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Supplemental Information

CXCR5-Overexpressing Mesenchymal Stromal Cells Exhibit Enhanced Homing and Can Decrease Contact Hypersensitivity

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Supplementary material

Figure S1. Lentiviral transduction, and experiments showing that forced expression of CXCR5 does not alter the characteristics of MSCs *in vitro*.

(a and b) Lentivirus were produced by virus package technique and condensed by ultracentrifugation. MSC^{CXCR5} and MSC^{eGFP} were cultured continuously after fluorescence activated cell sorting. The morphologies of MSC^{eGFP} and MSC^{CXCR5} were assessed by a bright field microscopy, and green fluorescence was monitored under fluorescence microscopy.

(c and d) The MSCs surface expression levels of CD29, CD44, CD73, CD90, CD105, CD166, CD34, and CD45, were analyzed by flow cytometry.

(e and f) Oil red O, Alizarin red S, and Toluidine blue O staining were used to assess the adipogenic, osteogenic, and chondrogenic differentiations, respectively, of transfected MSCs. Scale bar=200 μm.

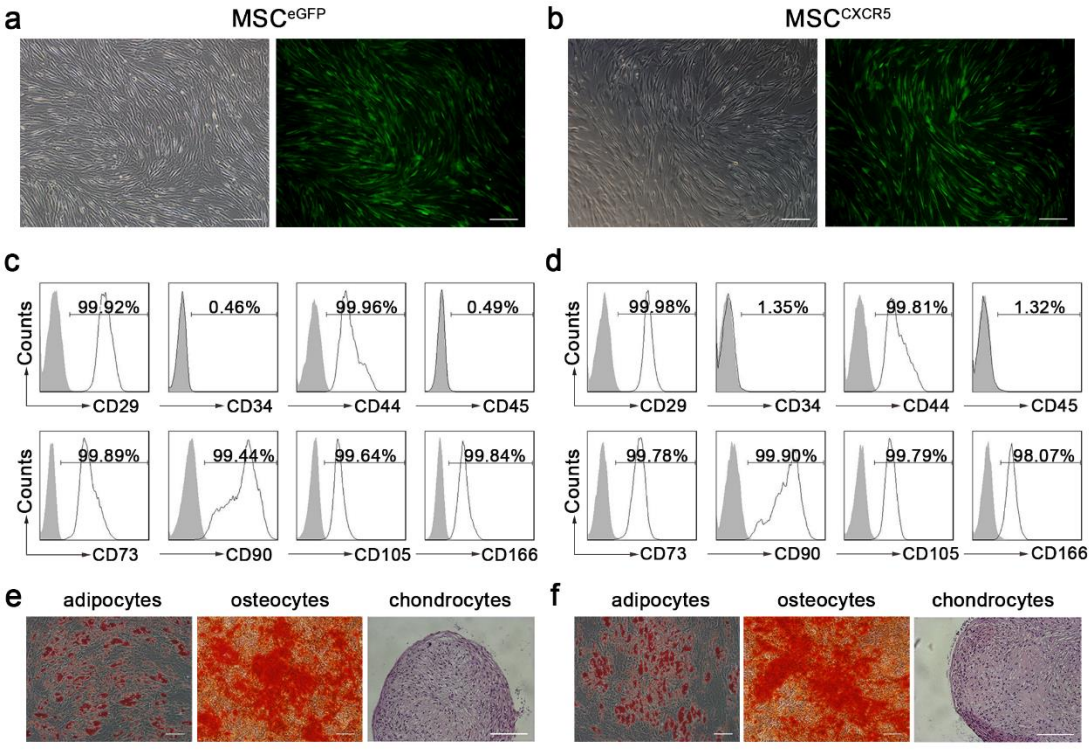

Figure S2. MSC^{CXCR5} gain the ability to migrate towards mCXCL13 and hCXCL13 *in vitro*.

(a) Cytoskeleton arrangement pattern of MSC^{CXCR5} in culture (Ⅰ) and reorganization after migration (Ⅱ), as examined by fluorescence microscopy. Actin filaments were exhibited with Phalloidin（red），and cell nucleus were exhibited with DAPI (blue). bar=100 μm.

(b) Relative number of MSC^{CXCR5} with lamellipodia in culture or after migration induced with mCXCL13; ***P<0.001.

