DNA encodes the blueprint for any organism, therefore damage to that crucial part of life must be repaired. Otherwise, unrepaired DNA damage caused by endogenous or exogenous sources can lead to mutations and devastating diseases, including cancer. To prevent accumulation of DNA damage, multiple DNA repair pathways have evolved in humans. Mutations within the DNA repair pathways increase the risk for multiple cancers, including breast and ovarian cancer.

DNA repair pathways are initiated after the recognition of DNA double strand or single strand breaks by poly-(ADP-ribose)-polymerase enzymes (PARP). PARP1 is a first responder at DNA damage sites, where it catalyzes mono- and poly-ADP-ribosylation (ADPr) of proteins. Thereby, PARP1 induces chromatin relaxation and DNA repair factor recruitment to enable the repair of DNA damage. The enzyme is recognized as an important therapeutic target in the clinic as its inhibition in cancer cells with mutations of specific DNA repair pathway proteins triggers synthetic lethality in these cancer cells. By exploiting this synthetic lethality, PARP1 inhibitors are used to treat, for example, breast cancer.

Even though PARP1 is an important clinical target, its induced pathways remain understudied due to technical difficulties when studying ADPr. Indeed, it was only recently discovered that the interaction of PARP1 with histone poly-ADP-ribosylation factor 1 (HPF1) shifts PARP1 catalysis from poly- to mono-ADPr and changes its preferred substrates from aspartate and glutamate to serine sites, which are the main ADPr sites during DNA damage. Enabled by new tools, we recently identified an intricate temporal component of PARP1-HPF1 catalyzed serine poly- and mono-ADPr during the DNA damage response occurring in two sequential waves. Subsequently, we showed that the second mono-ADPr wave recruits the ubiquitin E3 ligase RNF114 towards DNA damage sites.

Here, I aimed to further understand the second wave of ADPr and the role of RNF114 in the DNA damage response by screening for interactors of RNF114. During these studies I found direct cellular evidence of a recently discovered unconventional ubiquitylation of serine mono-ADPr, which is read by RNF114. To further study this unconventional ubiquitylation, the ADPr and ubiquitin binding domain of RNF114 were transformed into a tool detecting this modification. By mass spectrometry, the sites of this unconventional

ubiquitylated ADPr were identified and, for the first time, localized on serine residues of PARP1 and histones during DNA damage.