

# Novel antibody reagents for the characterization of protein ADP-ribosylation

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## INTRODUCTION

Poly-ADP-ribose polymerases (PARPs) catalyze the transfer of ADP-ribose from  $\beta$ -NAD<sup>+</sup> and release nicotinamide in the process. ADP-ribosylation predominantly occurs on amino acid side chains of proteins (such as lysine, arginine, glutamate, aspartate, cysteine, serine), but it has also been described to occur on protein amino termini as well as on DNA and tRNA.<sup>1</sup> The most widely studied PARPs (PARP1, 2, 5a and 5b) can synthesize linear or branched chains of up to ~200 ADP-ribose units (PARylation).<sup>2</sup> However, there are 13 additional PARPs which transfer only a single ADP-ribose unit to their target residue (MARylation). The best-known function of poly-ADP-ribose chains is to serve as a scaffold for the recruitment of DNA repair proteins that contain PAR-binding modules to sites of DNA damage. ADP-ribosylation is also involved in a variety of additional cellular processes, including cell stress responses, mitotic spindle formation, chromatin decondensation, retroviral silencing, RNA biology, and transcription.<sup>3</sup> Even though MAR/PARylation is of central importance to cellular function, there are no commercially available antibodies that recognize both MARylated and PARylated proteins. Therefore, novel rabbit monoclonal antibodies have been produced and characterized against this modification on proteins, and their utility for the detection of ADP-ribosylated proteins by ELISA, western blot, dot blot, and immunofluorescence assays has been validated.

## METHODS

Polyclonal antibodies were produced by modifying lysine residues on KLH using periodate chemistry. Rabbits were selected for monoclonal antibody development based on reactivity in ELISA and western blot assays. Rabbit monoclonal clones were then produced, tested, and selected for scale-up and additional testing. ELISA, Western blot, and immunofluorescence assays were performed as described.<sup>4</sup> Hydrogen peroxide treatment was performed at 500mM for 5 min. PDE1 treatment was performed at 0.5mg/mL for 4hr at 37°C. PARG treatment was performed at 5mM for 4hr at 37°C. The immunofluorescence PARylation assay was performed as described by Jones et al. and cells were imaged on a ImageXpress Micro XLS and quantified using MetaXpress.<sup>5</sup>

