

817 **Supplemental Fig. 1.** CCT2/CTGF promotes *ex vivo* morphogenesis of calvarial suture
818 in organ culture. The calvarial suture is patent by postnatal day 10 (p10) (A), showing
819 fibroblastic soft tissue between mineralized bones. The suture undergoes ectopic
820 mineralization or synostosis by ~p25. By p35, suture mesenchyme virtually
821 disappeared and was replacement by mineralizing tissue between existing mineralized
822 bone (B). Delivery of 50-ng/mL CCN2/CTGF to p10 explant for 25 days rescued the
823 calvarial suture from undergoing ectopic mineralization (C), showing the presence of a
824 fibroblastic, soft tissue interface between mineralizing bone. b: bone, s: suture. FSP1
825 and vimentin were expressed in the representative p10 innate calvarial suture and the

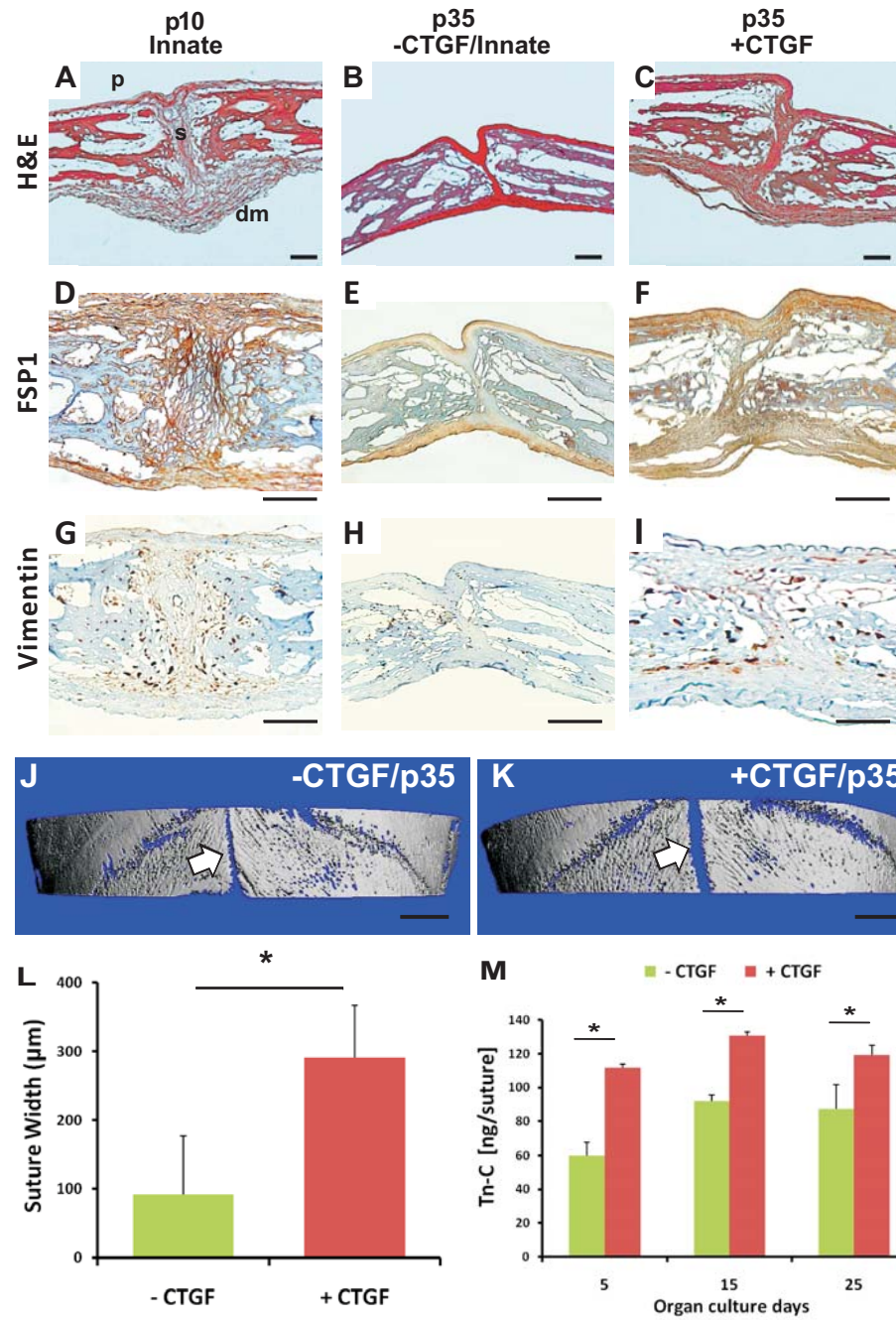
826 representative CCN2/CTGF-treated calvarial suture (D, F for FSP1) and (G, I for
827 vimentin), in comparison with faint FSP1 expression and virtual absence of vimentin
828 without CCN2/CTGF (E and H, respective). The width of the representative calvarial
829 suture by p35 without CCN2/CTGF delivery was narrow on 3D μ CT reconstructed
830 sample (J), in comparison with wide, patent suture with CCN2/CTGF treatment by p35
831 (K). Quantitatively, the average width of CCN2/CTGF-treated sutures was significantly
832 wider than without CCN2/CTGF (L) ($p < 0.05$; $N = 8$). Harvested soft tissue from
833 CCN2/CTGF-treated sutures showed significantly more Tn-C contents than
834 CCN2/CTGF-free sutures (M) ($p < 0.05$). Scale: 60 μ m.

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836 **Supplemental Fig. 2.** Calvarial suture mesenchymal cells showed multi-lineage
837 differentiation capacity. Cells isolated from native, patent calvarial sutures by p7 readily
838 differentiated into fibroblast-like cells that are highly positive to Masson's trichrome
839 staining upon 100-ng/mL CCN2/CTGF stimulation (A). In contrast, isolated calvarial
840 suture cells without CCN2/CTGF treatment continued to assume MSC morphology and
841 synthesized little collagen (E). Suture cells from p7 calvaria that was about to undergo
842 synostosis within 20-30 days readily differentiated into osteoblasts under osteogenic
843 stimulation with or without CCN2/CTGF (B,C), in comparison to isolated cells without
844 osteogenic stimulation (F). Also, isolated calvarial suture cells underwent adipogenic
845 differentiation under permissive conditions (D), in comparison with isolated suture cells
846 without adipogenic stimulation (G). Scale: 100 μ m (A, D-G), 50 μ m (B, C). **Data**
847 **represent mean \pm STDV.**

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Suppl Fig. 1 Lee et al.



Suppl Fig. 2 Lee et al.

Trichrome

Alizarin Red

Oil-red O

