Cell Systems, Volume *13*

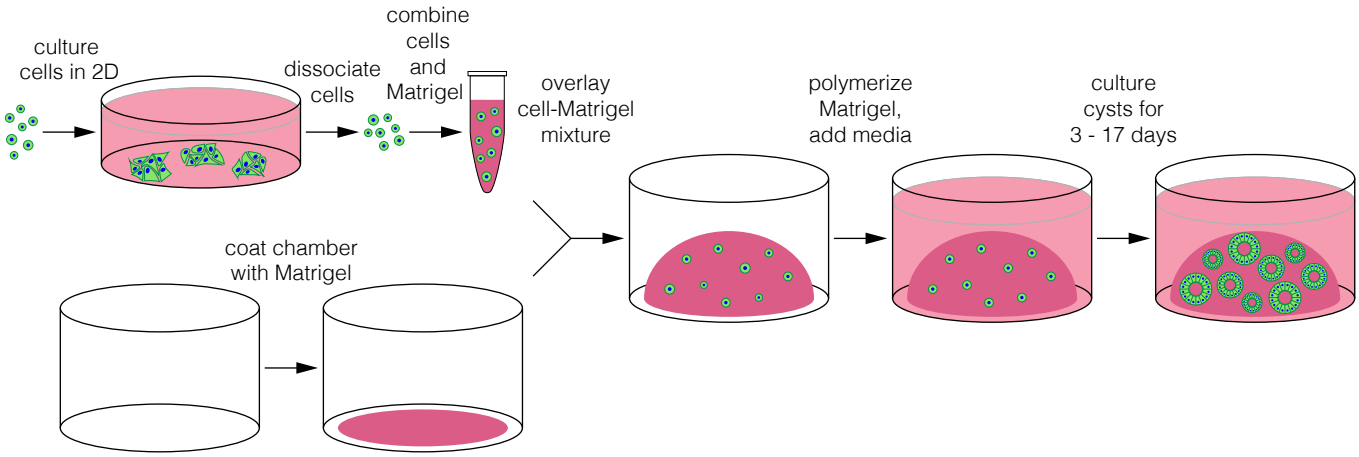
Supplemental information

Systematically quantifying morphological features reveals constraints on organoid phenotypes

Lauren E. Beck, Jasmine Lee, Christopher Coté, Margaret C. Dunagin, Ilya Lukonin, Nikkita Salla, Marcello K. Chang, Alex J. Hughes, Joseph D. Mornin, Zev J. Gartner, Prisca Liberali, and Arjun Raj

