

Appendix A Primers used in quantitative RT-PCR of *DIAGO* genes.

Genes ID/Accession number		Gene Name	Primers used in quantitative RT-PCR (5'→3')		T _m (°C)
1	EU330204	<i>Fe-SOD</i>	Forwards:	GGTCAGATGG TGAAGCCGTA GAG	58
			Reverse:	GTCTATGCCACCGATAACAACAAACCC	
2	AK073620	<i>eIF-4a</i>	Forwards:	TTGTGCTGGATGAAGCTGATG	58
			Reverse:	GGAAGGAGCTGGAAGATATCATAGA	
3	DQ471426	<i>EF-1a</i>	Forwards:	GATGATTCCCACCAAGCCCAT	58
			Reverse:	GGGTCCTTCT TCTCAACT CT	
4	–	<i>UBQ</i>	Forwards:	GCCGACTACAACATCCAGAAG	53
			Reverse:	GCTTGGTGTA GGTCTTCTTC TT	
5	Dl0031503	<i>DIAGO1</i>	Forwards:	GAACATCATTCGAGTGGGGC	58
			Reverse:	CCATATCCTCCACGGCCTC	
6	Dl0022883	<i>DIAGO2</i>	Forwards:	GAAGTCTGGTGGTGGTAATG	59
			Reverse:	GGGTGGAGGAGAAGAAGAAGA	
7	Dl0025430	<i>DIAGO4</i>	Forwards:	AAGAAGGAAATGGAGCGGAG	58
			Reverse:	GCCACGCCTAGACATTAGAAC	
8	Dl0026081	<i>DIAGO5-1</i>	Forwards:	GCCGGAAGAGGTAGAGGATC	57
			Reverse:	GCCAATGTAAGTTTCTGCGC	
9	Dl0027040	<i>DIAGO6</i>	Forwards:	ATCATGAGTAGGCGTGGAGT	60
			Reverse:	GCATCCTGAGCTTTAACCGA	
10	Dl0018449	<i>DIAGO7</i>	Forwards:	TCTCCACAAACCCTCTTCCA	59
			Reverse:	AATCAGGTCTCTTTGCAGCC	
11	Dl0002921	<i>DIAGO10</i>	Forwards:	AAACCCCACTTGCAAAACAC	58
			Reverse:	TGGTGAAGTTTGGTTGTGGT	
12	Dl0029681	<i>DIAGOMEL1</i>	Forwards:	GGTCTCACTCTCCAAACTCAG	57
			Reverse:	TTGAAGAAGCAGCTGTCTCC	

Appendix B RNA-seq data of the *DIAGO* family that were used in this study.

Gene ID	EC	IcpEC	GE
Dlo031503	230.30	259.47	201.41
Dlo022883	21.67	20.36	19.29
Dlo025430	321.52	294.69	392.05
Dlo026081	24.13	21.44	11.04
Dlo010130	0.17	0.17	0.01
Dlo010241	0	0	0
Dlo027040	81.12	81.37	38.44
Dlo018449	0.20	0.12	0.08
Dlo002921	8.78	12.65	33.78
Dlo029681	2.65	2.61	0.53

Appendix C The information for each conserved domain of *DIAGO*s

Protein Name	Gly-rich_Ago1		ArgoN		DUF1785		PAZ		ArgoL2		ArgoMid		Piwi	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
DIAGO1	81	189	201	336	345	397	402	534	538	584	594	674	687	1008
DIAGO2			138	274	283	335	340	474					632	930
DIAGO4			67	229	239	291	295	427	436	482	489	571	579	887
DIAGO5-1			126	259	268	320	325	455	459	506	516	596	613	915
DIAGO5-2			84	220	229	281	286	418	421	468	478	558	575	882
DIAGO5-3			81	201			316	377	365	406			472	779
DIAGO6			55	214	231	283	284	419	420	466			563	855
DIAGO7			162	309	318	370	378	509					666	979
DIAGO10			149	284	293	345	350	482	486	532	542	622	634	956
DIAGOMEL1			97	231	240	292	297	430	456	499	509	588	598	843

