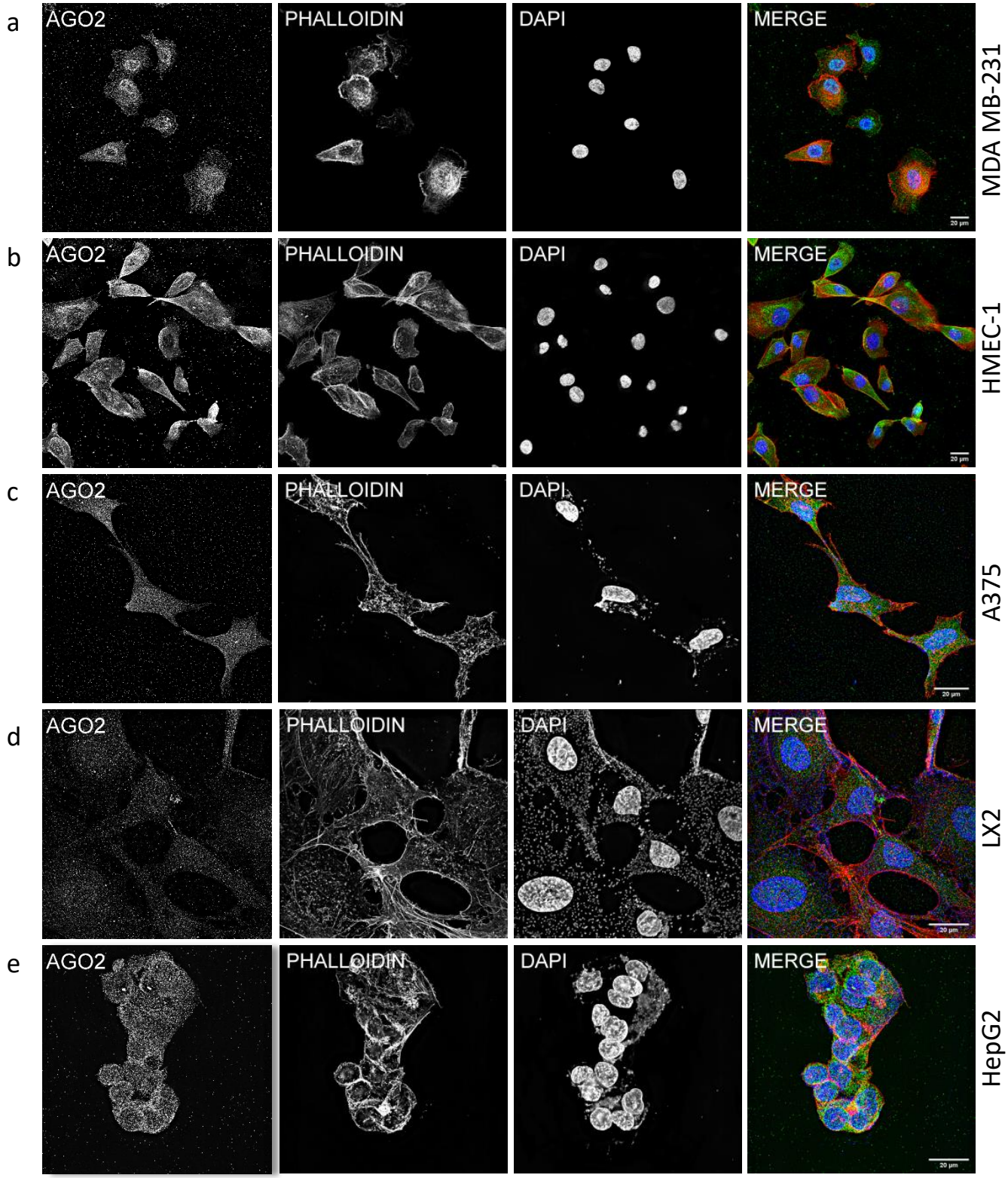



Figure S1: Subcellular distribution of AGO2 in different cell lines. Representative images of (a) MDA MB-231, (b) HMEC, (c) A375, (d) LX-2 and (e) HepG2 cells. AGO2 in green, F-Actin (phalloidin) in red and nuclei in blue (DAPI) (scale bar: 20µm). (f) Barplot of AGO2 mean signal intensity per cell in the respective cell lines measured by Imaris using 3D surface reconstruction of confocal images.

