Supplemental Methods

T Cell IFNγ Secretion assay

T cells were isolated from fresh spleens obtained from C57Bl/6 mice (Charles River Labs). The spleens were harvested, rinsed with PBS and placed into a 40µm cell strainer sitting in a sterile Petri dish. The spleens were gently homogenized using the top of a 3-mL syringe as a plunger. Splenocytes were collected by passing the contents through the cell strainer. The red blood cells were removed from the cell suspension by using ACK (ammonium-chloride-potassium) lysis buffer (Lonza). $CD8^+$ T cells were isolated using the mouse $CD8a^+$ T Cell Isolation Kit (Miltenyi Biotec). $CD8^+$ T cells were seeded into a polystyrene round bottom 96 well tissue culture plate (Corning, 29442-066) at $0.2x10^6$ cells/well, cultured in T cell complete medium consisting of RPMI-10% FBS with HEPES + 1x L-Glut, 1x pen/strep, 1x sodium pyruvate, 1x non-essential amino acids and 1x β2-ME with compounds at 37°C for 30 minutes, and then incubated with NECA (adenosine analogue, Sigma, E2387), 10ng/ml mouse IL-2 (R&D Systems, 402-ML) and 1.25ul/well Mouse CD3/CD28 Dynabeads (ThermoFisher) for 24 h. Supernatant were collected on day 2 and stored at -80°C. The concentration of IFNγ in the supernatants was assayed using the Mouse IFNγ ELISA Ready-SET-Go!® kit (Affymetrix eBioscience)

Mouse Macrophage Assays

Bone marrow cells were obtained from the femurs and tibiae of C57BL/6 mice (Charles River Labs) and treated with vehicle, NECA (Sigma, E2387) or AZD4635 (3µM). Cells were cultured with 20ng/ml mouse GM-CSF (R&D Systems) for total of 7 days. On day 4, an equal amount of fresh media with 10ng/ml mouse GM-CSF was added. On day 6, 100ng/ml LPS (Sigma) was added. On day 7, cells were collected for surface marker detection by flow cytometry using the antibodies indicated in the supplemental methods.

Supplemental Figure 1. AZD4635 reverses adenosine mediated immune suppression *in vitro*.
A. AZD4635 (10uM) reverses NECA mediated inhibition of IFNγ secretion by T cells as measured by ELISA (n=2, error bars = SEM) B. AZD4635 reverses NECA mediated inhibition MHCII expression in $CD11b^+F4/80^+$ macrophages as determined by flow cytometry. C. EC_{50} (µM) of AZD4635 in a mouse $CD8a^+$ T cell IFNγ secretion assay. The EC_{50}s of AZD4635 for reversal of NECA-mediated suppression of IFNγ secretion were determined across a range of NECA concentrations. The activity was calculated as ((Sample Data – Blank) / (Untreated Data - Blank))*100. EC_{50} was calculated as the compound concentration that gave half-maximal response. Error bars represent SD.

Supplemental Figure 2. Tumor growth curves of transplanted B16F10-OVA, MC38 and MCA205 tumors treated with AZD4635 and anti-PD-L1.

(A-E) Individual growth curves of subcutaneously implanted A. B16F10-OVA, B. MC38, C. MCA205 and D. MCA205 implanted into NSG animals E. MC38-OVA (n=9 for B16F10, n=9 for MC38; n=6-10 for MCA205

Supplemental Figure 3. Flow cytometry antibodies used

Supplemental Figure 4. AZD4635 reduces pCREB signaling in T cells and monocytes.

100µL of human whole blood or isolated mouse CD8+ T cells were added per sterile 5.0 mL round-bottom, capped polystyrene Falcon® (Corning) tubes and incubated for 1 hour at 37°C. AZD4635 at 3, 1 or 0.3 µM, or DMSO as control was added to blood and allowed to incubate for an additional 30 minutes prior to addition of 5 µM NECA (Tocris). Tubes were then incubated for 1 hour at 37°C. Following stimulation, blood was lysed with 1x BD Phosflow Lyse/Fix (BD Bioscience cat # 558049) and antibodies for discriminating live cells, monocytes, T cell subsets and phospho-CREB (Ser^{133}) (see **Suppl.Method #1**) were used for staining cells in conjunction with the eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set, cat # 00-5523-00, per the manufacturer's instructions.

Supplemental Figure 5 A-D. Sunburst plots indicating TIL analysis of lymphoid and myeloid populations from dissociated tumors from the efficacy study shown in 4A. Red arrows in G indicate SIINFEKL-specific CD8+ T cells, as determined with tetramer staining, as a proportion of the CD8+ T cell population in TIL of animals treated with the combination of AZD4635 + anti-PD-L1.

Supplemental Figure 6. Ex vivo analysis of AZD4635 effects on CD103+ cross-presentation of MC38-OVA tumor antigen.

Supplemental Figure 7. HLA-A2-restricted Melan-A T cell priming protocol schematic

Supplemental Figure 8. Gating strategy for murine CD103+ DCs

Supplemental Figure 9. Expression of markers of antigen presentation (MHCII) and CD86 on dendritic cells (left panels) and macrophages (right panels) in MC38 tumors