

Figure S1. Organization and gene expression patterns in the mouse embryo at E7.5. Lateral view of the mouse embryo at E7.5 with the anterior side to the left. The illustration on the left-hand side indicates the different tissue layers and structures of the embryo, and the one to the right depicts the main gene expression pattern in those regions. The sources for the outlines shown here can be found in Table S1 and (Edri et al., 2018).

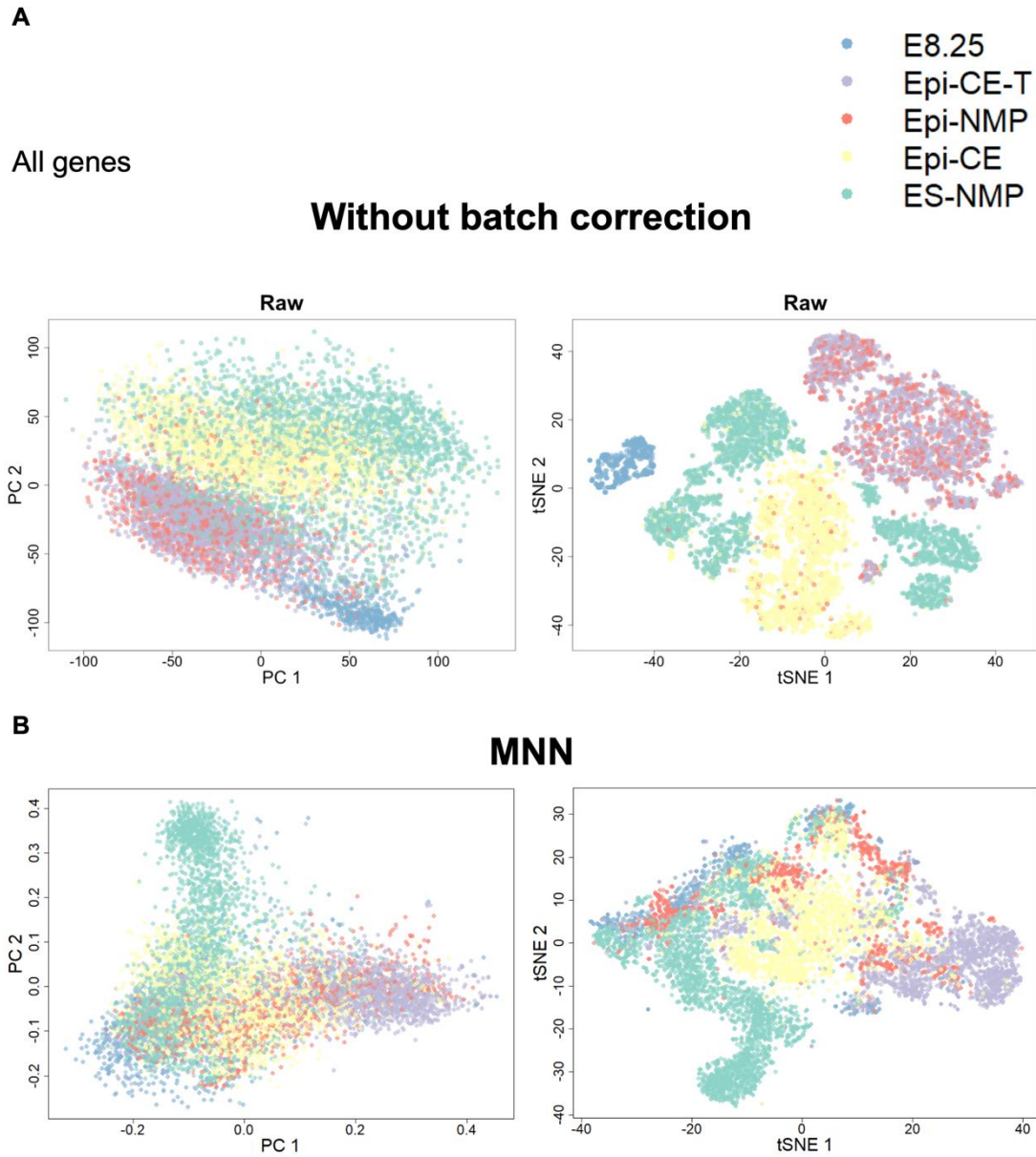
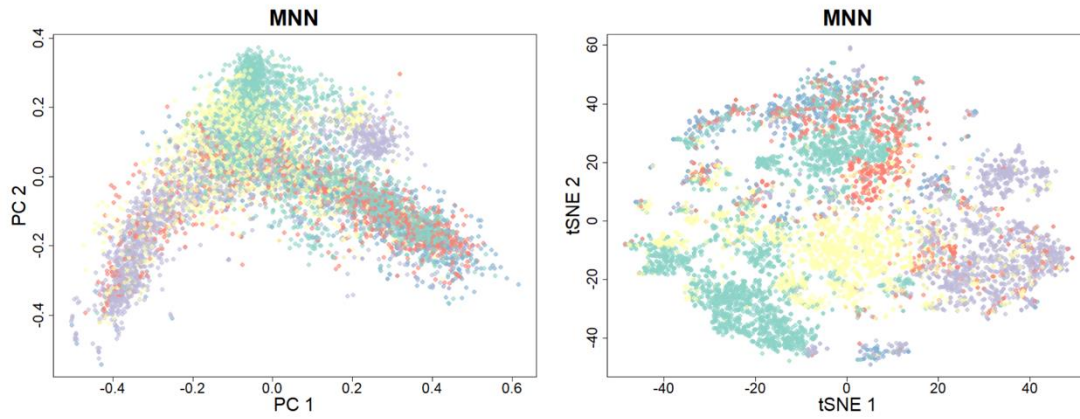


Figure S2. Correction for RNA-seq single cells technical variation. A. PCA and tSNE plots of the raw data after completing the quality control for the cells and genes. The plots were performed using all genes (14,822 genes) that passed the quality control. **B.** Batch effects correction using mutual nearest neighbour (MNN, (Haghverdi et al., 2018)).

