Supplementary Material

Cyclin-dependent kinases (CDKs) and the human cytomegalovirus-encoded CDK ortholog pUL97 represent highly attractive targets for synergistic drug combinations

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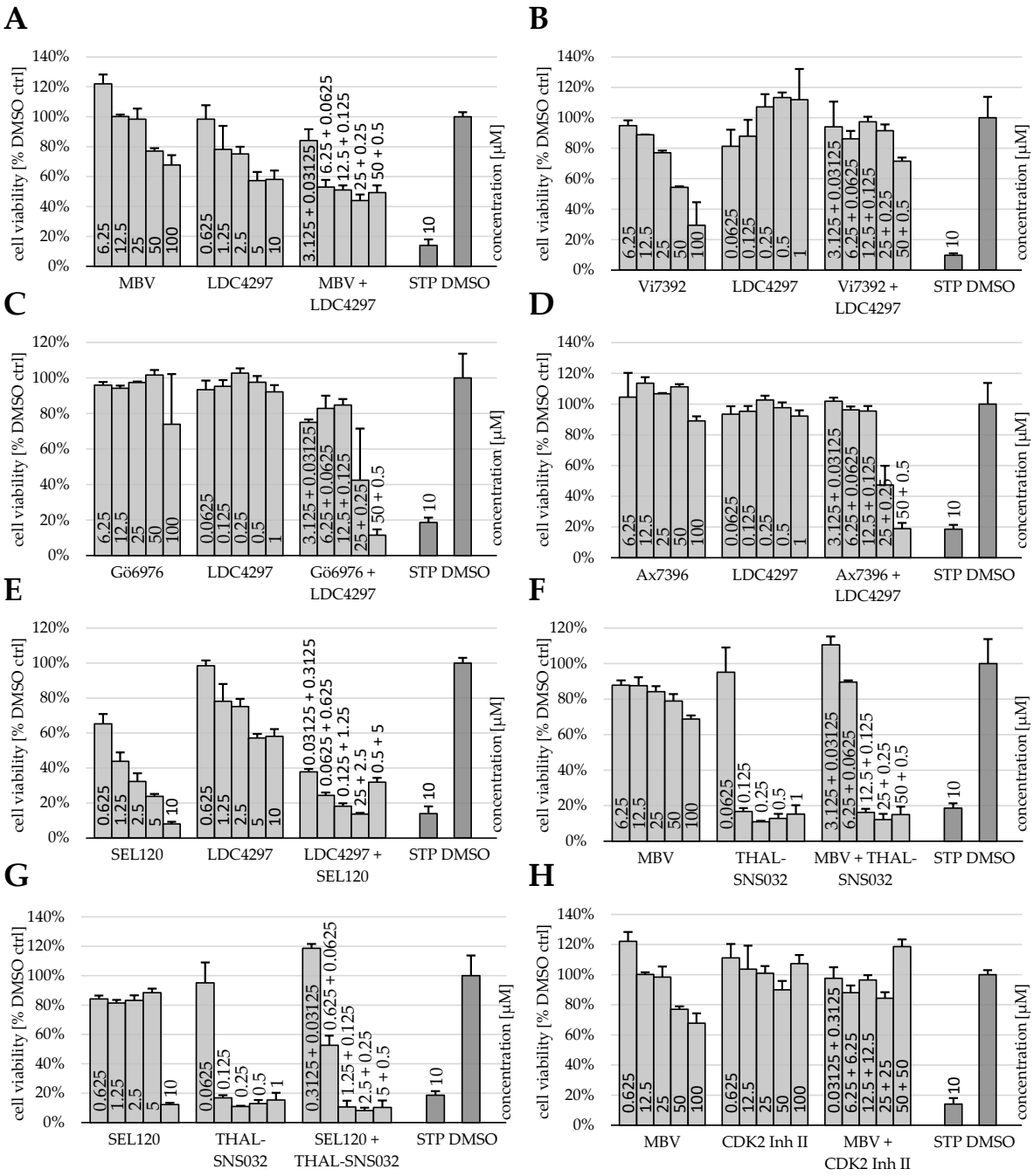
Supplementary Figures



