Supplementary tables

Supplementary table 1. Primary antibodies used for flow cytometry

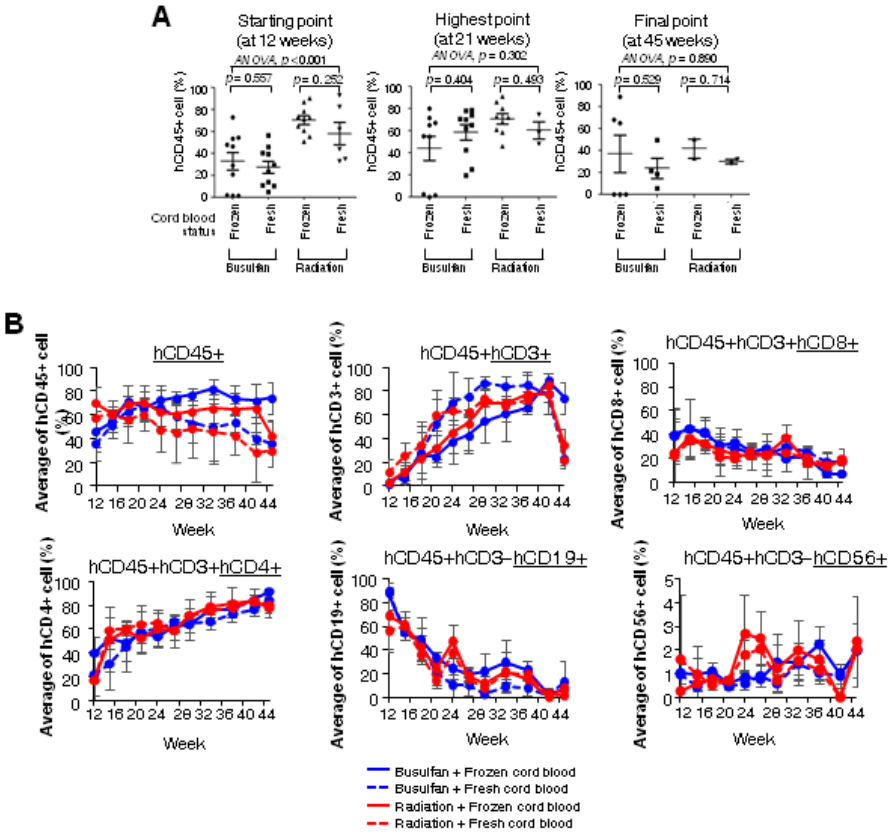
Antibody	Clone	Fluorescence	Company
Human CD45	HI30	FITC	e-Bioscience
Mouse CD45	20-F11	APC	e-Bioscience
Human CD3	BW264/56	PE	e-Bioscience
Human CD4	SK-3	PercP-eFluor710	e-Bioscience
Human CD8	SK1	APC	e-Bioscience
Human CD56	CMSSB	APC-eFluor780	e-Bioscience
Human CD19	SJ25C1	PercP-eFluor710	e-Bioscience
Human PD-1	MIH4	PE	BD bioscience
Ki67	SoLA15	PE	e-Bioscience

