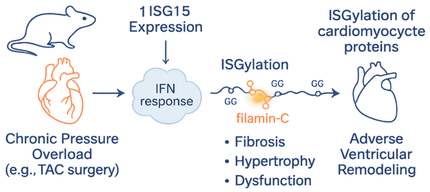
Mapping Cardiac ISGylation Under Pressure Overload Using diGLY Proteomics

Creative 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

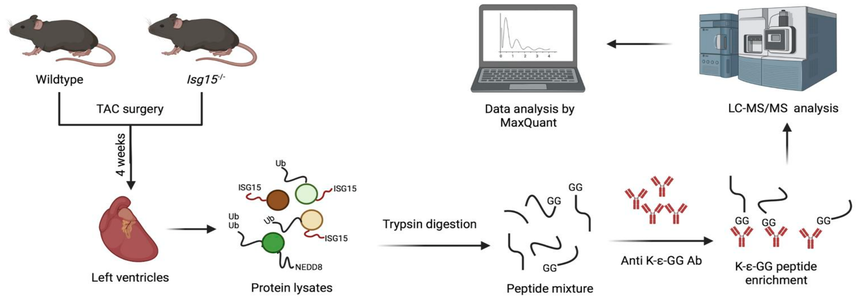
- detect ultra-low-stoichiometry ISGylation sites,
- distinguish ISG15 adducts from ubiquitin marks, and
- map thousands of sites in a single run.

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.

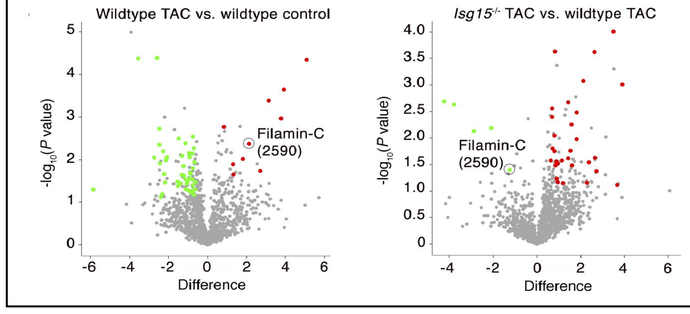
- Filamin-C as a Novel ISGylation Target

  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–/– 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.

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