SUPPLEMENTAL FIGURES

Supplement Figure 1: Validating the immunodeficiency in NSG/Col3.6GFPtpz mouse line. A: The retro-orbital sinus peripheral blood from wild type mice or NSG/Col3.6GFPtpz mice was collected and stained with listed antibodies as described in the Material and Method section. With forward scatter (FSC-A) and side scatter (SSC-A) separation, the nucleated cell population was defined for further antibody analysis (Left panels, circled region). With a combination of PE-Cy7 conjugated CD3 (for T cell) and APC conjugated B220 (for B cell) antibodies co-staining, 33.5% T cell and 42% B cells were identified in the nucleated cell population, and the co-staining with PE conjugated DX5 (for nature killer cell-NK) and eFluor-450 conjugated CD11b (for macrophage-MC), 8.02% NK and 57.1% MC cells were detected in the same cell population in wild type mouse sample (Middle and Right bottom panels). All these immune response cells were un-detected in the nucleated cell population of NSG/Col3.6GFPtpz mouse sample (Middle and Right top panels). B**:** Mouse tail genomic DNA was extracted from 2 NSG mice (NSG), 10 NSG/Col3.6GFPtpz mice (1 to 10), and 1 wild type mouse (Wt). Genomic PCR, using a primer design based on the suggestion of Jackson Laboratory, produced a PCR product of 349 bp for detecting $Il2rg^{tm1wj1}$ mouse, and 269 bp for wild type Il2rg gene.

Supplemental Figure 2: Alignment of 4 calvarial sections per tape, with two tapes per slide. Strips of calvaria that include the repair defect are embedded in the cryomold. Sections are captured with the Section Lab tape and noted as being the central or adjacent section set. The central section is used to establish the osteogenic landscape while the adjacent section can be used for fluorescent immunochemistry or special chomogenic stains. The entire area of each calvaria on the tape is distinguished and scanned as a separate sample and the position is maintained for the subsequent staining and imaging steps.

Supplemental Figure 3: Adjusting the fluorescent signal brightness (8.3 % of full image size). A. The example is the calcein mineralization signal that is recorded and illustrates the lack of an obvious

autofluorescent background from the accumulated mineral on the tissue section. To enhance the calcein signal, the Image->Adjustment->Levels command of Photoshop produces the histogram of the raw image A. The double peak is composed of the intensity of the background (sharp far-left peak) and the fluorescent signal (sloped right peak). In the B histogram, the left side slider is moved to the base of the fluorescent signal to eliminate background. The right side slider is moved to place the middle marker at the base of the fluorescent signal. In the C histogram, the resulting adjustment spreads the fluorescent signal across the histogram and the result is observed in the visual increase in the signal (panel A-> panel B) without an increase in the overall fluorescent background.

