

Understanding the eukaryotic pathways modulated by mycobacterial phosphatases: *Mtb*-PtpA interaction and activity on human TFP α , a key enzyme of host-lipid metabolism.

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BACKGROUND

Human macrophage proteins identified as putative PtpA partners

Biological process*	Protein accession	Protein name	Mascot score ^b	Mass [Da]	No. of matched ions ^b	No. of peptide sequences ^b
Lipid metabolism, fatty acid beta-oxidation	human TFP _{ECHA}	Trifunctional enzyme subunit alpha, mitochondrial	2422	82947	63	23
Sulfide oxidation, using sulfide:quinone oxidoreductase	SQRD_HUMAN	Sulfide:quinone oxidoreductase, mitochondrial	705	49929	28	10
Respiratory electron transport chain	ATPA_HUMAN	ATP synthase subunit alpha, mitochondrial	531	59714	16	7
Glycolysis	K6PP_HUMAN	6-phosphofructokinase, platelet type	922	85542	47	15

* Only the main biological processes with traceable author statement are shown, from UniProt (<http://www.uniprot.org/>) database (accessed on January 2014).
b The value of score, number of matched ions and peptide sequences is the best value obtained.

human Trifunctional protein: hTFP_{ECHA/ECHAB}

Fatty acid β -oxidation is the principal energy-yielding process in organisms ranging from bacteria to humans

PtpA acts on numerous eukaryotic proteins regulating different cell signaling pathways

Modulation of host lipid metabolism?

hTFP_{ECHA/ECHAB}
Mitochondrial Trifunctional enzyme
 β -oxidation-Lipid metabolism
(Margenat, M. et al. Sci Rep 5, 8819 (2015))

Inhibition of phagosome maturation

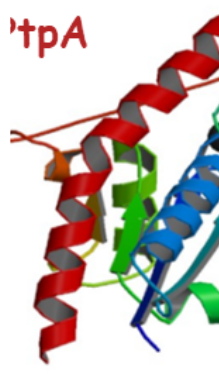
VPS33B
(and recruitment of H-VATPase)
(Bach et al., 2008 and Wong et al. 2011 and 2015)

Inhibition of Innate immunity

MAPK p38/Jnk and NF- κ B
TNF α , IL1 β , IL12 and NF- κ B
(Wang et al. 2015, 2017)

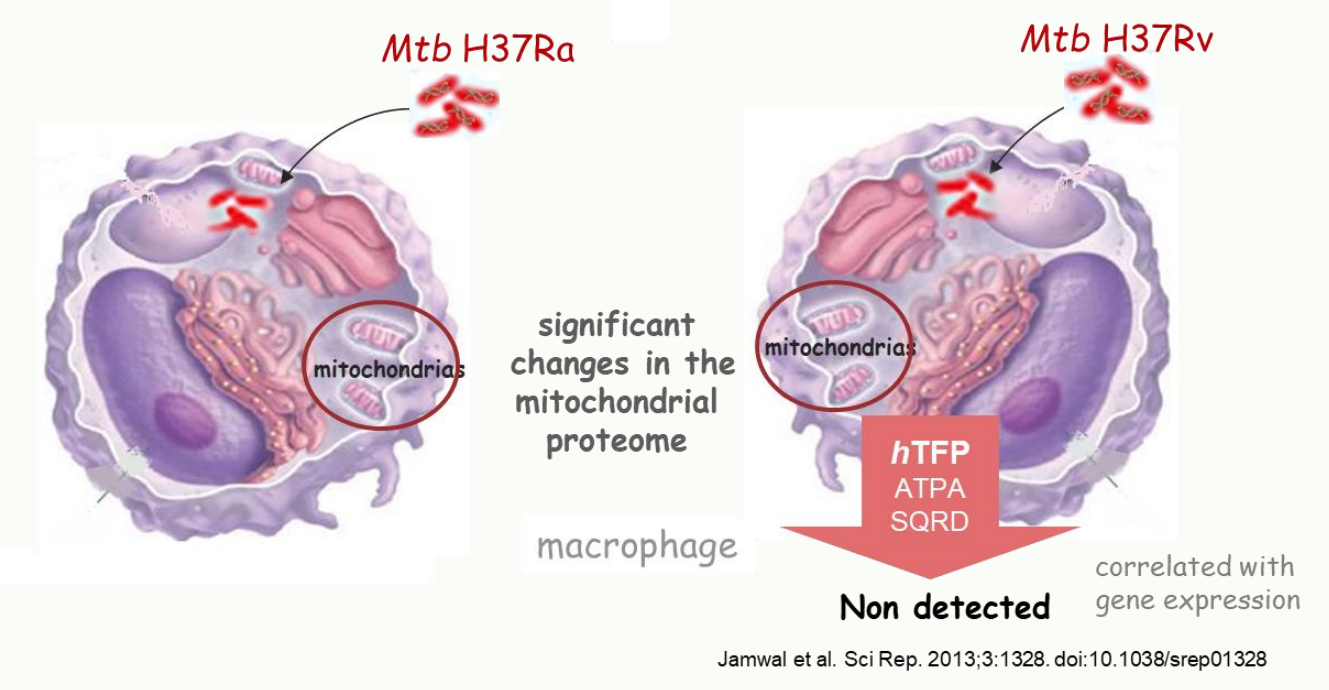
Inhibition of apoptotic pathway

GSK3 α
(glycogen synthase kinase 3-subunit α)
(Pomeroy et al. 2014)



