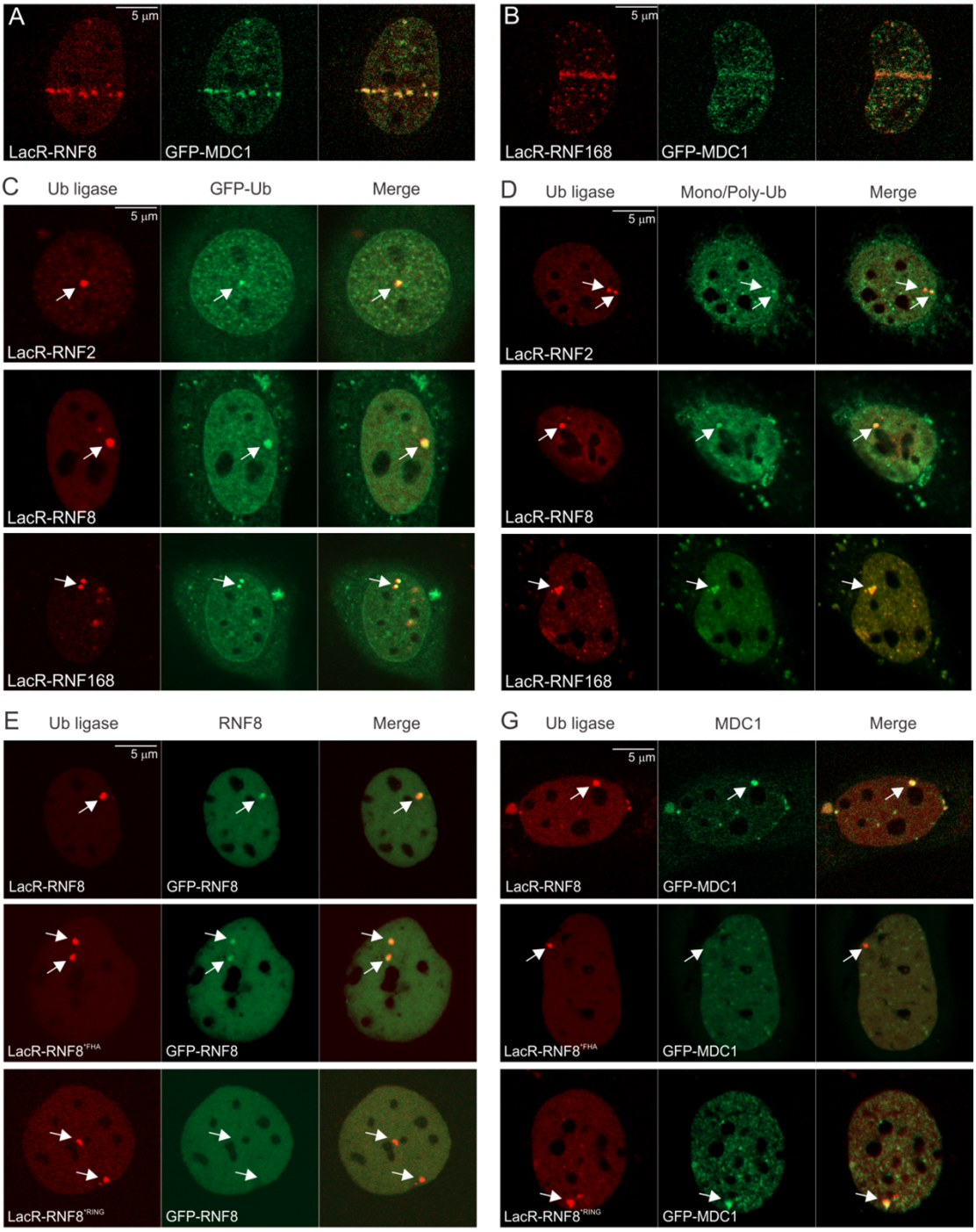
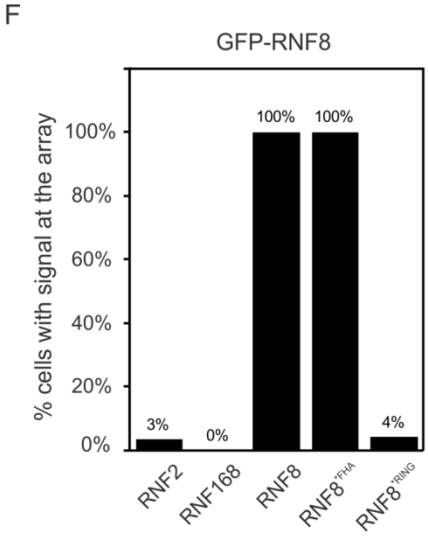
Figure S1


