

The role of LRRC8 in the hypotonic stress response of human keratinocytes

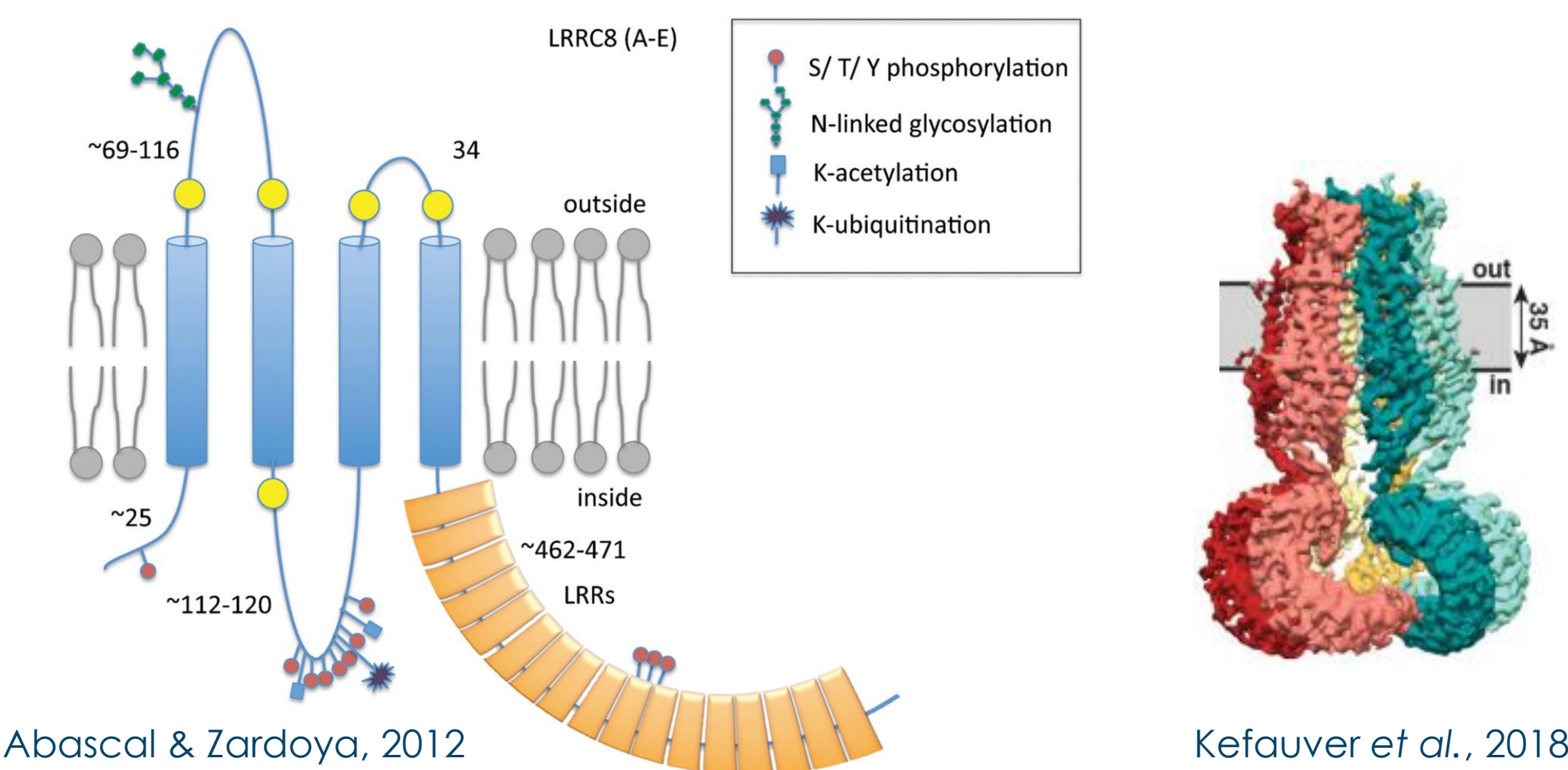
