

Supplemental information

**Multiscale integration of tissue
and chromatin context converts cell heterogeneity
into stable intestinal patterning**

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Supplementary Figure Legends

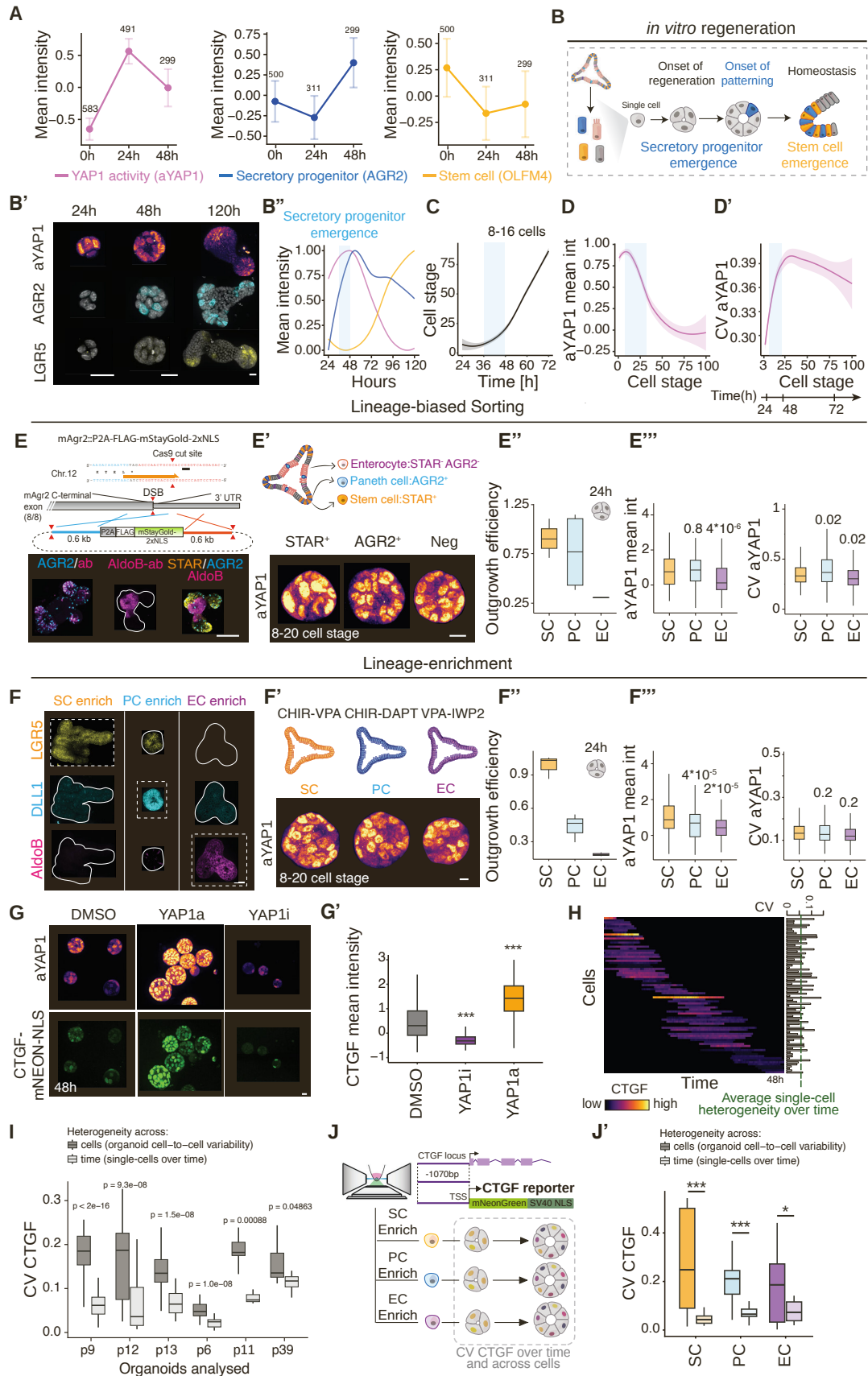


Figure S1. Changes of YAP1 activity and cell fate markers during *in vitro* and *in vivo* regeneration, related to Figure 1

(A) Data used to generate smoothened line plots for (Figure 1A''): Normalised mean intensities of cell type markers and YAP1 activity. N,n see Figure 1A'',A'''. Error bars $\pm$ s.e.m. **(B)** Scheme of *in vitro* regeneration. **(B')** MIPs of active YAP1, AGR2 staining, Lgr5::DTR-EGFP (stem cell marker) in organoids. **(B'')** Mean intensities of the cell type markers and YAP1 activity. Scale bar, 10 μ m. n=8,954 organoids. **(C)** Cell stage over time in DLL1 patterned organoids. N=3, n=5,210 organoids. **(D)** aYAP1 z-scored nuclear mean intensity over cell stage. N=3, n=5,091 organoids. **(D')** CV of aYAP1 over cell stage during *in vitro* regeneration. Scale (bottom), mapping of cell stages to the respective timepoints. N=3, n=1,727 organoids. **(E)** Targeting strategy to generate mouse Agr2::P2A-FLAG-mStayGold-2xNLS organoid line in the background of STAR-mTomato-NLS (top). MIPs of DAPI, STAR-mTomato-NLS (stem cells), Agr2::P2A-FLAG-mStayGold-2xNLS, AGR2 antibody (secretory cells) and Aldolase B (enterocytes). Scale bar, 50 μ m. **(E')** Dual-color line enables sorting of enterocytes (double negative), secretory cells (AGR2+), stem cells (STAR+, top). MIPs aYAP1 signal in 8-20 cell stage organoids grown from different single-cells (stem, secretory cells or enterocytes, bottom). Scale bar, 10 μ m. **(E'')** Boxplot of outgrowth efficiency of lineage-biased organoids at 24h. **(E''')** Boxplots of aYAP1 mean intensity and heterogeneity (coefficient of variation) of lineage-biased organoids. N=4 wells from 2 independent experiments, n: SC=284, PC=112, EC=175 cells. **(F)** MIPs of LGR5-DTR-eGFP signal (stem cells), DLL1 (secretory cells) and Aldolase B (enterocytes) in different compound-induced cell-fate enrichment conditions (see schemes on top). Scale bar, 50 μ m. **(F')** MIPs of aYAP1 signal in 8-20 cell stage organoids grown from conditions shown in (F). Scale bar, 10 μ m. **(F'')** Boxplot of outgrowth efficiency at 24h of different enrichment conditions. **(F''')** Boxplots of aYAP1 mean intensity and heterogeneity (coefficient of variation) of enrichment conditions. N = 3 wells from one representative experiment, SC enrich n=455, PC enrich n=131, EC enrich n=78 organoids. **(G)** MIPs of aYAP1, CTGF-mNEON-NLS signal in DMSO, YAP1 activator and inhibitor at 48h. Scale bar, 10 μ m. **(G')** Boxplot of CTGF mean intensity in YAP1 perturbations from one representative experiment, n: DMSO=440, YAP1i=213, YAP1a=347 organoids. **(H)** CTGF dynamics per single-cell. Single-cell CTGF trajectories over time (left). Time-heterogeneity component (CV over time) per cell (right). Dashed line, mean CV across all single-cell trajectories. N,n see Figure 1B'. **(I)** Relative contribution of cell-to-cell versus temporal heterogeneity to the overall CTGF heterogeneity in each organoid. **(J)** Long-term light sheet imaging to track CTGF levels and heterogeneity in space and time. **(J')** Boxplots of CTGF spatial versus temporal heterogeneity. n = 39 cells from N = 7 organoids. (C-D') Line plots, mean intensities $\pm$ 90% CI. (E'',E''',F'',F''',G',I,J') boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. Statistical significance is indicated with * (p < 0.05), ** (p < 0.01), *** (p < 0.001).

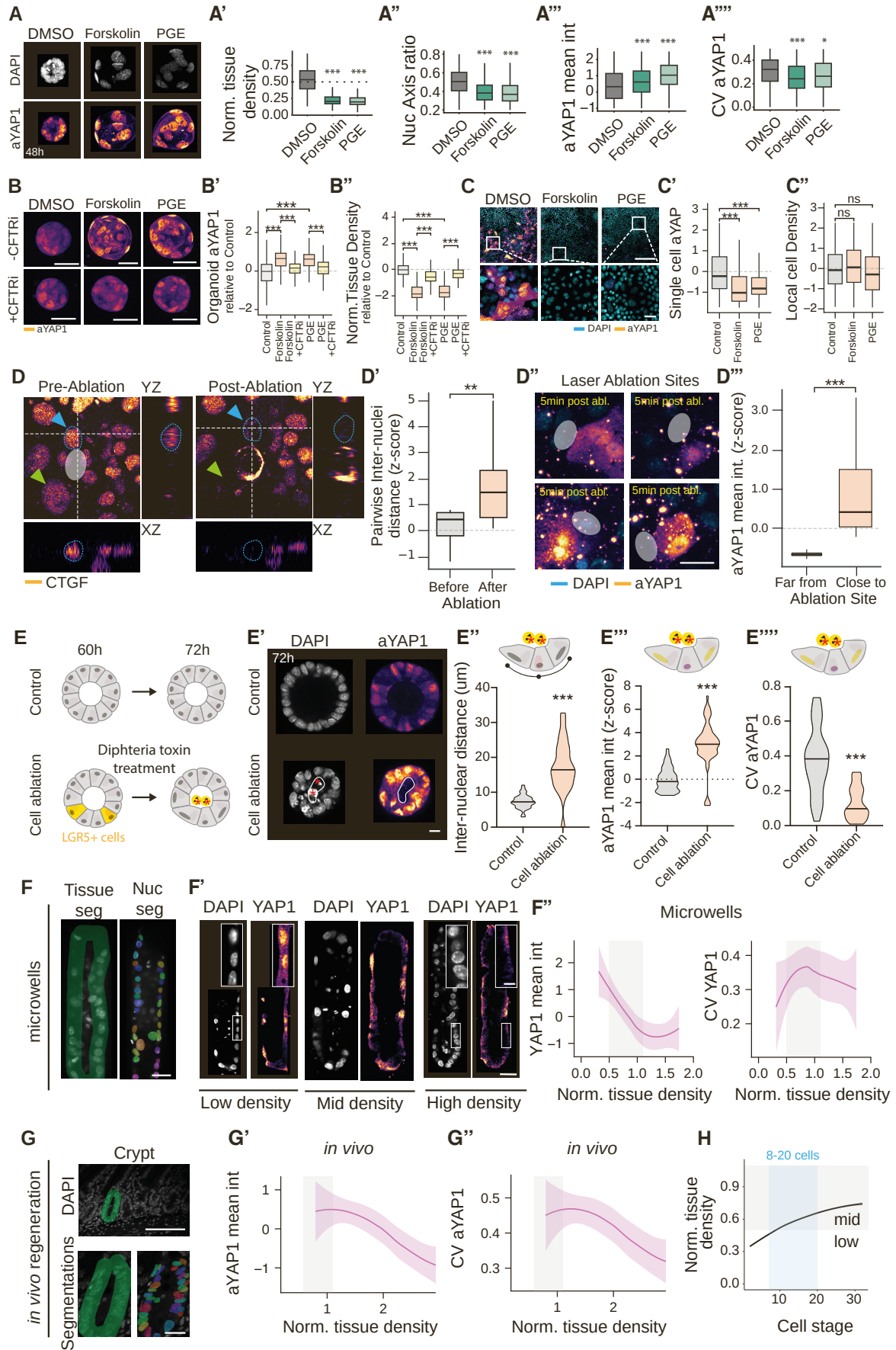


Figure S2. Tissue density gates a population-level window of YAP1 heterogeneity during regeneration, related to Figure 1

