Figure S1

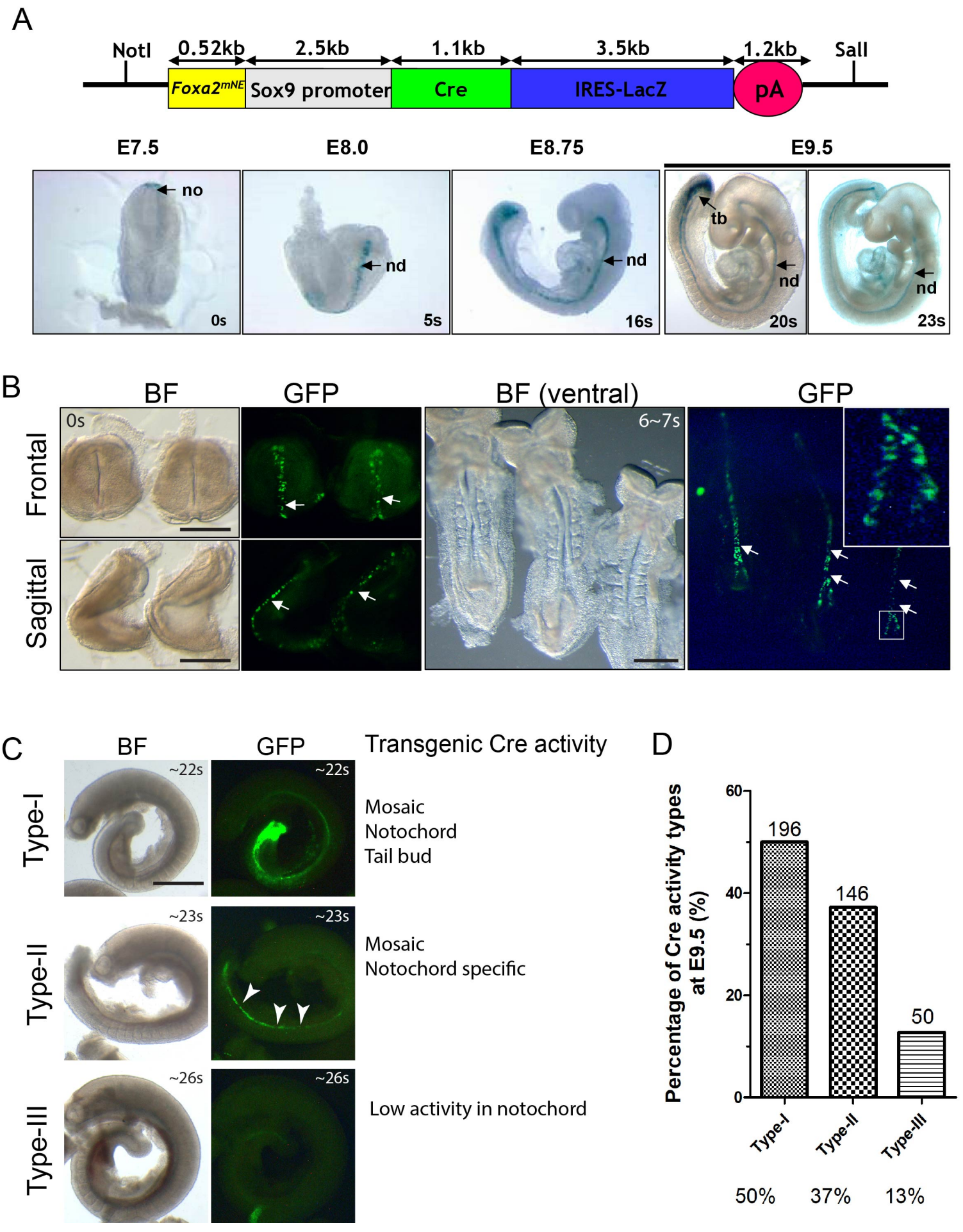
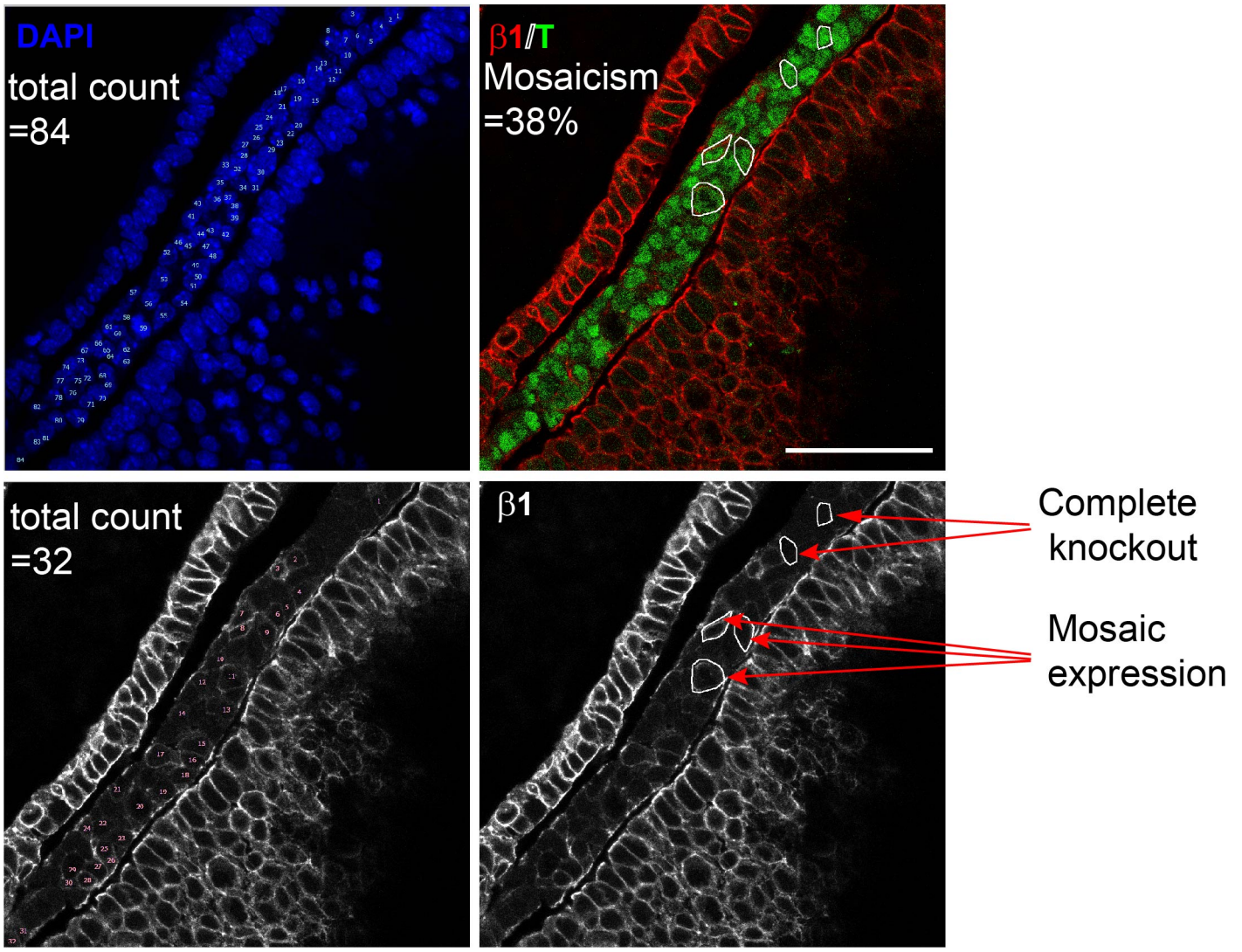


Figure S1: Transgenic notochord-specific Cre expression and activities. (A) Schematic representation of the *Foxa2mNE-Cre* construct. The Cre expression is driven by the *Sox9* promoter together with a notochord enhancer element. An IRES-*lacZ* gene was fused to mark the Cre expression. Shown here is the Cre expression from E7.5 to E9.5. Note that two expression patterns were observed at E9.5. One type with Cre expression in both tail bud (tb) and the notochord (nd), and another type with Cre expression restricted in notochord. (B) Closer look at the Cre activities marked by GFP signals at E8.0 and at E8.5. White arrows indicate the regions without Cre activities. No Cre activity in the pit cells within the center of the node (highlighted in white rectangle). Embryo stages are indicated with somite numbers. Bright field (BF) and GFP field images are shown. (C) Types of Cre activities observed at E9.5. BF and GFP field images are shown for each representative type. Cre activities for each type are described on the right hand side. Arrowheads: regions with no Cre activities. (D) Ratio of each type of Cre activities was quantified based on the observation on 392 embryos from different male breeders. The total count for each type is labelled on top and the ratio was plotted for each type. Scale bars: 1 mm.

