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Supplemental Information

Virus-Mediated Suppression of the Antigen Presentation Molecule MR1

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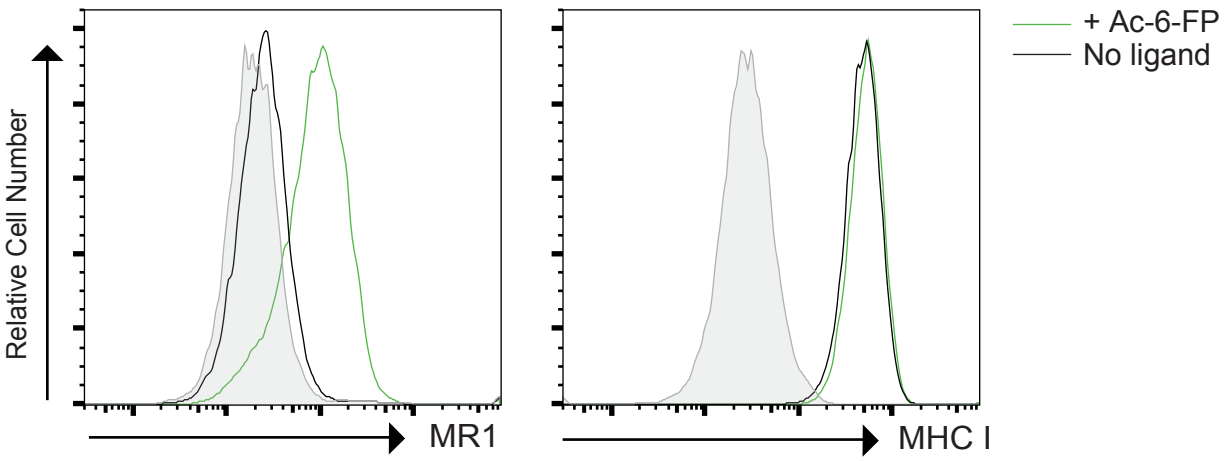