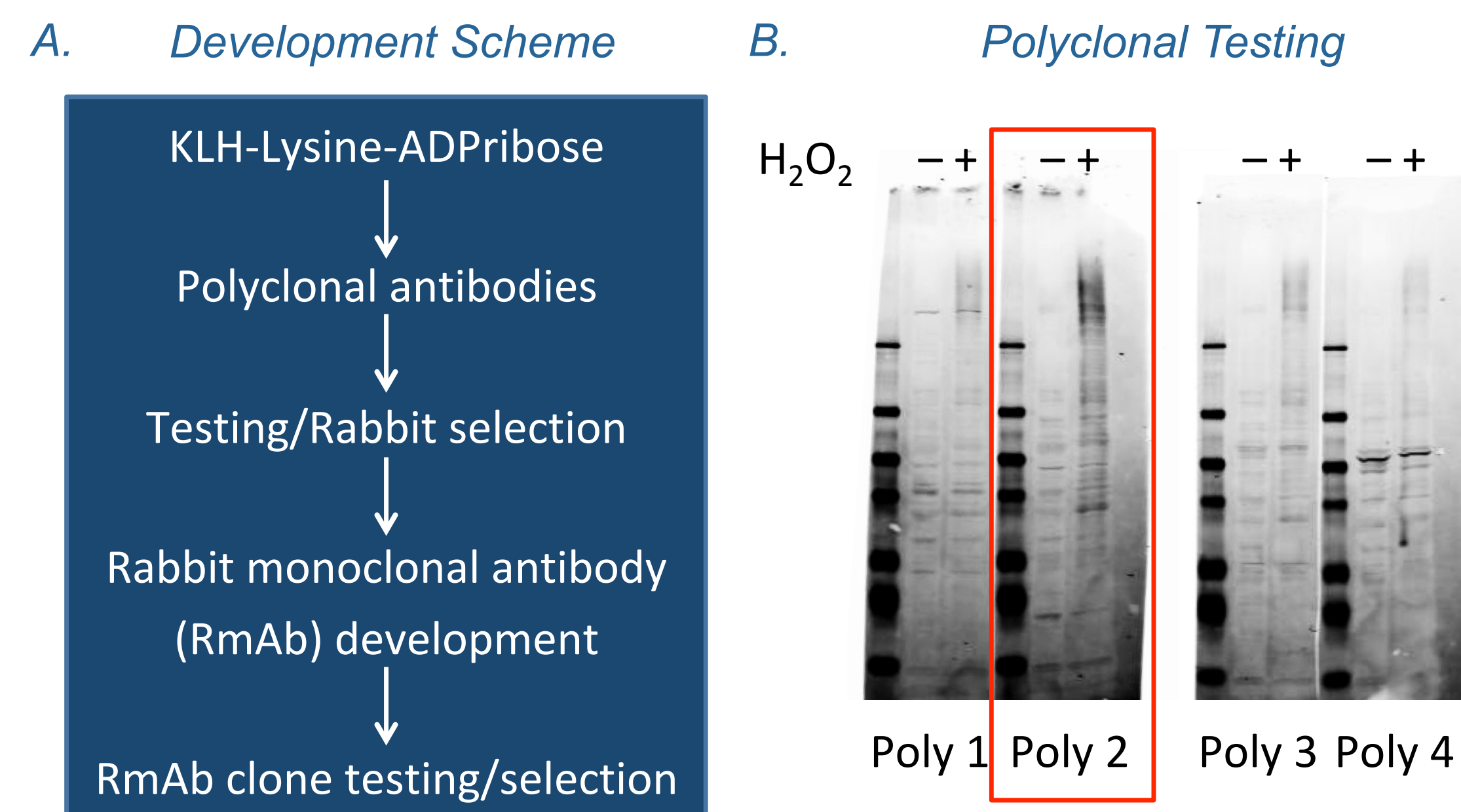
## CONCLUSIONS

Four antibody clones were isolated and characterized which showed distinct banding patterns and levels of induction of cellular ADP-ribosylation on Western blots of cells stimulated with PARylation-activating treatments such as hydrogen peroxide. Clone E6F6A which was selected for commercial release was validated for use in Western blot, dot blot, ELISA, and immunofluorescence assays. Initial studies also show that the antibody is a useful tool for the enrichment of MAR/PARylated proteins or peptides prior to LC-MS/MS analysis. Further work is aimed at the understanding of the specificity of the four antibody clones. Development of new tools to study this critical post-translational modification should facilitate new discoveries of MARylated/PARylated proteins and their function during growth, development, and in disease settings.

