

MS Maps TβRII-Dependent Phosphorylation Sites on Adrb2 in Cartilage Homeostasis

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Our role: Creative Proteomics performed mass spectrometry-based phosphorylation site analysis of Adrb2CT, identifying Thr398, Ser406, and Ser412 following incubation with cTβRII.

Background & Significance

Articular cartilage continuously renews its superficial membrane to withstand friction and mechanical loading. This study revealed that skeletal interoception regulates this process through a PGE2–hypothalamus–norepinephrine (NE)–Adrb2 signaling axis.

Mechanistically, NE signaling influences the interaction and internalization of Adrb2 and TGF-β type II receptor (TβRII), thereby modulating TGF-β activity in superficial-zone chondrocytes.

Study Objective

The study aimed to determine how sympathetic signaling regulates cartilage homeostasis and define the molecular mechanism connecting **Adrb2 with TβRII/TGF-β signaling**.

Specifically:

- Establish the role of Adrb2 in superficial-zone cartilage integrity
- Determine whether NE promotes Adrb2–TβRII interaction and internalization
- Identify phosphorylation events involved in the TβRII–Adrb2 regulatory mechanism

Case Focus

A key mechanistic question was whether TβRII can directly phosphorylate the cytoplasmic domain of Adrb2, and if so, which residues are modified.

Workflow

Cartilage & Signaling Phenotyping → Adrb2 Functional Analysis → Adrb2–TβRII Interaction → In Vitro Kinase Assay → MS-Based Phosphorylation Site Identification → Functional Validation

