**SUPPLEMENTAL MATERIAL**

*Preparation of rabbit anti-variola hyperimmune sera*. Within BSL-4 containment, four rabbits were immunized with purified variola, strain Bangladesh. Immunizations, intradermal (ID) and subcutaneous (SC) were made over 4 axillary and inguinal regions with Freund's adjuvant, at day 0, and 29. Equal volumes of Freund's and a final volume of 1 x $10^9$ pfu variola were used as the immunogen. Animals were euthanized and terminally bled 50 days post the second immunization; the terminal bleed was pooled with bleeds from 10 days, 20 days and 40 days post the second immunization to make the standard lot of pooled rabbit anti-variola hyperimmune sera. The sera was demonstrated to efficiently recognize variola, the 50% PRNT neutralization titer is extrapolated to be 1:1819 by the Kaber-Kogan method.

*Immunohistochemical staining of variola and vaccinia plaques.* Viral infected BSC-40 and VERO-E6 monolayers were fixed by adding 2 ml PBS/10% formaldehyde for 20 min at room temperature following by PBS rinse. The plates were then gamma-irradiated at the kill dose (4.4 x $10^6$ rads) and removed from the BSL-4 laboratory for immunohistochemical staining. The monolayers were blocked in PBS/1% FBS overnight at room temperature and then incubated with 1 ml of rabbit anti-variola primary antibody (1:2000 dilution in PBS/1% FBS) for 30 min with gentle rocking. After two rinses with PBS followed by two 15 min washes with 1X PBS/Tween on an orbital rocker, 2 mls of goat anti-rabbit horseradish peroxidase conjugated secondary antibody diluted 1:2000 in PBS/1% FBS was added to each well and incubated for 30 min at room temperature with gentle rocking. After two rinses of PBS followed by one 15 min wash and a second 30 min wash with 1X PBS/Tween, plaques were visualized by adding 1 ml of the TrueBlue Peroxidase Substrate (KPL) to the monolayer for 10 minutes until developed. The developer was then removed and the monolayers were rinsed with water.

Figure S1 Dose response effect of CI-1033 on vaccinia IHDJ, WR and VGF deletion mutant (vSC20) plaque and comet formation. Immunohistochemical staining of BSC40 and VERO-E6 cells was performed as in Figure 3. Effect of CI-1033 on vaccinia IHDJ plaque and comet

formation 48 hours post infection. Note the 5-10 fold greater sensitivity of VERO-E6 to comet inhibition by CI-1033. Inset: Effect of CI-1033 on vaccinia WR and VGF deletion mutant (vSC20) plaque and comet formation 72 hours post infection in BSC40 cells. Note the overall smaller plaque phenotype and reduced comet formation of the VGF mutant.

Figure S2 The lungs from VV-infected mice were fixed in 10% neutral buffered formaldehyde and then paraffin-embedded. Tissue sections (5 □m) were stained with hematoxylin and eosin. The scale bar represents 100 □m. Control (infected but untreated) and various treatment groups are indicated. Note necrosis of bronchiolar cells and eosinophilic, acellular exudates in the control lung. By contrast, cellular infiltrates are evident in the CI-1033, the anti-L1R, and the CI-1033 + anti-L1R treatment groups. In the latter double treatment group, the bronchiolar epithelia are well preserved.

Figure S3 Host immune response to vaccinia pneumonia challenge during therapy. A) Cytokine expression profile of whole lungs from uninfected or infected (4,6 or 8) mice at indicated days post-infection analyzed by RNase protection assay. Day 8 virus-infected controls are lacking because the mice died. 6 □g of total lung RNA from each mouse group was hybridized with the mCK-2b template set (BD Biosciences Pharmingen) probes which are shown in the left-most lane and protected fragments visualized on a DNA sequencing gel. Note that shorter exposure of GAPDH and L32 hybridizing bands confirmed equivalence of sample loading (data not shown). B) IFN-□ producing $CD8^+$ T cells in spleens of representative mice. Spleen cells from control, anti-L1R, CI-1033 or anti-L1R + CI-1033 treated infected mice (day 7) were stimulated in vitro by uninfected or VV-infected MC57G cells. Intracellular cytokine staining was performed and analyzed by flow cytometry. Numbers indicate the percentages of CD8 T cells. Methods for intracellular cytokine staining are as described (8).

