SUPPLEMENTAL METHODS

Derivation of hiPSCs. All the protocols for this study were approved by the Stanford University Institutional Review Board. Briefly, dermal fibroblasts were obtained via skin biopsy from 3 healthy patients in a previously described 7-patient family cohort[1]. These samples were reprogrammed using a lentiviral vector expressing OKSM and were characterized as described previously[1]. Three additional hiPSC lines were created from peripheral blood mononuclear cells using a Sendai virus vector expressing OKSM, as previously published[2]. Subsequently, hiPSC colonies were picked and cultured on feeder-free, growth factor-reduced Matrigel-coated tissue culture dishes (BD Biosciences, San Jose, CA) with E8 pluripotent stem cell growth medium (STEMCELL Technologies, Vancouver, Canada).

Immunofluorescence and laser confocal microscopy. Beating hiPSC-CM sheets were incubated in TrypLE for 5 minutes followed by mechanical dissociation using a 200 μL pipettor and plated on 0.1-0.2% gelatin-coated glass coverslips. Immunostaining was performed according to previously established protocols[3]. Primary antibodies consisted of mouse anti-human sarcomeric alpha-actinin (Sigma-Aldrich, St. Louis, MO), rabbit anti-human TNNT2 (Abcam, Cambridge, England), mouse anti-human TNNT (Abcam, Cambridge, England), rabbit anti-human alpha-smooth muscle actin (Abcam, Cambridge, England), mouse anti-enterovirus VP1 (Leica Microsystems, Buffalo Grove, IL), and rabbit anti-human CAR (Santa Cruz Biotechnology, Dallas, Texas). Goat anti-rabbit Alexa 488 and goat anti-mouse Alexa 594 (Invitrogen) were used as secondary antibodies. Imaging was performed using a DMIL -LED inverted tissue culture microscope (Leica Microsystems, Buffalo Grove, IL) or a Zeiss LSM 510Meta confocal microscope (Carl Zeiss AG, Oberkochen, Germany) using Zen imaging software.

Handling and processing of primary adult human left ventricle tissue samples. All samples were obtained according to the guidelines of Stanford University IRB (Internal Review Board). Protocol #19810 "*Collecting discarded tissue from organ donors and heart transplant recipients*". Left ventricle myocardial tissue samples were obtained from organ donors who have already been consented to use the excised tissue for scientific research purposes. Cardiac tissue was transported to the laboratory within 2 hours of procurement in ice-cold UW (University of Wisconsin) solution. Immediately, the specimen was transferred into a sterile container with UW solution for further processing. With clean forceps and scissors, the specimen was cut into < 500 mm^3 cubes and placed into pre-labeled sterile 1.5 mL conical Eppendorf tubes and placed into a pre-chilled coolbox (CoolRack M15 by BioCision, LLC, CA) filled with liquid nitrogen for 12-15 minutes. Following this snap freezing step, the tubes were stored in a liquid nitrogen tank until needed for further processing. Upon thawing of the cardiac tissue sample from liquid nitrogen, the sample was homogenized first using a TissueRuptor system (Qiagen) and QIAshredder (Qiagen). RNA was then extracted with the miRNeasy kit (Qiagen) and utilized for downstream qRT-PCR as detailed previously.

Culture of HL-1 Mouse Atrial Tumor Cardiac Cells. HL-1 cells were cultured in Claycomb Medium (Sigma) + 10% FBS, 0.1 mM norepinephrine, and 2 mM L-glutamine as described previously[4]. Medium was changed daily. Cells were passaged using 0.25% Trypsin/EDTA prior to replating for experiments.

SUPPLEMENTAL FIGURE LEGENDS

Online Figure I: hiPSCs express intracellular sarcomeric proteins and CAR at cell-cell junctions. A, Table illustrating cell types and methods used to create hiPSCs from 6 healthy control individuals. Three hiPSC lines were created from human skin fibroblasts and were reprogrammed using the lentivirus method as previously described[1]. Three hiPSC lines were created from human peripheral blood mononuclear cells and reprogrammed using the Sendai virus method[2]. **B,** Representative hiPSC colonies from one of these healthy control individuals expresses typical markers for pluripotency, such as SSEA-4, Nanog, Tra-1-81, and Oct4 **C,** Healthy control hiPSCs also express CAR at cell-cell junctions.

Online Figure II: Characterization of hiPSCs and hiPSC-CMs from patients exhibiting DCM. A, hiPSCs were previously produced from individuals in a 7-member family cohort afflicted with familial dilated cardiomyopathy (DCM)[5]. These individuals exhibit a mutation in *TNNT2*, encoding for a mutated version of cTnT (tryptophan in place of arginine at amino acid residue 173). hiPSCs from these individuals express typical markers for pluripotency, such as SSEA-4, Nanog, Tra-1-81, and Oct4. **B,** DCM hiPSCs express CAR at cell-cell junctions. **C,** DCM hiPSC-CMs exhibit abnormal sarcomeric organization, with "punctate" phenotypes for alpha-actinin and cTnT, as previously described[5]. **D,** DCM hiPSC-CMs, like healthy control hiPSC-CMs, express CAR at cell-cell junctions.

Online Figure III: HL-1 mouse atrial tumor cardiac cells express CAR but are less susceptible to CVB3-Luc infection than hiPSC-CMs. A, Immunofluorescence for CAR expression on HL-1 cells. Like hiPSC-CMs, HL-1 cells express CAR at cell-cell junctions. **B,**

Viral timecourse of HL-1 cells infected with MOI 5 CVB3-Luc. Immunofluorescence illustrates sparse VP1 staining in HL-1 cells, which has been previously described[6]. **C,** Representative 96-well plate containing hiPSC-CMs and HL-1 cells infected with CVB3-Luc at MOI 1. Each cell type was simultaneously plated at 40,000 cells and in quintuplicate. Cells were allowed to settle onto Matrigel-coated 96 wells for 24 hours before viral infection and bioluminescence imaging was conducted. **D,** Quantification of MOI 1 CVB3-Luc proliferation on hiPSC-CMs and HL-1 cells.

Online Figure IV: Coxsackievirus and adenovirus receptor (*CXADR*) gene expression in hiPSC-CMs, HL-1 cells, and adult human left ventricle sample. QRT-PCR results comparing expression of *CXADR* between day 15 hiPSC-CMs, HL-1 atrial tumor-derived cardiac cells, and primary adult human adult left ventricular (LV) myocardial tissue. Expression of *CXADR* in day 15 post-differentiation hiPSC-CMs is approximately 30-fold less than in LV tissue but is more than 10-fold greater than in HL-1 cells.

Online Figure V: CVB3-Luc gene expression correlates with luciferase bioluminescent signal after hiPSC-CM infection. QRT-PCR of CVB3-Luc gene expression after 0, 2, 4, 6, and 8 hours of MOI 5 infection on purified hiPSC-CMs. Primers were designed against coxsackievirus genes *VP2*, encoding for a viral capsid protein, and *3D*, encoding for a viral RNA polymerase. Data points are normalized to the 8 hour-post infection timepoint. Luminescence readings were simultaneously taken at each time point and were also normalized to the 8 hour-post infection timepoint.

Online Figure VI: hiPSC-CMs from healthy control patients do not exhibit patient-specific differences in terms of CVB3-Luc viral response. Quantification for onset of cytopathic effect in 6 different sets of day 30 post-differentiation hiPSC-CMs infected with CVB3-Luc at MOI 5. Three lines were created from OKSM lentivirus reprogramming of human skin fibroblasts to hiPSCs. Three lines were created from OKSM Sendai virus reprogramming of human peripheral blood mononuclear cells to hiPSCs. All hiPSC lines were differentiated using a previously published, high efficiency, small molecule differentiation protocol[7].

