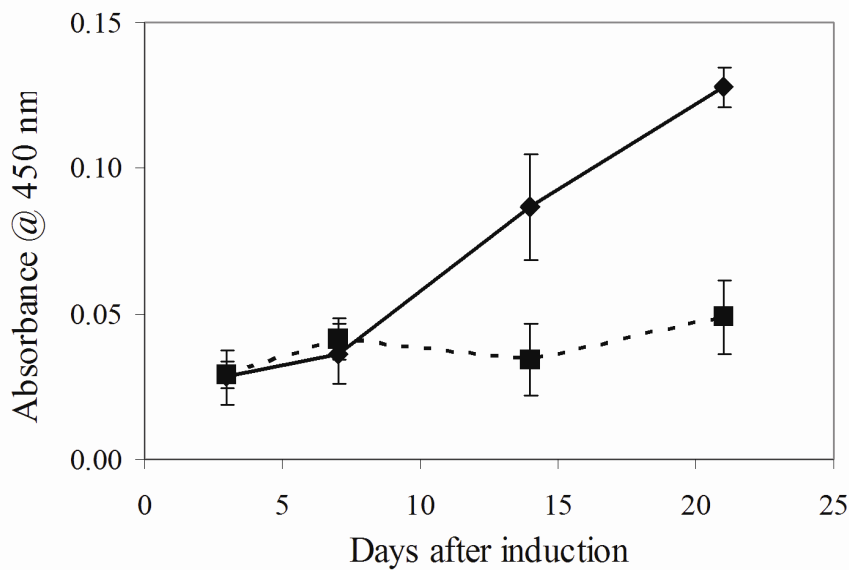


**Bone to pick: the importance of evaluating reference genes for RT-qPCR quantification of gene expression in craniosynostosis and bone-related tissues and cells**

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**Additional file 2**

**Induction of mineralisation in Kusa 4b 10 cells**

Cells were either uninduced (dotted line) or induced to differentiate into osteoblasts by addition of  $\beta$ -glycerophosphate (solid line). The progression of mineralisation over 21 days was measured by an increase in absorbance ( $A_{450}$ ) of Alizarin red S extracted from fixed and stained cells. After two weeks, induced cells at days 14 and 21 showed a significant increase in mineralisation compared to day 0 or uninduced cells (\* $P < 0.05$ , Student t-test).  $N=3$ .

Average  $\pm$  SD shown.