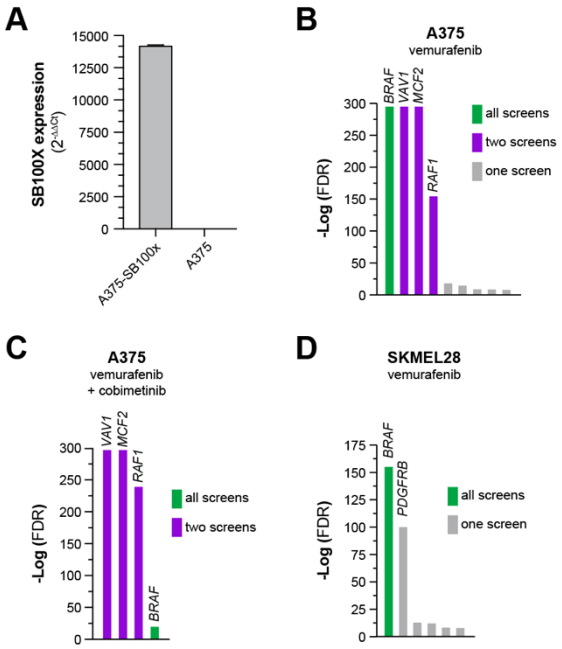


Figure S1. (**A**) Quantitative RT-PCR shows robust expression of the SB100x transposase in A375 cells. (**B-D**) Plots showing significance [-log(FDR)] for genes identified in the indicated resistance screen.

[Green bar = significant gene identified in all screens, purple bar = significant gene identified in two screens, gray bar = unique to the indicated screen].

