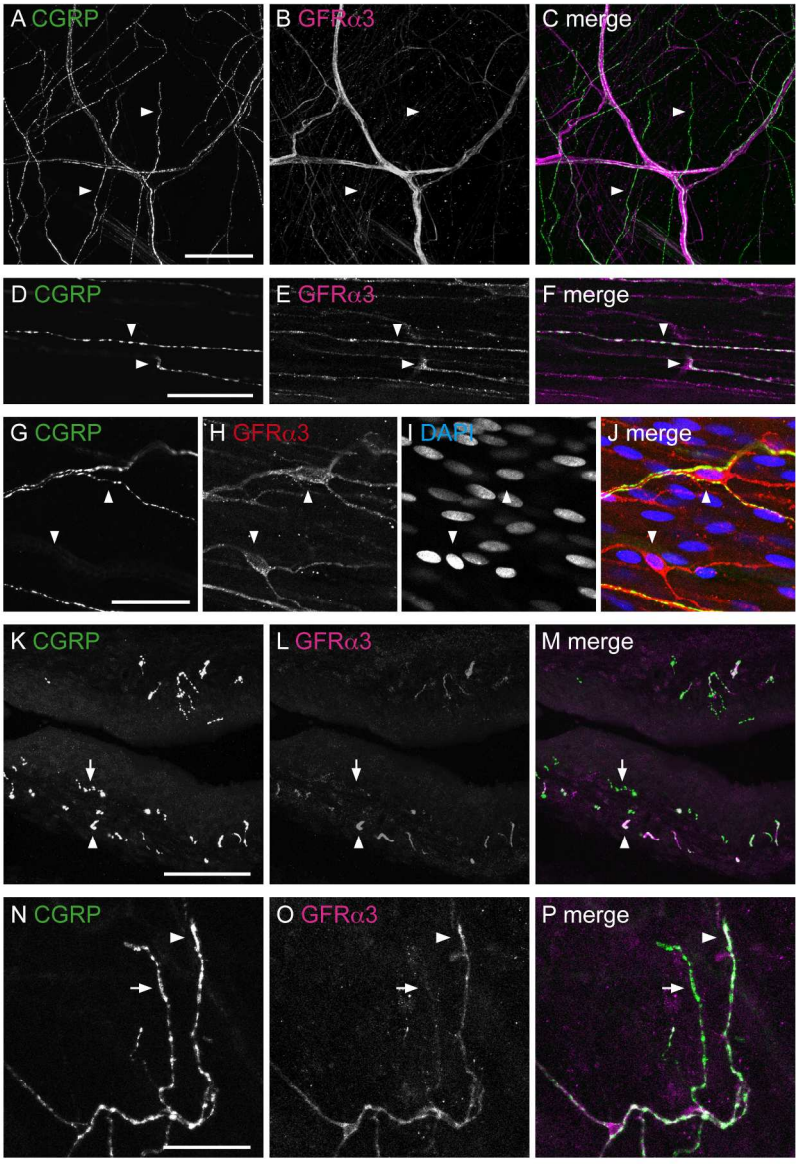
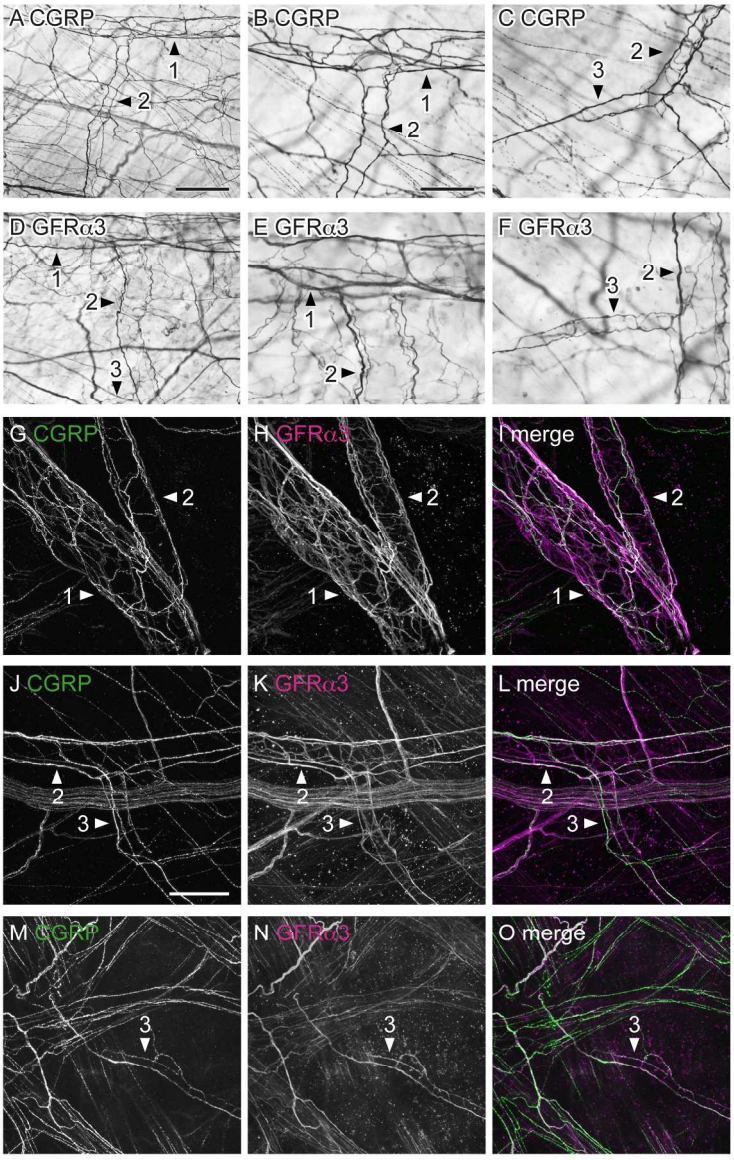


