# Repeated Intravenous Administration of hiPSC-MSCs Enhance the Efficacy of Cell-based Therapy in Tissue regeneration

Si-Jia Sun, MD;[1,2] Ming Dong, PhD;[3] Wing-Hon Lai, PhD;[1] Wai-In Ho, MPhil;[1] Rui Wei, PhD;[1] Wei-Hao Liang, MD;[4] Fei Li, Mphil;[1] Song-Yan Liao, PhD;[1,5*] Hung-Fat Tse, MD, PhD.[1, 5, 6, 7*]

[1]Cardiology Division, Department of Medicine, Queen Mary Hospital, the University of Hong Kong, Hong Kong SAR, China; [2]Division of Cardiology, Department of Medicine, The First Affiliated Hospital of Soochow University, Soochow University, Suzhou, China; [3]Bioland Laboratory, Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou, China; [4]Department of Cardiology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; [5]Shenzhen Institutes of Research and Innovation, the University of Hong Kong, Hong Kong SAR, China; [6]Hong Kong-Guangdong Joint Laboratory on Stem Cell and Regenerative Medicine, the University of Hong Kong and Guangzhou Institutes of Biomedicine and Health, China; [7]Department of Medicine, Shenzhen Hong Kong University Hospital, Shenzhen, China;

**Short title:** Immunomodulation of Repeated Cell Therapy

*SY Liao and HF Tse contributed equally to the supervision of this work and are co-corresponding authors.

**Address for correspondence:**

Hung-Fat Tse, MD, PhD; or Song-Yan Liao, PhD;

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China. Tel.: (852) 2255-4694; Fax: (852) 2818-6304

E-mail: hftse@ hku.hk; or lsy923@hku.hk

## Supplemental materials

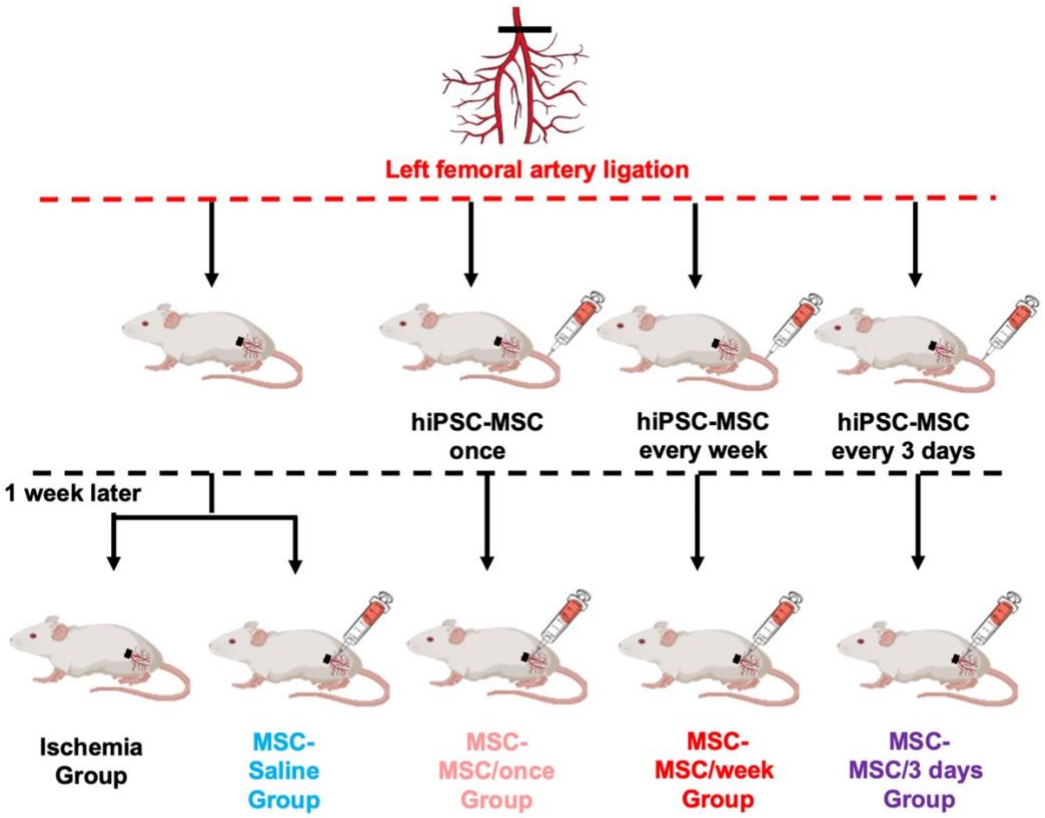
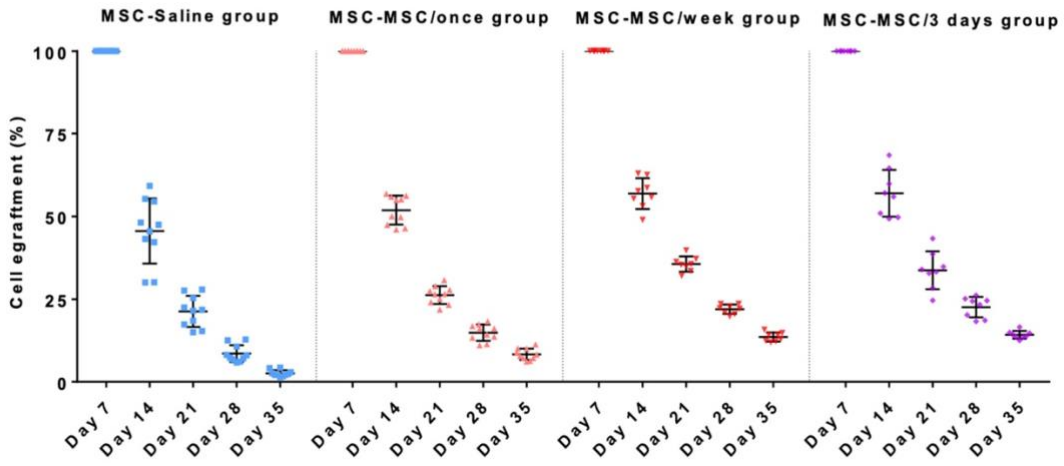



