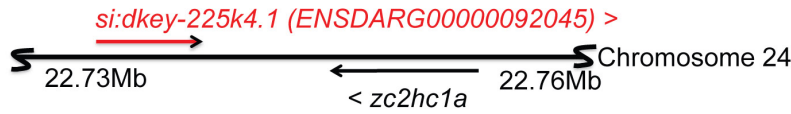


**Supporting Information for**

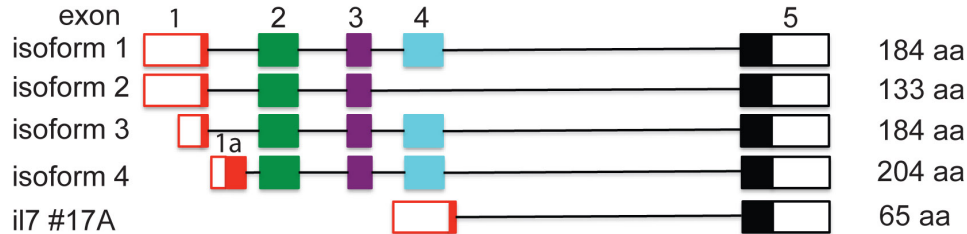
Evolutionary transition from degenerate to non-redundant cytokine signaling  
networks supporting intrathymic T cell development

Divine-Fondzenyuy Lawir, Isabell Hess, Katarzyna Sikora, Norimasa Iwanam, Iliana  
Siamishi, Michael Schorpp, and Thomas Boehm

## A Genomic location



## B Transcripts



## C Conceptual translation of il7 transcript isoforms

1: **M** HLPGTSVLTLLIILLPLAFPCDYNSNRITEDYRNIISIKLEHV  
 VNGIKQLNSNCTSRNVGVRARWQATN  
 AQQTIKKICRVNSKLEIFGSESRIINGELKVIFNALVKEMNESLGRCRYQK  
 KQQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMGNLRSKPEYSTWTPPTLQHSQDSGFD

2: **M** HLPGTSVLTLLIILLPLAFPCDYNSNRITEDYRNIISIKLEHV  
 VNGIKQLNSNCTSRNVGVRARWQATN  
 KQQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMGNLRSKPEYSTWTPPTLQHSQDSGFD

3: **M** HLPGTSVLTLLIILLPLAFPCDYNSNRITEDYRNIISIKLEHV  
 VNGIKQLNSNCTSRNVGVRARWQATN  
 AQQTIKKICRVNSKLEIFGSESRIINGELKVIFNALVKEMNESLGRCRYQK  
 KQQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMGNLRSKPEYSTWTPPTLQHSQDSGFD

4: **M** MRTLTLTSAPIAIIITGGFPVI  
 HLPGTSVLTLLIILLPLAFPCDYNSNRITEDYRNIISIKLEHV  
 VNGIKQLNSNCTSRNVGVRARWQATN  
 AQQTIKKICRVNSKLEIFGSESRIINGELKVIFNALVKEMNESLGRCRYQK  
 KQQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMGNLRSKPEYSTWTPPTLQHSQDSGFD

il7 #17A: **M** KQQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMGNLRSKPEYSTWTPPTLQHSQDSGFD

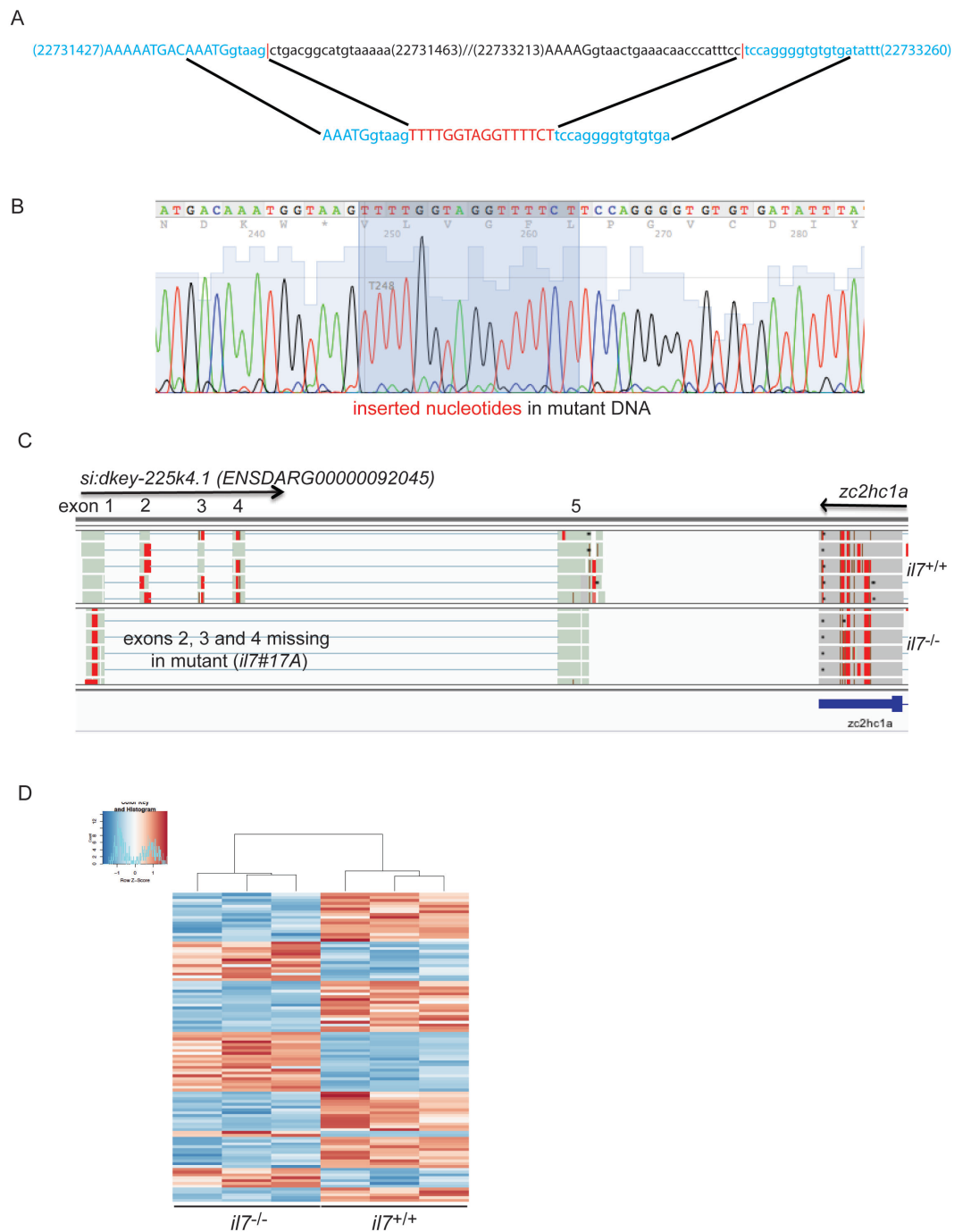
## D Sequence comparison of zebrafish and fugu il7 homologs