A



Highly variable genes detected by MNN algorithm:



B

Informative genes:

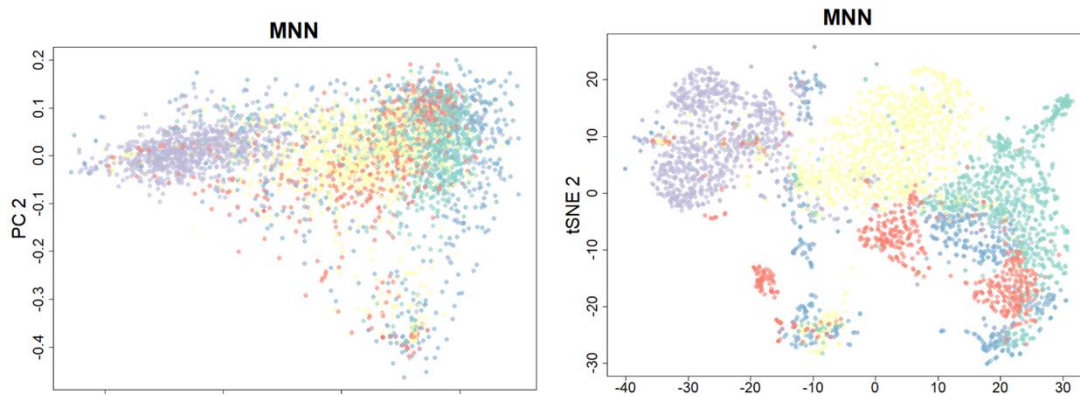


Figure S3. Correction for RNA-seq single cells technical variation for subset of genes. A. MNN batch effects correction using the highly variable genes detected by MNN. **B.** MNN batch effects correction using 1,342 genes of interest gathered from a literature review (see main text and Table S2).

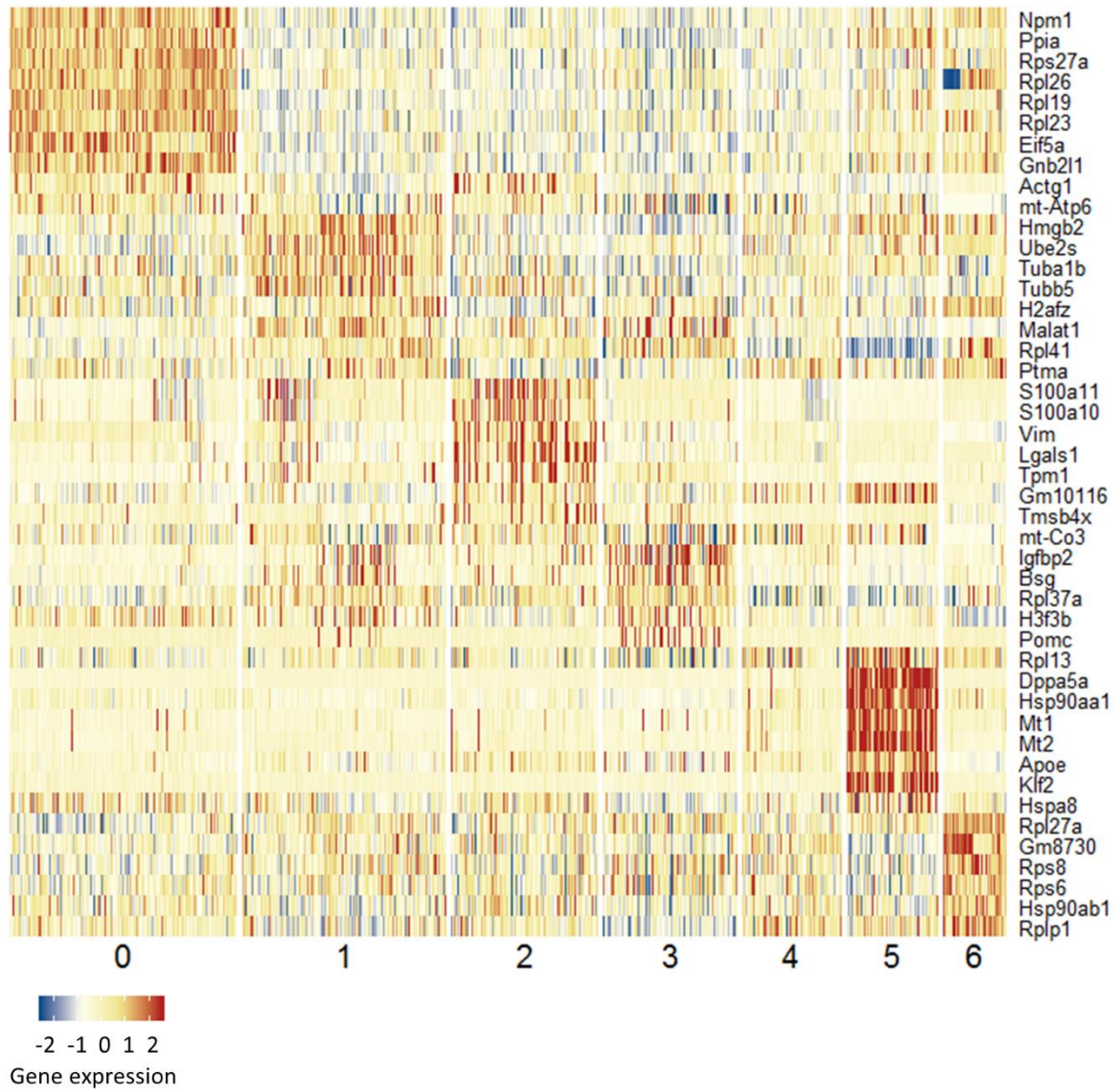


Figure S4. Clustering the embryo dataset and the *in vitro* populations after batch correcting process. Top 10 marker genes characterizing each of the 7 clusters obtained by Seurat package.

