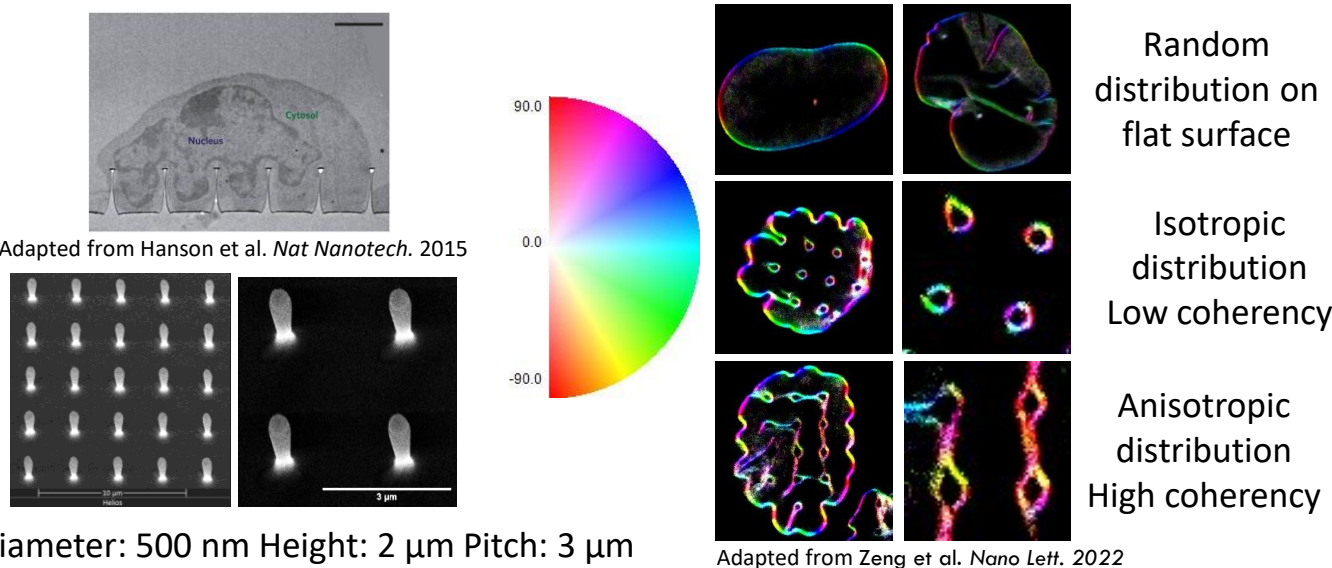


## 1. Introduction

Cellular senescence is a key driver of skin aging, one of the earliest and most visible manifestations of organismal aging<sup>1</sup>. At cellular level, one of the key markers is the abnormal nuclear morphology<sup>2,3</sup>. However, the subnuclear features are distributed randomly and difficult to quantify. Here we used vertically aligned nanopillar arrays to guide the regular nuclear deformation for senescence cells differentiation. Then we correlated the patterns with key senescence markers at single cellular level. To further investigate the mechanisms that cause the abnormal nuclear morphology, systematic mutants were employed for further characterization.

