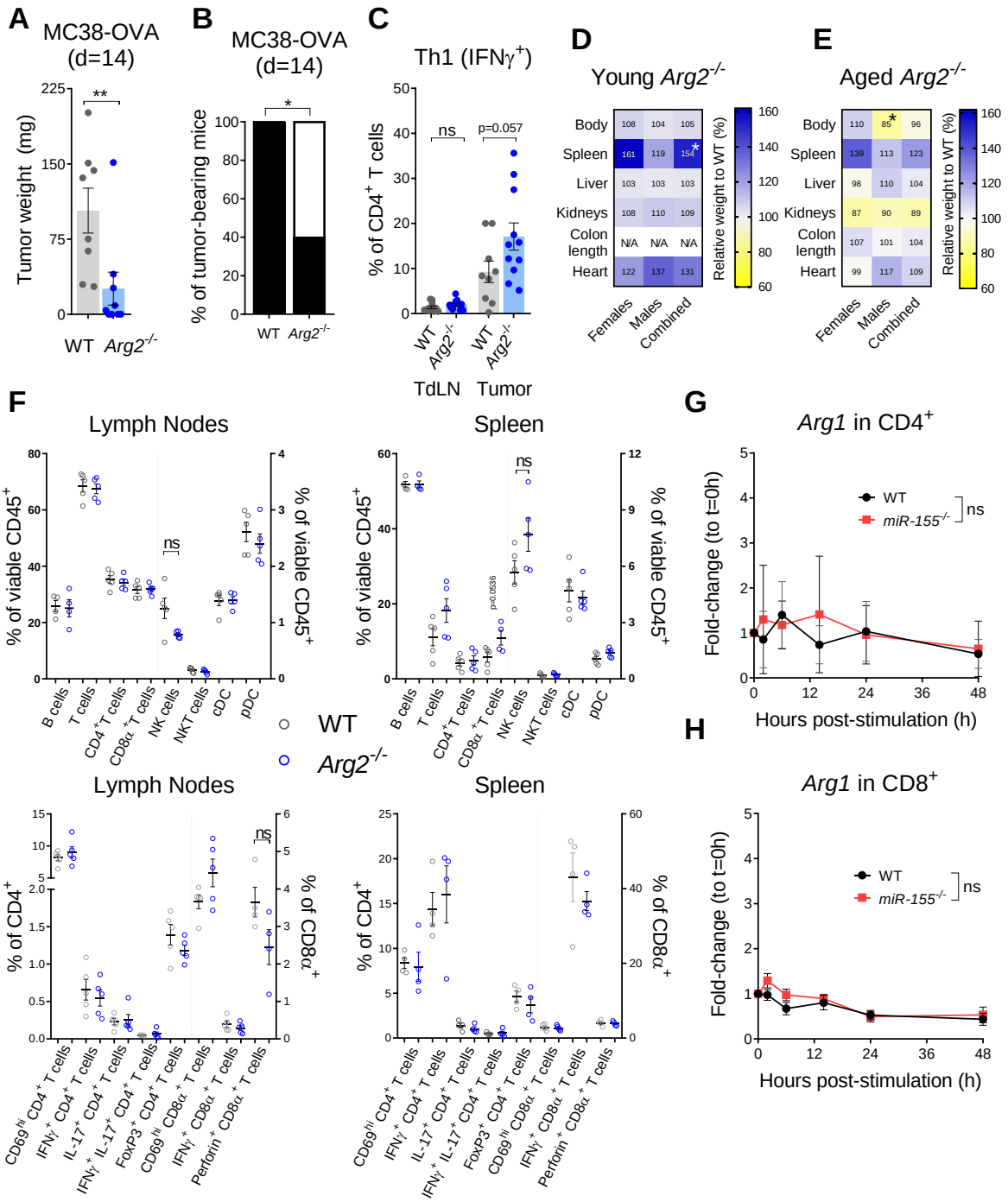
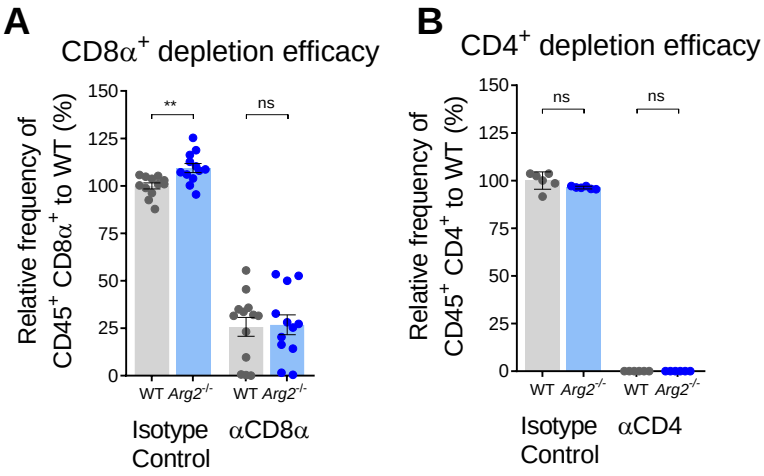
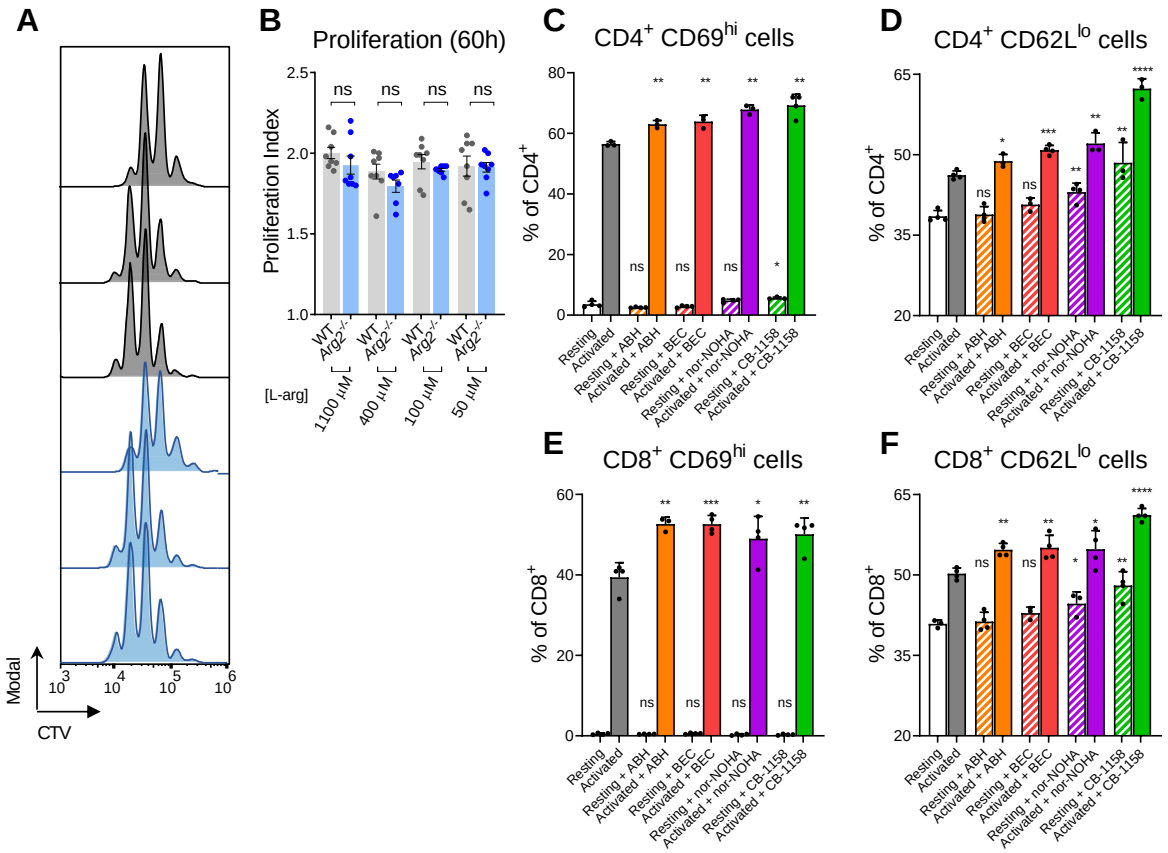
Supplemental Information



