

HIGH-THROUGHPUT SCREENING FOR DIPEPTIDYL PEPTIDASE III-INTERACTING PROTEINS

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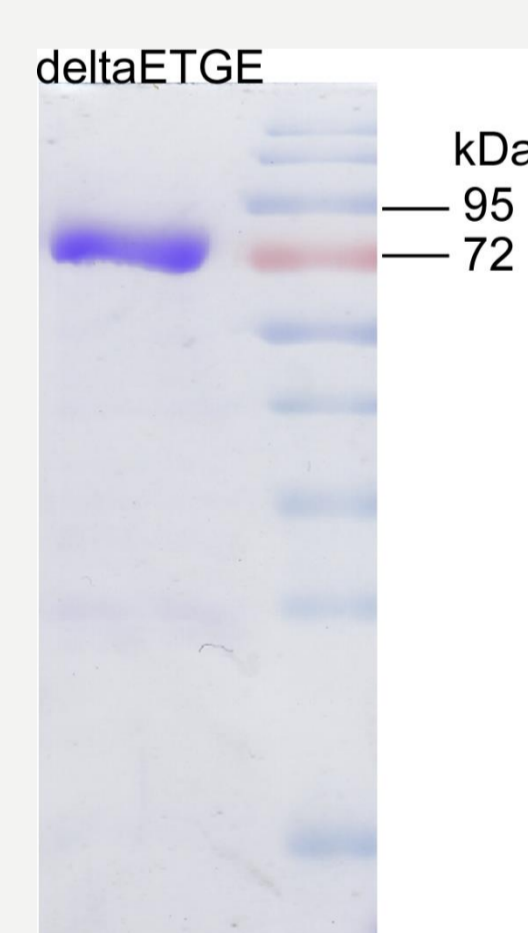
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INTRODUCTION

Dipeptidyl peptidase III (DPP III), the ubiquitously expressed member of the M49 family of metallopeptidases, cleaves dipeptides from the N-termini of 3 to 10 residues long peptides. Under *in vitro* conditions, DPP III displays restricted specificity on dipeptidyl-arylamide substrates, preferring *Arg-Arg-2-naphthylamide* (*Arg₂-2NA*). Increased expression of DPP III in the ovarian malignant tissues (1), as well as the proposed enrolment in the endogenous defense mechanisms against oxidative stress through direct binding to Keap1 (2), prompted us to investigate DPP III interactome in detail. Yeast two-hybrid (Y2H) screening of the universal standardized human cDNA library was set up to identify direct interactors of DPP III, whereas the SILAC-based immunoprecipitation of DPP III coupled to MS analysis was envisioned to identify the DPP III-containing protein complexes in the cell.

