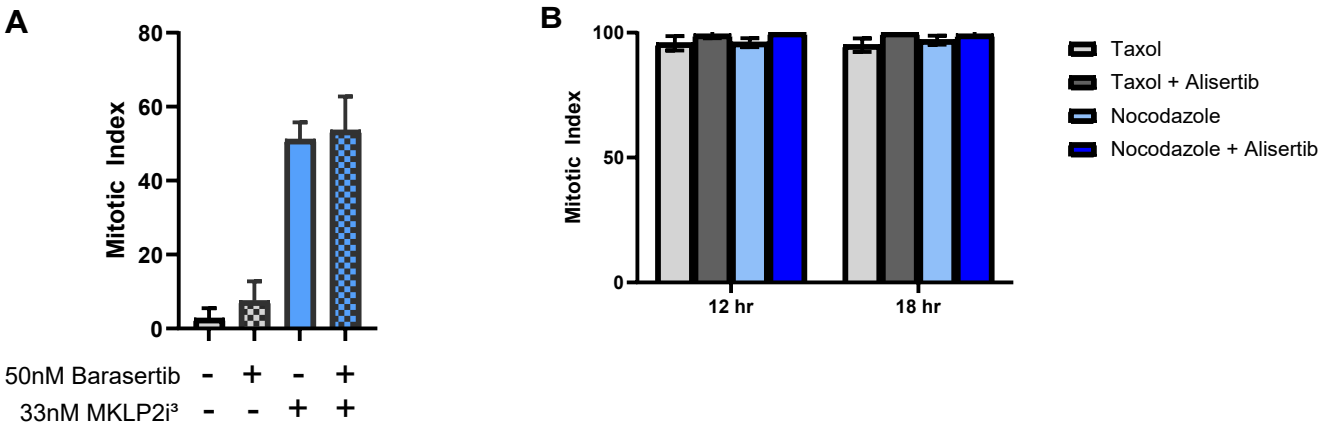
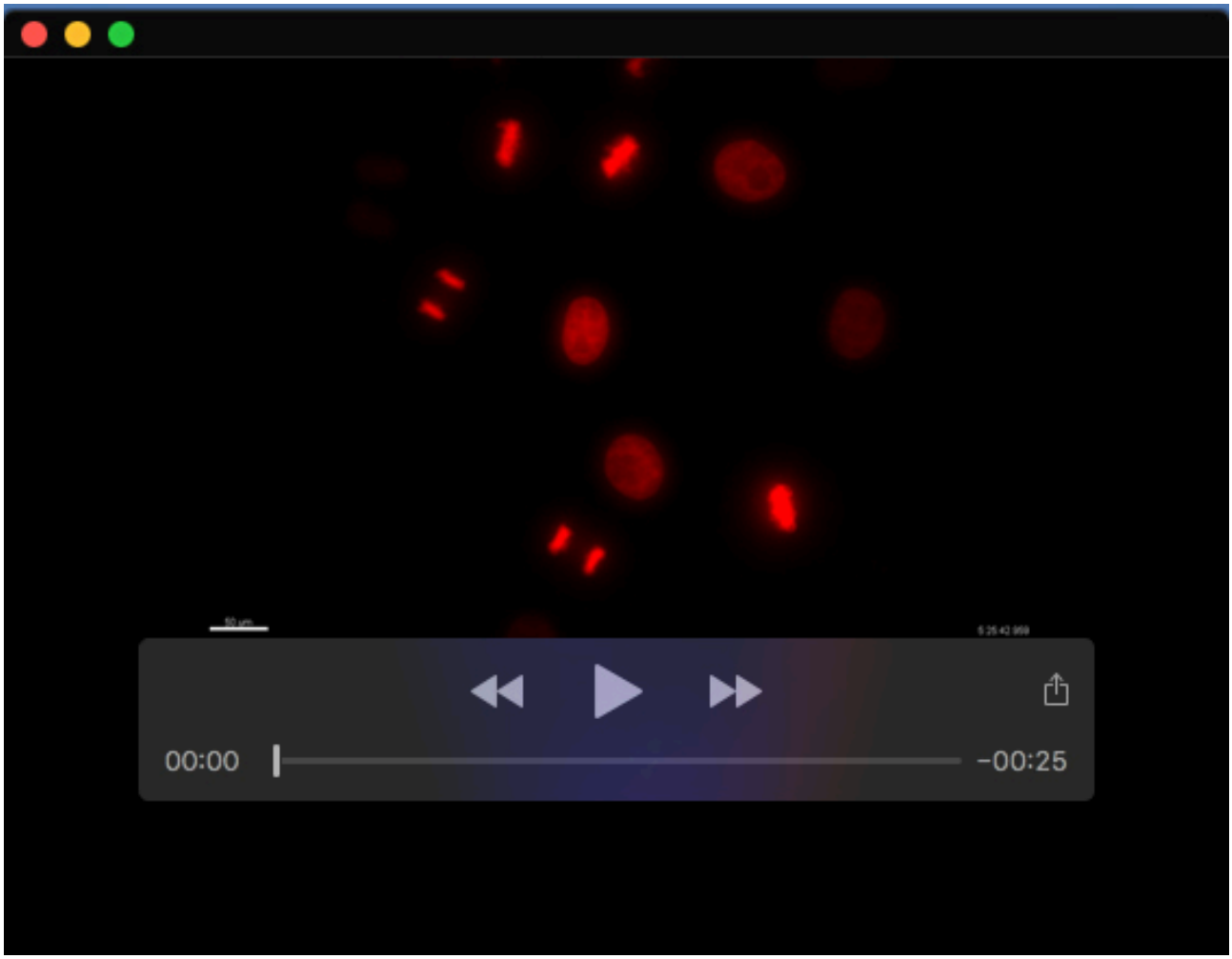


Fig. S1. A) Graph depicting mitotic index of HeLa cells 18 hrs after release from DTB then treated with DMSO, 50nM barasertib, 33nM MKLP2i3 or a combination (n= 100 cells, 3 independent experiments). B) Graph depicting mitotic index of HeLa cells 12 and 18 hrs after release from DTB into 100nM taxol, 100ng/µl nocodazole, 12.5nM alisertib, or a combination (n=100 cells).

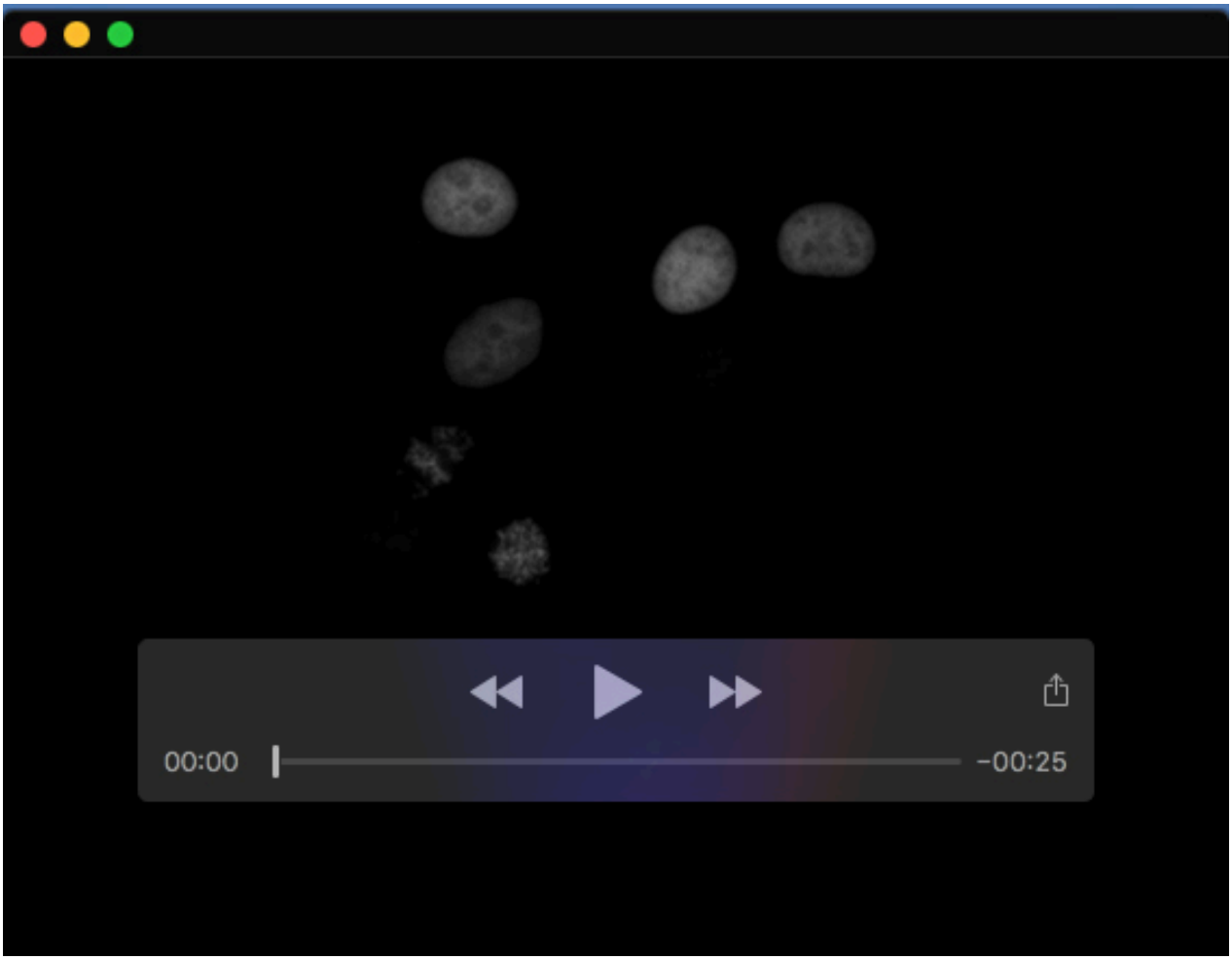
Table S1. KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Centromere protein antibody (CREST)	Antibodies Inc	Cat# 15-234 RRID:AB_2687472
ɣ-tubulin antibody	Millipore-Sigma	Cat# T5326 RRID:AB_ 532292
