Tran et al . Supplementary Materials

Supplemental Table 1. Primers for dsRNA generation, cloning or site-directed mutagenesis as indicated.

Supplemental Figure 1. Inhibition of S6K phosphorylation by PVR loss precedes decrease in protein levels.

Western blot analysis of Kc cells treated with indicated dsRNA for the stated number of days. Luc, luciferase; Ra, rapamycin.

Supplemental Figure 2. Ectopically expressed HA-d4E-BP reports on TORC1 activity in Kc cells.

Western blot analysis of S2 and Kc cells transfected (or not) with an empty vector (EV) or an HA-d4E-BP expressing vector, and treated (or not) with rapamycin (Ra).

Supplemental Figure 3. Repeat of experiments shown in Figure. 2.

Western blot analysis of Kc cells pretreated with Luc or PVR dsRNA, starved and stimulated with conditioned medium (A) or insulin (B) for the indicated amounts of time.

Supplemental Figure 4. Inactivation of Tsc1/Tsc2 complex is sufficient to activate TORC1 in S2 cells.

(A and C) Western blot analyses of Kc and S2 cells treated with the indicated dsRNA. (B) q-RT-PCR of *Lobe* expression (normalized to RpS17) in Kc and S2 cells. Data are means ± SD (*n*=2).

Supplemental Figure 5. TUNEL assay of Kc cells with knockdown of indicated genes.

Luc (blue), PVR (cyan), Drice (orange), Diap1 (red), Diap1 and Drice (green), PVR and Drice (pink).

Supplemental Figure 6. Western blot analysis of sunitinib timecourse in Kc cells.

Supplemental Figure 7. Sunitinib inhibits proliferation in Kc but not S2 cells.

BrudU incorporation assay of indicated cells treated the vehicle (Ve) or sunitinib (Su).

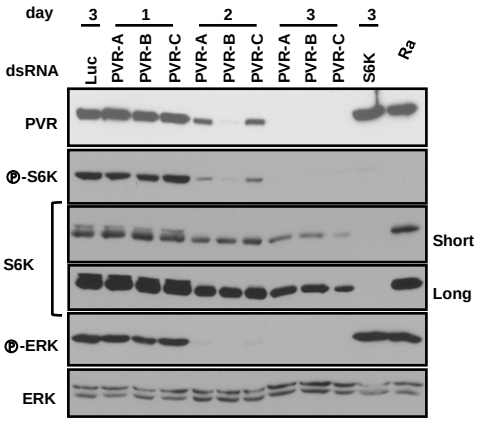
Supplemental Figure 8. Comparative analyses of sunitinib resistant mutations in inactive state KIT structure (A829P, left) and PVR model (G1166P, right).

Residues within the A-loop are colored comparatively: blue (identical) and light blue (differing), highlighting polarity by coloring individual elements: blue (nitrogen) and red (oxygen). Residues outside the A-loop are in cyan. Residues within 4Å of each mutation are displayed in stick. Mutated

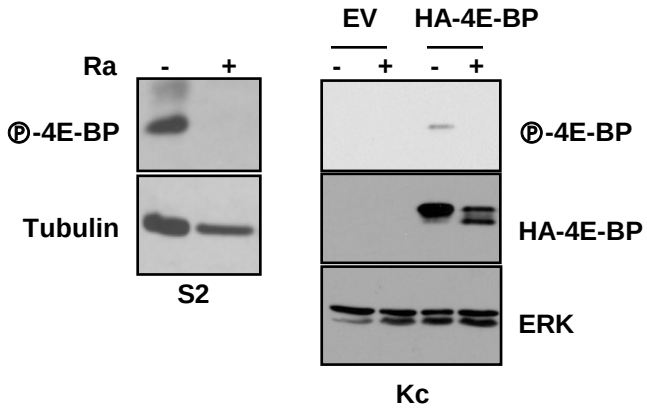
positions are depicted as magenta stick for wild-type side chains or with white stick surrounded by dots for mutated side chains. Hydrogen bonds are represented by black dots. G1166P mutation in PVR results in additional hydrogen bonds that maintain the inactive b-hairpin. Proline residue provides a greater hydrophobic area which is stabilized by the hydrophobic portion of E1164.