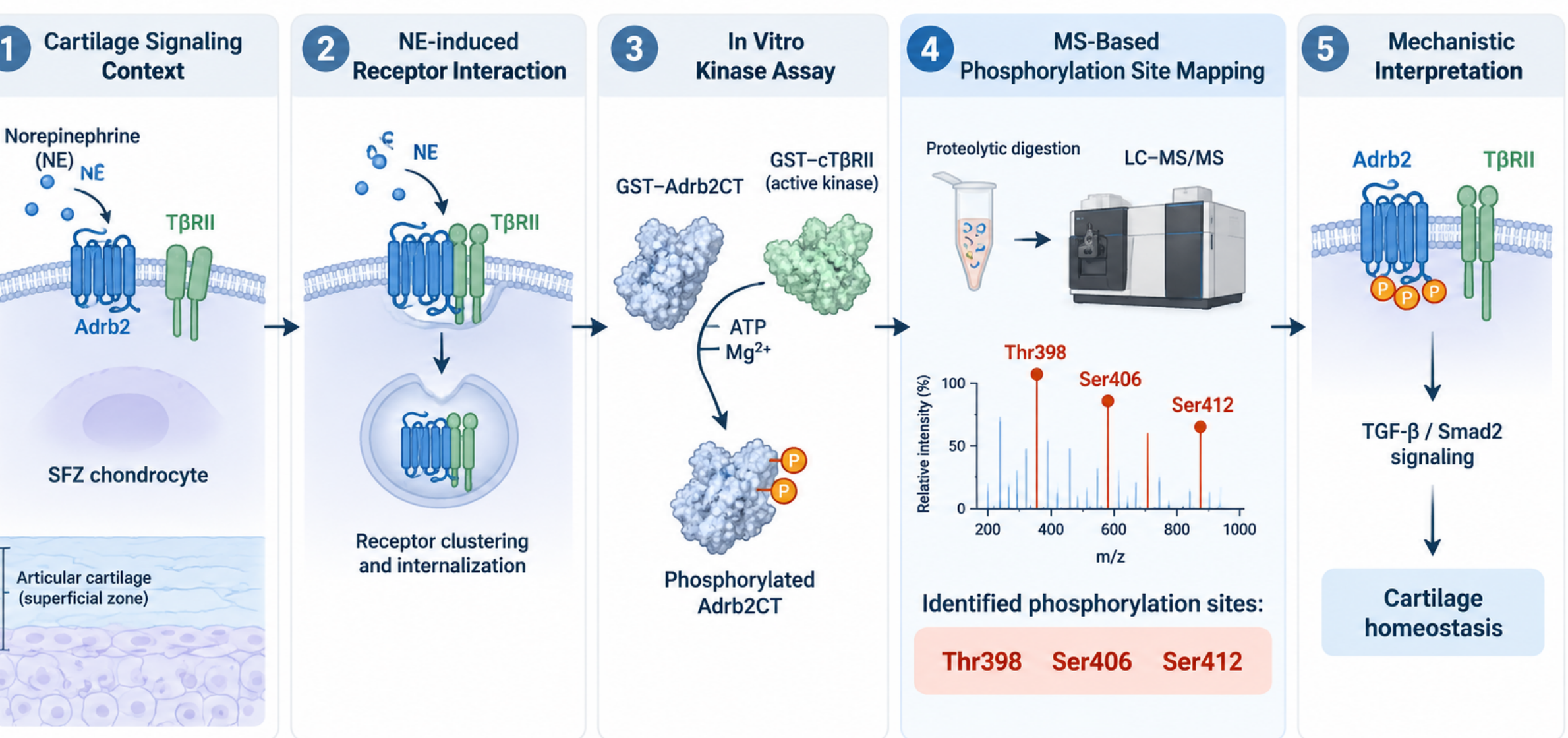


Fig. 1. Workflow for TβRII–Adrb2 interaction analysis and MS-based phosphorylation site mapping.

Key Findings

Finding 1 | Adrb2 Is Essential for Superficial-Zone Cartilage Integrity

Adrb2 was predominantly expressed in superficial-zone (SFZ) chondrocytes.

Conditional deletion of Adrb2 disrupted the superficial membrane, and mechanical loading by treadmill running further aggravated the phenotype, resulting in pronounced loss of the SFZ and reduced cartilage thickness.

These findings established Adrb2 as an important mediator of cartilage homeostasis.