Figure S2

A



B

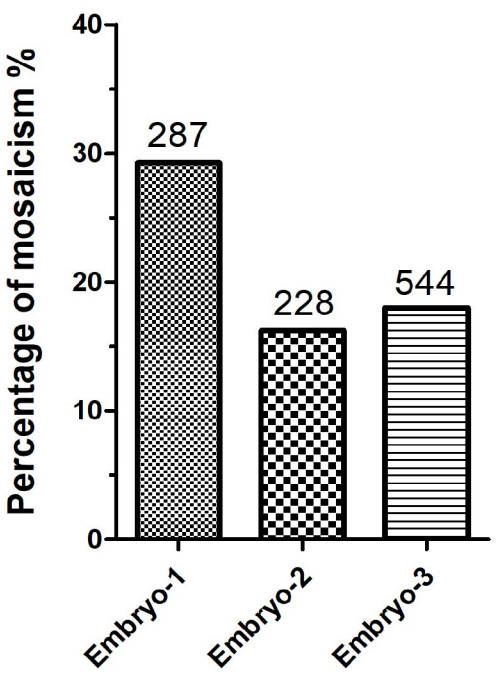


Figure S2: Variable mosaicism observed at E9.5 embryos. (A) Example images showing the calculation of the mosaicism in each image or each embryo. Cell numbers with mosaic β1 integrin expression were divided by the cell numbers that were DAPI/T +. Signal intensities were measured by ImageJ. Cells with an intensity <20 a.u. were grouped into "complete knockout", >20 a.u. were grouped into "mosaic expression". (B) Mosaicism counted for 3 different embryos at E9.5. Scale bar: 50 μm.

Figure S3

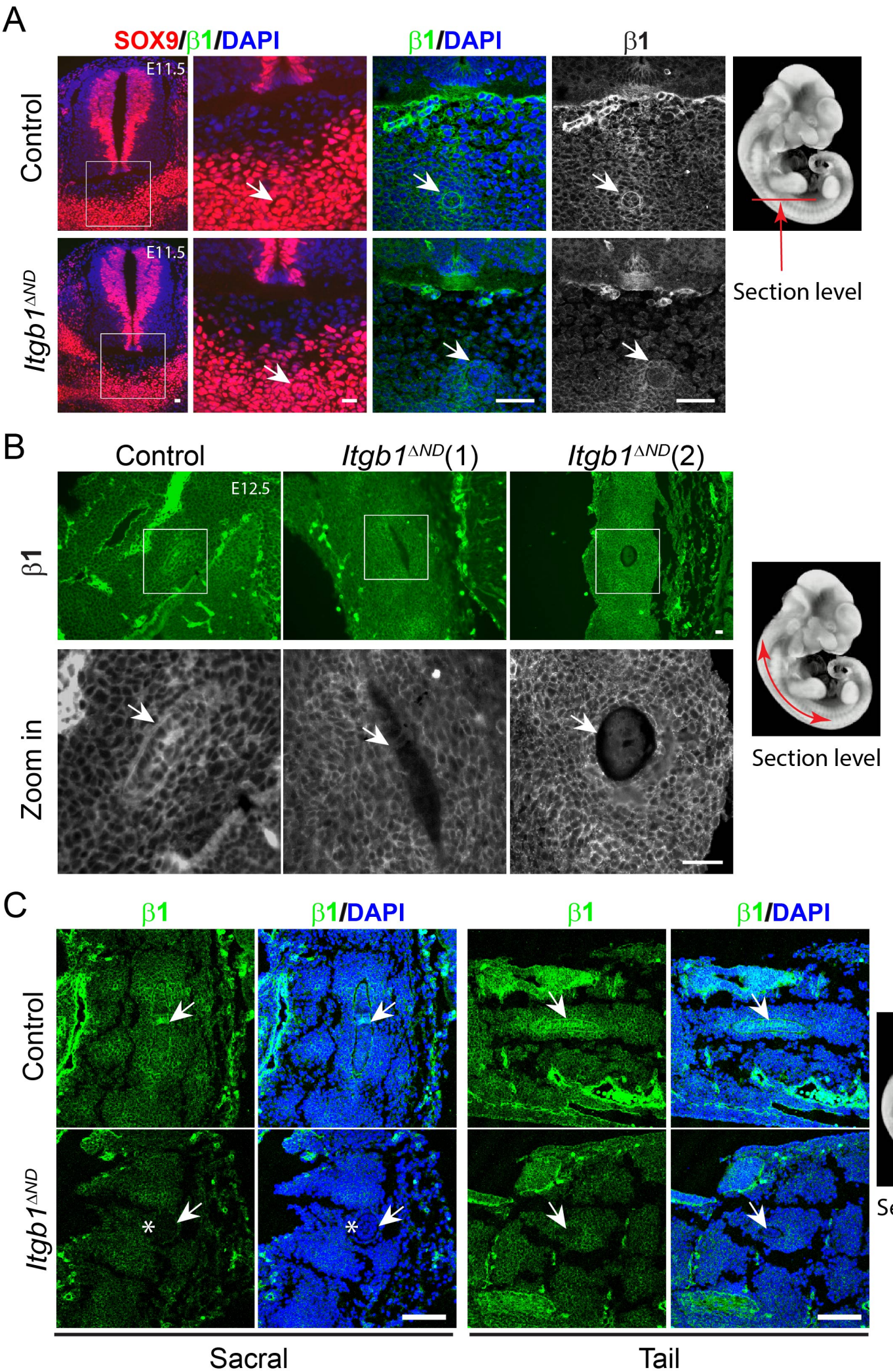


Figure S3: Specific ablation of β1 integrin expression from the mutant notochordal remnants. (A) Immunostaining against SOX9 (red), β1 integrin (green) and DAPI (blue) in the transverse sections from E11.5 embryos. Arrows: notochord. Noted that β1 integrin expression is significantly reduced comparing to the surrounding SOX9+ cells. (B-C) Immunostaining against β1 integrin (green) in the sagittal sections from E12.5 embryos. Noted that β1 integrin expression is specifically absent or reduced in the notochord descendants (arrows in B). (C) In the more posterior levels, such as sacral and tail vertebrae, β1 integrin expression is also slightly decreased in the regions surrounding the notochord. Arrows: notochord remnants. *: Note that the mutant notochord remnant was displaced to the lateral side. Scale bars: 50 μm for (A-B) and 100 μm for (C).

Figure S4

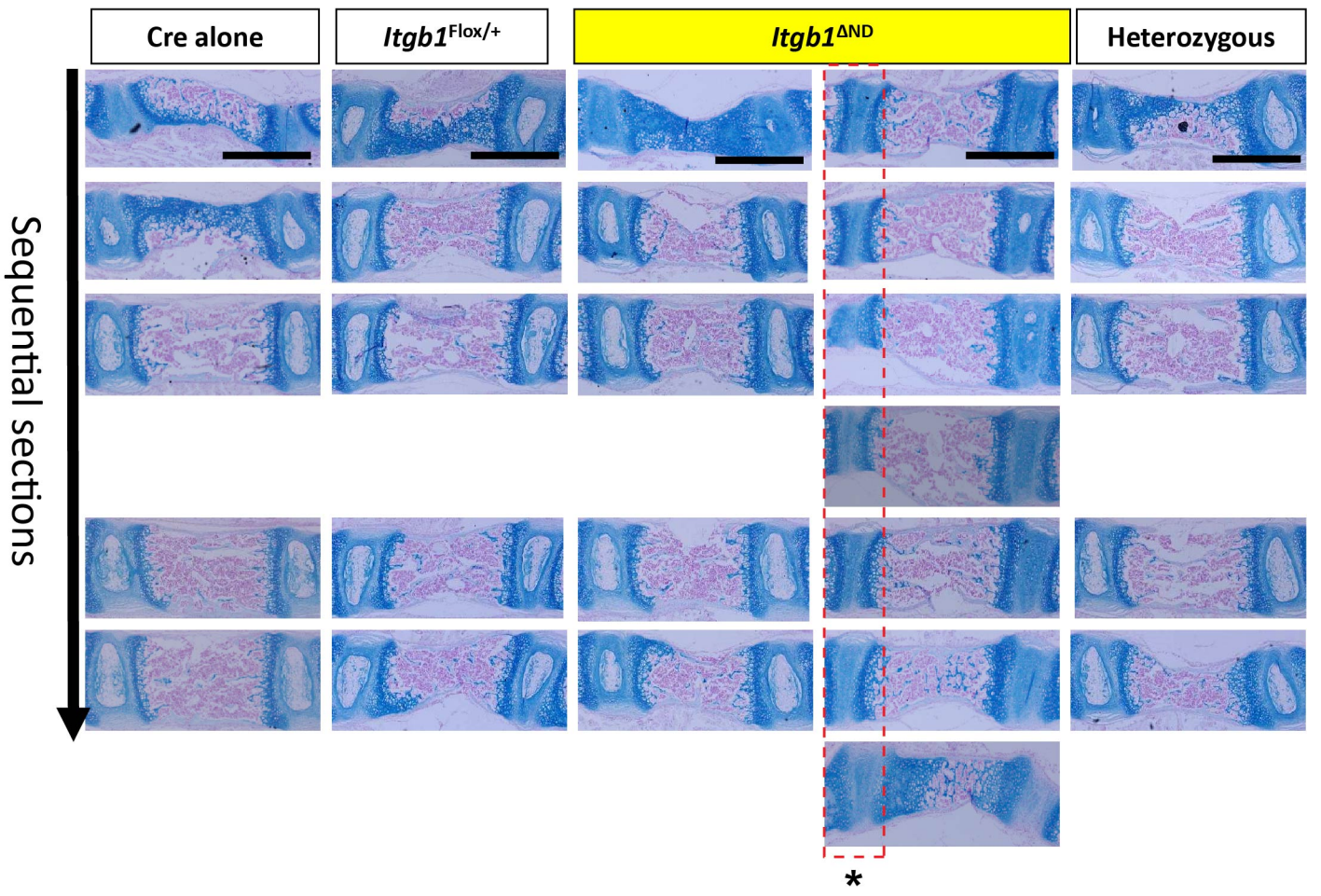


Figure S4: Histological analysis of the NP formation at postnatal stage (P10). Sequential sections were stained at every 5th section from the litter mates. *: Note that NP is not observed in all sections examined in the IVD highlighted in red. Scale bars: 200 µm.

