

SUPPLEMENTARY FIGURES 1-3, TABLES 1-2

JNK2 is required for tumorigenic properties of melanoma cells

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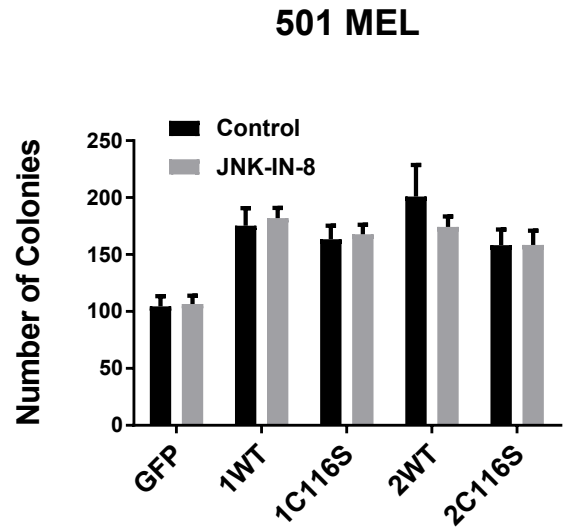
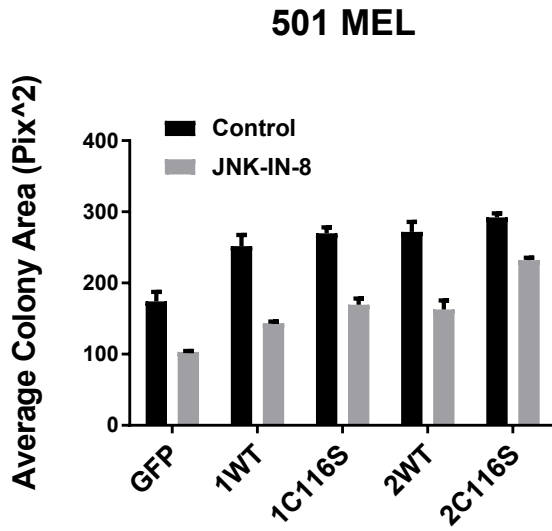
Supplementary Figure 1. JNK1/2^{C116S} mutants retain signaling to c-JUN in the presence of pan-JNK inhibition with JNK-IN-8.

Western blots for c-JUN activity (phospho-c-JUN) and total c-JUN are probed for each of the 501MEL cell lines transduced with JNK1^{WT} / JNK1^{C116S} (top) and JNK2^{WT} / JNK2^{C116S} (bottom). The lines were sham irradiated or UV-irradiated (18.5 kJ/m²; Newport, UV-SOL) in the presence or absence (DMSO) of 1 micromolar JNK-IN-8 and protein lysates procured 1 hour later.

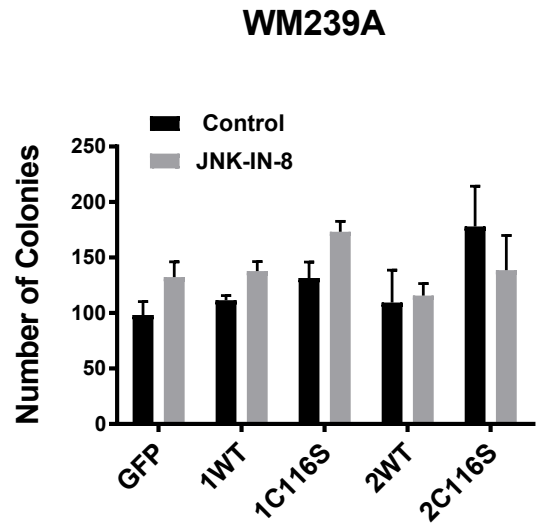
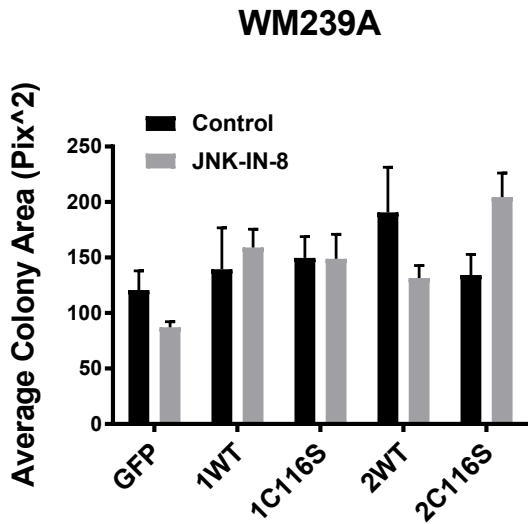
In the JNK1/2^{WT} transduced lines, phospho-c-JUN is suppressed by JNK-IN-8 following irradiation. Both JNK1^{C116S} and JNK2^{C116S} expressing cells continue to signal to c-JUN even in the presence of JNK-IN-8, showing that these mutants retain the ability to activate downstream targets even in the presence of the inhibitor.

Supplementary Figure 2

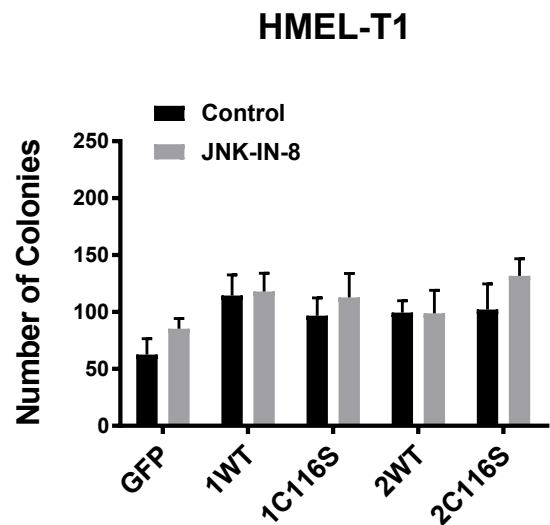
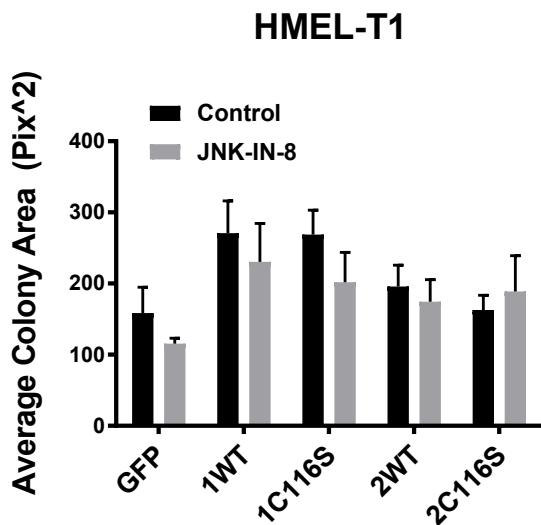
A.