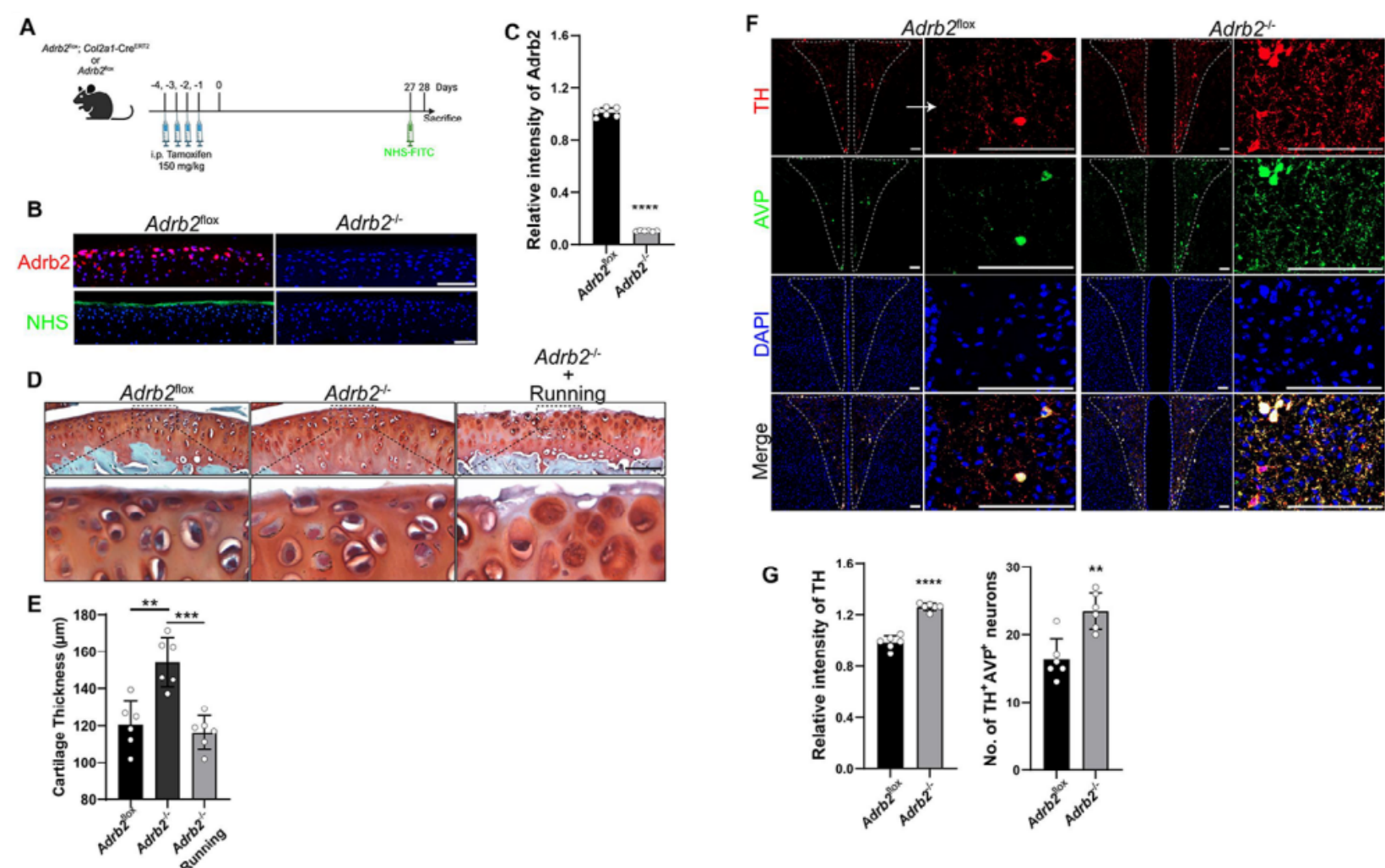


Fig. 2. Adrb2 is essential for superficial-zone cartilage integrity. Reproduced from Wang et al., Fig. 5.

