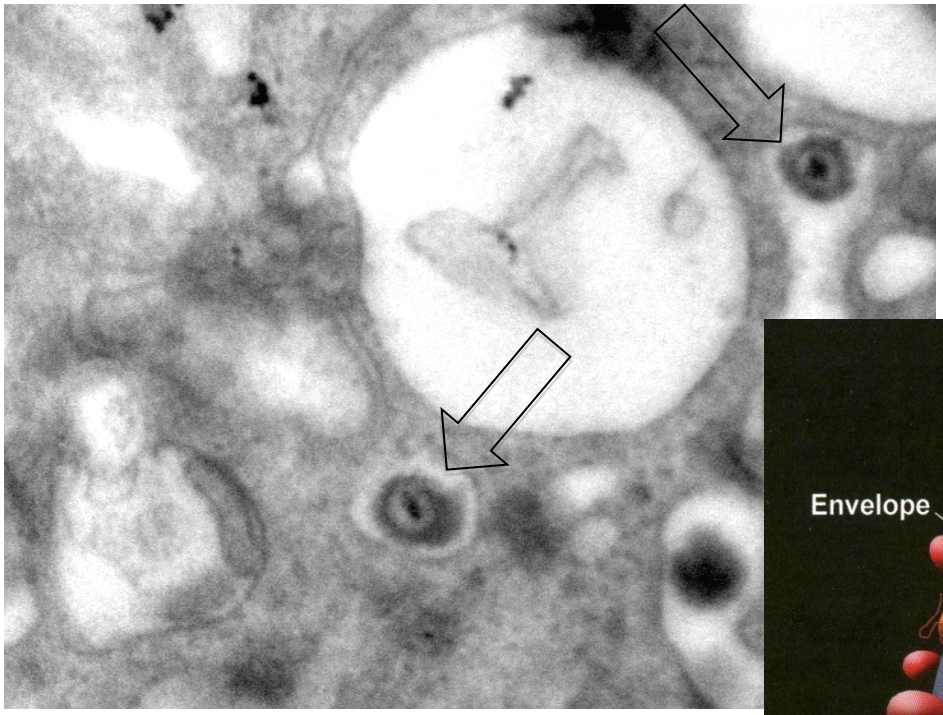
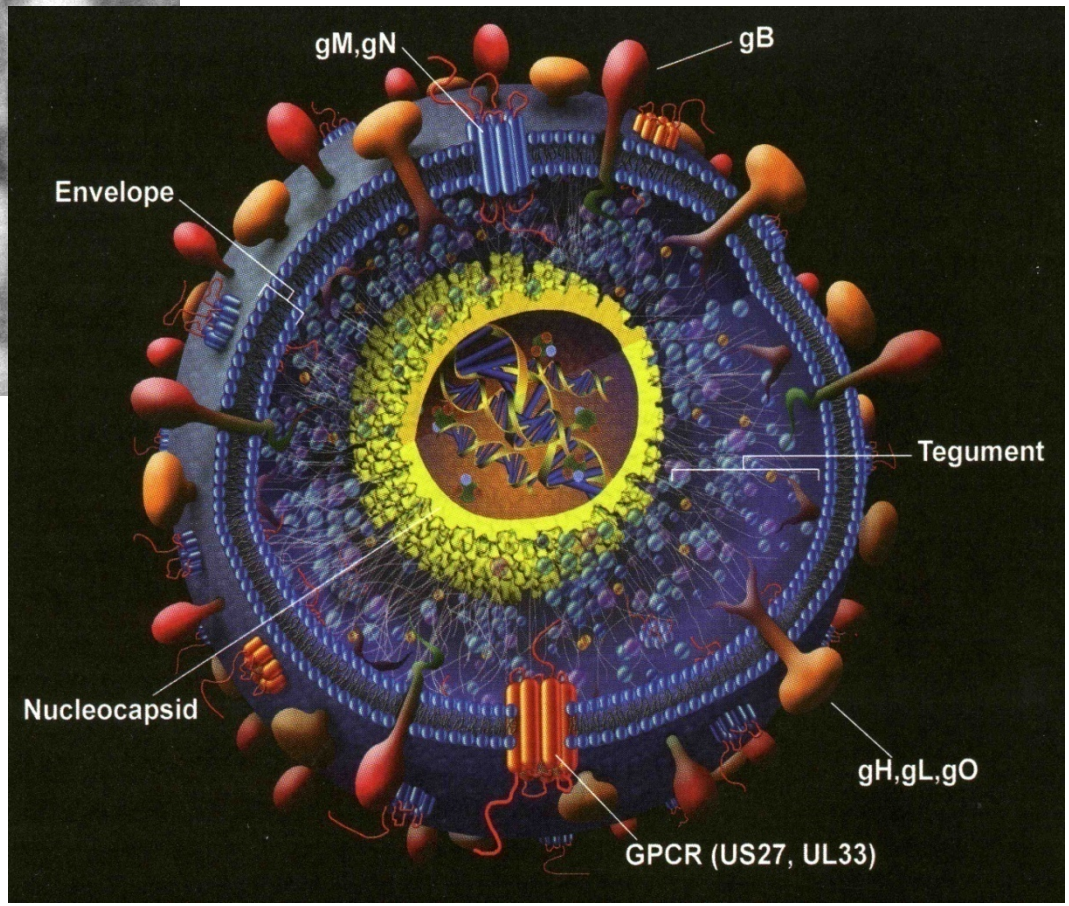
VIROLOGY

Generation and validation of genetically engineered indicator cell lines for the detection of infectious HCMV particles and the search of antiviral compounds: an academic lab scale example

Our main virus model: the Human Cytomegalovirus (HCMV)

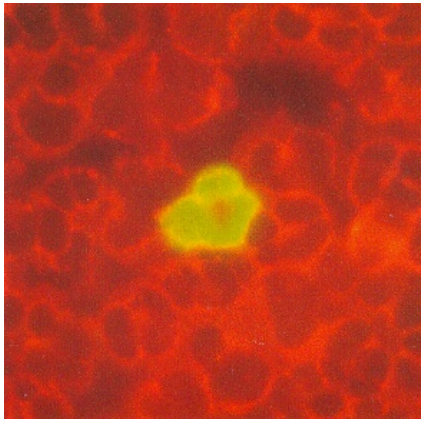


Luganini et al., J. Virol., 2017

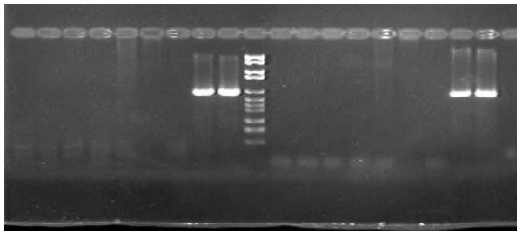


Diagnosis of HCMV infections

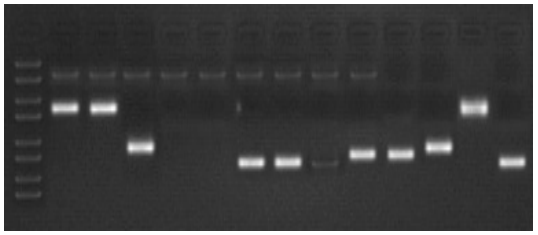
Antigenemia



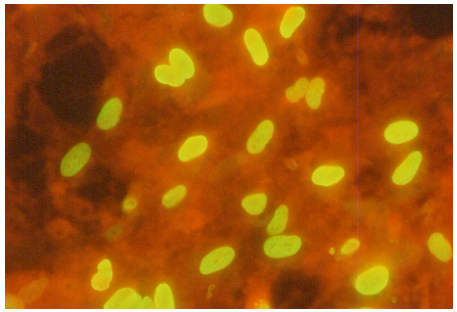
DNAemia



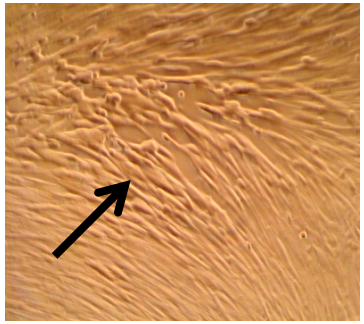
RNAemia



•Before validation of Real-time PCR DNAemia, the diagnostic gold standard of active infection was the infectious virus identification (viremia)



Viremia



• Quantitative determination of infectious viral particles correlates with clinical diseases and prognosis

