

Supplemental Material

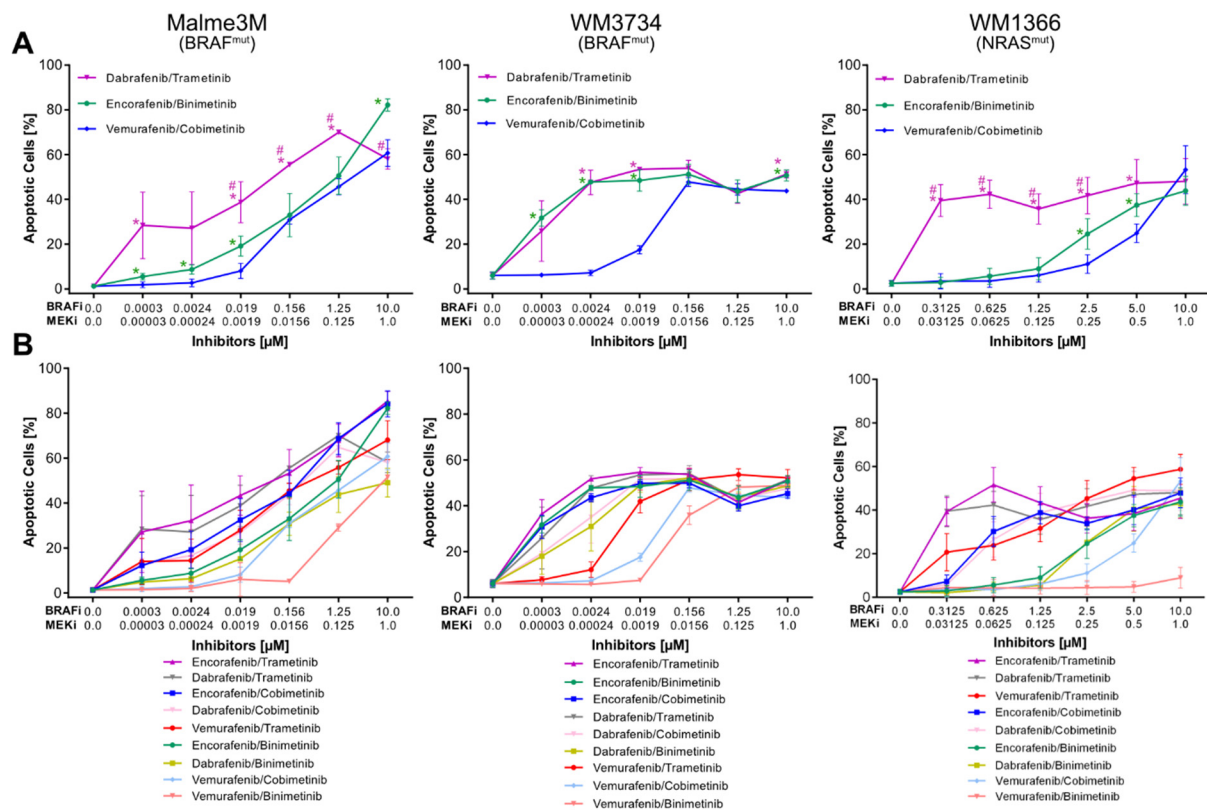


Figure S1: Encorafenib/trametinib represents the most efficient unconventional and dabrafenib/trametinib the best clinically approved inhibitor combination in BRAF^{mut} and NRAS^{mut} melanoma cells. Sub-G1 (apoptosis) of two BRAF^{mut} cell lines (Malme3M and WM3734) and one NRAS^{mut} cell line (WM1366) after treatment for 72 hours using different BRAFi/MEKi combinations with increasing concentrations of inhibitors (BRAF^{mut} 1:8 serial dilutions, NRAS^{mut} 1:2 serial dilutions). $n \geq 3$, error bars indicate standard deviation. (A) Panel compares approved inhibitor combinations for BRAF^{mut} melanoma, * indicates significantly different values relative to vemurafenib/cobimetinib, # labels data points significantly different relative to encorafenib/binimetinib. Significance defined as $p < 0.05$ based on two-tailed non-paired T-test. Table S3 shows complete list of p-values for all data points. (B) Panel summarizes all tested inhibitor combinations. Significances not shown.

