Supplementary materials

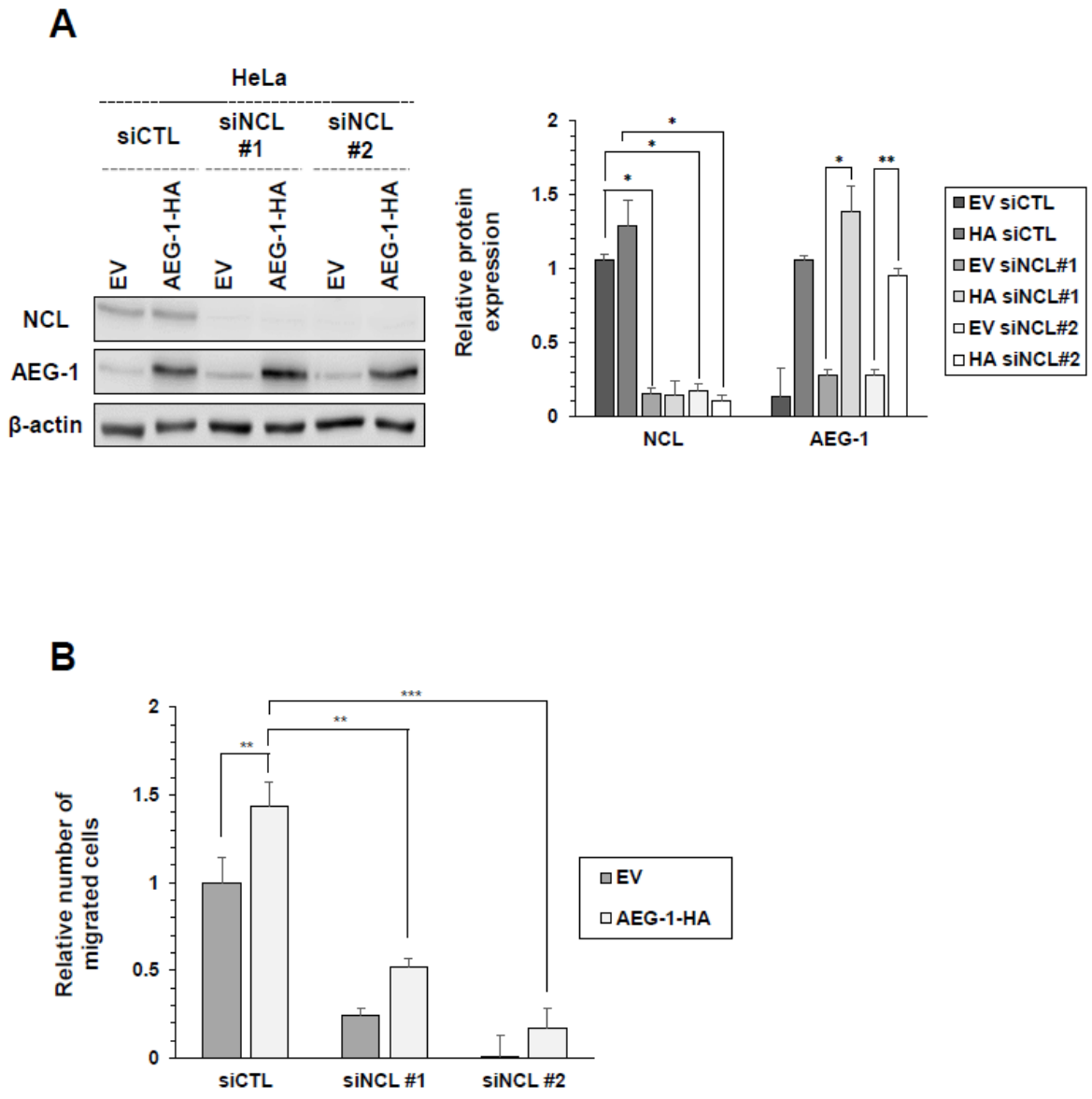
