Supplemental Methods

Isolation and culture of mouse BMMSCs and CXCR4 blocking

Mouse BMMSCs were isolated and cultured as described previously.[1] Bone marrow cells were flushed out from bone cavity of femurs and tibias with heat-inactivated 20% fetal bovine serum (FBS; Invitrogen) in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen). All nuclear cells (ANCs) were seeded at 10-20 × 10^6 into 100 mm culture dishes (Corning) and initially incubated for 3 days under 37^{o}C at 5% CO_2 condition. To eliminate the non-adherent cells, the cultures were washed with PBS twice. The attached cells were cultured for additional 7 days. Colonies-forming attached cells were passed once for further experiments. The BMMSCs were cultured with DMEM supplemented with 20% FBS, 2 mM L-glutamine (Invitrogen), and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Invitrogen). To confirm mesenchymal stem cell character, flow cytometric analysis were used to show these BMMSCs are positive for CD73, CD90, CD105, CD146, Stro-1 and SSEA-4 and negative for CD11b, CD34 and CD45.

Before neutralizing antibody blocking, BMMSCs was washed twice by PBS. Neutralizing anti-CXCR4 antibody (5μg/ml/5 × 10^5 cells, R&D Systems) was added and incubated in 37°C for 20 min. Then cells were harvested, washed twice by PBS and prepared for infusion.

BMMSCs in vitro differentiation assay

For osteo-induction *in vitro*, BMMSCs were cultured under osteogenic culture

condition containing 2 mM L-glycerophosphate (Sigma), 100 μM L-ascorbic acid 2-phosphate (Sigma) and 10 nM dexamethasone (Sigma). After the induction, the cultures were stained with alizarin red. For the adipo-induction *in vitro*, adipogenesis differentiation kit (Invitrogen) was used. Two weeks after the adipo-induction, the cultures were stained with Oil Red-O. The mineralized area and Oil Red-O positive cells were quantified by using Image Pro Plus 6.0. Total RNA were isolated from the mouse BMMSCs two weeks after induction. All experiments were repeated in three independent samples for each group.

T cell proliferation assay

Mouse splenocytes were isolated from ICR mice spleen and resuspended in rewarmed PBS at a final concentration of 5×10^6 then labeled by 5mM Carboxyfluorescein succinimidyl ester (CFSE; Invitrogen) for 10 min in PBS/0.1%BSA. To stop labelling, 5 volumes of ice cold PBS containing 1% BSA were added and washed 3 times with culture medium containing RPMI 1640 media (Invitrogen) with 10% FBS (Invitrogen), 2 mM L-glutamine (Invitrogen), and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Invitrogen). T cells were stimulated with immobilized 10 μg/ml anti-mouse CD3 antibody (BD Pharmingen), with BMMSCs from NOD/Ltj or ICR mice at the ratio of 1:1 for 4 days. For human cells, PBMCs were separated from 2 ml healthy human peripheral blood samples by Ficoll-Hypaque density centrifugation. After labeled by CFSE, human PBMCs were stimulated by 3 μg/ml immobilized anti-human CD3 antibody (BioLegend) and 2 μg/ml soluble anti-human

CD28 antibody (BioLegend) for 24 hours and then co-cultured with BMMSCs from SS patients or healthy human in the ratio of 1:1 for 4 days. Lymphocytes were then harvest and assayed by a flow cytometric. Proliferation index was calculated by Modfit LT 3.0 software, as the average number of cell divisions versus the original population.

Mice saliva flow rate test

For saliva flow rate test,[2] mice were weighed and mild anesthesia was induced with a solution of Ketamine (100 mg/ml, Beijing Double-Crane Pharmaceutical Co., China) and Xylazine (20 mg/ml; Sigma) in sterile water, given intraperitoneally (1 μl/g of body weight). Salivary secretion was stimulated using 0.1 ml/kg body weight of a pilocarpine solution (50 mg/ml, Beijing Double-Crane Pharmaceutical Co., China) subcutaneously. Saliva collection began within two minutes of pilocarpine administration. Animals were positioned with a 75 mm hematocrit tube placed in the oral cavity and whole saliva was collected into pre-weighed 0.75 ml Eppendorf tubes for 10 minutes. The amount of saliva collected was determined gravimetrically.