**Figure S1. Flow chart of the experiment.**

A




B

| | MSC-Saline | MSC-MSC/once | MSC-MSC/week | MSC-MSC/3 days |
|---|---|---|---|---|
| Day 7 | 100% | 100% | 100% | 100% |
| Day 14 | 45.47±3.11% | 51.89±1.38% | 56.88±1.65% | 56.99±2.49% |
| Day 21 | 21.28±1.49% | 26.22±0.85% | 35.39±0.82% | 33.71±2.03% |
| Day 28 | 8.54±0.81% | 14.87±2.49% | 21.97±1.41% | 22.58±3.09% |
| Day 35 | 2.59±0.31% | 8.33±0.54% | 13.56±0.49% | 14.23±0.42% |

**Figure S2. Repeated intravenous administration of hiPSC-MSCs prolonged the survival of transplanted hiPSC-MSCs.**

The estimated survival rates at day 14, 21, 28 or 35 was calculated as the percentage of the radiant efficiency at day 14, 21, 28 or 35 versus the percentage of the averaged radiant efficiency after intravenous transplantation. (**A**). At day 35, the estimated survival rates in MSC-Saline, MSC-MSC/once, MSC-MSC/week and MSC-MSC/3 days groups decreased to 2.59±0.31%, 8.33±0.54%, 13.56±0.49% and 14.23±0.42%, respectively (**B**).

