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Supplemental Information

Casein kinase 2 activity is a host restriction factor for AAV transduction

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Table S1. Antibodies used in immunofluorescent staining

target	clone	host	supplier
AAV8/9 intact capsid	ADK8/9	mouse	Progen
ATM	2C1	mouse	ThermoFisher Scientific
ATM/ATR phospho-substrate	2851S	rabbit	Cell Signaling
α-SMA	Polyclonal (ab5694)	rabbit	Abcam
BIP	polyclonal	rabbit	Proteintech
CANX	polyclonal	rabbit	Proteintech
CTNNT	13-11	mouse	ThermoFisher Scientific
CAV1	polyclonal	rabbit	Proteintech
CK2 (A1)	polyclonal	rabbit	Proteintech
COL1A1	Polyclonal (ab34710)	rabbit	Abcam
DDR2	AF2538	goat	R&D
MRE11	12D7	mouse	Abcam
NBS1	D6J5I	rabbit	Cell Signaling
p53	1C12	mouse	Cell Signaling
pATM (S1981)	polyclonal	rabbit	R&D systems
TCF21	polyclonal ab32981	rabbit	Abcam
PIH1D1	polyclonal	rabbit	Proteintech
anti-mouse IgG AlexaFluor-488	polyclonal	donkey	ThermoFisher Scientific
anti-mouse IgG AlexaFluor-568	polyclonal	donkey	ThermoFisher Scientific
anti-mouse IgG AlexaFluor-647	polyclonal	donkey	ThermoFisher Scientific
anti-rabbit IgG AlexaFluor-488	polyclonal	donkey	ThermoFisher Scientific
anti-rabbit IgG AlexaFluor-568	polyclonal	donkey	ThermoFisher Scientific
anti-rabbit IgG AlexaFluor-594	polyclonal	goat	ThermoFisher Scientific
anti-goat IgG AlexaFluor-488	polyclonal	donkey	ThermoFisher Scientific

Table S2. List of compounds used for AAV transduction modulation with concentrations used for iPSC-CMs and iPSC-CFs in the study. When applicable, concentrations used for iPSC-CFs correspond to those for HAECs.

compound	**target**	**concentration for iPSC-CMs**	**concentration for iPSC-CFs**	**supplier**
KU-55933	ATM	50 μM	20 μM	MedChemExpress
Selisistat	SIRT1	50 μM	20 μM	MedChemExpress
Silmitasertib	CK2	100 μM	20 μM	MedChemExpress
SR-3029	CK1	250 nM	250 nM	MedChemExpress
HA15	BIP	20 μM	20 μM	MedChemExpress

Table S3. List of primers with annealing temperatures specified for each pair

Target	Primers (5'--> 3'); forward and reverse	Ta [ºC]
ATF4	5'-TGCCCTGTTCCCGATTCTCT-3'	60
	5'-AGGGCATCCAAGTCGAACTC-3'	
ATM	5'-GGTTAGCAGAAACGTGCTTAGA-3'	60
	5'-CTTCATTCCGTCTCTCTTCATCAT-3'	
CAV1	5'-TACGTAGACTCGGAGGGACA-3'	60
	5'-CGGTGTGGACGTAGATGGAA-3'	
BIP	5'-TGGAGGTGGGCAAACAAAGA-3'	60
	5'-AGACACATCGAAGGTTCCGC-3'	
CHOP	5'-CCAGCCACTCCCCATTATCC-3'	60
	5'-GCAGGGTCAAGAGTGGTGAA-3'	
EEF2	5'-CGAGATCAAGGACAGTGTGG-3'	60
	5'-AAGGTAGATGGGCTCCATGA-3'	
GADD34	5'-TTATGCAAGACGCTGCACGA-3'	60
	5'-AGGAAGAAAGGGTGGGCATC-3'	
MATR3	5'-AGTAGGGCATCCTTCACCCA-3'	60
	5'-CGTGCAGTACCCTGGTTCAT-3'	
THRAP3	5'-GCGGGTGTTCTTTTGGGGTA-3'	60
	5'-GACCTTCTCTTTGGGGACCG-3'	
NCOA5	5'-AGAGACGCTTTGATGCCGAA-3'	60
	5'-AATCGGGCCAGGCAGAGAGT-3'	
TBP	5'-TATAATCCCAAGCGGTTTGC-3'	60
	5'-GCTGGAAAACCCAACTTCTG-3'	

Table S4. Antibodies used for western blotting/ co-IP

Antibody (host)	supplier	dilution	blocking buffer
CAV1 (rabbit)	Proteintech	1: 1000	5% milk in TBS
CK2 (A1)	Proteintech	1:1000	5% milk in TBS
α-tubulin (mouse)	SigmaAldrich	1: 1000	5% milk in TBS
eIF2α (rabbit)	Cell Signaling	1:1000	5% milk in TBS
p-eIF2α (rabbit)	Cell Signaling	1:1000	5% BSA in TBS
ATM/ATR phospho-substrate (rabbit)	Cell Signaling	1: 1000	5% BSA in TBS
AAV VP1/VP2/VP3 (rabbit)	Progen	1:500	5% milk in TBS
PIH1D1	Proteintech	1:1000	5% milk in TBS
Mre11	Abcam	1:500	5% milk in TBS
Goat anti-mouse IgG	BD	1: 10 000	As for primary antibody
Goat anti-rabbit IgG	Cell Signaling	1: 10 000	As for primary antibody

Table S5. Putative phosphorylation motifs in AAV9 VP1 protein based on NetPhos3.1 database search.

