Supplementary data
For:

Robinow Syndrome skeletal phenotypes caused by the *WNT5A*^{C83S} variant are due to dominant interference with chondrogenesis

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Supplementary Figures

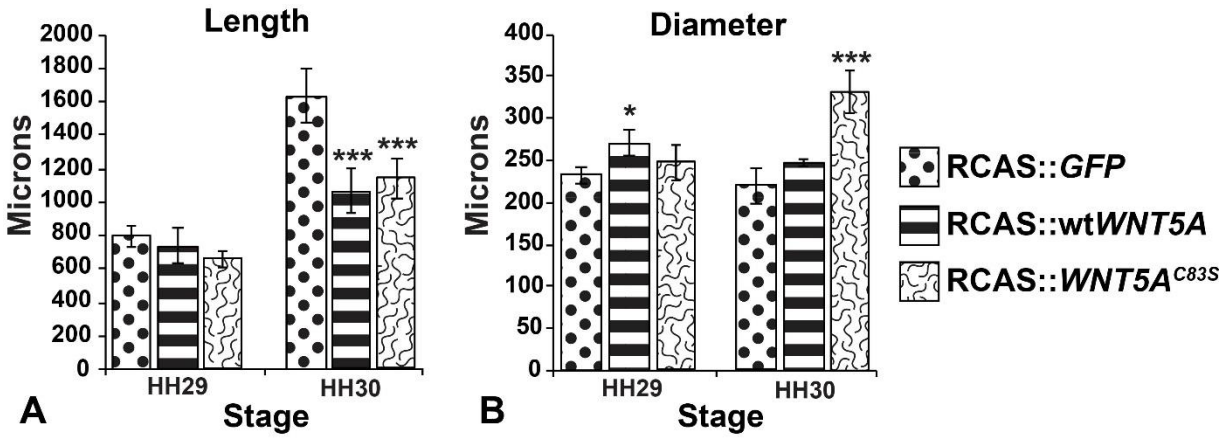

Figure S1: Phenotypic changes are first visible 4-5 days post infection with RCAS viruses The ulna was measured on sections used in Figure 4 (stage 30) and Figure S2 (Stage 29; For both stages n = 4 *GFP*; n = 5 wt*WNT5A* and *WNT5A*^{C83S}). **A)** The length of the ulna was significantly shorter in the treated limbs at stage 30. There was no difference in the length between wt*WNT5A* and *WNT5A*^{C83S}. **B)** The diameter of the ulna was significantly wider at stage 29 in wt*WNT5A* injected limbs. However at stage 30 there was already a large increase in diameter produced by the *WNT5A*^{C83S} virus. *p<0.05, ***p<0.001

