**SUPPLEMENTARY FILES**

**Inhibition of MICA and MICB Shedding Elicits NK cell-mediated Immunity against Tumors Resistant to Cytotoxic T cells**

Lucas Ferrari de Andrade1, 2, 3\*, Sushil Kumar1,2, Adrienne Luoma1, 2, Yoshinaga Ito1, 2, Pedro Henrique Alves da Silva3, Deng Pan1, 2, Jason W. Pyrdol1, 2, Charles H. Yoon4, 5, Kai W. Wucherpfennig1, 2, 6\*

1Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA.

2Department of Immunology, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA.

3Department of Oncological Sciences and Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY, 10029-6574, USA.

4Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA.

5Department of Surgery, Brigham and Women’s Hospital, 75 Francis Street, Boston, MA 02115, USA.

6Department of Neurology, Brigham & Women’s Hospital and Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA.

\*Corresponding authors: [Kai\_Wucherpfennig@dfci.harvard.edu](mailto:Kai_Wucherpfennig@dfci.harvard.edu); [Lucas.FerrarideAndrade@mssm.edu](mailto:Lucas.FerrarideAndrade@mssm.edu)

**SUPPLEMENTARY FIGURES**

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**Supplementary Figure 1. Characterization of *B2M* deficient A375 melanoma cells and inhibition of NK cell-mediated killing of melanoma cells by recognition of MHC-I.**

**(A)** Validation of efficiency of *B2M* gene inactivation in A375 melanoma cells. Control and *B2M*-KO cells were treated with the indicated concentration of IFN (1 ng/ml) or PBS for 24 hours, and surface HLA-A/B/C protein levels were quantified by flow cytometry.

**(B)** Control and *B2M*-KO A375 melanoma cells were treated with or without IFN (50ng/ml) for 24 hours. Western blots (20g of total protein per lane) were probed with antibodies specific for B2M and tubulin.

**(C)** NK cells isolated from a healthy donor were cultured for 24 hours in the presence of 1,000 U/ml of IL-2. Parental A375 melanoma cells were treated with 7C6-hIgG1 or isotype control antibodies (20 g/ml) for 24 hours prior to use in the cytotoxicity assay. NK cell-mediated killing of A375 cells was analyzed using a 4-hour 51Cr-release assay. Indicated KIR or isotype control antibodies were added to co-cultures at 10 g/ml.

Data are representative of three independent experiments (A-C). Statistical analysis was performed by two-way ANOVA, Bonferroni’s post-hoc test (C), \*\*\*p<0.001.

**Supplementary Figure 2. Characterization of B16F10-MICA cell lines and *in vivo* activity of MICA/B mAb.**

Control, *B2m*-KO or *Jak1*-KO B16F10-MICA cells were treated for 24 hours with the indicated concentrations of IFN

**(A)** Surface levels of H2-Kb (left) and MICA (right) were analyzed by flow cytometry. MFI = Mean Fluorescence Intensity.

**(B)** Surface levels of H2-Db were analyzed by flow cytometry. MFI = Mean Fluorescence Intensity.

**(C)** Control, *B2M*-KO or *Jak1*-KO B16F10 melanoma cells were treated with or without IFN (50ng/ml) for 24 hours. Western blots (20g of total protein per lane) were probed with antibodies specific for B2M, JAK1 or GAPDH (loading control).

Data representative of three independent experiments.

**Supplementary Figure 3. *B2m*-KO and *Jak*1-KO B16F10-MICA cells are resistant to CD8 T cell-mediated cytotoxicity.**

Control, *B2m*-KO and *Jak1*-KO B16F10 melanoma cells were pulsed overnight with Ova peptide (10nM), washed and added to 96 well plates (5,000 cells per well). Naïve OT-I T cells were added at different effector to target ratios (1:1, 2:1 and 5:1; the 0:1 condition contained no T cells). Cells were co-cultured for 48 hours (8-10 replicates per condition); wells were then washed to remove T cells as well as dead tumor cells, and adherent live tumor cells were counted using a Celigo Image Cytometer. Statistical significance was determined using a multiple t-test, error bars represent the standard deviation, \*\*\* p <0.0001.

