- 1 Supplementary Table 1: Embryos (separate file)
- 2 List of embryos, stage, sex, number of cells, mean number of genes and UMIs (per cell)
- 3 sequenced.
- 4 Supplementary Table 2: Single-cell barcodes and embryo IDs (separate file)
- 5 List assigning single-cell barcodes to embryo IDs.
- 6 Supplementary Table 3: Marker genes (separate file)
- 7 List of all marker genes and their expression profiles used for cell to cell state assignment.
- 8 Data is presented as processed with Cell Ranger v2 (n = 712) and v3 (n = 706) and includes
- 9 which genes are designated as top 30 marker genes for each state.
- 10 Supplementary Table 4: Percent of positive cells (separate file)
- Expression profile per state in percent of positive cells per gene.
- 12 Supplementary Table 5: Embryo proportions (separate file)
- List of embryos, stage, number of cells and fraction per cell state.
- 14 Supplementary Table 6: Differentially expressed genes (separate file)
- 15 Differentially expressed genes per cell state based on binary (percent positive cells)
- 16 expression measurement. Percent of cells per cell state in WT and KO expressing these genes,
- 17 their difference and log₂ fold-change.

18

- 19 Supplementary Table 7: Gene Ontology for recurrently up- and downregulated genes
- 20 (separate file)
- Associated q values for all ontologies with Q < 0.05 in at least one gene set. Columns include
- 22 the Q values for recurrently deregulated gene sets, either differentially up- or downregulated
- in ≥ 2 states of any given KO within either the embryonic or extraembryonic lineage. Here,
- 24 extraembryonic includes both the Xecto and Xendo states and Xmeso is part of the embryonic
- 25 lineage as it is specified within the posterior-proximal most region of the epiblast.

26

- 27 Supplementary Table 8: DNA methylation valleys (separate file)
- 28 DNA methylation valley (DMV) information, including the epiblast methylation of non-CpG
- 29 island CpGs across relevant PRC KOs and WT, cluster assignment as shown in Extended
- Data Fig. 5g, associated gene names, and enrichment for H3K27me3.
- 31 Supplementary Table 9: Hox gene expression (separate file)
- List of all detected *Hox* genes and the percent of positive cells per cell state in WT and KO.
- 33 Supplementary Table 10: sgRNA sequences (separate file)
- 34 Supplementary Table 11: mESC Nanostring expression data (separate file)
- 35 Supplementary Figure 1: Western blot for H3K27me3 and H4 in WT and Eed^{KO} mESCs
- 36 Images of the two membrane parts that were separately immunoblotted for H3K27
- methylation and for histone H4 which served as a loading control.