nature cell biology

Supplementary information

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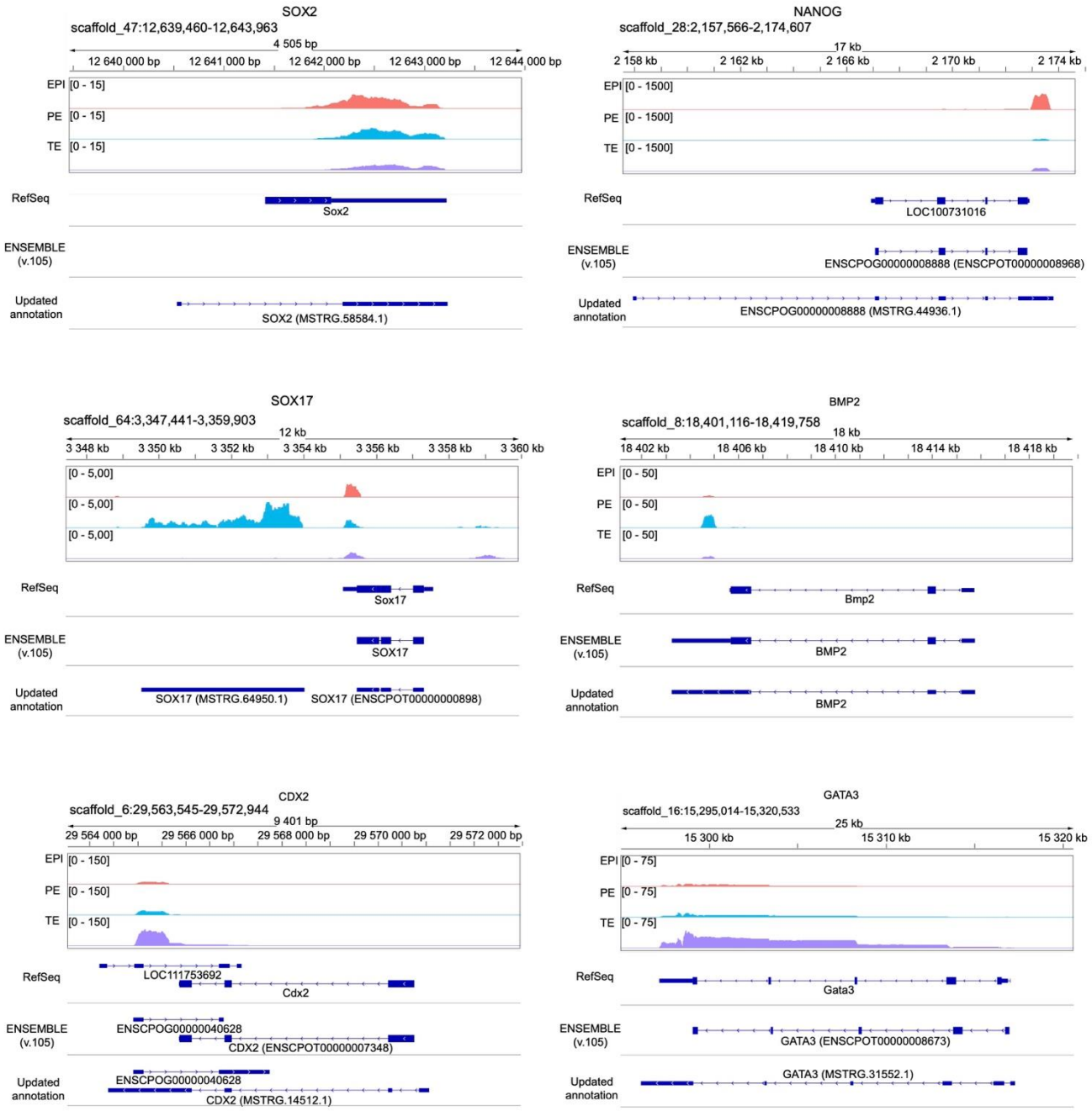
The guinea pig serves as an alternative model to study human preimplantation development

