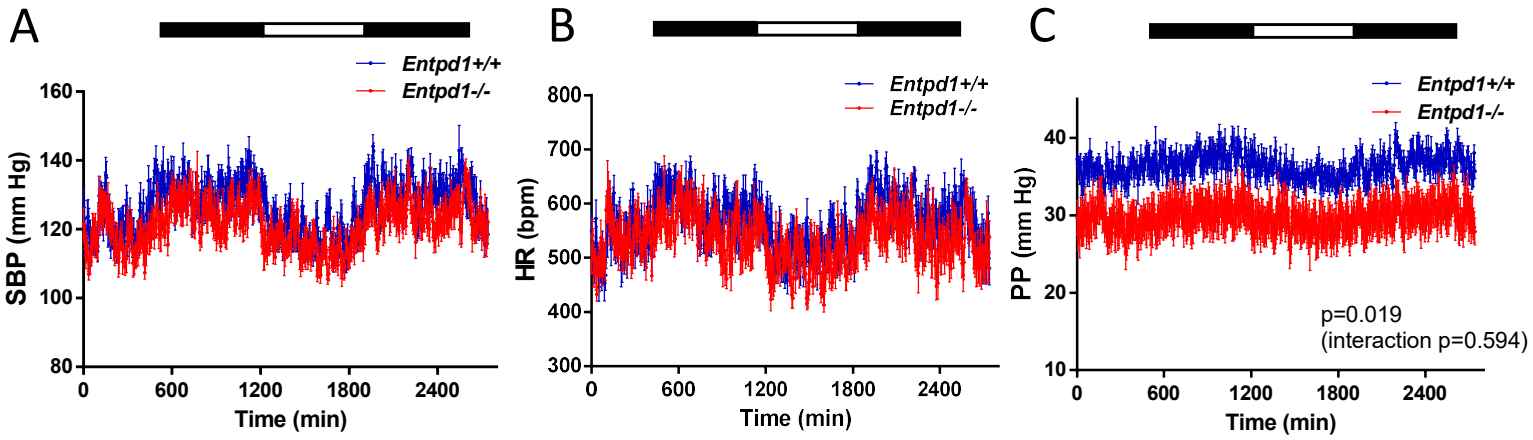
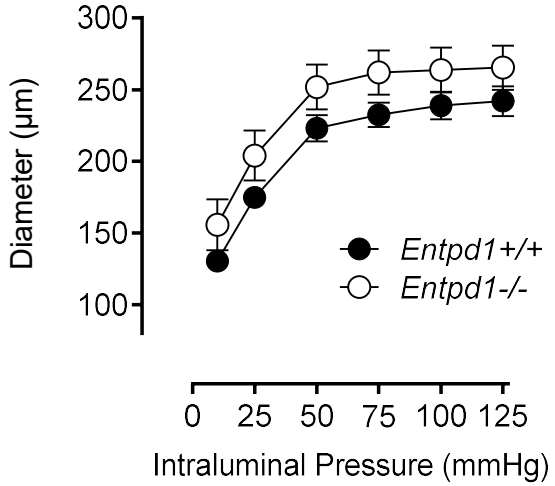
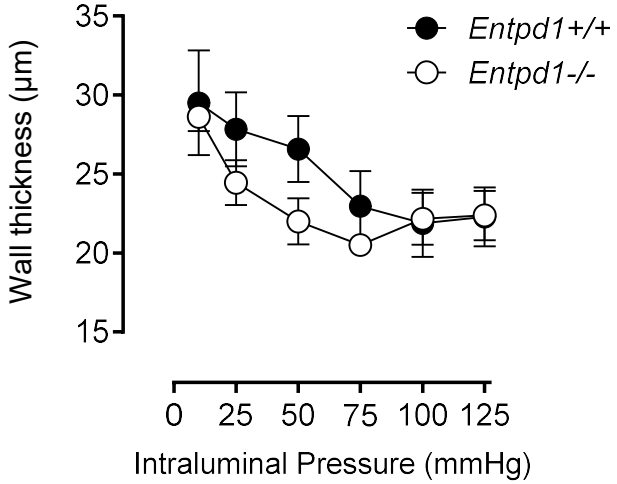
Supplementary Figure S1