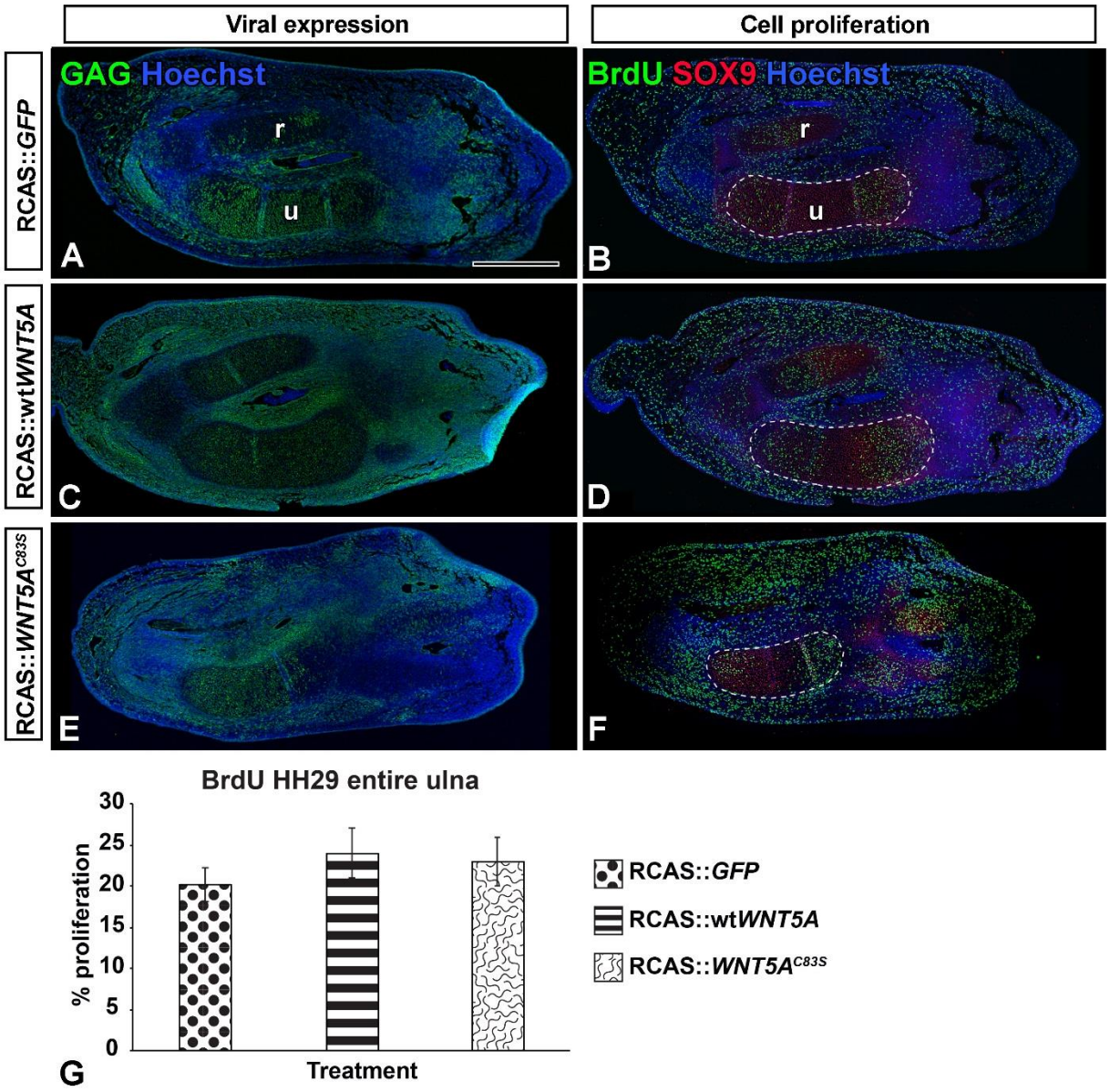

Figure S2: wt*WNT5A* or *WNT5A*^{C83S} injected forelimbs do not affect cell proliferation at HH29 in the developing ulna. Embryos were injected with viruses into the limb bud at stage 15 and fixed four days post-injection (stage HH29; (n = 4 *GFP*; n = 5 wt*WNT5A* and *WNT5A*^{C83S}). **(A-C)** Sagittal sections of the limbs show viral (anti-GAG) expression (green) and **(D-F)** neighboring sections probed for anti-BrdU labeling (green) and anti-SOX9 expression to detect chondrocytes (red). There were qualitatively fewer BrdU positive cells in the centre of the ulna or the future diaphysis (B,D,F). **(G)** BrdU positive cells in the entire ulna were quantified and normalized to control *GFP* virus No change in cell proliferation was detected. One-way Anova, Tukey's Posthoc test). Scale bar = 500 µm, Key: r - radius, u - ulna

