Supplementary Table 2

Structure	MEK1-ARAF with NST-628	MEK1-BRAF with NST-628	MEK1-CRAF with NST-628
PDB Entry	9AXM	9AXX	9AYA
Data collection			
Space group	$C222_1$	$P12_11$	C121
Cell dimensions			
a,b,c (Å)	80.209 173.429 187.769	72.23 82.40 121.57	149.725 58.202 162.347
α, β, γ (°)	90.000 90.000 90.000	90.00 105.69 90.00	90.00 95.59 90.00
Resolution (Å)	47.46-2.42 (2.50-2.42)	58.52-2.07 (2.11-2.07)	161.57-2.59 (2.69-2.59)
R_{merge}	0.107 (1.179)	0.068 (0.709)	0.075 (0.544)
I/σ(I)	17.7 (2.2)	17.6 (2.1)	13.0 (2.3)
Completeness (%)	100.0 (100.0)	99.2 (98.0)	99.9 (99.9)
Redundancy	13.6 (14.0)	7.1 (7.2)	4.5 (3.7)
Refinement			
R_{work}/R_{free}	0.2604/0.3006	0.2130/0.2480	0.2120/0.2566
R.m.s deviations			
Bond lengths (Å)	0.002	0.003	0.004
Bond angles (°)	0.442	0.557	0.565
Ramachandran statistics			
Favored (%)	96.13	97.58	97.09
Allowed (%)	3.87	2.24	2.73
Outliers (%)	0.00	0.18	0.18

Supplementary Table 2. Data collection and refinement statistics for crystal structures of NST-628 with MEK1 and active RAF. Values in parentheses represent the highest resolution shell.

Supplementary Table 3

Structure	**MEK1-KSR1 with NST-628**
PDB Entry	9AXH
Data collection	
Space group	C121
Cell dimensions	
a,b,c (Å)	249.87 67.08 74.40
α, β, γ (°)	90.00 90.25 90.00
Resolution (Å)	52.24-2.81 (2.96-2.81)
R_{merge}	0.051 (0.670)
I/σ(I)	18.0 (2.7)
Completeness (%)	99.9% (99.8%)
Redundancy	6.9 (7.1)
Refinement	
R_{work}/R_{free}	0.2031/0.2533
R.m.s deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.627
Ramachandran statistics	
Favored (%)	96.88
Allowed (%)	3.12
Outliers (%)	0.00

Supplementary Table 3. Data collection and refinement statistics for crystal structure of MEK1-KSR1 complex with NST-628. Values in parentheses represent the highest resolution shell.

Supplementary Table 4

Structure	MEK1-BRAF with NST-628	MEK1-CRAF with NST-628
PDB Entry	9AXY	9AY7
Data collection		
Space group	$P3_121$	$P2_12_12_1$
Cell dimensions		
a,b,c (Å)	117.097 117.097 129.424	60.030 107.950 116.240
α, β, γ (°)	90.00 90.00 120.00	90.00 90.00 90.00
Resolution (Å)	53.34-3.60(3.94-3.60)	48.95-2.41 (2.50-2.41)
R_{merge}	0.257 (0.918)	0.112 (1.148)
I/σ(I)	8.5 (2.4)	15.8 (2.2)
Completeness (%)	99.5% (98.2%)	100.0% (99.8%)
Redundancy	12.6 (8.7)	13.3 (13.8)
Refinement		
R_{work}/R_{free}	0.1879/0.2375	0.2012/0.2359
R.m.s deviations		
Bond lengths (Å)	0.003	0.002
Bond angles (°)	0.650	0.436
Ramachandran statistics		
Favored (%)	95.35	96.67
Allowed (%)	4.48	3.33
Outliers (%)	0.17	0.00

Supplementary Table 4. Data collection and refinement statistics for crystal structures of NST-628 with MEK1 and inactive RAF. Values in parentheses represent the highest resolution shell.

Supplemental Table 5

Structure	MEK1/CRAF/14-3-3/NST-628 (EMDB-43931, PDB 9AXA)	MEK1/CRAF/NST-628 focused (EMDB-43932, PDB 9AXC)
Data collection and processing		
Magnification	120,000x	120,000x
Voltage (kV)	200	200
Electron exposure ($e^-/Å^2$)	50	50
Defocus range (μm)	1 to 2.5	1 to 2.5
Pixel size (Å)	1.207	1.207
Symmetry	C1	C1
Initial particle images (no.)	1,270,842	1,270,842
Final particle images (no.)	83,248	83,248
Map resolution at FSC=0.143 (Å)	4.36	4.16
Refinement		
Initial model used	4IEA, MEK/CRAF active	MEK/CRAF active
Model resolution at FSC=0.143 (Å)	3.78	3.67
Model composition		
Non-hydrogen atoms	12423	8677
Protein residues	1553	1079
R.m.s deviations		
Bond lengths (Å)	0.004	0.004
Bond angles (°)	0.952	0.960
Validation		
MolProbity score	1.68	1.63
Clashscore	6.73	5.98
Poor rotamers (%)	0.44	0.21
Ramachandran plot		
Favored (%)	95.58	95.64
Allowed (%)	4.29	4.17
Disallowed (%)	0.13	0.19

Supplementary Table 5. Cryo-EM data collection, processing, and model statistics