Supplementary figure 3

Supplementary Figure 3. Co-expression of CGRP- and GFRα3-immunoreactivity (IR) in the detrusor and suburothelial plexus. All panels except K-M (cryosection) are from whole mount preparations. A-C: Confocal z-stack (34 µm thick, 1 µm step size) through the detrusor showed that many CGRP-IR axon bundles contained GFRα3-IR axons, but some single axons labeled for CGRP-IR were GFRα3-negative (arrowheads). D-F: Confocal image in the detrusor illustrating partial co-expression (arrowheads) between CGRP- and GFRα3-IR in axons. G-J: Confocal image in the detrusor showing two small, elongated GFRα3-IR cells (arrowheads) with their processes. These appeared to be innervated by CGRP axons. DAPI (blue) labels nuclei of GFRα3-IR cells and surrounding muscle cells. K-M: Confocal z-stack (15 µm thick, 0.5 µm step size) of a cryosection showing the majority of GFRα3-IR axons in the suburothelial plexus were immunoreactive for CGRP but relatively few CGRP-IR axons were immunoreactive for GFRα3. N-P: Confocal images in the suburothelial plexus of a whole mount demonstrating that most GFRα3-IR terminals were immunoreactive for CGRP, but some CGRP-IR terminals were not immunoreactive for GFRα3. In K-P, arrows indicate single-labeled CGRP-IR axons (K-M) and terminals (N-P), whereas arrowheads indicate a double-

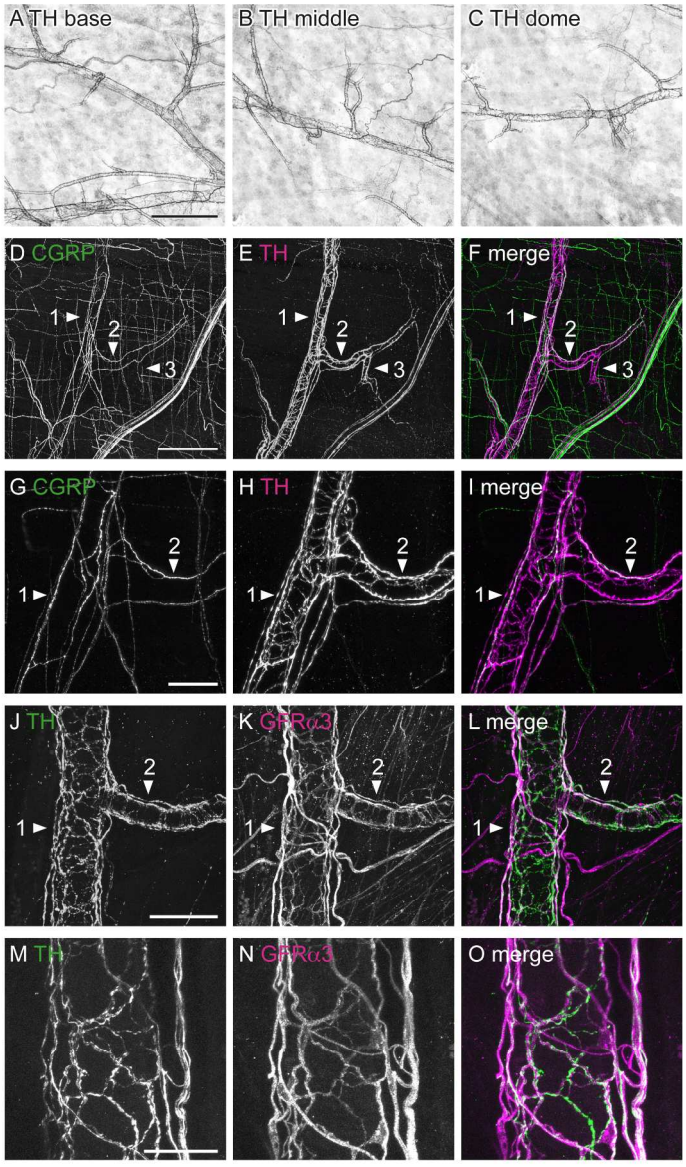
labeled GFRα3- and CGRP-IR axon (k-m), and terminal (n-p). Scale bar = 100 μm in (A) also applies to (B, C); 30 μm in (D) also applies to (E, F); 20 μm in (G) also applies to (H-J). Scale bar = 100 μm in (K) also applies to (L-M); 30 μm in (N) also applies to (O, P). CGRP, calcitonin gene-related peptide; GFRα3, glial cell line-derived neurotrophic factor family receptor α3.
171x253mm (300 x 300 DPI)



