

Supplementary Materials for
**Ly6C^{hi} monocytes balance regulatory and cytotoxic CD4 T cell responses to
control virus-induced immunopathology**

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The PDF file includes:

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Tables S1 and S2

Other Supplementary Material for this manuscript includes the following:

Data file S1
MDAR Reproducibility Checklist

Fig.S1. MHCII+ Sca-1+ MOs mobilized during MuHV-4 infection exhibit regulatory properties, related to Fig.1

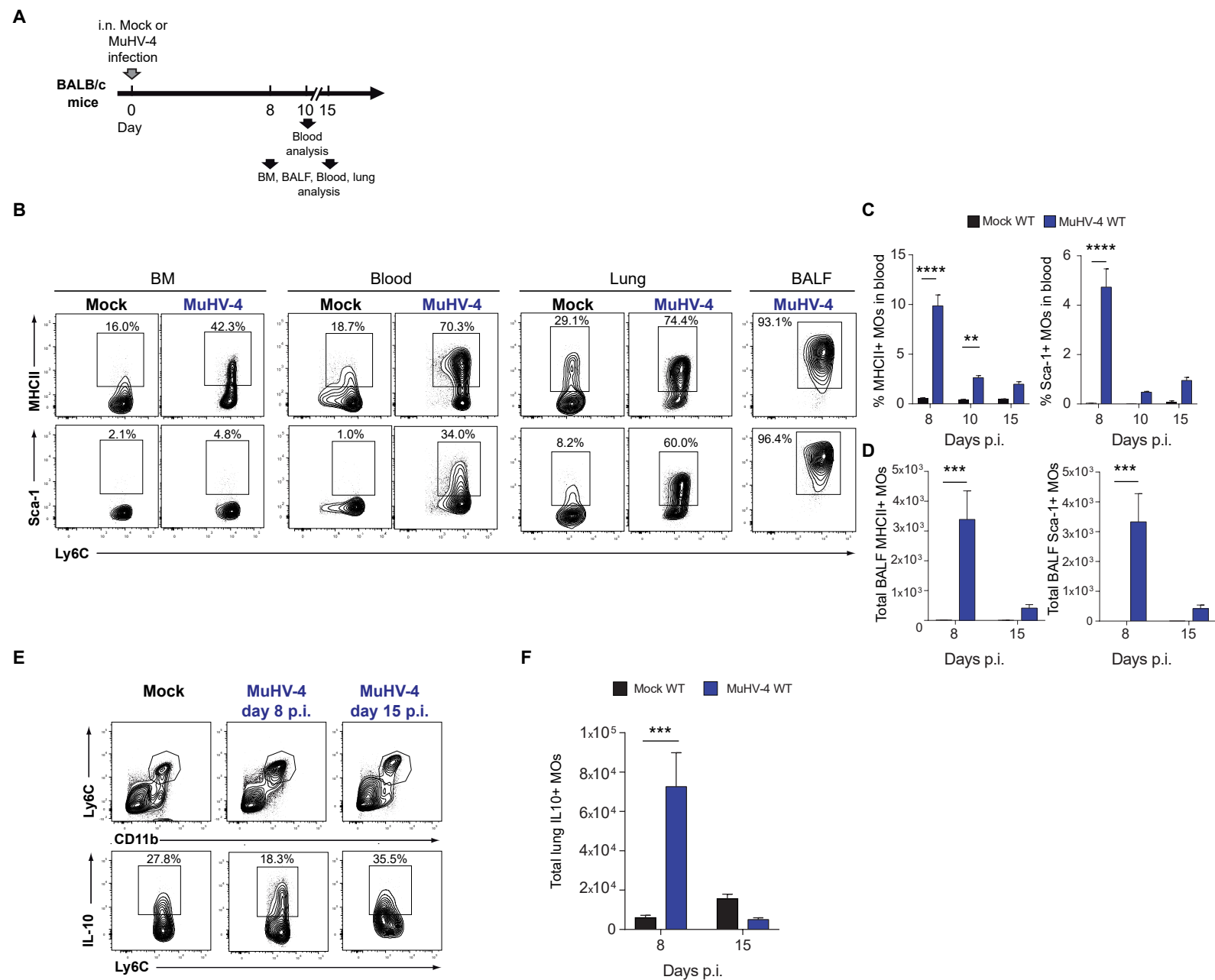


Fig.S1. MHCII+ Sca-1+ MOs mobilized during MuHV-4 infection exhibit regulatory properties, related to Fig.1 (A) Experimental layout of panels B-F (n= 5, three experiments). (B) FACS plots of MHCII and Sca-1 expression by MOs at day 8 p.i. BM and blood MOs were gated as Live, CD19⁻CD3⁻Ly6G⁻CD11b⁺Ly6C⁺CCR2⁺. Lung and BALF MOs were gated as Live, CD11c⁻non-autofluorescent, CD19⁻CD3⁻Ly6G⁻CD11b⁺Ly6C⁺CCR2⁺. (C-D) Percentages in blood (C) and numbers in BALF (D) of MHCII+ and Sca-1+ CCR2+ MOs from Mock or MuHV-4 infected mice. (E-F) FACS plots (E) and numbers (F) of lung MOs expressing IL-10. Numbers in gates (B-E) indicate mean percentages. Data were analysed by two-way ANOVA and Sidak's multiple-comparison test (***P < 0.001, ****P < 0.0001). Error bars represent SEM.

Fig.S2. Depletion of CCR2+ MOs in BALB/c mice worsens MuHV-4 associated disease, related to Fig.1

