

Supplementary Materials

Full T-cell activation and function in teleosts require collaboration of first and co-stimulatory signals

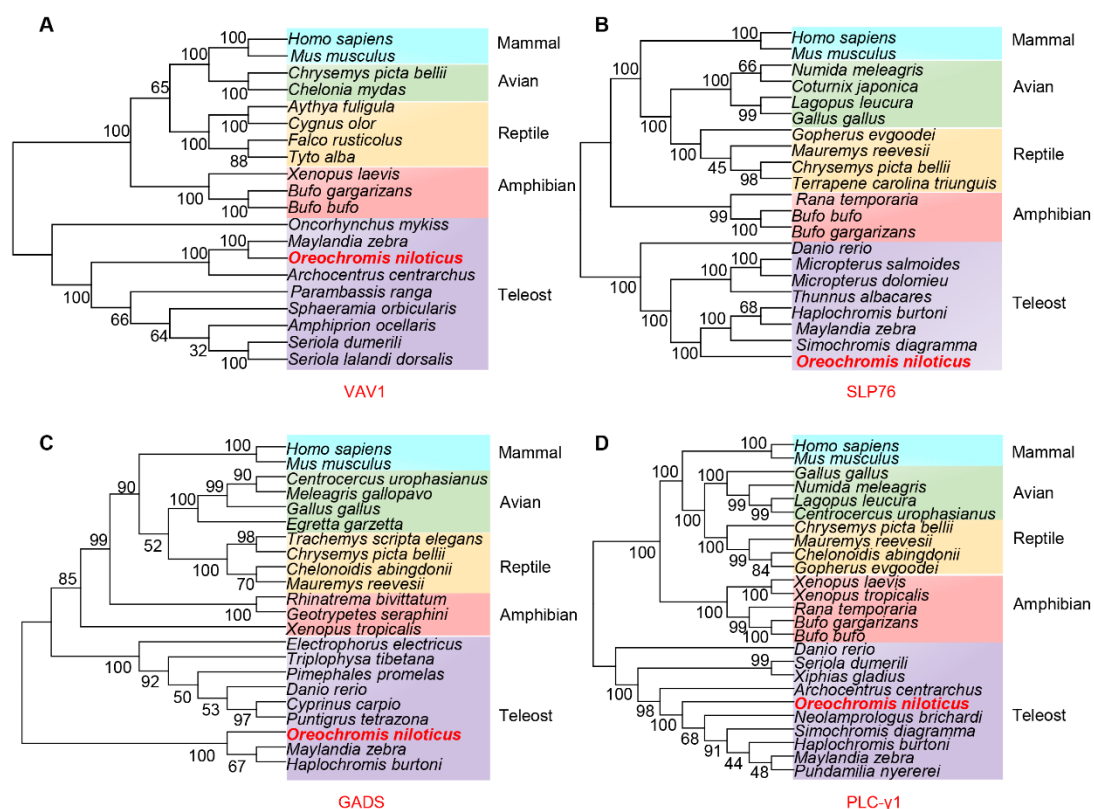
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Supplementary Figure S1 Phylogenetic trees of indicated molecules

A–D: Phylogenetic trees of VAV1 (A), SLP-76 (B), GADS (C), and PLC-γ1 (D) were constructed using MEGA v7.0 with the neighbor-joining method. Bootstrap values for 1 000 replicates (%) are indicated on each branch.

Supplementary Table S1. Information of genes used for sequence, phylogeny and structure analysis

