SUPPLEMENTAL DATA

Identification and characterization of intracellular proteins that bind oligonucleotides with phosphorothioate linkages

SUPPLEMENTAL MATERIALS

Antibodies

The following pprimary antibodies were purchased from Abcam: TCP1-β (ab92746), Ku70 (ab3114), Ku80 (ab119935), hnRNP K (ab32969), Lamp1 (ab25630), ADAT3A (ab67992), DDX6 (ab40684). RPL5 (ab86863), NCL1 (ab13541), NPM1 (ab24412), La/SSB (ab75927), VARS (ab72372), NARS (ab129162), Vimentin (ab20346), RHA (ab26271), hnRNP Q (ab10687), PC4/sub1 (ab72132), ILF3 (ab92355), ZNF9 (ab48027), ANXA2 (ab54771), ACLY (ab40793), eIF4H (ab77455), KCTD12 (ab124260), LRPPRC (ab97505), Rab7 (ab137029), and EEA1 (ab2900). The following antibodies were from Sigma: GAPDH (G8795), ACTB (A5316), and γ-tubulin (T6557). Antibodies for HSC70 (ab79857) and KHSRP (H00008570) were from Abnova. Anti-P54nrb (sc-376865) antibody was from Santa Cruz Biotechnology. GRSF1 antibody (ls-c80322) was from Lifespan. Anti-Lamp1 (9090S) was from Cell Signaling. ANXA2 (610069) antibody was from BD Biosciences. A rabbit RNase H1 antibody was kindly provided by Hongjiang Wu.

Anti-rabbit secondary antibody conjugated to HRP (170-6515) and anti-mouse secondary antibody conjugated to HRP (170-6516) were purchased from Bio-Rad. Anti-rabbit secondary antibodies conjugated to AF488 (ab150077) and AF647 (ab150079) as well as anti-mouse secondary antibodies conjugated to AF488 (ab150113) and AF647 (ab150115) were purchased from Abcam.

Oligonucleotides

ISIS116847 targets *PTEN*: 5'-C*T*G* C*T*A* G*C*C* T*C*T* G*G*A* T*T*T* G*A-3'; the underlined are 2'-*O*-MOE modified nucleotides and the * indicates phosphorothioate backbone.
ISIS386652 is of the same sequence and chemistry as 116847, but is labeled at the 5' end with biotin.
ISIS446654 is of the same sequence and chemistry as 116847, but is labeled at 5'end with Cy3.
ISIS256903 is of the same sequence and chemistry as 116847, but is labeled at 5'end with FITC.
XL180 is 5´- biotin-rArGrC rArGrU rUrCmU mCmAmA mAmUmC mCmAmG mAmGmG mCmUmA mGmCmA mG-3´, where r indicates ribo and m indicates 2'-O-methyl. XL180 is complementary to ISIS116847 and was used for affinity selection.
XL181 was used for affinity selection and has the sequence 5´- biotin- rArGrC rArGrU rUrCmG mUmGmA mUmGmG mGmUmU mUmGmU mCmUmG mCmGmC mU-3´.
XL279 is complementary to ASO116847 and has the sequence 5'-mUmCmA mAmAmU mCmCmA mGmAmG mGmCmU mAmGmC mAmG-3'.

ISIS110074 targets *NCL1*(ASO1): 5'- G*T*C* A*T*C* G*T*C* A*T*C* C*T*C* A*T*C* A*T-3'.
ISIS110080 targets *NCL1*(ASO2): 5'-C*G*T* C*G*T* C*G*T* C*A*T* C*C*T* C*G*T* C*C-3'.
ISIS395254 targets Malat1: 5'-G*G*C* A*T*A* T*G*C* A*G*A* T*A*A* T*G*T* T*C- 3'.
ISIS462026 targets U16: 5´-C*A*G* C*A*G* G*C*A* A*C*T* G*T*C* G*C*T* G*A-3´.
ISIS25690 targets *drosha*: 5´-A*T*C* C*C*T* T*T*C* T*T*C* C*G*C* A*T*G* T*G-3´.
ISIS286529 targets *La*: 5'-T*T*T* T*G*G* C*A*A* A*G*T* A*A*T* C*G*T* C*C-3'.
ISIS573658 targets *NPM1*: 5'-T*A*A* A*G*T* G*A*T* A*A*T* C*T*T* T*G*T* C*G-3'.

Deoxynucleotides probes used for northern hybridization:
XL057, 5'-CTCAGCCTCCCGAGTAGCTG-3', complementary to human 7SL RNA.
XL030, 5'-TCCTTCGAGCCGGAATTGA-3', complementary to human $tRNA^{Tyr}$.
XL011, 5'-TTGCTCAGTAAGAATTTTCG-3', complementary to human U16 snoRNA.

siRNAs:
The following siRNAs were purchased from Ambion or Life Technologies: La (HSS186106 and HSS186107), NPM1 (S9676 and S9677), Ku70 (2547 and 144693), Ku80 (139860 and 248391), KCTD12 (S41773 and S41774), eIF4H (HSS144459, HSS144457, and HSS144458), ANXA2 (S1383), DDX6 (121483 and 121484), DHX30 (S22643 and S22644), TUBB (S284), TCP1-β (S20756, S20757), LRPPRC (HSS115402, HSS115403), KHSRP (HSS112552, HSS189434), P54nrb (s9612 and s9614), NCL1 (S9312 and S9313), Vimentin (S14798 and S14799), VARS (139607 and 139608), PC4 (s21482 and 108088), NARS (117898 and 117899), ATAD3A (S30447 and S30448), GRSF1 (10948 and 144877), hnRNP Q (135712 and 135713), HSC70 (S6985), ILF3 (s7401), ZNF9/CNBP (S15020 and s230174), ACLY (S917).

siRNAs targeting U16 (5'- tcagcgacagttgcctgctg-3'), *RHA* (5'- gcataaaacttctgcgtct-3'), and *luciferase* (5'- cgtacgcggaatacttcga-3') were ordered from IDT. The sequences indicate the sense sequence of the genes. siRNA targeting *ANXA2* (sc-270151) was from Santa Cruz Biotechnologies.

Primer probe sets for qRT-PCR:
ACLY:
Forward: 5'- TCGTGGAAAACTTGGTCTCG
Reverse: 5'- AAGGGCTCGATCAGAAAGTTC
Probe: 5'- CTTCAGCCAGGACTTGACCCCAT

ANXA2:
Forward: 5'- GATGAGGTCACCATTGTCAACATT
Reverse: 5'- GGCGAAGGCAATATCCTGTCT

Probe: 5’- TGACCAACCGCAGCAATGCACA

ATAD3A: Hs01587333_m1, from Life Technologies.

DDX6: Hs00898915_g1.

DHX30:
Forward: 5’-AAAAGAGTTCCCACAGCCC
Reverse: 5’-GGCCATTTTATGTGCAGTGTG
Probe: 5’-AGTGTGATTGGAAGAGCCCTCGG

Drosha:
Forward: 5’- CAAGCTCTGTCCGTATCGATCA
Reverse: 5’- TGGACGATAATCGGAAAAGTAATCA
Probe: 5’-CTGGATCGTGAACAGTTCAACCCCGAT

eIF4H:
Forward: 5’- CAGGGCGACATAGATGCTATC
Reverse: 5’- CCCATCGTATGTCAAGGCTTC
Probe: 5’- AGGAGTGTACGGCTAGTCAGAGACAAA

GRSF1:
Forward: 5’- GGGAAGGCCACTGGAGAAG
Reverse: 5’- CGTGGGACCGATCCTTGA
Probe: 5’- CTTTGAGACCCATGAGGATGCTGTTGC

hnRNP Q: Hs03044160_g1 from Life Technologies.