Residue	Context	Score	Kinase
138 T	EAAKTAPGK	0.648	PKC
149 S	PVEQSPQEP	0.600	cdk5
181 S	GDTESVPDP	0.613	PKC
242 T	RVITTSTRT	0.682	PKC
244 T	ITTSTRTWA	0.611	PKC
251 T	WALPTYNNH	0.699	PKC
386 S	LNDGSQAVG	0.653	DNAPK
386 S	LNDGSQAVG	0.601	ATM
392 S	AVGRSSFYC	0.601	PKA
393 S	VGRSSFYCL	0.628	PKA
429 S	SYAHSQSLD	0.629	DNAPK
460 T	QNQQTLKFS	0.659	PKC
483 S	IPGPSYRQQ	0.621	PKC
490 S	QQRVSTTVT	0.702	PKC
492 T	RVSTTVTQN	0.721	PKC
508 S	PGASSWALN	0.648	PKC
516 S	NGRNSLMNP	0.728	PKA
548 T	GKQGTGRDN	0.758	PKC
561 T	KVMITNEEE	0.680	CKII

Fig. S1

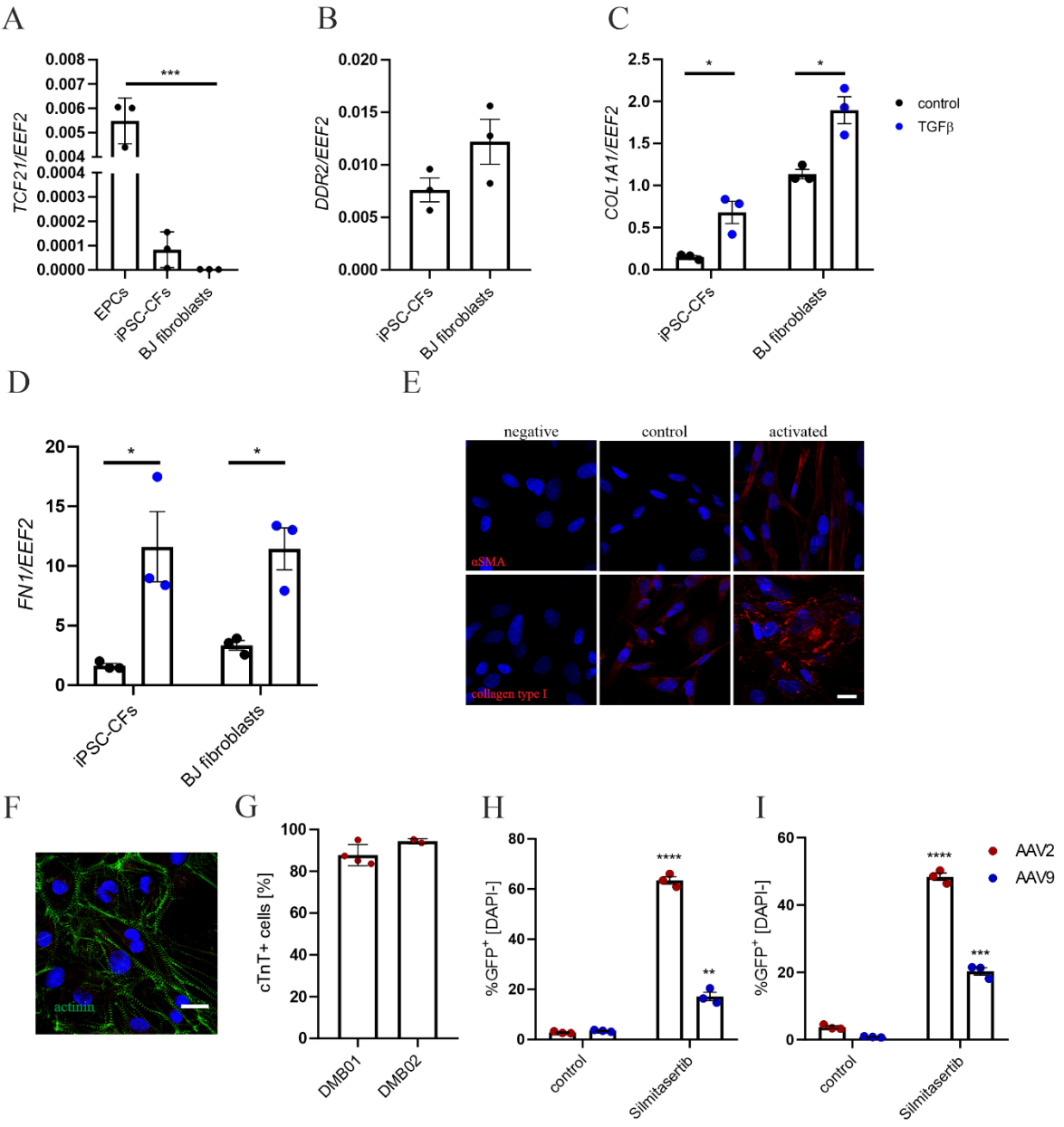

A
B
C
TCF21/EEF2
DDR2/EEF2
COL1A1/EEF2
EPCs
iPSC-CFs
BJ fibroblasts
control
TGFβ
D
E
FN1/EEF2
negative
control
activated
αSMA
collagen type I
F
G
H
I
actinin
cTnT+ cells [%]
DMB01
DMB02
%GFP+ [DAPI-]
Silmitasertib
AAV2
AAV9

Fig. S1 **Phenotyping of iPSC-CFs and iPSC-CMs used in the study.** A – comparison of expression of TCF21 in epicardial progenitor cells, iPSC-CFs and BJ fibroblasts; B – expression of DDR2 in iPSC-CFs and BJ fibroblasts; expression of COL1A1 (C) and FN1 (D) in quiescent and activated iPSC-CFs and BJs. Cells were activated using 5 ng/ml human recombinant TGFβ for 24h; Student's t-test vs appropriate control * $p<0.05$, ** $p< 0.01$, *** $p<0.001$; E – immunofluorescent staining of iPSC-CFs for collagen type I and α-SMA in control and activated (3 consecutive days of TGFβ stimulation) cells, scale bar = 10μm, F – immunofluorescent staining of iPSC-CMs for α-actinin, scale bar = 10μm; G – differentiation efficiency of DMB01 and DMB02 lines into iPSC-CMs based on percentage of cells positive for cardiac Troponin T assessed through flow cytometry; Transduction efficiency of scAAV2 and scAAV9 in primary human cardiac fibroblasts (H) and primary human fibroblasts from adventitia. Percentage of GFP+ cells was assessed by flow cytometry 5 days after exposure to the vectors. Two-way ANOVA vs control * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

