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Supplemental Information

Multimodal characterization of murine gastruloid development

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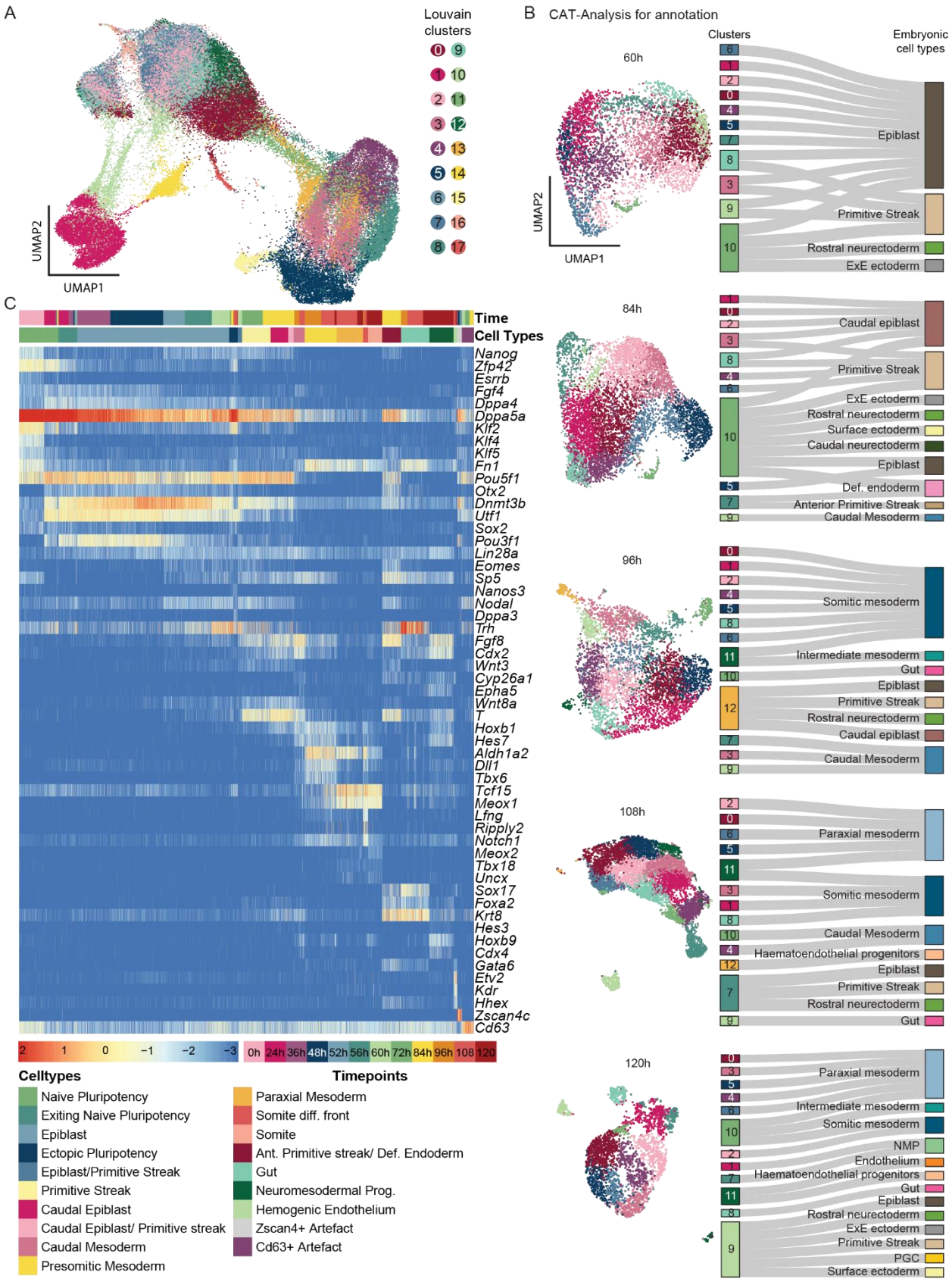
A
Louvain clusters
0 9
1 10
2 11
3 12
4 13
5 14
6 15
7 16
8 17
UMAP2
UMAP1
B
CAT-Analysis for annotation
Clusters
Embryonic cell types
60h
Epiblast
Primitive Streak
Rostral neurectoderm
ExE ectoderm
84h
Caudal epiblast
Primitive Streak
ExE ectoderm
Rostral neurectoderm
Surface ectoderm
Caudal neurectoderm
Epiblast
Def. endoderm
Anterior Primitive Streak
Caudal Mesoderm
96h
Somitic mesoderm
Intermediate mesoderm
Gut
Epiblast
Primitive Streak
Rostral neurectoderm
Caudal epiblast
Caudal Mesoderm
108h
Paraxial mesoderm
Somitic mesoderm
Caudal Mesoderm
Haematoendothelial progenitors
Epiblast
Primitive Streak
Rostral neurectoderm
Gut
120h
Paraxial mesoderm
Intermediate mesoderm
Somitic mesoderm
NMP
Endothelium
Haematoendothelial progenitors
Gut
Epiblast
Rostral neurectoderm
ExE ectoderm
Primitive Streak
PGC
Surface ectoderm
C
Time
Cell Types
Nanog
Zfp42
Esrrb
Fgf4
Dppa4
Dppa5a
Klf2
Klf4
Klf5
Fn1
Pou5f1
Otx2
Dnmt3b
Utf1
Sox2
Pou3f1
Lin28a
Eomes
Sp5
Nanos3
Nodal
Dppa3
Trh
Fgf8
Cdx2
Wnt3
Cyp26a1
Epha5
Wnt8a
T
Hoxb1
Hes7
Aldh1a2
Dll1
Tbx6
Tcf15
Meox1
Lfng
Ripply2
Notch1
Meox2
Tbx18
Uncx
Sox17
Foxa2
Krt8
Hes3
Hoxb9
Cdx4
Gata6
Etv2
Kdr
Hhex
Zscan4c
Cd63
2 1 0 −1 −2 −3
0h 24h 36h 48h 52h 56h 60h 72h 84h 96h 108 120
Celltypes
Naive Pluripotency
Exiting Naive Pluripotency
Epiblast
Ectopic Pluripotency
Epiblast/Primitive Streak
Primitive Streak
Caudal Epiblast
Caudal Epiblast/ Primitive streak
Caudal Mesoderm
Presomitic Mesoderm
Timepoints
Paraxial Mesoderm
Somite diff. front
Somite
Ant. Primitive streak/ Def. Endoderm
Gut
Neuromesodermal Prog.
Hemogenic Endothelium
Zscan4+ Artefact
Cd63+ Artefact

Figure S1 scRNA-seq time course of gastruloid development: Cell type annotation. ***Related to Figure 1***

(A) UMAP of single-cell transcriptomes from gastruloids highlighting Louvain clusters.