Supplementary figure 5

Supplementary Figure 5. CGRP- and GFRα3-immunoreactive (IR) axons associated with the bladder vasculature as viewed in whole mounts. Panels A-F show diaminobenzidine (DAB)-processed tissue viewed with conventional microscopy whereas G-O are confocal micrographs of immunofluorescence. Numbered arrowheads indicate primary, secondary and tertiary branches of the vascular tree. A-C: Many CGRP-IR para-vascular axons were found on the primary, secondary (A, B) and tertiary (C) branches of the vascular tree; peri-vascular axons were also found on the primary branch but were sparse on subsequent branches. D-F: Para- and peri-vascular GFRα3-IR axons were associated with all branches of the vascular tree. G-I: Many para-vascular CGRP- and GFRα3-IR axons were associated with the primary and secondary branches of the vascular tree; GFRα3-IR peri-vascular axons were more prevalent than CGRP-IR peri-vascular axons on these branches. J-I: CGRP- and GFRα3-IR para-vascular axons were associated with secondary and tertiary vessel branches; many GFRα3-IR peri-vascular axons were present in these branches. m-o: Neither CGRP- nor GFRα3-IR para-vascular axons extended to the most distal aspects of the vascular tree. Images (G-O) are confocal z-stacks (thickness: G-I, 45 μm; J-L, 28 μm; M-O, 30 μm; all with 1 μm step size). Scale

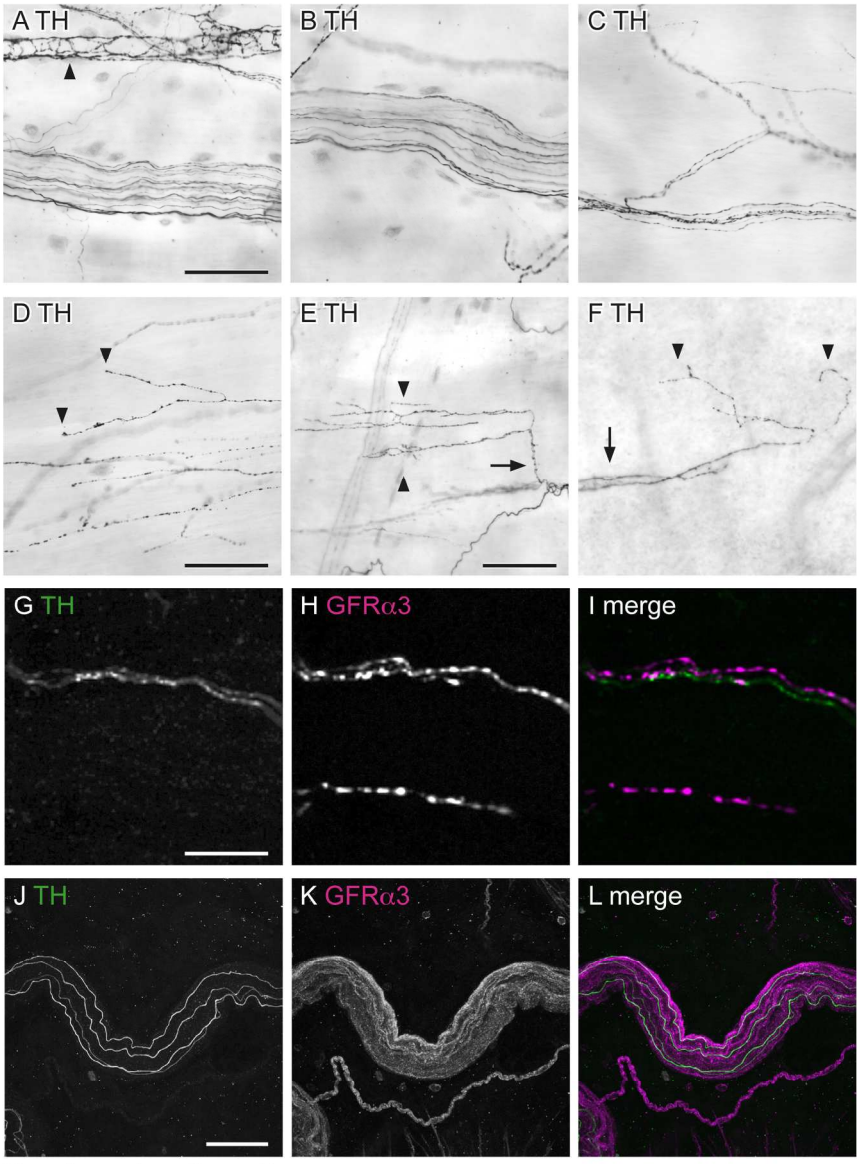
bar = 200 μm in (A) also applies to (D); 100 μm in (B) also applies to (C, E, F); 100 μm in (g) also applies to (H, I); 100 μm (J) also applies (K, L); 100 μm in (M) also applies to (N, O). CGRP, calcitonin gene-related peptide; GFRα3, glial cell line-derived neurotrophic factor family receptor α3.
164x262mm (300 x 300 DPI)