Mice splenectomy surgery

The splenectomy surgery of NOD/Ltj mice were carried out in a class II biological safety cabinets. Before the operation, animals were anesthetized and sterilize. Then an incision was made in the left side of the upper abdomen to expose the spleen. After ligated the splenic artery and vein, we cut the spleen and then sewn up the incision.

Real time PCR

Real-time PCR was performed using BioEasy SYBR Green I Real Time PCR Kit (Sunbio, China) with Line-gene Real-time PCR Detection System. Primers specific for real time PCR of the gene region are following: Actin: forward 5’-TGACGTGGACATCCGCAAAG-3’, reverse 5’-TGGAAGGTGGACAGCGAGG-3’; Gapdh: forward 5’-CGGAGCAAAAGGGTCATCATCTCCG-3’, reverse 5’-TGGTTCACACCCATCACAAACAT-3’; Alp: forward 5’-GCCTCAGCCTTCCATCTGTAAA-3’, reverse 5’-GTCCCCACGGTTGTTAATACCA-3’; Ranx2: forward 5’-CCTATGACCAGTCTTACCCCTCCTA-3’, reverse 5’-AGGTGGCAGTGTCATCATCTGA-3’; Pparg2: forward 5'-CTCAGACAGATTGTCACGGAACAC-3', reverse 5'-AGTGCAACTGGAAGAAGGGAAAT-3'; Lpl: forward 5’-AGGGAAAGCTGCCCACTTCTAG-3', reverse 5'-CAGGGCTTTGCTCTCCATCTC-3’; Cxcr4: forward 5'-CGGAAAACAAGGAACCCTGCTTCCG-3’, reverse 5’-GATGGTGGGCAGGAAGATCCTA-3’.

Flow cytometric analysis

For Treg cells staining, mononuclear cells from spleen of mice or peripheral blood of

patients (1 x 10^6/sample or subject) were stained with FITC-conjugated anti-CD4 antibody and APC-conjugated anti-CD25 antibody for 30 minutes at 4 ˚C, followed by staining with PE-conjugated anti-Foxp3 antibody using Foxp3 Staining Buffer Set (eBioscience) according to the manufacture's protocol for cell fixation and permeabilization. For Tfh cells staining, cells (1 x 10^6/sample) were stained with PE-conjugated anti-CXCR5 antibody and FITC-conjugated anti-CD4 antibody. For cytokine-producing cells (Th1, Th2, Th17 cells) staining, cells (1 x 10^6/sample) were stimulated with PMA (5 ng/ml; Sigma) and ionomycin (500 ng/ml; Sigma) in the presence of a protein transport inhibitor (GolgiStop, BD Bioscience) for 4 hours, and incubated with PerCP-conjugated anti-CD4 antibody. After fixation and permeabilization, cells were stained with anti-IFN-γ, anti-IL-4, anti-IL-13 or anti-IL17 antibodies. For GFP cells tracking, organs were crushed on nylon sieve (80 mesh), erythrocyte lysing solution (BD Bioscience) was used according to the manufacture's protocol to minimize non-specific fluorescence. Data were analyzed with FlowJo 6.0 software and Modfit LT 3.0 software.

Patient enrollment

All patients must fulfill all the following 2 items: (1) The classification criteria based on the revised American-European criteria (2002) for primary Sjögren's syndrome.[3] In brief, these criteria comprise subjective criteria: ocular symptoms and oral symptoms, and objective criteria: ocular signs, histopathological signs (focus-score ≥ 1) or parotid sialography, oral signs, and serological signs (presence of antinuclear

antibodies, anti-SSA or anti-SSB). Patients are diagnosis as primary Sjögren's syndrome if 4 of the 6 mentioned criteria are present, as long as histopathology or serology is positive, or if 3 of any 4 objective criteria are present. (2) Patients must be informed of the investigational nature of this study and given written informed consent. Exclusion criteria: (1) Active, uncontrolled infections; (2) End-stage organ failure.

