SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

RNA isolation, real-time polymerase chain reaction (RT-PCR) and quantitative RT-PCR

Cells were lysed and total RNA were isolated from cells using an RNeasy Mini kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. cDNA were generated using M-MuLV Reverse Transcription System (Fermentas Inc, Hanover, MD, USA), which were then amplified by PCR. RT-PCR was performed on an ABI PRISM 7700HT sequence detection system using a SYBR Green kit (Applied Biosystems, Foster City, CA, USA). GAPDH was amplified as an internal control.

IL-10 (2 ng/ml, R&D Systems, Minneapolis, MN, USA), TNF-α (25 ng/ml, R&D Systems), IL-10 blocking antibody (20 μg/ml, eBioscience, San Diego, CA, USA), TNF-α blocking antibody (20 μg/ml, eBioscience) or control IgG (20 μg/ml, eBioscience) were used to treat MCL cells. To induce B7-H1 expression, cells were cultured in the presence of ultra-pure LPS (1 μg/ml, Invivogen, San Diego, CA, USA) or IFN-γ (500 IU/ml, R&D Systems) for 24 hours.

Western blot analysis

Western blot analysis was used to detect B7-H1 protein expression in MCL cells, as described previously. Anti-human B7-H1 (R&D Systems) and β-actin (Sigma) monoclonal antibody were used.

Proliferation assays

Allogeneic $CD3^+$, $CD4^+$ and $CD8^+$ T cells were purified from peripheral blood mononuclear cells (PBMCs) from healthy donors using magnetic cell sorting (Miltenyi Biotec). $CD3^+$ T cells were labeled with 5(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE; 5 μM; Invitrogen,

Carlsbad, CA, USA) for 10 minutes at 37°C. After washing, T cells (5×10^4/100 μL/well) were seeded into 96-well U-bottomed tissue culture plates (Corning, New York, USA) and cocultured with irradiated MCL cells (5×10^3/100 μl/well) at 37°C for 7-10 days in 5% CO_2 in Aim-V medium supplemented with 10% pooled human serum (T-cell medium). Flow cytometry analysis was used to detect dilution of CFSE.

In some experiments, T cells (5×10^4/100 μL/well) were seeded into 96-well U-bottom tissue culture plates (Corning Incorporated, Corning, NY) and cocultured with irradiated MCL cells (5×10^3/100 μl/well) at 37°C for 5 days in 5% CO_2 in T-cell medium. T-cell proliferation was measured after overnight incubation with ^{3}[H]-thymidine (0.5 μCi/0.037 MBq/well). Results are expressed as mean count per minute (CPM) of triplicate cultures.

Cytokine ELISA and flow cytometry analysis

We measured the levels of various cytokines using the supernatants of T-cell cultures by using a commercially ELISA kits (R&D Systems) according to the manufacturer's instructions. T cells were stained with allophycocyanin (APC), phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (mAbs) and analyzed using a flow cytometer (FACS Calibur; BD Biosciences, Sparks, MD, USA).

Generation of tumor-reactive, alloantigen-specific cytotoxic T lymphocyte lines

Allogeneic $CD3^+$ T cells were cocultured in T-cell medium with irradiated SP53-wt, SP53-ctl or SP53-kd, Granta 519-wt, Granta 519-ctl, Granta 519-kd, respectively. After 7 days of coculture, $CD3^+$ T cells were harvested and restimulated with newly irradiated above described tumor cells. The cultures were fed with fresh T-cell medium containing recombinant IL-2 (10 IU/ml), IL-7 (5 ng/ml), and IL-15 (5 ng/ml) (all from R&D Systems). The frequencies of $CD3^+CD8^+$ T cells

were monitored every week by flow cytometry. After at least 4 repeated cycles of in vitro restimulation, T-cell lines were generated, and named CTL-SP53-wt, CTL-SP53-ctl, CTL-SP53-kd, CTL-Granta 519-wt, CTL-Granta 519-ctl and CTL-Granta 519-kd, based on their stimulatory MCL cells. T-cell lines were expanded in T-cell medium containing recombinant IL-2, IL-7, and IL-15 for 2 weeks and subjected to functional tests.

Cytotoxicity assay

The standard 4-hour ^{51}Cr-release assay was performed to measure cytolytic activity of the T-cell lines with target cells including SP53-wt, SP53-ctl or SP53-kd, Granta 519-wt, Granta 519-ctl, Granta 519-kd, primary tumor cells, PBMCs, and normal B cells isolated from MCL patients, and K562, as described previously. To determine whether the cytolytic activity was restricted by major histocompatibility complex (MHC) class I or II molecules, target cells were pretreated with 20 μg/ml mAbs against HLA-ABC (Serotec Ltd., Oxford, UK), HLA-DR (Immunotech, Marseilles, France), or control IgG (eBioscience).