promotes the survival of bacteria in infected cells

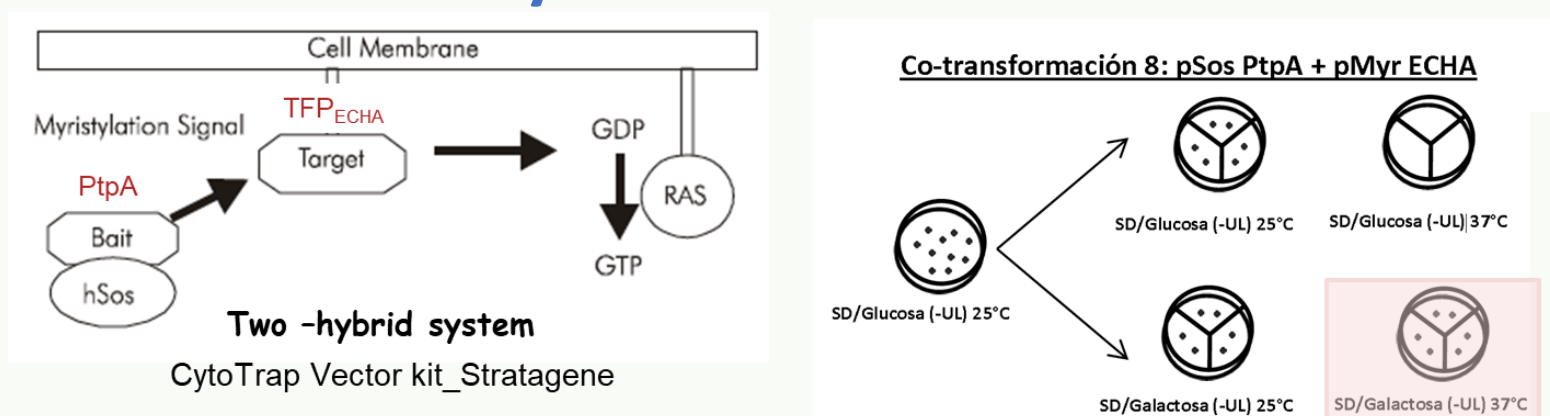
What about hTFP during *Mtb* infection?