Supplemental Figure 9 JMR region of insects.

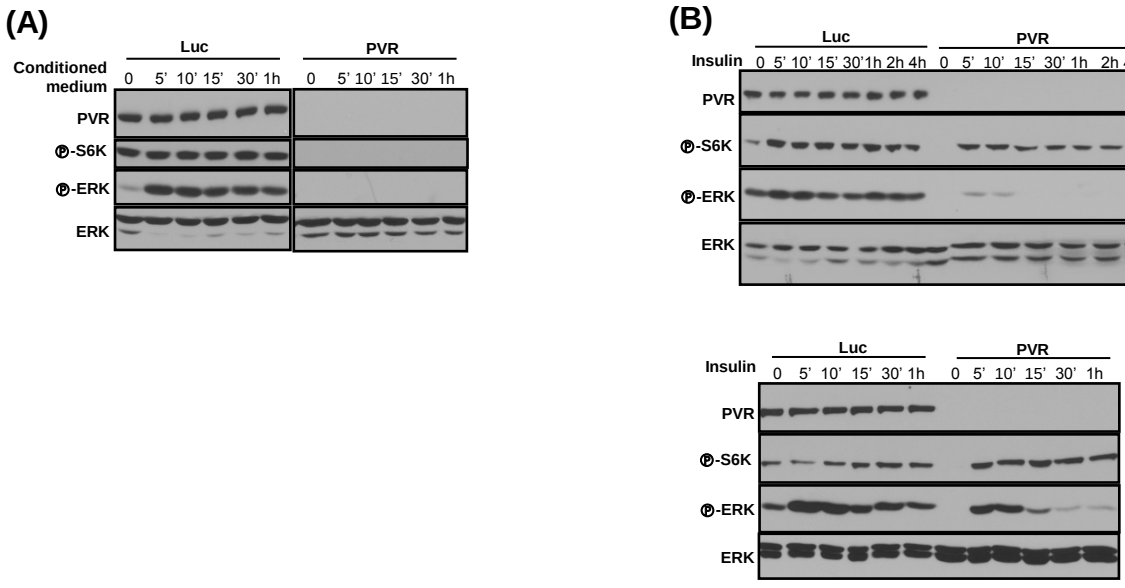
Alignment of the transmembrane helix and JMR of indicated insects.