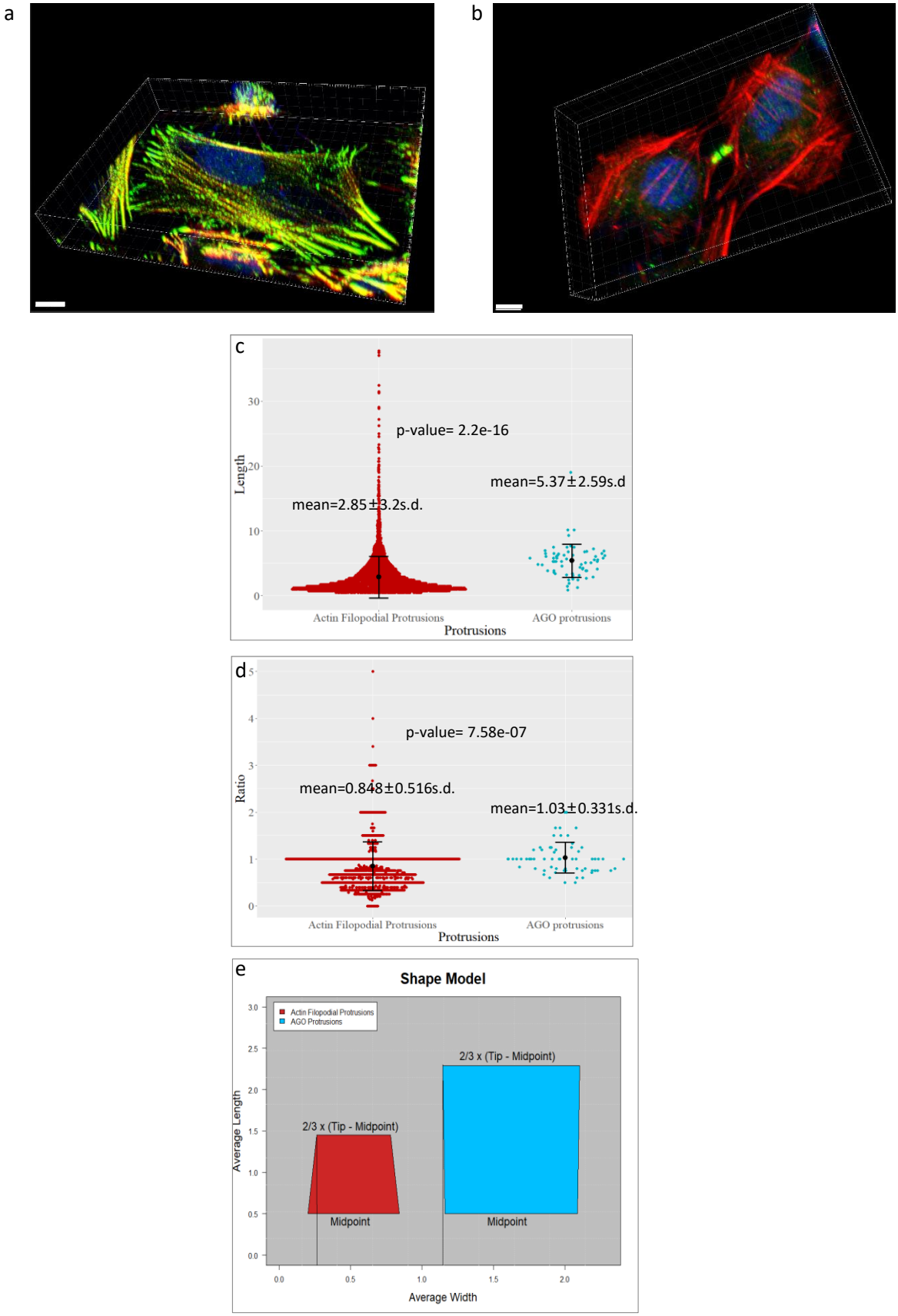



Figure S2: AGO2 close-ended structures differ from the typical Actin-filopodial protrusions. 3D signal reconstruction of representative images of NTHY ori 3-1 cells. (a) F-Actin filopodial (Actinin 4, green; F-Actin [Texas red-phalloidin], red, scale bar: 10 µm) and (b) AGO2 protrusions (AGO2 in green; F-Actin [phalloidin] in red, respectively, scale bar: 8 µm). Sina-plot of Actin-filopodial (at least n=3 biologically independent experiments, statistical test sample size n=3849 filopodial protrusions) and AGO2 protrusion (at least n=5 biologically independent experiments, statistical test sample size n=66 AGO2 protrusions) (c) lengths and (d) tip-to-midpoint width ratios. (e) Shape model based on protrusion mean value of tip-to-midpoint width ratios.

| Pairs for lineplots | | Pearson | Manders' | |
|---|---|---|---|---|
| | | R | M1 | M2 |
| AGO2 - CITK | mean | 0.126 | 0.221 | 0.336 |
| | p-value | 0.0007 | 0.0002 | 0.0007 |
| AGO2 - AURORA B | mean | 0.718 | 0.889 | 0.786 |
| | p-value | 8.00e-07 | 6.68e-07 | 4.62e-07 |
| AURORA B - CITK | mean | 0.172 | 0.323 | 0.461 |
| | p-value | 8.19e-06 | 9.43e-06 | 2.73e-05 |
| AGO2 - DICER | mean | 0.221 | 0.350 | 0.719 |
| | p-value | 0.0017 | 0.0007 | 0.0004 |
| AURORA B - DICER | mean | 0.233 | 0.342 | 0.593 |
| | p-value | 0.0181 | 0.0008 | 0.0004 |
| AGO2 - αTUB | mean | 0.770 | 0.786 | 0.958 |
| | p-value | 4.1e-08 | 1.36e-07 | 1.28e-09 |
| AGO2 - PHALLOIDIN | mean | 0.153 | 0.328 | 0.203 |
| | p-value | 0.0087 | 0.0099 | 0.0068 |
| αTUB - PHALLOIDIN | mean | 0.266 | 0.368 | 0.602 |
| | p-value | 0.0138 | 0.0096 | 0.0004 |
| αTUB - DICER | mean | 0.261 | 0.387 | 0.815 |
| | p-value | 0.0012 | 0.0006 | 3.15e-06 |
| DICER - PHALLOIDIN | mean | 0.077 | 0.291 | 0.148 |
| | p-value | 0.0088 | 0.0102 | 0.0306 |
| PHALLOIDIN - LAMIN A/C (non-colocalized area) | mean | 0.024 | 0.003 | 0.340 |
| | p-value | 0.0916 | 0.1257 | 0.0263 |

Table S1: Colocalization metrics (Pearson [R] and Manders' [M1 and M2] coefficients) and respective p-values of the paired proteins depicted in yellow ROIs in Fig. 3e-h, Fig. 4c-e, Fig. S4a-c.

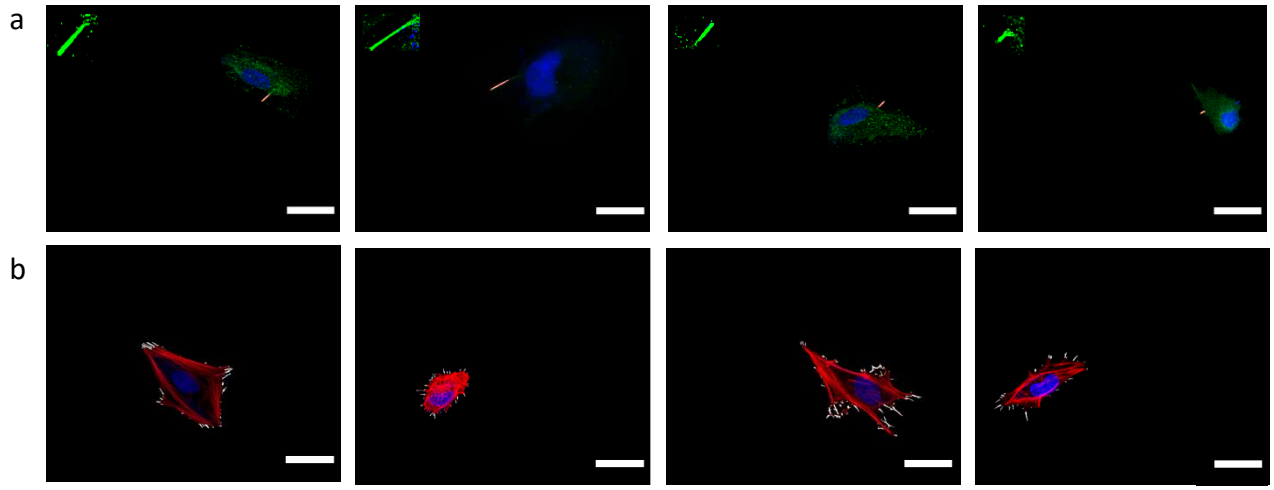



Figure S3: Examples of the output of the Matlab script used to detect, measure and yield statistics shown in Figure S2 from the comparative analysis of (a) AGO2 and (b) Actin-Filopodial protrusions. The identified protrusions are colored white. All measurements were made according to the ellipses created by Matlab's "regionprops" function [shown in red in (a)]. In the top-left corner of (a) the manually cropped protrusions used to extract the AGO2 protrusion among those identified by Matlab's "fibermetric" function are overlaid. Scale bar: 40μm.

