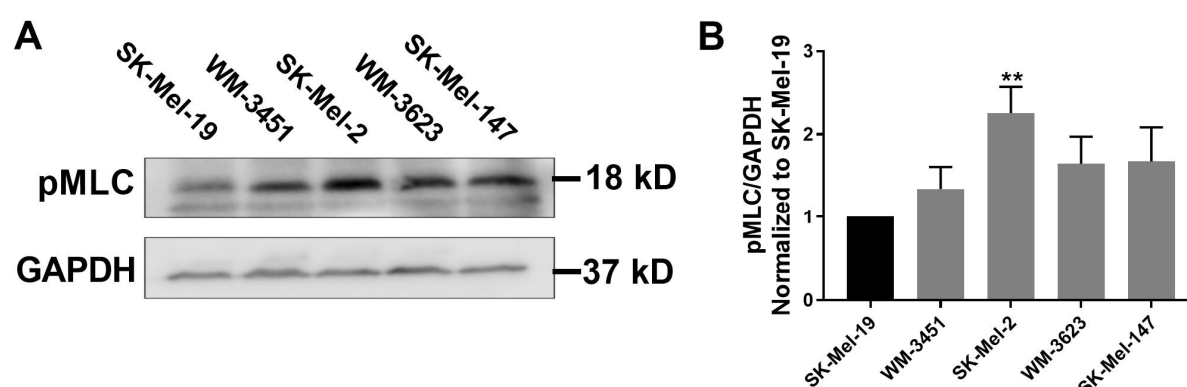
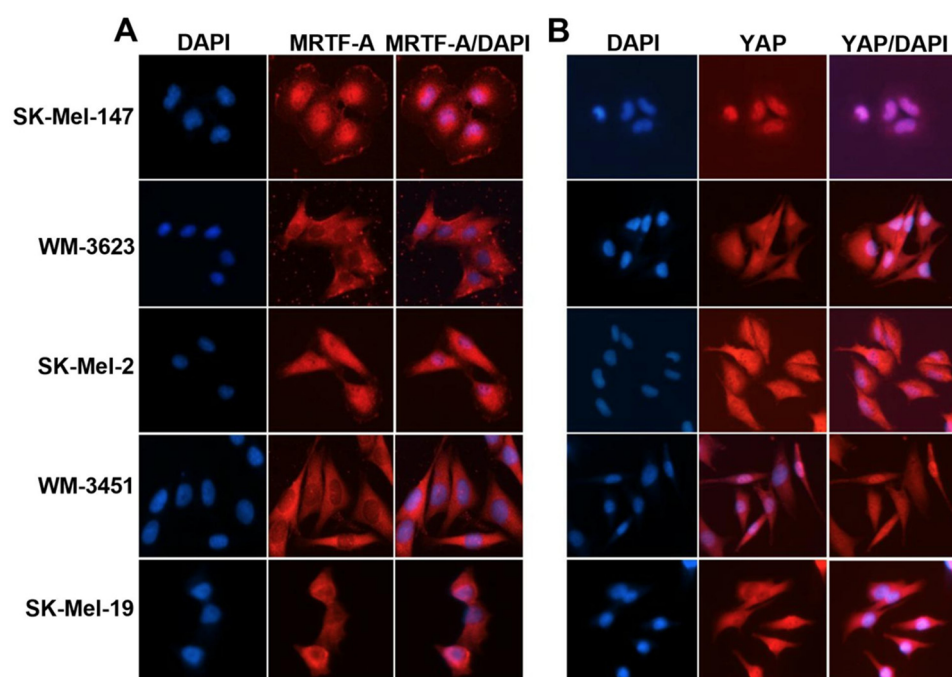


Inhibition of the Myocardin-Related Transcription Factor Pathway Increases Efficacy of Trametinib in *NRAS*-Mutant Melanoma Cell Lines