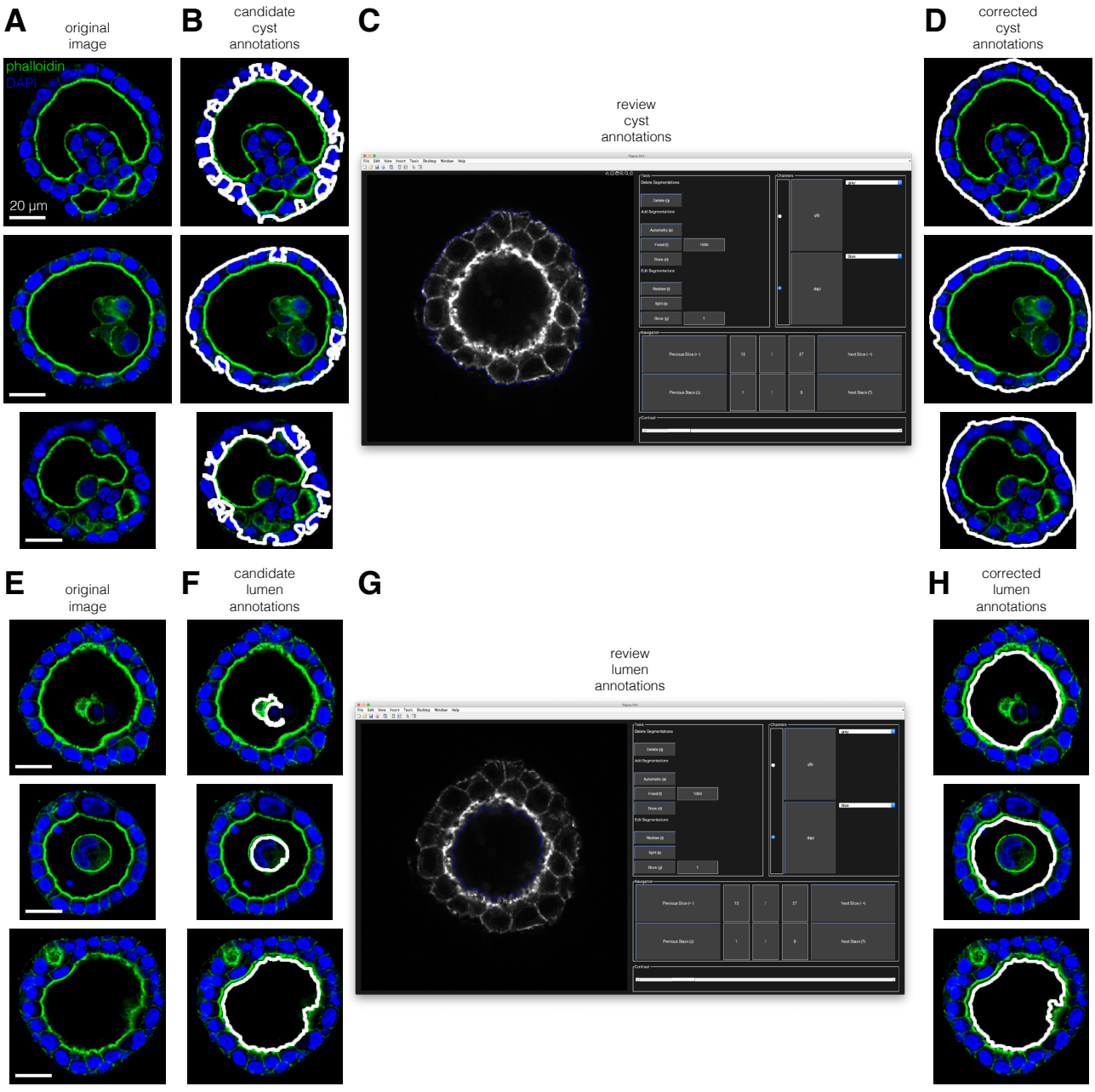
Supplemental Figure 1

A



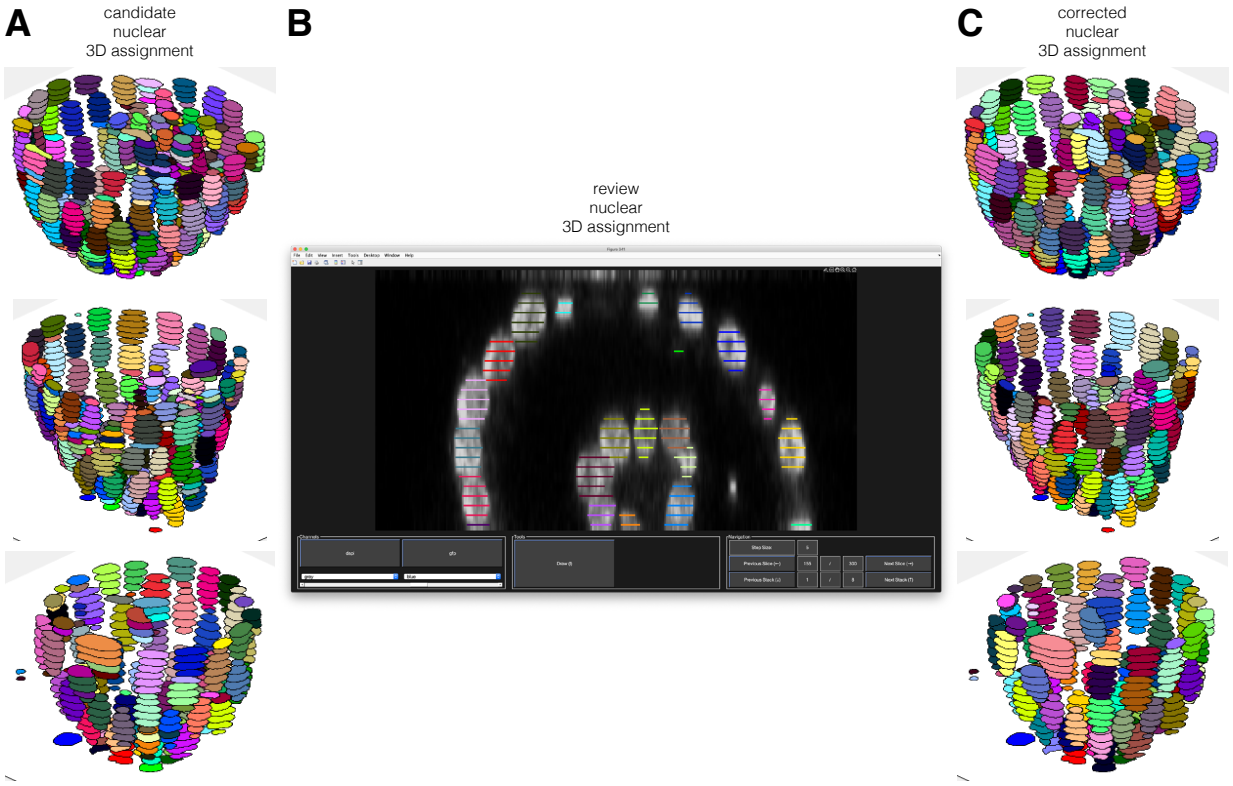
Supplemental Figure 1: Schematic for MDCK cyst culture technique.
A. MDCK cells are maintained in two-dimensional culture. When the cells are sufficiently confluent, they are dissociated into a single cell suspension. Cells are added to liquid Matrigel and the cell-Matrigel mixture is plated into a cell culture chamber already coated with pure Matrigel. After the Matrigel has polymerized, media can be added and the cysts can be cultured for at least 17 days. See methods for more information.

Supplemental Figure 2



Supplemental Figure 2: Manually correcting 2D cyst and lumen annotations.
A. Three example MDCK cysts, whose candidate annotations are representative of the range of annotations seen for all MDCK cysts. The scale bar represents 20 μm.
B. Example candidate annotations for the cyst boundary.
C. Our user interface for viewing and correcting 2D annotations.
D. Example annotations for the cyst boundary after correction.
E. Three example MDCK cysts, whose candidate annotations are representative of the range of annotations seen for all MDCK cysts. The scale bar represents 20 μm.
F. Example candidate annotations for the lumen boundaries.
G. Our user interface for viewing and correcting 2D annotations.
H. Example annotations for the lumen boundaries after correction.

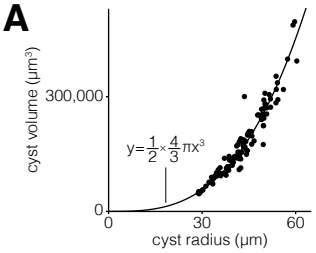
Supplemental Figure 3



Supplemental Figure 3: Manually correcting 3D nuclear annotations.
A. Example candidate nuclear annotations, color-coded by which 3D object they belong to.
B. Our user interface for viewing and correcting 3D nuclear annotations.
C. Example nuclear annotations after they have been manually corrected.

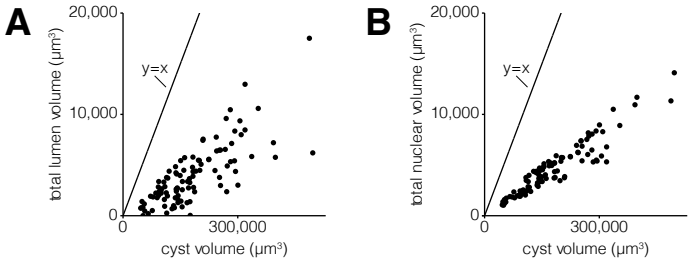
Supplemental Figure 4

A



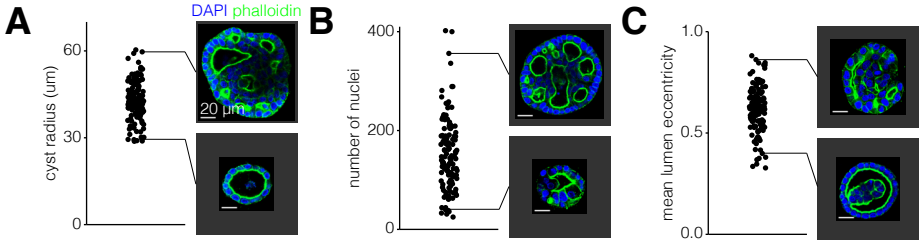
Supplemental Figure 4: Cyst volume scales with cyst radius to the third.
A. Cyst volume versus cyst radius for 102 7-11 day old MDCK cysts. Reference line indicates the relationship between radius and volume of half a sphere.

Supplemental Figure 5



Supplemental Figure 5: Total lumen and nuclear volumes are less than cyst volume.
A. Total lumen volume versus cyst volume for 102 7-11 day old MDCK cysts. Reference line indicates y = x.
B. Total nuclear volume versus cyst volume for 102 7-11 day old MDCK cysts. Reference line indicates y = x.

Supplemental Figure 6



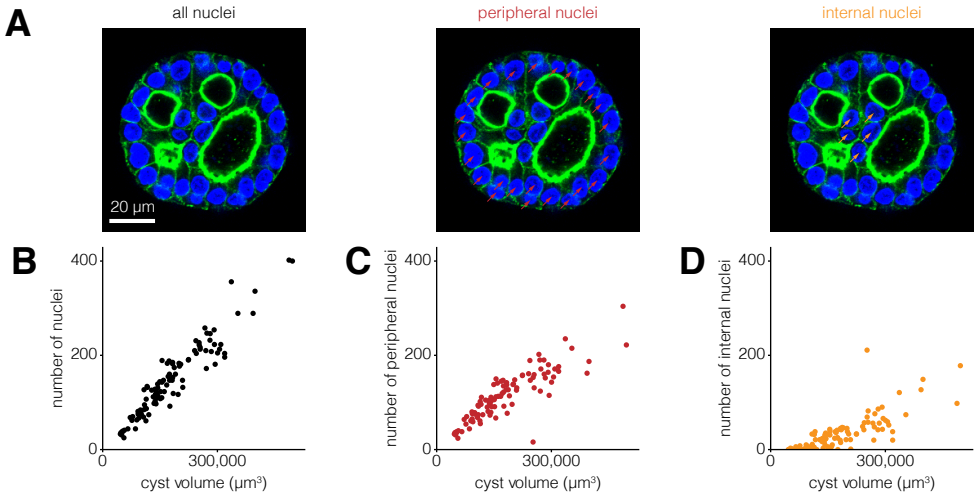
Supplemental Figure 6: Examples MDCK cysts with high and low values for cyst morphological features.

