Supplemental Material

A transgenic mouse model for HLA-B*57:01-linked abacavir drug tolerance and reactivity

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Supplemental Methods

In vitro culture assays.

In vitro T cell responses to ABC were measured in cultures of purified $CD8^+$ T lymphocytes and single-cell suspensions from spleen or pooled LN (cervical, axillary, brachial, inguinal, and mesenteric) of WT and/or HLA-B*57:01 Tg mice. Spleen and LN cell suspensions were prepared by macerating the lymphoid organs through 70-µm cell strainers and were used directly in in vitro culture assays, after lysis of RBC with Ammonium-Chloride-Potassium (ACK) lysing buffer (GIBCO), or as a cell source for the isolation of $CD8^+$ T lymphocytes using the mouse $CD8a^+$ T Cell Isolation Kit (Miltenyi Biotec). Cells were cultured for up to 14 days in complete RPMI (CRPMI) consisting of RPMI 1640 supplemented with 2 mM L-Glutamine, 1 mM Sodium Pyruvate, 1X MEM Non-Essential Amino Acids, 1X MEM Vitamin Solution, 10 mM Hepes, 0.05 mM $_2$-ME, 100 U/mL Penicillin, and 100 µg/mL Streptomycin (all from GIBCO). During the first 2 days of culture, CRPMI was completed with 0.5% heat-inactivated, normal mouse serum (Equitech-Bio, Inc.) to keep the background of cell activation low. Subsequently, media was replaced by fresh CRPMI containing 10% heat-inactivated, low-endotoxin FBS (Premium Select FBS, Atlanta Biologicals), as follows: half of the medium vol on days 2 and 9, and the complete medium vol on day 5. Cells were seeded in 48-well tissue culture treated plates (Cat# 3548, Corning) at 3.6 x 10^6 cells per well in 1.2 mL or in 96-well tissue culture treated plates (Cat# 3596, Corning) at 0.9 x 10^6 cells per well in 0.3 mL and incubated at 37°C and 5% CO_2. Enrichment of ABC-reactive $CD8^+$ T lymphocytes was obtained by adding 10 µg/mL ABC (Ziagen tablets, GlaxoSmithKline) to the cultures at day 0. Cultures without drug (None) served as a negative control. ABC tablets were dissolved at 10-20 mg/mL in Water For Injection (WFI) for Cell Culture (GIBCO) by vigorous shaking at RT. Drug solutions were filtered with 0.22 µm filters, aliquoted, and stored at -20°C. Aliquots of ABC solutions were only thawed once and drug concentration was verified according to the instructions reported in Matsyagiri et al. (1). Purified $CD8^+$ T lymphocytes were co-cultured at day 0 with irradiated (30 Gy) splenocytes from drug-naive mice of the same genotype at a 1:2 $CD8^+$ T cell to feeder ratio. Feeder

cells were pulsed with 10 µg/mL ABC overnight or left not pulsed to feed control $CD8^+$ T cell cultures without drug.

Morphological changes in cells were captured with bright-field microscope images using an inverted microscope (model IX81, Olympus). Secretion of IL-2, IFN-γ, and GZB in cell culture supernatants was measured by ELISA using commercial kits for mouse IL-2, IFN-γ, and GZB (eBiosciences). Production of IFN-γ and expression of PD-1, CD25, and 4-1BB in T lymphocytes as well as expression of PD-L1, CD86, CD80, CD40, and HLA in $CD11c^+MHC\text{-}II^+$ DCs were analyzed by flow cytometry as detailed in the Methods section "Flow cytometry, cell sorting, and in vivo proliferation assays". These analyses were performed within 5 days of primary in vitro stimulation, at the times specified in the figure legends, and prior to supplying the cell cultures with exogenous IL-2.

The ability of ABC-reactive $CD8^+$ T cells to respond to the drug after in vitro expansion and the requirement for CD8 and HLA-B*57:01 molecules in this response were evaluated in ELISpot experiments. In these experiments, ABC-reactive $CD8^+$ T cells were first generated by culturing purified $CD8^+$ T lymphocytes and irradiated feeders from Tg mice with 5 µg/mL ABC provided at day 0 and then expanded for up to 14 days with 50 IU/mL of recombinant IL-2 (Proleukin (aldesleukin), PROMETHEUS) supplied every other day, starting on day 5. Resulting cultures were then washed three times and tested in IFN-γ ELISpot microplates (R&D Systems) containing untreated or drug-pulsed irradiated splenocytes (at a 1:4 $CD8^+$ T cell to feeder ratio, 0.25×10^6 cells in 0.1 mL per well) and in the absence (None) or presence of 5 µg/mL ABC, as per manufacturer's instructions. Where indicated, ELISpot cell cultures were treated 1h prior to ABC with 10 µg/mL of: anti-CD8a mAb (clone 53.6.72, BioXcell), anti-HLA B/C mAb (clone B1.23.2, eBioscience), or the isotype controls rat IgG2a (clone 2A3, BioXcell) and mouse IgG2b (clone eBMG2b, eBioscience).

The role of costimulation in the activation of $CD8^+$ T lymphocytes by ABC in vitro was assessed using LN cell suspensions. Cell cultures were supplemented with 10 µg/mL of CTLA-4–Ig [ORENCIA (abatacept), Bristol-Myers Squibb], anti-CD80 mAb (clone 1G10, BioXcell), or the isotype control rat IgG2a (clone 2A3, BioXcell), 1h before drug exposure (day 0) and at day 2.

Treatment of mice.

WT and/or HLA-B*57:01 Tg mice were injected i.p., 5 days a week for up to 4 weeks, with 3 mg of ABC solution. Both ears were painted at the indicated times in Supplemental Figure 3 with a solution of 0.2 mg/ear of ABC in 70% sterile-filtered DMSO (Hybri-Max, SIGMA). The animal equivalent dose (AED) of ABC administered to mice was determined as described by Nair and Jacob (2), based on the equation AED (mg/kg) = Human dose (mg/kg) × K_m ratio, with the human equivalent dose (HED) of ABC = 600 mg/60 kg and the K_m ratio = 12.3. Control mice were treated with equal vol of Veh, corresponding to WFI for i.p. injection and WFI in 70% DMSO for topical painting. To deplete $CD4^+$ T cells, animals were injected i.p. with 0.25 mg of anti-CD4 mAb (clone GK1.5, BioXcell) 3 days prior to Veh or ABC treatment and then again at days 1, 4, and 7 during Veh or drug exposure (Supplemental Figure 3B). Depletion of $CD4^+$ T cells was verified by flow cytometry in pheripheral blood, spleen, and LN after 6 and 13 days of the initiation of the mAb treatment (days 3 and 10 from the start of Veh or ABC, data not shown). To block PD-1 in vivo, mice were injected i.p. with 0.2 mg of anti-PD-1 mAb (clone RPM1-14, BioXcell) every two days, starting at day 0, throughout the duration of Veh or drug exposure. In vivo neutralization of CD86 and/or CD80 was obtained by treating animals with 0.5 mg of CTLA-4–Ig [ORENCIA (abatacept), Bristol-Myers Squibb] or 0.25 mg of anti-CD80 mAb (clone 1G10, BioXcell) 3 days prior to ABC administration and then again every other day, starting at day 0, for the entire duration of drug exposure. In preliminary experiments, the isotype control antibodies rat IgG2b (clone LTF-2, BioXcell) and rat IgG2a (clone 2A3, BioXcell) were used in control treatment groups.