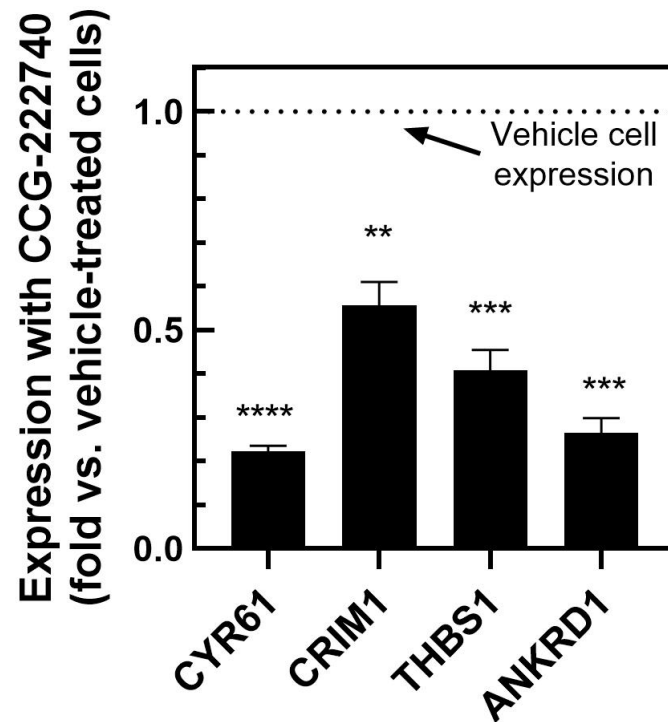
Kathryn M. Appleton, Charuta C. Palsuledesai, Sean A. Misek, Maja Blake, Joseph Zagorski, Kathleen A. Gallo, Thomas S. Dexheimer and Richard R. Neubig



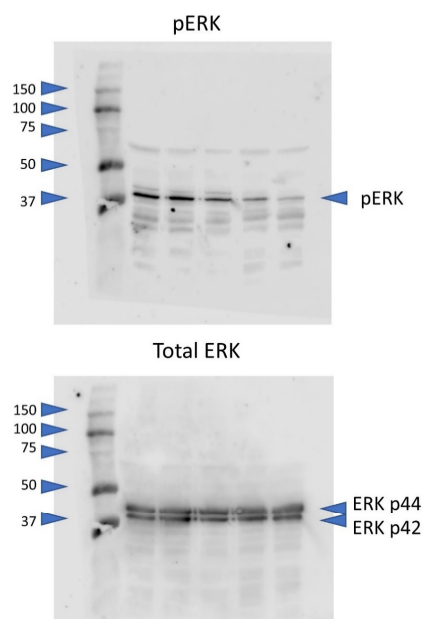
Supplementary Figure S1. Expression of phospho-myosin light chain (pMLC) in melanoma cell lines. **A.** Immunoblots were conducted across the melanoma cell line panel to detect pMLC or GAPDH as a protein loading control. **B.** Quantitative band density analysis was performed for each experiment comparing the intensity of pMLC relative to control GAPDH then normalized to the value for SK-Mel-19 from the same experiment. Results are expressed as the mean (\pm SEM) of five experiments ($n=5$, ** $p<0.01$ vs. SK-Mel-19, One-way ANOVA, with Dunnett's post-test).



Supplementary Figure S2. Localization of MRTF-A and YAP in melanoma cell lines. **A.** Representative images of the cellular localization of MRTF-A using immunofluorescence in melanoma cell lines (10% FBS). **B.** Representative images of the cellular localization of YAP using immunofluorescence in melanoma cell lines (10% FBS).

