Reviewer A

Comment 1: While the expression of annotated DELs, H19, miR155hg, LOC100910945 fluctuated from week 1 to week 4 post SCI, it was intriguing to note the gradual decline and increase in the expression of Miat and LOC100910973, respectively.

Reply 1: Thanks for the valuable comments. We also noticed these changes of DELs. The purpose of this study is to reveal the expression profiles and possible roles of lncRNAs in SCI rat NB. As shown in Figure 8 and Table S2, the fluctuation of lncRNAs is different from week 1 to week 4 post SCI. Specifically, the expression level of lncRNA Miat and LOC100911717 were gradually decreased, and that of Mir155hg was gradually increased. The expression level of LOC100910558 was the lowest in SCI–1 group, and then increased gradually. H19 was highest in SCI–1 group and then decreased gradually. The expression level of LOC100910945 and Mir155hg decreased overall but fluctuated between the SCI 1–3 groups (see Page 12, line 260–264). In summary, it will be very interesting and meaningful to investigate the relationship between the dynamic change of these DELs and the different stages of neurogenic bladder. Therefore, further in-depth studies should explore these interactions and their possible mechanisms.

Changes in the text: we have modified our text as advised (see Page 12, line 260–264).

Comment 2: Authors should discuss the relative importance of DELs as treatment targets, those DELs with expression fluctuating with time and those DELs whose expression progressively increasing or decreasing at time.

Reply 2: Thank you for the professional advice. As respectable reviewer requested, we discussed the relative importance of DELs as treatment targets in the revised manuscript. LncRNAs have been identified to exert both promotive and inhibitory effects on the multifaceted processes of fibrosis. A growing body of studies has revealed that lncRNAs are involved in fibrosis in various organs, including the liver, heart, lung, and kidney. We have discussed separately in the manuscript the regulatory mechanisms by which important DELs may be involved, such as "lncRNA Miat is involved in cardiac fibrosis via silencing of miR-214-3p" (see Page 16, line 348–349), "lncRNA H19 promotes epithelial–mesenchymal transition as a significant regulator both in vitro and in vivo" (see Page 17, line 367–368), and "LOC100910973 might exert an essential effect on the immune-inflammatory response following SCI" (see Page 17, line 378–379). H19 has been revealed to be involved in pneumonic, hepatic, renal and cardiac fibrosis with different mechanisms, indicating that H19 probably serves as a common therapeutic target for fibrosis. As lncRNAs have been increasingly identified, they have become promising targets for anti-fibrosis therapies. The results may help identify possible mechanisms of NB secondary to SCI from the perspective of lncRNAs and provide some new potential lncRNAs for the early diagnosis and treatment of human NB in the future.

Changes in the text: we have modified our text as advised (see Page 16, line 355–356).

Comment 3: The link between lncRNA and relaxin and TGFb signaling with fibrotic changes in SCI bladder is intriguing and authors should discuss their finding in light of the publication PMID: 32321501

Reply 3: Thank you for the professional suggestion. As respectable reviewer requested, we discussed the link between lncRNA and relaxin and TGFβ signaling pathway with fibrotic changes in SCI bladder (see Page 15, line 333–338). The objective of this study is to characterize relaxin receptor expression in the human bladder and assess the in-vitro effect of relaxin on expression of proteins involved in tissue remodeling and fibrosis in human bladder smooth muscle cells. This study has shown that the expression of relaxin receptors in the human bladder and that it localizes to both the urothelium, lamina propria, and muscularis propria and is expressed at both the trigone and dome of male and females of various age groups. Stimulation of normal human bladder smooth muscle cells with relaxin affects tissue remodeling and expression of fibrosis-related proteins via TGF-β/Smad signaling pathway. As revealed in Figure 6 in our manuscript, the target genes of DELs were predominantly enriched in the several pathways, including 'TGF-beta signaling pathway'. Therefore, we suspected these DELs might play a significant role in the process of bladder fibrosis. We validated TGF-β1 at the protein level (As shown in the Author's Response Figure 2). The results showed that the expression of TGF-β1 was significantly increased in the SCI 2–3 groups compared with the SCI–1 group and NC group. Moreover, the change trend of protein levels was the same as that of lncRNA LOC100910973 and LOC102554317. Then we suspected that these lncRNAs may act on this pathway through certain mechanisms. However, the specific mechanisms are not yet well known, so we did not put these results in the revised manuscript. We need more results in this area and this study is a beginning. As less study has been report on the link between lncRNA and relaxin and TGFβ signaling with fibrotic changes in SCI bladder, our study provides a novel strategy based on the signature of lncRNA expression, which derive for further study.