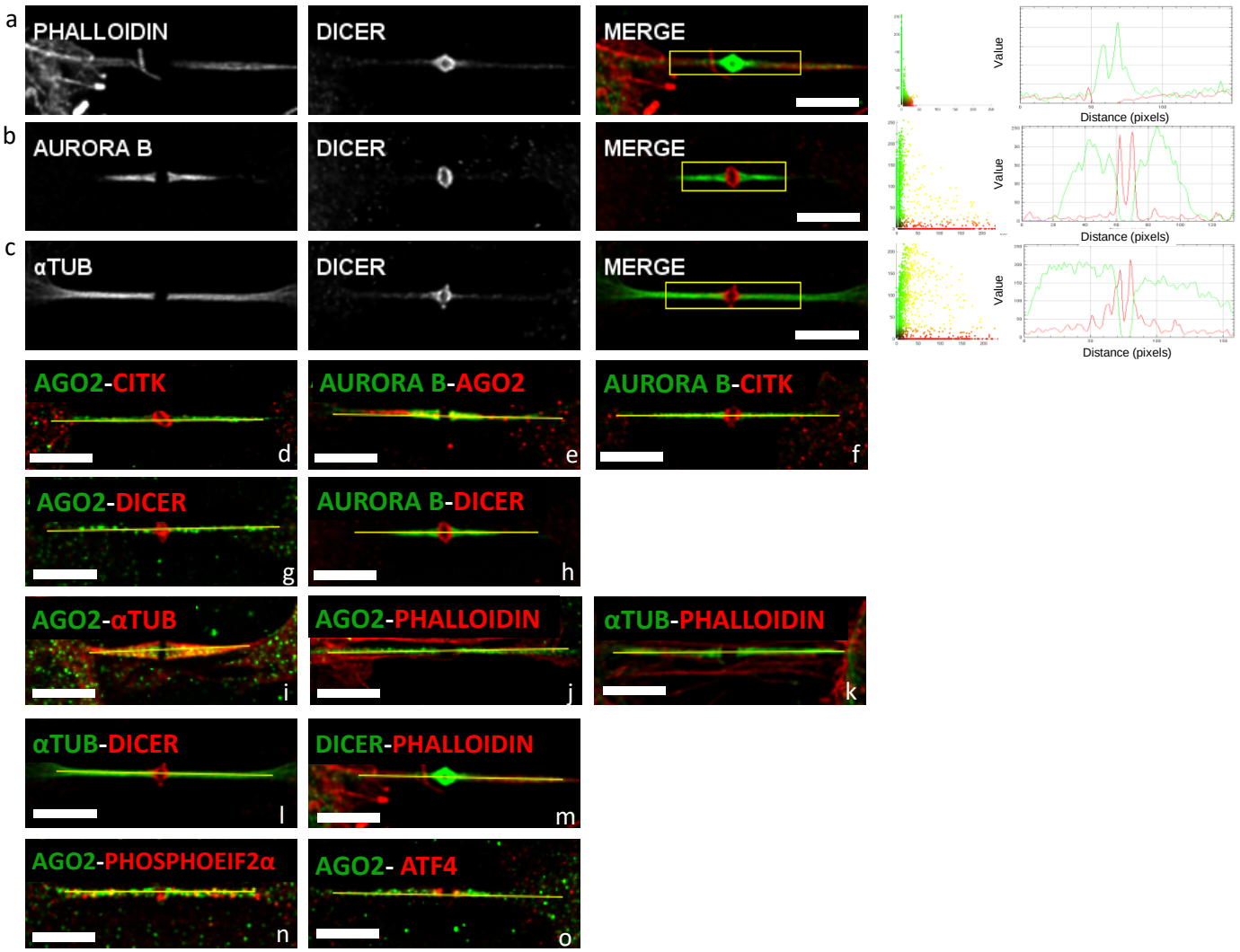



Figure S4: Colocalization analysis of the intercellular bridge content. (a-c) Representative images of NTHY ori 3-1 cells and visual assessment (merged images, scatterplots and intensity lineplots) of colocalization. The colocalization metrics (Pearson [R] and Manders' [M1 and M2] coefficients) and respective p-values are presented in Table S2 (n=6 biologically independent samples). (d-o) Lineplots indicate the signal intensity of Dicer and the paired protein along the lines. Line overlays on merged channels from all conditions shown in Fig. 3e-h, Fig. 4c-e, Fig. 10e-f and Fig. S4a-c used to obtain the corresponding lineplots. Scale bar: 5μm.

