Supplementary Figure Legends

Supplementary Figure 1. Characterization of Ad-siA20 vector. (**A**) Schematic diagrams of recombinant adenoviral vectors. The small hairpin mouse A20 siRNA oligo duplexes (5'-GATCCCCCAAAGCACTTATTGACAGATTCAAGAGATCTGTCAATAAGTGCTTTGTTTT TGGAAA-3') were first cloned into SpeI and ClaI-cut pSuper vector. The recombinant Ad vector Ad-siA20 was constructed to express siA20 by inserting the H1 RNA polymerase promoter-siA20 DNA fragment into the PacI-cut Ad-Easy shuttle vector. A control recombinant vector expressing siGFP under the H1 promoter was also constructed. (B) Transfection efficiency of BM-DCs with different MOIs (ifu) of a recombinant Ad viruses expressing GFP marker. (C) Downregulation of A20 mRNA in BM-DCs transduced with different MOIs (ifu) of Ad-siA20 with or without LPS stimulation (100 ng/ml for 18 hr) by qRT-PCR (Left panel). Mean fluorescent intensity of intracellular A20 protein staining with the anti-A20 antibody (Santa Cruz Biotechnology, #sc-32523) of Ad-siGFP and Ad-siA20-BM-DCs (MOI of 1000 ifu) with or without LPS stimulation (100 ng/ml for 18 hr) (Right panel).

Supplementary Figure 2. Comparison of Ad-siA20-BM-DC and gp120 protein/IFA systemic immunization. Groups of C57BL/6 mice (n=4-6) were systemically immunized with Ad-transduced BM-DCs pulsed with gp120 (10 μg/ml) or gp120 proteins formulated in IFA (10 μg/mouse) via footpads followed by i.p. administration of PolyI:C (50 μg/mouse) twice at a

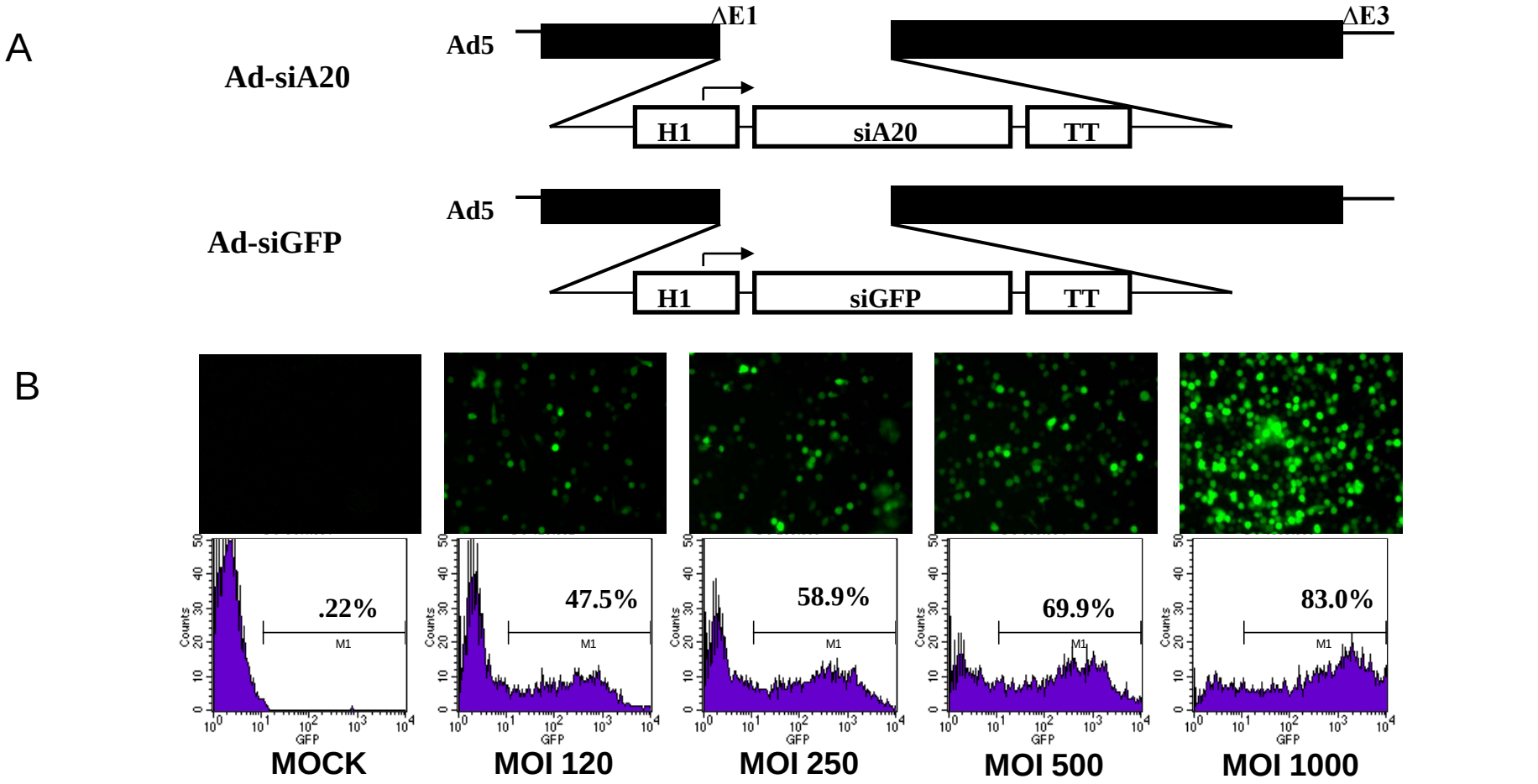
weekly interval. Two weeks after the second immunization, the mice were sacrificed and gp120-specific mucosal T cell responses were analyzed by ICS of mesenteric LN cell suspensions (**A**). Vaginal lavages were collected to determine gp120-specific IgA response by ELISA (**B**). The data are presented from one representative experiment of two independent experiments.