Changes in the text: we have modified our text as advised (see Page 15, line 333–338).

<mark>Reviewer B</mark>

Comment 1: The bladder wall contain different cell components and extra cellular matrices depending on its layers; especially, the bladder mucosa containing urothelium and lamina propria and the detrusor mostly consisting of smooth muscles, which could undergo different functional and molecular changes after SCI. Thus, the authors are encouraged to examine the lncRNA profiles in the separated layers of bladder mucosa and detrusor muscles, instead of using the whole bladder specimen.

Reply 1: Thank you for the professional comment. We have noticed this issue. Previous research mentioned that in animals, SCI triggers a cascade of events resulting in morphological and physiological changes in all components of the bladder (urothelium, smooth muscle, nerves)(1). For example, the urothelium, a stratified epithelium lining the lumen of the bladder, undergoes desquamation, partially losing the superficial umbrella cell layer, as early as 2–24 h post SCI in rodents. This is followed by rapid proliferation which in mice peaks around 3 days post SCI. However, even at 28 days post SCI, the urothelium retains abnormal features such as incomplete differentiation and altered cytokeratin markers. The detrusor smooth muscle (DSM) undergoes hypertrophy and sensitization to various agonists (e.g., purinergic, cholinergic) and ultimately becomes overactive. This is associated with increased spontaneous activity, which

can give rise to bladder overactivity(1,2). As respectable reviewer suggested, we will further explore the lncRNA profiles in the separated layers of bladder mucosa and detrusor muscles. We further discuss this issue in the revised limitation (see Page 19, line 421–422). We need more results in this area and this study is a beginning. Thus, further experimental studies are needed to verify the proposed interactions and their roles in the separated layers of bladder mucosa and detrusor muscles in the future.

Changes in the text: we have modified our text as advised (see Page 19, line 421–422).

Comment 2: This animal model has been used to study post–SCI detrusor–sphincter dyssynergia, bladder fibrosis and DO. However, any functional analysis to validate the animal model was not performed in this study, except for the description that "Animals were given manual bladder evacuations every 8 h until the spontaneous reflex of the bladder was re–established" (ln. 125–126). Also, additional histological information to confirm bladder fibrosis in this SCI model is needed as the results of this study suggest the link of altered lncRNA expression with tissue fibrosis.

Reply 2: Thank you for the professional advice. As respectable reviewer mentioned, bladder dysfunction after SCI involves bladder remodeling which was mainly shown as bladder fibrosis. Bladder fibrosis was characterized by the deposition of extracellular matrix and the increase of interstitial cells including fibroblasts and myofibroblasts. Indeed, our further study tentatively finds that deposition of collagens increases and bladder fibrosis gets worse progressively after SCI. Specifically, Author's Response Figure 1 shows that collagens are blue with microscopy by Masson staining and their deposition is more and more over time. Furthermore, as mentioned in the discussion, the levels of these DELs changed dynamically, which may indicate that they played predominant and dynamic role in different stages of bladder remodeling after SCI. For example, lncRNA LOC100910973, Mir155hg, LOC102554317, and H19 maintained a high level of expression after SCI and may keep functioning in the progression of bladder fibrosis. However, the specific mechanisms are not yet well known, so we did not put these results in the revised manuscript. Therefore, we will further investigate the relationship between the dynamic change of these DELs and the different stages of bladder remodeling.

Changes in the text: no change.

