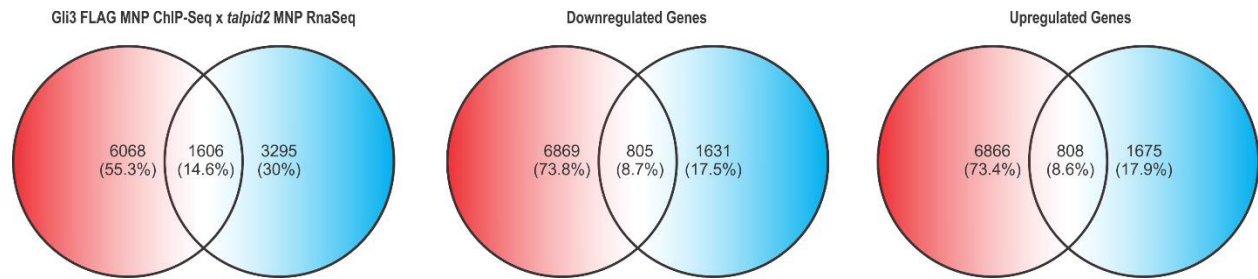
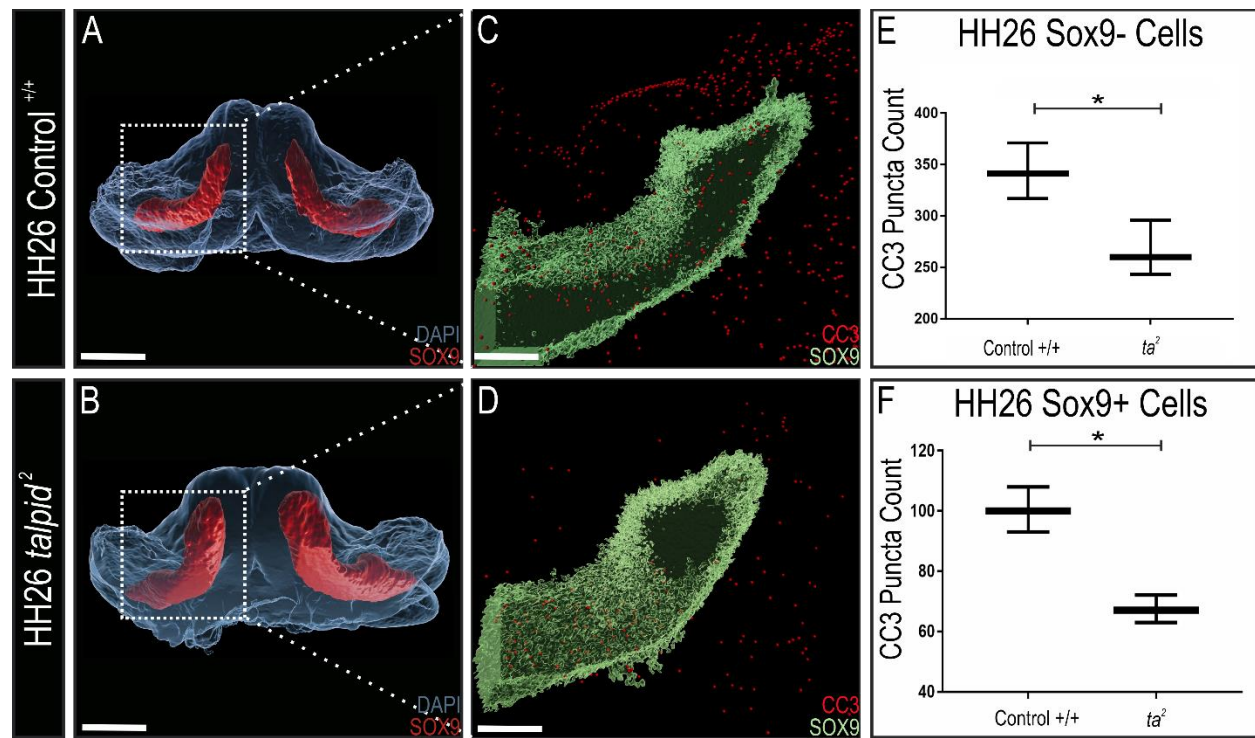


