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Supplementary Information for

Activating KRAS, NRAS, and BRAF Mutants Enhance Proteasome Capacity and Reduce Endoplasmic Reticulum Stress in Multiple Myeloma

Fazal Shirazi, Richard J. Jones, Ram K. Singh, Jianxuan Zou, Isere Kuiatse, Zuzana Berkova, Hua Wang, Hans C. Lee, Samuel Hong, Larry Dick, Nibedita Chattopadhyay, and Robert Z. Orlowski

Corresponding author
Robert Z. Orlowski
Email: rorlowski@mdanderson.org

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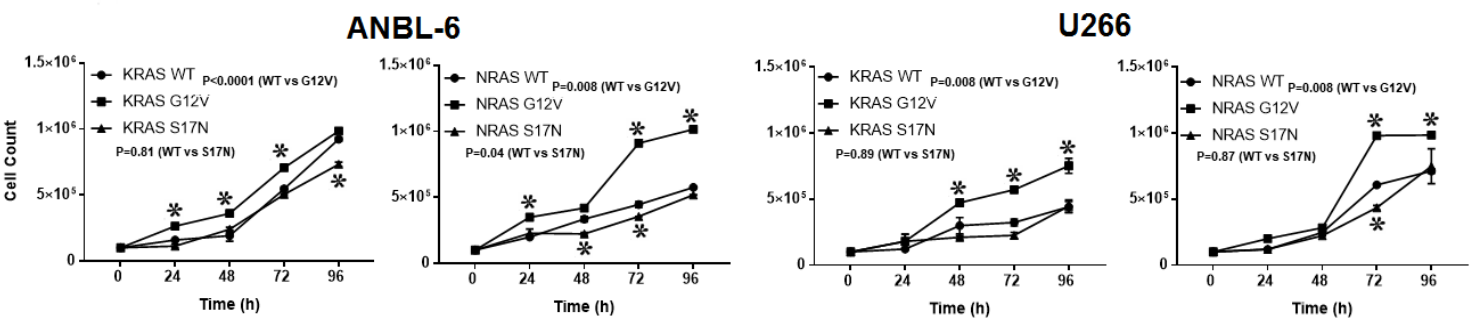
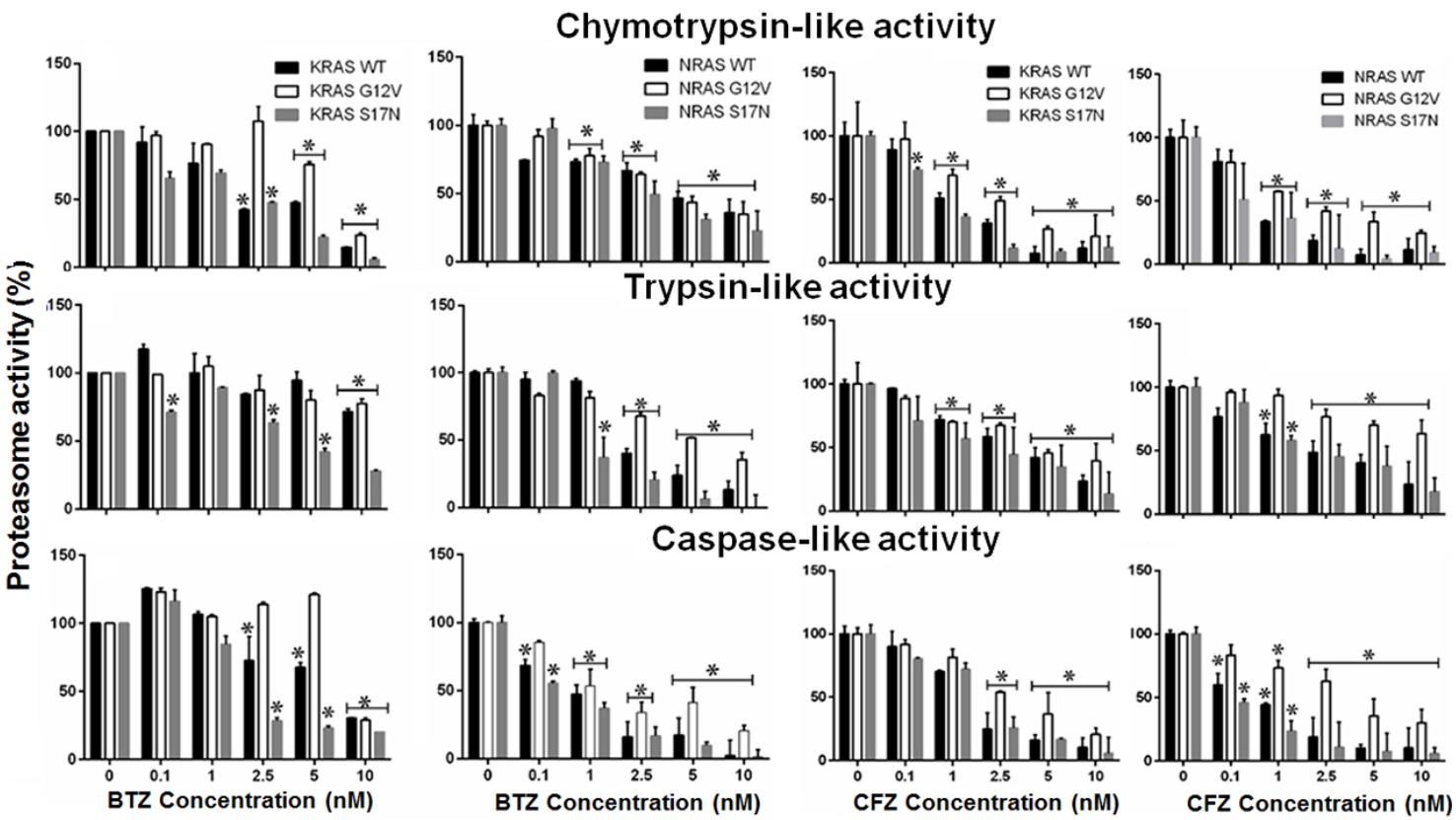
www.pnas.org/cgi/doi/10.1073/pnas.2005052117



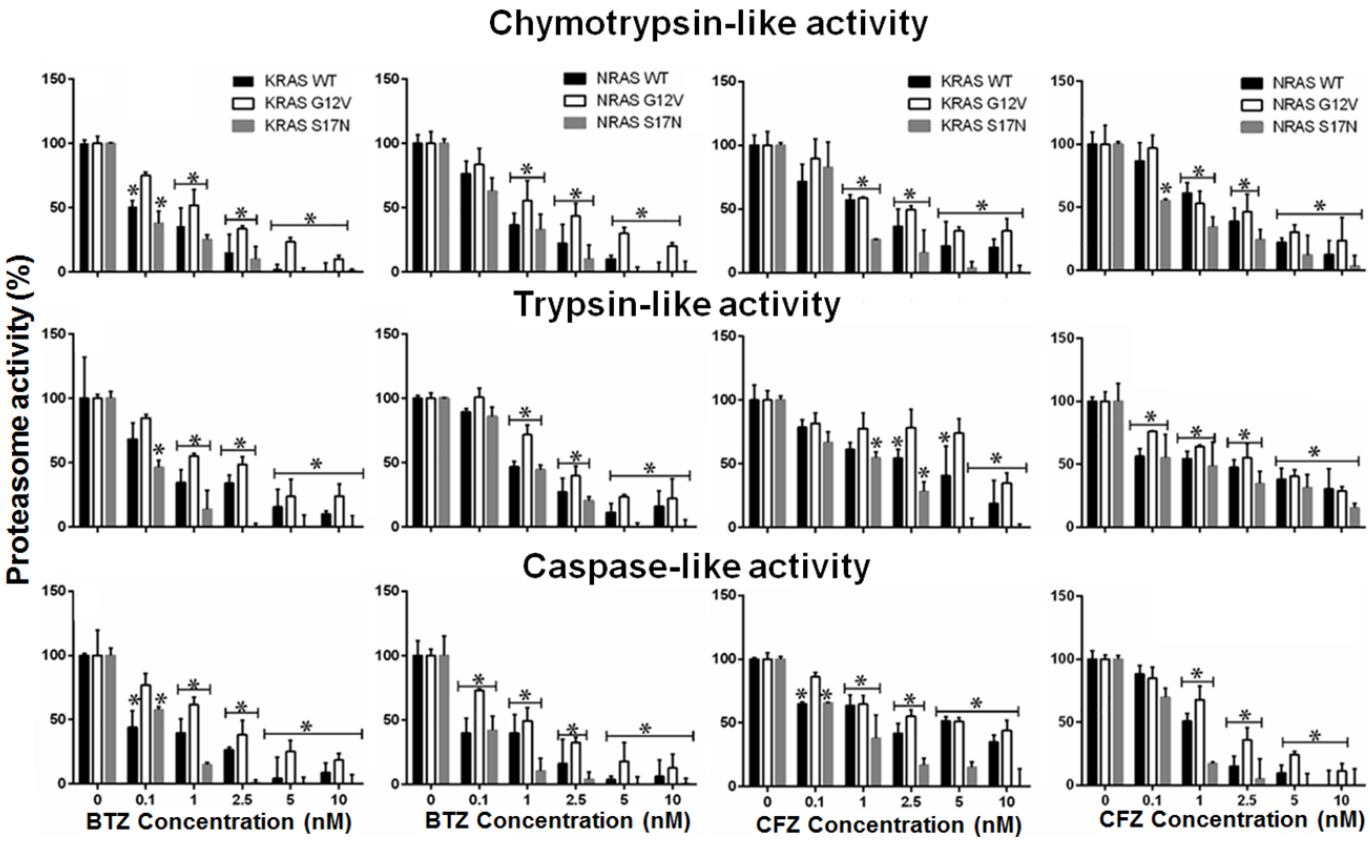
Supplementary Figure S1. ***KRAS* and *NRAS* mutants modulate myeloma cell proliferation**.

ANBL-6 and U266 cells expressing WT *RAS* or the indicated CA and DN mutants were monitored using the WST-1 assay, and data are presented as mean activity ± SD relative to vehicle from triplicate experiments, and asterisks indicate $p \leq 0.05$ in comparison to WT cells.