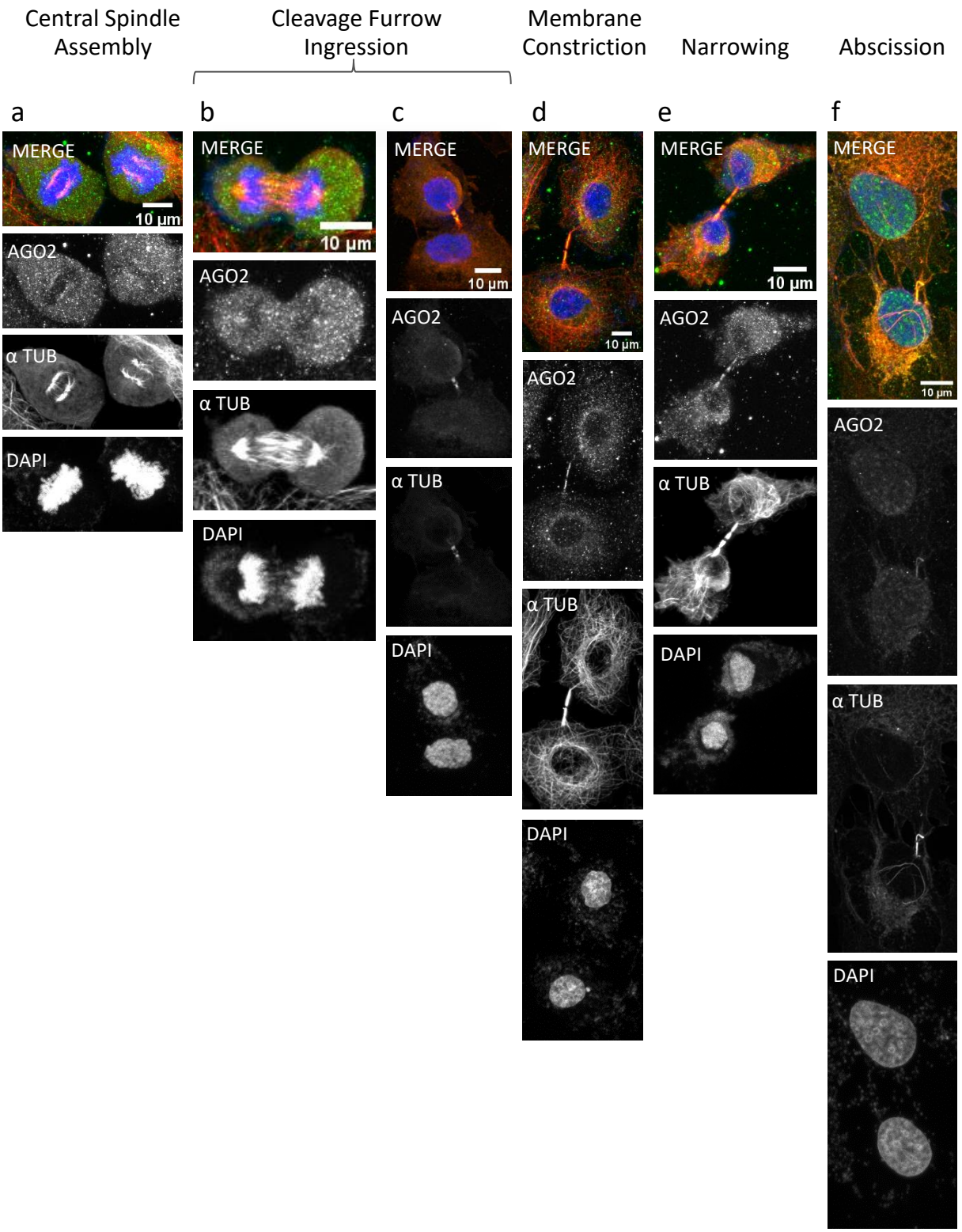



Figure S5: AGO2 distribution during cell division. Representative immunofluorescence images of NTHY ori 3-1 cells. AGO2 colocalization with *α*-Tubulin was visualized in a time-course manner. AGO2 in green. *α*-Tubulin in red and nuclei in blue (DAPI). Images depict (a) central spindle, (b,c) assembly cleavage furrow ingression, (d) membrane constriction, (e) narrowing and (f) abscission (scale bar: 10 µm).

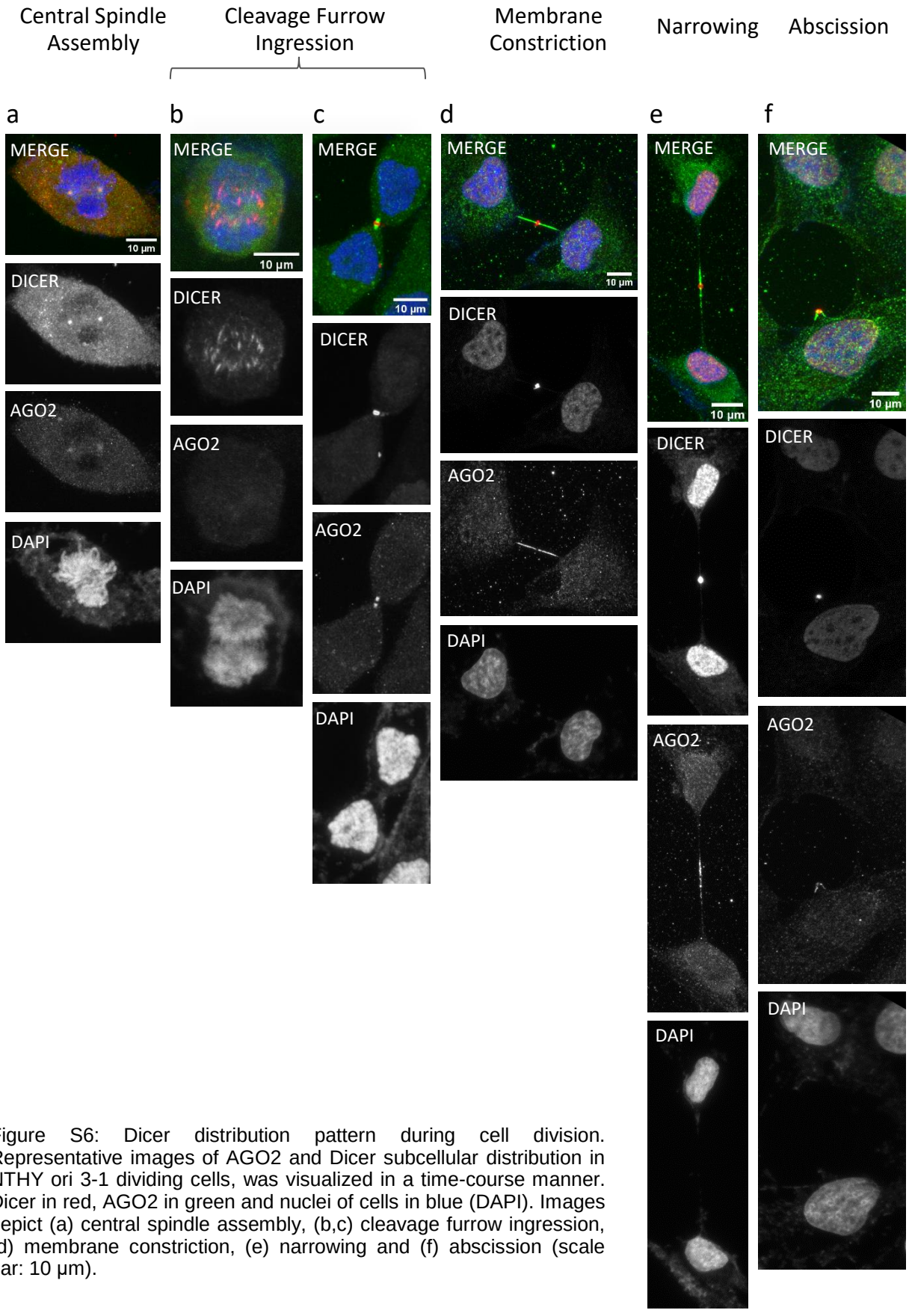



Figure S6: Dicer distribution pattern during cell division. Representative images of AGO2 and Dicer subcellular distribution in NTHY ori 3-1 dividing cells, was visualized in a time-course manner. Dicer in red, AGO2 in green and nuclei of cells in blue (DAPI). Images depict (a) central spindle assembly, (b,c) cleavage furrow ingression, (d) membrane constriction, (e) narrowing and (f) abscission (scale bar: 10 µm).