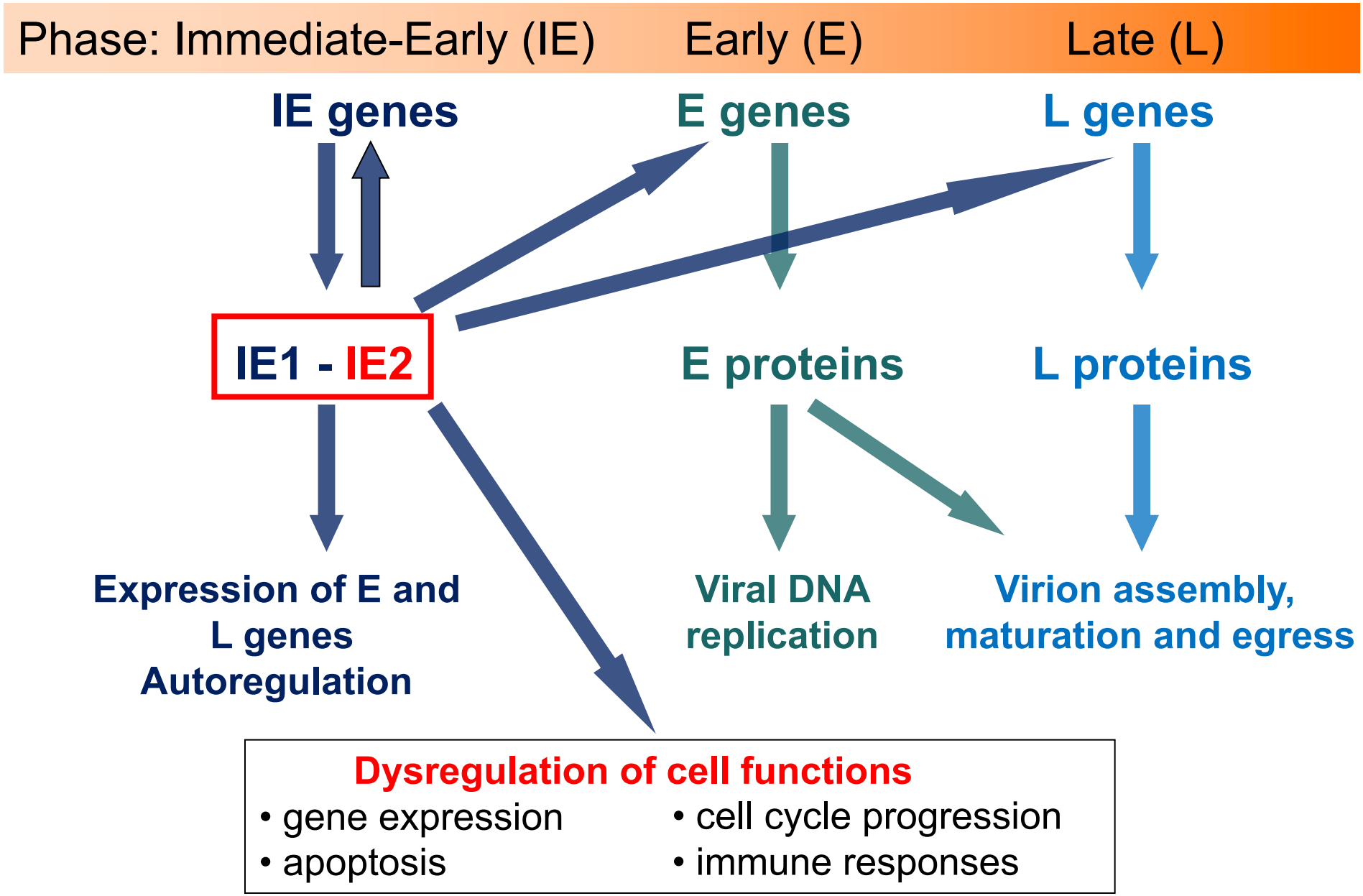
Aims of the research project

To generate and validate innovative indicator human cell lines suitable for:

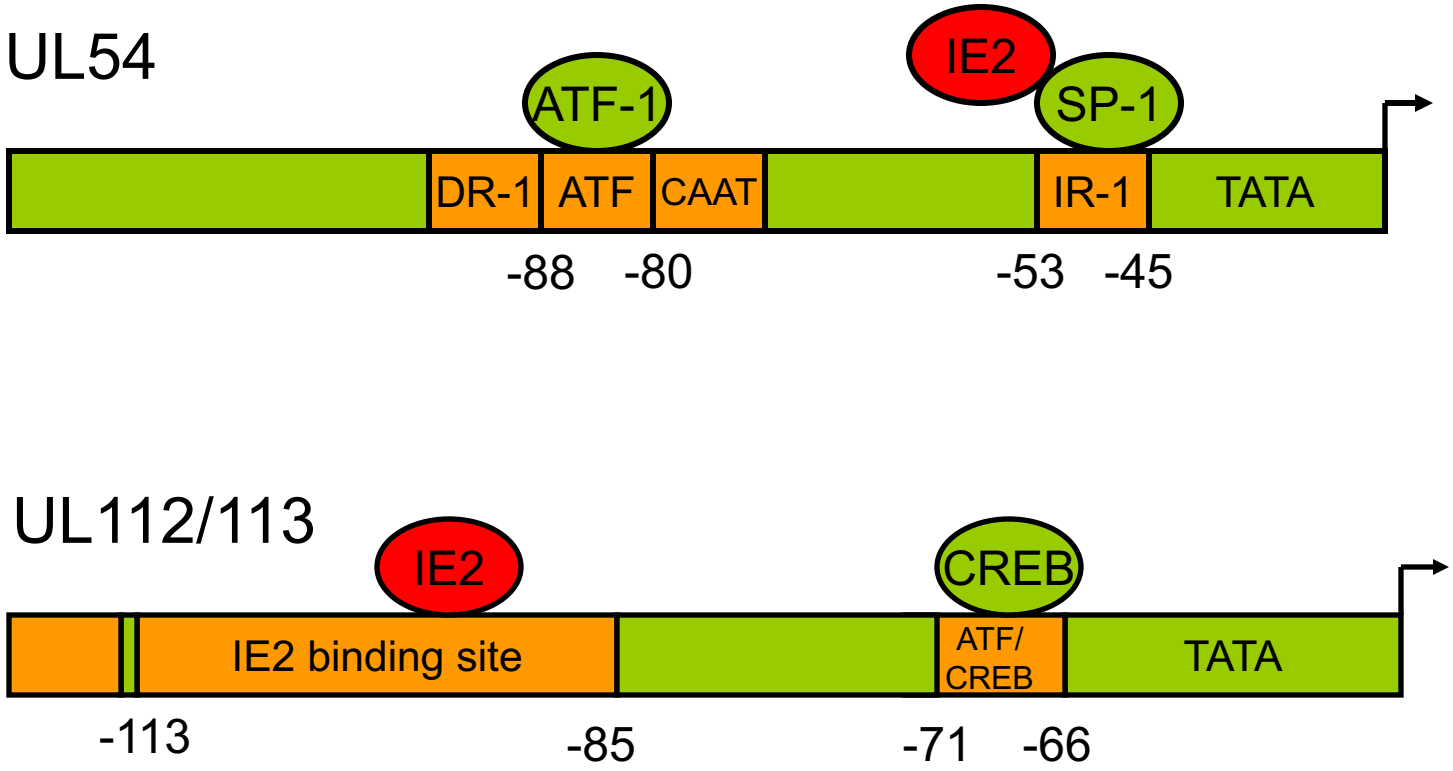
1) Detection of infectious HCMV particles.