Tubulin antibody [YL1/2]	Thermo Fisher Scientific	Cat# MA1-80017 RRID: AB_ 2210201
Hec1 (phospho Ser55) antibody	GeneTex	Cat# GTX70017 RRID:AB_11162004
Hec1 antibody [9G3.23]	GeneTex	Cat# GTX70268 RRID:AB_371632
Phospho-Aurora A (Thr288) (C39D8)	Cell Signaling	Cat# 3079S RRID:AB_2061481
Mouse Anti-IAK1 (Aurora A)	BD Biosciences	Cat# 610939 RRID:AB_398252
Phospho Aurora Kinase B (Thr232) Polyclonal antibody	Thermo Fisher Scientific	Cat# 600-401-677 RRID:AB_2061641
β-ACTIN	Millipore-Sigma	Cat# A3854, RRID:AB_262011
IRDye® 800CW Donkey anti-Mouse IgG Secondary Antibody	LI-COR Biosciences	Cat# 926-32212 RRID:AB_621847
IRDye® 800CW Donkey anti-Rabbit IgG Secondary Antibody	LI-COR Biosciences	Cat# 926-32213 RRID:AB_621848
Goat anti-rat secondary antibody, Alexa Fluor 546	Invitrogen	Cat# A-11081
Goat anti-mouse secondary antibody, Alexa Fluor 488	Invitrogen	Cat# A-32723
Donkey anti-rat secondary antibody, Alexa Fluor 488	Invitrogen	Cat# A-21208
Goat anti-mouse secondary antibody, Alexa Fluor 546	Invitrogen	Cat# A-11030
Donkey anti-rabbit secondary antibody, Alexa Fluor Plus 488	Invitrogen	Cat# A-32790
Goat anti-rabbit secondary antibody, Alexa Fluor 594	Invitrogen	Cat# A-11012
Goat anti-human secondary antibody, Alexa Fluor 488	Invitrogen	Cat# A-11013
Chemicals, peptides, and recombinant proteins		
Paprotrain (MKLP2i[1])	Tocris	Cat# 4813