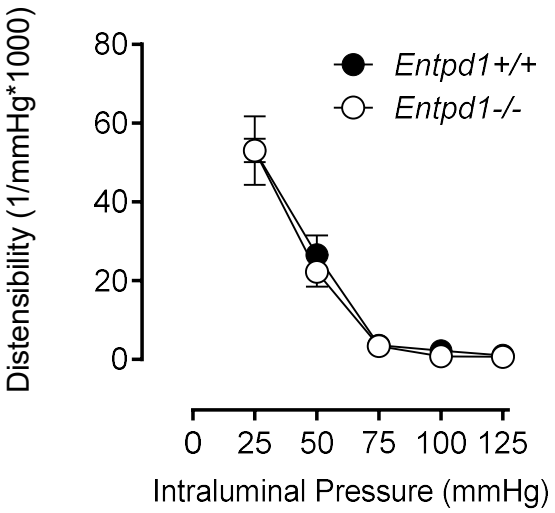


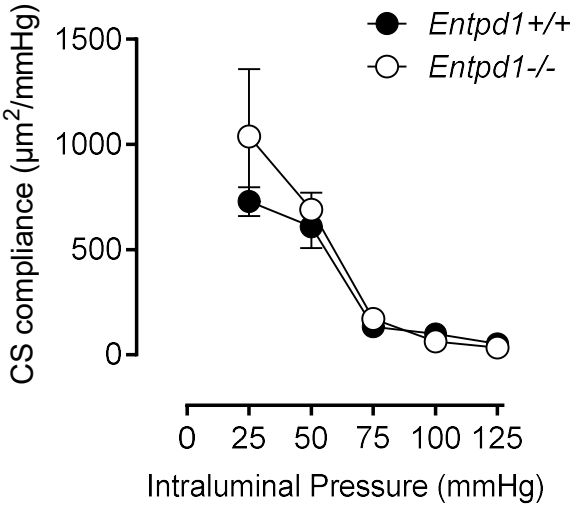
Supplementary Figure S1: *In vivo* hemodynamic parameters assessed by radiotelemetry in *Entpd1*$^{+/+}$ (blue) and *Entpd1*$^{-/-}$ (red) mice. Variations over periods (12h) of dark (black square) and light (white square). (A) Systolic blood pressure (SBP); (B) Heart rate (HR); (C) Pulse pressure (PP). Two-way ANOVA for repeated measurements was performed (*Entpd1*$^{-/-}$ versus *Entpd1*$^{+/+}$ mice) over 48h of measurements and significant p values are shown on graphs when significant.