(A'-A''') Boxplots of normalised tissue density, nuclear axis ratio (minor/major axis), organoid-level average of aYAP1 z-scored nuclear mean intensity, CV of aYAP1. N=2, n: DMSO=778, Forskolin=447, PGE=361 organoids for (A',A'',A'''). n: DMSO=8,966, Forskolin=2,213, PGE=1,861 nuclei for (A''). **(B)** MIPs of organoids treated with DMSO, Forskolin, or PGE in the absence (-CFTRI) or presence (+CFTRI) of CFTR inhibitor-172. Scale bar, 25 μ m (50 μ m Forskolin and PGE in -CFTRI). **(B')** Quantification of organoid YAP1 activity and **(B'')** normalized tissue density. Ctr: n = 37996 cells from N = 1537 organoids, Forskolin: n = 6013 cells from N = 223 organoids, Forskolin+CFTRI: n = 3504 cells from N = 279 organoids, PGE: n = 7654 cells from N = 294 organoids, PGE+CFTRI: n = 36125 cells from N = 260 organoids. **(C)** Representative images of 2D collagen/Matrigel monolayers treated with DMSO, Forskolin, or PGE. α YAP1 (inferno), DAPI (cyan). Insets show higher magnification of low density regions. Scale bar 100 μ m (25 μ m zoom-ins). **(C')** Quantification of single-cell α YAP1 nuclear intensity (z-score) and **(C'')** local cell density (z-score). Ctrl: n = 165547 cells, Forskolin: n = 220081 cells, PGE: n = 212231 cells. **(D)** Representative images of 2D organoids before and after single-cell laser ablation. CTGF (inferno). YZ and XZ views shown. **(D')** Quantification of pairwise inter-nuclear distance (z-score) before and after ablation. n = 64 from 16 laser ablation sites. **(D'')** Representative images of α YAP1 (inferno) and DAPI (cyan) at defined distances from the ablation site. **(D''')** Quantification of α YAP1 mean nuclear intensity (z-score) in cells close to versus far from the ablation site. n = 42 cells from 6 laser ablation sites. **(E)** Experimental setup for genetic LGR5::DTR ablation: Imaging LGR5-DTR-eGFP organoids before and after diphtheria toxin treatment enabled identification of organoids containing LGR5⁺ stem cells that underwent ablation versus negative control organoids. DT addition at 60h, readout at 72h. Only organoids that harbor LGR5⁺ cells at 60h will experience cell ablation. **(E')** MIPs of DAPI and aYAP1 staining at 72h. Scale bar, 10 μ m. **(E'')** Violin plots of internuclear distance [μ m] (measure of cell density in the ablation neighborhood), of **(E''')** aYAP1 mean intensity and **(E''')** heterogeneity (coefficient of variation). N=6 wells from 2 independent experiments, Ctrl: n=70, DT n=46 cells. **(F)** Z-plane of DAPI in microwells (left) and nuclear segmentation (right). Scale bar, 20 μ m. **(F')** Z-plane of DAPI and total YAP1 staining of engineered microwells with different tissue densities. Inset shows 4x zoom in. Scale bar, 10 μ m, 2.5 μ m (zoom-in). **(F'')** Plots show total YAP1 z-scored nuclear mean intensity (left) and CV of YAP1 over 2D normalised tissue density (right). N=3, n=27. **(G)** Z-plane of *in vivo* tissue section labelled with DAPI; green mask, manually segmented crypt. Scale bar, 100 μ m. Lower column: crypt and single-nuclei segmentation. Scale bar, 20 μ m. **(G',G'')** Plot of aYAP1 z-scored nuclear mean intensity (G') and CV of YAP1 (G'') over normalised tissue density. N=3 mice/time point, n=1,373 single-cells from 52 crypts. **(H)** Normalised tissue density over cell stage in 2-32 cell-stage organoids. Gray rectangle, intermediate density bin (0.5-1.1); blue rectangle, 8-20 cell stage. N=3, n=4,184 organoids. (A-A''',B',B'',C',C'',D',D''') boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. (F'',G'-H) Line plots, mean intensities $\pm$ 90% CI. Statistical significance is indicated with * (p < 0.05), ** (p < 0.01), *** (p < 0.001).

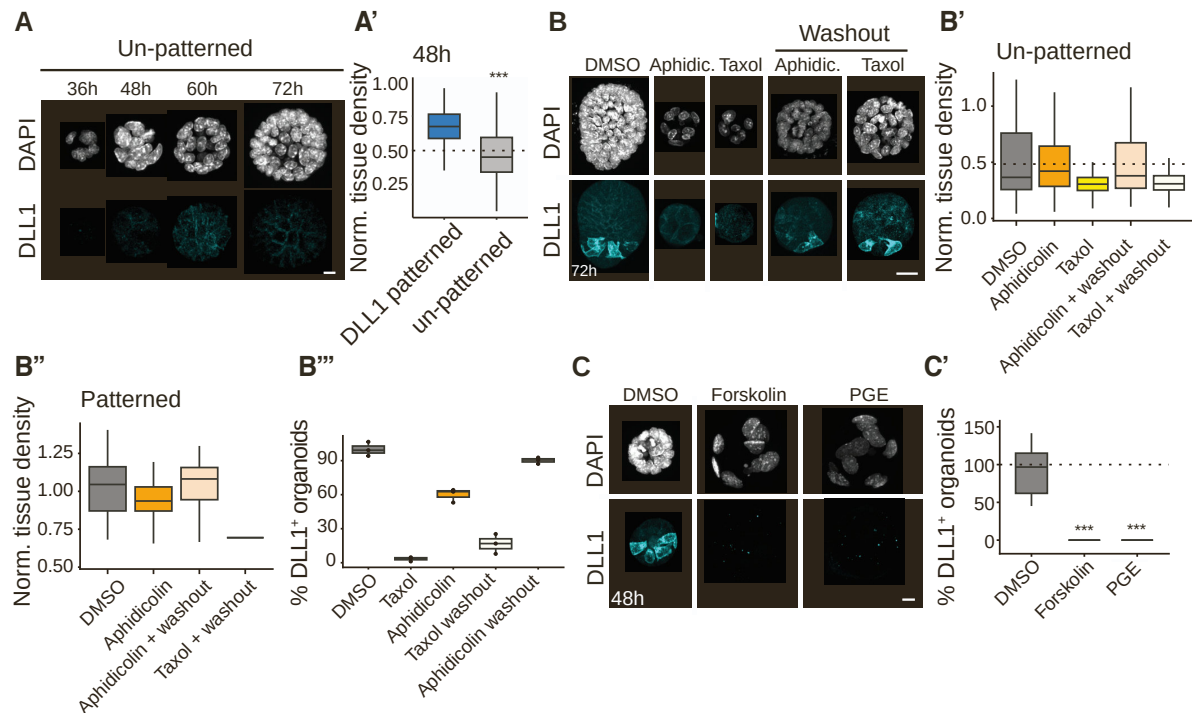


Figure S3. Optimal tissue density is necessary for fate patterning, related to Figure 1

(A) MIPs of DAPI and DLL1 in un-patterned organoids (no DLL1⁺ cell) at different time points of regeneration. Scale bar, 10µm. **(A')** Normalised tissue density for DLL1 patterned and un-patterned organoids at 48h. N=3, n=1,067 organoids. **(B)** MIPs of DAPI and DLL1 of DMSO control, Aphidicolin (0.25µM) and Taxol (5nM) treated organoids from 24-72 hours. Washout conditions, treatment window 24-48 hours, with Aphidicolin and Taxol are shown on the right. Scale bar, 20µm. **(B',B'')** Normalised tissue density for un-patterned (B') and DLL1 patterned organoids at 72h (B''). Line, transition from low to intermediate density. **(B''')** Percentage of DLL1⁺ organoids in conditions mentioned in (B). Data from one representative experiment. n=3 wells/condition. **(C)** MIPs of DAPI and DLL1 in DMSO control, Forskolin [10µM], PGE [0.5µM] at 48h. Scale bar, 10µm. **(C')** Percentage of DLL1⁺ organoids for (C). N=2, n: DMSO=782, Forskolin=482, PGE=375 organoids. (A',B'-B''',C') boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. Statistical significance is indicated with * (p < 0.05), ** (p < 0.01), *** (p < 0.001).

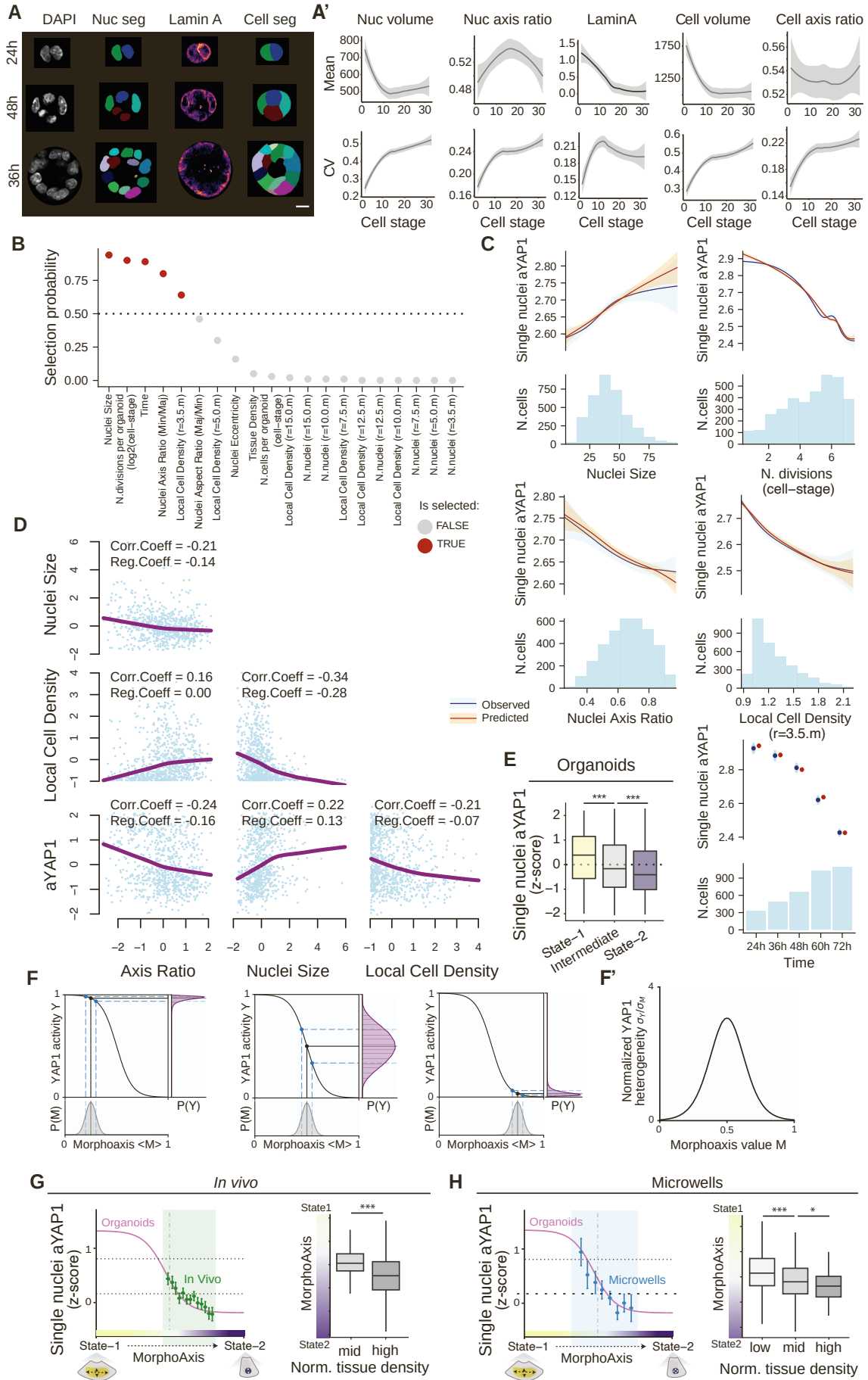


Figure S4. Statistical modeling of YAP1 heterogeneity, related to Figure 1

(A) Z-Planes of DAPI, Lamin A, nuclear and cell segmentation at different timepoints. Scale bar, 10 μ m. **(A')** Organoid-level averages of mean values (upper row) and CV (lower row) of nuclear volume [μ m³], nuclear axis ratio, Lamin A nuclear z-scored mean intensity, cell volume [μ m³], cell axis ratio over cell stages. Axis ratios, minor/major axis; mean values $\pm$ 90% CI. N=2 for Lamin A, N=3 for shape features, n: >900 organoids. **(B)** Feature selection probabilities for single-cell predictors of YAP1 activity, based on stability selection. **(C)** Observed versus predicted single-nuclei aYAP1 levels in test data, using an ordinary least squares (OLS) model trained on selected features from (A). For continuous predictors, line plots: mean $\pm$ 90% CI; for categorical predictors: means $\pm$ 90% CI as dots with error bars. Light-blue histograms, number of observations per predictor bin. **(D)** Pairwise scatterplots of model input features used in graphical Gaussian modeling. Shown are Pearson correlation coefficients and stabilised regression coefficients. **(E)** Single-nuclei aYAP1 in organoid cells grouped by morphoaxis position. **(F)** Relationship between the distribution of mechanical states P(M) (bottom) and the resulting distribution of YAP1 activities P(Y) (right), connected through the sigmoidal response curve Y(M) (center), shown for three representative mean mechanical states: (left) low <M> (early stage, uniformly high YAP1), (middle) intermediate <M> (heterogeneous YAP1), and (right) high <M> (late stage, uniformly low YAP1). Response curve is fitted to experiments using the generic sigmoidal function $Y(M) = 1 / (1 + \exp(k(M - M_0)))$ where $k=13.5$ sets the steepness of the response and $M_0=0.5$ is the inflection point (Fig 1E'). Solid and dashed lines indicate the mean and mean $\pm$ one standard deviation of M, M, respectively, with corresponding points on the response curve and their projections onto the YAP1 distribution. **(F')** Relative YAP1 heterogeneity σ_Y/σ_M as a function of mean mechanical state <M>, where σ_Y is the standard deviation of YAP1 activity, and σ_M is the standard deviation of the mechanical state. **(G)** Left, single-nuclei aYAP1 *in vivo*, plotted by morphoaxis position. In pink, sigmoidal function from organoid data is shown. Right, similarity of cells to State-1 or State-2 extremes, grouped by normalised tissue density of the crypts of origin. **(H)** Left, single-nuclei aYAP1 in microwell-grown cells, grouped by morphoaxis position. In pink, sigmoidal function from organoid data is shown. Right, similarity to State-1 or State-2 extremes, grouped by normalised tissue density of microwells of origin. (E,G,H) boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. (A',C,G,H) Line plots, mean intensities $\pm$ 90% CI. Statistical significance is indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

