Figure S1. Nasal-only inoculation. A: BALB/c mice were intranasally treated with 1% Evan Blue in PBS at the indicated volume per nostril (*X 2*; for the 50 µl group, only one nostril) under light anesthesia using isoflurane. Mice were euthanized 10 min later and bronchoalveolar fluids (BALF) obtained. The OD values at 610 nm were measured to detect aspirated Evan's blue in the lungs. N=6-9 per group except N=3 in the positive control group (50 µl). B: radiolabeled sulfur colloid solution (5 µl per nostril) was intranasally given to mice after light anesthesia using isoflurane. Radioactivity was then determined scintigraphically 20 min later. n=3. C: BALB/c mice were intranasally infected with MHV-1 (10^4 PFU in 2 µl MEM, 1 µl /nostril). The mice were then sacrificed on days 1, 2, 4, 6 and 8. Controls were mice treated with vehicle. Viral titers were measured in both nasal lavage fluids (NLF) and the lungs. dpi= day post infection. N=3 per group. **p<0.01 vs. all others. D: BALB/c mice were intranasally infected with MHV-1 (10^4 PFU in 2 µl MEM, 1 µl/nostril) or vehicle, and monitored for changes in weight. Positive controls were mice intratracheally infected with MHV-1 (10^4 PFU in 50µl MEM). **p<0.01, positive controls vs. mice treated intranasally with either 2 µl vehicle or MHV-1. n=4-6 per group. E: BALF cell numbers after nasal MHV-1 infection (10^4 PFU in 2 µl MEM, 1 µl /nostril). Controls were mice treated with vehicle. N=5-6 per group. F: Cell numbers in both the deep cervical lymph nodes and mediastinal lymph nodes 2 days after nasal infection. DCLN=deep cervical lymph nodes; MLN= mediastinal lymph nodes. **p<0.01, vs. controls in DCLN. N=3-4 per group. G-L: nasal associated lymphoid tissue (NALT) was isolated microscopically in mice after MHV-1 nasal-only inoculation and controls. Cervical lymph nodes (CLN, including both

