Supplemental Materials and Methods

Lentiviral infection of THP-1

Lenti-CSF2RB-shRNA and Lenti-nonsilencing RNAs were linearized and transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen) for virus packaging and propagation. Recombinant CSF2RB-shRNA-expressing lentivirus particles were used to infect THP-1 cells. More than 90% of lentivirus-transduced THP-1 cells expressed GFP during the first 48 h post-infection.

Cell fractionation and Western blotting

Cell fractionation experiments were performed as described previously. Briefly, confluent HBMEC were pretreated with 20 ng/ml rhGM-CSF (R&D Systems) for different time intervals, washed, extracted in Triton X-100 lysis buffer (25 mM HEPES, 150 mM NaCl, 4 mM EDTA, 1% Triton X-100), and centrifuged to obtain the soluble fraction. Pellets were dissolved in sodium dodecyl sulfate (SDS) lysis buffer (25 mM HEPES, 4 mM EDTA, 1% SDS) to obtain the insoluble fraction. Equal portions of the soluble and insoluble fractions were analyzed for occludin expression (Abcam, Cambridge, UK) by Western blotting.

In other experiments, HBMECs pretreated with rhGM-CSF or rhGM-CSF + MG-132 were collected at different time points, washed twice with ice-cold PBS, and prepared in radioimmunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS) containing a protease inhibitor cocktail (Roche, Basel, Switzerland). The samples were separated by SDS-PAGE and

transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA) using a Semi-Dry Transfer Cell apparatus (Bio-Rad, Hercules, CA, USA). The PVDF membrane was blocked with 5% non-fat milk and incubated separately with polyclonal antibodies against claudin-5, VE-cadherin, occludin (Abcam), or ZO-1 (Invitrogen) at 4°C overnight. The blots were incubated with a horseradish peroxidase (HRP) conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. Protein bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA). The relative signal densities were analyzed using a LAS-3000 imaging system (Fujifilm, Tokyo, Japan), and GAPDH densities were used as an internal control when comparing expression levels between samples.

Immunoprecipitation assay samples were subjected to Western blot analysis for ubiquitin (Abcam), myc (ABMART, Shanghai, China), or Flag (ABMART, Shanghai, China) after eluting the immune complex proteins in SDS sample buffer.

Proliferation assay

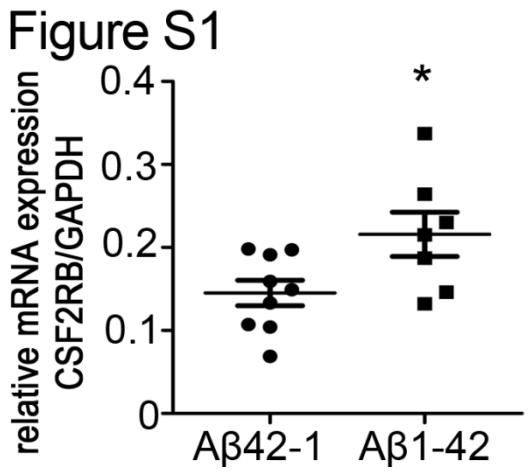
A density of 3×10^3 cells per well were seeded in the 96-well plates in MEM and then cultured at 37 ^{o}C in a humidified atmosphere with 5% CO_2 for 24 h. Different concentrations of samples in 10 μl solutions were added to each well, and the cells were incubated for additional 24 h. Then 10 μL of CCK-8 solutions was added to each well and cultured for another 4 h. The absorptions of each well was measured using a Microplate reader at a test wavelength of 450 nm and a reference wavelength of 690

nm, respectively. All the experiments were performed in triplicate.

