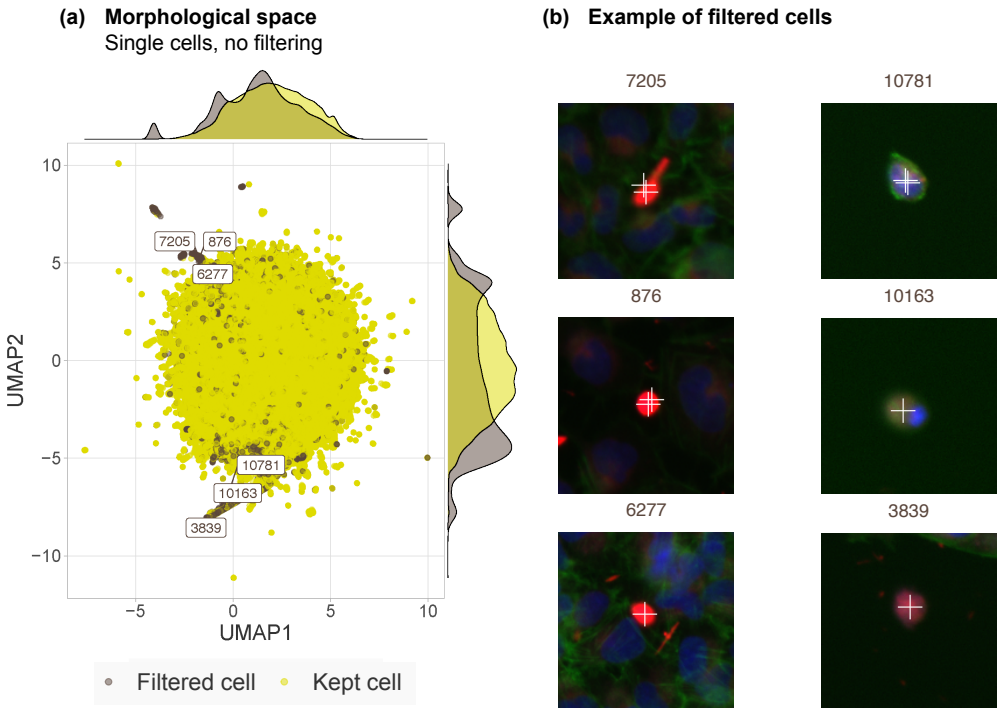
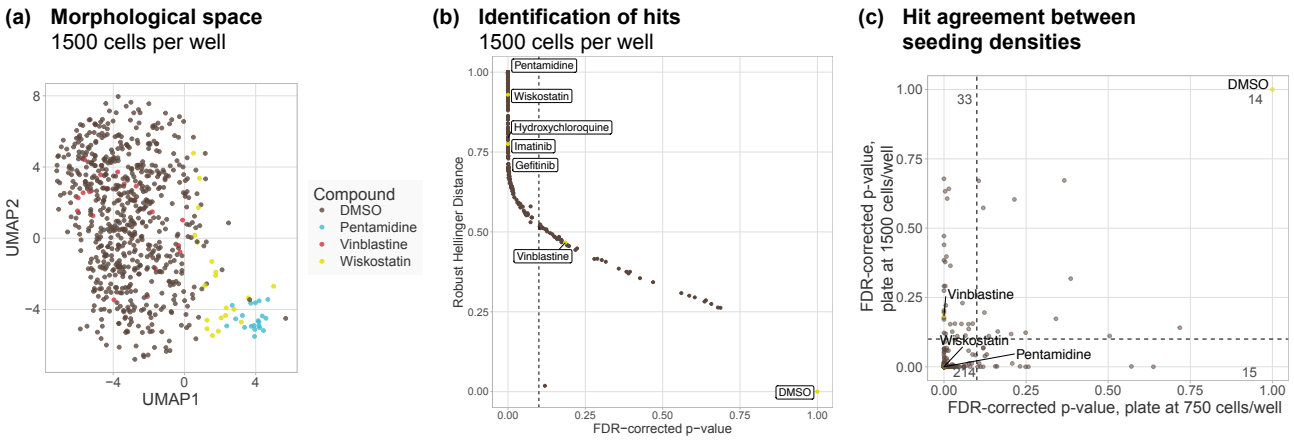
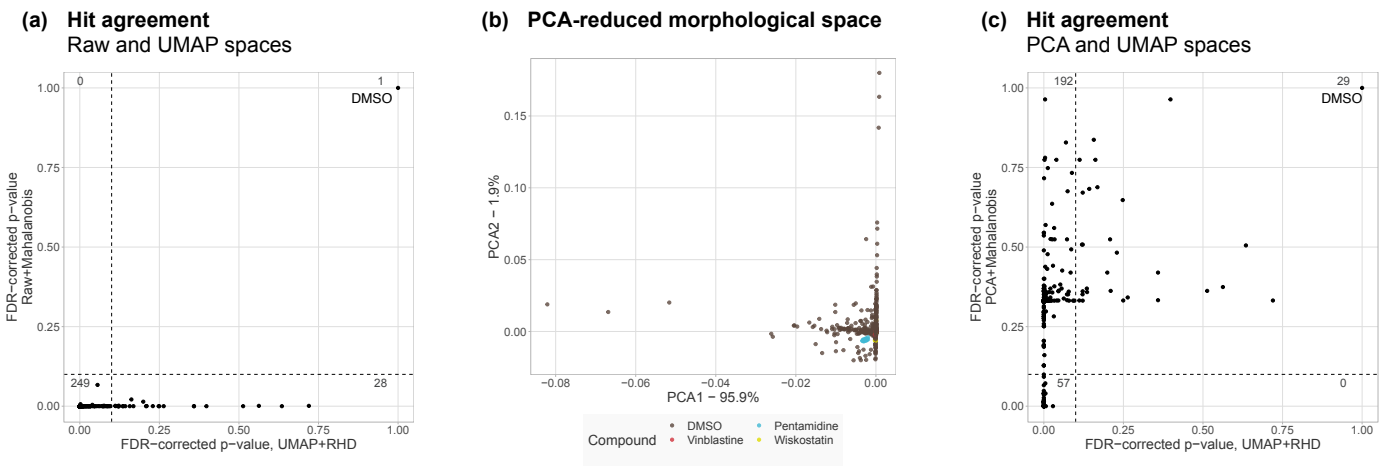