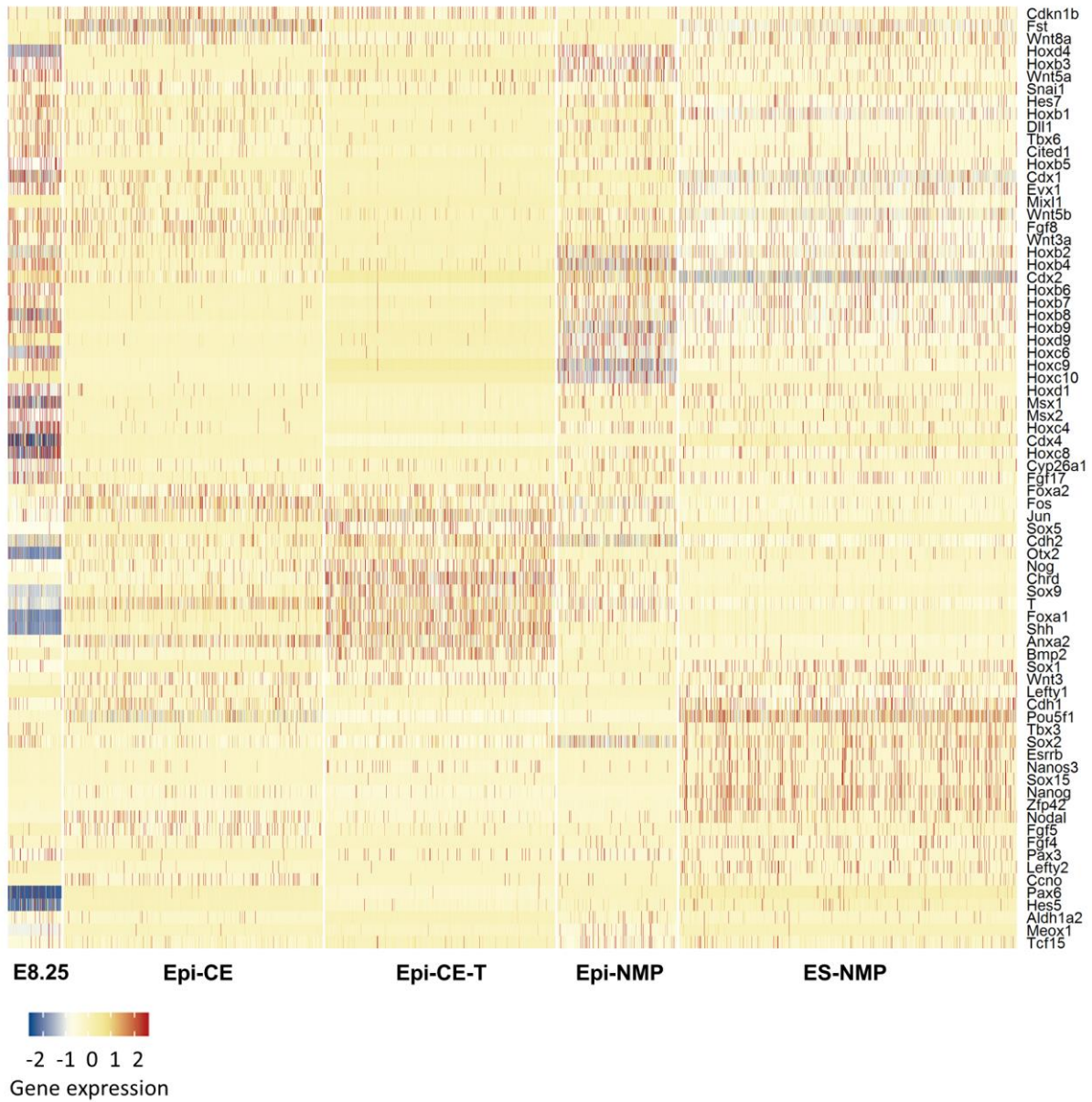


Figure S5. Visualization of gene expression in the *in vivo* and *in vitro* populations. Expression of chosen marker genes of pluripotent state, CE (E7.5), CLE, neural, mesoderm and the node along the different samples.

