Supplementary Figure 1

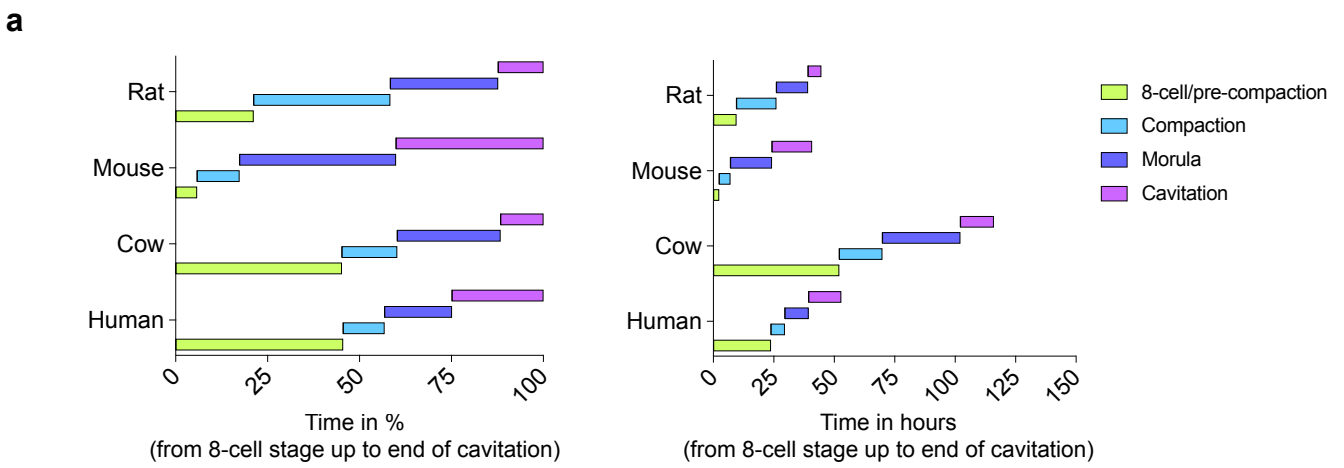


Fig. S1. Morphokinetic analysis.

a. Morphokinetic analysis of rat, mouse, cow and human pre-implantation development, showing relative time in percentage and in hours (from 8-cell stage to end of cavitation). *n* = 15 for rat, mouse and cow embryos, and *n* = 16 for human embryos.

b. Morphokinetic analysis of each of the rat pre-implantation embryos used in the analysis, showing relative time in percentage (from the 8-cell stage up to the end of cavitation).

c. Quantification of the number of individual cells that divided between 8-cell stage and start of compaction in rat, mouse, cow and human embryos. *n* =15 embryos for rat, mouse and human, *n* = 14 embryo for cow.

The data shown for mouse, cow and human embryos have already been reported in Gerri et al., Nature 2020. We are reporting them here again to compare them to the new data developed on rat embryos.

Supplementary Figure 2

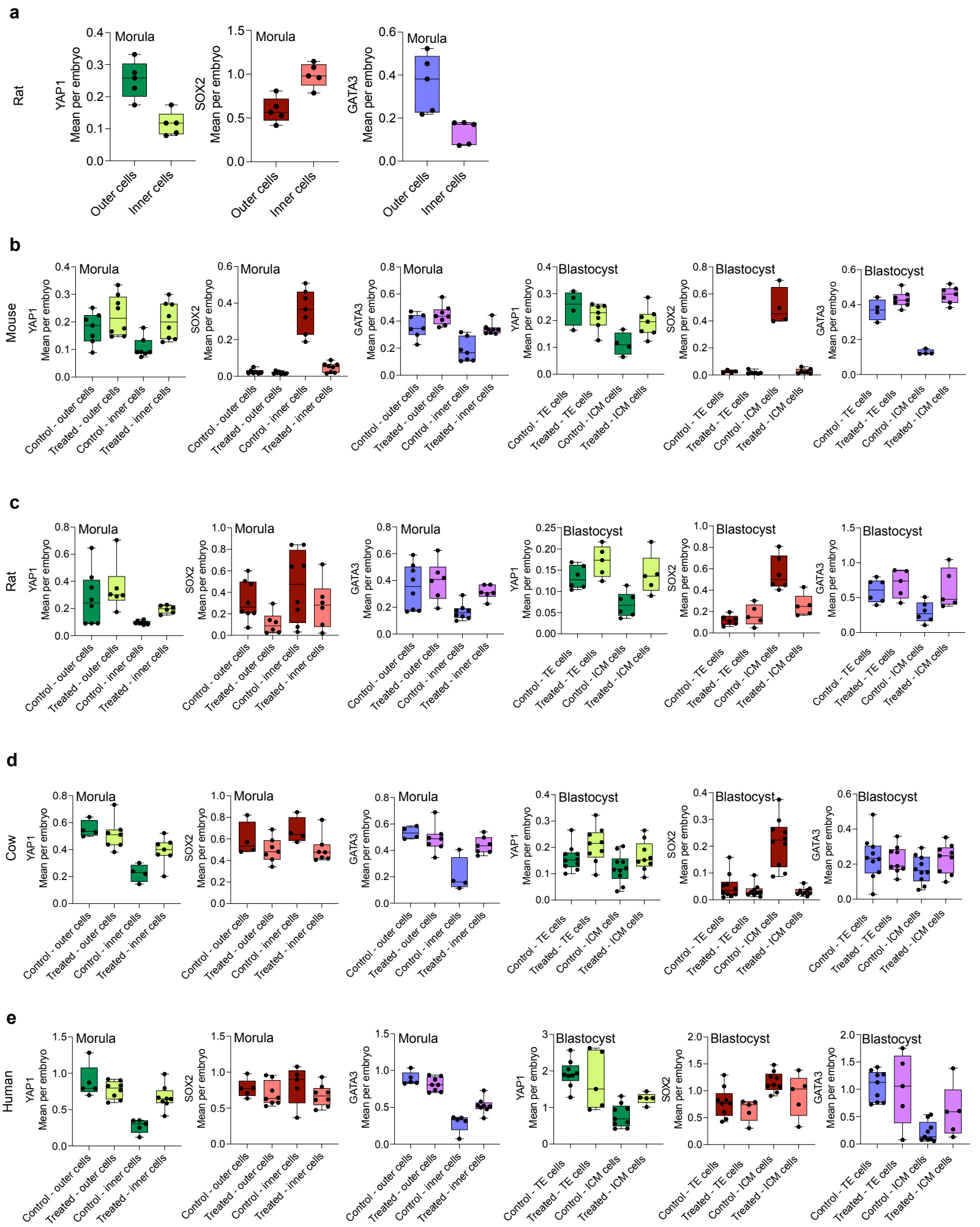


Fig. S2. Boxplots to show fate marker levels providing the mean values per embryo across experiments.

a. Boxplots showing mean values of YAP1, SOX2 and GATA3 for each embryo from Figure 1b.