A
5 μm
LacR-RNF8
GFP-MDC1
B
5 μm
LacR-RNF168
GFP-MDC1
C
Ub ligase
GFP-Ub
Merge
5 μm
LacR-RNF2
LacR-RNF8
LacR-RNF168
D
Ub ligase
Mono/Poly-Ub
Merge
5 μm
LacR-RNF2
LacR-RNF8
LacR-RNF168
E
Ub ligase
RNF8
Merge
5 μm
LacR-RNF8
GFP-RNF8
LacR-RNF8*FHA
GFP-RNF8
LacR-RNF8*RING
GFP-RNF8
G
Ub ligase
MDC1
Merge
5 μm
LacR-RNF8
GFP-MDC1
LacR-RNF8*FHA
GFP-MDC1
LacR-RNF8*RING
GFP-MDC1


F
GFP-RNF8
% cells with signal at the array
100%
80%
60%
40%
20%
0%
3%
0%
100%
100%
4%
RNF2
RNF168
RNF8
RNF8*FHA
RNF8*RING

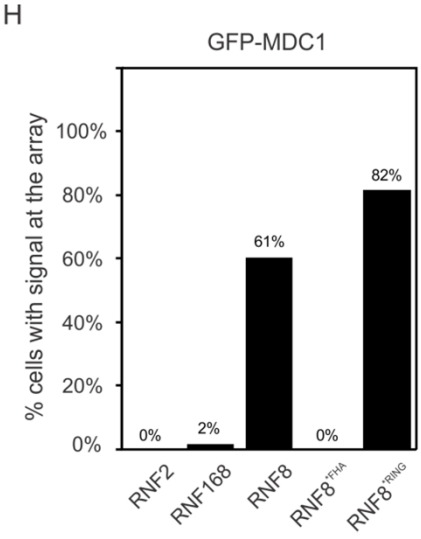
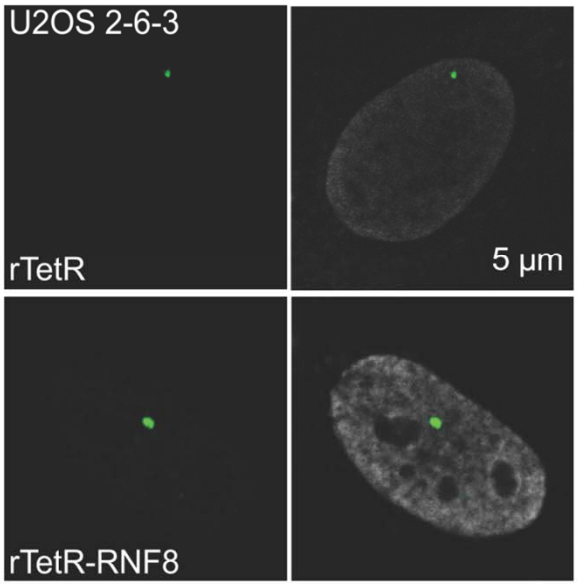

H
GFP-MDC1
% cells with signal at the array
100%
80%
60%
40%
20%
0%
0%
2%
61%
0%
82%
RNF2
RNF168
RNF8
RNF8*FHA
RNF8*RING