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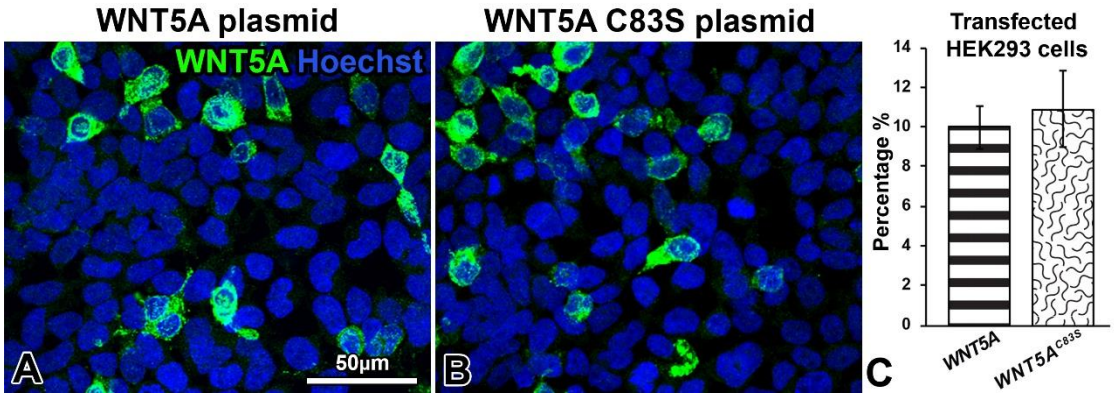

Figure S3: Immunocytochemistry with anti-WNT5A antibodies on transfected HEK293 cells.

HEK293 cells were transfected with wt*WNT5A* or *WNT5A*^{C83S} plasmids were transfected into HEK293 cells and grown for 48h prior to immunostaining (2.5 µg DNA transfected) with the anti-human WNT5A (the same antibody as that used in Fig. 9, main paper). The percentage of stained cells in 3 biological replicates was calculated and compared with a T-test. No significant difference was observed. Scale bar = 50 µm.

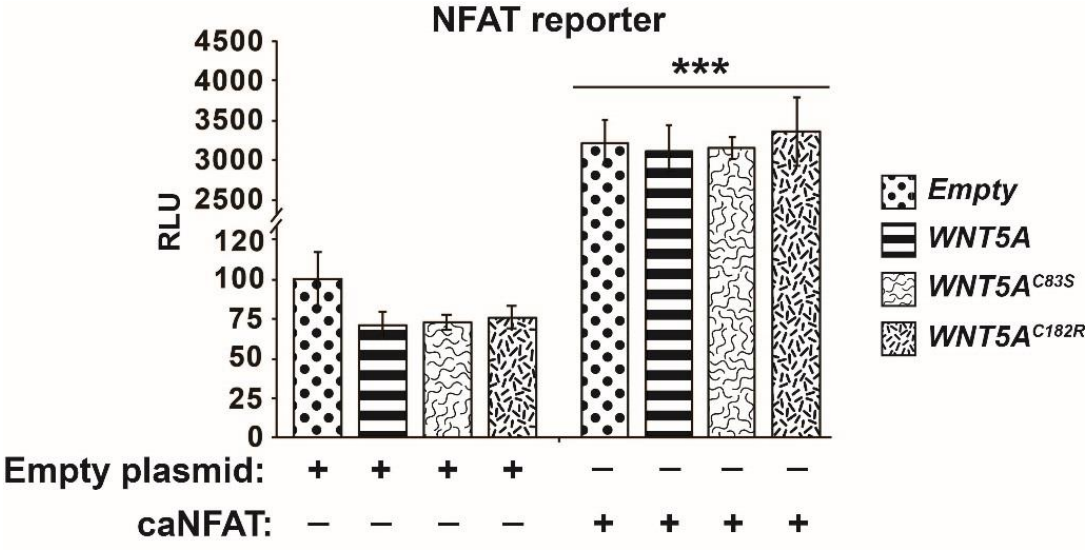

Figure S4. WNT5A constructs do not activate calcium Wnt signaling. NFAT luciferase reporter was used to assess non-canonical WNT Ca^{2+} signaling activity. HEK293 cells were transiently transfected (48h) with *WNT5A* plasmids (wild-type, *C83S*, and *C182R*). *WNT5A* plasmids failed to activate NFAT reporter (see left side of graph). The positive control, ca*NFAT*, activated NFAT reporter showing that the necessary signal transduction molecules are present in HEK293 cells. No further increase or decrease was seen with the addition of *WNT5A* constructs

