

Supplemental information

**AP-1 transcription factor network
explains diverse patterns
of cellular plasticity in melanoma cells**

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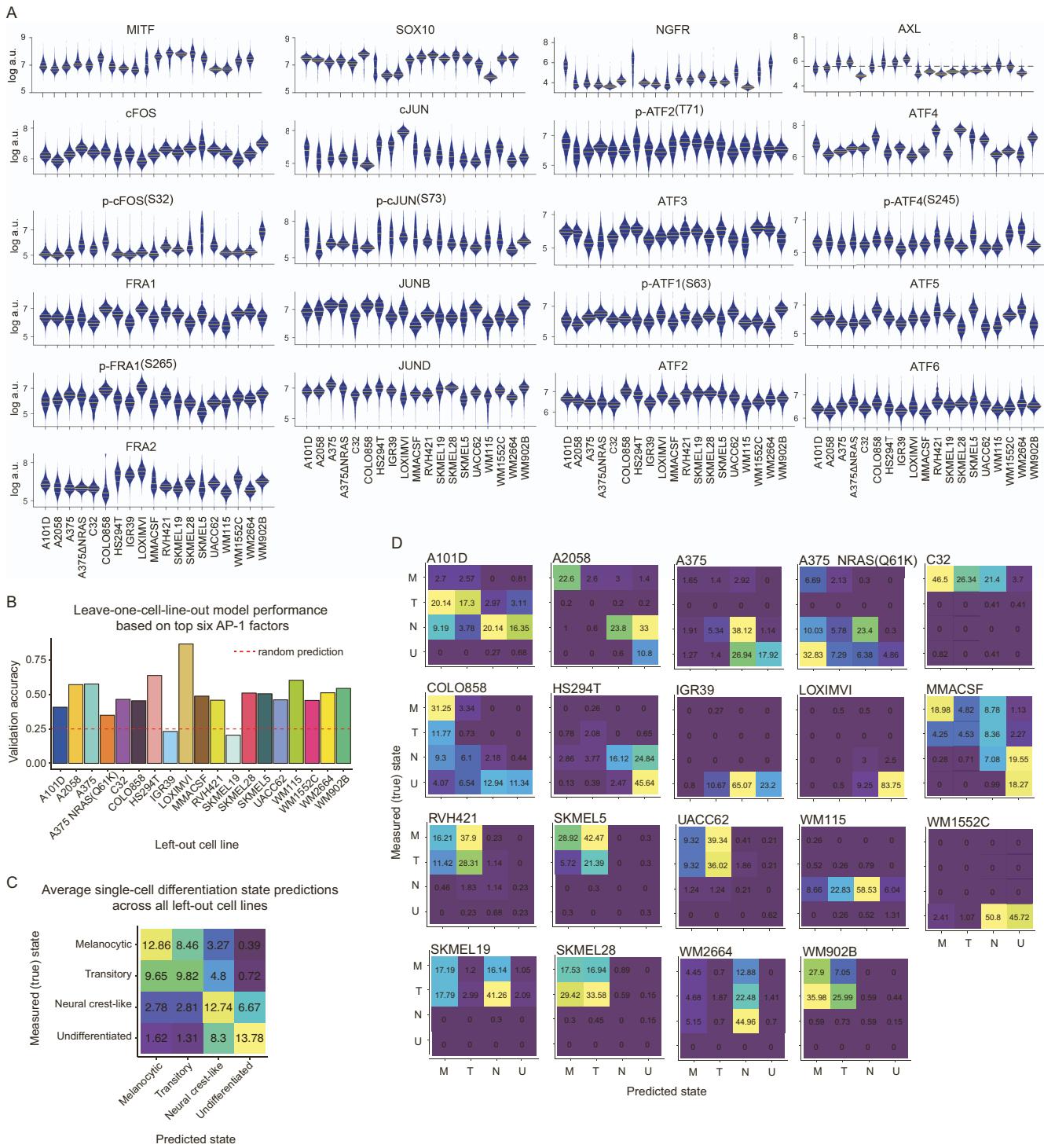
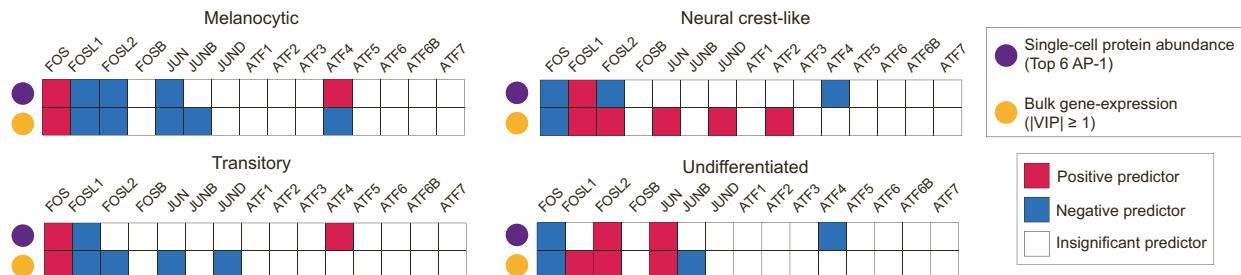


