Figure S1. Transcriptional characterization of axis elongation-related tissues (related to Figure 1):

(A) UMAP layout from Pijuan-Sala et al. (2019), highlighting clusters containing paraxial cell types used for further subclustering.
(B) Expression levels of Tcf15 in the Atlas layout.
(C) Expression levels of Meox1 in the Atlas layout.
(D) Atlas layout highlighting the newly defined axial elongation-related tissues present at E8.5
(E) Centred and scaled average expression levels of signature genes characterizing the newly identified elongation-related tissues present at E8.5.
(F) Left: diffusion map of elongation-related tissues present at E8.5 coloured by newly-identified subcluster (see corresponding legend in (D)); right: diffusion map of elongation-related tissues present at E8.5 colored by Diffusion Pseudotime value (i.e. position in the one-dimensional ordering).
(G) Centred and scaled average expression levels of Cdx and Hox transcription factors in the newly identified elongation-related tissues present at E8.5. Genes differentially expressed with FDR < 0.05 in at least one comparison are highlighted in bold font.
A

- Epiblast
- Primitive Streak
- Caudal epiblast
- Anterior Primitive Streak
- Nascent mesoderm
- Mixed mesoderm
- Intermediate mesoderm
- Pharyngeal mesoderm
- Somitic mesoderm

B

- E6.5 (18.4%)
- E6.75 (10.8%)
- E7.0 (26.1%)
- E7.25 (31.3%)
- E7.5 (49.2%)
- E7.75 (49.9%)
- E8.0 (46.0%)
- E8.25 (18.8%)
- E8.5 (5.4%)

C

- Vim log2 counts
- Epcam log2 counts

D

- Fraction of Vim+ Epcam+ cells

E

- Anterior vs Posterior Somitic trajectories:
  - HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
  - HALLMARK_KRAS_SIGNALING_UP
  - HALLMARK_MYOGENESIS
  - HALLMARK_MTORC1_SIGNALING
  - HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION

F

- Gene expression dynamics along trajectories:
  - Mef2c
  - Slc2a1
  - Slc2a3
  - Pdk1
Figure S2. Multiple transcriptional trajectories towards somites (related to Figure 2):

(A) Pijuan-Sala et al. (2019) annotation of cells belonging to the developmental trajectories for NMPs as well as for anterior vs posterior somites present at E8.5, predicted using Waddington Optimal Transport analysis (See Figure 2A, B).

(B) Expression dynamics of epithelial and mesenchymal signature genes along the three developmental trajectories. y-axis: mean log2(normalised counts).

(C) Scatterplots of log2(normalised counts) for $Epcam$ (epithelial marker) vs $Vim$ (mesenchymal marker) in NMP-fated cells along developmental time-points suggest a state of incomplete EMT. Titles indicate percentage of cells in which both genes were detected.

(D) Quantification of $Epcam/Vim$ double-positive cells in transcriptional trajectories towards NMP, Anterior Somitic tissues and Posterior somitic tissues throughout development suggest a high incidence of cells in a incomplete EMT state on the NMP trajectory.

(E) Top 5 Hallmark signatures resulting from Gene Set Enrichment Analysis with (left) the genes displaying different behaviours over time in the anterior and posterior somitic trajectories, and (right) the genes with expression dynamics specific to the NMP trajectory (these are genes overlapping between comparisons: NMP vs anterior somitic trajectories and NMP vs posterior somitic trajectories). See also Supplemental Table 1 and Methods.

