Longitudinal intravital imaging of transplanted mesenchymal stem cells elucidates their functional integration and therapeutic potency in an animal model of interstitial cystitis/bladder pain syndrome

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Running title: Intravital imaging of M-MSCs for treatment of IC/BPS

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This Supplementary Material file contains twelve Supplementary Figures, six Supplementary Movies and their legends.

SUPPLEMENTARY FIGURE LEGENDS

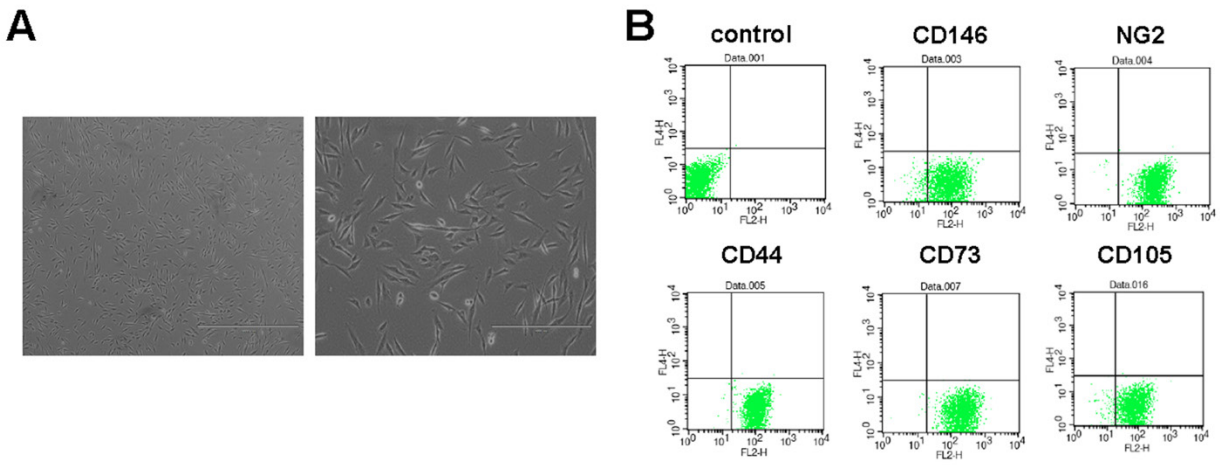

Figure S1. Characterization of hESC-derived M-MSCs.

(A) Morphological characterization of M-MSCs (left panel; magnification ×40, scale bar = 400 μm and right panel; magnification ×100, scale bar = 1,000 μm). **(B)** Analysis of surface antigen expression showing that M-MSCs were positive for markers of MSCs (CD44, CD73, and CD105) and pericytes (CD146 and NG2).