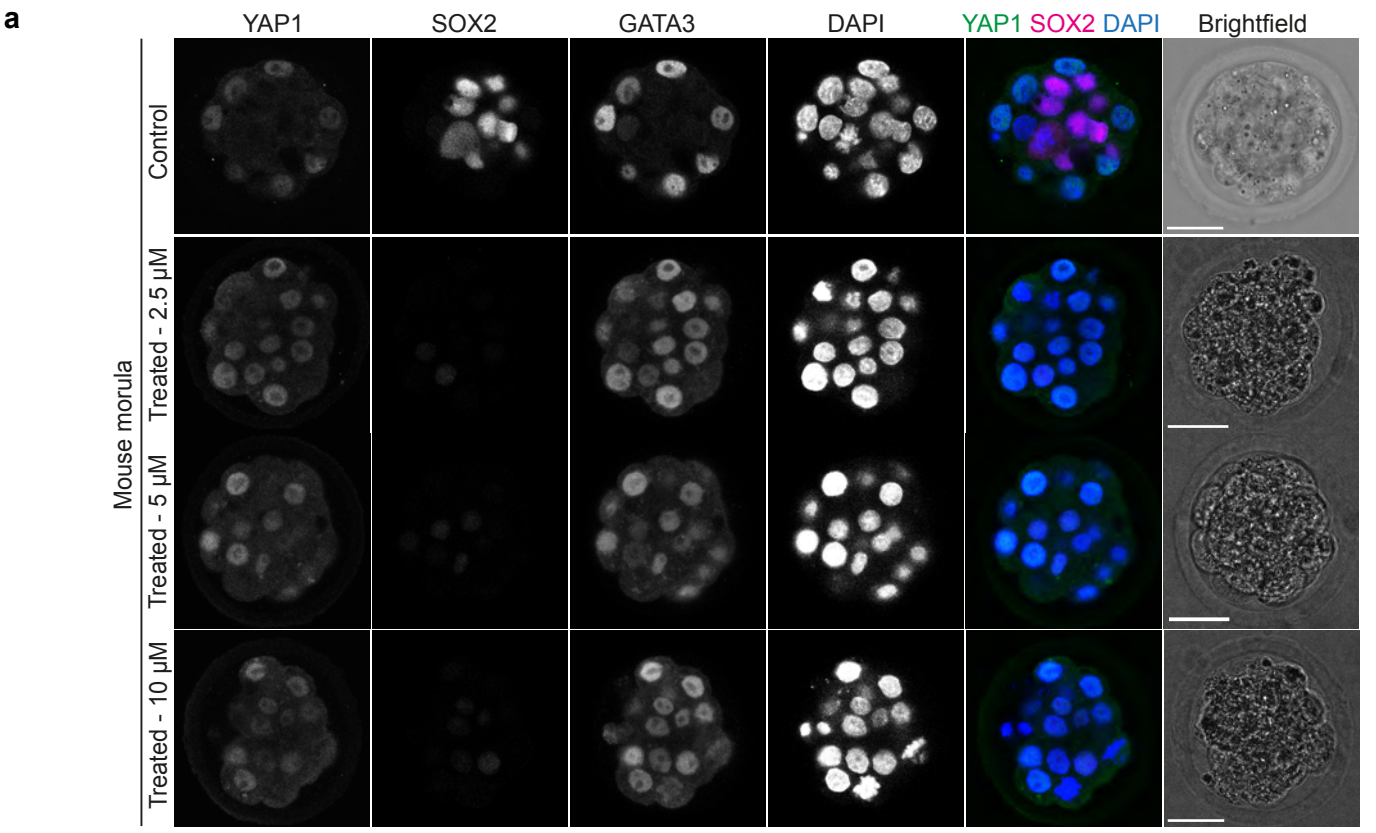
b. Boxplots showing mean values of YAP1, SOX2 and GATA3 for each embryo from Supplementary Figure 4c and 4e.

c. Boxplots showing mean values of YAP1, SOX2 and GATA3 for each embryo from Figure 2c and 2e.

d. Boxplots showing mean values of YAP1, SOX2 and GATA3 for each embryo from Figure 3c and 3e.

e. Boxplots showing mean values of YAP1, SOX2 and GATA3 for each embryo from Figure 4c and 4e.

Supplementary Figure 3



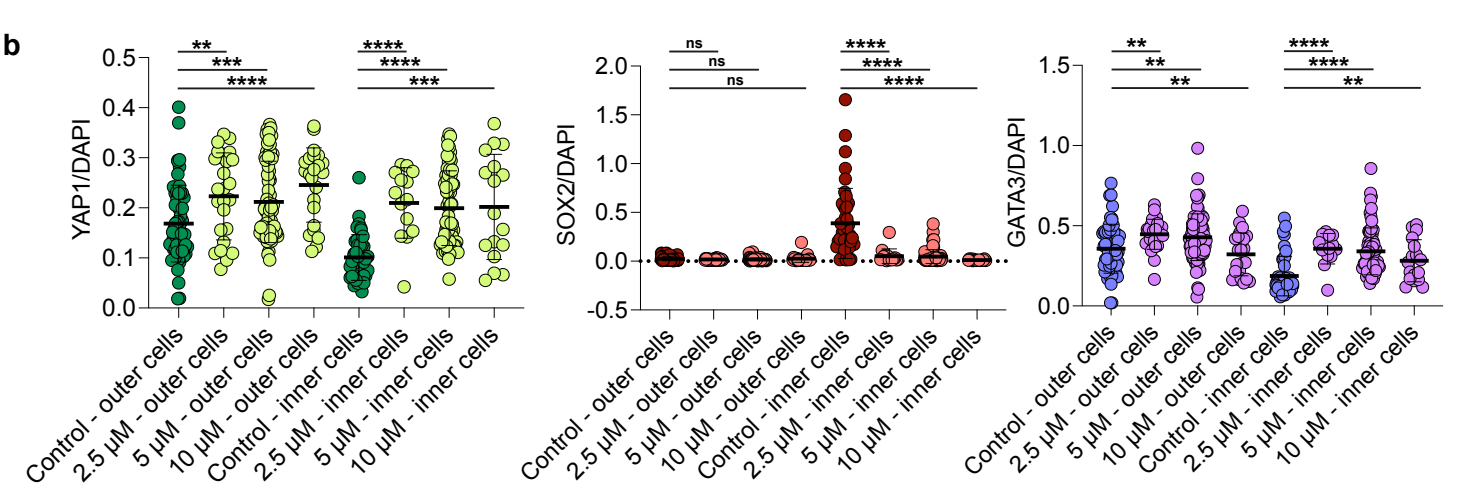


Fig. S3. LATS inhibitor dose response experiment in mouse embryos.

