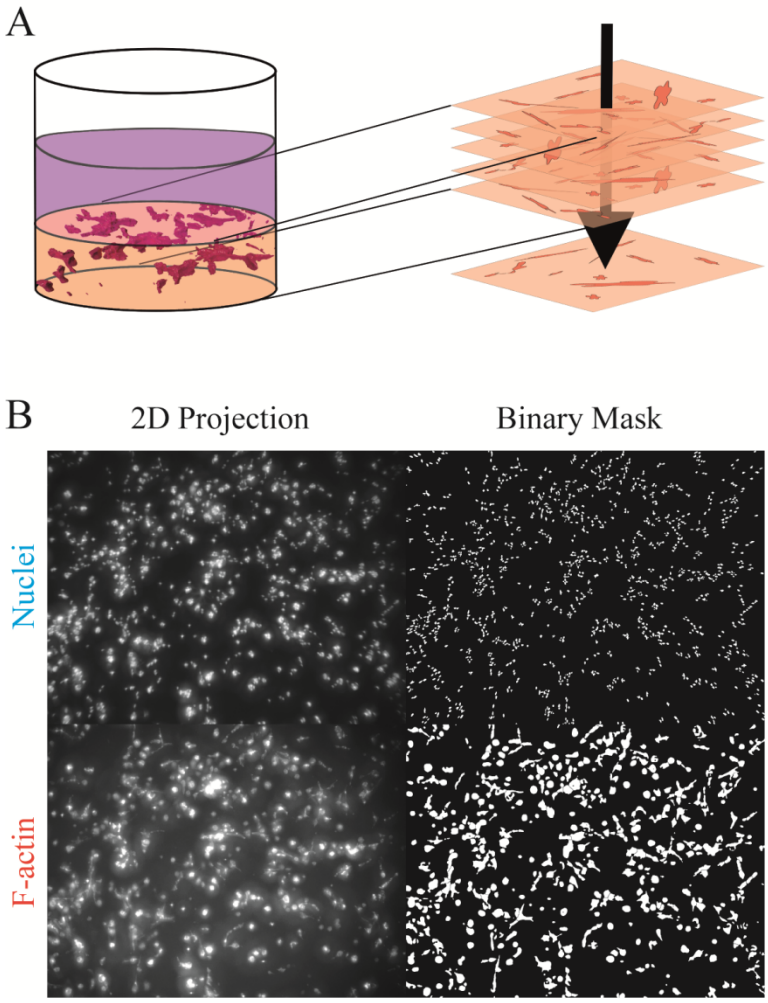
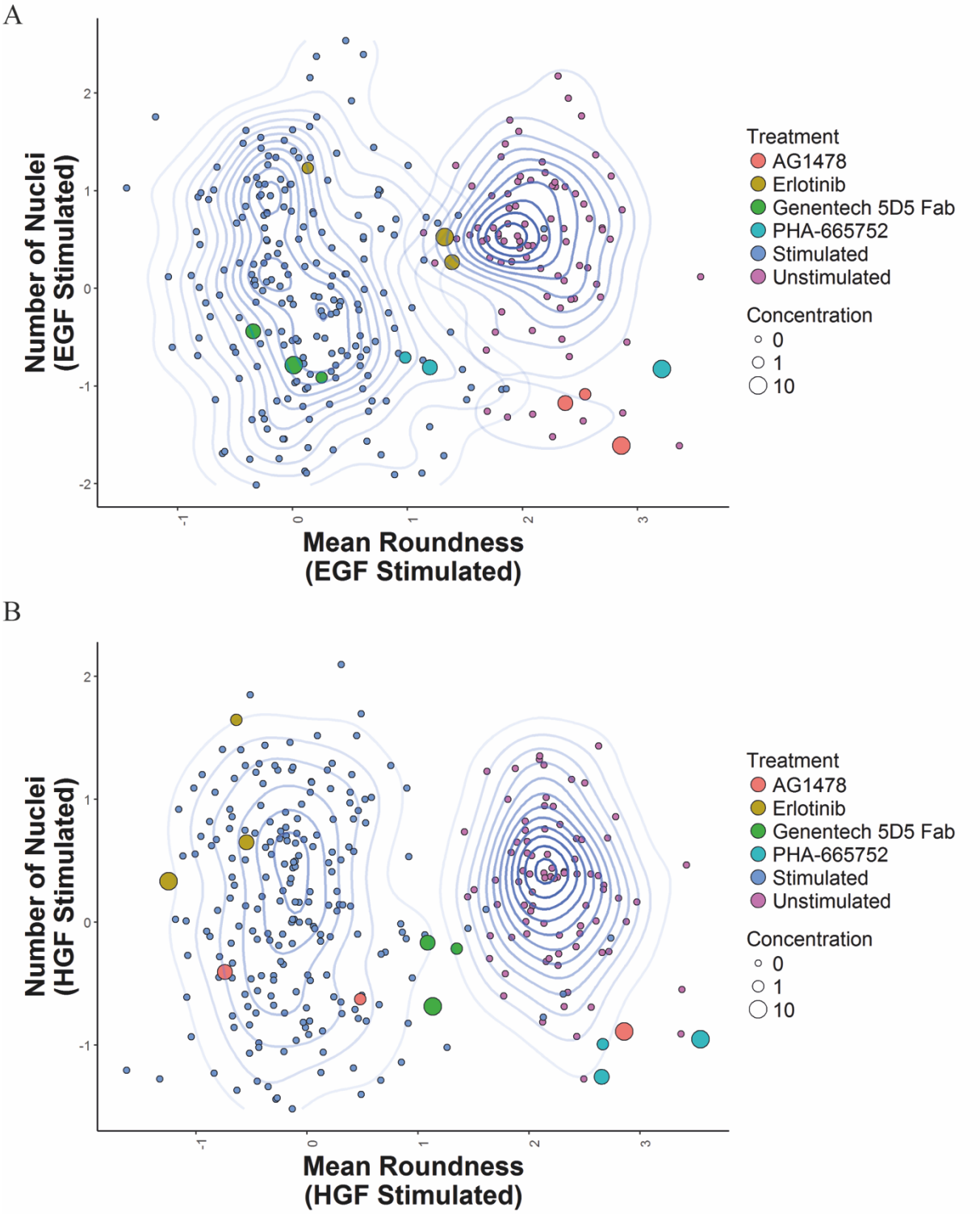


