Supporting Information

The orphan G-protein coupled receptor 182 is a negative regulator of definitive hematopoiesis through leukotriene B4 signaling

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Supplemental methods

Zebrafish genotyping

For RNA seq and qPCR analyses, we used zebrafish embryos and sorted ECs from adult wild-type and *gpr182* $^{-/-}$ incrosses, respectively. For WISH and confocal imaging, we used zebrafish embryos from *gpr182* heterozygous incrosses. The embryos were placed individually into PCR tubes with 100 µl of 50 mM NaOH. The samples were incubated by vortexing at 95°C for 20 minutes, then 10 µl of 1M Tris pH 8.0 was added to the solution. The extracted DNA was used for genotyping. The genotyping primers used are listed in Table S1.

Quantitative PCR

Quantitative PCR (qPCR) was performed using cDNA synthesized from total RNA. Total RNA was extracted using TRIZOL (Life Technologies) from sorted endothelial cells and non-endothelial cells (Figure 1R, S) from 30 hpf genotyped wild-type and *gpr182*$^{-/-}$ embryos (Figure 4B). DNase treatment was performed for 30 min at 37°C (Promega) followed by RNA purification with RNA Clean and Concentrator kit (Zymo Research). cDNA was synthesized using SuperScript II RT (Invitrogen) starting from 500 ng of total RNA. Bio-Rad and Thermo Real-Time PCR Systems were used for qPCR experiments. Gene expressions were normalized relative to that of the zebrafish and mouse housekeeping gene β-actin. All reactions were performed in three technical replicates and the results represent three independent biological samples. The qPCR primers used are listed in Table S1.

Establishing a Stable cell line.

HTLA, an HEK293 cell line stably expressing a tTA-dependent luciferase reporter and a β-arrestin2-TEV fusion gene, was maintained in DMEM supplemented with 10% FBS, 2 µg/ml puromycin and 100 µg/ml hygromycin B in a humidified atmosphere at 37°C in 5% CO2. HTLA cells expressing hGPR182-TANGO were established by transfecting cells with pcDNA hGPR182-TANGO using Lipofectamine2000 (Invitrogen) according to manufacturer's instructions. The cells were cultured for 2 weeks in DMEM media containing blasticidin (6 µg/ml). The cells that survived blasticidin selection were used as the cells stably expressing hGPR182-TANGO. Expression of hGPR182-TANGO was confirmed by immunoblot analysis using antiFlag and antiGPR182 antibodies (Sigma-Aldrich).

Plasmid

GPR182-Tango plasmid was a gift from Bryan Roth (Addgene plasmid # 66341 ; http://n2t.net/addgene:66341 ; RRID:Addgene_66341)[1].

Cell culture and transfection

Human embryonic kidney 293T (HEK293T) cells were maintained at 37^{o}C in a humidified atmosphere at 5% CO2 in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% (v/v) fetal bovine serum (Sigma Aldrich), 10 mM HEPES (Corning), and 750 µL gentamicin (Gibco)

