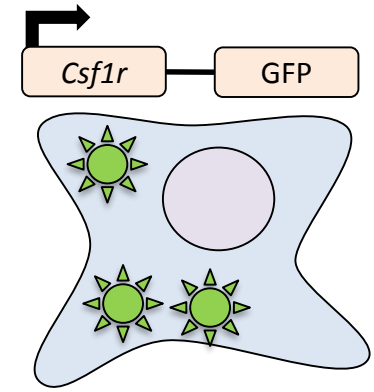
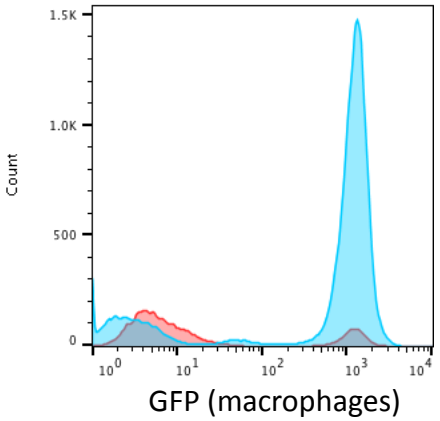
A



Macrophages / Dendritic Cells

B

***Csf1r* promoter driven GFP expression in peritoneal cells and enriched peritoneal macrophages:**

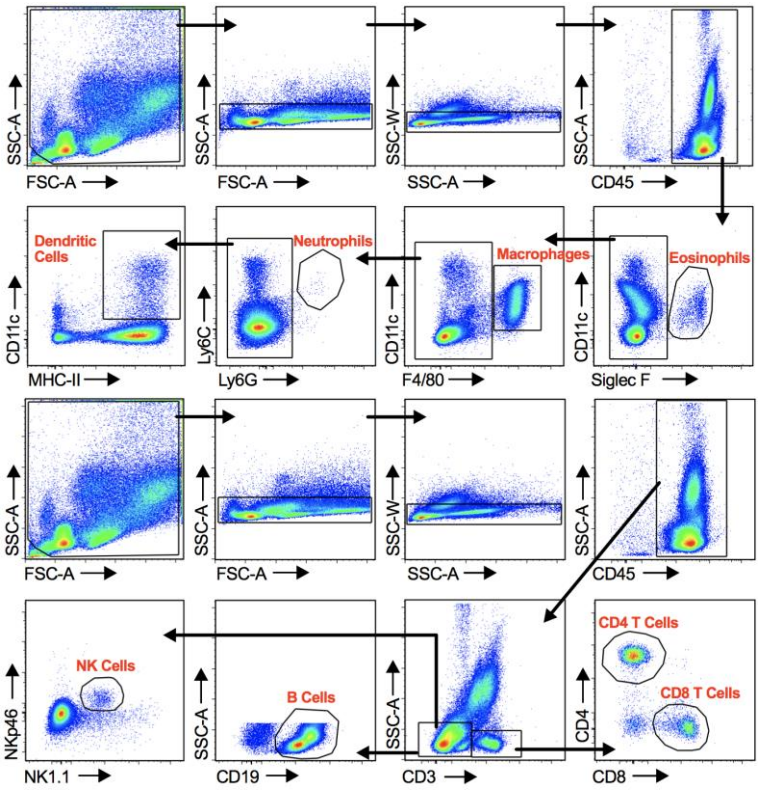


Red = total peritoneal cells
Blue = enriched pmacs

Figure S1: Relative numbers of peritoneal macrophages present in peritoneal cell or enriched peritoneal macrophage cultures from *MaFIA^{+}/Ifnar$^{-/-}$* mice.

A) Schematic of GFP expression of myeloid cells in *MaFIA^{+}/Ifnar$^{-/-}$* mice. GFP in the myeloid compartment is under control of the *Csf1r* promoter. B) Peritoneal cells were assessed for GFP by flow cytometry immediately after being collected from the peritoneal cavity (red filled histogram). Alternatively, cells were plated for 24 hours, washed to remove non-adherent cells, then lifted and assessed for GFP (blue filled histogram). This latter group of cells was termed enriched pmacs.

A.