A. Cyst radius for 102 7-11 day old MDCK cysts with example images. The scale bar represents 20 μm.

B. Number of nuclei for 102 7-11 day old MDCK cysts with example images. The scale bar represents 20 μm.

C. Mean lumen eccentricity for 102 7-11 day old MDCK cysts with example images. The scale bar represents 20 μm.

Supplemental Figure 7



Supplemental Figure 7: Peripheral scale sublinearly and internal nuclei scale superlinearly with cyst volume.

A. Example MDCK cyst (representative of 102 unperturbed MDCK cysts of ages 7-11 days) with peripheral nuclei annotated with a red dot and internal nuclei annotated with an orange dot. The scale bar represents 20 μm.

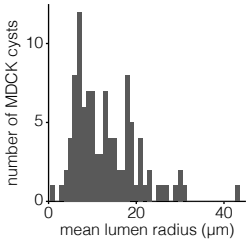
B. Number of nuclei versus cyst volume for 102 7-11 day old MDCK cysts.

C. Number of peripheral nuclei versus cyst volume for 102 7-11 day old MDCK cysts.

D. Number of internal nuclei versus cyst volume for 102 7-11 day old MDCK cysts.

Supplemental Figure 8

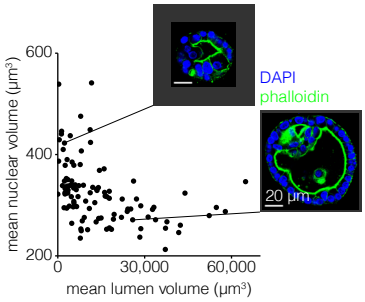
A



Supplemental Figure 8: MDCK cysts may have a minimum size for lumens.
A. Histogram (with a bin width of 1 um) of mean lumen radius for 102 MDCK cysts cultured for 7-11 days.

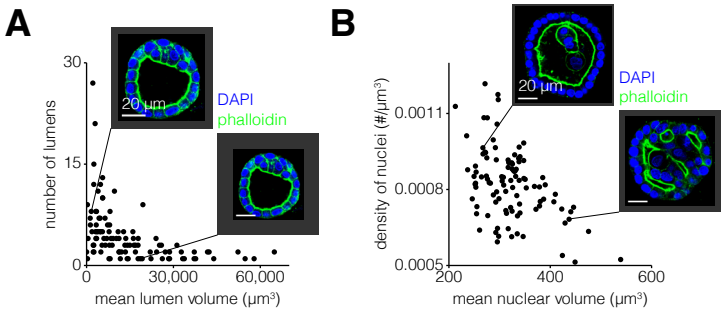
Supplemental Figure 9

A



Supplemental Figure 9: Mean nuclear size is inversely correlated with mean lumen size. **A.** Mean nuclear volume versus mean lumen volume for 102 7-11 day old MDCK cysts with example images. The scale bar represents 20 μm.

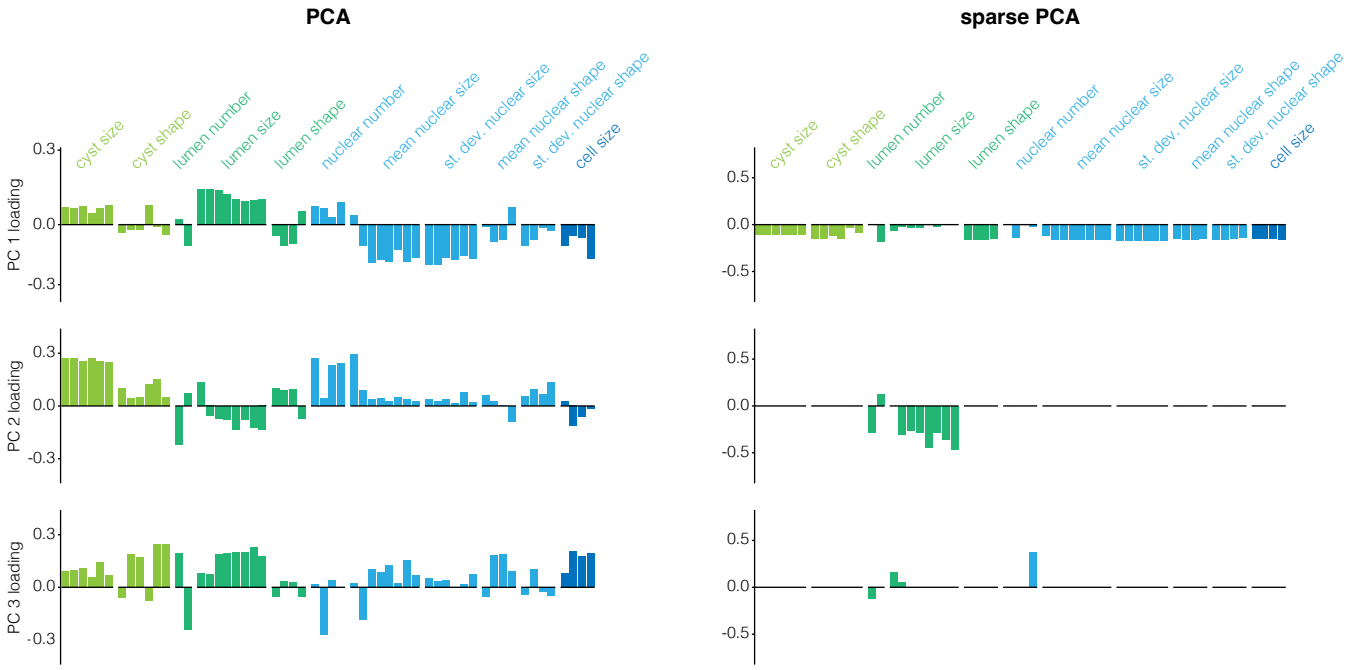
Supplemental Figure 10



Supplemental Figure 10: The number of lumens and nuclei are inversely correlated with their mean volume.
A. Number of lumens versus mean lumen volume for 102 7-11 day old MDCK cysts with example images. The scale bar represents 20 μm.
B. Density of nuclei versus mean nuclear volume for 102 7-11 day old MDCK cysts with example images. The scale bar represents 20 μm.