HSC70:
Forward: 5’- GTTTTTTGTGGCTTCCTTCGTT
Reverse: 5’- CAGGTCCCTTGGACATGGTT
Probe: 5’- TTGGAGCCAGGCCTACACCCCAG

ILF3:
Forward: 5’- CCTTCCAAGATGCCCAAGAA
Reverse: 5’- GGTGCTTGGTGGGATCTGA
Probe: 5’- CAAAGAATGAAAACCCAGTGGACTACACCG

KCTD12:
Forward: 5’- CTTCCTCTTCCGCTACATCC
Reverse: 5’- CTGGCAGCTCGAAGTACTC
Probe: 5’- CTGCCCGACTACTTCCCCGA

KHSRP:
Forward: 5’-TTCTCAACTTGGACCCATCC-3’
Reverse: 5’-CCACCGCAATCTGTACTTTG-3’

Probe: 5’-TGTTAATTTGTTCACCTCCTCTGCCCC-3’

Ku70:
Forward: 5’- TGTCGTCTTCTGTCCAAGTTG -3’
Reverse: 5’- GTAATATGACTCCCACCCTGAC -3’
Probe: 5’- TGTTGGCTACTGCTCACTTTGGCG -3’

Ku80:
Forward: 5’- CTCTTTCCGCTATCTGCCG -3’
Reverse: 5’- CACGTCCATACACAGCACAAC -3’
Probe: 5’- TTATTCCCCGACCGCACCATGT -3’

La:
Forward: 5’- GCGACTTCAATTTGCCACG -3’
Reverse: 5’- CTGCCTTGGATTTGCTCAATG -3’
Probe: 5’- ACCCAGCCTTCATCCAGTTTTATCTGTT -3’

LRPPRC:
Forward: 5’-TCTCACCAACTGATTTCCTGG-3’
Reverse: 5’-GAATACTGCCTCTGTAACTGGG-3’
Probe: 5’-CACTCGATTTGGTTGAATGTTTGCTTCC-3’

Malat1:
Forward: 5’- GCTTGGCTTCTTCTGGACTCA -3’
Reverse: 5’- TCGCGAGCTTCACCATGA -3’
Probe: 5’- CGCCACTTGTCCGCTTCACACTCC-3’

NARS: Hs00189846_m1 from Life Technologies.

NCL1:
Forward: 5’- GCTTGGCTTCTTCTGGACTCA -3’
Reverse: 5’- TCGCGAGCTTCACCATGA -3’
Probe: 5’- CGCCACTTGTCCGCTTCACACTCC-3’

NPM1:
Forward: 5’- TCCTGCGCGGTTGTTCTC-3’
Reverse: 5’- GGCGGCACGCACTTAGG -3’
Probe: 5’- CAGCGTTCTTTTATCTCCGTCCGCCT -3’

P54nrb:
Forward: 5´- GATTTGGCTTTATCCGCTTGG -3´
Reverse: 5´- ACACATACTGAGGAAGGTTTCG -3´
Probe: 5´- TTGGCAATCTCCGCTAGGGTTCG -3´

PC4/Sub1: Hs00970533_g1 from Life Technologies.

PTEN:
Forward: 5'-AATGGCTAAGTGAAGATGACAATCAT-3'
Reverse: 5'-TGCACATATCATTACACCAGTTCGT-3'
Probe: 5'-TTGCAGCAATTCACTGTAAAGCTGGAAAGG-3'

RHA:
Forward: 5'- CCACTTACTGATACTCCTGACAC-3'
Reverse: 5'- CAGGAACACCATAGCCAGAG-3'
Probe: 5'- TGCTTTGAGAGCCAGATGTGGAGG-3'

TCP1-β:
Forward: 5'-TTCTAAGCAGTGGACGAGATGCCT -3'
Reverse: 5'-ACGGTAACAGAGGTAGTGCCATCA -3'
Probe: 5'-ACATTGGTGTTGACAATCCAGCAGCT -3'

TUBB: Hs00742828_s1 from Life Technologies.

U16 snoRNA:
Forward: 5´- CTTGCAATGATGTCGTAATTTGC -3´
Reverse: 5´- TCGTCAACCTTCTGTACCAGCTT -3´
Probe: 5´- TTACTCTGTTCTCAGCGACAGTTGCCTGC -3´

VARS: Hs00389228_m1 from Life Technologies.

Vimentin:
Forward: 5'-TGCAGGAGGAGATGCTTCAGA-3'
Reverse: 5'-TGCCAGAGACGCATTGTCA-3'
Probe: 5'-AGAGGAAGCCGAAAACACCCTGCAA-3'

ZNF9: Hs01077102_g1 from Life Technologies.

Plasmids:
Plasmids for over-expression of La (EX-G0043-M02) and NPM1 (EX-Z2182-M02) were purchased from Genecopoeia.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. **Reduction of ACLY levels did not significantly alter ASO activity**. **A**) Levels of ACLY were reduced by siRNA treatment as confirmed for mRNA (left panel) and protein (right panel) by qRT-PCR and western analysis, respectively. VARS served as a loading control. UTC, mock transfected cells. **B-D**) Reduction of ACLY levels did not alter activity of ASOs targeting (B) *NCL1*, (C) *drosha*, or (D) Malat1 RNAs compared to control cells as determined by qRT-PCR. The error bars indicate standard deviations of three independent experiments.

Figure S2. **Reduction of eIF4H or KCTD12 levels did not significantly alter ASO activity**. **A**) Levels of eIF4H and KCTD12 were reduced by siRNA treatment as shown by qRT-PCR. UTC, mock transfected cells. **B-D**) Reduction of eIF4H or KCTD12 levels did not have a significant effect on the activity of ASOs targeting (B) *NCL1*, (C) *drosha*, or (D) Malat1 compared to control cells as determined by qRT-PCR. The error bars indicate standard deviations of three independent experiments.

Figure S3. Reduction of ATAD3A did not alter ASO activity. **A**) Reduction of ATAD3A by siRNA treatment was confirmed for mRNA (left panel) and protein (right panel) by qRT-PCR and western analysis, respectively. UTC, mock transfected cells. Tubulin served as a loading control for western. **B-D**) Reduction of ATAD3A levels did not significant alter the activity of ASOs targeting (B) *drosha*, (C) *NCL1*, or (D) Malat1 RNAs compared to control cells as determined by qRT-PCR. The error bars indicate standard deviation of three independent experiments.

Figure S4. Reduction in GRSF1 levels did not alter ASO activity. **A**) Reduction of GRSF1 was confirmed for mRNA (left panel) and protein (right panel) by qRT-PCR and western analysis, respectively. GAPDH served as a loading control for western. Mock transfected (UTC) and treatment with *luciferase* siRNA were used as controls. **B-D**) Reduction of GRSF1 did not alter activity of two different ASOs targeting *NCL1* or an ASO targeting Malat1 RNA compared to controls as determined by qRT-PCR. The error bars indicate standard deviation of three independent experiments.

Figure S5. Under the experimental conditions, reduction of La or NPM1 levels did not significantly impact their endogenous functions. **A**) Depletion of La protein by siRNA treatment for 48 hr did not significantly affect the levels of polymerase III transcribed 7SL RNA or $tRNA^{tys}$, as determined by northern hybridization. A polymerase II transcribed RNA, U16 snoRNA, was used as a control for loading. **B**) Reduction of NPM1 by siRNA treatment did not significantly affect the level of steady-state rRNAs as determined by gel electrophoresis and ethidium bromide staining. Levels of 7SL RNA from the same gel was determined by northern hybridization. **C**) Reduction of NPM1 did not significantly affect the subcellular distribution of RPL5 protein. Cytoplasmic and nuclear fractions were separated and the proteins were analyzed by western. hnRNP K and GAPDH served as nuclear and cytoplasmic markers, respectively, and ACTB was used as a loading control.

Figure S6. Reduction of NARS did not affect ASO activity. **A)** Reduction of *NARS* mRNA levels with either of two siRNAs was confirmed by qRT-PCR. **B-D**) Reduction of NARS levels had no significant effect on the activity of ASOs targeting (B) *NCL1*, (C) *drosha*, or (D) U16 RNAs as determined by qRT-PCR. UTC, mock transfected control cells. The error bars indicate standard deviations of three independent experiments.

Figure S7. Reduction of PC4 moderately reduced ASO activity. **A**) Reduction of *PC4* mRNA by siRNA treatment was confirmed by qRT-PCR. UTC, mock transfected cells. **B-D**) Reduction of PC4 levels moderately reduced the activities of ASOs targeting (B) *NCL1*, (C) *drosha*, or (D) U16 RNAs as determined by qRT-PCR. **E**) Reduction of PC4 using two different siRNAs was confirmed by western analysis. γ-tubulin served as a loading control. **F**) Reduction of PC4 using two different siRNAs led to similar effects on ASO activity, as exemplified for a *drosha*-targeting ASO. Where shown, the error bars indicate standard deviations of three independent experiments.

