Functional characterization of the circadian clock in the Antarctic krill, Euphausia superba

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SUPPLEMENTARY FIGURES



0.2

Fig. S1. *Es*CLOCK. (A) Multiple alignment of the CLOCK sequences corresponding to the *m*CLOCK's exon 19. (B) Phylogenetic relationships of the CLOCK protein family. *Es*CLK clusters with other crustaceans orthologues. The *D. melanogaster*'s bHLH-PAS protein TANGO isoform A has been used as outgroup. Bootstrap confidence values based on 1,000 replicates are shown at nodes. Scale bar indicates amino acid substitutions per site. The most relevant orthologues are indicated in bold.

		BCTR
Vertebrates	D. rerio (ARNT-like) X. laevis (BMAL1) G. gallus (ARNT-like 1) H. sapiens (BMAL1b) M. musculus (BMAL1b)	585 - M - EE PGSSSPSNDEAAMAVIMSLLEADAGLGGPVDFSDLPWPL - 626 585 - TETEHGSSSPSNDEAAMAVIMSLLEADAGLGGPVDFSDLPWPL - 627 591 - IDNDQGSSSPSNDEAAMAVIMSLLEADAGLGGPVDFSDLPWPL - 633 584 - IDNDQGSSSPSNDEAAMAVIMSLLEADAGLGGPVDFSDLPWPL - 626 584 - IDNDQGSSSPSNDEAAMAVIMSLLEADAGLGGPVDFSDLPWPL - 626 584 - IDNDQGSSSPSNDEAAMAVIMSLLEADAGLGGPVDFSDLPWPL - 626
	E. superba (CYC/BMAL)	623 A <mark>G</mark> D <mark>GGG</mark> ASDS <mark>DE</mark> AAMAVIMSLLEADAGLGGPVDFSHLPWPLP664
Crustaceans	P. leniusculus (BMAL1a) E. pulchra (ARNT-Ike 1) T. californicus (CYC) D. pulex (CYC) C. finmarchicus (CYC)	623 DLETDGTSDSDEAAMAVIMSLLEADAGLGGPVDFSHLPWPLP 664 605 DVESEVTSDSDEAAMAVIMSLLEADAGLGEPVDFSHLPWPLP 664 648 SSSEMDRGPKEGGNDEAAMAVIMSLLEADAGLGGPVDFSGLPWPLP 669 956 QSSSRENSSPGDGNDEAAMAVVMSLLEADAGLGGPVDFSGLPWPLP 633 642 - SPPSSSASSEGNDDAAMAVIMSLLEADAGLGGPVDFSGLPWPLP 663
Insects	D. melanogaster (CYC) B. mori (CYC-like factor b) D. plexippus (CYC) A. pemyi (CYC/BMAL) A. gambiae (CYC) T. castaneum (CYC) A. mellifera (ARNT-like isoform X1) T. domestica (CYC) G. bimaculatus (CYC)	665 L P S P L P L P L P L P L P L D G G P V S P L P L D G G P V S P L D G G Q V S P L D G G Q P V S P P P L G I G G Q V S L A A V S L E A A V N S L E A A V N S L E A A V N S L E A A V N S L E A A V N S L A A V N S L A A V N S L A A V N S L

Fig. S2. EsCYCLE/BMAL. Multiple alignment of the CYC/BMAL sequences corresponding to the mBMAL1's BCTR domain.

