The effect of DPP3 mutation found in cancer on the KEAP1-NRF2 Signaling Pathway

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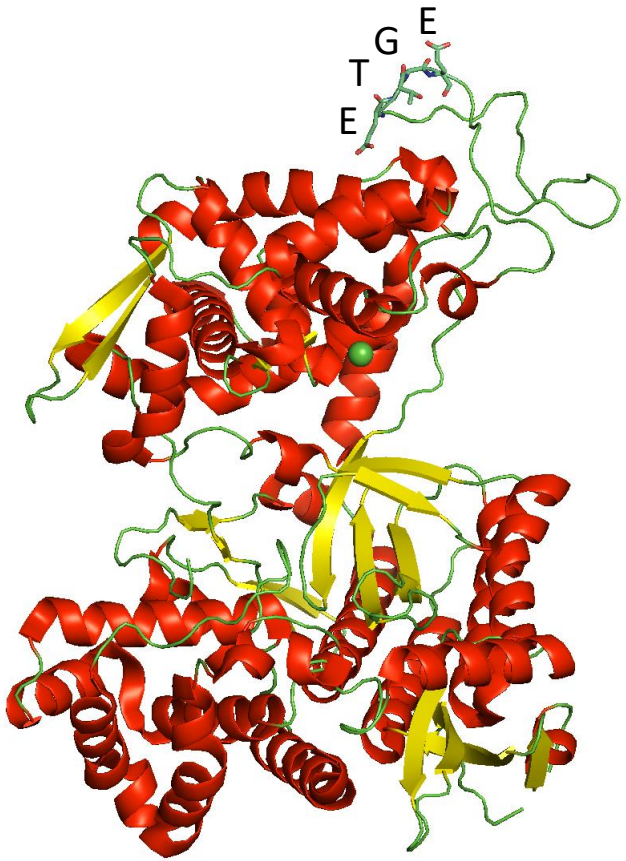
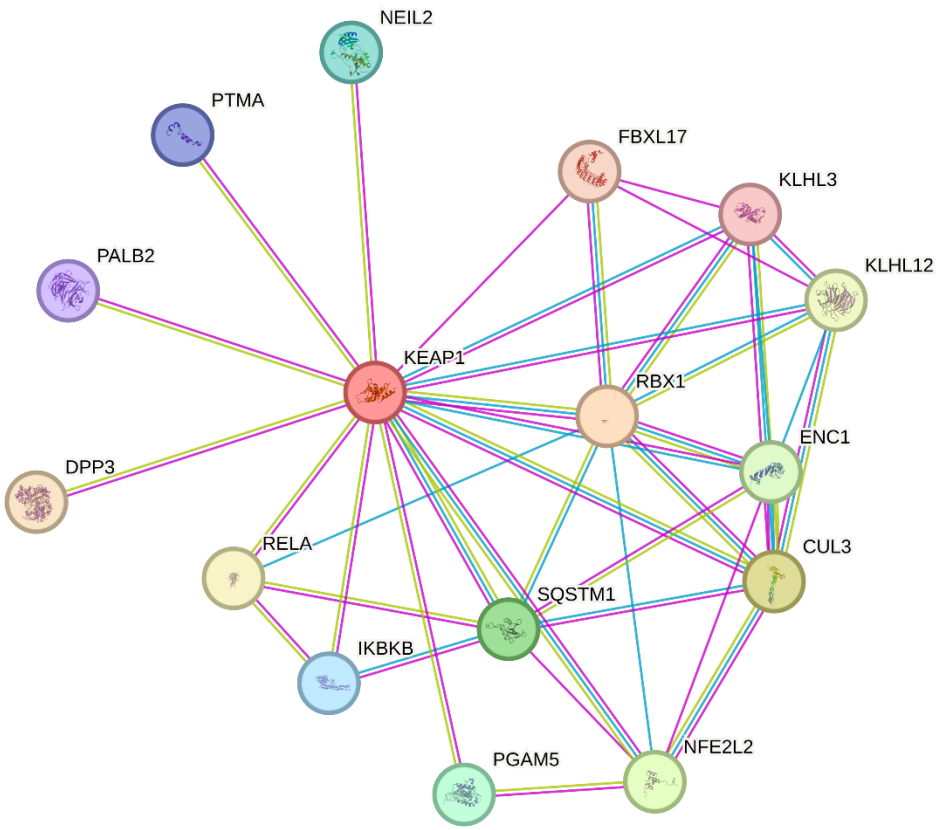
Dipeptidyl peptidase 3

