Metabolic tumor cell adaptation: tyrosine phosphorylation modulates cell surface expression of chloride cotransporters NKCC2 and KCC3

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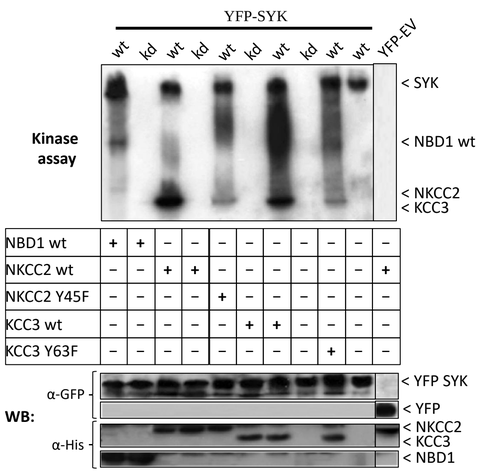
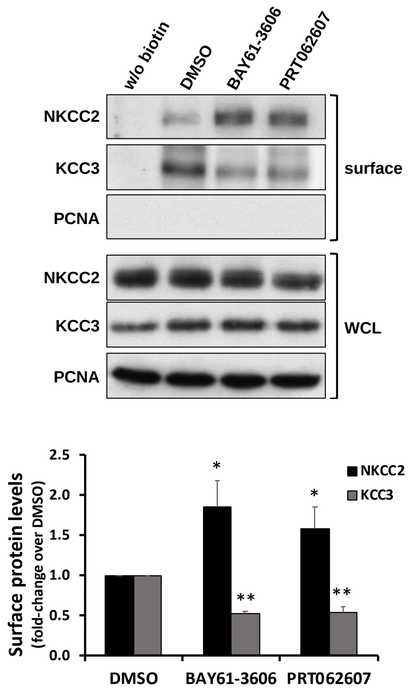
Introduction: Tumor cells require cellular chloride and potassium transport to adapt to a changing microenvironment, both for cell volume regulation and membrane potential maintenance. Cellular chloride and potassium entry or exit are mediated at the plasma membrane (PM) by cotransporter proteins of the solute carrier 12 family. For example, NKCC2 resorbs chloride with sodium and potassium ions at the apical membrane of epithelial cells in the kidney, whereas KCC3 releases chloride with potassium ions at the basolateral membrane. Their ion transport activity is regulated by protein phosphorylation in response to signaling pathways. An additional regulatory mechanism concerns the amount of cotransporter molecules inserted into the PM. **Methods:** Cotransporter constructs were transfected into HEK293 cells and the activity of spleen tyrosine kinase (SYK) modulated by incubation with SYK inhibitors or by cotransfection with siRNAs, kinase-dead, or constitutively active SYK mutants. Cotransporter abundance in the PM was analyzed by biotinylation of cell surface proteins. **Results:** Here, we describe that tyrosine phosphorylation of NKCC2 and KCC3 regulates their PM expression levels. We identified that SYK phosphorylates a specific N-terminal tyrosine residue in each cotransporter. Experimental depletion of endogenous SYK or pharmacological inhibition of its kinase activity increased the abundance of NKCC2 at the PM of human embryonic kidney cells. In contrast, overexpression of a constitutively active SYK mutant decreased NKCC2 membrane abundance. Intriguingly, the same experimental approaches revealed the opposite effect on KCC3 abundance at the PM, compatible with the known antagonistic roles of NKCC and KCC cotransporters in cell volume regulation.

