Supplements

Suppl. Table 1

Primary Antibody	Dilution			Host	Catalog Number	Supplier
	IHC	WB	MELC			
Anti-Claudin-1	1:200	1:500	-	rabbit	LS-B16533-50	LSBio, Seattle, USA
Anti-GAPDH, clone 6C5	-	1:1000	-	mouse	8245	Abcam, Cambridge, UK
Anti-VEGF-A, clone 56/1	-	1:500	-	mouse	MABC595	Sigma Aldrich, Merck, Deisenhofen, Germany
Anti-ZO-1	1:200	1:500	-	rabbit	61-7300	Invitrogen, ThermoFisher Scientific, Waltham, MA, USA
CD4 FITC, clone L3T4	-	-	1:200	rat	1540-02	SoutherBiotech, Birmingham, AL, USA
CD8a FITC, clone 53-6.7	-	-	1:200	rat	553030	BDPharmingen, San Diego, CA, USA
CD11b FITC, clone M1/70.15	-	-	1:800	rat	MCA74F	Bio-Rad Laboratories, Hercules, CA, USA
CD11c PE, clone N418	-	-	1:200	hamster	130-122-952	Miltenyi Biotec, Bergisch Gladbach, Germany
CD22 APC, clone Cy34.1	-	-	1:100	mouse	130-102-576	Miltenyi Biotec, Bergisch Gladbach, Germany
CD29 (GPIIa) APC, clone HMβ1-1	-	-	1:400	hamster	130-123-829	Miltenyi Biotec, Bergisch Gladbach, Germany
CD31 PE, clone MEC13.3	-	-	1:800	rat	553373	BDBiosciences, Franklin Lakes, NJ, USA
CD335 FITC, clone 29A1.4	-	-	1:200	rat	11-3351-82	Invitrogen, ThermoFisher Scientific, Waltham, MA, USA
F4-80 FITC, clone BM8	-	-	1:400	rat	123107	Biolegend, San Diego, CA, USA
Iba1 FITC, clone 1022-5	-	-	1:200	mouse	ab15691	Abcam, Cambridge, UK
Ly6C APC, clone HK1.4	-	-	1:400	rat	17-5932-82	eBioscience, ThermoFisher Scientific, Waltham, MA, USA
Ly6G (Gr-1) PE, clone RB6-8C5	-	-	1:400	rat	12-5931-82	eBioscience, ThermoFisher Scientific, Waltham, MA, USA
Propidium Iodide	-	-	1:16,000	-	P4170	Sigma Aldrich, Merck, Deisenhofen, Germany
Siglec F PE, clone E50-2440	-	-	1:200	rat	552126	BDBiosciences, Franklin Lakes, NJ, USA
Anti-mouse IRDye 800 CW	-	1:10000	-	goat	926-32210	LI-COR, Lincoln Nebraska, USA
Anti-mouse IRDye 680 RD	-	1:10000	-	goat	926-68070	LI-COR, Lincoln Nebraska, USA
Anti-rabbit IRDye 800 CW	-	1:10000	-	goat	926-32211	LI-COR, Lincoln Nebraska, USA
Anti-rabbit red IRDye 680 RD	-	1:10000	-	goat	926-68071	LI-COR, Lincoln Nebraska, USA
Anti-rabbit Cy3	1:100	-	-	goat	R9130	Sigma, Merck, Deisenhofen, Germany
Anti-rabbit FITC	1:100	-	-	goat	F1262	Sigma, Merck, Deisenhofen, Germany

Suppl. T1. List of antibodies.
Antibodies used for western blot (WB), immunohistochemistry (IHC) and multi-epitope ligand cartography (MELC) experiments are listed.