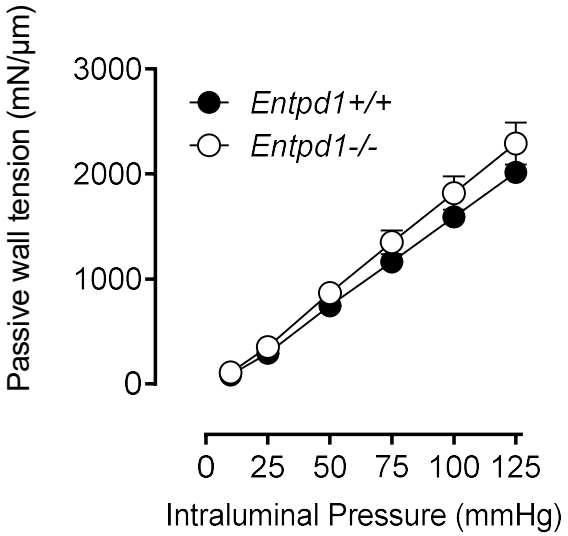
Supplementary Figure S2





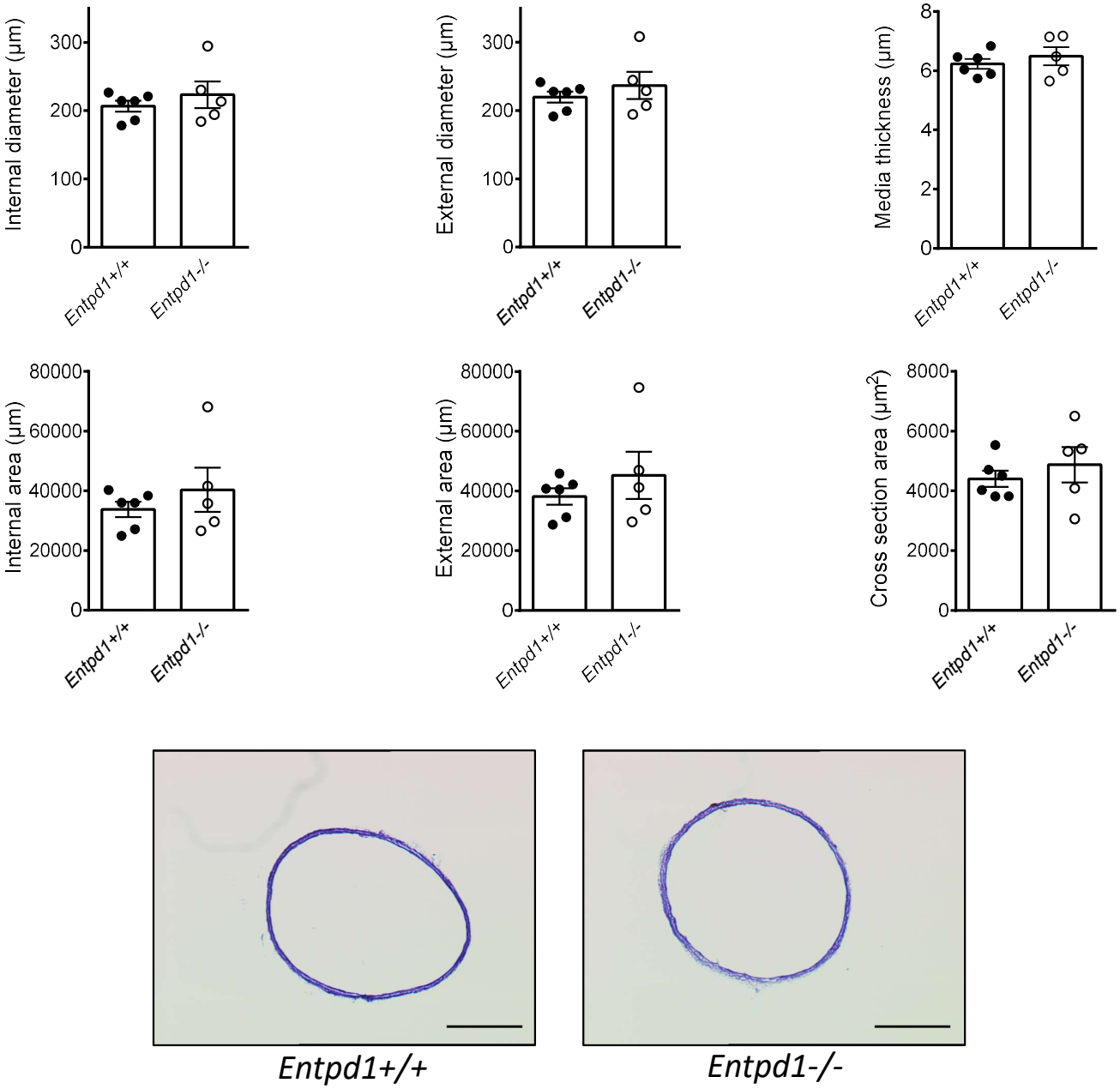






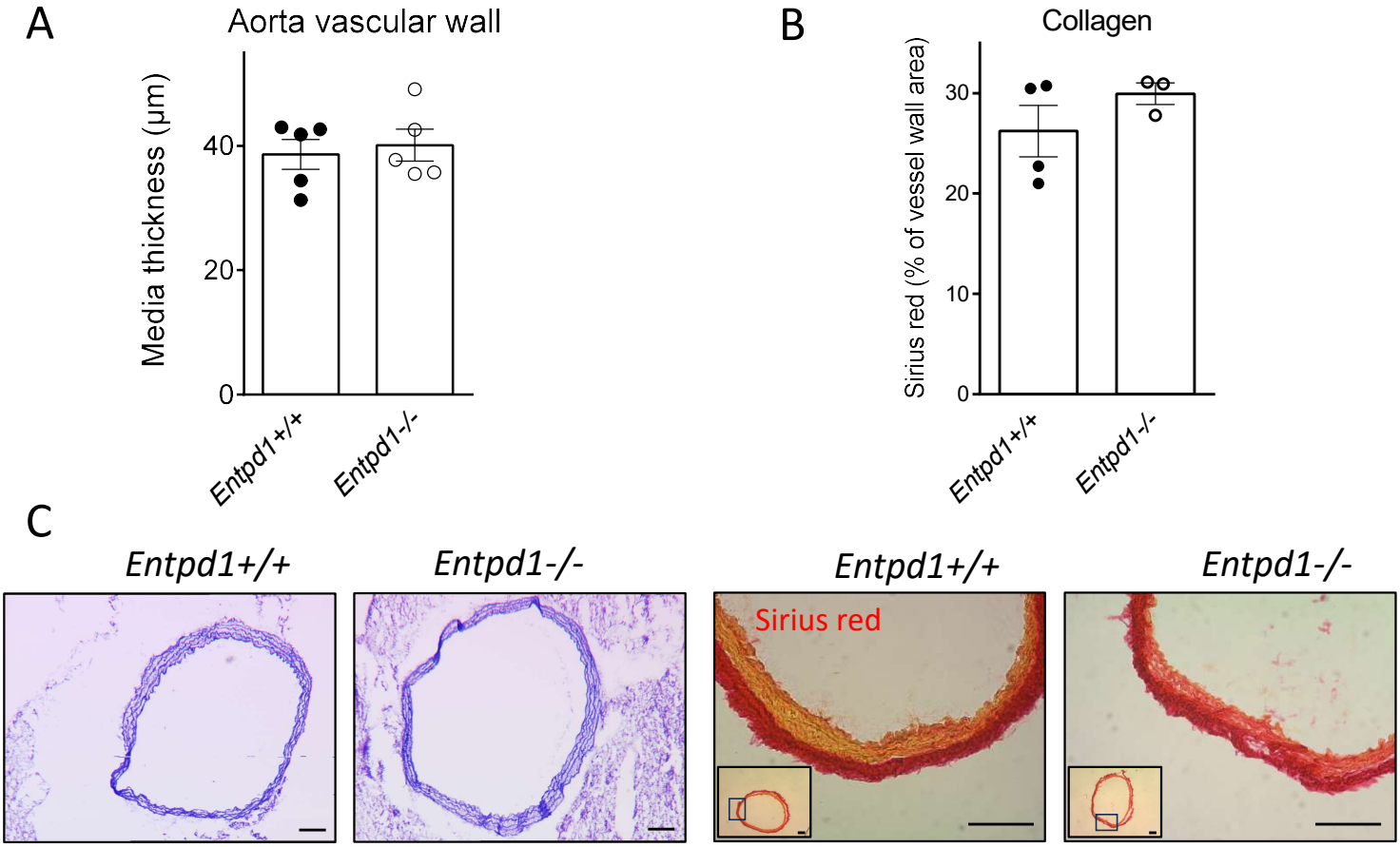
Supplementary Figure S2: Impact of Entpd1 deletion on mechanical properties assessed on isolated pressurized MRA. Passive changes in arterial diameter in response to the increase in internal pressure were measured on MRA superfused in PSS physiological solution without calcium with EGTA. Two-way ANOVA for repeated measurements was performed (*Entpd1*$^{-/-}$ versus *Entpd1*$^{+/+}$ mice, not significant).