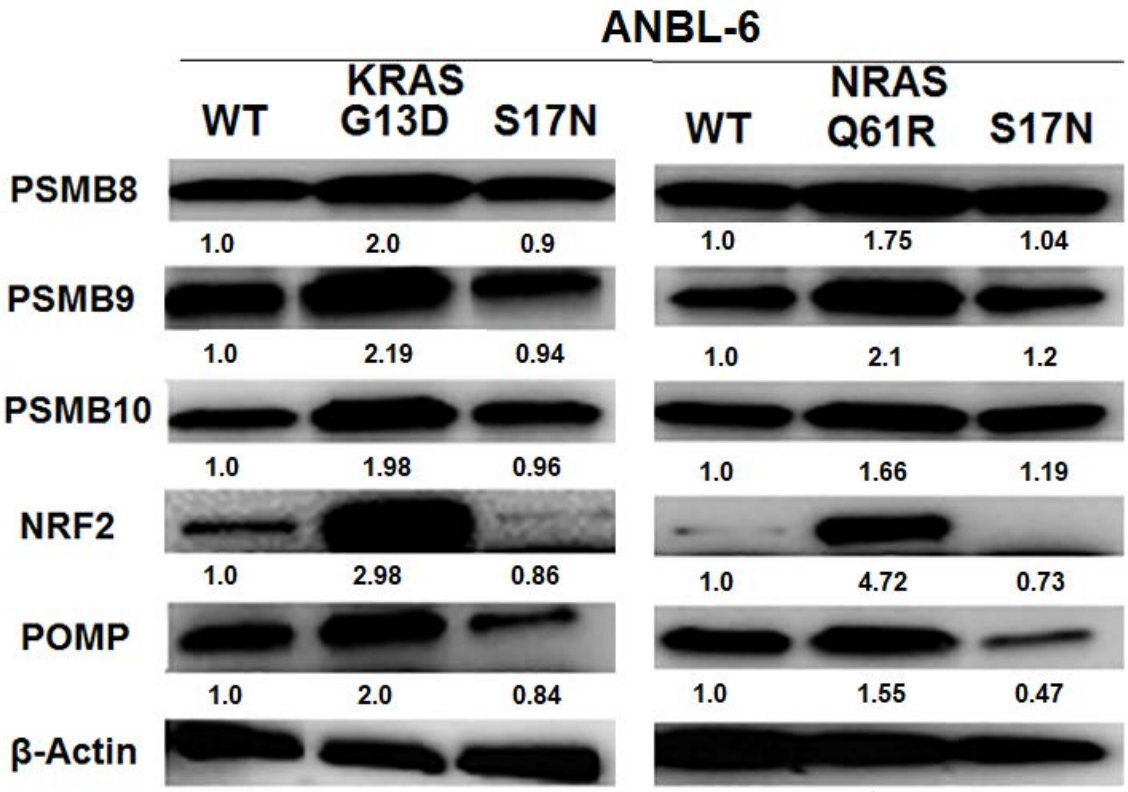
Supplementary Figure S2. *KRAS* and *NRAS* mutants modulate proteasome activity in ANBL-6 cells treated with bortezomib and carfilzomib.

ANBL-6 cells expressing WT *RAS* or the indicated CA and DN mutants were exposed to vehicle or a range of concentrations of bortezomib (BTZ) or carfilzomib (CFZ) as indicated. The chymotrypsin-, trypsin- and caspase-like proteasome activities were then measured in lysates using proteasome activity-specific fluorogenic substrates. Data are presented as mean activity ± SD relative to vehicle from triplicate experiments, and asterisks indicate $p \leq 0.05$ in comparison to WT cells.



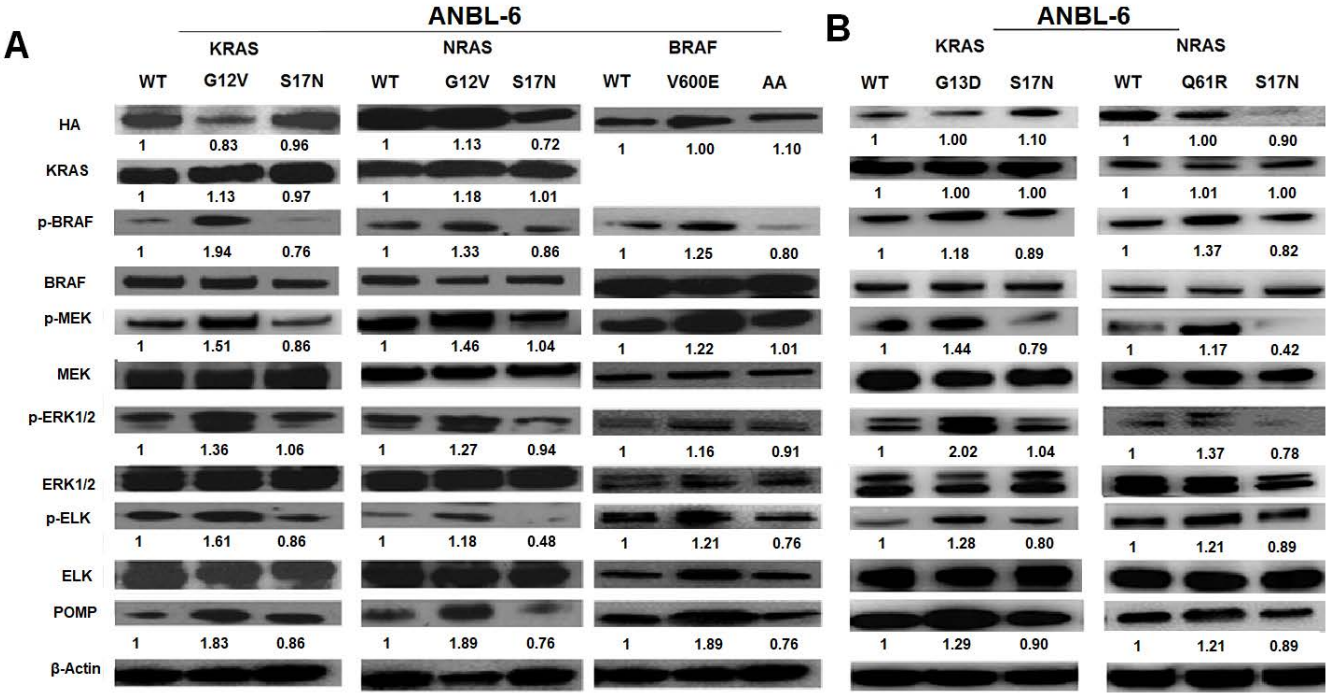
Supplementary Figure S3. *KRAS* and *NRAS* mutants modulate proteasome activity in U266 cells treated with bortezomib and carfilzomib.

U266 cells expressing WT *RAS* or the indicated CA and DN mutants were exposed to vehicle or a range of concentrations of bortezomib (BTZ) or carfilzomib (CFZ) as indicated. The chymotrypsin-, trypsin- and caspase-like proteasome activities were then measured in lysates using proteasome activity-specific fluorogenic substrates. Data are presented as mean activity ± SD relative to vehicle from triplicate experiments, and asterisks indicate $p \leq 0.05$ in comparison to WT cells.



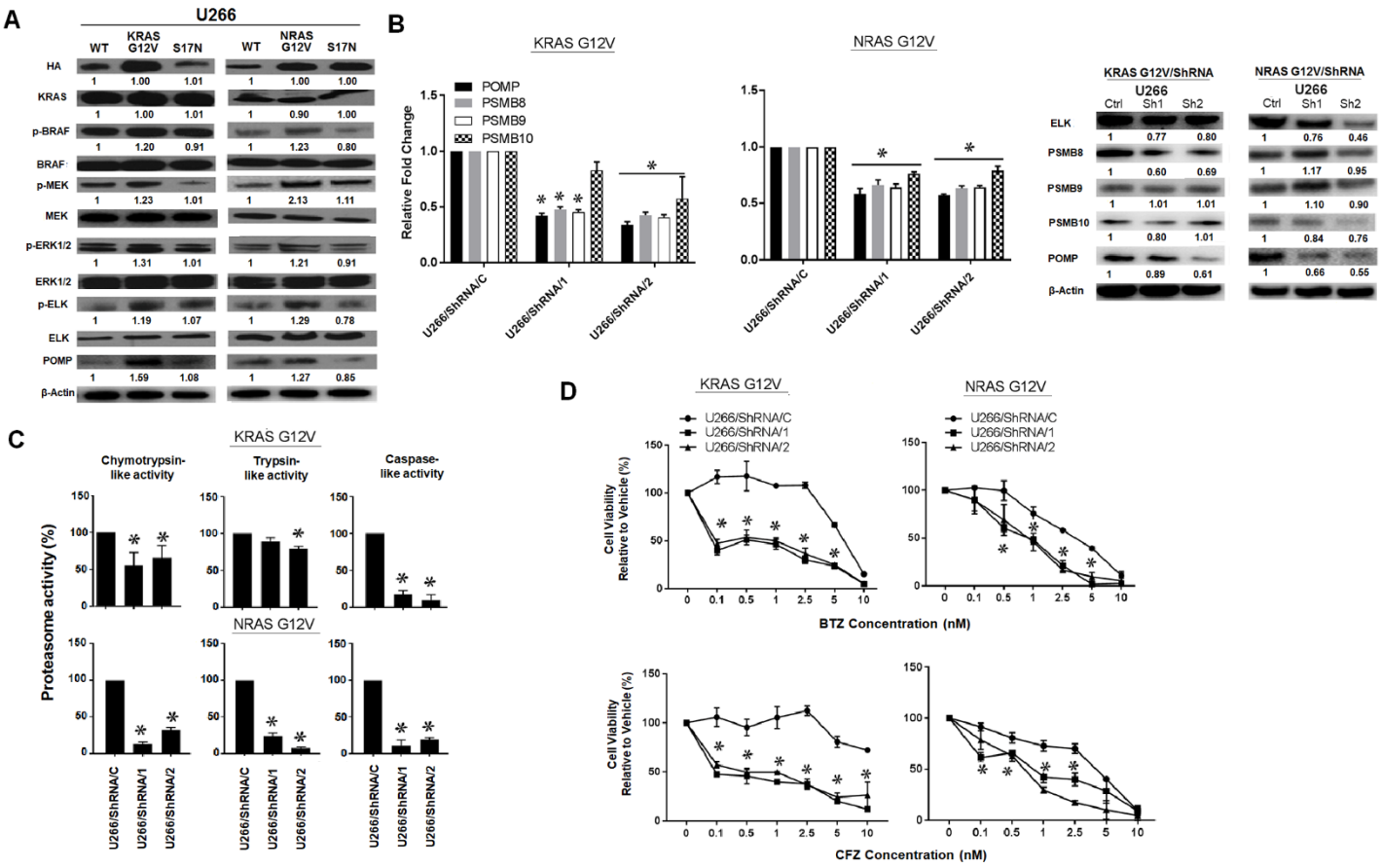
Supplementary Figure S4. Impact of additional *KRAS* and *NRAS* mutants on proteasome activity-related proteins in ANBL-6 cells.

Protein extracts from ANBL-6 cells expressing the indicated *RAS* mutants were analyzed for the levels of POMP, NRF2, PSMB8, PSMB9, and PSMB10 proteins by immunoblotting with β-Actin as a loading control. Densitometry of the Western blots was performed using Image J software, and data are shown which were generated first by normalizing all bands to β-Actin. Then, the G13D and Q61R mutants were evaluated in relationship to the WT controls, which were arbitrarily set to 1.0, and a representative set of blots is shown from one of two independent experiments.



Supplementary Figure S5. Constitutively active ANBL-6 *RAS* mutants activate downstream signaling intermediates.