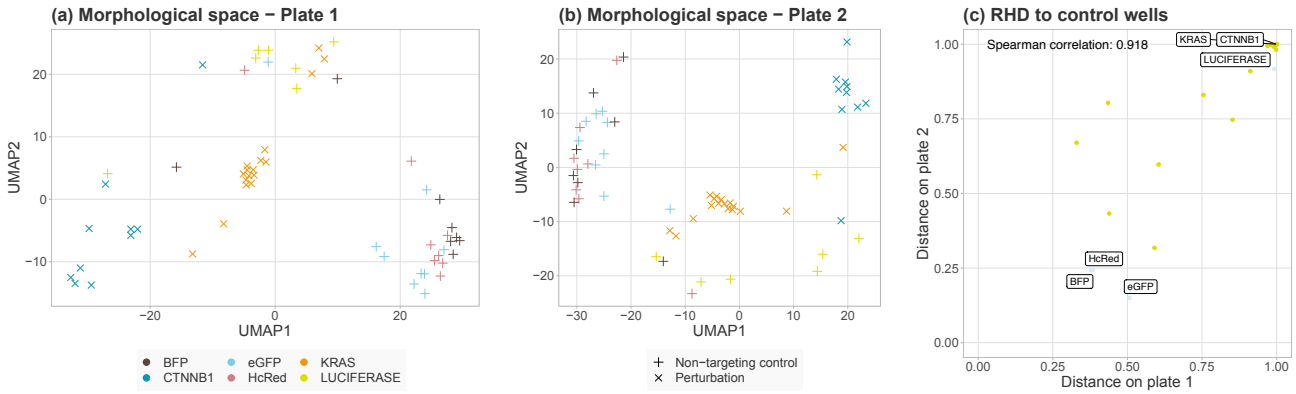
Supp. Figure 1: Filtering of artifacts and biological outliers is required for defining an informative morphological space. **(a)** UMAP embedding preserving the Euclidean distance between the morphological profiles of single cells in the plate seeded with 750 cells per well and without any filtering. Cells that we excluded from further analysis based on a set of feature thresholds (see Methods) are highlighted in brown. Six individual cells are labeled with a unique identifier. Marginal plots show relative density of filtered and kept cells. **(b)** Cropped images of six example cells discarded from further analyses along with their unique identifier. Channels are CellMask (red), phalloidin (green) and DAPI (blue). All images are shown at the same scale.



Supp. Figure 2: BioProfiling.jl profiles of plates seeded with 750 and 1500 cells per well show similarities. (a) UMAP embedding preserving the cosine distance between the morphological profiles aggregated per field of view in the plate seeded with 1500 cells per well. Two out of four dimensions are represented. **(b)** Robust Hellinger distance and Robust Morphological Perturbation Value (FDR-corrected p-value) of each compound in the plate seeded with 1500 cells per well compared to DMSO. Vertical dotted line indicates an FDR threshold of 0.1 and all compounds on its left are defined as morphological hits. **(c)** FDR-corrected p-value of the significance of morphological changes induced by each compound in both plates. Dotted lines indicate an FDR threshold of 0.1 and a number indicates the number of points in each of the regions delineated by the dotted lines.



Supp. Figure 3: BioProfiling.jl supports various approaches to hit detection. (a) FDR-corrected p-value of the significance of morphological changes induced by each compound based either on the robust Hellinger distance in the UMAP-reduced space or the Mahalanobis distance to the center of the DMSO profiles in the original space.**(b)** PCA embedding of the morphological profiles aggregated per field of view in the plate seeded with 750 cells per well. (**c)** FDR-corrected p-value of the significance of morphological changes induced by each compound based either on the robust Hellinger distance in the UMAP-reduced space or the Mahalanobis distance to the center of the DMSO profiles in the PCA space. In **(a)** and **(c)**, dotted lines indicate an FDR threshold of 0.1 and a number indicates the number of points in each of the regions delineated by the dotted lines.



Supp. Figure 4: BioProfiling.jl generates robust morphological profiles from CellPainting experiments. (a-b) UMAP embedding preserving the Euclidean distance between the morphological profiles aggregated per well in plate 1 (a) or plate 2 (b), subsetted to non-targetting controls and overexpression of CTNNB1 and KRAS. **(c)** Robust Hellinger Distance (RHD) between each perturbation and the non-targeting profiles in both plates. Data source: Cell Painting Image Collection (see Methods).

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
(+)-Butaclamol hydrochloride			0.2479	0	0.02
(+)-Cyclazocine			0.0288	0.0012	20
(+/-)-Sulfinpyrazone	["Uricosuric blocker"]	["ABCC1", "ABCC2", "FPR1", "SLC22A12"]	0.0019	0.0154	20
(-)-JQ1			0.0004	0	20
(-)-Perillic acid			0.157	0.0018	20
(-)-Quinpirole hydrochloride	["Dopamine receptor agonist"]	["DRD2", "DRD3", "DRD4", "DRD1", "HTR1A", "HTR2A", "HTR2B", "HTR2C"]	0.0016	0.1061	0.2
(-)-trans-(1S,2S)-U-50488 hydrochloride			0.0087	0.0278	20
(S)-(+)-Camptothecin				0	20
(S)-Propranolol hydrochloride	["Adrenergic receptor antagonist"]	["ADRB2", "ADRB3", "ADRB1", "CYP2C19", "HTR1A", "HTR1B"]	0.0287	0	2
(±)-Isoproterenol hydrochloride					20
(±)-Methoxyverapamil hydrochloride					0.2
(±)-Metoprolol (+)-tartrate					0.2
(±)-Octoclothepin maleate					20
(±)-SKF-38393 hydrochloride					20
(±)-Sulpiride					0.02
(±)-Verapamil hydrochloride					2
(±)-alpha-Lipoic Acid					2
1,10-Phenanthroline monohydrate			0.0208	0.0026	2
1,7-Dimethylxanthine			0.0558	0.1604	20
2,2'-Bipyridyl			0.0013	0.0308	20
2,3-Dimethoxy-1,4-naphthoquinone			0.0055	0.0642	0.02
2-Phenylaminoadenosine			0.1222	0	20
2-methoxyestradiol			0	0.1733	2
4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride			0	0.0015	20
4-Hydroxy-3-methoxyphenylacetic acid			0	0.1915	20
5-(N,N-hexamethylene)amiloride			0	0	20.000633
5-(N-Ethyl-N-isopropyl)amiloride			0	0	19.999068
5-Bromo-2'-deoxyuridine			0	0	20
5-Fluorouracil			0	0	20
5-azacytidine			0	0	19.99822
5HPP-33			0.398	0.3161	0.02
5alpha-Pregnan-3alpha-ol-20-one			0.0173	0	20
6,7-ADTN hydrobromide			0	0.3031	0.02
6-Nitroso-1,2-benzopyrone			0	0	0.02
7-Cyclopentyl-5-(4-phenoxy)phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamine			0.0016	0	20