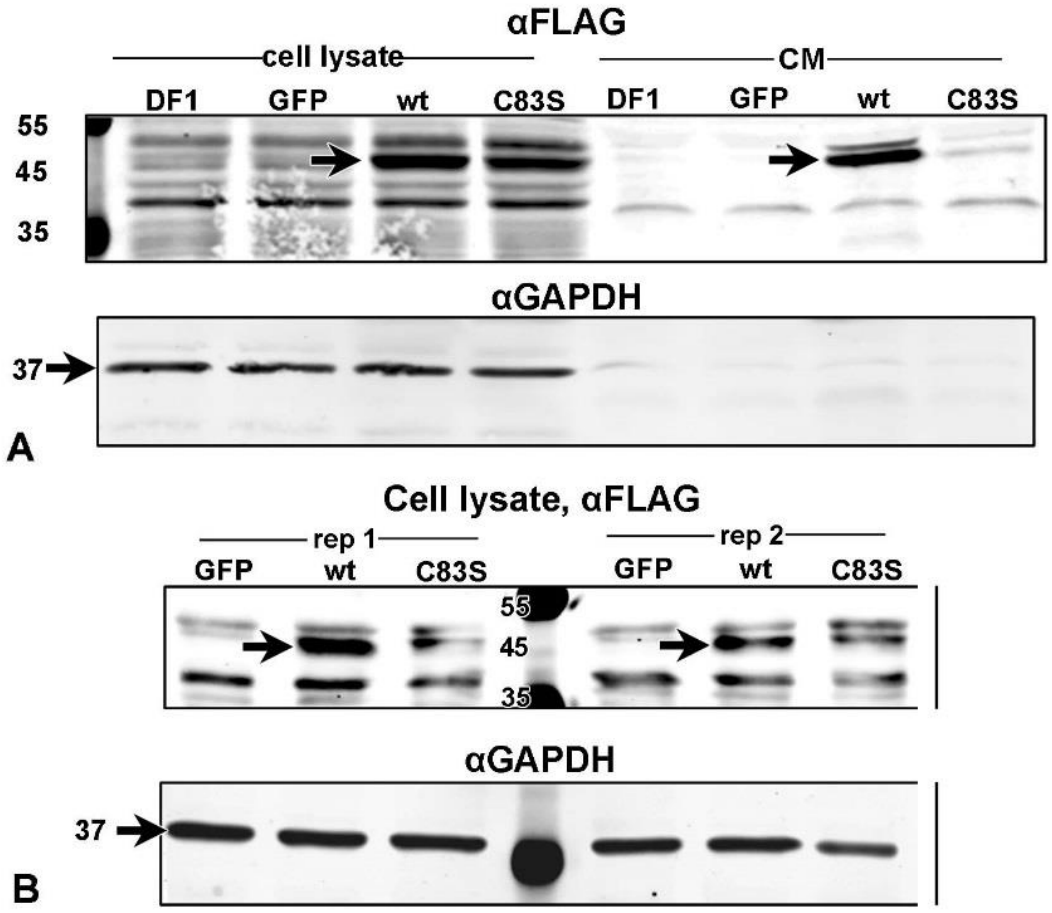

Figure S5.
Western blot analysis of FLAG protein. DF1 (chicken fibroblast) cells were transfected with RCAS viruses (FLAG tagged-*GFP*, -*WNT5A* or -*WNT5A*^{C83S}) or no virus, for 1 month. Heparin (100μg/ml) was added 24h prior to collecting conditioned media. After media was collected cells were lysed and protein extracted. Blots were probed with anti-FLAG polyclonal antibody, then stripped and reprobed with antibody to GAPDH. **A)** The lysate from wtWNT5A infected cells show a strong band at 45 kD and WNT5A^{C83S} lane shows a slightly lighter staining. The predicted size of WNT5A protein is 45kD. Secretion of wtWNT5A into media was detected but levels of WNT5A^{C83S} were considerably lower. Loading controls are showing below in the GAPDH stained blots. Key: CM – conditioned, concentrated media, DF1 – DF1 fibroblasts, parent cell line, GFP – cells infected with *GFP* virus, wt - wtWNT5A, C83S – WNT5A^{C83S}.