Fig. S2

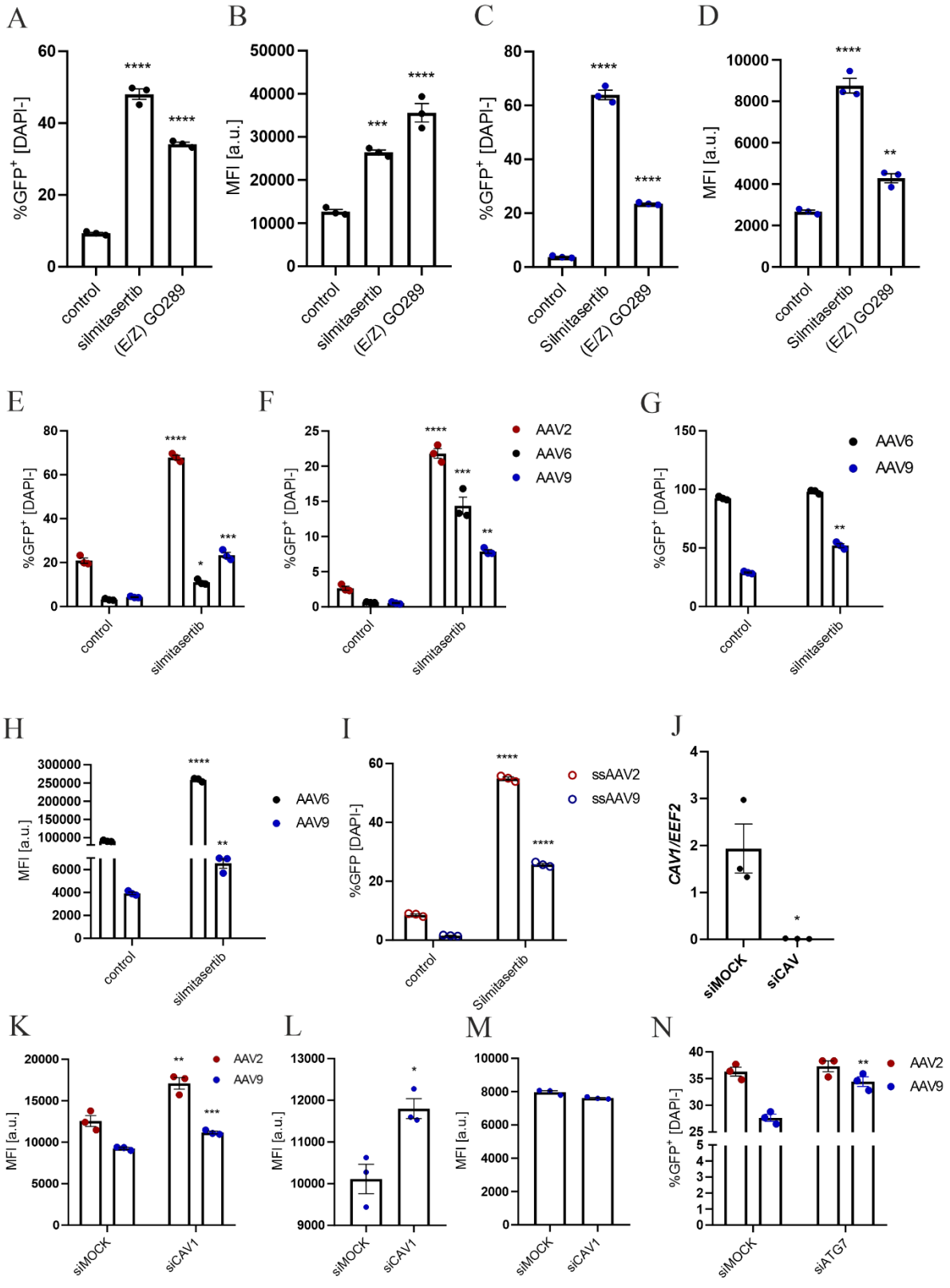

A
B
C
D
%GFP+ [DAPI-]
MFI [a.u.]
control
silmitasertib
(E/Z) GO289
Silmitasertib
E
F
G
AAV2
AAV6
AAV9
H
I
J
ssAAV2
ssAAV9
%GFP [DAPI-]
CAV1/EEF2
siMOCK
siCAV
K
L
M
N
siCAV1
siATG7

Fig. S2 **CK2 inhibition improves AAV transduction efficiency**. Transduction efficiency calculated as percentage of GFP+ cells and median fluorescence intensity in iPSC-CFs (A, B) and HAECs (C, D) exposed to scAAV9-CMV-GFP vectors in control conditions or in the presence of CK2 inhibitors (silmitasertib or E/Z-GO289); One-way ANOVA vs control; Transduction efficiency in iPSC-CFs obtained from alternative iPSC cell lines – DMB02 (E) and DMB04 (F) line in control conditions and after silmitasertib stimulation; Two-way ANOVA vs control. G - Transduction efficiencycalculated as percentage of GFP+ cells (G) and median fluorescence intensity (H) in iPSC-CMs obtained from DBM2 line using scAAV6 and scAAV9 in control conditions and after silmitasertib treatment; I – Transduction efficiency of ssAAV vectors in iPSC-CFs after silmitasertib treatment. J – efficiency of CAV1 silencing in iPSC-CFs 48h after transfection with siRNA, K – MFI in iPSC-CFs transduced with scAAV2 or scAAV9 after silencing of CAV1; L – MFI in iPSC-CMs transduced with scAAV9 after silencing of CAV1; M – MFI in HAECs transduced with scAAV9 after silencing of CAV1; Student's t-test vs siMOCK, * $p<0.05$, ** $p< 0.01$; N – Flow cytometry analysis of scAAV9 transduction efficiency in the presence of silmitasertib in iPSC-CFs following siATG7 transfection. Percentage of GFP+ cells was assessed by flow cytometry 5 days after exposure to the vectors. Two-way ANOVA vs control * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

