Table S2. Compounds excluded by image-based QC.

This table lists wells removed due to visually apparent imaging artefacts or unreliable staining patterns identified during image-based quality control. Exclusions were applied prior to computing well-level profiles (signed EMD) and downstream analyses. *QC category* summarizes the type of issue, and *QC rationale* provides a brief description.

Library	Well	Compound	QC category	QC rationale (English)
SCADS01	C02	Daunorubicin, HCl	Autofluorescence	Green autofluorescence in nuclei
SCADS01	D02	Doxorubicin, HCl	Autofluorescence	Green autofluorescence in nuclei
SCADS01	G10	Bisindolymaleimide I, HCl	Autofluorescence	Green autofluorescence in nuclei
SCADS02	A07	SB 218078	Autofluorescence	ER-like green autofluorescence
SCADS02	B04	NSC95397	Autofluorescence	Green autofluorescence in nuclei
SCADS02	H04	Sanguinarine	Autofluorescence	Green autofluorescence in nuclei
SCADS03	A04	Cdk4 inhibitor	Autofluorescence	Green autofluorescence in nuclei
SCADS03	C04	SB218078	Autofluorescence	ER-like green autofluorescence
SCADS03	F06	SU6656	Autofluorescence	Endosome-like green autofluorescence
SCADS03	F10	Bisindolymaleimide I, HCl	Autofluorescence	Green autofluorescence in nuclei
SCADS03	G09	SU11652	Autofluorescence	Endosome-like green autofluorescence
SCADS03	G10	Go7874	Autofluorescence	Endosome-like green autofluorescence
SCADS04	B11	BIO	Autofluorescence	Endosome-like green autofluorescence
SCADS04	F08	TMPyP4	Autofluorescence	Green autofluorescence in nucleoli
SCADS02	C06	A23187	Cell count instability	Unstable cell count
SCADS02	D02	Bafilomycin A1	Cell count instability	Unstable cell count
SCADS02	D06	Ionomycin	Cell count instability	Unstable cell count
SCADS04	G06	Crizotinib	Cell count instability	Unstable cell count
SCADS02	B09	Chetomin	Cytotoxicity	High fraction of dead cells
SCADS04	D05	PJ-34	Staining instability	Staining instability (observed in set4)
SCADS04	D06	Tretinoin	Staining instability	Staining instability (observed in set4)
SCADS04	D07	ABT-737	Staining instability	Staining instability (observed in set4)
SCADS04	D08	PCI-34051	Staining instability	Staining instability (observed in set4)
SCADS04	E05	Olaparib	Staining instability	Staining instability (observed in set4)
SCADS04	E06	Tamibarotene	Staining instability	Staining instability (observed in set4)

Table S3A. Image feature classes used in EGFR HCS profiling (summary).

This table summarizes the classes of CellProfiler features used for profiling. Object indicates the segmentation compartment (Cells, Cytoplasm, or Nuclei). Type and Subtype denote the feature family and the specific measurement. Markers lists the staining channels for which each measurement was computed; “NA” indicates measurements that are not marker-specific. Time-resolved features were computed across five stimulation time points (0, 5, 30, 60, and 180 min); for EGF and Tfn, the 0 min time point was excluded (four time points).

object	type	subtype	markers
Cells	AreaShape	Area	NA
Cells	AreaShape	Eccentricity	NA
Cells	AreaShape	FormFactor	NA
Cells	Granularity	Granularity	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cells	Intensity	IntegratedIntensity	EGFR, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cells	Intensity	MADIntensity	EGFR, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cells	Intensity	MedianIntensity	EGFR, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cells	Texture	AngularSecondMoment	pAkt, actin
Cells	Texture	Contrast	pAkt, actin
Cells	Texture	Correlation	pAkt, actin
Cells	Texture	Entropy	pAkt, actin
Cytoplasm	Intensity	IntegratedIntensity	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cytoplasm	Intensity	LowerQuartileIntensity	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cytoplasm	Intensity	MADIntensity	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cytoplasm	Intensity	MedianIntensity	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cytoplasm	Intensity	UpperQuartileIntensity	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, EGF, Tfn
Cytoplasm	RadialDistribution	FracAtD	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, actin, EGF, Tfn
Cytoplasm	RadialDistribution	MeanFrac	EGFR, pERK, pAkt, PI3P, PI4P, PIP2, actin, EGF, Tfn
Cytoplasm	RadialDistribution	RadialCV	pERK
Cytoplasm	Texture	AngularSecondMoment	EGFR, PI3P, PI4P, PIP2, EGF, Tfn, pERK
Cytoplasm	Texture	Contrast	EGFR, PI3P, PI4P, PIP2, EGF, Tfn, pERK
Cytoplasm	Texture	Correlation	EGFR, PI3P, PI4P, PIP2, EGF, Tfn, pERK
Cytoplasm	Texture	Entropy	EGFR, PI3P, PI4P, PIP2, EGF, Tfn, pERK
Nuclei	AreaShape	Area	NA
Nuclei	AreaShape	Eccentricity	NA
Nuclei	AreaShape	FormFactor	NA
Nuclei	Intensity	IntegratedIntensity	DNA, EGFR, pERK
Nuclei	Intensity	MADIntensity	DNA, EGFR, pERK
Nuclei	Intensity	MedianIntensity	DNA, EGFR, pERK
Nuclei	Texture	AngularSecondMoment	DNA
Nuclei	Texture	Contrast	DNA
Nuclei	Texture	Correlation	DNA
Nuclei	Texture	Entropy	DNA

Table S4A. Image feature classes used in Cell Painting profiling (summary).