Online Figure VII: hiPSCs are susceptible to infection with CVB3-Luc. hiPSCs, like hiPSC-CMs, are susceptible to CVB3-Luc infection. Over 24 hours, there is an increase in VP1 expression. Cells continue to proliferate during the course of infection.

Online Figure VIII: Characterization of long-term, low MOI CVB3-Luc infection on hiPSC-CMs. Brightfield images of 40,000 hiPSC-CMs after 120 hours of infection with decreasing MOI of CVB3-Luc. All hiPSC-CMs in wells receiving MOI 5 to MOI $5x10^{-5}$ CVB3-Luc succumbed to cytopathic effect by 120 hours post-infection. Asterisk represents the lowest MOI ($5x10^{-5}$) of CVB3-Luc on 40,000 hiPSC-CMs that is capable of inducing a cytopathic effect, suggesting that a single digit number of viral particles is able to propagate a CVB3-Luc infection to completely infect a well of hiPSC-CMs. Cytopathic effect is not observed at MOI $5x10^{-6}$ and less.

Online Figure IX: Treatment of CVB3-infected HL-1 cells with antiviral compounds PDTC and Ribavirin, but not IFNβ1, reduces CVB3-Luc proliferation. A, Representative 96-well plate containing HL-1 cells infected with CVB3-Luc and treated with 8 ng/mL IFNβ1 at 12 hours

and 0 hours prior to MOI 1 CVB3-Luc infection visualized over 12 hours using bioluminescence imaging. **B,** Quantification of CVB3-Luc proliferation on HL-1 cells treated with 8 ng/mL IFNβ1. **C,** Representative 96-well plate containing HL-1 cells infected with CVB3-Luc and treated with 800 μM Ribavirin 12 hours and 0 hours prior to MOI 1 CVB3-Luc infection visualized over 12 hours using bioluminescence imaging. **D,** Quantification of CVB3-Luc proliferation on HL-1 cells treated with 800 μM Ribavirin. **E,** Representative 96-well plate containing HL-1 cells infected with CVB3-Luc and treated with 800 μM PDTC at 12 hours and 0 hours prior to MOI 1 CVB3-Luc infection visualized over 12 hours using bioluminescence imaging. **F,** Quantification of CVB3-Luc proliferation on HL-1 cells treated with 800 μM PDTC.

Online Figure X: Addition of antiviral compounds on hiPSC-CMs after CVB3-Luc infection is moderately effective in reducing CVB3-Luc proliferation. A, Representative 96-well plate containing hiPSC-CMs infected with CVB3-Luc and treated with 8 ng/mL IFNβ1 at 0, 2, 4, and 6 hours post-MOI 1 CVB3-Luc infection visualized over 12 hours using bioluminescence imaging. **B,** Quantification of CVB3-Luc proliferation on hiPSC-CMs treated with 8 ng/mL IFNβ1. **C,** Representative 96-well plate containing hiPSC-CMs infected with CVB3-Luc and treated with 800 μM Ribavirin at 0, 2, 4, and 6 hours post-MOI 1 CVB3-Luc infection visualized over 12 hours using bioluminescence imaging. **D,** Quantification of CVB3-Luc proliferation on hiPSC-CMs treated with 800 μM Ribavirin. **E,** Representative 96-well plate containing hiPSC-CMs infected with CVB3-Luc and treated with 800 μM PDTC at 0, 2, 4, and 6 hours post-MOI 1 CVB3-Luc infection visualized over 12 hours using bioluminescence imaging. **F,** Quantification of CVB3-Luc proliferation on hiPSC-CMs treated with 800 μM PDTC.

Online Figure XI: PDTC treatment reduces CVB3-Luc proliferation on infected hiPSC-CMs in a concentration-dependent fashion. A, Representative 96-well plate containing hiPSC-CMs infected with CVB3-Luc and pretreated with PDTC for 12 hours, visualized over 12 hours using bioluminescence imaging. **B,** Quantification of CVB3-Luc proliferation on hiPSC-CMs pretreated with PDTC. **C,** WST-1 assay quantifying cellular metabolic output and viability following treatment with increasing amounts of PDTC. **D,** Stills from videos of hiPSC-CMs treated with 800 μM PDTC for up to 72 hours. These hiPSC-CMs continue beating after 72 hours of PDTC treatment.

SUPPLEMENTAL TABLES

Online Table I: Primers Utilized for qRT-PCR.

TAQMAN PRIMERS			
Gene Symbol	**Species**	**Gene Name**	**ABI Assay ID**
CXADR	Human	Coxsackievirus and Adenovirus Receptor	Hs00154661_m1
MYH6	Human	Myosin Heavy Chain 6, cardiac muscle, alpha	Hs00411908_m1
POU5F1	Human	POU class 5 homeobox 1	Hs00999632_g1
TNNT2	Human	Troponin T Type 2 (Cardiac)	Hs00165960_m1
18S	Human	Eukaryotic 18S rRNA	Hs99999901_s1
CXADR	Mouse	Coxsackievirus and Adenovirus Receptor	Mm00438355_m1
TNNT2	Mouse	Troponin T Type 2 (Cardiac)	Mm01290256_m1
18S	Mouse	Eukaryotic 18S rRNA	Mm03928990_g1
SYBR GREEN PRIMERS			
Gene Symbol	**Species**	**Primer**	**Sequence (5' to 3')**
VP2	Coxsackievirus	Forward	CGCTAGATTACTGCCCTGGG
		Reverse	CTGGTGCCCTGCTAAACGTA
3D	Coxsackievirus	Forward	TGATCGCATCGTACCCATGG
		Reverse	TAGGAAAGTGGCGTTGGTCC
18S	Human	Forward	AGAAACGGCTACCACATCCA
		Reverse	CACCAGACTTGCCCTCCA

Online Table II: Antiviral defense genes upregulated after IFNβ1 treatment in infected hiPSC-CMs

Gene	Description	Fold Change	ANOVA p-value
ISG15	ISG15 ubiquitin-like modifier	22.72	0.000804
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	21.06	0.002703
IFIH1	interferon induced with helicase C domain 1	10.37	0.002208
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa	10	0.035424
IFITM1	interferon induced transmembrane protein 1	9.49	0.001991
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	8.78	0.007341
IFI44L	interferon-induced protein 44-like	8.75	0.02334
STAT1	signal transducer and activator of transcription 1, 91kDa	7.88	0.003083
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	7.17	0.005251
ZC3HAV1	zinc finger CCCH-type, antiviral 1	7.07	0.00539
BST2	bone marrow stromal cell antigen 2	6.96	0.009572
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	6.82	0.000477
MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	6.77	0.008064
DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	5.59	0.003651
EIF2AK2	eukaryotic translation initiation factor 2-alpha kinase 2	5.47	0.004195
IRF9	interferon regulatory factor 9	5.24	0.000395
PLSCR1	phospholipid scramblase 1	4.15	0.000569

TRIM25	tripartite motif containing 25, microRNA 3614	3.82	0.003324
HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	3.35	0.013563
IFIT5	interferon-induced protein with tetratricopeptide repeats 5	2.93	0.004233
SAMHD1	SAM domain and HD domain 1	2.82	0.027551
IFITM3	interferon induced transmembrane protein 3	2.74	0.002204

SUPPLEMENTAL MOVIES

Online Movie I. Movie of beating sheet of hiPSC-CMs prior to purification via glucose starvation. These hiPSC-CMs spontaneously contract beginning at day 8-10 post-differentiation. Cell sheets consistently contain 85-95% hiPSC-CMs. Cells were recorded using a 10x objective.

Online Movie II. Movie showing a regularly beating monolayer of replated hiPSC-CMs following purification via glucose starvation. Cells were recorded using a 20x objective prior to MOI 5 CVB3-Luc infection.

