

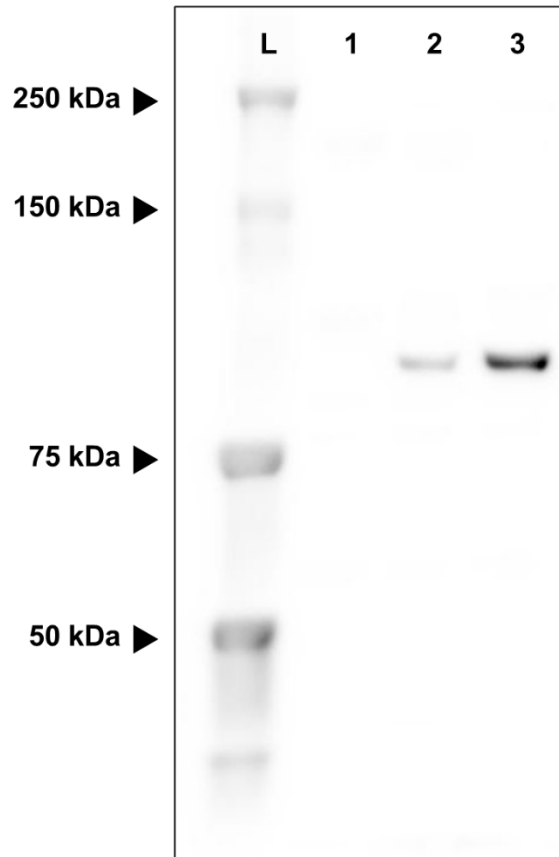


Fig. S1. Representative membrane from Western blot analysis. The complete lanes for chemiluminescent detection of sea lamprey Ano1 by the custom-made anti-sea lamprey Ano1 antibody are shown. Lane assignments are as follows: (1) FW-acclimated larvae, (2) FW-acclimated juvenile, (3) SW-acclimated juvenile. L, ladder.

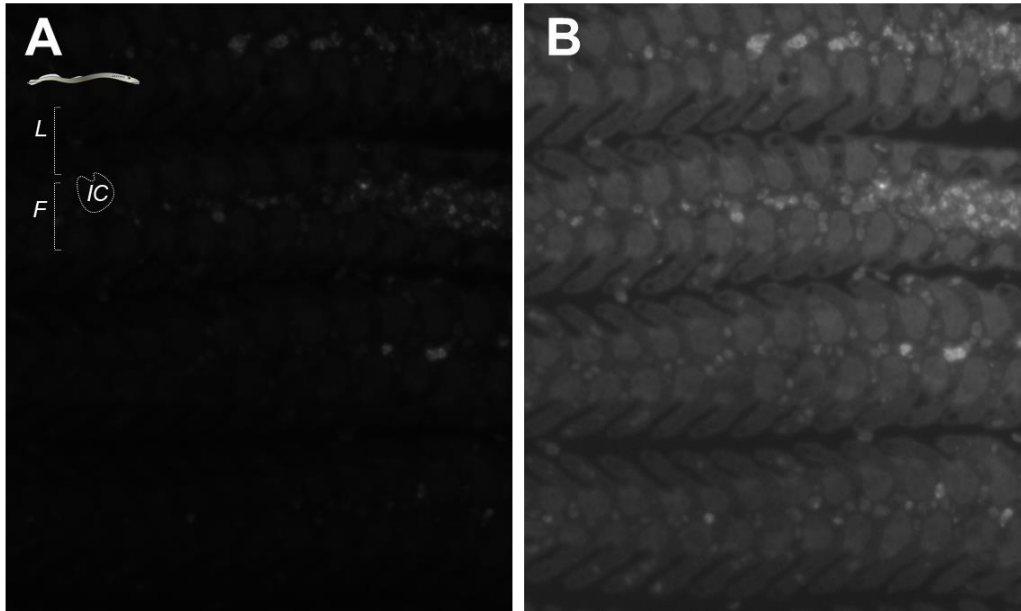


Fig. S2. Immunofluorescence microscopy image of secondary only control.

The gills of SW-acclimated juvenile sea lamprey incubated with secondary antibody only. The two panels are the same image, with (A) depicting the standard exposure, and (B) depicting a longer exposure (enhanced brightness) for clarity of visual reference. Ionocyte clusters (IC) are located along the filament (F) between lamellae (L).