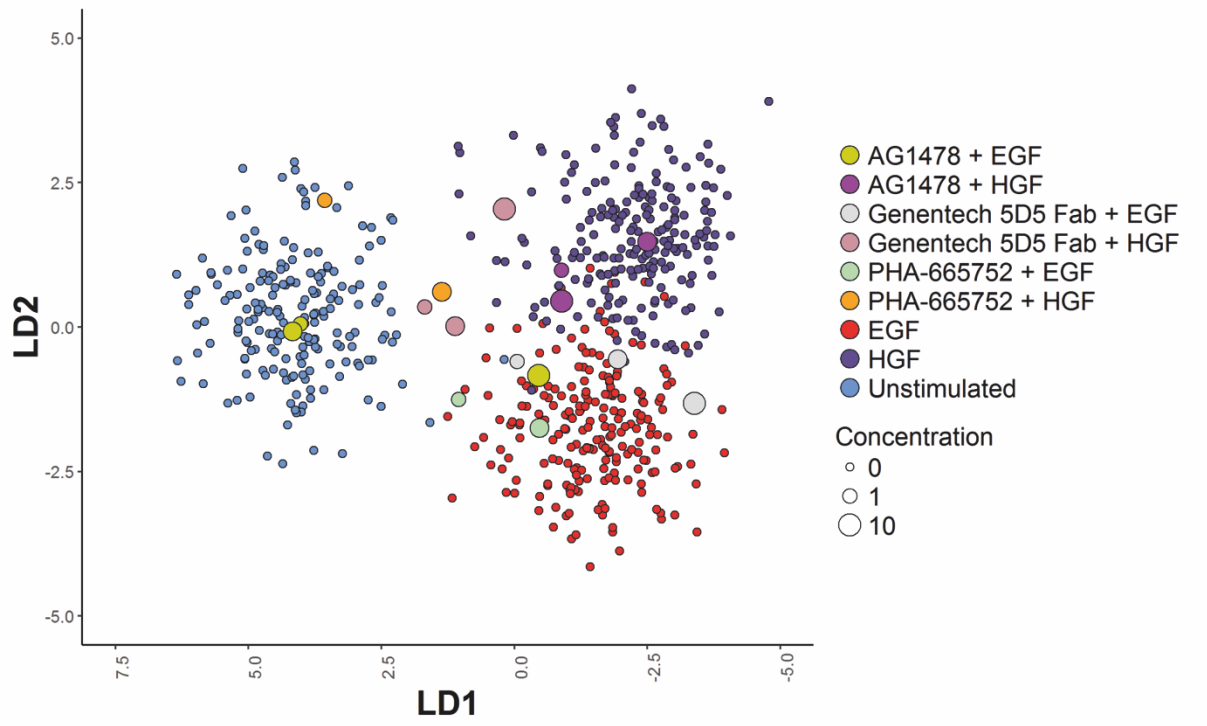
Supplemental Figure S1: 3D cell culture based screening platform pipeline.

