## Supplemental Figures

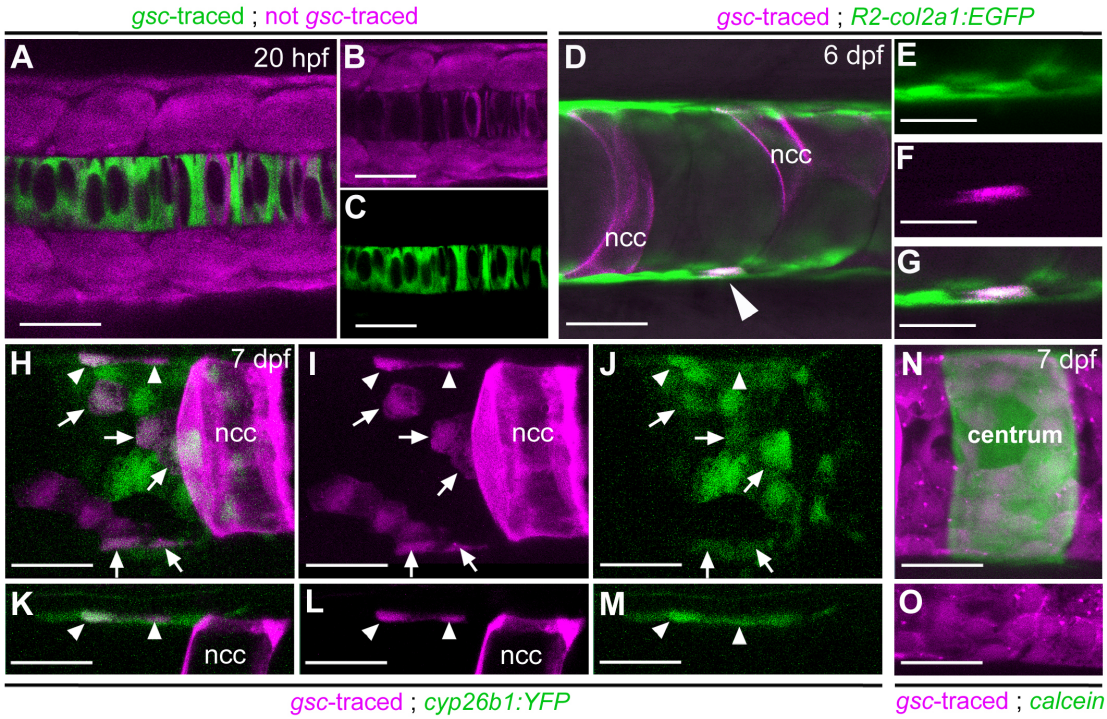


**Figure S1: Lineage tracing reveals that centra-associated *col2a1*- and *cyp26b1*-positive chordoblasts derive from the early notochord anlage.** (A-C) Characterization of the *gsc* driver used for the axial mesoderm lineage tracing experiments. Dorsal view of a *Tg(gsc:Cre); Tg(hsp70:loxp-dsRed-loxp-egfp)* double transgenic embryo at 15-somite stage (20 hours post fertilization; hpf), 2 hours post heatshock; confocal projection with merged red and green channels. (B) and (C) show the corresponding images of the single channels, respectively. Most notochordal cells express EGFP, whereas no EGFP-positive cells are present outside this structure, indicating the axial mesoderm specificity of the used *gsc* driver. In particular, sclerotomal cells of the somites are not labelled by the *gsc* driver. At the shown segmentation stage, notochordal cells have not segregated into outer chordoblasts and inner vacuolated cells as yet. Please note that compared to the endogenous *gsc* gene, the used *gsc* driver appears to lack negative cis-regulatory elements that usually repress expression within the notochord anlage, restricting expression to the prechordal plate (Doitsidou et al., 2002).
(D) Lateral view of the notochord of a 6 dpf *Tg(R2-col2a1a:EGFP)*; *Tg(bact2:loxP-AmCyanloxP-mCherry)* double transgenic larva injected with a