Assessment of skin reaction on treated ears.

IHC was performed by the Pathology/Histotechnology Laboratory, Laboratory Animal Sciences Program, Leidos Biomedical Research, Inc. Briefly, 10% neutral buffered formalin fixed skin samples from the edge of

the ear pinna were processed to paraffin blocks and sectioned at 5 microns onto positively charged slides. CD8a staining was performed using a Leica BondMax autostainer (Leica Biosystems). Heat-induced epitope retrieval was performed using Citrate buffer for 20 min at 100°C. The anti-CD8a mAb, clone 4SM16 (Cat# 14-0195-82, eBioscience), was used at a 1:50 dilution for 30 min. mAb visualization was accomplishing using the Bond Refine Polymer Detection Kit (Leica Biosystems). Isotype control was used alongside the primary mAb. Images of the stained sections were acquired with the Pannoramic Midi slide scanner (3DHISTECH) under identical acquisition settings and subsequently processed using the Pannoramic Viewer analysis software.

Ear thickness was measured with an electronic digital caliper before and at 3 weeks of treatment. To reduce the technical error, at each time point, measurements were repeated at least 3 times per mouse and then averaged to a single value per time point/animal before plotting.

For gene expression analysis, skin biopsies from the edge of the ear pinna, 2 mm in diameter (punches from Miltex, Inc), were immediately submerged in TRIzol (Invitrogen) upon excision, flash-frozen, and stored at -80°C until processing. Samples were thawed and mixed with 0.5 cm^3 of 2 mm Zirconia Beads (Biospec). Tissue was homogenized using a Precellys 24, Cryolys system (Bertin Technologies) with the following settings: 3 cycles at 6800 rpm, with 30s of alternate run and pause time. Homogenate was transferred to a clean 2 mL eppendorf tube and total RNA was extracted following the TRIzol protocol (Invitrogen) as per manufacturer's directions. RNA was then resuspended in 40 µl of DEPC treated water, assessed for quality, and measured using the NanoDrop 1000 (Thermo Scientific). 1 µg of total RNA per sample was reverse transcribed using the High Capacity cDNA RT Kit (Applied Biosystems) as per manufacturer's instructions. A fourth of the total cDNA solution of each sample was loaded in a mouse-specific TaqMan Low Density array (TLDA, Immune Array, Applied Biosystems) including a 96-gene panel. Alternatively, expression of genes was evaluated with individual gene expression assays (Applied Biosystems) and 1/20 of cDNA mix. The amplification reaction was conducted with 2x Universal Master Mix (Applied Biosystems) as per manufacturer's instructions in a Viia7 Real-Time system (Thermo Fisher Scientific) using the following conditions: 94°C for 10 min followed by 40 cycles of amplification at 94°C for

30 sec and 60°C for 1 min. Fold increase in gene expression was measured based on the $2^{-\Delta\Delta Ct}$ method, considering *Gapdh* as a housekeeping gene. Fold change in gene expression of skin biopsies harvested at the same time point and from mice of the same genotype belonging to the same treatment group were collapsed and plotted as log2-transformed data in a heat map generated using the GraphPad Prism 7.02 software (La Jolla, CA).

Flow cytometry, cell sorting, and in vivo proliferation assays.

Genotyping, expression analysis of the chimeric HLA-B*57:01 molecule in B cells and T lymphocytes as well as cell surface marker staining of blood, were performed using 50 µl of peripheral blood per mouse. The blood was collected in MiniCollect 0.8 mL LH Lithium Heparin Sep tubes (Cat# 450479, Greiner Bio-one GmbH) and transferred into 96-well sterile V-bottom plates (BRANDplates Cat# 781661, BRAND) for flow cytometry staining. Prior to staining, RBC were lysed with ACK lysing buffer as follows: one incubation with 75 µl of ACK/sample at RT for 5 min followed by a centrifugation (300xg) at 4°C for 3 min and subsequently by a second incubation with 150 µl of ACK/sample at RT for 5 min. Neutralization of the ACK was accomplished by washing leukocytes twice in cold PBS – pH 7.4 without Calcium Chloride and Magnesium Chloride (GIBCO) supplemented with 5% heat-inactivated FBS. To prevent non-specific Ab binding, cells were pre-incubated at 4°C for at least 15 min in 100 µl/sample of Stain Buffer (BD Biosciences) containing 10 µg/mL of anti-CD16/CD32 mAb (mouse Fc Block, clone 2.4G2, BD Biosciences). After blocking, samples were stained at 4°C for 30 min in the dark with 100 µl of a mixture constituted by 50% of Stain Buffer, 50% of Brilliant Stain Buffer (BD Horizon), and the appropriate diluted antibodies for cell surface markers. Once labeled, cells were washed by centrifugation at 4°C for 5 min with ACK Lysing buffer (150 µl per sample) and cold PBS supplemented with 5% heat-inactivated FBS (250 µl per sample) and finally resuspended in cold Stain Buffer prior to flow cytometry acquisition.

Intracellular staining was performed using the BD Cytofix/Cytoperm Plus kit (BD Biosciences) as per manufacturer's instructions. For intracellular detection of IFN-γ, cultures of 3.6 x 10^6 pooled LN cells or

purified $CD8^+$ T lymphocytes plus irradiated feeders were left untreated or incubated with 10 µg/mL of ABC for approximately 5 days as detailed in the Methods section "In vitro culture assays". During the last 6-12h of culture, cells were treated with Brefeldin A Solution (eBioscience) at a final dilution of 1/1000 and then tested. Viability was assessed by LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen) following manufacturer's directions while non-specific Ab binding was blocked with mouse Fc Block. After blocking, cells were stained for cell surface markers as indicated above, fixed, permeabilized, and finally stained at 4°C for 30 min in the dark with anti-mouse IFN-γ mAb. For intracellular detection of CTLA-4, LN cells were collected from mice treated for 10 days as indicated in the figure legends and in the Methods section "Treatment of mice". After LIVE/DEAD and cell surface marker staining in the presence of Fc Block, cells from individual mice were fixed, permeabilized, and then stained at 4°C for 30 min in the dark with anti-mouse CTLA-4 mAb. Once labeled, cells were washed twice with cold 1x BD Perm/Wash buffer (250 µl per sample) and finally resuspended in cold Stain Buffer prior to flow cytometry acquisition. For in vivo proliferation assay of $CD8^+$ T lymphocytes, Tg mice were injected i.p. with 2 mg of sterile-filtered BrdU (Sigma-Aldrich) in sterile saline, starting at day 0 of Veh or ABC administration and daily thereafter. At the time of euthanasia, LN cells from individual mice were collected and stained intracellularly for BrdU incorporation and Ki-67 expression after LIVE/DEAD and cell surface marker staining in the presence of Fc Block. Detection of BrdU and Ki-67 was accomplished using the BrdU Flow Kit (BD Pharmingen) as per manufacturer's instructions.

$CD8^+$ T cells were sorted from LN cells of individual mice and stained, after Fc Block, with cell surface marker antibodies at 4°C for 30 min and in PBS supplemented with 2% heat-inactivated FBS. Sorted $CD8^+$ T lymphocytes were collected in PBS supplemented with 2% heat-inactivated FBS.

