

A

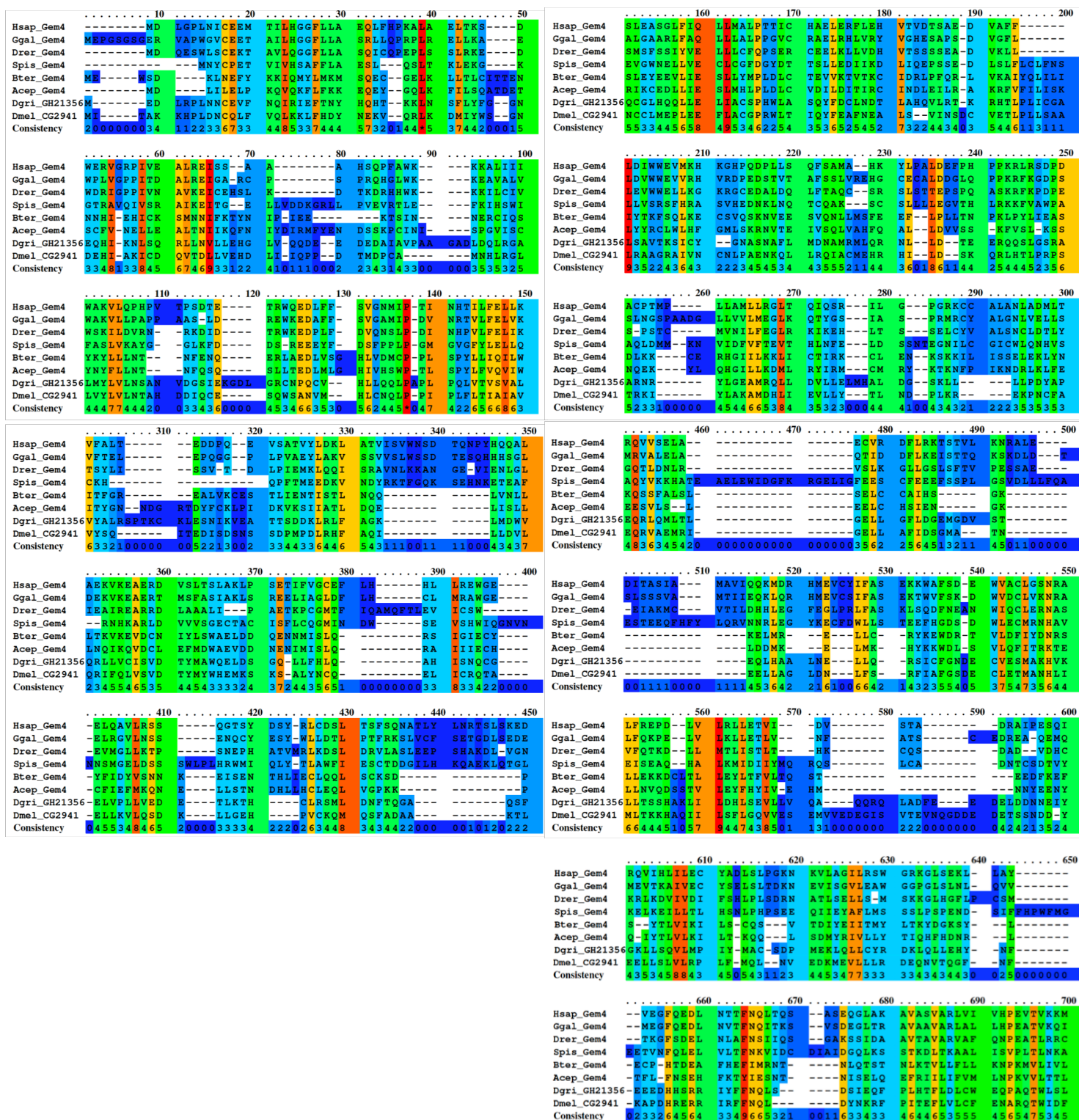


Fig. S1

B

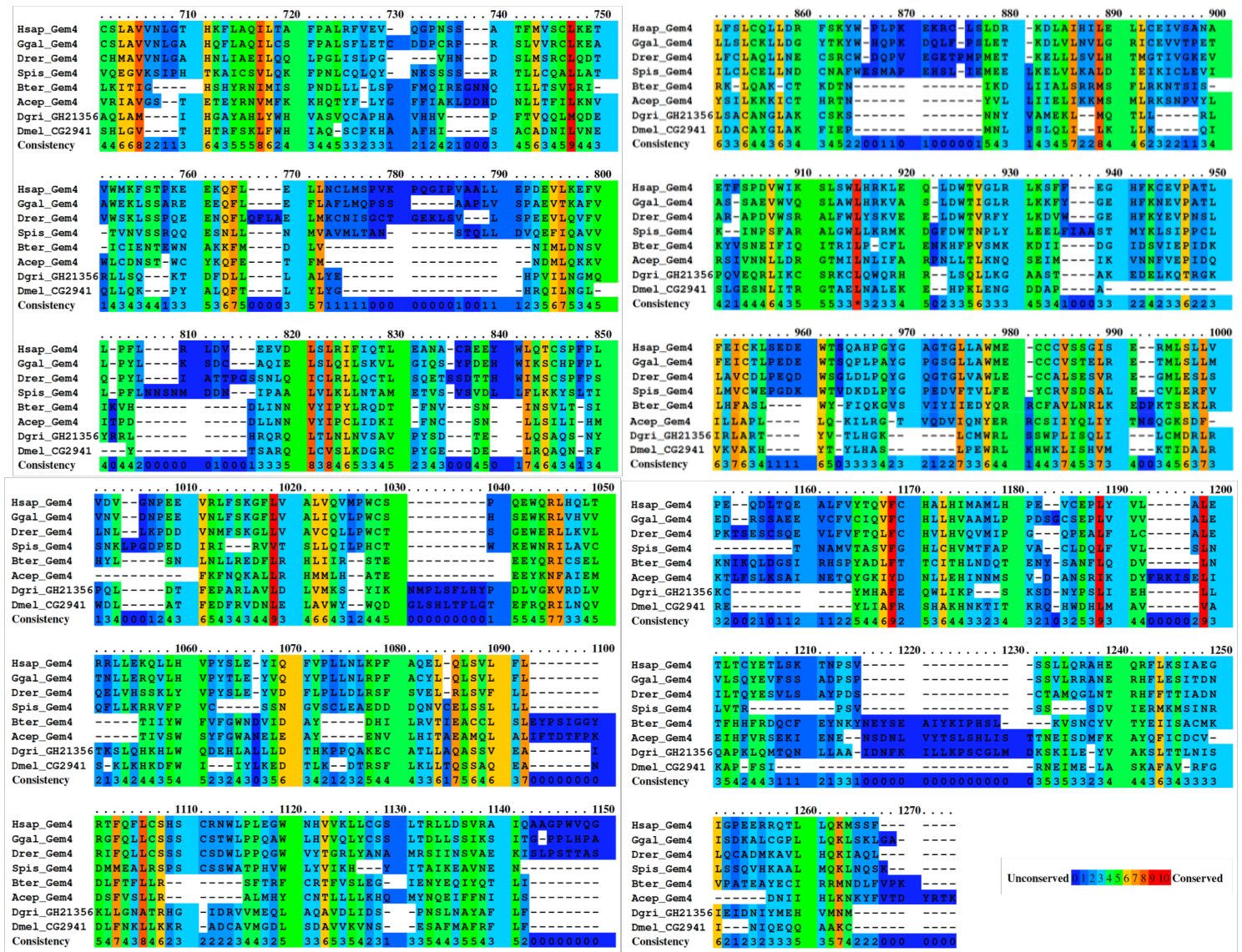


Figure S1. Amino acid alignment of Gemin4 orthologs from a variety of species, including: *Homo sapiens* (Hsap), *Gallus gallus* (Ggal), *Danio rerio* (Drer), *Stylophora pistillata* (Spis), *Bombus terrestris* (Bter), *Atta cephalotes* (Acep), *Drosophila grimshawi* (Dgri), and *Drosophila melanogaster* (Dmel). (A) Alignment of Dmel_CG2941 with residues 1-700 of metazoan Gemin4. (B) Alignment of Dmel_CG2941 with Gemin4 residues 701-1274. (A-B) The degree of conservation in the sequence is indicated by a coloration spectrum running from blue to red, with blue indicating regions of low conservation and red highlighting those that are highly conserved. (C) Predicted secondary structure alignment of Dmel_CG2941 with residues 1-700 of metazoan Gemin4. (D) Secondary structure alignment of Dmel_CG2941 with Gemin4 residues 701-1274. (C-D) Regions predicted to adopt an alpha-helical (red) vs. beta-stranded (blue) secondary structure are shaded accordingly.