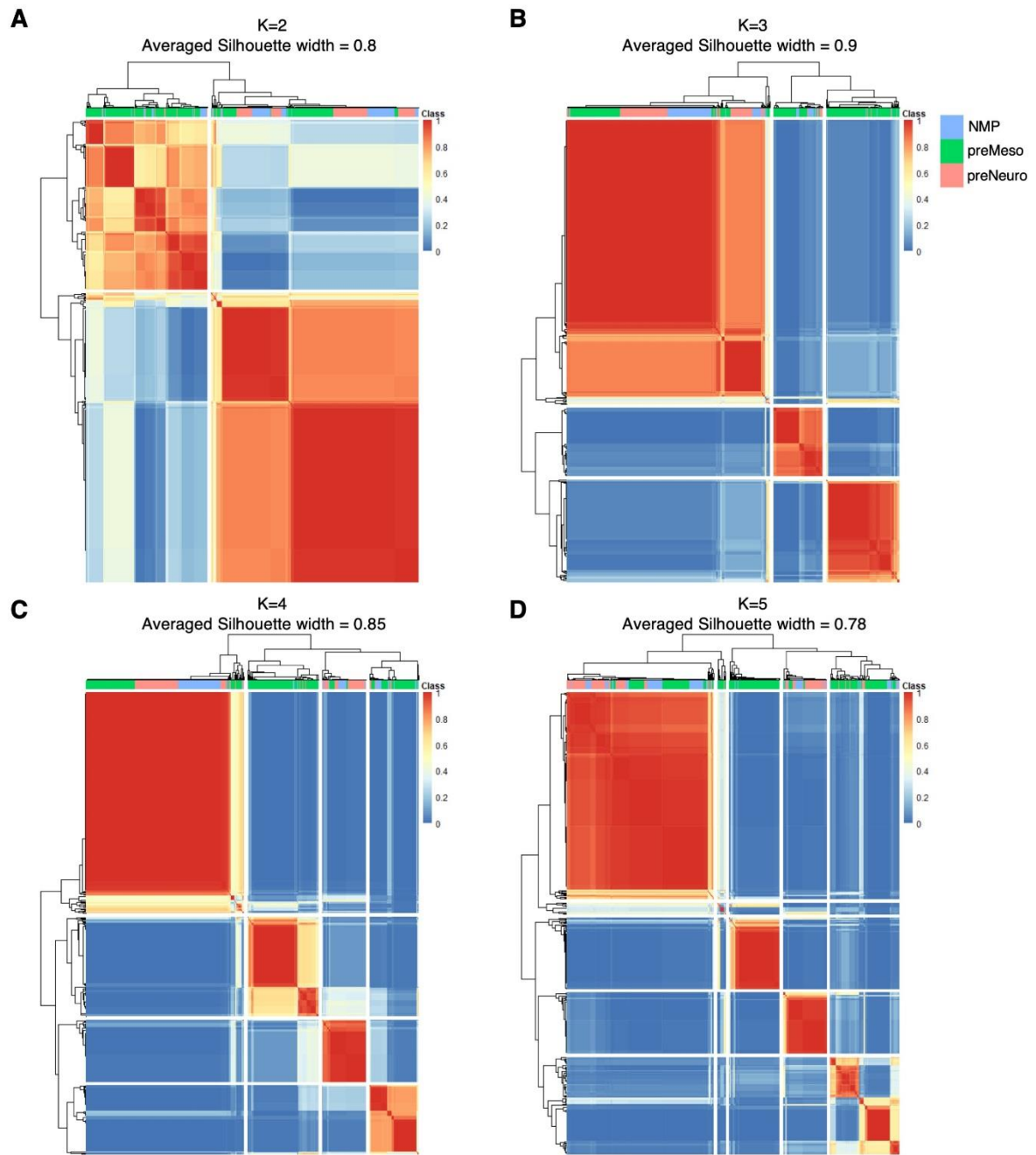


Figure S6. The averaged Silhouette width and the consensus matrices obtained for the embryo CLE for number of clusters: A. $k = 2$; B. $k = 3$; C. $k = 4$ and D. $k = 5$. Cells that were defined as NMP candidates, mesodermal progenitors (preMeso) and neural progenitors (preNeuro) are marked in blue, green and pink respectively.

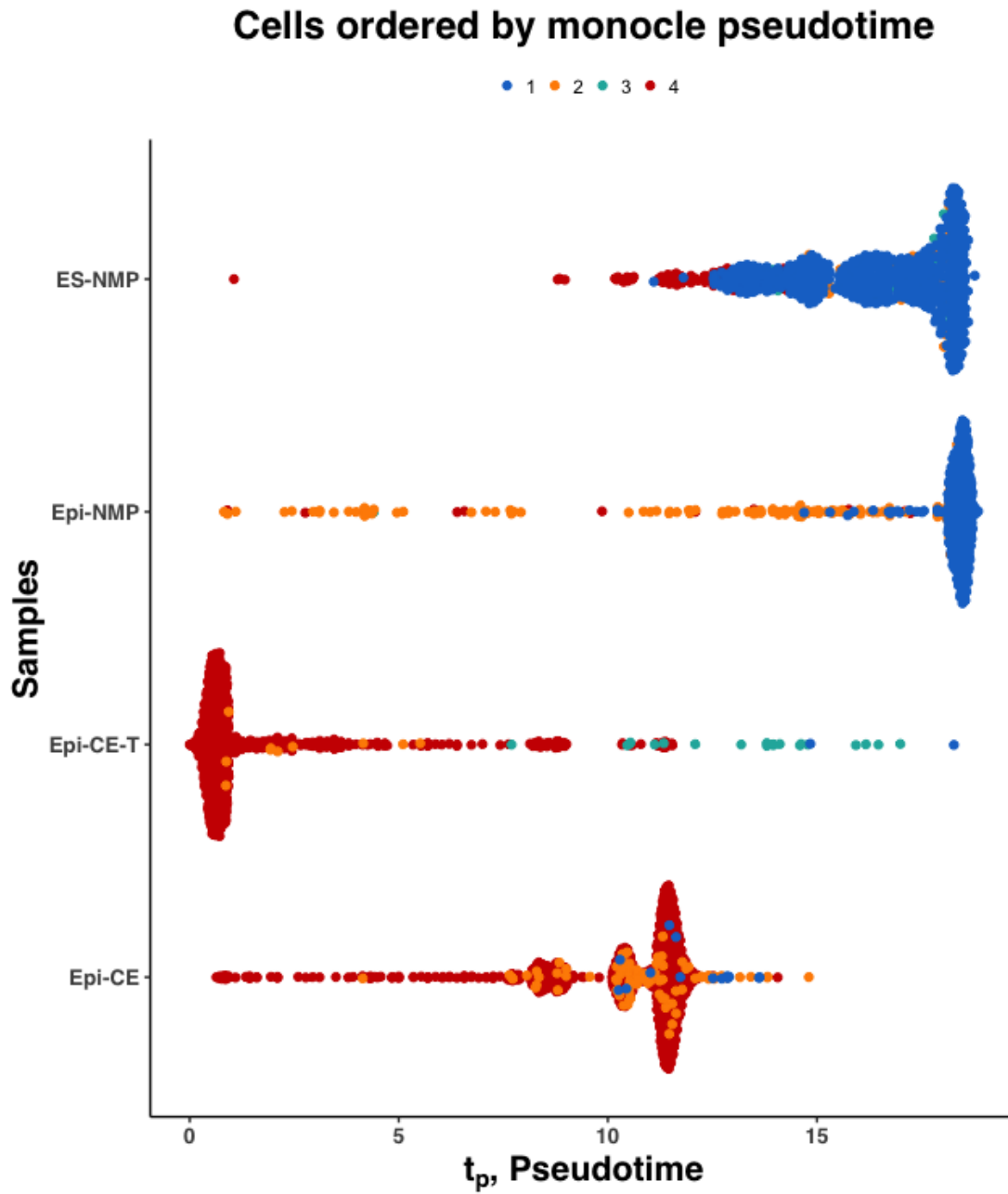


Figure S7. Monocle Pseudotemporal order of the *in vitro* cells. The cells are ordered along the pseudotime in the x-axis for every *in vitro* sample in the y-axis, where the cells that were classified to the 4 classes are coloured.