Antibodies used for cell surface marker staining were: anti-HLA B/C PE (clone B1.23.2, eBioscience), anti-H-2D[d] FITC (clone 34-2-12, BD Pharmingen), anti-CD3 APC/Cy7 (clone: 17A2, Biolegend), anti-CD4 BV605 (clone: RM4-5, BD Horizon), anti-CD8a BV711 (clone: 53-6.7, BD Horizon), anti-CD45R (B220) APC (clone: RA3-6B2, eBioscience), anti-CD19 Alexa Fluor 700 [clone: eBio1D3 (1D3), eBioscience], anti-CD11c APC (clone: N418, Biolegend), anti-CD62L Alexa Fluor 700 (clone: MEL-14, Biolegend), anti-CD44

PE (clone: IM7, Biolegend), anti-CD279 (PD-1) BV785 (clone: 29F.1A12, Biolegend), anti-CD223 (LAG-3) APC [clone: eBioC9B7W (C9B7W), eBioscience], anti-CD366 (TIM3) PE-Cy7 (clone: RMT3-23, eBioscience), anti-CD25 Alexa Fluor 488 [clone: eBio7D4 (7D4), eBioscience], anti-KLRG1 PE-Cy7 (clone: 2F1/KLRG1, Biolegend), anti-CD137 (4-1BB) APC (clone: 17B5, eBioscience), anti-CLA FITC (clone: HECA-452, Biolegend), anti-CD183 (CXCR3) APC (clone: CXCR3-173, Biolegend), anti-I-A^b ($A\beta^b$) FITC (clone: 25-9-17, Biolegend), anti-XCR1 BV650 (clone: ZET, Biolegend), anti-CD274 (B7-H1, PD-L1) BV421 (clone: 10F.9G2, Biolegend), anti-CD86 BV650 (clone: GL-1, Biolegend), anti-CD80 PE-Cy5 (clone: 16-10A1, eBioscience), and anti-CD40 PE-Cy7 (clone: 3/23, Biolegend). Antibodies used for intracellular staining were: anti-IFN-γ APC (clone: XMG1.2, eBioscience), anti-CD152 (CTLA-4) APC (clone: UC10-4B9, Biolegend), anti-Ki-67 PE (clone: 16A8, Biolegend), and anti-BrdU APC (clone: 3D4, BD Pharmingen). All antibodies were titrated and tested alongside their specific isotype controls and fluorescence minus one control samples in preliminary experiments.

Flow cytometry data acquisition and analysis were performed using LSR Fortessa X20-SORP and Diva 6.2/8.0.2 (BD Biosciences). Cell sorting was performed using BD FACS Aria Fusion and BD FACS Aria II SORP and Diva 8.0.2 (BD Biosciences). Daily calibration and QC were performed using CS&T Beads (Cat# 642412 and 655051, BD Biosciences).

NanoString sample preparation and data analysis.

NanoString nCounter gene expression profiling was performed on $CD8^+$ T lymphocytes sorted from LN cells of individual mice treated for up to 10 days as detailed in the figure legends and in the Methods section “Treatment of mice”. After sorting, lymphocytes were pelleted, flash-frozen in RLT-buffer (Qiagen) containing 1% $_2$-ME, and stored at -80°C until processing. Total RNA was extracted from thawed samples using the RNeasy Micro Kit (Qiagen) according to the manufacturer’s instructions and contaminant genomic DNA was digested during RNA isolation using the RNAse-free DNAse Set (Qiagen). RNA concentration and purity were determined using the NanoDrop 1000 (Thermo Scientific) and 100 ng of

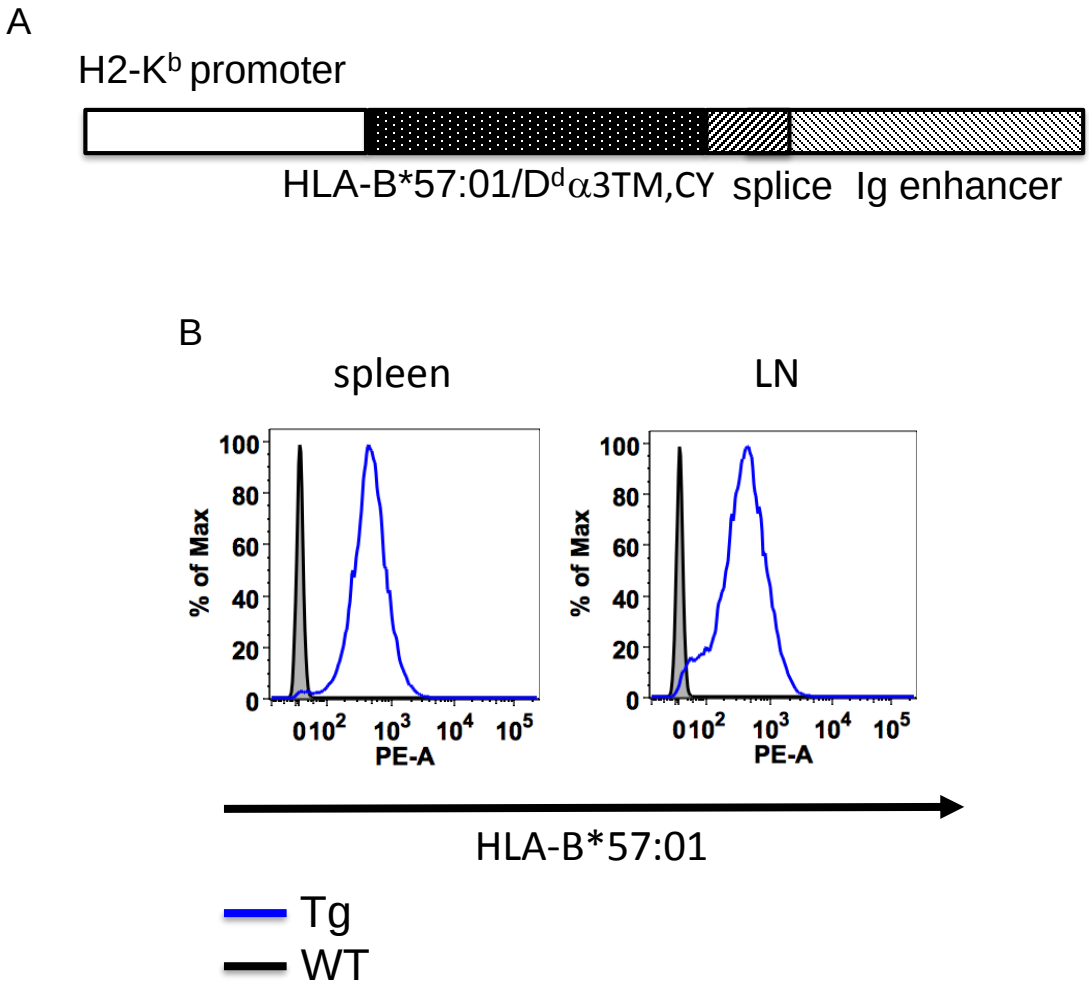
total RNA per sample was used for the gene expression analysis. RNA was hybridized with CodeSet and ProbeSet for 561 genes in the nCounter Mouse Immunology Panel + 30 additional genes in a custom nCounter Panel-Plus and CodeSet-Plus (Supplemental Table 4) (all from Nanostring Technologies) according to the manufacturer's instruction. Following hybridization and purification on nCounter Prep Station, transcripts were counted using the nCounter Digital Analyzer (Nanostring Technologies). Raw RNA counts were then assessed for quality and normalized according to the geometric mean of positive controls and the geometric mean of 5 housekeeping genes (*Gapdh*, *Tubb5*, *Tbp*, *Gusb*, and *G6pdx*) by using the nSolver analysis software version 3.0 (Nanostring Technologies). The heat map was generated with nSolver using Z-score–transformed expression values and the agglomerative hierarchical clustering method with the following clustering parameters: Euclidean Distance for the distance metric and Wards Minimum Distance for the linkage method. Genes included in the heat map were those with average of normalized counts for all samples above the geometric mean + 3 times the SD of negative controls. Few additional genes with a lower average were also included because of their relevance in lymphocyte dysfunction and/or effector responses and their high coefficient of variation due to the effect of treatment.

