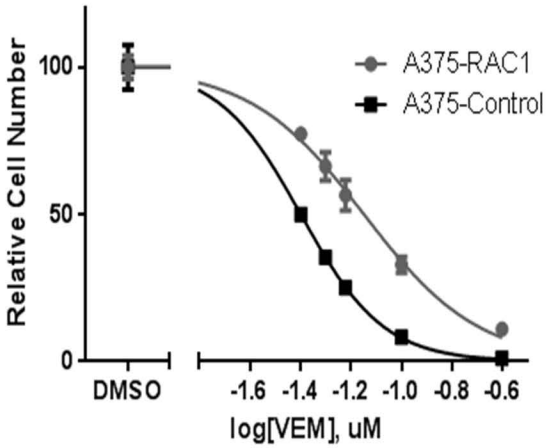
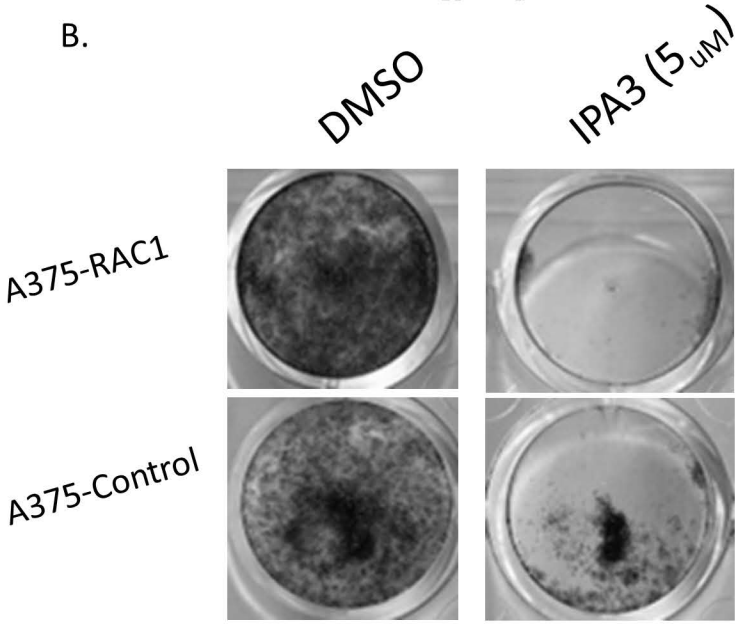
Supplementary Figure 1. The effects of activated RAC1 on cell response to pathway inhibitors.

(A) A375 cells were transduced with activated RAC1 ("A375-RAC1") or the corresponding empty vector ("A375-Control") and were treated for 5 days with the indicated doses of vemurafenib. The numbers of remaining cells were compared by the methylene blue staining and extraction method, and the results are plotted as a percentage of the values for the DMSO-treated cultures. (B) A375-RAC1and A375-Control cells were treated with IPA3 (5uM) or DMSO for 5 days and visualized by methylene blue staining. (C). A375-RAC1and A375-Control cells were treated for 5 days with the indicated doses of PF3758309. The numbers of remaining cells were compared and plotted as in (A).

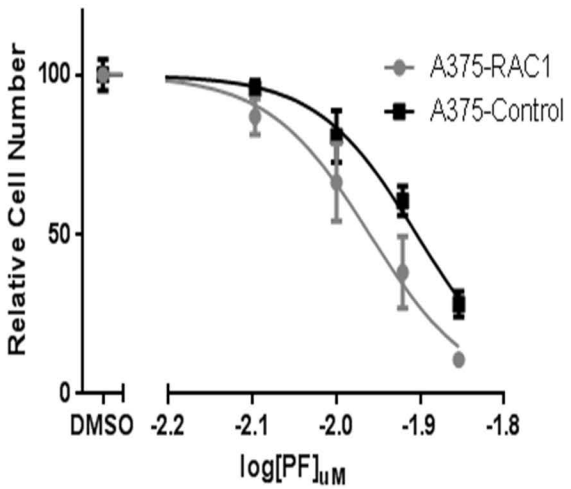
A.


Relative Cell Number
100
50
0
A375-RAC1
A375-Control
DMSO
-1.6 -1.4 -1.2 -1.0 -0.8 -0.6
log[VEM], uM

B.