Α	CRY	<u>β2</u>	<u>α2</u>	<u>α6</u>		α12	α15		
Vertebrates	M. musculus (CRY1) H. sapiens (CRY1) G. gallus (CRY1) D. rerio (CRY1A) X. laevis (CRY1)	34 VY I L DPW40 34 VY I L DPW40	64 ANL RKL N 70 64 ANL RKL N 70 64 ANL RKL N 70 64 ANL RKL N 70 64 ANL RKL N 70	145 QPPL T YKR 152 311 145 QPPL T YKR 152 311	GNPICVQIPWD GNPICVQIPWD GNPICVQIPWD GNPICVQIPWD GNPICVQIPWD	KNPEALAKWAE KNPEALAKWAE KNPEALAKWAE KNPEALAKWAE RNPEALAKWAE	G R 334 376 EGMKVF EEL G R 334 376 EGMKVF EEL	L 385 402 CSSFFQQFFHC 1385 402 CSSFFQQFFHC L 385 402 CSSFFQQFFHC L 385 402 CSSFFQQFFHC L 385 402 CSSFFQQFFHC L 385 402 CSSFFQQFFHC	YCPVG417 YCPVG417 YCPVG417 YCPVG417 YCPVS417 YCPVG417
Crustaceans	E. superba (CRY2) E. pulchra (CRY2) T. saltator (CRY2)	38 F DPW 44 46 F L DPW 52 82 Y L DPW 88	68TTLRKLN74 76RSLRTFN82 112NSLRNLN118	149 KLPLTYKT 156 314 157 KPPLTYKS 164 332 193 KPPLTYKS 200 358	GNP I CVQ I PWD	KNAEALAKWAH	G R 337 379 EGMKVFDEL GQ 345 387 EGMKVFDEP GQ 381 423 EGMKVFDEL	L 388 405 CSSFFQQFFHC L 396 413 CSSFFQQFFHC L 432 449 CSSFFQQFFHC	YCPVR420 YCPVR428 YCPVR464
Insects	D. pulex (CRY2) D. plexippus (CRY2) A. mellifera (CRY) B. mori (CRY2) T. castaneum (CRY)	42 TFTLDPW 48 60 VFTTDPW 66 52 VFVLDPW 58 64 VFTTDPW 70 48 VFVLDPW 54	90KNLRKLN96 82CSLRKLN88 94KSLKKLN100 78RSLRKLN84	153 KAPLTYRQ 160 318 171 KAPLTYHQ 77 336 163 KPPLTYHQ 69 328 175 KAPLTYHQ 81 340 159 RAPLTYHQ 85 324	GNPICVQIPWE GNPICVQIPWE GNPICVQIPWE GNPICVQIPWE GNPICVQIPWE	SNAEALAKWAN KNQEALKKWAN KNVEALAKWAN KNQDALAKWAN KNAEALAKWAN	GQ 341 383 EGMKVFEEL GQ 359 401 EGMKVFDEL GQ 351 383 EGMKVFDEL GQ 363 405 EGMKVFDEL GQ 347 389 EGMKVFEEL	L 392 409 CSSFFHQFFHC L 410 427 CSSFFQQFFHC L 402 419 CSSFFQQFFHC L 414 431 CSSFFQQFFHC L 398 415 CSSFFQQFFHC	YCPVR424 YCPVR442 YCPVR434 YCPVR446 YCPIK430
	M. musculus H. sapiens G. gallus D. rerio X. laevis	470 MV NHAEASR 470 MV NHAEASR 470 MV NHAEASR 470 MV NHAEASR 470 MV NHAEASR 470 MV NHAEASR	α22 * * * * * * * LNIERMKQIYO LNIERMKQIYO LNIERMKQIYO LNIERMKQIYO	2QL SRY 494 QQL SRY 494 QQL SRY 494 QQL SCY 494 QQL SRY 494					
	E. superba	473 MVDHVKQSQ	ANLMRMKQVY(QQLSHY 497					
	E. pulchra T. saltator D. pulex	481 MV DHSKQ SQ 517 MV DHG TQ SQ 477 MV NHL EV SQ	HN I E <mark>RMKQ VYO</mark> NN I E <mark>RMKQ VYO</mark> L N I E RMKQ VYO	QQLTHY 505 QQLAHY 541 QRLTQY 501					
	D. plexippus A. mellifera B. mori T. castaneum	495 I VDHTKASE 487 MVNHSKSSR 499 MVDHTKASQ 483 MVNHAAASR	INLERIKQVY/ INIERMKQVY/ INIERIKQVY/ VNIQRMKQVY/	QLAKF 519 QLNKY 511 QLARY 523 QLSNY 507					
					Period	C domain			
Vertebrates	PER M. musculus (PER2) H. sapiens (PER2) G. gallus (PER2) D. rerio (PER2) X. laevis (PER2)	1132 YVLQDPIVL 1254 CVLQDPIVL 1219 YVLQDPIVL 1322 YVLQDPIVL 1322 YVLQDPVVL	LMANT DDS IM LMADADSSVMM LMANT DDT VMM LMANV DED VMM LMANADEN VMM	ATYQL PSRDLQAVL ATYQL PSRNLEAVL ATYQL PSRNLEAVL ATYQL PSRDLETVL ASYQL PSRD I QKVL ASYQL PKRD I EVVL	KEDQEKLKLLQF KEDREKLKLLQF KEDKLKLKQMQF REDREKLRQMQF KEDRERLKSLQF	* RSQPRFTEGQR CLQPRFTESQK CLQPKFTEDQK CSQPRFTDEQK CLQPHFTEDQK	REL REVHPWVHTGGL QEL REVHQWMQTGGL REL I EVHPWIQQGGL REL ADVHPWMRRGGL KELGETHTWMHRGGL	PTAIDVTGCV PAAIDVAECV PKTVANSECI PKAIDIKACM PRVTEVTDCV	YCE121 YCE121 FCE130 GCE135 YCR140
Crustaceans	E. superba (PER) N. norvegicus (PER) C. finmarchicus (PER2 C. finmarchicus (PER1 D. pulex (PER) T. californicus (PER) E. pulchra (PER)	1119 PKLGGPSWL 1134 PVLNDPPWL)1103 PLLSEPFWN)1083 PVLSEPFWN 1111 TCKRIPPWL 1210 PVLQDPFWL 1155 PVLCEPPWI	ENVDVTPELLY EDVKVTPELLY ENVKLDDHLIY QRVKMTSDLIF EAVSVSNELVY QNVEVKSERFF EDVKVTPELLF	YKYQLETKELVDVL YKYQLEEEDIEDVL YKYQLEEEDIEDVL YYQMETQHLDDVL YQYQIPGRNSTDVL NYQMNTRKLADVL RYQLKTSELVDVL	RQDMDALKKFR RKDRAQFLKME LKDQEALADMQ EADRERLRLQ DEDRKHLQRMY RQDMDLLKNTK	- QPVCVDDQL - QPAWVDDQL - QPNLVDEQL - QPEIVDDQL - QPSLVTEQL - QVKKMNMYW	SFLYNEMEREGEST - SSLYNELELEGSST - TELLADLDFDYQGI - KELFTELEDGVELE - MQLYTEMEAEQRNGI YQLYEELAECGEKP - KACWIYK	KLHLEEGITS DUHLEEGITS DVLFDEDEDSDEETTS EFLVDFECPPTDD DLALDDSENSIS RSLLDESSSSLNINSS	SSSGEEGP 120 - SSEDQAGT 121 SADDS 118 FETSENE 117 SSSSESE - N - 128
Insects	D. plexippus (PER) A. mellifera (PER) B. germanica (PER) T. castaneum (PER)	1024 QKKVDPSWV 992 - RRKEPPWL 1061 - VLKEPPWM 998 RKRDPPWL	EEVCVTSELIN EGVHVTPELIN EDVHVTPDLVN EAVTITPEIN	YKYQIRTKSLEEVL YEYQIHPKTLNEVL YRYQMKDQTLKDVL YKYQMTVKSLDDIL	SEDKKKLETLE QADMNALKNFN QADLQALEKIH EADLHTLKKID	QPLLVSQQL QPLLVNDQL QPFLVNDQL QPMLVNDQL	GQLYLDLQLQGVAA- GQLYLDLEVEGFET- SQLYLDMELEGLST- NQLYIEMELEGLST-	RLTLEEGITS5 KLVLEDGITS5 KLKLEEGITS5 KLTLEEGITS5	SSS
B Vertebrates	CRY M. musculus (CRY2) H. sapiens (CRY2) G. gallus (CRY2) D. rerio (CRY3) X. laevis (CRY2)	Serine loop o	12 * * * * * NR 69 163 KPPLT NR 70 164 KPPLT NR 60 154 KPPLT NR 51 145 QSPLT NR 55 145 QSPLT NR 55 145 QSPLT	α6 * * * * * * * * * * * * * * * * * * *	* * * * PTE221 331 PICIO PTE222 332 PICIO PTE222 322 PICIO DTE203 315 PICIO PTE203 315 PICIO	VDRNPEAL IPWDRNPEAL IPWDRNPEAL IPWDRNAEAL	14 *** * *** *** KWAEGKT 353 385 RG DL KWAEGKT 354 386 RG DL KWAEGKT 344 376 RG DL KWAEAKT 337 398 RG DL KWAEAKT 337 398 RG DL	α17 * *** * * * * WVSWESGVRVFDE 401 WVSWESGVRVFDE 402 WISWESGVRVFDE 392 WISWEGGMKVFEE 395 WISWEGGMKVFEE 397	
				XKTEON 150 201 EL GE	ETE 207 216 PLCVC				
Crustaceans	E. pulchra (CRY2) T. saltator (CRY2) D. pulex (CRY2)	52 WF AG S S NVG VI 88 WF AG S S NVG VI 48 WF AG S S NVD I	NR63 157KPPLT NK99 193KPPLT NK59 153KAPLT	YKSFQN167 209 DLGF YKTFQN203 245 HLGF YRQFQN163 205 ELGF	DT D215 324 P I C V Q ET E 251 360 P I C V Q DT D211 320 P I C V Q	I PWDKNPEAL	AKWANGQT 346 378 RGDL AKWAHGQT 382 414 RGNL AKWANGQT 342 374 RGDL	WV SWEEGMK VF DE 394 WV SWEEGMK VF DE 430 WI SWEEGMK VF EE 390	
Insects	D. plexippus (CRY2) A. mellifera (CRY) B. mori (CRY2) T. castaneum (CRY)	66 WFASSSNVGI 58 WFAGSTNIGI 70 WFASSSNVGI 54 WFAGSSNVGI	NK 77 171 KAPLT NK 69 163 KPPLT NK 81 175 KAPLT NK 65 159 RAPLT	Y HQFQA 181 223 ELGF Y HQFQT 173 215 ELGF Y HQFQA 185 227 ELGF Y HQFLA 169 211 ELGF	DT E 229 338 P I C V Q DT E 221 330 P I C V Q E T E 233 342 P I C V Q DT E 217 326 P I C V Q	I PWEKNQEALI I PWDKNVEAL I PWEKNQDAL I PWDKNAEAL	KWANGQT 360 392 RGDL AKWANGQT 352 384 RGDL AKWANGQT 364 396 RGDL AKWANGQT 364 380 RGDL	WISWEEGMKVFDE 408 WISWEEGMKVFDE 400 WISWEEGMKVFDE 412 WLSWEEGMKVFEE 396	
	M. musculus H. sapiens G. gallus D. rerio X. laevis	420 CSAFFQQFFH 421 CSAFFQQFFH 411 CSAFFQQFFH 406 CSAFFQQFFH	* * * CYCPVG435482V CYCPVG436483V CYCPVG426473V CYCPVG4219466 CYCPVG421468I	The second secon	α22 * * * * * * * RLNIERMKQIYQ RLNIERMKQIYQ RLNIERMKQIYQ RNNIERMKQTYQ	* QL 509 QL 510 QL 500 QL 493 QL 495			
	E. superba	405 CSSFFQQFFH	CYCPVR 420 467 R			QL 494			
	E. pulchra T. saltator D. pulex	413 CSSFFQQFFH 449 CSSFFQQFFH 409 CSSFFHQFFH	CYCPVR428 475 I CYCPVR464 511Q CYCPVR424 471 K	DYPMPMVDHSKQSC HYPLPMVDHGTQSC DYPLPMVNHLEVSC	QHNIERMKQVYQ QNNIERMKQVYQ QLNIERMKQVYQ	QL 502 QL 538 RL 498			
	D. plexippus A. mellifera B. mori T. castaneum	427 CSSFFQQFFH 419 CSSFFQQFFH 431 CSSFFQQFFH 415 CSSFFQQFFH	CYCPVR442 489 K CYCPVR434 481 K CYCPVR436 493 R CYCPIK430 477 K	DYPMPIVDHTKASE DYSLPMVNHSKSSE DYPMPMVDHTKASC DYSLPMVNHAAASE	EINLERIKQVYA RINIERMKQVYQ QINIERIKQVYA RVNIQRMKQVYQ	QL 516 QL 508 QL 520 QL 504			
	PFR				Period	LC domain			
Vertebrates	M. musculus (PER2) H. sapiens (PER2) G. gallus (PER2) D. rerio (PER2) X. laevis (PER2)	1133 VLQDP IWLU 1255 VLQDP IWLU 1220 VLQDP IWLU 1323 VLQDP IWLU 1323 VLQDPVWLU	MANTDDS I MMT MADADSSVMMT MANTDDTVMMT MANVDEDVMMS MANADENVMMS	YQLPSRDLQAVLK YQLPSRNLEAVLK YQLPSRDLETVLK YQLPSRDIQKVLR YQLPSRDIQKVLR	EDQEKLKLLQRS EDREKLKLLQKL EDKLKLKQMQKL EDREKLRQMQKS EDRERLKSLQKI	QPRFTEGQRR QPRFTESQKQI QPKFTEDQKRI QPRFTDEQKRI QPHFTEDQKKI	ELREVHPWVHTGGLP ELREVHQWMQTGGLP ELIEVHPWIQQGGLP ELADVHPWMRRGGLP ELGETHTWMHRGGLP	TAIDVTGCV AAIDVAECV KTVANSECI KAIDIKACM RVTEVTDCV	
	E. superba (PER)	1120 KLGGPSWLEI	NVDVTPELLYK	VQLETKELVDVLR	Q DMDALKKFR	QPVCVDDQL SI	FLYNEMEREGEST - K	L HL EEG I T S	SSSGEEGP 1203
Crustaceans	N. norvegicus (PER) C. finmarchicus (PER2 C. finmarchicus (PER1 D. pulex (PER) T. californicus (PER) E. pulchra (PER)	1135 VLNDPPWLE[11104 LLSEPFWNE] 1104 VLSEPFWNG] 1112 CKRIPPWE 1211 VLQDPFWLQ 1156 VLCEPPWIE]	DVKVTPELLYK NVKLDDHLIYK RVKMTSDLIFN AVSVSNELVYC NVEVKSERFFN DVKVTPELLFF	YQMETKELVDVLR YQLEEEDIEDVLR YQMETQHLDDVLL YQIPGRNSTDVLE. YQMNTRKLADVLD YQLKTSELVDVLR	QDMDMLKRLR KDRAQFLKME KDQEALADMQ ADRERLRLLQ EDRKHLQRMY QDMDLLKNTK	QPAWVDDQLS QPNLVDEQLTI QPEIVDDQLKI QPVMLNHQLM(QPSLVTEQLY(QVKKMNMYWK)	SLYNELELEGSST - D ELLADLDFDYQGI - D ELFTELEDGVELE - E QLYTEMEAEQRNGID QLYEELAECGEKP - R ACWIYK	LHLEEGITS VLFDEDEDSDEETTSA FLVDFECPPTDD-TE LALDDSENSIS SLLDESSSSLNINSSS	SSEDQAGT 1218 DDS 1189 TSENE 1170 1190 SSESE - N - 1299 - 1214
Insects	D. plexippus (PER) A. mellifera (PER) B. germanica (PER) T. castaneum (PER)	1025 KKVDPSWVE 993 RRKEPPWLE 1062 VLKEPPWLE 999 RKRDPPWLE	EVCVTSELIYK GVHVTPELIYE OVHVTPDLVYF AVTITPEIIYK	YQIRTKSLEEVLS YQIHPKTLNEVLQ YQMKDQTLKDVLQ YQMTVKSLDDILE	EDKKKLETLE ADMNALKNFN ADLQALEKIH ADLHTLKKID	QPLLVSQQLG QPLLVNDQLG QPFLVNDQLS QPMLVNDQLN	QLYLDLQLQGVAA-R QLYLDLEVEGFET-K QLYLDMELEGLST-K QLYIEMELEGLST-K	L T L E EG I T S S S L V L E DG I T S S G L K L E EG I T S S S L T L E EG I T S S S	SSDSGSSN - 1103 SSDSGSSN - 1077 GSSGEELT 1147 1076

