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Supplemental Information

Essential Roles of Cohesin STAG2 in Mouse Embryonic Development and Adult Tissue Homeostasis

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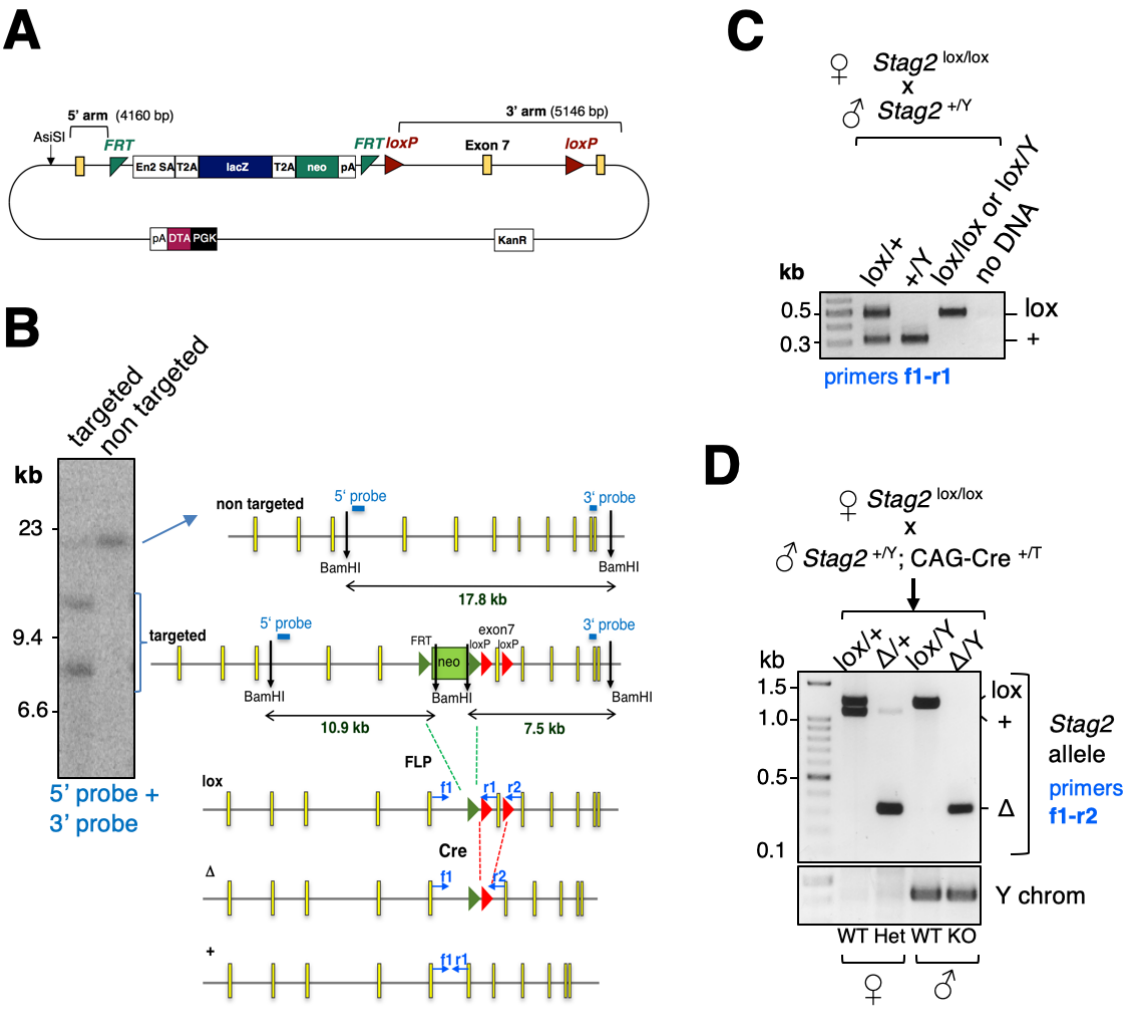

Figure S1. Generation of a *Stag2* cKO allele, related to STAR METHODS.