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
A-77636 hydrochloride					20.000382
AC-93253 iodide			0	0	0.2
AEG 3482			0	0	20
AMG 9810	["TRPV antagonist"]	["TRPV1"]	0.0314	0.0003	2
AZ191					20
Acepromazine maleate	["Dopamine receptor antagonist"]	["ADRA1A", "ADRA1B", "DRD1", "DRD2", "HTR1A", "HTR2A"]	0.1114	0.0097	0.02
Adaphostin					20
Albendazole	["Anthelmintic", "Tubulin inhibitor"]	["CYP1A2", "CYP2J2", "TUBA1A", "TUBB", "TUBB4B"]	0	0	20
Aliskiren	["Antihypertensive", "Peptidase inhibitor", "Protease inhibitor", "Renin inhibitor"]	["REN"]	0.0063	0.2234	20
Ammonium pyrrolidinedithiocarbamate				0	20
Amodiaquine	["Histamine receptor agonist"]	["HNMT", "CYP2C8"]	0	0	20
Amsacrine hydrochloride	["Topoisomerase inhibitor"]	["TOP2A", "KCNH2"]	0.2101	0.5859	2
Ancitabine hydrochloride			0	0	0.2
Apomorphine hydrochloride hemihydrate		["DRD2", "DRD5", "ADRA2A", "ADRA2B", "ADRA2C", "DRD1", "DRD3", "DRD4", "HTR1A", "HTR2A", "HTR2B", "HTR2C", "TRPA1", "CALY", "HTR1B", "HTR1D"]	0.0126	0.0012	1.9999762
Arbidol hydrochloride			0.0039	0.1244	2
Auranofin					20
Aurora-A Inhibitor I			0.1221	0.0731	20.000985
Aurothioglucose			0		20
Azatadine			0.1125	0.6573	20
Azithromycin	["Bacterial 50S ribosomal subunit inhibitor"]	["MLNR"]	0.1359	0.1024	2
BAY 61-3606 hydrochloride hydrate				0	20
BIO			0	0.0003	20
BIX 01294 trihydrochloride hydrate					20
BMS-193885				0	20
BRD3308			0	0	20
BTO-1			0.0842	0.0118	19.998522
BW 723C86	["Serotonin receptor agonist"]	["HTR2B", "HTR2A", "HTR2C"]	0.0126	0	0.02
Bay 11-7082					20
Bay 11-7085					19.999076
Benidipine hydrochloride	["Calcium channel blocker"]	["CACNA1C", "CACNA1G", "CYP3A5"]	0	0	20
Benoxathian hydrochloride			0.0004	0	20
Benzamil hydrochloride		["PKD2L1", "SCNN1A", "SCNN1B", "SCNN1G", "ASIC1", "SCNN1D", "SLC8A1"]	0.0004	0	20.000339
Benztropine mesylate			0	0	20.00068
Bortezomib					20

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Brefeldin A from Penicillium brefeldianum	["Protein synthesis inhibitor", "Brefeldin A inhibited guanine nucleotide exchange protein inhibitor", "Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor inhibitor"]	["ARF1", "ARFGEF1", "ARFGEF2", "CYTH2", "GBF1", "SAR1A"]	0	0	20
Brequinar sodium salt hydrate			0	0	0.2
Budesonide		["NR3C1", "CYP3A5", "CYP3A7"]	0	0	19.999503
CCCI-01			0	0	20
CCT137690				0	2
CGS-15943	["Adenosine receptor antagonist"]	["ADORA1", "ADORA2A", "ADORA2B", "ADORA3"]	0.1351	0.5604	2
CID 11210285 hydrochloride			0	0	20
CID2858522			0.0053	0	20
CP466722	["ATM kinase inhibitor"]	["ATM"]	0	0	20
CYM50358			0	0	0.2
Caffeic Acid	["Lipoxygenase inhibitor", "HIV integrase inhibitor", "NFkB pathway inhibitor", "Nitric oxide production inhibitor", "PPAR receptor modulator", "TNF production inhibitor", "Tumor necrosis factor production inhibitor"]	["ALOX5", "MIF", "RELA", "TNF"]	0	0.0062	0.0200001
Caffeic acid phenethyl ester		["RELA"]	0.0326	0	0.02
Calcimycin					20
Cantharidic Acid			0.0071	0.0097	20
Cantharidin		String[]	0.0053	0	20
Carmofur	["Thymidylate synthase inhibitor"]	["TYMS"]	0	0	20
Carvedilol	["Adrenergic receptor antagonist"]	["ADRB1", "ADRB2", "ADRA1A", "ADRA1B", "ADRA1D", "ADRA2A", "ADRA2B", "ADRA2C", "ADRB3", "CYP2C19", "CYP2E1", "GJA1", "HIF1A", "KCNH2", "NDUFC2", "NPPB", "RYR2", "SELE", "VCAM1", "VEGFA"]	0	0	20
Cerivastatin					20
Chloroquine	["Antimalarial"]	["CYP2C8", "GSTA2", "MRGPRX1", "TLR9", "TNF"]	0	0	20
Chlorpromazine hydrochloride	["Dopamine receptor antagonist"]	["DRD2", "ADRA2A", "ADRA2B", "ADRA2C", "DRD1", "DRD3", "DRD4", "DRD5", "HRH1", "HTR1A", "HTR2A", "HTR2C", "HTR6", "HTR7", "ADRA1A", "ADRA1B", "ADRA1D", "CALM1", "CHRM1", "CHRM3", "HRH4", "HTR2B", "KCNH2", "KIF11", "ORM1", "ORM2", "SMPD1", "TRPC5"]	0.0022	0.6397	0.02

