

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.

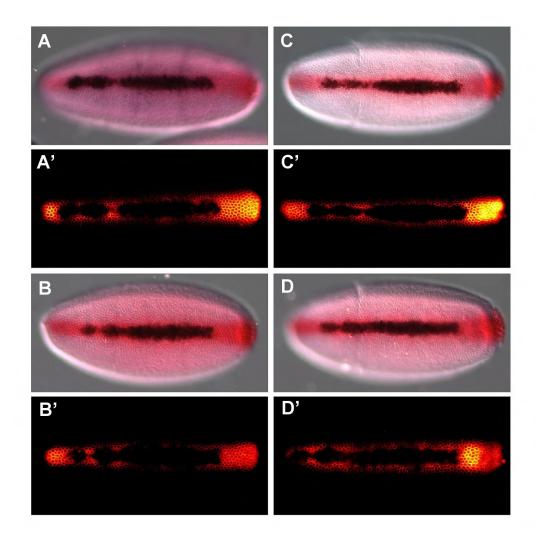


Fig. S2. pMad and *zen* **transcript in** *Drosophila melanogaster*. Double staining of pMad and *zen* transcript in *D. melanogaster* (**A-B**') at the cellular blastoderm stage and (**C-D**') during early gastrulation. All four embryos are shown in dorsal view with anterior to the left. *zen* transcript 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.

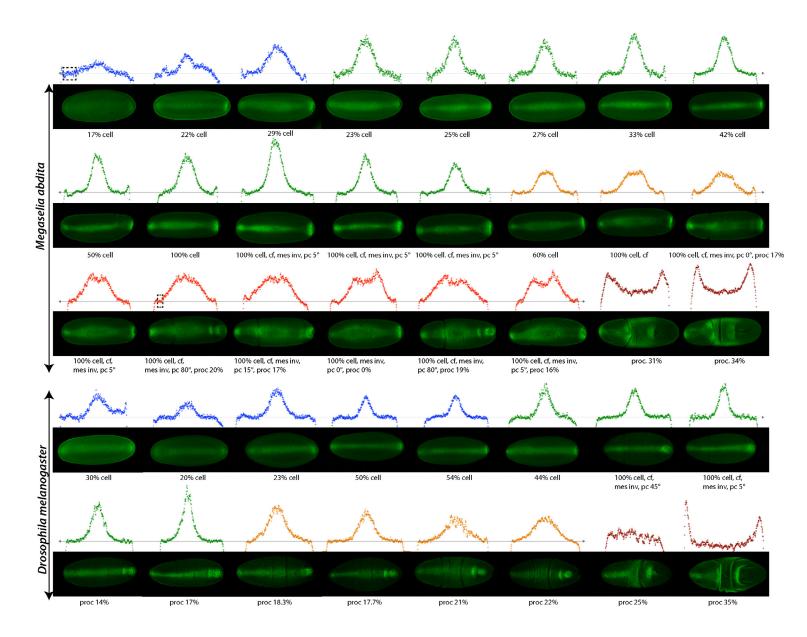
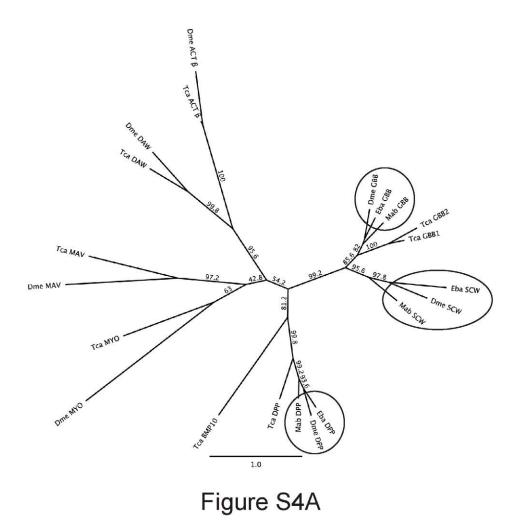


Fig. S3. pMad profiles of *M. abdita* **and** *D. melanogaster* **embryos.** Cross-sectional pMad profiles (at 50% egg length) of individual embryos are shown above dorsal projections of their confocal images. The embryos are shown in dorsal view with anterior to the left and grouped according to the 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).