a. Immunofluorescence analysis of YAP1, SOX2, GATA3 and DAPI nuclear staining in mouse morula embryos treated with different concentrations of LATS inhibitor (*n* = reported in Supplementary Table 1). Scale bars, 30 µm.

b. Quantification of YAP1, SOX2, GATA3 normalised fluorescence intensity in outer and inner cells in control and LATS-inhibitor-treated mouse morula stage embryos. *(n* = 100 cells from 7 control embryos, *n* = 42 cells from 3 2.5 µM-treated embryos, *n* =147 cells from 8 5 µM-treated embryos, and *n* = 40 cells from 3 10 µM-treated embryos).

Data are presented as mean ± s.d. Two-tailed Mann–Whitney U test, ns = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Supplementary Figure 4

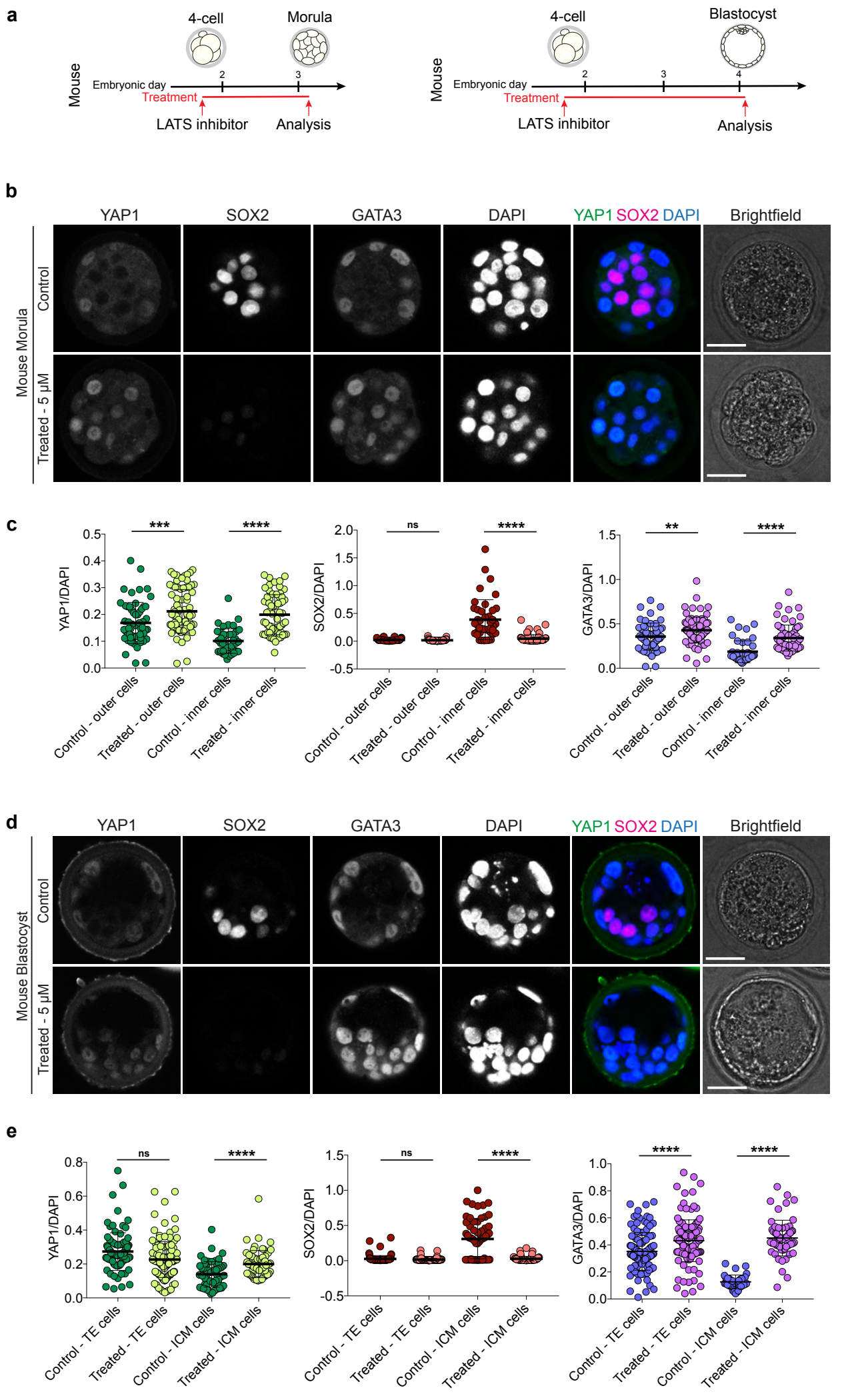


Fig. S4. LATS inhibitor treatment in mouse embryos.

a. Schematics of LATS inhibitor treatments in mouse embryos.

b. Immunofluorescence analysis of YAP1, SOX2, GATA3 and DAPI nuclear staining in control and LATS-inhibitor-treated mouse morula stage embryos.

c. Quantification of YAP1, SOX2, GATA3 normalised fluorescence intensity in outer and inner cells in control and LATS-inhibitor-treated mouse morula stage embryos. (*n* = 100 cells from 7 control embryos and 147 cells from 8 treated embryos).

d. Immunofluorescence analysis of YAP1, SOX2, GATA3 and DAPI nuclear staining in control and LATS-inhibitor-treated mouse expanded blastocyst stage embryos.

e. Quantification of YAP1, SOX2, GATA3 normalised fluorescence intensity in TE and ICM cells in control and LATS-inhibitor-treated mouse expanded blastocyst stage embryos. (*n* = 152 cells from 4 control embryos and 197 from 7 treated embryos).

Data are presented as mean ± s.d. Two-tailed Mann–Whitney U test, ns = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Scale bars, 30 μm.