Figure S8. Reduction of La, Ku70, and ANXA2 proteins in HEK293 cells also affected ASO activity. A) Reduction of La, Ku70, and ANXA2 by siRNA treatment was confirmed by qRT-PCR. UTC, control cells transfected with luciferase siRNA. siRNA treated HEK293 cells were reseeded and either transfected (panels B-D) or incubated by free uptake with an ASO targeting NCL1 mRNA (panel E). B) Reduction of La decreased the antisense activity of an ASO targeting NCL1 mRNA. C) Reduction of Ku70 increased the NCL1 ASO activity. D) Reduction of ANXA2 reduced the ASO activity. P-values were calculated based on unpaired *t* test (n=3), and the significance is indicated above the bars. "NS", not significant (P>0.05). "*", 0.01<P<0.05; "**", 0.001<p<0.01; "***", 0.0001<P<0.001; and "****", 0.00001<P<0.0001. E) Reduction of La, Ku70, or ANXA2 affected the ASO activity upon free uptake, as determined by qRT-PCR for the targeted NCL1 mRNA. P-values of the group difference were calculated based on two-tailed, paired *t* test, and is indicated beside the curves. The error bars indicate standard deviations of three independent experiments.

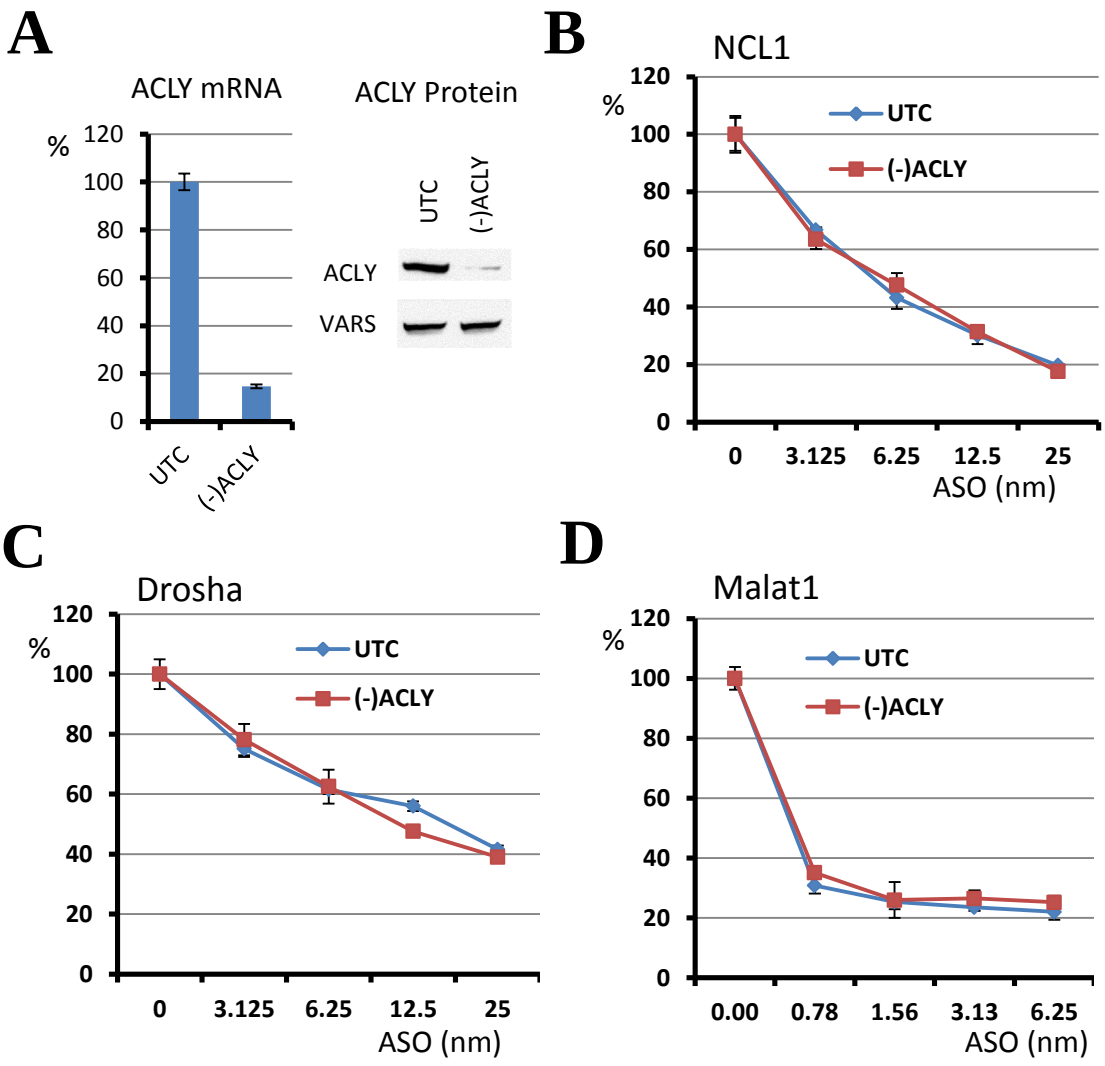
Figure S9. **A**) RNase H1 protein levels were not affected by treatment of cells with siRNAs targeting indicated genes as determined by western analysis. GRSF1 served as a loading control. **B**) Transfection of ASOs did not significantly affect the levels of RNase H1 or indicated ASO-binding proteins. HeLa cells were either mock transfected or transfected with 50 nM ASO116847; protein levels were detected by western analyses. **C**) La, NPM1, VARS, ANXA2, PC4, and Ku70/Ku80 proteins do not stably interact with RNase H1. Whole cell lysates prepared from Flag-H1 over-expressing cells were incubated with ASO116847 or ASO116847/XL279 duplex (20 nM/5 mg protein lysate/IP reaction) and subjected to immunoprecipitation using anti-Flag beads. The co-isolated proteins were eluted with Flag peptide and analyzed by western analysis.

Figure S10. A) Reduction of VARS altered lysosome patterns. The arrows indicate condensed lysosome structures. **B**) Reduction of VARS caused lysosome staining patterns to condense toward perinuclear structures in the absence of ASOs. HeLa cells were treated with two different siRNAs targeting VARS for 24 hrs and were stained for

Lamp1. Scale bars: 10 μm. **B**) ANXA2 co-localized with ASOs in cytoplasmic loci upon transfection. HeLa cells were transfected with 50 nM ASO446654 for 8 hr and stained for ANXA2. The co-localization of aggregated ANXA2 and ASO is exemplified by arrows. Scale bars: 20 μm. **C**) ANXA2 and ASO co-localized in Lamp1-containing structures upon transfection. HeLa cells transfected for 8 hr with 50 nM ASO446654 were co-stained for ANXA2 and Lamp1. The co-localization of ANXA2, ASO, and Lamp1 is indicated with arrows. Scale bars: 10 μm. D) ANXA2 co-localized with ASOs 4 hr after ASO incubation, but not at 2 hr after ASO incubation. Scale bars: 20 μm for 2hr and 10 μm for 4 hr images.

Figure S11. ANXA2 was not enriched in early endosomes upon ASO incubation. HeLa cells were incubated with 2 μM ASO446654 for 24 hr, fixed, and co-stained for ANXA2 and EEA1 proteins. The localization of ASOs in some EEA1-stained early endosomes is exemplified by arrows. Scale bars: 10 μm.

