**Apoptosis Assay**

The ApoTag Plus Fluorescein In Situ Detection Kit (Millipore) was used to detect apoptosis following the manufacturer’s instructions. Briefly, paraffin sections were deparaffinized at 65°C for 30 minutes, then incubated in two changes of xylene (10 minutes each). Sections were washed twice with 1x PBS for 5 minutes. Equilibration buffer (75 ml) was added immediately for 10 seconds, followed by 55 ml working strength terminal deoxynucleotidyl transferase enzyme, and incubated for 1 hour at 37°C. Working strength stop/wash buffer was added and incubated for 10 minutes at RT. Sections were washed three times before applying anti- digoxigenin conjugate and incubating for 30 minutes in the dark at RT. Sections were washed four times and Hoechst 33258 was added for 15 minutes before mounting with ProLong Gold and adding coverslips. Images were taken using a Zeiss LSM710 confocal microscope.

**Image analysis of macrophages and apoptosis**

CD68 and CD206 confocal raw images at 20x magnification (approximately 10 random fields per condition) were analysed using ImageJ software. The area of staining of nuclei, green and red positive cells (CD68 and CD206 respectively) were individually quantified using the ImageJ analysis tool and the percentage of green or red staining per cell was calculated for each condition. ApopTag Plus–treated cells were viewed at 20 x magnification and images analysed using ImageJ. Nuclei and green puncta (apoptotic bodies) were manually counted using the ImageJ counting tool and the percentage of apoptotic bodies per cell was calculated for each condition.

**Microcomputed tomography (microCT)**

Soft tissue was removed and tibiae were fixed in 70% ethanol at 4°C. The distal end of tibiae were scanned using a Skyscan 1172 microCT scanner (Bruker, Belgium) as previously described.22 The X-ray voltage and amperage were 80 kV and 124 µA, and a 1 mm aluminium filter was used. Images were acquired with an isotropic voxel size of 12 µm, with 180° of rotation, and a rotation step of 0.44°. After standardised reconstruction using NRecon software (Bruker, version 1.6.9.18), the datasets were analysed using CTAn software (Bruker, version 1.18.8.0). Standardised parameters of trabecular and cortical bone microstructure were measured.23 The trabecular region of interest was 1.07 mm and 0.95 mm distal to the growth plate in females and males respectively, and extended 3.58 mm in the distal direction. The cortical region of interest was 9.54 mm distal to the growth plate, and extended 2.38 mm in the distal direction in both sexes.