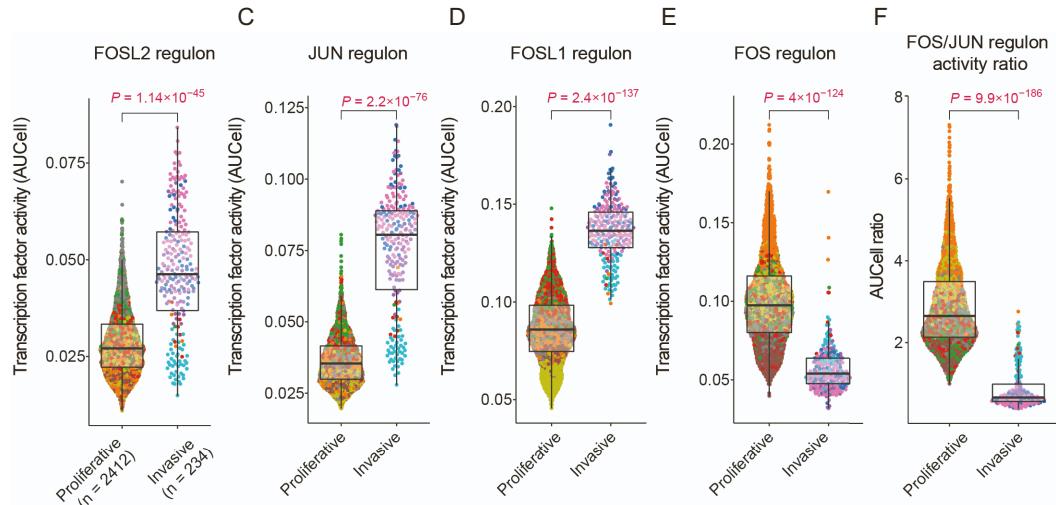
Figure S1. Single-cell AP-1 protein levels predict differentiation state heterogeneity in melanoma cells. Related to Figure 1. (A) Single-cell distributions of seventeen AP-1 factors and four differentiation state markers measured across 19 cell lines and shown by violin plots highlighting the median and interquartile (25% and 75%) ranges. (B) Random Forest model cross-validation performance (using the top six AP-1 factors) to predict the differentiation state of new cells from independent cell lines not included in model training. At each iteration, one cell line was removed, a model was built using randomly sampled cells from the remaining 18 cell lines, and then the trained model was used to predict the differentiation state of randomly selected cells from the left-out cell line. Red dash line indicates accuracy from random prediction (25%). (C) Confusion matrix showing the cross-validation performance of the random forest classifier. Numbers shown are in terms of average percentage of cells in the indicated categories. (D) Confusion matrices showing the model cross-validation performance for each independent cell line.

A

Summary of AP-1 protein and transcript contributions to differentiation state prediction