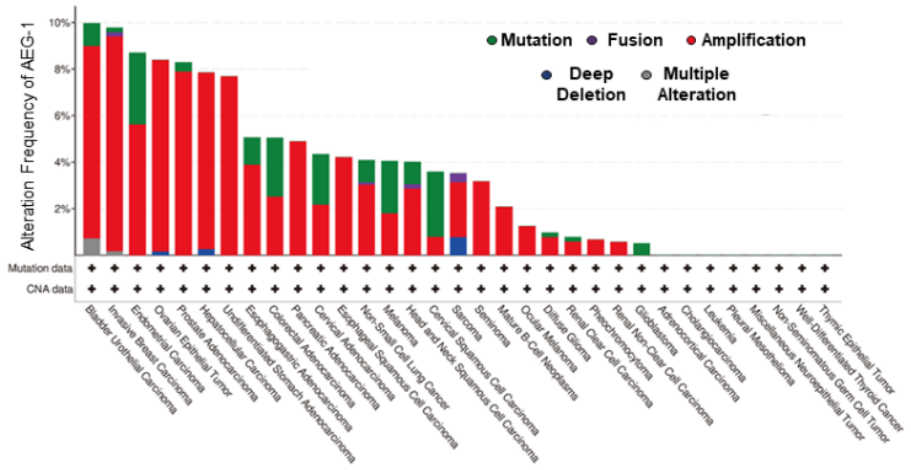
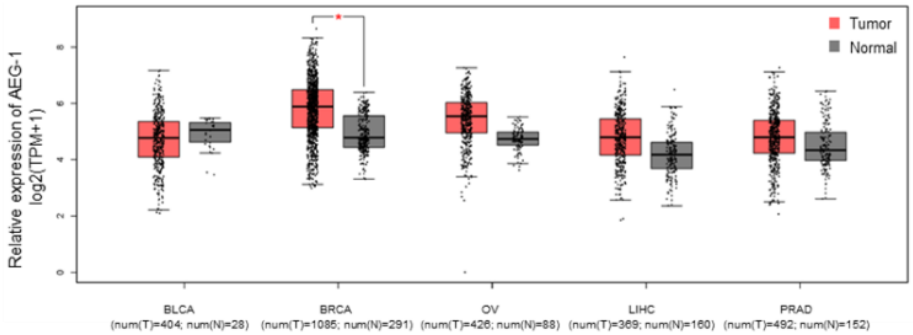