B.



C.

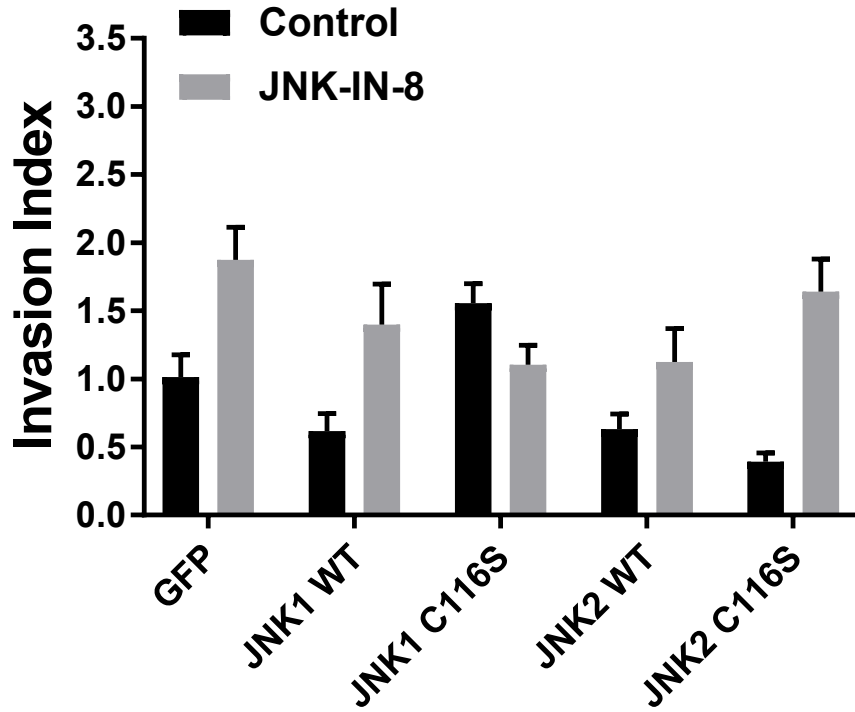


Supplementary Figure 2. Effects of JNK-IN-8 on long term colony proliferation in cells transduced with JNK1/2^{WT} and JNK1/2^{C116S} alleles.

Results of average colony area (left) and total colony counts (right) are shown for 501MEL (A), WM239A (B), and HMEL-T1 (C) cells. The number of colonies is not dramatically affected by inhibitor within each line tested, but the average colony area is most differentially affected in the responses to inhibitor in JNK1^{C116S} vs. JNK2^{C116S}-expressing cells, in which JNK1 and JNK2 activity are most effectively isolated. This is most apparent when expressed as a ratio between control-treated and inhibitor-treated cells (Figure 2) and the contrast between JNK2^{C116S}-expressing cells and the other lines is most apparent.

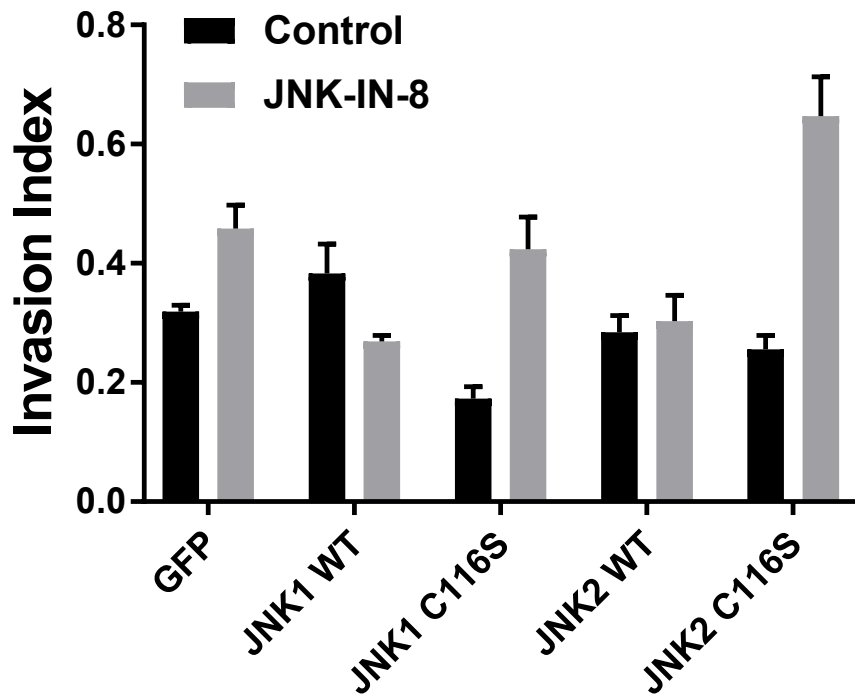
A.

501MEL



B.

HMEL-T1



Supplementary Figure 3. Effects of JNK-IN-8 on cellular invasiveness in cells transduced with JNK1/2^{WT} and JNK1/2^{C116S} alleles.

Results of cellular invasiveness (invasion index) are shown for 501MEL (A) and HMEL-T1 (B) cells. The invasiveness is variably affected by transduction of individual WT and mutant isoforms; however the effect of adding JNK-IN-8 leads to a baseline increase in invasiveness in control (GFP) cells. This may be due to the relative biological importance of JNK1 vs. JNK2 in this phenotype. Nevertheless, the invasion index is again most differentially affected in the responses to inhibitor in JNK1^{C116S} vs. JNK2^{C116S}-expressing cells, in which JNK1 and JNK2 activity are most effectively isolated. This is most apparent when expressed as a ratio between control-treated and inhibitor-treated cells (Figure 3) and the contrast between JNK2^{C116S}-expressing cells and the other lines is most apparent.