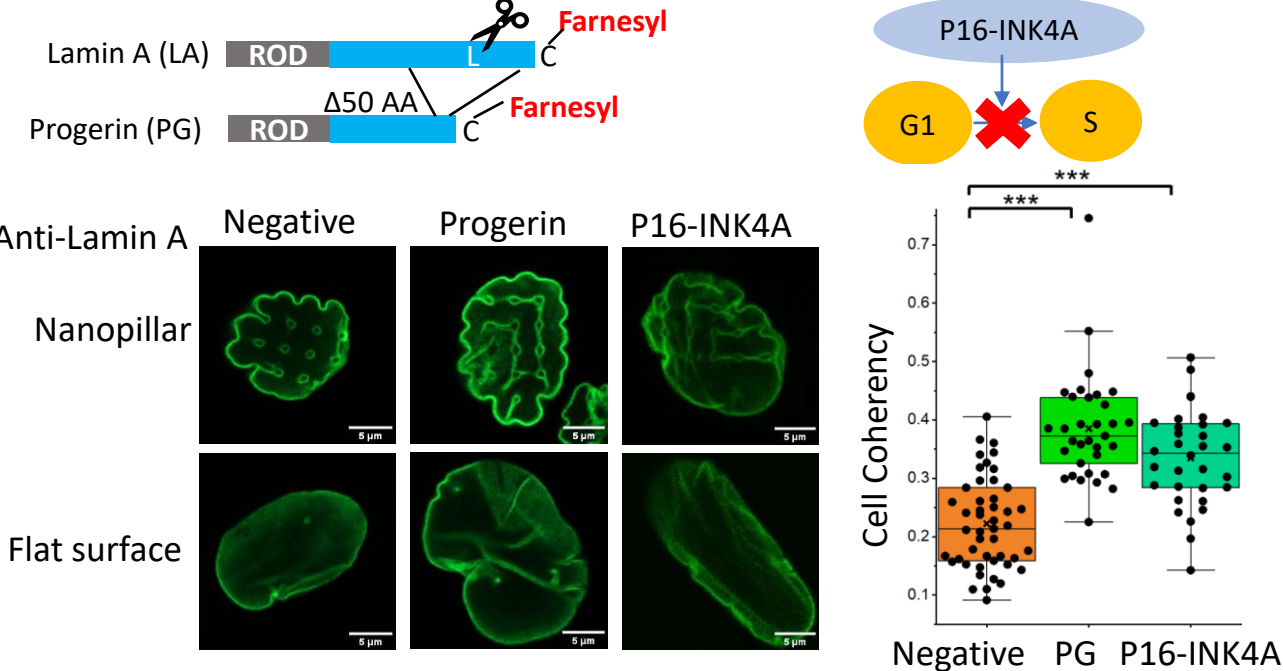
## 2. Nanopillar-induced nucleus deformation<sup>4,5</sup>