Supplementary Figure S3



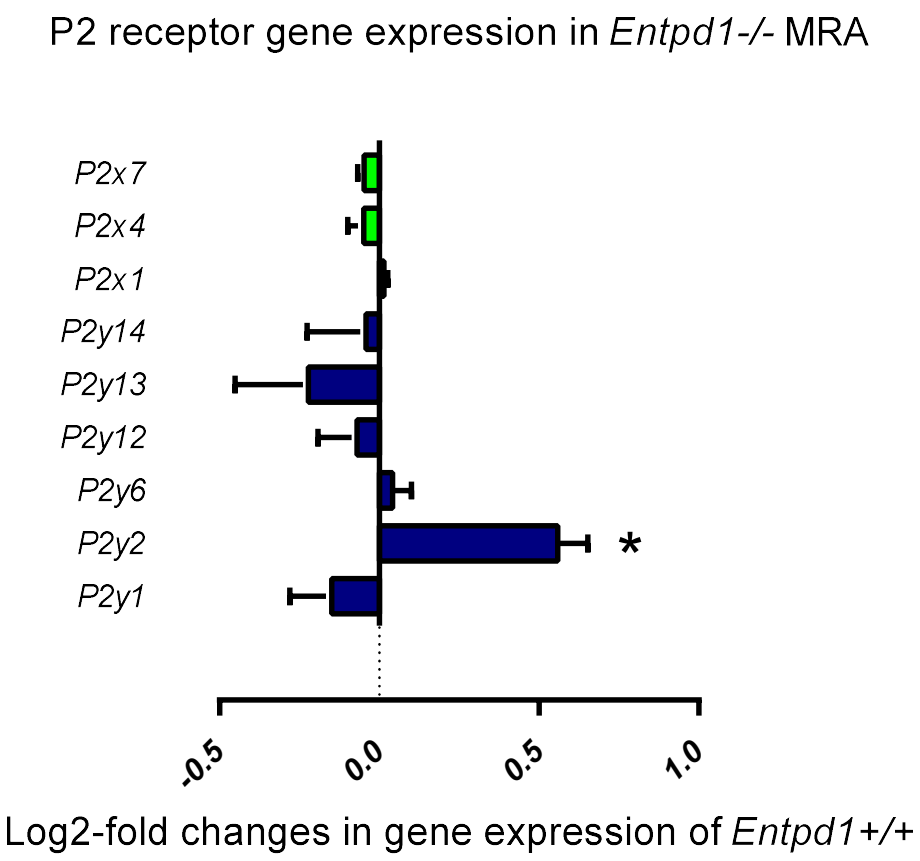
Supplementary Figure S3: Histomorphological analysis of pressurized mesenteric arteries. The MRA isolated from male *Entpd1-/-* and their wild-type littermates *Entpd1+/+* were cannulated and pressurized at 75 mmHg in physiologic solution without Ca^{2+} in order to obtain maximal passive diameter and the arteries were then fixed with PFA 4% before being embedded for histological staining with orcein. Scale bar 100 µm.

Supplementary Figure S4



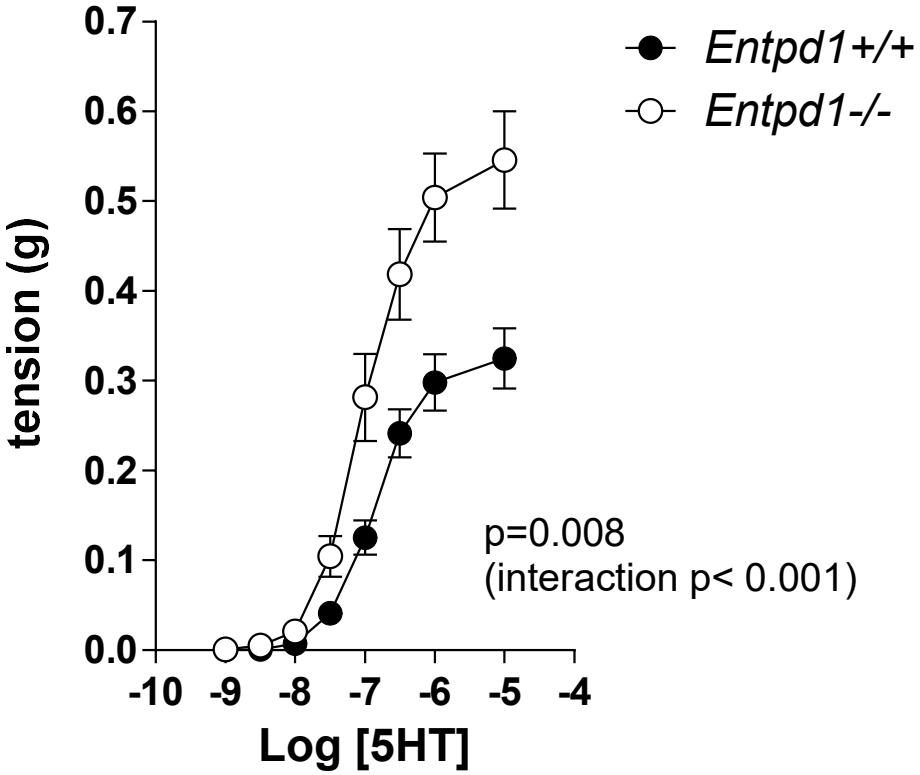
Supplementary Figure S4: Aorta vascular wall structure (A) and collagen content (B). Image J software was used to quantify media thickness (orcein staining) and collagen (Sirius red, expressed as % area of stained vessel wall). (C) Images illustrating histological analysis of thoracic aorta of *Entpd1-/-* and their control littermates *Entpd1+/+.* Different magnifications are shown for Sirius red staining. Scale bar 100 µm..

Supplementary Figure S5



Supplementary Figure S5: P2 receptor gene expression changes in Entpd1-/- MRA compared to WT littermates. Expressions were calculated using the $2^{\Delta Ct}$ method (housekeeping genes: Gusb, Hprt, Gapdh). * $p<0.05$ Mann-Whitney test *vs Entpd1+/+* (n=3-5).

