Figure S1

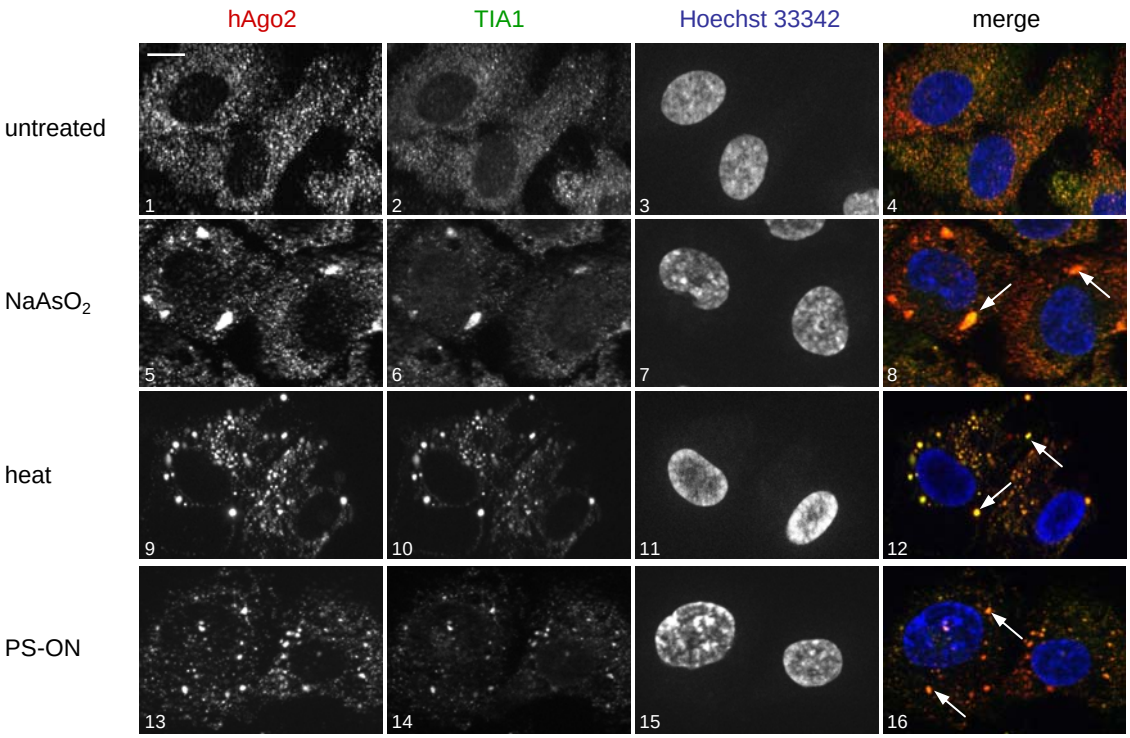
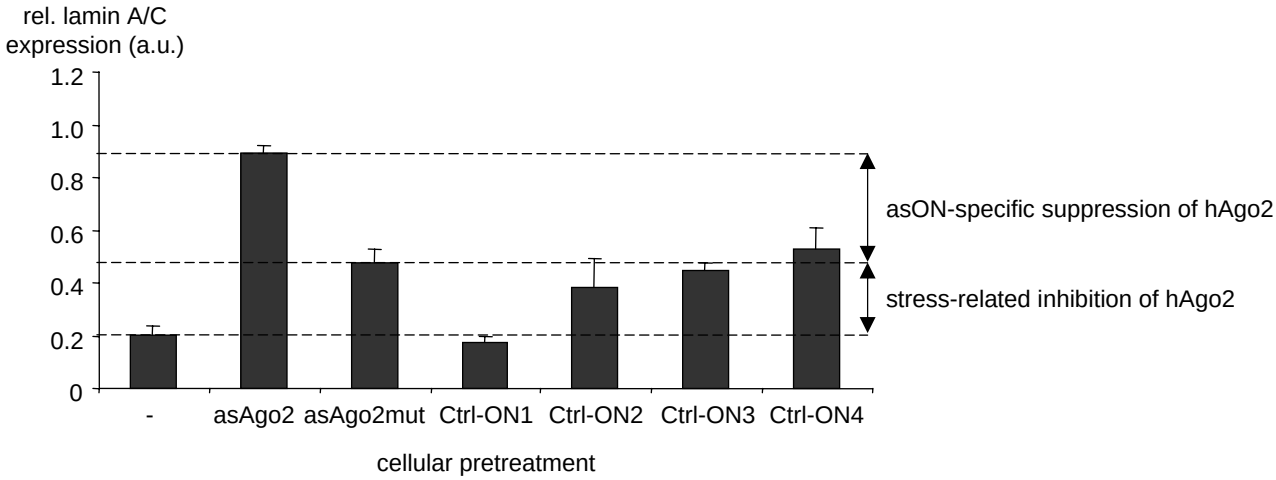