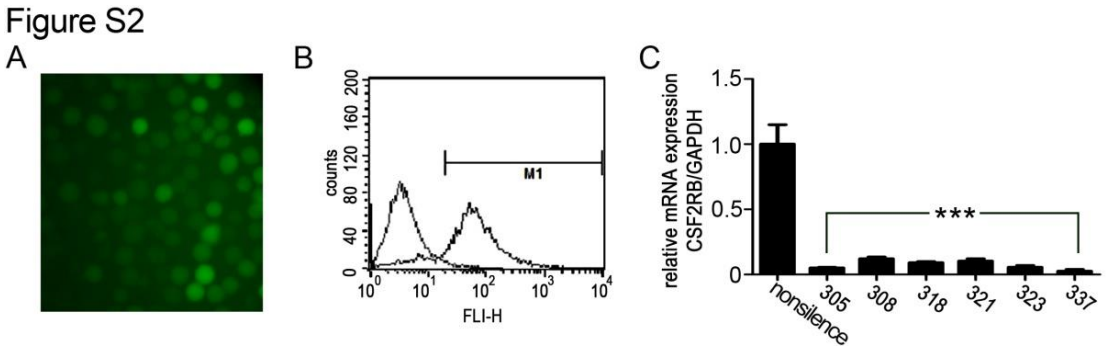
TUNEL assay

In order to determine the toxicity of GM–CSF on endothelial cell, one step TUNEL (C1089, Beyotime Institute) staining was performed. Briefly, confluent HBMEC were pretreated with 20 ng/ml rhGM–CSF for 24 or 48 h respectively. After fixing in 4% paraformaldehyde, the cells were rinsed with PBS, and permeabilized in 0.1% Triton X–100 for 2 min. Then, the TUNEL assay was done at 37 C for 1 h. Cy3 (Cyanine 3) –labeled TUNEL–positive cells were imaged under a fluorescence microscope at an excitation and emission of 488 nm and 530 nm, respectively. Cells with red fluorescence were defined as apoptotic cells to indicate the toxicity of GM–CSF. The positive control HBMEC was treated by DNase I for 10 minutes at 25 ºC to induce DNA strand breaks according to the instruction of the TUNEL assay kit.

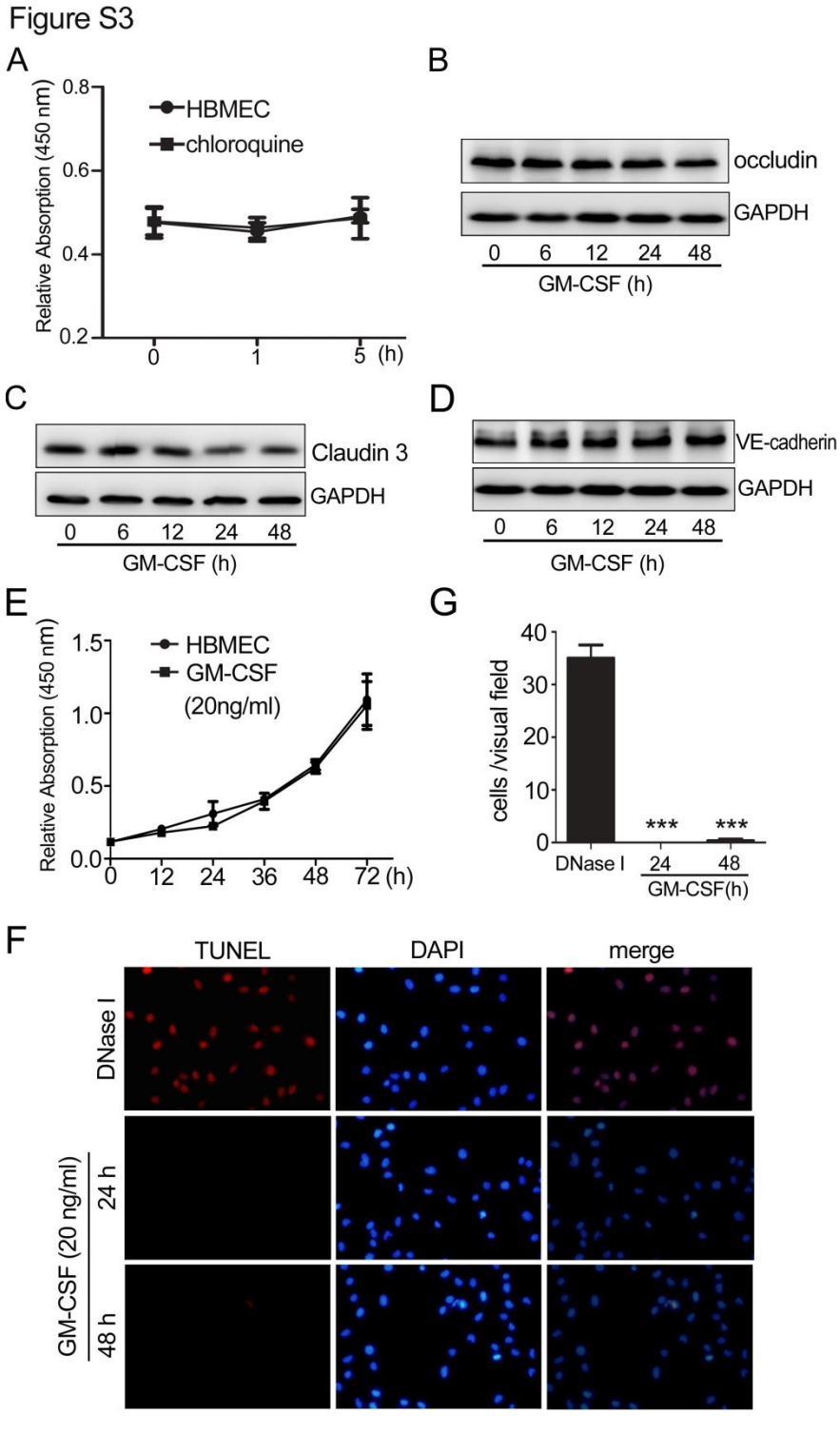
Supplemental data



Supplement S1. CSF2RB overexpression in monocytes from an experimental AD rat model. CSF2RB expression levels in experimental AD rat monocytes. CSF2RB expression levels in peripheral blood monocytes from 7 rats injected with Aβ1-42 and 9 rats injected with the reverse peptide Aβ42-1 (controls) were detected by real-time RT-PCR. *$p < 0.05$ vs. rats injected with reverse peptide Aβ42-1. CSF2RB/GAPDH and CSF2RA/GAPDH indicate the relative expression levels of CSF2RB and CSF2RA, respectively, after normalization to the expression level of GAPDH. Data are shown as means ± standard deviations.

