The Role of Beam Structure in Human Split Piezo-1

Semin Kim[1], Yeongdo Lee[+], Minsuk Kwak*, and Jinwoo Cheon*

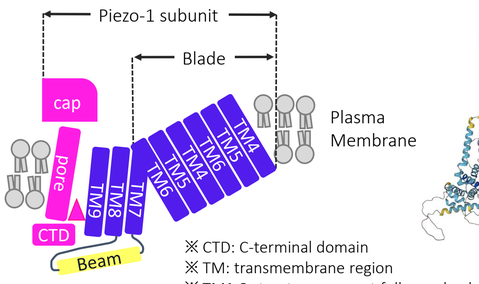
[1]Life Science and Biotechnology Department (LSBT), Underwood Division (UD), Underwood International College, Yonsei University, Sinchon, Seoul 03722, Korea
[+] * Center for Nanomedicine, Institute for Basic Science, Seoul 03722, Republic of Korea; Department of Nano Biomedical Engineering, Advanced Science Institute, Yonsei University, Seoul 03722, Republic of Korea

E-mail: minisemin@yonsei.ac.kr[1]

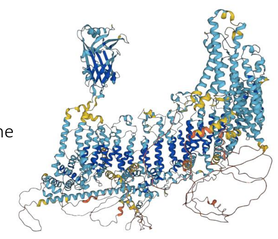
Introduction

Piezo-1, a mechanosensitive homotrimeric calcium ion channel, can be activated by mechanical strains on its blades or by chemical agents like Yoda-1. Piezo-1 was implicated in activating dormant degenerative neurons under mechanical stimuli, offering potential therapeutic applications for conditions like Parkinson's disease.[1] However, its **large size of 13.8kb** exceeds the loading capacity of FDA-approved viral vectors like AAV, crucial for long-term protein expression. As a solution, this study investigates **a bisected human Piezo-1** which has been demonstrated to be functional.[2] The **beam structure** of the Piezo-1 has been hypothesized to function as a **lever**, pivoting at the C-terminal domain (CTD), to couple mechanical sensing and pore opening. We questioned **if the segmented Piezo-1's beam structure retains this mechanism of signal transduction and mediates the reunion of the two segments**, as beam structure contains the binding site to the CTD. Thus, we examined the membrane localization and Yoda-1 activation of a beam-deleted split Piezo-1 construct. Additionally, we explored if the C terminus segment alone can be activated, potentially representing the **minimal functional unit of Piezo-1**.

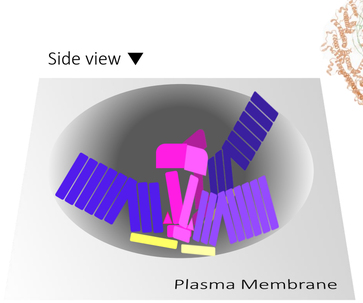
Human Piezo-1



※ CTD: C-terminal domain
※ TM: transmembrane region
※ TM1-3 structures are not fully resolved