Adoptive therapy in SCID mice

SCID mice (6-week old) were subcutaneously injected with MCL cell lines (1×10^6 /mouse). After tumor grew to 25 mm^2, mice were treated 3 times (1 week interval) with intravenous adoptive transfer of MCL-specific CTL cell lines (5×10^6/mouse) and with a high dose of rhIL-2 (100,000 IU/injection, daily for 3 days) to support the survival of transferred human T cells. Tumor sizes were recorded twice a week. Mice were sacrificed once tumors reached 225 mm^2.

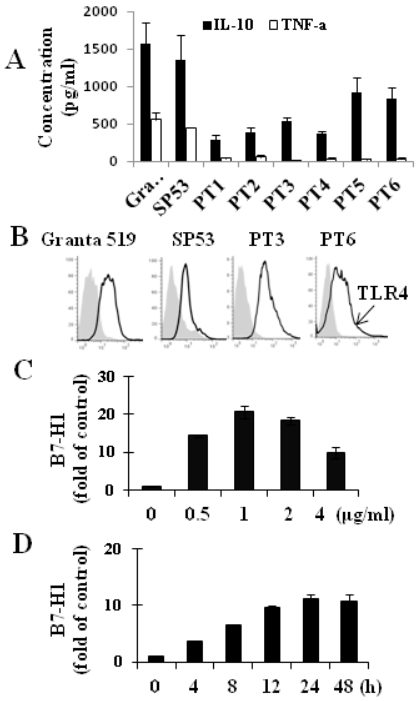
SUPPLEMENTAL FIGURES

sFigure 1. Expression of IL-10, TNF-α, TLR4 and B7-H1 in MCL. (**A**) ELISA assay showing the levels of IL-10 and TNF-α secreted by two MCL lines and purified primary MCLs from 6 patients. (**B**) Flow cytometry analysis of TLR4 expression on MCL cells. (**C**) B7-H1 gene expression in MCL cell line SP53 was increased in a dose-dependent manner after LPS exposure. SP53 cells were treated with different concentrations (0, 0.5, 1, 2 or 4 μg/ml) of LPS for 24 hours. (**D**) LPS induced time-dependent expression of B7-H1 gene in MCL cell line SP53. SP53 cells were treated with LPS (1 μg/ml) for 4, 8, 12, 24 or 48 hours. The mRNA levels are expressed as fold increase to the control. Representative results from one of three performed experiments are shown.

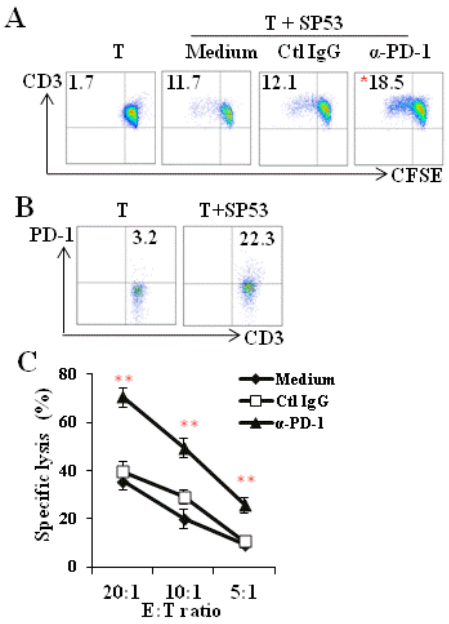
sFigure 2. Blockade of B7-H1/PD1 pathway leads to enhanced T-cell immune response. (**A**) Proliferation of $CD3^+$ T cells, measured by CFSE dilution assay, in culture with irradiated SP53 cells in the presence or absence of control IgG or anti-PD-1 mAb (20 μg/ml). (**B**) Surface expression of PD-1 in alloreactive T cells. (**C**) Cytotoxicity of CTLSP53-wt against SP53 cells (E:T ratio = 20:1) pre-treated with 20 μg/ml anti-PD-1(α-PD-1) mAb or control IgG (Ctl IgG). Representative results of 2 experiments are shown. $*P < 0.05$; $**P < 0.01$, compared with isotype control.

