Supporting Information

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SI Materials and Methods

CK1δ/ε Autophosphorylation Assay. K562 cells were plated at a cell density of 2×10^5 cells per milliliter and treated with DMSO, 1 μM PF670462, 10 μM CGP57380, 2.5 μM AST 487, or 20 μM cercosporamide for 24 h before addition of 50 nM of calyculin A (Sigma-Aldrich) to the culture media. Cells were harvested for Western blot analysis. Phosphorylation of casein kinase (CK)1δ/ε was measured by using anti-CK1δ and anti-CK1ε (gift from David M. Virshup, Duke-NUS Graduate Medical School, Singapore). Protein phosphatase 2A-A subunit (PP2A-A) was used as a loading control.

***siMNK1/2* Target Sequences.**

siMNK1 (no. 2) target sequence: CAAAGAGUAUGCCGUCAAA.

siMNK2 (no. 2) target sequence: GAACCGUUACUGUGAAUGA.

siMNK1 (no. 3) target sequence: GGGCACACAUCUCCAGUGA.

siMNK2 (no. 3) target sequence: GAAGAAACCAGCCGAACUU.

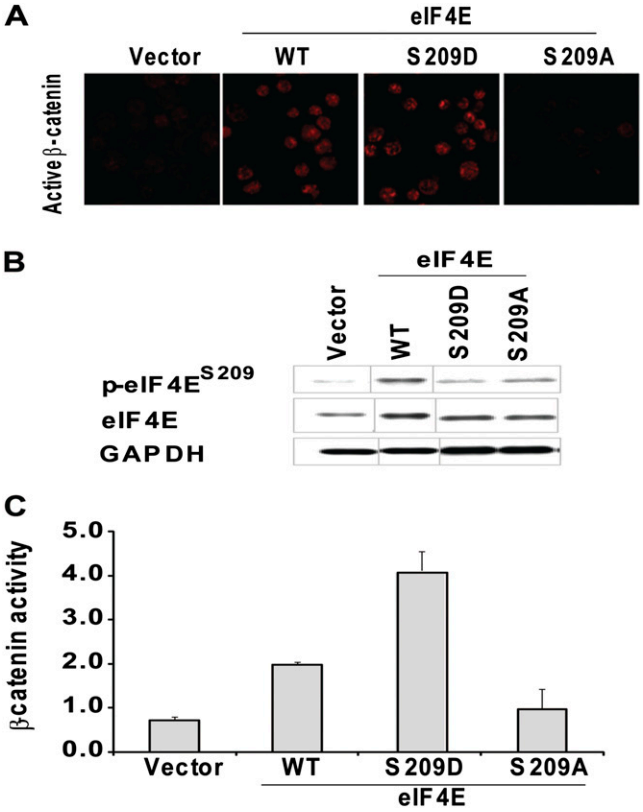

Fig. S1. Overexpression and phosphorylation of eIF4E activates β-catenin in KCL22 cell line. KCL22 cells were retrovirally transduced with an MSCV-based vector expressing vector only, eIF4E WT, eIF4E S209D, or eIF4E S209A. Cells were harvested for (*A*) immunofluorescent examination of nuclear β-catenin, (*B*) Western analysis for eIF4E protein, and (*C*) β-catenin transcriptional activity by using the SuperTOP/FOPflash assay (mean ± SEM from three independent experiments). The Western images are from the same gel and same exposures, and lanes were cropped for presentation purposes.