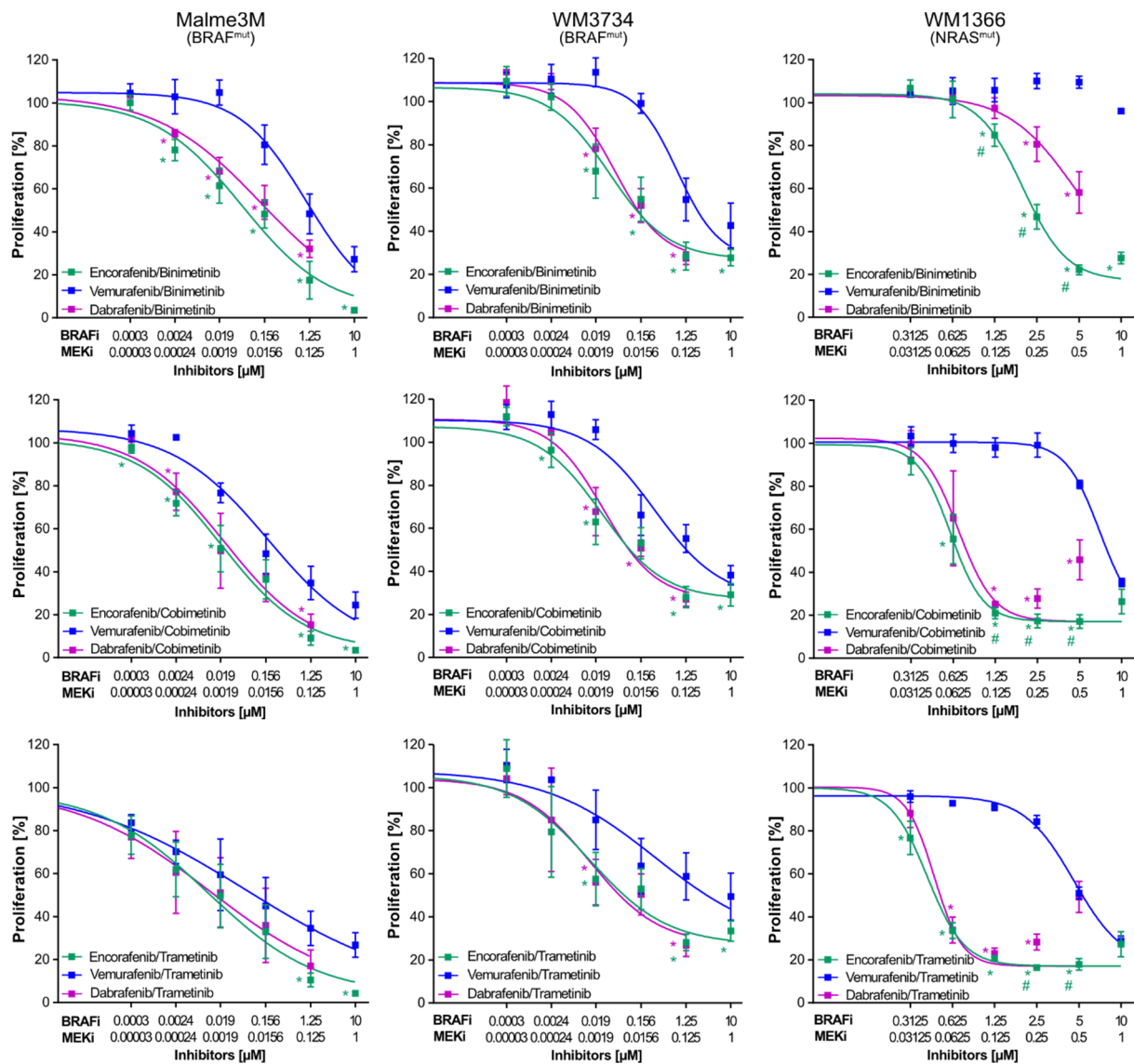


Figure S2: IC₅₀ curves modeled from MUH proliferation data displaying differences for BRAFi. $n \geq 3$, error bars indicate standard deviation, * indicates significantly different values relative to vemurafenib, # labels data points significantly different relative to dabrafenib. Significance defined as $p < 0.05$ based on two-tailed non-paired T-test. Table S5 shows a complete list of p-values for all data points.