Supplementary Table 1

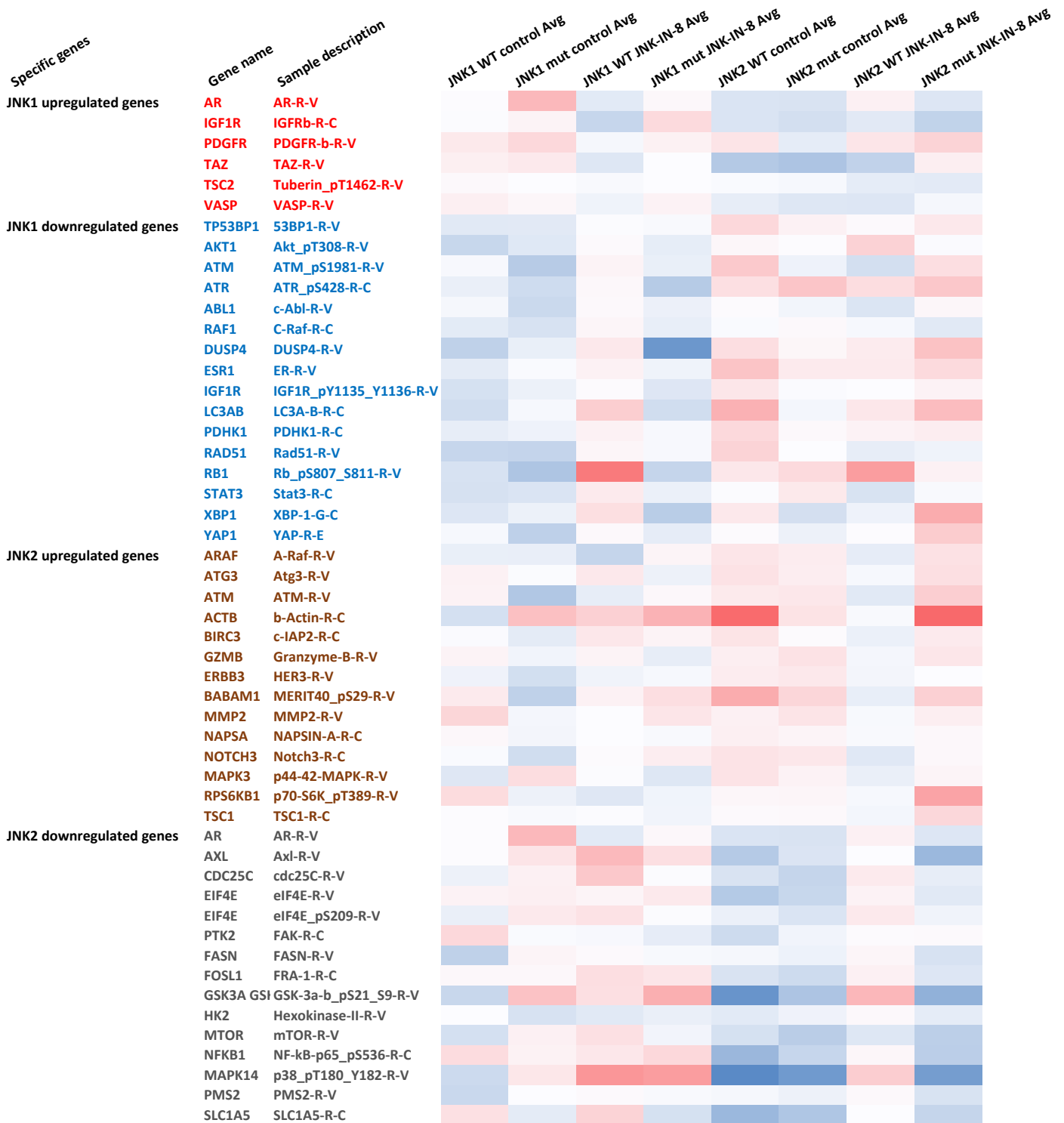
Gene name	Sample description	JNK1 WT	JNK1 mut	JNK1 WT	JNK1 mut	JNK2 WT	JNK2 mut	JNK2 WT	JNK2 mut
		control	control	JNK-IN-8	JNK-IN-8	control	control	JNK-IN-8	JNK-IN-8
		Avg	Avg	Avg	Avg	Avg	Avg	Avg	Avg
	4-Oct Oct-4-R-C	1.069079	0.991485	1.021365	1.03817	1.023362	0.974439	0.985093	1.056139
ABL1	c-Abl-R-V	0.991675	0.925113	1.013943	0.977489	1.009527	0.987535	0.951644	1.018352
ACACA	ACC1-R-C	0.935339	0.956979	0.948382	0.972683	1.091016	1.024019	1.042933	0.959996
ACACA	ACC_pS79-R-V	1.046736	1.053976	1.039574	0.928154	1.006983	1.076242	0.996058	0.99069
ACTB	b-Actin-R-C	0.94067	1.159465	1.115981	1.193975	1.377734	1.069087	0.997189	1.381859
AIM1	Aurora-B-R-V	1.029827	0.973996	1.08744	0.994243	0.927699	0.914045	0.929064	1.025483
AKT1	Akt-R-V	0.948368	1.013486	1.044168	0.991079	1.058485	0.971105	0.938695	1.034459
AKT1	Akt_pS473-R-V	0.955434	1.045755	1.015748	1.143739	1.03582	1.015599	1.129229	1.066087
AKT1	Akt_pT308-R-V	0.921315	0.958539	1.010914	0.969138	1.018562	1.006831	1.111559	0.999312
AKT1S1	PRAS40_pT246-R-V	0.988518	0.954225	1.068931	1.057597	0.998471	1.003816	1.031528	1.020958
AR	AR-R-V	1.005651	1.178069	0.963259	1.016131	0.949949	0.94809	1.033345	0.955493
ARAF	A-Raf-R-V	0.974004	0.97337	0.917542	1.023716	1.062886	1.043862	0.9689	1.072598
ARID1A	ARID1A-R-C	1.109039	0.992407	1.194834	1.112878	0.922278	0.896634	0.972755	1.048037
ATG3	Atg3-R-V	1.032635	0.999886	1.055879	0.979778	1.071086	1.041092	0.993985	1.07872
ATG7	Atg7-R-V	1.031168	0.986384	1.028928	0.93543	1.113566	1.147426	1.119345	1.155125
ATM	ATM-R-V	1.028803	0.886059	0.96972	1.016791	1.051085	1.058042	0.961209	1.12239
ATM	ATM_pS1981-R-V	0.995309	0.895926	1.02614	0.97385	1.135337	0.980662	0.939891	1.080206
ATR	ATR_pS428-R-C	0.974303	0.931747	1.01633	0.894177	1.077086	1.145438	1.08329	1.139085
ATRX	ATRX-R-C	0.968042	0.989249	0.984106	1.043191	1.028519	1.061569	1.030814	0.916986
AXL	Axl-R-V	1.00677	1.064622	1.17601	1.078262	0.892573	0.950927	1.002415	0.852548
BABAM1	MERIT40_pS29-R-V	1.048779	0.908628	1.031248	1.08117	1.210674	1.10096	0.972006	1.117162
BAD	Bad_pS112-R-V	1.046221	1.052627	1.005597	1.048756	1.036726	1.070059	1.039715	0.995127
BAK1	Bak-R-C	1.120283	0.969714	0.968651	1.041621	1.017192	1.008655	1.028876	0.986803
BAX	Bax-R-V	1.053889	0.980879	0.909628	0.989033	1.026575	1.103977	1.0069	1.099085
BCL2A1	Bcl2A1-R-V	1.022093	0.950169	1.019133	1.012735	1.030962	1.057357	1.012259	1.0258
BCL2L1	Bcl-xL-R-V	1.142747	1.124092	0.925734	0.993646	0.929034	1.069835	1.004505	1.03
BCL2L11	Bim-R-V	1.054095	1.092919	0.998498	1.034626	1.136613	1.12629	1.071691	1.133512
BECN1	Beclin-G-C	1.057224	1.04232	1.17018	1.058041	1.225514	1.137249	1.066892	1.579121
BID	Bid-R-C	1.127735	1.028025	1.124163	1.100765	0.969057	1.027059	0.994984	1.006529
BIRC3	c-IAP2-R-C	0.999619	0.96592	1.058296	1.026969	1.066432	1.008032	0.975742	1.050603
BRAF	B-Raf-R-V	0.98697	0.993786	0.930746	0.97832	0.919957	0.963855	0.971193	0.94268
BRAF	B-Raf_pS445-R-V	0.961179	1.057196	1.036783	0.974395	0.93406	1.019287	1.011896	0.970523
BRD4	BRD4-R-V	1.122337	1.029078	1.157608	1.08333	0.855526	0.895629	0.98694	1.188567
CASP3	Caspase-3-R-C	1.079043	0.989352	0.982804	0.98807	0.986636	1.007401	0.992085	1.034098
CASP7	Caspase-7-cleaved-R-C	1.289454	1.062973	0.997659	1.148316	1.228183	1.215233	0.986488	1.044161
CAV1	Caveolin-1-R-V	1.047417	0.886525	1.098516	1.129729	0.784642	0.882288	0.836614	1.140258
CCNB1	Cyclin-B1-R-V	1.06204	1.004181	1.150478	0.969344	0.884612	0.962595	0.938909	0.895214
CCND1	Cyclin-D1-R-V	1.000635	0.989925	1.012904	1.000697	1.120971	1.069812	1.008888	1.045391
CD274	PD-L1-R-C	1.318666	1.535693	3.054159	1.519197	1.443585	1.131378	1.292294	1.035061
CDC25C	cdc25C-R-V	0.979366	1.033888	1.137093	1.000646	0.948044	0.917615	1.050348	0.970543
CDH1	E-Cadherin-R-V	0.973167	1.596451	0.988034	1.566411	1.049367	2.090121	1.023635	1.001923
CDH2	N-Cadherin-R-V	1.038915	0.97486	0.994233	0.981214	0.931245	0.979967	0.951545	0.976049
CDH3	P-Cadherin-R-C	1.108432	0.920945	1.002164	0.988071	0.999567	1.450673	1.072355	1.049536
CDK1	Cdc2_pY15-R-C	0.940782	0.950386	1.019952	1.032403	1.020247	1.001659	0.99752	0.989899
CDK1	CDK1-R-C	1.121691	1.027811	1.286786	0.978428	0.954192	0.916754	0.960528	0.937704
CDKN1A	p21-R-V	0.981824	0.93397	1.004876	0.999564	0.986102	0.999054	1.013025	1.036731
CDKN1B	p27-Kip-1-R-V	1.01069	1.024718	0.988449	0.956261	1.000008	1.009064	1.014161	1.044059
CDKN1B	p27_pT198-R-V	1.027492	1.019947	0.999487	1.024225	1.025701	1.063635	1.042194	0.97961
CDKN2A	p16INK4a-R-V	0.853426	0.992478	0.877731	0.938822	0.850645	1.010702	1.039885	0.776443
CHEK1	Chk1_pS296-R-V	1.075685	1.04172	0.995629	1.125865	1.118648	1.296009	0.979348	0.958831