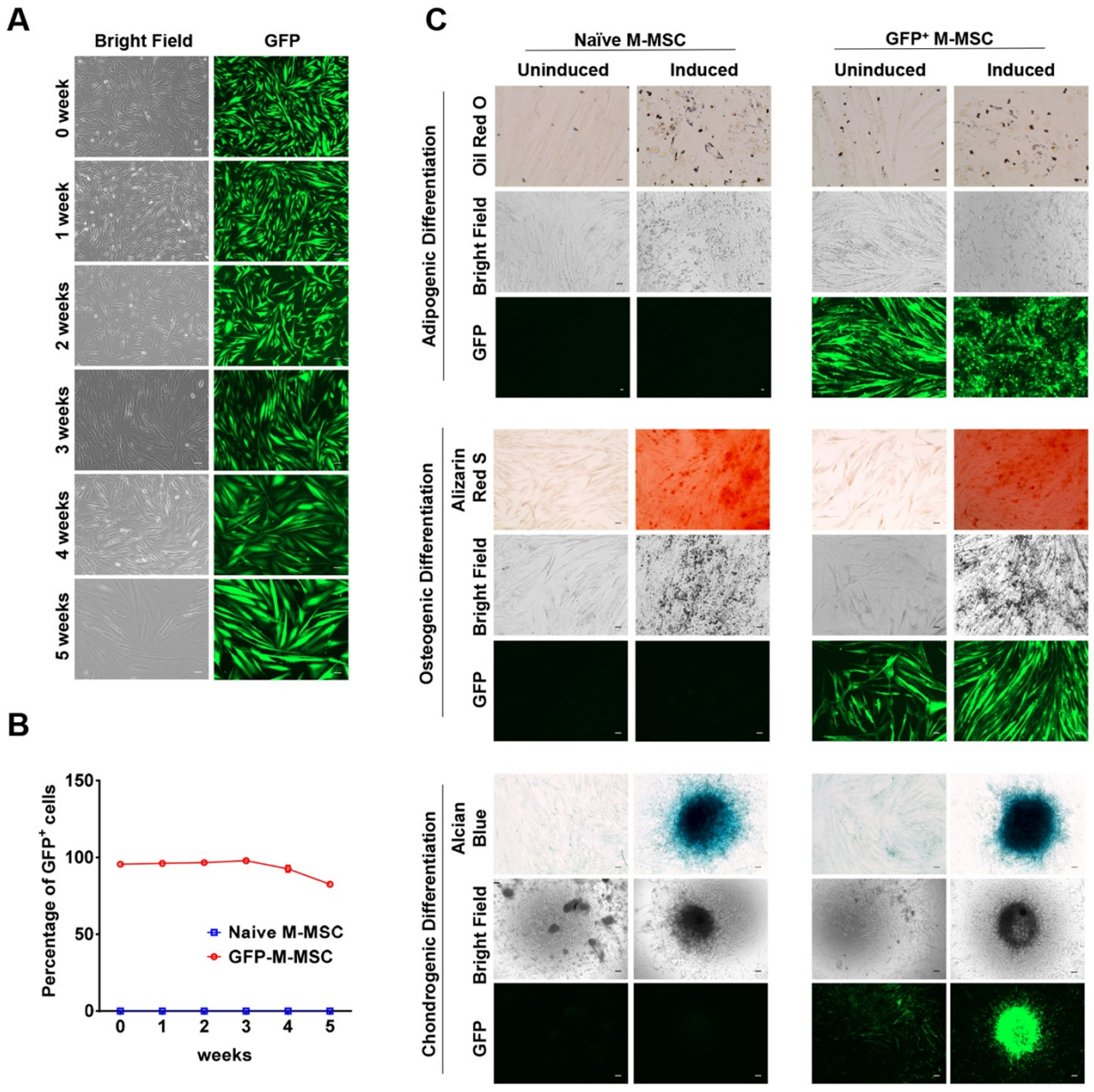

Figure S2. Stable expression of GFP in GFP$^+$ M-MSCs.

Expression of GFP in M-MSCs infected with a lentivirus containing a GFP-expressing cassette during long-term cultivation and multi-lineage differentiation. **(A and B)** Expression of GFP during cultivation of GFP$^+$ M-MSCs for 5 weeks was monitored using an inverted fluorescence

microscope (**A**; magnification ×100, scale bar = 100 μm) and by FACS analysis **(B)**. **(B)** The percentage of GFP-expressing cells determined by FACS analysis was quantified from three independent experiments. Data are presented as the mean ± SEM. **(C)** Expression of GFP during adipogenic (upper panel; ×200 magnification; scale bar = 20 μm), osteogenic (middle panel; ×200 magnification; scale bar = 40 μm), and chondrogenic (lower panel; ×40 magnification; scale bar = 200 μm) differentiation in control (Naïve) and GFP-expressing M-MSCs (GFP^+ M-MSCs). Adipogenic, osteogenic, and chondrogenic differentiation was characterized by Oil Red O, Alizarin Red S, and Alcian Blue staining, respectively.

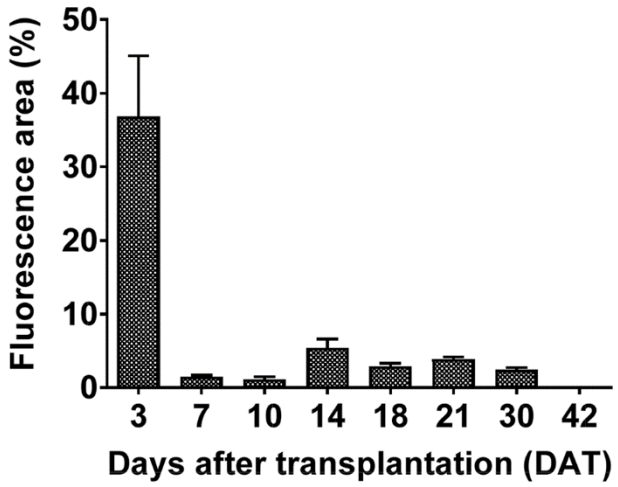

Figure S3. Quantitative assay of intravital micro-endoscopic imaging

Percentage of the fluorescent area in confocal endoscopic micrographs. Representative movies obtained in these experiments are available as **Supplementary Movies**.

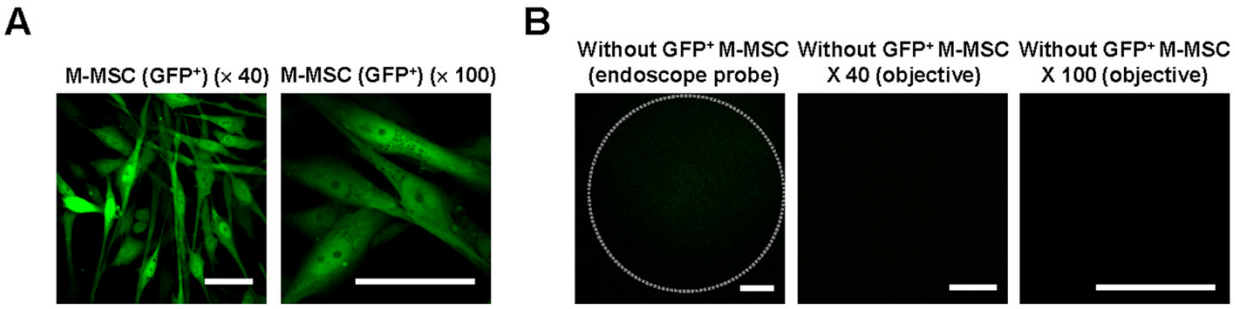

Figure S4. Low level of autofluorescence in intravital imaging.