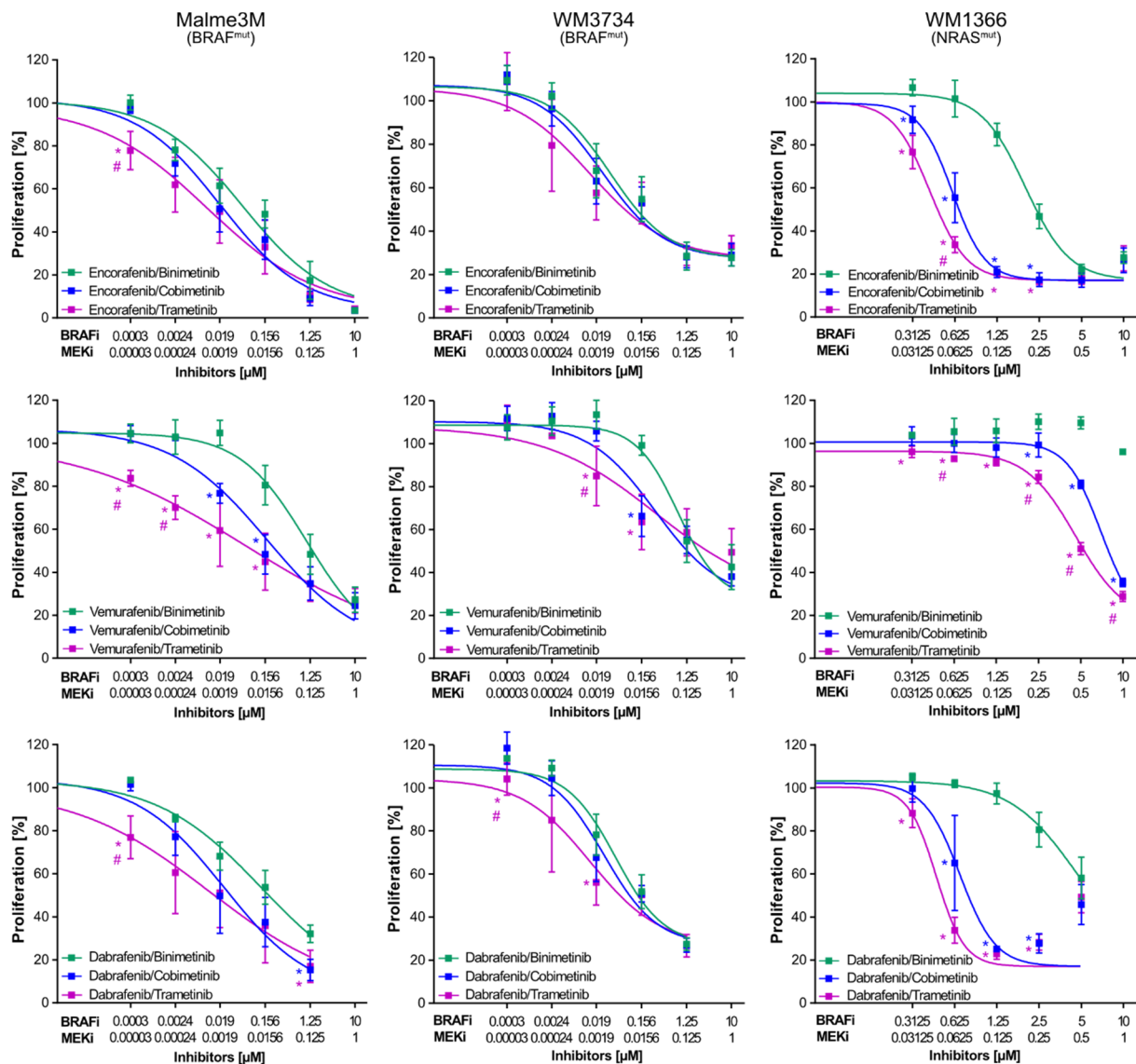


Figure S3: IC₅₀ curves modeled from MUH proliferation data displaying differences for MEKi. $n \geq 3$, error bars indicate standard deviation, * indicates significantly different values relative to vemurafenib, # labels data points significantly different relative to dabrafenib. Significance defined as $p < 0.05$ based on two-tailed non-paired T-test. Table S6 shows a complete list of p-values for all data points.

Table S1: List of p-values from subG1 analysis, related to Figure 1.

Malme3M (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EC/VC	EC/DC	VC/DC	ET/VT	ET/DT	VT/DT
0.0003/0.00003	0.00926	0.50751	0.00792	0.04350	0.90822	0.00362	0.33336	0.92797	0.23650
0.0024/0.00024	0.00996	0.12183	0.00708	0.02716	0.67199	0.01557	0.17214	0.72018	0.30830
0.019/0.0019	0.05842	0.20106	0.10122	0.01425	0.42710	0.00639	0.10195	0.57198	0.21777
0.156/0.0156	0.00774	0.73956	0.00103	0.10076	0.97737	0.01099	0.28965	0.73124	0.00756
1.25/0.125	0.01170	0.36204	0.03493	0.00759	0.46498	0.00498	0.05507	0.64993	0.00133
10/1	0.00004	0.00117	0.53419	0.00816	0.01218	0.67732	0.03543	0.00172	0.15356
WM3734 (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EC/VC	EC/DC	VC/DC	ET/VT	ET/DT	VT/DT
0.0003/0.00003	0.00029	0.05337	0.05807	0.00115	0.19782	0.13304	0.00151	0.29019	0.08136
