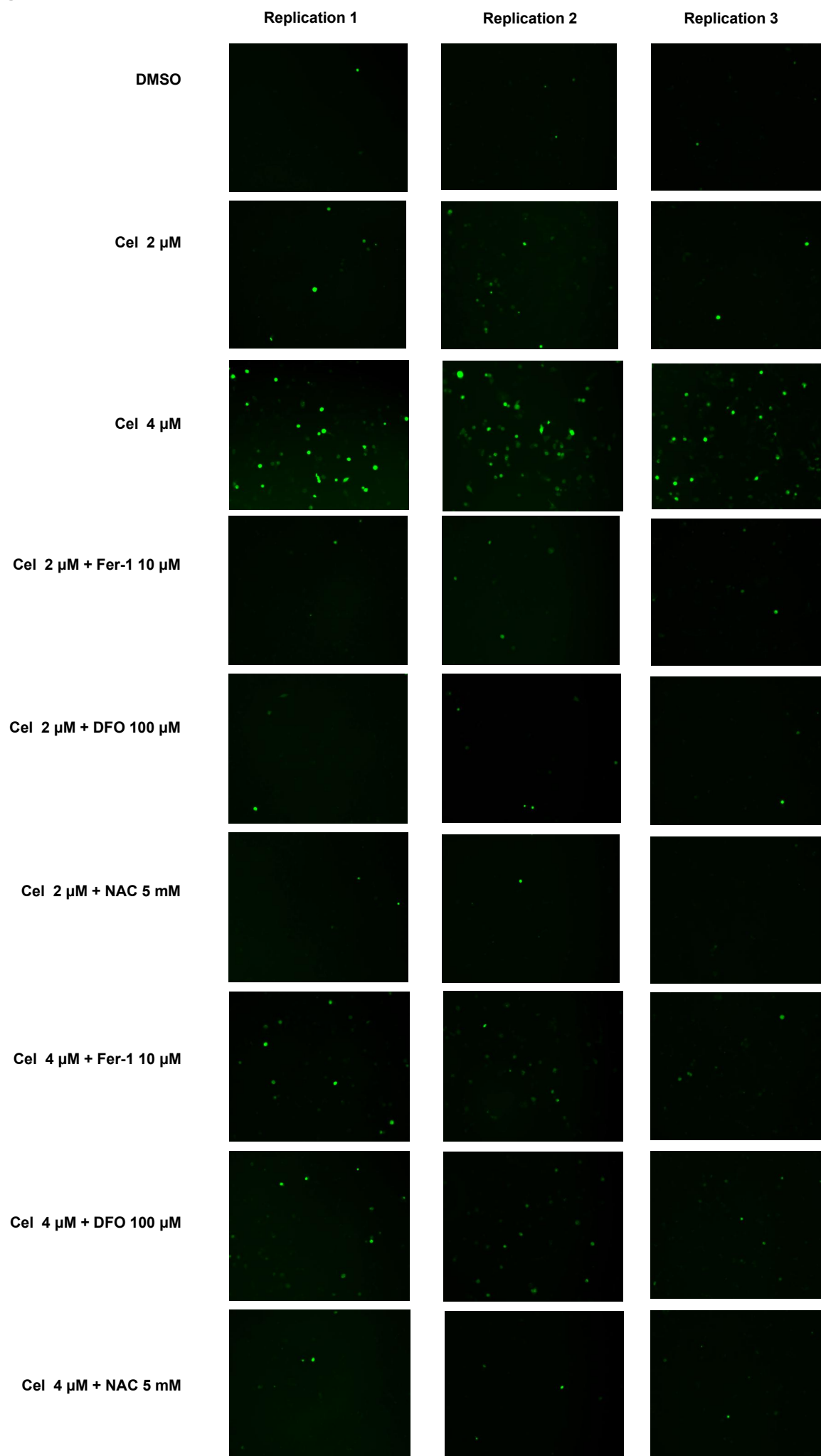


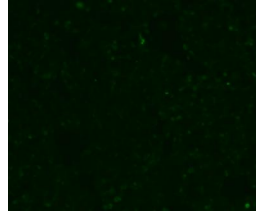
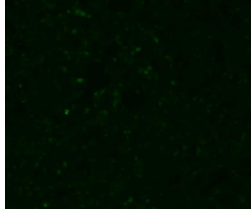
Figure S1. ROS raw data



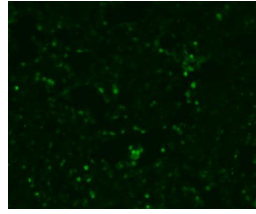
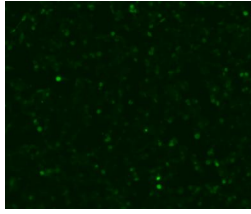
Replication 4

Replication 5

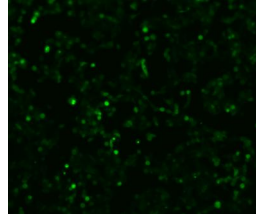
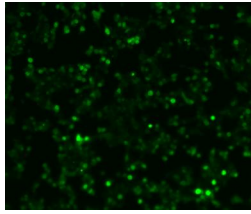
DMSO



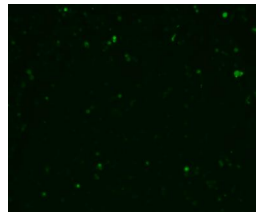
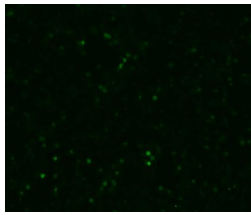
Cel 2 μ M



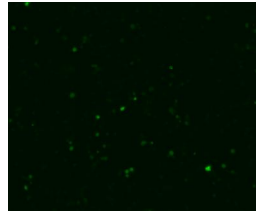
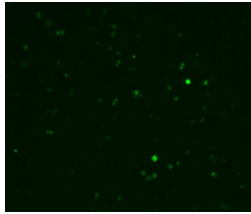
Cel 4 μ M



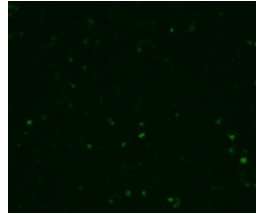
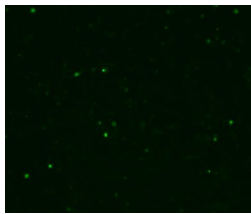
Cel 2 μ M + Fer-1 10 μ M



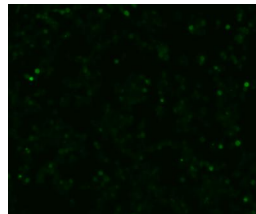
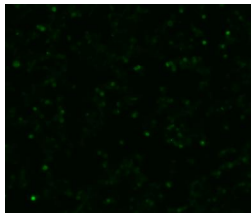
Cel 2 μ M + DFO 100 μ M



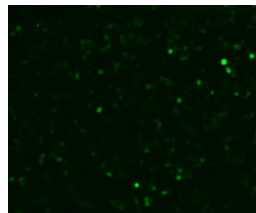
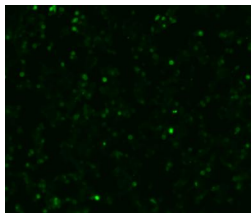
Cel 2 μ M + NAC 5 mM



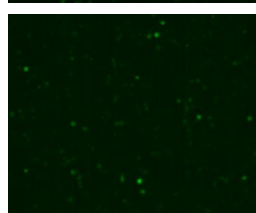
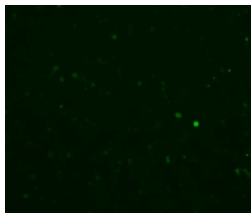
Cel 4 μ M + Fer-1 10 μ M

