

## SUPPLEMENTARY MATERIAL FOR:

### INACTIVATION OF *PATCHED1* IN THE LIMB HAS NOVEL INHIBITORY EFFECTS ON THE CHONDROGENIC PROGRAMME

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## INCLUDES:

**Legends for supplementary movies S1-S3**

**Supplementary figures 1-6 with legends**

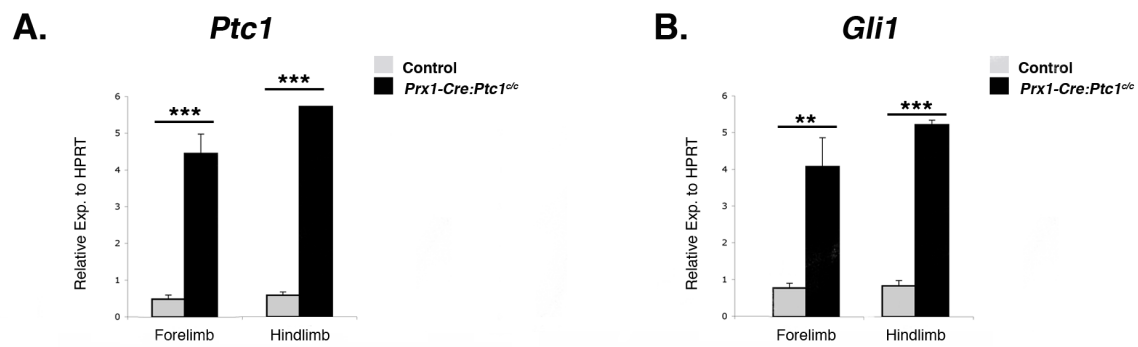
## SUPPLEMENTARY MOVIE LEGENDS

Movie S1. Control micromass day 2-3. Following 48hrs of culture, time-lapse imaging revealed control mesenchymal cells undergo rapid condensation and hypertrophy.

Movie S2. *Prx1-Cre:Ptc1<sup>c/c</sup>* micromass culture day 2-3. Following 48 hours, *Prx1-Cre:Ptc1<sup>c/c</sup>* micromass culture undergoes severe morphological disruption resulting in complete loss of mesenchymal cell condensations.

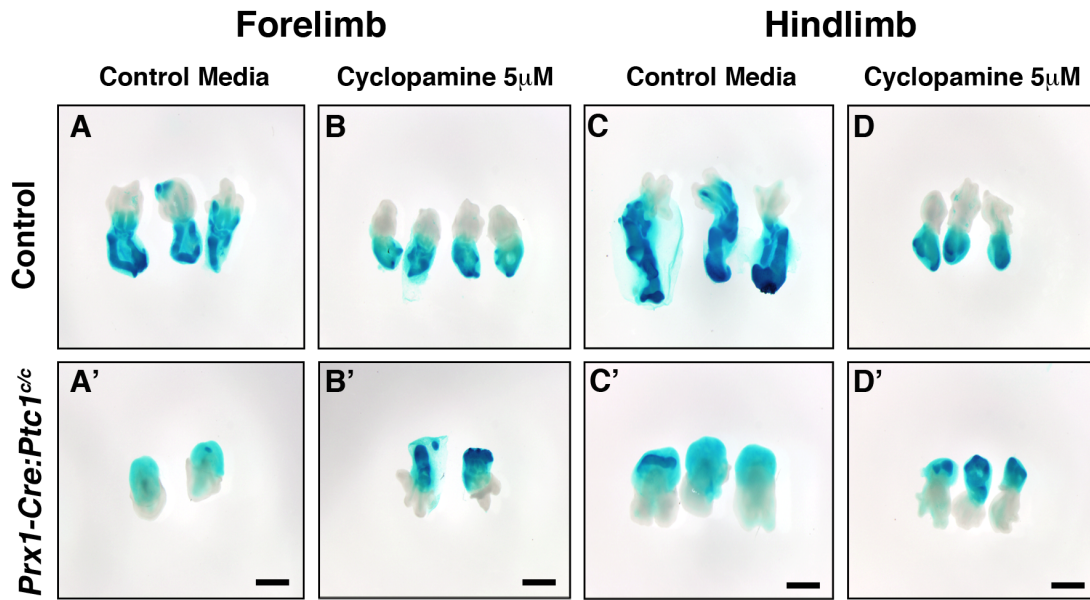
Movie S3. *Prx1-Cre:Ptc1<sup>c/c</sup>* micromass cultured in the presence of cyclopamine (day 2-3). Addition of cyclopamine (5µM) to *Prx1-Cre:Ptc1<sup>c/c</sup>* micromass completely inhibits the cell disruption observed in untreated *Prx1-Cre:Ptc1<sup>c/c</sup>* culture.