0.0024/0.00024	0.00000	0.05399	0.01496	0.00001	0.26774	0.01339	0.00004	0.26745	0.00068
0.019/0.0019	0.00012	0.89402	0.00002	0.00002	0.33596	0.00004	0.01561	0.40526	0.01722
0.156/0.0156	0.01223	0.74520	0.00311	0.28001	0.56392	0.26384	0.50961	0.90949	0.49747
1.25/0.125	0.24995	0.97658	0.09721	0.08291	0.15193	0.50204	0.00478	0.71378	0.01994
10/1	0.51134	0.24367	0.95457	0.30740	0.30355	0.06184	0.68383	0.84418	0.77401
WM1366 (NRAS ^{mut})									
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EC/VC	EC/DC	VC/DC	ET/VT	ET/DT	VT/DT
0.3125/0.03125	0.48307	0.61299	0.26616	0.14906	0.47553	0.28519	0.01365	0.96846	0.01501
0.625/0.0625	0.62789	0.46298	0.83763	0.00038	0.51007	0.00175	0.00179	0.11670	0.00719
1.25/0.125	0.13613	0.23894	0.50098	0.00003	0.99626	0.00044	0.05027	0.17498	0.40851
2.5/0.25	0.00177	0.90909	0.00213	0.00011	0.04996	0.00031	0.14343	0.34469	0.56205
5/0.5	0.00003	0.61931	0.00053	0.00867	0.14892	0.00222	0.01339	0.22404	0.26091
10/1	0.00013	0.84124	0.00014	0.42749	0.84743	0.56873	0.06183	0.71664	0.13577

E...encorafenib; V...vemurafenib; D...dabrafenib; B...binimetinib; C...cobimetinib; T...trametinib; bold numbers indicate significant values with p<0.05 as determined by two-tailed non-paired T-test.

Table S2: List of p-values from subG1 analysis, related to Figure 2.