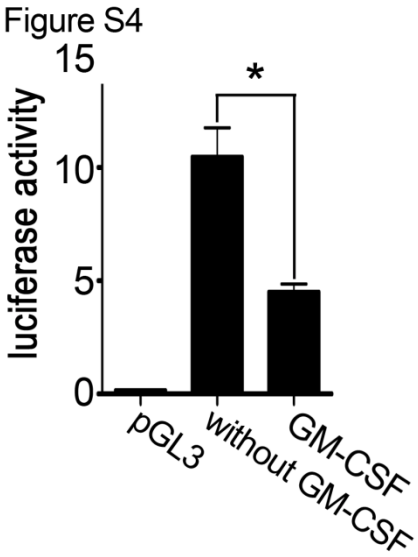


Supplement S2. CSF2RB gene silencing in THP-1 cell line. (A) THP-1 cells were transduced by lentivirus based CSF2RB-shRNA and efficiently expressed GFP. (B) 1×10^6 transduced THP-1 cells were analyzed by flow cytometry and GFP fluorescence intensity was measured on the FL1 channel. More than 90% of the THP-1 cells were GFP-positive. (C) Real-time RT-PCR was done to detect the expression of CSF2RB in different THP-1 clones transduced with lentivirus based CSF2RB-shRNA. CSF2RB expressions were significantly knocked down in all the clones. Data are shown as means ± SD ***$p < 0.0001$ vs. the nonsilence, the experiment was independently performed three times (n=3).

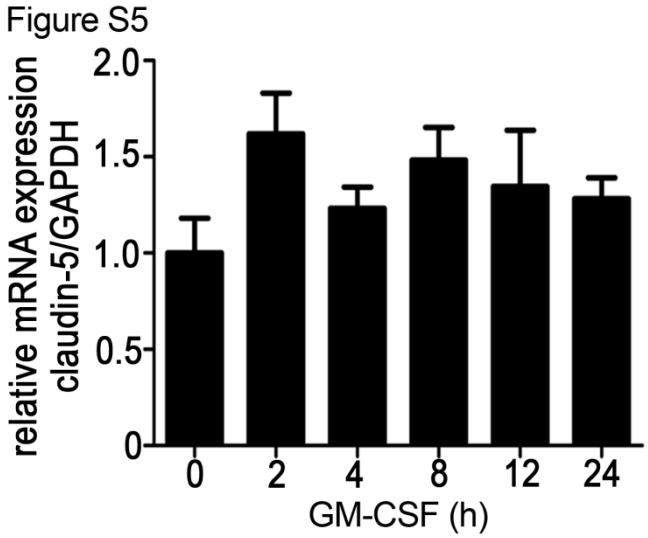


Supplement S3. GM-CSF stimulation does not affect tight junction protein occludin, Claudin-3 and adherine junction protein VE-Cadherin expression and GM–CSF did not induce endothelial cell growth retardationHBMEC were stimulated

with GM-CSF (20 ng/ml) in indicated time. Proliferation assay was performed to examine the effect of chloroquine on HBMEC (A). The expressions of occludin (B), Claudin-3 (C) and VE-cadherin (D) were examined. (E) CCK8 reagent was used to examine the HBMEC proliferation under stimulation of GM–CSF. Data are shown as means ± SD. The experiment was independently performed three times (n=3). (F) TUNEL assay was performed to determine the toxicity of GM–CSF on endothelial cell. DAPI was used to visualize cell nuclei (blue). Apoptotic cells were visualized by red fluorescence. Scale bar: 200 μm. (G) A statistical analysis of the TUNEL assay. The bar is means ± SD from 10 independent visual fields. *p<0.05 vs. GM-CSF stimulated HBMEC.



Supplement S4. GM-CSF inhibits ZO-1 promoter activity. Luciferase activities were normalized to Renilla activities. The bar is means ± SD from three independent experiments. *p<0.05 vs. pGL3-ZO-1 transfected HBMEC without the stimulation of GM-CSF.



Supplement S5. GM-CSF does not affect claudin-5 transcription. GM-CSF was incubated with HBMEC monolayer at the indicated time points, and the mRNA level was estimated by real-time RT-PCR analysis. Values are means±SD (n=3). There is no difference between the transcriptions of claudin-5 from GM-CSF stimulated HBEMC and normal HBMEC.