Compound 9a (MKLP2i[2])	OSU Medicinal Chemistry Shared Resource	(Labrière et al., 2016)
Compound 38 (MKLP2i[3])	Wuxi AppTec	(Pouletty, 2019)
Lipofectamine RNAiMAX	Thermo Fisher Scientific	Cat# 13778150
Thymidine	Millipore-Sigma	Cat# T1895
TransIT-LT1 Reagent	Mirus Bio	Cat# MIR 2304
RO-3306	Selleckchem	Cat# S7747
Alisertib	Selleckchem	Cat# S1133
Barasertib	Selleckchem	Cat# S1147
ProLong Gold Antifade Mountant with NucBlue Stain	ThermoFisher	Cat# P36981

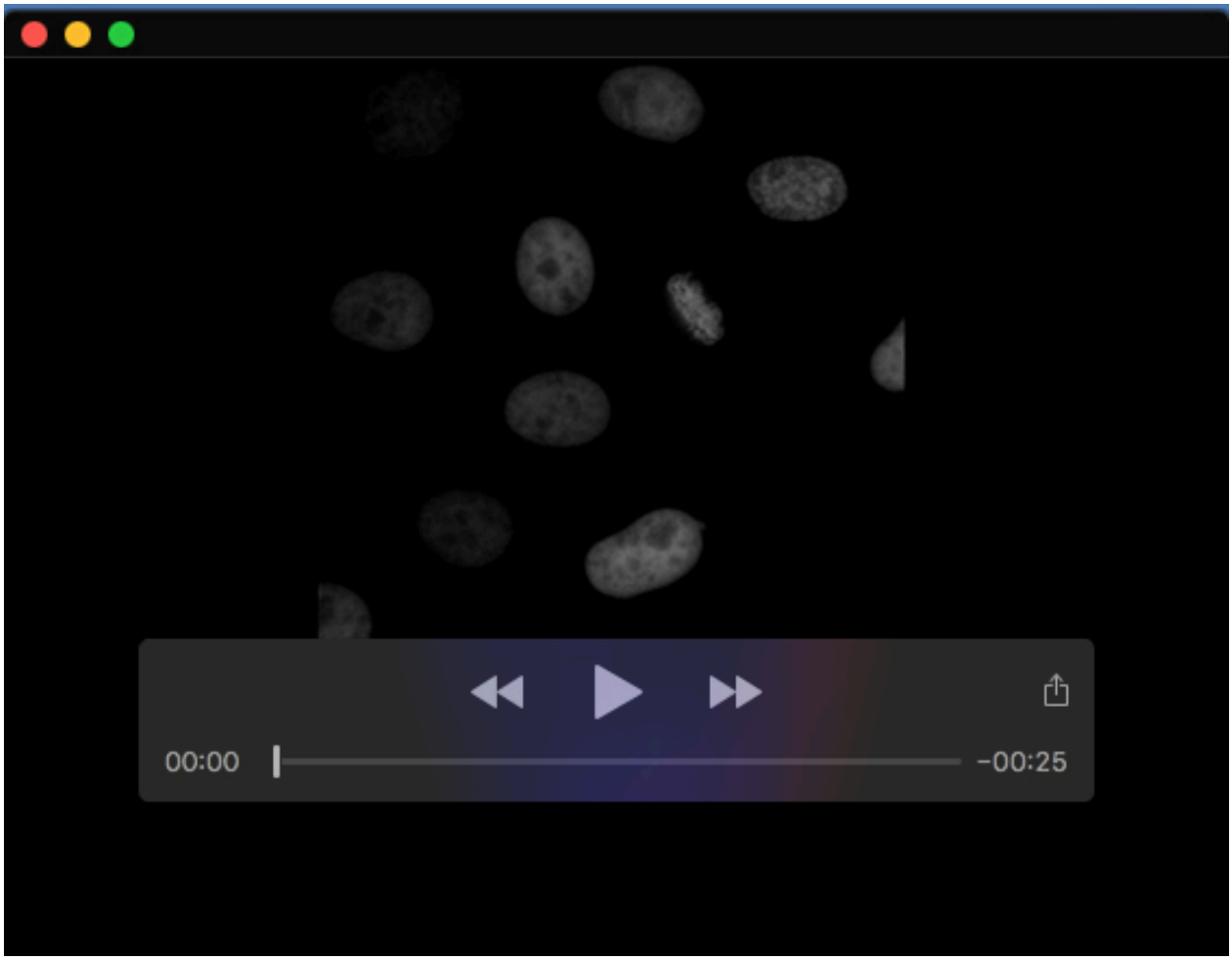
Fluoromount-G	ThermoFisher	Cat# 00-4958-02
Critical commercial assays		
Aquarius Enumeration Probes (Chr 15)	Oxford Gene Technology	Cat# LPE015R-A
Aquarius Enumeration Probes (Chr 2)	Oxford Gene Technology	Cat# LPE002G-A
