Supplementary Figure Legends

Supplementary Figure S1 Aurora-A expression levels in lentivirus-infected MCF10A. Protein extracts from BT-20, SKBR-3, ZR-75-1, ZR-75-30, MDA-MB157, Colo205, MCF10A**,** MCF-7, CAPAN1, MCF10A infected with empty lentiviral vector and MCF10A infected with Aurora-A expressing lentiviral vector were prepared and analysed in Western blot detection of Aurora-A protein levels Ezrin detection was used as a loading control.

Supplementary Figure S2 Repression of irradiation-induced RAD51 focus in mouse Embryonic Stem (ES) cells overexpressing Aurora-A. ES cells were transfected with plasmids carrying myc-tagged Aurora-A cDNA (myc-Aurora-A) or the corresponding empty vector (Empty vector). A 5-Gy X-ray treatment was delivered 24h after transfection, followed by 4h recovery. Non-irradiated cells were included as a control. (**A**) Detection of RAD51 and γ-H2AX, as indicated, by immunofluorecence. Control ES cells lacking functional BRCA2 were used as a control. (**B**) Protein lysates were prepared from ES cells treated as in (A), and analysed for Aurora-A expression level. Erzin detection was used for loading control. (**C**) Propidium Iodide DNA profiles of ES cells treated as in (A) were performed to assess the effect of Aurora-A overexpression on cell cycle.

Supplementary Figure S3 Repression of irradiation-induced RAD51 focus in MCF10A cells overexpressing Aurora-A is not affected by the Aurora-B inhibitor ZM447439. MCF10A cells were infected with a lentivirus construct driving the expression of GFP (Empty vector, left panel) or both GFP and Aurora-A (Aurora-A, right panel) and mixed in equal proportion with non-infected cells to provide an internal control. The mixed cells were pre-treated with the Aurora-B kinase inhibitor ZM447439 (+ZM447439) or with vehicle (-ZM447439) one hour before a 5-Gy X-ray treatment, followed by a 4h, 16h, 24h or 30h recovery, as indicated. RAD51 foci were then detected by immunofluorescence and RAD51 foci-positive nuclei were scored in uninfected (GFP negative) and infected (GFP positive) cells as described in Figure 1B. The average percentage of RAD51 foci positive cells from two independent experiments is shown. Error bars indicate SEM.

Supplementary Figure S4 Repression of irradiation-induced RAD51 focus in MCF10A requires Aurora-A kinase activity. MCF10A cells were infected with a lentivirus construct driving the expression of GFP (Empty vector), both GFP and Aurora-A (Aurora-A) or GFP and the kinase dead mutant Aurora-A D256A (Aurora-A D256A) and mixed in equal proportion with non-infected cells to provide an internal control. Cells were then exposed to 5-Gy X-ray, followed by a 4h, 16h, 24h or 36h recovery, as indicated. RAD51 foci were then detected by immunofluorescence and RAD51 foci-positive nuclei were scored in uninfected (GFP negative) and infected (GFP positive) cells as described in Figure 1B. The

average percentage of RAD51 foci positive cells from two independent experiments is shown. Error bars indicate SEM.

Supplementary Figure S5 Lentivirus-driven ectopic expression of Aurora-A does not increase aneuploidy of MCF10A cells. MCF10A cells were infected with a lentivirus construct driving the expression of GFP (Empty vector, top panel) or both GFP and Aurora-A (Aurora-A, bottom panel) and mixed in equal proportion with non-infected cells to provide an internal control. After two weeks, the DNA was stained with Hoechst 33342 and the DNA profile of the mixed cells was performed as described in material and methods. Single GFP positive and GFP negative single cells were selected for analysis based on Pacific Blue-W vs. Pacific Blue-A plot gating and their DNA content was analysed in parallel.

Supplementary Figure S6 Aurora-A overexpression does not affect the cell cycle profile of 293 cells. The 293 cell line bearing the HR repair substrate DR1*Bsd* was infected with an emtpy lentiviral vector (Empty vector), a vector driving the expression of Aurora-A (Aurora-A) or of a kinase inactive mutant (Aurora-A S361*) or Gadd45a (Gadd45a), or not infected, as described in Figure 3. A DNA profile was monitored by flow cytometry after Hoechst 33342 staining to verify that the exogenous expression of Aurora-A did not alter the cell cycle of 293 cells.

