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Supplemental Information

NOTCH1 Inhibits Activation of ATM by Impairing the Formation of an ATM-FOXO3a-KAT5/Tip60 Complex

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Supplementary inventory:

1)Supplementary experimental procedures

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Supplementary experimental procedures

Immunoblots and immunoprecipitations

Cells were harvested and lysed in the TEB150 lysis buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 5 mM EGTA pH 8, 1 mM dithiothreitol (DTT), 0.5% Triton X-100, 10% glycerol, protease inhibitor cocktail set III (Calbiochem)) followed by centrifugation. For immunoprecipitation 1mg or more of protein lysate was incubated with appropriated antibody or IGGs over night (Supplementary Table 1) followed by crosslinking with Protein G (Zymed Laboratories) and subsequently washed 3 times with the lysis buffer.

For pull down experiments 1mg of lysed cells or 1ug of recombinant proteins were incubated with the GST tagged proteins bound to glutathione sepharose beads (GE Healthcare) for 2h followed by 3 washes with the lysis buffer.

Obtained samples were subjected to SDS-PAGE followed by protein transfer. Nitrocelluose membranes were next blocked in 5% milk TBS-Tween (0.1%) solution and incubated with appropriated antibodies (Supplementary Table 1).

For the purpose of cell lysate fractionation cells were incubated with Nuclear isolation buffer (0.25M Sucrose, 10mM Tris HCl pH 7.4, 5mM $MgCl_2$ supplemented with protease inhibitor cocktail set III (Calbiochem)) washed and lysed in Nuclear lysis buffer (NLB) (150mM KCl, 25mM Tris HCl pH 7.4, 5mM $MgCl_2$ and 0.5% NP40, protease inhibitor cocktail set III (Calbiochem)). Next not soluble fraction (chromatin) was spun and lysed with NLB with Benzonase nuclease (Sigma) (1:300).

Immunofluorescence and PLA

Cells were fixed with Methanol-Acetone solution (1:1) for 2min at room temperature (RT). Next cells were blocked with PBG (0.2% cold-water-fish gelatin and 0.5% BSA in PBS) followed by incubation with primary antibody for 1h at RT. Samples were subsequently washed 3 times with PBS and incubated with secondary antibody, followed by DAPI counterstaining and washing (3 times). Images were acquired with the use of wide-filed microscope (Olympus).

PLA was done accordingly to the manufactures protocol. For this purpose cells were fix in 4% PFA solution in PBS for 10min. Cytospin of CUTLL1 cells, followed by the PLA was performed as described previously (Vermezovic et al., 2015). For the PLA between ATM and NOTCH1 anti-ATM and anti-N1IC antibody were used; anti-NOTCH1 antibody was used in parallel to detect NOTCH1 positive cells. The anti-KAT5 antibody used for all of the PLA assays was kindly provided by B. Amati (Italian Institute of Technology)(Frank et al., 2003).