Figure S2


U2OS 2-6-3
rTetR
5 µm
rTetR-RNF8

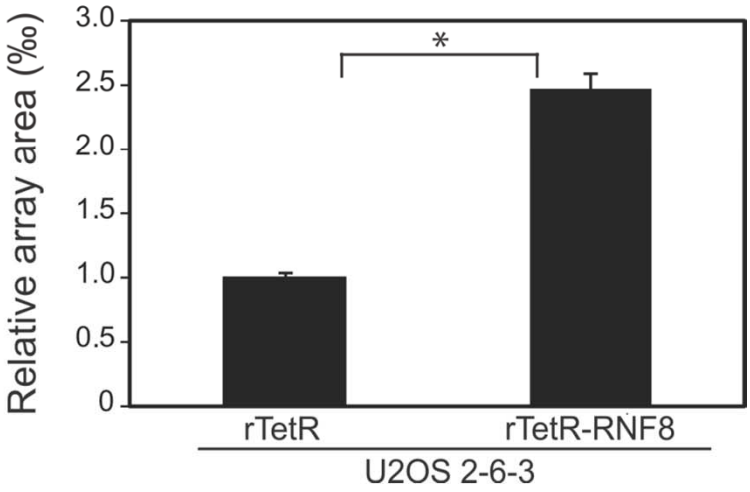

Relative array area (‰)
3.0
2.5
2.0
1.5
1.0
0.5
0
*
rTetR
rTetR-RNF8
U2OS 2-6-3

Figure S3

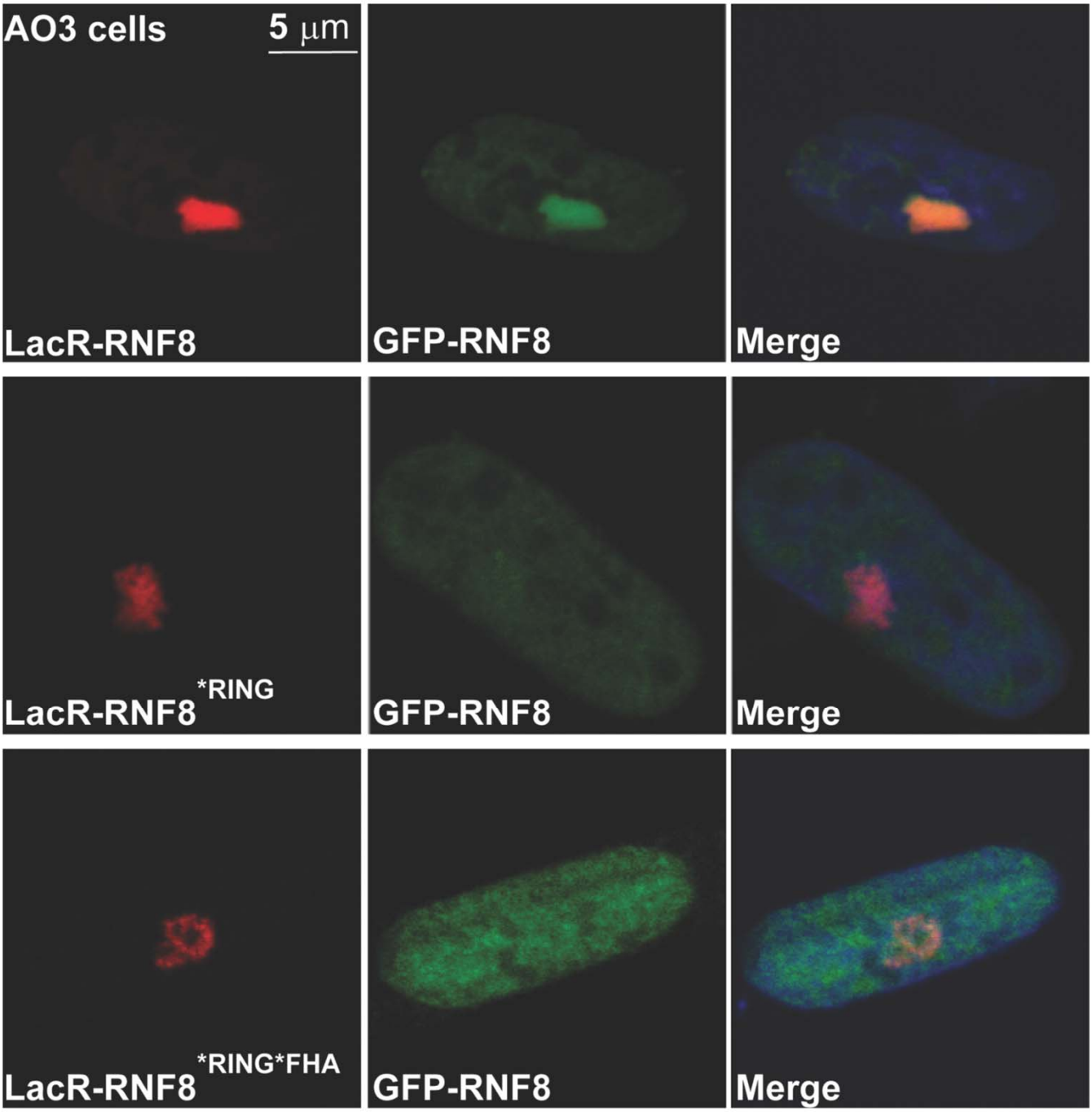

AO3 cells
5 μm
LacR-RNF8
GFP-RNF8
Merge
LacR-RNF8 *RING
GFP-RNF8
Merge
LacR-RNF8 *RING*FHA
GFP-RNF8
Merge