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Chlorprothixene hydrochloride	["Dopamine receptor antagonist"]	["DRD2", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "DRD1", "DRD3", "HRH1", "HTR2A", "HTR2B", "HTR2C"]	0	0	20
Cilengitide trifluoroacetic acid salt			0	0	20
Cilnidipine	["Calcium channel blocker"]	["CACNA1B", "CACNA1C"]	0.0007	0	20
Cinacalcet					20
Clemizole hydrochloride		String[]	0.2636	0	21
Clodronic acid		["SLC25A4", "SLC25A5", "SLC25A6"]	0.001	0.0635	20
Clofarabine	["Ribonucleoside reductase inhibitor"]	["RRM1", "POLA1", "RRM2", "SLC22A8"]	0.0332	0	2
Clomipramine hydrochloride	["Serotonin transporter inhibitor (SERT)"]	["SLC6A4", "SLC6A2", "CYP2C19", "GSTP1", "HTR2A", "HTR2B", "HTR2C", "SLC6A3"]	0.1222	0.041	2
Clotrimazole	["Cytochrome P450 inhibitor", "Imidazoline receptor ligand"]	["KCNN4", "CYP3A4", "CYP51A1", "NR1I2", "NR1I3", "TRPM2", "TRPM4", "TRPM8"]	0	0	20
Colchicine		["TUBB", "GLRA1", "GLRA2", "TUBB1"]	0.0167	0.6355	2
Cyclosporin A		["PPIA", "ABCB11", "CAMLG", "CYP3A5", "CYP3A7", "FPR1", "PPID", "PPIF", "PPP3CA", "PPP3R2", "SLC10A1", "SLCO1B1", "SLCO1B3"]	0	0	20
Cyproterone acetate	["Androgen receptor antagonist", "Progesterone receptor agonist", "Testosterone receptor antagonist"]	["AR", "ADORA1", "ESR1"]	0	0.0178	0.02
Cytarabine	["Ribonucleotide reductase inhibitor"]	["POLB", "POLA1"]	0	0	20
Cytosine-1-beta-D-arabinofuranoside hydrochloride				0	8.86
D-Cycloserine	["Bacterial cell wall synthesis inhibitor"]	["GRIN1"]	0.0791	0.0635	35.985971
D-ribofuranosylbenzimidazole			0.0045	0.0003	20
DCEBIO	["Potassium channel activator"]	["KCNN2", "KCNN3", "KCNN4"]	0	0.6868	20
Danshensu sodium salt			0.0039	0.2793	20
Dantrolene sodium	["Calcium channel blocker"]	["RYR1", "RYR3"]	0.635	0	2
Dequalinium chloride hydrate		["KCNN1", "KCNN3"]	0	0	2
Digitoxin					20
Dihydroartemisinin			0	0	20
Dihydroergotamine		["HTR1D", "HTR1B", "ADRA2A", "DRD2", "HTR1E", "HTR1F", "HTR2B", "HTR7"]	0.1428	0.0401	20
Dihydroergotamine methanesulfonate		["HTR1D", "HTR1B", "ADRA2A", "DRD2", "HTR1E", "HTR1F", "HTR2B", "HTR7"]	0.0034	0.007	20
Dihydroouabain			0.0053	0	2
Diphenyleneiodonium chloride	["Nitric oxide synthase inhibitor"]	["ALDH1A2", "ALDH2", "ALDH5A1", "ALDH7A1", "NOX3", "XDH"]	0	0	20

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Dipyridamole	["Phosphodiesterase inhibitor"]	["ADA", "PDE5A", "PDE10A", "PDE4A", "PDE7B", "PDE8A", "PDE8B", "SLC29A1"]	0.3587	0.6785	2
Docetaxel		["TUBB", "BCL2", "MAP2", "MAP4", "MAPT", "NR1I2", "TUBB1"]	0	0	2
Domperidone	["Dopamine receptor antagonist"]	["DRD2", "DRD3", "ABCG2", "CYP3A5"]	0	0	0.2
Doxazosin mesylate	["Adrenergic receptor antagonist"]	["ADRA1D", "ADRA1A", "ADRA1B", "CYP2C19", "KCNH2", "KCNH6", "KCNH7"]	0.0107	0.6036	2
Doxorubicin		["TOP2A"]	0	0	2
Doxycycline hydrochloride	["Bacterial 30S ribosomal subunit inhibitor", "Metalloproteinase inhibitor"]	["MMP8", "MMP1"]	0.0004	0	20
Droperidol	["Dopamine receptor antagonist"]	["DRD2", "ADRA1A"]	0.0045	0.3943	20
E-64			0	0.1733	20
Ebastine			0	0	20
Efavirenz	["HIV protease inhibitor"]	["CYP2B6", "CYP2C19", "CYP2C8", "CYP3A4", "CYP3A5"]	0.1994	0.014	0.0224
Ellipticine	["Topoisomerase inhibitor"]	["TOP2A", "TOP2B"]	0	0	2
Emetine dihydrochloride hydrate	["Protein synthesis inhibitor"]	["RPS2"]	0	0	20
Enclomiphene hydrochloride					20
Endoxifen				0	20
Eptifibatide			0	0.1203	20
Ethinyl Estradiol	["DNA directed DNA polymerase stimulant", "Estrogenic component in oral contraceptives", "Estrogen receptor agonist"]	["CYP2C8", "ESR1", "ESR2", "NR1I2"]	0.0958	0.0479	0.02
Etoposide		["TOP2A", "CYP2E1", "CYP3A5", "TOP2B"]	0	0	20
Flunarizine dihydrochloride	["Calcium channel blocker"]	["CACNA1G", "CACNA1H", "CACNA1I", "CALM1", "CYP2J2", "HRH1"]	0.0112	0	20
Fluoxetine hydrochloride	["Selective serotonin reuptake inhibitor (SSRI)"]	["SLC6A4", "ANO1", "CYP2C19", "HTR2B"]	0	0	20
Fluspirilene	["Dopamine receptor antagonist"]	["DRD2", "HTR2A", "CACNG1", "HRH1", "HTR1A", "HTR1D", "HTR1E"]	0.0206	0	20
Forskolin		["ADCY2", "ADCY5", "GNAS"]	0.0013	0.0003	2
Furamidine dihydrochloride			0	0	20
GANT61			0	0.0003	20
GBR-12909 dihydrochloride			0	0	20
GSK-650394		["SGK1", "SGK2"]	0	0.0107	0.02
GSK1210151A			0	0	20
GW2974			0	0.0095	20
GW9662			0.0048	0	20
Gefitinib	["EGFR inhibitor"]	["EGFR", "CYP2C19"]	0.0403	0.0024	0.02
Gemcitabine hydrochloride	["Ribonucleotide reductase inhibitor"]	["RRM1", "CMPK1", "RRM2", "TYMS"]	0	0	20