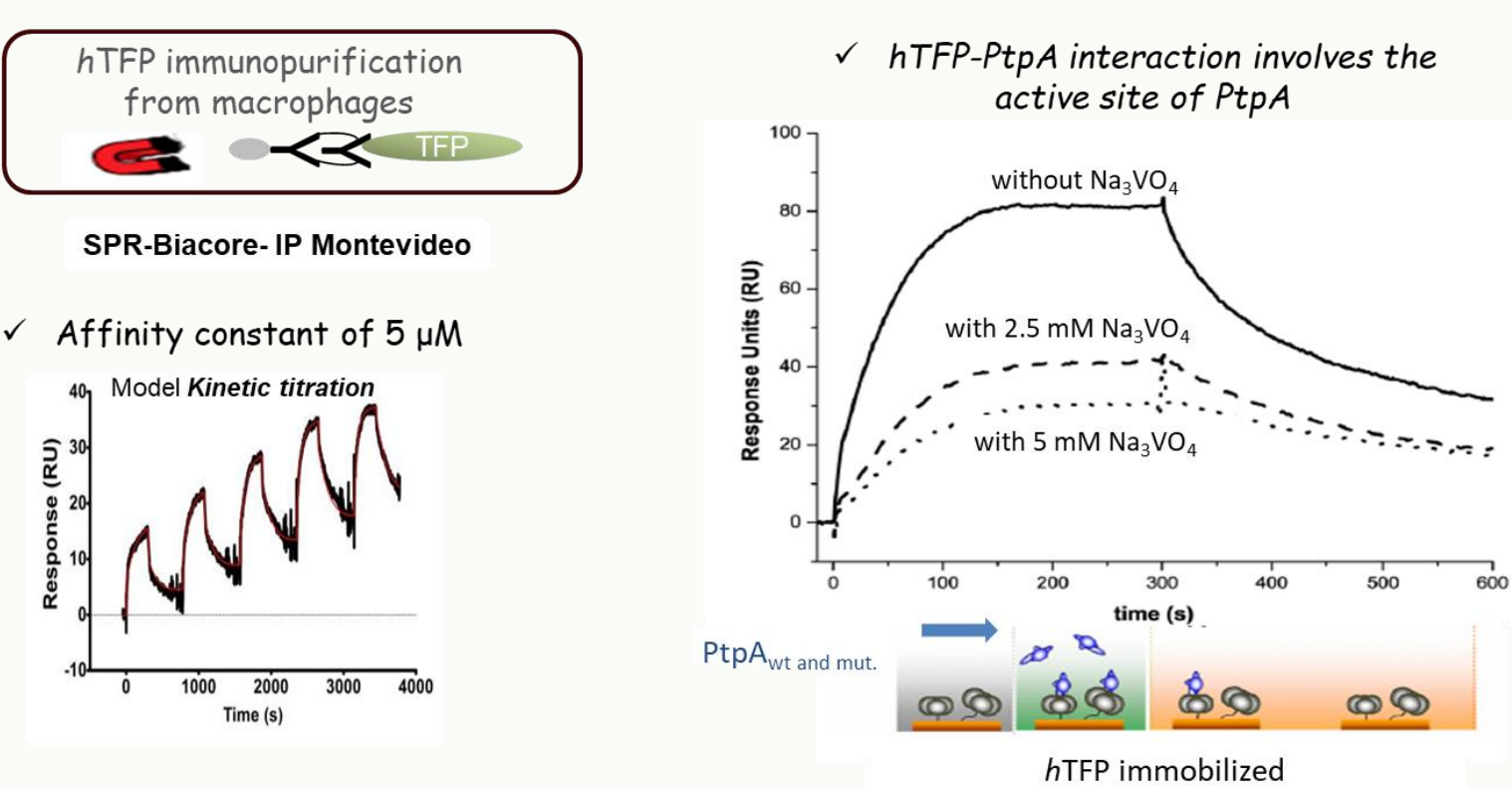
RESULTS

Mycobacterial PtpA and hTFP α form a stable complex that involves PtpA active site

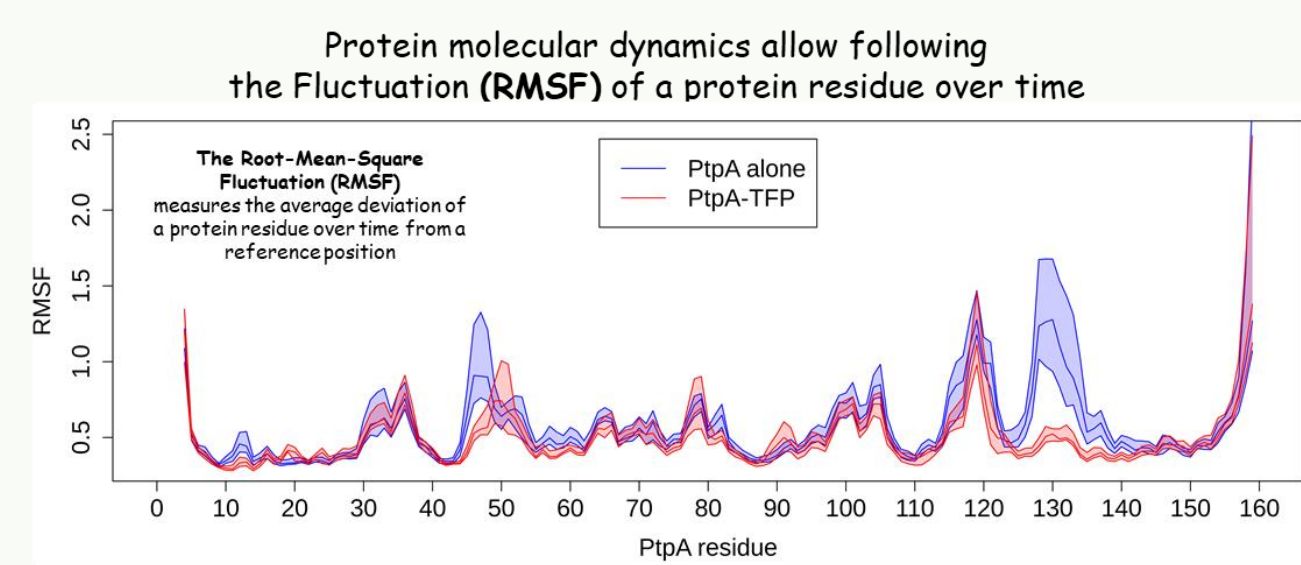
- hTFP-PtpA interaction was verified in an eukaryotic cellular context