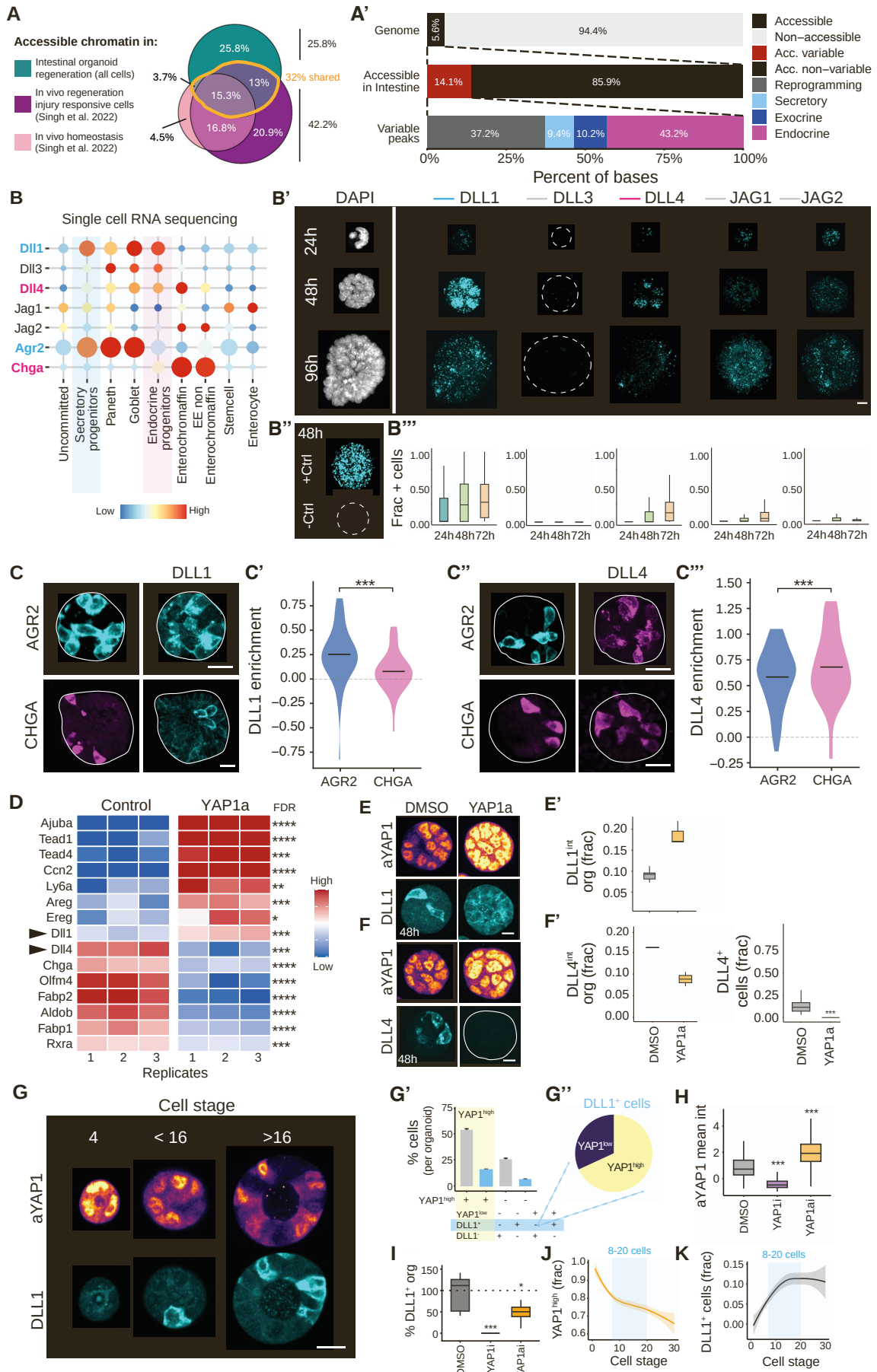


Figure S5. Notch ligands and YAP1 regulation of fate emergence, related to Figure 1

(A) Percentage of overlaps measured base by base in intestinal regeneration *in vivo* (pink and dark magenta) and in organoids (dark cyan). **(A')** Bar plots of genome accessibility in mouse intestinal organoid regeneration, showing percentage of total accessible chromatin (5%, top), and variable regions (14%, middle). Bottom row, distribution of variable peaks according to lineages. **(B)** Dot plot of single-cell RNA sequencing data showing mRNA expression across cell types. Dot size indicates fraction of expressing cells; color indicates mean expression level. **(B')** MIPs of DAPI and HCR-FISH of DLL1, DLL3, DLL4, JAG1, JAG2 during mouse intestinal regeneration of organoids across 24h, 48h, 72h timepoints. **(B'')** MIPs of positive control (Ubc) and negative control (Amplifier only) for HCR-FISH in organoids at 48h. **(B''')** Boxplots of the positive cell fractions of the respective HCR-FISH imaging shown in (B'). N=3 wells, n: DLL1, DLL4=1,864, DLL3=1,730, JAG1, JAG2=1,580 organoids from one representative experiment. Scale bar, 10 μ m. **(C)** Representative images of AGR2⁺ and CHGA⁺ cells stained for DLL1. **(C')** Quantification of DLL1 enrichment in AGR2⁺ versus CHGA⁺ cells. **(C'')** Representative images of AGR2⁺ and CHGA⁺ cells stained for DLL4. **(C''')** Quantification of DLL4 enrichment in AGR2⁺ versus CHGA⁺ cells. Scale bar 25 μ m. n = 267 organoids. **(D)** Heatmap of bulk RNA-seq data from regenerating organoids showing differential expression of YAP target genes and Delta ligands under Control and YAP1 activation conditions. **(E, F)** MIPs of aYAP1, DLL1 (E) or DLL4 (F) of DMSO control and YAP1a (activator, LATSi) at 48h. Scale bar, 10 μ m. **(E', F')** Fraction of DLL1int (DLL1 intermediate, E') or DLL4int (F') organoids, organoids that are enriched in DLL1/4int cell fraction. Boxplot of DLL4+ cell fraction per organoid in DMSO and YAP1 activator at 48h. N, n for (E') same as (Figure S5H). N=2, n: DMSO=105, YAP1a=68 organoids in (F'). **(G)** Z-planes of aYAP1, DLL1 staining at different cell stages. Scale bar, 10 μ m. **(G')** Percentage of YAP1^{high/low}, DLL1^{+/-} cells per organoid in 8-20 cell stage, DLL1 patterned organoids. N=3, n=4,907 cells. **(G'')** Percentage of YAP1^{high/low} signal in DLL1+ cells in 8-20 cell stage, DLL1 patterned organoids. N=3, n=1,016 cells. Mean $\pm$ s.e.m. **(H)** Boxplot of organoid-level average of aYAP1 nuclear mean intensity in YAP1 perturbations at 48h. N=3, n: DMSO=166, YAP1i=915, YAP1a=1,590 organoids. **(I)** Percentage of DLL1+ organoids in DMSO and YAP1 perturbations at 48h normalised to the average of DMSO control. N=3, n: DMSO=18, YAP1i=9, YAP1a=7 wells. **(J)** Mean aYAP1+ cell fraction in organoids with at least one aYAP1+ cell over cell stage. N=3, n=1,709 organoids. **(K)** Mean DLL1+ cell fraction in organoids with at least one YAP1+ cell over cell stage. N=3, n=5,210 organoids. (E', F', H, I) boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. (J, K) Line plots, mean intensities $\pm$ 90% CI. Statistical significance is indicated with * (p < 0.05), ** (p < 0.01), *** (p < 0.001).

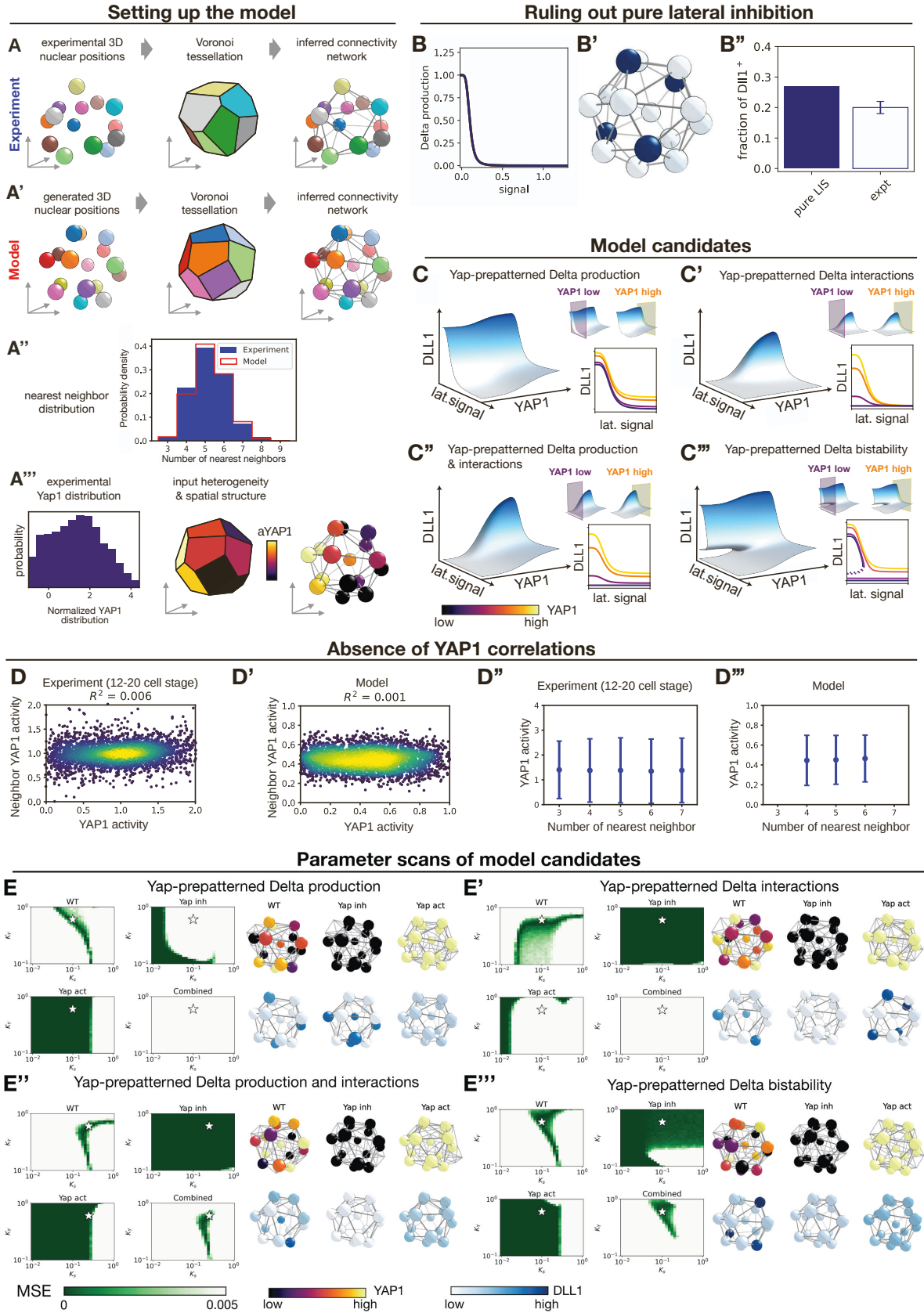


Figure S6. Tissue patterning model, related to Figure 2

(A) Experimental 3D nuclear positions (first panel) are used to perform a spherical Voronoi tessellation (by projecting points onto a unit sphere; second panel), allowing to infer the corresponding Delaunay triangulation

providing the inferred connectivity network (third panel). **(A')** In the model, equivalent steps are taken to generate 3D connectivity networks: cell positions are generated by placing points on a sphere, with a distribution that is fitted to match the nearest neighbor distribution in the experiment (panel A''). **(A'')** Distribution of the number of nearest neighbors per cell in experimental (blue) and model generated (red) connectivity networks. **(A''')** Left: Normalized experimental distribution of YAP1 activities in wildtype organoids. To determine the YAP1 distribution during patterning, only patterned organoids (i.e. at least one DLL1+ cell) are included, with cell numbers between 8-20 cells. Center: Model generated connectivity network with YAP1 heterogeneity generated by random sampling from the experimental distribution in (right). **(B)** DLL1 production as a function of lateral signal $s_i = \frac{1}{N_i} \sum_j K_{ij} D_j$ for the pure lateral inhibition model. **(B')** DLL1 intensity on model generated network. **(B'')** Pure lateral inhibition signaling predicts DLL1+ cell fraction of 0.25, compared to 0.2 ± 0.02 (SEM) observed experimentally. **(C-C''')** Analysis of stable manifolds for each model candidate. For each model, first the surface of stable DLL1 states as a function of lateral signal $s_i = \frac{1}{N_i} \sum_j K_{ij} D_j$ and YAP1 is shown. Cuts through the surface at low and high YAP1 are indicated, with the DLL1 vs lateral signal curve for different YAP1 values ($Y_i = 0.1, 0.4, 0.7, 1$) shown below. **(D)** Experimental correlation between YAP1 activity of single cells (x-axis) and the mean YAP1 activity of their nearest neighbors; plotted for organoids close to the 16-cell stage (organoids with 12-20 cells, in total 240 organoids, 3,743 cells). Neighborhood relations are inferred using a spherical Voronoi tessellation of experimental 3D nuclear positions. All YAP1 activities are normalized by the mean YAP1 activity of each organoid. **(D')** Equivalent plot for the model. **(D'')** Experimental YAP1 activity as a function of the number of nearest neighbors of each cell; plotted for organoids close to the 16-cell stage (organoids with 12-20 cells, in total 240 organoids, 3,743 cells). Neighborhood relations are inferred using a spherical Voronoi tessellation of experimental 3D nuclear positions. **(D''')** Equivalent plot for the model. **(E-E''')** Analysis of parameter spaces of the four model candidates, varying K_s and K_Y . Panel shows MSE as a function of parameters for each condition; the combined plot shows total MSE (Eq. 3). The star indicates the parameter combination used for generating network plots: **(E)** $K_s = 0.1, K_Y = 0.6$, **(E')** $K_s = 0.1, K_Y = 0.6$, **(E'')** $K_s = 0.25, K_Y = 0.6$, **(E''')** $K_s = 0.1, K_Y = 0.6$. In all panels, all other parameters are set to standard values (Table S3), i.e. noise amplitude $\sigma = 0.04$ and Hill exponents $h = 4$. In panel E''', the additional parameter $K_D = 0.2$ is used.