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Histamine, R(-)-alpha-methyl-, dihydrochloride			0.5132	0.1118	0.02
Hydroquinone			0.0728	0.1129	0.02
Hydroxychloroquine		["TLR7", "TLR9"]	0.0763	0	20
IKK-16 dihydrochloride	["IKK inhibitor"]	["IKBKB"]	0	0	0.02
IMS2186			0	0	20
IN-1130			0	0	20.000786
Icaritin			0	0.4267	20
Idarubicin		["TOP2A"]	0	0	20
Idazoxan hydrochloride		["NISCH"]	0	0	2
Imatinib	["BCR-ABL kinase inhibitor", "KIT inhibitor", "PDGFR receptor inhibitor"]	["ABL1", "KIT", "PDGFRA", "BCR", "CSF1R", "PDGFRB", "ABCG2", "CYP2C19", "CYP2C8", "CYP3A5", "DDR1", "NTRK1", "RET"]	0.1198	0	2
Imatinib mesylate	["BCR-ABL kinase inhibitor", "KIT inhibitor", "PDGFR receptor inhibitor"]	["ABL1", "KIT", "PDGFRA", "BCR", "CSF1R", "PDGFRB", "ABCG2", "CYP2C19", "CYP2C8", "CYP3A5", "DDR1", "NTRK1", "RET"]	0.0039	0	20
Imipramine hydrochloride	["Norepinephrine reuptake inhibitor", "Serotonin reuptake inhibitor"]	["SLC6A2", "SLC6A4", "CHRM2", "ADRA1A", "ADRA1B", "ADRA1D", "CHRM1", "CHRM3", "CHRM4", "CHRM5", "CYP2C19", "DRD1", "DRD2", "DRD5", "HRH1", "HTR1A", "HTR2A", "HTR2C", "HTR6", "HTR7", "KCND2", "KCND3", "KCNH1", "KCNH2", "SLC6A3"]	0	0	20
Iodoacetamide			0	0	20
Irinotecan				0	20
Isoproterenol			0.1221	0.0733	20
JFD00244			0	0	20
JS-K		String[]	0.0066	0.0012	20
K114			0	0	20
KB-R7493			0	0	20
KT203			0	0.0125	2
KU-55933	["ATM kinase inhibitor"]	["ATM", "PRKDC"]	0.0016	0.001	2
KY-05009			0	0	2.0001077
Kenpaullone	["CDK inhibitor", "Glycogen synthase kinase inhibitor"]	["GSK3B", "CDK1", "CDK5", "CCNB1", "CDK2", "LCK"]	0	0	20
Ketoconazole	["Sterol demethylase inhibitor"]	["AR", "CYP19A1", "CYP21A2", "CYP2C19", "CYP3A5", "CYP3A7", "KCNA10"]	0.0013	0	20
Ketotifen fumarate	["Histamine receptor agonist", "Histamine receptor ligand", "Leukotriene receptor antagonist", "Phosphodiesterase inhibitor"]	["HRH1", "PDE4A", "PDE4B", "PDE4C", "PDE4D", "PDE7A", "PDE7B", "PDE8A", "PDE8B", "PGD"]	0.0063	0.3895	20
L-703,606 oxalate salt hydrate			0.0861	0.168	2
L-741,626			0	0	20
L-Cycloserine			0.073	0.1592	0.2

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
L-Tryptophan			0.0007	0.0354	2
LDN-214117			0.026	0.0084	2
LP 12 hydrochloride hydrate			0.0007	0	20
LP44			0	0	20
LY-294,002 hydrochloride			0	0	20
Lasofoxifene tartrate					0.2
Lercanidipine hydrochloride hemihydrate			0.0019	0	20
Levetiracetam	["Calcium channel blocker"]	["SV2A", "CACNA1B", "SCN1A"]	0.0264	0.1362	20
Loperamide	["Opioid receptor agonist"]	["OPRM1", "OPRD1", "CACNA1A", "CALM1", "CYP2B6", "CYP2C8", "NPR2", "OPRK1", "POMC"]	0	0	20
Loperamide hydrochloride	["Opioid receptor agonist"]	["OPRM1", "OPRD1", "CACNA1A", "CALM1", "CYP2B6", "CYP2C8", "NPR2", "OPRK1", "POMC"]	0	0	20
Loratadine	["Histamine receptor antagonist"]	["HRH1", "CYP2C19", "CYP3A5"]	0.1217	0.1974	0.02
Lorcainide hydrochloride			0	0.0021	2
Lubeluzole dihydrochloride			0	0	20
M-110			0	0	20
MG 624			0	0	20
MK-677			0	0	0.2
ML-7	["Myosin light chain kinase inhibitor"]	["MYLK"]	0.0016	0	20
ML240			0.0882	0.0404	0.02
ML324			0	0	20
Maprotiline	["Norepinephrine reuptake inhibitor", "Tricyclic antidepressant"]	["SLC6A2", "ADRA1A", "ADRA1B", "ADRA1D", "ADRA2A", "ADRA2B", "ADRA2C", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "DRD2", "HRH1", "HTR2A", "HTR2C", "HTR7"]	0.0395	0.0015	2
Maprotiline hydrochloride	["Norepinephrine reuptake inhibitor", "Tricyclic antidepressant"]	["SLC6A2", "ADRA1A", "ADRA1B", "ADRA1D", "ADRA2A", "ADRA2B", "ADRA2C", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "DRD2", "HRH1", "HTR2A", "HTR2C", "HTR7"]	0	0	20
Metergoline					20
Methiothepin mesylate			0	0	20
Methoxamine hydrochloride		["ADRA1A", "ADRA1B", "ADRA1D"]	0.0013	0.0537	0.2
Metrazoline oxalate			0	0	20
Mibefradil dihydrochloride	["T-type calcium channel blocker"]	["CACNA1G", "CACNA1H", "CACNA1C", "CACNA1I", "ANO1", "CACNA1D", "CACNA1F", "CACNA1S", "CACNB1", "CACNB2", "CACNB3", "CACNB4", "CATSPER1", "CATSPER2", "CATSPER3", "CATSPER4", "CYP3A5", "CYP3A7", "SCN2A", "SCN4A", "SCN5A", "SCN9A"]	0	0	0.2

