Supplementary information

MATERIALS AND METHODS

Mice Wild-type BALB/c (H-2^d), C57BL/6 (B6, H-2^b), C3H (H-2^k) and FvB (H-2^q) mice were purchased from Vital River Laboratories (Beijing, China). Severe combined immune deficient (SCID, H-2^d) and Rag2 knockout (KO) mice (H-2^b) were purchased from Beijing HFK Bioscience Co. Ltd (Beijing, China). All mice were maintained in a specific pathogen-free facility and were experimented at 8-10 weeks old. All experimental manipulations were undertaken in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals, Institute of Zoology, Chinese Academy of Sciences (Beijing, China).

Reagents The following anti-mouse mAbs used for cell staining were purchased from eBioscience: fluorescein isothiocyanate (FITC)-anti-F4/80 (BM8; IgG2a), phycoerythrin-Cy5 (PE-Cy5)-Cy5-anti-CD11b (M1/70; IgG2b), phycoerythrin (PE)-anti-B220 (RA3-6B2; IgG2a). The following anti-mouse mAbs used for cell staining were purchased from Biolegend: PE-anti-CD8a (OX-8; IgG1), PE-anti-CD49b (DX5; IgM). The following anti-mouse mAbs used for cell staining were purchased from BD Biosciences Pharmingen (San Diego, CA, USA): PE-anti-CD4 (GK1.5; IgG2b), PE-anti-mouse Ly6G (1A8; IgG2a), PE-anti-CD11c (HL3; IgG1). Anti- mouse FcR mAb (2.4G2, IgG2b) was produced by 2.4G2 hybridoma (ATCC, Rockville, MD, USA) in our laboratory. Carboxyfluorescein succinimidyl ester (CFSE) (Cat# S8269) was purchased from Selleck Chemicals (Houston, TX, USA).

Priming mice with allogeneic splenocytes For allogeneic splenocytes immunized mice, splenocytes from allogeneic mice were injected into host mice (1×10^7 cells per mouse) via tail vein. At least 10 days or the indicated days after injection with allogeneic splenocytes, the immunized mice were used to isolate the primed macrophages.

Preparation of peritoneal macrophages Peritoneal macrophages (PEMs) were prepared as reported previously. Briefly, primary peritoneal cells were obtained from the peritoneal exudates of mice. To get the purified PEMs for adoptive transfer to immunodeficient mice, peritoneal cells were stained with F4/80-FITC, CD11b-PE-Cy5, CD4-PE, CD8-PE, B220-PE, Ly6G-PE, CD11c-PE, CD49b-PE and then $CD4^-CD8^-B220^-Ly6G^-CD11c^-CD49b^-F4/80^+CD11b^+$ PEMs were sorted using MoFlo XDP (the purity of the sorted PEMs were 99%).

***In vivo* rejection of allogeneic graft assay** For detecting rejection of allogeneic splenocytes, CFSE-labeled syngeneic splenocytes (0.2μM CFSE labeled, as $CFSE^{low}$ cells) and allogeneic splenocytes (2.5μM CFSE labeled, as $CFSE^{high}$ cells) were mixed together at 1:1 ratio. The CFSE-labeled targeted splenocytes were intraperitoneally injected into naïve or primed host mice (about 1×10^7 total CFSE-labeled cells per mouse), or a mixture with the sorted naïve or primed PEMs at 1:4 ratio were intraperitoneally injected into immunodeficient SCID or Rag2 KO mice (about 3×10^6

total cells consisting of sorted PEMs and CFSE-labeled targeted splenocytes per mouse), the survival of CFSE-labeled cells in host mice was determined by flow cytometry within 24 h after injection. For rejection of allogeneic skin graft assay, the sorted PEMs were adoptively transferred into SCID mice via tail vein ($0.5\text{-}1\times10^6$ per mice) the day before skin transplantation.

Flow cytometry For rejection of allogeneic splenocytes assay, peritoneal exudates containing CFSE-labeled targeted cells were washed twice with cold PBS and then assessed using flow cytometry. Data analysis was performed using CXP v2.2 software (Beckman Coulter). For cell sorting purpose, peritoneal cells or splenocytes were stained with fluorochrome-conjugated Abs at 4℃ for 30 min. The cells were washed twice with cold Hanks' solution with 0.1% NaN_3 and 0.5% BSA and then were sorted using MoFlo XDP.

Skin transplantation For rejection of skin allogeneic graft assay, skin grafts from allogeneic B6 or FvB mice were transplanted into SCID mice as reported previously. Since 7 days after transplantation, skin graft photos were taken every two days with a digital camera (Canon EOS450D; Canon, Tokyo, Japan) until the graft was rejected completely. Erythema, edema, and hair loss of allogeneic skin grafts were considered early signs of rejection, whereas ulceration, progressive shrinkage, and desquamation were considered to be the end point of rejection.

Statistical analysis All data are presented as the mean ± SD. Student's unpaired t-test was used to compare means between two groups. Two-way ANOVA analysis was used for comparison of means among multiple groups. A p value < 0.05 was considered significant.