Human Gingiva-Derived Mesenchymal Stem Cells Ameliorate Streptozoticin-induced T1DM in mice via Suppression of T effector cells and Up-regulating Treg Subsets

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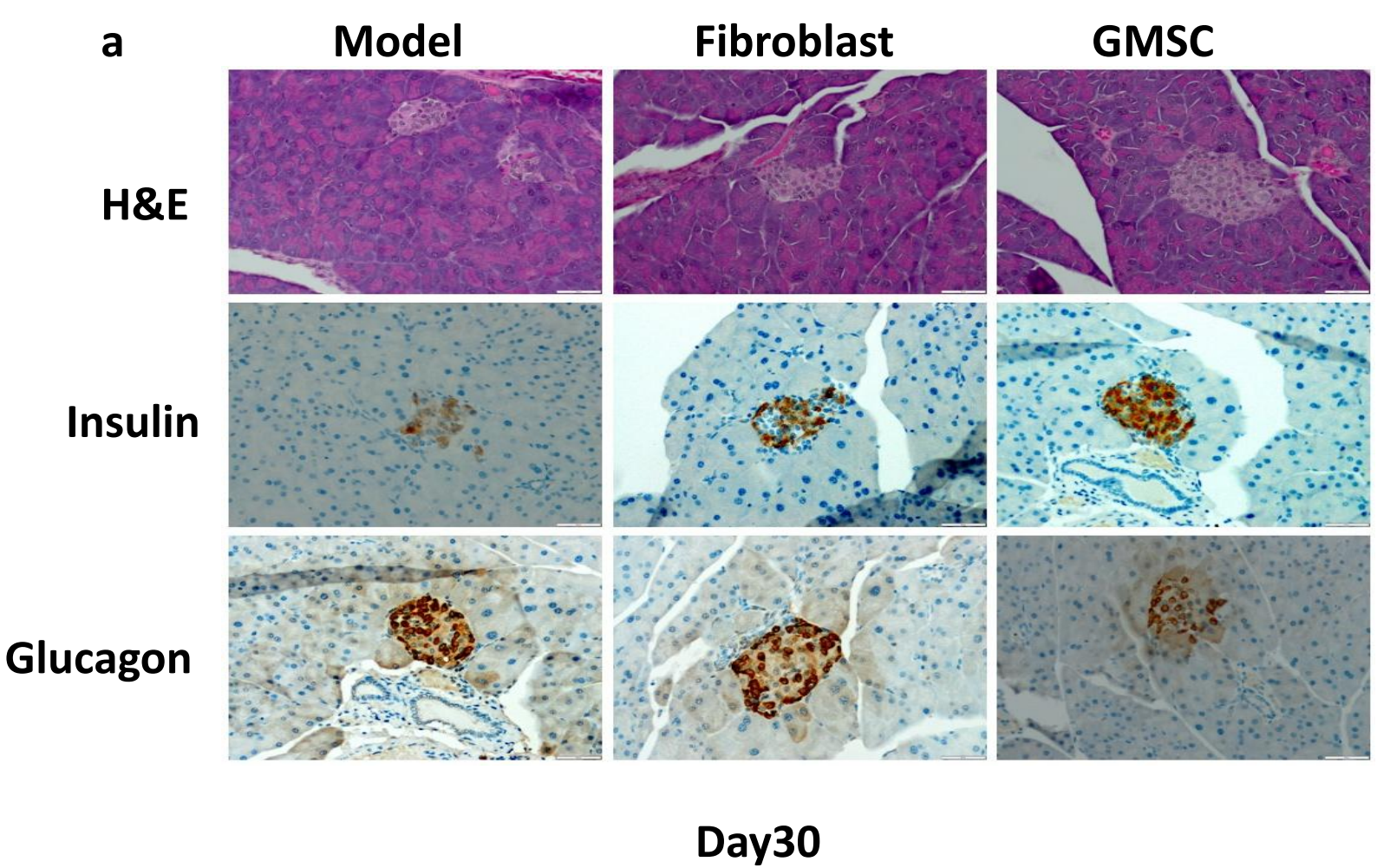
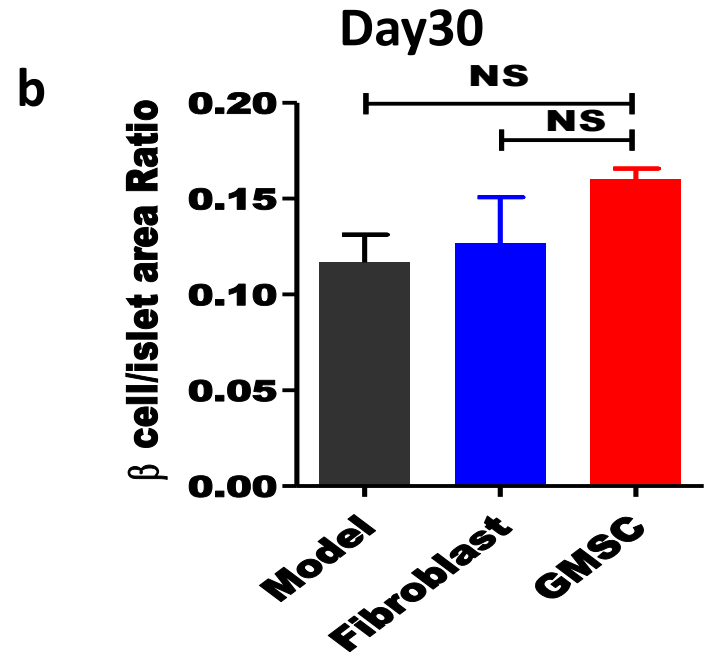
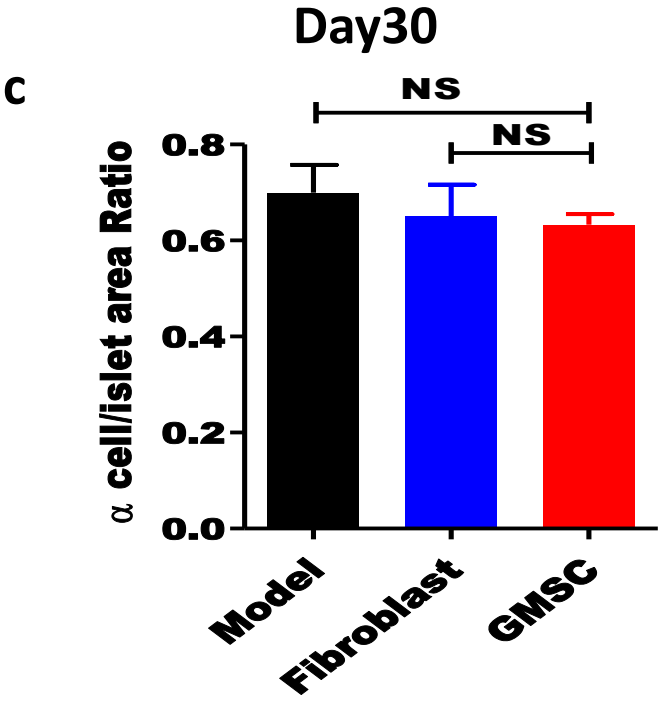
Supplementary Figure S1