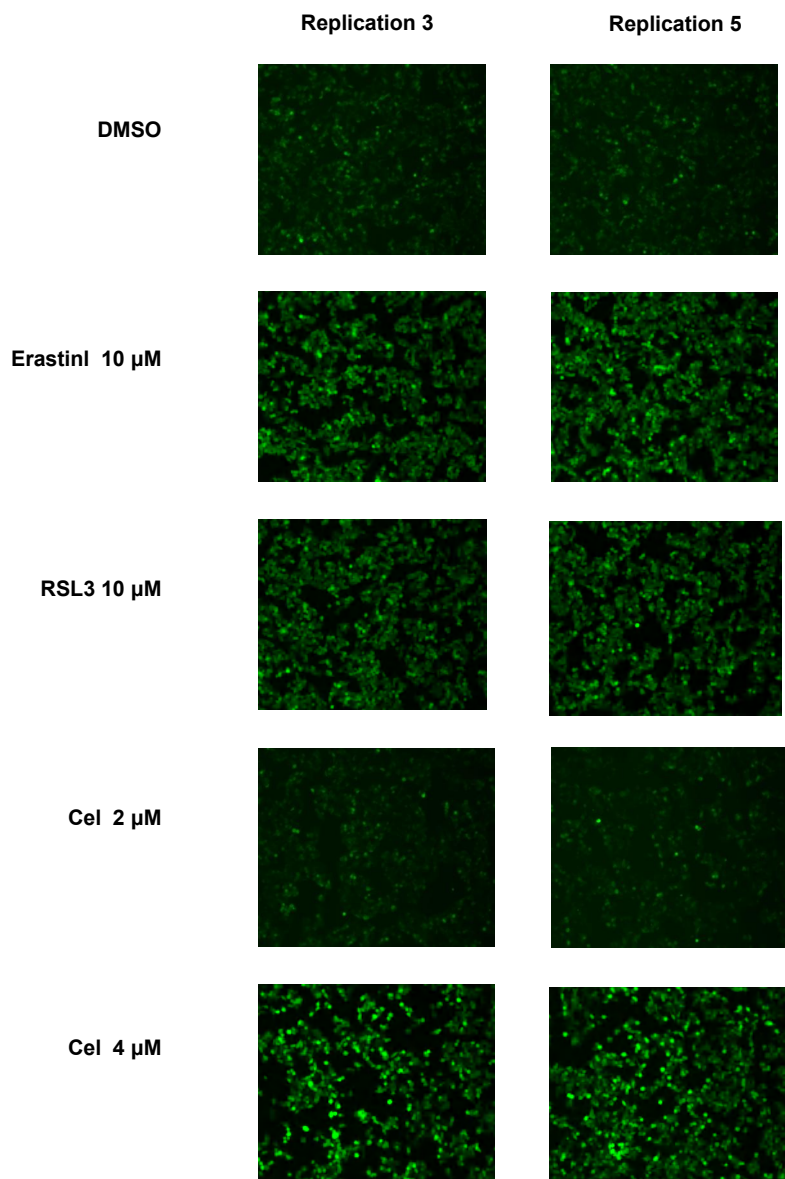
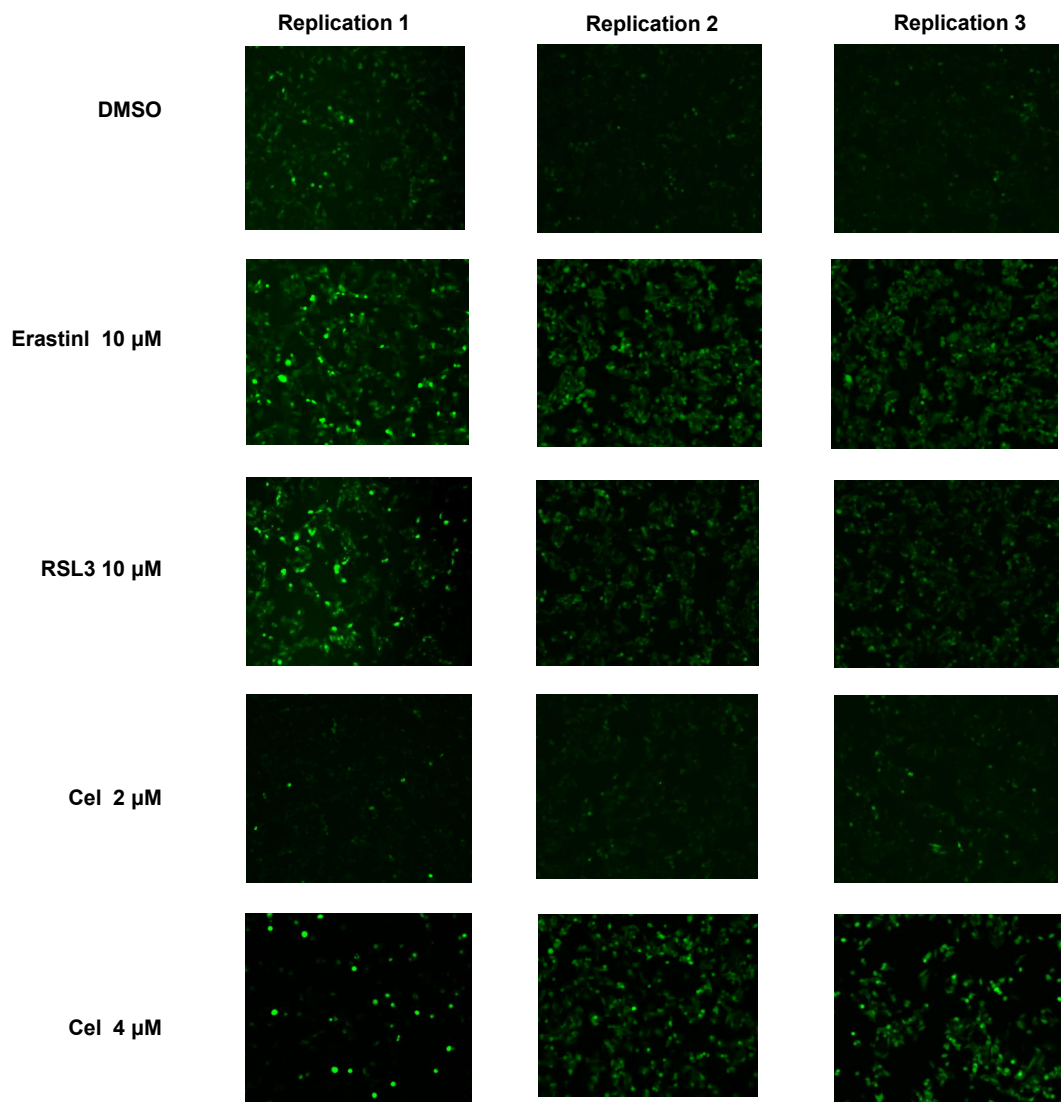


Cel 4 μ M + DFO 100 μ M



Cel 4 μ M + NAC 5 mM





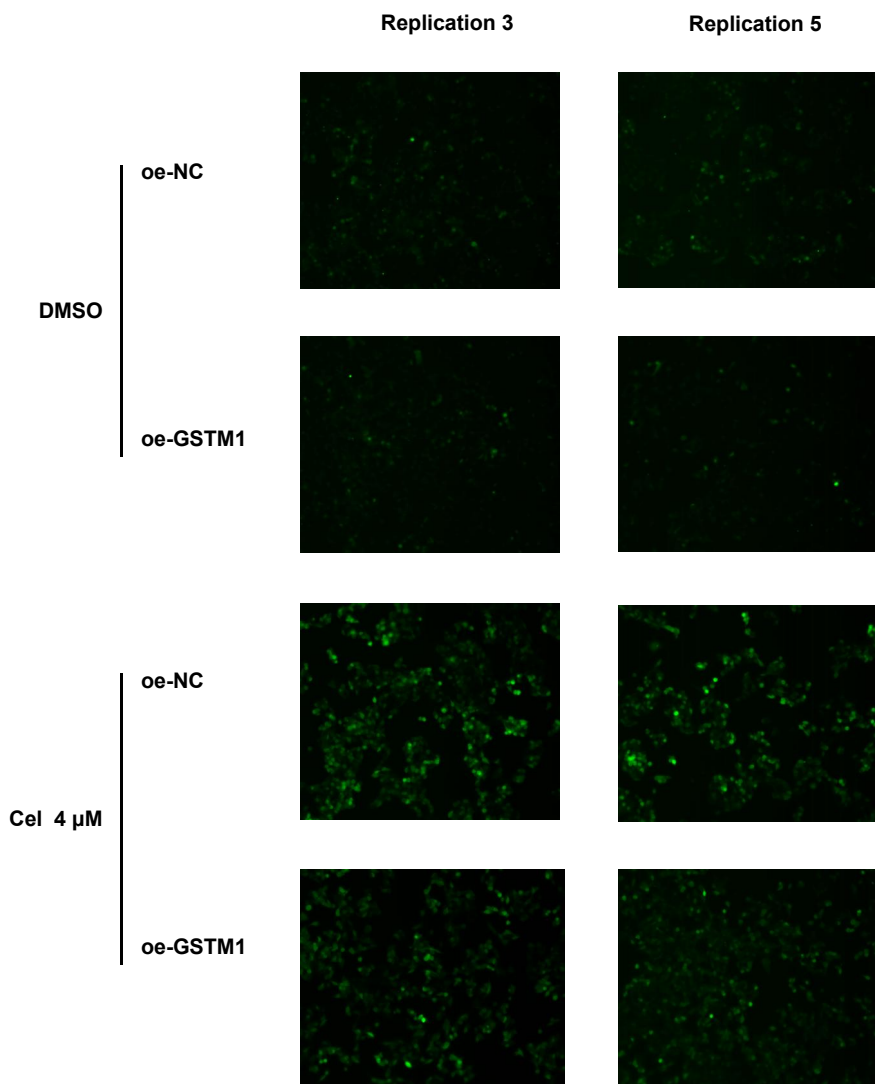
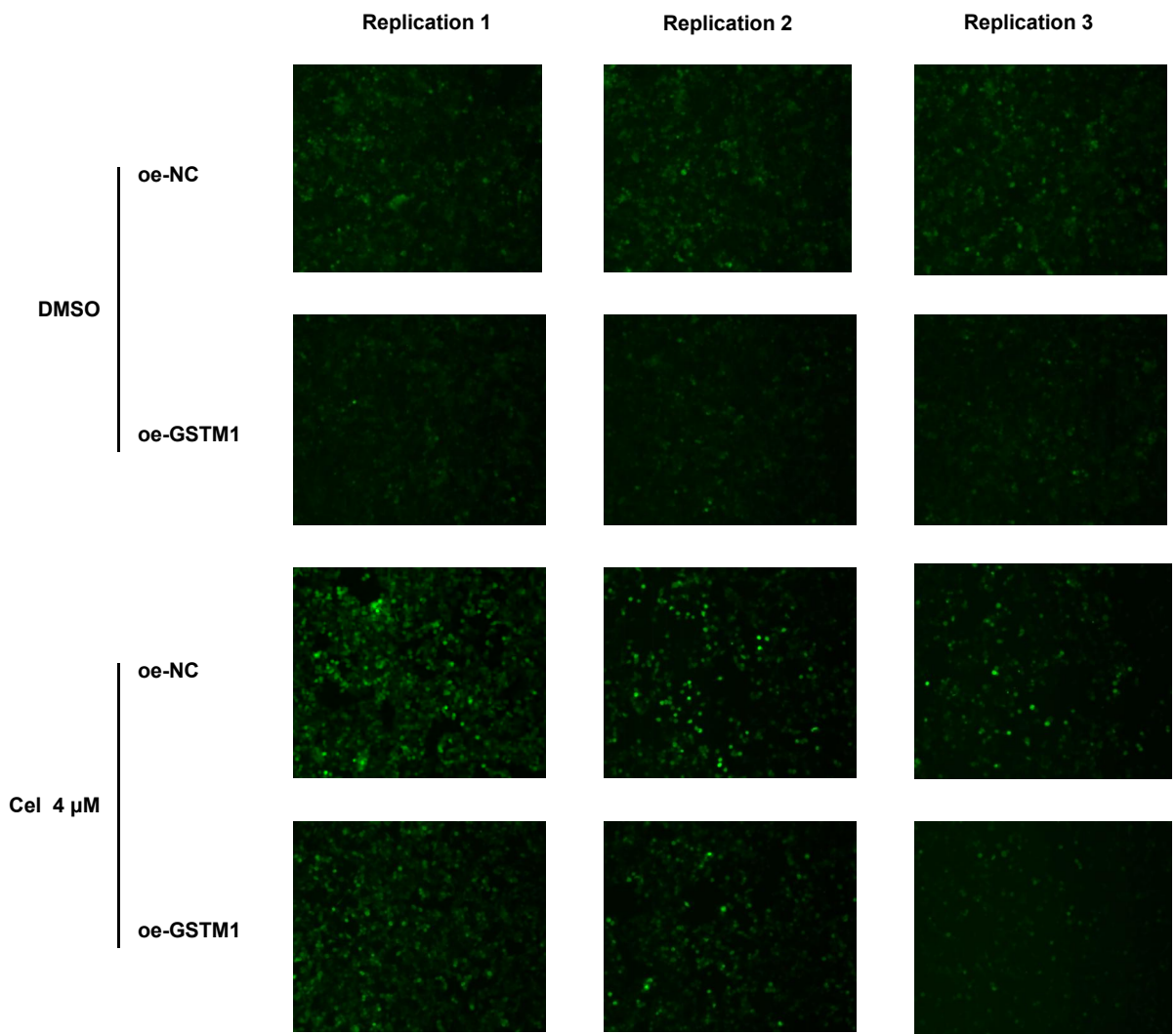


