Expanded View Figures

Figure EV1. No p53-MALAT1 interaction occurs in MCF7 and MCF10A breast cells.

A RIP assays performed in OVCAR-3 cells using an antibody directed to p53. Relative enrichment is calculated as folds over IgG sample, normalized on RPL19 mRNA level. Data are presented as mean ± SEM. Results from three biological replicates are shown.

B Fluorescence high-resolution images of fixed cells, labeled with DAPI (cell nuclei), Alexa Fluor 488 (p53 protein), and Quasar 570 (MALAT1 RNA). Merged images of Alexa Fluor 488 and Quasar 570 signals are shown. Scale bars, 10 μm.

C Western blot analysis of wt-p53 and its phosphorylated form p-p53-Ser15 in the indicated cell lines with or without 8 h treatment with 400 nM adriamycin.

D RT–qPCR analysis of MALAT1 RNA level in MCF10A and MCF7 cells treated or not with adriamycin (ADR) as in (A). Data are presented as mean ± SEM. Results from three biological replicates are shown.

E RIP assays performed in MCF10A and MCF7 cells treated or not with adriamycin as in (A) were performed using antibodies directed to p53 and ID4 proteins. Relative enrichment is calculated as folds over IgG sample, normalized on RPL19 mRNA level. Data are presented as mean ± SEM. Results from three biological replicates are shown.

F Fluorescence high-resolution images of the indicated fixed cell lines, labeled with DAPI (cell nuclei) and Quasar 570 (MALAT1 RNA). Scale bars, 10 μm.

G MALAT1 RNA abundance was evaluated by RT–qPCR in MDA-MB-468 cells depleted of SRSF1 using three different si-RNAs and normalized over GAPDH mRNA. Data are presented as mean ± SEM. Results from three biological replicates are shown. *$P \leq 0.05$ (two-tailed Student's *t*-test). Western blot on the left shows SRSF1 protein levels after si-SRSF1-3 transfection, while SRSF1-1 and SRSF1-2 are shown in Fig 1G.

Source data are available online for this figure.