Figure S5

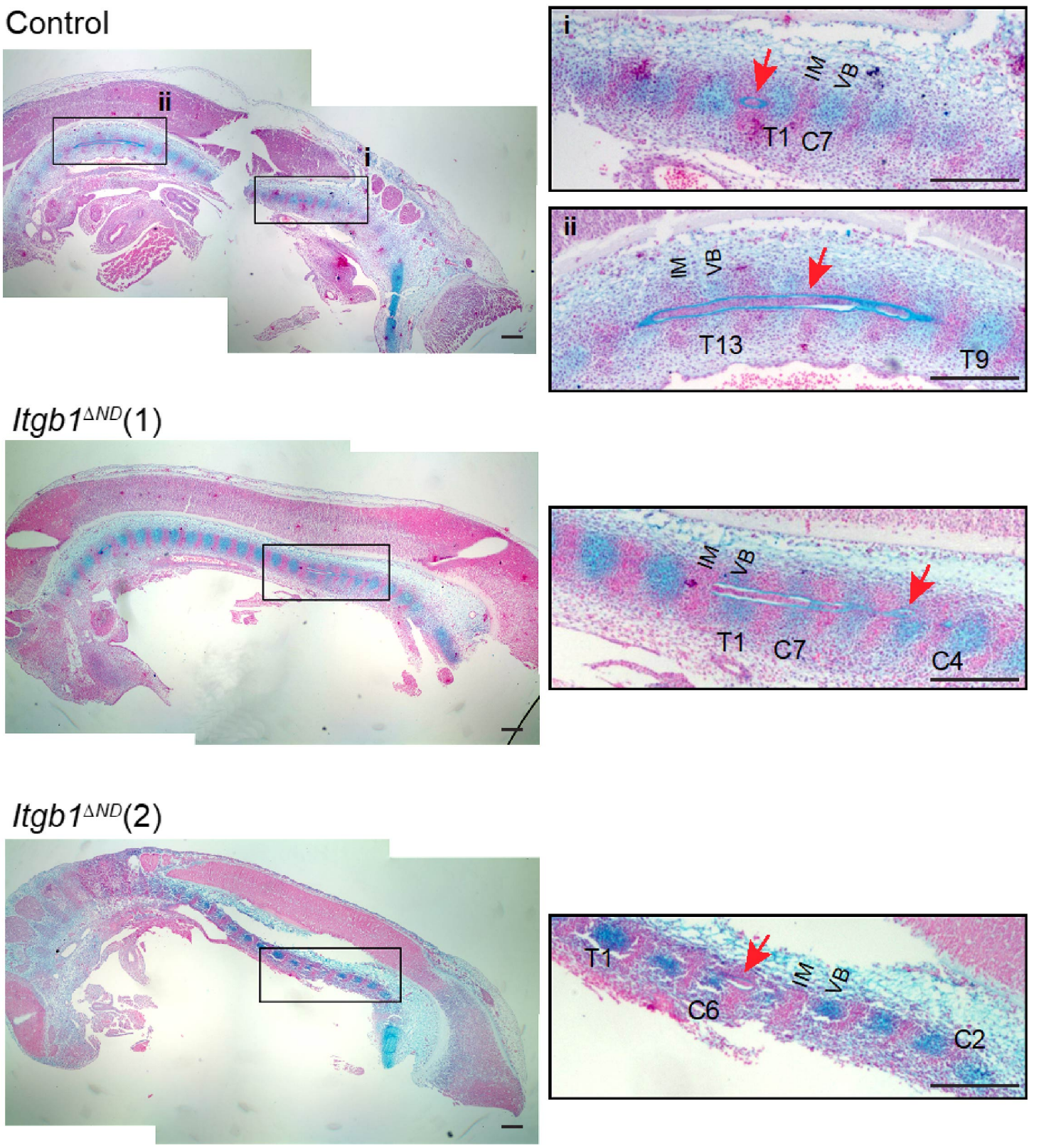


Figure S5: Histological analysis of the notochord remnants formation at E12.5. Note that notochord remnants (red arrows) are always centrally localized in control at different levels (i and ii) but are displaced from the midline in mutants. IM: intervertebral mesenchyme; VB: pre-vertebral region. Scale bars: 200 µm.

Figure S6

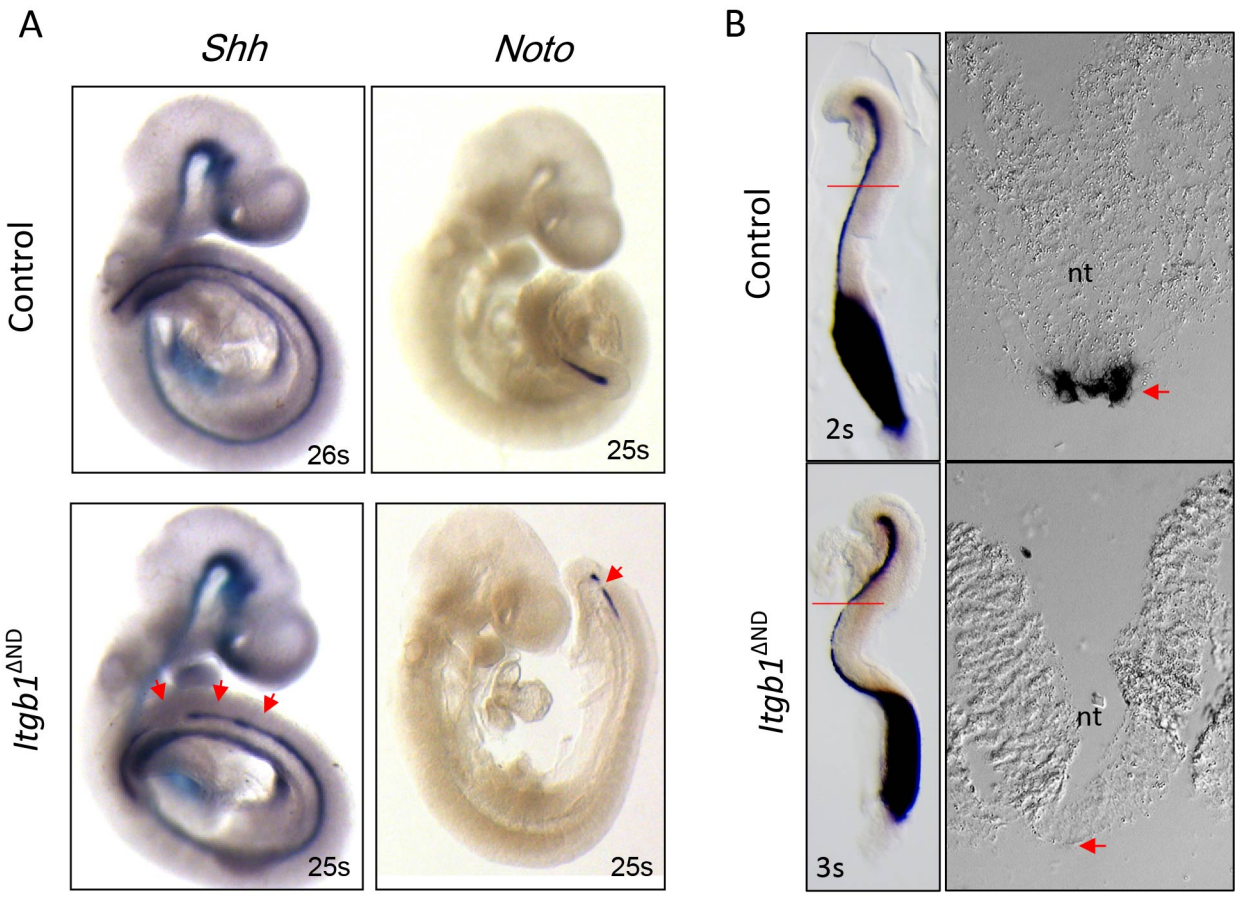


Figure S6: The notochord is interrupted in the *Itgb1*$^{\Delta ND}$ mutants. (A) *WISH* of *Shh* and *Noto* in the E9.5 embryos. Red arrows: interrupted gaps. (B) Detailed section analysis on the E8.5 embryos after *WISH* with *T* riboprobe. Red lines: section level. Red arrows: notochord. (C) Immunostaining against T (green) on the cross sections from embryos at E9.5. Nuclei are counter stained with DAPI. White arrows: notochord. (D) Immunostaining against SHH (green), NKX2.2, PAX6 or PAX7 (red) on the cross sections from embryos at E11.5. Arrows: notochord. Arrow heads: floor plate. nt: neural tube. da: dorsal aorta. Embryo stages are indicated with somite numbers. nt: neural tube. Scale bars: 50 μm.