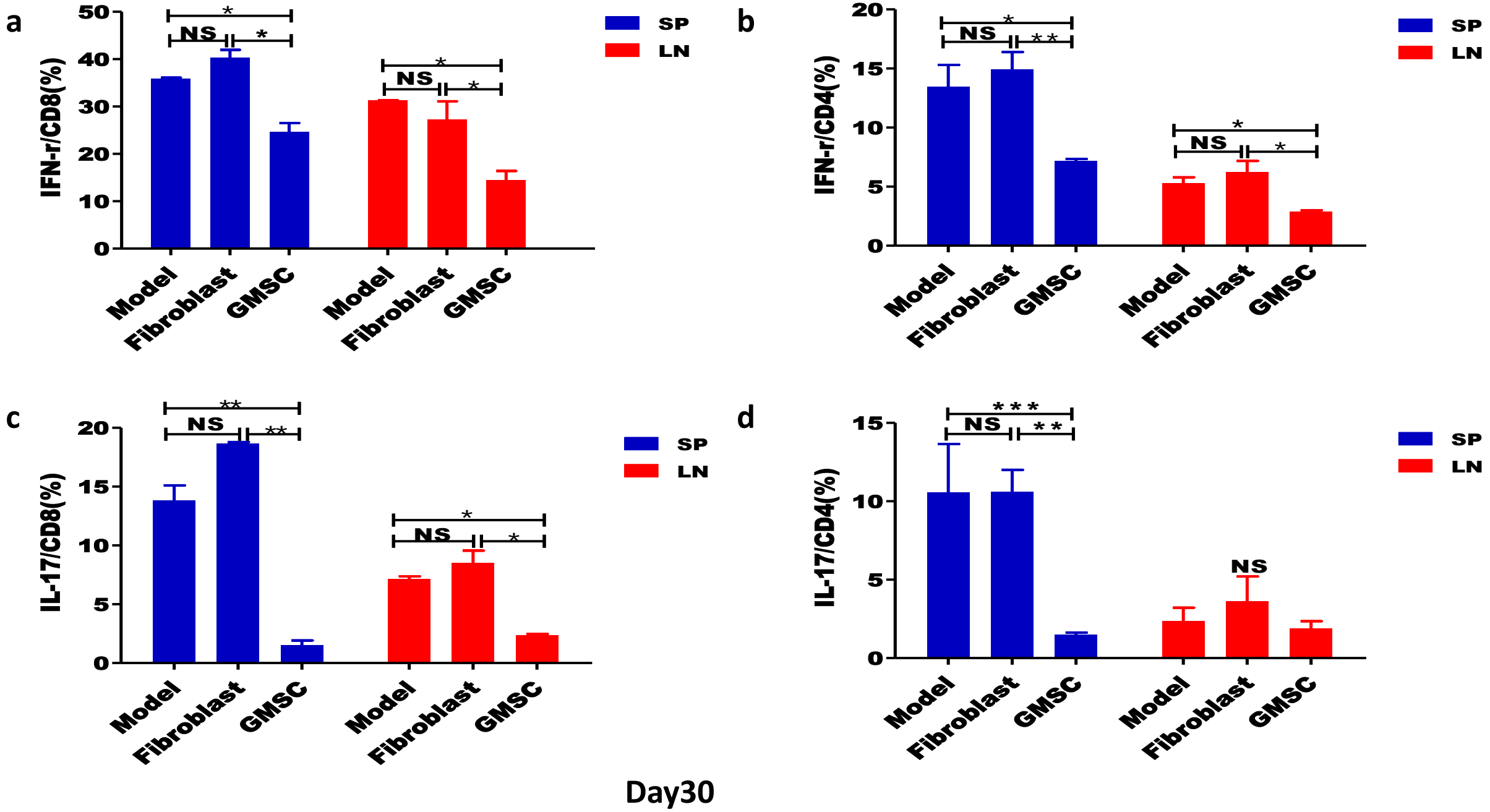


Supplementary Figure S1

GMSCs couldn’t ameliorate the severity of insulitis in mice model of T1DM on day 30.

Pancreas were harvested on day 30, fixed in 10% formalin, embedded in paraffin. Six sections per pancreas, at least three individual pancreases from each group, were evenly sectioned and separated by 200μm, stained with H&E, anti-insulin and anti-glucogon. a, Hematoxylin and eosin staining and immunohistochemistry examination of pancreas from three groups. b-c, Insulin-positive beta cells and glucogon-positive alpha cells in islets area were revealed by immunohistochemistry using the anti-insulin antibody and anti-glucogon antibody on day 30. Data are presented as the mean ± SEM from two independent experiments using at least three individual pancreases from each group. (NS: not significant)

