# STUDY OF THE INTERACTION OF DIPEPTIDYL PEPTIDASE 3 AND SH2 DOMAIN-CONTAINING PROTEIN 3C

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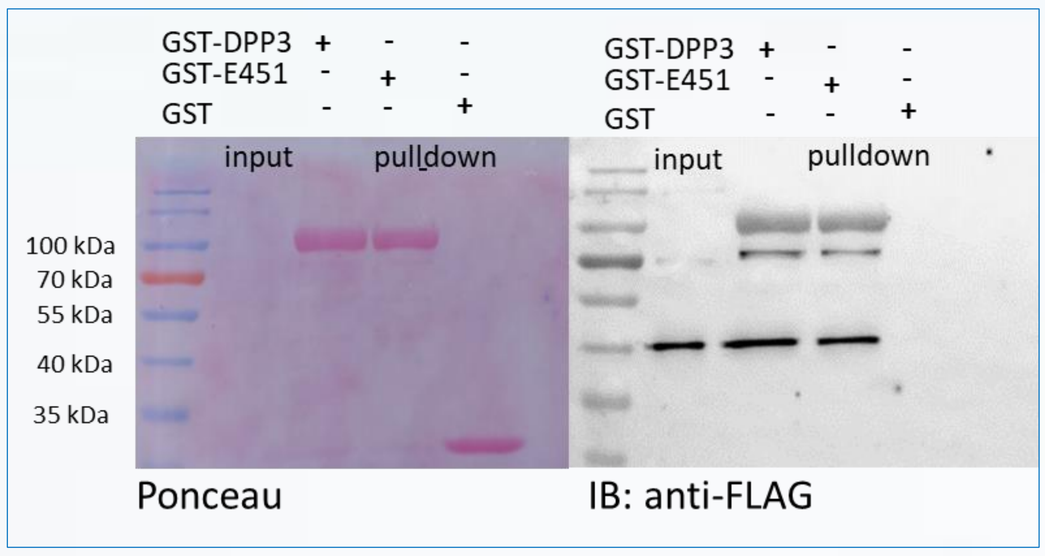
## Dipeptidyl peptidase 3

- DPP3, DPP III
- Peptidase from M49 family
- Substrates are 3-10 amino acids long peptides
  - Including bioactive peptides (angiotensins, enkephalins, endomorphins) – role in the regulation of blood pressure and pain?
- Ubiquitous – from prokaryotes to higher eukaryotes
  - Found in almost all human tissues
- Role in the protein turnover
- Interacts with KEAP1 protein – activates NRF2-KEAP1 pathway
- Indications that it is involved in cancer progression, but physiological role still unconfirmed

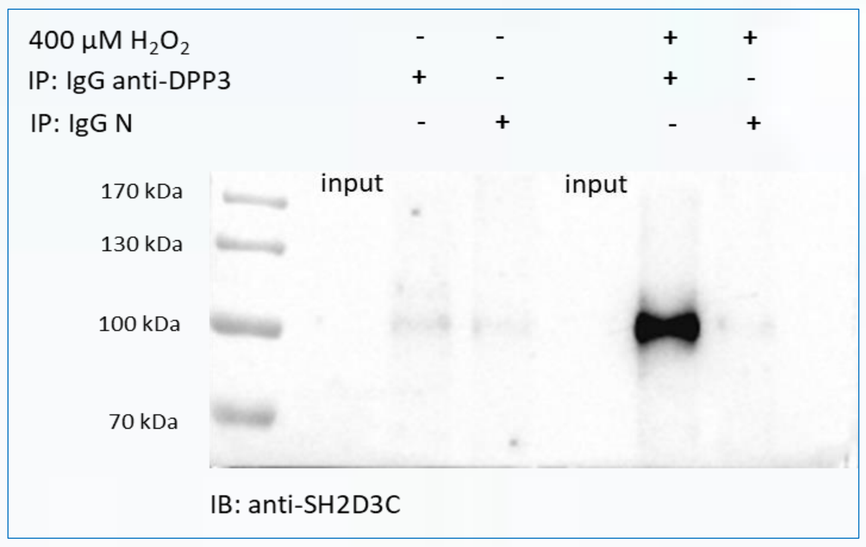
## SH2 domain-containing protein 3C

- SH2D3C, CHAT, NSP3, SHEP1
- one of the three members of the NSP family of proteins
  - contain both SH2 domain and Ras GEF-like domain
- interacts with phosphorylated cytoplasmic domain of EphB2, R-Ras and Rap1A small Ras family GTPases
  - does not show GTP exchange activity *in vitro*
- acts as an adapter protein involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response