This table summarizes the classes of CellProfiler image features used for Cell Painting profiling. Object indicates the segmentation compartment (Cells, Cytoplasm, or Nuclei). Type and Subtype denote the feature family and specific measurement. Markers lists the staining channels (AGP, DNA, ER, Mito, and RNA) for which each measurement was computed; "NA" indicates measurements that are not marker-specific. For correlation features, Markers2 indicates the second channel used to compute inter-channel correlation. A complete machine-readable list of feature names used in the analyses is provided as a separate CSV file (Table S5B).

object	type	subtype	markers1	markers2
Cells	AreaShape	Area		
Cells	AreaShape	Eccentricity		
Cells	AreaShape	FormFactor		
Cells	Correlation	Correlation	AGP,DNA,ER,Mito	DNA,ER,Mito,RNA
Cells	Correlation	RWC	AGP,DNA,ER,Mito,RNA	AGP,DNA,ER,Mito,RNA
Cells	Granularity	Granularity	AGP,DNA,ER,Mito,RNA	
Cells	Intensity	IntegratedIntensity	AGP,DNA,ER,Mito,RNA	
Cells	Intensity	LowerQuartileIntensity	AGP,DNA,ER,Mito,RNA	
Cells	Intensity	MADIntensity	AGP,DNA,ER,Mito,RNA	
Cells	Intensity	MedianIntensity	AGP,DNA,ER,Mito,RNA	
Cells	Intensity	UpperQuartileIntensity	AGP,DNA,ER,Mito,RNA	
Cells	Number	Object_Number		
Cells	RadialDistribution	FracAtD	AGP,DNA,ER,Mito,RNA	
Cells	RadialDistribution	MeanFrac	AGP,DNA,ER,Mito,RNA	
Cells	Texture	AngularSecondMoment	AGP,DNA,ER,Mito,RNA	
Cells	Texture	Contrast	AGP,DNA,ER,Mito,RNA	
Cells	Texture	Correlation	AGP,DNA,ER,Mito,RNA	
Cells	Texture	Entropy	AGP,DNA,ER,Mito,RNA	
Cytoplasm	AreaShape	Area		
Cytoplasm	AreaShape	Eccentricity		
Cytoplasm	AreaShape	FormFactor		
Cytoplasm	Correlation	Correlation	AGP,DNA,ER,Mito	DNA,ER,Mito,RNA
Cytoplasm	Correlation	RWC	AGP,DNA,ER,Mito,RNA	AGP,DNA,ER,Mito,RNA
Cytoplasm	Granularity	Granularity	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Intensity	IntegratedIntensity	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Intensity	LowerQuartileIntensity	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Intensity	MADIntensity	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Intensity	MedianIntensity	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Intensity	UpperQuartileIntensity	AGP,DNA,ER,Mito,RNA	
Cytoplasm	RadialDistribution	FracAtD	AGP,DNA,ER,Mito,RNA	
Cytoplasm	RadialDistribution	MeanFrac	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Texture	AngularSecondMoment	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Texture	Contrast	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Texture	Correlation	AGP,DNA,ER,Mito,RNA	
Cytoplasm	Texture	Entropy	AGP,DNA,ER,Mito,RNA	
Nuclei	AreaShape	Area		
Nuclei	AreaShape	Eccentricity		
Nuclei	AreaShape	FormFactor		
Nuclei	Correlation	Correlation	AGP,DNA,ER,Mito	DNA,ER,Mito,RNA
Nuclei	Correlation	RWC	AGP,DNA,ER,Mito,RNA	AGP,DNA,ER,Mito,RNA
Nuclei	Granularity	Granularity	AGP,DNA,ER,Mito,RNA	
Nuclei	Intensity	IntegratedIntensity	AGP,DNA,ER,Mito,RNA	
Nuclei	Intensity	LowerQuartileIntensity	AGP,DNA,ER,Mito,RNA	
Nuclei	Intensity	MADIntensity	AGP,DNA,ER,Mito,RNA	
Nuclei	Intensity	MedianIntensity	AGP,DNA,ER,Mito,RNA	
Nuclei	Intensity	UpperQuartileIntensity	AGP,DNA,ER,Mito,RNA	
Nuclei	RadialDistribution	FracAtD	AGP,DNA,ER,Mito,RNA	
Nuclei	RadialDistribution	MeanFrac	AGP,DNA,ER,Mito,RNA	
Nuclei	Texture	AngularSecondMoment	AGP,DNA,ER,Mito,RNA	
Nuclei	Texture	Contrast	AGP,DNA,ER,Mito,RNA	
Nuclei	Texture	Correlation	AGP,DNA,ER,Mito,RNA	

object	type	subtype	markers1	markers2
Nuclei	Texture	Entropy	AGP,DNA,ER,Mito,RNA	

Table S7. Duplicate compounds and their plate positions.