Comment 3: As described in the Discussion, it is important to identify "the expression of sensitive and specific biomarkers of NB secondary to SCI (ln. 262–263). However, the problem of this type of a biomarker searching study is to just provide with the open–end data without validation. It is not known if those identified lncRNAs in the post–SCI bladder are pathological causes, results of pathological outcomes, or just innocent bystanders. Thus, the authors should further discuss the limitation of this study with future directions for biomarker validation.

Reply 3: Thank you for the careful reminder. As respectable reviewer requested, we further discussed the limitation with future directions for biomarker validation. The main purpose of this study is to revealed the signature of lncRNA expression and the possible roles of lncRNAs and mRNAs in SCI rat NB through NGS analysis and qRT–PCR validation. We discussed separately in the manuscript the regulatory mechanisms by which important DELs may be involved, such as "lncRNA Miat is involved in cardiac fibrosis via silencing of miR-214-3p" (see Page 16, line 348–349), "lncRNA H19 promotes epithelial–mesenchymal transition as a

significant regulator both in vitro and in vivo" (see Page 16, line 367–368), and "LOC100910973 might exert an essential effect on the immune–inflammatory response following SCI" (see Page 17, line 378–379). The results may help identify possible mechanisms of NB secondary to SCI from the perspective of lncRNAs and provide some new potential lncRNAs for the early diagnosis and treatment of human NB in the future. As lncRNAs have been increasingly identified, they have become promising targets for anti–fibrosis therapies. Whether these DELs may also serve as possible NB biomarkers or as primary targets for therapeutic intervention requires further study (see Page 19, line 418–419). In summary, it will be very interesting and meaningful to investigate these DELs as possible NB biomarkers. As less study has been report on the link between lncRNAs and biomarkers with fibrotic changes in SCI bladder, our study provides a novel strategy based on the signature of lncRNA expression, which derive for further study.

Changes in the text: we have modified our text as advised (see Page 19, line 418–419).

Comment 4: The data analysis of this study rather focused on the changes of lncRNA profiles in SCI 1–3 groups together versus normal (especially in pathways analyses), but not much on the progress of SCI disease process of NB or fibrosis. For example, was there any difference or progressive pattern in lncRNA profiles such as Miat, Mir155hg, LOC100910973 and H19 in SCI rats at different time points? Because it is expected that SCI rats exhibit the initial areflexia phase more at 7 days, the emergence of spinal reflex–mediated micturition with DSD at 14 days or later, and more bladder fibrosis at 28 days vs. earlier dates, it is important to examine the correlation between post–SCI progressive changes in lncRNAs at different time points, if any, and functional/histological findings of post–SCI bladders.

Reply 4: Thanks for the valuable comments. As shown in Figure 8 and Table S2, the fluctuation of lncRNAs is different from week 1 to week 4 post SCI. Specifically, the expression level of lncRNA Miat and LOC100911717 were gradually decreased, and that of Mir155hg was gradually increased. The expression level of LOC100910558 was the lowest in SCI–1 group, and then gradually increased. H19 was highest in SCI–1 group and then decreased gradually. The expression level of LOC100910945 and Mir155hg decreased overall but fluctuated between the SCI 1–3 groups (see Page 12, line 260–264). As respectable reviewer requested, we reconciled the reported time dependent changes in voiding function after SCI with the lncRNA differences in SCI groups. Suprasacral SCI can abruptly disrupt intraspinal pathways and result in the "spinal shock" phase, during which the bladder is often atonic and areflexic and typically present with overflow incontinence(3). However, the relative concentration of collagen in rat bladders was reported to be significantly decreased in the first 10 days after SCI(4), which may be in agreement with the expression of lncRNA LOC100910973, Mir155hg, and LOC102554317 to a certain extent. Therefore, it is reasonable to presume that these lncRNAs might play a role in it. After spinal shock, hypermechanosensitive C-fiber bladder wall afferents were activated gradually and urodynamic findings were mainly characterized by detrusor overactivity or detrusor-sphincter dyssynergia. These lncRNAs may be involved in this stage of neurogenic bladder due to that it was significantly upregulated in the SCI–3 group compared to the SCI–1 group and SCI–2 group. Therefore, it will be very meaningful to investigate the relationship between the dynamic change of these DELs and the different stages of neurogenic bladder. The purpose of this study is to reveal the expression profiles and possible

roles of lncRNAs in SCI rat NB, further in-depth studies should explore the interactions between the dynamic change of these DELs and the different stages of neurogenic bladder and their possible mechanisms. For the correlation between the progressive changes in lncRNAs at different time points and functional/histological findings of post–SCI bladders, please refer to Reply 2.

