga SOG  K D D M G T M Y S T L N E Q N V V A T G R E S F H K K N L Y S F Y V S - - E R A A R E R S I O F V H A N G N I L E E H T L A L P S D E G R I S A Y O N
Cri SOG  R D E M L N N Y S T K N P O N L I A T G R L S F H K K N L Y S F Y V S - - E ? A S R P S A I O F I D N S G N I L E E H S L V I P N N - G P S S N Y O N
Tca SOG  - N D F R L M S N N L N K N N V V A T G R E T F H K K N L Y S F Y I S - - D K A A R P R S L O F V D S E C N I L E E F V L S R A G G - L V N S L Y O N

230       240       250       260       270       280       290       297
Dme SOG  A T G K I C G V W R R V P R D Y K R I L R D D R L H V V L L W G N - - - - K Q Q A E L A L A G K V A K Y T A L Q T E L F S S L L E - - - - -
Eba SOG
Mab SOG  S T G K I C G V W R R I P K D Y K R L L R D G R P H V V L L W R S T L L D T K Q V E L A L G G K T D K Y W A L S S E L F S S L L E
Aga SOG  G T G K V C G V W R R V P R E Y R L L R D E L V S V V L L W G S - - - - K H Q A E L A I A G P T A K Y P A L A H E L F S S L L E
Cri SOG  A T G K I C G V W R R V P R D Y R L L R E D O M N A V L I W N G - - - - K F G S D I A L A G P I S K Y P A L S T E L F S S L L E
Tca SOG  A T R K V C G V W K R T P H D Y R K R F R M E K I Y V A L V W G V - - - - K D Q A E F T L S G Q V M K Y A A L A T E N F S S L L E

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Fig. S6. Amino acid sequence alignment of Sog homologs. Truncated ClustalW alignment of predicted protein sequences of *Drosophila melanogaster* (*Dme*), *Episyrphus balteatus* (*Eba*), *Megaselia abdita* (*Mab*), *Chironomus riparius* (*Cri*) and *Tribolium castaneum* (*Tca*). Highlighting indicates similarity to the consensus sequence using Blosum62 similarity matrix and threshold of 1; black, 100%; dark gray, 80% to <100%; light gray, 60% to <80%; unmarked, <60%.

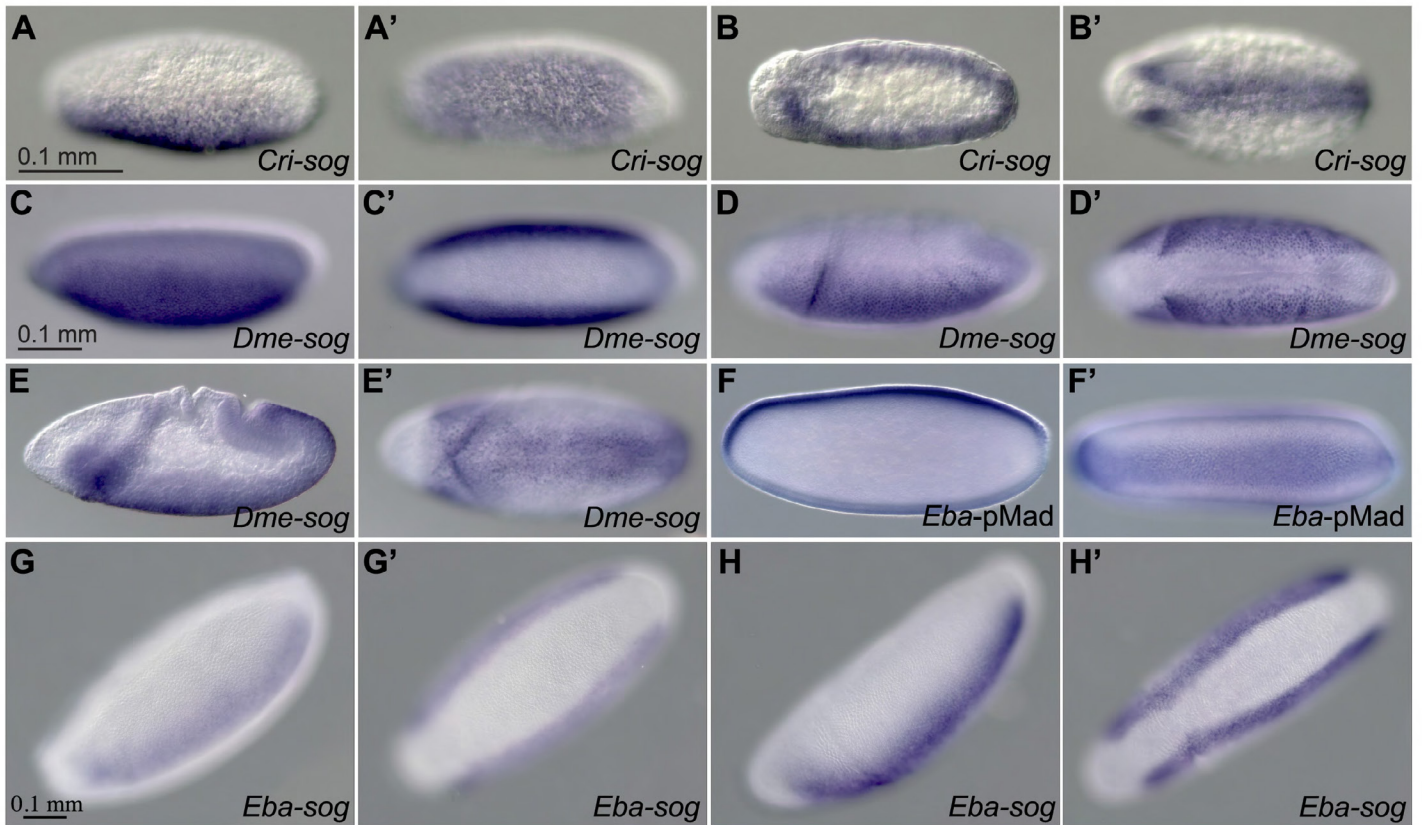


Fig. S7. Expression of *sog* in *Chironomus riparius* and *Drosophila melanogaster*, and pMad distribution and *sog* expression in *Episyrphus balteatus* (Syrphidae). (A-B') *sog* in situ hybridizations at the blastoderm stage (A,A') and the extended germband stage (B,B') in *C. riparius*. (C-E') *sog* in situ hybridizations at the blastoderm stage (C,C') and consecutive gastrulation stages (D-E') in *D. melanogaster*. (F,F') pMad at the blastoderm stage in *E. balteatus*. (G-H') *sog* in situ hybridizations at the blastoderm stage (G,G') and at the beginning of gastrulation (H,H') in *E. balteatus*. The embryos are shown in lateral view with the dorsal side up (A-H), or ventral view (A'-E',G',H') or dorsal view (F'). Anterior is left.