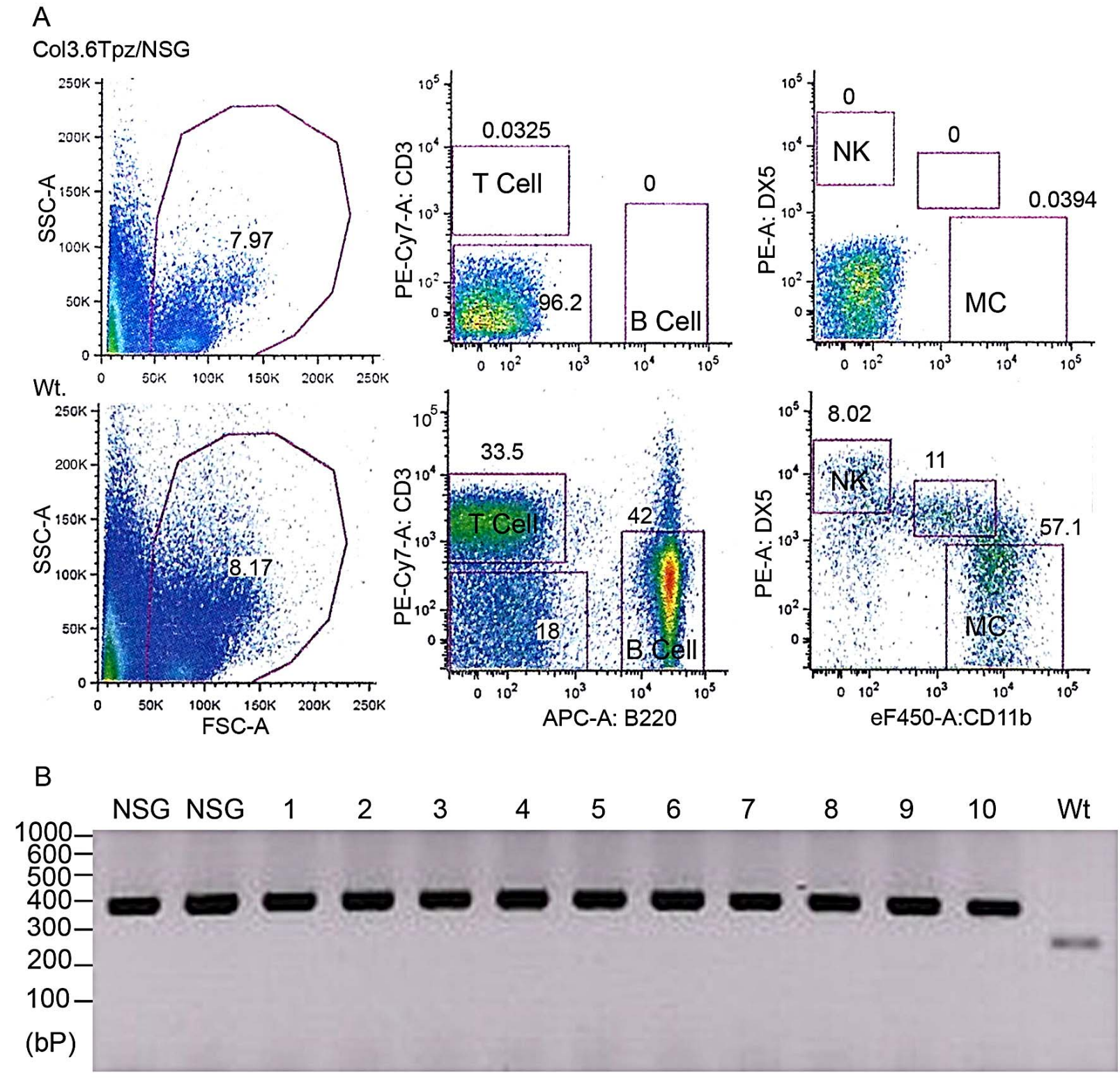
Supplemental Figure 4: Adjusting the opacity slider to merge chromogenic and fluorescent images. The screen setting of the layers window allows signals of each layer to be projected throughout all the layers. However the intense staining of the chromogenic layer still overpowers the fluorescent signals. The problem is solved by reducing the opacity slider of the chromogenic layer to ~50%. This is illustrated in the layer insert that controls the image. Strips 1 and 2 are the toluidine blue and merged fluorescent signal. Strip 3 is composed of duplicates of 1 and 2 that are layered with the opacity of the toluidine blue image reduced to 50% so that the fluorescent signal are balanced with the intensity of the toluidine blue image.

Supplemental Figure 5: File structure for image management. The graphic depicts the relationship for the root file and the subsequent composite image developed to illustrate cellular relationships within the repair field. The nomenclature includes the experiment and sample identification number followed by the file type (.psd stack, .psd composite or .jpg flat image) which is used to stamp the corresponding image. The raw root stack containing all the primary and unadjusted pseudocolored images, plus the photograph and X-ray of the defects region. A working version of the stack (_adj) is adjusted to equally increase the fluorescent signal intensity, align the individual layers, rotate the entire stack and crop out extraneous blank space. From the working stack, two tracks are possible. Creation of composite images from the working stack alone (salmon box), or creation of substacks that will be used to provide enlargements of a specific region (green box). From these stacks, individual or merged layers will be produced to generate a composite file.

Once all the elements are developed in a .psd format, the entire file is flattened and the size and resolution is adjusted for its intended purpose. Enlargement of a region is obtained by a composite file in which the image from the stack is reduced in size while the image from the sub-stack is not changed.

