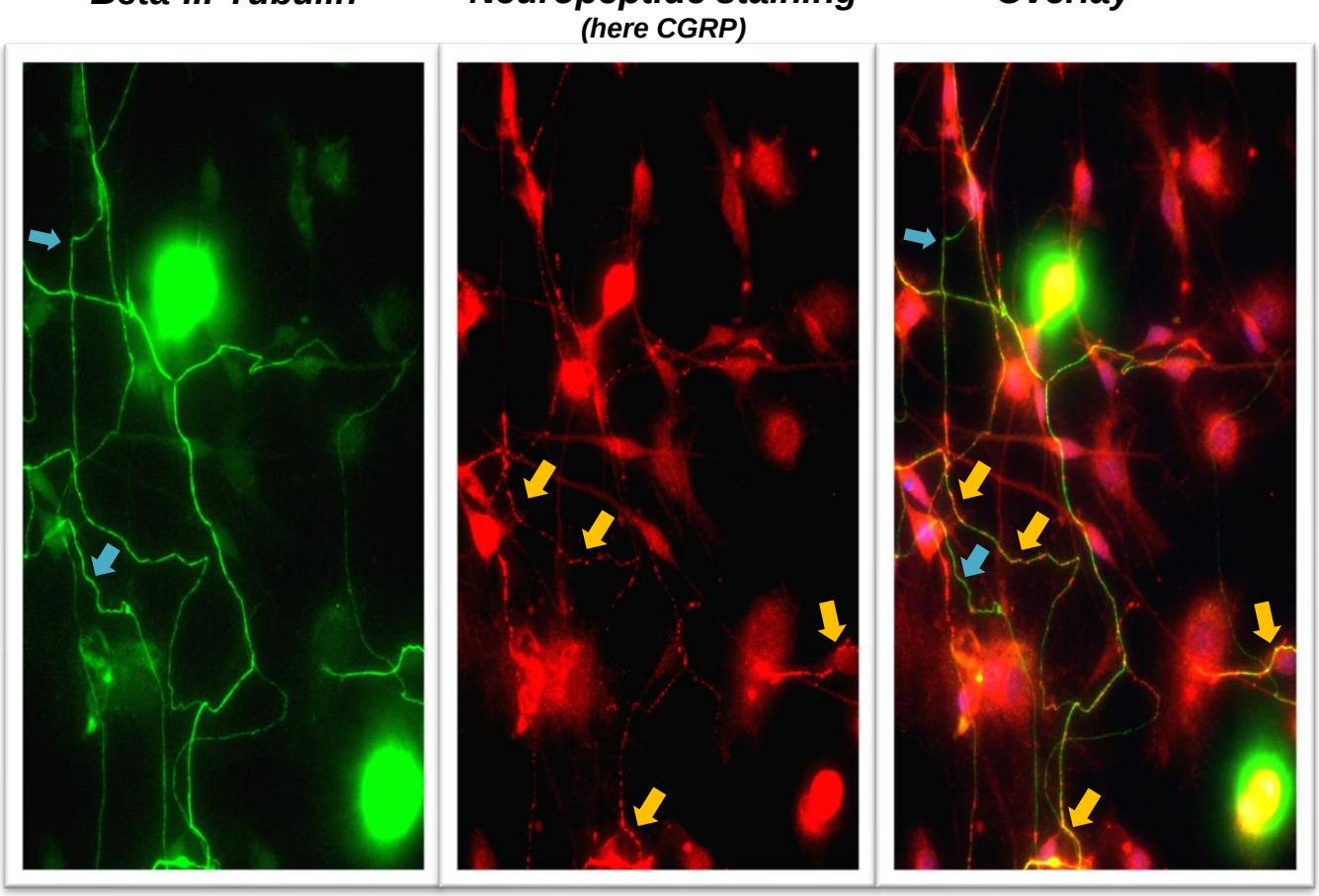
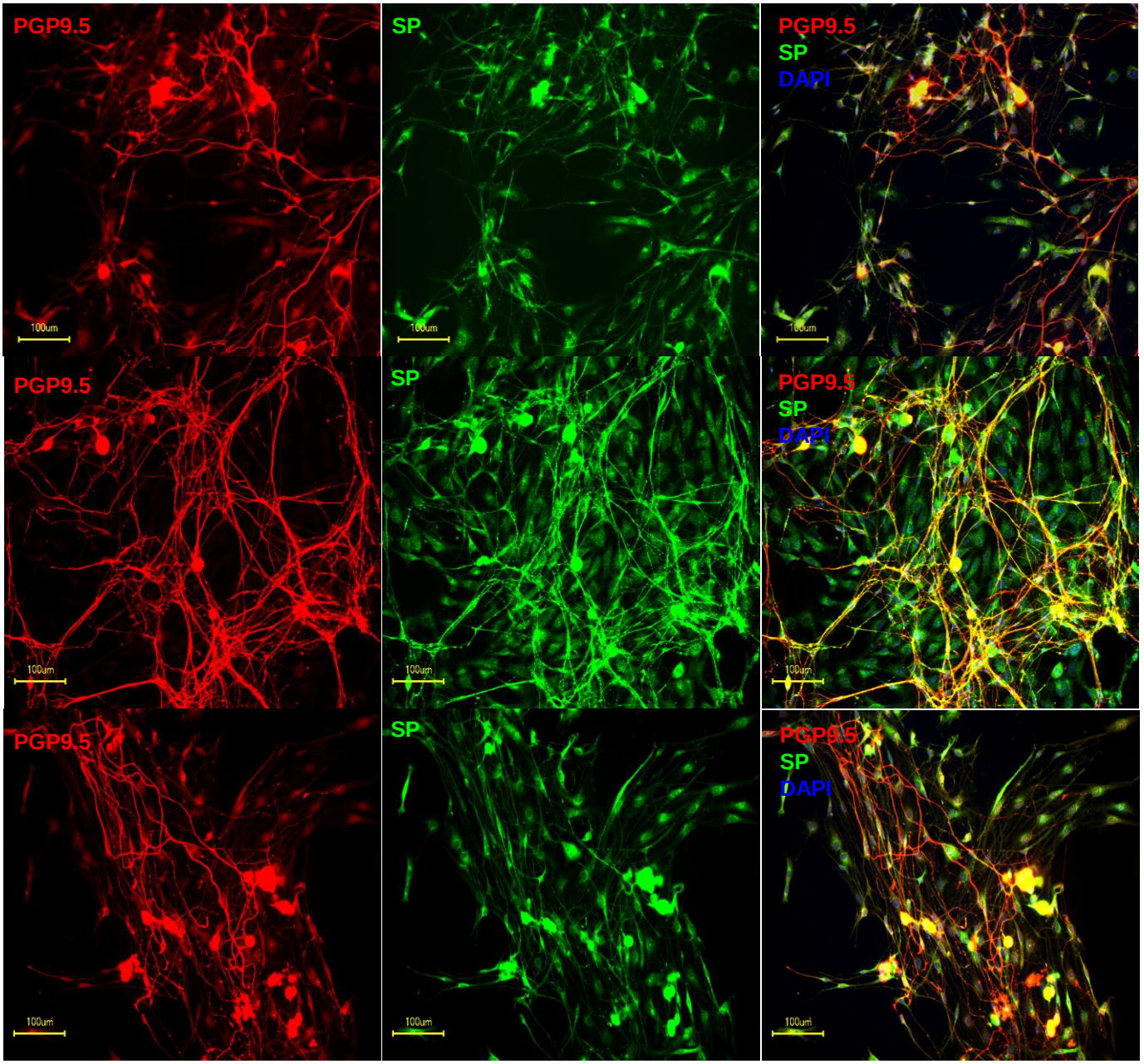
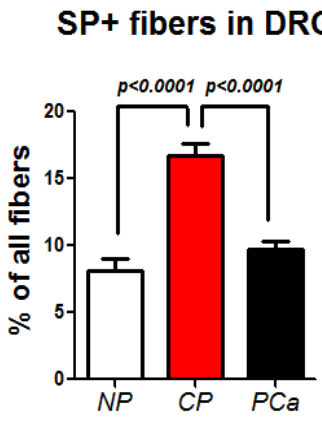