Online Movie III. Movie showing an irregularly beating monolayer of replated hiPSC-CMs 6 hours following MOI 5 CVB3-Luc infection. Cells were recorded using a 20x objective.

Online Movie IV. Movie showing an irregularly beating monolayer of replated hiPSC-CMs 12 hours following MOI 5 CVB3-Luc infection. Cells were recorded using a 20x objective.

SUPPLEMENTAL REFERENCES

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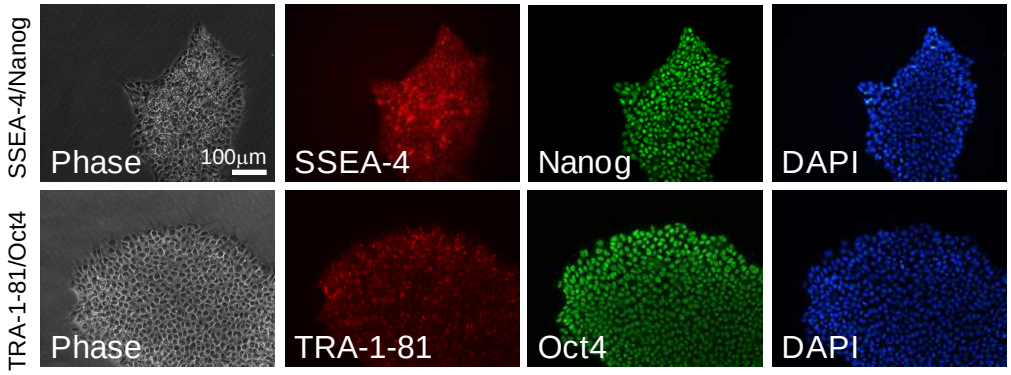
A

Cell Type Reprogrammed	Reprogramming Vector Used	# Control hiPSC Lines
Human Skin Fibroblasts	Lentivirus	3
Peripheral Blood Mononuclear Cells	Sendai virus	3

B



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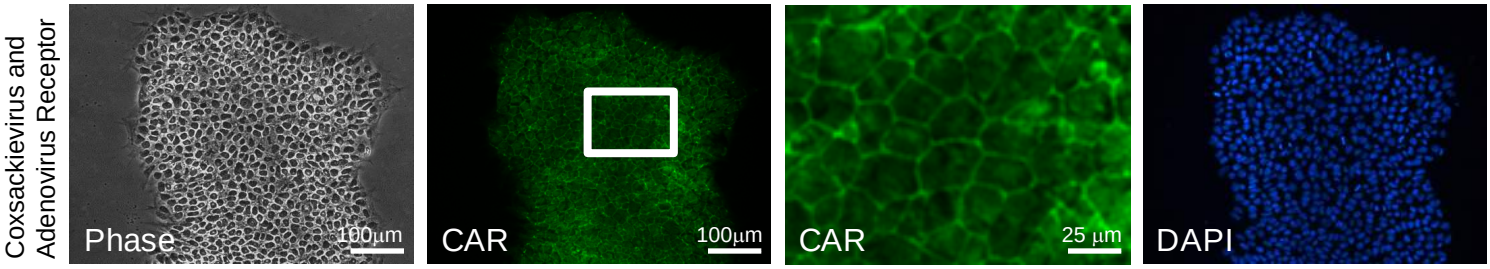

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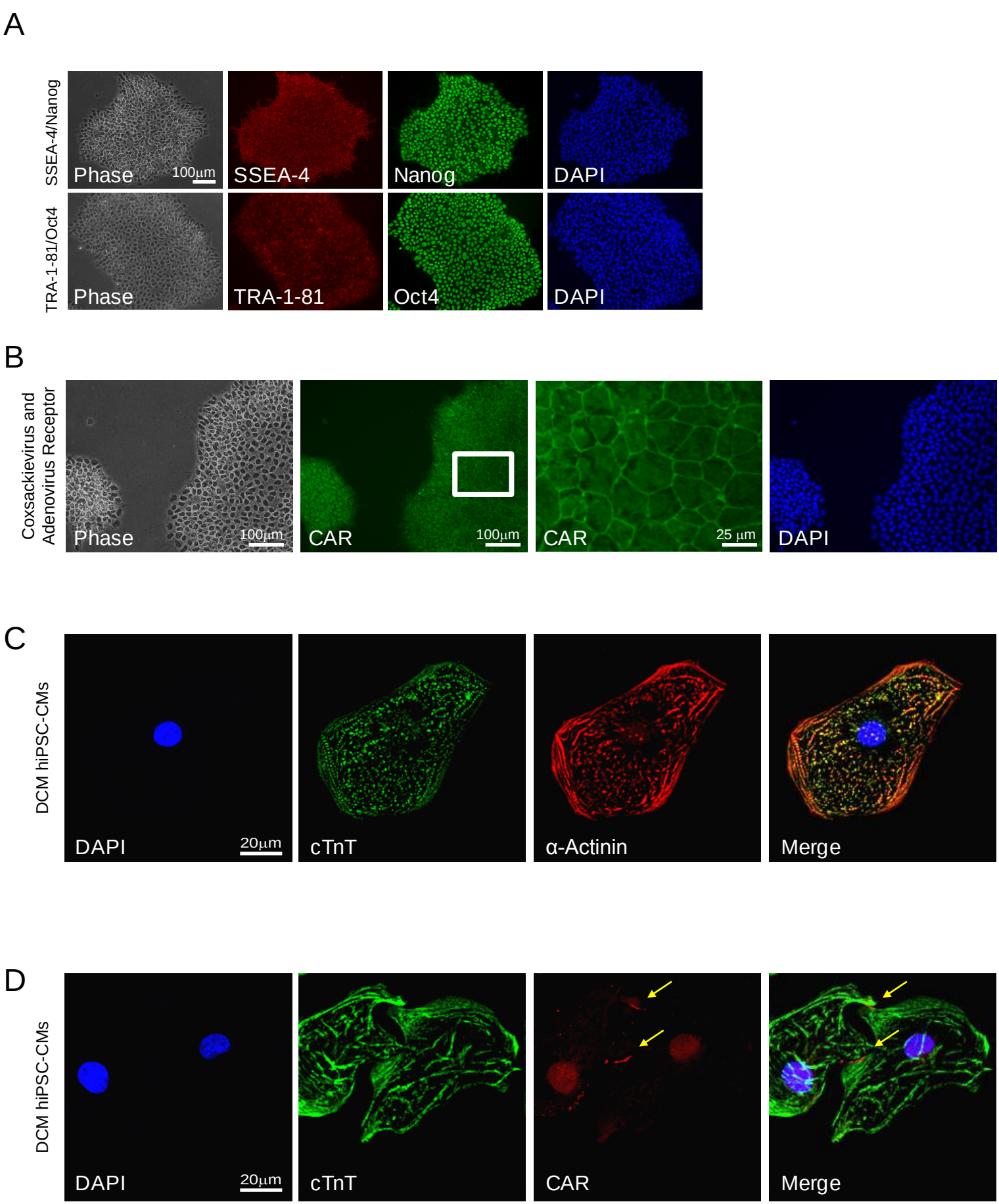