Table S1

Purpose	Primer name	Primer sequence (5' -3')	Species
Genotyping	gpr182 F	TTCTTGTTGACAGTGGATGATA	zebrafish
Genotyping	gpr182 R	AGCATTGCACAAGGCTAAACGA	zebrafish
qPCR	kdrl F	CAATGGCAGGATTCACTTTGAG	zebrafish
qPCR	kdrl R	GACCGGTGTGGTGCTAAAATG	zebrafish
qPCR	fli1rs F	CAGACCGAAGGGTCGTACAT	zebrafish
qPCR	fli1rs R	GACATAGGGGTGGAATGTGG	zebrafish
qPCR	gpr182 F	GGACTGGCAGAGAACACCAT	zebrafish
qPCR	gpr182 R	AGCTCACGTTGATGATGCAG	zebrafish
qPCR	cmyb F	TGATGCTTCCCAACACAGAG	zebrafish
qPCR	cmyb R	TTCAGAGGGAATCGTCTGCT	zebrafish
qPCR	b-actin F	TCTGTCCCATGCCAACCAT	zebrafish
qPCR	b-actin R	TGCCCTCGTGCTGTTTT	zebrafish
qPCR	LTA4H F	TGATTGCTTTGGTTGTTGGA	mouse
qPCR	LTA4H R	GCAGATTTCTCCACCTGCTC	mouse
qPCR	B-ACT F	CTGGCACCACACCTTCTACA	mouse
qPCR	B-ACT R	CTTTTCACGGTTGGCCTTAG	mouse
WISH	gpr182 *in situ* F (T7)	TAATACGACTCACTATAGGGA TGACGCATGACATTCACAACT	zebrafish
WISH	gpr182 *in situ* R (SP6)	GATTTAGGTGACACTATAGTG CGCCAATGTCAGAGTGACTT	zebrafish
WISH	cmyb *in situ* F (T7)	TAATACGACTCACTATAGGGAT GGCGAGGCGGCACAGACACA	zebrafish
WISH	cmyb *in situ* R (SP6)	GATTTAGGTGACACTATAGGC ATCCTTGCTCTAGGGATGGA	zebrafish
Genotyping	Gpr182 wild type F	CTGCAGCCTCCTGGCACTAACAGC	mouse
Genotyping	Gpr182 wild type R	CATTGTCCGGTTC CAAGGTGGAGAC	mouse
Genotyping	Gpr182tm2a(KOMP) WtsiTargeted F	GAGATGGCGCA ACGCAATTAAT	mouse
Genotyping	Gpr182tm2a(KOMP) WtsiTargeted R	GGGAGGATACCAC AGGGAAATAGAGC	mouse
Genotyping	Gpr182 lacZ Targeted F	TTCACTGGCCGT CGTTTTACAACGT	mouse
Genotyping	Gpr182 lacZ Targeted R	ATGTGAGCGAGTAAC AACCCGTCGGATTCT	mouse

Table S1: Primers used for qPCR, genotyping and WISH probe synthesis.

Table S2

gene	set	Ct (mean)	
		ECs	Non-ECs
ef1a	1st	22.187	22.573
	2nd	22.184	22.469
	3rd	22.256	22.469
	4th	23.169	23.329
	5th	23.258	23.130
	6th	23.140	23.072
kdrl	1st	27.994	33.714
	2nd	28.021	33.256
	3rd	27.963	33.665
	4th	29.026	33.956
	5th	28.905	34.279
	6th	28.719	34.174
fli1rs	1st	29.387	35.884
	2nd	29.298	37.083
	3rd	29.443	35.369
	4th	31.015	38.329
	5th	30.901	34.707
	6th	30.601	38.849
gpr182	1st	27.373	32.658
	2nd	27.388	32.976
	3rd	27.683	32.364
	4th	28.188	32.679
	5th	28.023	32.793
	6th	28.205	32.577

Table S2. Cycle threshold (Ct) values of candidate genes obtained via qPCR.
The cycle threshold (Ct) values were determined by qPCR analysis of *kdrl*, *fli1rs* and *gpr182* mRNA expression levels in isolated ECs and non ECs from 30 hpf wild-type *TgBAC(etsrp:EGFP)* zebrafish embryos. N = 6 biologically independent samples.