A. Map of the vector obtained from EUCOMM to target the murine *Stag2* gene.

B. Southern blot analysis (left) and strategy (right) to identify targeted ES clones.

C. PCR analyses to genotype the *Stag2* lox and wild type (+) alleles in the offspring of the indicated mating.

D. PCR analyses to genotype the embryos obtained from the indicated cross, including the Y chromosome.

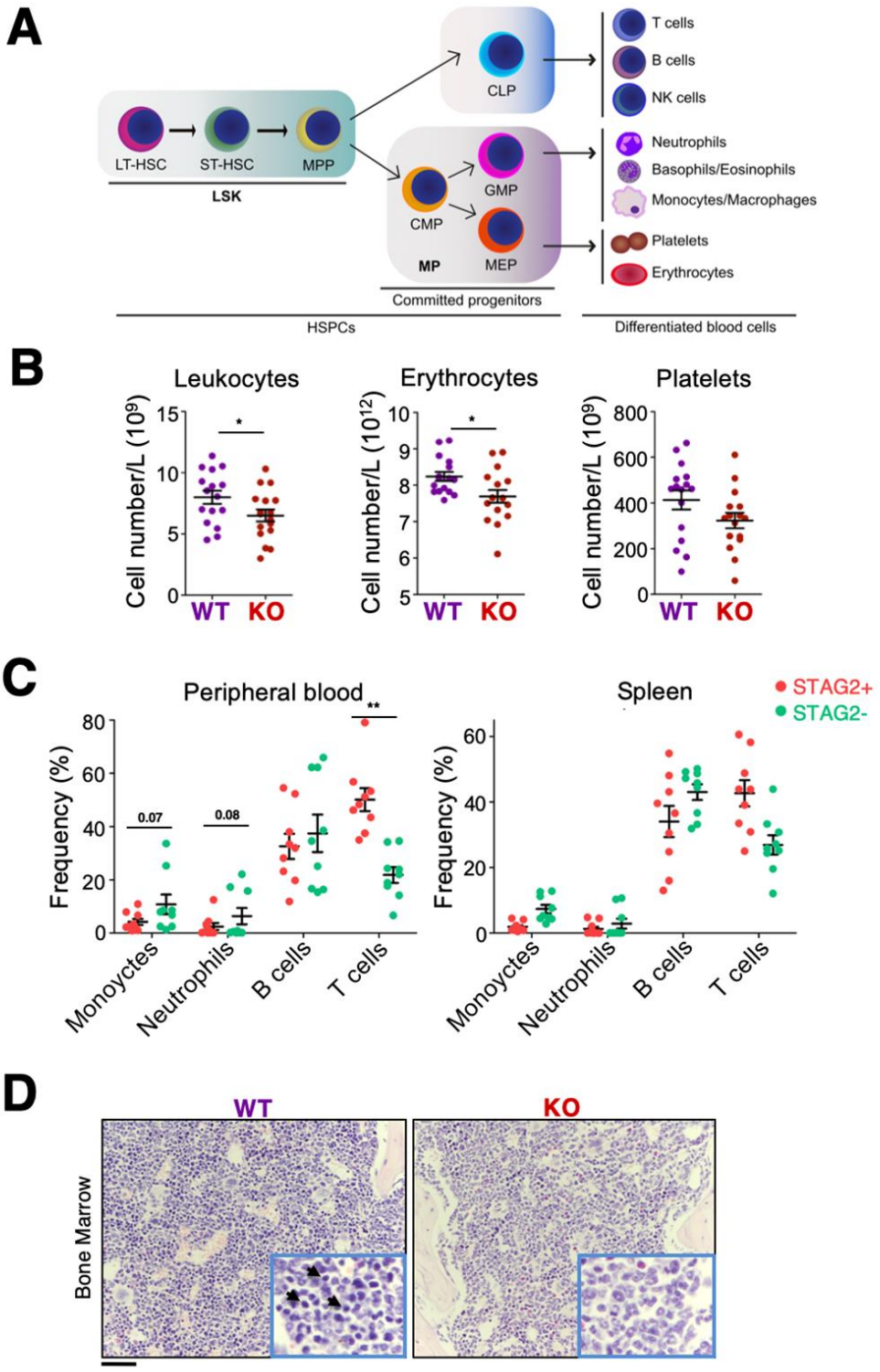

Figure S2. Requirement of STAG2 for normal adult hematopoiesis, related to Figure 2.

A. Scheme depicting normal hematopoiesis.

B. Peripheral blood counts of 12 week-old KO and WT mice (n=16 mice/genotype). Error bars indicate SEM. Two-sided Mann-Whitney U test; *P < 0.05.

