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Supplemental Information

A Distinct Role of the Autonomic Nervous System in Modulating the Function of Lymphatic Vessels under Physiological and Tumor-Draining Conditions

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Supplemental Figure 1

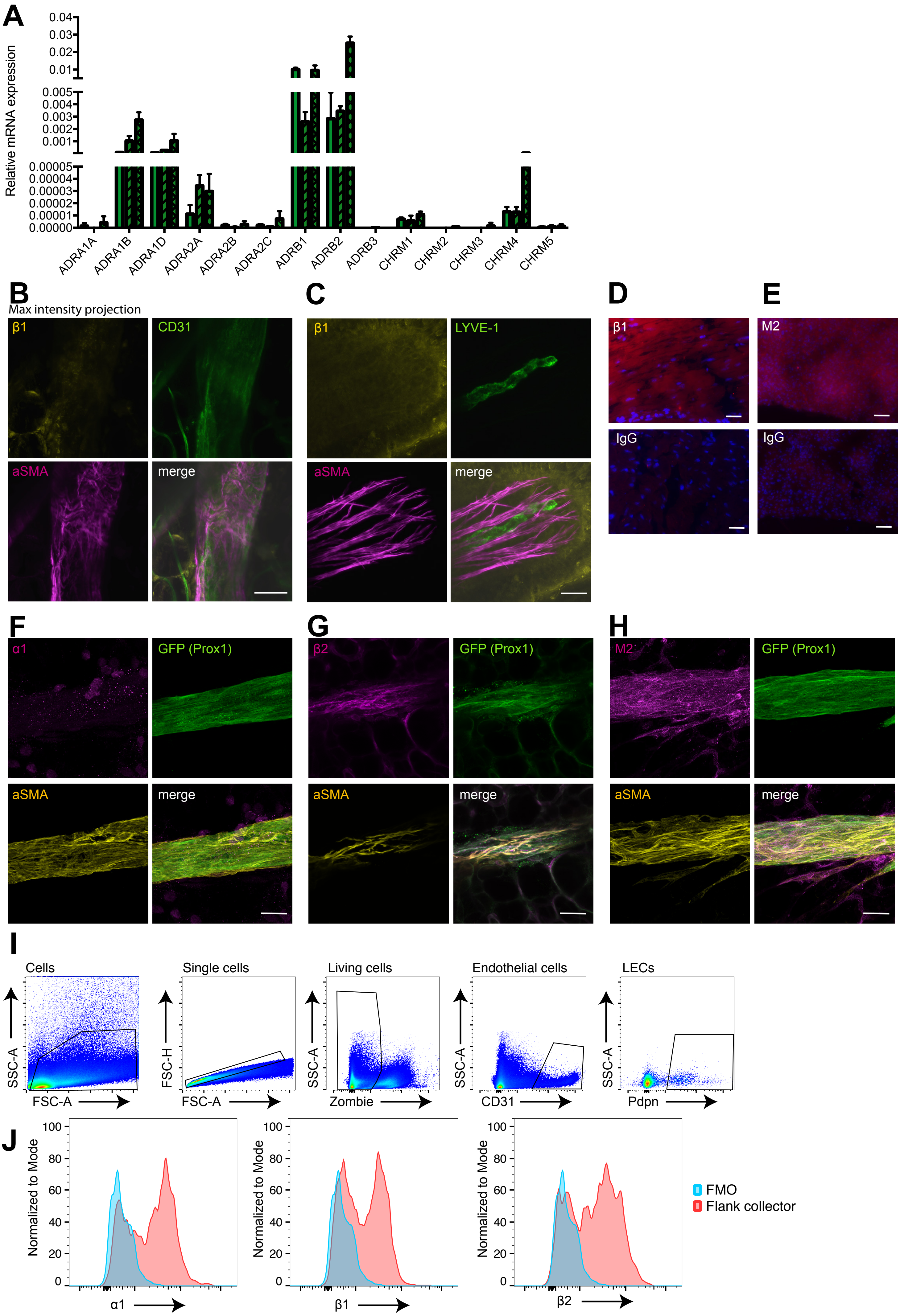

Figure S1. Related to Figure 1. Lymphatic vessels *in vivo* express low levels of beta 1 receptors. (A) qPCR-based detection of adrenergic β_1 and β_2 receptor mRNA in 3 additional primary human LEC lines, shown as differently filled columns, n=3 replicates (B and C) Fluorescent whole mount images show no expression of β1 receptors in CD31+ flank collector or LYVE-1+ lacteal with their aSMA+ smooth muscle cells. (D and E): Positive control stainings of sectioned of heart (D) and liver (E) tissue for β_1 and M_2 antibodies, including IgG controls. (F-H) Fluorescent whole mount images of mesenteric collectors show no expression of α_1 receptors, but presence of β_2 and M_2 receptors on both Prox1+ LECs and aSMA+ smooth muscle cells. (I) Gating strategy used to obtain living, CD31+ podoplanin+ LECs of flank collecting vessels. (J) Expression profiles of α_1, β_1 and β_2 receptors on flank collector LECs compared to their FMOs (fluorescence minus one), respectively, n = 1. Images were obtained using confocal microscopy. Scale bars: 50 µm (B and E), 25 µm (C, D, F-H). Data in (A) are represented as mean ± SD.