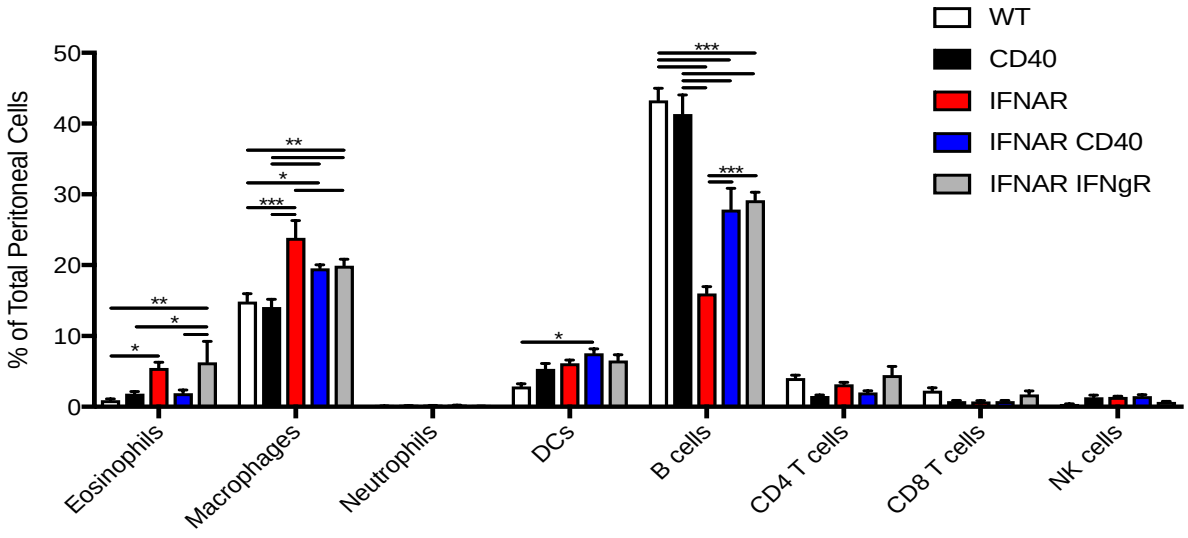


All populations: Live+, Singlet+, Singlet+, CD45+

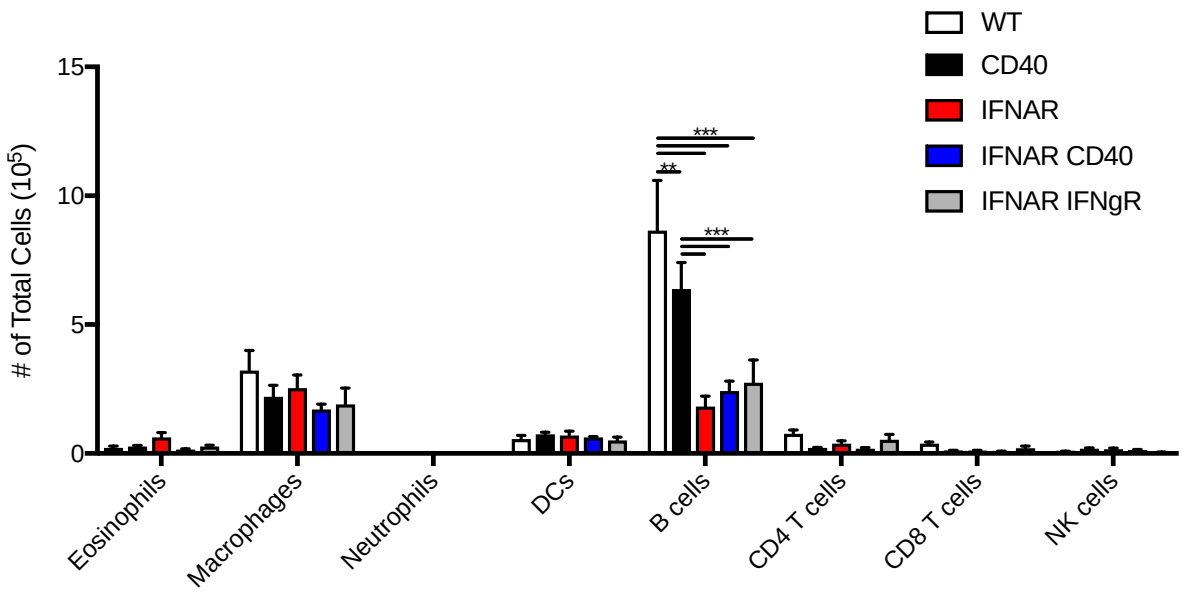
Eosinophils: Siglec F+ CD11c+
Macrophages: Siglec F- F4/80+
Neutrophils: Siglec F- F4/80- Ly6G+ Ly6C+
Dendritic Cells: Siglec F- F4/80- Ly6G- MHC-II+ CD11c+

