Immunogenetics

– Electronic Supplementary Material –

Differential expression and ligand binding indicate alternative functions for zebrafish polymeric immunoglobulin receptor (pIgR) and a family of pIgR-like (PIGRL) proteins

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Supplemental methods

Cloning full-length pigr and PIGRL cDNAs

Zebrafish *pigr* and various PIGRL cDNAs were PCR amplified and cloned by 3' or 5' RACE using RNA pooled from kidney and spleen, the GeneRacer kit (Invitrogen), Titanium Taq DNA polymerase (Clontech) and a series of primers (Table S1, below). Initial PIGRL primers were designed to be complementary to predicted Ig domains identified in the reference genome (Zv9). Resultant amplicons were ligated into the pGEM-T Easy plasmid (Promega) and sequenced.

Cloning *pigr* transcript.

A partial (5' and 3' truncated) transcript of zebrafish *pigr* was identified in GenBank (accession EF539183). A forward primer, pigrFP1, and a nested forward primer, pigrFP2, were designed from the D1 domain of this partial *pigr* sequence and used with RACE to identify the 3' sequence of the *pigr* transcript. Similarly, a reverse primer, pigrRP1, and a nested reverse primer, pigrRP2, were designed from the D2 domain of the partial *pigr* sequence and used with RACE to identify the 5' sequence of the *pigr* transcript. Then, a *pigr*-specific forward primer, pigrFP3, and a *pigr*-specific reverse primer, pigrRP3, were employed to directly amplify the entire *pigr* open reading frame from zebrafish intestine RNA (40 cycles, annealing at 60 °C).

Cloning *pigrl2.1*, *pigrl2.3* and *pigrl2.4* cDNAs.

Using 5' RACE, three leader sequences were obtained using degenerate primers, RP1 and the nested primer, RP2. One of these 5' sequences was used to design the forward primer FP1 and the nested primer FP2 which were used with 3' RACE to obtain full-length *pigrl2.1*^{3842}, *pigrl2.1*^{3843}, and *pigrl2.1*^{3844} cDNAs. The proteins encoded by these three transcripts differ by five or fewer amino acids. The forward primer FP3 and the nested primer FP4 were designed from the second 5' RACE sequence and used with 3' RACE to obtain full-length cDNA transcripts for *pigrl2.3*^{3822}, *pigrl2.3*^{3823}, *pigrl2.3*^{3824}, *pigrl2.4*^{4003}, and *pigrl2.4*^{4004}. The proteins encoded by the two *pigrl2.4* transcripts differ by two amino acids. The forward primer FP5 and the nested forward primer FP6 were designed from the third 5' RACE sequence and yielded the full length *pigrl2.3*^{3820} cDNA.

Cloning *pigrl3.5, pigrl3.4* and *pigrl3.10* cDNAs.

5' RACE was performed using the degenerate reverse primer RP3 and the nested reverse primer RP4 and revealed a leader sequence for the *pigrl3* family. Forwards primer FP7 and nested primer FP8 were then used with 3' RACE producing the *pigrl3.5*^{3960}, *pigrl3.5*^{3961}, *pigrl3.4/7*^{3962}, *pigrl3.4/7*^{4001} and *pigrl3.10*^{4002} cDNAs. Transcripts *pigrl3.10*^{5187} and *pigrl3.10*^{5188} subsequently were amplified using FP9 and RP8.

Cloning *pigrl3.8* and *pigrl3.9* cDNAs.
5' RACE with RP3 and RP4 also identified a second 5' sequence from which the forward primer FP9 and the nested forward primer F10 were designed and used with 3' RACE to clone the full-length *pigrl3.5*^{4021}, *pigrl3.8/11*^{4020}, *pigrl3.8/11*^{4023} and *pigrl3.9/12*^{4027} cDNAs. The proteins encoded by the two variants of *pigrl3.8/11* differ by five amino acids.

Cloning *pigrl2.5* and additional *pigrl2.3* cDNAs.
A third set of degenerate reverse primers, RP5 and RP6, when applied to 5' RACE revealed a new leader sequence from which the forward primer FP11 and nested forward primer FP12 were designed. 3' RACE with FP11 and FP12 identified the *pigrl2.3*^{3968}, *pigrl2.3*^{3969} and *pigrl2.5*^{3970} cDNAs.

Cloning a *pigrl4.2* cDNA.
5' RACE with RP1 and RP2 fortuitously amplified a *pigrl4.2* leader sequence, allowing for the design of forward primers FP13 and FP14. Using FP13 and the nested FP14 (that did not incorporate the translational start codon), 3' RACE was performed generating a cDNA encoding a predicted stop codon, from which reverse primer RP7 was designed. Using primers FP15 and RP7, located upstream of the translational start and downstream of the stop codon respectively, a full-length *pigrl4.2*^{4177} cDNA was obtained.

Supplemental Table S1. DNA primers for RACE and cloning full-length *pigr* and PIGRL cDNAs[1]

PIGRL cDNA	GenBank Accession	Forward Primer (FP) Name	Forward Primer Sequence	Reverse Primer (RP) Name	Reverse Primer Sequence
pigr^{4120} ***pigr***^{4121}	KF932324 KF932325	pigrFP1	TGTGAACATGCGCAACCTGAC	pigrRP1	GTTCTTATCATCATGGATGTGG
		pigrFP2	ACTGGTGCGGTGTGGAGCTTG	pigrRP2	CAGGCAGGAGTTCCAGTTTCC
		pigrFP3	GACCTCAACAATGAGTCTTCCGCTGCTTC	pigrRP3	CGTTGTTTTCAGTCCTCTGTTAGAGCATC
pigrl2.1^{3842}	KF932326	FP1	ATGCAATTGCTGCACATGC	RP1	AAWACCAKCCAGAATCAGA
		FP2	CATGCGGACGCAAAAATGT	RP2	ATCNMTRATCTGCACTGAT
pigrl2.1^{3843}	KF932327	FP1	ATGCAATTGCTGCACATGC	RP1	AAWACCAKCCAGAATCAGA
		FP2	CATGCGGACGCAAAAATGT	RP2	ATCNMTRATCTGCACTGAT
pigrl2.1^{3844}	KF932328	FP1	ATGCAATTGCTGCACATGC	RP1	AAWACCAKCCAGAATCAGA
		FP2	CATGCGGACGCAAAAATGT	RP2	ATCNMTRATCTGCACTGAT
pigrl2.3^{3820}	KF932329	FP5	AACCACTCAGAAGCTGACAGA	RP1	AAWACCAKCCAGAATCAGA
		FP6	GCTGACAGAGAAACAGAATGG	RP2	ATCNMTRATCTGCACTGAT
pigrl2.3^{3822}	KF932330	FP3	TGCTGACAGAGAAACAGAATGG	RP1	AAWACCAKCCAGAATCAGA
		FP4	GAGAAACAGAATGGCAGCGTA	RP2	ATCNMTRATCTGCACTGAT
pigrl2.3^{3823}	KF932331	FP3	TGCTGACAGAGAAACAGAATGG	RP1	AAWACCAKCCAGAATCAGA
		FP4	GAGAAACAGAATGGCAGCGTA	RP2	ATCNMTRATCTGCACTGAT
pigrl2.3^{3824}	KF932332	FP3	TGCTGACAGAGAAACAGAATGG	RP1	AAWACCAKCCAGAATCAGA
		FP4	GAGAAACAGAATGGCAGCGTA	RP2	ATCNMTRATCTGCACTGAT
pigrl2.3^{3968}	KF932333	FP11	CCCAATTAAACCACTCAGACAC	RP5	TCCAGGTTCCTCATAGYMACAGT
		FP12	GACAGAGAAACAGAATGACAGC	RP6	AAGAAACTCTGATCTGGATGATC
pigrl2.3^{3969}	KF932334	FP11	CCCAATTAAACCACTCAGACAC	RP5	TCCAGGTTCCTCATAGYMACAGT
		FP12	GACAGAGAAACAGAATGACAGC	RP6	AAGAAACTCTGATCTGGATGATC
pigrl2.4^{4003}	KF932335	FP3	TGCTGACAGAGAAACAGAATGG	RP1	AAWACCAKCCAGAATCAGA
		FP4	GAGAAACAGAATGGCAGCGTA	RP2	ATCNMTRATCTGCACTGAT
pigrl2.4^{4004}	KF932336	FP3	TGCTGACAGAGAAACAGAATGG	RP1	AAWACCAKCCAGAATCAGA
		FP4	GAGAAACAGAATGGCAGCGTA	RP2	ATCNMTRATCTGCACTGAT
pigrl2.5^{3970}	KF932337	FP11	CCCAATTAAACCACTCAGACAC	RP5	TCCAGGTTCCTCATAGYMACAGT
		FP12	GACAGAGAAACAGAATGACAGC	RP6	AAGAAACTCTGATCTGGATGATC
pigrl3.4^{3962}	KF932338	FP7	ACACACTTACTCCACTGCAG	RP3	GATCTCYACAGCGCACCAGTAA
		FP8	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.4^{4001}	KF932339	FP7	ACACACTTACTCCACTGCAG	RP3	GATCTCYACAGCGCACCAGTAA
		FP8	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.5^{3960}	KF932340	FP7	ACACACTTACTCCACTGCAG	RP3	GATCTCYACAGCGCACCAGTAA
		FP8	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.5^{3961}	KF932341	FP7	ACACACTTACTCCACTGCAG	RP3	GATCTCYACAGCGCACCAGTAA
		FP8	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.5^{4021}	KF932342	FP9	TCCACTGCAGACATGATTCAC	RP3	GATCTCYACAGCGCACCAGTAA
		FP10	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.8^{4020}	KF932343	FP9	TCCACTGCAGACATGATTCAC	RP3	GATCTCYACAGCGCACCAGTAA
		FP10	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.8^{4023}	KF932344	FP9	TCCACTGCAGACATGATTCAC	RP3	GATCTCYACAGCGCACCAGTAA
		FP10	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.9^{4027}	KF932345	FP9	TCCACTGCAGACATGATTCAC	RP3	GATCTCYACAGCGCACCAGTAA
		FP10	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.10^{4002}	KF932346	FP7	ACACACTTACTCCACTGCAG	RP3	GATCTCYACAGCGCACCAGTAA
		FP8	GCAGACATGATTCACACTCTG	RP4	TCTSCCATTCMACTGTAAA
pigrl3.10^{5187}	KF932347	FP9	TCCACTGCAGACATGATTCAC	RP8	CACATCTAGGTCTCTGACAG
pigrl3.10^{5188}	KF932348	FP9	TCCACTGCAGACATGATTCAC	RP8	CACATCTAGGTCTCTGACAG
pigrl4.2^{4177}	KF932349	FP15	CCGTCTGACCATCATTTACAG	RP7	CCCTTCTATATCAAAGGTCGTC
		FP13	GGAACTCTGAGCATGAAGACT	RP1	AAWACCAKCCAGAATCAGA
		FP14	GACTTTAGATCGTGTRMCTGT	RP2	ATCNMTRATCTGCACTGAT