*gsc:Cre* construct; confocal projection of merged green, red and transmitted light channels. Mosaic recombination events within the axial mesoderm result in mCherry labeling of some but not all cells deriving from the notochord anlage. The arrowhead points at an mCherry-expressing chordoblast identified as such by co-expression of the *R2-col2a1a:EGFP* transgene. (E-G) Magnification of the domain harboring the marked chordoblast from (D) in single and merged channels as indicated. In total, 28 chordoblasts positive for the *gsc* lineage tracer were observed in eight specimens. (H-M) Lateral view of the notochord of a 8 dpf *Tg(cyp26b1:YFP)*; *Tg(bact2:loxPAmCyan-loxP-mCherry)* double transgenic larva injected with the same *gsc:cre* construct; confocal projection of merged green and red (here magenta) channels (H,K) or the corresponding single channels (I,J,L,M); for better visualization, panels (K-M) show single confocal sections of the upper third from panels (H-J) with focus on marginal chordoblasts of the dorsal notochord border. Mosaic recombination within the axial mesoderm results in mCherry labeling of some chordoblasts as well as vacuolated notochordal cells (ncc). Some of the lineage-traced chordoblasts also coexpress the *cyp26b1:YFP* transgene (arrows and arrowheads), confirming their association with the centra inducing chordoblast subpopulation (seen in 11 centra of 4 specimens). mCherry-positive but YFP-negative chordoblasts most likely lie outside the RA target domain of the intercentral spaces. (N,O) Lateral view of a notochord at the level of a calcein-stained centrum (green) in a *Tg(gsc:Cre)*; *Tg(bact2:loxP-AmCyan-loxP mCherry)* double transgenic larva at 8dpf, confocal projection of merged red (here magenta) and green channels. Panel (O) shows the lower third of (N) in the single red (magenta) channel visualizing the continuous layer of notochord anlagen derived, mCherry-positive chordoblasts. Some of these cells are positioned directly underneath the mineralized matrix of the chordacentrum. Scale bars correspond to 20µm in (D-O) and to 50µm in (A-C).

