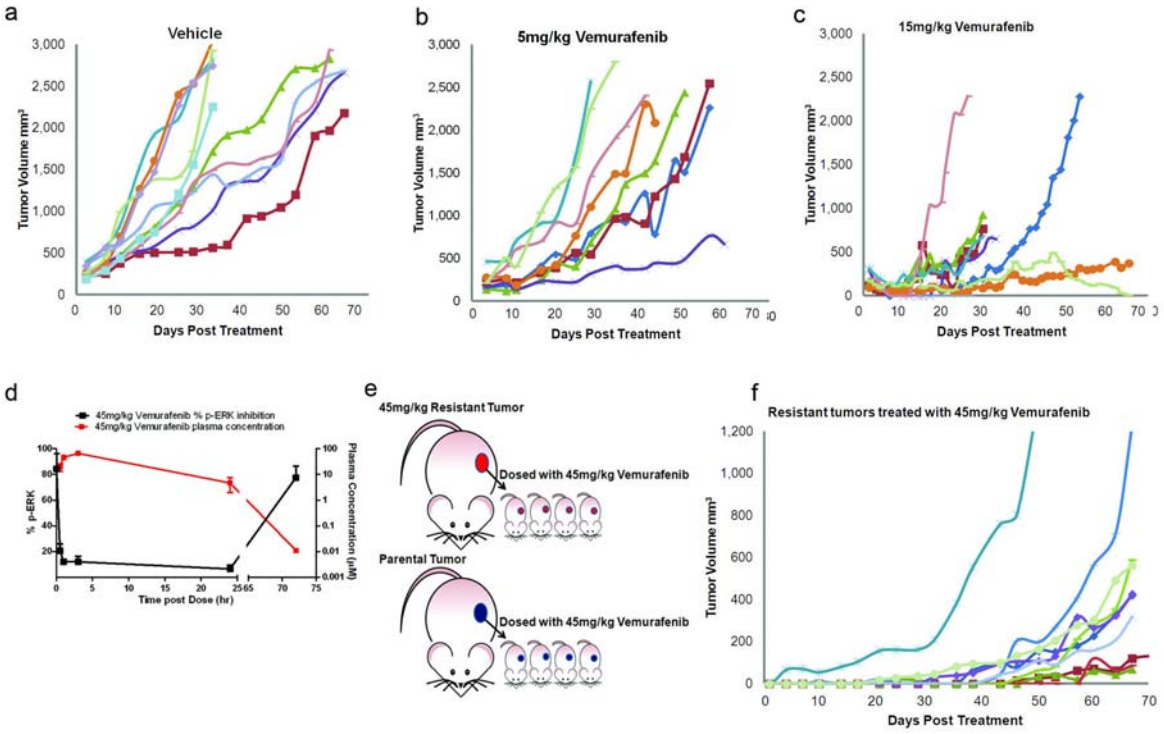
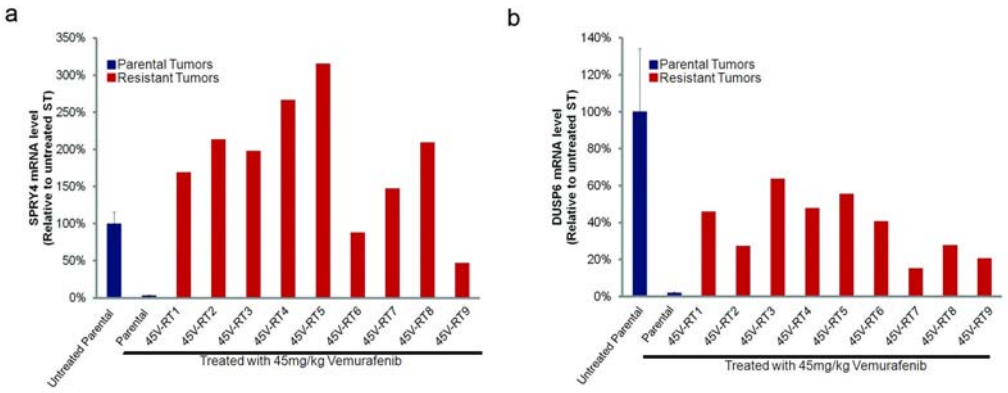
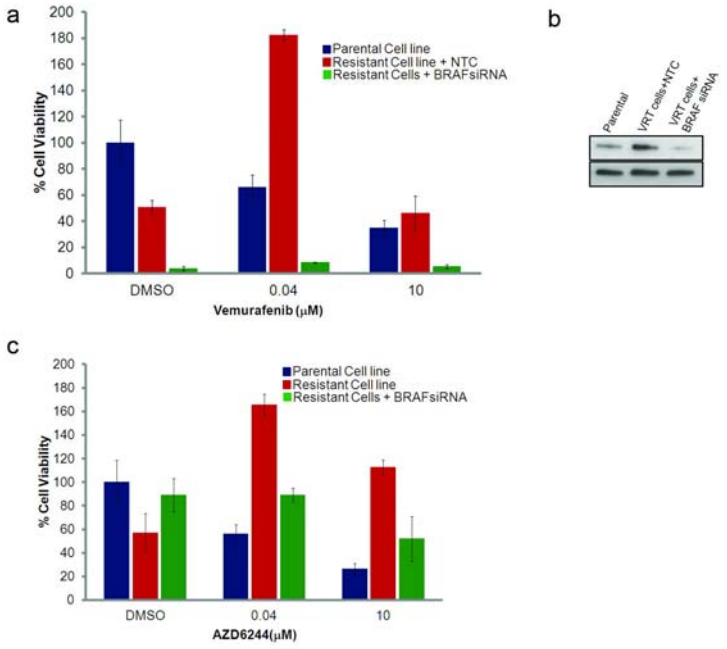
doi:10.1038/nature11814