Each row lists a compound present in duplicate and the two plate–well locations where it appears.

library_1	well_1	library_2	well_2	Compound
SCADS01	A08	SCADS03	B08	SP600125
SCADS01	A04	SCADS03	B03	Kenpaullone
SCADS01	A06	SCADS03	H12	SU1498
SCADS02	C11	SCADS03	A11	KT5823
SCADS01	D10	SCADS03	B10	LY294002
SCADS01	E10	SCADS03	C10	Wortmannin
SCADS01	E12	SCADS03	H11	PP1 analog
SCADS01	E05	SCADS03	H05	AG1478
SCADS02	A07	SCADS03	C04	SB218078
SCADS01	G03	SCADS03	G02	KN-93
SCADS01	G10	SCADS03	F10	Bisindolymaleimide I, HCl
SCADS01	C08	SCADS03	G08	PD98059
SCADS01	D08	SCADS03	H08	U-0126
SCADS02	H10	SCADS03	C09	ML-7
SCADS01	B04	SCADS03	C03	Purvalanol A
SCADS01	F10	SCADS03	D10	H-89
SCADS01	D12	SCADS03	F11	Y-27632
SCADS01	D04	SCADS03	D03	Olomoucine
SCADS02	G02	SCADS03	E02	LFM-A13
SCADS01	B08	SCADS03	D08	Damnacanthal
SCADS01	G06	SCADS03	B07	AG825
SCADS01	E04	SCADS03	H04	TBB
SCADS01	F03	SCADS03	D02	AG957
SCADS01	G07	SCADS03	A06	AG490
SCADS01	C10	SCADS03	F09	AG1296
SCADS01	D07	SCADS03	C07	AG1024
SCADS02	H02	SCADS03	F02	Terrific acid
SCADS02	F12	SCADS03	B12	SB431542

Table S9. Compounds selected as extreme responders for Fig. 3E.

This table lists the compounds selected as extreme responders for the four readouts used in Fig. 3E: pAkt (bottom 5% at 30 min), pERK (top 5% at 30 min), PIP2 (bottom 5% at 30 min), and EGFR granularity (top 5% at 180 min). Values are the representative signed-EMD feature used for ranking, and ID indicates the plate and well position in the format Library:Well. Each row corresponds to one well; the same compound may appear multiple times if present on multiple plates.

(A) pAkt bottom 5% (30 min)

id	compound	value
SCADS01:A11	Staurosporine	-1.000
SCADS01:B09	PD169316	-0.854
SCADS01:C09	SB 203580	-0.909
SCADS01:D10	LY-294002	-1.000
SCADS01:E05	AG1478	-0.865
SCADS01:E10	Wortmannin	-1.000
SCADS02:C11	KT 5823	-0.842
SCADS02:G08	LY 83583	-0.958
SCADS03:A11	KT 5823	-0.900
SCADS03:D01	Akt Inhibitor VIII, Isozyme-Sele	-0.895
SCADS03:E08	PP2	-0.838
SCADS03:G03	Cdk2/9 inhibitor	-0.914
SCADS03:G05	BPIQ-II	-0.831
SCADS03:H05	AG1478	-0.908
SCADS04:B03	erlotinib	-0.934
SCADS04:B10	Vandetanib	-0.874
SCADS04:D03	dasatinib	-0.851
SCADS04:H06	Torkinib	-0.912

(B) pERK top 5% (30 min)

id	compound	value
SCADS01:A01	none (DMSO)	0.528
SCADS01:B03	Cytochalasin D	0.541
SCADS01:B04	Purvalanol A	0.780
SCADS01:H06	Cycloheximide	0.563
SCADS01:H07	Cucurbitacin I	1.000
SCADS02:E06	Thapsigargin	1.000
SCADS02:F06	t-Butylhydroquinone (BHQ)	0.813
SCADS02:G08	LY 83583	0.934
SCADS02:H10	ML-7	0.889
SCADS03:A08	JAK3 Inhibitor VI	0.870
SCADS03:B11	PKR inhibitor	1.000
SCADS03:C01	Akt Inhibitor IV	0.740
SCADS03:F03	Cdk1/2 inhibitor III	0.525
SCADS04:C08	Tenovin-6	0.629
SCADS04:C09	ENMD-2076	0.843
SCADS04:E04	WP1066	1.000
SCADS04:F07	BIX01294	0.720
SCADS04:H06	Torkinib	0.530

(C) PIP2 bottom 5% (30 min)

id	compound	value
SCADS01:B03	Cytochalasin D	-1.000
SCADS01:B04	Purvalanol A	-0.563
SCADS01:D11	Cantharidin	-0.655
SCADS01:E10	Wortmannin	-0.580
SCADS01:E11	Cytostatin	-0.983
SCADS01:H07	Cucurbitacin I	-0.867
SCADS02:A08	Xanthohumol	-0.512
SCADS02:C02	Oligomycin	-0.705
SCADS03:C01	Akt Inhibitor IV	-0.551
SCADS03:C03	Purvalanol A	-0.482
SCADS03:C10	Wortmannin	-0.521
SCADS03:E06	cFMS Receptor Tyrosine Kinase Inhibitor (GW2580)	-0.912
SCADS03:E07	BMS-345541	-1.000
SCADS03:F05	TX-1918	-0.532
SCADS03:G01	ATM/ATR kinase inhibitor	-0.531
SCADS04:C09	ENMD-2076	-0.690
SCADS04:E04	WP1066	-0.935
SCADS04:E07	UNC0638	-0.904

(D) EGFR granularity top 5% (180 min)

id	compound	value
SCADS01:C09	SB 203580	0.454
SCADS01:E02	Tamoxifen, citrate	0.817
SCADS01:G03	KN-93	0.540
SCADS01:H07	Cucurbitacin I	1.000
SCADS02:E05	Nigericin	0.904
SCADS02:F04	Monensin	0.709
SCADS02:G08	LY 83583	0.434
SCADS03:C01	Akt Inhibitor IV	0.607
SCADS03:D09	SB202190	0.538
SCADS03:E08	PP2	0.462
SCADS03:G03	Cdk2/9 inhibitor	0.929
SCADS04:E04	WP1066	0.574
SCADS04:E07	UNC0638	0.882
SCADS04:E09	YM155	0.689
SCADS04:F04	5,15-DPP	0.399
SCADS04:F07	BIX01294	0.387
SCADS04:H06	Torkinib	0.915
SCADS01:C09	SB 203580	0.454

Supplementary Table S10. Representative signed-EMD features used for heatmaps in Figs. 3E, 4E, 5C and 5F.