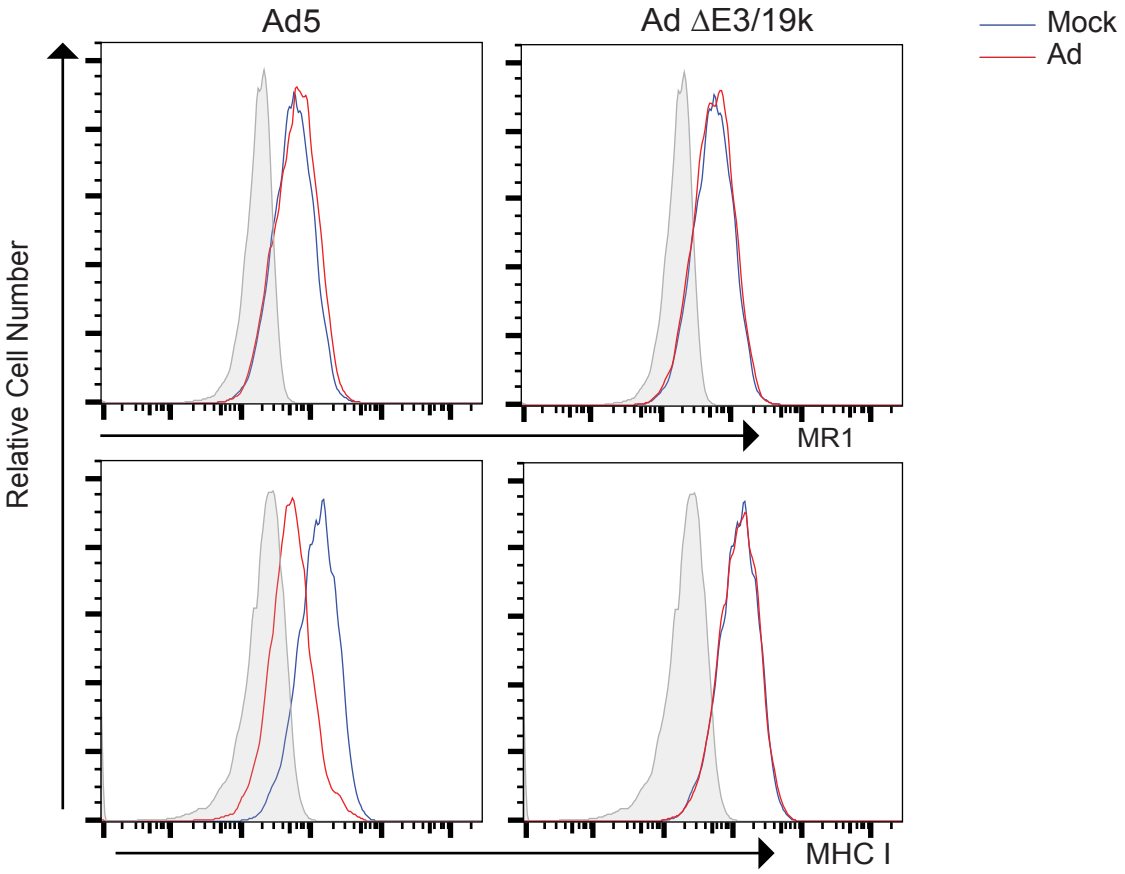
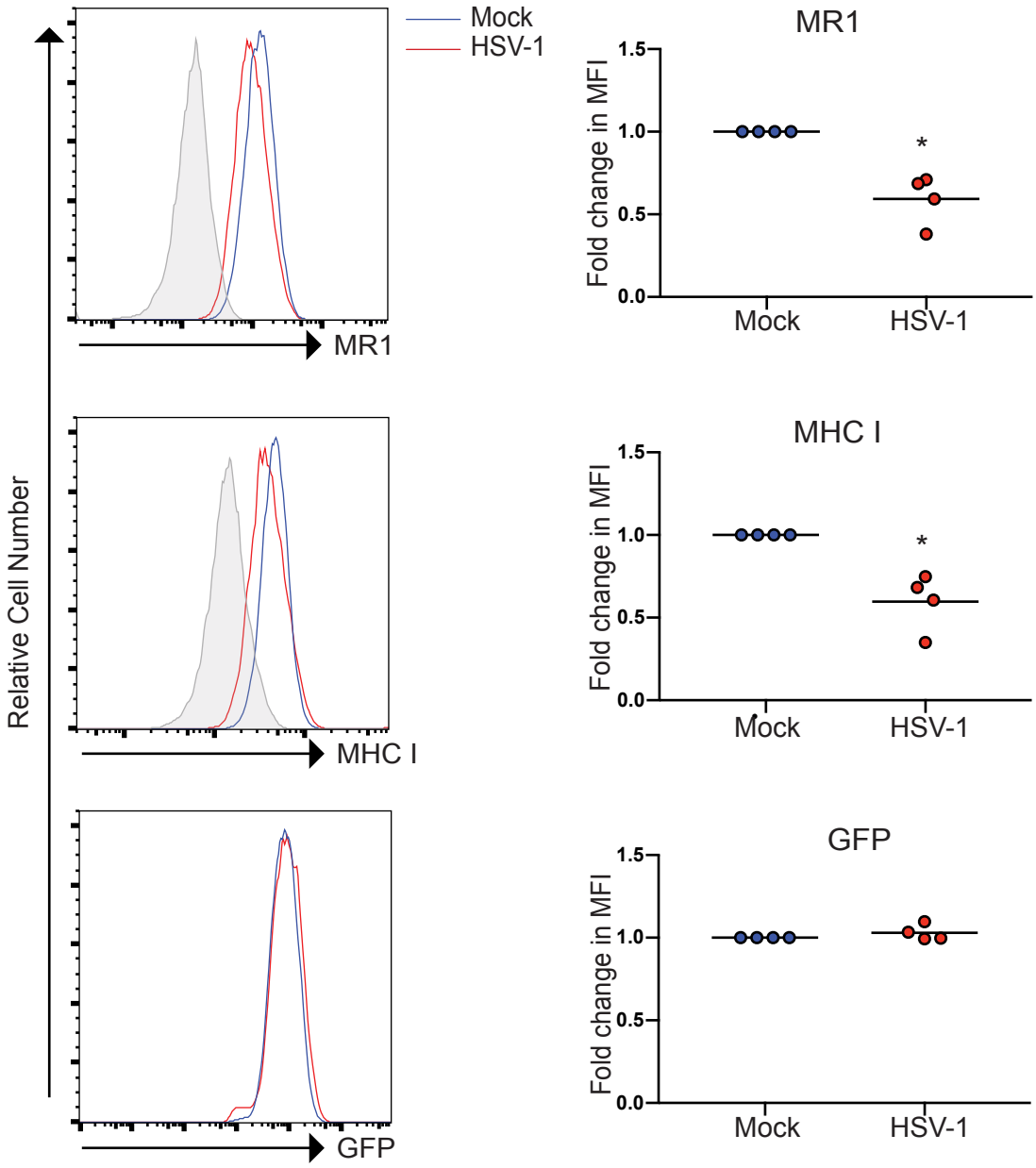


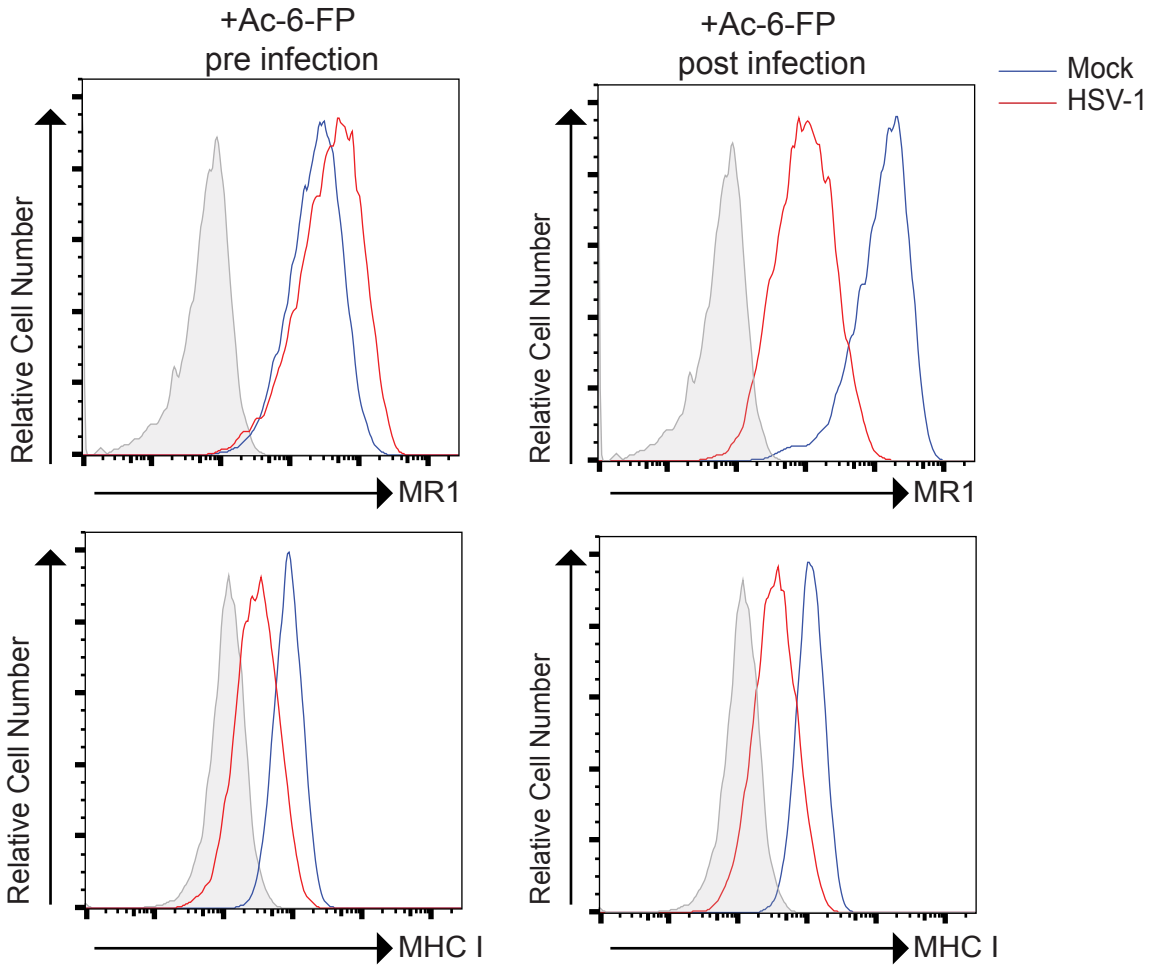
Supp Fig 1 : Treatment of HFFs with ligand drives surface MR1, related to Fig 1A. HFFs were left untreated or treated with Ac-6-FP (5 μM) for 16 h before staining for MR1, MHC I or isotype control (grey) as indicated. Data is representative of at least 3 independent experiments.