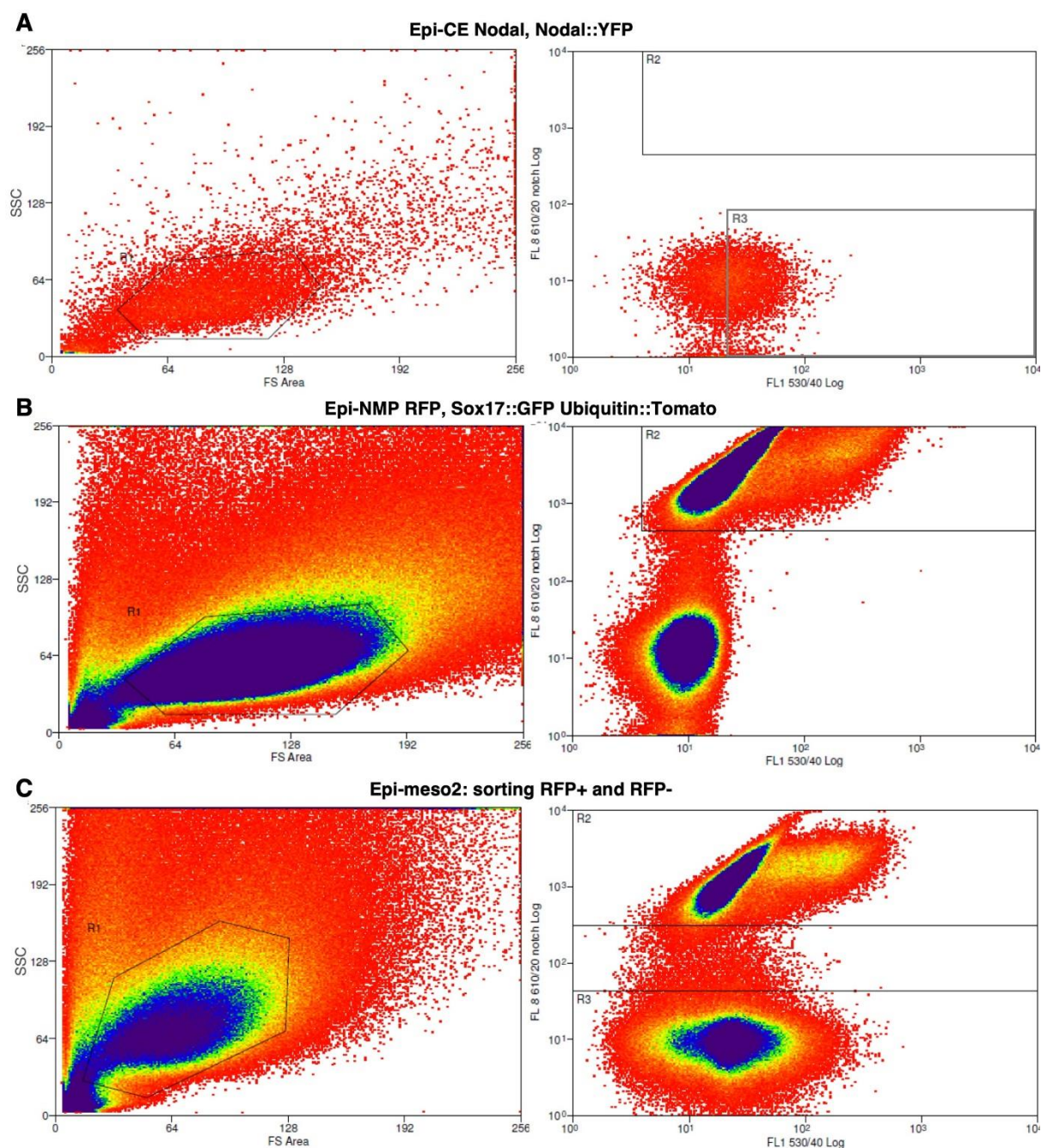


Figure S8. Node like cell with Epi-NMP mixture and sorting. Cells were sorted using a MoFlo sorter (Beckman Coulter). Single cells were collected according to the plot of SSC (side scattered light) versus FC (forward scattered light) Area (SSC versus FS area plots). The lasers that were used are 647nm laser with emission filter of 610/20 to collect the RFP positive cells and 488nm laser with emission filter of 530/40 to collect the YFP positive cells (FL8 610/20 versus FL1 530/40 log scale plots). **A.** Epi-CE Nodal sample composed of Nodal::YFP cells that were cultured under the Epi-CE protocol. Only the YFP positive cells (R3 rectangle) were used to make the mixture of Epi-meso2 (see Materials and Methods). **B.** Epi-NMP RFP sample composed of Sox17::GFP Ubiquitin::Tomato cells, were culture under the Epi-NMP protocol. Only the RFP positive cells (R2 rectangle) were used to make the Epi-meso2 mixture together with the Epi-CE Nodal YFP positive cells. **C.** After growing the mixture of Epi-meso2 for 4 days the cells were sorted based on their RFP fluorescence: RFP positive cells (R2 rectangle, sample named EM2-RFP+4d) and RFP negative cells (R3 rectangle, sample named EM2-RFP-4d). The sorted cells were quantified for their RNA of a chosen set of genes using RT-qPCR technique (Materials and Methods).