This table lists the representative image features used to summarize each marker in the heatmaps shown in Figs. 3E, 4E, 5C and 5F. For each marker, the heatmap value corresponds to the signed-EMD computed for the single feature listed here (CellProfiler measurement name), selected as a representative readout for that marker.

Marker	Representative feature (CellProfiler measurement)
EGFR	Cells_Granularity_Granularity_2_EGFR
EGF	Cells_Granularity_Granularity_3_EGF
c-Met	Cells_Granularity_Granularity_2_Met
Tfn	Cells_Granularity_Granularity_2_Tfn
PI3P	Cells_Intensity_IntegratedIntensity_PI3P
PI4P	Cells_Intensity_MADIntensity_PI4P
PIP2	Cells_Intensity_IntegratedIntensity_PIP2
pERK	Nuclei_Intensity_MedianIntensity_pERK
pAkt	Cells_Intensity_MedianIntensity_pAkt
actin	Cells_Texture_Texture_Correlation_Actin_5_mean_64

Table S11. Compounds selected as extreme responders for Fig. 4E.

This table lists the compounds selected as extreme responders for the four readouts used in Fig. 4E of the c-Met assay: pAkt (bottom 5% at 30 min), pERK (top 5% at 30 min), PIP2 (bottom 5% at 30 min), and c-Met granularity (top 5% at 180 min). Values are the representative signed-EMD feature used for ranking, and ID indicates the plate and well position in the format Library:Well. Each row corresponds to one well; the same compound may appear multiple times if present on multiple plates.

(A) pAkt bottom 5% (30 min)

id	compound	value
SCADS01:A11	Staurosporine	-1.000
SCADS01:B01	5-FU	-0.992
SCADS01:D10	LY-294002	-0.985
SCADS01:E10	Wortmannin	-1.000
SCADS01:G05	Manumycin A	-0.999
SCADS01:G12	Nocodazole	-0.608
SCADS02:C11	KT 5823	-0.715
SCADS02:G08	LY 83583	-0.908
SCADS03:A11	KT 5823	-0.879
SCADS03:B09	SU11274	-0.842
SCADS03:B10	LY-294002	-0.984
SCADS03:B11	PKR inhibitor	-0.658
SCADS03:C10	Wortmannin	-1.000
SCADS03:D01	Akt Inhibitor VIII, Isozyme-Sele	-1.000
SCADS03:D04	isogranulatimide	-0.603
SCADS03:G03	Cdk2/9 inhibitor	-1.000
SCADS04:H06	Torkinib	-1.000
SCADS04:H09	PF-04217903	-0.999

(B) pERK top 5% (30 min)

id	compound	value
SCADS01:B03	Cytochalasin D	0.702
SCADS01:H06	Cycloheximide	0.880
SCADS01:H07	Cucurbitacin I	1.000
SCADS02:A12	AZT	1.000
SCADS02:E03	Z-GLF-CMK	0.612
SCADS02:E06	Thapsigargin	0.769
SCADS02:F06	t-Butylhydroquinone (BHQ)	1.000
SCADS02:G08	LY 83583	0.962
SCADS02:H03	SB 225002	0.711
SCADS03:A02	Aurora kinase/cdk inhibitor	0.737
SCADS03:B11	PKR inhibitor	0.812
SCADS03:C01	Akt Inhibitor IV	1.000
SCADS03:D09	SB202190	0.631
SCADS03:H05	AG1478	1.000
SCADS04:A06	anisomycin	0.917
SCADS04:C11	TWS119	0.617
SCADS04:E04	WP1066	1.000
SCADS04:E09	YM155	0.674

(C) PIP2 bottom 5% (30 min)

id	compound	value
SCADS01:B03	Cytochalasin D	-1.000
SCADS01:B04	Purvalanol A	-0.517
SCADS01:E11	Cytostatin	-1.000
SCADS01:G04	Valeryl salicylate	-0.581
SCADS01:H07	Cucurbitacin I	-1.000
SCADS02:A08	Xanthohumol	-0.626
SCADS02:E06	Thapsigargin	-0.873
SCADS03:A11	KT 5823	-0.756
SCADS03:C09	ML-7	-0.618
SCADS03:C10	Wortmannin	-0.507
SCADS03:F02	Terreic acid	-0.624
SCADS03:F04	Chk2 inhibitor II	-0.529
SCADS03:F05	TX-1918	-0.545
SCADS03:H02	KN-62	-0.686
SCADS04:A06	anisomycin	-0.511
SCADS04:D02	temsirolimus	-0.760
SCADS04:E04	WP1066	-0.871
SCADS04:E09	YM155	-0.727

(D) c-Met granularity top 5% (180 min)

id	compound	value
SCADS01:A11	Staurosporine	1.000
SCADS01:B03	Cytochalasin D	0.745
SCADS01:E02	Tamoxifen, citrate	0.925
SCADS01:G03	KN-93	0.727
SCADS01:H07	Cucurbitacin I	0.790
SCADS02:A08	Xanthohumol	0.729
SCADS02:E02	HA 14-1	0.670
SCADS02:E05	Nigericin	0.897
SCADS02:F04	Monensin	1.000
SCADS03:D09	SB202190	1.000
SCADS03:G02	KN-93	0.663
SCADS03:G03	Cdk2/9 inhibitor	1.000
SCADS03:H01	ATM kinase inhibitor	0.753
SCADS04:C08	Tenovin-6	1.000
SCADS04:C09	ENMD-2076	0.644
SCADS04:D10	Vemurafenib	0.954
SCADS04:G09	OSI-906	0.634
SCADS04:H10	PD173074	0.773

Table S12. Empirically defined EGFR and c-Met degradation-inhibitor groups and excluded high-basal compounds (Fig. 5).