**Fig. 1. Measurements for *ta*<sup>2</sup> mandibles.**

(A) Volume measurements of HH36 control<sup>+/+</sup> and *ta*<sup>2</sup> splenial bone analyzed with the ectopic bone. (B-C) HH36 total mandible (B) volume and (C) surface area of control<sup>+/+</sup> and *ta*<sup>2</sup> mandibles measured in voxels (vx). (D-E) Alcian blue and Alizarin red staining of HH39 control<sup>+/+</sup> and *ta*<sup>2</sup> mandibular skeletons (n = 7). Black arrows in (E) denote abnormal growth in the cartilage of *ta*<sup>2</sup> mandibles. (F) Length measurements of HH39 control<sup>+/+</sup> and *ta*<sup>2</sup> ceratobranchials. (G-H) Alcian blue and Alizarin red staining of HH39 control<sup>+/+</sup> and *ta*<sup>2</sup> hyoid skeletons. Cb: Ceratobranchial. Error bars represent the mean data  $\pm$  s.d. Scale bars are .5cm (D, E, G, H). Statistical analysis was performed by Student's *t*-test (\* denotes  $P < 0.001$ ).

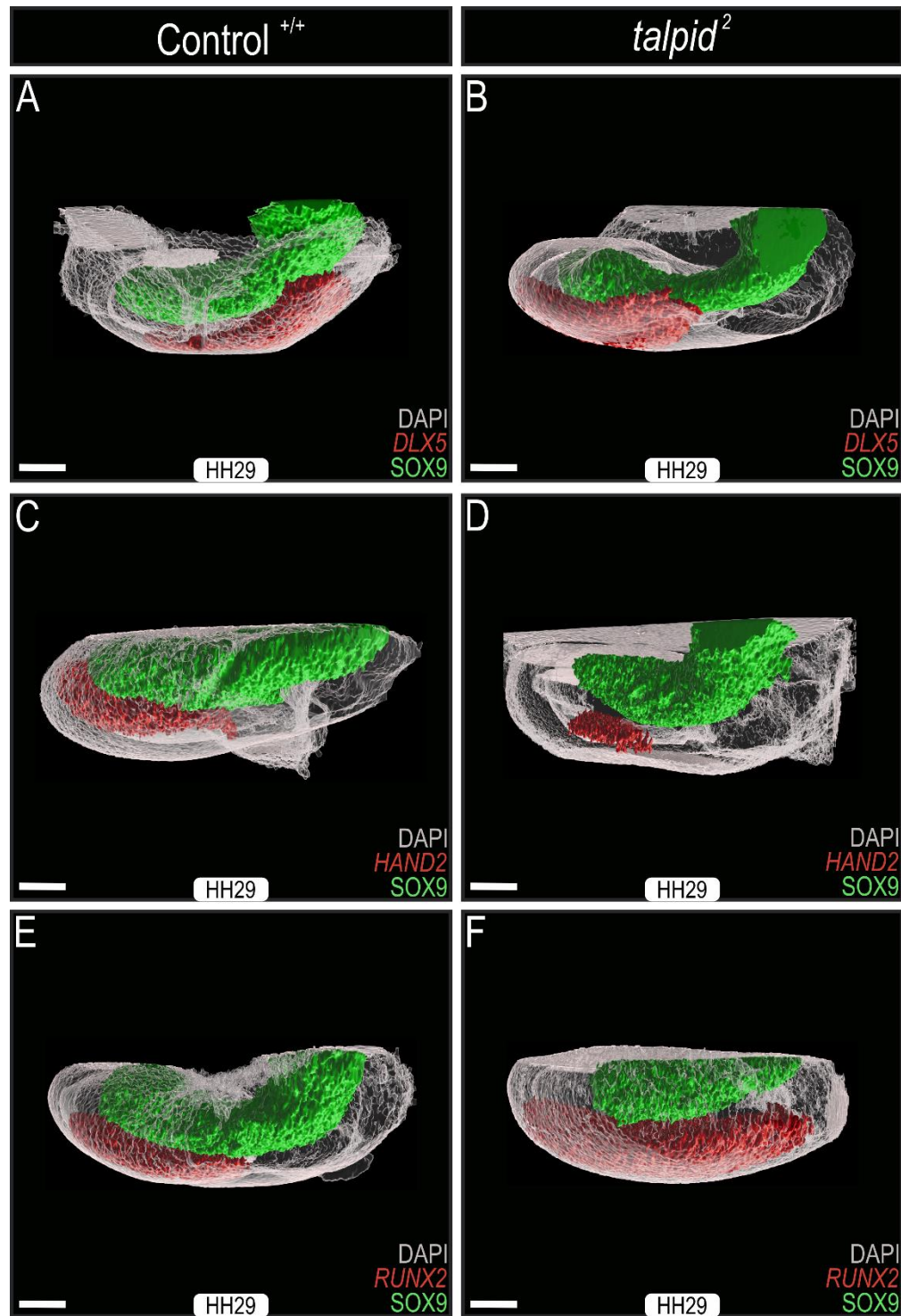