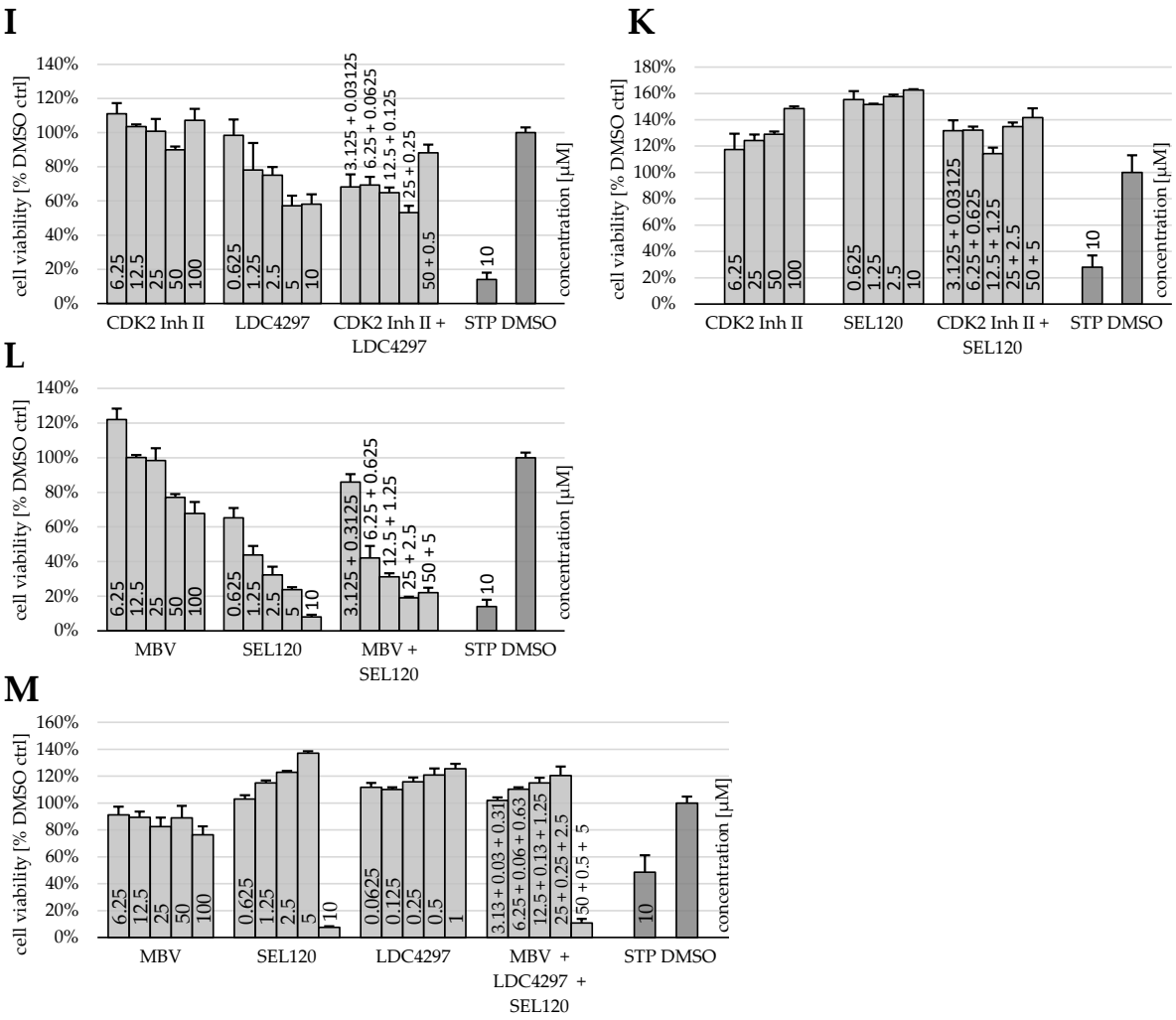


Figure S1. Assessment of cytotoxicity of various PKIs in single treatments and in combinations. **(A)** MBV + LDC4297, **(B)** Vi7392 + LDC4297, **(C)** Gö6976 + LDC4297, **(D)** Ax7396 + LDC4297, **(E)** SEL120 + LDC4297, **(F)** MBV + THAL-SNS032, **(G)** SEL120 + THAL-SNS032, **(H)** MBV + CDK2 Inh II, **(I)** CDK2 Inh II + LDC4297, **(K)** CDK2 Inh II + SEL120, **(L)** MBV + SEL120, **(M)** MBV + LDC4297 + SEL120. Staurosporine (STP) was used as a positive control (10 µM, added 3 h before harvesting); experiments were performed in biological triplicates and data are given as mean values + SD; ctrl, control.