Figure S1. Supporting data for figure 2. (A) AEG-1 overexpression and NCL knockdown in figure 2 were validated by western blot. Relative band intensities normalized by β-actin are shown below. EV: empty vector. HA: AEG-1-HA. (B) Quantification of transwell migration assay results presented in figure 2A. Average cell numbers counted from 5 independent microscopic fields are shown. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ with mean ± SE are shown.

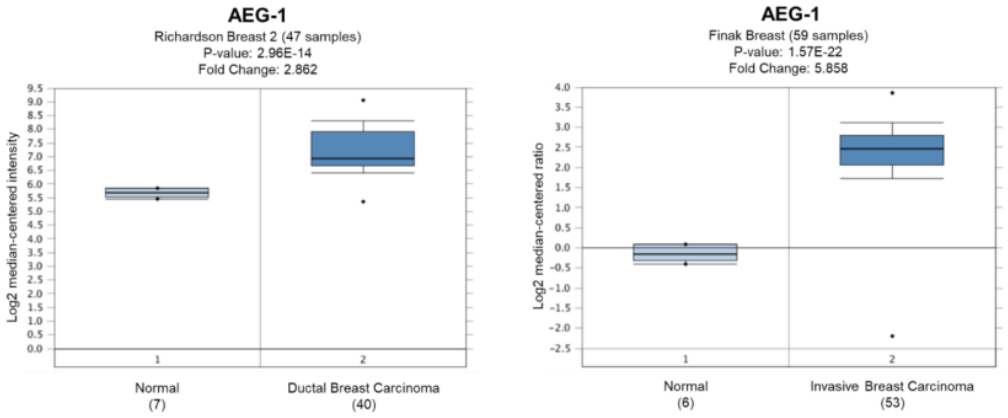
A


Alteration Frequency of AEG-1
10%
8%
6%
4%
2%
Mutation
Fusion
Amplification
Deep Deletion
Multiple Alteration
Mutation data
CNA data
Bladder Urothelial Carcinoma
Invasive Breast Carcinoma
Endometrial Carcinoma
Ovarian Epithelial Tumor
Prostate Adenocarcinoma
Hepatocellular Carcinoma
Undifferentiated Stomach Adenocarcinoma
Esophagogastric Adenocarcinoma
Colorectal Adenocarcinoma
Pancreatic Adenocarcinoma
Cervical Adenocarcinoma
Esophageal Squamous Cell Carcinoma
Non-Small Cell Lung Cancer
Melanoma
Head and Neck Squamous Cell Carcinoma
Cervical Squamous Cell Carcinoma
Sarcoma
Seminoma
Mature B-Cell Neoplasms
Ocular Melanoma
Diffuse Glioma
Renal Clear Cell Carcinoma
Pheochromocytoma
Renal Non-Clear Cell Carcinoma
Glioblastoma
Adrenocortical Carcinoma
Cholangiocarcinoma
Leukemia
Pleural Mesothelioma
Miscellaneous Neuroepithelial Tumor
Non-Seminomatous Germ Cell Tumor
Well-Differentiated Thyroid Cancer
Thymic Epithelial Tumor

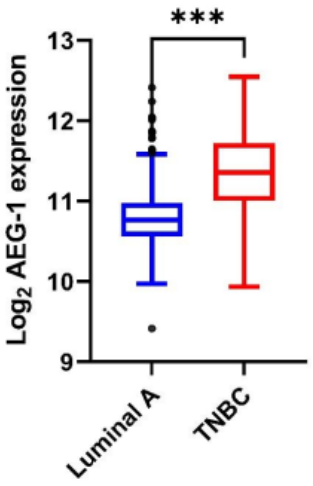
B


Relative expression of AEG-1
log2(TPM+1)
Tumor
Normal
BLCA
(num(T)=404; num(N)=28)
BRCA
(num(T)=1085; num(N)=291)
OV
(num(T)=426; num(N)=88)
LIHC
(num(T)=369; num(N)=160)
PRAD
(num(T)=492; num(N)=152)

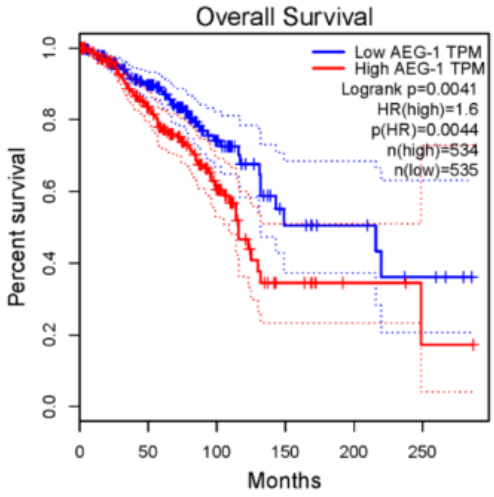
C


AEG-1
Richardson Breast 2 (47 samples)
P-value: 2.96E-14
Fold Change: 2.862
Log2 median-centered intensity
Normal
(7)
Ductal Breast Carcinoma
(40)
AEG-1
Finak Breast (59 samples)
P-value: 1.57E-22
Fold Change: 5.858
Log2 median-centered ratio
Normal
(6)
Invasive Breast Carcinoma
(53)

D


***
13
12
11
10
9
Log2 AEG-1 expression
Luminal A
TNBC

E


Overall Survival
Low AEG-1 TPM
High AEG-1 TPM
Logrank p=0.0041
HR(high)=1.6
p(HR)=0.0044
n(high)=534
n(low)=535
Percent survival
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
Months