Supplements

Suppl. Table 2

Triglycerides	WT		GPR40$^{-/-}$		P value (WT BL vs. 14d)	P value (GPR40$^{-/-}$ BL vs. 14d)	P value (WT BL vs. GPR40$^{-/-}$ BL)	P Value (WT 14d vs. GPR40$^{-/-}$ 14d
	BL n=19	14d n=10	BL n=7	14d n=5				
TG 42:2	0.0029	0.0036	0.0037	0.0057	n.s. p =0.4066	* p =0.0251	n.s. p =0.3290	** p =0.0090
TG 48:0	0.3658	0.5019	0.3804	0.6010	*** p =0.0008	*** p =0.0003	n.s. p >0.9999	n.s. p =0.0992
TG 48:1	0.4462	0.1848	0.5906	0.1714	* p =0.0393	* p =0.0260	n.s. p =0.4827	n.s. p >0.9999
TG 48:2	0.5250	0.1764	0.6676	0.1902	* p =0.0336	n.s. p =0.0558	n.s. p =0.7427	n.s. p >0.9999
TG 48:3	0.1137	0.0919	0.1616	0.0970	n.s. p =0.8665	n.s. p =0.2502	n.s. p =0.2639	n.s. p >0.9999
TG 48:4	0.0205	0.0363	0.0199	0.0349	n.s. p =0.0590	n.s. p =0.3202	n.s. p >0.9999	n.s. p >0.9999
TG 50:1	1.8154	2.0265	2.1586	1.6900	n.s. p >0.9999	n.s. p =0.9104	n.s. p =0.9375	n.s. p >0.9999
TG 50:2	4.2805	2.8420	6.1086	2.7820	n.s. p =0.2150	* p =0.0304	n.s. p =0.1440	n.s. p >0.9999
TG 50:3	1.7221	0.9702	2.6371	1.1450	n.s. p =0.0595	** p =0.0098	* p =0.0400	n.s. p >0.9999
TG 50:4	0.2603	0.2481	0.3834	0.2900	n.s. p >0.9999	n.s. p =0.4858	n.s. p =0.0908	n.s. p >0.9999
TG 52:1	0.5023	0.7906	0.6224	0.6770	n.s. p =0.1156	n.s. p >0.9999	n.s. p =0.9511	n.s. p >0.9999
TG 52:2	9.1826	9.7460	15.0286	9.6700	n.s. p >0.9999	n.s. p =0.0617	** p =0.0050	n.s. p >0.9999
TG 52:3	8.6668	12.3030	13.9143	13.1000	* p =0.0497	n.s. p >0.9999	* p =0.0101	n.s. p >0.9999
TG 52:4	3.9900	7.2540	5.5900	7.8600	** p =0.0064	n.s. p =0.3034	n.s. p =0.3601	n.s. p >0.9999
TG 52:5	0.7547	1.6949	0.8839	1.6792	** p =0.0036	n.s. p =0.1309	n.s. p >0.9999	n.s. p >0.9999
TG 54:1	0.1268	0.1271	0.0983	0.0902	n.s. p >0.9999	n.s. p >0.9999	n.s. p =0.8342	n.s. p =0.7953
TG 54:2	1.0194	1.4376	1.3906	1.2970	n.s. p =0.2511	n.s. p >0.9999	n.s. p =0.4536	n.s. p >0.9999
TG 54:3	1.0194	1.4376	1.3906	1.2970	** p =0.0028	n.s. p =0.5223	n.s. p =0.1240	n.s. p >0.9999
TG 54:4	3.7068	8.9790	4.8614	9.4160	** p =0.0011	n.s. p =0.0704	n.s. p =0.9353	n.s. p >0.9999
TG 54:5	3.2384	8.6840	3.7757	8.6740	** p =0.0014	n.s. p =0.0660	n.s. p >0.9999	n.s. p >0.9999
TG 54:6	1.5061	4.7780	1.6483	4.7500	** p =0.0018	n.s. p =0.0566	n.s. p >0.9999	n.s. p >0.9999
TG 56:1	0.0236	0.0337	0.0177	0.0275	n.s. p =0.3020	n.s. p =0.6917	n.s. p =0.9090	n.s. p >0.9999
TG 56:2	0.1374	0.1544	0.1078	0.1134	n.s. p >0.9999	n.s. p >0.9999	n.s. p =0.9017	n.s. p =0.8028
TG 56:3	0.3092	0.4287	0.4343	0.3504	n.s. p =0.2095	n.s. p =0.8818	n.s. p =0.2650	n.s. p =0.8838
TG 56:4	0.2454	0.3766	0.3497	0.3372	* p =0.0288	n.s. p >0.9999	n.s. p =0.1586	n.s. p >0.9999
TG 56:6	0.5105	1.1485	0.9024	1.3864	*** p =0.0005	n.s. p=0.0966	n.s. p =0.0697	n.s. p =0.5802
TG 58:1	0.0073	0.0117	0.0056	0.0095	n.s. p=0.1210	n.s. p=0.5103	n.s. p >0.9999	n.s. p =0.9937
TG 58:2	0.0259	0.0449	0.0206	0.0391	n.s. p=0.0810	n.s. p=0.3548	n.s. p >0.9999	n.s. p >0.9999
TG 58:3	0.0467	0.0753	0.0428	0.0656	n.s. p=0.1101	n.s. p=0.5974	n.s. p >0.9999	n.s. p >0.9999
TG 58:4	0.0346	0.0579	0.0352	0.0475	* p=0.0353	n.s. p=0.7754	n.s. p >0.9999	n.s. p =0.8655
TG 58:6	0.0561	0.1035	0.1051	0.1348	** p=0.0053	n.s. p=0.3712	* p=0.0111	n.s. p=0.2740

Suppl. T2. Plasma levels of triglycerides in naïve and STZ-treated WT and GPR40$^{-/-}$ mice.

Wild type and GPR40$^{-/-}$mice were analyzed for triglycerides in the plasma. Naïve mice and mice 14 days after STZ-injection were analyzed by LC-QTOFMS (values of chromatographic peak area) (WT BL n=19; 14d n= 10; GPR40 $^{-/-}$ BL n=7; 14d n=5). 2way ANOVA/Bonferroni post hoc test; p values are included in data table; n.s.= non-significant.