[1] Sequences corresponding to translational start and stop codons are underlined

Supplemental Table S2. DNA primers for RT-PCR

Gene(s)	**Primer sets**	**Forward primer** **Reverse primer**	**Annealing temp (°C)**	**Extension time (s)**	**No. of cycles**	**Amplicon length (bp)**
pigr	pIgR_intl-F	CAGCACAGTGACTACAATCG	61	30	35	430
	pIgR-D2_R	CTCACTCCACCTGAGGTTTTT				
PIGRL1 Family	PIGRL1-F	CAGRRTAYAAGAAATACTGGTGC	59.5	30	35	410
	PIGRL1-R	GARCTGTAGAAACACTGRACACT				
PIGRL2 Family	PIGRL2-F	ACTGTTAAGCCTGGAGGATCTGT	66	30	35	356
	PIGRL2-R	GARCTGTAGAAACACTGRACACT				
PIGRL3 Family	PIGRL3-F	ATATTACTGGTGCGCTGTGGA	66	30	35	159
	PIGRL3-R	GARCTGTAGAAACACTGRACACT				
PIGRL4 Family	PIGRL4-F	TGGACAATAACTGATTATCCA	61	30	35	227
	PIGRL4-R	GARCTGTAGAAACACTGRACACT				
β-actin	B-actin fwd	GGTATGGAATCTTGCGGTATCCAC	65	30	30	301
	B-actin rev	ATGGGCCAGACTCATCGTACTCCT				
mpx	MPX fwd	CCAGAACCAGTGAGCCTGAGACACG	70	30	35	639
	MPX rev	CAGTCTAACCATGGGCAGCGCTGCAC				
tcra	TCRα fwd	TCGTTTTCAATGTGCTGGTG	68	30	35	339
	TCRα rev	GATGATCTGGAATGGGATGC				

Supplemental Table S3. Human proteins most similar to zebrafish PIGRL proteins[1]

Query	Description	Alias	Max score	Total score	Query cover	E value	Ident	GenBank Accession
Pigrl2.4	polymeric immunoglobulin receptor	pIgR	64.7	226	54%	8e-11	23%	NP_002635.2
	CMRF35-like molecule 6 precursor	CD300C	50.8	94.7	53%	6e-07	29%	NP_006669.1
	trem-like transcript 2 protein	TREML2	50.8	50.8	20%	1e-06	39%	NP_079083.2
Pigrl3.5	polymeric immunoglobulin receptor	pIgR	60.8	229	58%	2e-09	27%	NP_002635.2
	CMRF35-like molecule 1	CD300LF	55.1	106	57%	5e-08	28%	NP_620587.2
	CMRF35-like molecule 7	CD300LB	52.4	102	72%	2e-07	29%	NP_777552.3
Pigrl4.2	polymeric immunoglobulin receptor	pIgR	70.1	232	87%	3e-13	24%	NP_002635.2
	natural cytotoxicity triggering receptor 2	NKp44	59.7	99.4	68%	4e-10	35%	NP_001186439.1
	CMRF35-like molecule 2	CD300E	55.1	102	65%	8e-09	33%	NP_852114.2

[1] Results are from BLASTp searches of the human reference protein database. Predicted sequences are excluded from results. Top three hits per query are shown.

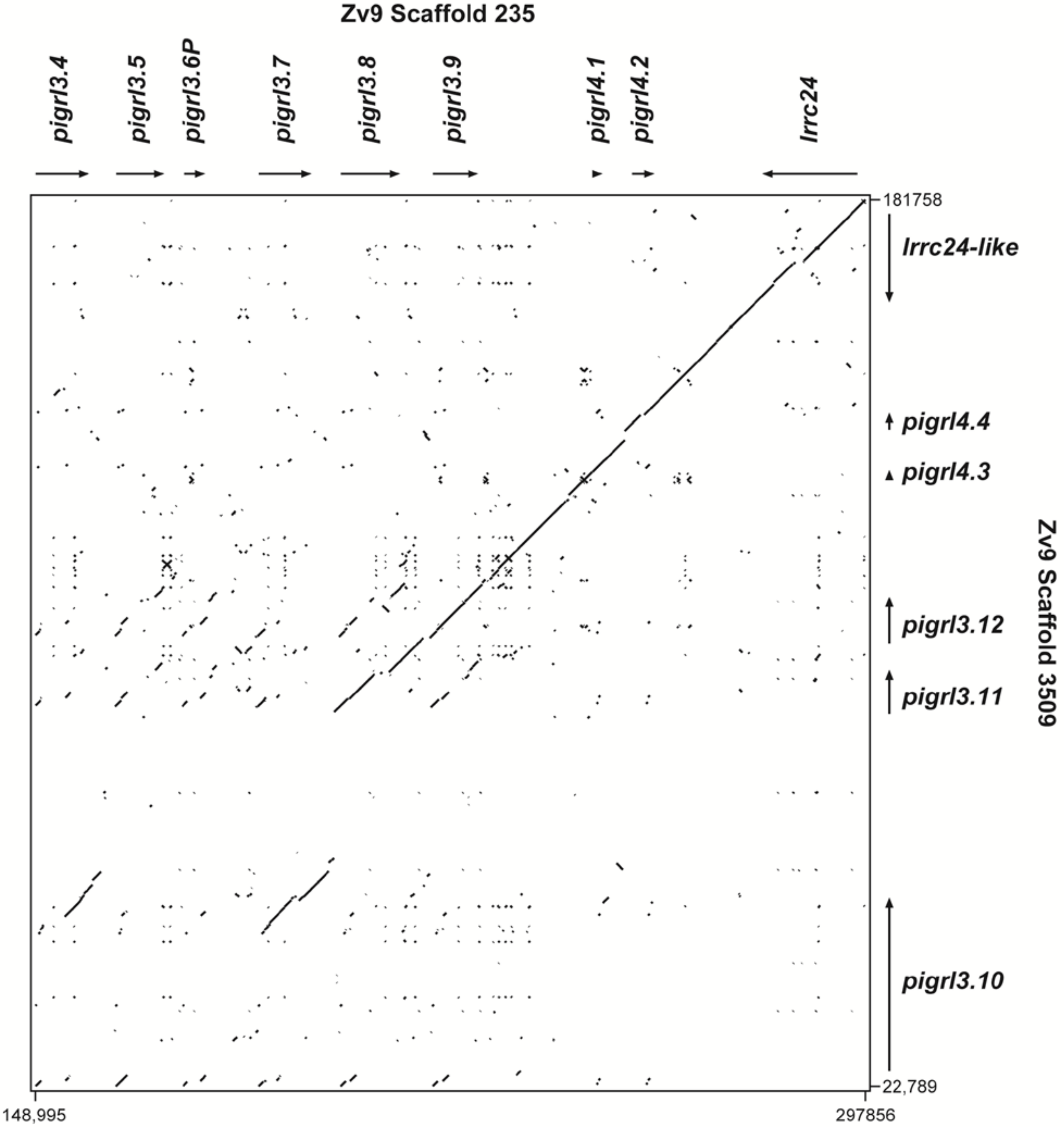

Figure S1. Dot-plot comparison of Zv9 scaffolds 235 and 3509. The region of scaffold 235 that encodes *pigrl3.8* through *lrrc24* shares a high degree of identity with the region of scaffold 3509 that encodes *pigrl3.11* through *lrrc24l* (see Figure 2). It remains to be determined if these scaffolds represent two genomic loci or haplotypic variation of a single locus. Graphic generated with PipMaker software (Schwartz et al. 2000. *Genome Research*. 10(4), 577-586).

