

## Table S1

### MK03 (ERK1)

HUMAN ILRGLKYIHSANVLHRDLKPSNLLINTTCDLKICDFGLAR  
BOVIN ILRGLKYIHSANVLHRDLKPSNLLINTTCDLKICDFGLAR  
MOUSE ILRGLKYIHSANVLHRDLKPSNLLINTTCDLKICDFGLAR  
RAT ILRGLKYIHSANVLHRDLKPSNLLINTTCDLKICDFGLAR  
MACMU ILRGLKYIHSANVLHRDLKPSNLLINTTCDLKICDFGLAR  
PIG ILRGLKYIHSANVLHRDLKPSNLLINTTCDLKICDFGLAR

### MK01 (ERK2)

HUMAN ILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLAR  
BOVIN ILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLAR  
MOUSE ILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLAR  
RAT ILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLAR  
MACMU ILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLAR  
PIG ILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLAR

\*These sequences were used to generate sequence logo shown in Figure 4B

Values used to generate dose-response curves shown in Figure 4D.  
Chemoproteomic analysis

ERK1		Ulixertinib (BVD-523)						
Concentration	log(concentration)	% Activity						
3 pM	-11.5	91	117					
300 pM	-9.5	91	99					
3 nM	-8.5	67	97					
10 nM	-8.0	63	60	63	60	63	63	58
50 nM	-7.3	25	16	25	16	24	20	
100 nM	-7.0	11	15	11	15	10	10	
500 nM	-6.3	5	2					
10 μM	-5.0	0	2					

Replicates per condition: 2-7

ERK2		Ulixertinib (BVD-523)						
Concentration	log(concentration)	% Activity						
3 pM	-11.5	103	110					
300 pM	-9.5	105	103					
3 nM	-8.5	85	91					
10 nM	-8.0	59	55	59	55	65	61	64
50 nM	-7.3	25	23	25	23	27	26	
100 nM	-7.0	5	10	5	10	18	12	
500 nM	-6.3	1	8					
10 μM	-5.0	5	6					

Replicates per condition: 2-7

ERK1		VX-11e		
Concentration	log (concentration)	% Activity		
1 nM	-9.0	94	97	
10 nM	-8.0	64	69	70
50 nM	-7.3	16	22	20
100 nM	-7.0	12	9	6
1 μM	-6.0	3	3	

Replicates per condition: 2-3

ERK2		VX-11e		
Concentration	log (concentration)	% Activity		
1 nM	-9.0	94	98	
10 nM	-8.0	68	69	63
50 nM	-7.3	16	31	24
100 nM	-7.0	14	23	15
1 μM	-6.0	8	10	

Replicates per condition: 2-3

Values used to generate dose-response curves shown in Figure 5.  
Cytotoxicity measurement (WST-1 assay)

DM122		Ulixertinib day4			
Concentration	log (concentration)	Viability (% of control)			
3 pM	-11.5	99	106	99	106
300 pM	-9.5	94	107	107	107
3 nM	-8.5	102	107	106	106
10 nM	-8.0	97	100	105	97
100 nM	-7.0	113	114	113	108
500 nM	-6.3	53	52	54	51
10 μM	-5.0	30	29	28	32

Biological replicates: 2

A549		Ulixertinib day4			
Concentration	log (concentration)	Viability (% of control)			
3 pM	-11.5	92	86	84	83
300 pM	-9.5	96	85	84	77
3 nM	-8.5	91	85	79	77
10 nM	-8.0	94	87	83	83
100 nM	-7.0	91	80	80	65
500 nM	-6.3	65	55	56	53
10 μM	-5.0	37	36	38	50

Biological replicates: 2

H82		Ulixertinib day4			
Conditions	Standardized absorbance value				
DMSO	1.0	1.1	0.9	1.0	
staurosporin (1μM)	0.4	0.4	0.5	0.5	
3 pM	1.0	0.9	1.0	1.2	
300 pM	0.9	1.0	1.1	1.1	
3 nM	0.8	0.9	1.1	1.1	
10 nM	0.9	0.9	1.0	1.1	
100 nM	0.9	1.1	1.1	1.1	
500 nM	1.0	1.1	1.1	1.1	
10 μM	0.7	0.7	0.7	0.3	

Biological replicates: 2

DM122		VX-11e day4			
Concentration	log (concentration)	Viability (% of control)			
1 nM	-9.0	91	90	89	100
10 nM	-8.0	93	90	93	98
50 nM	-7.3	82	85	82	90
100 nM	-7.0	77	77	79	85
1 μM	-6.0	52	56	54	57
10 μM	-4.3	39	36	34	41

Biological replicates: 2

A549		VX-11e day4			
Concentration	log (concentration)	Viability (% of control)			
1 nM	-9.0	86	87	94	110
10 nM	-8.0	87	86	92	106
50 nM	-7.3	81	82	85	105
100 nM	-7.0	77	82	81	103
1 μM	-6.0	50	49	51	61
10 μM	-4.3	23	23	21	21