## Supp. Figure 1



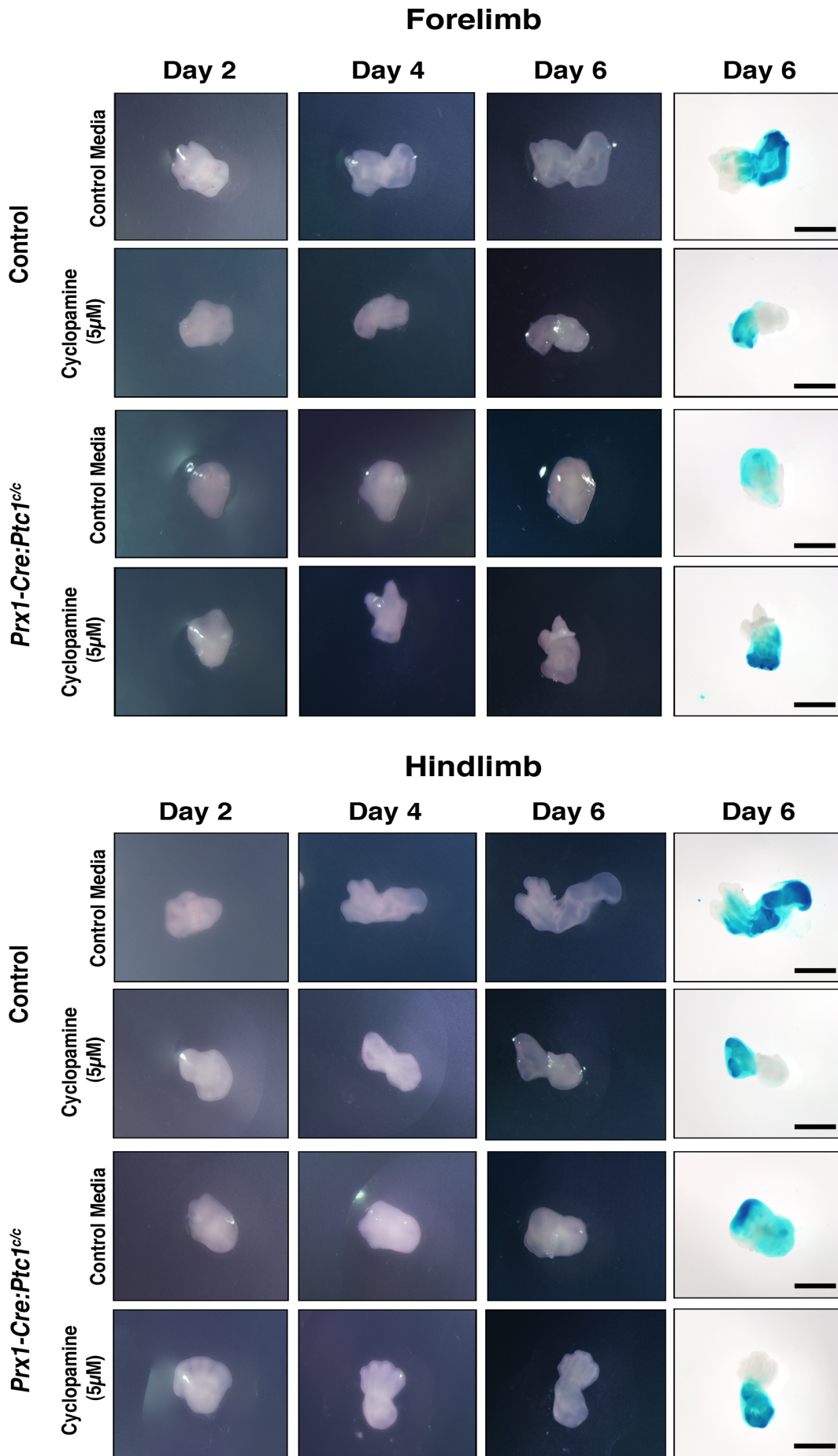
Supp. Figure 1. Hedgehog signalling activation in *Prx1-Cre:Ptc1<sup>c/c</sup>* autopods. At 12.5dpc expression of *Ptc1* and *Gli1* was significantly up regulated in *Prx1-Cre:Ptc1<sup>c/c</sup>* forelimb and hindlimbs (**A, B**). (\*\*P<0.01, \*\*\*P<0.001).