Changes in the text: we have modified our text as advised (see Page 12, line 260–264).

Comment: When the authors created the SCI rats model, manual bladder evacuation was performed every 8 hrs (meaning 3times per day). Previous research mentioned that post–injury management influence bladder function or molecular status (Neurourol Urodyn. 2017 Jun;36(5):1301–1305.). These profile of lncRNA could be changed depending on the management after SCI. Please could you give some comments about this point.

Reply: Thank you for the valuable comment. As respectable reviewer mentioned, the profile of lncRNA could be changed depending on the management after SCI. We read this article with great interest. This article examined how a different number of daily bladder squeezes (once to three times daily) affects storage and voiding dysfunction parameters during awake cystometry among SCI mice, and also compared bladder nerve growth factor (NGF) levels and expression of TRP and P2X receptor transcripts in lumbosacral dorsal root ganglia of spinal intact and SCI mice. The results of this study indicated that chronic SCI model animals would have different functional and molecular characteristics depending on the post–injury management methods. Post–injury bladder management strategy is one of important factors to maintain lower urinary tract function in a better condition for neurogenic lower urinary tract dysfunction due to SCI. In this study, in SCI mice receiving bladder squeezes twice or three times daily, the emergence of non–voiding contractions was less compared to SCI mice with once daily of squeezes. Similarly, post–void residual urine was significantly less and the voiding efficiency tended to be improved in twice or three times squeezed SCI mice compared to once daily squeezed SCI mice. It is probably because frequent bladder squeezes have advantage to prevent bladder overdistention and high bladder pressure during storage. In conclusion, the frequent bladder emptying after SCI can reduce bladder hypertrophy by avoiding bladder overdistention, and decrease the NGF expression in the bladder, resulting in improvements of storage and voiding dysfunctions. Our work has been greatly inspired by this research that SCI rats would have different functional, molecular characteristics, and lncRNA profiles depending on the post–injury management methods. This reference was also added in the revised manuscript (see Page 6, line 125). In summary, it will be very interesting and meaningful to investigate the relationship between the dynamic change of these differentially expressed lncRNAs and the different management after SCI. We need more results in this area and this study is a beginning. As less study has been report on the link between lncRNA and postoperative management with fibrotic changes in SCI bladder, our study provides a novel strategy based on the signature of lncRNA expression, which derive for further study.

Changes in the text: we have modified our text as advised (see Page 6, line 125).

Comment 1: Collection of bladders: Authors mentioned that "All rats were anesthetized by

isoflurane inhalation (3%)" in order to collect the bladder. So, does it mean that he tissue was collected while animals are alive but anaesthetized?

Reply 1: Thank you for the valuable comment. All rats were anesthetized and sacrificed by isoflurane inhalation (3%). Then we did a quick sample collection. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Xuanwu Hospital Capital Medical University (No.20190128), in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. We have added this data in the revised manuscript (see Page 6, line 130).

Changes in the text: we have modified our text as advised (see Page 6, line 130).

Comment 2: Line 136: "Three replicates of each sample were used for RNA sequencing (RNA–Seq)". does it mean that from each animal 3 samples were prepared and sequenced? Or you mean from each group?

Reply 2: Thank you for the valuable comment. A total of 20 rats were randomly divided into four groups: SCI 1–3 and a normal control (NC) (n = 5 per group). Three samples of each group and 3 replicates of each tissue were used for RNA sequencing. We have added this data in the revised manuscript (see Page 7, line 134).

Changes in the text: we have modified our text as advised (see Page 7, line 134).

Comment 3: Line 146: EdgeR is not a software! It is a package in R Program.