Figure S1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ASO (nm)
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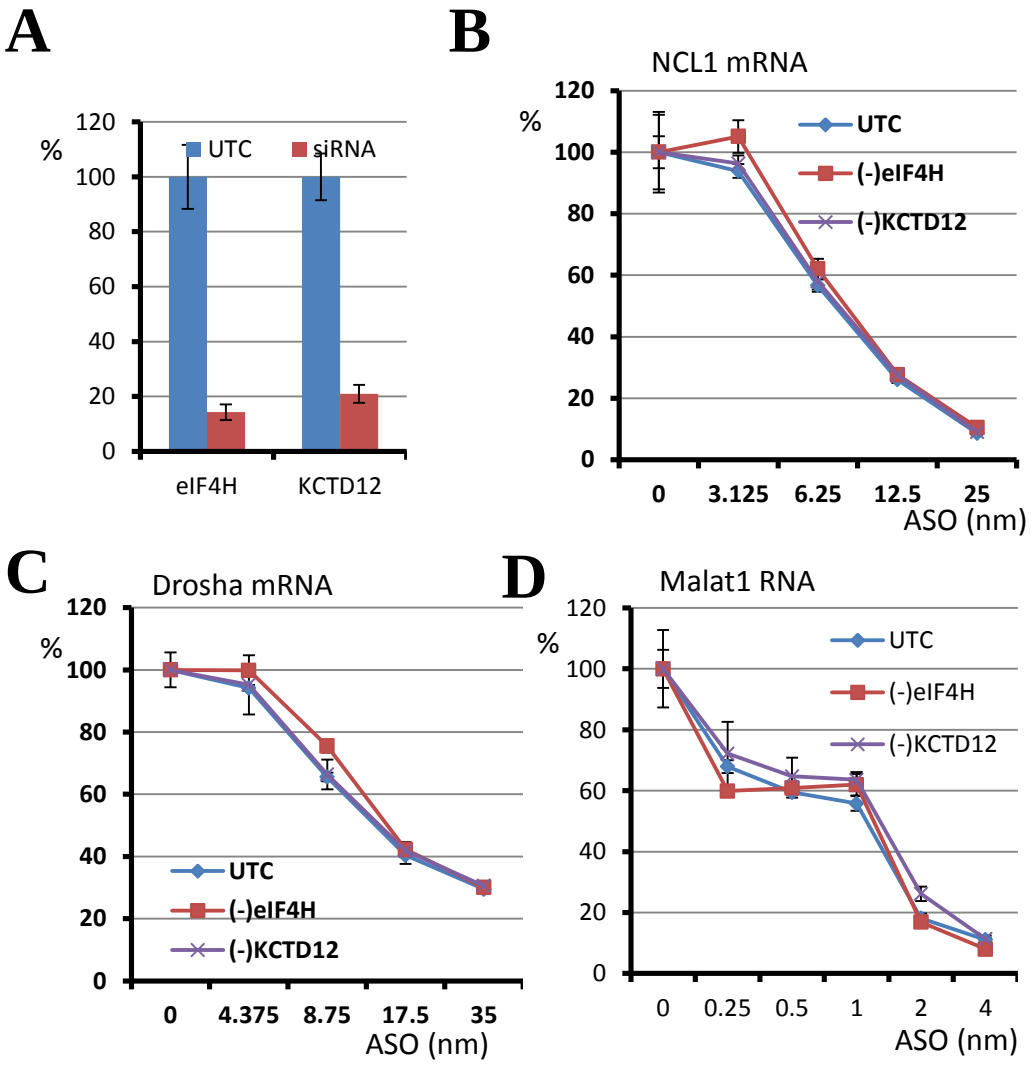
ASO (nm)

Figure S2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ASO (nm)

Figure S3

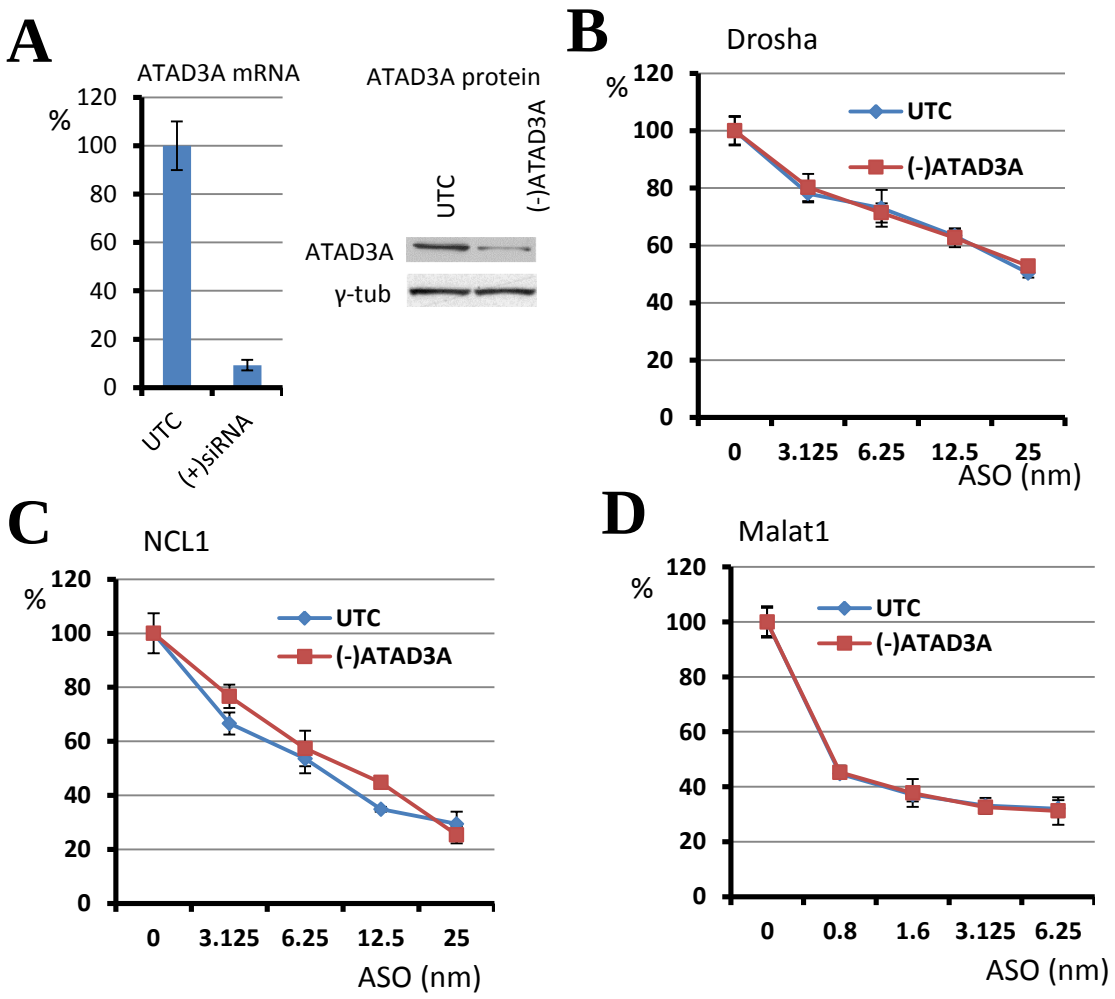
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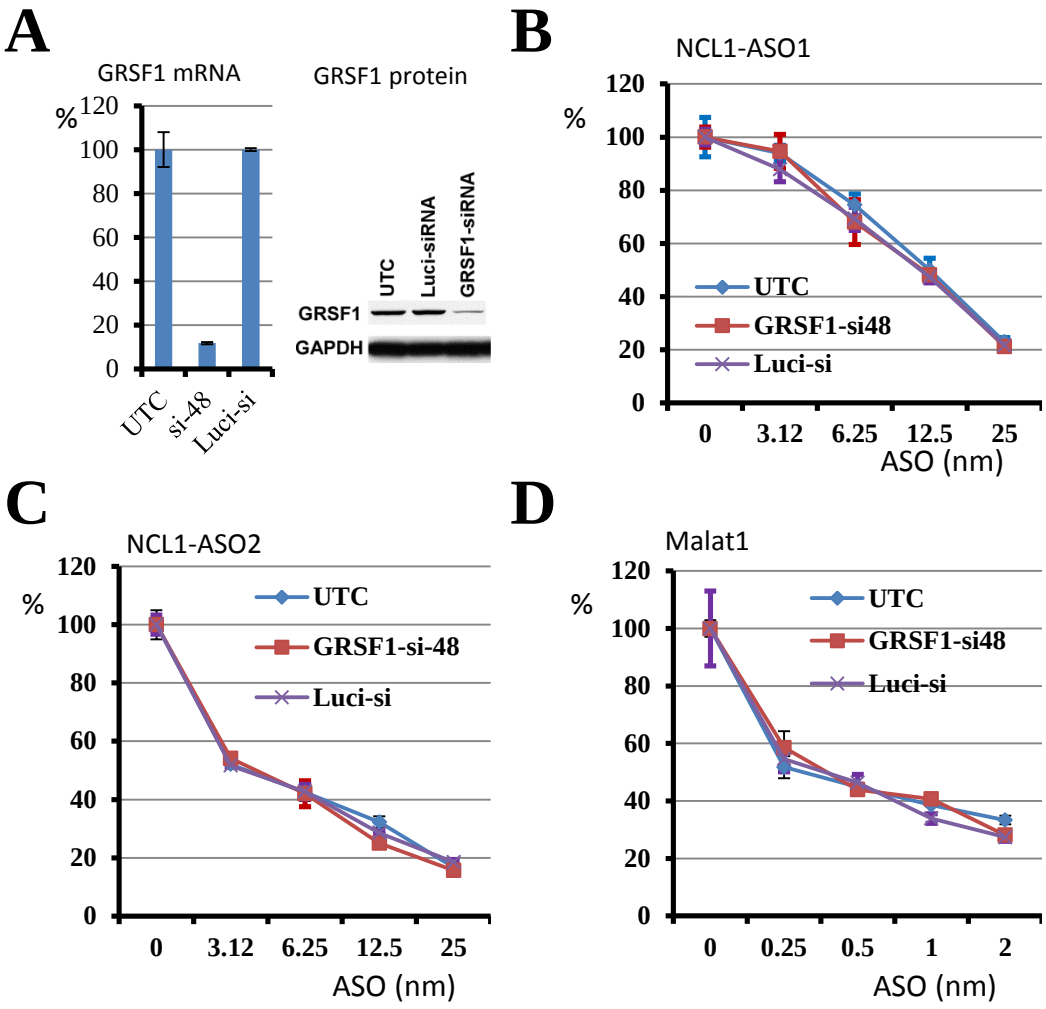
Figure S4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2
ASO (nm)

