# Nanoscale Membrane Curvature Modulates Activation-Associated Redistribution of PIEZO1 in Living Cells

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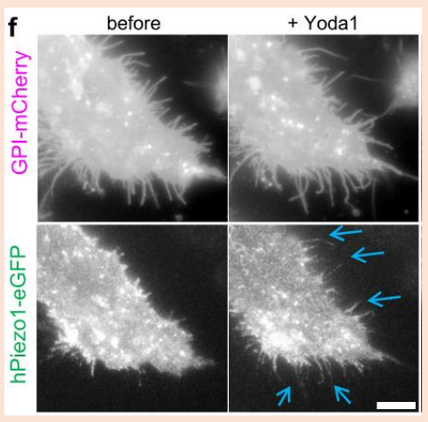
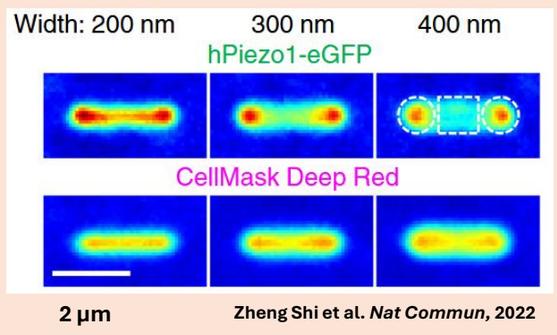
## Introduction

PIEZO1 is a mechanosensitive ion channel essential for cellular mechanotransduction and has been reported to localize at specialized subcellular structures. Its spatial distribution has also been implicated in diverse cellular. Recent studies further suggest that PIEZO1 preferentially associates with positively curved membrane regions and undergoes redistribution upon channel activation. However, **how activation alters PIEZO1 distribution on membranes with pre-existing curvatures, and whether this process is regulated by cellular components linked to PIEZO1 mechanosensitivity**, remains elusive. Here, we employed engineered nanotopography substrates to generate defined nanoscale membrane curvatures in living cells and quantitatively investigate PIEZO1 spatial organization during channel activation. Yoda1-induced PIEZO1 activation triggered curvature-dependent redistribution, and cellular components previously implicated in PIEZO1 mechanosensitivity modulated this activation-associated curvature-dependent distribution.