B



G

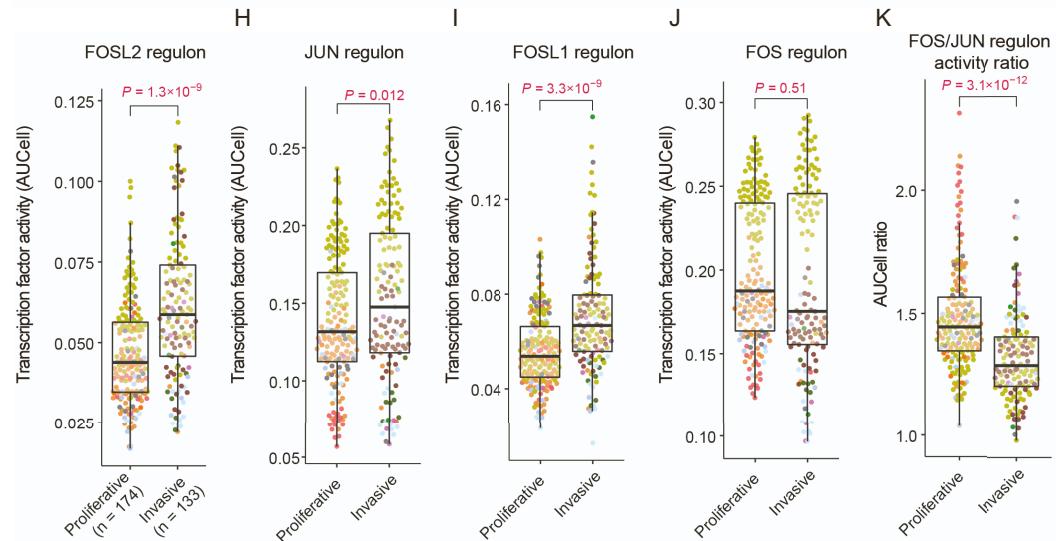


Figure S2. AP-1 protein, transcript levels and activities predict variations in differentiation state and phenotype across melanoma cell lines and single cells. Related to Figures 1-3. (A) Summary of contributions of 15 AP-1 factors in predicting each melanoma differentiation state based on analyses performed in this study on single-cell protein measurements and bulk gene expression data. Significant positive and negative predictors are highlighted. Significant AP-1 proteins were determined based on their SHAP importance in the random forest model. Significant AP-1 genes were determined based on the significance of their VIP scores (i.e., magnitude greater than 1) in the PLSR model. (B-F) Single-cell distributions of the activity of SCENIC regulons for FOSL2 (B), JUN (C), FOSL1 (D), and FOS (E) motifs, as well as the ratio of FOS and JUN regulon activities (F), measured using AUCell in individual cells (from 10 melanoma cell lines profiled by Wouters *et al*) across the two-class (“proliferative” versus “invasive”) melanoma phenotypes. The phenotype of individual cells was determined based on their gated levels of enrichment (quantified by AUCell) for the gene signatures as defined by Hoek *et al*. (G-K) Single-cell distributions of the AUCell activity of SCENIC regulons for FOSL2 (G), JUN (H), FOSL1 (I), and FOS (J) motifs, as well as the ratio of FOS and JUN regulon activities (K), quantified in individual cells from 12 treatment-naïve melanoma tumors as profiled by Tirosh *et al* and Jerby-Arnon *et al*. Statistical comparisons were performed using two-sided unpaired *t* test. Boxplot hinges correspond to the lower and upper quartiles, with a band at median. Whiskers indicate 1.5 times interquartile ranges.