Gene set enrichment analysis was performed using GSEA software (http://www.broadinstitute.org/gsea) as previously described (3) to compare established gene sets for T cell anergy, exhaustion, and effector function with the gene signature of sorted $CD8^+$ T lymphocytes from experimental mice. The following gene sets were exported from the Molecular Signatures Database (MSigDB, Broad Institute) and were used for: 1) anergy – SAFFORD_T_LYMPHOCYTE_ANERGY; 2) exhaustion – GSE9650_NAIVE_VS_EXHAUSTED_CD8_TCELL_DN, GSE9650_EXHAUSTED_VS_MEMORY_CD8_TCELL_UP, GSE30962_ACUTE_VS_CHRONIC_LCMV_PRIMARY_INF_CD8_TCELL_DN; 3) effector function – GSE9650_NAIVE_VS_EFF_CD8_TCELL_DN, GSE9650_EFFECTOR_VS_MEMORY_CD8_TCELL_UP, KAECH_DAY8_EFF_VS_MEMORY_CD8_TCELL_UP, KAECH_DAY15_EFF_VS_MEMORY_CD8_TCELL_UP, GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_UP.

A nominal *P* value < 0.05 was used to assess the significance of the enrichment score.

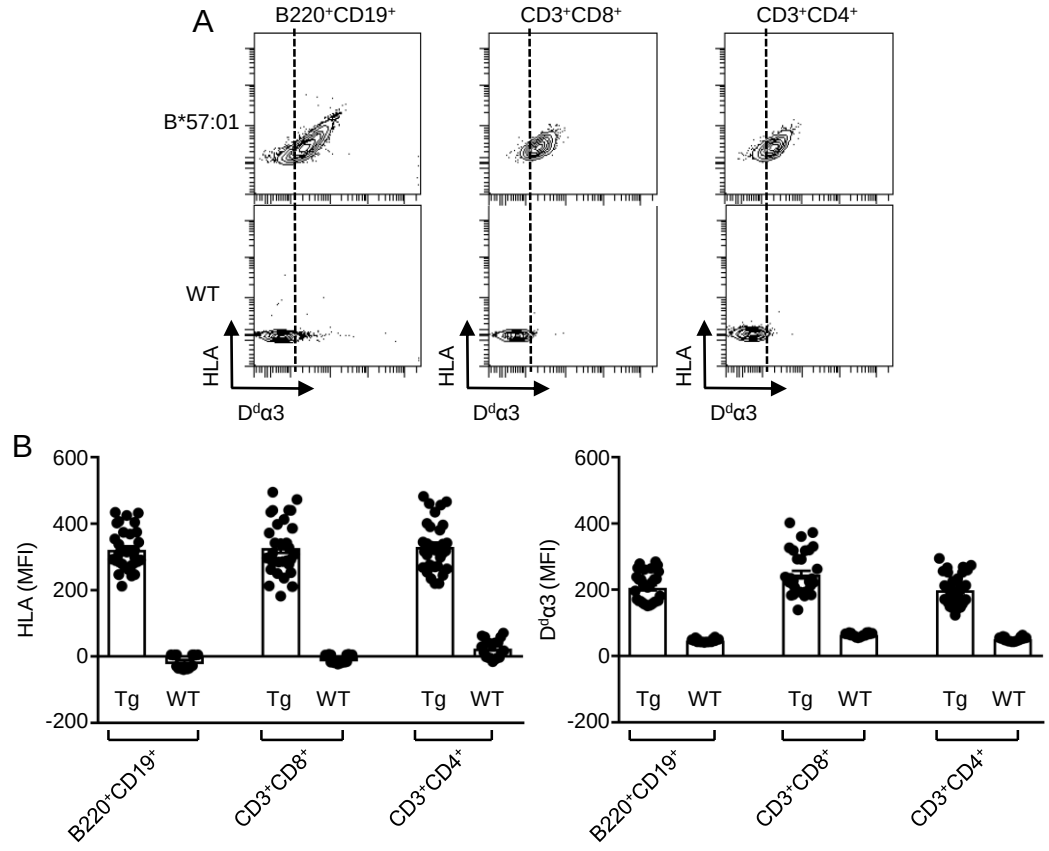
References

1. Matsyagiri L, Kumar VK, Santoshi T, Saritha B, Pranathi P. Spectrophotometric Method for the Estimation of Abacavir Sulphate in Bulk and Pharmaceutical Dosage Forms in Different Solvents. *VRI Phytomedicine* 2013.
2. Nair AB, and Jacob S. A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy.* 2016;7(2):27-31.
3. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences.* 2005;102(43):15545-50.



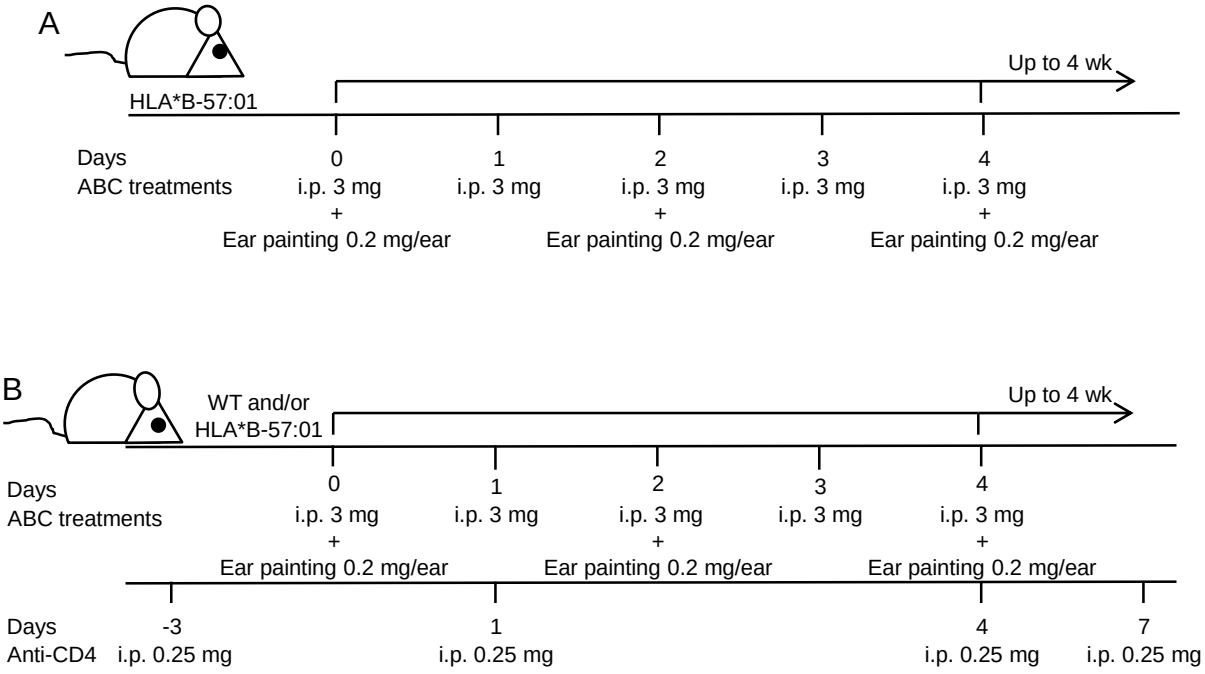
Supplemental Figure 1. Chimeric HLA-B*57:01-Tg construct and expression of the transgene on lymphoid organs