Reply 3: Thank you for the careful reminder. It was our mistake that we had written the wrong package. We have made modifications in the revised manuscript (see Page 7, line 147).

Changes in the text: we have modified our text as advised (see Page 7, line 147).

Comment 4: Please mention reads were aligned to which genome build and augmented with transcript information from which annotation (Ensebl? NCBI?) and which release.

Reply 4: Thank you for the valuable comment. The reads were mapped to the reference genome Rnor_6.0 and augmented with transcript information from NCBI. We have added this data in the revised manuscript (see Page 7, line 145).

Changes in the text: we have modified our text as advised (see Page 7, line 145).

Comment 5: Did Alignments were checked for evenness of coverage, rRNA content, genomic context of alignments (for example, alignments in known transcripts and introns), complexity, and other quality checks using FastQC, Qualimap?

Reply 5: Thank you for the valuable comment. We obtained raw data consisting of > 91441540 clean reads (97.32–97.74%) from the Illumina HiSeq 3000 platform. In brief, the working principle of Illumina HiSeq platform was as follows: we removed and linked the sequence fragments and low-quality fragments from the 3′ end dynamically based on the low mass fraction of illumina sequencing data was concentrated at the end of the distribution. Then sequencing read quality was inspected using the FastQC software. Adapter removal and read trimming were performed using Trimmomatic (v0.36). Sequencing reads were trimmed from the end (base quality less than Q20) and filtered by length (less than 25). Please refer to the Author's Response Excel 1 for detailed information.

Changes in the text: no change.

Comment 6: Please provide the following information for the sequencing: What is the mean quality score of all samples? How many reads per sample was yield? What is the percentage of mapped fragments over total? Some of these information is results part (line 187)

Reply 6: Thank you for the professional comment. The percentage of mapped fragments over total is 97.56%. Please refer to the Author's Response Excel 1 for detailed information.

Changes in the text: no change.

Comment 7: Line 146 mentions "Differentially expressed mRNAs (DEMs) and DELs were determined using EdgeR software (version 3.22.3)." but in line 176 it is mentioned "Differential expression analysis of lncRNAs was performed using DESeq2". Which package was finally used? DESeq or EdgeR?

Reply 7: Thank you for the careful reminder. It was our mistake that we had written the wrong package in line 146. Indeed, differential expression analysis of lncRNAs and mRNAs obtained from NGS was performed by the DESeq2 (version 1.24.0) which was an algorithm to examine differences between groups by using a generalized linear model and assuming a negative binomial distribution of RNA–Seq reads. All of DELs and DEMs were used to build heatmaps and volcano plot using R software (version 3.22.3). We have made corrections in the revised manuscript (see Page 7, line 147–148).

Changes in the text: we have modified our text as advised (see Page 7, line 147–148).

Comment 8: in line 178 it is mentioned that "We determined the statistical significance of the levels of lncRNAs verified by qRT–PCR using analysis of variance (ANOVA)"
Did you perform any normality test before choosing a parametric test?

Reply 8: Thank you for the professional comment. Shapiro–Wilk normality test was applied to examine whether data samples fit a normal distribution. All of the results conformed to a normal distribution. We have added this data in the revised manuscript (see Page 9, line 181–182).

Changes in the text: we have modified our text as advised (see Page 9, line 181–182).

Comment 9: with which tool did target prediction was performed (line 212)

Reply 9: There are two main ways for lncRNAs to regulate target genes: cis-regulation and trans-regulation. Cis-regulation in which lncRNA act on neighboring target genes (10kb upstream and downstream) using R software (version 3.22.3). Trans-regulation is the lncRNA–mediated transcriptional activation and expression regulation of coding genes on other chromosomes or distal to the same chromosome. The sequences of differentially expressed lncRNAs and mRNAs were extracted, and then screened using blast software and RNAplex software, so as to identify possible target genes of lncRNAs.

Changes in the text: no change.

Comment 10: Provide an excel sheet with full list of significant Long non coding RNA containing the read counts for individual samples, average, log2 FC, p value and adjusted pvalue.

