Identification of a novel putative interaction partner of dipeptidyl peptidase 3, SH2 domain-containing protein 3C

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Dipeptidyl peptidase 3 (DPP3)

- Cleaves dipeptides from the amino-termini of 3-10 amino acids long peptides → proposed roles in the final stages of protein turnover, and regulation of blood pressure and pain
- Only one confirmed protein interactor - KEAP1 - role in the regulation of Nrf2/KEAP1 oxidative response pathway

SILAC-MS analysis of DPP3 interactome

- 4 biological replicas
- 32 proteins found in at least 2 replicas (SILAC-MS DPP3/empty vector (EV) ratio from 1.5057 to 9.1937)
- 12 proteins chosen for further analysis → none of the interactions confirmed by co-IP
- SH2 domain-containing protein 3C (SH2D3C) found in only one SILAC-MS replica (DPP3/EV ratio 8.5492)

Co-IP

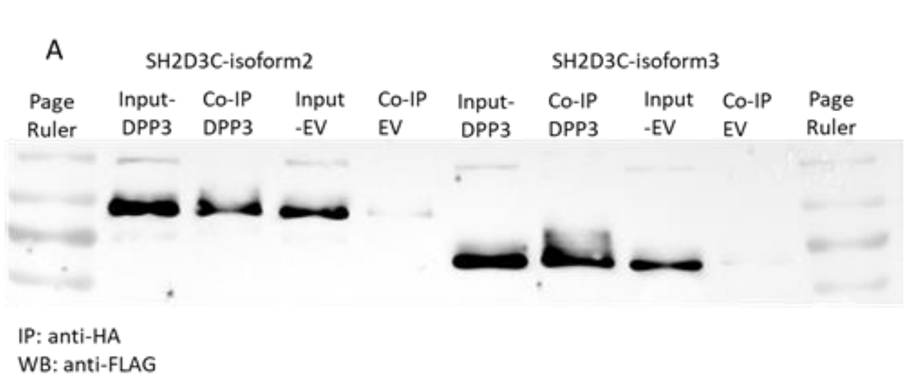

Figure 1. Co-immunoprecipitation; HA-DPP3 was co-immunoprecipitated from the TRex HEK293T cells stably expressing HA-DPP3 and transiently expressing FLAG-SH2D3C-isoform2 or 3; negative control – EV, TRex HEK293T cells stably transformed with empty vector (EV) and SH2D3C isoforms 2 or 3

Co-localization

Figure 3. Colocalization of EGFP-DPP3 and SH2D3C-mCherry in NIH 3T3 mouse fibroblast cells; Live cells were analysed on Laser Confocal Microscope Leica TCS SP8 X

SH2D3C

- Belongs to the family of proteins which contain both SH2 domain and Ras GEF-like domain
- Several different length isoforms expressed in different cell types
- Acts as an adapter protein involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response
- Interaction between overexpressed DPP3 and SH2D3C proteins confirmed by several methods

GST pulldown

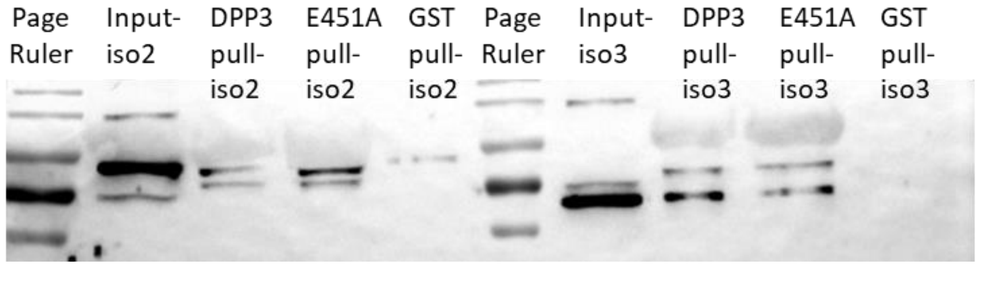

Figure 2. GST-pulldown with GST-DPP3, GST-DPP3-E451A (catalitically inactive variant) and GST (negative control) from cell lysates of HEK293T cells transiently overexpressing FLAG-SH2D3C-isoform 2 or -isoform 3

BiFC

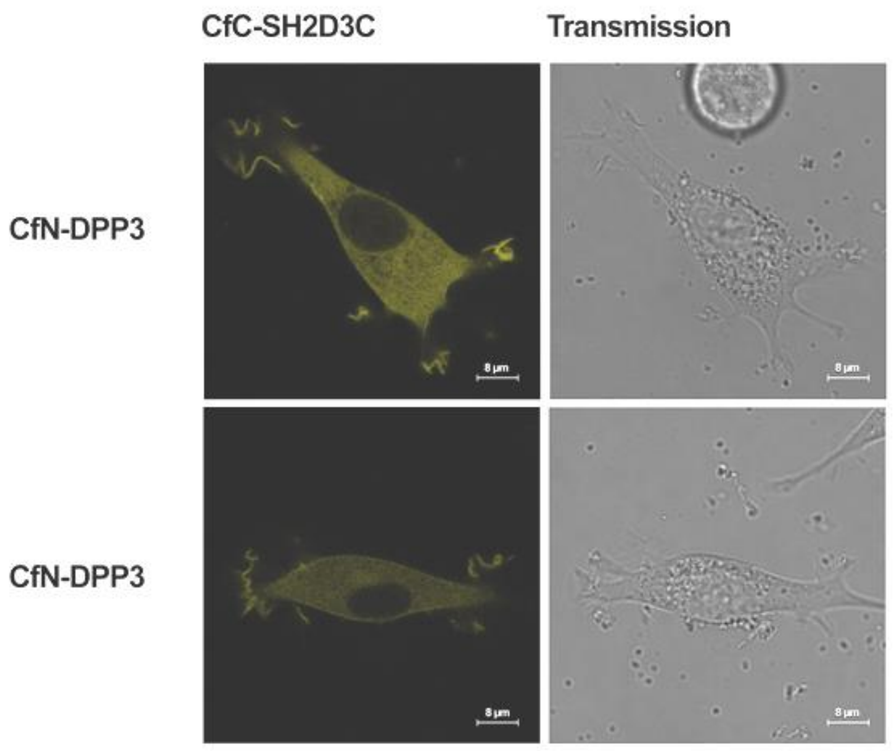

Figure 4. BiFC analysis of DPP3-VenusfN and SH2D3C-VenusfC; NIH 3T3 cells were transiently transfected with vectors expressing DPP3-VenusfN and SH2D3C-VenusfC. Live cells were analysed on Laser Confocal Microscope Leica TCS SP8 X

Conclusion

- Protein SH2D3C was identified as the putative interactor of DPP3 by SILAC-MS approach
- The interaction was confirmed on overexpressed proteins by several different methods
- Further studies are on the way in order to confirm the interaction on endogenous DPP3 and SH2D3C proteins and study the potential role of the interaction in the cells