CHEK2	Chk2_pT68-R-C	0.995355	1.029483	1.008341	0.962511	1.099224	1.033217	1.042833	1.093369
CLDN7	Claudin-7-R-V	0.997127	1.036828	0.937468	0.966537	0.943346	1.024891	0.955505	1.007516
CMC2	Cox2-R-C	1.363531	1.029263	1.034877	1.019853	1.041749	0.972098	1.000352	1.685494
CNST43	Connexin-43-R-C	1.206623	1.149834	0.9408	1.31516	1.010602	1.57755	1.147176	1.080753
COG3	COG3-R-V	0.990516	0.99823	0.994118	1.028342	1.036777	1.015138	1.001571	1.030447
COL6A1	Collagen-VI-R-V	0.970223	0.94893	0.964489	1.000491	1.005216	0.992351	0.983093	1.008592
CREB1	Creb-R-C	1.058123	1.025253	0.97476	1.025878	1.009244	1.004299	1.164829	1.027859
CTNNB1	b-Catenin-R-V	0.978588	1.280913	0.960312	1.044749	0.80802	1.032179	0.995776	0.906335
CTNNB1	b-Catenin_pT41_S45-R	0.994901	1.022772	1.03397	1.009114	1.028249	1.036895	1.039499	1.0157
DPP4	CD26-R-V	1.103579	0.925765	0.977203	1.114	0.925733	1.012987	0.868591	0.966593
DUSP4	DUSP4-R-V	0.908037	0.973485	1.054748	0.779942	1.080332	1.020101	1.04817	1.154169
EEF2	eEF2-R-C	0.988382	1.122267	1.120633	0.936306	0.87907	0.945779	0.939367	0.933633
EEF2K	eEF2K-R-V	0.943918	1.010566	1.032199	1.010959	0.905502	0.925857	0.996069	0.905378
EGFR	EGFR-R-V	1.065656	0.929291	0.987956	1.035343	0.988318	0.989248	0.985272	1.019123
EGFR	EGFR_pY1173-R-V	1.036077	1.013146	0.99843	0.992714	1.021258	1.043826	1.002432	0.984852
EIF4E	eIF4E-R-V	1.028201	1.036797	1.023151	1.052596	0.89193	0.921079	1.028833	0.960255
EIF4E	eIF4E_pS209-R-V	0.974322	1.051251	1.071179	1.001694	0.977157	0.949822	1.05291	0.984002
EIF4EBP1	4E-BP1-R-V	1.109197	1.090708	0.976164	0.928586	0.93995	1.042831	1.036274	1.116943
EIF4EBP1	4E-BP1_pS65-R-V	1.076697	1.033224	1.068254	1.054405	0.998496	1.086365	1.101288	1.025936
EIF4G1	eIF4G-R-C	0.970989	0.89273	1.182569	0.978726	0.942857	0.955451	0.996829	1.118789
ELK1	Elk1_pS383-R-C	1.08183	0.989736	1.048221	1.10281	0.966571	1.044625	0.975021	0.981161
ERBB2	HER2_pY1248-R-C	0.976285	1.01324	1.001655	1.037334	1.005412	1.017512	1.131384	0.963448
ERBB3	HER3-R-V	0.982093	0.938349	0.983806	0.993788	1.044495	1.05535	0.988409	1.003407
ERBB3	HER3_pY1289-R-C	1.038163	1.09832	0.993094	0.974046	0.980272	1.04304	1.053391	1.012085
ERCC5	ERCC5-R-C	1.000145	0.967645	1.081616	1.061475	0.895157	0.972402	0.961734	0.969803
ESR1	ER-R-V	0.966066	0.998602	1.030521	0.9828	1.148628	1.049679	1.049293	1.090151
ESR1	ER-a_pS118-R-V	1.022188	1.004106	1.002492	0.983731	0.979159	0.993284	1.00964	1.017373
ETS1	Ets-1-R-V	1.080966	1.047821	1.04524	0.975863	1.016978	0.970868	0.987395	0.970855
FASN	FASN-R-V	0.908189	1.025001	1.011519	0.99567	0.991487	0.980116	1.021212	0.945049
FOSL1	FRA-1-R-C	1.016404	1.015243	1.081156	1.063589	0.948802	0.929478	1.034517	0.955211
FOX3	FoxO3a-R-C	1.010211	0.981182	0.915639	1.006123	1.081545	1.067335	1.018444	1.080888
FOXO1	FoxO1-R-V	0.975597	0.979629	1.050323	1.024664	0.976356	0.931405	0.988773	0.945793
FOXO3	FoxO3a_pS318_S321-R	1.084881	1.039042	0.9952	0.988261	1.011739	1.05607	1.005858	1.076883
G6PD	G6PD-R-V	1.015373	0.960793	1.03984	0.983773	1.052386	0.986421	1.025439	1.071477
GAB2	Gab2-R-V	0.984914	1.012447	0.975514	1.046581	0.986044	0.951474	0.955964	0.935589
GCLM	GCLM-R-C	1.02463	0.925702	1.048531	0.993768	1.073222	0.992579	0.967608	1.054444
GLS	Glutaminase-R-C	0.961174	1.011682	1.015428	0.9528	1.006728	0.980383	0.989975	0.977744
GLUD	Glutamate-D1-2-R-C	1.013187	0.972924	1.020888	1.062815	1.077125	0.960232	0.934961	1.04907
GSK3A GSK3B	GSK-3a-b_pS21_S9-R-V	0.922592	1.152126	1.078796	1.202998	0.773848	0.879954	1.18051	0.837102
GYS1	Gys-R-V	0.999792	1.011095	1.291492	0.963434	1.203238	1.032072	1.219865	1.111869
GYS1	Gys_pS641-R-V	0.910752	1.056649	1.040636	1.046318	0.95548	1.006436	1.01689	0.969746
GZMB	Granzyme-B-R-V	1.027621	0.985575	1.027183	0.970054	1.03869	1.069818	0.990421	1.06034
H3K9ME2	DM-K9-Histone-H3-R-C	1.123933	1.033671	0.907476	0.981373	1.247401	1.317265	0.992049	1.066535
HES1	HES1-R-V	1.167551	1.275676	1.133594	1.329781	0.88739	1.104366	0.993514	1.034475
HIST3H3	Histone-H3-R-V	1.092672	1.17281	1.10228	0.910076	1.044228	1.207641	0.930151	1.017277
HISTH3	DM-Histone-H3-R-V	1.230614	1.072752	0.937285	1.00637	1.008689	1.079219	0.988399	1.050779
HK2	Hexokinase-II-R-V	1.004349	0.945831	0.961582	0.974906	0.962534	0.982172	1.013285	0.967915
HSBP1	HSP27_pS82-R-V	1.015617	0.996914	1.0429	0.951082	0.91118	0.887018	0.960965	0.93166
HSPA1A	HSP70-R-C	1.054929	1.029002	1.212229	0.994333	0.987386	1.175016	0.924071	0.893224
IGF1R	IGF1R_pY1135_Y1136-I	0.942855	0.978167	1.008018	0.95576	1.059376	0.999462	1.002943	1.029672
IGF1R	IGFRb-R-C	1.001235	1.026124	0.919145	1.091104	0.950131	0.939804	0.962192	0.912124
IGFBP2	IGFBP2-R-V	1.056553	1.077487	1.019093	1.007536	1.016933	1.059293	1.029099	1.020415
INPP4B	INPP4b-R-V	0.988146	1.016625	1.017959	1.018841	1.033028	0.987704	1.003277	0.991522
INSRB	IR-b-R-C	0.976052	1.086582	1.030002	1.023565	1.026507	0.982345	1.037833	1.053624
IRF1	IRF-1-R-C	0.99538	0.947762	0.911268	0.953485	1.140061	1.01348	1.042558	0.959633