Supplementary Figure 5

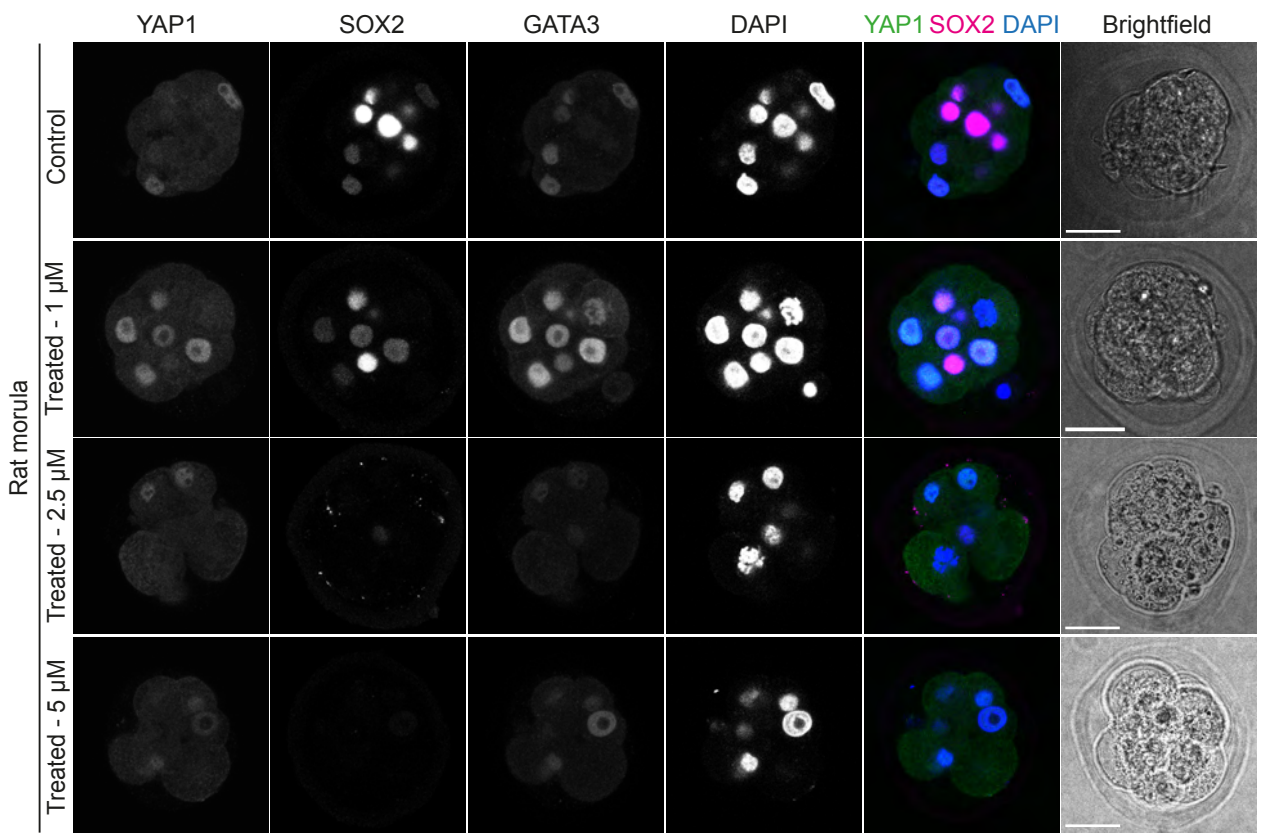


Fig. S5. LATS inhibitor dose response experiment in rat embryos.
Immunofluorescence analysis of YAP1, SOX2, GATA3 and DAPI nuclear staining in rat morula embryos treated with different concentrations of LATS inhibitor (*n* = reported in **Table S2**).
We did not quantify YAP1, SOX2 and GATA3 levels at 2.5 and 5 µM concentrations of LATS inhibitor as deemed to be toxic and they could create confusion to the data interpretation (**Table S2**). Quantifications for 1 µM LATS inhibitor-treated embryos are reported in main Figure 2.
Scale bars, 30 µm.

