Title

Purine nucleotide metabolism regulates expression of the human immune ligand MICA

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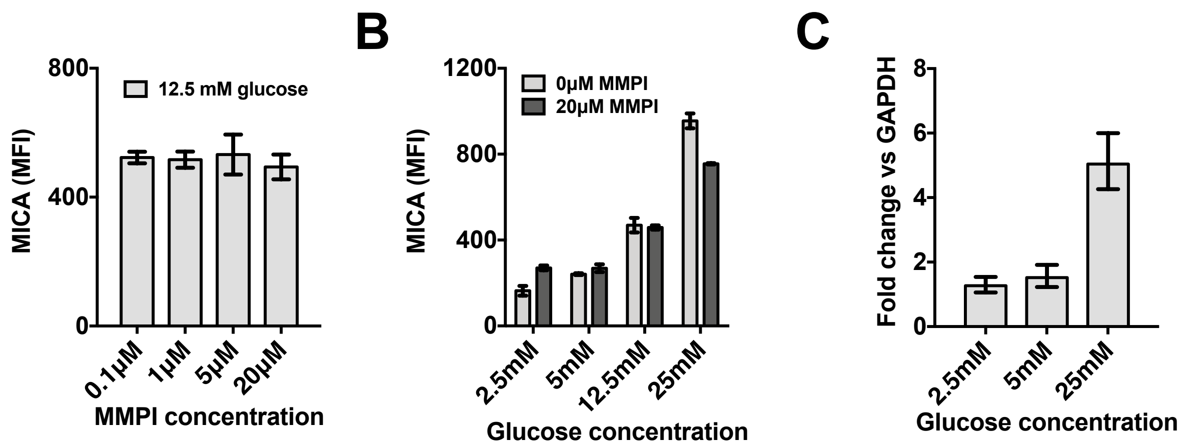

Figure S1. The effect of glucose on MICA expression is not mediated by protein shedding.

(A) HEK-293T cells cultured in 12.5 mM glucose demonstrated stable cell surface MICA expression with increasing concentrations of the matrix metalloprotease inhibitor MMPI III, measured by flow cytometry. (B) Cell surface glucose-induced MICA expression was observed in HEK-293T cells despite maximal matrix metalloprotease blockade. (C) MICA mRNA transcript levels are increased more than 4-fold in high glucose concentrations compared to GAPDH, measured by qPCR.

