Figure S1. Characterization of indel mutations in HIPK2-Cas9 cells. (A) Sequencing analysis of HIPK2-Cas9 cells displays a mixed sequence with double peaks, suggesting that a biallelic mutation was introduced as a result of CRISPR/Cas9 editing. (B) HIPK2-Cas9 DNA was subcloned by using the TOPO TA Cloning kit allowing to obtain the single allelic sequences (single peaked sequences). (C) Nucleotide Blast alignment of HIPK2-Cas9 allelic sequences with HIPK2 (sequence ID, NM_022740.4) shows a G insertion between 393 and 394 in one allele and a G deletion in 394 in the other. Frame mutations are encircled in red. HIPK2, homeodomain-interacting protein kinase 2.

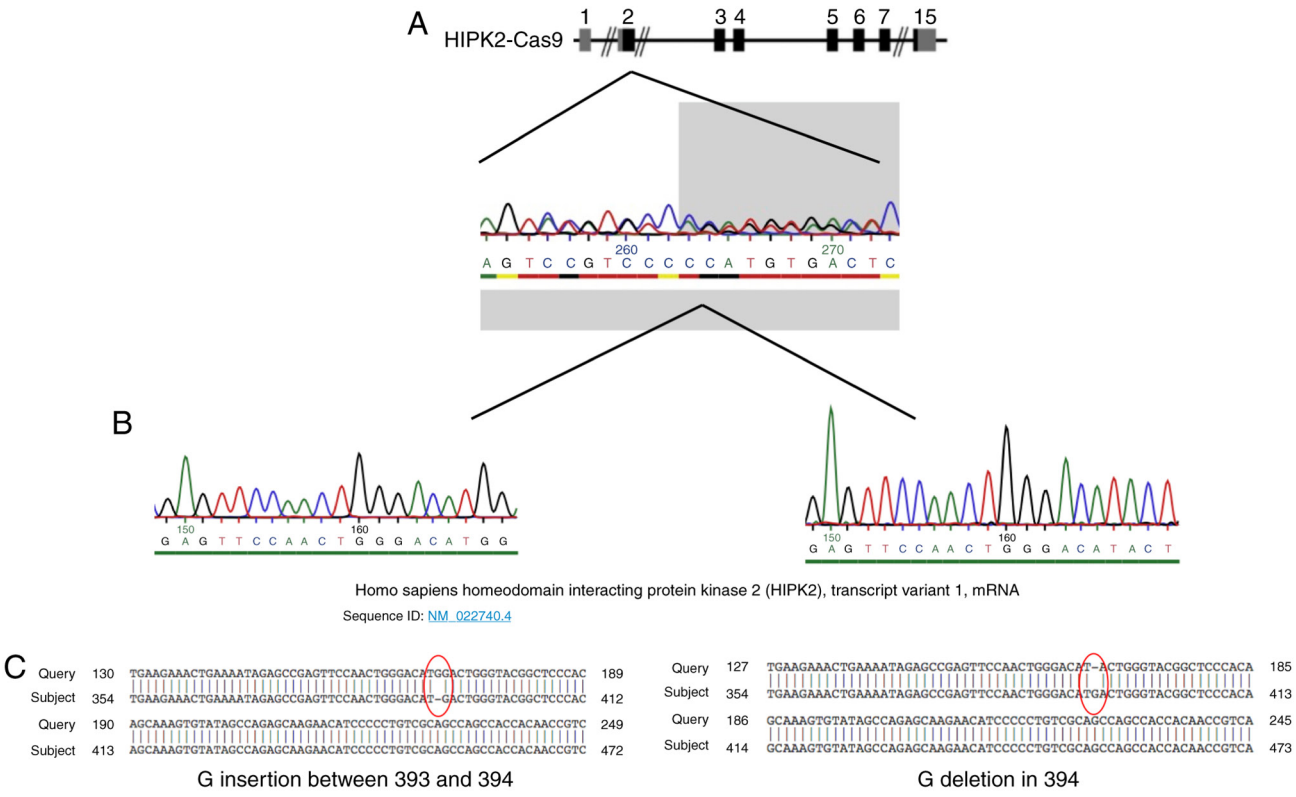

Figure S2. HIPK2-defective cells are resistant to 5-FU and OXA. (A) HeLa cells were previously obtained by CRISPR/Cas9, while (B) RKO cells were stably transfected using short hairpin RNAs. The indicated cells were exposed to increasing doses of 5-FU and OXA for 48 h, and cell viability was measured by XTT assay. For each point the percentage compared with the untreated sample was calculated. Each point represents the mean ± standard error of cell viability at each dose of 5-FU and OXA of three replicates in two independent experiments. *P<0.05; **P<0.01; ***P<0.001. XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfoph enyl)-2H-tetrazolium-5-carboxanilide; Ctrl, control; 5-FU, 5-fluorouracil; OXA, oxaliplatin; HIPK2, homeodomain-interacting protein kinase 2; i, inhibitor.