## 3. Nanopillar enabled quantitative differentiation of senescent skin cells across multiple models

### Model 1: Progerin induced senescence.

### Model 2: Replicative senescence



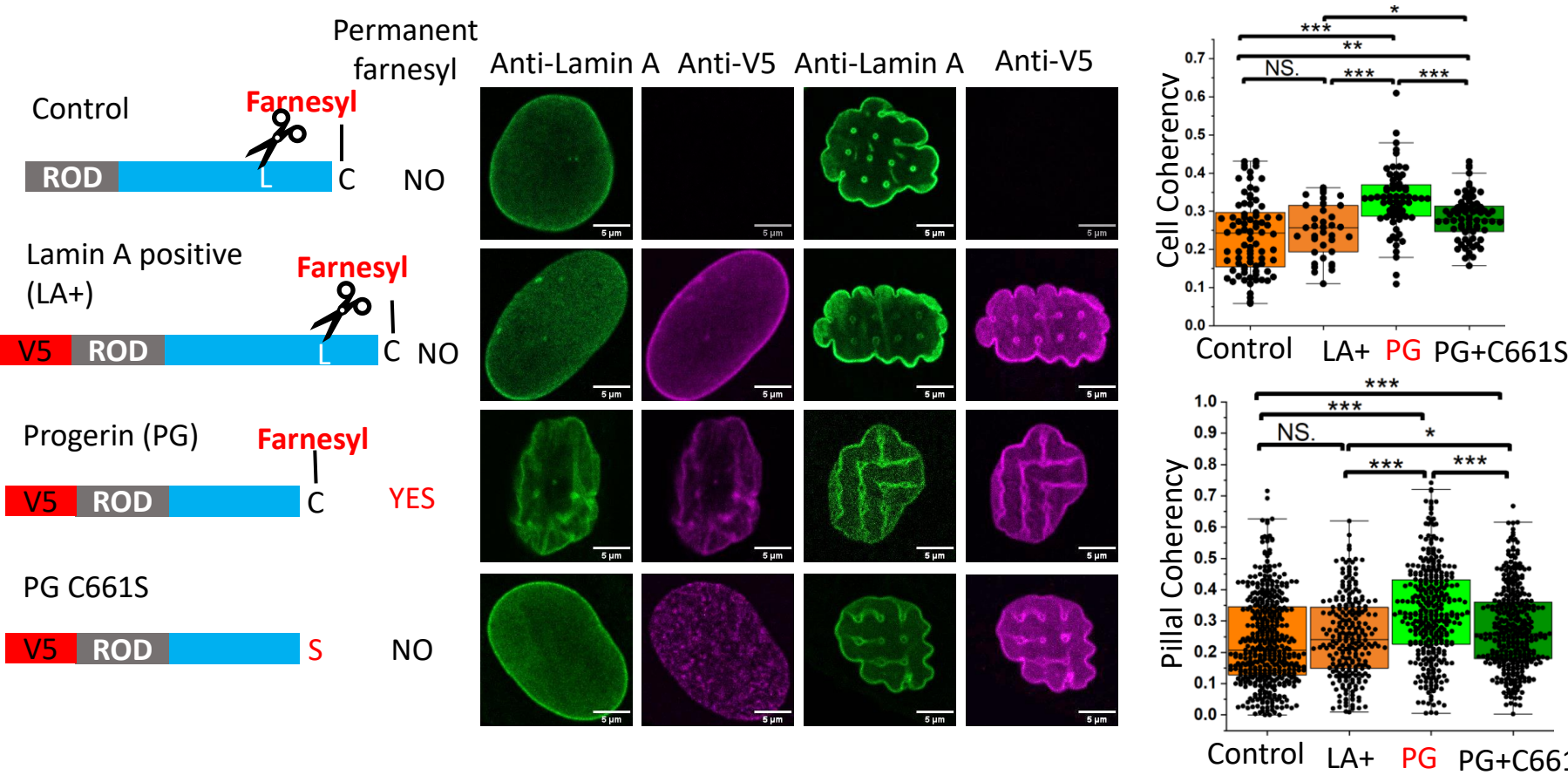
Senescent cells have higher coherency and more line patterns.

## 5. Permanent farnesylation results in more line patterns and the deterioration of the nuclear structures

### Systematic Lamin mutants separate the farnesylation and cleavage sites<sup>6</sup>

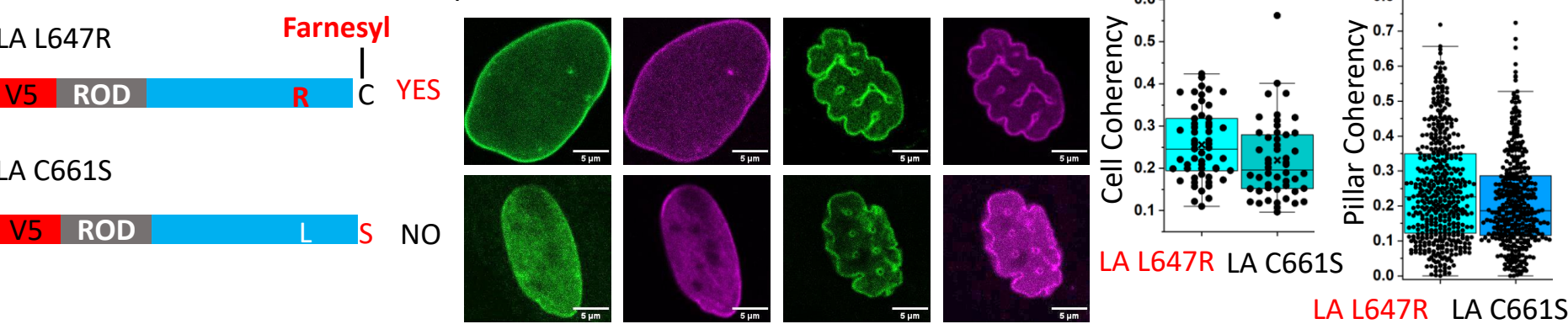
#### 1: Progerin vs PG C661S.