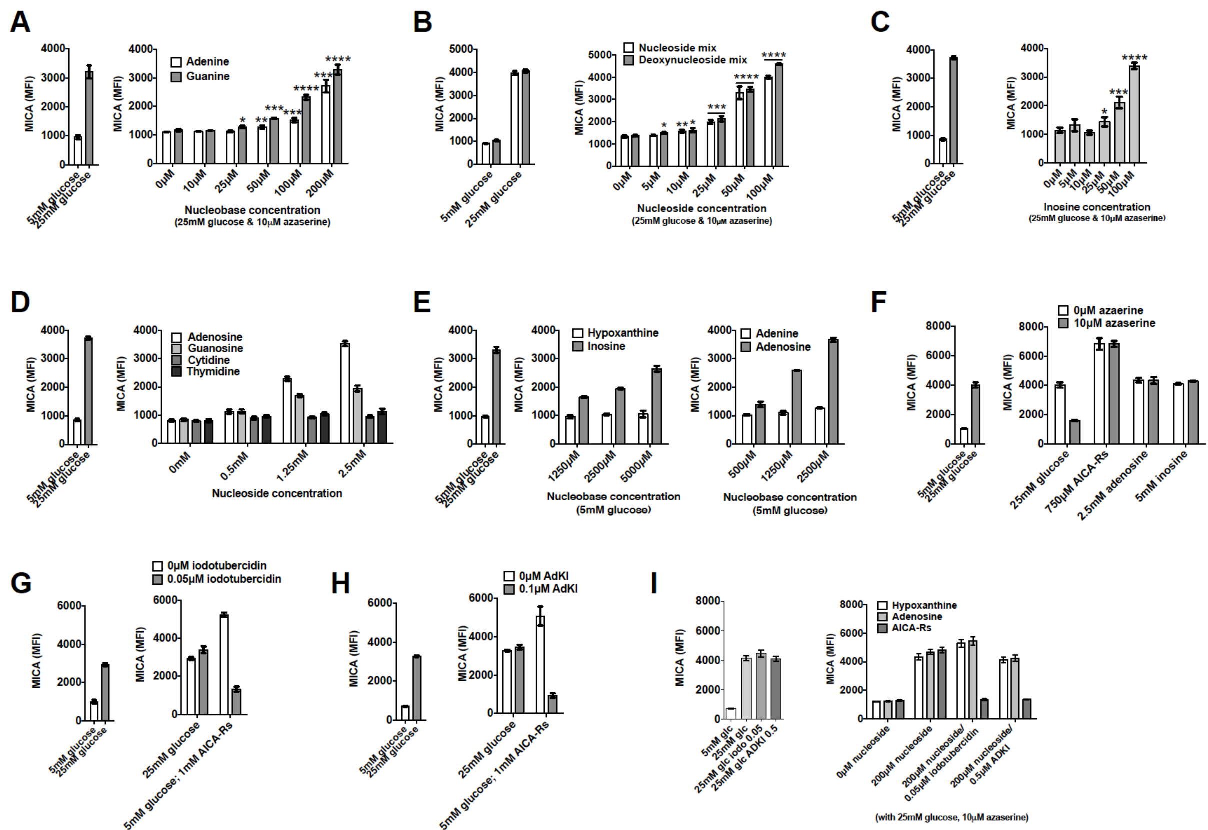

Figure S2. Purine nucleosides are necessary and sufficient for MICA induction.

(A) The purine nucleobases adenine and guanine rescue MICA expression in cells cultured in 25 mM glucose with the *de novo* purine pathway inhibitor azaserine. (B) Nucleosides and deoxynucleosides similarly salvage MICA expression. (C) The nucleoside inosine also induces MICA expression in 25 mM glucose with azaserine. (D) The purine nucleobases adenosine and guanosine induce MICA expression in a dose-dependent manner in cells cultured in 5 mM glucose, while the pyrimidine nucleobases cytidine and thymidine had no effect. (E) The purine nucleosides inosine and adenosine are sufficient to induce MICA expression in 5mM glucose, (F) while the equivalent nucleobases hypoxanthine and adenine did not alter MICA expression. Azaserine blocks GIME, but not MICA expression induced by the purine nucleosides AICA-Rs, adenosine and inosine. (G) The adenosine kinase inhibitor prevents AICA-Rs-induced MICA expression in low glucose. (H) AdKI, a second inhibitor of adenosine kinase, also blocks AICA-Rs-induced MICA expression. (I) Both inhibitors of adenosine kinase prevent AICA-Rs salvage of glucose expression in cells cultured in 25 mM glucose with azaserine, but not hypoxanthine or adenosine salvage of MICA expression in the same conditions.