Supplemental Figure 2

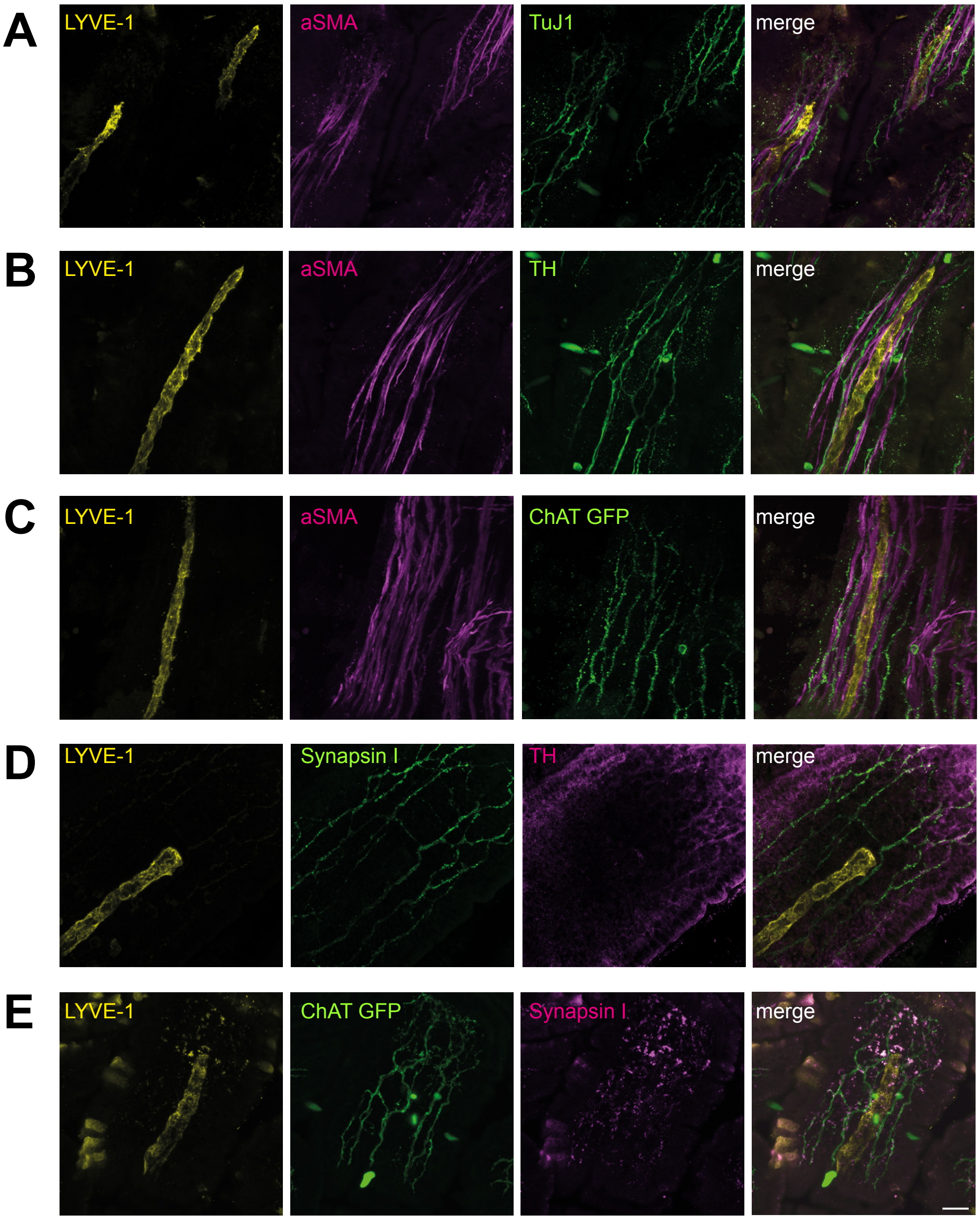

Figure S2. Related to Figure 2. Sympathetic and parasympathetic nerves form synapses close to lacteals. (A) Nerves (TuJ1+) are tightly associated with LYVE-1+ lacteals and their aSMA+ smooth muscle cells. (B and C) The innervation by sympathetic (tyrosine hydroxylase, TH+) and parasympathetic (cholinacetyl transferase, ChAT+) nerves. (D and E) Both TH+ and ChAT+ nerves form synapses (Synapsin I+) associated with the lacteal itself and with the smooth muscle cells. Images were obtained using confocal microscopy. Scale bar: 25 µm.