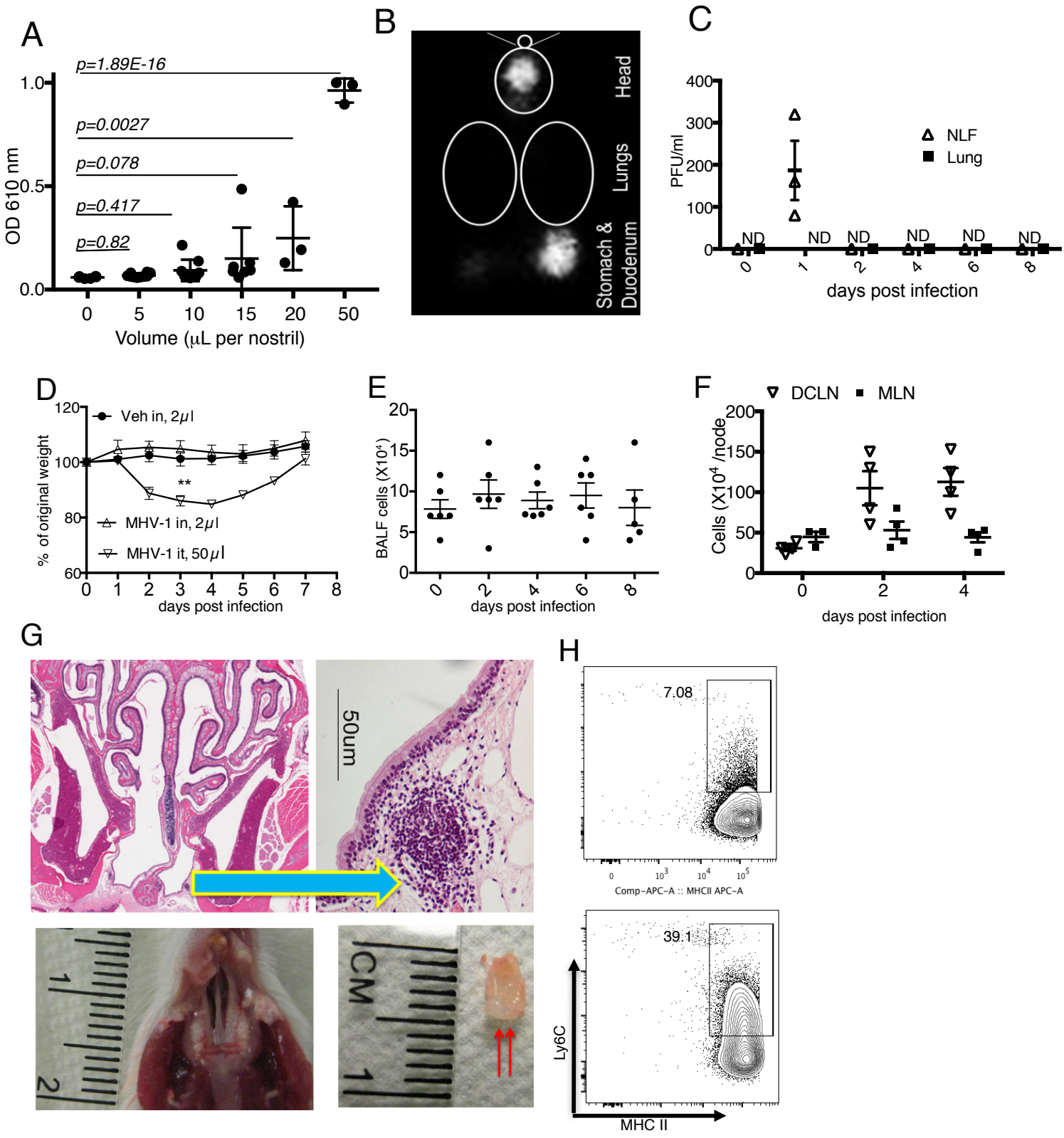
superficial and deep groups) were harvested as indicated. G: Histology and surgical isolation of the NALT. H: Increased expression of Ly6C in B cells in the NALT 12-15h after MHV-1 intranasal inoculation. Cells analyzed were $CD45^+CD19^+$ cells. I and J: T/B ratio in the SCLN, DCLN and NALT after MHV-1 inoculation at the indicated time points. Cells analyzed were $CD45^+$ cells. **P<0.01 vs. 12h and 24h p.i. by ANOVA. N=5=7 per group. K: Reduced B cell numbers in the NALT 12h and 24h after MHV-1 inoculation. **P<0.01 vs. 12h and 24h p.i. by ANOVA. n=5-7.

Figure S2. A: Reduced T and B cell frequencies and increased $Ly6C^+$ I.M. (inflammatory monocytes) in the lungs of mice after nasal-only inoculation. BALB/c mice were intranasally infected with MHV-1 (2 μl, 10^4 PFU). Their lungs were then analyzed on days 2 and 4 p.i.. The frequencies of $Ly6C^+$ I.M., T cells and B cells were analyzed. **p<0.01, *p<0.05, vs. controls (day 0). n=3-5 per group. B and C: NK cell infiltration in the lungs after nasal-only inoculation**.** NK cells in the lungs of mice with nasal-only MHV-1 inoculation were analyzed using flow cytometry. B: flow cytometric plots at p.i. Day 0, 2, 4 and 20. C: NK cell frequency at days 0, 2, 4 and 20 p.i. Data presented as % of CD45+ cells. n=3-10. D and E: MHV-JHM intranasal infection recruited $Ly6C^+$ I.M. into the lungs. BALB/c mice were intranasally infected with MHV-JHM (2 μl, $3x10^3$ PFU). Lungs were then analyzed on day 2 p.i. The frequencies of $Ly6C^+$ I.M. were analyzed. * p<0.05, vs. controls. N=3 per group

Figure S3. $Ly6C^+$ I.M. (inflammatory monocytes) lung infiltration in unilaterally vagotomized mice. Mice were anesthetized using isoflurane. Unilateral vagotomy was performed. Mice were then intranasally treated with MHV-1 (2 μl, 10^4 PFU) 14 days later. $Ly6C^+$ I.M. infiltration in the lungs was analyzed and compared between the vagotomized

vs. sham-treated sides in the same animals. Data were presented as % of the $CD45^+$ cells in the lungs. $P>0.05$.