Source	accession No.	Gene	Species	Application
GenBank	XP_013125310.1	LAT	<i>Oreochromis niloticus</i>	PA
GenBank	XP_021324861.1	LAT	<i>Danio rerio</i>	PA
GenBank	XP_043089930.1	LAT	<i>Puntigrus tetrazona</i>	PA
GenBank	XP_039543672.1	LAT	<i>Pimephales promelas</i>	PA
GenBank	XP_017310361.1	LAT	<i>Ictalurus punctatus</i>	PA
GenBank	XP_046721437.1	LAT	<i>Silurus meridionalis</i>	PA
GenBank	XP_036446287.1	LAT	<i>Colossoma macropomum</i>	PA
GenBank	XP_036825111.1	LAT	<i>Oncorhynchus mykiss</i>	PA
GenBank	XP_014047807.1	LAT	<i>Salmo salar</i>	PA
GenBank	XP_013125310.1	LAT	<i>Oreochromis niloticus</i>	PA
GenBank	XP_031749589.1	LAT	<i>Xenopus tropicalis</i>	PA
GenBank	XP_033799862.1	LAT	<i>Geotrypetes seraphini</i>	PA
GenBank	XP_039393239.1	LAT	<i>Mauremys reevesii</i>	PA
GenBank	XP_030404032.1	LAT	<i>Gopherus evgoodei</i>	PA
GenBank	XP_043373013.1	LAT	<i>Dermochelys coriacea</i>	PA
GenBank	XP_037754786.1	LAT	<i>Chelonia mydas</i>	PA
GenBank	XP_025912706.1	LAT	<i>Apteryx rowi</i>	PA
GenBank	XP_025978835.1	LAT	<i>Dromaius novaehollandiae</i>	PA
GenBank	NP_055202.1	LAT	<i>Homo sapiens</i>	PA
GenBank	XP_006507459.3	LAT	<i>Mus musculus</i>	PA
GenBank	XP_030591462.1	GRB2	<i>Archocentrus centrarchus</i>	PA
GenBank	XP_029945618.1	GRB2	<i>Salarias fasciatus</i>	PA
GenBank	XP_020514367.1	GRB2	<i>Labrus bergylta</i>	PA
GenBank	XP_024128392.1	GRB2	<i>Oryzias melastigma</i>	PA
GenBank	XP_033982964.1	GRB2	<i>Trematomus bernacchii</i>	PA
GenBank	XP_042360233.1	GRB2	<i>Plectropomus leopardus</i>	PA
GenBank	XP_037544829.1	GRB2	<i>Nematolebias whitei</i>	PA
GenBank	XP_028456091.1	GRB2	<i>Perca flavescens</i>	PA
GenBank	AAH59450.1	GRB2	<i>Danio rerio</i>	PA
GenBank	XP_003443139.1	GRB2	<i>Oreochromis niloticus</i>	PA, 3D, DOA, DP
GenBank	XP_012827143.1	GRB2	<i>Xenopus tropicalis</i>	PA
GenBank	NP_001084357.1	GRB2	<i>Xenopus laevis</i>	PA
GenBank	XP_043384508.1	GRB2	<i>Chelonia mydas</i>	PA
GenBank	XP_038227519.1	GRB2	<i>Dermochelys coriacea</i>	PA
GenBank	XP_025946144.1	GRB2	<i>Apteryx rowi</i>	PA
GenBank	XP_039356926.1	GRB2	<i>Mauremys reevesii</i>	PA
GenBank	NP_001385050.1	GRB2	<i>Gallus gallus</i>	PA
GenBank	XP_042658346.1	GRB2	<i>Tyto alba</i>	PA
GenBank	PKK25653.1	GRB2	<i>Columba livia</i>	PA
GenBank	XP_033923460.1	GRB2	<i>Melopsittacus undulatus</i>	PA
GenBank	AAH85254.1	GRB2	<i>Mus musculus</i>	PA, 3D, DOA, DP