Table S3

Gene ID	Gene name		ECs at 30 hpf				Whole embryos at 30 hpf				Whole embryos at 48 hpf			
			WT1	WT2	Mut1	Mut2	WT1	WT2	Mut1	Mut2	WT1	WT2	Mut1	Mut2
ENSDARG00000090783	mfap4	RPKM	7.906	2.139	24.764	16.804	1.410	1.139	0.729	0.887	0.406	0.243	1.236	1.065
		Z-score	-0.502	-1.082	1.193	0.392	1.236	0.328	-1.048	-0.516	-0.681	-1.016	1.025	0.673
ENSDARG00000067797	spi1a	RPKM	1.289	0.753	15.183	18.656	0.459	0.450	0.896	0.609	0.571	0.528	0.829	0.783
		Z-score	-0.827	-0.885	0.669	1.043	-0.694	-0.736	1.406	0.025	-0.711	-0.996	1.007	0.699
ENSDARG00000000767	spi1b	RPKM	11.418	12.123	26.243	28.393	2.312	3.138	2.672	2.849	1.508	2.066	2.336	2.245
		Z-score	-0.901	-0.822	0.742	0.981	-1.246	1.145	-0.205	0.307	-1.430	0.073	0.800	0.557
ENSDARG00000056407	irf8	RPKM	0.834	0.835	6.503	12.357	0.270	0.265	0.369	0.263	0.211	0.263	0.322	0.426
		Z-score	-0.780	-0.780	0.249	1.312	-0.419	-0.517	1.497	-0.561	-1.022	-0.461	0.175	1.308
ENSDARG00000055290	mpeg1.1	RPKM	0.840	0.934	9.389	7.337	0.435	0.426	0.248	0.387	0.354	0.706	0.935	0.894
		Z-score	-0.861	-0.839	1.084	0.617	0.698	0.604	-1.457	0.155	-1.389	-0.062	0.804	0.648
ENSDARG00000054610	coro1a	RPKM	14.909	8.546	68.067	82.228	2.841	3.923	2.877	3.070	2.985	2.923	4.234	3.273
		Z-score	-0.768	-0.939	0.663	1.044	-0.665	1.470	-0.593	-0.212	-0.608	-0.711	1.452	-0.133
ENSDARG00000041041	cxcr3.2	RPKM	1.558	3.378	12.405	15.247	0.202	0.297	0.148	0.147	0.049	0.098	0.200	0.299
		Z-score	-0.983	-0.712	0.635	1.060	0.048	1.398	-0.718	-0.727	-1.013	-0.571	0.349	1.235
ENSDARG00000102986	csf1ra	RPKM	1.267	0.267	4.542	4.018	2.226	2.057	1.087	0.982	2.583	2.800	3.009	2.940
		Z-score	-0.604	-1.085	0.970	0.719	0.990	0.728	-0.778	-0.941	-1.331	-0.173	0.936	0.568
ENSDARG00000053624	csf1rb	RPKM	0.679	2.380	2.331	5.290	0.725	0.776	0.483	0.609	0.838	0.996	0.950	0.976
		Z-score	-1.038	-0.151	-0.177	1.367	0.588	0.979	-1.267	-0.300	-1.445	0.792	0.136	0.517
ENSDARG00000023188	lcp1	RPKM	49.942	37.705	201.117	220.084	0.501	0.574	0.449	0.528	0.490	0.529	0.623	0.536
		Z-score	-0.799	-0.925	0.764	0.960	-0.224	1.163	-1.230	0.291	-0.974	-0.275	1.398	-0.149
ENSDARG00000045959	csf3r	RPKM	7.316	10.160	31.517	29.889	0.674	0.453	0.763	0.483	0.590	0.381	0.529	0.456
		Z-score	-0.973	-0.750	0.925	0.797	0.543	-0.940	1.132	-0.735	1.117	-1.193	0.442	-0.365
ENSDARG00000019521	mpx	RPKM	12.110	7.207	7.653	13.438	3.532	2.393	1.265	1.335	5.269	5.275	6.639	6.549
		Z-score	0.640	-0.923	-0.780	1.063	1.313	0.246	-0.812	-0.747	-0.869	-0.861	0.924	0.806
ENSDARG00000087646	runx1	RPKM	7.084	8.420	10.240	10.175	0.043	0.169	0.042	0.042	0.168	0.209	0.171	0.212
		Z-score	-1.248	-0.369	0.830	0.787	-0.488	1.500	-0.504	-0.507	-0.919	0.806	-0.809	0.922
ENSDARG00000009094	gata2b	RPKM	0.503	0.377	0.639	0.770	0.195	0.144	0.000	0.142	0.191	0.333	0.194	0.241
		Z-score	-0.410	-1.148	0.392	1.166	0.893	0.278	-1.434	0.263	-0.736	1.407	-0.691	0.019
ENSDARG00000053666	myb	RPKM	18.520	19.649	22.368	19.521	6.557	7.251	6.964	6.405	5.541	5.297	6.343	5.367
		Z-score	-0.907	-0.222	1.428	-0.299	-0.617	1.185	0.441	-1.010	-0.199	-0.706	1.465	-0.560
ENSDARG00000010317	gpr183a	RPKM	0.849	2.548	9.834	5.373	0.198	0.259	0.129	0.384	0.193	0.257	0.262	0.260
		Z-score	-0.968	-0.536	1.320	0.184	-0.411	0.149	-1.047	1.309	-1.497	0.415	0.565	0.517