Figure S7

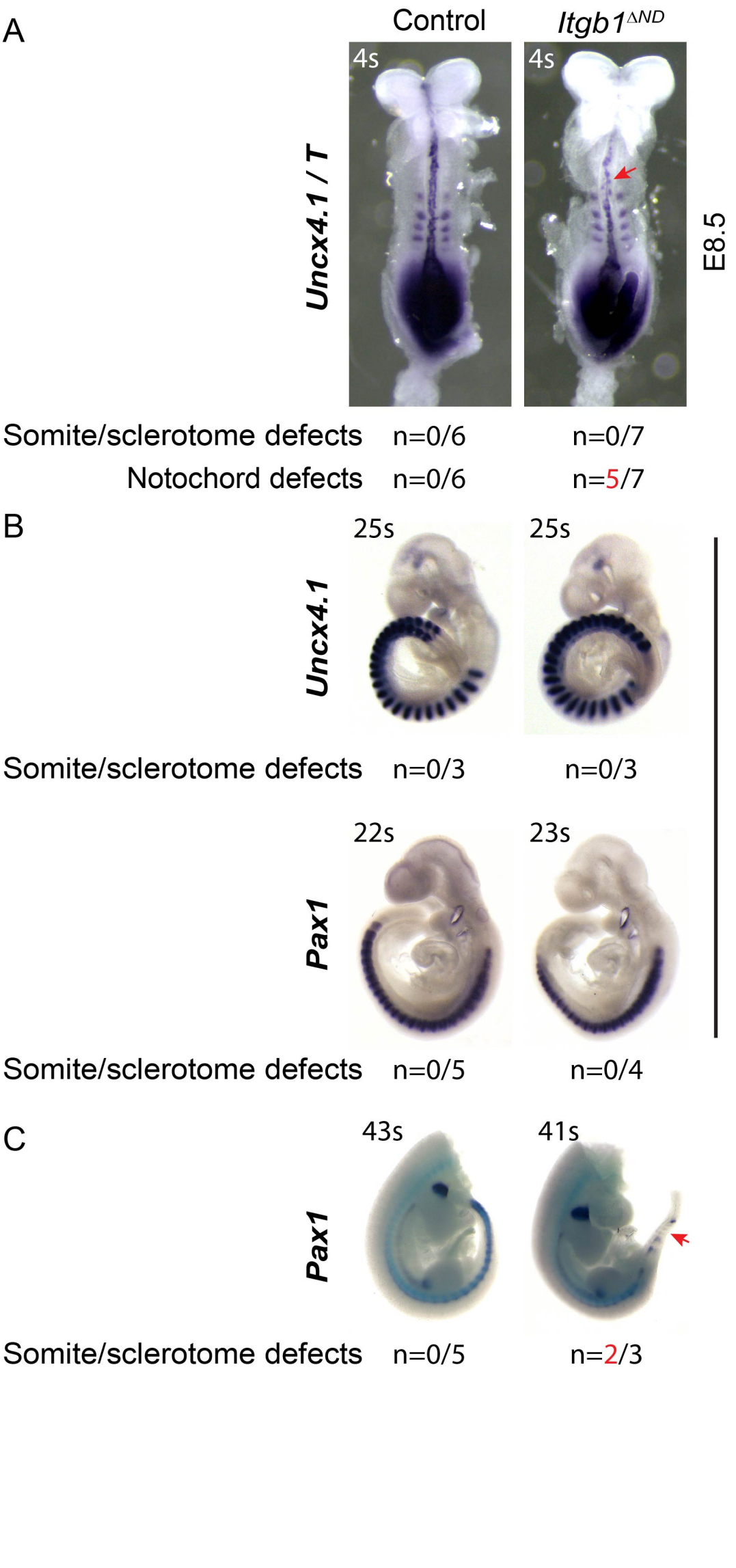


Figure S7: Sclerotome cell differentiation is defective in the *Itgb1*$^{\Delta ND}$ mutants from E10.5. (A-C) *WISH* of *Uncx4.1*, *T* and *Pax1* in the embryos at E8.5 (A), E9.5 (B) and E10.5 (C). n=number with defects/total number examined. Embryo age was indicated with somite numbers.

Figure S8

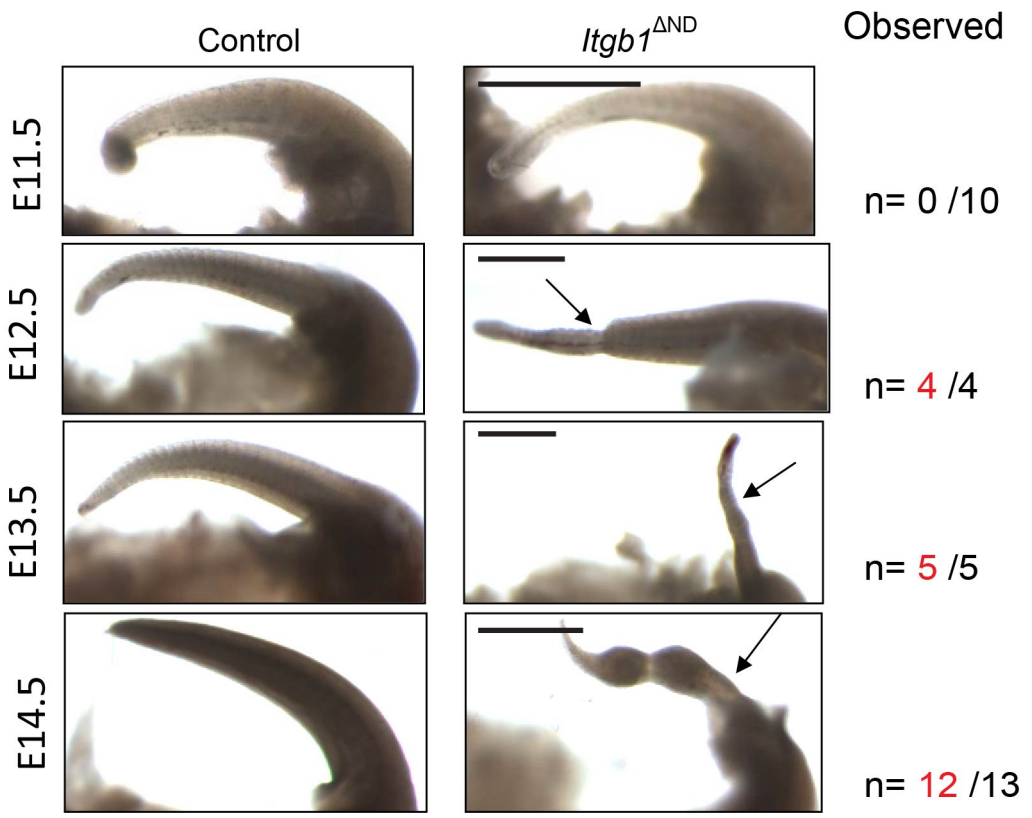
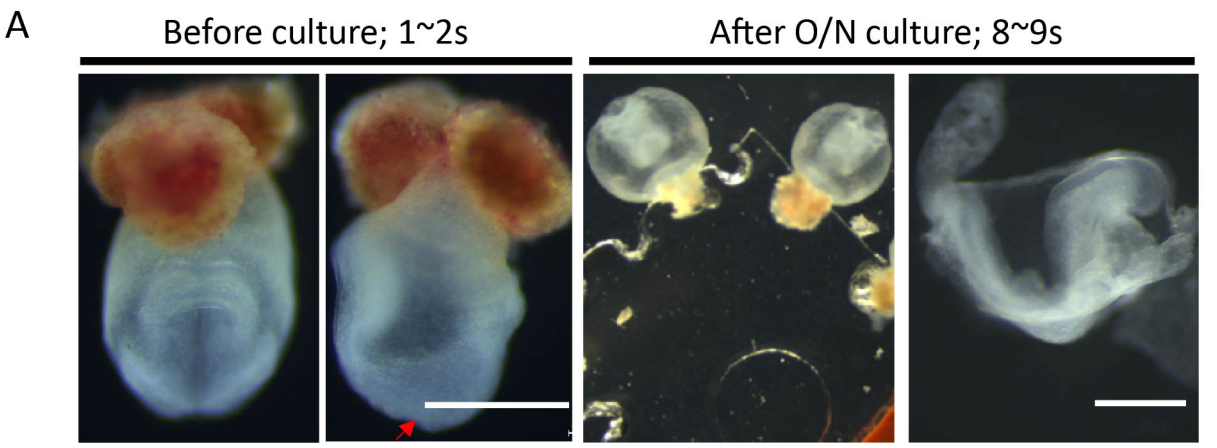
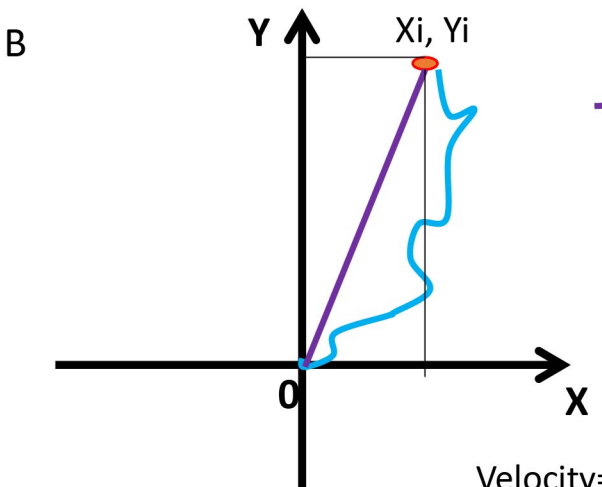


