# Identification and Characterization of Posttranslational Modifications of Myosin IC


**Ryan Schwab and Wilma A. Hofmann**

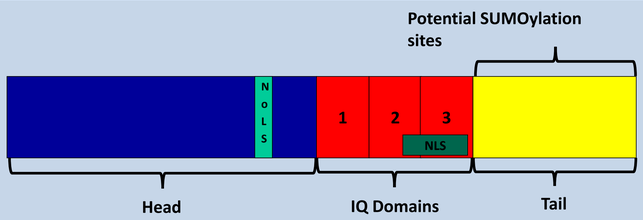
Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, New York


## Abstract:


Myosin IC (MyoIC) is a member of the myosin superfamily. It localizes to the nucleus where it plays important roles in transcription, intranuclear transport, and nuclear export. However, how the nuclear functions of MyoIC are regulated is not understood. We recently identified a novel, nucleus-specific posttranslational modification of MyoIC and showed that nuclear MyoIC is SUMOylated. SUMOylation of proteins is known to have a great number of consequences for the target protein such as changes in transcriptional activity, cellular translocations, and protein-protein interactions. Thus, SUMOylation could play an important role in the regulation of nuclear MyoIC functions. The goal of this project was to characterize this novel modification. Specifically, to identify the sites in MyoIC to which SUMO proteins are attached to and what SUMO protein is involved in this modification. Using in vivo SUMOylation assays, we showed that MyoIC is modified specifically by SUMO2. In addition, by using site directed mutagenesis in combination with in vivo SUMOylation assays, we identified the specific MyoIC amino acid sequence where SUMO is attached to, and created MyoIC mutant constructs that cannot get SUMOylated anymore. These data are an important first step in understanding the physiological consequences of this novel MyoIC modification.


## Background and Introduction:

**•Myosin IC illustration - characterizing its domains, including nucleolar localization signal (NoLS)[1]and nuclear localization signal (NLS)[2].**
**•Tail region depicted for potential SUMOylation sites (focus of study)**




Myosin IC (MyoIC) belongs to the myosin superfamily of molecular motor proteins. It has important functions in the nucleus where it is involved in transcription, intranuclear transport, and nuclear export[3]. While the nuclear functions of MyoIC have been indentified, it is not well understood how they are regulated. We recently identified the signal that targets MyoIC to the nucleolus[1] (see schematic). In the course of our studies, we noticed that only MyoIC constructs that accumulate in the nucleolus, show a posttranslational modification called SUMOylation. SUMOylation is the covalent attachment of SUMO proteins to a target protein and is known to have a great number of consequences for the target protein such as changes in transcriptional activity, cellular translocations, and protein-protein interactions[4]. Based on the role for SUMO modification for protein functions, we hypothesize that SUMOylation of MyoIC plays an important role in regulating the functions of MyoIC in the nucleolus. The objective of this study is to identify where SUMO is attached to MyoIC so that we can make mutants that cannot be modified anymore. These mutants can then be used in future experiments to determine the function of this modification by comparison to wild type proteins.


**References:**

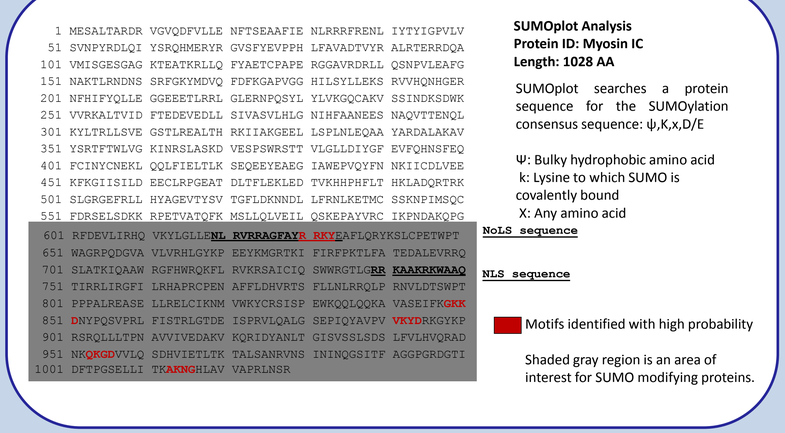
1. Schwab RS., Ihantovych I., Yunus S.,Domaradzki T., Hofmann WA. 2013. Identification of Isoform Specific Nucleolar Localization Signals in Myosin IC. Exp. Cell Res. 2013 Feb. 21.
2. Dzijak, R., S. Yildirim, M. Kahle, P. Novak, J. Hnilicova, T. Venit, and P. Hozak. 2012. Specific nuclear localizing sequence directs two myosin isoforms to the cell nucleus in calmodulin-sensitive manner. PLoS ONE. 7:e30529.
3. Hofmann WA, Johnson T, Klapczynski M, Fan JL, de Lanerolle P. From transcription to transport emerging roles for nuclear myosin I. Biochem Cell Biol. 2006 Aug;84(4):418-26.
4. Hay R.T. 2005. SUMO: a history of modification. Mol. Cell. 18:1–12.