Figure S1


A
p=1.89E-16
p=0.0027
p=0.078
p=0.417
p=0.82
OD 610 nm
Volume (μL per nostril)
B
Head
Lungs
Stomach & Duodenum
C
PFU/ml
NLF
Lung
ND
days post infection
D
% of original weight
Veh in, 2μl
MHV-1 in, 2μl
MHV-1 it, 50μl
**
days post infection
E
BALF cells (X10⁴)
days post infection
F
DCLN
MLN
Cells (X10⁴ /node
days post infection
G
50um
H
7.08
Comp-APC-A :: MHCII APC-A
39.1
Ly6C
MHC II

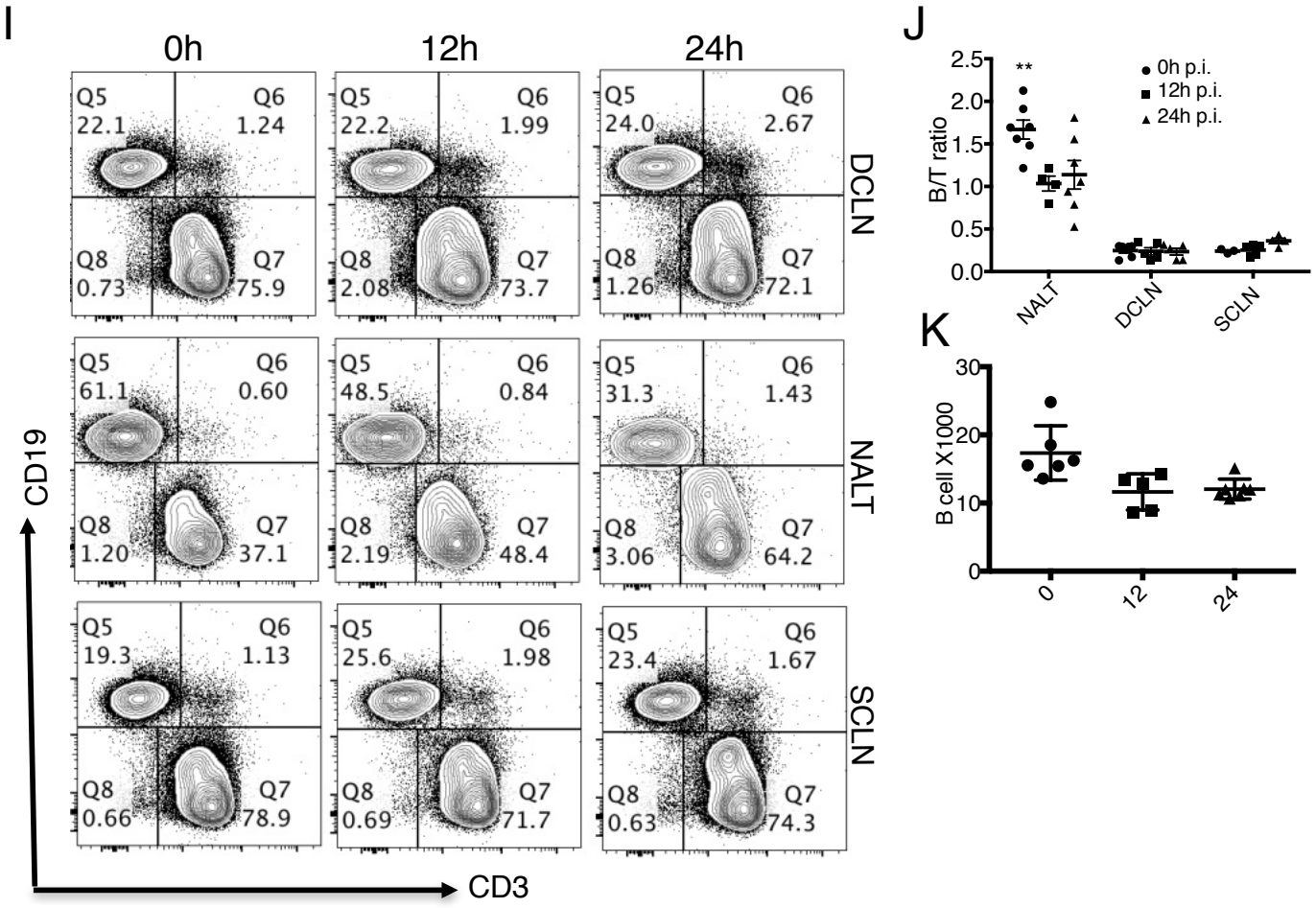

I
0h
12h
24h
DCLN
NALT
SCLN
CD19
CD3
Q5 22.1
Q6 1.24
Q8 0.73
Q7 75.9
Q5 22.2
Q6 1.99
Q8 2.08
Q7 73.7
Q5 24.0
Q6 2.67
Q8 1.26
Q7 72.1
Q5 61.1
Q6 0.60
Q8 1.20
Q7 37.1
Q5 48.5
Q6 0.84
Q8 2.19
Q7 48.4
Q5 31.3
Q6 1.43
Q8 3.06
Q7 64.2
Q5 19.3
Q6 1.13
Q8 0.66
Q7 78.9
Q5 25.6
Q6 1.98
Q8 0.69
Q7 71.7
Q5 23.4
Q6 1.67
Q8 0.63
Q7 74.3
J
B/T ratio
2.5
2.0
1.5
1.0
0.5
0.0
**
0h p.i.
12h p.i.
24h p.i.
NALT
DCLN
SCLN
K
B cell X1000
30
20
10
0
0
12
24