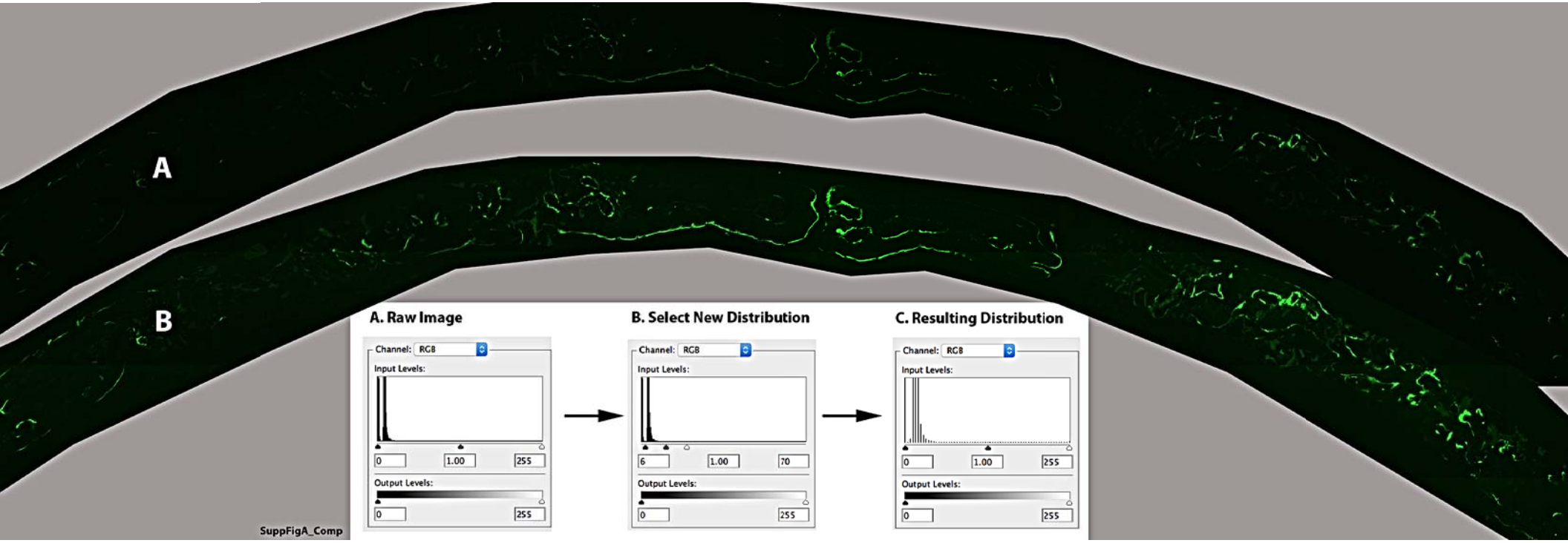
Supplemental Figure 6: Calvarial defect repaired (6 weeks) using mouse BMSCs from a donor carrying a Col3.6GFPsaph reporter (33.3% of full image size). Panel A demonstrates the cortical bone and included bone marrow that formed in both defect sites. SS is the sagittal suture. Panel A2 shows the digital X-ray and photograph of the repaired defect. Note the bright red color that is characteristic of a repair that is filled with bone marrow. Panel B enlarges the region boxed in panel A1. B1 shows hematoxylin and Col3.6GFPsaph together, panel B2 shows Col3.6GFPsaph and accumulated mineral, while panel B3 is hematoxylin only. The scale bar for A = 1000 µm and for B = 200 µm.

Supplemental Figure 7: Calvarial defect repaired (4 weeks) using mouse fetal calvarial cells carrying the Col3.6GFPcyan reporter (66.6% of full image size). Panel A. Hematoxylin stain of the entire repair field, which in this case extended over the calvarial bone and sagittal suture (SS). Please download and magnify the image to appreciate the absence of marrow. Panel B. Fluorescence signal of Col3.6GFPcyan (blue) and TRAP (yellow) obtained from an adjacent section. Despite the absence of marrow, osteoclasts are present within the fibrous tissue that separates the islands of developing bone. The scale bar = 1000 µm.