Aquarius Enumeration Probes (Chr 10)	Oxford Gene Technology	Cat# LPE010G-A
Aquarius Enumeration Probes (Chr 7)	Oxford Gene Technology	Cat# LPE007R-A
Experimental models: Cell lines		
HeLa	ATCC	CRM-CCL-2
HeLa mCherry H2B GFP tubulin	Katharine Ullman	n/a
Oligonucleotides		
G165E F-ACTAACTCAGaGAAAACCCAC	IDT	n/a
G165E R-GACTCCATATGTATAGATGAG	IDT	n/a
E413A F-GCTGGCTCAGcGCGCTGCAAA	IDT	n/a
E413A R-CAGATCACAGGGTGACAGCTCG	IDT	n/a
ON-TARGETplus Non-Targeting Pool	Horizon Discovery	D-001810-10-05
ON-TARGETplus KIF20A siRNA 3'UTR	Horizon Discovery	J-004957-06
ON-TARGETplus KIF20A siRNA 3'UTR	Horizon Discovery	J-004957-07
ON-TARGETplus KIF20A siRNA	Horizon Discovery	J-004957-08
ON-TARGETplus KIF20A siRNA 3'UTR	Horizon Discovery	J-004957-09
ON-TARGETplus KIF20A 3'UTR CCACCUAUGUAAUCUCAUGdTdT	Horizon Discovery	(Adriaans et al., 2020)
Recombinant DNA		
pCS2-TAP MKLP2	This Study	n/a
pCS2-TAP MKLP2 G165E	This Study	n/a
pCS2-TAP MKLP2 E413A	This Study	n/a
Software and algorithms		
FIJI		https://imagej.net/Fiji
Prism 9	GraphPad	https://www.graphpad.com
Incucyte ZOOM 2016B	Sartorius	https://www.sartorius.com/en



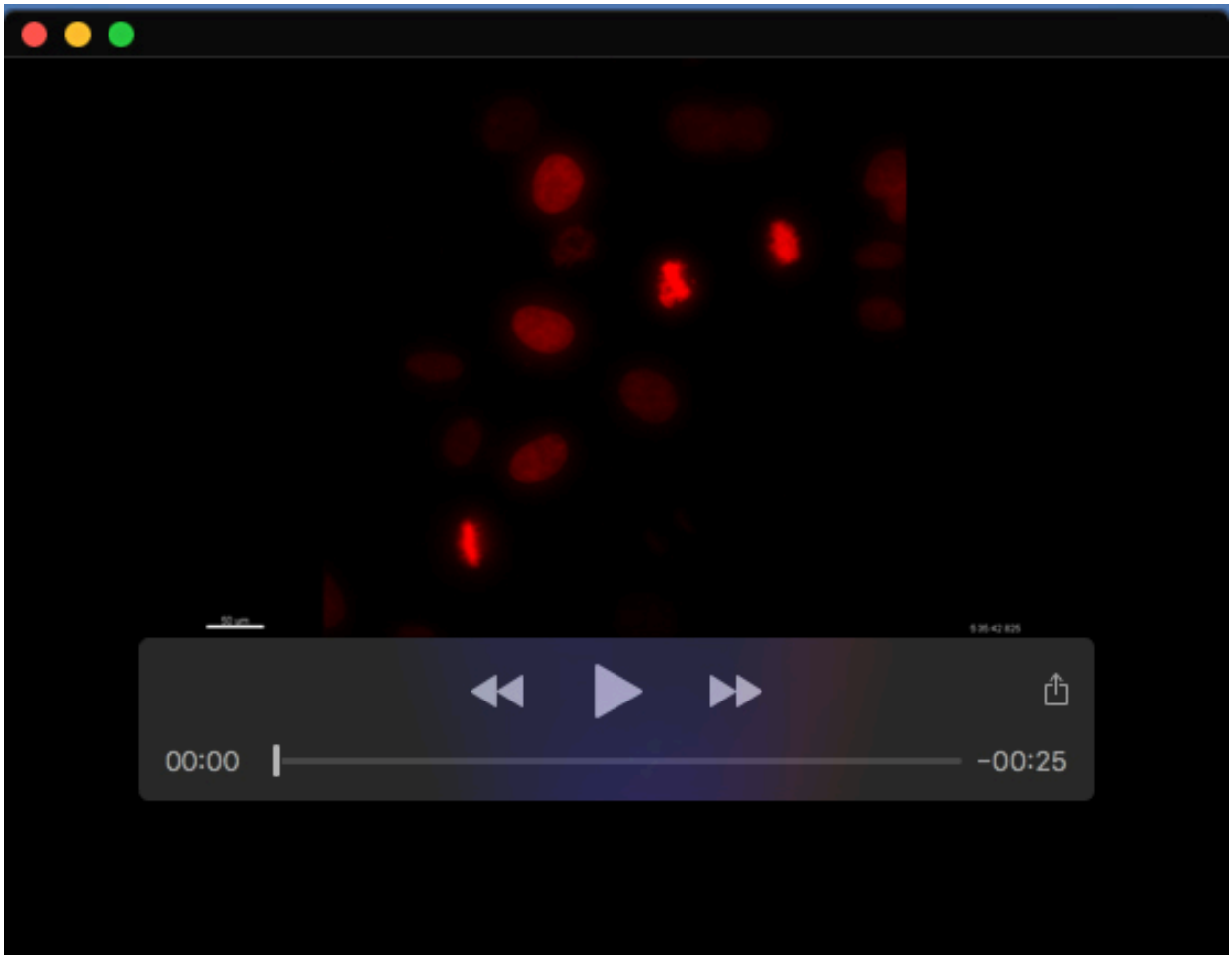
Movie 1. Epifluorescent images taken every 5 minutes of HeLa mCherry H2B GFP tubulin cells after DTB and treated with DMSO. Images were compiled into a time-lapse movie of SME projections.