hAgo2
TIA1
Hoechst 33342
merge
untreated
NaAsO2
heat
PS-ON
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Figure S2

(A)


rel. lamin A/C expression (a.u.)
1.2
1.0
0.8
0.6
0.4
0.2
0
asON-specific suppression of hAgo2
stress-related inhibition of hAgo2
-
asAgo2
asAgo2mut
Ctrl-ON1
Ctrl-ON2
Ctrl-ON3
Ctrl-ON4
cellular pretreatment

(B)

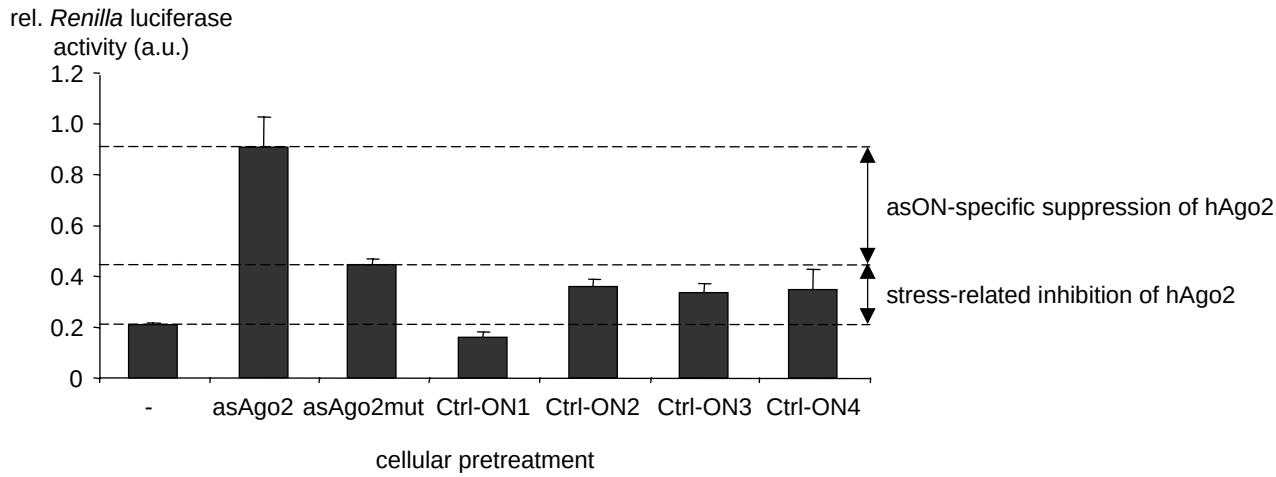

rel. Renilla luciferase activity (a.u.)
1.2
1.0
0.8
0.6
0.4
0.2
0
asON-specific suppression of hAgo2
stress-related inhibition of hAgo2
-
asAgo2
asAgo2mut
Ctrl-ON1
Ctrl-ON2
Ctrl-ON3
Ctrl-ON4
cellular pretreatment

