Supplementary figures

Figure S1) Unprocessed embryos of the corn snake and a caecilian.
A) Corn snake embryo 2 days after ovoposition. B) Caecilian embryo, Brauer stage 26.
Figure S2) Hox expression in snakes.

A-J) Additional data for Hox expression in snake embryos (2 days after ovoposition). Each figure is marked by the relevant Hox gene in the upper right corner. A-E, G, H, J) Wholemount or partially dissected embryos with indication of the anterior boundaries of Hox expression. Somitic boundaries of mesodermal Hox expression are indicated with black arrowheads and numbers referring to the pre-vertebra level. Expression limits in the lateral plate are indicated by red arrowheads. Neural tube or hindbrain expression is indicated by blue arrowheads at the

somite
- lateral plate
- neural
- spinal ganglia
- lateral somitic frontier
anterior most position of the neural domain visible in the images. Several examples of spinal ganglia expression are pointed out by green arrowheads. The anterior pre-vertebra starting from pre-vertebra 1 are marked with asterisks in panel C, D, H and J. “H?” in panel C marks a rod like structure in the branchial arch region which highly expresses HoxC5 and possibly consist of the hyoid. F, I) Transversal cryosections with indications of somites (black arrowheads), lateral plate (red arrowheads), neural tube (blue arrowheads), spinal ganglia (green arrowheads) and the lateral somitic frontier (yellow arrowheads). The HoxA6 transversal cryosection (F) is taken around pre-vertebra15 and shows robust expression in all tissue. The HoxB7 sections are taken around pre-vertebra 4 (I, 1) which is negative for HoxB7 expression in the somitic mesoderm and lateral plate, and around pre-vertebra 15 (I, 2) which shows robust HoxB7 expression in all tissues
Figure S3) HoxC6 expression in a 2 day snake embryo.
A) Whole mount in situ hybridization of HoxC6 shown in different orientations. It is clear from the whole mount views that HoxC6 is expressed strongly in the somitic mesoderm of the posterior but not anterior parts of the embryo, here roughly indicated by lines and the letters “a” (anterior) and “p” (posterior). A black asterisk is placed in the anterior somites where HoxC6 is not expressed and a white asterisk is placed posteriorly where HoxC6 is expressed robustly. For clarity the start of the neural tube expression domain is indicated with a blue arrowhead and as
an example expression in a spinal ganglion is indicated by a green arrowhead in the right most panel. B) Because of the spiral shape of the whole mount embryo it is difficult to visualize the gradual nature of the anterior expression limit of *HoxC6* in the somitic mesoderm. Therefore the embryo was partially dissected and flat mounted. The left panel shows the embryo from the posterior head region till approximately pre-vertebra 60, middle panel shows approximately pre-vertebrae 60-117, and right panel the approximate remaining 200 most posterior somites. In the left panel *HoxC6* expression can be seen increasing gradually posterior from pre-vertebrae 11, which is the most anterior position at which very faint expression can be detected in the somites (indicated black arrowhead marked “11”). The somitic domain, but now at a place of robust expression, is indicated again at pre-vertebrae 31 (black arrowhead marked “31”). It is clear that *HoxC6* expression is absent from the somitic mesoderm anterior to pre-vertebra 11. For clarity the start of the spinal cord expression domain is indicated with a blue arrowhead and the 2*nd* spinal ganglion is marked with a green arrowhead. C) Transversal cryosections of the partially dissected *HoxC6* stained embryo shown in the left panel of B this figure. Section 1 (indicated in B with a stripe and “1”) is taken around pre-vertebra 7 and shows absence of *HoxC6* expression in somitic (black arrowhead) and lateral plate mesoderm (red arrowhead). Section 2 (indicated in panel B with a stripe and “2”) is taken around pre-vertebra 50 and shows expression of *HoxC6* in somitic (black arrowhead) and lateral plate mesoderm (red arrowheads). In the sections spinal ganglion expression is indicated with a green arrowhead, neural tube expression with a blue arrowhead and the position of the lateral somitic frontier with a yellow arrowhead.
**Figure S4** *HoxC8 expression in 2 day snake embryo.*

