RESOLUTE — Research Empowerment on Solute Carriers

Live cell imaging of the Zinc solute carrier SLC30A8 (ZnT8)

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Introduction

- **In pancreatic β-cells, zinc is crucial for the synthesis and secretion of insulin,** which plays a key role in glucose homeostasis and **which deficiency is the cause of diabetes.**
- The accumulation of zinc in pancreatic cells is regulated by **the solute carrier transporter SLC30A8 (Zinc Transporter 8, ZnT8)**, which transports **zinc from cytoplasm in intracellular vesicles.**
- **Allelic variants of SLC30A8 gene have been linked to diabetes** (in particular seem to increase the risk of the development of **type 2 diabetes).**

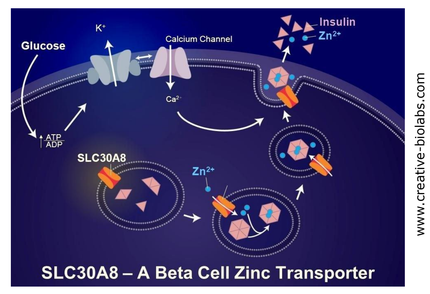


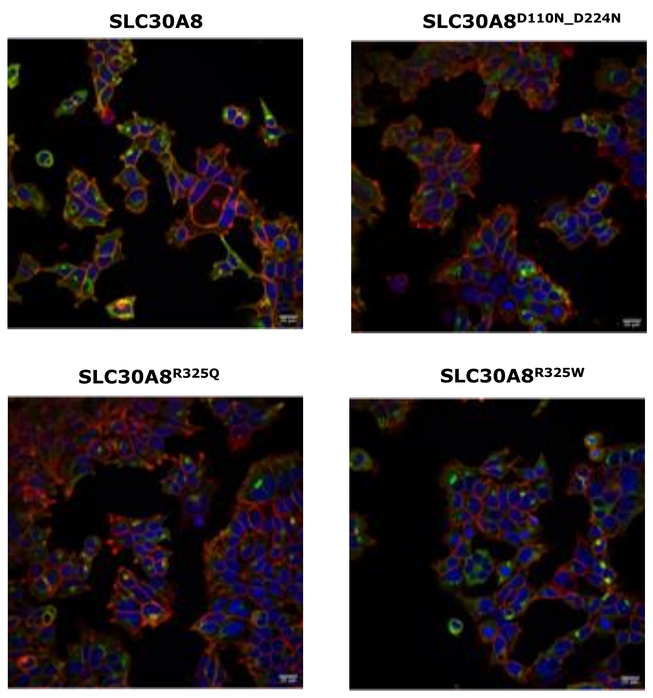
Figure 1 *– Solute carrier family 30 (zinc transporter), member 8.*

Challenges in developing an assay for SLC30A8	Solutions
SLC30A8 physiological expression in intracellular vesicles	SLC30A8 recombinant over-expression in plasma membrane
Cytotoxicity of SLC30A8 over-expression	Inducible system and optimization of induction condition
High background (endogenous Zinc transporters)	Imaging approach (single-cell analysis, high sensitivity)

A novel functional imaging- based assay specific for SLC30A8 was successfully developed in 384-well plate format:

- **Cell lines: HEK293-JI cells overexpressing SLC30A8, SLC30A8 D110N_D224N, SLC30A8 R325W or R325Q** (from CeMM, HA-tagged SLC; IF expression confirmed)
- **Read-out: Zinc (Zn^{2+}) influx measurements** using a Zinc-sensitive fluorescent dye (**Fluo Zin-3, AM, cell permeant,** by ThermoFisher)

Immunofluorescence for cellular localization of SLC30A8 over-expressed in HEK293 Jump-In T-REx cell lines

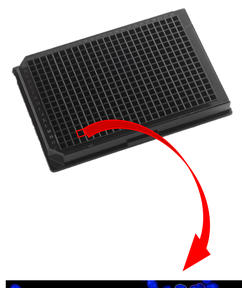


SLC30A8 D110N_D224N displayed the same expression levels and localization at the plasma membrane as SLC30A8.

The localization at the plasma membrane and overall expression of the diabetes variants (R325W or R325Q) was lower compared to SLC30A8 and the transport-dead mutant (D110N_D224N).

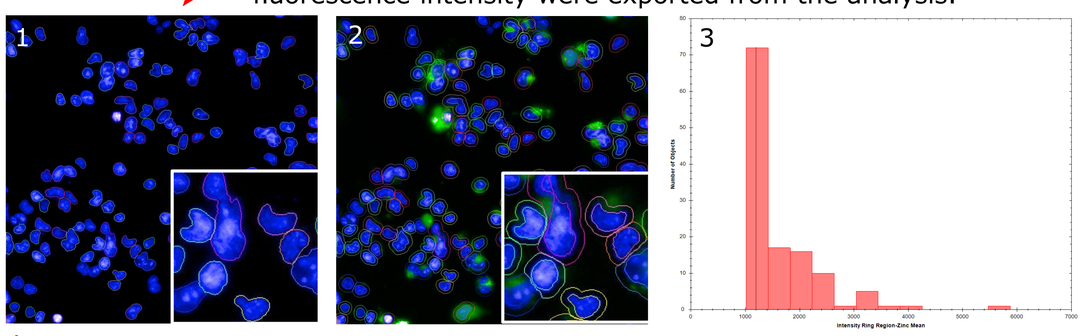
Figure 2 *– Merge images of staining with: HA antibody for SLC30A8 and mutants (green), SLC1A5 antibody for plasma membrane (red), nuclei (blue). Images were generated by CeMM.*

Image acquisition and image analysis



Cells stained with the FluoZin-3 (green) and counterstained with DAPI for nuclei (blue) were acquired by means of High Content Microscope (Opera Phenix, Revvity). Then cells were segmented to identify the nuclei using the DAPI channel.