Figure S5

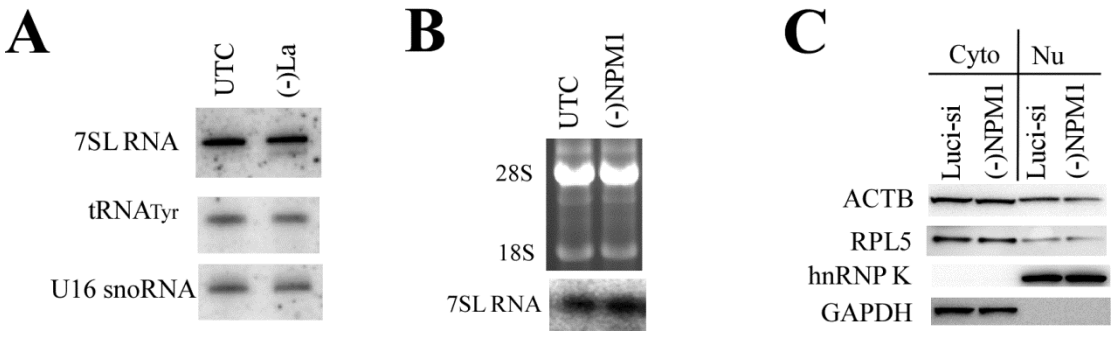
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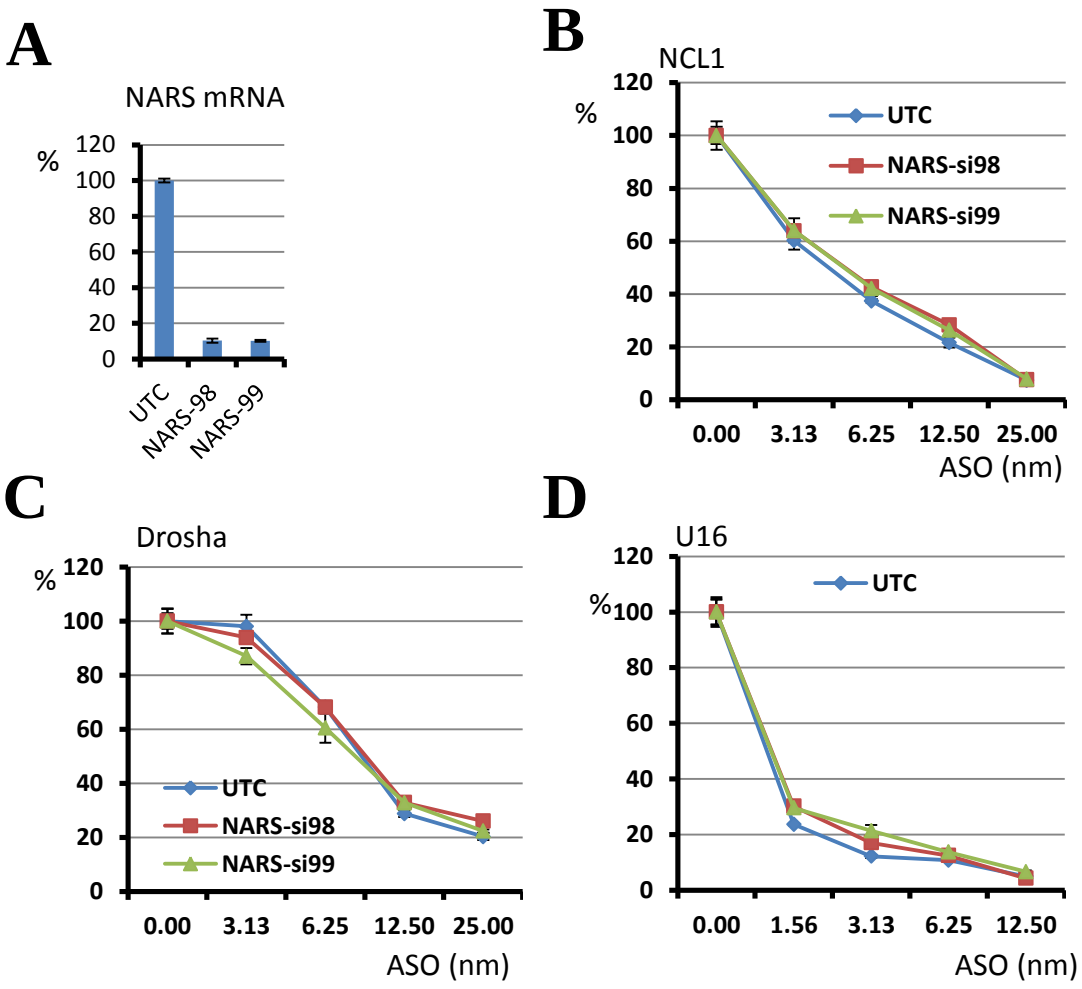
Figure S6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6.25
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25.00
ASO (nm)
C
Drosha
%
UTC
NARS-si98
NARS-si99
0.00
3.13
6.25
12.50
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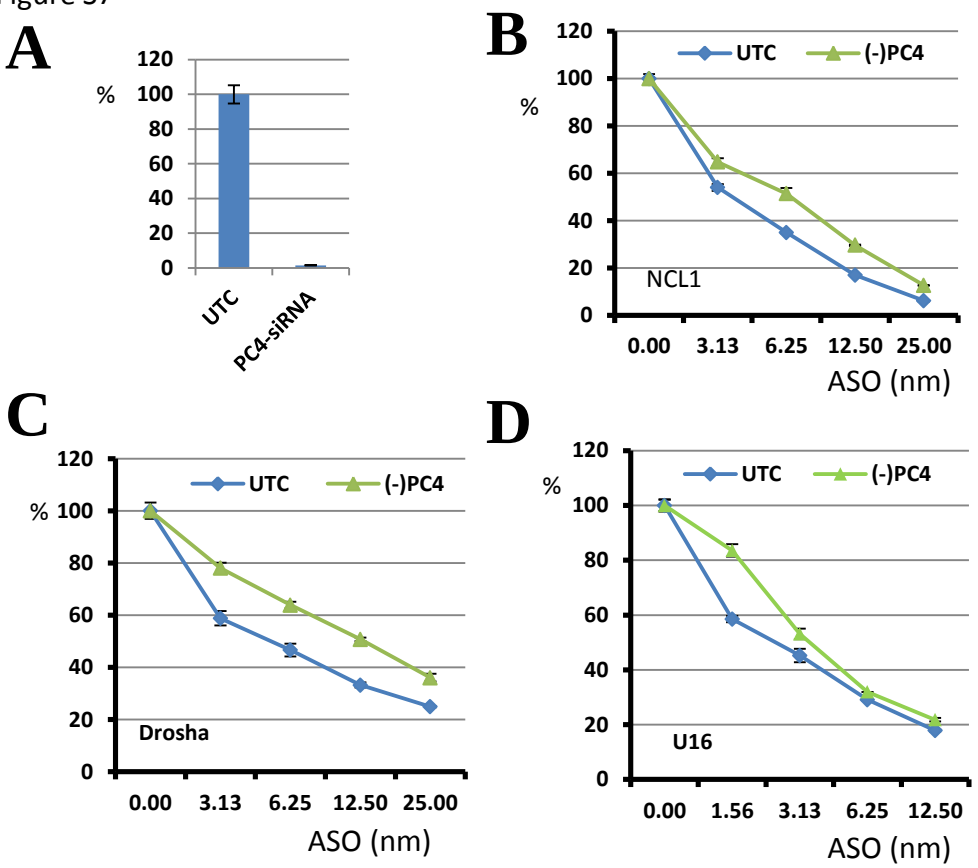
6.25
12.50
ASO (nm)