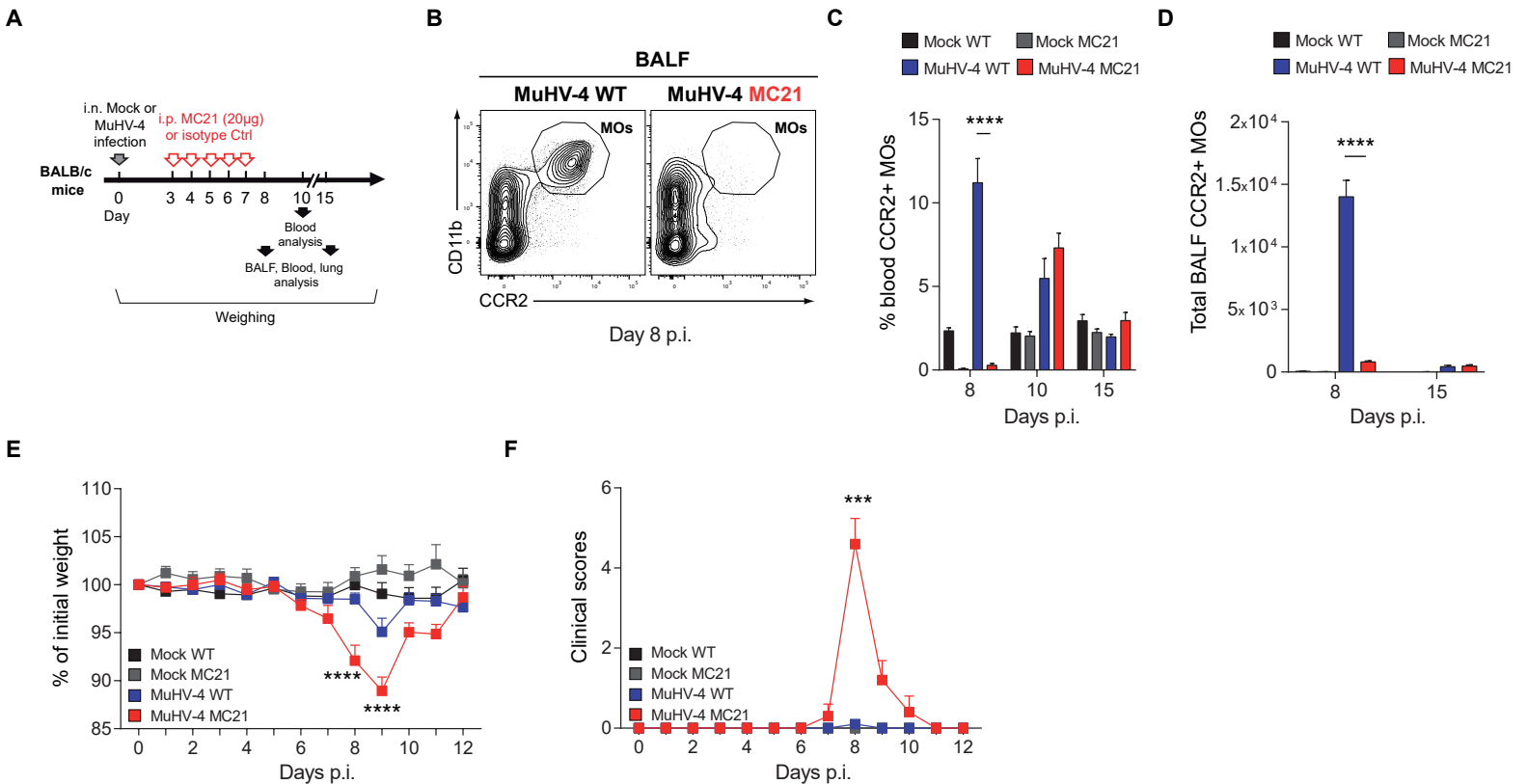


Fig.S2. Depletion of CCR2+ MOs in BALB/c mice worsens MuHV-4 associated disease, related to Fig.1 (A) Experimental layout (n= 4 to 10, three experiments). (B) FACS plots of MOs at day 8 p.i. in BALF of Mock-treated or MC21-treated MuHV-4 infected mice. (C-D) Percentages in blood (C) and numbers in BALF (D) of CCR2+ MOs from Mock or MuHV-4 infected mice. (E) Body weight as percentage of initial weight. (F) Clinical scores. Data were analysed by two-way ANOVA and Sidak or Tukey's multiple-comparison test (***P < 0.001, ****P < 0.0001). Error bars represent SEM.

Fig.S3. Alveolar niche is efficiently reconstituted after infection in a CCR2-independent manner, related to Fig.1

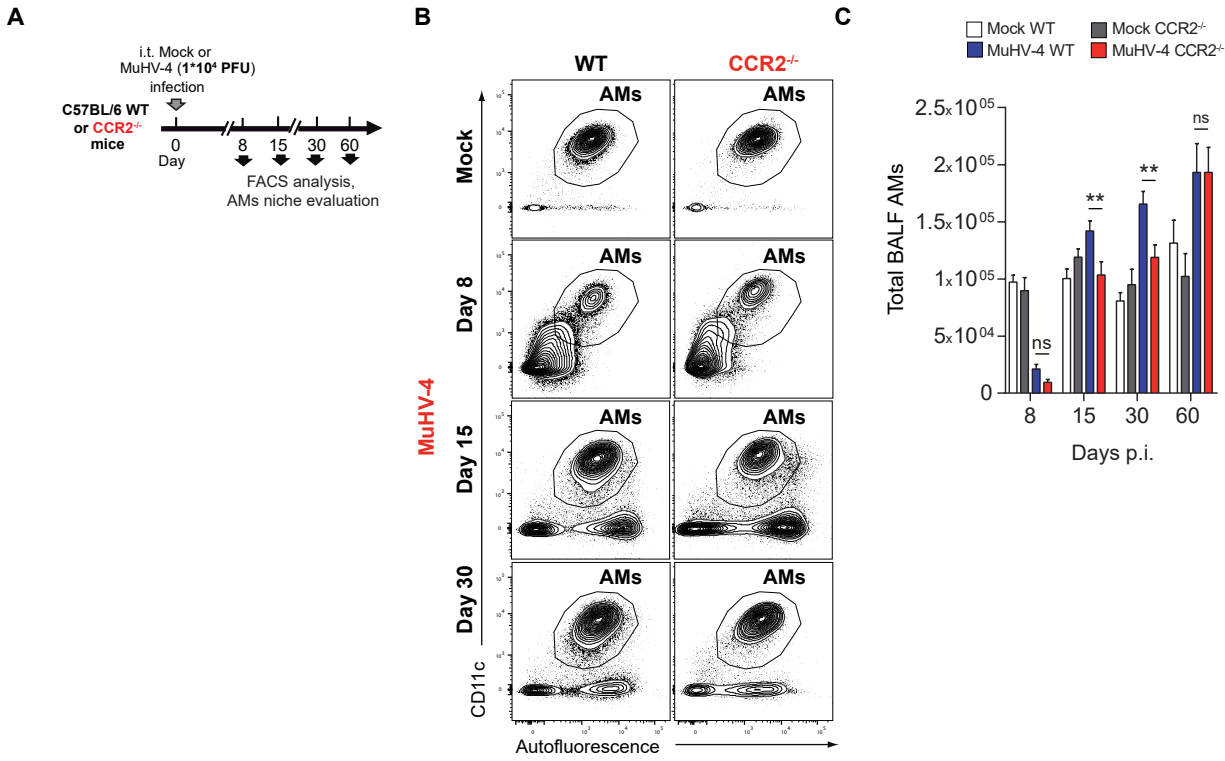
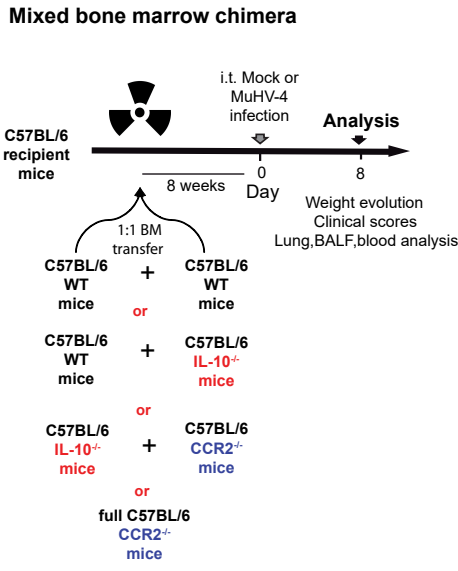


Fig.S3. Alveolar niche is efficiently reconstituted after infection in a CCR2-independent manner, related to Fig.1 (A) Experimental layout of panels B-C (n=4 to 10, three experiments) (B) FACS plots of BALF AMs in Mock and MuHV-4 infected WT and CCR2^{-/-} mice. (C) Numbers (at days 8,15,30,60 p.i.) of BALF AMs. Data were analysed by one-way ANOVA and Sidak's multiple-comparison test (ns, not statistically different, **P < 0.01). Error bars represent SEM.