MHLPGTSVLTLLIILLPLAFPCDYNSN--RITEDYRNIISIKLEHV  
 MpLlciS-LlvLLLLPqsltCDrNqllrdaaElynaIvktldLDnt  
 --VNGIKQLNSNCTSRNVGVRARWQATNAQQT--IKKICRVNSKLE  
 renisaslqeisCpqlrfkaenctpntsdelntlhKlACRmit---  
 IFGSESRIINGELKVIFNALVKEMNESLGRCRYQK-----  
 -----LNldq---tkrLtesvlrSIGCPdapattttktsvs  
 -----KQQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMG>  
 mktarpRkshKqktknwtrRLCKanaIvsSitecYEIlnSrIshs.

## E Sequence comparison of zebrafish and mouse il7 homologs

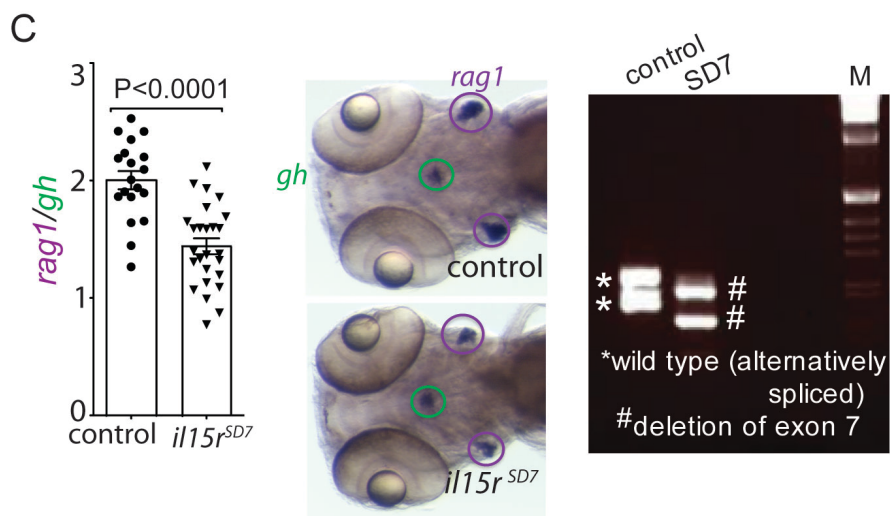
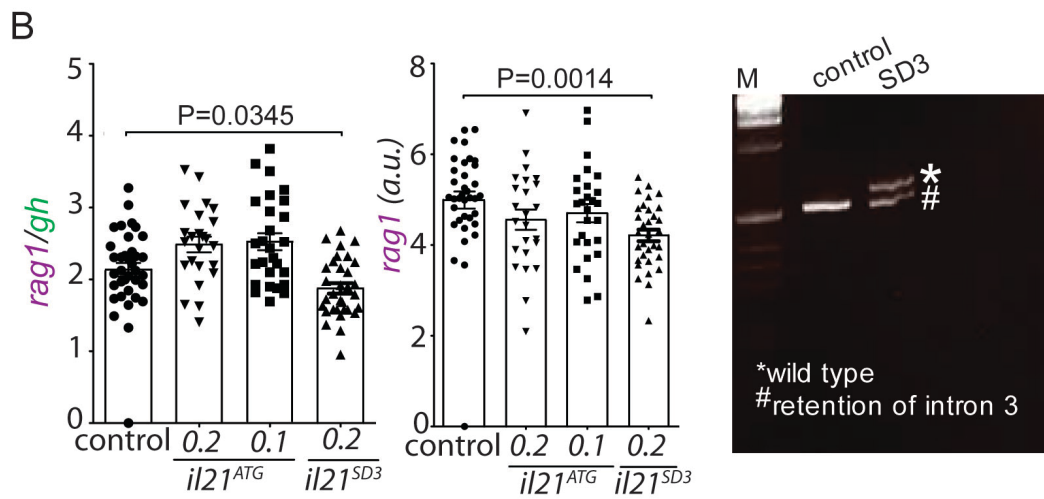
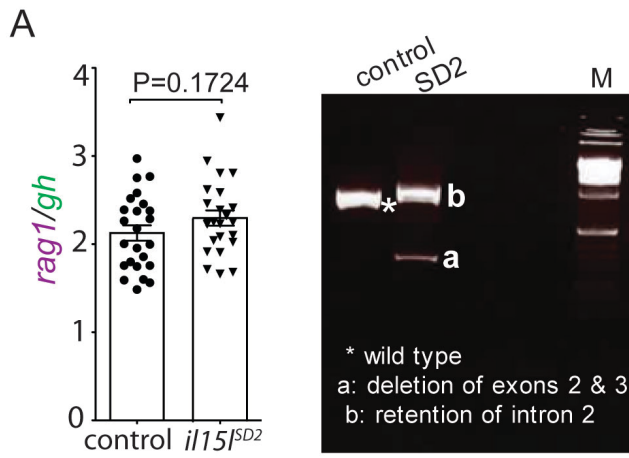
MHLPGTSVLTLLIILLPLAF-----PCDYNSNRITEDYRNI--ISIK-LEHVVNGIKQLNSNCTSRNVG  
 MfhvsfryIfgIppLiLvllpvtssChi-kdKegkaYesvlmISIdeLDkm----tgtDSNCpnnepn  
 VRARWQATNAQQTIKKICRVNSKLEIFGSESRIINGELKVIFNALVKEMNESL-----GRCRYQKKQQ  
 ffrKh-----vCddtkeaaflnraaRkLkqfLkMniseefnvhl1TvsggtqtlvnCtsKeek  
 KQKSPQNPQKWKLCVKVDILYSVQNYEYLHNTFQSMGNLRSKPEYSTWTPPTLQHSQDSGFD  
 --nvkeqkKndaCflKrLLreiktawnkilkgsi