Supplementary Figure 3. In vitro primed OT-II cells by Ad-siA20 DCs enhance migration to the secondary lymphoid tissues after adoptive transfer. The isolated OT-II cells were cocultured with OT-II peptide-pulsed DCs for 3-5 days. The cocultured OT-II cells ($5x10^6$) were harvested and labeled with 25 uM CFSE and adoptively transferred into naïve C57BL/6 mice by retro-orbital injection. 16 hrs after the adoptive transfer, migration of the labeled T cells to the mesenteric LNs, draining LNs, or spleen in the recipient mice was analyzed by flow cytometric assay.

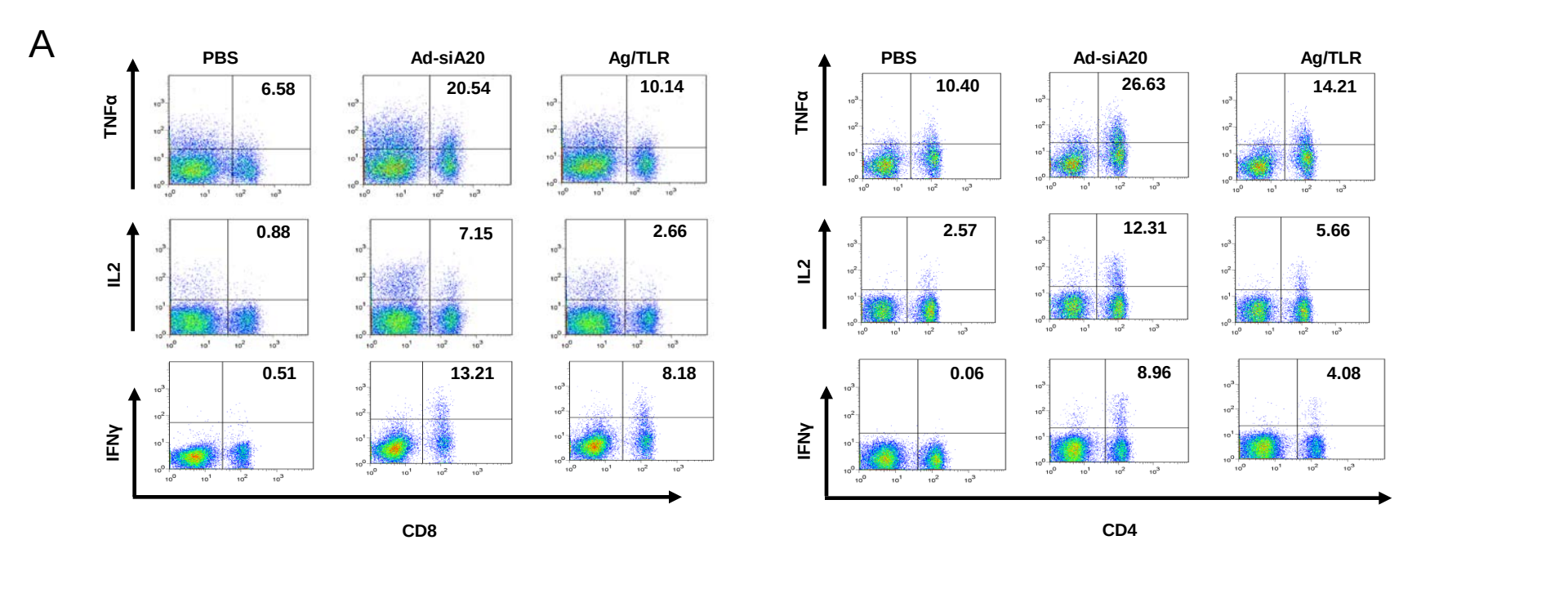
Supplementary Figure 4. Enhanced maturation of Ad-siA20-BM-DCs. Maturation markers on Ad-siA20 or Ad-siGFP transduced BM-DCs without or with LPS stimulation (100 ng/ml for 18 hrs) were examined by flow cytometric assays. The experiment was repeated twice.

Supplementary Figure 5. Survivability of Ad-siA20-transfected BM-DCs and endocytic activity of Ad-siA20-BM-DCs. A. Mouse BM-DCs were transduced with Ad-siGFP or Ad-siA20 at MOI of 500. 24 hrs after transduction, the DCs were cultured in the presence or absence of LPS (100 ng/ml) for 18 hrs. The data of PI and Annexin V (BD Bioscience) staining

are presented from one of three repeated experiments. **B**. Endocytic activity of Ad-siA20-BM-DCs and Ad-siGFP-BM-DCs was measured by analysis of Dextran-Texas-Red (Invitrogen) internalization at 90 min after incubating at **4**^{o}C or at 37^{o}C via flow cytometric assays.



Supplementary Fig. S1


A
PBS
Ad-siA20
Ag/TLR
TNFα
IL2
IFNγ
6.58
20.54
10.14
0.88
7.15
2.66
0.51
13.21
8.18
CD8
10.40
26.63
14.21
2.57
12.31
5.66
0.06
8.96
4.08
CD4