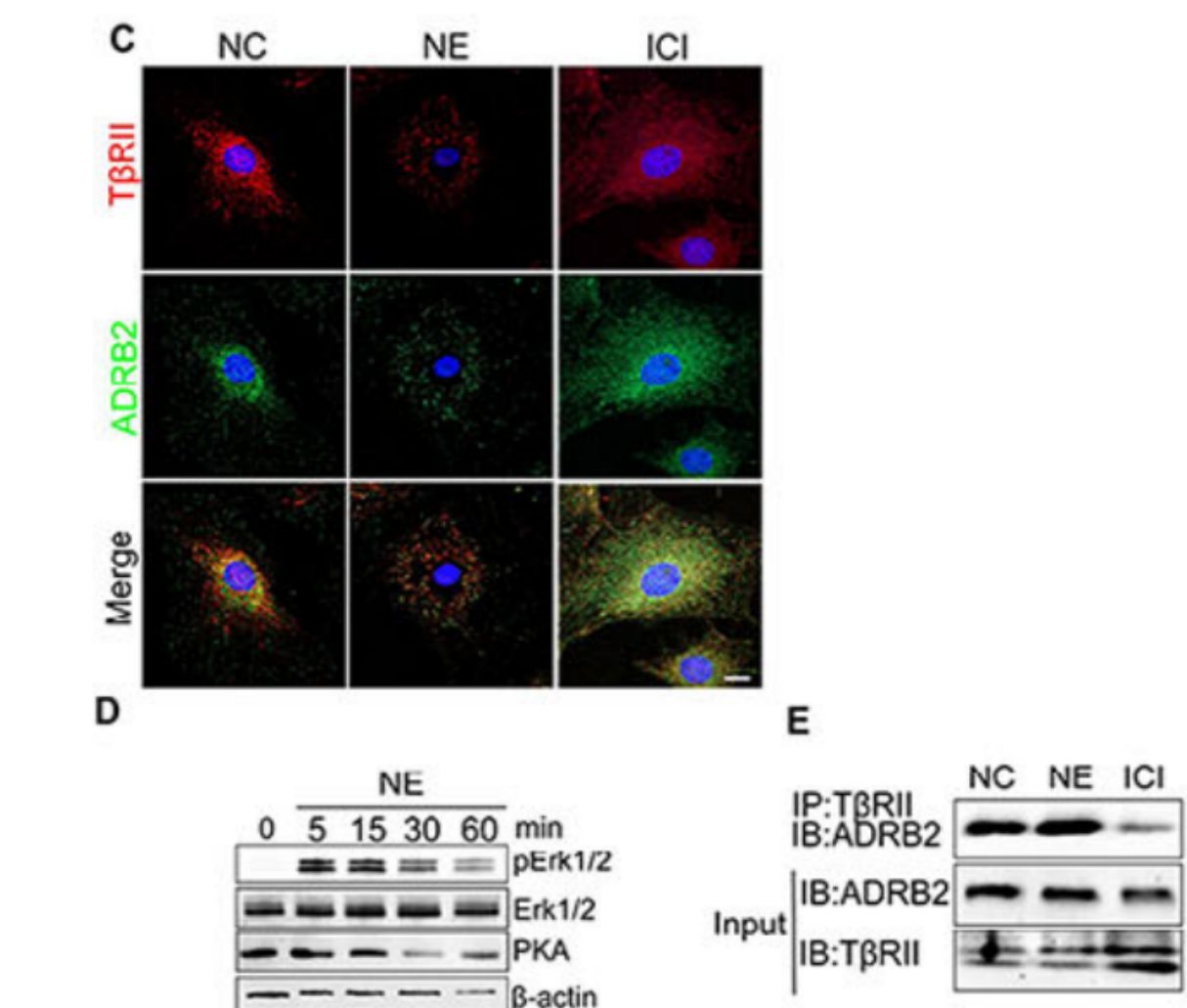


Fig. 3. NE promotes Adrb2–TβRII interaction and receptor internalization. Adapted from Wang et al., Fig. 6C–E.

Finding 2 | NE Promotes Adrb2–TβRII Interaction and Receptor Internalization

Norepinephrine treatment induced clustered internalization of Adrb2 and TβRII in SFZ chondrocytes.

Immunoprecipitation further showed increased interaction between the two receptors following NE treatment, whereas Adrb2 inhibition reduced this interaction.

Together, these results linked sympathetic signaling to coordinated regulation of the Adrb2–TβRII receptor complex.

Finding 3 | MS Identified Three TβRII-Dependent Phosphorylation Sites on Adrb2

Purified Adrb2 cytoplasmic domain (Adrb2CT) was incubated with constitutively active cTβRII in an in vitro kinase assay.

A shifted Adrb2CT band was observed after kinase incubation, and mass spectrometry localized phosphorylation to three residues:

Thr398 · Ser406 · Ser412

These residue-level data provided molecular evidence supporting phosphorylation of Adrb2CT by TβRII.