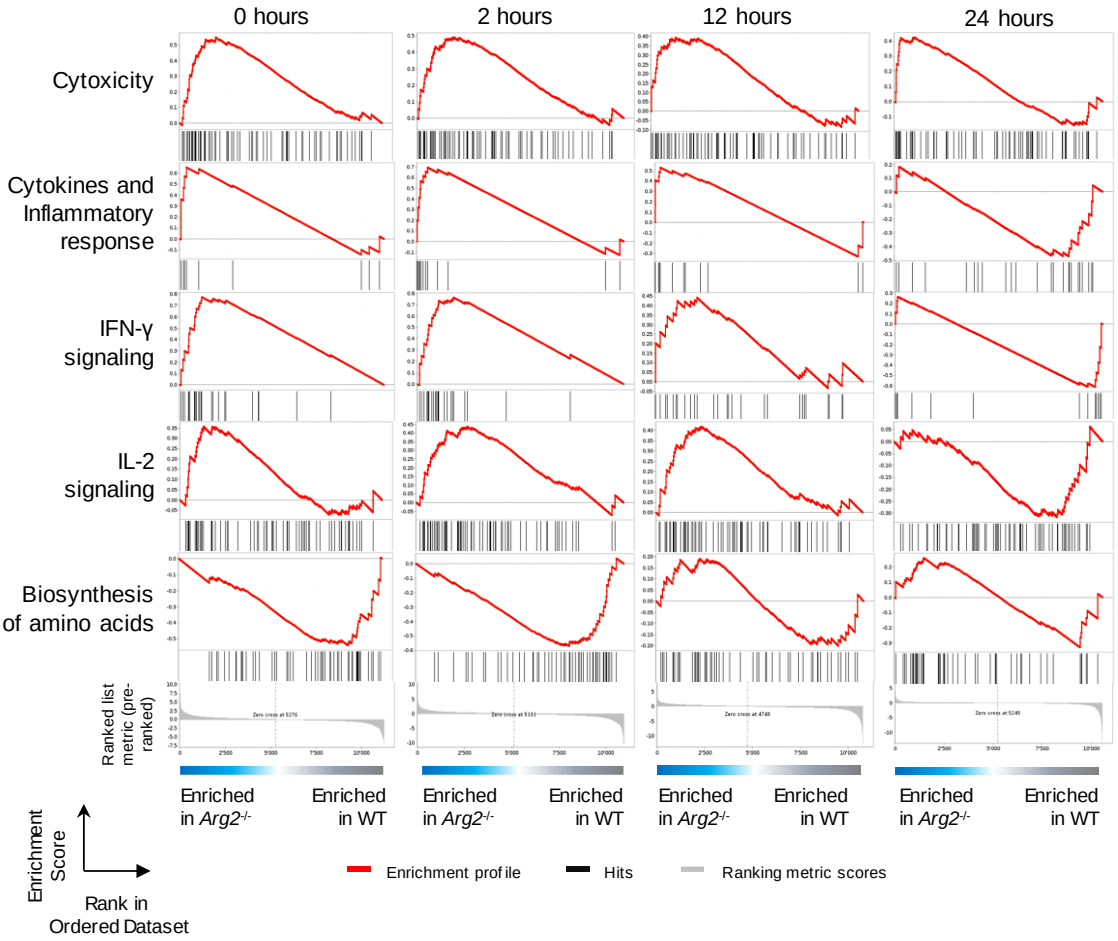
Supplementary Figure 1. Additional characterization of *Arg2*$^{-/-}$ mice. MC38-OVA tumor weight (**a**) and frequency of tumor-free mice at day 14 after tumor implantation (**b**) in male mice. (**c**) IFNγ^{+} Th1 cell frequencies were determined by flow cytometry in tumor-draining lymph nodes and tumor-infiltrating lymphocytes at day 9 after tumor implantation. (**d**,**e**) The heatmap represents changes in mean weights of major organs in young and aged *Arg2*$^{-/-}$ mice relative to WT mice. (**f**) Major DC and lymphocyte populations in the spleen and lymph nodes of WT and *Arg2*$^{-/-}$ mice were quantified by flow cytometry. (**g**,**h**) *Arg1* mRNA levels were determined by real-time qPCR in in vitro activated CD4^{+} (**g**) or CD8^{+} (**h**) T cells derived from WT or *miR155*$^{-/-}$ mice (n=6). Results are (**a**,**f**) representative of 3 independent experiments or (**c**,**g**,**h**) were pooled from two or three independent experiments. (**g**,**h**) Statistical analysis was done by two-way ANOVA test. Data is represented as mean ± s.e.m. throughout. *P < 0.05, **P < 0.01, and ***P < 0.001 (**a**, **c**, **d**, **f**: two-tailed Student's t test) (**b**: Fisher's exact test) (**g**,**h**: two-way ANOVA)