Appendix D The sequences of conserved domain of DIAGO proteins

Protein Name	Conserved domain						
	Piwi	ArgoN	PAZ	DUF1785	ArgoL2	ArgoMid	Gly-rich_Ago1
	LIVILPDNNGSLYGDLKRICETDLGLVSQ						
	CCLTKHVFRMSKQYLANVALKINVKVGGRN	RCIVKANHFFAELPDK					
	TVLLDAISRRIPLVSDRPTIIFGADVTHPHGE	DLHQYDVTITPEVTSR	DFVAQLLNRDVSARPLS				GGPSEYQGRGRGAS
	DSSPSIAAVVASQDWPEVTKYAGLVCAQAH	GVNRAVMEQLVKQHR	DADRVKIKKALRGVKV				
	RQELIQDLYKTWQDPVRGQVTGGMKELLIS	ESHLGKRLPAYDGRKS	EVTHRGNMRRKYRISGL	PVGRSFYSPDLGRR	EREVDIMQTVHHNA	PQVGQWNMMNKKMV	PQQGGRGGYTPARG
	FRRATGQKQRIIFYRDGVSEGQFYQVLLYEL	LYTAGPLPFISKEFRINL	TSQTTGELTFPVDESGTL	QPLGEGLESWRGF	YHNDPYAKEFGIKIS	NGGTVNHVICINFSRN	SAGMGGGGTSGGR
DIAGO1	DAIRKACASLEPNYQPPVTFVVVQKRHHTRL	LDEDDGQGGQRRERD	KSVVEYFYETYGFVIQH	YQSIRPTQMGLSLN	ERLASVEARILPAPW	VQDSVARGFCYELAQM	GGPSFGGQPRSSPIP
	FANNHHRNTIDRSGNILPGTVVDSKICHPT	FRVVIKLAARADLHHL	SQWPCLQVGNQQRPNY	IDMSSTAFI	LKY	CYVSGMAFNPEPVLPP	ELYQAQPASYPSPGV
	FDFYLCSHAGIQGTSRPAHYHVLWDENKFTA	GLFLQGRQADAPQEAL	LPMEVCKIVEGQRYSKR			SSRPEQVERVLKA	TPQASSEAGSSSQI
	DGLQSLTNNLCYTYARCTRSVSIVPPAYYAH	QVLDIVL	LNERQITA				VEH
	LAAFRARFYM						

	ILLCVMDKKHSGYKYLKWISETRVGVVTQC	TARLCVNHFPVKFNP	
	CLSSHANSARDQYLANLSIKINAKLGGSNVE	SIIRHYDVDVKPVVPSK	DFLSEHIRYFGQRNFRD
	LFDRLPYFEDEGHVMFVGADVNHPASRDTS	HGRPSKLSKSLAMIR	WGKVESALKGLKVTVT
	PSMAAVVATVNWPGANRYVARVRPQSHRT	NKLFEDDPAQFPLPMT	HRRTKQKFIIGGLTKQIT SVGRSFHPVHPYEE
DIAGO2	EKILNFGDMCMELIDSYARLNKVKPAKIVIFR	AYDGEKNIFSAVELPT	RQLSFLIEDPEEKAPPRQ DDLGFGLTASRGFQ
	DGVSEGQFDMVLNEELVPLKRAFRQRDYSPT	GNFNVKLEEDGRVQS	VRIIDYFREKYHTDIMW QGLKATSQGLALCL
	ITLIVAQKRHQTRLFPESRRDGGATGNVPPGT	FIFTIKLVNELRLGKLK	KDFPCLDLGRNGRKNY DYSVLAFRK
	VVDTTIVHPFEFDYLCSHYGLGTSKPTHYH	DYLTGNLSTIPRDILQG	VPMEFCELVGQIYQKD
	VLWDDHKFTSDQLQKLIYNMCFTFARCTKP	MDVVM	K
	VSLVPPVYYADLVAYRGR		
		QKIPLLTNHFKVNVNVTN	
	LLCLLPERKNCDIYGPWKRKNLADFGIVTQCI	VEGHFFHYSVSICYED	
	APTRVNDQYLTNVLLKINAKLGGLNQLLAIE	GRPVDGKGVGRRVIDR	DFLIANQNVDRPFSIDW
	RSPSIPVSKAPTIILGMDVSHGSPGQSDVPSIA	VHETYKAELD GK DFA	AKAKRVLKNLRIKTMPS
	AVVSSRHWPLISRYAAVRTQSPK VEMIDSL	YDGEKSLFTV GSLPRN	NQEYKITGLSEKSCKEQ VRQSFHNDPKNY ERMSALSNAKNSK PRNGRWNFNKKLVEP
DIAGO4	FKKVSDETEGIVRELLDFYTSSGKRKPEQII	KLEFTVVLEDVSSNRN	MFSLKQKNVKDDDGES ADVGGGVLGCRGF YDAEPMRSCGISIST TKIERWAVVNFSARCDI
	IFRDGVSESQFNQVMNIELDQMIEACKFLDE	NNGNASPDGQGSPNW	QNLEISVYDYFVNHRNI HSSFRTTQGGLSLNI NFAQVEGRVLSAPR RSLVRDLVKCGDMKGI
	KWNPKFVIIVAQKNHHTKFFQSGSPDNVPPG	GDRKRVRRPYQSKSYR	ELRYSGDLPCMNVGKP DVSTTMII LK QIDQ
	TVIDNRICHPKNND FYLCAHAGMIGTTRPTH	VEISFAAKIPMQAIANA	KRPTYIPLELCSLVSLQR
	YHVLYDEIGISADDLQELVHLSLYVYQRSTT	LRGQESSENSQEALRVL	YTKALSTLQRASL
	AISVVAPICYAHLAATQMGTFMK		
		DIIIL	

