Interplay between PFBC-associated SLC20A2 and XPR1 phosphate transporters requires inositol polyphosphates to control cellular phosphate homeostasis

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Supporting materials:

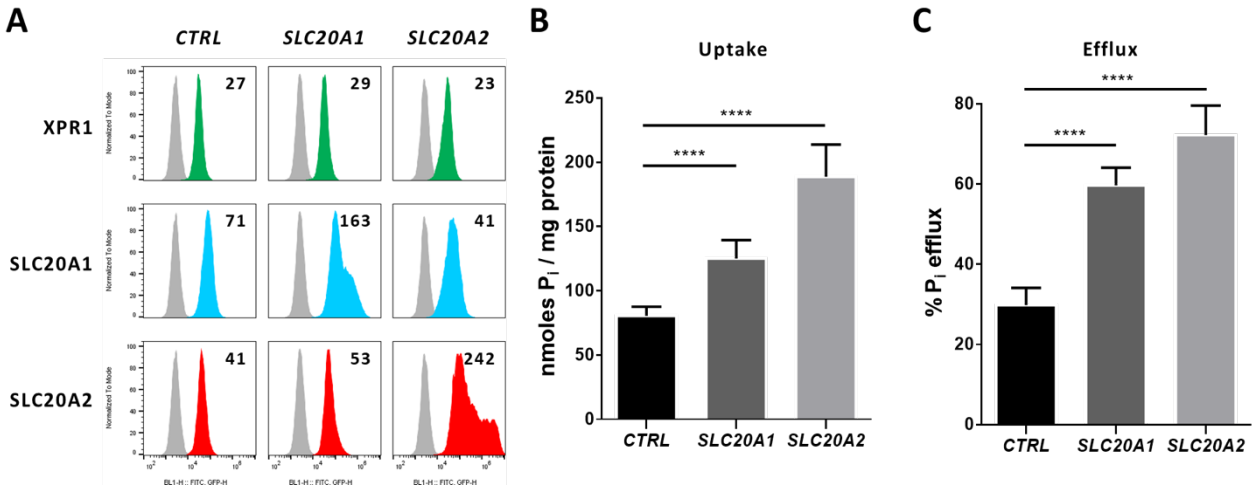
Supporting Fig. S1. SLC20A1 or SLC20A2 phosphate transporters increase both phosphate uptake and efflux activities

Supporting Fig. S2. XPR1 mutated in the lysine surface cluster (XPR1 KSC) is a functional and specific receptor for xenotropic-MLV (X-MLV) infection.