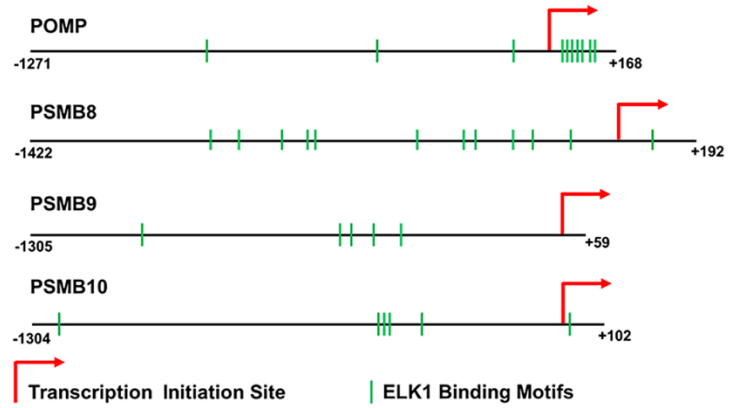
ANBL-6 cells expressing WT, G12V, or S17N RAS mutants, or WT, V600E, or AA *BRAF* mutants were analyzed by Western blotting (**A**) for the activation status of MAPK pathway intermediates. A similar approach was taken to compare ANBL-6 cells expressing either the CA *KRAS* G13D or *NRAS* Q61R (**B**) mutants to their DN or WT controls. Densitometry was performed and is represented as detailed above.



Supplementary Figure S6. Constitutively active *RAS* mutants activate downstream signaling and proteasome capacity in an ELK1-dependent fashion.

U266 cells expressing WT, G12V, or S17N *RAS* mutants were analyzed by Western blotting (**A**) for the activation status of MAPK pathway intermediates. Knockdown of *ELK1* by either of two different shRNAs (**B**) produced a reduction of mRNAs for *POMP*, *PSMB8*, *PSMB9*, and *PSMB10* in U266 cells, and also in the abundance of their respective proteins. *ELK1* suppression produced a reduction (**C**) in the proteasome chymotrypsin-, trypsin- and caspase like activities in both cells. This reduction was associated with enhanced sensitivity (**D**) to both bortezomib (upper panel) and carfilzomib (lower panel).

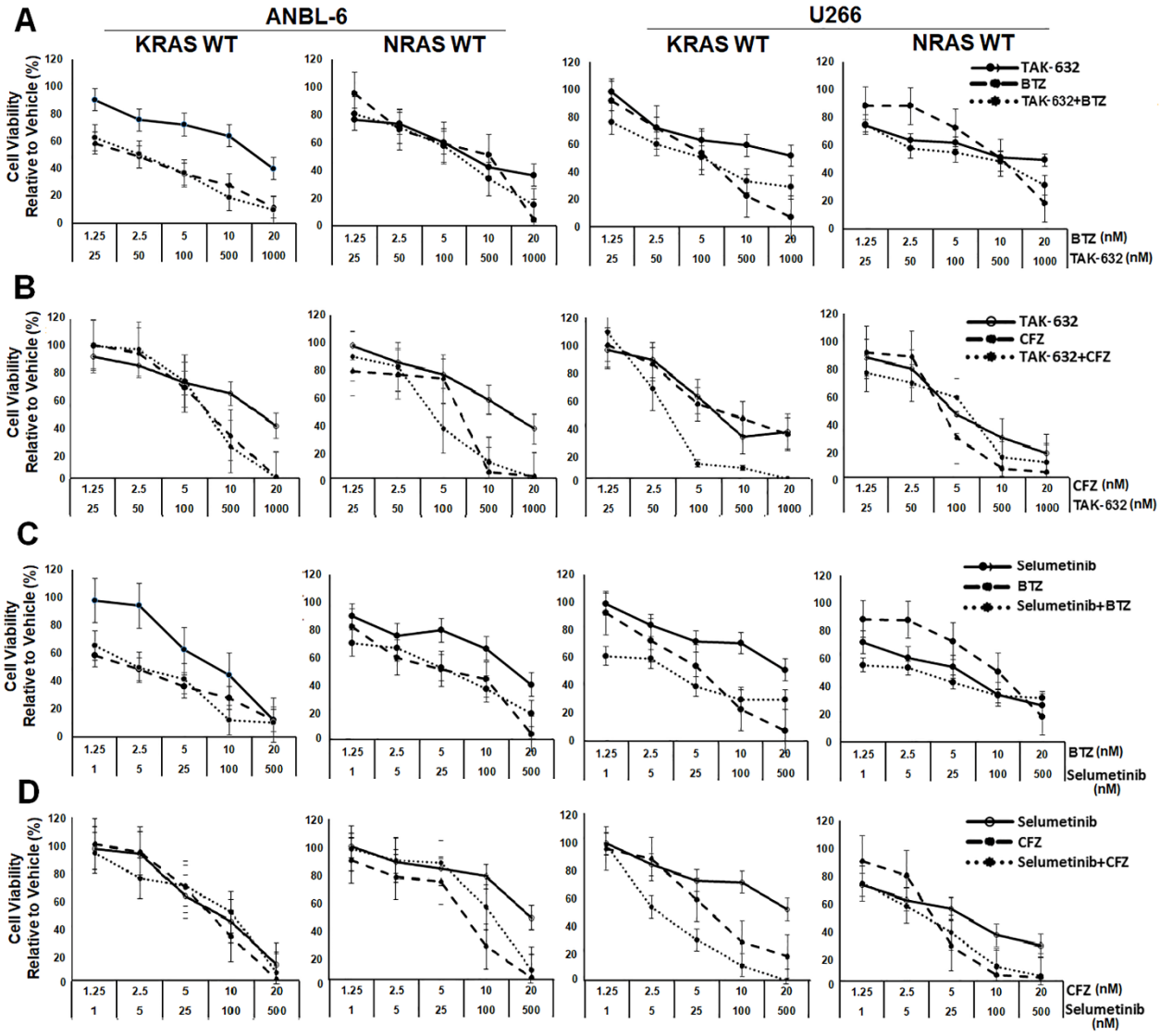
A



B

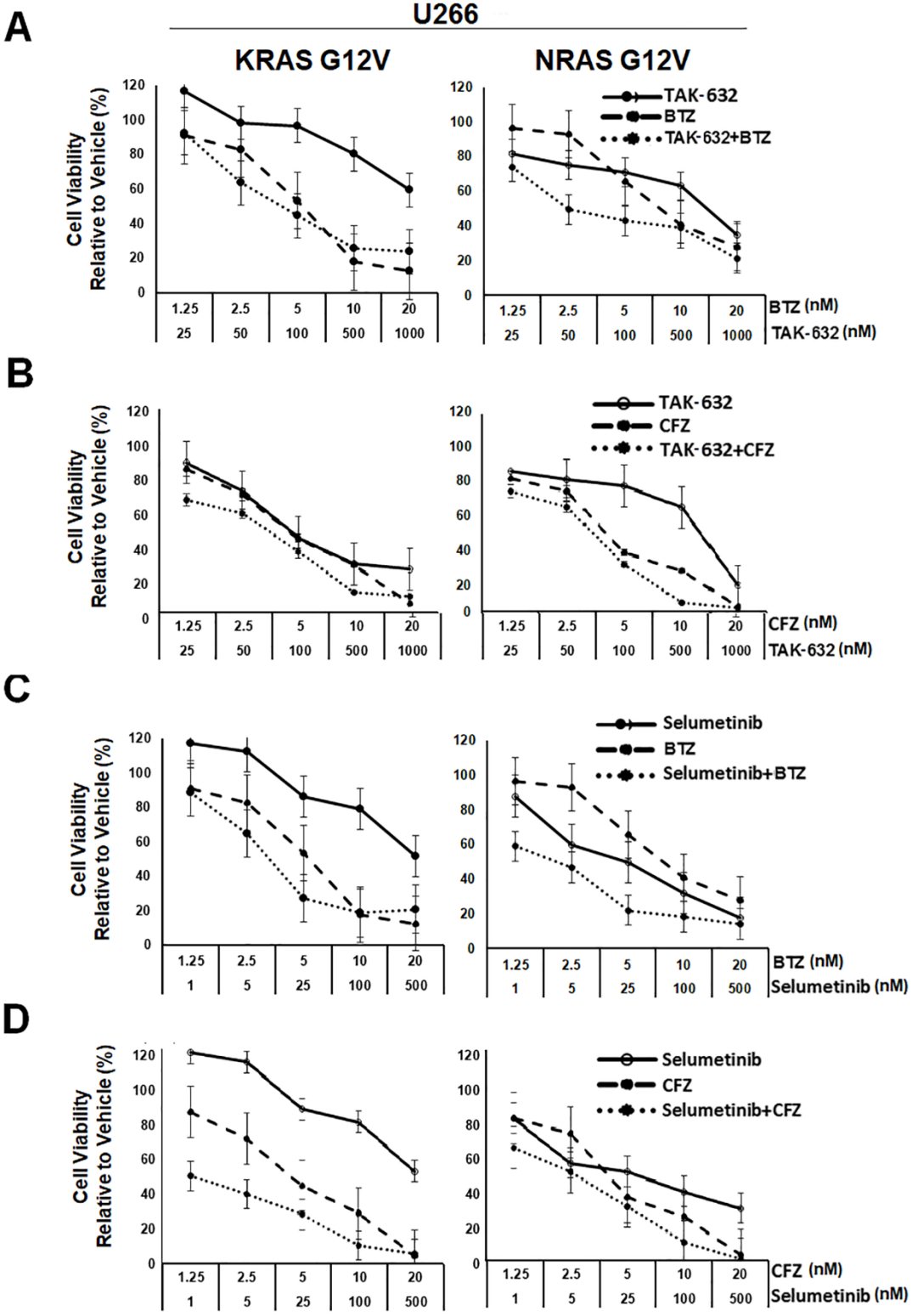
Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0028.1	ELK1	8.2943	0.887876781	PSMB10	1319	1328	+	AAGCAGGAAG
MA0028.2	ELK1	8.3839	0.87441739	PSMB10	1321	1330	+	GCAGGAAGTA
MA0028.1	ELK1	6.5733	0.830684261	PSMB10	64	73	+	AAACCGTAAA
MA0028.1	ELK1	6.5374	0.829493154	PSMB10	846	855	+	GAGAGGGAAG
MA0028.1	ELK1	6.5374	0.829493154	PSMB10	865	874	+	GAGATGGAAG
MA0028.1	ELK1	5.996	0.811499981	PSMB10	872	881	+	AAGATGGAAA
MA0028.1	ELK1	5.9888	0.811262815	PSMB10	959	968	+	GAGTTGGAAG
MA0028.1	ELK1	8.7894	0.904329667	PSMB9	839	848	+	GGGCCTGAAG
MA0028.1	ELK1	7.1761	0.850717819	PSMB9	907	916	+	CTCTCGGAAA
MA0028.1	ELK1	6.6551	0.833405814	PSMB9	270	279	-	GCTCTGGAAA
MA0028.1	ELK1	6.3046	0.821757428	PSMB9	757	766	+	GAGCGGGACA
MA0028.1	ELK1	6.0824	0.814371199	PSMB9	785	794	+	CACTCGGACG
MA0028.2	ELK1	15.551	0.974598812	POMP	1352	1361	-	GCCGGAAGTG
MA0028.1	ELK1	10.596	0.964347975	POMP	1354	1363	-	CCGCCGGAAG
MA0028.1	ELK1	9.8505	0.939590556	POMP	1383	1392	+	GAAACGGAAG
MA0028.2	ELK1	12.197	0.927715752	POMP	1385	1394	+	AACGGAAGTG
MA0028.1	ELK1	7.9911	0.877801197	POMP	424	433	-	GAACTGGAAA
MA0028.2	ELK1	7.9507	0.86836191	POMP	428	437	-	AACGGAACTG
MA0028.1	ELK1	7.1761	0.850717819	POMP	1377	1386	+	CCCTCGGAAA
MA0028.1	ELK1	7.0912	0.847894757	POMP	1342	1351	+	AGGACGGACG
MA0028.2	ELK1	6.2806	0.845016496	POMP	1359	1368	+	GGCGGATGTG
MA0028.1	ELK1	6.5187	0.828871439	POMP	1307	1316	-	ACGCCGGAGG
MA0028.1	ELK1	5.9811	0.811005778	POMP	1328	1337	-	AGGAAGGAAG
MA0028.1	ELK1	5.9087	0.808598703	POMP	1184	1193	-	GGGAGGGAAG
MA0028.1	ELK1	5.8724	0.807395585	POMP	430	439	-	AAAACGGAAC
MA0028.1	ELK1	5.7732	0.804098571	POMP	1314	1323	-	GCGCGGGACG
MA0028.1	ELK1	5.6781	0.800937611	POMP	848	857	-	ACTCCGGAGG
MA0028.1	ELK1	10.942	0.975869958	PSMB8	1165	1174	-	GTGCCGGAAA
MA0028.2	ELK1	8.423	0.874963732	PSMB8	1163	1172	-	GCCGGAAACA
MA0028.1	ELK1	7.3282	0.855771908	PSMB8	1046	1055	+	TGTACGGAAA
MA0028.1	ELK1	6.6611	0.833604443	PSMB8	689	698	-	CTCCCGGATA
MA0028.1	ELK1	6.5766	0.830794659	PSMB8	937	946	-	CTCCAGGAAG
MA0028.1	ELK1	6.5766	0.830794659	PSMB8	1505	1514	-	AGCCAGGAAG
MA0028.2	ELK1	5.1841	0.829688478	PSMB8	935	944	-	CCAGGAAGTC
MA0028.1	ELK1	6.5275	0.82916264	PSMB8	1076	1085	+	ATAACTGAAG
MA0028.1	ELK1	6.4068	0.825151989	PSMB8	501	510	+	GCCCTGGAAA
MA0028.1	ELK1	6.1308	0.815980479	PSMB8	689	698	+	TATCCGGGAG
MA0028.2	ELK1	3.8255	0.810697384	PSMB8	1307	1316	-	AGGGGAAGTG
MA0028.2	ELK1	3.7434	0.809549183	PSMB8	1214	1223	+	TGCGGATGTC
MA0028.2	ELK1	3.4842	0.805925973	PSMB8	606	615	-	AGCGGGAGTA
MA0028.1	ELK1	5.7581	0.803594306	PSMB8	433	442	+	GAATGGGAAA
MA0028.1	ELK1	5.6636	0.800453755	PSMB8	672	681	+	TTTCTGGAAG