RESULTS & DISCUSSION

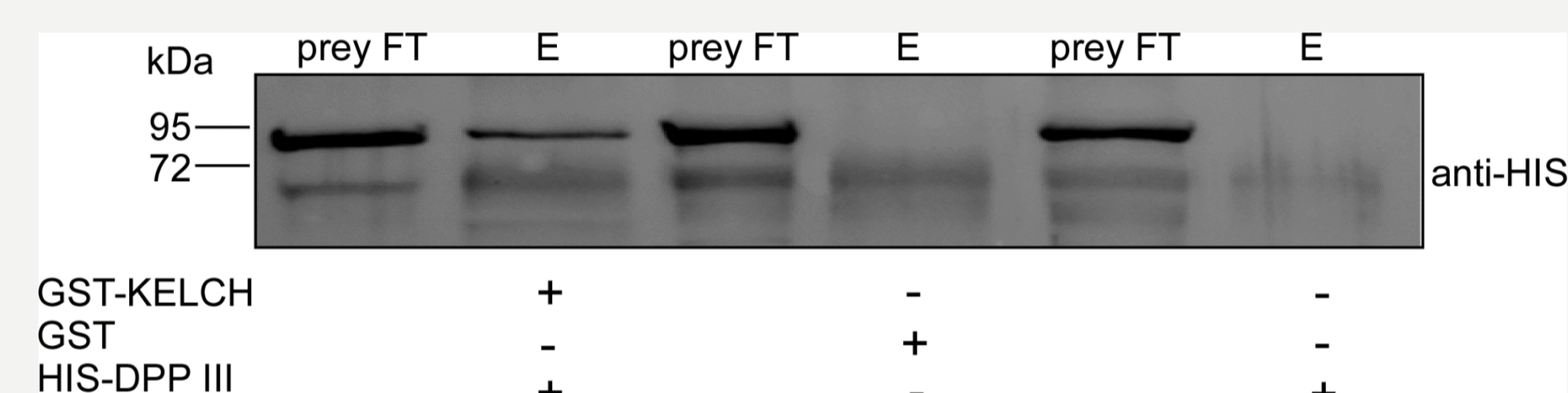
Perspective candidates will be further evaluated using downstream methods, such as one-to-one Y2H and GST pull-down assays, as well as the F-techniques. The GST pull-down assay will be set-up according to the established protocols using GST-tagged DPP III-interactive Keap1 domain, Kelch, as bait, and recombinant purified HIS-tagged DPP III (A), or doxycycline-inducible, overexpressed HA-tagged DPP III in HEK293T cell lysate (B), as prey. Recombinant purified human DPP III without the ETGE domain (deltaETGE; C), responsible for the interaction with Keap1, will serve as a negative control for binding. Interestingly, although ETGE domain does not contribute to the DPP III peptidase activity, the mutant displays slightly decreased catalytic properties (Table 1). DPP III is mainly located in the cell cytoplasm, however, nuclear signals for DPP III were reported as well (3). Our preliminary data suggest that there is indeed distinctive DPP III signal in the nuclear fraction, when compared to the DPP III KO mouse (D).



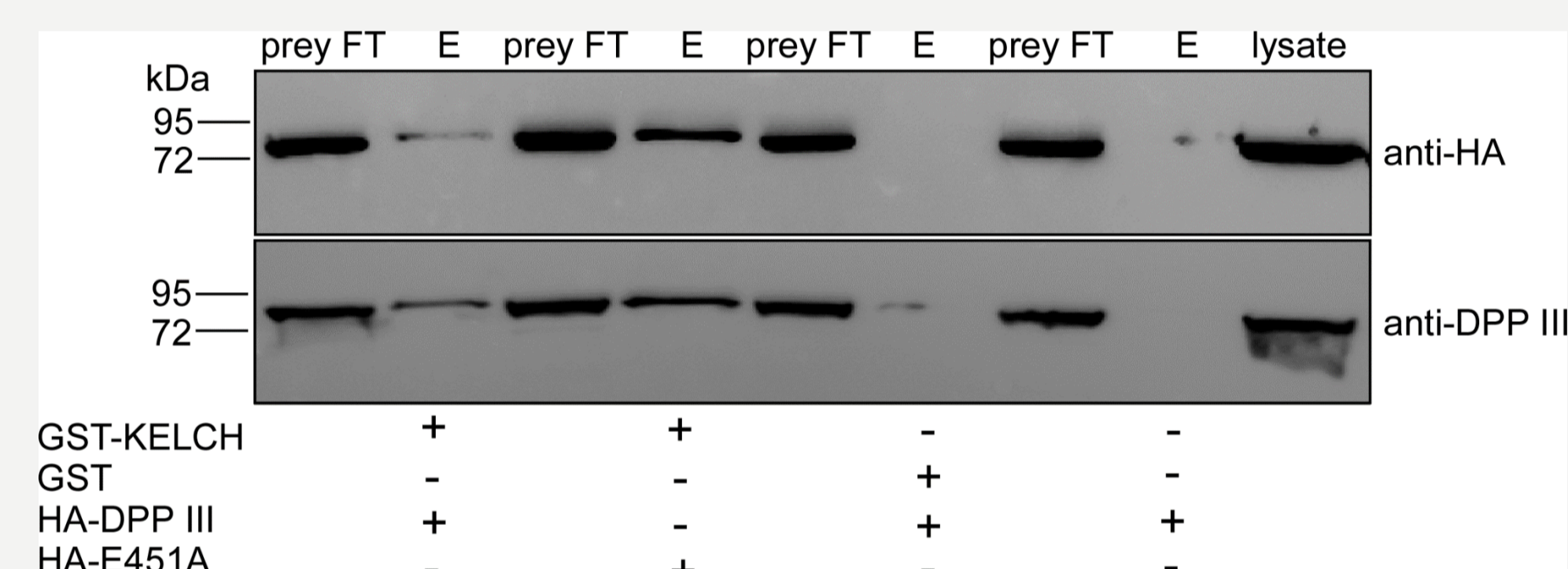
(C) deltaETGE purification from BL21 bacteria.

