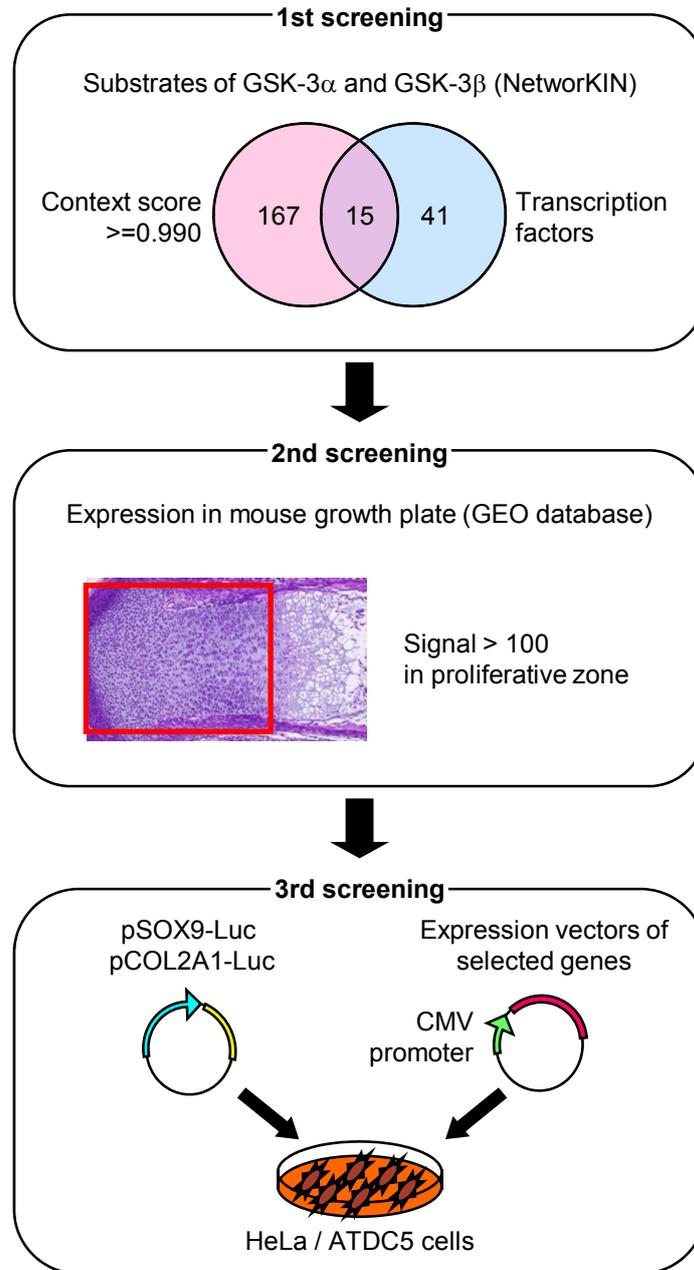
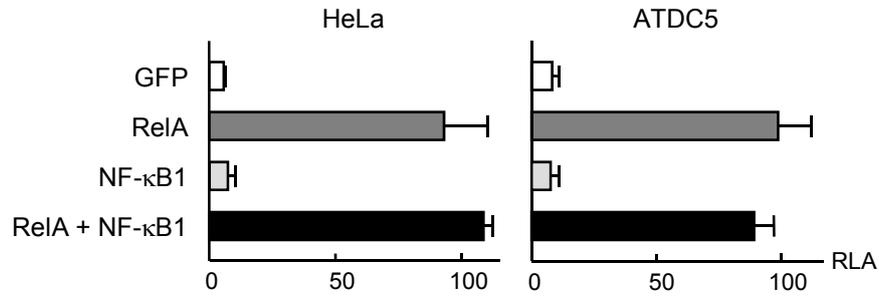


**Supplemental Figure 1.** Skeletal growth of wild-type (WT:  $Gsk3a^{+/+}; Gsk3b^{+/+}$ ) and  $Gsk3a^{-/-}; Gsk3b^{+/-}$  littermates after birth. A. Gross appearance (top) and plain radiograph (bottom) of the two genotype littermates at 12 weeks of age. Scale bars, 1 cm. B. Growth curves shown by naso-anal length and body weight of the two genotypes from 2 to 12 weeks of age. Data are expressed as means  $\pm$  s.d. \* $P < 0.05$  versus WT. C. Length of long bones (humerus, ulna, femur, and tibia) and vertebra (first to fifth lumbar spines) of the two genotype littermates at 12 weeks of age. Data are expressed as means  $\pm$  s.d. \* $P < 0.05$  versus WT.



**Supplemental Figure 2.** Schematic of the screening strategy of phosphorylation targets of GSK-3. We performed computational screenings using NetworkKIN (<http://networkin.info/search.php>) for transcription factors that are predicted to be substrates of both GSK-3 $\alpha$  and GSK-3 $\beta$  (first screening) and using Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/sites/GDSbrowser/>; GEO accession GSE7685) for expression in the proliferative zone of growth plate cartilage (second screening). The factors selected by the two screenings are shown in Supplemental Table 1. For the third screening, we measured transcriptional activity of the selected factors on the *SOX9* and *COL2A1* genes by the luciferase assay (Figure 4C).



**Supplemental Figure 3.** Luciferase activity after transfection with GFP, RelA, NF-κB1 or a combination of RelA and NF-κB1 into HeLa and ATDC5 cells containing the *SOX9* gene-reporter construct. Data are expressed as means  $\pm$  s.d. for 3 wells/group. There were no significant differences between RelA alone and the combination ( $P > 0.05$ ).

**Supplemental Table 1. Computational predictions of phosphorylation targets of GSK-3**

| Substrate     | 1st            |               | 2nd           |
|---------------|----------------|---------------|---------------|
|               | GSK-3 $\alpha$ | GSK-3 $\beta$ |               |
| <b>ATF2</b>   | 0.994          | 0.995         | <b>651.5</b>  |
| <b>Jun</b>    | 0.994          | 0.995         | <b>705.1</b>  |
| <b>JunB</b>   | 0.992          | 0.993         | <b>190.4</b>  |
| <b>JunD</b>   | 0.990          | 0.991         | <b>1130.8</b> |
| LEF1          | 0.995          | 0.998         | 35.3          |
| MITF          | 0.990          | 0.991         | 95.8          |
| <b>MYC</b>    | 0.993          | 0.994         | <b>283.5</b>  |
| NFATc2        | 0.990          | 0.991         | 61.6          |
| PML           | 0.994          | 0.995         | 54            |
| <b>RelA</b>   | 0.993          | 0.994         | <b>577.3</b>  |
| STAT1         | 0.992          | 0.993         | 82.4          |
| <b>STAT3</b>  | 0.994          | 0.995         | <b>361.6</b>  |
| STAT5A        | 0.992          | 0.993         | 75.4          |
| <b>STAT5B</b> | 0.992          | 0.993         | <b>157.6</b>  |
| <b>TCF8</b>   | 0.990          | 0.992         | <b>967.9</b>  |

Substrates with context scores of 0.99 or higher for both GSK-3 $\alpha$  and GSK-3 $\beta$  predicted by the first screening using NetworKIN are listed. Among them, those with scores above 100 for expression in the proliferative zone of growth plate cartilage selected by the second screening using the GEO database are shown in red.