Supplementary figure 6

Supplementary Figure 6. Comparison of different axon populations supplying blood vessels in bladder whole mounts. Panels A-C show diaminobenzidine (DAB)-processed tissue viewed with conventional microscopy whereas D-O are confocal micrographs of immunofluorescence. Numbered arrowheads indicate primary, secondary and tertiary branches of the vascular tree. A-C: TH-immunoreactive (IR) axons densely innervated all branches of the vascular tree that traversed the bladder (A: base; B: middle; C: dome). D-F: Many TH-IR axons and relatively few CGRP-IR axons were associated with distal portions of primary vessel branches. G-I: Higher magnification of the primary to secondary branch point in d-f that demonstrates relatively few CGRP-IR peri-vascular axons. J-L: Numerous TH- and GFRα3-IR para- and peri-vascular axons densely innervated the primary and secondary branches of the vascular tree. M-O: Many TH-IR para- and peri-vascular axons were immunoreactive for GFRα3. Panels (D-O) are confocal z-stacks (thickness: D-F, 50 µm; g-l, 30-32 µm; all with 1 µm step size). Scale bar = 500 µm in (A) also applies to (B, C); 200 µm in (D) also applies to (E, F); 50 µm in (G) also applies to (H-L); 40 µm in (M) also applies to (N-O). CGRP, calcitonin gene-related peptide; GFRα3, glial cell line-derived neurotrophic factor family receptor α3; TH,

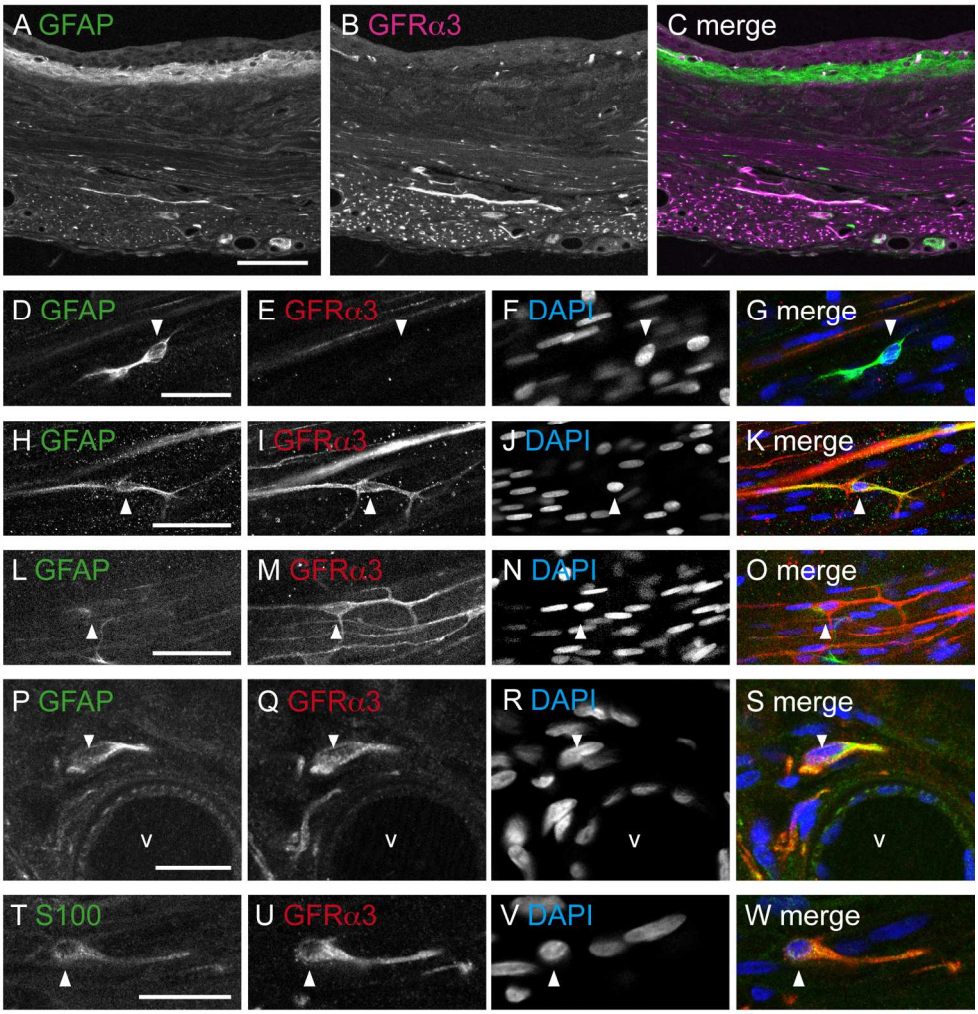
tyrosine hydroxylase.
156x268mm (300 x 300 DPI)