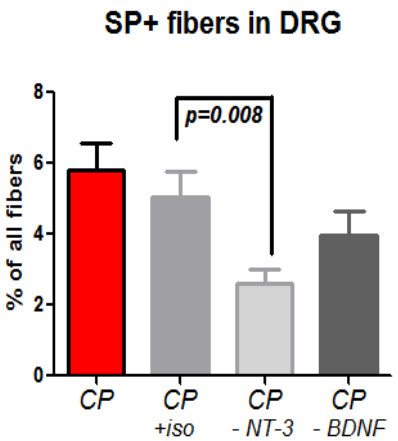
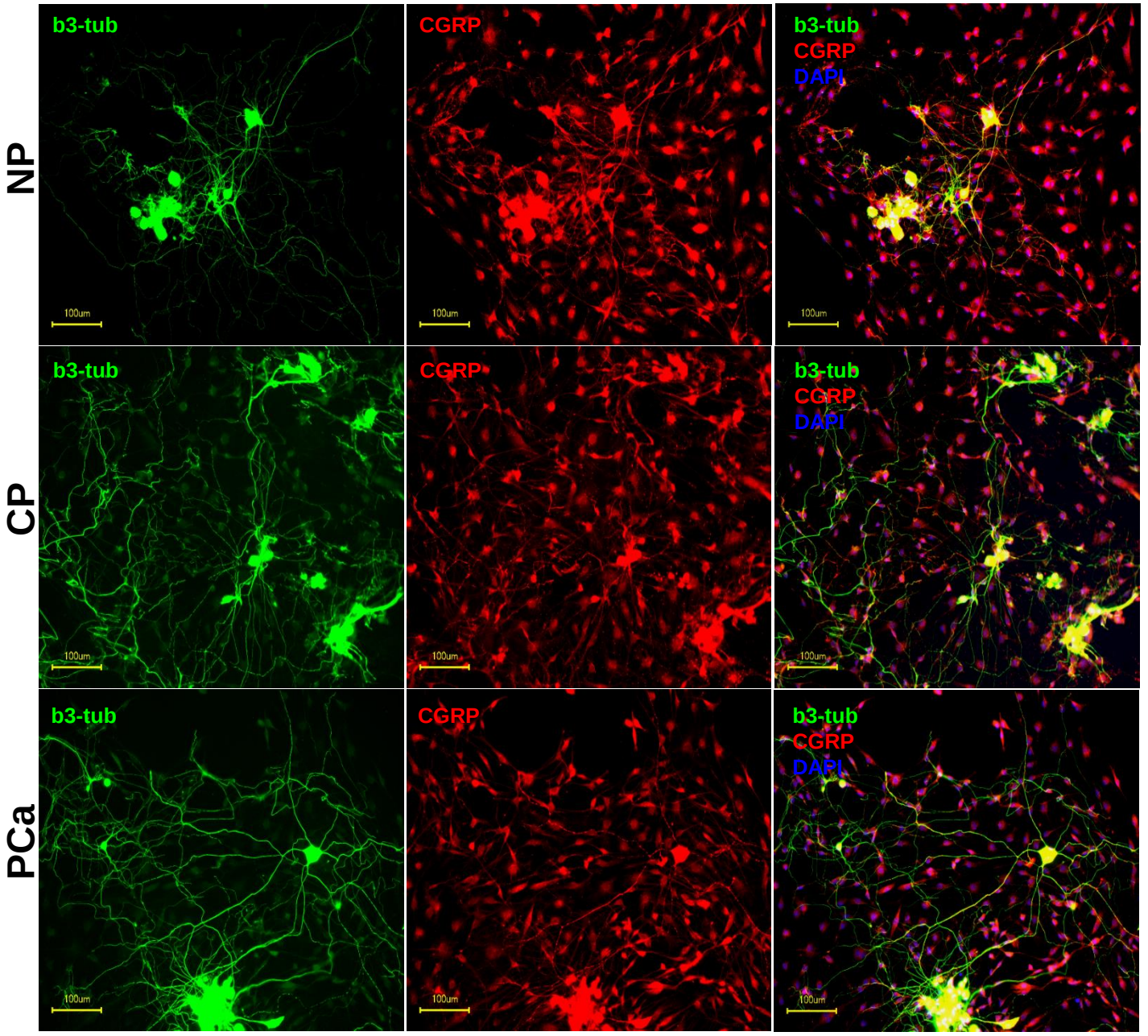