Figure S4

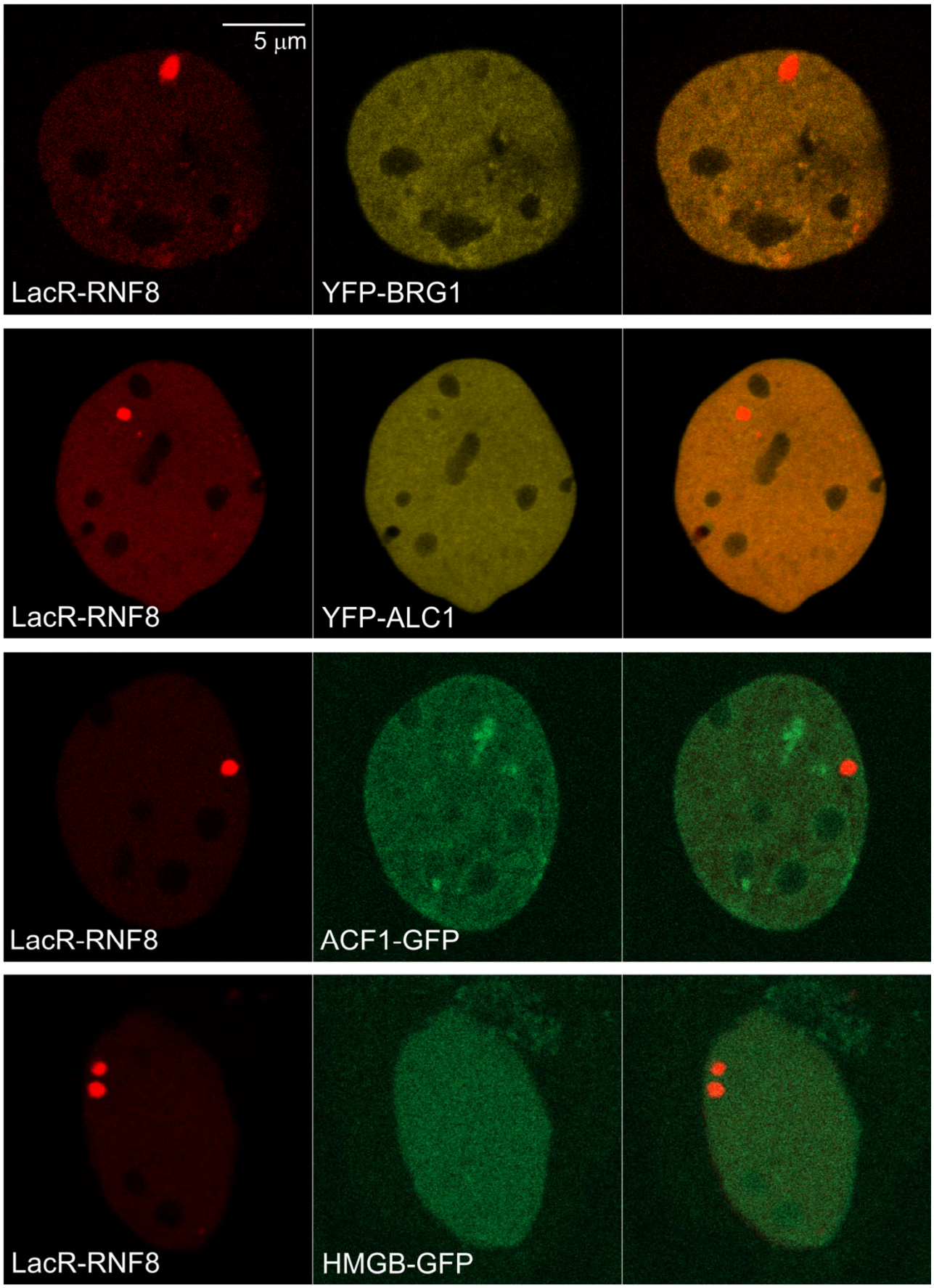
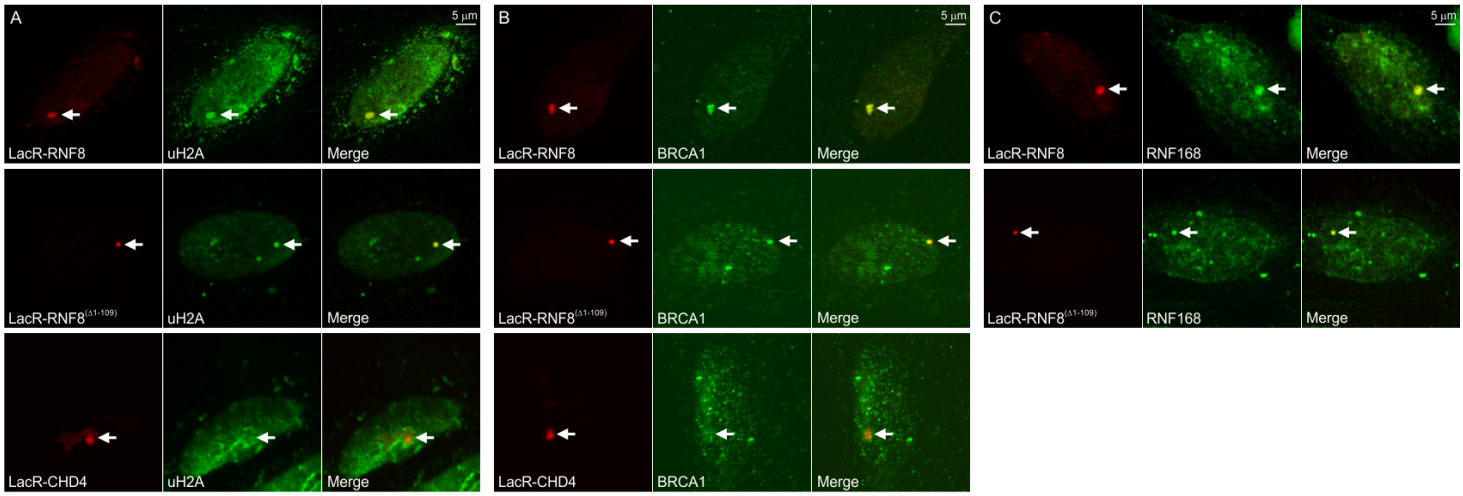
