Supplementary Materials

Figure 1: The activity of arginase in DCs ($CD11c^{+}MHC\,II^{+}$) and non-DCs ($CD11c^{-}MHC\,II^{-}$) from 6-day cultured BM cells was measured. Data are mean ± SD from 3 independent experiments. *: $p<0.05$, compared with control.

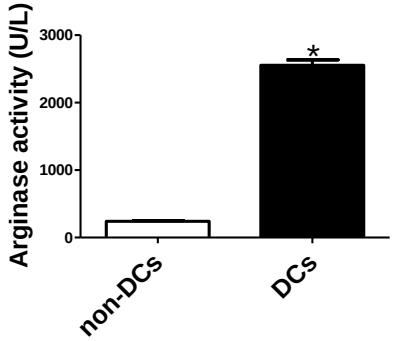

Figure 2: Enriched HPCs from mouse BM were transfected with lentiviral vectors expressing Arg1 cDNA or the GFP-only vectors, followed by culture in medium containing GM-CSF and IL4 for 6 days. The expression of Arg1 in GFP(+) cells were evaluated by western-blotting. Data are representative 3 independent experiments.

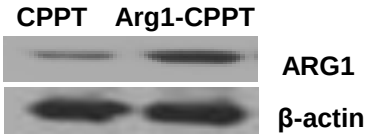

Figure 3: (A) BM cells cultured 24-well plate were transduced with lentiviral plasmids expressing Arg1 cDNA or shRNA, as well as empty vector controls, the total cell numbers in each well were counted. (B) The total numbers in cultured BM cells treated with TSA, SAHA or DMSO were counted. Data are mean ± SD from 4 independent experiments.

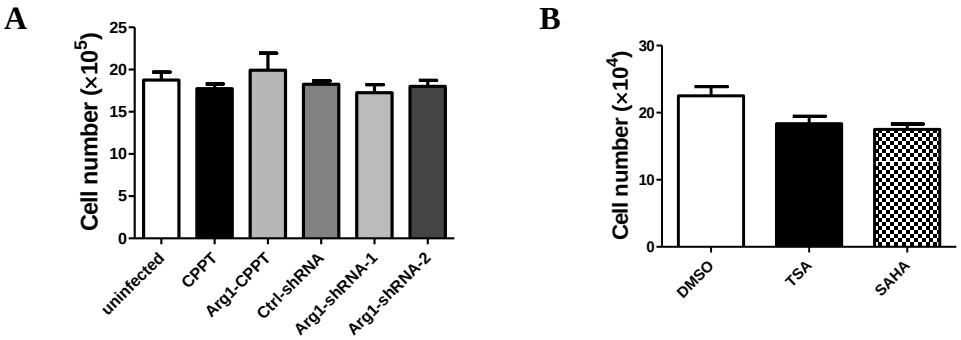

Figure 4: Enriched HPCs from mouse BM were cultured in medium containing GM-CSF and IL4, with the treatments of L-arginine, arginase inhibitor NOHA or vehicle for 6 days. The proportions of distinct populations were determined by flow cytometric analysis. *: $p<0.05$, **: $p<0.01$, compared with the controls.