2) Selection of inhibitors of IE2-dependent activities

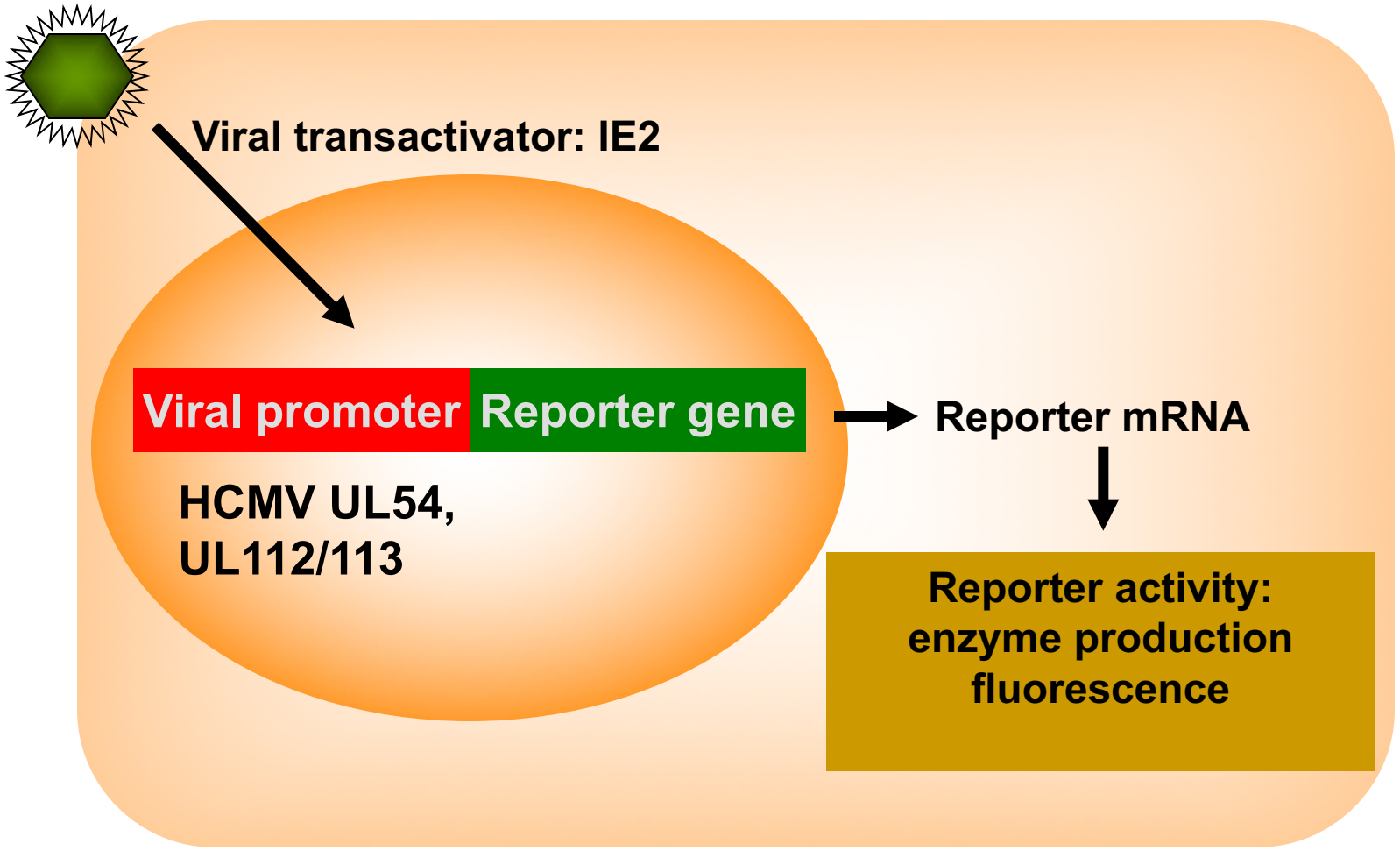
HCMV gene expression



Structure of HCMV UL54 and UL112/113 gene promoters: two prototypic IE2-activatable HCMV E genes



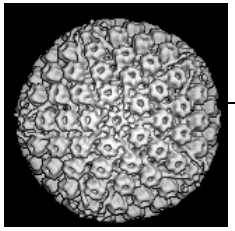
Genetically engineered cell lines to detect HCMV infectious viruses



Genetically engineered cell lines that facilitate Herpesvirus detection: critical issues?

- the viral promoter
- the cell type
- the reporter gene

Generation of IE2-activatable indicator cell lines: outline of the experimental procedure



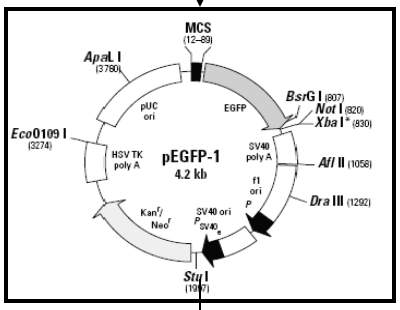
Purified HCMV AD169

DNA extraction and PCR

UL54 gene promoter

UL112/113 gene promoter

Cloning UL54 e UL112/113 gene promoters into pEGFP-1 plasmid

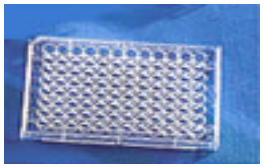


Recombinant selection, restriction mapping, sequencing and plasmids production

Transfection into U373-MG

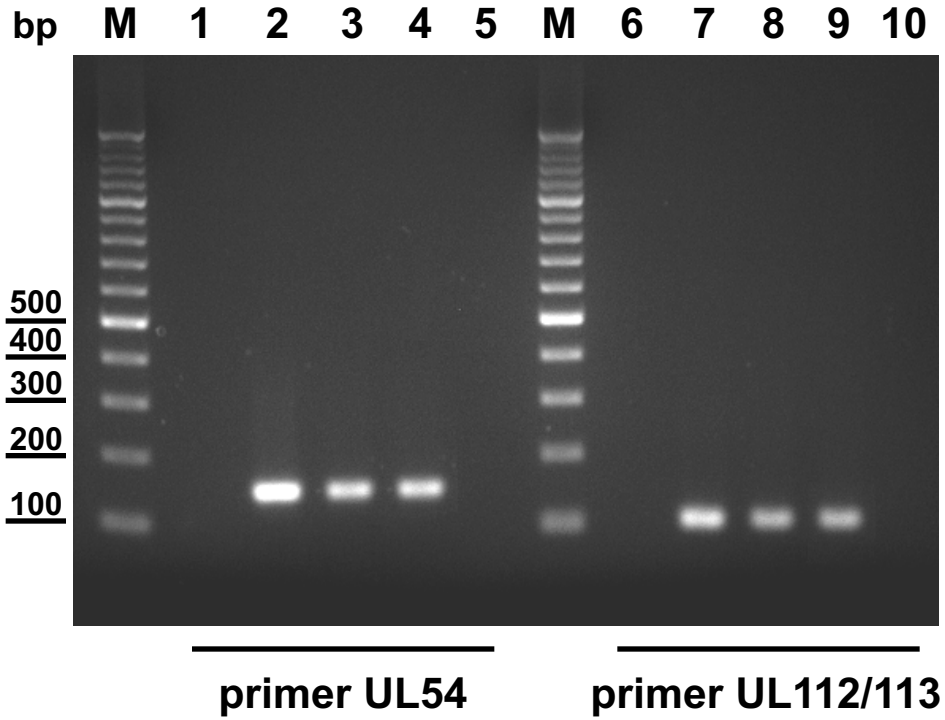
G418 selection

Cell cloning for limiting dilution



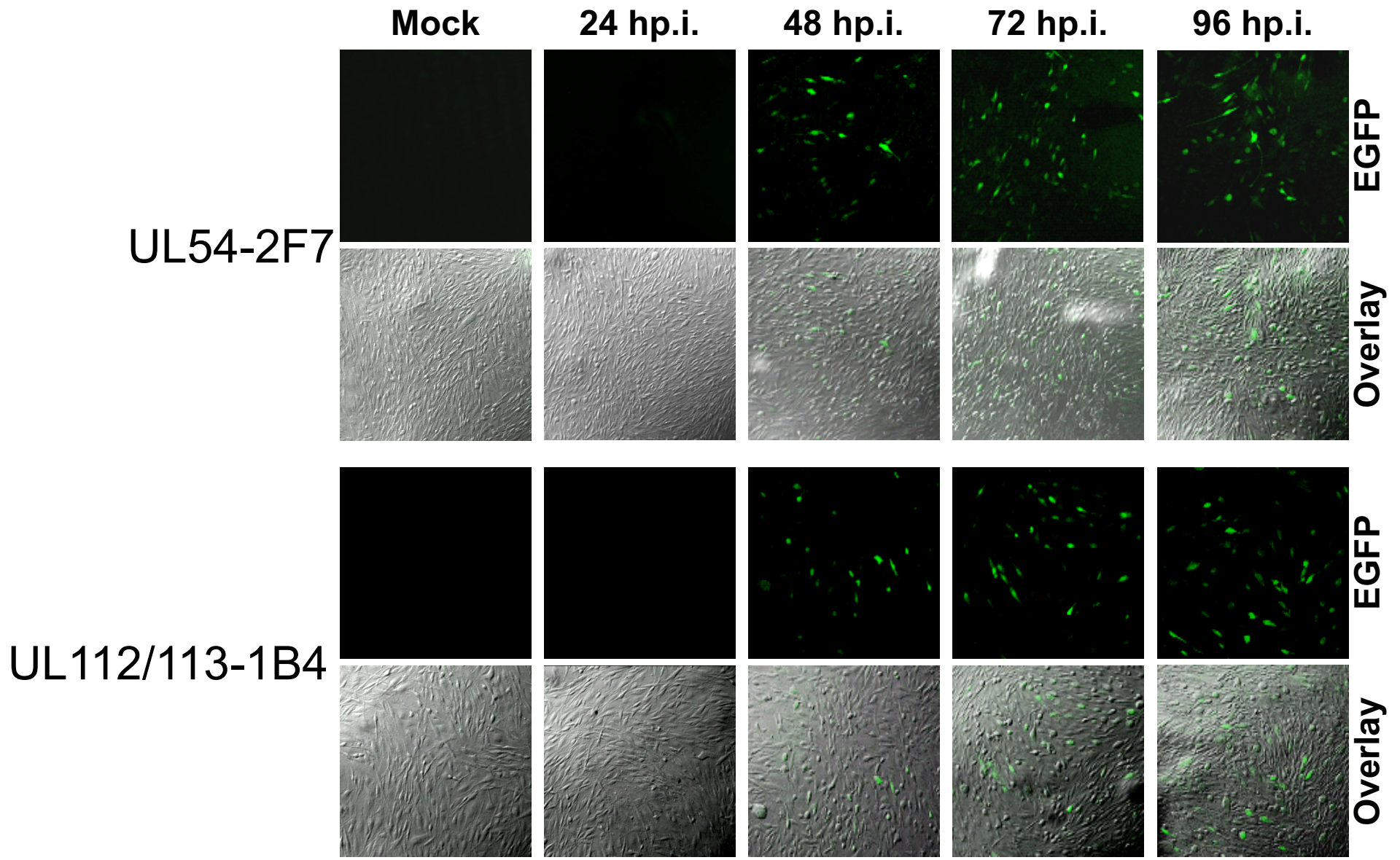
Functional characterization of selected clones