Fig.S4. IL-10 production by MOs is not the direct factor protecting against weight loss, related to Fig.3

A



B

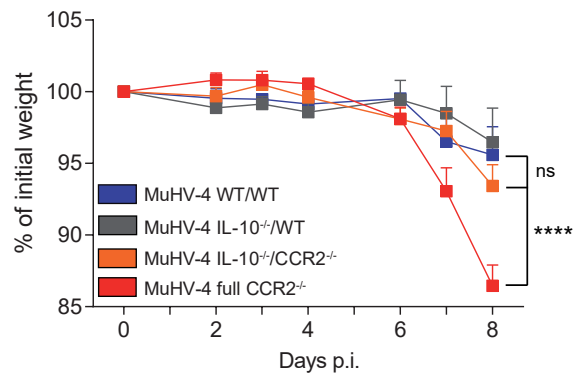


Fig.S4. IL-10 production by MOs is not the direct factor protecting against weight loss, related to Fig.3 (A) Experimental layout (n= 5 to 7, two experiments). (B) Body weight as percentage of initial weight. Data were analysed by two-way ANOVA and Tukey's multiple-comparison test (ns, not significant, **P < 0.0001). Error bars represent SEM.**

Fig.S5. Absence of MOs does not affect the priming of CD4 T cells but rather modulates local instruction in lung, related to Fig.3

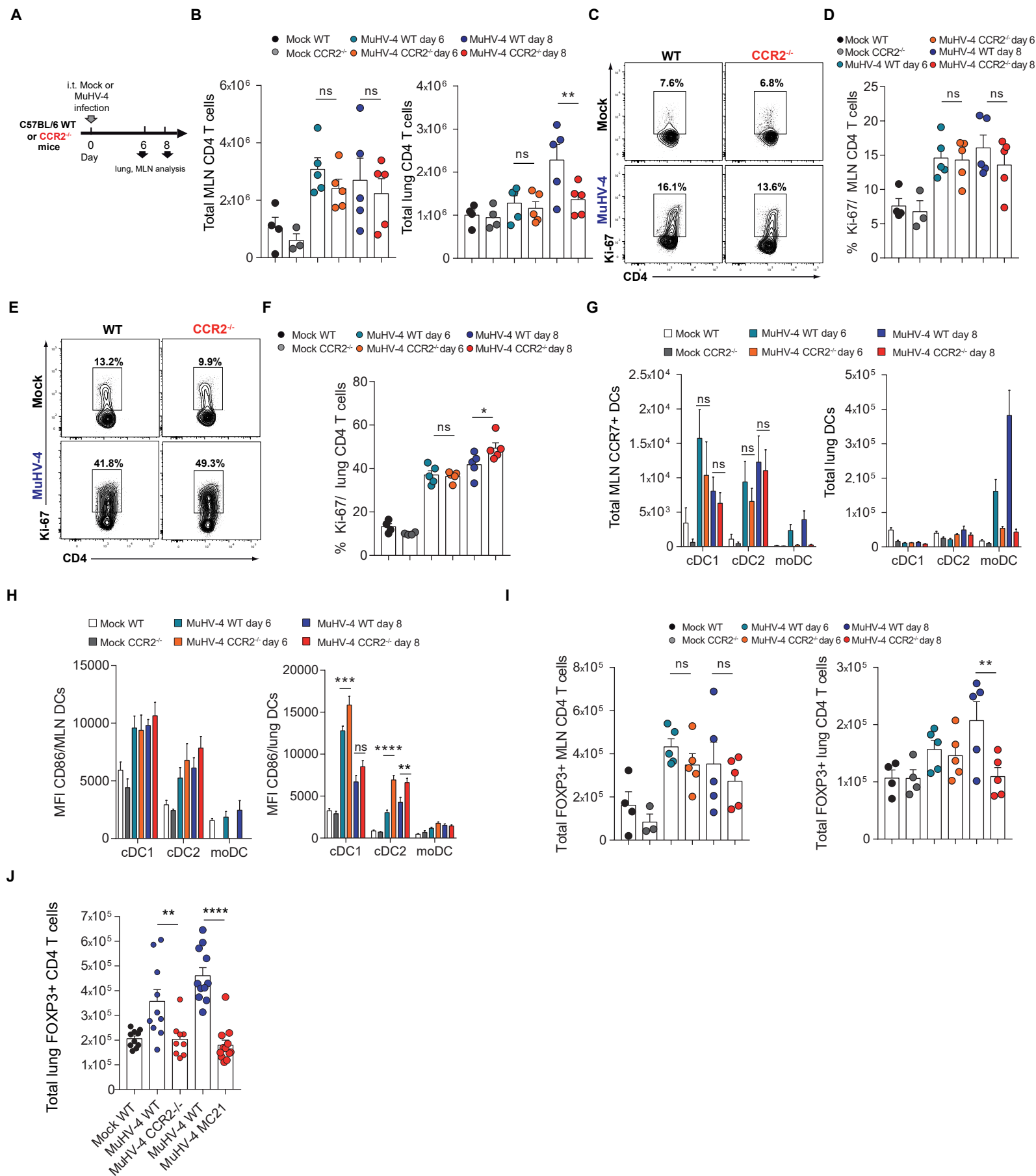


Fig.S5. Absence of MOs does not affect the priming of CD4 T cells but rather modulates local instruction in lung, related to Fig.3 (A) Experimental layout of panels B-I (n= 4 to 5, one experiment). (B) Numbers of CD4 T cells in MLN and lung. (C-F) FACS plots (C and E) and numbers (D and F) of Ki67+ CD4 T cells in MLN (C-D) and lung (E-F) of Mock and MuHV-4 infected mice. (G) Numbers of cDC1 (gated as Live, CD19-, CD11c+, MHCII+, CD103+,CD11b-), cDC2 (gated as Live, CD19-, CD11c+, MHCII+, CD103-,CD11b+, CD64-, Ly6C-) and moDC (gated as Live, CD19-, CD11c+, MHCII+, CD103-,CD11b+, CD64+, Ly6C+) in MLN and lung of Mock and MuHV-4 infected WT and CCR2^{-/-} mice. (H) MFI of CD86 staining on MLN and lung DCs. (I) Numbers of FOXP3+ CD4 T cells in MLN and lung of Mock and MuHV-4 infected mice. (J) Numbers of lung FOXP3-expressing CD4 T cells in Mock or MuHV4 WT or CCR2^{-/-} or MC-21 treated mice at day 8 p.i. (n=9 to 12 mice, one experiment). For comparisons between multiple groups, one-way or two-way ANOVA were used with multiple-comparison tests. (ns, not statistically different, *P < 0.05, **P < 0.01, ***P < 0.001,****P < 0.0001). Error bars represent SEM.

Fig.S6. Lung MOs and CD4 T cells were isolated from mock or MuHV-4 WT and CCR2^{-/-} infected mice for single-cell transcriptomic analysis, related to Fig.4

