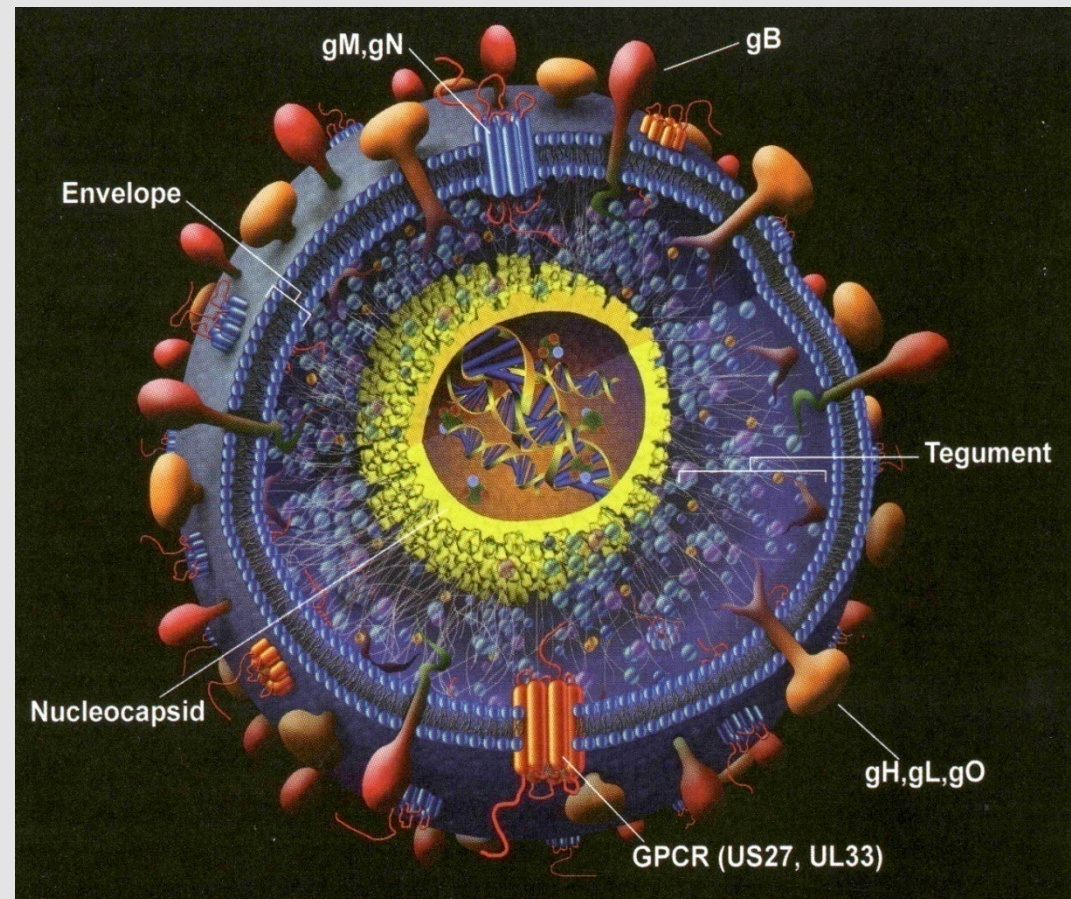
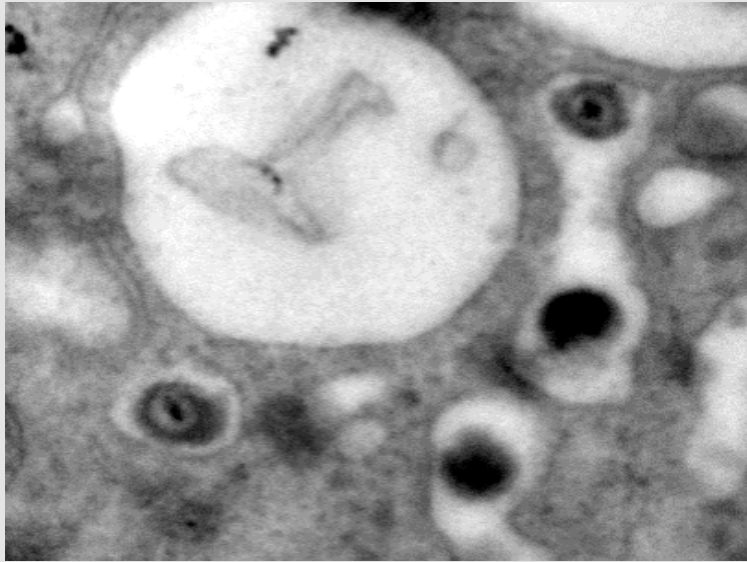


VIROLOGY

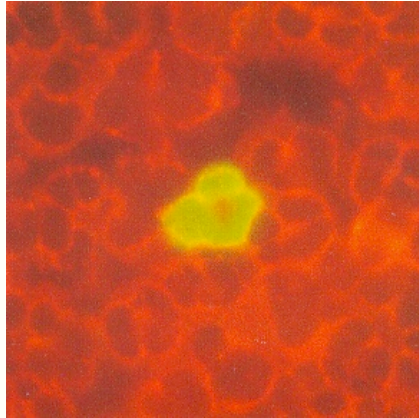
Generation and validation of genetically engineered indicator cell lines for the detection of infectious HCMV particles and the search of antiviral compounds: an academic lab scale example

Our main virus model: the Human Cytomegalovirus (HCMV)

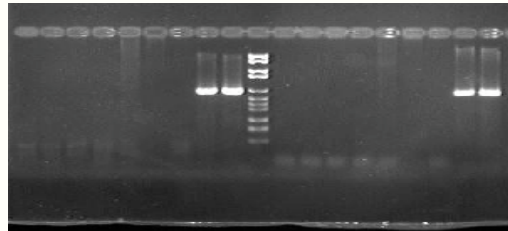


Diagnosis of HCMV infections

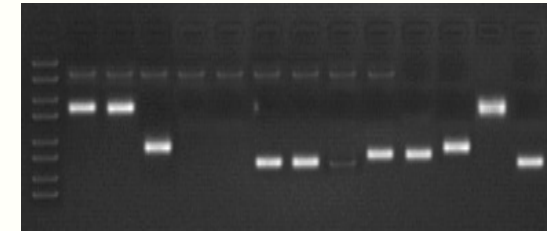
Antigenemia



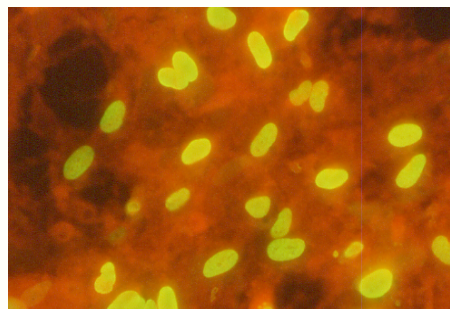
DNAemia



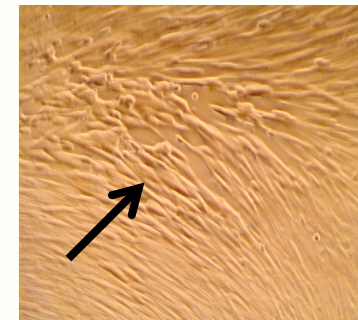
RNAemia



• 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

Phase: Immediate-Early (IE)

Early (E)

Late (L)