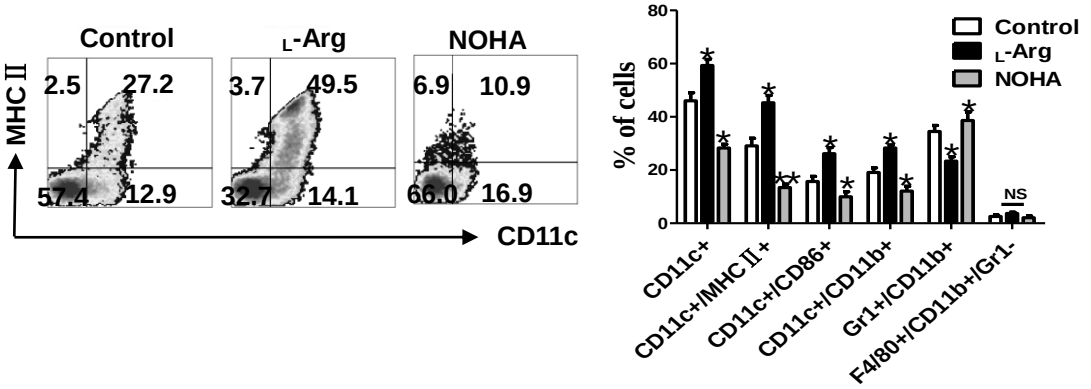

Figure 5: Allogeneic MLR: HPCs were transfected with lentiviral plasmids expressing Arg1 cDNA, Arg1-shRNA or empty vectors, followed by culture with GM-CSF for 6 days to induce DC differentiation. DCs were cocultured with allogeneic T cells at 1:10 ratio for 3 days, T cell proliferation was measured by CFSE dilution. The upper panels are from single representative experiment, the lower ones show mean ± SD from 3 independent experiments. *: $p<0.05$, compared with control.

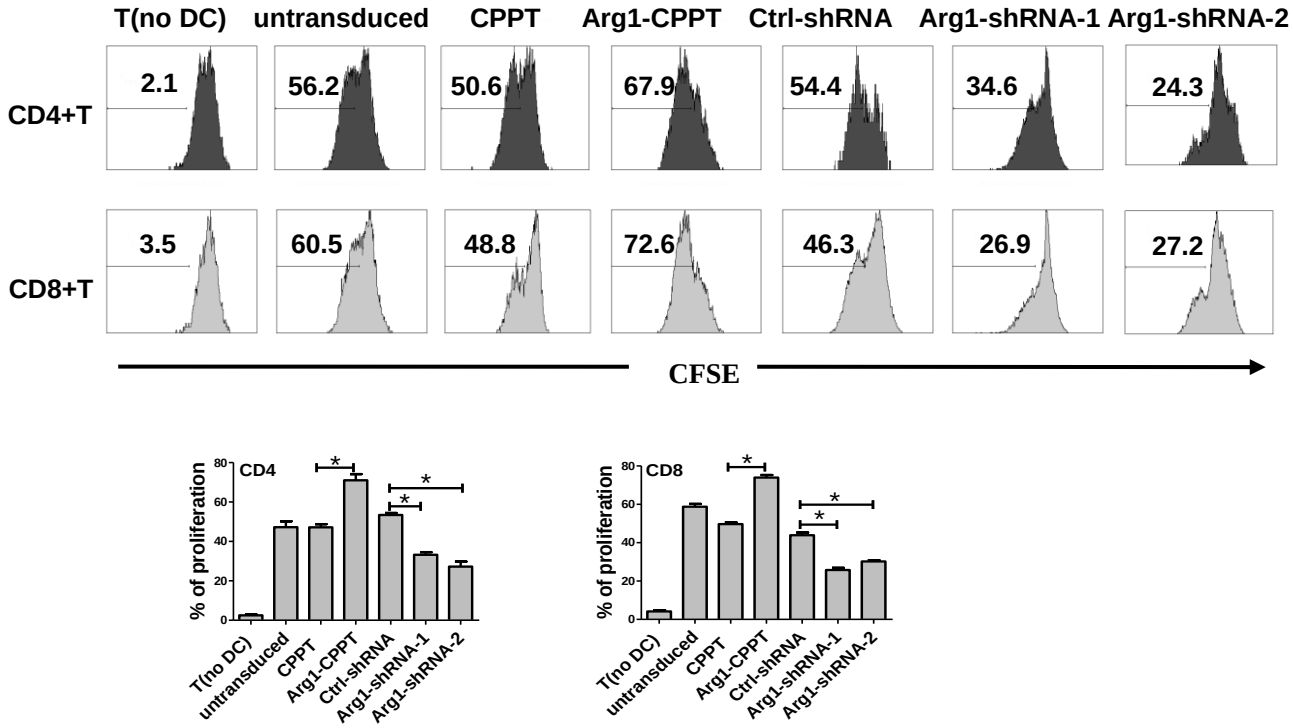

Figure 6: Human DC subsets, including cDC1($CD11c^+CD1c^+$), cDC2 ($CD11c^+CD141^+$) (equivalent to mouse $CD11b^+$DC, $CD8\alpha^+$DC respectively), and pDC ($CD123^+/CD303^+/CD304^+$), were isolated from pheripheral blood by flow cytometric sorting. Arg1 expression was determined by qRT-PCR. Results are mean ± SD from 3 independent experiments. *: $p<0.05$.

