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# Bone Marrow Transplantation Improves Autoinflammation and Inflammatory Bone Loss in SH3BP2 Knock-In Cherubism Mice

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## Abstract

Cherubism (OMIM#118400) is a genetic disorder in children characterized by excessive jawbone destruction with proliferation of fibro-osseous lesions containing a large number of osteoclasts. Mutations in the SH3-domain binding protein 2 (SH3BP2) are responsible for cherubism. Analysis of the knock-in (KI) mouse model of cherubism showed that homozygous cherubism mice (*Sh3bp2*$^{KI/KI}$) spontaneously develop systemic autoinflammation and inflammatory bone loss and that cherubism is a TNF-α-dependent hematopoietic disorder. In this study, we investigated whether bone marrow transplantation (BMT) is effective for the treatment of inflammation and bone loss in *Sh3bp2*$^{KI/KI}$ mice. Bone marrow (BM) cells from wild-type (*Sh3bp2*$^{+/+}$) mice were transplanted to 6-week-old *Sh3bp2*$^{KI/KI}$ mice with developing inflammation and to 10-week-old *Sh3bp2*$^{KI/KI}$ mice with established inflammation. Six-week-old *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells exhibited improved body weight loss, facial swelling, and survival rate. Inflammatory lesions in the liver and lung as well as bone loss in calvaria and mandibula were ameliorated at 10 weeks after BMT compared to *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells. Elevation of serum TNF-α levels was not detected after BMT. BMT was effective for up to 20 weeks in 6-week-old *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. BMT also ameliorated the inflammation and bone loss in 10-week-old *Sh3bp2*$^{KI/KI}$ mice. Thus our study demonstrates that BMT improves the inflammation and bone loss in cherubism mice. BMT may be effective for the treatment of cherubism patients.



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The authors have no conflicting financial interests to declare.

**Keywords**

Cherubism; SH3BP2; Autoinflammation; Bone loss; Bone marrow transplantation

## Introduction

Cherubism (OMIM#118400) is an autosomal dominant disorder characterized by bilateral multilocular cystic bone destruction in the maxilla and mandibula. The cystic bone lesions are filled with proliferative fibro-osseous tissues containing a large number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated giant cells, which lead to a disfiguring swelling of the lower face. Periodontal bone destruction causes “free-floating” teeth and chewing problems [1]. Swelling of submandibular lymph nodes is also observed [2]. Affected children seem normal at birth and manifest the characteristic facial appearance at 2 to 5 years old, and the cherubism lesions usually begin to regress after puberty by mechanisms that may involve Toll-like receptor (TLR)-MYD88 pathway in macrophages [3]. In some severe cases, the lesions expand into the orbital walls or upper airway [4–7], resulting in upward gazing, optic neuropathy [7], or respiratory disturbance [8]. Currently there is no established protocol for cherubism treatment.

We have previously discovered gain-of-function heterozygous mutations in the SH3-domain binding protein 2 (SH3BP2) responsible for cherubism [9]. SH3BP2 is a signaling adaptor protein that interacts with various proteins, such as SYK [10], VAV [11], PLCγ1 and PLCγ2 [10, 12]. SH3BP2 is expressed in hematopoietic cells including T and B lymphocytes, macrophages, neutrophils, mast cells, and osteoclasts [13–17]. We created a knock-in (KI) mouse model of cherubism by introducing the most common P418R amino acid substitution in cherubism patients into the mouse *Sh3bp2* gene (P416R in mice) [18]. The *Sh3bp2*$^{KI/KI}$ mice spontaneously develop systemic inflammation including in liver and lung with elevation of serum TNF-α levels and show periodontal and calvarial bone erosion caused by the inflammatory infiltrates, suggesting that cherubism is an inflammatory disorder. Inflammation in *Sh3bp2*$^{KI/KI}$ mice, which develops independently of T and B cells, is regarded as a new form of autoinflammation characterized by increased cytokine responsiveness of macrophages [19], and it starts to develop at around 1 week of age [20]. Genetic deletion of TNF-α in *Sh3bp2*$^{KI/KI}$ mice shows a significant rescue of inflammation, suggesting that TNF-α plays a key role in the pathogenesis of inflammation in *Sh3bp2*$^{KI/KI}$ mice. In fact, TNF-α is expressed in both multinucleated giant cells and stromal cells in human cherubism lesions [21, 22]. These results led us to investigate whether postnatal administration of anti-TNF-α drug is able to prevent or ameliorate the symptoms in *Sh3bp2*$^{KI/KI}$ mice, which is more relevant to clinical cases of human cherubism. We found that postnatal administration of a TNF-α blocker, etanercept, is able to prevent the development of inflammation when administered to the inflammation-free neonatal (1 week old) *Sh3bp2*$^{KI/KI}$ mice [20]. However, inflammation recurs after discontinuation of etanercept treatment. In contrast, administration of etanercept to 10-week-old *Sh3bp2*$^{KI/KI}$ mice with fully active inflammation showed only a limited effect [20]. These results suggest that anti-TNF-α therapy may be effective in young cherubism patients at the early stages of

the disease before the onset of lesion formation, but not in patients after the onset of active lesion formation.

Bone marrow transplantation (BMT) has been used for the treatment of a wide range of human diseases such as leukemia [23], lymphoma [24], aplastic anemia [25], Krabbe disease [26], Hurler syndrome [27–29], Gaucher disease [28], and Wiskott-Aldrich syndrome [30, 31]. Since our previous study on the *Sh3bp2*$^{KI/KI}$ mice demonstrated that cherubism is a hematopoietic disorder of myeloid lineage cells that causes autoinflammation [18] and abundant active inflammatory cells in human cherubism lesions are reported [32], we hypothesized that transplantation of wild-type (*Sh3bp2*$^{+/+}$) bone marrow (BM) cells may be able to ameliorate the actively growing or established inflammation in *Sh3bp2*$^{KI/KI}$ mice that cannot be ameliorated by etanercept treatment. Here, we show that transplantation of *Sh3bp2*$^{+/+}$ BM cells to *Sh3bp2*$^{KI/KI}$ mice rescues systemic inflammation and inflammatory bone loss in *Sh3bp2*$^{KI/KI}$ cherubism mice with actively growing and established inflammatory lesions.