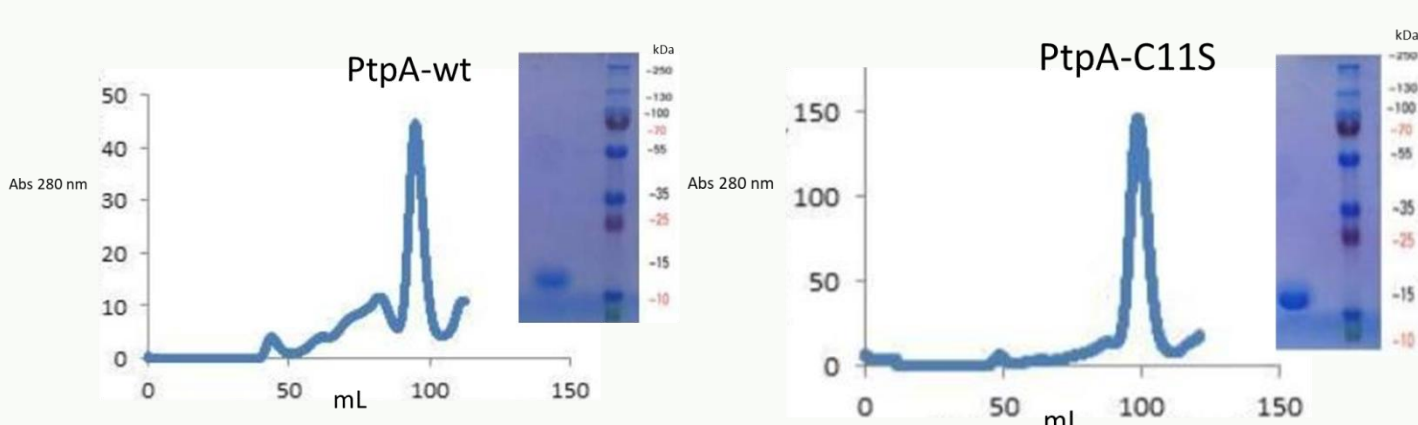
- PtpA-hTFP interacts through the active site of PtpA (affinity constant of 5 μ M)



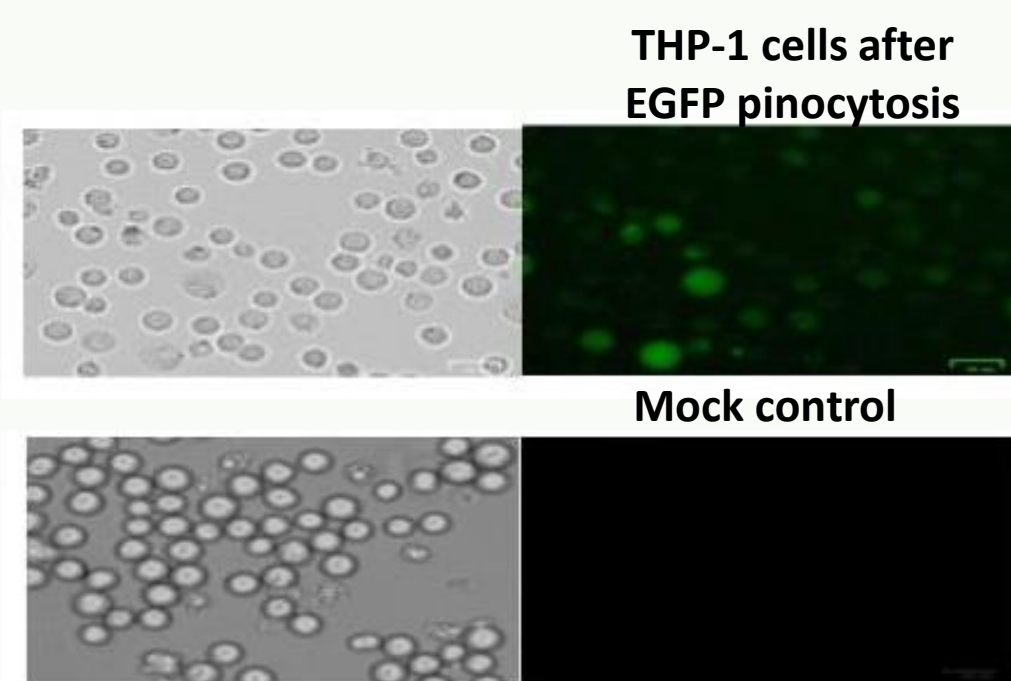
- The PtpA-hTFP complex is stable, detecting a PtpA catalytic D-loop stabilization