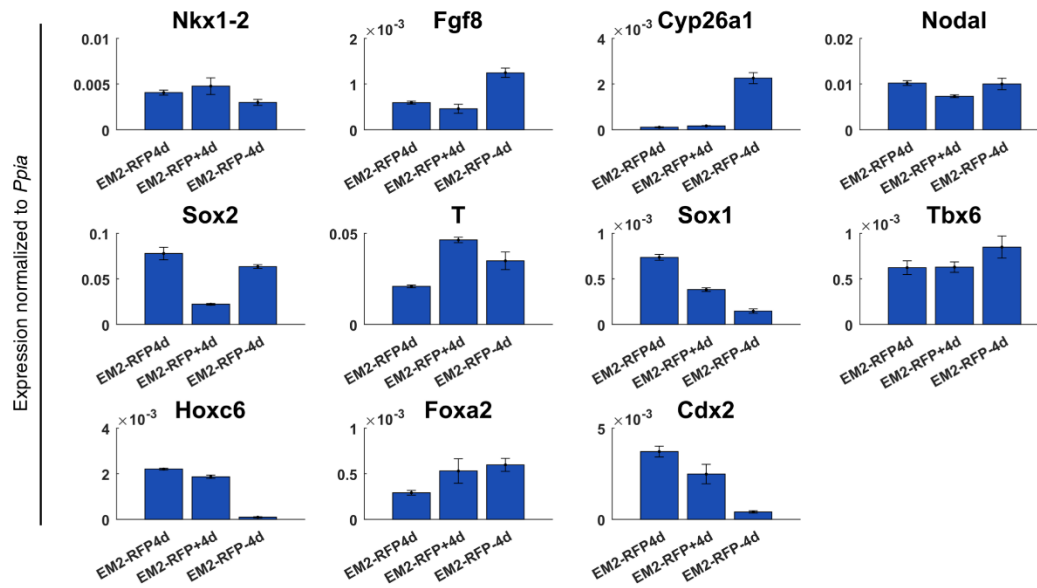


Figure S9. Gene expression of the sorted Epi-meso2 mixture and its control. The RNA expression of the sorted cells: EM2-RFP+4d and EM2-RFP-4d and the control sample EM2-RFP4d, was quantified for a chosen set of 11 genes using RT-qPCR technique. The bars represent the averaged gene expression normalized against the housekeeping gene *Ppia* across 3 technical replicas and the error bars indicate the standard deviation between the triplicates.

Table S1 - Genes expression in the mouse embryo

genes	Expression in the embryo E7.5 or E8.5	Category or pattern of expression	References
Nanog	-	Pluripotency and germ cells	(Chambers et al., 2003; Chambers et al., 2007)
Rex1	-	Pluripotency	(Toyooka et al., 2008)
Esrrb	-	Pluripotency	(Festuccia et al., 2012; Papp and Plath, 2012)
Fgf4	CE E7.5 and some expression in the CLE E8.5/9: the expression moves from the primitive streak to a region of the dorsal CE	Pluripotency and expression in the streak	(Niswander and Martin, 1992; Wright et al., 2003)
Sox15	-	Pluripotency	(Maruyama et al., 2005)
Nanos	-	Germ cells	(Tsuda et al., 2003)
Tbx3	-	Pluripotency and germ cells	(Pontecorvi et al., 2008; Russell et al., 2015)
Anxa2		Early epiblast	(Wang et al., 2015)
Fgf5	CE E7.5	Early epiblast	(Hebert et al., 1991; Khoa et al., 2016)
Otx2	CE E7.5	Early epiblast, primitive streak and node	(Acampora et al., 2009; Cajal et al., 2012)
Cdh1	CE E7.5	Pluripotency and early epiblast	(Basilicata et al., 2016; Cano et al., 2000; De Vries et al., 2004; Redmer et al., 2011)
Oct4 (Pou5f1)	CE E7.5	Pluripotency, early epiblast, caudal epiblast until E8.0	(Downs, 2008; Shi and Jin, 2010)
Pou3f1	Expression from E5.5 onward.	Early expression in the anterior epiblast, later expression in the nervous system (midbrain and forebrain) and in the peripheral nervous system. At	(Zhu et al., 2014)

		E8.0 Pouu3f1 expression is restricted to the anterior neuroectoderm.	
Pou4f1	E9.5	Cardiac development and development of cochleovestibular ganglion neurons in mouse inner ear.	(Deng et al., 2014; Maskell et al., 2017)
Tcf15	-	Pre gastrulation, primed pluripotency and somites.	(Davies et al., 2013)
Cdh2	CLE E8.5/9	Primitive streak and neural progenitors	(Basilicata et al., 2016)
Cdx1, Cdx2, Cdx4	Both in CE E7.5 and CLE E8.5/9	Caudal epiblast and tail bud (also posterior endoderm)	(Deschamps and van Nes, 2005)
Sox2	Both in CE E7.5 and CLE E8.5/9	Pluripotency, neural progenitors	(Henrique et al., 2015)
T	Both in CE E7.5 and CLE E8.5/9	Primitive Streak, caudal epiblast, tail bud and node.	(Henrique et al., 2015)
Cyp26a1	CLE E8.5/9	Caudal epiblast and tail bud from E8.0	(Sakai et al., 2001; Sirbu and Duester, 2006)
Nkx1-2	Both in CE E7.5 and CLE E8.5/9	Caudal epiblast and tail bud from E8.0; preneural spinal cord	(Henrique et al., 2015; Schubert et al., 1995)
Fgf8	Both in CE E7.5 and CLE E8.5/9	Caudal epiblast and tail bud	(Cunningham et al., 2015; Dunty et al., 2008; Sirbu and Duester, 2006)
Epha5	CLE E8.5/9	At E9.0 expression in the tail bud.	(Cooper et al., 2009)
Fos, Jun		Not known	
Fst1	CE E7.5	It is turned off in the CLE with the onset of somitogenesis.	(Cunningham et al., 2016)
Tbx6	Starts at CE E7.5	Paraxial mesoderm	(Chalamalasetty et al., 2011; Chalamalasetty et al., 2014; Dunty et