**Fig. S1.** Characterization of the *il7* locus (ENSEMBL ID: ENSDARG00000092045). (A) Schematic of the region on chromosome 24 (GRCZ11) containing the *il7* gene (*si:dkey-225k4.1*) relative to the flanking *zc2hc1a* gene. (B) Structure of four transcript isoforms arising from the *il7* locus as determined by cDNA cloning; for comparison, the structure of the transcript emanating from the mutant *il7#I7A* allele is also indicated. In wild-type fish, isoform 1 represents the most prevalent transcript species. (C) Conceptual translation of the transcripts shown in (B). (D) Sequence comparison of the conceptually translated proteins for zebrafish and fugu (Genbank accession number NP\_001129620.1) *il7* proteins. (E) Sequence comparisons of the conceptually translated proteins for zebrafish and mouse (Genbank accession number NP\_032397.1) *il7* proteins. For (D) and (E), identical amino acid residues are in bold face capital letters.



**Fig. S2.** Characterization of the *il7#17A* allele. (A) Sequence analysis of the breakpoint regions of the *il7#17A* allele. The nucleotide coordinates on chromosome 24 are indicated in brackets; exon sequences are in upper case, intron sequences are in lower case (compare to Fig. 1E). The 16 nucleotides of non-templated sequences joining the two ends of the deletion are indicated in red upper case. (B) Sanger sequence traces for the region across the deletion in the gene. (C) Kidney marrow RNA reads mapping to the *il7* genomic region, indicating that sequences corresponding to exons 2-4 are not present in transcripts of *il7*-deficient animals. By contrast, no differences in reads mapping to the *zc2hc1a* flanking gene are observed, as expected; n=5 each for each genotype. (D) Heat map displaying differentially expressed genes in wild-type and *il7*-deficient kidney marrow tissue (see Table S1).

Although only few changes are observed, they are highly reproducible across genotypes. When differential gene expression analysis was conducted using RNA from whole embryos at 5 dpf, no significantly expressed genes were found between wild-type and *il7*-deficient embryos, attesting to the negligible effects of the mutation in the context of the entire organism.



**Fig. S3.** Characterization of *il15l*, *il21*, and *il15r* morphants. (A) Unaffected thymopoietic activity in *il15l* morphants as determined by the thymopoietic index (left panel); each data point represents one fish; mean±SEM. RT-PCR analysis of *il15l* cDNA structures resulting from interference with splicing of the pre-mRNA transcripts by an anti-sense oligonucleotide targeting the splice donor site of exon 2 (SD2) (right panel). (B) Minor impairment of thymopoietic activity in *il21* morphants as determined by the thymopoietic index (left panel); each data point represents one fish; mean±SEM. RT-PCR analysis of *il21* cDNA structures resulting from interference with splicing of the pre-mRNA transcripts by an anti-sense oligonucleotide targeting the splice donor site of exon 3 (SD3). Note that the apparent reduction of the *rag1/gh* ratio in SD3 morphants is the result of slightly higher numbers of *gh*-expressing cells. The anti-sense oligonucleotides were also injected at higher concentrations than usual (0.2mM), as indicated. (C) Reduced thymopoietic activity in *il15r* morphants as determined by the thymopoietic index (left panel) derived from quantitative analysis of whole mount RNA *in situ* hybridization experiments (middle panel); each data point represents one fish; mean±SEM. RT-PCR analysis of *il15r* cDNA structures resulting from interference with splicing of the pre-mRNA transcripts by an anti-sense oligonucleotide targeting the splice donor site of exon 7 (SD7) (right panel).

## References

1. Kono T, et al. (2008) Characterization and expression analysis of an interleukin-7 homologue in the Japanese pufferfish, *Takifugu rubripes*. *FEBS J* 275:1213-1226.

**Table S1.** Differentially expressed genes in kidney marrow of wild-type and *il7*-deficient animals at 3 months of age. Gene ontology analysis indicates that no specific pathway is enriched among the differentially expressed genes. The results for *il7* (si:dkey-225k4.1) are highlighted in red font. Column headers (40) are as follows: (A) ENSEMBL gene ID; (B) mean values of normalized counts of all samples; (C) shrunken log<sub>2</sub> fold change; (D) unshrunken log<sub>2</sub> fold change; (E) error estimate for log<sub>2</sub> fold change; (F) Wald statistic (the ratio of log<sub>2</sub>FoldChange to lfcSE); (G) nominal p value from Wald test; (H) p value adjusted for multiple testing; (I) gene name; (J) gene description.