Isolation and culture of human UCMSCs

Fresh umbilical cords were obtained from informed, healthy mothers in local maternity hospitals after normal deliveries and processed as quickly as possible. The cords were rinsed twice by phosphate-buffered saline (PBS) in penicillin and streptomycin, the cord blood being removed during this process. The washed cords were cut into 1-mm^2-sized pieces and floated in Dulbecco's modified Eagle's medium with low glucose (DMEM-LG) containing 10% FBS. The pieces of cord were subsequently incubated at 37℃ in humid air with 5% CO_2. Non-adherent cells were removed by washing. The medium was replaced every 3 days after the initial plating. When well developed colonies of fibroblast-like cells appeared after 10 days, the cultures were trypsinised and passaged into a new flask for further expansion.

All the infused UC-MSCs were derived from passage 2 to passage 5, with rigorous purification and quality control. Cell viability of purified MSC was >92% (by trypan blue testing), and each preparation was negative for pathogenic microorganisms, including aerobic and anaerobic bacterium (by direct cultivation analysis), and

negative for HBsAg, HBcAb, HCVAb, HIVAb-I and II, CMV-IgM, and syphilis-Ab (by enzyme linked immunosorbent assay). Flow cytometric analysis showed that CD29, CD73, CD90 and CD105 expression was >95%, in parallel with CD45, CD34, CD14, CD79 and HLA-DR expression <2%. In addition, levels of alanine aminotransferase (by automatic biochemistry analyzer) and endotoxin (by tachypleus amebocyte lysate analysis) in supernatant of each cell preparation were strictly controlled within 40IU/L and 5EU, respectively. The capacity of MSC that differentiate along adipogenic and osteogenic lineages was also assayed.

Sialography and saliva flow rate measurements for patients

Sialography of parotid glands, using 1.0 ml of 60% Urografin® (Sine Pharmacy Co., Shanghai; China) as a contrast medium, were taken during the filling phase by a lateral projection. 1 ml citric acid was applied to tongue of the patients, a lateral emptying phase film was taken for excretory function examination 5 minutes later. The same volume and procedure of sialography were performed for follow-up examinations. For unstimulated saliva flow rate test, patients were comfortably seated and, after a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they produced for 10 minutes into a test tube through a funnel. For stimulated saliva flow rate test, patients were asked to chew 5 gram medical used white wax spit all the saliva they produced for 6 minutes, into a test tube through a funnel. The whole volume collected for 10 or 6 minutes was then measured (ml).[4,5]

Systemic improvements determination for patients

Patient's global assessment of the overall impact of disease on well-being was rated on a 0-10 visual analog scale (VAS), where 0 indicates no impact of disease and 10 indicates very severe impact of disease. The SS Disease Activity Index (SSDAI) score[6] was also used for systemic improvements determination, briefly, in this index the constitutional symptoms, change in salivary gland, articular symptoms, hematologic features, pleuropulmonary symptoms, change in vasculitis, active renal involvement and peripheral neuropathy were all scored.

Supplemental References

1. Sun L, Akiyama K, Zhang H, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells. 2009; 27:1421-1432.
2. Cotrim AP, Sowers A, Mitchell JB, Baum BJ. Prevention of irradiation-induced salivary hypofunction by microvessel protection in mouse salivary glands. Mol Ther. 2007; 15:2101-2106.
3. Vitali C, Palombi G, Baldini C, Benucci M, Bombardieri S, Covelli M, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002; 61:554-558.
4. Qi S, Liu X, Wang SL. Sialoendoscopic and irrigation findings in chronic obstructive parotitis. Laryngoscope. 2005; 115:541-545.
5. Wang SL, Zou ZJ, Yu SF, Zhu JR. Recurrent swelling of parotid glands and Sjogren's syndrome. Int J Oral Maxillofac Surg. 1993; 22:1362-1365.
6. Vitali C, Palombi G, Baldini C, et al. Sjogren's Syndrome Disease Damage Index and disease activity index: scoring systems for the assessment of disease damage and disease activity in Sjogren's syndrome, derived from an analysis of a cohort of Italian patients. Arthritis Rheum. 2007; 56:2223-2231.

