Supplementary information to

Mesenchymal Stem Cells Reversed Morphine Tolerance and Opioid-induced Hyperalgesia

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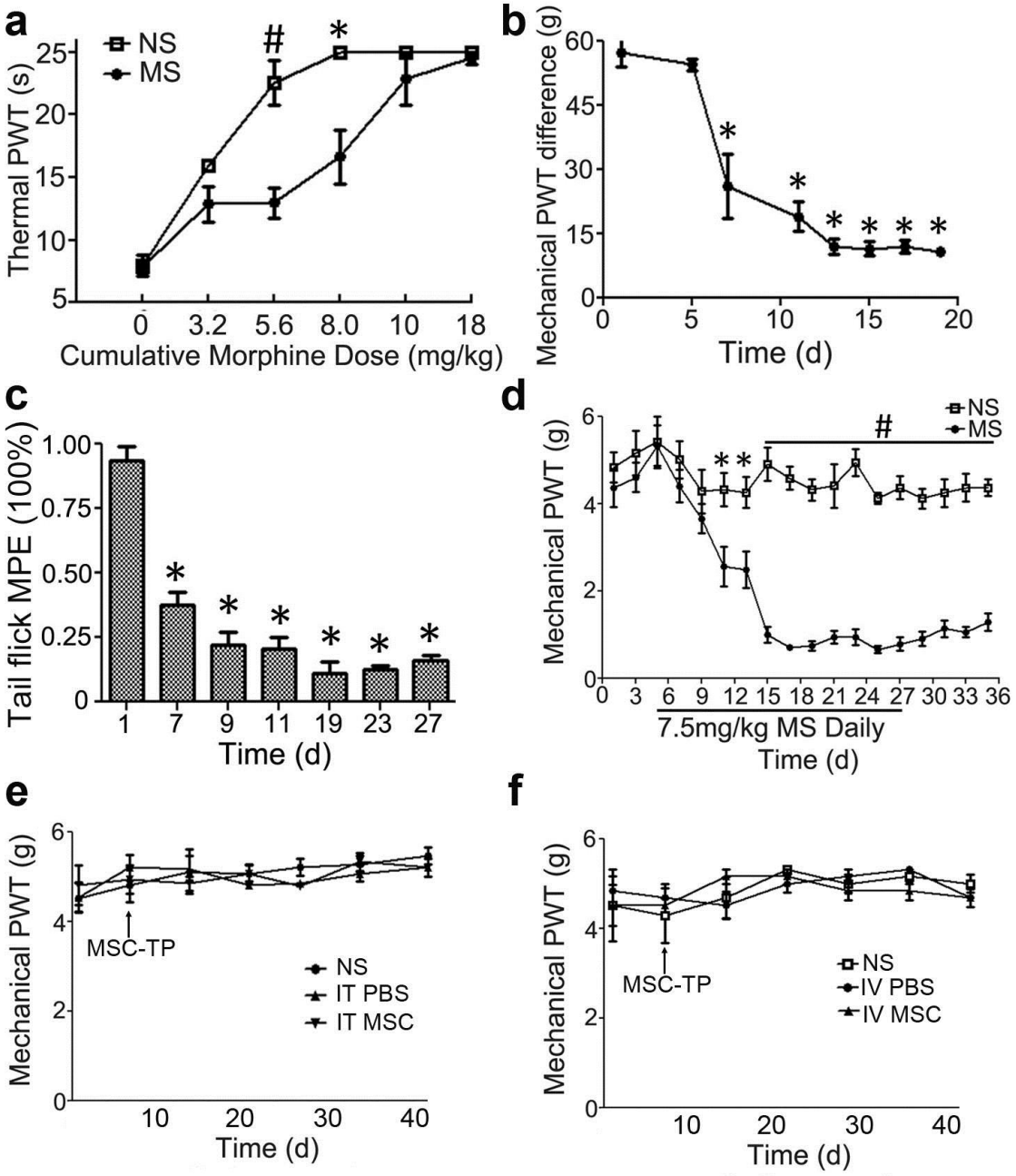
Introductory paragraph word count: 195; Text word count: 1499; Figures: 4; Supplementary Figures: 3; Table: 0; References: 34