Fig. S3. Multiple sequence alignment of domains responsible for the PER/CRYs interaction in mouse according to the *m*PER2:*m*CRY1 (A) and *m*PER2:*m*CRY2 (B) models, respectively. (A) *m*PER2:*m*CRY1 interaction model (7): 29/29 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 2/2 residues involved in zinc finger are conserved in *Es*CRY2; 15/28 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 0/2 residues involved in zinc finger are conserved in *Es*PER. (B) *m*PER2:*m*CRY2 interaction model (8): 32/36 residues involved in low energy interactions, 2/3 residues involved in salt bridges, and 0/2 residues involved in low energy interactions, 3/3 residues involved in *Es*CRY2; 18/34 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 0/2 residues involved in zinc finger are conserved in *Es*CRY2; 18/34 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 0/2 residues involved in zinc finger are conserved in *Es*CRY2; 18/34 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 0/2 residues involved in zinc finger are conserved in *Es*CRY2; 18/34 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 0/2 residues involved in zinc finger are conserved in *Es*CRY2; 18/34 residues involved in low energy interactions, 3/3 residues involved in salt bridges, and 0/2 residues involved in zinc finger are conserved in *Es*CRY2; 18/34, and *E*CRY2; 18/34 residues represent the conserved amino acids (identity in bold font style) involved in low energy interactions (blue), salt bridges (red), and zinc fingers (yellow). * = the residue is conserved among all the sequences aligned; $\alpha = \alpha$ helix position in *m*CRY sequence.