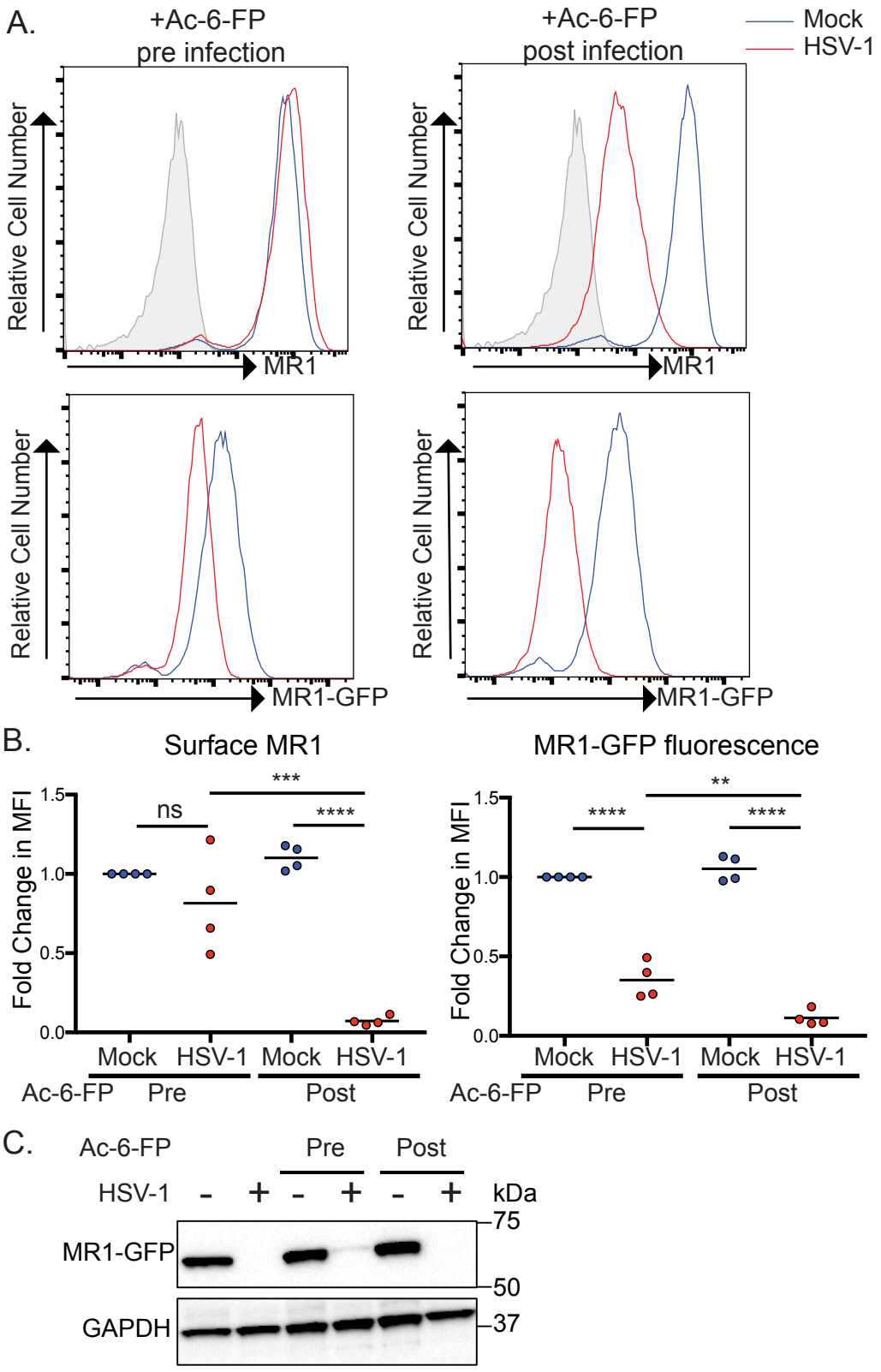
Supp Fig 2 : Ad infection inhibits MHC I but not MR1 surface expression, related to Fig 1A. 293 cells were infected with wild type Ad or ΔE3/19K at an MOI of 10 with Ac-6-FP (5 μM) added at 6 h p.i. before staining for surface MR1, MHC I or isotype control (grey) as indicated at 24 h p.i. Data is representative of 2 independent experiments.