Molecular characterization of U373-MG clones UL54-2F7 and UL112/113-1B4 stably transfected with reporter plasmids



1- 6 : H_20
2 : pUL54-EGFP
3 : UL54-2F7
4- 9 : DNA HCMV VR1814
5- 10: U373-MG
7 : pUL112/113-EGFP
8 : UL112/113-1B4

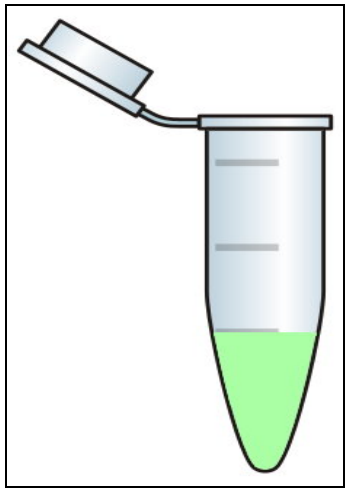
HCMV induces EGFP expression in UL54-2F7 and UL112/113-1B4 cells



Assay for quantitative EGFP expression by automated fluorometry

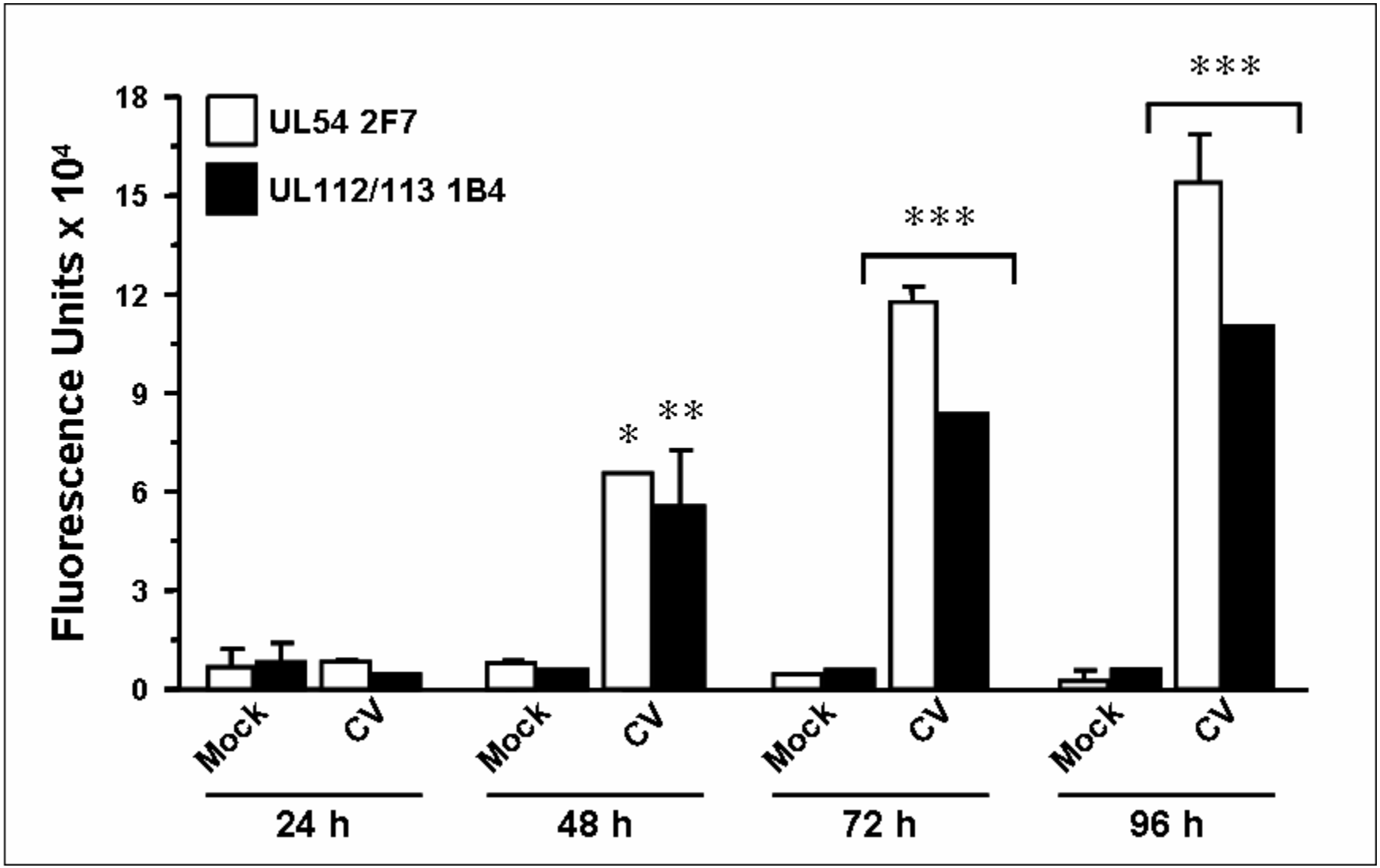
HCMV infection of 24- or 96-well plates

Preparation of total cell lysates at 48 h p.i.

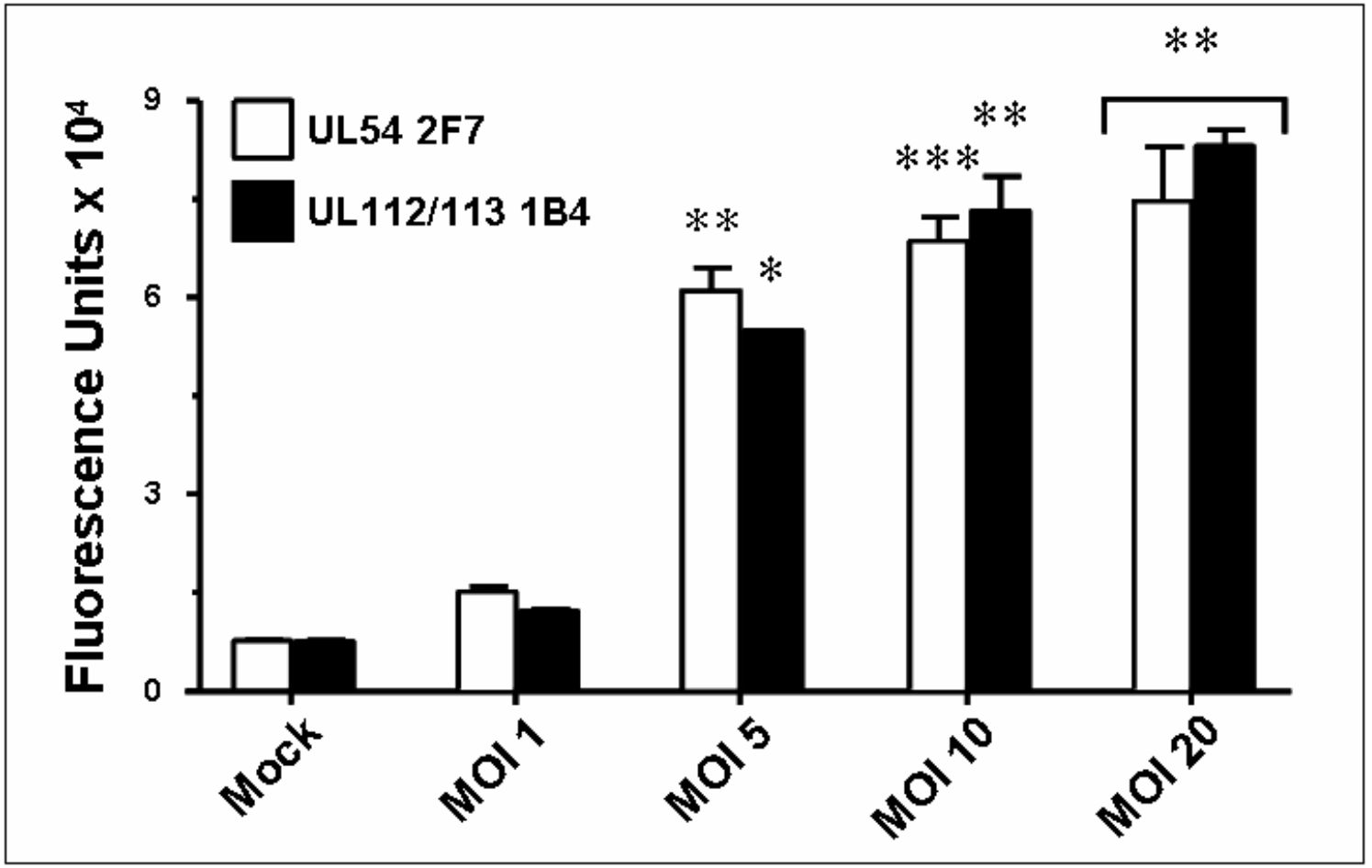


Analysis of EGFP content in a fluorescent microplate reader

Characteristics of UL54-2F7 and UL112/113-1B4 cells: HCMV infection induces EGFP expression in a *time-dependent manner*