Beta-III-Tubulin
Neuropeptide staining
(here CGRP)
Overlay
Neuropeptide-containing fibers
Neuropeptide-lacking fibers

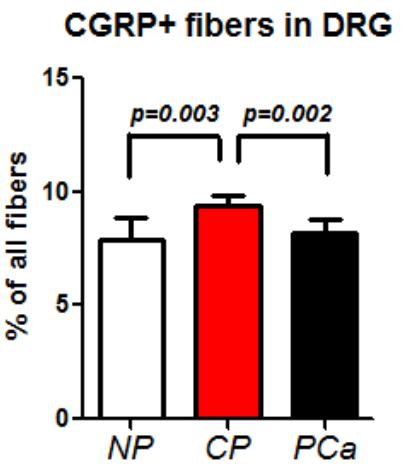

NP
CP
PCa
PGP9.5
SP
PGP9.5
SP
DAPI
100um


SP+ fibers in DRG
p<0.0001
p<0.0001
% of all fibers
20
15
10
5
0
NP
CP
PCa

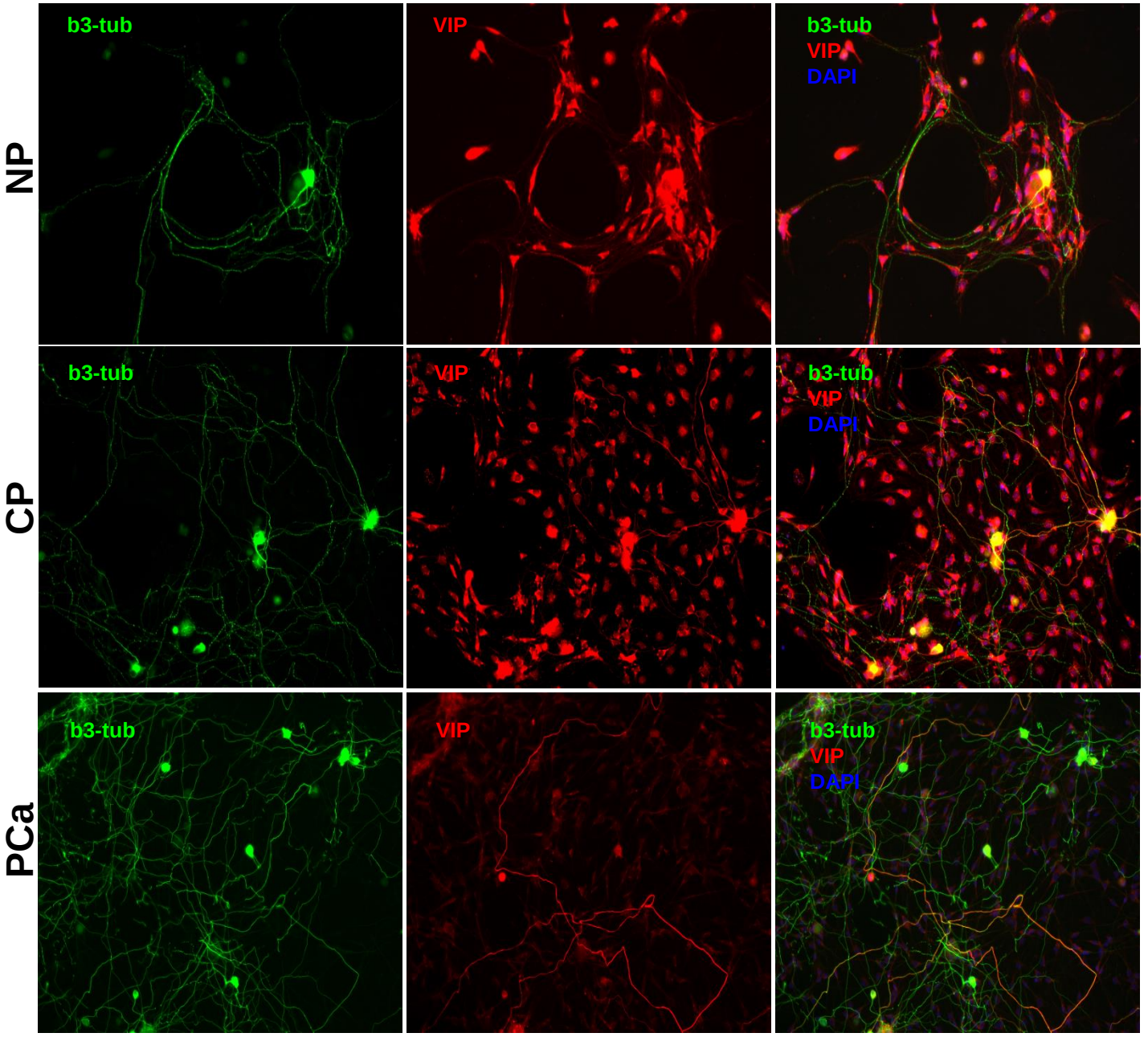

SP+ fibers in DRG
p=0.008
% of all fibers
8
6
4
2
0
CP
CP +iso
CP - NT-3
CP - BDNF

