Figure S1. Systemic transplantation of hMSCs improves serum hyper-autoantibody levels and renal dysfunction in MRL/lpr mice. (A) A schema of systemic transplantation (Tx) of hBMMSCs and SHED into MRL/lpr (lpr) or C57BL/6 (BL/6) mice. The mice were received the transplants at 16 weeks (wks) of age. The control mice were treated with PBS. (B) ELISA of serum autoantibody. ANA: anti-nuclear antibody. (C) Levels of urine protein and serum albumin and creatinine. B, C: n=5 for all group. Pre-MRL/lpr: pre-transplant MRL/lpr mice at 16 weeks of age; MRL/lpr, hBMMSC-T, SHED-T: non-, hBMMSC-, and SHED-transplanted MRL/lpr mice at 20 weeks of age, respectively. *P<0.05, **P<0.01, and ***P<0.005. The graph bars represent means±SD.
Figure S2. Systemic hMSC transplantation ameliorates the bone loss in MRL/lpr mice. (A) Representative histological images of trabecular bone structures (blue-dashed areas). (B) In vivo osteoblast activity assay. Real-time RT-PCR analysis of osteoblast-specific genes. *Alp*: alkaline phosphatase; *Gapdh*: Glyceraldehyde 3-phosphate dehydrogenase; *Ocn*: osteocalcin; *Runx2*: runt-related transcription factor 2. (C) In vivo osteoclast activity assay. TRAP staining of recipient tibias. A–C: n=5 for all groups. Pre-Tx (16w): pre-transplant stage at 16 weeks of mouse age; Post-Tx (20w): post-transplanted stage at 20 weeks of mouse age. B: *P<0.05 and ***P<0.005. Bar graphs show the means ±SD.
Figure S3. Systemic hMSC transplantation recovers dysregulation of recipient BMMSCs in MRL/lpr mice. (A–C) Stemness of recipient BMMSCs. Colony forming unit-fibroblasts (CFU-F) assay (A). Population doubling (PD) assay (B). Brd-U incorporation assay (C). A–C: n=5 for all groups. *P<0.05 and ***P<0.005. Bar graphs show the means±SD. MSC-Pre-MRL/lpr, MSC-MRL/lpr, MSC-hBMMSC, MSC-SHED: BMMSCs isolated from pre-transplant MRL/lpr mice at 16 weeks of age, and non-, hBMMSC-, and SHED-transplanted MRL/lpr mice at 20 weeks of age, respectively.
Figure S4. Systemic hMSC transplantation recovers dysregulation of osteoblastogenesis and osteoclastogenesis via recipient BMMSCs in MRL/lpr mice. (A) Osteogenic activity of recipient BMMSCs. Real-time RT-PCR analysis of osteoblast-specific genes at 1 week after osteogenic induction. (B) Osteoclast inductivity of recipient BMMSCs in co-culture with wild-type C57BL/6 mice-derived bone marrow cells (BMC-WT) under stimulation with 1α, 25-(OH)₂ vitamin D₃ and prostaglandin E₂ for 7 days. Real-time RT-PCR analysis of osteoclast-specific gene assay. Nfatc1: nuclear factor of activated T cells, cytoplasmic 1; Ctr: calcitonin receptor, CathK: cathepsin K; Gapdh: glyceraldehyde 3-phosphate dehydrogenase. A, B: n=5 for all groups. MSC-Pre-MRL/lpr, MSC-MRL/lpr, MSC-hBMMSC, MSC-SHED: BMMSCs isolated from pre-transplant, non-, hBMMSC-, and SHED-transplanted MRL/lpr mice, respectively. *P<0.05, **P<0.01, and ***P<0.005. Bar graphs show the means±SD.
**Figure S5.** Systemic transplantation of hMSCs suppresses peripheral Th17 cells in MRL/lpr mice. (A, B) Suppression of Th17 cell differentiation and IL-17 secretion by hBMMSCs and SHED in co-culture. Flow cytometry of CD4+IL-17+IFNγ+ cells (A). ELISA of IL-17 in conditioned medium (CM IL17) (B). (C, D) Peripheral levels of Th17 cells and IL-17 in recipient MRL/lpr mice. Flow cytometry of peripheral CD4+IL-17+IFNγ+ cells (C). ELISA of serum IL-17 (serum IL17) (D). *P<0.05, **P<0.01, and ***P<0.005. The graph bar represents means±SD. n=5 for all groups. A, B: Active T: human CD4+CD25− T cells activated with anti-CD3 and anti-CD28 antibodies.
Figure S6. Systemic hMSC transplantation suppresses the IL-17-enhanced environment in recipient bone marrow of MRL/lpr mice. (A) In vivo homing assay of hBMMSCs and SHED in recipient bone marrow. Cell tracing assay using the CSFE-labeling method. Nuclei were counterstained with DAPI. Day 1: Cell infusion after 1 day; Day 7: Cell infusion after 7 days. (B–D) Levels of IL-17 and Th17 cells in recipient bone marrow. Immunofluorescence of IL-17 (IL17). Nuclei were counterstained with DAPI. (B). Flow cytometric analysis of CD4+IL-17+IFNγ cells in bone marrow (BM CD4+IL-17+IFNγ cells) (C). ELISA of bone marrow IL-17 (BM IL17) (D). A–D: n=5 for all groups. C, D: **P<0.01 and ***P<0.005. Bar graphs show the means±SD.