- Zn metallopeptidase
- Ubiquitously expressed in organisms from bacteria to humans and in almost all human tissues
- Cleaves dipeptides from N-termini of 3 to 10 amino acids long peptides → protein turnover
- Cleaves bioactive peptides: angiotensins, enkephalines, endorphins → regulation of blood pressure and pain?
- Identified as interactor of KEAP1
- DPP3 KO mice have impaired bone development due to increased OS in osteoclasts
- Increased amount (and activity) in cancers of different etiology, ovarian, endometrial, lung, breast, colorectal, multiple myeloma
- Biomarker of the poor prognosis in septic, cardiogenic and vasodilatory shock



DPP3-KEAP1 interaction

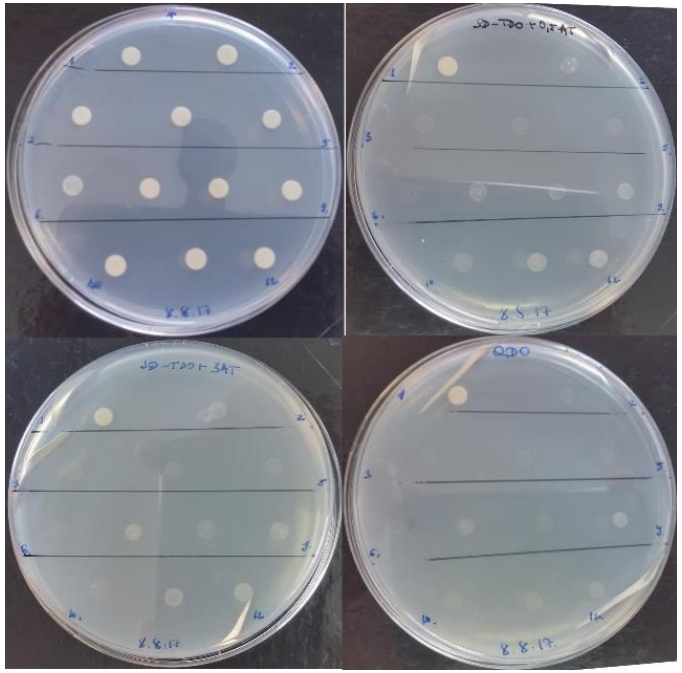
- Liu et al. PNAS 2007 – SQSTM1 (p62) and DPP3 activate ARE expression and induce NQO1 in NRF2-dependent manner
- Hast et al. Cancer Res 2013
  - DPP3 binds KEAP1 through ETGE
  - DPP3 ox. activated NRF2-mediated transcription and reduced NRF2 ubiquitination
  - DPP3 mRNA expression and copy number correlate with the NRF2 activity in squamous cell lung carcinoma
- Interaction confirmed by Lu et al. Cancer Res 2017
  - Interaction induced by oxidative stress
  - DPP3 ox. stabilizes KEAP1
  - DPP3 expression correlated with increased expression of NRF2 target genes and poor survival in ER+ breast cancer



STRING: KEAP1 physical interaction network (limited to 15 proteins)

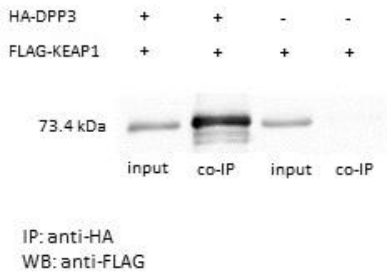
DPP3-KEAP1 interaction – confrimed by several methods

