SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell isolation and culture

CD4⁴ T cells were purified from whole blood by enrichment cocktail (STEMCELL Technologies) and memory CD4⁴ T cells with EasySep negative isolation kit. In some experiments, naïve (CD45RA⁻CD62L⁺), central memory (CM, CD45RA⁻CD62L⁻), effector memory (EM, CD45RA⁻CD62L⁻) and terminally differentiated effector (TEMRA, CD45RA⁺CD62L⁻) cells were sorted on an Aria III. Cells were stimulated with anti-CD3/CD28 Dynabeads (Life Technologies) at a ratio of 1 bead to 5 cells. In selected experiments, plate-immobilized anti-CD3/CD28 mAbs (10 and 5 μg/ml, respectively; BioLegend) or monocyte-derived dendritic cells (DC) at a T cell: DC ratio of 50:1 and superantigens SEB (0.1 ng/ml) and TSST-1 (0.1 ng/ml) were used as stimuli. To induce T follicular helper cells, CD4⁴ T cells were stimulated overnight with anti-CD3/CD28 Dynabeads, transferred into plates coated with anti-CD3 mAb (5 μg/ml) and cultured in the presence of soluble anti-CD28 mAb (1 μg/ml), IL-23 (25 ng/ml, PeproTech) and TGF-β (5 ng/ml, PeproTech) for another 3 days.

CD4⁴ T cells expressing activation-induced CD39 were separated from CD39⁻ cells by autoMACS using PE-labeled anti-CD39 mAb (A1 clone, BioLegend) plus anti-PE microbeads or biotinylated anti-CD39 mAb plus streptavidin microbeads (Miltenyi Biotec). B cells were purified by autoMACS with anti-CD19 microbeads. Subset purity was assessed by flow cytometry. CD19⁺ B cells, total CD4⁺ or memory CD4⁺ T cell subset purity exceeded 90%, while CD39⁺ T cell subset purity was between 70-90%.

Flow cytometry

For surface marker characterization of effector cell subsets, CD4⁺ T cells were activated with immobilized anti-CD3/CD28 mAbs for 4 or 5 days and stained with one of the following antibodies: anti-CD25 PE-Cy7, anti-CD26 PE, anti-HLA-DR PE, anti-OX40 Perp-Cy5.5, anti-PD-1 APC, anti-CTLA-4 PE, anti-CD127 APC, anti-KLRG1 PE, anti-CD73 APC or anti-CXCR5 Alexa Fluor 647 in addition to PE or FITC labeled anti-CD39 (BioLegend), Pacific blue or Perp-Cy5.5 labeled anti-CD4, PE or APC labeled anti-CD45RO and live/dead Aqua (Life Technologies). Cells were analyzed on an LSRII or a Fortessa. Antibodies were from BD Biosciences, eBioscience or BioLegend.

For cytoplasmic or nuclear staining, CD4⁺ T cells stimulated with anti-CD3/CD28 beads were collected on days 2 to 5 of culture, stained with live/dead Aqua, fixed (Cytofix from BD Biosciences), stained with FITC labeled anti-CD39 mAb, permeabilized with perm buffer III (BD Biosciences) and stained with one of the following antibodies: anti-BAX, anti-P21, anti-p-AMPK (T172), Pacific blue labeled anti-BCL2 Ab (all from Cell Signaling), or anti-BCL6 Perp-Cy5.5 (eBiosciences) followed by a cocktail of Pacific blue or Perp-Cy5.5 labeled anti-CD4, anti-CD45RO PE, and anti-CD25 PE-Cy7. Primary unlabeled rabbit antibodies (anti-BAX, anti-P21 and anti-p-AMPK) were detected using Alexa Fluor 647-labeled anti-rabbit IgG Ab (Life Technologies). To compensate for possible differences in cell sizes of CD39⁺ and CD39⁻ CD4⁺ T cells, geometric mean or median fluorescence intensities (MFI) were normalized to forward scatters.