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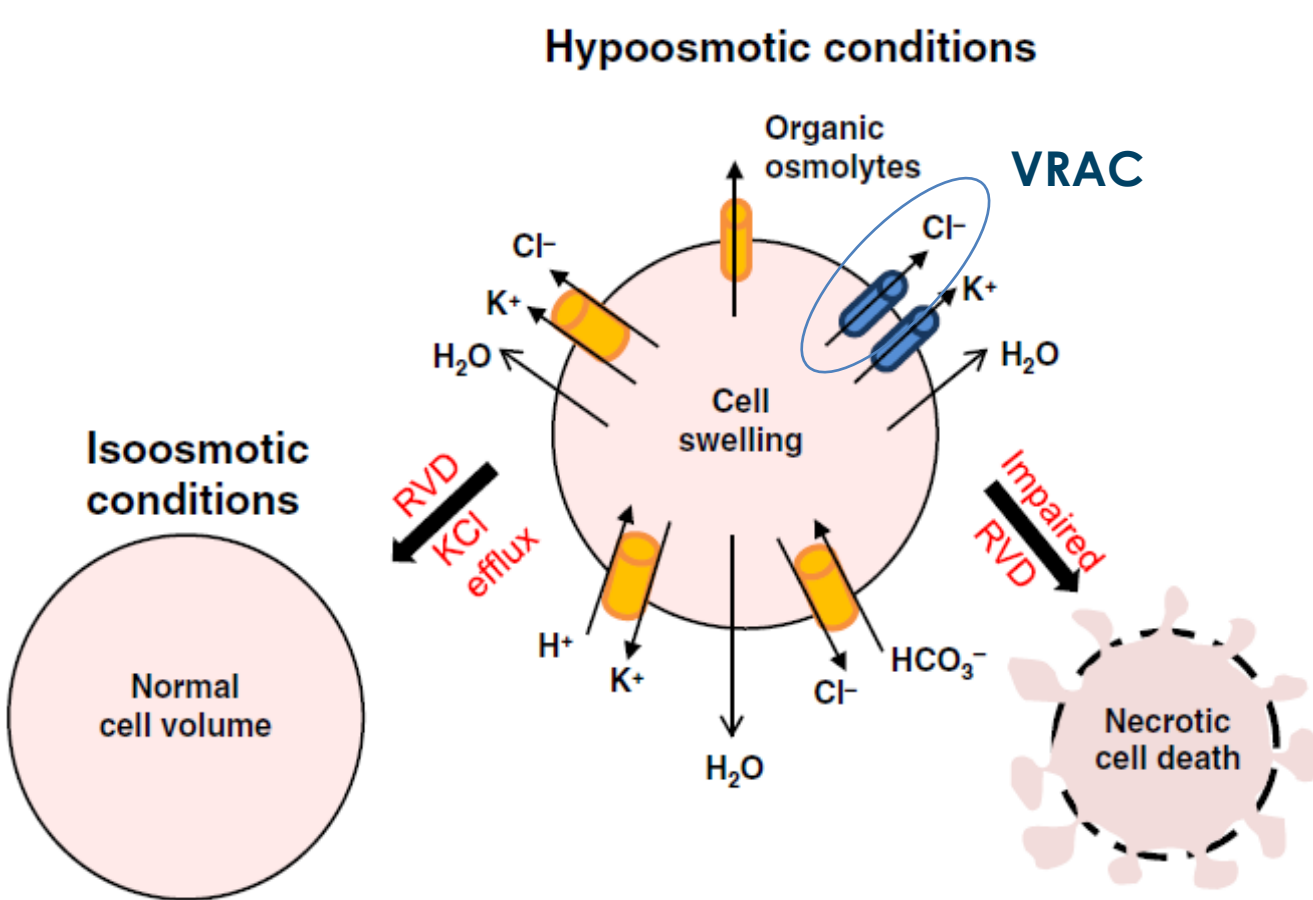
Introduction