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>pIgR-D1
GSHSTVTTIGDVAVLEGGSVTVPCHYNPQYISNVKYWCSGRMREFCSSLARTDDPESAPNGNRKVTIADDPTQHVFTVNMRNLTEDD
SGWYWCGVELGGMWVSDSTASLYISVVQ

>Pigrl1.1-D1
SEYKTASASRALTVQTGGSLVIPCYYDRYYTEYKKYWCFNAKGYFNSCSILAYTNETKGKVSLTDHPDQSFFTVTMRNLQHEDTGAY
WCAVEIEGFFKLDKREQLHLTVQS

>Pigrl1.3-D1
CKIGSASRALIIQTGGSVVIPCYYDRKYTGYKKYWCFNTNAAFNYCSILAYTKETKEKVSVIDHPDQSFFTVAMRNLQEEDTGYYWC
AVEIGGKLELDKNKQLYLSVQS

>Pigrl1.4-D1
CKIGSASRALTIQTGGSVVIPCYYDRKYTGYKKYWCFDAKTAFNYCSILAYTNETKGKVSVIDHPDQSFFTVTMRNLQHEDTGYYWC
AVEIEGFFKLDEKEQLHLTVQS

>Pigrl1.5-D1
SVKSVLTVQTGGSVVVPCYYDMKYAEYKKYWCFHANDLYSSCSILAYANETKGKVSVVDHPDQSFFTVTMRNLQHGYTGYYGCAVEI
GGIFELDQKKQLYLSVQS

>Pigrl1.6-D1
PECKTASAYSILTAHTGGSVVIQCFYDKYYAENKKYWCFYPTDSYSYCSILAYTNEYREKVSVTDYPYLSYFTVTMRNLQYKDSGYY
WCAVETGGIFVNDALEQYQLRIQS

>Pigrl1.7-D1
CKTAYVDPVLTVQTGGSVVIPCFYDKRYAEYKKYWCFYKTDSYWFCSALAYTNETKGKASVIDHPDQSFFTVTMRNLQHEDTGYYWC
AVEIGTILIRDETKKLQLRVQT

>Pigrl1.8-D1
PECKTAYVDPVLTVQTGGSVVIPCFYDKGYAEYKKYWCFYKTDSYRSCSILAYANETKGKVSVIDHPDQSFFTVTMRNLQHEDTGYY
WCAVEIGGIFKRDDEKKLHLIVQT

>Pigrl2.1-D1
VESFEGGSNHTITVKPGGSVTIPCYYDEKNPPQKKYWYSVIGESRKYTNTTEENLSVIDHPDQSLFTVTMRNLQENKHNGKYYCTVE
TGQKSNVTYELFLQ

>Pigrl2.2-D1
VESYSGLDHVLTINPGGSVTIPCHYNEETQWQMKFWFSEIFQLRSYTNTTEENLSVIDRPDQSLYTVTMRNIESRQSGFYYCVLESE
GKENTTYELFLM

>Pigrl2.3-D1
AESYDAFSSNRLTVKPGGSVTIPCYYDEKNTQLKYWFSVNDECNTYTNTTEENLSVIDHPDQSLVTVTMRNLQEKHTGEYHCGVVPG
GVIYKIYLQVQD

>Pigrl2.4-D1
YNEFSNQALTVQPGGSVTIPCHYNKQYTQLKKHCYLETDKYTNTREKNLLVIDHPDQSLFTVTMRNLRENQTGLCYCVVETGGTGTV
IYQFYLK

>Pigrl2.5-D1
AESLSCWSRCTITVQHGGSVTIPCYYDKKNPPQKKYWYTEIEEYHKSTNTTEKNLSVIDHPDQSLFTVTMRNLQDKQSGRHFCALET
GGQETVIYEFYLKVQF

>Pigrl2.6-D1
VESFSGGSNRTITVKPGGSVTIPCYYDEKNPPRRKYWFSEHGQSNKYTNTTEENLSVIDHPDQSLFTVTMRNLQENKHNGQYYCTVE
TGQKPNVTYVTYELYLKVHS
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>Pigrl2.7-D1
VESYTGGSNHIITVQHGGSVTIPCHYDEKYTLQNKYWFAEIDKTNKYTNTTEENLSVIDHPDQSLFTVTMRNLQYKHTGHYYCVVET
GEHPPIKVIYEPYIKIKY

>Pigrl3.1-D1
GSWPYNLNIRVKSGSPGIIPCQYEVKNKANRKYWCQGSVWSSCSVLAYANETRNKFSITDYPAQSVFTVEWQNLQPSDSGCYWCAVE
ISGTGTLDSGYYVYLTVQS

>Pigrl3.2-D1
EAWVYDVNIGVKSGSPGIIPCVYKEKLKANRKYWCQGSVWSSCTILAYANETRNKFSITDYPEQSVFTVEWQTLQPSDSGCYWCAVE
INGYGTVDDGYHMYLTVQS

>Pigrl3.3-D1
GAWIDKLNIGVKSGSPGIIPCQYEKQYQENHKYWCQGMFWSSCTILAFGNGPKSKFSITYYPAQSIFTVEWQKLQPSDSGYYWCAVE
IGGSGTLDAGYYVYLTVQS

>Pigrl3.4-D1
GAWVINQNIRVKSGSPGIIPCPYEEQYKEHQKFWCQGYFWSTCNLLAFGNETGKNFSITDYPAQSVFTVEWQNLQPSDSGHYWCAVE
IGGPGTLDAGYYVYLTVQS

>Pigrl3.5-D1
GAWIDKLNIGVKSGSPGIIPCLYKEQYKENHKYWCQGMFWSSCTILAFGNGPRSKFSITYYPAQSIFTVEWQNLQPSDSGYYWCAVE
IGDSGIPDARYYLYLKVQP

>Pigrl3.6P-D1
GAWIDKLNIKVKSGSPGIIPCLYDEQYKENHKYWCRGSVWSSCVIMAYANETSNTFSITDNPAQSIFTVEWQNLQPSDSGFYWCAVE
IGGSETLDAGYYMYLTVQS

>Pigrl3.7-D1
DGVWINKLNIGVKSGSPGIIPCLYDEQYKEHQKFWCWGTFFSTCSILAYVNETRNKFSITDYPAQSIFTVEWQNLQLSDSSYYWCVV
EIGGPGTLDAGYYLYLTVQS

>Pigrl3.8-D1
GAWVINPNIRVKSGSPGIIPCLYKEQYKEHQKFWCWGTFWSTCSILAYVNGTRNKFSITDYPAQSIFTVEWQNLQPSDSAYYWCAVE
IGGPGTLDAGYFLYLTVQS

>Pigrl3.9-D1
GVWINKLNIGVKSGSPGIIPCLYEEQYKEHQKYWCRGYIWSSCSILAYVNETRNKFSITDYPAQSIFTVEWQNLQESNSSYYWCAVE
IGGPGTLDAGYYLYLTVQS

>Pigrl3.10-D1
DGAWIDKLNIGVKSGFPGIIPCQYVEQYQENLKYWCQGMFWSSCTILAFGNGPRSKFSITDYPAQSIFTVEWQNLQPSDSGYYWCAV
EIGDSEKPDASYYLYLKVQS

>Pigrl3.11-D1
GAWIIRVKSGSPGNIPCLYEEQYKEHQKFWCQGYFWSTCAILTFVNETRNKFSITDYPAQSIFTVEWQNLQPSDSGYYWCAVEIGGA
ETLDAGYYVHLTVRS

>Pigrl3.12-D1
GVWINKLNIGVKSGSPGIIPCLYEEQYKEHQKYWCRGYIWSSCSILTFVNETRNKFSITDYPAQSIFTVEWENLQESNSSYYWCAVE
IGGPGTLDAGYYVYLTVQS

>Pigrl4.1-D1
TLSMKTLDRVTVSNGGSITIPCLYENKYKLDSKNWCKGKAWLTCKTIAHANHTGIWTITDHPADNIFTVTLNKAKPSNSGYYWCAAE
SDRTSLYLTVQEGKEGLFV

>Pigrl4.2-D1
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IDNHVDNSKYLYLTVQK
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>Pigrl4.3-D1
TLSMKTLDRVTVSNGGTITIPCLYENKYKLDSKNWCKGKAWLTCKTIAHANHTGIWTITDHPADNIFTVTLNKAKPSNSGYYWCAAE
SDRTSLYLTVQEGKEGLFV

>Pigrl4.4-D1
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IDNHVDNSKYLYLTVQK

