

An imaging-based assay for a challenging target: the case of SLC30A8

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Introduction

- **SLC30A8** (or **Zinc Transporter 8**) is expressed at a high level **only in the pancreas**, especially in **insulin-producing beta cells**
- it is a **zinc transporter** involved in the accumulation of **zinc from the cytoplasm into intracellular vesicles**
- it is involved in **intracellular zinc homeostasis** and signalling through compartmentation of the ion and regulation of its concentration
- it is thought to be required for **providing zinc to allow for proper maturation, storage and secretion of insulin**
- **diseases** associated with SLC30A8 include **Type 2 Diabetes Mellitus and Type 1 Diabetes Mellitus 5**

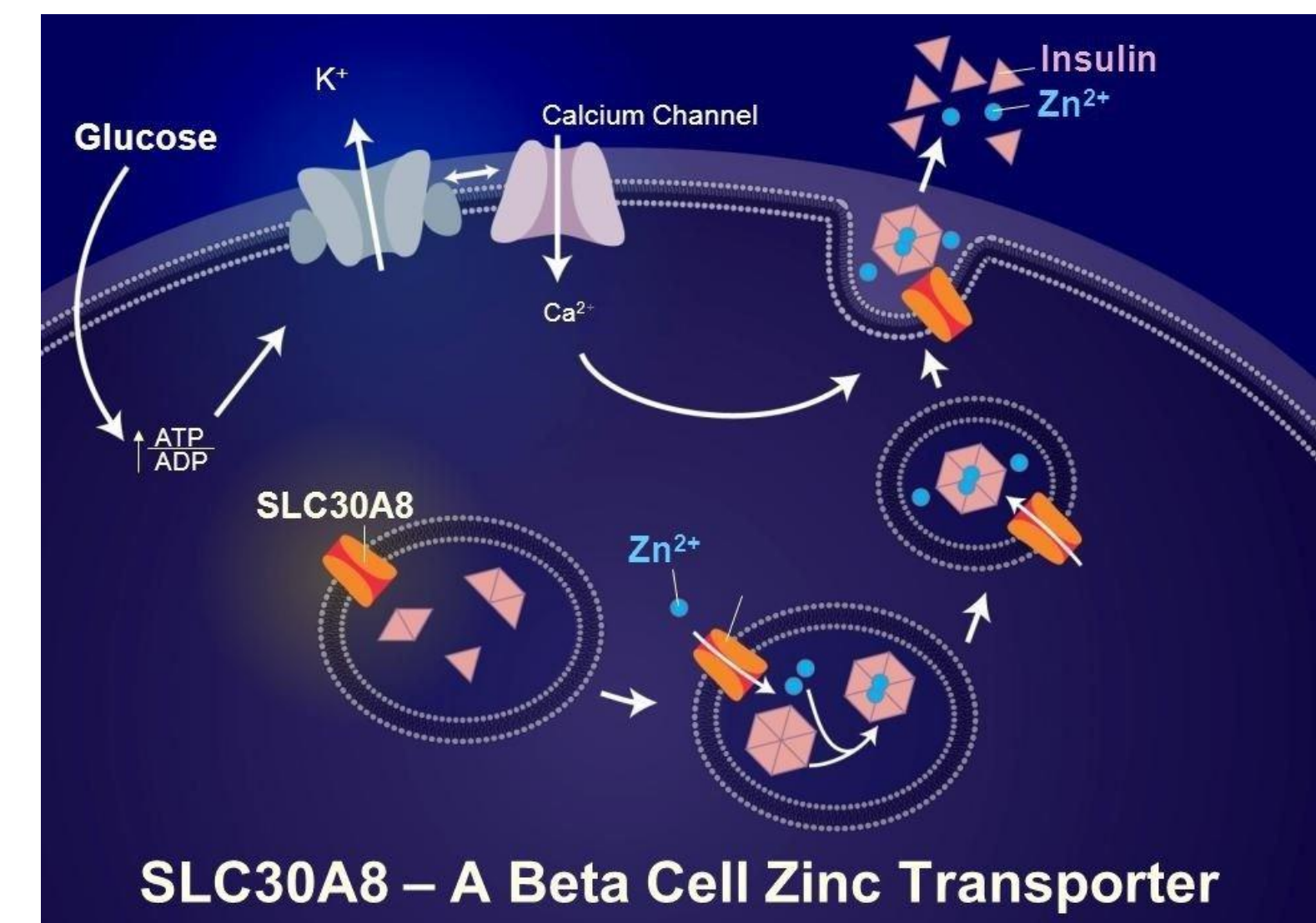


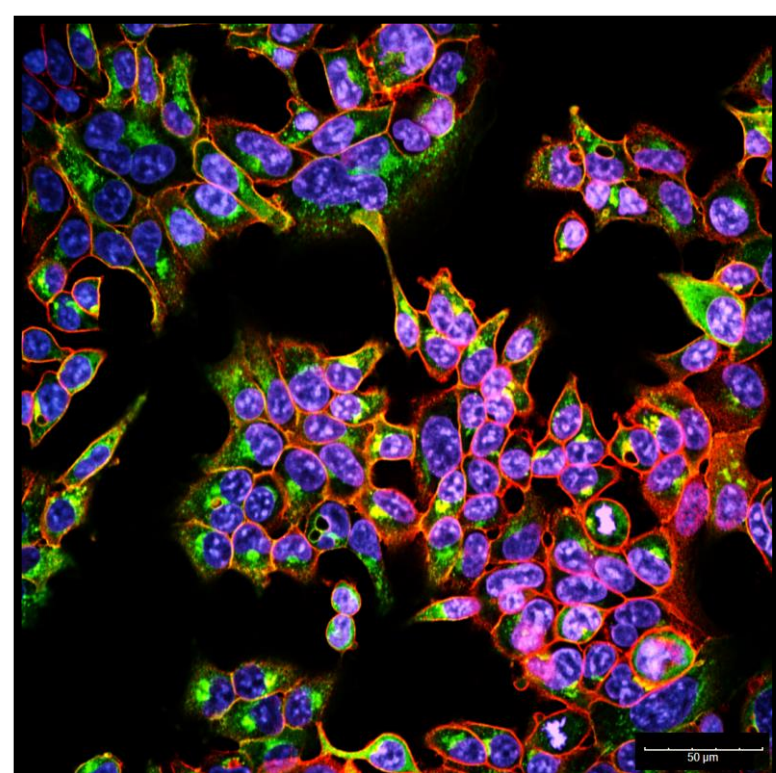
Figure 1 – Solute carrier family 30 (zinc transporter), member 8.

An imaging cell-based assay was successfully developed in 384-well plate format:

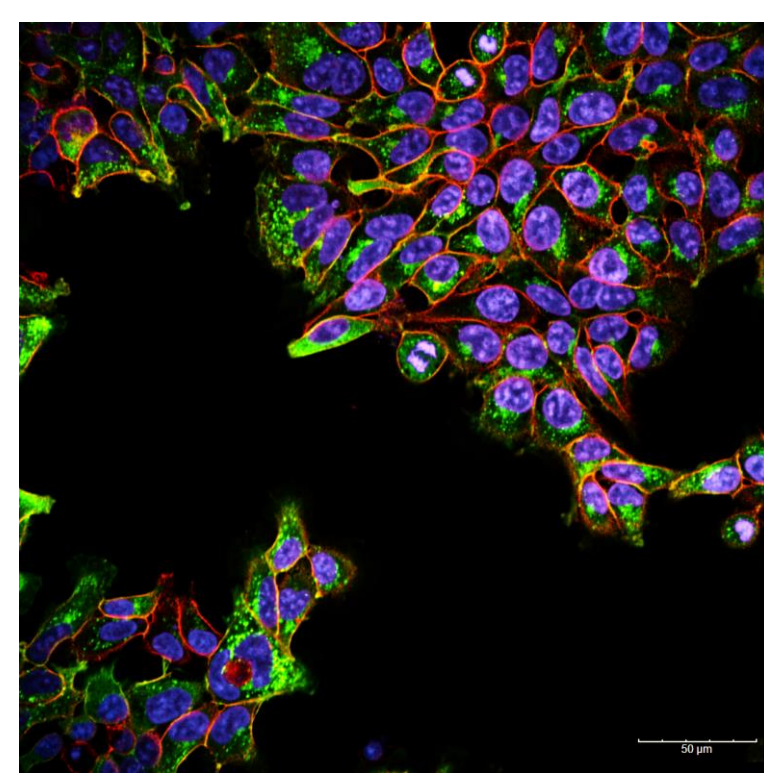
- **Cell line:** **HEK-293 Jump In T-REx/SLC30A8 WT or SLC30A8 D110N_D224N** (OE cell lines from CeMM, HA-tagged SLC; IF expression confirmed)
- **Read-out:** **Zinc (Zn²⁺) influx measurements** using a Zinc-sensitive fluorescent dye (**Fluo Zin-3, AM, cell permeant, by ThermoFisher**)