IE genes

E genes

L genes

IE1 - IE2

E proteins

L proteins

Expression of E and L genes
Autoregulation

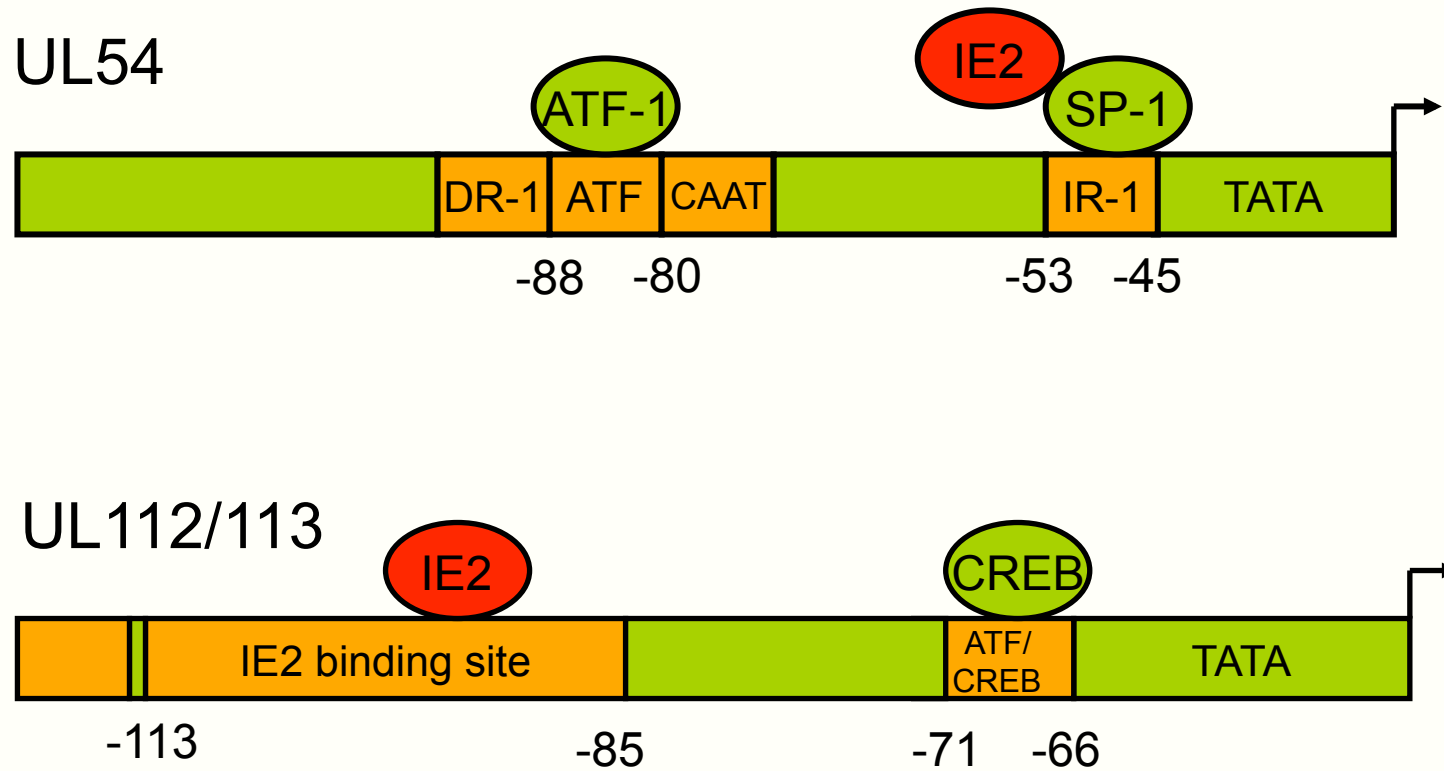
Viral DNA replication

Virion assembly, maturation and egress

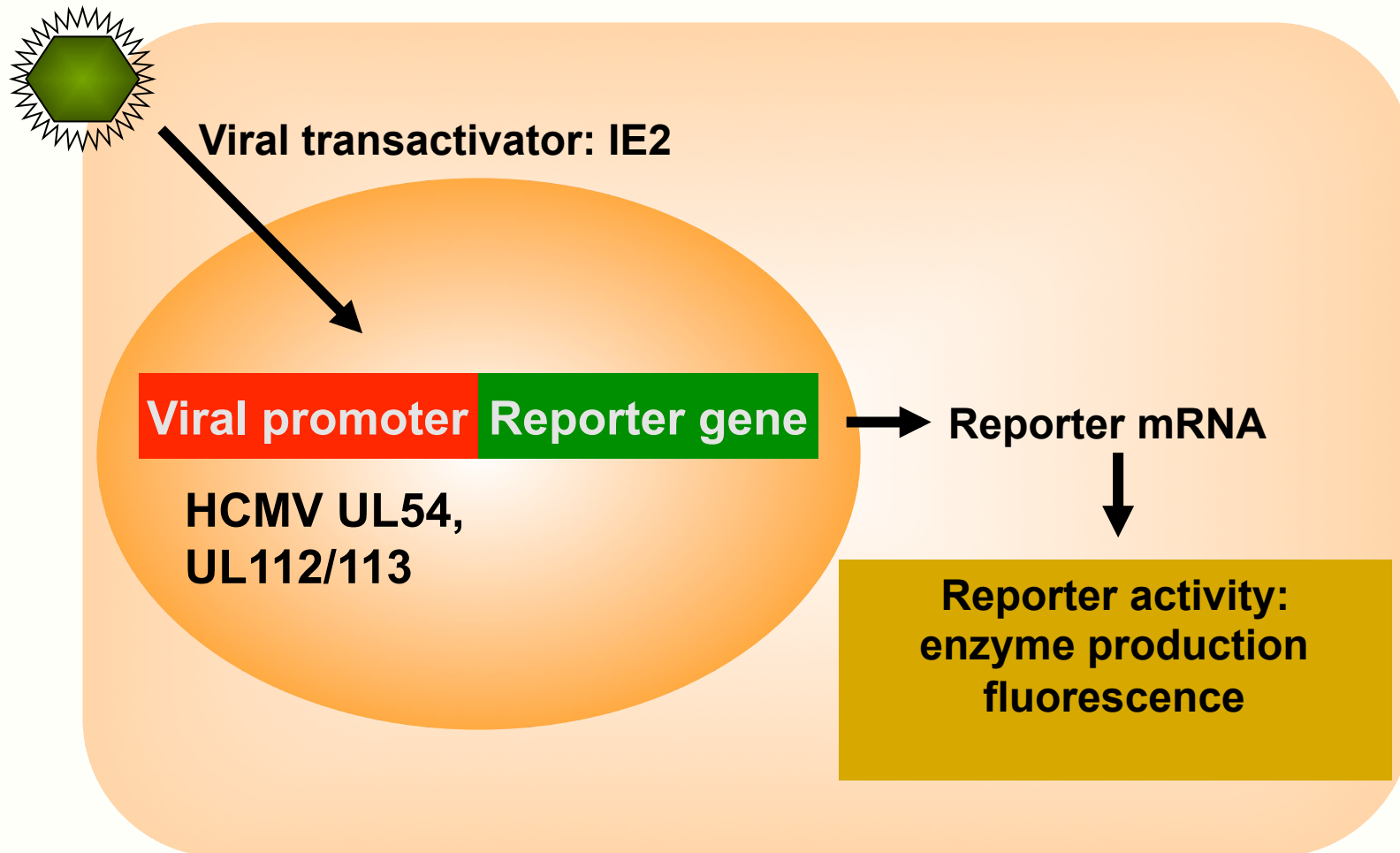
Dysregulation of cell functions

- gene expression
- apoptosis
- cell cycle progression