(A) Representative images of GFP in cultured GFP^+ M-MSCs (magnification ×40 and ×100). **(B)** Longitudinal imaging of living animals not transplanted with GFP^+ M-MSCs. Time-lapse images of the bladders of LPS-IC rats were obtained using a front-view GRIN optical probe endoscopically inserted into the bladder (left, magnification ×40) or an objective lens (middle; magnification ×40, right; magnification ×100). Baseline images from rats not injected with GFP^+ M-MSCs show low autofluorescence, and weak fluorescence signals overall are detected by intravital imaging. Scale bar = 50 μm.

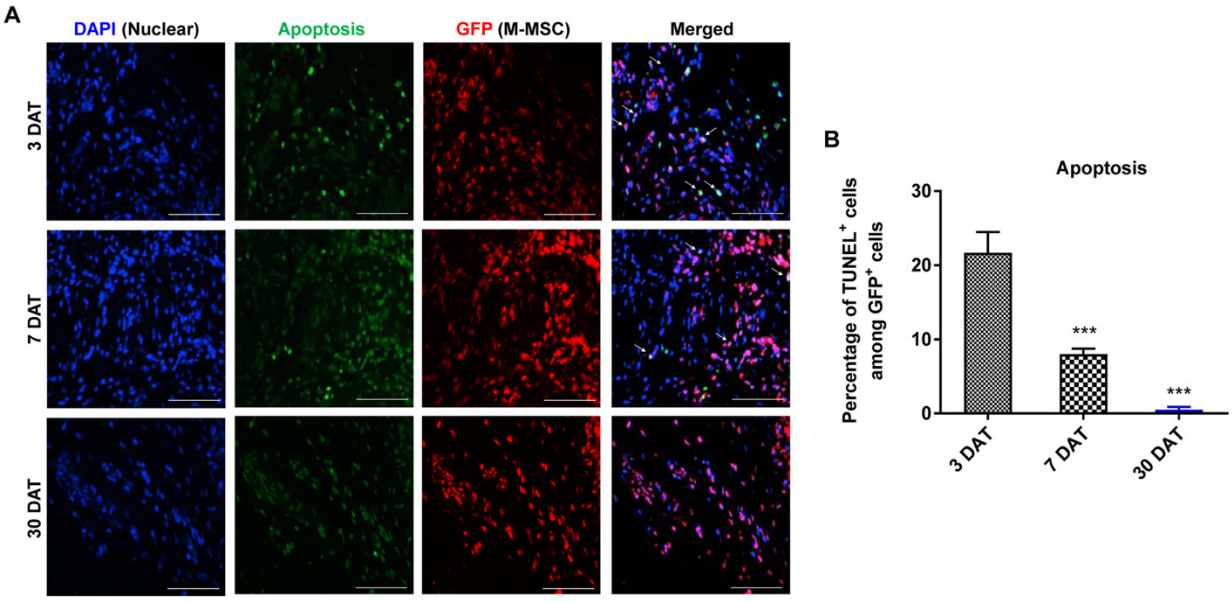

Figure S5. Apoptosis of transplanted M-MSCs.

(A) Representative images of staining to detect $TUNEL^+$ apoptotic cells (red) among transplanted GFP^+ M-MSCs (green) (magnification ×400, scale bar = 20 μm) in bladder tissues of LPS-IC + M-MSC rats at the indicated number of DAT. **(B)** Quantification of the staining results. Data show the percentage of GFP^+ cells that were $TUNEL^+$ (n = 8) and are presented as the mean ± SEM. ***$p<0.001$ compared with the 3 DAT group according to a one-way ANOVA with the Bonferroni post-hoc test.