(B) UMAPs highlighting gastruloid clusters from individual timepoints (Left). Sankey plots summarising CAT analysis at individual timepoints. Thickness of the matching bands is directly proportional to the Euclidean distance, i.e, the thicker the band, the greater the Euclidean distance.

(C) Heatmap showing the expression of cell type markers. Scalebar, log2-transformed normalised expression. Ant. Primitive streak/ Def. Endoderm (Anterior Primitive streak/ Definitive Endoderm).

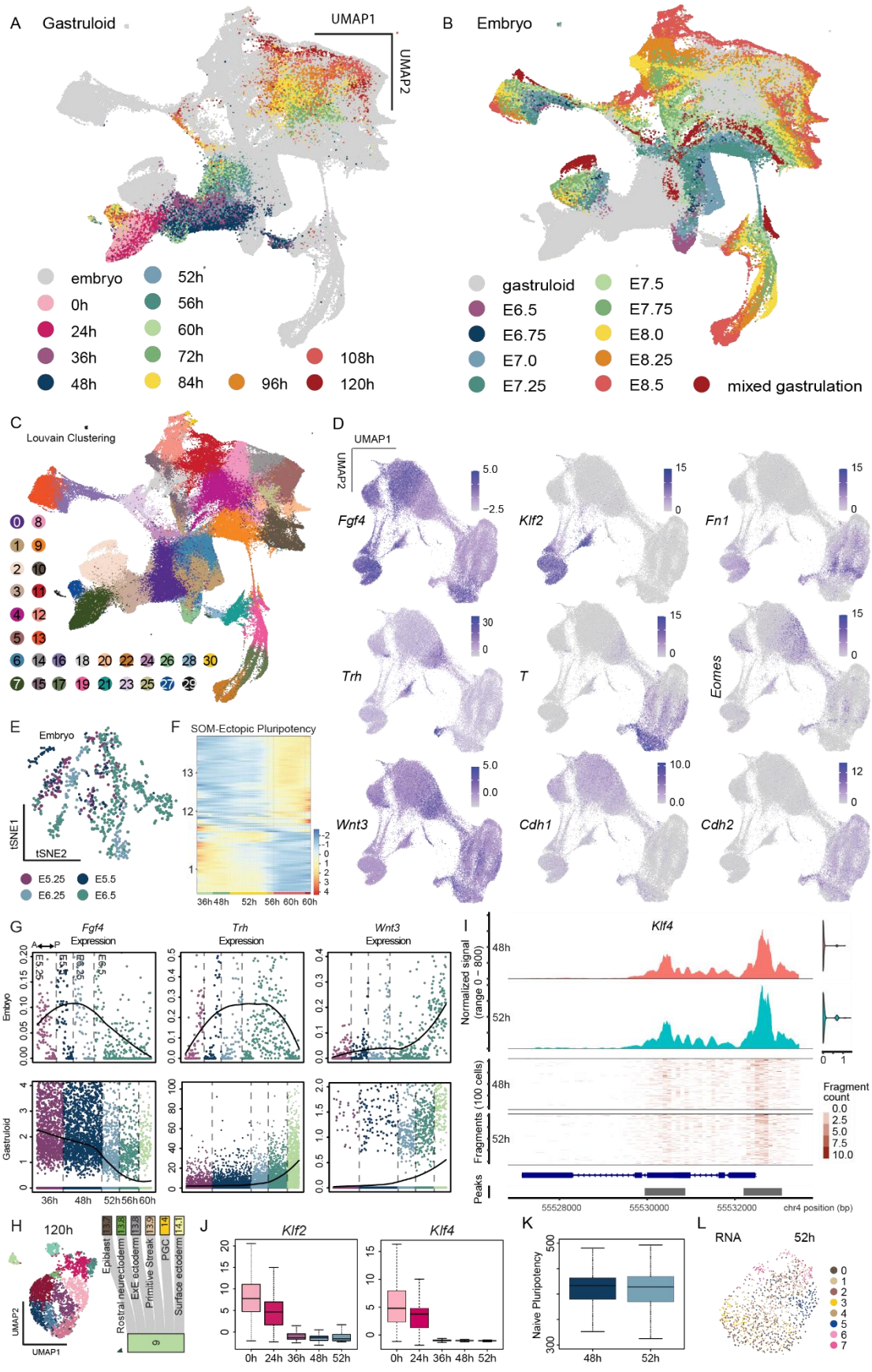
A
Gastruloid
UMAP1
UMAP2
embryo
0h
24h
36h
48h
52h
56h
60h
72h
84h
96h
108h
120h
B
Embryo
gastruloid
E6.5
E6.75
E7.0
E7.25
E7.5
E7.75
E8.0
E8.25
E8.5
mixed gastrulation
C
Louvain Clustering
D
UMAP1
UMAP2
Fgf4
Klf2
Fn1
Trh
T
Eomes
Wnt3
Cdh1
Cdh2
E
Embryo
tSNE1
tSNE2
E5.25
E5.5
E6.25
E6.5
F
SOM-Ectopic Pluripotency
36h 48h 52h 56h 60h 60h
G
Fgf4 Expression
Trh Expression
Wnt3 Expression
Embryo
Gastruloid
36h 48h 52h 56h 60h
I
Klf4
Normalized signal (range 0 – 800)
48h
52h
Fragments (100 cells)
Fragment count
Peaks
55528000
55530000
55532000
chr4 position (bp)
H
120h
UMAP1
UMAP2
Epiblast
Rostral neuroectoderm
ExE ectoderm
Primitive Streak
PGC
Surface ectoderm
J
Klf2
Klf4
0h 24h 36h 48h 52h
K
Naive Pluripotency
48h
52h
L
RNA
52h

Figure S2 *In vivo* comparison and characterization of different epiblast and pluripotency states. *Related to Figure 2*

(A) UMAP of the integration and co-embedding of gastruloid and embryonic cells highlighting gastruloid timepoints.

(B) UMAP of the integration and co-embedding of gastruloid and embryonic cells highlighting embryonic timepoints.

(C) UMAP of the integration and co-embedding of gastruloid and embryonic cells highlighting Louvain clusters.

(D) Expression UMAPs of *Fgf4, Trh, Wnt3, Klf2, T, Cdh1, Fn1, Eomes*, and *Cdh2*.

(E) t-SNE map of single-cell transcriptomes from Cheng *et al.* 2019[1] dataset highlighting sampling timepoints.

(F) SOM of temporal profiles for the ectopic pluripotency population. Bottom: Colour bar shows timepoints.