Supplemental Figure 3

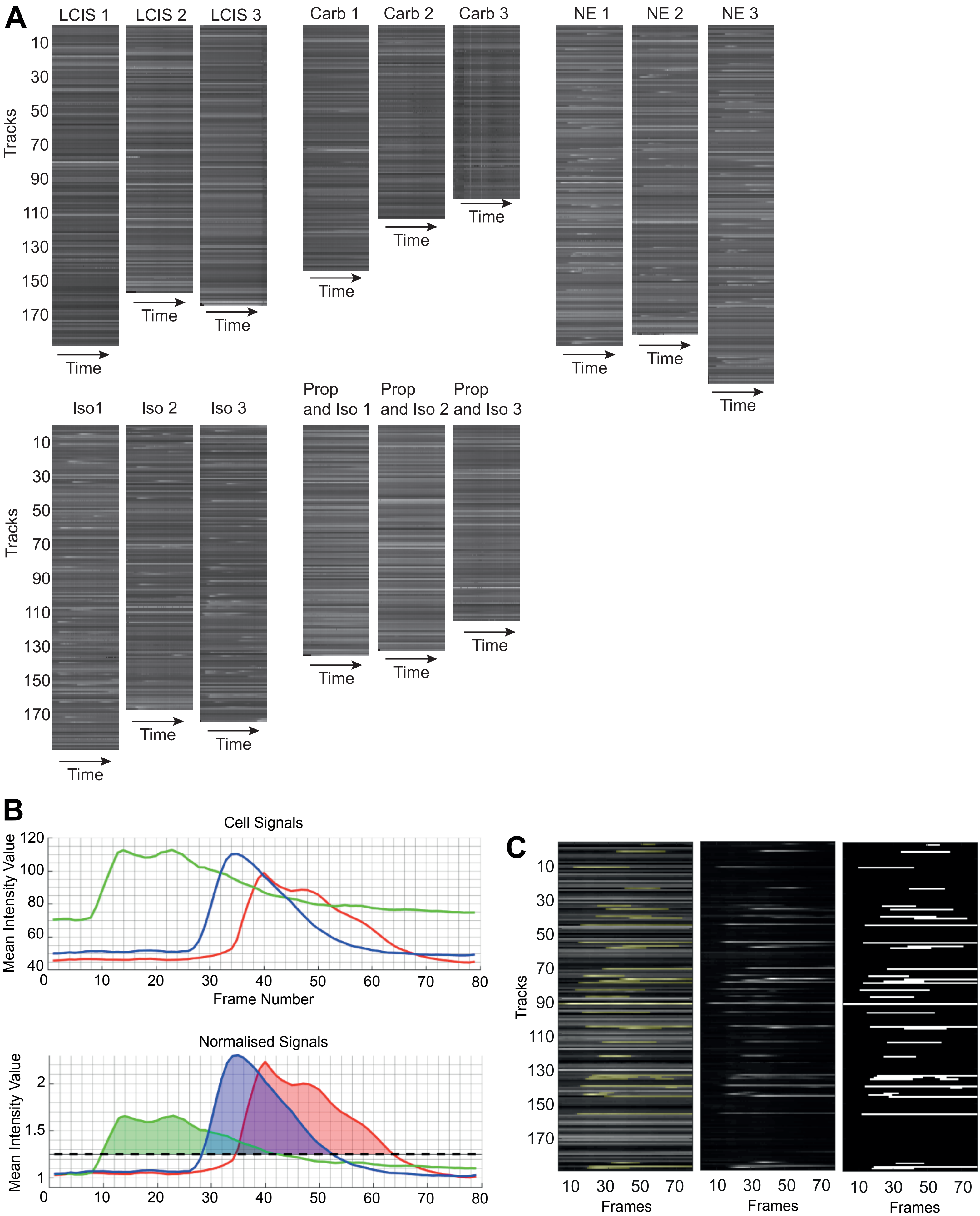

Figure S3. Related to Figure 3. Increased Ca^{2+} levels in cultured hLECs upon activation of β receptors. Raw data from *in vitro* Ca^{2+} imaging. Three independent replicates are shown for each treatment. Each horizontal line represents one tracked cell. Activation of cells can be seen by changes of the grey scale of a cell over time. LCIS=live cell imaging solution, Carb=carbachol, NE=norepinephrine, Iso=isoproterenol, Prop=propranolol.