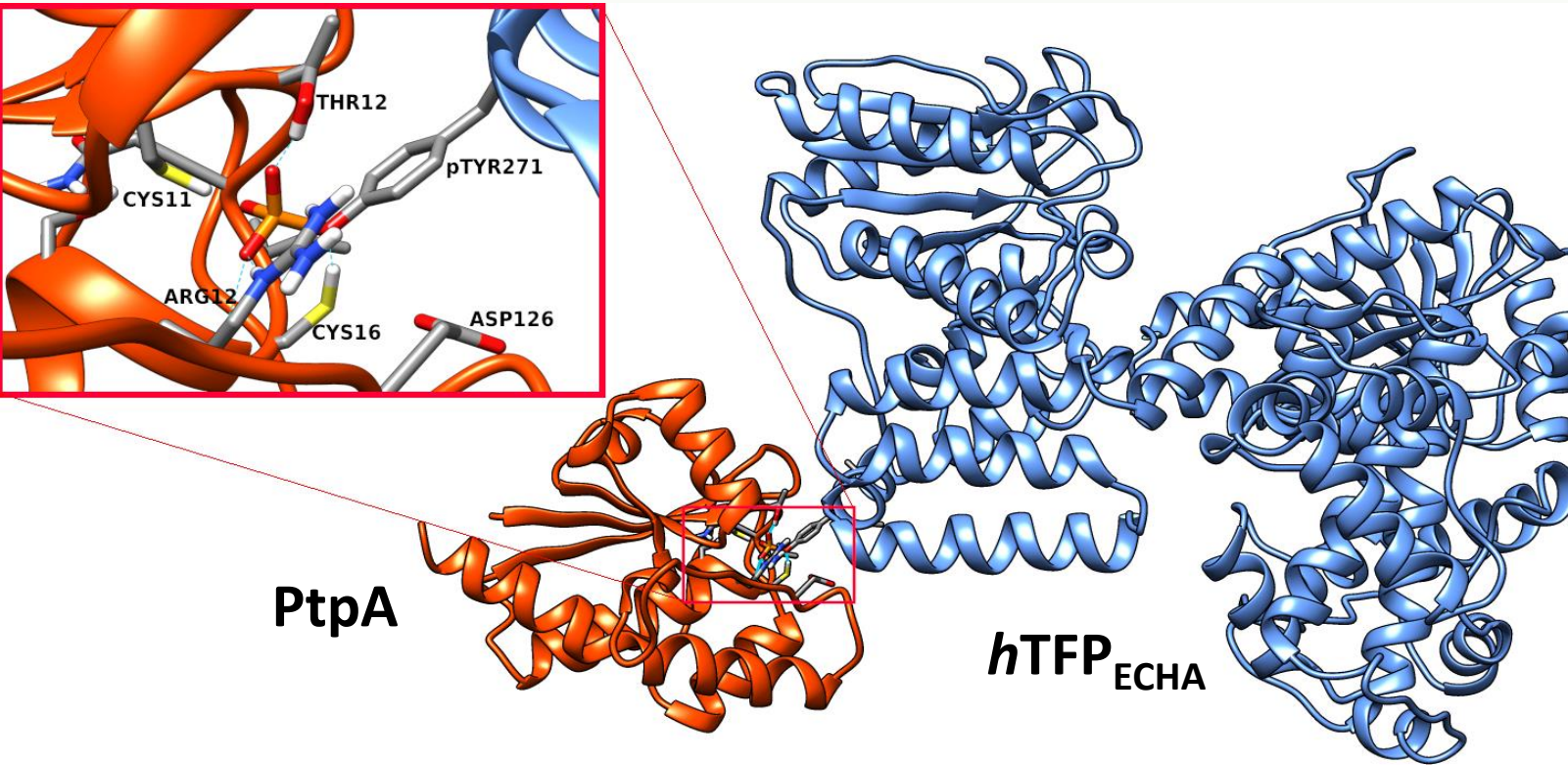
- Production of recombinant proteins



- Assays of pinocytosis



The P-Tyr 271 is the potential target of mycobacterial PtpA and is located in a helix relevant for human TFP localization and activity