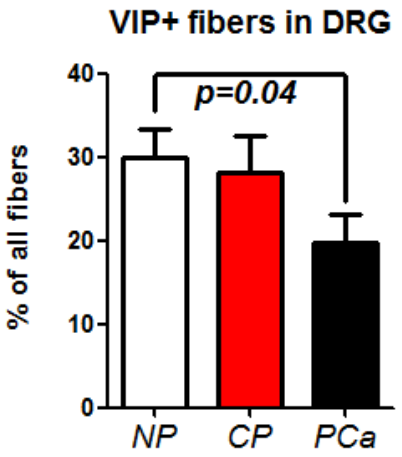
Supplementary fig. 3

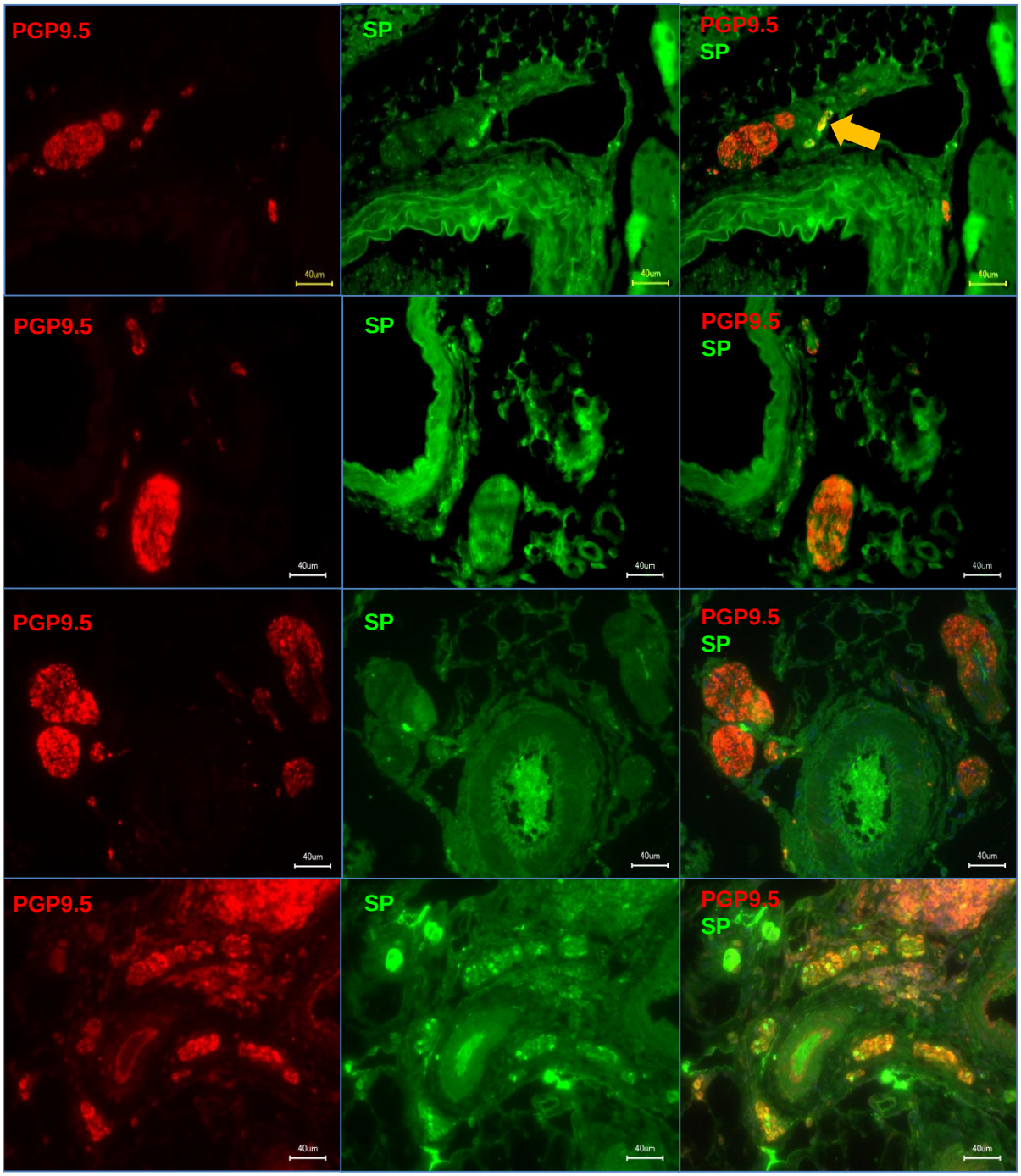


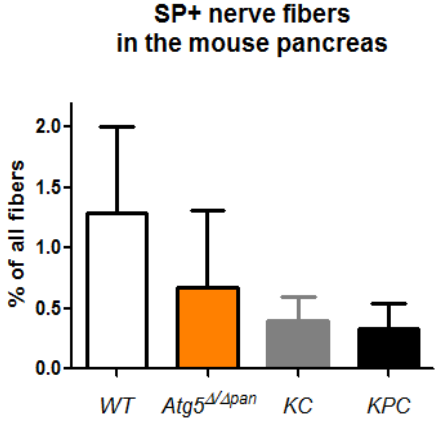


Supplementary fig. 4

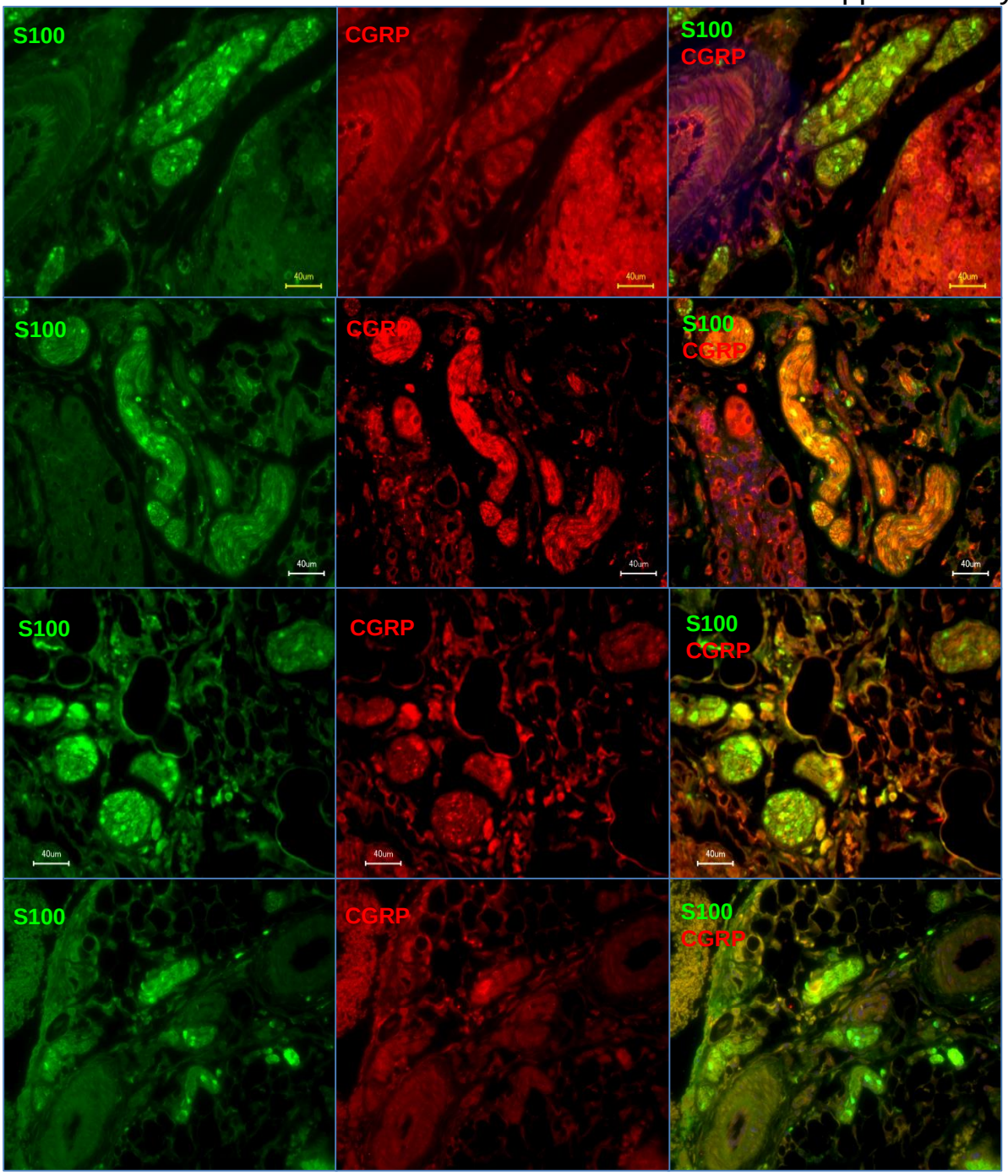


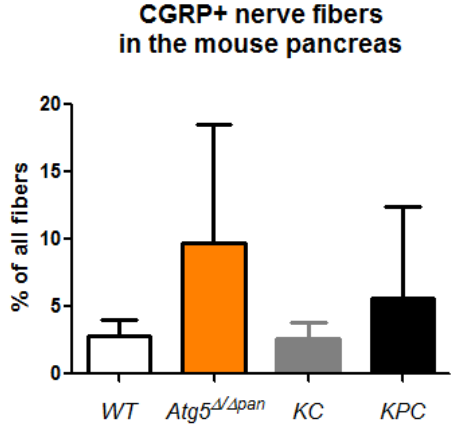


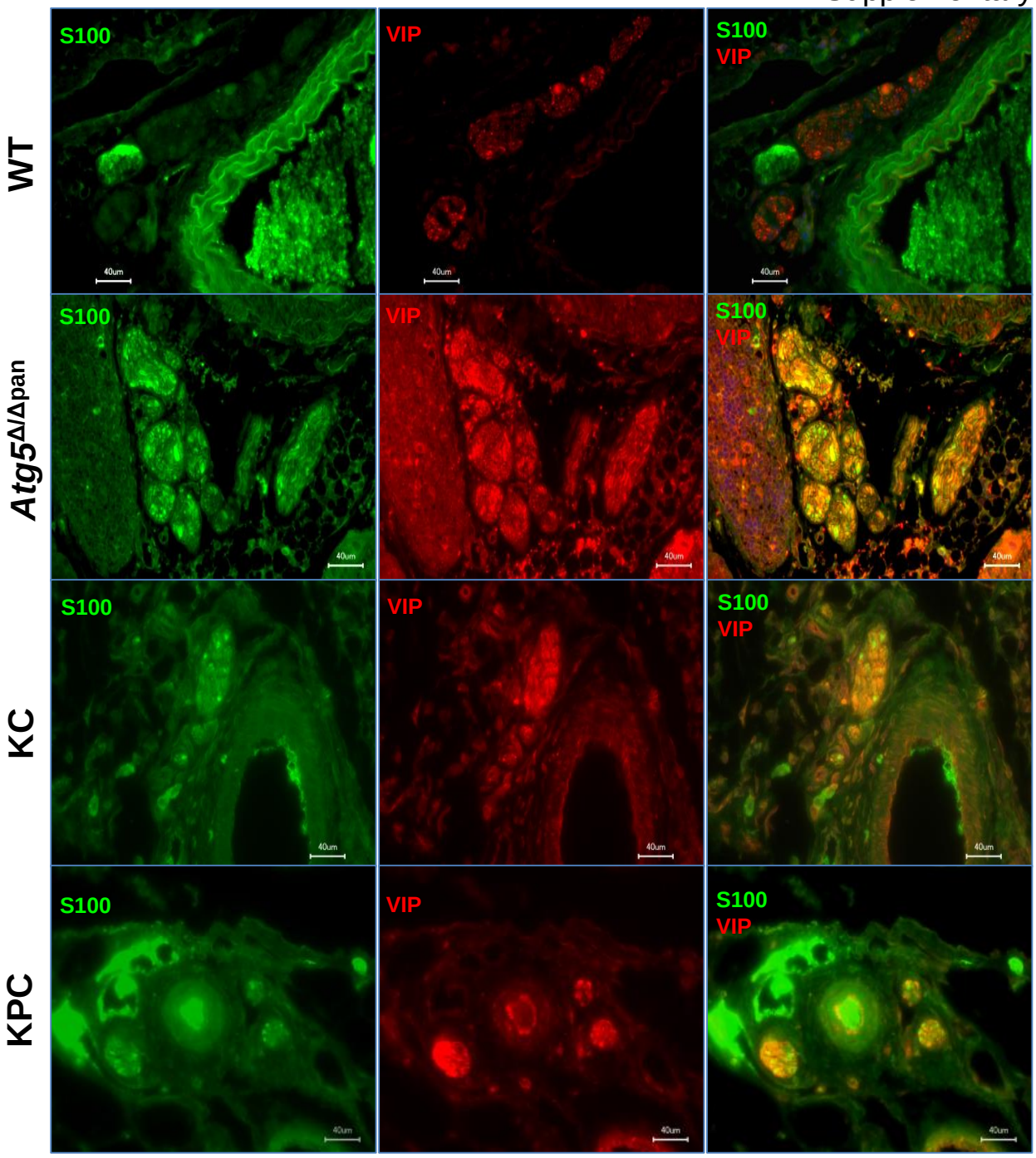

WT
Atg5Δ/Δpan
KC
KPC
PGP9.5
SP
PGP9.5
SP
40um

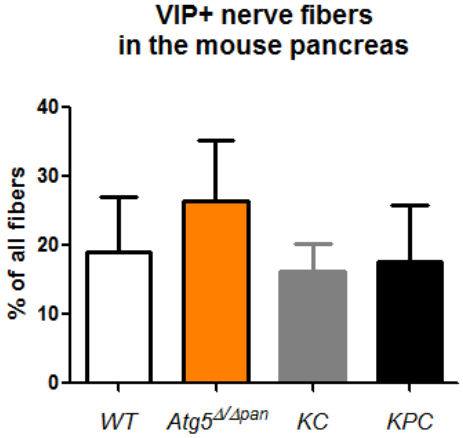

SP+ nerve fibers
in the mouse pancreas
% of all fibers
2.0
1.5
1.0
0.5
0.0
WT
Atg5Δ/Δpan
KC
KPC

Supplementary fig. 6






WT
Atg5Δ/Δpan
KC
KPC
S100
VIP
S100
VIP
40um


VIP+ nerve fibers
in the mouse pancreas
% of all fibers
40
30
20
10
0
WT
Atg5Δ/Δpan
KC
KPC

Supplementary figure legends

Supplementary figure 1. *Ex vivo*-simulation of neural sprouting and selective quantification of neuropeptide-containing neurites in DRG cultures. For mechanistic studies, newborn rat DRG neurons were cultivated in their growth media with tissue extracts