GenBank	NP_002077.1	GRB2	<i>Homo sapiens</i>	PA
GenBank	XP_005462712.1	VAV1	<i>Oreochromis niloticus</i>	PA, 3D, DOA, DP
GenBank	XP_036804677.1	VAV1	<i>Oncorhynchus mykiss</i>	PA
GenBank	XP_029994747.1	VAV1	<i>Sphaeramia orbicularis</i>	PA
GenBank	XP_028255743.1	VAV1	<i>Parambassis ranga</i>	PA
GenBank	XP_035802513.1	VAV1	<i>Amphiprion ocellaris</i>	PA
GenBank	XP_023286310.1	VAV1	<i>Seriola lalandi dorsalis</i>	PA
GenBank	XP_022593883.1	VAV1	<i>Seriola dumerili</i>	PA
GenBank	XP_030587173.1	VAV1	<i>Archocentrus centrarchus</i>	PA
GenBank	XP_004569054.1	VAV1	<i>Maylandia zebra</i>	PA
GenBank	XP_040280559.1	VAV1	<i>Bufo bufo</i>	PA
GenBank	XP_044142343.1	VAV1	<i>Bufo gargarizans</i>	PA
GenBank	XP_041440009.1	VAV1	<i>Xenopus laevis</i>	PA
GenBank	XP_037743954.1	VAV1	<i>Chelonia mydas</i>	PA
GenBank	XP_005312628.1	VAV1	<i>Chrysemys picta bellii</i>	PA
GenBank	XP_042658796.1	VAV1	<i>Tyto alba</i>	PA
GenBank	XP_037263351.1	VAV1	<i>Falco rusticolus</i>	PA
GenBank	XP_040398895.1	VAV1	<i>Cygnus olor</i>	PA
GenBank	XP_032060895.1	VAV1	<i>Aythya fuligula</i>	PA
GenBank	NP_035821.3	VAV1	<i>Mus musculus</i>	PA, 3D, DOA, DP
GenBank	XP_005259699.1	VAV1	<i>Homo sapiens</i>	PA
GenBank	XP_039900672.1	SLP-76	<i>Simochromis diagramma</i>	PA
GenBank	XP_004554606.2	SLP-76	<i>Maylandia zebra</i>	PA
GenBank	XP_005933653.1	SLP-76	<i>Haplochromis burtoni</i>	PA
GenBank	XP_038592975.1	SLP-76	<i>Micropterus salmoides</i>	PA
GenBank	XP_045926024.1	SLP-76	<i>Micropterus dolomieu</i>	PA
GenBank	XP_044228845.1	SLP-76	<i>Thunnus albacares</i>	PA
GenBank	XP_013121379.1	SLP-76	<i>Oreochromis niloticus</i>	PA, 3D, DOA, DP
GenBank	NP_998003.1	SLP-76	<i>Danio rerio</i>	PA
GenBank	XP_040217748.1	SLP-76	<i>Rana temporaria</i>	PA
GenBank	XP_018108932.1	SLP-76	<i>Xenopus laevis</i>	PA
GenBank	XP_040290702.1	SLP-76	<i>Bufo bufo</i>	PA
GenBank	XP_044152163.1	SLP-76	<i>Bufo gargarizans</i>	PA
GenBank	XP_039338899.1	SLP-76	<i>Mauremys reevesii</i>	PA
GenBank	XP_026506940.1	SLP-76	<i>Terrapene carolina triunguis</i>	PA
GenBank	XP_023956982.1	SLP-76	<i>Chrysemys picta bellii</i>	PA
GenBank	XP_030425893.1	SLP-76	<i>Gopherus evgoodei</i>	PA
GenBank	NP_990239.1	SLP-76	<i>Gallus gallus</i>	PA
GenBank	XP_042719000.1	SLP-76	<i>Lagopus leucura</i>	PA
GenBank	XP_015722666.1	SLP-76	<i>Coturnix japonica</i>	PA
GenBank	XP_021255732.1	SLP-76	<i>Numida meleagris</i>	PA
GenBank	NP_032554.2	SLP-76	<i>Mus musculus</i>	PA, 3D, DOA, DP
GenBank	NP_037446.1	SLP-76	<i>Homo sapiens</i>	PA
GenBank	XP_039543619.1	GADS	<i>Pimephales promelas</i>	PA