C

Hsap_Gem41020304050	Hsap_Gem4360370380390400
Ggal_Gem4	-----MD	LGPLNICEEM	TIHGGFLIA	EQLFHPKALA	ELTKS----	Ggal_Gem4	AEKVKEAERD	VSLTSLAKLP	SETIPVGCFF	LH-----	HL LREWGE----
Drer_Gem4	-----MD	VAPWGVCEET	AILHGGFLIA	SRLLQPRPLR	ELLKA-----	Drer_Gem4	DEKVKEAERT	MSFASIAKLS	REELIAGLDF	LH-----	CL MRWANG----
Spis_Gem4	-----MD	QESWLSCEKT	AVLQGGFLIA	SOICQPEPLS	SLRKE-----	Spis_Gem4	TEAIRREARRD	LAAAI-----	AETKPCGMTF	TQAMQFFLEV	ICSW-----
Bter_Gem4	-----MD	-----MNYCPET	VIVNSAFFLIA	ESL-----	QBLT KLEKG----	Bter_Gem4	-----RNHKKARID	VVVSGETC	ACISFLCQGMIN	DM-----	SE VSHWIGGNVN
Acep_Gem4	-----MD	-----KLNEFY	KKIQMYLMKM	SQEC-----	GELK LLTSCITTEN	Acep_Gem4	LTQVKKEVDGN	IYLSWAEADD	QENNMISLQ	-----	RS IGIECY----
Dgri_GH21356	-----MD	-----LILELP	KQVQKFLFKK	EQEY-----	GOLK FILSQAIDET	Dgri_GH21356	LNQIKKEVDCL	EEMDMAEVDD	NENNMISLQ	-----	RA IIEECY----
Dmel_CG2941	MI-----TAK	LRPLNNCEVP	NQRIEFTNY	HQHT-----	KKLN SLVYFG--GN	Dmel_CG2941	QRLIVLCISVD	TYMANCELDS	GG--LLFBLQ	-----	AH ISNQCQ----
		KHPLDNCQLF	VQLKKLFHDY	NEKV-----	QRLK DMIYWS--GN		QRIFQLVSVSD	TYMYNHEMK	KS--ALYNCO	-----	SL ICROTA----
Hsap_Gem460708090100	Hsap_Gem4410420430440450
Ggal_Gem4	-----MD	WERVGRPIVE	ALREIS--A	A-----A	HSQPFNWK--	Ggal_Gem4	-----ELQAVLRSS	-----QGTSY	DSY--RLCD	SLTSFSONATLY	LNRTSLSKED
Drer_Gem4	-----MD	WPIVGPPIITD	ALREIGA-RC	P-----S	PRQHGLW--	Drer_Gem4	-----ELRGVLNSS	-----ENQCY	ESY--WLHLL	TLPTFRKSLVCF	SETGDLSEDE
Spis_Gem4	-----MD	WDRIGPPIVN	AVKEIGESHL	K-----D	TKDRHWNK--	Spis_Gem4	-----VYMGMLKTP	-----SNEPH	ATVMRLKDSL	DRVLASIEEP	SHAKDL-VGN
Bter_Gem4	-----MD	GTRAVQIVSR	AIKEITG--E	LLVDDKGRLL	PVEVETLE	Bter_Gem4	-----NNSMGELDSS	SWLPLRNM	QLY--FLAWFI	ESCTDDGILH	KQAEKLTQGL
Acep_Gem4	-----MD	NNHT--EHICK	SMNNIFKTYN	IP--IEE--	-----KTSIN--	Acep_Gem4	-----YFIDYVSN	N--EISEN	THLIECLQQL	CKSD----	-----F
Dgri_GH21356	EQHT--KNLSQ	SCFV--NELLE	ALTNIIKQFN	IYDIRMFIYEN	DSSKPCINI--	Dgri_GH21356	-----CFIEFMKN	E--LLSTN	DHLLHCLEQL	VGPKK----	-----F
Dmel_CG2941	DHHT--AKICD	QVTDLLVEHD	LT--IQPP--E	TMDPCA-----	-----MNHLEGL	Dmel_CG2941	ELVPLLVLED	S--FLKTH	-----CLRSML	DNFTQGA--	-----QSF
Hsap_Gem4110120130140150	Hsap_Gem4460470480490500
Ggal_Gem4	-----MD	WAKVLPQHPV	TESDTE	TRWQEDLFF	SVGNMIP-TI	Ggal_Gem4	-----QVVSSELA	-----ECVR	DPLRKSSTVL	KNRALE--	-----T