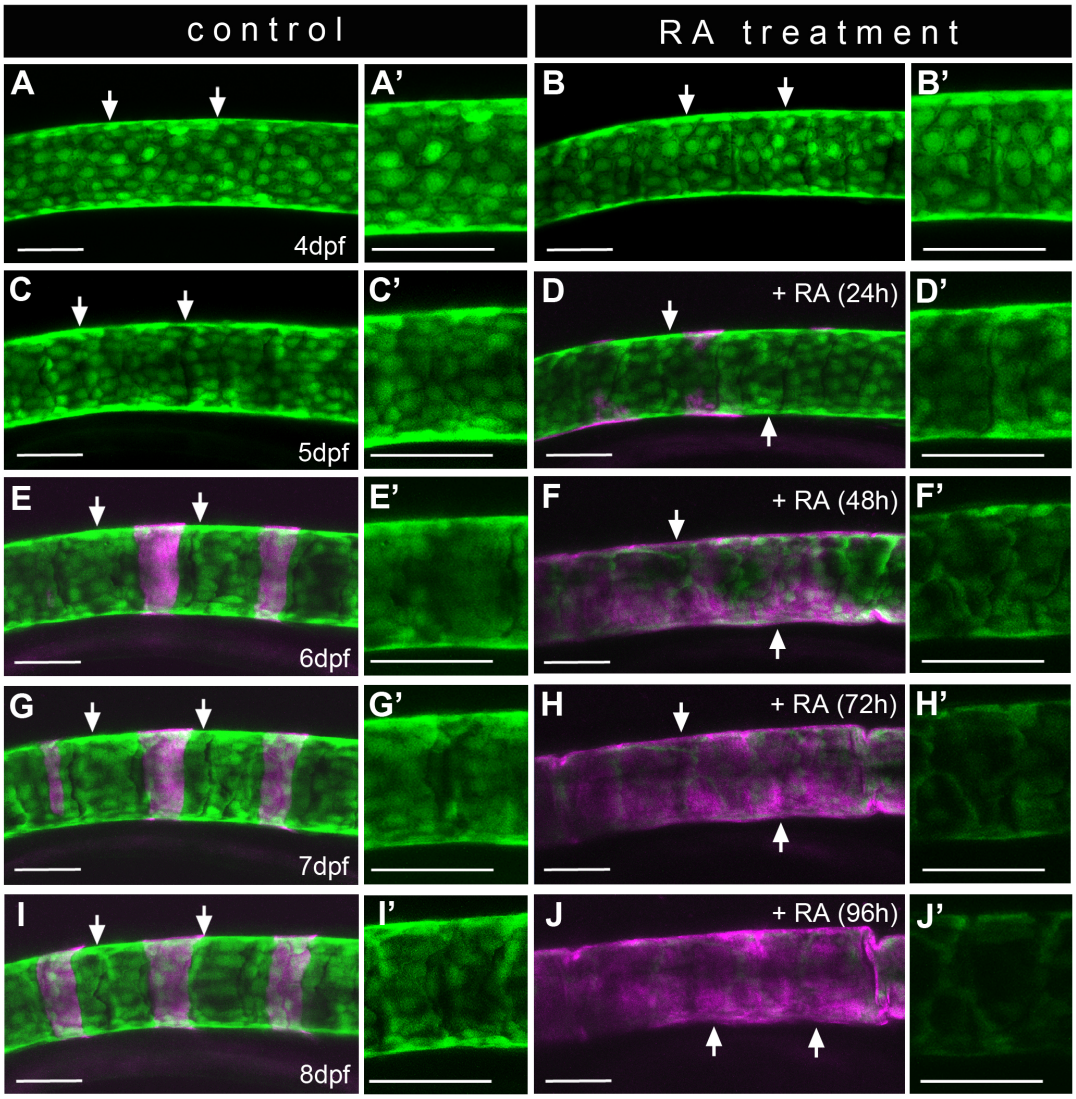


**Figure S2: Down-regulation of *Tg(R2-col2a1a:EGFP)* expression and alterations in chordoblast morphology upon RA treatment are progressive processes**

(A-J) Lateral views of notochords at the level of centra #2 to #4 in a representative control- (A,C,E,G,I; n=8) or RA-treated (B,D,F,H,J; n=12) *Tg(R2-col2a1a:EGFP)* larva in a time series over the indicated ages, anterior to the left; calcified extracellular matrix is labeled via Alizarin red (magenta). Time of RA incubation in the treated specimen (right column) is shown in brackets. Panels (A-J) show projections of confocal stacks with merged green and magenta channels. Panels (A'-J') show single green channel projections of the corresponding area framed by the two arrows in (A-J). Scale bars represent 50µm. Note that the decrease in transgene expression is

accompanied by a gradual change in chordoblast morphology, and that both processes occur in a progressive manner, with maximal effects only seen after three days of RA treatment.