Figure S2. clone formation raw data

Replication 1

Replication 2

Replication 3

Replication 4

DMSO

Cel 2 μ M

Cel 4 μ M

Cel 2 μ M + Fer-1 10 μ M

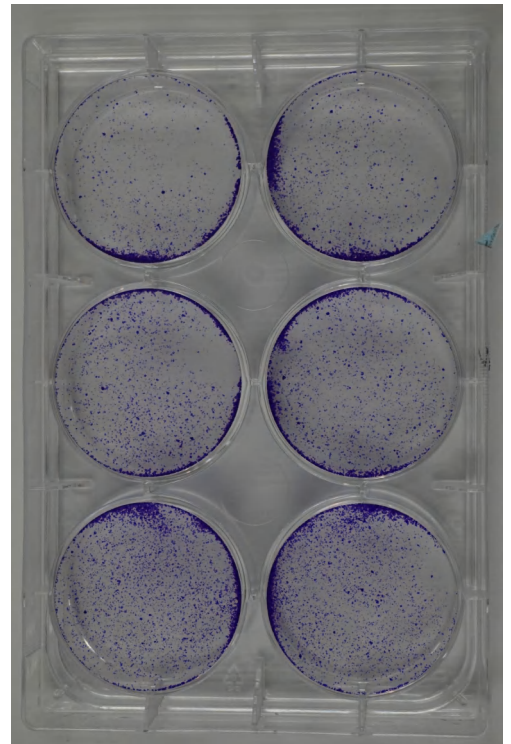
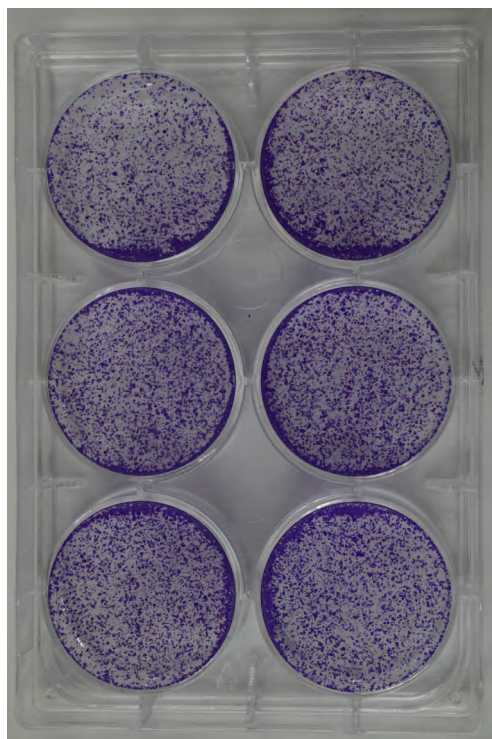
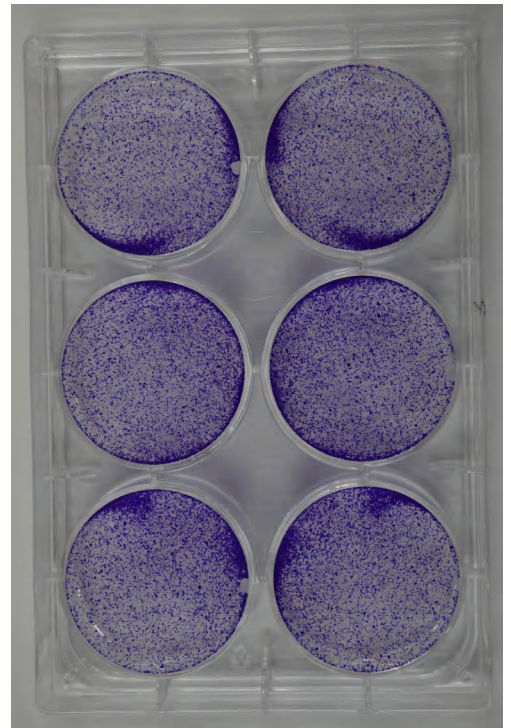
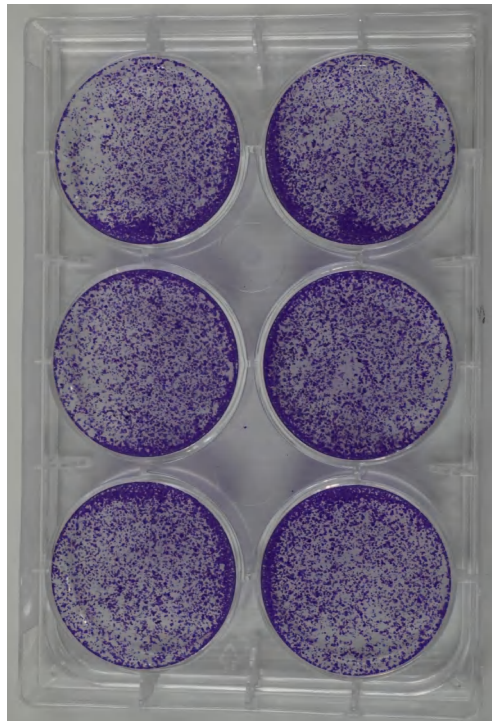
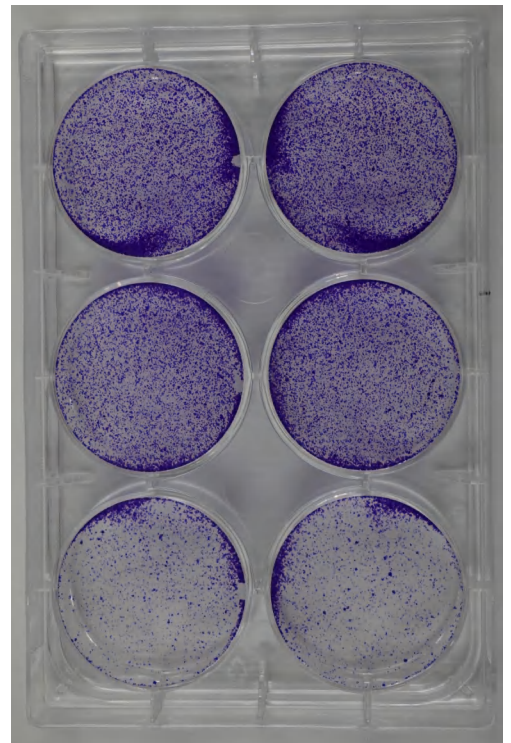
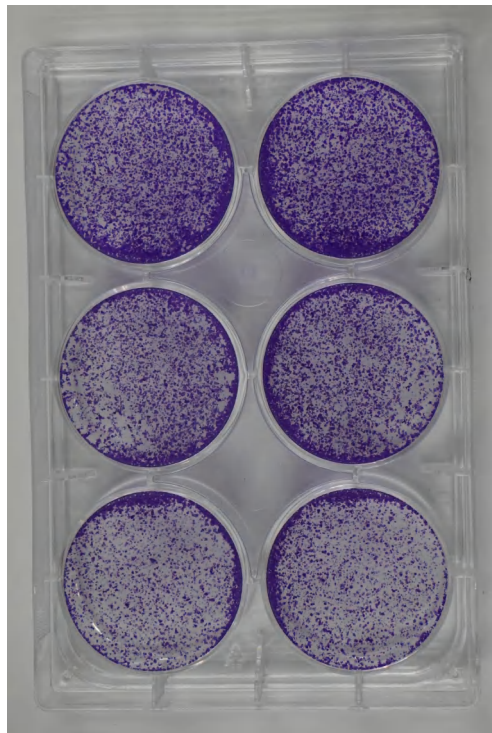
Cel 2 μ M + DFO 100 μ M