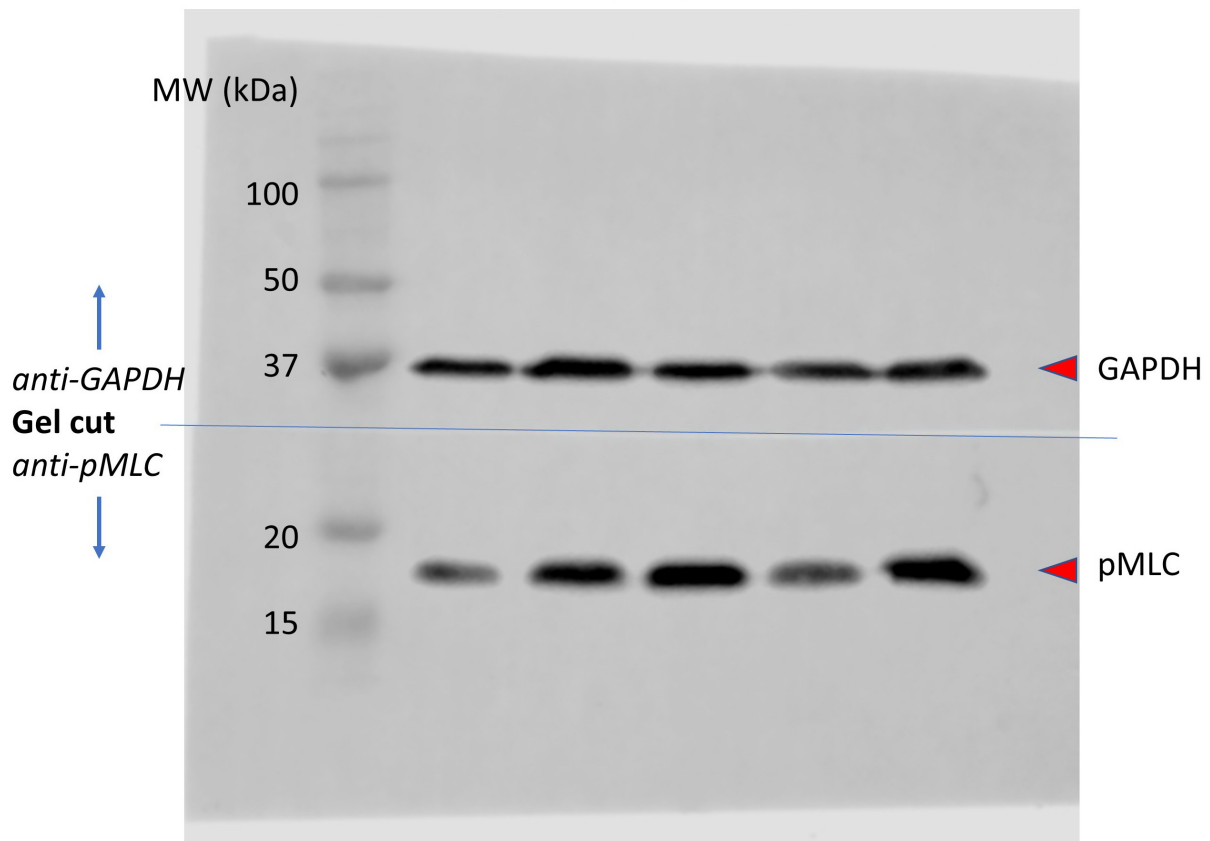


Supplementary Figure S3. Effect of CCG-222740 on expression of MRTF-target genes in SK-Mel-147 cells. CCG-222740 treatment reduces expression of MRTF target genes *CYR61*, *CRIM1*, *THBS1* and *ANKRD1*. SK-Mel-147 cells were treated for 24 hours with 10 μ M CCG-222740 or DMSO (0.02%) before isolation of RNA for qPCR analysis. Expression of target genes were normalized against *GAPDH* and results are expressed as the mean (\pm SEM) of triplicate experiments (n=3, *, $P < 0.01$, **, $P < 0.001$, ***, $P < 0.0001$, ****, $P < 0.00001$ vs. DMSO control).



Supplementary Figure S4 Full blots for ERK and pERK supporting Figure 1C & D

Western Blot of phospho-MLC2



Supplementary Figure S5 Full blot for pMLC and GAPDH supporting Figure S1

Supplementary Table S1. RNA-Seq analysis of differential gene expression in SK-Mel-147 cells upon 24-hour treatment with CCG-222740. Effects of 10 mM CCG-222740 in SK-Mel-147 cells was compared to untreated cells. See Methods section 4.8 of main manuscript for experimental details. Negative logFC values indicate genes downregulated upon CCG-222740 treatment, and positive values indicate genes upregulated upon CCG-222740 treatment. Raw RNA-Seq reads and processed HTSeq read counts are available on GEO under GSE134320.