Dme DPP Mab DPP	1 10 10 10 10 10 10 10 10 10 10 10 10 10
Eba DPP Tca DPP	UVUS TÕSTREKOLOPSTSTSSELSISSSASLASSISSO-18555550.19971DN101SNNKKSESDESKRRSGELLÄNNNÖMENNSSKLV-KDNTIEMOLITKOETSSSAD -NOSPDDDOVÕYKSEOYPS
Eba GBB Mab GBB	FCAYFTCTKRMYRKVIIKSDMBSQCVCDITAQLFVITLVLA-SQSGIYDDNG
MaD CBB Dree CBB Tca GBB1 Tca GBB2 Dree SCW Eba SCW Mab SCW	NGG-LRNTSEDAVAVLASLGLÖNVULMENAUTED-PAVDA-OGGGIVEDNG NNTVLVLSLVLGEDALGNGKAGIVEDNG MSUVVASTVLLLVVREVSTFLKSGIVEDNG NLNVVFLSUSTVRASTTIVNHEEN YNLYTLFHSVNIIEMIFKSVFIFLSGGVFFFFFFFFF
Dme ACT β Tca ACT β	NELEKLELSHKPRVSHELPKOFINEEEYRVDGSKNIPEENAFGSSKNIDOKTIGIKAFRSPOEELINGKGGKEOUGSSEREPSEEEYKSFPOFTPNISKNNVYGKVLKEKSIERIOKNSFLN-GWTENROLKINSOIASMPIEIESH
Dme DAW Tca DAW	QLQQQHHIRQQRSRQLXHLEDDPTSEEDDVDIGKREYYARIRRHVIHXRQHLLQQELSYMHPCSHSEQLKVPRLWQHLMEQDEKAHRHVSPPVELPIMYCDAPESSLSSDHFDDLSPLDFWRNELMAEDQQNQEXDLDLDLD MCTRAVLVLDIFLSVCRSDERSLS-VFCAIFWPCTQFSQS-ENS
Tca BMP10 Tca MYO	MVSREPLPOPESRELKSLVLLTLIEATPSEKE
Tca MAV Dme MAV Dme MYO	©WSGHGIFR©TSSSKEUVFSYQPKENNITFTN LQLQNDERNEAKRLETNQHPIIRAKSTPKMGLERNVFESFSKOSROSIYNESSNENYSQSKNFPOLFNKLSVQWINTVEINSROTRETRDIGLETKRHSKPSKRVDETRDEHL HRELMKKTRONTSDETVHIEVNSNELOFPESTRENDESKKINTGFPOKVILHREGNFRVTRVTVTVLULLSENVNAANPEDKESTSNSSSNNDSPENVNLSNETNNSEEKUGSSSTSADDKENTENNIGFPOKKVQSKPGKKST
Dme DPP	10 10 20 210 210 210 210 210 210 210 210
Mab DPP Eba DPP Tca DPP Eba GBB Mab GBB Dme GBB Tca GBB1 Tca GBB2 Dme SCW Eba SCW Mab SCW	VERY AND
Dme ACT β Tca ACT β	HNG数字→KELKS資料でRKVRG TN の運気MNEN型取KKSTYPIDINHSIDNK運用運気KRG型I=6回内2階YPNDYBYC回目の250Kマ型目の国际RYHGR貿SIGYQFMAIHNIEYEM(なな日田SFM)の方向日間型直日間のFFGM型QEIITFA層をGTQYRQYRI運行運営AQMに
Dme DAW Tca DAW	LDDQOPETPIEPELPLGERNUTISVKSSAGGCPKCESNROVEHITEBOLUHHLREGF-VEQQILEKLRLKESPEVSAVELPENPIFOGMT-LSHPDDSTKNKELDDYYARTSKKEILLNREEVECNKARDGKSNPSMCFTEENDDA VSOS-APPEAP