Fig. S3

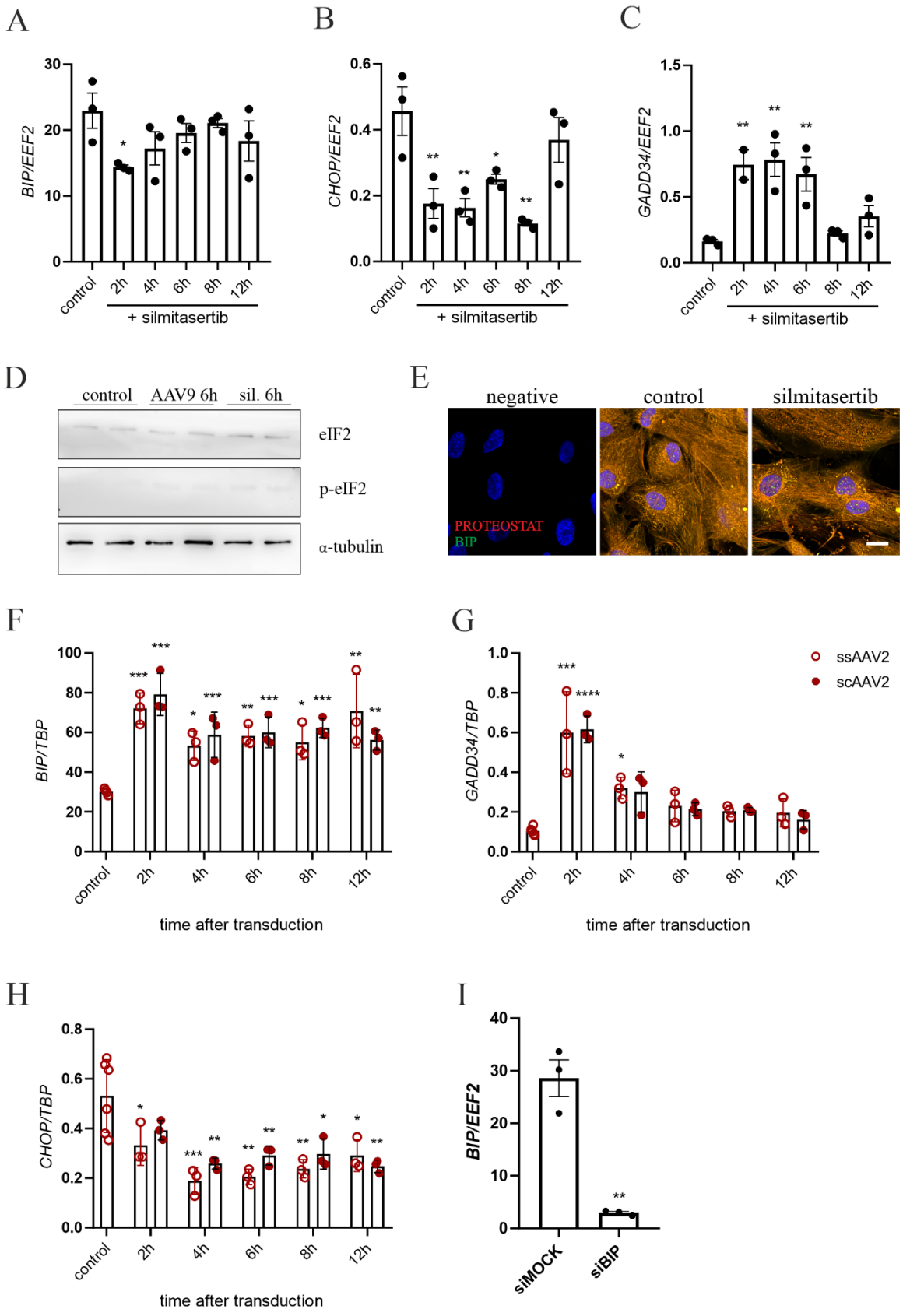
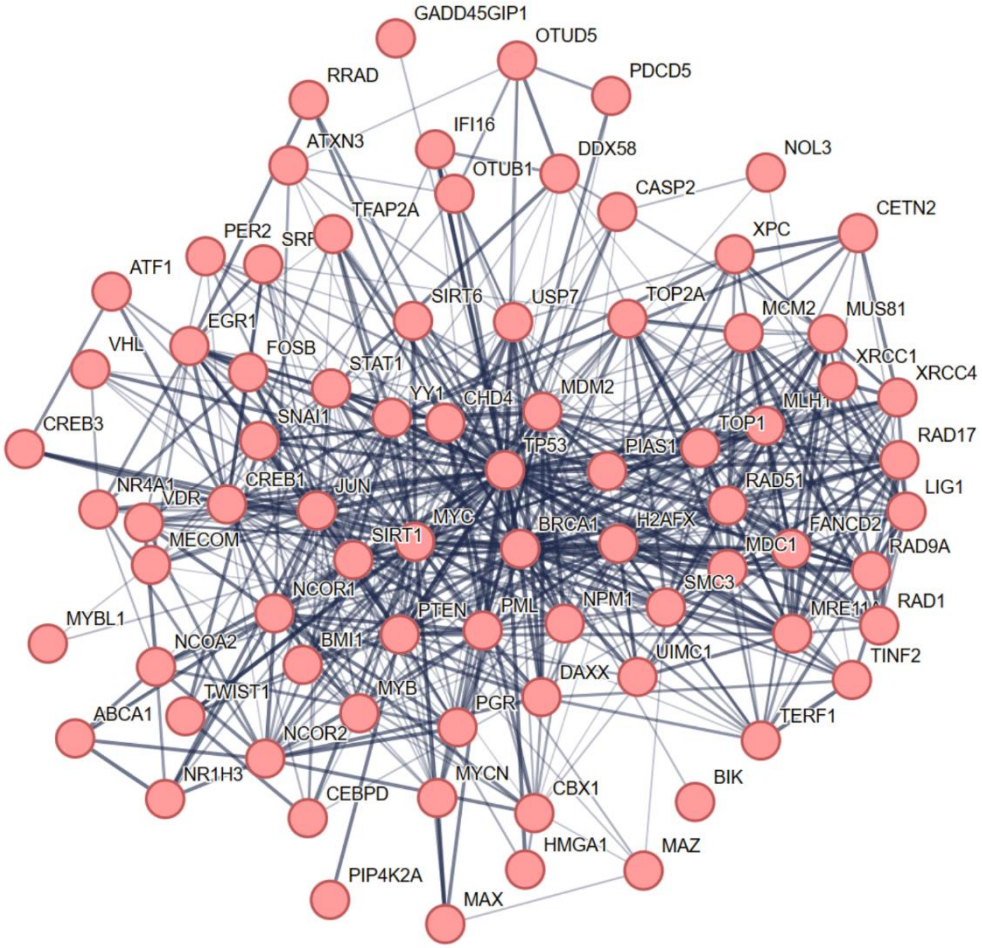