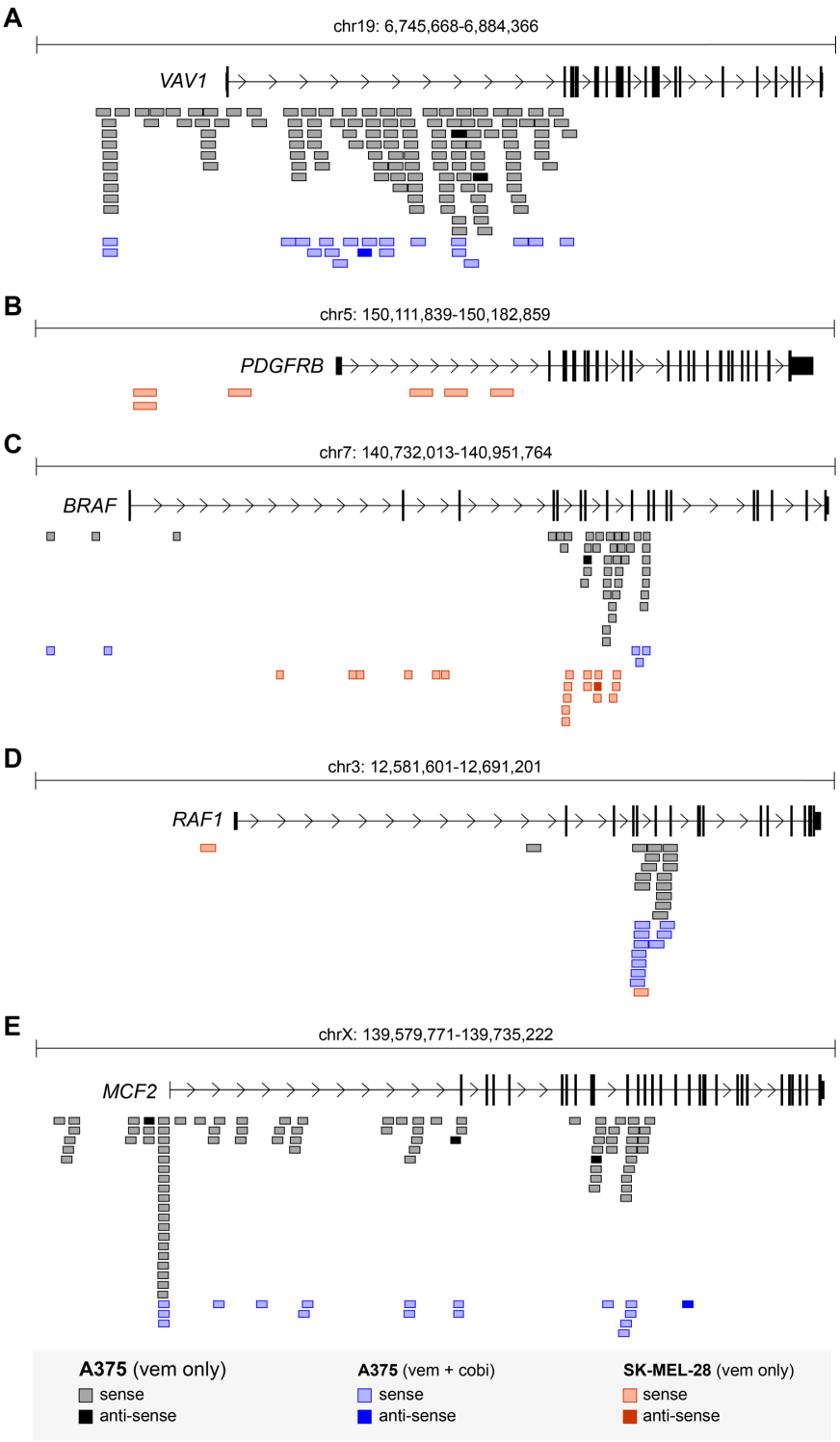


Figure S2. Distribution of transposon insertion events observed in (**A**) *VAV1*, (**B**) *PDGFRB* (**C**) *BRAF*, (**D**) *RAF1*, and (**E**) *MCF2*. In all cases, rectangles represent the position and orientation of transposon insertions identified in the various screens (see legend).

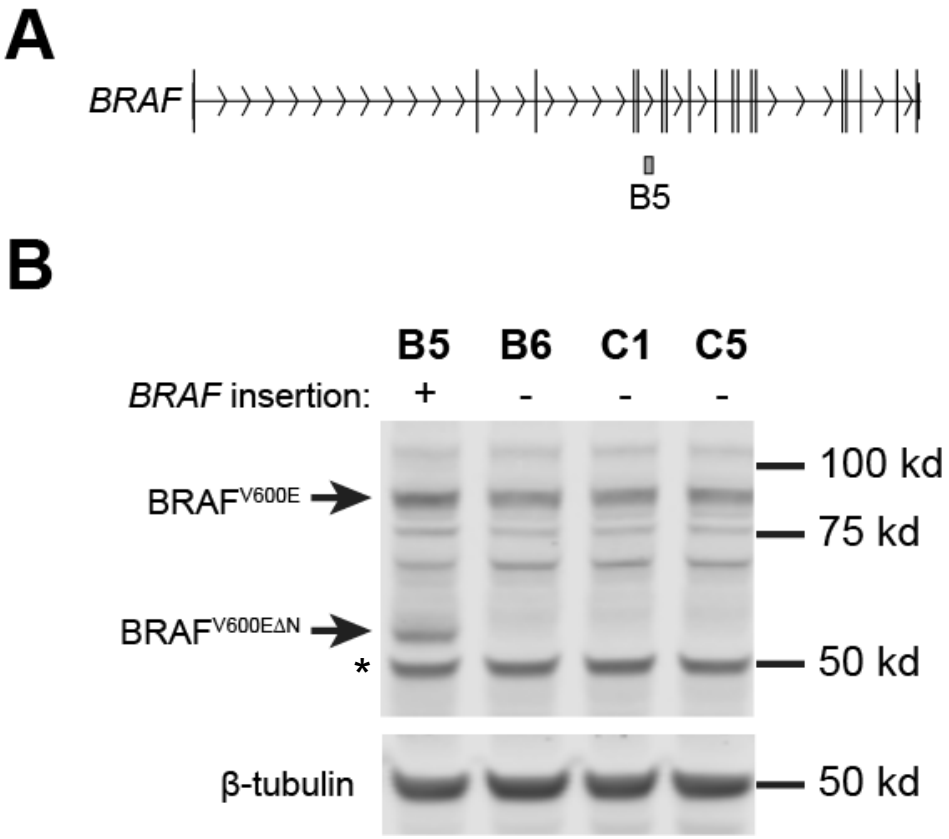


Figure S3. Transposon insertions express truncated $BRAF^{V600E}$ protein. (**A**) Clones of transposon-mutagenized, vemurafenib-resistant A375 cells were isolated. Genetic analysis identified a transposon insertion in the 3' end of the *BRAF* locus in clone B5. This insertion is predicted to drive expression of a 54 kD N-terminally-truncated protein. (**B**) Western blotting using an antibody against the $BRAF^{V600E}$ protein shows that clone B5 expresses both a full-length and truncated form of $BRAF^{V600E}$ while three independent clones lacking BRAF transposon insertions express only full-length $BRAF^{V600E}$. [* = background band detected by the $BRAF^{V600E}$ antibody]