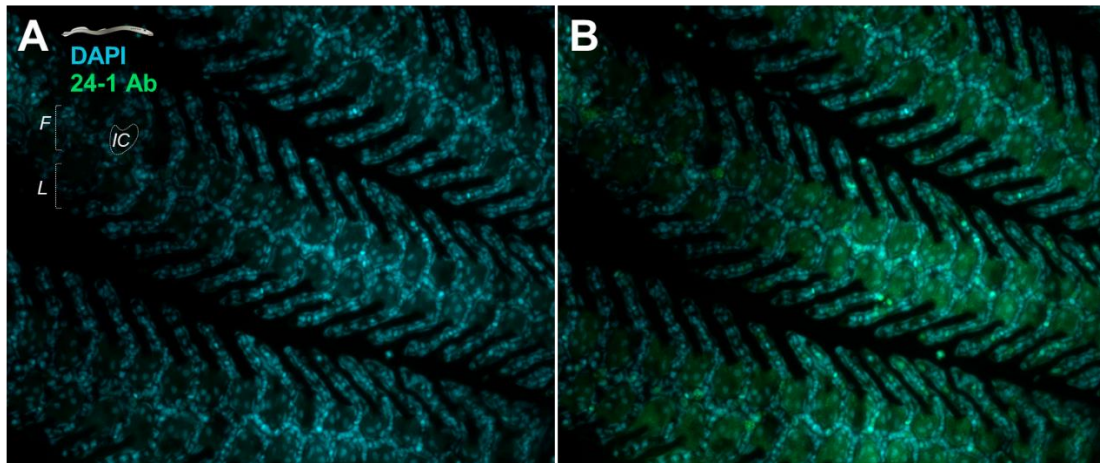


Fig. S3. Immunofluorescence microscopy image using the anti-Cftr antibody '24-1'. The gills of SW-acclimated juvenile sea lamprey incubated with the nuclear marker DAPI (blue) and the anti-Cftr antibody '24-1' (green). The two panels are the same image, with (A) depicting the standard exposure. A longer exposure (enhanced brightness) is depicted in (B) for visual clarity (no specific signal was detected). Ionocyte clusters (IC) are located along the filament (F) between lamellae (L).

Table S1. Raw data and differential expression analysis of an *a priori* and a *posteriori* selection of Cl⁻ or anion transporters in the branchial transcriptomes of larvae and juvenile (Juv) sea lamprey. Data available on Dryad: 10.5061/dryad.z34tmpgm3.