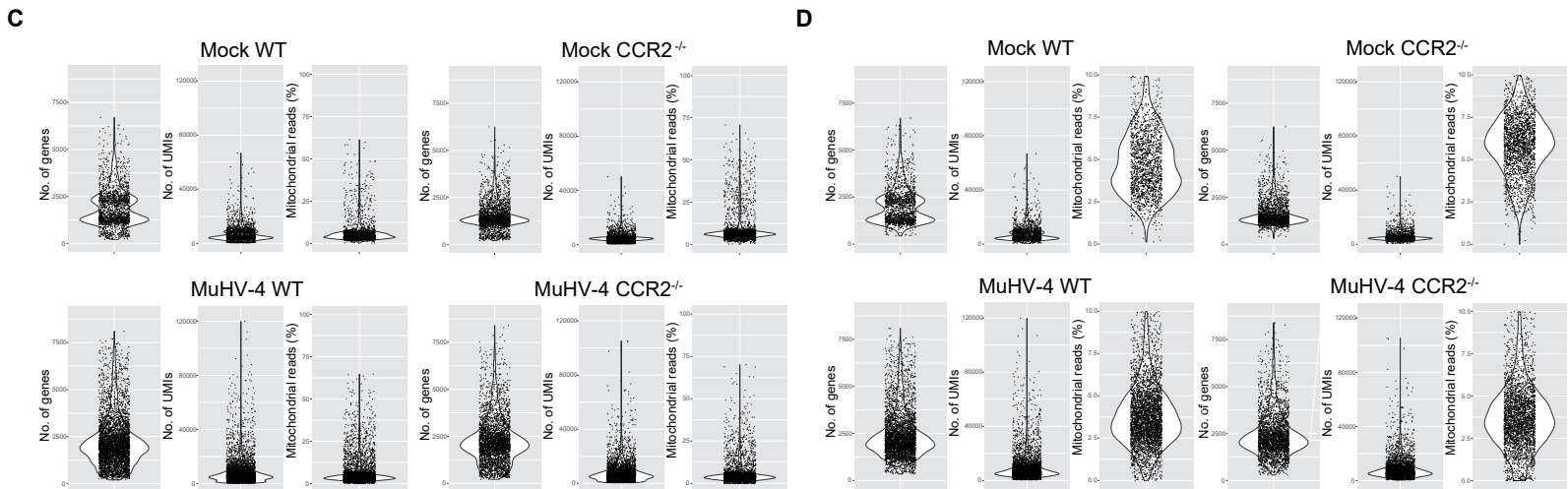
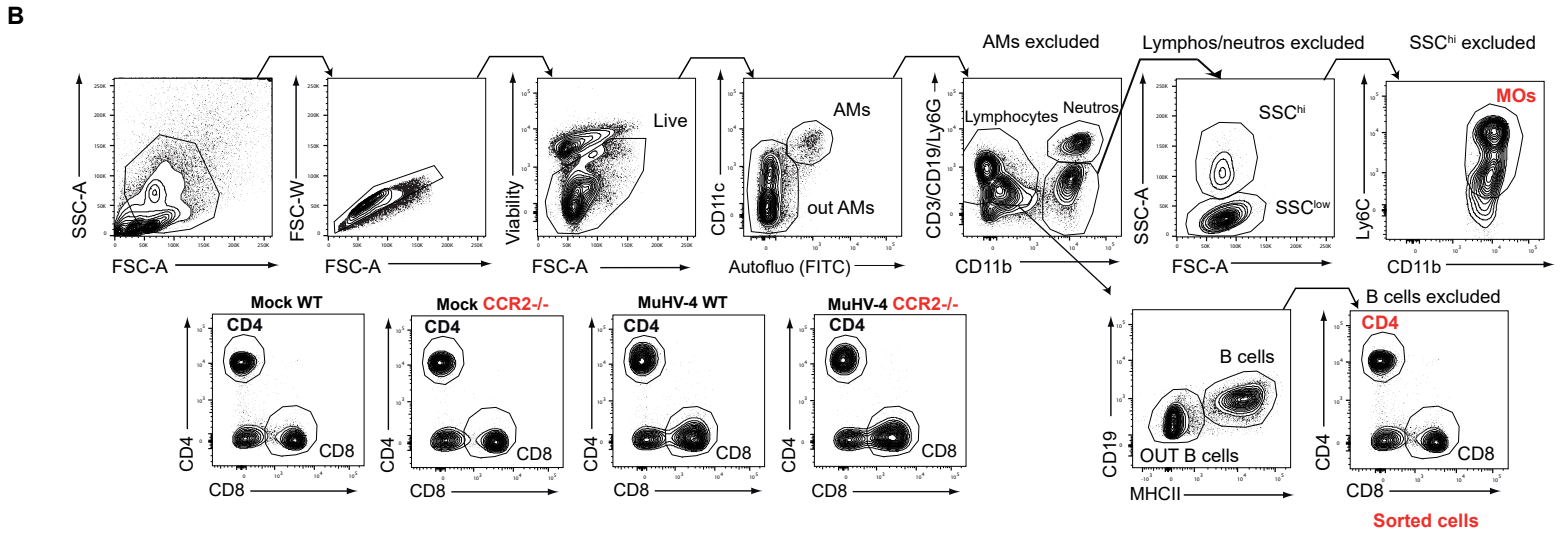
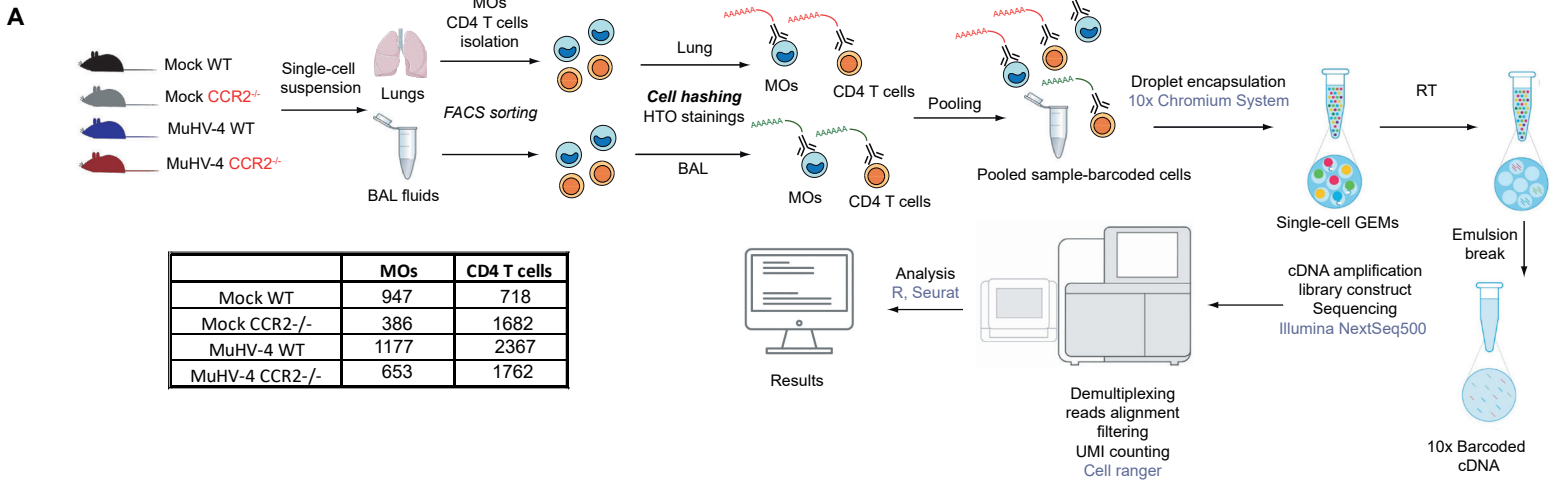


Fig.S6. Lung MOs and CD4 T cells were isolated from mock or MuHV-4 WT and CCR2^{-/-} infected mice for single-cell transcriptomic analysis, related to Fig.4 (A) Experimental layout (n= 4 pooled mice by group, one experiment). (B) Gating strategy for FACS sorting of BALF and lung MOs and CD4 T cells. Cells from a MuHV-4-infected mouse are shown. (C and D) Gene counts, Unique Molecular Identifiers (UMI) counts and percentage of mitochondrial genes detected in the indicated conditions before (C) and after (D) selection and filtering as described in the methods, presented as violin plots (individual dots representing individual cells).