## REFERENCES

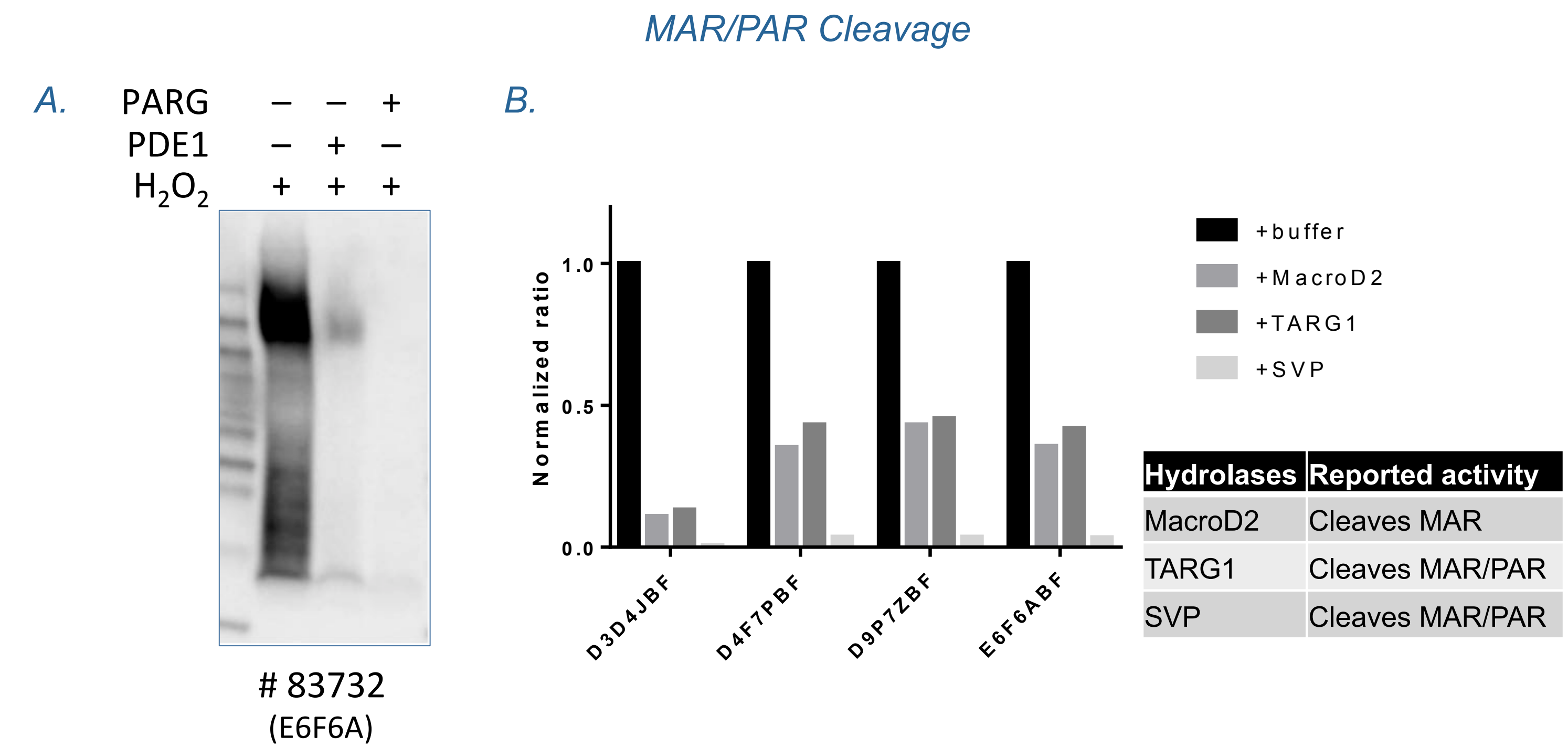
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## Antibody Development



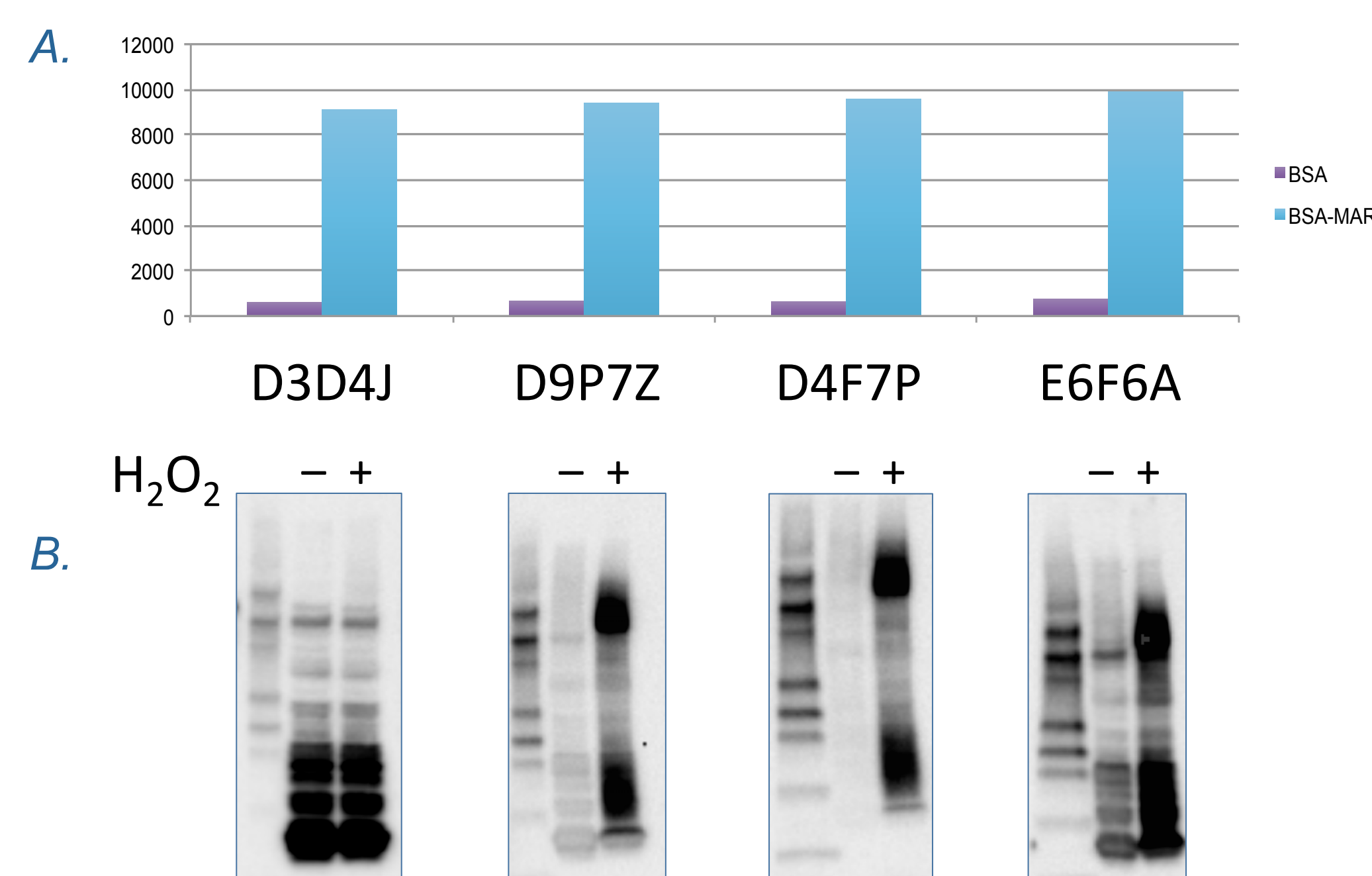
**Figure 1:** MAR/PAR antibody development. **A.** Antibody development scheme. Rabbits were injected with ADP-ribose modified KLH, tested, and reactive animals selected for rabbit monoclonal antibody development. Clones were validated and selected for production of final reagent. **B.** Western blot testing of ADP-ribosylation polyclonal antibodies against HeLa cells untreated or treated with H<sub>2</sub>O<sub>2</sub>. Red box indicates polyclonal selected for rabbit monoclonal development.