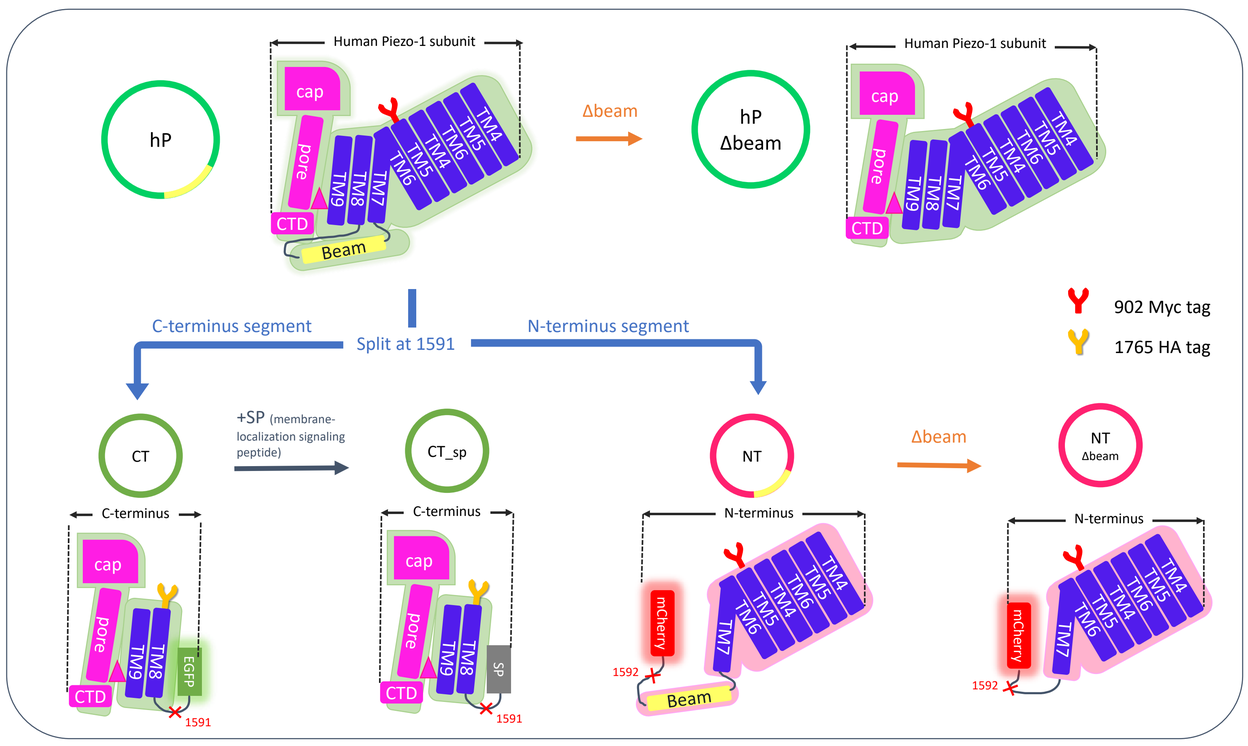


Human Piezo-1 trimer

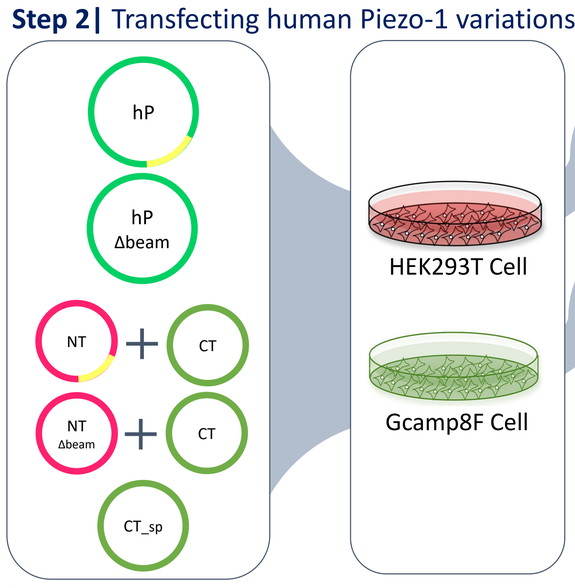


Experimental Methods

Step 1| Molecular cloning for human Piezo-1 variations



Step 2| Transfecting human Piezo-1 variations



Step 3| Expression and Localization Analysis

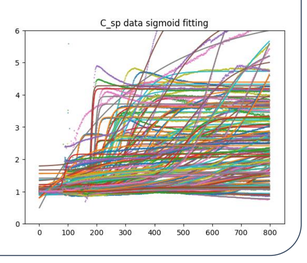