(c) Pull-down assay assessing Rac-1 activation in MSC^{CXCR5} cultured with and without mCXCL13. Representative blots and histogram showed the up-regulation of active Rac 1induced by mCXCL13.

(d) Cytoskeleton arrangement pattern of MSC^{CXCR5} in culture (Ⅰ) and reorganization after migration (Ⅱ), as examined by fluorescence microscopy. Actin filaments were exhibited with Phalloidin（red），and cell nucleus were exhibited with DAPI (blue). bar=100 μm.

(e) Relative number of MSC^{CXCR5} with lamellipodia in culture or after migration induced with hCXCL13; ***P<0.001.

(f) Pull-down assay assessing Rac-1 activation in MSC^{CXCR5} cultured with and without hCXCL13. Representative blots and histogram showed the up-regulation of active Rac 1induced by hCXCL13.

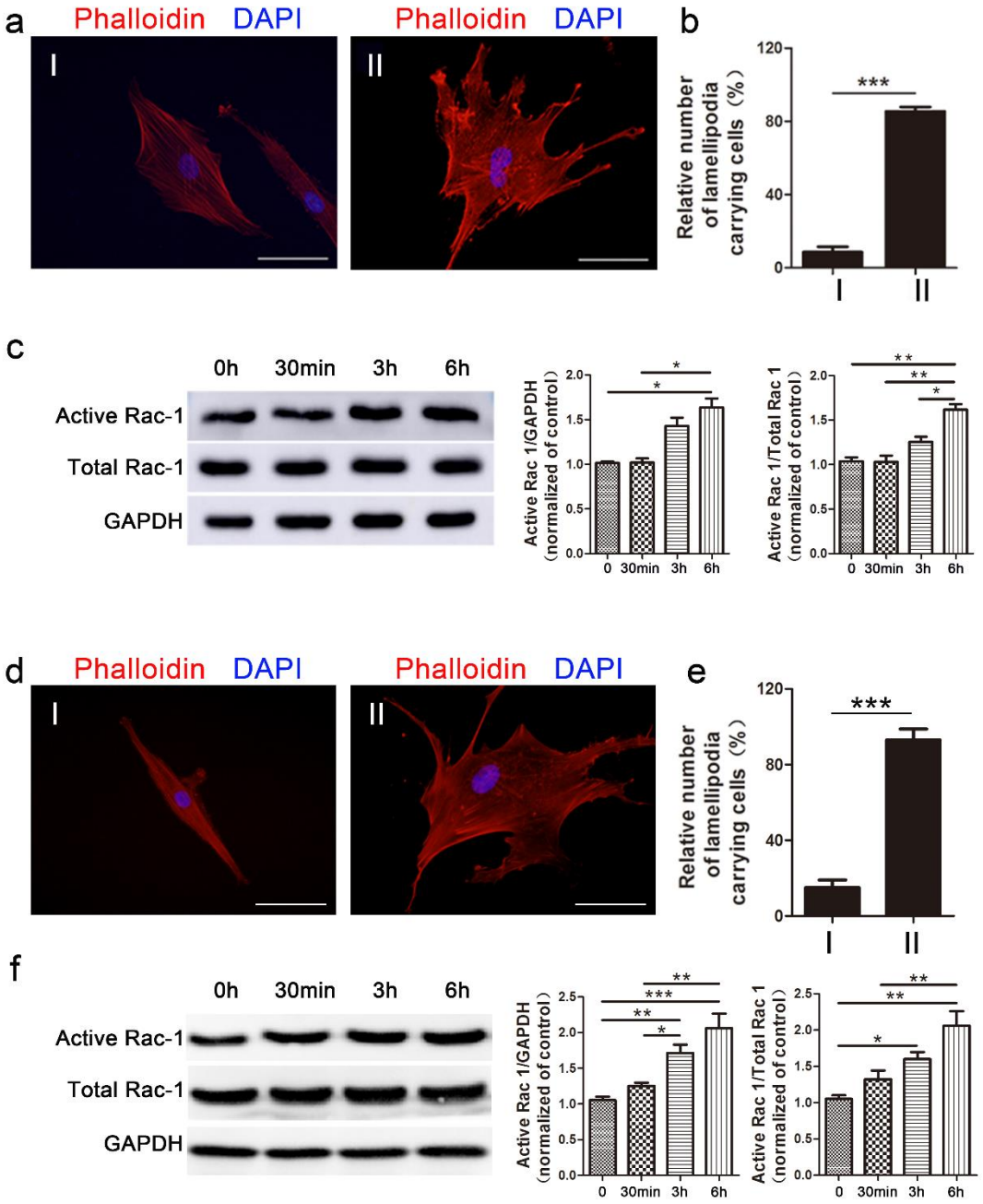
a
Phalloidin DAPI
I
Phalloidin DAPI
II
b
Relative number of lamellipodia carrying cells (%)
120
80
40
0
***
I
II
c
0h 30min 3h 6h
Active Rac-1
Total Rac-1
GAPDH
Active Rac 1/GAPDH (normalized of control)
2.0
1.5
1.0
0.5
0.0
*
*
0 30min 3h 6h
Active Rac 1/Total Rac 1 (normalized of control)
**
**
*
0 30min 3h 6h
d
Phalloidin DAPI
I
Phalloidin DAPI
II
e
Relative number of lamellipodia carrying cells (%)
120
80
40
0
***
I
II
f
0h 30min 3h 6h
Active Rac-1
Total Rac-1
GAPDH
Active Rac 1/GAPDH (normalized of control)
2.5
2.0
1.5
1.0
0.5
0.0
**
***
**
*
0 30min 3h 6h
Active Rac 1/Total Rac 1 (normalized of control)
**
**
*
0 30min 3h 6h

