## **Supporting Information**

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**Fig. S1.** (*A*) 4T1.2, E0771, and 67NR mouse breast-cancer cell lines were analyzed for CD73 expression by flow cytometry (black, unstained control; white, CD73). (*B*) HPLC analysis of extracellular adenosine production by 4T1.2 tumor cells in vitro. (*C*) TLC of conditioned media from 4T1.2 tumor cells treated with increasing doses of anti-CD73 mAb (TY/23) for 5 h. (*D*) Relative CD73 activity of 4T1.2 cells treated TY/23 for 5 h (\*, *P* < 0.05 by Mann–Whitney test; means  $\pm$  SEs of triplicates are shown). (*E*) Relative CD73 activity of 4T1.2 cells treated with 10 µg/mL TY/23 for the indicated time (\*, *P* < 0.05 by Mann–Whitney test; means  $\pm$  SEs of triplicates are shown). (*F*) Relative CD73 cell-surface expression as determined by flow cytometry on 4T1.2 tumor cells treated with 10 µg/mL TY/23 for the indicated time (one representative of two experiments is shown).



**Fig. S2.** Anti-CD73 mAb therapy inhibits spontaneous 4T1.2 lung metastasis. BALB/c mice were injected s.c. in the mammary fat pad with  $10^5$  4T1.2 cells and treated with 100 µg isotype control Ig (clone Mac4; *Upper*) or anti-CD73 mAb (clone TY/23; *Lower*) two times weekly from day 3. Spontaneous 4T1.2 lung metastases (as indicated by arrows) were counted at day 20 under a dissecting microscope. Ventral views of lungs after perfusion with Indian ink are shown. (Scale bar: 1 mm.)



**Fig. S3.** CD73 knockdown in 4T1.2 tumor cells. (A) 4T1.2 tumor cells were gene-modified to express a short-hairpin (sh) RNA against CD73 (CD73-shRNA) or control shRNA (Ctr-shRNA), and CD73 expression of gene-modified cells was measured by flow cytometry (gray, unstained; black, CD73-shRNA 4T1.2; white, Ctr-shRNA 4T1.2). (*B* and C) TLC of conditioned media from Ctr-shRNA 4T1.2 and CD73-shRNA 4T1.2 cells (\*, P < 0.05 by Mann–Whitney test; means  $\pm$  SEs of triplicates are shown).



**Fig. S4.** NK cell depletion in SCID mice. Female SCID mice were depleted of NK cells with rabbit anti–asialo-GM1 antibody 1 day before the inoculation of  $10^5$  4T1.2 cells. (A) Flow cytometry analysis of DX5+ spleen cells isolated from SCID mice treated with control rabbit serum or anti–asialo-GM1 antibody 5 days after injection (\*, P < 0.05 by Mann–Whitney test; means ± SEs of triplicates are shown). (B) Primary tumor growth of 4T1.2 tumor cells injected s.c. to SCID mice treated with anti–asialo-GM1 antibody 1 day before, 1 day after, and 7 days after tumor inoculation (P > 0.05 by Mann–Whitney test; means of six mice per group ± SEs are shown).



**Fig. S5.** Wild-type C57BL/6 mice were irradiated and injected i.v. with  $2 \times 10^6$  bone-marrow cells isolated from congenic wild-type or A2A-deficient mice. Reconstitution of hematopoiesis was assessed by flow cytometry on individual populations of peripheral blood mononuclear cells (PBMCs) after 8 weeks (ns, nonsignificant when P > 0.05 by Mann–Whitney test; means  $\pm$  SEs are shown).



**Fig. S6.** Female BALB/c mice were injected s.c. in the mammary fat pad with  $10^5 4T1.2$  cells, and tumor growth was monitored over time. (A) Mice were treated two times weekly with PBS 0.1% DMSO (Ctr) or NECA at 0.05 mg/kg diluted in PBS 0.1% DMSO intraperitoneally plus 0.05 mg/kg s.c. (P > 0.05 by Mann–Whitney test; n = 5 per group; means  $\pm$  SEs are shown). (B) Like in A, mice were treated two times weekly, but they were treated with DPCPX at 1 mg/kg diluted in PBS 0.1% DMSO intraperitoneally (P > 0.05 by Mann–Whitney test; n = 7 per group; means  $\pm$  SEs are shown). (C) Like in A, mice were treated two times weekly, but they were treated with PBS1115 at 10 mg/kg diluted in PBS 0.1% DMSO intraperitoneally (P > 0.05 by Mann–Whitney test; n = 6 per group; means  $\pm$  SEs are shown).

## Table S1. Generation of adenosine from extracellular AMP

Rate of adenosine generation, nmol/h/10<sup>6</sup> cells

4T1.2	377.9 ± 28.0
4T1.2 + APCP	36.4 ± 11.6*
67NR	0.0

Treatment of 4T1.2 tumor cells with alpha, beta-methylene ADP (APCP; 40  $\mu$ M) significantly decreased adenosine production.

\*P < 0.05 by Mann-Whitney test; means  $\pm$  SEs of triplicates are shown.