(**A**) Design of Tg HLA-B*57:01α1α2/D^{d}α3 vector construct. Schematic representation of gene construct in the pHSE' vector. Regions of the H2-K^{b} promoter, HLA-B*57:01 signal peptide, α1 and α2 domains, and H2-D^{d}α3 TM and CY domains, along with a splice sequence and Ig enhancer are indicated. (**B**) Expression of HLA-B*57:01 on spleen and LN cells. Ficoll-purified cells were isolated from chimeric Tg (blue), and WT (grey filled) mice, stained with anti-human HLA-B/C mAb (clone: B1.23.2) and analyzed by flow cytometry, as described in the Methods section. Flow cytometry data are from 1 representative experiment out of 4.



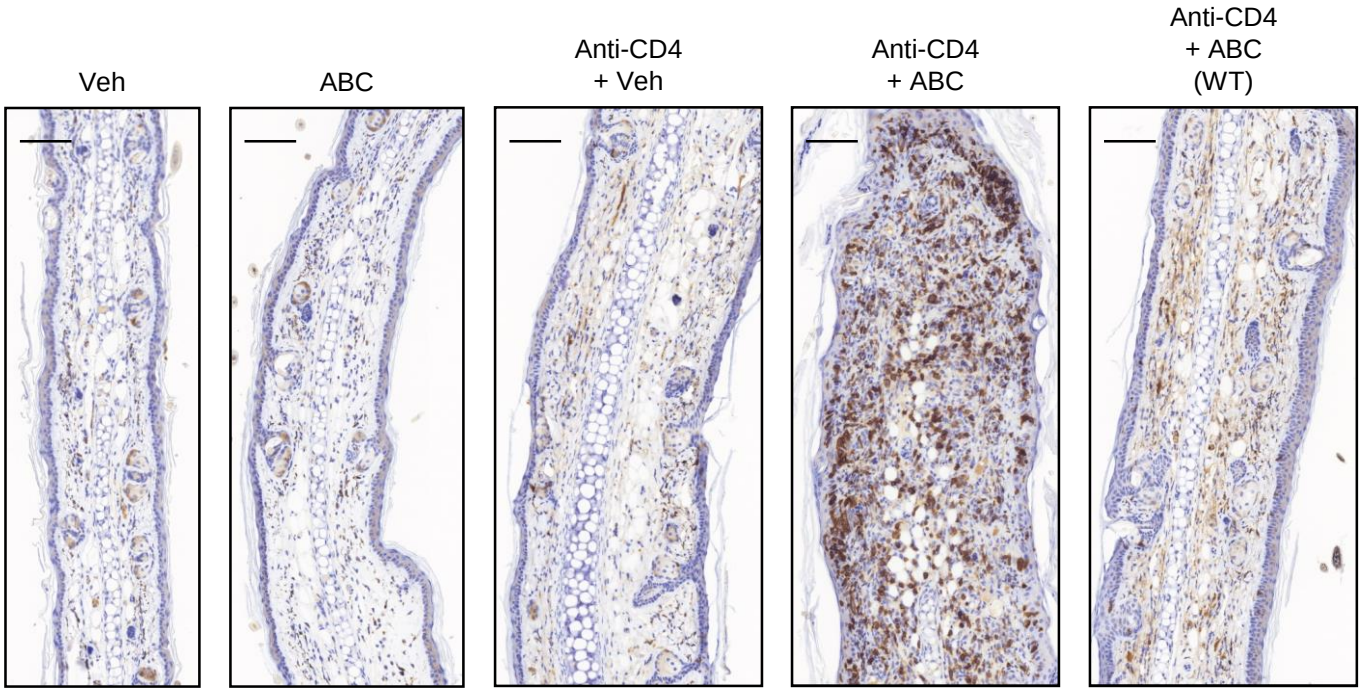
Supplemental Figure 2. Cell surface expression of HLA-B*57:01 on hematopoietic cell subsets

Blood from HLA-B*57:01-Tg or WT mice was stained for HLA-B*57:01 and $D^d\alpha3$ as detailed in the Methods section. (**A**) Plots show the coexpression of HLA-B*57:01 and $D^d\alpha3$ in $B220^+CD19^+$ cells, $CD3^+CD8^+$ T cells and $CD3^+CD4^+$ T lymphocytes from one representative mouse of each strain. (**B**) Bars represent mean ± SEM of median fluorescence intensity (MFI) for HLA-B*57:01 and $D^d\alpha3$ in the indicated cell subpopulation of each strain. Dots indicate values from individual mice (*n* = 32 Tg and 20 WT mice).



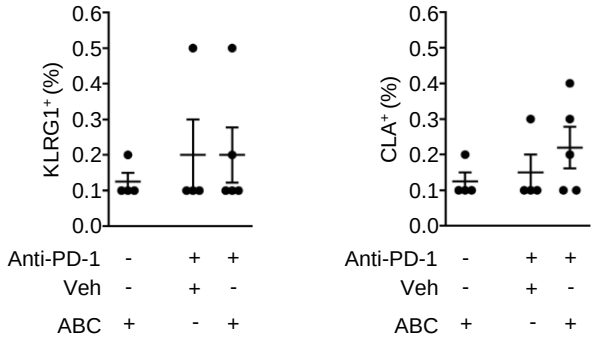
Supplemental Figure 3. Experimental layout of in vivo treatment of HLA-B*57:01-Tg and WT mice

WT and/or HLA-B*57:01-Tg mice were treated systemically (i.p. injection) and topically (ear painting) with ABC for up to 4 wk, in the absence (**A**) or presence (**B**) of a CD4-depleting mAb (Anti-CD4) as detailed in the Methods section. Control mice were treated with equal vol of Veh.