Y2H

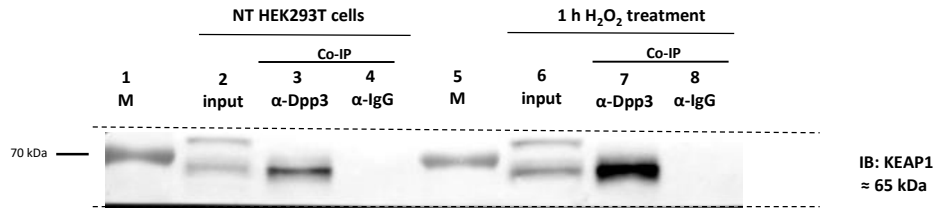


Co-IP

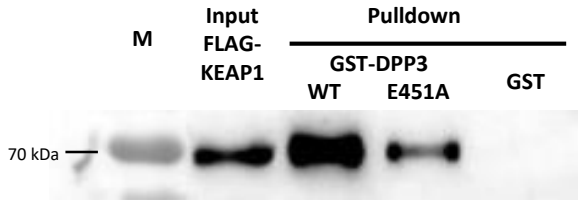
Ox. proteins



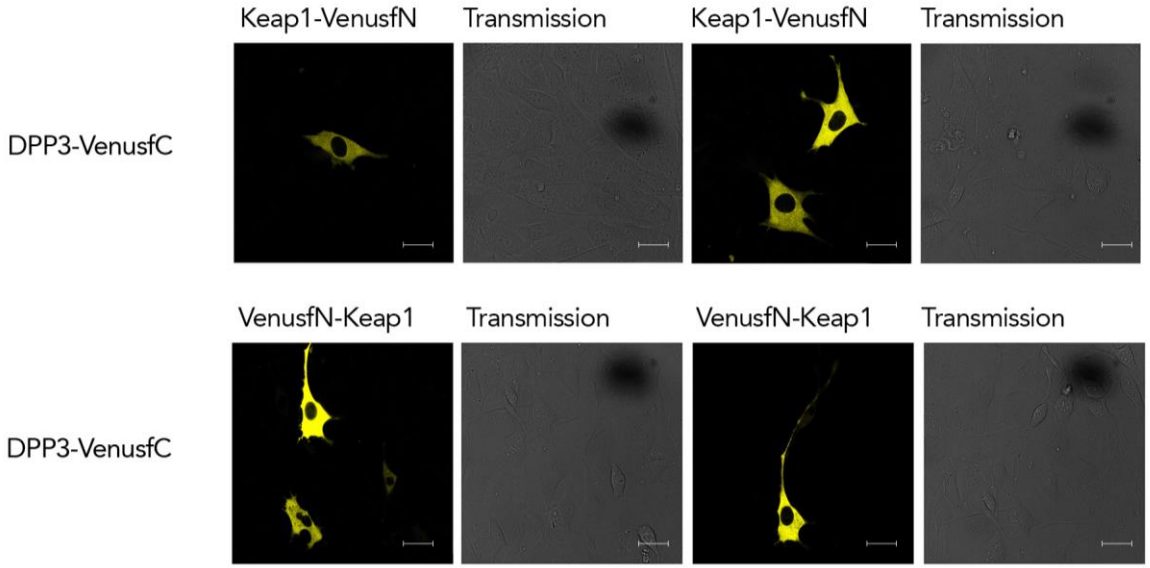
Endogenous proteins



GST-pulldown

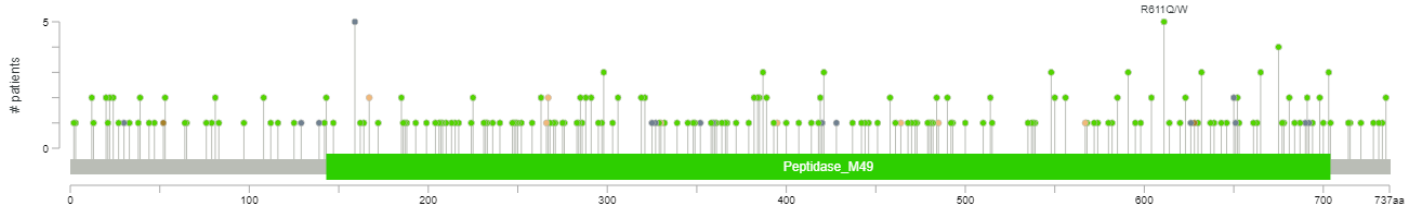


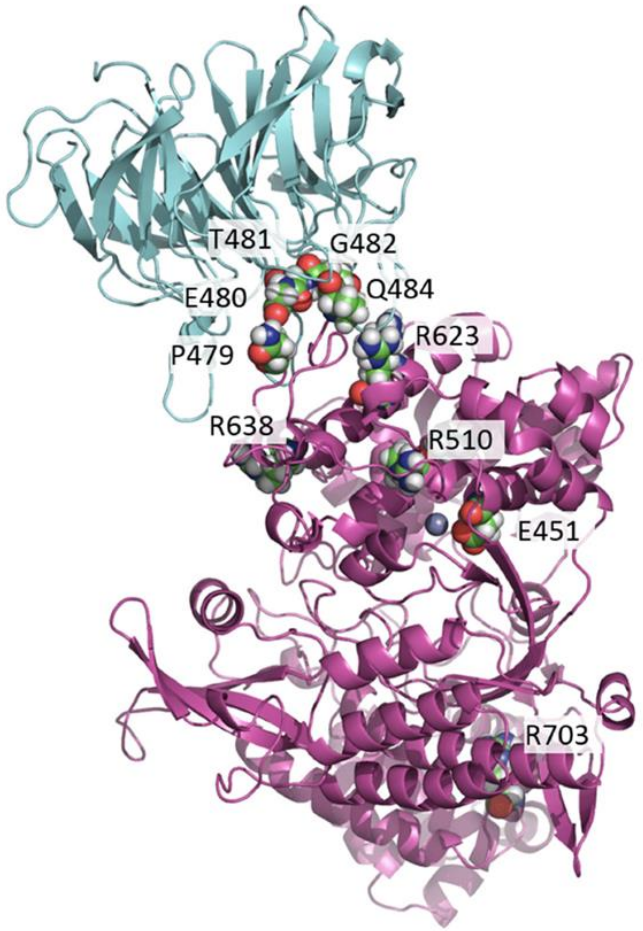
BiFC



DPP3 mutations in cBioPortal for cancer genomics

- DPP3 – around 280 mutations





- Enzyme kinetics analysis of mutant variants compared to the WT
- Analysis of the interaction with KEAP1

DPP3 mutants enzyme kinetics

	WT	P479S	E480Q	T481M	G482C	Q484H	R620C	R623W	R623L	R638L	R638W