Cell surface live staining

Step 4| Functionality Analysis

Yoda-1 stimulation
Live imaging
Curve fitting

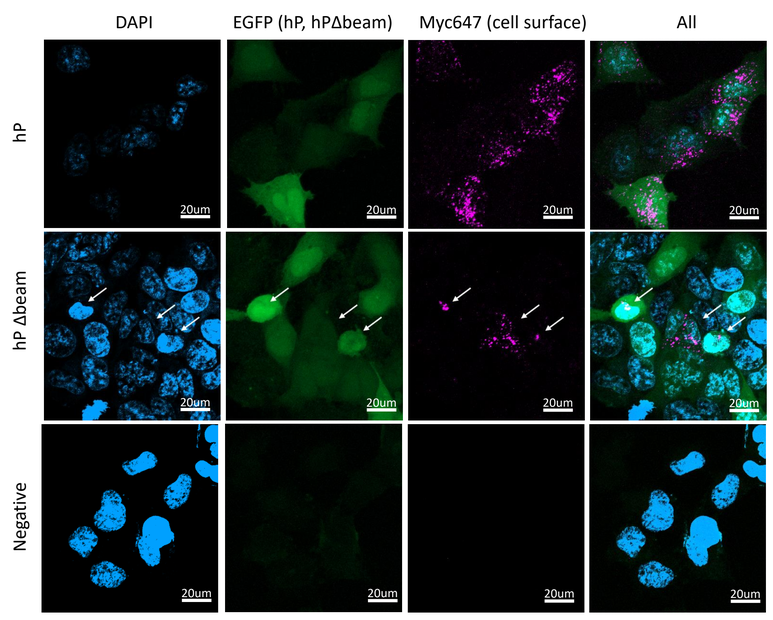
$$y = \frac{L}{1 + e^{-k(x-x0)}} + b$$

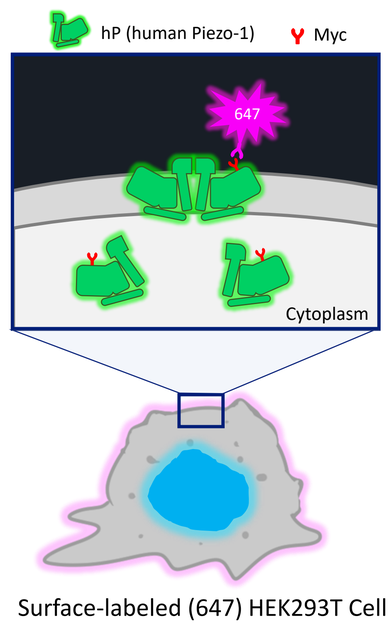
L : maximum value; k: steepness
x0: midpoint; b: offset



