

Supplemental Figure 1

Supplementary Figure 1. Changes in NGF expression pattern in the bladder after SCI in mice. (A) NGF immunoreactivity is detected in the urothelium (U) and in the detrusor muscle (M) of the bladder from naïve mice without SCI. At 7hr post-injury, there is a significant drop in NGF staining in the bladder. NGF is again detected beginning 1 to 5 days post-injury. L; lumen of the bladder. Scale bar, 150 μ m. (B) Quantification of NGF immunoreactivity using Image J (n=2-5 mice). The significance was calculated by repeated one-way ANOVA (p \leq 0.01), with pairwise comparisons were made by Tukey's multiple comparisons test. (C) Western blotting of the urothelium demonstrates a reduction in proNGF levels at 1 hr post-injury. Also shown are mature NGF blot. Uroplakin (UP) and α -actin are markers for the urothelium and detrusor muscle, respectively. (D) Western blotting of the detrusor muscle illustrates an increase in proNGF levels at 3-5 days post-injury.



Supplementary Figure 2. Various cell types express p75 during the period of urothelial regeneration after SCI in mice. (A) p75 is expressed both in the urothelium and detrusor muscle based on Western blotting. Uroplakin (UP) and α -actin are used as markers for expression in the urothelium and the detrusor muscle, respectively. Note that the control blots for uroplakin (UP) and α -actin are the same as those shown in Supplemental Figure 1. (B, C) Immunohistochemical analyses of p75 expression in the

urothelium (U) and the detrusor muscle (M) after SCI. L, lumen. P75 was expressed in UP^+ umbrella cells without injury (Ba, f, Ca). Note that the bright staining identified by the arrows is from nerve fibers and not from urothelial cells (Bf). At 1 day post-injury, a majority of umbrella cells that line the lumen surface is lost due to apoptosis. At 3 days post-injury when hyperplasic response is the greatest with thick urothelium, p75 was re-expressed among cells that are positioned on the contour of the urothelium (Bd, i). A majority of these cells are mitotic co-localizing with pH3 (Cb). After hyperplasia has subsided at 5 days post-injury, p75 expression was most robust among basal cells that are in contact with the laminin⁺ basal membrane (Be, j, Cc, arrow heads). P75⁺ cells are dividing during the period of hyperplasia after SCI, as indicated by BrdU incorporation during a 2 hr pulse prior to tissue harvest (Cd) and pH3⁺ immunoreactivity (Cf).





For Fig. 1G







50 -

37 -

For Supplemntal Fig. 1

Crothelial



WT SCI Bladder tossue

Muscle

For Supplemntal Fig. 2



For Supplemental Fig. 1 and 2

W: Flag (TrpV4)

W: Flag (TrpV4)