Supplemental Figure 4

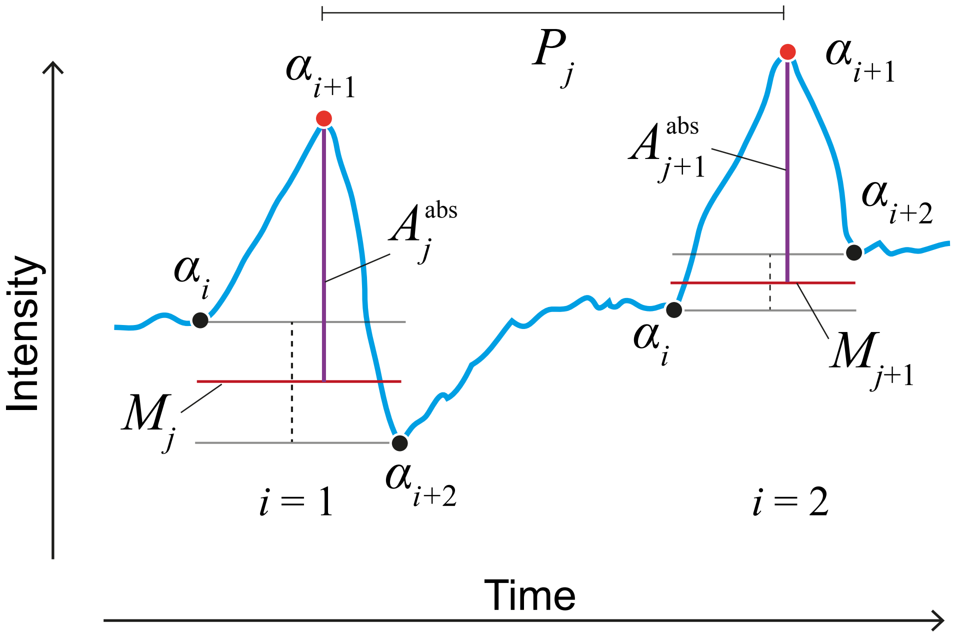

Figure S4. Related to Figure 5. Algorithm for quantification of *in vivo* contractility experiments. Visualization of a typical fluorescence signal (schematic) with the mathematical notations used by the signal processing method developed in Matlab. Each contraction is defined by three points (α_i, α_{i+1}, α_{i+2}) with its amplitude (A^{abs}) according to the mean (M_j) between the starting and ending point of the contraction. Frequency is defined as the time between two peaks (P_j).

