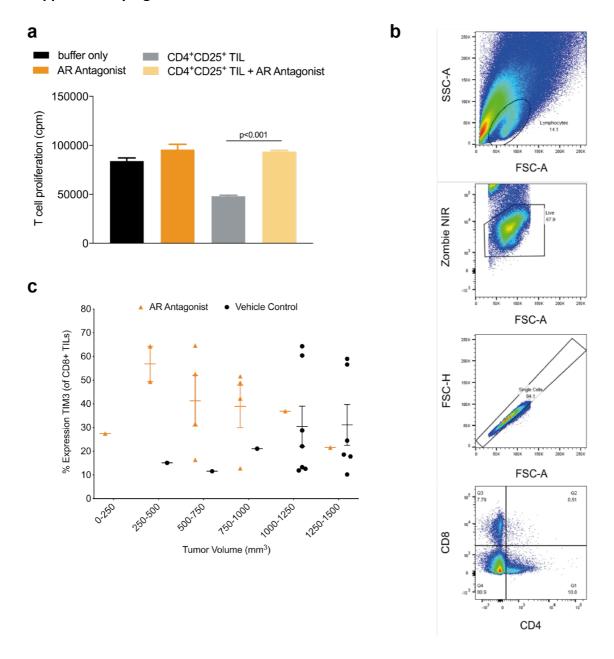
Supplementary information for

Adenosine 2a receptor and TIM3 suppress cytolytic killing of tumor cells via cytoskeletal polarization

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Supplementary figures 1 and 2 Supplementary movies 1 through 4 Figure source files for figures 2 through 7

Supplementary Figures



Supplementary figure 1 - supporting data for A2aR antagonist treatment

a CD4 $^+$ CD25 $^+$ TIL from RencaHA tumor-bearing BALB/c mice were co-cultured with naïve CD8 $^+$ CL4 T cells and irradiated mature dendritic cells and K d HA peptide with or without the A2aR antagonist ZM 241385 at 1.25 μ M for 3 days. 3 H-thymidine incorporation (cpm) is shown as mean \pm SEM. N=10 mice over two experiments (paired t-test). **b** FACS gating strategy for the identification of CD8 $^+$ TIL in sequential gates as indicated from the top to the bottom panel. **c** Percentage of CD8 $^+$ TIL from RencaHA tumor-bearing BALB/c mice treated with or without the A2aR antagonist ZM 241385 at 10 mg/kg i.p. expressing TIM3 are given as a function of tumor size as mean \pm SEM for N=6 experiments.

а Tumours treated with one of three treatments: AR-Antagonist + Anti-TIM3 mAb + ATT (28) AR-Antagonist + ATT (27) Vehicle + ATT (31) Responders Non-responders Stop Continued treatment Continued treatment treatment Flow staining Coupling Kill Assays Flow staining = 10 Rechallenge for presence for presence Fig. 5a-e Fig. 5f, g Thy1.1+ cells Thy1.1+ cells tumour cells = 5 Fig. 4a Fig. 4a = 13 Half of tumours taken Fig. 4c Flu challenge immunohistochemistry (FOXP3, CD8, Thy1) = 5 Fig. 5h,i Depletion Flow staining Flow for Thy1.1+ staining for of either CD8 or cells Thy1.1+ Thy1.1 cells cells Fig. 4a = 6 Fig. 4a Fig. 4d, e b anti-CD8-depleted control TCRbeta 35.7 CD8+ T cells 0.038 103 10⁴ 10⁵ CD8

Supplementary figure 2 – Supporting technical data for *in vivo* tumor growth experiments upon adoptive CTL transfer

a Numbers of mice used across the different parts of the experiments in Figs. 4 and 5 are given. **b** The efficiency of the depletion of CD8⁺ T cells in Fig. 4e is shown in representative FACS plots from blood samples two days after the injection of the depleting antibody or control.