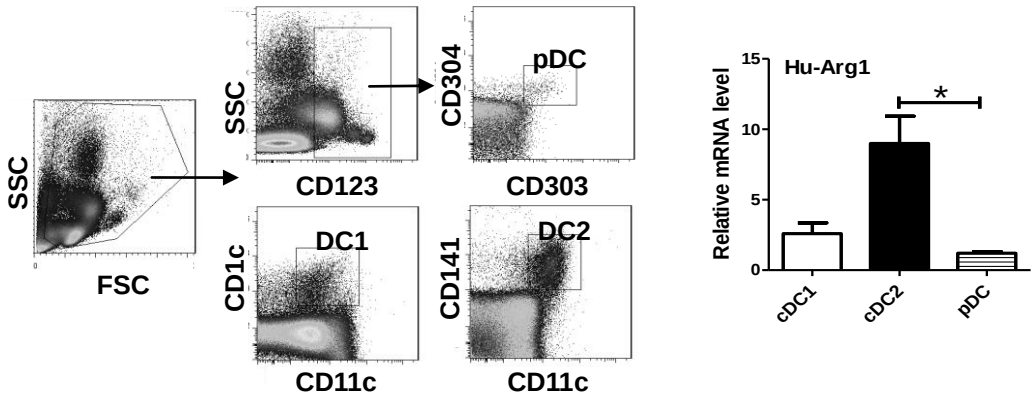

Figure 7: The levels of p-STAT6 and total STAT6 proteins in cultured BM cells were determined by western blotting.

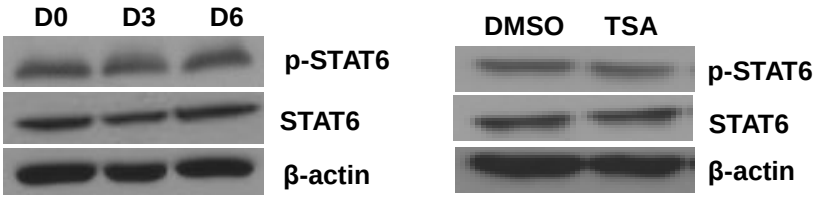

Figure 8: (A-B) C57BL/6 mice (n=3) were injected i.p. with TSA (5 mg/kg) or DMSO once a day for 3 consecutive days. The proportions of DC subsets (gated $CD11c^+$) (A) or IMC subsets (B) in spleen were evaluated by flow cytometric analysis 5 days at post-injection. (C) BM cells from naive mice were cultured in medium containing Flt3L for 9 days, in the presence of TSA or DMSO. the levels of DC subsets were determined by flow cytometric analysis. (A-C): Left panels show representative results from one single experiment, right panels are mean ± SD from 3 independent experiments. *: $p<0.05$, **: $p<0.01$, compared with the corresponding controls.