Reply 10: Thank you for the valuable comment. The excel sheet with full list of significant lncRNAs were added in the supplementary file. Please refer to the Author's Response Excel 2

for detailed information.

Changes in the text: no change.

Comment 11: Provide an excel sheet with full list of significant mRNAs containing the read counts for individual samples, average, log2 FC, p value and adjusted pvalue.

Reply 11: Thank you for the valuable comment. The excel sheet with full list of significant mRNAs were added in the supplementary file. Please refer to the Author's Response Excel 3 for detailed information.

Changes in the text: no change.

Comment 12: Provide a venn diagram for SCI1, 2, 3 exclusive and common DELs.

Reply 12: Thank you for the valuable comment. The venn diagram for SCI 1–3 exclusive and common DELs was added in the revised manuscript. Please refer to revised Figure 1.

Changes in the text: we have modified our text as advised (see Figure 1).

Comment 13: Provide a venn diagram for SCI1, 2, 3 exclusive and common DEMs.

Reply 13: Thank you for the valuable comment. The venn diagram for SCI 1–3 exclusive and common DEMs was added in the revised manuscript. Please refer to revised Figure 3.

Changes in the text: we have modified our text as advised (see Figure 3).

Comment 14: line 268 – Please add a reference.

Reply 14: Thank you for the careful reminder. The reference was added in the revised manuscript (see Page 13, line 278).

Changes in the text: we have modified our text as advised (see Page 13, line 278).

Comment 15: line 278 to 280 – based on what evidence authors made this argument?

Reply 15: Thank you for the professional comment. The suitability of the animal model and detection time period exert a determined role in experimental research. In general, it is accepted that contusion SCI is the classic SCI model because of its similarity to the clinical situation– which also comprises bony fragments or extruded disk materials in addition to spinal tissue destruction. However, because of variation in residual tissues, there are shortcomings of the contusion SCI model relating to the evaluation of nerve regeneration and functional recovery. These are not issues in the complete transection SCI model, a standard SCI model with identical severity. Secondary injury following SCI is the core of its debilitating pathologic progression, and provides the optimal time frame for therapeutic interventions. Necrotic and apoptotic cell death in the acute and subacute phase (about 2 weeks post–injury) are the most significant post– trauma changes, and these peak at 7 days after the initial mechanical insult(5). As research concerning lncRNAs in complete transection SCI has to our knowledge not been reported, we therefore conducted a transection SCI model and analyzed lncRNA expression profiles at 7, 14, 28 days post–injury to get a more extended understanding of lncRNAs pattern in SCI. This reference was added in the revised manuscript (see Page14, line 290).

Changes in the text: we added a reference (see Page14, line 290).

Comment 16: Discussion – redundant and descriptive information: A lot of sentences are

repetitive: such as : "A total of 366 DELs (25 annotated) contained 2845 target genes." Which is repeated in discussion (line 298) and in results part (line 214).

Reply 16: Thank you for the valuable comment. We have removed duplicate sentences from the Discussion section (see Page 14, line 301).

Changes in the text: we have modified our text as advised (see Page 14, line 301).

Comment 17: Discussion – line 299 to 302 does not provide any information!

Reply 17: Thank you for the valuable comment. We have removed duplicate sentences from the Discussion section (see Page 14, line 296 and line 301).

Changes in the text: we have modified our text as advised (see Page 14, line 296 and line 301).

Comment 18: Figure 1: how many LNCs where used to build these heatmaps

Reply 18: Thank you for the professional comment. All of differentially expressed lncRNAs were used to build these heatmaps.

Changes in the text: no change.

Comment 19: Figure 1: What clustering method have been used?

Reply 19: Thank you for the professional comment. A hierarchical clustering analysis was performed using R software (version 3.22.3).

Changes in the text: we have modified our text as advised (see Page 7, line 147–148).

Comment 20: Figure 1: Provide a combined figure showing controls and SCI animals for all time points with clustering

Reply 20: Thank you for the professional comment. We have added a combined figure showing controls and SCI animals for all time points with clustering. Please refer to revised Figure 1.

Changes in the text: we have modified our text as advised (see Figure 1).