In the format provided by the authors and unedited

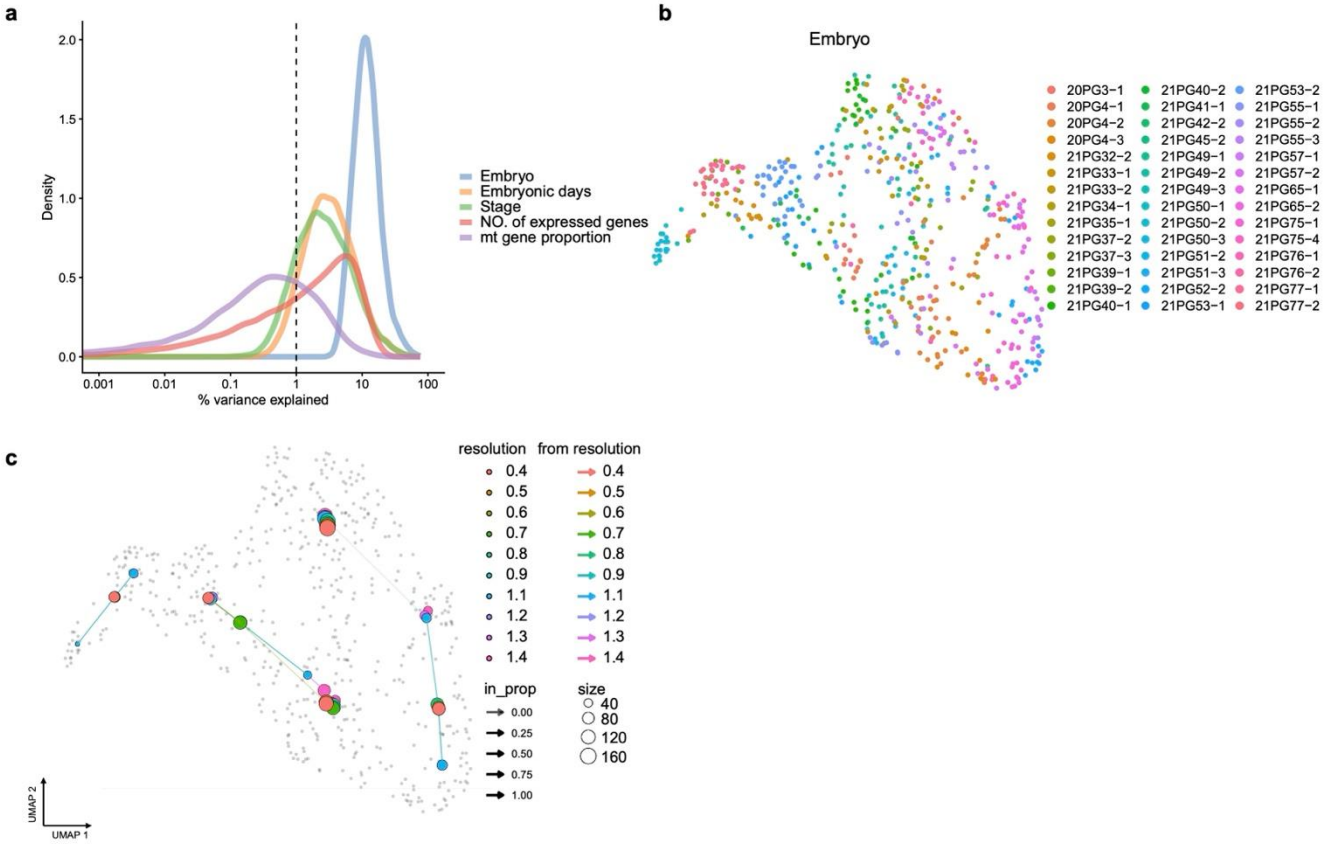
SUPPLEMENTARY INFORMATION

This file contains Supplementary Figs. 1-11

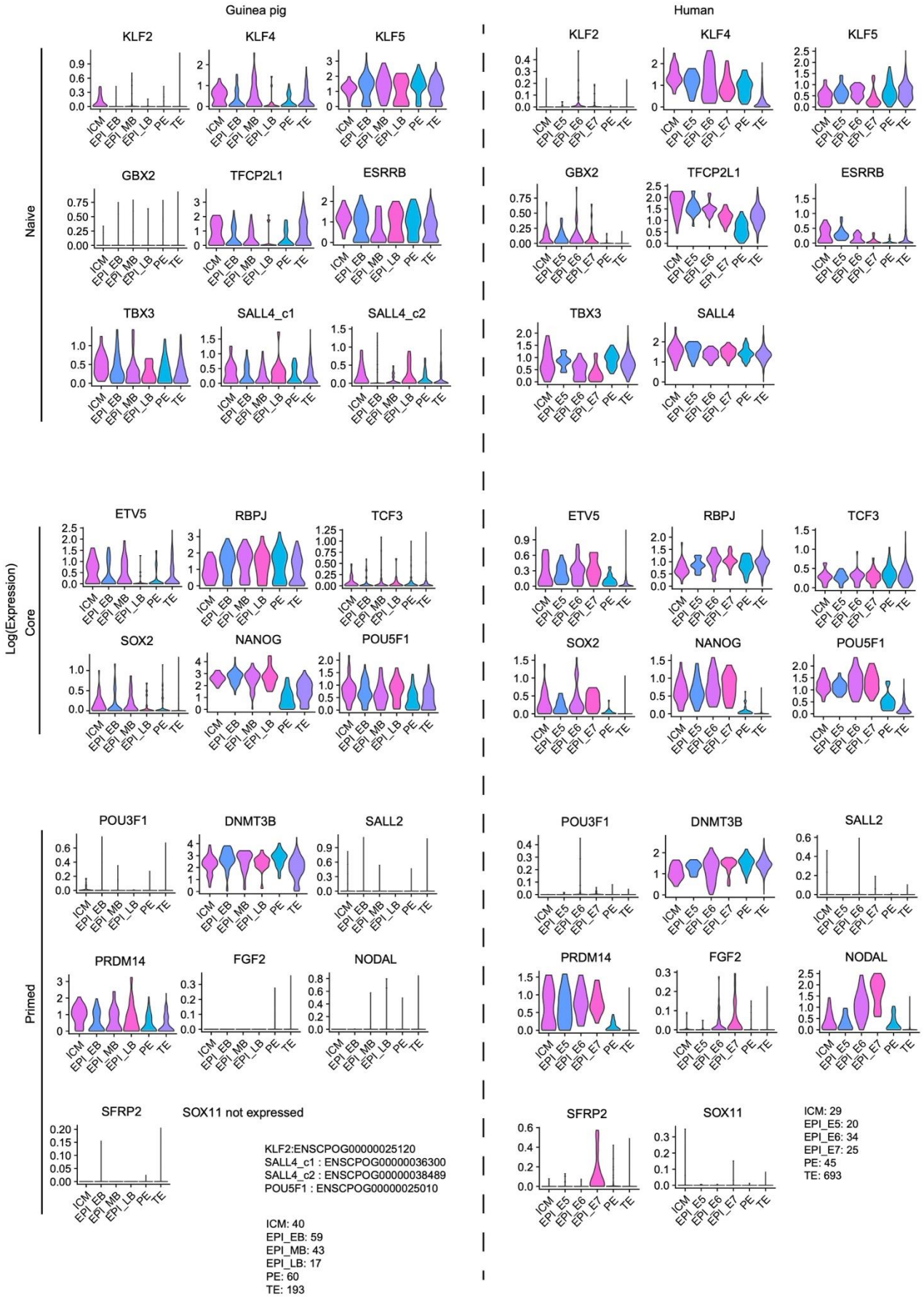
Source Data for Supplementary Figs. 5-8 and 11



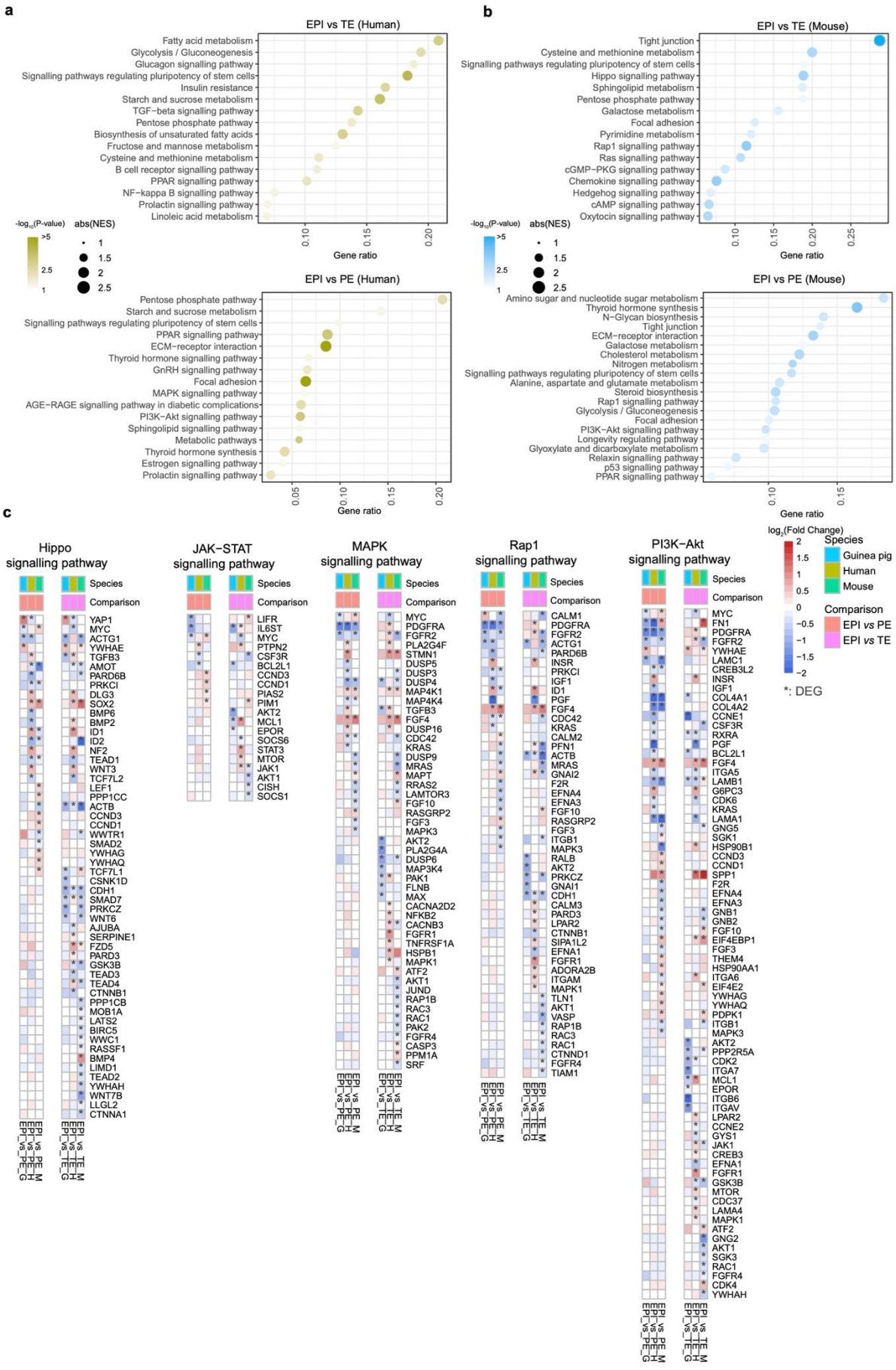
Supplementary Fig. 1 IGV snapshot displaying the read distribution from middle and late blastocyst EPI, PE, and TE cells for *SOX2*, *NANOG*, *SOX17*, *BMP2*, *CDX2*, and *GATA3*. Gene annotations from RefSeq, ENSEMBL, and updated annotations are listed.