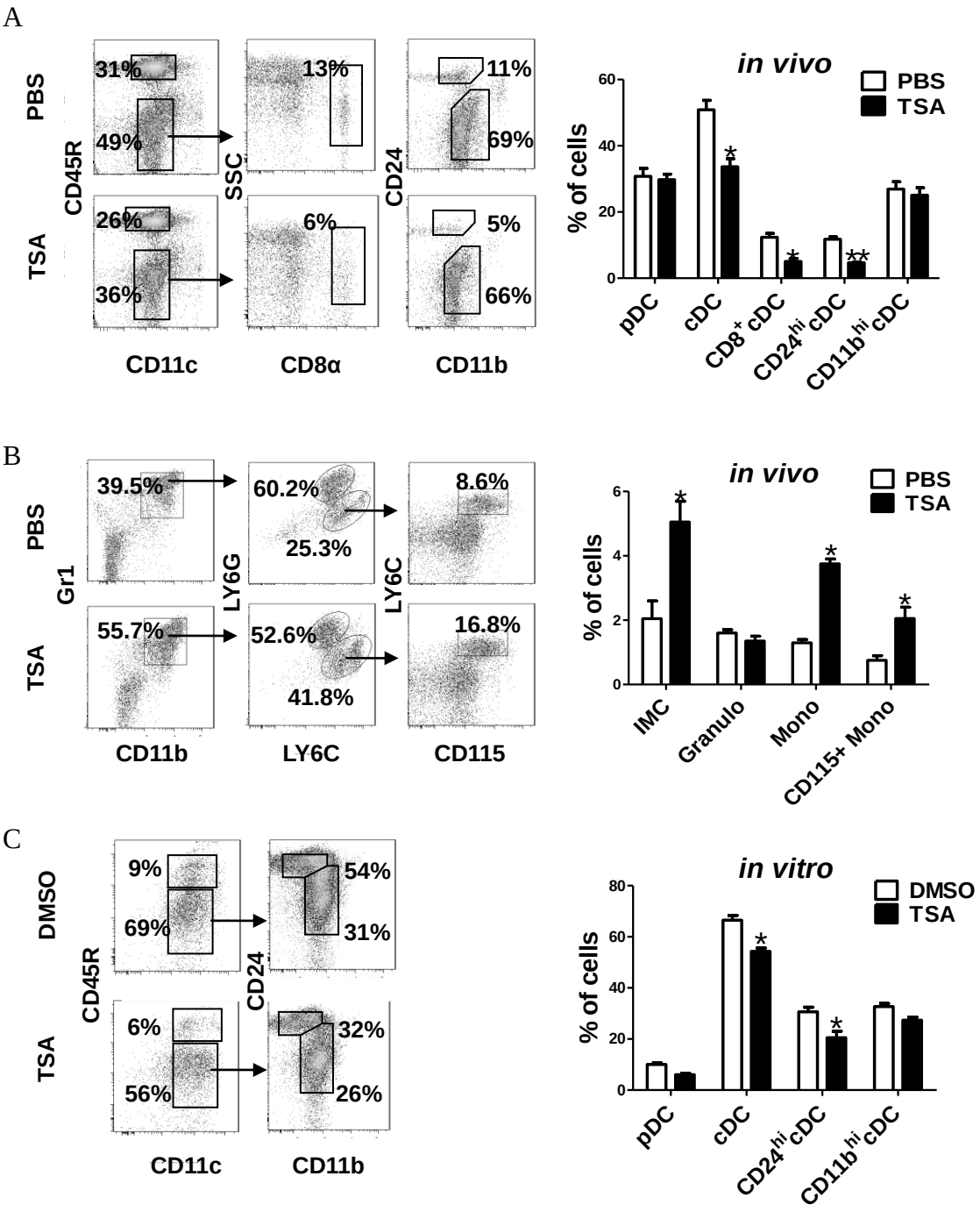

Figure 9: (A) Antigen-specific peritonitis mouse model was established by injection of methylated BSA, with administration of TSA or DMSO. PBS injection was used as control. Splenic $CD11c^{+}CD11b^{+}$ DCs were analyzed by flow cytometry 6 weeks at post-injection. (B) BM cells were cultured in medium containing GM-CSF and IL4 for 6 days, with TSA or DMSO treatment, the percentages of cell subpopulations were analyzed by flow cytometry. (A-B): Left panels show representative results from one single experiment, right panels are mean ± SD from 3 independent experiments. *:

p<0.05, **: p<0.01, compared with the corresponding controls.