A) Whole mount *in situ hybridization* with *HoxC8* shown in different orientations. From the whole mount views it is clear that *HoxC8* is expressed strongly in the somitic mesoderm of the posterior but not anterior parts of the embryo, here roughly indicated by lines and the letters “a”
(anterior) and “p” (posterior). A black asterisk is placed in the anterior somites where $HoxC8$ is not expressed and a white asterisk is placed posteriorly where $HoxC8$ is expressed robustly. For clarity the start of the neural tube expression domain is indicated with a blue arrowhead and as an example expression in a spinal ganglion is indicated by a green arrowhead in the left panel. B, C). Partially dissected embryo (approximately posterior head region till around pre-vertebra 83 in B and approximately pre-vertebra 33 till 73 in C) are shown in different orientations, anterior marked with “a”. $HoxC8$ expression is absent from anterior somites (marked with a black asterisk in B) and strongly expressed more posteriorly (marked with white asterisks in B and C). $HoxC8$ expression increases gradually posterior from approximately pre-vertebra 33 onwards (C left and middle panel, in the left panel the black asterisk is placed at the start of the somitic expression domain). The lateral plate expression domain shows a similar gradual increase in expression. The lateral plate is indicated with a red arrowhead in C middle and right panel. D) Cryosections of the partially dissected embryo shown in B and C show absence of anterior $HoxC8$ expression and a simultaneous gradual increase of expression in both somites and lateral plate mesoderm. Approximate positions of the sections are indicated in B and C by stripes and numbers referring to the respective sections; section 1 is taken around pre-vertebra 10 and shows absence of $HoxC8$ expression at this axial level in lateral plate and somitic mesoderm. Sections 2-6 are taken between pre-vertebra 33 and 73 in the region where $HoxC8$ expression is initiated (approximate positions are indicated in C). The sections show the gradual simultaneous increase of expression in somitic and lateral plate mesoderm. The dorsal somitic frontier is marked with a yellow arrowhead.
Figure S5) *HoxB9* expression in a 2 day snake embryo.

A-C) Whole mount *in situ* hybridization with *HoxB9* shown in different orientations. From the whole mount views it is clear that *HoxB9* is expressed strongly in the somitic mesoderm of the posterior but not anterior parts of the embryo, here roughly indicated by lines and the letters “a” (anterior) and “p” (posterior). A black asterisk is placed in the anterior somites where *HoxB9* is not expressed and a white asterisk is placed posteriorly where *HoxB9* is expressed robustly. For clarity the start of the neural tube expression domain is indicated with a blue arrowhead and as an example expression in a spinal ganglion is indicated by a green arrowhead in panel A and C.

D, E) Partially dissected embryo from head region till approximately pre-vertebra 47 with an
indication of the somitic and lateral plate boundaries of *HoxB9* expression. *HoxB9* expression can be seen increasing gradually in the somitic mesoderm (black arrowhead marked “17”) and lateral plate mesoderm (red arrowhead) posterior from pre-vertebrae 17 onwards, which is the most anterior position at which very faint expression can be detected. The somitic domain is again indicated around pre-vertebra 37 (black arrowhead marked “37”) in an area of more robust expression. It is clear that *HoxB9* expression is absent from the somitic and lateral plate mesoderm anterior to pre-vertebra 17. For clarity the start of the neural tube domain is indicated with a blue arrowhead and the 2nd spinal ganglion is indicated by a green arrowhead in panel D.

F) Partially dissected embryo, posterior of pre-vertebra 47 shows robust homogenous *HoxB9* expression in all tissues. G) Transversal cryosections of the partially dissected *HoxB9* stained embryo shown in D this figure. Section 1 is taken around pre-vertebra 10 and shows absence of *HoxB9* expression in somitic (black arrowhead) and lateral plate mesoderm (red arrowhead). Section 2 is taken around pre-vertebra 20 and shows gradually increasing expression of *HoxB9* in somitic (black arrowhead) and lateral plate mesoderm (red arrowhead). Section 3 is taken around pre-vertebra 37 and shows robust expression of *HoxB9* in somitic (black arrowhead) and lateral plate mesoderm (red arrowhead). Approximate positions where the sections were taken are indicated in D with stripes and numbers referring to the respective sections. In the sections spinal ganglion expression is indicated with a green arrowhead, neural tube expression with a blue arrowhead and the position of the lateral somitic frontier with a yellow arrowhead.
**Figure S6** *HoxA7* expression in a 2 day snake embryo.