## Antibody Characterization



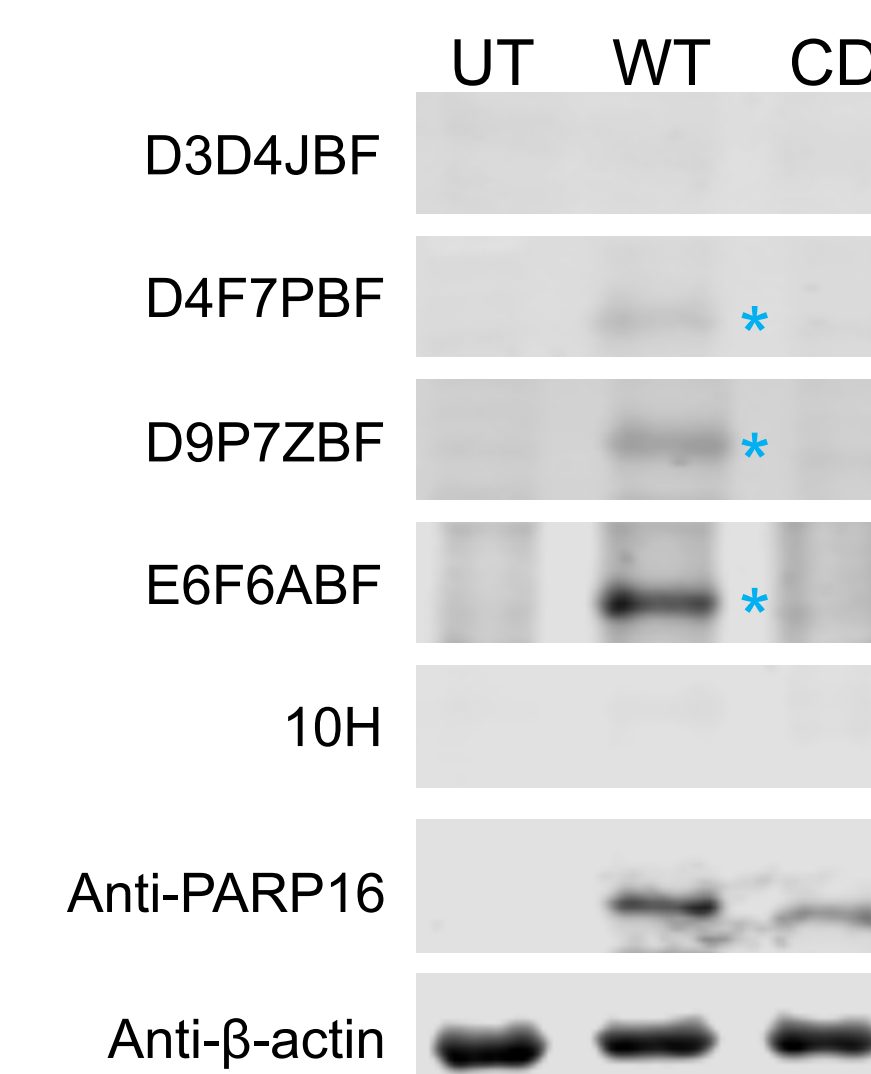
**Figure 4:** **A.** MAR/PAR antibody signal is abrogated by treatment of COLO 205 cell lysates with PDE1 or PARG. **B.** MAR/PAR antibodies show reduced signal against ADP-ribosylated PARP3 after incubation with hydrolases. Dot blot signal was normalized against buffer control.