Figure S1

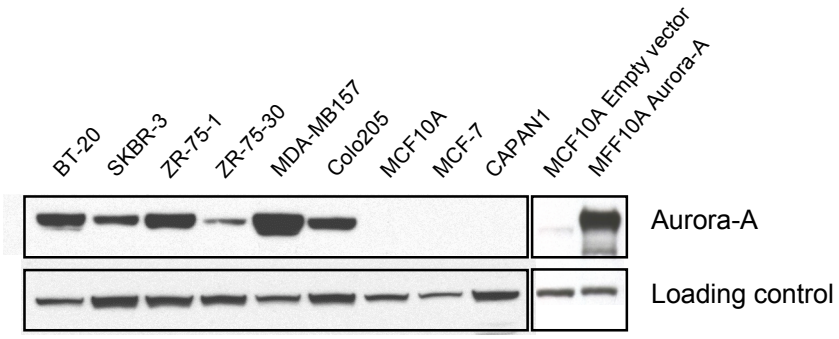

BT-20
SKBR-3
ZR-75-1
ZR-75-30
MDA-MB157
Colo205
MCF10A
MCF-7
CAPAN1
MCF10A Empty vector
MFF10A Aurora-A
Aurora-A
Loading control

Figure S2

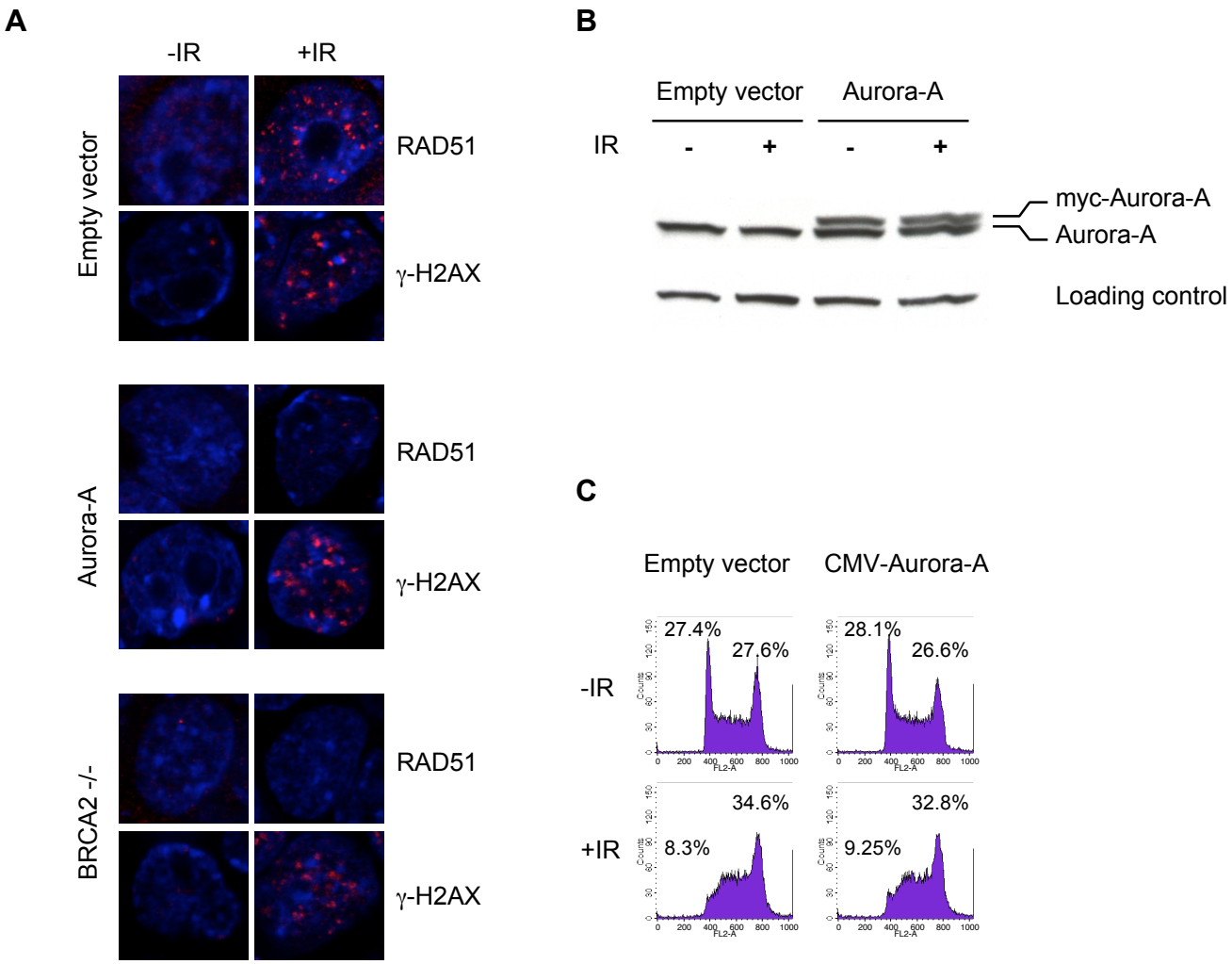

A
-IR
+IR
Empty vector
RAD51
γ-H2AX
Aurora-A
RAD51
γ-H2AX
BRCA2 -/-
RAD51
γ-H2AX
B
Empty vector
Aurora-A
IR
-
+
-
+
myc-Aurora-A
Aurora-A
Loading control