NK Cells: CD3- NK1.1+ NKp46+
B Cells: CD3- CD19+
CD8 T Cells: CD3+ CD8+
CD4 T Cells: CD3+ CD4+

B. Peritoneal cavity cell composition by percent total cells:



C. Total numbers of cell populations within the peritoneal cavity



D. Cell populations in the peritoneal cavity targeted by rVSV/EBOV GP

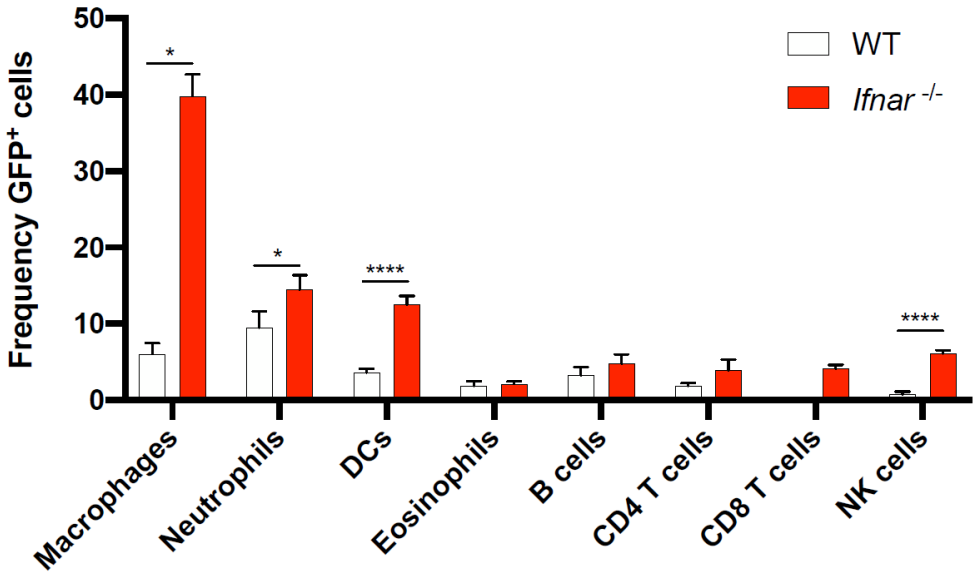


Figure S2: Cell populations present in the peritoneal cavity that become infected with rVSV/EBOV GP.

Total resident peritoneal cells were isolated and stained as outlined in the Materials and Methods. **A)** Gating strategy used. **B-C)** Quantification of cell populations for all five mouse strains used in this study shown as percent of cells or **(B)** as absolute numbers of cells **(C)**. **D)** Peritoneal cells were harvested from male mice of the indicated genotype 24 hours after infection with $2x10^6$ iu rVSV/EBOV GP. Cells were stained as outlined in the Materials and Methods and the gating strategy used is shown in A. Infected cells were quantified for the indicated populations. * indicates $p<0.05$.

