Supplemental Figure S1



Supplemental Figure S1: CPI-444 suppresses pCREB and restores pERK in human immune cells.

A) Three donors were assayed separately, NECA induced phosphorylated CREB in B cells (1.5 fold ± 0.1) was blocked by CPI-444 as well as the control A2AR antagonist ZM 241385. The reported CREB phosphorylation in each condition is normalized to the level in unstimulated cells (MFI in stimulated condition ÷ MFI in unstimulated condition). B) NECA and CGS21680 (both 1 µM) suppressed IL-2 and IFNγ 48 hours after T cell activation. Blockade of A2AR with CPI-444 (1 µM) neutralized adenosine signaling and restored IL-2 and IFNγ secretion. C) NECA inhibits TCR-mediated ERK phosphorylation and CPI-444 restores ERK phosphorylation. TCR crosslinking resulted in ERK phosphorylation in all donors. The level of phospho-ERK induction was reduced by NECA; this NECA effect was blocked by pre-treatment with CPI-444 (1 µM) or the positive control for A2AR antagonism ZM 241385 (1 µM).

Supplemental Figure S2



Supplemental Figure S2:

A-C) Spider plots of individual MC38 tumor bearing mice treated with vehicle control (red line) or CPI-444 at 100 mg/kg (A, blue line), 10 mg/kg (B, black line), or 1 mg/kg (C, green line) D) Percentage of mice in each treatment cohort that were tumor free at the conclusion of the experiment. The (number of tumor free mice) / (total number of mice in cohort) is also shown at the top of each treatment column. E-F) CPI-444 does not inhibit CT26 (E) or MC38 (F) tumor cell proliferation in vitro. Staurosporine, a well established inducer of apoptosis, was used as a positive control.

Supplemental Figure S3



Supplemental Figure S3: CPI-444 synergizes with anti-CTLA-4 mAb treatment.

A) Percentage of tumor free mice in each treatment cohort. B) CT26 tumor growth in mice treated with CPI-444 alone and in combination with low dose anti-CTLA-4

Supplemental Figure S4



Supplemental Figure S4: Specificity of anti-CD73 immunohistochemistry antibody.

A) Representative images of MDA-MB-231 (CD73High) parental cells (left panel) or MDA-MB-231 cells in which CD73 expression was eliminated by CRISPR-Cas9 (right panel) B) Representative images of HT1080 (CD73Neg) parental cells (left panel) or HT1080 cells designed to overexpress CD73-GFP (right panel). Antibody was used at 5 μg/ml. Images captured at 20x magnification. H&E images are presented on the bottom row.

Supplemental Table S1



Supplemental Table S1: Reagents used in CPI-444 radioligand displacement experiments

Supplemental Table S2



Supplemental Table S2: Binding affinity and selectivity of CPI-444 in radioligand binding assays.

Supplemental Table S3



Supplemental Table S3: Details regarding setup and dosing of animal models

Supplemental Table S4



Supplemental Table S4: Pharmacokinetic parameters of CPI-444 in C57BL/6 and Balb/c mice



Supplemental Table 4: Gene expression changes associated with CPI-444 + Anti-PD-L1 efficacy in MC38 tumors