Supplementary Figure 1

TM at 1 month

24 h

PF suture

Tomato DAPI
Supplementary Figure 1. Gli1⁺ cells in the posterior frontal (PF) suture.

Gli1-CreER^{T2}; Ai9 mice were administered TM at one month of age and harvested 24 hrs later.

Scale bar: 100 μm. Boxed region shown at a higher magnification to the right.
Supplementary Figure 2. Proliferation assays of different Gli1\(^+\) cell populations.

Gli1-CreER\(^{T2}\); Ai9 mice were administered TM at one month of age and injected EdU 24hrs later. Mice were sacrificed at 4 hrs after EdU injection. Scale bars: 100 \(\mu\)m.

(A) Fluorescence images of a longitudinal section through the distal end of femur. Red: tdTomato; green: EdU immunostaining; yellow: co-localization. Boxed areas of the growth plate ("GP") or the chondro-osseous junction ("J") are shown to the right at a higher magnification (B, C).

(D, E) Fluorescence images of a section through the distal femur containing the articular cartilage with (D) or without (E) the DAPI signal (blue). Note no co-localization of tdTomato and EdU among the articular chondrocytes.

(F) EdU labeling index among different Gli1\(^+\) populations (% tdTomato\(^+\)EdU\(^+\)/tdTomato\(^+\)) in mice at one (1m) or two (2m) months of age with TM at 1 month or 2 months. N.D.: not detectable. Labeling percentage derived from one representative section each of three mice at each age.
Supplementary Figure 3

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Images show the expression of Sox9 and tdTomato in various mouse brain regions. Images A, B, and C illustrate the expression patterns at 1 month and 1 year, respectively, with detailed views of the GP (Glia Progenitor) and J (Junction) regions. Images D and E highlight the expression of Nestin in tdTomato labeled cells, again at 1 month and 1 year.
Supplementary Figure 3  MMP does not express Sox9 or nestin.

Gli1-CreER<sup>T2</sup>; Ai9 mice were administered TM at one month (A-D) or one year (E) of age and harvested 24 hrs later.

(A) Sox9 immunostaining of a section through the distal growth plate of a femur. Boxed areas were shown to the right (B, C). Note that Sox9 (green) and tdTomato (red) co-localize (yellow) in growth plate ("GP") chondrocytes but not MMP at the chondro-osseous junction ("J").

(D, E) Nestin immunostaining of sections through the distal growth plate of femurs. Note little to no overlap between nestin (green) and tdTomato (red). Few MMP (red) remained in one-year-old mice (E). Scale bar: 100 µm.