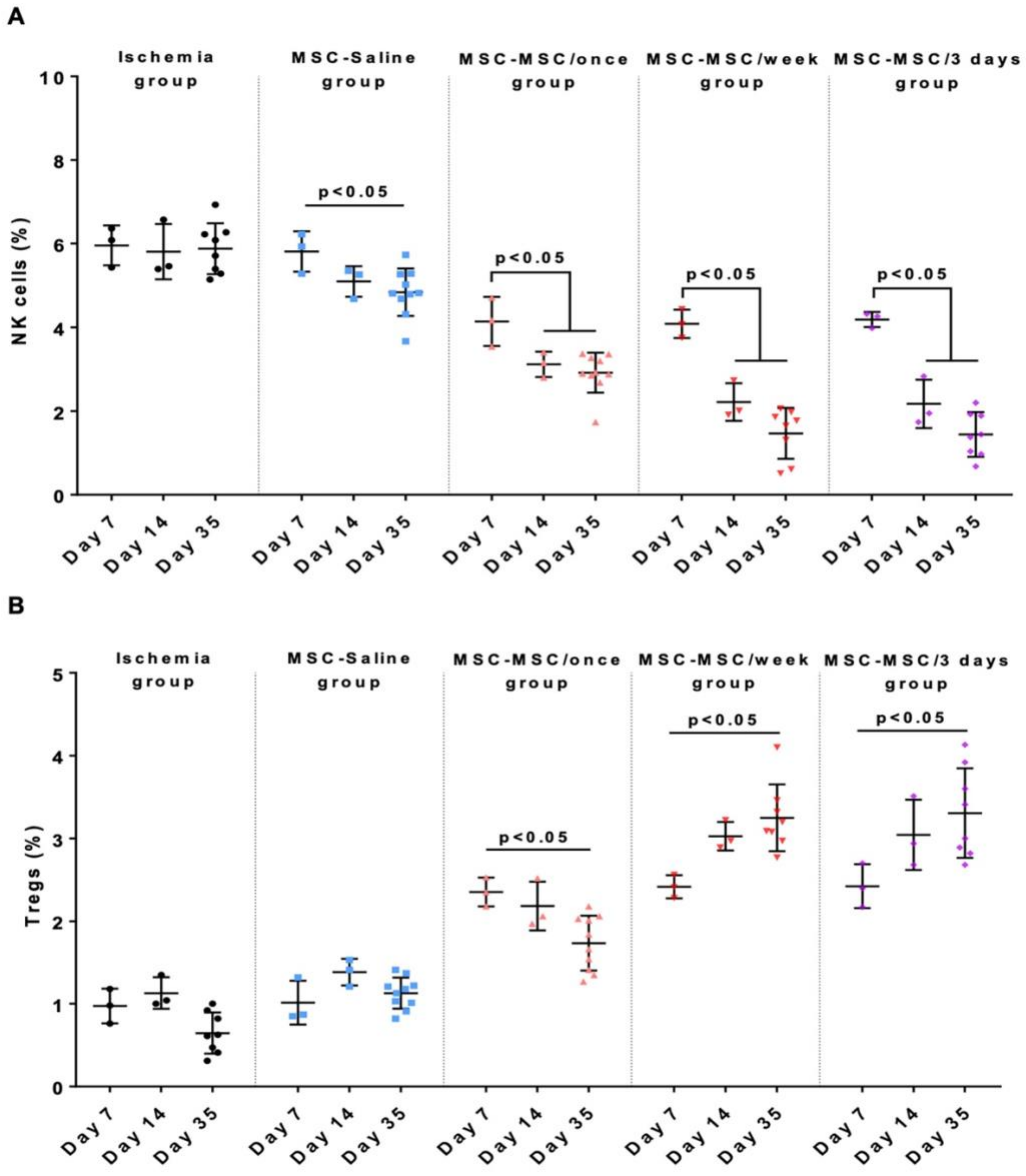



**Figure S3. Repeated intravenous administration of hiPSC-MSCs further reduced NK cells and continuously increased Tregs.**

The changes of splenic Tregs and NK cells within each group were showed in this figure. Splenic NK cells progressively decreased following intramuscular hiPSC-MSCs transplantation or intravenous hiPSC-MSCs infusion in MSC-Saline, MSC-MSC/once, MSC-MSC/week and MSC-MSC/3 days groups, whereas no significant difference between different time points in ischemia group (**A**). Splenic Tregs progressively decreased after reached the peak level at day 7 in MSC-MSC/once group, whereas these immunomodulatory cells continued to increase in MSC-MSC/week and MSC-MSC/3 days groups. No significant difference between different time points in ischemia and MSC-Saline groups (**B**).