(G) Temporal gene expression maps of *Fgf4*, *Trh*, and *Wnt3*, for embryonic and gastruloid epiblast cells. The y-axis: normalised expression.

(H) UMAP of single-cell transcriptomes from 120h highlighting clusters. Sankey plot highlighting results of the CAT analysis for EP cluster at 120h (cluster 9) in relation to the embryonic cell types. The thickness of the matching bands is directly proportional to the Euclidean distance. Greater thickness corresponds to greater Euclidian distance.

(I) Coverage plot showing the chromatin accessibility for *Klf4* and 1000 bp upstream region of transcription start site (TSS) containing the promoter for the 48h and 52h timepoints. Upper right panel: multiome RNA expression of *Klf4* in the same cells. Top panel: averaged frequency of sequenced DNA fragments within the genomic region for 48h and 52h timepoints. Middle panel shows frequency of sequenced fragments within the genomic region for single cells from 48h and 52h timepoints. The panel underneath shows gene annotation; arrows indicate the direction of transcription. Bottom panel shows peak coordinates.

(J) Boxplots showing scRNA-seq derived expression profiles of *Klf2* and *Klf4* at 0h, 24h, 36h, 48h, and 52h.

(K) Box plots showing the aggregated gene activity scores for the naive pluripotency signature in single cells.

(L) UMAP of single nucleus RNA-seq data/modality from the multiome highlighting clusters. The 52h timepoint is shown.

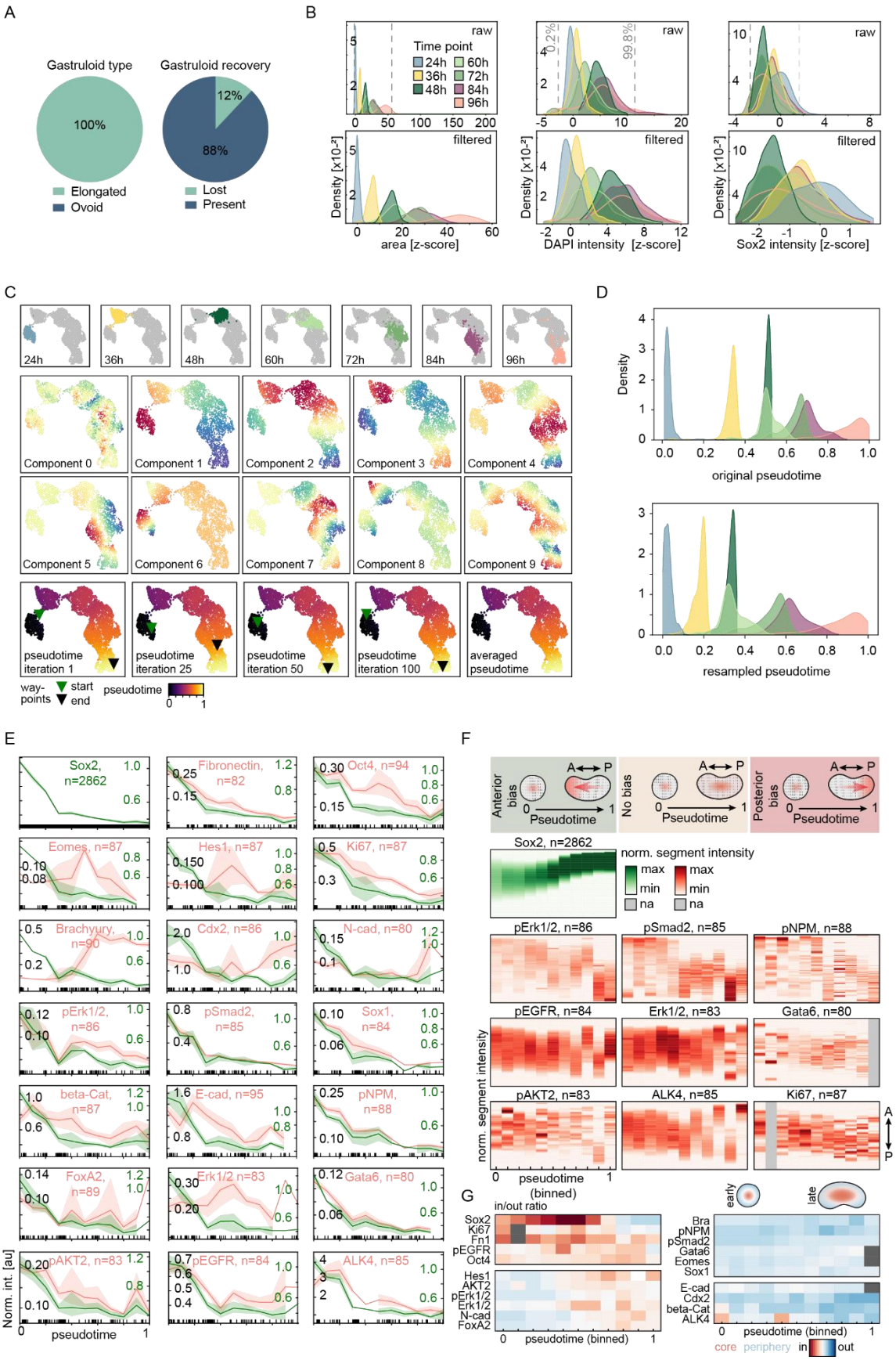
A
Gastruloid type
100%
Elongated
Ovoid
Gastruloid recovery
12%
88%
Lost
Present
B
raw
Time point
24h
36h
48h
60h
72h
84h
96h
filtered
Density [x10-3]
area [z-score]
DAPI intensity [z-score]
Sox2 intensity [z-score]
C
24h
36h
48h
60h
72h
84h
96h
Component 0
Component 1
Component 2
Component 3
Component 4
Component 5
Component 6
Component 7
Component 8
Component 9
pseudotime iteration 1
pseudotime iteration 25
pseudotime iteration 50
pseudotime iteration 100
averaged pseudotime
way-points
start
end
pseudotime
D
Density
original pseudotime
resampled pseudotime
E
Sox2, n=2862
Fibronectin, n=82
Oct4, n=94
Eomes, n=87
Hes1, n=87
Ki67, n=87
Brachyury, n=90
Cdx2, n=86
N-cad, n=80
pErk1/2, n=86
pSmad2, n=85
Sox1, n=84
beta-Cat, n=87
E-cad, n=95
pNPM, n=88
FoxA2, n=89
Erk1/2 n=83
Gata6, n=80
pAKT2, n=83
pEGFR, n=84
ALK4, n=85
Norm. int. [au]
pseudotime
F
Anterior bias
No bias
Posterior bias
A↔P
Pseudotime
Sox2, n=2862
norm. segment intensity
max
min
na
pErk1/2, n=86
pSmad2, n=85
pNPM, n=88
pEGFR, n=84
Erk1/2, n=83
Gata6, n=80
pAKT2, n=83
ALK4, n=85
Ki67, n=87
norm. segment intensity
pseudotime (binned)
G
in/out ratio
Sox2
Ki67
Fn1
pEGFR
Oct4
Hes1
AKT2
pErk1/2
Erk1/2
N-cad
FoxA2
pseudotime (binned)
early
late
Bra
pNPM
pSmad2
Gata6
Eomes
Sox1
E-cad
Cdx2
beta-Cat
ALK4
core
periphery
in
out

Figure S3. Trajectory inference of gastruloid imaging time course. ***Related to Figure 3.***

(A) Left: Assessment of elongation efficiency (elongated or ovoid) with high-throughput culture. n=329 gastruloids. Right: quantification of gastruloid-loss with high-throughput culture. n=2 plates, n=717 gastruloids.