Fig. S4. *Es*TIMELESS1. (A) Multiple sequence alignment of serine-rich domain, putative *Es*CRY1 binding site (CTT motif), and the conserved region of the C-terminal cytoplasmic localization domain (CLD core). (B) Phylogenetic relationships of the TIMELESS protein family that confirm the annotations of *Es*TIM1 and *Es*TIM2 as a TIMELESS and TIMEOUT proteins, respectively. The *N. crassa* timeless domain-containing protein TGO1 has been used as outgroup. Bootstrap confidence values based on 1,000 replicates are shown at nodes. Scale bar indicates amino acid substitutions per site. The most relevant orthologues are indicated in bold



Fig. S5. *Es*PERIOD. (A) Visual comparison between T/G (blue), S/G (yellow), and S/Q (green) repeats found in relevant crustaceans and insects. (B) Phylogenetic relationships of the PERIOD protein family. *Es*PER clusters with other crustaceans orthologues. The *H. sapiens*'s bHLH-PAS protein ARNT has been used as outgroup. Bootstrap confidence values based on 1,000 replicates are shown at nodes. Scale bar indicates amino acid substitutions per site. The most relevant orthologues are indicated in bold

		СТТ
	E. superba	482 IVIHKNASKENRRMMQDISAKLEMATPHVCPSNNVETRTFLRLPDQCQHNVC533
Crustaceans	D. nigrofasciatus D. magna D. pulex	487 I I DHQ LASEKNRSYMDE I RNRLM NPPPHCRPSSEK ET ROFMWF PDDCSEHSSQ 539 476 I V I HEEVL T RNQT WMK DF RRKFK ET PAHCQPSSNSE V YK F F CL PDDSL P F 525 476 MV V HEEVL PRNLEWMKEF RQKFK ET PAHCQPSSNSE V YK F F CL PDD SL P F 525
Insects	D. melanogaster A. gambiae D. plexippus A. pernyi B. mori	495 I I DL SMAVK RNML AMKSL RNSL I TPPPHC RPSNEE EV ROFFWL A DVVV

Fig. S6. EsCRY1. Multiple sequence alignment of CRY1's putative EsTIM1/EsCRY1 binding site (CTT motif) inside the CCE domain.