Figure S3

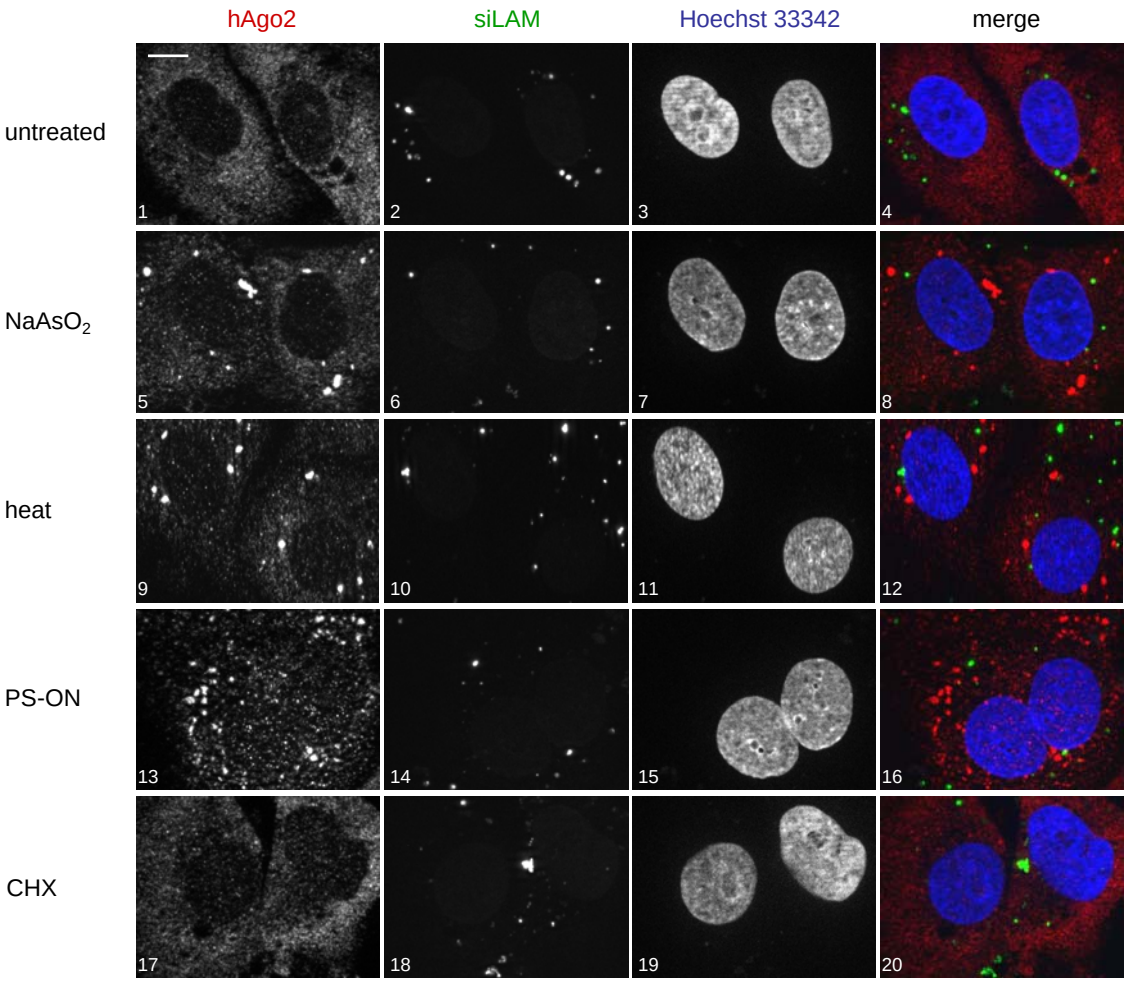