Table S3. Expression levels of myeloid and HE/HSC markers from the RNA seq data sets. z-score of reads per kilobase per million reads (RPKMs) of each gene in ECs isolated from 30 hpf wild-type and *gpr182* $^{-/-}$ embryos, and in whole embryos (30 and 48 hpf wild-type and *gpr182* $^{-/-}$).

Table S4

Mouse NO	Genotype	WBC (K/uL)	NEUT# (K/uL)	NEUT %(%)	LYMPH #(K/uL)	LYMPH %(%)	MONO #(K/uL)	MONO %(%)	EO #(K/uL)	EO %(%)	BASO #(K/uL)
WT1	wild type	6.68	1.99	29.8	4.33	64.8	0.14	2.1	0.16	2.4	0.06
WT2	wild type	6.28	1.23	19.5	4.74	75.5	0.15	2.4	0.15	2.4	0.01
WT3	wild type	5.1	1.57	30.8	3.32	65.1	0.05	1	0.16	3.1	0
WT4	wild type	3.53	0.41	11.6	3	85	0.04	1.1	0.08	2.3	0
WT5	wild type	5.07	0.71	14	4.19	82.6	0.07	1.4	0.1	2	0
WT6	wild type	3.87	0.47	12.1	3.2	82.7	0.05	1.3	0.14	3.6	0.01
WT7	wild type	3.69	0.53	14.3	2.98	80.8	0.04	1.1	0.12	3.3	0.02
WT8	wild type	5.29	0.87	16.5	4.2	79.4	0.06	1.1	0.15	2.8	0.01
NO.573	GPR182 KO	10.2	1.33	13	8.38	82.2	0.22	2.2	0.26	2.5	0.01
NO.600	GPR182 KO	14.46	7.91	54.7	5.47	37.8	0.24	1.7	0.82	5.7	0.02
NO.593	GPR182 KO	5.57	1.84	33	3.37	60.5	0.08	1.4	0.26	4.7	0.02
NO.634	GPR182 KO	6.25	1.92	30.7	3.64	58.2	0.33	5.3	0.36	5.8	0
NO.641	GPR182 KO	4.06	1.09	26.9	2.7	66.5	0.05	1.2	0.21	5.2	0.01
NO.653	GPR182 KO	6.95	3.18	45.8	3.49	50.2	0.08	1.2	0.19	2.7	0.01
NO.656	GPR182 KO	5.45	1.3	23.8	3.94	72.3	0.08	1.5	0.13	2.4	0

Mouse NO	BASO %(%)	RBC (M/uL)	HGB (g/dL)	RET# (K/uL)	RET% (%)	PLT (K/uL)	PDW (fL)	MPV (fL)	P-LCR (%)	PCT (%)
WT1	0.9	9.1	13.5	581.5	6.39	1500	8.5	8.4	10	1.26
WT2	0.2	10.17	14.8	616.3	6.06	1227	8.5	8.8	9	1.08
WT3	0	10.21	14.8	528.9	5.18	1514	7.4	8.6	4.2	1.3
WT4	0	9.83	14.8	505.3	5.14	1185	7.4	8.5	6.4	1.01
WT5	0	10.18	15.4	504.9	4.96	807	8	8.6	10.6	0.69
WT6	0.3	10.57	16	449.2	4.25	357	7.9	8.4	9.6	0.3
WT7	0.5	8.33	13.7	333.2	4	324	7.8	8.7	7	0.28
WT8	0.2	9.94	15.3	464.2	4.67	446	8.3	8.6	4.9	0.38
NO.573	0.1	8.99	13.5	766.8	8.53	1111	7.8	8.3	8.4	0.92
NO.600	0.1	9.57	14.9	1950.4	20.38	207	---	10.4	----	0.22
NO.593	0.4	10.16	15.5	809.8	7.97	1232	8	8.1	8.1	1
NO.634	0	9.95	14.9	690.5	6.94	757	8.6	8.5	6	0.64
NO.641	0.2	9.73	15	510.8	5.25	921	7.6	8	5.5	0.74
NO.653	0.1	10.88	16.4	478.7	4.4	1141	8.2	8.1	6.9	0.92
NO.656	0	10.33	15.8	477.2	4.62	676	8.2	8	8.4	0.54

Table S4. Results of complete blood count test.