Fig. S7. Schematic representation of the constructs used in the Fig. 3D's experiment.



Fig. S8. Full-length western blot images. (A) Co-immunoprecipitation of *Es*CLK-V5 and MYC-*Es*CYC/BMAL co-expressed in HEK293 cells; experiment 1 α V5+ α Myc, (B) experiment 2 α V5, and (C) experiment 2 α Myc (digital imaging). (D) Western blot of *Es*CRY1 protein after a 8 hours light pulse in *Drosophila* cells (film exposure) and Ponceau S staining used for total protein normalization (digital imaging). Dashed line rectangles mark bands displayed in the main text figures.

SUPPLEMENTARY TABLES

	Contig resulted from krill database mining		Transcript red by molecula appro	construction ar cloning ach	Deduced protein information		tblastx search statistics		Sequence
	Accession number	Length (nt)	GenBank accession number	Length (nt)	Protein length (aa)	Protein type	Score	E-value	status
CLOCK	ESS034514	1659	KY888674	4652	1329	F	650	0	Confirmed
CYCLE/BMAL	ESS133965	303	KY922996	2767	664	F	911	0	Confirmed
PERIOD	ESS133963	2339	KY922997	4127	1261	F	1117	0	Confirmed
TIMELESS 1	ESS040526	4047	KY922998	5300	1311	F	1094	0	Confirmed
CRYPTOCHROME 1	ESS023688	1819	KY922999	1844	533	F	662	0	Confirmed
DOUBLETIME	ESS096455	1694	KY923000	3342	345	F	667	0	Validated
SHAGGY	ESS074789	1350	KY923001	1966	415	F	666	0	Validated
VRILLE	ESS123359	1509	KY923002	2224	488	F	233	5e-69	Validated
SLIMB	ESS133964	1350	KY923003	1457	449	С	764	0	Validated
CASEIN KINASE 2α	ESS071625	1722	-	-	350	F	629	0	Validated
CASEIN KINASE 2β	ESS051147	805	-	-	244	N	422	3e-148	Validated
PROTEIN PHOSPHATASE 1A	ESS073680	2139	-	-	329	F	663	0	Validated
PROTEIN PHOSPHATASE 2A wbtB	ESS023456	3762	-	-	634	F	884	0	Validated
PDH	ESS011558	422	-	-	79	F	103	2e-27	Validated
PDH RECEPTOR	ESS048520	1035	-	-	344	I	359	7e-119	Predicted
CLOCKWORK ORANGE	ESS049812	2084	-	-	629	F	226	2e-62	Predicted
EZH 2	ESS108699	1719	-	2684	804	F	984	0	Predicted
HDAC 3	ESS014944	1677	-	4139	488	F	817	0	Predicted
CREB binding protein	ESS048098	2180	-	5322	1301	C	1387	0	Predicted
TIMELESS 2	ESS130297	2077	KY923004	4638	1364	F	966	0	Validated

 Table S1. Putative Euphausia superba circadian protein-encoding transcripts identified via in silico transcriptome mining.

Protein type: F = full coding sequence; N = amino-terminal partial protein; I = internal protein fragment; C = carboxy-terminal partial protein. Sequence status: Confirmed = functionally confirmed component; Validated = sequence validated by molecular cloning; Predicted = *in silico* predicted sequence.

	aa position	Sequence	Description
EsCLK NLS	27-58	RNLSEKKRRDQFNLLINELSVMVA ANNRKMDKST	highly conserved
NES	102-113	LEALDGFFLAI	highly conserved
EsCYC NLS	87-93	EKRRRDK	highly conserved
NES	112-121	LDKLTVLRM	highly conserved
EsPER NLS	523-532	EPAEKRRRTL	conserved among crustaceans
	704-739	RHNVDQQHKFVQKQRTHKHSKD RFKIKNI	 conserved in <i>D. melanogaster</i>, with only small effect
NES	744-765	KKATKRPHGLKRPGTIMDRECSAP KQIYM	 principal NLS in <i>D. melanogaster</i>, not detected by NLSmapper
	502-511	AQNYIVELLA	 principal NES in <i>D. melanogaster</i>, the distribution of the hydrophobic residues, albeit not the exact consensus sequence, is conserved in <i>E. superba</i>
CLD	426-476	GRPHRSKPYRFRTFNGSYVTLET EWLCFVNPWTMKIDSVIGQHRVIK GPED	highly conserved
EsTIM1 NLS	612-621	GPACKRPHHQ	• conserved in E. superba and E. pulchra
	643-651	RNVKRTKLL	 highly conserved among insects and crustaceans
EsCRY2 NLS	278-282	RKIKK	 conserved in <i>M. musculus</i>, with only small effect

 Table S2.
 Localization signals identified in the main components of krill circadian clock.
 Abbreviation: NLS = nuclear localization signal; NES = nuclear export signal; CLD = cytoplasmic localization domain.