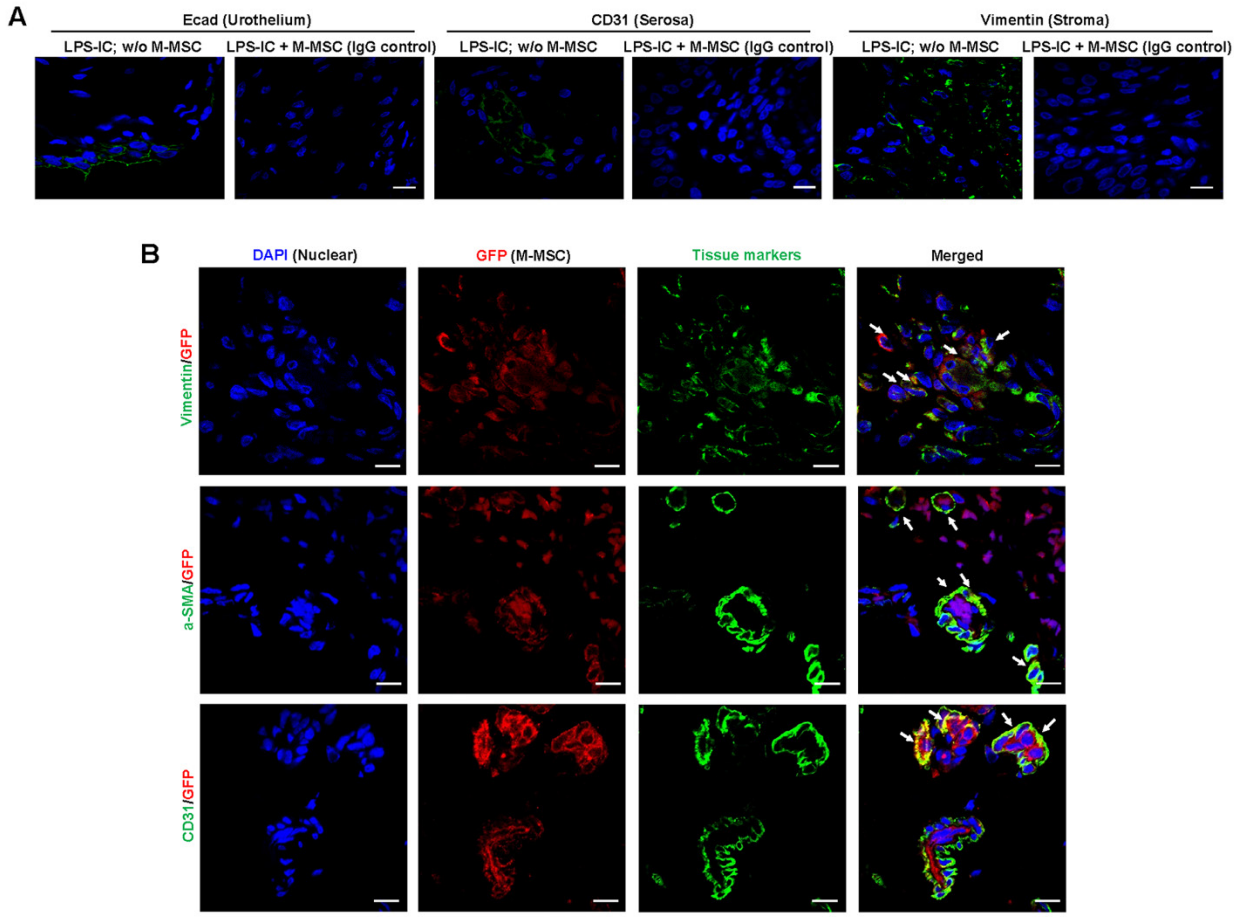

Figure S6. Immunostaining of transplanted M-MSCs.

(A) To rule out the possibility of non-specific staining, bladder tissues of LPS-IC + M-MSC rats were co-stained with mouse and rabbit IgG control antibodies, and bladder tissues of LPS-IC rats not injected with M-MSCs (LPS-IC; w/o M-MSC) were co-stained for the indicated markers (green) and GFP (red) (magnification ×1,000, scale bar = 10 μm) as two sets of negative controls. **(B)** Representative confocal micrographs of bladder sections of LPS-IC + M-MSC rats stained for GFP (red) and vimentin, α-SMA, or CD31 (green) at 30 DAT (magnification ×1,000, scale bar = 10 μm). Nuclei were stained with DAPI (blue).