Wells were selected if receptor granularity ranked within the top 5% at 180 min after ligand stimulation and were then excluded if ranked within the top 10% at 0 min to remove high-basal granularity phenotypes. Panels (A) and (C) list the criteria-defined selected wells for EGFR and c-Met, respectively, whereas panels (B) and (D) list wells excluded by the 0-min criterion. ID indicates the plate and well position in the format Library:Well, and Value denotes the granularity-derived feature value used for ranking. Each row corresponds to one well, and the same compound may appear multiple times if present on multiple plates. For the comparative analysis in Fig. 5A, one additional KN-93 well was added to the EGFR operational set to mirror the duplicate KN-93 present in the c-Met selected set.

(A) EGFR criteria-defined selected wells (top 5% granularity at 180 min; excluding top 10% at 0 min)

id	compound	value
SCADS01:C09	SB 203580	0.454
SCADS01:E02	Tamoxifen, citrate	0.817
SCADS01:G03	KN-93	0.540
SCADS02:E05	Nigericin	0.904
SCADS02:F04	Monensin	0.709
SCADS02:G08	LY 83583	0.434
SCADS03:D09	SB202190	0.538
SCADS03:G03	Cdk2/9 inhibitor	0.929
SCADS04:E09	YM155	0.689
SCADS04:F04	5,15-DPP	0.399
SCADS04:F07	BIX01294	0.387
SCADS04:H06	Torkinib	0.915

(B) EGFR excluded wells (top 5% granularity at 180 min but also top 10% at 0 min)

id	compound	value
SCADS01:A11	Staurosporine	1.000
SCADS01:H07	Cucurbitacin I	1.000
SCADS03:C01	Akt Inhibitor IV	0.607
SCADS03:E08	PP2	0.462
SCADS04:E04	WP1066	0.574
SCADS04:E07	UNC0638	0.882

(C) c-Met criteria-defined selected wells (top 5% granularity at 180 min; excluding top 10% at 0 min)

id	compound	value
SCADS01:E02	Tamoxifen, citrate	0.925
SCADS01:G03	KN-93	0.727
SCADS02:A08	Xanthohumol	0.729
SCADS02:E02	HA 14-1	0.670
SCADS02:E05	Nigericin	0.897
SCADS03:D09	SB202190	1.000
SCADS03:G02	KN-93	0.663
SCADS03:G03	Cdk2/9 inhibitor	1.000
SCADS03:H01	ATM kinase inhibitor	0.753
SCADS04:D10	Vemurafenib	0.954
SCADS04:G09	OSI-906	0.634
SCADS04:H10	PD173074	0.773

(D) c-Met excluded wells (top 5% granularity at 180 min but also top 10% at 0 min)

id	compound	value
SCADS01:A11	Staurosporine	1.000
SCADS01:B03	Cytochalasin D	0.745
SCADS01:H07	Cucurbitacin I	0.790
SCADS02:F04	Monensin	1.000
SCADS04:C08	Tenovin-6	1.000
SCADS04:C09	ENMD-2076	0.644

Supplementary Table S13. Rationale for assigning degradation-inhibiting compounds to candidate cellular process nodes (Fig. 6C).

Compounds that inhibited ligand-induced EGFR and/or c-Met degradation were connected to empirically defined cellular process nodes in Fig. 6C. Assignments and edges reflect plausible associations based on reported pharmacology, literature knowledge, and phenotype-level consistency (including in vitro kinase profiling where applicable), rather than definitive molecular targets. Primary node corresponds to the main candidate process (thick edges), whereas Secondary node / hypothesis indicates secondary or indirect associations (thin edges) or conservative hypotheses based on structural/pharmacological analogy (dashed edges). Compounds shown without edges in Fig. 6C are listed as Unassigned to avoid overinterpretation. Reference numbers correspond to the Supplementary References list.