Figure S3. Quantitative real-time PCR assay for identifying human DNA.

(a) Serial dilutions of genomic human DNA (hDNA) concentrations and comparable cell number with correspondingly Ct values were presented in tabular format.

(b) Standard curves derived from the log10 cell number (human MSCs) were plotted against the mean Ct values.

(c) Probable cell number of MSC^{eGFP} or MSC^{CXCR5} in 1μg genomic DNA of diseased ears. *n* = 6 mice per group from three independent experiments.

(d) Serial dilutions of genomic human cDNA concentrations and comparable cell number with correspondingly Ct values were presented in tabular format.

(e) Standard curves derived from the log10 cell number (human MSCs) were plotted against the mean Ct values.

(f) Probable cell number of MSC^{eGFP} or MSC^{CXCR5} in 1μg cDNA of diseased ears. *n* = 6 mice per group from three independent experiments.

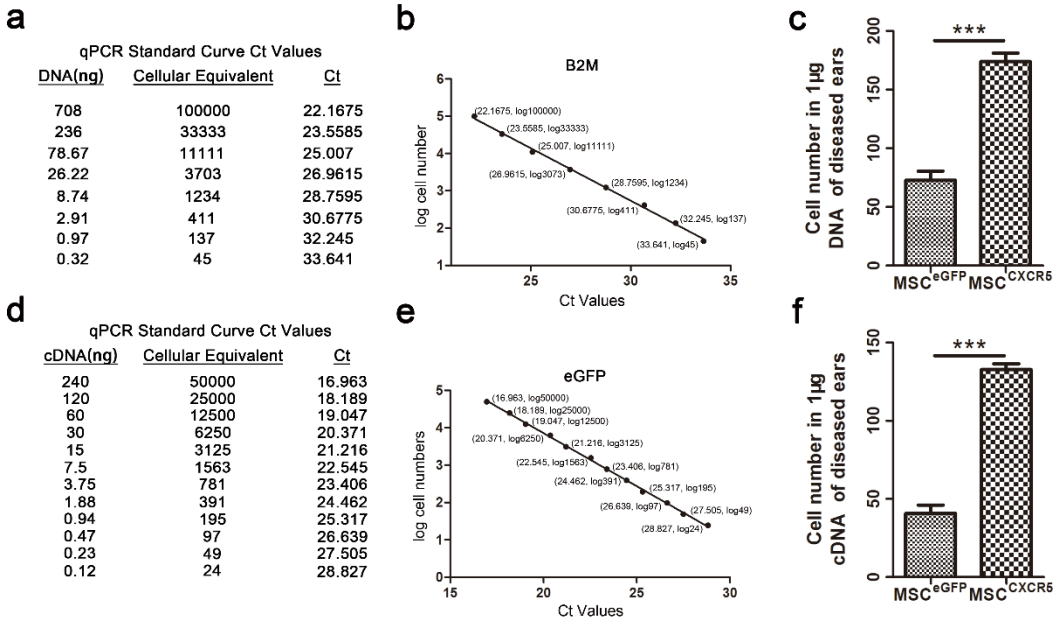

Figure S4. MSC^{eGFP} and MSC^{CXCR5} with T cells in the inflamed ears.