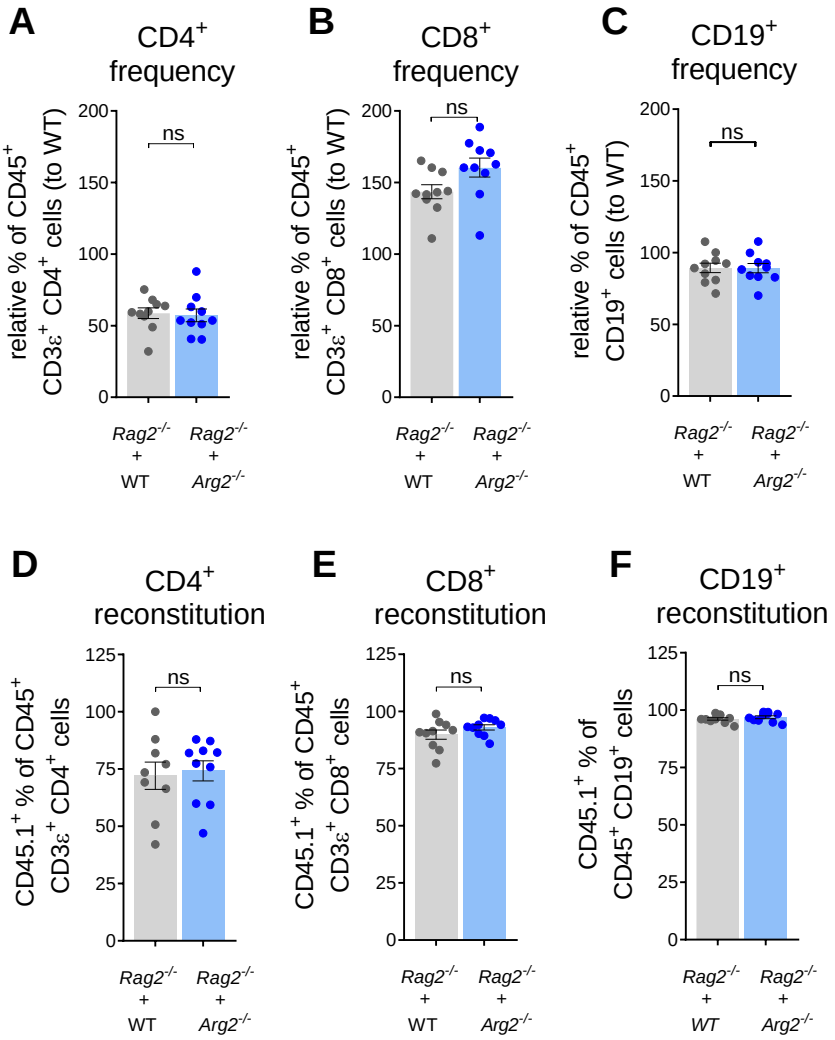
Supplementary Figure 2. Efficacy of T cell depletion in tumor-bearing *Arg2*$^{-/-}$ mice. T cell depletion efficacy in the blood was determined by flow cytometry for CD8$^+$ T cells (**a**) at day 30 after tumor implantation and for CD4$^+$ T cells (**b**) at day 14 after tumor implantation. Results were pooled from two independent experiments. Data is represented as mean ± s.e.m. throughout. **P < 0.01 (**a**, **b**: two-tailed Student's t test)