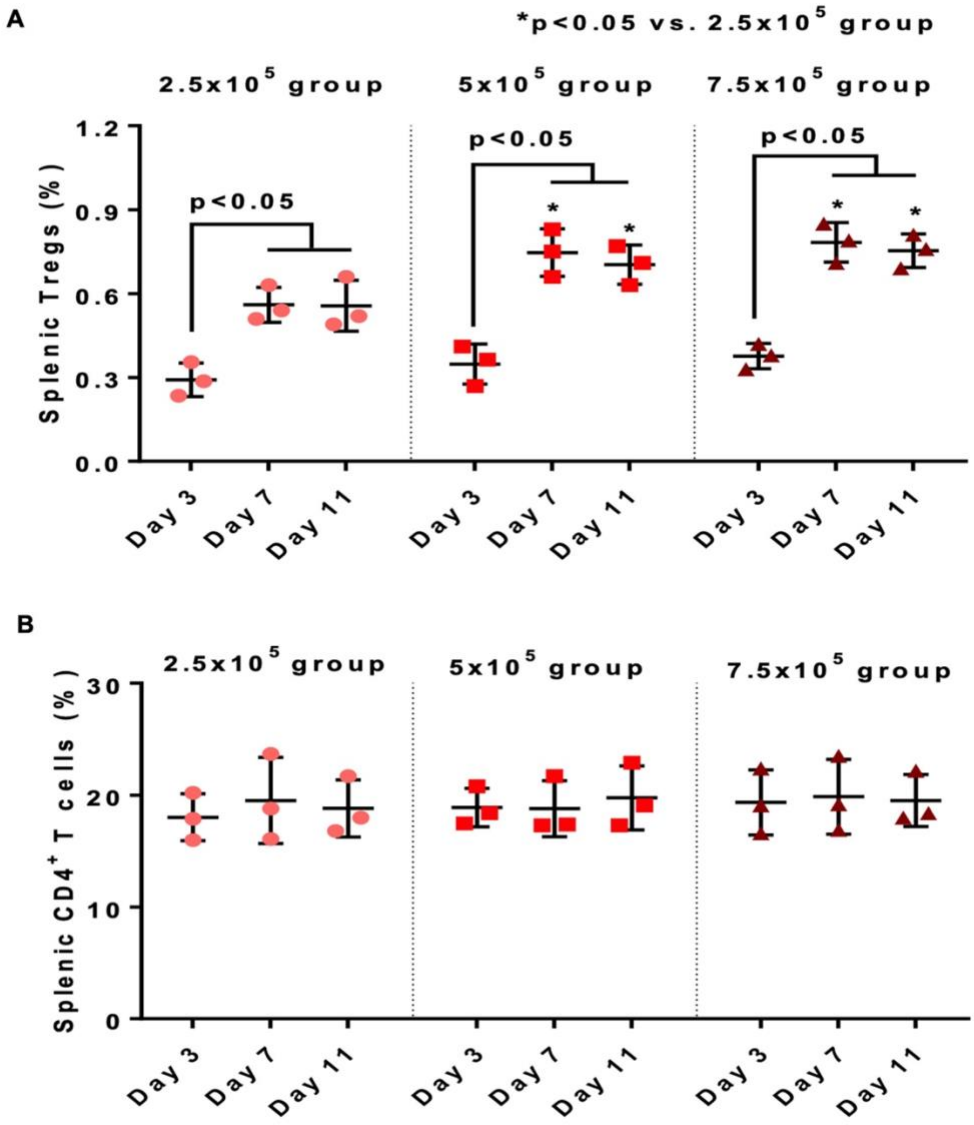



**Figure S4. The optimal time and dosage for systemic intravenous hiPSC-MSC infusion.** To define the optimal time and dosage for systemic intravenous hiPSC-MSC infusion, we compared the changes of splenic Tregs after a single intravenous administration of $2.5x10^5$, $5x10^5$ or $7.5x10^5$ hiPSC-MSCs in the $2.5x10^5$, $5x10^5$ or $7.5x10^5$ groups respectively. The results showed that after a single intravenous hiPSC-MSC infusion in the $2.5x10^5$, $5x10^5$ or $7.5x10^5$ groups, splenic Tregs progressively increased to their peak level at day 7. When comparison among $2.5x10^5$, $5x10^5$ and $7.5x10^5$ groups was performed, higher splenic Tregs level was observed in the $5x10^5$ or $7.5x10^5$ groups compared with the $2.5x10^5$ group. There was no significant difference between the $5x10^5$ and $7.5x10^5$ groups (**A**). There was no significant difference in splenic $CD4^+$ T cells within or between all three groups (**B**).