## Rabbit Monoclonal Testing



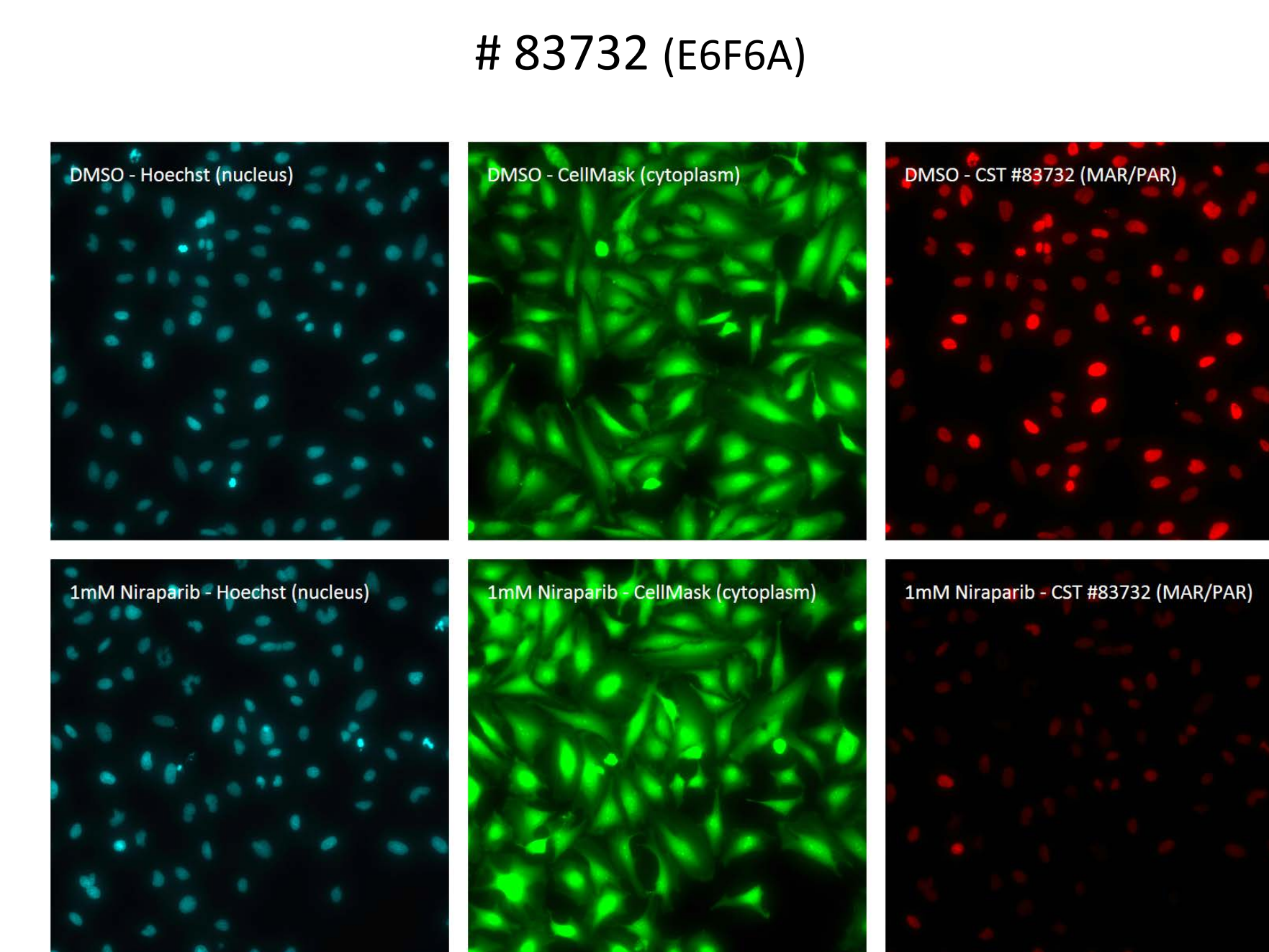
**Figure 2:** MAR/PAR rabbit monoclonal antibody validation. **A.** ELISA test of RmAb clones against BSA and ADP-ribosylated BSA. **B.** Western blot testing of ADP-ribosylation rabbit monoclonal antibodies against HeLa cells untreated or treated with H<sub>2</sub>O<sub>2</sub>.