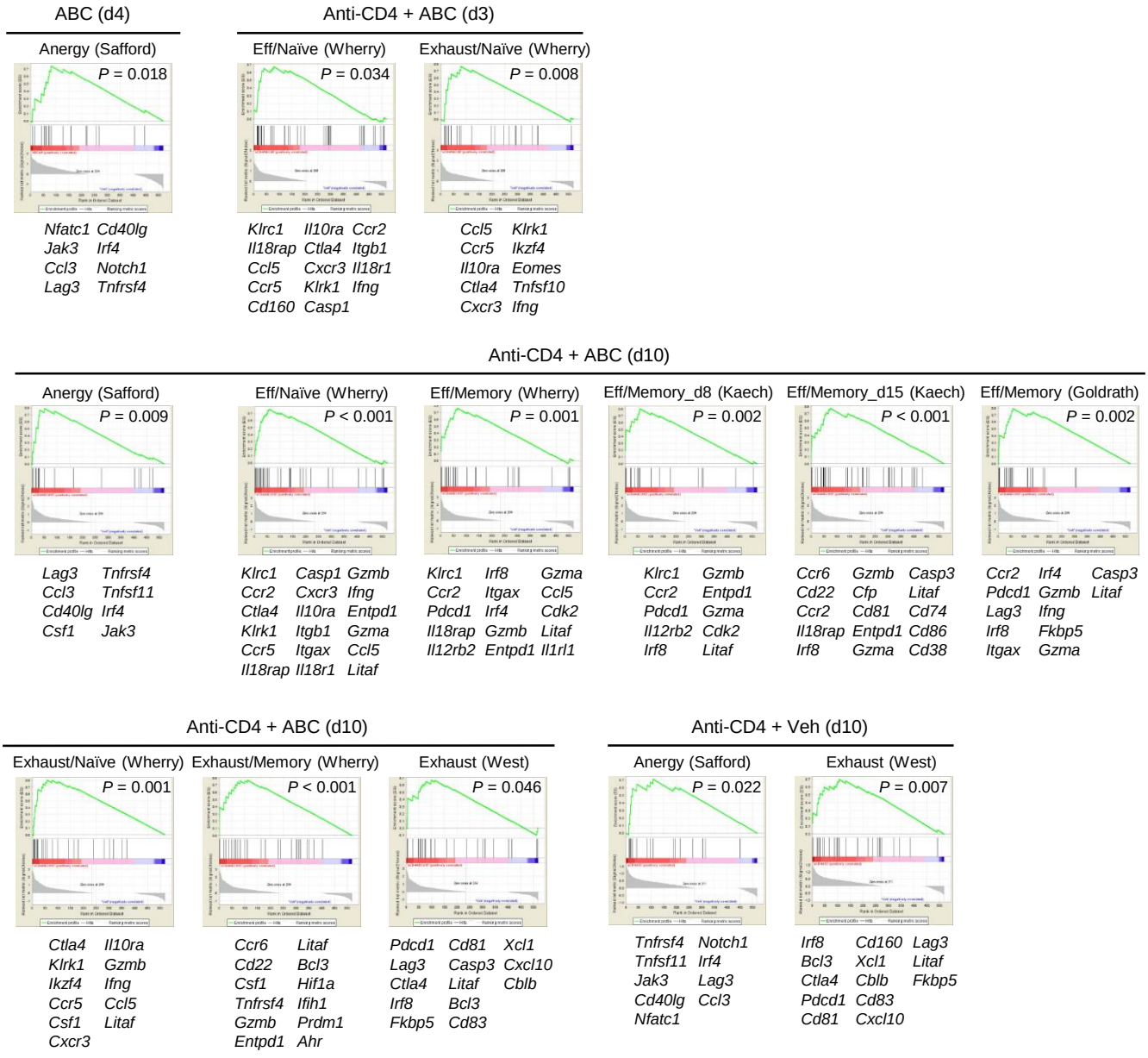
Supplemental Figure 4. IHC of ear skin of treated HLA-B*57:01-Tg and WT mice

Dermal and epidermal infiltration of $CD8^+$ cells in the skin of the ears of anti-CD4 + ABC Tg, but not WT, mice at 3 wk of drug exposure. Skin sections were stained for CD8α (IHC) and are representative of 2 independent experiments. Images of Veh, ABC, Anti-CD4 + Veh, and Anti-CD4 + ABC groups include the IHC image insets in (Figure 2, A and F). IHC scale bars = 100 μm.



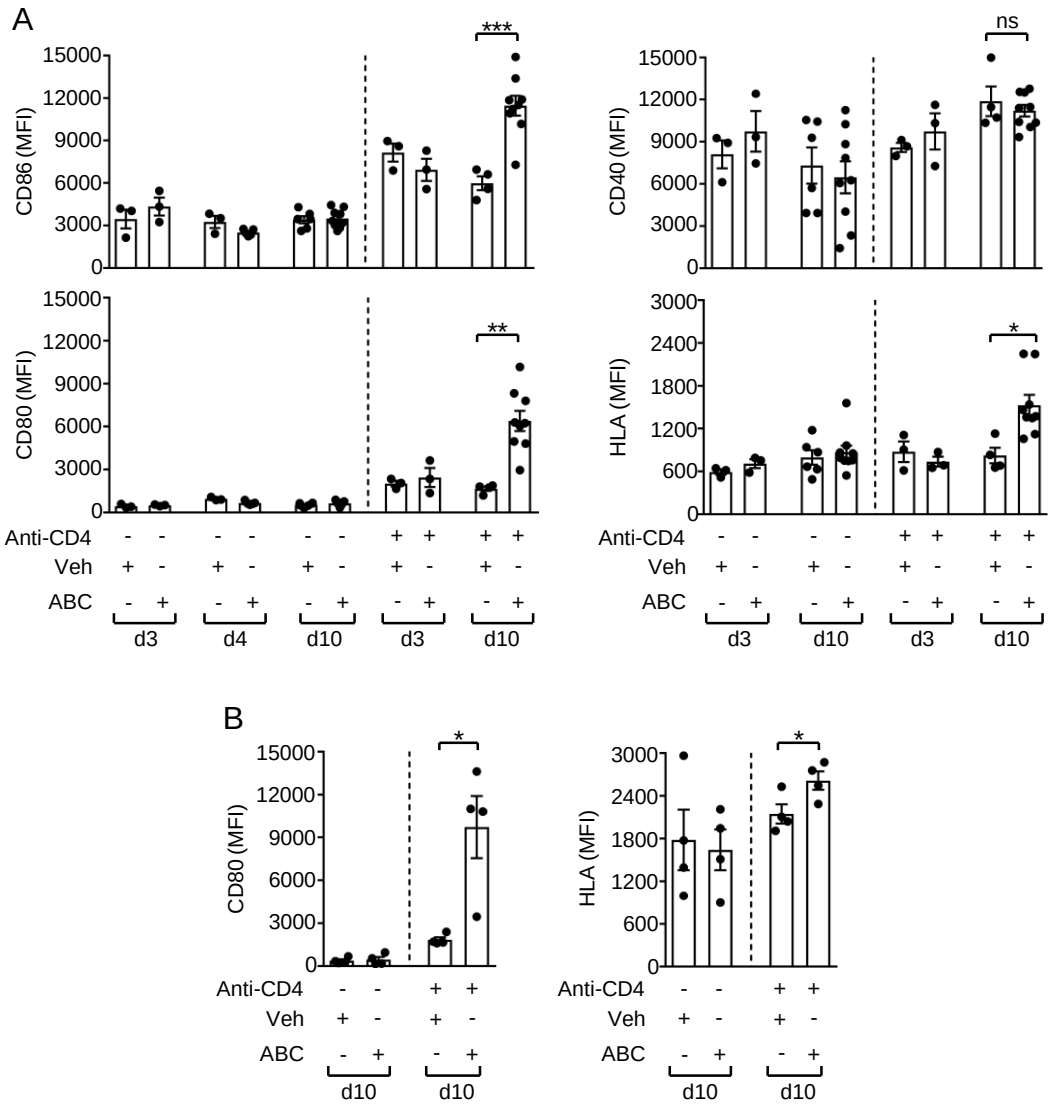
Supplemental Figure 5. Effect of PD-1 blockade on CD8⁺ T cells from ABC-treated Tg mice