Figure S7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25.00
ASO (nm)
C
%
UTC
(-)PC4
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3.13
6.25
12.50
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%
UTC
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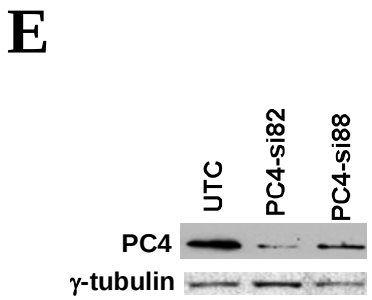
12.50
ASO (nm)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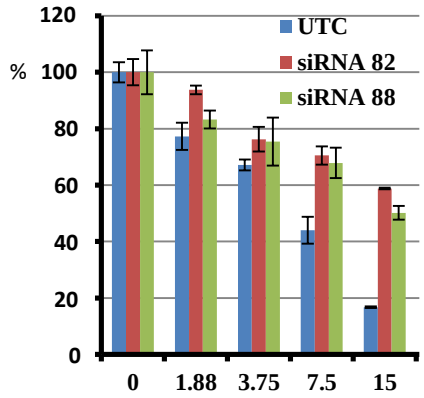
γ-tubulin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1.88
3.75
7.5
15

Figure S8

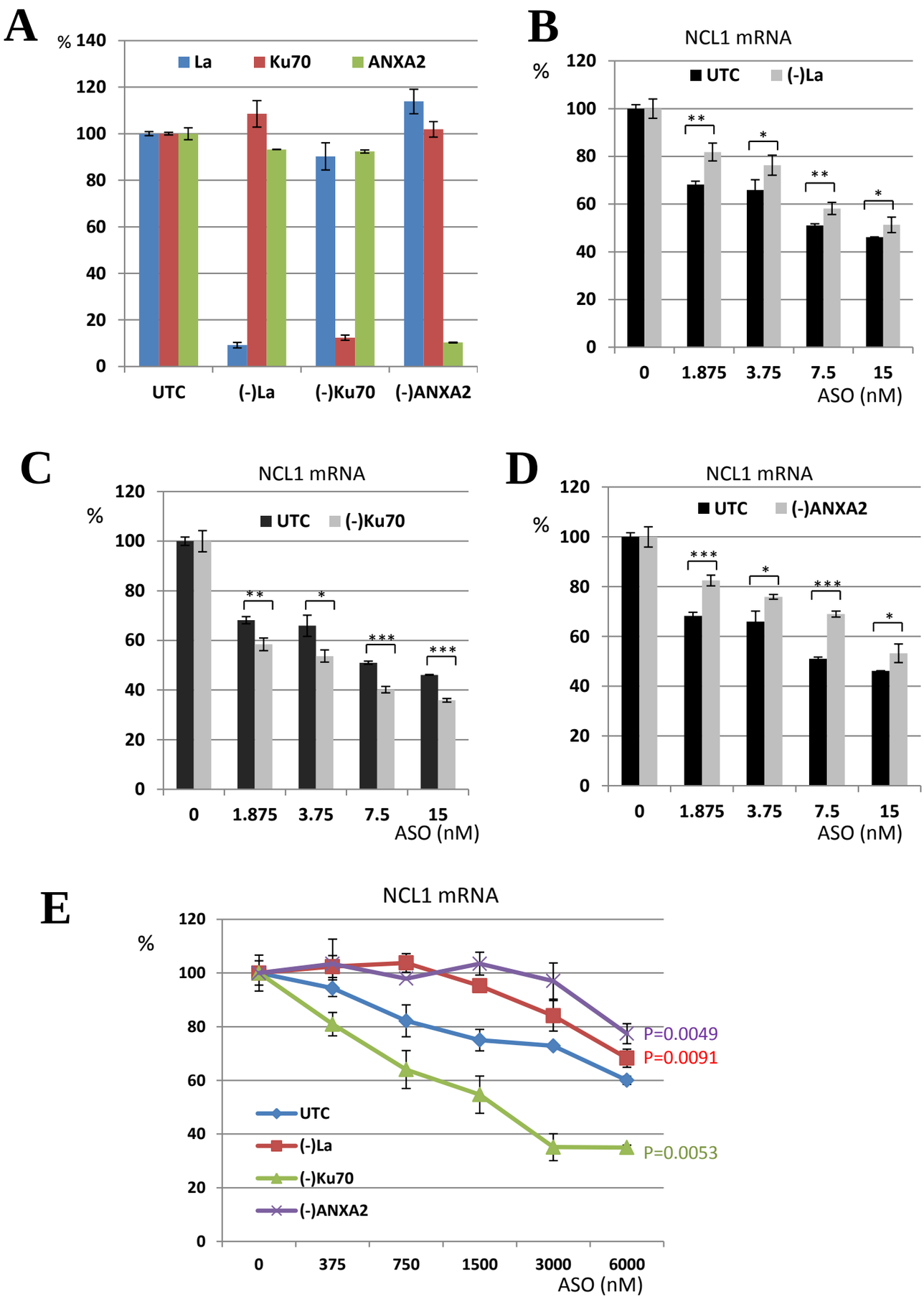

A
% 140
120
100
80
60
40
20
0
La
Ku70
ANXA2
UTC
(-)La
(-)Ku70
(-)ANXA2
B
NCL1 mRNA
%
120
100
80
60
40
20
0
UTC
(-)La
**
*
**
*
0
1.875
3.75
7.5
15
ASO (nM)
C
NCL1 mRNA
%
120
100
80
60
40
20
0
UTC
(-)Ku70
**
*
***
***
0
1.875
3.75
7.5
15
ASO (nM)
D
NCL1 mRNA
%
120
100
80
60
40
20
0
UTC
(-)ANXA2
***
*
***
*
0
1.875
3.75
7.5
15
ASO (nM)
E
NCL1 mRNA
%
120
100
80
60
40
20
0
UTC
(-)La
(-)Ku70
(-)ANXA2
P=0.0049
P=0.0091
P=0.0053
0
375
750
1500
3000
6000
ASO (nM)