Supplemental Figure 11

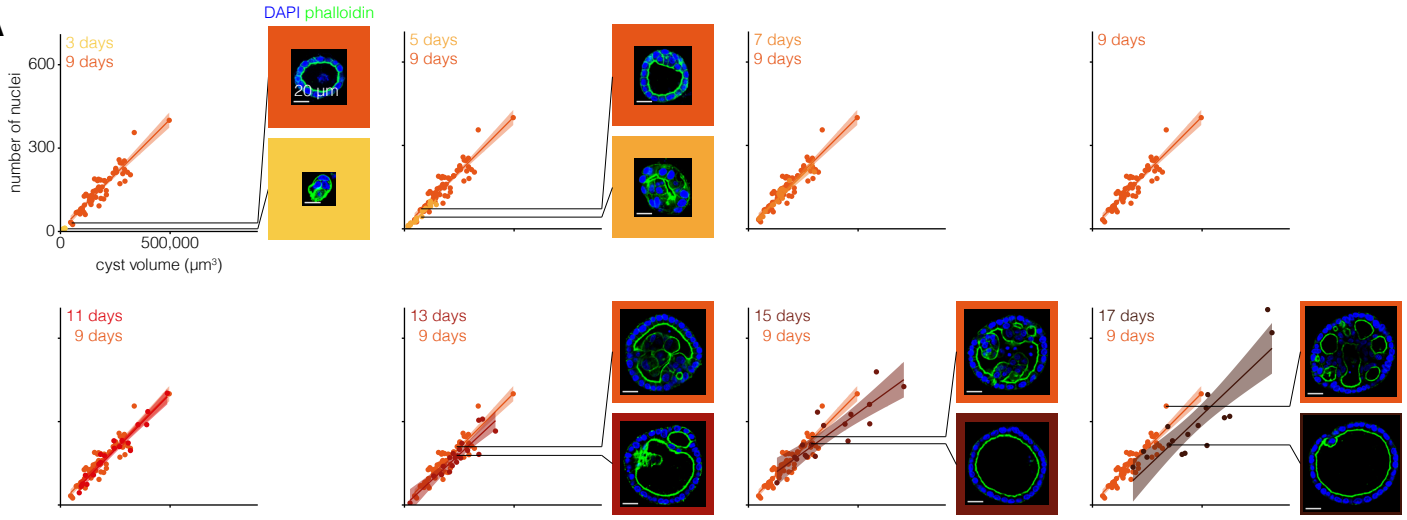
A



Supplemental Figure 11: Sparse PCA reveals similar constraints to traditional PCA.
A. Loading of each feature on principal components one through three for both standard PCA and sparse PCA. Each feature is color-coded by what structure (cyst, lumen, nucleus, or cell) it describes.

Supplemental Figure 12

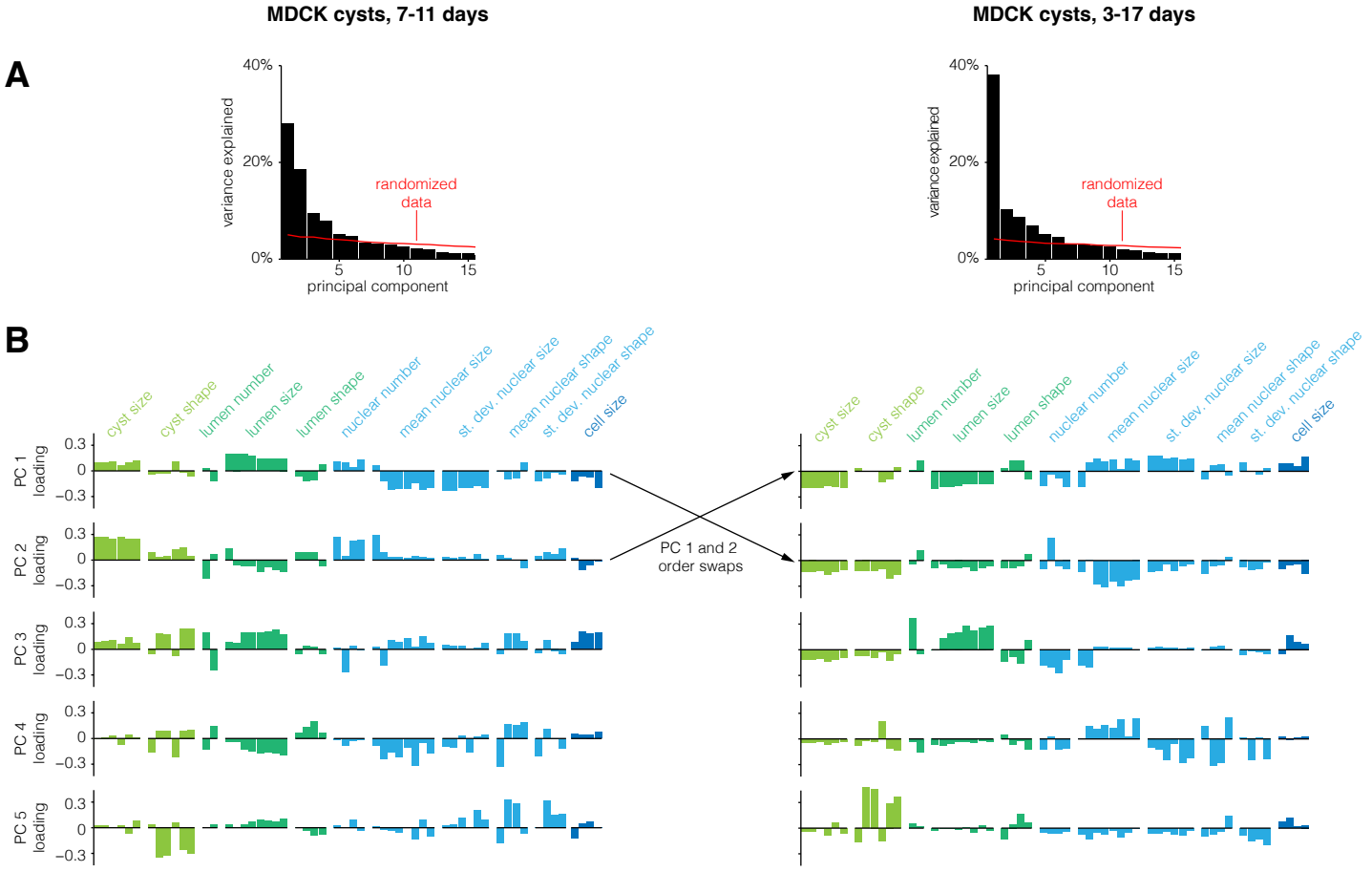
A



Supplemental Figure 12: Constraint on number of nuclei and cyst volume varies with MDCK cyst age.

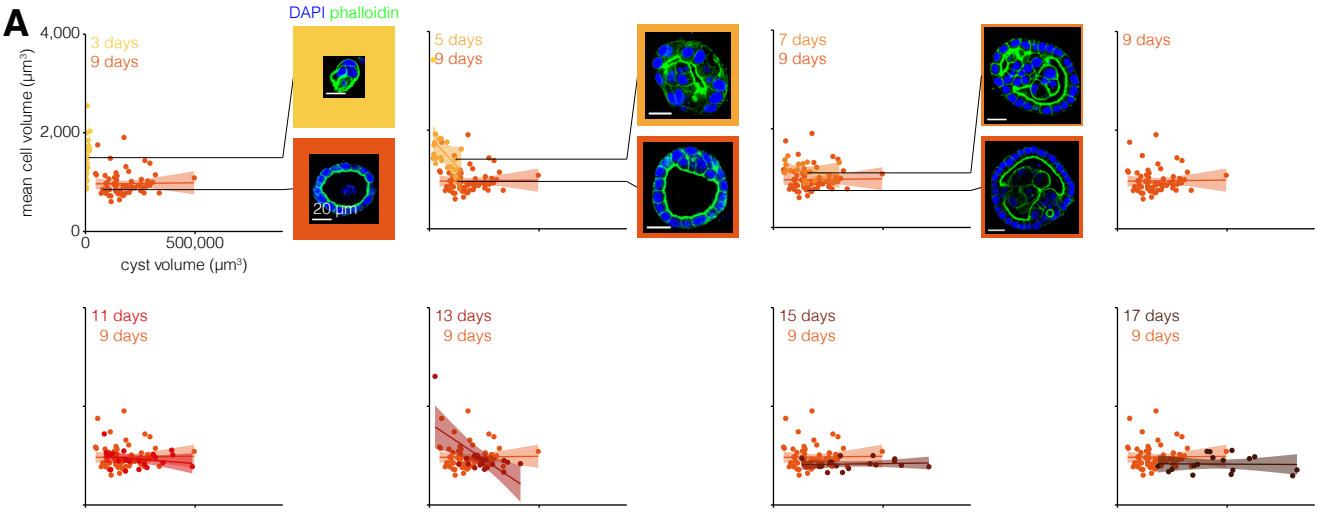
A. Number of nuclei versus cyst volume for 17-64 MDCK cysts of each age. Each age is represented by one color, and 9 day old MDCK cysts are repeated on each graph for reference. The line represents the line of best fit and the shaded area represents the 95% confidence interval. Example MDCK cysts of different ages with approximately the same volume and different numbers of nuclei are shown. The scale bar represents 20 μm.