Progerin with permanent farnesyl have more line patterns and higher coherency.



#### 2: LA L647R vs LA C661S.

LA L647R with permanent farnesyl have more line patterns and higher coherency.



## 6. Summary

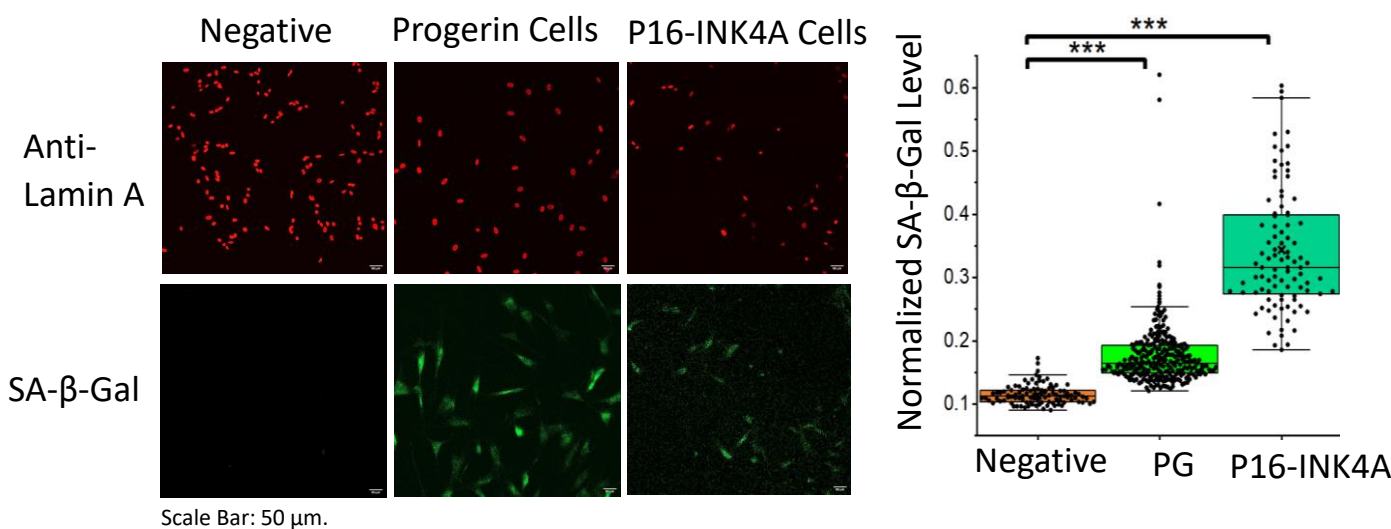
- Nanopillar is able to characterize cells with different senescence models of skin aging, senescent cells with abnormal nuclear morphology have more line patterns and higher cell coherency level.
- Cell coherency can be correlated with DNA methylation intensity at single cellular level in different senescence models.
- Systematic Lamin A mutants have similar nuclear morphology on flat surface but have different response on nanopillar.
- Mutations with permanent farnesyl have more line patterns and higher cell coherency, indicating that the permanent farnesyl is one of the key contributors to deteriorate the nuclear morphology.
- Mutants with permanent farnesyl prefers to accumulate more on line patterns.

**References:** (1) Park et al. *Biogerontology* 2022 (2) Sandre-Giovannoli et al. *Science* 2003 (3) Pathak et al. *Ageing Res. Rev.* 2021 (4) Hanson et al. *Nat Nanotech.* 2015 (5) Zeng et al. *Nano Lett.* 2022 (6) Foo et al. *Aging Cell.* 2024.