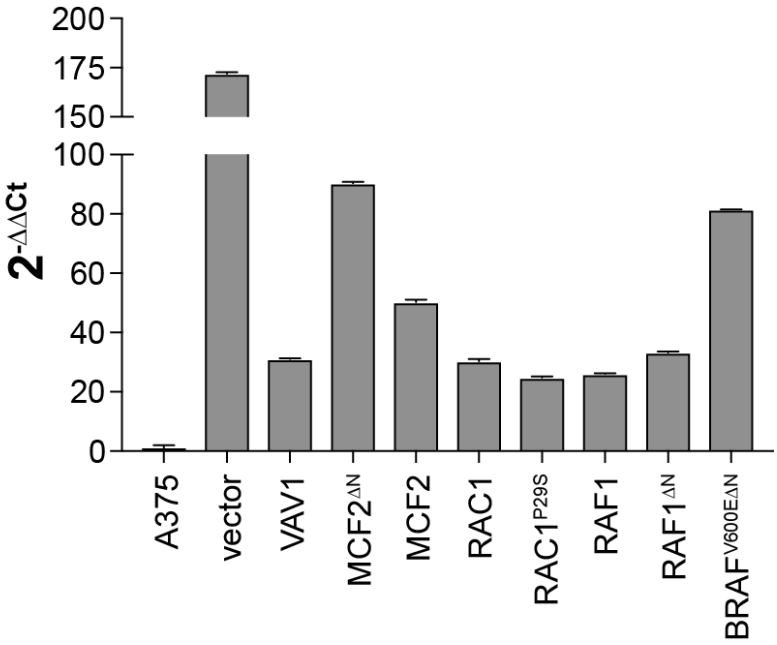


Figure S4. Quantitative RT-PCR analysis shows relative expression of each candidate gene in A375 cells. The relative expression values were obtained using primers to the puromycin resistance marker common to all constructs to allow for direct comparison across the various cell populations.