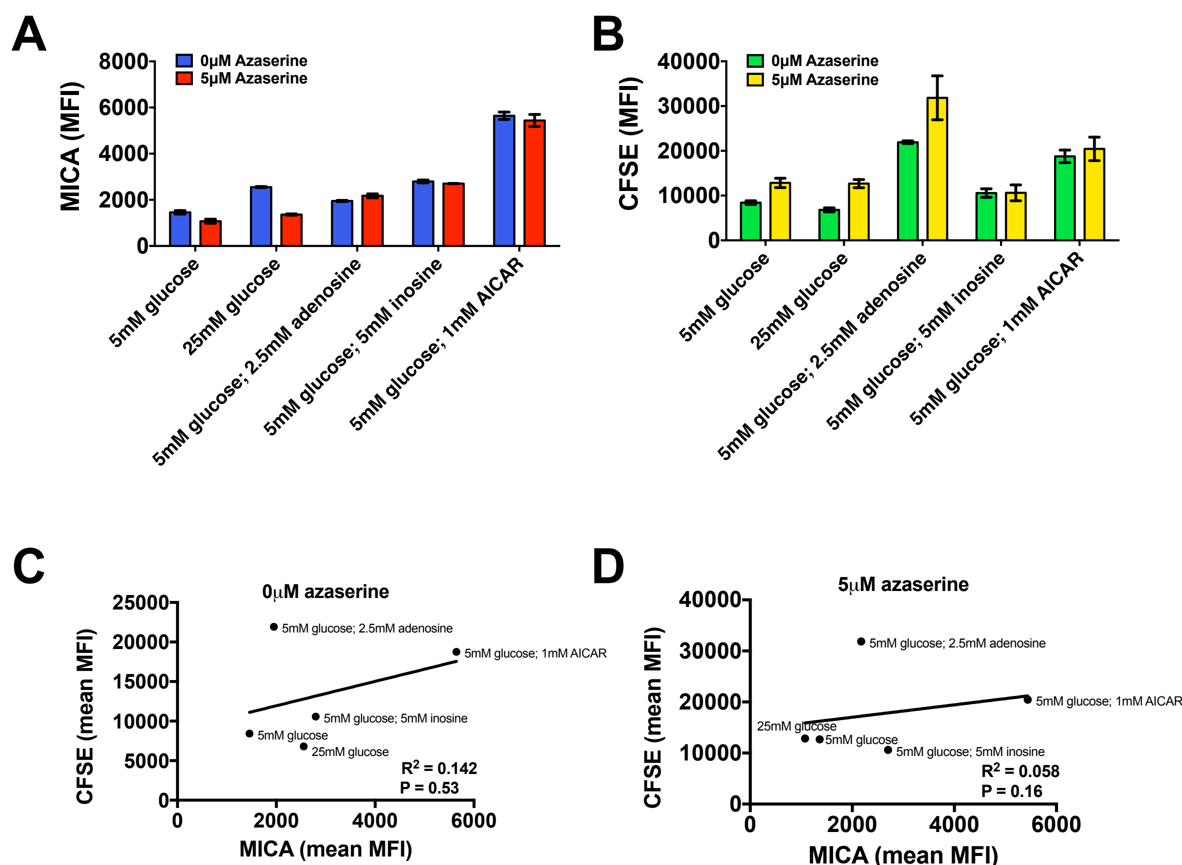

Figure S3. Cell proliferation does not correlate with MICA expression.
Experimental conditions that influence cell metabolism have varying effects on cell proliferation rate. (A) HEK293T cells were cultured with or without azaserine in a range of metabolic conditions as indicated. Cell surface MICA expression was measured after 48 hours. The *de novo* purine synthesis pathway inhibitor azaserine inhibited MICA expression in cells cultured in 5 mM or 25 mM glucose alone, but did not inhibit MICA expression in cells cultured in 5 mM glucose in the presence of the purine nucleosides adenosine, inosine or AICAR. (B) The cells shown in (A) were stained with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) at the beginning of the 48-hour culture period. CFDA-SE dilution occurs with cell division and as these cells divide continuously and asynchronously, the relative proliferation is indicated by the CFDA-SE mean fluorescence index (MFI) at 48 hours. Azaserine inhibited proliferation in cells cultured in 5 mM or 25 mM glucose. Adenosine, inosine and AICAR inhibited cell proliferation compared to cells cultured in glucose alone. (C and D) The mean CFDA-SE fluorescence intensity was plotted against mean MICA fluorescence intensity for the different culture conditions. No correlation was observed between relative proliferation and MICA expression for cells cultured without azaserine (C) or with azaserine (D).