Figure S8: Tail morphology at different embryonic stages. n=number with defects/total number examined. Arrows: tail abnormalities. Scale bars: 1mm.

Figure S9





Euclidean (Euc) distance

Accumulated (Acc) distance

Cell end point

Velocity= changed accumulated distance / time;
Directionality = Euc / Acc distance;
Directionality ~1 → more persistent cell movement;
Directionality ~0 → less persistent cell movement.

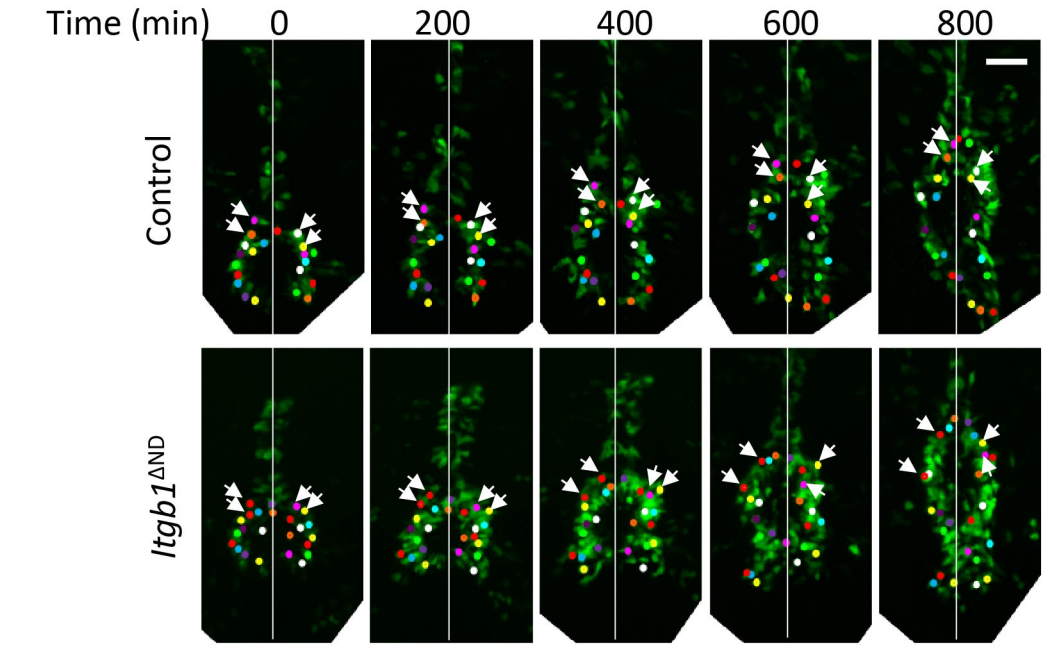


Figure S9: Embryo culture set up. (A) Embryos set up for time-lapse live imaging. Early head fold (EHF) stage (1~2s) embryo, shown before culture (frontal and lateral view). Embryos were then attached to the hand-made sticky edges and developed to 8~9s after ~17 hours culture and imaging. Red arrow indicates the position for O/N imaging. (B) Definitions of the parameters analyzed were illustrated. (C) Snapshot images of time-lapse movies. Cells tracked are indicated with color dotes. The cells highlighted with white arrows in the junction between the notochordal plate and the node were undergoing active convergent extension to the midline axis in the control embryo. This movement was less efficient in the *Itgb1*$^{\Delta ND}$ mutant embryo. A representative repeat was shown from 3 independent repeats (n=3 for each genotype). Scale bars: 500 μm for (A), 100 μm for (B).

Figure S10

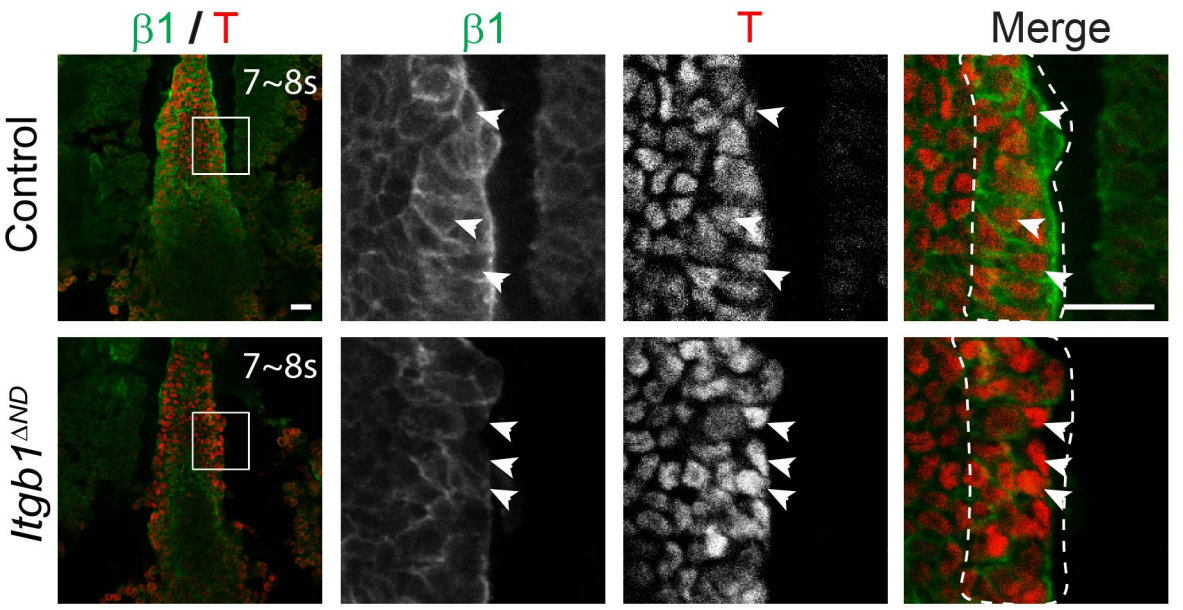
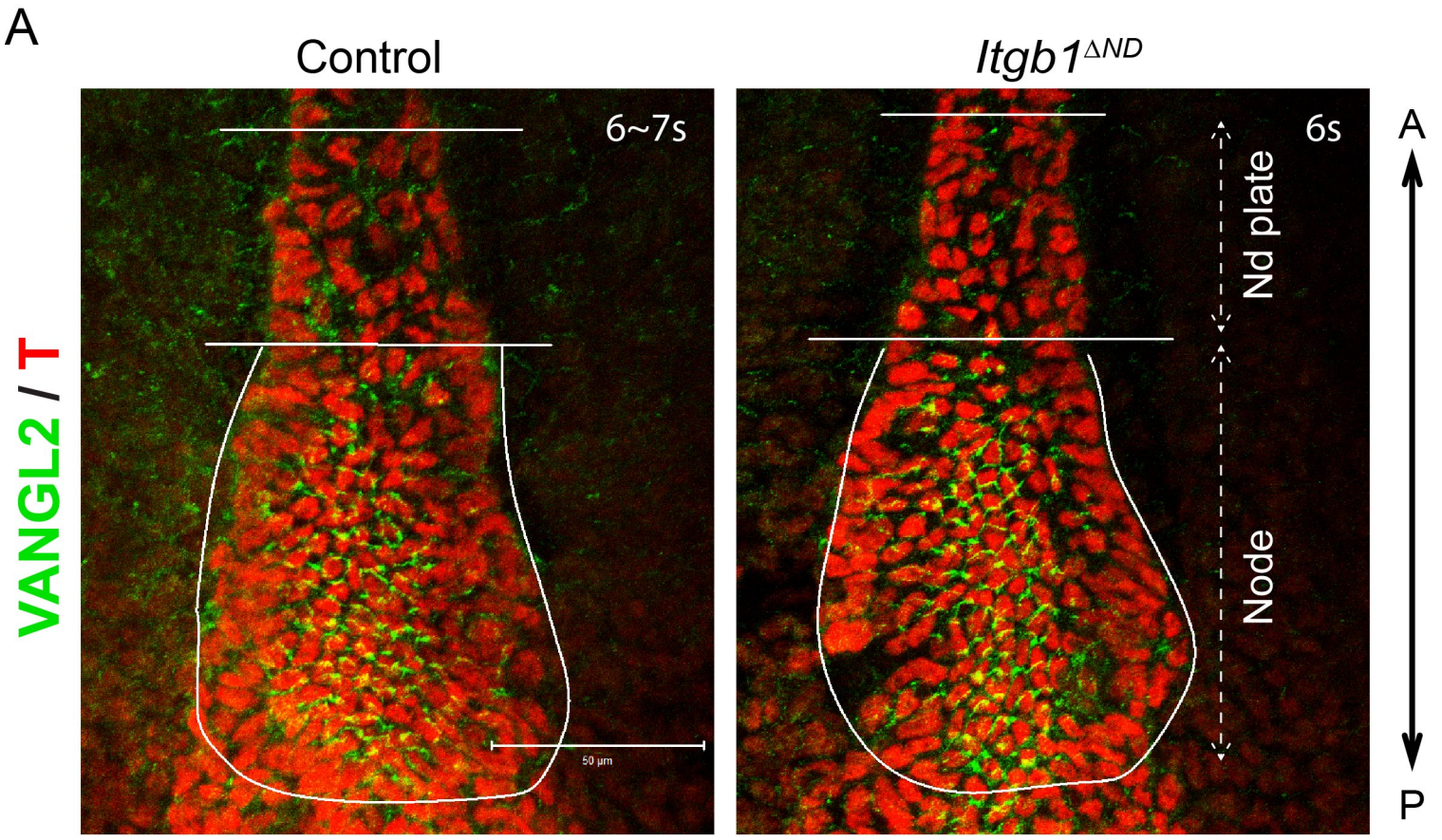