**Fig. 2. Venn diagram of differentially expressed Hh-targets in *ta*<sup>2</sup> MNPs.** Comparison of Gli bound loci and genes differentially expressed in control and *ta*<sup>2</sup> MNPs revealed 1606 potential Gli targets that were differentially expressed in the *ta*<sup>2</sup> MNPs.



**Fig. 3. Cell death is decreased in *ta*<sup>2</sup> MNPs.**

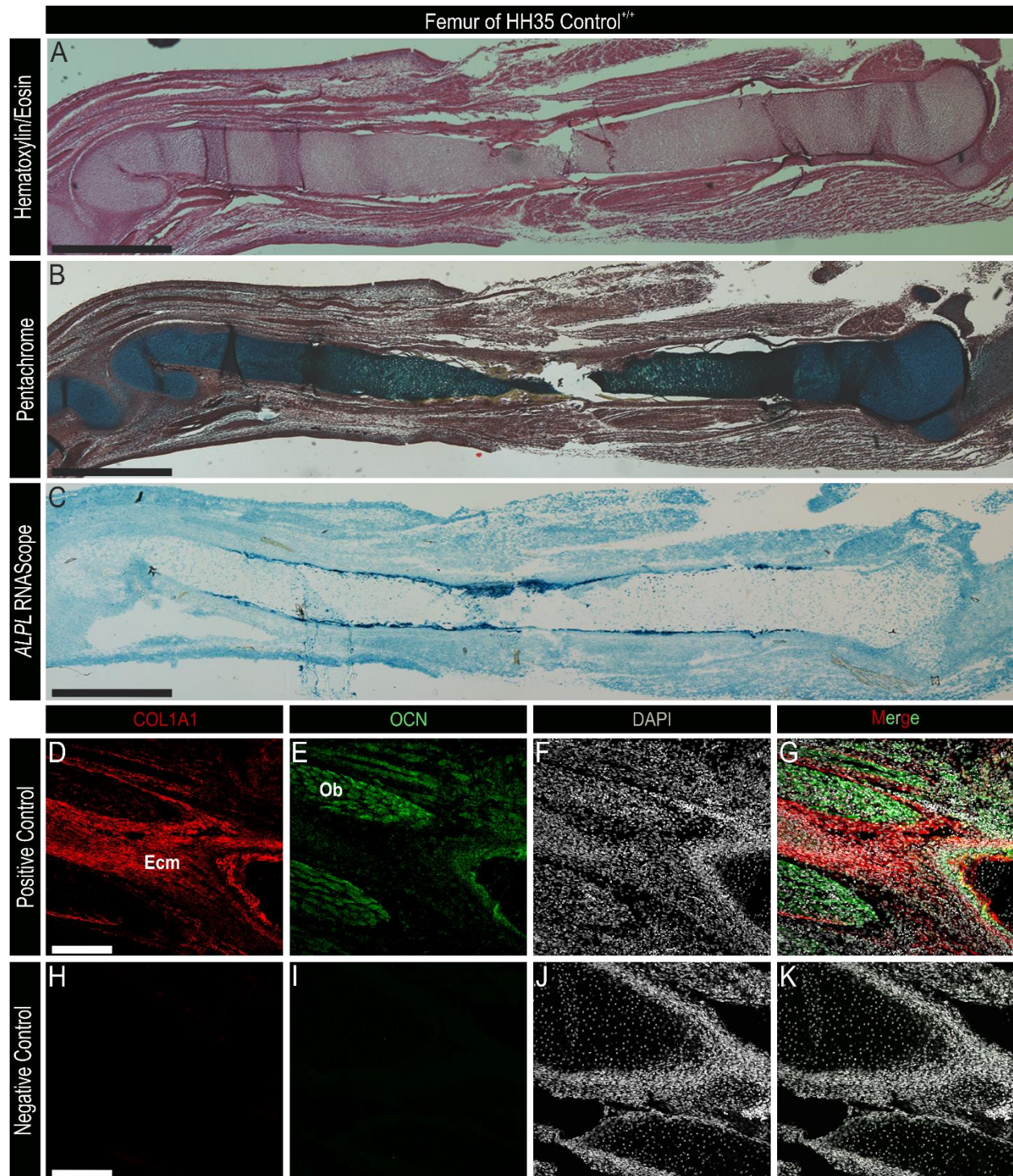
(A-B) Wholemount MNP staining for SOX9 (red) and DAPI (blue) in HH26 control<sup>+/+</sup> and *ta*<sup>2</sup> MNPs. (C-D) Puncta positive for CC3 (red) and SOX9 (green) in HH26 (C) control<sup>+/+</sup> and (D) *ta*<sup>2</sup> MNPs. (E, F) CC3 puncta count in the (E) SOX9- and (F) SOX+ cell population of control<sup>+/+</sup> and *ta*<sup>2</sup> MNPs (n = 3). Error bars represent the mean data ± s.d. Scale bars are 100µm (A, B) and 50µm (C, D). Statistical analysis was performed by Student's *t*-test (\* denotes *P* < 0.005).



