

***Suppressor of Fused* Restraint of Hedgehog Activity Level is Critical for Osteogenic Proliferation and Differentiation during Calvarial Bone Development**

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**Supplemental Data**

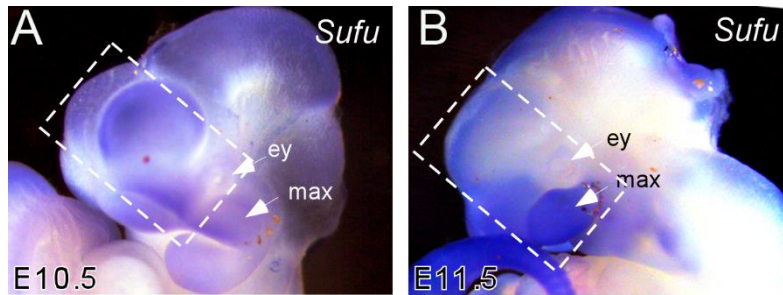
**Figure S1**

**Figure S2**

**Figure S3**

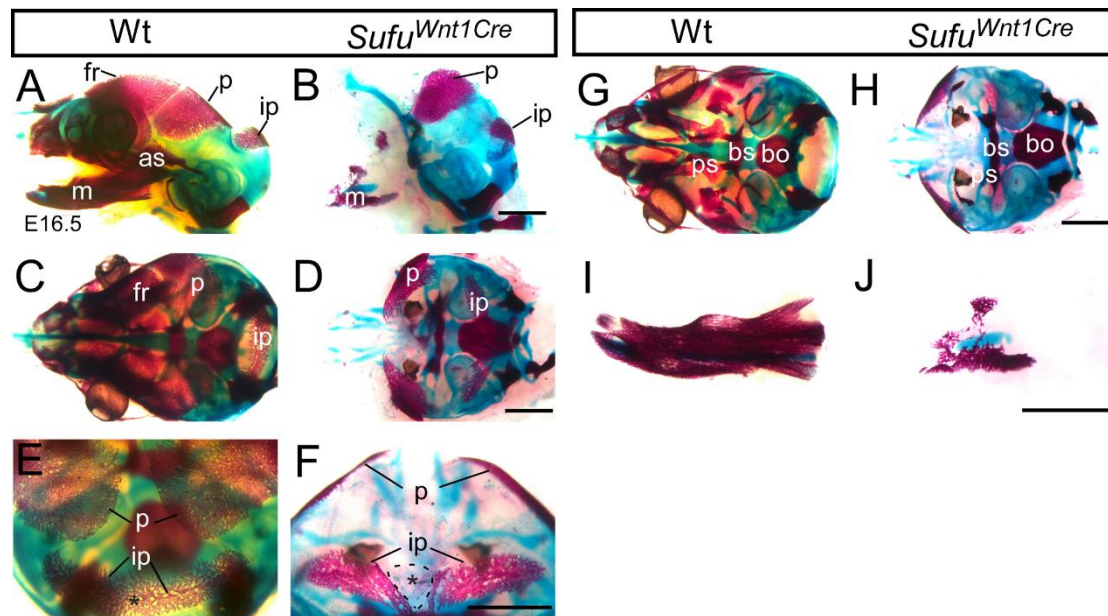
**Figure S4**

**Figure S5**



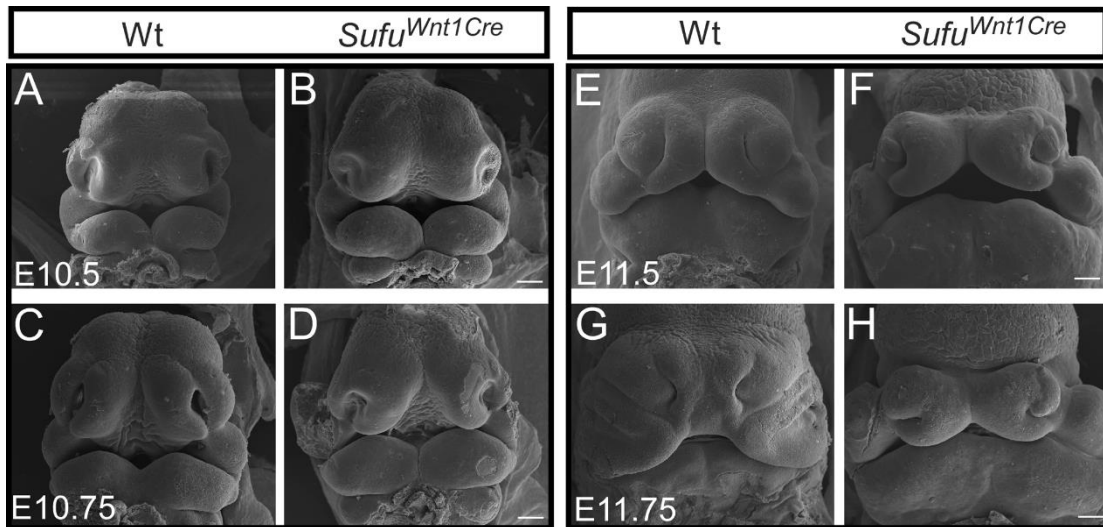
**Figure S1. Whole-mount in situ hybridization analysis of *Sufu* during embryo development.**

(A and B) Whole-mount in situ hybridization showing the global expression of *Sufu* in the wild type craniofacial primordia at E10.5 and E11.5. ey, eye; max, maxillary.



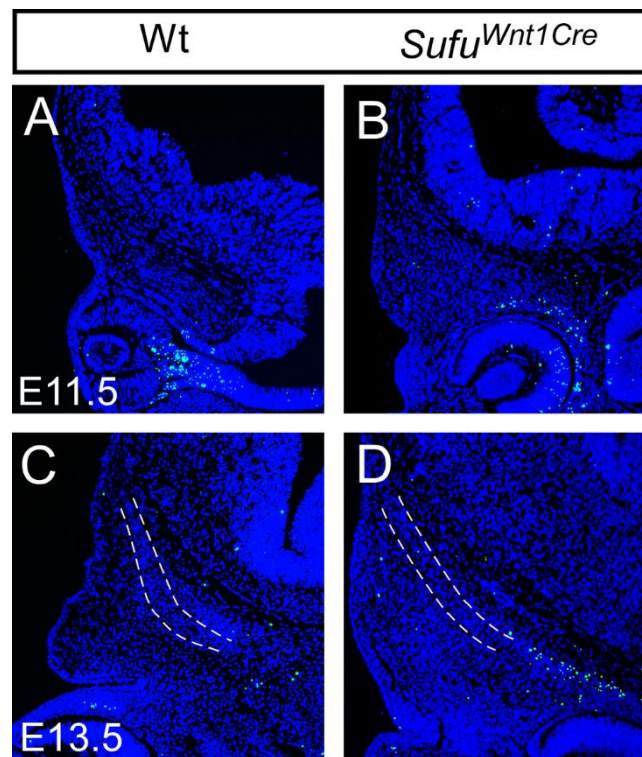
**Figure S2. Skeletal staining analysis of *Sufu*<sup>Wnt1Cre</sup> at E16.5.**

(A-J) Alcian blue/ Alizarin red staining showing severe developmental defects of CNC-derived bones in *Sufu*<sup>fx/fx</sup>; *Wnt1-Cre* mutants (n=3), including the frontal bones (A-D), the central portion of the interparietal bone (E and F), and the mandible bones (I and J). (A and B), the lateral view of skull bones; (C and D), the dorsal view of calvarial bones; (E and F), the back view of skull bones. The asterisks mark the central portion of the interparietal bone. (G and H), the ventral view of skull base bones; (I and J) the images of the mandible bones. as, alisphenoid bone; bo, basioccipital bone; bs, basisphenoid bone; fr, frontal bone; ip, interparietal bone; m, mandible; p, parietal bone; ps, presphenoid bone. *Scale bars*, 1 mm.