Results

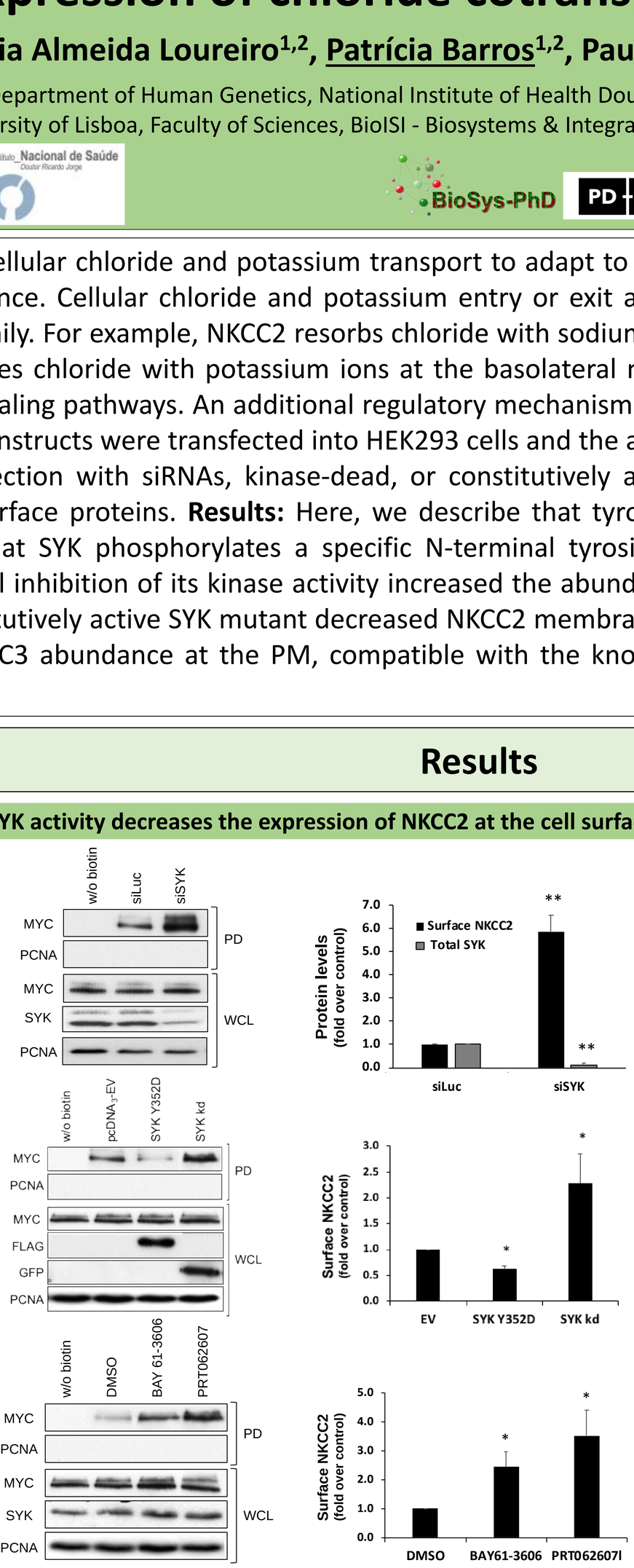
NKCC2 and KCC3 are a substrate for SYK protein kinase