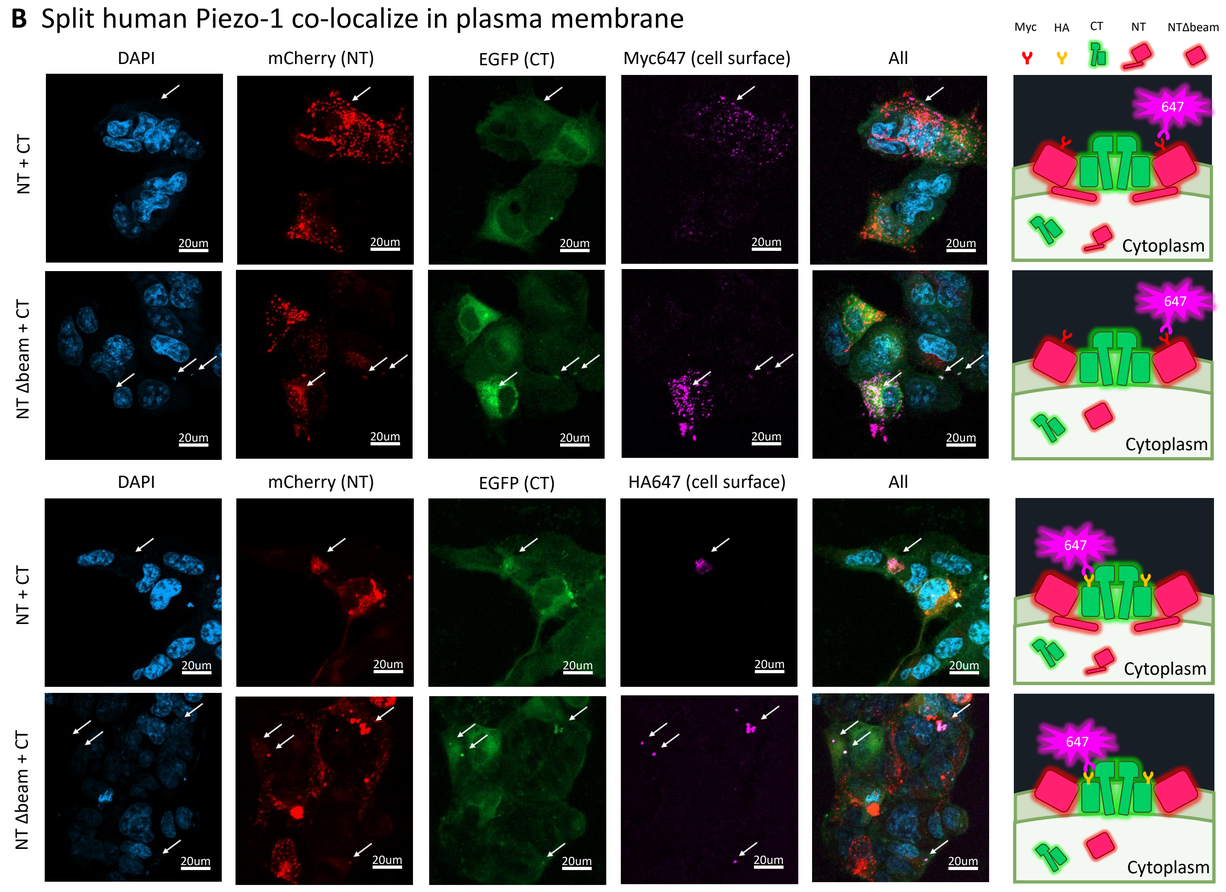
Results

A Whole human Piezo-1 localize in plasma membrane

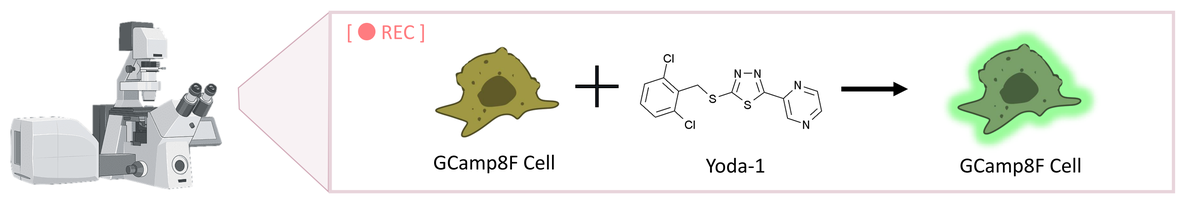




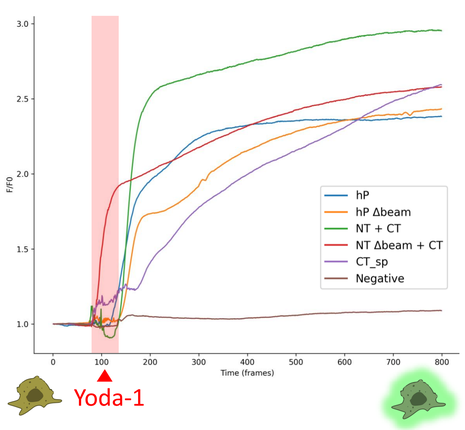
B Split human Piezo-1 co-localize in plasma membrane



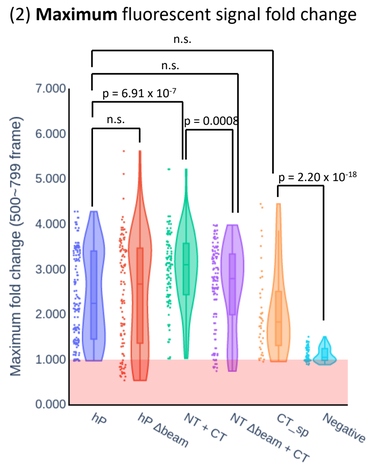
c Yoda-1 activation patterns of human Piezo-1 variations



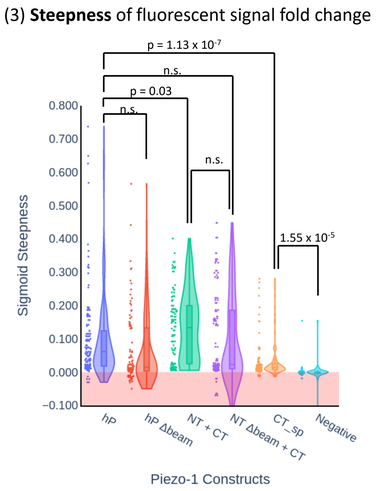
(1) **Average** fluorescent signal fold change