Supplementary Figure 6

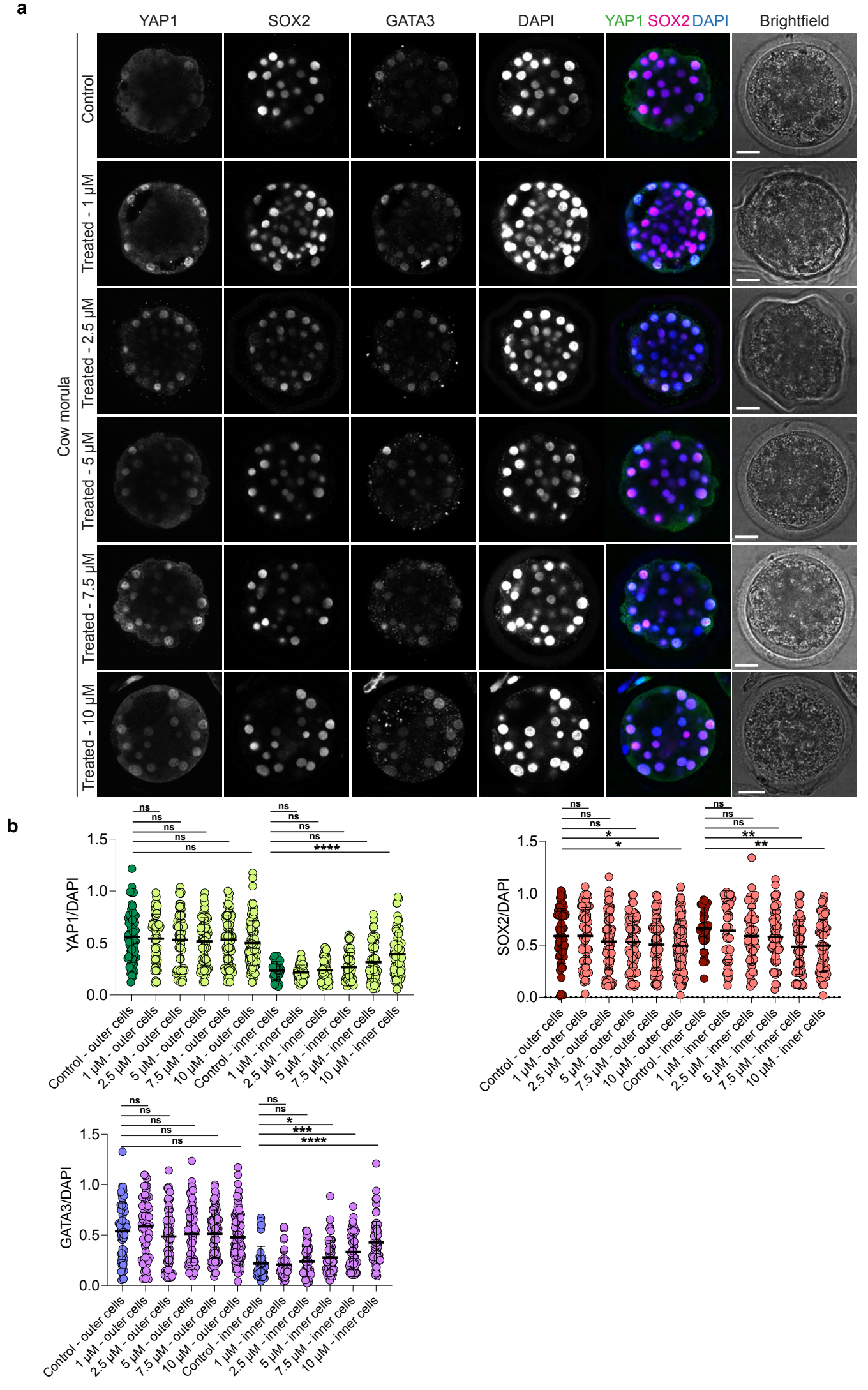


Fig. S6. LATS inhibitor dose response experiment in cow embryos.

a. Immunofluorescence analysis of YAP1, SOX2, GATA3 and DAPI nuclear staining in cow morula embryos treated with different concentrations of LATS inhibitor (*n* = reported in Table S3).
Scale bars, 30 µm.

b. Quantification of YAP1, SOX2, GATA3 normalised fluorescence intensity in outer and inner cells in control and LATS-inhibitor-treated cow morula stage embryos. (*n* = 93 cells from 4 control embryos, *n* = 110 cells from 3 1 µM-treated embryos, *n* = 125 cells from 3 2.5 µM-treated embryos, *n* = 125 cells from 35 µM-treated embryos, *n* = 131 cells from 37.5 µM-treated embryos, and *n* = 201 cells from 7 10 µM-treated embryos).
Data are presented as mean ± s.d. Two-tailed Mann–Whitney U test, ns = not significant, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001.

Supplementary Figure 7

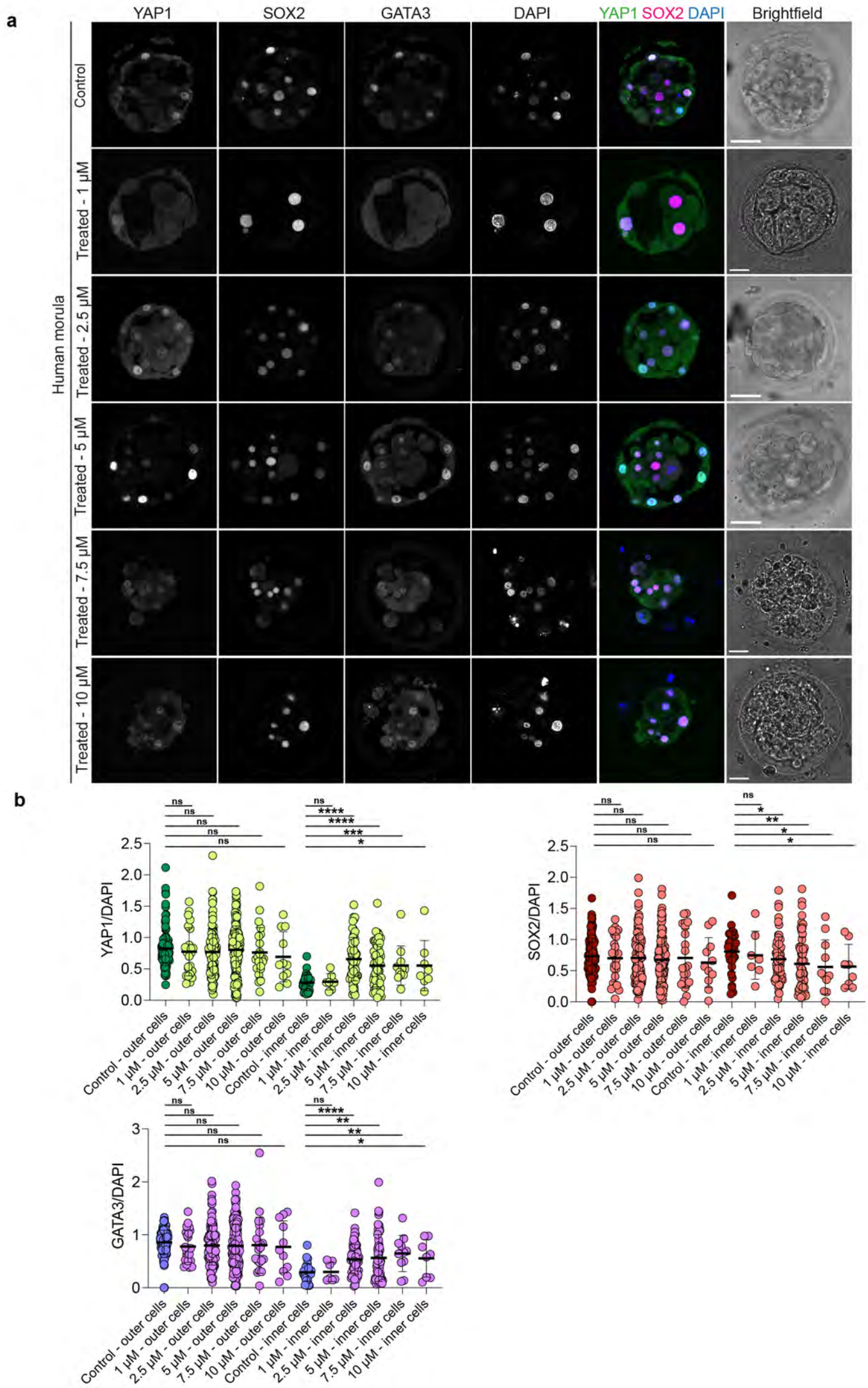


Fig. S7. LATS inhibitor dose response experiment in human embryos.

a. Immunofluorescence analysis of YAP1, SOX2, GATA3 and DAPI nuclear staining in human morula embryos treated with different concentrations of LATS inhibitor (*n* = reported in Table S4).
Scale bars, 30 µm.

b. Quantification of YAP1, SOX2, GATA3 normalised fluorescence intensity in outer and inner cells in control and LATS-inhibitor-treated human morula stage embryos. (*n* = 173 cells from 5 control embryos, *n* = 31 cells from 2 1 µM-treated embryos, *n* = 195 cells from 8 2.5 µM-treated embryos, *n* = 221 cells from 6 5 µM-treated embryos, *n* = 33 cells from 2 7.5 µM-treated embryos, and *n* = 19 cells from 1 10 µM-treated embryos).