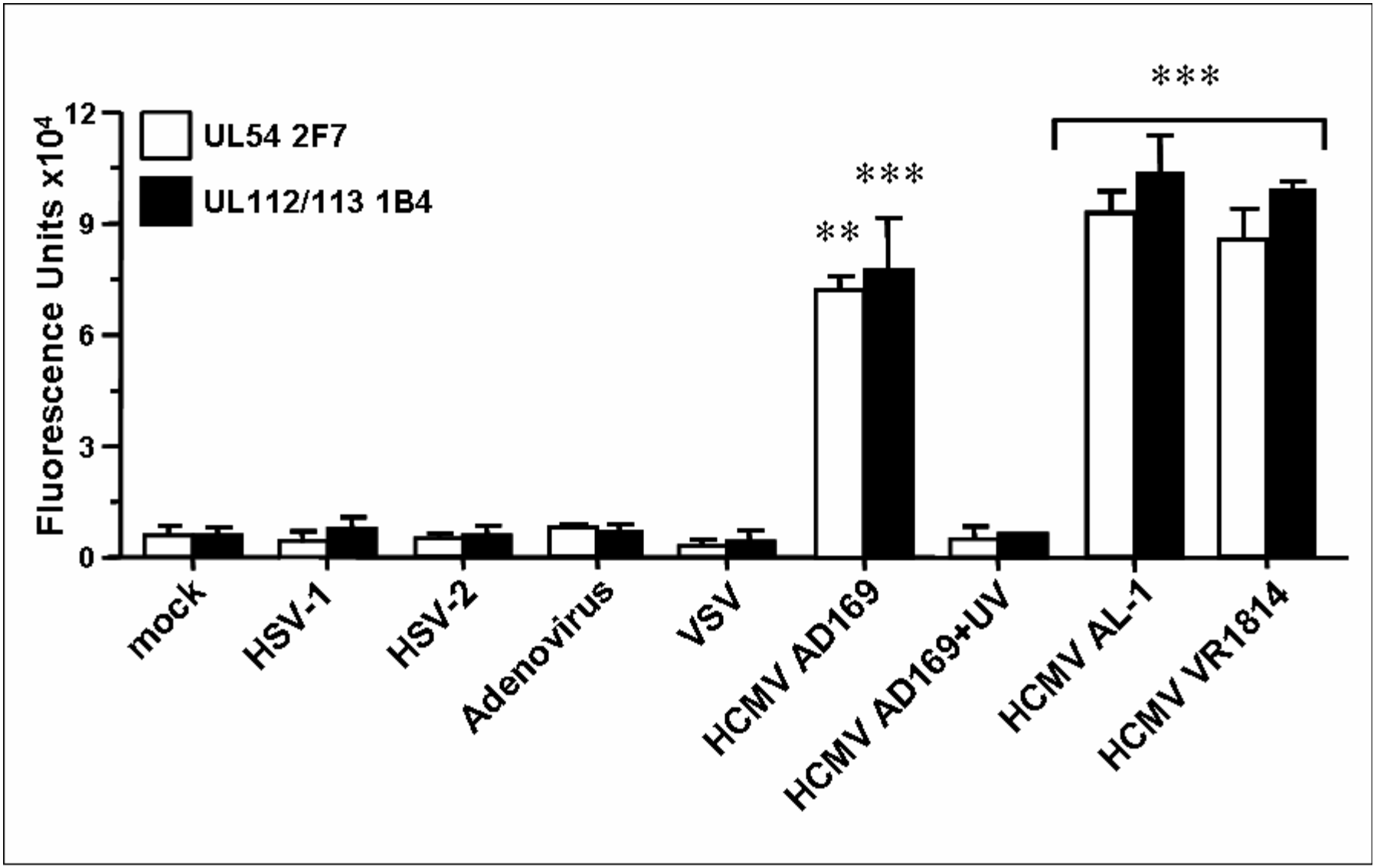


Characteristics of UL54-2F7 and UL112/113-1B4 cells: HCMV infection induces EGFP expression in a *dose-dependent manner*

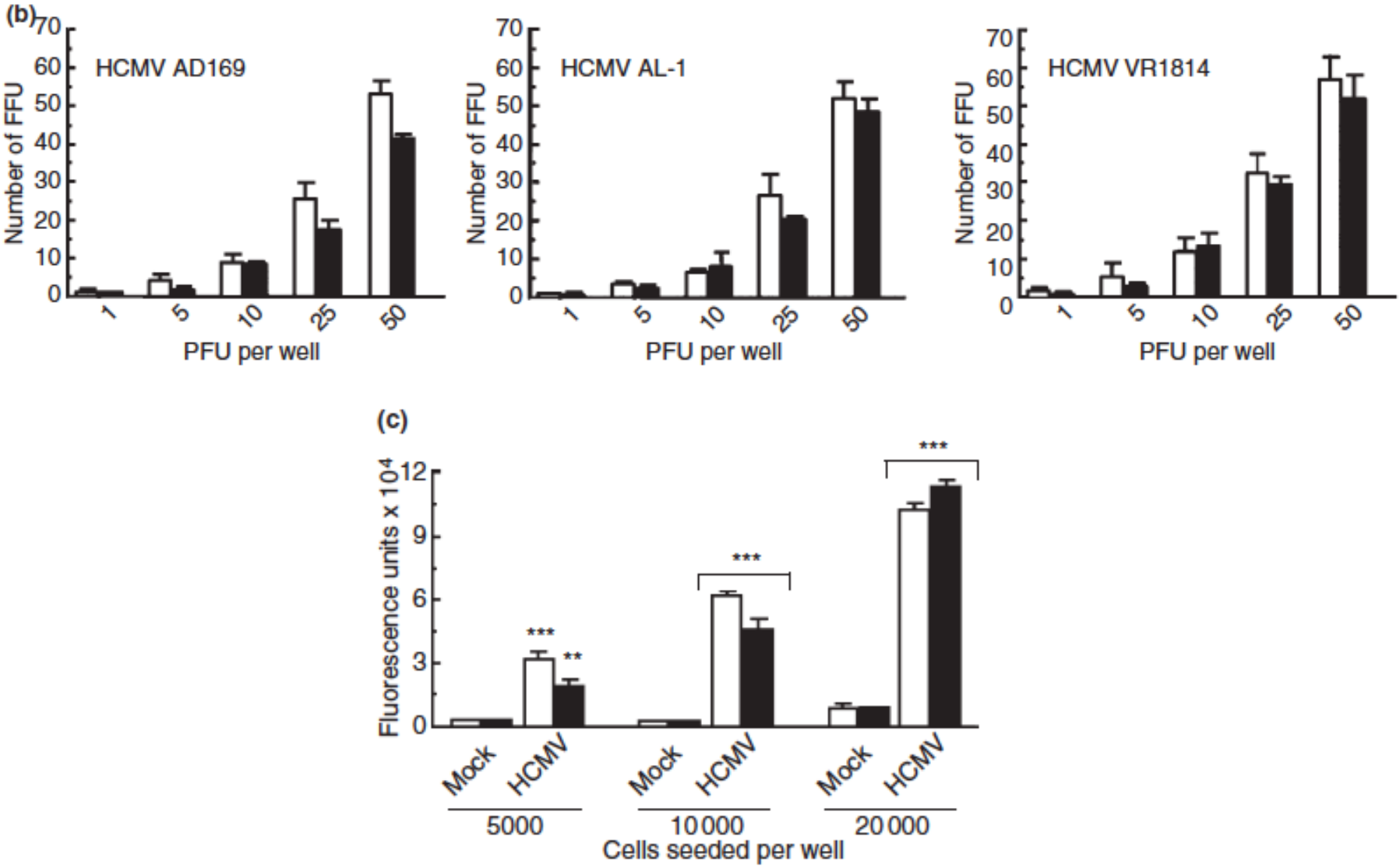


Characteristics of UL54-2F7 and UL112/113-1B4 cells:

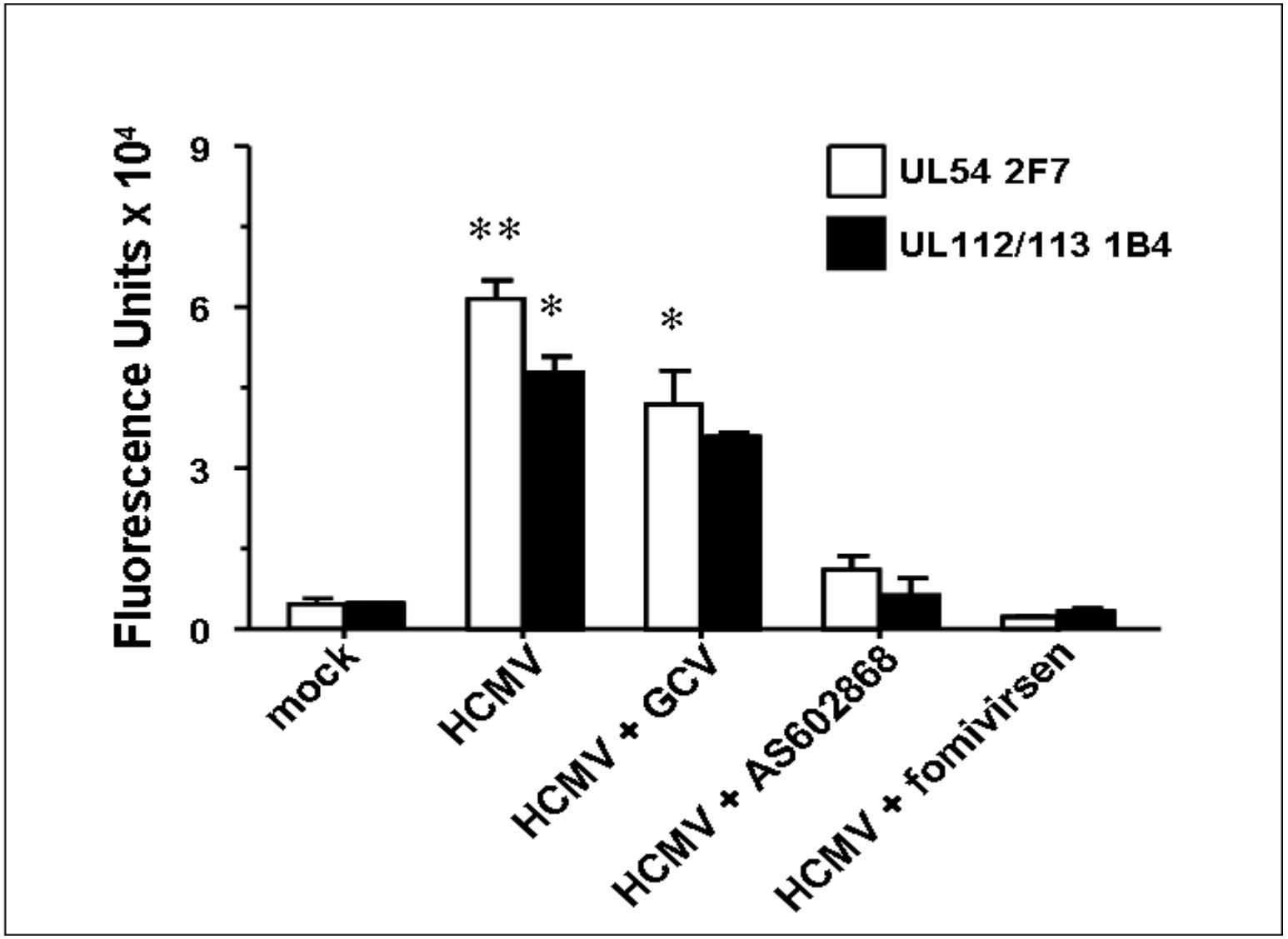
HCMV infection specifically stimulates EGFP signals



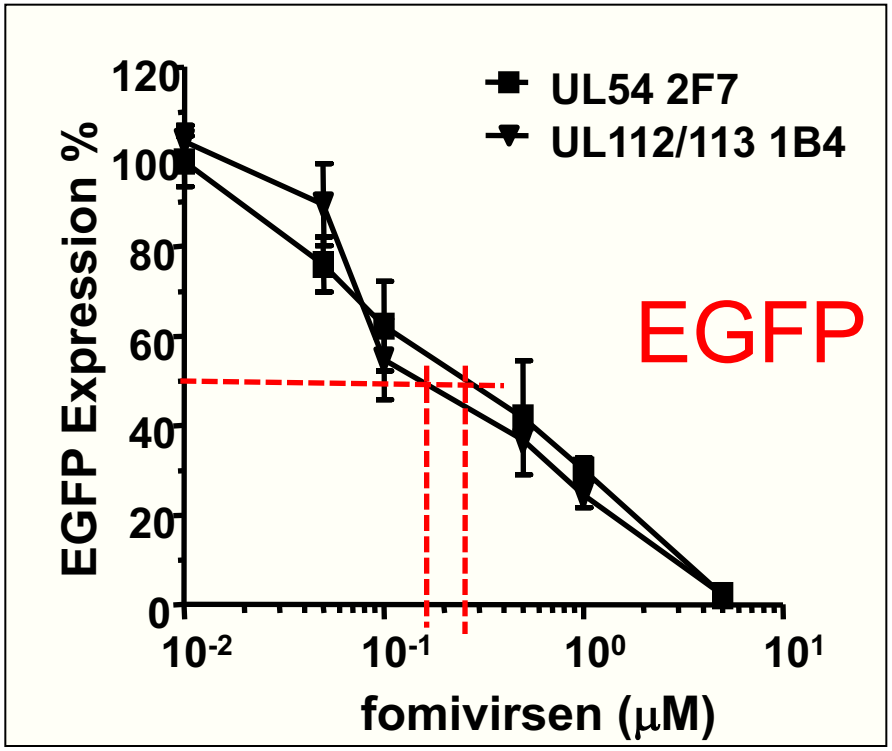
Characteristics of UL54-2F7 and UL112/113-1B4 cells: *sensitivity* of the EGFP cell based assay and its optimization for the 96-well format

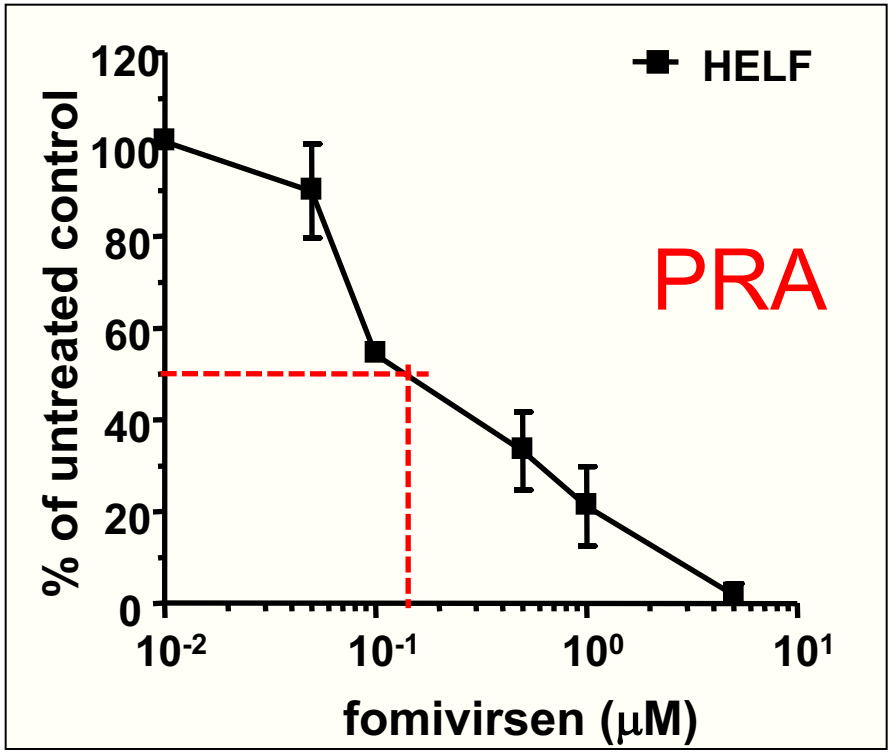


Effects of anti-HCMV drugs on EGFP expression in UL54-2F7 and UL112/113-1B4 cells