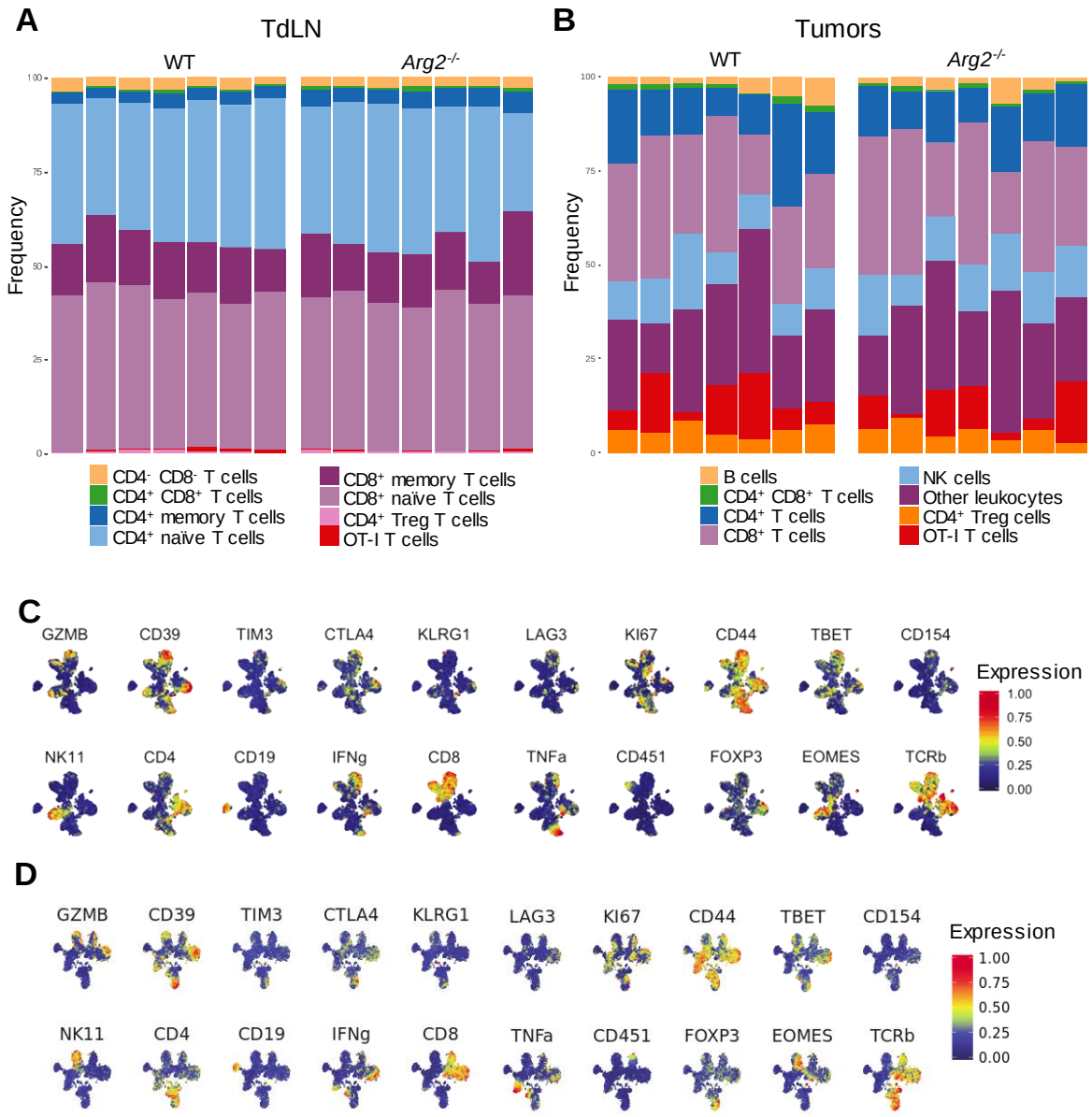
Supplementary Figure 3. Deletion of *Arg2* does not affect in vitro proliferation of CD8$^+$ T cells. (**a**) The results show representative dye dilution profiles for 60-hour in vitro activated WT (grey) and *Arg2*$^{-/-}$ (blue) CD8$^+$ T cells. (**b**) WT or *Arg2*$^{-/-}$ CD8$^+$ T cells were activated in vitro in RPMI containing the indicated concentrations of L-arginine. The proliferation index was calculated from dye dilution profiles obtained 60 hours after activation. (**c-f**) Total T cells were isolated from human PBMCs (n=4) and activated for 24 hours in the presence of the ABH, BEC, nor-NOHA and CB-1158 arginase inhibitors (30 µM). Statistic analysis of the comparison between untreated cells and inhibitor-treated cells is represented for each condition. Results were pooled from two independent experiments. Data is represented as mean ± s.e.m. throughout. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (**b-f**: two-tailed Student's t test)



