Ruolo della Protein Tirosin Phosphatasi-ε nella modulazione del fosfoproteoma del globulo rosso murino

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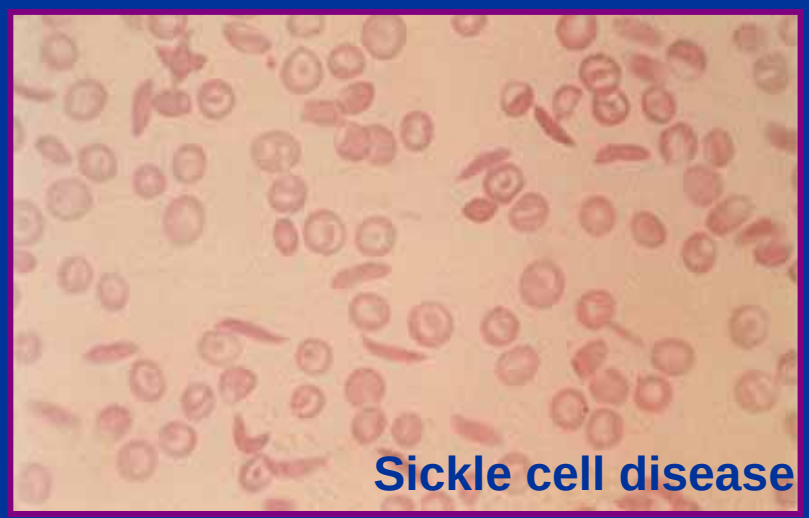
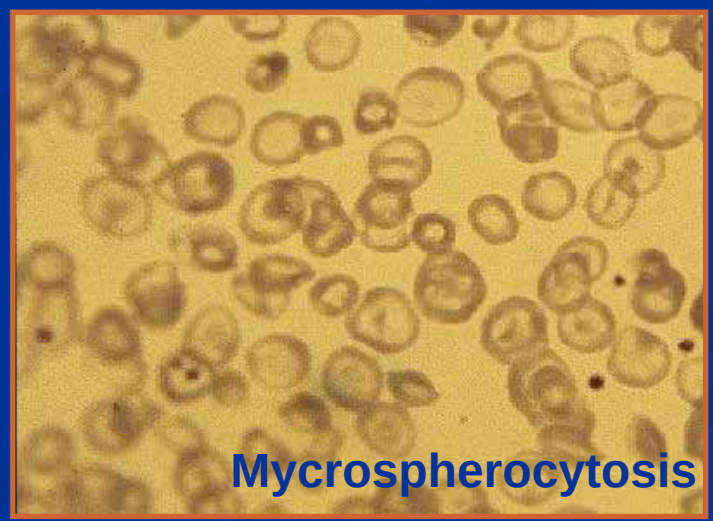
In red cells, the volume and the hemoglobin concentrations are dependent on the ions, water and hemoglobin content





In red cells, ion and water content are regulated by the activity of different membrane ion transport pathways and channels

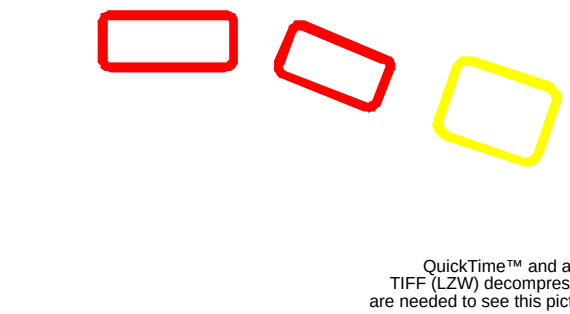


Fig. 1: Schematic diagram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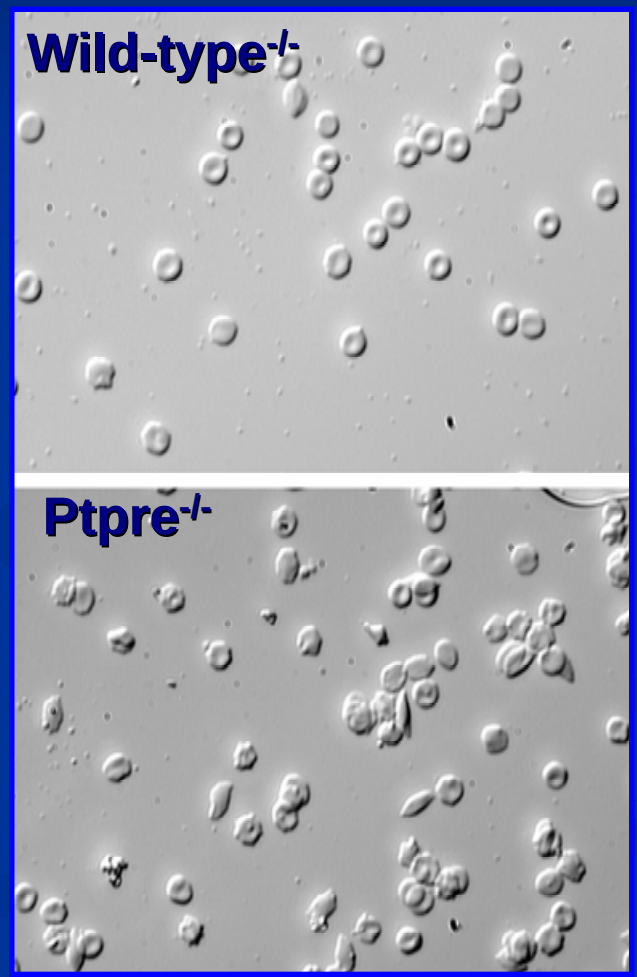
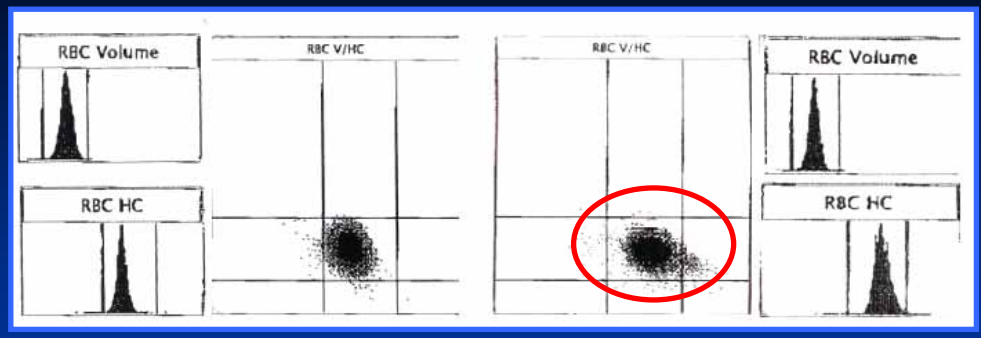
Red cells features in a mice lacking protein tyrosine phosphatase epsilon (Ptpre-/-)

