

DR. YINSHENG WAN'S LABORATORY

The Investigation of
Cell Signaling
Components Affected
by Microneedles in
Cultured Human Skin
Keratinocytes

Alexandra Delano and Angelika Kartsagoulis

Background



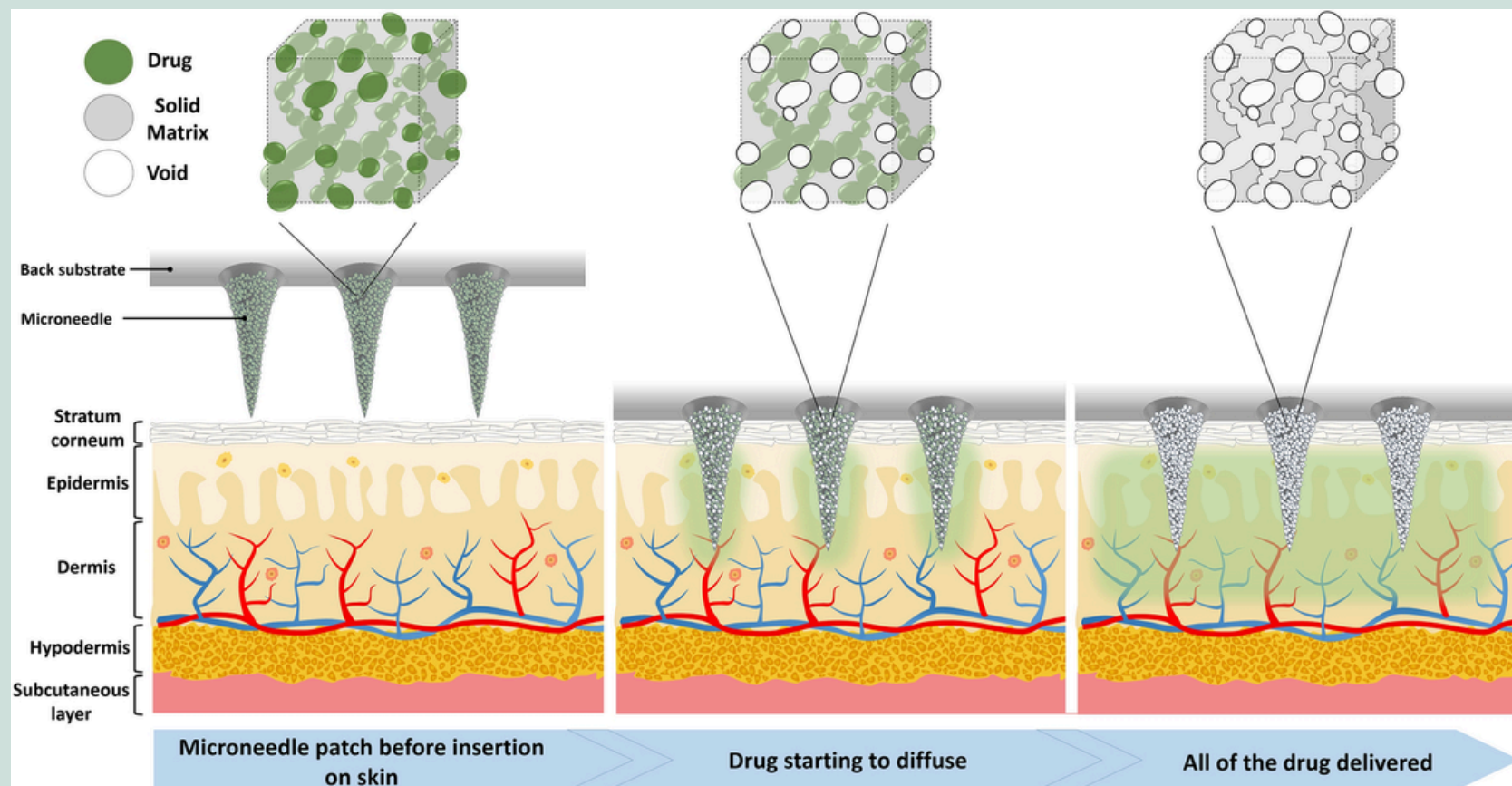
- Microneedles aid the delivery of drugs, genes, and vaccines through human skin.

- Within the dermatology field, microneedles have been in vogue in cosmetics.

- Wrinkles, aging, scarring, and much more has been shown to improve dramatically with microneedles.

- Previous studies have shown that microneedles do not alter skin appearance, barrier function, or cause infection or inflammation.