(F) Expression dynamics along the three developmental trajectories for genes featured in the “Myogenesis” and “mTorC1 signaling” Hallmark signatures in (E).
**A**

Genes enriched in E7.0 ancestors of Anterior Somitic tissues:

- Lfng
- Dact1
- Hes7
- Spry2
- Snai1

Genes enriched in E7.0 ancestors of Posterior Somitic tissues:

- Narp
- Axin2
- Dusp6
- Nkd1
- Sp5

**B**

Gene expression dynamics along trajectories:

- Anterior Somitic trajectory
- Posterior Somitic trajectory
- NMP trajectory

**C**

Average Z-score

**D**

Genes enriched in E7.0 ancestors of Anterior Somitic tissues:

- Phlda2
- Myl7
- Pdic
- Hand1
- Mpc2
- Dusp9
- Phlda1
- Apoe
- S100a10
- Mest

**E**

Genes enriched in E7.0 ancestors of Anterior Somitic tissues:

Overall Activity Score

**F**

Fraction NMP mass vs log2 FC (posterior vs anterior)

Trajectory:

- Anterior Somitic tissues
- Posterior Somitic tissues
Figure S3. Spatial segregation of somitic developmental trajectories (related to Figure 3):

(A) Expression dynamics of markers of the clock and wave-front somitogenesis model along the three developmental trajectories. y-axis: mean log₂(normalised counts).

(B) Gene expression dynamics of candidate regulators specific to the anterior somite development along the three developmental trajectories. y-axis: mean log₂(normalised counts).

(C) Levels of average Z-scores for the genesets retrieved from Peng et al. (2019), as enriched in E7.5 anterior mesoderm (left) and in E7.5 posterior mesoderm (right), in the Pijuan-Sala et al. (2019) dataset. To compare with Figure 2A. See also Supplemental Table 3 and Methods.

(D) Differential expression analysis between E7.0 cells present on the posterior somitic tissues trajectory vs E7.0 cells on the anterior somitic tissue trajectory.

(E) Schematics of the overall “activity score” for the genes significantly differentially expressed in each trajectory (from analysis in D), calculated using the eGastrulation tool for E7.0 spatial data (Peng et al., 2019) and expression levels in log₁₀(FPKM+1) for selected genes highlighted in bold font in (D). See also legend for Figure 3B. M: whole Mesoderm; L: Left lateral; R: Right lateral.

(F) Average fraction of optimal transport mass for the NMP lineage, in cells allocated to the anterior or posterior somite trajectories. In all comparisons, cells on the posterior somitic tissue trajectory have significantly higher NMP mass (adjusted p < 10⁻¹⁰, Student's t-test; error bars: standard deviation).
**A** CRISPR targeting of the T locus in tdTom+ mESCs

**B** Injection of tdTom+ T−/− mESCs into tdTom WT blastocysts E8.5 chimera harvest and dissociation

**C** Single-cell RNAseq

**D** FACS sorting of tdTom fractions

**E** Alkaline Phosphatase+ cell counts

**F** log2FC (injected T−/− vs injected WT) -2 0 2

**G** WT

**H** WT T−/−

**I** Alkaline Phosphatase+ cell counts

**J** Alkaline Phosphatase+ cell counts

**K** a

**L**

**M** cm ne
Figure S4. Dissecting the cell-autonomous role of $T$ (related to Figure 4):

(A) Schematic of sequencing results of the CRISPR-targeting of $T$ locus showing frameshift mutations in both analyzed clones. Het: heterozygous; hom: homozygous.

(B) Schematics of the predicted protein sequences resulting from mutations depicted in (A). All detected mutations result in an early stop codon and prevent the formation of DNA-binding and dimerization domains, precluding the existence of a functional $T$ protein.

(C) Schematic of experimental strategy for generation of E8.5 $T^{-/-}$ chimeras.

(D) Average detected levels of $T$ transcript in $T$-expressing cell types in $T^{-/-}$ (top) and WT control (bottom) chimeras.

(E) Confocal image of a $T^{-/-}$ chimeric embryo with high chimerism levels showing negative correlation between tdTomato and $T$ protein. Scale bar: 50μm.

(F) Differential abundance testing of embryonic cell types in $T^{-/-}$ chimeras compared to the WT chimera controls. Only cell types comprising more than 10 chimeric cells are displayed (excluded cell types: Mixed Mesoderm and Epiblast containing 3 and 1 cells respectively). *: BH-corrected p < 0.1, n = 4 independent experiments.