generated from the resected specimens of patients with CP, PCa or normal pancreas (NP). Cultures were fixed after 48hrs in 4% paraformaldehyde and double-immunolabeled with a pan-neuronal marker (beta-III-tubulin or PGP9.5) and with antibodies against each one of the four neuropeptides, SP, CGRP, VIP or nNOS. Neurites possessing the neuropeptides of interest (orange arrows) were detected adjacent to neurites that did not contain these neuropeptides (blue arrows). Density of neurites containing the neuropeptides of interest was quantified in relation to the total number of neurites.

Supplementary figure 2. Human CP tissue extracts induce sprouting of substance P/SP-containing neurites from murine dorsal root ganglia/DRG. Dissociated DRG cultures were treated in their medium with tissue extracts derived from resection specimens of patients with CP (n=3), PCa (n=3), or normal pancreas/NP (unallocated organ donors, n=3)). Forty eight hours after the incubation, the cultures were co-immunostained against SP and the pan-neural marker PGP9.5. Here, CP tissue extracts induced an increased sprouting of SP-containing neurites from DRG, similar to the rather higher SP content of nerves in human CP tissues (Figure 1b). Upon addition of neutralizing antibodies against neurotrophin-3 (NT-3), or against brain-derived-neurotrophic factor (BDNF), or of a control isotype-matched antibody ("iso"), the density of SP-containing neurites was diminished only in the presence of NT-3-neutralizing antibodies. Experiments were repeated three times. Unpaired t-test.

Supplementary figure 3. Human CP tissue extracts increase the density of calcitonin-gene-related-peptide/CGRP-containing neurites in murine dorsal root ganglia/DRG cultures. Treatment of newborn rat DRG cultures in their medium with tissue extracts derived

from resection specimens of patients with CP (n=3), PCa (n=3), or normal pancreas/NP (unallocated organ donors, n=3) resulted in the increased sprouting of CGRP-containing neurites from DRG, similar to the rather higher CGRP content of nerves in human CP tissues (Figure 1b). b3-tub: beta-III-tubulin, a pan-neural marker. Experiments were repeated three times. Unpaired t-test.

Supplementary figure 4. Human PCa tissue extracts suppress the density of vasocative intestinal peptide/VIP-containing neurites in murine dorsal root ganglia/DRG cultures. The density of VIPergic neurites in DRG cultures treated with human pancreatic tissue (CP: n=3, PCa: n=3, or normal pancreas/NP: n=3) was selectively measured and correlated to the overall neurite density (labeled via b3-tub: beta-III-tubulin, a pan-neural marker). Here, PCa tissue extracts lowered the density of VIPergic fibers in murine DRG cultures. Experiments were repeated three times. Unpaired t-test.