Supplemental Table

Table S1

Patient/ age/sex	Disease duration (months)	Clinical manifestations
1/63/F	144	Xerostomia, xerophthalmia, myelitis
2/38/F	78	Xerostomia, RTA, hypopotassaemia, thyroiditis, arthralgia
3/39/F	31	Xerostomia, xerophthalmia, ILD
4/67/F	19	Xerostomia, xerophthalmia, autoimmune thrombocytopenia,
5/39/F	18	Xerostomia, leukodystrophy
6/62/F	60	Xerostomia, xerophthalmia, peripheral nervous system involvement, type 2 diabetes
7/56/F	192	Xerostomia, RTA
8/39/F	6	Xerostomia, xerophthalmia, autoimmune hepatitis
9/34/F	36	Xerophthalmia, autoimmune thrombocytopenia, autoimmune hepatitis
10/46/F	126	Xerophthalmia, RTA, thyroiditis
11/56/F	120	Xerostomia, xerophthalmia, ILD, autoimmune hepatitis
12/30/F	27	Xerostomia, autoimmune hemolytic anemia
13/56/M	50	Xerostomia, ILD
14/27/F	16	Xerostomia, autoimmune thrombocytopenia
15/35/F	26	Autoimmune thrombocytopenia
16/43/F	50	Xerostomia, xerophthalmia, RTA
17/35/F	384	ILD, inflammatory bowel disease
18/45/F	3	Autoimmune hepatitis
19/62/F	31	Autoimmune hepatitis
20/56/F	124	Autoimmune hepatitis, femoral head necrosis
21/31/F	106	Autoimmune thrombocytopenia
22/40/F	75	Autoimmune hepatitis , liver cirrhosis
23/53/F	96	Autoimmune hepatitis
24/36/F	24	Autoimmune thrombocytopenia

Table S1. Demographic and clinical characteristics of SS patients. RTA, renal tubule acidosis; ILD, interstitial lung disease

Table S2

Patient	Treatment before UCMSCT	Treatment after UCMSCT‡
1	Pred 20mg/day, CYC 0.4gm/2wks	Pred 7.5mg/day, CYC 0.4gm/month,
2	Pred 15mg/day, CYC 0.6gm/month, LEF 20mg/day, HCQ 0.4gm/day	Pred 2.5mg/day, LEF 10mg/day, HCQ 0.2gm/day
3	Pred 5mg/day, CYC 0.4gm/2wks, HCQ 0.4gm/day	Pred 5mg/day, CYC 0.6gm/month, HCQ 0.4gm/day
4	MP 12mg/day, CYC 0.4gm/2wks, CsA150mg/day	MP 4mg/day
5	MP 24mg/day, CYC 0.4gm/wk, HCQ 0.4gm/day	MP 8mg/day, CYC 0.4gm/2wks, HCQ 0.4gm/day
6	MP 12mg/day, LEF 20 mg/day, CYC 0.4gm/wk	MP 6mg/day, CYC 0.6gm/month
7	MP 12mg/day, HCQ 0.4gm/day	MP 4mg/day, LEF 10mg/day
8	MP 12mg/day, MMF 2.0gm/day, HCQ 0.4gm/day	MP 2mg/day, HCQ 0.2gm/day
9	Pred 20mg/day, Vincristine 1mg/wk (3 times), CsA 150mg/day	Pred 10mg/day, CsA 100mg/day, HCQ 0.4gm/day
10	Pred 5mg/day, CYC 0.4gm/2wks, LEF 20mg/day, HCQ 0.4gm/day	LEF10mg/day, CYC 0.6gm/3months
11	Pred. 10 mg/day, MMF 2.0gm/day	Pred. 5mg/day, MMF 1.0gm/day
12	Pred 30mg/day, CsA 200mg/day, CYC 0.4gm/2wks	Pred 10mg/day, CsA 75mg/day
13	MP 12mg/day, CsA 200mg/day	MP 4mg/day, CsA 100mg/day
14	MP 20mg/day, CsA 200mg/day, MTX 10mg/wk	MP 4mg/day, CsA 75mg/day
15	MP 12mg/day, CYC 0.4gm/2wks	MP 4mg/day, CsA 100mg/day
16	MP 8mg/day, MTX 10mg/wk	MP 4mg/day
17	MP 24mg/day, CTX 0.6gm/month, MMF 2.0gm/day	MP 12mg/day, MMF 1.0gm/day
18	MP 12mg/day, HCQ 0.4gm/day	MP 4mg/day, HCQ 0.4gm/day
19	Pred 10mg/day	Pred 5mg/day
20	Pred 20mg/day, HCQ 0.4gm/day	Pred 10mg/day, HCQ 0.4gm/day
21	MP 16mg/day, CsA 150mg/day, CTX 0.4gm/2wks	MP 8mg/day, CsA 75mg/day
22	MP 12mg/day, MMF 2.0gm/day,	MP 4mg/day, MMF 1.0gm/day
23	MP 12mg/day	MP 4mg/day