- immune responses

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



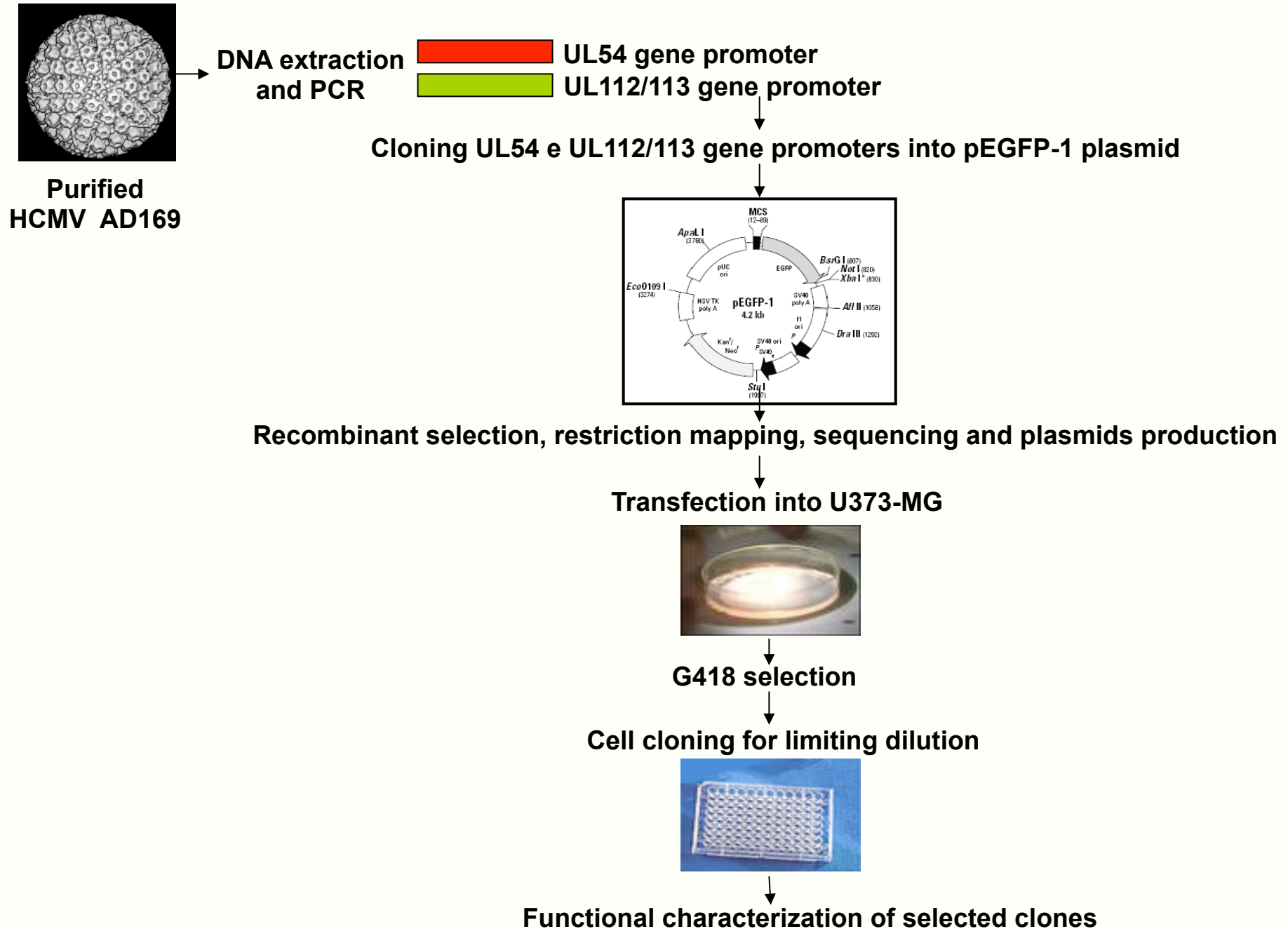
Genetically engineered cell lines to detect HCMV infectious viruses



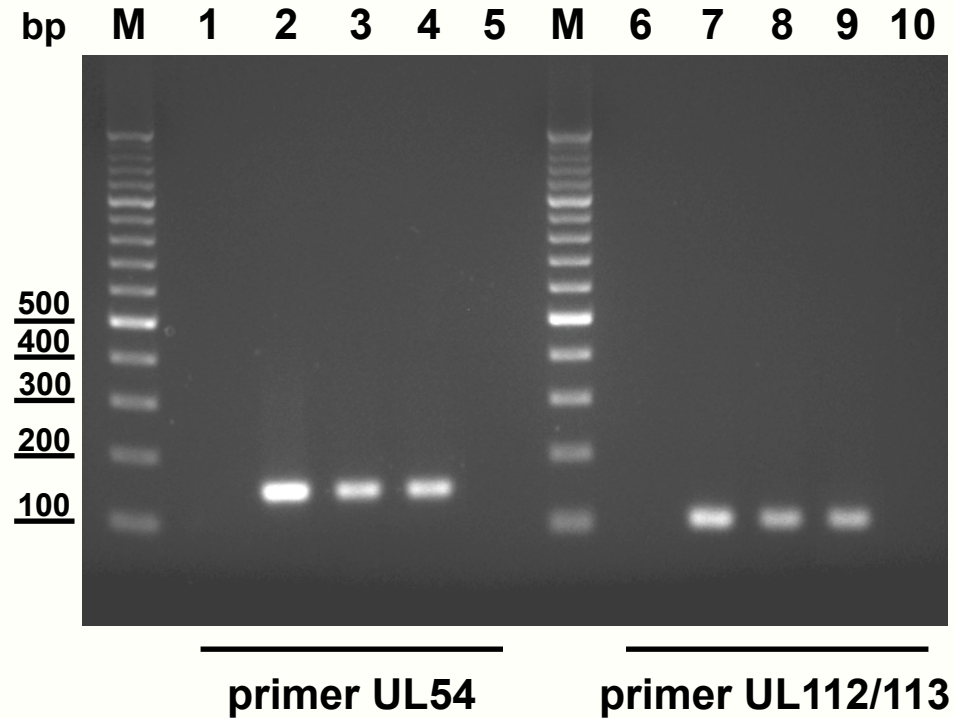
Genetically engineered cell lines that facilitate Herpesvirus detection: critical issues?