The human skin is constantly exposed to various stress factors such as temperature changes, mechanical stress, different humidity levels, air pollution or radiation. These factors can have a tremendous impact on the skin and can contribute to barrier disruption and inflammation, dry and fragile skin as well as premature ageing (Rosso *et al.*, 2016). Recent advances in different research areas point to an important role of LRRC8 volume regulated anion channels (VRACs) in a plethora of different physiological processes (Chen *et al.*, 2019). The function of LRRC8 has been characterized in human keratinocytes and in the native human epidermis (Trothe *et al.*, 2018) and the LRRC8 ion channel has been proposed to be a novel molecular target to modulate keratinocyte differentiation. LRRC8A (also named SWELL1) has been identified as the first essential component of VRACs in various cell types (Qui *et al.*, 2014; Voss *et al.*, 2014). LRRC8A is composed of four transmembrane domains and a C-terminal domain containing up to 17 leucine-rich repeats. Together with four additional LRRC8 family members (LRRC8B-E) it assembles into hetero-hexameric complexes (Qiu *et al.*, 2014; Voss *et al.*, 2014). The LRRC8 subunit composition differs between cell types and influences VRAC properties such as inactivation kinetics, voltage-dependence and selectivity of the transported osmolyte. The generation of LRRC8A^{-/-} knockout HaCaT keratinocytes has provided evidence for the essential function of LRRC8A in hypotonic stress response of human keratinocytes (Trothe *et al.*, 2018).