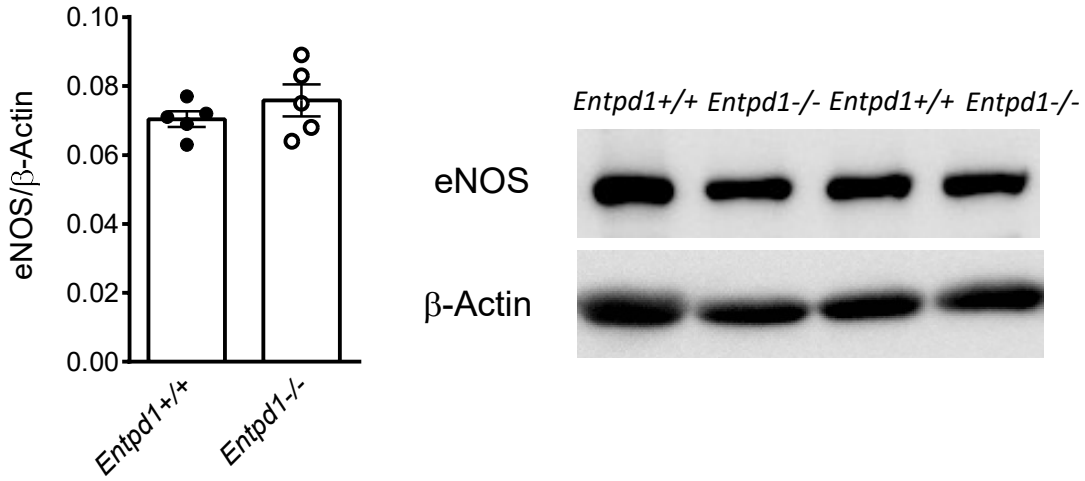
Supplementary Figure S6



Supplementary Figure S6: *Entpd1-/-* thoracic aortas show greater contraction in response to increasing concentrations of serotonin (5HT) compared to WT controls. Vascular reactivity of isolated aortas was assessed using a wire myograph.. Means ±SEM are shown, n=8-11. Two-way ANOVA for repeated measurements *Entpd1+/+* vs *Entpd1-/-,* p values are shown on graph.

Supplementary Figure S7

A

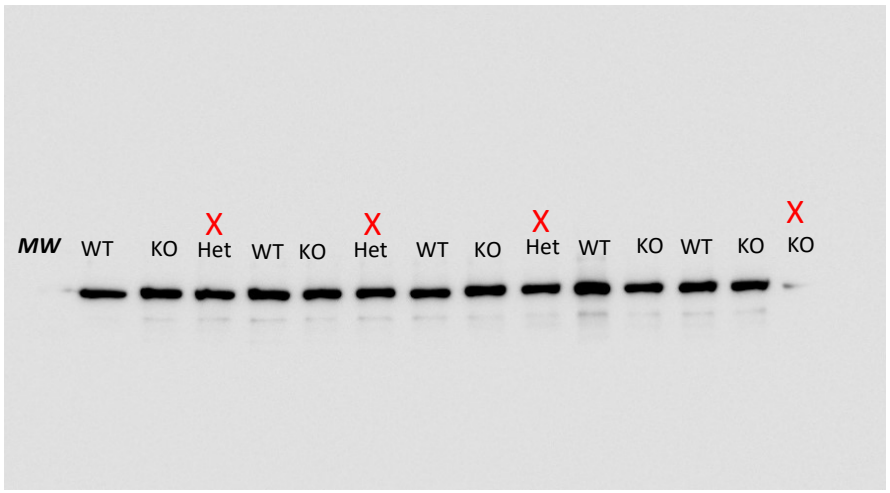


Supplementary Figure S7: Aortic protein eNOS expression. (A) Densitometric quantification (normalized to β-Actin) and representative western-blot analysis of eNOS protein expression (n=5; KO *Entpd1-/-* vs WT *Entpd1+/+* mice). Student t-test *p=0.325*

