SUPPLEMENTARY MATERIAL AND METHODS

Mouse strains and tumor cell lines
C57BL/6 (B6, H-2Kb), B10.BR (H-2Kk), BALB/c(H-2Kd), DBA/2(H-2Kd) and luciferase expressing B6 background mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B6 strain (H-2Kb, CD45.2, CD90.2) vasoactive intestinal peptide/peptide histidine isoleucine knockout (VIP-KO) mice were obtained from Dr. Waschek at the University of California (Los Angeles, CA) and bred at Emory University animal care facility (Atlanta, GA). Transgenic mice expressing enhanced green fluorescence protein (EGFP) from on a C57BL/6 background (strain designation: C57BL/6-Tg(Act-EGFP)C14-Y01-FM131 Osb) were developed by Dr. Masaru Okabe (Osaka University, Osaka, Japan) and were bred and maintained at the Emory University Animal Care Facility (Atlanta, GA). Male mice were used at 7-8 weeks of age and were housed in a pathogen-free facility in micro-isolator cages in Emory University. LBRM 33-5A4, a B10.BR T cell lymphoblastic lymphoma cell line, was obtained from ATCC and was transfected to express luciferase as we previously described. The luciferase-expressing P815-TGL (H-2d, CD45.2) DBA/2 mastocytoma cell line was obtained from Dr. Marcel van den Brink at Sloan Kettering. All procedures were approved by Emory University’s Institutional Animal Care and Use Committee (IACUC), following the ‘Guide for the Care and Use of Laboratory Animals.

Genotyping
VIP-KO homozygous mice were confirmed with two methods, both by Transnetyx and by internal QC procedures. Probes and primers was designed by Transnetyx (Vip-1 wild-type: Forward TTTGCTCCCTTGCTTTAGAAATGA, Reverse TCTGCTGTAATCGCTGGTGAAAA, Vip-KO: Forward CCCGGTAGAATTGATATCAAGCT, Reverse CCATCATGCTGCAGCAGT). In addition, DNA samples (DNeasy Blood & Tissue Kits, QIAGEN) were assayed by triple primer PCR with the use of the following primers: omVIPF4+ (V1) TTTCAAGGTTGAGGCTAGAGAT (0.5 μM), omVIPF5- (V2) TTACCTGATTCGTTTGCCAATGAGTGAC (1.0 μM), and opNEO10- (N1) GCCCGGAGATGAGGAAGAGGAGAACAG (0.5 μM). The PCR reaction was performed with Promega GoTaq® Master Mixes (Cat# M7122). A touchdown PCR procedure was used. After
samples were heated to 94°C for 5 min, they were subject to PCR as follows: denaturation at 94°C for 30s, annealing for 45s (starting at 65°C and decreasing by 0.5 degree each cycle until 60°C, then maintaining at 60°C for another 28 cycles), and extending at 72°C for 2 min. The reaction was finished with an extra 10-min elongation at 72°C and a 20-min period at 4°C. When using double primer method, the mutant allele gives 2 bands. The 2.1-kb band is generated by primers V1 and V2. The 708-bp band is generated by primers N1 and V2. The wild-type allele gives a product of 318 bp, generated by V1 and V2. With the triple primer method, the 2.1-kb band is sometimes faint or not seen, because the 2.1-kb and 318-bp bands both require primer V2, and the 318-bp band amplifies more efficiently in heterozygous. Also, the existence of other primers may disrupt larger band extension. Sanger sequencing and alignment (NCBI BLAST, Clustal Omega) of the large 2.1-kb band was done to confirm the neomycin cassette insertion site on VIP exon 4.

**RT-PCR**

Total RNA was isolated with Trizol reagent (Thermo Fisher Scientific) and the first-strand cDNA was prepared using an AMV RNA PCR kit (TaKaRa) from 1 mg total RNA. PCR amplification for VIP mRNA detection was carried out with an initial denaturing step at 95°C for 5 min, then 35 cycles of PCR (95°C for 30 s, 58°C for 45 s, and 72°C for 45 s) and a further extension at 72°C for 10 min. The PCR products (Promega) and 50bp ladder (Thermo Fisher) were loaded on 4% of agarose gel further underwent electrophoresis in TBE buffer. UV system was applied for imaging. Primers:

**GAPDH**

Forward AGGAGAGTGTTTCTCGTCCC
Reverse CAGATCCACGACGGACACAT

**VIP-CDS**

Forward AGGATGCCGTTTGAAGGAGC
Reverse GATGCTGCTGCTGATTCGTT

**Cell activation**

FACS-isolated pDCs were cultured in complete media (RPMI medium 1640 containing 10% (vol/vol) FBS, 2 mM L-glutamine, 100 units/mL penicillin and streptomycin, 1 mM sodium pyruvate, and 10 mM Hepes), activated using PMA/ionomycin (PMA: 10ng/ml, Ionomycin: 0.5ug/ml) for 24 hours before immunofluorescence staining.

**Cell culture**

FACS-sorted pDCs were preincubated with 100 ng/ml R848 (Sigma Aldrich) and 5uM CpG (ODN 1585 InvivoGen) in complete media containing 50 ng/ml FLT3-L (Thermo Fisher) for 24
hours. Activated pDCs were washed 3 times and then cocultured with syngeneic CFSE-labeled T cells for 72 hours, (T cell: 10^5 cells/well; pDCs-T ratio=1:10) in 96-well, flat-bottom tissue culture plates that had been previously incubated with 10 ug/ml anti-CD3 antibodies at 37°C for 2 hours. T cells were cultured in the presence of 30U/ml mouse recombinant IL-2(R&D System). Proliferation index was calculated as the total number of divisions divided by the number of cells that went into division and represents the fraction of cells that underwent at least one cell division. For analysis of pDC effects on T-cell intracellular cytokine production, pDCs were pre-activated with R848 and CpG in 48-well plates, then cocultured with syngeneic T-cells in the presence of 50 ng/ml FLT3-L for 72 hours. Leukocyte activation cocktail was added for the final 5 hours of culture, and cells stained as below.

**Flow cytometry analysis**

Splenocytes and bone marrow cells were harvested from recipients and were counted with the Beckman Coulter device (California U.S.A). Cells were stained with LIVE/DEAD Fixable Aqua (Thermo Fisher Scientific) according to manufacturer’s instructions. Donor cells were stained with antibodies listed in Supplementary Table 1. For intracellular cytokine expression staining, splenocytes or T cells were incubated with leukocyte activation cocktail (BD) for 5 hours, then stained with antibodies listed in Supplementary Table 1. FACS files were acquired with a FACS Aria cytometer (Beckon Dickinson, San Jose, CA) or an Aurora cytometer (Cytek Biosciences, Inc, Fremont, CA) and analyzed using FlowJo software (Tree Star, Inc).

**Histology analysis**

Representative samples of small intestine and large intestine were obtained from transplanted recipients and fixed in 10% neutral-buffered formalin. Samples were then embedded in paraffin, sectioned at 4 μm thickness and stained using hematoxylin and eosin (HE) or Periodic acid–Schiff (PAS). Histological sections were evaluated using an Olympus BX 41 light microscope (Olympus USA, Center Valley, PA) and histological images were prepared using an Olympus 25 digital camera and associated Olympus cellSens Standard software. Intestinal histopathological evaluation and all measurements were performed by a board-certified veterinary pathologist with experience in mouse pathology (T.N.) who was masked as to treatment group. The histological GvHD scores were evaluated based on the criteria listed in Supplementary Table 2 and graded into 10-points criteria. Apoptotic and non-apoptotic intestinal crypts, goblet cells were counted,
and crypt length was in 10× magnification images using tools in the cellSens Standard imaging software.

**Bioluminescent imaging in vivo and in vitro**

In the GvHD experiment, donor T cells were purified from Luciferase-expressing B6 mice. Transplanted recipient mice were anesthetized and injected intraperitoneally with luciferin (10 ug/g body weight). Five minutes after injection, bioluminescent imaging was performed for 180 seconds using an IVIS100 charge-coupled device imaging system (Xenogen). Thymus, lung, intestine, spleen, paw (as a representation of skin), and liver were quickly removed at necropsy for bioluminescent imaging (BLI) by placing dissected organs in a plastic petri dish with small amount of warmed PBS with 2% v/v FBS to keep them moist. The imaging stage was unheated. Imaging data were analyzed with Living Image Version 3.2 software (Xenogen).