Tg mice were treated systemically (i.p. injection) and topically (ear painting) with Veh or ABC, in the absence or presence of a PD-1-neutralizing mAb (Anti-PD-1) as detailed in the Methods section. PD-1 blockade failed to increase the accumulation of $CD8^+$ T cells expressing KLRG1 and CLA significantly in LN of ABC-treated Tg mice. Percentages of $KLRG1^+$ and CLA^+ cells within $CD8^+$ T lymphocytes were measured by flow cytometry at day 10 of drug administration. Data are mean ± SEM. Dots indicate values from individual mice (*n* = 4-5 per group).



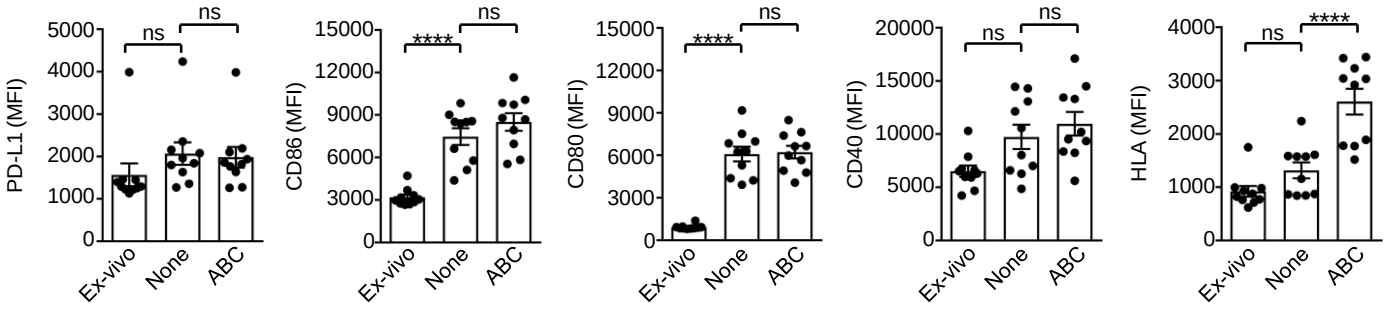
Supplemental Figure 6. Transcriptional traits of anergy, effector, and exhaustion in sorted CD8⁺ T cells from treated Tg mice

CD8⁺ T cells for gene expression analysis were sorted from LN of treated Tg mice as indicated in Figure 3. GSEA was performed using gene sets for T cell anergy, effector, and exhaustion listed in the Methods section and exported from the MSigDB (Broad Institute). Plots show the enriched profile of the indicated gene sets in CD8⁺ T cells sorted from treated Tg mice at the indicated times. Genes within the leading-edge subset of each gene set are listed. A nominal *P* value < 0.05 was used to assess the significance of the enrichment score.



Supplemental Figure 7. Expression of HLA-B*57:01 and costimulatory molecules in DCs of treated Tg mice

HLA-B*57:01-Tg mice were treated as specified in Figure 2. Expression of CD86, CD80, CD40, and HLA-B*57:01 on total CD11c$^+$MHC-II$^+$ DCs (**A**), as well as of CD80 and HLA-B*57:01 on the subpopulation XCR1$^+$ (**B**) from the LN of treated Tg mice. DCs were analyzed by flow cytometry at the indicated times of in vivo treatment. Bars represent mean ± SEM. Dots indicate values from individual mice (n = 3-9 for total CD11c$^+$MHC-II$^+$ DCs and n = 4 for the subpopulation XCR1$^+$, per group and time point). *P < 0.05, **P < 0.005, ***P < 0.0005, ns = not statistically significant using unpaired, two-tailed Student's t test.



Supplemental Figure 8. Ex-vivo and in vitro expression of HLA-B*57:01, coinhibitory, and costimulatory molecules in DCs of Tg mice

Expression of PD-L1, costimulatory molecules, and HLA-B*57:01 on total $CD11c^{+}MHC\text{-}II^{+}$ DCs in cultures of LN cells from drug-naive Tg mice. LN cells were analyzed before culture (ex-vivo) and after overnight culture without (None) or with 10 µg/mL ABC (ABC). Bars represent mean ± SEM. Dots indicate values from LN cell cultures of individual mice (n = 10 per group). ****P < 0.0001, ns = not statistically significant using one-way ANOVA with Tukey's multiple comparisons correction.

Supplemental Table 1

ABC				
Symbol	Gene reference ID	Expr Fold Change		
		Day 3	Day 4	Day 10
Bcl2	NM_009741		2.31	
Card9	NM_001037747		1.51	
Ccl25	NM_009138		1.95	
Ccr5	NM_009917		1.69	
Cd22	NM_001043317		1.88	
Cd274	NM_021893		1.82	
Cd40lg	NM_011616		1.65	
Cd53	NM_007651		1.50	
Cfp	NM_008823		2.24	
Cxcr4	NM_009911			3.27
Dgkz	NM_001166597		1.79	
Dpp4	NM_001159543		1.55	
Egr3	NM_018781		1.58	
Ikzf1	NM_001025597		1.57	
Ikzf2	NM_011770		2.30	
Ikzf4	NM_011772		1.66	
Il11ra1	NM_010549		3.13	
Il21r	NM_021887		1.95	
Il6st	NM_010560		1.50	
Irak2	NM_001113553		1.67	
Itga5	NM_010577		1.91	
Jak3	NM_010589		1.69	
Lag3	NM_008479		3.34	
Lcp2	NM_010696		1.91	
Lef1	NM_010703		1.79	
Litaf	NM_019980		1.54	
Lta	NM_010735		1.51	
Map4k2	NM_009006		1.58	
Mapk11	NM_011161		2.27	
Mbp	NM_010777		1.57	
Mx1	NM_010846		4.12	
Nfatc1	NM_016791		1.83	
Nfatc2	NM_001037177		2.13	
Nfkbiz	NM_030612		2.44	
Nos2	NM_010927			1.59
Pdcd1	NM_008798	2.53	2.65	
Plaur	NM_011113		1.89	1.60
Prkcd	NM_011103		1.52	
Runx3	NM_019732		2.40	
Selplg	NM_009151		2.30	
Ski	NM_011385		1.60	
Socs3	NM_007707		2.50	
Stat2	NM_019963		1.55	
Tgfbr2	NM_009371		1.61	
Tirap	NM_001177847		1.68	
Tnf	NM_013693		2.20	
Tnfrsf11a	NM_009399		1.65	
Tnfrsf1b	NM_011610		1.55	
Tnfsf8	NM_009403		1.64	
Traf4	NM_009423		1.71	
Trp53	NM_011640		1.56	
Zap70	NM_009539		1.75	
Arhgdib	NM_007486		-1.56	
C1qbp	NM_007573		-1.98	
Ccr7	NM_007719		-1.62	
Cd164	NM_016898		-1.82	
Cd3d	NM_013487		-1.51	
Ctss	NM_021281		-1.61	
Cxcr6	NM_030712		-1.99	
H2-DMa	NM_010386		-1.79	
Oaz1	NM_008753		-2.15	
Ppia	NM_008907		-1.52	
Psmb5	NM_011186		-1.62	
Psmb7	NM_011187		-1.80	
Rpl19	NM_009078		-1.80	
Runx1	NM_001111021		-1.66	
Sdha	NM_023281		-1.54	
Sh2d1a	NM_011364		-1.77	
Tcf7	NM_009331		-1.50	
Ube2l3	NM_009456		-1.65	