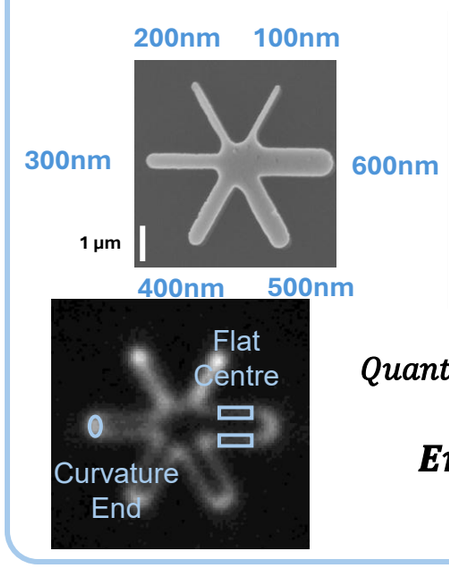


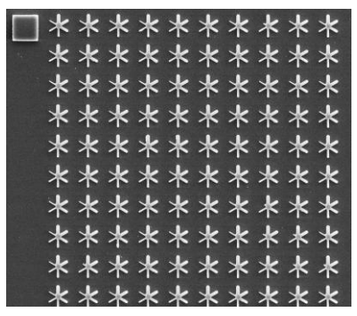

## Methodology

### NanoSnow Made by E-beam Lithography

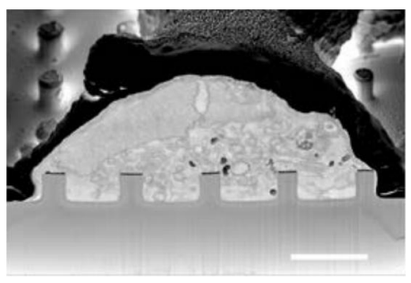
NanoSnow with gradient curvature sizes




Nanostructure array with precise shape and size



Membrane topography is manipulated by nanostructure



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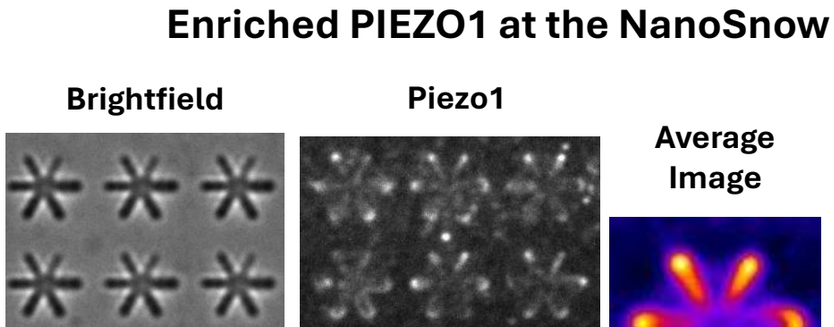
*Quantatative measurement*:

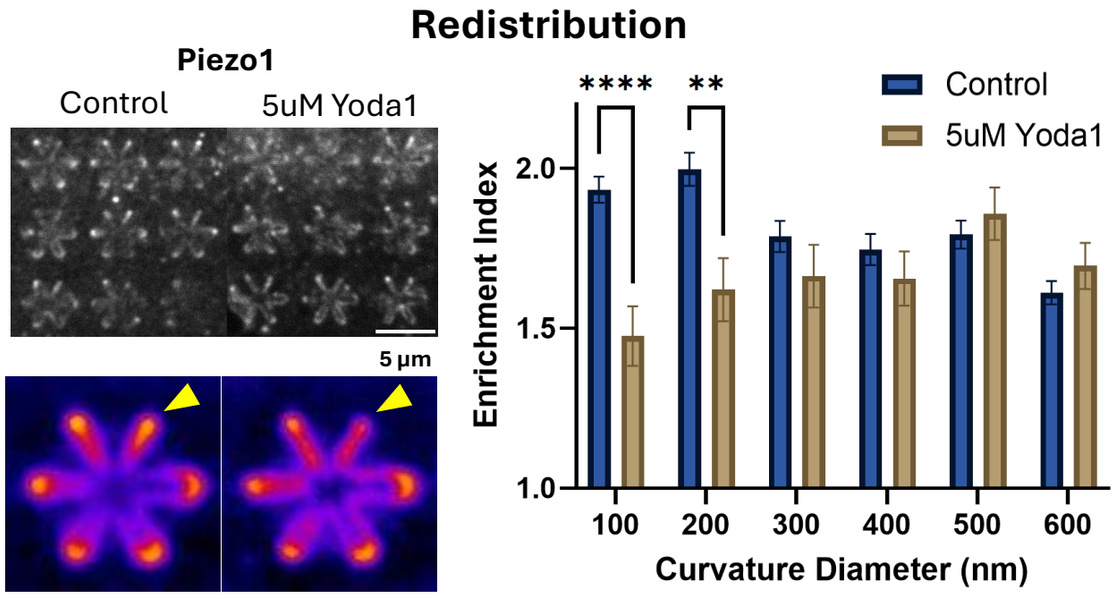
$$\textit{Enrichment Index} = \frac{\textit{End mean intensity}}{\textit{Center mean intensity}}$$