IRS1	IRS1-R-V	1.056981	0.999617	0.960163	0.995236	0.956619	0.954788	0.954538	1.037378
JAG1	Jagged1-R-V	1.06226	1.024966	1.011495	1.122358	1.075787	1.065419	0.998338	1.025244
JAK2	Jak2-R-V	0.965974	1.084306	1.118251	0.994965	1.00616	1.079728	1.027996	0.98154
JUN	c-Jun_pS73-R-V	0.984176	0.948822	1.106087	1.071014	1.023026	1.025385	1.058502	1.005552
KAT2A	GCN5L2-R-V	0.959297	1.029626	1.023474	0.994716	1.01641	1.05232	1.029333	0.961598
KDR	VEGFR-2-R-V	1.074743	1.000969	0.91669	0.966984	1.034853	1.044936	1.081608	1.089117
KIT	c-Kit-R-V	1.01614	1.029045	1.048424	0.933267	0.977755	0.950166	1.003411	1.054523
LC3AB	LC3A-B-R-C	0.934978	0.993398	1.122785	0.93445	1.196781	0.989095	1.058478	1.168514
LCK	Lck-R-V	0.981688	1.030387	1.002566	1.049892	1.238403	1.228269	1.041606	0.982012
LDHA	LDHA-R-C	1.053099	1.022578	1.205585	0.915491	0.991538	1.117685	1.085191	1.106905
LRP6	LRP6_pS1490-R-V	0.984966	0.979571	1.007334	1.07012	1.04741	1.005954	1.002104	1.014898
MAP2K1	MEK1-R-V	1.097221	0.932352	1.036104	1.157119	0.775029	0.769141	0.958605	0.864133
MAP2K1	MEK1_pS217_S221-R-V	0.948817	1.100638	1.066088	1.044879	0.977873	1.009796	1.040292	1.00454
MAPK14	p38-MAPK-R-V	0.910932	0.987311	0.981231	0.974115	0.932492	0.969706	0.978339	1.012669
MAPK14	p38_pT180_Y182-R-V	0.928228	1.058619	1.267379	1.249624	0.750871	0.786546	1.124068	0.793626
MAPK3	MAPK_pT202_Y204-R-V	0.896283	0.99355	1.041596	1.125684	0.979556	0.973448	1.077852	0.905937
MAPK3	p44-42-MAPK-R-V	0.957978	1.083903	1.001508	0.956409	1.06753	1.028506	0.974375	1.023242
MAPK8	JNK_pT183_Y185-R-V	1.014069	0.976343	0.994701	0.99205	1.035963	1.024781	1.022724	0.983648
MAPK9	JNK2-R-C	1.003294	1.006814	0.856555	0.973624	0.836908	1.139811	0.975847	1.040261
MCL1	Mcl-1-R-V	0.976061	1.170339	1.125842	1.047297	0.930832	0.927989	0.899324	0.953936
MDM2	MDM2_pS166-R-V	0.990122	1.860778	1.105984	2.171343	0.880904	1.966616	0.961744	0.914736
MET	c-Met_pY1234_Y1235-R-V	1.02229	1.007306	0.96261	1.006681	1.031957	1.056722	1.033949	1.017383
MIF	MIF-R-C	0.979346	0.961014	0.985555	0.921588	1.08827	1.066462	1.051553	1.128648
MKNK1	Mnk1-R-V	0.911067	1.012211	1.023859	0.976009	1.016188	0.964315	1.028482	0.934533
MMP2	MMP2-R-V	1.102749	0.990763	1.003917	1.061974	1.033225	1.065993	0.995583	1.040035
MSH6	MSH6-R-C	1.056774	0.97641	1.050437	1.01668	0.919718	0.922361	0.961215	0.996655
MSI2	MSI2-R-C	0.969564	1.06651	0.983591	0.976609	1.005269	0.979333	0.984397	1.065629
MTOR	mTOR-R-V	0.941419	1.034991	1.07527	0.986565	0.943119	0.899853	0.955821	0.90377
MTOR	mTOR_pS2448-R-C	0.970293	1.227335	1.071898	1.087804	0.892071	0.982738	1.034651	0.901649
MYC	c-Myc-R-C	0.975323	0.937744	0.984433	1.029174	1.019726	1.012194	1.016801	0.954299
MYH11	Myosin-11-R-V	1.03922	1.713902	1.413303	1.726363	1.712908	1.510167	1.250285	1.374309
MYO2A	Myosin-IIa_pS1943-R-V	0.98949	1.010455	1.246036	0.975619	0.868343	0.981308	1.038427	1.066248
MYT1	Myt1-R-C	0.977952	0.952959	0.988737	0.986391	1.075451	1.002871	1.004162	1.021997
NAPSA	NAPSIN-A-R-C	1.015112	0.988527	1.004406	0.997084	1.035675	1.024588	0.995964	1.017076
NDRG1	NDRG1_pT346-R-V	0.888807	0.890909	0.886342	0.876024	1.049455	0.950044	1.304986	1.274461
NF2	Merlin-R-C	1.009932	1.139071	1.062381	0.980203	1.022382	1.034864	1.063304	1.01853
NFKB1	NF-kB-p65_pS536-R-C	1.085195	1.030409	1.056778	1.095601	0.851888	0.918909	1.017769	0.901325
NOTCH1	Notch1-R-V	0.957989	1.71908	1.025962	1.853569	0.854686	1.776717	0.938033	0.883092
NOTCH3	Notch3-R-C	0.997932	0.93388	1.008563	1.042701	1.06868	1.057082	0.960056	1.016481
NRG1	Heregulin-R-V	1.105826	1.095	1.404855	1.143125	1.063531	1.085913	1.00604	1.011824
PAICS	PAICS-R-C	1.126357	0.983653	1.012803	0.957981	1.020961	1.125149	1.012667	1.226657
PAK1	PAK1-R-V	0.986884	0.954125	1.019658	1.020862	0.999704	1.013397	1.010326	1.074701
PAK4	PAK4-R-V	0.968474	1.005458	1.023701	0.971983	1.011549	0.986405	1.004703	0.98497
PAR	PAR-R-C	1.350641	1.131913	1.321331	1.680184	0.745873	0.882249	1.160371	1.113613
PARK7	DJ1-R-V	1.063256	1.077244	1.063736	0.945718	1.118781	1.189969	1.108459	1.201955
PARP1	PARP1-R-V	1.050838	0.988895	0.999345	1.026306	1.035447	1.058333	1.042799	0.928401
PAX8	PAX8-R-C	0.982112	1.007668	1.008463	0.989922	1.014388	0.973772	0.989091	0.990117
PDCD1	Pdcd-1L1-G-C	1.032464	0.84388	1.050398	1.01026	1.189037	0.981316	0.978435	1.380778
PDCD4	Pdcd4-R-C	0.899663	0.985431	0.897379	0.988009	1.075025	1.088353	1.030555	1.085161
PDGFR	PDGFR-b-R-V	1.053501	1.095862	0.991687	1.033038	1.06593	0.967366	1.063182	1.108978
PDHK1	PDHK1-R-C	0.96961	0.980418	1.032016	0.995249	1.094095	1.014811	1.028985	1.041232
PDK1	PDK1-R-V	1.063852	1.031043	1.170364	1.021512	1.023742	1.088809	1.052281	1.015143
PDK1	PDK1_pS241-R-V	0.931558	1.004857	0.966294	0.9247	1.081149	1.067421	1.044788	1.022166
PEA15	PEA-15-R-V	1.106114	1.127918	0.956666	0.97877	0.93715	0.905604	0.957493	1.067193
PEA15	PEA-15_pS116-R-V	0.962795	0.995471	0.944685	0.957068	1.019194	0.981819	1.086365	1.04379