BASO, basophils; EO, eosinophils; HGB, hemoglobin; LYMPH, lymphocytes; MONO, monocytes; MPV, mean volume platelet; NEUT, neutrophils; PLT, platelets; PCT, Procalcitonin; PDW, PLT distribution width; P-LCR, Platelet Large Cell Ratio; RBC, red blood cells; RET, Reticulocytes; WBC, white blood cells.

A

Gene ID	Gene Name		Endothelial cell			Hemogenic endothelium			Hematopoietic stem cells			length
			EC1	EC2	EC3	HE1	HE2	HE3	HSC1	HSC2	HSC3	
ENSDARG00000036616	*gpr182*	RPKM	70.821	57.606	68.995	86.482	104.135	98.239	0.109	0.000	0.083	1481
		z-score	0.390	0.083	0.348	0.755	1.165	1.028	-1.255	-1.258	-1.256	
ENSDARG00000053666	*cmyb*	RPKM	1049.605	2.575	729.997	1334.445	983.276	677.882	526.544	463.916	609.411	3317
		z-score	0.390	-1.834	0.055	1.625	0.713	-0.080	-0.473	-0.636	-0.258	
ENSDARG00000009094	*gata2b*	RPKM	2.578	4.600	0.075	2.904	4.027	8.738	0.083	0.062	0.000	1955
		z-score	0.390	0.690	-0.843	0.116	0.496	2.092	-0.840	-0.847	-0.868	
ENSDARG00000040080	*fli1a*	RPKM	60.538	48.221	68.205	81.536	83.624	86.629	1.986	0.150	1.506	2914
		z-score	0.390	0.005	0.543	0.902	0.959	1.040	-1.241	-1.290	-1.254	
ENSDARG00000054632	*fli1rs*	RPKM	37.490	42.606	34.489	75.431	65.151	84.870	1.480	0.764	1.485	3203
		z-score	0.390	0.136	-0.114	1.148	0.831	1.439	-1.132	-1.154	-1.131	

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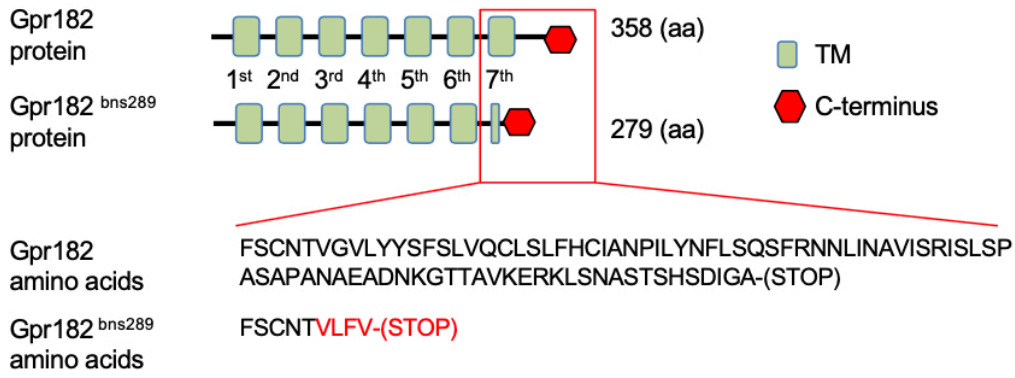
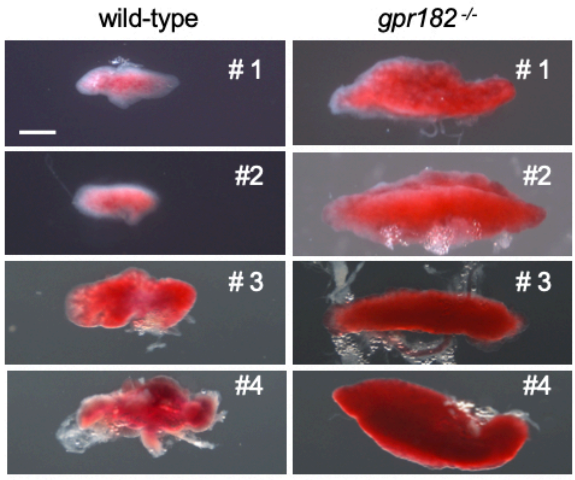
B