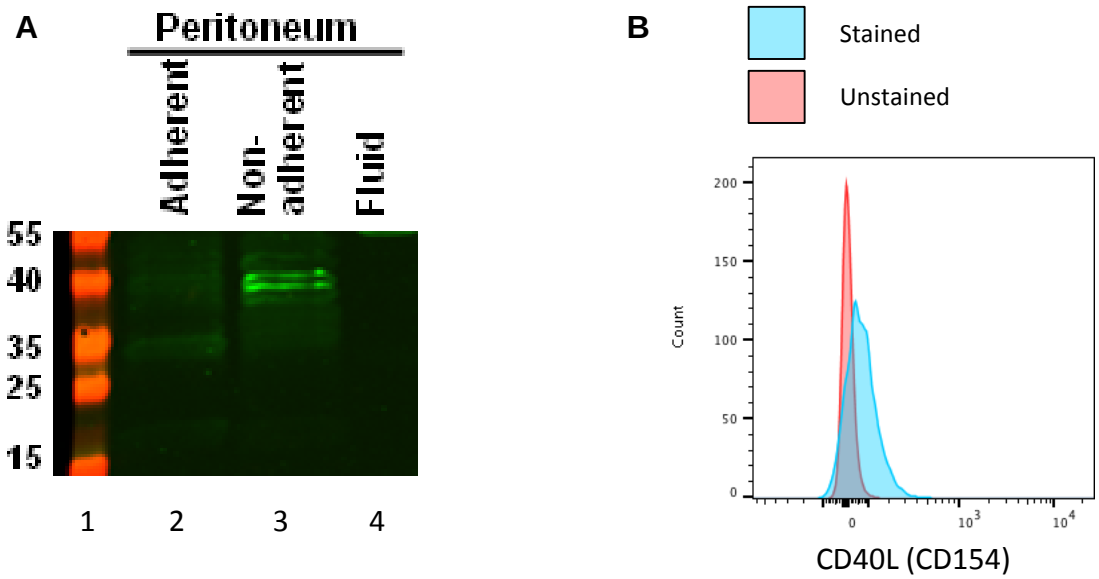


Figure S3: Peritoneal lymphocytes are the predominant source of CD154 within the compartment.

A) Naïve resident cells from the peritoneum *Ifnar*$^{-/-}$ mice were incubated in media overnight. The fluid from the peritoneum was also collected. Peritoneal fluid that had been diluted in PBS during the collection was concentrated 30-fold. ~10^6 cells non-adherent and adherent cells were analyzed. Cell were lysed in PBS and 1% SDS and treated with Pierce Universal Nuclease. Proteins from reduced and denatured lysates and peritoneal fluid were separated by SDS PAGE. Proteins were transferred to nitrocellulose, blocked with 5% milk and incubated with 8 µg/ml of anti-CD154 mAb MR-1 overnight at 4º C. Membrane was washed and imaged using a 790 nm fluor-conjugated goat anti-hamster secondary (1:100) on an Odyssey LiCor. **B)** Peritoneal cells were harvested from *Ifnar*$^{-/-}$ mice and stained with anti-CD154 antibody (APC-conjugated anti-CD154, Thermo Fischer Scientific #17-1541-81). Lymphocytes (determined by FSC-A and SSC-A) were gated on and CD154 levels within that population are displayed.

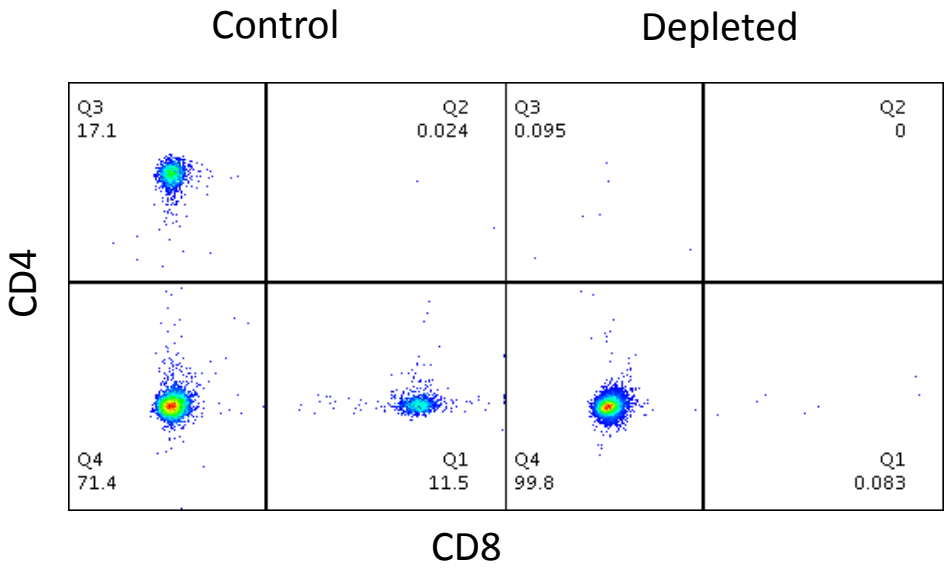


Figure S4: Evaluation of efficacy of *in vivo* cell depletions and the effect of T cell depletion.