Drer_Gem4	-----MD	WAKVLPQHPV	AAS--LD	REWKEDAF	SVGAMIP-DV	Drer_Gem4	-----MRVALELA	-----QTID	DPLKEISTTQ	KSKDLE--	-----T
Spis_Gem4	-----MD	WSKVLDVRN	-----SKDIT	TRNKEDPL	DVQNSLP-DI	Spis_Gem4	-----GQFLDNLR	-----VSLK	GLLGSLSTPV	PESSAL--	-----T
Bter_Gem4	-----MD	PASVVKAYG	-----GLFND	DS--REEEYF	DSFPPLP-GM	Bter_Gem4	-----AQVVKKHAT	AELEWIDGFK	GELIGFEES	CEEFESSP	GSVDLLLFQ
Acep_Gem4	-----MD	YKYLLENT--	-----NFENO	ERLAEDLVSG	HIVHMCPL	Acep_Gem4	-----KQSSPALS	-----SELK	CAIHS	-----	-----GK
Dgri_GH21356	LYNYLVNLSAN	-----NFQSO	SLTTEDLMIG	HIVHMCPL	PLSFYLLIQILW	Dgri_GH21356	-----EESVLS--	-----EELC	HSIN	-----	-----GK
Dmel_CG2941	LYNYLVNLSAN	-----NFQSO	SLTTEDLMIG	HIVHMCPL	PLSFYLLIQILW	Dmel_CG2941	-----EESVLS--	-----EELC	HSIN	-----	-----GK
Hsap_Gem4160170180190200	Hsap_Gem4510520530540550
Ggal_Gem4	-----MD	SLEASGLFIQ	LLMALPTTIC	HAELERFLEN	VTVDSAP--D	Ggal_Gem4	-----DITASTA--	NAVIQKMDR	HMEVCYIPAS	ERRWAFSD-E	NVACLGSNRA
Drer_Gem4	-----MD	ALGAARLEPAQ	LLMALPTTIC	HAELERFLEN	VTVDSAP--D	Drer_Gem4	-----SLSSSVA--	NTIIEQKLQR	HMEVCYIPAS	ERRWAFSD-E	NVACLGSNRA
Spis_Gem4	-----MD	SMSFSSIIYVE	LLMGFPQSEA	CEELKLLVDH	VTSSSSEA-D	Spis_Gem4	-----ETAKMC--	VTILDHNLG	FEGGLRPLAS	KESQDFNEAN	WICQLERNAS
Bter_Gem4	-----MD	VGVNNEELLVE	CLCGFDGYDT	TSLLIEDIHK	LQEPSSSE-D	Bter_Gem4	-----ESTEEQPHFY	LQRVNNRLEG	YKECFDWLLS	TEEHGSDS-D	WLECMRHHAV
Acep_Gem4	-----MD	SLEYEEVLIE	SLLMPLDLDC	TEVVKVTIRC	IRLRFPOR-L	Acep_Gem4	-----KELMR--	-----E--LNC	RYKENDR-T	VLDPIYDNRS	-----
Dgri_GH21356	CKGLHQQLLE	LIACSPHWLA	SOYFDCDNDT	LAHQVLRN-K	RHTFLPLICGA	Dgri_GH21356	-----LDDMK--	-----E--LNC	RYKENDR-T	VLDPIYDNRS	-----
Dmel_CG2941	NCCLMPELEE	FLAGGPRWLT	IQYFEAFNEA	LS--VINSD	YETFLPLISAA	Dmel_CG2941	-----EQLHAA	LNE--LIQ	-----RSTCFGNDE	CVESMAKHVK	-----
Hsap_Gem4210220230240250	Hsap_Gem4560570580590600
Ggal_Gem4	-----MD	LDVWWEVVMX	KGHPQDPLLS	QFSAMA--HK	YLPALDEFPH	Ggal_Gem4	-----LREPH--	LV--LRLLETV	-----DV	-----STA	-----DRAIPESQI
Drer_Gem4	-----MD	LDVWWEVVMX	VRDEPDSVT	AFSSLVREHG	CECALDDGLQ	Drer_Gem4	-----LQKKE--	LV--LKLLETV	-----NF	-----STA	-----EDREA-QEMQ
Spis_Gem4	-----MD	LEVWWEVVMX	KRGCEADLDQ	LFTAQ--E	SLSTTEPSPO	Spis_Gem4	-----VFQTKD--	LL--MTLISTLT	-----HK	-----QS	-----DAD--VDRC
Bter_Gem4	-----MD	LLVRSFPHRA	SVHECNKING	TCCAK--SC	SLILLEGVHE	Bter_Gem4	-----EISEAQ--	HA--LKMIDIIYMO	-----RQS	-----LCA	-----DNTCSDTYV
