Supplemental Figure Legends

Figure S1 Co-administration of MS-275 extends VSV-luc activity in B16F10 tumours.
Mice bearing 5-day-old B16-F10 intracranial melanomas received i.v. injections of $4x10^8$ pfu of VSV-Luc with or without co-treatment with MS-275. Each of the 4 days following treatment with VSV, mice were injected with 3 mg of d-luciferin (Molecular Imaging Products Company, Ann Arbor, MI) intraperitoneally. Under anesthetic, VSV infection was visualized with a 200 Series Imaging System (Xenogen Corporation, Hopkinton, MA). Data acquisition and analysis were performed using Living Image v2.5 software. Images were captured under identical exposure, aperture and pixel binning settings.

Figure S2 Structure of MS-275 and inactive analogue.
The active compound is displayed on the left, while the structure of a highly similar compound lacking HDAC inhibitory activity used in these studies is shown on the right.

Figure S3 PolyI:C induces a lymphopenia that is extended by MS-275 co-administration.
8-10 weeks old C57BL/6 female mice were treated with a single dose of PolyI:C (200 μg in 100 μl of phosphate-buffered saline, Sigma) and were treated with 0.1 mg of MS-275 once a day for 5 days via intraperitoneal injection as PolyI:C combination MS-275 treatment group. Mice treated with a single dose of PolyI:C only were regarded as PolyI:C treatment group, whereas mice treated with five doses of MS-275 for 5 days represent the drug only treatment group. Blood was taken from the periorbital sinus and red blood cells were lysed with ACK lysis buffer. Peripheral blood lymphocyte counts were assessed at 2h, 6h, 24h, 48h, 72h and 120h after PolyI:C injection (N=3). Data were collected by a FACSCanto flow cytometer with FACSDiva 5.0.2 software (BD Pharmingen) and analyzed with FlowJo Mac (Treestar, Ashland, OR).

Figure S4 Co-administration of VSV and MS-275 depleted immature lymphocyte precursors in bone marrow and thymus.
8-10 weeks old C57BL/6 female mice were infected with a single tail-vein injection (i.v.) dose of VSV (2×10^9 PFU VSV in 200 μl of phosphate-buffered saline) and were treated with 0.1mg of MS-275 via intraperitoneal injection once a day for 3 days as VSV combination MS-275 treatment group. Mice infected with a single dose of VSV only were regarded as VSV treatment group, whereas mice treated with three doses MS-275 or MS-275 analogue for 3 days were drug only or analogue only treatment groups respectively, naïve mice were not treated with virus or drug. (N=3) Lymphocytes from thymus or bone marrow (femur and tibia) were harvested 3 days after VSV injection. Cells were then treated with anti-CD16/32 and surface markers fluorescently labelled by antibodies for (A) CD4/CD8 or (B) B220/IgM (BD Pharmingen). Representative plots are shown. (C) Diagram indicating the location on the B cell plots occupied by B cell precursors.

Figure S5 Combination therapy reduces Tregs but increases effector T cells in the tumor.

Mice bearing 5-day-old B16-F10 tumors were primed with Ad-hDCT and then boosted with VSV-hDCT in the presence of MS-275. Tumor tissues were processed 5 days after boosting and the percentage of $FoxP3^+$ Tregs in $CD4^+CD25^+$ T cells or DCT-specific $CD8^+$ T cells was determined by flow cytometry.