24	Pred 15mg/day, CsA 200mg/day, Vincristine 1mg/wk (4 times)	Pred 5mg/day, CsA 100mg/day

Table S2. Treatments pre and post UCMSCT for each patient. UCMSCT, umbilical cord mesenchymal stem cell transplantation; Pred, prednisone; LEF, leflunomide; CYC, cyclophosphamide; MP, Methylprednisolone; CsA, cyclosporine A; MMF, mycophenolate mofetil; HCQ, hydroxychloroquine

‡Adjusted dose at the last follow up visit.

Supplemental Figure

Figure S1

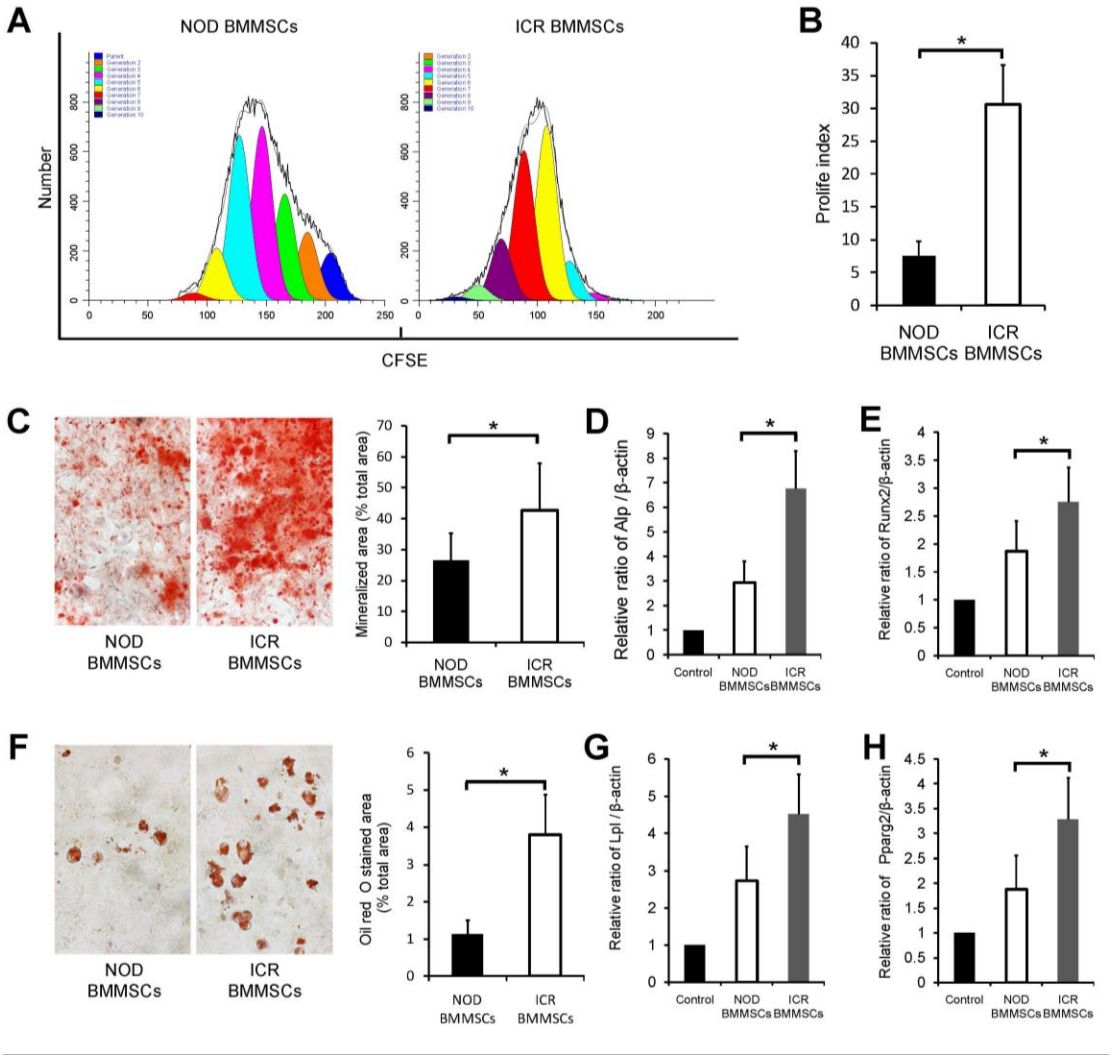

Figure S1. Impairment of immunological and biological properties of BMMSCs in NOD/Ltj mice. (A, B) The proliferation rate of BMMSCs from NOD/Ltj mice was significantly lower than that from ICR mice (P =1.691×10^{-11}, n=12). BMMSCs from NOD/Ltj mice also showed impairment of osteogenic and adipogenic differentiation. In osteo-inductive cultures, BMMSCs from NOD/Ltj mice showed a decreased mineralization (P =0.04, n=12) (C), and decreased levels of osteogenic genes expression, including alkaline phosphatase (ALP, D), runt-related transcription factor

2 (Runx2, E) as determined by Real time-PCR. In adipogenic inducing differentiation, impairment of BMMSCs from NOD/Ltj was shown by decreased numbers of lipid-specific Oil red O-positive cells (F) (P =5.049×10^{-8}, n=12) and reduced expression of adipocyte specific genes, lipoprotein lipase (LPL,G) and peroxisome proliferator-activated receptor γ2 (PPARγ2, H) were determined by real time-PCR.