>pIgR-D2
GMSVVNGMVSAEEGKSVSVQCLYSKNLRWSEKRWCRSGNWNSCLLTDSEGTFSGKNVHIHDDKNSVFTVTLQRLEMRDSGWYWCGAG
QQNVAVHVSVTRR

>Pigrl1.1-D2
PEVSVMNSSVSGHESGDVSVQCFYSSEYRNTEKRWCRYKDQICYAVERFNTSHNASVQIRDDGESSFTVLMTGLRLSDSGWYSCCVG
GKEALVQLTVTE

>Pigrl1.3-D2
PDVSVKSSRVSGHEGDHVRIQCFYSSGYRNTQLLSLYLHHIFCFTEKTTNTSQNSSVQISDDGESSFTVLMTGLTLSDSGWFSCCVG
KQETLVQLTVTE

>Pigrl1.4-D2
PDVFVKSSSESGHEGDDVSVQCFYSSGYKKKLKRWCRYKDRKCFREKKTDTSQSSSVQISDDGESSFTVLITGLRLSDSGWYFCSAG
NLQAPVQLTVTE

>Pigrl1.5-D2
SDVFVVSSSVSGHEGDDVSVQCFYSSRYKNKLKRWCRYKDQKCFREKKTDTSQNSSVQISDDGESSFTVLMTGLRLSDSGWYFCSAG
EQIILVQLTVTK

>Pigrl1.7-D2
PVVSVMSSSVSKHEGDNVTFQCLYSFGYRNSQKQWCRYKDQKCFYPEEKTDTSQSSSVQISDDGERCFTVLMTGLRLSDSGWYFCSA
GDRIIPVQLTVT

>Pigrl1.8-D2
PDVSVMSSSVSKHEGDNVTFQCLYSSGYRNSQKQWCRYKDQKCFNPEEKTDTSQNSSVQISDDGERCFTVLMTGLRLSDSGWYYCSA
GDRIIPVQLTVT

>Pigrl2.1-D2
PDVSVMNSSVSGHEGDDVSVQCFYSSGYKDKQKRWCRYKDQKCFSQKKTDTSQSSSVQISDDGESSFTVLMTGLRLSDSGWFFCSVG
HQTIPVKLTVTEDKIFS

>Pigrl2.2-D2
PDVSVLSSSVSGHESGNVSIQCFYSSKYRDTQKQWCRYKDQKCFRENKTNISQSSSVQISDDGENSFTVLMTGLRLSDSGWYFCSAG
NRIVPVQLTVTEAEP

>Pigrl2.3-D2
PDVSVKSSSVSGHEGGNVSVQCFYSSGYRDKQKRWCRFKDEKCFREKKTNTSKNSSVQISDDGESSFTVLMTGLTLSDSGWYYCSAG
EQILPLQLTVTTSES

>Pigrl2.4-D2
PDVSVMSSSVSGHEGDTVSVQCFYSSGYKNEQKQWCRYKNQKCFGEKKTDTSKSSSVQISDDGKSSFTVLMTGLTLSDSGWYFCSVG
NQMIPVQLTVTHTES

>Pigrl2.5-D2
PDVSVFSSVSGHEGGNVSVQCFYSSGYRTLQKQWCRYKDQKCFTEKKTDTSQNPSVQISDNGERCFTVLMTGLRLSDSGWYFCSAGD
RIIPVQLTVTTLES

>Pigrl2.6-D2
PDVSVMSSSVSGHEGGNVSVQCFYSSEYKKKLKRWCRYKDEKCFRKKTNTSQNSSVQISDDGENFFTVLMTGLTLSDSGWYYCSAGD
QIVPVQLTVTDALSCTVFFSNRLF
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>Pigrl2.7-D2
PDVSVMSSSVSGHEGDTVSVQCFYSSEYEKKLKRWCRYEDQKCFREKKTDTSQNSSVQISDDGESSFTVLMTGLTLSDSGWYYCSAG
EQIIPVQLTVTGAETST

>Pigrl3.1-D2
PAVSVMSSSVSAHEGDGVRVQCFYTSGYQNDFKHWCRYKDQRCFTKKKTDTSQNSSVQISDDGKSSFTVLMTGLTLSDSGWYFCSVG
DLQVHVHLIVTKPGPK

>Pigrl3.2-D2
YGVFVKSSSVSGHEGGDVSVQCIYSDIYRNTIKQWCRVKDKRCYAVERSDTSQNPSVQISDDGESSFTVLMTRLRLSDSGWYFCSVE
DLQVLVQLTVTKPGPK

>Pigrl3.3-D2
PDVFVRSSSVSGHEGDDVRVQCFYSSGYKAKNKQWCRVKDKSCFTEKKTDTSQNSSVQISDDGESSFTVLMTGLRLSDSGWYFCSVG
NLQVPVQLTVTKPRPK

>Pigrl3.4-D2
PDVSVMSSSVSGHEGGNVSVQCFYSSRYKAYNKQWCRVKDKSCYAVERSDTSQSSSVQISDDGESSFTVLMTGLRLSDSGWYFGSVG
NLQVPVQLTVTKPEHK

>Pigrl3.5-D2
PDVSVKSSSVSGHEGGNVSVQCFYSSEYHNKLKGWCRFKDKRCFKEEKTDTSQNPSVQISDDGESSFTVLMTGLTLSDSGWYFCSAG
NLQVPVRLTVTKPEPK

>Pigrl3.7-D2
PDLSVMNSSVSGHEGGNVSVQCFYSSGYKNKTKQWCRVKDKSCFPENKTDTFQNSSVQISDDGESCFTVLMTGLTLSDSGWYFCSAG
NLQVPVQLTVTKPEPK

>Pigrl3.8-D2
PDVSVKSSSVSGHEGGNVSVQCLYRSGYKTYNKQWCRVKDKSCFTEEKTDTSQNLSVQISDDGKSFFTVLMTGLNLSDSGWYFCSVG
DLQVPVQLTVTKPEPK

>Pigrl3.9-D2
PALSVLNSSVSGHEGGNVSVQCFYSSGYKAKTKQWCRFKDKSCFTEKKTDTFQNPSVQISDDGKSSFTVLMTGLRLSDSGWYFCSVG
DLQVPVQLTVTKPVPK

>Pigrl3.10-D2
PDVSVKSSSVSGHEGGNVSVQCFYSSGYKAKEKQWCKYKDQKCLYPKKNTGTSQNSSVQISDDGKSSFTVLMAGLRLSDSGWYFCSV
GDLQVPVRLTVTKPEPK

>Pigrl3.10-D2*
PDLSVMNSSVSGHVGGNVSVQCFYSSGYKNKTKQWCRVKDKSCFPENKTDTFQNSSVQISDDVESSFTVLMTGLRLSDSGWYFCSVG
DLQVPVQLTVTKPKPK

>Pigrl3.11-D2
PDVSVMNSSVSGHEGGNVSVQCFYSSGYKAYNKQWCRVKDKSCFTEEKMDTSQNLSVQISDDGKSFFTVLMTGLNLSDSGWYFCSVG
NLKVPVQLTVTKPEPK

>Pigrl3.12-D2
PAVSVMSSSVSGHEGDDVSVQCFYSSGYKAKTKQWCRFKDKSCFTEKKTDTSQNSSVQISDDVESSFTVLMTGLTLSDSGWYFCSVG
DLQVPVQLTVTKPEHK

>Pigrl4.1-D2
PDVSVVNSSVLGHEGDDVRVQCFYSSAYQNELKRWCRMKDQKCFTEKKSDASQNSSVQISDDGESFSVLMTGLRLSDSGWYFCSAGD
LQVPVRLTVTKNKA

>Pigrl4.2-D2
PDVSVLSSSVSGHKGDDVSVRCFYRSAYKNKLKQWCRIDDLTCFREKKTDTSQNSSVQISDDGESSFTVLMTGLRLSDSGWYFCSVG
NLQVPVQLTVHQGENKNKNTCKSFIQLLSENIKCIYLKL
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>Pigrl4.3-D2
PDVSVVNSSVLGHEGDDVQCFYSSAYQNELKRWCRMKDQKCFTEEKTNTSQSSSVQISDDGESFSVLMTGLRLSDSGWYFCSVGDLQ
VPVRLTVTKNKAGTSLTIILNVLLPFASSVSVMKMKSPDTIVYEQDSQM

>Pigrl4.4-D2
PDMSVLSSSVSGHKGDDVSVRCFYRSANQNKLKQWCRIDDLTCFREKKTDTSQNSSVQIIDDGESSFTVLMTGLRLSDSGWYYCSAG
NLQVPVQLTVHKGENKNKNTCKSFMQLPSDNIKCIYLKLVSYLYKTIHINIV

>orphan-D2
PDVSVMSSSVSGHEGGNLSAQCFYSSGYKAKNKQWCGYKDKKCFTEKKTDTSQNSSVQISDDGKSSFTVLMTGLRLSDSGWYFCSVG
NLQFPVQLTVSKPERKGKITNQSAVNNVKLLLHLKYPCCSFLYFPVLTTTPETE

Figure S2. Zebrafish pIgR and PIGRL Ig domains (D1 and D2). Ig domains encoded by Zv9 genomic scaffolds 234, 235 and 33509 are shown. * indicates a second D2 domain encoded by *pigrl3.10*. The orphan-D2 domain was identified in scaffold 3509.