(B) Kernel density estimation plots of selected features in gastruloids at indicated time points. Top: dashed lines show the 0.2 and 99.8 percentiles of the data distributions on the raw data. Bottom: data after clipping by percentiles. Z-score normalisation. n=3165 and 2862 individual gastruloids for raw and filtered data, respectively.

(C) UMAP plots for n=2862 individual gastruloids colour-coded by time point (top), diffusion components 0-9 (middle), or pseudotime (bottom). In bottom row, colour coding by pseudotime from individual iterations 1, 25, 50, and 100 or averaged pseudotime, respectively. For individual iterations, starting and terminal gastruloids are highlighted.

(D) Kernel density estimation plots of gastruloids from indicated time points over the original (top) and resampled (bottom) averaged pseudotime.

(E) Line plots of mean staining intensity for indicated markers and Sox2 from same gastruloids along pseudotime. n, number of gastruloids, see panel (F). Opaque interval shows standard deviation. Intensity of the marker labelled in red plotted on the main axis (left), intensity of Sox2 from the same gastruloids plotted on the secondary axes (right).

(F) Heatmaps depicting distribution of indicated stainings from anterior (top) to posterior (bottom) pole of gastruloids for indicated pseudotime intervals. n, number of gastruloids stained for indicated markers.

In E and F, rug plots depict positions of individual data points. A, anterior; a.u., arbitrary units; na, missing data point; Norm. int., normalised intensity; P, posterior; pt, pseudotime; see Table 2.

(G) Heatmaps depicting distribution of indicated stainings between inner and outer region of gastruloid middle plane (in/out ratio) at indicated pseudotime intervals.

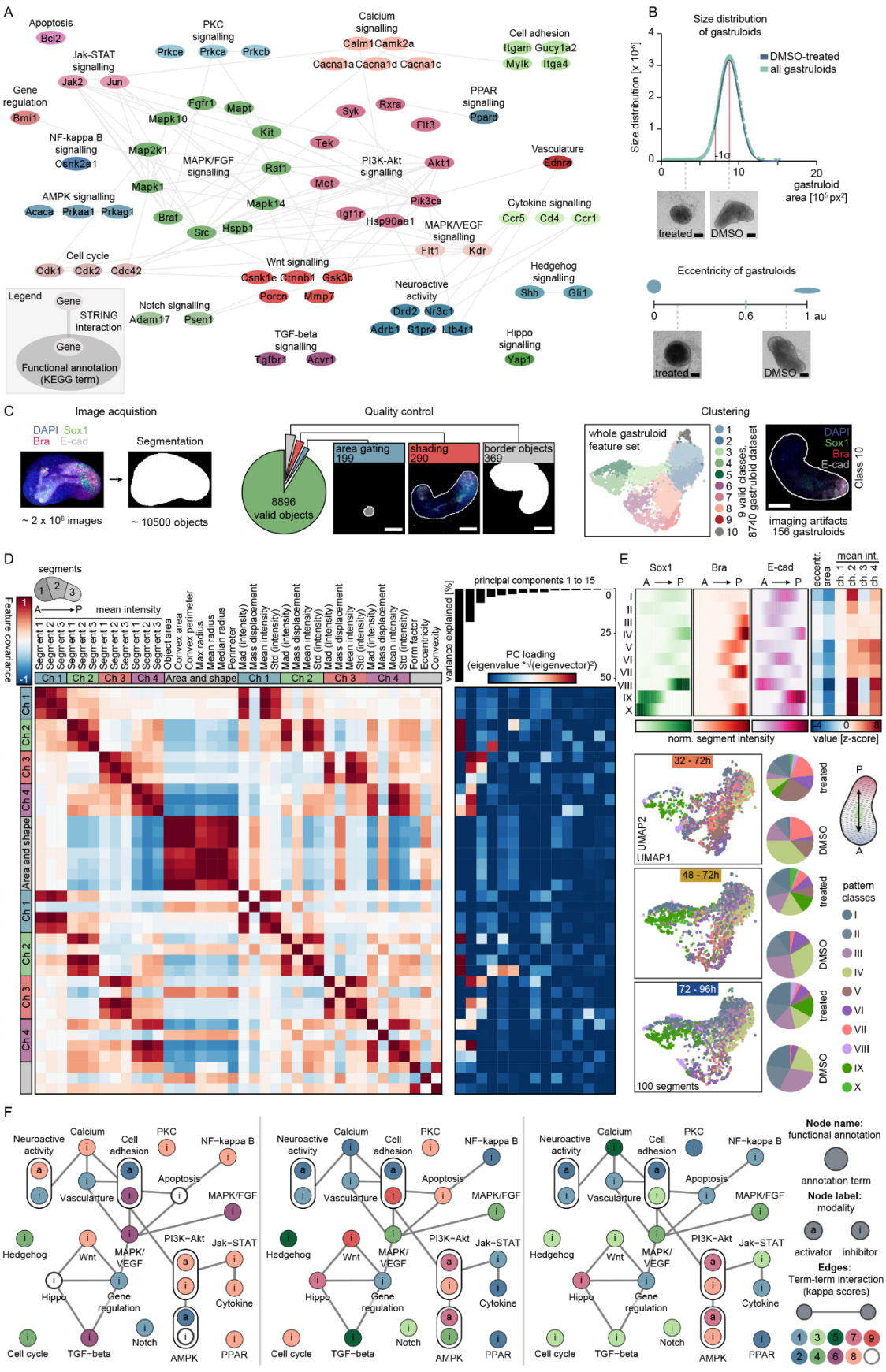
A
Apoptosis
Bcl2
PKC signalling
Prkce
Prkca
Prkcb
Calcium signalling
Calm1 Camk2a
Cacna1a Cacna1d Cacna1c
Cell adhesion
Itgam Gucy1a2
Mylk Itga4
Jak-STAT signalling
Jak2
Jun
Gene regulation
Bmi1
Fgfr1
Mapt
Mapk10
Syk
Rxra
PPAR signalling
Pparg
Flt3
Kit
Tek
NF-kappa B signalling
Csnk2a1
Map2k1
MAPK/FGF signalling
Raf1
PI3K-Akt signalling
Akt1
Vasculature
Ednra
Met
Mapk1
AMPK signalling
Acaca
Prkaa1
Prkag1
Mapk14
Pik3ca
Cytokine signalling
Ccr5
Cd4
Ccr1
Braf
Igf1r
Hsp90aa1
MAPK/VEGF signalling
Src
Hspb1
Flt1
Kdr
Cell cycle
Cdk1
Cdk2
Cdc42
Wnt signalling
Csnk1e
Ctnnb1
Gsk3b
Hedgehog signalling
Shh
Gli1
Porcn
Mmp7
Neuroactive activity
Drd2
Nr3c1
Adrb1
S1pr4
Ltb4r1
Legend
Gene
STRING interaction
Gene
Functional annotation (KEGG term)
Notch signalling
Adam17
Psen1
TGF-beta signalling
Tgfbr1
Acvr1
Hippo signalling
Yap1
B
Size distribution of gastruloids
DMSO-treated
all gastruloids
Size distribution [x 10⁻⁶]
gastruloid area [10⁵ px²]
treated
DMSO
Eccentricity of gastruloids
0
0.6
1 au
treated
DMSO
C
Image acquisition
DAPI Sox1
Bra E-cad
Segmentation
~ 2 x 10⁶ images
~ 10500 objects
Quality control
8896 valid objects
area gating 199
shading 290
border objects 369
Clustering
whole gastruloid feature set
9 valid classes, 8740 gastruloid dataset
imaging artifacts 156 gastruloids
Class 10
D
segments
mean intensity
principal components 1 to 15
variance explained [%]
PC loading (eigenvalue *√(eigenvector)²)
Ch 1
Ch 2
Ch 3
Ch 4
Area and shape
E
Sox1
Bra
E-cad
eccentr.
area
mean int.
norm. segment intensity
value [z-score]
32 - 72h
48 - 72h
72 - 96h
100 segments
UMAP1
UMAP2
treated
DMSO
pattern classes
F
Node name: functional annotation
annotation term
Node label: modality
activator
inhibitor
Edges: Term-term interaction (kappa scores)