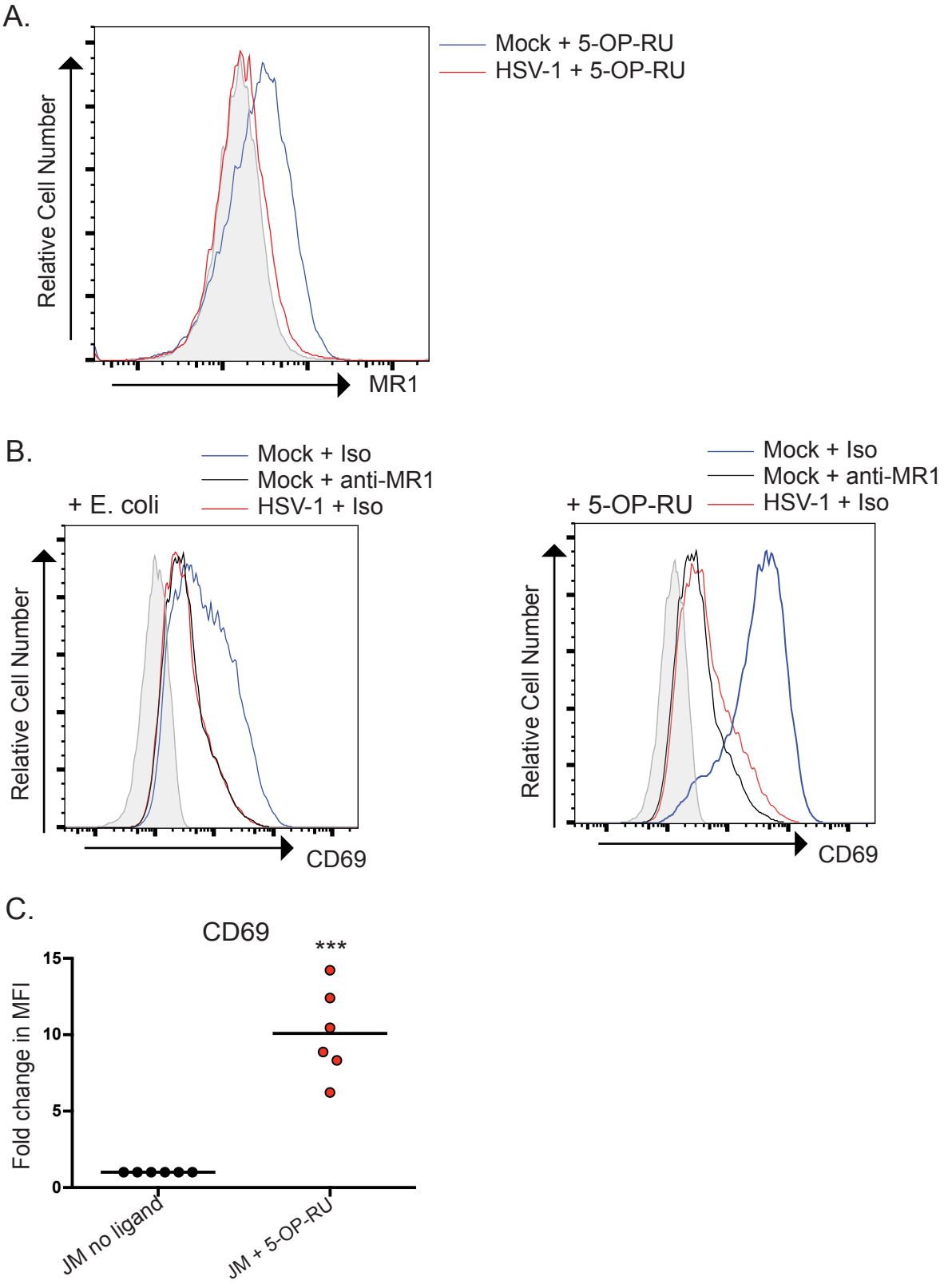
Supp Fig 3 : HSV-1 infection inhibits surface MR1 surface expression at 6 h p.i., related to Fig 1C. ARPE-19 MR1 were mock or HSV-1 infected before staining at 6 h p.i. for surface MR1, MHC I or isotype control (grey) as indicated. Fold change in MFI relative to mock infected cells is graphed. Statistical significance was calculated by paired Student's *t*-test * $p<0.05$, (n=4)