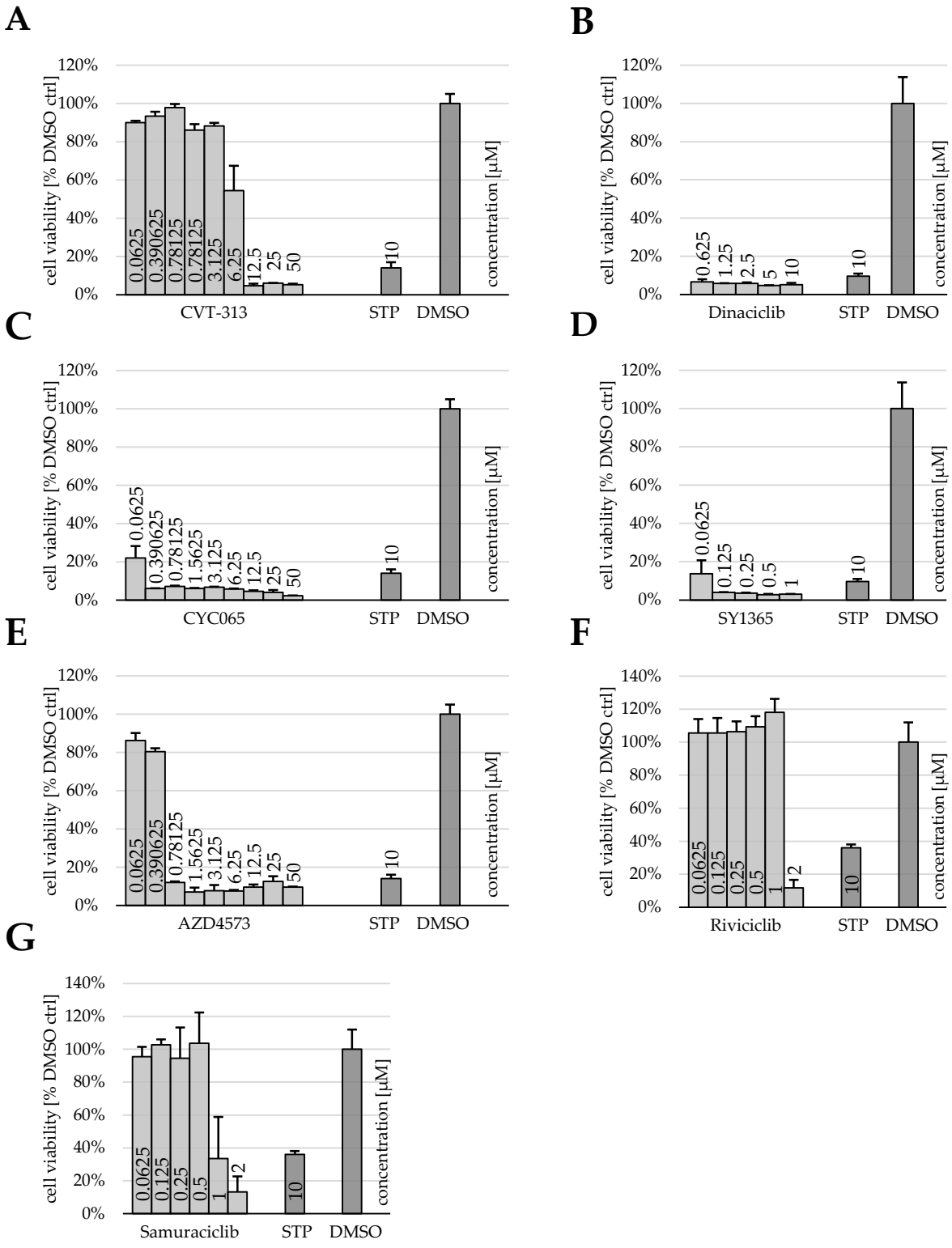


Figure S2. Assessment of cytotoxicity of distinct PKIs presently investigated in clinical trials. **(A)** CVT-313, **(B)** dinaciclib, **(C)** CYC065, **(D)** SY1365, **(E)** AZD4573, **(F)** riviciclib, **(G)** samuraciclib. Staurosporine (STP) was used as a positive control (10 µM, added 3 h before harvesting); experiments were performed in biological triplicates and data are given as mean values + SD; ctrl, control.