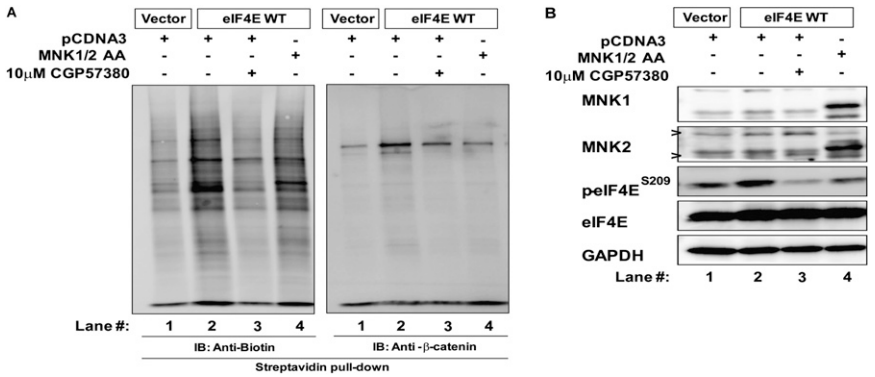

Fig. S2. MNK kinase activity regulates protein translation in K562 cells. K562 cells stably overexpressing eIF4E were treated with DMSO or the MNK1/2 inhibitor CGP57380 and transduced with empty vector or vector expressing dominant-negative MNK1/2 (AA). Cells were then deprived of methionine for 2 h before labeling with L-AHA for 1 h before harvesting for (*A*) Click-iT assay and (*B*) Western analysis. (*A*) Newly synthesized proteins were pulled down by using high-capacity streptavidin beads. Precipitated proteins were first probed with antibodies against β-catenin. Blots were stripped and reprobed with anti-biotin HRP to assess global protein translation. Blots are representative of four independent experiments.

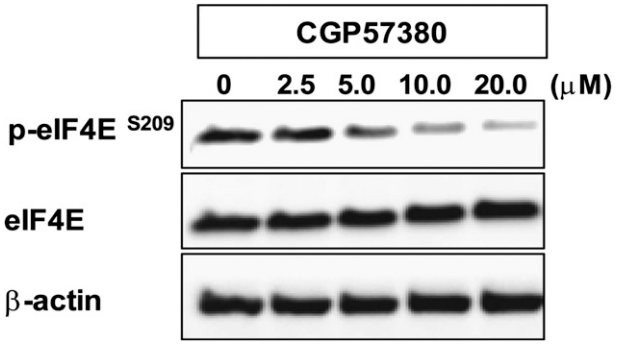

Fig. S3. Inhibition of eIF4E phosphorylation by CGP57380 in a dose-dependent manner. K562 cells (5×10^5 cells per milliliter) were treated with increasing concentrations of CGP57380. At 24 h after drug treatment, cells were harvested, and cell lysates were subjected to Western analysis to detect phosphorylated eIF4E. Representative Western blots (from at least three independent experiments) are shown.