Ensembl ID	Approved Symbol	logFC	logCPM	PValue	FDR
ENSG00000168003	<i>SLC3A2</i>	1.740277527	8.507639444	1.21E-14	7.71E-10
ENSG00000115380	<i>EFEMP1</i>	-1.706565216	3.530197807	9.55E-11	3.04E-06
ENSG00000091986	<i>CCDC80</i>	-1.495390124	4.347019002	4.67E-09	9.92E-05
ENSG00000181449	<i>SOX2</i>	2.140598107	0.239972409	3.44E-08	0.000547402
ENSG00000104419	<i>NDRG1</i>	1.363870834	5.978803232	4.66E-08	0.000586459
ENSG00000148677	<i>ANKRD1</i>	-1.397055415	4.627081746	5.59E-08	0.000586459
ENSG00000150938	<i>CRIM1</i>	-1.308149346	6.808500344	6.45E-08	0.000586459
ENSG00000224773	<i>HSPA8P7</i>	-1.98036841	2.305467038	1.86E-07	0.001477206
ENSG00000137801	<i>THBS1</i>	-1.185139541	10.08725598	2.85E-07	0.00201518
ENSG00000196878	<i>LAMB3</i>	1.137023364	7.353579658	9.04E-07	0.005469966
ENSG00000213820	<i>RPL13P2</i>	-2.378109738	2.097258073	9.45E-07	0.005469966
ENSG00000128564	<i>VGF</i>	1.413051246	1.555022873	1.3E-06	0.006602879
ENSG00000154734	<i>ADAMTS1</i>	-1.102716883	6.357359483	1.35E-06	0.006602879
ENSG00000236105	<i>PRELID3BP10</i>	-2.521855225	-0.667607432	1.89E-06	0.008593398
ENSG00000231445	<i>TIMM8AP1</i>	-2.03756353	-0.277981649	2.78E-06	0.01016252
ENSG00000129757	<i>CDKN1C</i>	1.19260645	3.011058803	2.85E-06	0.01016252
ENSG00000142871	<i>CYR61</i>	-1.036485735	9.347827715	2.87E-06	0.01016252
ENSG00000092969	<i>TGFB2</i>	-1.146679925	4.714269799	3.1E-06	0.010396226
ENSG00000052841	<i>TTC17</i>	1.075378747	6.169282608	5.58E-06	0.017771644
ENSG00000078401	<i>EDN1</i>	-1.202295537	3.121136369	5.94E-06	0.018013191
ENSG00000052802	<i>MSMO1</i>	1.079766996	5.379290384	7.15E-06	0.020689773
ENSG00000138798	<i>EGF</i>	-1.282835697	2.550065363	7.79E-06	0.021564115
ENSG00000170439	<i>METTL7B</i>	1.152224231	2.41811322	1.06E-05	0.028090638
ENSG00000114251	<i>WNT5A</i>	-1.050718673	5.271773994	1.39E-05	0.032740269
ENSG00000232480	<i>TGFB2-AS1</i>	-1.851166895	0.032554217	1.4E-05	0.032740269
ENSG00000168028	<i>RPSA</i>	-1.157332873	6.684989777	1.44E-05	0.032740269
ENSG00000145632	<i>PLK2</i>	-0.9729373	7.098792127	1.44E-05	0.032740269
ENSG00000151892	<i>GFRA1</i>	-1.241962153	1.757886426	1.67E-05	0.035350063
ENSG00000170523	<i>KRT83</i>	-1.706326252	0.010133492	2.25E-05	0.045127787
ENSG00000214274	<i>ANG</i>	1.837466378	-0.700807455	2.27E-05	0.045127787

Supplementary Table S2 Intensities for ERK and pERK supporting Figure 1C & D

Individual band ratios from Figure 1 – three independent experiments

	SK-Mel-19	WM-3451	SK-Mel-2	WM-3623	SK-Mel-147
Experiment 1					
<i>pERK</i>	4.22	4.38	2.92	1.64	1.09
<i>ERK1/2</i>	7.96	8.8	8.36	9.21	9.77
<i>pERK/ERK ratio</i>	0.530	0.498	0.349	0.178	0.112
norm to SK-Mel-19	1.000	0.939	0.659	0.336	0.210
Experiment 2					
<i>pERK</i>	4.85	4.86	3.36	2.73	1.18
<i>ERK1/2</i>	28.6	24.3	27.5	30.6	33.2
<i>pERK/ERK ratio</i>	0.170	0.200	0.122	0.089	0.036
norm to SK-Mel-19	1.000	1.179	0.720	0.526	0.210
Experiment 3					
<i>pERK</i>	4.92	5.21	4.44	1.86	1.48
<i>ERK1/2</i>	12.4	12.1	13.8	20.8	20.4
<i>pERK/ERK ratio</i>	0.397	0.431	0.322	0.089	0.073
norm to SK-Mel-19	1.000	1.085	0.811	0.225	0.183

Supplementary Table 3 Intensities ratios for pMLC/GAPDH vs SK-Mel-19 supporting Figure S1

Individual band ratios from Figure S1

	SK-Mel-19	WM-3451	SK-Mel-2	WM-3623	SK-Mel-147
GAPDH	1.07	1.26	1.06	1.27	1.06
pMLC	0.97	1.11	1.89	1.36	1.49
pMLC/GAPDH	0.902	0.881	1.783	1.071	1.406
Norm to SK-Mel-19*	1*	0.977	1.977	1.187	1.559

Normalized band ratios from 5 independent experiments

SK-Mel-19	WM-3451	SK-Mel-2	WM-3623	SK-Mel-147
1	1.242	2.092	1.271	1.880
1	1.143	2.009	2.344	0.765
1	2.385	3.488	2.510	3.116
1	0.954	1.721	0.917	1.047
1*	0.977	1.977	1.187	1.559

* Data from blot in Figure S1