**Table S2.** Characterization of cytokine gene morphants.



**Table S1.** Differentially expressed genes in whole kidney marrow of *il7* mutants.

ENSDARG	baseMean	log2FoldChange	lfcMLE	lfcSE	stat	pvalue	padj	external_gene_name	description
ENSDARG00000104174	365.562372	-3.652385252	-8.4705441	0.44963567	-8.1229882	4.55E-16	1.22E-12	CABZ01055168.2	
ENSDARG00000095615	499.606917	-3.146697949	-3.8575524	0.35033465	-8.9819775	2.66E-19	2.02E-15	si:dkeyp-86h10.3	si:dkeyp-86h10.3 [Source:ZFIN;Acc:ZDB-GENE-100922-65]
ENSDARG00000059125	240.590854	-3.142359103	-5.485564	0.4414443	-7.1183592	1.09E-12	2.13E-09	prkacbb	protein kinase, cAMP-dependent, catalytic, beta b [Source:ZFIN;Acc:ZDB-GENE-050904-4]
ENSDARG00000104099	57.2553027	-2.840196536	-5.5314123	0.44823562	-6.3363919	2.35E-10	3.37E-07	FP236513.1	
ENSDARG00000018491	2935.2848	-2.802967341	-3.6868129	0.38618335	-7.2581259	3.92E-13	9.37E-10	pdia4	protein disulfide isomerase family A, member 4 [Source:ZFIN;Acc:ZDB-GENE-030131-5493]
ENSDARG000000092780	60.7075222	-2.562764073	-4.3364727	0.44003615	-5.8239854	5.75E-09	6.86E-06	si:ch1073-170a4.1	si:ch1073-170a4.1 [Source:ZFIN;Acc:ZDB-GENE-030131-7012]
ENSDARG00000099002	339.942527	-2.482926247	-3.5668736	0.41501122	-5.982793	2.19E-09	2.94E-06	creb5a	cAMP responsive element binding protein 5a [Source:ZFIN;Acc:ZDB-GENE-120827-2]
ENSDARG00000104213	255.291001	-2.322481217	-2.7357581	0.325106	-7.143766	9.08E-13	1.95E-09	si:dkey-283b1.6	si:dkey-283b1.6 [Source:ZFIN;Acc:ZDB-GENE-120215-112]
ENSDARG000000095461	16.9745885	-2.100088539	-4.966897	0.45583781	-4.607096	4.08E-06	0.00224907	pimr171	Pim proto-oncogene, serine/threonine kinase, related 171 [Source:ZFIN;Acc:ZDB-GENE-141212-267]
ENSDARG00000092362	79.7775751	-2.085136911	-2.6729324	0.37447634	-5.5681406	2.57E-08	2.91E-05	hsp70.2	heat shock cognate 70-kd protein, tandem duplicate 2 [Source:ZFIN;Acc:ZDB-GENE-110405-1]
ENSDARG00000054667	24.3391503	-2.008014431	-3.8509562	0.45186574	-4.4438298	8.84E-06	0.0038741	adra2db	adrenergic, alpha-2D-, receptor b [Source:ZFIN;Acc:ZDB-GENE-021010-5]
ENSDARG00000069377	300.571195	-1.945363144	-3.3487352	0.44688581	-4.3531549	1.34E-05	0.0052719	si:dkey-242g16.2	si:dkey-242g16.2 [Source:ZFIN;Acc:ZDB-GENE-030131-8324]
ENSDARG00000055723	311.72777	-1.938413421	-2.9082653	0.42700554	-4.539551	5.64E-06	0.0028829	hsp70l	heat shock cognate 70-kd protein, like [Source:ZFIN;Acc:ZDB-GENE-050321-1]
ENSDARG00000094415	746.30401	-1.933387496	-4.2452213	0.45609534	-4.2389986	2.25E-05	0.00803818	si:ch211-264e16.2	si:ch211-264e16.2 [Source:ZFIN;Acc:ZDB-GENE-060503-320]
ENSDARG00000089202	160.566385	-1.852985007	-3.5810444	0.45408356	-4.0807137	4.49E-05	0.01321159	nkl3	NK-lysin tandem duplicate 3 [Source:ZFIN;Acc:ZDB-GENE-131127-147]
ENSDARG00000092283	20.8293396	-1.851475718	-5.2401014	0.45422614	-4.0761101	4.58E-05	0.01329365	cxl34b.11	CX chemokine ligand 34b, duplicate 11 [Source:ZFIN;Acc:ZDB-GENE-091204-33]
ENSDARG00000096153	46.525934	-1.836524679	-2.9970189	0.44006757	-4.1732789	3.00E-05	0.00992247	si:dkey-21h14.9	si:dkey-21h14.9 [Source:ZFIN;Acc:ZDB-GENE-110915-1]
ENSDARG00000089377	23.2723451	-1.800784766	-3.0772028	0.44456921	-4.0506286	5.11E-05	0.01402337	si:ch211-232p21.6	si:ch211-232p21.6 [Source:ZFIN;Acc:ZDB-GENE-131121-178]
ENSDARG00000076211	74.6838469	-1.798908392	-2.1163999	0.32378352	-5.5558985	2.76E-08	2.97E-05	CABZ01059914.1	
ENSDARG00000063518	32.1147812	-1.796665237	-3.3188226	0.45149999	-3.9793251	6.91E-05	0.01786907	zgc:153913	zgc:153913 [Source:ZFIN;Acc:ZDB-GENE-061103-397]
ENSDARG00000004262	1179.12843	-1.787259695	-1.9623243	0.25979336	-6.8795433	6.00E-12	1.07E-08	cyp46a1.4	cytochrome P450, family 46, subfamily A, polypeptide 1, tandem duplicate 4 [Source:ZFIN;Acc:ZDB-GENE-040426-1224]
ENSDARG00000075783	30.4859625	-1.766681148	-2.5423829	0.41572265	-4.249663	2.14E-05	0.00779478	hmgxb4a	HMG box domain containing 4a [Source:ZFIN;Acc:ZDB-GENE-070912-675]
ENSDARG00000098547	13.2212484	-1.766442171	-3.8269807	0.45573085	-3.8760645	0.00010616	0.02434187	RNF222	ring finger protein 222 [Source:HGNC Symbol;Acc:HGNC:34517]
ENSDARG00000004748	267.05039	-1.755449336	-2.5680736	0.42194912	-4.1603342	3.18E-05	0.01018848	zgc:100868	zgc:100868 [Source:ZFIN;Acc:ZDB-GENE-040801-33]
ENSDARG00000014967	55.3774229	-1.754692353	-3.4141113	0.45429144	-3.8624817	0.00011224	0.02534481	g6pcb	glucose-6-phosphatase b, catalytic subunit [Source:ZFIN;Acc:ZDB-GENE-081223-2]
ENSDARG00000025311	96.6939435	-1.750512266	-2.2737014	0.38153864	-4.5880341	4.47E-06	0.00240286	cuzd1.2	CUB and zona pellucida-like domains 1, tandem duplicate 2 [Source:ZFIN;Acc:ZDB-GENE-131119-30]