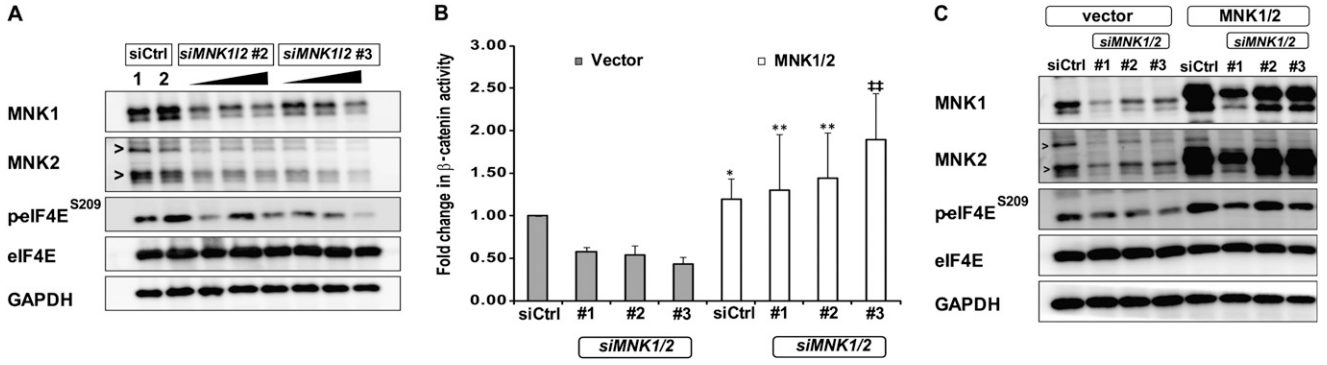

Fig. S4. Overexpression of MNK1/2 restores β-catenin signaling following siRNA-mediated MNK1/2 knockdown. (*A*) K562 cells were nucleofected with two different siRNA controls (siCtrl) or two different siRNA pairs (no. 2 and no. 3) targeting *MNK1* and *MNK2* at 20, 50, and 100 nM. Cells were harvested, and lysates were used for Western analysis. siRNA pair 1 is the pair used in Fig. 4. For *B* and *C*, K562 cells were nucleofected with siRNA controls or siMNK1/2 together with vector control or MNK1/2 expression plasmids. At 36 h post transfection, cells were harvested for (*B*) TOPFlash reporter assay and (*C*) Western analysis. (*B*) Bar chart shows an average of three independent experiments (mean ± SEM; $^{*}P \leq 0.05$, $^{**}P \leq 0.008$, and $^{\ddagger\ddagger}P < 0.001$).

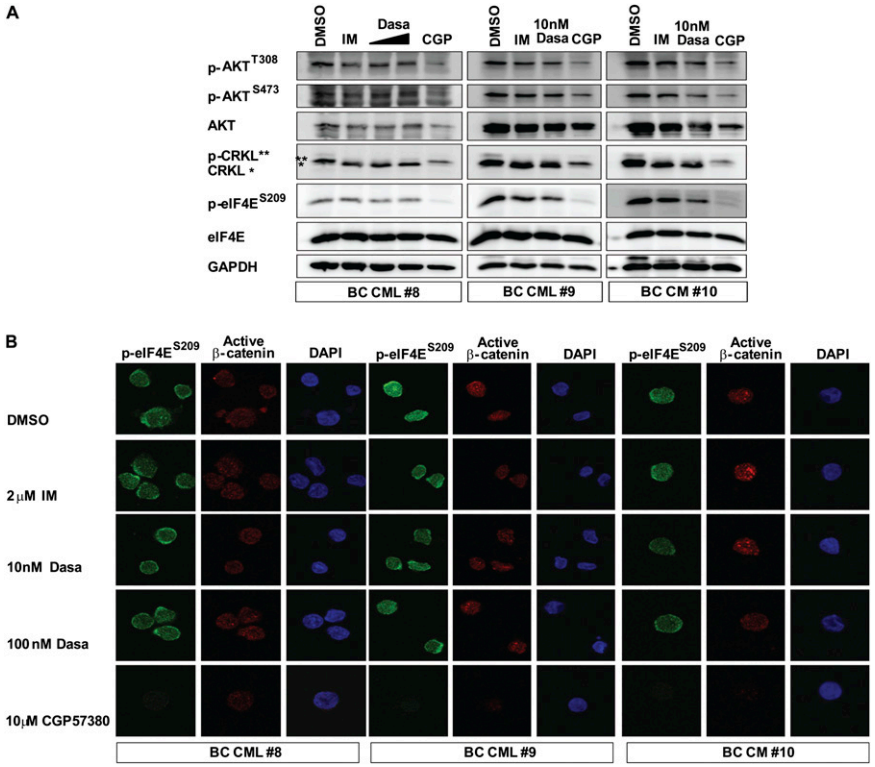

Fig. S5. CGP57380, but not tyrosine kinase inhibitor, treatment inhibits AKT activity and nuclear β-catenin translocation. $CD34^{+}$ enriched blast crisis (BC) chronic myeloid leukemia (CML) cells from three different patients with BC were treated with imatinib (IM), dasatinib (Dasa), or CGP57380 (CGP) for 48 h before harvesting for (*A*) Western analysis and (*B*) immunofluorescence analysis. (*A*) BC CML no. 8 was treated with 10 nM and 100 nM dasatinib. CGP57380 was included as a positive control.

