

Supplementary Information for

The human cytomegalovirus-encoded G protein-coupled receptor UL33 exhibits oncomodulatory properties

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Figs. S1 to S5

References for SI reference citations

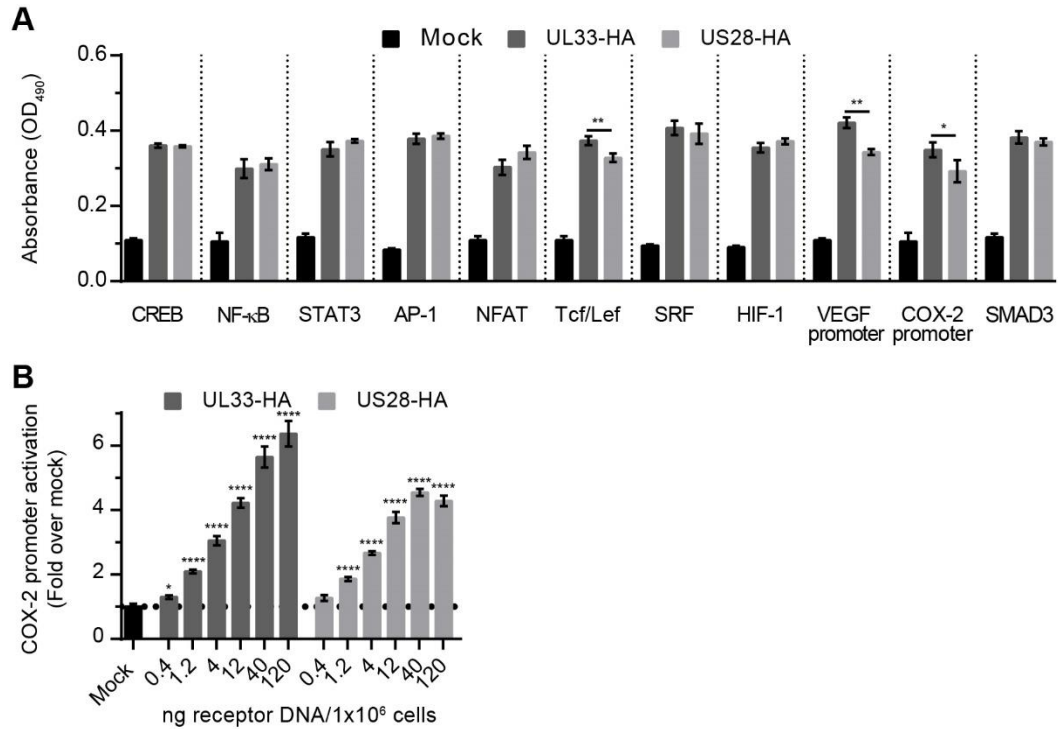


Figure S1. (A) Similar expression of UL33 and US28, as assessed by means of anti-HA ELISA, in HEK293T cells transfected with 12 ng UL33-HA or 40 ng US28-HA DNA per 10^6 cells in combination with the respective luciferase reporter gene constructs. UL33 and US28 were both C-terminally tagged with the HA-epitope. (B) Constitutive COX-2 promoter activation in HEK293T cells transiently transfected with the COX-2 promoter reporter gene and increasing amounts of DNA encoding UL33-HA or US28-HA. Graphs are representatives of at least three individual experiments performed in triplicate. Data are depicted as mean \pm S.D. * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$.

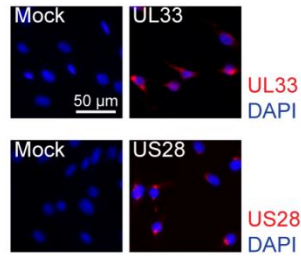


Figure S2. Expression of UL33 and US28 in NIH-3T3 cells. Immunofluorescence imaging of receptor expression in NIH-3T3 cells stably transfected with empty vector (Mock), UL33-eGFP or US28. UL33 and US28 were visualized using polyclonal antibodies raised against the receptors (1,2).

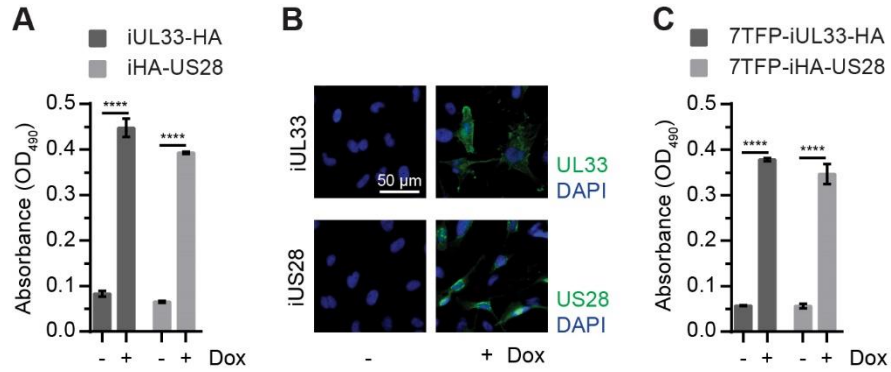


Figure S3. Doxycycline-dependent receptor expression in U251 iUL33-HA and U251 iHA-US28 cells (**A**), U251 iUL33 and U251 iUS28 cells (**B**), or U251-7TFP iUL33-HA and U251-7TFP iHA-US28 cells (**C**) as determined using anti-HA ELISA (**A and C**) and immunofluorescence microscopy using polyclonal antibodies raised against the respective receptor (1,2) (**B**). (**A and C**) The HA-epitope was located C-terminally of UL33 and at the N-terminus of US28. Graphs are representatives of three individual experiments performed in triplicate. Data are depicted as mean \pm S.D. **** $p < 0.0001$.

