## **Supporting Information**

## Kasamatsu et al. 10.1073/pnas.1001910107

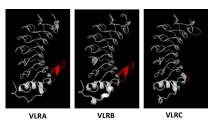
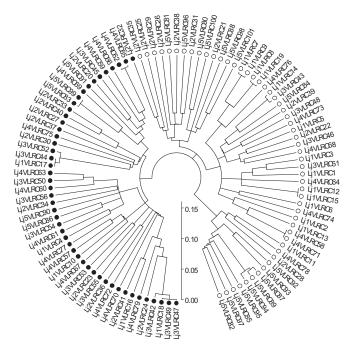


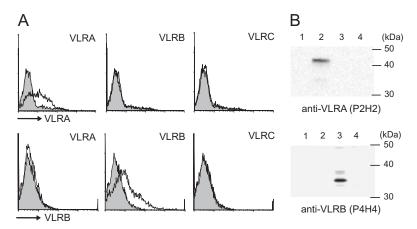
Fig. S1. Predicted 3D structures of lamprey variable lymphocyte receptor (VLR)A (accession no. AB021126) and VLRC (accession no. AB507271). Prediction was made using the crystal structure of lamprey VLRB (Protein Data Bank ID: 3E6J; *Middle*) (23) as a template. Protrusions located in the leucine-rich repeat C-terminal cap (LRRCT) of VLRA and VLRB are indicated in red. A loop at the corresponding region of VLRC is also indicated in red.

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Lj3VLRC55 Lj3VLRC56 Lj3VLRC53	ACLAVGRODICTCSNRTD ACLAVGRODICTCSNRTD	SSPETVDCSSKKLTAVPTGIPES7		TTO - I O HAR GVED DEDIGTING TENOL SONK - I O HARVGVED BININ IN BUBICIPNOL	SMSLOVER Q L Q SS I I E	I K I KTLINLQILYLNNNQLQSIPKGAFD I KI	TUTI SENT OSVPDGATDELAS TURI OTNOLOSVPNGATALITS	TINDIAN MOOASSDITTL TITUNIN DOOASSDITTL	RTTAK TOKISOME SACONTSAVA RTTAK TOKISOME SACONTSAVA RTTAK TOKISOME SACONTSAVA	222 045 - 2 100 PC : 222 045 - 2 100 PC : 246 045 - 2 100 PC : 222
Lj4VLRC70 Lj4VLRC57	ACLAVGROD I CTCSNATD ACLAVGROD I CTCSNATD	SSPETVDCSSKKLTAVPTGIPAS	TRUCKS NOT SUPANALKAL OF USIC	******	SIM POVIDR	I KTLINLQTLYLNINQLQSIEAGLFD I N		THEOP TOASD	RTELAKS TOKIAGHE SACON CTSTAVK RTELAKS TOKIAGHE SACONCTSTAVK	
Lj4VLRC59 Lj4VLRC66	ACLAVGKDDICTCSNKTD ACLAVGKDDICTCSNKTD	SSPETVDCSSKKLTAVPTGIPAS SSPETVDCSSKKLTAVPTGIPAS	THE POINT PERIOD AND PROPERTY AND A STORE	IN POLYMARY WED HONNEHRIDE NO ISSPOLYMARY OVER HONNEHRISSNIL	SMOPROTORITKI TY SISENOLOSIIK SMOPROTORITKI TY SISENOLOSIIK	A DAST	TROPANNICOSVPORTED DAS TUTI CONTOSVPORTED DAS	THE YAN MOOA SDUTYL	RTHAN TOKISME SACON TSHAW RTHAN TOKIAME SACON FTSHAW	
Lj4VLRC72 Lj4VLRC77	ACLAVGKD DICTCSNKTD ACLAVGKD DICTCSNKTD	SSPETVDCSSKKLTAVPTGIPAS SSPETVDCSSKKLTAVPGGIPAD	TEKLOPHLNOFTSUPG ABHSA PROTOSIT TEKLOPRETGIAKI SDTAPROLOKIA UNIK	IDAO - LOSLAVGVEDOUESI GOLHUTINOL MAQUEDOUESI GOLHUTINOL	SMISOVERSITELTED INVNOVOSTER		TUEL RINE LORV PROATDS IVIN TUM RONGLOSV PROATDS LAS	TUBLELS WIDOASSDITTEL	RTHAKITOKIAGMESACONPTPEAVK	222
Lj4VLRC79 Lj5VLRC86	ACLAVGED DICTCSNETD	SSPETVDCSSKKLTAVPTGIPAS SSPETVDCSSKKLTAVPTGIPAS	TERIALO NOLON PAGMEDOL BIGTICH TERIALE NOLTSI PD ARY CORD TOD	ytyk – iksinfryftyftyftoi tlinidrwyf Nyk – i prinfygyftoi fennodi rintwyf	SWEDGVED SMEPRVEDSIGTIONISIDA TOPOSITIE	IIII III	REDIZONOLOSVPDCAPDSLVA TUMI SINGLOSVPDCAPDSLVA	TIMPELS ADOACSDUTHL	RTYTAKNTOK I SOME SACON PT STAVK KTYTAKNTOK I SOME SACON PT STAVKI	198 198 198 198 198
Lj5VLRC90 Lj1VLRC17 Lj3VLRC44		SSPETVDCSSKKLTAVPTGIPAS SSPETVDCSSKKLTAVPTGIPAD SSPETVDCSSKKLTAVPTGIPAD	TRUCHTNOLANT PG A DHSU RUTUSH DKUMIMSTOLKSI SPT I ENHURD I CRUEH	DIN-PT-INVEND INVEDING DIVISION		VII	THAT RENGIOSVED CATOSING DURI STNULOSVED CATOSING		ARTEAN TOXI A POBLACIA	DWRPEDTOWPCN : 222 DWRTENTOWTCN : 222 DWRTENTOWTCN : 222
Lj3VLRC44 Lj3VLRC52	AGLAVEROD OTES	SSPETVDCSSKKLIAVPTGIPAD	THVIMSTOKSI SPT INNERDICEPT	STO-I ADDANIO VNIKEI YILL TOP	TSM POWER AT THE MENNING OF COLUMN	V	DIR STAROSVERCE ID ITS	MORO SI ANASDILL	RTTAKSTOKISOMESTOON	DUNTER IKNUTCH 222 DUNTER IKNUTCH 222