Ifnar$^{-/-}$ mice were treated with antibodies to selectively deplete T cells. Twenty-four hours after antibody administration, peripheral blood was collected and leukocytes were isolated, stained with the indicated antibodies, and evaluated by flow cytometry. Cells from untreated mice (left) were compared to cells from depleted animals (right). Populations evaluated were selected by SSC-A and FSC-A.

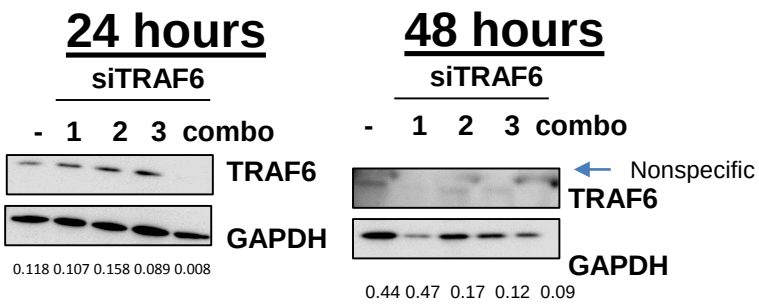
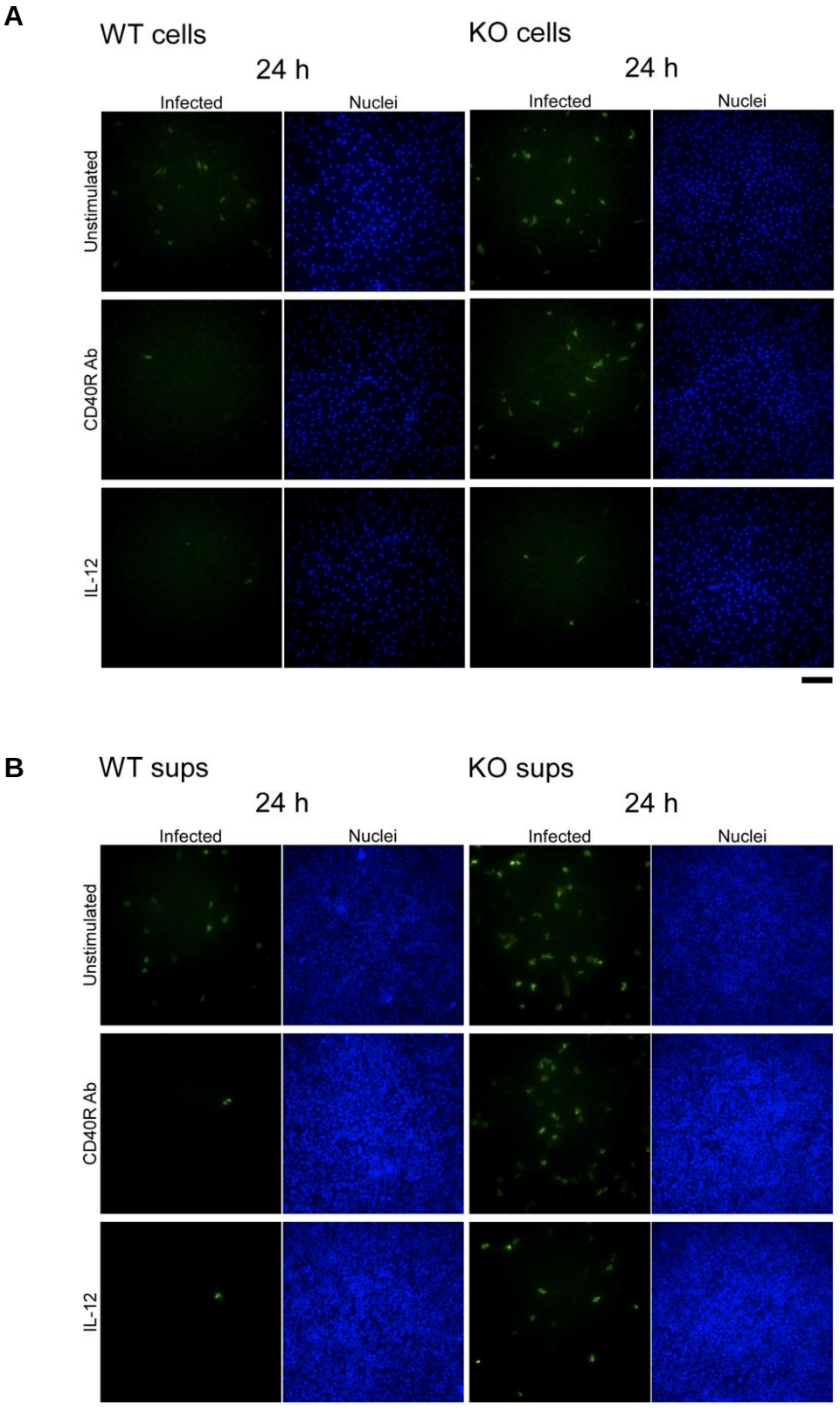


Figure S5. siRNA knockdown of TRAF6 in pmacs.