A) PC-3 cells were cultured in 384 well plate format embedded in ECM-protein hydrogels. Compound treatment was performed for 96 hours, after which the 3D cultures were stained for F-actin and nuclei . Images were obtained for both channels at different z-planes in the gel using a BD Pathway 855 imager. In-focus information of all Z-stacks was extracted by means of a 2D maximum intensity projection.

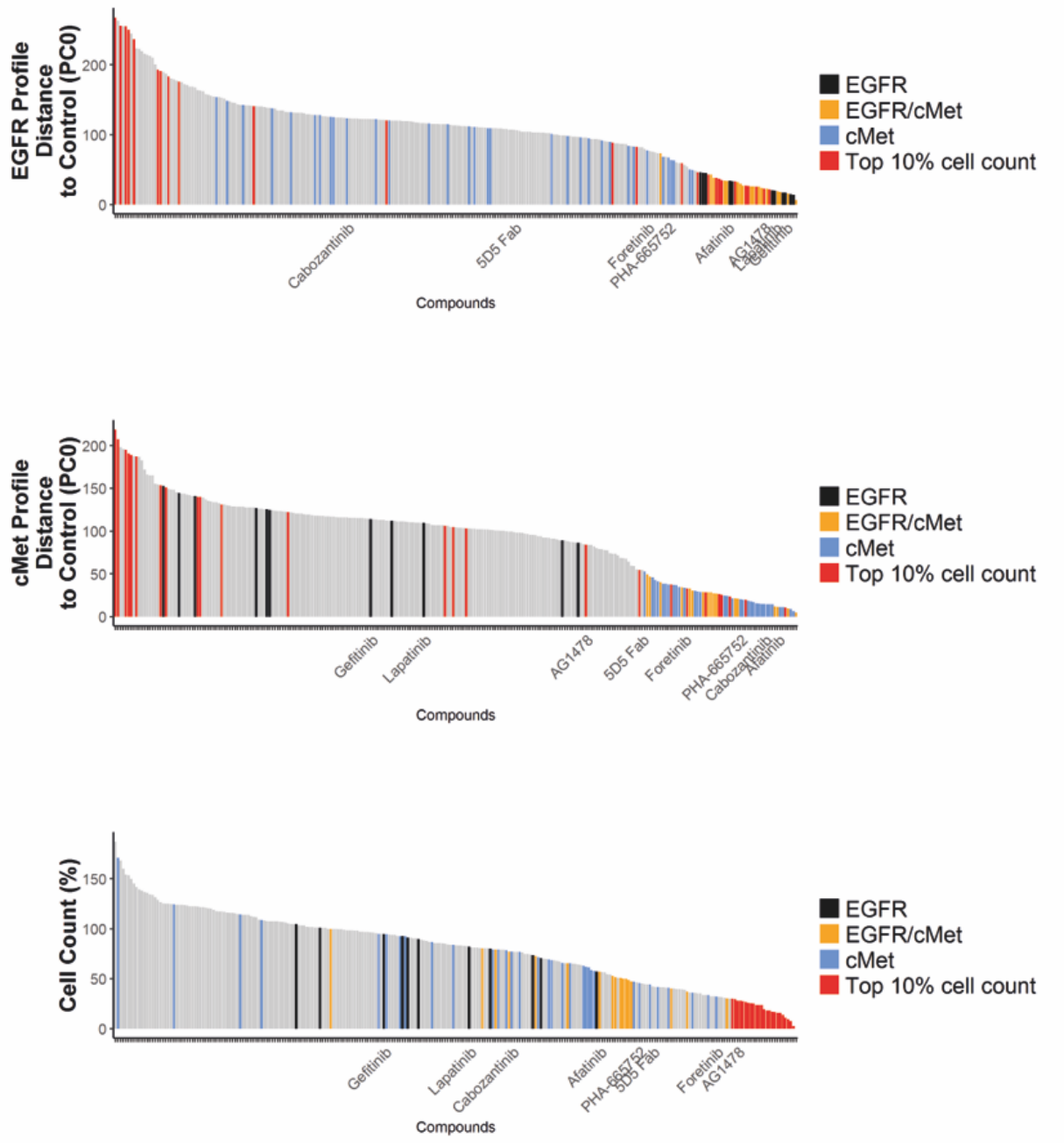
B) Binary black and white masks were generated to define the segmented objects and were used for shape measurements. Fluorescence intensity parameters were obtained from the segmented regions of the original micrograph projection.