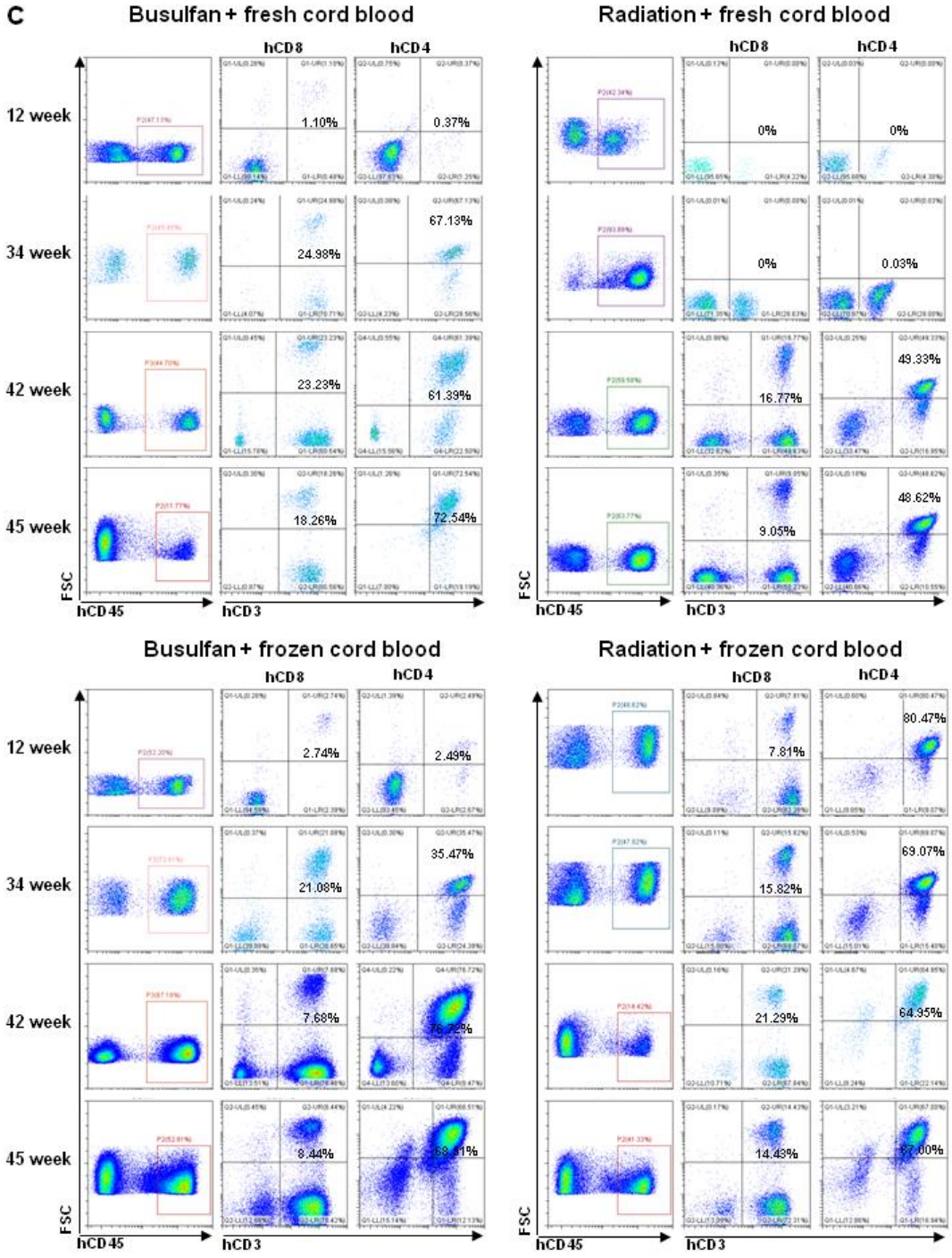
Supplementary table 2. Durability of humanized mouse model

No.	Duration	Myeloablation	Reference
1	24 week	Irradiation	Ishikawa et al., Blood, 2005;106:1565
2	13 week	Irradiation	Walcher et al., Immun Inflamm Dis. 2020;8:363
3	19 week	Irradiation	Verma et al., Curr Protoc Pharmacol. 2020;89:e77
4	20 week	Irradiation	Blumich, University of Zurich, 2020
5	4 week	Irradiation	Tanaskovic et al., Plos ONE, 2019;14:e0217345
6	19 week	Busulfan	McCarthy et al., JAIDS, 2019;82:407

Supplementary figures

Supplementary figure S1





Supplementary figure S1. Immune cell monitoring in humanized mice blood

(A) The proportion of cells in the peripheral blood was analyzed for the humanization marker