Tca BMP10 Tca MYO	NPNVFILVPLVILLULLSSLANSIQUDINTSTNAM-AGINTTTNAM-BURGENANTP
Tca MAV Dme MAV Dme MYO	NGWINNAAAAMTEPYLNQOVESÄBRGHSEN LESSAENGDVAKINISHE CYEKNNTUIDTROOHHEAIPDELGESIPVKNDNNEREKEPNE- VIEGGLGIKKEPDNHKVNISQAFFYSKVTEVISTRASVORKONSYVTENNINGASSERDIEPISITYNCFNDISNKKLIKHRSIGHT. LANDYSEHCDLSRVQSVS-LYRNTLENIEGENLQRQLERKAKVOSIESIKHHLLERHENLKLPNITKPISVPQNIIDNYYEVNASSKTVWNRNESIDSHLSINDYGDHEMEDPPDESSSOQODAN-TVHVILHULED JAG 300 J0 J
Dme DPP Mab DPP Eba DPP Tca DPP Eba GB8 Mab GB8 Dme GB8 Tca GB81 Tca GB82 Dme SCW Eba SCW Mab SCW	
Dme ACT β Tca ACT β Dme DAW Tca DAW	
Tca BMP10 Tca MYO Tca MAV Dme MAV Dme MYO	DEBGY VSTATEMENT LTLVEVKSGVKSGVKSGVKSG
Dme DPP Mab DPP	
Eba DPP Tca DPP	GUVRBRRDVSSRSSHHQQHPHQKRDKRRDKRRSDKKIAHHELGORKADYDDESENGRISDITEN. HKSRSARDMS-RPKRHHTKSHRKRDKDEIGRIBSDVGTADUGTADUGTADUGTADUGTADUGTADUGTADUGTADU
Eba GBB Mab GBB	KTTQRHTRRKRVGGSRSGENEHELLEGHHTGESKKSGOMGDUYMEDSKOPEDUIDTER BURGTER KTPRHNR-RNGGSKKENENEFEFERSKENE SKREGENE MYNDERINDER MENDINGENE FOR DAN DE ANDER DE FERINDER HANDA GMEL N
Dme GBB Tca GBB1 Tca GBB2	KATAHSEHHRSKRSASHPRK-RKKSVSENNVPLLEPMESTRSGOMOTEYHORMOFGMHOLFUA ECHOAFKGSGEGNPPLMAHMMANNE-AFMOTEYELLEPKKVEREOORDTRWORDPULYHINDNWIKE-KYRMIIUKEGGH OprRsirDlskrrvkrsmyvemmusomphygsewrupenson of modern of the same for predering and manne-Afvordeyelly provensor for opposite sources and the same sources of
Dme SCW Eba SCW	vkiOkirpkrdiskrra-GGGSpppppppdlyrpp0.GEEINBTVDEXENHHHDOVDAEKKDEAPGCG-GNFPLGTXMDAEDNE-AEDVOTEMEIKOPH-DEKEGONETVEGANTNEFT RIMBIHDD-KEMEGON
Mab SCW Dme ACT β Tca ACT β Dmc DAW Tca DAW Tca BMP10 Tca MYO Tca MYO Dme MAV Dme MAV	NF LIE SFROOPN-HROWAG AGAINET BUYGODDUDEL TO FERENCE STOCADUMAANNE-ADECTED A SUMAANNE-ADECTED SER THESE SER THESE THE STATUS - PX NITURE COORD TRR
	Figure SAP
	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%.