Figure S9

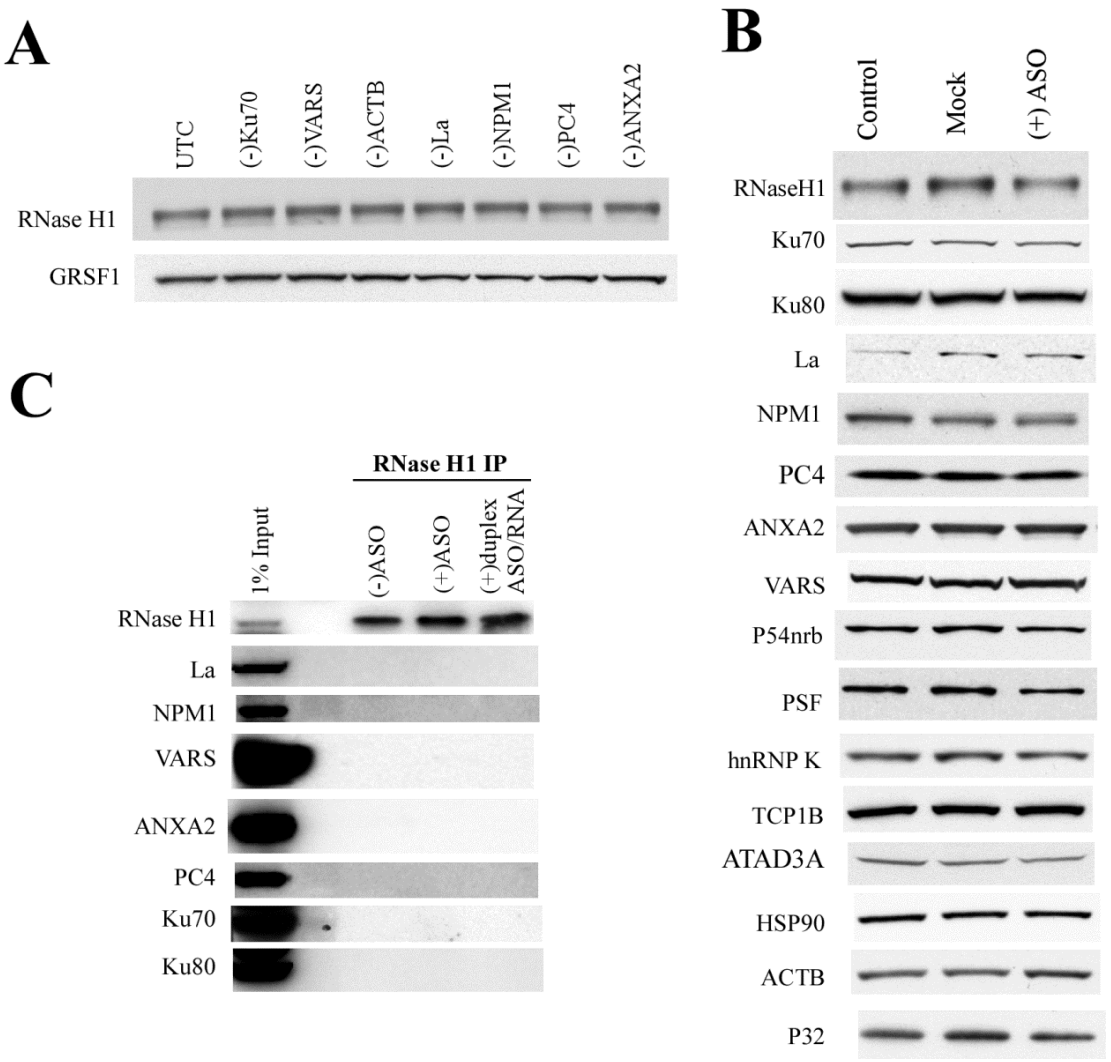

A
UTC
(-)Ku70
(-)VARS
(-)ACTB
(-)La
(-)NPM1
(-)PC4
(-)ANXA2
RNase H1
GRSF1
B
Control
Mock
(+) ASO
RNaseH1
Ku70
Ku80
La
NPM1
PC4
ANXA2
VARS
P54nrb
PSF
hnRNP K
TCP1B
ATAD3A
HSP90
ACTB
P32
C
RNase H1 IP
1% Input
(-)ASO
(+)ASO
(+)duplex ASO/RNA
RNase H1
La
NPM1
VARS
ANXA2
PC4
Ku70
Ku80

Figure S10

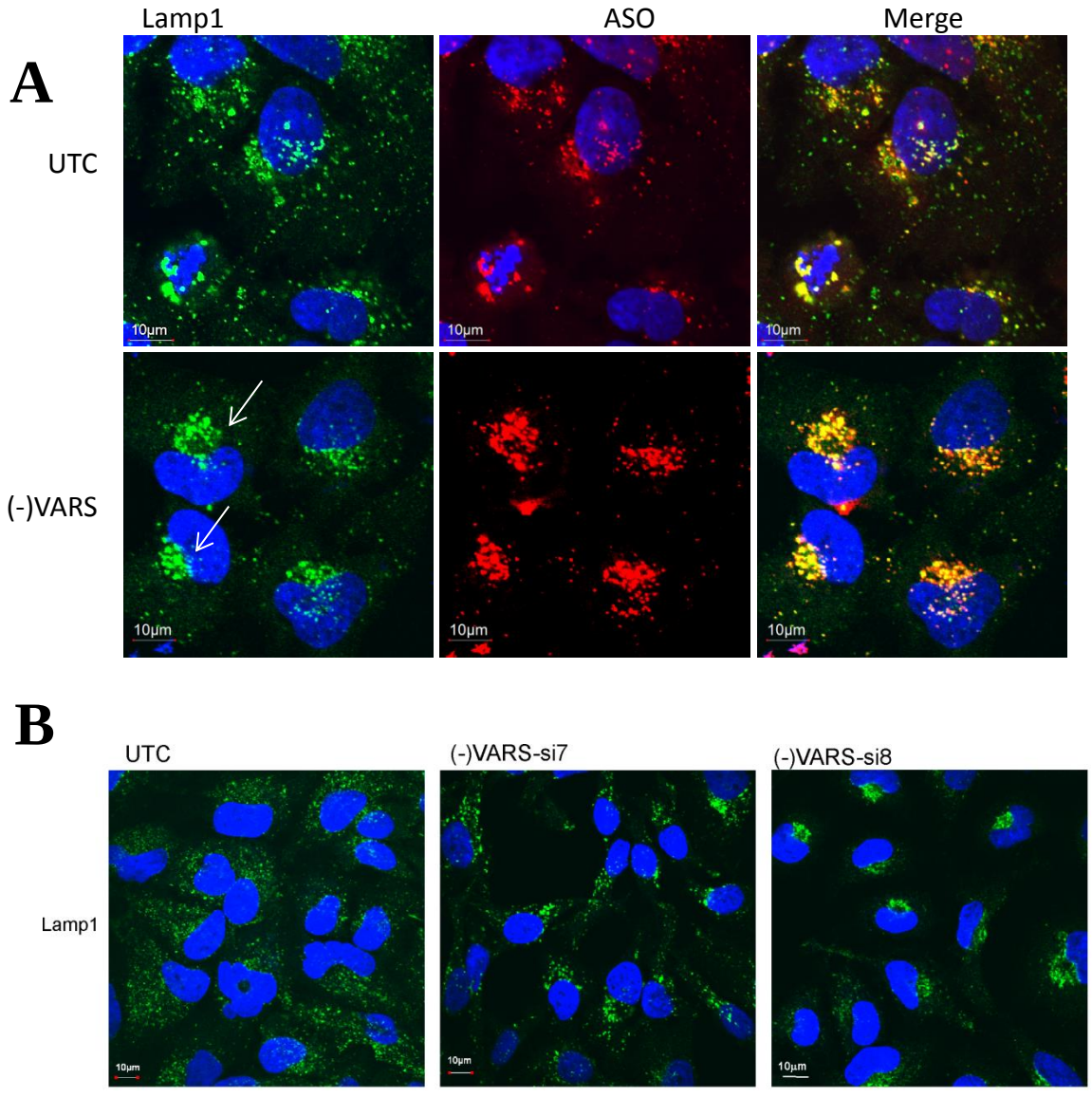
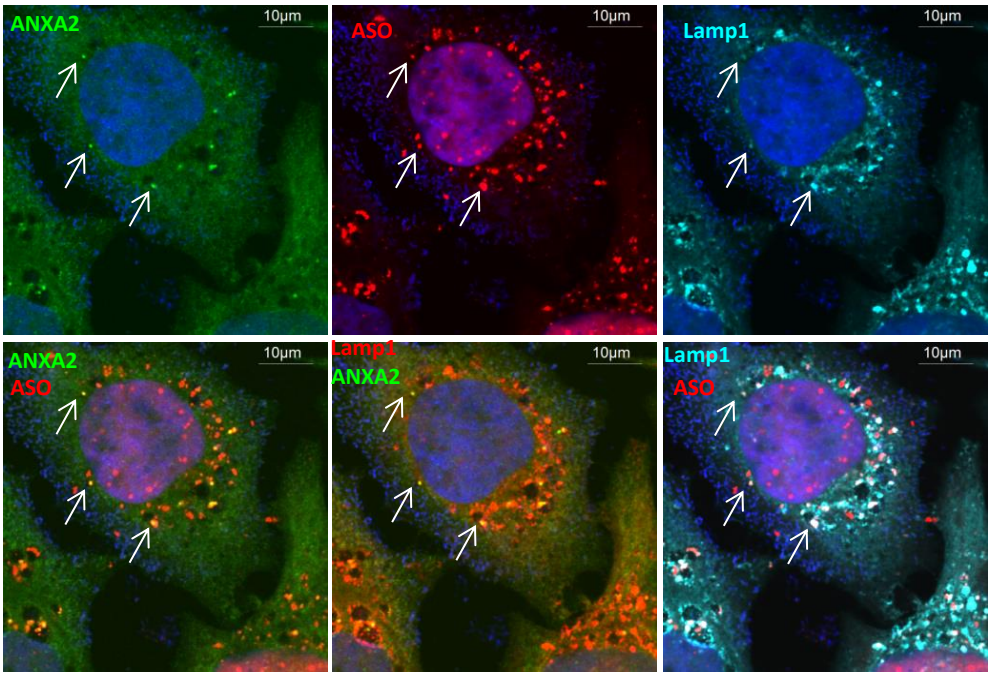