Supplementary Figure 4. Transcriptome analysis of in vitro activated WT and *Arg2*$^{-/-}$ CD8^{+} T cells. WT or *Arg2*$^{-/-}$ OT-I CD8^{+} T cells were activated in vitro with αCD3ε and αCD28 antibodies and their transcriptome was determined by RNA-seq at different time points. Gene set enrichment analysis were then performed to identify pathways that are upregulated in *Arg2*$^{-/-}$ cells relative to WT cells. The profiles represent the results of gene set enrichment analyses performed for the indicated gene sets at 0, 2, 12 and 24 hours post-activation. Results for the 6 hours time point are shown in Figure 4.



Supplementary Figure 5. Analysis of mixed bone marrow chimeras used as donors of WT and *Arg2*$^{-/-}$ OT-I CD8^{+} T cells. Irradiated WT mice were reconstituted with a mixture, in a 9:1 ratio, of bone marrow derived from Rag2$^{-/-}$ mice and either WT or *Arg2*$^{-/-}$ OT-I mice. (**a-c**) Reconstitution was documented by flow cytometry: the results show the relative percentages of CD4^{+} T cells (**a**), CD8^{+} T cells (**b**) and CD19^{+} B cells (**c**) within blood CD45^{+} cells. Frequencies were normalized relative to mean values measured in a comparable group of naive WT mice (data not shown). (**d-f**) Percentages of lymphocytes derived from donor CD45.1^{+} OT-I bone marrow were determined by flow cytometry for circulating CD4^{+} T cells (**d**), CD8^{+} T cells (**e**) and CD19^{+} B cells (**f**). Results were pooled from two independent experiments. Data is represented as mean ± s.e.m. throughout. *P < 0.05 (**a-f**: two-tailed Student's t test)



Supplementary Figure 6. High-dimensional flow cytometry analysis of lymphocyte populations in tumor-bearing mice. MC38-OVA tumor-bearing mice were adoptively transferred with WT or *Arg2*$^{-/-}$ CD8^{+} OT-I cells and immunized one day later with CpG-B and OVA$_{257-264}$. Seven days later, lymphocyte populations in TdLNs and tumors were analyzed using high-dimensional flow cytometry. (**a**) Barplots of major subpopulations amongst (**a**) TCRb^{+} T cells in the TdLNs or (**b**) CD45^{+} cells in each individual mouse. (**c**) tSNE plots showing stochastically selected T cells in TdLNs from both WT OT-I and *Arg2*$^{-/-}$ OT-I recipient mice. (**d**) tSNE plots showing stochastically selected CD45^{+} cells in tumors from both WT OT-I and *Arg2*$^{-/-}$ OT-I recipient mice.