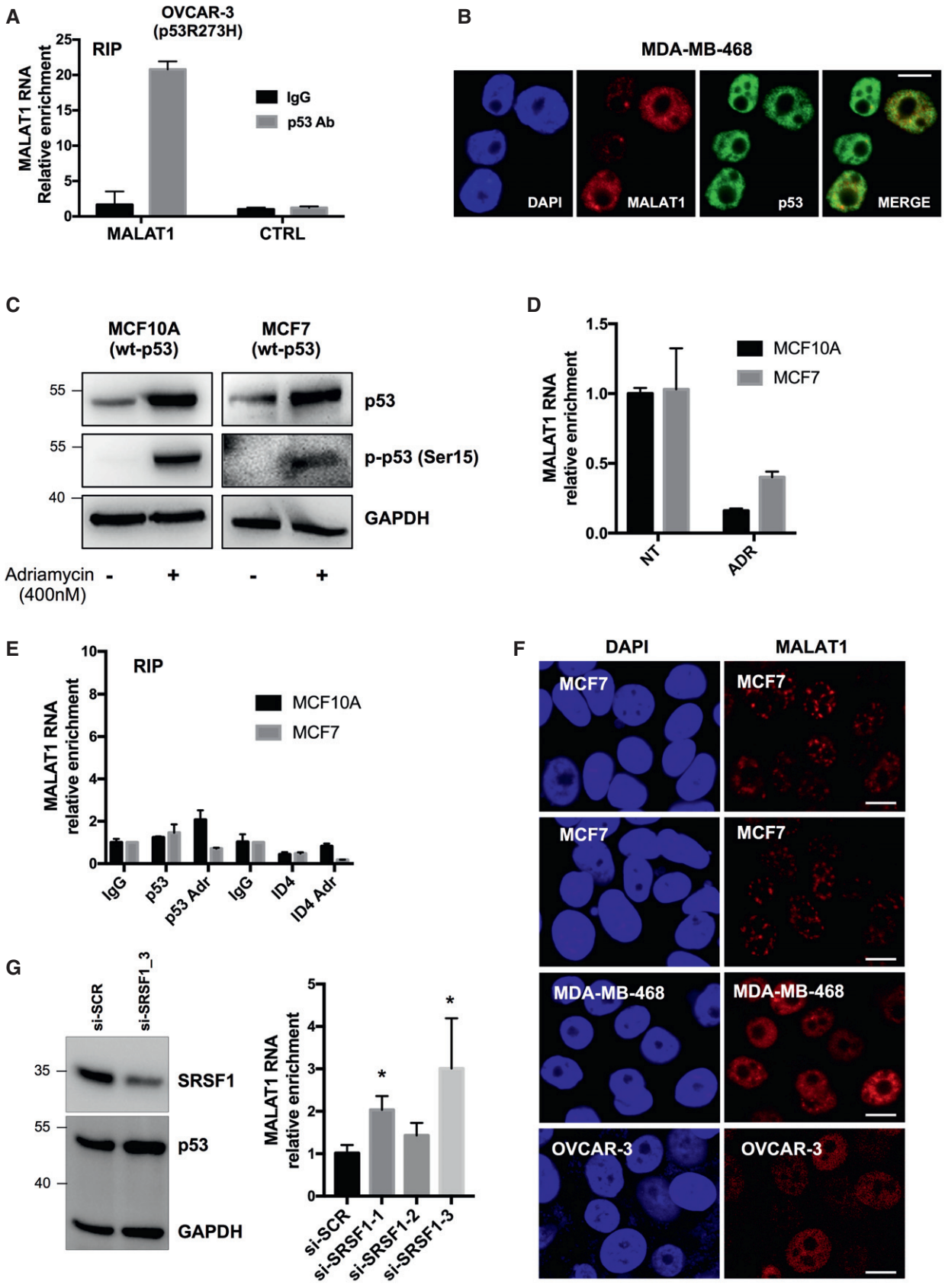

Figure EV1.

Figure EV2. The mutant p53-ID4 interaction is not affected by MALAT1 or SRSF1 depletion.

A, B PLA assays showing interactions SRSF1-p53, SRSF1-ID4, and p53-ID4 in SKBR3 cells depleted or not of MALAT1 RNA expression using two different siRNAs (A). MALAT1 and ID4 level after MALAT1 interference was evaluated by RT–qPCR (B, graphs); p53 and SRSF1 protein level after MALAT1 interference was evaluated by Western blot (B, panels). In (A) the horizontal line represents the median, the box represents the inter-quartile range and 10–90th percentile interval is shown in whiskers. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (two-tailed Student's *t*-test). Data in (B) are presented as mean ± SEM. Results from three biological replicates are shown.

C Representative images of PLA experiments are shown in Figs 2C–E and EV2A. Merged signals of DAPI and PLA are shown in the indicated cells. Scale bars, 0.01 mm.

D PLA assays to evaluate the interaction between p53 and ID4 proteins, performed in the indicated control and SRSF1-depleted cell lines. Results from three biological replicates are shown. The horizontal line represents the median, the box represents the inter-quartile range and 10–90th percentile interval is shown in whiskers.

E Interaction between p53 and ID4 proteins was evaluated by immunoprecipitation of ID4 followed by Western blot of p53 in the indicated cells, depleted or not of SRSF1 expression.

Source data are available online for this figure.