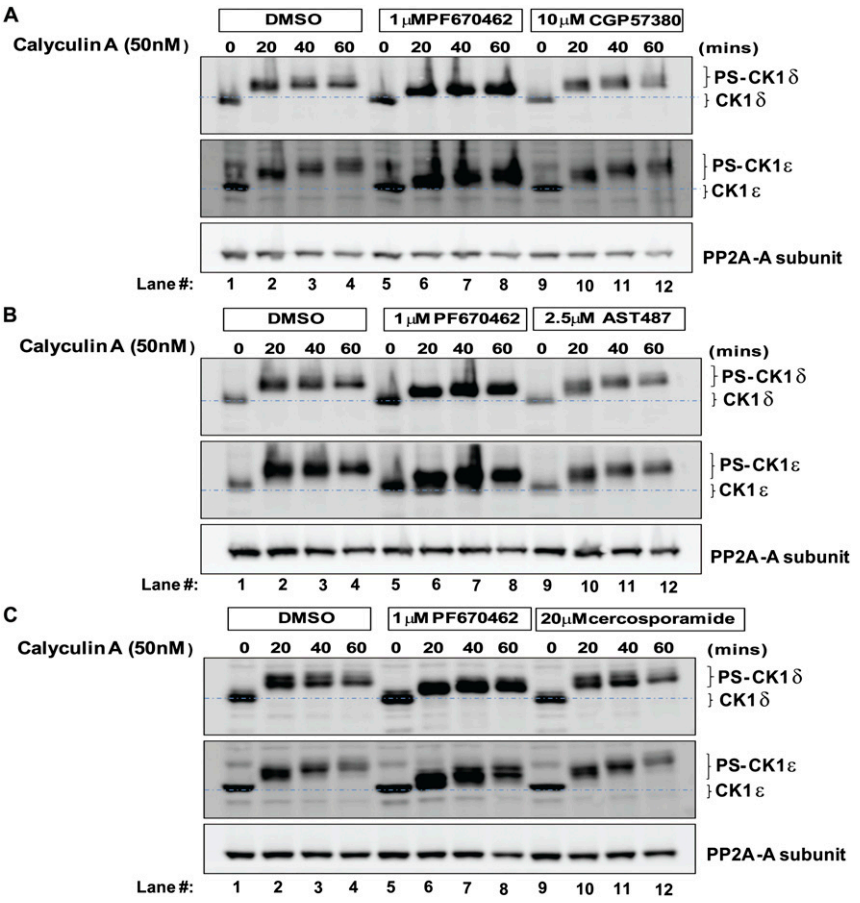

Fig. S6. CGP57380 does not inhibit CK1δ/ε kinase in vivo. K562 cells were treated with DMSO, the three MNK kinase inhibitors, or the specific CK1δ/ε inhibitor PF670462 for 24 h. Cells were then incubated with 50 nM calyculin A for the indicated time and harvested, and whole-cell lysates were analyzed for CK1 autophosphorylation by Western analysis. PS-CK1, phosphorylated serine-CK1. PP2A-A served as a loading control. (*A*) As seen in each of the DMSO control lanes (lanes 2–4), the bands specific for CK1 (*Top*, probed for CK1δ; *Middle*, probed for CK1ε) exhibit an upward shift compared with the band at time 0 (lane 1) that is characteristic of autophosphorylated CK1δ/ε (1). In contrast, treatment with PF670462 (lanes 6–8) results in a smaller upward shift compared with the DMSO-treated lanes (lanes 2–4). The degree of shift seen in the CGP57380-treated lanes (lanes 10–12) is similar to the DMSO control lanes (lanes 2–4), indicating a lack of CK1 kinase inhibition. (*B*) Cells were treated as in *A*, but with AST 487 instead of CGP57380. (*C*) Cells were treated as in *A*, but with cercosporamide instead of CGP57380.