C. Flow cytometry analysis of GFP+ (STAG2-) or Tomato+ (STAG2+) leukocyte populations in peripheral blood and spleen of 12 week-old KO mice (n=9). Monocytes (CD3- B220- CD11b+ Ly6G-); neutrophils (CD3- B220- CD11b+ Ly6G+); B cells (CD3- B220+); T cells (CD3+ B220-). Error bars indicate SEM. Unpaired t test; **$P < 0.01$.

D. H-E staining of bone marrow from 12 week-old WT and KO mice shows a decrease in the erythrocyte population (arrows) in KO mice. Scale bar, 50 μm.

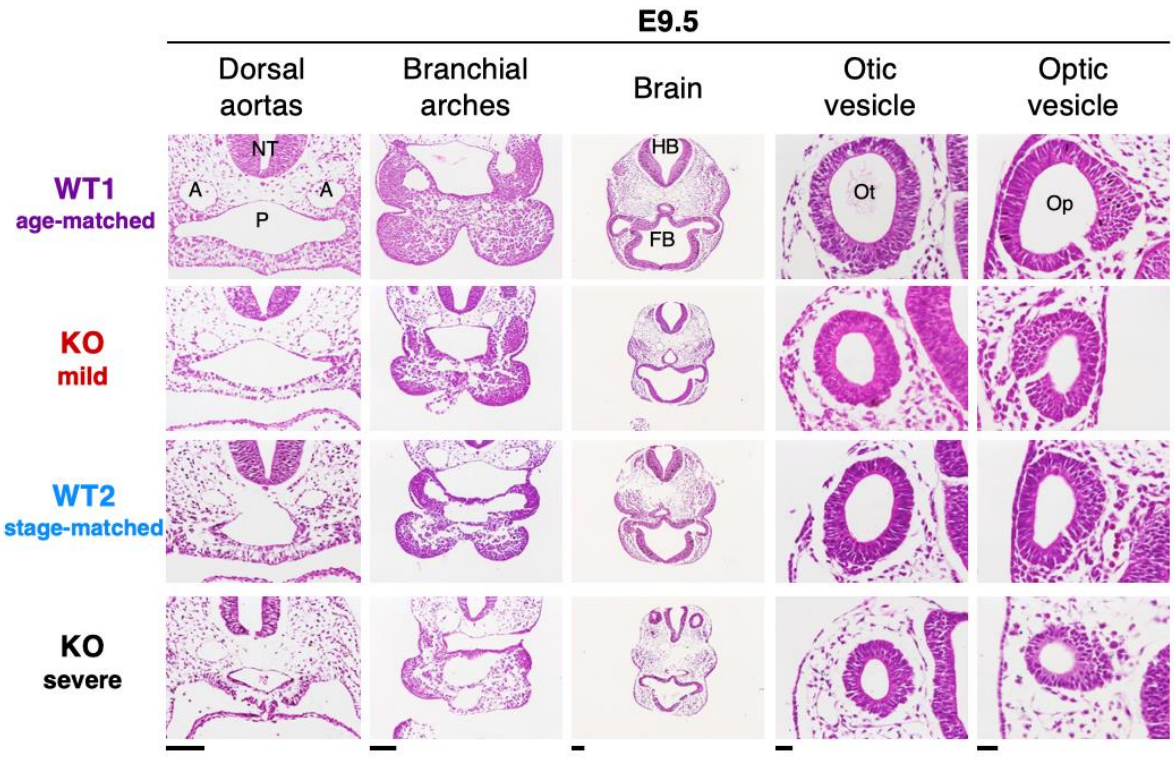

Figure S3. Global developmental defects in *Stag*2 null E9.5 embryos, related to Figure 5.

H-E stained transverse sections of *Stag2* null (KO, mild and severe), WT1 (age-matched control) and WT2 (stage-matched control) embryos extracted at E9.5. NT: neural tube. A: aorta. P: pharynx. HB: hindbrain. FB: forebrain. Ot: otic vesicle. Op: optic vesicle. Scale bars (for entire column): 100 μm for dorsal aortas, branchial arches and brain; 25 μm for otic and optic vesicles.