Supplements

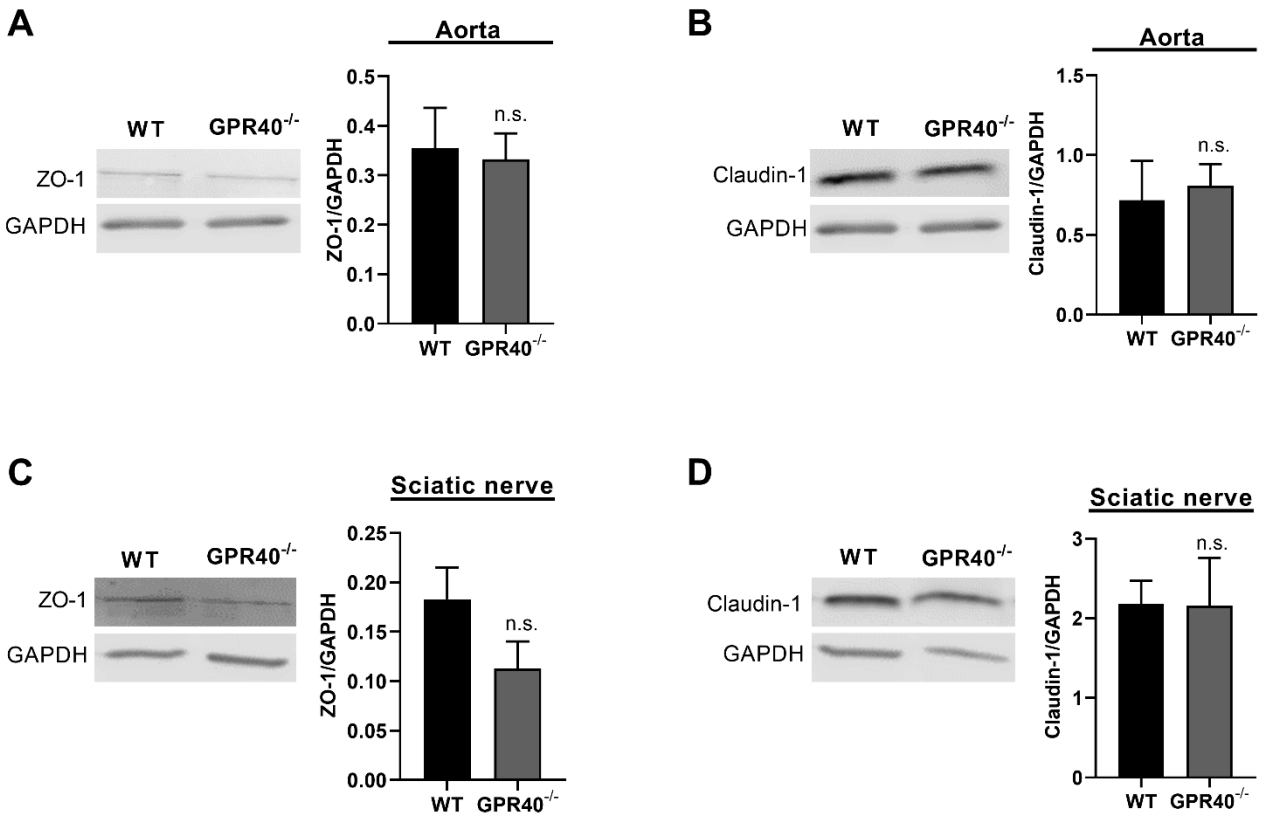
Suppl. Table 3

Free fatty acids	Experiment	WT		GPR40$^{-/-}$		P value (WT BL vs. 14d)	P value (GPR40$^{-/-}$ BL vs. 14d)	P value (WT BL vs. GPR40$^{-/-}$ BL)
		BL	14d	BL	14d			
Oleic acid 18:1	LC-QTOFMS	74.9889	94.5300	102.7000	128.5000	n.s. p =0.0919	n.s. p =0.3304	n.s. p =0.0642
Linoleic acid 18:2		42.7453	69.4400	42.6571	76.8000	**p =0.0067	*p =0.0161	n.s. p =0.9933
Linolenic acid 18:3		3.8732	5.9980	3.4857	5.9100	**p =0.0024	*p =0.0345	n.s. p =0.5707
Stearidonic acid 18:4		0.1179	0.1503	0.0972	0.1404	*p =0.0238	n.s. p =0.0825	n.s. p =0.1362
Eicosenoic acid 20:1		2.5078	2.3270	3.0371	2.8340	n.s. p =0.5883	n.s. p =0.7848	n.s. p =0.2060
Eicosadienoic acid 20:2		0.9532	1.0141	1.2700	1.4172	n.s. p =0.6065	n.s. p =0.4976	*p =0.0227
Arachidonic acid 20:4		27.8868	19.1000	32.9000	29.0600	n.s. p =0.5162	n.s. p =0.6211	n.s. p =0.7594
Eicosapentaenoic acid 20:5		0.9161	0.7967	0.7416	0.7550	n.s. p =0.6757	n.s. p =0.9349	n.s. p =0.6093
Docosatetraenoic acid 22:4		1.2243	1.8230	2.8571	3.0300	**p =0.0063	n.s. p =0.7498	****p <0.0001
Docosahexaenoic acid 22:6		12.1142	15.3900	18.8857	19.9000	n.s. p =0.0903	n.s. p =0.7484	**p =0.0065
Nervonic acid 24:1		0.2336	0.2330	0.2300	0.2204	n.s. p =0.9791	n.s. p =0.8077	n.s. p =0.9066
Linoleic acid 18:2	LC-MS/MS	2698.6000	4612.2000	2845.1430	4938.8000	**p =0.0025	*p =0.0226	n.s. p =0.8075
Arachidonic acid 20:4		519508.7000	407584.0000	631474.6000	591419.2000	n.s. p =0.5946	n.s. p =0.7355	n.s. p =0.6586
Docosahexaenoic acid 22:6		342557.4000	365095.8000	482105.7000	448055.6000	n.s. p >0.9999	n.s. p >0.9999	* p =0.0380
20-HETE		n.d.	n.d.	n.d.	n.d.			