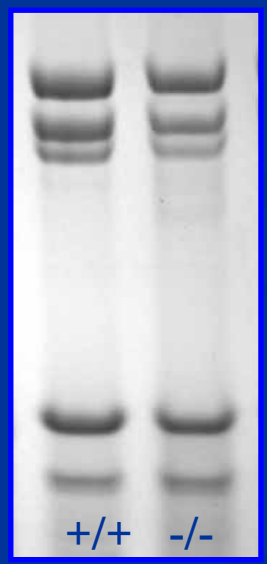
Ptpre$^{-/-}$ mice showed abnormal red cells (RBCs) morphology, characterized by micro-spherocytic acanthocytic, ovalocytic and fragmented red cells.



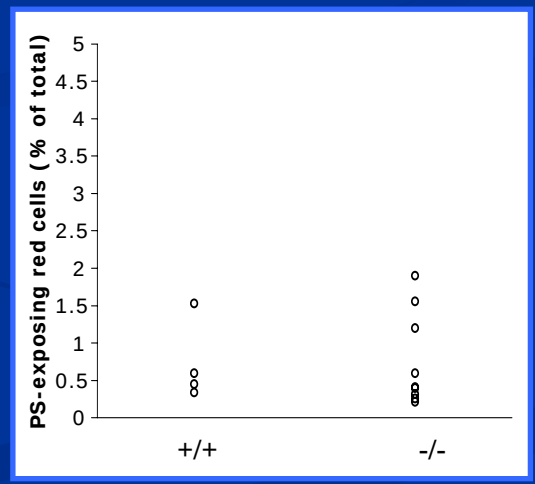
- Ptpre-/- red cells distribution was rightward shifted indicating the presence of dense, dehydrated red cells



- Red cell membrane proteins expression was similar in wild-type and Ptpre$^{-/-}$ mouse strains



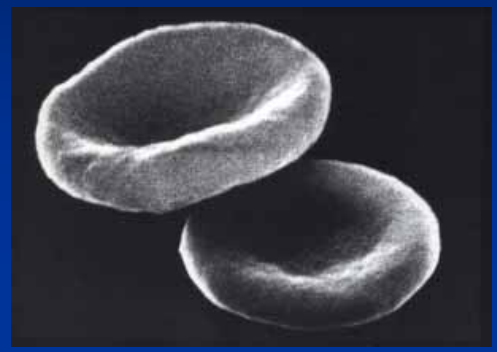
- No differences in the percentage of red cells exposing phosphatydilserine (PS) was present between wild-type and Ptpre $^{-/-}$ mice.

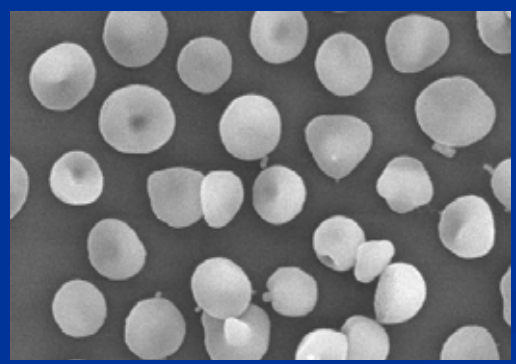


Mechanisms of red cells dehydration

Red cell dehydration is related to abnormalities in the activites of

- K-Cl cotransport
- Ca^{2+} activated K^{+} channel (Gardos channel)
- Cl^{-}conductance