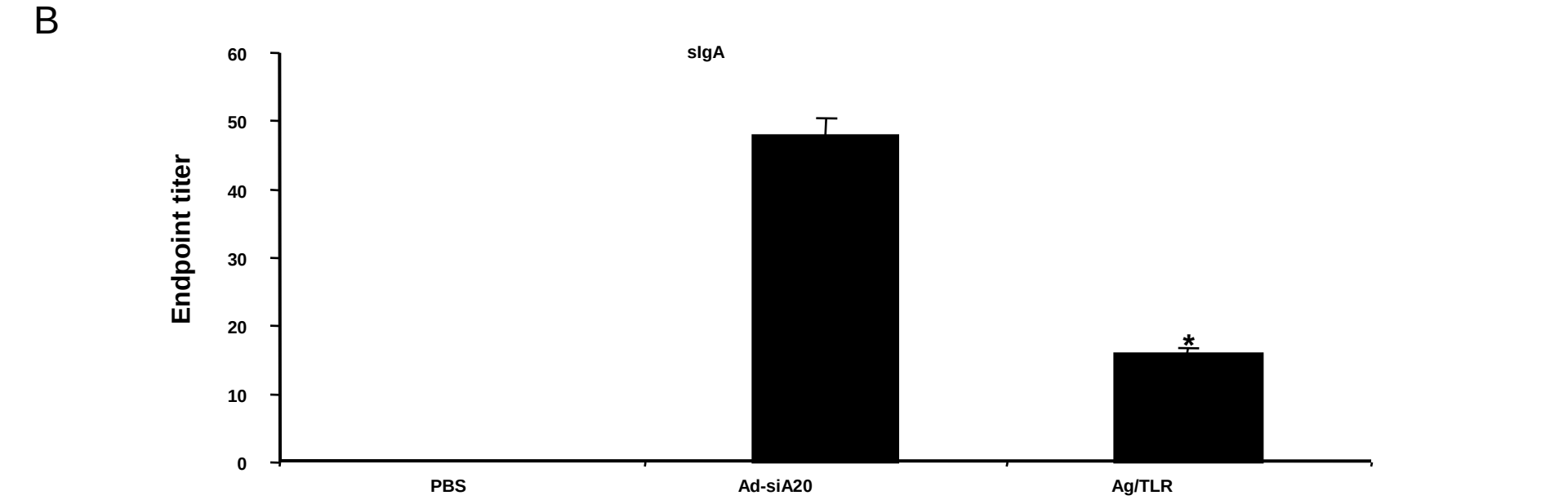

B
sIgA
Endpoint titer
60
50
40
30
20
10
0
*
PBS
Ad-siA20
Ag/TLR