Table S1. Lack of cytotoxicity of CI-1033 at various concentrations of BSC-40 monlayers.

| | **Experimental LDH Release ($OD_{490}$)** | **Maximum LDH Release ($OD_{490}$)** | **% Cytotoxicity** |
|---|---|---|---|
| **Untreated** | -0.0485 | 2.8824 | -1.6837 |
| **10 μM** | 0.0615 | 2.7341 | 2.2505 |
| **5 μM** | 0.0290 | 2.6394 | 1.0999 |
| **2.5 μM** | 0.0428 | 2.7960 | 1.5308 |
| **500 nM** | 0.0567 | 2.7315 | 2.0747 |
| **50 nM** | -0.0048 | 2.6015 | -0.1857 |

Cells were treated +/- CI-1033 at various concentrations for 48 hours and both supernatants and cell lysates were assayed for the presence of lactate dehydrogenase (LDH).

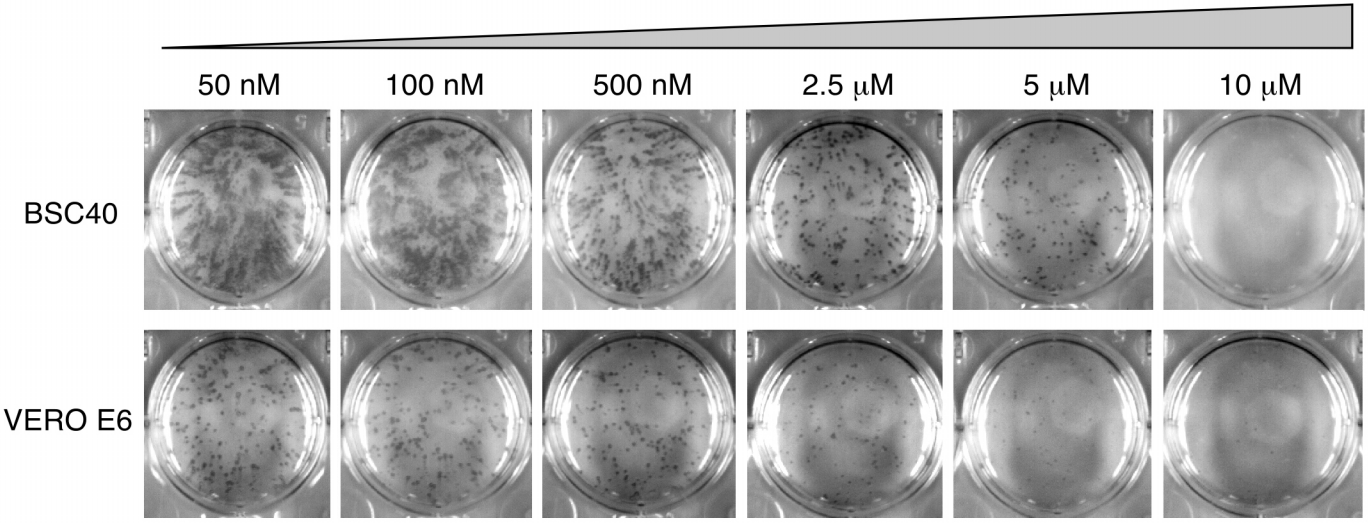
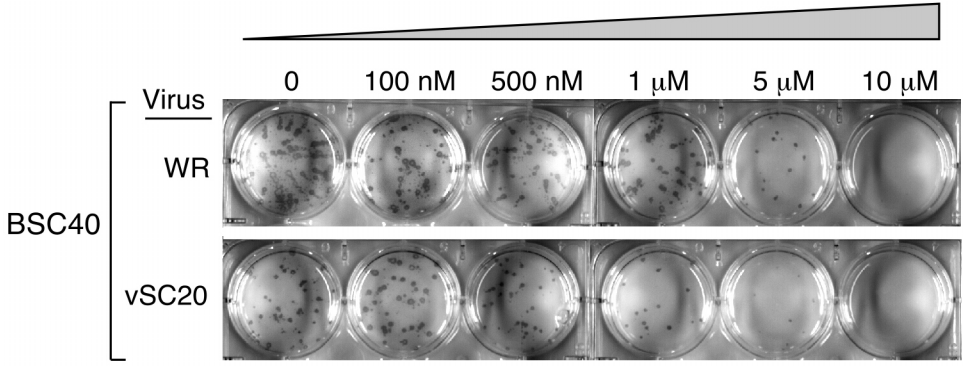