Figure S4. Library and phenotypic classes of gastruloid screen. ***Related to Figure 4.***

(A) STRING network of annotated genes of the screening library. Nodes are colour-coded by KEGG terms. Nodes show genes and edges show STRING interactions.

(B) Top: Size distribution of gastruloids treated with pre-screening library and representative images. Bottom: Spectrum of eccentricity and representative images. Compounds causing gastruloids to be one standard deviation (s) smaller than average and below eccentricity 0.6 were chosen for final screening library.

(C) Overview of image processing, quality control, and quantification. left: image of a representative gastruloid and corresponding segmentation mask. Middle: pie chart showing distribution of objects discarded by indicated quality control gating. right: UMAP plot colour-coded by whole gastruloid class, representative image of a gastruloid from discarded class 10.

(D) Left: covariance matrix of extracted features, grouped by feature type. Right: heat map showing principal component loading of the extracted features; bar chart above, variance explained by the first 15 principal components.

(E) Top: distribution of indicated stainings from anterior (left) to posterior (right) in indicated pattern classes, heatmap view of the mean feature values for the pattern classes. Below: UMAP plot colour-coded by pattern class for gastruloids from the indicated treatment regimens (left to right: 32h-72h, 48h-72h, 72h-96h). Pie charts display abundance of each class in the respective subset for DMSO controls and other conditions separately.

(F) Networks of functional interactions of all annotated terms colour-coded by most frequent whole gastruloid class for each annotation term in indicated treatment regime (left to right: 32h-72h, 48h-72h, 72h-96h). Node name, annotation term. Node label, modality (a, activator, i, inhibitor). Node colour, most frequent whole gastruloid phenotype. White node indicates no detected phenotype. Edges, term-term interaction.