(a) Sections were obtained from challenged ears one day after injections and stained with anti-mouse CD3 and anti-mouse TCR β Chain. eGFP expressing MSC^{eGFP} and MSC^{CXCR5} cells were observed in challenged ears. *n* = 6 mice per group from three independent experiments and the pictures were performed in at least six sections per tissue. CD3, white; mTCR, red; eGFP, green; DAPI, blue; Bar=100μm.

(b) Three days after injections, sections were obtained from challenged ears and stained with anti-mouse CD3 and anti-mouse CD4 or anti-mouse CD8. eGFP expressing MSC^{eGFP} and MSC^{CXCR5} cells were observed in challenged ears. *n* = 6 mice per group from three independent experiments and the pictures were performed in at least six sections per tissue. CD3, white; CD4, violet; CD8, red; eGFP, green; DAPI, blue; Bar=100μm.

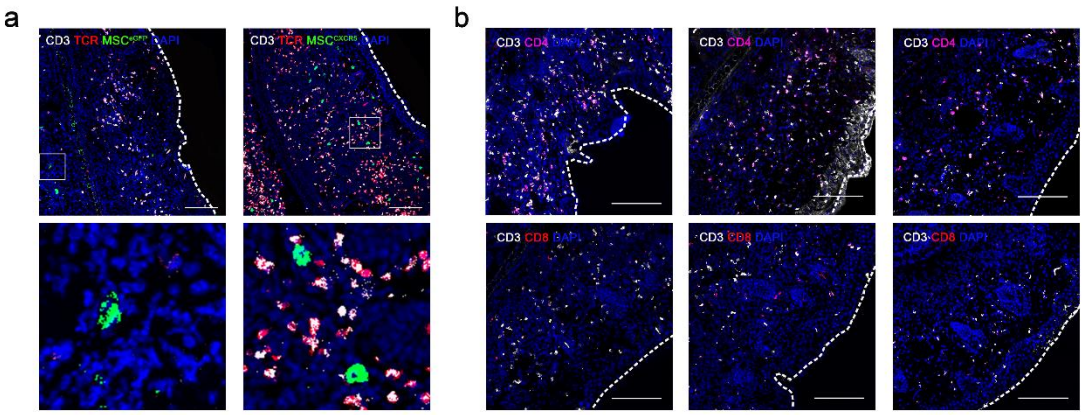

Figure S5. Construction of plasmids.

(a-c) Construction of pLV/Puro-EF1α-CXCR5-IRES-eGFP. The CXCR5-encoding plasmid was constructed using the multisite gateway technology.