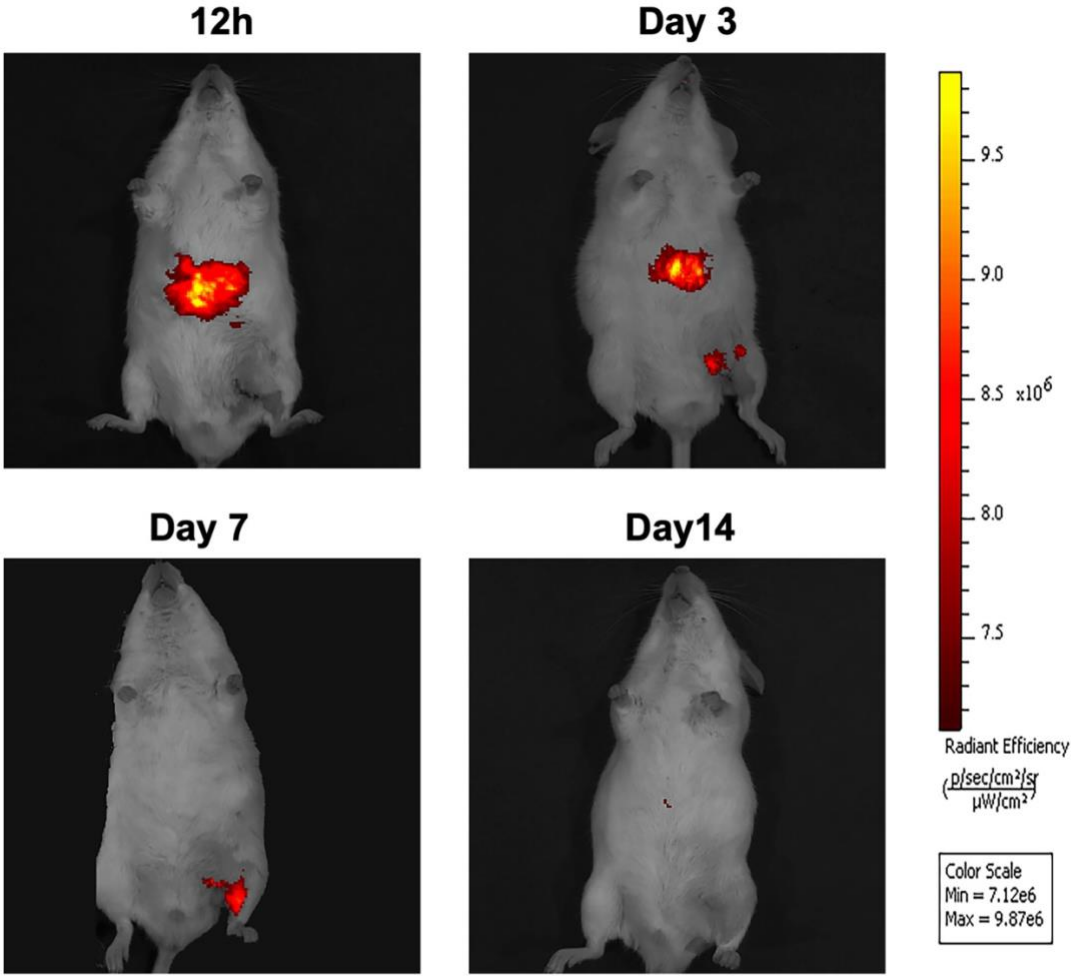



**Figure S5. Biodistribution of intravenously administrated hiPSC-MSCs.**

To determine cell engraftment after a single intravenous cellular infusion, 3 mice receiving intravenous DiR-labeled hiPSC-MSC infusion after induction of ischemia were scanned under epi-fluorescent imaging at 12h and on day 3, 7 and 14. Most hiPSC-MSCs engrafted into the liver 12h after intravenous cellular infusion. The engrafted hiPSC-MSCs gradually migrated into ischemic limbs at day 3 and had disappeared by day 14.