A
B
C
BIP/EEF2
CHOP/EEF2
GADD34/EEF2
control
2h
4h
6h
8h
12h
+ silmitasertib
D
control
AAV9 6h
sil. 6h
eIF2
p-eIF2
α-tubulin
E
negative
control
silmitasertib
PROTEOSTAT
BIP
F
G
BIP/TBP
GADD34/TBP
ssAAV2
scAAV2
time after transduction
H
I
CHOP/TBP
siMOCK
siBIP

Fig. S3 **Unfolded protein response upon silmitasertib treatment and AAV transduction**. Expression of *BIP* (A), *CHOP* (B), *GADD34* (C) in iPSC-CFs after silmitasertib stimulation; One-way ANOVA * $p<0.05$, ** $p< 0.01$; D – western blot for eIF2α and phosphorylated eIF2α in iPSC-CFs, 6h after transduction with AAV vectors or treatment with silmitasertib; E – immunofluorescent staining for BIP and protein aggregates (PROTEOSTAT) in iPSC-CFs treated with silmitasertib for 6h, scale bar =10μm; Expression of *BIP* (F), *GADD34* (G) and *CHOP* (H) in iPSC CFs transduced with ssAAV2 or scAAV2. One-way ANOVA * $p<0.05$, ** $p< 0.01$, *** $p<0.001$, **** $p< 0.0001$ vs control conditions; I – efficiency of BIP silencing in iPSC-CFs 48h after transfection with siRNA, Student's t-test vs siMOCK, * $p<0.05$, ** $p< 0.01$

Fig. S4

A


GADD45GIP1
OTUD5
RRAD
PDCD5
IFI16
ATXN3
DDX58
NOL3
OTUB1
CASP2
TFAP2A
CETN2
PER2
SRF
XPC
ATF1
SIRT6
USP7
TOP2A
MCM2
MUS81
EGR1
VHL
FOSB
STAT1
XRCC1
XRCC4
YY1
CHD4
MDM2
MLH1
CREB3
SNAI1
TOP1
RAD17
TP53
PIAS1
NR4A1
CREB1
JUN
RAD51
LIG1
VDR
MYC
BRCA1
H2AFX
FANCD2
MECOM
SIRT1
MDC1
RAD9A
NCOR1
SMC3
MYBL1
PTEN
PML
NPM1
MRE11
RAD1
NCOA2
BMI1
UIMC1
TINF2
DAXX
TWIST1
MYB
PGR
ABCA1
TERF1
NCOR2
NR1H3
MYCN
CBX1
BIK
CEBPD
HMGA1
MAZ
PIP4K2A
MAX

B

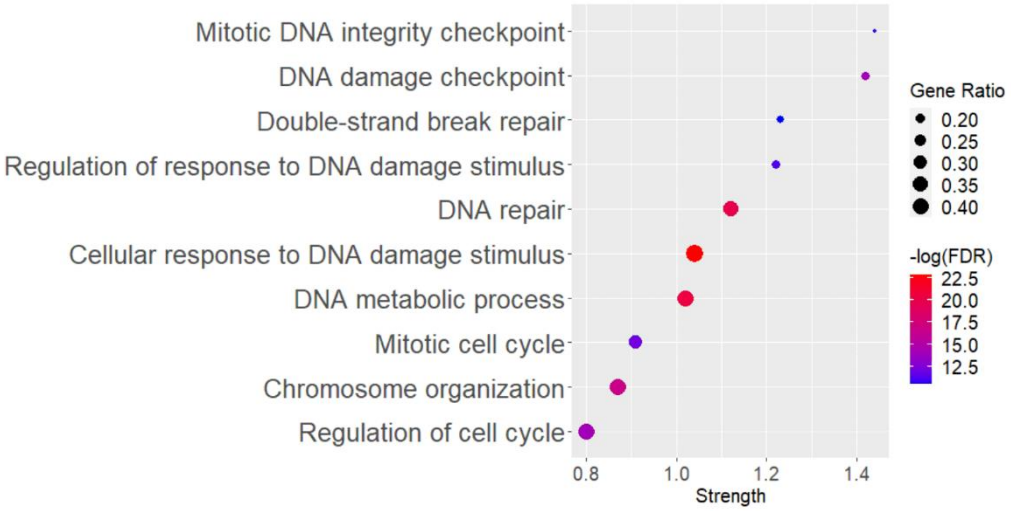

Mitotic DNA integrity checkpoint
DNA damage checkpoint
Double-strand break repair
Regulation of response to DNA damage stimulus
DNA repair
Cellular response to DNA damage stimulus
DNA metabolic process
Mitotic cell cycle
Chromosome organization
Regulation of cell cycle
0.8
1.0
1.2
1.4
Strength
Gene Ratio
0.20
0.25
0.30
0.35
0.40
-log(FDR)
22.5
20.0
17.5
15.0
12.5

