**Supplementary Information**

**Supplementary Methods**

***Mice, Antibodies and Chemicals***

C57BL/6 and BALB/c wild-type (WT) mice were purchased from the Walter and Eliza Hall Institute for Medical Research or ARC Animal Resource Centre. C57BL/6 CD11c.DOG mice were used as described previously (1). Diptheria toxin was obtained from Sigma, and clodronate liposomes were obtained from Michael Kershaw (Peter MacCallum Cancer Centre, Melbourne). Some mice additionally received α-CD4 (GK1.5), α-CD8β (53.5.8) to deplete T cell subsets and α-asialoGM1 to deplete NK cells as indicated.

***Chemosensitivity assay***

4000 MDA-MB-231 and 2000 4T1.2 cells were seeded in a 96 well plate. Cells were treated with doxorubicin 24 h after plating. On the 7th day, cells were treated with MTS reagent (1:10 dilution in media) and incubated at 37°C for 30 minutes and the OD was recorded at 490 nM (2).

***Metastatic Gene expression array***

cDNA was loaded on The Mouse Tumor Metastasis RT2 ProfilerTM PCR array 84 genes (Cat PAHS-028Z) on ABI Viia 7 (Applied Biosystems) and analyzed on SAS Biosciences as per manufacturers’ instructions.

***In vitro cell migration and invasion assays***

Cell migration and invasion (with BD growth factor reduced matrigel) rates were assessed using xCELLigence system (Roche Applied Sciences) as previously described (3).

***References***

1. Haynes NM, Hawkins ED, Li M, McLaughlin NM, Hammerling GJ, Schwendener R, et al. CD11c+ dendritic cells and B cells contribute to the tumoricidal activity of anti-DR5 antibody therapy in established tumors. J Immunol. 2010;185:532-41.

2. Kalimutho M, Minutolo A, Grelli S, Formosa A, Sancesario G, Valentini A, et al. Satraplatin (JM-216) mediates G2/M cell cycle arrest and potentiates apoptosis via multiple death pathways in colorectal cancer cells thus overcoming platinum chemo-resistance. Cancer Chemother Pharmacol. 2011;67:1299-312.

3. Dunne PD, McArt DG, Blayney JK, Kalimutho M, Greer S, Wang T, et al. AXL is a key regulator of inherent and chemotherapy-induced invasion and predicts a poor clinical outcome in early-stage colon cancer. Clin Cancer Res. 2014;20:164-75.

**Supplementary Figure Legends**

**Supplementary Figure 1. A2BRi anti-metastatic activity does not require immune cells**. **(A)** C57BL/6 wild type (WT) mice were injected intravenously with B16F10-CD73hi melanoma cells (105 cells) on day 0. On day 0 and 3 after tumor inoculation, mice were treated with intraperitoneal injections of vehicle or A2BRi (PSB1115, 1 mg/kg i.p) as indicated. Some groups of mice were depleted of NK cells or T cells, by treatment with α-asGM1, α-CD8 or α-CD4 and α-CD8 (100 g i.p.) on days -1, 0, and 7 as indicated. Metastatic burden was quantified in the lungs after 14 days by counting colonies on the lung surface. Means ± SEM of 5 mice per group are shown. Retained metastatic control by PSB1115 in NK cell depleted mice was statistically significant by ANOVA as indicated (\*\*\* P <0.001; \*\*\*\* P <0.0001). **(B)** Groups of 5 female BALB/c WT mice were injected in the mammary fat pad with the mammary carcinoma cell line 4T1.2 (5 x 104). On day 22, the primary tumor was resected and mice were treated i.p with vehicle or PSB1115 (1 mg/kg i.p.) as indicated on day 22, 25, 28, and 31. Some groups of mice were depleted of NK cells or T cells, by treatment with α-asGM1, α-CD8 or α-CD4 and α-CD8 (100 g i.p.) on days 21, 22, 29, and 36 as indicated. Survival of the mice was monitored. **(C)** C57BL/6 CD11c.DOG.Tg mice or **(D)** C57BL/6 WT mice were injected intravenously with B16F10-CD73hi melanoma cells (1 x 105 cells) on day 0. Mice were given intraperitoneal injections of either (C) diptheria toxin (400 ng/kg) daily from day -1 to day 7 or (D) clodronate liposomes (100 mg/kg) on day -2, 0, 2, 4 and 6 alongside PBS controls. On day 0 and 3 after tumor inoculation, mice were treated with intraperitoneal injections of PBS or PSB1115 (1 mg/kg) as indicated. Data pooled from two independent experiments (C, D). Mean ± SEM of 8-14 mice per group, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001; Mann-Whitney test.