## GST pulldown of SH2D3C-f539

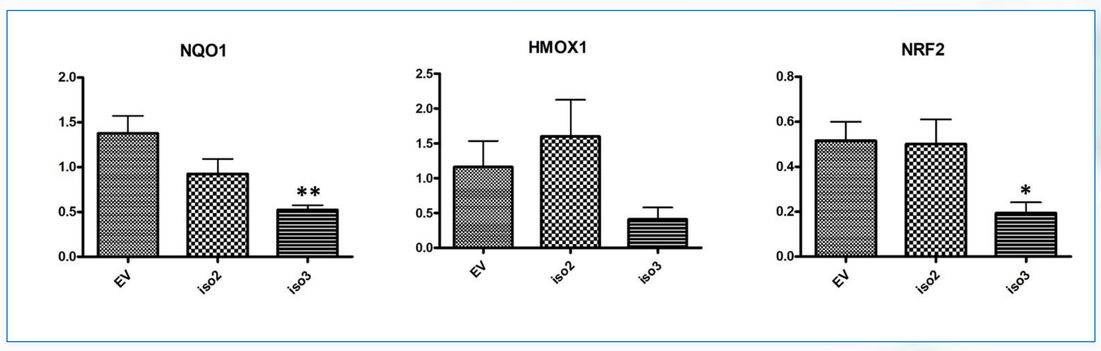



## Co-IP of endogenous proteins in HeLa cells

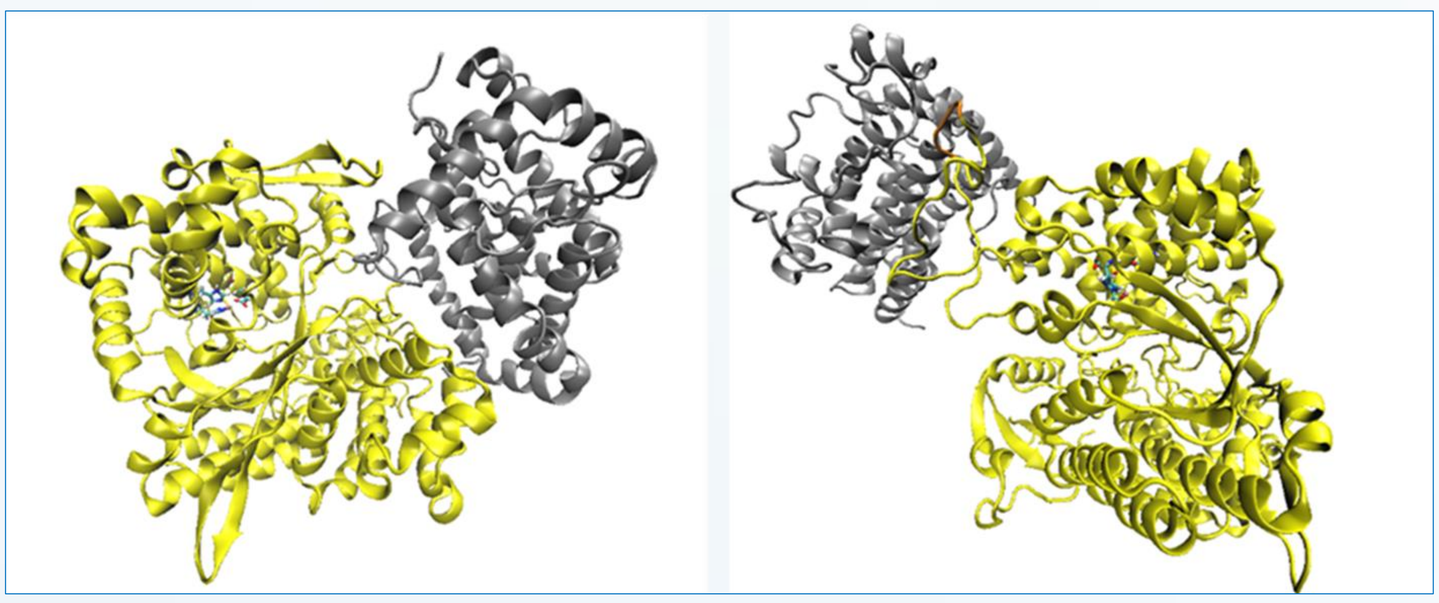



## qPCR analysis of the expression of NRF2-controlled genes

Relative expression of NQO1, HMOX1 and NRF2 genes in HEK293T cells transfected with empy vector (EV), SH2D3C-isoform2 and -isoform 3, respectively. The results represent the average of 4 biological replicates with standard error. Statistical analysis was performed using unpaired t-test (NQO1: ** p = 0.006, iso3 vs. EV; NRF2 * p = 0.0148 iso3 vs. EV).




## Representative models of binding determined by molecular docking



## CONCLUSION

SH2D3C was identified as a putative interactor of DPP3 by SILAC-MS approach. DPP3-SH2D3C interaction might represent a link between oxidative stress response mediated by NRF2-KEAP1 signaling pathway and the regulation of cell migration. The interaction was confirmed on overexpressed proteins by several methods and it was determined that C-terminal domain (f539) of SH2D3C is sufficient for binding by GST-pulldown. Co-IP of endogenous proteins in HeLa cells showed that the interaction is induced by $H_2O_2$ treatment, and the analysis of the expression of several NRF2-controlled genes showed that the overexpression of SH2D3C-isoform 3 decreases the expression of NQO1 and NRF2 genes. Molecular docking analysis produced two representative models of the interaction. The results gathered thus far, warrant the continuation of the investigation of DPP3-SH2D3C interaction and elucidation of its putative physiological role

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