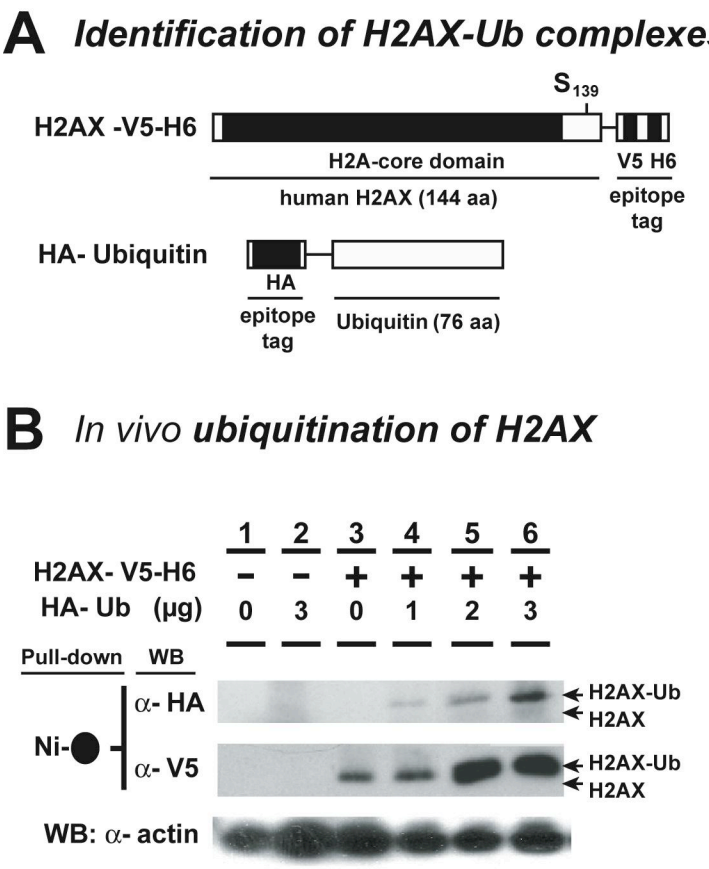
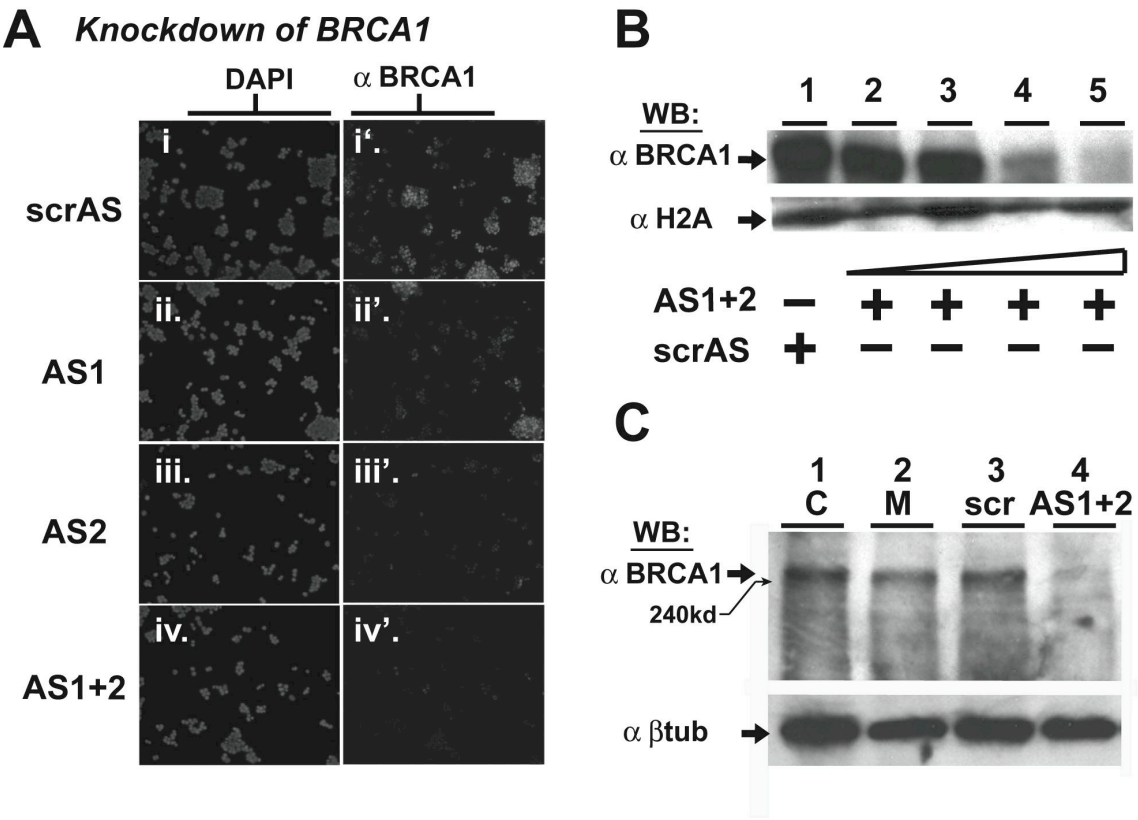
Supplemental Figures