C

5-months old adult fish

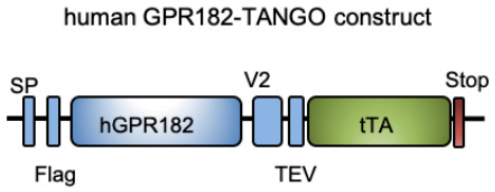
D



6-months old adult fish spleen

Figure S1. Adult *gpr182* mutant zebrafish present with bigger spleens than wild-type. (A) Table showing mRNA levels of *gpr182* as well as EC, HE and HSC marker genes from the RNA seq dataset. RNA seq dataset consists of mRNA isolated from non-hemogenic ECs (ECs, *kdrl*+/*runx1*−), specified HE (HE, *kdrl*+/*runx1*+), and HSCs (HSC, *kdrl*−/*runx1*+) from 28 hpf *Tg(kdrl:mCherry); Tg(runx1:EGFP)* embryos[2]. (B) Gpr182 and Gpr182^{bns289} proteins. The *bns289* mutation causes a premature stop codon at amino acid 279, resulting in the loss of the 7th putative trans membrane domain (TM) and the intracellular domain. (C) Brightfield images of 5-months old wild-type and *gpr182* $^{-/-}$ zebrafish. (D) Brightfield images of 6-months old wild-type and *gpr182* $^{-/-}$ zebrafish spleen. Scale bar: 1 mm (D). RPKMs, reads per kilobase per million reads; HE, hemogenic endothelium; HSC, hematopoietic stem cell.

A



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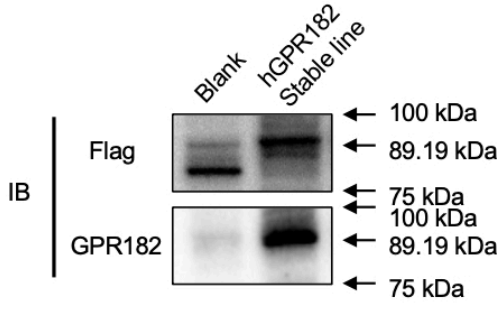
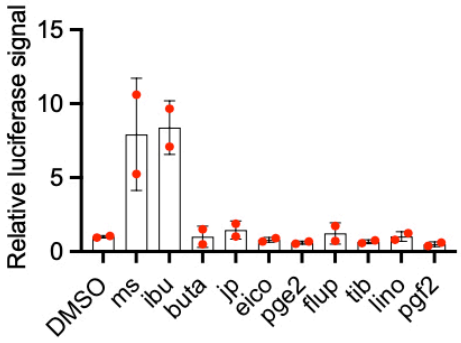