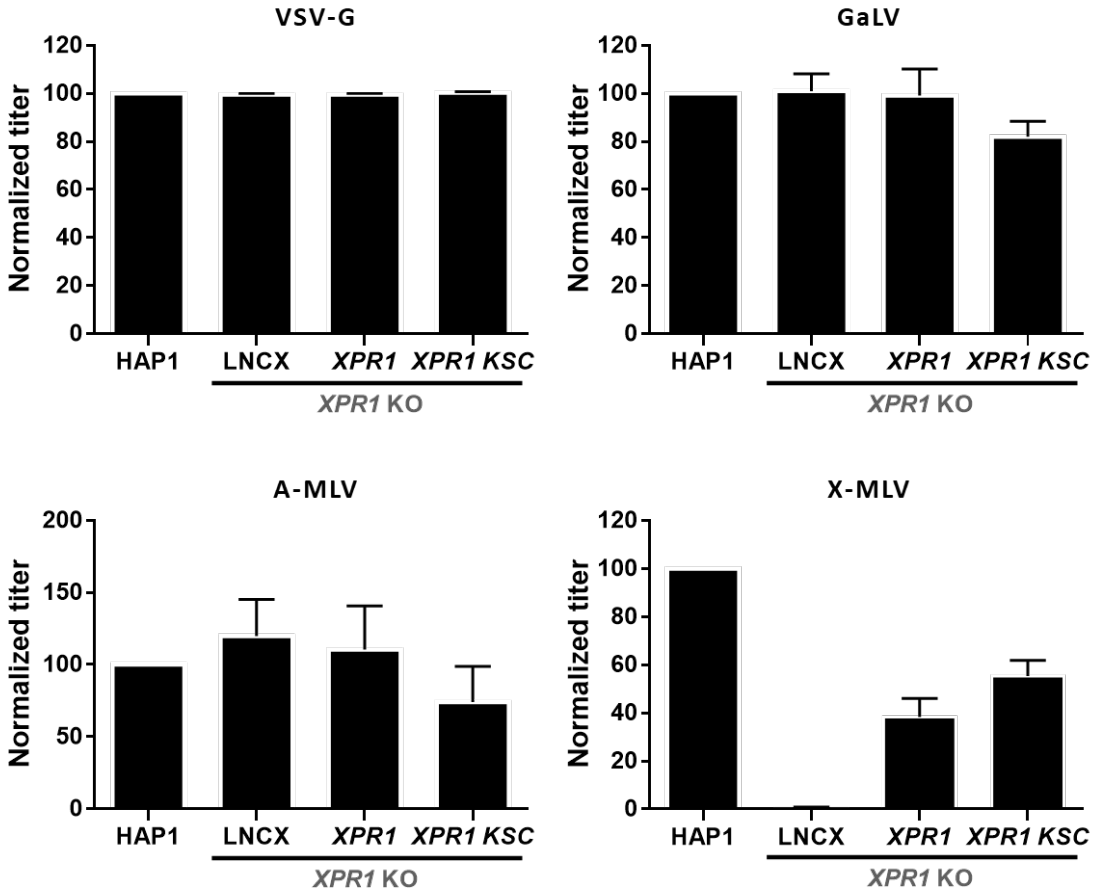
Supporting Fig. S3. Knockout of XPR1 alters levels of inositol polyphosphates

Supporting Fig. S4. Modulation of extracellular phosphate concentration alters inositol polyphosphates

Supporting Fig. S5. TNP treatment on IP6K DKO cells.



Supporting Fig. S1. SLC20A1 or SLC20A2 phosphate transporters increase both phosphate uptake and efflux activities. HEK293T cells transfected with either the pCHIX empty vector (control, *CTRL*), or expressing *SLC20A1* or *SLC20A2* genes were assayed for **(A)** cell surface expression of SLC20A1 (Blue histograms), SLC20A2 (red histograms) and XPR1 (green histograms) with cognate receptor-binding ligands, respectively. Numbers indicate the specific delta mean fluorescence intensity of a representative experiment (n=3), as compared to non-specific staining with the secondary IgG (grey histograms); **(B)** uptake and **(C)** efflux assays of [^{33}P] phosphate. Graphics show means ± SD from n=3 (uptake) and n=4 (efflux) experiments; ***P≤0.001. One-way ANOVA with Holm-Sidak's multiple comparison test; for data in A, F=89.38 (p<0.0001); for data in B, F=186.1 (p<0.0001).

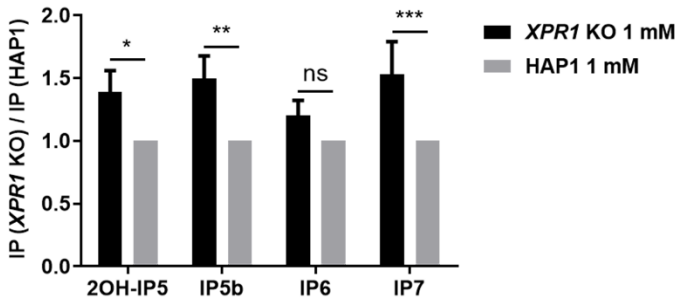


Supporting Fig. S2. XPR1 mutated in the lysine surface cluster (XPR1 KSC) is a functional and specific receptor for xenotropic-MLV (X-MLV) infection. Parental HAP1 WT cells and XPR1 KO derivative expressing empty LNCX retroviral vector or LNCX expressing the WT (*XPR1*) or *XPR1 KSC* genes were challenged with retroviral EGFP vectors pseudotyped with either of the VSV-G protein, the SLC20A1-specific gibbon ape leukemia virus Env (GaLV), the SLC20A2-specific amphotropic-MLV Env (A-MLV), or the XPR1-specific X-MLV Env. Graphics are average values from triplicate infection assays in one representative experiment (n=3).