Biological replicates: 2

H82		VX-11e day4			
Conditions	Standardized absorbance value				
DMSO	1.0	1.1	0.9	1.0	
staurosporin (1uM)	0.4	0.4	0.5	0.5	
1 nM	1.0	1.0	1.1	---	
10 nM	1.0	1.0	1.2	1.2	
50 nM	1.1	1.1	1.2	1.2	
100 nM	1.1	1.0	1.1	1.1	
1 μM	0.9	0.9	1.0	1.0	
10 μM	0.5	0.4	0.5	0.4	

Biological replicates: 2

\*technical replicates of each biological replicate are shown

Values used to generate bar graphs in Figure 6.  
Chemoproteomic analysis

<b>RSK</b>	<b>Ulixertinib (BVD-523)</b>				
<b>Concentration</b>	<b>log (concentration)</b>	<b>% Activity</b>			
<b>3 pM</b>	-11.5	104	108		
<b>300 pM</b>	-9.5	103	103		
<b>3 nM</b>	-8.5	101	108		
<b>10 nM</b>	-8.0	106	90	104	97
<b>50 nM</b>	-7.3	103	99	102	109
<b>100 nM</b>	-7.0	105	121	107	86
<b>500 nM</b>	-6.3	112			
<b>10 μM</b>	-5.0	88	87		
<b>ATP</b>	ATP (1mM)	1			

Replicates per condition: 1-4

<b>RSK</b>	<b>VX-11e</b>			
<b>Concentration</b>	<b>log (concentration)</b>	<b>% Activity</b>		
<b>1 nM</b>	-9.0	108		
<b>10 nM</b>	-8.0	118	108	
<b>50 nM</b>	-7.3	123	119	
<b>100 nM</b>	-7.0	121	114	
<b>1 μM</b>	-6.0	117		
<b>ATP</b>	ATP (1mM)	2		

Replicates per condition: 1-2

Values used to generate heat maps in Figure 7.  
Chemoproteomic analysis

MEK1/2		Ulixertinib (BVD-523)			
Concentration	log (concentration)	% Activity			
3 nM	-8.5	98	101	105	104
10 nM	-8.0	101	101	101	99
50 nM	-7.3	103	102	107	105
500 nM	-6.3	115	116		
10,000 nM	-5.0	113	116	110	109
ATP	ATP (1mM)	2	1	1	1

Replicates per condition: 2-4

MEK3		Ulixertinib (BVD-523)			
Concentration	log (concentration)	% Activity			
3 nM	-8.5	103	101		
10 nM	-8.0	97	87	115	
50 nM	-7.3	97	97	98	115
500 nM	-6.3	107			
10,000 nM	-5.0	102	101		
ATP	ATP (1mM)	0	0		

Replicates per condition: 1-4

MEK4		Ulixertinib (BVD-523)			
Concentration	log (concentration)	% Activity			
3 nM	-8.5	99	102		
10 nM	-8.0	98	94	98	93
50 nM	-7.3	96	96	92	
500 nM	-6.3	110			
10,000 nM	-5.0	106	99		
ATP	ATP (1mM)	0			

Replicates per condition: 1-4

MEK6		Ulixertinib (BVD-523)			
Concentration	log (concentration)	% Activity			
3 nM	-8.5	105	102		
10 nM	-8.0	84	99	84	91
50 nM	-7.3	95	105	95	98
500 nM	-6.3	109			
10,000 nM	-5.0	85	83		
ATP	ATP (1mM)	0	24		

Replicates per condition: 1-4

MEK1/2		VX-11e	
Concentration	log (concentration)		
1 nM	-9.0	103	
10 nM	-8.0	107	107
50 nM	-7.3	107	120
100 nM	-7.0	108	112
1,000 nM	-6.0	106	
ATP	ATP (1mM)	1	

Replicates per condition: 1-2

MEK3		VX-11e	
Concentration	log (concentration)	MP2K3	
1 nM	-9.0	98	
10 nM	-8.0	102	100
50 nM	-7.3	101	110
100 nM	-7.0	104	105
1,000 nM	-6.0	100	
ATP	ATP (1mM)	0	

Replicates per condition: 1-2

MEK4		VX-11e	
Concentration	log (concentration)	MP2K4	
1 nM	-9.0	102	
10 nM	-8.0	103	105
50 nM	-7.3	102	116
100 nM	-7.0	106	111
1,000 nM	-6.0	102	
ATP	ATP (1mM)	0	

Replicates per condition: 1-2

MEK6		VX-11e	
Concentration	log (concentration)	MP2K6	
1 nM	-9.0	92	
10 nM	-8.0	92	107
50 nM	-7.3	102	111
100 nM	-7.0	101	83
1,000 nM	-6.0	98	
ATP	ATP (1mM)	24	

Replicates per condition: 1-2