Compound	Primary node	Secondary node / Hypothesis	Literature rationale
Monensin	Endolysosomal pH/ions	—	Carboxylic ionophore that dissipates monovalent cation gradients, elevates lysosomal pH, and inhibits lysosomal proteolysis/autophagy. [1]
Nigericin	Endolysosomal pH/ions	—	K+/H+ ionophore used to equilibrate pH across membranes; rapidly elevates lysosomal pH and perturbs lysosome-dependent processes. [2]
Tamoxifen	Endolysosomal pH/ions	Ca2+/CaM	Reported to inhibit endolysosomal acidification independent of estrogen receptor signaling, potentially impacting receptor trafficking and turnover. [3]
SB202190	Endosomal lipids	Cell stress/signaling	Although widely used as a p38 inhibitor, pyridinyl-imidazole p38 inhibitors (e.g., SB202190/SB203580) can inhibit PIKfyve off-target and induce Rab7-dependent vacuolation. [4]
SB203580	Endosomal lipids	Cell stress/signaling	SB203580 is a pyridinyl-imidazole p38 MAPK inhibitor closely related to SB202190. In addition to p38 inhibition, this compound class has been reported to inhibit PIKfyve off-target, resulting in endosomal vacuolation and altered phosphoinositide-dependent trafficking. Stress-related signaling effects are treated as secondary. [4]
Cdk2/9 inhibitor	Endosomal lipids	Cell stress/signaling	Annotated as a CDK2/9 inhibitor in the library; in our phenotypic profiles it clusters with endosomal-lipid perturbations rather than nuclear/cell-cycle signatures, so a trafficking mechanism is treated as a working hypothesis. [16]
KN-93	Ca2+/CaM	—	Commonly used as a CaMKII inhibitor, but has CaMKII-independent inhibition of ion channels (including L-type Ca2+ currents and IKr), which can drive pleiotropic phenotypes. [5,6]
HA14-1	Cell stress/signaling	Ca2+/CaM	Originally described as a BCL-2 antagonist; has reported off-target effects on Ca2+ handling via SERCA2b and IP3 receptors. [7]
Xanthohumol	Cell stress/signaling	Endolysosomal pH/ions	Xanthohumol has been reported to directly inhibit p97/VCP, thereby impairing autophagosome maturation and lysosome-dependent degradation. We therefore interpret its phenotype primarily as a proteostasis/autophagy-linked system effect, while any endolysosomal pH/ion contribution remains a secondary hypothesis. [17]
Torkinib (PP242)	Cell stress/signaling	Endosomal lipids	ATP-competitive mTOR kinase inhibitor affecting both mTORC1 and mTORC2 outputs; expected broad downstream metabolic and trafficking consequences. [9]
PD173074	Cell stress/signaling	—	Selective FGFR inhibitor (pyrido[2,3-d]pyrimidine) with demonstrated inhibition of FGFR signaling in model systems. [10]
BIX01294	Cell stress/signaling	—	BIX01294 is a small-molecule inhibitor of the G9a histone methyltransferase and reduces H3K9me2, leading to broad transcriptional rewiring. Thus, we interpret its phenotype as a system-level state change rather than a dedicated trafficking target. [18]
Vemurafenib	Cell stress/signaling	—	BRAF V600E inhibitor that strongly rewires MAPK pathway activity; downstream effects are broad and context-dependent. [12]
YM155	Cell stress/signaling	—	YM155 toxicity is linked to SLC35F2-mediated uptake and DNA-damage–associated stress responses; likely reflects general cellular stress. [13]
ATM kinase inhibitor (e.g., KU-55933)	Unassigned	—	ATM kinase inhibition blocks core DNA damage response signaling; we keep this compound unassigned to the trafficking nodes in this figure. [14]
LY83583	Cell stress/signaling	—	LY83583 is widely used as a superoxide/ROS-inducing compound at micromolar concentrations and can elicit mitochondrial/oxidative stress responses. We therefore treat its phenotype as stress-driven and potentially indirect to the endolysosomal pathway. [19]
OSI-906 (Linsitinib)	Cell stress/signaling	—	OSI-906 (linsitinib) is a selective dual inhibitor of IGF-1R and the insulin receptor, suppressing downstream PI3K–Akt–mTOR signaling. We interpret its phenotype as an indirect consequence of growth/metabolic signaling blockade rather than a direct endocytic/lysosomal target. [20]
5,15-DPP (Stat3 inhibitor VIII)	Unassigned	—	5,15-DPP is sold as a STAT3 inhibitor, but direct STAT3 binding has been questioned; we therefore keep it unassigned. [15]

Compound	Primary node	Secondary node / Hypothesis	Literature rationale

Edge definition.
Thick edges indicate primary candidate cellular processes; thin edges indicate secondary or indirect associations; dashed edges denote hypotheses based on structural or pharmacological analogy. Compounds shown without edges were intentionally left unassigned to avoid overinterpretation.

Node definitions.
Endolysosomal pH/ions: regulation of endosomal/lysosomal acidification and ion homeostasis.
Endosomal lipids: phosphoinositide-dependent control of endosomal maturation and trafficking (e.g., PIKfyve–PI(3,5)P2 and PI3K/VPS34-related pathways).
Ca2+/CaM: Ca^{2+}- and calmodulin-dependent regulation potentially affecting endocytosis and trafficking.
Cell stress/signaling: pleiotropic cellular responses including stress signaling, DDR, transcriptional/translational stress, and broad cytoskeletal effects.

References for Supplementary Table S13

1. Grinde, B. (1983). Effect of carboxylic ionophores on lysosomal protein degradation in rat hepatocytes. Exp. Cell Res. 149, 27–35. https://doi.org/10.1016/0014-4827(83)90377-4.

2. Morgan, A.J., and Galione, A. (2021). Lysosomal agents inhibit store-operated Ca2+ entry. J. Cell Sci. 134, jcs248658. https://doi.org/10.1242/jcs.248658.

3. Altan, N., Chen, Y., Schindler, M., and Simon, S.M. (1999). Tamoxifen inhibits acidification in cells independent of the estrogen receptor. Proc. Natl. Acad. Sci. USA 96, 4432–4437. https://doi.org/10.1073/pnas.96.8.4432.

4. Wible, D.J., Parikh, Z., Cho, E.J., Chen, M.D., Mukhopadhyay, S., Dalby, K.N., Varadarajan, S., Bratton, S.B., and Jeter, C.R. (2024). Unexpected inhibition of PIKfyve by SB202190 leads to lysosomal dysfunction and apoptosis. Cell Death Dis. 15, 80. https://doi.org/10.1038/s41419-024-06423-0.

5. Gao, L., Blair, L.A.C., and Salinas, G.D. (2006). CaMKII-independent effects of KN93 and its inactive analog KN92 on L-type calcium channels. Biochem. Biophys. Res. Commun. 345, 1606–1610. https://doi.org/10.1016/j.bbrc.2006.05.066.