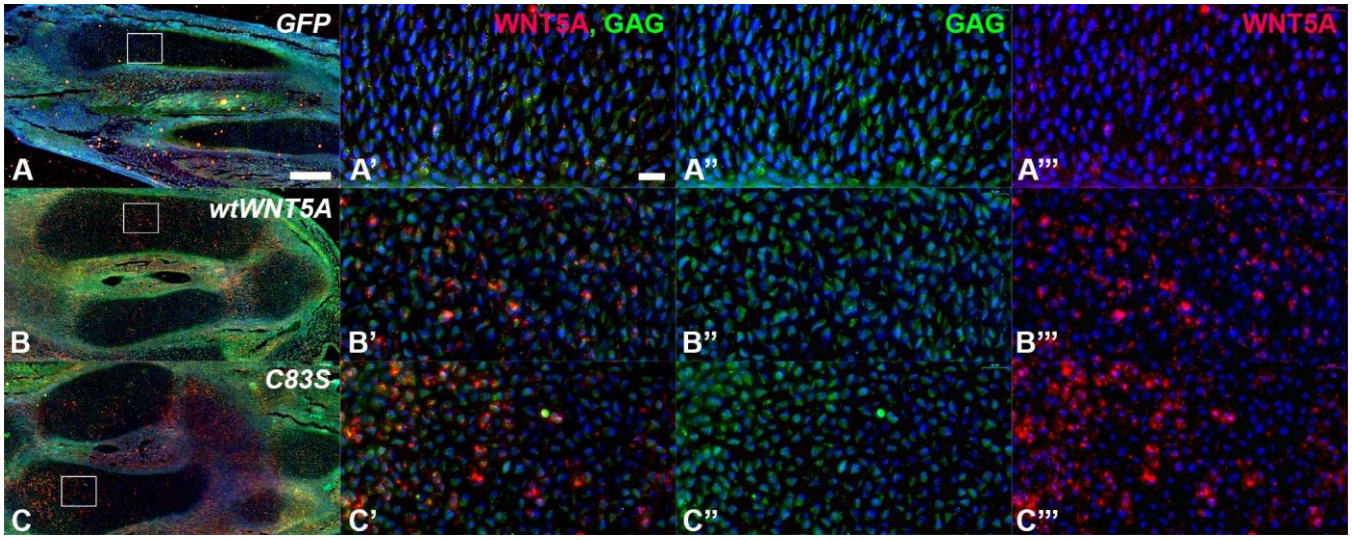

Figure S6.
Detection of virus-derived WNT5A and GAG proteins, 5 days post-injection (stage 30). A-A''') Lower expression of human WNT5A is seen in the GFP controls, demonstrating lack of cross reactivity with chicken protein. B-B''') Expression of wtWNT5A overlapping GAG staining is visible in the cartilage of the radius. C-C''') Similar expression of WNT5A and GAG is seen in the C83S variant-infected limbs. The antibody can recognize mutant or wild-type protein. Scale bars = 200 µm for low power views and 20 µm for high power views.