## Materials and Methods

### Animals

Cherubism mice (*Sh3bp2*$^{KI/KI}$) in C57BL/6 background were described previously [18]. Briefly, the most common mutation in human cherubism patients (P418R) was knocked-in to the mouse *Sh3bp2* gene (P416R in mouse) by homologous recombination. The *Sh3bp2*$^{+/+}$ or *Sh3bp2*$^{KI/KI}$ mice in C57BL/6 background mice were used as donors and recipients of BMT. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Missouri-Kansas City.

### Bone marrow (BM) cell transplantation

Twenty-four hours before transplantation, recipient mice were intraperitoneally injected with busulfan (25 mg/kg in 1:1 ratio of PBS/DMSO for 10-week-old recipients and 50 mg/kg for 6-week-old recipients, Sigma-Aldrich, St. Louis, MO) for myeloablation. BM cells were flushed out with Hank's Balanced Salt Solution (HBSS) from tibias and femurs of 6-week-old donor mice. The cells were filtrated with 70 μm nylon mesh (BD Biosciences, San Jose, CA), and red blood cells were lysed with RBC lysis solution (eBioscience, San Diego, CA). $1 \times 10^7$ cells in HBSS were injected intravenously to the busulfan-conditioned recipient mice. Water supplemented with sulfamethoxazole/trimethoprim (800 and 160 mg/l, respectively, Hi-Tech Pharmacal, Amityville, NY) was given for 2 weeks after busulfan treatment to prevent infections in the recipient mice.

### Body weight measurement, assessment of facial swelling, and enzyme-linked immunosorbent assay (ELISA)

Body weights were recorded after BMT. At 10 or 20 weeks after transplantation, facial swelling was assessed by two independent observers in a blinded manner as previously described [20]. Blood samples were collected at the time of necropsy. Serum TNF-α levels were measured by ELISA in accordance with the manufacturer's instruction (R&D, Minneapolis, MN).

### Evaluation of chimerism

Chimerism of BM cells in recipient mice at the time of analysis was determined by semi-quantitative genomic PCR detecting mouse *Sh3bp2* using genomic DNAs isolated from whole BM cells. Primers for *Sh3bp2* are as follows: Forward: 5′-CTTGGAGACTGGGCTTAAGAGGAC - 3′ and Reverse: 5′-TAATACTGGTAAGCAGGGGTGCTG - 3′ (product size: 262bp for wild-type, 303bp for mutant). Control samples for the standard curve creation were prepared by mixing homozygous mutant and wild-type genomic DNAs at various ratios (0 to 100% of homozygous mutant DNA with 10% increments). PCR products were separated on an agarose gel and a digital image was taken. The intensity of DNA bands (INT) for mutant ($INT^{KI}$), wild-type ($INT^{WT}$), and background ($INT^{Bgd}$) were acquired using ImageJ (NIH). The ratio (R) of adjusted INT for sample ($INTR^{Sample}$) and control ($INTR^{Control}$) were calculated by the following equation: $INTR = (INT^{KI} – INT^{Bgd})/(INT^{WT} – INT^{Bgd})$. Standard curves were made using values of $INTR^{Control}$ at different mix ratios. Percentage of chimerism in each genomic DNA from bone marrow cells was calculated from the standard curve and $INTR^{Sample}$. *Sh3bp2*$^{KI/KI}$ recipient mice transplanted with *Sh3bp2*$^{+/+}$ BM cells with less than 70% chimerism at the time of analysis were excluded from the study. When genotype of recipients is identical to donor cells, the chimerism percentage was regarded as 100%.

### Tissue preparation, staining, and histomorphometric analysis

Liver and lung tissues were fixed with 4% paraformaldehyde/PBS, embedded in paraffin, and 6-μm-thick sections were stained by hematoxylin and eosin (H&E). To quantify the inflammation in liver tissues, digital images of the stained liver sections were taken with a 4x objective lens. Areas occupied by inflammatory cells ($Area^{Inf}$) and by blood vessels ($Area^{Vessels}$) were measured with ImageJ software as pixels. Inflammatory area in the liver (%) was calculated by the following equation: (total inflammatory area) = 100 x (total $Area^{Inf}$)/((total image area) – (total $Area^{Vessels}$)). An averaged value from 3 to 4 images per sample was regarded as the representative value for each individual sample. Inflammatory lung lesions on H&E image were measured by ImageJ as pixels. The equation used for the calculation of inflammatory lung area (%) is as follows: (inflammatory area in lung) = 100 x (total inflammatory area)/(total lung area).

### MicroCT analysis

Paraformaldehyde-fixed mandibulae, calvariae, and tibiae were scanned with microCT (vivaCT 40, Scanco Medical, PA) with an X-ray energy of 55 kVp (145 μA). Resulting images were used to quantify bone loss. Two-dimensional images were taken with following thresholds: 300 (equivalent to 471.1 mg hydroxyapatite (HA)/$cm^3$) for jaw and calvarial bones, 220 (equivalent to 298.2 mg HA/$cm^3$) for trabecular bones to distinguish mineralized tissues from non-mineralized tissues, and they were processed for three-dimensional (3D) image reconstruction with a spatial resolution of 10 μm. To assess the proportion of erosion area on calvaria (%), microCT images (6 × 6 mm square) were used. The intersections of the coronal and sagittal sutures (bregma) were set as the reference positions of the square center [20]. Total area of bone erosions, including suture areas in the 6 mm × 6 mm square on

calvaria, was measured as pixels with ImageJ and divided by the total pixel number of the square. Distance between the cemento-enamel junction (CEJ) and alveolar bone crest (ABC) at the distal lingual surface of the mandibular first molar was measured using reconstructed 3D images according to our previous report [20] to assess periodontal bone loss. The region of trabecular bone comprised 100 slices of secondary spongiosa beginning just below primary spongiosa. Bone properties were analyzed using Scanco bone evaluation software. Micro-CT assessments were performed according to the international guidelines [33].