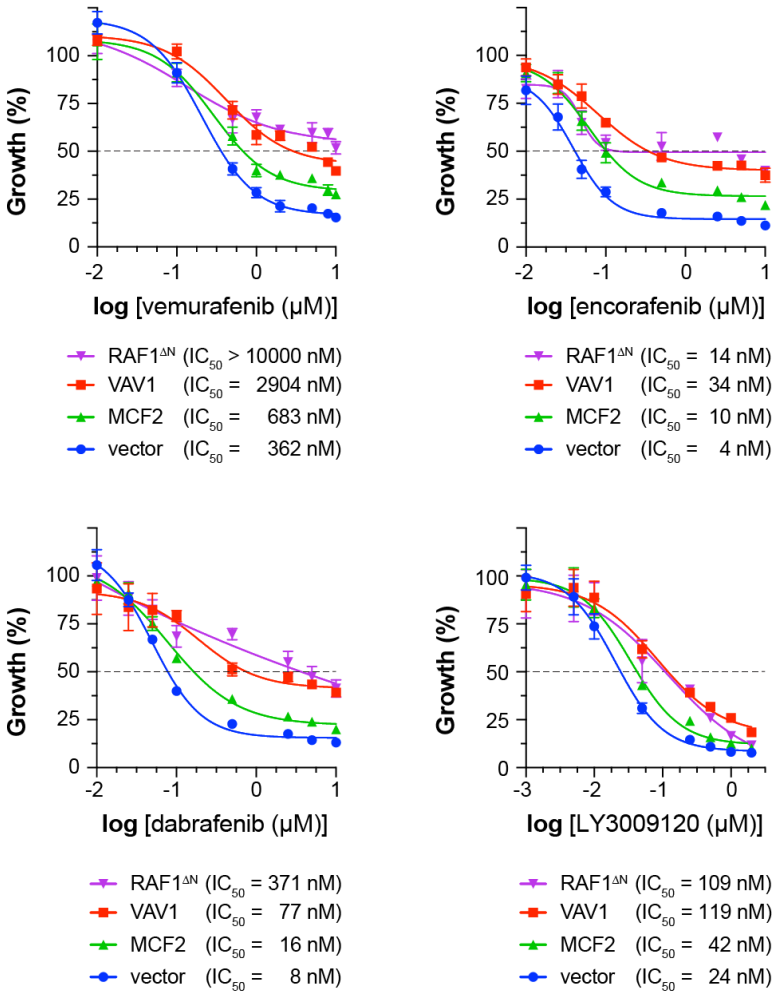


Figure S5. Dose response curves for candidate resistance drivers. Candidate resistance drivers ($RAF1^{\Delta N}$, VAV1, MCF2, empty vector) were stably expressed in A375 cells. Cell populations were then challenged with varying doses of vemurafenib, encorafenib, dabrafenib, or LY3009120. In all cases, expression of the candidate resistance driver increased the IC_{50} over that observed in cells expressing the empty vector.

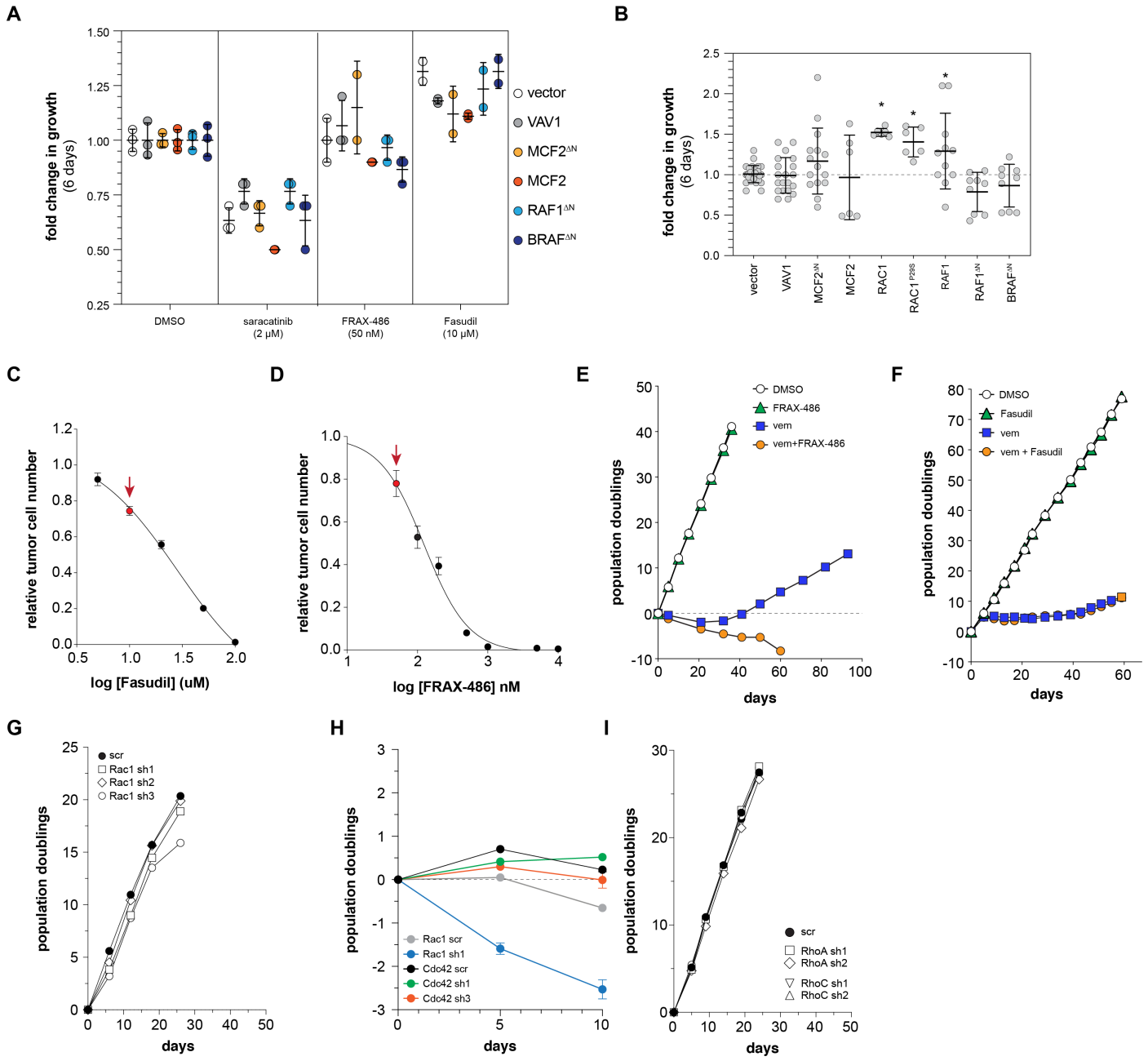
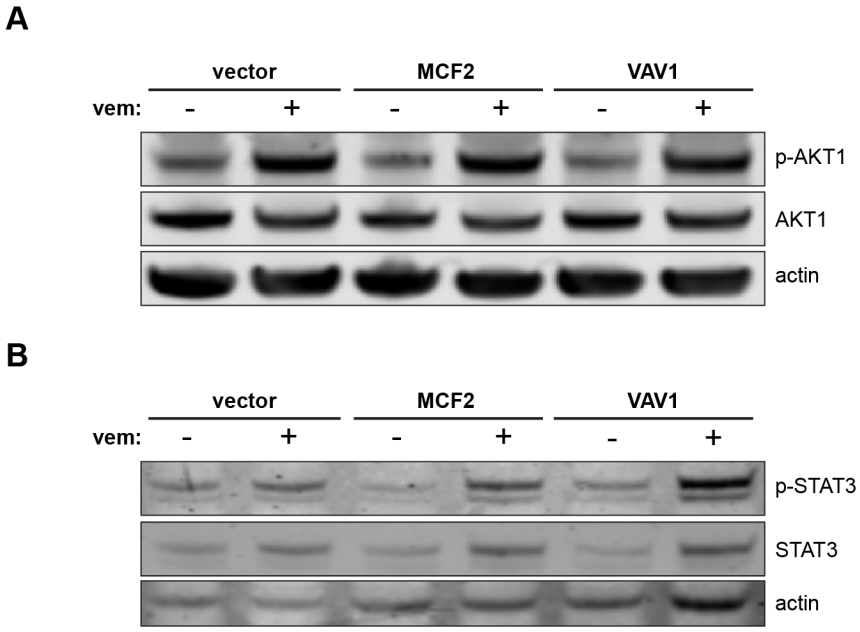