F

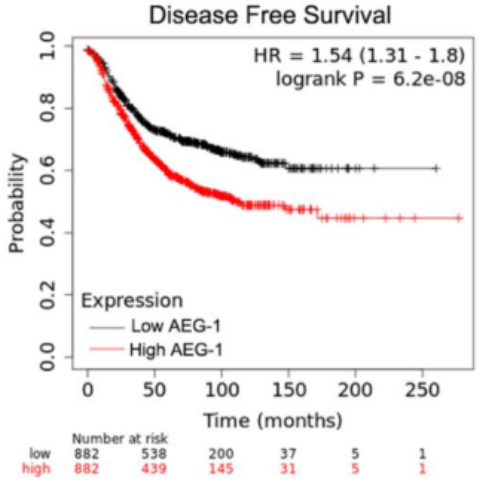
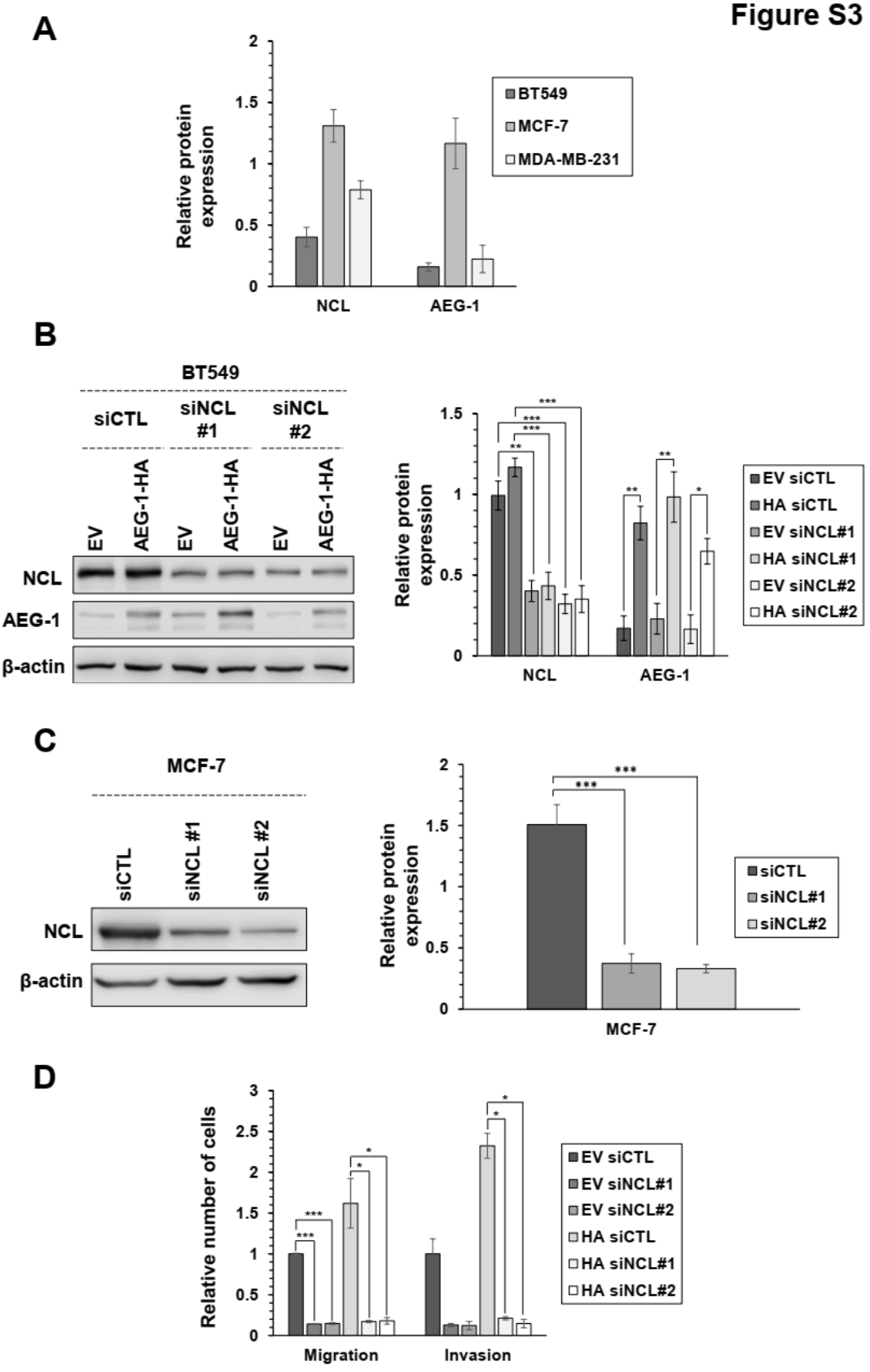
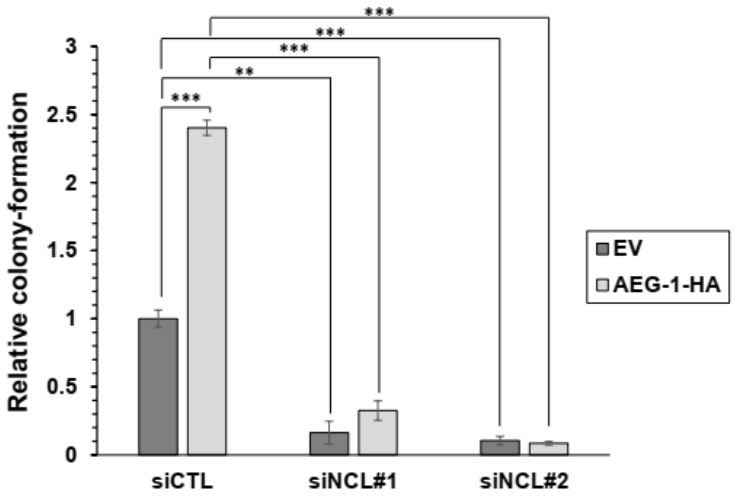