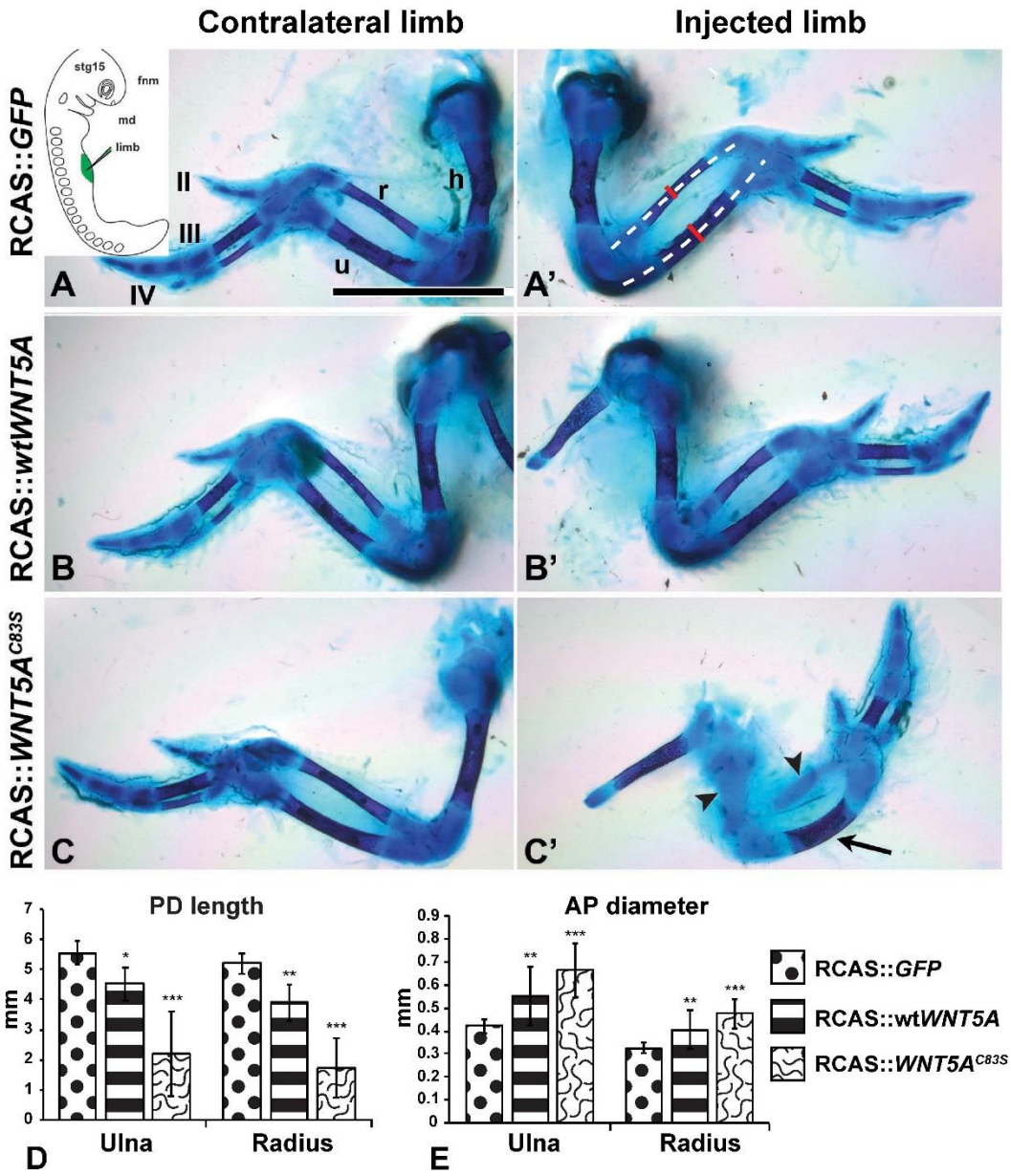

Figure S7. Colour version of Figure 1 in main manuscript.
Skeletal phenotypes caused by injection of RCAS viruses expressing *GFP*, wt*WNT5A* or *WNT5A*^{C83S}. The embryos were injected at stage 15 into the right forelimb field (inset, A) and fixed at stage 38. (A, A') Wholemount staining with Alcian blue (cartilage) and Alizarin red (bone) shows the normal bone length (dashed white lines) and normal AP diameter (red line). The expression of GFP does not affect patterning or size of the skeletal elements. (B, B') The right limb is slightly shorter than the contralateral side and shorter than the *GFP*-injected limbs. (C, C') The mutant virus has inhibited bone formation in the radius and humerus (arrowheads), while the ulna was still able to ossify (arrow). (D) Quantification of bone length and AP diameter from photographs. All bones are shorter and have increased AP diameter compared to GFP control limbs. Scale bar=5 mm. For sample size refer to Table 1. Key: fnm, frontonasal mass; h, humerus; md, mandibular prominence; r, radius; u, ulna; II, III, IV, digit numbers.