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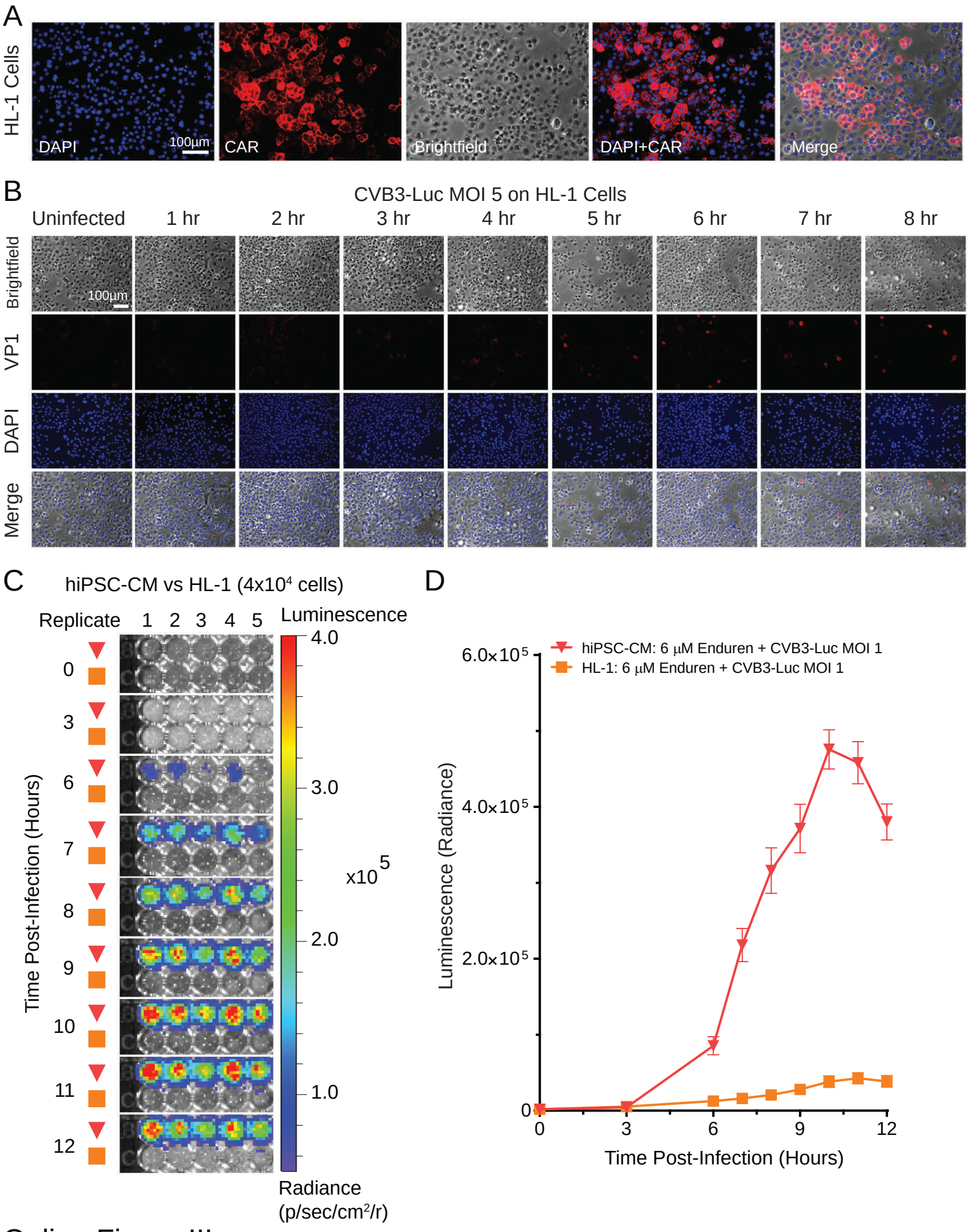

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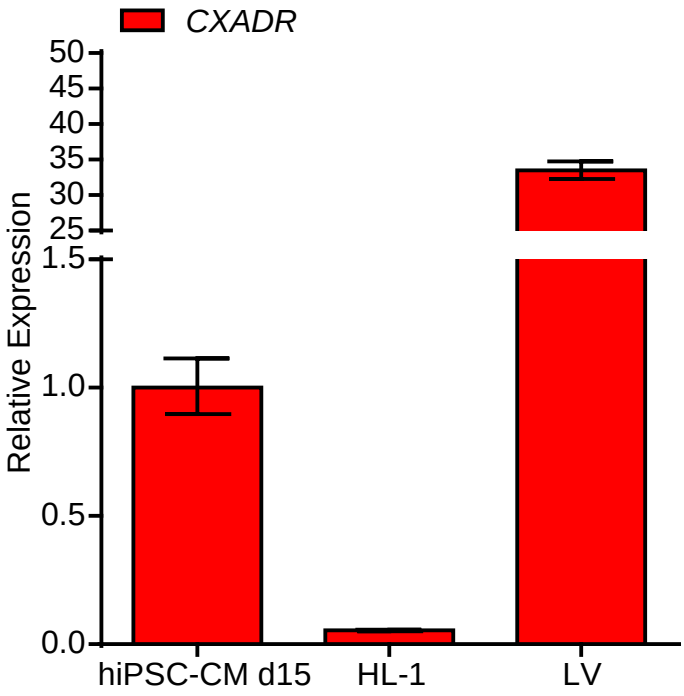

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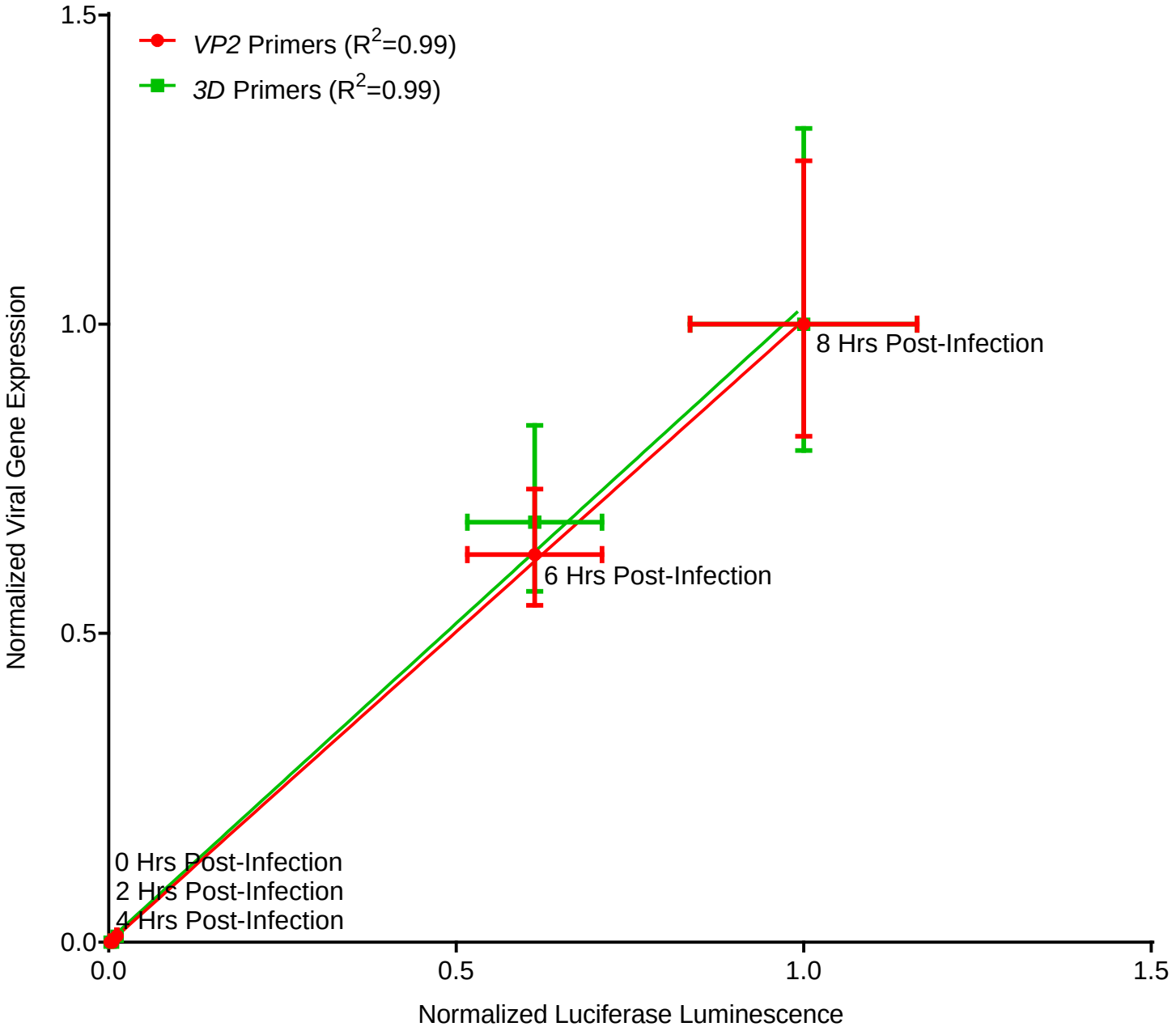