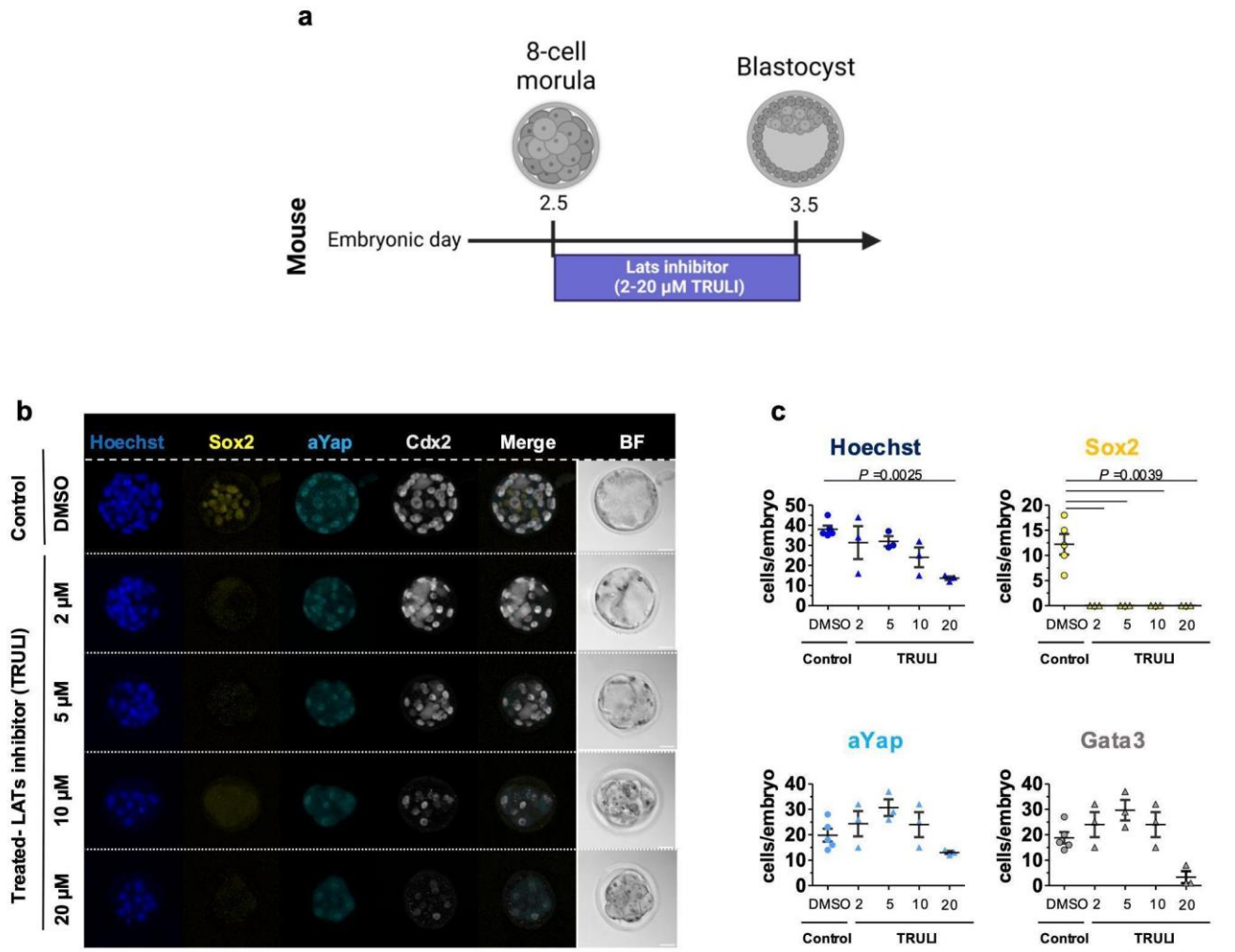
Supplementary Fig. 2 Variance and resolution selection in the guinea pig scRNA-seq dataset. a, Line plot showing the percentage of variance explained by factors "Embryo", “Embryonic days”, “Stages", “Number of expressed genes”, and "Mitochondrial gene proportion". **b**, Two-dimensional UMAP representation of 541 single-cell transcriptomes (from 42 embryos) from guinea pig preimplantation embryos, with colours indicating the embryo source. **c**, Cluster stability was analyzed with Clustree at various resolution values (0.4 to 1.4).


Guinea pig
Human
Naive
Primed
Log(Expression)
Core
KLF2
KLF4
KLF5
GBX2
TFCP2L1
ESRRB
TBX3
SALL4_c1
SALL4_c2
SALL4
ETV5
RBPJ
TCF3
SOX2
NANOG
POU5F1
POU3F1
DNMT3B
SALL2
PRDM14
FGF2
NODAL
SFRP2
SOX11
ICM
EPI_EB
EPI_MB
EPI_LB
EPI_E5
EPI_E6
EPI_E7
PE
TE
SOX11 not expressed
KLF2:ENSCPOG00000025120
SALL4_c1 : ENSCPOG00000036300
SALL4_c2 : ENSCPOG00000038489
POU5F1 : ENSCPOG00000025010
ICM: 40
EPI_EB: 59
EPI_MB: 43
EPI_LB: 17
PE: 60
TE: 193
ICM: 29
EPI_E5: 20
EPI_E6: 34
EPI_E7: 25
PE: 45
TE: 693

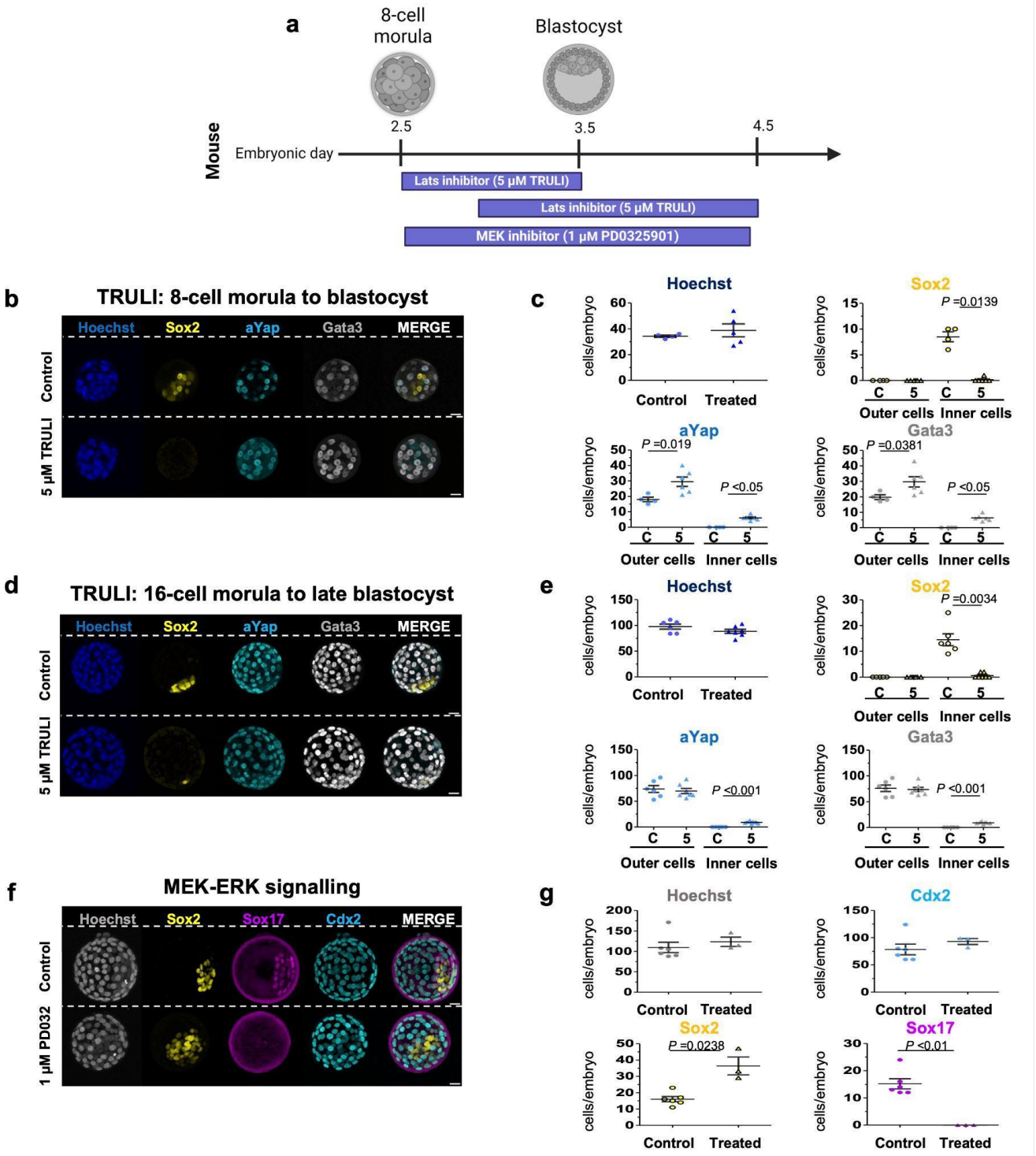
Supplementary Fig. 3 Pluripotency signatures in guinea pig and human preimplantation embryos. Violin plots showing the expression of selected pluripotency (naive, core and primed) genes in guinea pigs and humans, stratified by lineages. Published data for the human embryo was leveraged from Petropoulos et al., 2016. Cell numbers used to generate the violin plots are indicated for each lineage and species.