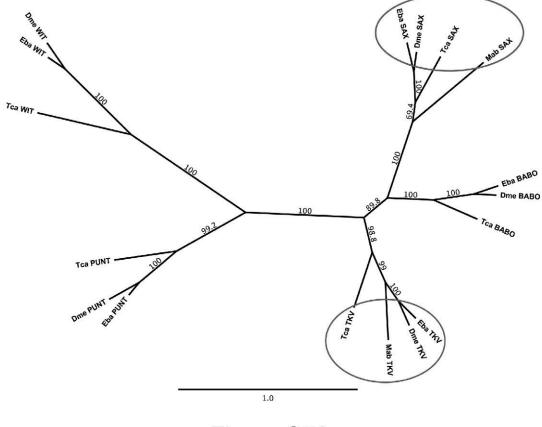


Figure S5A



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%.

	1 10	20	30	40	50	60	70
Dme 50G	LIVCLAGVTEGRR	HAPLMFEESI	TGRRSNRPAVT	COFGKVLRE	LGSTWYADLGP	PEGVMYCIKC	ECVAIPKKRRI
Eba SOG		MLVEDPE	-ARRPSRQG-A	COFGKTLHE	LGSTWWADLGP	PEGVMYCINC	ECVAVPKKRRI
Mab SOG						1997	
Aga SOG			VT	COFGKTIRE	LHTTWFADLGP	PIGVMYCIMC	ECVPFQKKRRV
Cri SOG							
Tca SOG	LTWGLFCVLVTLA	TARNKAPLL	DGTRT-RNKAA	CVFGKQVRE	LGSQWIPDLGV	PIGVLYCMMC	ECVPLOKKRRI
	80	90	100 110	12	0 130	140	150
Dme SOG	VARVQCRNIKNEC						
Eba SOG	VARVQCRNIKNEC						
Mab SOG			LPGKCCKTCPGI				
Aga SOG	VGRVQCRNIKNEC						
Cri SOG			LPGRCCKTCPGI				
Tca SOG	VARVQCRSIKNEC	PEPTCDEPVI	RPGRCCKTCPGI	DLYDSDIIOD	IVEON-VLDDO	EKSSTKHYAA	ERSSLVME
	160	170	180	390	200	210	220
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Eba SOG	GEEMKANYSTHN	ONVVATGREI	F#KK				
Eba SOG Mab SOG	GEEMKAMYSTHNE REDT-TVSSMHNE	ONVVATGRFI NNLIATGRFN	FHKK FOKKNLYYSFY	SANKOMGRE	TTIOFVNDVGT	LDBHNLTTS	TN-GPLSVXEK
Eba SOG Mab SOG Aga SOG	GEEMKANYSTHNE REDT-TVSSMHNE KDDMGTNYSTLNE	ONVVATGRFI NNLIATGRFN ONVVATGRFS	FHKK FOKKNLYYSFY FHKKNLYYSFY	/SANKOMGRE /SERAARE	TTIOFVNDVGT RSIQFVHANGN	ILEEHNL TTS ILEEHTLALE	TN-GPLSVYEK SDEGRLSAYON
Eba SOG Mab SOG Aga SOG Cri SOG	G DEMKAMYSTHN REDT-TVSSMHNE KDDMGTNYSTLN RDEMLNNYSTKN	ONVVATGRFI NNLIATGRFN ONVVATGRFS ONLIATGRLS	FHKK FOKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY	/SANKQMGRP /SERAARP /SE?ASRP	TTIOFVNDVGT RSIQFVHANGN SAIQFIDNSCN	ILEEHNLTTS ILEEHTLALE ILEEHSLVIE	TN-GPLSVYEK SDEGRLSAYON PNN-GPSSNYON
Eba SOG Mab SOG Aga SOG	GEEMKANYSTHNE REDT-TVSSMHNE KDDMGTNYSTLNE	ONVVATGRFI NNLIATGRFN ONVVATGRFS ONLIATGRLS	FHKK FOKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY	/SANKQMGRP /SERAARP /SE?ASRP	TTIOFVNDVGT RSIQFVHANGN SAIQFIDNSCN	ILEEHNLTTS ILEEHTLALE ILEEHSLVIE	TN-GPLSVYEK SDEGRLSAYON PNN-GPSSNYON
Eba SOG Mab SOG Aga SOG Cri SOG Tca SOG	GERKAMYSTHNE RET-TVSSMHNE KODMGTNYSTLN REMLNNYSTKNE -NEFRLNSNNLNK 230 240	ONVVATGRFI NNLIATGRFN ONVVATGRFS ONLIATGRLS NNVVATGRF7 250	FHKK FOKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY 260	/SANKQMGRP /SERAARP /SE?ASRP /SDKAARP 270	TTIOFVNDVGT RSIOFVHANGN SAIOFIDNSGN RSLOFVDSEGN 280	ILEEHNLTTS ILEEHTLALF ILEEHSIVIF ILEEFVLSRA 290	TN-GPLSVMER SDEGRLSAMON NN-GPSSNMON GG-LVNSLMON 297
Eba SOG Mab SOG Aga SOG Cri SOG Tca SOG Dme SOG	GERKAMYSTHNE RET-TVSSMHNE KODMGTNYSTLN REMLNNYSTKNE -NEFRLNSNNLNK 230 240	ONVVATGRFI NNLIATGRFN ONVVATGRFS ONLIATGRLS NNVVATGRF7 250	FHKK FOKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY	/SANKQMGRP /SERAARP /SE?ASRP /SDKAARP 270	TTIOFVNDVGT RSIOFVHANGN SAIOFIDNSGN RSLOFVDSEGN 280	ILEEHNLTTS ILEEHTLALF ILEEHSIVIF ILEEFVLSRA 290	TN-GPLSVMER SDEGRLSAMON NN-GPSSNMON GG-LVNSLMON 297