GenBank	XP_042576290.1	GADS	<i>Cyprinus carpio</i>	PA
GenBank	XP_004556406.1	GADS	<i>Maylandia zebra</i>	PA
GenBank	XP_005924260.1	GADS	<i>Haplochromis burtoni</i>	PA
GenBank	XP_043090808.1	GADS	<i>Puntigrus tetrazona</i>	PA
GenBank	XP_026858616.1	GADS	<i>Electrophorus electricus</i>	PA
GenBank	KAA0705765.1	GADS	<i>Triplophysa tibetana</i>	PA
GenBank	XP_005469094.1	GADS	<i>Oreochromis niloticus</i>	PA, DOA, DP
GenBank	NP_001017756.1	GADS	<i>Danio rerio</i>	PA
GenBank	XP_002933793.1	GADS	<i>Xenopus tropicalis</i>	PA
GenBank	XP_029446038.1	GADS	<i>Rhinatrema bivittatum</i>	PA
GenBank	XP_033785406.1	GADS	<i>Geotrypetes seraphini</i>	PA
GenBank	XP_039355280.1	GADS	<i>Mauremys reevesii</i>	PA
GenBank	XP_042699464.1	GADS	<i>Chrysemys picta bellii</i>	PA
GenBank	XP_034632001.1	GADS	<i>Trachemys scripta elegans</i>	PA
GenBank	XP_032643852.1	GADS	<i>Chelonoidis abingdonii</i>	PA
GenBank	XP_010709006.1	GADS	<i>Meleagris gallopavo</i>	PA
GenBank	XP_042693885.1	GADS	<i>Centrocerus urophasianus</i>	PA
GenBank	XP_035759102.1	GADS	<i>Egretta garzetta</i>	PA
GenBank	XP_040516551.1	GADS	<i>Gallus gallus</i>	PA
GenBank	NP_001276371.1	GADS	<i>Mus musculus</i>	PA, DOA, DP
GenBank	AAD04926.1	GADS	<i>Homo sapiens</i>	PA
GenBank	XP_006796378.1	PLC-γ1	<i>Neolamprologus brichardi</i>	PA
GenBank	XP_039863711.1	PLC-γ1	<i>Simochromis diagramma</i>	PA
GenBank	XP_005940312.1	PLC-γ1	<i>Haplochromis burtoni</i>	PA
GenBank	XP_004546027.1	PLC-γ1	<i>Maylandia zebra</i>	PA
GenBank	XP_005740473.1	PLC-γ1	<i>Pundamilia nyererei</i>	PA
GenBank	XP_022597680.1	PLC-γ1	<i>Seriola dumerili</i>	PA
GenBank	XP_040008522.1	PLC-γ1	<i>Xiphias gladius</i>	PA
GenBank	XP_030588968.1	PLC-γ1	<i>Archocentrus centrarchus</i>	PA
GenBank	XP_005448876.1	PLC-γ1	<i>Oreochromis niloticus</i>	PA, 3D, DOA, DP
GenBank	AAI62663.1	PLC-γ1	<i>Danio rerio</i>	PA
GenBank	XP_012808222.3	PLC-γ1	<i>Xenopus tropicalis</i>	PA
GenBank	XP_044153895.1	PLC-γ1	<i>Bufo gargarizans</i>	PA
GenBank	XP_040290757.1	PLC-γ1	<i>Bufo bufo</i>	PA
GenBank	XP_040186336.1	PLC-γ1	<i>Rana temporaria</i>	PA
GenBank	BAF64273.1	PLC-γ1	<i>Xenopus laevis</i>	PA
GenBank	XP_030389275.1	PLC-γ1	<i>Gopherus evgoodei</i>	PA
GenBank	XP_032623244.1	PLC-γ1	<i>Chelonoidis abingdonii</i>	PA
GenBank	XP_023960838.1	PLC-γ1	<i>Chrysemys picta bellii</i>	PA
GenBank	XP_039354654.1	PLC-γ1	<i>Mauremys reevesii</i>	PA
GenBank	XP_042739599.1	PLC-γ1	<i>Lagopus leucura</i>	PA
GenBank	XP_042690487.1	PLC-γ1	<i>Centrocerus urophasianus</i>	PA
GenBank	XP_021272307.1	PLC-γ1	<i>Numida meleagris</i>	PA
GenBank	XP_040506711.1	PLC-γ1	<i>Gallus gallus</i>	PA

GenBank	AAH65091.1	PLC- γ 1	<i>Mus musculus</i>	PA, 3D, DOA, DP
GenBank	AAI36467.1	PLC- γ 1	<i>Homo sapiens</i>	PA

Note:

PA: phylogenetic analysis; 3D: 3D structure analysis; DOA: domain organization analysis; DP: domain prediction.