In the GvT experiments, DBA/2J (Jackson) mice underwent 9 Gy lethal irradiation on day -2 followed by subcutaneous inoculation with luciferase positive mouse P815-TGL mastocytoma cells on day -1. In the first experiment, each mouse received $5 \times 10^5$ tumor cells suspended in 100ul of PBS followed by transplantation on day 0 with $5 \times 10^3$ FACS-sorted HSCs, $3 \times 10^5$ MACS-enriched splenic T cells with $5 \times 10^4$ FACS-purified pDCs from wild-type or VIP-KO C57BL/6J mice. In the second experiment, each mouse received $1 \times 10^5$ tumor cells in the same volume of PBS followed by same transplant graft except a lower number of T cells ($1 \times 10^5$). Transplanted recipient mice were imaged once a week. Mice were anesthetized and injected intraperitoneally with 200 ul of luciferin solution (15mg/ml). Eight minutes after injection, bioluminescent imaging was performed using an IVIS100 charge-coupled device imaging system (Xenogen). Imaging data were analyzed with Living Image Version 3.2 software (Xenogen).

**Serum cytokine analysis**

Blood samples were collected by submandibular vein bleeding. Serum was obtained and stored at -80°C until analysis. Cytokine levels were measured in 50 ul of serum by Multiplex cytometric bead array (Multiplex) on a Luminex 38 Analyzer or using V-PLEX Proinflammation Panel 1 (mouse) Kit (Catalog number: K15048D-1, Meso Scale Discovery, Rockville, MD) according to the manufacturer’s protocol.
**Cells and tissue and immunofluorescence staining**

Cells were sorted and prepared (activated or not) as per the protocol above. Cells were suspended in 0.5% BSA DPBS, loaded into a cytology funnel (Thermo Fisher Scientific), and centrifuged at 1000 rpm for 3 minutes on a Shandon Cytospin 3 cytocentrifuge (Thermo Fisher Scientific); For tissue preparation, mice intestines were removed and immediately embedded in Optimal cutting temperature compound (OCT compound) and processed for frozen sections. Tissue sections and cytospins were fixed with acetone at -20 °C for 10 minutes. After three washes with DPBS, the samples were blocked by 10% Fetal Bovine Serum containing 0.1% Triton-X100 (Sigma) for 1 hour. For direct fluorescent GFP signal detection, fixation and permeabilization with Triton-X100 were not performed. Samples were then incubated with monoclonal anti-VIP (Origene), and anti-PDCA-1, anti-CD45.1 and anti-GFP primary antibodies (Thermo Fisher Scientific) diluted in 0.5% FBS PBS overnight at 4°C. Dilution ratios were 1:150, 1:500, 1:200, and 1:100, respectively. A variety of different anti-pre-pro-VIP Abs were used to detect VIP expression. For pDC sourced from human PBMC, the anti-BDCA-2 antibody is a FITC-conjugated anti-human CD303 (BDCA-2) antibody (MACS). After rinsing with DPBS, anti-mouse IgG-AF488 (1:500), anti-rat IgG-AF568(1:500) (Thermo Fisher), anti-mouse IgG-AF555 (1:500)(Abcam), and anti-rat IgG-AF647(1:500)(BD Biosciences) antibodies were added to the slides, which were then cultured at room temperature for 1 hour and washed 3 times with DPBS. DAPI or Hoechst was added to stain the nucleus before washing with DPBS, and the slides were treated with mounting media. (Thermo Fisher Scientific, ProLong Antifade Mountants). After sealing the slides, the samples were imaged and photographed with a confocal microscope (Olympus FV1000, Japan). Images were analyzed using ImageJ (version:2.0.0-rc-69/1.52p).

**RNA sequencing analysis**

T cells were isolated from the spleen cells of recipient mice using FACS sorter (BD AriaII). Immediately afterwards, they were frozen and stored at -80 °C. Cellular RNA was stabilized using 1/3 RLT buffer (Qiagen). After extraction of RNA using Qiagen RNeasy Micro kit, Nanodrop and Agilent 2100 were used to quantify, and quality check the RNA. RNA analyzed using the nCounter® Myeloid Innate Immunity Panel (Nanostring Technologies, Seattle, WA). Raw NanoString data (n=24) from both batches was exported from NSolver. Data quality was assessed with the help of QC plots using R package NanoStringNorm⁵. Three potential outlier samples were excluded from the analysis. The remaining 21 samples were imported and
analyzed using R package NanoStringClustR. Data was normalized using quantile normalization after background subtraction of mean2sd, and count thresholding. Differential expression analysis was carried out using NanoStringClustR and genes were identified as significant with an FDR adjusted p-value of less than or equal to 0.05. Functional enrichment and network analysis for the principal comparison of interest was carried out using an ordered query on gProfiler.