a
EPI vs TE (Human)
Fatty acid metabolism
Glycolysis / Gluconeogenesis
Glucagon signalling pathway
Signalling pathways regulating pluripotency of stem cells
Insulin resistance
Starch and sucrose metabolism
TGF−beta signalling pathway
Pentose phosphate pathway
Biosynthesis of unsaturated fatty acids
Fructose and mannose metabolism
Cysteine and methionine metabolism
B cell receptor signalling pathway
PPAR signalling pathway
NF−kappa B signalling pathway
Prolactin signalling pathway
Linoleic acid metabolism
Gene ratio
EPI vs PE (Human)
Pentose phosphate pathway
Starch and sucrose metabolism
Signalling pathways regulating pluripotency of stem cells
PPAR signalling pathway
ECM−receptor interaction
Thyroid hormone signalling pathway
GnRH signalling pathway
Focal adhesion
MAPK signalling pathway
AGE−RAGE signalling pathway in diabetic complications
PI3K−Akt signalling pathway
Sphingolipid signalling pathway
Metabolic pathways
Thyroid hormone synthesis
Estrogen signalling pathway
Prolactin signalling pathway
Gene ratio
-log10(P-value)
abs(NES)
b
EPI vs TE (Mouse)
Tight junction
Cysteine and methionine metabolism
Signalling pathways regulating pluripotency of stem cells
Hippo signalling pathway
Sphingolipid metabolism
Pentose phosphate pathway
Galactose metabolism
Focal adhesion
Pyrimidine metabolism
Rap1 signalling pathway
Ras signalling pathway
cGMP−PKG signalling pathway
Chemokine signalling pathway
Hedgehog signalling pathway
cAMP signalling pathway
Oxytocin signalling pathway
Gene ratio
EPI vs PE (Mouse)
Amino sugar and nucleotide sugar metabolism
Thyroid hormone synthesis
N−Glycan biosynthesis
Tight junction
ECM−receptor interaction
Galactose metabolism
Cholesterol metabolism
Nitrogen metabolism
Signalling pathways regulating pluripotency of stem cells
Alanine, aspartate and glutamate metabolism
Steroid biosynthesis
Rap1 signalling pathway
Glycolysis / Gluconeogenesis
Focal adhesion
PI3K−Akt signalling pathway
Longevity regulating pathway
Glyoxylate and dicarboxylate metabolism
Relaxin signalling pathway
p53 signalling pathway
PPAR signalling pathway
Gene ratio
c
Hippo signalling pathway
JAK−STAT signalling pathway
MAPK signalling pathway
Rap1 signalling pathway
PI3K−Akt signalling pathway
Species
Comparison
log2(Fold Change)
Guinea pig
Human
Mouse
EPI vs PE
EPI vs TE
*: DEG

Supplementary Fig. 4 Signalling pathways related to lineage segregation in guinea pig, human, and mouse. a and **b**, Dot plots of KEGG terms showing the results from Gene Set Enrichment Analysis (GSEA) for the EPI-TE comparison and EPI-PE comparison in human (a) and mouse (b), with P values (one-sided, permutation test) less than 0.05 indicated. The dot size indicates the absolute values of normalized enrichment score (NES), colour represents the P value, and the x-axis shows the ratio of DEGs corresponding to each KEGG term. **c**, Gene regulation associated with the Hippo signalling pathway, JAK-STAT signalling pathway, MAPK signalling pathway, Rap1 signalling pathway, and PI3K-AKT signalling pathway in guinea pigs, humans, and mice. The log2(fold change) of genes from the EPI-TE and EPI-PE comparisons is represented by colour, with DEGs identified by Bonferroni adjusted P values (two-sided, Wilcoxon test) less than 0.05 and log2(fold change) more than 0.25 marked by an asterisk (*). Species and lineage comparisons are indicated in the heatmap.



Supplementary Fig. 5 Dose-response of LATs inhibitor in mouse embryos. a, Schematic of TRULI treatments in mouse embryos. **b**, Representative immunofluorescence images of Sox2 (yellow), Cdx2 (gray), aYAP (cyan) and Hoechst nuclear staining (blue) in control (DMSO) and LATs inhibitor-treated mouse embryos with different concentrations of TRULI inhibitor (2-20 µM) from 8 cell E2.25 to blastocyst E3.5 (n = 5 for control and n = 3 for each TRULI dose). **c**, Scatter plot showing the number of cells per embryo for the indicated markers, P values stated in each figure (Kruskal-Wallis test). Schematic created in BioRender.com. Scale bars: 20 µm.



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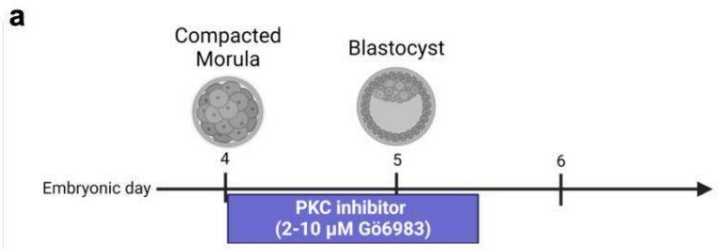
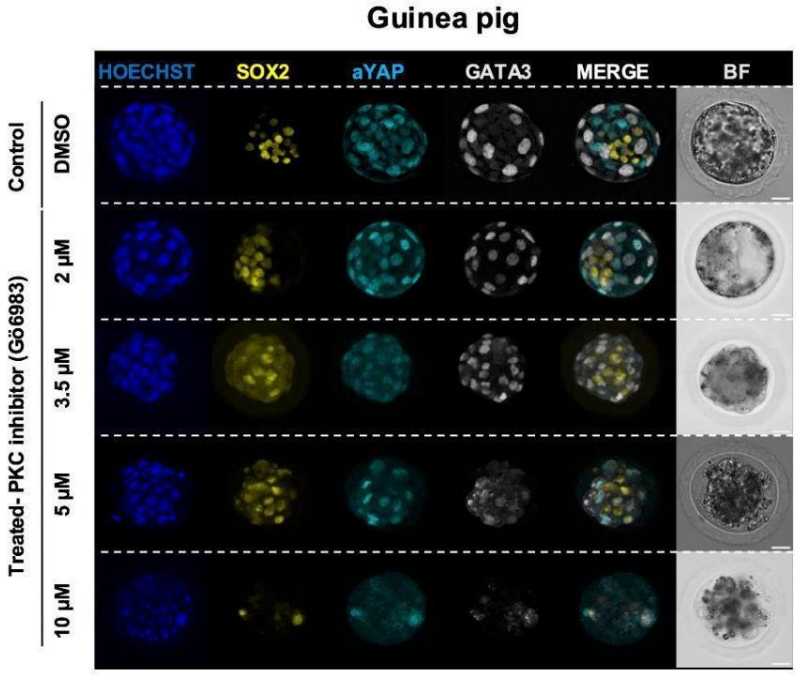
78 **Supplementary Fig. 6 Hippo and MEK-ERK signalling pathways in mouse embryos. a**, Schematic of the treatment
79 protocol for individual small molecules used with mouse embryos. **b**, Representative immunofluorescence image of Sox2
80 (yellow), aYAP (cyan), Gata3 (gray) and Hoechst nuclear staining (blue) in control (DMSO) and LATS inhibitor (5 μM of
81 TRULI) treated mouse embryos from 8-cell compacted morula to mid-blastocyst. **c**, Scatter plot showing the total number of
82 cells per embryo (Hoechst stained) and the number of cells per embryo for the indicated markers in control (n = 4) and TRULI
83 (n = 5) treated embryos. P values stated in each figure (two-tailed Mann-Whitney test). **d**, Representative immunofluorescence