Supplementary figure 7

Supplementary Figure 7. Non-vascular noradrenergic innervation of the detrusor, as viewed in whole mount preparations. Panels A-F show diaminobenzidine (DAB)-processed tissue viewed with conventional microscopy whereas G-L are confocal micrographs of immunofluorescence. A-C: TH-immunoreactive (IR) axon bundles of varying thickness and axons associated with a blood vessel (arrowhead, A). D-F: Three types of TH-IR axon terminals (arrowheads) were found in the detrusor: non-specialised simple endings (D), specialised multi-branched endings (E), and those that branched off the vascular tree, originating from para-vascular axons (F). Arrows indicate a branch point from a single specialised TH-IR axon (E) and TH-IR para-vascular axons on a distal portion of the vascular tree (F). G-I: TH- and GFRα3-IR were rarely co-expressed in axons in the detrusor. J-L: Few TH-IR axons travelled within the large diameter bundles of GFRα3-IR axons; these TH-IR axons did not co-express GFRα3-IR. Images (G-O) are confocal z-stacks (thickness: G-I, 10 µm; J-L, 20 µm; all with 1 µm step size). Scale bar = 100 µm in (A) also applies to (B, C); 100 µm in (D) also applies to (E, F); 10 µm in (G) also applies to (G, I); 50 µm in (J) also applies to (J, K, L). GFRα3, glial cell line-derived neurotrophic factor family receptor α3; TH, tyrosine hydroxylase.

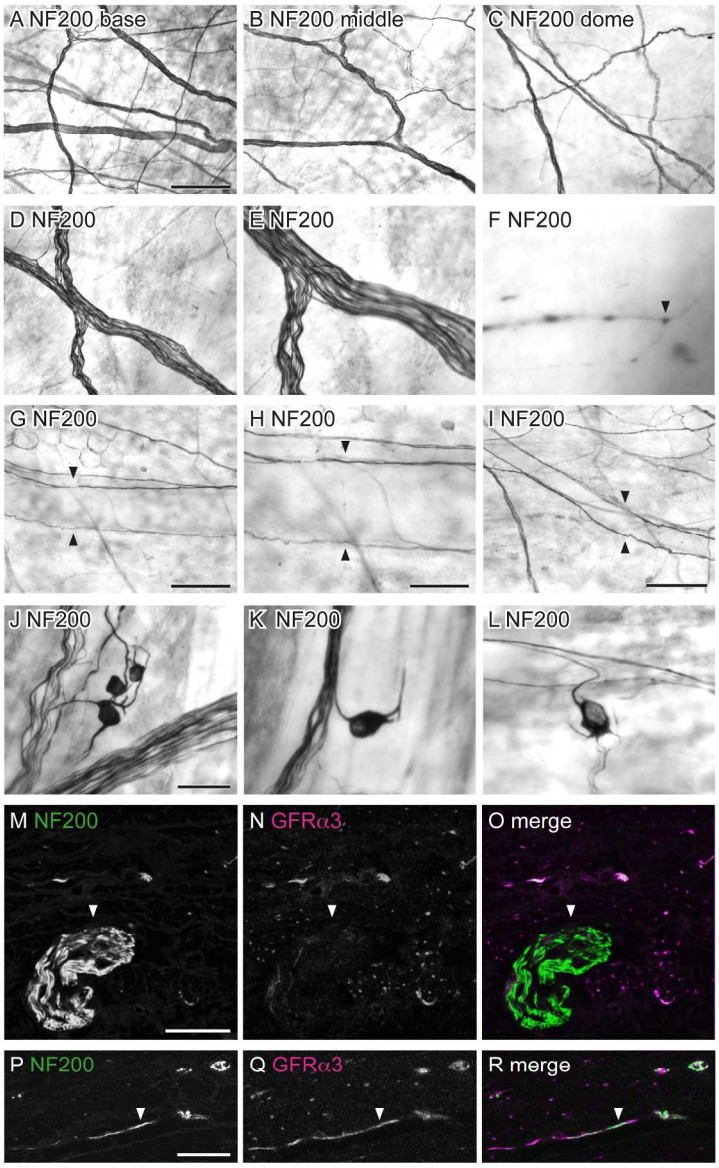
167x228mm (300 x 300 DPI)