Supplementary Table S2. Information and sequence of the primers used in present study

Accession No.	Primer name	Primer sequence
NM_001309405.1	RT-LCK	Forward: GAGATTCCTCGTGAGTCCCTGA Reverser: TCTTGATTGCCACTTTCCTGTC
XM_003445128.4	RT-ZAP-70	Forward: GCGGACGGCAAGTTTCTAA Reverser: GTTTGTTCCCTCGGGCATA
XM_013269856.3	RT-LAT	Forward: ACAATCCGGAGGAGAGCAAA Reverser: CCTATTGGTGCCACGTTCTG
XM_003445827.5	RT-ITK	Forward: GTCTAACGGACTACCTGCGA Reverser: GTCCGATACCTTCACCTCGT
XM_005469037.3	RT-GADS	Forward: AGTTTGTGATCCGAGGGTGT Reverser: CCTCTCTTGTCCTCAGCAA
XM_003443091.4	RT-GRB2	Forward: ATCAGAGAGAGCGAGAGTGC Reverser: ACTGAGTTCAAGCGGTGGTA
XM_013265925.2	RT-SLP-76	Forward: AGATTATGTCCAACCCAGCA Reverser: CATTATGAGCCAAAGGCAGTGT
XM_005448819.3	RT-PLC- γ 1	Forward: GGAAGGCTCTTTTGAATCGC Reverser: TGGCTTGGACTTAGACACGGTT
XM_005453731.4	RT-IKK α	Forward: CAAGGACGCTGACAACACCG Reverser: TGCCGACTGGGACCACTGA
XM_003445438.5	RT-IkBa	Forward: GACACGCCTCTTCACATTGCC Reverser: GCTGCGACCATTATGCTGCTC
XM_005463160.3	RT-NF- κ B p65	Forward: CAGAGCAGACAAAGGTAGAAGCC Reverser: AGCGATGGTAGAGGAGACGGT
XM_019361943	RT-h-Ras	Forward: AAGCAGGTTGTGATAGACGGGGA Reverser: AGACTTGATTGTTGATGGCGAA
XM_003438987	RT-c-Raf	Forward: TGAAAAGGTTGGTGGCTGACTG Reverser: CGGTTTATCTTAGGGAGGGCAT
XM_003440445.4	RT-MEK1	Forward: TGGGTCTGTCACTGGTTGAAATG Reverser: CACTGGGAAGCCGAAAATCTGT
XM_003453986.5	RT-Erk1	Forward: CTGCTCTGGACAACCTGACA Reverser: CCCTGATGATGTCGTTGATG
XM_005453315.3	RT-AKT1	Forward: CGTCCTCCCAGCAATCGTCT Reverser: CCACACAGGTATGTTGTCTCGG
XM_003454192.4	RT-S6	Forward: ATGAAAAGGTTGGTGGCTGACTG Reverser: ACTGCGGTTTATCTTAGGGAGGG
XM_003444360.5	RT-4E-BP1	Forward: CATCCCAGGAGTGACCAGCC