## PARP16 Over-Expression



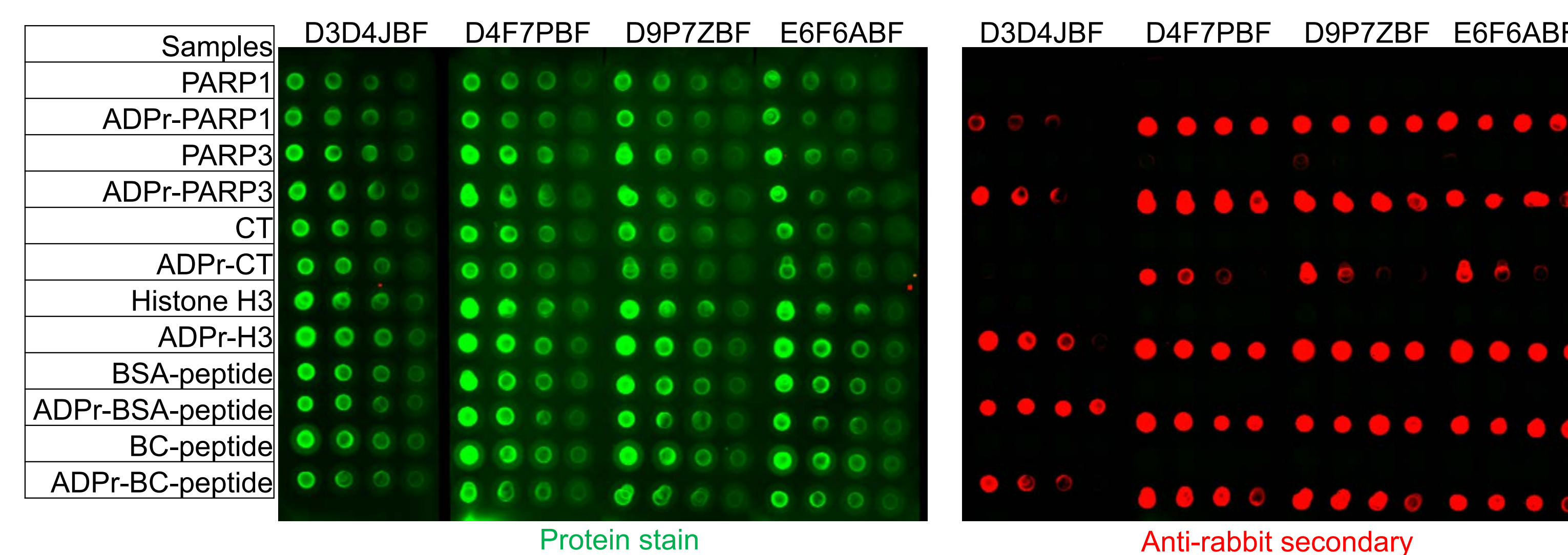
**Figure 5:** MAR/PAR antibodies detect mono-ADP-ribosylated PARP16. Lysates from HeLa PARP16 -/- cells left untransfected (UT) or transfected with wild-type SBP-tagged PARP16 (WT) or catalytically dead SBP-tagged PARP16 (CD). (\*) ADP-ribosylated PARP16