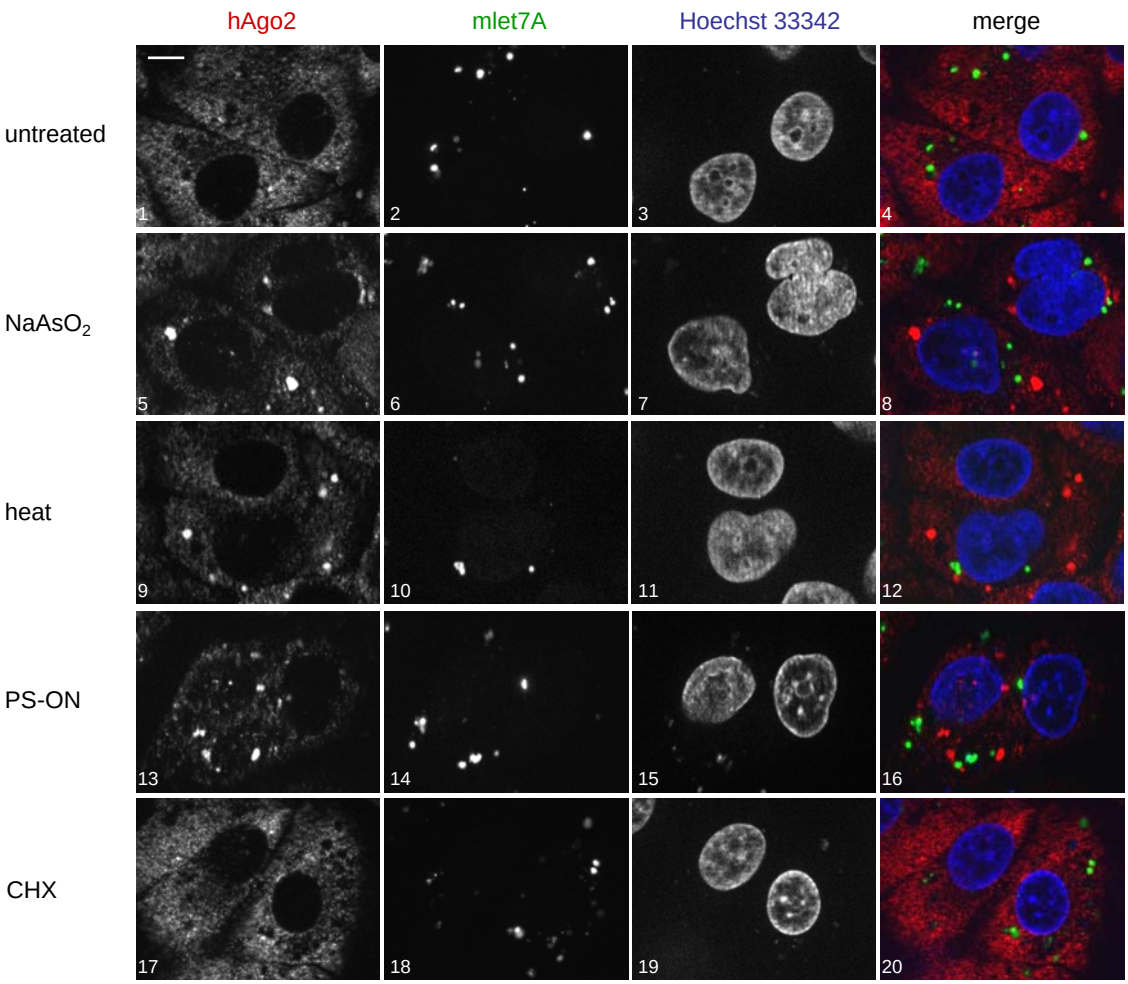

