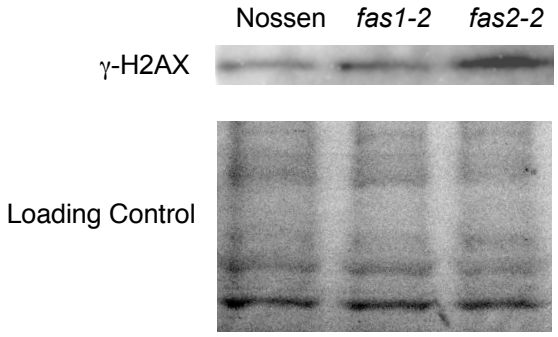

Western blot analysis of γ-H2AX in wild-type Nossen and *fas* mutants.

Increment of γ-H2AX was detected in *fas* mutants.

Methods
Nuclear protein extracts
Nuclear proteins were extracted using a CelLytic™ PN kit (SIGMA, St. Louis, Missouri USA) as described by the manufacturer. Protein samples were quantified using the Bio-Rad Protein Assay (Bio-Rad Laboratories), using bovine serum albumin (BSA) to create a standard curve. Five percent 2-mercaptoethanol, 5% 1.5M Tris-HCl pH8.8, and 5x sample loading buffer (10% SDS, 0.5% Bromophenol Blue, 313mM Tris-HCl pH6.8, 50% glycerol) were then added to each sample. Samples were boiled for 5 minutes and stored at -20°C until used for immunoblotting.

Immunoblotting
Approximately 7.5μgs of protein samples were loaded into each well of a 4-20% Tris-glycine-SDS gradient precast polyacrylamide gel (BIO-RAD, California, USA) prior to electrophoresis. Gels were transferred to PVDF membranes (Immobilon-P), (Millipore, Massachusetts, USA) for 2.5 hours at 300mA, 4 °C with Tris/Glycine/Methanol buffer (25mM Tris-HCl pH8.3, 192mM Glycine, 20% v/v methanol). Blots were incubated in 3% BSA in 1x TBS-T (0.1% final concentration Tween-20) at room temperature for 3 hours on a rotating platform. Blots were then incubated overnight at 4°C in rabbit anti-plant γ-H2AX primary antibody (Friesner *et al*., 2005) diluted 1:5000 in 3% BSA in 1x TBS-T. After rinsing briefly 3 times with 1x TBS-T, blots were washed once for 15 minutes and 3 times for 5 minutes each in a large volume of 1x TBS-T. Blots were then incubated with anti-rabbit Ig horseradish peroxidase linked secondary antibody (PIERCE, Illinois, USA) at a dilution of 1: 1000 in 3% BSA/ 1x TBS-T for 1 hour at room temperature on a rotating platform. Blots were washed as described above and incubated with enhanced chemiluminescence reagent, SuperSignal West Femto Maximum Sensitivity Substrate (PIERCE) for 5 minutes at room temperature. Luminescence was detected by LumiVision PRO (AISIN, Japan). After luminescence detection, blots were stained by MemCode Reversible Protein Stain Kit (PIERCE) to visualize proteins and estimate protein loading.

Reference
Friesner JD, Liu B, Culligan K, Britt AB (2005) Ionizing radiation-dependent gamma-H2AX focus formation requires ataxia telangiectasia mutated and ataxia telangiectasia mutated and Rad3-related. *Mol Biol Cell.* **16**, 2566-2576

Supplementary Figure S3