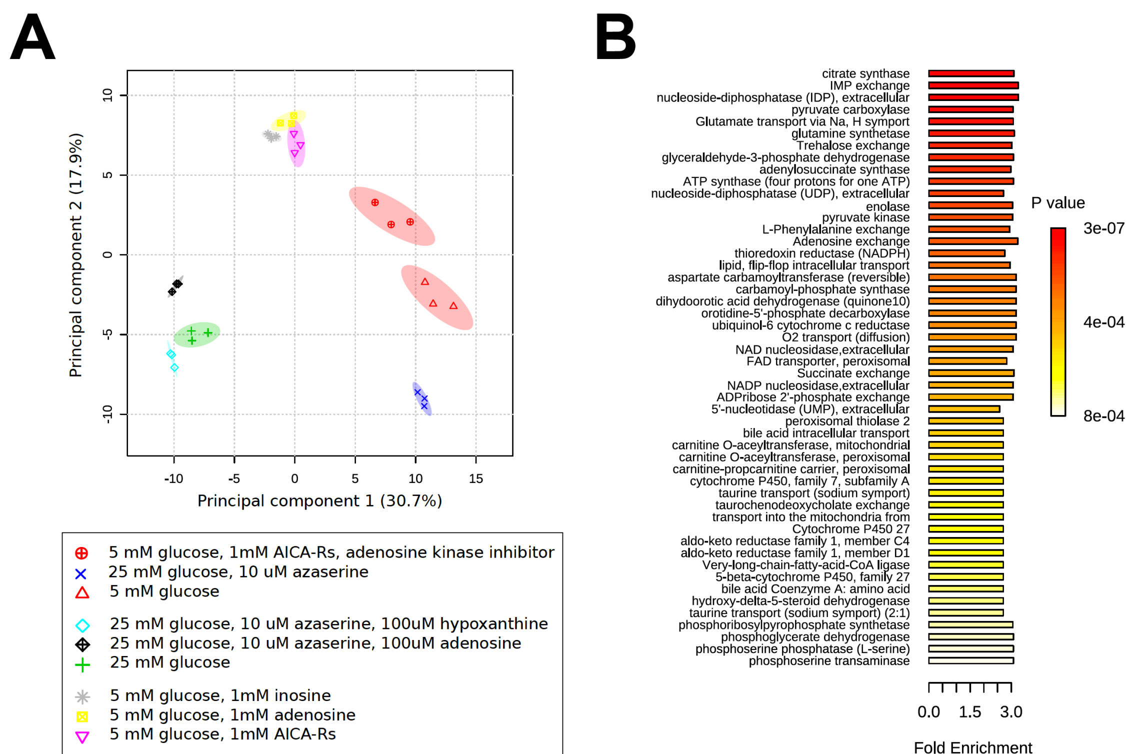

Figure S4. Principal component and metabolite enrichment analysis of metabolomic data.

The complete dataset of measurable intracellular metabolites was interrogated further using the algorithms implemented in Metaboanalyst 3.0. (A) The first principal component (30.7% of variation), showed clustering according to MICA expression. Low MICA groups (right-hand side; 5 mM glucose, 25 mM glucose and azaserine, 5 mM glucose with AICA-Rs and an adenosine kinase inhibitor) clustered independently of the two high MICA groups: a low glucose-purine nucleoside group, and an active purine synthesis group (left-hand side) containing both the high glucose dataset (*de novo* purine synthesis) and the active purine salvage pathway groups. (B) Metabolite enrichment analysis demonstrated significant enrichment across our datasets for pathways closely associated with support for high-energy purine synthesis, including nucleoside diphosphatases and ATP synthase pathways.

Figure S5. Metabolite concentration heatmap [see separate file].

Ward clustering analysis of all measureable metabolites in our dataset showed segregation into high and low MICA expression groups. The concentrations of high energy purine nucleotides correlated with MICA expression.