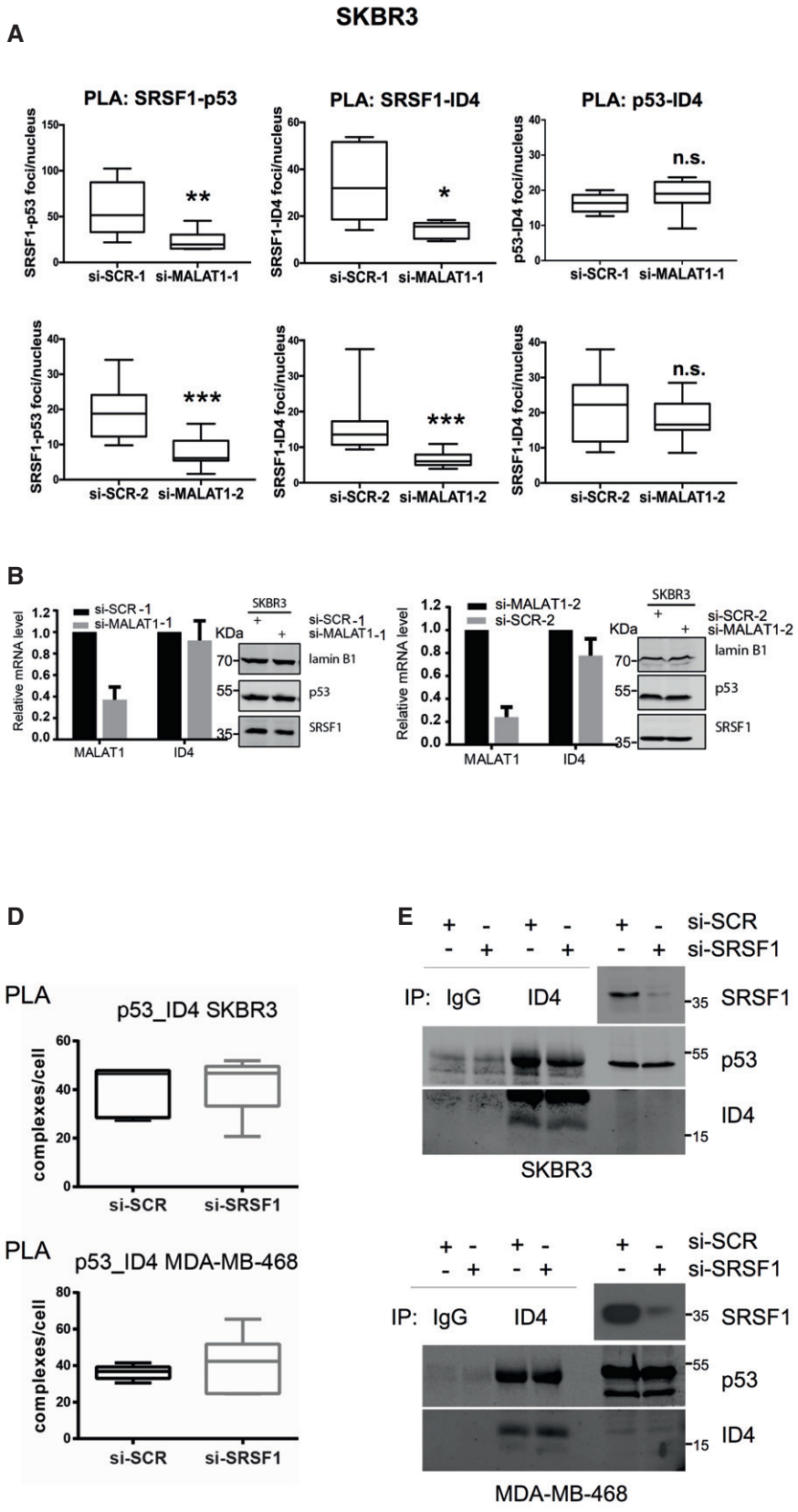
A
SKBR3
PLA: SRSF1-p53
PLA: SRSF1-ID4
PLA: p53-ID4
SRSF1-p53 foci/nucleus
SRSF1-ID4 foci/nucleus
p53-ID4 foci/nucleus
si-SCR-1
si-MALAT1-1
si-SCR-2
si-MALAT1-2
n.s.
B
Relative mRNA level
MALAT1
ID4
KDa
lamin B1
p53
SRSF1
C
si-SCR
si-MALAT1
MDA-MB-468
SKBR3
SRSF1_p53
SRSF1_ID4
p53_ID4
0.01 mm
D
PLA
p53_ID4 SKBR3
p53_ID4 MDA-MB-468
complexes/cell
E
IP: IgG
ID4
SRSF1
p53
SKBR3
MDA-MB-468

Figure EV2.

