

P059 Constitutive phosphorylation of MDC1 physically links the MRE11–RAD50–NBS1 complex to damaged chromatin
Christoph Spycher¹, Edward S. Miller³, Kelly Townsend³, Lucijana Pavic¹, Nicholas A. Morrice⁴, Pavel Janscak², Grant S. Stewart³, and Manuel Stucki¹

¹ Institute of Veterinary Biochemistry and Molecular Biology and ² Institute of Molecular Cancer Research, University of Zürich, 8057 Zürich, Switzerland ³, School of Cancer Sciences, Birmingham University, Birmingham B15 2TT, England, UK ⁴ Medical Research Council Protein Phosphorylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK

The MRE11–RAD50–Nijmegen breakage syndrome 1 (NBS1 [MRN]) complex accumulates at sites of DNA double-strand breaks (DSBs) in microscopically discernible nuclear foci. Focus formation by the MRN complex is dependent on MDC1, a large nuclear protein that interacts with phosphorylated H2AX. We have identified a region in MDC1 that is essential for the focal accumulation of the MRN complex at sites of DNA damage. This region contains multiple conserved motifs that are constitutively phosphorylated *in vivo*. These motifs are efficiently phosphorylated by casein kinase 2 (CK2) *in vitro* and directly interact with the N-terminal forkhead-associated domain of NBS1 in a phosphorylation-dependent manner. Mutation of these motifs in MDC1 or depletion of CK2 by siRNA disrupts the interaction between MDC1 and NBS1 and abrogates accumulation of the MRN complex at sites of DNA DSBs *in vivo*. Our data reveal the mechanism by which MDC1 physically couples the MRN complex to damaged chromatin.