Differential effects of jump versus running exercise on trabecular architecture during remobilization after suspension-induced osteopenia in growing rats

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Ju Y-I, Sone T, Ohnaru K, Choi H-J, Fukunaga M. Differential effects of jump versus running exercise on trabecular architecture during remobilization after suspension-induced osteopenia in growing rats. J Appl Physiol 112: 766–772, 2012. First published December 8, 2011; doi:10.1152/japplphysiol.01219.2011.—High-impact exercise is considered to be very beneficial for bones. We investigated the ability of jump exercise to restore bone mass and structure after the deterioration induced by tail suspension in growing rats and made comparisons with treadmill running exercise. Five-week-old male Wistar rats (n = 28) were randomly assigned to four body weight-matched groups: a spontaneous recovery group after tail suspension (n = 7), a jump exercise group after tail suspension (n = 7), a treadmill running group after tail suspension (n = 7), and age-matched controls without tail suspension or exercise (n = 7). Treadmill running was performed at 25 m/min, 1 h/day, 5 days/wk. The jump exercise protocol consisted of 10 jumps/day, 5 days/wk, with a jump height of 40 cm. Bone mineral density (BMD) of the total right femur was measured by dual-energy X-ray absorptiometry. Three-dimensional trabecular bone architecture at the distal femoral metaphysis was evaluated using microcomputed tomography. After 5 wk of free remobilization, right hindlimb muscle weight, and body weight returned to age-matched control levels, but trabeculae remained thinner and less connected. Although both jump and running exercises during the remobilization period increased trabecular bone mass, jump exercise increased trabecular thickness, whereas running exercise increased trabecular number. These results indicate that restoration of trabecular bone architecture induced by jump exercise during remobilization is predominantly attributable to increased trabecular thickness, whereas running adds trabecular bone mass through increasing trabecular number, and suggest that jumping and running exercises have different mechanisms of action on structural characteristics of trabecular bone.

Tail suspension (unloading); remobilization

MECHANICAL STRESS IS REGARDED as an important determinant of the structural and functional integrity of the skeletal system in humans and other animals. Skeletal unloading causes rapid and marked bone loss, as has been demonstrated in humans following spaceflight or prolonged bed rest (19, 40), and has been extensively modeled in animals (10, 24).

Previous studies of skeletal reloading after prolonged skeletal unloading have indicated incomplete recovery of bone mass and architectural parameters, even after recovery periods longer than the duration of unloading (20, 29, 37). Loss of trabecular bone induced by skeletal unloading is apparently more pronounced and remains longer after reloading than that of cortical bone (31, 37). A previous study indicated that trabecular bone mass showed insufficient recovery during the reloading period after tail suspension in rats, although reloading for 14 days restored the reduced trabecular bone formation and suppressed increased trabecular bone resorption (28). Even if subsequent normal weight bearing after a period of unloading results in restoration of bone formation to baseline values, reconnecting the trabecular bone architecture after initial disruption remains difficult.

Mechanical loading is now widely accepted to be beneficial to skeletal bone. Different sports activities are known to result in high peak bone mass, particularly at loaded bone sites (25), and of the various types of exercise regimen, high-impact exercise such as jumping seems more beneficial for bones than more moderate impact exercise such as running (36). Furthermore, several animal studies suggest that bone mass gained by running exercise is usually lost when exercise is completely ceased (11, 41, 42). In contrast, bone mass gained by jump exercise is relatively maintained after exercise cessation (9, 35). It is generally accepted that turnover rate of cancellous bone is more rapid than that of cortical bone, because of its greater surface area (18). Bone loss rate could differ between cancellous bones with different trabecular structure even if both have the same amount of bone mass. Accordingly, the difference in bone loss rate between jump and running exercises after detraining may be attributed to the difference in trabecular bone structure. However, little is known about relationships between different types of mechanical loading and their effects on trabecular bone structure. The objective of this study was to compare the differential effects of jumping vs. treadmill running exercise on three-dimensional (3D) trabecular architecture after the deterioration induced by tail suspension in growing rats.