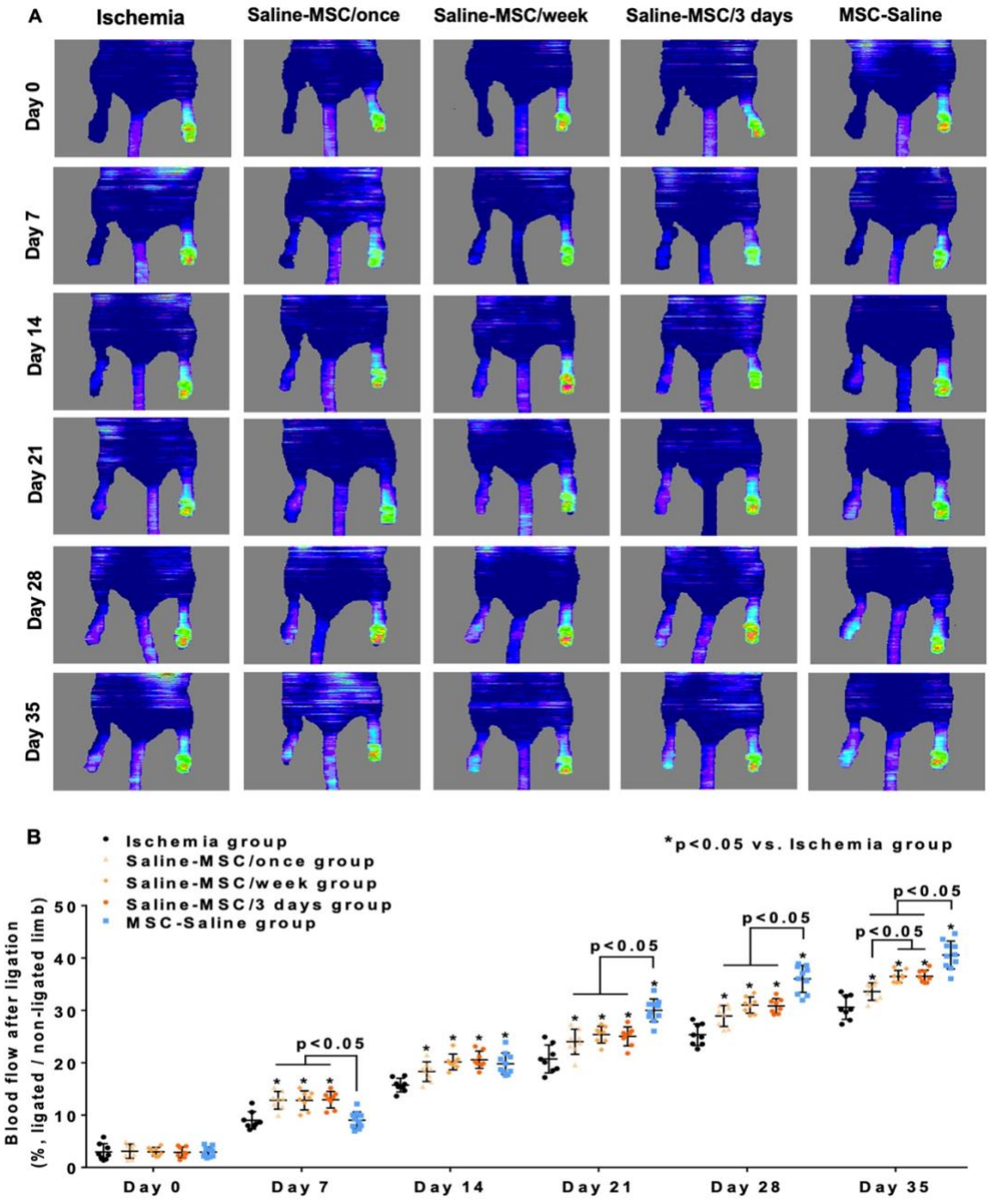



**Figure S6. Intravenous administration of hiPSC-MSCs without local cellular delivery improved blood perfusion.**

To evaluate blood perfusion in the groups that received intravenous hiPSC-MSCs infusion without intramuscular hiPSC-MSCs transplantation, Laser Doppler imaging analysis was performed immediately and every week following femoral artery ligation **(A)**. A single or repeated intravenous administration of hiPSC-MSCs in the Saline-MSC/once, Saline-MSC/week or Saline-MSC/3 days groups significantly increased blood perfusion from day 7 onwards compared with the ischemia group. Moreover,

repeated intravenous hiPSC-MSCs infusion further improved blood perfusion at day 35. Nonetheless intramuscular hiPSC-MSC transplantation in the MSC-Saline group showed a superior beneficial effect over repeated intravenous hiPSC-MSC infusion in the Saline-MSC/week and Saline-MSC/3 days groups (**B**).