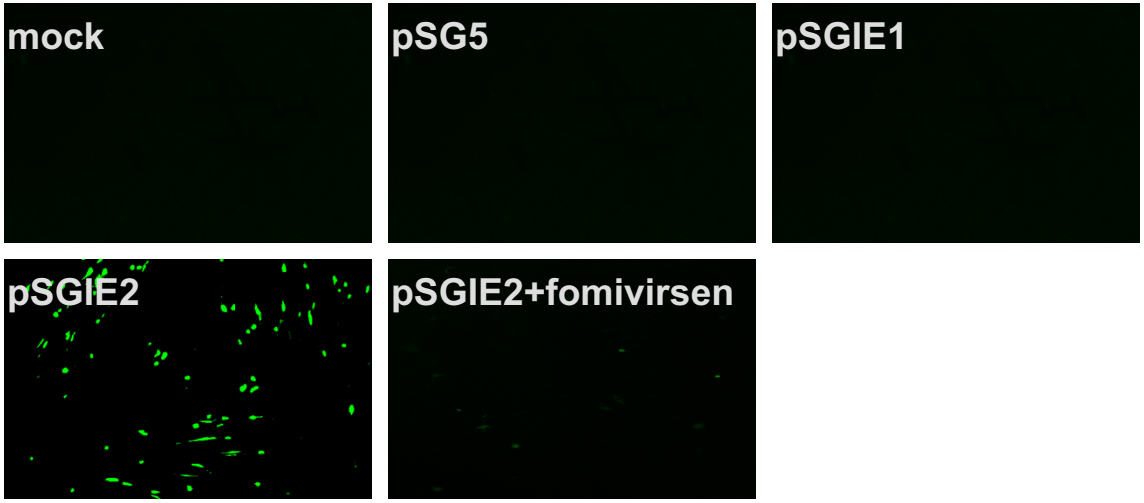


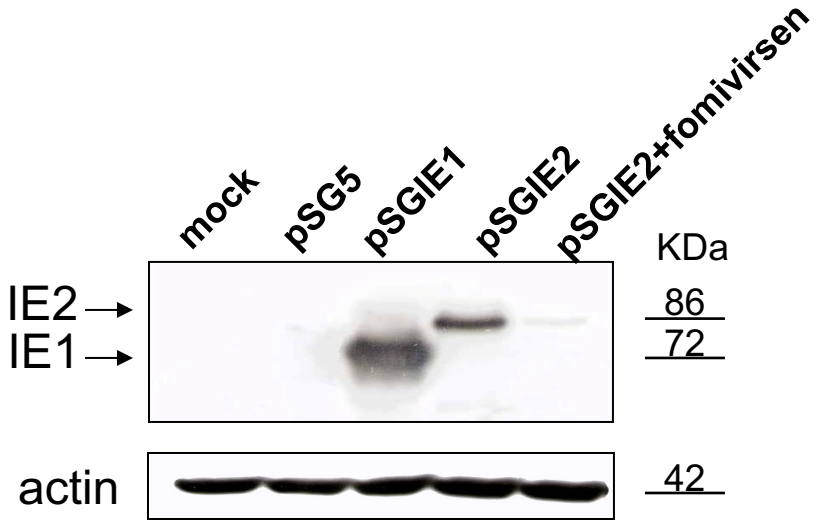
Validation of UL54-2F7 and UL112/113-1B4 cells as reliable tools for assessing the *antiviral activity* of a test compound



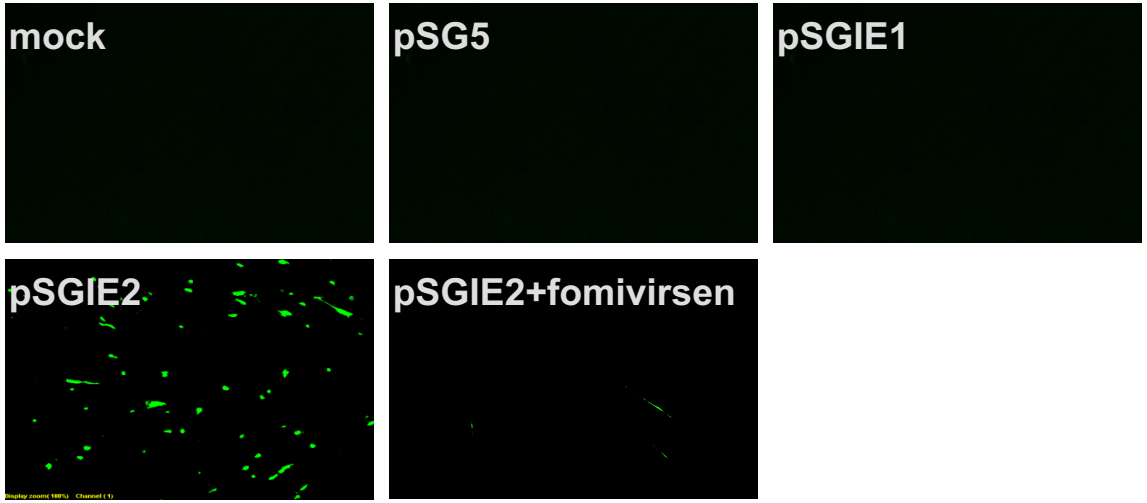


The *IE2 protein stimulates* EGFP expression in UL54-2F7 cells and this induction is prevented by fomivirsen

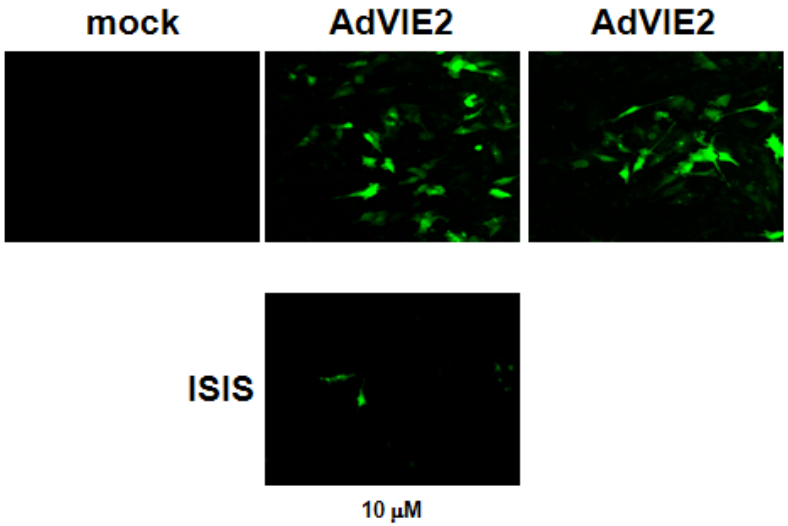


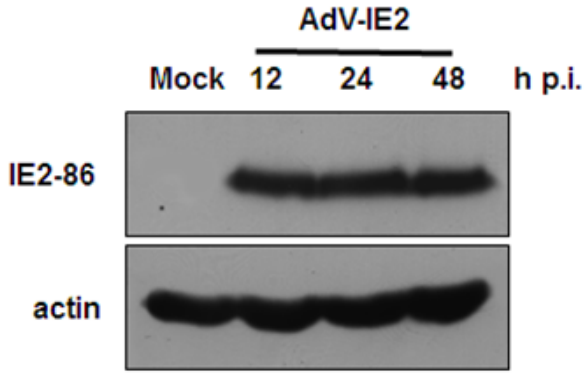


The *IE2 protein stimulates* EGFP expression in UL112/113-1B4 cells and this induction is prevented by fomivirsen



Adenoviral-mediated IE2 expression induces EGFP in UL54-2F7 cells and this induction is prevented by fomivirsen





Cells: U373_UL54-2F7
Infection: AdVIE2 (MOI=20)
Assay: 72 hp.i.
Treatment: 2 h.p.i

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

New cell-based indicator assays for the detection of human cytomegalovirus infection and screening of inhibitors of viral immediate-early 2 protein activity

A. Luganini[1], P. Caposio[1], M. Mondini[2], S. Landolfo[1] and G. Gribaudo[1]

1 Department of Public Health and Microbiology, University of Torino, Torino, Italy
2 Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy

Conclusions:

1) UL54-2F7 and UL112/113-1B4 indicator cell lines warrant quantitative detection of infection within 48 h p.i. compared to 7 d by conventional plaque assay.

2) UL54-2F7 and UL112/113-1B4 cells are specific for HCMV, both laboratory and low-passages clinical strains.

3) The sensitivity of UL54-2F7 and UL112/113-1B4 cells for detecting antiviral activity (48 p.i.) is comparable to that of the standard plaque reduction assay (PRA) (7 d).