Supplemental Figure S2: Performance of individual phenotypic parameters to identify selective c-Met or EGFR inhibitors. A) Z-score normalized roundness and number of nuclei of tumouroids shown for EGF stimulated condition. B) Z-score normalized roundness and number of nuclei of tumouroids shown for HGF stimulated condition.



Supplemental Figure S3: EGF-and HGF-induced phenotypes are both characterized by invasion into the ECM, but can be differentiated using linear discriminant analysis (LDA). LDA training was performed using KNIME and RStudio (MASS package). Linear correlation filter was applied to all parameters (R^2<0.85) prior to training LDA on EGF, HGF and unstimulated groups, in order to filter out the most highly collinear variables. Approximately 250 phenotypic descriptors are required to separate both phenotypes. LDA was subsequently applied to the complete dataset. Individual data points for EGF, HGF and unstimulated shown. Mean data shown for AG1478, Genentech 5D5 Fab and PHA-665752.



Supplemental Figure S4: Hit selection based on cell count does not identify selective c-Met and EGFR inhibitors. Ranking performed on cell count and on c-Met and EGFR profile, as in figure 4C, show different results.

Supplemental table 1: Vichem compound information

Compound ID	**References**	**Core structure**
VCC030450:22	D.W.Fry et al. 1994 Science 265, 1093-95.	quinazoline
VCC613596:10	WO 2009104027 A1	benzothieno[2,3-d]pyrimidine
VCC912492:07	WO 2012080727 A2	benzothieno[2,3-d]pyrimidine
VCC740005:11	Cancer Res, Vol.65 Nr., 379-382, 2005.	quinazoline
VCC407451:10	Bioorg Med Chem Lett, Vol.14 Nr.1, 111-114, 2004.	quinazoline
VCC075648:03	J. Med. Chem., 1999, 42, 5120-5130, Cancer Research (2003), 63(17), 5462-5469	indol-2-one
VCC475979:01	unpublished	indol-2-one
VCC884444:01	unpublished	indol-2-one
VCC833029:24	Bioorg Med Chem Lett, Vol.16 Nr.17, 4686-4691, 2006.	quinazoline
VCC502987:01	unpublished	indol-2-one
VCC370686:02	unpublished	indol-2-one
VCC703724:02	unpublished	indol-2-one
VCC260084:01	unpublished	indol-2-one
VCC458127:03	Bioorg. Med. Chem.Lett. 2001,11,2867-2870	4-(1H-imidazol-5-yl)pyrimidine
VCC930986:01	Tetrahedron Letters (2008), 49(7), 1269-1273.	quinazoline
VCC996608:06	Current Medicinal Chemistry (2014), 21(17), 1938-1955.	quinoline
VCC398520:05	Preparation of quinoline derivatives as AXL kinase inhibitors WO 2009127417 A1	quinoline
VCC285946:01	Molecular Cancer Therapeutics (2009), 8(12), 3181-3190.	[1,2,4]triazolo[4,3-b]pyridazine
VCC055393:01	2008 Oncogene 27 (34), pp. 4702-4711	quinazoline
VCC131028:02	unpublished	quinoline
VCC098211:02	unpublished	**quinoline**
VCC999628:01	unpublished	quinoline
VCC376189:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC967844:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC617235:01	unpublished	quinoline
VCC395122:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC883733:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC868449:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC155409:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC048363:02	Clinical Cancer Research 2009, 15, 5040, Cancer Res 2007;67(24):11924–32	quinazoline
VCC957400:02	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC716837:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC656237:01	unpublished	quinoline
VCC378728:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC461663:01	unpublished	quinoline
VCC325112:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC109756:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303	quinoline
VCC497510:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC444414:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC960450:01	Clin Cancer Res. 2010 Dec 15;16(24):5936-41.	quinoline
VCC450892:17	Cancer Res. 2009 Oct 15;69(20):8009-16.	quinoline
VCC528301:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC778672:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC466812:01	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC444508:05	ACS Medicinal Chemistry Letters (2014), 5(4), 298-303.	quinoline
VCC590177:01	unpublished	quinoline
VCC221701:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC979277:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one