### Statistical analysis

Statistical analysis was performed using SPSS ver. 20 (IBM, Chicago, IL). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied for the comparisons among groups. Statistical significance was defined as $P < 0.05$.

## RESULTS

### Transplantation of *Sh3bp2*$^{+/+}$ bone marrow cells to 6-week-old *Sh3bp2*$^{KI/KI}$ mice with actively growing inflammation rescues facial swelling, body weight loss, mortality, and elevation of serum TNF-α levels

We examined whether BMT rescues the *Sh3bp2*$^{KI/KI}$ mice that have actively developing inflammation. Six-week-old *Sh3bp2*$^{KI/KI}$ mice were selected as recipient mice, because *Sh3bp2*$^{KI/KI}$ mice start to die after 8 weeks of age due to severe systemic inflammation, which is supposed to occur after reaching the full active inflammatory phase [18]. We intravenously injected wild-type (*Sh3bp2*$^{+/+}$) BM cells from 6-week-old mice to 6-week-old *Sh3bp2*$^{KI/KI}$ mice (Fig. 1A). The *Sh3bp2*$^{KI/KI}$ recipients transplanted with *Sh3bp2*$^{+/+}$ BM cells were followed up for 10 or 20 weeks and compared to *Sh3bp2*$^{+/+}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells (non-inflamed controls) and *Sh3bp2*$^{KI/KI}$ inflamed control mice. As inflamed controls, we used *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells for a 10-week follow-up and *Sh3bp2*$^{KI/KI}$ mice without treatment for a 20-week follow-up because our pilot study showed that all three *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells died during the 20-week follow-up period (Fig. 1D), which is consistent with the previous report that more than 70% of *Sh3bp2*$^{KI/KI}$ mice die by 30 weeks of age due to systemic inflammations [18].

At 10 weeks after BMT, all *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells showed a rescue of facial swelling with open eyelids (Fig. 1B right, top, n = 9/9), while none of *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells showed a similar rescue (Fig. 1B center, top, n = 0/8). The rescue of facial swelling was observed at 20 weeks after the BMT in 88.9% of *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells (Fig. 1B right, bottom, n = 8/9), while none of 26-week-old *Sh3bp2*$^{KI/KI}$ mice without BMT exhibited an improvement of severe eyelid closure (Fig. 1B center, bottom, n = 0/9). Consistent with these observations, loss of body weight was improved in the *Sh3bp2*$^{KI/KI}$ mutants transplanted with *Sh3bp2*$^{+/+}$ BM cells in both males and females (Fig. 1C, red lines) compared to *Sh3bp2*$^{KI/KI}$ mice without BMT (Fig. 1C, green lines).

All *Sh3bp2*$^{KI/KI}$ mutants transplanted with *Sh3bp2*$^{+/+}$ BM cells survived for 20 weeks after BMT (26 weeks of age, n = 9/9, Fig. 1D, red line), while 56.3 % of non-transplanted *Sh3bp2*$^{KI/KI}$ mice survived to 26 weeks of age (n = 9/16, Fig. 1D, green line). Serum TNF-α levels in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells at 10 and 20 weeks after BMT were significantly reduced and practically undetectable in all cases compared to *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells or *Sh3bp2*$^{KI/KI}$ mice without transplantation, respectively (Fig. 1E). Taken together, these results demonstrate that transplantation of wild-type BM cells rescues facial swelling, body weight loss, reduction in survival rate, and elevation of serum TNF-α levels in *Sh3bp2*$^{KI/KI}$ mice by 10 weeks after BMT, and the rescue continues for at least 20 weeks after BMT.

### Transplantation of *Sh3bp2*$^{+/+}$ bone marrow cells to 6-week-old *Sh3bp2*$^{KI/KI}$ mice with actively growing inflammation improves liver and lung inflammation

To evaluate whether inflammation is rescued in internal organs in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells, liver and lung tissue sections were stained by H&E, and the inflammatory lesions were quantified at 10 and 20 weeks after BMT. Liver inflammatory lesions in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells were significantly reduced at 10 weeks after BMT (Fig. 2A, right, top) compared to *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells (Fig. 2A, center, top), and the reduced liver inflammation continued until 20 weeks after transplantation (Fig. 2A, right, bottom). Histomorphometric analysis confirmed the reduction of liver inflammation (Fig. 2B). Consistent with the improvement of liver lesions, inflammatory lesions in lung tissues were significantly reduced in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells (Fig. 2C), which was verified by histomorphometric analysis (Fig. 2D). These results indicate that transplantation of *Sh3bp2*$^{+/+}$ BM cells is effective for the reduction of inflammatory lesions in liver and lung in *Sh3bp2*$^{KI/KI}$ mice.

### BMT with *Sh3bp2*$^{+/+}$ cells to 6-week-old *Sh3bp2*$^{KI/KI}$ mice rescues inflammatory bone loss

To examine the effect of BMT on bone loss in *Sh3bp2*$^{KI/KI}$ mice, we evaluated calvarial and periodontal bones using 3D reconstructed microCT images. Inflammatory bone loss on the surface of calvaria was detected as multiple erosion pit formation in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells (Fig. 3A, center panel, arrowheads). Quantitation of areas of calvarial bone erosion showed that *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells exhibit a significant reduction in erosion pit formation at both 10 and 20 weeks after BMT (Fig. 3B).

Next, we assessed the periodontal bone loss in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells using reconstructed 3D microCT images. Consistent with the rescue from calvarial bone erosion in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells, transplantation of *Sh3bp2*$^{+/+}$ BM cells reduced erosion pit formation on the surface of mandibula at 10 weeks after BMT (Fig. 3C, right, bottom). To quantitate the periodontal bone loss, the distance between CEJ and ABC at the distal lingual surface of the first molar was measured. Average CEJ-ABC distance in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells at 10 and 20 weeks after BMT was also reduced compared to 16-week-old *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells (10 weeks after BMT) or 26-

week-old *Sh3bp2*$^{KI/KI}$ mice without transplantation (20 weeks after BMT), respectively (Fig. 3D). Consistent with these results, transplantation of wild-type BM cells partially, but effectively, prevented trabecular bone loss at proximal tibia (bone volume per total volume: BV/TV) in *Sh3bp2*$^{KI/KI}$ mice at 20 weeks after BMT (Fig. 3E).