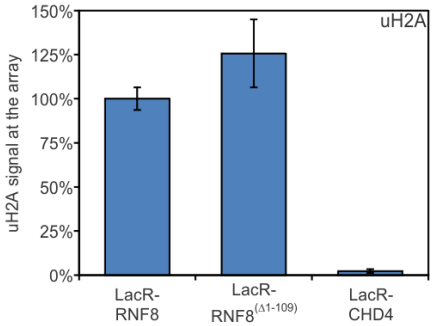
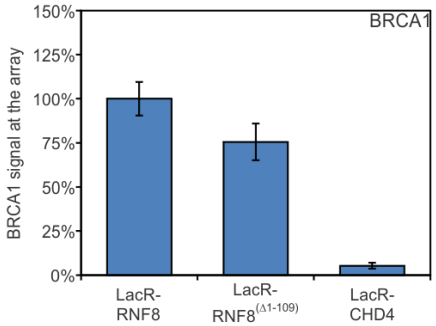
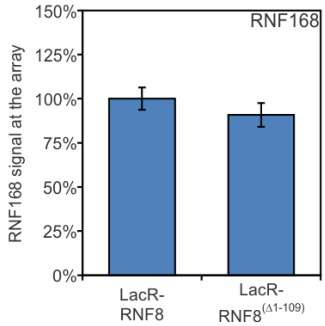