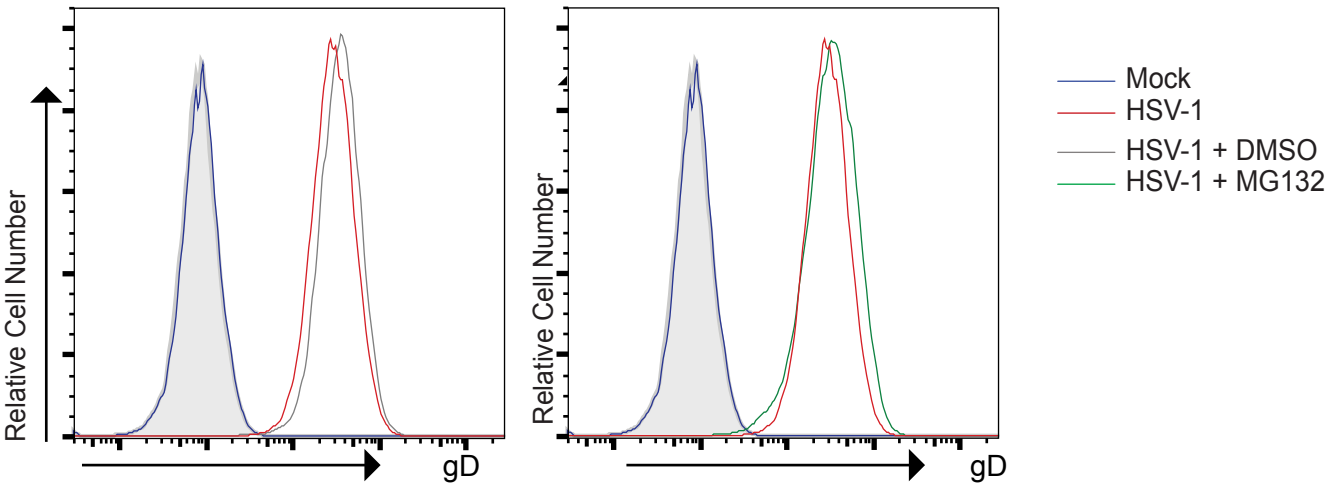
Supp Fig 4: Ligand binding blocks HSV-1 dependent targeting of surface MR1, related to Fig 2. HFFs overexpressing MR1 were mock or HSV-1 infected in parallel. Cells were either treated with Ac-6-FP (5 μM) for (A) 24 h prior to infection (pre) or (B) at 6 h post infection (post) before staining for surface MR1, MHC I or isotype control (grey) as indicated at 24 h p.i. Data is representative of 2 independent experiments.



