SUPPLEMENTAL MATERIAL

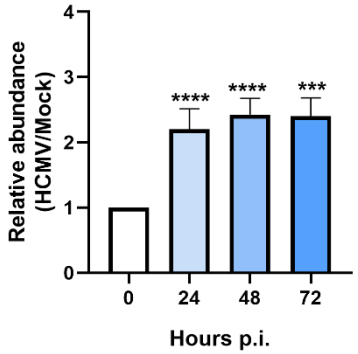

FIG S1 Quantification of hCYP51 induction in HFF cells infected with HCMV. Upregulation of host hCYP51 protein was analyzed in HFF cells infected with HCMV AD169 by Western blot and densitometry analysis was performed with ImageJ software. hCYP51 signal was normalized by its respective β-actin signal and plotted with respect to uninfected sample (Mock). Graph represents the mean ± SD of n = 4 independent experiments. Data were analysed by a one-way ANOVA followed by Dunnett's multiple comparison tests. ***p = 0.0001; ****p<0.0001, compared to control (mock sample).

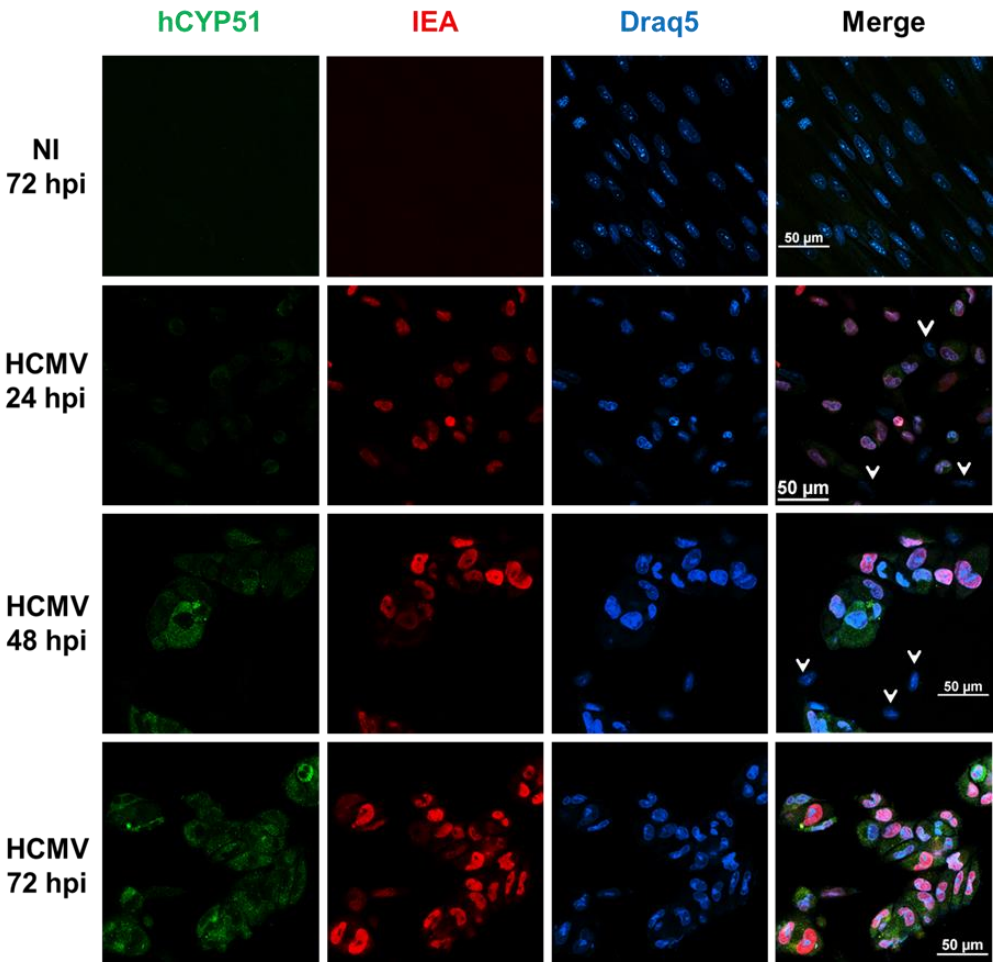

FIG S2 Analysis of hCYP51 and viral proteins expression during HCMV infection in living cells. Host hCYP51 protein and viral IE antigens (IEA) were detected in living cells by immunofluorescence analysis both in uninfected HFFs (NI) and in HFFs infected with HCMV at an MOI = 0.25 PFU/cell at the indicated h p.i. Draq5 was used to stain the cell nuclei. White arrows in the merge panels at 24 and 48 h p.i. indicate the nuclei of uninfected HFF cells in the same field with HCMV-infected cells. Progression of HCMV cycle is confirmed by appearance of kidney-shaped nuclei of infected cells containing viral replication compartments from 48 h p.i.

Table S1. List of the oligonucleotides used in this study

hCYP51 primer set	Primer FOR	CAGGTTGGCTGCCTTTGC
	Primer REV	CTTGATTTCCCGATGAGCTCTGT
UL54 primer set	Primer FOR	GACACTGTACGGCAGAAAAGCCGGCTC
	Primer REV	ACAGCATTCGTGCGC
GAPDH primer set	Primer FOR	AGCCACATCGCTCAGACAC
UL122 primer set	Primer FOR (CM-5T)	TCATCCACACTAGGAGAGCAGACT
	Primer REV (CM-3T)	GCCAAGCGGCCTCTGAT
	Probe	ACTGGGCAAAGACCTTCATGCAGATCTC
β-globin primer set	Primer FOR	AGGGCCTCACCACCAACTT
	Primer REV	GCACCTGACTCCTGAGGAGAA

Table S2. List of the antibodies used in this study

Antigen	**Supplier**	**Clone ID**	**Usage**
IE1/IE2	Argene-Biosoft	E13	WB (1:400); IF(1:500)
hCYP51	Sigma	polyclonal	WB (1:2,000); IF (1:500)
Actin	Sigma	AC-74	WB (1:8,000)
HRP-Rabbit IgG (H+L)	Santa Cruz	-	WB (1:2,000)
HRP-Mouse IgG (H+L)	Santa Cruz	-	WB (1:2,000)
Alexa FluorTM 488 Rabbit IgG (H+L)	Invitrogen	-	IF (1:2,000)
Alexa FluorTM 546 Mouse IgG (H+L)	Invitrogen	-	IF (1:2,000)

WB: Western Blot; IF: immunofluorescence.

Table S3. Simulated Dose Reduction Index for GCV and PCZ against HCMV

Viral inhibition effect (%)	GCV[a] (µM)	PCZ[a] (µM)	DRI[b] GCV	DRI[b] PCZ
50	2.91 ± 0.76	3.13 ± 0.42	3.86 ± 0.72	2.79 ± 0.16
75	6.77 ± 1.08	6.36 ± 0.10	6.22 ± 0.74	3.93 ± 0.22
90	15.82 ± 0.91	13.08 ± 2.14	10.05 ± 0.51	5.57 ± 0.95
95	28.27 ± 0.35	21.48 ± 5.63	13.96 ± 0.06	7.09 ± 1.75

[a] Drug concentration required for the inhibition of viral replication at the indicated extent as determined by PRAs in infected HFF cells. Data represent the mean ± SD of n = 3 independent experiments in triplicate.

[b] Dose reduction index, i.e., the simulated fold of reduction of each drug in combination compared to the dose required for the drug alone to obtain the same inhibitory effect.