- **There are almost no reports on the mechanisms through which microneedles induce cellular rejuvenation.**



Objectives

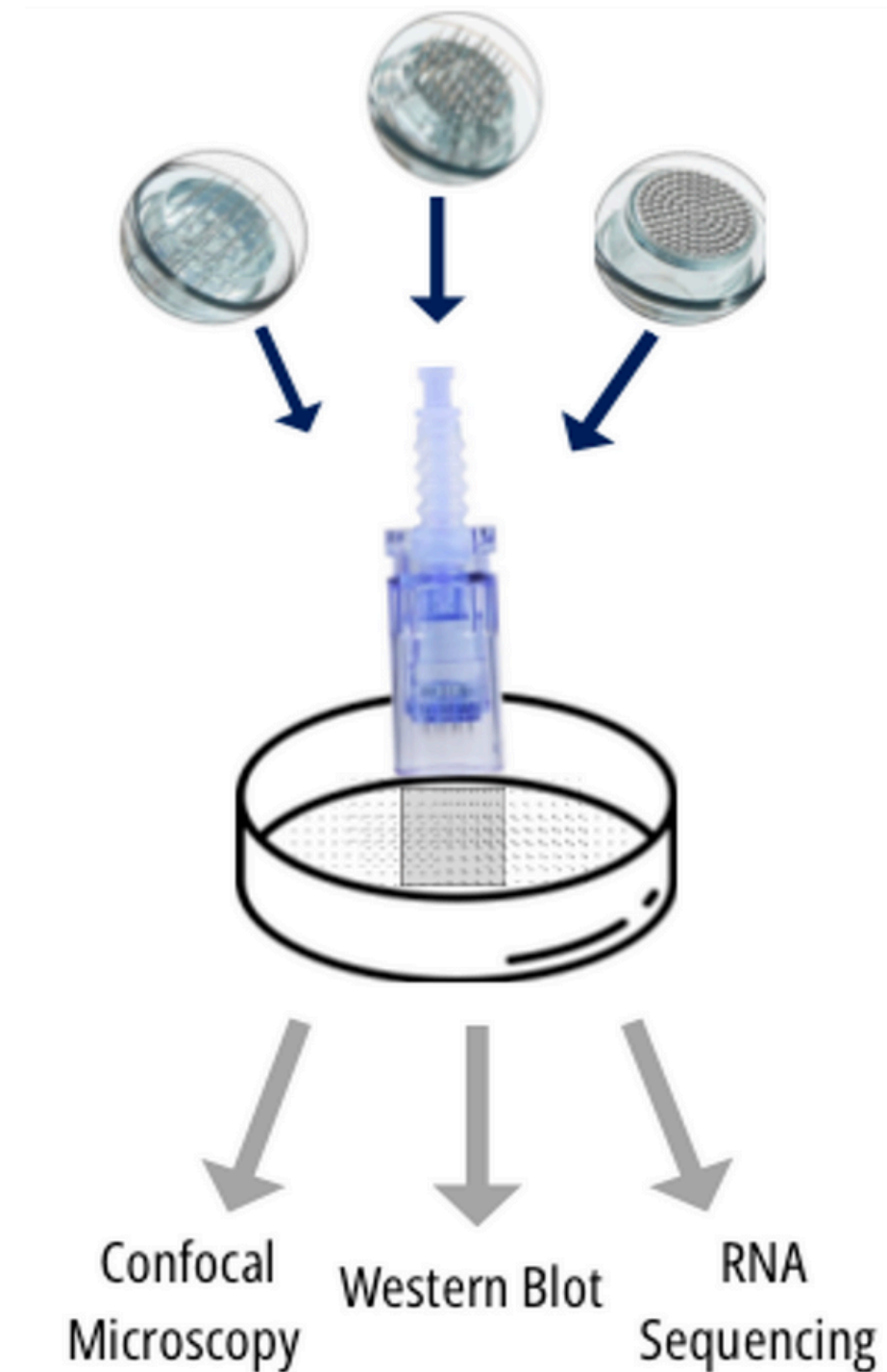
1. *To study the cell surface and mitochondrial proteins*
2. *To investigate autophagy related pathways*
3. *To study chromatin remodeling and its associated proteins*
4. *To investigate the differentiated gene expression, specifically with SMAD6 and CHCHD10.*

Given that microneedles exert beneficial effects on human skin, we hypothesize that microneedles induce skin cell rejuvenation via modulation of cell signaling pathways.