MATERIALS AND METHODS

Animals

Twenty-eight male Wistar rats (4 wk old) were purchased from CLEA Japan (Osaka, Japan) and acclimatized for 1 wk. Rats were housed individually in 35 × 35 × 35 cm metal grill cages and provided with commercial standard diet (MF containing 1.15% calcium, 0.88% phosphorus; Oriental Yeast, Chiba, Japan). Body weight and food intake were measured 3 times/wk. Food intake for these groups of rats was monitored, and control rats were pair-fed throughout the experiment. All study protocols were approved by the Animal Care and Use Committee at Kawasaki University of Medical Welfare, and all animals were treated in accordance with the guidelines set forth in the Care and Use of Laboratory Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan (http://www.soc.nii.ac.jp/psj/psj/doubutu.html). At the end of the experiment, rats were anesthetized with intraperitoneal pentobarbital sodium (0.1 ml/100 g body wt) and killed by exsanguination from the abdominal aorta. After death, right hindlimb muscles were collected from each rat and immediately weighed. The right...
femur was excised from each rat and cleaned of soft tissue. Femoral length was measured using digital calipers. The femur was stored at -40°C until needed for further measurements.

**Experimental Design**

Rats were habituated to the diet and new environment for 1 wk. At the end of the week, rats were randomly assigned into four groups: a spontaneous recovery group after tail suspension (S+RCON, n = 7); a jump exercise group after tail suspension (S+RJUMP, n = 7); a treadmill running group after tail suspension (S+RRUN, n = 7); and age-matched controls (CON, n = 7) without tail suspension or exercise.

**Tail Suspension**

The tail suspension procedure was performed in accordance with the recommendations of Morey-Holton and Globus (22), with slight modification. In brief, traction tape was loosely wrapped around the tail in a helical pattern starting at 1 cm from the base of the tail. A strip of traction tape 1 cm wide was preattached to a metal connector, attached to two-thirds the length of the tail along the lateral sides, and then secured by two strips of filament tape. One strip of filament tape was placed around the end of the body side of the traction tape, and a second strip was added halfway up the tape. The filament tape was loosely applied to allow normal blood circulation, while remaining tight enough to prevent the traction tape peeling away from the tail. The metal connector was connected by a wire to a swivel mounted at the top of the cage, allowing free 360° rotation. Rats were maintained at this final level of speed and duration for the remainder of the training program. When rats ran, electric stimulus was not applied because compressed air was blown from behind instead of electrical stimulation. The front half of the treadmill was covered in black paper to keep the area dark, as rats are more active in darkness.

**Exercise Protocols**

**Jumping exercise.** The jumping exercise protocol was implemented according to the methods previously reported by Umemura et al. (36). Rats in the jump exercise group were individually placed at the bottom of a special wooden box surrounded with boards. The height of this box could be adjusted. Rats were initially forced to jump by electric stimulus and to grasp the top of the board with the forelimbs and climb up the board. The rat was then returned to the floor of the cage to repeat the procedure. As rats became accustomed to the jump exercise, electric stimulus was used less frequently. The jump exercise program was performed 10 times/day, 5 days/wk for 5 wk. Height of the box was initially 25 cm, gradually increased to 40 cm during the first week. The time required for 10 jumps was about 1 min.

**Running exercise.** After removal from the tail suspension apparatus, rats rested for 24 h. Rats in the running exercise group were given a program of running on a motor-driven treadmill (KN. 73 Tread-Mill RM. 5; Natume, Tokyo, Japan) for 5 wk. Treadmill speed and the duration of each running session were gradually increased from 10 m/min for 10 min to 18 m/min for 50 min during the first week and to a final level of 25 m/min for 60 min within the following week. Rats were maintained at this final level of speed and duration for the remainder of the training program. When rats ran, electric stimulus was not applied because compressed air was blown from behind instead of electrical stimulation. The front half of the treadmill was covered in black paper to keep the area dark, as rats are more active in darkness.

**Bone Mineral Density Measurements**

Bone mineral density (BMD) of the femur was measured by dual-energy x-ray absorptiometry (DXA) using a QDR-2000 unit (Hologic, Waltham, MA) in small animal ultra-high resolution scan mode, starting at the distal end of the femur. For subregional analysis, the total femoral region was divided along the femoral long axis into seven equal regions, as reported previously (12): R1, distal epiphysis; R2, distal metaphysis; R3, distal diaphysis; R4, mid-diaphysis; R5, proximal diaphysis; R6, intertrochanter; and R7, including the femoral head, neck, and greater trochanter.