Figure S10: Reduction of β1 integrin expression in the mutant crown cells. Immunostaining against T (red) and β1 integrin (green) on the node region. Crown cells at the peripheral of the node were shown in higher magnification. Scale bars: 20 μm.

Figure S11



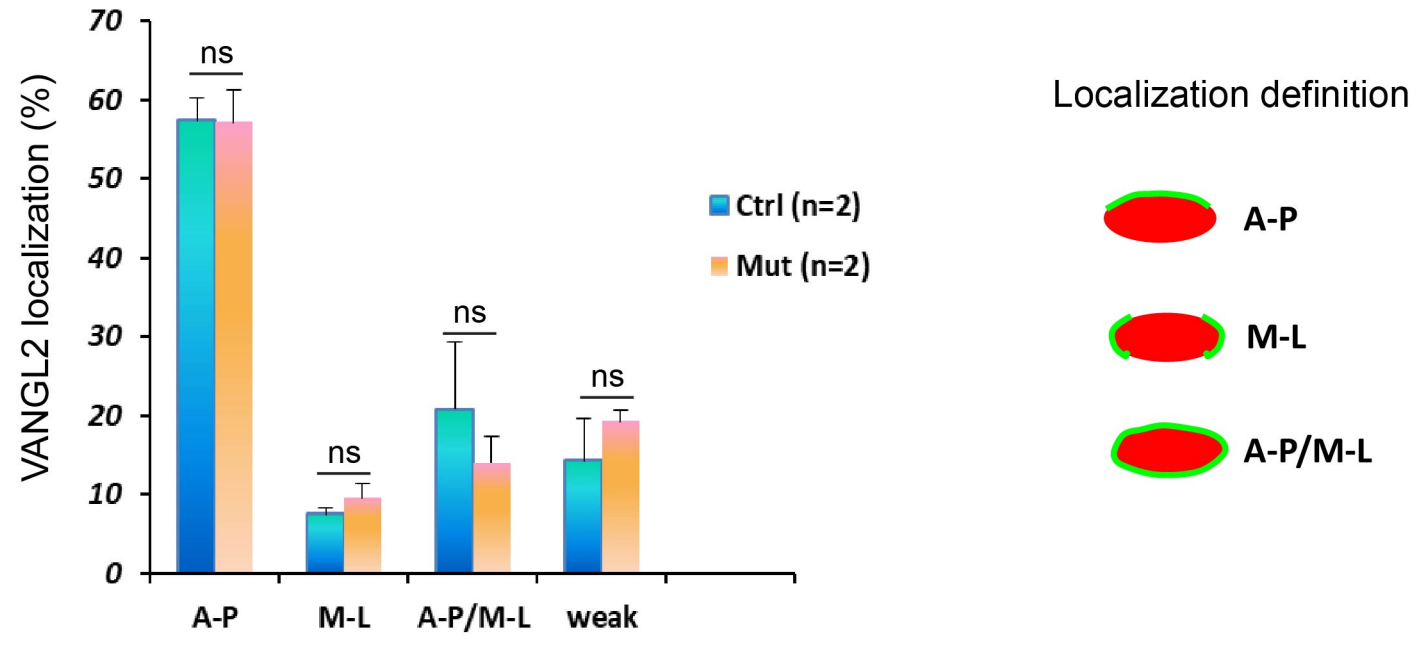
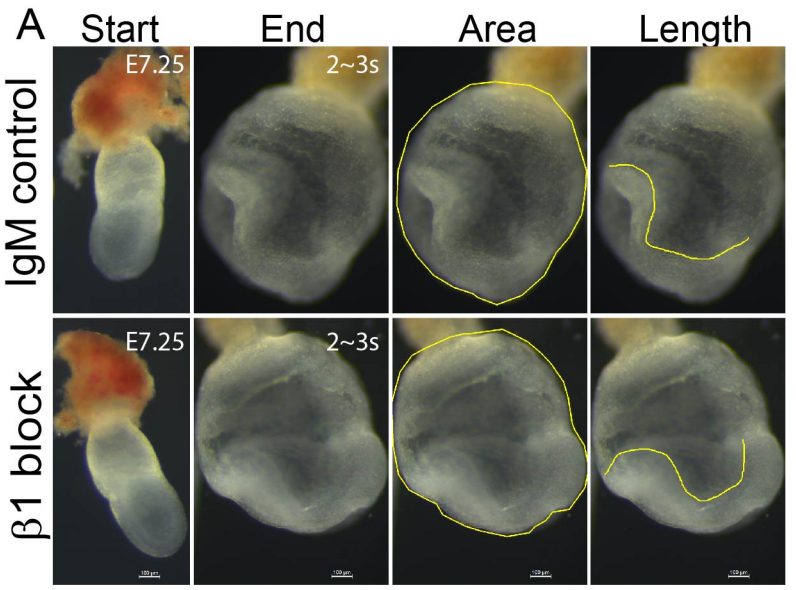
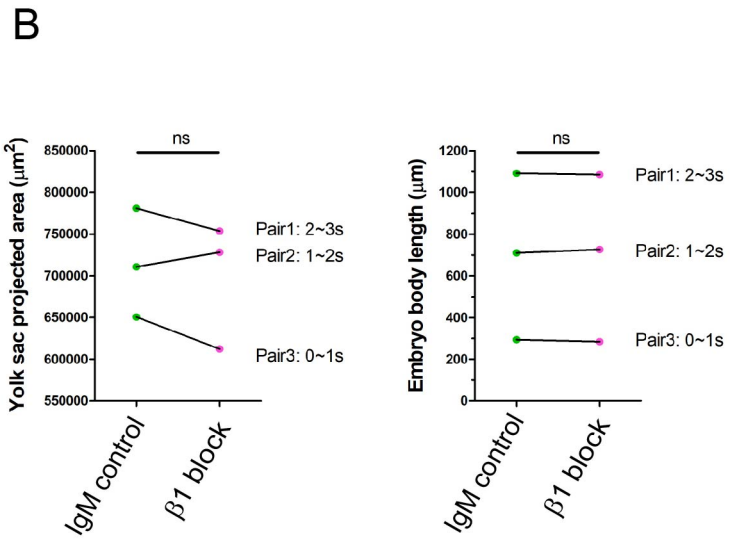
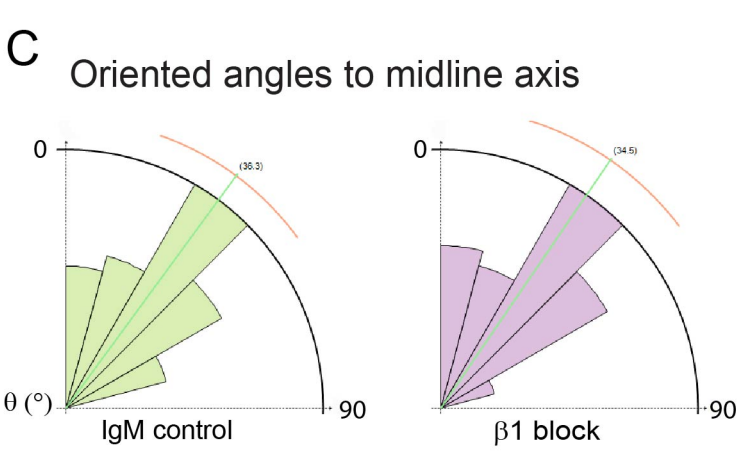