Supplementary figure 9

Supplementary Figure 9. Comparison of glial markers (GFAP- and S100-) with GFRα3-immunoreactive (IR) distribution in the bladder. Panels show confocal images of immunofluorescence in bladder sections (A-C, P-S) and wholemount preparations (D-O, T-W). DAPI labelling of nuclei was performed to indicate general cellular distribution within each tissue and to show that none of the other markers were found within the nucleus. A-C: Comparison of GFAP- and GFRα3-IR distribution in the bladder at low magnification. D-G: Many, but not all GFAP-IR cells in the detrusor were immunoreactive for GFRα3. H-K: Example of a double labelled GFAP- and GFRα3-IR glial cell in the detrusor. L-O: The majority of GFRα3-IR cells with elongated processes were not immunoreactive for GFAP. P-S: Many GFRα3-IR cells associated with the vasculature were immunoreactive for GFAP. T-W: Many S100-IR cells in the detrusor were immunoreactive for GFRα3. Arrowheads indicate single-labeled GFAP-IR cell (D-G), single-labeled GFRα3-IR cell (L-O), and double-labeled cells (H-K, P-S, T-W). The lumen of the blood vessel in panels P-S is indicated (V). Scale bar = 200 µm in (A) also applies (B, C); 20 µm in (D, H, L, T) also applies to (E-G, I-K, M-O, U-W); 10 µm in (P) also applies to (Q-S). GFAP, glial fibrillary acidic protein; GFRα3, glial cell line-derived neurotrophic factor family receptor α3.

173x182mm (300 x 300 DPI)



Supplementary figure 10

Supplementary Figure 10. Distribution of NF200-immunoreactive (IR) axons in the bladder. Panels A-L show DAB-processed whole mounts viewed with conventional microscopy whereas M-R are confocal micrographs of immunofluorescence in cryosections. A-C: Many NF200-IR axons were found in the detrusor and were distributed evenly throughout the bladder (a: base; b: middle; c: dome). D-E: The majority of NF200-IR axons travelled in large diameter axon bundles. F: NF200-IR terminals were occasionally observed in the detrusor (arrowhead). G-I: Few NF200-IR axons were associated with the vasculature; these were para-vascular (arrowheads) and only observed on primary (G, H) and secondary (I) vascular branches. J-L: NF200-IR neurons were observed in the detrusor; many had short processes and were found individually (K, L) or in small clusters (J). M-O: Large diameter NF200-IR axon bundles (arrowheads) on the serosal surface were not immunoreactive for GFRα3. P-R: Some NF200-IR axons in the detrusor were immunoreactive for GFRα3 (arrowheads); it was difficult to determine if these comprised single or multiple axons. The serosal surface of the bladder is orientated to the bottom in panels m-r. Scale bar = 200 μm in (A) also applies to (B, C); 100 μm in (D) also applies to (E); 20 μm in (F); 200 μm in (G); 100 μm in (G); 200 μm in (I), 50

μm in (J) also applies to (K, L); 20 μm in (M) also applies to (N, O); 10 μm in (P) also applies (Q, R). GFRα3, glial cell line-derived neurotrophic factor family receptor α3; NF200, neurofilament, 200 kDa.
168x274mm (300 x 300 DPI)

