Supporting Information

Hu et al. 10.1073/pnas.1102554108

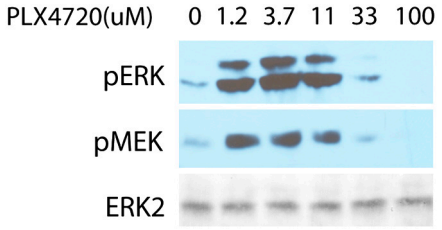

Fig. S1. Dose response of PLX4720 on extracellular signal regulated kinase (ERK) and mitogen activated protein kinase kinase (MEK) phosphorylation in HCT116 cells. HCT116 cells were treated for 1 h with the indicated dose of PLX4720. Cell lysates were analyzed by SDS-PAGE and after transfer to nitrocellulose membranes were immunoblotted with antibodies specific for phosphorylated ERK (pERK) or phosphorylated MEK (pMEK).

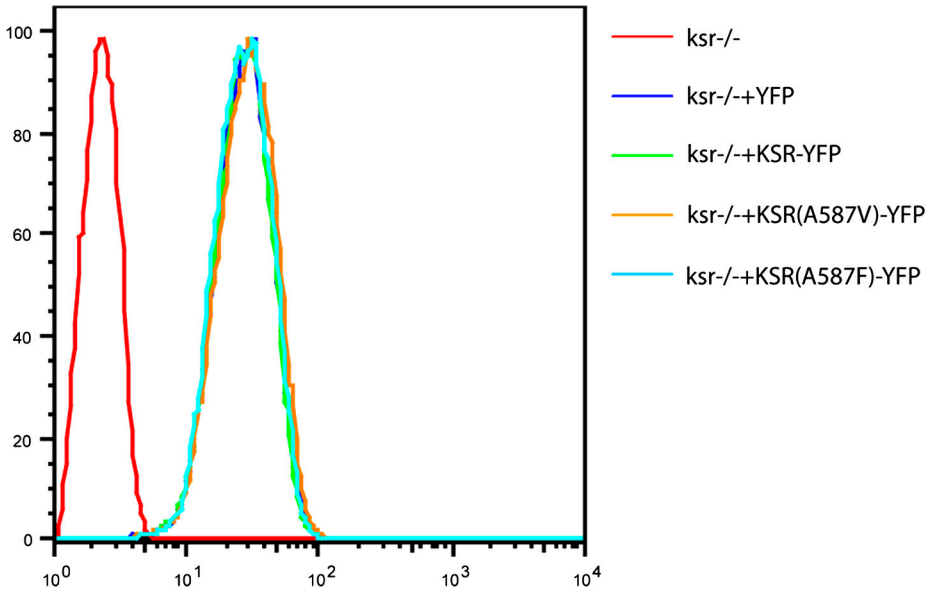

Fig. S2. Expression levels of transfected WT or mutant mutated kinase suppressor of Ras's (KSR)1 in ksr1-/- mouse embryonic fibroblasts (MEFs). Wild-type or mutant KSR-GFP constructs were transfected into KSR-/- MEFs and selected using neomycin resistance. Neomycin resistant cells were sorted multiple times to achieve equivalent levels of GFP levels.

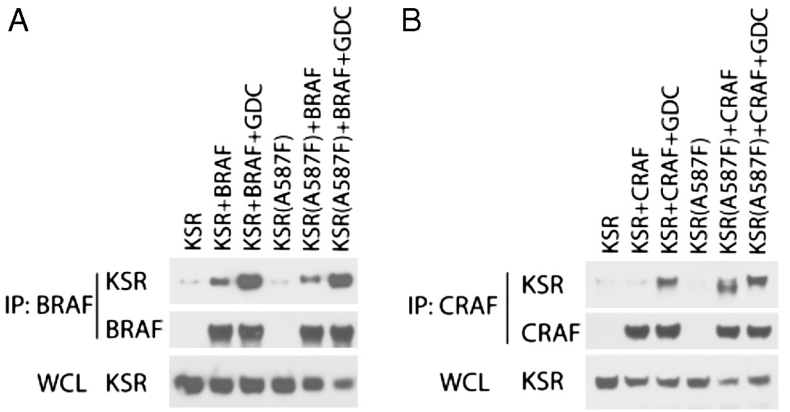

Fig. S3. The images in Figs. 1 *B* and *C* and 2 *D* and *E* were performed in the same experiment. So that the experiments can be comparable to each other, we include the complete blots for each experiment. Experimental details are in the figure legends for Figs. 1 and 2.