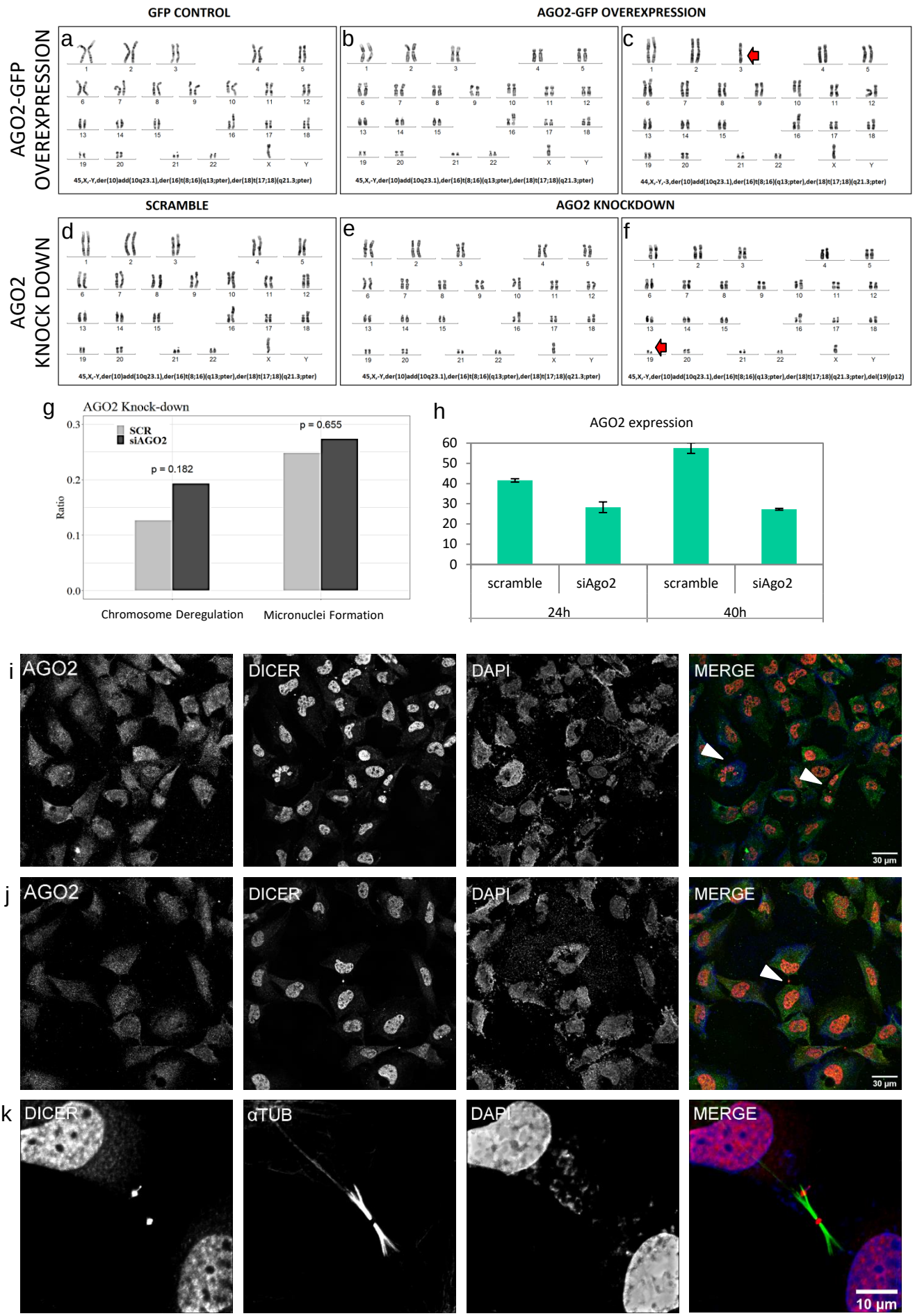

GFP CONTROL
AGO2-GFP OVEREXPRESSION
AGO2-GFP OVEREXPRESSION
a
b
c
45,X,-Y,der(10)add(10q23.1),der(16)t(8;16)(q13;pter),der(18)t(17;18)(q21.3;pter)
45,X,-Y,der(10)add(10q23.1),der(16)t(8;16)(q13;pter),der(18)t(17;18)(q21.3;pter)
44,X,-Y,-3,der(10)add(10q23.1),der(16)t(8;16)(q13;pter),der(18)t(17;18)(q21.3;pter)
SCRAMBLE
AGO2 KNOCKDOWN
AGO2
KNOCK DOWN
d
e
f
45,X,-Y,der(10)add(10q23.1),der(16)t(8;16)(q13;pter),der(18)t(17;18)(q21.3;pter)
45,X,-Y,der(10)add(10q23.1),der(16)t(8;16)(q13;pter),der(18)t(17;18)(q21.3;pter)
45,X,-Y,der(10)add(10q23.1),der(16)t(8;16)(q13;pter),der(18)t(17;18)(q21.3;pter),del(19)(p12)
g
AGO2 Knock-down
SCR
siAGO2
p = 0.182
p = 0.655
Ratio
0.3
0.2
0.1
0.0
Chromosome Deregulation
Micronuclei Formation
h
AGO2 expression
60
50
40
30
20
10
0
scramble
siAgo2
scramble
siAgo2
24h
40h
siAGO2
i
AGO2
DICER
DAPI
MERGE
30 µm
j
AGO2
DICER
DAPI
MERGE
30 µm
k
DICER
αTUB
DAPI
MERGE
10 µm

Figure S7: AGO2 over-expression and down-regulation effect on cell division. Karyotypes of HCT116 (a) GFP-control cells and (b,c) AGO2-GFP over-expressing cells. Karyotypes of HCT116 (d) cells transfected with scramble control and (e,f) AGO2-knocked down cells. (g) Bar plot of the effects of AGO2 down-regulation in HCT116 cells on numerical chromosome instability and micronuclei formation. z-test was used to evaluate their significance. Bar height indicates the ratio of deregulated chromosomes (n=2 biologically independent experiments, statistical sample size n1=103 SCR cells, n2=114 siAGO2 cells) and cells with micronuclei formation (n=2 biologically independent experiments, statistical sample size n1=117 SCR cells, n2=117 siAGO2 cells), respectively (h) Bar plot indicates AGO2 expression levels in 24 and 40h following siAGO2 transfection determined by quantitative PCR analysis. (i-k) Representative images of NTHY ori 3-1 AGO2 knocked-down cells. White arrowheads indicate abnormalities such as (i) abnormal nucleus (scale bar: 30 μm), (i,j) malformed intercellular bridges (scale bar: 30 μm) and (k) double midbody ring (scale bar: 10 μm). (i,j) AGO2 and (k) *α*-Tubulin in green and (i-k) Dicer in red. Nuclei in blue (DAPI).