ENSDARG00000069632	198.679812	-1.728959838	-2.8299878	0.44208447	-3.9109264	9.19E-05	0.02219127	emp1	epithelial membrane protein 1 [Source:ZFIN;Acc:ZDB-GENE-070822-2]
ENSDARG00000076142	493.34555	-1.722546718	-2.5375238	0.42379539	-4.0645716	4.81E-05	0.01360108	trib1	tribbles pseudokinase 1 [Source:ZFIN;Acc:ZDB-GENE-091207-4]
ENSDARG00000098036	139.588758	-1.710673023	-2.2288024	0.38336018	-4.4623128	8.11E-06	0.0037352	si:ch211-6910.4	si:ch211-6910.4 [Source:ZFIN;Acc:ZDB-GENE-131127-259]
ENSDARG00000093258	31.4124399	-1.708650144	-2.6378735	0.43108635	-3.9635914	7.38E-05	0.01844133	igl3v1	immunoglobulin light 3 variable 1 [Source:ZFIN;Acc:ZDB-GENE-060503-880]
ENSDARG00000004746	4356.19391	-1.706406995	-2.1267265	0.36243364	-4.7081916	2.50E-06	0.0014128	hsp90aa1.2	heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 2 [Source:ZFIN;Acc:ZDB-GENE-031001-3]
ENSDARG00000093316	115.024016	-1.690630948	-2.2981004	0.40024571	-4.2239827	2.40E-05	0.00827462	adgrf8	adhesion G protein-coupled receptor F8 [Source:ZFIN;Acc:ZDB-GENE-130531-41]
ENSDARG00000063375	281.475539	-1.650943534	-1.927263	0.3189389	-5.176363	2.26E-07	0.00019441	pter	phosphotriesterase related [Source:ZFIN;Acc:ZDB-GENE-060825-190]
ENSDARG00000091317	113.436982	-1.643743075	-2.2169669	0.39746681	-4.135548	3.54E-05	0.01117802	hunk	hormonally up-regulated Neu-associated kinase [Source:ZFIN;Acc:ZDB-GENE-050309-240]
ENSDARG00000037495	1040.53621	-1.631467826	-2.2910637	0.41142594	-3.9653986	7.33E-05	0.01844133	rtt4rl2b	reticulon 4 receptor-like 2b [Source:ZFIN;Acc:ZDB-GENE-040310-2]
ENSDARG00000079004	159.322383	-1.619908537	-1.9948748	0.35528718	-4.5594342	5.13E-06	0.00268731	tanc2b	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2b [Source:ZFIN;Acc:ZDB-GENE-030131-7067]
ENSDARG00000092045	41.2459884	-1.602423221	-2.454429	0.43095581	-3.7183005	0.00020057	0.04026535	si:dkey-225k4.1	si:dkey-225k4.1 [Source:ZFIN;Acc:ZDB-GENE-091204-219]: il7
ENSDARG00000054343	114.481007	-1.556045474	-2.1212791	0.40153511	-3.8752414	0.00010652	0.02434187	slc7a8b	solute carrier family 7 (amino acid transporter light chain, L system), member 8b [Source:ZFIN;Acc:ZDB-GENE-030616-586]
ENSDARG00000020224	28.2161641	-1.526120095	-2.0622913	0.39667407	-3.8472898	0.00011943	0.02606999	ERGIC2 (1 of many)	si:ch211-225b10.3 [Source:ZFIN;Acc:ZDB-GENE-130531-53]
ENSDARG00000043531	4356.19225	-1.50108755	-1.7361376	0.31183605	-4.8137075	1.48E-06	0.00099454	jun	jun proto-oncogene [Source:ZFIN;Acc:ZDB-GENE-030131-7859]
ENSDARG00000059055	108.973872	-1.483135294	-1.6705553	0.28716866	-5.1646837	2.41E-07	0.00019899	ft2	alpha(1,3)fucosyltransferase gene 2 [Source:ZFIN;Acc:ZDB-GENE-990715-18]
ENSDARG00000042940	74.9045507	-1.476760558	-2.0246467	0.40333374	-3.6613861	0.00025085	0.04811252	nab1a	NGFI-A binding protein 1a (EGR1 binding protein 1) [Source:ZFIN;Acc:ZDB-GENE-050208-574]
ENSDARG00000060070	934.473929	-1.457255143	-1.669544	0.30362204	-4.7995697	1.59E-06	0.00103504	adcy7	adenylate cyclase 7 [Source:ZFIN;Acc:ZDB-GENE-040713-1]
ENSDARG00000105100	217.400284	-1.412677452	-1.7029216	0.3422761	-4.1273038	3.67E-05	0.01126345	si:dkey-3h2.2	si:dkey-3h2.2 [Source:ZFIN;Acc:ZDB-GENE-070705-464]
ENSDARG00000099401	1052.41063	-1.412251786	-1.6038207	0.2955863	-4.7777986	1.77E-06	0.00110442	ccl33.3	chemokine (C-C motif) ligand 33, duplicate 3 [Source:ZFIN;Acc:ZDB-GENE-081104-131]
ENSDARG00000099466	83.8234093	-1.410026379	-1.7897326	0.37150751	-3.7954182	0.0001474	0.03123682	si:dkey-190j3.2	si:dkey-190j3.2 [Source:ZFIN;Acc:ZDB-GENE-050208-399]
ENSDARG00000033587	177.833527	-1.397339768	-1.7272323	0.35777675	-3.9056193	9.40E-05	0.02243198	CABZ01088134.1	
ENSDARG00000077473	3340.72322	-1.393131989	-1.5487659	0.27413091	-5.0819953	3.73E-07	0.00029715	mych	myelocytomatosis oncogene homolog [Source:ZFIN;Acc:ZDB-GENE-030219-51]
ENSDARG000000040777	188.963549	-1.334914331	-1.6555102	0.35975191	-3.7106525	0.00020673	0.04070414	inhbb	inhibin, beta B [Source:ZFIN;Acc:ZDB-GENE-990415-2]
ENSDARG00000018263	845.649887	-1.31505892	-1.457374	0.27065143	-4.8588657	1.18E-06	0.00081808	pdia2	protein disulfide isomerase family A, member 2 [Source:ZFIN;Acc:ZDB-GENE-040426-705]
ENSDARG00000013576	183.480549	-1.270090807	-1.4509216	0.30086232	-4.2215017	2.43E-05	0.00827462	gad45bb	growth arrest and DNA-damage-inducible, beta b [Source:ZFIN;Acc:ZDB-GENE-050223-1]
ENSDARG000000041339	367.117883	-1.25022754	-1.3982657	0.28040918	-4.4585657	8.25E-06	0.0037352	KRT18 (1 of many)	zgc:92380 [Source:ZFIN;Acc:ZDB-GENE-040801-221]
ENSDARG00000098171	305.624215	-1.210563123	-1.3870966	0.30366171	-3.9865517	6.70E-05	0.01777898	zgc:162193	zgc:162193 [Source:ZFIN;Acc:ZDB-GENE-030131-2035]