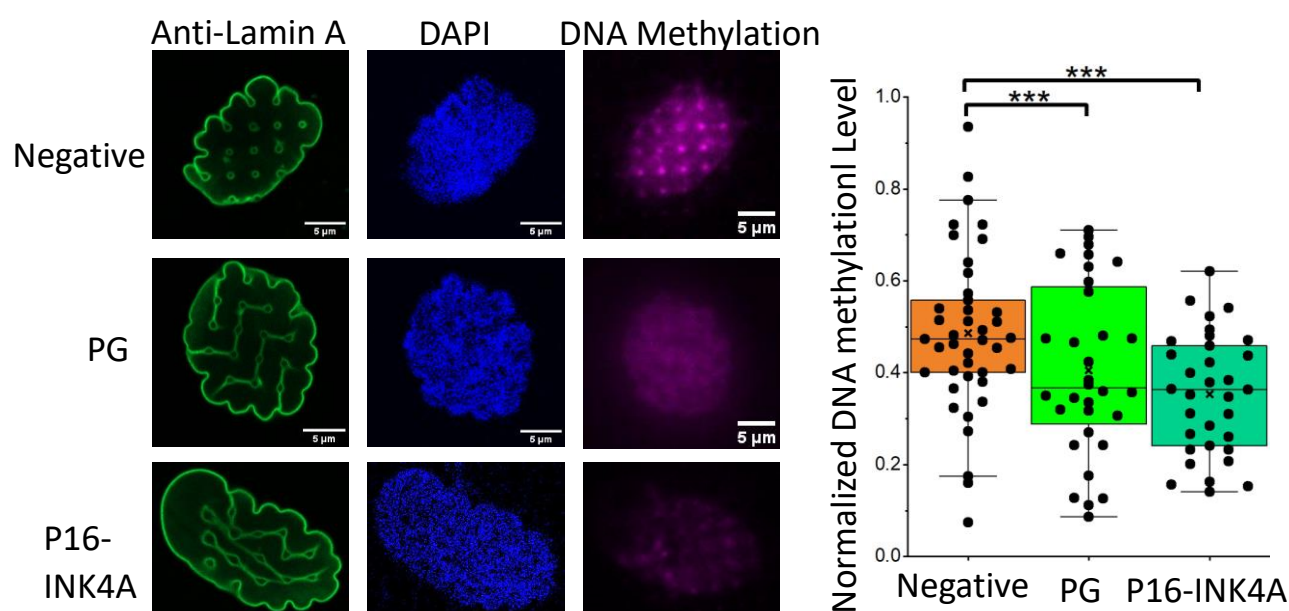
**Acknowledgements:** This work is supported by the Singapore Ministry of Education (MOE), MOH-HLCA, IDMxS, Centre for Disruptive Photonic Technologies and Nanyang NanoFabrication Center. MFAC is supported by the NTU Presidential Postdoctoral Fellowship.

## 4. Nanopillar-guided subnuclear deformation patterns correlate with key senescence markers.

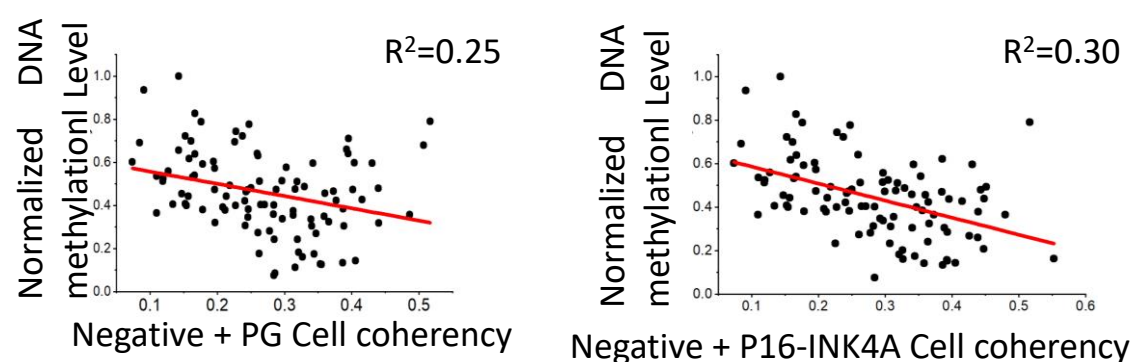
At overall population level, senescent cells have higher level of SA- $\beta$ -Gal.