Cel 2 μ M + NAC 5 mM

Cel 4 μ M + Fer-1 10 μ M

Cel 4 μ M + DFO 100 μ M

Cel 4 μ M + NAC 5 mM



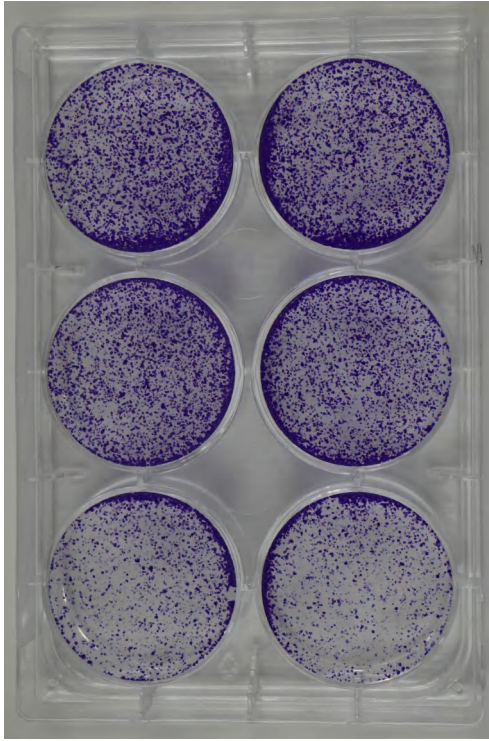
Replication 5

Replication 6

DMSO

Cel 2 μ M

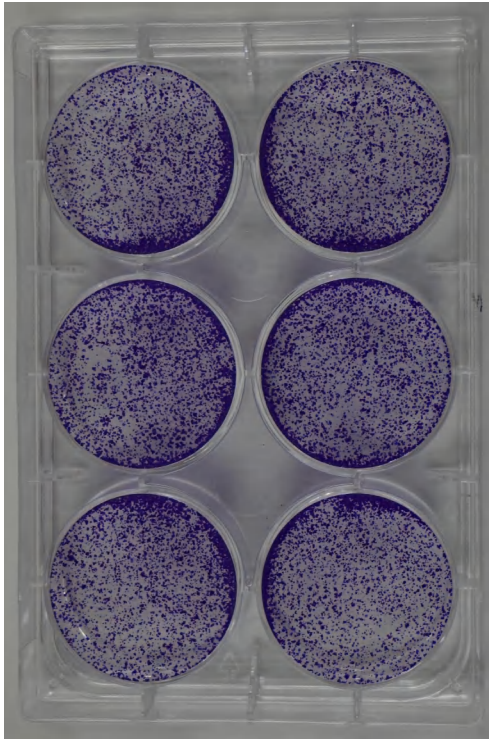
Cel 4 μ M



Cel 2 μ M + Fer-1 10 μ M

Cel 2 μ M + DFO 100 μ M

Cel 2 μ M + NAC 5 mM



Cel 4 μ M + Fer-1 10 μ M

Cel 4 μ M + DFO 100 μ M

Cel 4 μ M + NAC 5 mM

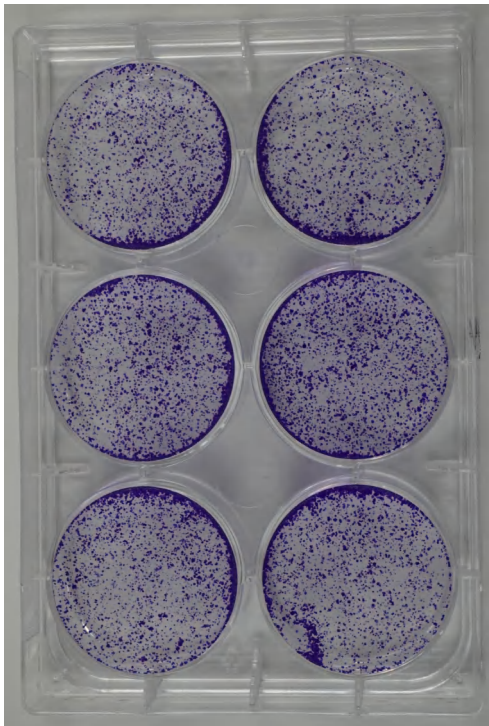
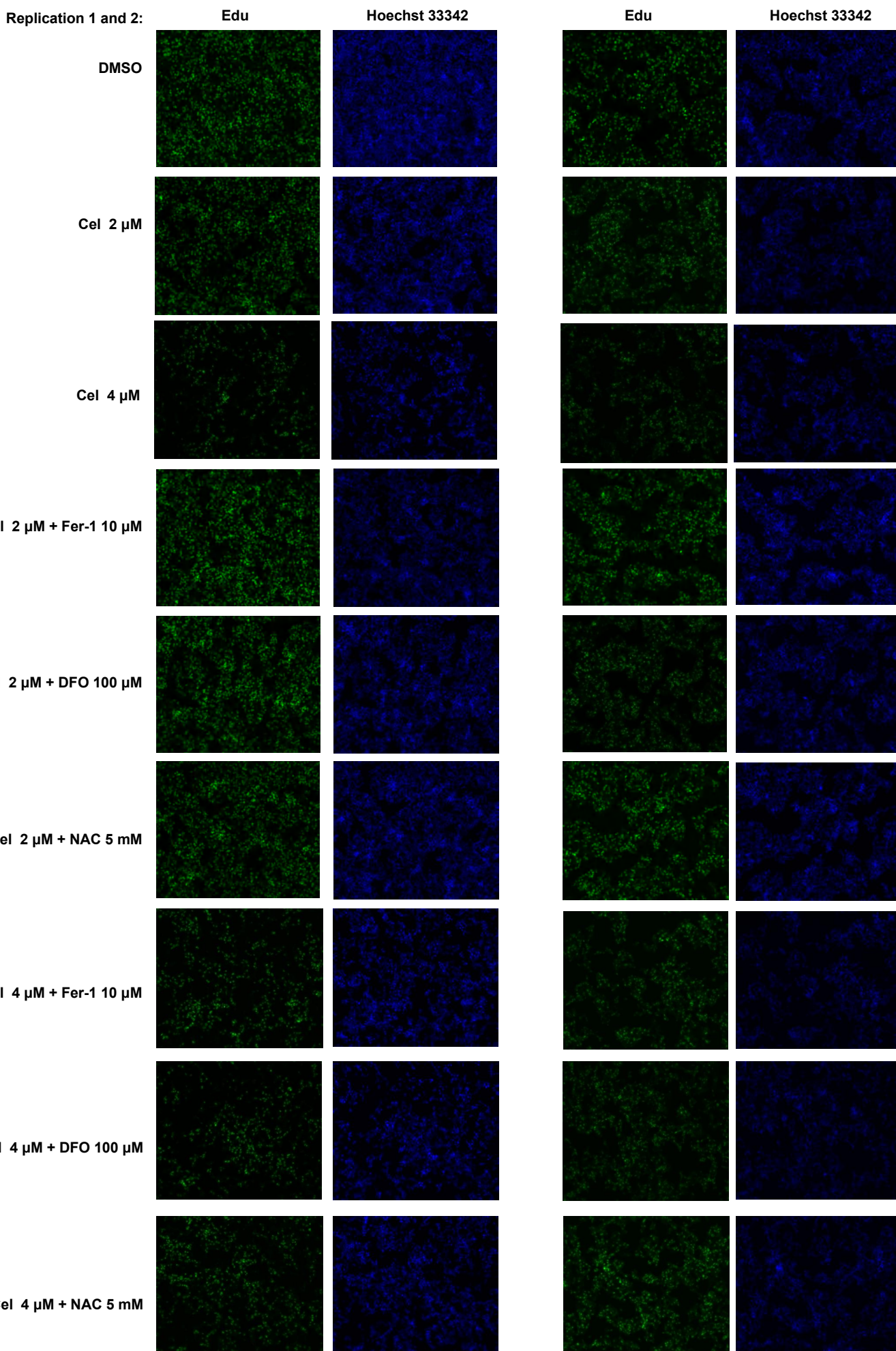


Figure S3. EDU raw data



Replication 3 and 4:

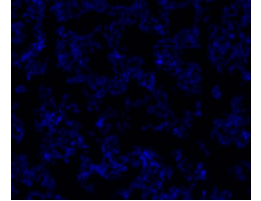
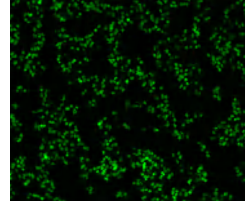
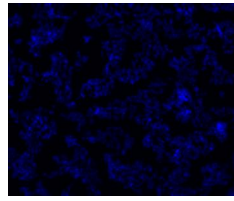
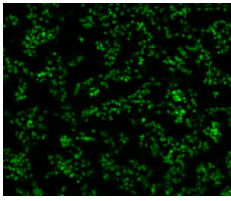
Edu

Hoechst 33342

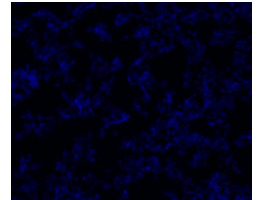
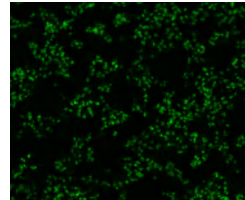
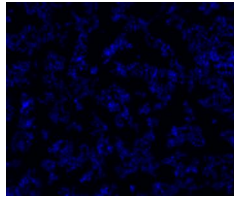
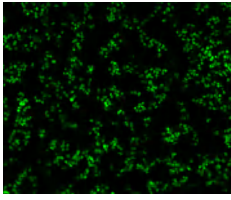
Edu

Hoechst 33342

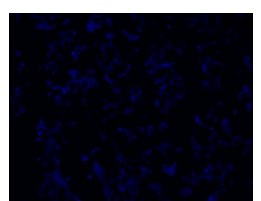
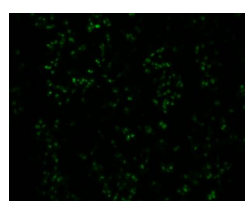
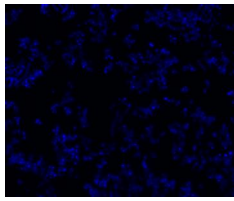
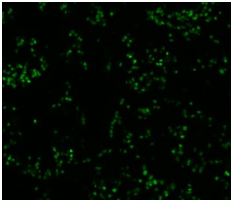
DMSO



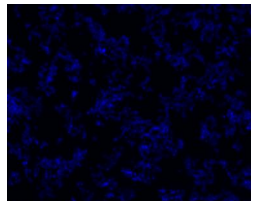
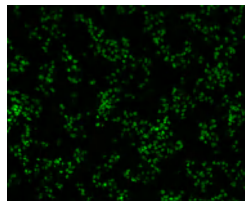
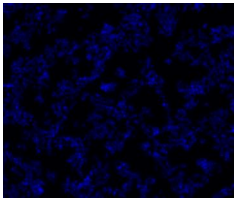
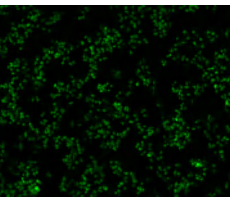
Cel 2 μ M



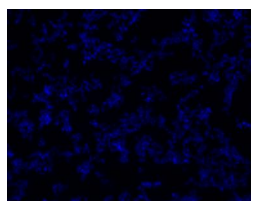
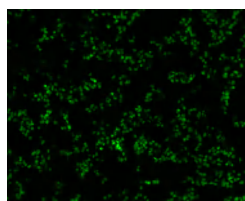
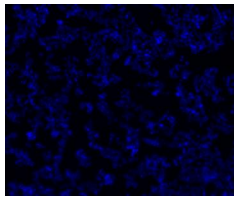
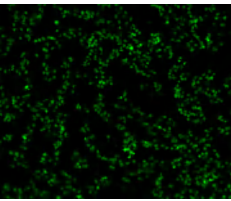
Cel 4 μ M



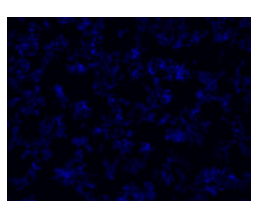
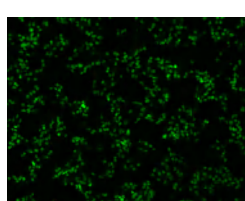
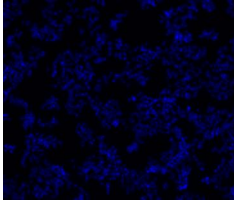
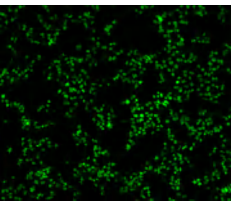
Cel 2 μ M + Fer-1 10 μ M



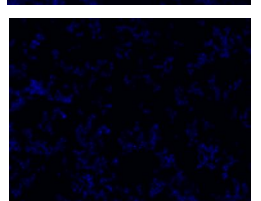
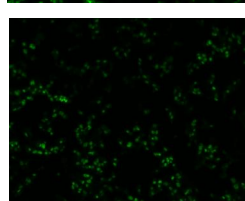
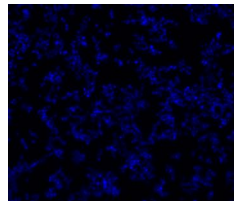
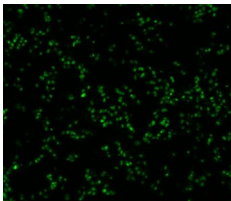
Cel 2 μ M + DFO 100 μ M