Fig. S2

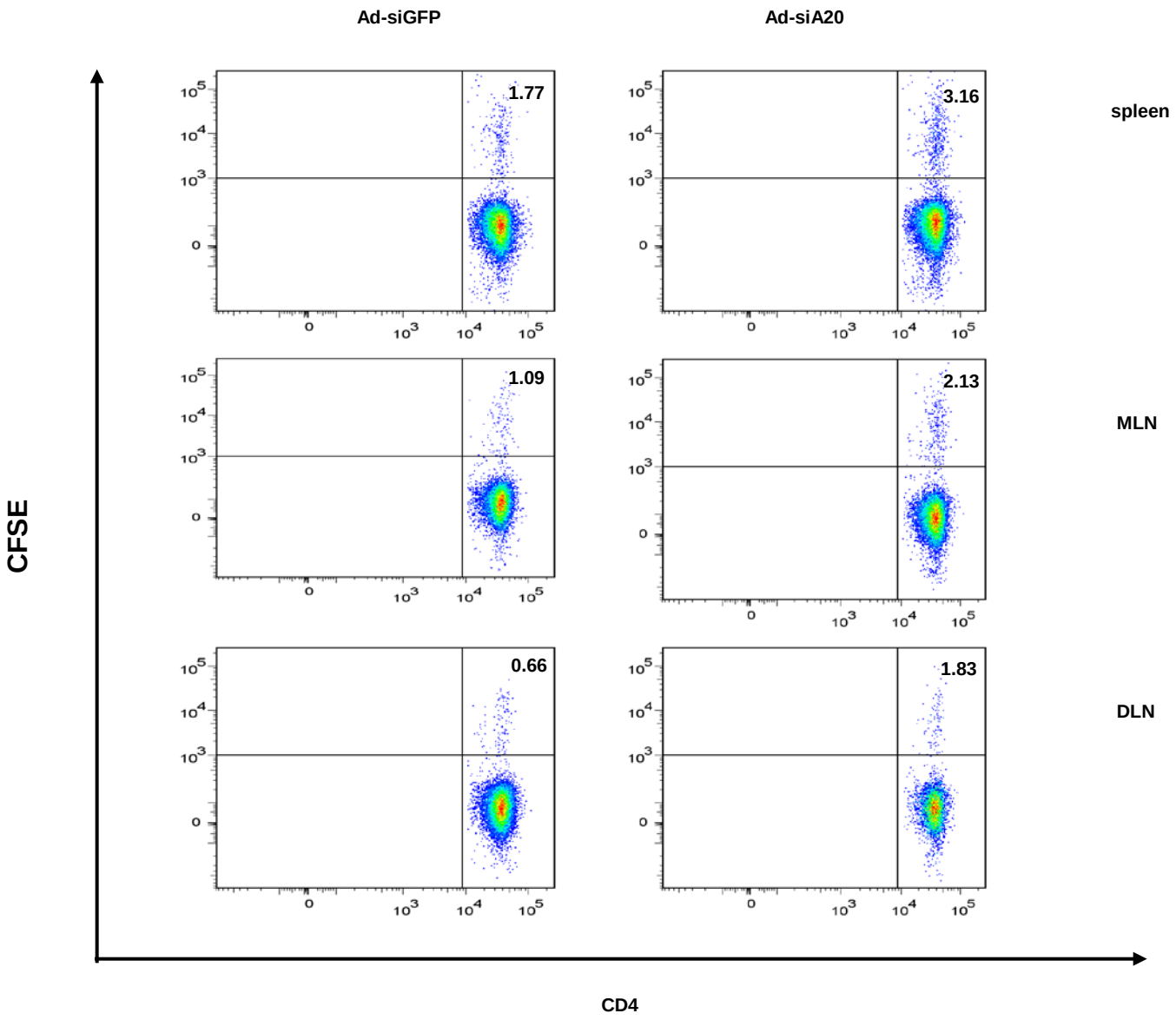

Ad-siGFP
Ad-siA20
spleen
MLN
DLN
1.77
3.16
1.09
2.13
0.66