Disease Free Survival
HR = 1.54 (1.31 - 1.8)
logrank P = 6.2e-08
Probability
0.0
0.2
0.4
0.6
0.8
1.0
Expression
Low AEG-1
High AEG-1
0
50
100
150
200
250
Time (months)
Number at risk
low 882 538 200 37 5 1
high 882 439 145 31 5 1

Figure S2. AEG-1 is aberrantly expressed in breast cancer and is associated with a poor prognosis. (A) The TCGA database shows multiple abnormalities of the AEG-1 gene present in most cancers, including breast cancer. (B) The GEPIA database shows that AEG-1 protein levels are significantly upregulated in breast cancer tissues compared to normal (|Log2FC| Cutoff: 1, p-value Cutoff: 0.01). (C) The Oncomine database shows that AEG-1 mRNA expression levels are upregulated in breast cancer. (D) The GENT2 database shows AEG-1 expression level was upregulated in TNBC (n=252) than luminal A (n=380) subtype samples ($Log_2FC = 0.558$). *** : $p < 0.001$. (E) The GEPIA database shows that high AEG-1 expression is associated with low overall survival (OS) in breast cancer patients. (F) The KM-plotter database shows that high AEG-1 expression is associated with low disease free survival (DFS) in breast cancer patients.

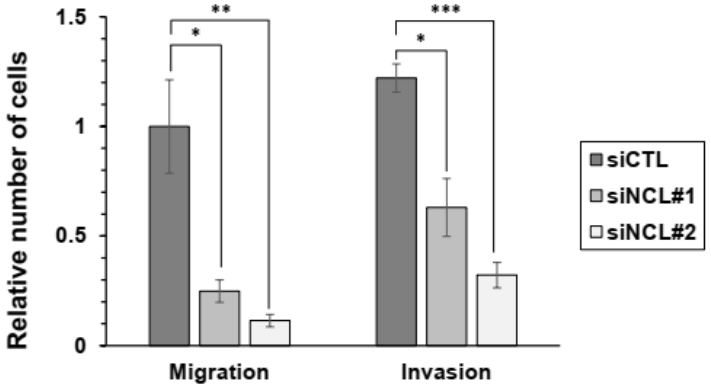
Figure S3


A
Relative protein expression
2
1.5
1
0.5
0
BT549
MCF-7
MDA-MB-231
NCL
AEG-1
B
BT549
siCTL
siNCL #1
siNCL #2
EV
AEG-1-HA
EV
AEG-1-HA
EV
AEG-1-HA
NCL
AEG-1
β-actin
Relative protein expression
1.5
1
0.5
0
***
***
***
**
**
**
*
NCL
AEG-1
EV siCTL
HA siCTL
EV siNCL#1
HA siNCL#1
EV siNCL#2
HA siNCL#2
C
MCF-7
siCTL
siNCL #1
siNCL #2
NCL
β-actin
Relative protein expression
2
1.5
1
0.5
0
***
***
MCF-7
siCTL
siNCL#1
siNCL#2
D
Relative number of cells
3
2.5
2
1.5
1
0.5
0
***
***
*
*
*
*
Migration
Invasion
EV siCTL
EV siNCL#1
EV siNCL#2
HA siCTL
HA siNCL#1
HA siNCL#2

E


Relative colony-formation
3
2.5
2
1.5
1
0.5
0
***
***
***
**
***
siCTL
siNCL#1
siNCL#2
EV
AEG-1-HA

F


Relative number of cells
1.5
1
0.5
0
**
*
***
*
Migration
Invasion
siCTL
siNCL#1
siNCL#2

G

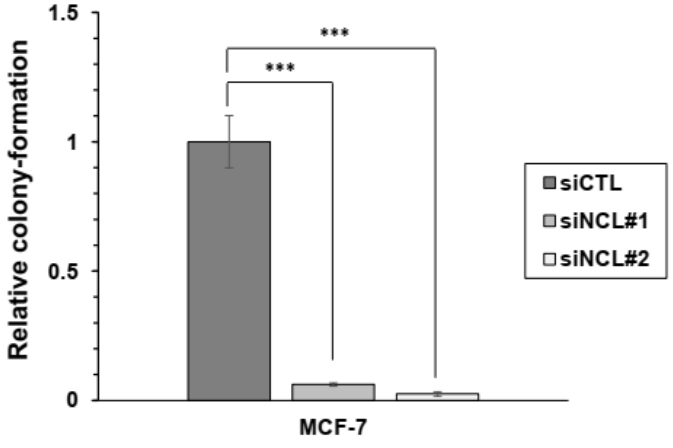

Relative colony-formation
1.5
1
0.5
0
***
***
MCF-7
siCTL
siNCL#1
siNCL#2

Figure S3. Supporting data for figure 3. (A) Quantification of western blot results presented in figure 3B. Relative band intensities normalized by β-actin are shown. (B and C) Validation of AEG-1 overexpression and NCL knockdown in BT549 (B) and MCF-7 (C) cells by western blot. Relative band intensities normalized by β-actin are shown. EV: empty vector. HA: AEG-1-HA. (D-G) Quantification of results presented in figures 3C (D), 3D (E), 3F (F) and 3G (G). *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ with mean ± SE are shown.