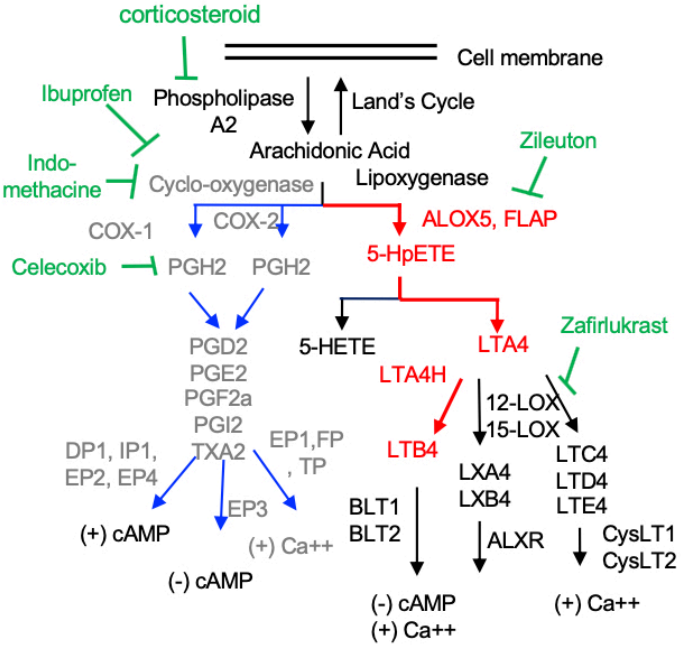
C

No	Name (function)
1	MS-275 (A HDAC1, HDAC3 inhibitor)
2	Ibuprofen (Cyclooxygenase (COX) inhibitor)
3	Butaprost (an EP2 selective agonist)
4	JP83 (an irreversible fatty acyl amide hydrolase inhibitor)
5	5,8,11-Eicosatriynoic Acid
6	8-iso Prostaglandin E2 isopropyl ester
7	9-keto Fluprostenol isopropyl ester
8	5(Z),11(Z),14(Z)-Eicosatrienoic Acid
9	Tibolone
10	alpha.-Linolenoyl Ethanolamide
11	13,14-dihydro-15-keto Prostaglandin F2.alpha. isopropyl ester

D



E



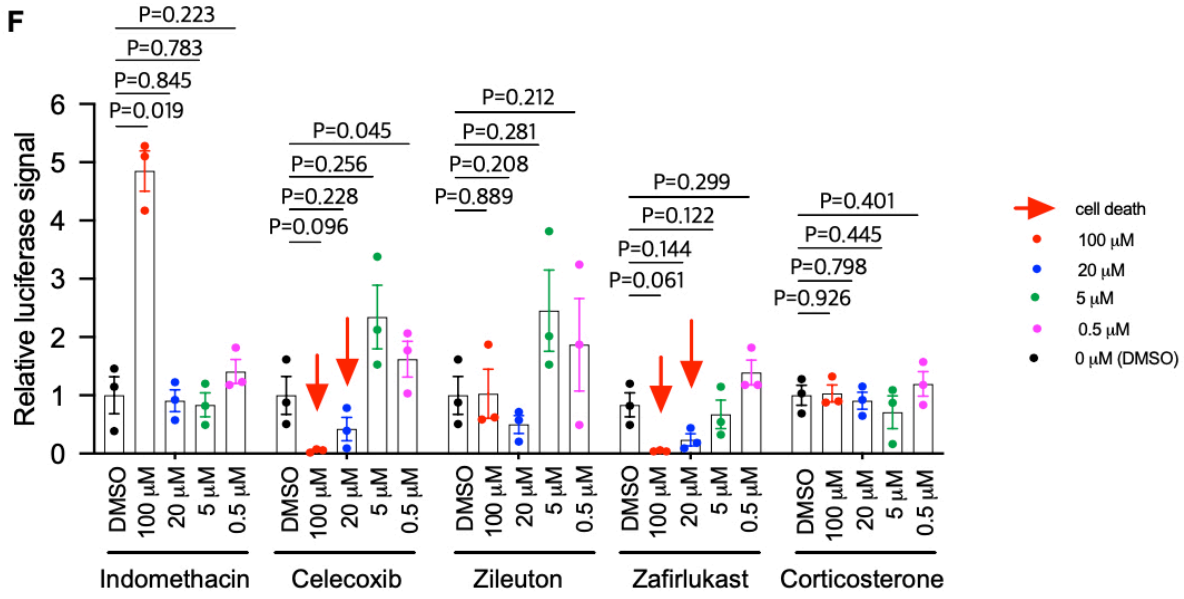
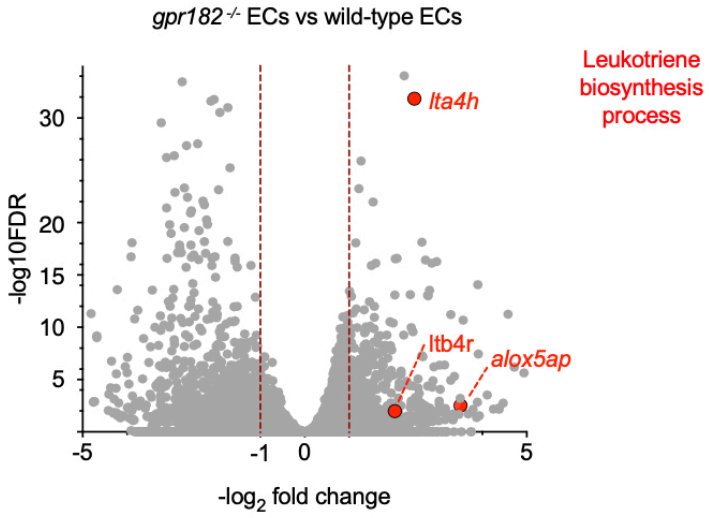