Malme3M (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/EC	EB/ET	EC/ET	VB/VC	VB/VT	VC/VT	DB/DC	DB/DT	DC/DT
0.0003/0.00003	0.13393	0.10823	0.24608	0.56391	0.10224	0.11435	0.00993	0.05117	0.14475
0.0024/0.00024	0.09811	0.06342	0.27715	0.60258	0.09217	0.10817	0.03495	0.09032	0.34912
0.019/0.0019	0.09649	0.01367	0.22261	0.68431	0.03099	0.02283	0.01850	0.01148	0.12348
0.156/0.0156	0.25303	0.07165	0.34600	0.00002	0.00003	0.00289	0.03055	0.00123	0.01289
1.25/0.125	0.04594	0.05294	0.90242	0.00193	0.00016	0.01943	0.01694	0.00477	0.12677
10/1	0.62594	0.34050	0.78321	0.05494	0.02942	0.29196	0.22047	0.11662	0.98670
WM3734 (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/EC	EB/ET	EC/ET	VB/VC	VB/VT	VC/VT	DB/DC	DB/DT	DC/DT
0.0003/0.00003	0.80307	0.33424	0.29694	0.51316	0.09424	0.16057	0.88814	0.43371	0.55842
0.0024/0.00024	0.02353	0.01095	0.00217	0.11515	0.03106	0.07854	0.67705	0.07498	0.15781
0.019/0.0019	0.69042	0.11063	0.03500	0.00066	0.00030	0.00141	0.29951	0.06642	0.24514
0.156/0.0156	0.66666	0.45381	0.11214	0.01059	0.01330	0.29782	0.91802	0.46549	0.57790
1.25/0.125	0.30807	0.53416	0.50663	0.17565	0.06851	0.01232	0.82298	0.75291	0.85798
10/1	0.04460	0.81549	0.01538	0.08502	0.34559	0.01959	0.41249	0.07043	0.08010
WM1366 (NRAS ^{mut})									
BRAFi/MEKi [μM]	EB/EC	EB/ET	EC/ET	VB/VC	VB/VT	VC/VT	DB/DC	DB/DT	DC/DT
0.3125/0.03125	0.06662	0.00005	0.00011	0.69475	0.01225	0.00967	0.04172	0.00006	0.00010
0.625/0.0625	0.00073	0.00004	0.00661	0.74687	0.00240	0.00148	0.00197	0.00004	0.02175
1.25/0.125	0.00016	0.00024	0.34235	0.38142	0.00019	0.00032	0.00035	0.00015	0.59852
2.5/0.25	0.04686	0.05199	0.54440	0.04445	0.00009	0.00032	0.01270	0.02427	0.68364
5/0.5	0.55919	0.85258	0.74865	0.00017	0.00000	0.00011	0.24622	0.39733	0.78858
10/1	0.42509	0.78017	0.69431	0.00028	0.00002	0.42301	0.31539	0.41892	0.90006

E...encorafenib; V...vemurafenib; D...dabrafenib; B...binimetinib; C...cobimetinib; T...trametinib; bold numbers indicate significant values with p<0.05 as determined by two-tailed non-paired T-test.

Table S3: List of p-values from subG1 analysis, related to Figure S1A.

Malme3M (BRAF ^{mut})				WM3734 (BRAF ^{mut})			WM1366 (NRAS ^{mut})			
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EB/VB	EB/DB	VB/DB	BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB
0.0003/0.00003	0.03246	0.05644	0.03679	0.00030	0.50912	0.06629	0.3125/0.03125	0.48307	0.61299	0.26616
0.0024/0.00024	0.01939	0.12323	0.06077	0.00000	0.96149	0.00024	0.625/0.0625	0.62789	0.46298	0.83763
0.019/0.0019	0.02677	0.02979	0.00562	0.00047	0.14939	0.00001	1.25/0.125	0.13613	0.23894	0.50098
0.156/0.0156	0.73636	0.01638	0.00004	0.29814	0.44901	0.05649	2.5/0.25	0.00177	0.90909	0.00213
1.25/0.125	0.40243	0.01598	0.00035	0.80294	0.79512	0.54429	5/0.5	0.00003	0.61931	0.00053
10/1	0.00471	0.00151	0.58050	0.00990	0.72283	0.00262	10/1	0.00013	0.84124	0.00014

E...encorafenib; V...vemurafenib; D...dabrafenib; B...binimetinib; C...cobimetinib; T...trametinib; bold numbers indicate significant values with p<0.05 as determined by two-tailed non-paired T-test.