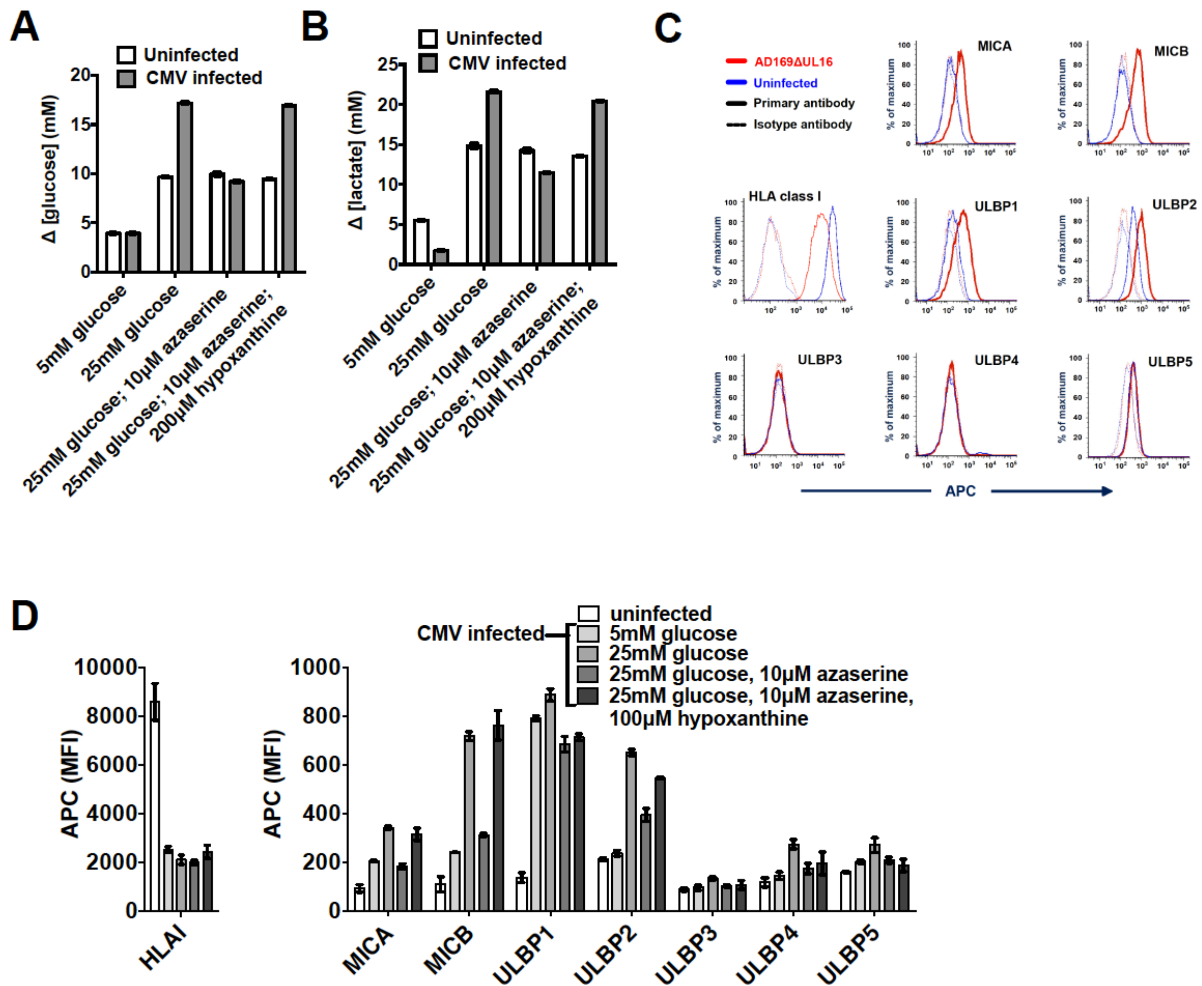

Figure S6. Metabolism affects NKG2D ligand expression during infection of primary human cells with cytomegalovirus.

(A) Glucose consumption is increased in primary human fibroblasts infected with human cytomegalovirus (CMV). The addition of azaserine prevents the increase in glucose consumption. Glucose consumption is restored with the addition of the salvage pathway substrate hypoxanthine. (B) Similarly, lactate production is increased in CMV-infected primary fibroblasts cultured in 25 mM glucose. The loss of lactate production in cells cultured with azaserine is recovered by the addition of hypoxanthine. (C) Infection of primary fibroblasts with CMV leads to induction of MICA, MICB, ULBP1 and ULBP2 at the cell surface, measured by flow cytometry. Reduced cell surface HLA class I, indicating productive infection, is also observed in CMV-infected cells. (D) Azaserine limits NKG2D ligand expression in high glucose for the NKG2D ligands MICA, MICB and ULBP2. NKG2D ligand expression is rescued by the addition of salvage pathway substrate hypoxanthine. Reduction in cell surface HLA class I expression is noted in all conditions, indicating productive infection. Error bars represent the 95% confidence interval.