DMSO
IPA3 (5uM)
A375-RAC1
A375-Control

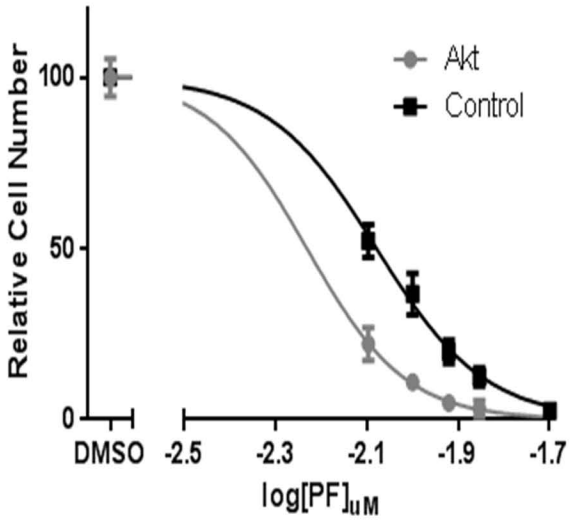
C.


Relative Cell Number
100
50
0
A375-RAC1
A375-Control
DMSO
-2.2
-2.1
-2.0
-1.9
-1.8
log[PF]uM

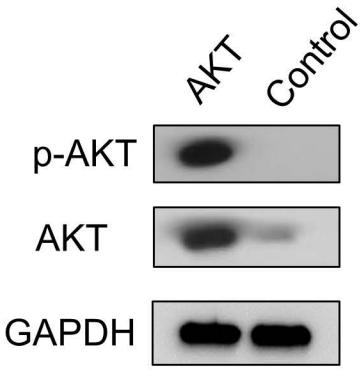
Supplementary Figure 2. The effects of constitutively active AKT on the response of A375 cells to PF-3758309.

(A) A375 cells transduced with constitutively active AKT ("Akt") or the corresponding empty vector ("Control") were treated for 5 days with the indicated doses of PF3758309. The numbers of remaining cells were compared using methylene blue staining and extraction method and are plotted as percentages of the corresponding DMSO-treated populations. (B) The levels of total and activated (phosphorylated) AKT were compared by immunoblotting for the cells used in (A). GAPDH immunoreactivity is shown as a loading control.

A.


Relative Cell Number
100
50
0
Akt
Control
DMSO
-2.5
-2.3
-2.1
-1.9
-1.7
log[PF]uM

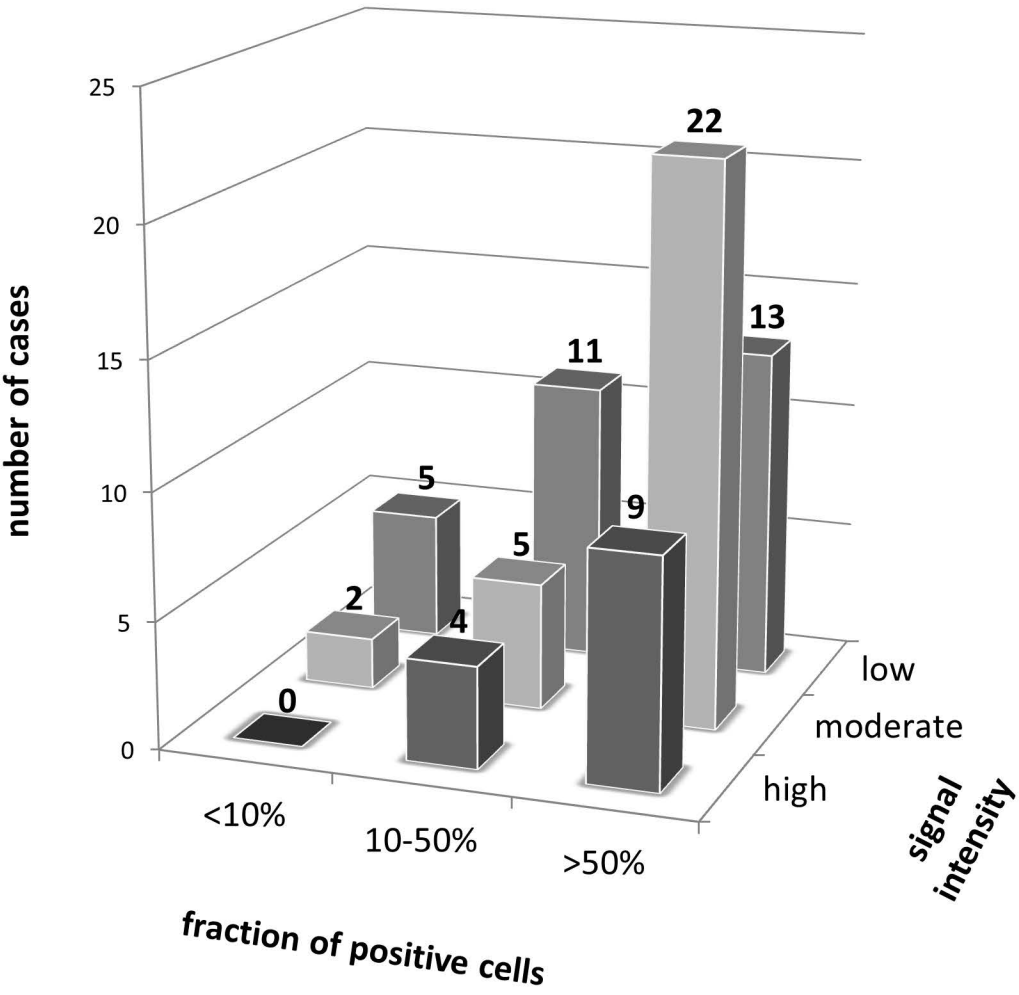
B.