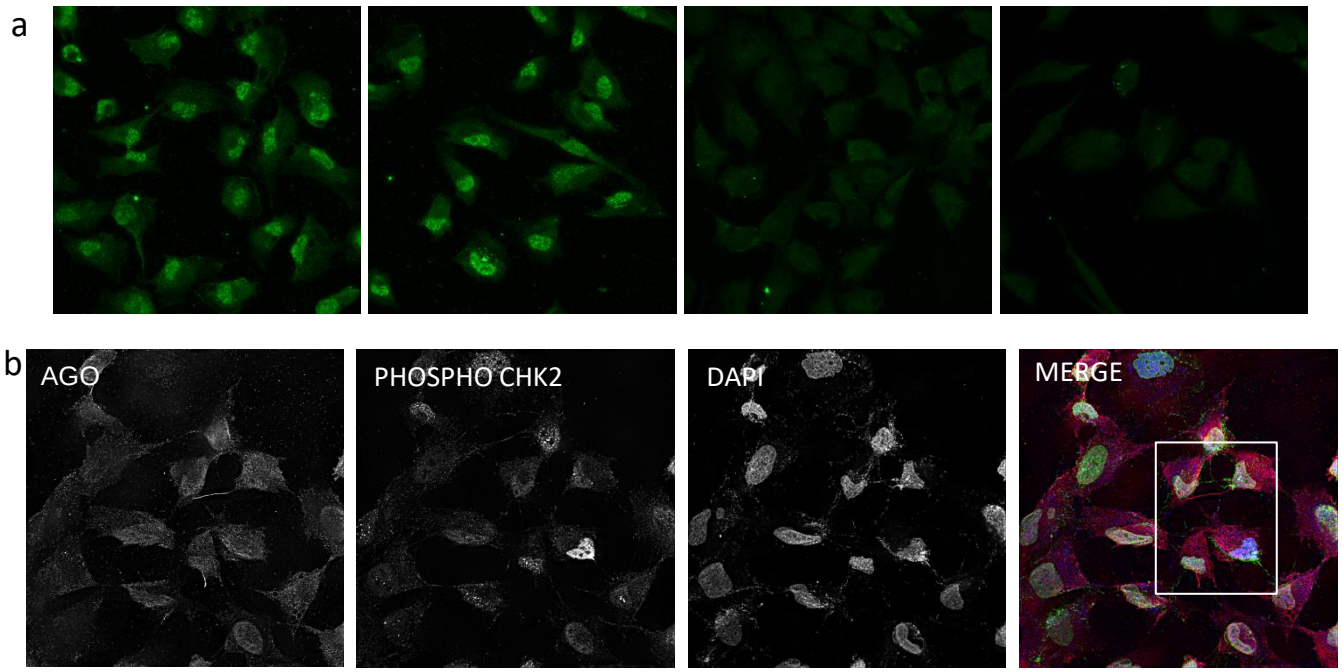
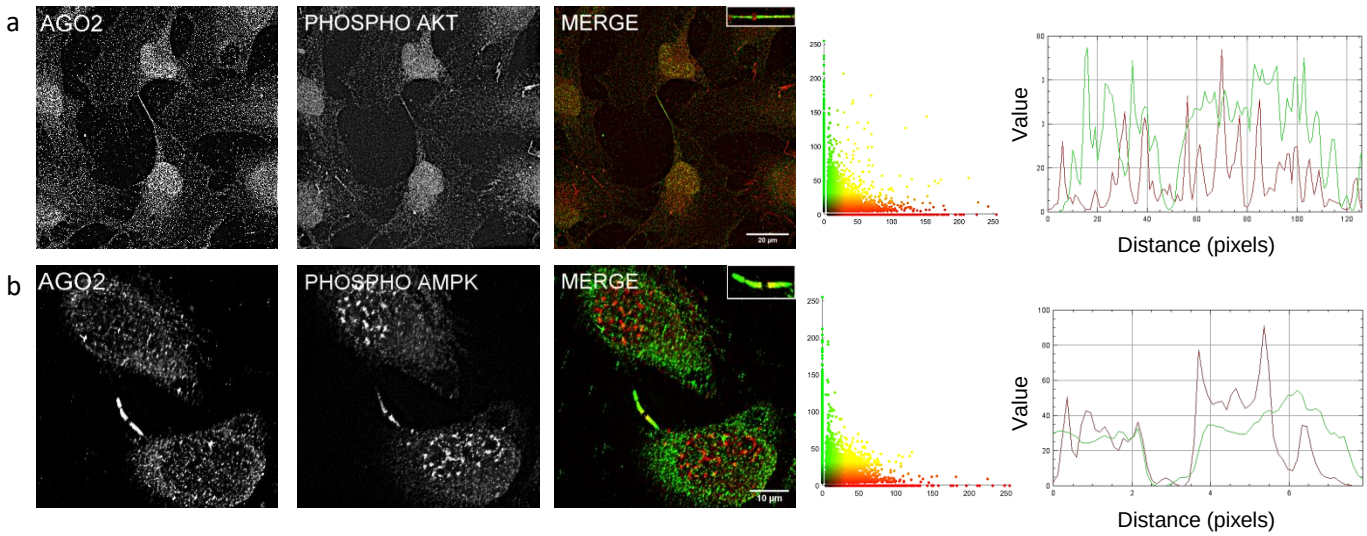



Figure S8: Negative controls. Representative images of NTHY ori 3-1 cells. Lack of colocalization distribution pattern of AGO2-phospho CHK2 across the intercellular bridge. (a) Differential signal intensities of AGO2 in siAGO2 cells compared to the scrambled ones, (b) AGO2 (green), phospho-CHK2 (red), nuclei (blue).