Table S4: Comparison of IC₅₀ values for single and combined inhibitor treatment.

Malme3M (BRAF^{mut})				
IC₅₀ (μM)	Single BRAFi	Binimetinib	Cobimetinib	Trametinib
Single MEKi		2.12400	0.08621	0.00922
Encorafenib	0.0841	0.0537 0.00537	0.0194 0.00194	0.0107 0.00107
Vemurafenib	3.0400	1.0780 0.10780	0.1684 0.01684	0.0620 0.00620
Dabrafenib	0.3286	0.1294 0.01294	0.0227 0.00227	0.0119 0.00119
WM3734 (BRAF^{mut})				
IC₅₀ (μM)	Single BRAFi	Binimetinib	Cobimetinib	Trametinib
Single MEKi		3.75800	0.48130	0.10690
Encorafenib	0.0466	0.0356 0.00356	0.0247 0.00247	0.0143 0.00143
Vemurafenib	2.6450	0.8345 0.08345	0.2885 0.02885	0.3022 0.03022
Dabrafenib	0.0546	0.0458 0.00458	0.0271 0.00271	0.0145 0.00145
WM1366 (NRAS^{mut})				
IC₅₀ (μM)	Single BRAFi	Binimetinib	Cobimetinib	Trametinib
Single MEKi		NC	12.08000	4.41400
Encorafenib	NC	1.9830 0.19830	0.5985 0.05985	0.4139 0.04139
Vemurafenib	NC	NC NC	6.9750 0.69750	4.6110 0.46110
Dabrafenib	NC	4.6610 0.46610	0.6812 0.06812	0.4631 0.04631

IC₅₀...half maximum inhibitory concentration, NC...not converged

Table S5: List of p-values from MUH proliferation data, related to Figure S2.

Malme3M (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EC/VC	EC/DC	VC/DC	ET/VT	ET/DT	VT/DT
0.0003/0.00003	0.23484	0.18011	0.69762	0.04834	0.10860	0.38831	0.34917	0.90818	0.32922
0.0024/0.00024	0.01038	0.06477	0.02239	0.00090	0.42776	0.00739	0.36330	0.92152	0.44996
0.019/0.0019	0.00170	0.31932	0.00197	0.01879	0.93199	0.06008	0.48216	0.90218	0.57006
0.156/0.0156	0.00768	0.40761	0.01865	0.18787	0.90605	0.27080	0.31847	0.82795	0.51007
1.25/0.125	0.01406	0.06191	0.04905	0.00614	0.14929	0.02160	0.00876	0.23519	0.05049
10/1	0.00227	ND	ND	0.00405	ND	ND	0.00251	ND	ND
WM3734 (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EC/VC	EC/DC	VC/DC	ET/VT	ET/DT	VT/DT
0.0003/0.00003	0.70308	0.26181	0.08809	0.94424	0.18218	0.20030	0.88113	0.57058	0.35288
0.0024/0.00024	0.11532	0.09949	0.74236	0.01767	0.19744	0.15623	0.11083	0.74191	0.24507
0.019/0.0019	0.00066	0.23365	0.00093	0.00029	0.55804	0.00075	0.04004	0.86969	0.02539
0.156/0.0156	0.00022	0.67198	0.00005	0.07104	0.60394	0.02388	0.25891	0.72112	0.17495
1.25/0.125	0.00445	0.77767	0.00189	0.00056	0.73079	0.00024	0.00318	0.68895	0.00343
10/1	0.03813	ND	ND	0.03774	ND	ND	0.04398	ND	ND
WM1366 (NRAS ^{mut})									
BRAFi/MEKi [μM]	EB/VB	EB/DB	VB/DB	EC/VC	EC/DC	VC/DC	ET/VT	ET/DT	VT/DT
0.3125/0.03125	0.27420	0.56233	0.28905	0.05770	0.19300	0.45232	0.01494	0.12138	0.13772
0.625/0.0625	0.55579	0.92364	0.41584	0.00342	0.53766	0.05522	0.00001	0.94300	0.00007
1.25/0.125	0.00903	0.03923	0.12049	0.00001	0.04340	0.00001	0.00000	0.24061	0.00000
2.5/0.25	0.00009	0.00397	0.00442	0.00003	0.02914	0.00007	0.00000	0.00639	0.00003
5/0.5	0.00000	0.00340	0.00094	0.00001	0.00713	0.00315	0.00012	0.00219	0.69943
10/1	0.00000	ND	ND	0.06481	ND	ND	0.69309	ND	ND