Fig. S4 **CK2 interactome analysis**. A – Graphical representation of DNA repair-associated cluster identified in total CK2 interactome analysis based on STRING protein-protein interaction network; B – analysis of GO-term pathway enrichment in the DDR cluster based on STRING analysis;

Fig. S5

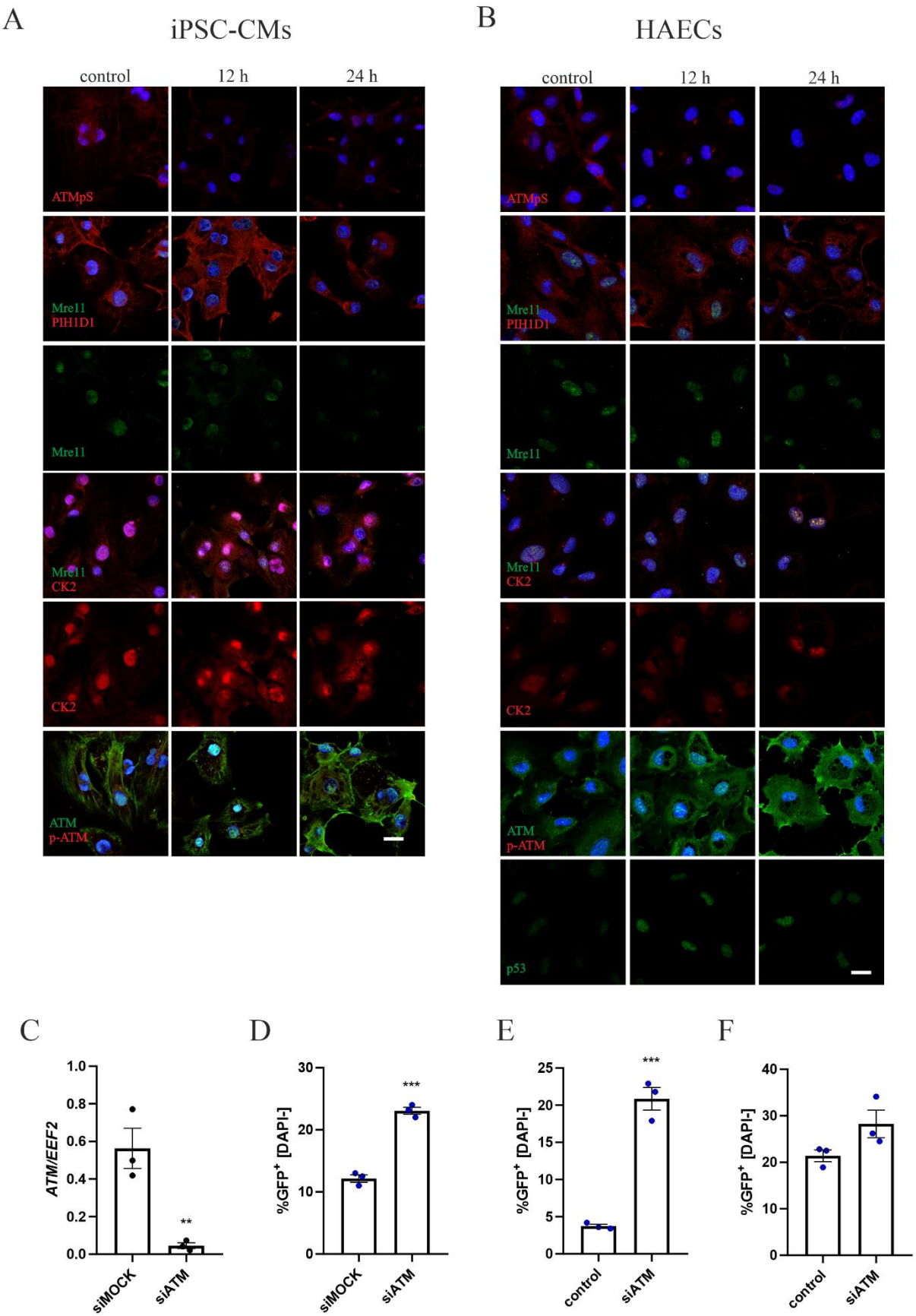

A
iPSC-CMs
control
12 h
24 h
ATMpS
Mre11
PIH1D1
Mre11
Mre11
CK2
CK2
ATM
p-ATM
B
HAECs
control
12 h
24 h
ATMpS
Mre11
PIH1D1
Mre11
Mre11
CK2
CK2
ATM
p-ATM
p53
C
ATM/EEF2
siMOCK
siATM
D
%GFP+ [DAPI-]
siMOCK
siATM
E
%GFP+ [DAPI-]
control
siATM
F
%GFP+ [DAPI-]
control
siATM