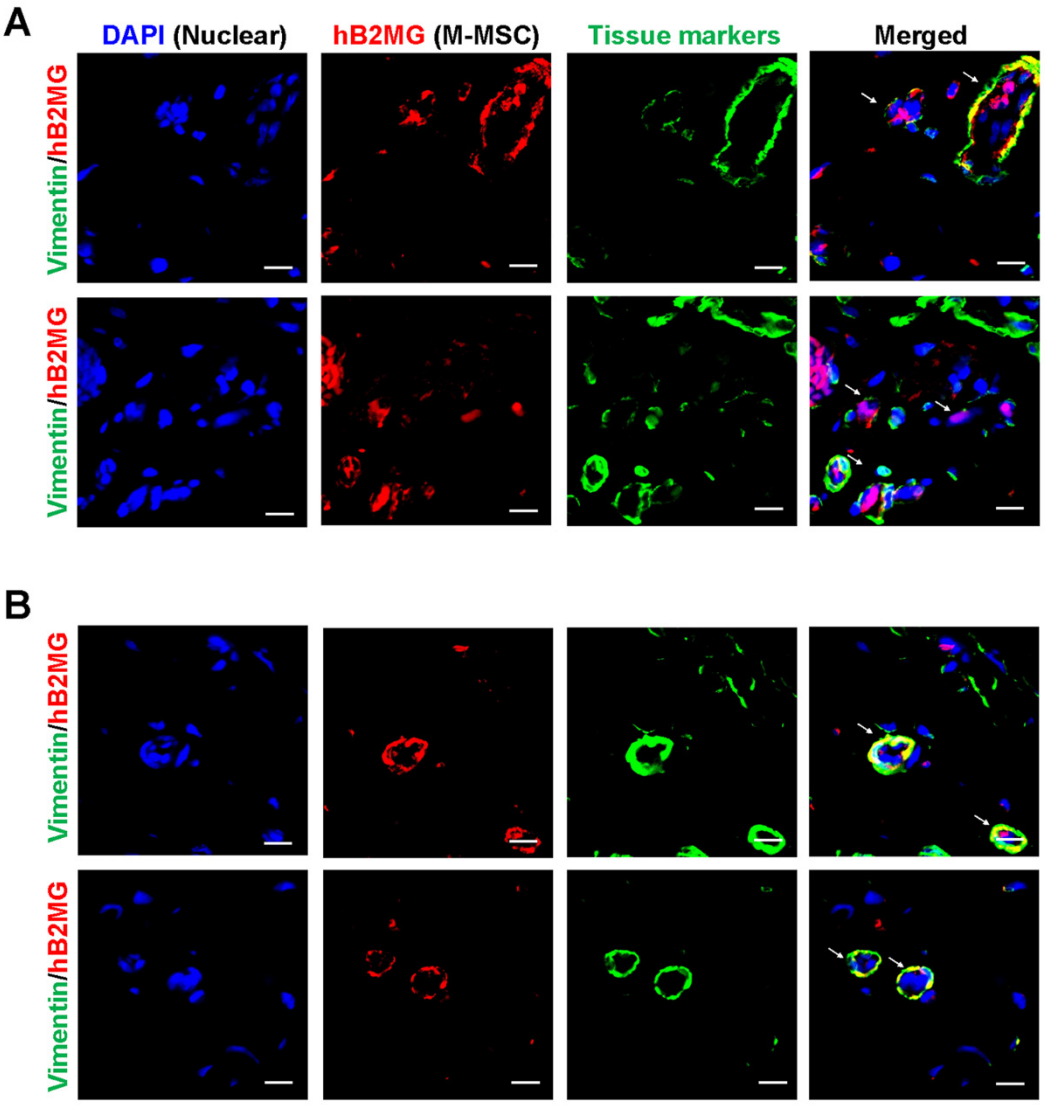

Figure S7. Co-expression of vimentin and human antigens in transplanted M-MSCs.

Representative confocal micrographs of bladder sections of LPS-IC + M-MSC rats stained for hB2M (red) and vimentin at 7 DAT (upper panel) and 30 DAT (lower panel) in blood vessel-like structures (magnification ×1,000, scale bar = 10 μm). Nuclei were stained with DAPI (blue).

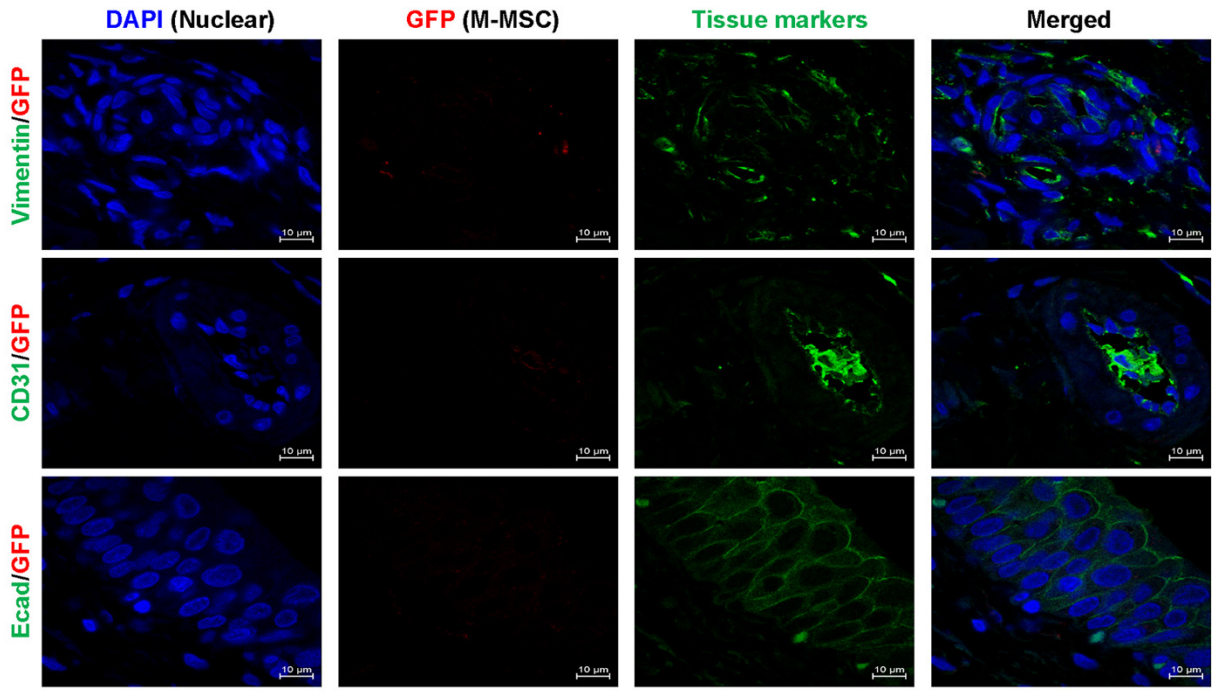

Figure S8. Disappearance of transplanted M-MSCs at 1 month after transplantation.

Representative confocal micrographs of bladder sections of LPS-IC + M-MSC rats stained for GFP (red) and vimentin, E-cadherin (Ecad), or CD31 (green) at 42 DAT (magnification ×1,000, scale bar = 10 μm). Nuclei were stained with DAPI (blue).