Supplemental Figure 13



Supplemental Figure 13: Constraints on MDCK cysts are consistent across cysts of all ages.
A. Variance explained by each principal component. The red line indicates how much variance is explained when the data is randomized before PCA (see methods for details).
B. Loading of each feature on principal components one through five. Each feature is color-coded by what structure (cyst, lumen, nucleus, or cell) it describes.

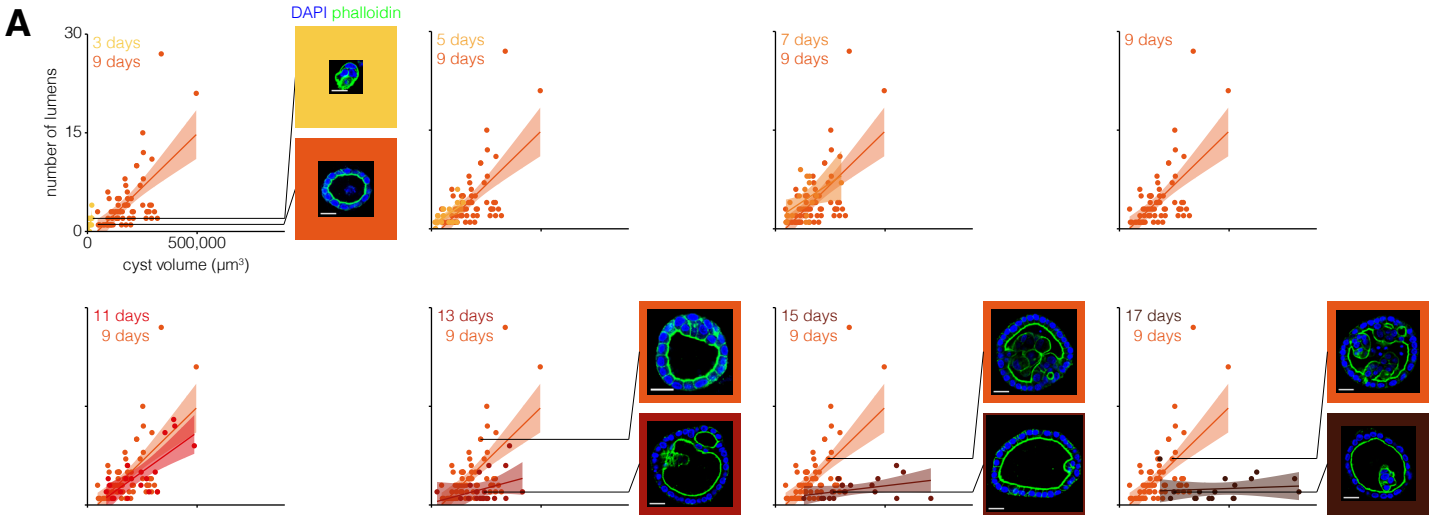
Supplemental Figure 14



Supplemental Figure 14: Constraint on cell volume and cyst volume varies with MDCK cyst age.

A. Mean cell volume versus cyst volume for 17-64 MDCK cysts of each age. Each age is represented by one color, and 9 day old MDCK cysts are repeated on each graph for reference. The line represents the line of best fit and the shaded area represents the 95% confidence interval. Example MDCK cysts of different ages with approximately the same cyst volume and different mean cell volume are shown. The scale bar represents 20 μm.

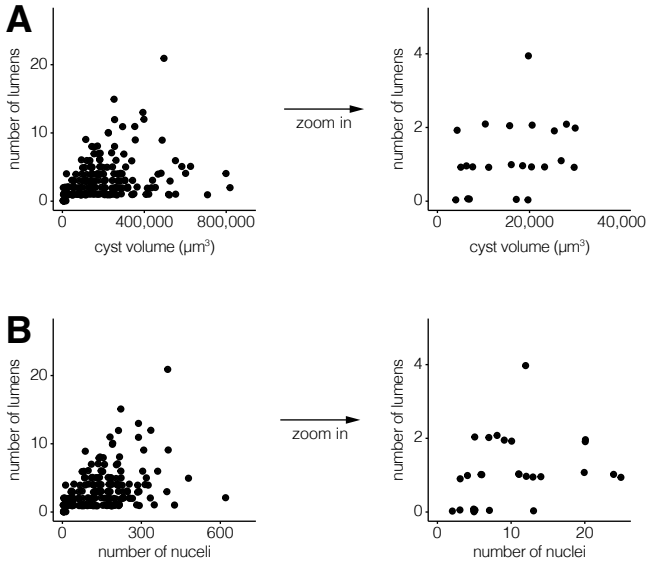
Supplemental Figure 15



Supplemental Figure 15: Constraint on number of lumens and cyst volume varies with MDCK cyst age.

A. Number of lumens versus cyst volume for 17-64 MDCK cysts of each age. Each age is represented by one color, and 9 day old MDCK cysts are repeated on each graph for reference. The line represents the line of best fit and the shaded area represents the 95% confidence interval. Example MDCK cysts of different ages with approximately the same cyst volume and different numbers of lumens are shown. The scale bar represents 20 μm.

Supplemental Figure 16

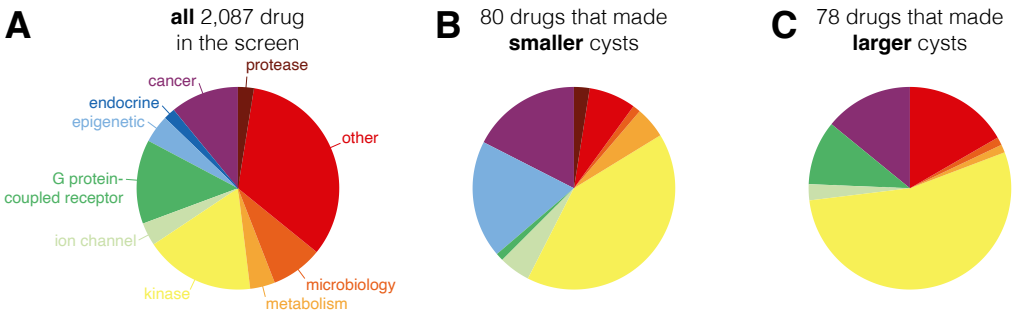


Supplemental Figure 16: MDCK cysts develop a lumen when they have 7 cells and are 10,000 μm^3.

A. Number of lumens versus cyst volume for 196 MDCK cysts with 3-17 days of growth.

B. Number of lumens versus number of cells for 196 MDCK cysts with 3-17 days of growth.

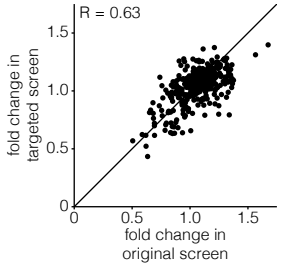
Supplemental Figure 17



Supplemental Figure 17: Proportions of drug categories for drug screen.
A. Proportion of drugs categories for all 2,088 drugs screened.
B. Proportion of drug categories amongst drugs found to decrease cyst area.
C. Proportion of drug categories amongst drugs found to increase cyst area.