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Mifepristone	["Glucocorticoid receptor antagonist", "Progesterone receptor antagonist"]	["PGR", "NR3C1", "AR", "CYP2B6", "CYP2C8", "CYP3A5", "CYP3A7", "NR1I2"]	0.0727	0.0049	20
Mitotane	["Antineoplastic"]	["CYP11B1", "CYP11A1", "CYP3A4", "ESR1", "FDX1"]	0.0165	0.1653	20
Mitoxantrone	["Topoisomerase inhibitor"]	["TOP2A", "PIM1"]	0.7198	0.1515	2
Mycophenolic Acid	["Dehydrogenase inhibitor", "Inositol monophosphatase inhibitor"]	["IMPDH1", "IMPDH2"]	0	0	20
N-p-Tosyl-L-phenylalanine chloromethyl ketone			0.0004	0.0015	2
NG-Monomethyl-L-arginine acetate			0.0577	0	20
Nestorone			0.0007	0.0594	19.998523
Nicardipine hydrochloride	["Calcium channel blocker"]	["CACNA1C", "ADORA3", "ADRA1A", "ADRA1B", "ADRA1D", "CACNA1D", "CACNA2D1", "CACNB2", "CALM1", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "PDE1A", "PDE1B"]	0	0.0764	0.2
Niclosamide	["DNA replication inhibitor", "STAT inhibitor"]	["STAT3"]	0.0457	0	2
Nisoldipine	["Calcium channel blocker"]	["CACNA1C", "CACNA1D", "CACNA1S", "CACNA2D1", "CACNB2", "CYP3A5"]	0	0	20
Nitidine chloride					20
Nocodazole	["Tubulin inhibitor"]	["HPGDS"]	0	0	20
Nortriptyline hydrochloride	["Tricyclic antidepressant"]	["KCNJ10", "SLC6A2", "SLC6A4", "ADRA1A", "ADRA1B", "ADRA1D", "ADRA2A", "ADRA2B", "ADRA2C", "ADRB1", "ADRB2", "ADRB3", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "CYP2C19", "DRD2", "HRH1", "HTR1A", "HTR2A", "HTR2C", "HTR6", "PGRMC1", "PIK3CD", "SIGMAR1"]	0	0	20
Olanzapine	["Dopamine receptor antagonist", "Serotonin receptor antagonist"]	["DRD2", "HTR2A", "HTR2C", "DRD1", "DRD3", "DRD4", "HRH1", "HTR1A", "HTR1B", "HTR1D", "HTR1E", "HTR6", "HTR7", "ADRA1A", "ADRA1B", "ADRA2A", "ADRA2B", "ADRA2C", "ADRB1", "ADRB2", "ADRB3", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "CYP2C8", "DRD5", "GABRA1", "GABRA2", "GABRA3", "GABRA4", "GABRA5", "GABRA6", "GABRB1", "GABRB2", "GABRB3", "GABRD", "GABRE", "GABRG1", "GABRG2", "GABRG3", "GABRP", "GABRQ", "HRH2", "HRH4", "HTR1F", "HTR2B", "HTR3A", "HTR5A"]	0.0076	0.0777	20

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Ouabain	["ATPase inhibitor"]	["ATP1A1", "ATP1A2", "ATP1A3", "ATP1A4", "ATP1B1", "ATP1B2", "ATP1B3", "ATP1B4", "FXYD2"]	0	0	20
PAPP			0	0	0.2
PD-407824				0	20
PD153035 hydrochloride			0.0068	0.0057	19.998253
PF-429242 dihydrochloride			0	0	20
PMEG hydrate				0	20
Palonosetron hydrochloride	["Serotonin receptor antagonist"]	["HTR3A"]	0	0.004	0.02
Paroxetine hydrochloride hemihydrate (MW = 374.83)	["Selective serotonin reuptake inhibitor (SSRI)"]	["SLC6A4", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "HTR2A", "SLC6A2"]	0	0	20.000106
Parthenolide					20
Pazopanib	["KIT inhibitor", "PDGFR receptor inhibitor", "VEGFR inhibitor"]	["KDR", "KIT", "FLT1", "FLT4", "PDGFRB", "PDGFRA", "BRAF", "CSF1R", "CYP2B6", "CYP2C8", "CYP2E1", "DDR2", "FGF1", "FGFR1", "FGFR3", "ITK", "SH2B3"]	0.0037	0	20
Pentamidine		["TRDMT1"]	0	0	20
Pentamidine isethionate		["TRDMT1"]	0		20
Pergolide methanesulfonate	["Dopamine receptor agonist"]	["DRD1", "DRD2", "ADRA2A", "ADRA2B", "ADRA2C", "DRD3", "DRD4", "DRD5", "HTR1A", "HTR1B", "HTR1D", "HTR2A", "HTR2B", "HTR2C", "ADRA1A", "ADRA1B", "ADRA1D", "KCNA5"]	0	0.0512	2
Perphenazine	["Dopamine receptor antagonist"]	["DRD2", "CALM1", "DRD1", "HRH1", "HTR2A", "HTR2C", "HTR6", "HTR7"]	0.0039	0.0125	0.02
Phenamil methanesulfonate	["TRPV antagonist"]	["PKD2L1"]	0.0325	0.1496	2
Pheniramine maleate	["Histamine receptor antagonist"]	["HRH1"]	0.0169	0.0024	20
Phorbol 12-myristate 13-acetate	["PKC activator"]	["CD4", "KCNT2", "PRKCA", "TRPV4"]	0	0	2
Pifithrin-mu	["HSP inhibitor"]	["HSPA1A", "TP53"]	0	0.0003	2
Pimozide	["Dopamine receptor antagonist"]	["DRD2", "DRD3", "CACNA1I", "CALM1", "HRH1", "HTR1A", "HTR2A", "KCNA10", "KCNH2"]	0.001	0.0174	20
Piperlongumine	["Glutathione transferase inhibitor"]	String[]	0.0747	0.0144	2
Podophyllotoxin	["Microtubule inhibitor", "Tubulin inhibitor"]	["IGF1R", "CASP3", "TOP2A", "TUBA4A", "TUBB"]	0.001	0	2
Prazosin hydrochloride	["Adrenergic receptor antagonist"]	["ADRA1A", "ADRA1B", "ADRA1D", "ADRA2A", "ADRA2B", "ADRA2C", "CDK1", "KCNH2", "KCNH6", "KCNH7"]	0	0.0003	0.2
Progesterone		["PGR", "CYP17A1", "NR3C2", "CATSPER1", "CATSPER2", "CATSPER3", "CATSPER4", "CYP2C19", "ESR1", "OPRK1", "TRPC5"]	0	0	20