Supplementary Figure S7

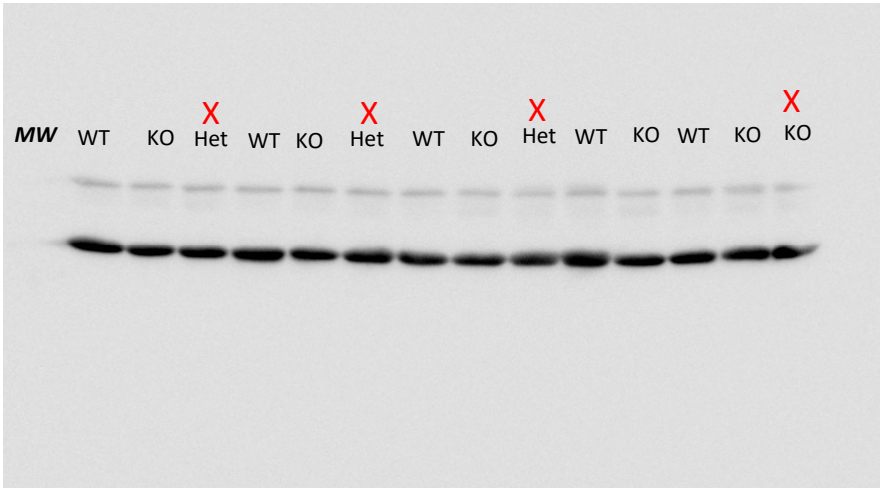
B

eNOS

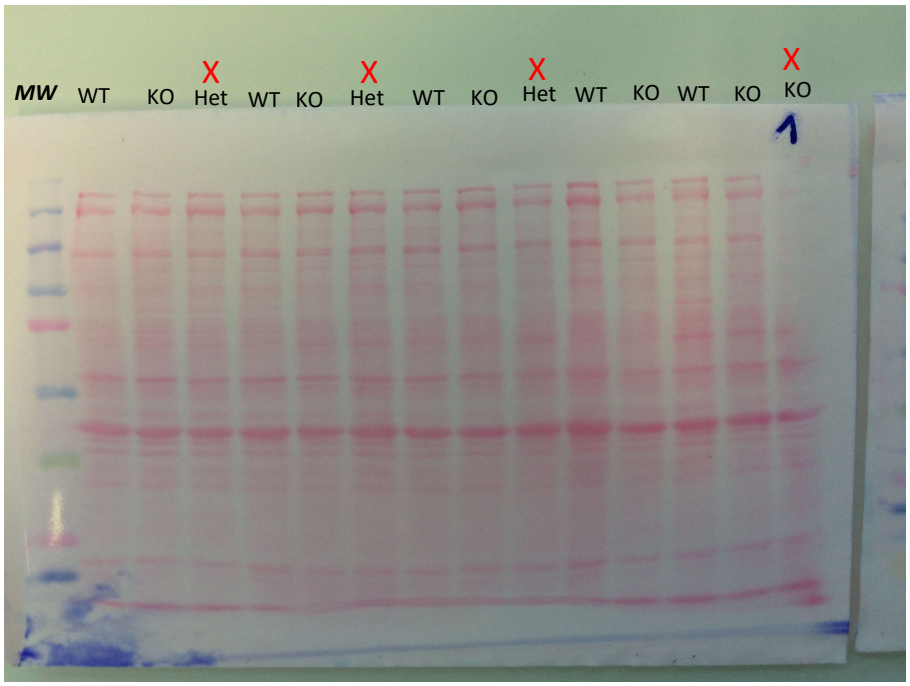


X Not used or discarded of quantification

β-Actin

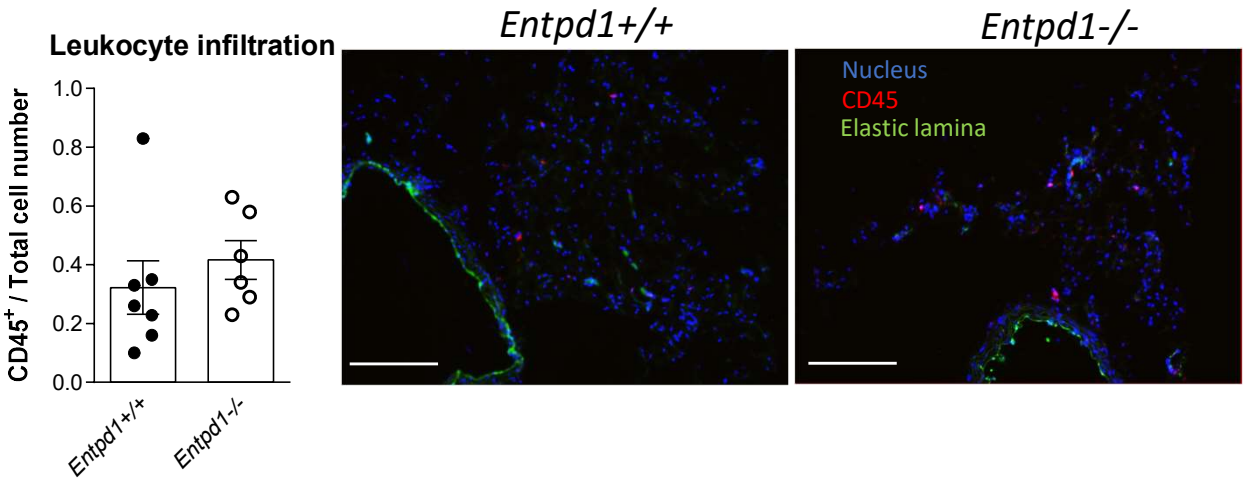


Ponceau



Supplementary Figure S7: (B) Uncropped gels used for western-blot analysis of aortic protein expression of eNOS (140 kD) normalized to β-Actin (42 kD) labeled with primary antibodies anti-eNOS (endothelial nitric oxide synthase; BD Biosciences, Franklin Lakes, NJ; #610297) and anti–β-actin (Sigma, St. Louis, MO; A5316). Control of protein loading and transfer is visualized with ponceau red staining of the nitrocellulose membrane. MW: molecular weight.

Supplementary Figure S8



Supplementary Figure S8: Leukocyte infiltration in perivascular area of thoracic aorta. CD45+ cells stained in red were counted and expressed as a ratio of total cell number stained with Dapi in blue. The green autofluorescence represents multilayered lamina. Scale bar 100μm.