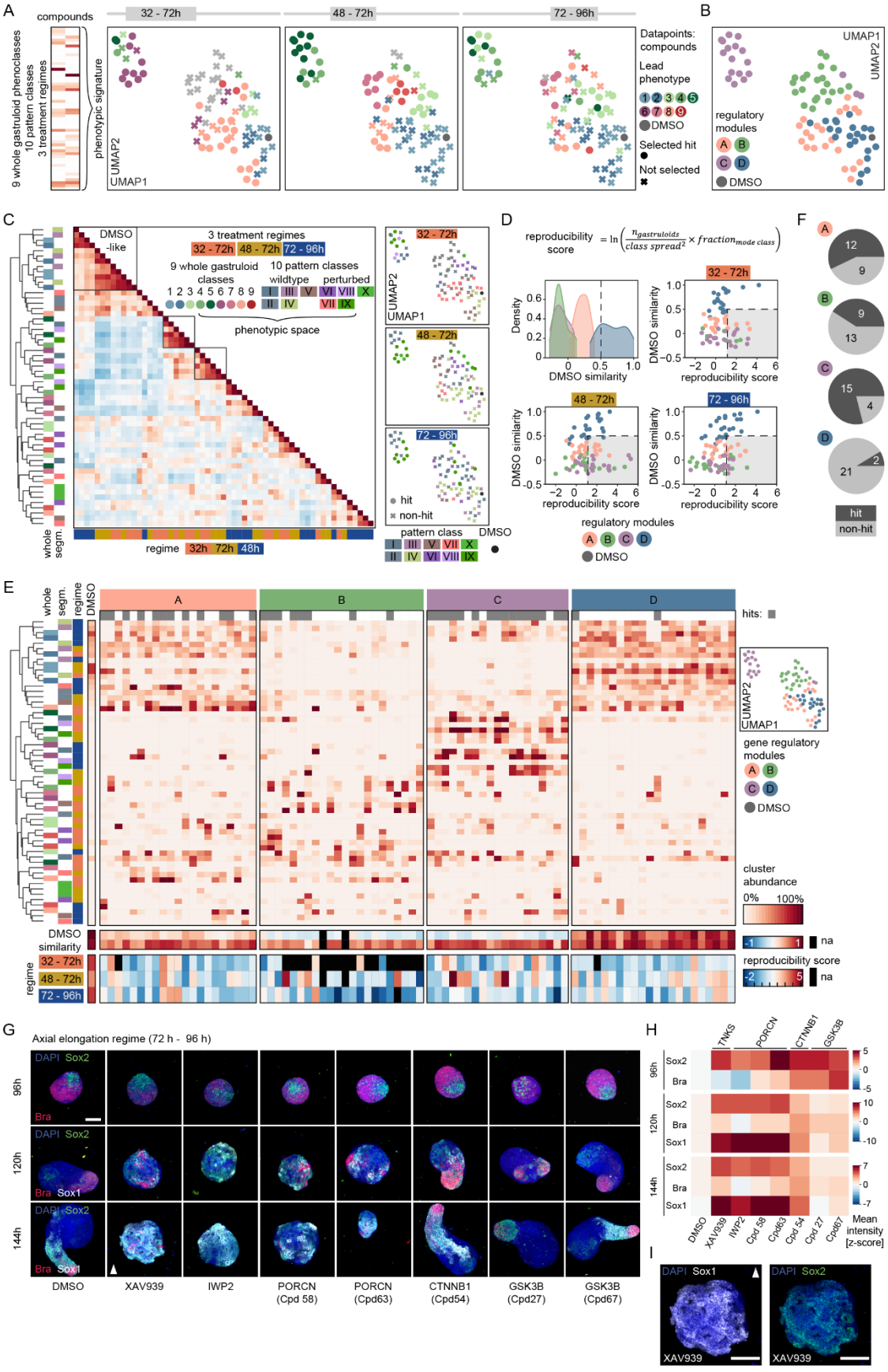
A
compounds
32 - 72h
48 - 72h
72 - 96h
9 whole gastruloid phenoclasses
10 pattern classes
3 treatment regimes
phenotypic signature
UMAP2
UMAP1
Datapoints: compounds
Lead phenotype
1 2 3 4 5
6 7 8 9
DMSO
Selected hit
Not selected
B
UMAP1
UMAP2
regulatory modules
A B
C D
DMSO
C
DMSO -like
3 treatment regimes
32 - 72h 48 - 72h 72 - 96h
9 whole gastruloid classes
1 2 3 4 5 6 7 8 9
10 pattern classes
wildtype
perturbed
I III V VI VIII X
II IV VII IX
phenotypic space
hit
non-hit
whole segm.
regime 32h 72h 48h
pattern class
DMSO
D
reproducibility score
$= \ln\left(\frac{n_{gastruloids}}{class\ spread^2} \times fraction_{mode\ class}\right)$
Density
DMSO similarity
reproducibility score
regulatory modules
A B C D
DMSO
F
A 12 9
B 9 13
C 15 4
D 21 2
hit
non-hit
E
whole segm.
regime
DMSO
A
B
C
D
hits:
gene regulatory modules
A B
C D
DMSO
cluster abundance
0%
100%
DMSO similarity
-1
1
na
regime
32 - 72h
48 - 72h
72 - 96h
reproducibility score
-2
5
na
G
Axial elongation regime (72 h - 96 h)
96h
120h
144h
DAPI Sox2
Bra
Sox1
DMSO
XAV939
IWP2
PORCN (Cpd 58)
PORCN (Cpd63)
CTNNB1 (Cpd54)
GSK3B (Cpd27)
GSK3B (Cpd67)
H
TNKS
PORCN
CTNNB1
GSK3B
Sox2
Bra
Sox1
Mean intensity [z-score]
I
DAPI Sox1
DAPI Sox2
XAV939

Figure S5. Regulatory modules of gastruloid screen. ***Related to Figure 5.***

(A) Left: depiction of combined phenotypic space (see S5D). Right: UMAP plots colour-coded by most frequent whole gastruloid phenotype in indicated treatment regime. Marker type specifies compounds selected as hits in indicated regimes.

(B) UMAP plot colour-coded by regulatory modules, DMSO condition depicted separately. In UMAP plots, data points are individual compounds, n=85.

(C) Left: heatmap representation of hierarchically clustered covariance matrix between whole gastruloid and pattern classes, heatmap rows and columns are abundances of indicated classes in indicated treatment regimes. Right: UMAP plot colour-coded by most frequent pattern class in compound treatment in indicated treatment regime (top to bottom: 32h-72h, 48h-72h, 72h-96h). Marker type indicates compounds selected as hits in indicated regimes. DMSO controls highlighted separately.

(D) Definition of reproducibility score. Top left: kernel density estimation plot of phenotypic similarity to DMSO for compounds from indicated similarity clusters (see Figure 5). Top right and bottom row: scatter plots of phenotypic similarity to DMSO and reproducibility score for the compound library colour-coded by compound-level similarity clusters. Highlighted areas correspond to hit selection criteria, thresholds for DMSO similarity, and reproducibility score shown as dashed lines.

(E) Top: heatmap representation of the 57-dimensional combined phenotypic space for all compounds, grouped by compound-level similarity cluster. Heatmap rows are grouped by covariance, see panel (A). Colour coding by whole gastruloid and pattern classes and treatment regime as in panel (A). Middle: correlation of phenotypic signature to that of DMSO controls. Bottom: reproducibility score of individual compounds in indicated regimes (top to bottom: 32h-72h, 48h-72h, 72h-96h). Compounds selected as hits indicated by grey highlight above the heatmap.

(F) Pie charts depicting abundance of selected hits in regulatory modules.
In UMAP and scatter plots, data points are individual compounds, n=85. na, missing data point; segm., pattern class; whole, whole gastruloid class.

(G) Representative images of gastruloids treated with Wnt pathway compounds in the “Axial elongation regime” (48h-72h) corresponding to H. MIPs of confocal z-stacks, DAPI and antibody stainings. Compounds indicated below were used at 5µM. White arrowhead depicts gastruloid shown in I. Scale bar, 150 µm.

(H) Heatmaps of z-scored mean intensity of Sox2, Sox1 and Bra of gastruloids treated with indicated small compounds at 72h and fixed at indicated time points. n = minimum of 23 per timepoint and condition.

(I) Enlarged images of XAV939 treated gastruloids fixed at 144h shown in G. DAPI, Sox1 and Sox2 are shown. Scalebar, 200µm.