E...encorafenib; V...vemurafenib; D...dabrafenib; B...binimetinib; C...cobimetinib; T...trametinib; ND...not determined; bold numbers indicate significant values with p<0.05 as determined by two-tailed non-paired T-test.

Table S6: List of p-values from MUH proliferation data, related to Figure S3.

Malme3M (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/EC	EB/ET	EC/ET	VB/VC	VB/VT	VC/VT	DB/DC	DB/DT	DC/DT
0.0003/0.00003	0.29511	0.01589	0.02120	0.95532	0.00324	0.00272	0.36441	0.01017	0.01504
0.0024/0.00024	0.23360	0.10863	0.28399	0.94449	0.00436	0.00060	0.16801	0.08452	0.23952
0.019/0.0019	0.24524	0.28623	0.90821	0.00289	0.01144	0.15881	0.15961	0.16493	0.92187
0.156/0.0156	0.14502	0.13535	0.71754	0.01269	0.01847	0.73149	0.11439	0.18000	0.89552
1.25/0.125	0.19667	0.26341	0.63154	0.12361	0.12402	0.97069	0.01111	0.03714	0.76095
10/1	0.91088	0.08211	0.05383	0.60273	0.91426	0.66938	ND	ND	ND
WM3734 (BRAF ^{mut})									
BRAFi/MEKi [μM]	EB/EC	EB/ET	EC/ET	VB/VC	VB/VT	VC/VT	DB/DC	DB/DT	DC/DT
0.0003/0.00003	0.55416	0.93331	0.66520	0.35930	0.64264	0.77152	0.25710	0.04641	0.03625
0.0024/0.00024	0.29233	0.08428	0.18482	0.63917	0.14101	0.05638	0.33315	0.09244	0.17180
0.019/0.0019	0.57584	0.28861	0.52877	0.10934	0.01454	0.03417	0.20502	0.02109	0.18266
0.156/0.0156	0.80420	0.80173	0.97327	0.00077	0.00331	0.76684	0.82688	0.81828	0.92817
1.25/0.125	0.93005	0.92718	0.99647	0.92636	0.62501	0.60976	0.85754	0.82579	0.93268
10/1	0.69534	0.11824	0.27196	0.46664	0.44221	0.11655	ND	ND	ND
WM1366 (NRAS ^{mut})									
BRAFi/MEKi [μM]	EB/EC	EB/ET	EC/ET	VB/VC	VB/VT	VC/VT	DB/DC	DB/DT	DC/DT
0.3125/0.03125	0.02483	0.00383	0.05993	0.89550	0.00929	0.06674	0.22332	0.01353	0.09701
0.625/0.0625	0.00536	0.00024	0.03684	0.28948	0.02607	0.04390	0.04536	0.00005	0.07769
1.25/0.125	0.00005	0.00003	0.90252	0.13312	0.01192	0.06563	0.00001	0.00002	0.19692
2.5/0.25	0.00146	0.00084	0.65062	0.04903	0.00069	0.01513	0.00056	0.00050	0.91116
5/0.5	0.08528	0.10404	0.74835	0.00015	0.00001	0.00011	0.18918	0.26927	0.65211
10/1	0.75555	0.92560	0.86538	0.00000	0.00000	0.01922	ND	ND	ND

E...encorafenib; V...vemurafenib; D...dabrafenib; B...binimetinib; C...cobimetinib; T...trametinib; ND...not determined; bold numbers indicate significant values with p<0.05 as determined by two-tailed non-paired T-test.