Supp. Figure 2



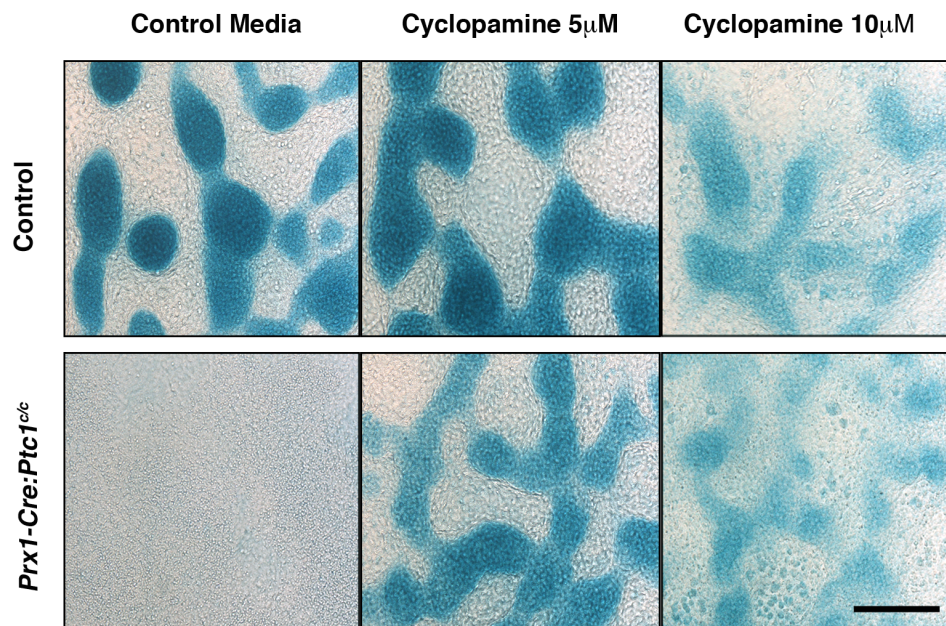
Supp. Figure 2. Explant culture of 11.5dpc control and *Prx1-Cre:Ptc1<sup>cl/c</sup>* autopods. Additional examples of the *ex-vivo* growth of autopods grown in the presence or absence of 5 $\mu$ M cyclopamine. Scale 400 $\mu$ m