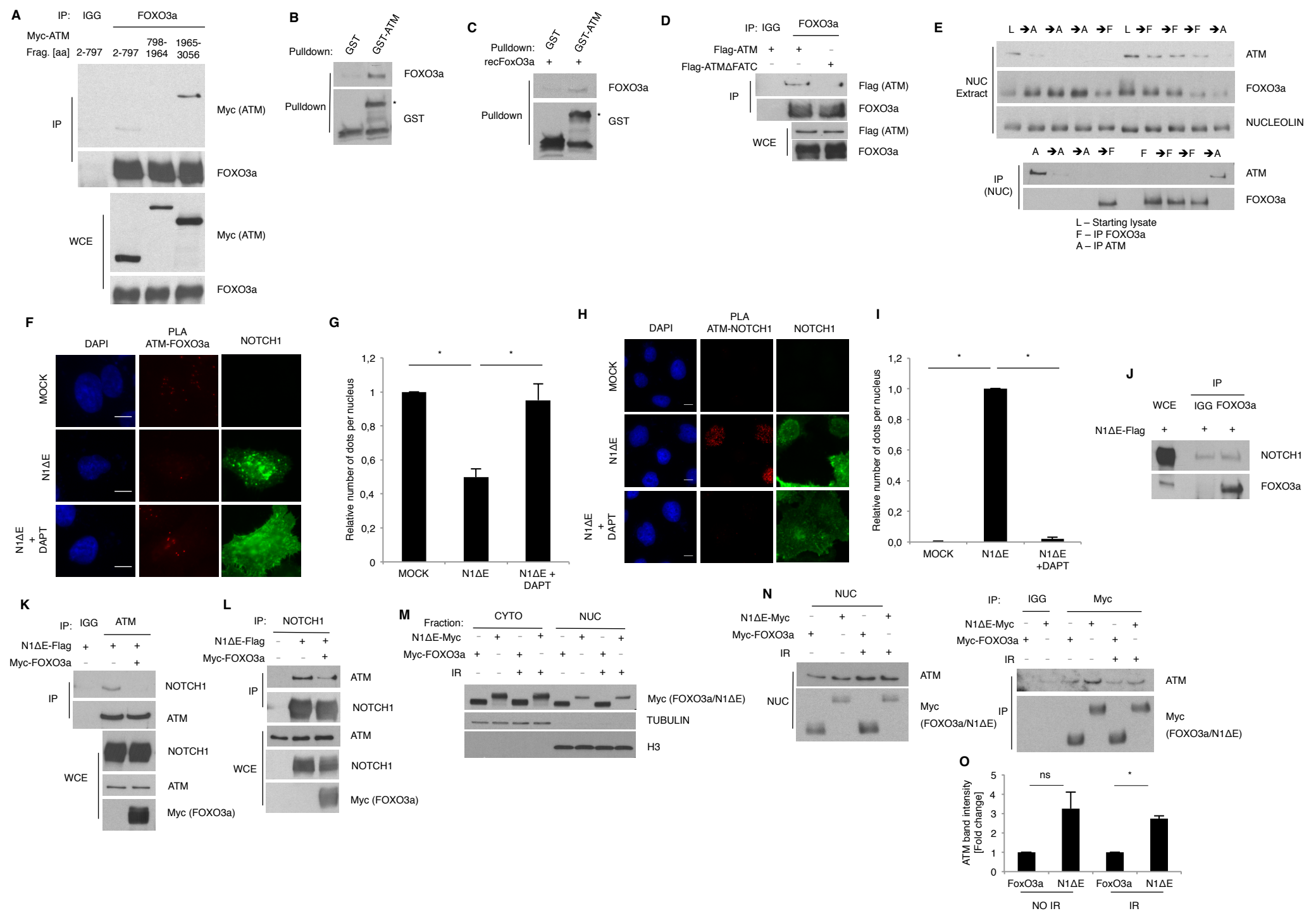
Constructs and protein purification

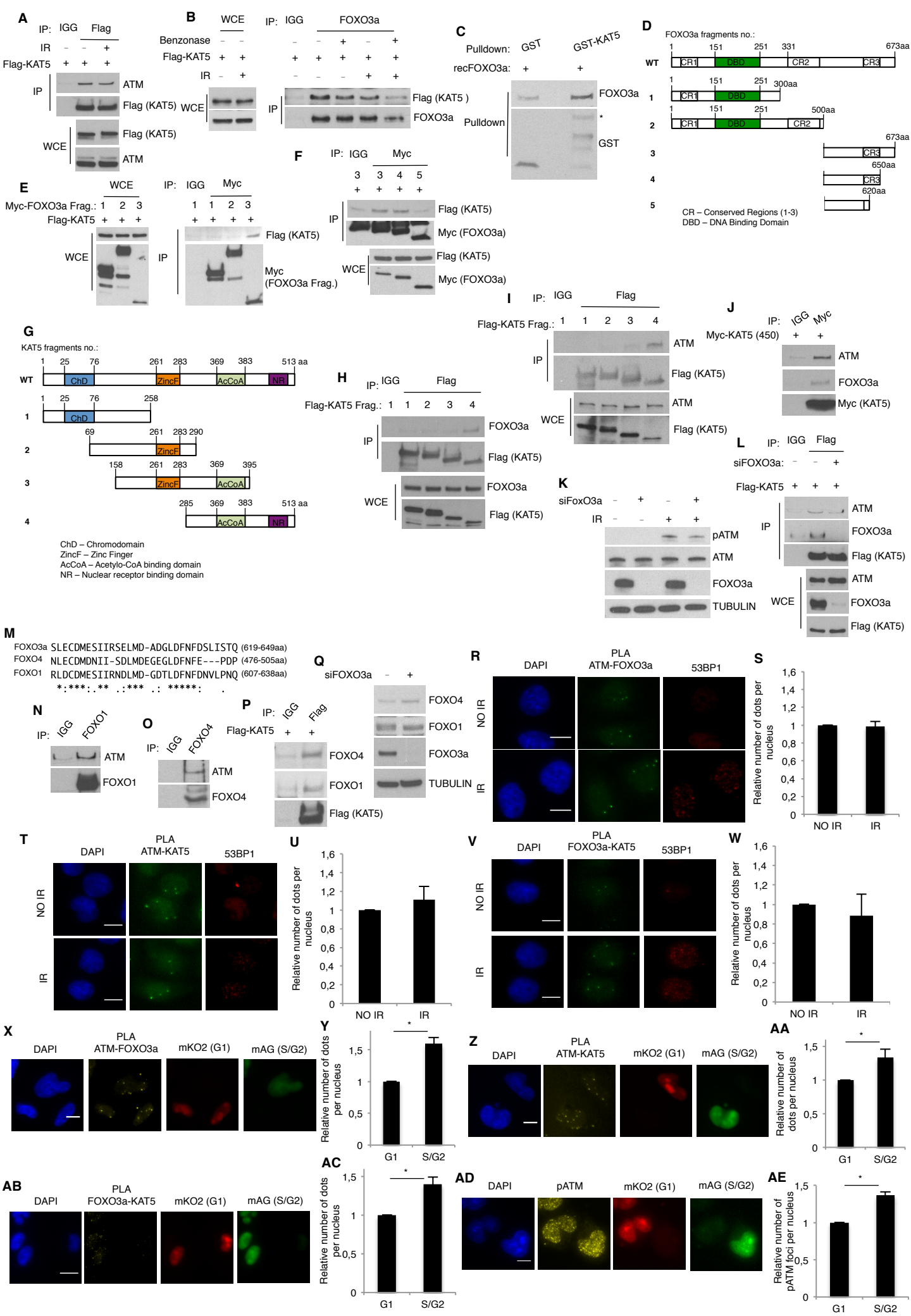
Human N1IC-GFP and EGFP constructs were a kind gift of A. Sarin (National Centre for Biological Sciences). Human N1ΔE-Flag construct were a kind gift of P.P. Di Fiore (Istituto Europeo di Oncologia). Human N1ΔE-Myc construct was a kind gift of G. Del Sal (University of Trieste). Human Flag-KAT5 construct was a kind gift of S.P. Jackson (Gurdon Institute)(Kaidi and Jackson, 2013). Human Myc-FOXO3a construct was a kind gift of K. Yamamoto (Nagasaki University) (Wang et al., 2008). Mouse 3xFlag-KAT5 fragments were a kind gift of H. S. Park (Chonnam National University)(Kim et al., 2007). Human Flag-ATM construct was a kind gift of M. Kastan (Duke cancer institute). Human Flag-ATMΔFATC (1-2992aa) was cloned by IFOM Biochemistry unit. Human Myc-ATM fragments were a kind gift of S. J. Kim (CHA University)(Park et al., 2015). GST-ATM fragments were kind gift of A. Behrens (Francis Crick Institute)(Khanna et al., 1998). Myc-KAT5 450-513aa and Myc-FOXO3a fragments (1-300aa; 1-500aa; 500-673aa; 500-650aa and 500-620aa) were amplified with the use of indicated primers (Supplementary Table 2) and cloned into BamHI and XhoI sites of pcDNA-Myc (Wang et al., 2008). GST-KAT5 and GST-FOXO3a were amplified with the use of indicated primers (Supplementary Table 2) and cloned into pGex 2rbs.