Fig. S5 **The impact of CK2 activity inhibition on DNA damage response machinery.** Immunofluorescent staining of iPSC-CMs (A) and HAECs (B) for Mre11, PIH1D1, CK2, ATM/ATR phosphorylated substrate (abbreviated as ATMpS in the figure), ATM, p-ATM and p53 at 12 and 24 h after silmitasertib treatment; Scale bar = 10 μm; C – efficiency of ATM silencing in iPSC-CFs 48h after transfection with siRNA, Student's t-test vs siMOCK, * $p<0.05$, ** $p< 0.01$; Flow cytometry analysis of AAV2 transduction efficiency in iPSC-CFs (D) and AAV9 in HAECs (E) and iPSC-CMs (F) following siATM transfection. Transduction efficiency was assessed 5 days after exposure to AAV vectors; Student's t-test vs appropriate control * $p<0.05$, ** $p< 0.01$, *** $p<0.001$;

Fig. S6

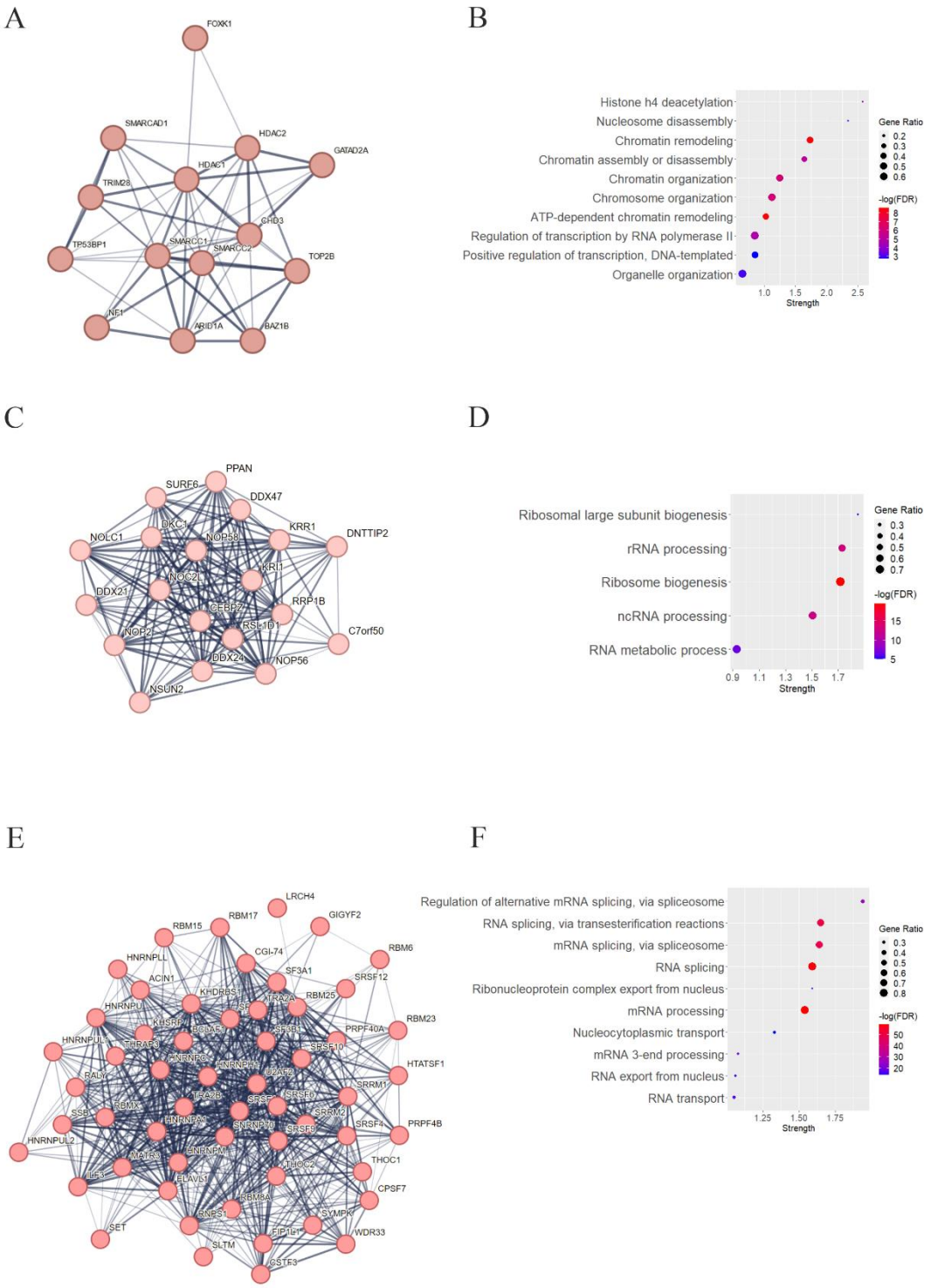

A
FOXK1
SMARCAD1
HDAC2
GATAD2A
HDAC1
TRIM28
CHD3
TP53BP1
SMARCC1
SMARCC2
TOP2B
INF1
ARID1A
BAZ1B
B
Histone h4 deacetylation
Nucleosome disassembly
Chromatin remodeling
Chromatin assembly or disassembly
Chromatin organization
Chromosome organization
ATP-dependent chromatin remodeling
Regulation of transcription by RNA polymerase II
Positive regulation of transcription, DNA-templated
Organelle organization
1.0 1.5 2.0 2.5
Strength
Gene Ratio
0.2 0.3 0.4 0.5 0.6
-log(FDR)
8 7 6 5 4 3
C
PPAN
SURF6
DDX47
DKC1
KRR1
DNTTIP2
NOLC1
NOP58
KRI1
NOC2
DDX21
CEBPZ
RRP1B
RSL1D1
NOP2
C7orf50
DDX24
NOP56
NSUN2
D
Ribosomal large subunit biogenesis
rRNA processing
Ribosome biogenesis
ncRNA processing
RNA metabolic process
0.9 1.1 1.3 1.5 1.7
Strength
Gene Ratio
0.3 0.4 0.5 0.6 0.7
-log(FDR)
15 10 5
E
LRCH4
RBM17
GIGYF2
RBM15
RBM6
HNRNPLL
CGI-74
SF3A1
ACIN1
SRSF12
KHDRBS1
TRA2A
RBM25
HNRNPUL1