**Microcomputed Tomography Imaging and 3D Architectural Indexes**

The bone microarchitecture of the right femur was evaluated using a microcomputed tomography (micro-CT) system (Ele Scan mini; Nittetsu Elek, Tokyo, Japan). This apparatus is based on a fan-beam tomography and is able to function in multislice mode. An X-ray tube with a microfocus (spot size of 6 × 8 μm) is used, and maximum resolution of 4 μm (in pixel size) is attainable. Parameters selected for this study include a source energy of 30 kVp and 100 mA to obtain the best contrast between bone and soft tissue. The sample area selected for scanning was positioned at distance of 4–5.5 mm proximal from the distal femoral end, including the border between the distal metaphysis and growth plate. The distal femur was selected over the proximal femur because of the larger volume of cancellous bone available for 3D analysis in the distal femur. A total of 300 consecutive tomographic slices with a slice thickness of 14.1 μm (~4.2 mm) were acquired. Digital data were reconstructed to obtain CT images with a pixel size of 17.6 μm in 512 × 512 matrices. After micro-CT scanning, the original image data were transferred to a workstation, and structural indexes were calculated using 3D image analysis software (TRI/3D-BON; Ratoc System Engineering, Tokyo, Japan). The volume of interest was defined as the 160 slices above the most proximal portion of growth plate (Fig. 1). The resulting gray-scale images were segmented using a 3 × 3 median filter to remove noise and a fixed threshold of 120 (0–255 range) to extract the mineralized bone phase. Isolated small particles in the marrow space and isolated small holes in bone were removed using a cluster-labeling algorithm. Cortical and trabecular bone were subsequently separated and structural indexes were calculated. Bone surface area (BS) and bone volume (BV) were calculated using a tetrachedron meshing technique generated using the marching cubes method, and total tissue volume (TV) was calculated as the volume of the entire scanned sample. Trabecular bone volume fraction (BV/TV) was then calculated from BV and TV. Trabecular thickness (Th.Th), trabecular number (Th.N), and trabecular separation (Th.Sp) were calculated by measuring 3D distances directly in the trabecular network (8). Connectivity density

![Fig. 1. Volumes of interest in the femur (sagittal section of distal metaphysis of the femur).](image-url)
Table 1. Body weight, hindlimb muscle weight, and femoral length in experimental rats

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>S + RCON</th>
<th>S + RRUN</th>
<th>S + RJUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt before tail suspension, g</td>
<td>114.01 ± 0.88</td>
<td>113.59 ± 5.62</td>
<td>111.76 ± 4.72</td>
<td>111.59 ± 3.36</td>
</tr>
<tr>
<td>Body wt after tail suspension, g</td>
<td>203.03 ± 11.88</td>
<td>177.43 ± 9.89</td>
<td>169.04 ± 9.08</td>
<td>165.04 ± 1.72</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>317.77 ± 12.43</td>
<td>313.06 ± 9.15</td>
<td>295.57 ± 17.49</td>
<td>308.11 ± 14.49</td>
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<tr>
<td>Hind limb muscles wt, g</td>
<td>1.70 ± 0.11</td>
<td>1.67 ± 0.05</td>
<td>1.61 ± 0.17</td>
<td>1.67 ± 0.13</td>
</tr>
<tr>
<td>Femoral length, mm</td>
<td>34.86 ± 0.66</td>
<td>35.15 ± 0.51</td>
<td>34.54 ± 0.60</td>
<td>35.04 ± 0.55</td>
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All values represent mean ± SD. *Number of rats in each group: CON, age-matched controls without tail suspension; S + RCON, spontaneous recovery after tail suspension; S + RRUN, treadmill running exercise after tail suspension. Significant difference vs. CON group: *P < 0.05; †P < 0.001.

(B1/TV) (6) and trabecular bone pattern factor (TBPf) (7) were calculated directly from segmented voxel representations.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 16.0 software (SPSS, Chicago, IL). Comparisons among treatment groups were statistically processed by one-way ANOVA with Tukey’s post hoc analysis. All data were expressed as means ± SD. Values of P < 0.05 were considered statistically significant.