			al., 2008; White et al., 2003)
Meox1	CLE E8.5/9	Presomitic and somatic mesoderm	(Jukkola et al., 2005)
Aldh1a2	Both in CE E7.5 and CLE E8.5/9	Paraxial mesoderm at E7.5, presomitic and somatic mesoderm.	(Duester, 2008; Sirbu and Duester, 2006)
Cited1	CLE E8.5/9	Mesoderm. Early kidney development, progenitors of the heart, limb, axial skeleton and placenta.	(Boyle et al., 2007; Dunwoodie et al., 1998; Rodriguez et al., 2004)
Mesp1	From gastrulation E6.5 onwards.	Initially Mesp1 was expressed at the onset of gastrulation in the primitive streak and then in cardiac mesoderm. At E7.5 expression in the base of the allantois. At E8.0 Mesp1 is expressed on both sides of the node.	(Saga et al., 1996; Saga et al., 1997)
Mesp2	Initially detected at E8.0	At E8.0 expression was detected on both sides of the node at the same locations as for Mesp1. From E8.5 expressed in the PSM until E12.5.	(Saga et al., 1997)
Ifitm1	CLE E8.5/9	At E7.5 expression in the allantois. At E8.5 expression in the brain and in the neural tube. At E9.5 expression in the brain, primordial gut, the somites and the PSM. Expression in primordial germ cells (PGCs).	(Klymiuk et al., 2012; Lange et al., 2003)

Fn1		At E10 fibronectin matrix enriched at intersomitic borders.	(Bajanca et al., 2004)
Snai1	CE E7.5	EMT.	(Carver et al., 2001)
Bmp2	Both in CE E7.5 and CLE E8.5/9	IM, extra embryonic tissue (yolk sac and allantois), at E8.25 expression in the neural fold and LPM.	(Danesh et al., 2009)
Bmp4	Both in CE E7.5 and CLE E8.5/9	embryonic tissue (yolk sac and allantois), posterior part of the embryo, at E8.25 high expression in the posterior LPM, neural tube and in the caudal mesoderm of the tail.	(Danesh et al., 2009; Lawson et al., 1999; Zakin and De Robertis, 2004)
Evx1	Both in CE E7.5 and CLE E8.5/9	Posterior primitive streak. At E8.5 expression in the tail bud and in the allantois.	(Cambray and Wilson, 2007; Kojima et al., 2014; Schubert et al., 1995)
Mixl1	CE E7.5 and E8.5/9	Primitive streak, progenitors of endoderm. At E8.5 some expression in the tip of the tail bud and in the allantois, also expression in the crown cells of the node.	(Dunty et al., 2014; Hart et al., 2002; Kojima et al., 2014; Pearce and Evans, 1999; Robb et al., 2000; Wolfe and Downs, 2014)
Foxb1	E8.5	At E8.5 expression in the somites, presomitic mesoderm and in the neural plate	(Zhao et al., 2007).
Fgf3	Both in CE E7.5 and CLE E8.5/9	Fgf3 together with Fgf8 and Fgf17 are expressed in the in the primitive streak, PSM and tail bud. Later (E9.5) expressed in the ectoderm	(Bachler and Neubüser, 2001; Wahl et al., 2007)

		covering the midfacial region.	
Fgf9	Detected from E9.5.	At E9.5 together with Fgf3, Fgf8 and Fgf17, is expressed in the ectoderm covering the midfacial region. At E10.5 expressed the epithelium of the developing lung.	(Bachler and Neubüser, 2001; del Moral et al., 2006)
Fgf15	Expression detected at E9.5	Expressed in the developing CNS	(Fischer et al., 2011)
Fgf17	Low expression in the primitive streak at E7.5 and in the CLE E8.5	expressed in prestreak- and streak-stage embryos. Lower expression than Fgf8 in the posterior primitive streak and then in the presumptive mid/hindbrain junction, presomitic mesoderm and developing heart.	(Maruoka et al., 1998; Sun et al., 1999)
Msx1, Msx2	Starts at E7.5	At E7.5 expression in the embryonic and extraembryonic mesoderm (including the allantois), at E8.0 expression in the LPM and in the visceral endoderm together with the primordial germ cells, expression in the neural crest.	(Ishii et al., 2005; Sun et al., 2016)
Osr1	E8.5	At E8.5 expression in IM. Later expression in the developing heart, limb, lung, and craniofacial structures.	(Lan et al., 2011; Wang et al., 2005)
Phlda2	E8.0-8.5	yolk sac endoderm (expression in the	(Hou et al., 2007; Lefebvre, 2012)

