Supplemental Methods

**Mice.** B6 strain (H-2K\(^b\), CD45.2, CD90.2) vasoactive intestinal peptide/peptide histidine isoleucine (VIP/PHI)-knockout (KO) mice (VIP-KO) were bred from heterozygous (+/-) mice and VIP-KO (-/-) and WT (+/+) progeny typed by PCR as previously described\(^1\). WT syngeneic littermates were used for controls in transplant experiments. B10BR (H-2K\(^k\), CD45.2, CD90.2) and CB6 F1 mice (H-2K\(^b/d\), CD45.2, CD90.2) were purchased from JAX Lab. B6 mice (H-2K\(^b\), CD45.1\(^+\)/CD45.2\(^+\), CD90.2), and all of the other mice were 8-10 weeks old and bred at the Emory University Animal Care Facility (Atlanta, GA). Procedures conformed to the *Guide for the Care and Use of Laboratory Animals*, and were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

**Analysis of Blood Samples.** Blood samples were obtained from submandibular vein at various times after Lm-MCMV vaccination or mCMV infection. Leukocytes, red blood cells and platelets were counted using a Beckman Coulter automated counter. Blood samples were depleted of red blood cells by ammonium chloride lysis and washed twice. Donor T-cell subsets were enumerated using H-2K\(^b\) (positive population in B6→B10BR) or H-2K\(^d\) (negative population in B6→F1) FITC, CD3 PE/PE-Cy7/FITC, CD4 PE-Alexa610/PE-Alexa700, CD8 PE-Cy7/Per-CP, CD62L FITC/APC, CD25 APC-Cy7, CD44 PE-Cy5, B220 APC, and NK1.1 PE (Pharmingen). APC labeled mCMV M45 aa-985~993-peptide-HGIRNASFI-H-2D\(^b\) tetramer was obtained from the Emory Tetramer Core Facility. All samples were analyzed on a FACS Canto (Beckon Dickinson, San Jose, CA) and list mode files were analyzed using FlowJo software (Tree Star, Inc. 2007). Samples for flow cytometric analysis of mCMV-M45 epitope peptide-MHC-I tetramer\(^+\) CD8\(^+\) T-cells (tetramer\(^+\) CD8 T-cells) were gated for lymphocytes in the area of FSC and SSC, using a gate setting on CD8\(^+\) T-cells for tetramer\(^+\) T-cells such that 0.01% of control (non-immune) CD8\(^+\) T-cells were positive\(^2,3\).

**In vivo Killing Assay.** Naive splenocytes were harvested from CD45.1\(^+\)/CD45.2\(^+\) heterozygous C57BL/6 mice and pulsed with 3 µM mCMV M45 aa-985~993- HGIRNASFI peptide in RPMI 1640 containing 3% FBS for 90 min at 37°C, and washed three times with ice-cold media. MCMV peptide-
pulsed target splenocytes and non-pulsed splenocytes from CD45.1+ B6 congenic mice were mixed together in equal parts. 40 × 10⁶ total target cells per mouse were injected i.v. into CD45.2+ VIP-KO or WT C57BL/6 mice that had been infected 9 days earlier with low-dose (LD10) mCMV, or injected into non-infected WT control mice. Sixteen hours following injection of target cells, recipients were sacrificed, splenocytes harvested, and the numbers of mCMV peptide-pulsed CD45.1+/CD45.2+ and non-pulsed CD45.1+ target cells quantified by FACS analysis. Immune mediated killing of mCMV peptide pulsed targets was calculated by dividing the percentage of peptide-pulsed or non-pulsed targets recovered from the spleen of mCMV-immune mice with the mean percentage of the corresponding population of peptide-pulsed or non-pulsed targets from non-immune mice (ratio of immune killing). The specific anti-viral in vivo lytic activity for individual mice were calculated by the formula: (1- (ratio of immune killing mCMV-peptide pulsed-target cells/ ratio of immune killing non-pulsed target cells)) × 100.

**Determination Of Liver Viral Load.** Viral loads were analyzed as previously described. Briefly, livers were collected from CMV-infected recipients, homogenized, and centrifuged (10,000 rpm, 10 min, 4 °C). Serially diluted supernatants were added to 3T3 confluent monolayers in 24-well tissue culture plates and incubated for 90 minutes at 37°C and 5% CO2, then over layered with 1 mL 2.5% methylcellulose in DMEM. After 4 days, the methylcellulose was removed and the 3T3 confluent monolayers were stained with methylene blue. MCMV plaques were directly counted under a light microscope (Nikon, Melville, New York) and the PFUs in each tissue sample were calculated.