RESULTS

Body Weight, Hindlimb Muscle Weight, and Femoral Length

Body weight before and after tail suspension, final body weight, hindlimb muscle weight, and femoral length of rats from each group are shown in Table 1. Body weight of rats subjected to tail suspension for 14 days was significantly lower than that of sedentary control rats (S + RCON 13%, S + RJUM 17%, and S + RRUN 19% difference, respectively; P < 0.01). Final body weight in S + RCON and S + RRUN groups rapidly improved to the same level as the CON group. However, the S + RJUM group was significantly lower compared with CON group (7% difference; P < 0.05). Exercises did not affect hindlimb muscle weight or femoral length.

BMD

BMDs with respect to anatomical location along the length of the femur are shown in Figs. 2 and 3. After 5 wk of free remobilization, BMDs for regions R1, R2, R3, R4, and R5 and total femoral BMD in the S + RCON group were restored to the same level as the CON group. Conversely, BMD at R6 and R7 in the S + RCON group was not sufficiently recovered after 5 wk of reloading (7% and 6% difference, respectively; P < 0.05). In the S + RRUN group, BMDs at R4, R6, and R7 and total femoral BMD were significantly increased compared with the S + RCON group (5–11% difference; P < 0.05–0.001), but BMDs at R1, R2, R3, and R5 were not. Total femoral BMD and BMDs at R2, R3, R4, R6, and R7 were significantly higher in the S + RJUM group than in the S + RCON group (5–13% difference; P < 0.05–0.001), but not at R1 and R5. Compared with the CON group, total femoral and R6 BMD were significantly higher only in the S + RJUM group (5–7% difference; P < 0.05–0.01). No significant differences in femoral BMD were observed between S + RJUM and S + RRUN groups.

Microstructural Properties

Results for 3D microstructural parameters in the distal metaphysis of the femur are shown in Fig. 4. After 5 wk of spontaneous recovery, BS, BV/TV, and Tb.N were 27%, 27%, and 24% lower, respectively, whereas Tb.Sp and TBPf were 13% and 34% higher, respectively, in the S + RCON group compared with the CON group. No significant differences in Tb.Th or B1/TV were observed between S + RCON and CON groups. When rats were jump exercised during the recovery period, BV/TV and Tb.Th were 35% and 22% increased, respectively, and Tb.Sp and TBPf were 16% and 38% decreased, respectively, when compared with the S + RCON group. In the S + RRUN group, BV/TV, Tb.N, and B1/TV were 32%, 32%, and 27% higher, and TBPf was 33% lower than in the S + RCON group. Tb.Th was 14% and 15% higher in the S + RJUM group than in the CON and S + RRUN groups. There was a trend for higher Tb.N in S + RRUN group than CON and S + RJUM groups, although the difference was not statistically significant (P = 0.399 and P = 0.396, respectively). Figure 5 shows typical features of 3D trabecular microstructure in the distal femoral metaphysis for a rat from each group. These images demonstrate that the unloading elicited marked decreases in trabeculae and that these decreases were recovered by both exercises.

DISCUSSION

The major finding in the present study was that jump and running exercises during remobilization have beneficial effects on the recovery of suspension-induced osteopenia at the femur.

Fig. 2. Bone mineral density (BMD) in the subregions of femur measured by dual-energy X-ray absorptiometry (DXA). All values represent mean ± SD. CON, age-matched controls without tail suspension; S + RCON, spontaneous recovery after tail suspension; S + RRUN, treadmill running exercise after tail suspension. Significant difference vs. S + RCON group: *P < 0.05; †P < 0.01; ‡P < 0.001. Significant difference vs. CON group: *P < 0.05; †P < 0.01.
calcaneus following 12 wk of spaceflight may be bone mineral content in normally loaded bones such as the caused by skeletal unloading has also been reported in humans.

morphology (28, 38). Incomplete recovery of bone mass unloading have not demonstrated complete restoration of bone Several studies investigating recovery potential after skeletal bone mass following mechanical unloading remains unclear.