Supplemental Figure 18

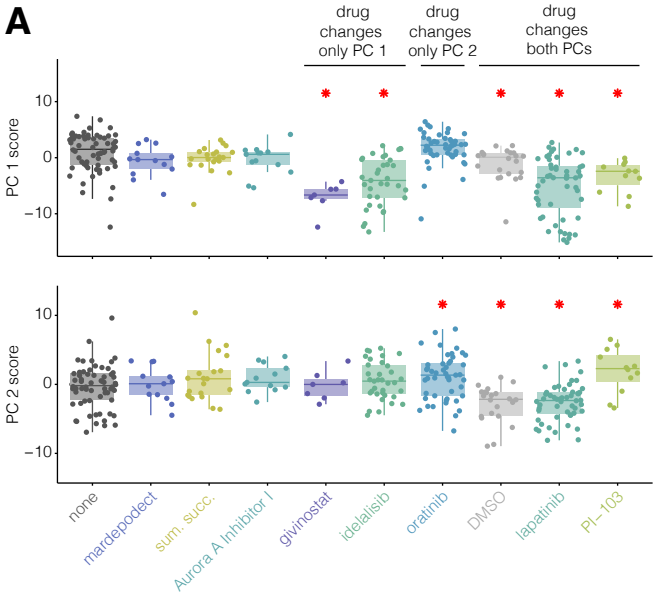
A



Supplemental Figure 18: Correlation between original and targeted drug screen.
A. For all 2,088 drugs screened in replicate (1/7th of all drugs screen), the fold change in the targeted screen versus the fold change in the original screen.

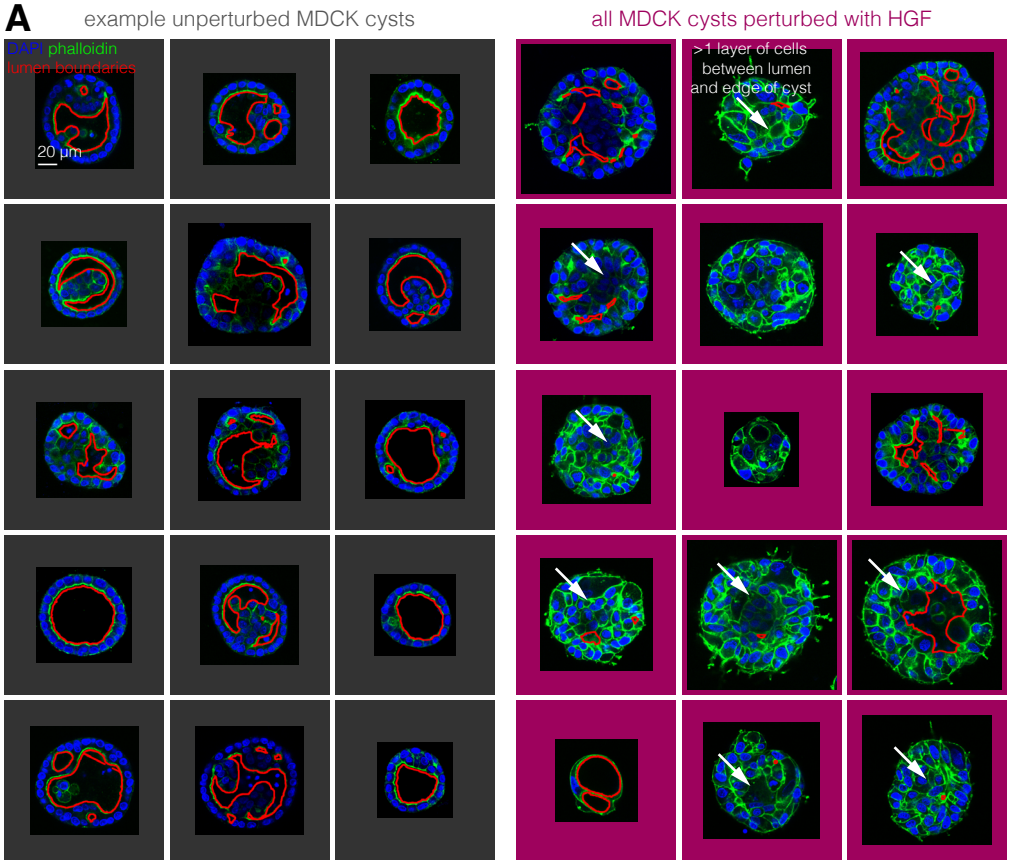
Supplemental Figure 19

A



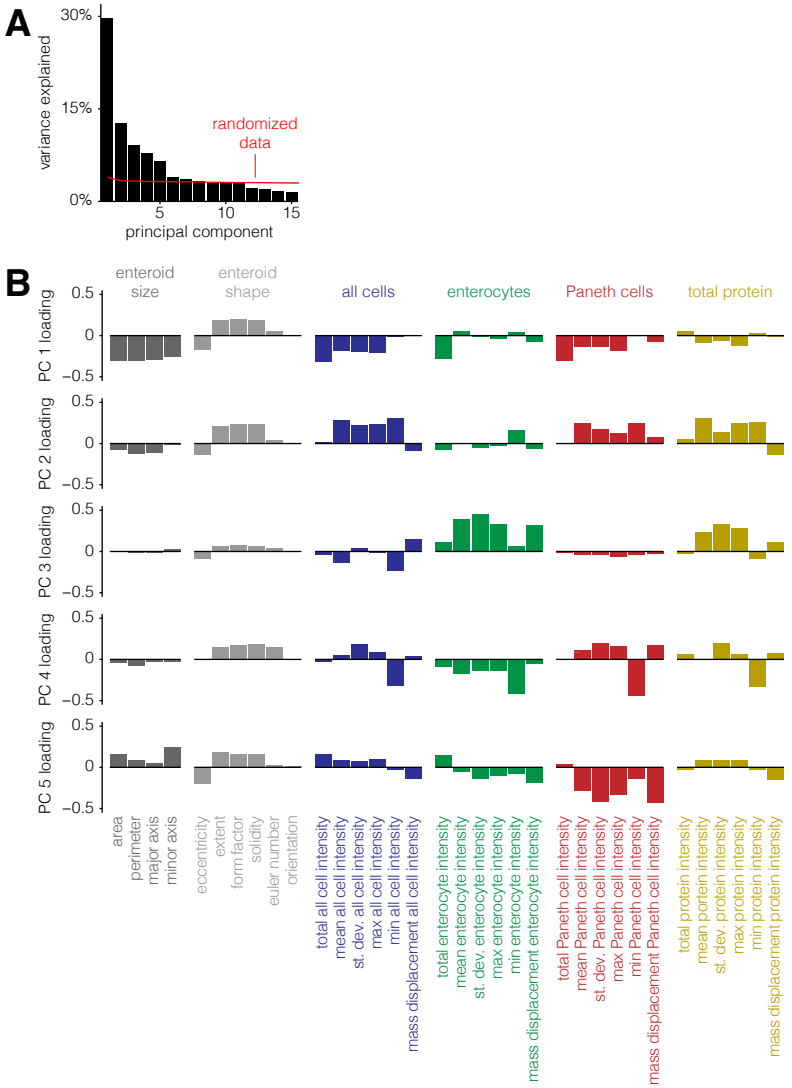
Supplemental Figure 19: Perturbations can shift MDCK cysts along more than one principal component.
A. Scores of 7-65 MDCK cysts (of various perturbations) when projected into PC space calculated using unperturbed MDCK cysts.