hAgo2
siLAM
Hoechst 33342
merge
untreated
NaAsO2
heat
PS-ON
CHX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Figure S4


hAgo2
mlet7A
Hoechst 33342
merge
untreated
NaAsO2
heat
PS-ON
CHX
1
2
3
4
5
6
7
8
9
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12
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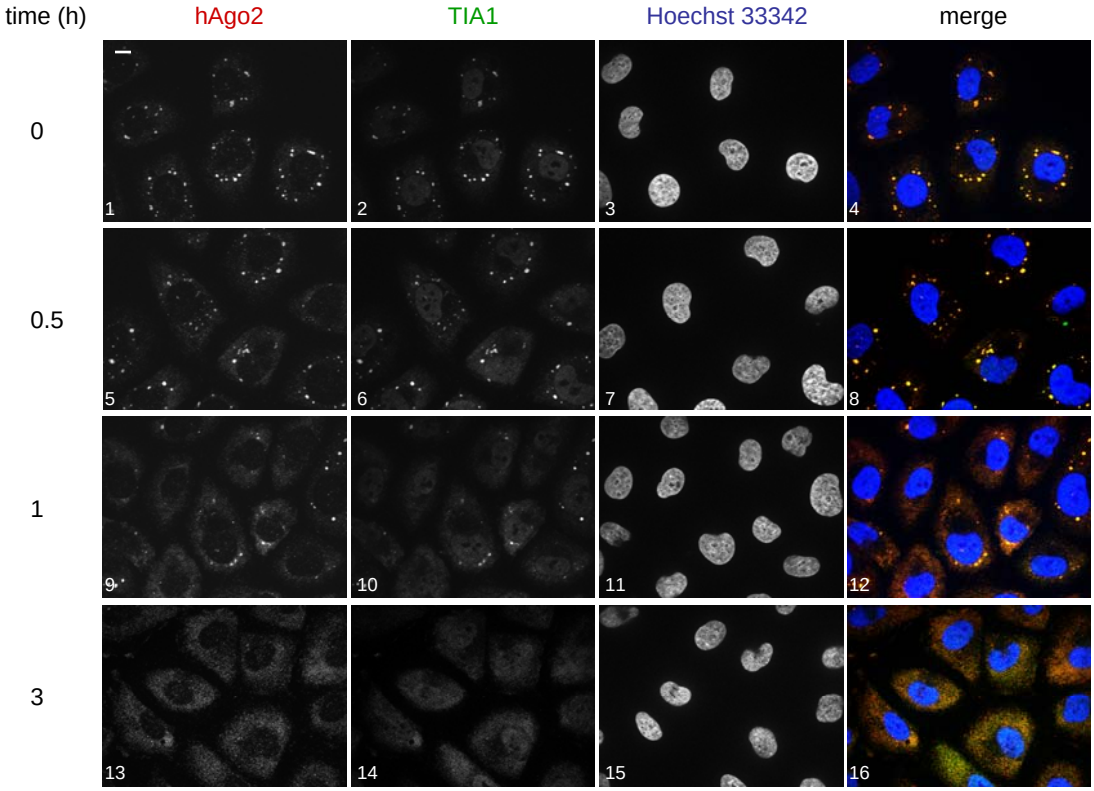
17
18
19
20

Figure S5

(A) time-dependent localization of hAgo2 with respect to SGs upon $NaAsO_2$ treatment



(B) time-dependent RNAi activity of hAgo2 upon $NaAsO_2$ treatment

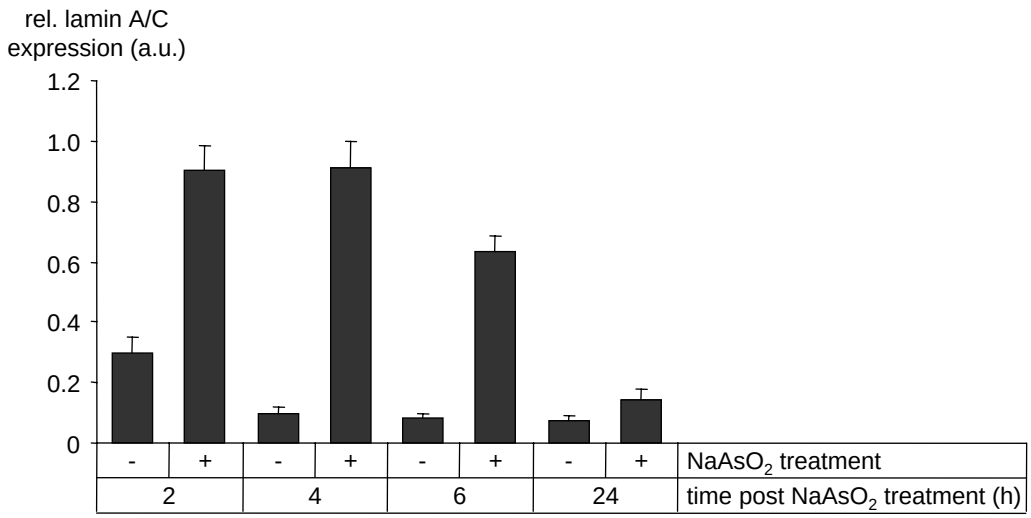
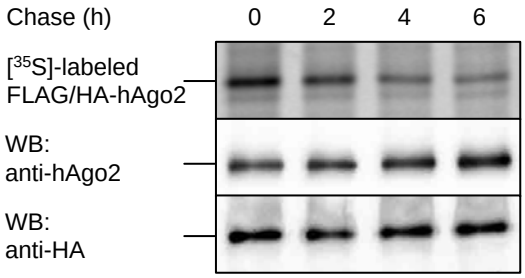