k_{cat} (s^{-1})	70.2 ± 3.6	53.9 ± 1.5	52.3 ± 1.6	26.5 ± 0.9	62.5 ± 1.0	45.1 ± 1.8	63.0 ± 1.6	41.1 ± 0.6	55.7 ± 1.8	15.6 ± 0.4	8.6 ± 0.3
K_M(μM)	12.7 ± 1.8	8.3 ± 0.8	10.4 ± 1.0	10.0 ± 1.1	8.8 ± 0.4	6.6 ± 1.0	11.2 ± 0.8	7.6 ± 0.4	9.8 ± 1. 0	3.5 ± 0.42	3.5 ± 0.5
k_{cat}/K_M (s^{-1} μM^{-1})	5.5	6.5	5.0	2.6	7.1	6.8	5.6	5.4	5.7	4.5	2.5

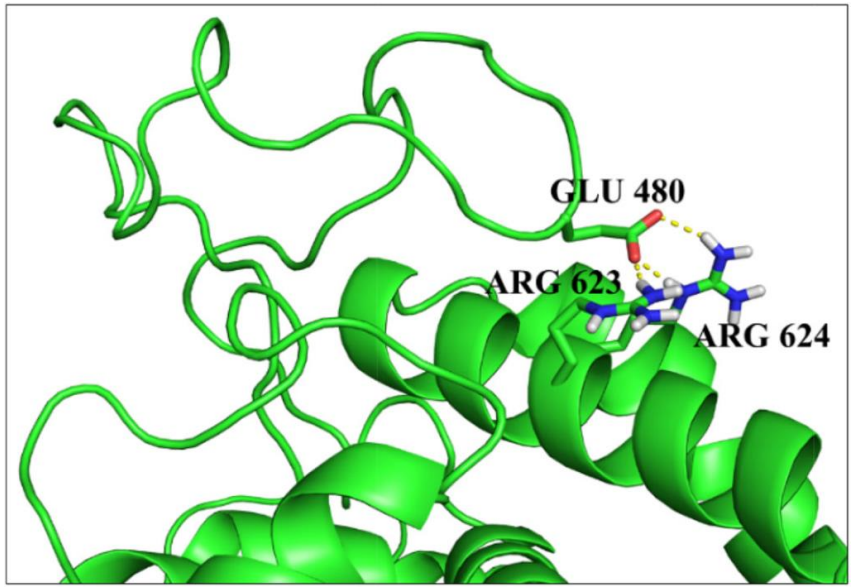
- Measure the enzyme kinetics towards synthetic substrate Arg_2-β-naphthylamide
- WT has the highest catalytic activity (k_{cat}), however, differences are relatively small
- Affinity towards the substrate (K_M) and specific activity (k_{cat}/K_M) similar in WT and all tested mutants