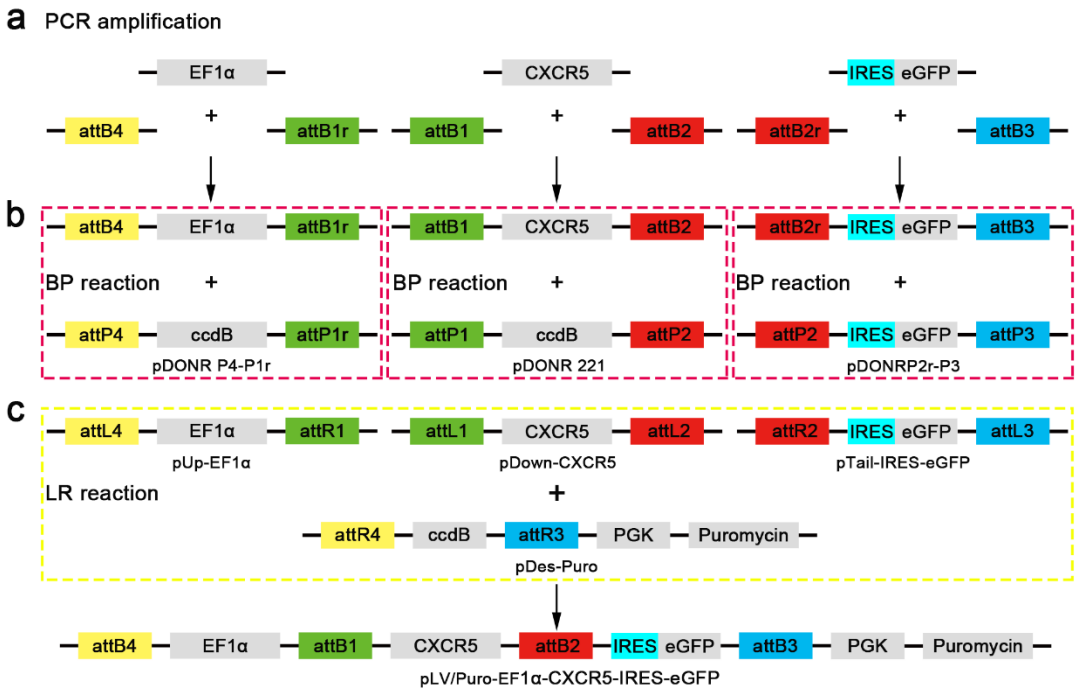

Figure S6. Construction of plasmids.

(a-c) Construction of pLV/Puro-EF1α-eGFP. The control plasmid which only express eGFP was constructed by multisite gateway technology.

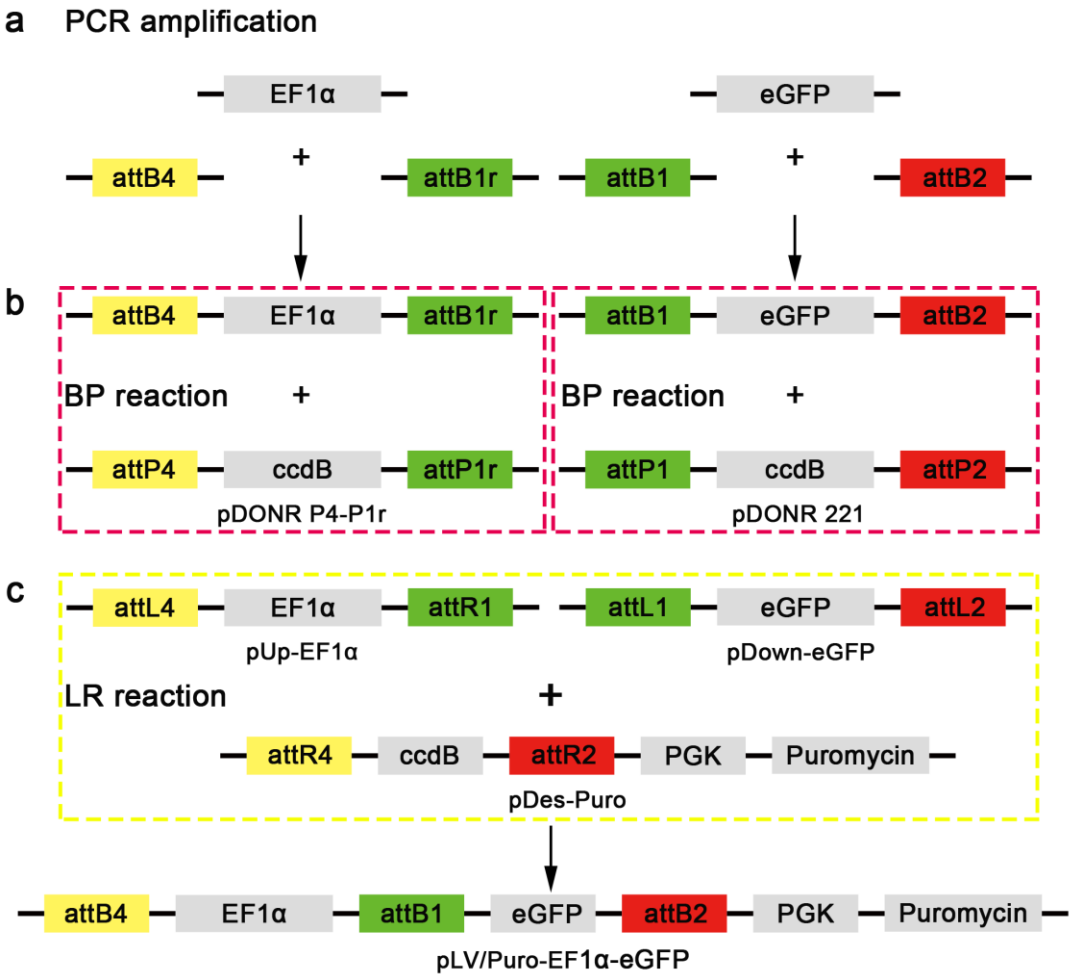

Figure S7. The transduction efficiency of MSC^{CXCR5} and MSC^{eGFP}.