Young adults – 12 weeks old

	Animals affected		Significance
	WT	*Arg2-/-*	
ADRENALS			
Accessory	1		Background
Cortical nodular hyperplasia	1		Background
Subcapsular cell hyperplasia		1	Background
SPLEEN			
Accessory		1	Background
GALLBLADDER			
Cystic dilation	1		Background
LIVER			
Oval cell hyperplasia		1	Incidental
Single cell necrosis	1	1	Incidental
Extramedullary hematopoiesis	2	2	To be judged with CBC** ante mortem
SEMINAL VESICLES			
Dilatation	1		Background
TRICUSPID VALVE			
Melanosis		1	Background, common in black coated
HEART			
Interstitial fatty vacuolation	1		Incidental
CERVICAL LYMPH NODES			
Sinus histiocytosis	2	2	Background
Hemorrhage	1		Iatrogenic (due to cervical dislocation)
THYMUS			
Hemorrhage	1		Iatrogenic (due to cervical dislocation)
KIDNEY			
Lymphocytic infiltrate (corticomedullary)	4	3	Background/Reactive
Tubular epithelial basophilia		1	Incidental
Tubular hyaline cast		1	Incidental
EPYDIDYMIS			
Giant sperms	1		Background
STOMACH			
Glandular mineralization	1		Incidental
Epithelial eosinophilic cytoplasmic inclusions	1		Background
Neutrophilic infiltrate	1		Incidental

**bone marrow cellularity and ratios comparable between groups

Aged adults – 21 months old

	Animals affected		Significance
	WT	*Arg2-/-*	
SPLEEN			
Extramedullary hematopoiesis	9	9	Background
Hemosiderosis	9	9	Background
Hyperplasia, lymphocytic	3	1	Background/ Reactive
Lymphoma, pleomorphic	2	3	Neoplasia
Amyloidosis		3	
CERVICAL AND MEDIASTINAL LYMPH NODES			
Hyperplasia, lymphocytic	2	2	Background/ Reactive
Lymphoma, pleomorphic	1	1	Neoplasia
Hemosiderosis	2	1	Background
Plasmacytosis		1	Background
Proliferation/Infiltration, plasmacellular, histiocytic		4	Reactive
Infiltration, histiocytic, pigment containing	3	2	Background/Reactive
MESENTERIC AND PANCREATIC LYMPH NODES			
Lymphoma, pleomorphic	2	2	Neoplasia
Hyperplasia, lymphocytic	4		Background/ Reactive
Extramedullary hematopoiesis	1		Background
Proliferation/Infiltration, plasmacellular, histiocytic		1	Background/ Reactive
Infiltration, histiocytic, pigment containing	1	1	
BONE MARROW			
Hemosiderosis	1		Neoplasia
HEART			
Amyloidosis including Purkinje fibers and arterial walls		1	
BLOOD VESSELS			
Arteries: Tunica media thickening / hyalinization		1	Background
Amyloidosis of liver arteries		1	
MANDIBULAR SALIVARY GLAND			
Lymphoma, pleomorphic	1		Neoplasia
Infiltration, lympho-histiocytic and plasmacellular	1	4	Background/Age related
Infiltration, lymphocytic	4		Background/Age related
PAROTID SALIVARY GLAND			
Lymphoma, pleomorphic	1		Neoplasia
Infiltration, lymphocytic	1		Background/Age related
STOMACH			
Amyloidosis	3	3	Amyloidosis
SMALL INTESTINE			
Amyloidosis	3	3	
Lymphoma, pleomorphic	1		Neoplasia
Crypt abscess	1		Incidental
LARGE INTESTINE			
Pinworm infestation	1	5	Background /Parasitosis
Amyloidosis	3	2	
Infiltration lymphocytic and plasmacellular		2	Background/ Reactive
PANCREAS			
Vacuolation acinar cell	1	1	Background
Focal hyperplasia		1	Background /Proliferative
LIVER			
Extramedullary hematopoiesis	6	9	Background
Lymphoma, pleomorphic	2	2	Neoplasia
Hemosiderosis	1		Background
Infiltration, lympho-histiocytic and plasmacellular		3	Background/Reactive
Hepatocellular carcinoma	1		Neoplasia
Hepatocellular vacuolation, large vacuolar		2	Background
TRACHEA			
Ectasia/Eosinophilic crystal deposition in submucosal glands	2	2	Background
LUNG			
Hemosiderosis	1	1	Background
Adenoma, pulmonary		1	Neoplasia
Hemosiderosis	1		Incidental
Acidophilic macrophage pneumonia	1	3	Background
Lymphoma, pleomorphic	1	1	Neoplasia
Infiltration, lympho-histiocytic and plasmacellular	2	4	Reactive
Infiltration, histiocytic	1	1	Reactive
Infiltration, mixed cellular	2		Reactive/Incidental
KIDNEYS			
Infiltration, lympho-histiocytic	4	6	Background/ Reactive
Amyloidosis		1	
Lymphoma, pleomorphic	1	1	Neoplasia
Glomerulosclerosis	1		Incidental
URINARY BLADDER			
Infiltration, lymphocytic	1		Incidental
Lymphoma, pleomorphic	1		Neoplasia
ADRENALS			
Pigment deposition, cortical	2		Background/Age related
Amyloidosis		1	
SKIN			
Infiltration, lymphocytic	1		Background/Reactive
BRAIN			
Epidermoid cyst	1		Background
OVARY			
Atrophy and pigmentation	4	5	Background/Age related
OVIDUCT			
Infiltration, lymphocytic	1	1	Background/Reactive
Atrophy, epithelial	1		Background/Age related
UTERUS			
Hyperplasia, cystic	3	3	Background/Age related
Dilation		1	Background/Age related
MAMMARY GLAND			
Hyperplasia alveolar epithelium	1		Background/Age-related
TESTIS			
Tubular atrophy/degeneration	2		Background/Age related
PREPUTIAL GLANDS			
Inflammation, neutrophilic	1	1	Background/Inflammator
Cystic dilation	1		Background