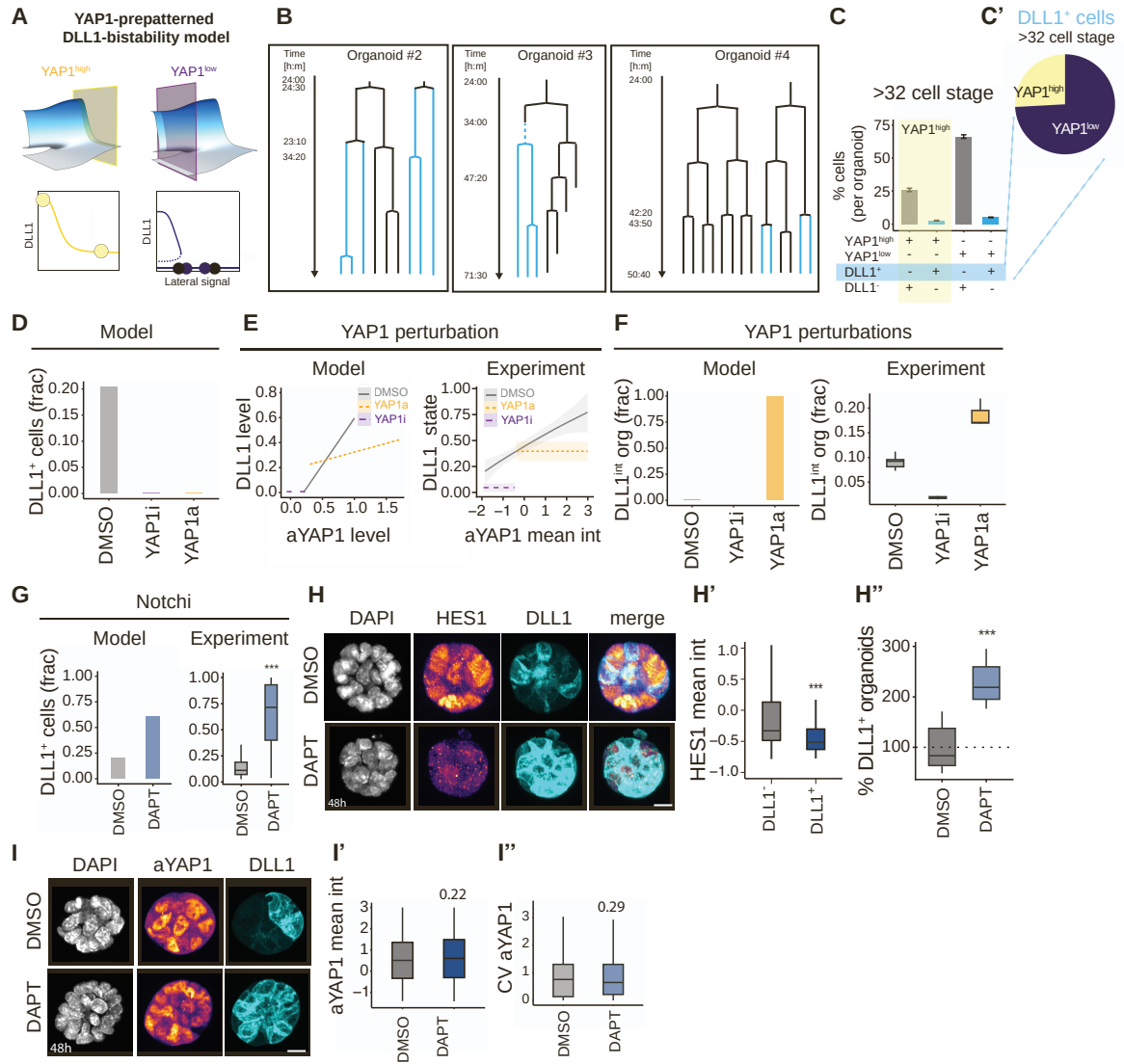


Figure S7. Experimental validation of tissue patterning model, related to Figure 2

(A) Cuts through the attractor surface at high and low YAP1 activity, respectively, with locations of low and high YAP1 cells from Figure 2C indicated. **(B)** Zoom into lineage trees of three different organoids with single-cell tracks of DLL1⁺ cells (solid blue line), DLL1 intermediate levels (dotted blue line) and DLL1⁻ cells (black solid line). Organoids were imaged from 24-72 hours. N=3, n=3 organoids. **(C)** Percentage of YAP1^{high/low} / DLL1^{+/-} cells per organoid at >32 cell stage, DLL1 patterned organoids. N=3, n=38,603 cells. **(C')** Percentage of YAP1^{high/low} signal in DLL1⁺ cells in 8-20 cell stage, DLL1 patterned organoids. N=3, n=1,062 cells. **(D)** Average DLL1⁺ cell fraction in DMSO and YAP1 perturbation experiments for the YAP1-prepatterned DLL1-bistability model (Figure 2C). **(E)** Correlation of single-cell DLL1 levels in model (left) and experiments (right) with aYAP1 (nuclear), shown for organoids from 8-20 cell stage for control, YAP1 perturbation experiments. Mean $\pm$ 90% CI, linear fit. n: DMSO=18, YAP1i=79, YAP1a=44 organoids. **(F)** Fraction of DLL1^{int} (DLL1 intermediate) organoids, organoids that are enriched in DLL1^{int} cell fraction for model (left) and experiment (right). N, n same as (Figure S5H). **(G)** Fraction of DLL1⁺ cells in model (left) and in experiment (right). N=3, n: DMSO=351, DAPT=838 organoids. **(H)** MIPs of DAPI, HES1 (Notch target), DLL1 and a merge of HES1 and DLL1 of DMSO and DAPT treated organoids, fixed at 48h. Scale bar, 10 μ m. **(H')** Boxplot of HES1 z-scored nuclear mean intensity in DLL1⁺ and DLL1⁻ cells. N,n same as (Figure S5H). **(H'')** Boxplot of % DLL1⁺ organoids. N=3, n: DMSO=351, DAPT=838 organoids. **(I)** MIPs of DAPI, aYAP1 and DLL1 of DMSO and DAPT treated organoids, fixed at 48h. **(I')** Boxplot of aYAP1 mean intensity. N=3, n: DMSO=351, DAPT=838 organoids. **(I'')** Boxplot of CV aYAP1. N=3, n: DMSO=351, DAPT=838 organoids.

as in (G). **(H'')** Percentage of DLL1+ organoids in DMSO control compared to DAPT at 48h normalised to average of DMSO. N,n same as in (G). **(I)** MIPs of DAPI, active YAP1 and DLL1 of DMSO and DAPT treated organoids, fixed at 48h. Scale bar, 10µm. **(I')** Boxplot of aYAP1 z-scored nuclear mean intensity and **(I'')** CV of aYAP1 in DMSO control and DAPT at 48h. N=3, n: DMSO=5,319, DAPT=5,945 nuclei. (F,G,H',H'',I',I'') boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. Statistical significance is indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

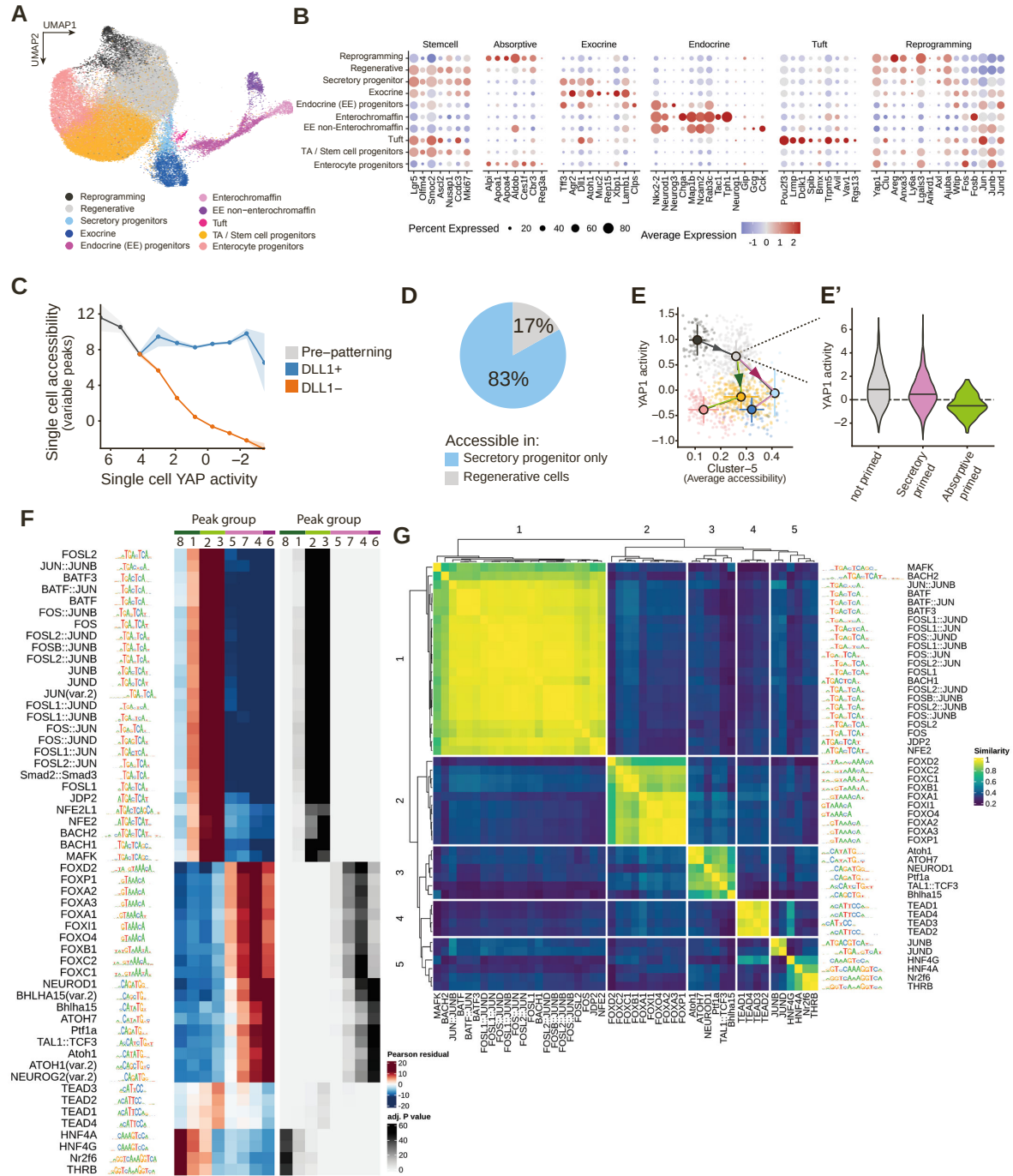


Figure S8. Characterisation of chromatin accessibility changes during patterning, related to Figure 3

(A) UMAP embedding coloured by cell type annotation, including endocrine sub-types. **(B)** Dot plot of predicted expression for known intestinal markers. **(C)** Mean single-cell accessibility at variable ATAC peaks as a function

of single-cell YAP activity, stratified by pre-patterning (cells before fate commitment), DLL1⁺, and DLL1⁻ populations. **(D)** Pie chart of secretory progenitor peaks (groups 5-7-4) showing number of peaks uniquely accessible in secretory progenitor cells (groups 7-4, light blue), and number of peaks with accessibility in regenerative cells (group 5). **(E)** Scatter plot of YAP1 activity and average accessibility in peak cluster 5. Points are coloured according to cell type annotation. For robust estimates, single-cells are grouped in meta-cells (average of 30 single cells), respecting cell type and experimental time point boundaries. Cell type centroids and error bars are calculated as median $\pm$ MAD. Connecting segments, lineage progression according to developmental time and predicted gene expression. **(E')** Violin plots of YAP1 activity in regenerative cells at 36-48 hours. Regenerative cells are stratified in secretory primed (highest accessibility in peak group 5, magenta), absorptive primed (highest accessibility in peak group 8 and low accessibility in peak group 3, green), and not primed (all others). **(F)** Heatmap of motif enrichment analysis in peak groups of interest. **(G)** Heatmap of similarity in transcription factor motifs.