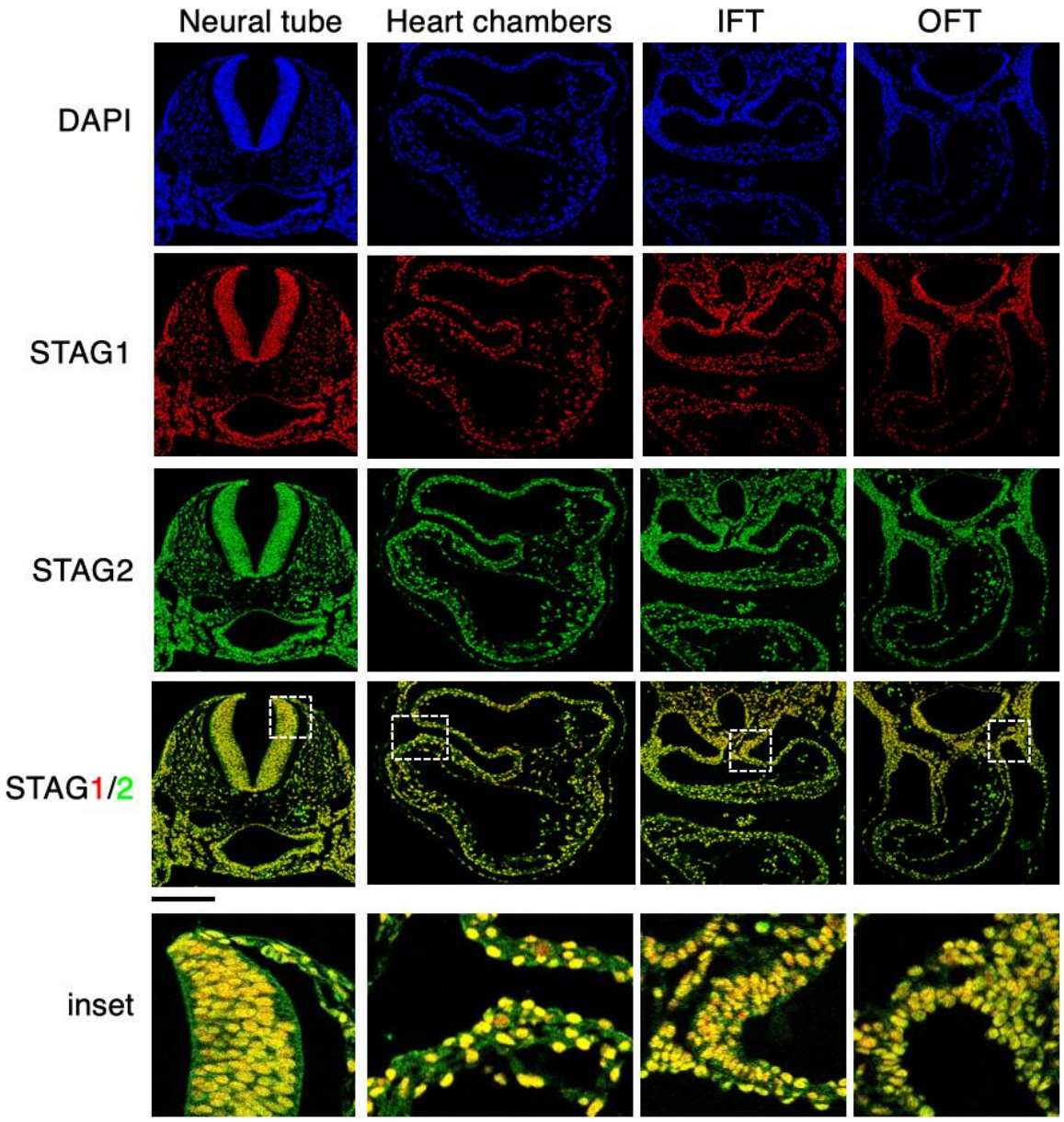

Figure S4. Distribution of cohesin variants in the E9.5 embryo, related to Figure 6.

Immunofluorescence co-staining of STAG1 (red), STAG2 (green) and DAPI (blue) in transverse sections containing the heart of wild type E9.5 embryos. Scale bar, 200 μm.