Figure S6. Characterization of drug and shRNA reagents used to interrogate the mechanism downstream of the DBL GEFs. (**A**) Cell growth assays using each drug at the selected concentration shows either no significant effect or slight growth reduction over a 6-day assay. (**B**) Expression of some candidates did increase proliferation significantly in the absence of treatment (*i.e.* DMSO)[* indicates a corrected p-value < 0.05 after ANOVA analysis]. A dose response curve was generated for Fasudil (**C**) and FRAX-486 (**D**) to identify a drug concentration that is well tolerated by A375 cells (red dot = selected dose). (**E**) The addition of the PAK inhibitor, FRAX-486, showed synergy with vemurafenib to block A375 proliferation. (**F**) The addition of the ROCK inhibitor, Fasudil (7 μM), did not show synergy with vemurafenib in A375 cells. (**G**) Knockdown of Rac1 does not impact growth of A375 under standard culture conditions. (**H**) Knockdown of Cdc42 did not alter the vemurafenib response of A375 as was observed with Rac1 knockdown. (**I**) Knockdown of neither RhoA nor RhoC impacts growth of A375 under standard culture conditions.



F**igure S7.** Vemurafenib treatment does not significantly alter levels of phosphorylated Akt1 (**A**) or Stat3 (**B**) in A375 cells expressing empty vector (*i.e.* vector), MCF2, or VAV1. A375 cells expressing an empty vector, MCF2, or VAV1 were treated for 48 hours with 5 μM vemurafenib. Total lysates were collected and analyzed via western blotting using the indicated antibody.