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
Proguanil	["Dihydrofolate reductase inhibitor"]	["CYP2C19", "DHFR"]	0.0016	0	20
Promazine hydrochloride	["Dopamine receptor antagonist"]	["CHRM5", "DRD2", "ADRA1A", "ADRA1B", "ADRA1D", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "DRD1", "DRD3", "DRD4", "HRH1", "HTR2A", "HTR2C"]	0.0243	0	2
Propafenone hydrochloride	["Antiarrhythmic"]	["KCNH2", "SCN5A", "ADRB1", "ADRB2", "KCNA5", "KCNK2", "KCNK3"]	0	0	20
Propionylpromazine hydrochloride			0	0	20
Protriptyline hydrochloride	["Tricyclic antidepressant"]	["SLC6A2", "SLC6A4"]	0	0	20
Psoralidin			0	0	20
Pyridostatin trifluoroacetate salt			0	0.1911	20
Quinacrine dihydrochloride					20
Quinidine sulfate				0	20
RN-9893			0.2081	0.1042	0.02
RU-SKI 43 maleate			0	0	20
Rabeprazole sodium	["ATPase inhibitor", "Gastrin inhibitor"]	["ATP4A", "CYP2C19"]	0.0919	0	20.000126
Raloxifene hydrochloride	["Estrogen receptor antagonist", "Selective estrogen receptor modulator (SERM)"]	["ESR1", "ESR2", "ACVRL1", "ENG"]	0	0	20
Ranolazine dihydrochloride	["Sodium channel blocker"]	["SCN9A", "SCN10A", "SCN5A", "SLC22A2"]	0.0022	0.0209	2
Reserpine	["Vesicular monoamine transporter inhibitor"]	["SLC18A2", "SLC18A1"]	0	0	20
Ro 11-1464			0.0028	0	20
Ro 90-7501	["Beta amyloid inhibitor"]	["APP"]	0	0.0012	20
Roscovitine	["CDK inhibitor"]	["CDK2", "CDK9", "CDK7", "CDK1", "CDK5"]	0	0	20
Rotenone		["MT-ND1"]	0.0004	0	20
Ruthenium red			0.0627	0	20
S-(+)-Fluoxetine hydrochloride			0	0.2889	2
S-Methylisothiourea hemisulfate			0.0518	0.2182	20
SB 202190	["p38 MAPK inhibitor"]	["MAPK14", "AKT1", "ALOX5", "CHEK1", "GSK3B", "LCK", "MAPK1", "MAPK11", "MAPK12", "MAPK8", "PRKCA", "ROCK1", "RPS6KB1", "SGK1"]	0	0	2.0000599
SB 415286	["Glycogen synthase kinase inhibitor"]	["GSK3B", "GSK3A", "RPS6KB1"]	0	0.0012	20
SB743921 hydrochloride			0	0	20
SID 3712249			0.0025	0	20
SKF 83959 hydrobromide			0.0457	0	20
SMER28			0	0	20
SP600125			0	0	20
SR 59230A oxalate	["Adrenergic receptor antagonist"]	["ADRB3", "ADRB1", "ADRB2"]	0.0107	0	20
SR9243			0.0694	0.0012	20
SU 5416			0.0212	0.3581	2