Supplemental Figure 20



Supplemental Figure 20: Spindle-like extensions are more common in MDCK cysts perturbed with HGF.
A. 15 randomly-chosen unperturbed MDCK cysts (representative of 64 unperturbed MDCK cysts of age 9 days) and all 15 HGF-perturbed cysts of age 9 days. The boundary of the lumen is outlined in red. Spindle-like extensions are denoted with a white arrow. The scale bar represents 20 μm.

Supplemental Figure 21



Supplemental Figure 21: Enteroids obey a similar set of constraints to MDCK cysts.
A. Variance explained by each principal component. The red line indicates how much variance is explained when the data is randomized before PCA (see methods for details).
B. Loading of each feature on principal components one through five. Each feature is color-coded by what structure (enteroid size, enteroid shape, all cells, enterocytes, Paneth cells, or total protein) it describes.

Supplemental Table 1

Feature	Quantities Measured	Method
volume (μm^3)	cyst volume mean lumen volume mean nuclear volume standard deviation nuclear volume	1. Calculate the number of voxels inside the object. 2. Multiply by the voxel volume.
cell volume (μm^3)	mean cell volume	1. Subtract the volume of all lumens from the cyst volume. 2. Divide by the number of nuclei.
fraction of cyst volume	lumen fraction of cyst volume nuclear fraction of cyst volume	1. Sum the volume of all lumens/nuclei. 2. Divide by the cyst volume.
surface area (μm^2)	cyst surface area mean lumen surface area mean nuclear surface area standard deviation nuclear surface area	1. Use MATLAB's regionprops function to calculate the perimeter of the object on each image slice. 2. Sum the perimeters over all image slices. 3. Multiply by the size of the voxel in XY and the size of the voxel in Z.
XY radius (μm)	cyst XY radius mean lumen XY radius mean nuclear XY radius standard deviation nuclear XY radius	1. Calculate the image slice where the object has the largest area. 2. Calculate the center of the object on this slice. 3. Measure the distance between all boundary points (on this slice) and the center. 4. Take the mean.
Z radius (μm)	cyst Z radius mean lumen Z radius mean nuclear Z radius standard deviation nuclear Z radius	1. Calculate the maximum z coordinate of the object. 2. Subtract the minimum z coordinate of the object.
Z radius:XY radius	cyst Z radius:XY radius mean lumen Z radius:XY radius mean nuclear Z radius:XY radius standard deviation nuclear Z radius:XY radius	1. Divide the Z radius by the XY radius.
3D radius	cyst 3D radius standard deviation cyst 3D radius coefficient of variation	1. Calculate the image slice where the object has the largest area. 2. Calculate the center of the object on this slice. 3. Measure the distance between all boundary points (on this slice) and the center. 4. Take the standard deviation or coefficient of variation.
external cell height (μm)	external cell height	1. For every cyst coordiante, calculate the distance to the nearest lumen coordinate. 2. Take the mean.
external cell width (μm)	mean external cell width standard deviation external cell width	1. Calculate the center of each nucleus. 2. For each nucleus, calculate the distance to the nearest nucleus center.
major axis (μm)	cyst major axis mean lumen major axis mean nuclear major axis standard deviation nuclear major axis	1. Calculate the image slice where the object has the largest area. 2. Use MATLAB's regionprops function to calculate the major axis of the object on that slice. 3. Divide by 2. 4. Multiply by the size of the voxel in XY.
minor axis (μm)	cyst minor axis mean lumen minor axis mean nuclear minor axis standard deviation nuclear minor axis	1. Calculate the image slice where the object has the largest area. 2. Use MATLAB's regionprops function to calculate the minor axis of the object on that slice. 3. Divide by 2. 4. Multiply by the size of the voxel in XY.
major:minor axis	cyst major:minor axis mean lumen major:minor axis mean nuclear major:minor axis standard deviation nuclear major:minor axis	1. Divide the major axis by the minor axis.
solidity	cyst solidity mean lumen solidity mean nuclear solidity standard deviation nuclear solidity	1. Use MATLAB's regionprops function to calculate the solidity of the 3D object.
eccentricity	cyst eccentricity mean lumen eccentricity mean nuclear eccentricity standard deviation nuclear eccentricity	1. Calculate the image slice where the object has the largest area. 2. Use MATLAB's regionprops function to calculate the eccentricity of the object on that slice.
number	number of lumens number of nuclei number of internal nuclei number of external nuclei	1. Count the number of objects.
density	density of lumens density of nuclei	1. Divide the number of objects by the cyst volume.

Supplemental Table 1: MDCK Cyst Morphological Features.

Supplemental Table 2

Drug	Concentrations
selinexor	0.1, 0.3 μM
lapatinib	40, 130, 400 nM
givinostat	0.1 μM
PI-103	0.1, 0.3 μM
idelalisib	3, 10, 30 μM
mardepodect	0.1, 1.0 μM
sumatriptan succinate	0.1, 0.3, 1.0 μM
Aurora A Inhibitor I	0.1, 0.3, 1.0 μM
orantinib	0.5, 1.6, 5.0 μM
Y-27632	10 μM
NSC23766	30 μM
blebbistatin	10 μM
HGF	5, 20 ng/mL

Supplemental Table 2: Perturbation Drugs and Concentrations.

Supplemental Table 3

	Drug	Selleckchem Catalog Number	Target(s)
✔	Indicates used for follow-up analysis		
	PI3K/AKT/mTOR		
	A66	S2636	PI3K
	CZC24832	S7018	PI3K
	NU7026	S2893	DNA-PK
✔	PI-103	S1038	PI3K,Autophagy,DNA-PK,mTOR
	PP242	S2218	mTOR,Autophagy
	YM201636	S1219	PI3K
	CCT128930	S2635	Akt
	MK-2206 2HCl	S1078	Akt
	DNA Damage		
	Caffeic Acid Phenethyl Ester	S7414	NF-kB
	VE-822	S7102	ATM/ATR
	Capecitabine	S1156	DNA/RNA Synthesis
	Costunolide	S1319	Telomerase
	Aurora Kinase		
✔	Aurora A Inhibitor I	S1451	Aurora Kinase
	CCT137690	S2744	Aurora Kinase
	CYC116	S1171	Aurora Kinase,VEGFR
	Bcr-Abl		
	DCC-2036 (Rebastinib)	S2634	Bcr-Abl
	GZD824	S7194	Bcr-Abl
	Nilotinib (AMN-107)	S1033	Bcr-Abl
	CDK		
	AT7519	S1524	CDK
	MK-8776 (SCH 900776)	S2735	CDK,Chk
	Palbociclib (PD-0332991) HCl	S1116	CDK
	RAF/MEK/ERK		
	AZ 628	S2746	Raf
	GW5074	S2872	Raf
	SB590885	S2220	Raf
	Adrenergic Receptor		
	Clorprenaline HCl	S4135	Beta2 receptor
	Xylazine HCl	S2516	Adrenergic Receptor
	Histamine		
	Cimetidine	S1845	Histamine Receptor
	Ketotifen Fumarate	S2024	Histamine Receptor
	JAK		
	CYT387	S2219	JAK
	Ruxolitinib (INCB018424)	S1378	JAK
	PDE		
✔	PF-2545920	S2687	PDE
	Pentoxifylline	S4345	PDE

