Supplementary Data for Reviewers

Figure legends

Figure R1: Asf1b levels reflect the proliferating status of breast cell lines.

A. Western blot analysis of total extracts from tumoral (T) and normal (Bst) mammary cell lines. We revealed Asf1(a+b) with a mix of the specific Asf1 antibodies and CAF-1 p60 is shown for comparison. Increasing amounts of cell extracts (x) are loaded. Memcode is a loading control. M: molecular weight marker. The percentage of cells in S phase (Figure 2A) as well as the number of CAF-1 positive cells (Figure 2E) is indicated below the Western Blot.

B. Representative quantification of Asf1a and Asf1b protein levels in tumoral (T) and normal (Bst) mammary cell lines. We normalized levels to the Memcode staining and set levels in normal cells to 100%.

Figure R2: Transcriptional signature of Asf1b depleted cells reflects proliferation defects.

mRNA extracted from human U-2-OS cells depleted of Asf1a, Asf1b or Asf1(a+b) for 48h were hybridized to Affymetrix HG-U133-Plus2 oligonucleotide microarrays. mRNA expression levels obtained from the Affymetrix hybridisation are expressed as a log2(fold change) relative to the control siRNA depletion. Error bars represents data from two independent experiments.

A. mRNA expression levels of MRAS, RAS8B, RRAS2, MAPK1, ABL1 and JUN involved in cellular proliferation.

B. mRNA expression levels of MMP1, MMP9 and TIMP3 involved in breast cancer metastasis.

Figure R3: Single depletion of Asf1b slightly alters cell cycle progression.

Flow cytometry analysis of the cell cycle distribution of HeLa cells (left panel) or Hs578T cells (right panel) depleted of Asf1a, Asf1b or Asf1(a+b) for 48h by siRNA treatment. Overlay of the 4 different conditions underlines that the single depletion of Asf1b slightly alters S phase progression with an increase in the number of cells in S/G2 phases. As described previously, the double knockdown of Asf1(a+b) strongly impairs S phase progression (Groth et al., 2007).

Figure R4: Asf1a and Asf1b expression levels in breast cell lines.

Quantitative RT-PCR analysis of Asf1a and Asf1b mRNA levels in normal (HMEC) and tumoral (MDA-MB-231, SKBR3, ZR75.1) mammary cell lines. We normalized levels to the reference gene RPLPO. While Asf1a is expressed at low levels in all cell lines, Asf1b is specifically overexpressed in breast cancer cell lines. Error bars represent s.d. from 2 independent experiments.

Figure R5: Transcriptional signature of Asf1b depleted cells does not show any bias towards apoptotic genes.

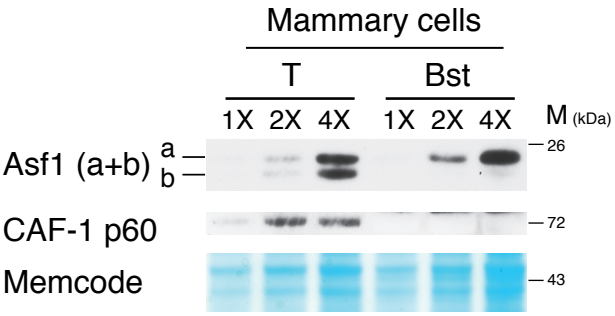
mRNA extracted from human U-2-OS cells depleted of Asf1a, Asf1b or Asf1(a+b) for 48h were hybridized to Affymetrix HG-U133-Plus2 oligonucleotide microarrays. mRNA expression levels obtained from the Affymetrix hybridisation are expressed as a log2(fold change) relative to the control siRNA depletion. Error bars represents data from two independent experiments.