		Entpd1+/+		*Entpd1-/-*		
Protein	Gene	Mean	SEM	Mean	SEM	*p*
Inflammation						
CD45	*Ptprc*	2,54E-03	1,85E-04	3,51E-03	4,56E-04	*0,078*
F4/80	*Emr1*	8,72E-03	1,17E-03	8,44E-03	1,08E-03	*0,861*
Cxcr3	*Cxcr3*	6,72E-03	2,09E-03	1,79E-02	4,69E-03	*0,074*
Il-6	*Il-6*	5,07E-03	8,88E-04	1,12E-02	5,22E-03	*0,270*
Mcp1	*Ccl2*	3,97E-02	1,69E-02	1,86E-02	3,66E-03	*0,253*
Cxcl2	*Cxcl2*	2,09E-02	7,51E-03	1,23E-02	2,12E-03	*0,297*
Cox2	*Ptgs2*	1,05E-02	3,58E-03	2,47E-02	4,99E-03	*0,054*
Inducible nitric oxide synthase	*Nos2*	3,75E-03	8,52E-04	3,94E-03	8,19E-04	*0,875*
Oxidative stress						
Gp91-phox	*Cybb*	1,19E-02	2,97E-03	1,42E-02	1,96E-03	*0,524*
Superoxide dismutase-1	*Sod1*	1,02E+01	1,00E+00	1,02E+01	9,48E-01	*1,000*
Superoxide dismutase-2	*Sod2*	9,66E+00	1,07E+00	9,08E+00	1,45E+00	*0,752*
NADPH oxidase 4	*Nox4*	4,69E-02	6,13E-03	5,08E-02	9,02E-03	*0,730*
Cell adhesion						
Vascular cell adhesion molecule-1	*Vcam1*	3,38E-02	5,96E-03	3,29E-02	2,99E-03	*0,901*
Intercellular adhesion molecule-1	*Icam1*	1,45E-02	3,04E-03	1,89E-02	3,86E-03	*0,395*
E-selectin	*Sele*	7,27E-03	1,84E-03	1,30E-02	3,44E-03	*0,172*
Matrix proteolysis						
Matrix metalloproteinase-2	*Mmp2*	3,61E-02	8,44E-03	3,34E-02	4,73E-03	*0,786*
Matrix metalloproteinase-9	*Mmp9*	1,53E-03	2,78E-04	1,13E-03	1,54E-04	*0,265*
Tissue inhibitor of metalloproteinase-1	*Timp1*	2,88E-02	9,14E-03	2,84E-02	3,48E-03	*0,969*
Tissue inhibitor of metalloproteinase-2	*Timp2*	6,77E-01	1,36E-01	6,65E-01	1,12E-01	*0,945*
	Mmp9/Timp1	8,28E-02	3,14E-02	3,65E-02	3,68E-03	*0,217*
	Mmp2/Timp2	5,40E-02	5,55E-03	5,16E-02	4,78E-03	*0,744*
Plasminogen activator Inhibitor-1	*Serpine1*	2,73E-02	8,25E-03	3,17E-02	1,46E-02	*0,799*
Neutrophil elastase	*Elan*	9,05E-03	4,48E-03	7,35E-03	2,79E-03	*0,746*
Cathepsin G	*Ctsg*	1,72E-02	3,87E-03	6,60E-03	2,40E-03	*0,055*
Thromboregulation						
Thrombomodulin	*Thbd*	2,60E-02	5,27E-03	2,79E-02	4,79E-03	*0,793*
Heparan sulfate proteoglycan	*Hspg2*	6,16E-02	1,16E-02	7,51E-02	1,66E-02	*0,521*
Fibrosis						
Connective tissue growth factor	*Ctgf*	6,09E-01	8,88E-02	4,56E-01	5,26E-02	*0,168*
Tissue growth factor-beta-1	*Tgfb*	5,71E-02	1,10E-02	6,33E-02	7,57E-03	*0,653*
Collagen-1	*Coll1A1*	8,81E-02	1,44E-02	8,42E-02	1,76E-02	*0,866*
Vascular homeostasis						
Endothelial nitric oxide synthase	*Nos3*	5,07E-02	9,51E-03	5,35E-02	8,19E-03	*0,827*
Endothelin-1	*Edn1*	3,19E-02	9,97E-03	2,22E-02	3,08E-03	*0,377*
Vascular endothelial growth factor receptor-2	*Kdr*	1,76E-02	2,45E-03	1,91E-02	2,84E-03	*0,688*
Vascular endothelial growth factor-α	*Vegfa*	1,22E-01	2,35E-02	1,28E-01	1,33E-02	*0,815*
Hypoxia inducible factor	*Hif1a*	1,47E-01	1,34E-02	1,67E-01	2,14E-02	*0,458*

Supplementary Table S1: Expression of genes likely to modulate vascular function. mRNA expression was determined in thoracic aorta with perivascular fat of *Entpd1-/-* and *Entpd1+/+* mice (n = 5-6) by RT-qPCR. Data are presented as means ± SEM. *p* represents Student's *t*-test analysis vs. Controls. Methods: Tissues from *Entpd1-/-* and *Entpd1+/+* controls were stored at − 20 °C in RNAlater Stabilization Reagent (Qiagen). RNA extraction was performed with the RNeasy® Micro Kit (Qiagen). RNA extract was used to synthesize cDNA. RT-qPCR was performed with SYBR® Select Master Mix (Applied Biosystems) by using a LightCycler 480 Real-Time PCR System (Roche). mRNA levels were expressed as 2(Ct target gene – Ct mean of reference genes). Reference genes represent the 3 housekeeping genes *Gapdh*, *Gusb* and *Hprt*. Sequences of primer pairs are represented in Supplementary Table S2.