1.83
CFSE
CD4

Fig. S3

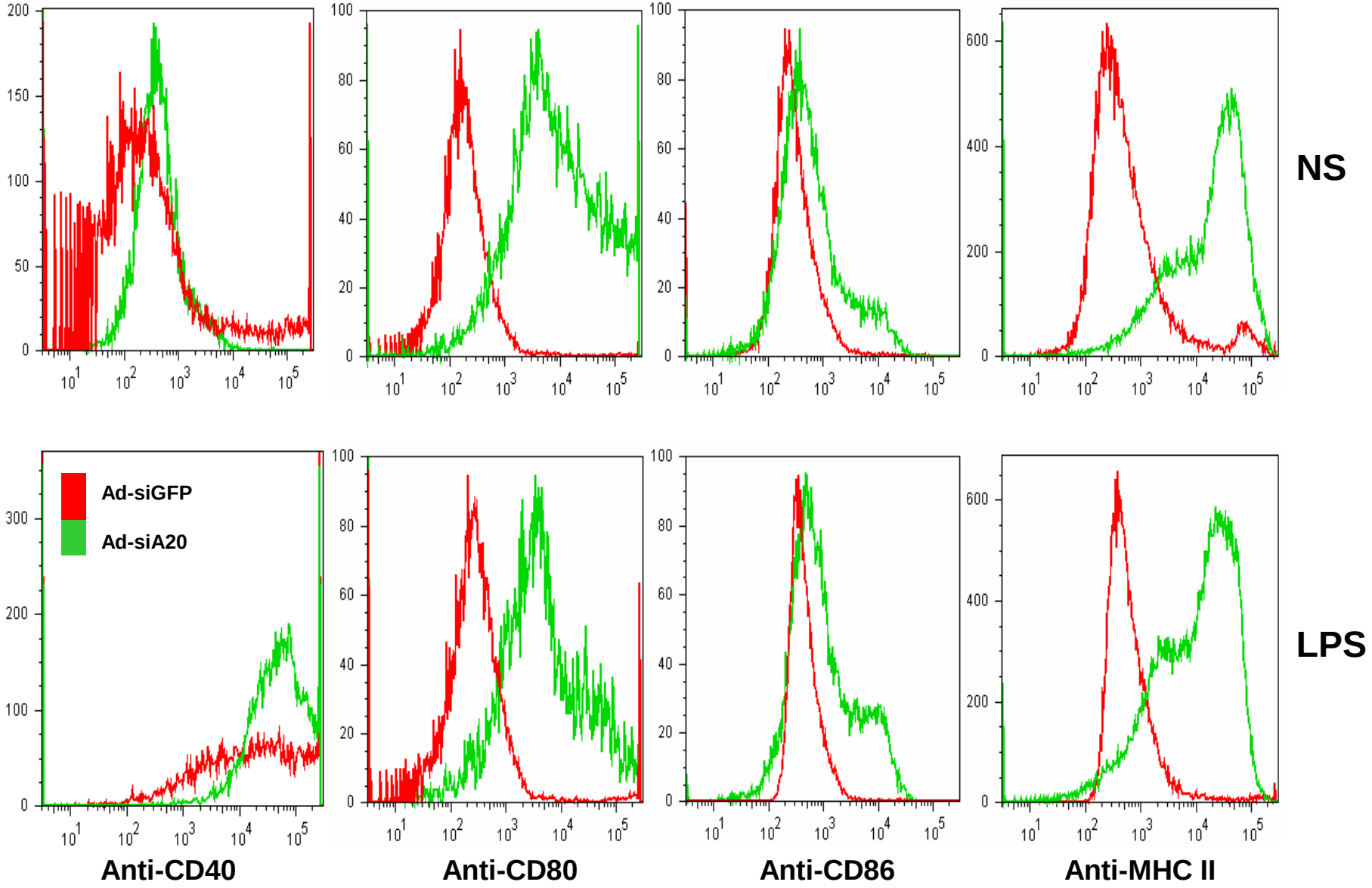
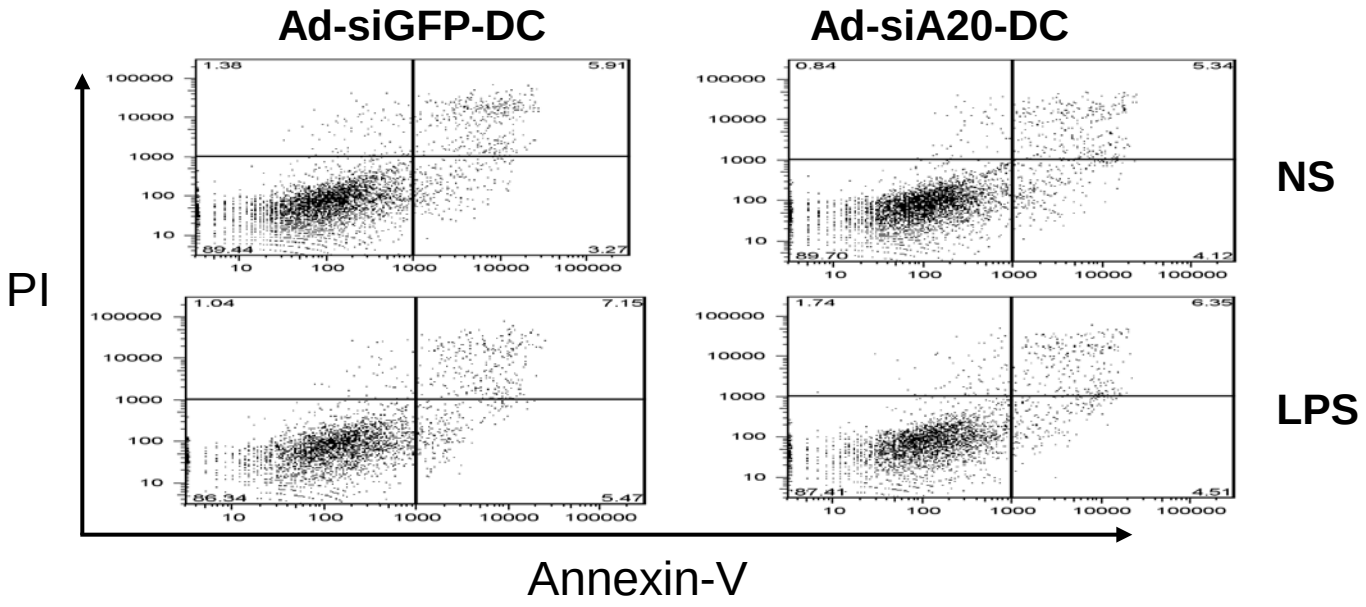