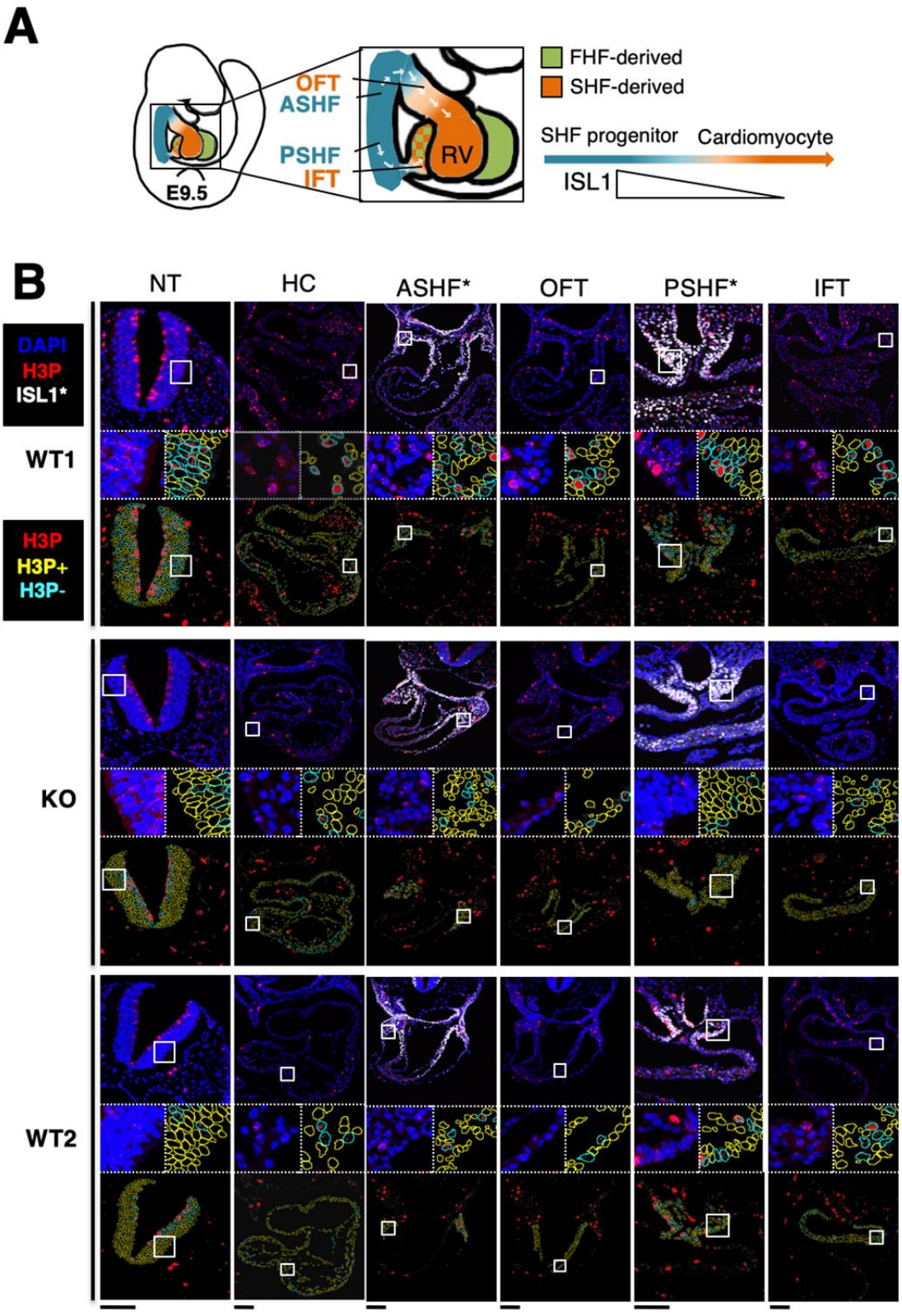

Figure. S5. Decreased proliferation in *Stag2* null embryos, related to Figure 6.

A. Scheme depicting the migration of second heart field (SHF) progenitors into the heart tube of an E9.5 embryo. Early differentiating cardiac cells termed the first heart field (FHF) form the primitive heart tube (not depicted). Starting at E8, additional cardiac progenitors are progressively added into the heart chambers: anterior SHF (ASHF) progenitors add onto the arterial pole through the outflow tract (OFT) and posterior SHF (PSHF) progenitors migrate into the venous pole, through the inflow tract (IFT). As they migrate into the heart they differentiate into cardiomyocytes, which correlates with progressively lower expression of the transcription factor Islet 1 (ISL1) (Kelly *et al.* 2014). While the left ventricle derives from the FHF (green), the right ventricle derives from the SHF (orange).

B. Representative images of H3P staining and image processing in E9.5 WT1 (age-matched control), KO (mild phenotype) and WT2 (stage-matched control) embryos. For each region and genotype are shown the original immunofluorescence signals (top) of DAPI (blue), H3P (red) and ISL1 (white, only shown in regions marked with *), and the binary H3P signal (bottom), with corresponding insets. Scale bars, 100 μm.