	LIVILPDVSGSYGRIKRV CETELGIVTQCCQPR	RPCMVRANHFLVEVA			
	QASRLNMQYFENVALKINVKVGGRNTVLND	DRDIHHYDVTISPEITS	NMLSNALSDQDRVKVK		
	AIQRKIPHVSDHPTIIIIGADVTHPQPGEDSSPSI	KKINREVMAQVTRLY	KALRGIKVALNHMEfvK		
	AAVVASMDWPEVTKYRGIVSAQTHREEIIKD	GESHLGRRIPAYDGRK	SYKITGITSEPLSQLTFPL	VGRSFFSPQLGPKG	DRENNIKRMVLSNN PSFGQWNMINKRMING
	LYTTHQDAQRGVIHGGMIRELLRAFRMSTGH	SLYTAGPPFDSKEFVV	DDNVRTSVVKYFRDKY	ELGDGIEFWRGFYQ	YNRDKLANEEFGIH GRVEFWTCVCFSTRVS
DIAGO5-1	KPHRIIFYRDGVSEGQFSQVLLHEVDAIRQAC	DLEQDAGARTRRDRK	RIVLKYTALPALLAGSE	SLRPAQMGLSFNIDI	VGDDLTLCEARILPS RELPSQFCEQLANMCTS
	TSLEAEYMPFITFVVVQKRHHTRLFPVDHRN	FKVTIKLASKPDLFTLQ	ARPVYLPMELSRIVEGQ	SARGFYE	PMLNY KGMAFNPRPVIITANP
	RQMTDKSGNILPGTVVDTKICHPTEFDYFVLS	QFLRGRVLDSPQEVIQ	RYSKKLNERQVTAL		NQIERALTDVN
	HAGIQGTSRPTHYHVLFDENKFSADELQVLT	VLDVVL			
	NNLCYTYARCTRSVSI				
	VGGCYGRIKRV CETELGIVTQCCRPQQALKL	KKCLVRANHFLVQVA			
	NMQYFENVALKINVKRRIPHVS DHPTIIIIGAAI	DRDIHHYHVTITPWAA	FVKKYMYNKNLSNPLS		
	THPHPGEDSSASIAAVVASMDWPEVTKYRCT	EKIKRDVMAQVTRMY	NQDLVKVKKALRGVKV		
	VSAQPPRLEIIKDLYKTHQDAQRGVVHGGMI	GESYLGKRIIAYDGRK	APTHMEYVKSYRITGIS	VRRSFFSPHLGPKG	DRESNITRVVLSNNY PSFGQWNMINKKMING
	RELLAAFKMSTGCKPHRIIFYRDGVSEGQFSK	SLYTAGPPFDSKEFIV	KSYVVQYYREKYGIVL	ELGDGIEFWRGFYQ	NQVKLANEEFGIHV GRVEFWTCVCFSKRVN
DIAGO5-2	VLLHEVDAIRQACASLEAEYMPFITFVVVQK	NLEQDFRGSSTGTRIE	KYTALPALQAGSEAKPI	VSARGFYE	MLNY GELPSLFCEQLVMCKS
	RHHTRLFPADHQNEQMTDRSGNILPGTVVDT	RKFEVAIKLASKLDFIG	YLPMELSRIVEGQQYSK		KGVEFSPPVPIITANP
	KICHPKEFDYFVLSHAVSQGTSRPTHYHVLV	LQQFLRQGHLDFRQEV	RLNEGQEIA		CQMEKALTDVHR
	DENRFTADELQLLTNNLCYTYARCTRSVSVV	IQVL			
	PPAYYAHLAAIRACY				