**Statistical analyses**

Data were analyzed in GraphPad Prism 8 for Mac and Microsoft Excel for Mac. Data were presented as mean and SD. Survival curve differences between groups were analyzed with the Kaplan-Meier log-rank test in a pairwise manner. Differences in serial clinical measurements of GvHD were determined by using Two-way ANOVA test. Differences in GvHD histological scores were determined with the nonparametric two-tailed Mann-Whitney U test. The degree of T cell expansion, percentages of T cell with different immune polarizations, and frequencies of T cells expressing specific cytokines were analyzed with the nonparametric Mann-Whitney U test or two-tailed unpaired two-sample Student’s t test. The organ-specific migration of pDCs and T cells as assessed by bio-luminescent imaging was analyzed using power linear regression. Values < .05 were considered significant.
## Supplementary Table 1: Flow cytometry antibodies and the other reagents

| Target                  | Clone          | Fluorochrome(s)                  | Vendor          |
|-------------------------|----------------|----------------------------------|-----------------|
| LIVE/DEAD               | N/A            | Aqua                             | Thermo Fisher   |
| CD3                     | 145-2C11       | APC                              | Thermo Fisher   |
| CD4                     | RM4-5          | Pacific Blue                     | Thermo Fisher   |
| CD8                     | H35-17.2       | BV650                            | BD Biosciences  |
| CD25                    | PC61           | BV480                            | BD Biosciences  |
| FoxP3                   | FJK-16s        | PerCP-Cyanine5.5                 | Thermo Fisher   |
| IFN-γ                   | XMG1.2         | PE                               | BD Biosciences  |
| TNF                     | MP6-XT22       | BV421                            | BD Biosciences  |
| IL-4                    | 11B11          | PE-Cyanine7                      | BD Biosciences  |
| IL-10                   | JES5-16E3      | APC/Cyanine7                     | Biolegend       |
| IL-17                   | TC11-18H10     | Alexa Fluor® 700                 | BD Biosciences  |
| GM-CSF                  | MP1-22E9       | APC                              | Thermo Fisher   |
| ICOS                    | 7E.17G9        | PE-Cyanine5                      | Thermo Fisher   |
| RORγt                   | Q31-378        | BV421                            | BD Biosciences  |
| T-bet                   | eBio4B10 (4B10)| PE                               | Thermo Fisher   |
| CXCR5                   | L138D7         | APC/Cyanine7                     | Biolegend       |
| CCR3                    | 83103          | BV480                            | BD Biosciences  |
| CCR5                    | HM-CCR5 (7A4)  | APC                              | Thermo Fisher   |
| CD146                   | P1H12          | PE-Cyanine7                      | Thermo Fisher   |
| Lineage                 | N/A            | APC                              | BD Biosciences  |
| CD11c                   | HL3            | FITC                             | BD Biosciences  |
| B220                    | RA3-6B2        | PerCP-Cyanine5.5                 | Biolegend       |
| CD317                   | eBio927        | eFluor 450                       | Thermo Fisher   |
| CD117                   | ACK2           | APC                              | Thermo Fisher   |
| Ly-6A/E                 | D7             | PE-Cyanine7                      | BD Biosciences  |
| CD80                    | 16-10A1        | BV421                            | Biolegend       |
| CD86                    | GL1            | PE-Cyanine7                      | Thermo Fisher   |
| ICOSL                   | HK5.3          | PE                               | Thermo Fisher   |
| H2Kd                    | SF1-1.1.1      | APC                              | Thermo Fisher   |
| H2Kk                    | SF1-1.1        | FITC                             | Biolegend       |
| H2Kb                    | 28-8-6         | PerCP-Cyanine5.5                 | Biolegend       |
| PD-1                    | J43            | APC-R700                         | BD Biosciences  |
| Fc Block                | 2.4G2          | N/A                              | BD Biosciences  |
| VIP                     | OT11C3         | N/A                              | Origene         |
| CPG ODN 1585            | N/A            | N/A                              | Invivogen       |
| Resiquimod (R848)       | N/A            | N/A                              | Sigma Aldrich   |
| FLT3-Ligand             | N/A            | N/A                              | Thermo Fisher   |
| Leukocyte Activation    | N/A            | N/A                              | BD Biosciences  |
| Cocktail with GolgiPlug | N/A            | N/A                              | BD Biosciences  |
Supplementary Table 2: Intestinal Histological GvHD Evaluation Criteria