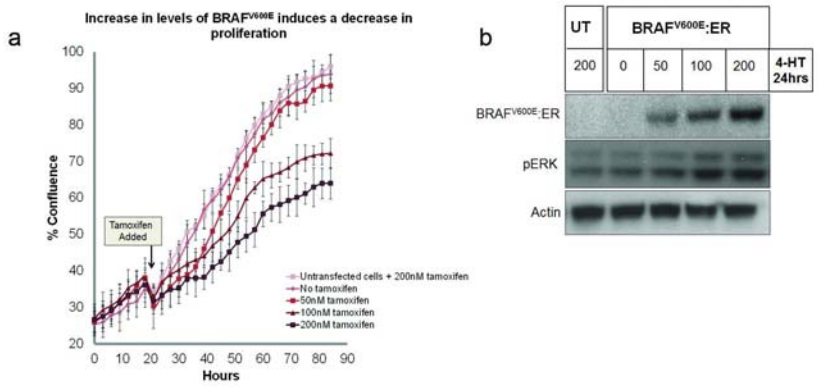
Supplementary Figure 1: Efficacy of vemurafenib in a primary human melanoma xenograft model. HMEX1906 tumor-bearing nude mice were dosed with vehicle **a**, 5 mg/kg bid vemurafenib **b**, or 15 mg/kg bid vemurafenib **c**, and tumor volume was measured for 70 days. **d,** RAF-MEK-ERK MAPK pathway inhibition was determined by measuring pERK1/2 levels using MSD analysis at various time points (30min, 1hr, 3hrs, 24hrs and 72hrs) after one single dose of 45 mg/kg. Corresponding plasma concentrations of vemurafenib at each time point are also plotted on the right axis (mean percentage ± s.e.m., n=4). **e**, Resistant tumor implants (red) were dosed immediately following implants, and sensitive parental tumors were dosed once with vemurafenib before tumors were harvested. **f,** Growth kinetics of the 45mg/kg vemurafenib resistant tumor from Figure 1c which was fragmented and re-implanted in mice that were dosed with 45mg/kg bid vemurafenib immediately after implantation.