## Immunofluorescence



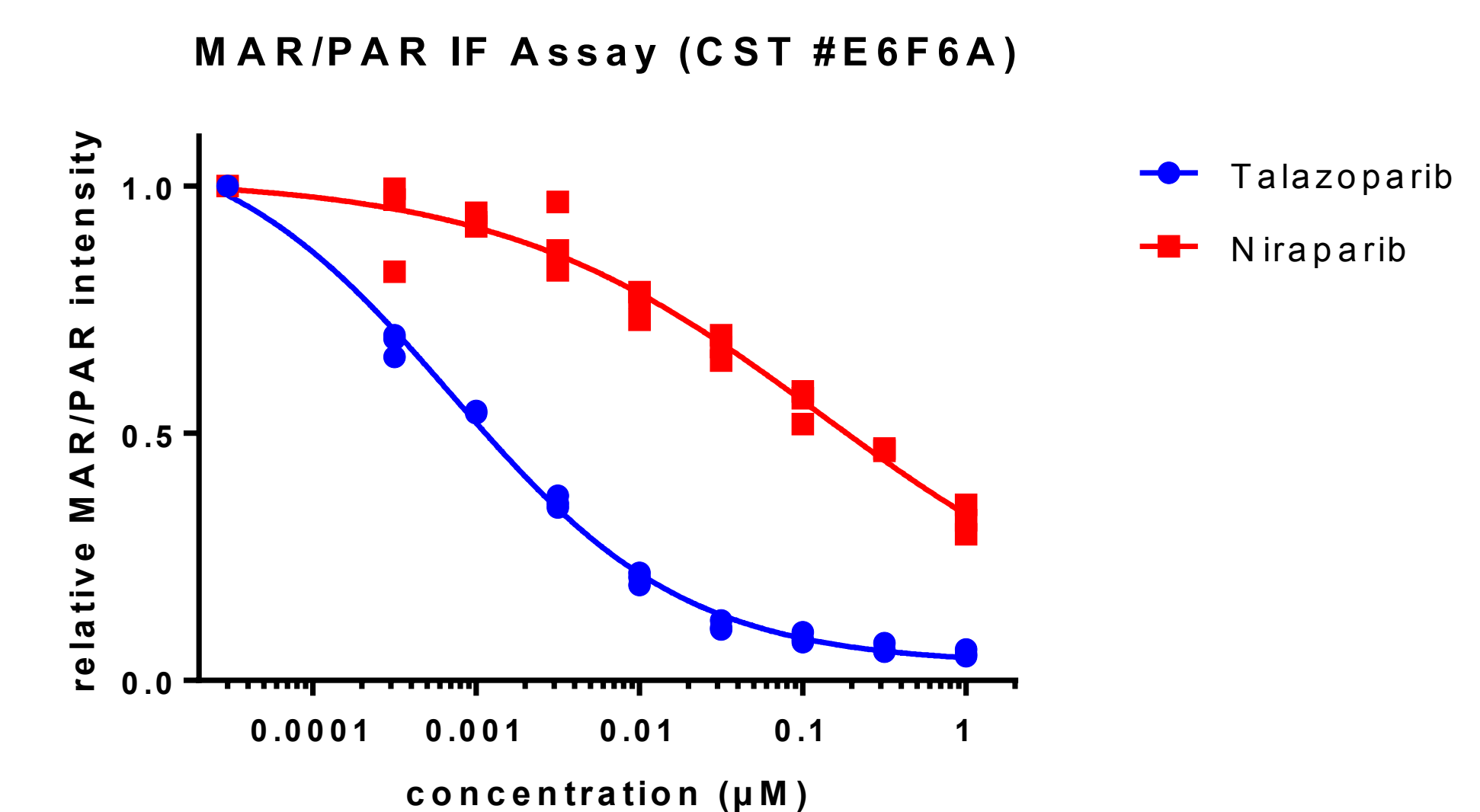
**Figure 6:** Immunofluorescence staining of HeLa cells stimulated with hydrogen peroxide and treated without and with Niraparib.

## Dot Blot Testing



**Figure 3:** MAR/PAR antibodies detect both mono- and poly-ADP-ribosylated recombinant proteins by dot blot. The four clones show different levels of sensitivity toward modified proteins and do not bind to unmodified proteins.

## Compound Screening



**Figure 7:** MAR/PAR antibody (E6F6A) detects dose-dependent inhibition of PARylation by talazoparib and niraparib in HeLa treated with hydrogen peroxide.