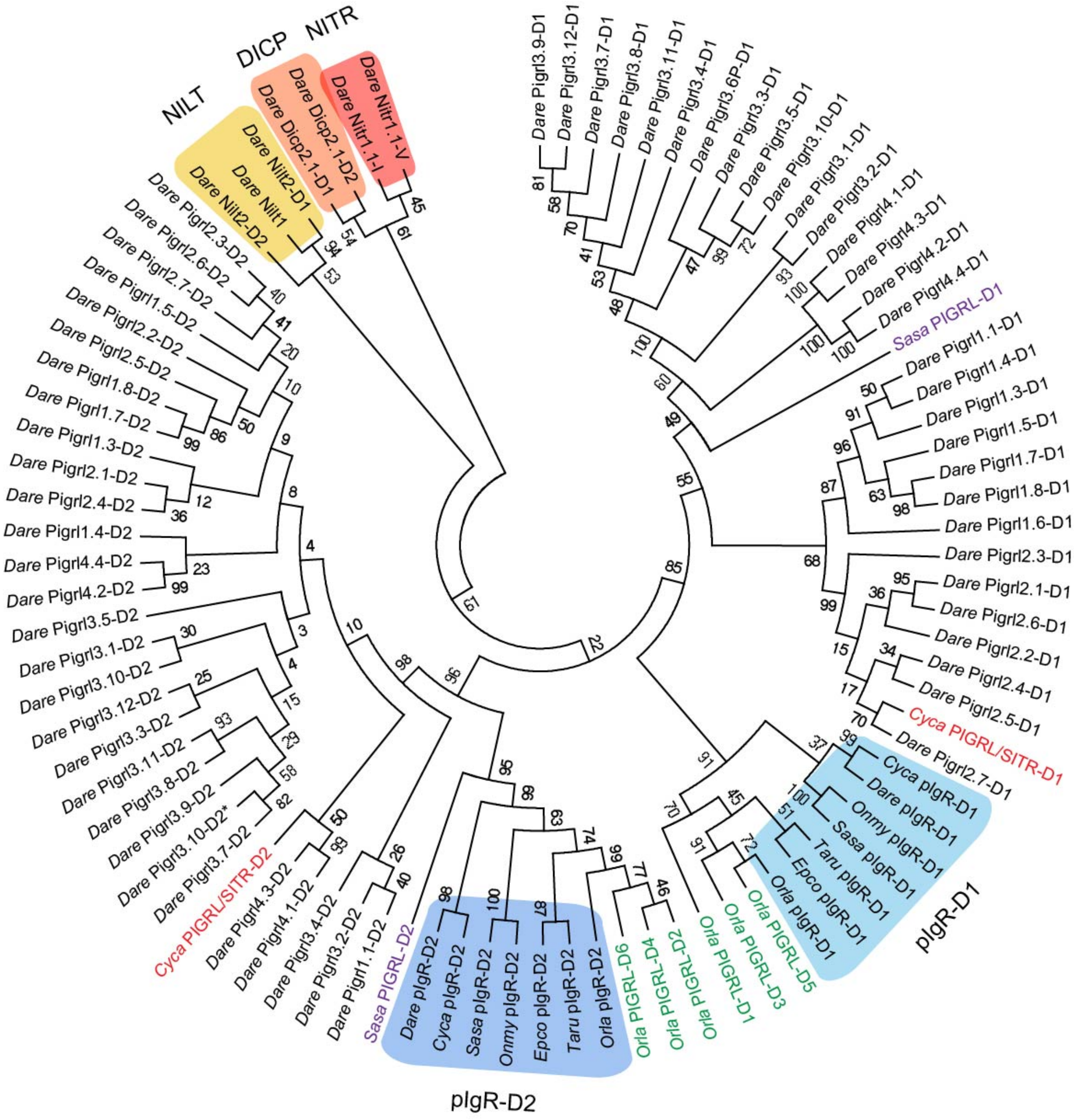

Figure S3. Phylogenetic comparison of pIgR and PIGRL Ig domains to representative NILTs, NITRs and DICPs. pIgR (blue shading) and PIGRL Ig domains from multiple fish species (see Figure S2 and methods) were aligned with Ig domains from zebrafish Nilt1 and Nilt2 (yellow shading, GenBank CAR82065, CAR82066), Nitr1i (red shading, GenBank NP_571721) and Dicp2.1 (orange shading, GenBank NP_001245155). Single PIGRL sequences previously reported from carp (red text, GenBank ADL59933) and salmon (violet text, GenBank ADM18014) as well as predicted pIgR (GenBank XM_004079122) and PIGRL sequences (green text, GenBank XM_004079121) from medaka were included. Species include: zebrafish (*Danio rerio*, *Dare*), carp (*Cyprinus carpio*, *Cyca*), fugu (*Takifugu rubripes*, *Taru*), orange-spotted grouper (*Epinephelus coioides*, *Epco*), Atlantic salmon (*Salmo salar*, *Sasa*), rainbow trout (*Oncorhynchus mykiss*, *Onmy*) and medaka (*Oryzias latipes*, *Orla*). * indicates a second D2 domain present in *pigrl3.10*. The NITR D1 and D2 domains are variable (V) and intermediate (I) Ig domains, respectively.

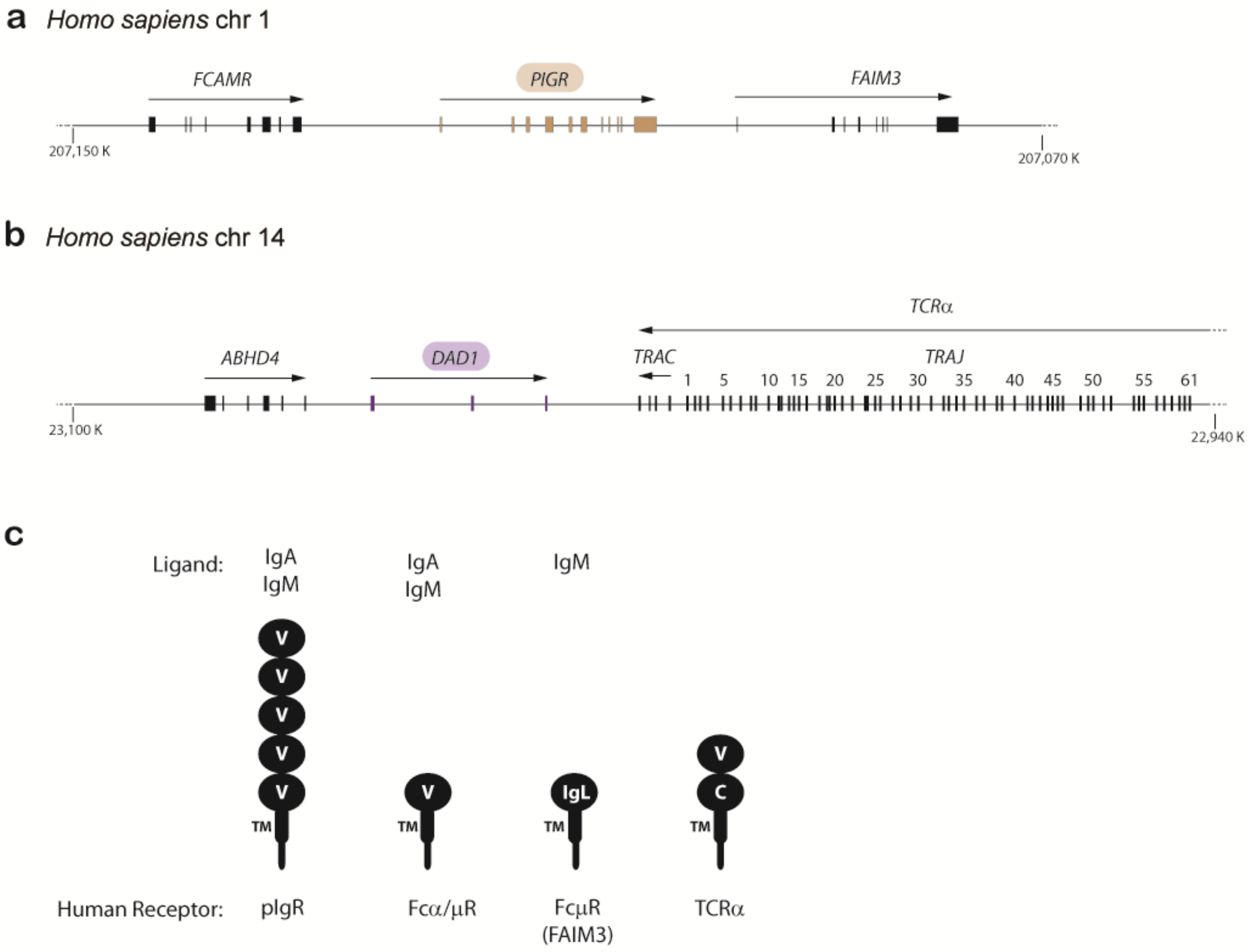

Figure S4. Genomic organization of the human *PIGR* and *DAD1* loci. (a-b) The regions of human chromosomes 1 and 14 that encode *PIGR* and *DAD1* are shown. **(c)** The protein structures and ligands of human pIgR, Fcα/μR, FcμR and TCRα are illustrated for comparison. Receptor domains include Ig-like (IgL), variable-type (V), and constant (C) as defined by UniProt (http://www.uniprot.org/).