Fig.S7. MuHV-4 imprinted MOs preferentially infiltrate the alveoli and establish a reciprocal dialogue with recruited CD4 T cells, related to Fig.4

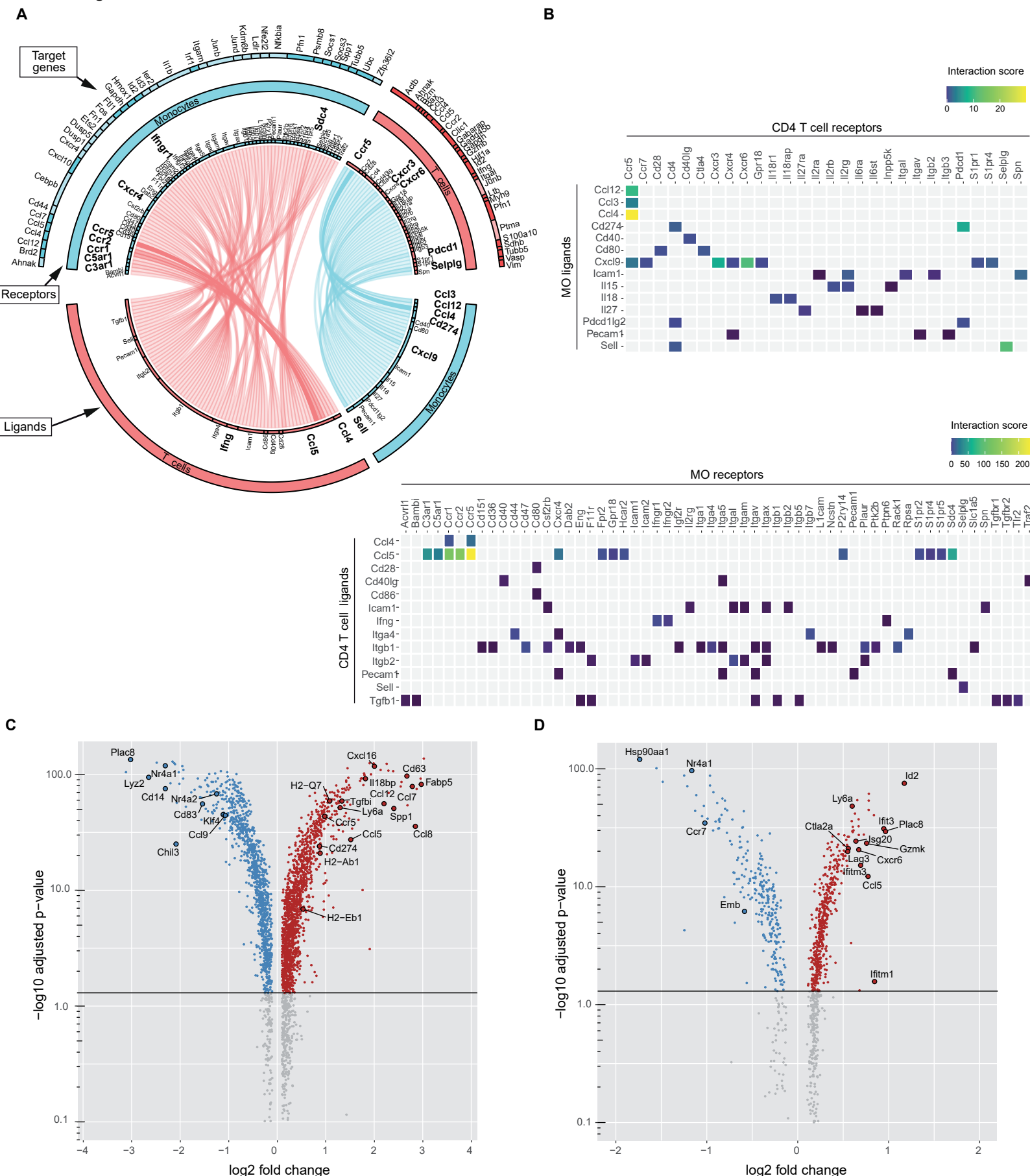


Fig.S7. MuHV-4 imprinted MOs preferentially infiltrate the alveoli and establish a reciprocal dialogue with recruited CD4 T cells, related to Fig.4 (A) Circular plot of the putative interactions between ligands (bottom) on MOs and CD4 T cells based on their expression at day 8 post-MuHV-4 infection. Opacity of the link correlates with the interaction score (alpha from 0,25 to 1). (B) Heatmap of the interaction scores between ligands and receptors expression by MOs and CD4 T cells after MuHV-4 infection. (C) Volcano plot of DE genes between MOs from alveoli or lung parenchyma of MuHV4 infected mice. Only genes with a FDR < 1 are plotted. The horizontal line represents the FDR of 0.05 threshold. Blue and red genes are overexpressed in lung parenchyma or alveolar MOs respectively. (D) Volcano plot of DE genes between CD4 T cells from alveoli or lung parenchyma of MuHV-4 infected mice. Only genes with a FDR < 1 are plotted. The horizontal line represents the FDR of 0.05 threshold. Blue and red genes are overexpressed lung parenchyma or alveolar CD4 T cells respectively.

Fig.S8. Absence of MuHV-4 imprinted MOs increases the numbers of pre-armed cytotoxic CD4 T cells in the airways, related to Fig.5