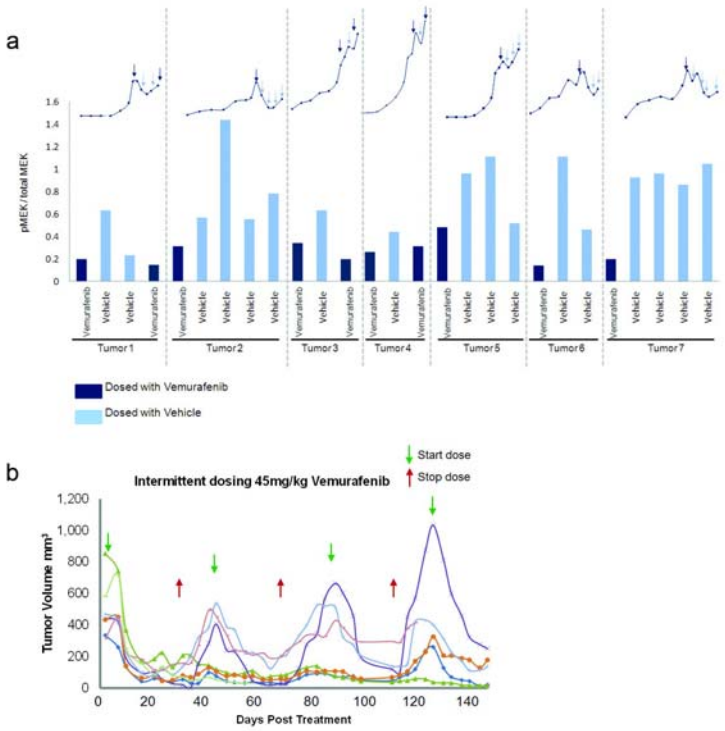
Supplementary Figure 2: MAPK pathway target genes are not suppressed in vemurafenib resistant tumors. a, SPRY4 and **b**, DUSP6 mRNA was quantified by qRT-PCR in parental and resistant tumors 3 hours following 45mg/kg vemurafenib treatment. a, b (n=3 untreated and treated independent parental tumors, mRNA levels ± s.e.m).



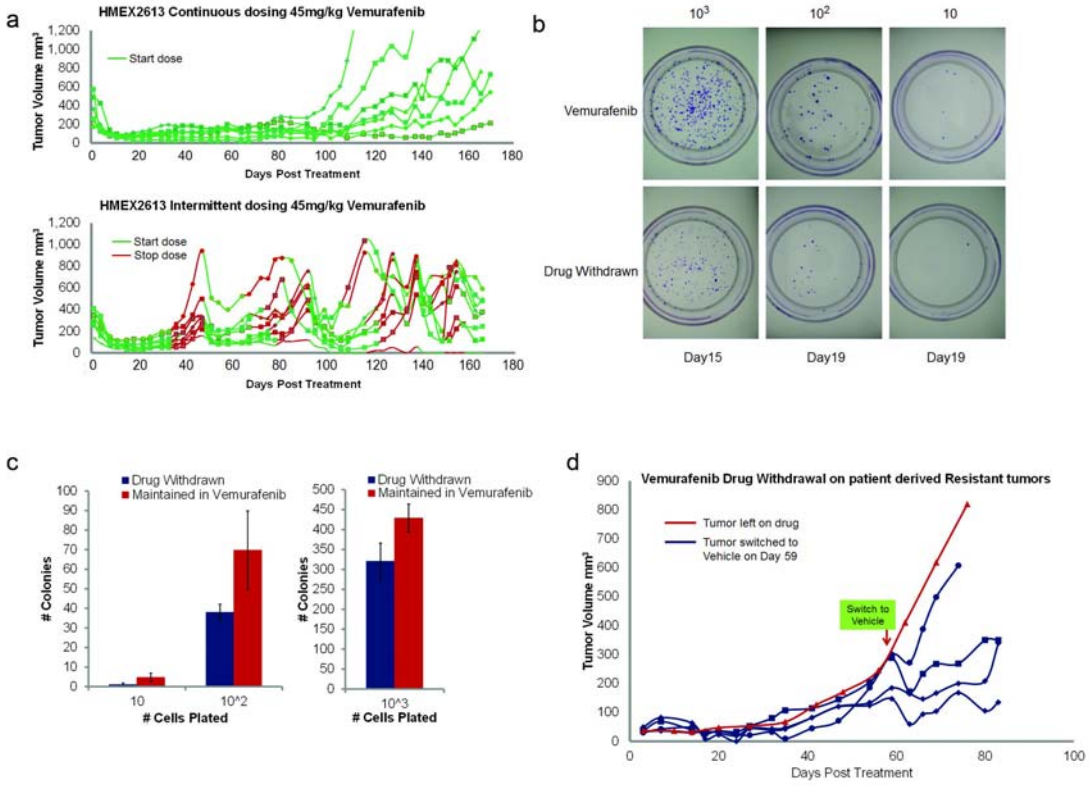
Supplementary Figure 3: Complete BRAF knockdown by siRNA decreases viability of sensitive and resistant tumor cells. a, Cell viability 72 hours following strong BRAF knock down in parental and resistant cells in the presence or absence of the indicated concentration of vemurafenib. **b,** BRAF Western blot parental and resistant tumor cell lysates following BRAF siRNA or non-targeting siRNA (actin as loading control). **c** Resistant and parental tumor cells from Figure 3e were subjected to BRAF siRNA, treated with AZD6244 or control (DMSO) and cell viability was determined by Cell Titer-glo assay following 3 days of culture. **a, c** Mean percentage ± s.e.m., n=6.