A-C) Whole mount *in situ hybridization* with *HoxA7* shown in different orientations. From the whole mount views it is clear that *HoxA7* is expressed strongly in the somitic mesoderm of the posterior but not anterior parts of the embryo, here roughly indicated by lines and the letters “a” (anterior) and “p” (posterior). A black asterisk is placed in the anterior somites where *HoxA7* is not expressed and a white asterisk is placed posteriorly where *HoxA7* is expressed robustly. For clarity the start of the neural tube expression domain is indicated with a blue arrowhead and as an example expression in a spinal ganglion is indicated by a green arrowhead in panel B. D) Partially dissected embryo from head region till approximately pre-vertebra 47 with an indications of the somitic and lateral plate boundaries of *HoxA7* expression. *HoxA7* expression can be seen increasing gradually in the somitic mesoderm (black arrowhead marked “32”) and
lateral plate mesoderm (red arrowhead) posterior from pre-vertebrae 32 onwards. The somitic domain is again indicated around pre-vertebra 43 (black arrowhead marked “43”) in an area of more robust expression. It is clear that $HoxA7$ expression is absent from the somitic and lateral plate mesoderm anterior to pre-vertebra 32. For clarity the start of the neural tube domain is indicated with a blue arrowhead and as an example, expression in a spinal ganglion is indicated by a green arrowhead. E) Partially dissected embryo, posterior of pre-vertebra 47 shows robust homogenous $HoxA7$ expression in all tissues.
Figure S7) *HoxC10* expression in the corn snake including early pre-somitic mesodermal expression.

A, B) Corn snake embryo (~5 days after ovoposition) posterior view (A) and side view (B). *HoxC10* expression is observed in the somitic mesoderm far anterior from the cloaca (indicated “C”) (expression within the somitic domain indicated with white asterisks). *HoxC10* is thus expressed in somites that will develop into rib-bearing vertebrae. In B neural tube staining is indicated with a blue arrowhead. C-F) Corn snake embryo 1.5 days after ovoposition (C, D) and corn snake embryo 0.5 days after oviposition (E, F), shown in wholemounts (C, E) and whole embryo flatmounts (D, F). *HoxC10* is expressed in the pre-somitic mesoderm in pre-vertebrae located anterior to the cloaca. In D expression of *HoxC10* can be detected in the pre-somitic mesoderm at the position of forming somite 216 marked with a black arrowhead (in the somitic mesoderm *HoxC10* is expressed more anteriorly at this stage). In F starting expression in the
tailbud is marked with a black arrowhead. The embryo at this stage has formed approximately 156 somites (indicated by the most posterior asterisk and number). In the flat mounts every tenth somite is indicated with an asterisk.
Figure S8) Tbx5 expression in chicken and snake embryos.

A) Chicken embryo, approximately Hamburger and Hamilton (HH) stage 25, in situ hybridized with Tbx5 shown in lateral view. At this stage Tbx5 is strongly expressed in the chicken forelimb field and forelimb but not in the rest of the lateral plate mesoderm (expression indicated with red arrowhead). B, C) Corn snake embryo in situ hybridization with Tbx5. The embryo is 4 days after ovoposition which approximately equals the chicken stage shown. Lateral view is shown in panel B, dorsal view in panel C. In the snake Tbx5 expression is expressed strongly throughout the pre-cloacal lateral plate somatopleure and does not show a regionalization to a putative forelimb domain. The anterior start of expression at the head-trunk transition is indicated with a red asterisk, the general expression domain is indicated with a red arrowhead. Although weak flank Tbx5 expression in the rostral flank region bordering the forelimb has been reported in early stage chicken embryos e.g.22 this expression has disappeared at this developmental stage (as also shown in22).