		placenta), LPM and ventral definitive endoderm	
Krt18, Kr8		Extra embryonic endoderm	(Maurer et al., 2008)
Cldn6, Cldn7	At E6.5-E7.5 Cldn6 expressed in the epiblast excluding the primitive streak. At E8.5 expression begins to be restricted to the endoderm.	Definitive endoderm, kidney development	(Anderson et al., 2008; Khairallah et al., 2014)
Sparc	E9.0	At E9.0 present in extra embryonic tissues, later expressed in the parietal endoderm.	(Howe et al., 1988)
Pax6	E8.0 in the neuroepithelium	Spinal cord	(Bel-Vialar et al., 2007)
Pax3	First detected at E8.5	Expressed in the developing spinal cord, later (10-12 day) in the neural crest. Also, marker of limb muscle progenitor cells and expression in the dorsal neural tube.	(Goulding et al., 1991; Relaix et al., 2004)
Sox1	Detected at E7.5 and continue to be expressed at E9.5	Spinal cord: neural plate and neural tube	(Pevny et al., 1998; Wood and Episkopou, 1999)
Sox3	Sox2 and Sox3 expressed early in the embryo at E6.5 and at E8.5.	Both Sox2 and Sox3 are expressed throughout the epiblast. At E7.5 Sox3 is expressed in the posterior epiblast. At E8.5 Sox3 shows anteroposterior gradients of expression with bias to the posterior region.	(Wood and Episkopou, 1999)
Sox9	Expression starts at E9.5	Involved in chondrogenesis, formation of	(Barrionuevo et al., 2006; Wright et al., 1995)

		cardiac valves, and neural crest, testis and spinal cord development. Also expressed in the notochord.	
Sox5	Later expression than E9.0	Involved together with Sox6 in chondrogenesis.	(Akiyama et al., 2002)
Sox6	Later expression than E9.0	Expressed in wide range of tissues amongst them: the central nervous system (brain and spinal cord), somites, notochord, limb buds and liver.	(Akiyama et al., 2002; Hagiwara, 2011)
Sema3a	Expression detected at E9.5	Expression in the peripheral nervous system (PNS) and in the somites.	(Fujisawa and Kitsukawa, 1998; Schwarz et al., 2009)
Hes3	Both in CE E7.5 and CLE E8.5/9	At E7.5 expressed in the primitive streak, at E8.5 expression in the hindbrain, the neural plate and primitive streak.	(Lobe, 1997)
Hes5	First detected at E8-8.5	Neuroepithelium: midbrain, hindbrain and neural tube.	(Hitoshi et al., 2011)
Hes7	CLE E8.5	Expression in the PSM.	(Bessho et al., 2001a; Bessho et al., 2001b)
Ptn		Expressed in the central and peripheral nervous system	(Rosenfield et al., 2012)
Crabp2		At E10.5 can be detected in the spinal cord.	(Colbert et al., 1995; Duester, 2008)
Wnt3	First expressed at E5.5 and at E7.5 expression is evident in the epiblast.	Expression in the posterior visceral endoderm (E5.5) and later in the adjacent posterior epiblast.	(Tortelote et al., 2013)(Stuckey et al., 2011)
Wnt3a, Wnt8a	Both in CE E7.5 and CLE E8.5/9	Caudal epiblast and tail bud.	(Cambray and Wilson, 2007; Cunningham et al., 2015; Girós et al.,

			2011; Parr Brian A. et al., 1993)
Wnt5a	CLE E8.5/9	Caudal epiblast and tail bud.	(Yamaguchi et al., 1999)
Nodal	CE E7.5	Expression in the node.	(Lu and Robertson, 2004)
Foxa2	CE E7.5	Marker of the node and anterior mesendoderm. Also expressed in the extraembryonic tissue.	(Kojima et al., 2014; Lu and Robertson, 2004)
Ccno	CE E7.5-E8.0	Expression in the node and in the posterior tip of the embryo.	(Funk et al., 2015)
Zic1, Zic2, Zic3	From E8.0 to E16.5. Expressed in the CLE.	Expression in the CNS, somites, limb (not Zic1) and eye. Around E8.0, all the three expressed in the dorsal spinal cord and in the somites, Zic1 is evenly distributed along the AP axis but no expression was detected in the tail bud. Zic2 and Zic3 are highly expressed in the head and in the tail bud, but weakly in the trunk.	(Nagai et al., 1997)
Hox5-10	CLE E8.5 – E10.5	Anterior – posterior patterning, specify segment identity. Expression from the last cervical vertebrae (ribs) till the first sacral vertebrae (pelvis).	(Myers, 2008)
Hoxc10	Expression at E10.5		(Lee et al., 1999)

Table S2 – List of genes that were used to cluster the embryo cells (attached xls file).

[Click here to Download Table S2](#)

Table S3 – The marker genes of the 4 clusters of the CLE embryo data (attached xls file).

[Click here to Download Table S3](#)

Table S4 – The 82 informative genes that were used to build the SVM and classify the cells to the 4 clusters

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Table S5 – The highly expressed genes in the 2 pseudotime ranges of class 4 (attached xls file).

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Table S6 – Primer sequences used for qRT-PCR.

	Gene	Forward primer sequence	Reverse primer sequence
1	<i>Cdx2</i>	TCCTGCTGACTGCTTTCTGA	CCCTTCCTGATTTGTGGAGA
2	<i>Cyp26a1</i>	TCTGGGACCTGTACTGTGTGA	AAGCCGTATTTCTGCGCTT
3	<i>Fgf8</i>	AGGACTGCGTATTCACAGAGAT	CATGTACCAGCCCTCGTACT
4	<i>Foxa2</i>	CATTACGCCTTCAACCACCC	GGTAGTGCATGACCTGTTCG
5	<i>Hoxc6</i>	CCCTCTCTTCTCCCTTGCTC	CCACGTCTGACTCCCTGTTT
6	<i>Nkx1-2</i>	ACAACCACACAAGCCACTGA	CCATCCTGGGAACCCTTATT
7	<i>Nodal</i>	AGCCACTGTCCAGTTCTCCAG	GTGTCTGCCAAGCATACATCTC
8	<i>Sox1</i>	AGACAGCGTGCCTTTGATTT	TGGGATAAGACCTGGGTGAG
9	<i>Sox2</i>	CATGAGAGCAAGTACTGGCAAG	CCAACGATATCAACCTGCATGG
10	<i>T</i>	CTGGGAGCTCAGTTCTTTTCG	GTCCACGAGGCTATGAGGAG
11	<i>Tbx6</i>	CCAGAACCCTAGGATCACACA	CCCGAAGTTTCCTCTTCACA

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