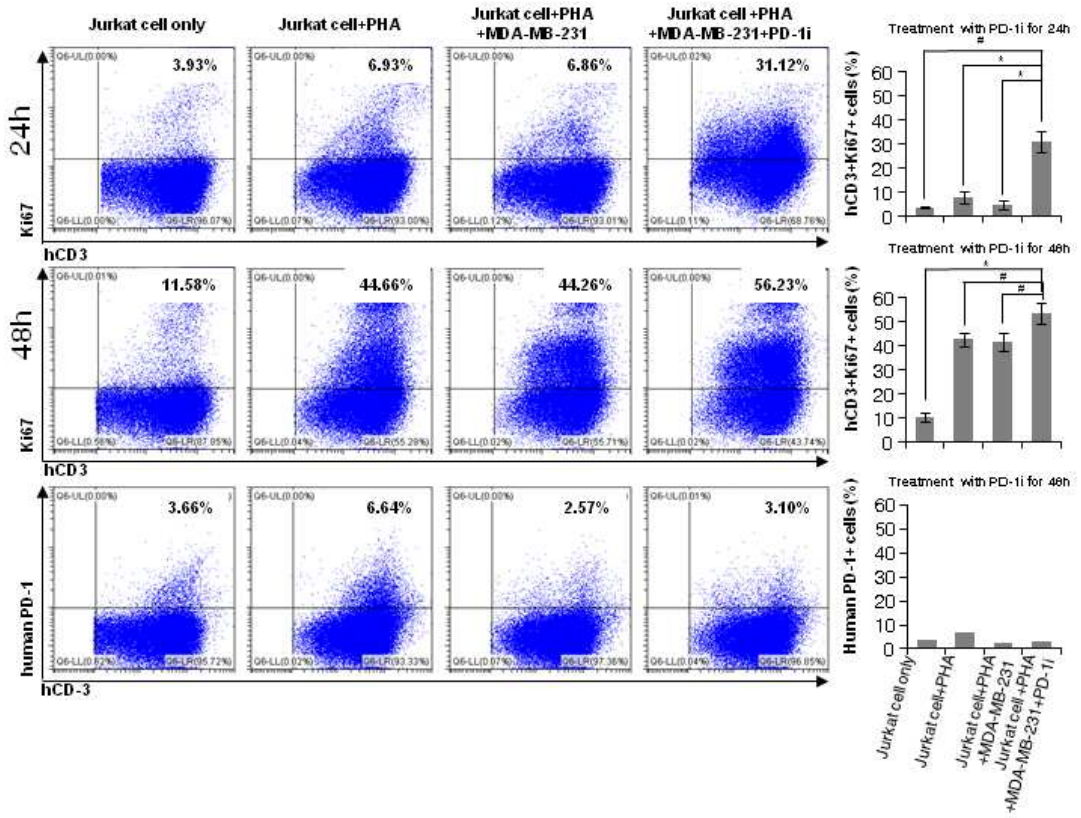
hCD45+ by flow cytometry over 12 weeks to 45 weeks from the injection of hCD34+ HSCs. Percentages of hCD45+ cells were demonstrated at three time points (starting point, highest point, and final point) according to the myeloablative method and cord blood status.

(B) Various immune cell markers, including hCD45, hCD3, hCD4, hCD8, hCD19, and hCD56 in the peripheral blood were analyzed by flow cytometry over 12 weeks to 45 weeks from the injection of hCD34+ HSCs by myeloablative method and cord blood status. *P*-values were calculated by ANOVA test, and post-hoc *P*-values were calculated by LSD test. Data are presented as mean ± standard deviation.

(C) Representative FACS plots of hCD3, hCD4, and hCD8 analyses according to the myeloablative method and cord blood status were shown. Those representative plots were selected at starting point (12 weeks), highest point (hCD3 and hCD4; 42 weeks, and hCD8; 34 weeks), and final point (45 weeks).