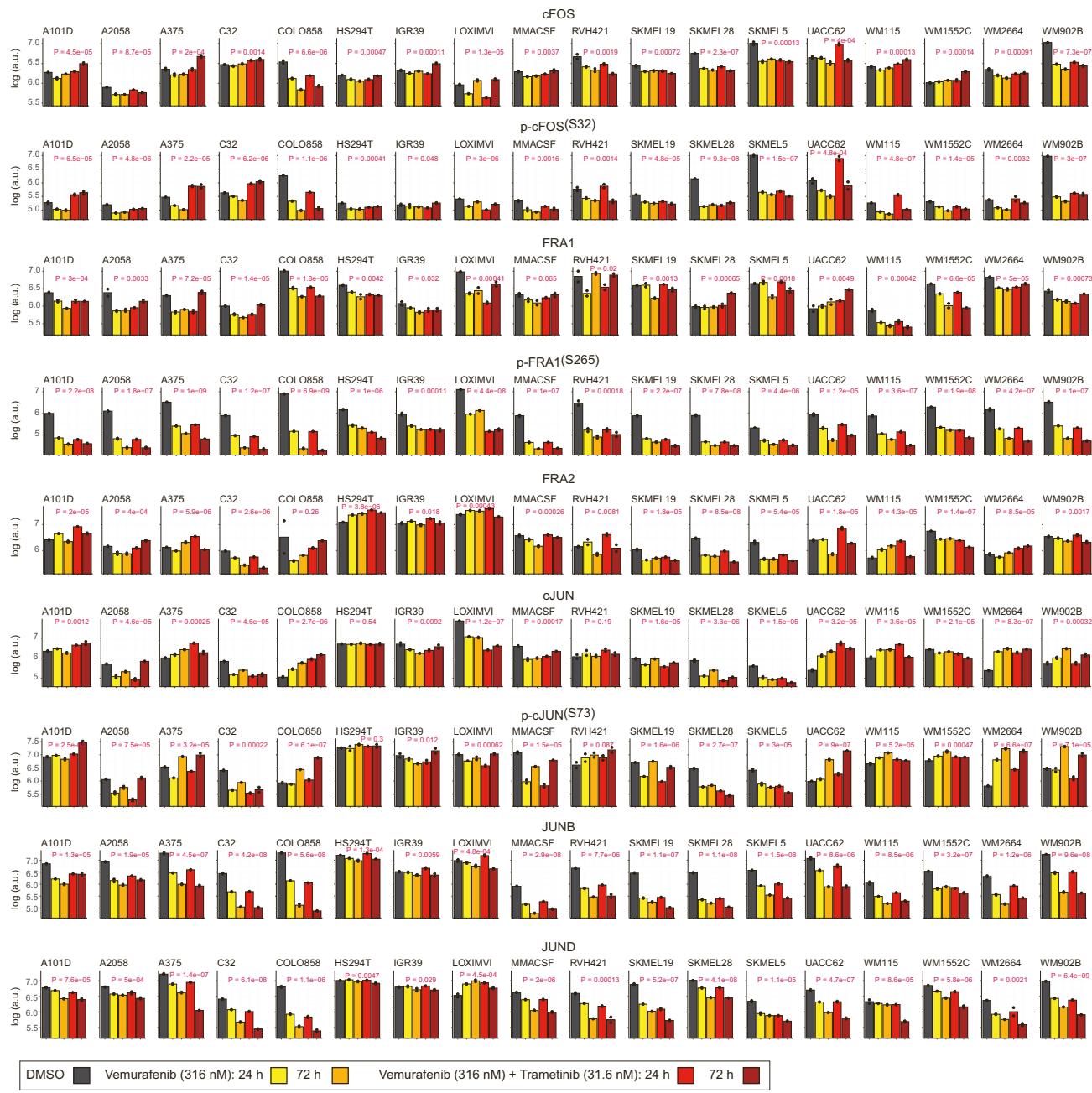


Figure S3. AP-1 protein measurements (including FOS and JUN subfamily members), measured in 18 cell lines before and following treatment with MAPK inhibitors for 24 and 72 h. Related to Figures 4 and 5. Bar height indicates mean values between two replicates shown as black dots. P-values show the statistical significance of treatment condition effect on indicated protein levels based on one-way ANOVA test.