Figure	Experin	nental comparison	t-test adjusted p-value
		E-Box-Luc + EsCyc/Bmal	0.027
Fig. 4B	E-Box-Luc	E-Box-Luc + EsClk	0.272
		E-Box-Luc + EsCyc/Bmal + EsClk	0.004
		E-Box-Luc + EsCyc/Bmal	0.008
Fig. 4B	E-Box-Luc	E-Box-Luc + EsClk	0.016
•		E-Box-Luc + EsCyc/Bmal + EsClk	0.011
		E-Box-Luc + EsCyc ¹⁻⁶²⁹ + EsClk	1.7E-04
		E-Box-Luc + EsCyc + EsClk ¹⁻¹¹¹⁷	1.6E-04
	E-Box-Luc + EsCyc/Bmal + EsClk	E-Box-Luc + EsCyc + EsClk ¹⁻⁸⁶³	1.8E-04
Fig. 4D		E-Box-Luc + EsCyc + EsClk ¹⁻⁶⁸⁵	1.5E-04
FIG. 4D		E-Box-Luc + EsCyc + EsClk ¹⁻⁵⁰⁰	1.8E-04
		E-Box-Luc + EsCyc + EsClk ¹⁻⁸⁶³	0.025
	E-Box-Luc + EsCyc + EsClk ¹⁻¹¹¹⁷	E-Box-Luc + EsCyc + EsClk ¹⁻⁶⁸⁵	0.051
		E-Box-Luc + EsCyc + EsClk ¹⁻⁵⁰⁰	0.935
	E Box Luc + $E_0C_{1/2}/B_{mol}$ + $E_0C_{1/2}$	E-Box-Luc + dCyc + <i>Es</i> Clk	9.9E-05
Fig. 4F	E-BOX-LUC + ESCYC/BITIAI + ESCIK	E-Box-Luc + EsCyc/Bmal + dClk	9.4E-06
Fig. 4E		E-Box-Luc + dCyc + <i>E</i> sClk	1.2E-05
		E-Box-Luc + EsCyc/Bmal + dClk	1.1E-04
	E Bay Luc L Eccur/Brad L Eccur	E-Box-Luc + <i>m</i>Bmal1 + <i>Es</i> Clk	9.4E-04
Fig. 4F	E-BOX-LUC + ESCYC/BITIAI + ESCIK	E-Box-Luc + EsCyc/Bmal + mClock	0.007
Fig. 4F	E Poy Luo + $mPmol1 + mClock$	E-Box-Luc + <i>m</i>Bmal1 + <i>Es</i> Clk	2.0E-04
	E-BOX-LUC + IIIBIIIAIT + IIIGIOCK	E-Box-Luc + EsCyc/Bmal + mClock	0.008
Fig 54	Light	Light E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk + <i>Es</i> Cry1	0.120
	E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk	Light E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk + <i>Es</i> Cry2	0.002
1 lg. 3A	Dark	Dark E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk + EsCry1	0.050
	E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk	Dark E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk + <i>Es</i> Cry2	9.4E-04
Fig 5B	E-Box-Luc + EsCvc/Bmal + EsClk	E-Box-Luc + EsCyc/Bmal + EsClk + EsCry1	1
Tig. ob		E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2	0.010
Fig. 5C	Dark	Light	0.007
		E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2	0.002
		E-Box-Luc + EsCyc/Bmal + EsClk + EsPer	0.062
	E-Box-Luc + EsCvc/Bmal + EsClk	E-Box-Luc + EsCyc/Bmal + EsClk + EsTim1	0.184
		E-Box-Luc + EsCyc/Bmal + EsClk + EsPer + EsTim1	0.002
		E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2 + EsPer	0.002
		E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2 + EsTim1	0.002
	E-Box-Luc + EsCvc/Bmal + EsClk + EsCrv2	E-Box-Luc + EsCyc/Bmal + EsClk + EsPer + EsTim1	0.035
		E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2 + EsPer	0.018
Fig. 5D	E-Box-Luc + EsCyc/Bmal + EsClk + EsPer + EsTim1	E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2 + EsPer	0.335
		E-Box-Luc + EsCyc/Bmal + EsClk + 1:20 EsCry2 + EsPer	3.8E-04
	E-Box-Luc + EsCyc/Bmal + EsClk + 1:20 EsCry2	E-Box-Luc + EsCyc/Bmal + EsCik + 1:20 EsCry2 + EsTim1	0.337
		E-Box-Luc + EsCyc/Bmal + EsClk + 1:20 EsCry2 + EsPer + EsTim1	8.8E-06
	E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk + 1:20 <i>Es</i> Cry2 + <i>Es</i> Per	E-Box-Luc + <i>Es</i> Cyc/Bmal + <i>Es</i> Clk + 1:20 <i>Es</i> Cry2 + <i>Es</i> Per + <i>Es</i> Tim1	0.002
	E-Box-Luc + EsCyc/Bmal + EsClk + 1:20 EsPer + 1:20	E-Box-Luc + EsCyc/Bmal + EsClk + 1:20 EsPer + EsTim1	0.421
	EsTim1	E-Box-Luc + EsCyc/Bmal + EsClk + EsPer + 1:20 EsTim1	5.2E-05
		E-Box-Luc + EsCyc/Bmal + EsClk + EsCry2	0.003
Fig. 5E	E-Box-Luc + EsCyc/Bmal + EsClk	E-Box-Luc + EsCyc/Bmal + EsClk + EsPer	0.442
		E-Box-Luc + EsCyc/Bmal + EsClk + EsPer + EsTim1	0.015
Fig. 5F	IgG control	A-V5	0.002

 Table S3. Student's t-test Bonferroni-corrected p-values for all the experimental comparisons discussed.