Supplementary Figure S7. **ELK1 binding sites in key promoter regions**. Putative ELK1 binding sites in the promoters of *POMP*, *PSMB8*, *PSMB9*, and *PSMB10* are shown (**A**). Their locations were identified through an analysis utilizing JASPAR, which also assigned relative binding scores of ELK1 for these sites (**B**).



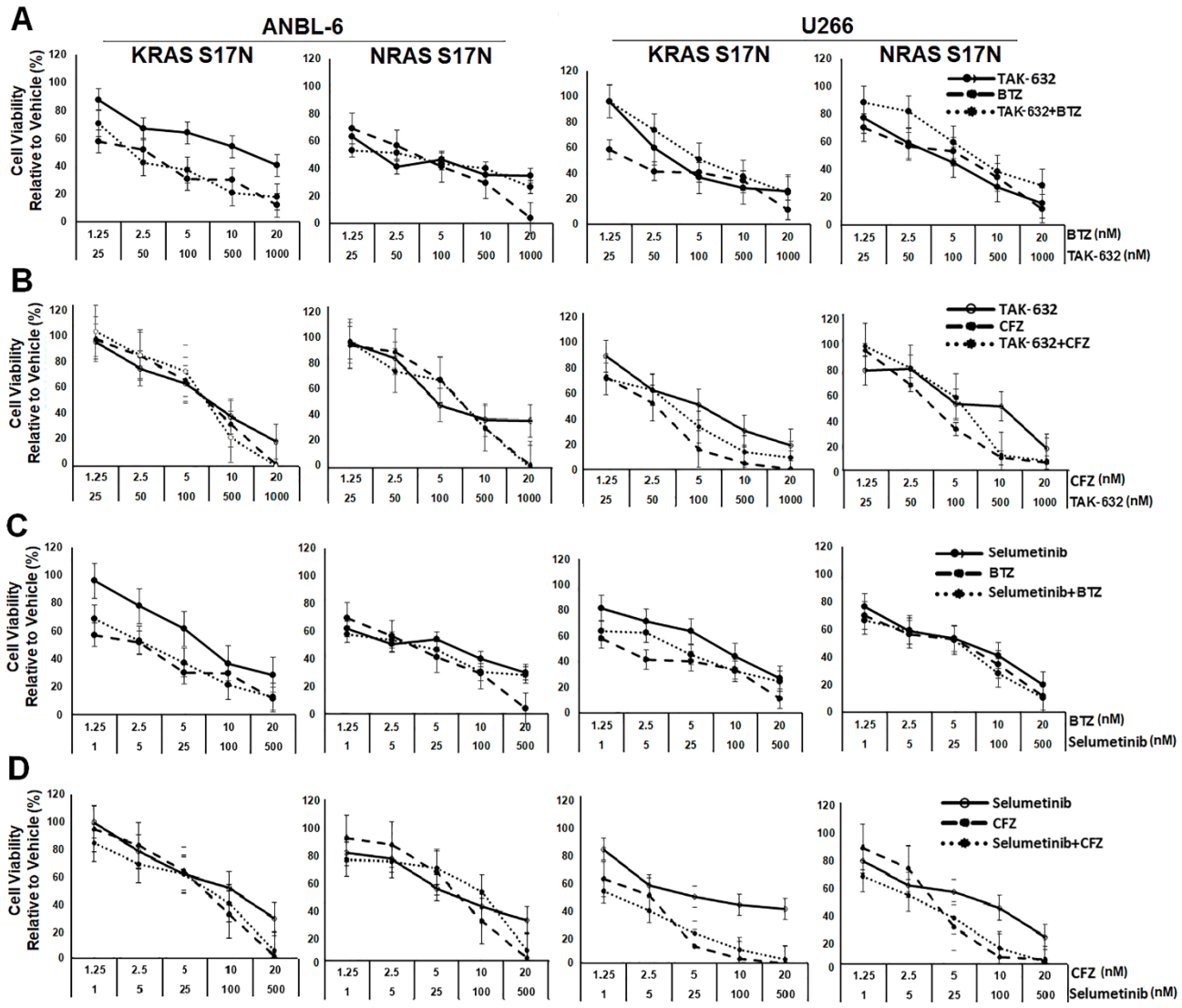
Supplementary Figure S8. **Bortezomib with TAK-632 or selumetinib, and carfilzomib with TAK-632 or selumetinib, in myeloma cells over-expressing WT *RAS*.**

ANBL-6 (left two columns) and U266 cells (right two columns) expressing WT RAS were incubated with the pan-RAF inhibitor TAK 632, bortezomib (BTZ), or the combination (**A**), and cellular viability was determined after 72 hour incubation. Data were collected from triplicate experiments and are plotted as the mean viability ± SD, while combination indices are provided in Supplementary Tables S3 and S4. Carfilzomib was also combined with TAK-632 in these same cell lines (**B**), and the data were collected, analyzed, and presented as detailed earlier. Next, either bortezomib (**C**) or carfilzomib (**D**) were added to selumetinib, and the data were collected, analyzed, and presented as detailed earlier.


A
U266
KRAS G12V
NRAS G12V
Cell Viability
Relative to Vehicle (%)
TAK-632
BTZ
TAK-632+BTZ
BTZ (nM)
TAK-632 (nM)
B
TAK-632
CFZ
TAK-632+CFZ
CFZ (nM)
TAK-632 (nM)
C
Selumetinib
BTZ
Selumetinib+BTZ
BTZ (nM)
Selumetinib (nM)
D
Selumetinib
CFZ
Selumetinib+CFZ
CFZ (nM)
Selumetinib (nM)

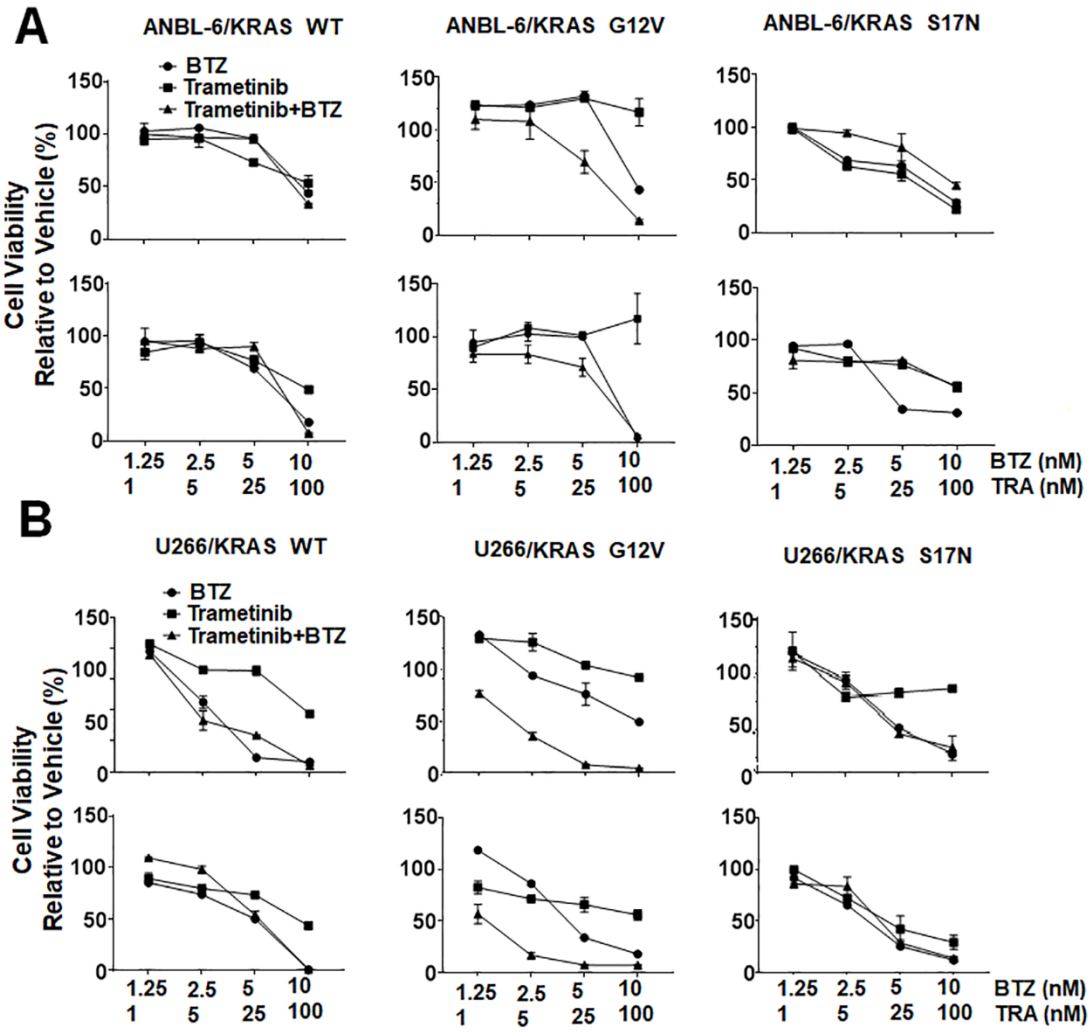
Supplementary Figure S9. **TAK-632 with bortezomib or carfilzomib, or selumetinib with bortezomib or carfilzomib, in U266 cells over-expressing CA *RAS***.