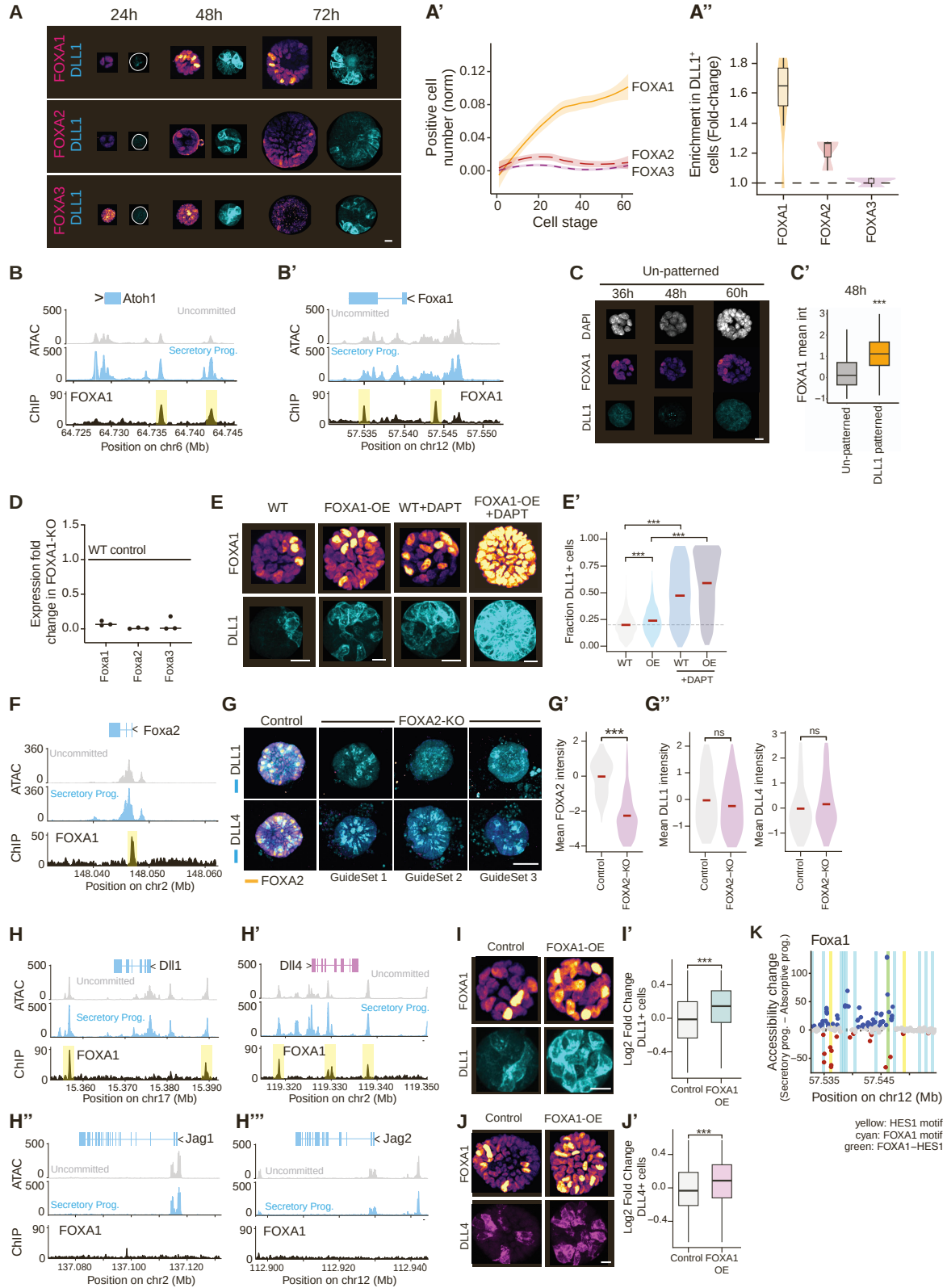


Figure S9. Role of FOXAs factors in secretory fate commitment, related to Figure 3

(A) MIPs of FOXA1 (first row), FOXA2 (second row), FOXA3 (third row) in magenta and DLL1 in cyan across different time points in regeneration (24h, 48h, 72h). Scale bar, 10 μ m. **(A')** Normalised positive cell number of different FOXA factors over cell stages. **(A'')** Boxplots of FOXA factor enrichment in DLL1⁺ cells during intestinal

regeneration (24h till 72h). FOXA1: N=9 wells from 3 independent experiments, FOXA2,3: N=3 wells from one representative experiment, (A') n=3,780 cells each. (A'') FOXA1: n=960 organoids, FOXA2: n=480 organoids, FOXA3: n= 413 organoids. **(B,B')** Representative ATAC and ChIP sequencing signal in regenerative (grey) and secretory progenitor cells (blue) around *Atoh1* (B) and *Foxa1* (B') region. **(C)** MIPs of DAPI, FOXA1, DLL1 of un-patterned organoids (no detectable DLL1 signal) at different time points. Scale bar, 10µm. **(C')** Organoid-level FOXA1 mean intensity at 48h. N=3, n: DLL1 patterned=1,269, un-patterned=3,618 organoids. **(D)** Expression fold-change of *Foxa1*, *Foxa2*, *Foxa3* genes in FOXA1-KO for three different monoclonal organoid lines (single dots) normalised to the expression level of housekeeping gene (Elongation factor 1 alpha). Fold-change of 1 indicates WT control and values below indicate decreased expression levels. **(E)** Representative images of FOXA1 and DLL1 staining in WT, FOXA1-OE, WT + DAPT, and FOXA1-OE + DAPT organoids. Scale bar, 25 µm. **(E')** Quantification of DLL1⁺ cell fraction under the indicated conditions. WT: n=40789 cells from 634 organoids, FOXA1-OE: n=26420 cells from 351 organoids, WT+DAPT: n=42592 cells from 641 organoids, FOXA1-OE + DAPT: n=30077 cells from 388 organoids. **(F)** Representative ATAC and ChIP sequencing signal in regenerative (grey) and secretory progenitor cells (blue) around *Foxa2* region. **(G)** Representative images of Control and FOXA2-KO organoids stained for DLL1 or DLL4 (cyan) and FOXA2 (inferno). Three different sets of guides are shown (see Methods). Scale bar 25µm. **(G')** Quantification of FOXA2, **(G'')** DLL1 and DLL4 mean intensity. Ctrl: n = 132 organoids, FOXA2-KO: n = 304 organoids. **(H-H''')** ATAC-seq and FOXA1 ChIP-seq tracks at the *Dll1*, *Dll4*, *Jag1*, and *Jag2* loci. **(I, J)** Representative MIPs of FOXA1 with DLL1 (I) or DLL4 (J) in Control and FOXA1-overexpressing (FOXA1-OE) organoids. **(I', J')** Quantification of log2 fold change in DLL1⁺ (I') and DLL4⁺ (J') cell fractions in Control versus FOXA1-OE organoids. Ctrl: n=70864 cells from 1323 organoids, FOXA1-OE: n=45410 cells from 737 organoids. (A'',C',I',J') boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. (A') Line plot, mean intensities ± 90% CI. Statistical significance is indicated with * (p < 0.05), ** (p < 0.01), *** (p < 0.001). **(K)** Accessibility changes at *Foxa1* locus in secretory progenitors (blue) or absorptive progenitors (red). HES1 motif (yellow), FOXA1 motif (cyan), overlap of HES1 and FOXA1 (green).

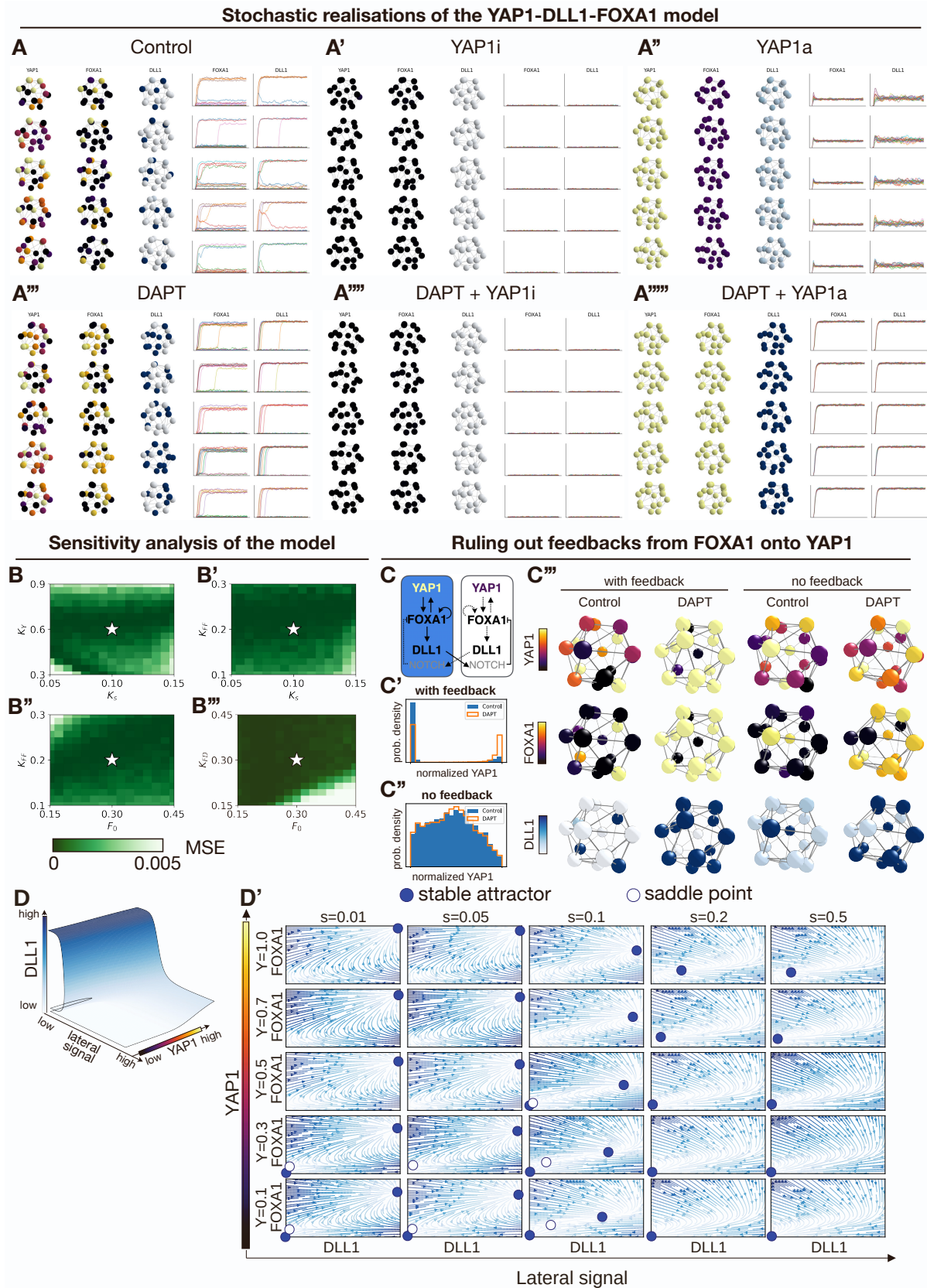


Figure S10. Stochastic realisations of the YAP1-DLL1-FOXA1 model, related to Figures 4

(A-A'') Dynamics of control (A), YAP1 inhibition (A'), YAP1 activation (A''), DAPT (A'''), DAPT and YAP1 inhibition (A''') and DAPT and YAP1 activation (A''') condition simulation. Left to right: 3D network plots of YAP1 state,

and FOXA1 and DLL1 final states; graphs display the temporal evolution of FOXA1 and DLL1 for each cell in the organoid (each cell is assigned a random color which is consistent between the FOXA1 and DLL1 graphs). **(B'-B''')** Sensitivity analysis of model parameters in the YAP1-FOXA1-DLL1 model. Panels show prediction mean-square-error (MSE, Eq. 3) evaluated over predicted DLL1+ fractions. Each parameter in the model is varied by $\pm 50\%$ of its standard value; white stars indicate standard values. For each pairwise parameter sweep, all other model parameters are held fixed at standard values as given in Table S4. **(C-C''')** Model with positive regulation of YAP1 by FOXA1. **(C)** Schematic network of the model. **(C')** YAP1 distributions of control and DAPT simulations. **(C'')** The same plot as in **(C')**, but for our standard model (Eq. 9). **(C''')** Organoid network plots of YAP1, FOXA1 and DLL1 for control and DAPT simulations. Throughout this figure, additional parameters $K_{F \rightarrow Y} = 0.6$ is used and a Hill coefficient of 4 are used; all other parameters are set to the standard values given in Table S4. **(D)** Dynamical systems and attractor manifold of the YAP1-FOXA1-DLL1 model. Each panel shows the phase portrait in (F_i, D_i) -space. Blue dots: stable fixed points (attractors); open dots: saddle points. The variation of these phase portraits as a function of YAP1 and lateral signal fully defines the dynamics of the system. **(D')** 3D attractor manifold showing the fixed points of DLL1 as a function of lateral signal and YAP1, derived from the YAP1-FOXA1-DLL1 model (STAR Methods).

