

# Functional proteomics of FANCA: Identifying novel molecular functions of FA proteins in the endocrine pancreas

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## Introduction

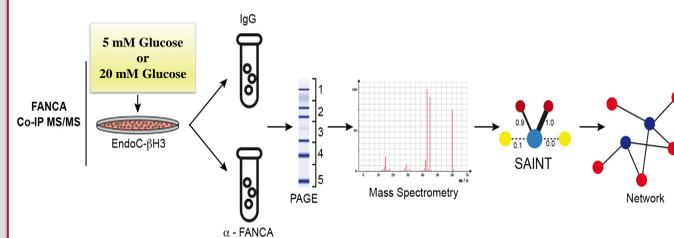
Fanconi anemia (FA) is a hereditary disorder characterized by pancytopenia and cancer predisposition as the result of impaired DNA damage repair of interstrand cross links. Around 80% of FA patients also exhibit endocrinopathies, including diabetes, for which the underlying molecular mechanisms specifically affecting endocrine cells are unknown. *FANCA* mutations are the most common cause of FA, therefore, we optimized the co-immunoprecipitation-coupled mass spectrometry (Co-IP-MS) approach to analyze interaction networks of endogenous *FANCA* protein-protein interactions to evaluate the molecular pathways regulating pancreatic islet  $\beta$ -cell function. With this method, we identified a large network of previously unknown *FANCA* interactors and discovered novel functions of FA proteins that likely contribute to pancreatic endocrinopathies in FA patients.

## Funding

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2. CCSG – Fred & Pamela Buffet Cancer Center
3. University of Nebraska Medical Center
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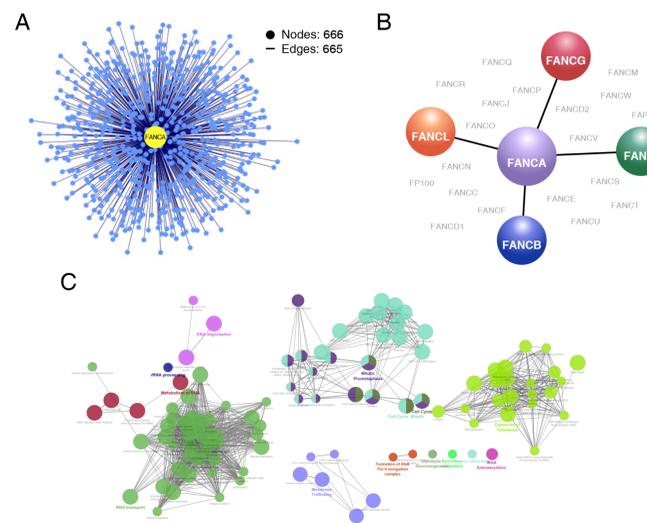
## Methods

Human embryonal kidney 293FT cells were used to optimize the conditions for Co-IP-MS analysis of endogenous *FANCA*. Cells were lysed with NETN buffer containing protease inhibitors, and *FANCA* and its associated interactors were isolated with antibody-coupled magnetic beads. IgG coupled beads were used as a negative control. After the in-gel digestion, samples were analyzed on the Orbitrap Fusion Lumos. Human pancreatic EndoC- $\beta$ H3 cells were stimulated with both lower (5 mM) and higher (20 mM) glucose concentrations and endogenous *FANCA* was isolated using the optimized experiment conditions developed with 293FT cell. Characterization of *FANCA* interactors was done using Scaffold (4.5.1) and SAINT. Molecular interaction networks and functional enrichment analysis were performed using Clue-GO plug-in in Cytoscape (3.4.0).



## Results

The optimum conditions for Co-IP of *FANCA* and its interaction partners for further large-scale analysis were investigated, and the cleanest fractions were isolated when coupling 3 mg of the magnetic beads to 50  $\mu$ g of the antibody. The total protein amount of 2.5 mg was necessary to pool down enough quantity of endogenous proteins to be successfully analyzed by mass spectrometry. Instead of using glycine for the elution of *FANCA* from the beads, we efficiently eluted the specific protein fraction with a salt-free 0.5 M  $\text{NH}_4\text{OH}/0.5$  mM EDTA solution, pH 11.0.

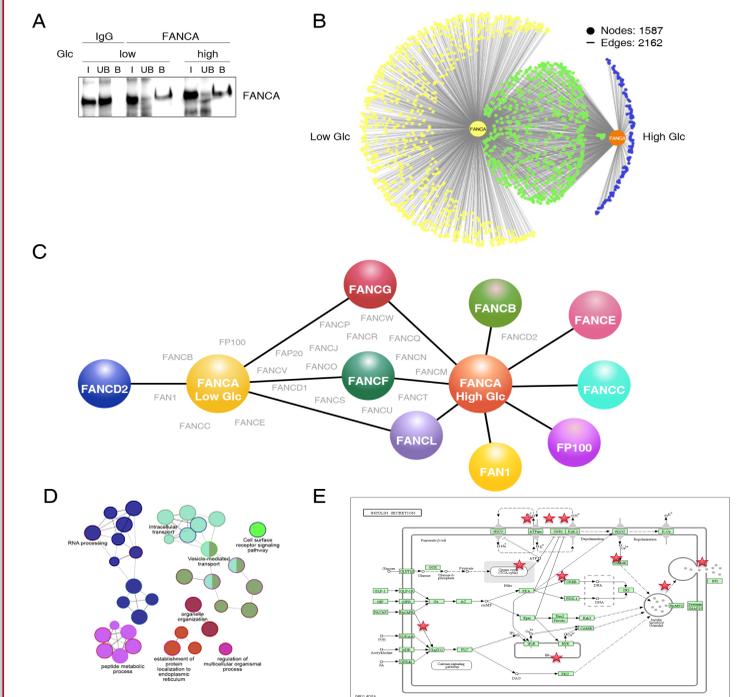


**Figure 1 - Endogenous *FANCA* interactome optimization in 293FT cells**

- A - Protein interaction network with *FANCA*  
B - Confirmed interactions within FA protein family in the data set  
C - ClueGO analysis of enriched GO terms and KEGG pathways

## Conclusion

*FANCA* interactome analysis revealed many novel pathways regulated by glucose stimulation, suggesting that regulation of insulin secretion by FA proteins could be through associations with vesicle-mediated transport. This work also reveals that *FANCA* protein interaction network changes rapidly in response to a glucose-induced DNA damage in human pancreatic  $\beta$ -cells.



**Figure 2 - Overview of the workflow used for the analysis of endogenous *FANCA* interactome and functional pathways in EndoC- $\beta$ H3 cells**

- A - Co-IP analysis of *FANCA* in the input (I), unbound (UB) and bound (B) fraction, after the stimulation with both lower (5 mM) and higher (20 mM) glucose concentration (Glc)  
B - Protein interaction network with *FANCA* after the stimulation with low and high Glc concentration  
C - Confirmed interactions within FA protein family in the data set  
D - ClueGO analysis of enriched gene ontology terms for biological processes  
E - The analysis of *FANCA* protein interactions in the KEGG insulin secretion pathway