Responding cells were selected based on a fluorescence intensity of FluoZin-3. The number of responding cells and relative mean fluorescence intensity were exported from the analysis.



4 Image Analysis Results

Results | Summary | Properties Nuclei

Population: Nuclei	Value						
Number of Objects	124						
Property	**Mean**	**CV %**	**StdDev**	**Median**	**Max**	**Min**	**Sum**
Intensity Ring Region-Zinc Mean	1631.14	43.9881	717.508	1353.12	5873.24	1017.17	202261
Zinc positive cells	0.709677	64.2197	0.455753	1	1	0	88

Figure 3 *–Image analysis process: 1. Nuclei segmentation with DAPI channel; 2. Cytoplasm ring region extended from nuclei segmentation; 3. Selection on responding cells; 4. Data extraction. Image analysis is performed with Harmony 4.9 (Revvity). Image acquired by 20x magnification.*

Live imaging analysis of SLC30A8

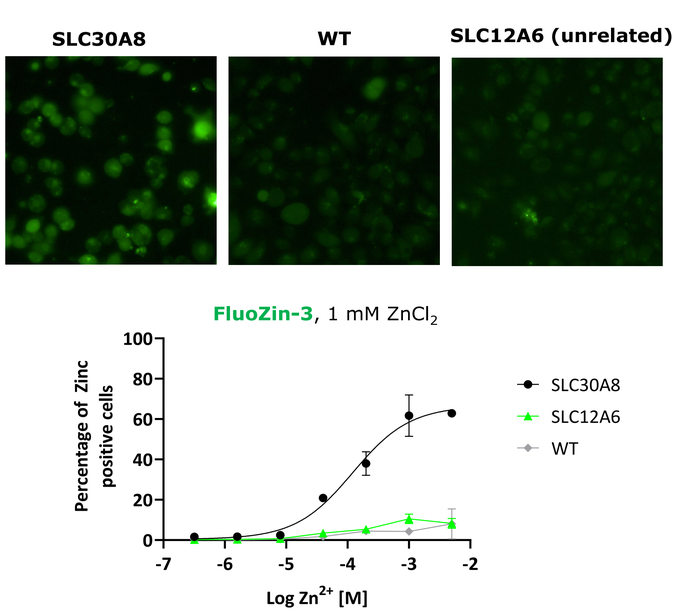


Figure 4 *– Representative images of FluoZin-3 staining after stimulation of induced cells with 1 mM $ZnCl_2$ (upper side); dose-response curves expressed as percentage of Zinc positive cells (lower side). Image acquired by 20x magnification.*

- Cells induction: 24h (10 ng/mL doxycycline).
- Fluorescent dye loading: 45 mins at 37°C (in Ca^{2+} -Mg^{2+} -phosphate-free HBSS w 5mM EDTA).
- Automatic washing (3X) w Ca^{2+} -Mg^{2+} -phosphate-free HBSS .
- Stimulation w Zn^{2+} dose-response (as Zinc Chloride) up to 5 mM.
- Reading at Operetta CLS (Revvity).

- **Zinc internalization** was **increased in SLC30A8 compared to unrelated transporter (SLC12A6) or WT** cell line.

Comparison between $ZnCl_2$ transport activity across SLC30A8 mutants

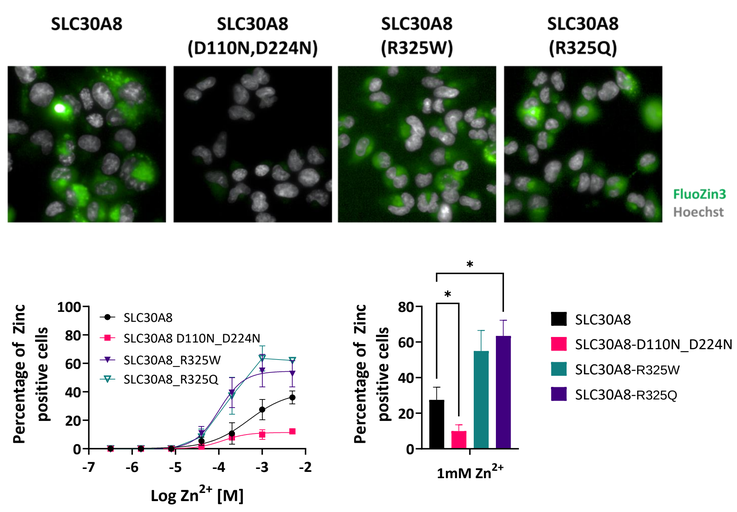


Figure 5 *– Representative images of FluoZin-3 staining after stimulation of induced cells with 1 mM $ZnCl_2$ (upper side); dose-response curves expressed as percentage of Zinc positive cells (lower side on the left); histograms representing percentage of Zinc positive cells at 1mM $ZnCl_2$. Statistical analysis: Brown-Forsythe and Welch ANOVA tests, p-values <0.05 = *, <0.005=**, <0.001=*** (lower side on the left). Image acquired by 20x magnification.*

- Cytosolic **accumulation** of **$ZnCl_2$** is **greatly impaired** in **SLC30A8 D110N_D224N** mutant and significantly lower than SLC30A8.
- Mutations associated with **T2DM** (**SLC30A8 R325W** and **R325Q**) display **alterations** in **$ZnCl_2$ transport (higher n° of cells accumulating $ZnCl_2$ compared to SLC30A8).**
- This **cellular-based assay** represents a **novel and valuable tool to screen modulators of SLC30A8** transporter activity.

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