Microscale thermophoresis (MST) analysis of the binding affinity

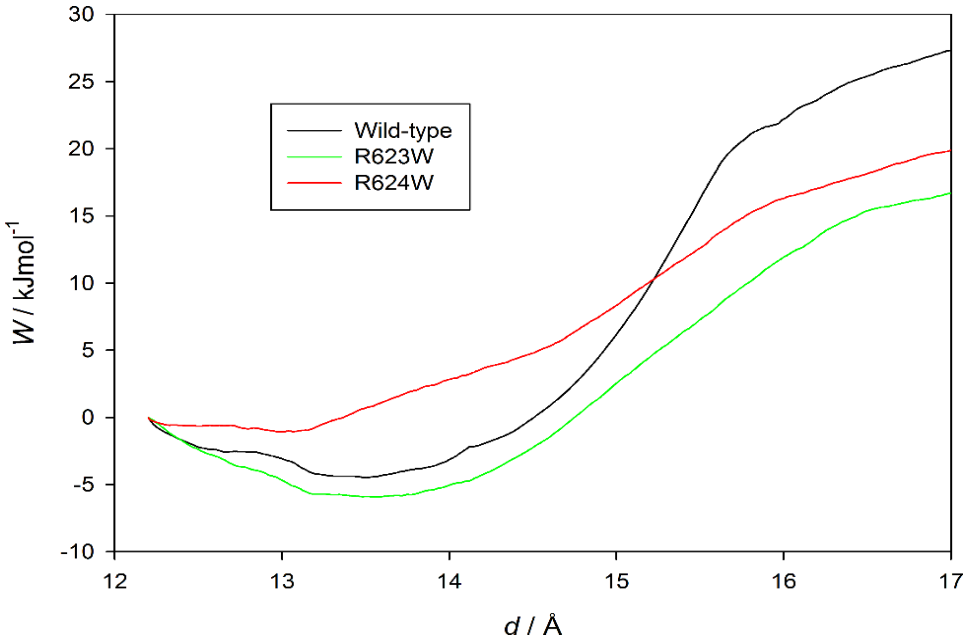
DPP III	K_d (WT)/K_d(mut)
WT	1.0
E451K	2.1
P479S	**18.4**
E480Q	0.1
T481M	0.1
G482C	0.8
Q484H	2.1
R510W	0.3
R623W	**160.0**
R638L	2.0
R638W	2.0

- MST analysis - interaction of DPP3 mutant variants found in cBioPortal with Kelch domain
- P479S around 20 times higher affinity for Kelch than the WT - NRF2 has Glu at the same position
- R623W - more than 100 times higher affinity for the Kelch domain than the WT