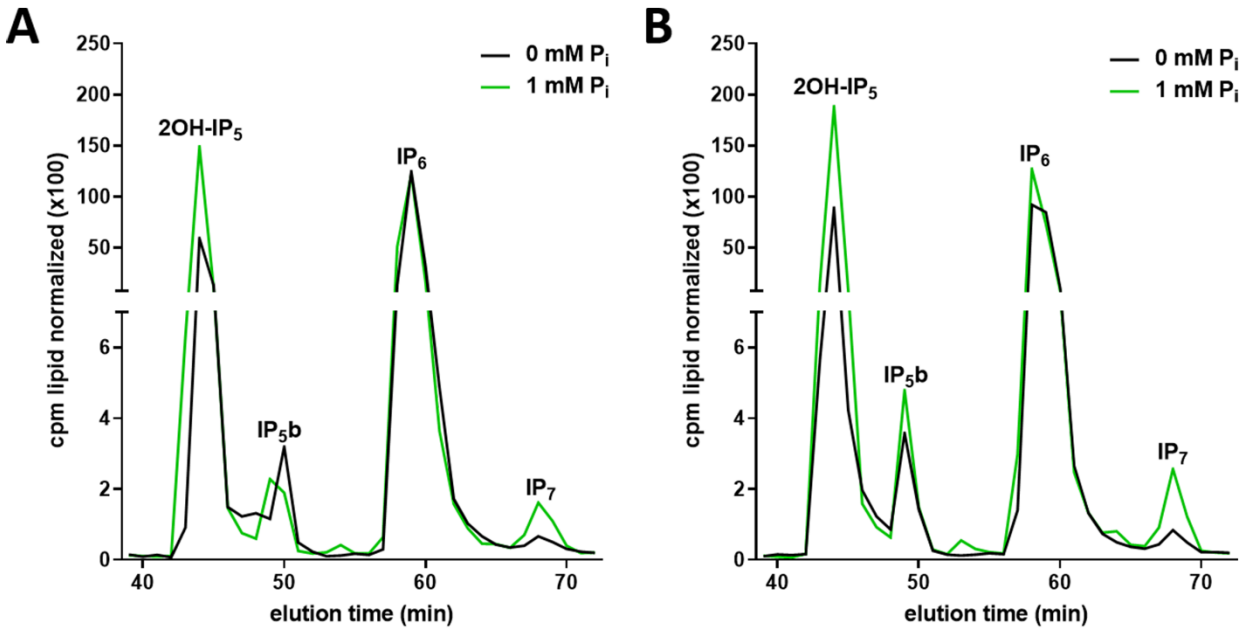
A

	HAP1 1 mM P_i		*XPR1* KO 1 mM P_i	
IP	Mean	SD	Mean	SD
2OH-IP5	135,0	38,8	189,6	1,9
IP5b	4,1	0,3	6,2	0,1
IP6	198,7	20,9	238,7	2,4
IP7	3,5	1,1	5,3	0,1

B



Supporting Fig. S3. Knockout of XPR1 alters levels of inositol polyphosphates. (A) Values were obtained from SAX-HPLC performed on WT and XPR1 KO HAP1 cells grown for 4 days in Myo-[^{3}H]-inositol DMEM. Data show means ± SD of cpm counts normalized to lipids from 3 experiments. A representative HPLC profile is shown in figure 3F **(B)** HPLC data from A were normalized to 1mM phosphate in the indicated HAP1 cells and shown as bar charts. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001; Two-way ANOVA with Sidac's multiple comparison test. F([Pi]) =1.814 (p=0.1851); F(cells)=54.19 (p<0.0001); F(interaction)=1.814 (p=0.1851). ns: non-significant. Pi: inorganic phosphate.