### Enriched PIEZO1 at the NanoSnow Curvature




## Result

### 1. Activation-associated PIEZO1 Curvature Dependent Redistribution

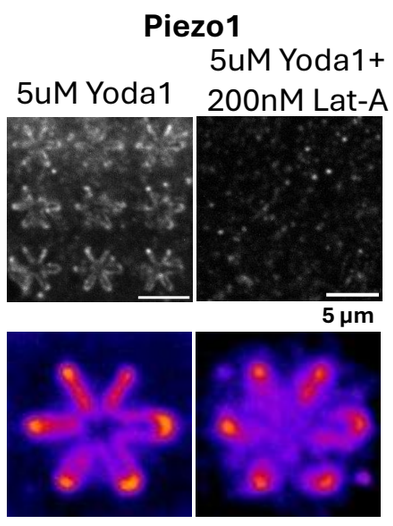


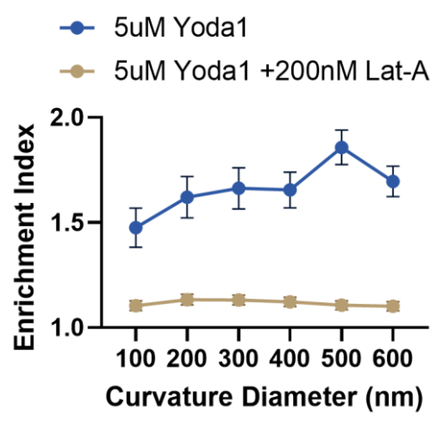

Yoda1-induced PIEZO1 redistribution is curvature-size-dependent, with stronger redistribution at smaller membrane curvature.

## Result

### 2. Cellular Components Regulate Activation-Associated PIEZO1 Distribution

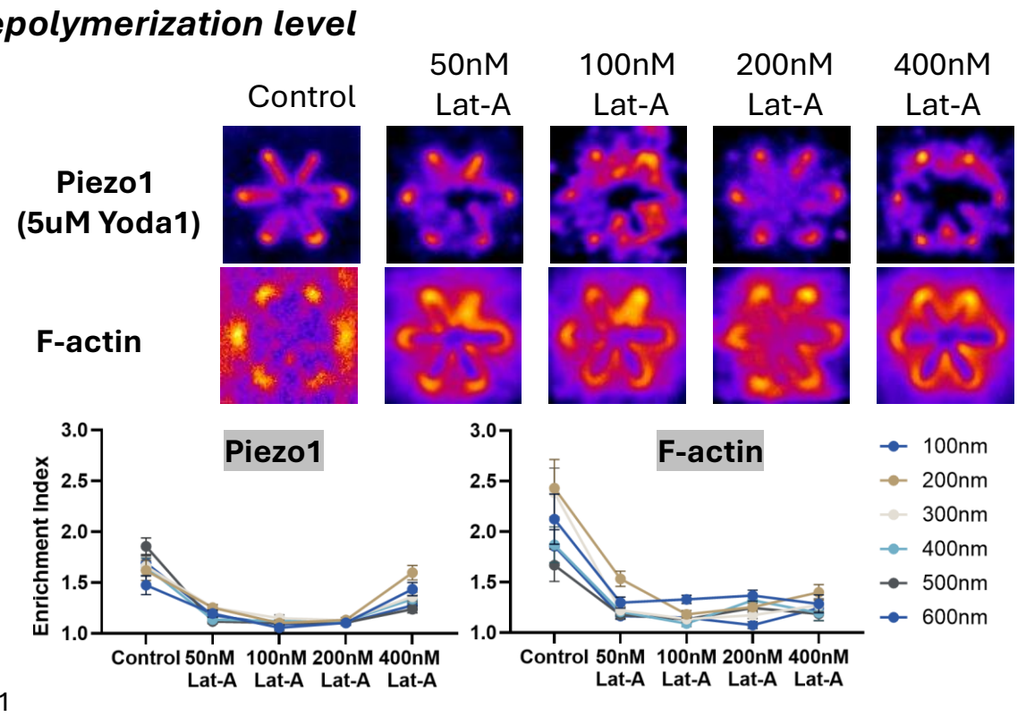
#### *a. Actin Cytoskeleton Remodeling Regulates Activation-Associated PIEZO1 Distribution*