AKT
Control
p-AKT
AKT
GAPDH

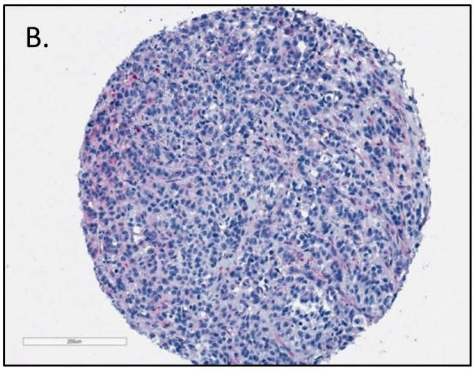
Supplementary Figure 3. Detection of activated PAK1 in metastatic melanoma samples.

(A) Metastatic melanoma tissue microarray was probed for the presence of PAK1 phosphorylated at threonine 423 and scored for the abundance and signal intensity of the stained cells. The graph shows the distribution of positively stained samples. In addition, no signal was detected in 21 samples (scored as “negative” by the fraction of positive cells and signal intensity). (B) An example of a sample scored as “negative”. (C) An example of a samples scored as “>50% positive cells” and “high intensity”.

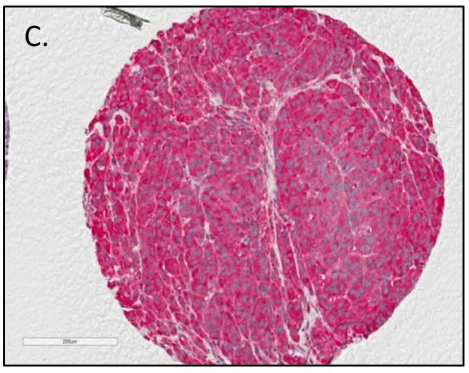
A.


25
20
15
10
5
0
number of cases
22
13
11
9
5
5
4
2
0
low
moderate
high
signal intensity
<10%
10-50%
>50%
fraction of positive cells

B.



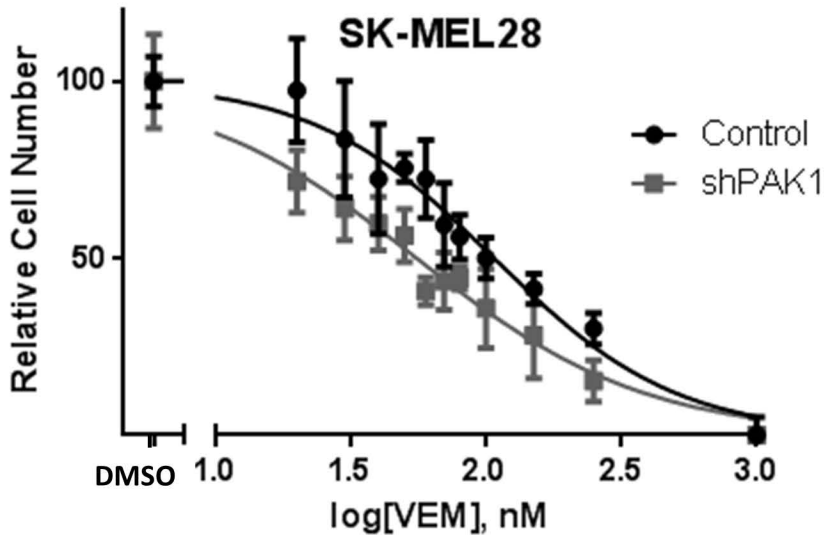
C.