Figure S2. Leukotriene signaling induces the activation of GPR182-TANGO assay.
(A) Schematic showing human GPR182-TANGO (hGPR182-TANGO) construct. (B) Western blots for hGPR182 expression in cells stably expressing the hGPR182-TANGO construct. (C) Table showing 11 potential hit compounds selected from primary small molecule screening. (D) Retesting of 11 initial hit compounds using hGPR182-TANGO. Assay run in duplicate. Negative control (1% DMSO). (E) Schematic illustration of prostaglandin and leukotriene biosynthesis pathways. Pathways blocked by Ibuprofen marked in blue; pathways upregulated in *gpr182* $^{-/-}$ zebrafish ECs marked in red. (F) Testing hGPR182-TANGO activation by treatment with inhibitors blocking prostaglandin and leukotriene biosynthesis pathways. Negative control (1% DMSO). N = 3 biologically independent samples. DMSO treated samples were set at 1. Data are mean ± s.d., and a two-tailed Student's t-test was used to calculate P values. Red arrows indicate cell death in the inhibitor treated condition. tTA, tetracycline transactivator; SP, signal peptide; TEV, Tobacco Etch Virus nuclear inclusion an endopeptidase.

A



B

Ensembl gene id	Ensembl gene name	fold change HE vs EC (Zhang)	logCPM	P Value	FDR	fold change (gpr182 -/- vs wild-type)
ENSDARG00000054755	*alox5ap*	6.035	3.800	1.18E-10	3.89E-09	3.512
ENSDARG00000006029	*lta4h*	2.612	8.384	2.07E-29	2.03E-27	2.474
ENSDARG00000032631	*ltb4r*	6.985	5.879	8.07E-11	2.71E-09	2.032

Figure S3. Transcriptomic analysis supports the hypothesis that *gpr182* mutant zebrafish ECs upregulate leukotriene signaling pathway genes compared to wild-type ECs.
(A) Volcano plot showing the relative mRNA expression in the transcriptomic analysis of 30 hpf *gpr182* $^{-/-}$ ECs compared to wild-type ECs. Genes involved in the leukotriene biosynthesis pathway are marked in red. (B) Table showing the z-score of reads per kilobase per million reads (RPKMs) of each gene in ECs from 30 hpf wild-type and *gpr182*-/- embryos. RPKMs, reads per kilobase per million reads.

Reference

(1) Kroeze, W. K.; Sassano, M. F.; Huang, X.-P.; Lansu, K.; McCorvy, J. D.; Giguère, P. M.; Sciaky, N.; Roth, B. L. PRESTO-Tango as an Open-Source Resource for Interrogation of the Druggable Human GPCRome. *Nature Structural and Molecular Biology* **2015**, *22* (5), nsmb.3014. https://doi.org/10.1038/nsmb.3014.

(2) Zhang, P.; He, Q.; Chen, D.; Liu, W.; Wang, L.; Zhang, C.; Ma, D.; Li, W.; Liu, B.; Liu, F. G Protein-Coupled Receptor 183 Facilitates Endothelial-to-Hematopoietic Transition via Notch1 Inhibition. *Cell research* **2015**, *25* (10), 1093–1107. https://doi.org/10.1038/cr.2015.109.