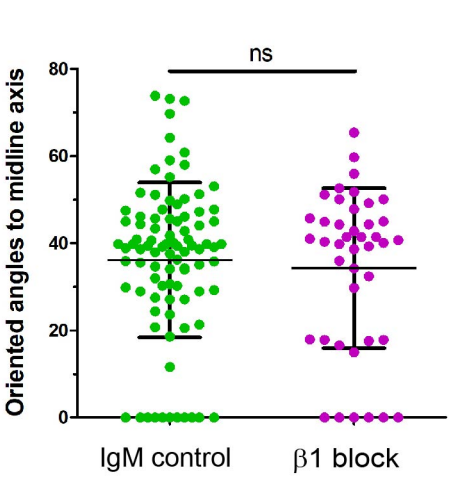
Figure S11: VANGL2 asymmetric localization analysis. (A) Whole embryo staining with T (red) and VANGL2 (green). VANGL2 localization within the node was analyzed and quantified. A-P: anterior-posterior axis. (B) Statistical graphs showing the percentage of the localizations are the data of mean±S.D from 2 pairs of control and mutants (two-tailed student t-test). A-P: anterior-posterior localized localized; M-L: medial-lateral localized; A-P/M-L: both directions; weak: weak signals. Scale bar: 50 μm.

Figure S12









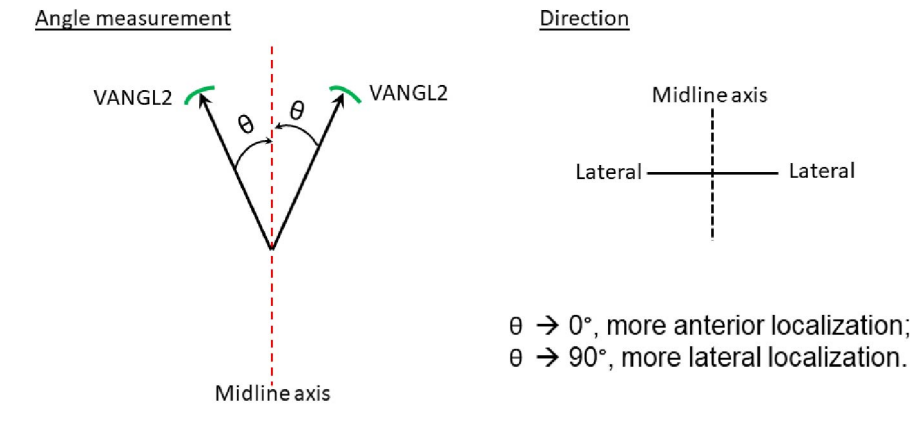


Figure S12: Embryo culture with blocking antibodies. (A) Embryo morphology before and after ~24 hours rotating culture in the presence of either IgM control or β1 specific functional blocking antibody. Age at the starting point and at the end point were indicated. The corresponding region and the embryo length applied for quantification are highlighted with yellow lines. Image shown is one representative from 3 independent repeats. (B) No difference of the projected yolk sac area and the embryo length at the end of culture. Statistical graphs are plotted in a paired manner. ns: no significance (paired student t-test). (C) VANGL2 orientation with respect to midline axis were measured and plotted in ROSE diagram. Quantification for all 3 pairs shown in (D). ns: no significance (two-tailed student t-test). (E) The orientations of the asymmetric VANGL2 signals to the embryo midline axis were measured and expressed with the angles from 0 to 90 degree. Scale bars: 100 μm.

Figure S13

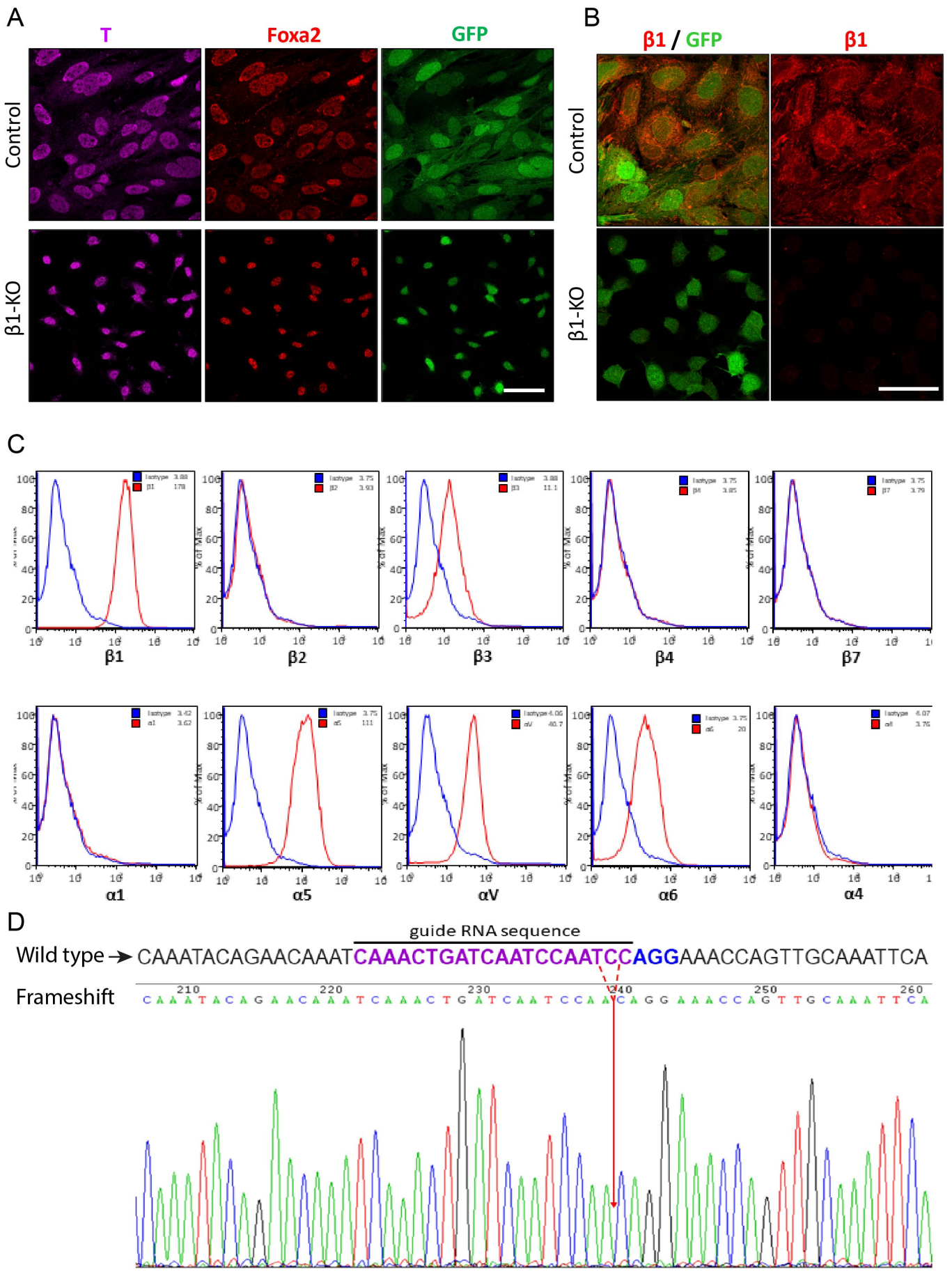


Figure S13: Generation and characterisation of the β1-KO notochordal cells. (A) The sorted notochordal cells immortalized by SV40 were stained with Foxa2 (red) and T (green). (B) *β1* integrin gene knockout was verified by immunofluorescence with β1 integrin antibody (red). GFP signals marking the notochordal cell lineages. (C) Integrin expression profile analysis in the immortalized notochordal cells. Isotype control shown in blue and integrin levels shown in red. (D) *β1* integrin gene knockout was verified by sequencing the frameshift. Red arrow indicates the 2 nucleotides (TC) deletion in the knockout cells. Guide RNA sequence and PAM sequence shown in magenta and blue, respectively. Scale bars: 50 μm.