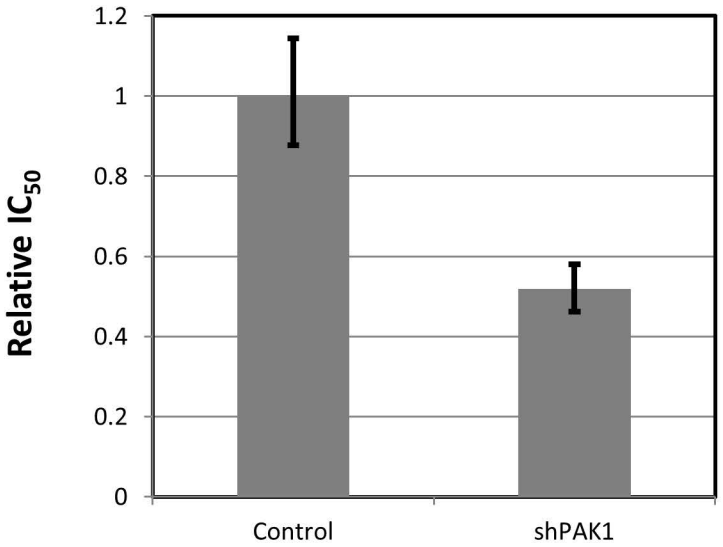
Supplementary Figure 4. Knockdown of PAK1 sensitizes SK-MEL-28 cells to vemurafenib.

(A) SK-MEL-28 cells transduced with an shRNA targeting PAK1 (“shPAK1”) or a non-targeting control shRNA (“control”) were treated for 5 days with the indicated doses of vemurafenib, and the numbers of the remaining cells were compared using methylene blue staining and extraction method. The values are plotted as percentages of those for the DMSO-treated populations. The error bars represent standard deviations. (B) IC50 values for the experiment shown in (A) are plotted relatively to that of the Control population. The error bars represent 95% confidence intervals.

A.


SK-MEL28
Relative Cell Number
100
50
Control
shPAK1
DMSO
1.0
1.5
2.0
2.5
3.0
log[VEM], nM

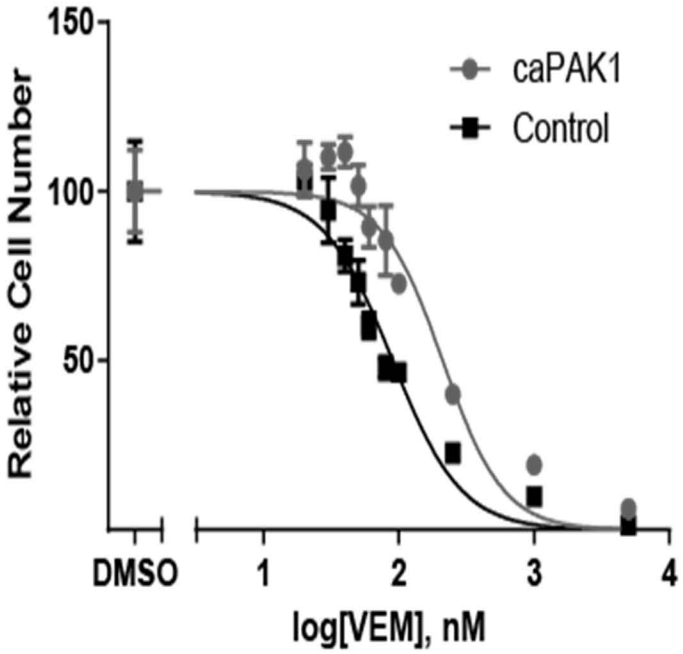
B.