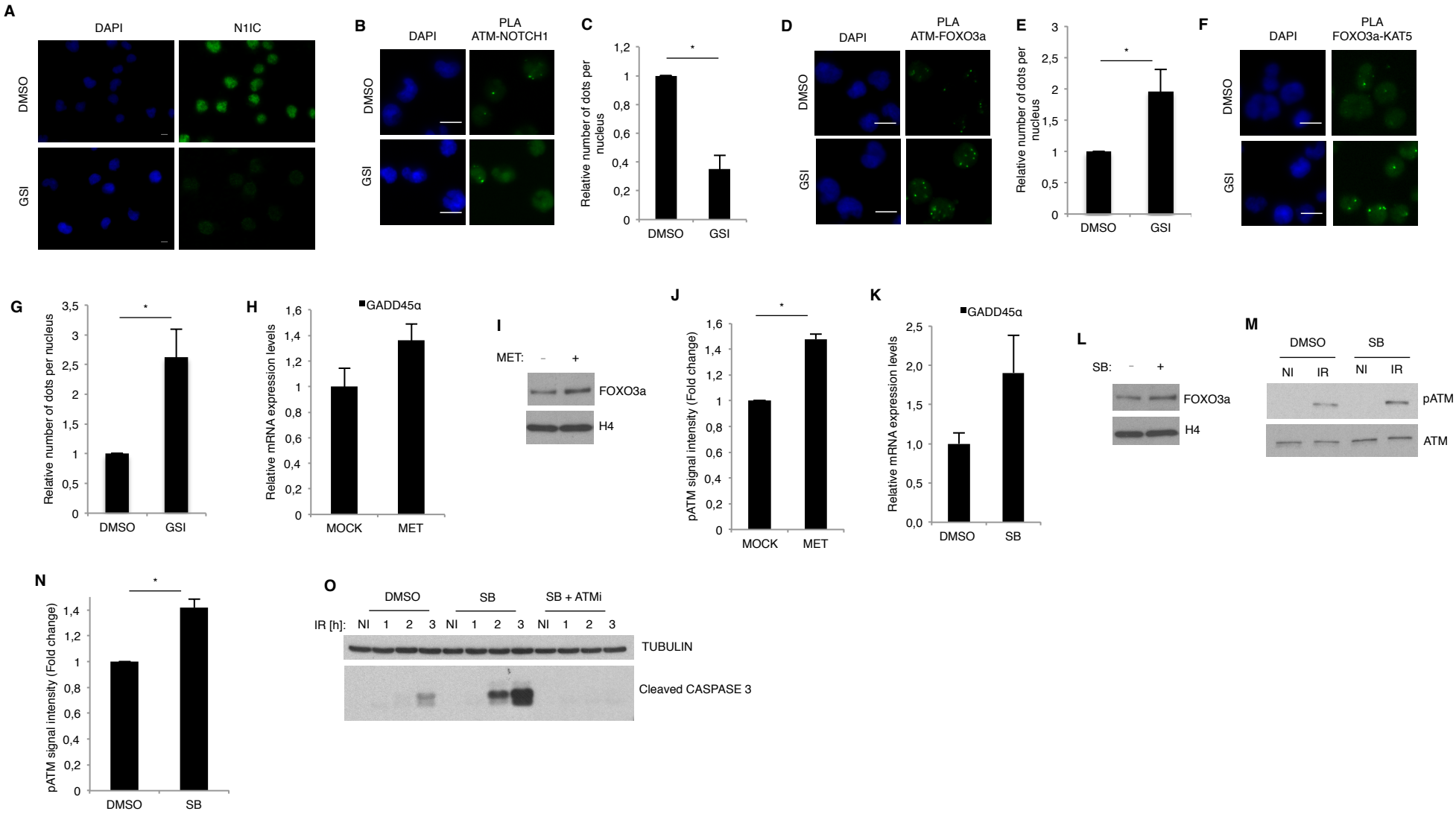
Recombinant proteins: GST-ATM fragments, GST-KAT5 and GST-FOXO3a were expressed and purified from *Esherichia Coli* BL21 bacteria with the use of Gluthatione sepharose beads (GE Healthcare). For the purpose of some experiments GST tag was cleaved with the use of Precission protease.

qRT-PCR

Total RNA was extracted from cells with the use of QIAGEN RNA extraction kit, according to manufacture procedures. RNA was subsequently retrotranscribed with the use of SuperScript Vilo kit (Invitrogen). Next cDNA was used in the RT-qPCR reaction with the use of indicated primers (Supplementary Table 2) and GoTaq q-PCR Master Mix (Promega).


A
IP: IGG FOXO3a
Myc-ATM
Frag. [aa] 2-797 2-797 798-1964 1965-3056
IP
Myc (ATM)
FOXO3a
WCE
Myc (ATM)
FOXO3a
B
Pulldown: GST GST-ATM
Pulldown
FOXO3a
GST
C
Pulldown: GST GST-ATM
recFoxO3a + +
Pulldown
FOXO3a
GST
D
IP: IGG FOXO3a
Flag-ATM + + –
Flag-ATMΔFATC – – +
IP
Flag (ATM)
FOXO3a
WCE
Flag (ATM)
FOXO3a
E
L →A →A →A →F L →F →F →F →A
NUC Extract
ATM
FOXO3a
NUCLEOLIN
A →A →A →F F →F →F →A
IP (NUC)
ATM
FOXO3a
L – Starting lysate
F – IP FOXO3a
A – IP ATM
F
DAPI
PLA ATM-FOXO3a
NOTCH1
MOCK
N1ΔE
N1ΔE + DAPT
G
Relative number of dots per nucleus
1,2
1
0,8
0,6
0,4
0,2
0
*
*
MOCK
N1ΔE
N1ΔE + DAPT
H
DAPI
PLA ATM-NOTCH1
NOTCH1
MOCK
N1ΔE
N1ΔE + DAPT
I
Relative number of dots per nucleus
1,2
1,0
0,8
0,6
0,4
0,2
0,0
*
*
MOCK
N1ΔE
N1ΔE +DAPT
J
IP
WCE IGG FOXO3a
N1ΔE-Flag + + +
NOTCH1
FOXO3a
K
IP: IGG ATM
N1ΔE-Flag + + +
Myc-FOXO3a – – +
IP
NOTCH1
ATM
WCE
NOTCH1
ATM
Myc (FOXO3a)
L
IP: NOTCH1
N1ΔE-Flag – + +
Myc-FOXO3a – – +
IP
ATM
NOTCH1
WCE
ATM
NOTCH1
Myc (FOXO3a)
M
Fraction: CYTO NUC
N1ΔE-Myc – + – + – + – +
Myc-FOXO3a + – + – + – + –
IR – – + + – – + +
Myc (FOXO3a/N1ΔE)
TUBULIN
H3
N
NUC
N1ΔE-Myc – + – +
Myc-FOXO3a + – + –
IR – – + +
NUC
ATM
Myc (FOXO3a/N1ΔE)
IP: IGG Myc
N1ΔE-Myc – + – + – + – +
Myc-FOXO3a + – + – + – + –
IR – – – – + + + +
IP
ATM
Myc (FOXO3a/N1ΔE)
O
ATM band intensity [Fold change]
5
4
3
2
1
0
ns
*
FoxO3a N1ΔE FoxO3a N1ΔE
NO IR
IR