Figure EV3. Subcellular localization of MALAT1 in breast cancer cells.

A RIP assay performed in control and ID4-depleted MDA-MB-468 cells crosslinked with formaldehyde using an antibody directed to SRSF1 (A96, Santa Cruz). Results from three biological replicates are shown. Data are presented as mean ± SEM. Enrichment of SRSF1 protein on BCL2L1 and BIM pre-mRNAs was evaluated using primers encompassing the junction between exon 2 and intron 2.

B Representative images of PLA assays are shown in Fig 3E analyzing the interaction between SRSF1 and mutant p53. Merged signals of DAPI and PLA are shown in the indicated cell lines. Scale bars, 0.01 mm.

C Representative images of MALAT1 staining obtained in RNA FISH analysis using fluorescence microscopy showing the presence of cells with "classical" speckled localization and cells showing a diffused staining in addition to the speckled localization of MALAT1. Scale bars, 10 μm.

D, E Western blot analysis of cell extracts derived by fractionation of lysates from MDA-MB-468 and SKBR3 cells, to obtain cytoplasmic, nuclear-soluble, and chromatin-associated nuclear fractions, performed using the indicated antibodies.

Source data are available online for this figure.

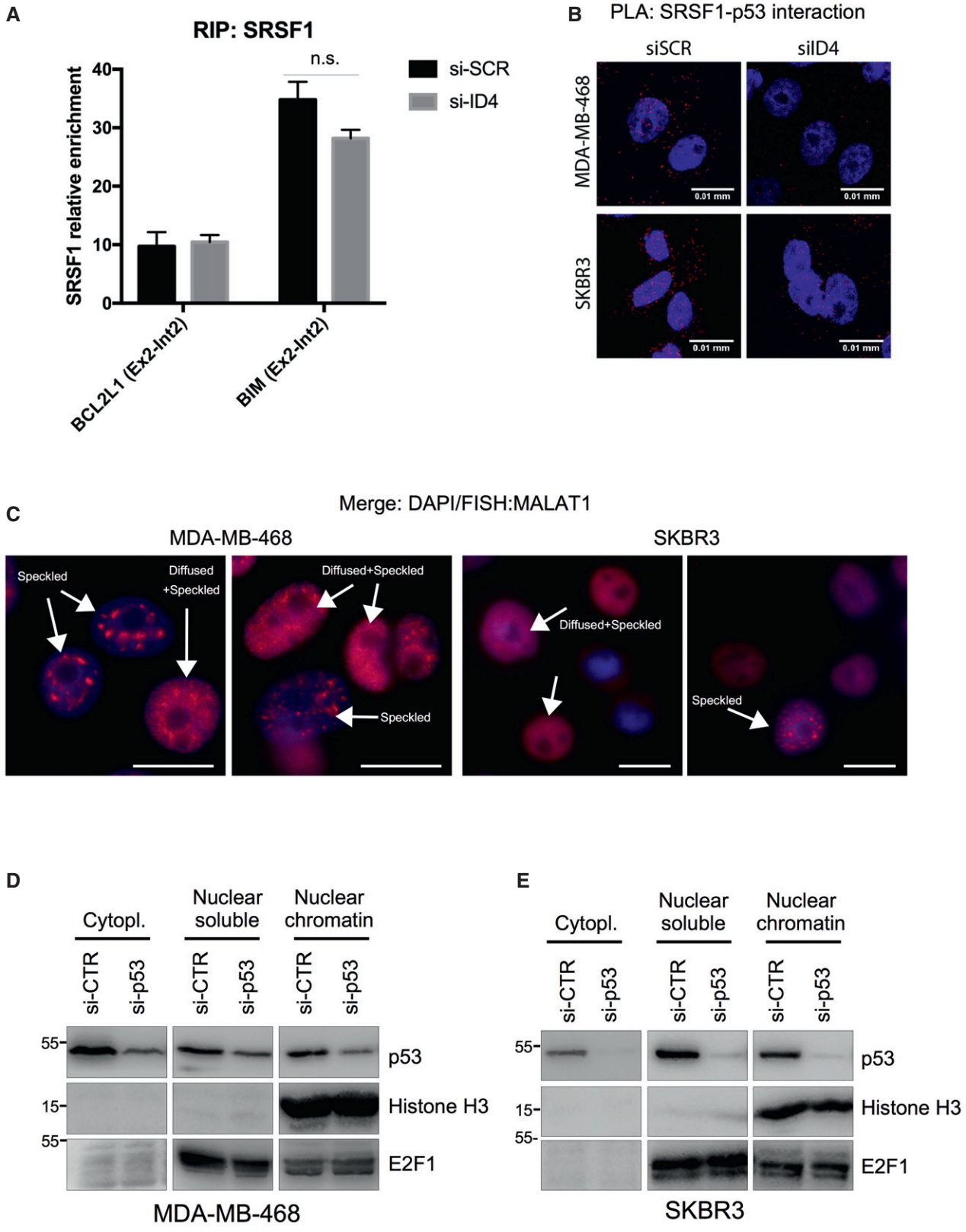
A
RIP: SRSF1
n.s.
si-SCR
si-ID4
SRSF1 relative enrichment
40
30
20
10
0
BCL2L1 (Ex2-Int2)
BIM (Ex2-Int2)
B
PLA: SRSF1-p53 interaction
siSCR
siID4
MDA-MB-468
SKBR3
0.01 mm
C
Merge: DAPI/FISH:MALAT1
MDA-MB-468
SKBR3
Speckled
Diffused +Speckled
Diffused+Speckled
Speckled
Diffused+Speckled
Speckled
D
Cytopl.
Nuclear soluble
Nuclear chromatin
si-CTR
si-p53
55
15
55
p53
Histone H3
E2F1
MDA-MB-468
E
Cytopl.
Nuclear soluble
Nuclear chromatin
si-CTR
si-p53
55
15
55
p53
Histone H3
E2F1
SKBR3