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                      50   55             70 72
                      GXGXXG              AXK                  91
PKA       40 DQFDRIKTLGTGSFGRVMLVKHKESGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVNF
BRAF     492 GQITVGQRIGSGSFGTVY--KGKWHGD-VAVKMLNVTAPTP-QQLQAFKNEVGVLRKTRH
CRAF     347 SEVMLSTRIGSGSFGTVY--KGKWHGD-VAVKILKVVDPTP-EQLQAFRNEVAVLRKTRH
KSR1     561 EQVELGEPIGQGRWGRVH--RGRWHGE-VAIRLLEMDGHNQ-DHLKLFKKEVMNYRQTRH


PKA          PFLVKLEFSF-KDNSNLYMVMEYVAGGEMFSHLRRI-GRFSEPHARFYAAQIVLTFEYLH
BRAF         VNIL-LFMGY-STKPQLAIVTQWCEGSSLYHHLHIIETKFEMIKLIDIARQTAQGMDYLH
CRAF         VNIL-LFMGY-MTKDNLAIVTQWCEGSSLYKHLHVQETKFQMFQLIDIARQTAQGMDYLH
KSR1         ENVV-LFMGACMNPPHLAIITSFCKGRTLHSFVRDPKTSLDINKTRQIAQEIIKGMGYLH
                  165                 184                        208
                  HRD                 DFG                        APE
PKA          SLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKG---------RTWTLCGTPEYLAPEI
BRAF         AKSIIHRDLKSNNIFLHEDLTVKIGDFGLATVKSRWSGSH----QFEQLSGSILWMAPEV
CRAF         AKNIIHRDMKSNNIFLHEGLTVKIGDFGLATVKSRWSGSQ----QVEQPTGSVLWMAPEV
KSR1         AKGIVHKDLKSKNVFYD-NGKVVITDFGLFGISGVVREERREN-QLKLSHDWLCYLAPEI

                                220  225
PKA          ILS---------KGYNKAVDWWALGVLIYEMAAGYPPFFADQPIQIYEKIVSGKVRFPSH
BRAF         IRM------QDKNPYSFQSDVYAFGIVLYELMTGQLPYSNINNRDQIIFMVGRGYLSPDL
CRAF         IRM------QDDNPFSFQSDVYSYGIVLYELMAGELPYAHINNRDQIIFMVGRGYSKVRS
KSR1         VREMIPGRDEDQLPFSKAADVYAFGTVWYELQARDWPFKHQPAEALIWQIGSLSPDLGEG

                                           280
PKA          -----------FSSDLKDLLRNLLQVDLTKRFGNLKNGVNDIKNHKWFATT
BRAF         ----------NCPKAMKRLMAECLKKKRDERPLFPQILASIELLARSLPKI
CRAF         ASPDLSRLYKNCPKAMKRLVADCVKKVKEERPLFPQILSSIELLQHSLPKI
KSR1         --VRRVLASVSLGKEVGEILSACWAFDLQERPSFSLLMDML----ERLPKL
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Fig. S4. Alignment of the "kinase" domains of protein kinase A (PKA), BRAF, CRAF, and kinase suppressor of Ras (KSR)1. The kinase core sequences are aligned and the well-known conserved motifs are in bold. V600 and S602 in the activation segment of BRAF are in red. V600 is mutated to E in many cancers and thus serves as a phosphomimietic for S602 which is a key phosphorylation site. The K to R mutation in KSR that has categorized it as a pseudokinase is shown in magenta.