Data are presented as mean ± s.d. Two-tailed Mann–Whitney *U* test, ns = not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Supplementary Figure 8

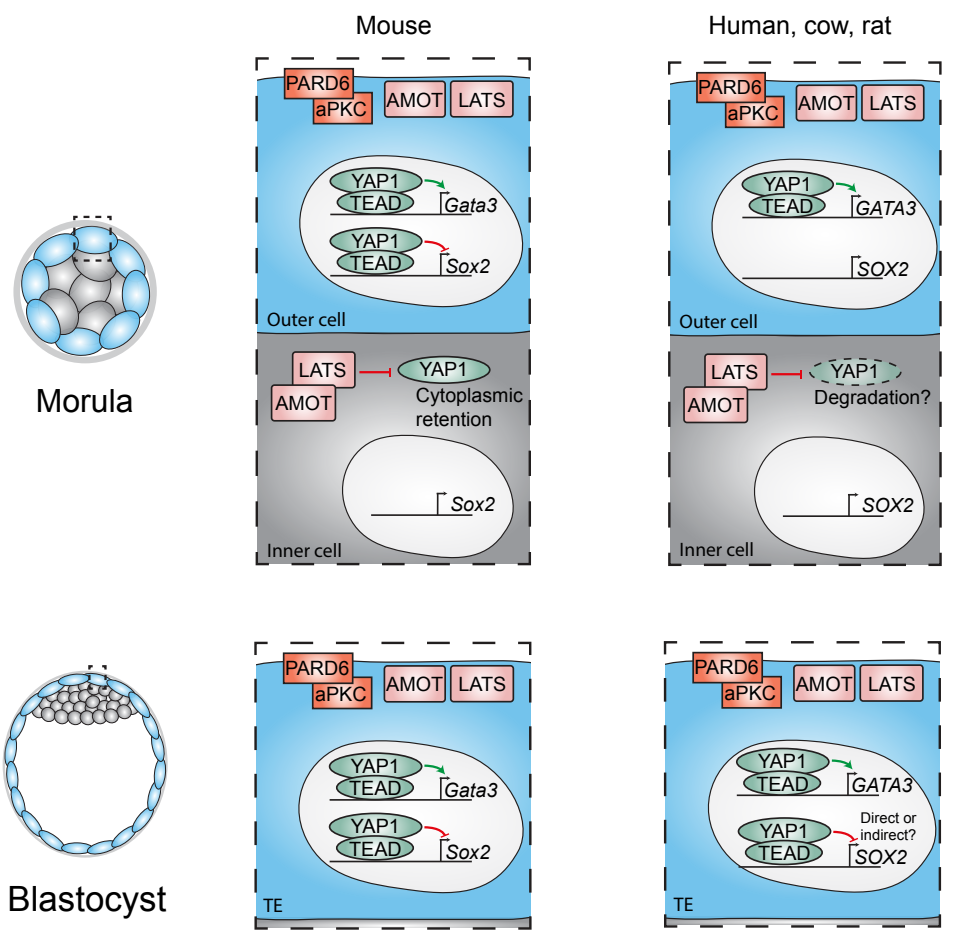


Fig. S8. Model for similarities and differences in TE initiation and specification across species. TE = trophectoderm.

Table S1. LATS inhibitor concentrations tested in mouse embryos. List of concentrations of the LATS inhibitor tested in mouse embryos, treating from the 4-cell to morula or to expanded blastocyst stage. We report the total number of embryos treated, the number of embryos arrested during the treatment and number of embryos that reached the analysed stage.

LATS inhibitor treatment from 4-cell to morula stage in mouse embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of morula embryos**
DMSO control (volume matched)	50	3 (6%)	47 (94%)
2.5 µM	25	1 (4%)	24 (96%)
5 µM	50	4 (8%)	46 (92%)
10 µM	25	10 (40%)	15 (60%)

LATS inhibitor treatment from 4-cell to blastocyst stage in mouse embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of blastocyst embryos**
DMSO control (volume matched)	50	3 (6%)	47 (94%)
5 µM	50	2 (4%)	48 (96%)

Table S2. LATS inhibitor concentrations tested in rat embryos.

List of concentrations of the LATS inhibitor tested in rat embryos, treating from the 4-cell to morula or to expanded blastocyst stage. We report the total number of embryos treated, the number of embryos arrested during the treatment and number of embryos that reached the analysed stage.

LATS inhibitor treatment from 4-cell to morula stage in rat embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of morula embryos**
DMSO (volume matched)	50	5 (10%)	45 (90%)
1 µM	50	6 (12%)	44 (88%)
2.5 µM	25	11 (44%)	14 (56%)
5 µM	25	16 (64%)	9 (36%)

LATS inhibitor treatment from 4-cell to blastocyst stage in rat embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of blastocyst embryos**
DMSO (volume matched)	45	5 (11%)	40 (89%)
1 µM	45	3 (7%)	42 (93%)

Table S3. LATS inhibitor concentrations tested in cow embryos.

List of concentrations of the LATS inhibitor tested in cow embryos, treating from pre-compaction to morula or to expanded blastocyst stage. We report the total number of embryos treated, the number of embryos arrested during the treatment and number of embryos that reached the analysed stage.

LATS inhibitor treatment from pre-compaction to morula stage in cow embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of morula embryos**
DMSO (volume matched)	50	3 (6%)	47 (94%)
1 µM	22	2 (9%)	20 (91%)
2.5 µM	18	1 (6%)	17 (94%)
5 µM	45	3 (7%)	42 (93%)
7.5 µM	23	1 (4%)	22 (96%)
10 µM	50	5 (10%)	45 (90%)

LATS inhibitor treatment from pre-compaction to blastocyst stage in cow embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of blastocyst embryos**
DMSO (volume matched)	40	2 (5%)	38 (95%)
10 µM	40	3 (7%)	37 (93%)

Table S4. LATS inhibitor concentrations tested in human embryos.