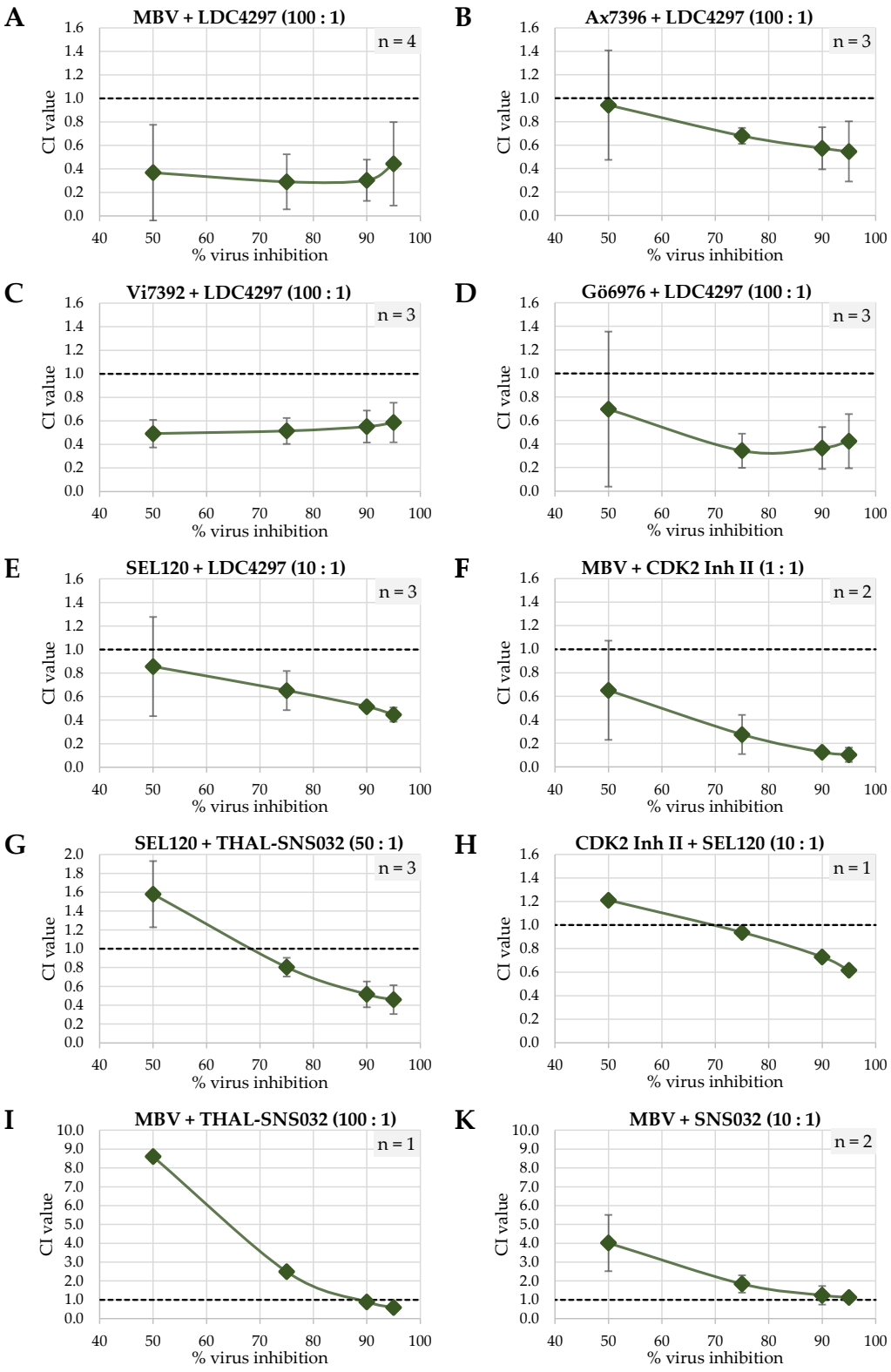


Figure S3. Assessment of PKI drug interactions utilizing the Loewe additivity fixed-dose assay. **(A)** MBV + LDC4297 (ratio 100 : 1), **(B)** Ax7396 + LDC4297 (100 : 1), **(C)** Vi7392 + LDC4297 (100 : 1), **(D)** Gö6976 + LDC4297 (100 : 1), **(E)** SEL120 + LDC4297 (10 : 1), **(F)** MBV + CDK2 Inh II (1: 1), **(G)** SEL120 + THAL-SNS032 (100 : 1), **(H)** CDK2 Inh II + SEl120 (10 : 1), **(I)** MBV + THAL-SNS032 (100 : 1), **(K)** MBV + SNS032 (10 : 1). Data are presented as mean CI values ± SD, as extrapolated at 50%, 75%, 90% and 95% virus inhibition across the number of individual experiments (n=1 to n=4). Individual experiments were performed as biological duplicates; CI, combination index.