ENSDARG00000068851	1131.18029	-1.207714306	-1.3362706	0.26888141	-4.4916244	7.07E-06	0.00345073	rnf183	ring finger protein 183 [Source:ZFIN;Acc:ZDB-GENE-060929-1090]
ENSDARG00000074378	1367.14604	-1.189104776	-1.3603555	0.30244839	-3.9315957	8.44E-05	0.0205983	junba	jun B proto-oncogene a [Source:ZFIN;Acc:ZDB-GENE-040426-2172]
ENSDARG00000069787	338.515089	-1.183897287	-1.3099252	0.2687689	-4.4048894	1.06E-05	0.00454701	tmc6a	transmembrane channel-like 6a [Source:ZFIN;Acc:ZDB-GENE-081114-3]
ENSDARG00000104502	133.207963	-1.183224126	-1.3727404	0.31433392	-3.7642267	0.00016707	0.03450701	si:dkey-264f17.5	si:dkey-264f17.5 [Source:ZFIN;Acc:ZDB-GENE-120703-23]
ENSDARG00000021444	127.759635	-1.16236149	-1.3297152	0.30224013	-3.8458212	0.00012015	0.02606999	prfl1.2	perforin 1.2 [Source:ZFIN;Acc:ZDB-GENE-111212-3]
ENSDARG00000074466	814.332087	-1.14470246	-1.230031	0.23168936	-4.9406778	7.79E-07	0.00057666	gdpd3a	glycerophosphodiester phosphodiesterase domain containing 3a [Source:ZFIN;Acc:ZDB-GENE-040426-2279]
ENSDARG00000036074	10482.9159	-1.143535976	-1.212726	0.21156178	-5.4052107	6.47E-08	6.05E-05	cebpa	CCAAT/enhancer binding protein (C/EBP), alpha [Source:ZFIN;Acc:ZDB-GENE-020111-2]
ENSDARG00000103980	3416.16554	-1.084132763	-1.1927201	0.26235181	-4.1323624	3.59E-05	0.01117802	ets2	v-ets avian erythroblastosis virus E26 oncogene homolog 2 [Source:ZFIN;Acc:ZDB-GENE-050522-552]
ENSDARG00000077726	1627.7123	-1.057026908	-1.1866961	0.28448574	-3.7155708	0.00020275	0.0403257	nocta	nocturnin a [Source:ZFIN;Acc:ZDB-GENE-050208-306]
ENSDARG00000020788	247.053945	-1.044011877	-1.1487573	0.26240436	-3.9786376	6.93E-05	0.01786907	sla2	Src-like adaptor 2 [Source:ZFIN;Acc:ZDB-GENE-080204-98]
ENSDARG00000067741	2852.57841	-1.036620565	-1.1514317	0.2732365	-3.7938583	0.00014832	0.03123682	itpkcb	inositol-trisphosphate 3-kinase Cb [Source:ZFIN;Acc:ZDB-GENE-080225-27]
ENSDARG00000077862	211.481611	4.144400758	6.38737769	0.41678422	9.94375636	2.69E-23	5.77E-19	si:dkey-169i5.4	si:dkey-169i5.4 [Source:ZFIN;Acc:ZDB-GENE-030131-8695]
ENSDARG00000094550	54.4677971	3.868616434	8.30265427	0.43545681	8.88404156	6.45E-19	3.46E-15	BX649490.2	
ENSDARG00000103982	38.6472731	3.637726645	6.34406382	0.42458505	8.56772201	1.06E-17	4.53E-14	AL929185.1	
ENSDARG00000097247	193.417597	3.520053805	5.58205852	0.42729854	8.23792616	1.75E-16	6.27E-13	BX322555.3	
ENSDARG00000100939	41.3706602	3.492372942	5.99580837	0.4267318	8.18399974	2.75E-16	8.43E-13	CABZ01117603.1	
ENSDARG00000093570	96.1090932	3.281431213	4.1798187	0.36560226	8.97541286	2.82E-19	2.02E-15	si:dkey-25o1.7	si:dkey-25o1.7 [Source:ZFIN;Acc:ZDB-GENE-091204-375]
ENSDARG00000051912	159.17244	2.936811263	4.7830739	0.43523434	6.74765529	1.50E-11	2.48E-08	HPX (1 of many)	zgc:152945 [Source:ZFIN;Acc:ZDB-GENE-060929-800]
ENSDARG00000096579	36.1545157	2.625400411	4.75828439	0.44440756	5.90764121	3.47E-09	4.39E-06	si:dkey-9c18.3	si:dkey-9c18.3 [Source:ZFIN;Acc:ZDB-GENE-121214-321]
ENSDARG00000045758	69.2920642	2.55360694	3.47825917	0.39593944	6.44948862	1.12E-10	1.72E-07	cracr2ab	calcium release activated channel regulator 2Ab [Source:ZFIN;Acc:ZDB-GENE-041210-94]
ENSDARG00000038770	51.5925112	2.465289324	7.34277656	0.45603771	5.4058892	6.45E-08	6.05E-05	zgc:103625	zgc:103625 [Source:ZFIN;Acc:ZDB-GENE-040912-116]
ENSDARG00000104185	233.056767	2.215258312	3.01424934	0.39948939	5.5452244	2.94E-08	3.00E-05	AL831726.2	
ENSDARG00000086831	54.4465584	2.01667722	2.84724884	0.40974211	4.9218208	8.57E-07	0.00061395	zmp:0000001228	zmp:0000001228 [Source:ZFIN;Acc:ZDB-GENE-140106-188]
ENSDARG00000092542	26.858509	1.9799767	4.29794332	0.45569711	4.34494024	1.39E-05	0.00534392	BX005068.1	
ENSDARG00000042677	109.551621	1.972754557	2.65594554	0.39578853	4.98436517	6.22E-07	0.00047692	cadm1b	cell adhesion molecule 1b [Source:ZFIN;Acc:ZDB-GENE-080327-34]
ENSDARG00000070138	20.1862819	1.66007827	3.12247969	0.43355572	4.53461395	5.77E-06	0.0028829	si:dkey-84o3.3	si:dkey-84o3.3 [Source:ZFIN;Acc:ZDB-GENE-091113-21]
ENSDARG00000096775	43.2614042	1.959134257	3.54484733	0.44970936	4.3564454	1.32E-05	0.005279	BX119992.1	
ENSDARG00000068370	179.174824	1.873104886	2.81675469	0.42759341	4.38057474	1.18E-05	0.00498556	ralgps1	Ral GEF with PH domain and SH3 binding motif 1 [Source:ZFIN;Acc:ZDB-GENE-070720-16]