VCC935482:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC443915:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC051013:02	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC228833:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC685240:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC804481:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC365775:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC744093:03	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC323972:01	WO 2014022116 A2 20140206	4-phenoxypyridine
VCC353749:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC317779:01	WO 2014022116 A2 20140206	4-phenoxypyridine
VCC415997:02	WO 2008102870 A1	4-phenoxypyridine
VCC055876:02	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	4-phenoxypyridine
VCC429285:02	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC372550:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC290183:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC031393:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC346202:02	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC563740:02	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC180368:02	unpublished	quinoline
VCC239215:01	unpublished	4-phenoxypyridine
VCC898902:01	unpublished	4-phenoxypyridine
VCC733981:01	unpublished	4-phenoxypyridine
VCC656576:02	unpublished	4-phenoxypyridine
VCC034014:01	unpublished	4-phenoxypyridine
VCC132459:01	unpublished	4-phenoxypyridine
VCC692601:01	unpublished	4-phenoxypyridine
VCC581800:03	unpublished	quinoline
VCC232089:02	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC650454:03	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one
VCC227946:01	Preparation of benzopyrrolidone derivatives for use as GRK5 modulators WO 2015022437 A1	indol-2-one

Supplemental table 2: Hit selection phenotypic screen

Vichem ID	1µM	3.16 µM	10 µM
VCC030450:22	EGFR	EGFR	EGFR
VCC031393:01	Top 10% cell count	None	cMet
VCC034014:01	None	None	None
VCC048363:02	EGFR	EGFR	EGFR/cMet
VCC051013:02	Top 10% cell count	Top 10% cell count	None
VCC055393:01	EGFR/cMet	EGFR/cMet	EGFR/cMet
VCC055876:02	None	None	None
VCC075648:03	None	None	None
VCC098211:02	None	None	None
VCC109756:01	cMet	cMet	cMet
VCC131028:02	None	None	None
VCC132459:01	None	None	None
VCC155409:01	None	None	EGFR/cMet
VCC180368:02	None	None	None
VCC221701:01	None	None	None
VCC227946:01	None	None	EGFR/cMet
VCC228833:01	None	None	Top 10% cell count
VCC232089:02	None	None	Top 10% cell count
VCC239215:01	None	None	None
VCC260084:01	None	None	None
VCC285946:01	None	cMet	cMet
VCC290183:01	None	None	None
VCC317779:01	None	None	None
VCC323972:01	None	None	None
VCC325112:01	None	None	None
VCC346202:02	Top 10% cell count	Top 10% cell count	Top 10% cell count
VCC353749:01	None	None	None
VCC365775:01	None	None	cMet
VCC370686:02	None	None	None
VCC372550:01	None	None	None
VCC376189:01	cMet	cMet	EGFR/cMet
VCC378728:01	None	EGFR	None
VCC395122:01	None	None	cMet
VCC398520:05	None	None	None
VCC407451:10	None	EGFR	EGFR
VCC415997:02	cMet	cMet	cMet
VCC429285:02	None	None	EGFR/cMet
VCC443915:01	Top 10% cell count	None	cMet
VCC444414:01	cMet	cMet	cMet
VCC444508:05	None	None	Top 10% cell count
VCC450892:17	cMet	cMet	cMet
VCC458127:03	None	None	EGFR/cMet
VCC461663:01	None	None	None
VCC466812:01	None	None	None
VCC475979:01	None	None	Top 10% cell count
VCC497510:01	cMet	cMet	EGFR/cMet
VCC502987:01	None	None	EGFR/cMet
VCC528301:01	None	None	EGFR/cMet
VCC563740:02	None	Top 10% cell count	None
VCC581800:03	cMet	cMet	cMet
VCC590177:01	None	None	None
VCC613596:10	None	None	None
VCC617235:01	None	None	None
VCC650454:03	Top 10% cell count	Top 10% cell count	Top 10% cell count
VCC656237:01	None	None	None
VCC656576:02	None	None	None
VCC685240:01	None	None	cMet
VCC692601:01	None	None	None
VCC703724:02	None	None	None
VCC716837:01	None	None	Top 10% cell count
VCC733981:01	None	None	None
VCC740005:11	None	EGFR	EGFR
VCC744093:01	None	None	None
VCC744093:03	None	Top 10% cell count	EGFR/cMet
VCC778672:01	None	None	None
VCC804481:01	None	None	Top 10% cell count
VCC833029:24	None	None	EGFR
VCC868449:01	None	None	EGFR/cMet
VCC883733:01	None	None	None
VCC884444:01	None	None	Top 10% cell count
VCC898902:01	None	None	None
VCC912492:07	None	None	None
VCC930986:01	None	None	None
VCC935482:01	None	None	None
VCC957400:02	None	None	Top 10% cell count
VCC960450:01	cMet	cMet	cMet
VCC967844:01	None	None	None
VCC979277:01	None	None	EGFR/cMet
VCC996608:06	None	None	Top 10% cell count
VCC999628:01	None	None	None