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

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

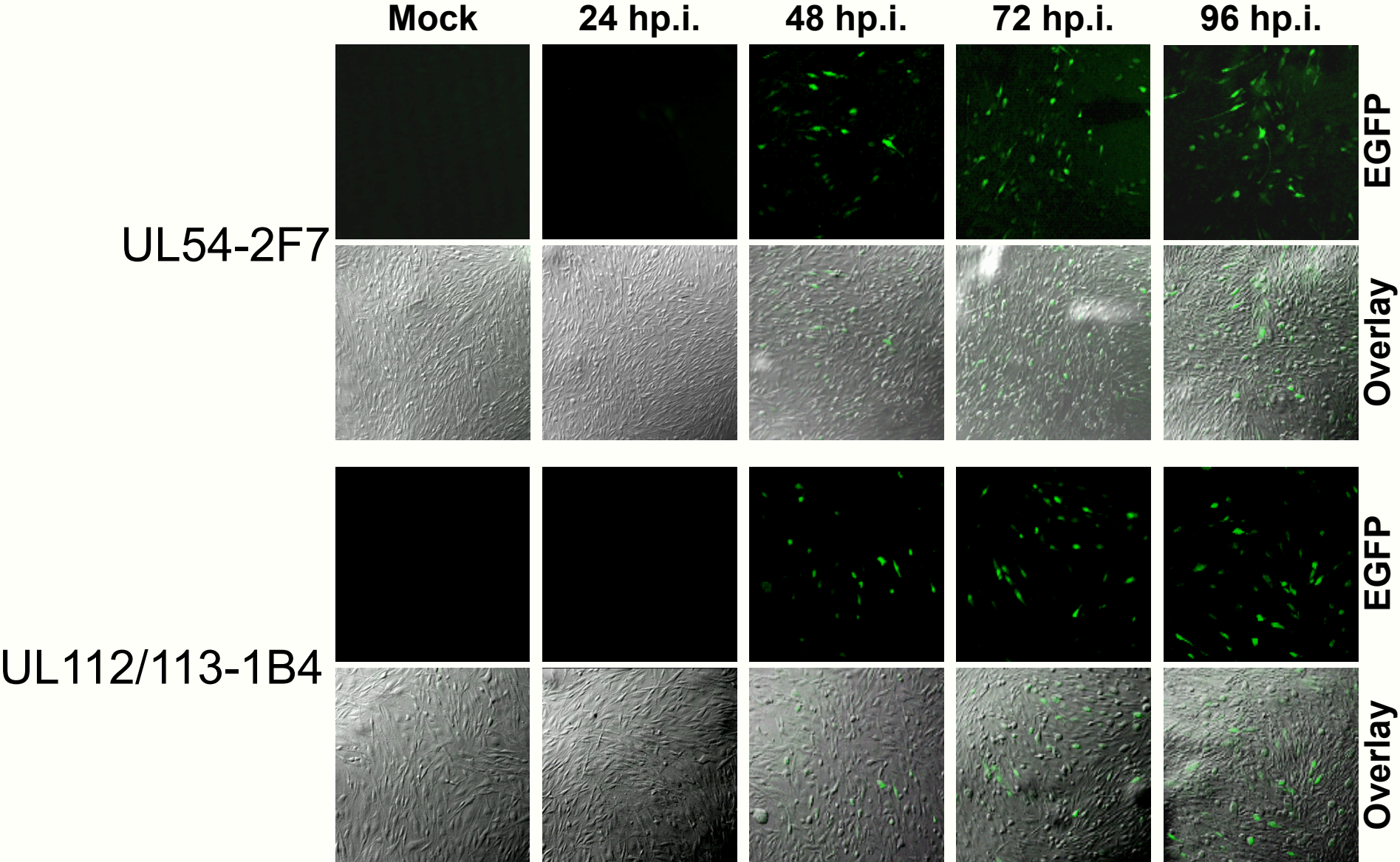


Molecular characterization of U373-MG clones UL54-2F7 and UL112/113-1B4 stably transfected with reporter plasmids



- 1- 6 : H₂O
- 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

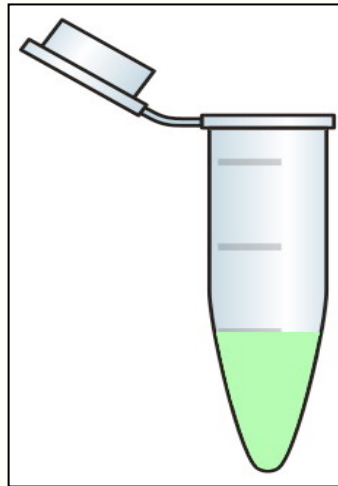


Assay for quantitative EGFP expression by automated fluorometry



HCMV infection of 24- or 96-well plates

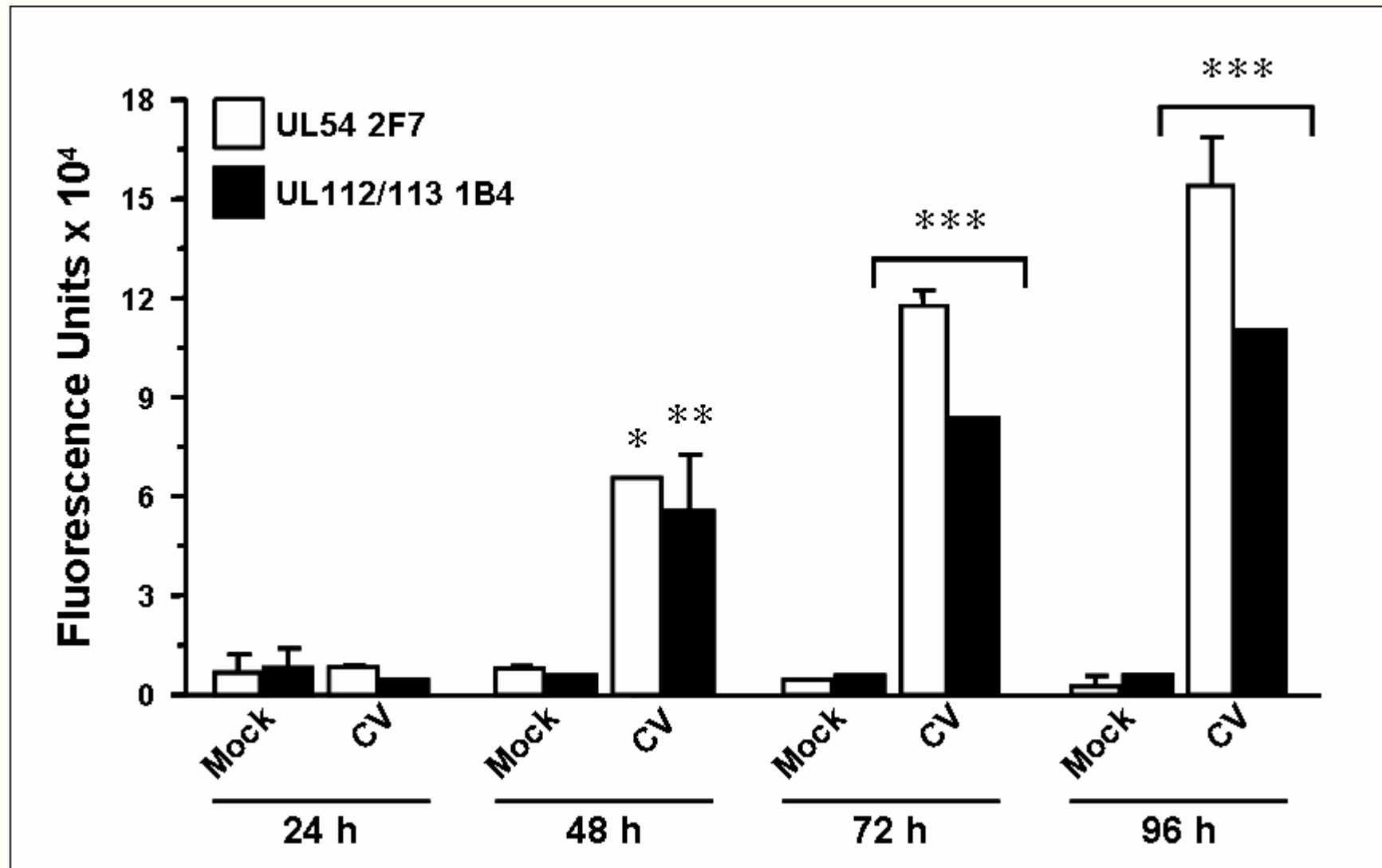
Preparation of total cell lysates at 48 h p.i.