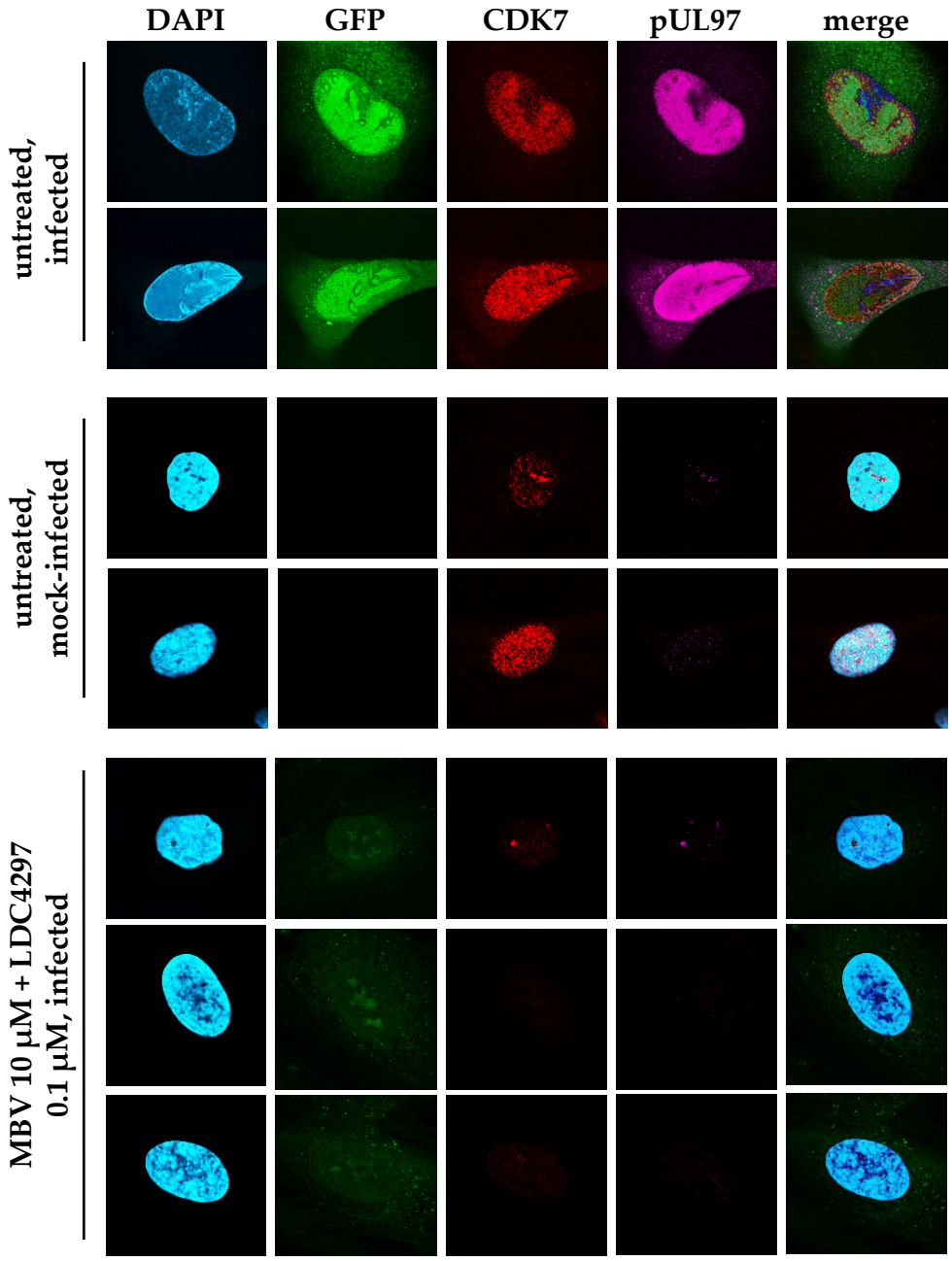


Figure S4. Assessment of the intracellular localization of CDK7 and pUL97 using confocal laser-scanning microscopic analysis. HFFs were seeded on glass slides, used for HCMV infection (AD169-GFP, MOI of 0.25 GFP-FU/ml) one day later or remained mock-infected and treated with the indicated concentrations of compounds for 7 days before fixation and staining. Cells were analyzed under the following conditions: drug-untreated, HCMV-infected (upper section); untreated, mock-infected (middle section); HCMV-infected, treated with a combination of MBV and LDC4297 (concentrations of 10 µM and 0.1 µM; lower section). Each section presents 2-3 exemplary cell samples to illustrate typical patterns of intracellular localization and the degree of visual variation.

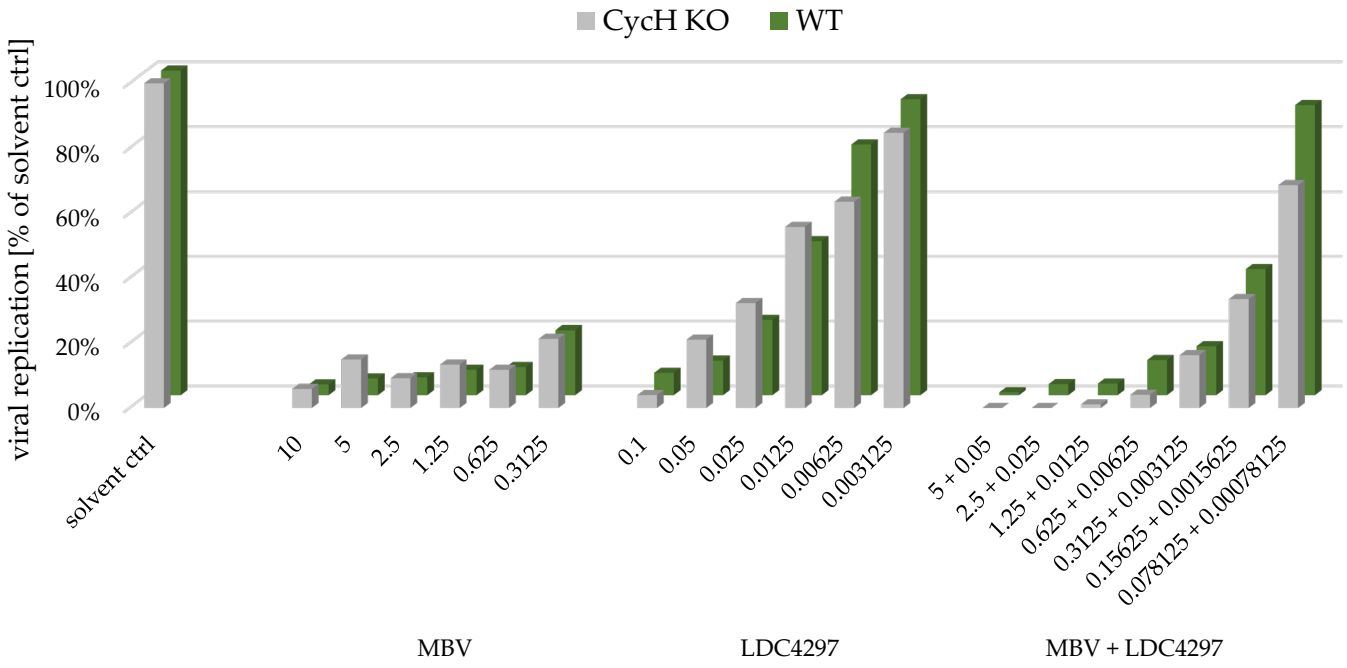


Figure S5. Assessment of antiviral efficacy of MBV, LDC4297 and the combination treatment MBV + LDC4297 in wild-type HFFs (WT) and partial cyclin H knock-out HFFs (CycH KO). HFFs were seeded in 12-well culture plates and treated with lentiviral supernatants to achieve a CRISPR/Cas9-mediated transient cyclin H knock-down. Subsequently, cells were directly infected with HCMV AD169-GFP for 90 min, then fresh medium, supplemented with indicated concentrations of compounds, was added. Cells were harvested and lysed 7 d post-infection and viral GFP reporter signals were measured in quadruplicate. Data are presented as mean values of biological duplicates, adjusted in percent to the respective solvent DMSO control (ctrl).