Comment 21: Figure 2: increase the x–axis to 10.

Reply 21: Thank you for the professional comment. We have revised the Figure 2 following the reviewers' comments.

Changes in the text: we have modified our text as advised (see Figure 2).

Comment 22: Figure 3: the legend says this is a heat map. But it is a Volcano plot.

Reply 22: Thank you for the careful reminder. It was our mistake that we misplaced the order of the Figures. Actually, Figure 3 is a heat map. We have made modifications in the revised manuscript (see Figure legends, Figure 3).

Changes in the text: we have modified our text as advised (see Figure legends, Figure 3).

Comment 23: Figure 3: increase the x–axis to 10 and y–axis to 20 (a lot of points are not shown)

Reply 23: Thank you for the professional comment. We have revised the Figure 4 following the reviewers' comments.

Changes in the text: we have modified our text as advised (see Figure 4).

Comment 24: Figure 3: Line 550 mentioned that this is lncRNA but this is about mRNAs

Reply 24: Thank you for the careful reminder. It was our mistake that we had written the wrong Figure legends. Actually, Figure 3 is about mRNAs. We have made modifications in the revised manuscript (see Figure legends, Figure 3).

Changes in the text: we have modified our text as advised (see Figure legends, Figure 3).

Comment 25: Figure 4: the legend says this is a volcano plot. But these are heatmaps

Reply 25: Thank you for the careful reminder. It was our mistake that we misplaced the order of the Figures. Actually, Figure 4 is a volcano plot. We have made modifications in the revised manuscript (see Figure legends, Figure 4).

Changes in the text: we have modified our text as advised (see Figure legends, Figure 4).

Comment 26: Figure 4: Line 557 mentioned that this is lncRNA but this is about mRNAs

Reply 26: Thank you for the careful reminder. It was our mistake that we had written the wrong Figure legends. Actually, Figure 4 is about mRNAs. We have made modifications in the revised manuscript (see Figure legends, Figure 4).

Changes in the text: we have modified our text as advised (see Figure legends, Figure 4).

Comment 27: Figure 5: The font for "Negative regulation of extrinsic apoptotic …." Is different. Why?

Reply 27: Thank you for the careful reminder. This item is so long that we have to narrow down the font, but we have unified the font size in the revised Figure 5.

Changes in the text: we have modified our text as advised (see Figure 5).

Comment 28: Figure 5: the color od molecular function and cellular components are very similar. Change that to something distinguishable.

Reply 28: Thank you for the careful reminder. We have changed the color in the revised Figure 5.

Changes in the text: we have modified our text as advised (see Figure 5).

Comment 29: Figure 5: Which time point is used to build this figure? (7, 14, 28 days ??)

Reply 29: Thank you for the professional comment. Figure 5 was built at the time point of 28 days post SCI. We have added this data in the revised manuscript (see Page 11, line 226–227).

Changes in the text: we have modified our text as advised (see Page 11, line 226–227).

Comment 30: Figure 5: Is this based on DEL target mRNAs?

Reply 30: Thank you for the valuable comment. Yes, Figure 5 was built based on DELs target mRNAs.

Changes in the text: no change.

Comment 31: Figure 6: Which time point is used to build this figure? (7, 14, 28 days ??)

Reply 31: Thank you for the professional comment. Figure 6 was built at the time point of 28 days post SCI. We have added this data in the revised manuscript (see Page 11, line 235–236).

Changes in the text: we have modified our text as advised (see Page 11, line 235–236).

Comment 32: Figure 6: Is this based on DEL target mRNAs?

Reply 32: Thank you for the valuable comment. Yes, Figure 6 was built based on DELs target mRNAs.

Changes in the text: no change.

Comment 33: Figure 8: the information of RNA seq (fold change) has to be shown to. Be able to compare it with PCR data.

Reply 33: Thank you for the valuable comment. The information of RNA seq (fold change) is shown in revised Table S2.

Changes in the text: we have modified our text as advised (see Table S2).

Comment 34: All the RNA seq data (Fasta file) needs to be provided in GEO or ENA with the accession number.