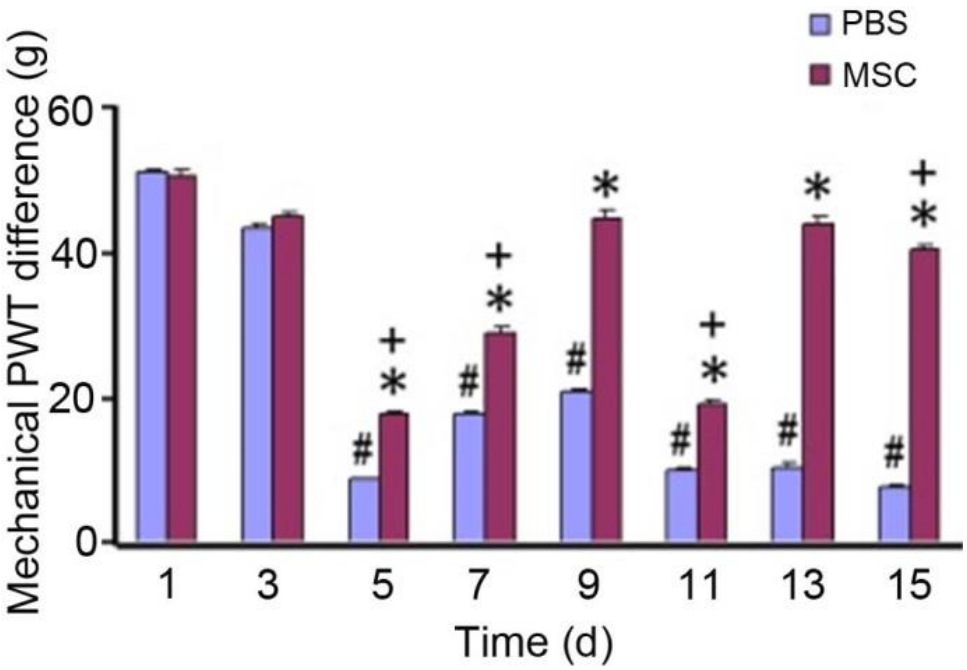
Supplement Figure 1



Supplement Figure 1. **Induction of opioid tolerance (OT) and opioid-induced hyperalgesia (OIH). (a)** Acute tolerance. Agonist dose-response curves were constructed with increasing doses of morphine (MS) (0–18 mg/kg) in rats that had received 3 days of daily injections of normal saline (NS) or MS (7.5mg/kg). The Plantar test was used to construct the dose-response curves 30 min after third day of daily NS or MS injection. (MS: n=5 and NS: n=3, thermal

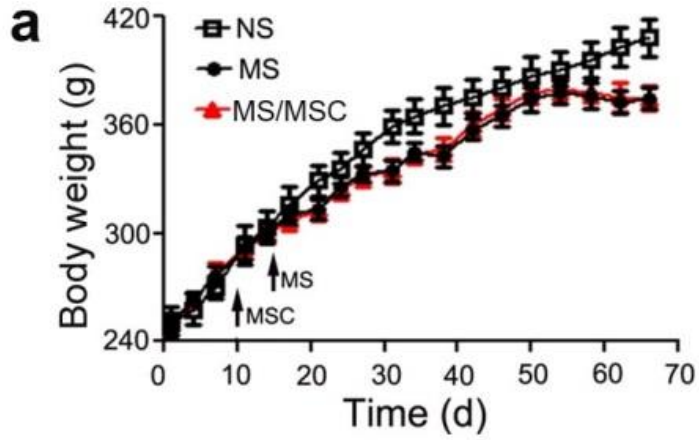
response cutoff time: 25s; #P<0.01; *P<0.05 between groups). **(b)** Chronic tolerance assessed by mechanical stimulation. Rats were treated with MS daily for 3-4 weeks. Paw withdrawal thresholds to mechanical stimulation by von Frey filaments were tested before and 50 min after MS injection. The difference between the two measurements indicates responsiveness to MS. A large difference indicates low or no tolerance while a small difference indicates high tolerance (n=8, *P<0.05 compared the mean value recorded in Day 1 of MS injection). **(c**) Chronic tolerance assessed by tail flick test. Maximum possible effect (MPE) of MS was used to indicate tolerance. The lower the MPE (%) the higher the tolerance (n=12, *P < 0.05 compared the mean value recorded in Day 1 of MS injection). (**d**) Chronic OIH. PWTs were assessed by von Frey filament before daily MS injections. The progressive declining of PWTs indicates hyperalgesia in response to daily morphine injections (n=6, #P<0.01; *P<0.05 compare to the NS control group). (**e, f**) In normal rats, PWTs did not change in response to intrathecal (**e**) or intravenous (**f**) injection of PBS or MSCs ($0.5x\ 10^6$) (n=6-8). Data: mean$\pm$ s.e. IT, intrathecal; IV, intravenous; MPE, maximal possible effect; MS, morphine sulfate; NS, normal saline; PWT, paw withdrawal threshold.

Supplement Figure 2



Supplement Figure 2. MSC-TP reversed OT induced by daily morphine injections in rats. Decreasing PWT difference between the measurements made before and 30 min after MS injection indicates OT. * P<0.05 compared with same day PBS; # P<0.05 compared with PBS Day 1; + P<0.05 compared with MSC Day 1. n=12 in each group. Please note the sham control group (injection with saline) was not included for clarity. The effect of morphine injection is reflected by increased PWTs (larger Y axis scale).

Supplement Figure 3



b

Group	ALT(U/L)	Cholesterol (mg/dL)	Glucose(mg/dL)	BUN(mg/dL)	Creatinine(mg/dL)
NS	63.7±3	92.7±3	155.0±9	18.0±1.1	0.2±0
MS	70.3±3	91.3±7	151.7±10	18.6±2.2	0.2±0
IT MSC	60.0±9	86.3±3	143.3±10	18.4±2.5	0.3±0.1
IV MSC	75.8±6	96.3±5	152.8±21	18.6±2.8	0.2±0.2

Supplement Figure 3. Long term safety of MSC transplantation (MSC-TP). (**a**) Body weight gain was not affected by MSC-TP. Daily MS injections slightly but significantly reduced body weight gain compared to the NS control group ($P<0.05$ in many time-points between Days 24 and 68). However, there were no significant differences between the MS group and the MS+MSC group ($P>0.05$). NS group n=6, MS group n=11, MS+MSC group n=12. Data mean± s.e. (**b**) Normal liver and kidney functions after long-term MSC-TP. Blood plasma was collected from rats at the end of the experiments (55 days after MSC-TP). Biochemical tests for liver function (ALT, cholesterol and glucose) and kidney function (BUN, creatinine) were performed. Data: mean± s.e. $P>0.05$. n=6 each group. MS, morphine sulfate; MSC, mesenchymal stem cell; NS: normal saline.