50 nM
100 nM
500 nM
2.5 μM
5 μM
10 μM
BSC40
VERO E6

Fig. S1


0
100 nM
500 nM
1 μM
5 μM
10 μM
Virus
WR
vSC20
BSC40

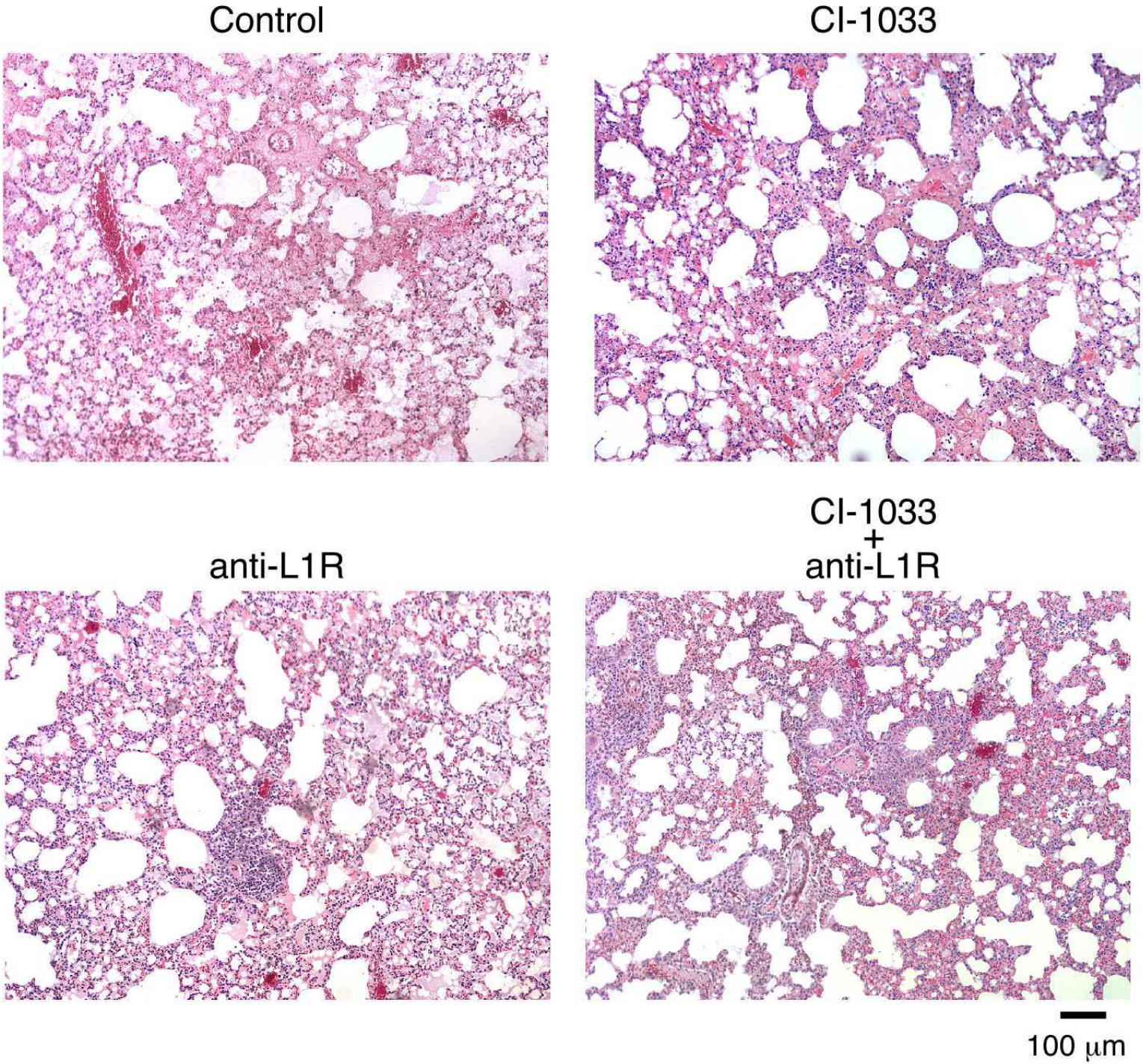



Fig. S2

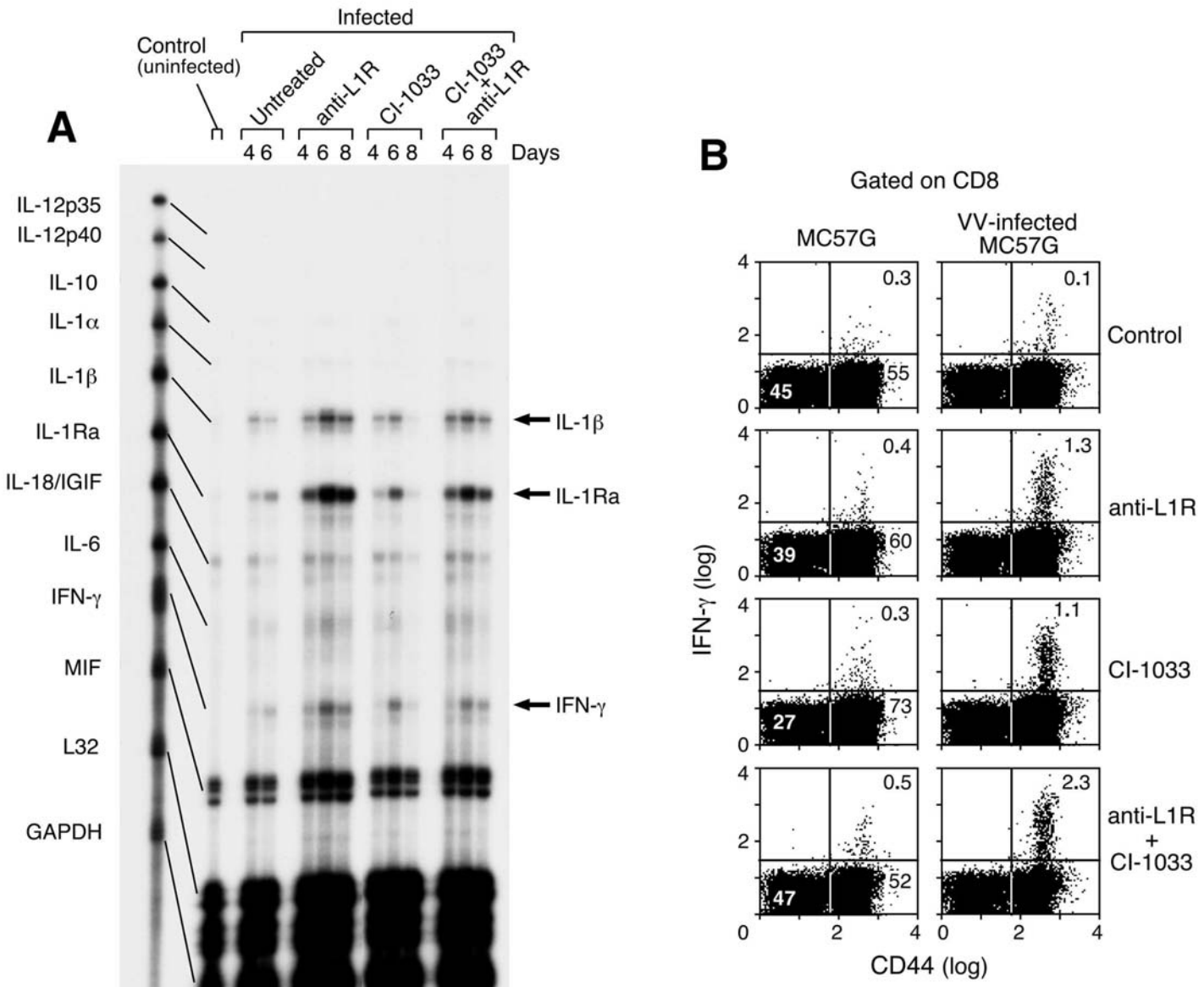

A
Infected
Control (uninfected)
Untreated
anti-L1R
CI-1033
CI-1033 + anti-L1R
4 6
4 6 8
4 6 8
4 6 8
Days
IL-12p35
IL-12p40
IL-10
IL-1α
IL-1β
IL-1Ra
IL-18/IGIF
IL-6
IFN-γ
MIF
L32
GAPDH
IL-1β
IL-1Ra
IFN-γ
B
Gated on CD8
MC57G
VV-infected MC57G
0.3
0.1
45
55
Control
0.4
1.3
39
60
anti-L1R
0.3
1.1
27
73
CI-1033
0.5
2.3
47
52
anti-L1R + CI-1033
IFN-γ (log)
CD44 (log)
0
2
4

Fig. S3