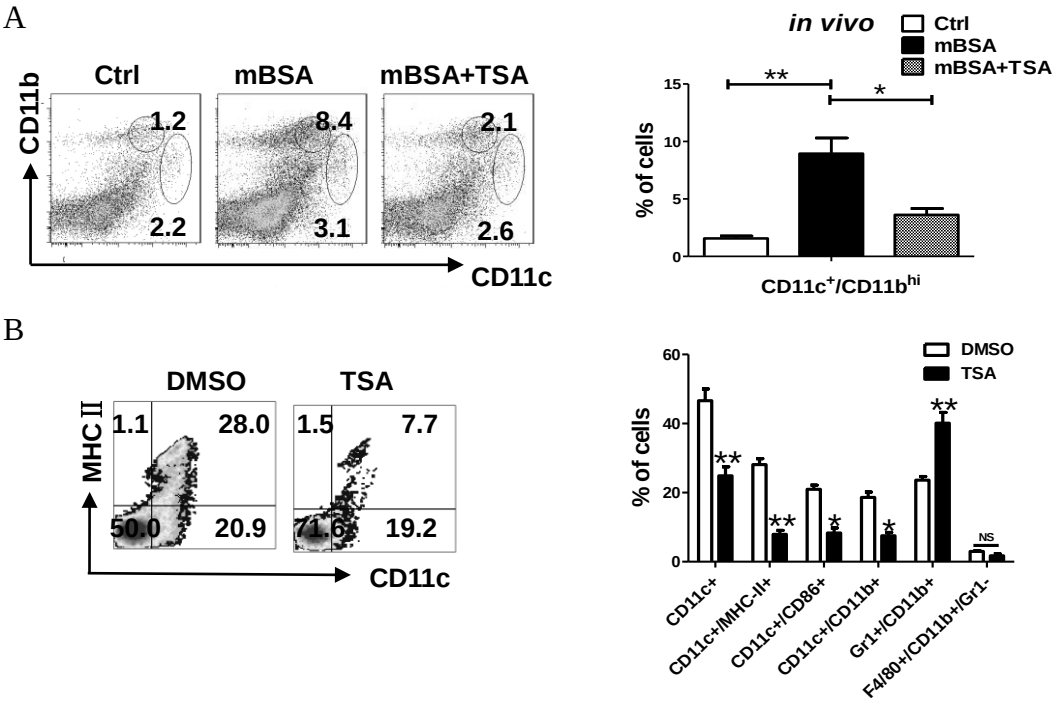

Figure 10: CD8α+ DCs were isolated from mouse spleen or FLT3L cultured BM, by flow cytometric sorting. Arg1 expression was determined by qRT-PCR, LPS-stimulated macrophage (24h) was used as positive control. Results are mean ± SD from 3 independent experiments. *: p<0.05, compared with the corresponding controls.

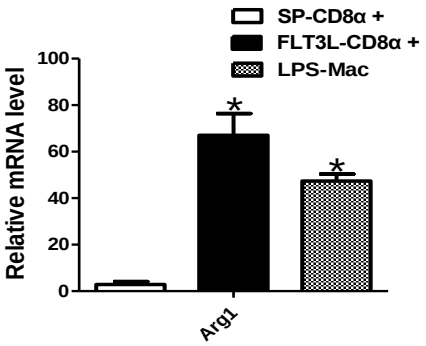

Figure 11: BM cells were stimulated with IL4, GM-CSF, or both for 12 hours , the level of p-STAT6 and total STAT6 proteins in cultured were determined by western blotting, results are representative of 3 independent experiments..

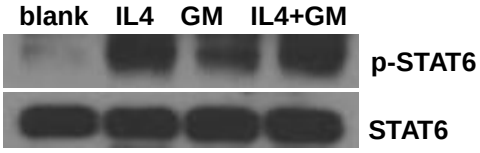

Figure 12: Mouse BM cells were transfected with lentiviral vectors expressing 2 independent STAT6-shRNAs, or the vector (with GFP tag), cells were then cultured in medium containing GM-CSF for 48hs and the expression of Arg1 in sorted GFP(+) cells were evaluated by qRT-PCR, STAT6 was used as positive control, NOS2 was used as negative control. Results are mean ± SD from 3 independent experiments. *: $p<0.05$, compared with the corresponding controls.

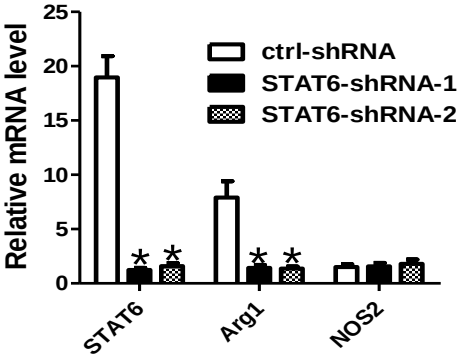

Figure 13: Enriched HPCs from mouse BM cells were transfected with lentiviral vectors expressing Arg1 cDNA or CPPT vector (with GFP tag), GFP+ cells were isolated by flow cytometric sorting. (A) mRNA profiling was performed to screen potential targets of Arg1, results show the expression of some genes related to DC differentiation. (B) The changes were confirmed by qRT-PCR. Arg1 was used as positive control. Results are mean ± SD from 3 independent experiments. *: $p<0.05$, **: $p<0.01$ compared with the corresponding controls.