Taken together, these results indicate that transplantation of *Sh3bp2*$^{+/+}$ BM cells is effective for improving inflammatory bone loss in *Sh3bp2*$^{KI/KI}$ mice that have actively growing inflammatory lesions.

## Transplantation of *Sh3bp2*$^{+/+}$ bone marrow cells to 10-week-old *Sh3bp2*$^{KI/KI}$ mice with fully established inflammation rescues facial swelling, body weight loss, mortality, and elevation of serum TNF-α levels

In human, cherubism is usually diagnosed during the full active phase in which rapid expansion of jaws occurs. Therefore, we questioned whether BMT is able to relieve the fully established phenotypes in *Sh3bp2*$^{KI/KI}$ mice, which presumably simulate the situation in human cherubism treatment. For this aim, 10-week-old *Sh3bp2*$^{KI/KI}$ mice were used as recipients for BMT, and they were followed up for 10 weeks after BMT (Fig. 4A). At 10 weeks of age, *Sh3bp2*$^{KI/KI}$ mice show swelling in the face with closed eyelids, which becomes more severe at 20 weeks of age (Fig. 4B, center). In contrast, transplantation of *Sh3bp2*$^{+/+}$ BM cells to 10-week-old *Sh3bp2*$^{KI/KI}$ mice resulted in a partial rescue of facial swelling after 10 weeks of BMT in 42.9% (n = 3/7) of *Sh3bp2*$^{KI/KI}$ mice (Fig. 4B, right). Furthermore, 10-week-old *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells showed a significant improvement in a gain of body weight in both males and females (Fig. 4C, red lines) compared to *Sh3bp2*$^{KI/KI}$ mice without BMT (Fig. 4C, green lines). The improved weight gain in 10-week-old *Sh3bp2*$^{KI/KI}$ mice started at around 4 weeks after BMT, whereas such improvement started at around 2 weeks after BMT in 6-week-old *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. All *Sh3bp2*$^{KI/KI}$ mice (n = 7) transplanted with *Sh3bp2*$^{+/+}$ BM cells survived for 10 weeks after BMT (data not shown). Significant reduction of serum TNF-α levels was observed in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells at 10 weeks after BMT compared to *Sh3bp2*$^{KI/KI}$ mice without transplantation (Fig. 4D).

## Transplantation of *Sh3bp2*$^{+/+}$ bone marrow cells to 10-week-old *Sh3bp2*$^{KI/KI}$ mice with fully established inflammation improves inflammation in liver and lung as well as inflammatory bone loss

We examined inflammation in liver and lung in *Sh3bp2*$^{KI/KI}$ mice that were transplanted with *Sh3bp2*$^{+/+}$ BM cells at 10 weeks of age. The transplantation greatly reduced the infiltration of inflammatory cells in both liver and lung at 10 weeks after BMT (Fig. 5A, right), whereas *Sh3bp2*$^{KI/KI}$ mice without BMT exhibited inflammatory cell accumulation (Fig. 5A, center, arrowheads). Histomorphometric analysis confirmed the significant rescue of liver and lung inflammation in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells (Fig. 5B).

Next, we investigated whether transplantation of *Sh3bp2*$^{+/+}$ BM cells to 10-week-old *Sh3bp2*$^{KI/KI}$ mice was able to rescue the bone destruction in calvarial and periodontal

regions. MicroCT images showed that the number of erosion pits on calvaria was reduced in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells (Fig. 5C, right, top) compared to *Sh3bp2*$^{KI/KI}$ mice without transplantation (Fig. 5C, center, top). Erosion pit formation on the surface of mandibula was reduced in the *Sh3bp2*$^{KI/KI}$ mice (Fig. 5C, right, bottom). Quantitative analysis revealed that the rescue of calvarial bone loss in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells is not statistically significant, but showed a trend of reduction ($P = 0.11$), compared to *Sh3bp2*$^{KI/KI}$ mice without transplantation. (Fig. 5D, left). Measurement of the CEJ-ABC distance showed a significant rescue of periodontal bone loss in *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells compared to *Sh3bp2*$^{KI/KI}$ mice without transplantation (Fig. 5D, right). Trabecular bone loss was also ameliorated in *Sh3bp2*$^{KI/KI}$ mice that underwent wild-type BMT (Fig. 5E). Collectively, these results suggest that transplantation of *Sh3bp2*$^{+/+}$ BM cells effectively ameliorates the systemic inflammation associated with elevated serum TNF-α levels as well as inflammatory bone loss in *Sh3bp2*$^{KI/KI}$ mice that have fully established inflammation.

## DISCUSSION

At present, standard management of cherubism symptoms is generally limited to supportive care including observation of the disease course with the expectation that lesions may regress after puberty [34]. While lesions in human cherubism are usually localized to the jaw region [35–37] and functional or cosmetic problems during the active phase of the disease differ between affected children, aggressive cases involving nasal obstruction, proptosis, or severe facial deformities have been reported [38–42]. To correct upward gazing and visional disturbance, orbital surgery may be required [43]. Corrective surgery by curettage with or without bone grafting is sometimes performed for extensively enlarged jaws [44]. Generally, such surgical intervention is considered only in severe cases and most of the surgical interventions are performed after puberty when cherubic lesions may become inactive, although mechanisms of the age-dependent phenotype regression in human cherubism patients, but not in cherubism mice, remain to be further elucidated [3]. Age-associated change in immune responses by TLRs [45, 46] might be a cause of the regression [3]. Prosthetic treatment could be applied for the missing or floating teeth [47].