Supplemental Figure 1



H2AX is ubiquitinated *in vivo*. In order to identify critical residues in human H2AX, an epitope tagged version of the protein was created. (A) Key features of the H2AX-V5-H6 and HA-ubiquitin expression vectors are illustrated. HA and V5 represent unique antibody recognition sites. H6 represents a Histidine (His)-tag allowing purification by Ni^{++}-affinity chromatography. (B) Wild type H2AX-V5-H6 was transfected into 293T cells along with 0, 1, 2 or 3 µg of HA-tagged ubiquitin. H6 tagged proteins were purified from chromatin fractions directly immunoblotted (WB) for the presence of HA-ubiquitin. The blot was stripped and reprobed for the presence of the V5 tag on H2AX. 2% of the cell lysate was probed for the presence of β-actin.

Supplemental Figure 2



Knockdown of BRCA1 expressions with antisense morpholino oligos. (A) Cells treated with a scrambled antisense morpholino (scrAS) or α-BRCA1 antisense morpholino oligos (AS1 and AS2), separately or in combination (AS1+2) were stained with DAPI (i-iv) to identify DNA, or immunostained for the presence of BRCA1 (i'-iv'). Black and white photomicrographs were captured with a 10x objective (magnification, 100x). (B) Cells treated with scrambled antisense morpholino (lane 1, scrAS)) or increasing amounts of both antisense morpholino oligos (AS1+2; lanes 2-5) were lysed, separated by SDS-PAGE, and probed (WB) for the presence of BRCA1 or H2A. (C) Cells treated with nothing (lane 1), media and morpholino delivery solution only (no morpholino, M, lane 2), scrambled antisense morpholino (lane 3, scrAS)) or antisense morpholino oligos (AS1/2; lane 4) were lysed and separated by SDS-PAGE and probed (WB) for the presence of BRCA1 or ß-tubulin.

Supplemental Data:

SUPPLEMENTAL Table 1

	Variant[1]	Template[2]	Primers used for cloning or point mutagenesis[3]	Orientation[4]
1.	H2AX	RNA	5'- CACCATGTCGGGCCGCGGCAAGACTG[†]	S
			5'- TCCTCCGTACTCCTGGGAGGCCTGGGTG	AS
2.	H2AX-A_{139}	RNA	5'- TCCTCCGTACTCCTGGGCGGCCTGGGTG	AS
3.	H2AX-E_{139}	RNA	5'- TCCTCCGTACTCCTGCTCGGCCTGGGTG	AS
4.	H2AX-E_{139}-R_{118}	H2AX-E_{139}	5'- GCTGCTGCCCAGGAAGACCAGCGCC	S
5.	H2AX-E_{139}-R_{119}	H2AX-E_{139}	5'- TGCTGCCCAAGAGGACCAGCGCCAC	S
6.	H2AX-E_{139}-R_{128}	H2AX-E_{139}	5'- CGTGGGGCCGAGGGCGCCCTCGG	S
7.	H2AX-E_{139}-R_{133}	H2AX-E_{139}	5'- CTCGGGCGGCAGGAAGGCCACCC	S
8.	H2AX-E_{139}-R_{134}	H2AX-E_{139}	5'- GGGCGGCAAGAGGGCCACCCAGG	S
9.	H2AX-E_{139}-$RR_{118/9}$	H2AX-E_{139}	5'- GCTGCCCAGGAGGACCAGCGCCA	S
10.	H2AX-E_{139}-$RR_{133/4}$	H2AX-E_{139}	5'- GGGCGGCAGGAGGGCCACCCAGG	S

[1]RT-PCR was used to generate human 1) H2AX, 2) H2AX-A_{139}, and 3) H2AX-E_{139}, Point mutagenesis was then performed to generate a series of mutations (4-10) within the conserved carboxy-terminus of the H2AX-E_{139} variant protein.
[2]The template for PCR represented RNA or cloned cDNA as listed.
[3]The primers used for PCR reactions are listed. All reactions required primer pairs. The first three variants were created with the sense strand primer indicated in 1[†] and the indicated reverse (AS) primer. Reactions 4-10 used the indicated S primer and a second AS primer that was exactly complementary to it.
All products were sequenced to confirm identity with the intended product and then cloned into pcDNA3.1DV5-H6 (Invitrogen) to create epitope tagged fusions suitable for expression in human cells.
[4]The orientation of the listed primer: Sense (S) or Antisense (AS).