Materials & Methods

Cell Culture of Human keratinocytes (HaCaT cells) :

- Cultured in DMEM on gelatin-coated glass cover slips
- 6 and 12 well plates and treated with various microneedle pins (16, 36, or Nano)



Materials and Methods Cont.

Confocal Microscopy

Laser scanning microscope that collects images from a thin cross section of a slide or large sample.

Western Blot

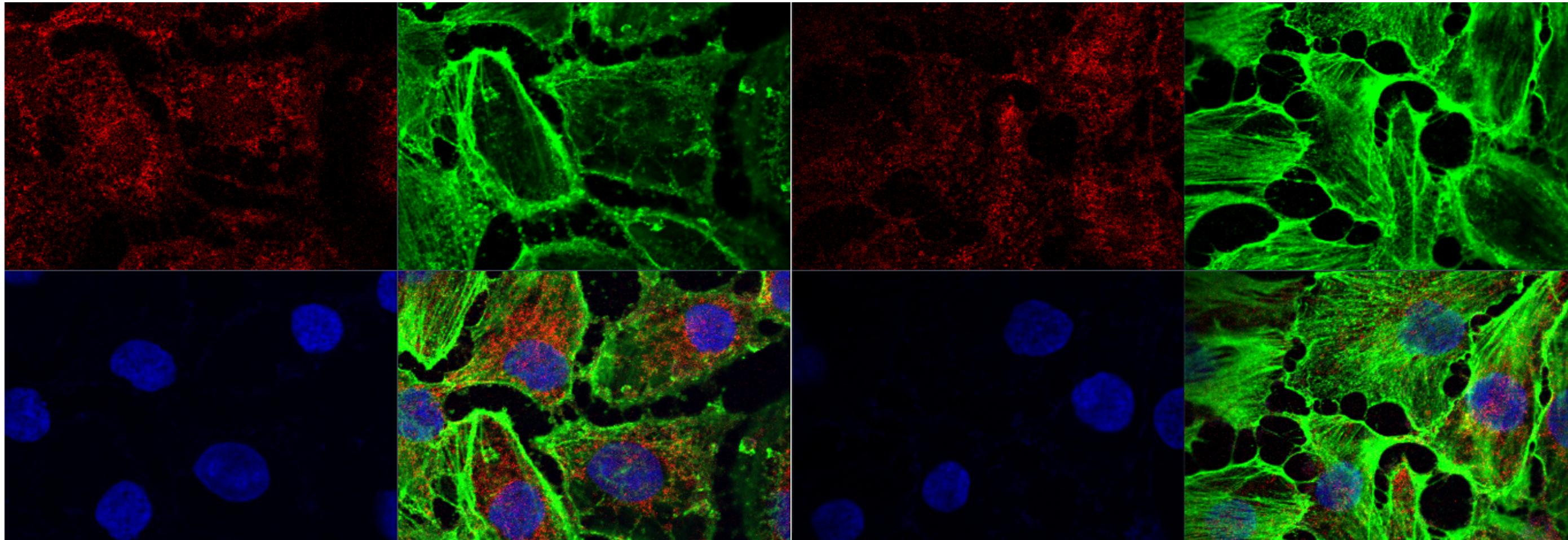
Analytical technique used to separate proteins based on molecular weight.

RNA Sequencing

Technique used to study aspects of gene expression in a given biological sample.

Results

Cytoskeleton



Control

36 Pins

Fig. 2: Confocal microscopy images of HaCaT cells with mitochondria protein mito-fusin (red) and cytoskeleton protein actin (green).