Negative controls

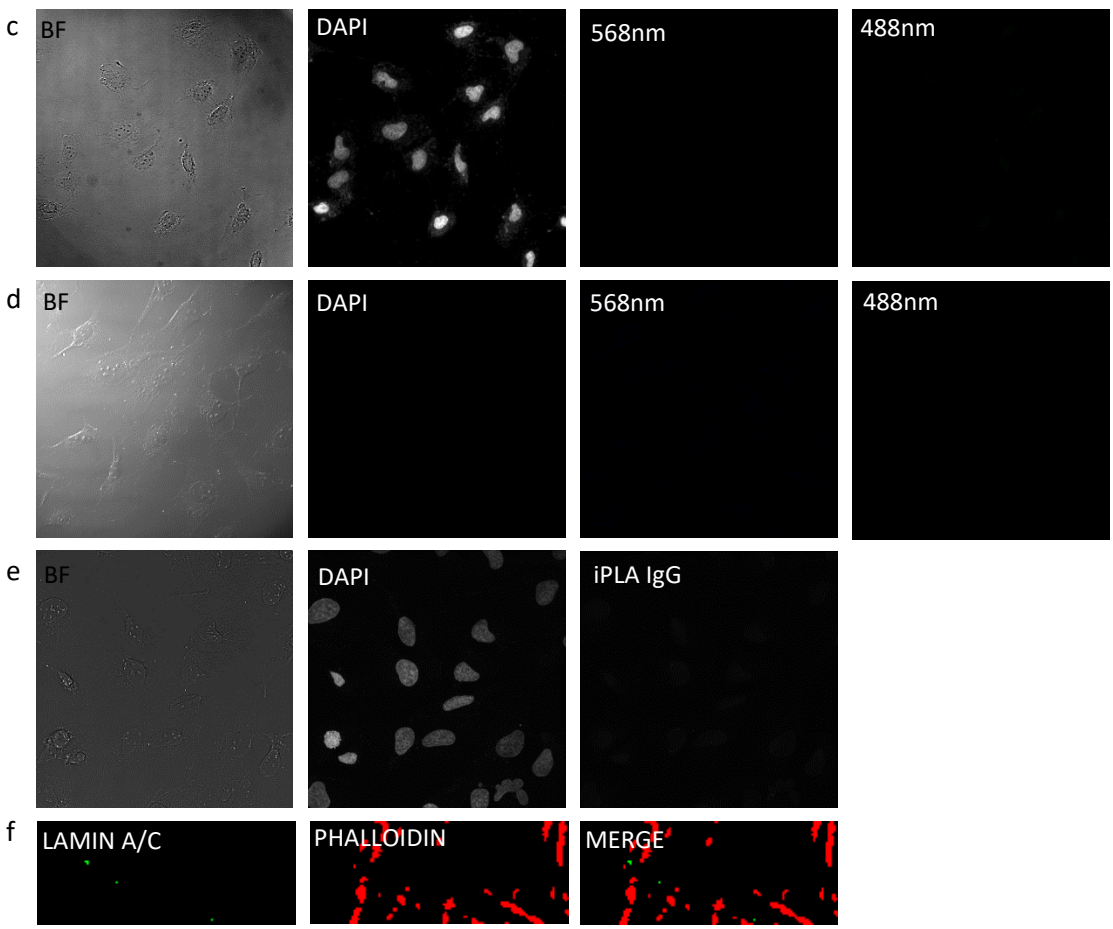



Figure S9

Negative controls

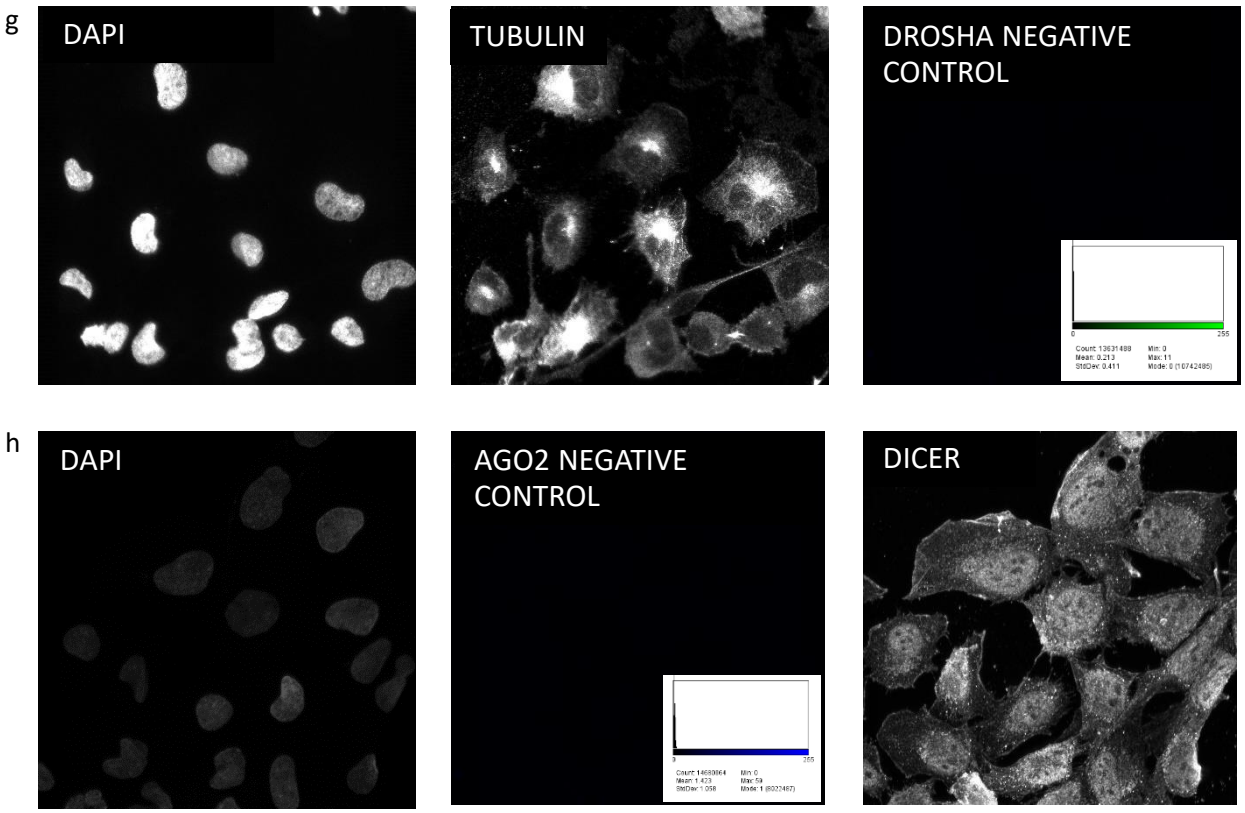



Figure S9: Phospho-Akt and phospho-AMPK kinase profiling follow the AGO2 distribution in the midbody structure. Representative images of NTHY ori 3-1 cells. (a) AGO2 (green) and phospho-Akt (red) (scale bar: 20 μm), (b) AGO2 (green) and phospho-AMPK (red) (scale bar: 10 μm). Nuclei in blue (DAPI). Visual assessment (merged images, scatterplots and intensity lineplots) of colocalization. Lineplots indicate the signal intensity of the two paired proteins. (c) Negative control of NTHY ori 3-1 cells stained with DAPI and secondary antibodies (without primary antibodies), (d) Negative control for autofluorescence (only fixed cells), (e) Negative control for PLAi experiment (IgG control), (f) Colocalization control, cytoplasmic region where Lamin A/C (green) and phalloidin (Texas-red) are known to anti- colocalize. (g) Negative control for red secondary antibody, *α*-Tubulin (green) and nuclei (blue). In the red channel anti-Drosha primary antibody staining without red secondary antibody. (h) Negative control for green secondary antibody, Dicer stained red and nuclei (blue) by DAPI. In the green channel anti-AGO2 primary antibody staining without green secondary antibody. (g,h) Signal histograms in the negative channels.