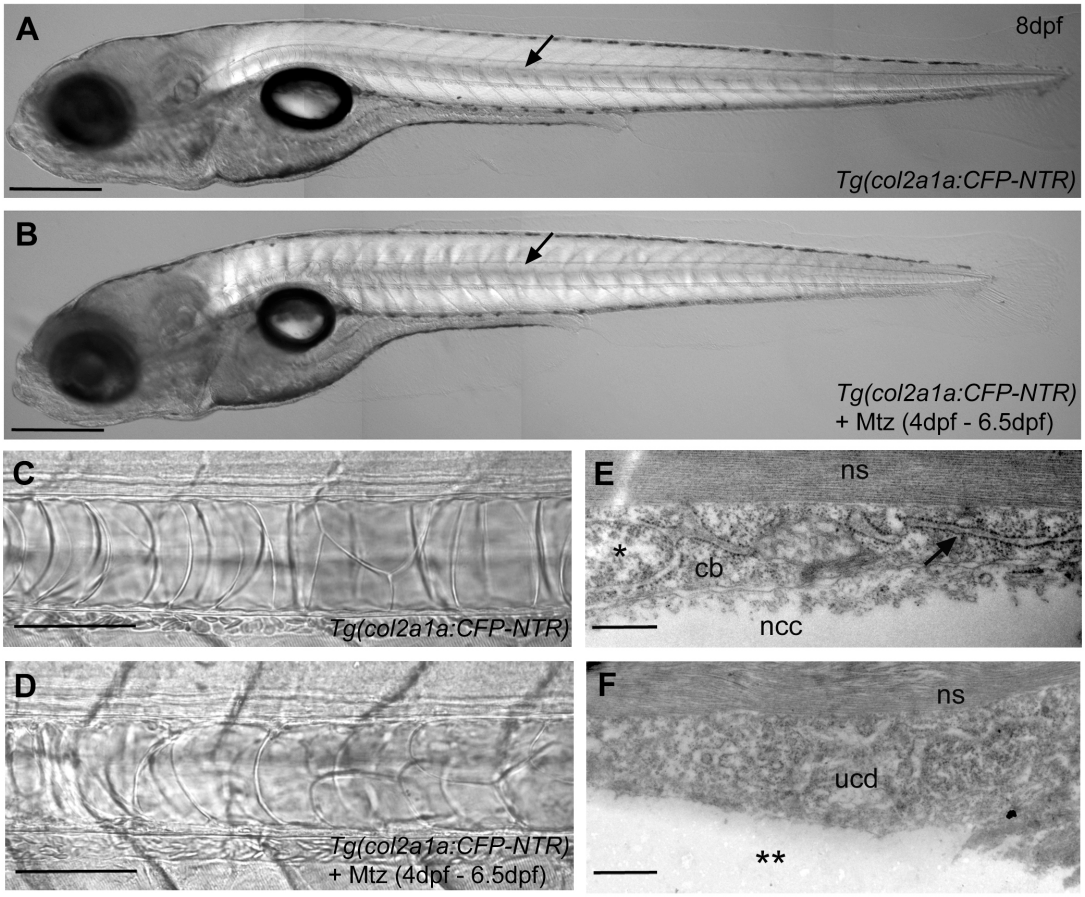


**Figure S3: Ablation of chordoblasts does not impair overall notochord integrity.**

(A,B) Lateral view of an untreated (A; n=42) and a Mtz treated *Tg(col2a1a:CFP-NTR)* transgenic larva (B; n=63) at 8dpf, anterior is to the left. Even though the chordoblast ablated animal is a bit shorter than the untreated control, it has an obvious notochord (arrow) and an infladed swim bladder, indicating that it does not suffer from retarded development. Panels (C,D) depict magnifications of a stretch of notochord of the same specimens as in (A,B). As in the untreated larva (C), vacuolated notochordal cells persist after chordoblast ablation upon Mtz incubation from 4dpf to 6.5dpf (D). (E,F) TEM micrographs of transverse sections at the level of chordacentra through the notochordal sheath and adjacent chordoblast layer in an untreated control *Tg(col2a1a:CFP-NTR*) (D; n=5) and an Mtz-treated specimen of the same genotype (F; n=6) at 8dpf. The shown chordoblast (cb) of the untreated control (E) contains an intact nucleus (*) and clearly recognizable rER (arrow), and is directly attached to a proximally positioned notochordal cell (ncc). In

the chordoblast-ablated larva (F), no distinct chordoblasts can be found; rather material without any obvious cellular structures designated as “undefined cellular debris” (ucd) can be detected at cognate positions. Underscoring the acellular nature of this material, no directly attached notochordal cells can be observed (**). It is most likely this ucd that gives rise to the remaining rather weak uniform fluorescence seen around the notochord of chordoblast-ablated larvae (compare with Fig.4C,D). Scale bars correspond to 500µm in (A,B), 100µm in (C,D) and 0.5µm in (E,F). Other abbreviations: ns, notochord sheath.