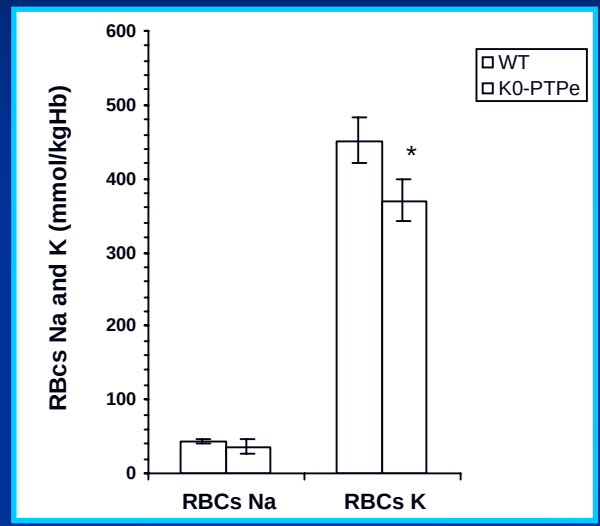


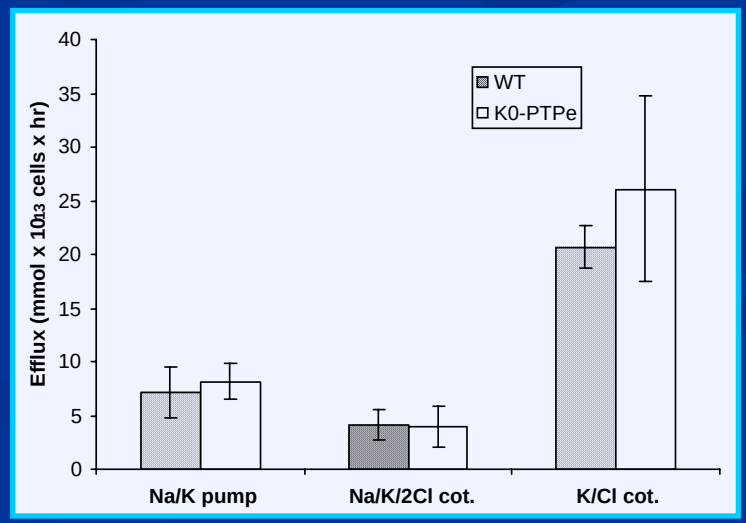


RBCs ions content and main cation transport pathways

- **The RBCs K^+ content is significantly decreased in Ptpre$^{-/-}$ mice** compared to wild-type mice (*P*<0.05; *n*=20).

- No differences in red cell **Na/K ATPase pump, Na/K/2Cl cot and K/Cl cot** maximal rates are present between the two mouse strains.

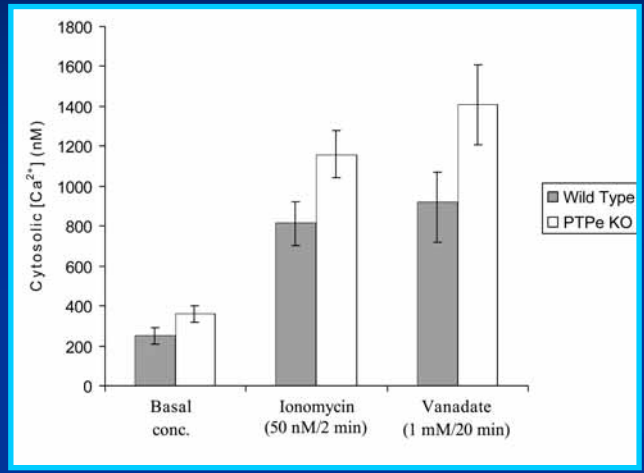


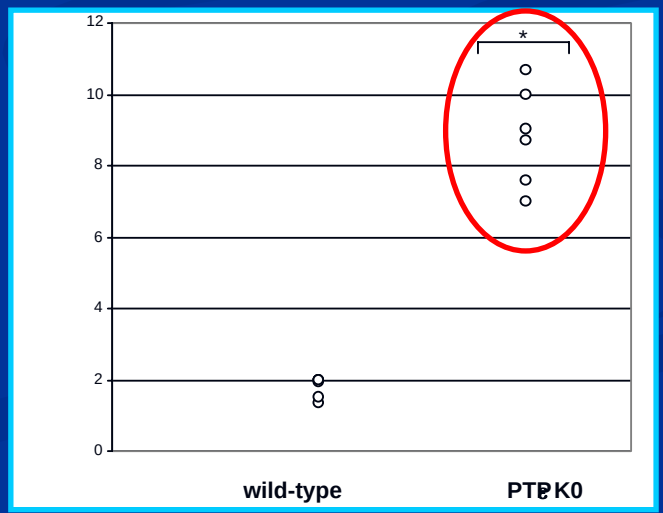


Gardos channel activity in Ptpre-/- mouse red cells

- **Red cell $[Ca^{2+}]_i$ is higher in Ptpre$^{-/-}$than in wild-type mouse**

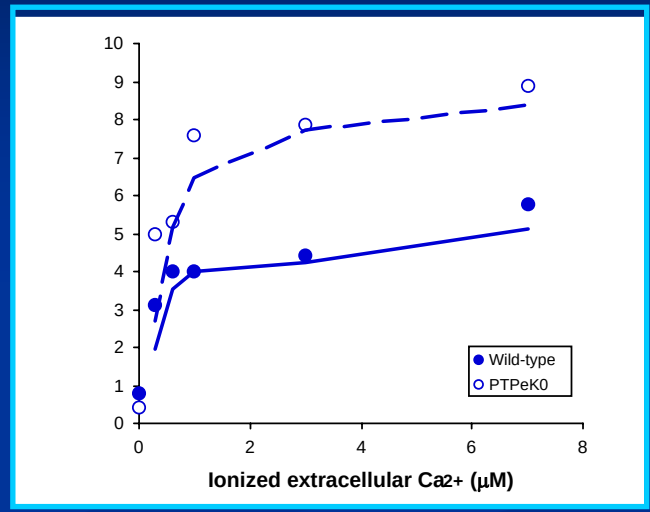
- The **Ca^{2+} activated K^+ channel** activity is significantly increased in Ptpre$^{-/-}$ mouse red cells compared to wild-type erythrocytes (1.7 ± 0.2 vs 8.8± 1.3 mmol/L cell x min; ***P***<0.05; *n*=6).