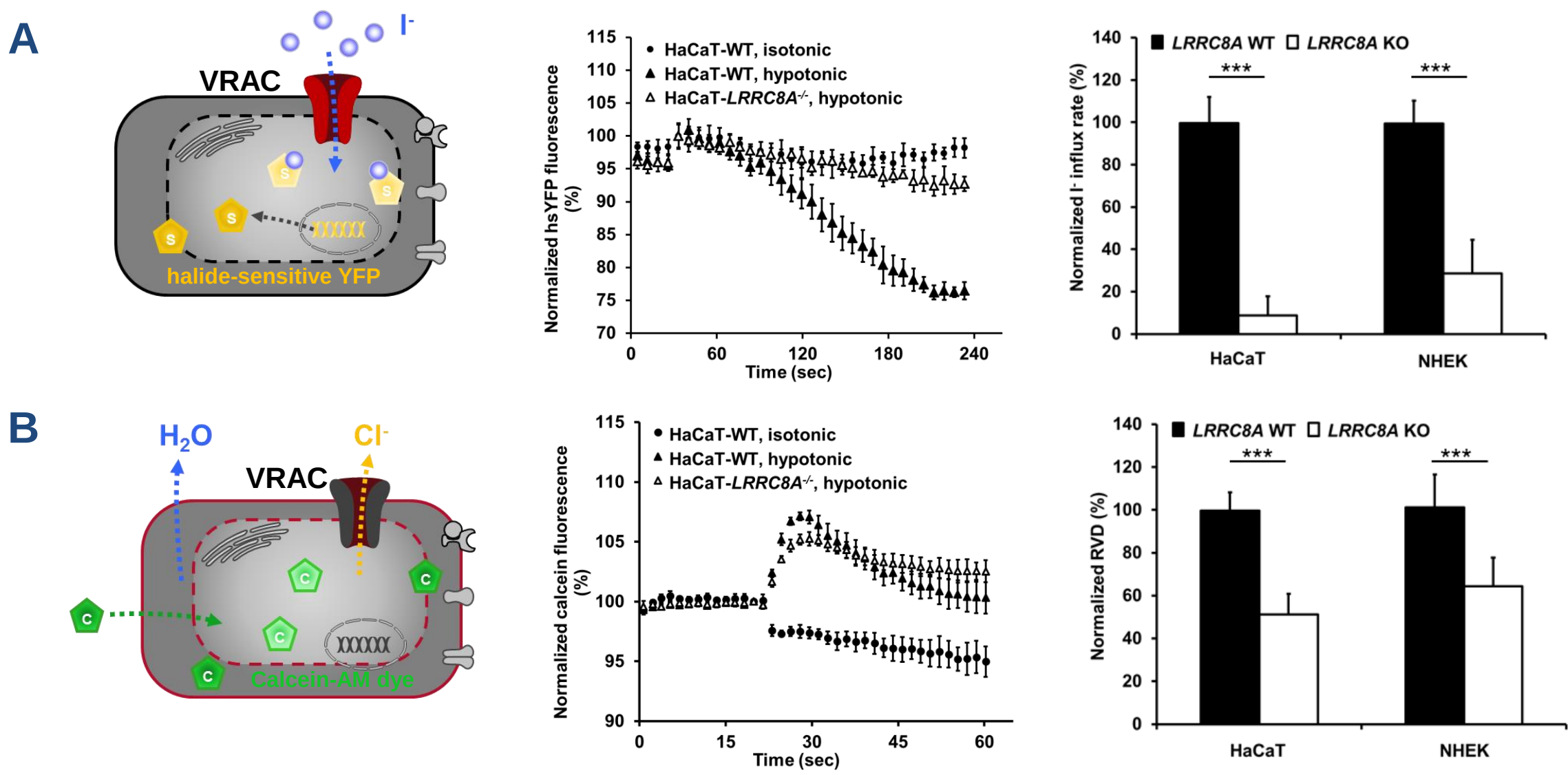
VRAC channels regulate cell volume

Hypoosmotic conditions result in cell swelling, which is counteracted by regulatory volume decrease (RVD) to prevent necrotic cell death. RVD involves activation of volume-regulated anion channels (VRACs), which allow efflux of chloride (Cl⁻). Subsequent efflux of osmotically driven water restores the initial cell volume.