Supplementary Figure 4: Excessive amounts of BRAF^{V600E} are deleterious to the cell. a, Percent confluence was measured in HMEX1906 cell lines that were retrovirally transfected with BRAF^{V600E}:ER construct and given increasing amounts of 4-HT (mean percentage ± s.e.m., n=6). **b,** BRAF and p-ERK Western blot of cell lysates from S4a (actin as loading control).



Supplementary Figure 5: Drug withdrawal leads to a spike in p-MEK levels that corresponds with tumor regression. a, Lysates collected from FNA (Figure 4c) were analyzed for p-MEK . Bars represent p-MEK levels from 7 different tumors (separated by dotted grey lines) in the presence (dark blue bars) or in the absence of vemurafenib (light blue bars). The growth kinetics of each tumor is represented by the line graph above the p-MEK bars and the time points of FNA collection are depicted by arrows. **b,** Tumor growth kinetics from HMEX1906 tumor-bearing mice dosed with 45mg/kg bid vemurafenib on an intermittent dosing schedule (4 weeks on drug and 2 weeks off drug).



Supplementary Figure 6: Resistance free regression can be prolonged by intermittent dosing in HMEX2613 and 2 other vermurafenib resistant models also appear to be drug-addicted. a, Tumor growth kinetics of naïve parental HMEX2613 tumors with 7 tumors dosed continuously (top) and 8 tumors dosed intermittently (bottom). Dosing of vemurafenib with intermittent dosing carried out on an individualized on drug (green line) and off drug (red line) schedule with 45 mg/kg bid vemurafenib. Tumors were kept on drug until an individual tumor showed no further regression for 2 consecutive time points (3 days apart) before being switched of off dose. **b**, Skmel239–C3 cells were plated at the indicated cell densities in the presence or absence of vemurafenib and stained with crystal violet on the days indicated. **c,** The images from Fig.S6b are quantified (mean number ± s.e.m., n=4). **d**, The patient biopsy (M120214) was taken from the a lymphnode/skin met increasing in size during systemic Vemurafenib therapy. The patient-derived vemurafenib-resistant tumors were implanted into nude mice and dosed with 45mg/kg b.i.d vemurafenib immediately post implant. Once tumors reached a volume of ~150-300 mm3, mice were switched from vemurafenib to vehicle-control (blue lines) on day 59, while one mouse remained on vemurafenib (red line).

Patient Biopsy	Age	Site of Biopsy	Final patient Diagnosis	Clinical History	Mutational Status
HMEX1906	61F	Lymph node Met (left groin)	Six of sixteen lymph nodes showed malignant neoplasm consistent with metastatic malignant melanoma.	Left groin mass dissection and history of melanoma	$BRAF^{V600E}$
HMEX2613	44M	Excision from skin on right leg	Malignant melanoma present in subcutaneous tissue.	Recurrent melanoma	$BRAF^{V600E}$
M120214	65F	Lymph node Met (left side of neck)	Neck lymph node was infiltrated with micro metastases. Patient developed resistance to vemurafenib.	Chemotherapy and postop radiotherapy at the left side of the neck.	$BRAF^{V600E}$

Table 1: Clinical characteristics of the melanoma patients whose tumors were implanted into mice to study drug resistance