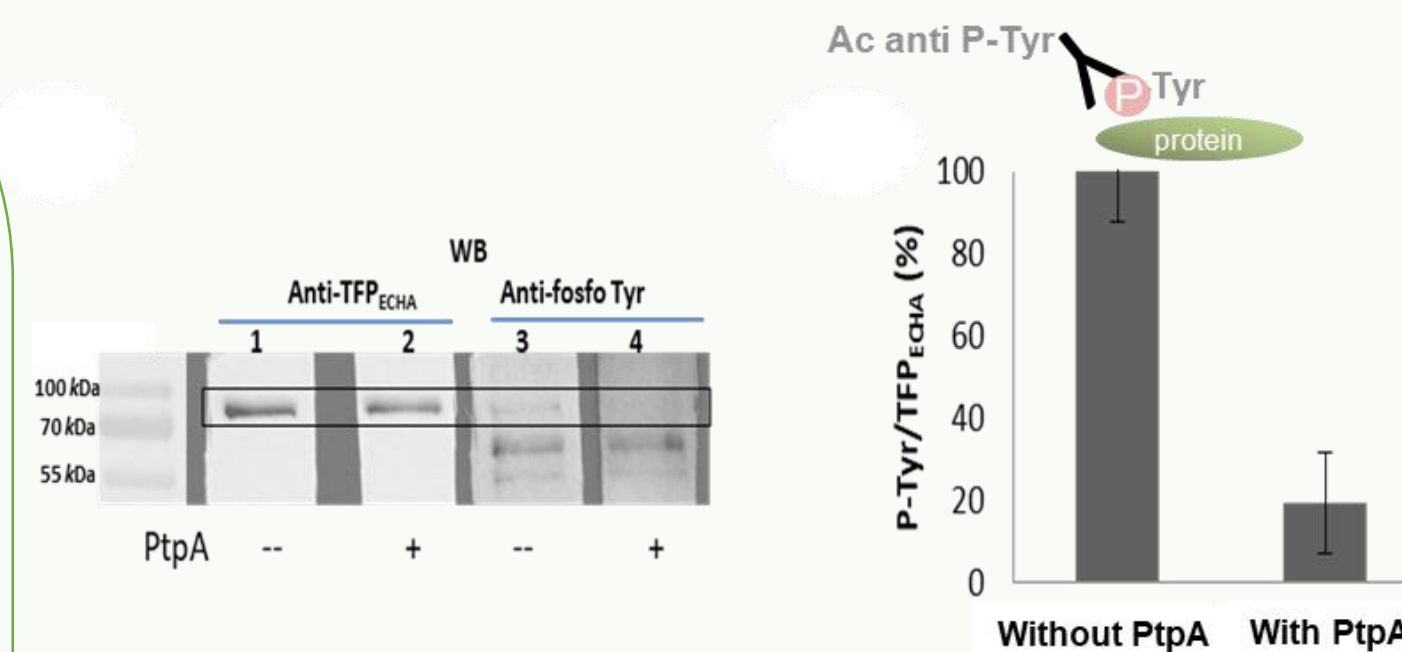
Phosphotyrosine	Total number of clusters	Number of members for:	Electrostatic binding energy (Kcal/mol) for:
p-Tyr 43	10	67	42
p-Tyr 239	7	92	122.4620.1
p-Tyr 271	3	173	193.1418.1
p-Tyr 435	7	49	163.948.9
p-Tyr 637	8	64	17.368.2
p-Tyr 639	8	75	121.7922.7
p-Tyr 724	11	34	99.9204.4
p-Tyr 762	10	42	151.1221.0
			14.7135.1
			88.2132.7
			52.5441.9
			30.632
			2.229

- P-Tyr 271 and Helix-10 are absent in bacterial-TFP homologues

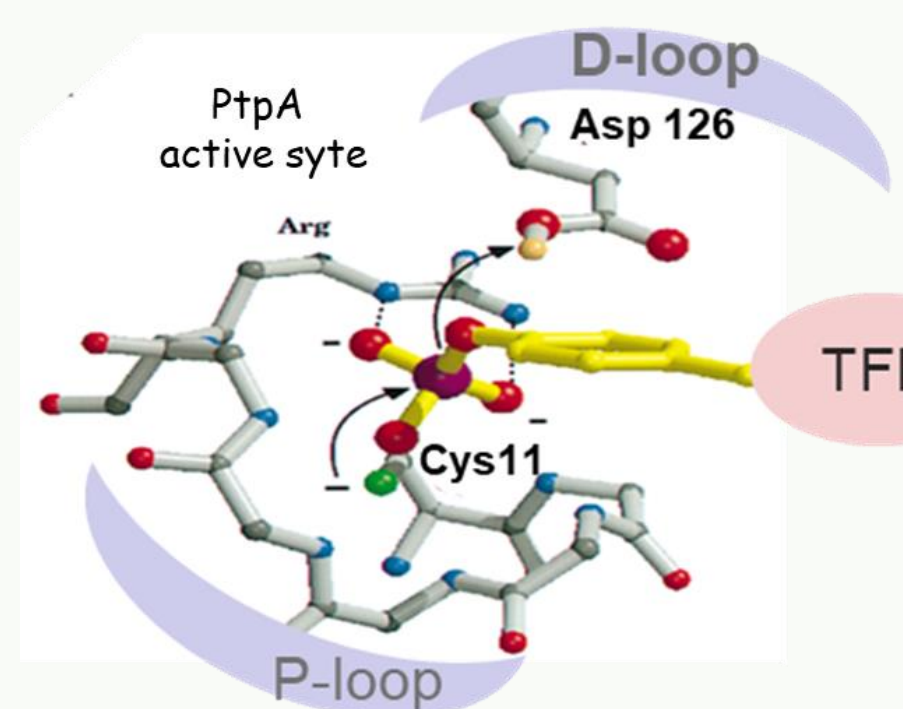
Sequence	240	250	260	270	280	290
ECHA_HUMAN	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI
M. canettii	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI
M. bovis	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI
M. bovis BCG	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI
M. microti	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI
M. tuberculosis	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI
M. tuberculosis	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI	ELLEEVITFARKGLADKKI