Supplemental Figure 5

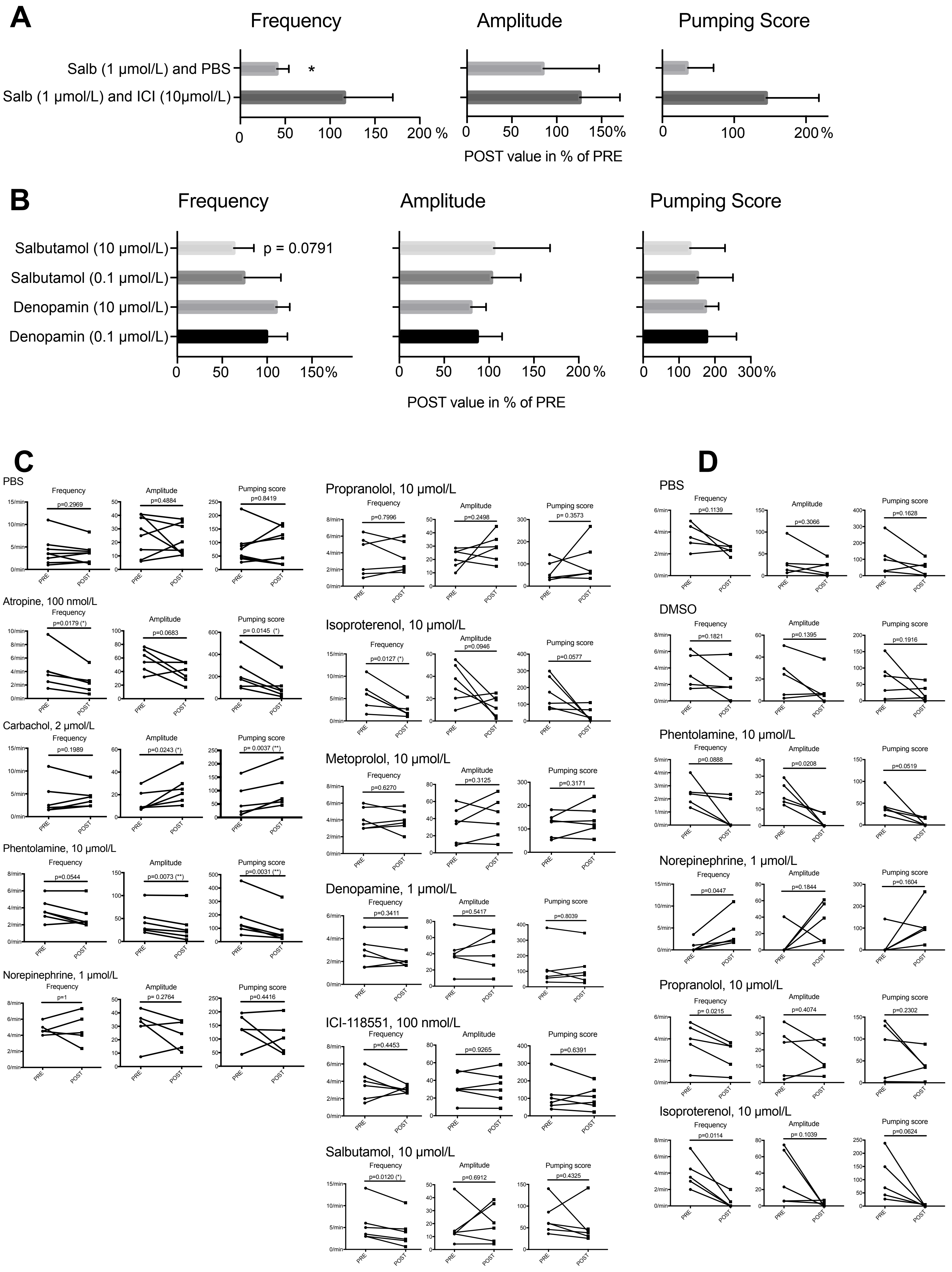

Figure S5. Related to Figure 5. Raw data of *in vivo* contractility experiments (flank assays). (A) Specificity testing of Salbutamol by a second treatment of the exposed vessel with either PBS or ICI. Shown are the post treatment values in % of pre-treatment values of mice under injection anesthesia (ketamine, xylazine, acepromazine), n=4 vessels. (B) Additional concentrations of β_1 and β_2 agonists in mice under injection anesthesia, shown are post treatment values in % of pre-treatment values, n=5 vessels. (C) Raw data of flank assay experiments using injection anesthesia. All pre (minute 0-2) and post treatment (minutes 4-7) values are shown; n=6 vessels per treatment. (D) Raw data for flank assay experiments using isoflurane anesthesia; n=5 vessels per treatment. Two-tailed, paired Student's t-test.