Relative IC50
1.2
1
0.8
0.6
0.4
0.2
0
Control
shPAK1

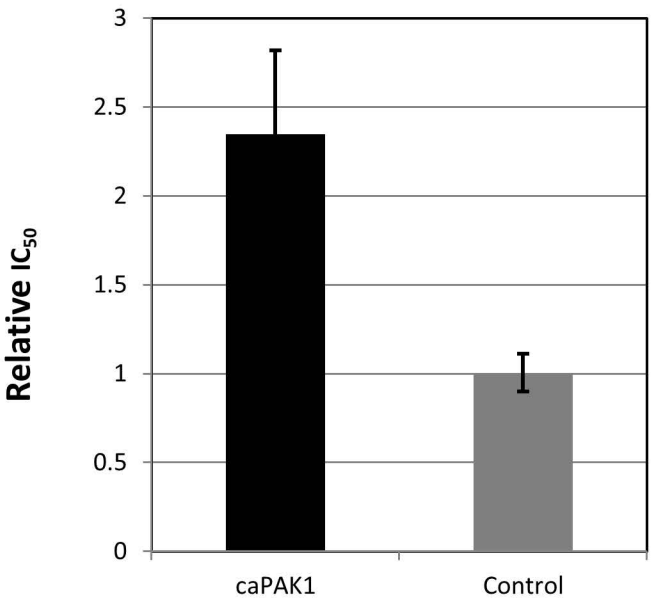
Supplementary Figure 5. Activated PAK1 increases vemurafenib tolerance of Colo205 cells.

(A) Colo205 cells transduced with a constitutive active PAK1 (“caPAK1”) or the corresponding empty vector (“Control”) were treated for 5 days with the indicated doses of vemurafenib and the numbers of the remaining cells were compared using methylene blue staining and extraction method. The values are plotted as percentages of those for the DMSO-treated populations. The error bars represent standard deviations. (B) IC50 values for the experiment shown in (A) are plotted relatively to that of the Control population. The error bars represent 95% confidence intervals.

A.

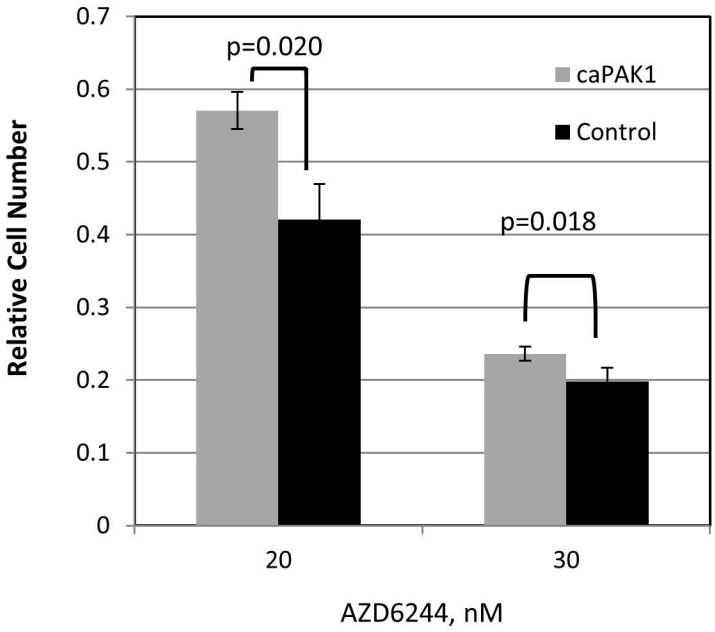

150
100
50
Relative Cell Number
caPAK1
Control
DMSO
1
2
3
4
log[VEM], nM

B.


3
2.5
2
1.5
1
0.5
0
Relative IC50
caPAK1
Control

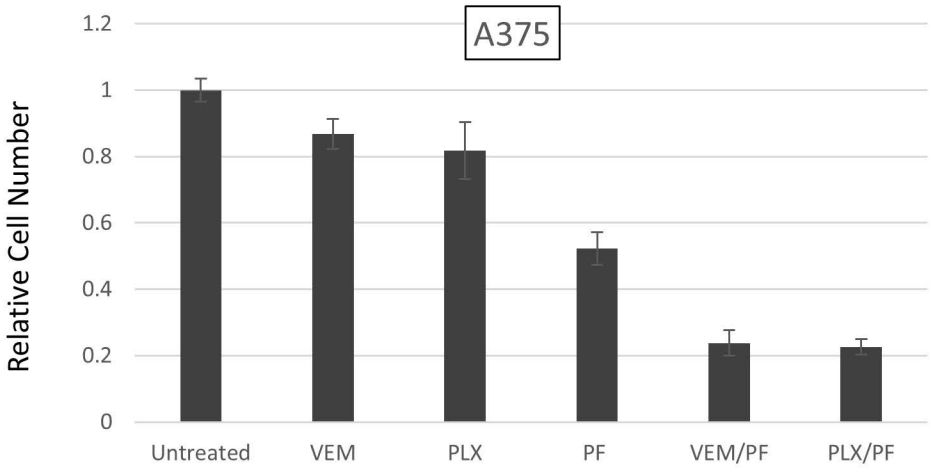
Supplementary Figure 6. The effect of activated PAK1 on the response of A375 cells to MEK inhibitor AZD6244.

