**Supplemental data**

Inhibition of CD39 enzymatic function at the surface of tumor cells alleviates their immunosuppressive activity by Bastid J. *et al*.

**Supplementary Material and methods**

CD39 expression by flow cytometry: HEK-293T cells overexpressing or not human CD39 were stained with anti-human CD39 antibody OREG-103/BY40 at 1µg/ml for 30min at 4°C. Cells were washed and then stained with PE conjugated Goat anti-mouse (BD, 555589) for 30min at 4°C. After washes, cells were resuspended in 300 µL of PBS containing 2% BSA (10 g/L) and 0.2% NaN3 and were analyzed by flow cytometry.

CD39 immunoblotting: Whole protein extracts from HEK-293T overexpressing or not human CD39 were prepared using the M-PER Mammalian protein extraction reagents kit (Thermo Scientific) and were analyzed by Western blotting using the anti-human CD39 primary antibody 22A9 (Ab49580, Abcam) or β-actin (A2066; Sigma-Aldrich). Signals were revealed using a horseradish peroxidase–conjugated secondary antibody (Jackson ImmunoResearch) and enhanced chemiluminescence (ECL-Plus; Perkin Elmer) according to the manufacturer’s instructions.

CD39 immunohistochemistry. Cell pellets of HEK-293T cells overexpressing or not human CD39 were fixed with 2% paraformaldehyde, embedded in paraffin and sectioned. Three-micrometer sections were deparaffinized and rehydrated in graded alcohols. Following epitope retrieval treatment in EDTA buffer pH8 and neutralization of endogenous peroxidase, cell pellet sections were incubated 30 minutes at room temperature with the anti-human CD39 antibody 22A9 (Ab49580, Abcam). Immunohistochemical labeling was revealed using EnVision FLEX/HRP (DAKO cytomation) and 3,3'-Diaminobenzidine (DAKO cytomation) as a substrate. Sections were counterstained with hematoxylin. Images were taken using Axiovision software. ACKNOWLEDGMENTS: We thank the Réseau d’Histologie Expérimentale de Montpellier (RHEM) for histology facilities.

ATPase activity measured by phosphate release: Cells were cultured in adequate medium for 16h. Cells were then washed in phosphate free buffer (10 mM glucose, 20 mM Hepes, 5 mMKCl, 120 mM NaCl, 2 mM CaCl2) and resuspended in the same buffer supplemented with 100 µM ATP for 15 min. Phosphate concentration in supernatants was measured after addition of Malachite green/polyvinylalcohol /ammonium mobylate solution for 20 min by a spectrophotometer at 620 nm and compared against a standard curve.

**Supplementary figure legends**

Figure S1: Validation of CD39 IHC (clone 22A9). (A) Control HEK-293T cells (HEK-293T WT) or cells overexpressing the human CD39 (HEK-293T huCD39) were generated and stained with anti-human CD39 antibody (OREG-103/BY40) and CD39 expression was analyzed by flow cytometry. Note the significant expression of human CD39 in HEK-293T huCD39 and the absence of CD39 expression in HEK-293T WT cells. Results are representative of at least 3 independent experiments. (B) The anti-human CD39 antibody, clone 22A9, detects a single band by Western blotting of HEK-293T cells overexpressing human CD39. Whole protein extracts of HEK-293T WT and HEK-293T huCD39 were analyzed by western blot using anti-CD39 (clone 22A9) and anti-β-actin antibodies. The 22A9 antibody detected a single band at the predicted size in HEK-293T huCD39 but not in HEK-293T WT cells. (C) The anti-human CD39 antibody, clone 22A9, specifically stains HEK-293T cells overexpressing human CD39 and does not stain wild type cells. CD39 immunochemistry was performed on paraformaldehyde fixed and paraffin embedded pellets of HEK-293T WT and HEK-293T huCD39 using the 22A9 anti-human CD39 antibody. Results show that the 22A9 anti-human CD39 antibody specifically stains CD39-expressing cells.

Figure S2: Representative IHC staining of CD39 expression in vascular endothelial cells and lymphocytes from various normal tissues. CD39 expression was assessed via IHC staining in formalin-fixed, paraffin-embedded tissues using an anti-CD39 antibody (clone 22A9, Abcam).

Figure S3: Representative IHC staining of CD39 expression in vascular endothelial cells and lymphocytes from various normal tissues using the anti-human CD39 antibody clone HPA014067 (IHC data from the Human Protein Atlas).

Figure S4: Representative IHC staining of CD39 expression in human normal and tumor tissues (IHC data from the Human Protein Atlas). “S” and “T” indicate stromal and tumor regions, respectively.

Figure S5: CD39 ATPase activity can be assessed by measuring exogenous ATP consumption or phosphate release. (A) CD39 induces the degradation of ATP into ADP and ADP into AMP. Each enzymatic reaction leads to the generation of one phosphate. (B) To measure the remaining ATP, 5x104 BJAB, BL41 or B104 lymphoma cells were cultured in complete RPMI medium for 30 min in the presence of 10 µM ATP, and the levels of non-hydrolyzed ATP were determined using ATPlite. To measure phosphate release, 5x104 BJAB, BL41 or B104 lymphoma cells were cultured in phosphate-free buffer supplemented with 100 µM ATP for 15 min, and the phosphate concentration was measured after addition of Malachite green/polyvinyl alcohol/ammonium molybdate solution for 10 min. The results are expressed as the means of 2 independent experiments.