Continued
Figure S9

| Antibodies, Chemicals, Drugs | Company | Code | Host | Concentrations |
|---|---|---|---|---|
| 16% paraformaldehyde | Thermoscientific | 28906 | | |
| Triton-X 100 | Sigma/Aldrich, St. Louis, MO, USA | 11332481001 | | |
| Bovine Serum Albumin | Sigma/Aldrich, St. Louis, MO, USA | B2064 | | |
| anti-α actinin 4 | Abcam, Cambridge, MA, USA | ab198608 | rabbit | (1/50) |
| anti-argonaute-2 | Abcam, Cambridge, MA, USA | ab57113 | mouse | (1/80) |
| anti-α-tubulin | Abcam, Cambridge, MA, USA | ab18251 | rabbit | (1/1000) |
| anti-α-tubulin | Cell Signaling | DM1A#3873s | mouse | (1/100) |
| anti-Citron kinase | Novus | NBP2-38592 | rabbit | (1/100) |
| anti-Argonaute-2 | Abcam, Cambridge, MA, USA | ab186733 | rabbit | (1/50) |
| anti-Drosha | Abcam, Cambridge, MA, USA | ab12286 | rabbit | (1/100) |
| anti-DGCR8 | Novus | NBP1-30115 | rabbit | (1/100) |
| anti-Dicer | Novus | NBP1-06520 | rabbit | (1/120) |
| anti-ATF4 | GenScript | Ab-245 | rabbit | (1/100) |
| anti-Aurora B | BD Biosciences | 611082 | mouse | (1/200) |
| anti-Staufen/STAU1 | Abcam, Cambridge, MA, USA | ab50914 | rabbit | (1/100) |
| anti-Phospho-p38 MAPK (Thr180/Tyr182) | Cell Signaling | 9211 | rabbit | (1/100) |
| anti-phospho-AMPK1 (Ser485)/AMPK 2 (Ser491) | Cell Signaling | 4185 | rabbit | (1/100) |
| anti-Phospho-MEK1/2 (Ser217/221) (41G9) | Cell Signaling | 9154 | rabbit | (1/100) |
| anti-Phospho-p38 MAPK (Thr180/Tyr182) | Cell Signaling | 9211 | rabbit | (1/100) |
| anti-Phospho-Akt (Thr308) | Cell Signaling | 9275 | rabbit | (1/100) |
| anti-Phospho-sAPK/JNK (Thr183/Tyr185) | Cell Signaling | 9251 | rabbit | (1/100) |
| Donkey Anti-Rabbit IgG (H + L) Secondary Antibody Fluor®488 conjugate | Invitrogen, Waltham, MA, USA | | | (1/250) |
| Goat anti-Rabbit IgG (H + L) Secondary Antibody Alexa Fluor 594 | Invitrogen, Waltham, MA, USA | | | (1/250) |
| Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody Alexa Fluor 488 | Invitrogen, Waltham, MA, USA | | | (1/250) |
| Goat Anti-mouse IgG Dylight 594 Conjugated | Invitrogen, Waltham, MA, USA | | | (1/250) |
| Rhodamine - Phalloidin | Biotium | | | (5/200) |
| VECTASHIELD® Mounting Medium with DAPI | Vector Laboratories, Inc., 30 Ingold Road, Burlingame, CA 94010 USA | | | |
| phospho-ago kit[Argonaute 2 (a.a. 389-398), Argonaute 2 (Tyr-393) Argonaute 2 (Ser-387)] | ECM BIOSCIENCES | AK6970 | rabbit | (1/50) |
| Upf1 antibody (D15G6) | Cell Signaling | 12040 | rabbit | (1/100) |
| TIAR antibody | Cell Signaling | 8509 | rabbit | (1/100) |
| Dynactin antibody | Cell Signaling | 69399 | rabbit | (1/100) |
| lipofectamine 2000 | invitrogen | 11668-027 | | |
| CF 488 anti-streptavidin | Biotium | #29034 | | (1/250) |
| biotinylated oligo(dT) probe | biotrans | Z5261 | | (1/50) |
| DsiRNAs and TriFECTa Kits in tubes | IDT | | | according to the manufacturer's protocol |
| Phospho-eIF2α (Ser51) (D9G8) | Cell Signaling | 3398 | rabbit | (1/100) |
| Duolink™ proximity ligation assay (PLA) | Merck | 92101 | | according to the manufacturer's protocol |
| anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) | Cell Signaling | 9101 | rabbit | (1/100) |
| demecolcine | Sigma/Aldrich, St. Louis, MO, USA | D7385 | | 0,4μg/ml |
| cytochalasin D | Sigma/Aldrich, St. Louis, MO, USA | C8273 | | 10μM |
| anti-chk2 (1C12) | Cell Signaling | 3440 | mouse | (1/100) |
| anti-chk1 | Cell Signaling | 2345 | rabbit | (1/100) |
| anti-phospho-Chk1 (Ser345) | Cell Signaling | 2341 | rabbit | (1/100) |
| anti-phospho-Chk2 (Thr68) | Cell Signaling | 2661 | rabbit | (1/100) |
| anti-lamina/c | Abcam, Cambridge, MA, USA | ab108595 | rabbit | (1/400) |
| anti-Wee1 | Cell Signaling | 4936 | rabbit | (1/100) |
| anti phospho Wee1 (Ser642) (D47G5) | Cell Signaling | 4910 | rabbit | (1/100) |
| anti-phosphop53 | Cell Signaling | 9286 | mouse | (1/100) |
| p53 | Cell Signaling | 2433 | rabbit | (1/100) |
| TRI Reagent | Sigma/Aldrich | T9424 | | |

Table S2: Reagents, drug compounds and antibodies (primary and secondary). Details of the materials used in the methodology of the manuscript.