Supplementary Table 1. Histopathologic analyses of young and aged Arg2-deficient mice. A total of (**a**) 16 or (**b**) 20 C57BL/6J mice (Charles River), aged 12 weeks were sent for examination. The mice were composed of 4 balanced groups composing of (**a**) four or (**b**) five arginase 2 knockout mice (*Arg2*$^{-/-}$) and (**a**) four or (**b**) five control (*Arg2*$^{+/+}$) animals for each gender. The analyzed organs included: spleen, cervical and mediastinal lymph nodes, mesenteric and pancreatic lymph nodes, thymus, bone marrow, heart, blood vessels, mandibular salivary gland, sublingual salivary gland, parotid salivary gland, oral cavity, tongue, esophagus, stomach, small intestine, large intestine, pancreas, liver, nasal cavity, trachea, lung, kidneys, urinary bladder, ovary/testis, oviduct/epididymis, uterus/prostate/ coagulative gland/ seminal vesicles, vagina/penis, clitoral glands/ preputial glands, mamma, adrenals, thyroid, skin, muscle, knee joint, bone, brain, spinal cord, eye and ear.

Gene	Assay	Sense	Sequence
mRag2	PCR	Fw	CAGCGCTCCTCCTGATACTC
		Rev	TGCATTCCTAGAGCGTCCTT
		Rev Mut	GGTCATCCTTTGCAACACAG
mArg2	PCR	Fw	CTCACTGTAACCCAGCCTCAG
		Rev	CATGAGCATCAACCCAGATG
		Rev Mut	CAGTCATAGCCGAATAGCC
mBIC	PCR	Fw	GCCCTGGCTGTACCCCCTATCTTG
		Rev	CATACAGCCTTCAGCAAGCCTCCA
		Mutant	GAAATGCGTAGGAAACGTGGGTCTC
mArg2	qRT-PCR	Fw	CTGTGTCACCATGGGAGGAG
		Rev	GCATGAGCATCAACCCAGAT
mArg1	qRT-PCR	Fw	ATGAAGAGCTGGCTGGTGTG
		Rev	GCCAGAGATGCTTCCAACTG
mBIC	qRT-PCR	Fw	AACCAGGAAGGGGAAGTGTG
		Rev	TTCAGCAAGCCTCCAGCA
18S	qRT-PCR	Fw	CTTAGAGGGACAAGTGGCG
		Rev	ACGCTGAGCCAGTCAGTGTA
mGAPDH	qRT-PCR	Fw	CCCGTAGACAAAATGGTGAAG
		Rev	AGGTCAATGAAGGGGTCGTTG
mIFNg	qRT-PCR	Fw	GGATGCATTCATGAGTATTG
		Rev	CTTTTCCGCTTCCTGAGG
mIL2	qRT-PCR	Fw	TTCAATTGGAAGATGCTGAGAA
		Rev	TCATCGAATTGGCACTCAAA
mNOS2	qRT-PCR	Fw	GCCCTCCCTCTGGAAAGAC
		Rev	AGGACTCTGAGGCTGTGTGG