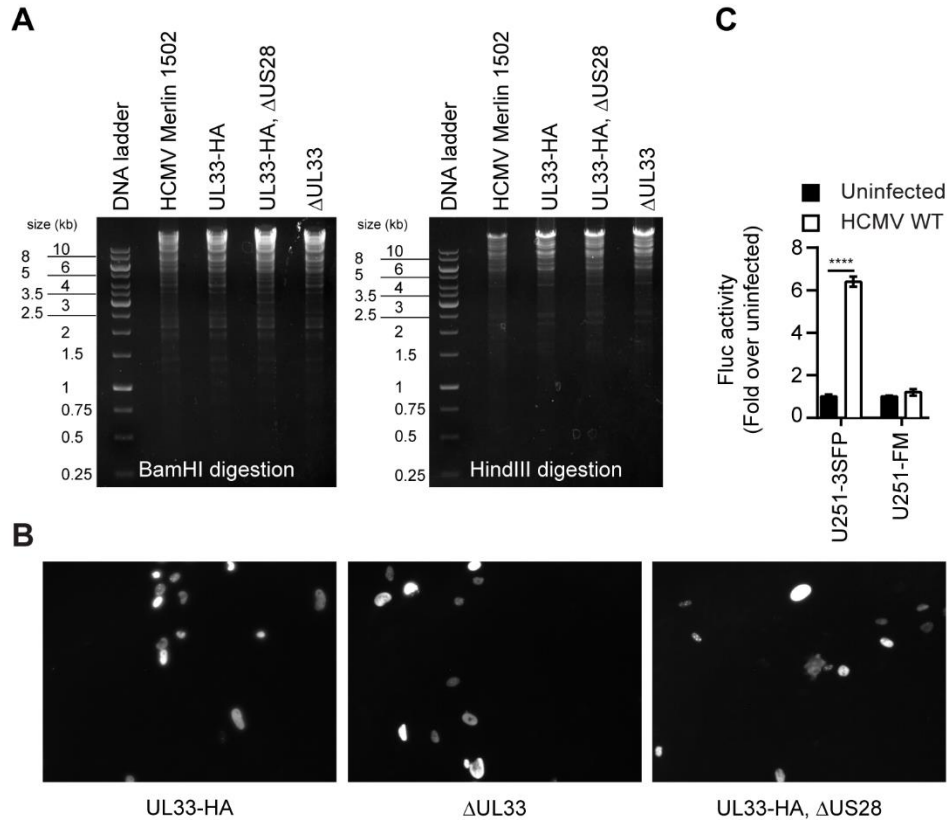


Figure S4. (A) BamHI and HindIII digestion patterns of HCMV Merlin 1502 (starting material), WT (UL33-HA), US28 knockout (UL33-HA, ΔUS28) and UL33 knockout (ΔUL33) BAC DNA. (B) Similar infection rate of U251-3SFP cells infected with wild type (UL33-HA), UL33 knockout (ΔUL33) or US28 knockout (UL33-HA, ΔUS28) Merlin virus, as determined by IE1 staining at five days post infection. Pictures show representatives of 6 frames per well. (C) Effect of HCMV infection on STAT3-driven transcription (U251-3SFP) vs constitutive transcriptional activity (U251-FM), at five days post infection at MOI 6. Graphs are representatives of three individual experiments performed in triplicate. Data are depicted as mean ± S.D. ****p<0.0001.

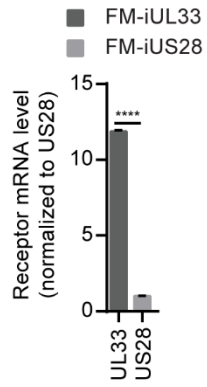


Figure S5. Relative receptor mRNA expression in U251-FM-iUL33 and U251-FM-iUS28 cell lines. Receptor mRNA abundance was determined by RT-qPCR for their shared 3' UTR. Graph is representative of three individual experiments performed in triplicate. Data are depicted as mean \pm S.D. ****p<0.0001.

References

1. Bongers, G., Maussang, D., Muniz, L. R., Noriega, V. M., Fraile-Ramos, A., Barker, N., Marchesi, F., Thirunarayanan, N., Vischer, H. F., Qin, L., Mayer, L., Harpaz, N., Leurs, R., Furtado, G. C., Clevers, H., Tortorella, D., Smit, M. J., and Lira, S. A. (2010) The cytomegalovirus-encoded chemokine receptor US28 promotes intestinal neoplasia in transgenic mice. *J Clin Invest* **120**, 3969-3978
2. Margulies, B. J., Browne, H., and Gibson, W. (1996) Identification of the human cytomegalovirus G protein-coupled receptor homologue encoded by UL33 in infected cells and enveloped virus particles. *Virology* **225**, 111-125