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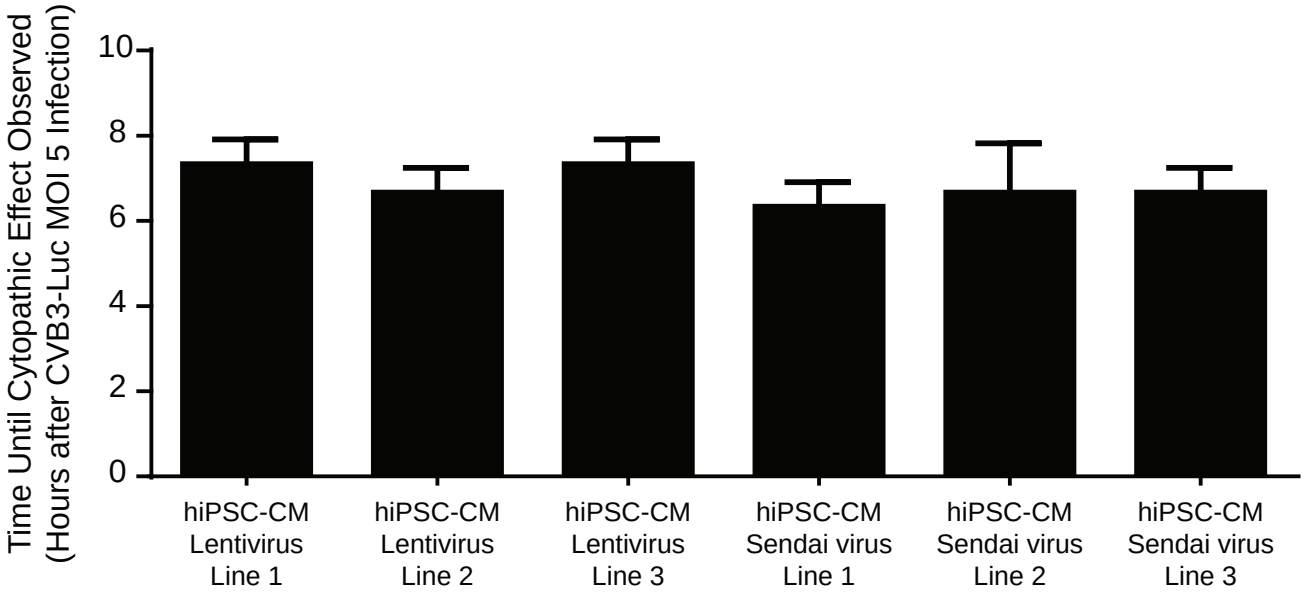

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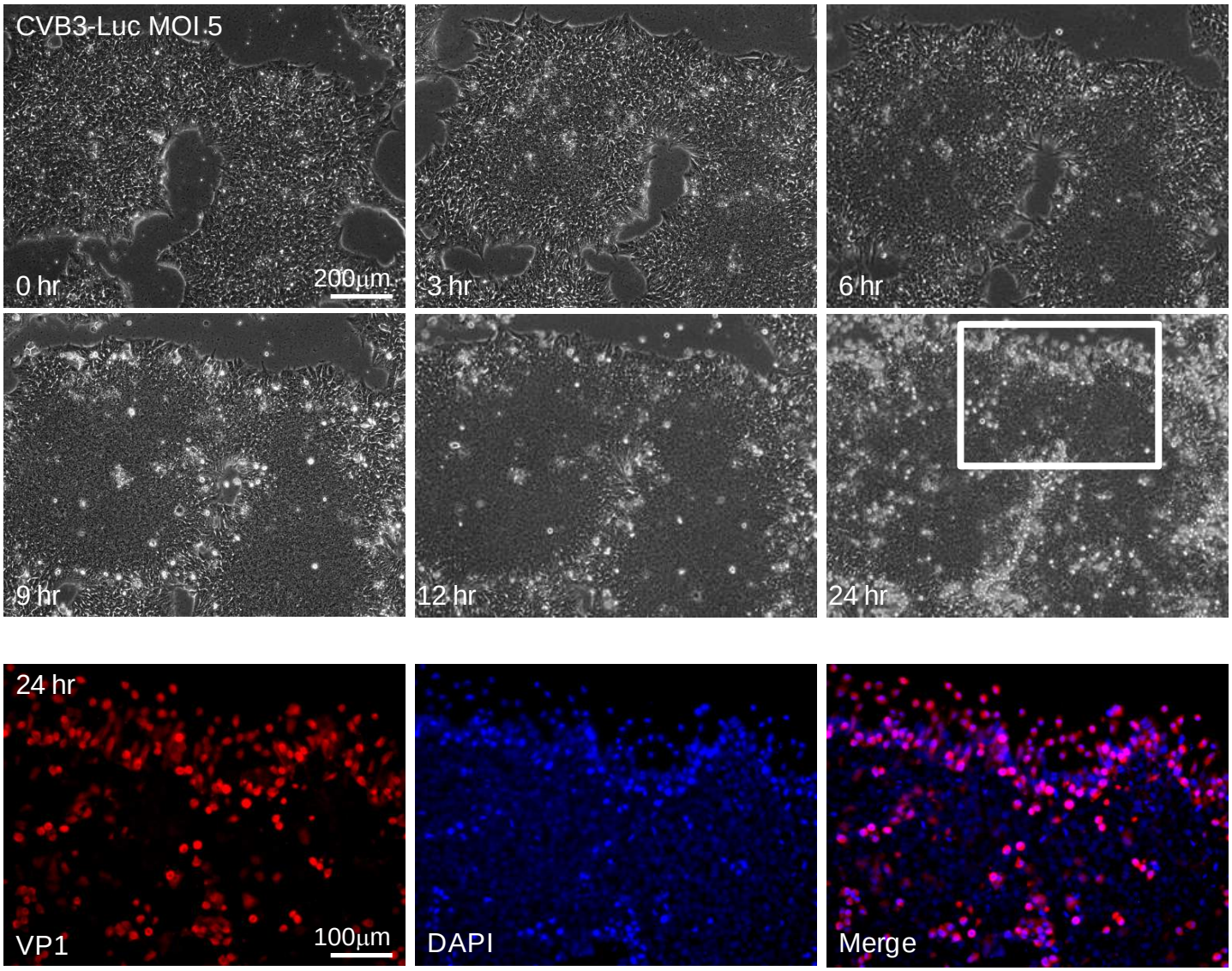