**Supplementary Figure 4. *In vivo* inhibition of MICA shedding by 7C6 antibody in the B16F10 metastasis model.**

B cell deficient (*Ighm*-/-) mice were inoculated i.v. with 7x105 control, *B2m*-KO or *Jak1*-KO B16F10-MICA cells. When metastases were established (day 7), mice were treated with MICA/B or isotype control mAbs (200 g on days 7, 8 and 12). Shed MICA in plasma samples was quantified using a sandwich ELISA. Data were pooled from two independent experiments. Statistical analyses were performed by two-way ANOVA, Bonferroni’s post-hoc test, \*\*\*p<0.001.

**Supplementary Figure 5. Characterization of control and *B2m*-KO LLC1-MICA cell lines.**

**(A)** Control and *B2m*-KO LLC1-MICA cell lines were cultured for 24 hours with the indicated concentrations of IFN and surface expression of H2-Db was analyzed by flow cytometry.

**(B)** Control or *B2M*-KO LLC1-MICA cells were treated with or without IFN (50ng/ml) for 24 hours. Western blots (20g of total protein per lane) were probed with antibodies specific for B2M or tubulin (loading control).

**Supplementary Figure 6. Expression of NKG2D and CD16 receptors on NK cells infiltrating the lungs of mice with B16F10 metastases.**

WT mice were inoculated intravenously with control, *B2m*-KO or *Jak1*-KO B16F10-MICA cells and treated with the indicated antibodies as described in Figure 4A and B. Surface expression of NKG2D and CD16 receptors was analyzed on lung-infiltrating NK cells by flow cytometry. Data were pooled from two independent experiments. \*p<0.05, calculated with two-tailed unpaired Student’s t-test.

**Supplementary Figure 7. Effect of panobinostat on gene expression by A375 cells.**

**(A-B)** A375 cells were treated for 24 hours with panobinostat (50 nM) or PBS and analyzed by bulk RNA-seq, as described in Figure 5A. Key differentially expressed genes in cells treated with panobinostat or solvent control (A) and key immunological pathways (B) upregulated in panobinostat compared to control treated A375 cells. FDR, false discovery rate; q-val, q-value.

**Supplementary Figure 8. Effect of panobinostat treatment on MICA/B expression by melanoma cells.**

Representative histograms for data on primary melanoma cell lines shown in Figure 5E.

**Supplementary Figure 9. Effect of panobinostat and 7C6 antibody on MICA/B expression by a diverse panel of tumor cell lines.**

**(A-F)** The indicated human tumor cell lines were treated with the indicated antibodies (20 g/ml) and increasing concentrations of the HDAC inhibitor panobinostat for 24 hours. MICA/B surface levels were quantified by flow cytometry using PE-labeled 6D4 mAb (left graphs in A, B and graphs C-F). Shed MICA in supernatants was quantified by sandwich ELISA (right graphs in A and B). 7C6 antibodies with different Fc regions were used based on the availability of such antibodies at the time of the assays; the Fc region of this antibody does not affect inhibition of MICA/B shedding, as previously reported (*23*). Data representative of three independent experiments.

**Supplementary Figure 10. Specificity of ELISA assay for MICA compared to MICB.**

The supernatants of B16F10 cell lines transduced with human MICA (allele 009) or MICB (allele 005) cDNAs were analyzed using an ELISA for MICA (Abcam, Ab59569) that was used throughout this study. These B16F10 cell lines were described previously (*23*). The ELISA detected soluble MICA shed by the B16F10-MICA cell line, but not shed MICB released by B16F10-MICB cells.

**Supplementary Figure 11. Panobinostat did not inhibit reconstitution of NSG mice with human NK cells and synergized with 7C6 mAb to enhance surface MICA/B expression on melanoma metastases.**

NSG mice were injected i.v. with 2x106 *in vitro* expanded human NK cells from healthy donors. Immediately following NK cell inoculation, mice were treated with IL-2 (7.5x104 units) to support NK cell survival; mice also received panobinostat (10 mg/kg in PBS) or PBS as a control. 24 hours later, blood NK cells were analyzed by flow cytometry.

**(A)** Number of circulating NK cells identified as CD45+ CD56+ CD3- viable cells.

**(B, C)** Percentage of blood NK cells labeled with CD16a (B) or NKG2D (C) mAbs.

Data pooled from two independent experiments (A-C).

**(D)** Representative histograms of the data shown in Figure 6A.



**Supplementary Table. The sequences of the gRNAs used for gene editing.**

The indicated genes were edited via Cas9 using the indicated gRNAs. More details in the ‘Material and Methods’ section.