Serotonin

✔ Sumatriptan Succinate	S1432	5-HT Receptor
Trazodone HCl	S2582	5-HT Receptor

Src

Bosutinib (SKI-606)	S1014	Src
PP1	S7060	Src

Unique Targets

Glipizide	S1715	Potassium channels
Amfenac Sodium Monohydrate	S4149	COX
Ferulic Acid	S2300	
Formononetin	S2299	
Meprednisone	S1689	glucocorticoid
Mometasone furoate	S1987	corticosteroid
Penicillin G Sodium	S4160	antibiotic
Rifaximin	S1790	RNA polymerase
Thiamet G	S7213	O-GlcNAcase
Triamcinolone Acetonide	S1628	corticosteroid
VGX-1027	S7515	TLR4
(-)-Blebbistatin	S7099	ATPase
Cyclopamine	S1146	Hedgehog
DMXAA (Vadimezan)	S1537	VDA
Dexamethasone acetate	S3124	interleukin receptor
Fasudil (HA-1077) HCl	S1573	ROCK,Autophagy
Fingolimod (FTY720) HCl	S5002	S1P Receptor
GDC-0152	S7010	IAP
GSK3787	S8025	PPAR
GW788388	S2750	TGF-beta/Smad
Griseofulvin	S4071	Microtubule Associated
Indirubin	S2386	GSK-3
Isradipine	S1662	Calcium Channel
Mifepristone	S2606	Estrogen/progestogen Receptor
Mubritinib (TAK 165)	S2216	HER2
AG-1024	S1234	IGF-1R
PHA-665752	S1070	c-Met
PX-478 2HCl	S7612	HIF
SB216763	S1075	GSK-3
SKLB1002	S7258	VEGFR
Sitaxentan sodium	S3034	Endothelin Receptor
Sotrastaurin	S2791	PKC
Thioguanine	S1774	DNMT1
VX-745	S1458	p38 MAPK
Thiamine HCl (Vitamin B1)	S3211	Vitamin B
Disodium Cromoglycate	S1911	antiallergic drug

Non-Specific Targets

Cabozantinib (XL184, BMS-907351)	S1119	FLT3,Tie-2,c-Kit,c-Met,VEGFR,Axl
Dovitinib (TKI-258, CHIR-258)	S1018	FGFR,FLT3,c-Kit,VEGFR,PDGFR
Golvatinib (E7050)	S2859	VEGFR,c-Met
NVP-BHG712	S2202	Raf,Src,Bcr-Abl,VEGFR,Ephrin receptor
Ponatinib (AP24534)	S1490	PDGFR,FGFR,VEGFR,Bcr-Abl
TG101209	S2692	JAK,FLT3,c-RET

Supplemental Table 3: Hits for Larger MDCK Cysts from Drug Screen, Grouped by Target.

Supplemental Table 4

	Drug	Selleckchem Catalog Number	Target(s)
✔	Indicates used for follow-up analysis		
	EGFR/HER2		
	AEE788 (NVP-AEE788)	S1486	HER2,VEGFR,EGFR
	AZD8931 (Sapitinib)	S2192	HER2,EGFR
	Afatinib (BIBW2992)	S1011	EGFR,HER2
	Canertinib (CI-1033)	S1019	EGFR,HER2
	Dacomitinib (PF299804, PF299)	S2727	EGFR
	Gefitinib (ZD1839)	S1025	EGFR
✔	Lapatinib (GW-572016) Ditosylate	S1028	HER2,EGFR
	Neratinib (HKI-272)	S2150	HER2,EGFR
	Pelitinib (EKB-569)	S1392	EGFR
	HDAC		
	AR-42	S2244	HDAC
	Belinostat (PXD101)	S1085	HDAC
	Entinostat (MS-275)	S1053	HDAC
✔	Givinostat (ITF2357)	S2170	HDAC
	M344	S2779	HDAC
	Mocetinostat (MGCD0103)	S1122	HDAC
	PCI-24781 (Abexinostat)	S1090	HDAC
	Pracinostat (SB939)	S1515	HDAC
	Scriptaid	S8043	HDAC
	PI3K/AKT/mTOR		
	AZD8055	S1555	mTOR
	Everolimus (RAD001)	S1120	mTOR
	GDC-0980 (RG7422)	S2696	mTOR,PI3K
	WYE-125132 (WYE-132)	S2661	mTOR
	GSK2126458 (GSK458)	S2658	PI3K,mTOR
	INK 128 (MLN0128)	S2811	mTOR
	Torin 2	S2817	ATM/ATR,mTOR
	Aurora Kinase		
	AMG-900	S2719	Aurora Kinase
	Barasertib (AZD1152-HQPA)	S1147	Aurora Kinase
	GSK1070916	S2740	Aurora Kinase
	Hesperadin	S1529	Aurora Kinase
	PF-03814735	S2725	Aurora Kinase, FAK
	SNS-314 Mesylate	S1154	Aurora Kinase
	Topoisomerase		
	Idarubicin HCl	S1228	Topoisomerase
	SN-38	S4908	Topoisomerase
	Topotecan HCl	S1231	Topoisomerase
	Camptothecin	S1288	Topoisomerase
	Mitoxantrone HCl	S2485	Topoisomerase
	Teniposide	S1787	Topoisomerase
	RAF/MEK/ERK		
	AZD8330	S2134	MEK
	PD0325901	S1036	MEK
	Pimasertib (AS-703026)	S1475	MEK

	TAK-733	S2617	MEK
	Trametinib (GSK1120212)	S2673	MEK
	CRM1		
	KPT-185	S7125	CRM1
	KPT-276	S7251	CRM1
✔	KPT-330	S7252	CRM1
	Epigenetic Reader Domain		
	(+)-JQ1	S7110	Epigenetic Reader Domain
	CPI-203	S7304	Epigenetic Reader Domain
	GSK1324726A (I-BET726)	S7620	Epigenetic Reader Domain
	Microtubule Associated		
	Vinblastine	S1248	Microtubule Associated
	Nocodazole	S2775	Microtubule Associated,Autophagy
	Fosbretabulin Disodium	S7204	Microtubule Associated,Autophagy
	CDK		
	Flavopiridol HCl	S2679	CDK
	PHA-793887	S1487	CDK
	DHFR		
	Pralatrexate	S1497	DHFR
	Methotrexate	S1210	DHFR
	Unique Targets		
	LB42708	S7467	Ftase
	MPI-0479605	S7488	Kinesin
	NSC697923	S7142	E2
	ONX-0914 (PR-957)	S7172	Proteasome
	OTX015	S7360	BET
	Olanzapine	S2493	5-HT Receptor,Dopamine Receptor
	Oligomycin A	S1478	ATPase
	RG108	S2821	Transferase,DNA Methyltransferase
	Raltitrexed	S1192	DNA/RNA Synthesis
	Roflumilast	S2131	PDE
	Volasertib (BI 6727)	S2235	PLK
	Tipifarnib	S1453	Transferase
	Erastin	S7242	Ferroptosis
	A-769662	S2697	AMPK
	BIIB021	S1175	HSP (e.g. HSP90)
	BMN 673	S7048	PARP
	Cephalomannine	S2408	Taxol
	Evodiamine	S2382	
	Flubendazole	S1837	antihelminic
	Y-320	S7516	
	Guanethidine Sulfate	S4328	
	Nanchangmycin	S1450	
	Acetanilide	S2538	
	Non-Specific Targets		
	AT9283	S1134	JAK,Aurora Kinase,Bcr-Abl
	Danusertib (PHA-739358)	S1107	c-RET,FGFR,Bcr-Abl,Aurora Kinase

Supplemental Table 4: Hits for Smaller MDCK Cysts from Drug Screen, Grouped by Target.