Suppl. T3. Plasma levels of free fatty acids in naïve and STZ-treated mice.
Wild type and GPR40$^{-/-}$mice were analysed for free fatty acid concentrations in the plasma. Naïve mice and mice 14 days after STZ-injection were analysed by LC-QTOFMS (values of chromatographic peak area) or quantitative LC-MS/MS (values in pg/ml) (WT BL n=19; 14d n= 10; GPR40$^{-/-}$ BL n=7; 14d n=5). Unpaired two-tailed t-test; p values are included in data table; n.s. = non-significant; n.d. = not detectable

Supplements

Figure S1



Suppl. Fig. S1. Western Blot analysis of TJ proteins ZO-1 and Claudin-1.

A-C: Representative Western Blot and data analysis of TJ proteins ZO-1 and Claudin-1 in aorta tissue (panel a+b) or in sciatic nerve (panel c+d) from naive wild type and GPR40$^{-/-}$ mice, Data normalized to GAPDH signal (n=6). Unpaired two-tailed t-test, n.s.= non-significant.

Supplements

Figure S2

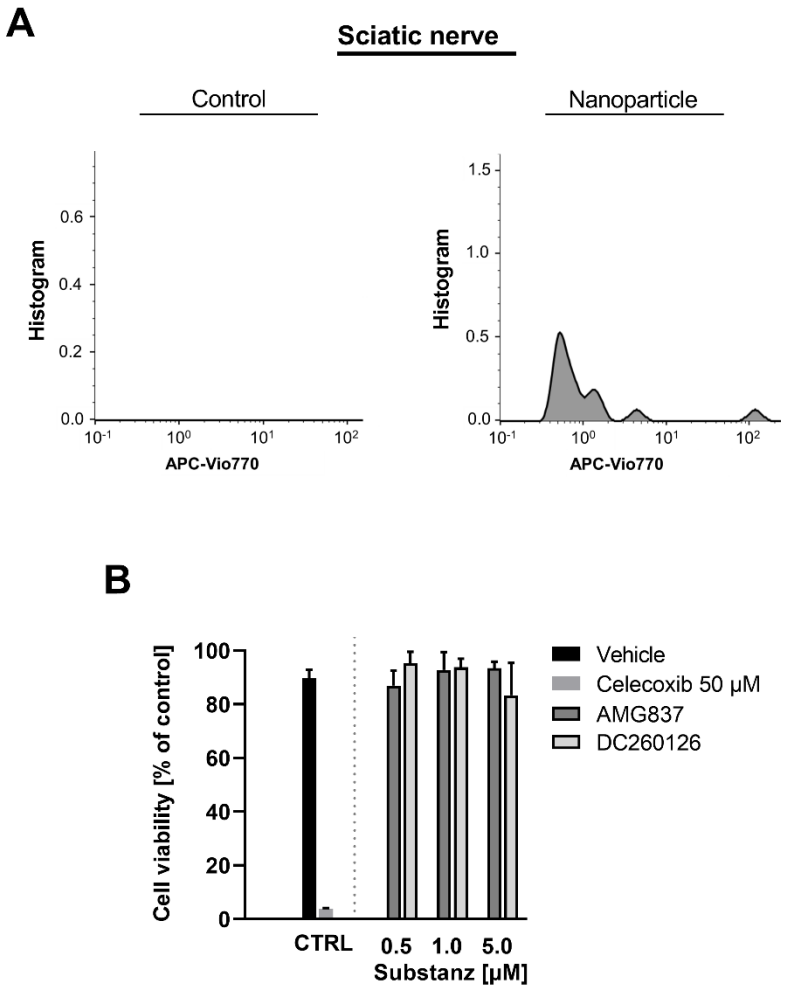
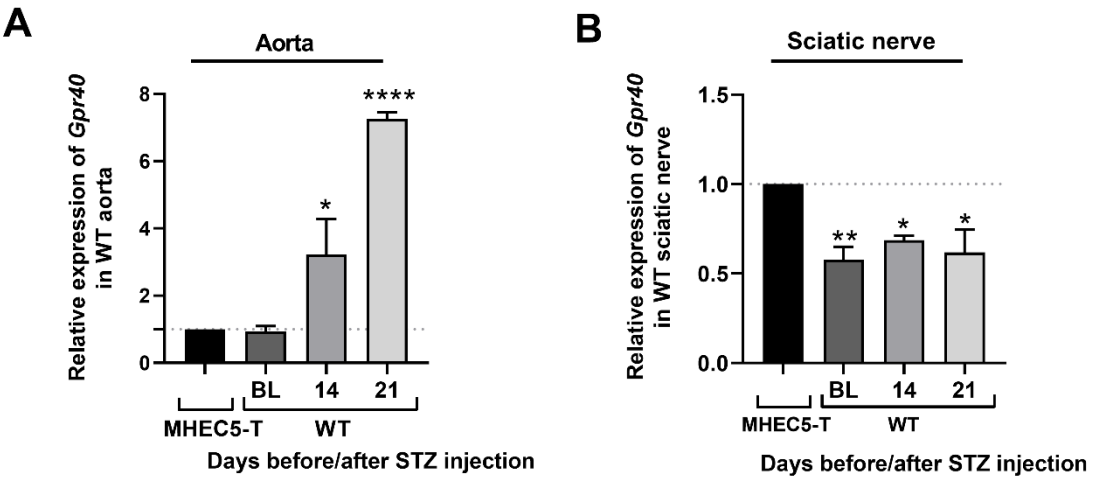