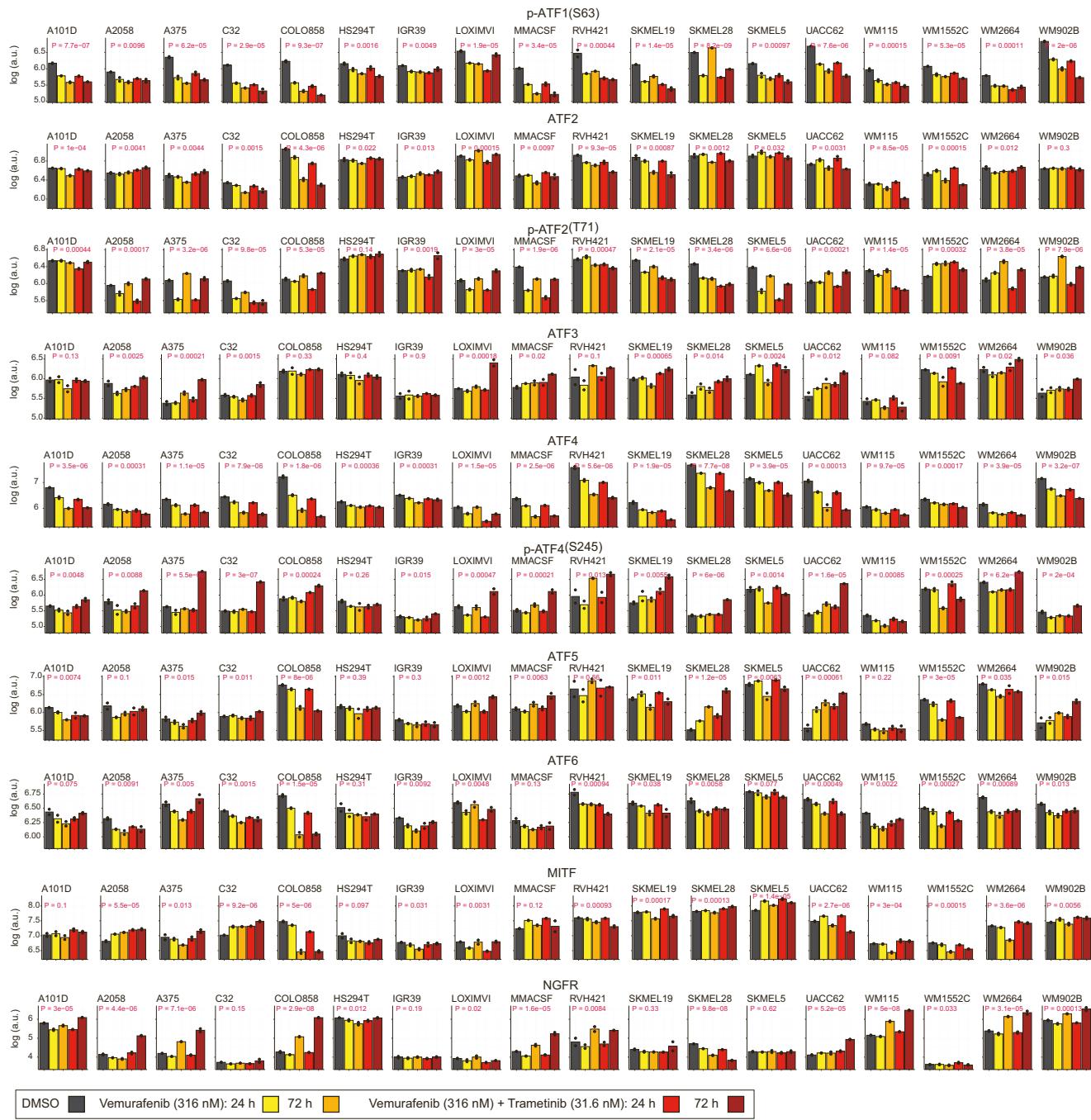
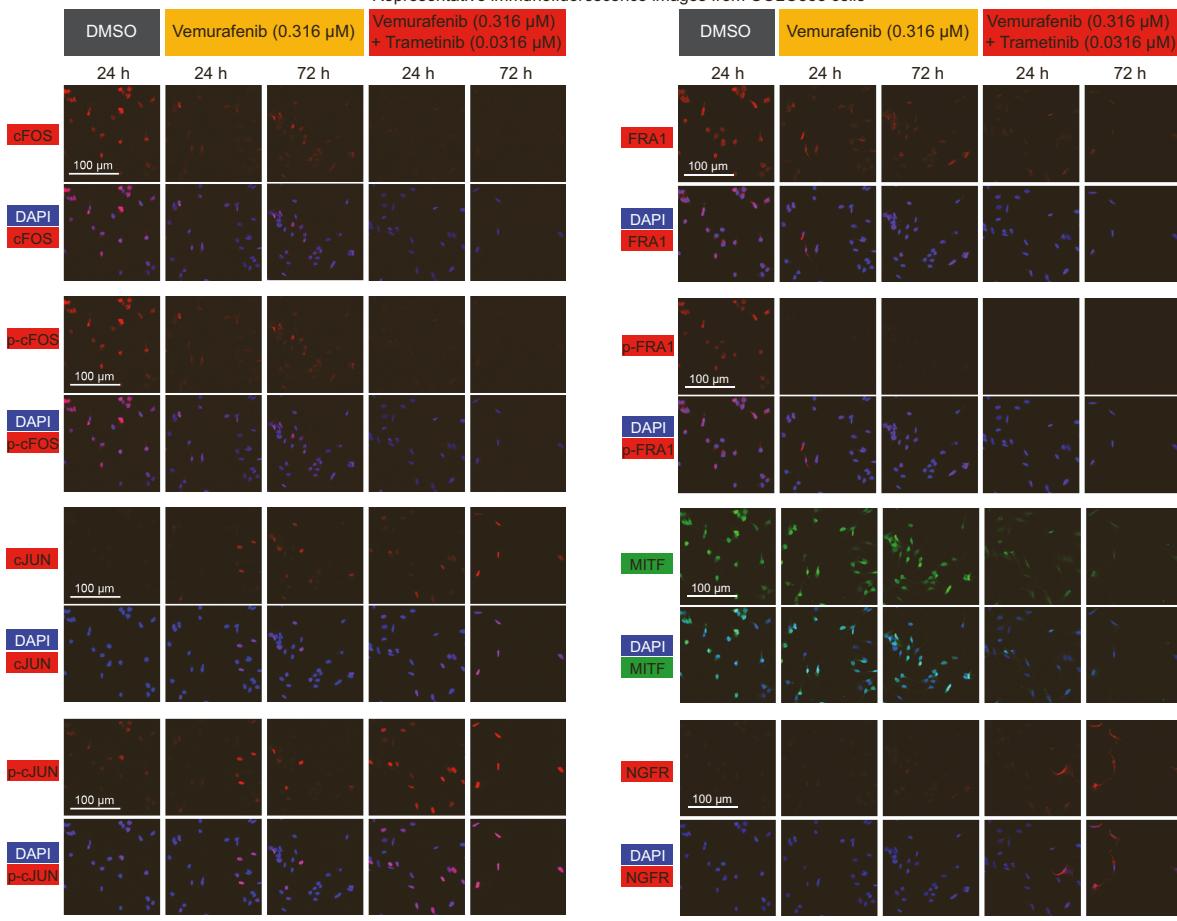