Ad-siGFP
Ad-siA20
NS
LPS
Anti-CD40
Anti-CD80
Anti-CD86
Anti-MHC II

Fig. S4

A


Ad-siGFP-DC
Ad-siA20-DC
1.38
5.91
89.44
3.27
0.84
5.34
89.70
4.12
1.04
7.15
86.34
5.47
1.74
6.35
87.41
4.51
NS
LPS
PI
Annexin-V

B

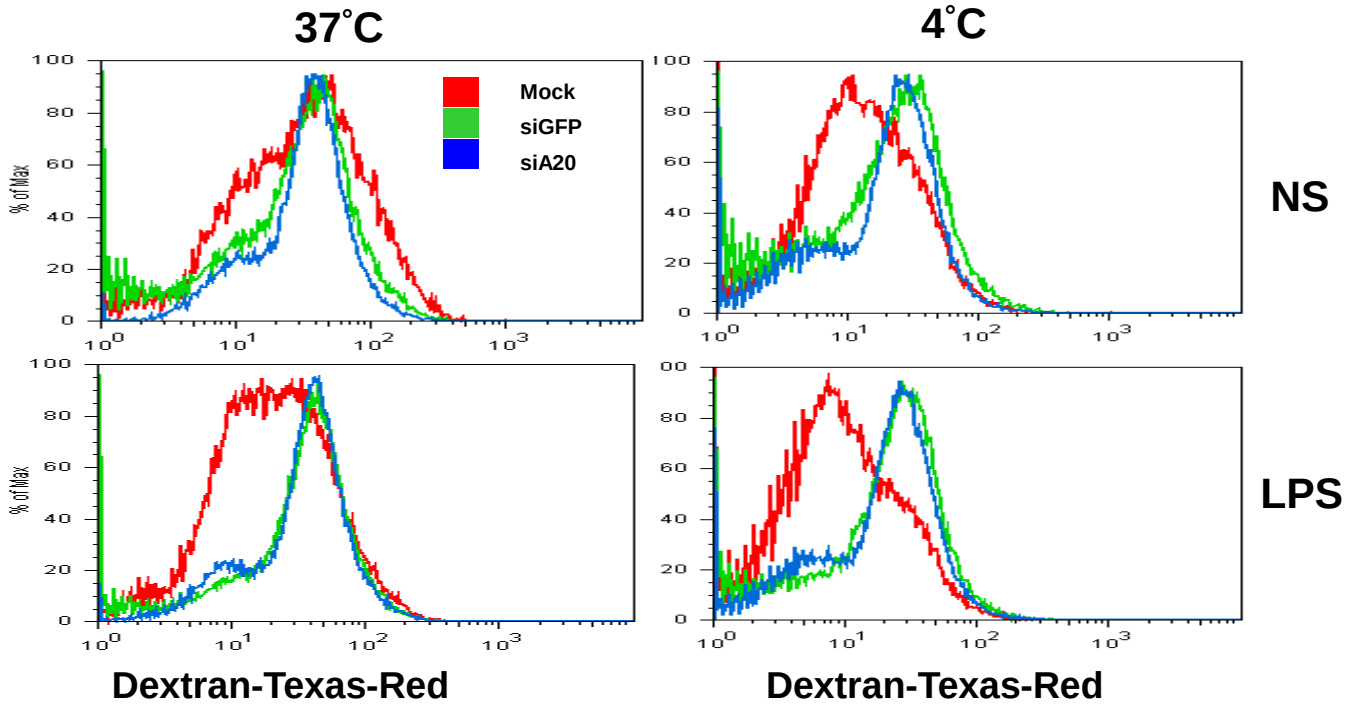

37˚C
4˚C
Mock
siGFP
siA20
% of Max
NS
LPS
Dextran-Texas-Red
Dextran-Texas-Red

Fig. S5