	CLOCK	CYCLE /BMAL	PERIOD	TIME- LESS	TIME- OUT	Vertebrate- like CRY	Drosophila -like CRY	Other CRYs	(6-4)- photolya se	Outgroup
Aedes aegypti	-	AEX32872.1	-	AAY40757. 1	XP_00166 0177.1	-	-	-	-	
Anopheles gambiae	XP_315720. 4	XP_556301.3	XP_321212.4	XP_001689 006.1	XP_00168 9129.1	ABB29887.1	XP_321104.4		-	
Antheraea pernyi	AAR14936. 1	AAR14937.1 (BMAL)	Q17062.1	AAF66996. 1	-	ABO38435.1	AAK11644.1		-	
Apis mellifera	XP_394233. 4	2 (ARNT-like 1)	NP_001011596.1	-	XP_00656 5496.1	NP_001077099.1	-	-	-	
Arabidopsis thaliana		-		-	-	-		-	-	NP_567341.1 (Plant CRY)
Bactrocera tryoni	-	-	-	-	-	-	AAU14170.1	-	-	
Blattella germanica	-	-	AAN02439.2	-	-	-	-	-	-	
Bombus impatiens	-	-	-	-	-	NP_001267051.1	-	-	-	
Bombyx mori	-	NP_001036982. 1 (CYC-like b)	BAD93177.1	NP_001037 622.1	-	NP_001182627.1	NP_001182628.1			
Calanus finmarchicus	comp76772 _cl_seq1	compl60482_c0 _seq1	compl71214_c0_ seq1 (PER1); comp205215_c0_ seq1 (PER2)	comp88114 _c0_seq1	-	-	comp37700_c0_seq 1	-	-	
Ceratitis capitata		-	ABB20914.1	-	-	-		-	-	
Danaus plexippus	EHJ69324.1	AAR13012.1	EHJ74075.1	EHJ67997. 1	EHJ76705 .1	ABA62409.1	AAX58599.1	-	-	
Danio rerio	AAI63244.1	NP_571652.1 (ARNT-like 1)	AAO38747.1 (PER2)		NP_00126 5529.1	NP_0010/0765.2 (CRY1a); NP_571865.4 (CRY1b); BAA96848.1 (CRY2a); BAA96849.1 (CRY2b); BAA96850.1 (CRY3)		AAH44385.1 (CRY4)	AAH44204.1 (CRY5)	
Daphnia magna	-	-	-	JAN75617. 1	JAN88373 .1	-	KZS20490.1	-	-	
Daphnia pulex	NCBI_GNO _118274	NCBI_GNO_11 44014	NCBI_GNO_2124 04	NCBI_GNO _238244	NCBI_GN O_88254	EFX82092.1	EFX77441.1	-	-	
nigrofasciatus	- NP 523964.			-		-	BAF45421.1	-		
Drosophila melanogaster	2 (CLK isoform A)	NP_524168.2	NP_525056.2	AAC46920. 1	AAF73481 .1	-	NP_732407.1	-	NP_477188. 1	NP_731308.1 (TGO isoform A)
Eurydice pulchra	AGV28720. 1 (CLK5)	AGV28715.1 (ARNT-like 1)	AGV28714.1	AGV28716. 1	-	AGV28717.1	-	-	-	
Gallus gallus	AAL98708.1	NP_001001463. 1	NP_989593.1 (PER2)	-	-	NP_989576.1 (CRY1); NP_989575.1 (CRY2)	-	NP_00103468 5.1 (CRY4)	-	
Gryllus bimaculatus	BAM76759. 1	BAN28450.1	BAG48878.1	BAJ16356. 1	-	-	-	-		
Homo sapiens	AAF13733.1	BAA19935.1 (BMAL1b)	NP_073728.1 (PER2)		BAA36499 .1	NP_004066.1 (CRY1); NP_001120929.1 (CRY2)		-	-	NP_001612.1 (ARNT)
Macrobrachium rosenbergii	AAX44045. 1	-	-		-	-	-	-	-	
Mamestra brassicae	-	-	-	-	-	-	AAY23345.1	-	-	
Mus musculus	NP_031741. 1 (CLOCK isoform 1)	BAD26600.1 (BMAL1b)	NP_035196.2 (PER2)	-	NP_00112 9554.1	NP_031797.1 (CRY1); NP_034093.1 (CRY2)	-	-	-	
Nephrops norvegicus	-	-	ALC74274.1	-	-	-	-	-	-	
Neurospora crassa	-	-	-	-	-	-	-	-	-	XP_958771.1 (TOF1)
Pacifastacus Ieniusculus	-	AFV39705.1 (BMAL1a)	-	-	-	-	-	-	-	
Talitrus saltator			-		-	AFV96168.1			-	
domestica		BAJ16354.1	-	-	-	-	-	-	-	
californicus	-	JW523145	JW535312	JW538330	-	-	-	-	-	
Tribolium castaneum	XP_008199 501.1 (CLK isoform X1)	NP_001107795. 1	NP_001106933.1	NP_001106 934.1	XP_00820 1051.1	NP_001076794.1	-	-	-	
Xenopus laevis	NP_001083 854.2	AAW80970.1 (BMAL1)	NP_001081098.1 (PER2)	-	XP_01810 2910.1	NP_001081129.1 (CRY1); NP_001082139.1 (CRY2a); AAK94667.1 (CPY2b)	-	-	_	

Table S4. Accession numbers for protein sequences included in the phylogenetic analyses. Protein sequences of the molecular clock components from *Drosophila melanogaster*, *Apis mellifera*, *Antheraea pernyi*, *Danaus plexippus*, *Daphnia pulex*, *Macrobrachium rosenbergii*, *Euridice pulchra*, and *Tigriopus californicus* were used to query the database. Orthologues to *E. superba* clock components were retrieved from NCBI databases (https://www.ncbi.nlm.nih.gov). For *Daphnia pulex*, protein sequences were downloaded from FleaBase (http://wfleabase.org). For *Calanus finmarchicus* we used the contigs published by Christie *et al.* (9). Legend: - = not used or not available.