Figure S4. AP-1 protein measurements (including ATF subfamily members) and differentiation state markers MITF and NGFR, measured in 18 cell lines before and following treatment with MAPK inhibitors for 24 and 72 h. Related to Figures 4 and 5. Bar height indicates mean values between two replicates shown as black dots. P-values show the statistical significance of treatment condition effect on indicated protein levels based on one-way ANOVA test.

A

Representative immunofluorescence images from COLO858 cells



B

LOXIMVI

COLO858

SKMEL28

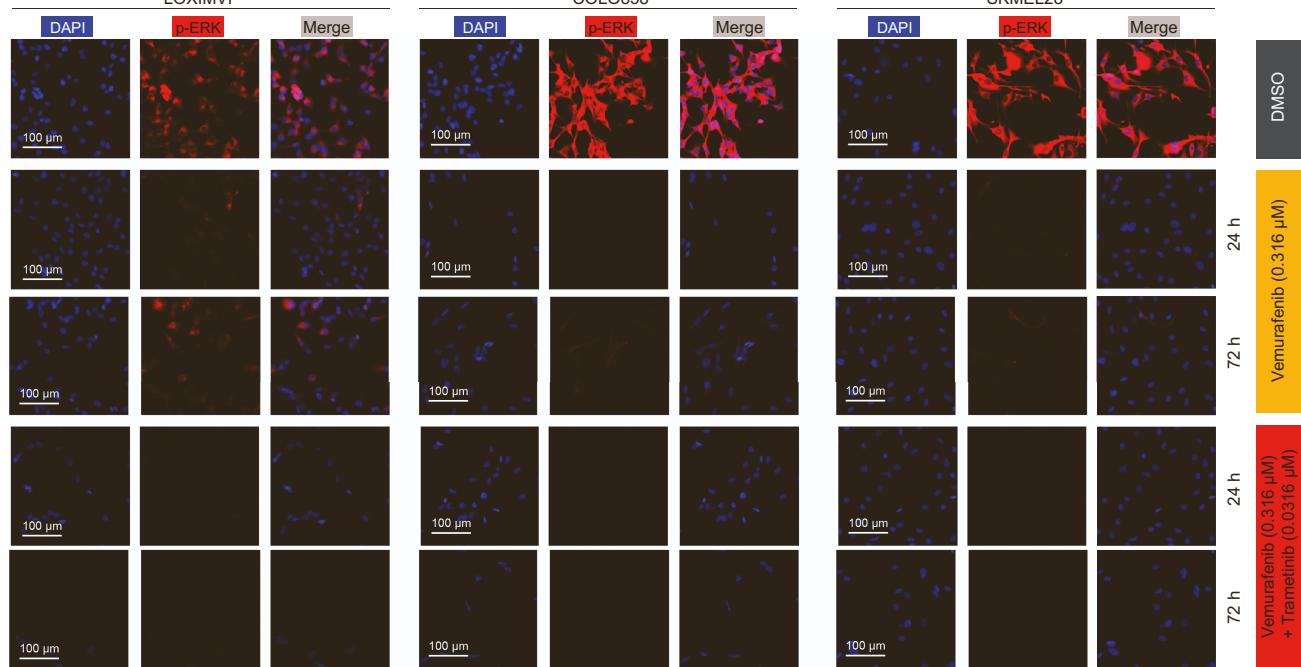


Figure S5. Representative immunofluorescence images of AP-1 proteins, differentiation state markers and p-ERK levels following MAPK inhibitor treatment. Related to Figures 5, 6. (A) Immunofluorescence images of AP-1 proteins cFOS, p-cFOS, cJUN, p-cJUN, FRA1, p-FRA1 and differentiation state markers MITF and NGFR in COLO858 cells before and after treatment with indicated inhibitors at indicated doses and timepoints. Each experiment was repeated twice with similar result. Scale bars represent 100 μm. (B) Immunofluorescence images of p-ERK in representative cell lines (LOXIMVI, COLO858 and SKMEL28) before and after treatment with indicated inhibitors at indicated doses and timepoints. Each experiment was repeated twice with similar result. Scale bars represent 100 μm.

