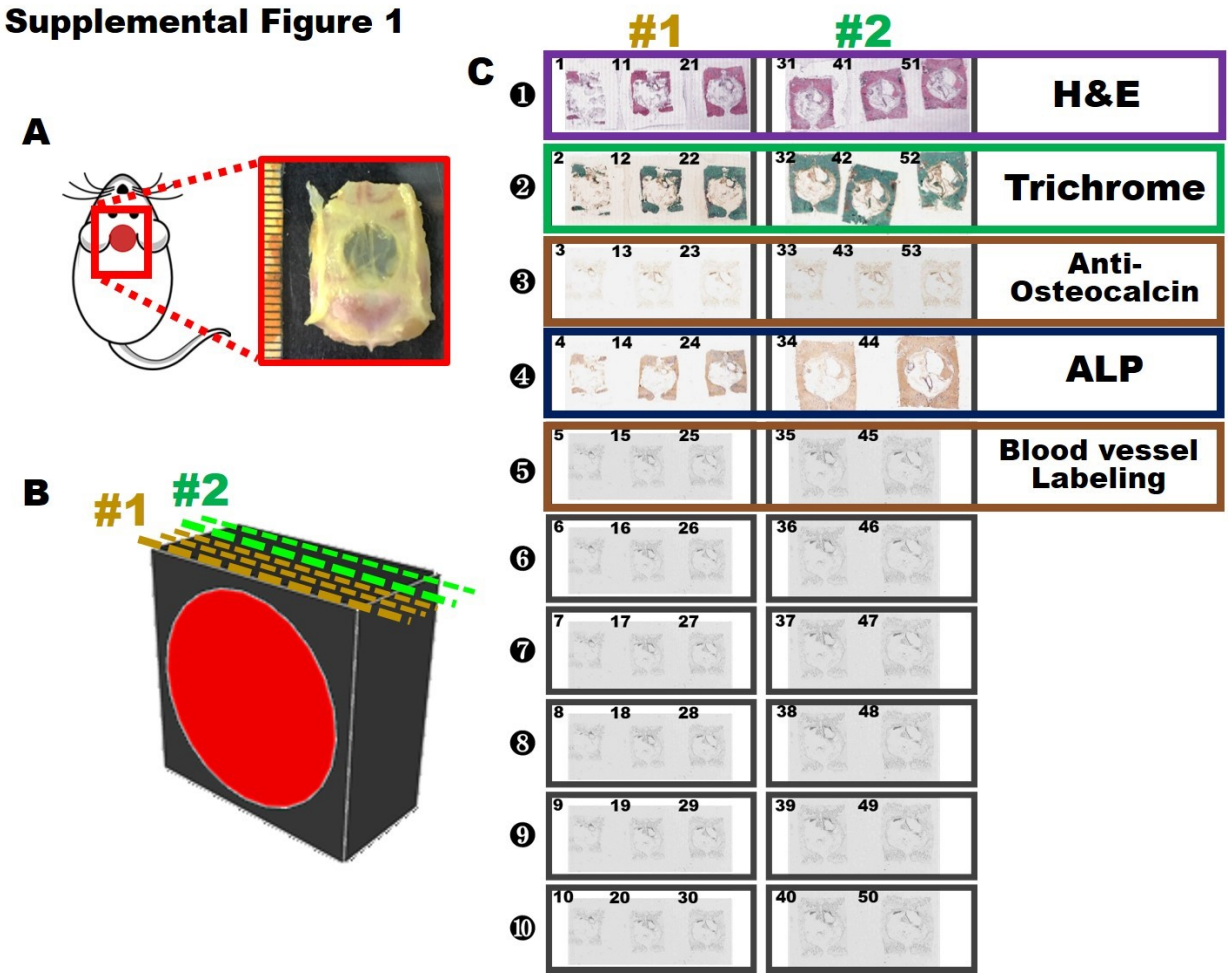

Supplementary Figure 1. Experimental procedure for paraffin embedding, horizontal sectioning, and labeling of slides of calvarial tissues

At five months following surgery of bone defect, the rats were perfused and the calvarial tissues were obtained (A). A schematic illustration of serial horizontal sections was shown (B). Calvaria was horizontally and consecutively sectioned from the top part, each slice with a thickness of 7 μm. Ten serial slices numbered from 1 to 10 were placed on slides numbered ❶ to ❿, respectively. Slices numbered 11 to 20 and 21 to 30 were also placed on the slides. The first 30 tissue sections were labeled as the first set (#1) of sections, representing the superficial region of calvaria close to scalp. Subsequently, calvarial sections numbered 31 to 40, 41 to 50, and 51 to 53 were sequentially placed on slides until the tissue was completely sectioned. The second set (#2)

of sections was counted from the 31st tissue section onward and was considered the inner region of calvaria close to brain. The first slide (i.e., slide ❶) was subjected to H&E staining. The second slide (i.e., slide ❷) was used for Goldner's trichrome staining to mark the distribution of collagen. The third slide (i.e., slide ❸) was subjected to immunohistochemical staining with anti-osteocalcin antibody to show the bone matrix. The fourth slide (i.e., slide ❹) was used for ALP tissue staining to label the distribution of osteoblasts. The fifth slide (i.e., slide ❺) was subjected to GSL1-B4 tissue staining to label the distribution of blood vessels. The rest of the slides were kept as spares.