1. Cheong JK, et al. (2011) IC261 induces cell cycle arrest and apoptosis of human cancer cells via CK1δ/ε and Wnt/β-catenin independent inhibition of mitotic spindle formation. *Oncogene* 30(22):2558–2569.

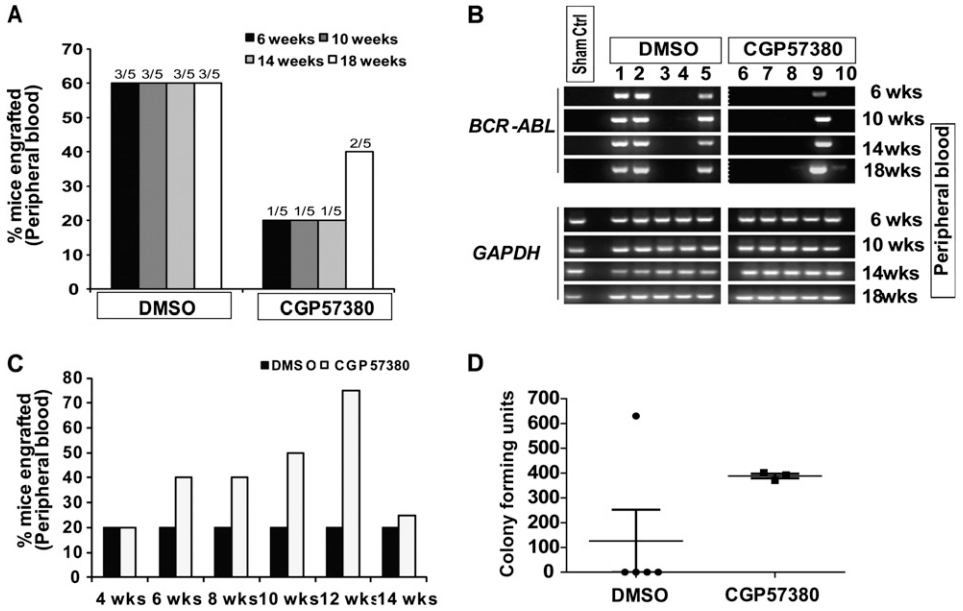

Fig. S7. MNK inhibitors decrease eIF4E phosphorylation and β-catenin activity and reduce leukemia cell burden. (*A*) $CD34^+$ cells from an individual with BC CML were cultured for 48 h in the presence of DMSO or 10.0 μM CGP57380, following which they were transplanted into NSG mice via intrafemoral injection. At 4 wk posttransplantation, peripheral blood was obtained for flow cytometric analysis every 2 wk for a total of 14 to 18 wk. The percentage of mice engrafted are shown in a bar chart. (*B*) RT-PCR analysis for *BCR-ABL1* transcripts in peripheral blood from mice treated in *A* were performed at 2-wk intervals. (*C*) $CD34^+$ cord blood cells were treated as in *A* and injected into NSG mice, and engraftment was evaluated as in *A*. (*D*) Human $CD45^+$ cells were obtained from the mice in *C* at the time of euthanasia, and committed progenitors were enumerated at 2 wk by using a CFC assay.