| Section                        | Criteria                                                                 | Point |
|--------------------------------|--------------------------------------------------------------------------|-------|
| Extent of inflammation         | Superficial inflammation (only the mucosa and submucosa are affected)    | 1     |
|                                | Transmural inflammation (the inflammation is present in more layers than the mucosa and submucosa) | 2     |
| Severity of inflammation       | Mild                                                                      | 1     |
|                                | Moderate                                                                 | 2     |
|                                | Marked                                                                   | 3     |
|                                | Severe                                                                   | 4     |
|                                | Mild                                                                      | 1     |
| Severity of the crypt epithelial necrosis \(^2\), or for the severity of the colonic gland necrosis \(^3\) | Moderate                                                                 | 2     |
|                                | Marked                                                                   | 3     |
|                                | Severe                                                                   | 4     |

\(^1\) Total histological GvHD score is the sum of the score from each section; \(^2\) for small intestine; \(^3\) for colon.
Supplementary Table 3: Serum Cytokines (pg/ml) on days 3, 8, and 15 after BMT.

| Cytokine | Day 3 post-transplant | Day 8 post-transplant |
|----------|-----------------------|-----------------------|
|          | VIP-KO pDC | WT pDC | No pDC | VIP-KO pDC | WT pDC | No pDC |
| IL10     | 1.1±0 | 1.1±0 | 1.1±0 | 28.8±23.9 | 31.1±25.9 | 34.9±28.2 |
| IL12P40  | —     | —     | —     | 13±4.5 | 21.3±7.7 | 17.8±5 |
| IL12P70  | 0.7±0.1 | 0.4±0 | 0.5±0 | 3.9±2.4 | 1.1±0.4 | 2.1±1 |
| IL13     | 0.4±0.2 | 0.3±0.3 | 0.2±0.3 | 2.3±1.7 | 0.5±0.4 | 0.5±0.4 |
| IL15/IL15R | 1.3±0.7 | 1.1±0.4 | 1.3±0.3 | 6.7±2.3 | 2.3±1.4 | 2.7±1.6 |
| IL17A    | 0.1±0.2 | 0.2±0.3 | 0.3±0.4 | 1.5±0.5 | 1.6±0.4 | 1.1±0.3 |
| IL18     | 467±64.7 | 544.9±63.5 | 460.4±28 | 585.8±152 | 375.1±68.8 | 455.4±57.7 |
| IL1A     | 27.3±15 | 24.1±10.9 | 11.1±1.5 | 27.9±7.3 | 19.5±1.6 | 21.6±4.5 |
| IL1B     | 1.1±0.3 | 1.3±0.5 | 1.1±0.1 | 1.5±0.5 | 1.6±0.4 | 1.1±0.3 |
| IL2      | 28.3±13.6 | 34.7±7 | 15.3±4.7 | 13.6±19 | 3.8±1.9 | 15.8±26.6 |
| IL22     | 60.8±31.3 | 126.8±87.6 | 82.1±37.9 | 53.2±11.7 | 26.2±9.7 | 29.1±12.6 |
| IL23     | 4.6±1.8 | 3.5±1.1 | 5.1±1 | 6.7±0.3 | 6.7±0.5 | 6.3±1.8 |
| IL27     | 12.5±2.1 | 16.6±3 | 8.1±1.5 | 50.1±79.5 | 3.1±1.5 | 9.5±10.7 |
| IL28     | 36.9±0 | 36.9±0 | 36.9±0 | 37.4±0 | 37.4±0 | 37.4±0 |
| IL3      | —     | —     | —     | 0.2±0.1 | 0±0 | 0±0 |
| IL31     | —     | —     | —     | 3.7±1.3 | 1.2±0.8 | 0.2±0.1 |
| IL4      | 2.7±0.2 | 2.9±0.2 | 2.7±0.1 | 1.9±1.5 | 1.7±0.8 | 1.4±0.9 |
| IL5      | 14.3±3 | 23.2±9.2 | 17.9±4.5 | 24.6±11.2 | 31±13.2 | 27.7±11.2 |
| IL6      | 5.2±0.8 | 6.5±1.2 | 6.9±10.4 | 543.4±266.9 | 493.5±401.8 | 450.2±393.9 |
| IL9      | 7.7±0.2 | 7.7±0 | 7.7±0.1 | 27.3±38.2 | 8.1±0 | 8.1±0.2 |