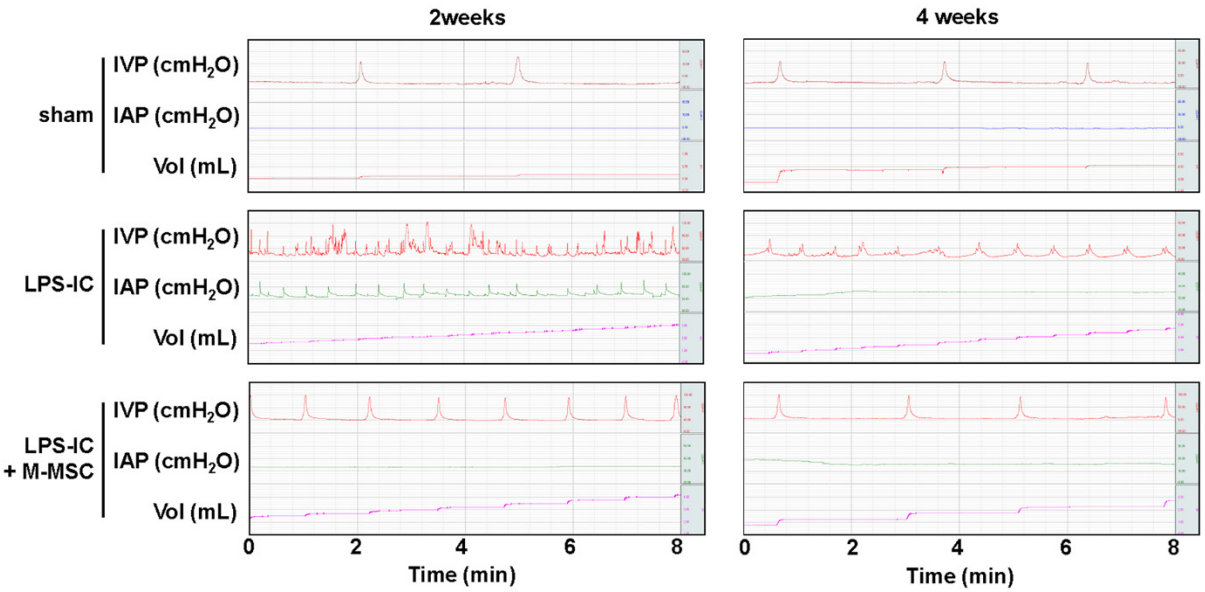

Figure S9. Long-term therapeutic effects of M-MSCs on bladder function in LPS-IC rats.

Representative awake cystometry results at 2 weeks (left) and 4 weeks (right) after injection of 1×10^6 M-MSCs (LPS-IC + M-MSC) or PBS (LPS-IC) into LPS-IC rats. Sham: sham-operated.

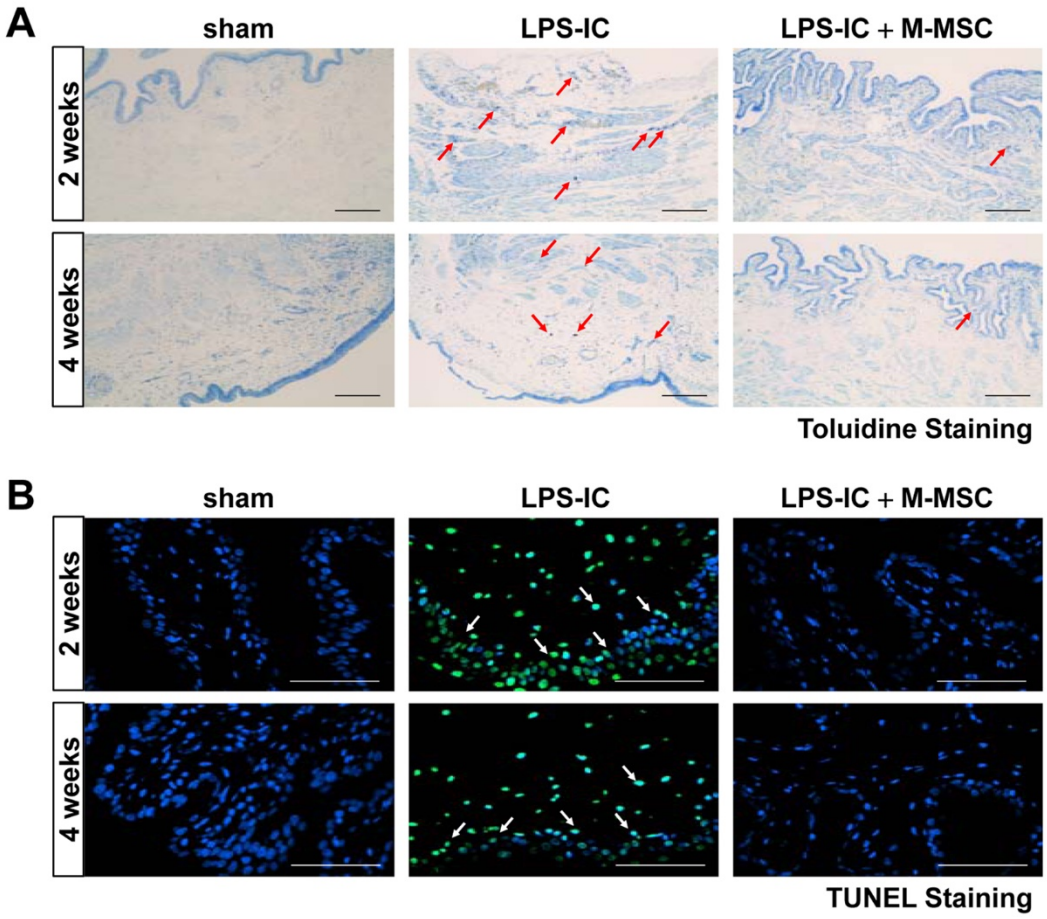

Figure S10. Therapeutic effects of M-MSCs on chronic bladder injury in LPS-IC rats.