- p-Tyr 271 detected by MS
- P-Tyr 271 is located in the Helix-10 of hTFP, a helix required for the correct mitochondrial membrane interaction and total TFP activity (Chuanwu Xia et al, PNAS 2019; 116:13:6060-6074)

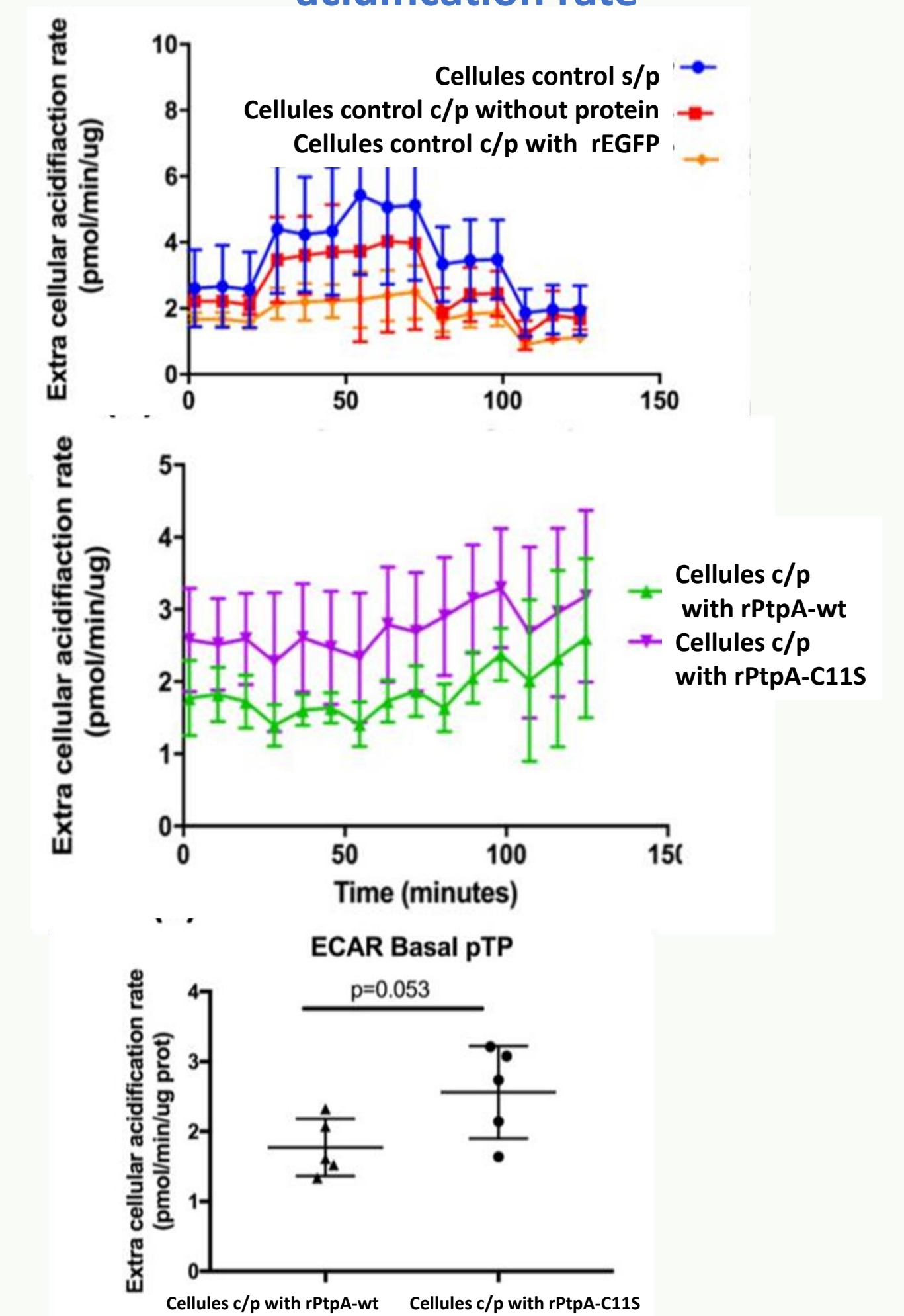
In vitro PtpA dephosphorylates hTFP



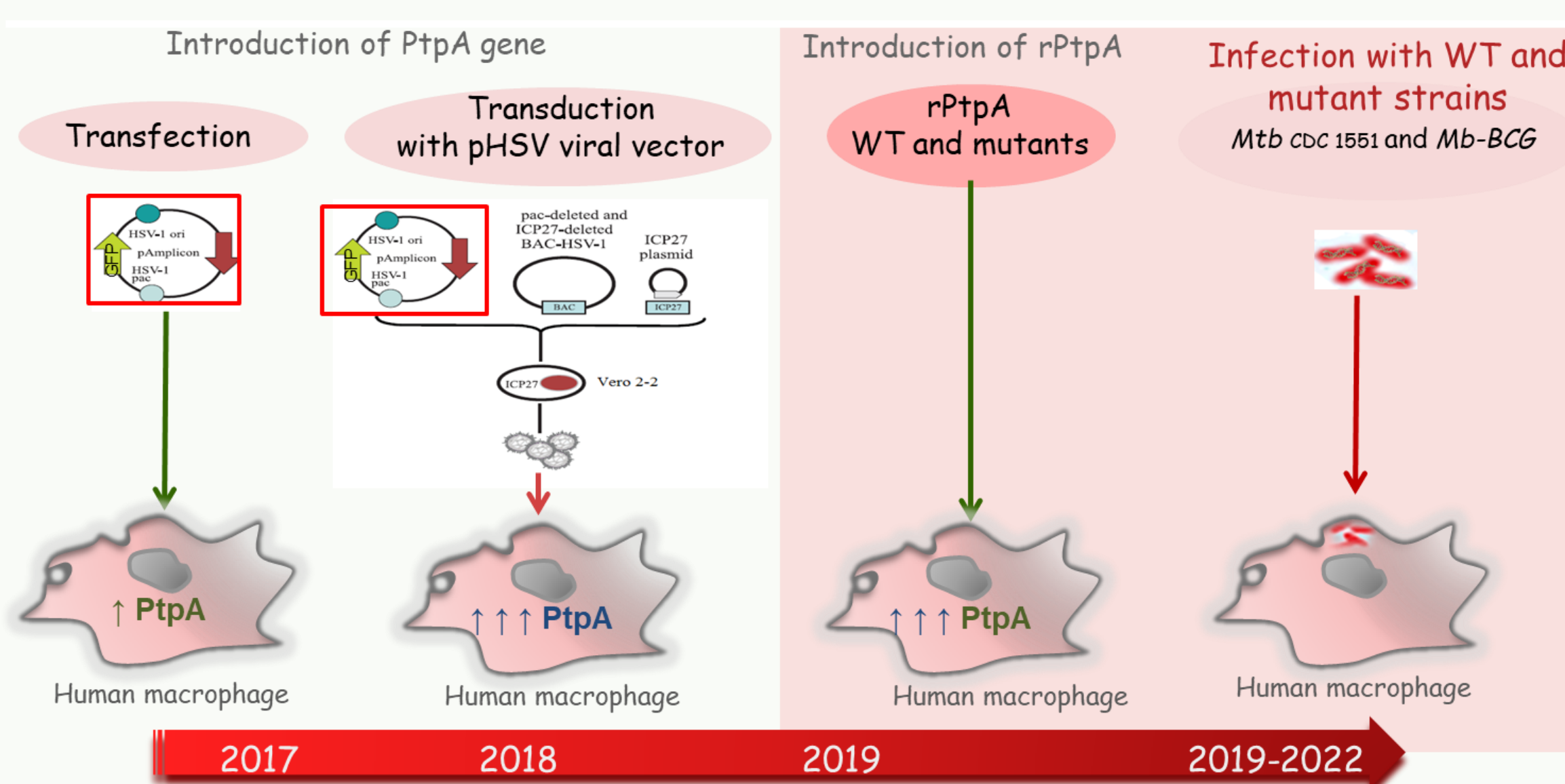
- hTFP dephosphorylation was dependent on PtpA dose



- In THP1-cells containing PtpA-wt but not the inactive mutant PtpA-C11S we observed a decrease in the extracellular acidification rate



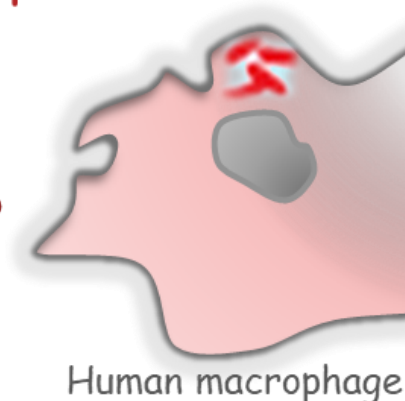
What occurs in macrophages?



CHALLENGES

Using the infection model
we attempt to answer these questions

Is the hTFP activity or
localization affected by PtpA?



PtpA activity correlates with
glycolysis inhibition acting on
PFK (K6PP) potential
substrate?

PtpA correlates with
FAs/TAGs accumulation in
the cytosol?

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