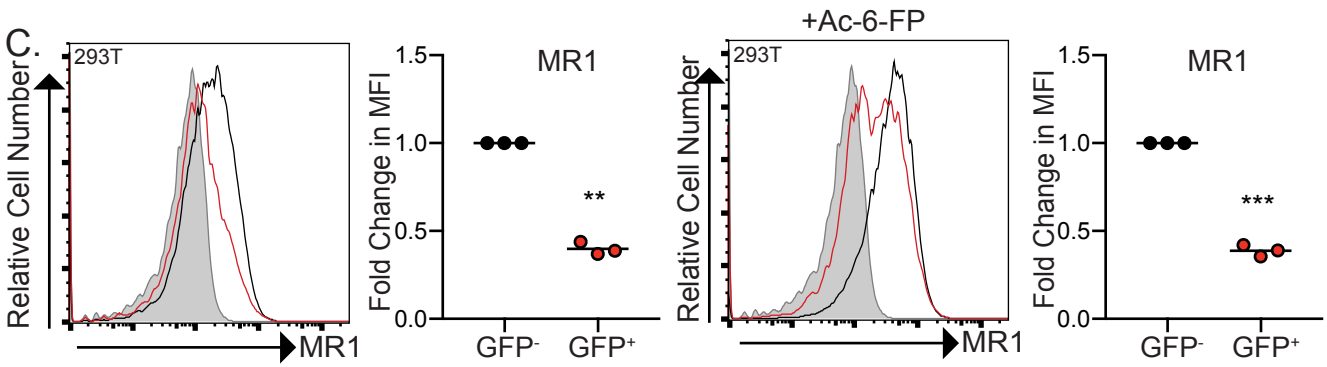
Supp Fig 5. HSV-1 can inhibit MR1-GFP surface expression in a ligand dependent manner, related to Fig 2. ARPE-19 MR1-GFP were mock or HSV-1 infected in parallel. Cells were either treated the Ac-6-FP (5 μM) for 24 h prior to infection (pre) or at 14 h post infection (post) before (A) staining for surface MR1, MHC I at 18 h p.i. and analysis by flow cytometry. (B) Fold change relative to mock infected cells is graphed. Statistical significance was calculated by ANOVA * $p<0.05$, ** $p<0.005$, *** $p<0.0005$, **** $p<0.0001$ (n=4) (C) Cell lysates from mock or HSV-1 infected ARPE-19 MR1 cells treated with Ac-6-FP (5 μM) for 24 h prior to infection (pre) or at 14 h p.i. (post) were harvested at 18 h p.i. and immunoblotted for GFP and GAPDH. Data is representative of 2 independent experiments.



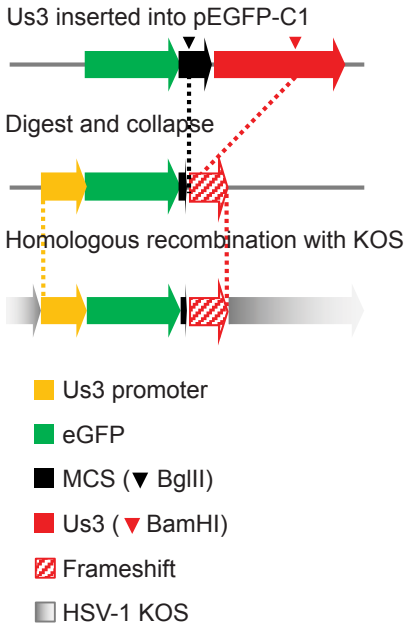
Supp Fig 6: TCR-mediated activation of Jurkat MAIT cells by ligand treatment and HSV-1 infection, related to Fig 4. (A) Mock or HSV-1 infected HFFs were treated with 5-OP-RU (10 μM) at 14 h p.i. before staining for surface MR1 or isotype control (grey) at 18 h p.i. (B) CD69 expression on Jurkat MAIT (JM) cells incubated with mock or HSV-1 infected cells treated with *E. coli* or 5-OP-RU before blocking with anti-MR1 or isotype control as indicated (C) Fold change in CD69 expression on JM left untreated or treated with 5-OP-RU is graphed. Statistical significance was calculated by paired Student's *t*-test *** $p<0.001$, (n=6)



Supp Fig 7: Late viral gene expression is not blocked by proteasomal inhibition, related to Fig 5. ARPE-19 cells were infected with HSV-1 Strain F (MOI=5) or mock infected in parallel, and stained for the viral protein gD or isotype control (grey) at 18 h p.i. Cells were treated with MG132 (5 μM at 0 h p.i.) or DMSO, or left untreated as indicated.



Supp Fig 8. HSV-1 Us3 expression downregulates surface MR1, related to Fig 7A. 293T cells transduced with lentivirus expressing HSV-1 Us3 were treated with Ac-6-FP (5 μM) for 6 h prior to staining for surface MR1, analysis by flow cytometry, with gating on GFP expression to identify Us3-expressing GFP^+ cells. Fold change relative to GFP^- cells is graphed. Statistical significance was calculated by paired Student's *t*-test ** $p<0.005$, *** $p<0.0005$, (n=3).



Supp Fig 9: Construction of HSV-1 Us3 mutant, related to STAR methods. HSV-1 KOS Us3 coding sequence was inserted downstream of eGFP in pEGFP-C1 plasmid before digesting with BglII and BamHI to collapse Us3. Mutation introduced into parental virus through homologous recombination to generate ΔUs3.