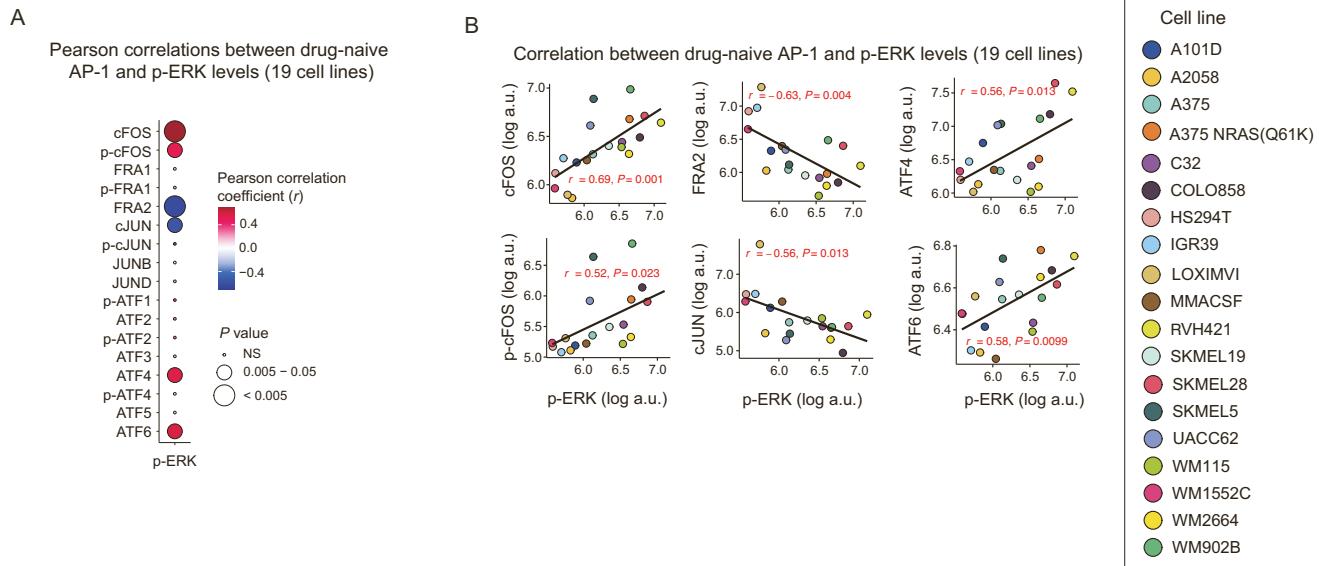


Figure S6. Correlation analysis between drug-naïve (baseline) p-ERK and AP-1 protein levels across melanoma cell lines.

Related to Figure 6. (A-B) Pearson's correlations (evaluated across 19 drug-naïve cell lines) and associated P values between each of the 17 AP-1 measurements and p-ERK levels. Each data-point in (B) represents population-averaged measurements across all cells pooled from two replicates for each cell line.