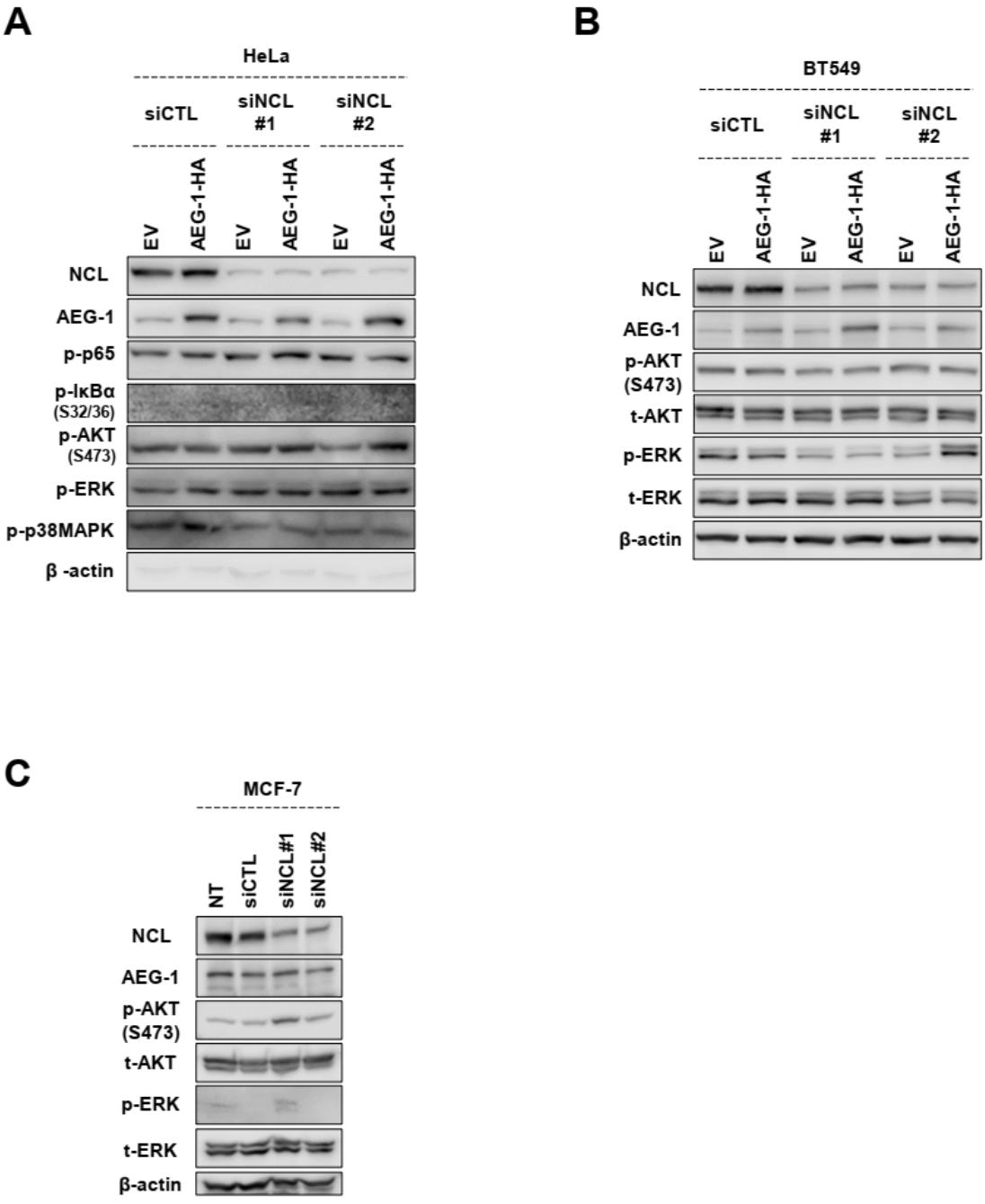


Figure S4. Analysis of AEG-1 downstream phosphoproteins. (A and B) HeLa (A) and BT549 (B) cells were transfected with the indicated siRNAs, then AEG-1-HA or empty vector (EV) plasmids were transfected 2 days later. The phosphorylation of p65, IκBα, AKT, ERK, and p38MAPK was then analyzed by western blot. (C) MCF-7 cells were transfected with siNCL or control siRNA before the phosphorylation of AKT and ERK were analyzed by western blot. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ with mean ± SE are shown.