For intracellular cytokine staining, CD4⁺ T cells were restimulated on day 4 after the initial stimulation with 2.5 ng/ml PMA and 500 ng/ml ionomycin (Life Technologies) in presence of Golgi plug/Golgi stop (BD Biosciences). Two hours later, cells were stained with live/dead Aqua and FITC-labeled anti-CD39 Ab, fixed and permeabilized and stained with antibodies specific for cytokines [anti-IL-2 Perp-Cy5.5, anti-IL-17A Pacific blue, anti-IL-4 Alexa Fluor 647 (all BioLegend); anti-IFN-γ PE, anti-IL-9 PE and anti-IL-21 Alexa Fluor 647 (all BD Biosciences)] and cell surface molecules (Pacific blue or Perp-Cy5.5 anti-CD4, PE-Cy7 anti-CD25 and APC anti-CD45RO).

Frequencies of apoptotic cells were determined by staining with APC-labeled Annexin V and 7-AAD according to manufacturer’s instructions (BD Biosciences).

To assess mitochondrial function, activated CD4⁺ T cells were stained with live/dead Aqua, anti-CD39 biotin plus APC streptavidin and anti-CD4 Pacific blue followed by staining with JC-1 at an optimized concentration of 0.46 μM (Sigma-Aldrich).

ELISpot assays

Serial dilution of PBMC obtained on days 0 and 28 after vaccination were cultured in duplicate cultures in 200 μL complete RPMI 1640 in anti-IFN-γ Ab-coated plates (Mabtech) for 16 hours with or without antigen (influenza virus, 5 HA units/ml, MedImmune or peptide pool from VZV IE63 protein, 67 peptides of 15 amino acids in length with overlaps of 11 amino acids, JPT Peptide Technologies). Spots were developed with an HRP-conjugated anti-IFN-γ antibody and TMB substrate. Plates were read on an Immunospot reader and quantified using Immunospot software.
T cell coculture assays
To compare survival of CD39⁺ and CD39⁻ CD4⁺ T cells, CD39⁺ CD4⁺ T cells were purified from anti-CD3/CD28 Abs-activated cultures on day 5, stained with CFSE (Life Technologies), and cultured with unstained CD39 T cells at a 1:1 ratio without any further restimulation. Relative percentages of each subset were determined at days 1, 3 and 5. To assess the proliferative capacity of both T cell subsets, CD39⁺ and CD39⁻ CD4⁺ T cells were stained with CFSE and Claret (Sigma-Aldrich), respectively, mixed at a 1:1 ratio and restimulated with DC cells and 0.1 ng/mL SEB and 0.1 ng/mL TSST-1 in the presence or absence of 20 U/mL IL-2 or 20 U/mL IL-2 and 10 ng/ml IL-7.

Treg assay
CD4⁺ T cells from donors representing the different CD39 SNP genotypes (A/A, A/G and G/G) were stimulated for 4 days to induce CD39 expression. Activated cells were then cocultured with CFSE-stained fresh naïve CD4⁺ T cells for 6 days on immobilized anti-CD3/CD28 Abs in the presence or absence of 200 μM ARL. CFSE dilutions were assessed by flow cytometry. In other cultures, activated CD4⁺ T cells from donors with the different SNP genotypes were cultured with fresh CD4⁺ T cells to determine whether they could inhibit the differentiation into Th1 or Th17 effector cells, or with effector cells to determine whether CD39 contributes to the inhibition of cytokine production by effector cells.

T-B cell coculture assay
CD39⁺ and CD39⁻ T cells were purified from CD4⁺ T cell cultures stimulated with anti-CD3/CD28 beads, treated with 30 μg/ml mitomycin for 30 min at 37°C, then cocultured with autologous B cells at a ratio of 2:1 on plate-immobilized anti-CD3/CD28 Abs for additional 6 days. Frequencies of plasmablasts defined as CD3⁻ IgD/IgD⁺CD38⁺ cells were determined by flow cytometry.

Transcript quantification by qPCR
RNA from activated CD39⁺ and CD39⁻ CD4⁺ T cells was extracted using RNasy Plus mini Kit (QIAGEN), reversed transcribed with AMV-reverse transcriptase and random hexamer primers (both from Roche) and amplified in triplicates in 384 well plates using the ABI 7900HT system. Primer sequences are given in Supp. Table 2. Results were normalized to 18sRNA and are presented as delta Ct or transcript numbers. Transcript numbers were calculated from gene-specific standard curves generated by qPCR of serially diluted plasmids containing the specific cDNA sequence.