**Acknowledgements:** We would like to thank Tera Domaradzki for help with the methods and procedures. This study was funded in part by an Undergraduate Research Award from the University at Buffalo's Center for Undergraduate Research & Creative Activities (CURCA) to R.S.S. and in part by the Department of Defense CDMRP (PC111523 to W.A.H.)


## Methods:

1) Analysis of MyoIC protein sequence for potential SUMOylation sites using the SUMOplot™ program (Abgent).




**NoLS sequence**

**NLS sequence**

**SUMOplot Analysis**
**Protein ID: Myosin IC**
**Length: 1028 AA**

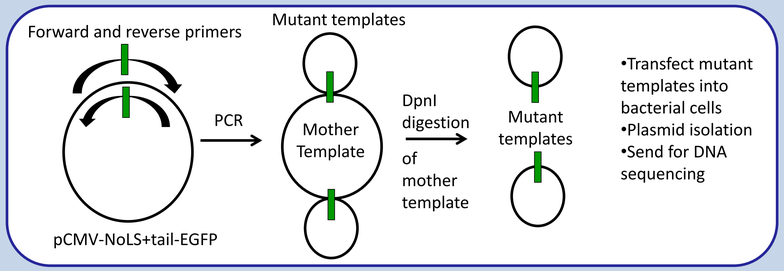
SUMOplot searches a protein sequence for the SUMOylation consensus sequence: ψ,K,x,D/E

Ψ: Bulky hydrophobic amino acid
k: Lysine to which SUMO is covalently bound
X: Any amino acid

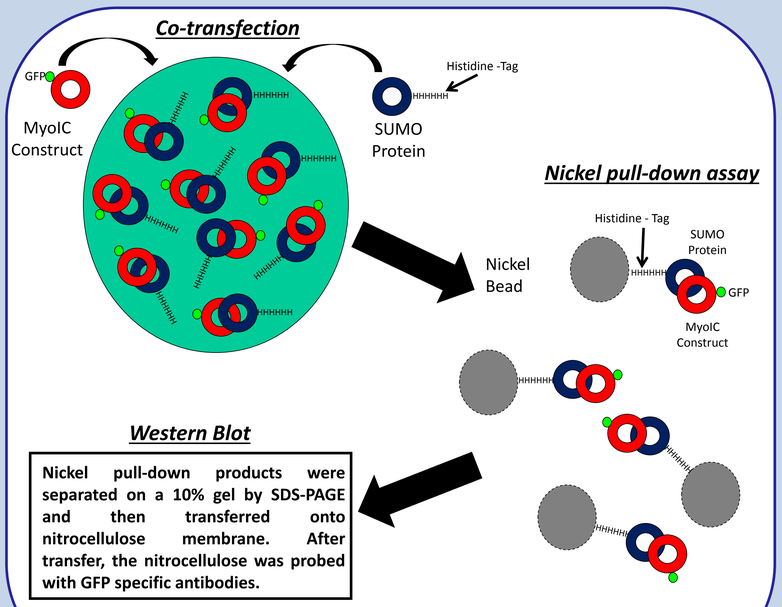
Motifs identified with high probability

Shaded gray region is an area of interest for SUMO modifying proteins.

2) Introduction of point mutations at potential SUMOylation sites into the DNA template that codes for amino acids 619-1028 of MyoIC (pCMV-NoLS+tail-EGFP; abbr: BNoLS+T) At SUMOylation site, SUMO is covalently attached to the amino acid K. To identify the site(s) of SUMOylation, K in each identified potential SUMOylation motif was changed to R by site directed mutagenesis.