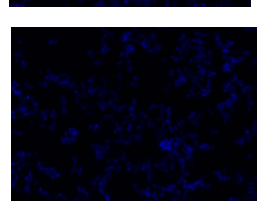
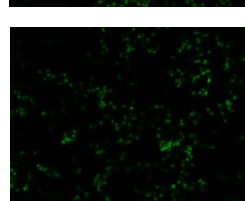
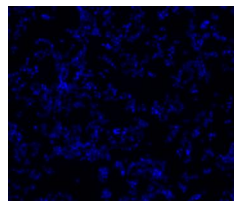
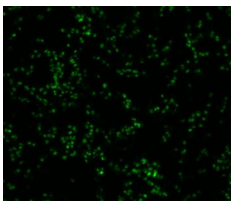
Cel 2 μ M + NAC 5 mM



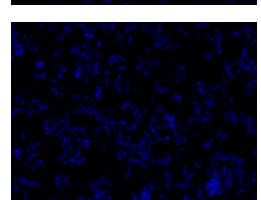
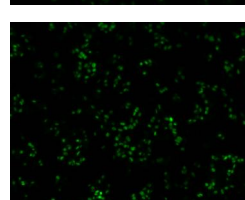
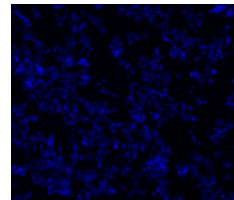
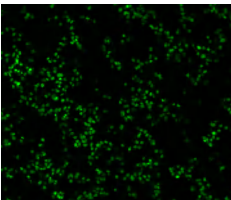
Cel 4 μ M + Fer-1 10 μ M



Cel 4 μ M + DFO 100 μ M



Cel 4 μ M + NAC 5 mM

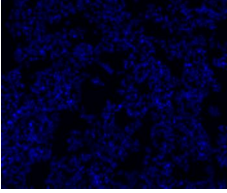
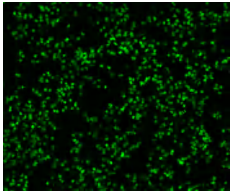


Replication 5:

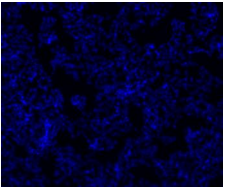
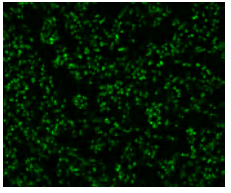
Edu

Hoechst 33342

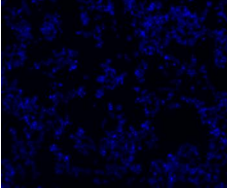
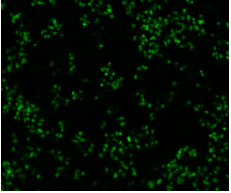
DMSO



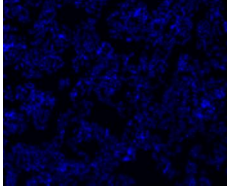
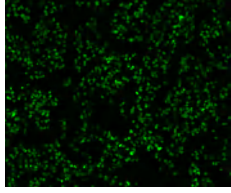
Cel 2 μ M



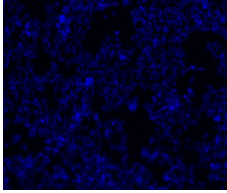
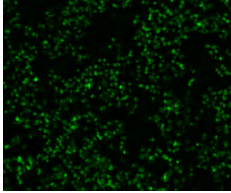
Cel 4 μ M



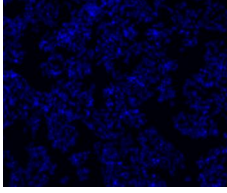
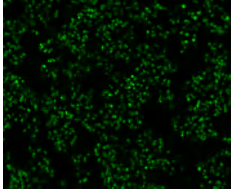
Cel 2 μ M + Fer-1 10 μ M



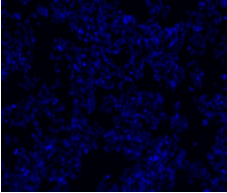
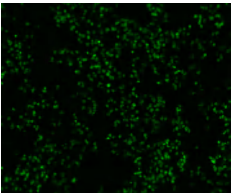
Cel 2 μ M + DFO 100 μ M



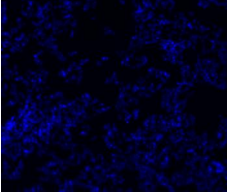
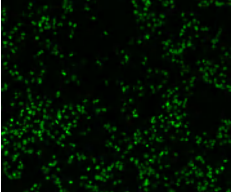
Cel 2 μ M + NAC 5 mM



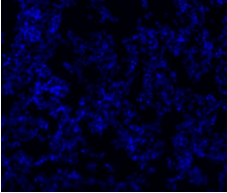
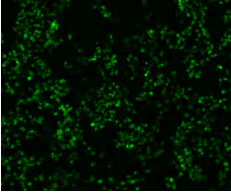
Cel 4 μ M + Fer-1 10 μ M



Cel 4 μ M + DFO 100 μ M



Cel 4 μ M + NAC 5 mM



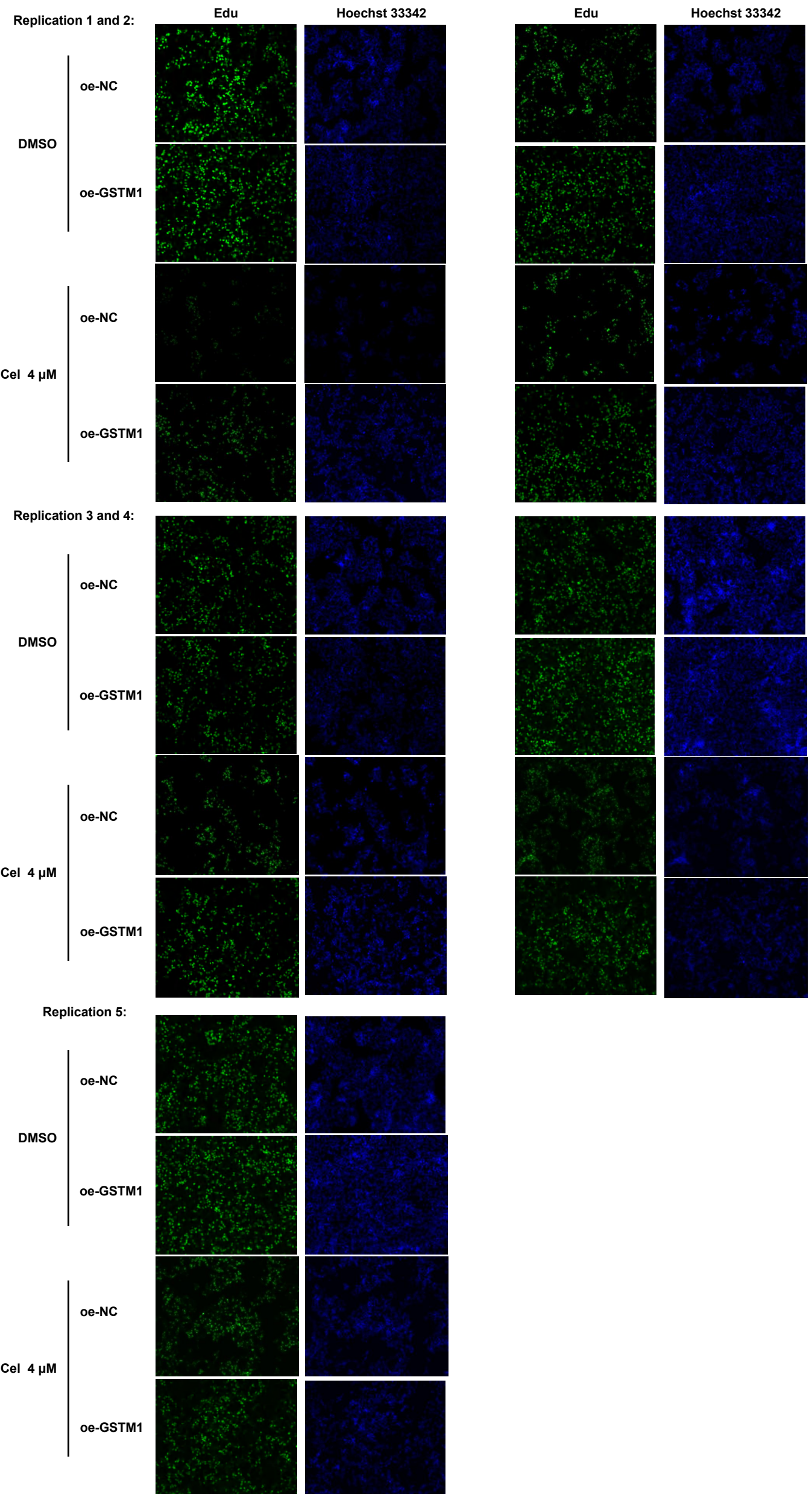
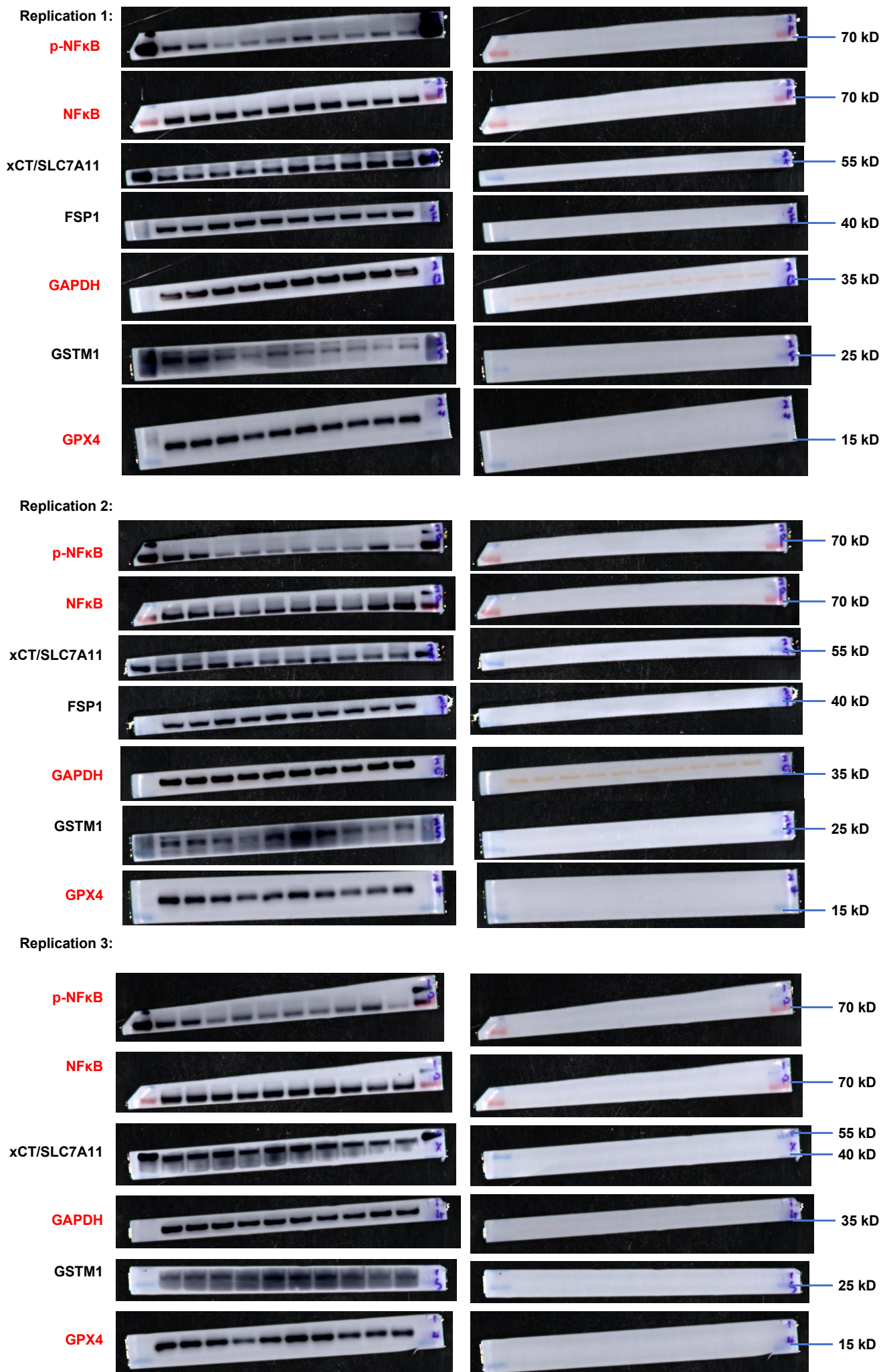