Supplementary Figure Legends

Supplementary Figure 3. Co-expression of CGRP- and GFRα3-immunoreactivity (IR) in the detrusor and suburothelial plexus. All panels except K-M (cryosection) are from whole mount preparations. **A-C:** Confocal z-stack (34 μm thick, 1 μm step size) through the detrusor showed that many CGRP-IR axon bundles contained GFRα3-IR axons, but some single axons labeled for CGRP-IR were GFRα3-negative (arrowheads). **D-F:** Confocal image in the detrusor illustrating partial co-expression (arrowheads) between CGRP- and GFRα3-IR in axons. **G-J:** Confocal image in the detrusor showing two small, elongated GFRα3-IR cells (arrowheads) with their processes. These appeared to be innervated by CGRP axons. DAPI (blue) labels nuclei of GFRα3-IR cells and surrounding muscle cells. **K-M:** Confocal z-stack (15 μm thick, 0.5 μm step size) of a cryosection showing the majority of GFRα3-IR axons in the suburothelial plexus were immunoreactive for CGRP but relatively few CGRP-IR axons were immunoreactive for GFRα3. **N-P:** Confocal images in the suburothelial plexus of a whole mount demonstrating that most GFRα3-IR terminals were immunoreactive for CGRP, but some CGRP-IR terminals were not immunoreactive for GFRα3. In K-P, arrows indicate single-labeled CGRP-IR axons (K-M) and terminals (N-P), whereas arrowheads indicate a double-labeled GFRα3- and CGRP-IR axon (k-m), and terminal (n-p). Scale bar = 100 μm in (A) also applies to (B, C); 30 μm in (D) also applies to (E, F); 20 μm in (G) also applies to (H-J). Scale bar = 100 μm in (K) also applies to (L-M); 30 μm in (N) also applies to (O, P). CGRP, calcitonin gene-related peptide; GFRα3, glial cell line-derived neurotrophic factor family receptor α3.

Supplementary Figure 5. CGRP- and GFRα3-immunoreactive (IR) axons associated with the bladder vasculature as viewed in whole mounts. Panels **A-F** show diaminobenzidine (DAB)-processed tissue viewed with conventional microscopy whereas **G-O** are confocal micrographs of immunofluorescence. Numbered arrowheads indicate primary, secondary and tertiary branches of the vascular tree. **A-C:** Many CGRP-IR para-vascular axons were found on the primary, secondary (A, B) and tertiary (C) branches of the vascular tree; peri-vascular axons were also found on the primary branch but were sparse on subsequent branches. **D-F:** Para- and peri-vascular GFRα3-IR axons were associated with all branches of the vascular tree. **G-I:** Many para-vascular CGRP- and GFRα3-IR axons were associated with the primary and secondary branches of the vascular tree; GFRα3-IR peri-vascular axons were more

prevalent than CGRP-IR peri-vascular axons on these branches. **J-I:** CGRP- and GFRα3-IR para-vascular axons were associated with secondary and tertiary vessel branches; many GFRα3-IR peri-vascular axons were present in these branches. **m-o:** Neither CGRP- nor GFRα3-IR para-vascular axons extended to the most distal aspects of the vascular tree. Images (G-O) are confocal z-stacks (thickness: G-I, 45 μm; J-L, 28 μm; M-O, 30 μm; all with 1 μm step size). Scale bar = 200 μm in (A) also applies to (D); 100 μm in (B) also applies to (C, E, F); 100 μm in (g) also applies to (H, I); 100 μm (J) also applies (K, L); 100 μm in (M) also applies to (N, O). CGRP, calcitonin gene-related peptide; GFRα3, glial cell line-derived neurotrophic factor family receptor α3.

Supplementary Figure 6. Comparison of different axon populations supplying blood vessels in bladder whole mounts. Panels **A-C** show diaminobenzidine (DAB)-processed tissue viewed with conventional microscopy whereas **D-O** are confocal micrographs of immunofluorescence. Numbered arrowheads indicate primary, secondary and tertiary branches of the vascular tree. **A-C:** TH-immunoreactive (IR) axons densely innervated all branches of the vascular tree that traversed the bladder (A: base; B: middle; C: dome). **D-F:** Many TH-IR axons and relatively few CGRP-IR axons were associated with distal portions of primary vessel branches. **G-I:** Higher magnification of the primary to secondary branch point in d-f that demonstrates relatively few CGRP-IR peri-vascular axons. **J-L**: Numerous TH- and GFRα3-IR para- and peri-vascular axons densely innervated the primary and secondary branches of the vascular tree. **M-O:** Many TH-IR para- and peri-vascular axons were immunoreactive for GFRα3. Panels (D-O) are confocal z-stacks (thickness: D-F, 50 μm; g-l, 30-32 μm; all with 1 μm step size). Scale bar = 500 μm in (A) also applies to (B, C); 200 μm in (D) also applies to (E, F); 50 μm in (G) also applies to (H-L); 40 μm in (M) also applies to (N-O). CGRP, calcitonin gene-related peptide; GFRα3, glial cell line-derived neurotrophic factor family receptor α3; TH, tyrosine hydroxylase.