Supplementary figure S2

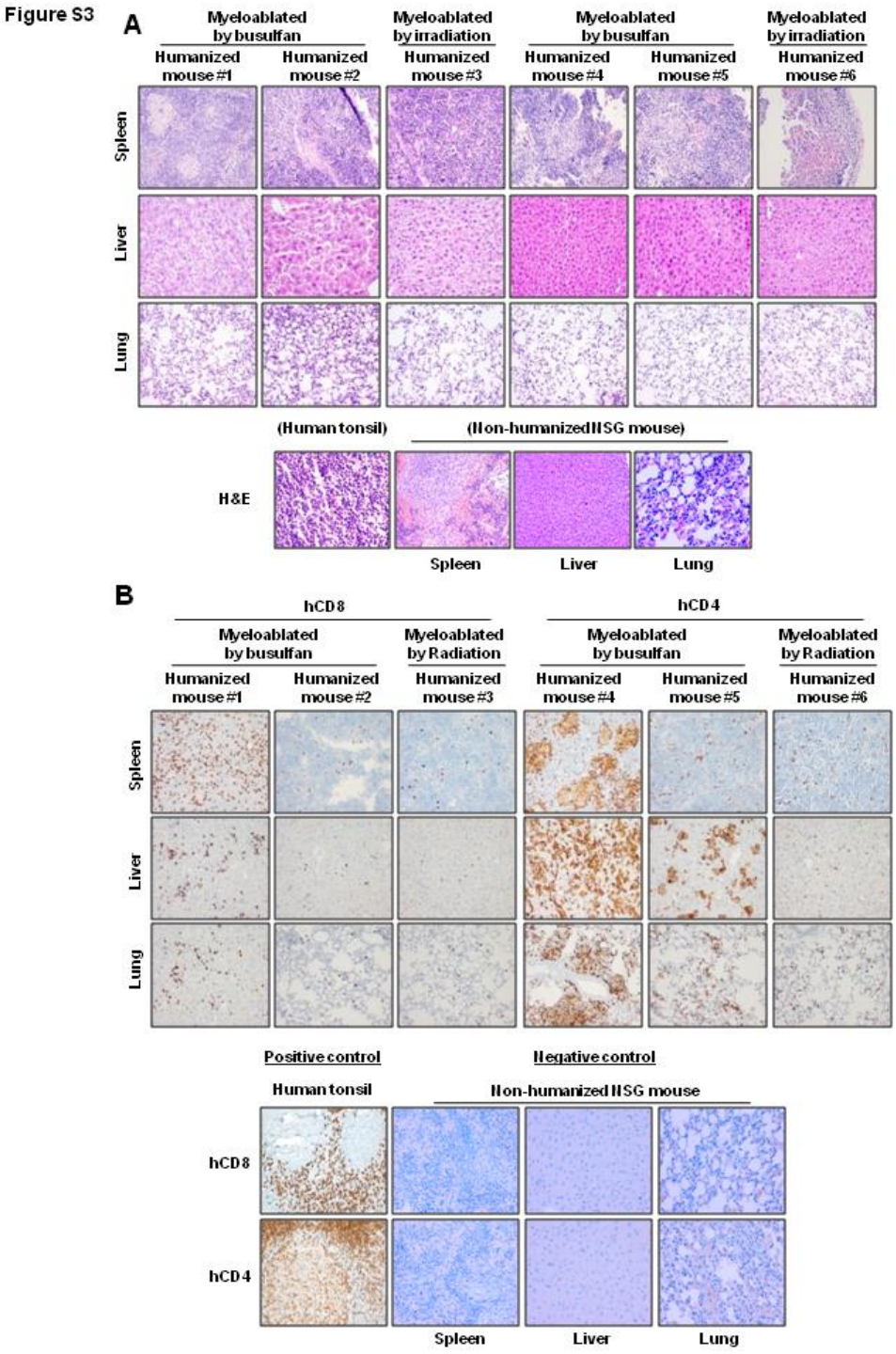
Figure S2



Supplementary figure S2. Confirmation of inhibition efficiency of human PD-1 inhibitor

The expressions of Ki67 or PD-1 on CD3+ Jurkat cells after PD-1 inhibitor treatment were analyzed by flow cytometry. Representative flow cytometry plots from incubation of hCD3+ Jurkat cells with T cell activator PHA, MDA-MB-231 cells, or PD-1 inhibitor for 24 h and 48 h are shown. *P*-values were calculated by Student's t- test (*$p<0.05$ and $^{\#}p>0.05$). Data are presented as mean ± standard deviation.