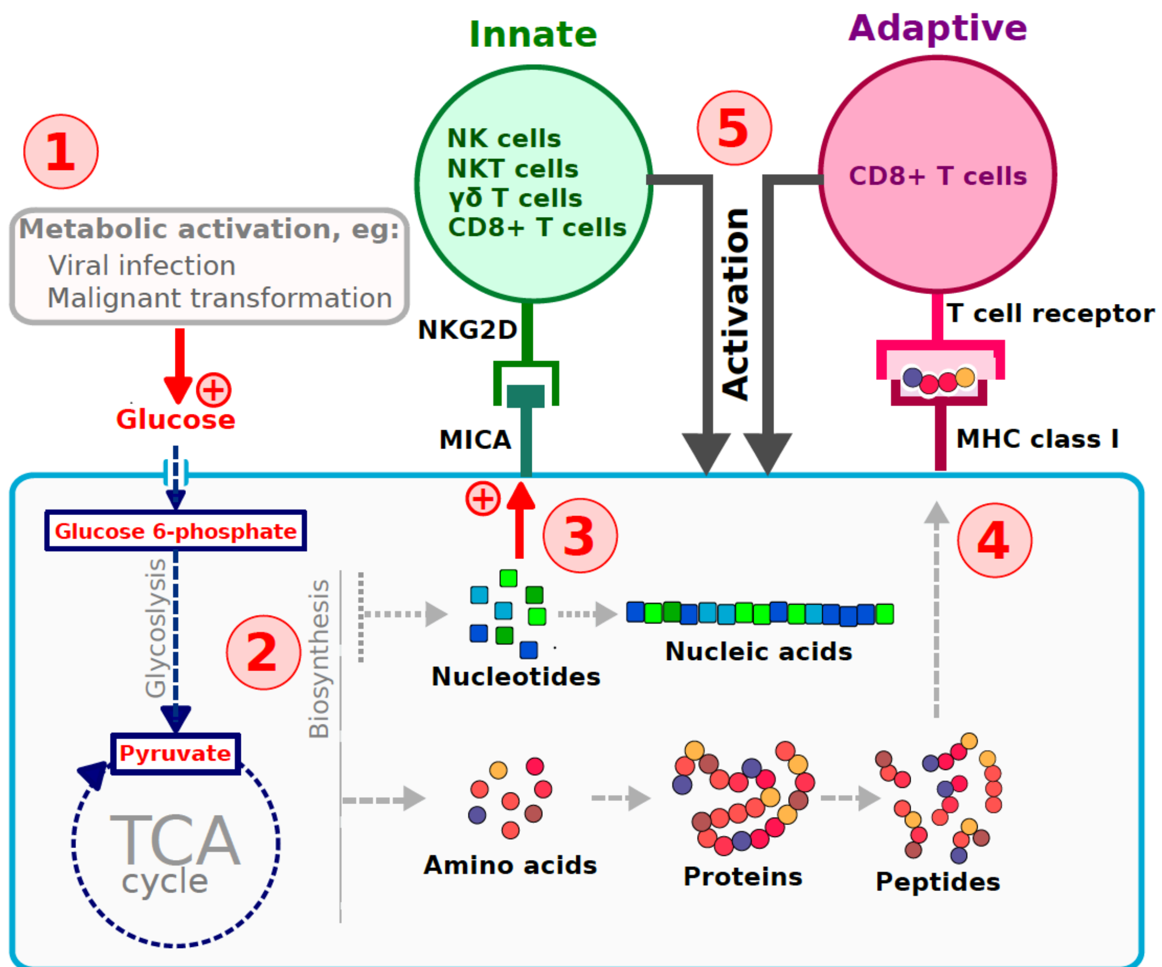

Figure S7. A schematic representation of NKG2D and TCR mediated oversight of nucleotide biosynthesis and the cellular proteome.

(1) Cellular perturbance by stimuli such as viral infection or malignant transformation induces an ‘activated’ or Warburg metabolism, characterized by increase glucose uptake and metabolism. (2) Increased glycolytic flux generates metabolic intermediates necessary for biosynthesis of molecules including nucleic acids and proteins. (3) Increased intracellular nucleotide concentrations drive cell surface expression of MICA, leading to susceptibility to NKG2D-based innate immunity. (4) Continuous cytosolic sampling of cellular proteins, by degradation and presentation at the cell surface in the peptide binding cleft of MHC class I molecules allows T cell oversight of the cellular proteome. (5) Activation of NKG2D or T cell responses can lead to direct cellular cytotoxicity, cytokine secretion or co-stimulation, depending on the immune context.