Reply 34: Thank you for the valuable comment. We have uploaded all the RNA seq data to GEO, and the accession number is GSE19754.

Changes in the text: no change.

Comment 35: Since for the first time point (7 days) animals are still in spinal shock (can authors comment why they chose this time point?

Reply 35: Thank you for the professional comment. It is expected that SCI rats exhibit the initial areflexia phase more at 7 days, the emergence of spinal reflex–mediated micturition with detrusor sphincter dyssynergia (DSD) at 14 days or later, and more bladder fibrosis at 28 days vs. earlier dates, it is important to examine the correlation between post–SCI progressive changes in lncRNAs at different time points and functional/histological findings of post–SCI bladders. Secondary injury following SCI is the core of its debilitating pathologic progression, and provides the optimal time frame for therapeutic interventions. Necrotic and apoptotic cell death in the acute and subacute phase (about 2 weeks post–injury) are the most significant post–trauma changes, and these peak at 7 days after the initial mechanical insult(5). As research concerning lncRNAs in SCI has to our knowledge not been reported, we therefore conducted the SCI model and analyzed lncRNA expression profiles at 7, 14, 28 days post–injury to get a more extended understanding of lncRNAs pattern in SCI. We need more results in this area and this study is a beginning. As less study has been report on the link between the dynamic change of these DELs and the different stages of NB, our study provides a novel strategy based on the signature of lncRNA expression, which derive for further study.

Changes in the text: no change.

Comment 36: Comparing these 3 time points what do we conclude? Which mRNAs are mostly regulated for which time point?

Reply 36: Thank you for the professional comment. In this study, we investigated the DELs and DEMs between groups by NGS and qRT–PCR. Furthermore, our results revealed the dynamic change of these DELs and DEMs in the SCI rat. As shown in Figure 8 and Table S2, the fluctuation of lncRNAs is different from week 1 to week 4 post SCI. Specifically, the expression level of lncRNA Miat and LOC100911717 were gradually decreased, and that of Mir155hg was gradually increased. The expression level of LOC100910558 was the lowest in SCI–1 group,

and then gradually increased. H19 was highest in SCI–1 group and then decreased gradually. The expression level of LOC100910945 and Mir155hg decreased overall but fluctuated between the SCI 1–3 groups (see Page 12 line 260–264). The results may help identify possible mechanisms of NB secondary to SCI from the perspective of lncRNAs and provide some new potential lncRNAs for the early diagnosis and treatment of human NB in the future. However, the interactions between lncRNAs and mRNA were not explored. We discuss this issue in the limitations: "First, the interactions between lncRNAs and mRNAs in NB were not investigated in detail." (see Page 19, line 417–418). We need more results in this area and this study is a beginning. Thus, further experimental studies are needed to verify the lncRNA–mRNA interactions and their roles in neurogenic bladder in the future.

Changes in the text: no change. we have modified our text as advised (see Page 12, line 260–264, and Page 19 line 417–418).

Comment 37: Can authors provide any protein level validation to prove the regulation of discussed pathways?

Reply 37: Thank you for the professional advice. As mentioned in the manuscript, "The TGF-β1 signaling pathway is critically involved in the development and progression of bladder fibrosis. Recent studies have highlighted that overexpression of TGF-β1 could activate the Smad signaling pathway, a critical molecular mechanism contributing to smooth muscle cell apoptosis and bladder fibrosis (44)" (see Page 18, line 402–404). We validated TGF-β1 at the protein level (As shown in the Author's Response Figure 2). The results showed that the expression of TGF-β1 was significantly increased in the SCI 2–3 groups compared with the SCI–1 group and NC group. Moreover, the change trend of protein levels was the same as that of lncRNA LOC100910973 and LOC102554317. Then we suspected that these lncRNAs may act on this pathway through certain mechanisms. However, the specific mechanisms are not yet well known, so we did not put these results in the revised manuscript. As less study has been report on the link between lncRNA and related pathway regulation with fibrotic changes in SCI bladder, our study provides a novel strategy based on the signature of lncRNA expression, which derive for further study.

Changes in the text: no change.