Figure S6


Chase (h)
0
2
4
6
[35S]-labeled
FLAG/HA-hAgo2
WB:
anti-hAgo2
WB:
anti-HA

LEGENDS TO SUPPLEMENTARY FIGURES

Fig. S1: **Different kinds of cell stress direct hAgo2 to stress granules in HUVEC.** This experiment was performed as described in detail in the legend to Fig. 1. Briefly, ECV-304 cells were left untreated (picture 1-4) or stressed by 250 µM $NaAsO_2$ for 90 min at 37°C (picture 5-8), incubated at 42°C for 16 h (picture 9-12), or transfected with Lipofectamine™ 2000 and 100 nM PS-modified ON 24h prior to fixation (picture 13-16). Subsequently, cells were co-stained with a rat anti-ago2 antibody (red color, left column) and an anti-TIA-1 antibody (stress granule marker, green color, second left column). All preparations were also co-stained with Hoechst 33342 dye (blue color). Merged images indicate co-localization of hAgo2 and SGs (right column). The white arrows in picture 8,12 and 16 indicate the staining of SGs in an exemplary manner. Optical sections are displayed either as merged images or as greyscale images of the respective red, green or blue channel. The white bar in the upper left corner represents a 10 µm scale bar.

Fig. S2: **Influence of the nucleotide sequence of transfected PS-ON on the extent of siRNA- or miRNA-induced RNAi.** ECV-304 cells were transfected with 100 nM fully PS-modified ON in the use of Lipofectamine™ 2000 as the transfectant. After 24 h cells were tested for their capability to perform siRNA- or miRNA-mediated RNAi. Briefly, cells were transfected **(A)** with 50 pM lamin A/C directed siLAM or **(B)** with 50 nM *Renilla* luciferase directed mlet-7A, RL-Hmga2m7 target vector and pGL3 control vector. As control, Ctrl-RNA transfected ECV-304 cells were used and set 1. Indicated are mean values ± standard deviations. Increased target levels (y-axis) indicate the perturbing effect of the transfection of fully PS-modified ON on small RNA silencing activity. The lower dashed line in each panel indicates the siRNA- or miRNA-mediated gene silencing activity in the absence of PS-induced cell stress. The sequence-specific effect of asAgo2 versus its control asAgo2mut is indicated by a vertical arrow between both bars.

Fig. S3: **No influence of cell stress on the intracellular localization of transfected siRNA**. As indicated on the left margin, ECV-304 cells were left untreated (picture 1-4) or stressed by 250 µM $NaAsO_2$ for 90 min at 37°C (picture 5-8), incubated at 42°C for 16 h (picture 9-12), transfected with Lipofectamine™ 2000 and 100 nM PS-modified ON 24 h prior to the following treatment (picture 13-16), or treated with 20 µg/ml CHX for 24 h (picture 17-20). Afterwards cells were transfected with 50 nM Alexa488-labelled siLAM (green color, second left column) and

subsequently stained with a rat anti-hAgo2 antibody (red color, left column). All preparations were also co-stained with Hoechst 33342 dye (blue color, second right column). Merged images are shown on the right column and do not indicate a modified siLAM localization in stressed cells compared to unstressed cells. By contrast the induction of cell stress by $NaAsO_2$, heat or PS-ON is related to an accumulation of hAgo2 in irregularly shaped intracellular structures previously characterized as SGs (compare Fig. 1 (A)). Optical sections are displayed either as merged images or as greyscale images of the respective red, green or blue channel. The white bar in the upper left corner represents a 10 µm scale bar.