45 Supplementary figure S3



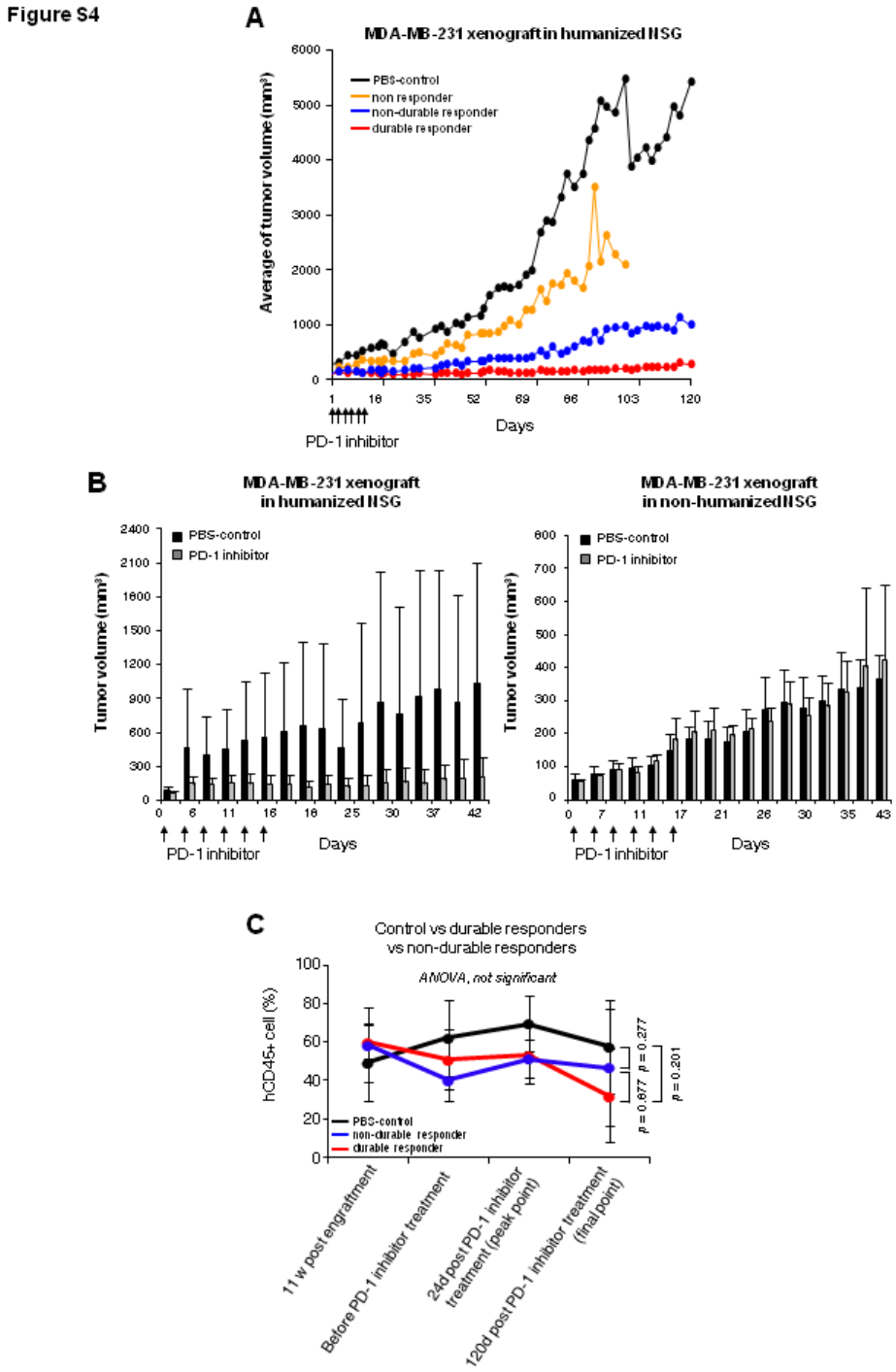
46

47 **Supplementary figure S3. H&E staining and IHC staining for hCD4+ and hCD8+ T**

cells

H&E (A) and IHC staining for hCD4+ and hCD8+ T cells (B), using normal organs such as spleens, livers, and lungs, collected from 45-week humanized mice with MDA-MB-231 xenograft. At 43 days post-implantation of MDA-MB-231 cells, mice were sacrificed and normal organs were collected from those mice. IHC images were displayed by myeloablative method. The human tonsil was used for positive control, and tumor tissue and organs (spleen, liver, and lung) from non-humanized mouse was used for negative control. Scale bars, 5 mm. Images were photographed at ×400 magnification. In humanized mouse #1 #2 and #3, frozen cord blood was used as a HSC source; In humanized mouse #4, #5, and #6 fresh cord flood was used as a HSC source.

68 Supplementary figure S4



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70

Supplementary figure S4. *In vivo* efficacy test of PD-1 inhibitor in MDA-MB-231 xenografted humanized mice

(A) In the 17 humanized mice with MDA-MB-231 xenograft, 5 mice were PBS- treatment control and 12 mice received PD-1 inhibitor. Eleven of 12 mice treated with PD-1 inhibitor, showed response. In 8 of 11 responders, response was durably sustained up to 120 days (i.e., durable responder), whereas in 3 of 11 responders, tumors started to grow again from around day 35 (i.e., non-durable responder). One case did not respond to PD-1 inhibitor (i.e., non-responder).