SF3B1
RBM23
KHSRP
PRPF40A
HNRNPUL2
THRAP3
SRSF10
HNRNPC
HNRNPH1
HTATSF1
RALY
U2AF2
SRRM1
U2AF1
SRSF9
SSB
RBMX
SRRM2
HNRNPA1
SNRNP70
SRSF5
SRSF4
PRPF4B
HNRNPUL2
HNRNPM
AATF
THOC1
DDX3X
ELAVL1
THOC2
CPSF7
RBM8A
RNPS1
SYMPK
SET
FIP1L1
WDR33
SLTM
CSTF3
F
Regulation of alternative mRNA splicing, via spliceosome
RNA splicing, via transesterification reactions
mRNA splicing, via spliceosome
RNA splicing
Ribonucleoprotein complex export from nucleus
mRNA processing
Nucleocytoplasmic transport
mRNA 3-end processing
RNA export from nucleus
RNA transport
1.25 1.50 1.75
Strength
Gene Ratio
0.3 0.4 0.5 0.6 0.7 0.8
-log(FDR)
50 40 30 20

Fig. S6 **Impact of silmitasertib on DNA-damage and RNA processing-related signalling**. A – The STRING protein-protein interaction network representing DNA repair-associated cluster, B – analysis of GO-term pathway enrichment in the cluster based on STRING analysis; C – The STRING protein-protein interaction network representing rRNA processing cluster, D – analysis of GO-term pathway enrichment in the cluster based on STRING analysis; E – The STRING protein-protein interaction network representing mRNA processing cluster, F – analysis of GO-term pathway enrichment in the cluster based on STRING analysis;

Fig. S7

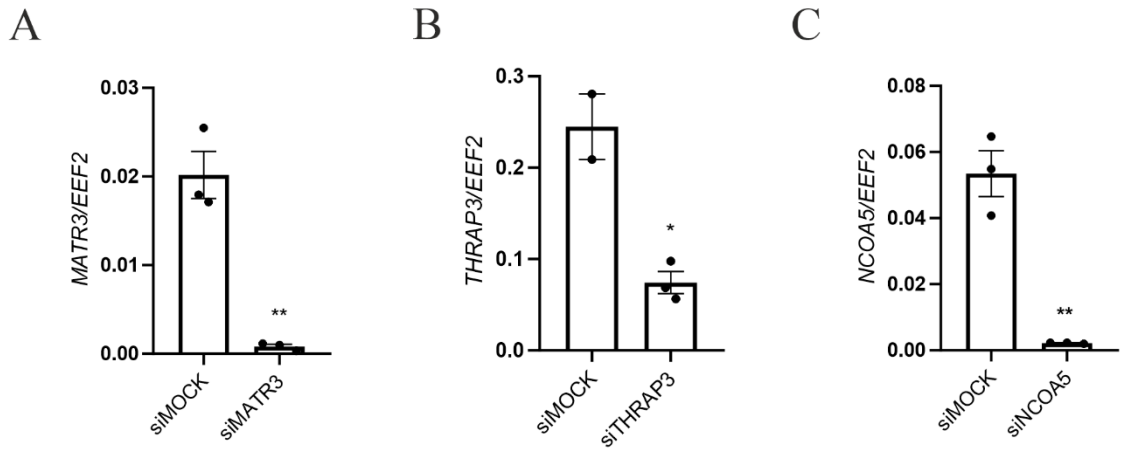


Fig. S7 **Knock-down efficiency in iPSC-CFs.** Efficiency of MATR3 (A), THRAP3 (B) and NCOA5 (C) silencing in iPSC-CFs 48h after transfection with siRNA, Student's t-test vs siMOCK, * $p<0.05$, ** $p< 0.01$;

Fig. S8

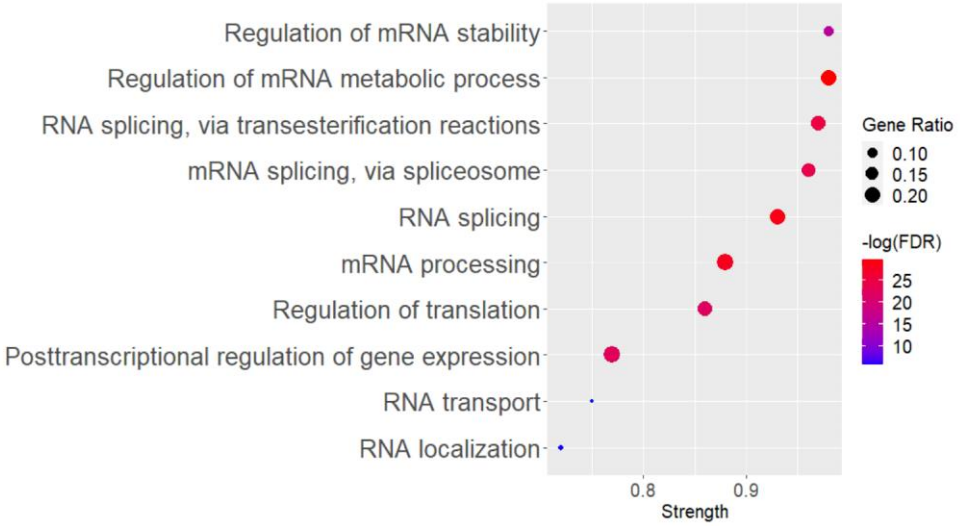


Fig. S8 **AAV interactome analysis from study by Chandran et al.**, Analysis of GO-term pathway enrichment based on STRING analysis