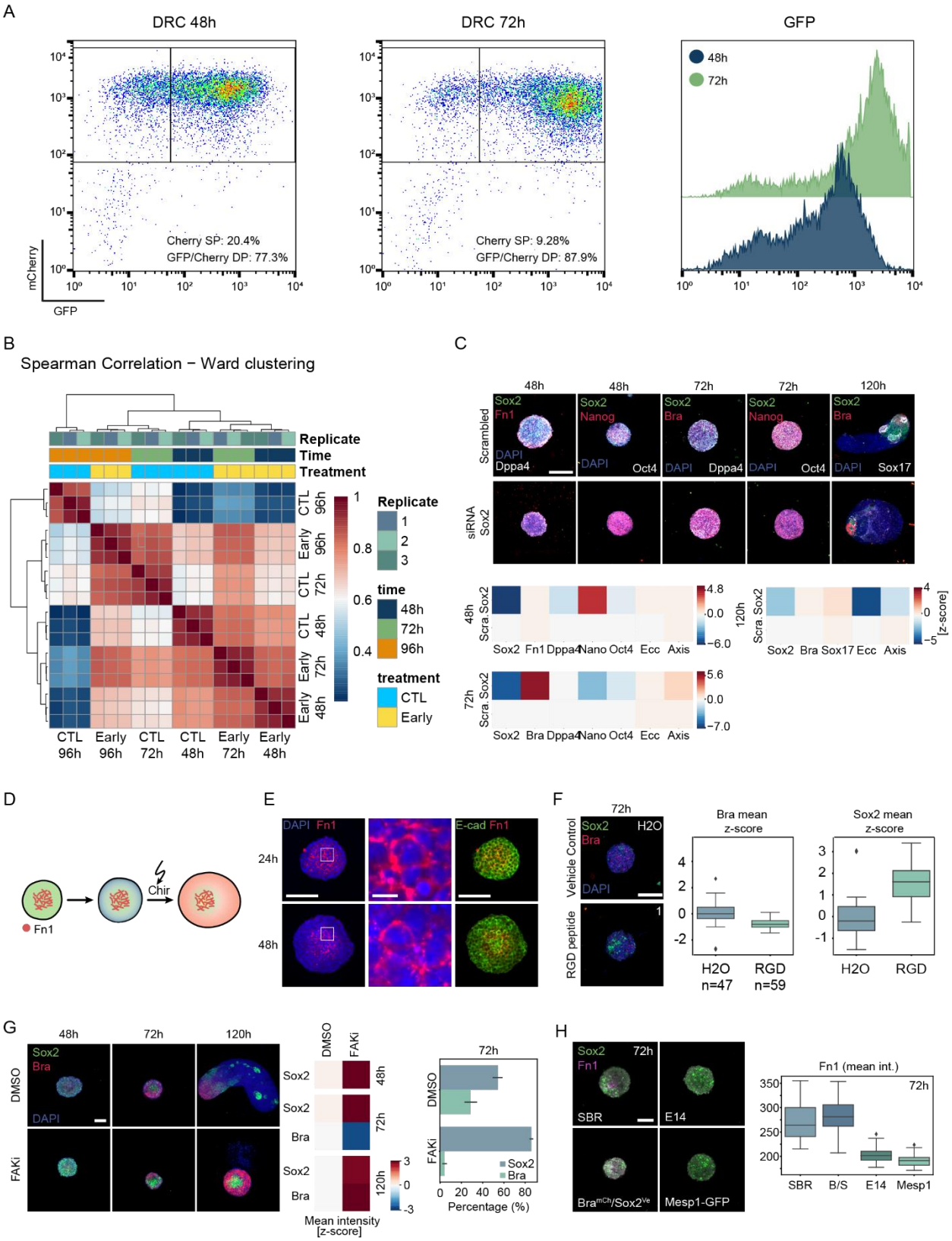
A
DRC 48h
DRC 72h
GFP
Cherry SP: 20.4%
GFP/Cherry DP: 77.3%
Cherry SP: 9.28%
GFP/Cherry DP: 87.9%
mCherry
GFP
48h
72h
B
Spearman Correlation − Ward clustering
Replicate
Time
Treatment
CTL 96h
Early 96h
CTL 72h
CTL 48h
Early 72h
Early 48h
Replicate
time
treatment
CTL
Early
C
Scrambled
siRNA Sox2
Sox2 Fn1 DAPI Dppa4
Sox2 Nanog DAPI Oct4
Sox2 Bra DAPI Dppa4
Sox2 Nanog DAPI Oct4
Sox2 Bra DAPI Sox17
Scra. Sox2
Sox2 Fn1 Dppa4 Nano Oct4 Ecc Axis
Sox2 Bra Sox17 Ecc Axis
[z-score]
Sox2 Bra Dppa4 Nano Oct4 Ecc Axis
D
Chir
Fn1
E
DAPI Fn1
E-cad Fn1
24h
48h
F
Vehicle Control
RGD peptide
Sox2 H2O DAPI
Bra mean z-score
Sox2 mean z-score
H2O n=47
RGD n=59
H2O
RGD
G
DMSO
FAKi
Sox2 Bra DAPI
Mean intensity [z-score]
Percentage (%)
H
Sox2 Fn1
SBR
E14
Bra^mCh/Sox2^Ve
Mesp1-GFP
Fn1 (mean int.)
SBR
B/S
E14
Mesp1

Figure S6. Core characterization and perturbation. ***Related to Figure 6.***

(A) Flow cytometry results of 48h and 72h DRC gastruloids. Left and middle panels depict scatterplots of single cells. x-axis depicts GFP intensities, y axis depicts mCherry intensities. The gatings chosen to differentiate mCherry single positive (SP) and GFP/mCherry double positive (DP) as well as relative cell proportions are shown. Right panel compares GFP histograms of the 48h and 72h timepoints.

(B) Spearman Correlation Matrix showing similarities between bulk RNA-seq samples using the 2000 most variable genes across all samples. Respective replicates, timepoints and treatments are indicated (right).

(C) siRNA KD experiment: representative MIP images of gastruloids at 48h, 72h and 120h. MIP of confocal z-stacks showing antibody stainings for Sox2, Fn1, Dppa4, Oct4, Sox17, Bra and Nanog. Scale bar, 200 µm. Bottom: Heatmaps of z-scored mean intensity of Sox2, Fn1, Dppa4, Nanog (Nano) and Oct4 as well as z-scored eccentricity (Ecc.) and major axis length (Axis) of gastruloids treated either with siRNA targeting Sox2 (Sox2) or scrambled control (Scra.) fixed at indicated time points. For siRNA KD 129 (120h), 138 (72h) and 141 (48h) gastruloids were analysed. For the scrambled control 99 (120h), 116 (72h) and 138 (48h) gastruloids were analysed.

(D) Scheme depicting Fn1 expression pattern.

(E) Representative images of gastruloids at indicated time points. Images are middle z-plane showing DAPI and antibody stainings for Fn1 and E-cadherin. Images in second column show ROI with Fn1 localisation. Scale bars, 100 µm (left and right) or 25 µm (middle).

(F) Left: Representative MIP images of gastruloids treated either with RGD peptide or vehicle control, fixed at 72h and stained for Bra, Sox2 and DAPI. Scale bar, 200µm. Right: Boxplots showing z-scored mean intensities of Bra and Sox2 stainings of gastruloids treated with 1mg/ml RGD peptide or control. Number of gastruloids (n) are indicated.