Acep_Gem4	-----MD	IYTKFSQLEK	CSVQSKNVEE	SVQNLMSFE	ES--PLLTN	Acep_Gem4	-----LEKKKDCLT	LEYLTPVITQ	-----S	-----	-----EEDPKF
Dgri_GH21356	LSAVTKSICY	-----GNASNAFL	MDNAMRLQR	NL--LD--TE	ERQSSIGSRA	Dgri_GH21356	-----LLNVQDSSTV	LEYFYHYV-E	-----HM	-----	-----NNYEENY
Dmel_CG2941	LRAAGRAIVN	CNLP--ENKQL	LROIACMEHR	HT--LD--SK	QRLHTLPRPS	Dmel_CG2941	-----LLTSSHAKLI	LDHLEVLV	QA--QQRQ	-----LADFE	-----EDELDDNNEIY
Hsap_Gem4260270280290300	Hsap_Gem4610620630640650
Ggal_Gem4	-----MD	ACPTT--	ELAMLLRGLT	QIQSR--IL	G--PGRKC	Ggal_Gem4	-----QVVIHILILEC	YADLSLPCKN	KVLAGILBSW	GRKGLSEK	-----LAV
Drer_Gem4	-----MD	SLNGSPAA--	LLVVLMEGLK	QTYGS--IA	S--PRMKC	Drer_Gem4	-----MEVTKAIVEC	YSELSTDRN	EVISGVLEAW	GGPGLSLNL	-----QVV
Spis_Gem4	-----MD	S-PS--	MYNLFPEGLR	KIKER--AT	S--SELVCY	Spis_Gem4	-----KRLKDVIVDI	FHHLPLSDRN	ATLSBELL--M	SKKGLHGLFP	-----CSN
Bter_Gem4	-----MD	ALDDMM--KN	VIDPVFTEVT	HLNFE--LD	SSNTEGKIC	Bter_Gem4	-----KELKEILLTL	HNNLPHPSSE	QIIEYAFIMS	SSLSPEND	-----SIFHPWFMS
Acep_Gem4	-----MD	DLKK--CE	RHGILLKKLI	CTIRK--CL	EN--KSKKIL	Acep_Gem4	-----S--YTLVIKI	LS--CS--V	TDIYIITMY	LTKYDGRKSV	-----L
Dgri_GH21356	ARNR--	YL	QHGILLKMDL	RYIRM--CM	RY--TKNFP	Dgri_GH21356	-----Q--YTLVLIK	LT--KQ--L	SDMYRIVILY	TIQHFHDNR	-----N
Dmel_CG2941	TRKI--	YLAAMDHIL	EVLLY--TL	ND--PLKR	-----EKPNCFA	Dmel_CG2941	-----GKLLSQVLMR	IY--MAC--SDP	MEKLLQLCYR	DKLQLLHY	-----NF
Hsap_Gem4310320330340350	Hsap_Gem4660670680690700
Ggal_Gem4	-----MD	VEANT--	-----EDDPQ--E	VSAIVYLDKL	ATVIVSWNSD	Ggal_Gem4	-----VEGQFQEDL	NTIFNQITQS	-----ASEGGLAK	AVASVARLVI	VHPFVTVKRM
Drer_Gem4	-----MD	VFTET--	-----EPQGG--P	LPVAEYLAHV	SSVSLWSSD	Drer_Gem4	-----MEGQFQEDL	NVTFNQITQS	-----VSGELTR	AVASVARLVI	VHPFVTVKRM
Spis_Gem4	-----MD	TSYI--	-----SSV--T--D	LPKEMLQOI	SRAVNLKRN	Spis_Gem4	-----TKGFSDEL	NLAFNSIQS	-----GAKSSIDA	AVTAVARLVI	QNEEATLRRC
Bter_Gem4	-----MD	CKH--	-----QFFTMEEDKV	NDYRKTEGK	SEHNKETEAF	Bter_Gem4	-----ETVNFQLEL	VLTFNKVIDC	DIADIGGLKS	STKDLTKAAL	ISVPLTKKA
Acep_Gem4	-----MD	ITFG--	-----EALVKCES	TLIENTISTL	NQ--	Acep_Gem4	-----ECP--HTDEA	FHEFIMNNT	-----NLQST	NKTVLLFL	LNKPMVLI
Dgri_GH21356	YVY--	RTDYFCKLP	DKVKSIIATL	DQE--	-----LVNLL	Dgri_GH21356	-----TFI--NSENH	FKTYIESNT	-----NISELO	EFRIILIFVM	LNKPMVLI
Dmel_CG2941	YYSQ--	ITEDISDSNS	SDPMPDLRHF	AQI--	-----LMDV	Dmel_CG2941	-----KAPDHRERR	IRFFNQL--	-----DYNKRF	PITEFLVLCP	ENARQTWIDF