Currently there is no established pharmacological treatment for cherubism, but administration of calcitonin or a TNF-α antagonist has been applied to control the disease progression during the active phase. de Lange and colleagues have reported a considerable regression of the cherubism lesions in one 11-year-old boy after daily administration of calcitonin for 15 months [48]. Etoz and colleagues have also reported one case in which cherubism lesions in the mandible significantly regressed after 30 months of calcitonin therapy [49]. On the other hand, Lannon and Earley have reported no obvious improvement by calcitonin administration [38]. Therefore, efficacy of calcitonin for cherubism treatment seems to be still controversial. Our recent study in *Sh3bp2*$^{KI/KI}$ mice demonstrated that the anti-TNF-α drug, etanercept, is able to prevent disease manifestation when administration begins at 1 week of age [20]. However, symptoms recurred after discontinuation of the etanercept treatment, suggesting that long-term anti-TNF-α therapy will be required until puberty to keep the non-active status in human cherubism patients, which may adversely cause side effects of anti-TNF-α treatment [50]. In addition, anti-TNF-α treatment was not

effective for 10-week-old *Sh3bp2*$^{KI/KI}$ mice with fully active inflammation, suggesting that anti-TNF-α treatment initiated after manifestation of the symptoms may not be effective in cherubism treatment and that inflammatory cytokines other than TNF-α such as IL-1α/β are involved in the later phase of the disease [20]. In agreement with the results in *Sh3bp2*$^{KI/KI}$ mice, Hero and colleagues have reported that treatment with a TNF-α inhibitor, adalimumab, failed to introduce the regression of lesions in two cherubism patients with actively growing lesions [21]. Likewise, a case of unsuccessful treatment with adalimumab in combination with bisphosphonate has been reported [51]. Therefore, besides current treatment options, alternative approaches have been demanded.

In this study, we elucidated that BMT can be a future potential treatment option for cherubism by using a cherubism mouse model. We found that transplantation of *Sh3bp2*$^{+/+}$ BM cells to *Sh3bp2*$^{KI/KI}$ recipient mice is able to cure both inflammation and bone loss in *Sh3bp2*$^{KI/KI}$ mice. Notably, a single injection of wild-type BM cells could induce this remission. Furthermore, BMT was effective even in treating fully inflamed *Sh3bp2*$^{KI/KI}$ mice, which were not ameliorated with anti-TNF-α therapy in mice and humans [20, 21], suggesting that wild-type BMT also corrected inflammatory cytokine levels other than TNF-α. We have previously reported that cherubism is a hematopoietic disorder of myeloid lineage cells caused by hyperactive macrophages and osteoclasts [3, 18, 22, 52]. Therefore, we assume that replacement of the mutant macrophages and osteoclasts with those carrying wild-type SH3BP2 is a main cause of the improved symptoms in *Sh3bp2*$^{KI/KI}$ mice, which resulted in the comprehensive reconstitution of the expression profile of inflammatory cytokines and osteoclast-mediated bone resorption activity in *Sh3bp2*$^{KI/KI}$ recipients. Effective rescue by BMT further suggests that contribution of non-hematopoietic *Sh3bp2*$^{KI/KI}$ stromal cells to the inflammation and bone loss is limited. Interestingly, improvement in the trabecular bone loss appears to be smaller than that in calvarial bone and jawbone loss, indicating that dysfunction of osteoblasts that is responsible for impaired mineral and matrix properties of trabecular bone in *Sh3bp2*$^{KI/KI}$ mice [53] was not rescued by BMT and that BMT primarily rescues bone loss that is associated with inflammation in *Sh3bp2*$^{KI/KI}$ mice.

We found that BMT in 6-week-old *Sh3bp2*$^{KI/KI}$ mice has more therapeutic effect when compared to 10-week-old *Sh3bp2*$^{KI/KI}$ mice in the improvement of body weight gain and liver inflammation, suggesting that BMT in the earlier phase of cherubism may be more effective. We did not see any gender-dependent differences in the BMT responses (data not shown). Because of the potential adverse effects of BMT such as graft-versus-host or graft-versus-leukemia disease [54, 55], infections [56], thrombocytopenic hemorrhage [57], and toxicities of busulfan [58], current application of BMT to humans is limited to malignant hematopoietic or severe life-threatening genetic disorders. Therefore, it is important to weigh the risks and benefits if BMT is applied to cherubism patients. Since high levels of serum TNF-α increase the risk of complications of BMT [59], a combination of BMT and anti-TNF-α therapy may also contribute to the successful treatment of cherubism. With novel genome editing technologies [60, 61], we may be able to correct the cherubism mutation in induced pluripotent stem (iPS) cells [62, 63] that are derived from cherubism patients. Transplantation of hematopoietic stem cells differentiated from patient's own iPS

cells in which cherubism mutation has been corrected would reduce the risk of graft-versus-host disease and contribute to the improvement of the benefit/risk balance in the treatment of cherubism.

In conclusion, we showed that transplantation of *Sh3bp2*$^{+/+}$ BM cells to *Sh3bp2*$^{KI/KI}$ mice is able to improve inflammation and inflammatory bone loss in *Sh3bp2*$^{KI/KI}$ mice with developing inflammation as well as *Sh3bp2*$^{KI/KI}$ mice with fully established inflammation. Our results propose that BMT is a future potential therapeutic option for human cherubism patients, particularly for those individuals with aggressive and severe symptoms.

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## Highlights

- Bone marrow transplantation rescues adult cherubism mice from autoinflammation
- Bone marrow transplantation improves inflammatory bone loss in adult cherubism mice
- Bone marrow transplantation may be a therapeutic option for cherubism treatment