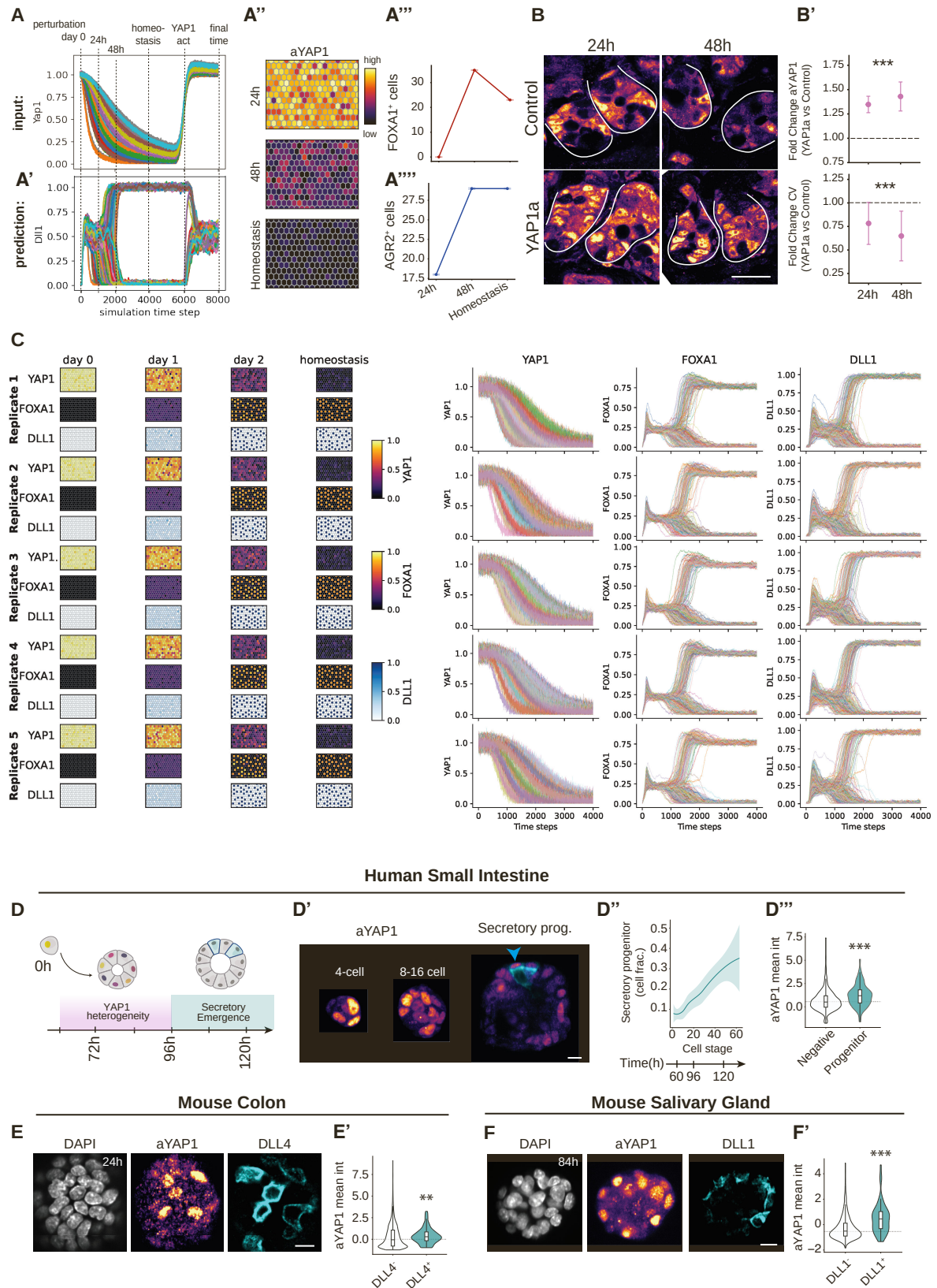


Figure S11. YAP1 heterogeneity and fate patterning *in vivo* and across tissues, related to Figures 4 and 5

(A) YAP1 trajectories are generated as input, modeling initial heterogeneous downregulation to homeostasis. Beyond 6000 time-steps, rapid increase of YAP1 models the YAP1 activation experiment, in which homeostatic

tissue is perturbed. **(A')** The model predicts the expected output DLL1 pattern as a function of time, as visualized in Figure 5A. **(A'')** Model predictions for *in vivo* regenerative time-course experiments. The YAP1-levels evolved similarly to experiment (uniform high to heterogeneously downregulated to uniform low, see STAR Methods, Figure 5A-B'). **(A''',A''')** Data used to generate smoothened line plots for Figure 5B'. N, n same as in Figure 5B'. **(B)** Z-planes of α YAP1 staining in Control and YAP1a conditions at 24 h and 48 h. **(B')** Fold change in mean α YAP1 intensity and CV relative to control. Data is mean $\pm$ s.e.m. **(C)** Results of five representative tissue simulations (approach to homeostasis, without YAP1 activation). From left to right, we show 2D spatial Voronoi structures (YAP1, FOXA1, DLL1), at four time-points. Graphs show time trajectories (YAP1, FOXA1, DLL1), where each curve corresponds to a single cell. **(D)** Schematic of human intestinal organoid regeneration from single cells. **(D')** MIPs of α YAP1 and secretory progenitor fate marker (MUC2, intermediate levels) of human intestinal organoids at 60-96h after single-cell plating. Scale bar, 10 μ m. **(D'')** Fraction of secretory progenitor cells over cell stage (x-axis) and mapped developmental time (bottom). N=6 wells from one representative experiment, n: 309 organoids. **(D''')** Box- and violin plot of α YAP1 z-scored nuclear mean intensity in negative (MUC2-negative cells) versus secretory progenitors (MUC2-intermediate cells). N=2 wells from one representative experiment, n: MUC2^{neg}=710, MUC2^{int}=163 cells, from 99 organoids. **(E)** MIPs of DAPI, α YAP1, DLL4 immunostaining of mouse colon organoids 24h after mechanical split. Scale bar, 10 μ m. **(E')** Box- and violin plot of α YAP1 z-scored nuclear mean intensity in DLL4^{neg/pos} cells. N=3 wells from one representative experiment, n: DLL4^{neg}=3,090, DLL4^{pos}=152 cells, from 210 organoids. **(F)** MIPs of DAPI, α YAP1, DLL1 immunostaining of mouse salivary gland organoids at 84h after single-cell plating. Scale bar, 10 μ m. **(F')** Box- and violin plot of α YAP1 z-scored nuclear mean intensity in DLL1^{neg/pos} cells. N=3 wells from one representative experiment, n: DLL1^{neg}=861, DLL1^{pos}=56 cells, from 57 organoids. (D''',E',F') boxplots: median, upper and lower quartiles; whiskers, 1.5x interquartile range. (D'') Line plot, mean intensities $\pm$ 90% CI. Statistical significance is indicated with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

Supplementary Tables

Model	DMSO	YAP1i	YAP1a	Total
YAP1-DLL1 (A)	6e-04	0	7e-02	7e-02
YAP1-DLL1 (B)	5e-04	7e-02	0	7e-02
YAP1-DLL1 (C)	1e-04	0	4e-07	1e-04
YAP1-DLL1 (D)	6e-05	2e-04	0	2e-04
YAP1-FOXA1-DLL1	8e-05	0	1e-07	8e-05

Table S1. MSE evaluation of the conditions to which model parameters are fitted, for the four model candidates using best fit parameters for each model, related to Figure 2. (A) YAP1-prepatterned DLL1 production model, $K_S = 0.1, K_Y = 0.6$, (B) YAP1-prepatterned DLL1 interaction model, $K_S = 0.1, K_Y = 0.6$, (C) YAP1-prepatterned DLL1 production and interaction model, $K_S = 0.25, K_Y = 0.6$, (D) YAP1-prepatterned DLL1 bistability model, $K_S = 0.1, K_Y = 0.6, K_D = 0.2$.

Model	DAPT	YAP1i+DAPT	YAP1a+DAPT	Total (all conds)
YAP1-DLL1 (A)	2e-01	0	2e-01	5e-01
YAP1-DLL1 (B)	6e-02	1	2e-01	1
YAP1-DLL1 (C)	2e-01	0	2e-01	4e-01
YAP1-DLL1 (D)	2e-02	2e-04	2e-01	3e-01
YAP1-FOXA1-DLL1	5e-02	0	2e-01	3e-01

Table S2. MSE evaluation of the conditions predicted by the model, for the four YAP1-DLL1 model candidates using best fit parameters for each model, as well as the YAP1-FOXA1-DLL1 model, related to Figure 4. (A) YAP1-prepatterned DLL1 production model, $K_S = 0.1, K_Y = 0.6$, (B) YAP1-prepatterned DLL1 interaction model, $K_S = 0.1, K_Y = 0.6$, (C) YAP1-prepatterned DLL1 production and interaction model, $K_S = 0.25, K_Y = 0.6$, (D) YAP1-prepatterned DLL1 bistability model, $K_S = 0.1, K_Y = 0.6, K_D = 0.2$.

Name	Symbol	Value
Hill coefficient lateral signal	K_S	0.1
Hill coefficient YAP1 activation	K_Y	0.6
Hill coefficient DLL1 self-activation	K_D	0.2
Intermediate DLL1 level	D_0	0.3
Hill exponent DLL1	h_D	4
Hill exponent YAP1	h_Y	4
Noise amplitude	σ	0.04

Table S3. Overview of parameters used in the YAP1-prepatterned DLL1 bistability model, in units with non-dimensionalized time and concentration coordinates, related to Figure 2. For direct comparison to experiment, predicted DLL1 levels are classified into low, intermediate, and high states using thresholds of 0.2 and 0.7.

Name	Symbol	Value
Hill coefficient lateral signal	K_S	0.1
Hill coefficient YAP1 activation	K_Y	0.6
Hill coefficient FOXA1	$K_{F \rightarrow F}$	0.2
Hill coefficient activation of DLL1 by FOXA1	$K_{F \rightarrow D}$	0.3
Intermediate FOXA1 level	F_0	0.3
Hill exponent DLL1	h_D	4
Hill exponent YAP1	h_Y	4
Hill exponent FOXA1	h_F	4
Noise amplitude	σ	0.02

Table S4. Overview of parameters used, in units with non-dimensionalized time and concentration coordinates, related to Figure 4. For direct comparison to experiment, predicted DLL1 levels are classified into low, intermediate, and high states using thresholds of 0.2 and 0.7.

Primer name	Primer sequence
mFOXA1-gRNAoligo15_fw, targeting exon2	5'- CACCG ACAGCGGGGCCACGGCGACA-3'
mFOXA1-gRNAoligo15_rv, targeting exon2	5'- AAACT GTCGCCGTGGCCCCGCTGTC-3'
mFOXA1-gRNAoligo18_fw, targeting 5' UTR	5'- CACCG GATGGTTGTGTGCGCCGGGC-3'
mFOXA1-gRNAoligo18_rv, targeting 5' UTR	5'- AAAC GCCCGGCCGACACAACCATCC-3'
mFOXA2-gRNA3_oligo_fw, targeting exon3	5'- CACCG ATGTCTTGGGGTCCCGAGCG-3'
mFOXA2-gRNA3_oligo_rv, targeting exon3	5'- AAAC CGCTCGGGACCCCAAGACATC-3'
mFOXA2-gRNA4_oligo_fw, targeting exon3	5'- CACCG CGGTTCCGGCAACATGAGCG-3'
mFOXA2-gRNA4_oligo_rv, targeting exon3	5'- AAAC CGCTCATGTTGCCGGAACCGC-3'
mFOXA2-gRNA5_oligo_fw, targeting exon3	5'- CACCG GTTGAAGGCGTAATGGTGCT-3'
mFOXA2-gRNA5_oligo_rv, targeting exon3	5'- AAAC AGCACCATTACGCCTTCAACC-3'
mFOXA2-gRNA6_oligo_fw, targeting exon2	5'- CACCG AAGGGCACGAGCCATCCGAC-3'
mFOXA2-gRNA6_oligo_rv, targeting exon2	5'- AAAC GTCGGATGGCTCGTGCCCTTC-3'
EF1A1-fw	5'-TCCACTTGGTCGCTTTGCT-3'
EF1A1-rv	5'-CTTCTTGTCCACAGCTTTGATGA-3'
FOXA1_qPCR_fw1	5'-GCCTTACTCCTACATCTCGCTC-3'
FOXA1_qPCR_rv1	5'-CTGCTGGTTCTGGCGGTAATAG-3'
FOXA2_qPCR_fw1	5'-CGAGCACCATTACGCCTTCAAC-3'
FOXA2_qPCR_rv1	5'-AGTGCATGACCTGTTCGTAGGC-3'
FOXA3_qPCR_fw1	5'-GTCCACACCTTATTTCAAGCGGC-3'
FOXA3_qPCR_rv1	5'-CACATCCAGTTTGAAGGTGTCG-3'

Table S5. Primers used in this study, related to Figure 3, Figure S1 and Figure S9. List of primers used to validate lines generated in this study.

Methods S1 - Development of a mathematical model of heterogeneity-driven intestinal organoid patterning

1. Model setup

To determine the interplay of YAP1 heterogeneity and DLL1-mediated lateral inhibition, we developed a minimal model of intestinal organoid patterning (Fig. S6A-A'''). To this end, we focused on signaling at the 16-cell stage during which patterning is initiated. Based on experimental findings that both YAP1 and DLL1 are critical for patterning, our goal was to identify the minimal regulatory network that links them. Such a network can be written as a general dynamical system for the change of DLL1 concentration D_i in each cell i :

$$\frac{dD_i}{dt} = f(D_i, s_i, Y_i) - D_i + \sigma \xi(t) \quad (1)$$

where $s_i = \frac{1}{N_i} \sum_j K_{ij} D_j$ is the lateral inhibition signal sent by neighboring cells, N_i is the number of nearest neighbors of cell i , and K_{ij} is the adjacency matrix determining the connectivity of the tissue. Thus, the lateral signal is given by the average DLL1 level of the nearest neighbors of each cell. This implementation builds on previous work on modeling lateral inhibition signaling^{16–18} in which DLL1-Notch interactions are implemented through a phenomenological neighbourhood signal (see Fig. S6B-B'' and section on the model including Notch below). The second term in Eq. (1) models the degradation dynamics of DLL1. Throughout, we used non-dimensionalized units such that the degradation constant of DLL1 is unity, to set the time unit. For more details, see the parameter overview (section 8). $\xi(t)$ is a Gaussian white noise process modelling the stochastic nature of cell signaling, with $\langle \xi(t) \rangle = 0$ and $\langle \xi(t) \xi(t') \rangle = \delta(t - t')$ and amplitude σ .

