Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S5
Supplementary Figure S6
Supplementary Figure S7
Supplementary Figure S8

Elution Volume (ml)

Absorbance at 280 nm

WT
WT (0.2mM MgCl₂)
D432A
D432A (0.2mM MgCl₂)
Supplementary Figure S9

A bar chart showing the net 57Co²⁺ uptake for various constructs. The constructs are labeled as follows:

- WT
- Δ1-129
- D226N/D250A
- D259N
- E258Q
- L324A
- N329A
- N332A
- Q333A
- E359A
- D432A
- D432N

The y-axis represents the net 57Co²⁺ uptake, ranging from 0 to 80,000.
Supplementary Figure S10
Supplementary Figure S11

A

Marker

Trypsin

+Na⁺ (mM)

0 50 100 200 300

Wild type

kDa

66

45

36

B

Low intracellular Na⁺ (1 mM)

90 (mM)

0.2

Mg²⁺

High intracellular Na⁺ (300 mM)

90 (mM)

0.2

Mg²⁺
Supplementary Figure S12
Supplementary Figure S13
Supplementary Figure S15

A. Open dwell time

WT

\( \tau_{fast} = 1.8 \pm 0.09 \text{ ms} \)
\( \tau_{slow} = 7.9 \pm 0.5 \text{ ms} \)

B. Open dwell time

D226N/D250A

\( \tau_{fast} = 3.3 \pm 0.09 \text{ ms} \)
\( \tau_{slow} = 16.4 \pm 1.0 \text{ ms} \)

C. Open dwell time

E258Q

\( \tau_{fast} = 4.1 \pm 0.17 \text{ ms} \)
\( \tau_{slow} = 17.4 \pm 2.3 \text{ ms} \)

D. Open dwell time

D259N

\( \tau_{fast} = 4.0 \pm 0.35 \text{ ms} \)
\( \tau_{slow} = 33.7 \pm 2.2 \text{ ms} \)

E. Open dwell time

\( \Delta 1-129 \)

\( \tau_{fast} = 3.7 \pm 0.5 \text{ ms} \)
\( \tau_{slow} = 211.7 \pm 15.1 \text{ ms} \)

F. Closed dwell time

WT

\( \tau_{fast} = 1.6 \pm 0.07 \text{ ms} \)
\( \tau_{slow} = 17.6 \pm 0.5 \text{ ms} \)

G. Closed dwell time

D226N/D250A

\( \tau_{fast} = 4.2 \pm 0.14 \text{ ms} \)
\( \tau_{slow} = 73.3 \pm 4.5 \text{ ms} \)

H. Closed dwell time

E258Q

\( \tau_{fast} = 1.5 \pm 0.08 \text{ ms} \)
\( \tau_{slow} = 21.0 \pm 0.7 \text{ ms} \)

I. Closed dwell time

D259N

\( \tau_{fast} = 4.7 \pm 0.12 \text{ ms} \)
\( \tau_{slow} = 75.1 \pm 5.1 \text{ ms} \)

J. Closed dwell time

\( \Delta 1-129 \)

\( \tau_{fast} = 5.6 \pm 0.4 \text{ ms} \)
\( \tau_{slow} = 258.2 \pm 27.7 \text{ ms} \)
Supplementary Figure S16