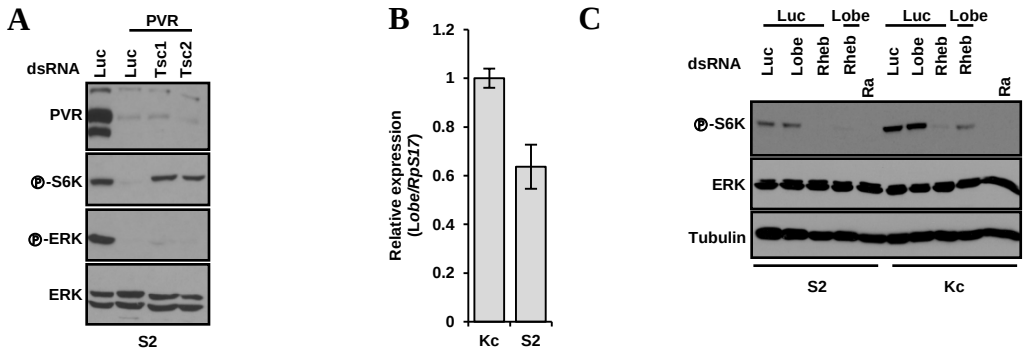
Supplemental Figure 1



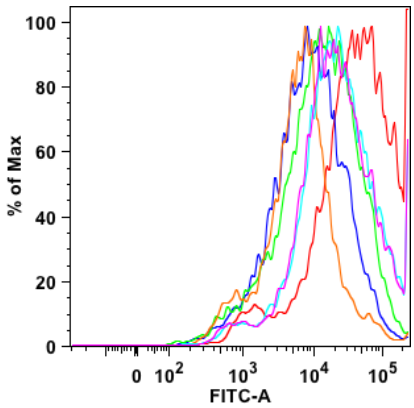
Supplemental Figure 2



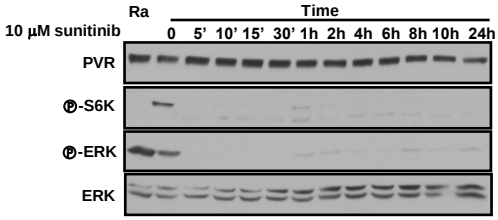
Supplemental Figure 3



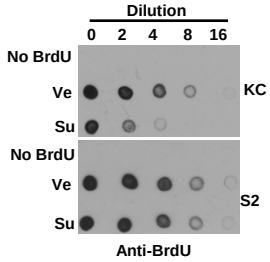
Supplemental Figure 4



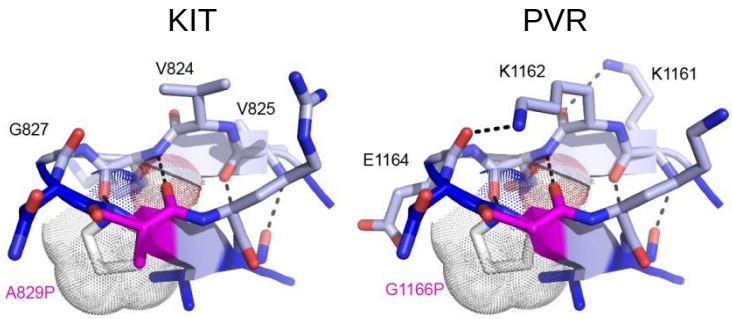
Supplemental Figure 5



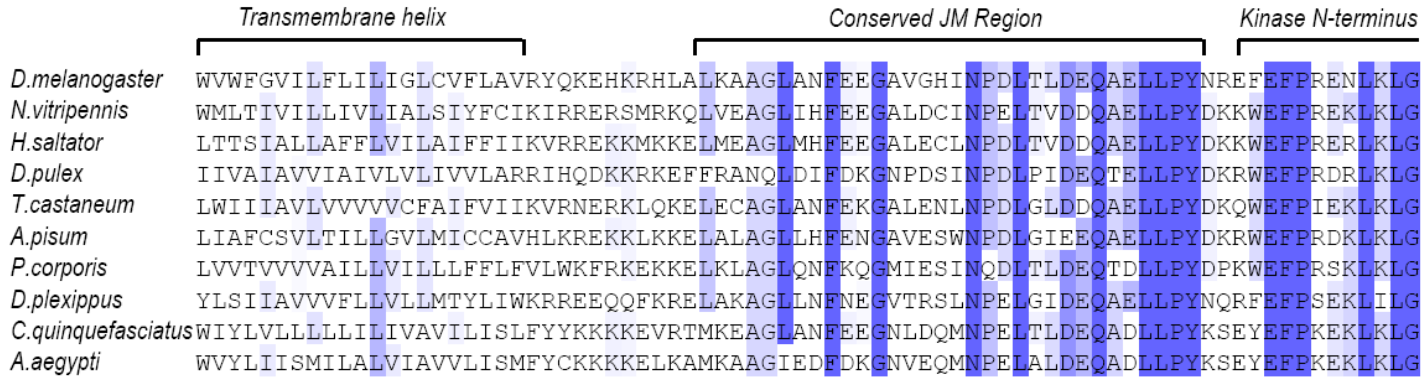
Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 9