U266 cells expressing CA G12V *KRAS* (left column) or G12V *NRAS* (right column) mutants were incubated with the pan-RAF inhibitor TAK-632, bortezomib (BTZ), or the combination (**A**), and cellular viability was determined after 72-hour incubations. Data were collected from triplicate experiments and are plotted as the mean viability ± SD, while combination indices are provided in Supplementary Tables S3 and S4. Carfilzomib was also combined with TAK-632 in this cell line (**B**), and the data were collected, analyzed, and presented as detailed earlier. Next, either bortezomib (**C**) or carfilzomib (**D**) were added to selumetinib, and the data were collected, analyzed, and presented as detailed earlier.



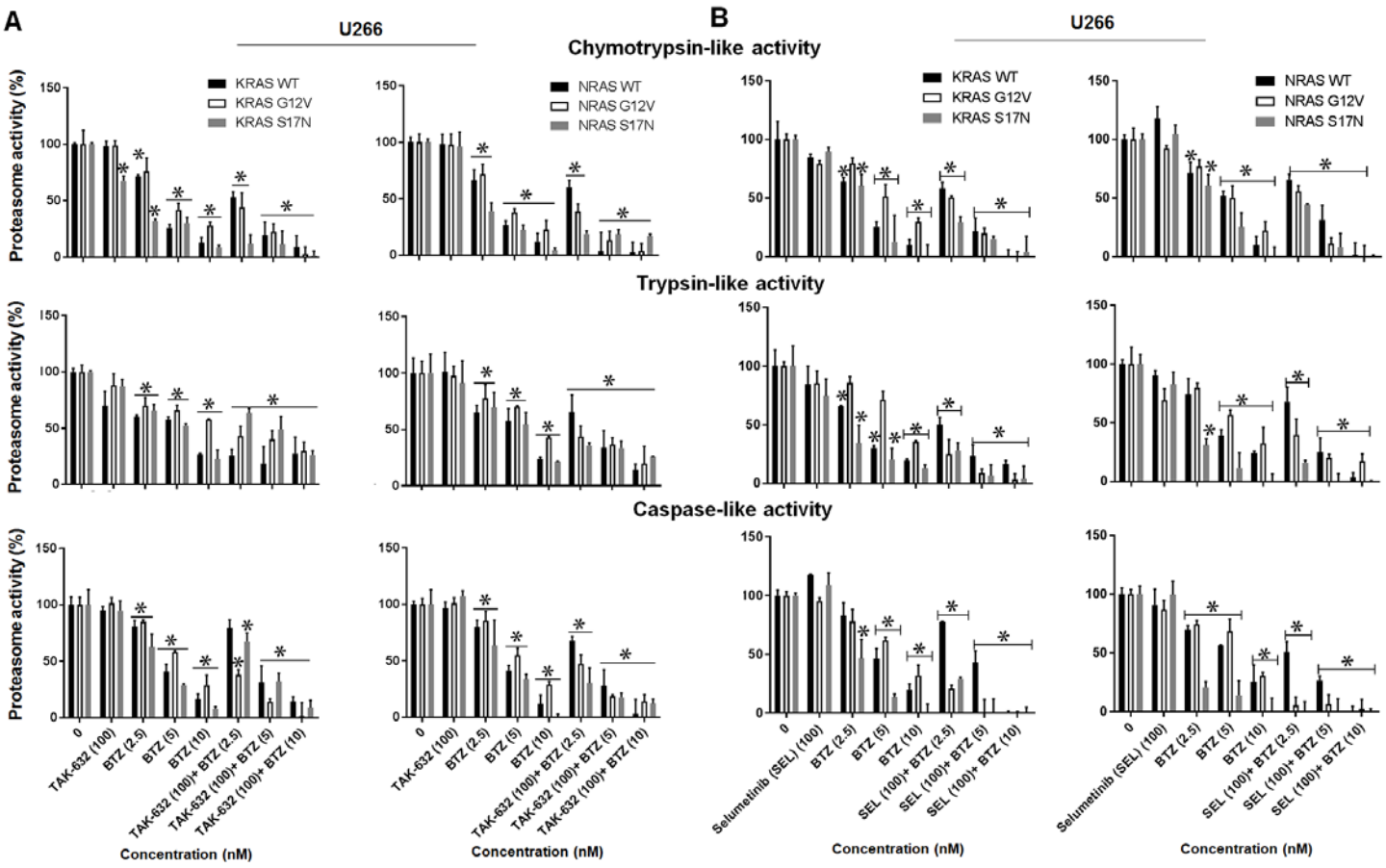
Supplementary Figure S10. TAK-632 with bortezomib or carfilzomib, or selumetinib with bortezomib or carfilzomib, in myeloma cells over-expressing DN *RAS*.

ANBL-6 (left two columns) and U266 cells (right two columns) expressing DN *RAS* variants as indicated were incubated with the pan-RAF inhibitor TAK-632, bortezomib (BTZ), or the combination (**A**), and cellular viability was determined after 72 hour incubations. Data were collected from triplicate experiments and are plotted as the mean viability ± SD, while combination indices are provided in Supplementary Tables S3 and S4. Carfilzomib was also combined with TAK-632 in these same cell lines (**B**), and the data were collected, analyzed, and presented as detailed earlier. Next, either bortezomib (**C**) or carfilzomib (**D**) were added to selumetinib, and the data were collected, analyzed, and presented as detailed earlier.



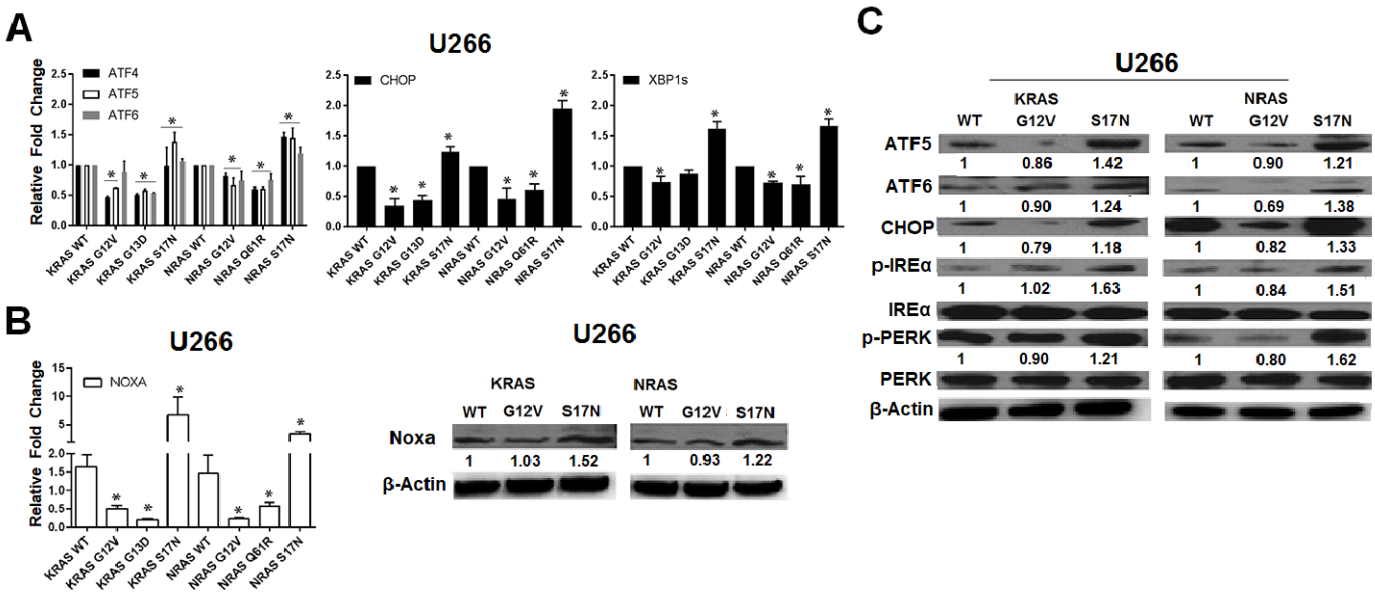
Supplementary Figure S11. **Bortezomib with trametinib in myeloma cells overexpressing WT, CA and DN *RAS***.

ANBL-6 (**A**) and U266 cells (**B**) expressing WT, CA G12V and DN S17N *KRAS* mutants were incubated with the MEK inhibitor trametinib (TRA), bortezomib (BTZ), or the combination, and cellular viability was determined after 72-hour incubations. Data were collected from triplicate experiments and are plotted as the mean viability ± SD, while combination indices are provided in Supplementary Table S5.