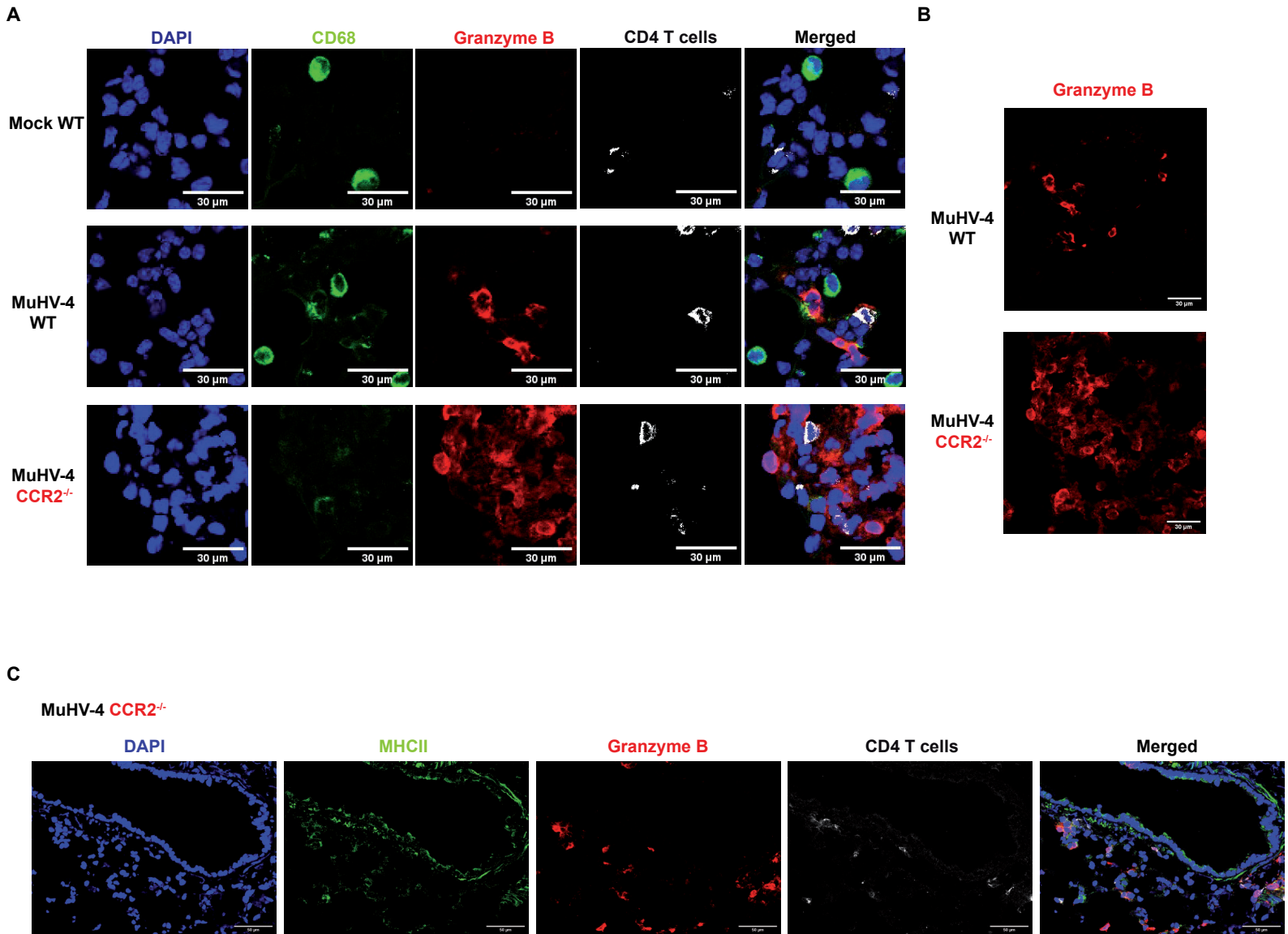


Fig.S8. Absence of MuHV-4 imprinted MOs increases the numbers of pre-armed cytotoxic CD4 T cells in the airways, related to Fig.5 (A) Confocal microscopy intracellular stainings of CD4, CD68 and GrzB in lung of Mock or MuHV-4 infected WT or CCR2^{-/-} mice at day 8 p.i. (B) Confocal microscopy stainings of GrzB in lung of MuHV-4 infected mice at day 8 p.i. (C) Confocal microscopy intracellular stainings of CD4, MHC-II and GrzB in lung of MuHV-4 infected CCR2^{-/-} mice at day 8 p.i. Pictures are representative of more than 6 lungs analysed from 5 different mice.

Fig.S9. Gating strategy of lung MO and CD4 T cell populations identified by flow cytometry isolated from mock or MuHV-4 infected mice, related to Materials and Methods

A

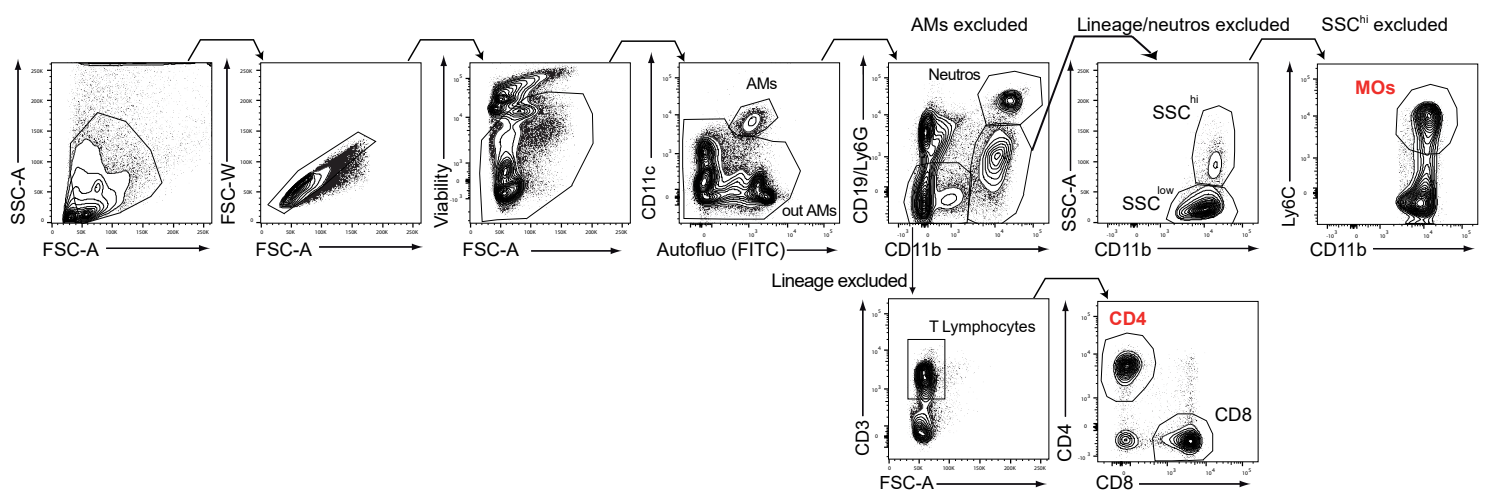


Fig.S9. Gating strategy of lung MOs and CD4 T cells identified by flow cytometry isolated from mock or MuHV-4 infected mice. (A) BALF and lung MOs are gated as described with the representative FACS plots (Live, CD11c non-autofluorescent, CD19-Ly6G-CD11b⁺SSC^{low}Ly6C⁺). BALF and lung CD4 T cells are gated as described with the representative FACS plots (Live, CD11c, non-autofluorescent, CD19-Ly6G-CD11b-CD3⁺CD4⁺).

Table S1. Key resources used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-goat IgG NL557-conjugated Antibody	R&D systems	Cat#NL001, RRID: AB_663766
Anti-mouse CCR2 PE (clone 475301)	R&D Systems	Cat# FAB5538P; RRID: AB_10718414
Anti-mouse CD103 Brilliant Violet 711 (clone: 2E7)	BioLegend	Cat# 121435, RRID: AB_2686970
Anti-mouse CD103, FITC, eBioscience™ (clone: 2E7)	Thermo Fisher Scientific	Cat# 11-1031-82, RRID: AB_465176
Anti-mouse CD107a APC (clone: 1D4B)	BioLegend	Cat# 121613, RRID:AB_2134486
Anti-mouse CD11b Brilliant Violet 605 (clone M1/70)	BioLegend	Cat# 101237, RRID:AB_11126744
Anti-mouse CD11b Brilliant Violet 711 (clone M1/70)	BioLegend	Cat# 101241, RRID:AB_11218791
Anti-mouse CD11c Alexa Fluor 700 (clone N418)	Thermo Fisher Scientific	Cat# 56-0114-82, RRID:AB_493992
Anti-mouse CD16/32 Fc block (clone 93)	BioLegend	Cat# 101301, RRID:AB_312800
Anti-mouse CD172α PerCP/Cyanine 5.5 (clone: P84)	BioLegend	Cat# 144009, RRID: AB_2563547
Anti-mouse CD183 APC (clone CXCR3-173)	BioLegend	Cat# 126511, RRID: AB_1088994
Anti-mouse CD183 FITC (clone CXCR3-173)	BioLegend	Cat# 126535, RRID: AB_2566564
Anti-mouse CD19 Alexa Fluor 700 (clone 6D5)	BioLegend	Cat# 115528, RRID: AB_493735
Anti-mouse CD19 APC/Cyanine7 (clone 6D5)	BioLegend	Cat# 115529, RRID:AB_830706
Anti-mouse CD197 (CCR7) BV421 (clone: 4B12)	BD Biosciences	Cat# 562675, RRID:AB_2737716
Anti-mouse CD24 APC/Cyanine7 (clone:M1/69)	BioLegend	Cat# 101849, RRID:AB_2892262
Anti-mouse CD25 Alexa Fluor 700 (clone PC61)	BioLegend	Cat# 102024, RRID:AB_493709
Anti-mouse CD26 FITC (clone :H194-112)	BD Biosciences	Cat# 559652, RRID: AB_397295
Anti-mouse CD274 APC (clone 10F.9G2)	BioLegend	Cat# 124311, RRID:AB_10612935
Anti-mouse CD279 APC/Fire750 (clone 29F.1A12)	BioLegend	Cat# 135240, RRID:AB_2629768
Anti-mouse CD3 APC (clone 17A2)	BioLegend	Cat# 100236, RRID: AB_2561456
Anti-mouse CD31 PE (clone: MEC13.3)	BioLegend	Cat# 102507, RRID:AB_312914
Anti-mouse CD326 (Ep-CAM) Alexa Fluor 488 (clone: G8.8)	BioLegend	Cat# 118210, RRID:AB_1134099
Anti-mouse CD3e APC-Cy7 (clone 145-2C11)	BD Biosciences	Cat# 561042, RRID:AB_2034003
Anti-mouse CD3e FITC (clone 145-2C11)	BioLegend	Cat# 100306, RRID:AB_312671
Anti-mouse CD4 APC (clone RM 4-5)	BioLegend	Cat# 100515, RRID:AB_312718
Anti-mouse CD4 Brilliant Violet 421 (clone GK1.5)	BioLegend	Cat# 100438, RRID:AB_11203718