5 μm
LacR-RNF8
YFP-BRG1
LacR-RNF8
YFP-ALC1
LacR-RNF8
ACF1-GFP
LacR-RNF8
HMGB-GFP

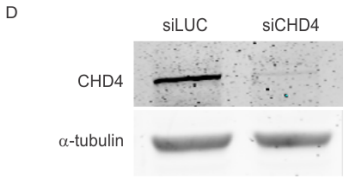
Figure S5

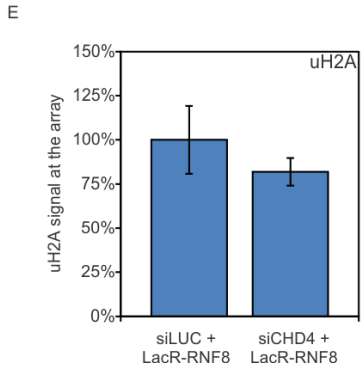

A
5 μm
LacR-RNF8
uH2A
Merge
LacR-RNF8(Δ1-109)
uH2A
Merge
LacR-CHD4
uH2A
Merge
B
5 μm
LacR-RNF8
BRCA1
Merge
LacR-RNF8(Δ1-109)
BRCA1
Merge
LacR-CHD4
BRCA1
Merge
C
5 μm
LacR-RNF8
RNF168
Merge
LacR-RNF8(Δ1-109)
RNF168
Merge

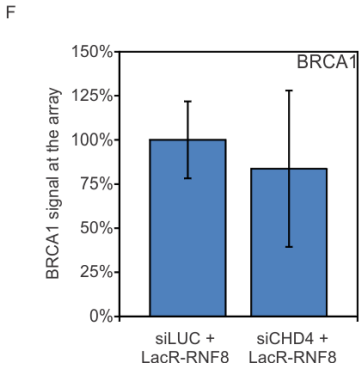

uH2A
uH2A signal at the array
150%
125%
100%
75%
50%
25%
0%
LacR-RNF8
LacR-RNF8(Δ1-109)
LacR-CHD4


BRCA1
BRCA1 signal at the array
150%
125%
100%
75%
50%
25%
0%
LacR-RNF8
LacR-RNF8(Δ1-109)
LacR-CHD4


RNF168
RNF168 signal at the array
150%
125%
100%
75%
50%
25%
0%
LacR-RNF8
LacR-RNF8(Δ1-109)


D
siLUC
siCHD4
CHD4
α-tubulin


E
uH2A
uH2A signal at the array
150%
125%
100%
75%
50%
25%
0%
siLUC + LacR-RNF8
siCHD4 + LacR-RNF8


F
BRCA1
BRCA1 signal at the array
150%
125%
100%
75%
50%
25%
0%
siLUC + LacR-RNF8
siCHD4 + LacR-RNF8