Fig. S2. Nanoparticle FACS control and TEER cytotoxicity assay.

A: Histograms of the nanoparticle FACS data of sciatic nerve cells (left, control) or of fluorescent nanoparticles (0.06 µm) in sciatic nerves (right). ***B*:** To test for cell toxicity HUVECs were incubated with the compounds for 24 hours. Orang reagent (Cambridge biosciences, UK) was added for 1 hour before determining absorbance at 450 nm (Enspire, PerkinElmer, Waltham, USA). OrangU cell toxcicity test of HUVEC cells treated with DC260126, AMG837, celecoxib or DMSO (n=3).

Supplements

Figure S3

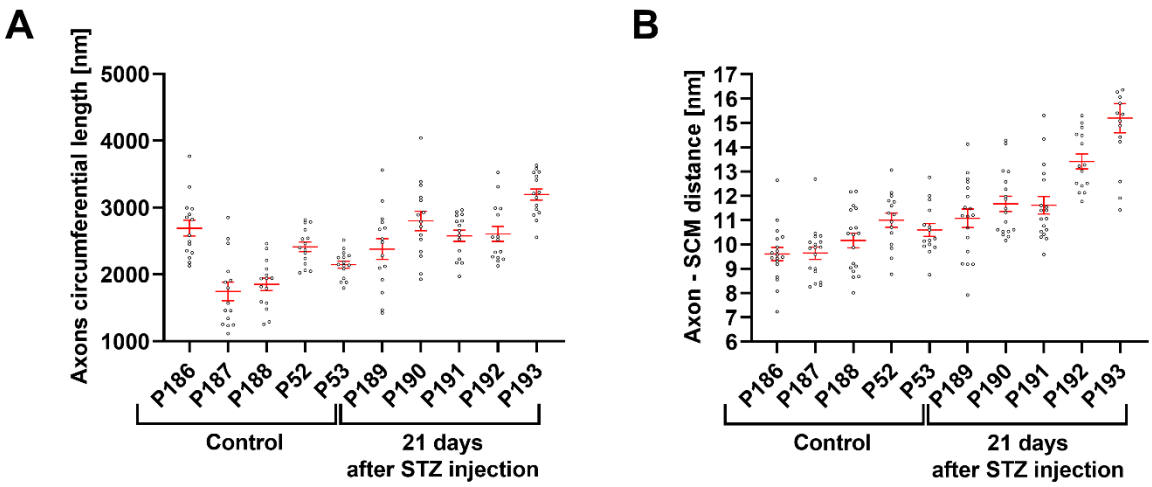


Suppl. Fig. S3. Gpr40 mRNA is expressed in MHEC5-T, aorta and sciatic nerves.

Gpr40 mRNA expression in MHEC5-T compared to WT aorta (A) or sciatic nerves (B) (n=4). One-way ANOVA/Bonferroni post hoc test; *p<0.05; **p <0.01 compared to MHEC5-T.