A375 cells ectopically expressing constitutive active PAK1 (“caPAK1”) or the corresponding empty vector control (“Control”) were exposed to AZD6244 (20nM or 30nM) for 5 days. The numbers of the remaining cells were compared using methylene blue staining and extraction method. The values are plotted as percentages of those for the corresponding untreated populations. The error bars represent standard deviations for quadruplicate cultures.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative Cell Number
p=0.020
p=0.018
caPAK1
Control
20
30
AZD6244, nM

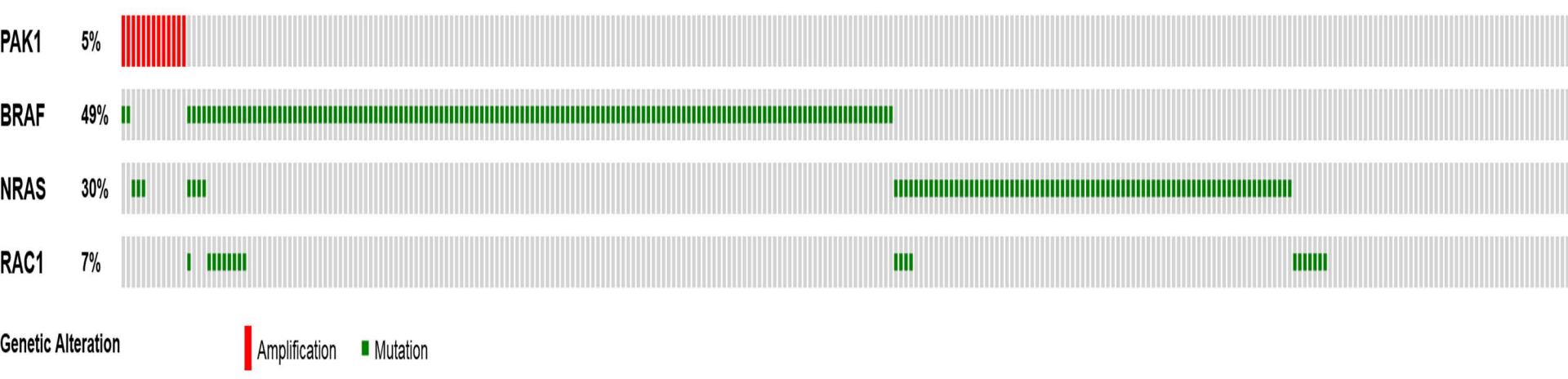
Supplementary Figure 7. Vemurafenib and PLX4720 exhibit similar toxicity in combination with PF3758309.

A375 cells were plated and treated the next day with the indicated drugs or drug combination. Vemurafenib and PLX4720 were both used at 40nM. PF3758309 was used at 8nM. After five days, the numbers of the remaining cells were compared to those in parallel untreated cultures using methylene blue staining and extraction method. Error bars represent standard deviations for quadruplicate cultures.


A375
Relative Cell Number
1.2
1
0.8
0.6
0.4
0.2
0
Untreated
VEM
PLX
PF
VEM/PF
PLX/PF

Supplementary Figure 8. Co-occurrence of certain oncogenic genetic alterations in human melanomas.

Co-occurrence between amplification of PAK1 and point mutations in BRAF, NRAS and RAC1 was examined using the TCGA Provisional dataset and the analytical tools available through the cBio Portal for Cancer Genomics (www.cbioportal.org). Note that all four types of events have a tendency towards mutual exclusivity. In particular, mutations in BRAF are mutually exclusive with mutations in NRAS ($p=2.16x10^{-26}$) and amplification of PAK1 ($p=0.01$).


PAK1
5%
BRAF
49%
NRAS
30%
RAC1
7%
Genetic Alteration
Amplification
Mutation