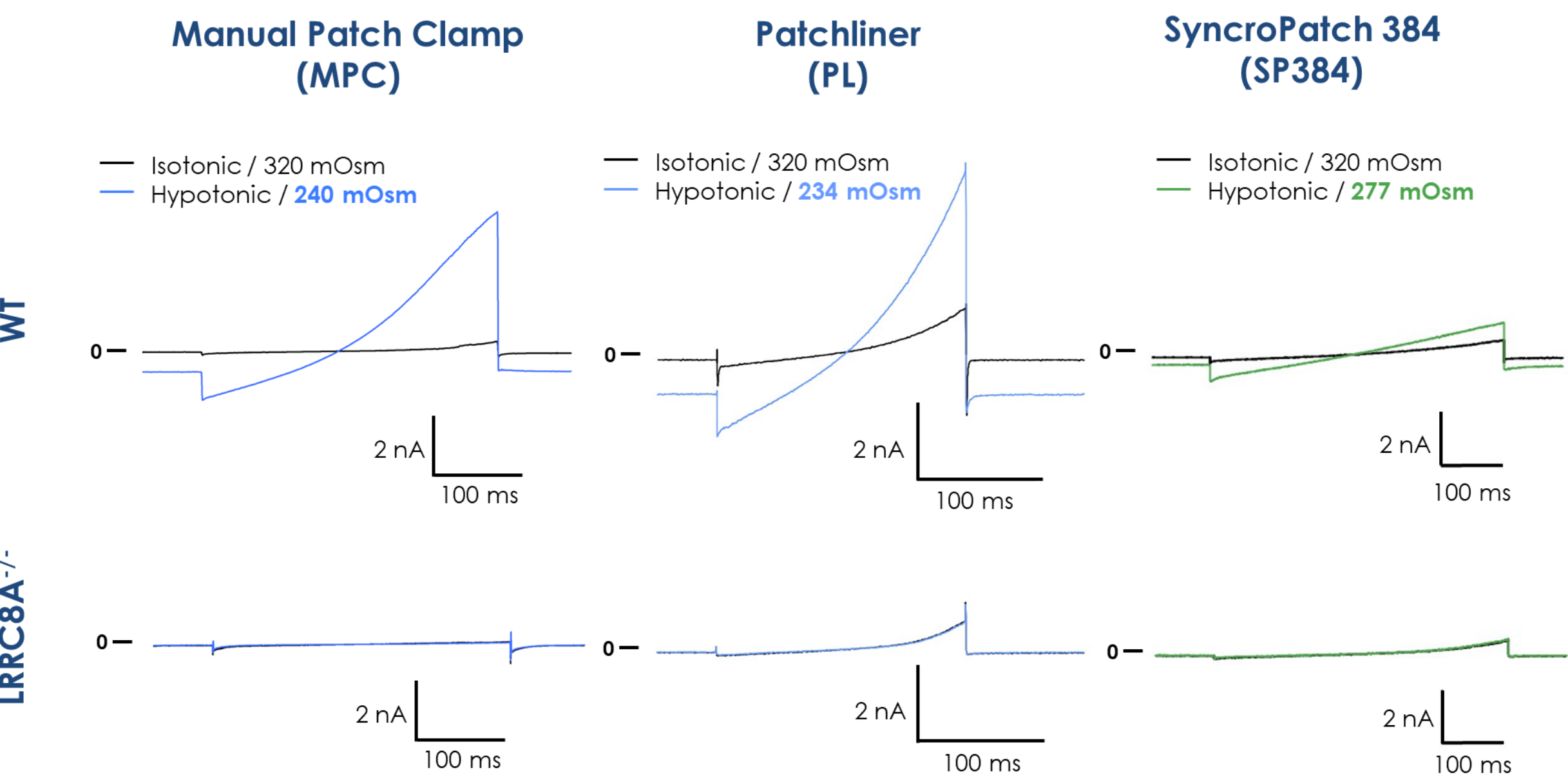
Adapted from El-Chami *et al.*, 2014

LRRC8A mediates VRAC activity and RVD



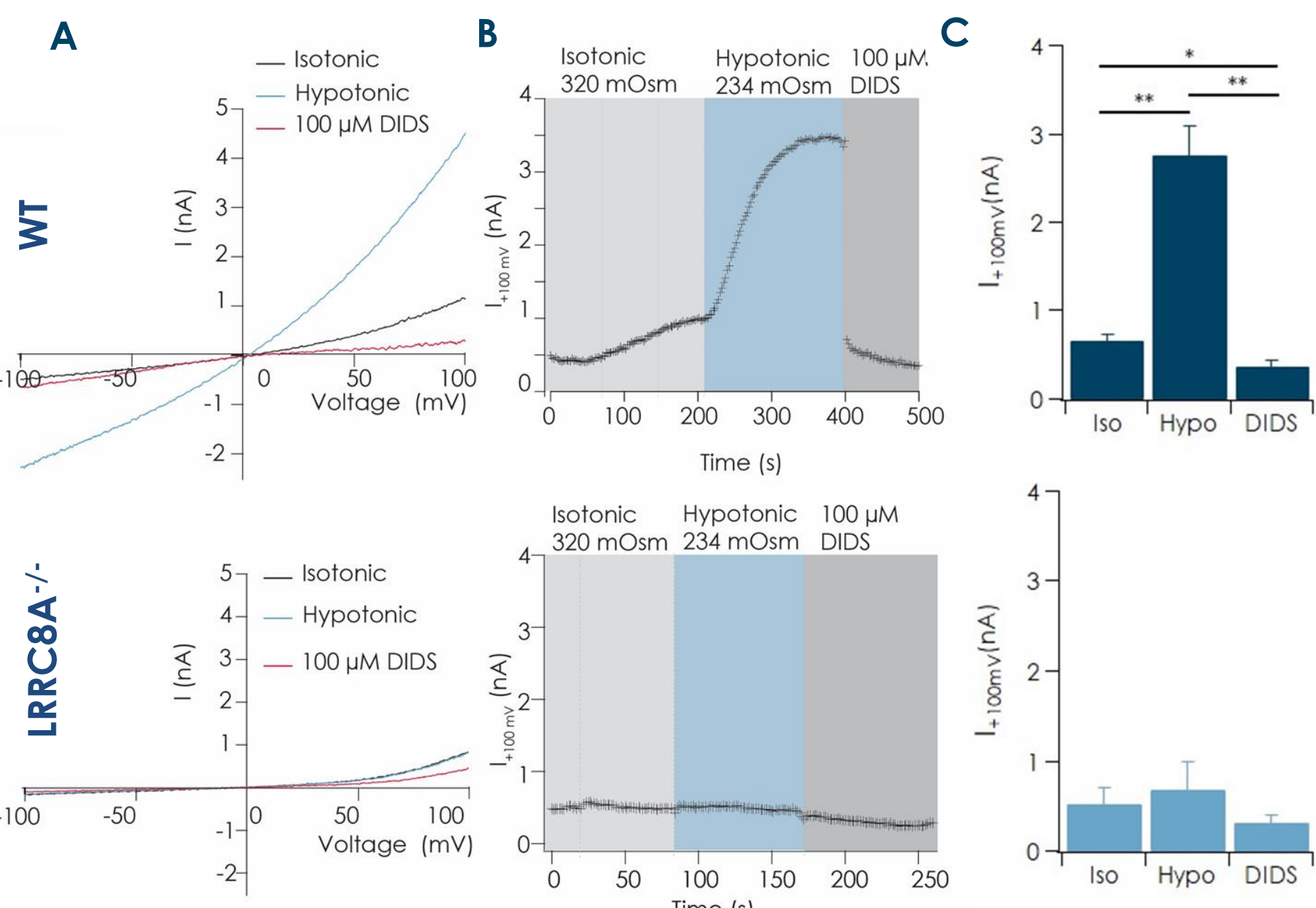
(A) Keratinocytes were engineered to express the I⁻ sensor hYFP. Hypotonic stimulation of HaCaT WT, but not of HaCaT LRRC8A^{-/-} triggered an I⁻-dependent quenching of hYFP fluorescence. VRAC activity was drastically reduced in LRRC8A-deficient HaCaTs as well as NHEKs. **(B)** Keratinocytes were loaded with volume-sensitive dye calcein-AM. Reduction of calcein fluorescence upon hypotonic stimulation of HaCaT LRRC8A^{-/-} cells was reduced compared to HaCaT WT cells. HaCaT LRRC8A^{-/-} and NHEK LRRC8A KO populations showed decreased RVD compared to the respective WT cells (Trothe *et al.*, 2018).