Supplements

Figure S4

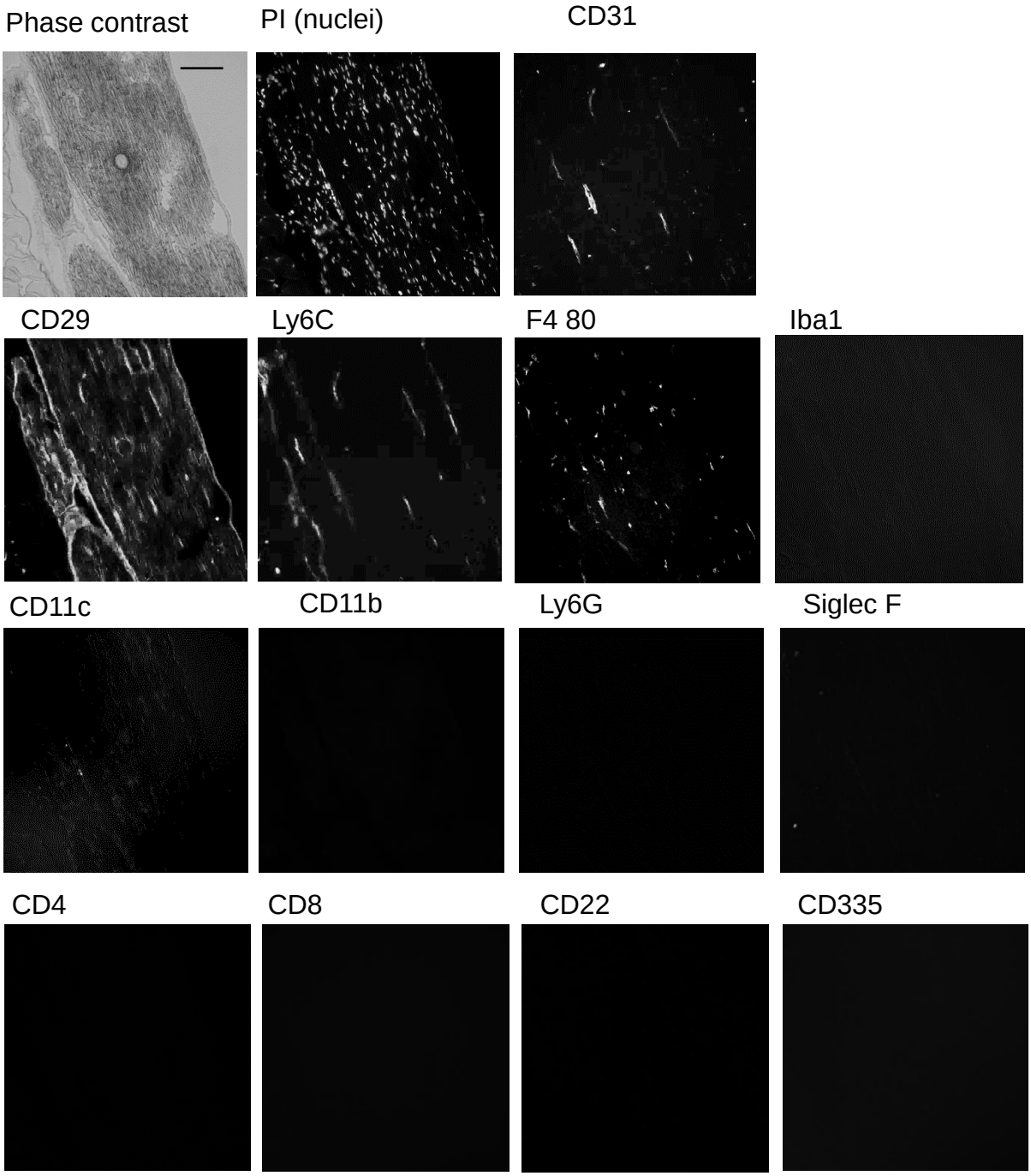


Suppl. Fig. S4. Diabetes- induced swelling of axons and intercellular space.

Quantitative analysis of unmyelinated and myelinated axons in individual naive mice or 21 days after STZ injection. The nonmyelinated axons of the nerve in mice with diabetes are swollen (panel A, 15 axons/mouse) and the intercellular spaces between axons and Schwann cells as well as between axon are enlarged compared (panel B, 18 axons/mouse).

Supplements

Figure S5



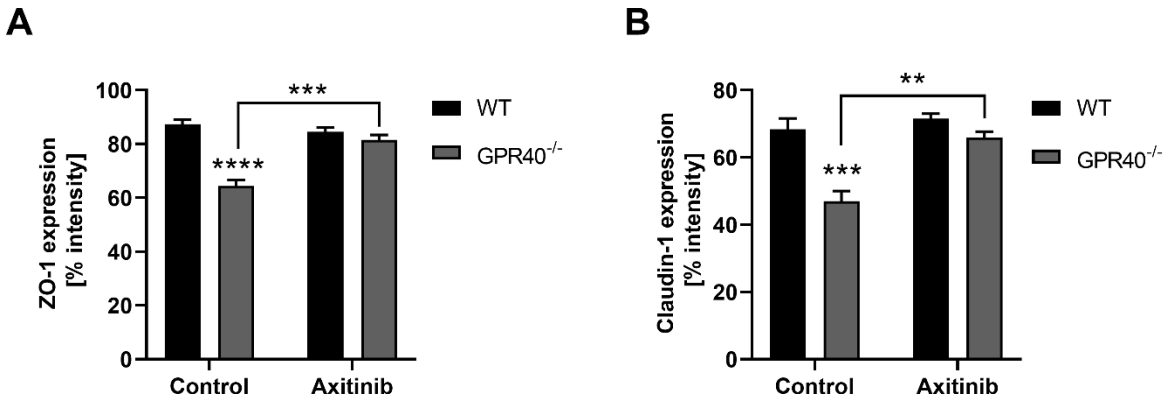
Suppl. Fig. S5. Immune cells are not recruited to the sciatic nerve 3 weeks after STZ-injection.