Figure S4. WB raw data

DMSO	-	+	-	-	-	-	-	-	-
Cel 2 μ M	-	-	+	-	+	+	+	-	-
Cel 4 μ M	-	-	-	+	-	-	-	+	+
Fer-1 10 μ M	-	-	-	-	+	-	-	+	-
DFO 100 μ M	-	-	-	-	-	+	-	-	+
NAC 5 mM	-	-	-	-	-	-	+	-	+

Marker: Thermo Fisher #26616



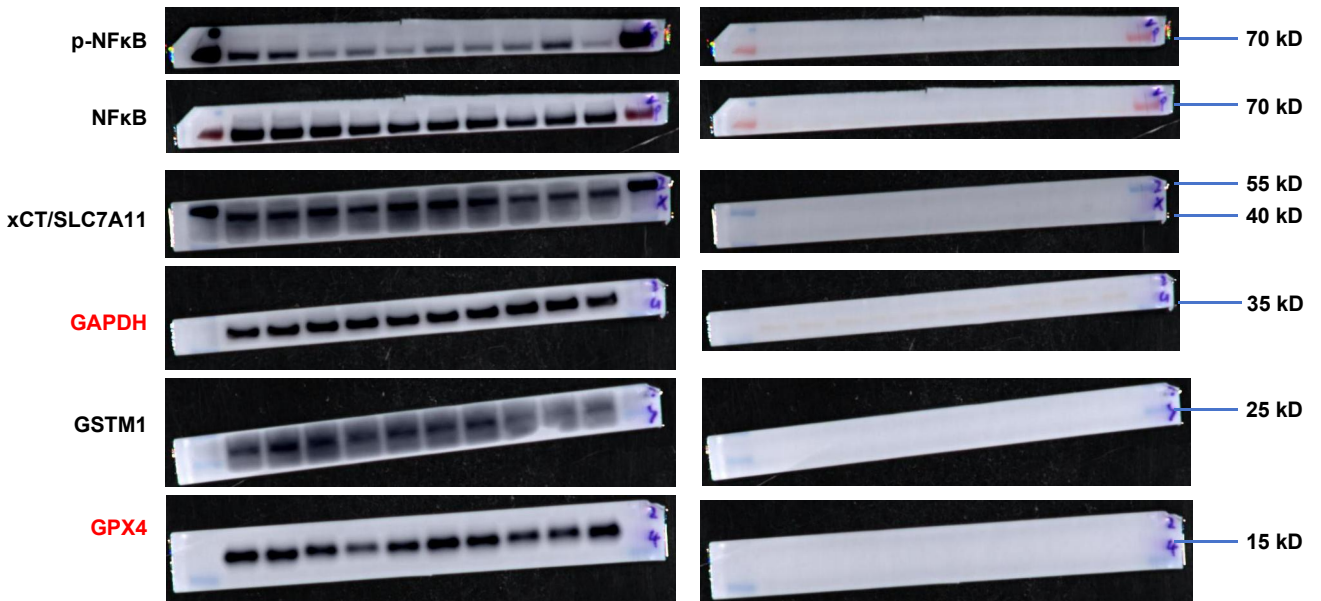
DMSO	-	+	-	-	-	-	-	-	-	-
Cel 2 μ M	-	-	+	-	+	+	+	-	-	-
Cel 4 μ M	-	-	-	+	-	-	-	+	+	+
Fer-1 10 μ M	-	-	-	-	+	-	-	+	-	-
DFO 100 μ M	-	-	-	-	-	+	-	-	+	-
NAC 5 mM	-	-	-	-	-	-	+	-	-	+

Marker: Thermo Fisher #26616

Replication 4:



Replication 5:

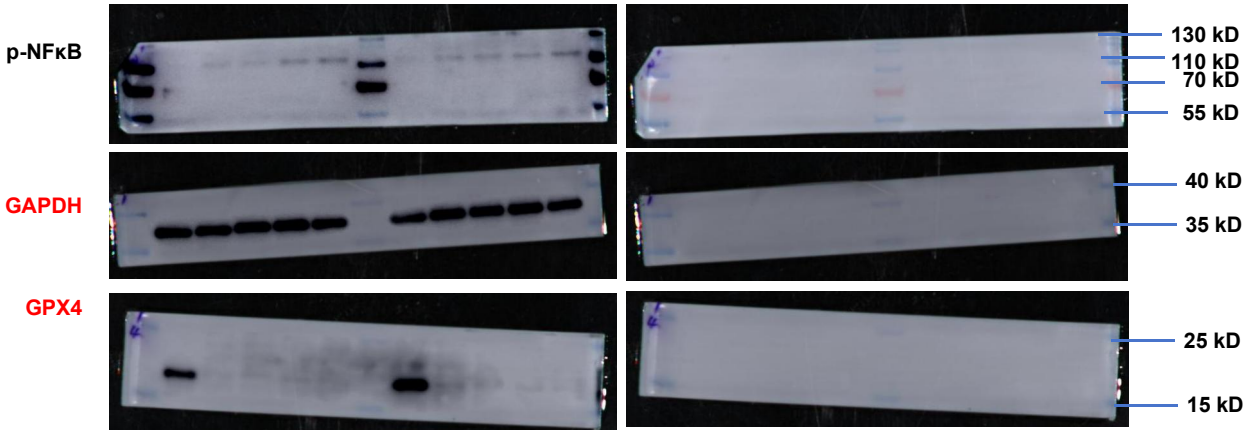


DMSO	+	-	-	-	-
Erastin 10 μ M	-	+	-	-	-
RSL3 10 μ M	-	-	+	-	-
Cel 2 μ M	-	-	-	+	-
Cel 4 μ M	-	-	-	-	+

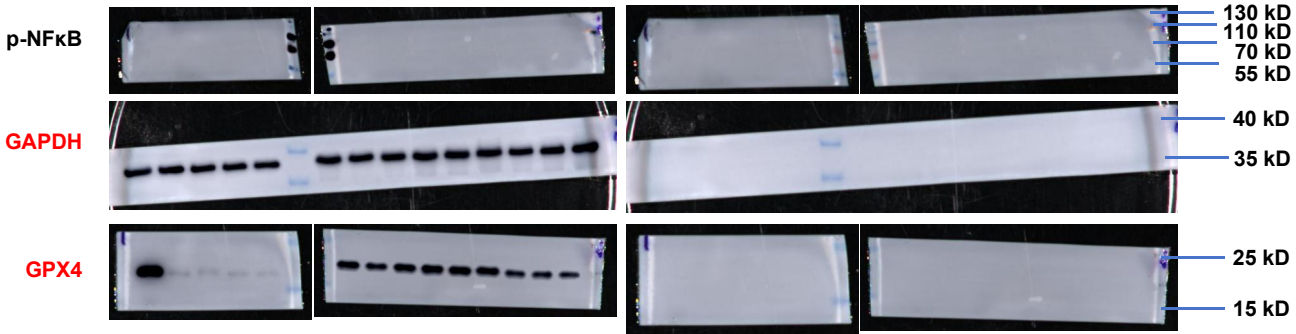
Marker: Thermo Fisher #26616

Replication 1:

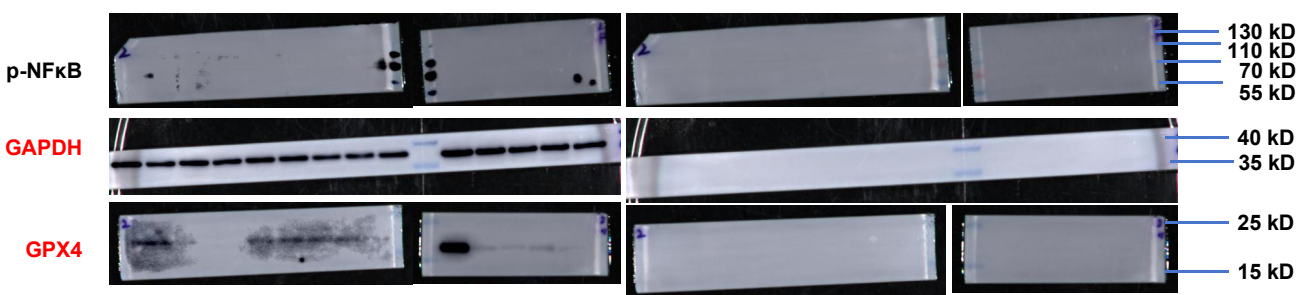
Replication 2:



Replication 3:

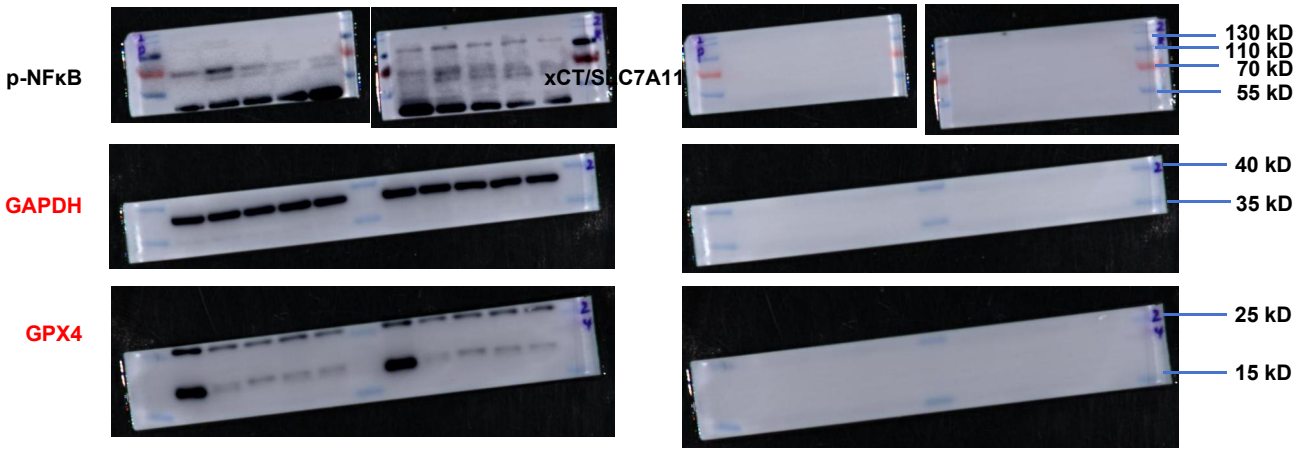


Replication 4:



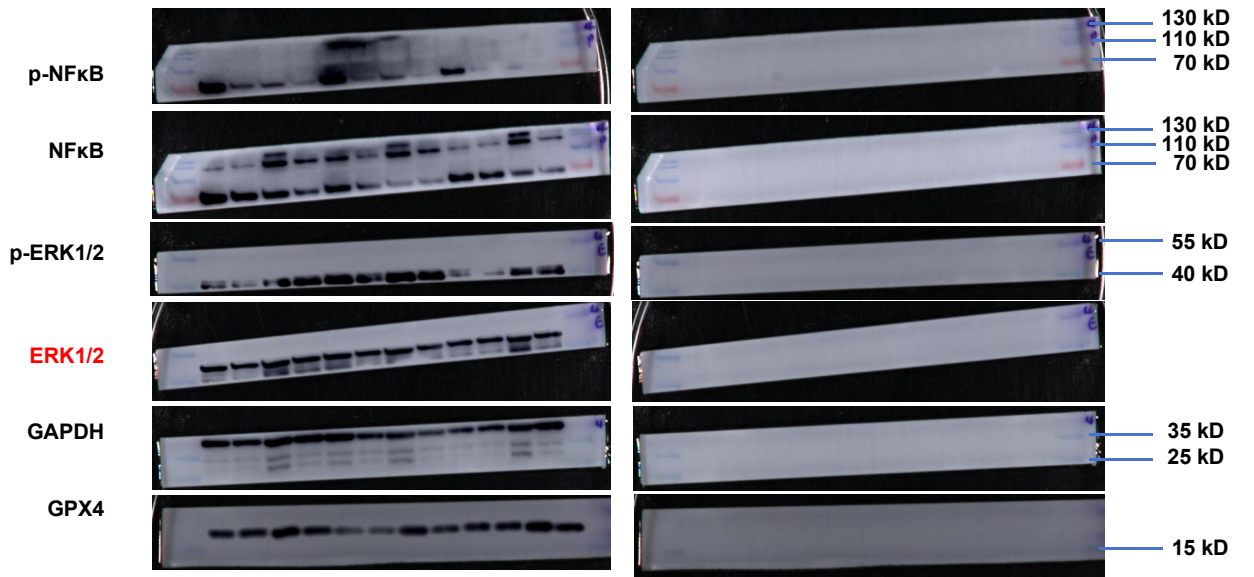
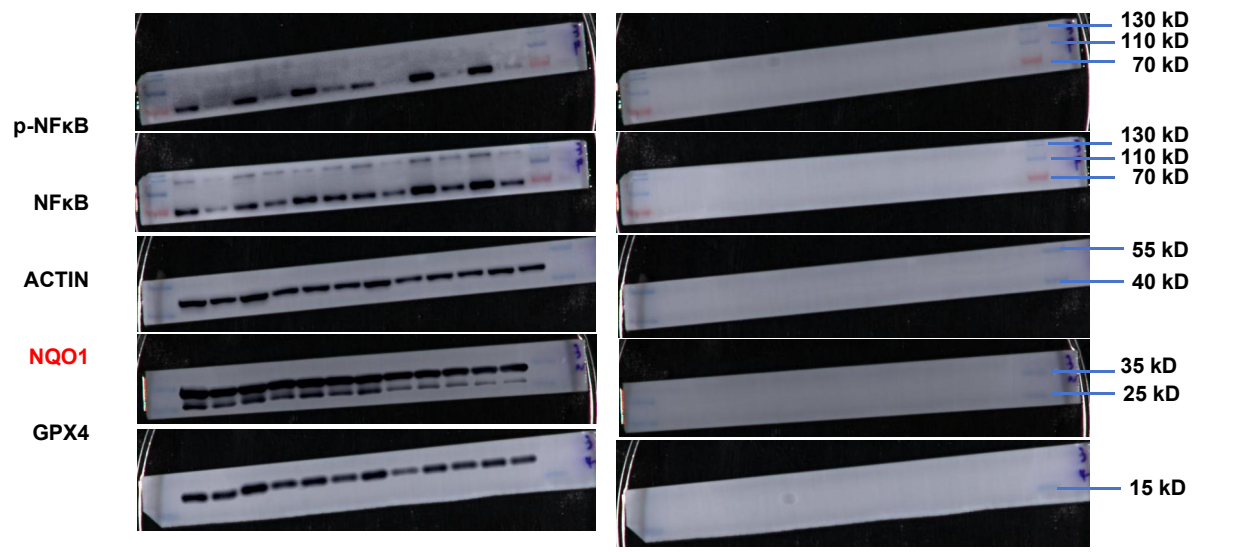
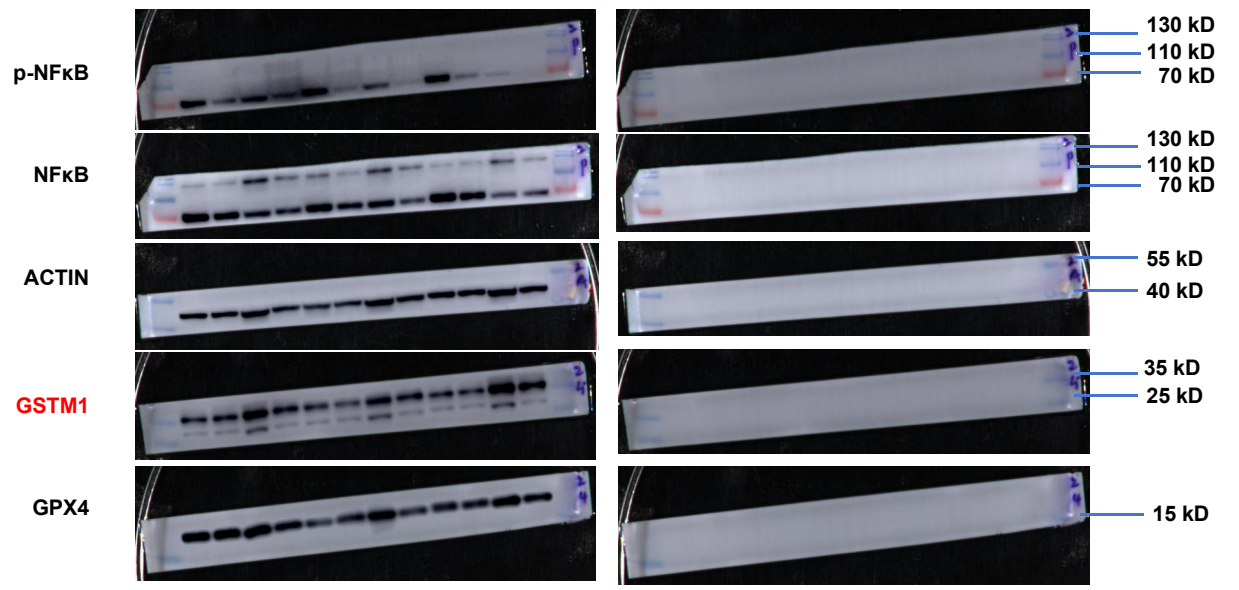
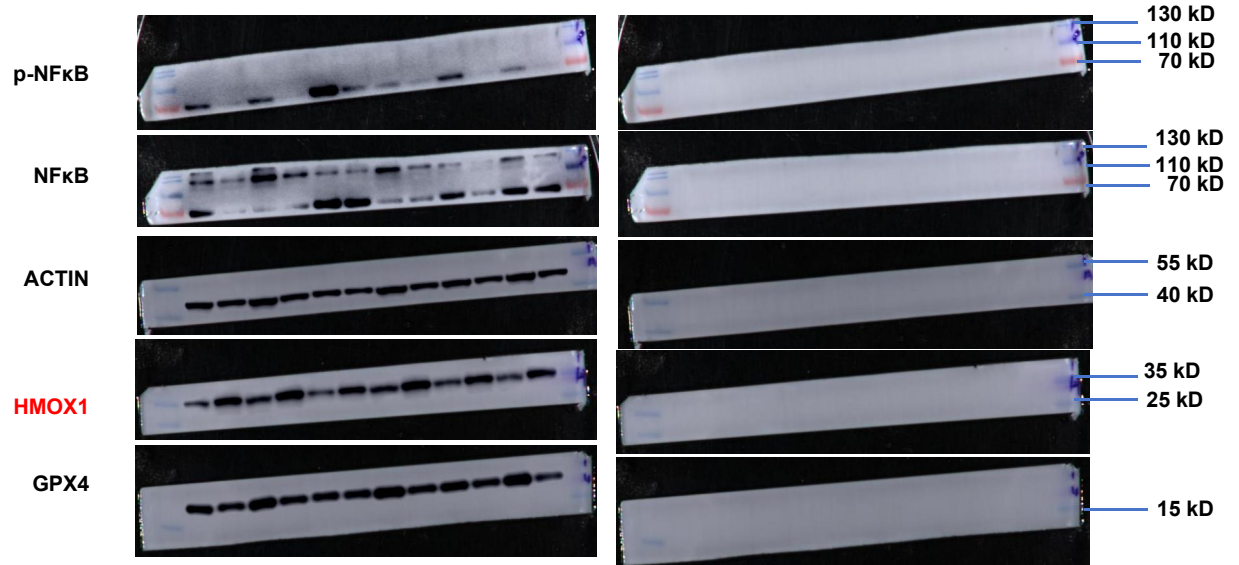
Replication 5:

Replication 6:



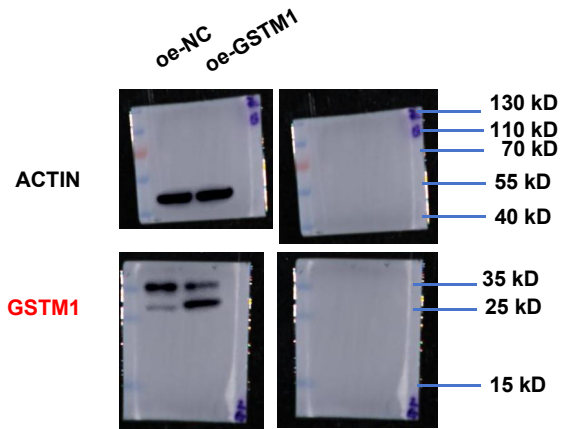
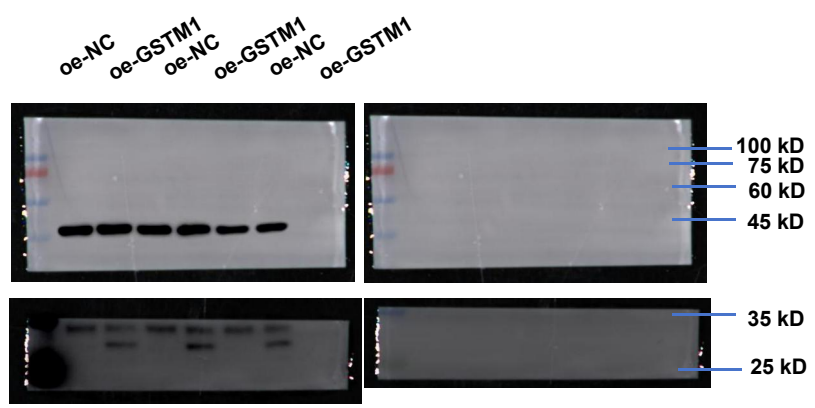
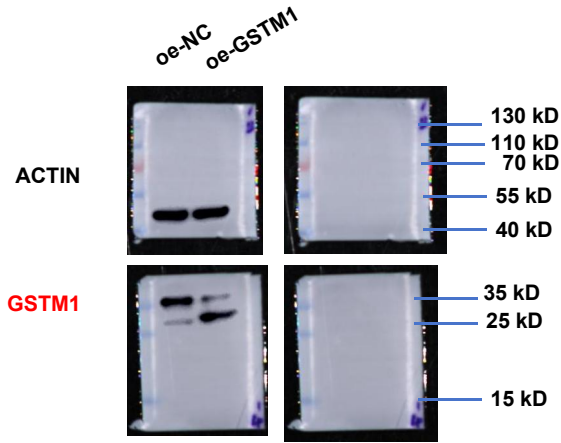
DMSO + - + - + - + - + - + -
 Cel 4 μ M - + - + - + - + - + - +

Marker: Thermo Fisher #26616

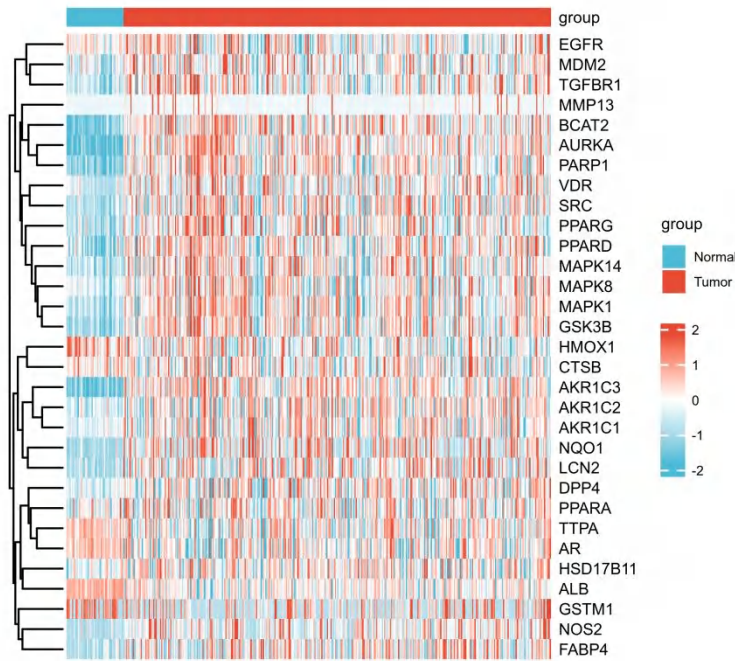


Marker: Thermo Fisher #26616

Marker: ABclonal #RM19001



Supplementary File 1. Differential expression heat maps of 31 candidate genes in HCC and adjacent tissues and OS survival analysis forest maps of these genes



Characteristics	Total(N)	HR (95% CI)		P value
VDR	373	1.193 (1.006 - 1.416)		0.043
PPARD	373	1.295 (1.072 - 1.566)		0.007
NOS2	373	0.974 (0.750 - 1.266)		0.845
MMP13	373	1.221 (0.888 - 1.678)		0.219
PPARA	373	1.002 (0.831 - 1.209)		0.98
MDM2	373	1.116 (0.889 - 1.400)		0.345
DPP4	373	1.014 (0.888 - 1.158)		0.833
HSD17B11	373	1.031 (0.850 - 1.250)		0.758
MAPK8	373	1.306 (0.961 - 1.775)		0.088
BCAT2	373	1.137 (0.958 - 1.349)		0.142
PPARG	373	1.268 (1.074 - 1.498)		0.005
AKR1C2	373	1.050 (0.955 - 1.153)		0.313
AURKA	373	1.282 (1.112 - 1.477)		< 0.001
SRC	373	1.206 (1.055 - 1.378)		0.006
MAPK14	373	1.167 (0.900 - 1.513)		0.245
CTSB	373	1.310 (1.060 - 1.620)		0.013
AKR1C1	373	1.087 (0.974 - 1.213)		0.138
FABP4	373	0.901 (0.816 - 0.995)		0.039
TGFB1	373	1.210 (0.976 - 1.500)		0.082
LCN2	373	1.013 (0.958 - 1.072)		0.641
AKR1C3	373	1.323 (1.119 - 1.564)		0.001
ALB	373	0.926 (0.858 - 1.000)		0.049
TTPA	373	0.927 (0.845 - 1.018)		0.112
AR	373	0.872 (0.783 - 0.972)		0.014
GSK3B	373	1.577 (1.149 - 2.163)		0.005
PARP1	373	1.310 (1.051 - 1.633)		0.017

Supplementary File 2. The Proof of Ethical Exemption

证 明

我院中心实验室研究生蔡邦兰的开题项目“网络药理学结合体外实验验证雷公藤红素通过铁死亡治疗肝细胞癌的作用”。该课题计划自2021年11月28日至2024年6月30日执行。

经审查，该课题不涉及到人的临床研究，免于伦理审查。

特此证明！

上海市浦东新区公利医院伦理委员会
医学伦理委员会
二〇二四年二月四日

Given that our Proof of Ethical Exemption is in Chinese, we have translated it for readability, as follows.

Certificate

This is to certify that the research project titled "Integrating network pharmacology with in vitro experiments to validate the efficacy of celastrol against hepatocellular carcinoma through ferroptosis," initiated by graduate student Banglan Cai in our Shanghai Health Commission Key Lab of Artificial Intelligence (AI)-Based Management of Inflammation and Chronic Diseases, is scheduled to be executed from November 28, 2021, to June 30, 2024. Upon review, it has been determined that the project does not involve clinical research on human subjects and is thereby exempt from ethical review.

Ethics Committee,
Pudong Gongli Hospital,
Shanghai, China
2024.2.4

Supplementary Table S1

Antibody	Company	Catalog number
p-NFκB p65	Cell signaling technology	# 3033S
NFκB	Cell signaling technology	# 8242S
GPX4	Cell signaling technology	#52455S
xCT/SLC7A11	Proteintech	26864-1-AP
FSP1	Cell signaling technology	#24972S
GSTM1	Sangon Biotech	D126782-0025
HMOX1	Sangon Biotech	D220756-0025
NQO1	Sangon Biotech	D161049-0025
Phospho-p44/42 MAPK (Erk1/2)	Cell signaling technology	#9101S
p44/42 MAPK (Erk1/2)	Cell signaling technology	#9102S
GAPDH	Sangon Biotech	D190090-0200

Supplementary Table S2

Ligand Interactions Report

Thu Apr 20 09:27:03 2023 (MOE 2022.02)

7BEU: TRANSFERASE / 7BEU Browser

Ligand			Receptor			Interaction	Distance	E (kcal/mol)
0	1	OD1	ASN	58	(B)	H-donor	3.15	-1.3
0	3	NF2	HIS	107	(B)	H-donor	3.03	-2.6
0	1	NE1	TRP	45	(B)	H-acceptor	3.15	-0.5
0	2	NE1	TRP	45	(B)	H-acceptor	3.30	-1.4
0	2	NZ	LYS	49	(B)	H-acceptor	3.01	-2.5

Supplementary materials' legends

Supplementary fig.1. ROS raw data.

Supplementary fig.2. Clone formation raw data.

Supplementary fig.3. Edu raw data.

Supplementary fig.4. WB raw data.

Supplementary file.1. Differential expression heat maps of 31 candidate genes in HCC and adjacent tissues and OS survival analysis forest maps of these genes

Supplementary file.2. The Proof of Ethical Exemption in Chinese and English.

Supplementary Table.1. Antibodies information used in Western blot.

Supplementary Table.2. Molecular docking results between celastrol and GSTM1.