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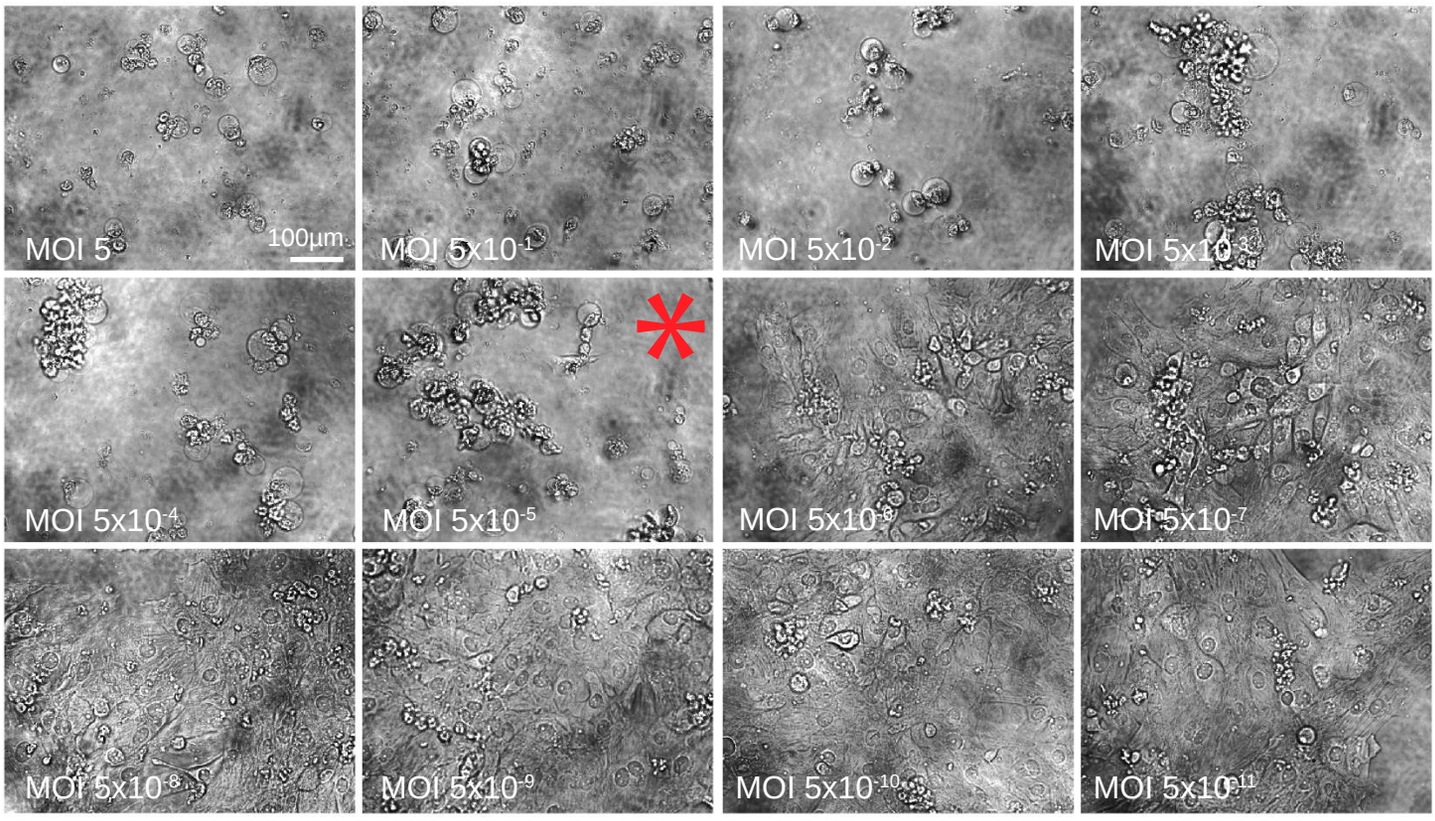

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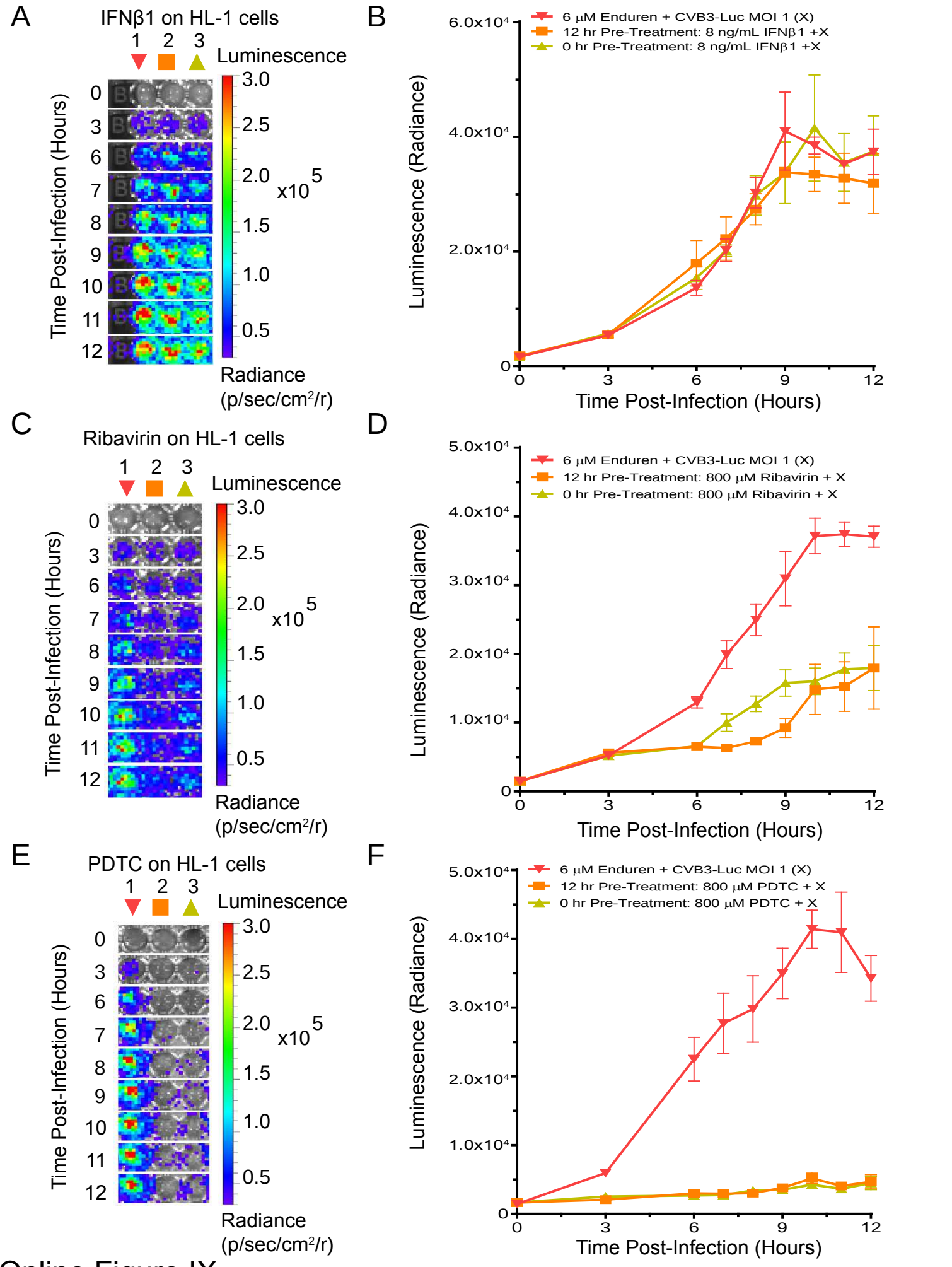