sFigure 3. Phenotype of MCL-reactive T-cell lines. (**A**) Increased expression of CD45RO, CD28, and CD62L by $CD8^+$ T cells in the SP53-kd CTL lines, compared to the SP53-wt CTL lines. (**B**) Increased expression of CD45RO by $CD4^+$ T cells in the SP53-kd CTL lines, compared to the SP53-wt CTL lines. (**C**) ELISA assay showing the increased levels of IFN-γ production by the SP53-kd CTL lines, compared to the SP53-wt CTL lines (**D**) Intracellular cytokine staining showing the percentages of IFN-γ-, IL-4-, IL-17- or CD107a-expressing $CD4^+$

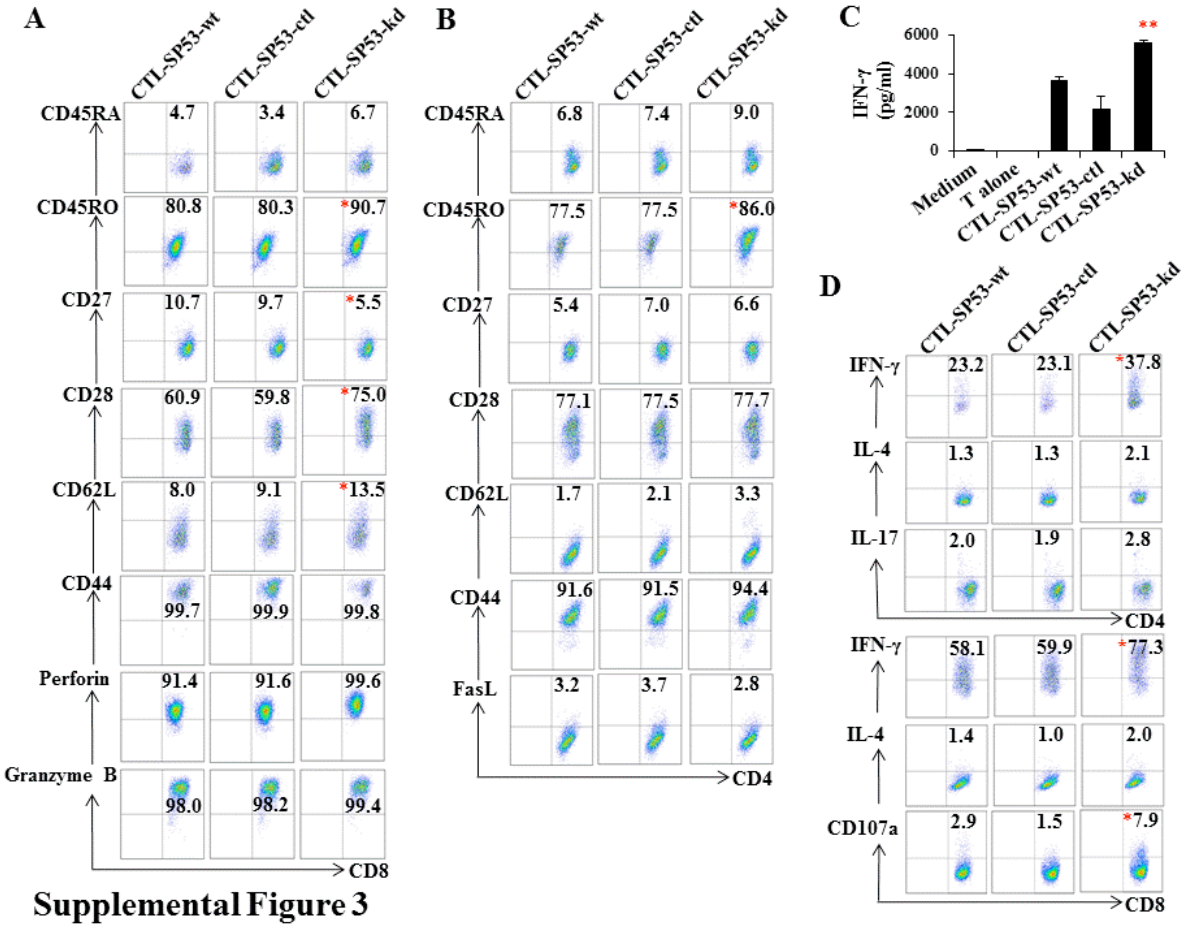
or CD8$^+$ T cells in the T-cell lines after restimulation with irradiated tumor cells. Representative results of 3 experiments are shown. Similar results were obtained with T-cell lines generated from other healthy donors. *$P < 0.05$; **$P < 0.01$, compared with shRNA control T cells.



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3