## Improvement in bladder dysfunction after bladder transplantation of amniotic fluid stem cells in diabetic rats

Ching-Chung Liang<sup>1, 2</sup>, Sheng-Wen Steven Shaw<sup>2, 3, 4</sup>, Yung-Hsin Huang<sup>1</sup>,

Yi-Hao Lin<sup>1, 2</sup> & Tsong-Hai Lee \*, 2, 5

<sup>1</sup>Female Urology Section, Department of Obstetrics and Gynecology, Chang Gung

Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan

<sup>2</sup>College of Medicine, Chang Gung University, Taoyuan, Taiwan

<sup>3</sup>Division of Obstetrics, Department of Obstetrics and Gynecology, Chang Gung

Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan

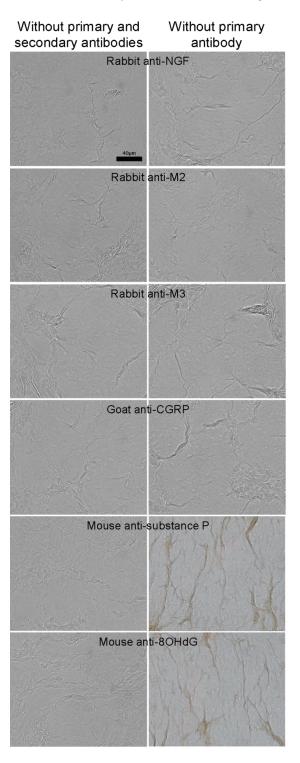
<sup>4</sup>Prenatal Cell and Gene Therapy Group, Institute for Women's Health, University

College London, London, UK

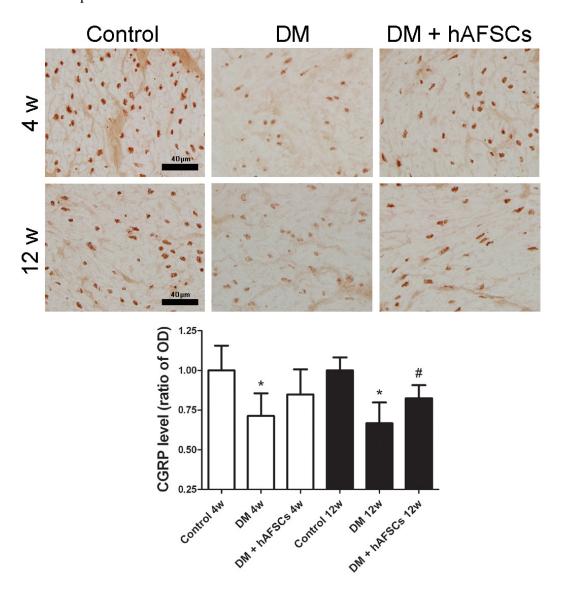
<sup>5</sup>Stroke Center and Department of Neurology, Chang Gung Memorial Hospital Linkou

Medical Center, Taoyuan, Taiwan

**Supplemental Fig. 1.** The negative control studies are performed without primary antibody and without primary and secondary antibodies for nerve growth factor (NGF), M2-muscarinic receptor (M2) and M3-muscarinic receptor (M3), calcitonin gene-related peptide (CGRP), substance P and 8-hydroxy-20-deoxyguanosine (8OHdG). No or mild immunoreactivity can be seen in the negative studies.



**Supplemental Fig. 2.** Temporal expressions of calcitonin gene-related peptide (CGRP) immunoreactivity in the bladder of control, STZ-induced diabetic rats (DM) and diabetic rats + hAFSCstransplantation (DM + hAFSCs). The immunoreactivity of CGRP can be seen in control rats (A), diabetic rats (B), and diabetic rats + hAFSCs transplantation (C) at 4 weeks, and in control rats (D), diabetic rats (E), and diabetic rats + hAFSCs transplantation (F) at 12 weeks. When compared to the controls, the expressions of CGRP in the diabetic rats are decreased at 4 and 12 weeks, but can be recovered to near the control level after hAFSCs transplantation. OD= optical density. Bar indicates 40  $\mu$ m. \*P < 0.05 vs. control group. #P < 0.05 vs. diabetic rat. N = 10 in each time point.



**Supplemental Fig. 3.** Temporal expressions of Substance P immunoreactivity in the bladder of control, STZ-induced diabetic rats (DM) and diabetic rats + hAFSCs transplantation (DM + hAFSCs). The immunoreactivity of Substance P can be seen in control rats (A), diabetic rats (B), and diabetic rats + hAFSCs transplantation (C) at 4 weeks, and in control rats (D), diabetic rats (E), and diabetic rats + hAFSCs transplantation (F) at 12 weeks. When compared to the controls, the expressions of substance P in the diabetic rats are decreased at 4 weeks, but can be recovered to near the control level after hAFSCs transplantation. There is no significant difference at 12 weeks. OD= optical density. Bar indicates 40  $\mu$ m. \*P < 0.05 vs. control group. N = 10 in each time point.

