

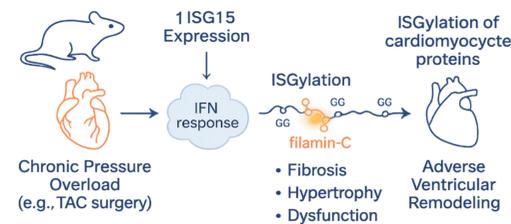
# Mapping Cardiac ISGylation Under Pressure Overload Using diGLY Proteomics



Pressure overload induces ISG15 to facilitate adverse ventricular remodeling and promote heart failure. *J Clin Invest.* 2024;134(10):e161453. Doi:10.1172/JCI161453.

## Background & Significance

Heart-failure patients pass through a silent yet lethal phase of ventricular remodelling. Clinicians can measure wall thickening, but the underlying molecular switches—especially stress-induced **post-translational modifications (PTMs)**—remain largely invisible.



**Clinical model.** Chronic pressure overload (e.g., TAC surgery in mice) mimics human hypertension, driving hypertrophy, fibrosis and contractile decline.

**Knowledge gap.** Interferon-stimulated ISG15 is rapidly up-regulated by stress, but:

- Does pressure overload dynamically regulate cardiac ISG15 expression and conjugation?
- Does ISGylation actively worsen remodelling and heart-failure progression?
- What proteins make up the cardiac ISGylome, and what are the functional consequences?

**Technology need.** To answer these questions Yerra et al. required a platform that could

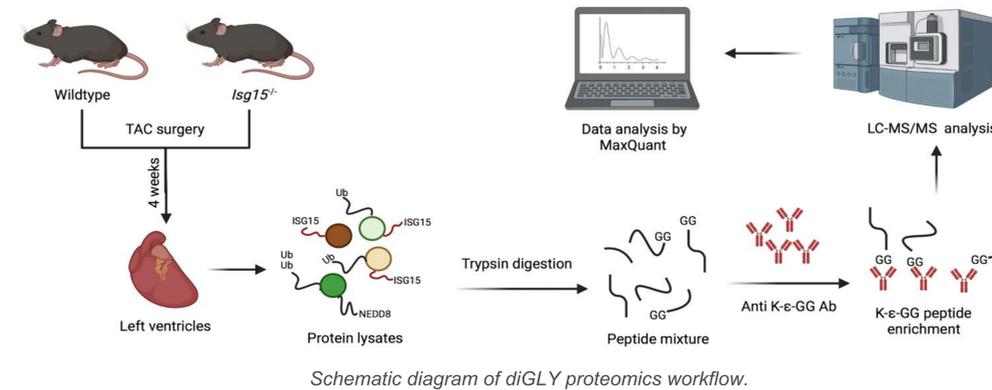
- detect ultra-low-stoichiometry ISGylation sites,
- distinguish ISG15 adducts from ubiquitin marks, and
- map thousands of sites in a single run.

Conventional LC-MS/MS without enrichment misses over 90% of diglycine-tagged peptides, making advanced enrichment strategies such as Creative Proteomics' diGLY proteomics essential for comprehensive analysis.

## Workflow for diGLY Proteomics

1. Tissue or cell lysate → trypsin digest
2. Anti-K-ε-GG antibody captures all diglycine-tagged peptides
3. nanoLC-Q Exactive HF Orbitrap MS (120 min gradient)
4. MaxQuant + PTM-Navigator™ AI separates ISG15 vs. ubiquitin signatures
5. Comprehensive technical report, MS raw data, and figures suitable for publication

Contact Us



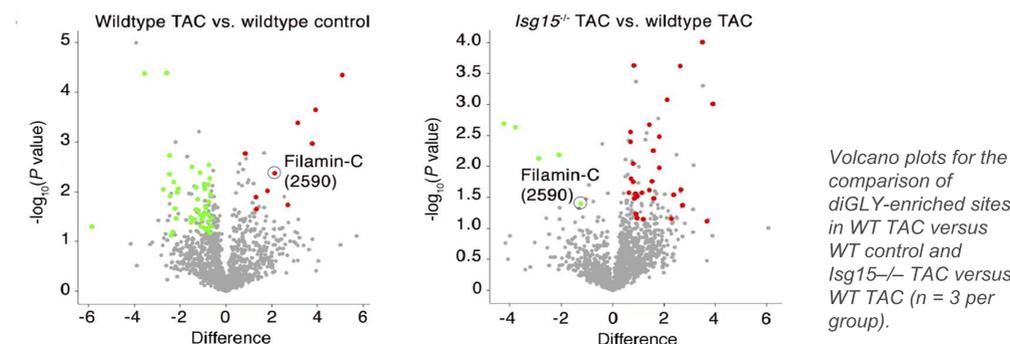
Schematic diagram of diGLY proteomics workflow.

## Key Discoveries Enabled by Our Service

Using diGLY proteomics, Yerra et al. (*JCI* 2023) systematically mapped the cardiac ISGylome under pressure overload conditions.

### Main discoveries include:

- Widespread Upregulation of ISGylation  
Pressure overload led to increased expression of ISG15 and accumulation of ISG15-conjugated proteins in the heart.
- Identification of ISGylated Proteins  
1,426 diGLY-modified lysine sites identified across 562 proteins.  
Highlights the broad impact of ISGylation on cardiac cellular processes.
- Filamin-C as a Novel ISGylation Target  
Filamin-C, an actin-binding protein involved in sarcomeric integrity, was identified as a target of ISGylation at lysine 2590.  
ISGylation of filamin-C was observed at intercalated discs and may contribute to cytoskeletal disorganization during remodelling.
- Functional Implications  
Mice lacking ISG15 showed preserved cardiac function under pressure overload, suggesting that ISGylation contributes to maladaptive remodelling.



Volcano plots for the comparison of diGLY-enriched sites in WT TAC versus WT control and Isg15<sup>-/-</sup> TAC versus WT TAC (n = 3 per group).

## Scientific Significance

- Provides the first comprehensive map of the cardiac ISGylome in pressure overload.
- Identifies novel PTM targets potentially involved in cardiac pathophysiology.
- Opens new avenues for investigating how inflammation-driven PTMs contribute to cardiac disease mechanisms.

## Platform At-a-Glance

### Technical Expertise Applied:

- High-stringency diGLY enrichment enables specific isolation of ISG15-conjugated peptides.
- nanoLC-MS/MS provides deep coverage of low-abundance PTMs.
- Integrated bioinformatics pipeline ensures confident site identification and pathway analysis.

### Applications

Mapping ISGylation, ubiquitination, and NEDDylation in stress and disease models.

Understanding protein turnover, cytoskeletal regulation, and signalling pathways.

Supporting functional studies and biomarker discovery.

## Why Choose Us:

**High Specificity:** Separates ISG15 from ubiquitin/NEDD8, reducing false positives by >90%.

**Deep Coverage:** Identifies >1,500 diGLY sites per sample, as shown in Yerra et al. (*JCI* 2023).

**High Sensitivity:** Detects PTMs at sub-femtomole levels missed by standard MS.

**Collaborative Support:** Integrates findings into biological context for meaningful interpretation

## Interested in Exploring PTMs in Your System?

Contact us to discuss how diGLY proteomics can support your research on ISGylation, ubiquitination, or other PTMs in health and disease models.

Web: [www.creative-proteomics.com](http://www.creative-proteomics.com)

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