Fig. S2. Sequences of the VLRC diversity region [LRR N-terminal cap (LRRNT) to LRRCT] amplified by RT-PCR from Japanese lamprey leukocytes. Diversity of VLRC was assessed by PCR in five lampreys using leukocyte cDNA as templates (primer sequences: 5'-AGTGTTGGTCCGTGCGAGC-3' and 5'-GGTGGGA-GACGATGCTGTAA-3'). The whole diversity region, from LRRNT to LRRCT, was amplified. PCR products were cloned into vectors using the pGEM-T Easy vector system (Promega) or TOPO TA cloning system (Invitrogen) and their sequences determined using an automated sequencer. A total of 101 sequences (accession nos. AB507273–AB507373) were aligned with the Clustal X program and adjusted by eye to maximize sequence similarity. Note that the region encoding LRR1, LRRV, LRRVe, and connecting peptide (CP) exhibits high levels of diversity, whereas LRRNT and 5'-LRRCT show only limited diversity. Clones were named as described in the legend to Fig. 2.



**Fig. S3.** Graphic representation of VLRC diversity. The neighbor-joining tree was constructed using the 101 diversity region sequences shown in Fig. S2. Filled circles stand for VLR proteins with 5'-LRRCT sequences identical or nearly identical to that of the germline *VLRC* gene, whereas open circles stand for VLR proteins with 5'-LRRCT sequences identical to that of module 1 or 2. Clones were named as described in the legend to Fig. 2.



**Fig. 54.** Preparation of mAbs specific for VLRA or VLRB. Invariant 3' terminus-encoding sequences of VLRs were PCR amplified using KOD DNA polymerase (Toyobo) with the following primer pairs: 5'-CGCGGATCCGCGAACCAGTGGAACTGCAGCAG-3' and 5'-CCCAAGCTTGGGTTCTGTGGGCGCACTCACTGC-3' for VLRA and 5'-CGCGGATCCGCGGTCCTGGTACCAATACCCCCG-3' and 5'-CCCAAGCTTGGGGCAGGCGGGCTTTCCGCAGT-3' for VLRB. Resultant DNA fragments were cloned into pQE-30 (Qiagen) and expressed in *Escherichia coli*. His-tagged recombinant VLRA and VLRB proteins were purified under denaturing conditions with Ni-NTA agarose (Qiagen) and injected into the footpads of BALB/c mice. One week after booster immunization, popliteal lymph node cells were collected and fused with P3-X63-Ag8.653 myeloma cells. Hybridomas specifically reacting with VLRA (clone P2H2) or VLRB (clone P4H4) were selected by ELISAs against immunogens. The specificities of mAbs were evaluated by testing their reactivity against a panel of HEK293T cells expressing FLAG-tagged VLRA, VLRB, or VLRC proteins by flow cytometry (A) or Western blotting (B). In A, transfectants were stained with VLRA-specific mAb (*Upper*) or VLRB-specific mAb (*Lower*). Lanes 1–4 in *B* indicate mock, VLRA, VLRB, and VLRC transfectants, respectively.