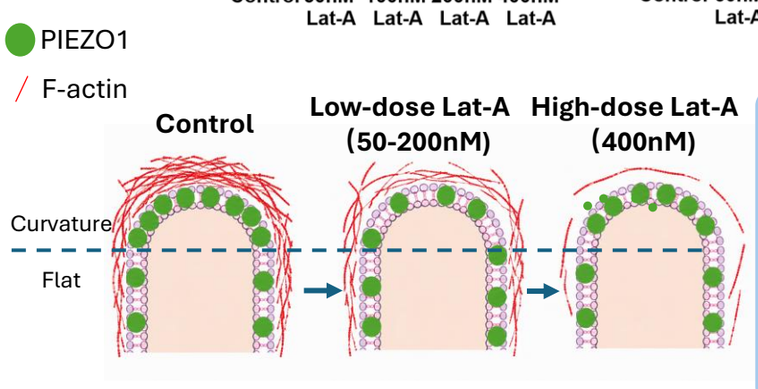



F-actin disruption reduces activation-associated PIEZO1 curvature enrichment, indicating that actin remodeling regulates PIEZO1 distribution upon activation.

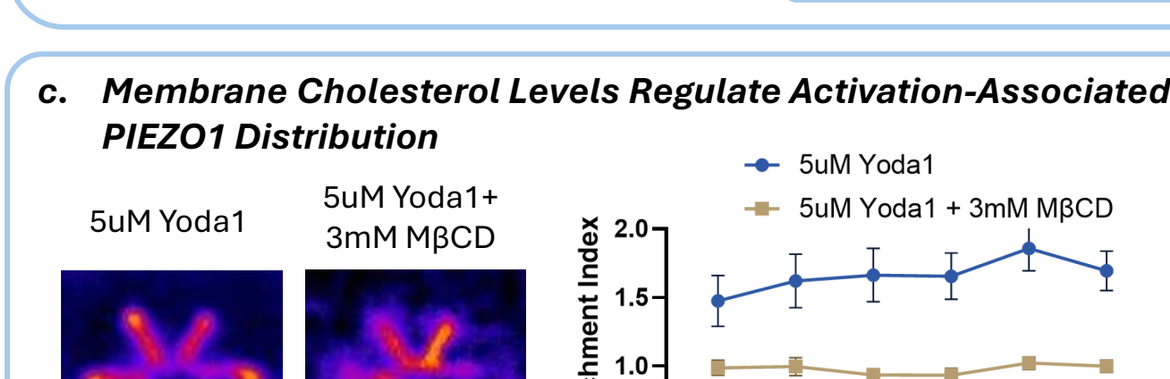
#### *b. PIEZO1 Distribution was Modulated by Actin Cytoskeleton Depolymerization level*




Actin-mediated diffusion confinement may biphasically modulates PIEZO1 curvature enrichment under Lat-A induced cytoskeleton depolymerization.

#### *c. Membrane Cholesterol Levels Regulate Activation-Associated PIEZO1 Distribution*




Membrane cholesterol depletion significantly suppresses activation-associated PIEZO1 curvature enrichment, suggesting that cholesterol is important for maintaining curvature-dependent PIEZO1 distribution.

## Conclusion

In this work, our results demonstrate the pre-existing membrane geometry spatially biases activation-associated PIEZO1 redistribution, and perturbing of the actin cytoskeleton and membrane cholesterol altered the curvature-dependent distribution of PIEZO1 following activation. Collectively, our findings reveal that PIEZO1 redistribution upon activation is **governed by a curvature-dependent spatial framework shaped by both nanoscale membrane geometry and the local membrane environment**. This work establishes engineered nano-topography as a powerful platform to dissect the spatial mechanobiology of mechanosensitive ion channels in living cells.

## Acknowledgement

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