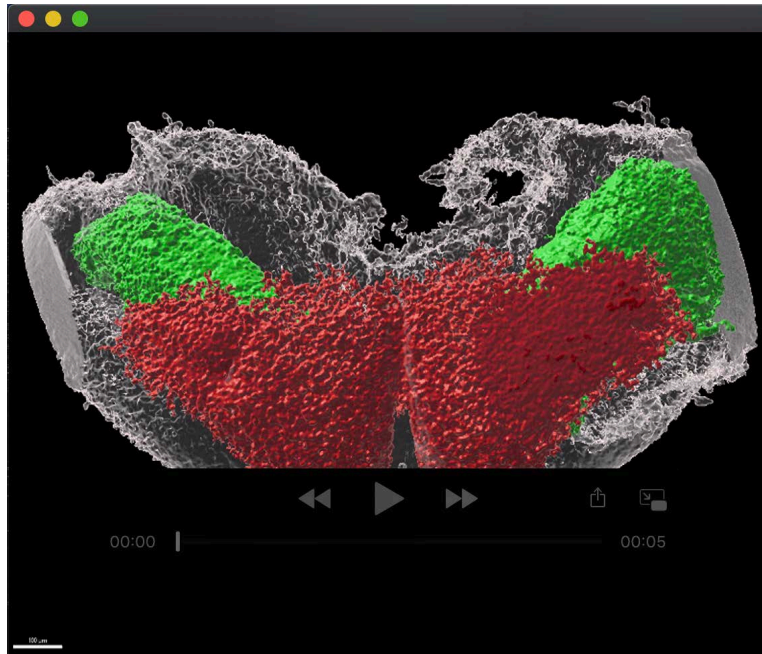
**Fig. 4. Orthogonal projections of HH29 control<sup>+/+</sup> and *talpid*<sup>2</sup> MNPs.**

Lateral view of (A, B) *DLX5* and *SOX9*, (C, D) *HAND2* and *SOX9*, (E, F) *RUNX2* and *SOX9* stained control<sup>+/+</sup> and *talpid*<sup>2</sup> MNPs at HH29. Z-stacks were taken at every 5µm, ranging from 600 to 800µm. Scale bars are 100µm.





**Fig. 5. Quality control testing in long bones for antibodies and probes used in the MNP.** Section of long bone (femur) from HH35 control<sup>+/+</sup> embryo stained for (A) H&E, (B) Pentachrome and (C) RNAscope *in situ* hybridization for ALPL. (D-G) Immunofluorescence of COL1A1 (red) and OCN (green) antibodies in HH35 control<sup>+/+</sup> long bones with (positive control) and (H-K) without (negative control) primary antibody. Ecm: Extracellular Matrix, Ob: Osteoblasts. Scale bars are 1mm (A-C) and 200μm (D-K).



**Movie 1. 3D-rendering of *RUNX2* expression in control MNPs.**

Rendering of *RUNX2* expression in red and SOX9 in green. DAPI is in light cyan. Scale bar is variable.

Table S1

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