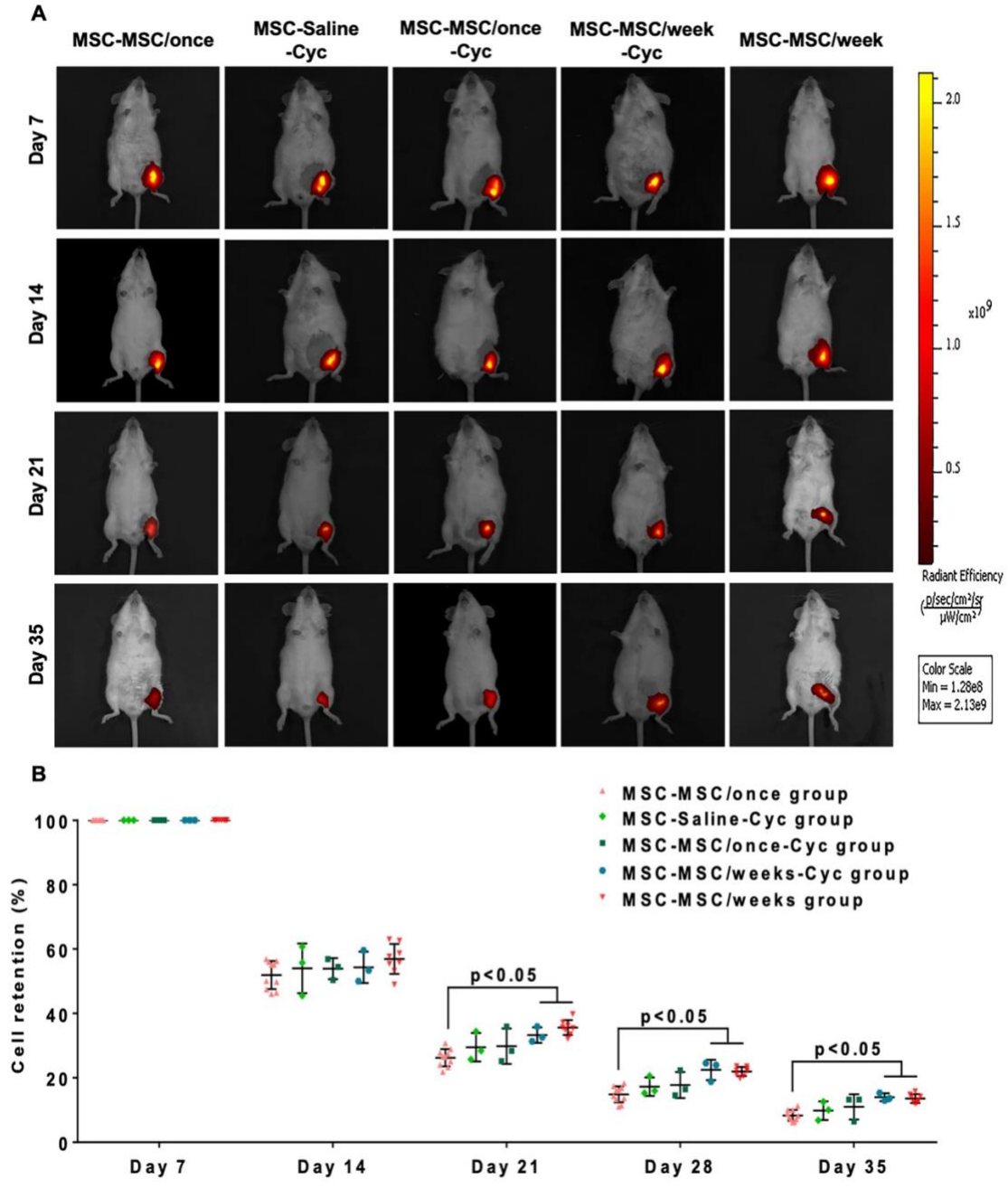



**Figure S7. The cell survival and engraftment have no difference between subcutaneous administration of cyclosporine A and single or repeated intravenous hiPSC-MSC infusion.**

To evaluate cell engraftment in the groups that received daily subcutaneous administration of cyclosporine A treatment, a series of fluorescent images of ischemic hind limb was obtained immediately and every week following intramuscular transplantation of hiPSC-MSCs **(A)**. There was no significant difference among the MSC-MSC/once, MSC-Saline-Cyc and MSC-MSC/once-Cyc groups. Repeated

intravenous infusion of hiPSC-MSCs with or without subcutaneous administration of cyclosporine A significantly increased cell engraftment compared with the MSC-MSC/once group (**B**).