Anti-mouse CD4 Monoclonal Antibody, eBioscience™ (clone GK1.5)	Thermo Fisher Scientific	Cat# 14-0041-82, RRID:AB_467063
Anti-mouse CD44 PE/Cyanine 7 (clone IM7)	BioLegend	Cat# 103030, RRID: AB_830787
Anti-mouse CD45 Brilliant Violet 510 (clone 30-F11)	BioLegend	Cat# 103137, RRID:AB_2561392
Anti-mouse CD45 PE/Cyanine7 (clone 30-F11)	BioLegend	Cat# 103114, RRID:AB_312979
Anti-mouse CD45.1 APC (clone A20)	BioLegend	Cat# 110713, RRID:AB_313502
Anti-mouse CD45.2 Brilliant Violet 510 (clone 104)	BioLegend	Cat# 109837, RRID:AB_2561393
Anti-mouse CD49b APC (clone DX5)	BioLegend	Cat# 108910, RRID:AB_313417
Anti-mouse CD62L, eFluor450, eBioscience™ (clone: MEL-14)	Thermo Fisher Scientific	Cat#48-0621-82, RRID: AB_1963590
Anti-mouse CD64 Alexa Fluor 647 (clone: X54-5/7.1)	BioLegend	Cat# 139322, RRID:AB_2566561
Anti-mouse CD68 purified (clone FA-11)	Thermo Fisher Scientific	Cat# 14-0681-82, RRID:AB_2572857
Anti-mouse CD80 Brilliant Violet 421 (clone: 16-10A1)	BioLegend	Cat# 104725, RRID:AB_10900989
Anti-mouse CD86, PE, eBioscience™, (clone: GL1)	Thermo Fisher Scientific	Cat# 12-0862-81, RRID: AB_465767
Anti-mouse CD8a APC (clone 53-6.7)	BioLegend	Cat# 100711, RRID:AB_312750
Anti-mouse CD8α Brilliant Violet 785 (clone 53-6.7)	BioLegend	Cat# 100749, RRID:AB_11218801
Anti-mouse CD8α PE (clone 53-6.7)	BioLegend	Cat# 100708, RRID: AB_312747
Anti-mouse CD8α PerCP/Cyanine5.5 (clone 53-6.7)	BioLegend	Cat# 100733, RRID:AB_2075239
Anti-mouse CXCL9 Alexa Fluor 674 (clone MIG-2F5.5)	BioLegend	Cat# 515606, RRID: AB_1877135
Anti-mouse FcεR1, PE , eBioscience™(clone: MAR-1)	Thermo Fisher Scientific	Cat# 12-5898-82, RRID: AB_466028
Anti-mouse FOXP3, APC, eBioscience™ (clone: FJK-16S)	Thermo Fisher Scientific	Cat#17-5773-80, RRID: AB_469456
Anti-mouse FOXP3, PE, eBioscience™ (clone: FJK-16S)	Thermo Fisher Scientific	Cat#12-5773-80, RRID: AB_465935
Anti-mouse Granzyme A PE (clone: 3G8.5)	BioLegend	Cat# 149703, RRID: AB_2565309
Anti-mouse Granzyme B antibody	R&D systems	Cat#AF1865, RRID: AB_2294988
Anti-mouse Granzyme B FITC (clone: GB11)	BioLegend	Cat# 515403, RRID: AB_2114575
Anti-mouse I-A/I-E Brilliant Violet 785 (clone: M5-114.15.2)	BioLegend	Cat# 107645, RRID:AB_2565977
Anti-mouse I-A/I-E PE/Cyanine7 (clone M5/114.15.2)	BioLegend	Cat# 107630, RRID:AB_2069376
Anti-mouse IFN-γ Brilliant Violet 711 (clone XMG1.2)	BioLegend	Cat# 505836, RRID:AB_2650928
Anti-mouse IL-10 Alexa Fluor 674 (clone:JES5-16E3)	BioLegend	Cat# 505014, RRID: AB_493511
Anti-mouse IL-10 PE (clone: JES5-16E3)	BD Biosciences	Cat# 554467, RRID: AB_395412
Anti-mouse Ki-67 Alexa Fluor 488 (clone: 16A8)	BioLegend	Cat# 652417, RRID:AB_2564236

Anti-mouse Ly6A/E FITC (clone D7)	BioLegend	Cat# 122506, RRID:AB_756191
Anti-mouse Ly6C Brilliant Violet 785 (clone HK1.4)	BioLegend	Cat# 128041, RRID:AB_2565852
Anti-mouse Ly-6C PE/Cyanine 7 (clone HK1.4)	BioLegend	Cat# 128017, RRID: AB_1732093
Anti-mouse Ly-6G Alexa Fluor 700 (clone 1A8)	BioLegend	Cat# 127622, RRID:AB_10643269
Anti-mouse Ly6G APC-Cy7 (clone 1A8)	BD Biosciences	Cat# 560600, RRID:AB_1727561
Anti-mouse Perforin, PE, eBioscience™ (clone: eBioOMAK-D)	Thermo Fisher Scientific	Cat# 12-9392-80, RRID: AB_466242
Anti-mouse XCR1 Brilliant Violet 421 (clone: ZET)	BioLegend	Cat# 148216, RRID: AB_2565230
Anti-rat IgG2a heavy chain Alexa Fluor 488	abcam	Cat# ab172332 RRID:AB_2893134
Anti-rat IgG2b Alexa Fluor 647 Antibody (clone: MRG2b-85)	BioLegend	Cat#408208, RRID: AB_2715912
Goat Anti-Mouse IgG (H+L), BIOT	SouthernBiotech	Cat# 1036-08 RRID:AB_2794349
Goat Anti-Mouse IgG2a, BIOT	SouthernBiotech	Cat# 1080-08 RRID:AB_2794479
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555	Thermo Fisher Scientific	Cat# A-21428, RRID:AB_2535849
MHC Class II (I-Ab) Polyclonal Antibody	Thermo Fisher Scientific	Cat#PA5-116876, RRID: AB_2893126
Purified anti-mouse CD16/32 Antibody	BioLegend	Cat#101302, RRID: AB_312801
Purified anti-mouse CD28 Antibody	BioLegend	Cat#102102, RRID: AB_312867
TotalSeq™-A0305 anti-mouse Hashtag 5 Antibody	BioLegend	Cat#155809, RRID: AB_2750036
TotalSeq™-A0306 anti-mouse Hashtag 6 Antibody	BioLegend	Cat#155811, RRID: AB_2750037
Ultra-LEAF™ Purified anti-mouse CD3ε Antibody	BioLegend	Cat#100340, RRID: AB_11149115
Bacterial and Virus strains		
MHV-68 pH43 strain (MuHV-4)	Stevenson Laboratory, Gillet Laboratory	Adler et al., 2000
Luciferase-expressing MuHV-4	Stevenson Laboratory, Gillet Laboratory	Milho et al., 2009
Chemicals, peptides, and recombinant proteins		
1X RBC Lysis Buffer	Thermo Fisher Scientific	Cat# 00433357
AKP Streptavidin	BD Biosciences	Cat# 554065
Brefeldin A Solution	BioLegend	Cat# 420601
Collagenase D	Roche	Cat# 11088866001
cOmplete™ Protease Inhibitor Cocktail	Roche	Cat# 11697498001
Corn oil	Sigma-Aldrich	Cat# C-8267
DAPI (4',6-Diamidino-2-Phenylindole, Dilactate)	BioLegend	Cat# 422801
Dextran, Tetramethylrhodamine, 3000 MW, Anionic	Thermo Fisher Scientific	Cat# D3307