	VGGCYGRIKRV CETQLGIVSQCCRPQQALKL		MVKKVLRGVK VAPTH	
	NMQYFENVALKINVKRRIPHVSDHPTIIIGAAI			
	THPQPGEDSSPSIAAVVASMDWPEVTKYRCT	AAEKIKRDVMVQVIR	MEFVKSYRITGISNQPLS	
	VSAQPHRLEIHKDLYKTHQDAQRGVVHGGMI	MYGESYLGKRIIAYDG	QITIFEDFRGQGIDELWK	
	RELLAAFKMSTGCKPHRIIFYRDGVSEGQFSK	GESLYTAGPPFFYSKEF	KVRFWGSVWASISINFK	VLSNNYNEVKQANE
DIAGO5-3	VLLHEVDAIRQACGSLEAEYMPFITFVVVQK	VVNLEQDFHASSSTGT	NTYLSLRTSPALRRLGV	EFGIHVGD DLTLCD A
	RHHTRLFPADHKNEQMTDRSGNILPGTVVDT	RKERKFEVAIKLASKL	PLTSMVYMFMLNDKVA	RILPSPMLNY
	KICHPKEFD FYLNSHAASQGTSRPTHYHVL F	DFIGLQQFLRQGH LDF	KKSVIQYYREKYGIALK	
	DENRFTSDELQLLTNTLCYTYARCTRSVS VV	HQEV IQVL	YTALPALQAGSEANIYL	
	PPAYYARLAAIRARYYM		PMEVV	
	FILCVLAERKNSDIYGPWKKICLTDMGIPTQC	RIGLLTNHFKVSVKAQ		
	ISPTRINDQYL TNVLLKINSKLGGINSL LAME	DAVFYQYTVTISGDDK	IDFLTLNQNVQDPRHID	
	HSSHPLIKDTP TLILGMDVSHGSPGRSDASIA	RGVEGKGIGRKL LDRL	WAKAKKMLKNLRVKP	
	AAVRTQSSKMEMIDALYKPLADGTDDGIIRE	YQMYS AELAHKKFAY	RHRNMEFKIIGLSERPCN	VRQSFHDDSKNFV
	LLMDFYQTSNQRKPRQIIVFRDGVSESQFSQV	DGEKSLYTVGALPQNN	QQLFTMKKEGDTVEITV	DVGGGVTVGVRGFH
DIAGO6	LNIELDQIVKAYQHLGEADV PKFTVVVAQKN	LEFIVVLDESRAKPKN	YDYFKNHC GIELTRSAY	SSFRPTQGGLSLNM
	HHTKLFQSNGTENVPPGT VVDTKVVHPRNY	GSPSADDSASDAGKRS	LPCLDVGKPNRPIYLPLE	DTSTTMIV
	DFYMCAQAGMIGTSRPAHYHVLLDEIGFSPD	KVSFRSKTFKVDISYSA	LCSLISLQRYTKSLSLMQ	
	ILQNLHLSLYVYQRSTTAIAIVAPICYAHLAA	KIPLKSIALALKGSEVD	RASL	
	AQMGQFIK	SGTQDALRVLDIAL		