Cells were transfected with one of three different siRNAs or in combination. Transfected cells were harvested at 24 or 48 hours and lysed with RIPA buffer. Lysates were subjected to SDS PAGE and immunoblotting and probed with antibodies against TRAF6 (MBL 597) and GAPDH (Santa Cruz sc-32233). Values below the images are densitometry readings determined by normalizing TRAF6 to GAPDH for each sample in Fujifilm Multi Gauge.


A
WT cells
KO cells
24 h
24 h
Infected
Nuclei
Infected
Nuclei
Unstimulated
CD40R Ab
IL-12
B
WT sups
KO sups
24 h
24 h
Infected
Nuclei
Infected
Nuclei
Unstimulated
CD40R Ab
IL-12

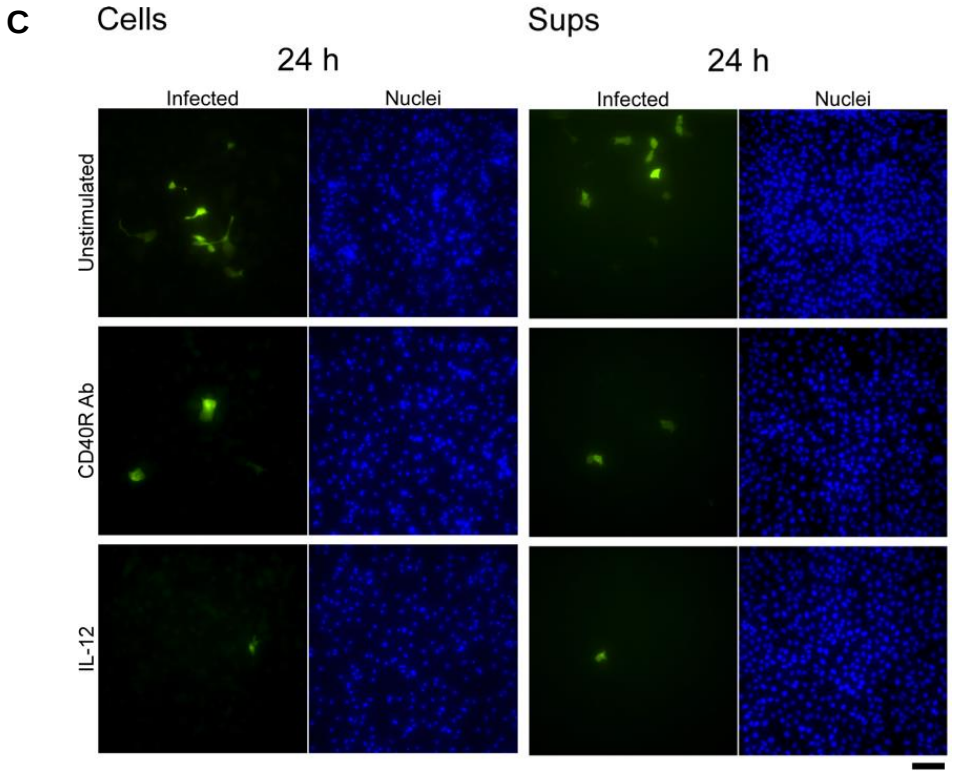


Figure S6: Representative images of EBOV infection of pmac cultures treated with agents noted.

A-B) Peritoneal macrophages (A) or supernatants (Sups) (B) from WT or *CD40*$^{-/-}$ (KO) C57BL/6 mice stimulated with agonistic CD40 mAb or IL-12 (corresponding Figures 1E, 5A and 6B). **C)** Human MDMs or supernatants stimulated with CD40 mAb or IL-12 (corresponding Figures 1F, 5B and 6C). Scale bar = 100 µm.