Figure S1
Figure S1. Neurovascular alignment

(A-D) Confocal images of the back skin of a midgestation mouse embryo stained with the indicated antibodies. Note alignment of TH+ sympathetic fibers and TuJ1+ sensory nerves with Flk1+ arteries. (E-L) Low magnification (E-H) and high magnification (I-L) images of mesentery and mesenteric artery at P2. TuJ1+ and TH+ axon bundles and fibers colocalise (arrows) illustrating that the majority of axons present are sympathetic axons. (M-P) Confocal images of the mesentery of a Wnt-1-Cre:mTmG mouse at postnatal day 5. mGFP (M) and TH immunostaining (N) co-localize in the majority of neuronal fibers surrounding the artery (O, P). Merged images containing red CD31+ vascular labeling are shown (H, L, P). Confocal images of the innervation of P10 circle of Willis (Q, R) and P2 cutaneous artery (S, T). TH+ sympathetic fibers (J, S) start to make contact with CX40+ arteries (S, T) at P2. a: artery, v: vein, l: lymphatic. Scale bars: 100 µm (A-H); 50 µm (M-P); 25 µm (I-L and Q-T)
Figure S2

A

B

E15.5 Circle of Willis  
Adult Circle of Willis

C

Exon 2  
loxP  
Exon 3  
loxP  
ATG  
Exon 4

Met1  
(ntrn-1 -604 aa)  
(ntrn-1 -421 aa)

D

Mesenteric Arteries

Ntn-1 F/F  
SM22 Cre-  
Ntn-1 F/F  
SM22 Cre+

E

P3 Mesentery

F

Mesenteric Arteries

Ntn-1 +/+  
Ntn-1 +/-

G

OD 450-550

DCC-Fc  
UNC5B-Fc

DCC blocking Ab concentration (µg/ml)
Figure S2. Expression of Netrin-1 in innervated arteries.

(A) qPCR measurement of Netrin-1 levels in cDNA prepared from mesenteric arteries at the indicated stages, showing an increase of Netrin-1 expression at P2, when arterial innervation is initiated (N=6 animals/stage). (B) X-gal staining of Ntn-1+/− mice at the indicated stages. Note expression in the E15.5 brain, including the floorplate and the ventral telencephalon, but absence of staining of arteries of the Circle of Willis (higher magnification in bottom images, arrows), while arteries of the Circle of Willis in adult mice express Netrin-1. (C) Schematic representation of the floxed Ntn-1 allele showing the targeted region with loxP sites surrounding the first coding exon containing initiator Methionine (58). (D) Netrin-1 expression measured by qPCR using cDNA from mesenteric arteries of Ntn-1 F/F SM22 Cre + or − mice. (E) Visualization of SM22 Cre-recombination in mesenteric arteries using mT/mG reporter. (F) Netrin-1 expression measured by qPCR using cDNA from mesenteric arteries of wild-type or Ntn-1+/− mice (F). (N=5 Ntn-1 F/F SM22 Cre + and 4 Ntn-1 F/F SM22 Cre −; N=8 for Ntn-1 +/− and control littermates). (G) Elisa results show that increasing concentrations of DCC blocking antibody block Netrin-1 binding to DCC-Fc but not to Unc5B-Fc.

A: Artery; V: Vein.

Scale bars: 1 mm (B-lower panel); 50 µm (E).
Figure S3. Effects of Netrin-1 blockade on sympathetic nerves.

(A, B): Quantification of the diameter of mesenteric artery (A) and adjacent sympathetic nerve bundle (B) in Ntn-1 deficient mice and control littermates. N=6 mice per group, 8-10 images per mesentery (see Fig.3).

(C) Anti-TH staining of the heart from adult wild-type (C) and Ntn-1\(^{+/}\) mice (D). Note similar innervation of cardiac muscle in both mice.

(E-H) Co-cultures of Superior Cervical Ganglion (SCG) of P1 wild-type pups with mesenteric arteries of P8 wild-type (E-F) and Ntn-1\(^{+/}\) littermates (G-H) stained with anti-TH and anti-SMA antibodies. Axons extend from SCG and wrap around arteries originating from wild-type pups, whereas arteries from Ntn-1\(^{+/}\) pups failed to attract SCG axons. (I) Quantification of sympathetic innervation \textit{in vitro} (n=10 pictures per condition, 3 animals per genotype). *** P<0.001 using unpaired two-tailed t test.

Scale bars: 2 mm (C-D); 25 \(\mu\)m (E-H)
Figure S4

G

H
Figure S4. Lack of Netrin-1 expression in adult mice impairs innervation and vasoconstriction, but not SMC differentiation.

(A-F) Confocal microscopy of arterioles in the oesophagus shows reduced innervation in $Ntn^{-1/0}$ compared to wildtype littermates.

(G-H) qPCR mesurements showed no significant difference in expression of arterial differentiation markers including Myosin Heavy Chain (MHC) between Ntn-1 +/− and WT and between Ntn-1 F/F SM22 Cre + or − using cDNA prepared from mesenteric arteries of adult mice (N=5 and 4 respectively).

Scale bars: 35 μm (A-F).