Table 1.
Kinetic characterization of wild-type and DPP III deltaETGE mutant.

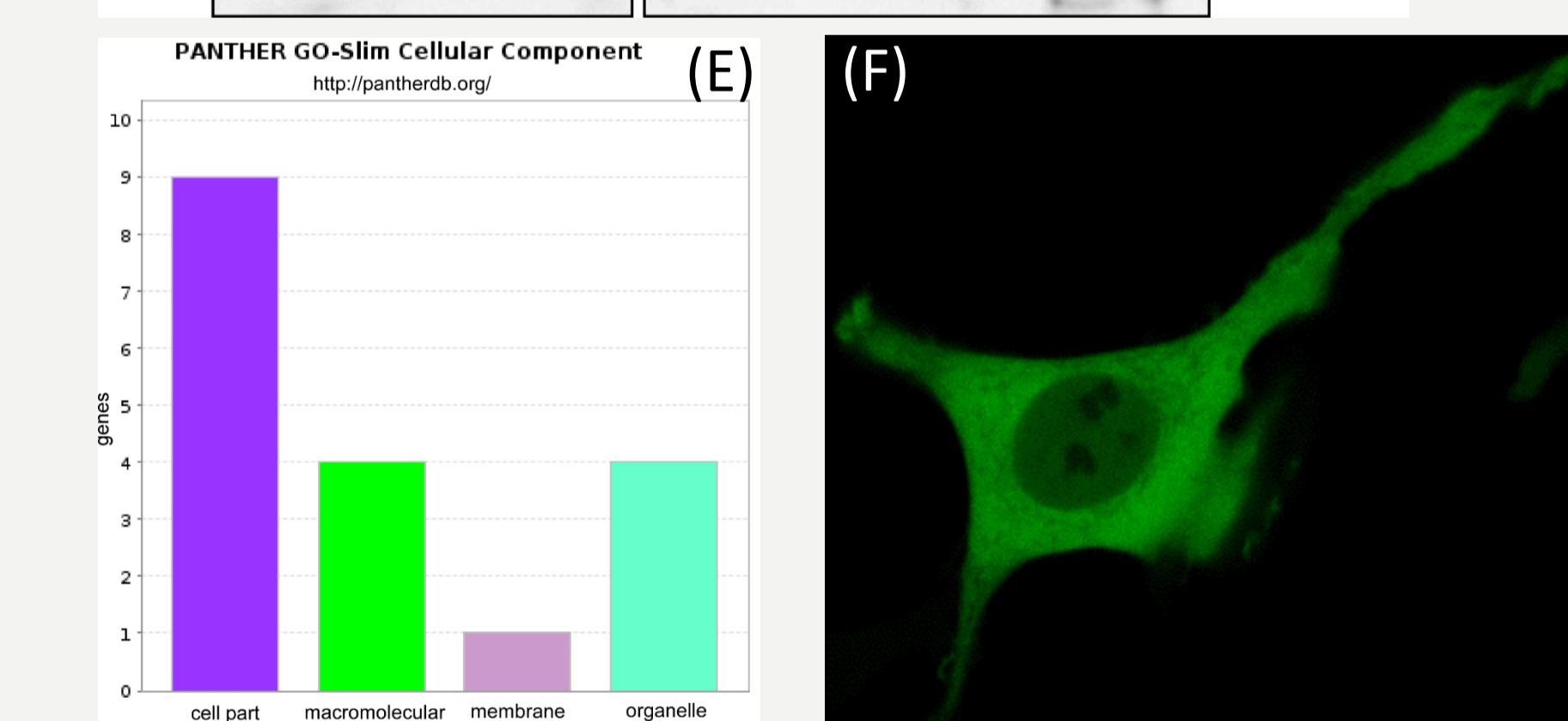
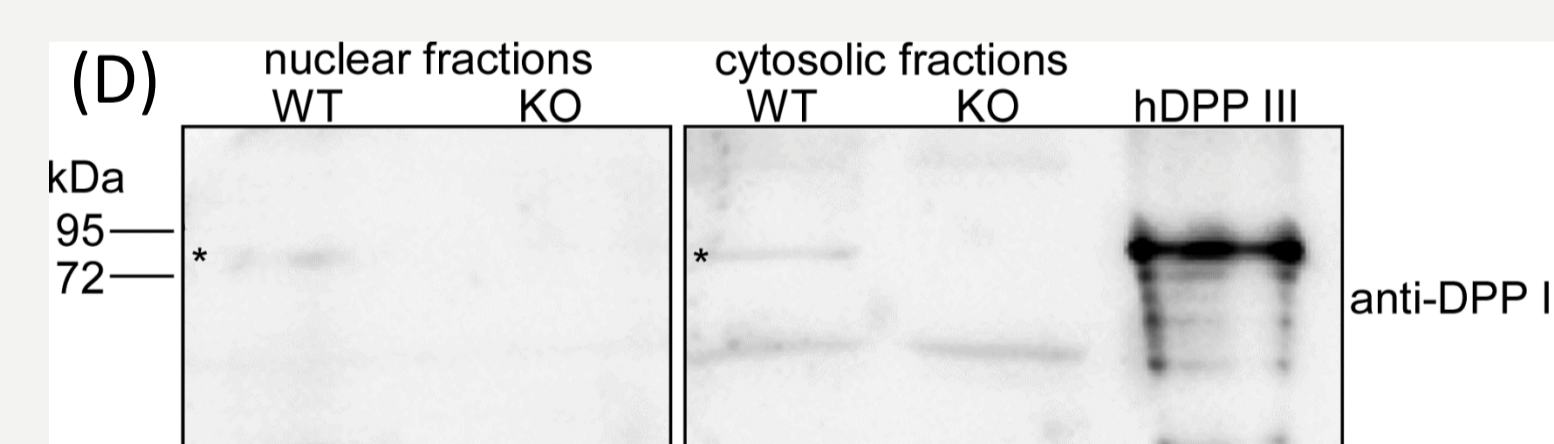
pH 8,6; T=25 °C	Wild-type	deltaETGE
<i>Arg₂-2N</i>		
K_m (μM)	5.4 ± 0.4	2.2 ± 0.6
k_{cat} (s^{-1})	35 ± 5	6.3 ± 0.5
k_{cat}/K_m ($\text{mM}^{-1} \text{s}^{-1}$)	6470	3000



(A) GST pull-down assay using HIS-tagged hDPP III as prey. FT, flow-through; E, eluate.



(B) GST pull-down assay using HA-tagged hDPP III from HEK293T lysate as prey. E451A, DPP III catalytically inactive mutant, unimpaired in Keap1 binding; FT, flow-through; E, eluate.



(D) hDPP III localization at the cellular level. Samples are generous gift from Dr Sobočanec, IRB. (E) Certain SILAC candidates are located in the nucleus. (F) Visualisation of the hDPP III-EGFP signal in HeLa cells (cell culture manipulation by Dr Tomašić Paić and Leica SP8 X FLIM confocal microscopy by Dr Filić Mileta, IRB).

FUTURE PERSPECTIVES

We aim to discover the novel DPP III protein interactors and to confirm these interactions by several complementary approaches. Once confirmed, novel interactors might open new directions in the investigation of the DPP III physiological roles in the future.

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