Functional Characteristics of the Gardos Channel in Ptpre$^{-/-}$ Mouse Red Cells

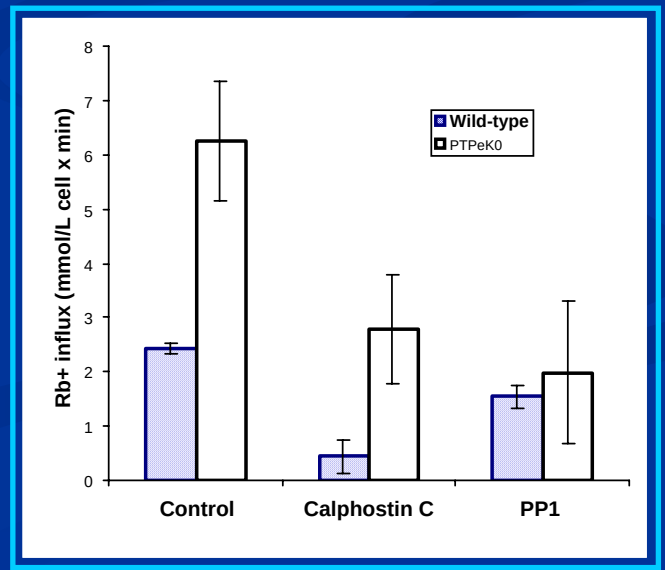
- In Ptpre$^{-/-}$ red cells, the flux kinetic analysis of the Gardos channel activity, shows increased V_{max} and decreased Ca^{2+} $K_{0.5}$ compared to wild-type mice



- In both Ptpre$^{-/-}$ and wild-type mouse red cells, the Gardos channel activity is significantly inhibited by:

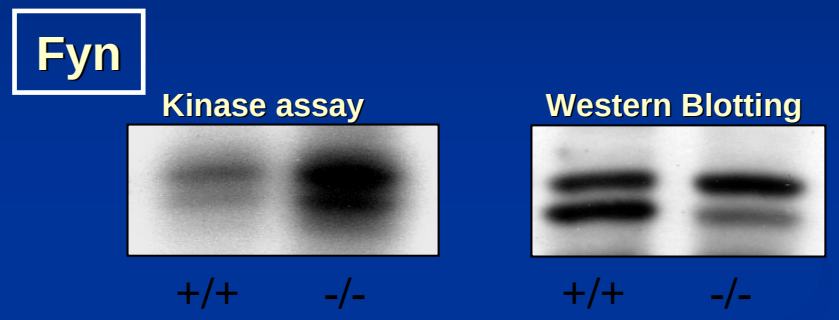
  PKC inhibitor: Calphostin C (10 μM)

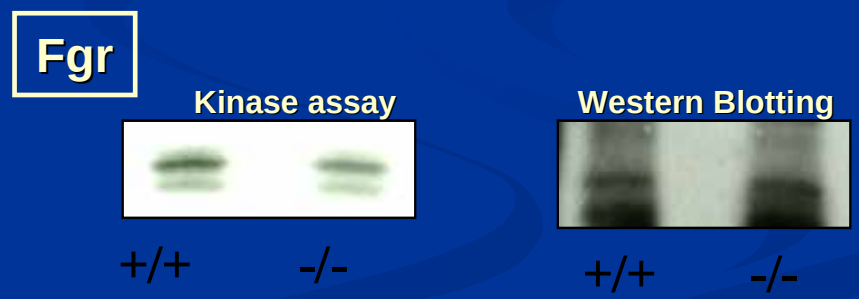
  Src kinase inhibitor: PP1 (10 μM)



Src-family kinase activity in Ptpre-/- mouse red cells

- Src-family kinases have been involved in functional regulation of Ca^{2+} channels in various cell types
- Src-family kinase Fgr and Fyn are similarly expressed in wild-type and Ptpre-/- red cells
- Fyn kinase activity is increased in Ptpre-/- red cells compared to wild-type