(G) Left: Representative images of gastruloids treated either with FAK inhibitor or DMSO, fixed at 48h, 72h and 120h and stained for Bra, Sox2 and DAPI. Scale bar, 100µm. Middle: Heatmaps of z-scored mean intensity of Sox2 and Bra of gastruloids treated with FAK inhibitor or DMSO and fixed at indicated time points. Right: Percentage of super pixels positive for indicated markers in indicated conditions at 72h. For FAKi 149 (120h), 151 (72h) and 165 (48h) gastruloids were analysed. For the DMSO control 140 (120h), 150 (72h) and 152 (48h) gastruloids were analysed.

(H) Left: Representative middle z-stack images of 72h gastruloids generated from SBR, E14, BramCh/Sox2Ve and Mesp1-GFP cell lines. Gastruloids were stained for Fn1, Sox2 and DAPI. Scale bar, 150µm. Right: Boxplots showing mean intensity of Fn1 stainings of gastruloids generated from indicated cell lines.

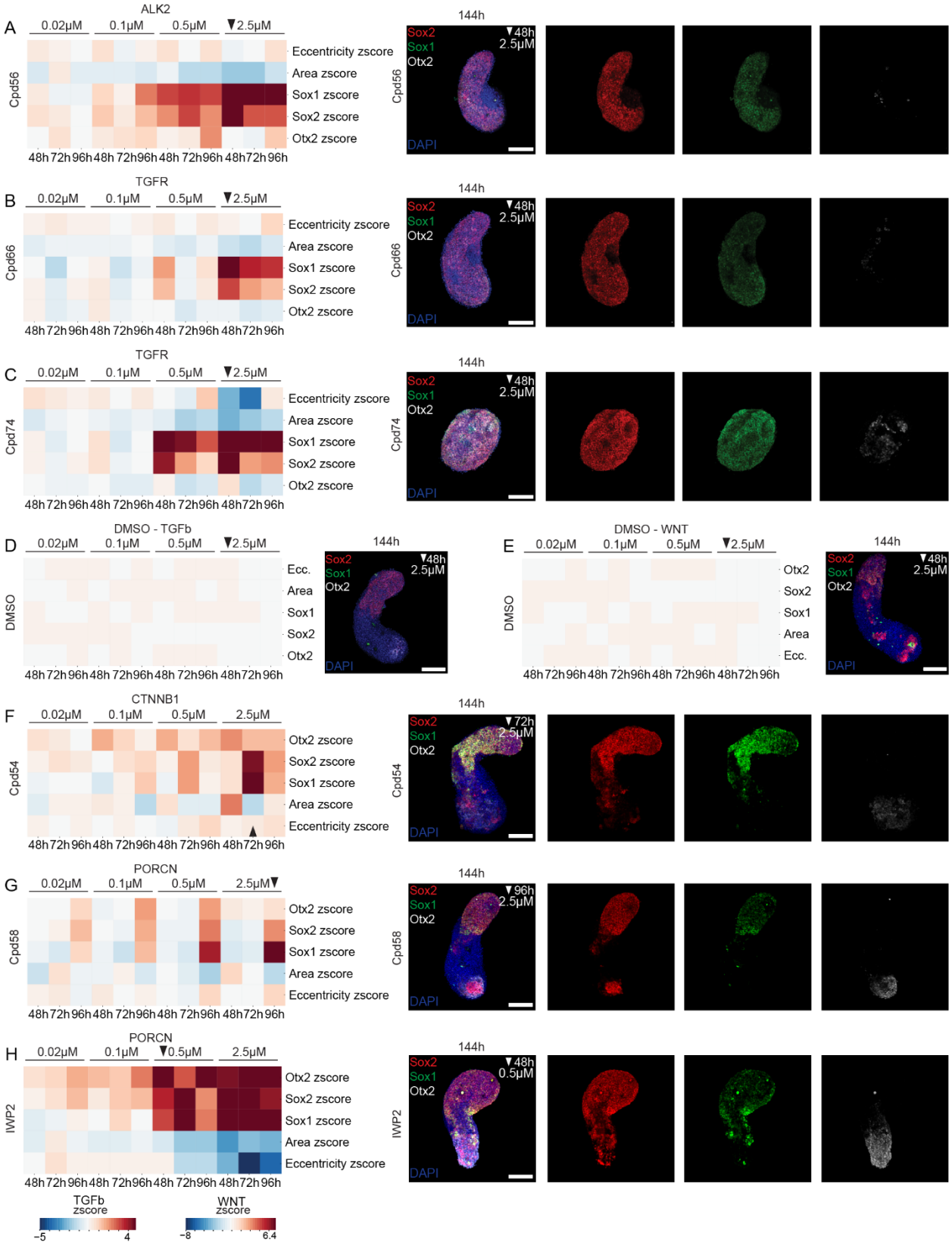
A
ALK2
0.02μM
0.1μM
0.5μM
▼2.5μM
Cpd56
Eccentricity zscore
Area zscore
Sox1 zscore
Sox2 zscore
Otx2 zscore
48h 72h 96h 48h 72h 96h 48h 72h 96h 48h 72h 96h
144h
Sox2
Sox1
Otx2
DAPI
▼48h
2.5μM
B
TGFR
Cpd66
C
Cpd74
D
DMSO - TGFb
DMSO
Ecc.
Area
Sox1
Sox2
Otx2
E
DMSO - WNT
F
CTNNB1
Cpd54
▼72h
G
PORCN
Cpd58
▼96h
H
IWP2
▼48h
0.5μM
TGFb
zscore
−5
4
WNT
zscore
−8
6.4

Figure S7. Limiting posterior gradients. ***Related to Figure 7.***

(A-H) Left: Heatmaps of z-scored mean intensity of Sox2, Sox1 and Bra as well as eccentricity (Ecc.) and area of treated gastruloids or DMSO controls at indicated timepoints and fixed at 144h. Legends for both Wnt and TGFb superfamily related heatmaps in the left corner of Figure S7. Black arrow indicates condition of which a representative gastruloid is shown. Right: MIP (144h) of z-stack, showing DAPI and antibody stainings for indicated markers. Gastruloids were fixed at 144h. Respective condition is depicted in the upper right corner of the RGB image. Per condition and timepoint 24 gastruloids were used. Scale bar, 200 µm.

References

1. Cheng, S., Pei, Y., He, L., Peng, G., Reinius, B., Tam, P.P.L., Jing, N., and Deng, Q. (2019). Single-Cell RNA-Seq Reveals Cellular Heterogeneity of Pluripotency Transition and X Chromosome Dynamics during Early Mouse Development. CellReports *26*, 2593-2607.e2593. 10.1016/j.celrep.2019.02.031.