Supplementary figure 5. Analysis of the SP content of intrapancreatic nerves in genetically induced mouse models of CP and PCa. Murine intrapancreatic nerves were quantified for their neuroimmunoreactivity for SP in genetically induced CP (atrophic CP upon pancreas-specific ablation of the autophagy-related protein *Atg5*, i.e. *Ptf1a-Cre;Atg5*$^{fl/fl}$, termed herein *Atg5*$^{\Delta/\Delta pan}$, 18 week-old), in KC mice (*Ptf1a-Cre;*LSL-*Kras*^{G12D}) that contain the precursor (PanIN) lesions of PCa, and in KPC mice (*Ptf1a-Cre;*LSL-*Kras*^{G12D}*;p53*$^{fl/fl}$) at the age of 4-6 weeks that have overt PCa. In a similar tendency to human PCa (Figure 1b), the genetically induced murine cancer models tended to have lower SP content in intrapancreatic nerves. PGP9.5: pan-neural marker. WT: wildtype mice.

Supplementary figure 6. The *Atg5*$^{\Delta/\Delta pan}$ model of CP tends to contain more CGRP in intrapancreatic nerves. Intrapancreatic nerves of the genetically induced *Atg5*$^{\Delta/\Delta pan}$, KC and KPC mouse models were quantitatively compared for their CGRP content to WT mice. The

Atg5$^{\Delta/\Delta pan}$ and PKC mouse models tended to have more CGRP+ nerve fibers in the pancreas. S100: pan-neural marker. WT: wildtype mice.

Supplementary figure 7. Comparison of VIPergic fibers in the intrapancreatic nerves of genetically induced mouse models of CP and PCa. When the intrapancreatic nerves of the genetically induced *Atg5*$^{\Delta/\Delta pan}$, KC and KPC mouse models were quantitatively compared for their VIP content to WT mice, no differences were detected in the amount of VIP in intrapancreatic nerves, which is similar to the analysis of human VIPergic fiber content (Figure 1b). S100: pan-neural marker. WT: wildtype mice.

Supplementary methods

Antibodies & recombinant proteins

For a complete list of the applied antibodies and recombinant proteins, please refer to Table 1b.

Ex vivo neuroplasticity assay & dorsal root ganglia (DRG) cultures

DRG neurons of newborn (i.e., postnatal day 2-10) Wistar rats were cultivated in neuronal media containing human pancreatic tissue extracts (100µg protein/ml medium) derived from the surgical pancreas specimens of CP and PCa patients [13]. Neurons were seeded at 10,000 cells/well on poly-D-lysine-coated (40mg/m^2, Sigma-Aldrich, Taufkirchen/Germany) 13mm coverslips in 24-well plates (NUNC, Langenselbold/Germany). To assess the impact of neurotrophin-3 (NT-3) or BDNF in pancreatic tissue extracts on DRG neurite density, tissue extracts were treated with specific neutralizing antibodies (Table 1b). Untreated/native DRG neurons cultivated in Neurobasal medium and non-immunized mouse IgG_1 isotype antibody (Sigma-Aldrich)-supplied wells were used as negative control (NC). All experiments were repeated three times and in triplicates.

After 24hrs of cultivation, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline, double-immunostained with a pan-neuronal marker (i.e. β-III Tubulin or PGP9.5) and with antibodies against SP, CGRP, VIP or nNOS. The specific density of SP, CGRP, VIP or nNOS-containing nerve fibers was proportioned to the overall neurite density (labelled by one of the two pan-neuronal markers). The measurements were performed as described previously [13].

For nNOS induction upon tryptase treatment, mast cell tryptase (cat. number T7063, Sigma-Aldrich, Taufkirchen, Germany) was supplied into the growth medium of mouse DRG

cultures at increasing concentrations. The mRNA from the cultures was extracted 24 hours after treatment for the measurement of nNOS expression.

Real-time Light Cycler® Quantitative-Polymerase-Chain-Reaction (QRT-PCR)

Extraction of mRNA from human and murine tissues, cDNA synthesis and QRT-PCR for brain-derived-neurotrophic-factor (*Bdnf*, Gene ID:627) and nNOS (*Nos1*, Gene ID: 18125) were essentially performed as described previously [14]. The primer sequences are indicated in Table 1c.

Mechanical abdominal hyperalgesia (von Frey filaments)

Mechanical hypersensitivity was tested by applying von Frey filaments (Stoelting Co., IL, USA) of increasing tactile stimulus intensity 10 consecutive times from the bottom of a grid on the abdomen of the mice. The response was scored as 0 = no response, 1= mild response (licking of abdomen, slow abdominal withdrawal), and 2 = heavy response (jumping, kicking out). The sum of response scores from 10 applications of each filament at 10-second intervals represented the von Frey score, as described previously [40].

Statistics

Results are expressed as mean ± standard deviation (SD). Two-group analyses were performed using the unpaired t-test. More than two-group analyses were conducted using the one-way analysis of variance (ANOVA) test followed by Bonferroni's multiple comparison test. All tests were two-sided, and a p value of < 0.05 was considered to indicate statistical significance.

Study approval

The study was approved by the ethics committee of the Technical University of Munich, Germany (Approval-Nr.: 550/16s). All animal experiments were carried out in accordance with the regulations of the Government of Upper Bavaria (Approval Nrs. 55.2-1-54-2532-223-2015 and 55.2-1-54-2532-20-2015).