A
IP: IGG Flag
IR
Flag-KAT5
IP
ATM
Flag (KAT5)
WCE
Flag (KAT5)
ATM
B
WCE
Benzonase
Flag-KAT5
IR
WCE
IP: IGG FOXO3a
IP
Flag (KAT5)
FOXO3a
C
Pulldown: GST GST-KAT5
recFOXO3a:
Pulldown
FOXO3a
*
GST
D
FOXO3a fragments no.:
WT
CR1
DBD
CR2
CR3
673aa
300aa
500aa
650aa
620aa
CR – Conserved Regions (1-3)
DBD – DNA Binding Domain
E
WCE
Myc-FOXO3a Frag.:
Flag-KAT5
WCE
IP: IGG Myc
IP
Flag (KAT5)
Myc (FOXO3a Frag.)
F
IP: IGG Myc
IP
Flag (KAT5)
Myc (FOXO3a)
WCE
Flag (KAT5)
Myc (FOXO3a)
G
KAT5 fragments no.:
WT
ChD
ZincF
AcCoA
NR
513 aa
ChD – Chromodomain
ZincF – Zinc Finger
AcCoA – Acetylo-CoA binding domain
NR – Nuclear receptor binding domain
H
IP: IGG Flag
Flag-KAT5 Frag.:
IP
FOXO3a
Flag (KAT5)
WCE
FOXO3a
Flag (KAT5)
I
IP: IGG Flag
Flag-KAT5 Frag.:
IP
ATM
Flag (KAT5)
WCE
ATM
Flag (KAT5)
J
IP: IGG Myc
Myc-KAT5 (450)
ATM
FOXO3a
Myc (KAT5)
K
siFoxO3a
IR
pATM
ATM
FOXO3a
TUBULIN
L
IP: IGG Flag
siFOXO3a:
Flag-KAT5
IP
ATM
FOXO3a
Flag (KAT5)
WCE
ATM
FOXO3a
Flag (KAT5)
M
FOXO3a SLECDMESIIRSELMD-ADGLDFNFDSLISTQ (619-649aa)
FOXO4 NLECDMDNII-SDLMDEGEGLDFNFE---PDP (476-505aa)
FOXO1 RLDCDMESIIRNDLMD-GDTLDFNFDNVLPNQ (607-638aa)
*:***:.** .:*** .: *****: .
N
IP: IGG FOXO1
ATM
FOXO1
O
IP: IGG FOXO4
ATM
FOXO4
P
IP: IGG Flag
Flag-KAT5
FOXO4
FOXO1
Flag (KAT5)
Q
siFOXO3a
FOXO4
FOXO1
FOXO3a
TUBULIN
R
DAPI
PLA ATM-FOXO3a
53BP1
NO IR
IR
S
Relative number of dots per nucleus
NO IR
IR
T
DAPI
PLA ATM-KAT5
53BP1
NO IR
IR
U
Relative number of dots per nucleus
NO IR
IR
V
DAPI
PLA FOXO3a-KAT5
53BP1
NO IR
IR
W
Relative number of dots per nucleus
NO IR
IR
X
DAPI
PLA ATM-FOXO3a
mKO2 (G1)
mAG (S/G2)
Y
Relative number of dots per nucleus
*
G1
S/G2
Z
DAPI
PLA ATM-KAT5
mKO2 (G1)
mAG (S/G2)
AA
Relative number of dots per nucleus
*
G1
S/G2
AB
DAPI
PLA FOXO3a-KAT5
mKO2 (G1)
mAG (S/G2)
AC
Relative number of dots per nucleus
*
G1
S/G2
AD
DAPI
pATM
mKO2 (G1)
mAG (S/G2)
AE
Relative number of pATM foci per nucleus
*
G1
S/G2