LOC#	GeneID	Gene Name	Normalized Transcript Counts				DESeq2 Analysis		Wald Test Comparison				Sig?
			Larvae FPM	SE	Juv FPM	SE	baseMean	Log ₂ FC	SE	Statistic	P		
116950729	nkcc	solute carrier family 12 member 2	38.22	4.65	2393.49	266.73	20656.57	-6.17	0.23	-26.48	1.72E-154	9.65E-151	TRUE
116956436	ano1	anoctamin 1	18.76	1.25	52.89	4.32	565.91	-1.59	0.18	-9.03	1.69E-19	5.18E-18	TRUE
116950414	clcn2	chloride channel protein 2-like	1.11	0.13	6.2	0.9	51.17	-3.41	0.44	-7.74	1.04E-14	1.89E-13	TRUE
116949658	tyh2	protein tweety homolog 2-like	8.02	0.84	17.17	1.9	193.05	-1.37	0.27	-5.16	2.48E-07	1.76E-06	TRUE
116951572	tyh1	protein tweety homolog 1-like	5.28	0.6	11.38	1.31	138.23	-1.19	0.24	-5.01	5.60E-07	3.76E-06	TRUE
116949466	slc26a6	solute carrier family 26 member 6-like	19.33	1.16	17.85	0.68	13.61	-3.16	0.63	-5.01	5.59E-07	3.76E-06	TRUE
116942811	irrc8d	volume-regulated anion channel subunit LRRC8D-like	5.58	0.65	11.14	0.75	116.85	-1.13	0.24	-4.71	2.46E-06	1.48E-05	TRUE
116943351	clcc1	chloride channel CLIC like 1	18.63	0.95	27.58	0.4	286.35	-0.66	0.15	-4.40	1.09E-05	5.84E-05	TRUE
116946045	clcn7	chloride voltage-gated channel 7	41.28	0.78	59.51	3.15	700.66	-0.56	0.14	-4.15	3.34E-05	1.63E-04	TRUE
116946971	clcnkb	chloride channel protein ClC-Kb-like	16.42	2.97	26.62	5.08	252.79	-1.48	0.41	-3.64	2.70E-04	1.08E-03	TRUE
116953150	cllc5	chloride intracellular channel protein 5-like	18.6	0.84	28.74	2.03	234.75	-0.70	0.20	-3.55	8.82E-04	1.46E-03	TRUE
116936995	irrc8a	volume-regulated anion channel subunit LRRC8A-like	8.34	0.3	11.91	0.75	70.60	-0.84	0.24	-3.48	4.94E-04	1.85E-03	TRUE
116951979	tsnd	barttin CLCNK type accessory subunit beta	10.91	1.01	20.54	2.68	68.71	-1.10	0.32	-3.40	6.76E-04	2.45E-03	TRUE
116938942	irrc8a	volume-regulated anion channel subunit LRRC8A-like	45.48	1.69	57.92	1.82	792.41	-0.39	0.12	-3.25	1.17E-03	3.98E-03	TRUE
116951305	slc26a6	solute carrier family 26 member 6-like	49.65	2.37	55.76	3.18	320.66	-0.86	0.31	-2.83	4.73E-03	1.37E-02	TRUE
116958216	irrc8c	volume-regulated anion channel subunit LRRC8C-like	6.95	0.52	8.57	0.55	93.42	-0.46	0.21	-2.17	2.98E-02	6.76E-02	FALSE
116947243	pacc1	proton activated chloride channel 1	17.12	0.35	20.84	1.53	296.39	-0.36	0.17	-2.13	3.34E-02	7.47E-02	FALSE
116938665	ccc9	solute carrier family 12 member 8	45.92	2.22	53.44	2.53	589.84	-0.31	0.15	-2.04	4.11E-02	8.90E-02	FALSE
116949685	slc26a11	solute carrier family 26 member 11	20.38	2.14	26.06	1.81	321.22	-0.33	0.20	-1.68	9.36E-02	1.75E-01	FALSE
116952042	kcc2	solute carrier family 12 member 5-like	0.86	0.25	1.48	0.37	11.32	-0.85	0.57	-1.50	1.35E-01	2.34E-01	FALSE
116944991	clca1	calcium-activated chloride channel regulator 1-like	6.68	0.45	6.39	0.59	58.38	-0.46	0.32	-1.42	1.56E-01	2.63E-01	FALSE
116937505	slco2a1	solute carrier organic anion transporter family member 2A1	50.26	6.88	30.4	2.24	34.99	-0.64	0.49	-1.31	1.91E-01	3.09E-01	FALSE
116955765	best2	bestrophin-2-like	52.94	3.86	56.02	3.87	800.05	-0.20	0.17	-1.20	2.32E-01	3.58E-01	FALSE
116957883	tyh1	protein tweety homolog 1-like	15.62	0.17	20.12	0.8	11.58	-0.64	0.54	-1.17	2.41E-01	3.69E-01	FALSE
116937120	irrc8a	volume-regulated anion channel subunit LRRC8A-like	2.07	0.32	2.49	0.27	22.44	-0.49	0.53	-0.92	3.59E-01	4.99E-01	FALSE