LICIMERKHKGYADLKRIAETSVGVVQCCL
 ISLLANHFLVQFDSSQR
 YSNLGLSSQFLANLALKINAKVGGCTVALY LEFLKDLLQRKTRGLSG
 IFQYNVEITPNPSKEVA
 NSLPSQIPRLFRPDEPVIFMGADVTHPHPLDD EERKEVEKALKNMRFV
 RKIKQKLVGNNSVVL
 FSPSVAAVVGS MNWPAANKYVSRMRSQTHR CHRETVQRYRVYGLTEE PVGRSLYSSSMEGT
 GADPAYDGRKNLYSP
 QEIIDLGAMVGELDDFYNEENKLPKRIFF ATENLWFPDRDGKSLRL KEIGGGVVGLRGYF
 VEFQNRLEFFISLPITT
 RDGVSETQFYKVLQEELQAIKEACSRFPGYSP VSYFKDHYNYNIQFRNL QSLRPTQQGLALNV
 NKSLLPHGEVNESKEK
 PITFAVVQKRHHTRLFPFENNDPSSSTQNQNQF PCLQISRKPCYLPMELC DSSVSAFH
 HQHKLFRINIKLVSKF
 LDENIPPGTVVDTVITHPREDFYLC SHWGV MICEGQKFLGKLSDDQT
 DGKDLNSYLSKEGDD
 KGTSRPTHYHILWDENHFTSDELQKLVYNLC ARI
 WIPLQDYLHALDVVL
 YTFVRCTKPVSLVPPAYYAHLAAYRGR
 LLAILPDNNGSLYGDLKRICETDLGLISQCCL
 TKHVFRISKQYLANVSLKINVKMGRNTVLL KCIVKANHFFAELPDK
 DAISCRIPLVSDIPTIFGADVTHPENGE DSSPSI DLNQYDVTITPEVASR CRTLS DSDRVKIKKALR
 AAVVASQDWPEVTKYAGLVCAQHRQELIQ TVNRAIMAELVRLYKE GVKVEVTHRGNVRRKY
 DRENDILQTVQHNA PQVGQWNMMNKKMIN
 DLYKTWHD PVRG TGSGMIRDLLISFRKATG SDLGMRLPAYDGRKSL RVSGLTSQPTREL VFPV PIGRSFFSPDIRTPQ GMTVNRWACINF SRSV
 YDQDPYAKEFGIKIS
 DIAGO10 QKPLRIIFYRDGVSEGFYQVLLYELDAIRKA YTAGELPFAWKEFKIK DDNSTMKS VVEYFQEM RLG DGLESWCGFY QESVARGFCNELA QMC
 EKLASVEARILPAPW
 CASLEPNYQPPVTFIVVQKRHHTRLFANNHR LVDEDDGINGPKRERE YGFTIQHTHL PCLQVGN QSIRPTQMGLSLNI QVSGMEFNPDPIPIYN
 LKY
 DRSSDTSKSGNVLP GTV VDSKICHPTFE DFYL YKVVIKFVARANMHH QKKANYLPMEACKIVE DMSAAFI ARPEQVEKALKHVY
 CSHAGIQGTSRPAHYHVLWDENNFTADGIQS LGQFLAGKRADAPQE GQRYTKRLNERQITA
 LTNNLCYTYARCTRSVSVVPPAYYAHLA AFR ALQILDIVL
 ARFY

RCMIRANHFLVELSDR
DLHHYDISITPEVTSRG ASRPLSEQDLIKLKKVL
IIIPLDLTGSYGRIKRV CETELGIVSQCCQPKH
LNRAVMRELLDKEGK RGVKVEVCHGEAKRYK PRVGQWNMINAKMYH
ARKCSPQYLENVALKINVKAGGQNTVLEDA VGRSFFSAGLDTKT
TVFDERKPAYDGRKGF VTGITSAPTNQLKFLAE QIAQFNYYQNDSLA GAKVKFWTCLNFSTVS
FYRRIPLLSDIPTIIFGADVTHPPPAGEDSSPSIA EIGSGVECWRFYQ
DIAGOMELI YTAGALPFTSKDFVVK GKKEKLVIQYFREKYNI KEFGVNVKSELVCID RQMAAGFCQDLINMCR
AVVASMDWPVVTTYRGQVYAQKHRQEIIQD SLRPTQMGMMLNM
LIDRDKKKGSTIKQRDF SLRYASWPSLQSGNDSK GRVLPPPVLKY NKGMDVNPSPFPVWS
LYKEKGLVGAGMVRELLIAFKRATNQKPQRI DVSATSFYE
IFYRDGISEGQFSQVLHCEMDAIH
KVTIKLASRTDLKHLK PIFLPMELCTIVEGQRYS SNSSQIERALSDVHR
EFLTSRQEEAPHETIQV KKLNEKQVAA
LDVVV

Appendix E The information for each motif of DIAGOs.

Motif	E-value	Sites	Width	Best possible match
Motif1	3.3E-277	10	50	EFDLYLCSHAGIQGTSRPTHYHVLWDENKFTADELQKLTNNLCYTYARCT
Motif2	3.2E-196	9	50	GADVTHPHPGEDSSPSIAAVVASMDWPEVTKYRGLVSAQSHRQEIIQDLY
Motif3	1.6E-118	9	29	RIIFYRDGVSEGQFSQVLLHELDAIRKAC
Motif4	8.1E-85	10	22	YGRJKRICETDLGIVSQCLPK
Motif5	1.7E-125	9	41	GDGVEGWRFYQSLRPTQMGLSLNIDVSATAFYEPJPVIDF
Motif6	3.1E-81	6	33	EPEYMPPITFVVVQKRHHTRLFPADHRBRQMTD
Motif7	1.4E-97	6	41	DARILPSPMLKYHETGKEKRVNPSVGQWNMINKKMINGGRV
Motif8	1.8E-83	9	27	RSVSVVPPAYYAHLAAYRARYYMEGET
Motif9	5.3E-88	10	29	SELGKRIPAYDGRKSLYTAGPLPFBSKEF
Motif10	1.7E-76	10	21	NDQYLENVALKINVKLGRNT