Western blotting
Activated CD39⁺ and CD39⁻ CD4⁺ T cells cells (stimulated with anti-CD3/CD28 beads for 4 or 5 days) were lysed in RIPA buffer containing phosphatase inhibitor (sodium orthovanadate), proteinase inhibitor and PMSF (all from Santa Cruz Biotechnology) for 30 min on ice. Lysates were cleared by centrifugation (12,000g at 4°C for 10 min). For reducing conditions, supernatants were boiled in loading buffer containing β-ME for 10 min. For nonreducing SDS-PAGE, supernatants were mixed with loading buffer without β-ME. Proteins were separated on 4-15% precast gels (Bio-Rad), transferred to PDVF membranes (Millipore) and developed with antibodies to BAX, p-AMPK, p-p53, PUMA, BIM, β-actin (all Cell Signaling Technology) and p21, A2AR (Abcam) using Pierce ECL Western blotting substrate (Thermo Scientific).

Quantification of intracellular ATP
CD39⁺ and CD39⁻ T cells purified on day 3 from anti-CD3/CD28 stimulated CD4⁺ T cells were lysed and cytoplasmic ATP was quantified using the CellTiter-Glo kit (Promega). Briefly, the premade mix including luciferase and its substrate luciferin was incubated with diluted supernatants from cell lysates for 10 min at room temperature. The luminescence was read using Turner Biosystems and ATP concentrations were calculated from a standard curve.

Statistical analysis
Statistical analysis was performed in Prism (Graphpad) or R 3.1.1 (2014 The R foundation for Statistical Computing). Two-sample comparisons are based on nonparametric Wilcoxon rank sum test. For comparing vaccination response between different genotype groups (e.g., AA vs AG) adjusting for the confounding effect from the CMV status, the analysis of covariance was used with the log-transformed postvaccination fold change as the response variable. The trend test was performed similarly with the number of copies of genotype “A” (0, 1 or 2) as the independent variables of interest. All the tests are two-sided with the significance level of 0.05.
Supplemental Table S1 refers to Figure 1. CD39 SNP frequencies in young and old adults.

| SNP | 20-35y, n=35 | %   | 65-85y, n=44 | %   |
|-----|-------------|-----|-------------|-----|
| AA  | 11          | 31.43 | 12          | 27.27 |
| AG  | 18          | 51.43 | 26          | 59.09 |
| GG  | 6           | 17.14 | 6           | 13.64 |

Supplemental Table S2: List of primers

| Gene name | Forward primers (5’-3’) | Reverse primers (5’-3’) |
|-----------|-------------------------|-------------------------|
| 18sRNA    | AGGAATTCCCATGTAAGTGCG   | GCCTCACTAAACCATCCAA     |
| A2AR      | TCTTCAGTCTCTGGCCATC     | CAATGATGCCCTTCCACTCC    |
| BAX       | GGACGAACCTGGACAGTAACATGG| GCAAGTAGGAAAGGAGGCACAAC |
| BCL2      | TGTGGCCCTTCTTTGAGTTTGG  | CCGTACAGTTCCACAAAGGCA   |
| CD39      | AGCAGCTGAATATGCTGGC     | GAGACAGTATCTGCCGAAAGTCC |
| DUSP4     | TGGCAATAGAACTCCGAATA    | GGATCTGTGTTGGTTATTCATC  |
| EGR1      | AGCCCTACGACCGACCTGAC    | GTTGGCTGCGTGAATTTGCT    |
| FOXP3     | GAAAACACAGCACATCCAGATTC | ATGGGCCACCCGATGAG       |
| GLUT1     | GCAGTTTGGCTCAAACACTGG   | TTTGCCGAAGCCCAGATGCAC   |
| p21       | GGAAGACCATGTTGGACCCTGT  | GCCGGTTTGGAGTGTTAGAGGA  |
| PUMA      | GGACGACCTCAACGCACAGTA   | GCCAGGTCCCATGAGTGAAGA   |
| T-bet     | GATGTTTGTTGGACGTGTTCCTTG| CTTGCCACACTGCACCCACTT   |
| TIGAR     | GGACAAAGCAGACCATGCATG   | ACCCGTATTTCCTTCCCGA     |