Figure EV3.

Figure EV4. Depletion of ID4, SRSF1, mutant p53 or MALAT1 leads to increased $VEGFA_{xxx}b$ protein levels.

A Western blot analysis of MDA-MB-468 cells interfered or not for hnRNP A1 expression using two different concentrations of siRNAs.

B RT–qPCR analysis of two isoforms of aldolase A mRNA (ALDOA) differing for the inclusion/exclusion of exon 2. Ratio of exon 2 excluding isoform versus exon 2 including isoform is presented. Results from three biological replicates are shown. Data are presented as mean ± SEM. $^{**}P \leq 0.005$ (two-tailed Student's *t*-test).

C–H Representative Western blot experiments of SKBR3 (C, E, G), MDA-MB-231 (D), and MDA-MB-468 (F, G) cells transfected with the indicated siRNAs to p53, ID4, SRSF1, or MALAT1. Numbers indicate ratio between $VEGFA_{165}b$ protein densitometry values in interfered cells (si-p53, si-ID4, si-SRSF1, si-MALAT1) over si-SCR sample normalized to total VEGFA protein levels. MALAT1 and ID4 depletion was assessed by RT–qPCR (H). Different siRNAs were used for each factor.

Source data are available online for this figure.

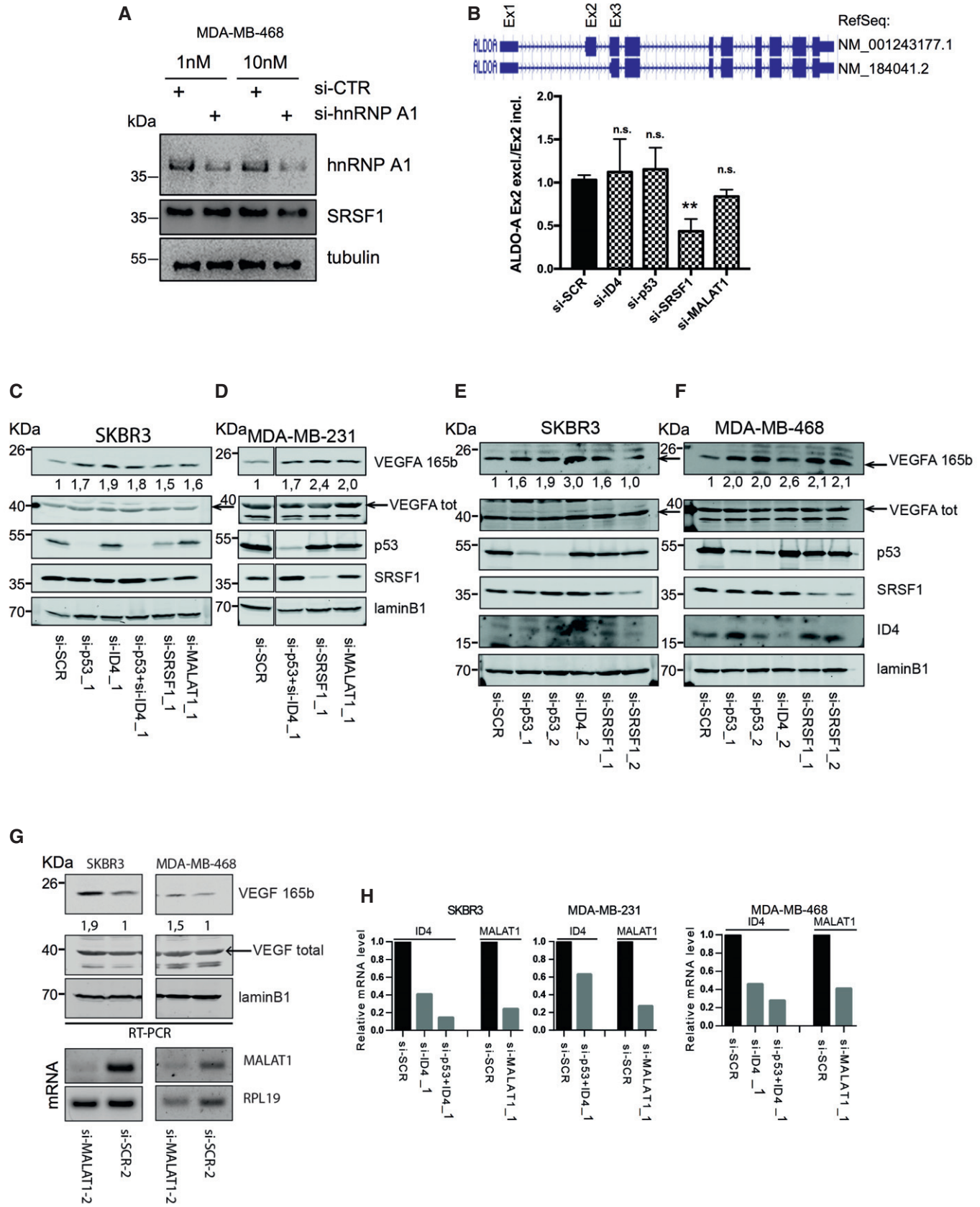
A
MDA-MB-468
1nM
10nM
si-CTR
si-hnRNP A1
kDa
hnRNP A1
SRSF1
tubulin
B
Ex1
Ex2
Ex3
RefSeq:
NM_001243177.1
NM_184041.2
ALDO-A Ex2 excl./Ex2 incl.
n.s.
**
si-SCR
si-ID4
si-p53
si-SRSF1
si-MALAT1
C
SKBR3
VEGFA 165b
VEGFA tot
p53
SRSF1
laminB1
si-SCR
si-p53_1
si-ID4_1
si-p53+si-ID4_1
si-SRSF1_1
si-MALAT1_1
D
MDA-MB-231
E
SKBR3
si-p53_2
si-ID4_2
si-SRSF1_2
F
MDA-MB-468
ID4
G
SKBR3
MDA-MB-468
VEGF 165b
VEGF total
laminB1
RT-PCR
mRNA
MALAT1
RPL19
si-MALAT1-2
si-SCR-2
H
Relative mRNA level

Figure EV4.