ENSDARG00000010610	467.4476	1.849353392	3.34896102	0.45110128	4.09964416	4.14E-05	0.01251922	sarm1	sterile alpha and TIR motif containing 1 [Source:ZFIN;Acc:ZDB-GENE-040219-1]
ENSDARG00000090722	78.9799493	1.842122372	4.61259154	0.45502701	4.0483803	5.16E-05	0.01402337	leg1.1	liver-enriched gene 1, tandem duplicate 1 [Source:ZFIN;Acc:ZDB-GENE-030131-1178]
ENSDARG00000079441	9.82573281	1.840144388	5.81440161	0.45315703	4.06072124	4.89E-05	0.01364779	BX649448.1	
ENSDARG00000100793	43.2521232	1.786542352	2.30109443	0.37693108	4.73970556	2.14E-06	0.0012771	CR450785.1	
ENSDARG00000045444	1298.42372	1.784147135	2.62970002	0.42382137	4.20966771	2.56E-05	0.00858389	fdz8a	frizzled class receptor 8a [Source:ZFIN;Acc:ZDB-GENE-000328-3]
ENSDARG00000018048	509.905502	1.738049281	2.64725355	0.43076445	4.03480203	5.46E-05	0.01467377	zgc:171704	zgc:171704 [Source:ZFIN;Acc:ZDB-GENE-070820-16]
ENSDARG00000097299	209.832094	1.733594488	2.58088348	0.42585234	4.07088165	4.68E-05	0.01341432	FLRT2 (1 of many)	fibronectin leucine rich transmembrane protein 2 [Source:HGNC Symbol;Acc:HGNC:3761]
ENSDARG00000101324	579.259794	1.711002804	3.30279113	0.45448226	3.76472961	0.00016673	0.03450701	apoa1b	apolipoprotein A-1b [Source:ZFIN;Acc:ZDB-GENE-050302-172]
ENSDARG00000098550	1089.99044	1.68996073	2.39161057	0.41357614	4.08621424	4.38E-05	0.0130816	F0904917.1	
ENSDARG00000102825	112.708263	1.658906966	2.38282417	0.41715581	3.9767083	6.99E-05	0.01786907	olfm2b	olfactomedin 2b [Source:ZFIN;Acc:ZDB-GENE-040426-2402]
ENSDARG00000055026	239.715615	1.655150881	2.14636829	0.38202565	4.33256482	1.47E-05	0.0055424	ptch2	patched 2 [Source:ZFIN;Acc:ZDB-GENE-980526-44]
ENSDARG00000040065	158.191272	1.649689307	2.15217135	0.38430853	4.29261691	1.77E-05	0.00653984	gja5a	gap junction protein, alpha 5a [Source:ZFIN;Acc:ZDB-GENE-040407-3]
ENSDARG00000102972	41.7261439	1.644747449	2.00857556	0.34918757	4.71021194	2.47E-06	0.0014128	CABZ01075125.1	
ENSDARG00000021250	527.63317	1.644015736	2.34899386	0.41633626	3.94876906	7.86E-05	0.01939564	slc25a48	solute carrier family 25, member 48 [Source:ZFIN;Acc:ZDB-GENE-040718-60]
ENSDARG00000105518	12.2684861	1.619643329	7.12136176	0.44181285	3.66590366	0.00024647	0.04769686	CABZ01078388.1	
ENSDARG00000008133	105.50819	1.617541979	2.10212803	0.38234095	4.23062708	2.33E-05	0.00820647	foxl1	forkhead box L1 [Source:ZFIN;Acc:ZDB-GENE-040426-1181]
ENSDARG00000054418	636.352991	1.608578179	1.85619983	0.30976993	5.19281578	2.07E-07	0.0001854	ssuh2.2	ssu-2 homolog, tandem duplicate 2 [Source:ZFIN;Acc:ZDB-GENE-060421-4694]
ENSDARG00000068934	147.245427	1.514644773	2.12547082	0.41110861	3.68429353	0.00022934	0.04478551	cyp1b1	cytochrome P450, family 1, subfamily B, polypeptide 1 [Source:ZFIN;Acc:ZDB-GENE-030902-1]
ENSDARG00000028163	750.108666	1.478038367	1.77419756	0.33965604	4.35157394	1.35E-05	0.005279	prg4b	proteoglycan 4b [Source:ZFIN;Acc:ZDB-GENE-041014-324]
ENSDARG00000030723	179.435722	1.402835163	1.64134004	0.32085746	4.37214448	1.23E-05	0.00508241	cldn11b	claudin 11b [Source:ZFIN;Acc:ZDB-GENE-010328-13]
ENSDARG00000045544	71.0848191	1.349656806	1.6492801	0.35045221	3.85118651	0.00011755	0.02603119	hgfa	hepatocyte growth factor a [Source:ZFIN;Acc:ZDB-GENE-041014-2]
ENSDARG00000070360	225.268356	1.323826732	1.59305431	0.34124879	3.87935954	0.00010473	0.02434187	fam212aa	family with sequence similarity 212, member Aa [Source:ZFIN;Acc:ZDB-GENE-060512-186]
ENSDARG00000097891	123.646235	1.296809061	1.54282313	0.33327394	3.89112056	9.98E-05	0.02355411	CR751227.2	
ENSDARG00000030844	2129.13483	1.289459296	1.53490845	0.33403454	3.86025742	0.00011327	0.02534481	kif11a	Kruppel-like factor 11a [Source:ZFIN;Acc:ZDB-GENE-030131-3568]
ENSDARG00000040926	1312.99666	1.278658907	1.47092688	0.30728756	4.16111514	3.17E-05	0.01018848	nox2f	nuclear receptor subfamily 2, group F, member 2 [Source:ZFIN;Acc:ZDB-GENE-990415-252]
ENSDARG00000030125	626.446510	1.110514879	1.26319793	0.29718131	3.73682617	0.00018636	0.03812523	srz7	SRZ (sex determining region Y)-box 7 [Source:ZFIN;Acc:ZDB-GENE-040109-4]
ENSDARG00000090752	364.540252	1.047438538	1.11629232	0.21937126	4.77473007	1.80E-06	0.00110442	plk2a	polo-like kinase 2a (Drosophila) [Source:ZFIN;Acc:ZDB-GENE-060810-71]