Patch-clamp recordings of HaCaT WT and LRRC8A^{-/-} cells under isotonic and hypotonic conditions



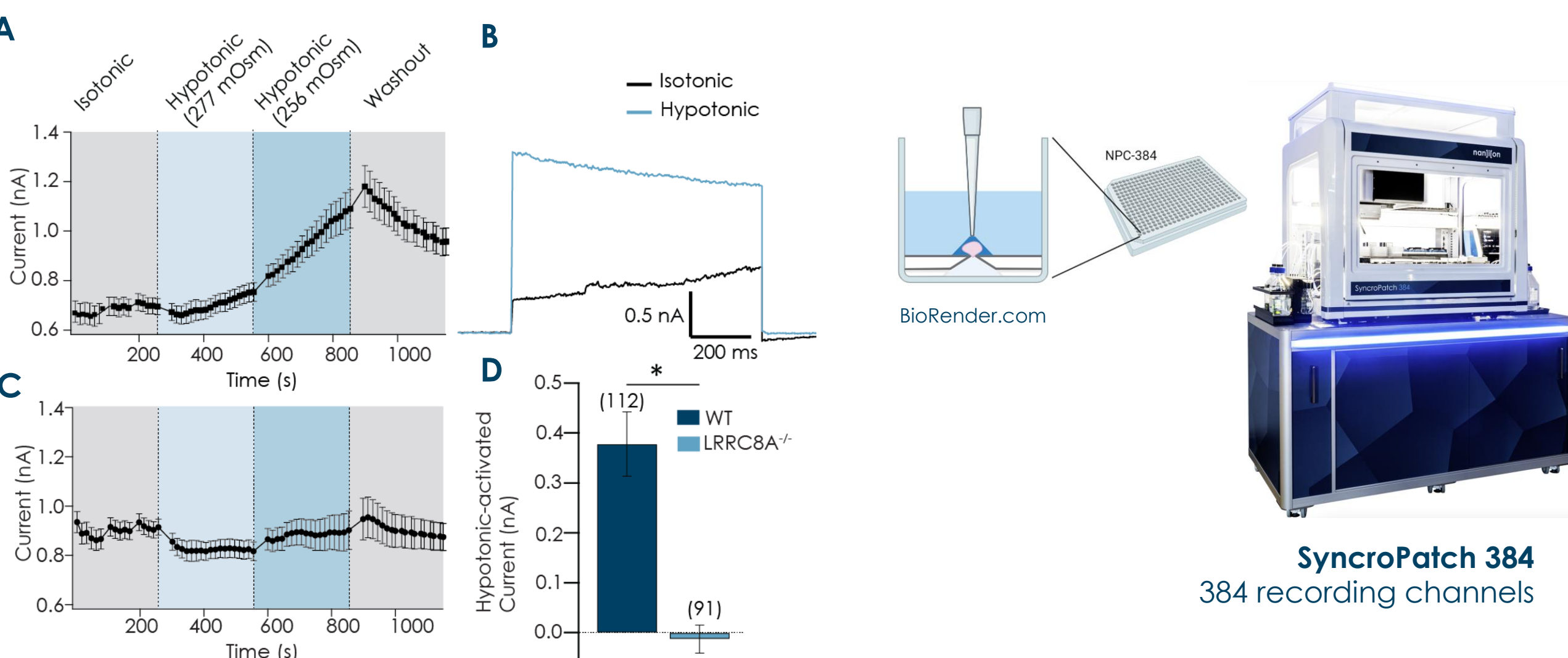
Representative whole-cell current traces of HaCaT WT and LRRC8A^{-/-} example cells in isotonic external solution (320 mOsm/kg) and after hypotonic shock, evoked by a voltage-ramp protocol ranging from -130 to +110 mV, V_{hold} -50 mV (MPC) or -100 to +100 mV, V_{hold} -40 mV (PL and SP384).