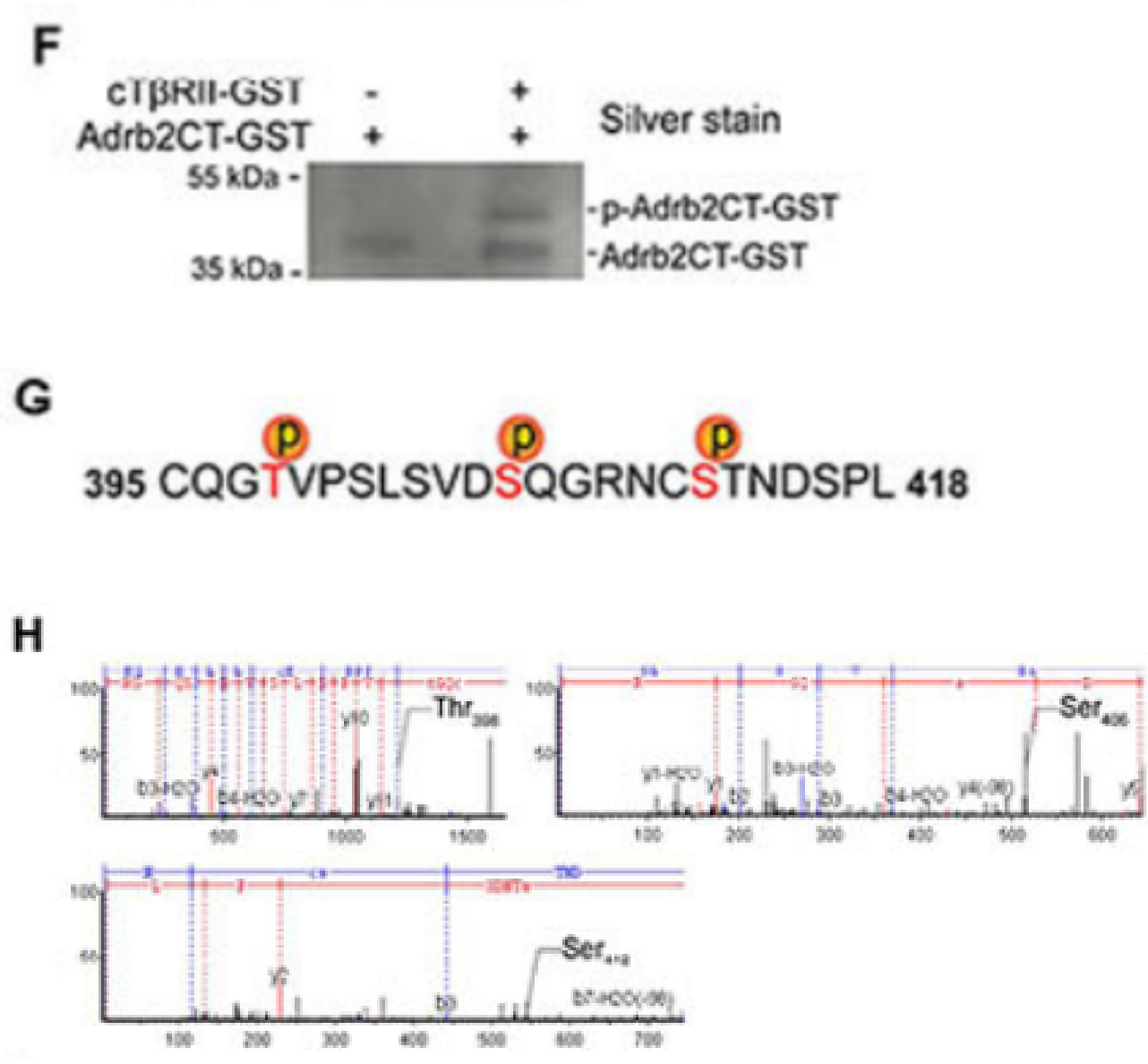


Fig. 4. MS identifies TβRII-dependent phosphorylation sites on Adrb2CT. Adapted from Wang et al., Fig. 6F–H.

Our Contribution (Creative Proteomics)

Creative Proteomics provided mass spectrometry-based phosphorylation site identification for the Adrb2–TβRII mechanistic study.

The analysis:

- Localized phosphorylation to Thr398, Ser406, and Ser412
- Converted biochemical evidence of phosphorylation into residue-level site information
- Supported interpretation of a defined kinase–substrate regulatory mechanism
- Provided candidate residues for downstream mechanistic and functional investigation

Why Site-Level Resolution Matters

Phosphorylation at different residues can produce distinct regulatory effects. Site-level MS helps:

- Pinpoint exact modified residues within a defined protein region
- Distinguish site-specific regulatory events beyond a general phosphorylation signal
- Provide testable phosphosites for mutagenesis and functional validation

Why Choose Creative Proteomics

- Residue-Level Localization: Identify specific Ser, Thr, or Tyr phosphorylation sites rather than reporting only protein-level modification.
- Kinase–Substrate Insight: Connect kinase activity with exact modified residues on a defined substrate.
- Follow-Up Ready Sites: Provide precise phosphosite candidates for mutagenesis and functional validation.

Need to Map Protein PTMs at Site Level?

Creative Proteomics provides **MS-based PTM proteomics for site-specific characterization of phosphorylation and other protein modifications**, supporting studies of protein regulation, signaling, and molecular mechanisms.

From targeted site identification to broader PTM profiling, our workflows help connect modification sites with downstream biological function.

Contact us to discuss the most suitable PTM analysis strategy for your project.

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