Construct Name	Vector	Enzymatic digestion	Tag	Tag position	Expressed proteins	Primers (5'->3')
p <i>Es</i> Clk	pAc5.1/V5-HisA	EcoRI; Notl	FLAG	C-ter	EsCLOCK/FLAG	F:CAGTGTGGTGGAATTCATGTACGAAGAAGAAGCATT R:TAGACTCGAGCGGCCGCTTACTTGTCGTCATCGTCTTTG TAGTCCTTCTTGGGTGGTAGCTGTGG
p <i>Es</i> Cyc/Bmal	pAc5-STABLE2-neo	EcoRI; Notl	His	C-ter	<i>Es</i> CYCLE/His; GFP; neo	F:CAGTGTGGTGGAATTCATGTTCGGTCTGGGGAATTAT R:GCGTCCTTCGCGGCCGCCGTGGTGGTGGTGGTGGTGGTG GGCAGTGGCCATGGCAGATG
pdClk	pAc5.1/V5-HisA	EcoRI; Notl	FLAG	C-ter	dCLOCK/FLAG	F:CAGTGTGGTGGAATTCATGGACGACGAGAGGGGCGACGAC R:TAGACTCGAGCGGCCGCTTACTTGTCGTCATCGTCTTTG TAGTCTTGACTACTGCCTGGGGCTGT
p <i>d</i> Cyc	pAc5-STABLE2-neo	EcoRI; Notl	His	C-ter	dCYCLE/His; GFP; neo	F:CAGTGTGGTGGAATTCATGGAAGTTCAGGAGTTCTGC R:GCGTCCTTCGCGGCCGCCGTGGTGGTGGTGGTGGTGGTGTGA AGAAGACGGAGTTCTTGGC
p <i>Es</i> Clk ¹⁻¹¹¹⁷	pAc5.1/V5-HisA	EcoRI; Notl	FLAG	C-ter	<i>Es</i> CLOCK ¹⁻¹¹¹⁷ / FLAG	F:CAGTGTGGTGGAATTCATGTACGAAGAAGAAGCATT R:TAGACTCGAGCGGCCGCTTACTTGTCGTCATCGTCTTTG TAGTCTTGGGGTAGCTGCACGGACAA
p <i>Es</i> Clk ¹⁻⁸⁶³	pAc5.1/V5-HisA	EcoRI; Notl	FLAG	C-ter	<i>Es</i> CLOCK ¹⁻⁸⁶³ / FLAG	F:CAGTGTGGTGGAATTCATGTACGAAGAAGAAGCATT R:TAGACTCGAGCGGCCGCTTACTTGTCGTCATCGTCTTTG TAGTCGCTGACCATTGATCCCATAAG
p <i>Es</i> Clk ¹⁻⁶⁸⁵	pAc5.1/V5-HisA	EcoRI; Notl	FLAG	C-ter	EsCLOCK ¹⁻⁶⁸⁵ / FLAG	F:CAGTGTGGTGGAATTCATGTACGAAGAAGAAGCATT R:TAGACTCGAGCGGCCGCTTACTTGTCGTCATCGTCTTTG TAGTCGTCTCCTCTTGATGGCAGGTG
p <i>Es</i> Clk ¹⁻⁵⁰⁰	pAc5.1/V5-HisA	EcoRI; Notl	FLAG	C-ter	EsCLOCK ¹⁻⁵⁰⁰ / FLAG	F:CAGTGTGGTGGAATTCATGTACGAAGAAGAAGCATT R:TAGACTCGAGCGGCCGCTTACTTGTCGTCATCGTCTTTG TAGTCGTCCGCTATGGATCTACGTTG
p <i>Es</i> Cyc/ Bmal ¹⁻⁶²⁹	pAc5-STABLE2-neo	EcoRI; Notl	His	C-ter	<i>Es</i> Cyc/Bmal ¹⁻⁶²⁹ / His; GFP; neo	F:CAGTGTGGTGGAATTCATGTTCGGTCTGGGGAATTAT R:GCGTCCTTCGCGGCCGCCGTGGTGGTGGTGGTGGTGGTGC GCCCCGCCCCATCACCTGC
p <i>Es</i> Per	pAc5-STABLE2-neo	EcoRI; Notl	Мус	C-ter	EsPERIOD/Myc; GFP; neo	F:CAGTGTGGTGGAATTCATGACTGAAAATGAGAGATCT R:GCGTCCTTCGCGGCCGCCCAGATCCTCTTCTGAGATGA GTTTTTGTTCTGAGTCGGAGGAGTCTCTACC
p <i>Es</i> Tim	pAc5.1/V5-HisA	EcoRI; Notl	V5	C-ter	<i>Es</i> TIMELESS/ V5	F:CAGTGTGGTGGAATTCATGGAGTGGATGATGATGAAC R:TAGACTCGAGCGGCCGCTTACGTAGAATCGAGACCGAG GAGAGGGTTAGGGATAGGCTTACCGTGTAAGTGAGCTGGC GCAGG
pEsCry1	pAc5.1/V5-HisA	EcoRI; Notl	НА	C-ter	EsCRY1/HA	F:TCCAGTGTGGTGGAATTCATGACCAACACTGGTTGTGTA G R:CGGCCGCCACTCGAGTTAAGCGTAATCTGGAACATCGTA TGGGTAACAGACATTGTGTTGACATTGATC
pEsCry2	pAc5.1/V5-HisA	EcoRI; Notl	Мус	C-ter	EsCRY2/Myc	F:TCCAGTGTGGTGGAATTCATGACGAGAGGAGGAGAGAA ACATG R:CGGCCGCCACTCGAGTTAAGCGTAATCTGGAACATCGTA TGGGTAGGCCGGCTGCATTTGCTGTGT

Table S5. Main features for the generation of the constructs used for the functional characterization of the krill circadian clock components.

Transcript	Forward Primer (5'->3')	Reverse Primer (5'->3')
EsClock	GCTGCAGCAAATGATAATGC	TGCCATATTGGGCCATAACT
EsCycle	TTTTGATTTAAGGCATTTTTCATCTA	CCCAGTCTCACGATTCTAGCTACT
<i>Es</i> Period	GCAGCTATGCCCAACTTTAATC	GGACTAGCAACAGGGACATTTT
EsTimeless1	TTGAAGACTCTCCTACAATGATGG	TACAGAGGCCGCATGACA
EsCryptochrome2	TCATGAACCATGGACTGCAC	GGTGGACACGACTTCAACAA
Spike	AATCCCCGACTCAGACCCAT	GGCTTCGGGAAATCAGCTGA

Spike sequence	TGATGGATGCCACCAATATGCCAGCTGTCAAAGCCATCATATATCAGTATATGGAAGAGATTTATCATAGAA
	TCCCCGACTCAGACCCATCTTCTAGCAAAACAAGGCAGATCATCTCCACCATCCGGACGCAGAATCTTCCC
	AACTGTCAGCTGATTTCCCGAAGCCACTACTCCCCCATCTACCTGTCGTTTGTCA

 Table S6. Primers for quantitative real-time PCR experiments and spike synthetic oligoribonucleotide sequence.