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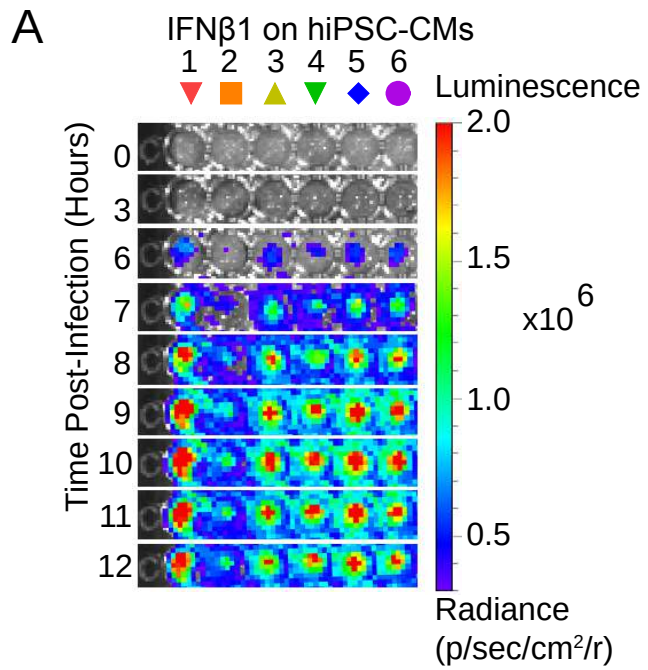