116946982	ae2	anion exchange protein 2-like	194.36	22.27	210.25	8.16	3419.32	-0.15	0.17	-0.89	3.75E-01	5.16E-01	FALSE
116948778	ccc8	solute carrier family 12 member 9	93.03	7.34	100.76	5.74	1598.34	-0.14	0.16	-0.86	3.90E-01	5.30E-01	FALSE
116946972	clcnkb	chloride channel protein ClC-Kb-like	9.4	0.8	9.9	0.57	61.24	-0.26	0.31	-0.84	4.01E-01	5.41E-01	FALSE
116954894	clcn6	chloride transport protein 6-like	6.78	0.85	7.07	0.54	41.06	-0.30	0.36	-0.84	4.03E-01	5.43E-01	FALSE
116937805	nkcc	solute carrier family 12 member 2-like	43.34	8.89	49.28	3.79	692.41	-0.22	0.28	-0.79	4.33E-01	5.72E-01	FALSE
116937010	irrc8c	volume-regulated anion channel subunit LRRC8C-like	1.02	0.13	1.32	0.16	10.07	-0.29	0.56	-0.51	6.11E-01	7.27E-01	FALSE
116938813	slc26a10	solute carrier family 26 member 10-like	1.98	0.18	2.04	0.27	0.31	-1.63	3.49	-0.47	6.40E-01	NA	N/A
116949463	pds	pendrin-like	1.83	0.31	3.08	0.15	5.70	-0.22	0.78	-0.28	7.79E-01	8.55E-01	FALSE
116944961	cllc5	chloride intracellular channel protein 5-like	72.23	13.52	72.68	4.27	1098.44	-0.04	0.27	-0.14	8.88E-01	9.32E-01	FALSE
116945081	clca1	calcium-activated chloride channel regulator 1-like	0.07	0.04	0.11	0.03	0.56	-0.36	2.58	-0.14	8.89E-01	NA	N/A
116951316	cftr	CF transmembrane conductance regulator	3.1	0.42	3.53	0.15	0.56	-0.33	2.58	-0.13	8.98E-01	NA	N/A
116944545	ano1	anoctamin-1-like	1.14	0.15	1.51	0.06	5.53	-0.09	0.84	-0.10	9.19E-01	9.51E-01	FALSE
116946215	tyh1a	protein tweety homolog 1-A-like	129.1	6.25	124.06	2.66	1969.35	0.02	0.10	0.25	8.01E-01	8.71E-01	FALSE
116937300	irrc8e	volume-regulated anion channel subunit LRRC8E-like	0.39	0.01	0.39	0.04	0.11	0.01	3.53	0.29	7.76E-01	NA	N/A
116954669	ae2	anion exchange protein 2-like	6.19	1.1	10.33	2.36	67.90	0.11	0.35	0.31	7.55E-01	8.36E-01	FALSE
116945083	clca1	calcium-activated chloride channel regulator 1-like	3.85	0.29	4.06	0.12	0.61	0.86	2.53	0.34	7.35E-01	NA	N/A
116955745	best2	bestrophin-2-like	9.82	0.54	11.03	1.01	65.02	0.12	0.24	0.50	6.16E-01	7.31E-01	FALSE
116939820	ano1	anoctamin-1-like	3.58	0.42	3.45	0.36	0.77	1.34	2.12	0.63	5.27E-01	NA	N/A
116957008	kcc2	solute carrier family 12 member 5-like	4.52	0.21	4.88	0.26	3.31	0.77	0.94	0.81	4.17E-01	5.57E-01	FALSE
116954190	kcc4	solute carrier family 12 member 7	39.14	1.99	34.95	2.64	532.23	0.15	0.17	0.87	3.85E-01	5.25E-01	FALSE
116938201	ano6	anoctamin-6-like	5.05	0.92	2.8	0.38	1.49	1.69	1.79	0.94	3.46E-01	4.87E-01	FALSE
116957515	clcn3	chloride voltage-gated channel 3	33.81	3.81	31.4	1.59	338.22	0.18	0.16	1.12	2.65E-01	3.98E-01	FALSE
116945082	clca1	calcium-activated chloride channel regulator 1-like	0.27	0.12	0.17	0.04	1.48	2.47	1.86	1.33	1.84E-01	3.00E-01	FALSE
116955412	cllc5	chloride intracellular channel protein 5-like	79.45	6.24	62.73	3.39	835.93	0.39	0.14	2.80	5.19E-03	1.49E-02	TRUE
116953033	cllc5	chloride intracellular channel protein 5-like	14.01	2.33	8.12	1.22	153.45	0.97	0.35	2.81	4.96E-03	1.43E-02	TRUE
116944987	clca1	calcium-activated chloride channel regulator 1-like	3.48	0.63	1.94	0.09	23.38	2.15	0.46	4.64	3.52E-06	2.05E-05	TRUE
116940953	ndcbe	sodium-driven chloride bicarbonate exchanger-like	74.33	4.69	29.93	1.5	708.64	1.35	0.14	9.64	5.27E-22	2.06E-20	TRUE
116957041	plccc	chloride channel protein C-like	34.36	3.88	11.11	0.44	181.37	2.67	0.27	9.95	2.42E-23	1.07E-21	TRUE