Figure S2

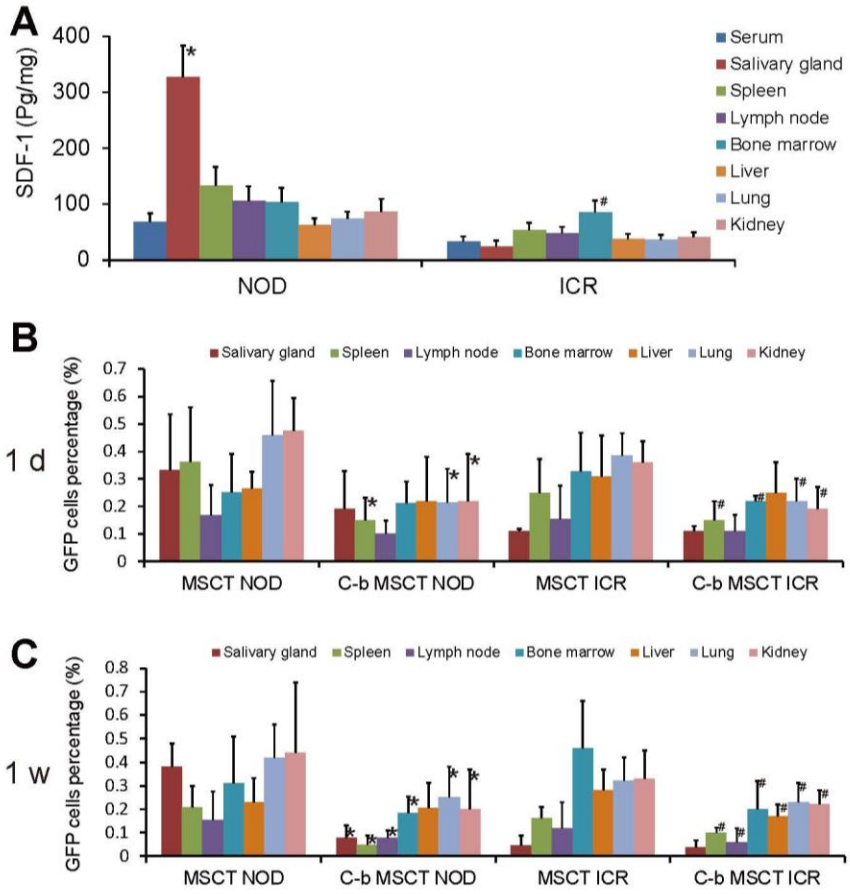

Figure S2. SDF-1 concentration and SDF-1/CXCR4 mediated MSCs trafficking in NOD/Ltj and ICR mice. (A) In NOD/Ltj mice, salivary gland has the highest concentration of SDF-1 (* versus other organs in NOD/Ltj mice, all P <0.05), while in ICR, the highest expression was observed in bone marrow ($^{\#}$ versus other organs in ICR mice, all P <0.05). (B, C) Distribution of GFP MSCs or CXCR4-blocked GFP MSCs in different organs 1 day and 1 week after infusion into NOD/Ltj or ICR mice. (B) Kidney and lung contained the highest number of GFP MSCs at 1 day post-infusion both in NOD/Ltj and ICR. Spleen and liver also contained a higher number of GFP cells both in NOD/Ltj and ICR. In NOD/Ltj mice, observed higher numbers of GFP^{+} BMMSCs in spleen, lung and kidney (MSCT NOD) were

suppressed by the CXCR4-blocking (C-b MSCT NOD) 1 day post-transplantation (*: MSCT NOD group versus C-b MSCT NOD group, all P <0.05, n=12; $^{\#}$:MSCT ICR group versus C-b MSCT ICR group, all P <0.05, n=12). (C) At 1 week post infusion, the number of GFP cells in kidney, spleen and lung decreased both in NOD/Ltj and ICR, while GFP cells in salivary gland of NOD/Ltj and bone marrow of ICR remained at the high level. In NOD/Ltj mice, higher numbers of GFP^+ BMMSCs were detected in salivary glands post GFP MSCs infusion group (MSCT NOD) compared to the CXCR4-blocked BMMSCs group (C-b MSCT NOD) 1 week post-transplantation (* $P = 4.5 \times 10^{-9}$, n=12). A similar trend was observed for GFP^+ BMMSCs in spleen, bone marrow, lymph node, lung and kidney in both NOD/Ltj (* MSCT NOD group versus C-b MSCT NOD group, all P <0.05, n=12) and ICR mice ($^{\#}$ MSCT ICR group versus C-b MSCT ICR group, all P <0.05, n=12).