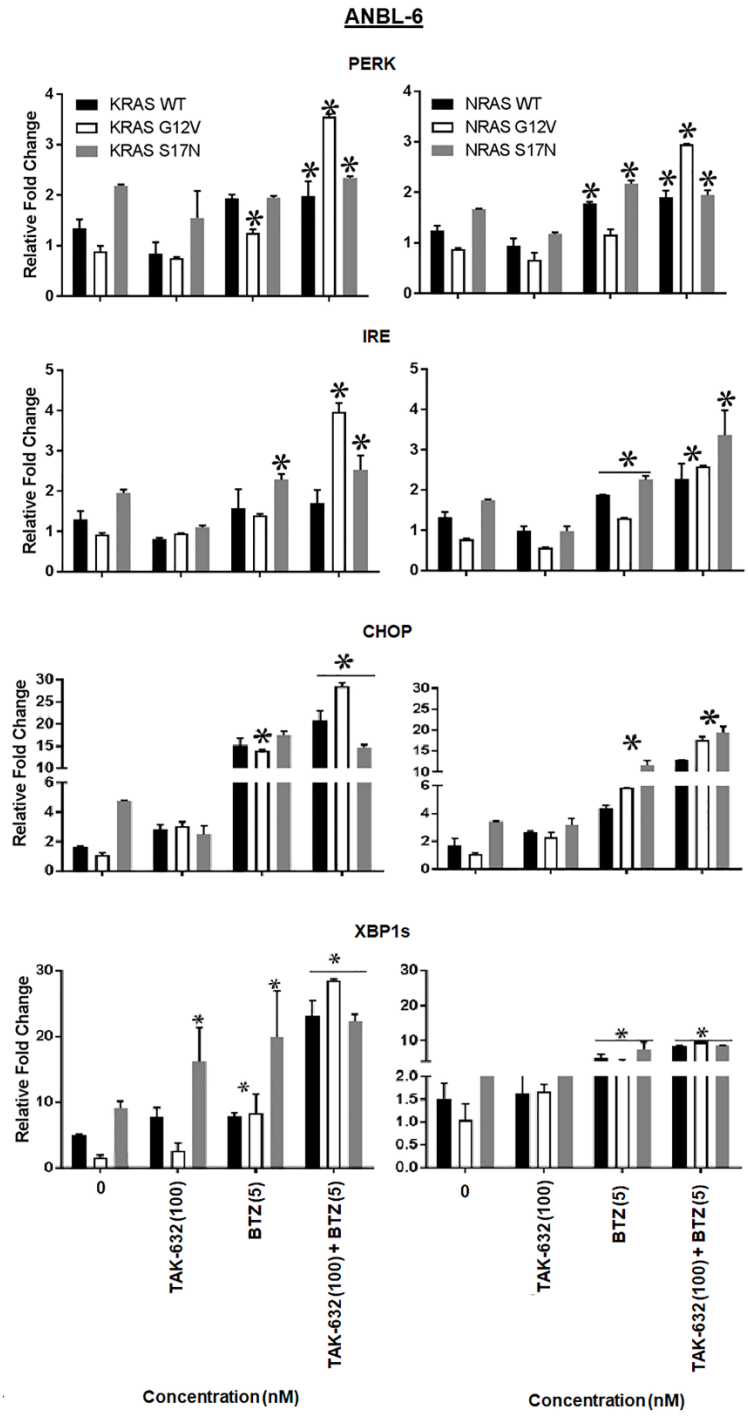
Supplementary Figure S12. Bortezomib with TAK-632 or selumetinib and proteasome inhibition in myeloma cells.

The chymotrypsin-, trypsin- and caspase-like proteasome activities were measured in lysates from U266 (**A**) expressing the indicated *RAS* mutants that had been treated with the indicated concentrations of TAK-632, bortezomib (BTZ), or both. These activities were then measured in U266 (**B**) with the indicated concentrations of selumetinib (SEL), BTZ, or both. Incubations were for 24-hours in all of the panels, and data are presented as the mean ± SD of triplicates.



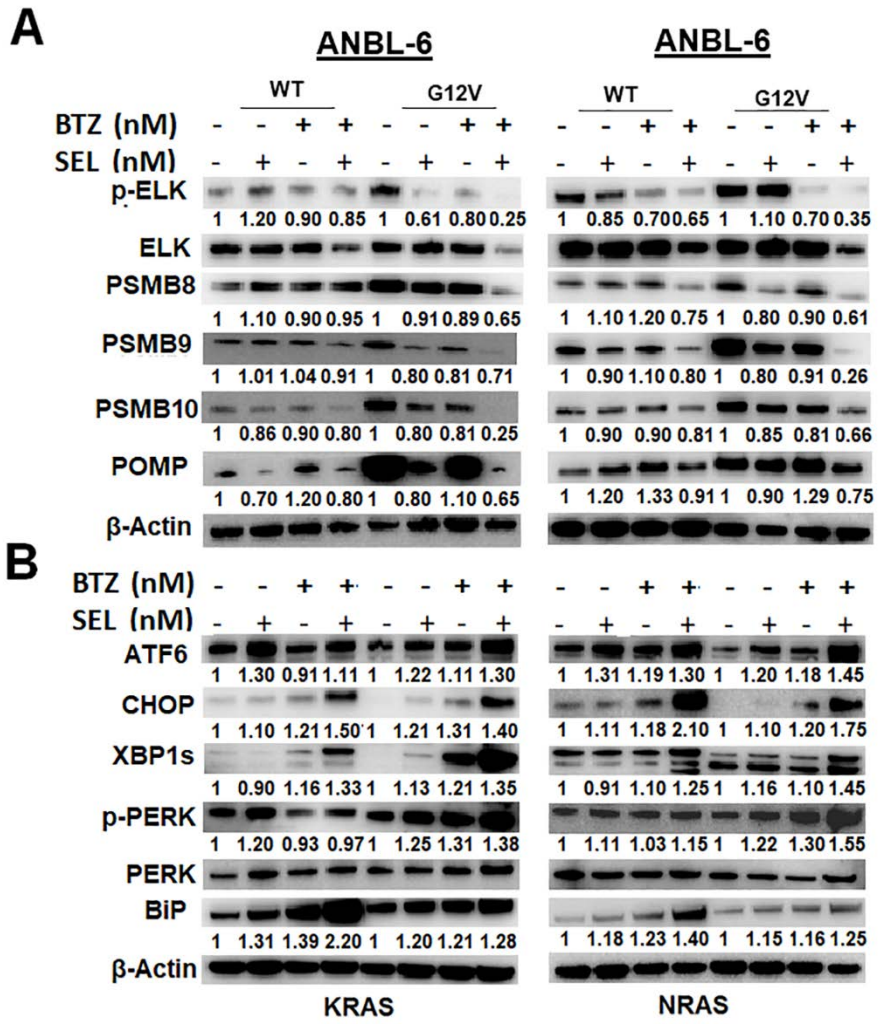
Supplementary Figure S13. Constitutively active *KRAS*, *NRAS* and *BRAF* mutants attenuate the unfolded protein response.

Expression of the UPR-related genes *ATF4*, *ATF5*, *ATF6*, *CHOP*, and spliced *XBP1* at the mRNA levels was assessed by qRT-PCR in U266 cells (**A**) expressing the indicated *KRAS* and *NRAS* mutants. NOXA mRNA expression levels were then assessed by qRT-PCR (**B**; **left panel**) and by Western blotting (**B**; **right panel**) in U266 cells. Lysates from U266 cells expressing *KRAS* and *NRAS* mutants were analyzed for the levels of the UPR proteins ATF5, ATF6, CHOP, and phosphorylation/activation of UPR receptors IREα and PERK (**C**) by immunoblotting.



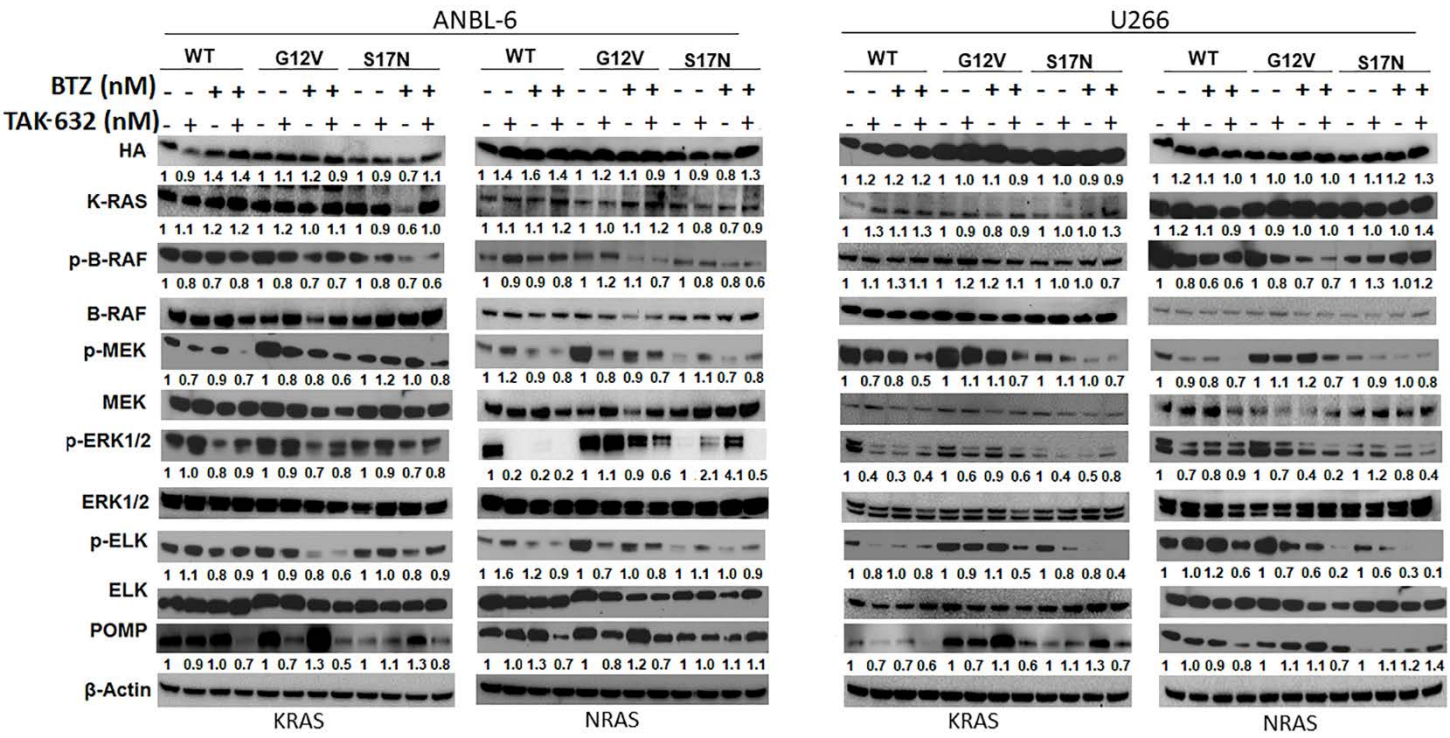
Supplementary Figure S14. *KRAS* and *NRAS* mutants and the unfolded protein response in the setting of drug therapies.

Transcription of *PERK*, *IRE1α*, *CHOP*, and *XBP1s* in ANBL-6 cells exposed to vehicle, TAK-632, bortezomib, or the combination was evaluated by qPCR, and data were analyzed and are displayed as detailed earlier.