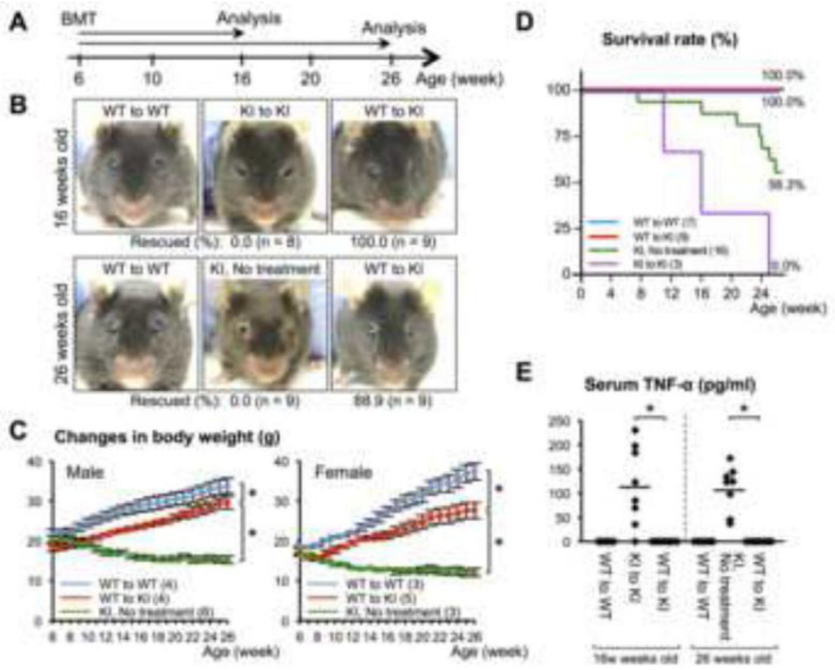


**Fig. 1.**

Transplantation of *Sh3bp2*$^{+/+}$ bone marrow (BM) cells to 6-week-old *Sh3bp2*$^{KI/KI}$ mice improves facial swelling, loss of body weight, survival rate, and elevation of serum TNF-α levels. **(A)** Experimental procedure of bone marrow transplantation (BMT). Six-week-old *Sh3bp2*$^{+/+}$ and *Sh3bp2*$^{KI/KI}$ mice were intravenously injected with BM cells isolated from 6-week-old mice. Ten or 20 weeks after BMT, namely at 16- or 26-week-old, respectively, recipient mice were analyzed. **(B)** Facial appearance of the recipient mice at 10 (top) or 20 weeks (bottom) after BMT. Twenty-six-week-old *Sh3bp2*$^{KI/KI}$ mice without BMT were used as controls for *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells that were followed-up for 20 weeks (center, bottom). **(C)** Body weight changes after BMT. **(D)** Survival rate after BMT. **(E)** TNF-α levels in serum from recipient mice followed up for 10 weeks (left) or for 20 weeks (right). Numbers in parentheses represent the number of mice measured. Error bars in (C) represent ± SEM. Horizontal bars in (E) represent the mean values. **P* < 0.05. WT to WT: *Sh3bp2*$^{+/+}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. KI to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells. WT to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells.

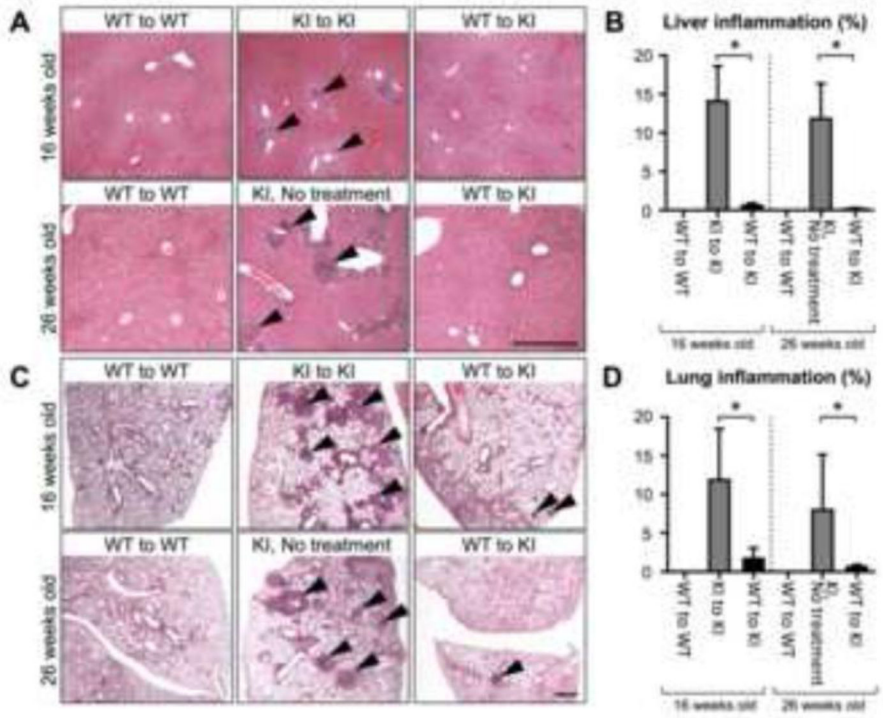


**Fig. 2.**

Transplantation of *Sh3bp2*$^{+/+}$ BM cells to 6-week-old *Sh3bp2*$^{KI/KI}$ mice reduces infiltration of inflammatory cells in liver and lung. **(A)** Images of liver tissue in recipient mice at 16-week-old (10 weeks after BMT, top) and 26-week-old (20 weeks after BMT, bottom). Arrowheads indicate the inflammatory cell infiltration. Scale bar = 500 μm. H&E staining. **(B)** Quantitative analysis of inflammation in liver. Data from 10-week follow-up (left) and 20-week follow-up (right) (n = 7–9). **(C)** Images of lung tissue. Panel order corresponds to (A). Arrowheads indicate the nodule formation by inflammatory cells. Scale bar = 500 μm. H&E staining. **(D)** Quantitative analysis of inflammation in lung. Data from 10-week follow-up (left) and 20-week follow-up (right) (n = 7–9). Error bars represent SD. **P* < 0.05. WT to WT: *Sh3bp2*$^{+/+}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. KI to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells. WT to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells.