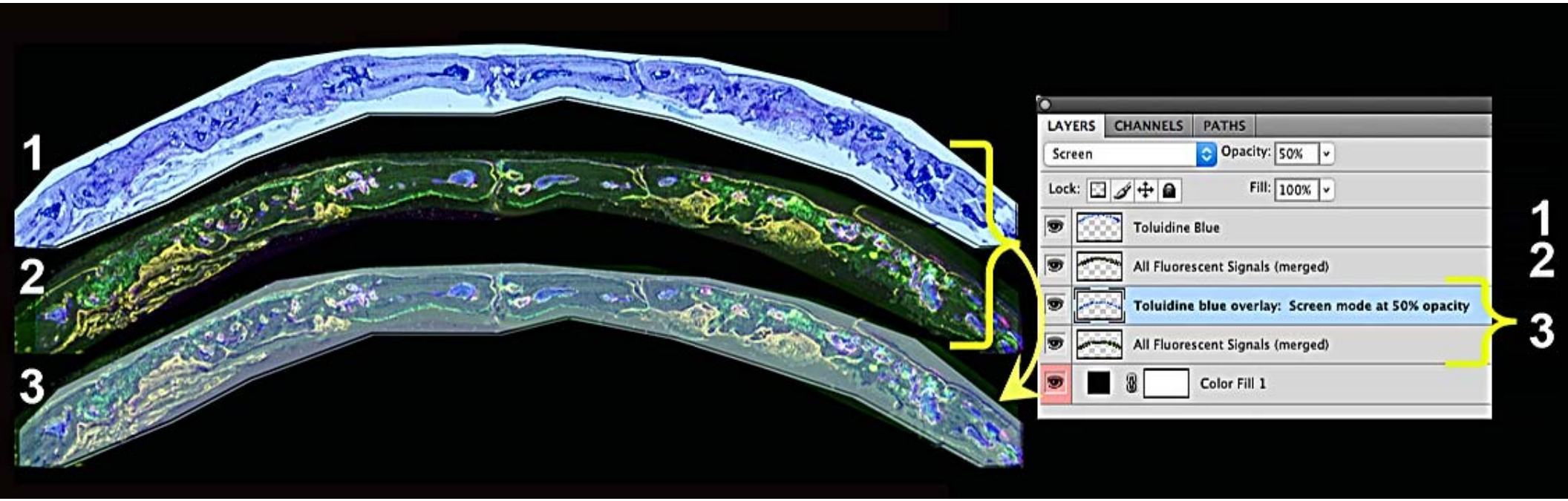


Supplemental Figure 1

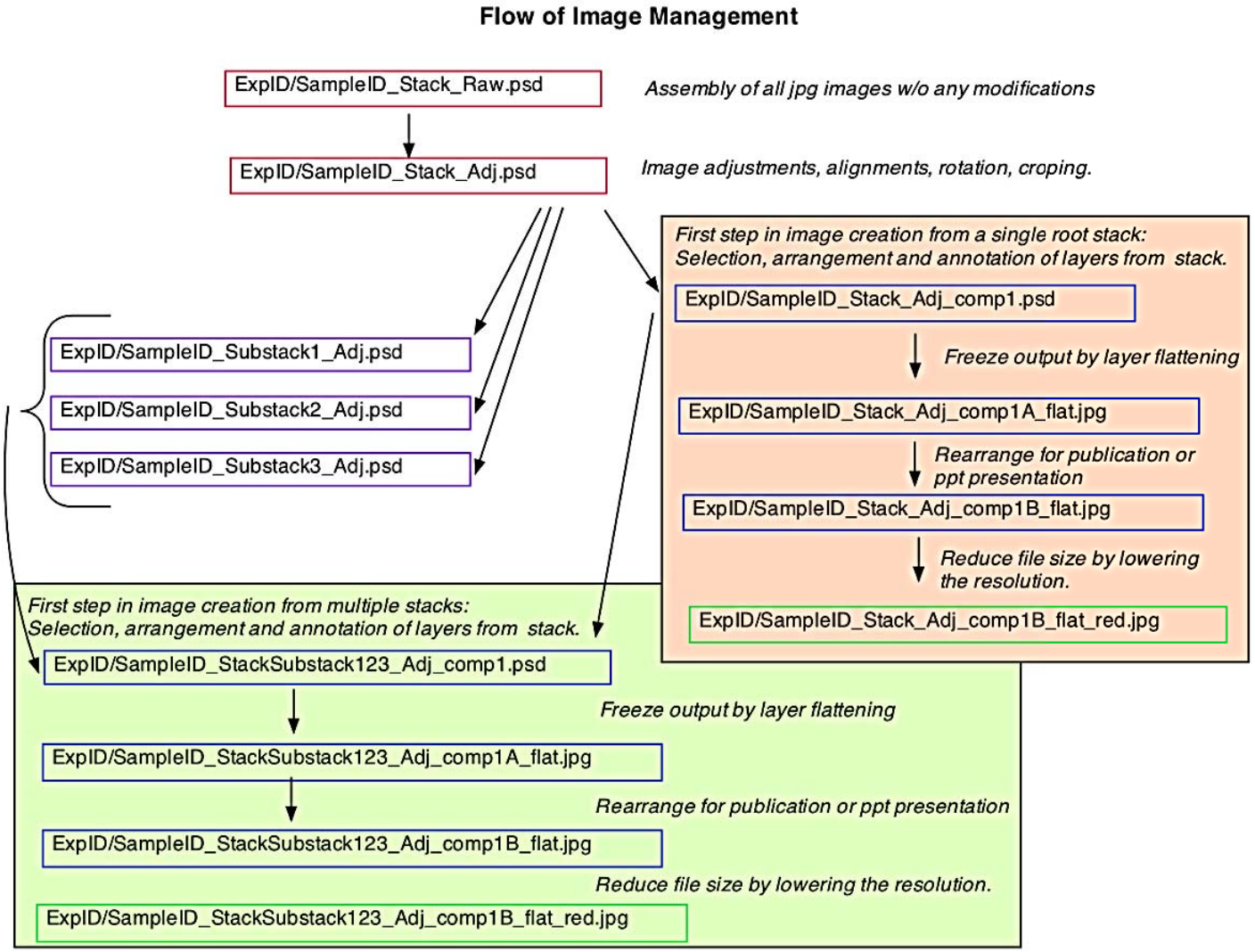
