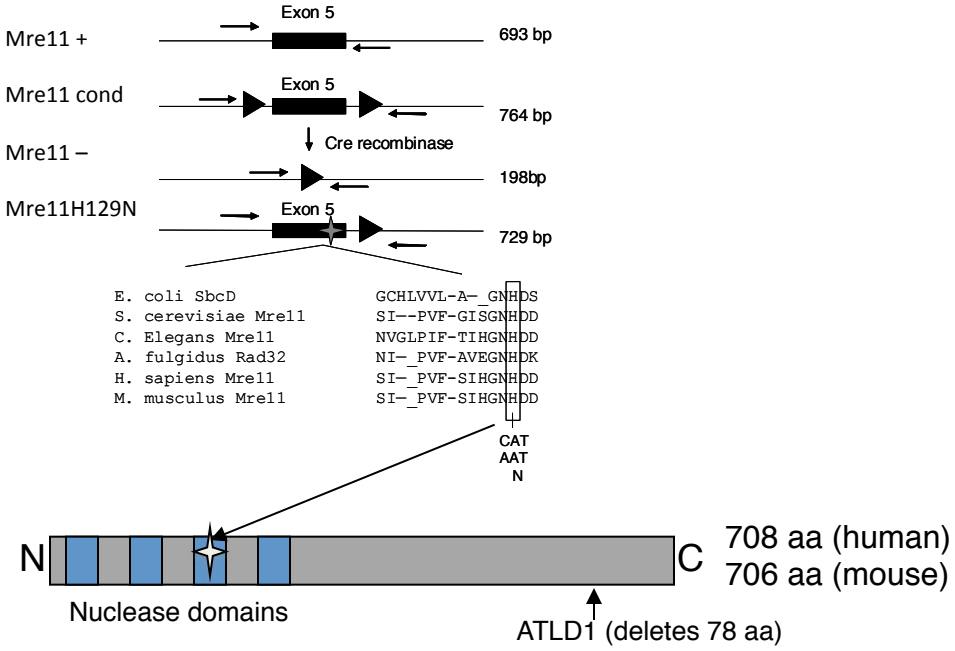
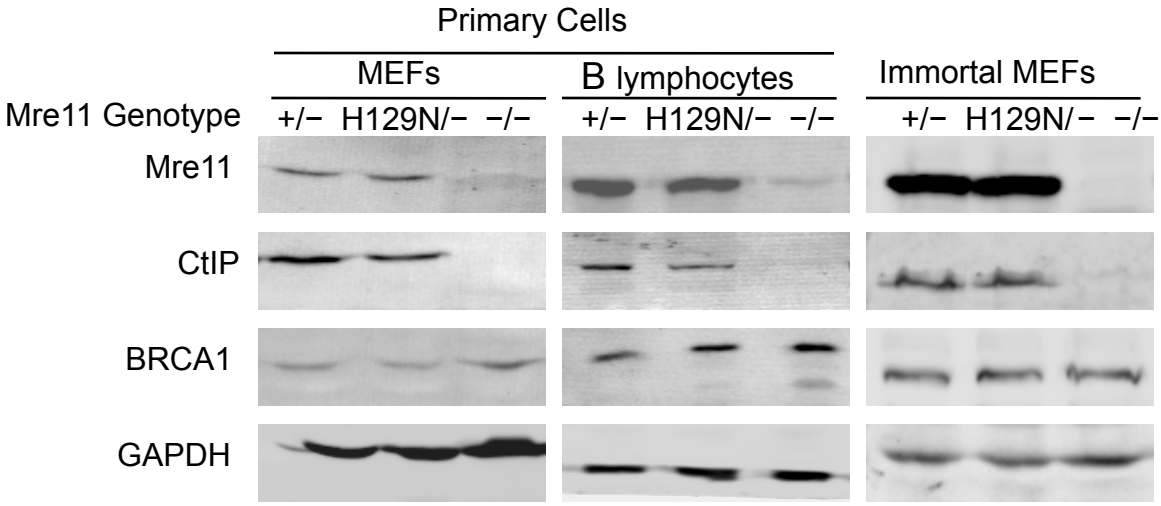
Supplemental Figure 1 Buis et al.