(B) Comparison of results of PD-1 inhibitor efficacy tests in our humanized versus non-humanized NSG mice. Paired bar graphs (black and gray bars) indicated average tumor sizes in the PBS-control (gray bar) and PD-1 inhibitor treatment (black bar) groups at the same time point. In the humanized mice, PD-1 inhibitor treatment group showed tumor growth halt unlikely the PBS-control group showing progressive tumor growth by time, whereas, in non-humanized mice, similar pattern of progressive tumor growth was seen in both PBS-control and PD-1 inhibitor treatment groups. Data are presented as mean ± standard deviation.

(C) Comparison of hCD45+ cells in the peripheral blood based on response durability to PD-1 inhibitor in humanized mice. The levels of hCD45+ cells at indicated time points [11w post engraftment with hCD34+ HSCs, before PD-1 inhibitor treatment, 24 days post PD-1 inhibitor treatment (peak point), and 120 days post PD-1 inhibitor treatment (final point)] were represented. *P*-values were calculated at indicated time points by ANOVA with post hoc LSD test. At all time points, *P*-values were not significant but only *P*-values at the final time point were marked in the graph. Data are presented as mean ± standard deviation.

Supplementary methods

Cell lines

The MDA-MB-231 human breast cancer cell line was purchased from the American Type Culture Collection (ATCC, VA, USA #HTB-26). The cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium and supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and were incubated at 37°C, 5% CO_2 in an incubator.

Hematoxylin & eosin (H&E) staining and immunohistochemistry (IHC)

Immune cell infiltration in the tumor was determined by IHC using 4% paraformaldehyde-fixed, paraffin-embedded tissues. All paraffin sections were cut at a 3 μm thickness, deparaffinized through xylene, and dehydrated with graded ethanol. For H&E staining, slides were stained with Harris hematoxylin solution and eosin Y solution. For IHC analysis, heat-induced antigen retrieval with 0.01 M citrate buffer (pH 6.0) was used for indicated antibodies. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol, and primary incubations were performed with human CD8 (1:500) or CD4 (1:500) antibody (Abcam, MA, USA) overnight (4°C). Subsequently, sections were incubated with secondary antibody (HRP-conjugated) for 1 h at room temperature, visualized with 3,3-diaminobenzadine tetrahydro-chloride (DAB; Thermo fisher Scientific, USA) for chromogenic development, washed, and counterstained with hematoxylin. The slides were dehydrated with graded ethanol and mounted with Canada balsam (Junsei, Japan). For quantification of IHC staining, positively stained cells were counted in five random ×400 microscopic fields for each tissue section. A total of five different sections were counted and the average percentage with standard deviation of positive cells per section is shown.

TUNEL assay

Paraffin-embedded tissue sections were cut at a 3-μm thickness, deparaffinized through xylene, and dehydrated with graded ethanol. Multiple sections were stained using the Apoptag Plus Peroxidase In Situ Apoptosis Detection Kit (EMD Millipore Corp, CA, USA). Slides were treated with proteinase K for 15 min at room temperature, and TdT for 1 h at 37°C. The slides were incubated with an anti-dioxigenin-peroxidase secondary substrate at room temperature and 37°C, and slides were counterstained with hematoxylin. The slides were dehydrated with graded ethanol and mounted with Canada balsam (Junsei, Japan). For quantification of IHC staining, positively stained cells were counted in five random ×400 microscopic fields for each tissue section. A total of five different fields were counted and the average percentage of positive cells per section with standard deviation is shown.

In vitro T cells proliferation assay

MDA-MB-231 cells (1.5 x 10^5 cells/well) were seeded in 6 well plates. Next day, Jurkat cells (1.5 x 10^5 cells/well) were co-cultured with MDA-MB-231 cells by adding T cell stimulator, 10 μg/ml of phytohemagglutinin (PHA) in the media. Then, those cells were treated with the human PD-1 inhibitor for 24 and 48 h. Suspended cells were collected and stained with indicated antibodies. Stained cells were analyzed by flow cytometry.