PGR	PR-R-V	1.112315	1.073455	0.99094	1.076898	1.039847	1.064765	1.00726	1.034582
PIK3C2A	PI3K-p110-a-R-C	0.99185	1.004587	1.024691	0.951275	1.024182	1.086798	1.031904	1.090813
PIK3R1	PI3K-p85-R-V	0.978181	0.970791	0.983625	0.958317	1.067884	1.103136	1.007461	1.063387
PKM2	PKM2-R-C	1.074474	1.290118	1.103493	0.988783	1.083049	1.088734	1.115131	1.158991
PLCG2	PLC-gamma2_pY759-R-	0.973576	0.963552	0.953141	1.020892	1.003371	0.975341	0.986093	0.97661
PLK1	PLK1-R-C	1.097213	1.098898	1.346585	1.179484	0.910999	0.863887	0.996231	0.851887
PMS2	PMS2-R-V	0.923055	1.003405	1.011793	0.997175	0.992295	0.978903	1.013573	0.947218
PREX1	PREX1-R-V	0.931274	0.918586	0.940901	0.891073	1.027895	1.005824	1.0446	1.106204
PRKAA1	AMPKa-R-C	1.08031	0.997085	1.008198	1.090584	0.925123	1.037217	0.953602	0.922635
PRKAA1	AMPKa_pT172-R-C	1.146611	1.204647	1.167827	1.076134	0.969718	1.011963	0.961278	1.012834
PRKAA2	AMPK-a2_pS345-R-V	1.01998	1.013733	1.004934	0.995072	1.010583	1.055475	1.015332	1.004234
PRKAR1A	PKA-a-R-V	0.938373	0.980368	0.999312	0.904243	0.861707	0.865051	0.978646	0.890096
PRKCA	PKC-a_pS657-R-C	0.974892	1.03141	0.9551	0.950713	1.118283	1.097259	1.131727	1.095477
PRKCB	PKC-b-II_pS660-R-V	0.829372	0.95762	0.989423	1.001505	1.039591	1.015462	1.029996	0.939972
PRKCD	PKC-delta_pS664-R-V	1.002788	0.9872	0.972855	0.978406	1.015453	1.023177	1.018041	1.023229
PTEN	PTEN-R-V	1.010816	1.111543	1.034649	1.040755	0.914828	0.93089	0.972087	0.890526
PTGS3	Cox-IV-R-V	1.223499	0.975798	0.866259	1.209878	0.946175	1.101653	0.926294	1.179583
PTK2	FAK-R-C	1.095186	0.998062	0.994316	0.9688	0.929814	0.985164	1.008389	1.010814
PTK2	FAK_pY397-R-V	1.023605	0.976708	1.032471	1.196028	0.882878	0.894584	1.140886	0.924833
PTPN11	SHP-2_pY542-R-C	0.942575	0.942816	1.063096	1.104703	0.937509	0.99757	1.372143	0.980865
PXN	Paxillin-R-C	1.054581	0.950749	1.015051	1.00855	0.975697	1.053023	1.066576	1.114751
RAB11A	Rab11-R-E	1.022363	0.991452	0.943965	1.003899	1.009069	0.972217	0.966119	1.076675
RAB25	Rab25-R-V	1.012516	1.057193	1.01178	1.038848	1.040145	1.008275	1.009004	1.035431
RAD51	Rad51-R-V	0.919622	0.915378	1.022153	0.994345	1.109812	1.00299	0.970366	0.983336
RAF1	C-Raf-R-C	0.964813	0.94582	1.021611	0.972585	0.998098	1.013981	0.993451	0.960377
RAF1	C-Raf_pS338-R-V	1.01902	1.023139	1.057354	1.038487	1.035582	1.084501	1.023472	1.009707
RB1	Rb_pS807_S811-R-V	0.945053	0.881224	1.336879	0.917567	1.057953	1.090145	1.248469	1.030754
RBM15	RBM15-R-V	0.971232	0.994982	1.023314	1.005015	1.071826	1.06394	1.025944	1.010206
RICTOR	Rictor-R-C	0.964494	1.013557	0.97064	1.048119	0.97733	0.931402	0.998242	0.983558
RICTOR	Rictor_pT1135-R-V	1.020633	0.970958	0.992538	1.04578	0.984294	0.993576	0.973378	0.955802
RIP	RIP-R-C	0.930815	1.023723	0.991421	0.94486	0.987507	0.941889	1.00299	1.001029
ROCK1	Rock-1-R-C	0.968799	1.070527	1.247206	0.943013	1.116855	1.003183	1.085258	1.153896
RPA2	RPA32_pS4_S8-R-C	0.91229	0.900222	1.044892	0.932074	0.890956	0.939207	0.989416	0.937212
RPS6	S6_pS235_S236-R-V	0.931621	1.079099	1.524552	1.243025	1.071062	1.032852	1.138579	1.019633
RPS6	S6_pS240_S244-R-V	0.918942	1.120212	1.367842	1.183214	0.988895	1.021056	1.063544	0.968332
RPS6K	p90RSK_pT573-R-C	0.877293	0.970777	1.120581	1.145387	0.897416	0.931185	1.031386	0.866379
RPS6KA1	RSK-R-C	0.98532	0.98374	0.97081	1.008211	1.060272	1.051565	1.029365	1.016179
RPS6KB1	p70-S6K1-R-V	1.073425	1.058392	0.990985	1.056521	1.01287	1.112441	1.02655	1.062584
RPS6KB1	p70-S6K_pT389-R-V	1.08677	0.979302	0.957354	0.984434	1.018788	1.022192	0.993729	1.232492
RPTOR	Raptor-R-V	1.024263	1.035329	1.012247	0.993511	0.988687	0.998513	0.960089	0.95982
SDHA	SDHA-R-V	1.018392	1.01882	0.868464	0.993913	1.00203	1.044538	0.977864	1.058139
SHC1	Shc_pY317-R-V	0.979384	1.012139	0.98566	0.961584	0.983429	0.947876	0.986318	1.001578
SLC16A4	MCT4-R-V	1.004761	0.932682	0.873612	0.839771	1.009342	0.968821	1.114225	1.074789
SLC1A5	SLC1A5-R-C	1.075824	0.966047	1.109612	0.942736	0.852659	0.883285	1.003641	0.918039
SMAD1	Smad1-R-V	1.061435	1.146834	1.022926	1.102575	0.886048	0.912207	0.95789	0.913299
SMAD3	Smad3-R-V	1.100694	0.986572	0.991626	1.004983	0.980388	1.024208	0.961505	1.009331
SOX2	Sox2-R-V	1.2418	1.069425	1.238525	1.048101	0.978616	0.87472	0.936772	0.961059
SRC	Src_pY416-R-V	1.064255	1.012522	1.305688	1.585576	0.879116	1.03243	1.993558	0.897734
SRC	Src_pY527-R-V	0.959258	0.956685	1.125279	1.066348	0.969486	1.089332	1.2621	0.968128
STAT	Stat_pY694-R-N	0.98427	1.046618	1.017309	1.034609	1.138486	1.049198	1.097032	1.07505
STAT3	Stat3-R-C	0.943195	0.949839	1.048937	0.975958	1.001699	1.053431	0.947695	0.99704
STAT3	Stat3_pY705-R-V	0.960036	1.125685	0.973066	1.032438	1.039376	1.104244	1.070186	0.991933
STAT5A	Stat5a-R-V	0.987371	1.022071	0.980668	0.972184	0.980457	1.01322	0.996988	0.980654
STMN1	Stathmin-1-R-V	1.042716	0.962439	1.048468	1.004372	1.033891	1.035552	1.100918	1.122729
TAZ	TAZ-R-V	1.038054	1.051999	0.956792	1.00317	0.89113	0.880739	0.911377	1.039519