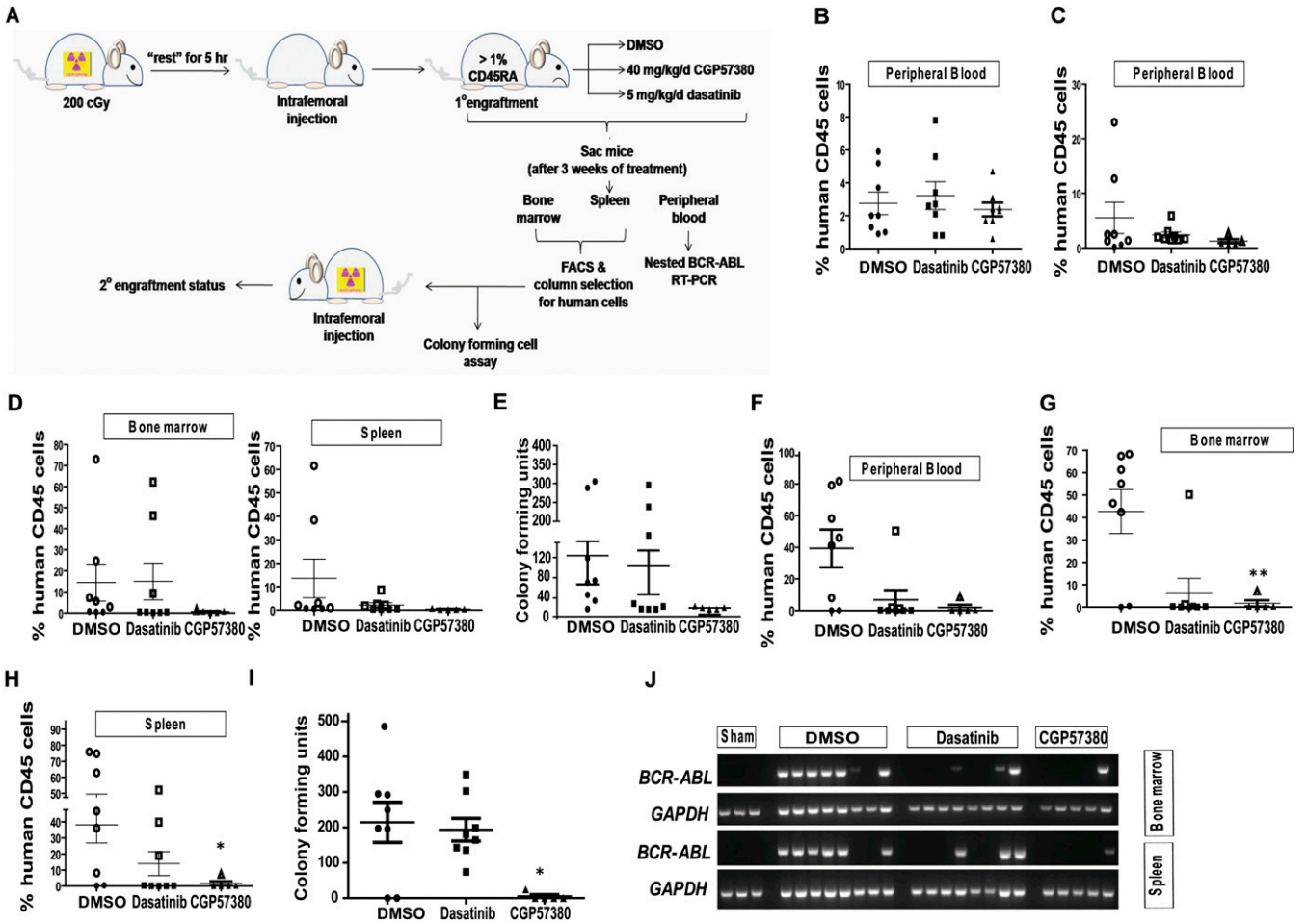

Fig. S8. MNK1/2 inhibition reduces engraftment and serial transplantation capacity of CD34$^+$ BC CML cells. (*A*) BC cells were injected intrafemorally into sublethally irradiated NSG mice and allowed to engraft, after which animals were treated with DMSO or drugs. Mice were then killed, and human cells were isolated and transplanted into a secondary transplant recipient. (*B*) CD34$^+$ BC cells (5×10^5) were injected femorally into sublethally irradiated NSG mice, which were divided into three treatment groups (DMSO, dasatinib, and CGP57380). At 4 wk after transplantation, and before drug treatment, the percent engraftment in peripheral blood was assessed. (*C*) Percent human cell engraftment in mice in *B* after 3 wk of treatment with DMSO, CGP57380 (40 mg·kg^{-1}·d^{-1}), or dasatinib (5 mg·kg^{-1}·d^{-1}). Open circles, squares, and triangles denote the presence of greater than 1% human CD45 cells. (*D*) Mice were killed after treatment, and the percent engraftment in bone marrow and spleen after 3 wk of treatment assessed by flow cytometry. (*E*) Human CD45$^+$ cells obtained at the time of *D* were also plated in a CFC assay using an input of 1×10^5 cells, and colonies were counted at 2 wk. (*F–H*) All the remaining human CD45$^+$ cells isolated from the primary transplants were injected into secondary recipients. Secondary transplant recipients were killed at week 10, and peripheral blood, bone marrow, and spleen were isolated for FACS analysis. (*I*) Human cells (1×10^5) isolated from secondary transplants were plated for a CFC assay, and colonies were counted at 2 wk. (*J*) RT-PCR analysis for BCR-ABL1 transcripts were performed on hematopoietic organs isolated from secondary transplants (*$P \leq 0.03$ and **$P \leq 0.01$).