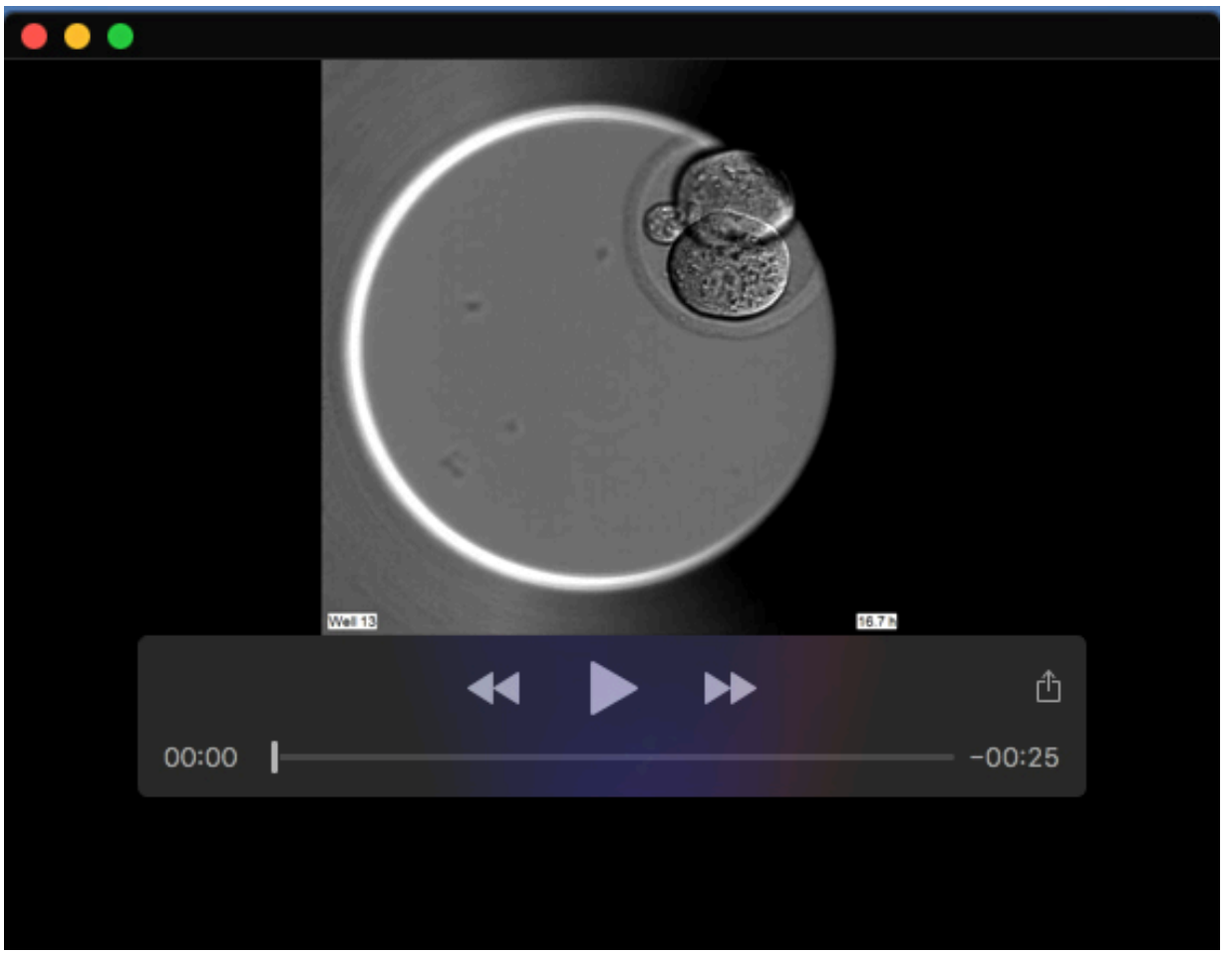
List of concentrations of the LATS inhibitor tested in human embryos, treating from pre-compaction to morula or to expanded blastocyst stage. We report the total number of embryos treated, the number of embryos arrested during the treatment and number of embryos that reached the analysed stage.

LATS inhibitor treatment from pre-compaction to morula stage in human embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of morula embryos**
DMSO (volume matched)	10	0 (0%)	10 (100%)
1 µM	2	0 (0%)	2 (100%)
2.5 µM	9	1 (11%)	8 (89%)
5 µM	13	4 (30%)	9 (70%)
7.5 µM	4	2 (50%)	2 (50%)
10 µM	1	0 (0%)	1 (100%)

LATS inhibitor treatment from pre-compaction to blastocyst stage in human embryos			
LATS inhibitor concentration	**Number of embryos**	**Number (percentage) of arrested embryos**	**Number (percentage) of blastocyst embryos**
DMSO (volume matched)	9	0 (0%)	9 (100%)
2.5 µM	5	0 (0%)	5 (100%)