| IP10     | 31.8±2.6 | 30.5±7.9 | 33±7.6 | 148.5±27.8 | 97.6±9.2 | 127.6±29.3 |
| LIF      | 11.3±1.8 | 12±1.7 | 12.8±1 | 10.5±1.5 | 7.1±1.2 | 7.5±1 |
| LIX      | 1058±169.1 | 1097.9±126 | 1077.9±94.3 | 149.8±32.1 | 64.8±17.5 | 60.1±19.6 |
| MCSF     | 0.1±0.1 | 0.2±0.1 | 0.1±0.1 | 0.7±0.2 | 0.4±0.1 | 0.5±0.1 |
| MCP1     | 50.5±13.9 | 44.2±23.3 | 61.9±12.7 | 385±110.9 | 182.8±46.3 | 111.6±4.6 |
| MCP2     | 296.4±14.6 | 295.6±18.8 | 298±9.2 | 329±36.2 | 271.5±11.7 | 284±4.3 |
| MIP1A    | 1.6±0.3 | 1.7±0.1 | 1.7±0.1 | 4.9±3.9 | 1.9±0.4 | 1.9±0.5 |
| MIP1B    | 3.4±0.7 | 3.2±0.7 | 3.7±1 | 18.6±2 | 12.5±2.4 | 7.5±0.7 |
| MIP2     | 16.4±0.9 | 17.2±0.6 | 16.8±0.1 | 71.7±55.4 | 26.8±13.4 | 17.6±5.7 |
| RANTES   | 23.2±2.6 | 26.4±3.9 | 23.7±3.8 | 64.2±31.8 | 40.8±5.5 | 36.5±2.3 |
| TGFB     | 70.2±11.7 | 94.3±19.2 | 67.9±15.8 | 9±2.2 | 5.9±1.2 | 5.1±3.7 |
| TNFA     | 3.1±0.6 | 2.8±1.6 | 3.2±0.4 | 51±24.8 | 59.1±31.9 | 61.1±46.9 |
| VEGF     | 10.3±3.8 | 8.1±0.6 | 8.2±0.4 | 9.8±3 | 5.4±1.1 | 6.9±1.8 |
| EOTAXIN  | 549.5±28.4 | 559.6±32 | 509.2±26.2 | 586.6±109.5 | 143.1±59.3 | 439.2±77.9 |
| GSCF/CSF3| 16±6.1 | 14.6±3.3 | 18.6±7.6 | 228.6±40.7 | 143.1±21.5 | 160.5±5.5 |
| GMCSF    | 1.8±0.4 | 0.4±0 | 1.3±0.9 | 5.2±1.7 | 1.7±1 | 1.4±1.2 |
| GROA     | 26.1±6.2 | 31.1±7.3 | 31.8±9.1 | 1513.9±1170.4 | 1181±1116.5 | 1247.6±1060.7 |
| Cytokine | VIP-KO pDC | WT pDC | No pDC |
|---------|-----------|--------|--------|
| IFNG    | 53.32±22.48 | 34.01±24.33 | 56.73±42.74 |
| IL-10   | 50.39±8.05  | 42.89±7.74  | 65.01±20.85  |
| IL1B    | 14.81±3.73  | 12.34±6.03  | 6.62±3.12    |
| IL2     | 3.39±1.18   | 3.27±1.42   | 4.96±0.2     |
| IL-4    | 1.46±0.68   | 0.58±0.3    | 0.39±0.35    |
| IL5     | 71.96±66.79 | 19.83±6.77  | 18.03±13.27  |
| IL6     | 97.35±30.55 | 101.33±60.57| 606.33±802.25|
| KC/GRO  | 197.42±53.32| 172.2±84.63 | 250.07±79.52 |
| TNF-alpha| 88.48±10.8  | 59.89±19.25 | 74.45±41.43  |
Supplementary Figure 1. VIP gene structure of VIP and VIP KO mice, pDC flow cytometry analysis, expression of immuno-reactive pre-pro VIP in pDC from wild-type and VIP KO marrow and spleen, and purity of sorted pDC. (A) Genomic structure of the wild-type PHI-VIP gene and pre-pro-VIP polypeptide with proteolytic processing sites. (B) Genomic structure of the VIP KO mice showing insertion in reverse orientation of a neomycin gene cassette into exon 4 of the VIP gene. The nucleotide sequence encoding the PHI and VIP peptides are shown bolded in salmon color, with the Sph1 restriction site into which the neomycin cassette is inserted bolded in burnt orange color. (C-F) Flow cytometry gating strategy for pDC and staining for expression of pre-pro VIP in pDC from marrow of wild-type and VIP KO mice using a directly conjugated Origene anti-pre-pro-VIP monoclonal antibody. Gating of total nucleated cells was based upon forward and side-scatter and removing doublets. pDCs are defined as B220+CD11c+ lineage-PDCA-1+ cells. The threshold for VIP staining is based upon an isotype control antibody and VIP KO marrow cells. (G) Flow cytometric re-analysis of FACS-purified pDC showing >90% purity of the population gated on single-cell leukocytes. (H) Expression patterns for B220, CD11c, lineage and PDCA-1 on bone marrow cells are shown with B220 and Lineage marker expression based upon single live cells, CD11c expression in single live mononuclear cells, and PDCA-1 expression in single live B220+ cells.