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>pIgR encoded by cDNA 4120, transcript variant 1 (GenBank KF932324)
MSLPLLLTALVLGGLPGCHSTVTTIGDVAVLEGGSVTVPCHYNPQYISNVKYWCSGRMREFCSSLARTDDPESAPNGNRKVTIADDP
TQHVFTVNMRNLTEDDSGWYWCGVELGGMWVSDSTASLYISVVQGMSVMNGMVSAEEGKSVSVQCLYSKNLRWSEKRWCRSGNWNSC
LLTDSEGTFSGKNVHIHDDKNSVFTVTLQRLEMRDSGWYWCGAGQQNVAVHVSVTRRSPTPVSTAFPVHNKTAGNLSVTSSNESYSR
PVWESPLVMCGVVLLVMTACVALWKLQQQCKKKHKPRETSDMSDNLAICPWREGDCKNASVIFLNTPAQQVQML

>pIgR encoded by cDNA 4121, transcript variant 2 (GenBank KF932325)
MSLPLLLTALVLGGLPGSHSTVTTIGDVAVLEGGSVTVPCHYNPQYISNVKYWCSGRMREFCSSLARTDDPESAPNGNRKVTIADDP
TQHVFTVNMRNLTEDDSGWYWCGVELGGMWVSDSTASLYISVVQGMSVVNGMVSAEEGKSVSVQCLYSKNLRSSEKRWCRSGNWNSC
LLTDSEGTFSGKNVHIHDDKNSVFTVTLQRLEMRDSGWYWCGAGQQNVAVHVSVTRRSPTPVSTASPVHNKTAGNLSVTSSNESYSR
PVWESPLVMCGVVLLVMTACVALWKLQQQCKKKHKPRETSDMSDNLAICPWREGDCKNASVIFLNTPAQQVQML

>Pigrl2.1 encoded by cDNA 3842, transcript variant 1 (GenBank KF932326)
MFYITAGFWLILGVESFEGGSNHTITVKPGGSVIIPCYYDEKNPPQKKYWFSEHDQSNKYTNTTEENLSVIDHPDQSLFTVTMRNLQ
ENKHNGQYYCTVETEQKTNVTYELYLQVHSAPDVSVMNSSVSGHEGDDVSVQCFYSSGYKDKQKRWCRYKDQKCFSEEKTDTSQNSS
VQISDDGESSFTVLMTGLTLSDSGWYFCSVGHQTIPVQLTVTEDGVSAFHTSTVFCTST-

>Pigrl2.1 encoded by cDNA 3843, transcript variant 2 (GenBank KF932327)
MFYITAGFWLILGVESFEGGSNHTITVKPGGSVIIPCYYDEKSPPQKKYWFSEHDQSNKYTNTTEENLSVIDHPDQSLFTVTMRNLQ
ENKHNGQYYCTVETGQKTNVTYELYLQVHSAPDVSVMNSSVSGHEGDDVSVQCFYSSGYKDKQKRWCRYKDQKCLSEEKTDTSQNSS
VQISDDGESSFTVLMTGLTLSDSGWYFCSVGHQTIPVQLTVTEDGVSAFHTSTVFCTST-

>Pigrl2.1 encoded by cDNA 3844, transcript variant 3 (GenBank KF932328)
MFYITAGFWLILGVESFEGGSNHTITVKPGGSVIIPCYYDEKSPPQKKYWFSEHDQSNKYTNTTEENLSVIDHPDQSLFTVTMRNLQ
ENKHNGQYYCTVETGQKTNITYELYLQVHSAPDVSVMNSSVSGHEGDDVSVQCFYSSGYKDKQKRWCRYKDQKCFSEEKTDTSQNSS
VRISDDGESSFTVLMTGLTLSDSGWYFCSVGHQTIPVQLTVTEDGVFAFHTSTVFCTST-

>Pigrl2.3 encoded by cDNA 3820, transcript variant 1 (GenBank KF932329)
MAAYAAAKILYLSVGFWLILGAESYDAFSSNRLTVKPGGSVTTPCYYDEKNTQLKYWFSVNDECNTYTNTTEENLSVIDHPDQSLVT
VTMRNLQEKHTGEYHCGVVPGGVIYKIYLQVQDVPDVSVKSSSVSGHEGGNVSVQCFYSSGYRDKQKRWGRFKDEKCFREKKTNTSK
NSSVQISDDGESSFTVLMTGLTLSDSGWYYCSAGEQILPLQLTVTTSESVSVNTDTGTHPDRWRFCLSHS-

>Pigrl2.3 encoded by cDNA 3822, transcript variant 2 (GenBank KF932330)
MAAYAAAKILYLSVGFWLILGAESYDAFSSNRLTVKPGGSVTIPCYYDEKNTQLKYWFSVNDECNTYTNTTEENLSVIDHPDQSLVT
VTIRNLQEKHTGEYHCGVVPGGVIYKIYLQVQDVPDVCVKSSSVSGHEGGNVSVQCFYSSGYRDKQKRWCRFKDEKCFREKKTNTSK
NSSVQISDDGESSFTVLMTGLTLSDSGWYYCSAGEQILPVQLTVTTSESVSVNTDPGTHPDRWRFCL-

>Pigrl2.3 encoded by cDNA 3823, transcript variant 3 (GenBank KF932331)
MAAYAAAKILYLSVGFWLILGAESYDAFSSNRITVKPGGSVTIPCYYDEKNTQLKYWYSVNDECNTYTNTTEENLSVIDHPDQSLVT
VTMRNLQEKHTGEYHCGVVPGGVIYKIYLQVQDVPDVSVKSSSVSGHEGGNVSVQCFYSSGYRDKQKRWCRFNDEKCFREKKTNTSK
NSSVQISDDGESSFTVLMTGLTLSDSGWYYCSAGEQILPLQLTVTTSESVSVNTDTGTHPDRWRFCLSHS-

>Pigrl2.3 encoded by cDNA 3824, transcript variant 4 (GenBank KF932332)
MAAYAAAKILYLSVGFWLILGAESCDAFSSNRLTVKPGGSVTIPCYYDEKNTQLKYWFSVNDECNTYTNTTEENLSVIDHPDQSLVT
VTMRNLQEKHTGEYHCGVVPGGVIYKIYLQVQDVPDVSVKSSSVSGHEGGNVSVQCFYSSGYRDKQKRWCRFKDEKCFREKKTNTSK
NSSVQISDDGESSFTVLMTGLTLSDSGRYYCSAGEQILPLQLTVTTSESVSVNTDTGTHPDRWRFCLSHS-

>Pigrl2.3 encoded by cDNA 3968, transcript variant 5 (GenBank KF932333)
MTAYAAARILYLSVGFWLILGAESYDAFSSNRLTVKPGESVTIPCYYDEKNTQLKKYWFSVNDKCNTYTNTTEENLSVIDHPDQSLV
TVTMRNLQEKHTGEYHCGVVPGGVIYKIYLQVQDVPDVSVKSSSVSGHEGGNASVQCFYSSGYRDTQKRWCRFKDEKFFREKKTNTS
KNSSVQISDDGESSFTVLMTGLRLSDSGWYFCSAGEQILPLQLTVTTLESVSVNTDTGTHPDRWRFCLSHS

>Pigrl2.3 encoded by cDNA 3969, transcript variant 6 (GenBank KF932334)
MTAYAAARILYLSVGFWLILGAESYDAFSSNRLTVKPGGSVTIPCYYDEKNTQLKKYWFSVNDKCNTYTNTTEENLSVIDHPDQSLV
TVTMRNLQEKHTGEYHCGVVPGGVIYKIYLQVQDVPDVSVKSSSVSGHEGGNASVQCFYSSGYRDTQKRWCRFKDEKFFREKKTNTS
KNSSVQISDDGESSFTVLMTGLRLSDSGWYFCSAGEQILPLQLTVTTLESVSVNTDTGTHPDRWRFCLSHS
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>Pigrl2.4 encoded by cDNA 4003, transcript variant 1 (GenBank KF932335)
MAAYATATILYISIGFWFILSADSYNEFSNHALTVPPGGSVTIPCHYNEQYTQLKKHCYLETDKYTNTREKNLLVIDHPDQSLFTVT
MRNLRENQTGLYYCVVETGGTRTVIYEFYLKVQYVPDVSVMSSSVSGHEGDTVSVQCFYSSGYKNEQKQWCRYKNQKCFGEKKTDTS
KSSSVQISDDGKSSFTVLMTGLRLSDSGWYFCSVGNQMIPVQLTVTHTESDRNNKDRDEKLSPVWFFALTPVLLISLILVGVFIFKW
RRRPKQDEHQLKERNDSSTTDEISSKPDDLAVYCSINDETPYSISPLDPNKNMIYSTIDYIPGSEAKPPAGEDVYSSVGPH