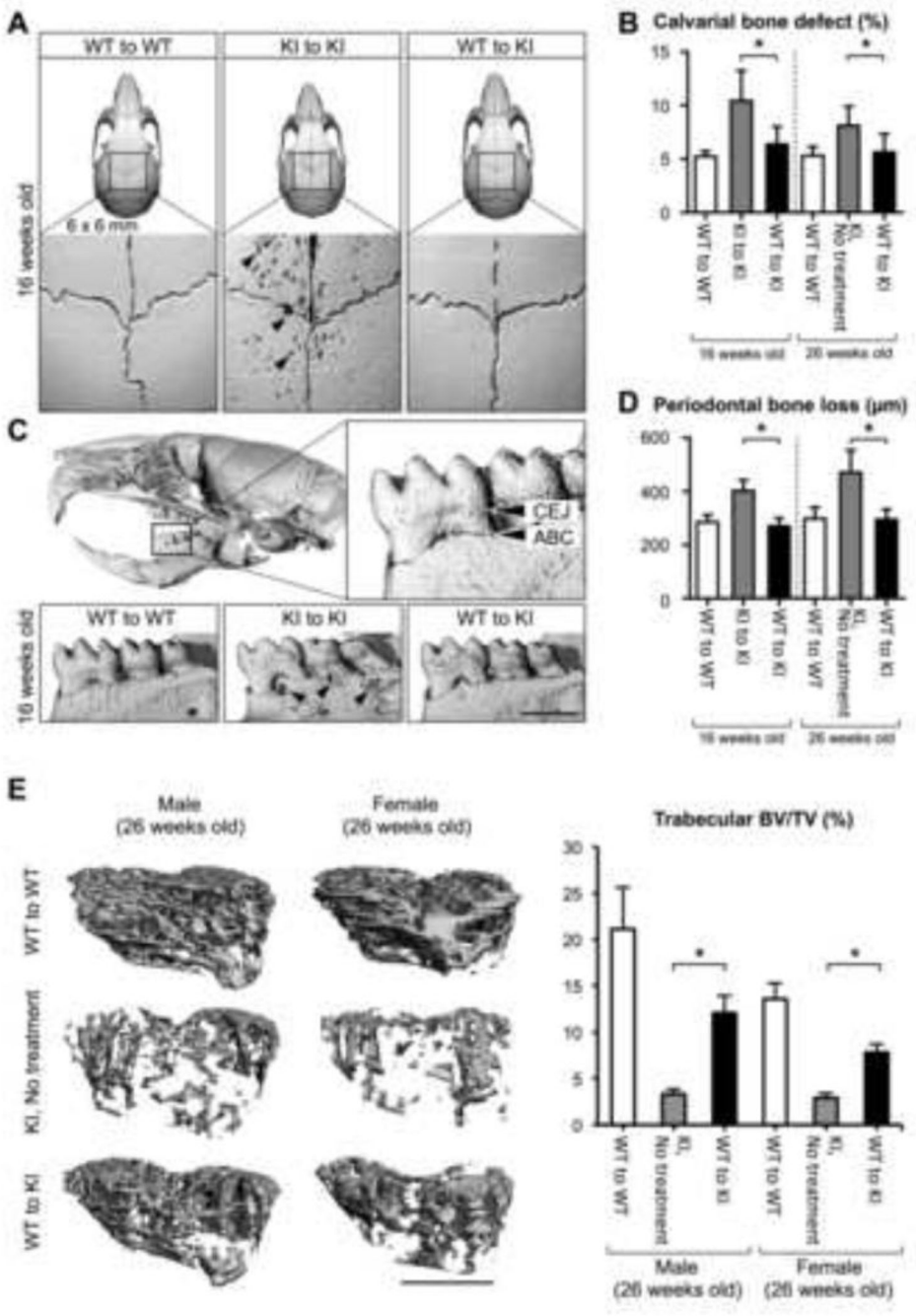


**Fig. 3.**

Transplantation of *Sh3bp2*$^{+/+}$ BM cells to 6-week-old *Sh3bp2*$^{KI/KI}$ mice improves inflammatory bone loss. **(A)** MicroCT images of calvaria. Upper panels: gross images of dorsal skull of the recipient mice at 10 weeks after BMT (16-week-old). Lower panels: enlarged 6 × 6 mm area on calvaria with the bregma at center. Arrowheads indicate erosion pits. **(B)** Quantitative measurement of calvarial bone defects. Data from 10-week follow-up (left) and 20-week follow-up (right) (n = 6–9). **(C)** MicroCT images. Top: Gross image of jaw and craniofacial bones from lingual side (left) and location of the CEJ and ABC (right)

(16-week-old *Sh3bp2*$^{+/+}$ mouse). Bottom: Images of periodontal bone in 16-week-old recipient mice (10 weeks after BMT). Arrowheads indicate erosion pits. Scale bar = 500 μm. **(D)** Quantitative measurement of periodontal bone loss. Distance between CEJ and ABC at the distal lingual side of the first molar was measured. Data from 10-week follow-up (left) and 20-week follow-up (right) (n = 6–9). **(E)** Reconstructed microCT images of trabecular bone and quantitative measurement of trabecular bone volume per total volume (BV/TV) at proximal tibia at 20 weeks after BMT (n = 3–4). Scale bar = 1 mm. Error bars represent SD. *$P < 0.05$. WT to WT: *Sh3bp2*$^{+/+}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. KI to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{KI/KI}$ BM cells. WT to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. CEJ: cementoenamel junction. ABC: alveolar bone crest.