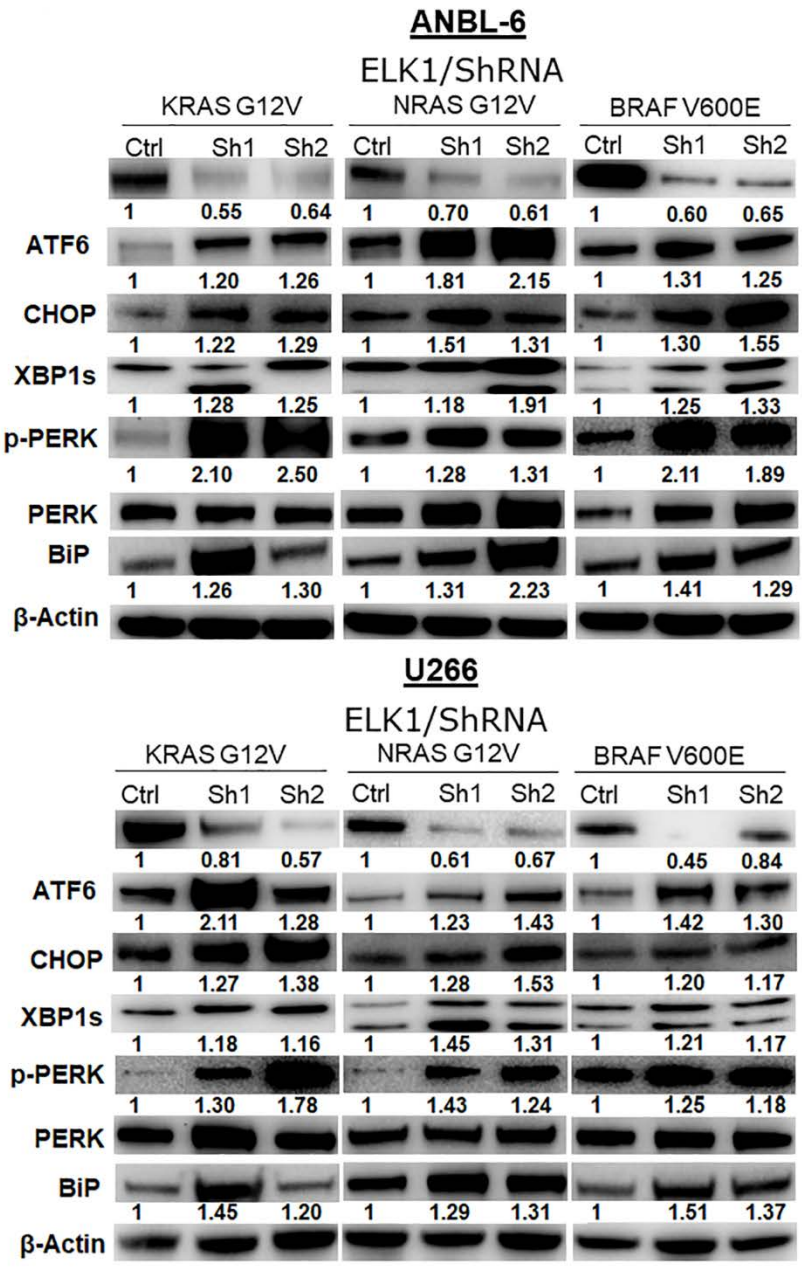
Supplementary Figure S15. *KRAS* and *NRAS* mutants and the unfolded protein response in the setting of drug therapies.

ANBL-6 cells expressing the indicated WT and G12V CA *KRAS* and *NRAS* mutations were exposed to bortezomib or selumetinib. Extracts were probed for the expression and activation status of ELK1, and for the expression levels of POMP and the three immunoproteasome subunits (**A**). These same extracts were then probed for the abundance of UPR gene products (**B**), including ATF6, CHOP, spliced XBP-1, PERK and BiP.



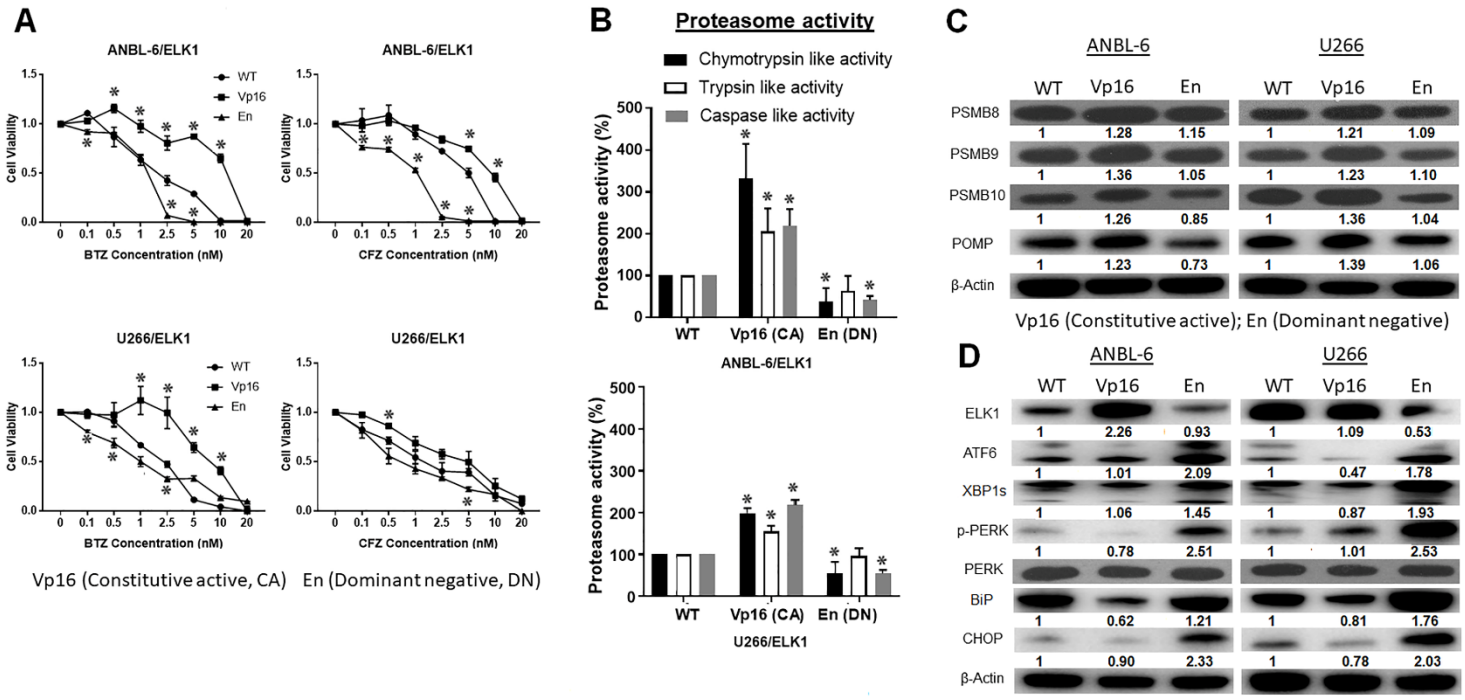
Supplementary Figure S16. *KRAS* and *NRAS* mutants and downstream signaling in the setting of drug therapies.

ANBL-6 and U266 cells expressing the indicated WT, CA, and DN *KRAS* and *NRAS* mutations were exposed to bortezomib, TAK-632, or the combination. Extracts were then probed for the expression and activation status of ELK1, for the expression levels of POMP and for multiple intermediate signaling proteins.



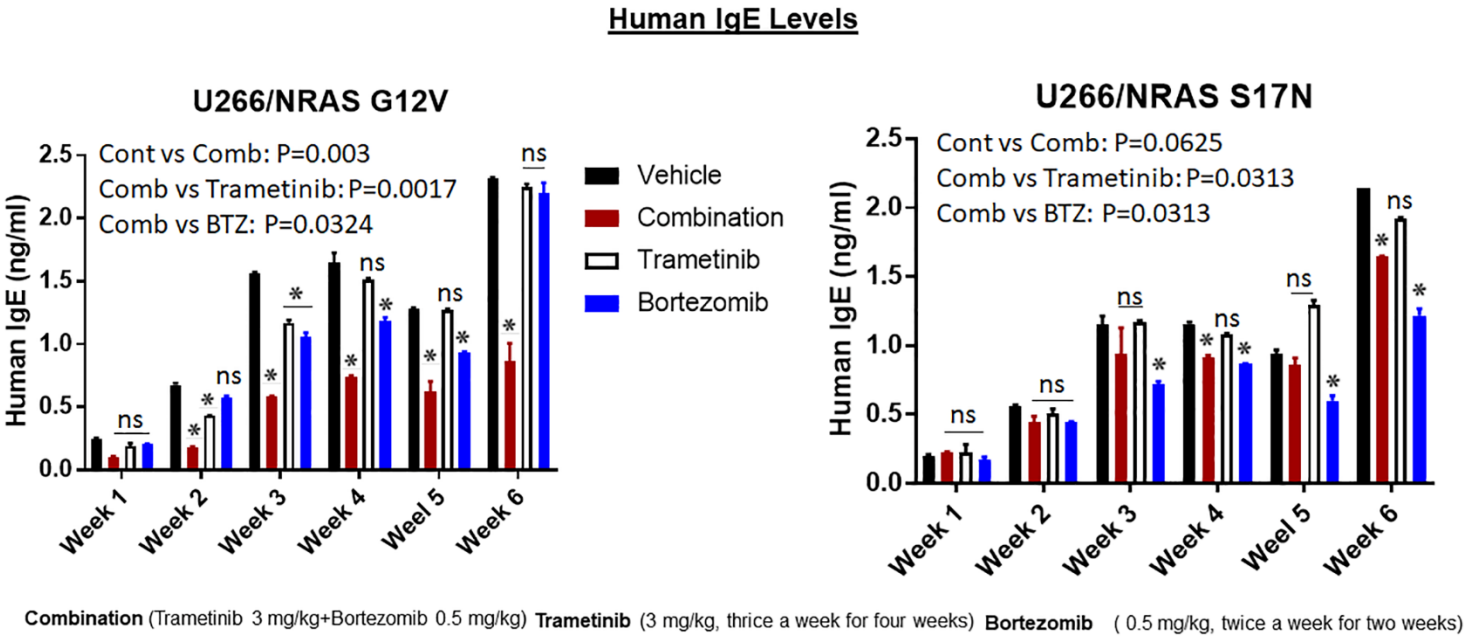
Supplementary Figure S17. Suppression of *ELK1* and its impact on UPR protein levels.

ANBL-6 (top panel) and U266 cells (bottom panel) cells expressing WT, CA, or DN *ELK1* variants were evaluated by Western blotting for the levels of UPR pathway intermediates.



Supplementary Figure S18. Impact of CA (Vp16) and DN (En) *ELK1* mutants on cell viability, proteasome capacity, and the UPR.

ANBL-6 (upper panel) and U266 cells (lower panel) expressing WT, CA, or DN *ELK1* mutants were analyzed for their cell viability (**A**) after treatment with BTZ (top row) or CFZ (bottom row). Proteasome chymotrypsin-, trypsin- and caspase-like activities were then evaluated (**B**) in both cell lines. Expression levels of POMP and the immunoproteasome subunits were then examined by Western blotting (**C**), and their impact on UPR proteins was probed as well (**D**).



Supplementary Figure S19. Biochemical disease parameters in mice harboring U266 based cells with CA or DN *RAS* and treated with drug regimens.