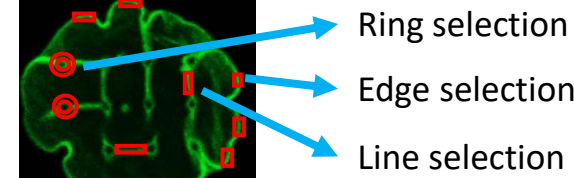
At overall population level, senescent cells have lower level of DNA methylation.



At single cellular level, coherency is correlated with DNA methylation level.

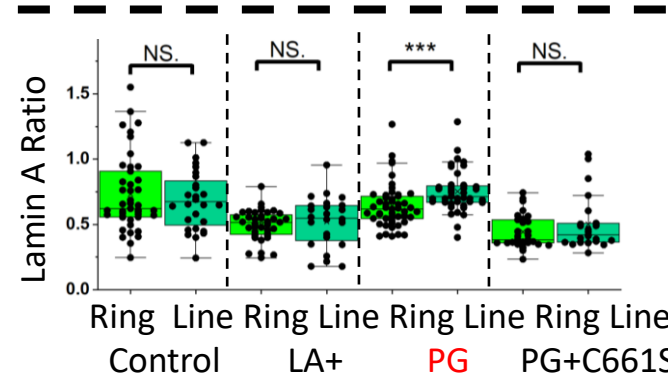


### Quantification of enrichment

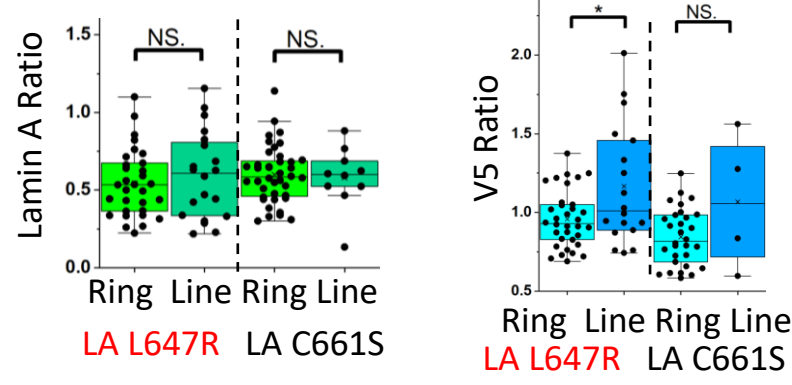
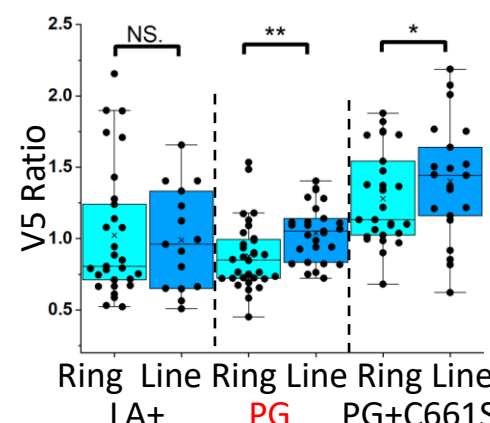


$$\text{Ratio} = \frac{\text{Intensity of ring or line}}{\text{Intensity of edge}}$$

|                     |      | Intensity | Ratio |
|---------------------|------|-----------|-------|
| Ring, line features | Edge | 15121     | N.A.  |
|                     | Ring | 11139     | 0.737 |
|                     | Line | 15595     | 1.031 |
| Ring features only  | Edge | 15278     | N.A.  |
|                     | Ring | 8361      | 0.548 |
|                     | Line | N.A.      | N.A.  |



Progerin with permanent farnesylation relatively accumulate more on line patterns.



NS. Non significant, \* p<0.05, \*\* p<0.01\*\*\* p<0.001