A
DAPI
N1IC
DMSO
GSI
B
DAPI
PLA ATM-NOTCH1
DMSO
GSI
C
Relative number of dots per nucleus
1,2
1
0,8
0,6
0,4
0,2
0
*
DMSO
GSI
D
DAPI
PLA ATM-FOXO3a
DMSO
GSI
E
Relative number of dots per nucleus
3
2,5
2
1,5
1
0,5
0
*
DMSO
GSI
F
DAPI
PLA FOXO3a-KAT5
DMSO
GSI
G
Relative number of dots per nucleus
3,5
3
2,5
2
1,5
1
0,5
0
*
DMSO
GSI
H
GADD45α
Relative mRNA expression levels
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
MOCK
MET
I
MET: – +
FOXO3a
H4
J
pATM signal intensity (Fold change)
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
*
MOCK
MET
K
GADD45α
Relative mRNA expression levels
2,5
2,0
1,5
1,0
0,5
0,0
DMSO
SB
L
SB: – +
FOXO3a
H4
M
DMSO
SB
NI
IR
NI
IR
pATM
ATM
N
pATM signal intensity (Fold change)
1,4
1,2
1
0,8
0,6
0,4
0,2
0
*
DMSO
SB
O
DMSO
SB
SB + ATMi
IR [h]: NI 1 2 3 NI 1 2 3 NI 1 2 3
TUBULIN
Cleaved CASPASE 3

Supplementary figure legends:

Supplementary Figure 1 - Related to Figure 2. NOTCH1 competes with FOXO3a for binding to the FATC domain of ATM.

(A) Immunoblot analysis of the coIP of the Myc-ATM fragments with FOXO3a.

(B) Immunoblot analysis of the pulldown experiment with the GST or GST-ATM (2842-3056aa) incubated with the lysate of HEK293T cells (asterisk indicates correct band).

(C) Immunoblot analysis of the pulldown experiment with GST or GST-ATM (2842-3056aa) incubated with the recombinant FOXO3a (asterisk indicates correct band).

(D) Immunoblot analysis of the coIP of Flag-ATM and Flag-ATMΔFATC with FOXO3a.

(E) Immunoblot analysis of the sequential immunodepletion of either ATM or FOXO3a. HEK293T cells were lysed and the nuclear fraction was subjected to sequential immunodepletions (L - starting lysate; F - immunodepletion of FOXO3a; A - immunodepletion of ATM).

(F) Immunofluorescence analysis of the PLA assay between ATM and FOXO3a in HeLa cells, expressing NOTCH1 and treated with GSI (Scale bar, 10μm).

(G) Analysis of the PLA shown in Fig.S1F. (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

(H) Immunofluorescence analysis of the PLA assay between ATM and NOTCH1 in HeLa cells, expressing NOTCH1 and treated with GSI (Scale bar, 10μm).

(I) Analysis of the PLA shown in Fig.S1H (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

(J) Immunoblot analysis of the coIP of NOTCH1 with FOXO3a.

(K) Immunoblot analysis of the coIP of NOTCH1 with ATM in cells over-expressing or not FOXO3a.

(L) Immunoblot analysis of the coIP of ATM with NOTCH1 in cells over-expressing or not FOXO3a.

(M) Immunoblot analysis of the expression levels of Myc-FOXO3a or N1ΔE-Myc. Cell lysate fractions: cytosolic (CYTO) and nuclear (NUC).

(N) Immunoblot analysis of the coIP of ATM with FOXO3a or NOTCH1, in cells expressing NOTCH1 or over-expressing FOXO3a from the nuclear fraction (NUC).

(O) Quantification of the immunoblots shown in Fig.S1N. ATM signal was normalized to Myc signal (Mean ± S.E.M.; n=2; two tailed Student's t-test; p value ≤0.05).

Supplementary Figure 2 - Related to Figure 3. FOXO3a bridges KAT5 binding to ATM.

(A) Immunoblot analysis of the coIP of ATM with KAT5.

(B) Immunoblot analysis of the coIP of KAT5 with FOXO3a.

(C) Immunoblot analysis of the pulldown experiments with GST or GST-KAT5 incubated with recombinant FOXO3a (asterisk indicates correct band).

(D) Scheme of the Myc-FOXO3a fragments used in this study.

(E) Immunoblot analysis of the coIP of KAT5 with Myc-FOXO3a fragments (1-3).

(F) Immunoblot analysis of the coIP of KAT5 with Myc-FOXO3a fragments (3-5).

(G) Scheme of Flag-KAT5 fragments used in this study.

(H) Immunoblot analysis of the coIP of FOXO3a with Flag-KAT5 fragments (1-4).

(I) Immunoblot analysis of the coIP of ATM with Flag-KAT5 fragments (1-4).

(J) Immunoblot analysis of the coIP of FOXO3a and ATM with Myc-KAT5 fragment (450-513aa).

(K) Immunoblot analysis of the ATM activation (pATM) in HeLa cells transfected with siRNA against *FOXO3a* or *LUCIFERASE* (2G; 1h).