Table S1

Random distribution of absent NP in mutant intervertebral discs

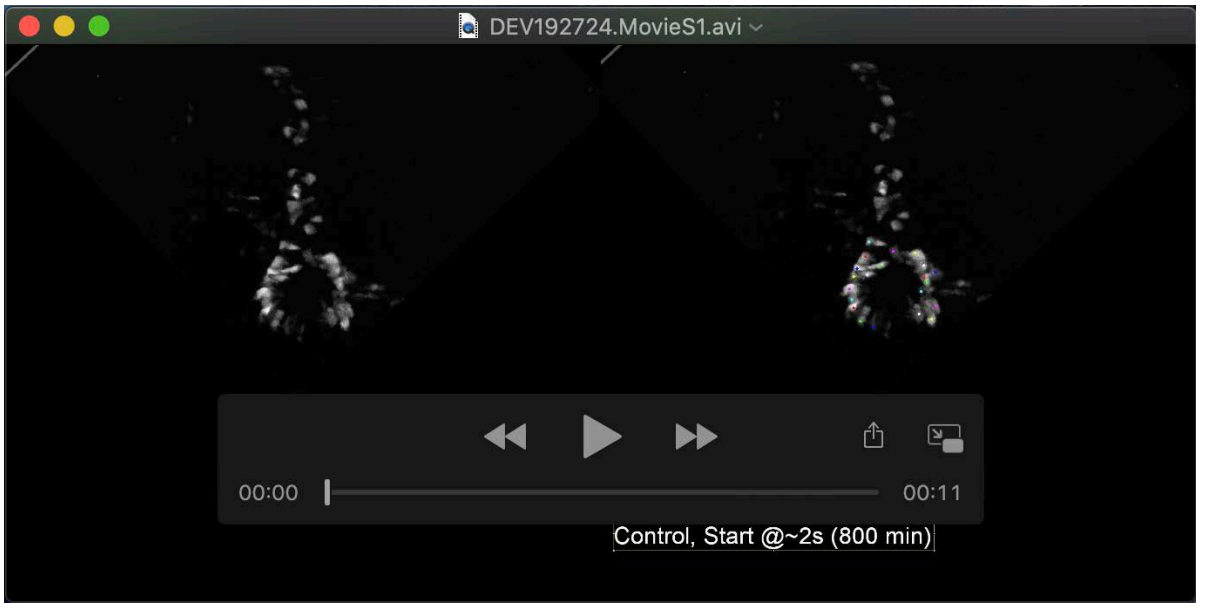
Genotype												
Control	S-N.	S-30	S-35	S-40	S-45	S-50						**Summary**
	T1	Y	0	0	0	0						Y
	T2	Y	Y	Y	Y	0						Y
	T3	Y	Y	Y	Y	0						Y
	T4	Y	Y	Y	Y	Y						Y
	T5	0	Y	Y	Y	Y						Y
	T6	0	0	Y	Y	Y						Y
	T7	0	0	Y	Y	Y						Y
	T8	0	0	Y	Y	Y						Y
	T9	Y	Y	Y	Y	Y						Y
	T10	Y	Y	Y	Y	Y						Y
	T11	Y	Y	Y	Y	Y						Y
	T12	Y	Y	Y	Y	Y						Y
	T13	N/A	N/A	N/A	N/A	N/A						N/A
Mutant-1	S-N.	S-5	S-10	S-15	S-20	S-25	S-30	S-35	S-40	S-45	S-50	S-55	**Summary**
	T1	0	0	0	Y	0	0	0	0	0	0	0	Y
	T2	0	0	0	0	Y	Y	Y	0	0	0	0	Y
	T3	0	0	0	0	0	0	Y	0	0	0	0	Y
	T4	0	0	0	0	0	0	0	0	0	0	0	0
	T5	0	0	0	0	0	0	0	0	0	0	0	0
	T6	0	0	0	0	0	0	0	0	0	0	0	0
	T7	0	0	0	0	0	0	0	0	0	0	0	0
	T8	0	0	0	0	0	0	0	0	0	Y	0	Y
	T9	0	0	0	0	0	0	0	0	0	0	0	0
	T10	0	0	0	0	0	0	0	0	0	0	0	0
	T11	0	0	0	0	0	0	0	0	0	0	0	0
	T12	0	0	0	0	0	0	0	0	0	0	0	0
	T13	0	0	0	0	0	0	0	0	0	0	0	0
Mutant-2	S-N.	S-5	S-10	S-15	S-20	S-25	S-30	S-35	S-40	S-45	S-50	S-55	**Summary**
	T1	0	0	0	0	0	0	0	0	0	0	0	0
	T2	0	0	0	0	0	0	0	0	0	0	0	0
	T3	0	0	0	0	Y	0	Y	0	0	0	0	Y
	T4	0	0	0	0	Y	0	Y	0	0	0	0	Y
	T5	0	0	0	0	0	Y	Y	Y	Y	Y	0	Y
	T6	0	0	0	0	0	0	0	0	0	0	0	0
	T7	0	0	0	0	0	0	0	0	0	0	0	0
	T8	0	0	0	Y	Y	Y	Y	Y	0	0	0	Y
	T9	0	0	0	0	0	0	0	0	0	0	0	0
	T10	0	0	0	0	0	0	0	0	0	0	0	0
	T11	0	0	0	0	0	0	0	0	0	0	0	0
	T12	0	0	Y	Y	Y	0	0	0	0	0	0	Y
	T13	0	0	0	0	0	0	0	0	0	0	0	0

S-N.: section ID number; T1-T13: the corresponding thoracic levels;
0: NP not found; Y: NP found
N/A: the level was not included in the sections.

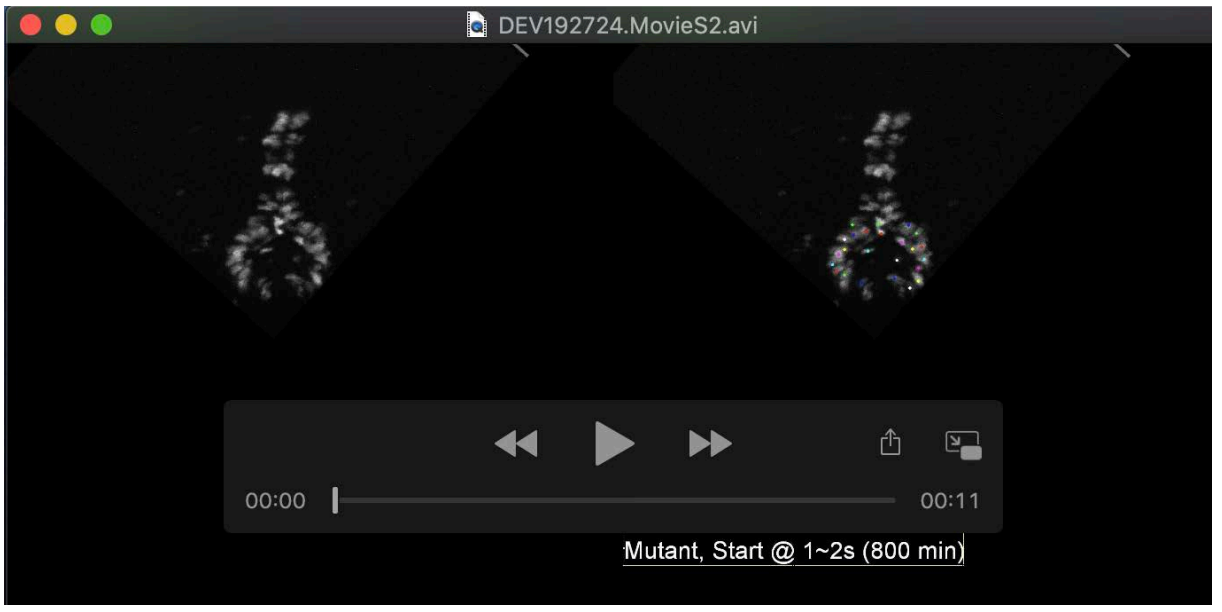
Table S2

Phenotype penetrance at different embryonic stages

Mutant Stage	E8.0-E8.5	E8.5	E8.75-E9.5		E10.5
Method	WISH	DoubleWISH	WISH	WIHC	WISH
Analyzed	12	7	5	8	5
Interrupted	8	5	4	4	5
Overall penetrance of interrupted notochord: 26/37= ~70% (wt: 1/49=2%)					
Mutant (Section IFC)	Stage	~E9.0 14-17s	E9.25 18-21s	E9.5 22-25s	
	Analyzed	5	5	6	
	D-V defects	1	4	0	
Penetrance of notochord D-V displacement from midline: 5/16=~31% (wt: 1/21=4.7%)					
Mutant (Section IFC)	Stage	~E9.0 14-17s	E9.25 18-21s	E9.5 22-25s	E8.75-E9.5 WIHC
	Analyzed	5	5	6	8
	L-R defects	2	0	2	2
Penetrance of notochord L-R displacement from midline: 6/24=~25% (wt: 1/28=3.6%)					



Movie 1: Representative time-lapse movie of the node and notochordal cell movement in control embryo (*Foxa2mNE-Cre* X zEG). Left: original movie; Right: overlaid with cell trajectory. Frames were captured every 10 minutes. Repeats n =3.



Movie 2: Representative time-lapse movie of the node and notochordal cell movement in mutant embryo (*Itgb1*$^{\Delta ND}$ X zEG). Left: original movie; Right: overlaid with cell trajectory. Frames were captured every 10 minutes. Repeats n =3.