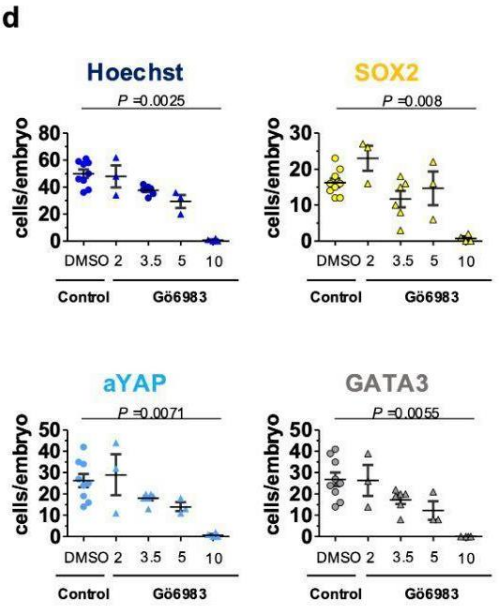
image of Sox2 (yellow), aYAP (cyan), Gata3 (gray) and Hoechst nuclear staining (blue) in control (DMSO) and LATS inhibited (5 µM of TRULI) treated mouse embryos from 16-cell compacted morula to late blastocyst. **e**, Scatter plot showing the total number of cells per embryo (Hoechst stained) and the number of cells per embryo for the indicated markers in control (n = 6) and TRULI (n = 7) treated embryos, P values stated in each figure. **f**, Representative immunofluorescence image of Sox2 (yellow), Sox17 (magenta), Cdx2 (cyan) and Hoechst nuclear staining (gray) in control (DMSO) and MEK-ERK inhibitor (1 µM of PD0325901) mouse embryos. g, Scatter plot showing the total number of cells per embryo (Hoechst stained) and the number of cells per embryo for the indicated markers in control (n = 6) and PD0325901 (n = 3) treated embryos. P values stated in each figure (two-tailed Mann–Whitney test for a two-group comparison). Schematic created in BioRender.com. Scatter plots with mean ± s.e.m. Scale bars: 20 µm.

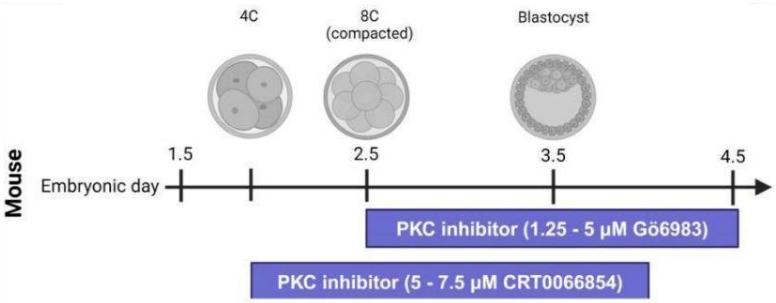


b

Medium	N	Number (%) of arrested embryos	Number (%) of morula	Number (%) of blastocyst
DMSO	12	1 (8)	2 (17)	9 (75)
2 µM Gö6983	3	0 (0)	1 (33)	2 (67)
3.5 µM Gö6983	6	0 (0)	6 (100)	0 (0)
5 µM Gö6983	3	0 (0)	3 (100)	0 (0)
10 µM Gö6983	4	3 (75)	0 (0)	1 (25)