Fig S1

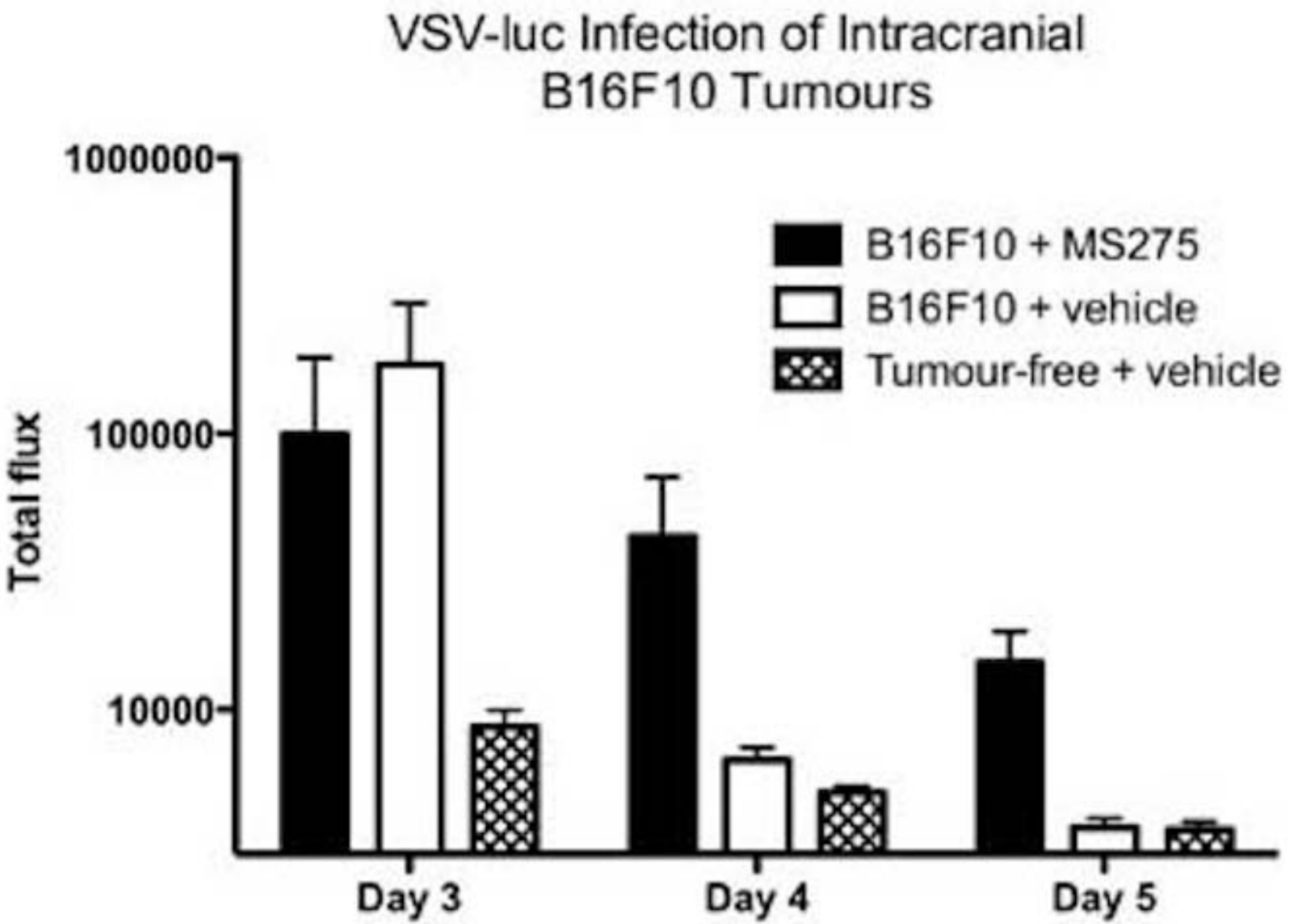
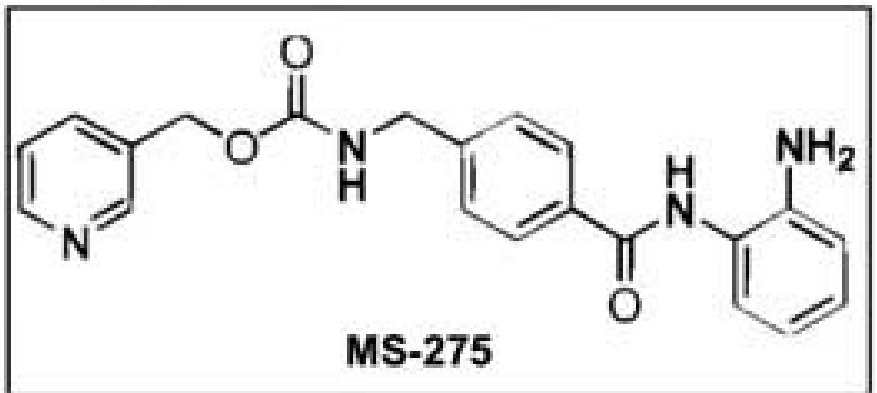
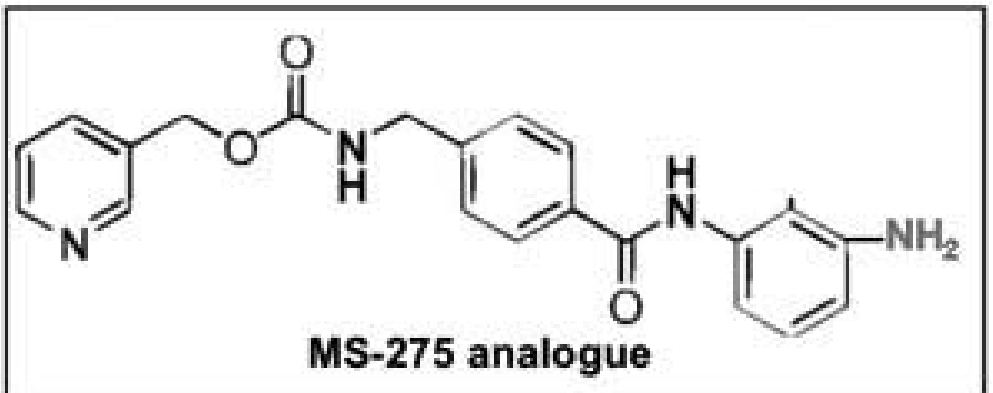