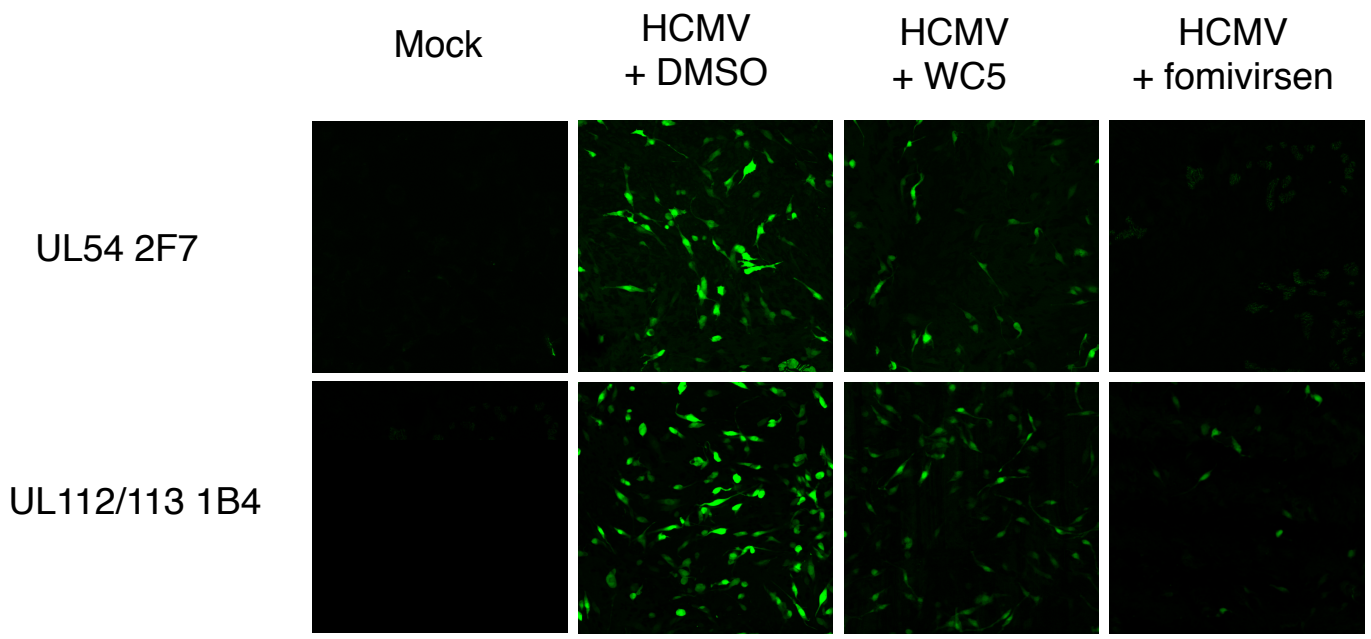
4) UL54-2F7 and UL112/113-1B4 cells could be used as cell-based assays for screening of molecules able to interfere with the activities of the essential IE2 protein of HCMV.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2010, p. 1930–1940 Vol. 54, No. 5
0066-4804/10/$12.00 doi:10.1128/AAC.01730-09

The 6-Aminoquinolone WC5 Inhibits Human Cytomegalovirus Replication at an Early Stage by Interfering with the Transactivating Activity of Viral Immediate-Early 2 Protein▽†

Arianna Loregian,[1]*‡ Beatrice Mercorelli,[1]‡ Giulia Muratore,[1] Elisa Sinigalia,[1] Silvana Pagni,[1] Serena Massari,[2] Giorgio Gribaudo,[3] Barbara Gatto,[4] Manlio Palumbo,[4] Oriana Tabarrini,[2] Violetta Cecchetti,[2] and Giorgio Palù[1]

Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, 35121 Padua,[1] Department of Chemistry and Technology of Drugs, University of Perugia, 06123 Perugia,[2] Department of Public Health and Microbiology, University of Turin, Turin,[3] and Department of Pharmaceutical Sciences, University of Padova, via Marzolo 5, 35131 Padua,[4] Italy

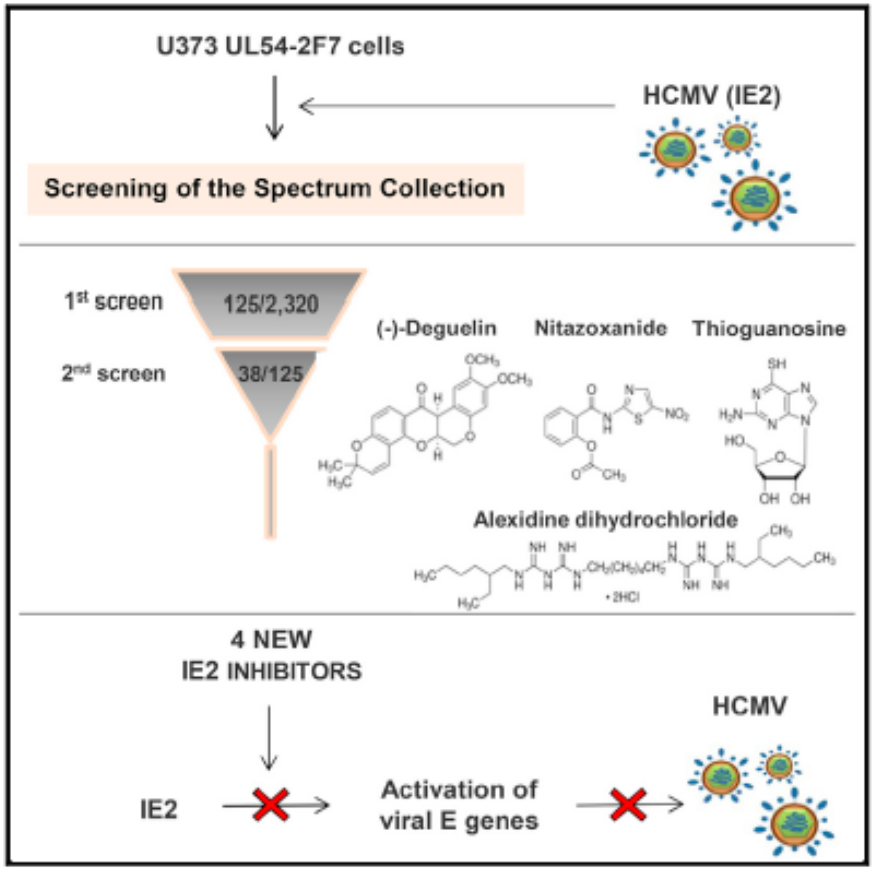


Please cite this article in press as: Mercorelli et al., Drug Repurposing Approach Identifies Inhibitors of the Prototypic Viral Transcription Factor IE2 that Block Human Cytomegalovirus Replication, Cell Chemical Biology (2016), http://dx.doi.org/10.1016/j.chembiol.2015.12.012

Drug Repurposing Approach Identifies Inhibitors of the Prototypic Viral Transcription Factor IE2 that Block Human Cytomegalovirus Replication

Beatrice Mercorelli,[1,4] Anna Luganini,[2,4] Giulio Nannetti,[1] Oriana Tabarrini,[3] Giorgio Palù,[1] Giorgio Gribaudo,[2,5] and Arianna Loregian[1,5,*]

[1]Department of Molecular Medicine, University of Padua, 35121 Padua, Italy
[2]Department of Life Sciences and Systems Biology, University of Turin, 10123 Turin, Italy
[3]Department of Pharmaceutical Sciences, University of Perugia, 06123 Perugia, Italy
[4]Co-first author
[5]Co-senior author
*Correspondence: arianna.loregian@unipd.it
http://dx.doi.org/10.1016/j.chembiol.2015.12.012



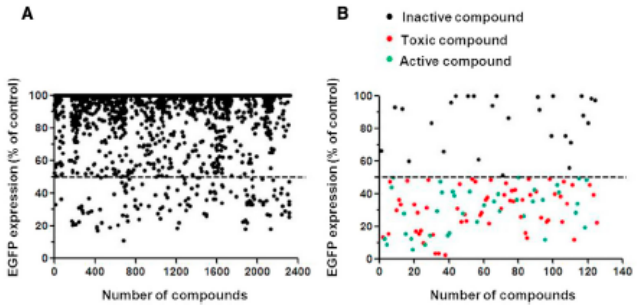


Figure 1. Cell-Based Screening Data
Each circle represents the mean % of EGFP expression for a given compound tested at 10 μM in duplicate during the primary (A) and secondary screen (B). Infected and DMSO-treated cells were considered as exhibiting 100% of EGFP expression. The dashed line represents the arbitrary hit cutoff (50% of EGFP expression). In (B), among the compounds with two replicates below 50% of the EGFP expression threshold, active (green) compounds were distinguished from toxic (red) compounds. Compounds with the means above 50% were considered inactive (black).