For instance, Modlesky et al. (21) reported that the distal femur was even more rapid in younger rats. In previous experiments with this model and 3D micro-CT of the distal femoral metaphysis, we found that the loss of cancellous bone volume in the femur induced by skeletal unloading was primarily associated with a tendency toward decreased Tb.N (−51%), with no significant decreases in Tb.Th (−21%) (13). Basso et al. (3) also showed that 14 days of unloading resulted in reductions of 50% in BV/TV and Tb.N, 25% in Tb.Th, and a 179% increase in Tb.Sp in the proximal tibia isolated from 6-wk-old rats. Furthermore, similar findings have been confirmed in humans subjected to unloading caused by spinal cord injury (21, 32). For instance, Modlesky et al. (21) reported that the distal femur and proximal tibia of spinal cord-injured men had 27% and 20% lower app BV/TV, 21% and 20% lower app Tb.N, and 44% and 33% higher app Tb.Sp, respectively, than able-bodied men.

The length of time required to achieve complete recovery of bone mass following mechanical unloading remains unclear. Several studies investigating recovery potential after skeletal unloading have not demonstrated complete restoration of bone morphology (28, 38). Incomplete recovery of bone mass caused by skeletal unloading has also been reported in humans. For instance, the period required to restore calcium balance and bone mineral content in normally loaded bones such as the calcaneus following 12 wk of spaceflight may be ≥5 yr (33). A human study has also shown that the unloading-induced deficit in bone mass was not completely recovered after 17 wk of bed rest and 6 mo of reambulation (20). In the present study, although the results of DXA analysis indicated recovery of total femoral BMD in spontaneous recovery of rats after 5 wk of remobilization, micro-CT analysis revealed that the micro-architecture of the distal femoral metaphysis had not completely recovered. Unlike BMD, reloading for 5 wk was not sufficient to achieve full recovery of the trabecular bone architecture. This supports the findings of a previous study (15) in which trabecular disappearance could not be restored during remobilization. The reloading period might be too short for sufficient recovery or increased trabecular perforation attributable to rapid trabecular thinning might cause deterioration of the trabecular bone packet and a decrease in possible sites for bone formation (28).

The risk of osteoporosis later in life is determined by the peak bone mass attained during childhood and adolescence (1, 5). However, there are conflicting accounts about the effects of exercise cessation on bone mass. Yeh and Aloia (42) demonstrated that bone mass gained through treadmill running was lost when exercise was completely ceased in young growing rats. Wu et al. (41) also showed that bone mass gained through treadmill running exercise was lost after short-term exercise cessation in rats. On the other hand, the pace of bone loss after exercise cessation seems to be different among exercise types. Recently, Umemura et al. (35) and Honda et al. (9) found that bone mass gained by jump exercise was maintained for at least 17 wk of detraining in ovariectomized rats and young rats. Warden et al. (39) also reported that the effects of 7 wk of loading using an axial compression loading device on ulnar bone strength were preserved in rats, accompanied by bone structural changes in ulnar diaphysis, for 92 wk after exercise cessation. One possible explanation for these contradictory findings is the difference in the effects of exercise loading on trabecular micro-architecture.

To our knowledge, the effect of different exercises modality on the 3D architectural changes in trabecular bone of rats was not clarified. In the histomorphometric studies, Notomi et al. (27) found that Tb.Th in the proximal tibial metaphysis was significantly increased following 4 and 8 wk of resistance exercise training in growing rats, whereas Tb.N was unaffected. They also reported that jump exercise for 8 wk induced significant increases in vertebral Tb.Th without altering vertebral Tb.N (26). In contrast, Yeh et al. (43) reported that Tb.N in the proximal tibial metaphysis was increased after 16 wk treadmill running exercise, but Tb.Th was unaffected. Resistance and aerobic exercise might have different influences on trabecular bone architecture. In the present study, jump exercise during the remobilization period induced significant increases in Tb.Th compared with the other three groups. Conversely, treadmill running exercise induced increased Tb.N when compared with the spontaneous recovery group, leading to total increases in cancellous bone mass. The effect of jump exercise on cancellous bone mass of the distal femur was thus primarily achieved by significant alterations in Tb.Th, whereas treadmill running exercise induced effects by increasing Tb.N. In support of this hypothesis, we found that BS tended to be lower in jump compared with treadmill running group, although the difference was not statistically significant. Collectively, these results suggest that jumping and running exercises have different mechanisms of action on structural characteristics of trabecular bone. Further studies are necessary to define
whether this phenomenon occurs even in normally growing rats.