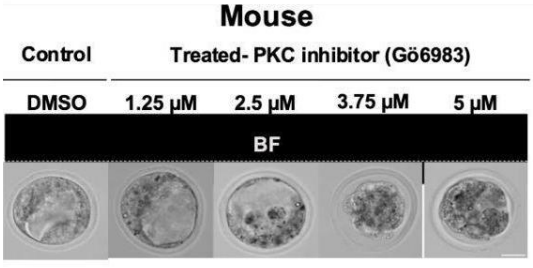


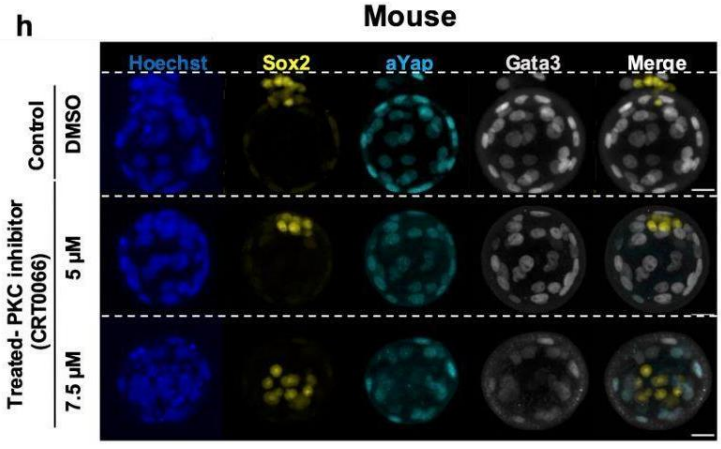




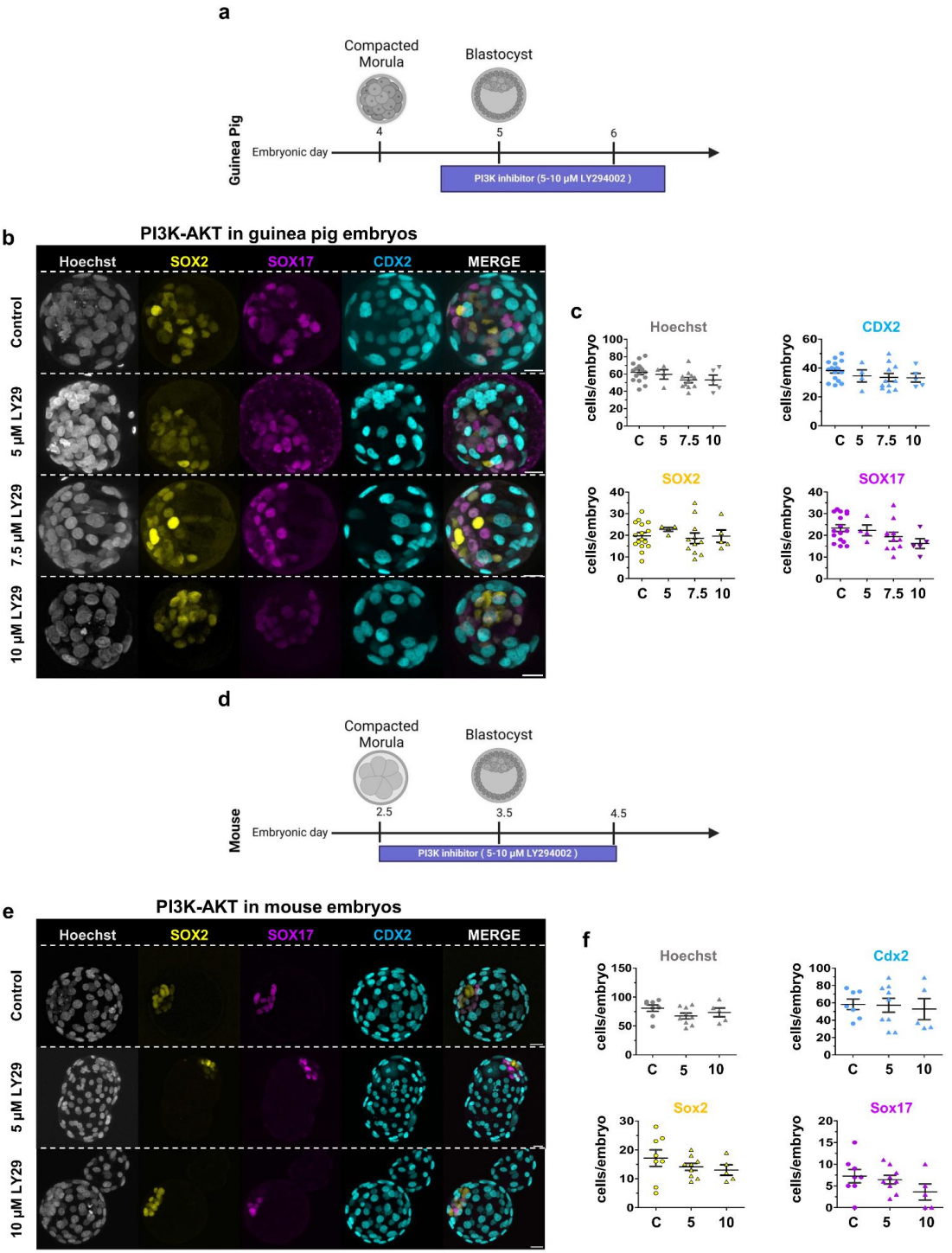
f

Medium	N	Number (%) of arrested embryos	Number (%) of morula	Number (%) of blastocyst
DMSO	8	0 (0)	2 (25)	6 (75)
1.25 µM Gö6983	5	0 (0)	2 (37)	3 (63)
2.5 µM Gö6983	7	0 (0)	2 (29)	5 (71)
3.75 µM Gö6983	5	0 (0)	5 (100)	0 (0)
5 µM Gö6983	5	5 (100)	0 (0)	0 (0)
DMSO	3	0 (0)	0 (0)	3 (100)
5 µM CRT0066854	3	0 (0)	1 (33)	2 (67)
7.5 µM CRT0066854	4	1 (25)	3 (75)	0 (0)



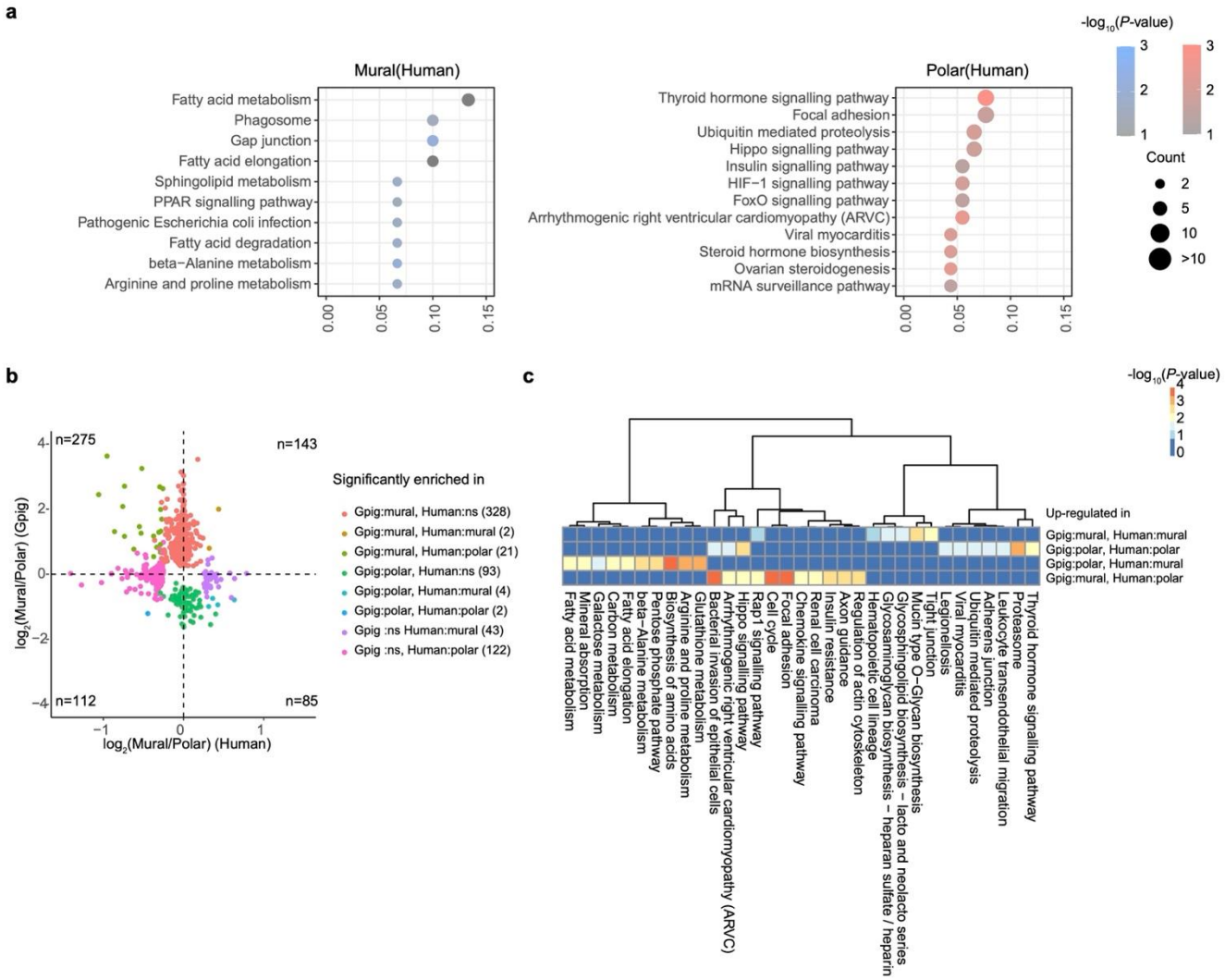


Supplementary Fig. 7 Dose-response of PKC inhibitor treatment in guinea pig and mouse embryos. a, Schematic of dose-response of PKC inhibitor (Gö6983) in guinea pigs. **b**, The percentage of guinea pig embryos either developing to form a blastocyst or arrested morula in control and Gö6983 treated embryos. **c**, Representative immunofluorescence image of SOX2 (yellow), aYAP (cyan), GATA3 (gray) and Hoechst nuclear staining (blue) in control (DMSO) and PKC inhibitor (Gö6983) treated guinea pig embryos from 16-cell compacted morula to mid-blastocyst. **d**, Scatter plot showing the total number of cells per embryo (Hoechst stained) and the number of cells per embryo for the indicated markers in control (n = 12) and Gö6983 treated embryos at 2 µM (n = 3), 3.5 µM (n = 6), 5 µM (n = 3) and 10 µM (n = 4). **e,** Schematic of dose-response of PKC inhibitors (Gö6983 and CRT0066854) in mice. **f**, The percentage of guinea pig embryos that developed to blastocyst or arrested at the morula stage in control and PKC inhibitors-treated embryos. **g,** Bright-field representative images of mouse embryos at E3.5 for control (n = 8) and Gö6983 treated at 1.25 µM (n = 5), 2.5 µM (n = 7), 3.75 µM (n = 5) and 5 µM (n = 5) µM. **h,** Representative immunofluorescence image of Sox2 (yellow), aYAP (cyan), Gata3 (gray) and Hoechst nuclear staining (blue) in control (n = 3) and CRT066854 treated mouse embryos at 5 µM (n = 3) and 7.5 µM (n = 4). P values stated in each figure (Kruskal-Wallis test with Dunn's post-test). Scatter plots with mean ± s.e.m. Schematic created in BioRender.com. Scale bars: 20 µm.