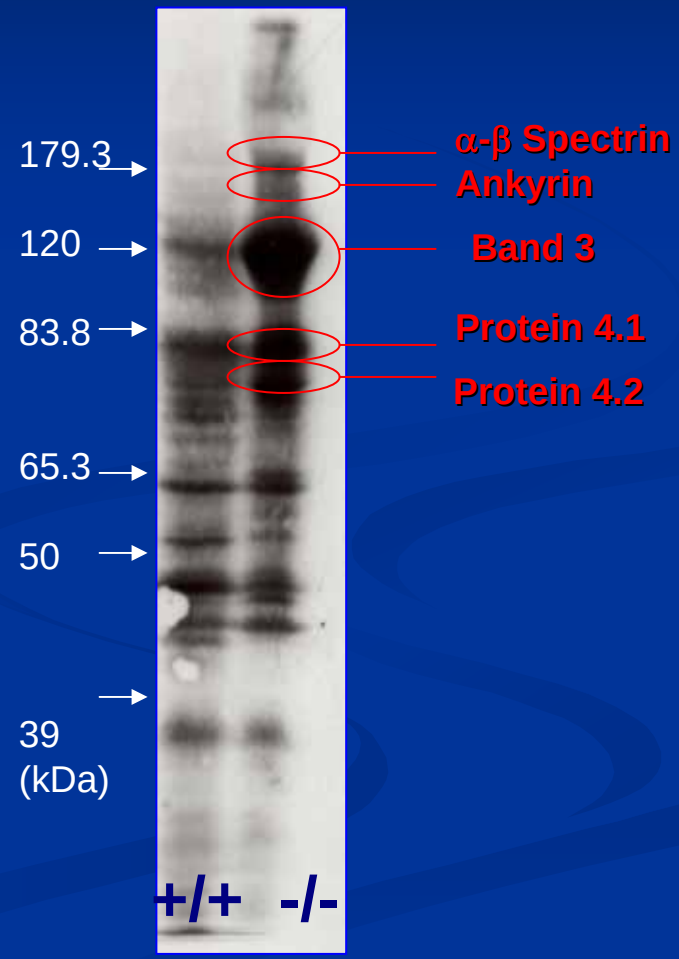




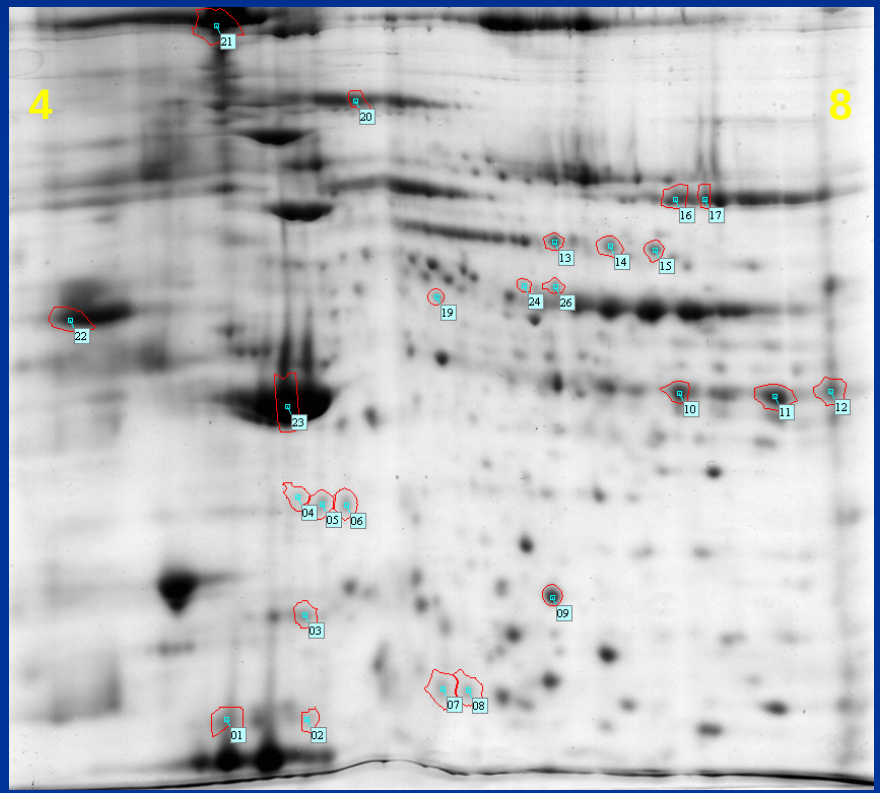
Inhibition of PTPs and red cell membrane proteins tyrosine phosphorylation pattern

- In vitro inhibition of PTPs by Na vanadate affects red cell membrane proteins tyrosine phosphorylation state

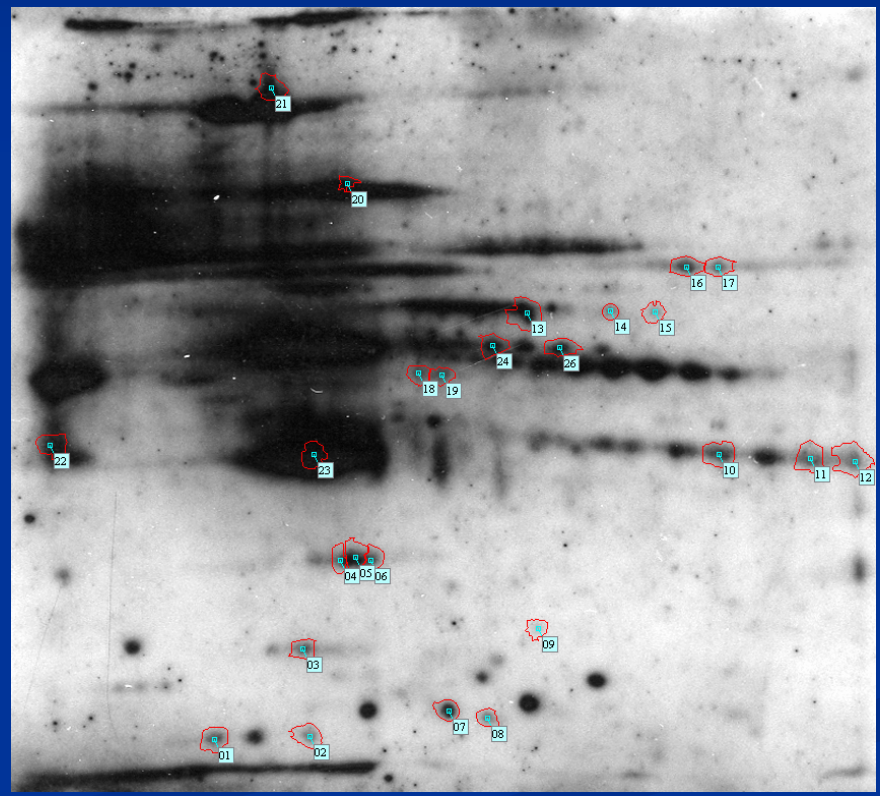
- Red cells lacking PTP-ε show a different membrane proteins tyrosine phosphorylation pattern compared to wild-type mouse erythrocytes.