Figure S2

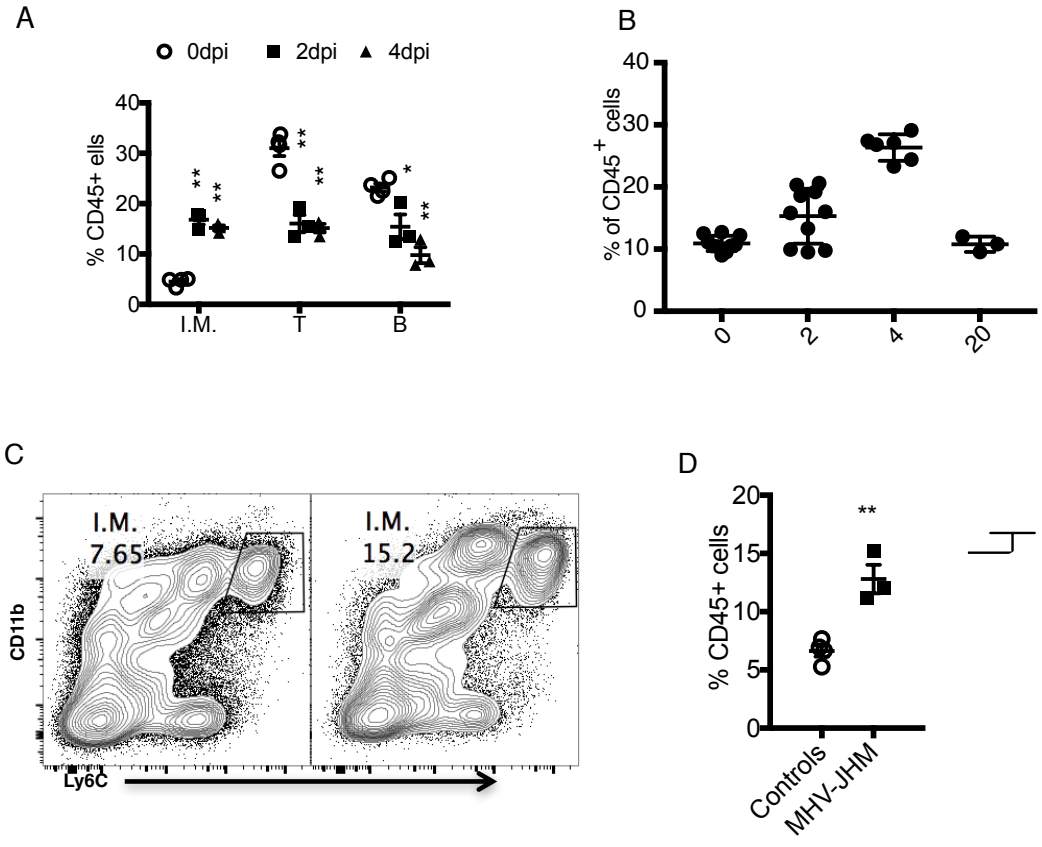
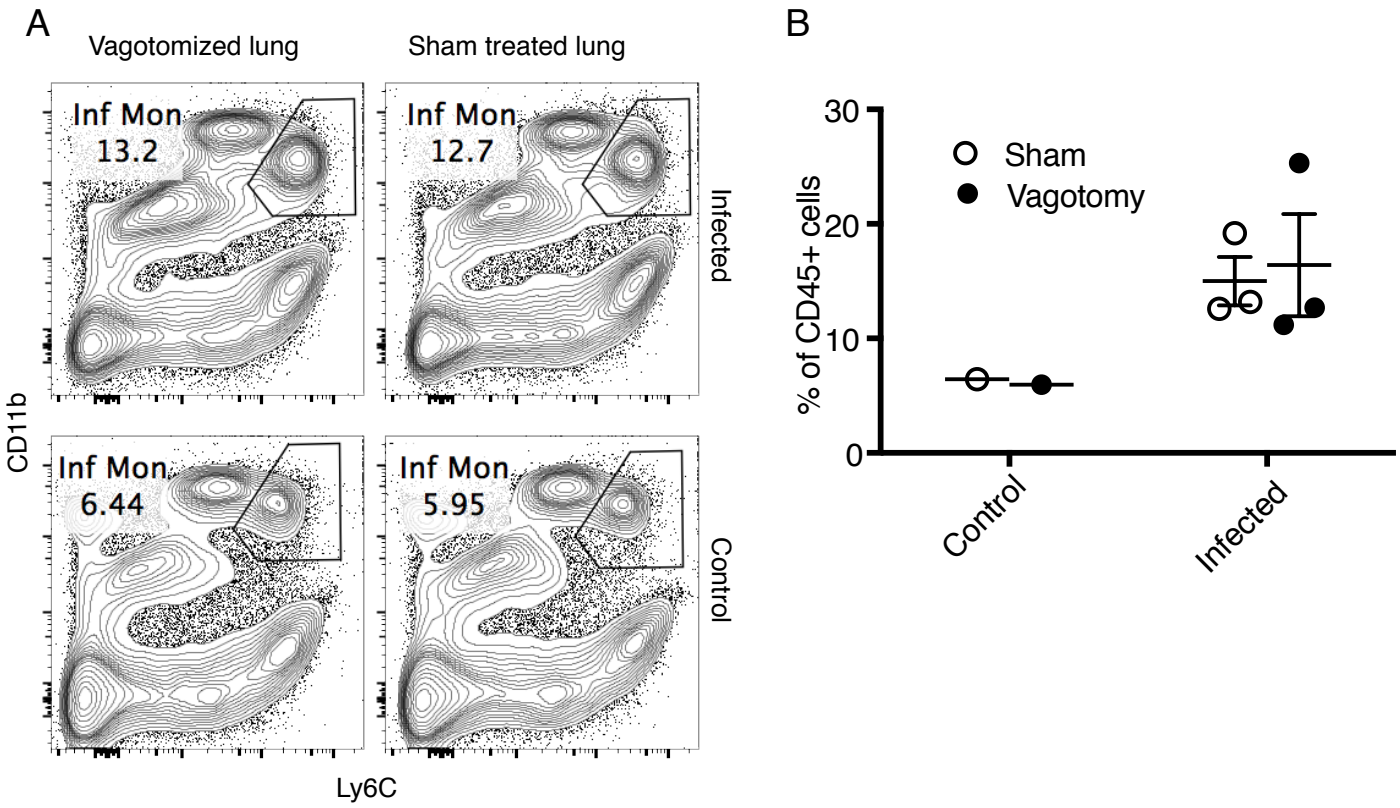

A
0dpi
2dpi
4dpi
% CD45+ ells
40
30
20
10
0
I.M.
T
B
B
% of CD45+ cells
40
30
20
10
0
0
2
4
20
C
I.M.
7.65
I.M.
15.2
CD11b
Ly6C
D
% CD45+ cells
20
15
10
5
0
**
Controls
MHV-JHM

Figure S3


A
Vagotomized lung
Sham treated lung
Inf Mon
13.2
Inf Mon
12.7
Inf Mon
6.44
Inf Mon
5.95
Infected
Control
CD11b
Ly6C
B
% of CD45+ cells
30
20
10
0
Sham
Vagotomy
Control
Infected