To simulate the model, we specified two inputs based on experimental data: the cell connectivity matrix K_{ij} and the YAP1 heterogeneity distribution Y_i . Our goal was to identify and characterize the dynamical system $f(D_i, s_i, Y_i)$ that captures the experimental observations on DLL1 expression in the tissue, and to make new predictions for perturbation phenotypes and quantitative correlations in the experiments. To this end, we systematically add terms to screen possible model candidates for this more general dynamical system (see Fig. S6C-C''' and section 3).

Firstly, K_{ij} is determined by the spherical connectivity of the tissue. To this end, we generated 3D spatial cell positions consistent with the experimental data using the following approach (Figure S6A-A'''): First, we placed points (cell nuclei) equidistantly on a sphere, and added a random noise with amplitude A . We then performed spherical Voronoi tessellation using *scipy*¹¹⁵ to identify the neighbourhood matrix. This gave access to the distribution of the

number of nearest neighbours for each cell. To match the statistics to our experimental data, we similarly performed spherical Voronoi tessellation on 3D segmentation of the cell nucleus positions of intestinal organoids. We fitted the noise amplitude A for these distributions to match quantitatively (Figure S6A''). This provided a procedure to generate organoid neighbourhood maps with the same statistics as those in the experiment. The resulting neighbourhood maps are then plotted by visualizing the 3D cell positions with the neighbourhood matrix visualized as connecting edges, providing a neighbourhood graph that preserves 3D spatial structure (Figure S6A''').

Secondly, we took YAP1 levels to be heterogeneously distributed among different cells. Based on our experimental finding that downstream YAP1 targets exhibit dynamics that are slow compared to the growth of the organoid (Figure 1B-B''), we first took YAP1 levels to be constant in time, to better understand their influence on DLL1 patterning in the model, before implementing slow (although still heterogeneous) decay in YAP1 activity. Based on observations that YAP1 activity is uncorrelated among neighbouring cells (Figure S6D-D'''), we then randomly sampled YAP1 activities Y_i for each cell i from the experimentally measured YAP1 distribution (Figure S6A'''). To make model parameters interpretable, we used normalized YAP1 values throughout, meaning we used the normalized experimental YAP1 distribution such that YAP1 activities are scored between 0 (min activity) and 1 (max activity).

Together with the initial condition of no DLL1 expression at the beginning of the patterning process, $D_i(t = 0) = 0$, this fully specified the setup for the simulations. We then used this model to predict the expected DLL1 pattern for different choices of the dynamical system f . This allowed us to systematically constrain f and thereby identify the regulatory network coupling YAP1 heterogeneity, DLL1 production and lateral inhibition signalling.

2. Ruling out pure lateral inhibition signalling

Using our model, we first simulated the expected dynamics of pure lateral inhibition, i.e. no coupling to YAP1 heterogeneity. In this case, the dynamical system is given by¹⁶

$$f(D_i, s_i, Y_i) = I(s_i; K_s) \quad (2)$$

where the first term is a production term of DLL1 that is inhibited by the signal s_i and the second term is a degradation term. Throughout, we used activation and inhibition functions modelled as Hill functions; which have been shown in multiple works to reproduce well the key aspects of lateral inhibition^{16–18}, i.e.

$$I(x; K) = \frac{1}{1 + \left(\frac{x}{K}\right)^h} \quad \text{and} \quad A(x; K) = \frac{\left(\frac{x}{K}\right)^h}{1 + \left(\frac{x}{K}\right)^h}$$

where h are the Hill exponents setting the steepness of the curve, and K is the Hill threshold parameter setting the location of the steepest response (at $x=K$).

Thus, for pure lateral inhibition we could visualize the DLL1 production term as a function of the signal received by each cell, which is a monotonically decreasing curve as a function of signal (Figure S6B). This already demonstrates that, by construction, the DLL1 production of each cell only depends on the received lateral inhibition signal, and would be independent of YAP1 heterogeneity, which is in contrast to the experimental observation that DLL1 patterning is critically affected by YAP1 perturbations. Furthermore, the pure lateral inhibition model predicts an average of 4 DLL1+ cells per 16-cell organoid, i.e. a DLL1+ fraction of 0.25 (Figure S6B', B''). This prediction is independent of parameters, as it only depends on the connectivity of the tissue, which we have constrained experimentally. It contrasts with the expected fraction of $1/3 \approx 0.33$ DLL1+ cells in a 2D hexagonal lattice. This is because in a 2D hexagonal system, all cells have 6 nearest neighbours, which is in contrast to the nearest neighbor distribution in our spherical organoids with a high fraction of cells with 5 nearest neighbors (Figure S6A'). However, this prediction exceeds the experimentally measured fraction of 0.2 ± 0.02 (SEM). Taken together, these results suggest that a pure lateral inhibition model is unable to capture the experiments.

3. Identifying a regulatory network constrained by perturbation phenotypes

We next systematically screened possible model candidates coupling YAP1 heterogeneity and lateral inhibition signaling. Our goal was to identify a model that is able to capture the following experimental observations: (1) The proportion of DLL1+ cells matches the experimental measurement, i.e. $f_{\text{exp}}^{\text{control}} = 0.2 \pm 0.02$. (2) Under YAP1 inhibition, the system fails to pattern: $f_{\text{exp}}^{\text{iYAP1}} = 0$. (3) Under YAP1 activation, the system fails to pattern: $f_{\text{exp}}^{\text{aYAP1}} = 0$. To incorporate these observations into an objective function evaluating fit quality, we evaluate the total mean-square-error (MSE) over predicted DLL1+ fraction f summed over conditions i , and seek model candidates that minimize the MSE:

$$\begin{aligned} \text{MSE} &= \sum_i (f_{\text{pred}}^i - f_{\text{exp}}^i)^2 \\ &= (f_{\text{pred}}^{\text{control}} - f_{\text{exp}}^{\text{control}})^2 + (f_{\text{pred}}^{\text{iYAP1}} - f_{\text{exp}}^{\text{iYAP1}})^2 + (f_{\text{pred}}^{\text{aYAP1}} - f_{\text{exp}}^{\text{aYAP1}})^2 \end{aligned} \quad (3)$$

Note that the MSE function only evaluates DLL1+ fractions, and we do not consider DLL1-intermediate fractions as part of the fitting procedure. Simulated DLL1-intermediate fractions are therefore predictions rather than fitting targets.

As model candidates, we constructed proposed dynamical systems $f(D_i, s_i, Y_i)$ by combining activation and inhibition functions (Eq. 3) of the key variables D_i, s_i, Y_i . We systematically

increased the complexity of the model by first considering combinations of two regulatory functions, before considering combinations of three functions. Rather than explicitly going through all possible combinations, we here present the predicted dynamics of the most promising model candidates, specifically those that combine the functions $I(s_i)$ to model lateral inhibition and $A(Y_i)$, motivated by the observed positive correlation between DLL1 and YAP1. Each of these models can be characterized using the curve of DLL1 production as a function of the lateral signal s_i , plotted for different values of YAP1 Y_i . Thus, in these models, each cell has a different DLL1 vs. lateral signal curve which is determined by its YAP1 level. For each model, we performed computational parameter screens of the key parameters that determine these curves (Figure S6E-E''').

We first considered multiple possible implementations of a scenario in which YAP1 positively regulates DLL1 (which has been proposed in several other experimental scenarios^{116,117}. To this end, we combine the functions $I(s_i)$ and $A(Y_i)$ in different ways. We first consider the two possible combinations of just these two functions, in which YAP1 regulates DLL1 either cell-autonomously, or through interactions.

3.1. Ruling out simple models: pre-patterned DLL1 production or interactions

First, we considered a model in which YAP1 regulates a cell-autonomous production of DLL1:

$$f(D_i, s_i, Y_i) = \frac{1}{1 + D_0} [D_0 A(Y_i; K_Y) + I(s_i; K_s)] \quad (4)$$

We termed this model the **YAP1-prepatterned DLL1 production model**, since in this model, YAP1 generates a baseline activation of DLL1, on top of which additional DLL1 production can occur depending on the lateral signal. The additional factor of $1/(1 + D_0)$ is simply for normalization of DLL1 levels to a maximum of 1, which does not affect the results. We visualized the steady-state DLL1 levels of the model as a function of the YAP1 level Y_i and the received lateral signal s_i , defined by

$$D_i = f(D_i, s_i, Y_i) \quad (5)$$

This showed that as a function of increasing YAP1, all cells have a higher background-level of DLL1, independent of the lateral signal, but the DLL1+ state ($D_i = 1$) is only reached for cells that receive low lateral signal (Figure S6C). For parameters $K_s < D_0$, this model captures the YAP1 activator experiment through an interesting effect: because YAP1 prepatterns DLL1, each cell receives sufficient signals from the neighbors to block any further DLL1 production through lateral signal; thus, patterning is prevented (Figure S6E-E'''). However, this model fails because it predicts patterning in the YAP1 inhibitor experiment. Specifically, while for low YAP1, there is no prepatterning (for low YAP1, $A(Y_i; K_Y) = 0$), but the second term in Eq. (5)

leads to DLL1 patterning through lateral inhibition interactions, which is not perturbed by YAP1 inhibition. Importantly, these findings are independent of parameters: for none of the tested combinations of (K_s, K_Y) , all three conditions (wildtype, YAP1 inhibition, YAP1 activation) are captured (Figure S6E). Note that in those regions of parameter space in which both YAP1 inhibition and YAP1 activation are captured, there is also no patterning in the wildtype scenario, which means these combinations are also inconsistent with experiment.

Second, we considered a model in which YAP1 regulates the DLL1 production term that is controlled through lateral inhibition, i.e. it modulates how cells respond to external signal received through interactions with neighboring cells:

$$f(D_i, s_i, Y_i) = A(Y_i; K_Y)I(s_i; K_s) \quad (6)$$

We termed this model the **YAP1-prepatterned DLL1 interaction model** (Figure S6C'). Here, only YAP1+ cells are able to become DLL1+: for low YAP1, $A(Y_i; K_Y) = 0$, and thus no DLL1 production occurs, irrespective of the received lateral signal. This model does not capture the experiment since it predicts a 25% fraction of DLL1+ cells in a YAP1 activation experiment (Figure S6E). This is because for high YAP1, $A(Y_i; K_Y) = 1$, and thus the model simply recovers the pure lateral inhibition case (Figure S6B-B''). Again, we find similar behavior across parameter combinations: for none of the tested combinations of (K_s, K_Y) , all three conditions (wildtype, YAP1 inhibition, YAP1 activation) are captured (Figure S6E').

3.2. Model with combined YAP1-prepatterned DLL1 production and interactions

We next explored a model that combines these two options:

$$f(D_i, s_i, Y_i) = \frac{1}{1 + D_0} [D_0 A(Y_i; K_Y) + A(Y_i; K_Y)I(s_i; K_s)] \quad (7)$$

which we termed the **YAP1-prepatterned DLL1 production and interaction model** since it combines the previous two models (Figure S6C''). This combination exhibits the two key features that allowed the previous models to capture the perturbations: first, like the YAP1-prepatterned DLL1 production model, it exhibits a DLL1 intermediate state at high YAP1, leading to blocking of patterning in the YAP1 activation experiment (Figure S6C''). Second, like the YAP1-prepatterned DLL1 interaction model, steady-state DLL1 levels vanish for low YAP1 cells independent of the lateral signal, leading to absence of patterning in the YAP1 inhibition experiment (Figure S6C''). Thus, this model successfully predicted the absence of patterning in both YAP1 inhibitor and activator experiments, while capturing the correct proportion of DLL1 positive cells in the wildtype (Figure S6E'').

However, this model also predicted that if the YAP1 level of a cell is downregulated, it can no longer be DLL1 positive. This is fundamentally inconsistent with the observed time-progression in the experiment, in which YAP1 is heterogeneously downregulated in time, leading to YAP1 heterogeneity being only transient, with uniformly low YAP1 activity at late times. This leads to patterning during the heterogeneous phase, but DLL1 positive cells are lost at later stages when YAP1 activity is low. This led us to hypothesize that in addition to the DLL1- stable state for YAP1-low cells, a second DLL1+ state should exist for these cells, separated by a barrier. This suggested bistability as a function of the received lateral signal, which is the simplest mechanism allowing an inductive signal to be first critical and then dispensable⁷⁴. Interestingly, such bistability has been previously proposed in the context of DLL1-Notch signalling in other systems¹⁸, making it a promising model candidate.

3.3. YAP1-prepatterned DLL1 bistability model

A model in which YAP1 levels determine a cell-intrinsic bistability of DLL1 is implemented by making the interaction term depend not directly on YAP1 levels Y_i as in the YAP1-prepatterned DLL1 production and interaction model (Eq. 7), but on DLL1 itself:

$$f(D_i, s_i, Y_i) = \frac{1}{1 + D_0} [D_0 A(Y_i; K_Y) + A(D_i; K_D) I(s_i; K_S)] \quad (8)$$

which we termed the **YAP1-prepatterned DLL1 bistability model** (Figure S6C'''). In contrast to the previous models, in which the signal-response curves were always monostable, low YAP1 cells now exhibit stable states for both DLL1+ and DLL1-, with the preferred state depending on signalling history. This bistability is controlled by the YAP1 activity of the cell, which gives the cellular landscapes the following structure (Figure S6C'''): YAP1-low cells are bistable at low lateral signal, meaning they can remain in a low-DLL1 state; at high lateral signal, there is only the low DLL1 state available. YAP1-high cells have a single stable DLL1-high state at low signal, and an intermediate DLL1 state at high signal due to the pre patterning term. Thus, in the YAP1 inhibitor, cells are stuck in the low DLL1 state. In the YAP1 activator, all cells exhibit intermediate DLL1 and thus are blocked from becoming DLL1+ by the lateral signal of the neighbours. In numerical simulations, this model again is able to predict the YAP1 inhibition and activation experiments, while also capturing the experimentally measured DLL1+ fraction in wildtype (Figure S6E'''). Throughout these models, we include a moderate level of intermediate noise in the chemical kinetics equations ($\sigma = 0.04$, see parameter table), which is low enough that spontaneous stochastic transitions across the barrier in the bistable regime are rare.