2-DE and Western-blotting analysis of red cell membrane protein tyrosine phosphorylation pattern

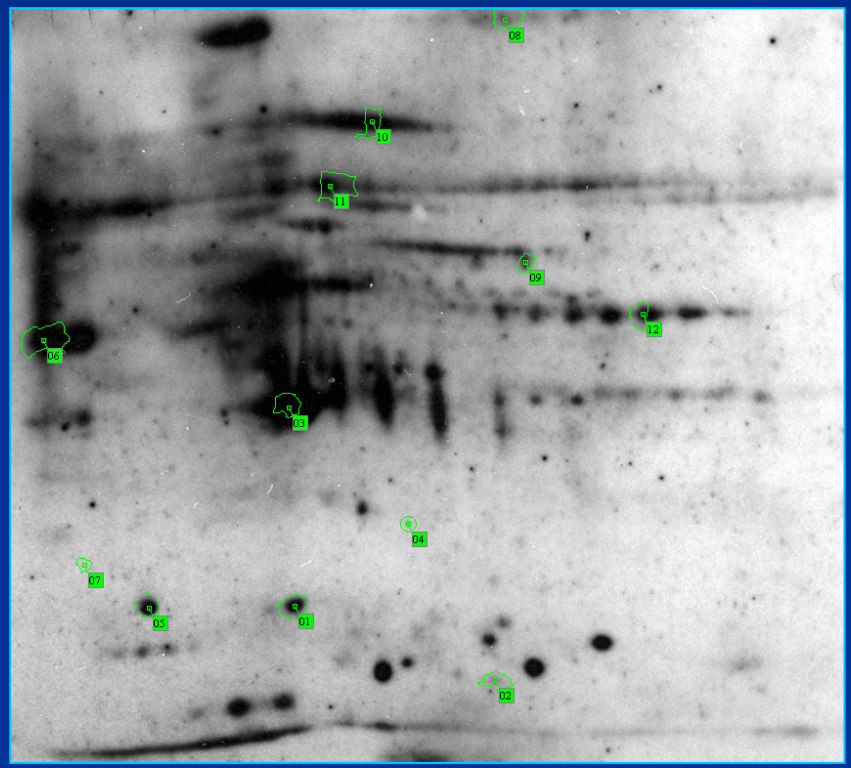


2-DE

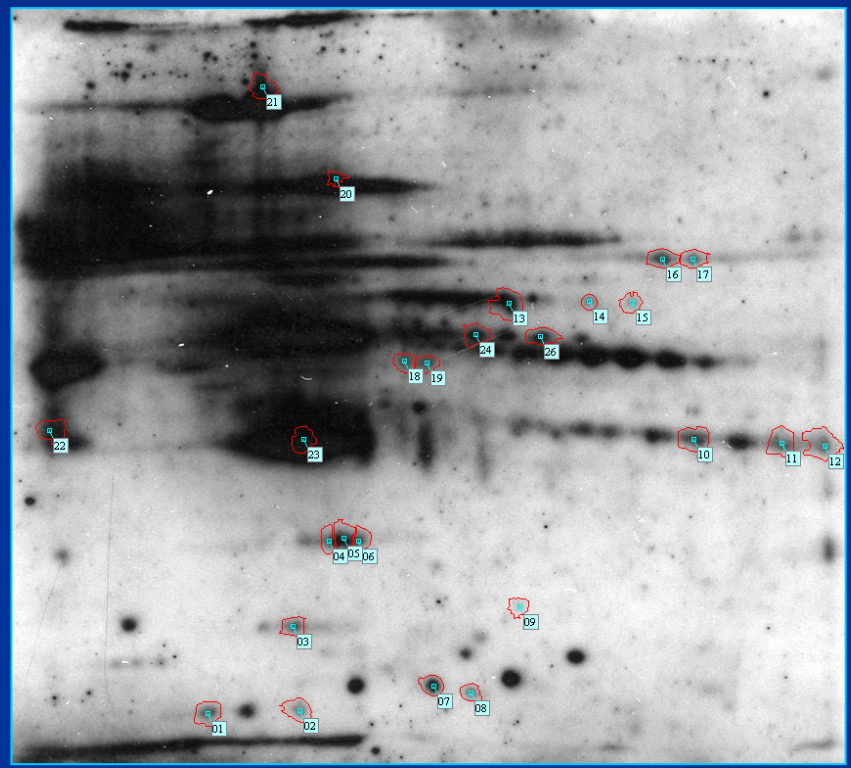


Western-blotting

Membrane Tyrosine Phosphorylation Pattern in Red Cells lacking PTP-ε