Supplemental table 3: Enzyme activity measurement (% inhibition)

Vichem Compound-ID	EGFR Inh (%) 10µM	EGFR Inh (%) 1µM	EGFR Inh (%) 0.1µM	c-Met Inh (%) 10µM	c-Met Inh (%) 1µM	c-Met Inh (%) 0.1µM
VCC030450:22	101	99	97	4	2	0
VCC031393:01	11	2		16	0	
VCC034014:01	18	3		2	5	
VCC048363:02	101	100	97	1	-2	-1
VCC051013:02	16	0		41	6	
VCC055393:01	99	100	100	3	3	-4
VCC055876:02	-2	-9		22	-1	
VCC075648:03	66	13		41	6	
VCC098211:02	47	7		26	1	
VCC109756:01	43	7	2	93	61	7
VCC131028:02	30	-2		12	-4	
VCC132459:01	6	0		7	0	
VCC155409:01	76	34	10	25	2	-2
VCC180368:02	92	83		35	7	
VCC221701:01	22	4		22	1	
VCC227946:01	8					
VCC228833:01	16	1	0	4	0	-2
VCC232089:02	69					
VCC239215:01	17	4		16	4	
VCC260084:01	8	3		2	1	
VCC285946:01	32	0	9	84	49	8
VCC290183:01	16	4		27	3	
VCC317779:01	3	-1		82	18	
VCC323972:01	-2	-8		19	4	
VCC325112:01	59	16		44	5	
VCC346202:02	3	1		25	2	
VCC353749:01	5	1		26	1	
VCC365775:01	13	4		36	5	
VCC370686:02	-4	-8		2	-3	
VCC372550:01	-12	-9		2	-2	
VCC376189:01	12	2	-2	83	56	9
VCC378728:01	85	41	8	72	16	0
VCC395122:01	93	69	10	52	2	-3
VCC398520:05	98	55		87	30	
VCC407451:10	93	92	73	7	0	-4
VCC415997:02	-11	-11	-7	95	77	14
VCC429285:02	3	2	0	6	-2	2
VCC443915:01	23	3		27	1	
VCC444414:01	7	-4	-6	90	47	3
VCC444508:05	100	93		41	8	
VCC450892:17	32	10	5	85	39	4
VCC458127:03	95	62		3	0	
VCC461663:01	88	43		51	7	
VCC466812:01	102	84		67	13	
VCC475979:01	20	10		14	1	
VCC497510:01	20	10	2	90	54	9
VCC502987:01	19	9	1	4	1	0
VCC528301:01	94	82	29	37	7	1
VCC563740:02	15	10		25	5	
VCC581800:03	50			100		
VCC590177:01	94	67		56	3	
VCC613596:10	91	55		0	-1	
VCC617235:01	80	25		37	4	
VCC650454:03	39					
VCC656237:01	81	43		43	8	
VCC656576:02	-2	-7	-13	0	-4	-4
VCC685240:01	10	-6		4	1	
VCC692601:01	5	4		20	7	
VCC703724:02	9	9		7	4	
VCC716837:01	48	10	1	41	6	0
VCC733981:01	9	5		8	1	
VCC740005:11	105	102	93	11	1	3
VCC744093:03	77	25	3	27	-1	-1
VCC778672:01	101	85		56	9	
VCC804481:01	3	5		12	0	
VCC833029:24	97	90	37	-3	0	0
VCC868449:01	95	89	37	56	14	3
VCC883733:01	81	40		42	12	
VCC884444:01	2	-9		3	-2	
VCC898902:01	7	4		5	0	
VCC912492:07	87	47		2	-1	
VCC930986:01	92	49		0	-2	
VCC935482:01	-3	-2		-1	-3	
VCC957400:02	83	37		25	0	
VCC960450:01	-11	-7	-10	87	50	11
VCC967844:01	87	33		39	6	
VCC979277:01	12	5		17	3	
VCC996608:06	96	58		81	26	
VCC999628:01	26	0		14	1	