(G) UMAP representation of the scRNAseq data of chimeric embryos, split by genotype. See (F) for colour code of the different cell types. Arrowheads highlight the cell types with observed significant increased or decreased representation in the $T^{-/-}$ cell fraction as depicted in Figure 4A, corresponding to NMPs, somitic mesoderm, Intermediate mesoderm. Notochord and PGCs (depleted but below statistical significance) are also highlighted.

(H) Close-up of the definitive endoderm and notochord lineages in the UMAP in (G), displaying expression levels for key notochord genes dysregulated in the $T^{-/-}$ fraction. Colour scale represents Log$_2$(normalized counts) for each transcript.

(I) Counts of Alkaline Phosphatase-positive PGCs in $T$-expressing mouse embryos increase exponentially throughout development. NP: Neural Plate (E7.5); HF: Headfold (E7.75); s: somite stage.

(J) Counts of Alkaline Phosphatase-positive PGCs in $T$-expressing and $T^{-/-}$ mouse embryos at the headfold stage showing a significant decrease in numbers in homozygous mutant embryos (p-value = 0.0018, Student's t-test).

(K) Confocal image of the caudal region of an E8.5 chimeric embryo with low chimerism showing accumulation of tdTom$^+$ cells within the remnant primitive streak region (arrowhead). a: allantois, containing tdTom$^+$ cells. Scale bar: 50μm.

(L) Confocal image of the heart region of an E8.5 chimeric embryo showing contribution of tdTom$^+$ cells to cardiac tissue (white arrowheads), foregut diverticulum (asterisk) and extra-embryonic mesoderm (blue arrowhead). Scale bar: 100μm.

(M) Confocal image of the cranial region of an E8.5 chimeric embryo with high chimerism, showing contribution of tdTom$^+$ cells to cranial tissues, including cranial mesenchyme (cm) and neurectoderm (ne). Scale bar: 100μm.
Figure S5. Lineage contribution of $T^-$ cells in E7.5 chimaeras (related to Figure 5):

(A) Representative images of E7.5 $T^-$ chimeric embryos, bright field (top) and tdTomato fluorescence (bottom). Scale bar: 500μm.

(B) Differential abundance testing of embryonic cell types in E7.5 $T^-$ chimeras compared to the WT chimera controls. No statistically significant deviations in relative abundance were observed ($n = 3$ independent experiments).

(C) UMAP representation of the scRNAseq data of chimeric embryos, split by genotype. See (B) for colour code of the different cell types.
Figure S6. Molecular analysis of T\(^{-}\) chimaeras (related to Figure 6):

(A) UMAPs of E8.5 T\(^{-}\) chimera cells, split by genotype with expression levels of NMP marker genes. Arrowhead: NMP cells. See also Figure S4G.
(B) Scatterplots of log\(_2\) (normalised counts) for T and Sox2 in single E8.5 NMP-mapped cells in WT into WT chimeras (left) and T\(^{-}\) chimeras (right). Although T and Sox2 appear generally anti-correlated, Sox2 is not up-regulated in the absence of T (lower-right), arguing against cross-antagonism of these two regulators in NMPs.
(C) Violin plots of genes featuring a neural signature in T\(^{-}\) chimera NMP-mapped cells, showing no up-regulation in the T\(^{-}\) fraction.
(D-F) Genes significantly deregulated in E7.5 T\(^{-}\) chimera cells mapping to anterior somitic tissues (D), posterior somitic tissues (E), NMP (F) trajectories plotted by adjusted p values corresponding to expression levels comparison of tdTom\(^{+}\) injected cells and tdTom\(^{-}\) host cells in WT (x-axis) and T\(^{-}\) chimeras (y-axis). Highlighted are genes excluded from Figure 6b analysis due to significant deregulation in WT chimeras (plotted in grey) or high correlation with the tdTomato transcript (plotted in brown) over whole WT chimeras. These highlighted genes are likely technical artefacts of the chimera generation procedure (e.g. imprinted genes).