(L) Immunoblot analysis of the coIP of ATM and FOXO3a with KAT5 in HEK293T cells transfected with siRNA against *FOXO3a* or *LUCIFERASE* and Flag-KAT5 construct.

(M) Protein alignment of the human FOXO3a, human FOXO4 and human FOXO1 done in clustal X. "*" - fully conserved residue. ":" - strongly similar properties of the residues. "." - weakly similar properties of the residues

(N) Immunoblot analysis of coIP of ATM with FOXO1.

(O) Immunoblot analysis of coIP of ATM with FOXO4.

(P) Immunoblot analysis of the coIP of FOXO1 and FOXO4 with KAT5. HEK293T cells were transfected with Flag-KAT5 construct.

(Q) Immunoblot analysis of the proteins levels (FOXO1 and FOXO4) in HEK293T cells transfected with siRNA against *FOXO3a* or *LUCIFERASE.*

(R) Immunofluorescence analysis of the PLA assay between ATM and FOXO3a in HeLa cells (Scale bar, 10μm).

(S) Analysis of the PLA shown in Fig.S2R. (Mean ± S.E.M.; n=3).

(T) Immunofluorescence analysis of the PLA assay between ATM and KAT5 in HeLa cells (Scale bar, 10μm).

(U) Analysis of the PLA shown in Fig.S2T. (Mean ± S.E.M.; n=3).

(V) Immunofluorescence analysis of the PLA assay between FOXO3a and KAT5 in HeLa cells (Scale bar, 10μm).

(W) Analysis of the PLA shown in Fig.S2V. (Mean ± S.E.M.; n=3).

(X) Immunofluorescence analysis of the PLA assay between ATM and FOXO3a in HeLa Fucci cells (Scale bar, 10μm).

(Y) Analysis of the PLA shown in Fig.S2X. (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

(Z) Immunofluorescence analysis of the PLA assay between ATM and KAT5 in HeLa Fucci cells (Scale bar, 10μm).

(AA) Analysis of the PLA shown in Fig.S2Z. (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

(AB) Immunofluorescence analysis of the PLA assay between FOXO3a and KAT5 in HeLa Fucci cells (Scale bar, 10μm).

(AC) Analysis of the PLA shown in Fig.S2AB. (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

(AD) Immunofluorescence analysis of the ATM activation (pATM) in HeLa Fucci cells (Scale bar, 10μm).

(AE) Analysis of the immunofluorescence shown in Fig.S2AD. (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

Supplementary Figure 3 - Related to Figure 4. Induction of FOXO3a nuclear localization sensitizes T-ALL cells to DNA damage induced cell death.

(A) Immunofluorescence analysis of the NOTCH1 localization in CUTLL1 cells treated with GSI (Scale bar, 10μm). N1IC - nuclear form of NOTCH1.

(B) Immunofluorescence analysis of the PLA assay between ATM and NOTCH1 in CUTLL1 cells treated GSI (Scale bar, 10μm).

(C) Analysis of the PLA shown in Fig.S3B. (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05)

(D) Immunofluorescence analysis of the PLA assay between ATM and FOXO3a in CUTLL1 cells treated with GSI (Scale bar, 10μm).

(E) Analysis of the PLA shown in Fig.S3D. (Mean ± S.E.M.; n=4; two tailed Student's t-test; p value ≤0.05)

(F) Immunofluorescence analysis of the PLA assay between FOXO3a and KAT5 in CUTLL1 cells treated with GSI (Scale bar, 10μm).

(G) Analysis of the PLA shown in Fig.S3F. (Mean ± S.E.M.; n=4; two tailed Student's t-test; p value ≤0.05).

(H) qRT-PCR quantification of the mRNA expression levels of *GADD45α* in TALL-1 cells treated with MET. Values were normalized to the expression levels of *B2M* (unrelated gene).

(I) Immunoblot analysis of the FOXO3a nuclear localization upon treatment of TALL-1 cells with MET. TALL-1 cell were lysed and chromatin fraction of the lysate was extracted.

(J) Quantification of the signals shown in Fig.4A. pATM signal was normalized to ATM (Mean ± S.E.M.; n=3; two tailed Student's t-test; p value ≤0.05).

(K) qRT-PCR quantification of the mRNA expression levels of *GADD45α* , in TALL-1 cells treated with SB. Values were normalized to the expression levels of *B2M* (unrelated gene).

(L) Immunoblot analysis of the FOXO3a nuclear localization upon treatment of TALL-1 cells with SB. TALL-1 cell were lysed and chromatin fraction of the lysate was extracted.

(M) Immunoblot analysis of the ATM activation (pATM) in TALL-1 cells pretreated with SB.

(N) Quantification of the signals shown in Fig.S3M. pATM signal was normalized to ATM (Mean ± S.E.M.; n=4; two tailed Student's t-test; p value ≤0.05).