DNase I	Roche	Cat# 11284932001
Emdotrim 10% SOL	Emdoka	N/A
Evan's Blue	Sigma-Aldrich	Cat# E2129
Fixable Viability Dye eFluor 780	Thermo Fisher Scientific	Cat# 65-0865-14
H-2D(b) Murid herpesvirus 4 ORF6 487-495 AGPHNDMEI Brilliant Violet 421-Labeled Tetramer	NIH Tetramer Core Facility	N/A
H-2K(b) Murid herpesvirus 4 ORF61 524-531 TSINFVKI Brilliant Violet 421-Labeled Tetramer	NIH Tetramer Core Facility	N/A
InVivoMAb anti-mouse Ly6G	BioXCell	Cat# BE0075-1
InVivoMAb anti-mouse PD-1 (CD279)	BioXCell	Cat# BE0146
InVivoMAb anti-mouse PD-L1 (B7-H1)	BioXCell	Cat# BE0101
Lipopolysaccharides from Escherichia coli O55:B5	Sigma-Aldrich	Cat# L2880
LiveBLAzer™ FRET-B/G Loading Kit with CCF4-AM	Thermo Fisher Scientific	Cat# K1095
Monensin Solution	BioLegend	Cat# 420701
Probenecid, Water Soluble, Invitrogen™	Thermo Fisher Scientific	Cat# P36400
ProLong™ Gold Antifade Mountant mounting media	Thermo Fisher Scientific	Cat# P10144
Scigen O.C.T compound cryostat embedding medium	Fisher scientific	Cat# 23-730-625
SIGMAFAST™ p-Nitrophenyl phosphate Tablets	Sigma-Aldrich	Cat# N-1891
Tamoxifen	Sigma-Aldrich	Cat# T-5648
Tuerk solution	Sigma-Aldrich	Cat# 93770
XenoLight D-Luciferin - K+ Salt Bioluminescent Substrate	PerkinElmer	Cat# 122799
Zombie Aqua Fixable Viability Kit	BioLegend	Cat# 423101
Zombie Violet Fixable Viability Kit	BioLegend	Cat# 423113
β-mercaptoethanol	Sigma-Aldrich	Cat# 3148
Critical commercial assays		
FoxP3/transcription factor staining buffer kit	Thermo Fisher Scientific	Cat# 00-5523-00
GentleMACS C tube	Miltenyi	Cat# 130-093-237
iQ™ Supermix	Bio-Rad	Cat# 170-8860
LEGENDplex™ Customized Mouse Anti-Virus Response Panel (13-plex) with V-bottom Plate	BioLegend	N/A
LEGENDplex™ Mouse Anti-Virus Response Panel (13-plex) with V-bottom Plate	BioLegend	Cat#740622

Mouse Granzyme B ELISA Ready-SET-Go	Thermo Fisher Scientific	Cat# 88-8022-88, RRID:AB_2575171
Mouse IFN gamma ELISA Ready-SET-Go	Thermo Fisher Scientific	Cat# 88-7314-88, RRID:AB_2575070
QIAamp DNA mini kit	Qiagen	Cat# 513004
Experimental models: Cell lines		
Baby hamster kidney (BHK)-21 cells	ATCC	ATCC Cat# CCL-10, RRID:CVCL_1915
Experimental models: Organisms/strains		
Mouse : C57BL/6J (JAX™)	Charles River	Cat# JAX:000664, RRID:IMSR_JAX:000664)
Mouse: B6 Albino	Charles River	Cat#CRL:493, RRID:IMSR_CRL:493
Mouse : BALB/cAnNCrI	Charles River	Cat# CRL:028, RRID:IMSR_CRL:028
Mouse : C57BL/6 CD45.1 ⁺	Charles River	Cat# CRL:494, RRID:IMSR_CRL:494
Mouse : C57BL/6 CD45.1 ⁺ .2 ⁺	GIGA	N/A
Mouse : C57BL/6 CCR2 ^{-/-}	The Jackson Laboratory	Cat# JAX :004999, RRID:IMSR_JAX:004999
Mouse : C57BL/6 IL10 ^{-/-}	The Jackson Laboratory	Cat# JAX :002251, RRID:IMSR_JAX:002251
Mouse : C57BL/6 IL10 ^{lox-lox}	GIGA	N/A
Mouse : C57BL/6 CD4 Cre	GIGA	N/A
Mouse: C57BL/6 CD274tm1Pfal (PD-L1fl/fl)	Prof P .Fallon	Schwartz et al., 2018
Mouse : IL-10-β-lactamase reporter C57BL/6	GIGA	Bouabe et al., 2011
Mouse: Ccr2-creER ^{T2} mKate2	Prof B. Becher (Zurich University)	Croxford et al., 2015
Oligonucleotides		
MuHV-4 ORF25 fwd : 5'-atggtatagccgcctttgtg-3'	Eurogentec	N/A
MuHV-4 ORF25 reverse : 5'-acaagtgatgaagggttc-3'	Eurogentec	N/A
Software and algorithms		
FlowJo software v10	Three Star	https://www.flowjo.com
GraphPad Prism 7	GraphPad	https://www.graphpad.com/scientific-software/prism/
Fiji software	ImageJ	https://imagej.net/software/fiji/
R (v.4.1.0), R package Seurat (v.4.0.3)	The R Foundation	http://www.r-project.org/
Living image software v4.1	PerkinElmer	https://www.perkinelmer.com/
OlyVIA Software 3.2	Olympus	https://olyvia.software.informer.com/
Other		

Table S2. Clinical evaluation grid

Parameters	0	1	2	3
Activity	Active	Isolated	Inactive, depressed	Moribund
Posture	Normal	Slightly hunched	Hunched	Hunched and trembling
Gait	Normal	Awkward	Reluctance to move, incoordination, tiptoe walking	Very slow movement or paralysis or decubitus
Coat	Well groomed	Not well groomed	Rough hair coat	Very rough hair coat, self-mutilating
Breathing	Normal	Rapid and shallow breathing,	Rapid and abdominal breathing	Dyspnea, Tachypnea, irregular rhythm
Alertness	Normal	Mildly agitated	Nonresponsive when coaxed	Violent reaction to stimuli or unconsciousness
Weight	Normal, body score condition (BCS) 3,4	Weight loss < 10 %	Weight loss >10% BCS 2	Weight loss > 20%, BCS 1
Dehydration	Hydration	Decreased elasticity of the skin	Increased skin fold	Severe, eyes sunken, persistent skin fold