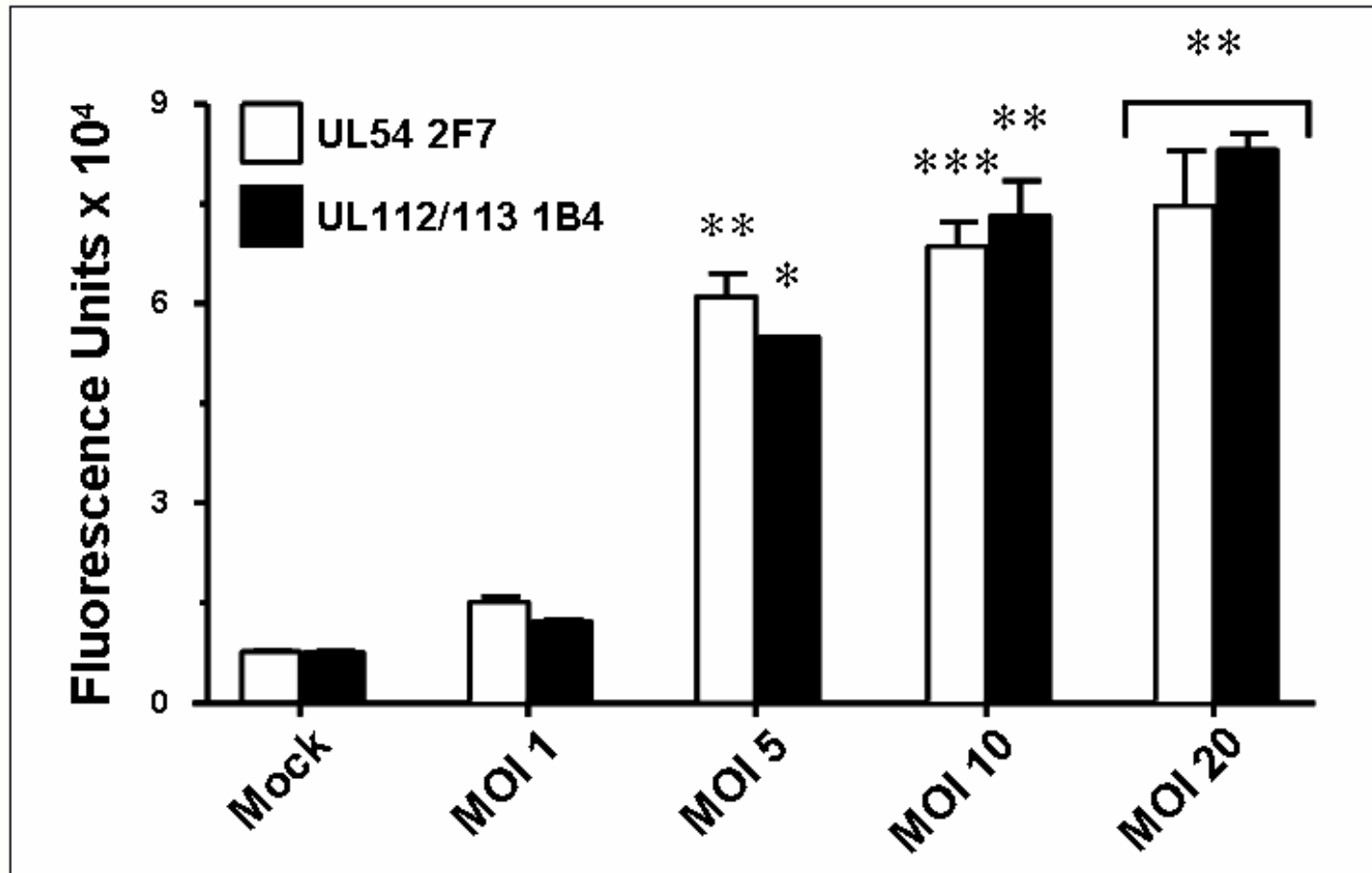
Analysis of EGFP content in a fluorescent microplate reader



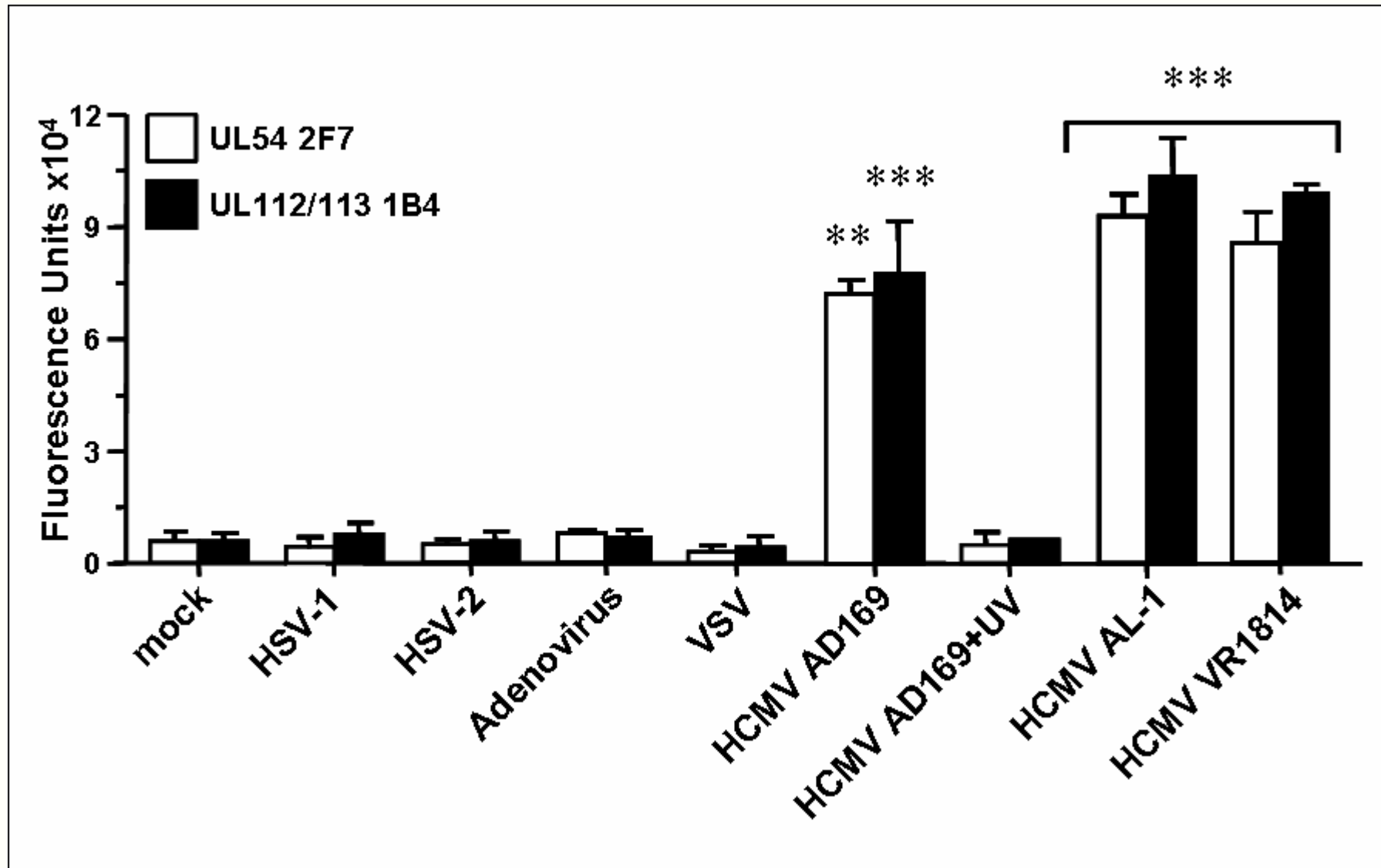
Characteristics of UL54-2F7 and UL112/113-1B4 cells: HCMV infection induces EGFP expression in a time-dependent manner