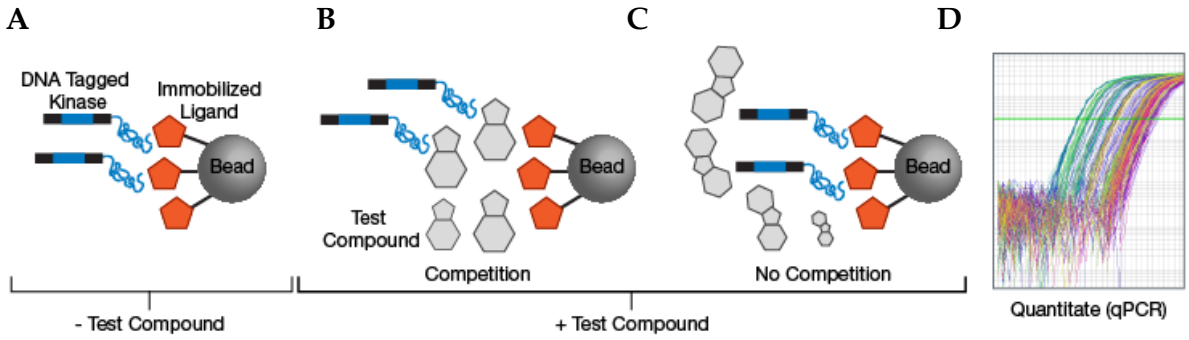


Figure S6. Assay principle of the DiscoverX KINOMEscan™. The immobilized ligand binds to the target kinase, which has been tagged with DNA (**A**). If the test compound (Vi7392) binds the kinase active site either directly (sterically) or indirectly (allosterically), kinase binding to the immobilized ligand will be reduced, lowering the amount of kinase captured on the bead (**B**). Conversely, if the tested kinase is not bound by Vi7392, no effect on the amount of kinase captured on the bead is detected (**C**). Screening hits are identified by measuring the amount of kinase captured in test versus control samples by using a quantitative, precise and ultra-sensitive qPCR method that detects the associated DNA label (**D**). In a similar manner, dissociation constants (Kds) for test compound-kinase interactions are calculated by measuring the amount of kinase captured on the solid support as a function of the test compound concentration. For methodological details, see *https://www.discoverx.com/services/drug-discovery-development-services/kinase-profiling/kinomescan*.

Supplementary Tables

Table S1. Assessment of secondary cellular targets of pUL97 inhibitor Vi7392 by commercial DiscoverX KINOMEscan™ Profiling. Vi7392 was applied at a concentration of 3 μM. Data indicate two levels of cellular targets of Vi7392 on the basis of a drug competition-reduced binding, at a stringency of ≤ 10% (S10; see dark shadings) or lower stringency of ≤ 35% (S35; see light shadings). The following candidate kinases binding to Vi7392 were considered relevant: CDKL1 and PIP5K2C (S10); CDKL1, PIP5K2C, EGFR, MAPK2K5, MKNK2 and PRKG2 (S35; cancer-specific kinase mutants of EGFR and FLT3 without a known relevance for HCMV infection were not taken into consideration). For methodological details, see *https://www.discoverx.com/services/drug-discovery-development-services/kinase-profiling/kinomescan*.