A
Lamp1
ASO
Merge
UTC
(-)VARS
10μm
B
UTC
(-)VARS-si7
(-)VARS-si8
Lamp1
10μm

Figure S10

C



D

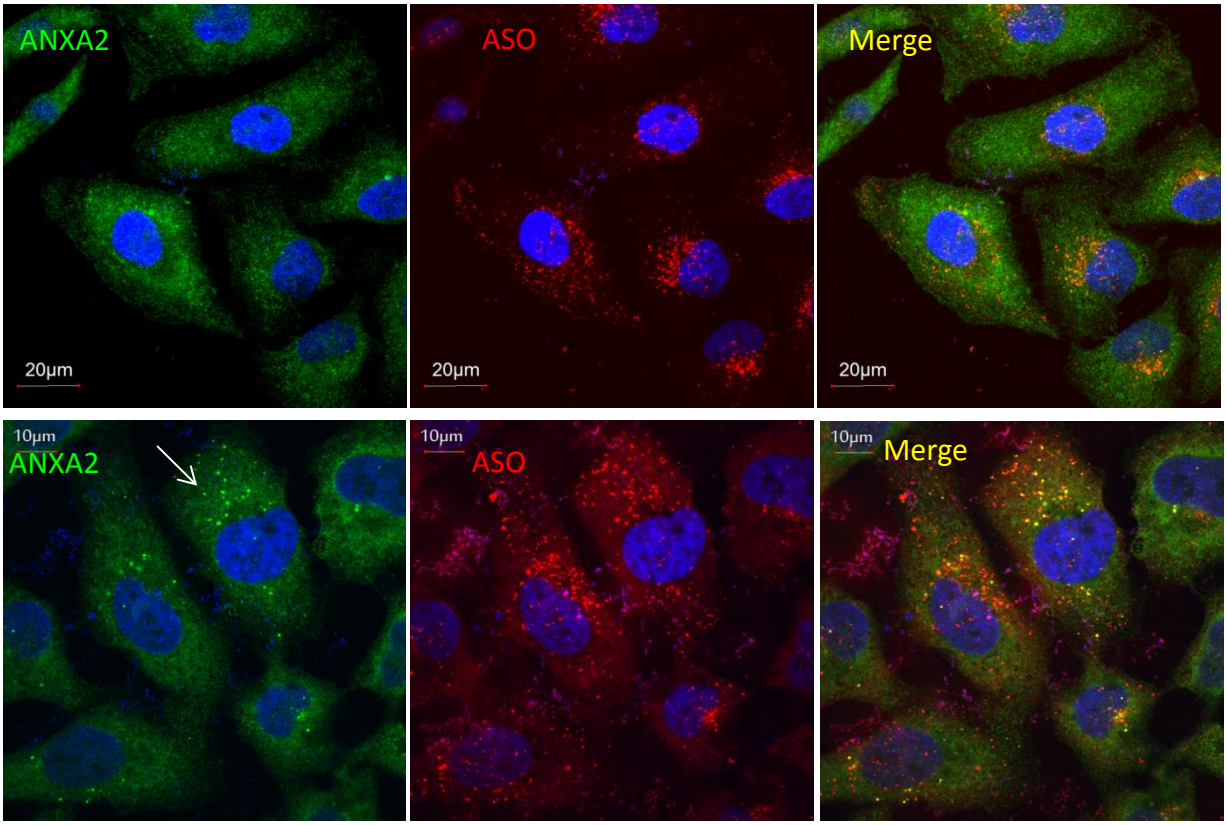
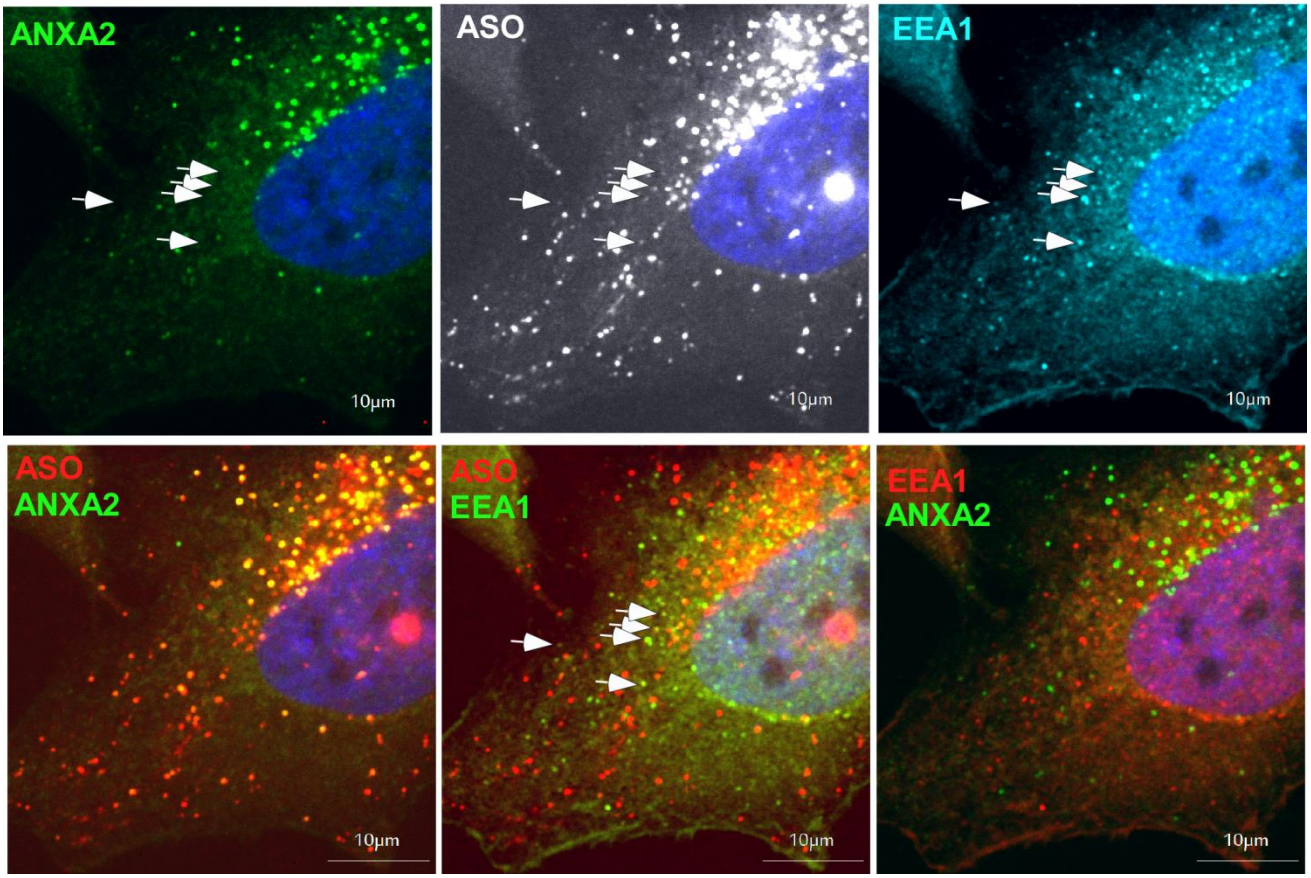


Figure S11


ANXA2
ASO
EEA1
10μm
10μm
10μm
ASO
ANXA2
ASO
EEA1
EEA1
ANXA2
10μm
10μm
10μm