TFAM	TFAM-R-V	1.112136	0.928589	0.939872	1.084099	0.99862	0.985939	0.954646	1.2164
TFRC	TFRC-R-V	1.118271	0.932168	1.151877	1.17562	0.94858	1.346546	0.83634	0.813875
TIGAR	TIGAR-R-V	1.028254	1.090833	1.027105	0.982485	1.026936	0.997098	0.987897	0.976983
TP53	p53-R-C	1.051784	1.04178	1.004503	1.07606	1.032962	0.938715	0.893713	0.997788
TP53BP1	53BP1-R-V	0.9609	0.962368	1.000882	0.996879	1.096747	1.032839	1.012224	1.054239
TRIM25	TRIM25-R-C	1.036474	0.926527	0.975327	0.981092	0.986231	1.063371	1.043916	1.101322
TSC1	TSC1-R-C	1.005906	0.997142	1.001517	0.986424	1.010294	1.013328	0.98951	1.098595
TSC2	Tuberin-R-V	0.912213	0.978696	0.921904	0.943145	0.909952	0.954948	1.011134	0.997068
TSC2	Tuberin_pT1462-R-V	1.014872	1.002998	0.996114	1.002974	0.997805	0.993942	0.96771	0.964165
TTF1	TTF1-R-V	1.076552	0.995674	1.072897	1.015842	0.995452	0.972492	0.976793	0.982951
TUBA1A	D-a-Tubulin-R-V	1.155317	1.120476	0.957415	1.107581	1.095842	1.134902	0.992725	1.145366
TUFM	TUFM-R-V	0.946302	1.011706	1.059823	0.990027	1.031472	0.964934	0.975498	1.066351
TYRO3	Tyro3-R-V	0.991842	0.998426	0.968183	0.999222	0.99937	1.039682	0.99777	0.955203
UBAC1	UBAC1-R-V	1.151327	1.046591	1.051964	1.020879	0.921612	0.961452	0.967442	1.035815
ULK1	ULK1_pS757-R-C	0.98151	1.116275	1.047449	0.94918	0.998627	1.006893	0.98035	0.999663
VASP	VASP-R-V	1.035989	1.0178	0.983387	1.028158	0.969585	0.956973	0.954753	0.991773
VTCN1	B7-H4-R-C	0.948329	1.061588	1.057588	1.01611	1.10609	1.05672	1.102746	1.059894
WEE1	Wee1-R-C	0.950997	0.924727	0.989951	0.977585	1.014368	0.992224	1.003196	0.976269
WIPI1	WIPI1-R-C	0.915829	0.982794	1.046674	1.056311	1.060251	0.980756	1.061343	0.952759
WIPI2	WIPI2-R-C	0.953468	1.03576	0.927222	0.93252	0.92508	0.912755	1.04705	1.294715
XBP1	XBP-1-G-C	0.955304	0.978443	1.079112	0.898542	1.05514	0.940129	0.98076	1.210101
XRCC1	XRCC1-R-C	1.009484	1.030772	0.993237	0.970252	0.993294	0.974061	0.998741	1.055963
YAP1	YAP-R-E	0.993684	0.907218	1.01452	0.968016	1.007673	0.979642	1.004744	1.128608
YAP1	YAP_pS127-R-E	1.022268	0.983659	1.011046	0.969642	0.99592	1.077889	1.139211	1.323482
YBX1	YB1_pS102-R-V	1.051744	0.984728	1.071757	1.02142	0.962543	1.010605	0.977308	1.003726
YWHAB	14-3-3-beta-R-V	1.06474	1.019279	1.043003	1.010208	1.053866	1.035405	0.996302	1.186256
YWHAZ	14-3-3-zeta-R-V	1.015863	1.045188	1.049055	0.979129	1.132682	1.126908	1.001068	1.122446
ZAP70	ZAP-70-R-C	0.985663	0.957963	1.021957	1.052013	1.219512	1.275236	1.017241	1.054687