Eba SOG Mab SOG Aga SOG Cri SOG Tca SOG Dme SOG Eba SOG	GEEMKAMYSTHNE REDT-TVSSMHNE KDDMGTNYSTLNE RDEMLNNYSTKNE -NDFRLNSNNLNK 230 240 ATGKICGVWRRVE	ONVVATGREI NNLIATGREN ONVVATGRES ONLIATGRES NNVVATGRES NNVVATGRES ZSO RDYKRILRDI	FHKK FOKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY 260 DRLHVVLLWGN-	/SANKQMGR /S- ERAAR /S- ERAAR /S- DKAAR 220 KQQAELA	T TI OFVN DVG T R SI OFVHANGN SAI OFI DN SGN R SL OFVDS EGN 280 LAGKVA KY TAL	LEEHNLTTS LEEHTLALF LEEFVIS RA 290 DTELESSALF	TN - GPLSVMEK SDEGRLSAMON NN - GPSSNMON GG - LVNSLMON 297
Eba SOG Mab SOG Aga SOG Cri SOG Tca SOG Dme SOG Eba SOG Mab SOG	GEEMKAMYSTHNE RET-TVSSMHNT KDDMGTNYSTLNE RDEMLNNYSTKNE -NDFRLNSNNLNK 230 ATGKICGVWRRIE STCKICGVWRRIE	ONVVATGREI NLIATGREN ONVVATGRES ONLIATGRES NNVVATGRES RDYKRILRDI KDYKRILRDI	FHKK FQKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY 260 ORLHVVLLWGN RPHVVLLWRSTI	ISAN KOMGRE IS- ERAARE IS- ERAARE IS- DKAARE 270 KOQAELA LLDTKOVELA	T TI OFVN DVG T R SI OFV HANGN SALOFI DNSGN R SLOFVDS EGN 280 HAGKVAKY TAH	LEEHNLTTS LEEHTLALF LEEHSLVIF LEEFVLSRA 290 DTELFSSLLF SSELFSSLLF	TN - GPLSVYEK SDEGRLSAMON NN - GPSSNYON GG - LVNSLYON 297
Eba SOG Mab SOG Aga SOG Cri SOG Tca SOG Dme SOG Eba SOG Mab SOG Aga SOG	GEEMKAMYSTHNE RET-TVSSMHNT KDDMGTMYSTLNE RDEMLNNYSTKNE -NDFRLMSNNLNK 230 240 ATGKICGVWRRVE STGKICGVWRRIE GTGKVCGVWRRVE	ONVVATCREI NLIATCREN ONVVATCRES ONLIATCRLS NNVVATCRE 250 RDYKRILRDI KDYKRLLRDC REYRRLLRDE	FHKK FQKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY CORLHVVLLWGN RPHVVLLWRSTI LVSVVLLWGS	ISAN KOMGRE ISERAARE ISERAARE 270 KQQAELA LDTKQVELA KHQAELA	T TI OFVN DVG T R SI OFV HANGN S AI OFI DN SGN R SL OFV DS EGN 280 HAGKVAKY TAH IGGKI DKYWAT IAG PI AKY PAH	LLEEHNLTTS LLEEHTLALE LLEEHTLALE LLEEFVLSRA 290 QTELFSSLLE SSELFSSLLE AHELFSSLLE	TN - GPLSVYEK SDEGRLSAYON NN - GPSSNYON GG - LVNSLYON 297
Eba SOG Mab SOG Aga SOG Cri SOG Tca SOG Dme SOG Eba SOG Mab SOG	GEEMKAMYSTHNE RET-TVSSMHNT KDDMGTNYSTLNE RDEMLNNYSTKNE -NDFRLNSNNLNK 230 ATGKICGVWRRIE STCKICGVWRRIE	ONVVATCREI NLIATCREN ONVVATCRES ONLIATCRES NNVVATCRES RDYKRILRDI KDYKRLLRDC REYRRLLRDC REYRRLLRDC	FHKK FOKKNLYYSFY FHKKNLYYSFY FHKKNLYYSFY 260 RLHVVLLWGN RPHVVLLWRSTI LVSVVLLWGS OMNAVLIWNG	ISAN KQMGRE IS- ERAARE IS- DKAARE 270 KQQAELA LDTKQVELA KHQAELA KHQAELA	T TIOFVNDVGT RSIOFVHANGN SAIOFIDNSGN RSIOFVDSEGN 280 HAGKVAKYTAI IGGKIDKYWAH TAGPTAKYPAT HAGPISKYPAT	ILEEHNUTTS ILEEHTVIF ILEEFVISRA 290 OTELFSSLLF SSELFSSLLF AHELFSSLLF STELFSSLLF	TN - GPLSVMER SDEGRLSAMON NN - GPSSNMON GG - LVNSLMON 297