3.4. Simulation of different cell stages in the YAP1-prepatterned DLL1 bistability model

In Figure 2, we indicate how the dynamical landscape is putatively explored during different cell stages. Based on the fact that the dynamics of organoid growth is relatively slow (tens of hours), we assume that our dynamical equations for signalling are in the quasi-static regime. Thus, we perform simulations at fixed cell number, but inputting for each stage the YAP1 distribution from experimental measurements (YAP1 homogeneously high in 4-cell stage, heterogeneous in 16-cell stage, homogeneously low in >32-cell stage). We then visualize simulated steady-state outcomes for the combination of YAP1, lateral signal, DLL1 values (see also section 8 for the *in vivo* dynamics).

4. YAP1-FOXA1-DLL1 model

4.1. Model development

To enable a mechanistic interpretation of the dynamical system identified by our model screen, we considered how the DLL1 cell-intrinsic bistability and the YAP1 pre patterning could be implemented mechanistically through interaction of molecular pathways.

From a theoretical perspective, a minimal implementation of bistability is a positive autoregulation, for instance if DLL1 could activate an intermediate species X which in turn positively regulates DLL1 production. However, in the canonical model of Delta-Notch signalling, Delta has no signalling pathway linking back to the nucleus and therefore does not directly control gene regulation¹¹⁸. An alternative (mathematically equivalent) formulation is that the species X is trans-inhibited by the DLL1 of the neighbouring cell. Mechanistically, this could function through trans-activation of Notch by DLL1 combined with cis-inhibition of X by Notch (see next section on an explicit model including Notch dynamics). The intermediate species X could furthermore provide a link from YAP1 to DLL1, if YAP1 activates X and X activates DLL1. As discussed in the main text, based on single-cell ATAC sequencing, we identified the transcription factor FOXA1 as a candidate for this intermediate species (Figure 3), and therefore included its dynamics in our model.

A minimal extension of our YAP1-prepatterned DLL1 bistability model (Eq. 8) which includes FOXA1 dynamics is given by the following set of coupled equations:

$$\begin{aligned} \frac{dF_i}{dt} &= \frac{1}{1 + F_0} [F_0 A(Y_i; K_Y) + A(F_i, K_{F \rightarrow F}) I(s_i; K_S)] - F_i + \sigma \xi(t) \\ \frac{dD_i}{dt} &= A(F_i, K_{F \rightarrow D}) - D_i + \sigma \xi(t) \end{aligned} \quad (9)$$

This model is equivalent to the identified dynamical system from the previous section, under the assumption that the dynamics of DLL1 production are fast compared to those of FOXA1:

if we approximate the first equation to be in steady-state, i.e. $dD_i/dt = 0$, and linearize the Hill function, we obtain $D_i \approx F_i$. Substituting into the second equation recovers the YAP1-prepatterned DLL1 bistability model (Eq. 8). Similar to the YAP1-DLL1 models, we fitted the parameters of this model to simultaneously predict patterning of FOXA1 and DLL1, given an input distribution of YAP1 (Figure S6A'', A'''). Our model then defines how varying lateral signal s_i and YAP1 activity Y_i modulate the dynamical system governing FOXA1 and DLL1 dynamics. To visualize this, we plot the phase portraits in (F_i, D_i) -space as a function of lateral signal and YAP1 activity, showing the location of attractors and flow fields that determine the dynamics of the system (Figure S10A, A'). Plotting the fixed points of DLL1 as a function of YAP1 and lateral signal from the YAP1-FOXA1-DLL1 model (Eq. 9) recovers the attractor manifold of this model, demonstrating its equivalency to the YAP1-prepatterned DLL1 bistability model (Eq. 8).

The bistability at low YAP1 and low lateral signal gives rise to hysteresis behaviour, meaning that the system's state depends not only on present inputs but also on its history. Throughout this work, we use the term 'memory' to describe the consequence of this hysteresis, namely the ability of cells to retain their fate state (DLL1⁺ or DLL1⁻) after the transient YAP1 signal has diminished. The bistable FOXA1–DLL1 regulatory feedback creates this hysteresis, enabling transient YAP1 heterogeneity to be converted into stable fate outcomes. We use the term 'memory' in the main text because it more directly reflects the biological interpretation of this hysteretic behaviour in the context of cell fate decisions.

4.2. Parameter fitting and model evaluation

To quantitatively evaluate model performance, we evaluate the MSE function (Eq. 3) for the best fit parameters of the YAP1-FOXA1-DLL1 model and compare it to the other considered model candidates. First, we consider the three conditions to which model parameters are fitted (DMSO, YAP1i, YAP1a), showing that the MSE is indeed minimized by the YAP1-prepatterned DLL1 bistability model (model D) which captures all three conditions, and an even better fit is achieved by our final YAP1-DLL1-FOXA1 model (Table S1). As expected, model C performs equally well as model D on these metrics as it also captures all three conditions (Figure S6C''). Second, we evaluate model performance on the additional conditions that are predicted by the model without fitting the parameters to them (DAPT, YAP1i+DAPT, YAP1a+DAPT), and find that consistently, the YAP1-prepatterned DLL1 bistability model and the YAP1-FOXA1-DLL1 models perform the best out of all models (Table S2).

4.3. Stochastic dynamics

Since simulations are stochastic, we show the patterning dynamics of multiple replicate simulated organoids for each condition in the following set of figures: Figure S10A-A'''''

4.4. Sensitivity analysis of the model parameters

To test the sensitivity of our predictions to the exact choice of parameters, we perform sweeps of the YAP1-FOXA1-DLL1 model around the vicinity of the chosen values for pairwise combinations of parameters, while all other parameters are held fixed (Figure S10B-B'''). We find that predictions are not highly sensitive to the exact choice of parameter values, as the experimentally observed DLL1+ fraction is captured in a broad region around the standard parameter values. Throughout the study, we set all Hill exponents to $h = 4$, although the results are not sensitive to this choice as long as a sufficiently high value is chosen.

5. Model extensions

5.1. Approximating a model with Notch interactions

At a molecular level, the lateral inhibition signalling described by our model is implemented by trans-membrane interactions of DLL1 and Notch. Throughout our modelling approach, to reduce the number of parameters in the model, we neglected the dynamics of Notch expression as we assume these to be strongly anti-correlated to those of DLL1, and therefore used the inhibition by neighbourhood signal as an effective implementation of DLL1-Notch dynamics throughout. Furthermore, we primarily predict steady-state patterns, which are not sensitive to additional temporal delays introduced by Notch dynamics.

To demonstrate how our model (Eq. 9) is implemented at the molecular level, we first wrote a complete model including DLL1-Notch trans-membrane interactions, as indicated schematically in Figure 4A. Specifically, we assumed that the inhibition of FOXA1 by DLL1 of neighbouring cells is implemented the following way: DLL1 leads to an increase of activated (i.e., cleaved) Notch in neighbouring cells (i.e. trans-activation, neglecting alternative or additional mechanisms such as cis-inhibition¹⁷ and cis-activation¹¹⁹), and the activated Notch subsequently inhibits FOXA1 (e.g., through the HES1). Adding this additional step in our model formulated in Eq. 9 leads to the following set of equations:

$$\begin{aligned}\frac{dN_i}{dt} &= A(s_i) - N_i \\ \frac{dD_i}{dt} &= A(F_i) - D_i \\ \frac{dF_i}{dt} &= A(Y_i) + A(F_i)I(N_i) - F_i\end{aligned}\tag{10}$$

Where N_i is Notch in cell i . As before, $s_i = \frac{1}{N_i} \sum_j K_{ij} D_j$ is the DLL1 level of neighboring cells. If Notch is in steady-state (i.e., its dynamics evolve faster than those of FOXA1 and DLL1), i.e. $dN_i/dt = 0$, and linearizing the Hill functions, then Notch effectively tracks the lateral signal,

i.e. $N_i \approx s_i$, which consequently maps Eq. 10 to Eq. 9. Therefore, this more detailed molecular model including Notch directly maps onto our simplified model, in which the neighbor DLL1 signal inhibits FOXA1.

5.2. Ruling out positive regulation of YAP1 by FOXA1

An additional potential feedback mechanism in the system are feedbacks from the FOXA1-DLL1 loop onto YAP1. Indeed, previous work⁷² suggests that YAP1 is positively regulated by FOXA1 in liver cancer cells. To test the implications of this possibility in our system, we consider a minimal model in which YAP1 activity is not just an upstream variable, but treated as a dynamic process with feedbacks. Specifically, we assume that YAP1 exhibits heterogeneous baseline activation with rate a_i to generate initial heterogeneity, and is activated by FOXA1 (Figure S10C). This is implemented using the following set of coupled equations:

$$\begin{aligned} \frac{dY_i}{dt} &= a_i + A(F_i, K_{F \rightarrow Y}) - Y_i \\ \frac{dF_i}{dt} &= \frac{1}{1 + F_0} [F_0 A(Y_i; K_Y) + A(F_i, K_{F \rightarrow F}) I(s_i; K_S)] - F_i + \sigma \xi(t) \\ \frac{dD_i}{dt} &= A(F_i, K_{F \rightarrow D}) - D_i + \sigma \xi(t) \end{aligned} \quad (11)$$

Here, a_i is again drawn from a random distribution given by the experimental distribution of YAP1 heterogeneity. As intuitively expected, this leads to a much stronger correlation of YAP1 with FOXA1 and DLL1, leading to a bimodal distribution of YAP1, contrary to our experimental observations (Figure S10C'-C'''). The discrepancy between model and experiments is further highlighted by the predicted YAP1 pattern in the DAPT experiment: here, a much higher fraction of cells are predicted to be YAP1+, leading to a change of YAP1 distribution relative to the control simulation (Figure S10C'). This is again contrary to our experimental measurements (Figure S7I-I''), and unlike our standard model (Eq. 9) without feedback (Figure S10C''). Therefore, we conclude that positive regulation of YAP1 by FOXA1 does not play a significant role in the patterning process of intestinal organoids.

6. Prediction of in vivo regeneration dynamics

To make predictions for the expected dynamics of FOXA1 and DLL1 emergence during regeneration *in vivo*, we generalized our model to larger tissues and to a temporally modulated YAP1 heterogeneity. As before, we will use a distribution of YAP1 states among cells as an input, and predict the expected output, namely FOXA1 and DLL1 patterns.

To this end, we simulated signalling dynamics on a large 2D tissue with a hexagonal lattice of cells. We focus on the dynamics in the bulk of the tissue to avoid boundary effects. We then used a minimal implementation of the dynamics of YAP1 activity observed *in vivo*. Initially, cells are uniformly active in YAP1, and we therefore set all cells to uniform maximal $Y=1$. In homeostasis, YAP1 is uniformly inactive, and the final state is therefore with all cells distributed around uniform $Y=0$. To interpolate between these two states, and given our *in vivo* observation of YAP1 heterogeneity in static snapshots, we generate YAP1 trajectories in single cells that heterogeneously reduce their YAP1 activity at randomly chosen rates (Figure S11A-A''). Given that the *in vivo* experiments, and YAP1 downregulation, occur on time scales of days, we use a time-scale for YAP1 dynamics that is much slower than the time-scale of equilibration of the dynamical system, such that the FOXA1 and DLL1 dynamics are in quasi-steady-state. This is therefore consistent with the implementation of YAP1 heterogeneity in the modelling results above. The model then predicts the expected output patterns of FOXA1 and DLL1, in which patterning of FOXA1 and DLL1 emerges during the heterogeneous downregulation of YAP1 (Figure S11A-B' and Figure 5). Multiple runs of these simulations give consistent results (Figure S11C).

Furthermore, to model the *in vivo* YAP1 activation experiment, we rapidly increase YAP1 after homeostasis was reached in the previous simulations. In our model, such YAP1 activation brings all cells back to the intermediate state, thus destroying the DLL1 spatial pattern. This prediction is nontrivial as it demonstrates that YAP1 continues to control DLL1 patterning even in homeostatic states, consistent with experiments.

7. Numerical implementation

The dynamical system (Eq. 1) is numerically integrated using custom-written python code using numpy¹²⁰. The time derivative is approximated using Euler forward differences. Specifically, the following scheme for each cell i is used:

$$D_i(t + \delta t) = D_i(t) + [f(D_i(t), s_i(t), Y_i) - D_i(t)]\delta t + \sigma r_t \sqrt{\delta t}$$

$$s_i(t) = \sum_j K_{ij} D_j(t)$$

where r_t is a Gaussian random number with mean zero and unit variance.

8. Parameter overview

The parameters of the YAP1-prepatterned DLL1 bistability model (Eq. 8, Figure S6C''') are summarized in Table S3. The parameters of the final YAP1-FOXA1-DLL1 model (Eq. 9) are summarized in Table S4.

9. Methods S1 references

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