MELC analysis of sciatic nerves 3 weeks after STZ-injection shows no recruitment of macrophages, dendritic cells, neutrophils, eosinophils CD4- and CD8-Tcells, B-cells or NK-cells. Resident macropgages (F4 80+/Ly6C-) do not express the activation marker IB4. The black bar represents 100 µm.

For desciption of the MELC method for sequential immunohistology please see the supplementary methods. All anibodies are listed in the supplementary data as table T1.

Supplements

Figure S6

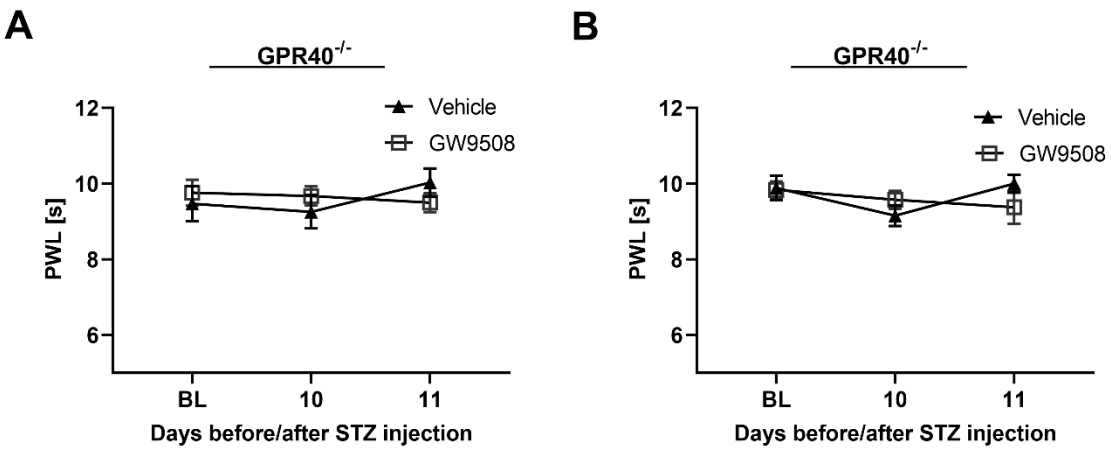


Suppl. Fig. S6. Tight junction formation is recovered by Axitinib in naïve GPR40$^{-/-}$ mice.

A: ZO-1 expression in sciatic nerve from naïve or Axitinib treated treated (two days in a row) wild type and GPR40$^{-/-}$ mice (control n=3, Axitinib n=4; 6 sections per mouse). 2way ANOVA/Bonferroni post hoc test; ****p<0.0001, ***p<0.001. ***B:*** Same as panel a with Claudin-1 expression (control n=3, Axitinib n=4; 6 sections per mouse). 2way ANOVA/Bonferroni post hoc test; ***p<0.001, **p<0.01.

Supplements

Figure S7

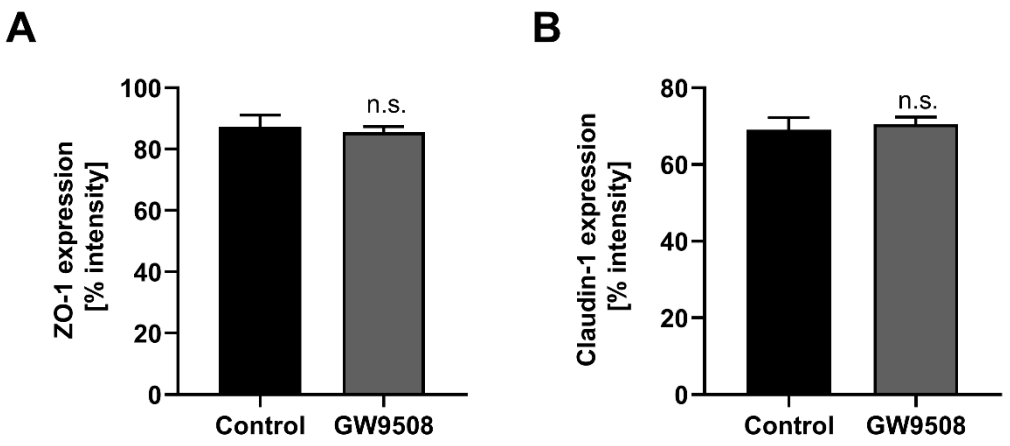


Suppl. Fig. S7. The GPR40 agonist GW9508 does not influence thermal and mechanical hypersensitivities in GPR40$^{-/-}$ mice.