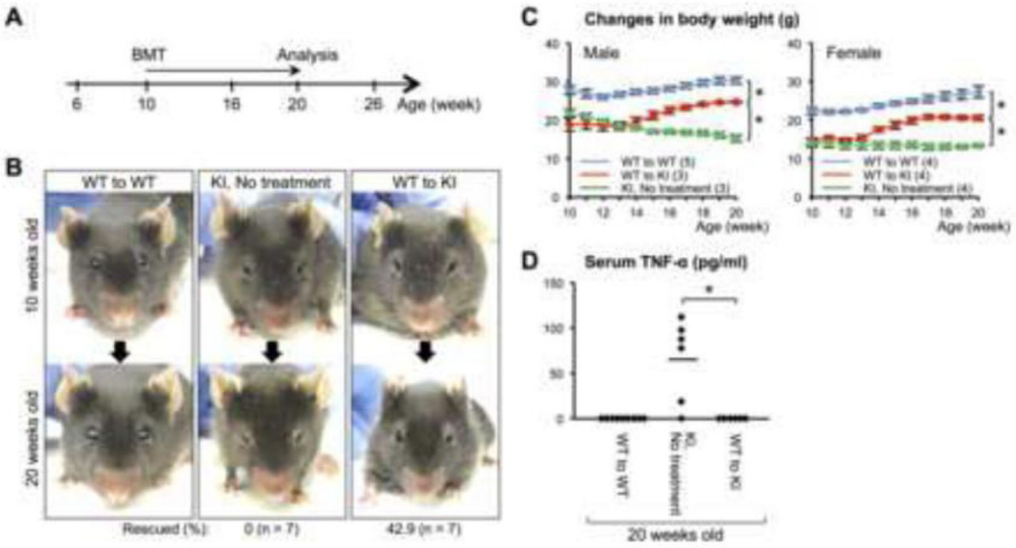


**Fig. 4.**

Transplantation of *Sh3bp2*$^{+/+}$ BM cells to 10-week-old fully inflamed *Sh3bp2*$^{KI/KI}$ mice improves facial swelling, loss of body weight, and elevated serum TNF-α levels. **(A)** Experimental procedure of BMT. Ten-week-old *Sh3bp2*$^{+/+}$ or *Sh3bp2*$^{KI/KI}$ mice were transplanted with BM cells and analyzed 10 weeks later. **(B)** Facial appearance of recipient mice (WT to WT, and WT to KI) before (top) and after (bottom) BMT. *Sh3bp2*$^{KI/KI}$ mouse transplanted with *Sh3bp2*$^{+/+}$ BM cells (right) shows a partial rescue of the inflammation around eyelids compared to *Sh3bp2*$^{KI/KI}$ mouse without BMT (center). **(C)** Body weight changes after BMT. Numbers in parentheses represent the number of mice measured. Error bars represent ± SEM. **(D)** Serum TNF-α levels at 10 weeks after BMT (20 weeks old). Transplantation of *Sh3bp2*$^{+/+}$ BM cells to fully inflamed *Sh3bp2*$^{KI/KI}$ mice reduces the TNF-α levels to undetectable levels comparable to those in “WT to WT” mice. *$P < 0.05$. WT to WT: *Sh3bp2*$^{+/+}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. WT to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells.

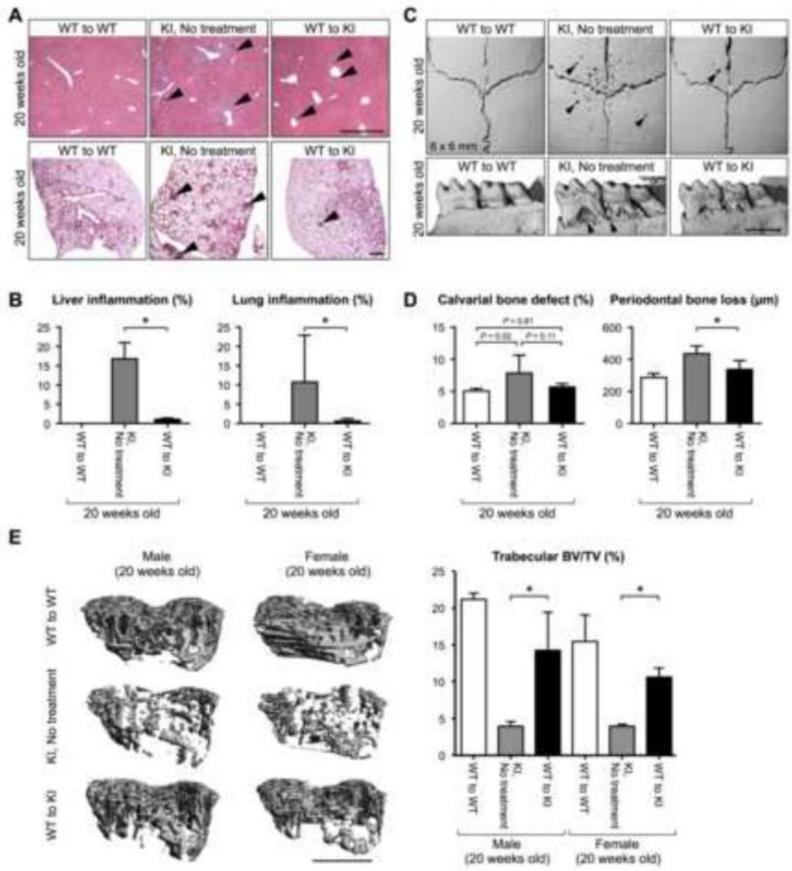


**Fig. 5.**

Transplantation of *Sh3bp2*$^{+/+}$ BM cells to 10-week-old fully inflamed *Sh3bp2*$^{KI/KI}$ mice reduces inflammation in liver and lung, and improves inflammatory calvarial and periodontal bone loss. **(A)** Liver (top) and lung (bottom) tissue images at 10 weeks after BMT. Arrowheads indicate the inflammatory cell infiltration. Scale bar = 500 μm. H&E staining. **(B)** Quantitation of inflammation in liver (left, n = 6–9) and lung (right, n = 6–9) tissues. **(C)** MicroCT images of calvarial (top) and periodontal (bottom) bones after BMT. Arrowheads indicate calvarial and jaw bone erosions. Scale bar = 500 μm. **(D)** Quantitative measurement of calvarial (left, n = 5–7) and periodontal (right, n = 5–7) bone loss. Distance between CEJ and ABC at the distal end of the first molar on lingual side was measured for the evaluation of periodontal bone loss. **(E)** Reconstructed microCT images of trabecular bone and quantitative measurement of trabecular bone volume per total volume (BV/TV) at proximal tibia at 10 weeks after BMT (n = 3–4). Scale bar = 1 mm. Error bars represent SD. **P* < 0.05. WT to WT: *Sh3bp2*$^{+/+}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. WT to KI: *Sh3bp2*$^{KI/KI}$ mice transplanted with *Sh3bp2*$^{+/+}$ BM cells. CEJ: cementoenamel junction. ABC: alveolar bone crest.