XM_013276256.3	RT-NFAT1	Reverser: GGAGCAGCGGTGTTGTTGTTC Forward: TCCAACCCACCAACTTCTAACCA Reverser: GCAGGGATACCGACTCGTGATGT
XM_025898776.1	RT-c-Fos	Forward: GTGCAGCAGCCTCCTTTGTT Reverser: TTTCGGCCCTTTCCTGTTGC
XM_005478937.4	RT-c-JUN	Forward: CCAAAGCGCTGAAACACAAC Reverser: ATGATCAGCCTCTCCAGCTC
XM_019360337.1	RT-CD122	Forward: GGAGGTGTGTTTGGCAAGTT Reverser: ATGCCCTCATCCTGACTCAG
ENSONIT00000018373	RT-CD44	Forward: CTTTGGACTTTGGCATGGCT Reverser: GCGATGCTCGTATTGCTGAT
XM_003451768.5	RT-Caspase-3	Forward: CTATTCCACCGCTCCAGGAT Reverser: GAGGACTCGAAGTAGCGTGA
XM_019359973.2	RT-Caspase-6	Forward: GGTACATCCAGGCTCTGTGT Reverser: AGCTGTGGGTCACAGGATTT
XM_025900112.1	RT-Caspase-8	Forward: GCTGGAAATAGCAGCGGAAA Reverser: TATTTGGGCTCCGGCATTG
XM_025901776.1	RT-Caspase-9	Forward: TATTTGGGCTCCGGCATTG Reverser: CCTGAGTCTCTCGCCAAGAA
ENSONIT00000028270.2	RT-IL-2	Forward: ATGTCGAGACCCAGGGAAAC Reverser: CAGGCCACAGGTGACAGTTA
XM_025902124.1	RT-TNF- α	Forward: TCCTCCTGGCATTACACTT Reverser: TGCCATTCCACTGAGGTCTT
XM_003448658.4	RT-T-bet	Forward: ACCTCGGTCACCCAACTAAATC Reverser: CACCCACACCTCCCTCAAAT
XM_001287402.1	RT-IFN- γ	Forward: GGGTGGTGTTTTGGAGTCGT Reverser: GTAGCGAGCCTGAGTTGTTGGTG
XM_005475852.4	RT-GATA3	Forward: CTTCCCACCAAGTAGCCTGA Reverser: CGCAGGCGTTACACAGATAG
XN_005469217.1	RT-Perforin A	Forward: TTCTCCGACAGGAAGACCAAG Reverser: TGTAGTCACTGATGGCGTCTC
XM_003439678.4	RT-Granzyme B	Forward: GAGCATTTTGTGGTGACTGCTGC Reverser: CACGGTCTGCGAGAGGAATAGGT
KJ126772.1	RT- β -actin	Forward: CGGAATCCACGAAACCACCTA Reverser: CCAGACGGAGTATTTACGCTCA
XM_013269856.3	LAT-Flag	Forward: CCCTCGAGCTATGGACTTCTCCTGG TTCCTCCT Reverser: CGGGATCCTTACTTATCGTCGTCATC CTTGTAATCGTGGTTGACTAGTCCCATAGGT
XM_003445827.5	ITK-HA	Forward: CCCTCGAGCTATGTTCCCCAGGGTG ATTTTGAA Reverser: CGGGATCCTTAAGCGTAGTCTGGGA CGTCGTATGGGTAGGCGTCTGAAAGGGAGGC C

XM_003443091.4	GRB2-HA	Forward: CGGAATTCTATGGAGGCCATTGCCA AATACGA Reverser: CGGGATCCTTAAGCGTAGTCTGGGAC GTCGTATGGGTACATGTTCTGACTGACAGGC
XM_005462655.2	VAV1-HA	Forward: CCCTCGAGCTATGGAGCTGTGGCG GCAGTGCGC Reverser: CGGAATTCTTAAGCGTAGTCTGGGA CGTCGTATGGGTAGCAGTAGTCAGAGTAATCC
XM_013265925.2	SLP-76-HA	Forward: CCCTCGAGCTATGAACCTGCCGT CCAGAGAGGA Reverser: CGGAATTCTTAAGCGTAGTCTGGGAC GTCGTATGGGTAGGGCTTCACTGGGTAGCAT
XM_005469037.3	GADS-HA	Forward: CCCTCGAGCTATGGAAGCCAGAGGA AAGTTTGA Reverser: CGGAATTCTTAAGCGTAGTCTGGGA CGTCGTATGGGTACAGCGGTCTTGTGGCATT
XM_005448819.3	PLC- γ 1 -HA	Forward: CCCTCGAGCTATGGCTGGGACCT CTGGCTTCTT Reverser: CGGAATTCTTAAGCGTAGTCTGGGA CGTCGTATGGGTAGACACGGTTCTCCACTCTG