6. Hegyi, B., Chen-Izu, Y., Jian, Z., Shimkunas, R., Izu, L.T., and Banyasz, T. (2015). KN-93 inhibits IKr in mammalian cardiomyocytes. J. Mol. Cell Cardiol. 89, 173–176. https://doi.org/10.1016/j.yjmcc.2015.10.004.

7. Akl, H., Vervloessem, T., Kiviluoto, S., Bittremieux, M., Parys, J.B., De Smedt, H., and Bultynck, G. (2013). HA14-1, but not the BH3 mimetic ABT-737, causes Ca2+ dysregulation in platelets and human cell lines: caution in using HA14-1 as a BH3 mimetic. Haematologica 98, e49–e51. https://doi.org/10.3324/haematol.2012.080598.

8. Honkanen, R.E. (1993). Cantharidin, another natural toxin that inhibits the activity of serine/threonine protein phosphatases types 1 and 2A. FEBS Lett. 330, 283–286. https://doi.org/10.1016/0014-5793(93)80889-3.

9. Feldman, M.E., Apsel, B., Uotila, A., Loewith, R., Knight, Z.A., Ruggero, D., and Shokat, K.M. (2009). Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. 7, e38. https://doi.org/10.1371/journal.pbio.1000038.

10. Mohammadi, M., Froum, S., Hamby, J.M., Schroeder, M.C., Panek, R.L., Lu, G.H., Eliseenkova, A.V., Green, D., Schlessinger, J., and Hubbard, S.R. (1998). Crystal structure of an angiogenesis inhibitor bound to the FGF receptor tyrosine kinase domain. EMBO J. 17, 5896–5904. https://doi.org/10.1093/emboj/17.20.5896.

11. Harrington, E.A., Bebbington, D., Moore, J., Rasmussen, R.K., Ajose-Adeogun, A.O., Nakayama, T., Graham, J.A., Demur, C., Hercend, T., Diu-Hercend, A., et al. (2004). VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. Nat. Med. 10, 262–267. https://doi.org/10.1038/nm1003.

12. Chapman, P.B., Hauschild, A., Robert, C., Haanen, J.B., Ascierto, P., Larkin, J., Dummer, R., Garbe, C., Testori, A., Maio, M., et al. (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N. Engl. J. Med. 364, 2507–2516. https://doi.org/10.1056/NEJMoa1103782.

13. Winter, G. E., Radic, B., Mayor-Ruiz, C., *et al.* (2014). *The solute carrier SLC35F2 enables YM155-mediated DNA damage toxicity.* **Nature Chemical Biology**, 10, 768–773. https://doi.org/10.1038/nchembio.1590

14. Hickson, I., Zhao, Y., Richardson, C.J., Green, S.J., Martin, N.M.B., Orr, A.I., Reaper, P.M., Jackson, S.P., Curtin, N.J., and Smith, G.C.M. (2004). Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res. 64, 9152–9159. https://doi.org/10.1158/0008-5472.CAN-04-2727.

15. Mtwebana, S. S., & Prinsloo, E. (2020). *The commercially available STAT3 inhibitor 5,15-diphenylporphyrin (5,15-DPP) does not directly interact with STAT3 core residues 127–722.* **BMC Research Notes**, 13, 345. https://doi.org/10.1186/s13104-020-05189-w

16. Tanabe, K. (2026). This study.

17. Sasazawa, Y., Kanagaki, S., Tashiro, E., Nogawa, T., Muroi, M., Kondoh, Y., Osada, H., & Imoto, M. (2012). *Xanthohumol impairs autophagosome maturation through direct inhibition of valosin-containing protein.* **ACS Chemical Biology**, 7, 892–900. https://doi.org/10.1021/cb200492h

18. Kubicek, S., O'Sullivan, R. J., August, E. M., *et al.* (2007). *Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase.* **Molecular Cell**, 25, 473–481. https://doi.org/10.1016/j.molcel.2007.01.017

19. Cheng, J. C., Xie, Y., *et al.* (2013). *ROS-mediated downregulation of MYPT1 in vascular smooth muscle cells: a potential mechanism for vascular dysfunction.* **Laboratory Investigation**, 93, 422–433. https://doi.org/10.1038/labinvest.2013.40

20. Mulvihill, M. J., Cooke, A., Rosenfeld-Franklin, M., *et al.* (2009). *Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor.* **Future Medicinal Chemistry**, 1, 1153–1171. https://doi.org/10.4155/fmc.09.89

Table S14A. Image feature classes used in the HGF/c-Met assay (summary).

This table summarizes the classes of CellProfiler features used for profiling in the HGF/c-Met assay. *Object* indicates the segmentation compartment (Cells, Cytoplasm, or Nuclei). *Type* and *Subtype* denote the feature family and specific measurement. *Markers* lists the staining channels for which each measurement was computed; "NA" indicates measurements that are not marker-specific.