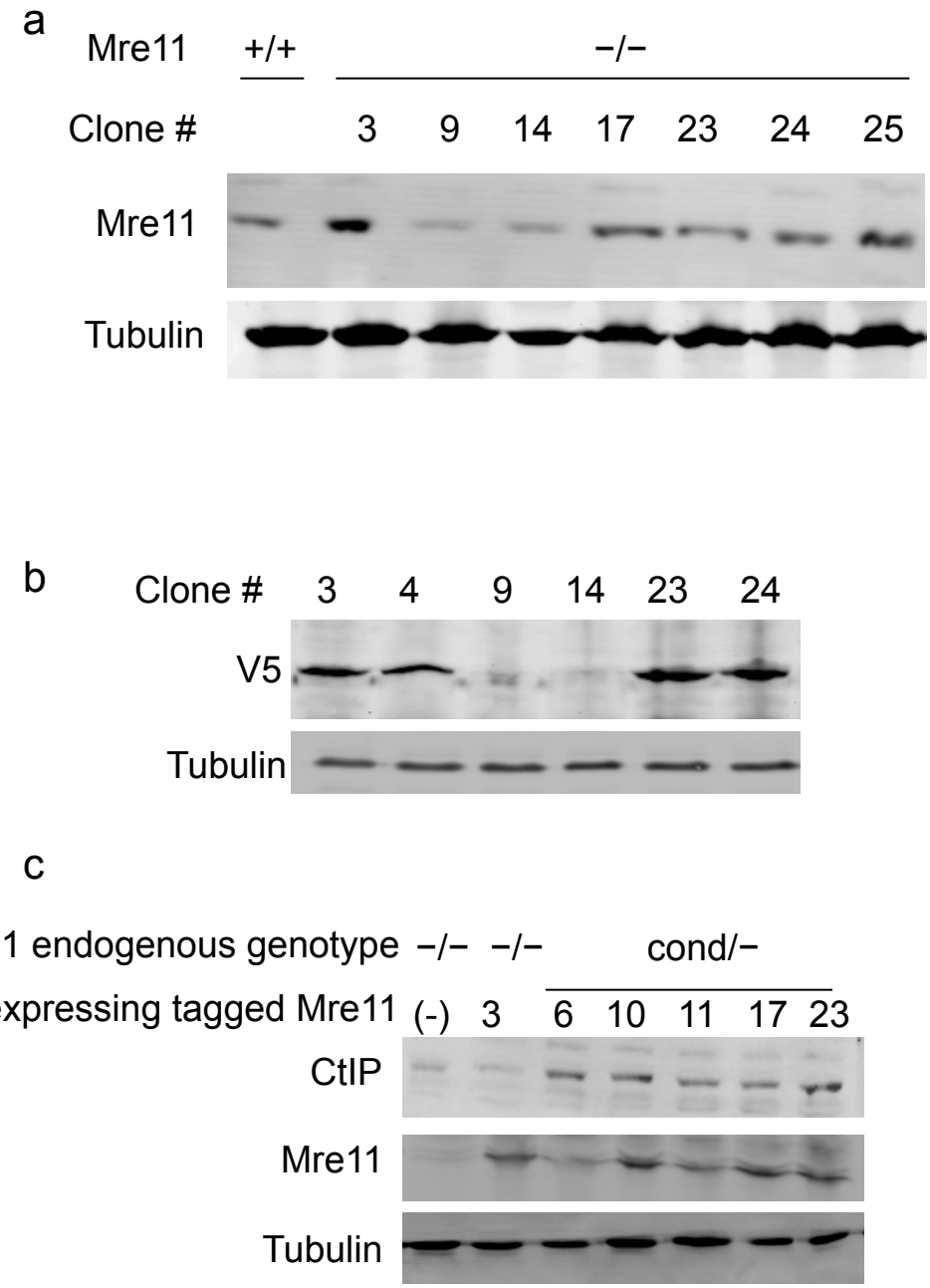
Supplemental Figure 1. Four mouse germline alleles of Mre11.
Mre11^{+} (wild–type), *Mre11*^{cond} (Cre/LoxP conditional), *Mre11*$^{-}$ (null) and *Mre11*^{H129N} (nuclease deficient). Cre mediated recombination of LoxP sites (triangles) converts *Mre11*^{cond} to *Mre11*$^{-}$. Arrows depict location of two PCR primers used to distinguish the four alleles, using PCR product sizes indicated at right (bp). *Mre11*$^{-/-}$ cells lack the entire MRN complex, whereas *Mre11*$^{-/H129N}$ cells possess an intact complex with Mre11 lacking endo/exonuclease activities due to targeted mutation of the invariant histidine residue (star). Mre11 protein shown at bottom depicts four highly conserved nuclease motifs, mouse Mre11^{H129N} mutation (star), and Mre11^{ATLD1} responsible for ataxia telangiectasia like disorder.

Supplemental Figure 2 Buis et al.