Gene	Protein	Gene ID	Amplicon size	Forward	Reverse
Ccl2 bis	**MCP1**	NM_011333.3	75	gcctgctgttcacagttgc	caggtgagtggggcgtta
Col1a1	**Col1a1**	NM_007742.3	94	catgttcagctttgtggacct	gcagctgacttcagggatgt
Ctgf	**Ctgf**	NM_010217.2	112	tgacctggaggaaaacattaaga	agccctgtatgtcttcacactg
Ctsg	**Cathepsin G**	NM_007800.2	91	acggttctggaaagatgcag	tctcggcctccaatgatct
Cxcl2 ter	**MIP2**	NM_009140.2	102	aacatccagagcttgagtgtga	ttcagggtcaaggcaaactt
Cxcr3	**Cxcr3 (CD183)**	NM_009910	89	gcagcacgagacctgacc	ggcatctagcacttgacgttc
Cybb	**Gp91-phox**	NM_007807.4	71	gaggttggttcggttttgg	gttttgaaagggtgggtgac
Edn1	**Endothelin-1**	NM_010104.2	87	tgctgttcgtgactttccaa	gggctctgcactccattct
Ela-2	**Neutrophil Elastase**	NM_015779.2	94	tggaggtcatttctgtggtg	ctgcactgaccggaaatttag
Emr1	**F4/80**	NM_010130	113	gcaaggagaatgagtccattaac	tgagacaaaagccactcctg
Entpd1	**CD39**	NM_009848.3	76	ctcctgcaaggctataacttcac	gcgttgctgtctttgatcttg
Gapdh	**GAPDH**	NM_008084.2	121	ccggggctggcattgctctc	ggggtgggtggtccagggtt
Gusb	**GUSB**	NM_010368.1	72	ctctggtggccttacctgat	cagttgttgtcaccttcacctc
Hif1a	**HIF1α**	NM_010431.2	104	gcactagacaaagttcacctgaga	cgctatccacatcaaagcaa
Hprt	**HPRT**	NM_013556.2	125	ggggtgggtggtccagggtt	aagacattctttccagttaaagttgag
Hspg2	**Hspg2**	NM_008305.3	95	caccttcgctggctcaag	tggcagctctggtctaagttc
Icam1	**ICAM**	NM_010493.2	80	gctaccatcaccgtgtattcg	aggtccttgcctacttgctg
Il6	**IL-6**	NM_031168.1	84	gatggatgctaccaaactgga	ccaggtagctatggtactccagaa
Kdr	**VegfR2**	NM_010612.2	65	cagtggtactggcagctagaag	acaagcatacgggcttgttt
Mmp2	**MMP2 (gelatinase A)**	NM_008610.2	74	gtgggacaagaaccagatcac	gcatcatccacggtttcag
Mmp9	**MMP9 (gelatinase B)**	NM_013599.2	108	ttctggcacacgcctttc	ccatagtaagtggggatcacg
Nos2	**iNOS**	NM_010927.3	65	ctttgccacggacgagac	tcattgtactctgagggctgac
Nos3	**eNOS**	NM_008713.4	65	ccagtgccctgcttcatc	gcagggcaagttaggatcag
Nox4	**NOX4**	NM_015760.4	69	ctggaagaacccaagttcca	aaaggcacaaaggtccagaa
Nt5e	**Cd73**	NM_011851.4	76	ccattgatgagaagaacaatggta	gtcaaatgtccctccaaagg
P2rx1	**P2X1**	NM_008771.3	86	ccgaagccttgctgagaa	ggtttgcagtgccgtacat
P2rx4	**P2X4**	NM_011026.2	89	ccaacacttctcagcttggat	tggtcatgatgaagagggagt
P2rx7	**P2X7**	NM_011027.2	76	ctggttttcggcactgga	ccaaagtaggacagggtgga
P2ry1	**P2Y1**	NM_008772.4	60	ctgtgtggaccccattcttt	tcgggacagtctccttctga
P2ry12	**P2Y12**	NM_027571.3	94	gagacactcatatccttcagattcag	tcgggacagtctccttctga
P2ry13	**P2Y13**	NM_028808.3	60	atgtgtgagatggggaaagg	gtcccaggggagaaggtg
P2ry14	**P2Y14**	NM_001008497.2	59	ctttgcctccagaggtgaga	ggctggtttggagggtct
P2ry2	**P2Y2**	NM_008773.3	127	tgcgctgatgaactttgttt	ggcaggaaacaggaagaaca
P2ry6	**P2Y6**	NM_183168.1	80	tcttccatcttgcatgagaca	ggatggtgccattgtcct
Panx1	**PANX1**	NM_019482.2	61	agaccaagggagaggacca	gctgctcaggtccaaatctt
Ptgs2 bis	**COX2**	NM_011198.3	109	gggagtctggaacattgtgaa	gcacattgtaagtaggtggactgt
Ptprc	**CD45**	NM_011210	111	cgggatgagacagttgatga	gtattctgcgcacttgttcct
Sele	**E-selectin**	NM_011345.2	67	tcctctggagagtggagtgc	ggtgggtcaaagcttcacat
Serpine1	**Serpine1 (PAI1)**	NM_008871	80	ggcacctttgaatactcagga	tttcccagagaccagaacca
Sod1	**SOD1**	NM_011434.1	78	caggacctcattttaatcctcac	tgcccaggtctccaacat
Sod2	**SOD2 (MnSOD)**	NM_013671.3	68	gacccattgcaaggaacaa	gtagtaagcgtgctcccacac
Tgfb1	**Tgf-beta1**	NM_011577.1	70	tggagcaacatgtggaactc	cagcagccggttaccaag
Thbd	**Thrombomodulin**	NM_009378.3	62	tggcgataaccacaccagt	agagtggagccgtctggtc
Timp1	**TIMP1**	NM_011593.2	93	catggaaagcctctgtggat	gatgtgcaaatttccgttcc
Timp2	**TIMP2**	NM_011594	60	ttttgcaatgcagacgtagtg	ggaatccacctccttctcg
Vcam1	**VCAM1**	NM_011693.2	110	tgattgggagagacaaagca	aacaaccgaatccccaactt
Vegfa	**Vegfa**	NM_001025250.3	74	aaacgaaagcgcaagaaatc	atgctttctccgctctgaac

Supplementary Table S2: Sequences of primer pairs used for RT-qPCR.
Housekeeping genes: *Gapdh, Gusb, Hprt.*