Supp. Figure 3



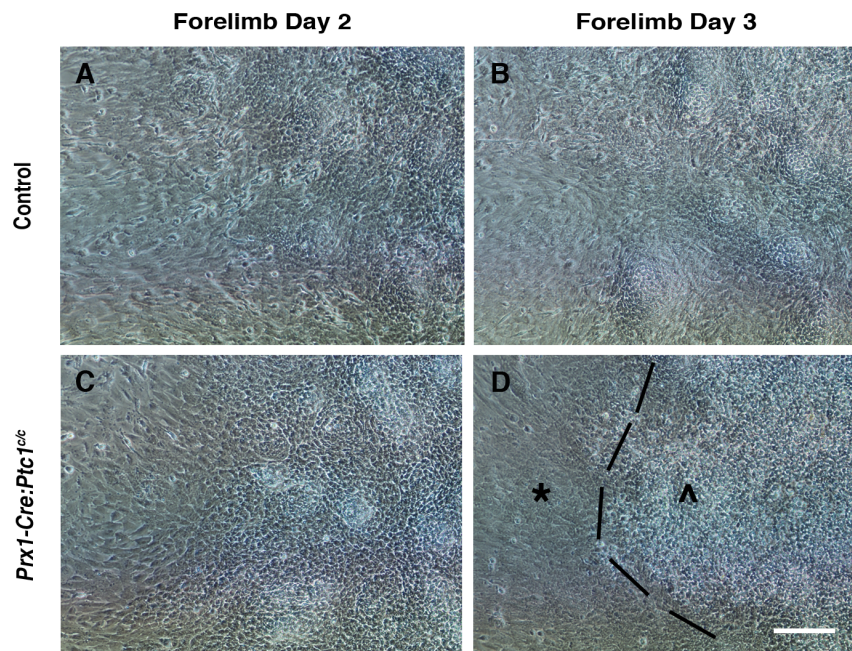
Supp. Figure 3. Explant development of 11.5dpc control and *Prx1-Cre:Ptc1<sup>c/c</sup>* autopods. Forelimb and hindlimb autopods were maintained for 6 days in the presence or absence of cyclopamine (5μM). Following 6 days of culture the tissue was harvested and stained with Alcian blue to identify chondrogenic elements. Scale 400μm

## Supp. Figure 4



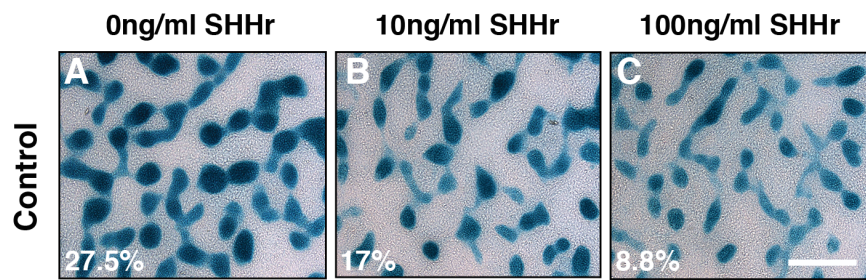
Supp. Figure 4. Cyclopamine concentration alters the chondrogenic response in micromass culture. Cyclopamine was added at 0μM, 5μM, and 10μM to micromass cultures derived from control and *Prx1-Cre:Ptc1<sup>c/c</sup>* 11.5dpc limbs. Alcian blue staining was undertaken following 6 days of culture. Scale 200μm.

## Supp. Figure 5



Supp. Figure 5. Phase contrast imaging reveals loss of chondrogenic and stromal cell morphology within the inner disrupted zone (^) compared to the outer intact zone (\*) in *Prx1-Cre:Ptc1<sup>f/c</sup>* micromass from day 3 (margin defined by dotted line; **D**). Scale 200 $\mu$ m.

## Supp. Figure 6



Supp. Figure 6. Alcian blue staining was decreased in a dose dependent manner by SHH (A-C). ImageJ analysis shows the Alcian blue stained area as a percentage of the total micromass area. (\*P<0.05), Scale 500 $\mu$ m.