Wild-type

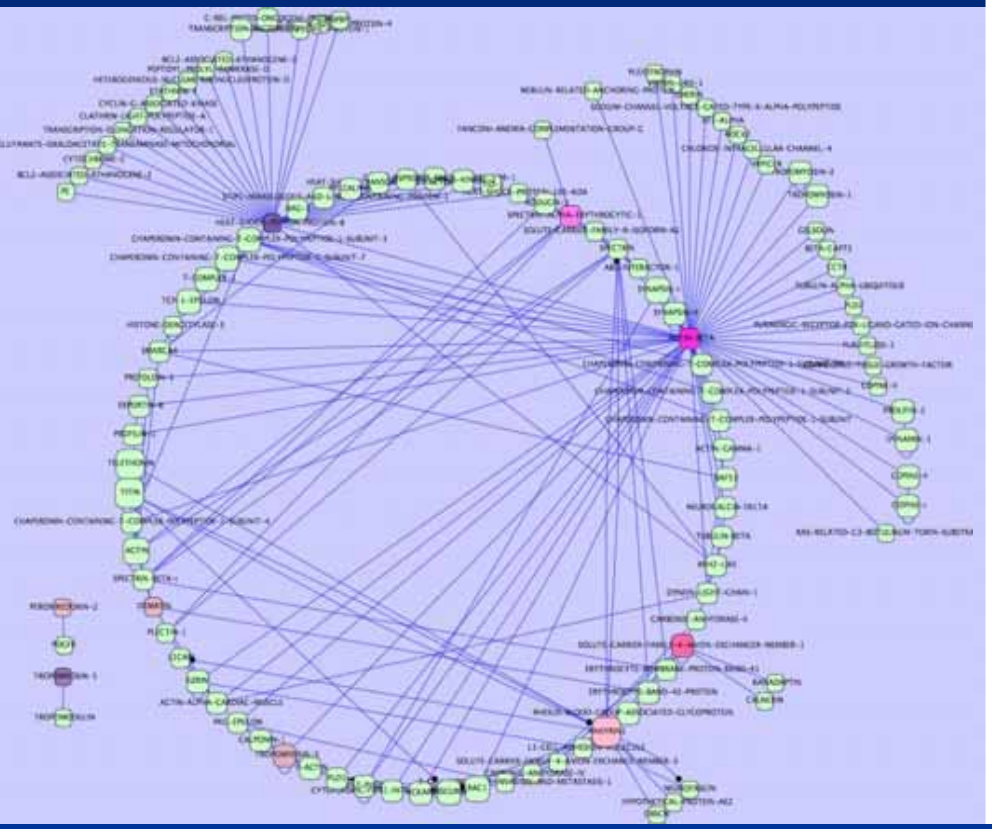


Ptpre-/-

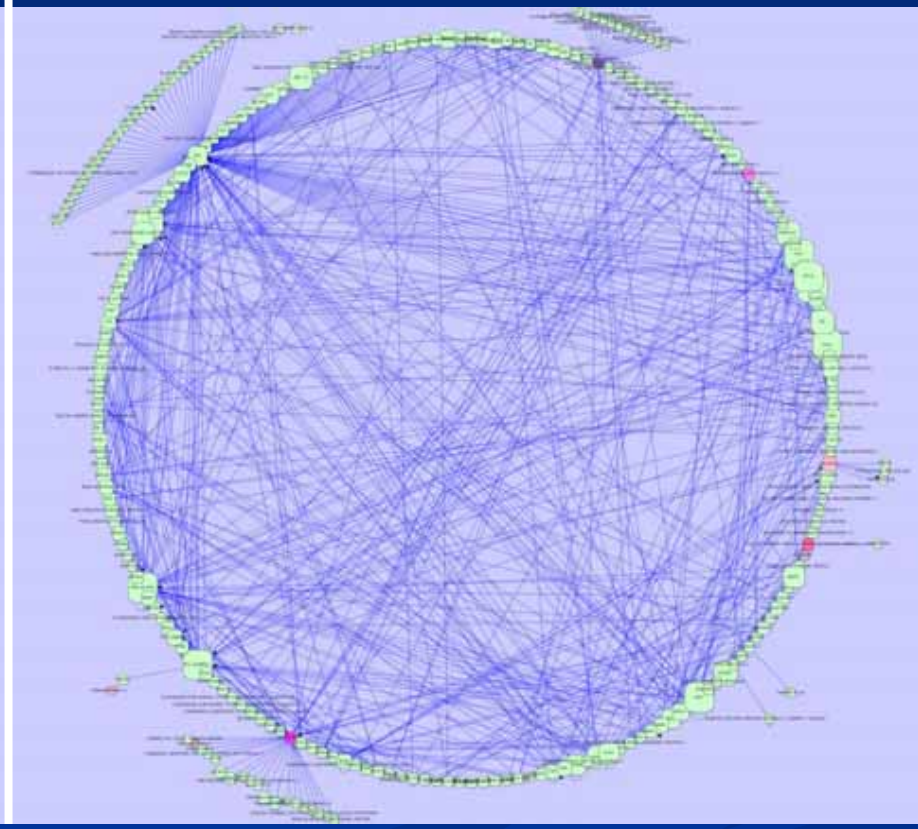
Identification of red cell membrane proteins presenting different tyrosine phosphorylation state by MALDI-TOF analysis