VSV-luc Infection of Intracranial
B16F10 Tumours
1000000
100000
10000
Total flux
B16F10 + MS275
B16F10 + vehicle
Tumour-free + vehicle
Day 3
Day 4
Day 5

Fig S2



MS-275



MS-275 analogue

Fig S3

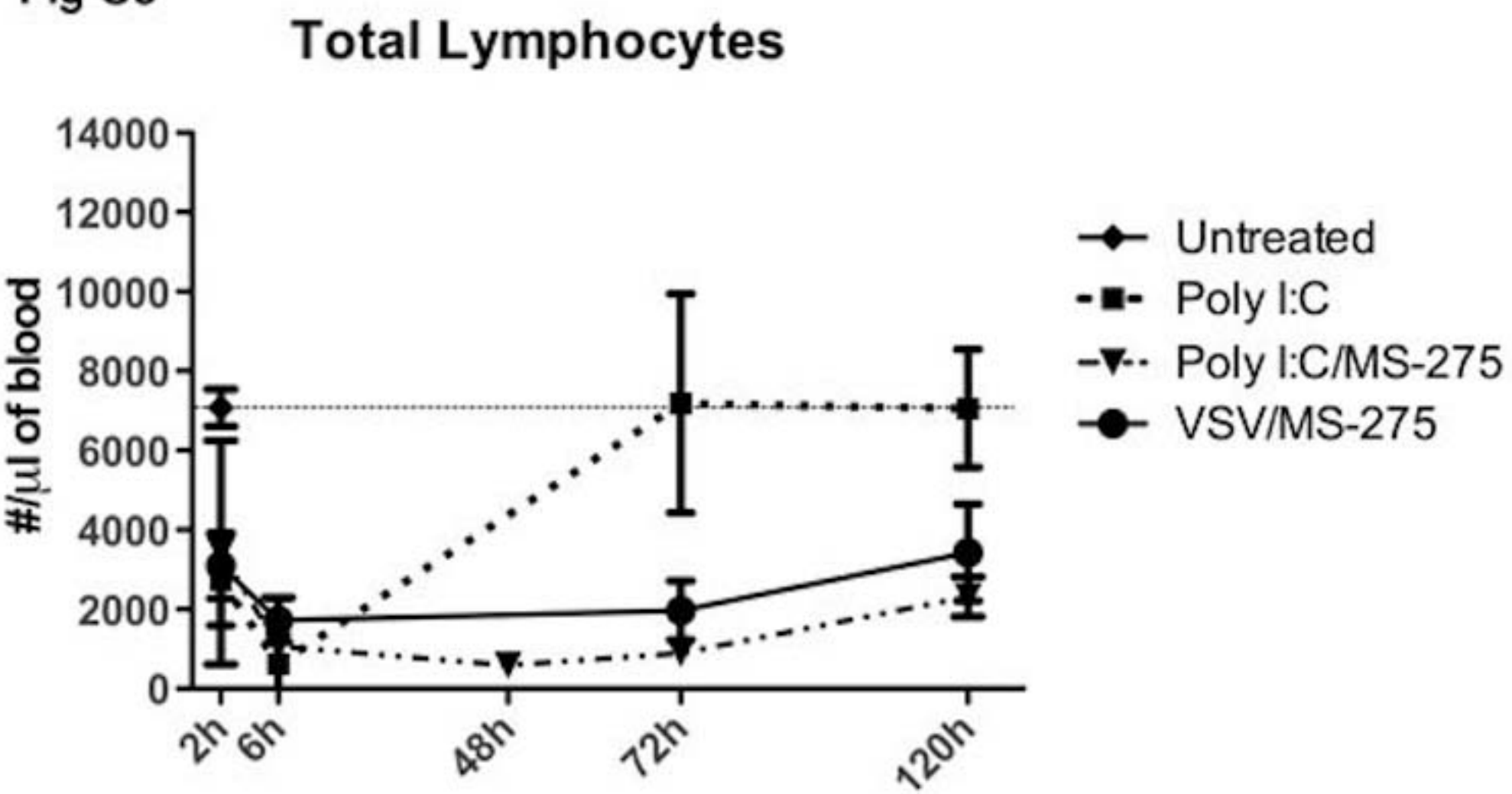
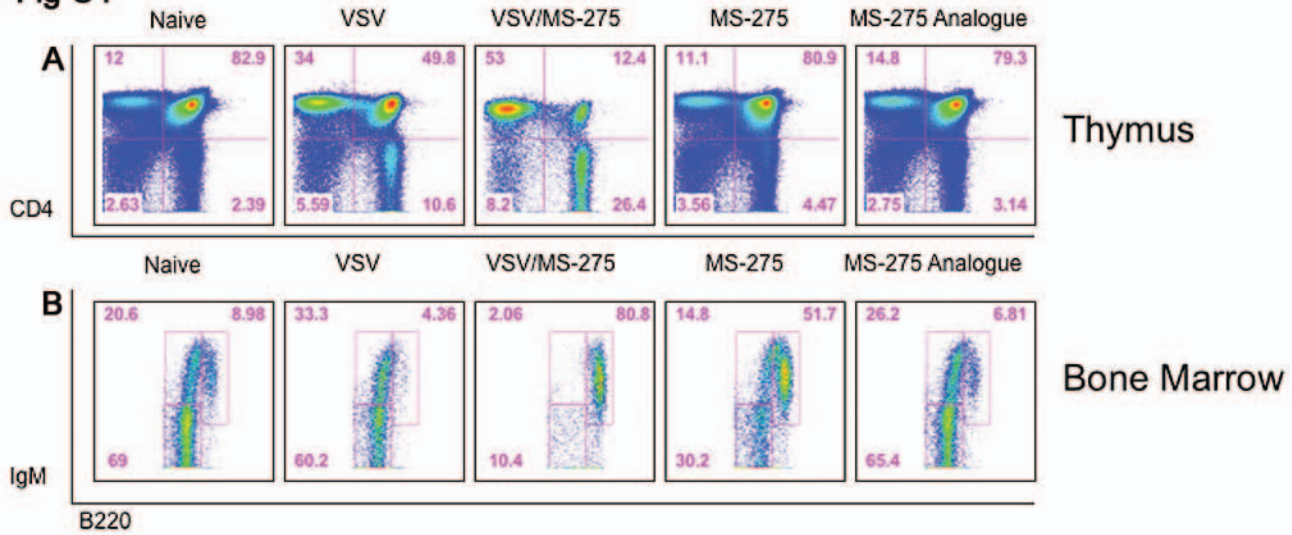