Supplemental Table 2

Anti-CD4 + ABC			
Symbol	Gene reference ID	Expr Fold Change	
		Day 3	Day 10
Ahr	NM_013464		2.43
Bst2	NM_198095	1.52	
Casp1	NM_009807		2.92
Ccl3	NM_011337		3.34
Ccl4	NM_013652		2.39
Ccl5	NM_013653	1.70	
Ccr2	NM_009915		5.75
Ccr5	NM_009917		2.38
Ccr6	NM_001190333		12.85
Cd160	NM_001163496	1.63	
Cd22	NM_001043317	2.00	3.59
Cd274	NM_021893	1.80	
Cd40lg	NM_011616		2.58
Cd44	NM_009851		2.02
Cd80	NM_009855		5.87
Cfp	NM_008823		1.59
Csf1	NM_001113530		5.49
Ctla4	NM_009843		5.33
Cxcl10	NM_021274		1.99
Cxcr3	NM_009910		1.91
Ebi3	NM_015766		4.27
Entpd1	NM_009848		2.64
Fasl	NM_010177		1.57
Foxp3	NM_054039		7.48
Gzma	NM_010370		7.44
Gzmb	NM_013542		21.33
Havcr2	NM_134250		13.73
Icos	NM_017480		2.06
Ifih1	NM_027835	1.99	
Ifitm1	NM_001112715		7.44
Ikzf2	NM_011770	2.41	3.31
Ikzf4	NM_011772		2.01
Il10ra	NM_008348		1.93
Il12rb1	NM_008353		2.10
Il12rb2	NM_008354		5.33
Il18r1	NM_001161842		1.60
Il18rap	NM_010553	1.70	2.02
Il1r2	NM_010555		10.44
Il21	NM_021782		2.87
Il2ra	NM_008367	2.83	8.18
Il2rb	NM_008368		1.55
Irf4	NM_013674		1.92
Irf7	NM_016850	2.10	
Irf8	NM_008320		1.81
Irgm1	NM_008326	1.50	
Itga1	NM_001033228		3.04
Itgax	NM_021334		1.94
Itgb1	NM_010578		2.53
Klra7	NM_001110323	1.82	
Klrc1	NM_001136068	2.51	5.33
Klrk1	NM_001083322		2.47
Lag3	NM_008479	4.45	11.03
Lilrb4	NM_013532		4.31
Mx1	NM_010846	3.65	
Pdcd1	NM_008798	3.44	8.37
Pdcd1lg2	NM_021396		1.75
Pdgfb	NM_011057	1.75	1.83
Phlpp1	NM_133821		1.66
Rorc	NM_011281		3.39
Slamf7	NM_144539	1.83	1.94
Stat2	NM_019963	2.01	
Tbx21	NM_019507		1.90
Tigit	NM_001146325		7.78
Tnfrsf4	NM_011659		2.83
Tnfsf11	NM_011613	2.47	5.65
Xcl1	NM_008510	1.62	
Zbtb7b	NM_009565		3.42
C1qbp	NM_007573		-1.55
Ccr9	NM_009913		-1.50
Itga6	NM_008397		-1.69

Supplemental Table 3

Anti-CD4 + Veh			
Symbol	Gene reference ID	Expr Fold Change	
		Day 3	Day 10
Bcl2	NM_009741	2.03	
Bcl3	NM_033601		1.83
Casp1	NM_009807		2.03
Ccr4	NM_009916		2.81
Cd274	NM_021893	2.51	1.88
Cd53	NM_007651	1.79	
Cfp	NM_008823	1.95	1.65
Foxp3	NM_054039		4.26
Ikzf2	NM_011770	1.75	
Ikzf4	NM_011772		1.64
Il12rb1	NM_008353	2.04	
Il15ra	NM_008358	1.83	
Il2	NM_008366		2.56
Il21r	NM_021887	2.14	1.73
Il2ra	NM_008367		4.94
Irf8	NM_008320		1.96
Irgm1	NM_008326	1.71	1.51
Lag3	NM_008479		2.93
Lef1	NM_010703	1.59	
Mx1	NM_010846	4.42	
Nfkbiz	NM_030612	1.65	
Notch1	NM_008714	1.61	
Pdcd1	NM_008798		3.29
Runx3	NM_019732	1.92	
Socs3	NM_007707		1.77
Stat1	NM_009283	1.95	
Stat2	NM_019963		1.74
Tnfrsf11a	NM_009399	1.57	1.62
Tnfrsf4	NM_011659	1.55	
Tnfsf11	NM_011613		3.01
Trp53	NM_011640	1.52	
Ccr7	NM_007719	-1.61	
H2-Aa	NM_010378	-1.90	
Id2	NM_010496	-1.50	
Itga6	NM_008397	-1.53	
Oaz1	NM_008753	-1.96	
Psmb7	NM_011187	-1.66	
Rpl19	NM_009078	-1.62	

List of upregulated (red, fold change ≥ 1.5) and downregulated (blue, fold change ≤ -1.5) genes with normalized RNA counts above the geometric mean + 3 SD of negative controls. Genes regulated in $CD8^+$ T cells from LN of ABC (Supplemental Table 1), anti-CD4 + ABC (Supplemental Table 2), and anti-CD4 + Veh (Supplemental Table 3) Tg mice as compared to the Veh group. Days indicate the length of treatment.

Supplemental Table 4

Symbol	Gene reference ID
Cblb	NM_001033238.1
Cd38	NM_007646.5
Cdk2	NM_183417.3
Cdk4	NM_009870.3
Cma1	NM_010780.3
Dgka	NM_016811.2
Dgkz	NM_001166597.1
Egr2	NM_010118.3
Egr3	NM_018781.3
Fos	NM_010234.2
Foxo1	NM_019739.3
Foxp1	NM_053202.2
Foxp2	NM_053242.4
Havcr2	NM_134250.2
Hdac9	NM_001271386.1
Id2	NM_010496.3
Ing4	NM_133345.2
Itch	NM_008395.3
Itga1	NM_001033228.3
Jun	NM_010591.2
Klrg1	NM_016970.1
Lag3	NM_008479.2
Lat	NM_010689.3
Lep	NM_008493.3
Lgals3	NM_001145953.1
Mef2a	NM_001033713.2
Nhlh2	NM_178777.3
Nr4a1	NM_010444.2
Pbx3	NM_016768.2
Rnf128	NM_023270.5

Genes and reference ID in the nCounter Panel-Plus and CodeSet-Plus (NanoString)