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%.

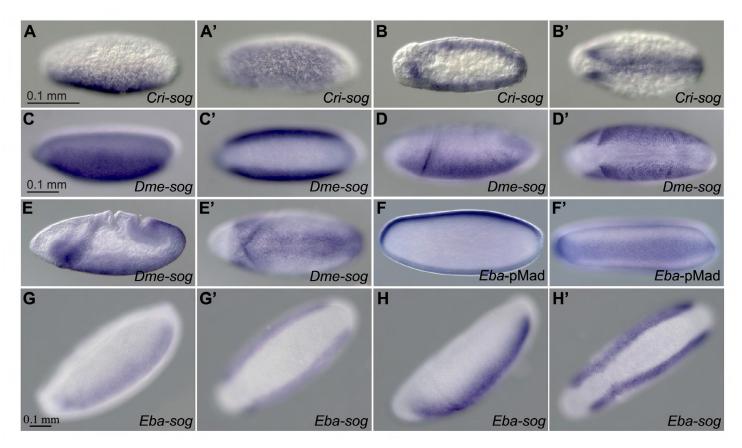


Fig. S7. Expression of sog in Chironomus riparius and Drosophila melanogaster, and pMad distribution and sog expression in Episyrphus balteatus (Syrphidae). (A-B') sog in situ hybridizations at the blastoderm stage (A,A') and the extended germband stage (B,B') in C. riparius. (C-E') sog in situ hybridizations 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.