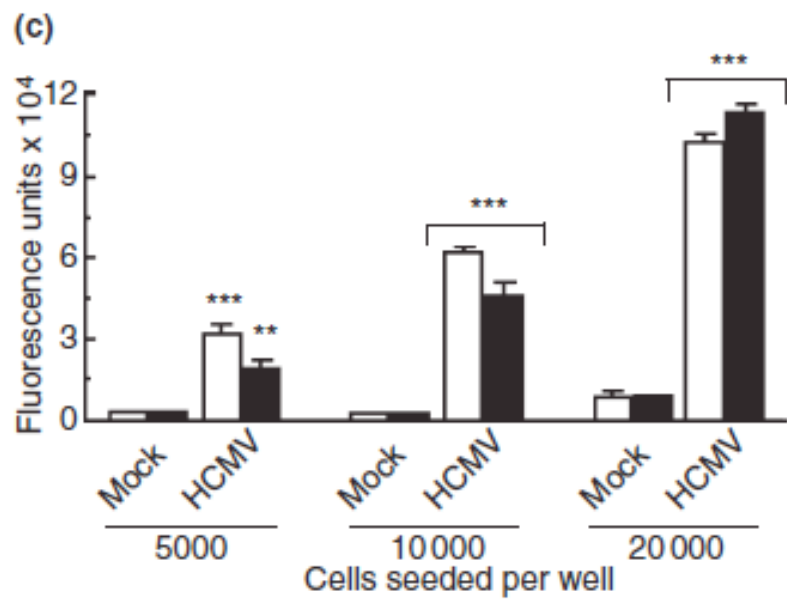
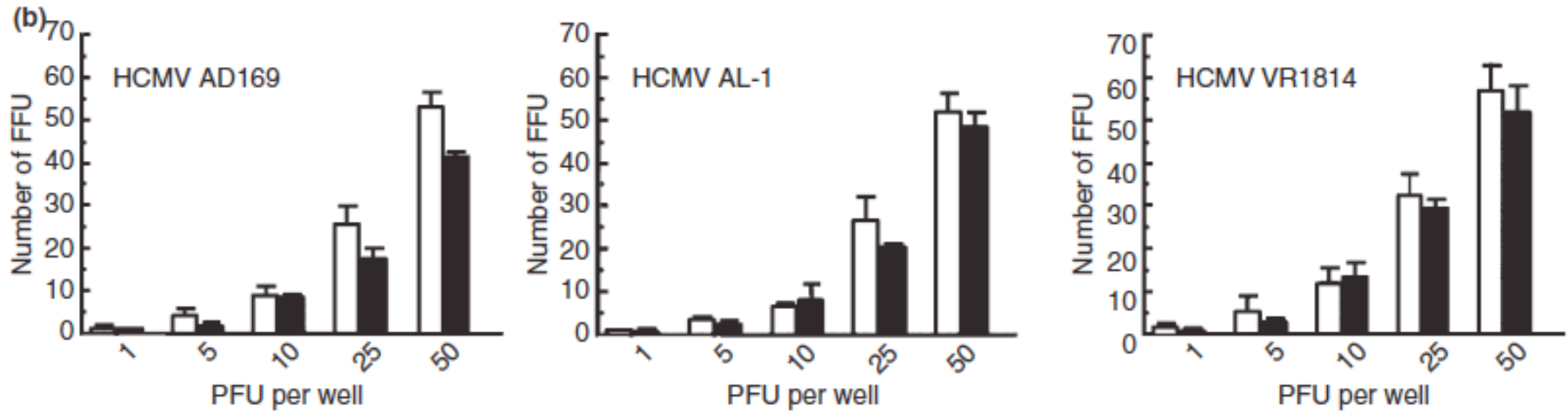
Characteristics of UL54-2F7 and UL112/113-1B4 cells: HCMV infection induces EGFP expression in a dose-dependent manner



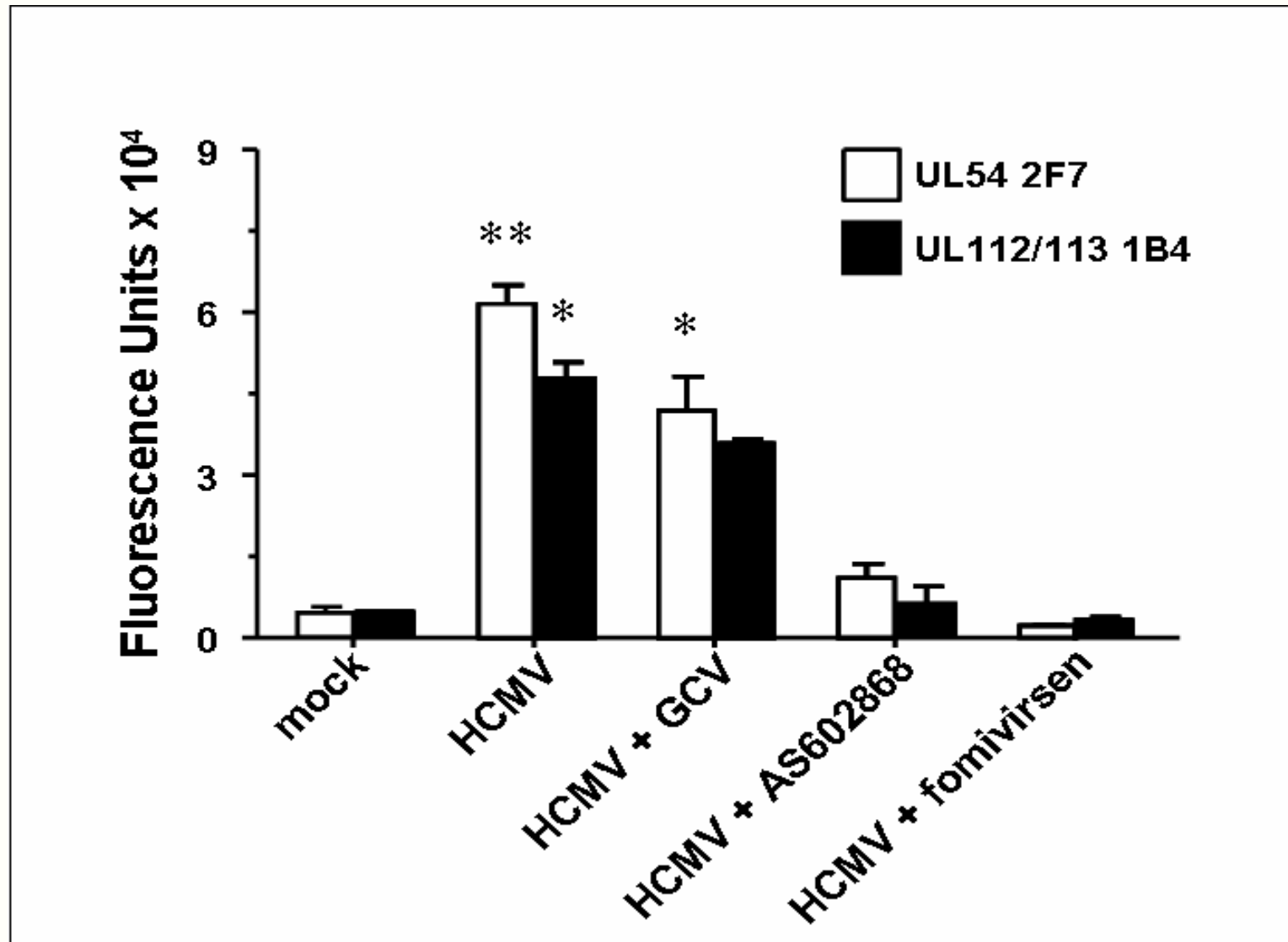
Characteristics of UL54-2F7 and UL112/113-1B4 cells: HCMV infection specifically stimulates EGFP signals