HaCaT WT and LRRC8A^{-/-} cells recorded on the PL, a microfluidic-based APC system



(A) LRRC8A WT and KO currents were recorded using a voltage ramp protocol from -100 to +100 mV over 200 ms. Hypotonic solution had an osmolarity of 234 mOsm, isotonic solution had an osmolarity of 320 mOsm. **(B)** Time course of the experiment shown in (A). After addition of hypotonic solution, there was a large increase in current at both +100/-100 mV (not shown) followed by block by 100 μM DIDS, only for WT cells. There was no activation of LRRC8 currents in the knockout keratinocytes. **(C)** Average current size in isotonic, hypotonic and in DIDS for WT (n=11; dark blue) and LRRC8A^{-/-} (n=7; light blue) HaCaTs. *P<0.05; **P<0.001, paired Student's t-test.

HaCaT WT and LRRC8A^{-/-} cells recorded on the SP384, a well-based APC system

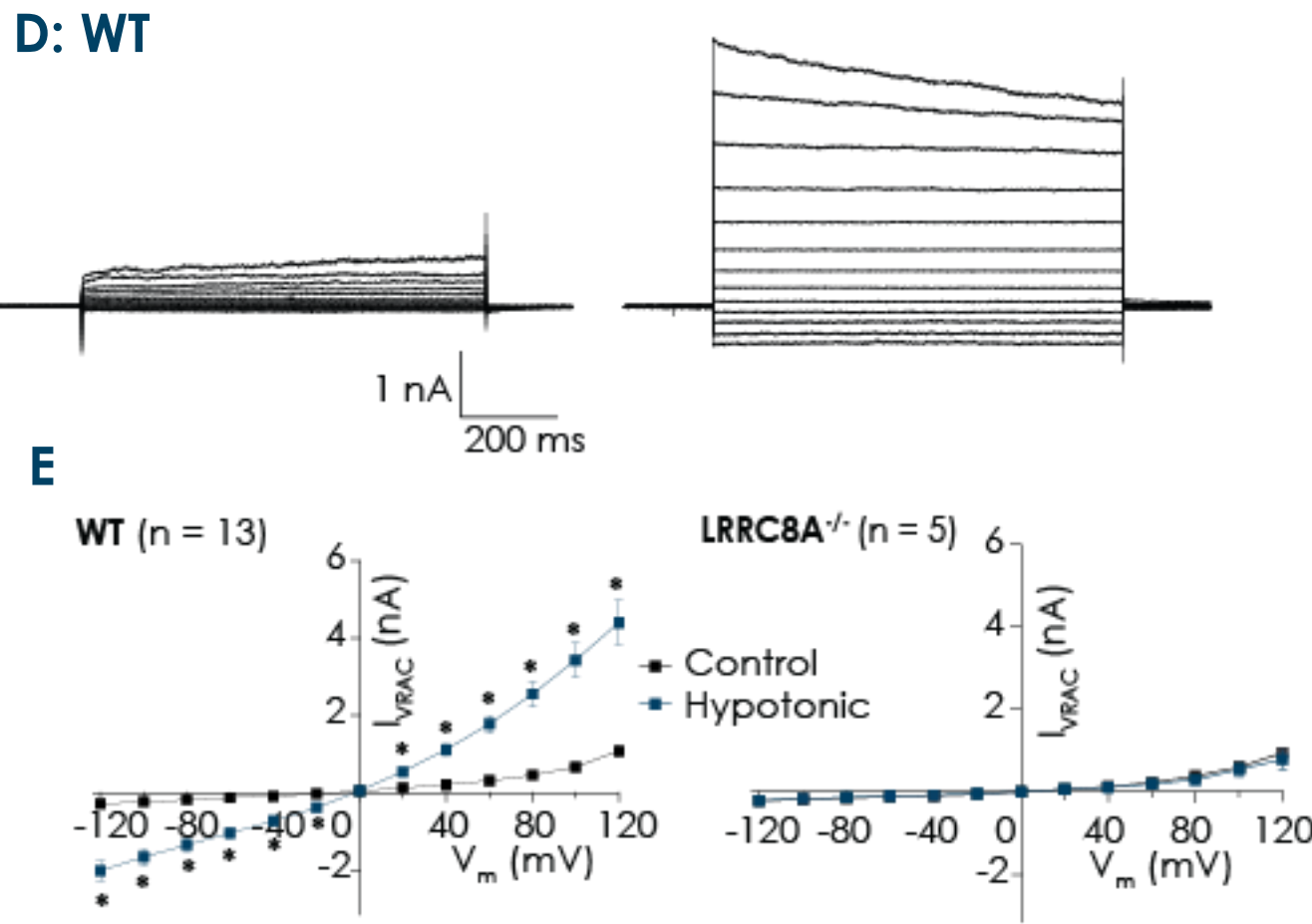


(A) Current amplitude elicited using a voltage step to +100 mV (V_{hold} -40 mV) in isotonic (grey) and hypotonic (blue) solution of WT HaCaT keratinocytes. **(B)** Example traces from a WT cell in isotonic (black) and hypotonic (blue) solution. **(C)** Current amplitude elicited using a voltage step to 100 mV in isotonic (grey) and hypotonic (blue) solution of LRRC8A^{-/-} HaCaT keratinocytes. **(D)** Average hypotonic-activated current amplitude at +100 mV for WT (dark blue) and LRRC8A^{-/-} (light blue) keratinocytes is shown as a bar graph, number of cells shown in brackets. The hypotonic-activated current was significantly larger in WT vs. LRRC8A^{-/-} cells (P<0.05, unpaired Student's t-test).



Patchliner Dynamite⁸
4 to 8 recording channels

(D) Traces from a representative cell in isotonic (left) and hypotonic (right) conditions recorded using a voltage-step protocol ranging from -120 to +120 mV, 20 mV steps, for 840 ms (V_{hold} -40 mV). **(E)** Current voltage-plot for an average of 13 WT cells (left) and 5 LRRC8A^{-/-} cells (right) (P<0.05; paired Student's t-test).



Conclusions & Acknowledgements

The Patchliner and SyncroPatch 384 automated patch clamp instruments can be used to record VRAC channels in keratinocytes which, in combination with WT and KO genotypes, could elucidate the physiological and pathological role of VRAC diseases, ischemia and even cancer. This work was supported by the German Federal Ministry of Education and Research through NatLife2020 (FKZ031B0089A).