Supplementary Figure 2. Wild type pDCs induce Treg generation in vitro while VIP knock-out pDC enhance expression of perforin and granzyme B. (A-B) The pDCs isolated from the B6 mouse and VIP-KO mouse were stimulated with R848 and CpG for 24 h, followed by co-culturing with syngeneic T cells for 72 h in the presence of FLT-3L. pDCs: T=1:10. Cytokine profiles for CD4⁺ T cells (A) and CD8⁺ T cells (B) were analyzed by flow cytometry after intracellular staining. N=8 technical replicates from 2 independent experiments for each group. (C) Perforin and granzyme B expression in CD4⁺ and CD8⁺ T cells. Statistics: nonparametric Mann-Whitney U test, Data presented as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 3. pDCs did not change gastrointestinal tract architecture and histological GvHD at day 15 after transplant. (A-B) B10.BR mice were transplanted with 5 × 10³ HSCs, 1 × 10⁶ splenic T cells and 5 × 10⁴ pDCs from C57BL/6J or VIP-KO B6 mice. Mice
were sacrificed 15 after transplantation and their small intestine, including duodenum, jejunum, and ileum were scored for histological GvHD. Every dot represents a segment of small intestine, N=4 mice in each group, with 3 technical replicates screening different gut sections per mouse. (C-D) Sections of colon scored for histological GvHD. N=4 in each group. (E-H) Colons were collected at necropsy 7 days after B10.BR mice were transplanted with $5 \times 10^3$ HSCs, $2 \times 10^6$ splenic T cells and $5 \times 10^4$ pDCs from wild-type or VIP-KO C57BL/6J donors. Formalin-fixed paraffin-embedded sections were stained with H & E and PAS and evaluated by a pathologist masked to treatment group. Crypt lengths were measured from the bottom of the crypt to the crypt-villi junction with 6 or 7 biological replicates for each group. (G-H) Crypts were counted with 6 or 7 biological replicates in each group. Bar indicates 100 microns at 40 × magnification.
Supplementary Figure 4. VIP produced by pDCs does not abrogate the GvL/GvT activity of donor T cells. (A-B) B10.BR recipients were injected with $2 \times 10^6$ LBRM cells on day -1 prior to transplantation with $5 \times 10^3$ FACS-sorted HSCs, $1 \times 10^6$ MACS-enriched splenic T cells from C57BL/6J mice, with $5 \times 10^4$ FACS-purified pDCs from wild-type or VIP-KO mice. Survival and clinical GvHD scores of mice were monitored biweekly for the first 30 days after transplant and weekly afterward. N=10 per group. In the first GvT study, (C-E) DBA/2J recipients were injected subcutaneously with $5 \times 10^5$ P815-TGL cells a day before transplant with $5 \times 10^3$ FACS-sorted HSCs, $3 \times 10^5$ MACS-enriched splenic T cells from C57BL/6J mice, and $5 \times 10^4$ FACS-purified pDCs from either wild-type or VIP-KO mice. Mice were imaged once a week using IVIS100 and analyzed with Living Image Version 3.2 software (Xenogen). Survival was monitored every day. N=6 mice per group. In the second GvT experiment (F-H) the same experimental design was used except transplant doses of T cells and tumor cells were both reduced to $1 \times 10^5$ cells. N=5 mice per group. ANOVA was performed. *p<0.05, **p<0.005. BLI scale: Day -1 from $1 \times 10^6$ to $1 \times 10^7$; the BLI scale for remainder of the time points was from $1 \times 10^6$ to $1 \times 10^7$. 