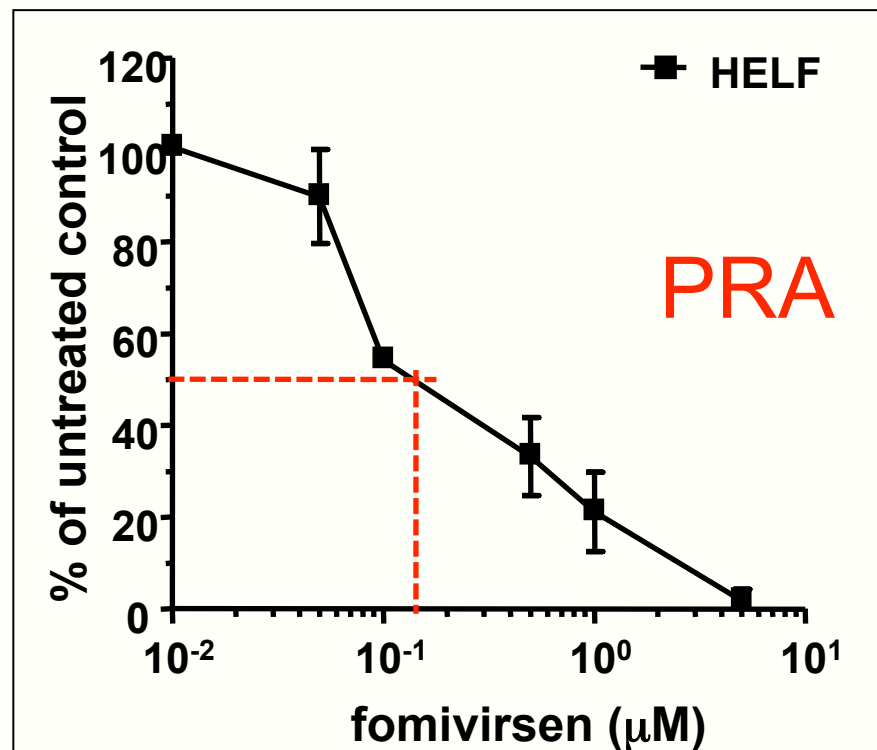
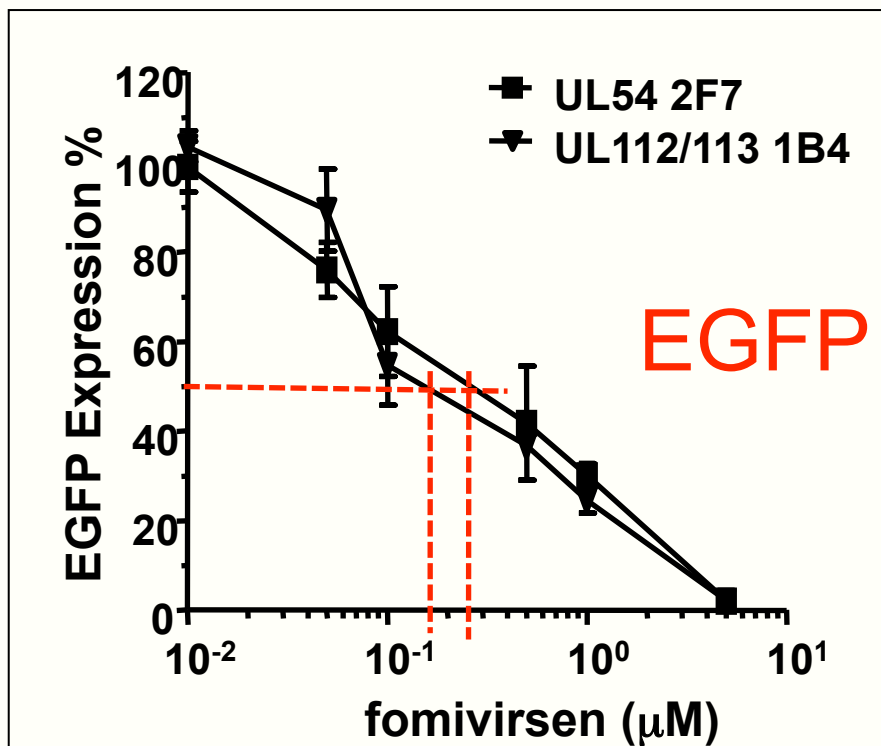
Characteristics of UL54-2F7 and UL112/113-1B4 cells: sensitivity of the EGFP cell based assay and its optimization for the 96-well format



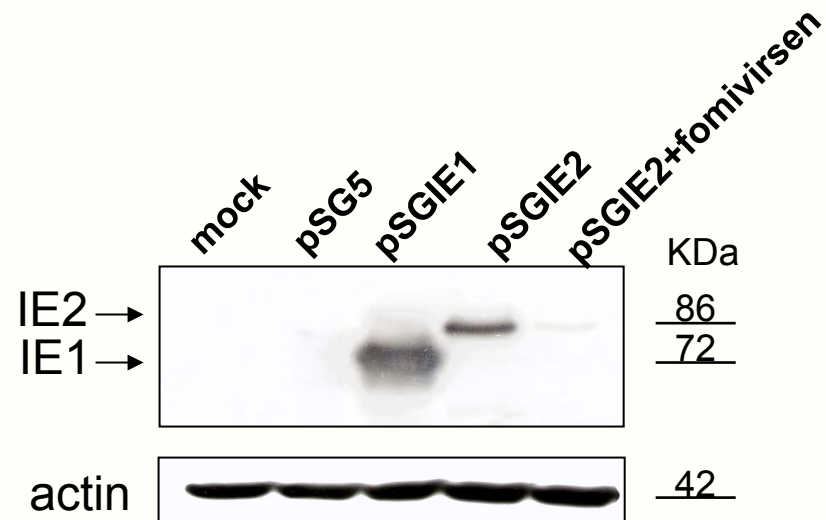
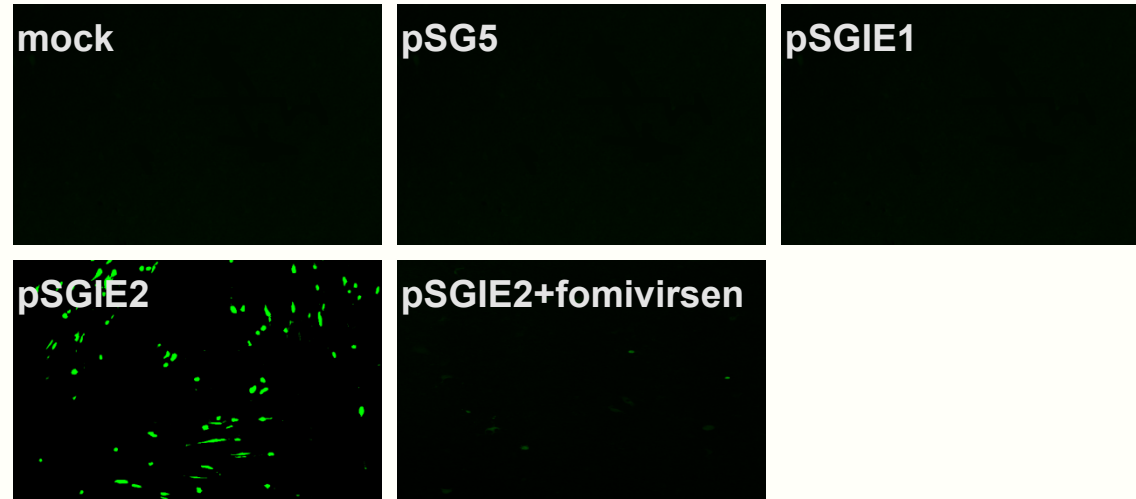
Effect of anti-cytomegaloviral drugs on EGFP expression in UL54-2F7 and UL112/113-1B4 cells