A. mRNA expression levels of BCL2, BCL2L1 (Bcl-x) and BIRC2 (c-IAP1) involved in negative regulation of apoptosis.

B. mRNA expression levels of BAD, CASP9, CASP3, BAX and AIFM1 involved in positive regulation of apoptosis.

Figure R1

A **Western Blot**



	T	Bst
% S phase	25%	13%
% CAF-1 positive cells	72%	37%

B **Quantification of the Western Blot**

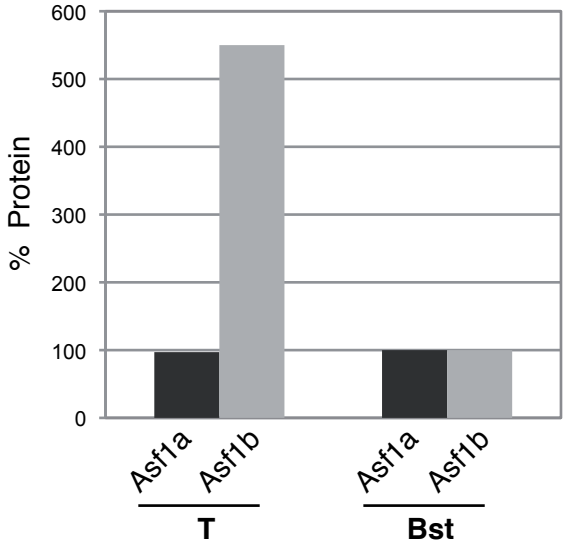


Figure R2

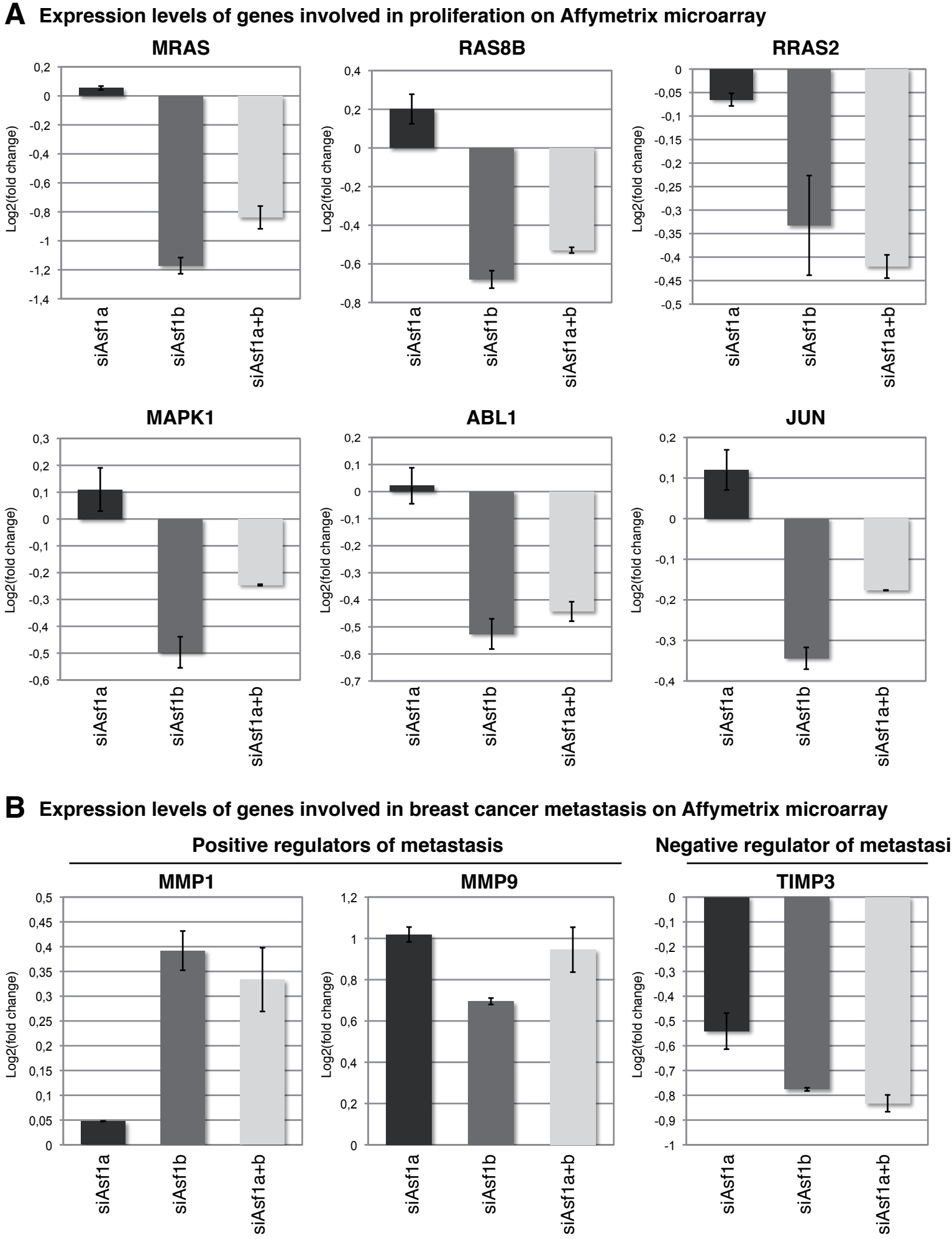

A
Expression levels of genes involved in proliferation on Affymetrix microarray
MRAS
RAS8B
RRAS2
MAPK1
ABL1
JUN
Log2(fold change)
siAsf1a
siAsf1b
siAsf1a+b
B
Expression levels of genes involved in breast cancer metastasis on Affymetrix microarray
Positive regulators of metastasis
Negative regulator of metastasis
MMP1
MMP9
TIMP3