C
Empty vector
CMV-Aurora-A
-IR
27.4%
27.6%
28.1%
26.6%
+IR
34.6%
8.3%
32.8%
9.25%

Figure S3

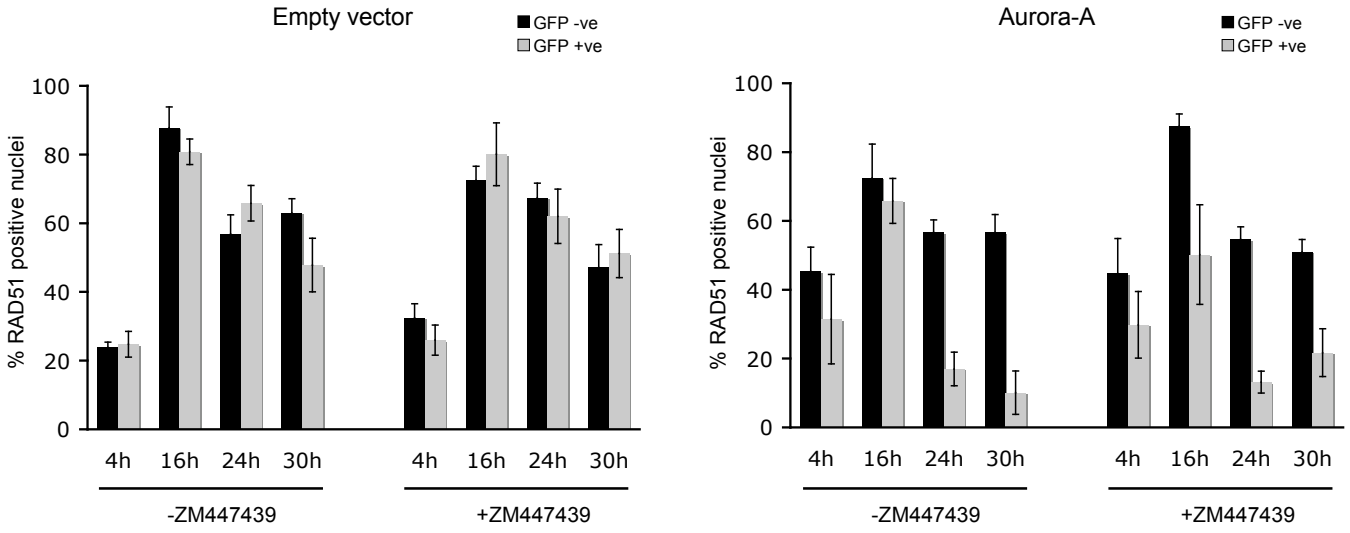

Empty vector
GFP -ve
GFP +ve
% RAD51 positive nuclei
100
80
60
40
20
0
4h
16h
24h
30h
4h
16h
24h
30h
-ZM447439
+ZM447439
Aurora-A
GFP -ve
GFP +ve
% RAD51 positive nuclei
100
80
60
40
20
0
4h
16h
24h
30h
4h
16h
24h
30h
-ZM447439
+ZM447439