**Supplementary Figure 2. Mechanism of A2BRi.** C57BL/6 wild type (WT) mice were injected intravenously with 2 x 105 EO771 cells on day 0. On day 0 and 3 after tumor inoculation, mice were treated with injections of vehicle or A2BRi (PSB1115, 1 mg/kg i.p) as indicated. Some groups of mice were depleted of NK cells and T cells, by treatment with α-asGM1, α-CD8 and α-CD4 (100 g i.p.) on days -1, 0, and 7 as indicated. Metastatic burden was quantified in the lungs after 14 days by counting colonies on the lung surface. Means ± SEM of 5 mice per group is shown (ANOVA, \*\*P <0.01, \*\*\*\*P<0.0001).

**Supplementary Figure 3. Expression of adenosine receptors in tumor cells. (A)** RT-PCR expression of A2BR on mouse tumor cell lines. Expression of adenosine receptors A1, A2A, A2B and A3 in **(B)** EO771 and **(C)** 4T1.2 breast cancer cells. Ribosomal protein RPL32 was used as a reference gene. Means ± SEM, n = 3-5.

**Supplementary Figure 4. Effect of A2BR KD on primary tumor growth. (A)** 5 x 105 EO771 control (ctrl sh) and A2BR KD (sh3) cells were injected subcutaneously in C57BL/6 WT mice and primary tumor growth was monitored. **(B)** 5 x 104 4T1.2 control and A2BR KD (sh1) cells were implanted in the fourth mammary fat pad of BALB/c WT mice and primary tumor growth was monitored. RT-PCR expression of mouse A2BR after lentiviral KD of A2BR in **(C)** SM1WT1-LWT1 (LWT1) mouse melanoma cells and **(D)** RM1 mouse prostate carcinoma cells with three different small hairpins. **(E)** Immunoblot analysis of A2BR expression in control and A2BR KD LWT1 and RM1 cells. β-actin was used as the loading control.

**Supplementary Figure 5. ADORA2B expression in human breast cancer. (A)** ADORA2B gene expression analysis on triple-negative (TN – Basal A and Basal B), luminal, HER2 and hormone receptor (HR)-positive human breast cancer cells. **(B)** Relative gene expression of ADORA2B in individual human breast cancer cell lines. Data analysis was done by GOBO gene set analysis – neve database.

**Supplementary Figure 6. A2BRi therapy of human breast cancer xenografts. (A)** 1 x 106 SUM159PT cells were injected intravenously in Balb/c.Rag2-/-γc-/- immunodeficient mice and mice were treated intraperitoneally with vehicle or A2BRi **(**10 mg/kg) on day 0 and day 3. Metastatic burden was quantified in the lungs after 5 weeks by counting colonies on the lung surface. **(B)** Migration and **(C)** invasion of EO771 breast adenocarcinoma cells were assessed using Roche xCELLiegence system. **(D)** RT-PCRexpression of human A2BR and FXDY5 in MDA-MB-231 sh control (control) and A2BR KD cells.Human HPRT was used as a reference gene. **(E)** RT-PCRexpression of mouse A2BR and FXDY5 in EO771 sh control (control) and A2BR KD cells. Mouse RPL32 was used as a reference gene. Means ± SEM, n = 5-6 mice/group, n = 4-9 mRNA samples/group, \*P<0.05, \*\*P<0.01; Mann-Whitney test.

**Supplementary Figure 7. Effect of A2BR KD on cell viability.** Relative cell viability of **(A)** mouse 4T1.2 control and sh1 A2BR KD cells and **(B)** human MDA-MB-231 control and sh3 A2BR KD cells in the presence or absence of different concentration of doxorubicin (means ± SD).

**Supplementary Table 1.**

The list of genes upregulated and downregulated after A2BR KD in EO771 cells as determined by Mouse Tumor Metastasis RT2 ProfilerTM PCR array of 84 genes (Cat PAHS-028Z). β-Actin was used as a reference gene and the samples were analyzed as per manufacturer’s instructions (SAS Biosciences).

**Supplementary Table 2.**

List of Sybr Green primers used for RT-PCR reaction.