(O) Immunoblot analysis of the DNA damage-induced cell death (cleaved CASPASE 3) in TALL-1 cells pretreated with SB or SB and ATM inhibitor (ATMi).

Supplementary Table 1

Antibody:	Provider:	Use:	Cat. No.:	Species:
ATM	Abcam	WB 1:6000 5% Milk	ab32420	Rabbit
ATM	Sigma	WB 1:6000 5% Milk IP 1: 500 IF 1:200	A1106	Mouse
53BP1	Bethyl	IF 1:4000	A303906A	Goat
Celavaded CASPASE 3	Cell Signaling	WB: 1:1000 5% BSA	9661	Rabbit
pATM (S1981)	Rockland	WB 1:6000 5%Milk IF 1:200	200-301-400	Mouse
NOTCH1	Santa Cruz	WB 1:1000 5% Milk IF: 1:200	sc-6014	Goat
N1IC	Cell Signaling	IF: 1:200	4147S	Rabbit
GFP	Santa Cruz	WB 1:6000 5% Milk	sc-9996	Mouse
Flag	Sigma	WB 1:6000 5% Milk IP 1:500	F1804	Mouse
Flag	Cell Signaling	WB 1:6000 5% Milk	2368	Rabbit
Myc	Santa Cruz	WB 1:6000 5% Milk IP 1:500	sc-40	Mouse
Myc	Cell Signaling	WB 1:6000 5% Milk	2272	Rabbit
FOXO3a	Cell Signaling	IF 1:200	2497	Rabbit

FOXO3	Sigma	IF 1:200	SAB1403829	Mouse
FOXO3a	Abcam	WB 1:6000 5% Milk	ab109629	Rabbit
FOXO3a	Santa Cruz	IP 1:50	sc-11351	Rabbit
FOXO1	LifeSpan BioSciences	WB 1:8000 5% Milk IP 1:250	LS-C287207	Rabbit
FOXO4	Abcam	WB 1:8000 5% Milk IP 1:500	Ab128908	Rabbit
KAT5	Santa Cruz	WB 1:1000 5% Milk IP 1:30	sc-5725	Goat
KAT5	Kind gift of B. Amati	WB 1:1000 5% Milk IF 1:200	x	Rabbit
KAT5	LifeSpan BioSciences	WB 1:5000 5% Milk	LS-C109474	Rabbit
GST	Biochemistry Facility, IFOM-IEO Campus	WB 1:8000 5% Milk	x	Rabbit
NBS1	Novus	WB 1:1000 5% Milk IP 1:500	NB 100-143	Rabbit
TUBULIN	Sigma	WB 1:8000 5% Milk	T6074	Mouse
H3	Abcam	WB 1:8000 5% Milk	ab10799	Mouse
H4	Abcam	WB 1:8000 5% Milk	Ab10158	Rabbit
NUCLEOLIN	Novus	WB 1:8000 5% Milk	NB 600-241	Rabbit

WB - Western Blot; IF - Immunofluorescence; IP - Immunoprecipitation

Supplementary Table 2

Primers used:	Sequence (5'->3'):
qRT-PCR:	
GADD45α	Fr TTTGCAATATGACTTTGGAGGA
	Rv CATCCCCCACCTTATCCAT
B2M	Fr TTCTGGCCTGGAGGCTATC
	Rv TCAGGAAATTTGACTTTCCATTC
Cloning:	
GST-KAT5	Fr GATCGGATCCATGGCGGAGG
	Rv GATCGTCGACTCACCACTTCCC
Myc-FoxO3a 1-300	Fr GATCGGATCCATGGCAGAGGCACCGG
	Rv GATCCTCGAGTCAACTGCTGCGTGACGTGGG
Myc-FoxO3a 1-500	Fr GATCGGATCCATGGCAGAGGCACCGG
	Rv GATCCTCGAGTCACACAGCGGTGCTGGCC
Myc-FoxO3a 500-673	Fr GATCGGATCCATGTCTGCCCAGAATTCCC
	Rv GATCCTCGAGTCAGCCTGGCACCCAG
Myc-FoxO3a 500-650	Fr GATCGGATCCATGTCTGCCCAGAATTCCC
	Rv GATCCTCGAGTCAATTCTGTGTGGAGATGAGGG
Myc-FoxO3a 500-620	Fr GATCGGATCCATGTCTGCCCAGAATTCCC
	Rv GATCCTCGAGTCACAAGCTCCCATTGAAC
Myc-KAT5 450-513	Fr GATCGGATCCAAGAAGGAGGATG
	Rv GATCCTCGAGTCACCACTTCCC
GST-FOXO3a	Fr GATCGGATCCATGGCAGAGGCAC
	Rv GATCGTCGACTCAGCCTGGCAC

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