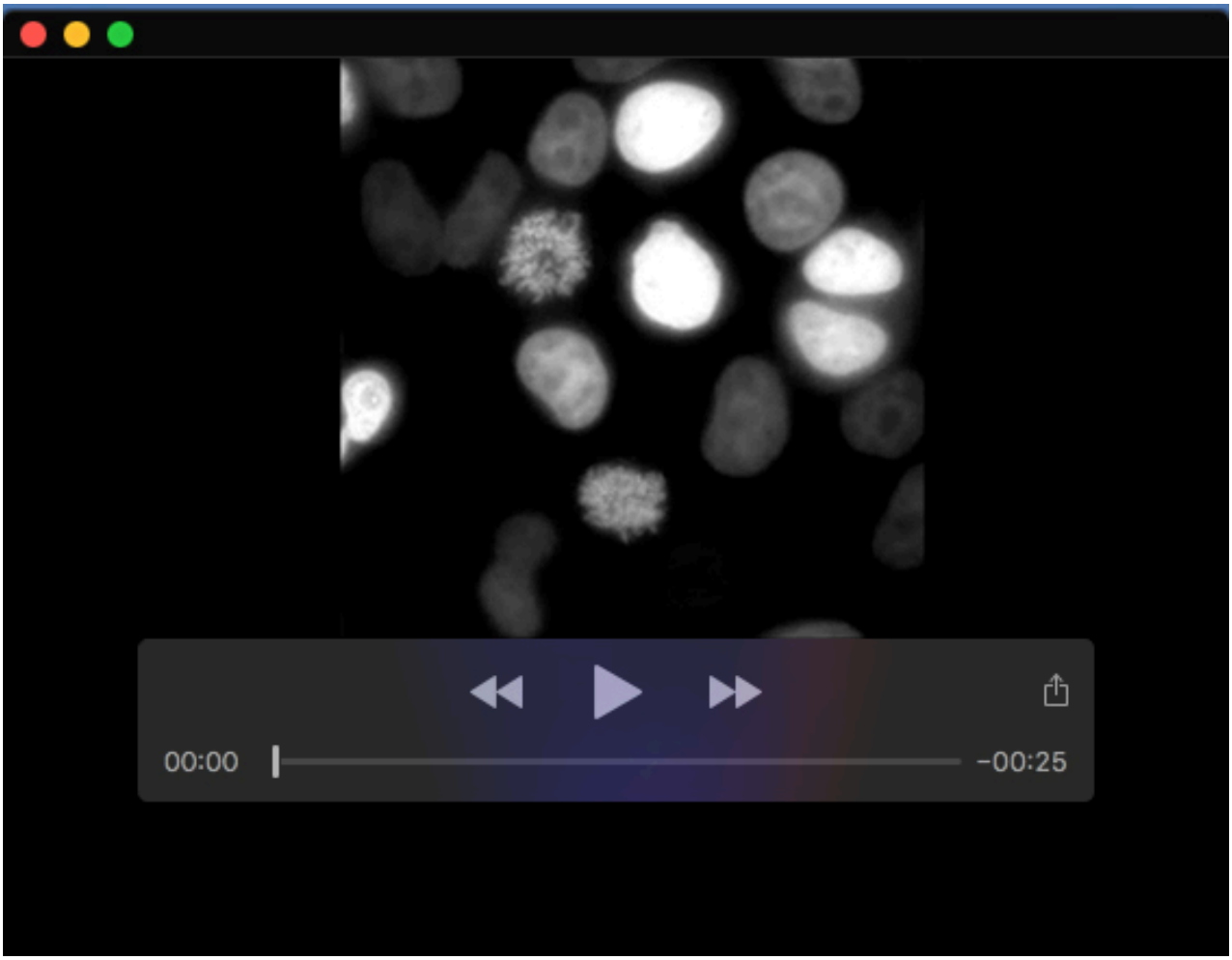
Movie 2. Epifluorescent images taken every 5 minutes of HeLa mCherry H2B GFP tubulin cells after DTB and treated with 33nM MKLP2i[3]. Images were compiled into a time-lapse movie of SME projections.



Movie 3. Epifluorescent images taken every 5 minutes of HeLa mCherry H2B GFP tubulin cells after DTB and treated with 3.7nM MKLP2i[3]. Images were compiled into a time-lapse movie of SME projections.



Movie 4. Epifluorescent images taken every 5 minutes of HeLa mCherry H2B GFP tubulin cells after DTB and transfected with siMKLP2 + pCS2 TAP KIF20A. Images were compiled into a time-lapse movie of SME projections.



Movie 5. Epifluorescent images taken every 5 minutes of HeLa mCherry H2B GFP tubulin cells after DTB and transfected with siMKLP2 + pCS2 TAP KIF20A E413A. Images were compiled into a time-lapse movie of SME projections.