Molecular dynamics analysis of DPP3/Kelch interaction

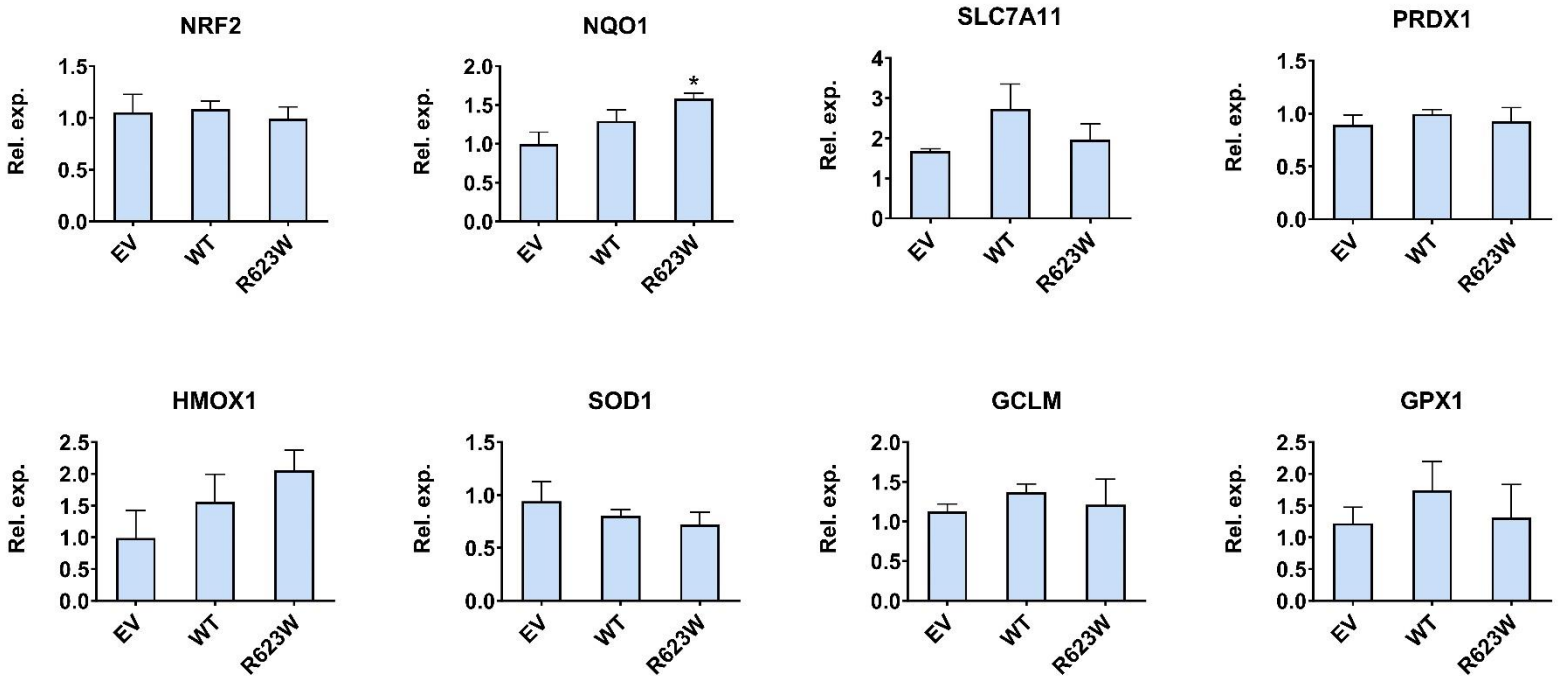


- Binding of DPP3 to Kelch is preceeded by the release of ^{480}ETGE^{483} loop from DPP3 protein body