Figure S3

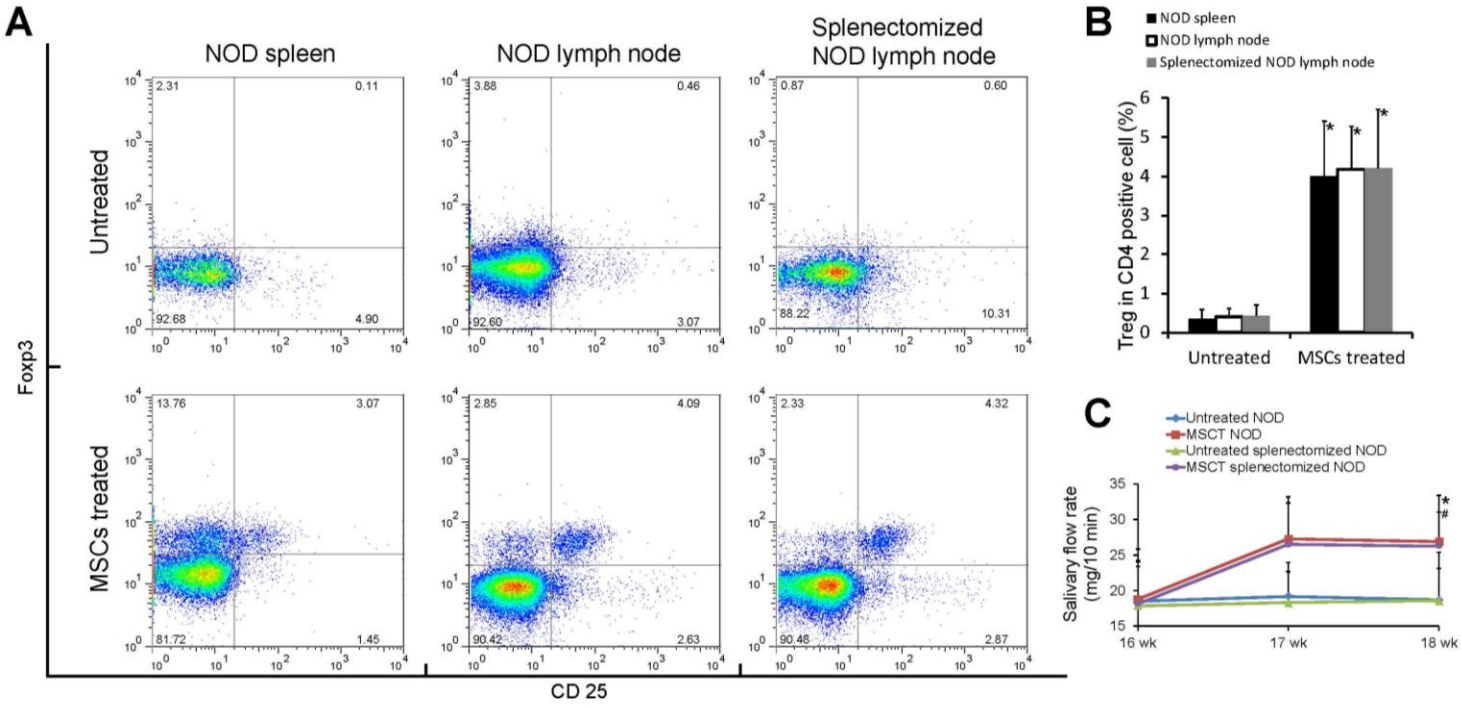

Figure S3. Restoration of salivary gland function and Treg expression in submendibular lymph nodes by MSCs treatment in splenectomized NOD/Ltj mice. (A, B) MSC treatment resulted in Treg generation in spleen and lymph node of NOD/Ltj mice (* all P <0.05), and splenectomized of NOD/Ltj mice did not affect Treg development in submendibular lymph nodes (P =0.83, n=6). (C) Salivary flow rate (n=6) of