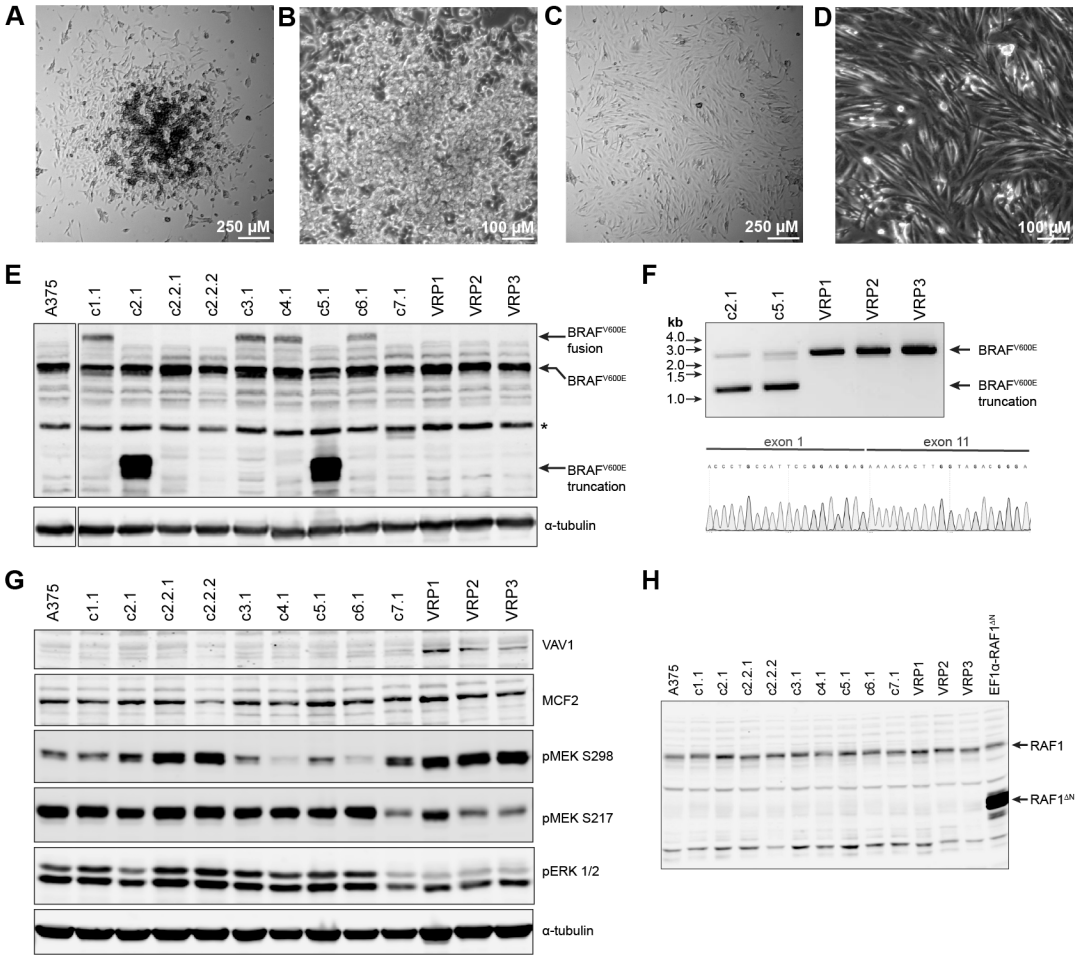


Figure S8. Characterization of spontaneous vemurafenib resistance in A375 melanoma. Resistant clones with distinct morphologies eventually emerged from long-term cultures of A375 cells in 3 µM vemurafenib. Some colonies showed a compact morphology (**A**,**B**) while others showed a more diffuse colony morphology (**C**,**D**). (**E**) Western blotting using an anti-BRAF^{V600E} antibody shows frequent alterations of BRAF^{V600E} protein in spontaneously resistant A375 populations compared to parental A375 cells [*denotes a non-specific band]. (**F**) RT-PCR analysis of the BRAF transcript shows an aberrant splice event between exons 1 and 11 in clones that express the truncated BRAF^{V600E} protein. (**G**) While MCF2 protein levels do not appear to change in resistant populations, VAV1 protein levels do appear to be increased in three independent cell populations. MEK phosphorylation on S217 (Raf site) and S298 (Pak site) also appear to be variable across the populations. (**H**) Western blotting using an anti-RAF1 antibody did not detect expression of an N-terminally-truncated form of RAF1.

[Colonies beginning with a “c” showed a compact morphology (**A**,**B**) while those labelled “VRP” showed the diffuse morphology (**C**,**D**).]