Supplementary Figure S2

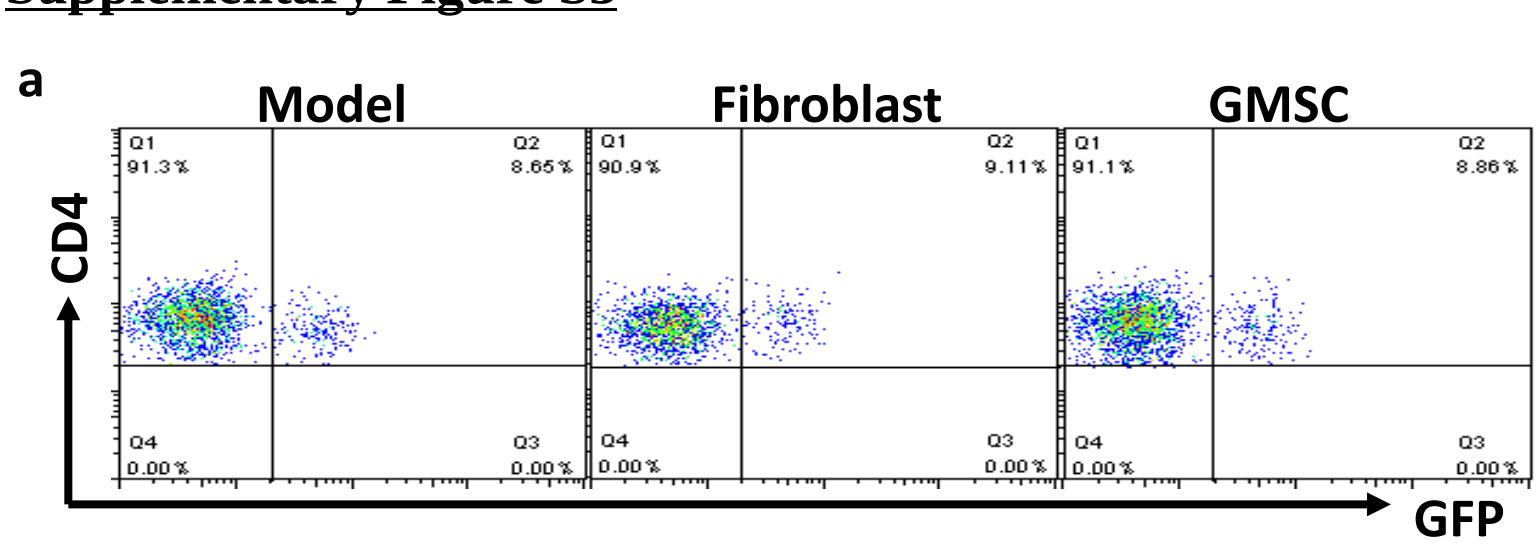


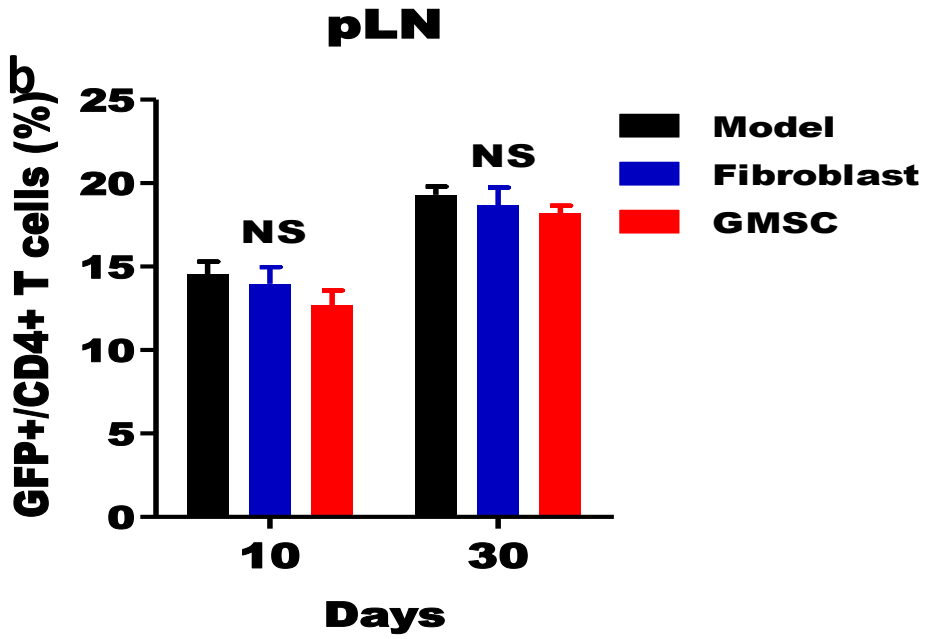
Supplementary Figure S2

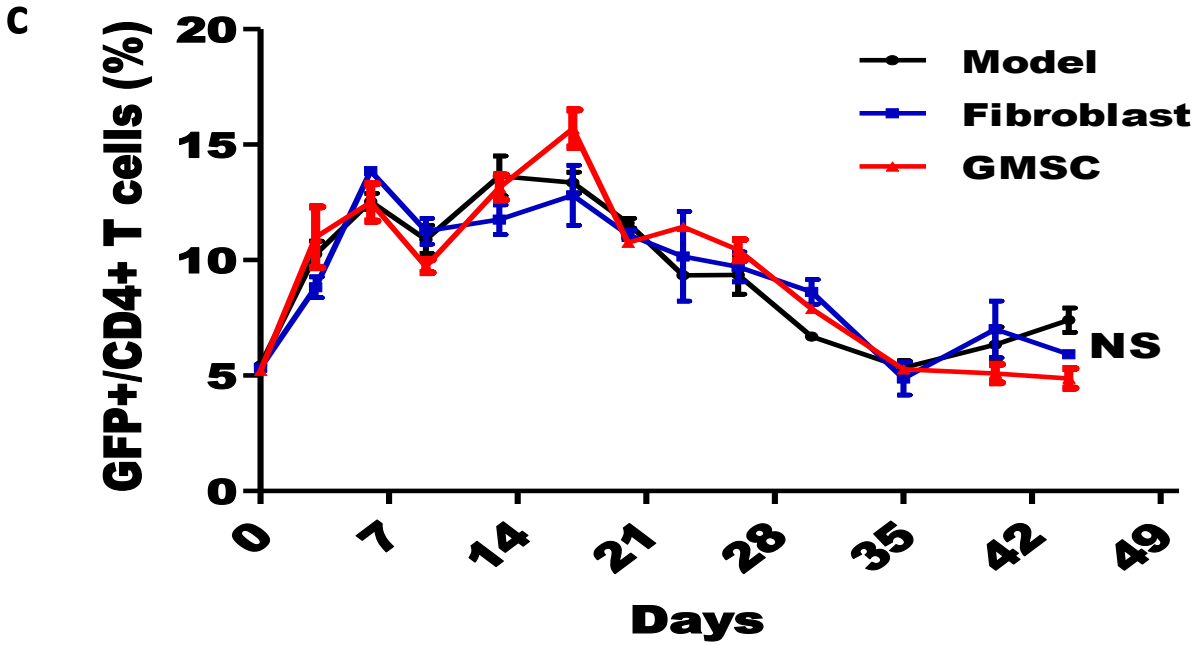
GMSCs could also down-regulated IL-17 and IFN-γ expression in spleen and lymph nodes in STZ-induced T1DM model on day 30.

T1DM was induced using multiple low dose STZ injection and 1 ×10^6 GMSCs or fibroblast cells were injected into mice via *i.p.* on day 0,7,14,21,28. Spleen and other lymph nodes like MLN were harvested on day 30. Lymphocytes were isolated and then stimulated in vitro with PMA (50 ng/ml) and ionomycin (500 ng/ml) for 5 hours, with brefeldin A (10μg/ml) added in the last 4 hours, and intracellular expression of IFN-γ and IL-17 on $CD4^+$ and $CD8^+$ T cells was analyzed by flow cytometry. a-b, Expression of IFN-γ on $CD8^+$ T and $CD4^+$ T cells in spleen (blue) and LN (red). c-d, Expression of IL-17 on $CD8^+$ T and $CD4^+$ T cells in spleen (blue) and LN (red). Data are presented as the mean ± SEM from two independent experiments (n=3). *P<0.05, **P<0.01, ***P<0.01, versus the fibroblast or model group.

Supplementary Figure S3







Supplementary Figure S3

GMSCs has no effect on the proportions of $CD4^+$ Tregs circulating in the peripheral blood and those residing in pLN of T1DM mice.

C57BL/6 $Foxp3^{gfp}$ reporter mice were injected via intraperitoneal route with GMSCs (1×10^6). $CD4^+$ $Foxp3^+$ (GFP^+) Tregs percentage in the peripheral blood and those residing in pLN of T1DM mice were tested after GMSC injection. a, Representative flow data of $CD4^+$ $Foxp3^+$ frequency in day 30 pLN in each groups. Cells were gated on $CD4^+$ subset. b, statistics analysis of $CD4^+$ $Foxp3^+$ (GFP^+) Tregs percentage in pLN on day10 and day 30. Each group in each time points had three mice. Data were representative of two separate experiments and mean ± SEM of each group was shown. c, statistics analysis of $CD4^+Foxp3^+(GFP^+)$ Tregs percentage in the peripheral blood dynamically after GMSCs injection. Data were representative of two separate experiments and mean ± SEM of each group (n=3) was shown.