Vi7392 (3 μM)					
Gene Symbol	**%Ctrl**	**Gene Symbol**	**%Ctrl**	**Gene Symbol**	**%Ctrl**
AAK1	100	BIKE	100	CDKL3	100
ABL1(E255K)-phosphorylated	80	BLK	91	CDKL5	100
ABL1(F317I)-nonphosphorylated	85	BMPR1A	100	CHEK1	100
ABL1(F317I)-phosphorylated	100	BMPR1B	100	CHEK2	100
ABL1(F317L)-nonphosphorylated	100	BMPR2	100	CIT	91
ABL1(F317L)-phosphorylated	100	BMX	96	CLK1	75
ABL1(H396P)-nonphosphorylated	92	BRAF	100	CLK2	100
ABL1(H396P)-phosphorylated	100	BRAF(V600E)	100	CLK3	90
ABL1(M351T)-phosphorylated	100	BRK	100	CLK4	90
ABL1(Q252H)-nonphosphorylated	76	BRSK1	100	CSF1R	100
ABL1(Q252H)-phosphorylated	100	BRSK2	100	CSF1R-autoinhibited	100
ABL1(T315I)-nonphosphorylated	100	BTK	100	CSK	100
ABL1(T315I)-phosphorylated	100	BUB1	100	CSNK1A1	64
ABL1(Y253F)-phosphorylated	100	CAMK1	100	CSNK1A1L	100
ABL1-nonphosphorylated	50	CAMK1B	100	CSNK1D	100
ABL1-phosphorylated	51	CAMK1D	100	CSNK1E	63
ABL2	66	CAMK1G	100	CSNK1G1	100
ACVR1	100	CAMK2A	96	CSNK1G2	100
ACVR1B	100	CAMK2B	88	CSNK1G3	84
ACVR2A	100	CAMK2D	100	CSNK2A1	95
ACVR2B	100	CAMK2G	100	CSNK2A2	100
ACVRL1	100	CAMK4	100	CTK	100
ADCK3	100	CAMKK1	100	DAPK1	98
ADCK4	100	CAMKK2	100	DAPK2	100
AKT1	98	CASK	93	DAPK3	90
AKT2	100	CDC2L1	98	DCAMKL1	99
AKT3	100	CDC2L2	100	DCAMKL2	100
ALK	100	CDC2L5	97	DCAMKL3	100
ALK(C1156Y)	100	CDK11	89	DDR1	59
ALK(L1196M)	100	CDK2	100	DDR2	100
AMPK-alpha1	100	CDK3	100	DLK	100
AMPK-alpha2	97	CDK4	100	DMPK	100
ANKK1	100	CDK4-cyclinD1	100	DMPK2	100
ARK5	100	CDK4-cyclinD3	100	DRAK1	86
ASK1	86	CDK5	100	DRAK2	100
ASK2	99	CDK7	100	DYRK1A	100
AURKA	100	CDK8	79	DYRK1B	78
AURKB	83	CDK9	100	DYRK2	100
AURKC	92	CDKL1	6.2	EGFR	17
AXL	100	CDKL2	100	EGFR(E746-A750del)	33

colour legend		
x < 10	10 ≤ x < 35	x ≥ 35

Table S1. cont.

Gene Symbol	%Ctrl	Gene Symbol	%Ctrl	Gene Symbol	%Ctrl
EGFR(G719C)	7.8	GAK	76	MAP3K15	90
EGFR(G719S)	9.6	GCN2(Kin.Dom.2,S808G)	100	MAP3K2	100
EGFR(L747-E749del, A750P)	30	GRK1	100	MAP3K3	100
EGFR(L747-S752del, P753S)	38	GRK2	100	MAP3K4	92
EGFR(L747-T751del,Sins)	24	GRK3	100	MAP4K2	100
EGFR(L858R)	30	GRK4	96	MAP4K3	100
EGFR(L858R,T790M)	93	GRK7	100	MAP4K4	78
EGFR(L861Q)	24	GSK3A	100	MAP4K5	100
EGFR(S752-I759del)	28	GSK3B	96	MAPKAPK2	100
EGFR(T790M)	89	HASPIN	73	MAPKAPK5	100
EIF2AK1	78	HCK	89	MARK1	100
EPHA1	100	HIPK1	54	MARK2	100
EPHA2	100	HIPK2	100	MARK3	99
EPHA3	100	HIPK3	97	MARK4	100
EPHA4	100	HIPK4	81	MAST1	100
EPHA5	100	HPK1	91	MEK1	78
EPHA6	100	HUNK	86	MEK2	89
EPHA7	100	ICK	100	MEK3	80
EPHA8	100	IGF1R	100	MEK4	88
EPHB1	100	IKK-alpha	100	MEK5	15
EPHB2	100	IKK-beta	100	MEK6	75
EPHB3	100	IKK-epsilon	70	MELK	96
EPHB4	100	INSR	100	MERTK	100
EPHB6	100	INSRR	100	MET	100
ERBB2	44	IRAK1	100	MET(M1250T)	97
ERBB3	100	IRAK3	100	MET(Y1235D)	71
ERBB4	99	IRAK4	100	MINK	58
ERK1	100	ITK	67	MKK7	100
ERK2	100	JAK1(JH1domain-catalytic)	100	MKNK1	95
ERK3	100	JAK1(JH2domain-pseudokinase)	79	MKNK2	25
ERK4	94	JAK2(JH1domain-catalytic)	100	MLCK	74
ERK5	100	JAK3(JH1domain-catalytic)	100	MLK1	84
ERK8	82	JNK1	97	MLK2	100
ERN1	100	JNK2	100	MLK3	100
FAK	86	JNK3	100	MRCKA	98
FER	100	KIT	100	MRCKB	100
FES	100	KIT(A829P)	100	MST1	100
FGFR1	100	KIT(D816H)	86	MST1R	100
FGFR2	97	KIT(D816V)	100	MST2	100
FGFR3	100	KIT(L576P)	100	MST3	100
FGFR3(G697C)	99	KIT(V559D)	93	MST4	100
FGFR4	100	KIT(V559D,T670I)	100	MTOR	98
FGR	100	KIT(V559D,V654A)	100	MUSK	100
FLT1	99	KIT-autoinhibited	81	MYLK	98
FLT3	100	LATS1	100	MYLK2	100
FLT3(D835H)	86	LATS2	79	MYLK4	91
FLT3(D835V)	18	LCK	100	MYO3A	100
FLT3(D835Y)	100	LIMK1	100	MYO3B	100
FLT3(ITD)	97	LIMK2	100	NDR1	87
FLT3(ITD,D835V)	100	LKB1	92	NDR2	100
FLT3(ITD,F691L)	100	LOK	95	NEK1	92
FLT3(K663Q)	100	LRRK2	100	NEK10	100
FLT3(N841I)	100	LRRK2(G2019S)	100	NEK11	100
FLT3(R834Q)	100	LTK	100	NEK2	100
FLT3-autoinhibited	100	LYN	100	NEK3	100
FLT4	100	LZK	100	NEK4	100
FRK	82	MAK	100	NEK5	100
FYN	91	MAP3K1	100	NEK6	100