Supplemental Figure 2



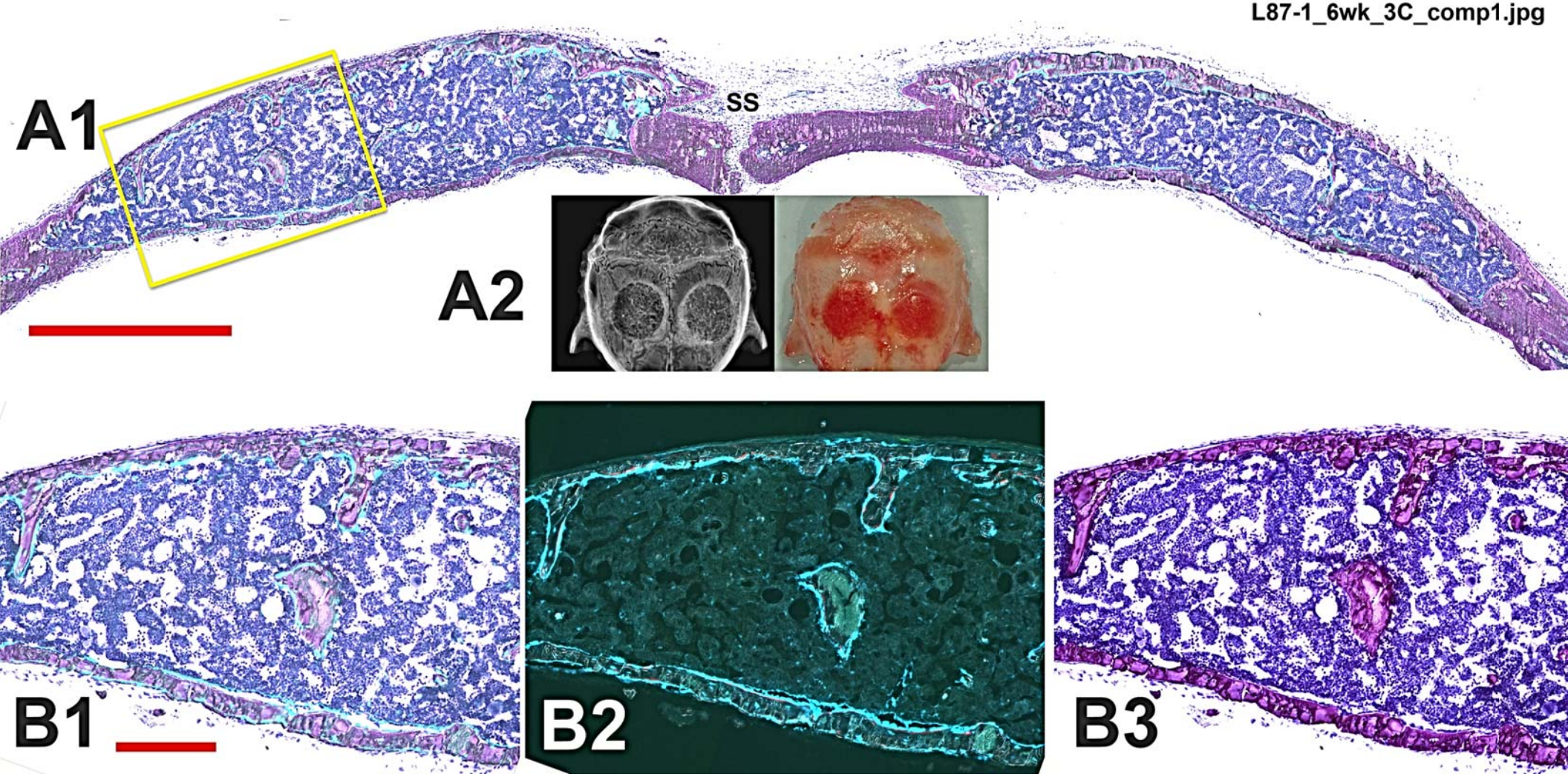
Supplemental Figure 3