Representative immunostaining 4i images for proteins in COLO858 cells

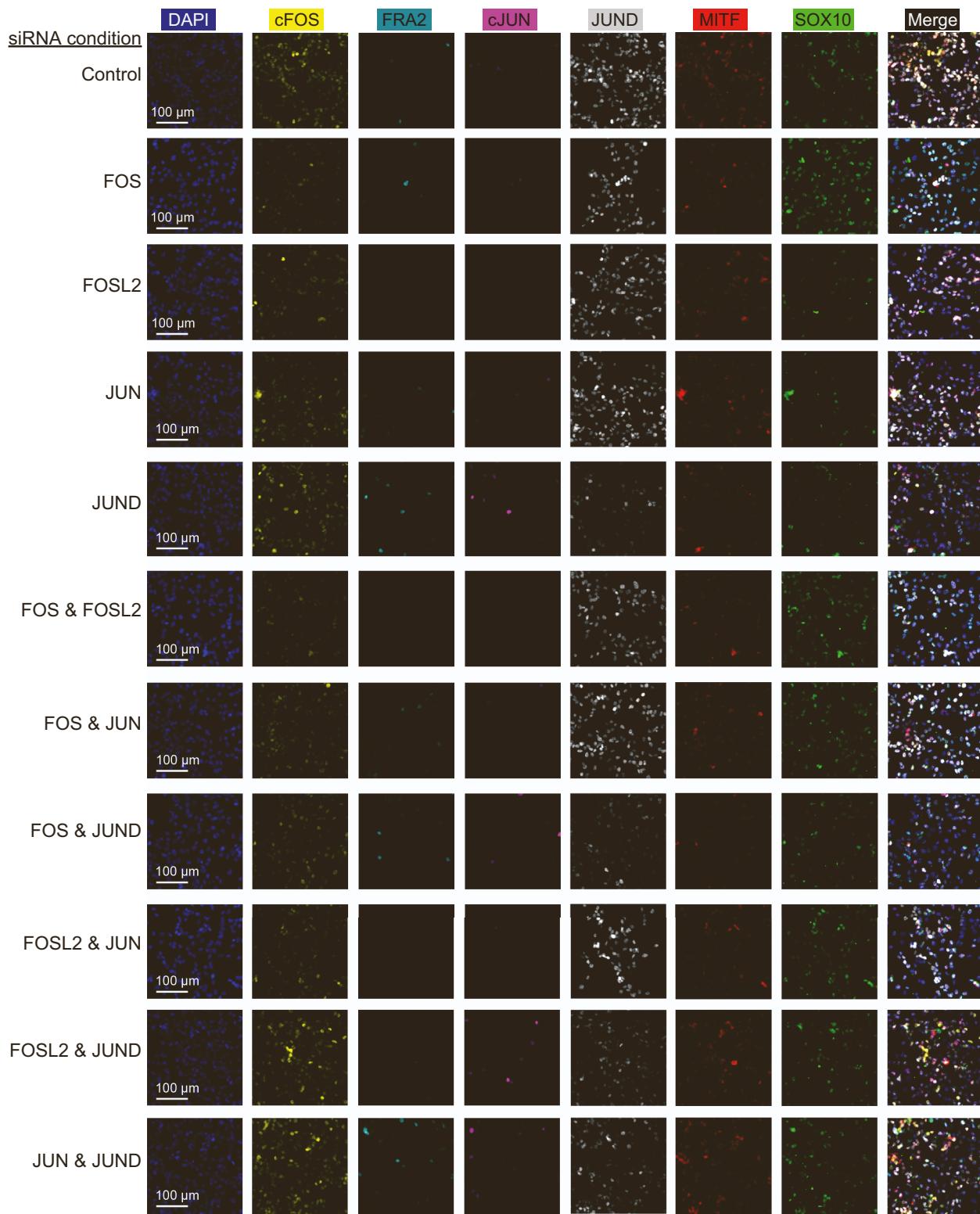


Figure S7. Representative immunofluorescence images of AP-1 proteins, differentiation state markers following different AP-1 knockdown conditions. Related to Figure 7. Immunofluorescence images (by 4i) of AP-1 proteins cFOS, FRA2, cJUN, JUND, and differentiation state markers MITF and SOX10 in COLO858 cells before and after treatment with indicated siRNA conditions for 96 h. Each experiment was repeated three times with similar result. Scale bars represent 100 μ m.

Table S1. The siRNA sequences used in this study. Related to Figure 7.

Target Gene	Dharmacon Cat#	siRNA sequence
Non-targeting control	D-001810-10-05	UGGUUUACAUGUCGACUAA, UGGUUUACAUGUUGUGUGA, UGGUUUACAUGUUUUCUGA, UGGUUUACAUGUUUUCUA
FOS	J-003265-09	GGGAUAGCCUCUCUUACUA
	J-003265-10	ACAGUUAUCCUCCAGAAGAA
	J-003265-12	GCAAUGAGCCUCCUCUGA
FOSL1	J-004341-06	GAGCUGCAGUGGAUGGUAC
	J-004341-07	AAUCUGGGCUGCAGCGAGA
	J-004341-08	GAGUAAGGCCGCGAGCGGAA
FOSL2	J-004110-13	GGCCCAGUGUGCAAGAUUA
cJUN	J-003268-10	GAGCGGACCUUAUGGUAC
	J-003268-12	GAAACGACCUUCUAUGACG
JUND	J-003900-12	GAAACACCCUUCUACGGCG
	J-003900-13	CCGACGAGCUCACAGUCC
	J-003900-14	UCAAGAGUCAGAACACCGGA
	J-003900-15	GUUCGAUUCUGCCUAUUU