Loss of trabecular connectivity is known to be associated with reductions in the physical strength of trabecular bone (30). Our previous study showed that both TBPf and \( \beta/TV \) indicated significant decreases in trabecular connectivity after tail suspension. In the present study, spontaneous recovery did not result in significantly increased trabecular connectivity. When exercise was applied during the recovery period, jump exercise significantly changed TBPf alone, whereas running exercise changed both TBPf and \( \beta/TV \). The decrease in TBPf seen with jump exercise might reflect changes in concavity of the trabecular surface without changes in connectivity. Increased trabecular connectivity is usually coupled with increased bone strength (17). Ascertaining whether cancellous bone strength differs according to the exercise modality would be of interest.

The effectiveness of exercise on bone mass and strength depends on the loading magnitude and rate on bone while exercising. Judex and Mernicke (14) compared the mechanical loading characteristics produced by running and drop jumps and found that drop jumping involved much larger maximal strain rates than running protocols in growing roosters.
izing loading conditions among different modes of exercise is thus difficult. In the present study, exercise protocols were designed so that equal levels of gain in total femoral BMD could be expected from running and jump exercise. Indeed, total BMD and trabecular bone volume were similar in the S/RJUM and S/RRUN groups.

We are aware of several limitations of this study. First, we used young growing rats (5 wk old) in the experimental design because it has been shown that in young growing rats (6 wk old), 14 days of tail suspension results in a marked decrease (−50% to −80%) in tibial metaphyseal bone volume as well as decreases in trabecular number and thickness but remained unaffected in adults (2), and growing animals usually have greater potential for bone recovery after immobilization (34). Also, some animal experiments studying the effect of exercise on intact bones have suggested a better loading response in young individuals, but others have found no clear age dependence (16). Therefore, our results might quantitatively differ if adult rats are used with tail suspension model. Second, sample size used in present study is comparatively small. In the present study, the S/RJUM group gained trabecular thickness relative to the other groups, whereas the S/RRUN group exhibited trends toward increased trabecular number compared with the CON and S/RJUM groups, but the differences were not statistically significant except in the S/RCON group. Perhaps a larger sample size could confirm this finding. Finally, the rat is an established model for many aspects of human bone metabolism, but it has some limitations because cortical structure and bone-modeling patterns are different with humans. Consequently, direct extrapolation of data from quadrupedal rats to humans is inappropriate. Nonetheless, the results obtained from the present study using 3D micro-CT analysis suggest the advantage of exercise intervention to prevent trabecular bone loss that is induced by immobilization and microgravity. Further studies are clearly needed for a more complete understanding of the relationship between altered mechanical usage and trabecular bone alterations, especially using the larger sample size, normally growing rats, other measurement sites, and methodical techniques.

In conclusion, we demonstrated that suspension-induced trabecular deterioration persists after remobilization and that jumping and running exercises during the remobilization period can restore the integrity of the femoral trabecular architecture in young growing rats. However, the effects on cancellous bone mass differed between jumping and running exercises in that jumping exercise increased trabecular bone volume by thickening existing trabeculae, whereas treadmill running exercise added bone by creating new trabeculae and increasing trabecular connectivity. These results suggest that functional adaptation of the trabecular architecture to mechanical loading takes place differently depending on several factors: strain rate, strain magnitude, cycle number, loading direction, etc.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: Y.-I.J., T.S., K.O., H.-J.C., and M.F. conception and design of research; Y.-I.J. and H.-J.C. performed experiments; Y.-I.J., K.O., and H.-J.C. analyzed data; Y.-I.J., T.S., K.O., and M.F. interpreted results of experiments; Y.-I.J., K.O., and H.-J.C. prepared figures; Y.-I.J. and T.S. drafted manuscript; T.S. and M.F. edited and revised manuscript; T.S. and M.F. approved final version of manuscript.
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