>Pigrl2.4 encoded by cDNA 4004, transcript variant 2 (GenBank KF932336)
MAAYATATILYISIGFWFILSADSYNEFSNHALTVQPGGSVTIPCHYNEQYTQLKKHCYLETDKYTNTREKNLLVIDHPDQSLFTVT
MRNLRENQTGLYYCVVETGGTRTVIYEFYLKVQYVPDVSVMSSSVSGHEGDTVSVQCFYSSGYKNEQKQWCRYKNQKCFGEKKTDTS
KSSSVQISDDGKSSFTVLMTGLRLSDSGRYFCSVGNQMIPVQLTVTHTESDRNNKDRDEKLSPVWFFALTPVLLISLILVGVFIFKW
RRRPKQDEHQLKERNDSSTTDEISSKPDDLAVYCSINDETPYSISPLDPNKNMIYSTIDYIPGSEAKPPAGEDVYSSVGPH

>Pigrl2.5 encoded by cDNA 3970 (GenBank KF932337)
MTAYATTKINYILVGFWLILGAESYFDWLSSRITIKPGGSVTIPCLYEKNPMQLKYWYSVNNPSNKFTNTKEENLSVIDHPDQSLFT
VTMRNLQENQTGEYHCVVKTGVTGGVTYRVYLDIRYDPDVSLKGRSLSGHEGGNVSVQCFYSSAYRNSQKQWCRYKDEKCFTERKTD
ISQNSSVQISDDGESPFTVLMTGLTLSDSGWYFCSAGEQIIPVQLTITHTESVNINTDLNNKDGAEELPPVWFLASALVLLISLILV
GVFIWRRRPKQDEHQLKESNNSRTTDEISSKPDDLAVYCSINDETPYSISPLDPNKNMIYSTIDYIPGSEAKSPAGEDAYCTVGPH

>Pigrl3.4 encoded by cDNA 3962, transcript variant 1 (GenBank KF932338)
MIHTLILTGVLLHIGDGAWIDKLNIGVKSGSPGIIPCQYVEQYQENLKYWCQGMFWISCTILAFGNGPRSKFSITDYPAQSIFTVEW
QNLQPSDSGYYWCAVEIGDSEKPDASYYLYLKVQSAPDVSVKSSSVSGHEGGNVSVQCFYSSGYKAKEKQWCKYKDQKCFYPKKNTG
TSQNSSVQISDDGKSSFTVRMAGLRLSDSGWYFCSVGNLQVPVQLTVSKPDPKDLYTTQPSAKQIPTSVLTTVSRPGTNILNKEHFC
LSET

>Pigrl3.4 encoded by cDNA 4001, transcript variant 2 (GenBank KF932339)
MIHTLILTGVLLHIGDGAWIDKLNIGVKSGSPGIIPCQYVEQYQENLKYWCQGMFWISCTILAFGNGPRSKFSITDYPAQSIFTVEW
QNLQPSDSGYYWCAVEIGDSEKPDASYYLYLKVQSAPDVSVKSSGVSGHEGGNVSVQCFYSSGYKAKEKQWCKYKDQKCFYPKKNTG
TSQNSSVQISDNGESSFIVLMTGLNLSDFGWYFCSVGNLQVPVQLTVSKPDPKDLYTTQPSAKQIPTSVLTTVSRPGTNILNKEHFC
LSET

>Pigr3.5 encoded by cDNA 3960, transcript variant 1 (GenBank KF932340)
MIHTLILAGVLLHIGNGAWIDKLNIGVKSGSPGIIPCLYKEQYKENHKYWCQGMFWSSCTILAFGNGPRSKFSITYYPAQSIFTVKW
QNLQPSDSGYYWCAVEIGDSGIPDARYYLYLKVQPVPDVSVKSSSVSGHEGGNVSVQCFYSSEYHNKLKGWCRVKDKRCFKEEKTDT
SQNPSVQISDDGESSFTVLMAELRLSDSGWYFCSVGDLQVPVRLTVTKPEPKAAMTTGSRPKSRVEDPPTTDSEINLGKDSKDEQNK
YDMILIMCLVVTLALLLLVALFTIIIRRMRKNPEGDPTREERFNSSTMNTMPSENQMTSISPAEADSSADDSSMVYSPVSFTKTCLS
SVDPEPDVLYSAVMKDRKTV

>Pigr3.5 encoded by cDNA 3961, transcript variant 2 (GenBank KF932341)
MIHTLILAGVLLHIGNGAWIDKLNIGVKSGSPGIIPCLYKEQYKENHEYWCQGMFWSSCTILAFGNGPRSKFSITYYPAQSIFTVKW
QNLQPSDSGYYWCAVEIGDSGIPDARYYLYLKVQPVPDVSVKSSSVSGHEGGNVSVQCFYSSEYHNKLKGWCRVKDKRCFKEEKTDT
SQNPSVQISDDGESSFTVLMAELRLSDSGWYFCSVGDLQVPVRLTVTKPEPKAAMTTGSRPKSRVEDPPTTDSEINLGKDSKDEQNK
YDMILIMCLVVTLALLLLVALFTIIIRRMRKNPEGDPIREERFNSSTMNTMPSENQMTSISPAEADSSADDSSMVYSPVSFTKTCLS
SVDPEPDVLYSAVMKDRKTV

>Pigr3.5 encoded by cDNA 4021, transcript variant 3 (GenBank KF932342)
MIHTLILTGVLLHIGNGAWIDKLNIGVKSGSPGIIPCLYKEQYKENHKYWCQGMFWSSCTILAFGNGPRSKFSITYYPAQSIFTVKW
QNLQPSDSGYYWCAVEIGDSGIPDARYYLYLKVQPVPDVSVKSSSVSGHEGGNVSVQCFYSSEYHNKLKGWCRVKDKRCFKEEKTDT
SQNPSVQISDDGESSFTVLMAELRFSDSGWYFCSVGDLQVPVRLTVTKPEPKAAMTTGSRPKSRVEDPPTTDSEINMGKDSKDEQNK
YDMILIMCLVVTLALLLLVALFTIIIRRMRKNPEGDPIREERFNSSTMNTMPSENQMTSISPAEADSSADDSSMVYSLVPFTKTCLS
SVDPEPDVLYSAVMKDRKTV

>Pigrl3.8 encoded by cDNA 4020, transcript variant 1 (GenBank KF932343)
MIHTLILAGVLLHIGDGAWVINPNIRVKSGSPGIIPCLYKEQHKEHQKYLCRGRFWSSCTILAYVNETGKKFFITDYPAQSIFTVKW
QNLQESDYGYYWCAVEIGGPGTLDDSYYFYLMVQSASALSVLSSSVSGHEGGNVSVQCFYSSGYKAYNKQWCRVKDKSCFTEKKTDT
SQNSSVQISDDGKSSFTVLMTGLTLSDSGWYFCSVEYLQDPVQLSVTKPEPKVFPVLTTPTCTPEEKNEHNTTFQQYNFNKYASNDK
QR

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>Pigrl3.8 encoded by cDNA 4023, transcript variant 2 (GenBank KF932344)
MIHTLILAGVLLHIGDGAWVINPNIRVKSGSPGIIPCLYKEQHKEHQKYLCRVRFWSSCTILAYVNETGKKFFITDYPAQSIFTVKW
QNLQESDYGCYWCAVEIGGPGTLDDSYYFYLMVQSASALSVLSSSVSGHEGGNVSVQCFYSSGYKAYNKQWCRVKDKSCFTEKKTDT
SQNSSVQISDDGKSSFTVLMTGLTLSDSGWYFCSVEYLQDPVQLTVTKPEPKVFPVLTTPTCTPEEKNEHNTTLQQYNFNKHASNDK
QR

>Pigrl3.9 encoded by cDNA 4027 (GenBank KF932345)
MIHTLILAGVLLHIGDGVWINKLYIGVKSGSPGIIPCLYEEQYKEHQKYWCRGYIWSSCSILAYVNETRNKFSITDYPAQSIFTVEW
QNLQESNSSYYWCAVEIGGPGTLDAGYYLYLTVQSDPAVSVMNSSVSGHEGGNVSVQCFYSSRYKAKNKQWCRVKDKSCFTEKKTDT
SQNSSVQISDDDESSFTVLITGLTLSDSGWYFCSVEDLQVPVQLTVTKPEPKEMNTTQLSTNTTSAKMHSTINTLCLLIQG