Supplemental Figure 6

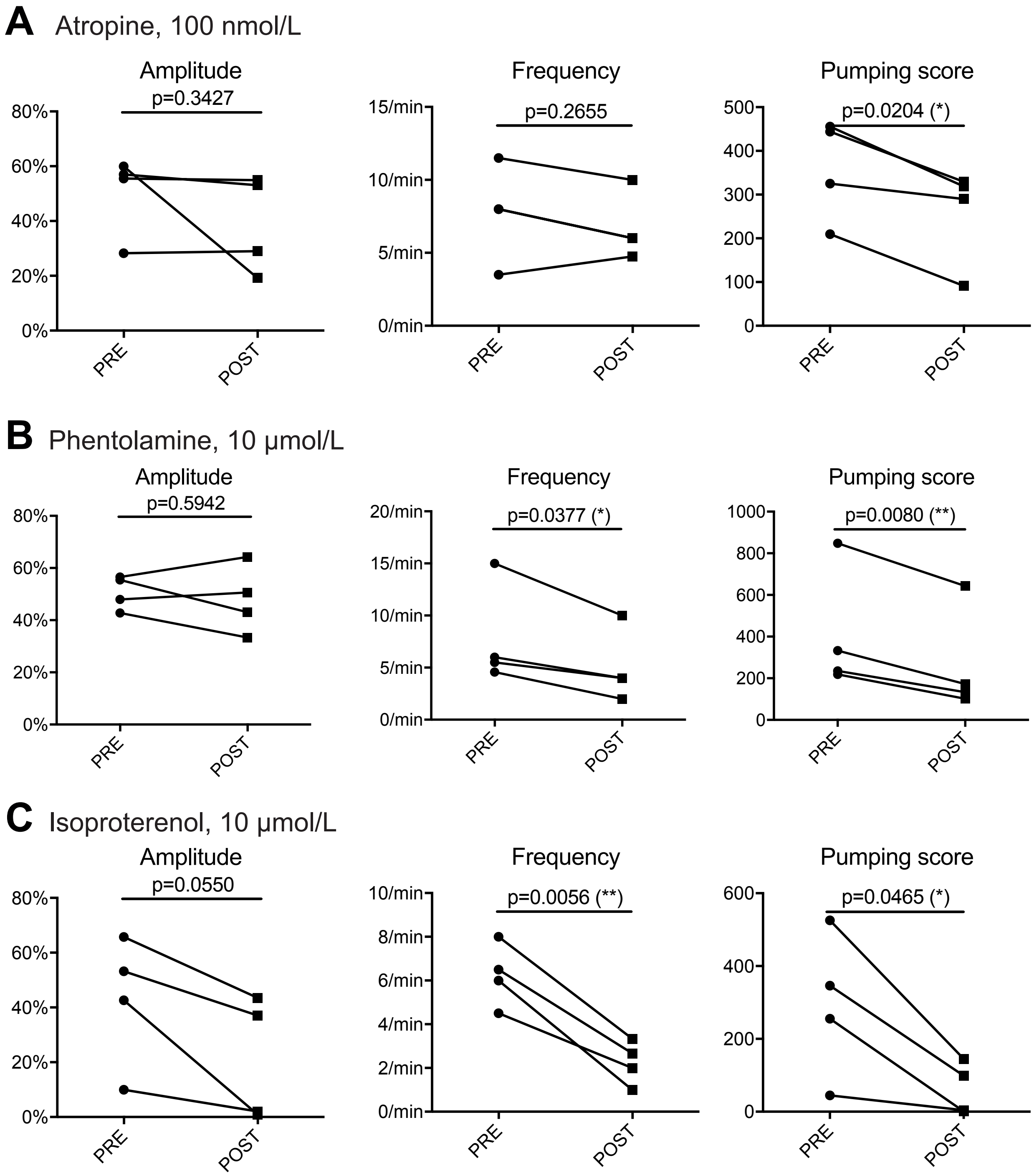

Figure S6. Related to Figure 6. Raw data of *in vivo* contractility experiments in tumor bearing mice. All pre (minute 0-2) and post treatment (minutes 4-7) values are shown; n=4 vessels per treatment, atropine (A), phentolamine (B), isoproterenol (C). Two-tailed, paired Student's t-test.

Table S1: Antagonists and agonists used in imaging and in vitro assays. Related to Figure 3-6.

Compound	Company	Order number
DL-Norepinephrine hydrochloride	Sigma-aldrich	A7256
Phentolamine hydrochloride	Sigma-aldrich	P7547
Atropine sulfate salt monohydrate	Sigma-aldrich	A0257
Carbachol	Millipore	212385
(−)-Isoproterenol hydrochloride	Sigma-aldrich	I6504
(±)-Propranolol hydrochloride	Sigma-aldrich	P0884
R(-)-Denopamine	Sigma-aldrich	D7815
(±)-Metoprolol (+)-tartrate salt	Sigma-aldrich	M5391
Salbutamol	Sigma-aldrich	S8260
ICI-118,551 hydrochloride	Sigma-aldrich	I127

Table S2: Primary antibodies used. Related to Figure 1, 2, 5, 6, S1, S2.