Figure S7. Systemic hMSC transplantation shows no effect to the bone loss and IL-17 levels in C57BL/6 mice. (A, B) Morphological analyses of mouse tibiae. Bone mineral density (BMD) (A). Trabecular bone parameter assay. BV/TV: bone volume ratio to tissue volume; Tb.Th: trabecular thickness; Tb.N: trabecular number; Tb.Sp: trabecular separation (B). (C, D) In vivo osteoclast activity assay. ELISA of mouse serum. CTX: C-terminal telopeptides of type I collagen; sRANKL: soluble RANKL (C). Histological analysis of recipient tibias by TRAP staining. TRAP+ cells: TRAP-positive osteoclast-like cells (D). (E) In vivo osteoblast activity assay. Real-time RT-PCR analysis of osteoblast-specific genes. Alp: alkaline phosphatase; Gapdh: Glyceraldehyde 3-phosphate dehydrogenase; Ocn: osteocalcin; Runx2: runt-related transcription factor 2. (F) IL-17 levels in serum and bone marrow. ELISA. A–F: n=5 for all groups. BL/6, BL/6-hBM-MSC-T, BL/6-SHED-T: non-, hBM-MSC-, and SHED-transplanted C57BL/6 mice. Bar graphs show the means±SD.
Figure S8. Effects of IL-17 on osteogenic capacity and osteoclast differentiation. (A, B) Alizarin Red staining 4 weeks after the osteogenic induction of wild type mouse-derived BMMSCs (MSC-WT) under recombinant mouse IL-17 (IL17) (A) or conditioned medium of MRL/lpr mice-derived BMCs (CM-MRL/lpr) (B) in the presence and absence of anti-mouse IL-17 antibody (Anti-IL17 Ab) or the control antibody (Cont Ab). (C, D) TRAP staining after co-culture of wild type mouse-derived BMCs (BMC-WT) and calvarial cells (Calvaria-WT) stimulated by 1α, 25(OH)₂ vitamin D₃ and prostaglandin E₂ under IL17 (C) or CM-MRL/lpr (D) in the presence and absence of Anti-IL17 Ab. n=5 for all groups. *P<0.05, ***P<0.005. The graph bars show means±SD.
Figure S9. Systemic treatment of anti-IL-17 antibody improves bone loss in MRL/lpr mice. (A) A schema of systemic treatment of anti-IL-17 antibody (Anti-IL17 Ab) into MRL/lpr (lpr) mice. The antibody (1 mg/ml) was intraperitoneally injected twice a week for 4 weeks to MRL/lpr mice at 16 weeks (wks) of age. The control mice were treated with the isotype-matched IgG2a or PBS. (B–D) Morphological analyses of mouse tibiae. Representative microCT images of trabecular bone structures stage of MRL/lpr mice at 20 weeks of age (yellow-dashed areas) (B). Bone mineral density (BMD) (C). Trabecular bone parameter assay. BV/TV: bone volume ratio to tissue volume; Tb.Th: trabecular thickness; Tb.N: trabecular number; Tb.Sp: trabecular separation (D). B–D: n=5 for all group. MRL/lpr, IgG2a, Anti-IL17 Ab: non-, IgG2a and Anti-IL17 Ab-treated MRL/lpr mice at 20 weeks of age, respectively. *P<0.05. ns: no significance. The graph bars represent means±SD.
Figure S10. Systemic treatment of anti-IL-17 antibody regulates osteoblastogenesis and osteoclastogenesis via recipient BMMSCs in MRL/lpr mice. (A) In vivo osteoblast differentiation assay. Real-time RT-PCR analysis of osteoblast-specific genes in recipient long bones of MRL/lpr mice at 20 weeks of age. Alp: alkaline phosphatase; Gapdh: Glyceraldehyde 3-phosphate dehydrogenase; Ocn: osteocalcin; Runx2: runt-related transcription factor 2. (B) In vivo osteoclast differentiation assay. Real-time RT-PCR analysis of osteoclast-specific gene assay in recipient long bones of to MRL/lpr mice at 20 weeks of age. Nfatc1: nuclear factor of activated T cells, cytoplasmic 1; Ctr: calcitonin receptor, CathK: cathepsin K. (C) In vivo osteoclast activity assay. ELISA analyses of mouse serum. CTX: C-terminal telopeptides of type I collagen; sRANKL: soluble RANKL. A-C: n=5 for all groups. MRL/lpr, IgG2a, Anti-IL17 Ab: non-, IgG2a and Anti-IL17 Ab-treated MRL/lpr mice at 20 weeks of age, respectively. *P<0.05 and ***P<0.005. ns: no significance. Bar graphs show the means±SD.