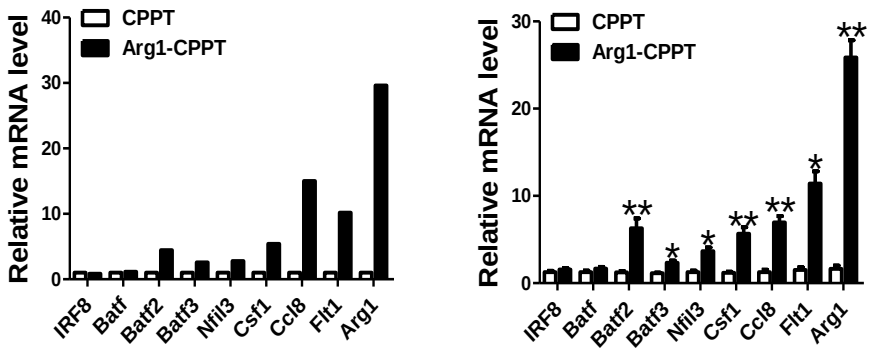

Table 1: Sequences of primers used in this study.

Primer	Sequence
Arg1-for	5′-ATTATCGGAGCGCCTTTCTC-3′
Arg1-rev	5′-ACAGACCGTGGGTTCTTCAC-3′
Arg2-for	5′-TGGATCAAACCTTGCCTCTC-3′
Arg2-rev	5′-GGATTGGCCTCTGCCTTT-3′
NOS2-for	5′-CACCTTGGAAGAGGAGCAAC-3′
NOS2-rev	5′-AAGGCCAAACACAGCATACC-3′
CAT2-for	5′-TTCATGGTCCCGTTCTTACC-3′
CAT2-rev	5′-GGCATCCTCATCGTCTTCTT-3′
HDAC4-for	5′-GGAGAAGGGCAAAGAGAGTG-3′
HDAC4-rev	5′-GGAAATGCAGTGGTTCAGGT-3′
WT-for	5′-GTAGGTACCCAGCTTGCAGCTAGACCACGATGC-3′
WT-rev	5′-CGACTCGAGTCTCACTTTCCCCAGAACTTGAAGCCTTGA-3′
Mutant-rev	5′-CGACTCGAGTCTCACTTTCCCCGCGCCTTGAAGCCTTGA-3′
CHIP-Arg1-for	5′-GGGTGTCTCTTACTTCCCTCCT-3′
CHIP-Arg1-rev	5′-AACCAGCCCCATGCTTTC-3′
Arg1-shRNA-1-for	5′-AATTGCCTTTGTTGATGTCCCTAATCTCGAGATTAGGGACATC AACAAAGGCTTTTTTTAT-3′
Arg1-shRNA-1-rev	5′-AAAAAAAGCCTTTGTTGATGTCCCTAATCTCGAGATTAGGGAC ATCAACAAAGGC-3′
Arg1-shRNA-2-for	5′-AATTGCCTTTGTTGATGTCCCTAATCTCGAGATTAGGGACATC AACAAAGGCTTTTTTTAT-3′
Arg1-shRNA-2-rev	5′-AAAAAAAGCCTTTGTTGATGTCCCTAATCTCGAGATTAGGGAC ATCAACAAAGGC-3′
Arg1-cDNA-for	5′-ATCGACCGGTATGAGCTCCAAGCCAAAGTC-3′
Hu-Arg1-for	5′-TCCAAGGTCTGTGGGAAAAG-3′
Hu-Arg1-rev	5′-TGGTTGTCAGTGGAGTGTTG-3′