3) In-vivo SUMOylation assay of mutated constructs. A three step process:
- •Co-transfection of pCMV-NoLS+tail-EGFP (wt or mutant) and SUMO constructs
- • Nickel pull-down assay 48hours after transfection
- • Western blot for protein analysis




## Results:

### 1) Myosin IC is SUMOylated by SUMO2 but not by SUMO1

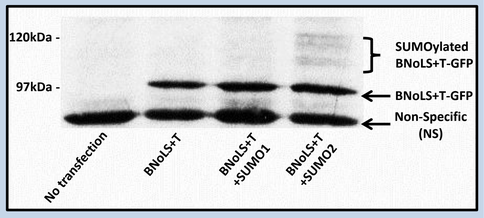



***Figure 1: Immunoblot analysis of in vivo SUMOylation assays.***

COS-7 cells were co-transfected with DNA constructs coding for the GFP-tagged myosin IC tail region (including one of the recently identified NoLS; BNoLS+T-GFP) and the indicated His-tagged SUMO proteins.
BNoLS+T-GFP is modified in vivo by SUMO2 but not by SUMO1 as indicated by the appearance of higher molecular weight bands that represent SUMOylated BNoLS+T-GFP.

### 2) Identification of K849R as a site of SUMOylation

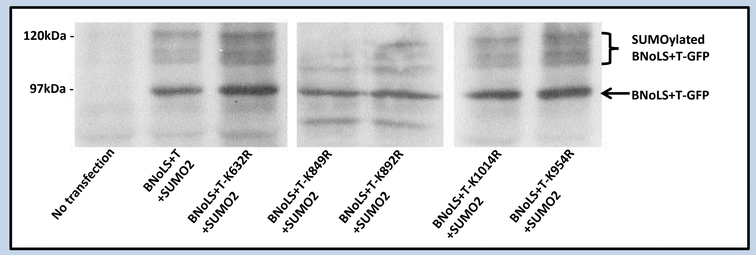
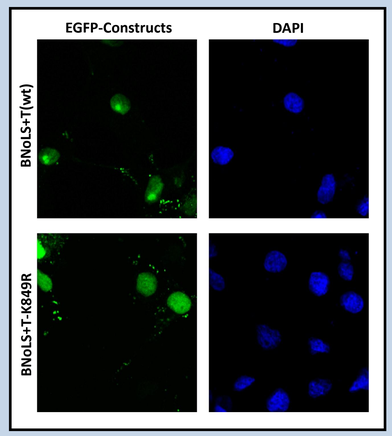



***Figure 2: In vivo SUMOylation assays using various BNoLS+T-GFP mutant constructs.***
COS-7 cells were co-transfected with His-SUMO2 and wild type BNoLS+T-GFP or BNoLS+T-GFP mutants containing the indicated K to R amino acid substitutions. Note that the K849R amino acid substitution eliminates one of the higher molecular weight bands that has been identified as a SUMOylated form of BNoLS+T-GFP (Fig. 1). This suggests that K489 is a site that is modified by SUMO.

### 3) SUMOylation of myosin IC is involved in nucleolar localization



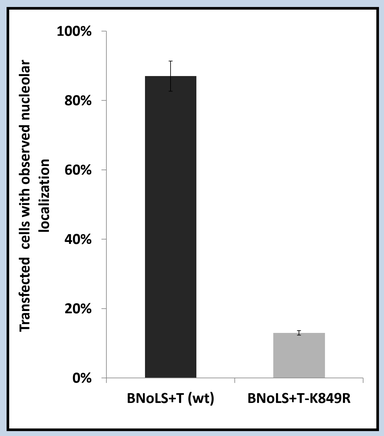




***Figure 3a:*** COS-7 cells transfected with wild type protein (BNoLS+T) and mutant (BNoLS+T-K849R)

***Figure 3b:*** After transfection of wild type protein and mutation (BNoLS+T-K849R), it is shown by a cell count that the mutant significantly reduces nucleolar localization .

## Conclusion:

- We have identified a novel posttranslational modification of myosin IC. We show for the first time that the tail region of Myosin IC is SUMOylated.
- In vivo SUMOylation assays demonstrate that the tail region of myosin IC is SUMOylated by SUMO2 but not by SUMO1 (Fig.1).
- We have identified, so far, one of at least two SUMOylation sites at amino acid K849 (Fig.2).
- Analysis of myosin IC tail constructs with a mutation in this SUMOylation site show a significantly reduced nucleolar localization when compared to the wild type (Fig.3). These data strongly suggest that SUMOylation facilitates the nucleolar localization of myosin IC.