**Table S2.** Generation and characterization of cytokine gene morphants.

Gene	ATG morpholino (5'>3')	Splice morpholino (5'>3')	final concentration	target site in gene	Analysis primers				aberrant cDNA structure
					First round forward	First round reverse	Second round forward	Second round reverse	
<i>i17</i>	TGCATTTGTCAATTTTGAGGCATGT		0.1						
<i>i17</i>		GGAAATGGGTTGTTTCAGTTACCTT	0.1	SD exon 4	CAGTTACGCACTTGACGTTCT	CCTGAATCTTGTGAATGTTGCA	CGAAGCGATATAGCCCCATC	TTGTGAATGTTGAAAAGTGGGT	see Fig. 1D
<i>i12</i>	AGAGCAGACATCCTCATCATCA		0.1		n/a				
<i>i12</i>		CAACCCTACAGAGAAACAGAGACAG	0.1	SA exon 3	CGCACACACTGATGATGATGA	TTCTGCCTCCATTGCTTCATC	AGGATGTCTGCTCTACACTG	ACGTTCTCAGGAACGTCATG	see Fig. 5C
<i>i115</i>	AGAGGGTCACTGAAATCATCGAAGC		0.1		n/a				
<i>i115</i>		GTTCTCTGCAAGAAATCACACACAC	0.1	SD exon 2	CGCTTCGATGATTCAGTGAC	GGATGCCTGCTTGACCTTC	TCGATGATTCAGTGACCTTC	GTCCTGCTTGACCTTCAGTC	see Fig. 5F
<i>cr1f2</i>	ACACAACTCCAGTTGATCATGACT		0.2		n/a				
<i>cr1f2</i>		TCTGCATGAAAGCAAAACAGCCTGT	0.2	SA exon 3	GCAGTACAAGCGCCAAATGTG	CTGAACTGCTCAGTGTCCAT	GCCAATGTGAAAGACTGG	TGGTACTTCAGGAAGAAGCAG	cryptic splice site in exon 3 used.
<i>cr1f2</i>		ACAGACATCTGTGACAAAATCTT	0.2	SA exon 4	dito				exon 4 is skipped.
<i>i1151</i>		AGTAAATCATGTTGTACCACATTTG	0.2	SD exon 2	CAACAATGAGCGGGTGACGA	ACTCTGCACTTGGATTTCCAC	ACAATGAGCGGGTGACGACT	GTAATTGTGGACTTGGAGCAG	see Fig. Fig. S3A
<i>i121</i>	ACACGCCTTCATGTTTAAGATGTAC		0.2		n/a				
<i>i121</i>		CATGTAATAAACACACACTCACGAT	0.2	SD exon 3	ACGGCCTCTCGATTACATC	GAGATTCACCACCGGTGG	AGGTGATCGAGCACCTGTGT	ACGCTTGACGGGCATAGATC	see Fig. Fig. S3B
<i>i115r</i>	CATGTCACCTTGTCCAGAGAATCC		0.1		n/a				
<i>i115r</i>		ATCTGAGCTGATGTTACTCACATTT	0.1	SD exon 7	CAATTGCCAGCAGCATCGCT	TCTCGACGCTTGACCTCCA	AGCAGCATCGCTACCAGACA	ACGCTTGACCTCCACCAGA	see Fig. Fig. S3C