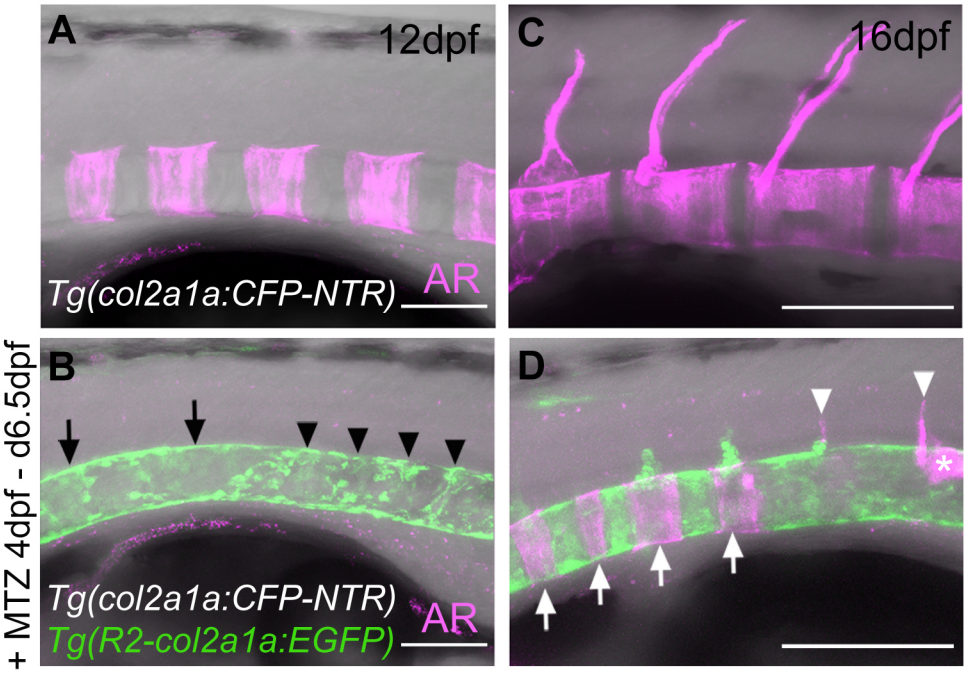


**Figure S4: Chordoblast-ablated zebrafish larvae show partial and severely delayed recovery of centra formation.**

Lateral view of a representative untreated *Tg(col2a1a:CFP-NTR)* larva (A,C; n=32) and an Mtz-treated *Tg(col2a1a:CFP-NTR)*; *Tg(R2-col2a1a:EGFP)* double transgenic larva (B,D; n=18) at 12 dpf (A,B) and 16 dpf (C,D; same individuals as at 12 dpf), anterior is to the left. Mineralized matrix is stained via Alizarin red (magenta). All panels represent confocal projections of merged red (here shown in magenta), green and transmitted light channels. Compared to control larvae (A) that show robust iteratively formed centra at 12 dpf none of such structures can be found in siblings of the same age in which chordoblasts have been ablated in a time window between 4 and 6.5 dpf (C).

The *col2a1a:EGFP* transgene in the treated larvae indicates that a few chordoblasts have already recovered in some domains of the notochord (arrows) while other regions are still devoid of distinct chordoblasts (arrowheads). (B) Four days later (16 dpf), the untreated zebrafish larva has massively gained in growth and thus size of the centra, which in the meantime have formed neural arches in most rostral segments. (D) By now, also a rather regular sequence of centra has been formed in the Mtz treated specimen at those positions along the notochord where proper chordoblasts

did recover earlier (white arrows). Additionally, mineralized neural arch anlagen (white arrowheads) and some associated mineralized matrix (white asterisks) can be found in adjacent caudal segments where the integrity of the chordoblast layer is still not completely given as indicated by the less intense and irregular chordoblast derived EGFP signal. Further histological analyses would have to reveal whether those mineralized matrices are of chorda- or autocentra nature. Despite this partial axial recovery, treated larvae rarely survive beyond 20dpf, possibly due to malformations of the mandibular apparatus that also expressed the *col2a1a:CFP-NTR* transgene during Mtz treatment. Scale bars correspond to 100µm in (A,B) and 200µm in (C,D).