Figure R3

FACS analysis at 48h of siRNA

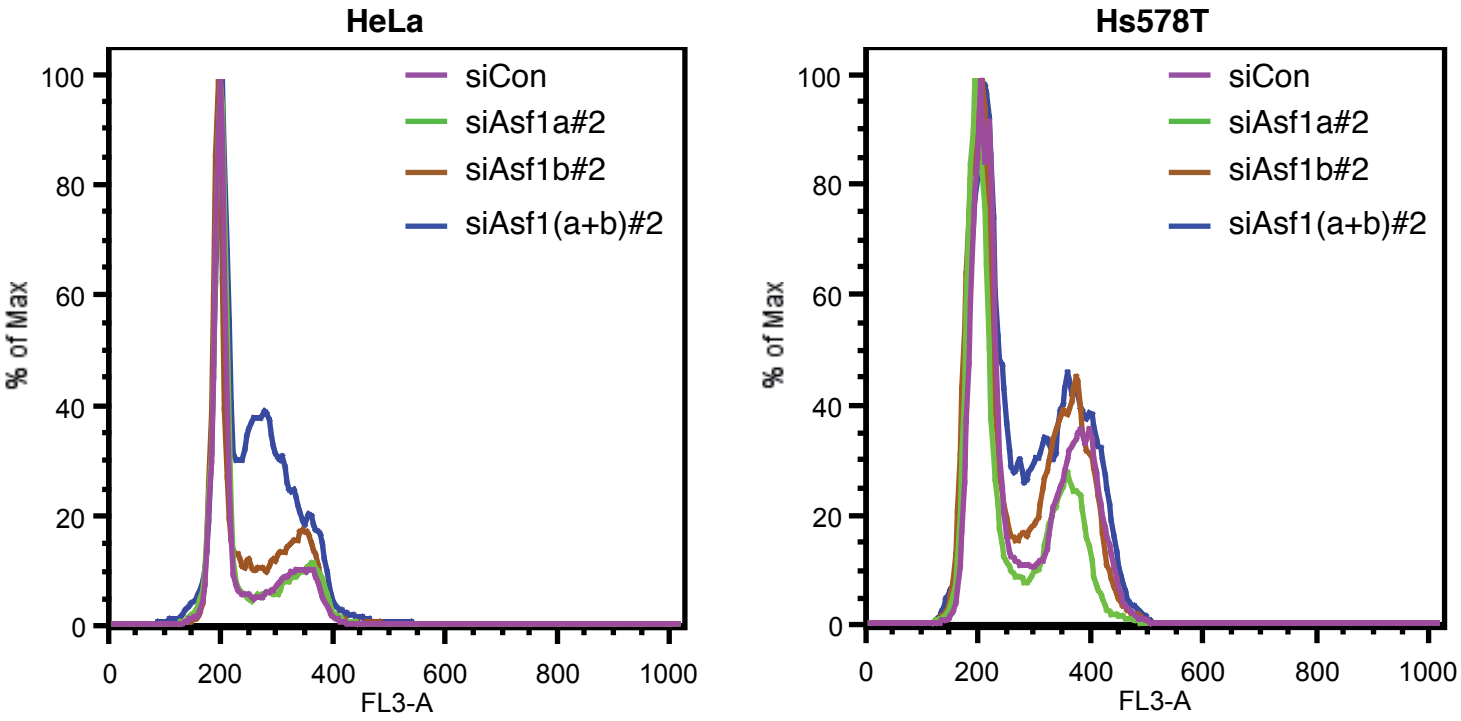


Figure R4
Asf1a and Asf1b expression levels in breast cell lines

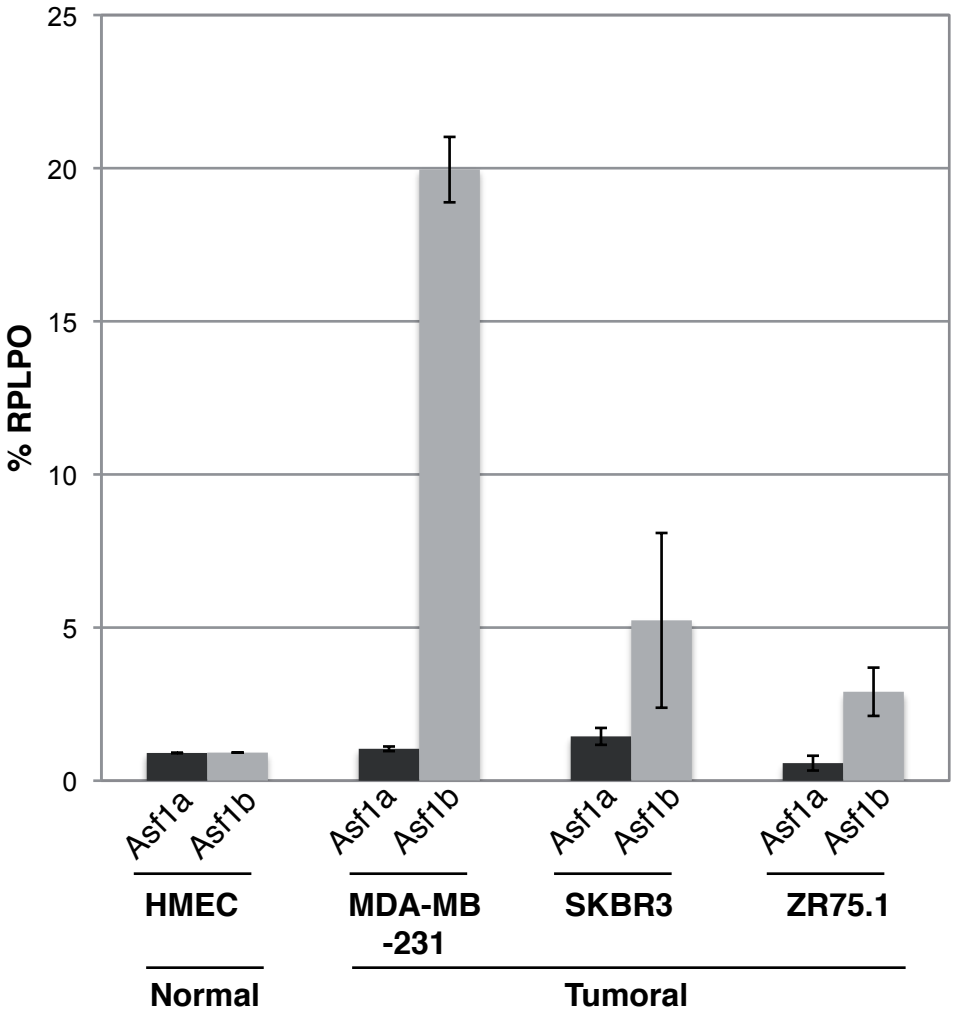
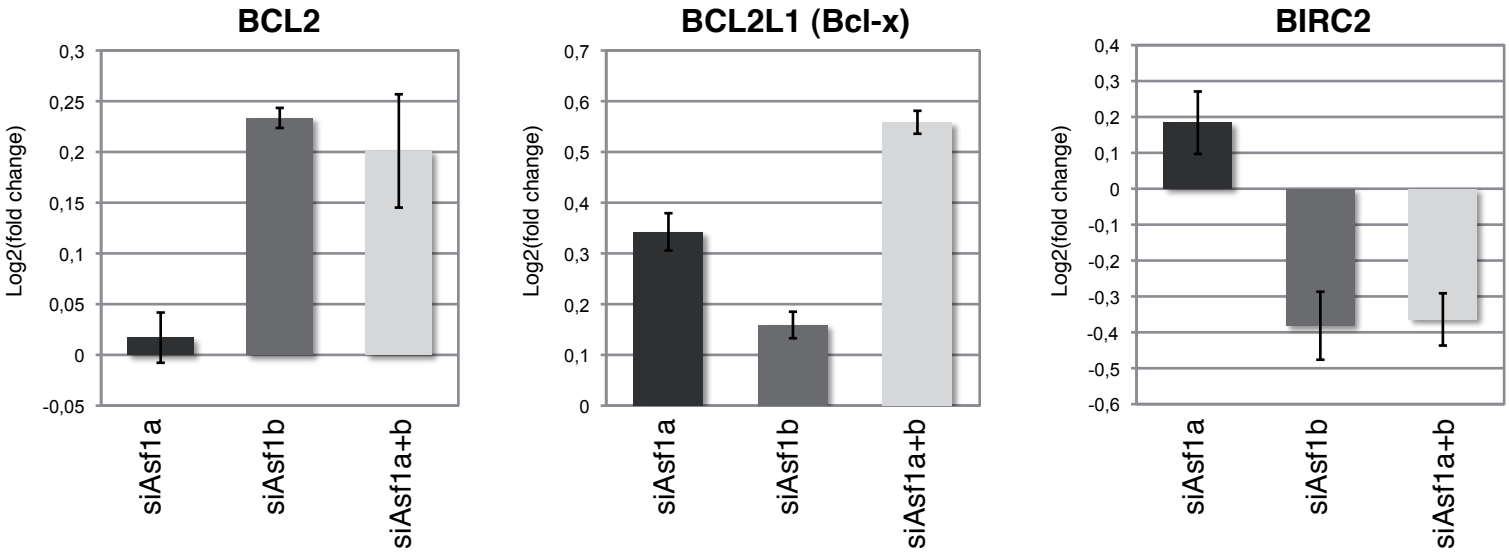


Figure R5

A Expression levels of genes involved in negative regulation of apoptosis on Affymetrix microarray



B Expression levels of genes involved in positive regulation of apoptosis on Affymetrix microarray