#	Protein ID	Protein Name	Matching peptide	Coverage (%)	Tyr- P state (arbitrary units)
Wild-type					
1	gi53043	Erythrocyte membrane protein band 3	10	26	42.2
2	gi6671509	Actin beta	10	34	18.4
3	gi208097509	Tropomyosin 3, gamma	12	27	4.35
4	gi1168457	Erythrocyte ankyrin	18	33	5.74
5	gi22477509	Epb 4.9	15	31	4.4
6	gi19526481	Spectrin alpha 1; alpha spectrin 1 erythroid	11	29	8.8
7	gi476850	Heat Shock Protein 70kDa	11	28	2.3
8	gi2499469	Peroxiredoxine 2	9	36	4.62
9	gi111212	Tropomyosin 5	8	48	3.012
10	gi6678925	Fyn-SrcFfamily Kinase	10	13	3.01
Ptpre-/-					
1	gi53043	Erythrocyte membrane protein band 3	10	33	90.6
2	gi1168457	Erythrocyte ankyrin	12	33	23.07
3	gi19526481	Alpha Spectrin- alpha spectrin 1, erythroid	11	29	20.05
4	gi2256784	Erythrocyte membrane protein 4.1	10	32	20
5	gi809561	Actin, gamma	10	34	18
6	gi485736	Erythrocyte membrane protein 4.2	11	26	18
7	gi6671509	Actin beta	15	31	18.4
8	gi2119258	Beta Spectrin	10	42	18.3
9	gi31419342	Beta adducin	8	28	16.4
10	gi6678925	Fyn-SrcFfamily Kinase	6	18	15
11	gi15488600	Membrane protein palmitoylated	22	34	15
12	gi45592932	Proteosoma beta subunit	12	26	14.8
13	gi22477509	Epb4.9	11	29	10.3
14	gi2458654	Peroxiredoxine 5 related sequence 1	10	48	7.29
15	gi2499469	Peroxiredoxine 2	9	38	3.86
16	gi56385	Heat Shock Protein 8	12	30	5.77

Network analysis in wild-type and Ptpre$^{-/-}$ mouse red cells

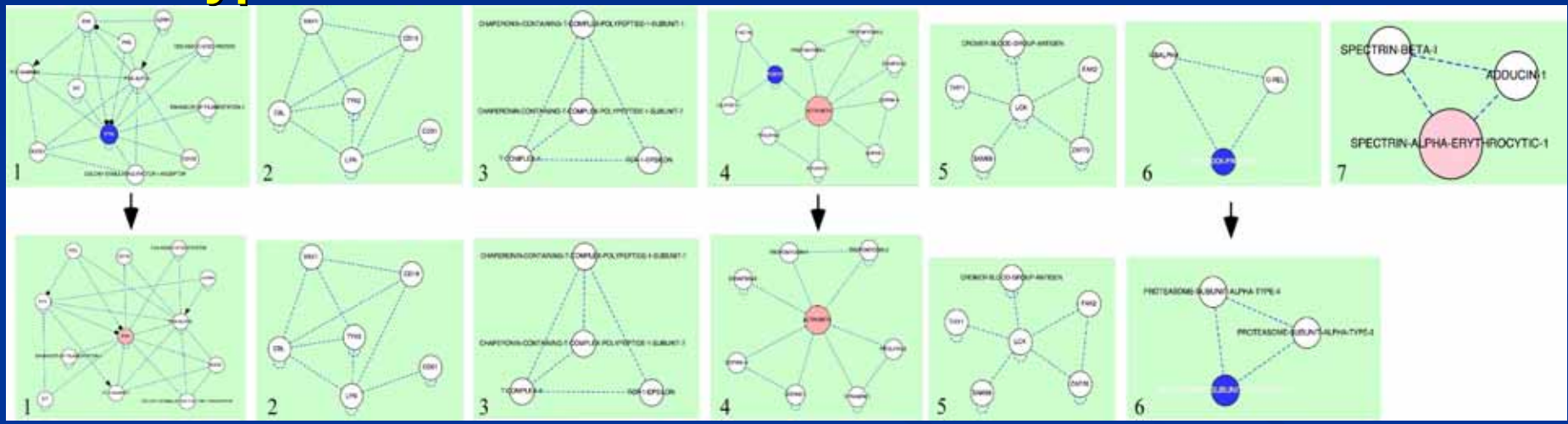


Wild-type



Ptpre-/-

Wild-type +/+



Ptrpe -/-

CONCLUSIONS-I

- Red cells lacking PTPε showed abnormal morphology, reduced red cell K+ content and increase Gardos channel activity
- Gardos channel activity is inhibited by Src-family kinases (SFK) blocker, PP1
- In Ptpre-/- the activity of SFK-Fyn is markedly increased compared to wild-type red cells

CONCLUSIONS-II

- Red cells lacking PTPε showed changes in membrane proteins tyrosine phosphorylation pattern compared to wild-type mouse red cells

- The differences in membrane proteins tyrosine phosphorylation pattern between wild-type and Ptpre-/- mice generated two different signaling sub-networks

- These data indicate the presence of a complex scenario for red cell volume regulation, involving cell signaling pathways through Src-kinase and PTPs.

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