Antigen	Host	Company	Order number
Immunofluorescence			
alpha-1 adrenergic receptor	goat	Santa Cruz	sc-1477
beta-1 adrenergic receptor	rabbit	abcam	ab3442
beta-2 adrenergic receptor	chicken	abcam	ab13989
muscarinic acetylcholine receptor 2	rabbit	abcam	ab109226
muscarinic acetylcholine receptor 3	rabbit	Santa Cruz	sc-9108
Synapsin-1	rabbit	invitrogen	51-5200
Tyrosine Hydroxylase	sheep	R&D	AF7566
Tyrosine Hydroxylase	rabbit	invtrogen	P21962
III beta Tubulin	rabbit	BioLegend	801201
GFP	chicken	Aves	GFP-1010
CD31	rat	BD Bioscience	550274
alpha smooth muscle actin	mouse	Sigma-Aldrich	C6198
LYVE-1	rabbit	AngioBio	11-034
LYVE-1	rat	ReliaTech	103-M130
Western Blot			
eNOS	rabbit	ThermoFisher	PA1-037
Phospho-eNOS (Ser1177)	rabbit	CellSignaling	9571
beta-1 adrenergic receptor	rabbit	abcam	ab3442
beta-2 adrenergic receptor	chicken	abcam	ab13989
Tubulin	mouse	Sigma-Aldrich	T9026V
FACS			
alpha-1 adrenergic receptor	rabbit	abcam	ab137123
beta-1 adrenergic receptor	rabbit	abcam	ab3442
beta-2 adrenergic receptor	rabbit	abcam	ab61778
CD31-APC	rat	BD Bioscience	551262
Podoplanin-PE-Cy7	hamster	eBiosience	25-5381-82

Table S3: Secondary antibodies used. Related to Figure 1, 2, 5, 6, S1, S2.

Antigen	Conjugate	Host	Company	Order number
Immunofluorescnece				
goat	Alexa 594	donkey	invitrogen	A-11058
rabbit	Alexa 488	donkey	invitrogen	A-21206
rabbit	Alexa 647	donkey	invitrogen	A-31573
chicken	Alexa 488	donkey	Jackson ImmunoResearch	703-545-1551
sheep	Alexa 594	donkey	invitrogen	A-11016
rat	Alexa 488	donkey	invitrogen	A-21208
Western Blot				
rabbit	HRP	donkey	GE Healthcare	NA9340V
mouse	HRP	sheep	GE Healthcare	NA931
FACS				
rabbit	PE	donkey	BioLegend	406421

Table S4: Used primers of neurotransmitter receptors. Related to Figure 1, S1.

Gene	Primer	
	fwd	rev
ADRA1A	TAGTCATGCCCATTGGGTC	AGCTGTTTAGATATCCGAGCC
ADRA1B	CTTGGCTCCTTGTTCTCCA	AGCTGTTGAAGTAGCCCAG
ADRA1D	TTTCTTCTTTGTCCTGCCG	AGATGACCTTGAAGACGCC
ADRA2A	TTCTTCTGGTTCGGCTACTG	GGTTGAAGATGGTGTAGATGAC
ADRA2B	TTCTTCTGGATCGGCTACTG	GGTTGAAGATGGTGTAGATAACAG
ADRA2C	TTCCTCATCGTCTTCACCG	CCAGGAAGAGGTTCTGTGG
ADRB1	ATCATGGCCTTCGTGTACC	TCGATCTTCTTCACCTGCT
ADRB2	GTCTACTCCAGGGTCTTTCAG	CCTCAGATTTGTCAATCTTCTGG
ADRB3	TCAACCCGCTCATCTACTG	TCAGGCCTAAGAAACTCCC
CHRM1	CTTTCCCTTTCTCCCTCCC	TGTTCATGGTGGCTAGGTG
CHRM2	GATCAAGGGAGAAAGAGAACC	AGTCCTTTGTGGGTTCAGG
CHRM3	CAGTTCCTGGTAGATTACTATGTC	TGTTTGGAAACAAAGGCGA
CHRM4	GAAGGAGAAGAAAGCCAAGAC	GACGCTCTGCTTCATTAGTG
CHRM5	GTCTCGAAGCTTACTATTTCCA	AATGATTTCCTAGGTTCCTGG
GAPDH	GAA ATC CCA TCA CCA TCT TCC AGG	GAG CCC CAG CCT TCT CCA TG
Cx40	AAT ACA ACG GCT ATC ACG	AGC TTC TGG CTT TCT TTA C