Tran et al. Supplementary Table 1.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
β-gal	TAATACGACTCACTATAGGTTATCGATGAGCGTGGTGGTTATGC	TAATACGACTCACTATAGGGCGCGTACATCGGGCAAATAATATC
Crk	TAATACGACTCACTATAGGGGAAAACTACCTGCATACGCC	TAATACGACTCACTATAGGGGCATATTTCTGTGGAGTTTTTG
ELMO	TAATACGACTCACTATAGGGGGAGAACATTGTTATGTGCAG	TAATACGACTCACTATAGGGGCGGCGTCTCAATGAAAT
Lobe	TAATACGACTCACTATAGGGTGATCTCGTGCAAATGCTC	TAATACGACTCACTATAGGGGCTTAAAGAACTCCATGATATG
Luc	TAATACGACTCACTATAGGGAGATGGAACCGCTGGAGAGC	TAATACGACTCACTATAGGGGACTCTGGCACAAAATCG
Mbc	TAATACGACTCACTATAGGGAAATCCCAGCTTTCCCAAAA	TAATACGACTCACTATAGGGCTTGTCGATGCGCTGAAC
PTEN	TAATACGACTCACTATAGGGACACATCAACCAATTCAAAAAAT	TAATACGACTCACTATAGGCAATCTTCCTCGCCATCTT
PVRA	TAATACGACTCACTATAGGGGAAGAAGGTCACGATAGCCG	TAATACGACTCACTATAGGGTCGGGTCATTACAACGTTCA
PVRB	TAATACGACTCACTATAGGGTGTGATCCTTATCGAAGCCA	TAATACGACTCACTATAGGG TTTTTGCGCACAATTACCAA
PVRC	TAATACGACTCACTATAGGCAGCAGCCGGGAGATAG	TAATACGACTCACTATAGGGTCAGATCGGGATTGATGTG
PVR-3'UTR	TAATACGACTCACTATAGGGGACGTCCCGGAGCCATTAGAT	TAATACGACTCACTATAGGGCCCATTGTATTTGAGTAGTCG
Ras85D	TAATACGACTCACTATAGGGCTTGCCTGCTCGTTGTT	TAATACGACTCACTATAGGG ACCTGCCTGCTGGACATC
Rheb	TAATACGACTCACTATAGGGCCAAGATCGAGCGTGTAAA	TAATACGACTCACTATAGGGCATTACGTCGAGCAGTTTTT
S6K	TAATACGACTCACTATAGGGCAATCGCTCCAGCCTTTAGA	TAATACGACTCACTATAGGGGTTTCACCTACGTTGCACCC
TOR	TAATACGACTCACTATAGGACCACAAACGAACTACGAAC	TAATACGACTCACTATAGGTACCTTGTGAGCAGACCTTC
Tsc1	TAATACGACTCACTATAGGCAGCCTGCCAGAAATTAACT	TAATACGACTCACTATAGGCCAGTCCTTCCACCGTCT
Tsc2	TAATACGACTCACTATAGGCTTCCAGCGGCGGAATT	TAATACGACTCACTATAGGCACGGATCCCGACGAAG
Diap1	TAATACGACTCACTATAGGGGCCACCGTATCGATATAGAG	TAATACGACTCACTATAGGGCCAACGACTCGACGCT
Drice	TAATACGACTCACTATAGGTGCATCCAAGCTTTTCACAG	TAATACGACTCACTATAGGCTTACGGTAGCTGGACGAGG
CG32406 RNAi	TAATACGACTCACTATAGGGCCGCCACAATGATAACCAAC	TAATACGACTCACTATAGGGCGCGTGCGTGAAGAGT
Lobe-RT	CGCAGGTGTTCCAGCAGA	CTTCCAGGGTGAGACCATACAAC
RpS17	GAGCAACATAATGGGTCGC	CAGGGGCTTGGTGGGAAT
PVR-pet	GGAGATATACATATGGTAGCCAGCCACTACCTG	TTAGCAGCCGGATCCCTATCAGTGATGGTGATGGTGATGCCCTCCATACCTTCGTTGCTCCTT
PVR-G1166P	CCCAAATTGCCCATCAAGTGG	ATTCTCTGACTTCTTGTAGTT
PVR-Y1160D	GACAAGAAGTCAGAGAATGGC	GTTATCACCTCGATACATGGA

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Name	Forward primer (5'-3')	Reverse primer (5'-3')
PVRN1159Y	CGATCCATGTATCGAGGTGATTACTACAAGAAGTCAGAGAATGGC	GCCATTCTCTGACTTCTTGTAGTAATCACCTCGATACATGGATCG
$Ras85D^{V12}$	ATATATGAATTCCAACATGACGGAATACAAACTGGTCGTCGTTGGAGCC GTCGGCGTGGGCAAGTCCGCGCTC	ATATATGCGGCCGCTTAGAGCATTTTACATTTAAATCTACG
CG32406 cloning	ATATATAGATCTATGCCCAAAAGCGAGGCC	ATATATGCGGCCGCCTACTGCTGCTGCTCCGTCTC