(a) Three days after transduction, eGFP of MSC^{CXCR5} and MSC^{eGFP} were detected by flow cytometric analyses. MSC^{CXCR5} and MSC^{eGFP} were purified by fluorescence activated cell sorting (Influx, BD). The results are representative of three experiments.

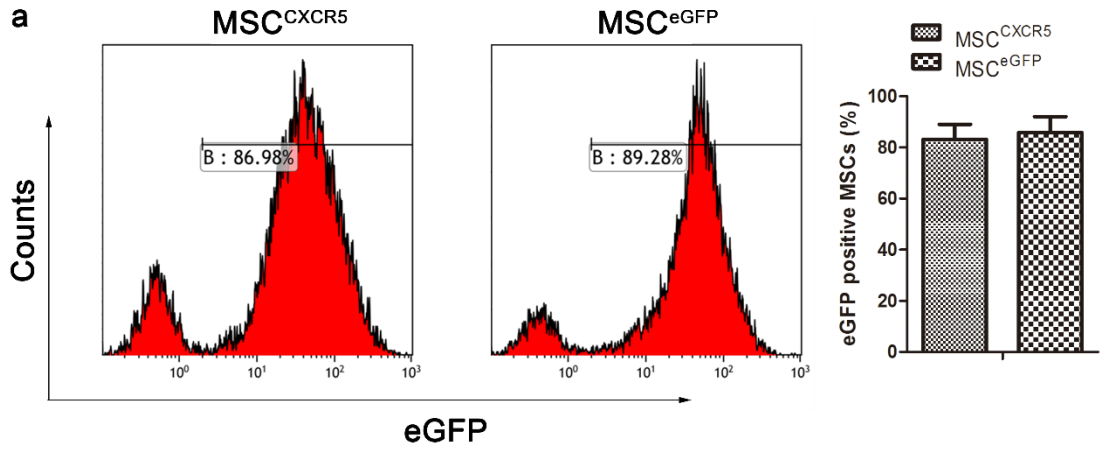

Table S1. Sequence of Specific Primers Used for q-PCR Analysis:

Human:

Genes	**Forward Sequence**	**Reverse Sequence**
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
CCR1	TGCATCCCCATAGTCAAACTC	CAGAAAGCCCCAGAAACAAA
CCR2	GACTTCTTCACCGCTCTCGT	CTGAGACAAGCCACAAGCTG
CCR3	CAACTCAGCAGTGAAATGTGC	TCTTCTTGTGCTTATCCGGG
CCR4	CTTTCATCGAGGGTGGTGTC	CACAGACCTTCCTCAGAGCC
CCR5	CTGCGATTTGCTTCACATTG	TGAGACATCCGTTCCCCTAC
CCR6	AAATTCATTGATTCCCCGCT	TGAAGGGAGTGGATCAGAGC
CCR7	TCTCCGATGTAATCGTCCGT	CAGCCTTCCTGTGTGGTTTT
CCR8	TCACAGGGGCTTGAGAAGAT	CCTCCAGAACAAAGGCTGTC
CCR9	AGGGCTTGTGAAGTCTGTGG	CAGAGAGCAACCCAGCTCTT
CCR10	GTCAGGGAGACACTGGGTTG	GACGGAGGCCACAGAGC
CXCR1	GGCATGCCAGTGAAATTTAG	TACTGTTGGACACACCTGGC
CXCR2	TCTTCAAAGCTGTCACTCTCCA	AGCAGGTCACAGCTGCTCTT
CXCR3	CTCGGCGTCATTTAGCACTT	AACCACAAGCACCAAAGCAG
CXCR4	CTTGTCCGTCATGCTTCTCA	GAACCCTGTTTCCGTGAAGA
CXCR5	CCTTGAAGGAGGCCATGAG	TAACGCTGGAAATGGACCTC
CXCR6	GCAGGAAGTCTTGATGCTCC	TGAGCAAGCTCATCTCTGGA
CXCR7	CAGATCCATCGTTCTGAGGC	GCAGAGCTCACAGTTGTTGC
B2M	GGTTGCTCCACAGGTAGCTC	TTTCCCCCAAATTCTAAGCA

eGFP	AGGACGACGGCAACTACAAG	AAGTTCACCTTGATGCCGTTC

Mouse:

Genes	**Forward Sequence**	**Reverse Sequence**
GAPDH	TCAATGAAGGGGTCGTTGAT	CGTCCCGTAGACAAAATGGT
IL-4	GGTGTTCTTCGTTGCTGTGA	TCTCGAATGTACCAGGAGCC
IL-6	TGGTACTCCAGAAGACCAGAGG	AACGATGATGCACTTGCAGA
IL-10	AGACACCTTGGTCTTGGAGC	TTTGAATTCCCTGGGTGAGA
IL-17	AGAATTCATGTGGTGGTCCAG	ACTACCTCAACCGTTCCACG
TNF-α	GGTCTGGGCCATAGAACTGA	CAGCCTCTTCTCATTCCTGC
IFN-γ	TGAGCTCATTGAATGCTTGG	AGGCCATCAGCAACAACATA
CCL2	TCTCCAGCCTACTCATTGGG	AGGTCCCTGTCATGCTTCTG
CCL8	CTCGTAGCTTTTCAGCACCC	TTCTTTGCCTGCTGCTCATA
CCL17	ATCCCTGGAACACTCCACTG	TGCTTCTGGGGACTTTTCTG
CCL19	ACATCGTGAAAGCCTTCCGCTA	TCCTTCTGGTGCTGTTGCCTT
CCL21	ACCAAGTTTAGGCTGTCCCAT	ACTTAGAGGTTCCCCGGTTC
CCL27	GGTACAGTCCCTTGGAGCCT	GACTGTCACCTCCAGGCTGT
CXCL2	CATCAGGTACGATCCAGGCT	CCTGGTTCAGAAAATCATCCA
CXCL10	CTTCCCTATGGCCCTCATTC	AAGTGCTGCCGTCATTTTCT
CXCL12	AGGGCACAGTTTGGAGTGTT	GCGCTCTGCATCAGTGAC
CXCL13	ATTCTGGAAGCCCATTACACA	TTTGGCACGAGGATTCACAC

Table S2. Primary Antibodies Used for Immunofluorescence Staining

Antigen	Host	Source	Dilution	Clone
eGFP	Rabbit	Bioss Antibodies	1:50	bs-2194R
Mouse CXCL13/BLC/BCA-1	Goat	R&D system	1:50	AF470
CD4	Rat-AF647 Rat-AF594	Biolegend	1:50	100424 100446
CD8a	Rat-AF594	Biolegend	1:50	100758
CD3	Rat-AF647	Biolegend	1:50	100209
TCR β Chain	Armenian Hamster	BD biosciences	1:50	561081

Table S3. Secondary Reagents Used for Immunofluorescence Staining

	Conjugate(s)	Source	Dilution
Anti-rabbit	Alexa Fluor 488	Invitrogen	1:200
Anti-goat	Alexa Fluor 594	Invitrogen	1:600

Table S4.Antibodies for Flow Cytometry:

Antigen	Source	Dilution	Clone
Anti-human CXCR5 (APC)	Biolegend	1:50	356908
Anti-human CD90 (PE-Cy7)	BD biosciences	1:50	561558
Anti-human CD29 (APC)	BD biosciences	1:50	559883
Anti-human CD34 (PE)	BD biosciences	1:50	550761
Anti-human CD44 (APC)	BD biosciences	1:50	559942
Anti-human CD45 (PE)	BD biosciences	1:50	560975
Anti-human CD73 (PE)	BD biosciences	1:50	550257
Anti-human CD90 (PE-Cy7)	BD biosciences	1:50	561558
Anti-human CD105 (PerCP-Cy5.5)	BD biosciences	1:50	560819
Anti-human CD166 (PE)	BD biosciences	1:50	559263
Anti-human CD3 (V450)	BD biosciences	1:50	561812
Anti-human IFN-γ (APC)	BD biosciences	1:50	551385
Anti-human TNF-α (PE)	BD biosciences	1:50	557647
AnnexinV (APC)	BD biosciences	1:20	550475
propidium iodide (PI)	BD biosciences	1:50	556463
Anti-mouse CD3 (PE-Cy7)	BD biosciences	1:100	561100
Anti-mouse CD4 (PE)	BD biosciences	1:100	553653
Anti-mouse CD8 (PB)	BD biosciences	1:100	558106
Anti-mouse IFN-γ (APC)	BD biosciences	1:100	554413
Anti-mouse IL-17 (AF700)	Biolegend	1:100	506914