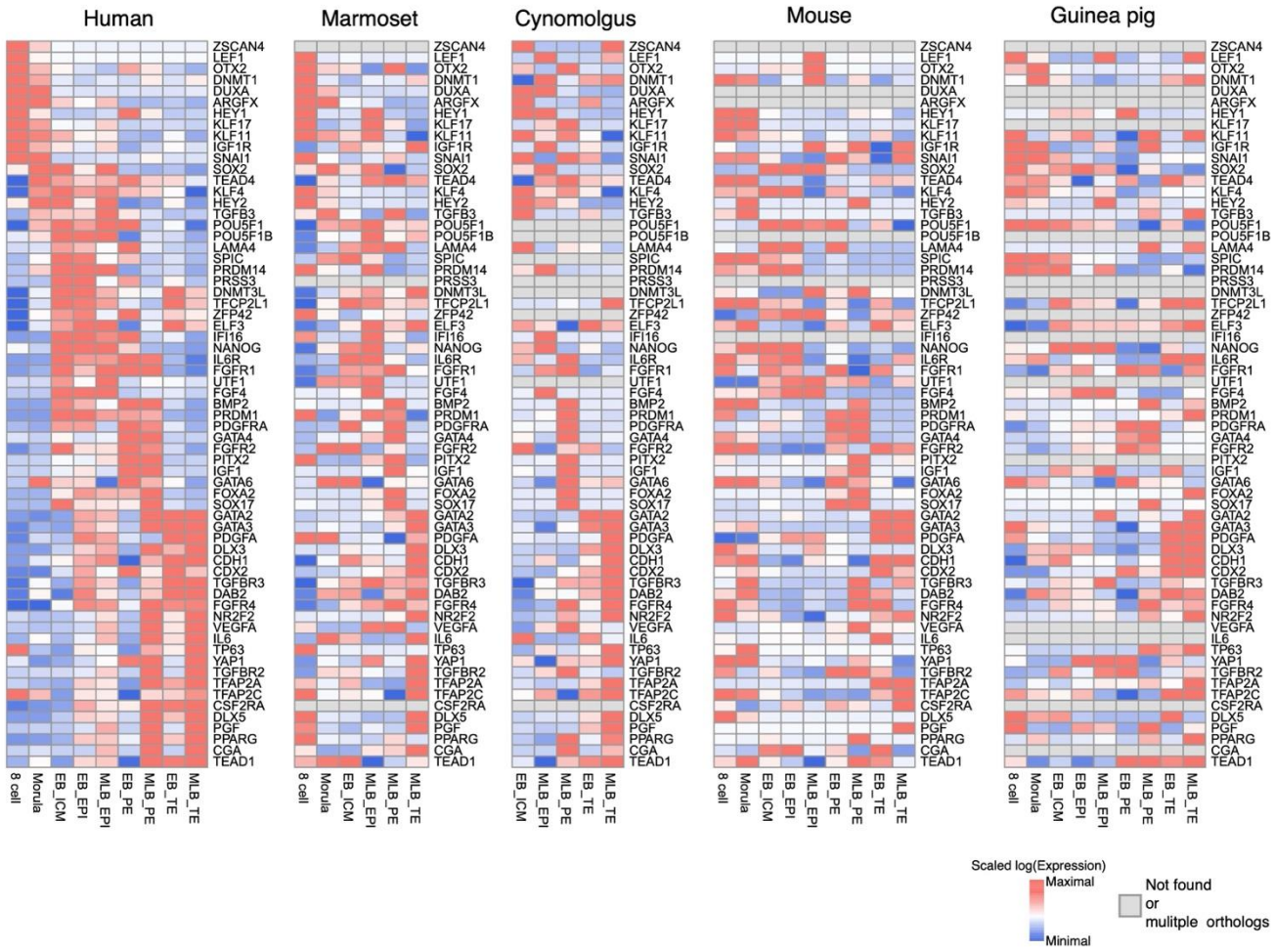


Supplementary Fig. 8 Dose-response of PI3K-AKT inhibitor in guinea pig and mouse embryos. **a**, Schematic of PI3K inhibitor (LY294002) treatment in guinea pig embryos. **b**, Representative immunofluorescence image of SOX2 (yellow), SOX17 (magenta), CDX2 (cyan) and Hoechst nuclear staining (gray) in control (DMSO) and LY29 treated guinea pig embryos for 48 h. **c**, Scatter plot showing the total number of cells per embryo (Hoechst stained) and the number of cells per embryo for the indicated markers in control embryos (n = 16) and LY29 treated embryos at 5 µM (n = 4), 7.5 µM (n = 11) and 10 µM (n = 5). **d**, Schematic of dose-response of PI3K inhibitor (LY294002) in mice. **e,** Representative immunofluorescence image of Sox2 (yellow), Sox17 (magenta), Cdx2 (cyan) and Hoechst nuclear staining (gray) in control (DMSO) and LY29 treated mouse embryos. **f**, Scatter plot showing the total number of cells per embryo (Hoechst stained)

and the number of cells per embryo for the indicated markers in control (n = 8) and LY29 treated embryos at 5 µM (n = 9) and 10 µM (n = 5). No significant differences (Kruskal-Wallis test). Schematic created in BioRender.com. Scatter plots with mean ± s.e.m. Scale bars: 20 µm.



Supplementary Fig. 9 Expression of mural and polar DEGs in guinea pig and human mural and polar cells. **a**, Dot plot illustrating the enriched KEGG pathways for DEGs between mural and polar TE cells from Human (P < 0.05, one-sided, permutation test). Colour and size indicate the significance and number of differentially expressed genes in each pathway. **b**, Log2 fold change between mural and polar cells in human and guinea pig (x-axis and y-axis, respectively). Colours indicate the differential expression status in the guinea pig and human. The number of genes is indicated behind the colour legend. "n" in each corner represents the total number of genes in each quadrant. "ns" denotes "not significant." **c**, Heatmap illustrating the significance of enriched KEGG pathways for genes associated with differential expression status in guinea pig and human mural and polar cells.



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133 **Supplementary Fig. 10: Heatmap depicting the pseudo-bulk expression of selected genes, categorized by stages and**
134 **lineages, across human, marmoset, cynomolgus monkey, guinea pig, and mouse species.** Gene selection was adapted
135 from Meistermann et al., 2021 gene module analysis. Genes that could not be detected or with multiple orthologs in the
136 corresponding species are coloured as gray. Preimplantation embryo data was leveraged from Petropoulos et al., 2016
137 (human), Nakamura et al., 2016 (cynomolgus monkey), Boroviak et al., 2018 (marmoset), Bergmann et al., 2022 (marmoset),
138 Deng et al., 2014 (mouse), Nowotschin et al.,2019 (mouse), guinea pig data generated in this study.

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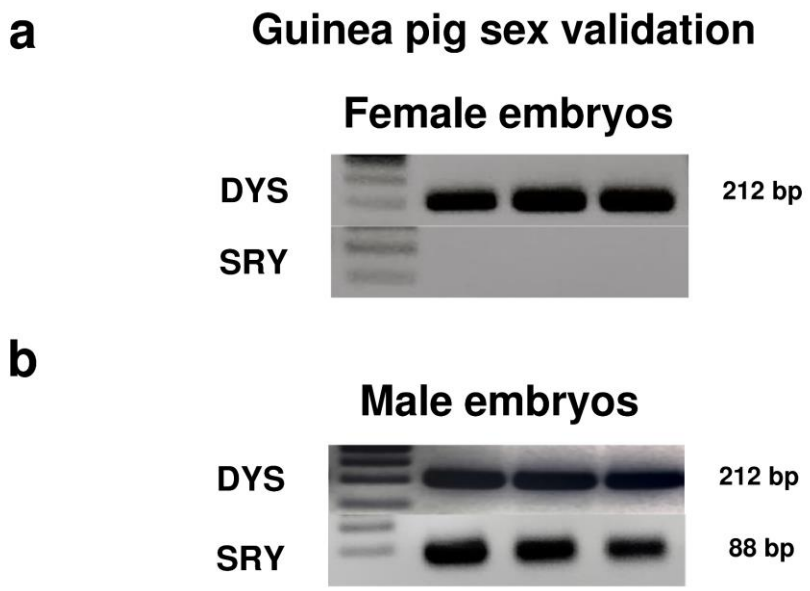

Supplementary Fig. 11: Guinea Pig Blastocysts Sex Validation. a and **b** Representative image of agarose gel following PCR of DYS and SRY genes for sex determination of three female (E1, E2 and E3) and three male (E4, E5 and E6) embryos used for H3Kme27 analysis. Amplicon of DYS is 212 bp and of SRY 88 bp.