(A and B) Representative Toluidine blue staining (**A**; magnification ×100, scale bar = 100 μm), and TUNEL (**B**; magnification ×400, scale bar = 100 μm) in bladder tissues of LPS-IC rats at 2 and 4 weeks after injection of 1×10^6 M-MSCs (LPS-IC + M-MSC) or PBS (LPS-IC). Nuclei were stained with Mayer's hematoxylin **(A)** or DAPI (blue, **B**). Arrows indicate infiltrated mast cells **(A)** and apoptotic cells **(B)**. Sham: sham-operated.

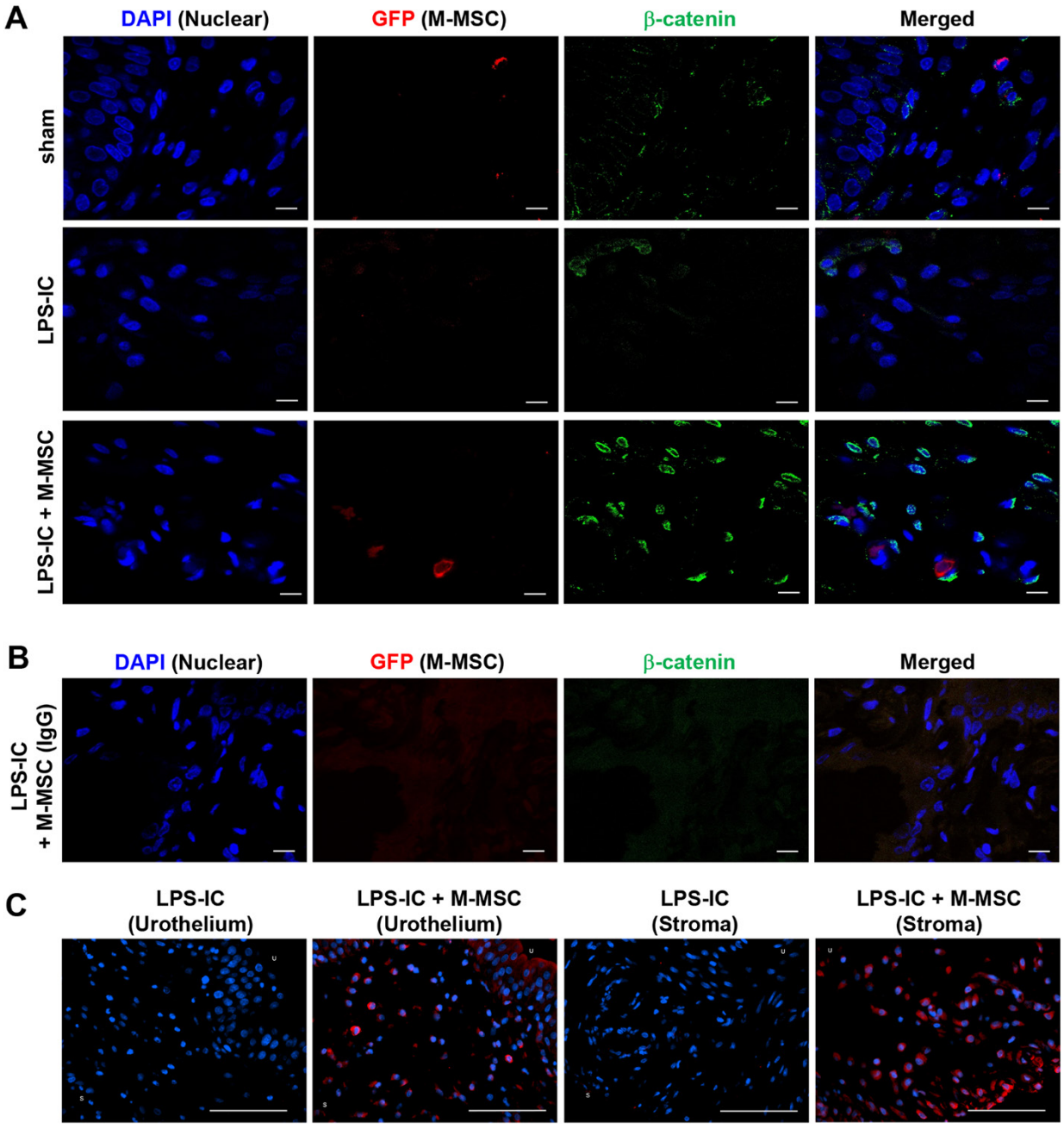

Figure S11. Immunofluorescence analysis of WNT signaling and engraftment of M-MSCs.

(A) Representative confocal micrographs of bladder sections from the indicated groups of rats co-stained for GFP (red) and β-catenin (green) (magnification ×1,000, scale bar = 10 μm). Nuclei were stained with DAPI (blue). **(B)** Bladder sections of LPS-IC + M-MSC rats were

stained with mouse and rabbit IgG control antibodies as a negative control in experiments assessing the nuclear localization of β-catenin and engraftment of GFP^+ cells (magnification ×1,000, scale bar = 10 μm). **(C)** Bladder tissues of LPS-IC rats not injected with GFP^+ M-MSCs (LPS-IC; w/o M-MSC) were stained as a negative control in experiments assessing the engraftment of GFP^+ cells (magnification ×200, scale bar = 200 μm). Nuclei were stained with DAPI (blue). U: urothelium; S: serosa; sham: sham-operated.