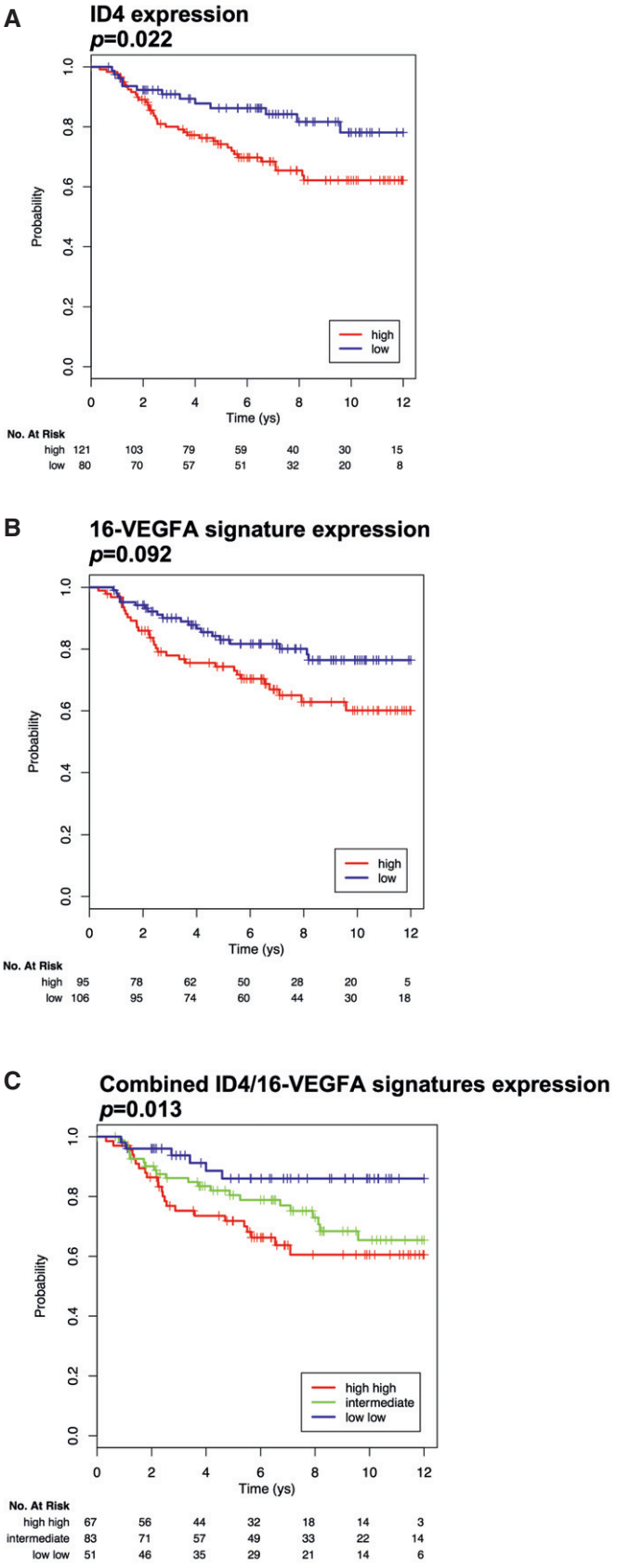

Figure EV5. Association between ID4 or VEGFA signature expression and survival in breast cancer.

A–C Kaplan–Meier analyses representing the correlation between the expression of ID4 mRNA (A), 16-VEGFA signature (B), or their combination (C), and overall survival in 201 basal-like breast cancer patients from the Breast Cancer Compendium Cohort. Tumors were divided into high- or low-ID4 expression categories based on the median of ID4 expression in the series.