Immunofluorescence for cellular localization of SLC30A8 WT and SLC30A8 D110N_D224N over-expressed in HEK293 Jump-In T-REx cell line

HEK-293 Jump In T-REx
SLC30A8 WT



HEK JumpIn T-REx
SLC30A8 D110N_D224N



Both SLC30A8 and SLC30A8 D110N_D224N are partially localized in plasma membrane (localization also in the ER and Golgi)

Figure 2 – Merge images of staining with: HA antibody for SLC30A8 wt and D110N_D224N mutant (green), SLC1A5 antibody for plasma membrane (red), nuclei (blue). Images were generated by CeMM.

Ubiquitous endogenous expression of Zinc transporters (SLC30 and SLC39 families)

The endogenous expression of different Zinc transporters both in HEK293 and other cell lines makes SLC30A8 characterization very challenging due to high unspecific background

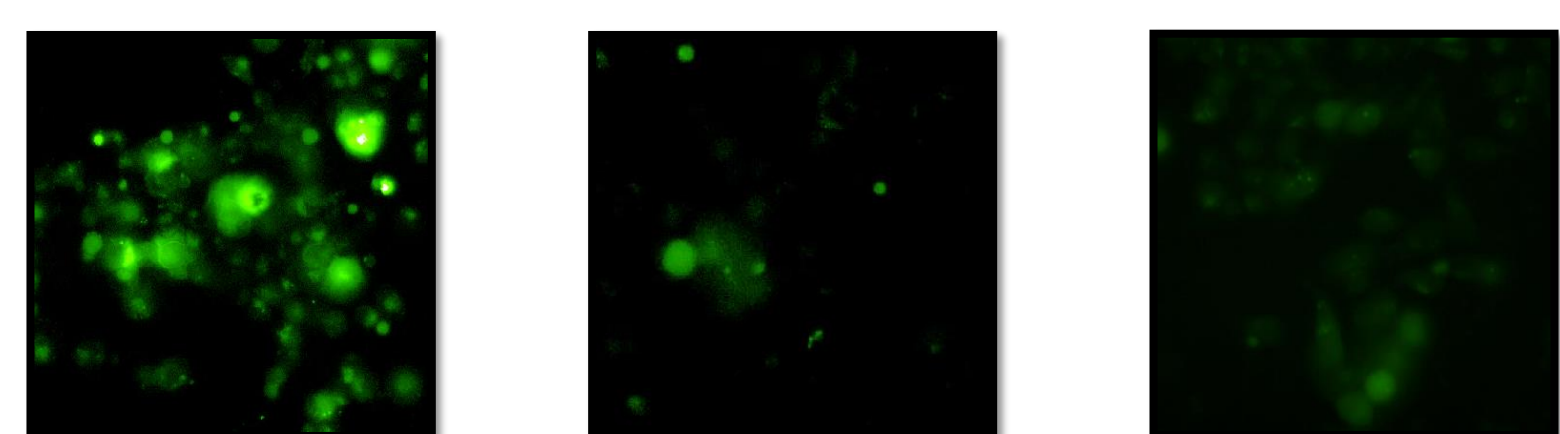
| SLC | Endogenous expression |
|----------|-----------------------|
| SLC30A1 | yes |
| SLC30A10 | yes |
| SLC30A2 | no |
| SLC30A3 | yes |
| SLC30A4 | yes |
| SLC30A5 | yes |
| SLC30A6 | yes |
| SLC30A7 | yes |
| SLC30A8 | no |
| SLC30A9 | yes |
| SLC39A1 | yes |
| SLC39A10 | yes |
| SLC39A11 | yes |
| SLC39A12 | no |
| SLC39A13 | yes |
| SLC39A14 | yes |
| SLC39A2 | no |
| SLC39A3 | yes |
| SLC39A4 | yes |
| SLC39A5 | no |
| SLC39A6 | yes |
| SLC39A7 | yes |
| SLC39A8 | yes |
| SLC39A9 | yes |