Serum drawn from mice at the indicated time points was evaluated for the concentration of human IgE secreted by U266 cells in xenografts with either a CA or DN *NRAS* after treatment with vehicle, bortezomib, trametinib, or the combination. Data are presented as the mean ± SD of triplicates with asterisks indicating $p < 0.05$ when compared to the vehicle controls.

Supplementary Table S1. Primers used in the study

Name		Sequence
Site directed mutagenesis primers		
KRAS G13D	FORWARD	CTTGTGGTAGTTGGAGCTGGTGACGTAGGCAAGAGTGCCTTGACG
	REVERSE	CGTCAAGGCACTCTTGCCTACGTCACCAGCTCCAACTACCACAAG
NRAS Q61R	FORWARD	CTCATGGCACTGTACTCTTCTCTTCCAGCTGTATCCAGTATGTCCAACAAACAG
	REVERSE	CTGTTTGTTGGACATACTGGATACAGCTGGAAGAGAAGAGTACAGTGCCATGAG
Braf	FORWARD	ACCTGGCTCACTAACTAACG
	REVERSE	GGCACTCTGCCATTAATCTC
ELK1	FORWARD	GCTCTAGAGCCACC ATGGACCCATCTGTGACGCTGTGGCAGTTT
	REVERSE	CTAGCTAGCTAGTTACTTGTCATCGTCGTCCTTGTAGTC TGGCTTCTGGGGCCCTGGGGAGAGCA
ELK1-VP16	FORWARD	GCTCTAGAGCCACC ATGGACCCATCTGTGACGCTGTGGCAGTTT
	REVERSE	CTAGCTAGCTAGTTACTTGTCATCGTCGTCCTTGTAGTC CCCACCGTACTCGTCAATTCCAAGGGCAT
ELK1-EN	FORWARD	GCTCTAGAGCCACC ATGGACCCATCTGTGACGCTGTGGCAGTTT
	REVERSE	CTAGCTAGCTAGTTA CTTGTCATCGTCGTCCTTGTAGTCGGATCCC
Syber green primers used		
sXBP1		CTGAGTCCGAATCAGGTGCAG
		ATCCATGGGGAGATGTTCTGG
usXBP1		CAGCACTCAGACTACGTGCA
		ATCCATGGGGAGATGTTCTGG
Total XBP1		TGGCCGGGTCTGCTGAGTCCG
		ATCCATGGGGAGATGTTCTGG

Supplementary Table S2: Primary antibodies

Target Protein	Species	Clone (Target)	Manufacturer	Cat#
ATF5	Rabbit	H-83	Santa Cruz	Sc-99205
ATF6	Mouse	F-7	Santa Cruz	Sc-166659
B-Raf	Rabbit	55C6	Cell Signaling	9433
p-B-Raf	Rabbit	S445	Cell Signaling	2696
CHOP	Mouse	B-3	Santa Cruz	Sc-7351
ELK	Rabbit		Cell Signaling	9182
p-ELK	Rabbit	S383	Cell Signaling	9181
IREα	Rabbit	14C10	Cell Signaling	3294
p-IREα	Rabbit		Abcam	ab48187
HA	Rabbit	Y-11	Santa Cruz	Sc-805
KRAS	Rabbit	C-terminal	Abgent	AP16005b
MEK1/2	Rabbit		Cell Signaling	9122
p-MEK1/2	Rabbit	S217/221	Cell Signaling	9154
p44/42 MAPK	Rabbit	137F5	Cell Signaling	4695
p-p44/42 MAPK	Rabbit	T202/Y204	Cell Signaling	4370
NRF2	Rabbit	EP1809Y	Abcam	ab76026
NRAS	Rabbit	C-terminal	Thermo Scientific	PA5-14833
Noxa	Rabbit		Abcam	ab36833
POMP	Rabbit	D2x9s	Cell Signaling	15141
PERK	Rabbit	D11A8	Cell Signaling	5683
p-PERK	Rabbit	T980	Cell Signaling	3179
PSMB8	Rabbit	D1K7X	Cell Signaling	13635
PSM9	Rabbit	EPR13785	Abcam	ab184172
PSM10	Rabbit	EPR14902	Abcam	ab183506
p53	Rabbit	DO-1	Santa Cruz	Sc-126

Supplementary Table S3. CI index values for TAK-632+BTZ/CFZ in ANBL-6 and U266 cells

			ANBL-6		U266	
Combination Dosage	**TAK-632 (nM)**	**BTZ/CFZ (nM)**	**CI with BTZ**	**CI with CFZ**	**CI with BTZ**	**CI with CFZ**
KRAS WT						
1	25	1.25	1.13	1.50	**0.66**	**0.95**
2	50	2.5	1.21	1.94	**0.78**	**0.64**
3	100	5	1.18	1.99	**0.92**	**0.39**
4	500	10	0.78	1.00	1.71	**0.57**
KRAS G12V						
1	25	1.25	1.11	**0.80**	1.35	**0.80**
2	50	2.5	**0.58**	**0.93**	**0.68**	1.02
3	100	5	**0.59**	**0.89**	**0.84**	**0.86**
4	500	10	1.63	**0.8**	1.01	**0.94**
KRAS S17N						
1	25	1.25	2.01	1.80	4.24	1.35
2	50	2.5	0.85	2.00	5.45	2.05
3	100	5	1.27	2.53	2.97	1.87
4	500	10	0.96	1.81	3.89	2.14
NRAS WT						
1	25	1.25	1.99	1.41	2.12	1.03
2	50	2.5	1.78	2.75	**0.54**	1.15
3	100	5	1.65	**0.53**	**0.88**	2.24
4	500	10	1.96	1.14	1.59	1.56
NRAS G12V						
1	25	1.25	1.43	**0.44**	1.03	**0.89**
2	50	2.5	0.53	**0.97**	**0.28**	1.00
3	100	5	0.61	**0.84**	**0.46**	**0.90**
4	500	10	0.88	2.02	**0.84**	**0.54**
NRAS S17N						
1	25	1.25	1.14	1.41	7.22	2.52
2	50	2.5	1.99	1.33	7.20	3.05
3	100	5	2.01	2.07	3.75	2.30
4	500	10	4.33	1.95	4.20	1.08

*****CI Values 01-0.3: very strong synergism; 0.3-0.7: synergism; 0.7-0.9: moderate to slight synergism; 0.9-1.1: additive; 1.1-1.45: moderate to slight antagonism; 1.45-3.3: antagonism; 3.3->10: strong to very strong antagonism (Chou TC. Synergism and Antagonism in Chemotherapy, Academic Press, San Diego, CA, 1991, pp. 61-102; Chou TC. Pharmacol Rev 58:621–681, 2006)

Supplementary Table S4. CI index values for Selumetinib+BTZ/CFZ in ANBL-6 and U266 cells

			ANBL-6		U266	
Combination Dosage	**Selumetinib (nM)**	**BTZ/CFZ (nM)**	**CI with BTZ**	**CI with CFZ**	**CI with BTZ**	**CI with CFZ**
KRAS WT						
1	1	1.25	1.24	**0.81**	**0.33**	1.93
2	5	2.5	1.18	**0.83**	**0.65**	**0.62**
3	25	5	1.73	2.02	**0.83**	**0.82**
4	100	10	0.56	3.22	1.33	1.00
KRAS G12V						
1	1	1.25	**0.89**	**0.57**	**0.86**	**0.30**
2	5	2.5	**0.71**	**0.58**	**0.69**	**0.47**
3	25	5	**0.91**	**0.70**	**0.52**	**0.70**
4	100	10	1.09	1.69	**0.79**	**0.67**
KRAS S17N						
1	1	1.25	1.56	**0.59**	1.33	**0.67**
2	5	2.5	1.43	**0.86**	2.67	1.38
3	25	5	1.38	1.17	2.10	1.67
4	100	10	1.02	2.57	2.01	2.83
NRAS WT						
1	1	1.25	0.45	2.62	**0.24**	1.65
2	5	2.5	1.02	2.06	**0.66**	1.47
3	25	5	1.11	4.21	**0.98**	1.58
4	100	10	0.85	3.21	1.33	1.57
NRAS G12V						
1	1	1.25	2.35	1.14	**0.25**	**0.66**
2	5	2.5	**0.62**	**0.78**	**0.41**	**0.86**
3	25	5	**0.60**	**0.81**	**0.34**	**0.90**
4	100	10	1.19	1.90	**0.69**	**0.79**
NRAS S17N						
1	1	1.25	**0.92**	**0.77**	**0.98**	**0.77**
2	5	2.5	1.76	1.12	1.66	1.06
3	25	5	2.41	5.33	2.92	1.36
4	100	10	2.16	4.33	1.41	1.25

*****CI Values 01-0.3: very strong synergism; 0.3-0.7: synergism; 0.7-0.9: moderate to slight synergism; 0.9-1.1: additive; 1.1-1.45: moderate to slight antagonism; 1.45-3.3: antagonism; 3.3->10: strong to very strong antagonism (Chou TC. Synergism and Antagonism in Chemotherapy, Academic Press, San Diego, CA, 1991, pp. 61-102; Chou TC. Pharmacol Rev 58:621–681, 2006)

Supplementary Table S5. CI index values for Trametinib+BTZ in ANBL-6 and U266 cells

			ANBL-6	U266
Combination Dosage	**Trametinib (nM)**	**BTZ (nM)**	**Trametinib +BTZ**	**Trametinib +BTZ**
KRAS WT				
1	1	1.25	2.61	1.39
2	5	2.5	2.25	0.88
3	25	5	4.18	1.32
4	100	10	**0.31**	**0.91**
KRAS G12V				
1	1	1.25	1.43	**0.25**
2	5	2.5	1.74	**0.19**
3	25	5	**0.92**	**0.09**
4	100	10	**0.23**	**0.10**
KRAS S17N				
1	1	1.25	3.21	3.01
2	5	2.5	3.51	1.31
3	25	5	2.87	1.07
4	100	10	4.85	1.35
NRAS WT				
1	1	1.25	1.99	1.67
2	5	2.5	1.78	2.89
3	25	5	1.65	2.29
4	100	10	1.96	**0.71**
NRAS G12V				
1	1	1.25	**0.03**	**0.29**
2	5	2.5	**0.02**	**0.28**
3	25	5	**0.05**	**0.39**
4	100	10	**0.48**	**0.78**
NRAS S17N				
1	1	1.25	**0.54**	**0.95**
2	5	2.5	**0.89**	2.08
3	25	5	2.21	1.34
4	100	10	2.43	2.02

*****CI Values 01-0.3: very strong synergism; 0.3-0.7: synergism; 0.7-0.9: moderate to slight synergism; 0.9-1.1: additive; 1.1-1.45: moderate to slight antagonism; 1.45-3.3: antagonism; 3.3->10: strong to very strong antagonism (Chou TC. Synergism and Antagonism in Chemotherapy, Academic Press, San Diego, CA, 1991, pp. 61-102; Chou TC. Pharmacol Rev 58:621–681, 2006)