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
SU1498			0	0	20
Sanguinarine chloride					20
Sertaconazole nitrate	["Sterol demethylase inhibitor"]	String[]	0.0119	0	20
Stattic					20
Sunitinib	["FLT3 inhibitor", "KIT inhibitor", "PDGFR receptor inhibitor", "RET tyrosine kinase inhibitor", "VEGFR inhibitor"]	["FLT3", "KDR", "KIT", "FLT4", "FLT1", "PDGFRA", "PDGFRB", "RET", "CSF1R", "FGFR1"]	0	0.3832	20
Supercinnamaldehyde			0	0.0144	20
Suprafenacine			0	0	20
T0070907			0	0	20
TBBz			0.001	0.0026	2
TIC10 angular			0	0	20
Tacrine	["Acetylcholinesterase inhibitor", "Acetylcholine release stimulant", "Butyrylcholinesterase inhibitor", "Potassium channel antagonist"]	["ACHE", "BCHE"]	0	0	20
Tamoxifen citrate					20
Taurine			0	0.4691	20
Testosterone	["Androgen receptor agonist"]	["AR", "CYP19A1", "CYP2C19", "CYP2C8", "CYP3A5"]	0.0126	0.0554	20
Tetraethylthiuram disulfide					20
Thiabendazole			0.0103	0.0012	20
Thiocolchicine			0	0	20
Tirapazamine			0.0007	0.0021	2.2628831
Tizanidine hydrochloride	["Adrenergic receptor agonist"]	["ADRA2A", "ADRA2B", "ADRA2C", "CYP1A2", "NISCH"]	0	0	20
Tolazoline	["Adrenergic receptor antagonist"]	["ADRA2A", "ADRA2B", "ADRA2C", "ADRA1A", "HRH1", "HRH2"]	0.0338	0.1042	20
Topotecan hydrochloride hydrate	["Topoisomerase inhibitor"]	["TOP1", "TOP1MT"]	0	0	2
Torin2			0	0	20
Trifluperidol hydrochloride			0.0007	0	20
Triflupromazine hydrochloride	["Dopamine receptor antagonist"]	["HTR2B", "CHRM1", "CHRM2", "CHRNA7", "DRD1", "DRD2"]	0	0	20
Trihexyphenidyl		["CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5"]	0.0467	0.1044	0.2
Trilostane			0	0	20
Trimipramine maleate	["Norepinephrine reuptake inhibitor", "Tricyclic antidepressant"]	["SLC6A2", "SLC6A4", "SLC6A3", "ADRA1A", "ADRA1B", "ADRA2A", "ADRA2B", "ADRB1", "ADRB2", "ADRB3", "CHRM1", "CHRM2", "CHRM3", "CHRM4", "CHRM5", "DRD1", "DRD2", "DRD5", "HRH1", "HTR1A", "HTR1D", "HTR2A", "HTR2C", "HTR3A"]	0.0216	0	20
Tyrphostin AG 879			0	0	20
U-101958 maleate			0.3587	0.1733	2

Compound name	MOA	Targets	RMPV750	RMPV1500	Conc.
U-62066			0.5627	0.0015	20
U0126		["JAK2", "MAP2K1", "MAP2K2", "MAP3K1", "MAP3K2"]	0	0	20
UNC0379 trifluoroacetate salt			0.0013	0	2
Vinblastine	["Microtubule inhibitor", "Tubulin inhibitor"]	["TUBB", "JUN", "TUBA1A", "TUBD1", "TUBE1", "TUBG1"]	0	0.1859	20
Vincristine sulfate		["TUBB", "TUBA4A"]	0.0004	0	20
Vorinostat					20
WIN 62,577			0.0101	0.0021	2
WZ4003			0	0	0.2
Wiskostatin	["Neural Wiskott-Aldrich syndrome protein inhibitor"]	["WAS", "WASL"]	0	0	20
XL388			0	0	20
Y-27632 dihydrochloride	["Rho associated kinase inhibitor"]	["ROCK1", "ROCK2", "LRRK2", "PKIA", "PKN2", "PRKACA", "PRKCE"]	0.0268	0.0057	0.2
Yoda1			0	0.0443	2
alpha-Lobeline hydrochloride			0.0107	0.0347	0.02
beta-Lapachone					20

Supp. Table 1: Compound list in chemical HCS experiment. Description of the name of the screened compounds (CompoundName), their mechanisms of action (MOA) and genetic targets (Targets), their robust morphological perturbation value (FDR-corrected p-value for a comparison to matching DMSO controls) computed on the plate seeded with 750 cells (RMPV750) or 1500 cells (RMPV1500), as well as their concentration in µM (Conc.). For a machine-readable version of this table, see FigShare dataset (DOI: 10.1101/2021.06.18.448961). For accessing the data programatically see the GitHub repository (DOI: 10.5281/zenodo.5659932).

Rank	Feature	Type	Object
1	Granularity_10_CorrCM_median	Granularity, CellMask	Cell
2	Granularity_9_CorrCM_median	Granularity, CellMask	Cell
3	AreaShape_FormFactor_1_median	Shape	Nucleus
4	AreaShape_Solidity_median	Shape	Cell
5	AreaShape_Zernike_3_1_1_median	Shape	Cell
6	Intensity_MassDisplacement_CorrDNA_1_median	Intensity distribution, DAPI	Nucleus
7	Granularity_7_CorrActin_median	Granularity, Phalloidin	Cell
8	AreaShape_Compactness_median	Shape	Cell
9	AreaShape_Compactness_1_median	Shape	Nucleus
10	RadialDistribution_RadialCV_CorrActin_2of3_median	Intensity distribution, Phalloidin	Cell

Supp. Table 2: Top 10 most variable features for the plate seeded with 750 cells per well. Features are ranked by decreasing median absolute deviation. As the data is normalized on the DMSO controls, this corresponds to the features varying the most in response to chemical perturbations across the plate.

MOA	Feature 1	Feature 2	Feature 3	Feature 4
Glycogen synthase kinase inhibitor	RadialDistribution_ZernikeMagnitude_CorrDNA_7_3_median	AreaShape_Zernike_8_6_1_median	RadialDistribution_ZernikeMagnitude_CorrCM_9_3_median	Intensity_MADIntensity_CorrCM_median
CDK inhibitor	AreaShape_Zernike_8_6_1_median	RadialDistribution_ZernikeMagnitude_CorrCM_9_3_median	Intensity_MaxIntensity_CorrCM_median	AreaShape_Zernike_4_0_1_median
Histamine receptor antagonist	AreaShape_Zernike_4_2_2_median	AreaShape_Zernike_4_0_1_median	RadialDistribution_ZernikeMagnitude_CorrCM_9_3_median	Intensity_MaxIntensity_CorrCM_median
Selective serotonin reuptake inhibitor	AreaShape_Zernike_4_2_2_median	AreaShape_Zernike_4_0_1_median	RadialDistribution_ZernikeMagnitude_CorrDNA_7_3_median	Granularity_1_CorrActin_median
Norepinephrine reuptake inhibitor	AreaShape_Zernike_4_2_2_median	AreaShape_Zernike_4_0_1_median	RadialDistribution_ZernikeMagnitude_CorrDNA_7_3_median	AreaShape_Zernike_9_3_2_median

Supp. Table 3: Top 4 most distinctive features (absolute log-fold change of mean field-of-view profiles) as compared to DMSO controls, for selected Mechanisms Of Actions (MOAs) for the plate seeded with 750 cells per well. Recurrent features are colored consistently across MOAs.