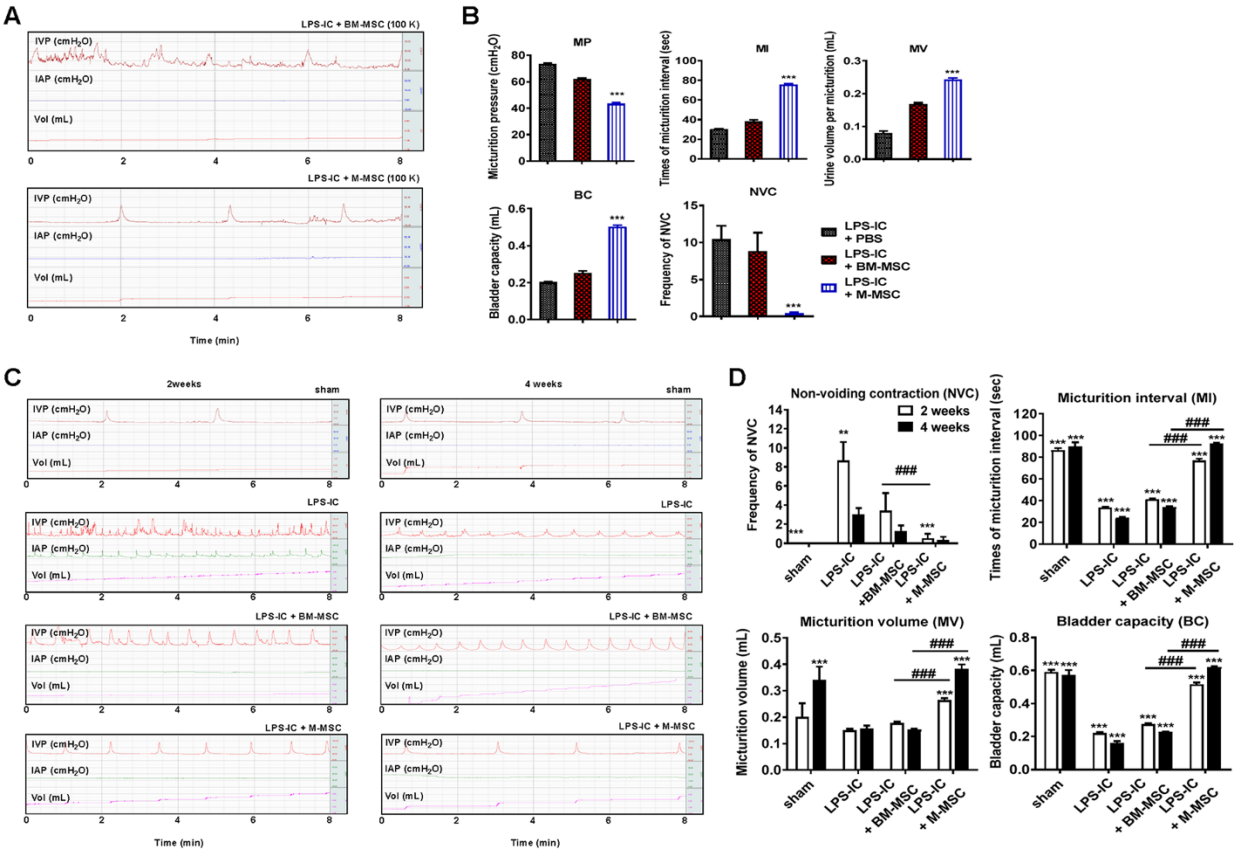

Figure S12. The therapeutic efficacy of M-MSCs is superior to that of BM-MSCs.

(A and C) Representative awake cystometry at 1 week **(A)** or 2 or 4 weeks **(C)** after injection of M-MSCs or BM-MSCs (1×10^5 cells; 100 K) into the bladders of LPS-IC rats. **(B and D)** The non-voiding contraction (NVC), micturition interval (MI), micturition volume (MV), and bladder capacity (BC) were quantified from the voiding pattern analysis. All data are presented as the mean ± SEM (five independent animals per group). Data were statistically analyzed using a one-way **(B)** or two-way **(D)** ANOVA and the Bonferroni post-hoc test. ***p<0.001 compared with the LPS-IC group; ###p<0.001 for the M-MSC vs. BM-MSC groups.

SUPPLEMENTARY MOVIES AND LEGENDS

Movie S1. Intravital endoscope imaging at 3 DAT (**Figure 2**)

Movie S2. Intravital endoscope imaging at 30 DAT (**Figure 2**)

Movie S3. Intravital microscope 3 DAT with 40×magnification (**Figure 3A**)

Movie S4. Intravital microscope 3 DAT with 100×magnification (**Figure 3B**)

Movie S5. Intravital microscope 30 DAT with 40×magnification (**Figure 3A**)

Movie S6. Intravital microscope 30 DAT with 100×magnification (**Figure 3B**)

Supplementary Movies at other days after transplantation would be available at website.

These videoclips can be also freely downloaded from FTP server (ftp://sdmlab.iptime.org). The login ID and password are is "ID: FTP_Guest and PW: Guest1".