Supplementary Table 1. Reverse Phase Protein Array (RPPA) analysis suggests distinct JNK1 vs JNK2-regulated proteins

Normalized RPPA data is presented in Tab 1. To assess whether intact JNK1 and JNK2 activity was individually associated with expression of distinct sets of proteins and phospho-proteins within tumors. For this analysis, we identified proteins that were up- or down-regulated in both WT and C116S mutants of JNK1/2 in the absence of JNK-IN-8. Proteins that changed in common between JNK1 and JNK2 were then removed from further consideration. We then focused on proteins for which levels changed in control-treated tumors in a manner that could be suppressed by JNK-IN-8 administration, but retained in JNK1/2^{C116S} expressing tumors in the context of JNK-IN-8. This is a significantly smaller set of proteins, but JNK1-specific changes were observed in 22 proteins and JNK2-specific changes in 29 proteins. The heatmap of normalized levels for these proteins is shown in Tab 2.

Supplementary Table 2. The role of JNK1 vs. JNK2 in BRAFi resistance

M229R	DMSO		JNK-IN-8	
	Log(IC50)	Emax	Log(IC50)	Emax
GFP	-7.36 ± 0.28	0.14 ± 0.01	-7.80 ± 0.19 **	0.26 ± 0.02 ****
JNK1 ^{WT}	-7.80 ± 0.23	0.25 ± 0.02	-7.90 ± 0.17	0.30 ± 0.02 ****
JNK1 ^{C116S}	-7.47 ± 0.18	0.24 ± 0.01	-7.93 ± 0.17 ***	0.31 ± 0.02 ****
JNK2 ^{WT}	-7.99 ± 0.28	0.19 ± 0.02	-7.23 ± 0.17 ****	0.28 ± 0.02 ****
JNK2 ^{C116S}	-7.84 ± 0.21	0.22 ± 0.01	-8.14 ± 0.28	0.20 ± 0.02

SK-MEL28R	DMSO		JNK-IN-8	
	Log(IC50)	Emax	Log(IC50)	Emax
GFP	-5.65 ± 0.19	0.61 ± 0.06	-7.56 ± 0.23 ****	0.53 ± 0.03
JNK1 ^{WT}	-6.82 ± 0.18	0.49 ± 0.03	-8.75 ± 0.17 ****	0.52 ± 0.02
JNK1 ^{C116S}	-6.77 ± 0.14	0.54 ± 0.02	-8.26 ± 0.08 ****	0.55 ± 0.01
JNK2 ^{WT}	-7.24 ± 0.19	0.45 ± 0.03	-8.50 ± 0.18 ****	0.58 ± 0.03 ***
JNK2 ^{C116S}	-6.66 ± 0.07	0.61 ± 0.01	-6.79 ± 0.12	0.56 ± 0.04

* $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.001$, **** $P \leq 0.0001$, JNK-IN-8 versus DMSO within groups.
Log(IC50): Log value of half maximal inhibitory concentration (IC50)

BRAFi-resistant melanoma cell lines transduced with JNK1/2^{WT} and JNK1/2^{C116S} were treated with increasing concentrations of dabrafenib in the presence or absence of JNK-IN-8 for 72 hours as described under *Materials and Methods*. Full dose response curves were fit in GraphPad using a standard four parameter logistic regression fit in which both IC50 and Emax were estimated. In the m229R cells, which exhibited the highest level of phospho-JNK and phospho-c-JUN, JNK-IN-8 increased the Emax in all derivative lines with the exception of the one expressing JNK2^{C116S}. In the SK-MEL28R cells, JNK-IN-8 decreased the IC50 in all derivative lines with the exception of the one expressing JNK2^{C116S}.