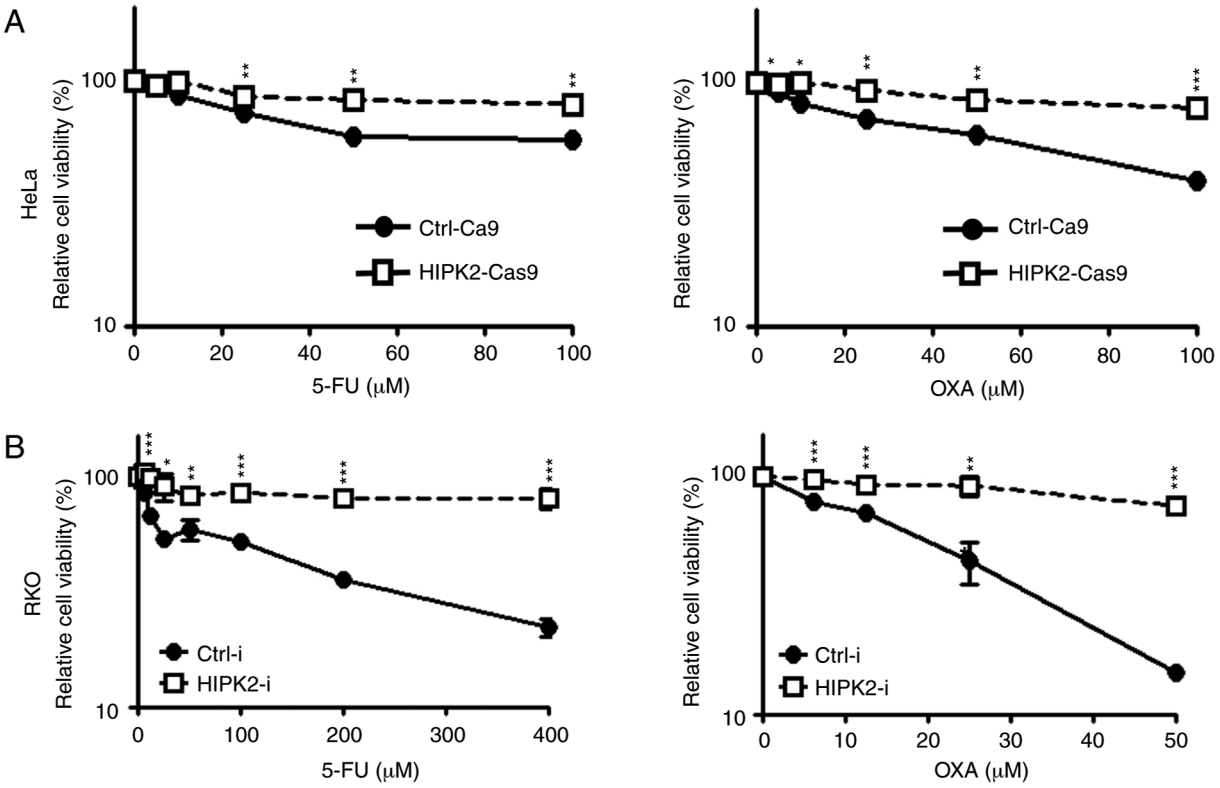

Figure S3. HIPK2-depleted RKO cells are resistant to brusatol. (A) RKO-Ctrl-i and HIPK2-i cells were treated with increasing doses of brusatol, and cell viability was assessed by XTT after 48 h. Each point represents the percentage (mean ± SE) of cell viability compared with the untreated sample. Brusatol induced an increase in chemotherapy response only when HIPK2 was expressed. (B) RKO-Ctrl-i and (C) RKO-HIPK2-i cells were treated with increasing doses of 5-FU (left panels) and OXA (right panels) for 48 h in the presence or absence of brusatol (15 nM) added 4 h before the other drugs. Two doses of brusatol (15 and 40 nM) were employed on the latter cells due to their resistance. *P<0.05; **P<0.01; ***P<0.001. XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; Ctrl, control; 5-FU, 5-fluorouracil; OXA, oxaliplatin; HIPK2, homeodomain-interacting protein kinase 2; i, inhibitor.