Supplementary Figure 5. pDCs facilitate HSC engraftment. (A-B) Balb/c recipients were injected with $5 \times 10^3$ FACS sorted luciferase positive HSC, with or without $5 \times 10^4$ wild-type C57BL/6J pDCs. (A) Bioluminescence images taken day +10 and day +17 after transplant. (B) mean Log(10) values of Total Flux for HSC engraftment are shown. N=5 mice per group. Statistics: $p=0.002$ by Student t-test.
Supplementary Figure 6. No difference in survival of allo-HSCT BALB/c recipients based upon donor pDC in the graft. (A-B) Survival of Balb/c mice transplanted with $5 \times 10^3$ FACS-sorted HSCs, $5 \times 10^4$ MACS-enriched splenic T cells from C57BL/6J mice with $5 \times 10^4$ FACS-purified pDCs from wild-type or VIP-KO mice. Data are from 2 independent experiments using HSC alone, n=14 mice; HSC, T cells, & wild-type pDCs group, n=14 mice; HSC, T cells, & KO pDCs group, n=14 mice; and HSC & T cells (no PDC) group, n=12 mice.
Supplementary Figure 7. B6 T cells tended to proliferate more in B10.BR recipient mice transplanted with VIP-KO pDCs. Bioluminescent imaging images of luc+ T in lethally irradiated allogeneic (H2K^d) B10.BR recipients. C57BL/6J T cells were isolated from C57BL/6J (H2K^b) luciferase mice and combined with 3 × 10^3 FACS-sorted HSC, 1 x 10^6 C57BL/6J T cells, and 5 x 10^4 donor pDCs from either C57BL/6 wild-type or VIP-KO B6 donor mice. (A) Bioluminescent imaging images of donor luc+ T cells in anesthetized animals and isolated organs taken on day +15 post-transplant; representative images of organs are shown. (B) Mean + SD of bioluminescent radiance (p/s/cm²/sr) of samples of gut, lung, and liver from B10.BR euthanized on day +8 and day +15 post-transplant, n=4 mice per group.
Supplementary Figure 8. Donor T co-transplanted with VIP-KO pDC have an inflammatory cytokine profile. (A-D) B10.BR mice were lethally irradiated and transplanted with $5 \times 10^3$ HSCs and $10^6$ T cells from C57B/6J donors with $5 \times 10^4$ FACS-purified pDCs either from wild-type or VIP-KO C57BL/6J donors. (A-B) Percentage of donor CD4 T cells and CD8 T cells expression of cytokine and chemokine on day 8 post-transplant. N=4 biological replicates (C-D) Percentage of donor CD4 T cells and CD8 T cells expression of cytokine and chemokine on day 15 post-transplant. Statistics: nonparametric Mann-Whitney U test, *p<0.05, ***p<0.001.
Supplementary Figure 9. VIP produced by pDCs affects the molecular interaction network of T cells. (A) Donor T cells from recipients of C57BL/6 sourced $5 \times 10^3$ FACS-sorted HSCs and $10^6$ T cells, with $5 \times 10^4$ FACS-purified pDCs either from B6 or VIP-KO B6 donors. Cell RNA was extracted and acquired using nCounter® Myeloid Innate Immunity Panel, and GProfiler2 was run using an ordered query for functional profiling. Adjusted P<0.1; No log2 fold change threshold used.
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