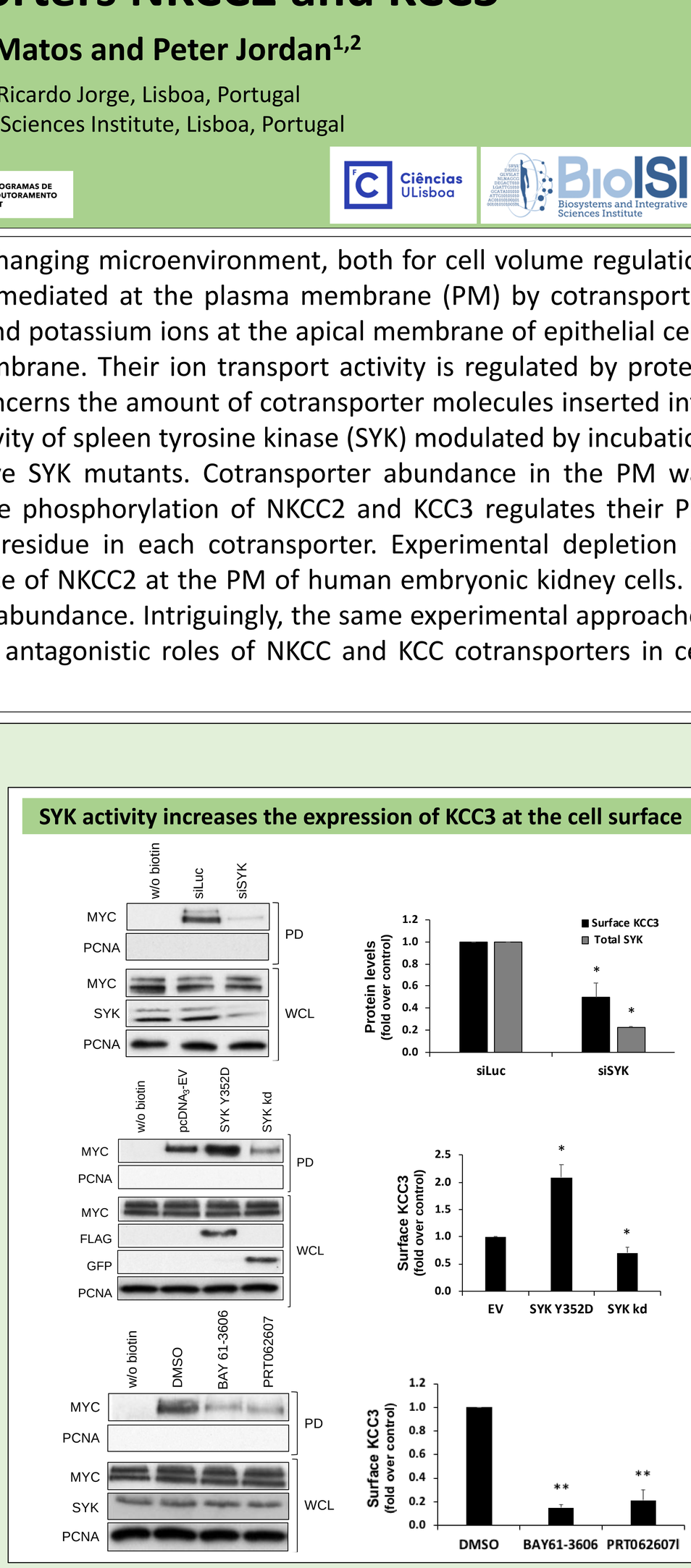
SYK activity modulates the cell surface expression of NKCC2 and KCC3 in opposite ways in the same cell

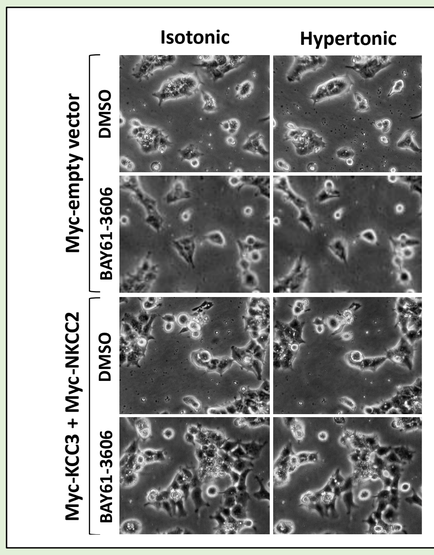


SYK activity decreases the expression of NKCC2 at the cell surface

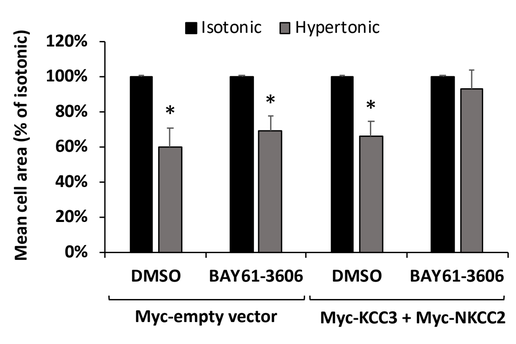


SYK activity increases the expression of KCC3 at the cell surface





SYK activity contributes to cell volume regulation



Conclusions

- ✓ Identification of a novel pathway modulating the cell surface expression of NKCC2 and KCC3 with opposite functional outcomes for the two cotransporters;
- ✓ New insights on how tumor cells may respond to microenvironmental changes that affect their cell volume or metabolic crosstalk.

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