A,B: Mechanical (panel a) and thermal (panel b) paw withdrawal latencies (PWL) during GW9508 treatment of GPR40$^{-/-}$ mice after 10/11 days after STZ injection (Vehicle n=7; GW9508 n=8). N-way repeated 2way ANOVA/Bonferroni post hoc test; n.s., non-significant.

Supplements

Figure S8

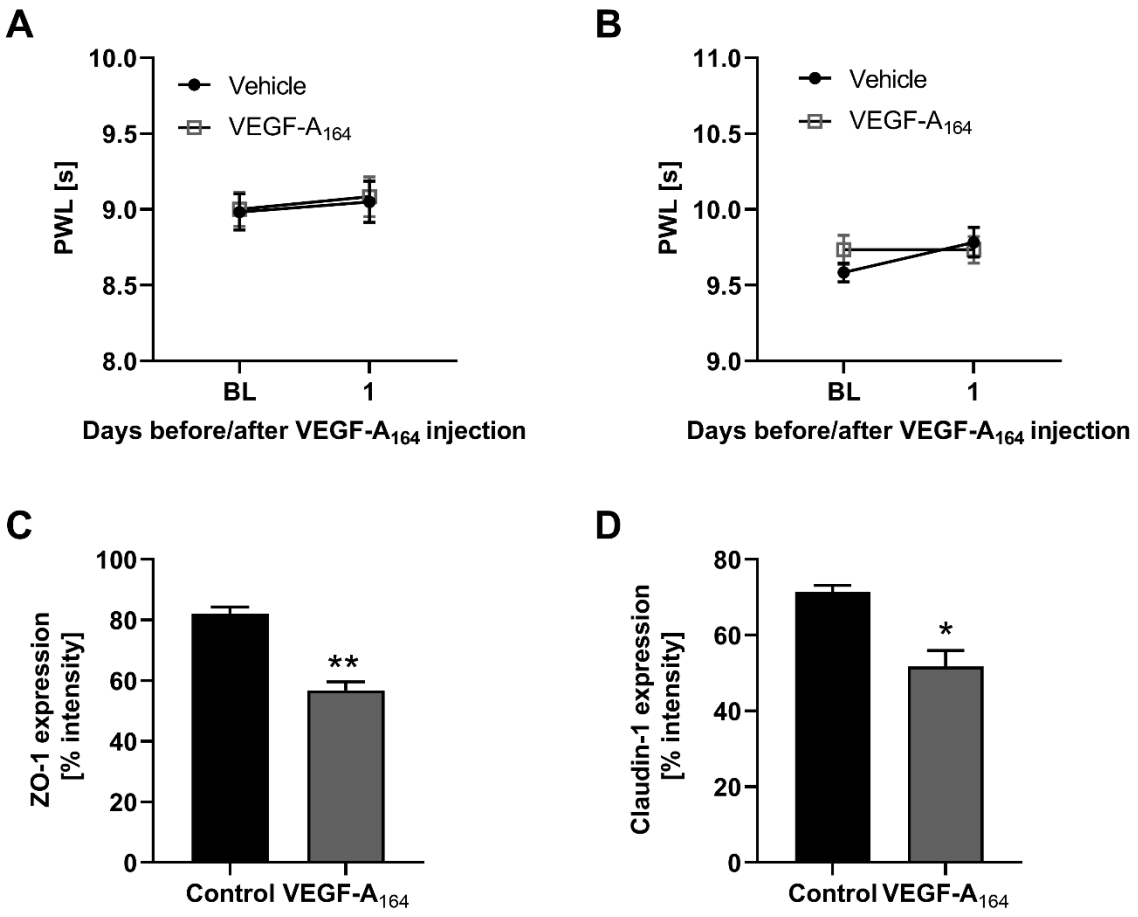


Suppl. Fig. S8: Tight junction formation is not influenced by GW9508 treatment in WT mice

A: ZO-1 expression in sciatic nerve from naïve or GW9508 treated (two days in a row) wild type mice (control n=3, GW9508 n=4; 6 sections per mouse; two-tailed unpaired t-test, n.s., non-significant). ***B:*** Same as panel a with Claudin-1 expression (control n=3, GW9508 n=4; 6 sections per mouse). Unpaired two-tailed t-test, n.s.= non-significant.

Supplements

Figure S9



Suppl. Fig. S9. Behavior is not influenced by VEGF-A_{164} protein treatment, but tight junction formation is disturbed in WT mice.

A,B: Mechanical (panel a) and thermal (panel b) paw withdrawal latencies (PWL) after VEGF-A_{164} injection of WT mice (Vehicle n=6; VEGF-A_{164} n=6). N-way repeated 2way ANOVA/Bonferroni post hoc test; n.s.= non-significant. ***C:*** ZO-1 expression in sciatic nerve from naïve or VEGF-A_{164} treated wild type mice (control n=3, VEGF-A_{164} n=4; 6 sections per mouse) Unpaired two-tailed t-test, n.s.= non-significant). ***D:*** Same as panel c with Claudin-1 expression (control n=3, VEGF-A_{164} n=4; 6 sections per mouse). Unpaired two-tailed t-test, n.s.= non-significant.