## Supplementary Materials and Methods

### Partial *entpd5a* cDNA sequence used for probe synthesis

5’GTGGAAGTCAGGGACTTCAAAAAGAAAGCCAAAGAAGTGTGCAATAAGATGACTAAAT
ACCGCCCGATCAGCCCCTACTTATGCATGGACATGACGTACATCACATGCCTACTGAAA
GACGGATTTGGCTTCAAGGACAGCACTGTTTTGCAGCTTGCTAAAAAGGTGAATAATGT
GGAGACAAGCTGGGCTCTCGGCGCCATATTTGATCATTTCCACAATTTCAGCATTCAGT
AAAGCTTGCGGTCTCATGGAAACCAGTATGAGGTGGAACACAGGGGAGCAGGAGGAA
AATCTCAAGTTATCAAGCAACCCTTCCTATTCCTCCTTCCCGTAACTCTATAGTGAGTTG
ACAAACCTGCTCTGCACTGCAGTCCTGTATCACAGCGCTCTCCTCTTTCACCAACTAAA
CACCCAAAGAACTGTTCGCGAAGAAATCACTATATTTTTGTAAAGCCAGTCTGCTATTTC
TACTGTATGTTTACATTTTTTGTCTGTCGCCTCACCTGAGACACTGTCCAAGTTGGCAAG
TTCTAATCTAAAATTCATCAATTTGAAGTAATTTATGCAGTTGTGCAGGATATTGTGCAGT
GGGATACGTGTAAAAACATTGCGTTAAGTAGTGTAGCCTCCATACCCTTTCTGCAGTAA
TGGGATAGTAGTAGTGCTTGCACAATAGTGTAGTGCTGTTTTAGGTTGTGATAAATTAAT
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GAATCAACCTTGGCTACTTGGCTACTTTGGCTAAATGGCTACTTTACATTGCCACATGAT
TGTGGATTTATGTATTATTAAATAATGCATGTATTTATGCAATAAATGCTAGGTTTGCATA
GACTCAAGGTGGAACATCTTTTAGATGAAGTTCATTAACTAGGTGCTTATTTTTTAAACC
GAGGTATAACCAGGTTTTTAACACTGAATGGCATGCAGTTGAATTAATTTTTACAGCATC
CATTATCAAATTAAATAGGTGCAAATGCTTACGTGTTATATCCTATAAATACTTTGATAAA
TATAACTACTGGTCAGATTATTCGTAGACATTAGGCATTCCATGTTACTGTAGATTTTTTT
AGGTCAACTTGCTAAGAGTGCCTTCAACCTCCTTTATGTCAAGTTGGGTTGGTTATTGT
GGA3’

## Table S1. Cloning primers

| | |
|---|---|
| R2 fragment-attb-F | **GGGGACAACTTTGTATAGAAAAGTTG**CCTCTGACACCTGATGCCAATTGC |
| R2 fragment-R | 5Phos/CAGGGATATGTGTATGTGTGTGATCG |
| E1B fragment-forward | 5Phos/TCTAGAGGGTATATAATGGATCC |
| E1B fragment-attb1-R | **GGGGACTGCTTTTTTGTACAAACTTG**TGTGGAGGAGCTCAAAGTGAGGC |
| CFP-ntr-attB1-F | **GGGGACAAGTTTGTACAAAAAAGCAGGCT**CCGCCACCATGGTGAGCAAGGGCGAGGAGCTGT |
| CFP-ntr-attB2-R | **GGGGACCACTTTGTACAAGAAAGCTGGGT**ATTACACTTCGGTTAAGGTGATGTT |
| EGFP- attB1-F1 | **GGGGACAAGTTTGTACAAAAAAGCAGGCT**TTCACCATGGTGAGCAAGGGCGAGGAGCTG |
| dnRARa- attB2-R | **GGGGACCACTTTGTACAAGAAAGCTGGGT**TTCACGGGATCTCCATCTTCAGCGT |
| EGFP-linker-R | GGTGAGGTCGCCCAAGCTCTCCTTGTACAGCTCGTCCAT |
| dnRARa-linker-F | GAGAGCTTGGGCGACCTCACCGCCAGCAACAGCAGCTCCT |
| cre-attb4-F | **GGGGACAACTTTGTATAGAAAAGTTG**CCACCATGTCCAATTTACTGACCGT |
| cre-attb1-R | **GGGGACTGCTTTTTTGTACAAACTTG**CTAATCGCCATCTTCCAGCA |
| entpd5-F | GTGGAAGTCAGGGACTTCAA |
| entpd5-R | TCCACAATAACCAACCCAACT |
| gsc-prom-F | GATTTGTCACAATGCATTT |
| gsc-prom-R | GCGAATTCTGTTTGTTTCCTG |
| bact2_attB4f | GGGGACAACTTTGTATAGAAAAGTTGAATTACAGTGGTCATGAATATT |
| bact2_attB1r | GGGGACTGCTTTTTTGTACAAACTTGGGCTAAACTGGAAAAGAAC |