| SLC | LS180 | HCT116 | HuH-7 | 1321-N1 | MDA-MB-41 | SK-MEL-28 |
|----------|-------|--------|-------|---------|-----------|-----------|
| SLC30A1 | 12.6 | 6.7 | 11.6 | 21.0 | 11.4 | 19.9 |
| SLC30A10 | 0.0 | 0.1 | 23.6 | 0.0 | 0.0 | 5.3 |
| SLC30A2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 |
| SLC30A3 | 0.1 | 13.3 | 0.5 | 6.6 | 0.2 | 0.5 |
| SLC30A4 | 0.6 | 1.3 | 0.3 | 1.8 | 2.3 | 2.4 |
| SLC30A5 | 43.6 | 43.1 | 38.3 | 23.1 | 42.5 | 51.9 |
| SLC30A6 | 14.9 | 22.8 | 16.5 | 31.4 | 25.4 | 22.0 |
| SLC30A7 | 12.1 | 10.1 | 11.0 | 19.6 | 13.5 | 19.2 |
| SLC30A8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| SLC30A9 | 66.4 | 42.2 | 37.0 | 25.9 | 38.8 | 31.9 |
| SLC39A1 | 89.2 | 60.2 | 101.3 | 119.0 | 200.9 | 103.8 |
| SLC39A10 | 57.2 | 18.7 | 13.2 | 15.4 | 6.3 | 24.3 |
| SLC39A11 | 13.6 | 39.1 | 25.7 | 24.8 | 55.0 | 48.1 |
| SLC39A12 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 |
| SLC39A13 | 10.6 | 13.9 | 18.8 | 37.2 | 15.5 | 30.5 |
| SLC39A14 | 98.8 | 142.8 | 554.2 | 209.7 | 25.6 | 197.3 |
| SLC39A2 | 0.0 | 0.0 | 0.1 | 0.0 | 1.7 | 0.0 |
| SLC39A3 | 57.4 | 53.1 | 51.5 | 72.2 | 44.4 | 78.1 |
| SLC39A4 | 111.9 | 103.3 | 4.3 | 10.8 | 32.5 | 9.9 |
| SLC39A5 | 2.5 | 0.2 | 112.0 | 0.4 | 0.1 | 0.2 |
| SLC39A6 | 40.8 | 92.7 | 29.1 | 25.4 | 22.4 | 77.2 |
| SLC39A7 | 23.1 | 19.2 | 27.4 | 22.6 | 24.7 | 21.7 |
| SLC39A8 | 30.3 | 14.9 | 7.5 | 7.1 | 20.5 | 17.5 |
| SLC39A9 | 48.7 | 44.6 | 38.5 | 33.9 | 33.2 | 25.1 |

Figure 3 – Information from Resolute WP1 dashboard

Imaging-based high-content analysis of SLC30A8

HEK JumpIn T-REx SLC30A8 | HEK JumpIn T-REx WT | HEK JumpIn T-REx SLC12A6 (unrelated)