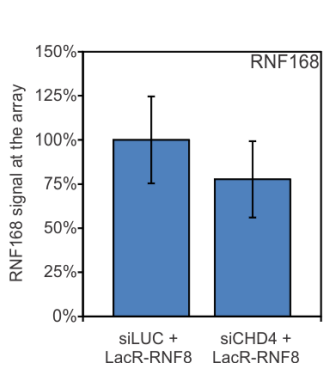

G
RNF168
RNF168 signal at the array
150%
125%
100%
75%
50%
25%
0%
siLUC + LacR-RNF8
siCHD4 + LacR-RNF8

Fig S1. Ubiquitin ligase fusion proteins are functional and mediate ubiquitin conjugation and ubiquitin ligase cross-recruitment. Recruitment of **A**) mCherry-LacR-RNF8 (red) and **B**) mCherry-LacR-RNF168 (red) to DSBs inflicted by laser micro-irradiation following BrdU sensitization. The sites of DNA damage are marked by GFP-MDC1 (green). **C-D**) Tethering mCherry-LacR-RNF2, mCherry-LacR-RNF8 or mCherry-LacR-RNF168 (shown in red) causes accumulation of **C**) GFP-Ub and **D**) ubiquitin conjugates (FK2) (green) at the array. **E**) Recruitment of GFP-tagged RNF8 (green) triggered by tethering mCherry-LacR-RNF8 and mCherry-LacR-RNF8^{*FHA}, but not by mCherry-LacR-RNF8^{*RING}. **F**) Quantification of the percentage of cells with GFP-RNF8 at the array after tethering the indicated mCherry-LacR-RNF fusion proteins. Values represent the mean of two independent experiments (n=50 cells). **G**) Recruitment of GFP-tagged MDC1 (green) triggered by tethering mCherry-LacR-RNF8 and mCherry-LacR-RNF8^{*RING}, but not by mCherry-LacR-RNF8^{*FHA}. **H**) Quantification of the percentage of cells with GFP-MDC1 at the array after tethering the indicated mCherry-LacR-RNF fusion proteins. Values represent the mean of two independent experiments (n=50 cells). The scale bar is 5 μm.

Fig S2. Immobilization of RNF8 with the rTetR to TetO in U2OS-2-6-3 cells causes decondensation. U2OS 2-6-3 cells harbouring ~200 copies of a TetO-containing cassette integrated in the genome were transfected with either GFP-rTetR or GFP-rTetR-RNF8 after which the binding of the rTetR fusion proteins was induced by treatment with dox for 24hours. The RNF8-mediated chromatin decondensation is quantified as the mean size of the array (in ‰ of nuclear area) next to the images.

Fig S3. Immobilization of RNF8, but not RNF8*RING, triggers recruitment of GFP-RNF8 to the unfolded array in AO3 cells. Hamster AO3 cells, harbouring a 90 Mbp array interspersed with LacO sequences, were transfected with the indicated LacR-RNF8 fusion proteins together with GFP-RNF8. While all RNF8 fusion proteins unfold the array, only LacR-RNF8^{WT} is capable of recruiting GFP-RNF8 to the array, while both LacR-RNF8^{*RING} and LacR-RNF8^{*RING*FHA} fail to do so.

Fig S4. Various chromatin modifiers are not recruited when tethering RNF8. NIH2/4 cells were transfected with mCherry-LacR-RNF8 together with the indicated fluorescent protein-tagged chromatin modifiers. No apparent recruitment of BRG1, ALC1, ACF1 or HMGB1 by immobilized RNF8 was observed.

Fig S5. CHD4 is not required to initiate the DDR upon tethering RNF8 to chromatin. A-C) U2OS-2-6-3 cells were transfected with the indicated LacR-fusion proteins and stained for **A**) uH2A, **B**) BRCA1, and **C**) RNF168. Quantification of the percentage of cells with positive signals at the array after tethering the indicated fusion proteins is shown below the images. D) Western blot analysis of U2OS-2-6-3 cells transfected with CHD4 siRNAs. Membranes were probed with antibodies to CHD4 and Tubulin. **E-G**) Quantification of the percentage of cells with positive signals at the array after tethering LacR-RNF8 in cells transfected with siLUC or siCHD4 and stained for **E**) uH2A, **F**) BRCA1, and **G**) RNF168. Values represent the mean of two independent experiments (n=20 cells).