Supplementary Table 2. List of primers used for PCR or qRT-PCR.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
BV650 Hamster Anti-Mouse CD154 (MR1)	Becton Dickinson (BD) Biosciences	Cat#740480
BUV661 Rat Anti-Mouse CD19 (1D3)	BD Biosciences	Cat#565076
PerCP-eFluor 710 Rat- Anti-Mouse CD39 (24DMS1)	Thermo Scientific™	Cat#46-0391-82
BUV496 Rat Anti-Mouse CD4 (GK1.5)	BD Biosciences	Cat#564667
Brilliant Violet 570™ Anti-mouse/human CD44 (IM7)	BioLegend	Cat#103037
BUV563 Rat Anti-Mouse CD45 (30-F11)	BD Biosciences	Cat#565710
Brilliant Violet 785™ Mouse Anti-mouse CD45.1 (A20)	BioLegend	Cat#110743
BUV805 Rat Anti-Mouse CD8a (53-6.7)	BD Biosciences	Cat#612898
APC-R700 Hamster Anti-Mouse CD152 (UC10-4F10-11)	BD Biosciences	Cat#565778
PE-eFluor 610 Rat Anti-Mouse Eomes (Dan11mag)	Thermo Scientific™	Cat#61-4875-82
PE Rat Anti-Mouse FoxP3 Monoclonal Antibody (FJK-16s)	Thermo Scientific™	Cat#12-5773-82
FITC Mouse Anti-human/mouse Granzyme B (GB11)	BioLegend	Cat#515403
BUV737 Rat Anti-Mouse IFN-γ (XMG1.2)	BD Biosciences	Cat#564693
BV480 Mouse Anti-Ki-67 (B56)	BD Biosciences	Cat#566109
APC Syrian hamster Anti-mouse/human KLRG1 (2F1/KLRG1)	BioLegend	Cat#138412
Brilliant Violet 421™ Rat Anti-mouse CD223 (C9B7W)	BioLegend	Cat#125221
Biotin Mouse Anti-Mouse NK-1.1 (PK136)	BD Biosciences	Cat#553163
Brilliant Violet 605™ Rat Anti-mouse CD279 (29F.1A12)	BioLegend	Cat#135220
PE-Cy7 Mouse Anti-Mouse T-bet (eBio4B10)	Thermo Scientific™	Cat#25-5825-82
PE-Cy5 Armenian hamster anti-mouse TCRβ chain(H57-597)	BioLegend	Cat#109210
APC Rat Anti-mouse CD366 (RMT3-23)	BioLegend	Cat#119706
Brilliant Violet 711™ Rat Anti-mouse TNF-α (MP6-XT22)	BioLegend	Cat#506349
PerCP/Cyanine5.5 Rat Anti-mouse TCR Vα2 (B20.1)	BioLegend	Cat#127814
APC Mouse Anti-mouse CD45.2 (104)	BioLegend	Cat#109814
PE Mouse anti-mouse TCR Vβ5.1, 5.2 (MR9-4)	BioLegend	Cat#139504
APC/Cy7 Rat Anti-mouse CD4 (RM4-5)	BioLegend	Cat#100526
FITC Rat Anti-mouse CD3 (17A2),	Thermo Scientific™	Cat#11-0032-80
APC Rat Anti-Mouse (eBioOMAK-D), APC	Thermo Scientific™	Cat#17-9392-80
PE Rat Anti-mouse Eomes (Dan11mag)	Thermo Scientific™	Cat#12-4875-82
PerCP-Cyanine5.5 Rat Anti-mouse IFNg (XMG1.2),	Thermo Scientific™	Cat#45-7311-82
FITC Armenian hamster Anti-mouse CD69 (H1.2F3)	Thermo Scientific™	Cat#11-0691-82
PerCP-eFluor 710Rat Anti-mouse CD279 (RMP1-30)	Thermo Scientific™	Cat#46-9981-82
PE Rat Anti-mouse anti-mouse IL-17A (TC11-18H10.1)	BioLegend	Cat#506904
PerCP/Cyanine5.5 Mouse Anti-mouse NK-1.1 (PK136)	BioLegend	Cat#108728
APC Rat Anti-mouse CD62L (MEL-14)	Thermo Scientific™	Cat#17-0621-82
Rat Anti-mouse PerCP/Cy5.5 anti-mouse Siglec H (551)	BioLegend	Cat#129614
InVivoMAb anti-mouse CD4	BioXCell	Cat#BE0003-1
InVivoMAb rat IgG2b isotype control, anti-KLH	BioXCell	Cat#BE0090
InVivoMAb rat IgG2a isotype control, anti-trinitrophenol	BioXCell	Cat#BE0089
InVivoMAb anti-mouse CD8α	BioXCell	Cat#BE0004-1
InVivoMAb anti-mouse PD-1 (CD279)	BioXCell	Cat#BE0146
InVivoMAb anti-mouse CD3ε	BioXCell	Cat#145-2C11
Purified NA/LE Hamster Anti-Mouse CD28	BD Biosciences	Cat#553294
Arginase II Antibody (H-64)	Santa Cruz Biot.	Cat#sc-20151
β-Actin Antibody (C4)	Santa Cruz Biot.	Cat#sc-47778
BV421 Mouse Anti-Human CD62L (DREG-56)	BioLegend	Cat#304827
FITC Mouse Anti-Human CD8 (HIT8a)	BD Biosciences	Cat#560960
PE-Cy7 CD4 Mouse Anti-Human (SFCI12T4D11)	Beckman Coulter	Cat#737660
PE Mouse Anti-Human CD69 (FN50)	BioLegend	Cat#310906
APC/Fire™ Mouse Anti-Human CD3 (SK7)	BioLegend	Cat#344840

Supplementary Table 3. List of antibodies used in flow cytometry, Western Blot and in vivo experiments