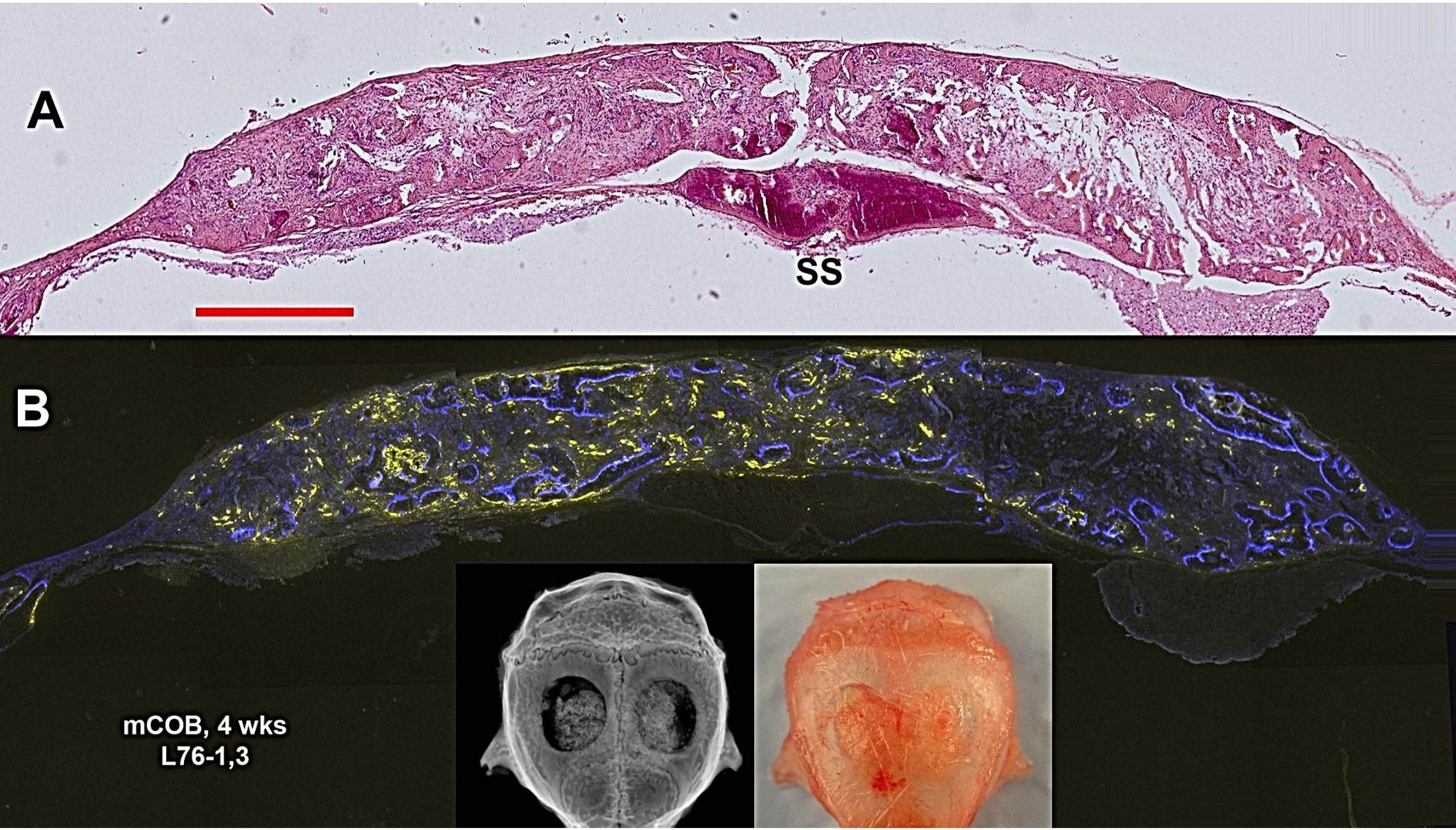
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7