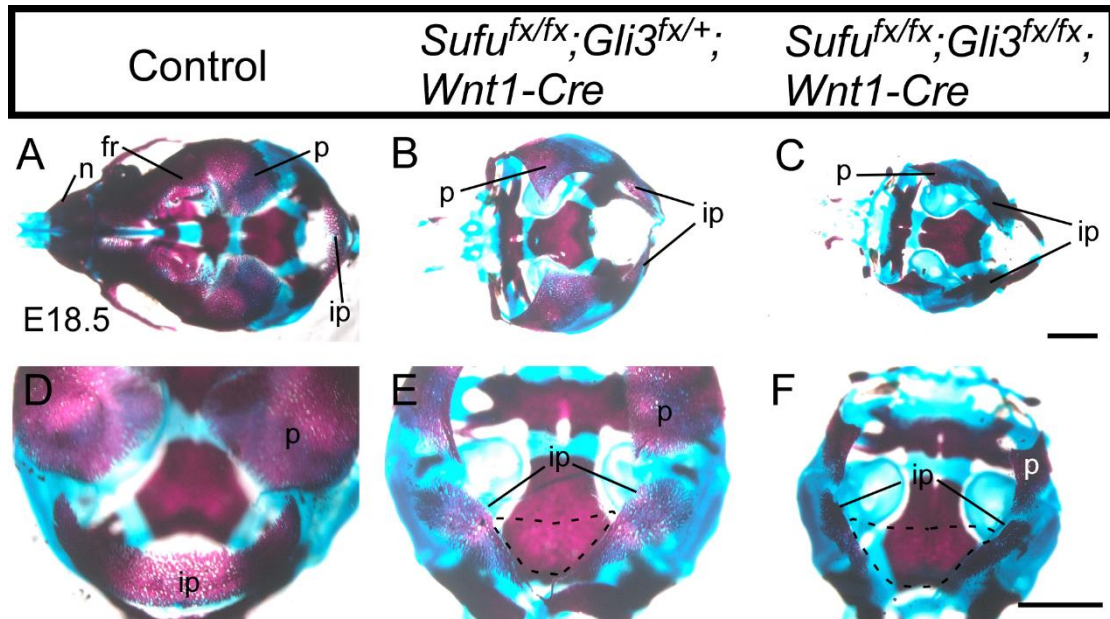
**Figure S3. Scanning electron microscope (SEM) analysis of *Sufu<sup>Wnt1Cre</sup>* at early developmental stages.**

(A-H) Developmental defects of facial primordia in *Sufu<sup>Wnt1Cre</sup>* mutants is manifested as early as E10.5. Scale bars, 200  $\mu$ m.



**Figure S4. TUNEL analysis of *Sufu<sup>Wnt1Cre</sup>* at early developmental stages.**

(A-D) TUNEL assay on the histological sections shows comparable cell death signal in the presumptive frontal primordia between wild type and *Sufu<sup>Wnt1Cre</sup>* (n=3). Dash lines outline the presumptive frontal primordium.



**Figure S5. Skeletal staining analysis of compound mutations of *Sufu* and *Gli3* using *Wnt1-Cre* mice.**

Alcian blue/Alizarin red staining showing more severe craniofacial defects in *Sufu<sup>fx/fx</sup>;Gli3<sup>fx/fx</sup>;Wnt1-Cre* than in *Sufu<sup>fx/fx</sup>;Gli3<sup>fx/+</sup>;Wnt1-Cre* mice at E18.5 (n=3).

(A-C) Vertical view of the cranial vault; (D-F) Back view of the cranial vault. Dash lines outline the missing of CNC-derived interparietal bone. n, nasal bone; fr, frontal bone; ip, interparietal bone; p, parietal bone. *Scale bars*, 1 mm.