**Statistical Analyses.** Survival differences among groups were calculated with the Kaplan-Meier log-rank test in a pair wise fashion. Differences in tetramer responses were compared by 1-way analysis of variance.
Supplemental Figure Legends.

Supplementary Figure 1. Allo-BMT recipients treated with VIP antagonist or transplanted with VIP-KO donors did not significantly increased GvHD. Allogeneic recipients (B10BR) were transplanted with grafts from either VIP-KO or WT C57BL/6 mice. Transplant recipients were treated with 7 daily s.c. injections of 10 μg of VIPhyb starting from one day before transplantation. Mice were examined daily for survival and clinical signs of GvHD as previously performed4,5. (A) Survival of B6→B10BR recipients of 5 x 10^6 TCD-BM plus various doses of splenocytes either from B6 WT donor (thin, medium, and thick solid lines with black filled circle, n=17 for all of the WT groups with 1 x 10^6, 3 x 10^6, or 8 x 10^6 splenocytes, respectively), or from B6 VIP-KO donors with 1 x 10^6 (thin dashed line, n=23), 3 x 10^6 (medium dashed line, n=17), and 8 x 10^6 (thick dashed line, n=22). Gray dashed line with gray circle represents recipients of 1 x 10^6 splenocytes from WT donors treated with VIPhyb (n=10), identified with an arrowhead. Data are pooled from 2-5 replicate experiments with 4-5 mice per group. (B) GvHD clinical scores for the B6→B10BR groups transplanted in A5-7.

Supplementary Figure 2. Specific anti-viral CD8 T-cells from recipients of VIP-KO allo-grafts and VIPhyb-treated recipients of WT allo-grafts expressed lower levels of PD-1. CB6 F1 mice were transplanted with grafts from either VIP-KO or WT C57BL/6 mice. Transplant recipients were treated with 7 daily s.c. injections of 10 μg of VIPhyb or PBS (100μL, per mouse). Allo-BMT recipients were infected with 5 x 10^3 PFU mCMV on day 8 post-transplant. Peripheral blood was collected and CD3, CD4, CD8, CD69, H-2K^d, PD-1, and mCMV-peptide MHC-tetramer^+ T-cells analyzed by flow cytometry. The gate for PD-1^+ population was set with PD-1^+ < 0.05 % in an isotype-matching control. Data are from 3 independent experiments with a total of n=12 mice at each time point for every group. A. PD-1 expression on the tetramer^+ CD8^+ T-cells. B. CD69 expression on the tetramer^+ CD8^+ T-cells. C-E. The dot plots of CD69 and PD-1 expression gated on H-2Kd^+, CD3^+CD8^+tetramer^+ T-cells combined from list-mode FACS files obtained from 4 individual mice in the same treatment of group
Supplementary Figure 3. Allo-BMT recipients treated with VIP antagonist or transplanted with VIP-KO donors had increased cytolytic activity following mCMV infection. CB6 F1 mice were transplanted with grafts from either VIP-KO or WT C57BL/6 mice. Transplant recipients were treated with 7 daily s.c. injections of 10 μg of VIPhyb or PBS. Allo-BMT recipients were infected with 1 x 10^4 PFU mCMV on day 35 post-transplant. In vivo specific cellular lytic activity against mCMV-peptide pulsed (both CD45.1^+ and CD45.2^+) or control-peptide pulsed (CD45-1^+) targets were tested by flow cytometry^3,8. Data are means ± SEM from two replicate experiments with 10 mice per time-point per group. * p<0.05, signifies significant differences between recipient of VIP-KO or VIPhyb-treated mice vs. PBS-treated recipients of WT.

Supplemental References:


**Tetramer^+ CD8 T-cells**

**A**

- % PD-1 positive cells over days post-transplantation with mCMV, VIPhyb or PBS.
- **B**
  - % CD69 positive cells with mCMV (1 x 10^3 pfu, ip), VIPhyb or PBS.

**C, D, E**

- ViPhyb, VIP-KO, and WT panels showing PD-1 and CD69 expression with corresponding counts.

Suppl Figure 2
Supp Figure 3