Hu-IRF4-for	5′-ATGACAACGCCTTACCCTTC-3′
Hu-IRF4-rev	5′-GTCACCTGGCAACCATTTTC-3′
Hu-Arg1-shRNA-1-for	5′-AATTCTCATAGTTAATGGCATAATTCTCGAGAATTATGCCATTAACTATGAGTTTTTTTAT-3′
Hu-Arg1-shRNA-1-rev	5′-AAAAAAACTCATAGTTAATGGCATAATTCTCGAGAATTATGCCATTAACTATGAG-3′
Hu-Arg1-shRNA-2-for	5′-AATTCCTGTATATCTGCCAAGGATACTCGAGTATCCTTGGCAGATATACAGGTTTTTTAT-3′
Hu-Arg1-shRNA-2-rev	5′-AAAAAACCTGTATATCTGCCAAGGATACTCGAGTATCCTTGGCAGATATACAGG-3′
IDO-for	5′-CCTGAAAGCATTGGAAAAGG-3′
IDO-rev	5′-ATATGCGGAGAACGTGGAAA-3′
BATF3-for	5′- TTTCGAAGCTGAAGGAGGAG-3′
BATF3-rev	5′-ACCGAAGCTGCACAAAGTTC-3′
IRF4-for	5′-GGCCCAACAAGCTAGAAAGA-3′
IRF4-rev	5′-GGAAACTCCTCACCAAAGCA-3′
IRF8-for	5′-ACGAGGTTACGCTGTGCTCT-3′
IRF8-rev	5′-CCCAGCTTGCATTTTTGTTC-3′
STAT6-shRNA-1-for	5′-AATTCCGGGATCTTGCTCAGTTAAACTCGAGTTTAACTGAGCAAGATCCCGGTTTTTTTAT-3′
STAT6-shRNA-1-rev	5′-AAAAAAACCGGGATCTTGCTCAGTTAAACTCGAGTTTAACTGAGCAAGATCCCGG-3′
STAT6-shRNA-2-for	5′-AATTGGCTTTCCGGAGTCACTATAACTCGAGTTATAGTGACTCCGGAAAGCCTTTTTTTAT-3′
STAT6-shRNA-2-rev	5′-AAAAAAAGGCTTTCCGGAGTCACTATAACTCGAGTTATAGTGACTCCGGAAAGCC-3′
BATF2-for	5′- TCTGCATCATCACCTTCTGG-3′
BATF2-rev	5′- CGGCAAATGCTCTCTGTTCT-3′

Nfil3-for	5′- GCACGTATTCCACCTCCATC-3′
Nfil3-rev	5′- GGACCTGTTGTTCGTCTTCC-3′
Csf1-for	5′- CTTCATGCCAGATTGCCTTT-3′
Csf1-rev	5′- GTTAGCATTGGGGGTGTTGT-3′
Ccl8-for	5′- CCTGCTGCTCATAGCTGTCC-3′
Ccl8-rev	5′- ATGGGGCACTGGATATTGTT-3′
Flt1-for	5′- CGAACTCCACCTCCATGTTT-3′
Flt1-rev	5′- CCGCCTCCTTGCTTTTACTC-3′

Reagents: The following reagents, including Trichostatin A (TSA), 5-aza-2′-deoxycytidine (AZA), suberoylanilide hydroxamic acid (SAHA), valproic acid (VPA), Freund's Adjuvant, Complete (FCA), Methylated bovine serum albumin (mBSA), 5-fluorouracil (5-FU), were purchased from Sigma-Aldrich (St. Louis, MO). The recombinant mouse cytokines, including GM-CSF, IL4, FLT3L, IL-3, IL-6, and SCF, were obtained from Peprotech (Rocky Hill, NJ). Ortho-phthaldialdehyde (OPA) and 3-mercaptopropionic acid were obtained from Fluka (St. Louis, MO). HPLC grade acetonitrile and methanol were from BDH (Atlanta, GA). Oasis MCX cation-exchange SPE columns were supplied by Waters (Milford, MA). The antibodies against ARG1, ARG2, NOS2, STAT3, STAT6, p-STAT6, Acetylated-lysine, β-actin and HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies against Acetyl-H3 were purchased from Millipore (Bedford, MA). N-hydroxy-nor-L-arginine (nor-NOHA) was obtained from BioVsion (Milpitas, CA). The following fluorescein-conjugated anti-mouse antibodies: CD11c-PE-Cy5, MHC-Ⅱ-PE, CD86-PE, Gr-1-PE-Cy5, Gr-1-PE, Ly-6C-PerCP-Cyanine5.5, CD45R-PE, CD11b-FITC, CD11b-PE-Cy7, F4/80-PE, CD24-eFluor®450, CD115-APC, CD4-PE, CD8a-PE-Cy5, CD8a-PE-Cy7 and the corresponding isotype antibodies, the anti-human antibodies: CD1a-PE, CD209-PE-Cy7, CD80-APC, CD86-PE, CD123-PerCP-Cyanine5.5, CD303-eFluor®450, CD304-APC, CD11c-PE-Cy7, CD1c-FITC, CD141-PE, and