colour legend		
x < 10	10 ≤ x < 35	x ≥ 35

Table S1. cont.

Gene Symbol	%Ctrl	Gene Symbol	%Ctrl	Gene Symbol	%Ctrl
NEK7	100	PLK1	100	SRMS	100
NEK9	100	PLK2	100	SRPK1	100
NIK	100	PLK3	72	SRPK2	100
NIM1	100	PLK4	100	SRPK3	90
NLK	100	PRKCD	100	STK16	100
OSR1	100	PRKCE	100	STK33	100
p38-alpha	100	PRKCH	100	STK35	100
p38-beta	100	PRKCI	84	STK36	100
p38-delta	100	PRKCQ	100	STK39	100
p38-gamma	84	PRKD1	100	SYK	100
PAK1	100	PRKD2	100	TAK1	100
PAK2	100	PRKD3	100	TAOK1	100
PAK3	100	PRKG1	99	TAOK2	100
PAK4	100	PRKG2	14	TAOK3	100
PAK6	100	PRKR	72	TBK1	55
PAK7	100	PRKX	100	TEC	100
PCTK1	100	PRP4	92	TESK1	100
PCTK2	100	PYK2	94	TGFBR1	100
PCTK3	100	QSK	100	TGFBR2	100
PDGFRA	92	RAF1	96	TIE1	100
PDGFRB	100	RET	100	TIE2	100
PDPK1	100	RET(M918T)	100	TLK1	100
PFCDPK1(P.falciparum)	87	RET(V804L)	100	TLK2	75
PFPK5(P.falciparum)	100	RET(V804M)	100	TNIK	100
PFTAIRE2	99	RIOK1	100	TNK1	100
PFTK1	100	RIOK2	97	TNK2	100
PHKG1	98	RIOK3	100	TNNI3K	100
PHKG2	86	RIPK1	100	TRKA	100
PIK3C2B	100	RIPK2	63	TRKB	100
PIK3C2G	100	RIPK4	100	TRKC	100
PIK3CA	100	RIPK5	100	TRPM6	98
PIK3CA(C420R)	89	ROCK1	100	TSSK1B	100
PIK3CA(E542K)	85	ROCK2	100	TSSK3	58
PIK3CA(E545A)	62	ROS1	100	TTK	100
PIK3CA(E545K)	65	RPS6KA4(Kin.Dom.1-N-terminal)	94	TXK	96
PIK3CA(H1047L)	91	RPS6KA4(Kin.Dom.2-C-terminal)	100	TYK2(JH1domain-catalytic)	100
PIK3CA(H1047Y)	66	RPS6KA5(Kin.Dom.1-N-terminal)	100	TYK2(JH2domain-pseudokinase)	100
PIK3CA(I800L)	87	RPS6KA5(Kin.Dom.2-C-terminal)	100	TYRO3	100
PIK3CA(M1043I)	100	RSK1(Kin.Dom.1-N-terminal)	100	ULK1	100
PIK3CA(Q546K)	97	RSK1(Kin.Dom.2-C-terminal)	98	ULK2	100
PIK3CB	100	RSK2(Kin.Dom.1-N-terminal)	100	ULK3	92
PIK3CD	72	RSK2(Kin.Dom.2-C-terminal)	100	VEGFR2	100
PIK3CG	100	RSK3(Kin.Dom.1-N-terminal)	100	VPS34	100
PIK4CB	86	RSK3(Kin.Dom.2-C-terminal)	98	VRK2	100
PIKFYVE	74	RSK4(Kin.Dom.1-N-terminal)	100	WEE1	100
PIM1	98	RSK4(Kin.Dom.2-C-terminal)	100	WEE2	100
PIM2	97	S6K1	100	WNK1	100
PIM3	100	SBK1	100	WNK2	100
PIP5K1A	98	SGK	100	WNK3	100
PIP5K1C	68	SgK110	100	WNK4	100
PIP5K2B	100	SGK2	100	YANK1	100
PIP5K2C	4.6	SGK3	100	YANK2	88
PKAC-alpha	100	SIK	100	YANK3	100
PKAC-beta	97	SIK2	100	YES	100
PKMYT1	100	SLK	100	YSK1	76
PKN1	83	SNARK	100	YSK4	100
PKN2	100	SNRK	81	ZAK	100
PKNB(M.tuberculosis)	100	SRC	100	ZAP70	100

colour legend		
x < 10	10 ≤ x < 35	x ≥ 35