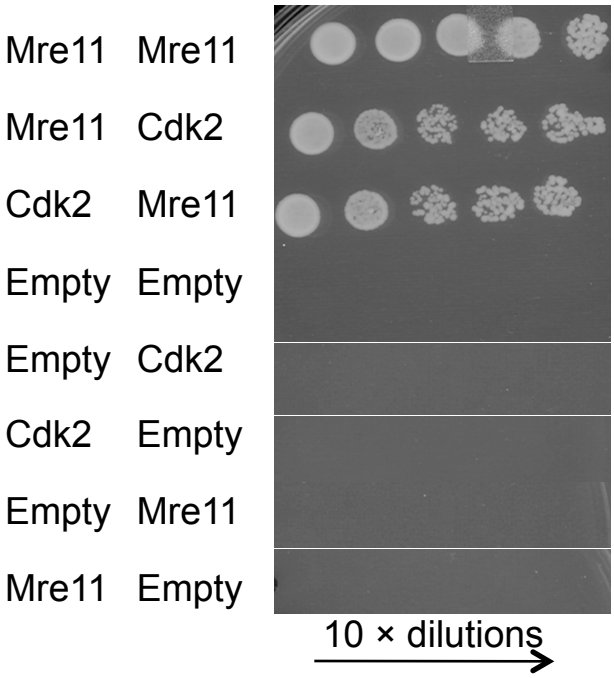
Supplemental Figure 2. MRN deficiency causes CtIP deficiency. Western blot analyses for the indicated proteins (left) performed on extracts from cells of the indicated *Mre11* genotypes (top). *Mre11* alleles are wild–type (+), nuclease deficient (H129N) or null (–). GAPDH is protein loading control.

Supplemental Figure 3 Buis et al.



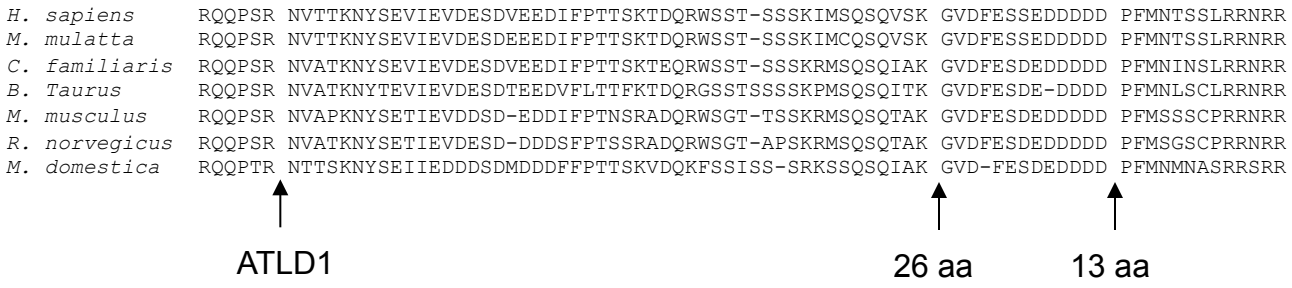
Supplemental Figure 3. Stable expression of Mre11 cDNA.
(a,b) Western blots of representative Mre11$^{-/-}$ clones stably expressing Mre11 cDNA with a 54 amino acid C–terminal tag comprised of 6his–V5–6his. Tubulin is protein loading control.
(a) Western blot using anti–Mre11 antibody. Clones 23 and 24 express ectopic Mre11 at levels similar to endogenous (left lane), and were used for further studies.
(b) Western blot using anti–V5 antibody identifying Mre11 encoded by cDNA . Representative clones are shown.
(c) Western blots of representative Mre11$^{+/-}$ clones stably expressing Mre11 cDNA with a 54 amino acid C–terminal tag comprised of 6his–V5–6his. No impact on CtIP Clevels is observed when endogenous wild–type Mre11 is present.

Supplemental Figure 4 Buis et al.



Supplemental Figure 4. Yeast two hybrid analyses ruling out auto–activation by Mre11 or CDK2.
pGBK (bait) and pGAD (prey) plasmids expressed Mre11 or CDK2 as indicated (left). Mre11 dimerization is positive control. Empty vectors are negative control.

Supplemental Figure 5 Buis et al.



Supplemental Figure 5. Alignment of Mre11 C-termini from representative mammalian species. The locations of ATLD1 and the 26 and 13 amino acid deletions are indicated (arrows). The final 13 amino acids show 69% identity between human and mouse. BLAST searches do not identify statistically significant similarity to anything other than mammalian Mre11, nor were any conserved domains recognized (Conserved Domain database – NCBI).