FluoZin-3, 1 mM ZnCl₂

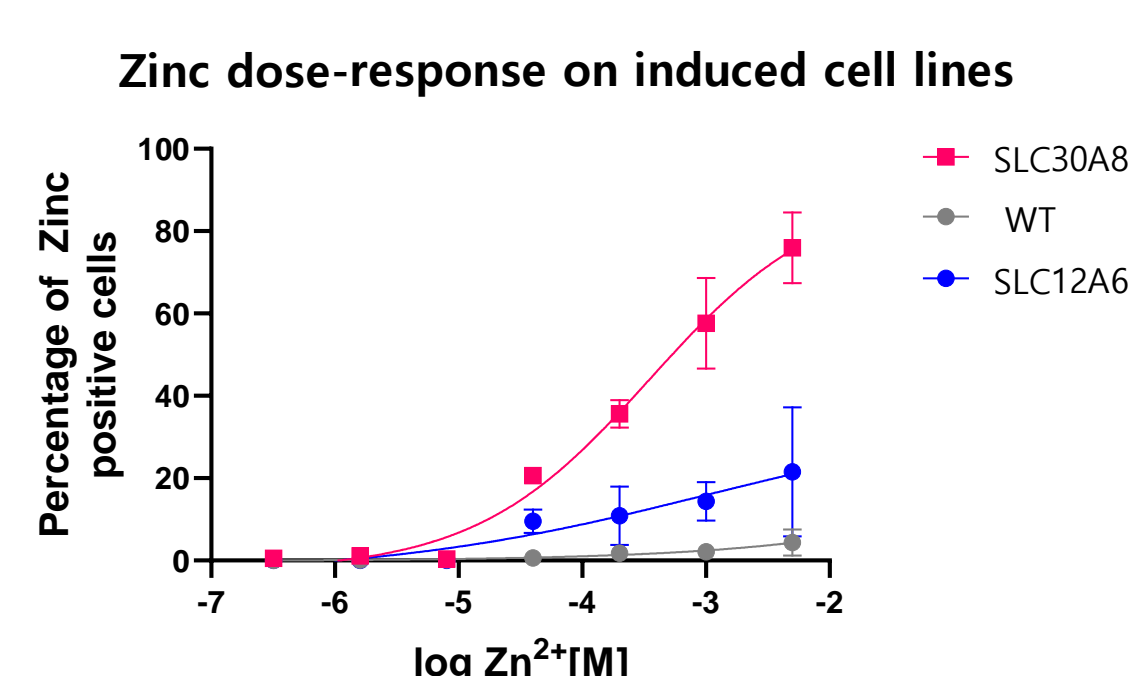


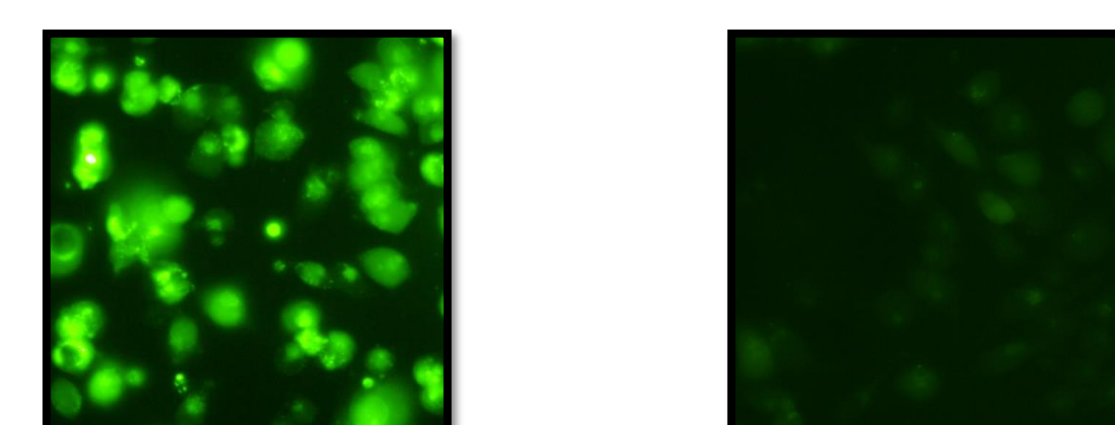
Figure 4 – Representative images of FluorZin-3 staining after stimulation of induced cells with 1 mM ZnCl₂ (upper side); dose-response curves expressed as percentage of Zinc positive cells (lower side)

- Cells induction: **24h** (1 µg/mL doxycycline)
- Fluorescent dye loading: **45 mins at 37°C** (in Ca²⁺ -Mg²⁺ -phosphate-free HBSS w 5mM EDTA)
- Automatic washing (3X) w Ca²⁺ -Mg²⁺ -phosphate-free HBSS
- Stimulation w Zn²⁺ dose-response (as Zinc Chloride) up to 5 mM
- Reading at Operetta CLS (PerkinElmer)