Fig. S1 (cont'd)

		710	720	730	740	750			1060	1070	1080	1090	1100									
Hsap_Gem4		CSLAVVNLGT	HKFLAQILTA	FPALRFVVE	QDPNRS	A	TPMVSCILKET		Hsap_Gem4	RRLLRQQLLH	VPYSLR	YIQ	PVPLNLNPKP	AQEL	QLSVL	FL	----					
Ggal_Gem4		CSLAVVNLGT	HQFLAQILCS	FPALSELETC	DDPCR	R	SLVVRCLKEA		Ggal_Gem4	TNLLRRQVLH	VPYTLR	YVQ	YVPLNLNPKP	ACYL	QLSVL	FL	----					
Drer_Gem4		CHMAVVNLGA	HNLAIEILQQ	LPGLISLPG	----	VHN	D	SLMRSCLQDT		Drer_Gem4	QELVHSKSLY	VPYSLR	YVD	PLPLDLNLR	SVEI	RLSVF	LI	----				
Spis_Gem4		VOEGVKSIPH	TKAICSVLQK	FPNMLCQLQY	NKSSSS	R	TLLCQALLAT		Spis_Gem4	QFLILRRRVFP	VC	----	SSN	GVSCLEAEDD	DQNVCLSLSL	LI	----					
Bter_Gem4		LKITITG	----	BSHYRNIMSS	PNDLIL	LSF	FMQIREGNQ	ILTSVLRI		Bter_Gem4	----	TIYIW	YVFGWNDVID	AV	----	DHI	LRVTIACCL	SLEYPISGG	F			
Accep_Gem4		VRIAVGS	----	ETERYRVMFK	KHQTYY	LYG	FFIAKLDDHD	ILLTFILKNV		Accep_Gem4	----	TIYSW	YVFGWANELE	AV	----	ENV	LHITAEAMQL	ALTFIDTFPK				
Dgri_GH21356		AQLAM	----	HGAYAHLYWH	VASVQCAPHA	VHHV	----	P	FTVQQLMQDE		Dgri_GH21356	KSLQ	RRKHLH	QDEHALLLD	THKPPQAKEC	ATLLAQASSV	EA	----	I			
Dmel_CG2941		SHLGV	----	HTRFSKLFWH	IAQ	SCP	KHA	AFHI	----	Dmel_CG2941	S	KLKHKDFW	T	----	LYLKE	TLK	----	DTRSF	LKLLTQSSAQ	EA	----	N
		760	770	780	790	800				1110	1120	1130	1140	1150								
Hsap_Gem4		VMMRFSTERE	EKQPL	----	E	LLNCIMSPVK	PQGI	VAALL	E	FDDEVLEKPV												
Ggal_Gem4		AMEKISSARE	EKQPL	----	E	FLAFIMQPSF	----	AAPLV	S	PAEVRTAFV												
Drer_Gem4		VMSKLSSPQE	ENQPLQPLAE	LMKCNISGCT	GKELSV	----	S	PSBEVLQGVF														
Spis_Gem4		RVNVSSRQQ	ESNLL	----	N	VMVAMITAN	----	STQLL	D	VQEFIQAVV												
Bter_Gem4		ICIENTENWE	AKKFM	----	D	LV	----	NIMIDNSI														
Accep_Gem4		WICDNS	----	KC	YKQPE	----	T	FM	----	NMDLQKKV												
Dgri_GH21356		RLLSQ	----	KT	PDFLL	----	L	ALYE	----	HPVILNGMQ												
Dmel_CG2941		QLLQK	----	PY	ALQET	----	L	YLYG	----	HRQLNLGI												
		810	820	830	840	850				1160	1170	1180	1190	1200								
Hsap_Gem4		R	PFH	----	R	LDV	----	EEVD	LSLRIFIQTL	EANA	CREXY	WLOTCSPFPL										
Ggal_Gem4		R	PYH	----	K	SDC	----	AQIE	LSLQILSKVL	GLQS	YPDEH	WIKSCHFFPL										
Drer_Gem4		R	PYH	----	I	ATTPGSSNLQ	ICGLRLQCTL	QETSSDTH	WMSCSPPFRS													
Spis_Gem4		R	PFLNNSNM	DD	----	IPAA	LVKLINTAM	ETVS	VSMDL	FLKKYSLTI												
Bter_Gem4		IKVH	----	DLIIN	VYTFYLRQDT	FNV	----	SN	----	INSVL	SI											
Accep_Gem4		ITPD	----	DLIIN	VYTFPCIDKI	LN	----	SN	----	LSILI	NM											
Dgri_GH21356		YRRV	----	HRQRQ	LTNLNVSAV	PYSD	----	TE	----	LQSAQS	NY											
Dmel_CG2941		----	----	TSARQ	LCVSLKDGR	PYGE	----	DE	----	LRQAQV	RF											
		860	870	880	890	900				1210	1220	1230	1240	1250								
Hsap_Gem4		LFSLCQLLDG	FSKYW	PLPK	----	EKKR	LSLDR	----	KDLAIRLE	LLCEIVSANA												
Ggal_Gem4		LLSLCQLLDG	YTKYW	QPK	----	QDLF	PSLET	----	KDLVNLVLG	RICEVVTPT												