Source Data:

Supplementary Figure 5c

Mouse TRULI dose-response
(Number of cells per embryo)

Hoechst				
DMSO	**2 µM TRULI**	**5 µM TRULI**	**10 µM TRULI**	**20 µM TRULI**
45	44	29	15	15
35	16	37	32	12
36	34	30	25	14
36				
38				

Sox2				
DMSO	**2 µM TRULI**	**5 µM TRULI**	**10 µM TRULI**	**20 µM TRULI**
18	0	0	0	0
10	0	0	0	0
6	0	0	0	0
12				
15				

aYap				
DMSO	**2 µM TRULI**	**5 µM TRULI**	**10 µM TRULI**	**20 µM TRULI**
28	32	26	15	14
23	15	37	32	12
18	26	29	25	13
14				
16				

Gata3				
DMSO	**2 µM TRULI**	**5 µM TRULI**	**10 µM TRULI**	**20 µM TRULI**
27	32	23	15	1
17	15	37	32	8
20	25	29	25	1
14				
16				

Supplementary Figure 6c

Mouse

Hippo signalling inhibition with optimal dose at two different time-points

From 8C to Blastocyst E3.5

(Number of cells per embryo)

Hoechst	
DMSO	**5 µM TRULI**
35	37
36	54
32	30
34	27
	46

Gata3			
Outer		**Inner**	
DMSO	**5 µM TRULI**	**DMSO**	**5 µM TRULI**
17	43	0	10
18	32	0	5
20	23	0	4
24	21	0	6
	33		7
	26		6

Sox2			
Outer		**Inner**	
DMSO	**5 µM TRULI**	**DMSO**	**5 µM TRULI**
0	0	10	0
0	0	6	0
0	0	8	0
0	0	10	0
	0		1
	0		0

aYap			
Outer		**Inner**	
DMSO	**5 µM TRULI**	**DMSO**	**5 µM TRULI**
17	23	0	4
15	32	0	5
18	40	0	9
22	21	0	6
	35		6
	26		6

Supplementary Figure 6e

From 16C to Blastocyst E4.5

(Number of cells per embryo)

Hoechst	
DMSO	**5 µM TRULI**
105	72
96	88
84	103
84	86
106	98
111	82
	90

Gata3			
Outer		**Inner**	
DMSO	**5 µM TRULI**	**DMSO**	**5 µM TRULI**
75	63	0	6
80	80	0	8
59	95	0	8
58	72	0	10
88	68	0	12
96	65	0	10
	73		9

Sox2			
Outer		**Inner**	
DMSO	**5 µM TRULI**	**DMSO**	**5 µM TRULI**
0	0	16	2
0	0	13	0
0	0	11	2
0	0	25	0
0	0	9	0
0	0	13	0
	0		0

aYap			
Outer		**Inner**	
DMSO	**5 µM TRULI**	**DMSO**	**5 µM TRULI**
74	62	0	8
71	93	0	7
60	82	0	6
53	62	0	10
89	56	0	13
96	63	0	10
	71		9

Supplementary Figure 6g

MAPK signalling inhibition (PD0325901) with optimal dose

(Number of cells per embryo)

Hoechst	
DMSO	**1 µM PD032**
171	146
105	109
88	115
90	
108	
95	

Cdx2	
DMSO	**1 µM PD032**
124	99
78	98
60	82
60	
80	
68	

Sox2	
DMSO	**1 µM PD032**
23	47
11	29
16	33
17	
14	
15	

Sox17	
DMSO	**1 µM PD032**
24	0
16	0
12	0
13	
14	
12	

Supplementary Figure 7b

Medium	N	Number (%) of arrested embryos	Number (%) of morula	Number (%) of blastocyst
DMSO	12	1 (8)	2 (17)	9 (75)
2 µM Gö6983	3	0 (0)	1 (33)	2 (67)
3.5 µM Gö6983	6	0 (0)	6 (100)	0 (0)
5 µM Gö6983	3	0 (0)	3 (100)	0 (0)
10 µM Gö6983	4	3 (75)	0 (0)	1 (25)

Supplementary Figure 7d

Guinea pig Dose-response of PKC inhibitor (Gö6983)

(Number of cells per embryo)

Hoechst				
DMSO	**2 µM**	**3.5 µM**	**5 µM**	**10 µM**
58	62	35	20	2
57	48	39	32	0
59	34	40	36	1
50		38		0
45		42		
36		32		
46				
38				
61				

SOX2				
DMSO	**2 µM**	**3.5 µM**	**5 µM**	**10 µM**
16	26	3	6	2
12	27	8	16	0
16	16	15	22	1
18		16		0
23		18		
12		10		
14				
15				
20				

aYAP				
DMSO	**2 µM**	**3.5 µM**	**5 µM**	**10 µM**
25	32	18	13	2
42	44	20	18	1
35	11	13	11	0
27		19		0
19		18		
14		20		
24				
16				
34				

GATA3				
DMSO	**2 µM**	**3.5 µM**	**5 µM**	**10 µM**
25	26	22	8	0
39	39	20	21	0
35	14	8	8	0
27		19		0
21		20		
14		15		
24				
16				
41				

Supplementary Figure 7e

Mouse PKC inhibitor Dose-response

Medium	N	Number (%) of arrested embryos	Number (%) of morula	Number (%) of blastocyst
DMSO	8	0 (0)	2 (25)	6 (75)
1.25 µM Gö6983	5	0 (0)	2 (37)	3 (63)
2.5 µM Gö6983	7	0 (0)	2 (29)	5 (71)
3.75 µM Gö6983	5	0 (0)	5 (100)	0 (0)
5 µM Gö6983	5	5 (100)	0 (0)	0 (0)

DMSO	3	0 (0)	0 (0)	3 (100)
5 µM CRT0066	3	0 (0)	1 (33)	2 (67)
7.5 µM CRT0066	4	1 (25)	3 (75)	0 (0)

Supplementary Figure 8c

Guinea Pig PI3K Dose-response
Inhibition (LY294002) for 48h
(Number of cells per embryo)

Hoechst			
DMSO	**5 µM LY29**	**7.5 µM LY29**	**10 µM LY29**
81	69	75	68
78	44	61	44
72	65	45	38
42	60	46	64
66		48	51
64		38	
65		46	
58		57	
62		56	
61		58	
69		52	
67			
53			
56			
46			
52			

CDX2			
DMSO	**5 µM LY29**	**7.5 µM LY29**	**10 µM LY29**
40	37	50	32
43	24	45	28
50	44	31	27
29	33	28	36
39		28	43
31		25	
39		41	
33		26	
28		39	
40		24	
37		31	
47			
29			
45			
37			
44			

SOX2			
DMSO	**5 µM LY29**	**7.5 µM LY29**	**10 µM LY29**
31	24	35	30
27	20	31	16
18	24	12	17
22	23	19	14
26		11	21
26		14	
8		9	
15		18	
20		16	
25		23	
17		17	
12			
17			
21			
16			
15			

SOX17			
DMSO	**5 µM LY29**	**7.5 µM LY29**	**10 µM LY29**
31	29	34	24
32	17	28	15
20	21	14	15
21	22	21	10
24		15	17
31		14	
22		10	
15		18	
17		18	
30		21	
31		20	
28			
18			
22			
16			
15			

Supplementary Figure 8f

Mouse PI3K Dose-response
Inhibition (LY294002) for 48h
(Number of cells per embryo)

Hoechst		
DMSO	**5 µM LY29**	**10 µM LY29**
89	70	76
82	87	60
91	85	54
92	51	81
49	56	96
67	65	
81	63	
95	46	
	82	

Cdx2		
DMSO	**5 µM LY29**	**10 µM LY29**
72	64	37
73	79	32
52	72	31
56	89	75
42	26	89
36	41	
77	41	
	26	
	77	

Sox2		
DMSO	**5 µM LY29**	**10 µM LY29**
28	15	12
16	9	19
24	13	9
23	10	10
5	18	15
16	15	
18	20	
7	16	
	11	

Sox17		
DMSO	**5 µM LY29**	**10 µM LY29**
15	7	3
10	8	0
5	10	0
7	11	10
0	5	5
5	3	
9	2	
7	6	
	6	

Supplementary Figure 11