(2) **Maximum** fluorescent signal fold change



(3) **Steepness** of fluorescent signal fold change



Conclusion & Further Study

Expression

- Splitting human Piezo-1 or deleting the beam domain still yields functional Piezo-1 mutants (Figure A-**C**)

Localization

- Split Piezo-1
  - Segments successfully co-localize on the cell membrane (Figure **B**: Myc647 and HA647 pannels)
- Beam deletion
  - Does not inhibit the membrane localization (Figure **A**: hPΔbeam pannels, Figure **B**: NT Δbeam + CT pannels)
    - ➔ Beam domain doesn't mediate co-localization between N-terminus and C-terminus segments

Activation by Yoda-1

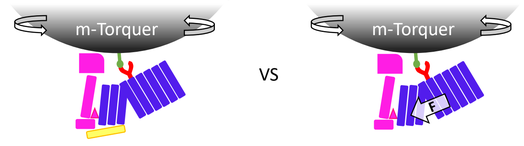
- Split Piezo-1
  - Calcium uptake is significantly increased (Figure **C** NT+CT)
- Beam deletion
  - Calcium uptake is reduced in split Piezo-1, but not in the whole Piezo-1 (Figure **C**-2 lane 1 vs 2, lane 3 vs 4)
- C-terminus segment
  - Yoda-1 can activate C-terminus segment without N-terminus segment[3]
    - ➔ Yoda-1's binding site is located in the C terminus segment
  - Calcium uptake is consistently increasing, but in a much slower rate (we haven't checked if the signal saturated after a long time)
    - ➔ N terminus segment is necessary for faster calcium uptake, and possibly for controlling the rate of influx

- Piezo-1 structural mutants have different calcium uptake patterns
  - Amount: whole < C-term < N-term + C-term
  - Rate: C-term < whole < N-term + C-term / Δbeam == beam
    - Bigger or longer pore openings can increase the calcium influx (we need to check if the same number of Piezo-1 mutants are expressed on the membrane)
    - Unstable pore openings can reduce the calcium influx
      - ➔ It is the blade, not the beam that stabilizes the pore opening

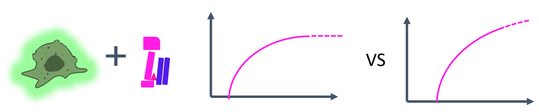
Further Study

m-Torquer or patch clamp experiment is necessary to confirm whether the whole and split Piezo-1 without beam domain maintains their natural mechanosensitive characteristics. Testing m-Torquer-induced activation on CT_sp construct would prove if C-terminus segment is the minimal functional unit.

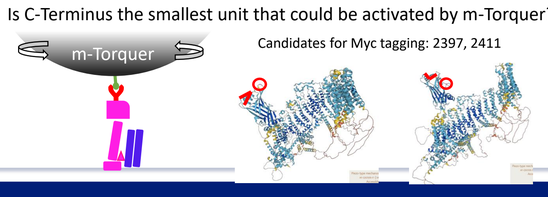
1. Is beam structure necessary to transduce mechanical stimuli?



2. Does C-terminus-only construct have uncontrolled calcium influx?



3. Is C-Terminus the smallest unit that could be activated by m-Torquer?



Reference

[1] Shin, W., Lee, Y., Lim, J., Lee, Y., Lah, J. D., Lee, S., … Cheon, J. (2023). Nanoscale magneto-mechanical-genetics of deep brain neurons reversing motor deficits in Parkinsonian Mice. *Nano Letters*, 24(1), 270–278. doi:10.1021/acs.nanolett.3c03899

[2] Bae, C., Suchyna, T. M., Ziegler, L., Sachs, F., & Gottlieb, P. A. (2016). Human Piezo1 ion channel functions as a split protein. *PLOS ONE*, 11(3). doi:10.1371/journal.pone.0151289

[3] Nosyreva, Elena. D., Thompson, D., & Syeda, R. (2021). Identification and functional characterization of the Piezo1 Channel Pore Domain. *Journal of Biological Chemistry*, 296, 100225. doi:10.1074/jbc.ra120.015905