Supplementary Figure 7. Non-vascular noradrenergic innervation of the detrusor, as viewed in whole mount preparations. Panels **A-F** show diaminobenzidine (DAB)-processed tissue viewed with conventional microscopy whereas **G-L** are confocal micrographs of immunofluorescence. **A-C:** TH-immunoreactive (IR) axon bundles of varying thickness and axons associated with a blood vessel (arrowhead, A). **D-F:** Three types of TH-IR axon

terminals (arrowheads) were found in the detrusor: non-specialised simple endings (D), specialised multi-branched endings (E), and those that branched off the vascular tree, originating from para-vascular axons (F). Arrows indicate a branch point from a single specialised TH-IR axon (E) and TH-IR para-vascular axons on a distal portion of the vascular tree (F). **G-I:** TH- and GFRα3-IR were rarely co-expressed in axons in the detrusor. **J-L:** Few TH-IR axons travelled within the large diameter bundles of GFRα3-IR axons; these TH-IR axons did not co-express GFRα3-IR. Images (G-O) are confocal z-stacks (thickness: G-I, 10 μm; J-L, 20 μm; all with 1 μm step size). Scale bar = 100 μm in (A) also applies to (B, C); 100 μm in (D) also applies to (E, F); 10 μm in (G) also applies to (G, I); 50 μm in (J) also applies to (J, K, L). GFRα3, glial cell line-derived neurotrophic factor family receptor α3; TH, tyrosine hydroxylase.

Supplementary Figure 9. Comparison of glial markers (GFAP- and S100-) with GFRα3-immunoreactive (IR) distribution in the bladder. Panels show confocal images of immunofluorescence in bladder sections (A-C, P-S) and wholemount preparations (D-O, T-W). DAPI labelling of nuclei was performed to indicate general cellular distribution within each tissue and to show that none of the other markers were found within the nucleus. **A-C:** Comparison of GFAP- and GFRα3-IR distribution in the bladder at low magnification. **D-G:** Many, but not all GFAP-IR cells in the detrusor were immunoreactive for GFRα3. **H-K:** Example of a double labelled GFAP- and GFRα3-IR glial cell in the detrusor. **L-O:** The majority of GFRα3-IR cells with elongated processes were not immunoreactive for GFAP. **P-S:** Many GFRα3-IR cells associated with the vasculature were immunoreactive for GFAP. **T-W:** Many S100-IR cells in the detrusor were immunoreactive for GFRα3. Arrowheads indicate single-labeled GFAP-IR cell (D-G), single-labeled GFRα3-IR cell (L-O), and double-labeled cells (H-K, P-S, T-W). The lumen of the blood vessel in panels P-S is indicated (V). Scale bar = 200 μm in (A) also applies (B, C); 20 μm in (D, H, L, T) also applies to (E-G, I-K, M-O, U-W); 10 μm in (P) also applies to (Q-S). GFAP, glial fibrillary acidic protein; GFRα3, glial cell line-derived neurotrophic factor family receptor α3.

Supplementary Figure 10. Distribution of NF200-immunoreactive (IR) axons in the bladder. Panels **A-L** show DAB-processed whole mounts viewed with conventional microscopy whereas **M-R** are confocal micrographs of immunofluorescence in cryosections. **A-C:** Many

NF200-IR axons were found in the detrusor and were distributed evenly throughout the bladder (a: base; b: middle; c: dome). **D-E:** The majority of NF200-IR axons travelled in large diameter axon bundles. **F:** NF200-IR terminals were occasionally observed in the detrusor (arrowhead). **G-I:** Few NF200-IR axons were associated with the vasculature; these were para-vascular (arrowheads) and only observed on primary (G, H) and secondary (I) vascular branches. **J-L:** NF200-IR neurons were observed in the detrusor; many had short processes and were found individually (K, L) or in small clusters (J). **M-O:** Large diameter NF200-IR axon bundles (arrowheads) on the serosal surface were not immunoreactive for GFRα3. **P-R:** Some NF200-IR axons in the detrusor were immunoreactive for GFRα3 (arrowheads); it was difficult to determine if these comprised single or multiple axons. The serosal surface of the bladder is orientated to the bottom in panels m-r. Scale bar = 200 μm in (A) also applies to (B, C); 100 μm in (D) also applies to (E); 20 μm in (F); 200 μm in (G); 100 μm in (G); 200 μm in (I), 50 μm in (J) also applies to (K, L); 20 μm in (M) also applies to (N, O); 10 μm in (P) also applies (Q, R). GFRα3, glial cell line-derived neurotrophic factor family receptor α3; NF200, neurofilament, 200 kDa.