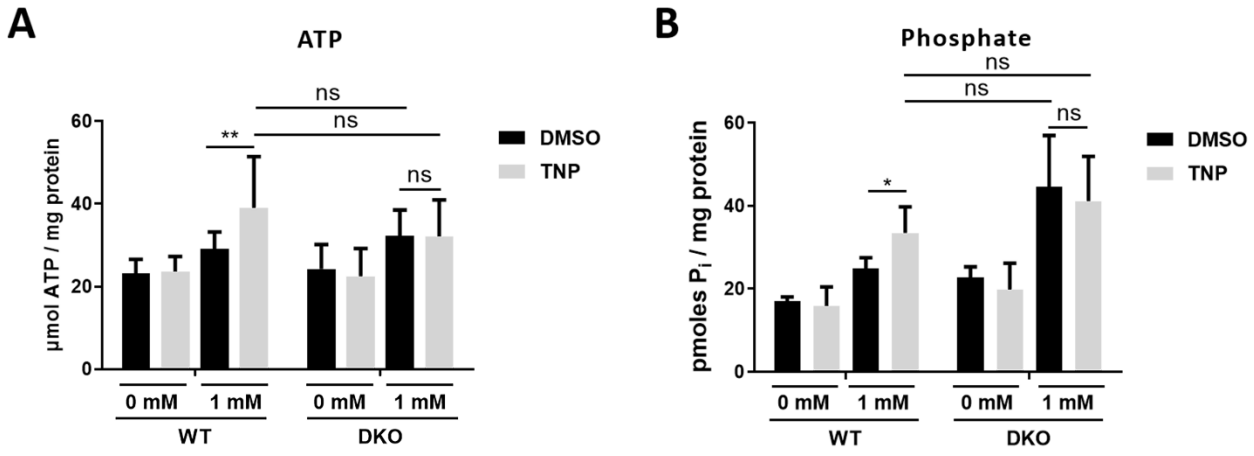


C

	HAP1			
	0 mM P_i		1 mM P_i	
IP	Mean	SD	Mean	SD
2OH-IP5	72,7	10,6	118,9	43,7
IP5b	6,3	2,9	5,5	2,4
IP6	227,2	48,7	220,6	42,0
IP7	2,1	1,2	4,0	1,3

	XPR1 KO			
	0 mM P_i		1 mM P_i	
IP	Mean	SD	Mean	SD
2OH-IP5	96,2	12,6	171,2	63,6
IP5b	6,0	0,6	6,2	0,9
IP6	281,5	63,6	254,8	35,5
IP7	2,6	1,2	5,8	1,3

Supporting Fig. S4. Modulation of extracellular phosphate concentration alters levels of inositol polyphosphates. Representative SAX-HPLC profile obtained on WT **(A)** and XPR1 KO **(B)** HAP1 cells either incubated in 0 mM or in 1 mM phosphate (n=4). **(C)** Comparison of IPs species from experiments performed in A and B. Data are means ± SD of cpm counts normalized to lipids from 4 independent experiments.



Supporting Fig. S5. TNP treatment on IP6K DKO cells. **(A)** Levels of intracellular ATP and **(B)** phosphate in HCT116 parental cells or IP6K DKO cells treated with either DMSO or with 10 µM of TNP for 48h; Data are shown as means ± SD from 6 experiments; *P≤0.05, **P≤0.01. Two-way ANOVA with Tukey's multiple comparison test. For (A), $F(TNP+P_i)=0.5546$ (p=0.458), F(cells)=21,7 (p=0.0001), F(interaction)=2.872 (p=0.0396). For (B), $F(TNP+P_i)=18.21$ (p=0.0001), F(cells)=21.51 (p<0.0001), F(interaction)=3.063 (p=0.0378). ns=not significant