Total Lymphocytes
#/µl of blood
14000
12000
10000
8000
6000
4000
2000
0
2h
6h
48h
72h
120h
Untreated
Poly I:C
Poly I:C/MS-275
VSV/MS-275

Fig S4


A
Naive
VSV
VSV/MS-275
MS-275
MS-275 Analogue
12
82.9
2.63
2.39
34
49.8
5.59
10.6
53
12.4
8.2
26.4
11.1
80.9
3.56
4.47
14.8
79.3
2.75
3.14
CD4
Thymus
B
Naive
VSV
VSV/MS-275
MS-275
MS-275 Analogue
20.6
8.98
69
33.3
4.36
60.2
2.06
80.8
10.4
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26.2
6.81
65.4
IgM
B220
Bone Marrow

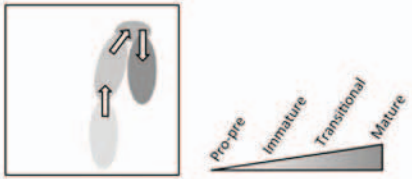
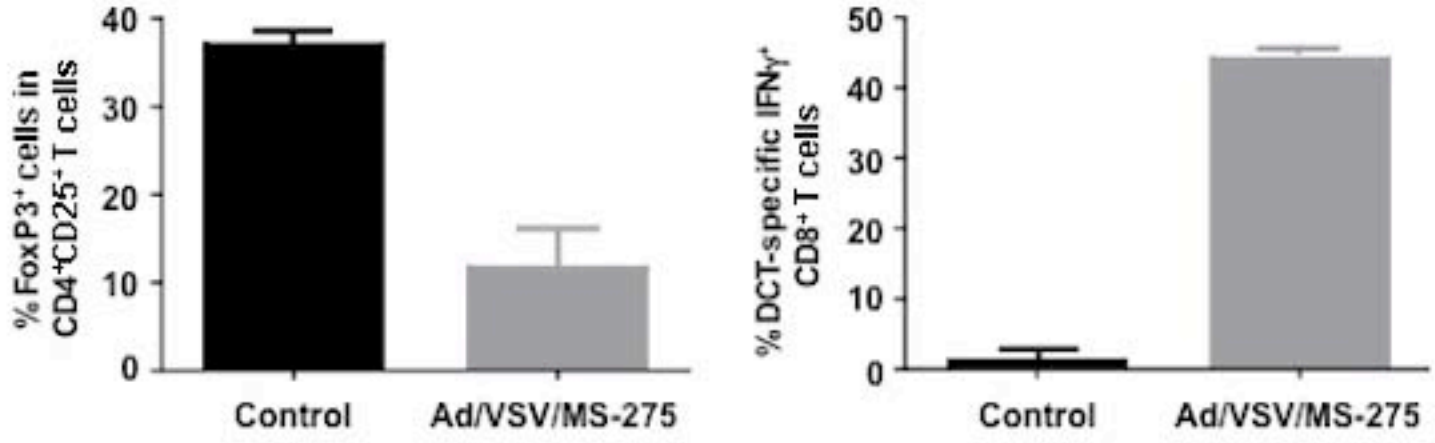

C
Pro-pre
Immature
Transitional
Mature

Figure S5


% FoxP3+ cells in CD4+CD25+ T cells
40
30
20
10
0
Control
Ad/VSV/MS-275
% DCT-specific IFNγ+ CD8+ T cells
50
40
30
20
10
0
Control
Ad/VSV/MS-275