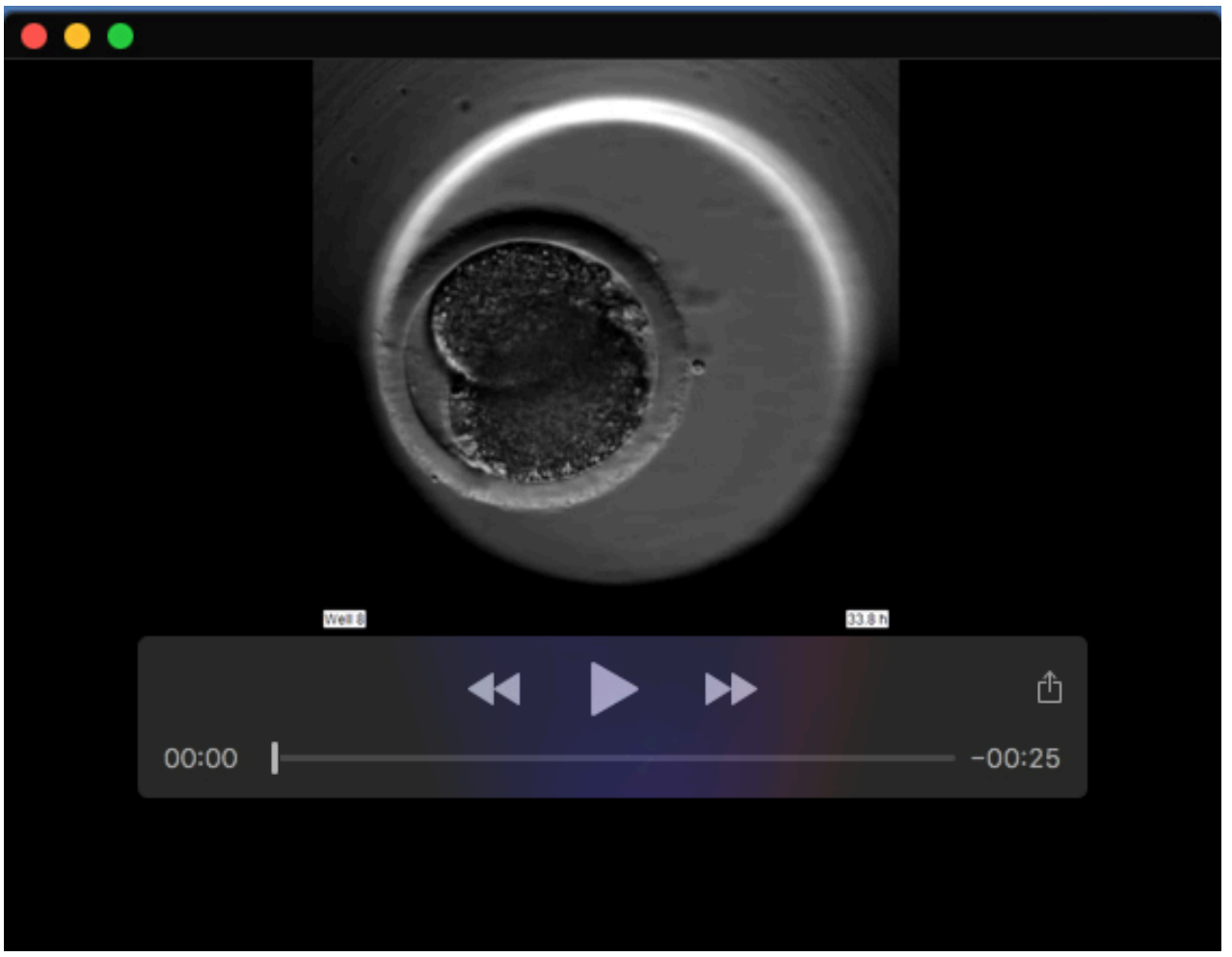
Table S5. Antibodies used in this study.

Click here to download Table S5

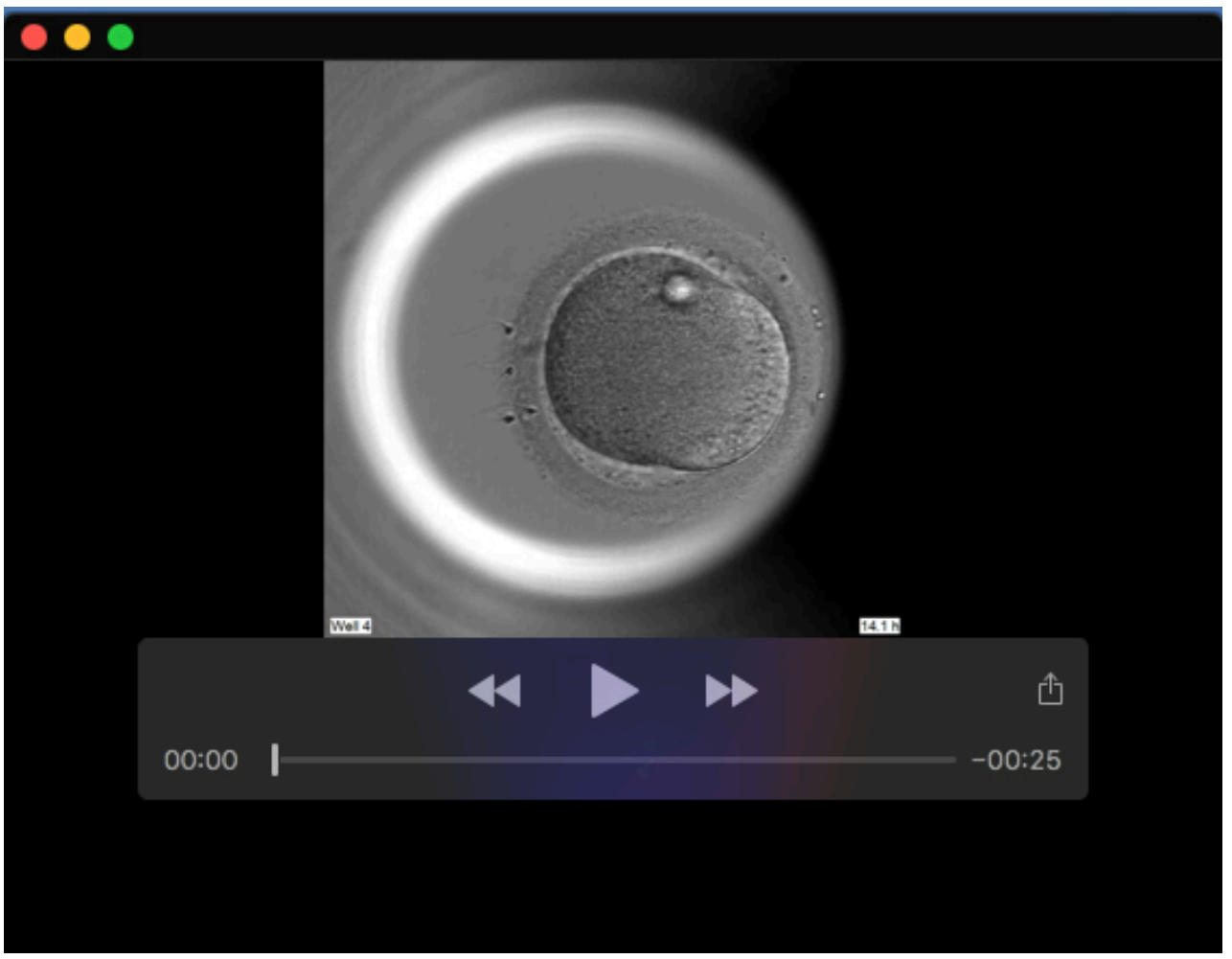


Movie 1. Mouse pre-implantation development. Time-lapse Embryoscope+ video of a mouse pronuclear stage zygote developing to the expanded blastocyst stage.

Movie 2. Rat pre-implantation development. Time-lapse Embryoscope+ video of a rat 2-cell stage embryo developing to the expanded blastocyst stage.



Movie 3. Cow pre-implantation development. Time-lapse Embryoscope video of a cow 2-cell stage embryo developing to the expanded blastocyst stage.



Movie 4. Human pre-implantation development. Time-lapse Embryoscope+ video of a human pronuclear stage zygote developing to the expanded blastocyst stage.