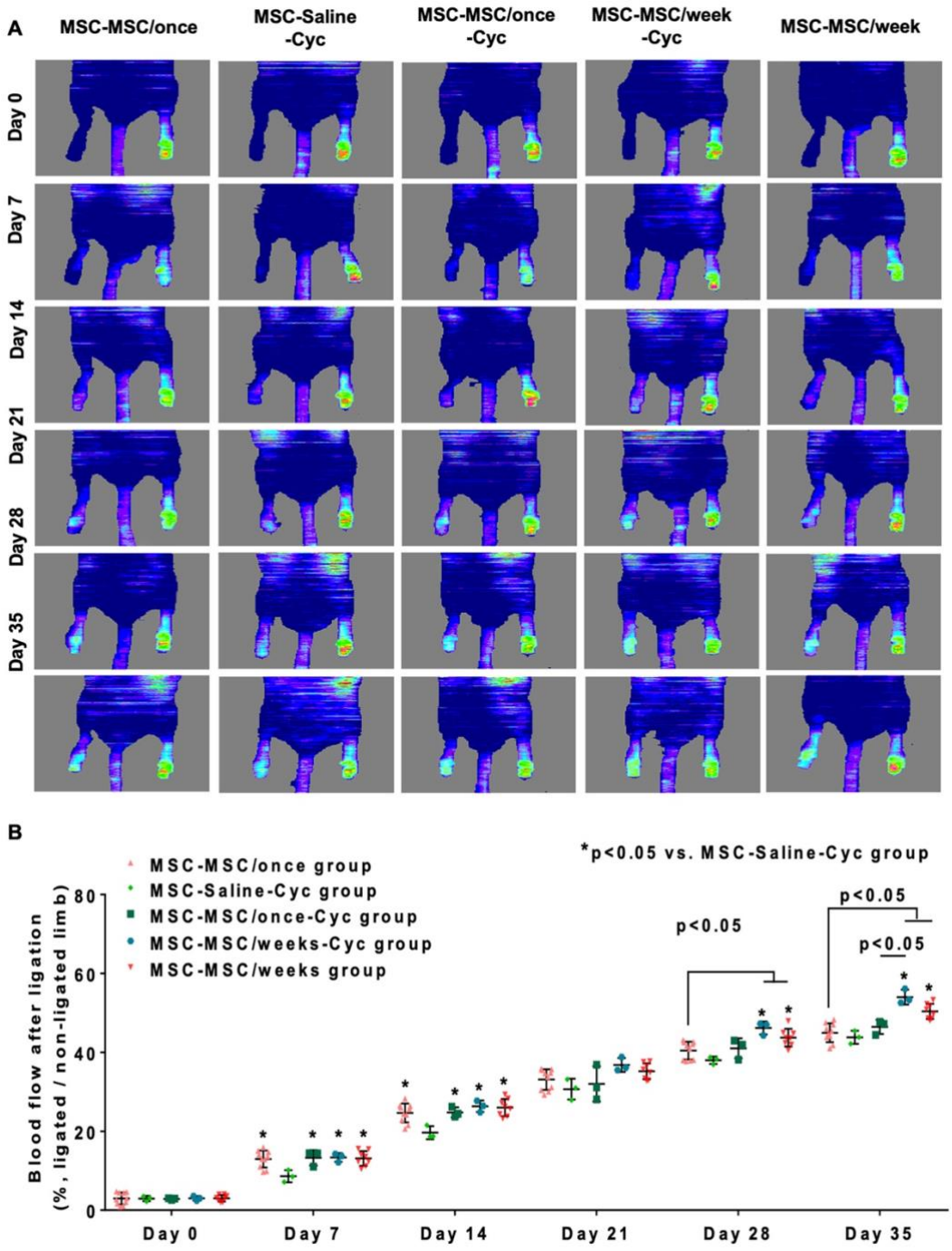



**Figure S8. Subcutaneous administration of cyclosporine A did not improve the blood perfusion without intravenous hiPSC-MSC infusion.**

To evaluate blood perfusion in the groups that received subcutaneous administration of cyclosporine A, Laser Doppler imaging analysis was performed immediately and every week following femoral artery ligation **(A)**. A single intravenous administration of hiPSC-MSCs in the MSC-MSC/once, MSC-MSC/week, MSC-MSC/once-Cyc or MSC-MSC/week-Cyc groups significantly increased blood perfusion during first two weeks

compared with the MSC-Saline-Cyc group. Nonetheless, by the end of experiment, no significant difference was observed among the MSC-MSC/once, MSC-Saline-Cyc and MSC-MSC/once-Cyc groups. Repeated intravenous infusion of hiPSC-MSCs with or without subcutaneous administration of cyclosporine A significantly increased therapeutic efficacy relative to the MSC-MSC/once group (**B**).