>Pigrl3.10 encoded by cDNA 4002, transcript variant 1 (GenBank KF932346)
MIHTLILTGVLLHIGDGAWIDKLNIGVKSGSPGIIPCQYVEQYQENLKYWCQGMFWISCTILAFGNGPRSKFSITDYPAQSIFTVEW
QNLQPSDSGYYWCAVEIGDSEKPDASYYLYLKVQSAPDVSVKSSSVSGHEGGNVSVQCFYSSGYKAKEKQWCKYKDQKCFYPKKNTG
TSQNSSVQISDDGKSSFTVRMAGLRLSDSGWYFCSVGDLQVPVRLTVTKPEPKAPDLSVMNSSVSGHVGGNVSVQCFYSSGYKNKTK
QWCRVKDKSCFPENKTDTSQNSSVQISDNGESSFIVLMTGLNLSDSGWYFCSVGNLQVPVQLTVSKPDPKDLYTTQPSAKQIPTSVL
TTVSRPGTNILNKEHFCLSET

>Pigrl3.10 encoded by cDNA 5187, transcript variant 2 (GenBank KF932347)
MIHTLILTGVLLHIGDGAWIDKLNIGVKSGFPGIIPCQYVEQYQENLKYWCQGMFWSSCTILAFGNGPRSKFSITDYPAQSIFTVEW
QNLQPSDSGYYWCAVEIGDSEKPDASYYLYLKVQSAPDVSVKSSSVSGHEGGNVSVQCFYSSGYKAKNKQWCGYKDKKCFYPKKNTG
TSQNSSVQISDDGKSSFTVLMAGLRLSDSGWYFCSVGDLQVPVRLTVTKPEPKDPDLSVMNSSVSGHVGGNVSVQCFYSSGYKNKTK
QWCRVKDKSCFPENKTDTSQNSSVQISDDGESSFIVLMTGLNLSDSGWYFCSVGNLQVPVQLTVSKPDPKDLYTTQPSAKQIPTTVL
TTVSRPGTNILNKEHFCLSET

>Pigrl3.10 encoded by cDNA 5188, transcript variant 3 (GenBank KF932348)
MIHTLILTGVLLHIGDGAWIDKLNIGVKSGFPGIIPCQYVEQYQENLKYWCQGMFWSSCTILAFGNGPRSKFSITDYPAQSIFTVEW
QNLQPSDSGYYWCAVEIGDSEKPDASYYLYLKVQSAPDVSVKSSSVSGHEGGNVSVQCFYSSGYKAKNKQWCGYKDKKCFYPKKNTG
TSQNSSVQISDDGKSSFTVLMAGLRLSDSGWYFCSVGDLQVPVRLTVTKPEPKDPDLSVMNSSVSGHVGGNVSVQCFYSSGYKNKTK
QWCRVKDESCFPENKTDTSQNSSVQISDDGESSFIVLMTGLSLSDSGWYFCSVGNLQVPVQLTVSKPDPKDLYTTQPSAKQIPTTVL
TTVSRPGTNILNKEHFCLSET

>Pigrl4.2 encoded by cDNA 4177 (GenBank KF932349)
MAHPSLLIAVLFCTAGTLSMKTLDRVPVIEGETITIPCLYDNKYKLNKKYWCNGNTWLGCSVVAYANHTGKWTITDYPDHNMFTVTL
NNSTSSDSGHYWCAVEIDHHVDNSKYLYLTVQKAPDVSVLSSSVSGHKGDDVSVRCFYRSAYQNKLKQWCRIDDLTCFREKKTDTSQ
NSSVQISDDGESSFTVLMTGLRLSDSGWYFCSVGNLQVPVQLTVYQGENKNKNTCKSFIQLPSDKI
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Figure S5. Full-length zebrafish pIgR and PIGRL proteins. Predicted proteins encoded by zebrafish *pigr* and PIGRL transcripts are shown. Transcript numbers corresponding to Figure 5 are listed and GenBank accession numbers are in parentheses. Predicted peptide leader sequences and transmembrane domains were predicted by SMART software (Letunic et al. 2012. *Nucleic Acids Res*. **40 (D1)**: D302-D305) and are in boxed text. Cytoplasmic ITIMs (S/I/V/LxYxxI/V/L) are shaded orange, ITIM-like sequences are shaded yellow and additional cytoplasmic tyrosines are shaded green.

pIgR^{4121}-D1 and pIgR^{scaffold234}-D1

pIgR^{4121}	GSHSTVTTIGDVAVLEGGSVTVPCHYNPQYISNVKYWCSGRMREFCSSLARTDDPESAPNGNRKVTIADDPTQHVFTVNMRNLTEDDSGWYWCGVELGGMWVSDSTASLYISVVQ
pIgR^{scf234}	GSHSTVTTIGDVAVLEGGSVTVPCHYNPQYISNVKYWCSGRMREFCSSLARTDDPESAPNGNRKVTIADDPTQHVFTVNMRNLTEDDSGWYWCGVELGGMWVSDSTASLYISVVQ

Pigrl1.1^{231}-D1 (GenBank KF932318) and Pigrl1.1^{scaffold234}-D1

1.1^{231}	PEYKTASASRALTVQTGGSLVIPCYYDRYYTEYKKYWCFNAKGYFSSCSILAYANETKGKVSVTDHPDQSFFTVTMRNLQHEDTGAYWCAVEIKEFFNLDKREQLHLTVQS
1.1^{scf234}	SEYKTASASRALTVQTGGSLVIPCYYDRYYTEYKKYWCFNAKGYFNSCSILAYTNETKGKVSLTDHPDQSFFTVTMRNLQHEDTGAYWCAVEIEGFFKLDKREQLHLTVQS

Pigrl1.7^{251}-D1 (Genbank KF932319) and Pigrl1.7^{scaffold234}-D1

1.7^{251}	CKTAYVDPVLTVQTGGSVVIPCFYDKRYAEYKKYWCFIKTDSYRFCSALAYTNETKGKVSVIDHPDQSFFTVTMRNLQHEDTGYYWCAVEIGTRLTKDETKKLQLRVQT
1.7^{scf234}	CKTAYVDPVLTVQTGGSVVIPCFYDKRYAEYKKYWCFYKTDSYWFCSALAYTNETKGKASVIDHPDQSFFTVTMRNLQHEDTGYYWCAVEIGTILIRDETKKLQLRVQT

Pigrl2.6^{1926}-D1 (Genbank KF932320) and Pigrl2.6^{scaffold235}-D1

2.6^{1926}	VESFSGGSNRTITVKPGGSVTIPCYYDEKNPPRRKYWFSEHGQSNKYTNTTEENLSVIDHPDQSLFTVTMRNLQENKHNGQYYCTVETGQKPNVTYVTYELYLKVHS
2.6^{scf235}	VESFSGGSNRTITVKPGGSVTIPCYYDEKNPPRRKYWFSEHGQSNKYTNTTEENLSVIDHPDQSLFTVTMRNLQENKHNGQYYCTVETGQKPNVTYVTYELYLKVHS

Pigrl3.2^{1933}-D1 (GenBank KF932321) and Pigrl3.2^{scaffold235}-D1

3.2^{1933}	EAWVYDVNIGVKSGSPGIIPCVYKEHLKANRKYWCQGSVWSSCTILAYANETRNKFSITDYPEQSVFTVEWQNLQPSDSGCYWCAVEINXYGTVDDGYHMYLTVQS
3.2^{scf235}	EAWVYDVNIGVKSGSPGIIPCVYKEKLKANRKYWCQGSVWSSCTILAYANETRNKFSITDYPEQSVFTVEWQTLQPSDSGCYWCAVEINGYGTVDDGYHMYLTVQS

Pigrl3.2^{1934}-D2 (GenBank KF932322) and Pigrl3.2^{scaffold235}-D2

3.2^{1934}	YGVFVKSSSVSGHEGGDVSVQCIYSDIYRNTIKQWCRVKDKRCYAVERSDTSQNPSVQISDDGESSFTVLMTGLRLSDSGWYFCSVEDLQVLVQLTVTKPGPK
3.2^{scf235}	YGVFVKSSSVSGHEGGDVSVQCIYSDIYRNTIKQWCRVKDKRCYAVERSDTSQNPSVQISDDGESSFTVLMTRLRLSDSGWYFCSVEDLQVLVQLTVTKPGPK

Pigrl4.1^{1910}-D2 (GenBank KF932323) and Pigrl4.1^{scaffold235}-D2

4.1^{1920}	PVVSVMNSSVFGREGDDVRVQCFYSTAYQNELKQWCRMKDQKCFTEEKTDTSQSSSVQISDDGESSLTVLMTGLRLSDSGWYFCSAGDLQVPVQLTVT
4.1^{scf235}	PDVSVVNSSVLGHEGDDVRVQCFYSSAYQNELKRWCRMKDQKCFTEKKSDASQNSSVQISDDGES-FSVLMTGLRLSDSGWYFCSAGDLQVPVRLTVT

Figure S6. pIgR and Pigrl domains employed as Fc fusions. Alignments of Ig domains from pIgR-hFc and PIGRL-hFc fusion proteins with Ig domains encoded by corresponding genomic sequences. Allele designation is shown as superscript after the gene.