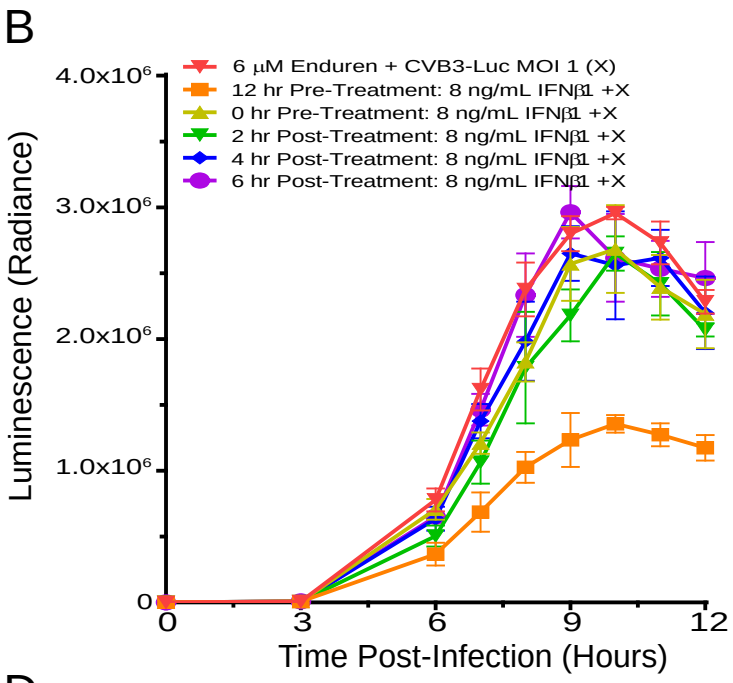

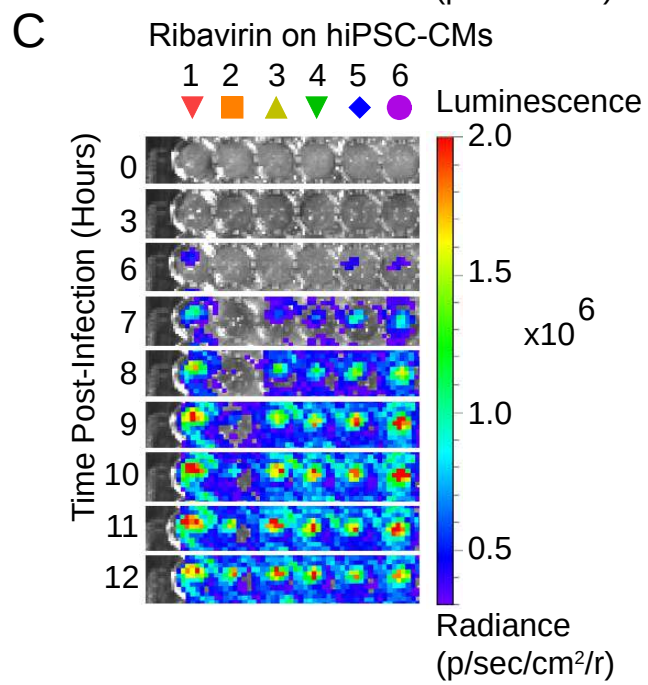

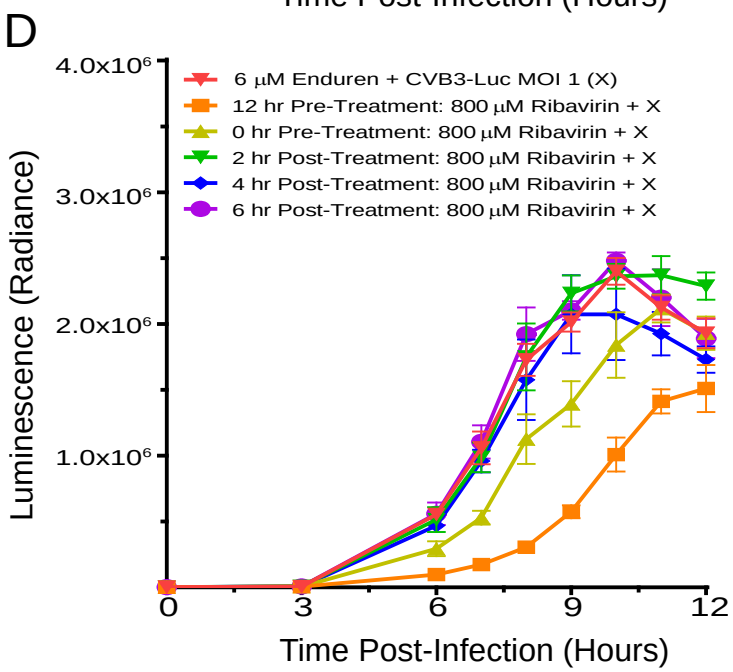

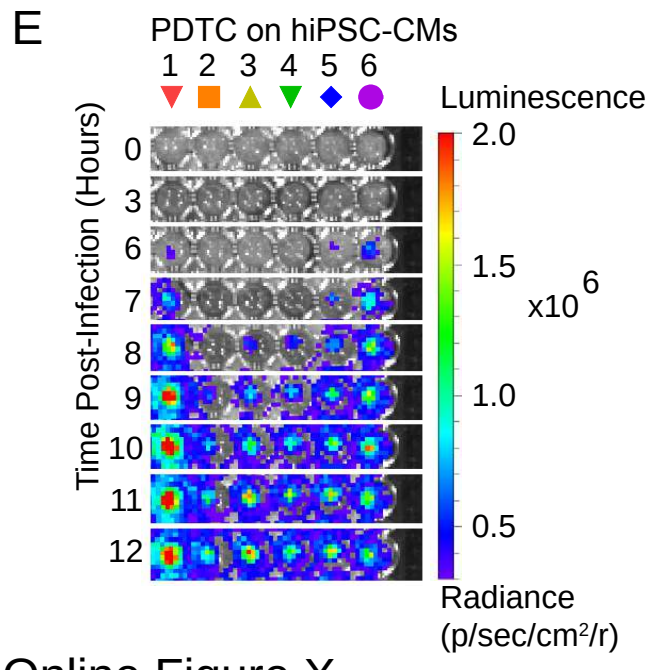

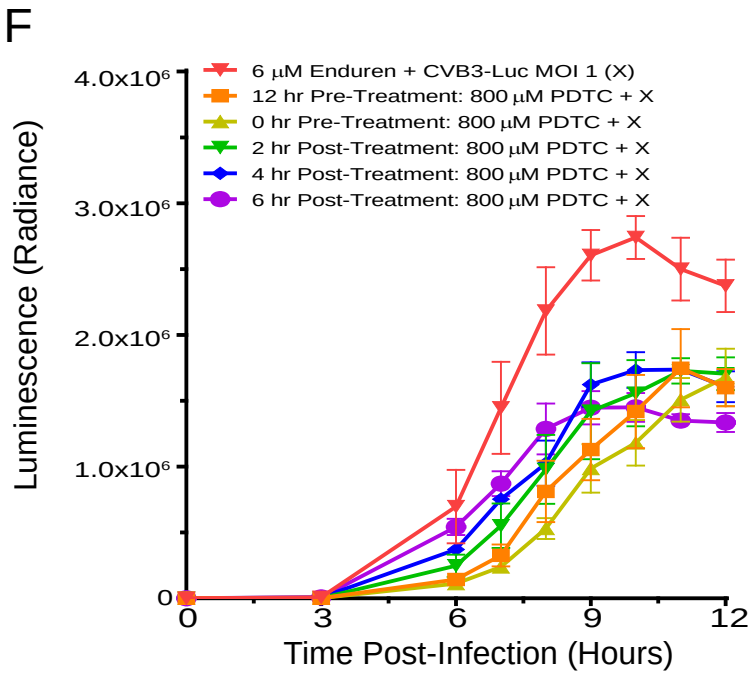

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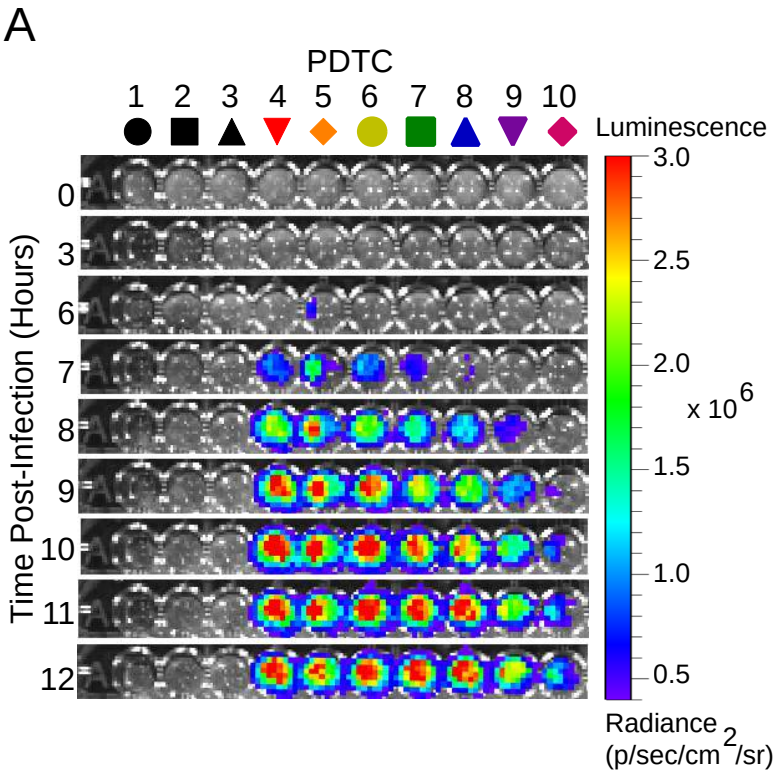

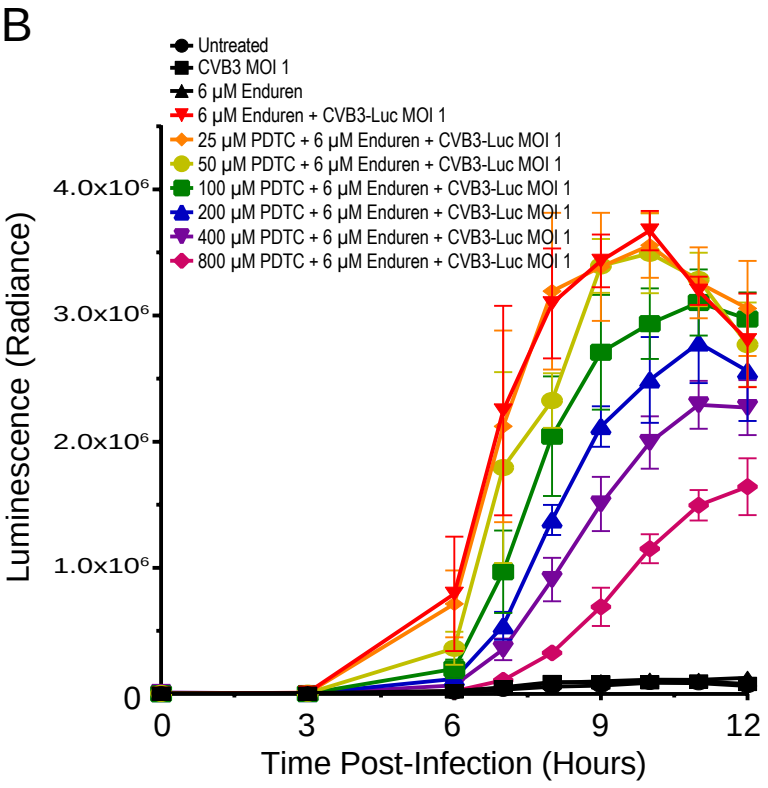

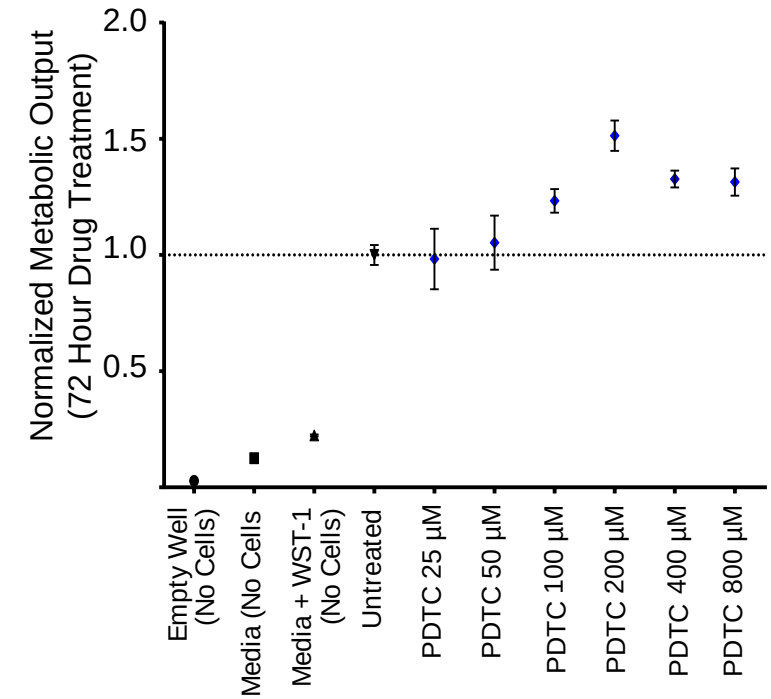

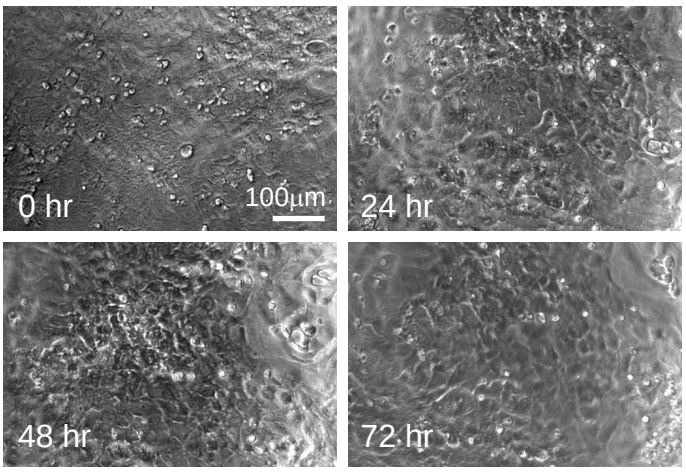

Online Figure XI