Figure S4

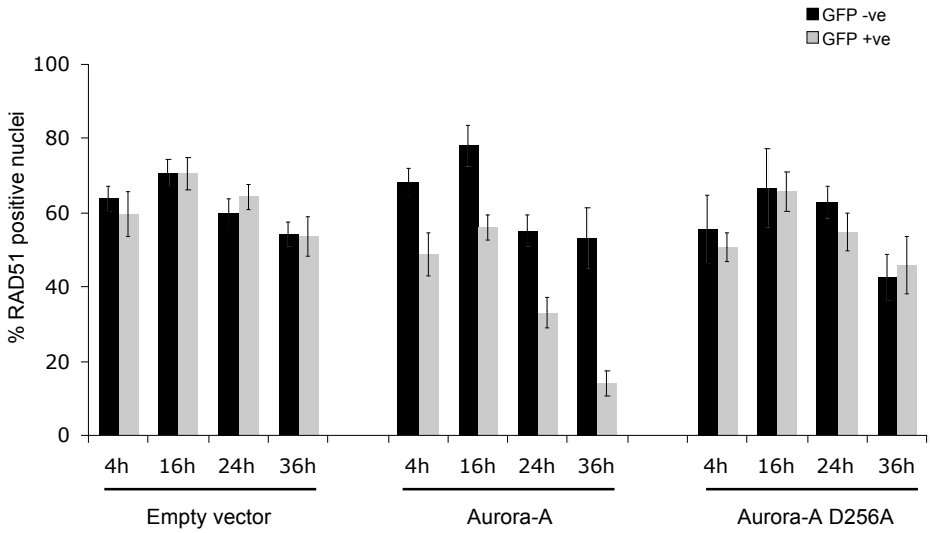

GFP -ve
GFP +ve
% RAD51 positive nuclei
100
80
60
40
20
0
4h
16h
24h
36h
4h
16h
24h
36h
4h
16h
24h
36h
Empty vector
Aurora-A
Aurora-A D256A

