**Temporal modulation of the NF-κB RelA network in response to different types of DNA damage**

Amy E. Campbell1†, Catarina Ferraz Franco1†, Ling-I Su2, Emma K. Corbin2, Simon Perkins3, Anton Kalyuzhnyy3, Andrew R. Jones3, Philip J. Brownridge1, Neil D. Perkins2, Claire E. Eyers1\*

1 Centre for Proteome Research, Department of Biochemistry & Systems Biology, Institute of Institute of Systems, Molecular & Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, UK

2 Faculty of Medical Sciences, Biosciences Institute, Newcastle University, Newcastle Upon Tyne, NE2 4HH, UK

3 Department of Biochemistry & Systems Biology, Institute of Institute of Systems, Molecular & Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, UK

† These authors contributed equally to this manuscript

\* Correspondence to ceyers@liverpool.ac.uk

**Supplementary Table Legends**

**Supplementary Table 1. Kmeans clustering of phosphoproteins in the RelA interactome network. RelA associated p**roteins were inputted into STRING and clustered using the Kmenas algorithm. Proteins associated with each of the 9 clusters are listed along with the categories as depicted in Figure 2.

**Supplementary Table 2. Temporal phosphopeptide response in the RelA network to cellular treatment with etoposide (ETO).** List of peptides (and the proteins from which they derived) identified by LC-MS/MS in response to cellular exposure of HA-RelA U2OS cells to 50 μM ETO for 30 min, 60 min or 120 min (or control treatment with DMSO), followed by HA-RelA immunoprecipitation and TiO2-based phosphopeptide enrichment. Peptide identification metrics, protein modifications, as well as the confidence of phosphosite localisation (ptmRS score) are detailed for each entry. Peptide quantification was performed following VSN normalisation of peptide ion abundances. ANOVA was performed to evaluate statistical changes across all time points. Paired t-test was used to compare two individual time points. Confidently localised phosphorylation sites were evaluated using NetPhorest to predict kinase family (group) and kinase. Also detailed is the prevalence of prior observation of the identified phosphopeptides in either PhosphositePlus (PSP) or Peptide Atlas (PA) – see Methods for detailed information.

**Supplementary Table 3. Temporal phosphopeptide response in the RelA network to cellular treatment with hydroxyurea (HU).** List of peptides (and the proteins from which they derived) identified by LC-MS/MS in response to cellular exposure of HA-RelA U2OS cells to 2 mM HU for 30 min, 60 min or 120 min (or control treatment with DMSO), followed by HA-RelA immunoprecipitation and TiO2-based phosphopeptide enrichment. Peptide identification metrics, protein modifications, as well as the confidence of phosphosite localisation (ptmRS score) are detailed for each entry. Peptide quantification was performed following VSN normalisation of peptide ion abundances. ANOVA was performed to evaluate statistical changes across all time points. Paired t-test was used to compare two individual time points. Confidently localised phosphorylation sites were evaluated using NetPhorest to predict kinase family (group) and kinase. Also detailed is the prevalence of prior observation of the identified phosphopeptides in either PhosphositePlus (PSP) or Peptide Atlas (PA) – see Methods for detailed information.

**Supplementary Figure Legends**

**Supplementary Figure 1. Time course of treatment of HA-RelA U2OS cells with etoposide or hydroxyurea.** HA-RelA U2OS cells were treated for the specified times with either etoposide (A) or hydroxyurea (B) as indicated to assess DNA damage. Cell lysates were separate by SDS-PAGE; total protein was visualised with Ponceau S staining. Gels were then subjected to immunoblotting with antibodies against either γH2AX as a marker of DNA damage, or RelA/p65. MM: molecular markers; WCL: whole cell lysate; CL: cleared lysate; Elution: eluant from anti-HA beads; CNT: control. Control cells for hydroxyurea treatment were exposed to DMSO for 6 h to match longest treatment time.

**Supplementary Figure 2 GIFs. Animated dynamic network map of RelA interacting phosphoproteins.** Significant changes across either (A) etoposide and/or hydroxyurea, or (B) both treatments as a function of time (using data presented in Fig. 2).

**Supplementary Figure 3. Schematic of RelA protein coverage.** Domains and quantified sites of phosphorylation are indicated. Blue phosphorylation sites were quantified in ETO only, red were quantified in both ETO and HU. Novel phosphorylation sites are represented with an asterix. Protein sequence coverage is represented by the boxes (and amino acid residue numbers) in the bottom panel. TA = transactivation domain; NLS – nuclear localisation sequence*.*