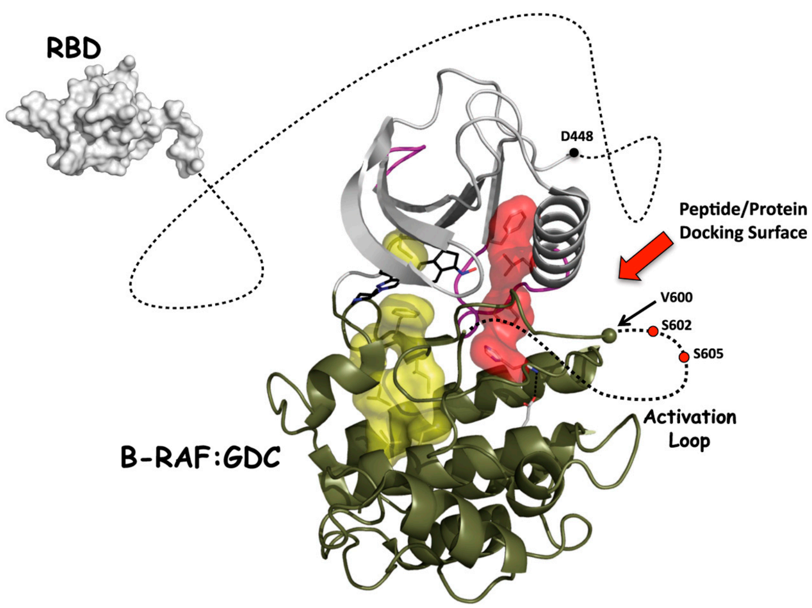

Fig. S5. Analysis of the hydrophobic spines in the structure of BRAF bound to GDC0879. The positions of residues making up the catalytic and regulatory hydrophobic spines were analyzed in the structure of BRAF bound to GDC0879 analogue (1). This analysis shows the assembly of both hydrophobic spines by this type I inhibitor. Catalytic spine residues are in yellow and regulatory sine residues in red. Loops that are associated with the dimer interface are shown on the back in magenta. This image also shows the position of D448 within the SSDD sequence (446–449) of BRAF which is important in uncoupling the inhibitory amino-terminal domain from the kinase domain. It also shows the position of V600E in the activation loop close to the activation loop phosphorylation sites, S602 and S605. Replacing A481 with Phe appears to stabilize this same active conformation where the Phe replaces the adenine ring of ATP. In the GDC0879 bound structure, the drug completes the C spine. Activation of mitogen activated protein kinase kinase (MEK) and extracellular signal activated kinase (ERK) by the V600E mutation requires RAS activation. Dimer formation and inhibition by GDC0879 is also RAS dependent. Surprisingly, the A/F mutation leads to downstream activation of MEK and ERK that is independent of RAS, suggesting that in this mutant the inhibitory N-terminal segment containing the RAS binding domain (RBD) is constitutively uncoupled from the kinase domain.

1 Hansen JD, et al. (2008) Potent and selective pyrazole-based inhibitors of B-Raf kinase. *Bioorg Med Chem Lett* 18:4692–4695.

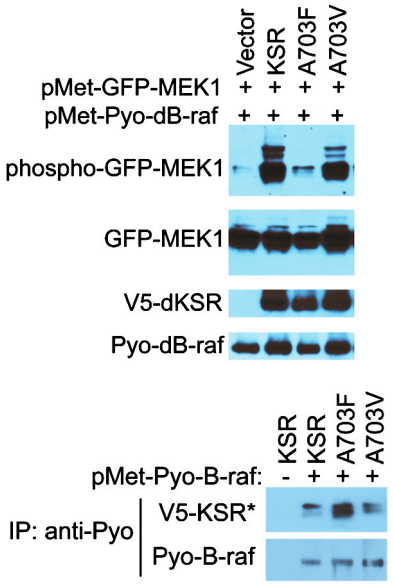

Fig. S6. Mutated *Drosophila* KSR (dKSR) functions similarly to mammalian KSR. A703 of dKSR is analogous to A587 of mouse KSR1, which was mutated to phenylalanine or valine using site-directed mutagenesis. Wild-type or mutated dKSR was coexpressed with dMEK in *Drosophila* S2 cells and activation of mitogen activated protein kinase kinase (MEK) was assessed using a phospho-MEK antibody. In the lower panel, the ability of wild-type and mutated dKSR to form complexes with BRAF was assessed. Wild-type and mutated dKSR was overexpressed in S2 cells with dBRAF. The dBRAF immunoprecipitates and coprecipitating dKSR were assessed by immunoblotting.