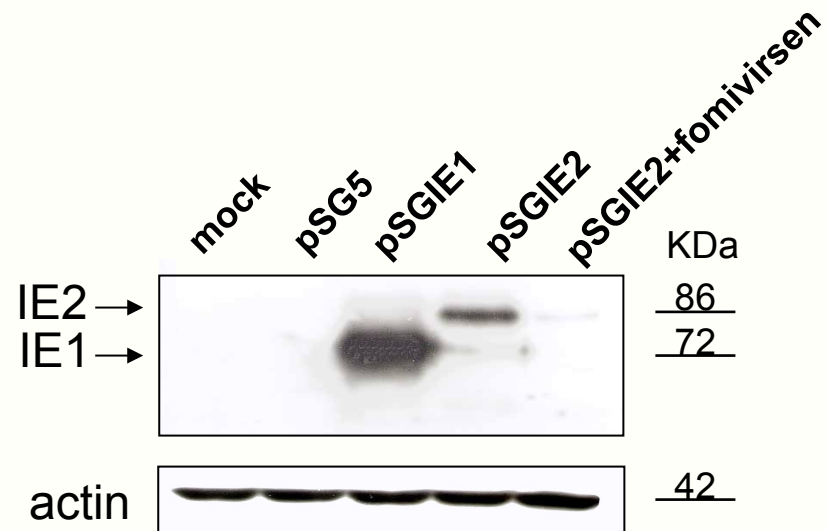
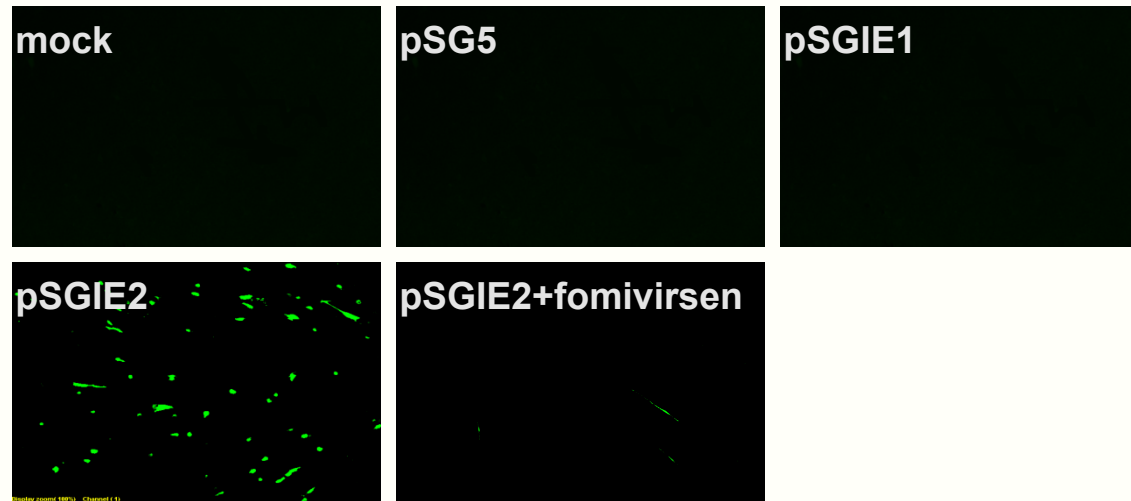
Validation of UL54-2F7 and UL112/113-1B4 cells as reliable tools for assessing the antiviral activity of a test compound



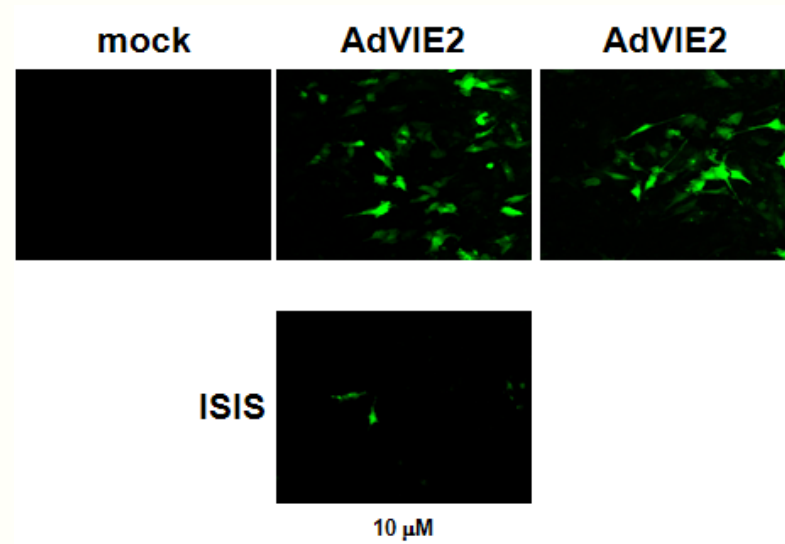
HCMV IE2 stimulates EGFP expression in UL54-2F7 cells and this induction is prevented by fomivirsen



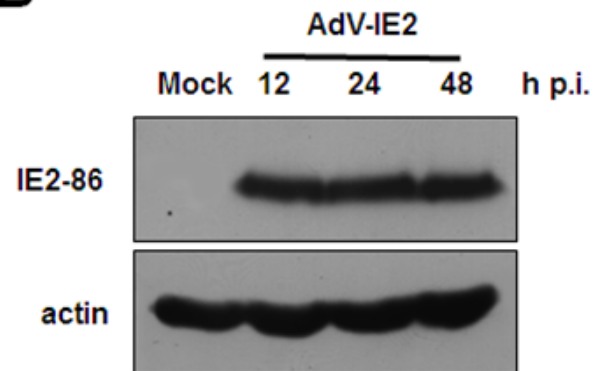
HCMV IE2 stimulates EGFP expression in UL112/113-1B4 cells and this induction is prevented by fomivirsen



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



B



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

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

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² Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy

Conclusions:

- 1) UL54-2F7 and UL112/113-1B4 indicator cells 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 indicator cell lines 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 plaquereduction 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.

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
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