Figure S5

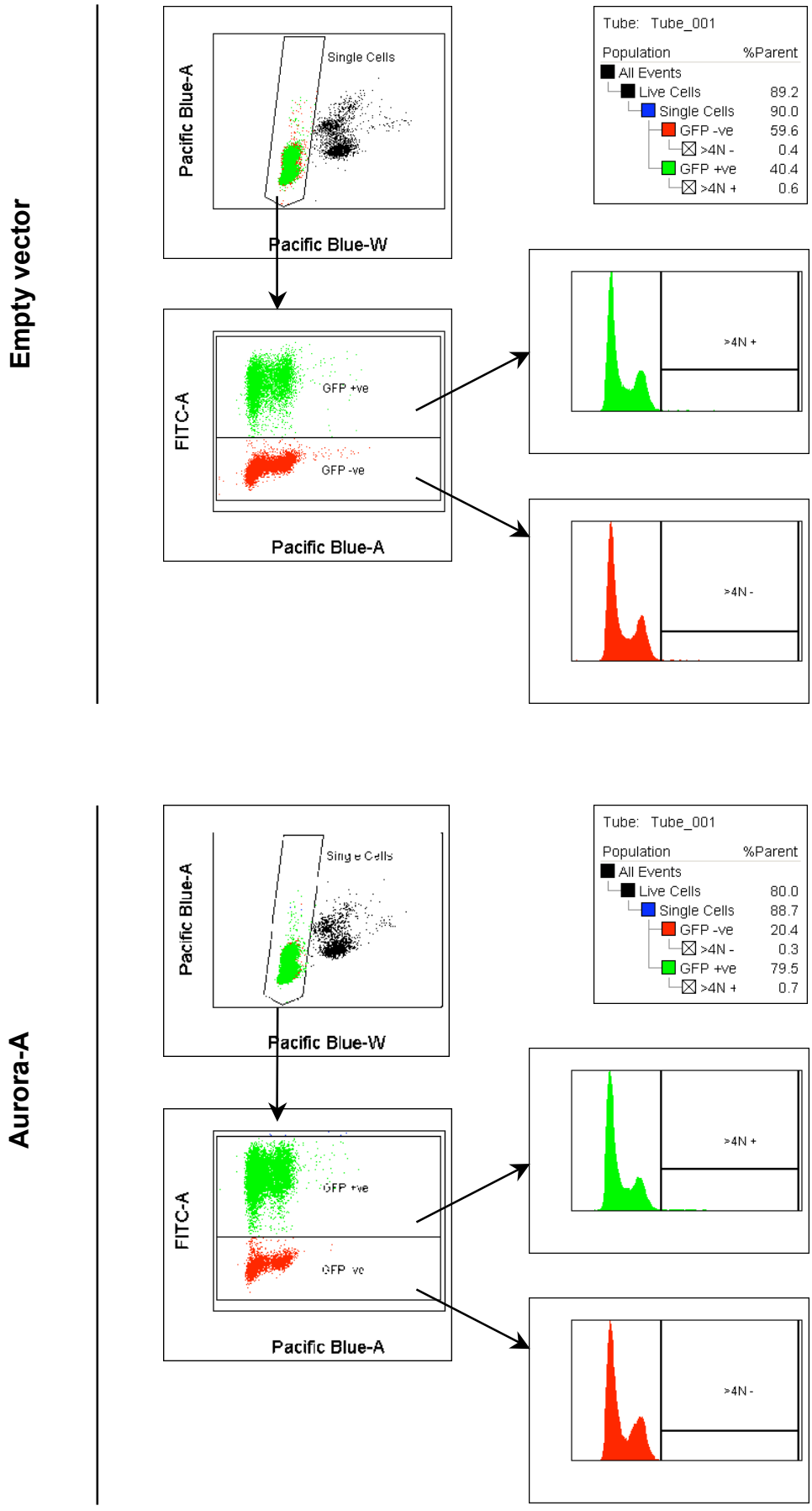
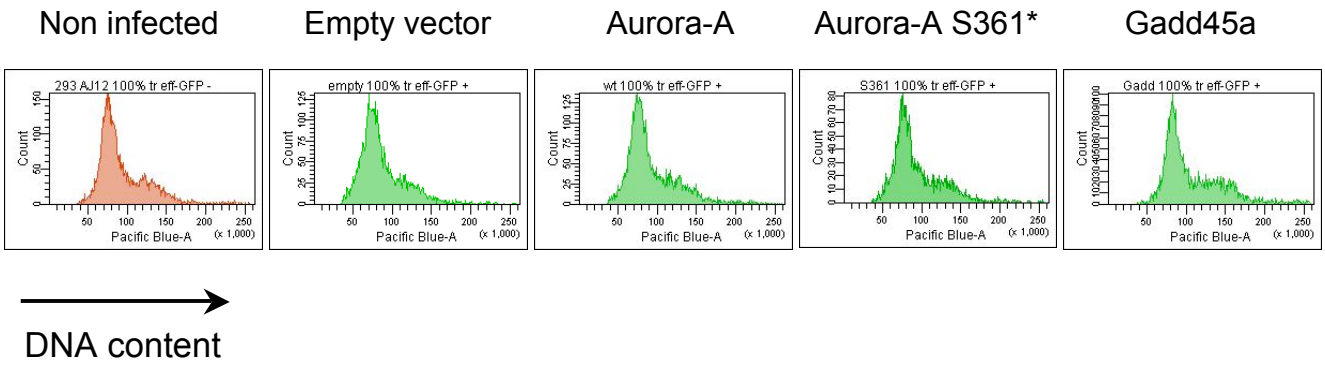

Empty vector
Pacific Blue-A
Single Cells
Pacific Blue-W
Tube: Tube_001
Population %Parent
All Events
Live Cells 89.2
Single Cells 90.0
GFP -ve 59.6
>4N - 0.4
GFP +ve 40.4
>4N + 0.6
FITC-A
GFP +ve
GFP -ve
Pacific Blue-A
>4N +
>4N -
Aurora-A
Pacific Blue-A
Single Cells
Pacific Blue-W
Tube: Tube_001
Population %Parent
All Events
Live Cells 80.0
Single Cells 88.7
GFP -ve 20.4
>4N - 0.3
GFP +ve 79.5
>4N + 0.7
FITC-A
GFP +ve
GFP -ve
Pacific Blue-A
>4N +
>4N -

Figure S6


Non infected
Empty vector
Aurora-A
Aurora-A S361*
Gadd45a
293 AJ12 100% tr eff-GFP -
empty 100% tr eff-GFP +
wt 100% tr eff-GFP +
S361 100% tr eff-GFP +
Gadd 100% tr eff-GFP +
Count
Pacific Blue-A
(x 1,000)
DNA content