Zn²⁺ D/R: Zinc internalization was **increased in induced SLC30A8** compared to **unrelated transporter (SLC12A6) or WT** cell line

Imaging-based high-content analysis of SLC30A8 WT versus SLC30A8 D110N_D224N double mutant

HEK JumpIn T-REx SLC30A8 ind | HEK JumpIn T-REx SLC30A8 D110N_D224N ind



FluoZin-3, 1 mM ZnCl₂

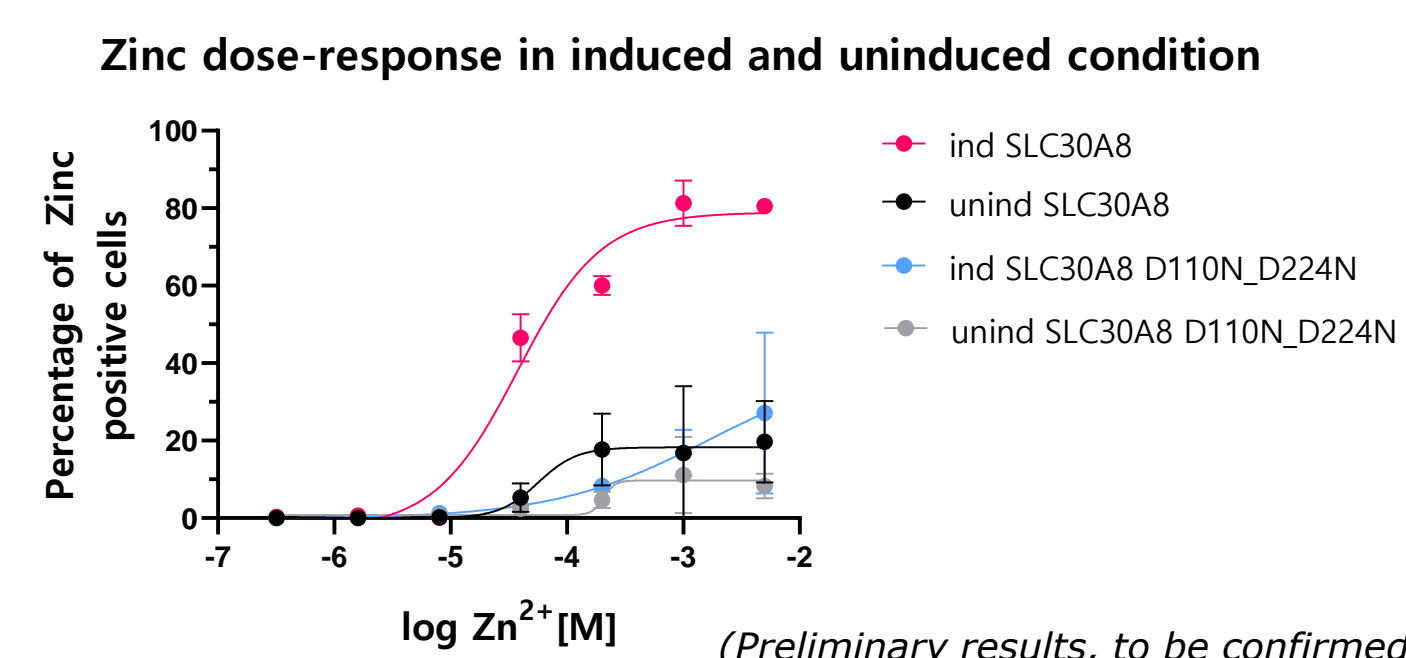


Figure 5 – Representative images of FluorZin-3 staining after stimulation of induced cells with 1 mM ZnCl₂ (upper side); dose-response curves expressed as percentage of Zinc positive cells (lower side)

- SLC30A8 Asp110/Asp224 mutations resulted in a reduction of Zn²⁺ uptake (Xue et al., eLife 2020)
- Same protocol described for Figure 4 applied for imaging

Zn²⁺ D/R: Zinc internalization was **increased in induced SLC30A8 WT** in comparison to **induced SLC30A8 D110N_D224N**; significant difference between **induced SLC30A8 WT and uninduced condition**