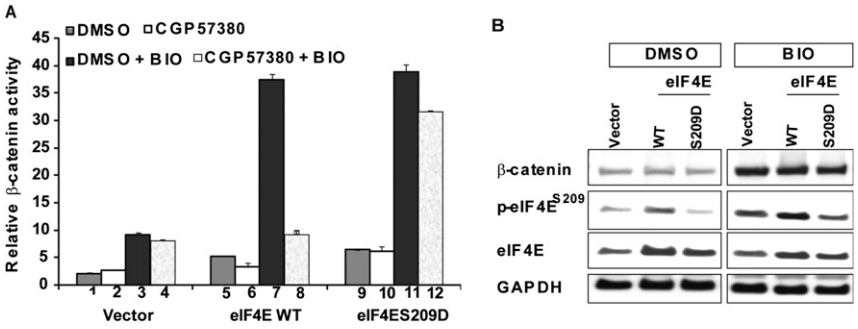

Fig. S9. GSK3β inactivation and eIF4E phosphorylation cooperate to activate β-catenin signaling, an effect inhibited by CGP57380. (*A*) K562 vector, K562-eIF4EWT, and K562-eIF4ES209D cells were pretreated with 0.5 μM BIO for 1 h before treatment with 10 μM CGP57380 for 24 h. Results shown are representative of three independent experiments. Exposure of vector-only K562 cells to BIO resulted in a fourfold increase in SuperTopFlash activity (compare bar 1 vs. bar 3), whereas, in K562-eIF4EWT and K562-eIF4ES209D cells, we observed a sevenfold increase in SuperTopFlash activity (compare bar 5 vs. bar 7 and bar 9 vs. bar 11). CGP57380 treatment did not significantly inhibit the BIO-induced increase in SuperTopFlash activity in control K562 cells (compare bar 3 vs. bar 4), but completely prevented the cooperative effect of eIF4E overexpression and BIO treatment on SuperTopFlash activity in K562-eIF4EWT cells (compare bar 7 vs. bar 8). In contrast, CGP57380 was not able to completely abolish the BIO-induced increase in SuperTopFlash activity in K562-eIF4ES209D cells (compare bar 11 vs. bar 12). Bars indicate mean ± SEM obtained from three independent experiments. (*B*) K562 vector, eIF4E WT, and eIF4E S209D cells treated with DMSO or 0.5 μM BIO for a total of 24 h were harvested for Western analysis. A representative blot from at least three independent experiments is shown.

Table S1. K_d values for binding of CGP57380, AST 487, and cercosporamide to the active sites of CK1δ, CK1ε, MNK1, and MNK2

	K_d, nM		
Kinase	CGP57380	AST 487	Cercosporamide
CK1δ	450	7,600	11,000
CK1ε	960	10,000	9,300
MNK1	900	24	5,300
MNK2	250	1.2	46

K_d values provide a direct measddure of kinase binding activity, and use a competitive kinase active-site dependent competition binding assay (KINOME*scan*) (1).

1. Karaman MW, et al. (2008) A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* 26(1):127–132.