their isotype control antibodies, were obtained from eBioscience (San Diego, CA). Fluorescein-conjugated anti-mouse antibodie Ly-6G-APC was purchased from BD Biosciences (San Jose, CA), The siRNAs for HDAC4 and the control siRNA, as well as the antibodies against HDAC members, were purchased from Cell Signaling Technology (Beverly, MA). $OVA_{323-339}$ peptide， Lipofectamine 2000, 5,6 carboxyfluorescein diacetate succinimidyl ester (CFSE) and the reagents for cell culture were from Invitrogen (Carlsbad, CA). CD11c MicroBeads, mouse were from Miltenyi Biotec (Teterow, Germany).

Co-immunoprecipitation and Western Blotting: Lysates from cultured cells were containing 100 μg protein were incubated with 2 μg of antibodies agianst interested proteins for 18 hrs at 4℃, followed by incubation with protein A/G agarose for 1 hr at 4℃. The immunoprecipitates were then washed with PBS and heated at 95℃ for 5 min, followed by centrifugation at 5000 g for 1 min. The supernatants were then subjected to western blotting analysis. Briefly, target molecules were then detected using specific primary antibodies and HRP-conjugated secondary antibodies, followed by detection using an ECL HRP Chemiluminescent Substrate Reagent kit (Invitrogen, Carlsbad, USA).

Plasmid Constructs and Transfection Assays: The 5'-regulatory sequence of the mouse Arg1 gene was amplified by PCR using the primers listed in Supplemental Table1. The wild type or mutated Arg1 promoter fragments (+143 ~ -2399) were cloned into pGL3-Basic vector (Promega), and the recombinations were confirmed by DNA sequencing. Transient transfections of reporter plasmid were performed on 32D cells using Lipofectamine 2000 following manufacturer's instructions. The luciferase activity was measured at 48 hr post-transfection.

Methylated BSA-induced peritonitis: mBSA-induced peritonitis was induced as previously described (33,34), Mice were immunized intradermally (i.d.) in two separate regions at the base of the tail with a total volume of 100 ul of mBSA (1 mg/ml in FCA) and was 'boosted' 2 weeks later with the same treatment. At least 7 d later, mice were injected intraperitoneally with 100 ug mBSA for the induction of inflammation. meanwhile, mice were injected intraperitoneally with or without TSA

(5 mg/kg). Then, 3 d later, spleen suspensions were analyzed by flow cytometry.

mRNA genomic profiling: Enriched HPCs from mouse BM cells were transfected with lentiviral vectors expressing Arg1 cDNA or CPPT vector (with GFP tag), GFP+ cells were isolated by flow cytometric sorting 48 h at post-infection, and total RNA was extracted using Trizol reagent and was further purified using Qiagen RNeasy Mini Kit according to the manufactures' instructions. The RNA quality was assessed by formaldehyde agarose gel electrophoresis and was quantitated spectrophotometrically. Affymetrix GeneChip microarrays (Affymetrix, Santa Clara, CA, USA) were prepared, hybridized and scanned by the authorized Affymetrix service provider (capitalbio, Inc., Beijing, China).

PBMC and human DC isolation: Peripheral blood were collected from healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll centrifugation, the distinct DC subsets were purified by flow cytometric sorting.