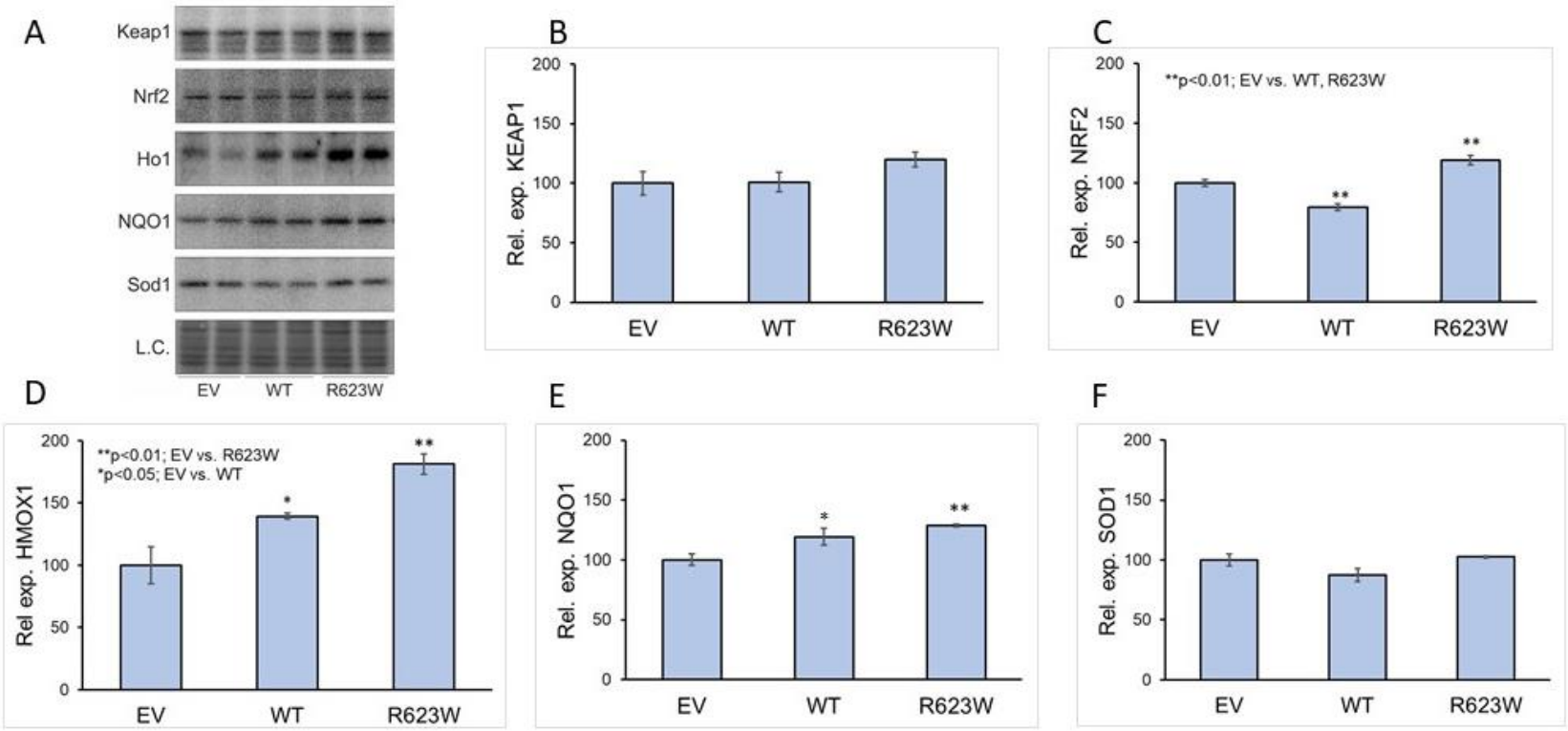
- Work required to detach the ETGE loop is lower in R623W and R624W mutant variants – possible explanation for lower Kd of R623W for Kelch binding

qPCR analysis of the expression of NRF2-controlled genes



- HEK293T cells transiently transformed with EV, WT-DPP3 or DPP3-R623W and treated with 400 µM H_2O_2
- qPCR analysis of the expression of 8 NRF2-controlled genes – reference gene TUBG1
- Only the expression of NQO1 in cells overexpressing DPP3-R623W significantly higher than in EV transfected cells (t-test; N=3; $p<0.05$)

Western blot analysis of the expression of NRF2-controlled genes



- HEK293T cells transiently transformed with EV, WT-DPP3 or DPP3-R623W and treated with 400 µM H_2O_2
- DPP3-R623W ox. Increases the expression of NRF2, HMOX1 and NQO1 ($p<0.01$)
- WT DPP3 ox. Increases the expression of HMOX1 and NQO1 ($p<0,05$) and decreases the expression of NRF2 ($p<0.01$) (?)

Summary

- Interaction of DPP3 and KEAP1 confirmed by several methods
- DPP3 mutants analyzed have lower or similar enzymatic activity as the WT
- MST analysis showed that DPP3-R623W mutant (found in cancer) has more than 100 fold higher affinity for Kelch domain than the WT
- MD simulations of DPP3-Kelch interaction: DPP3-Keap1 binding is a two step process, the 1st step is detachment of ETGE loop from protein body – lower work required to detach R623W
- Overexpression of DPP3-R623W in HEK293T cells induces the expression of NQO1 mRNA, and NQO1, HMOX1 and NRF2 proteins
- Overexpression of WT DPP3 increases HMOX1 and NQO1, also, but decreases the expression of NRF2 (?)

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