object	type	subtype	markers
Cells	AreaShape	Area	NA
Cells	AreaShape	Eccentricity	NA
Cells	AreaShape	FormFactor	NA
Cells	Granularity	Granularity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cells	Intensity	IntegratedIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cells	Intensity	LowerQuartileIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cells	Intensity	MADIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cells	Intensity	MedianIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cells	Intensity	UpperQuartileIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cells	Texture	AngularSecondMoment	pAkt, actin
Cells	Texture	Contrast	pAkt, actin
Cells	Texture	Correlation	pAkt, actin
Cells	Texture	Entropy	pAkt, actin
Cytoplasm	AreaShape	Area	NA
Cytoplasm	AreaShape	Eccentricity	NA
Cytoplasm	AreaShape	FormFactor	NA
Cytoplasm	Intensity	IntegratedIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Intensity	LowerQuartileIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Intensity	MADIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Intensity	MedianIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Intensity	UpperQuartileIntensity	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Cytoplasm	RadialDistribution	FracAtD	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, actin, Tfn
Cytoplasm	RadialDistribution	MeanFrac	c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, actin, Tfn
Cytoplasm	Texture	AngularSecondMoment	c-Met, EGFR, pERK, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Texture	Contrast	c-Met, EGFR, pERK, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Texture	Correlation	c-Met, EGFR, pERK, PI3P, PI4P, PIP2, Tfn
Cytoplasm	Texture	Entropy	c-Met, EGFR, pERK, PI3P, PI4P, PIP2, Tfn
Nuclei	AreaShape	Area	NA
Nuclei	AreaShape	Eccentricity	NA
Nuclei	AreaShape	FormFactor	NA
Nuclei	Intensity	IntegratedIntensity	DNA, c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Nuclei	Intensity	LowerQuartileIntensity	DNA, c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Nuclei	Intensity	MADIntensity	DNA, c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Nuclei	Intensity	MedianIntensity	DNA, c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Nuclei	Intensity	UpperQuartileIntensity	DNA, c-Met, EGFR, pERK, pAkt, PI3P, PI4P, PIP2, Tfn
Nuclei	Texture	AngularSecondMoment	DNA
Nuclei	Texture	AngularSecondMoment	DNA
Nuclei	Texture	Contrast	DNA
Nuclei	Texture	Correlation	DNA
Nuclei	Texture	Entropy	DNA

Table S16. Microscope acquisition settings for the EGF and HGF/c-Met time-course assays and the Cell Painting assay.

(A) Imaging parameters for the EGF stimulation time-course assay. For each staining panel (Set1–Set4), the channel assignment, marker, exposure time, and z-offset are listed. (B) Imaging parameters for the HGF stimulation time-course assay. In (A) and (B), exposure times are reported in milliseconds. Z offsets (μm) denote the per-channel focus offsets relative to the Hoechst channel (Ch1), which was used as the reference focus. (C) Imaging parameters for the Cell Painting assay. For each channel, the main dye(s), excitation/emission filter sets (center/bandpass, nm), exposure time (ms), stage z-position used for acquisition, and binning are shown.

(A) Imaging parameters for EGF stimulation assays.

Panel (set)	Ch.	Marker	Exposure (ms)	Z offset (μm)
EGF Set1	Ch1	Hoechst	17.4	0.00
EGF Set1	Ch2	GFP–EGFR	601.8	1.96
EGF Set1	Ch3	pERK	508.1	2.00
EGF Set1	Ch4	Alexa647–EGF	480.0	-3.57
EGF Set2	Ch1	Hoechst	17.4	0.00
EGF Set2	Ch2	GFP–EGFR	699.0	-0.04
EGF Set2	Ch3	PI3P	282.3	2.00
EGF Set2	Ch4	PI4P	263.7	-5.57
EGF Set3	Ch1	Hoechst	17.4	0.00
EGF Set3	Ch2	GFP–EGFR	699.0	2.96
EGF Set3	Ch3	PIP2	104.4	5.00
EGF Set3	Ch4	actin (phalloidin)	419.5	-2.57
EGF Set4	Ch1	Hoechst	17.4	0.00
EGF Set4	Ch2	GFP–EGFR	699.0	0.96
EGF Set4	Ch3	pAkt	685.0	2.00
EGF Set4	Ch4	Alexa647–Tfn	565.0	-2.57

(B) Imaging parameters for HGF stimulation assays.

Panel (set)	Ch.	Marker	Exposure (ms)	Z offset (μm)
HGF Set1	Ch1	Hoechst	39.6	0.00
HGF Set1	Ch2	GFP–EGFR	672.9	3.56
HGF Set1	Ch3	pERK	513.4	4.56
HGF Set1	Ch4	c-Met	647.5	-1.44
HGF Set2	Ch1	Hoechst	111.1	0.00
HGF Set2	Ch2	PI4P	83.2	4.12
HGF Set2	Ch3	c-Met	463.2	4.56
HGF Set2	Ch4	PI3P	211.3	-1.44
HGF Set3	Ch1	Hoechst	23.5	0.00
HGF Set3	Ch2	PIP2	374.0	1.56
HGF Set3	Ch3	c-Met	458.0	1.00
HGF Set3	Ch4	Alexa647–Tfn	341.6	-6.00
HGF Set4	Ch1	Hoechst	23.5	0.00
HGF Set4	Ch2	actin (phalloidin)	61.7	0.56
HGF Set4	Ch3	pAkt	402.5	0.00
HGF Set4	Ch4	c-Met	560.8	-6.00

(C) Imaging parameters for the Cell Painting assay (ImageXpress Micro).

Ch.	Main markers (dyes)	Excitation (nm)*	Emission (nm)*	Exposure (ms)	Stage z-position†	Binning
Ch1	Hoechst 33342 (nuclei)	377/50	447/60	5	10839.8	1×1
Ch2	ConA Alexa Fluor 488 (endoplasmic reticulum / plasma membrane)	482/35	536/40	11	10838.5	1×1
Ch3	SYTO 14 (nucleoli / cytoplasmic RNA)	543/22	593/40	5	10838.5	1×1
Ch4	WGA Alexa Fluor 555 + Phalloidin Alexa Fluor 568	562/40	624/40	13	10838.5	1×1
Ch5	MitoTracker Deep Red 633 (mitochondria)	628-631/40	692/40	22	10838.5	1×1

*Excitation/emission filter sets are shown as center/bandpass (nm).
†Stage z-position is the microscope focus coordinate used for image acquisition.