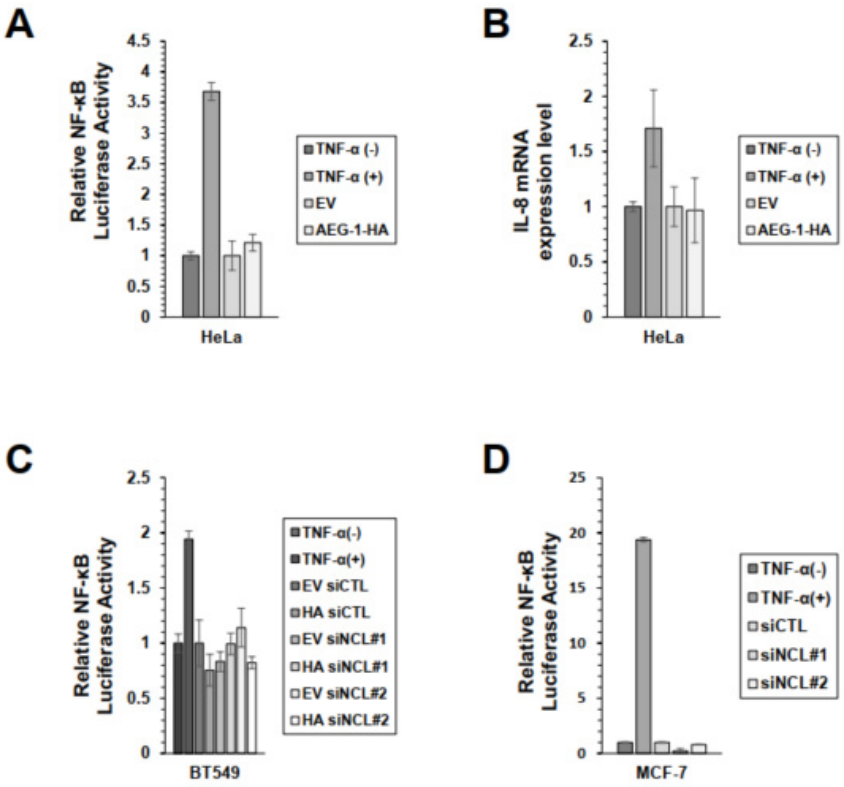


Figure S5. Analysis of NF-κB activity in cells transfected with AEG-1-HA and siNCL. (A and B) NF-κB activation was assessed by luciferase reporter assay (A) and quantitative RT-PCR for human IL-8 (B) in HeLa cells transfected with the AEG-1-HA plasmid. TNF-α treatment was used as a positive control. EV: empty vector. HA: AEG-1-HA. (C and D) NF-κB activation was assessed by luciferase reporter assay in BT549 (C) and MCF-7 (D) cells transfected with the indicated plasmids or siRNAs. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ with mean ± SE are shown.

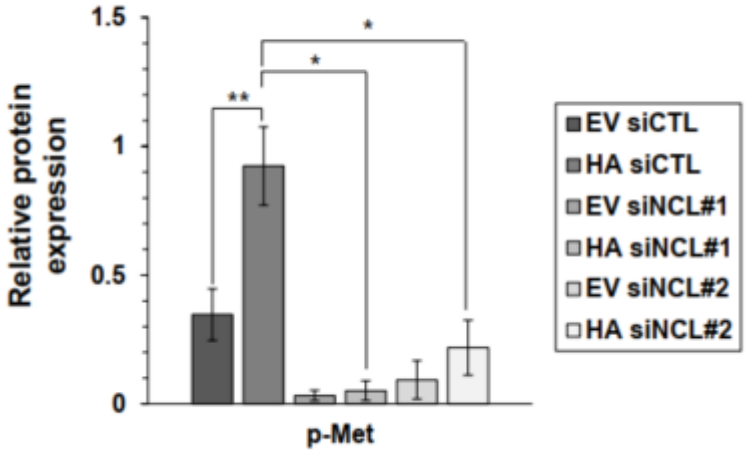


Figure S6. Supporting data for figure 4. Quantification of western blot results presented in figure 4B. Relative band intensities normalized by c-Met are shown. EV: empty vector. HA: AEG-1-HA. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ with mean ± SE are shown.