HELIX (H) STRAND (E)

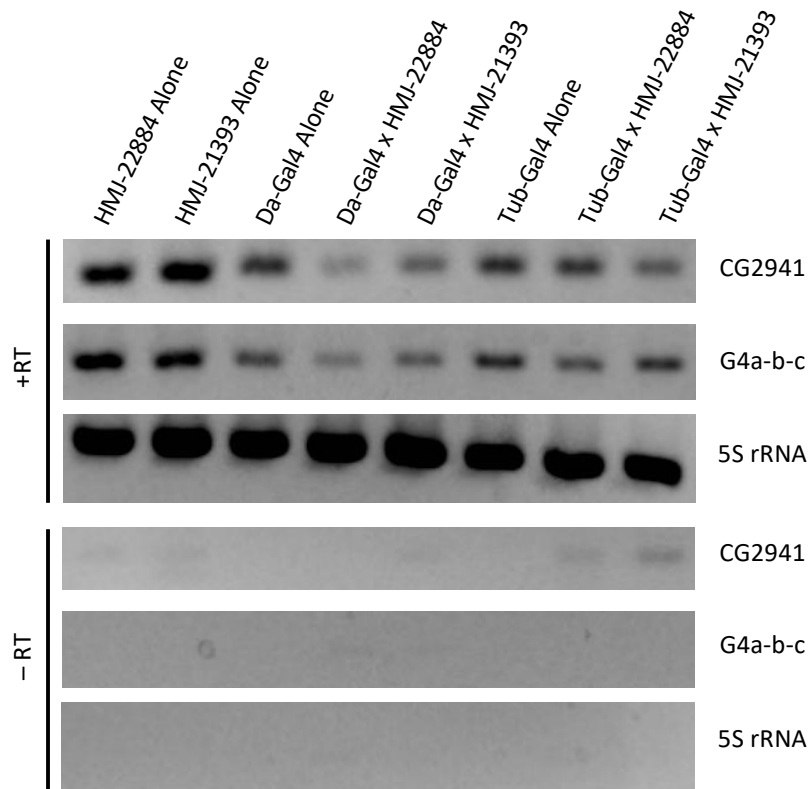


Figure S2. RT-PCR analysis of total RNA isolated from the early third instar larvae in Fig. 7C. Following reverse transcription, 35 cycles of PCR were performed using primers designed to analyze the expression of control RNA (5S rRNA), CG2941 mRNA only, or all three Gemin4 mRNA paralogs (G4a-b-c) are shown. Crosses as per Fig. 7.