Fig. S4: **No influence of cell stress on the intracellular localization of transfected miRNA**. As indicated on the left margin, ECV-304 cells were left untreated (picture 1-4) or stressed by 250 µM $NaAsO_2$ for 90 min at 37°C (picture 5-8), incubated at 42°C for 16 h (picture 9-12), transfected with Lipofectamine™ 2000 and 100 nM PS-modified ON 24 h prior to the following treatment (picture 13-16), or treated with 20 µg/ml CHX for 24 h (picture 17-20). Afterwards cells were transfected with 50 nM Alexa488-labelled mlet-7A (green color, second left column) and subsequently stained with a rat anti-hAgo2 antibody (red color, left column). All preparations were also co-stained with Hoechst 33342 dye (blue color, second right column). Merged images are shown on the right column and do not indicate a modified mlet-7A localization in stressed cells compared to unstressed cells. By contrast the induction of cell stress by $NaAsO_2$, heat or PS-ON is related to an accumulation of hAgo2 in irregularly shaped intracellular structures previously characterized as SGs (compare Fig. 1 (A)). Optical sections are displayed either as merged images or as greyscale images of the respective red, green or blue channel. The white bar in the upper left corner represents a 10 µm scale bar.

Fig. S5: **Cell stress-induced accumulation of hAgo2 in SGs is reversible.** ECV-304 cells were either left untreated or incubated with 250 µM $NaAsO_2$ for 90 min at 37°C. **(A)** At the indicated time points, cells were co-stained with a rat anti-hAgo2 antibody (red color, left column) and a goat anti-TIA-1 antibody (SG marker, green color, second left column). All preparations were also co-stained with Hoechst 33342 dye (blue color, second right column). Merged images are shown on the right column. Co-staining of hAgo2 and the SG marker TIA1 is detectable up to 1 h upon removal of $NaAsO_2$, after 3 h a complete re-localization of hAgo2 to a diffuse cytoplasmiatic localization can be observed. Optical sections are

displayed either as merged images or as greyscale images of the respective red, green or blue channel. The white bar in the upper left corner represents a 10 µm scale bar. **(B)** Subsequently to the $NaAsO_2$ treatment ECV-304 cells were transfected with lamin A/C-directed siRNA (250 pM, siLAM). At the indicated time points the siLAM-mediated suppression of lamin A/C mRNA was quantified by RT-qPCR. The siRNA-mediated gene silencing activity in $NaAsO_2$-stressed cells (indicated by a '+') was compared to cells, which were cultured in the absence of cellular stress (indicated by a '-'). In both cases as control, Ctrl-RNA transfected ECV-304 cells were used and set 1. Indicated are mean values ± standard deviations.

Fig. S6: **Half life time of hAgo2 in normal growing cells.** Stably FLAG/HA-hAgo2 expressing HeLa cells were pulsed with DMEM medium (Met depleted; Sigma-Aldrich, Taufkirchen, Germany) containing 100 µCi/ml [^{35}S]-labeled methionine (Hartmann Analytics, Braunschweig, Germany), incubated with nonradioactive chase medium, and harvested at the indicated time points. FLAG/HA-hAgo2 was immunoprecipitated with anti-HA MicroBeads (Miltenyi Biotech, Bergisch-Gladbach, Germany). The immunoprecipitate was analyzed for [^{35}S]-labeled FLAG/HA-hAgo2 (upper panel) and for total FLAG/HA-hAgo2 and total hAgo2 levels by western analysis (the two lower panel).