BMMSCs treated NOD/Ltj mice was significantly higher than untreated mice (* P =0.029); when NOD/Ltj mice were splenectomized, allogeneic MSCs treatment had similar therapeutic effects ($^{\#}$ P =0.041).

Figure S4

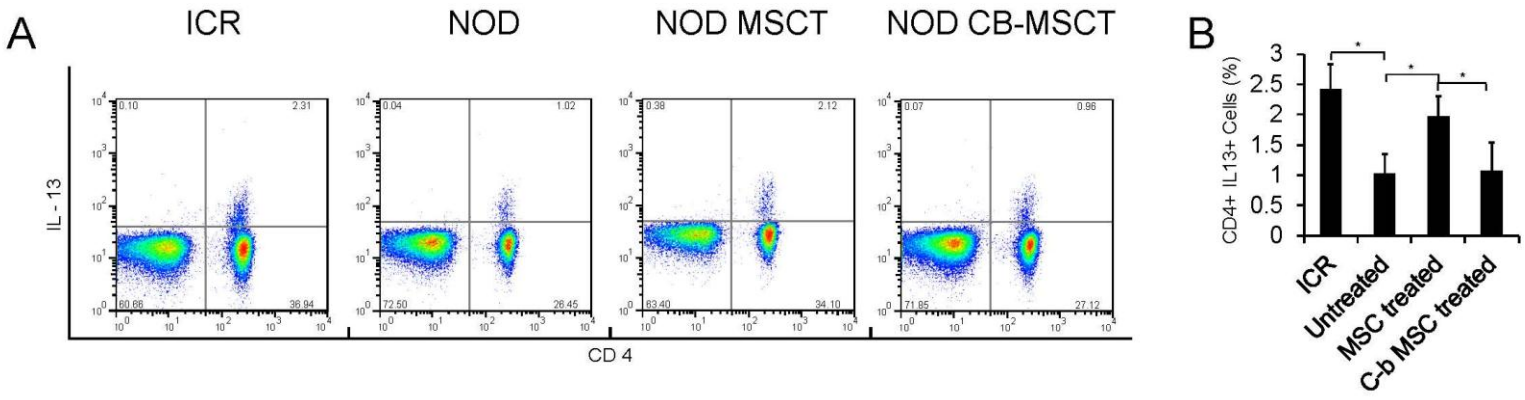

Figure S4. MSC treatment favored Th2 (CD4+, IL13+) in NOD/Ltj mice. (A, B) Allogeneic BMMSC treatment increased the percentage of Th2 cells, and CXCR4-blocked BMMSCs failed to do so (*all P <0.05).