Results

Mitochondrial Activity

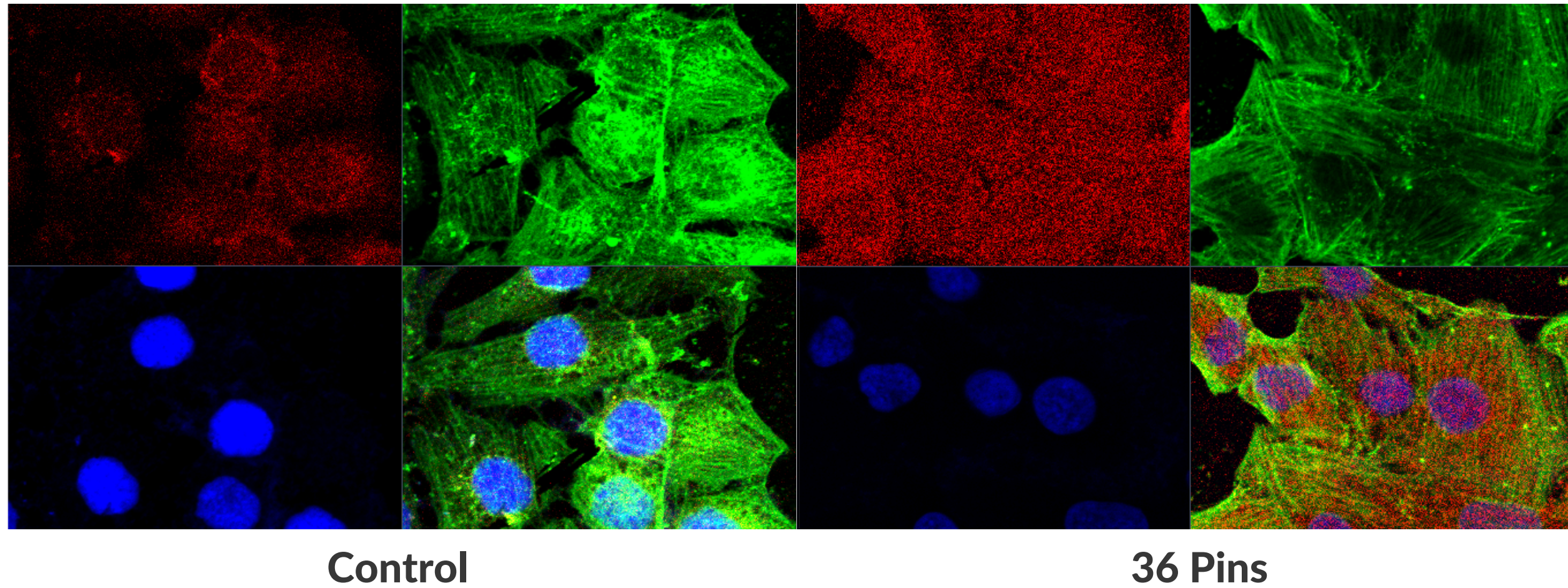


Fig. 3: Confocal microscopy images of HaCaT cells with autophagy protein ATG-5 (red).

Results

Nuclear Activity

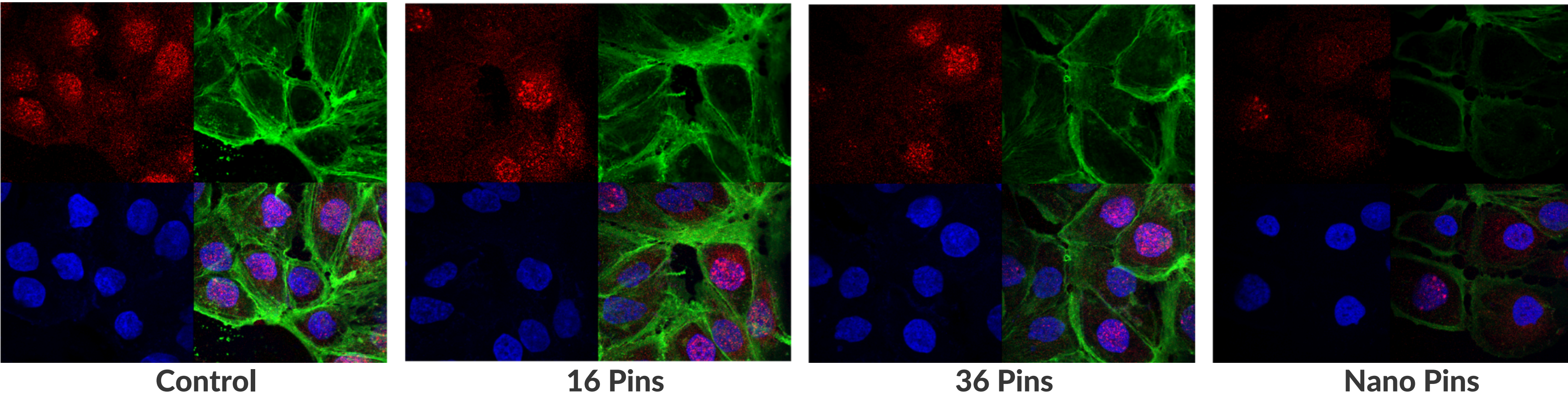


Fig. 5: Confocal microscopy images of HaCaT cells with p-Histone-3. A time course of 30 minutes showed increased p-Histone-3 with 16 and 36 pin treatment but decreased activity with nano pin treatment.

Results

Autophagy

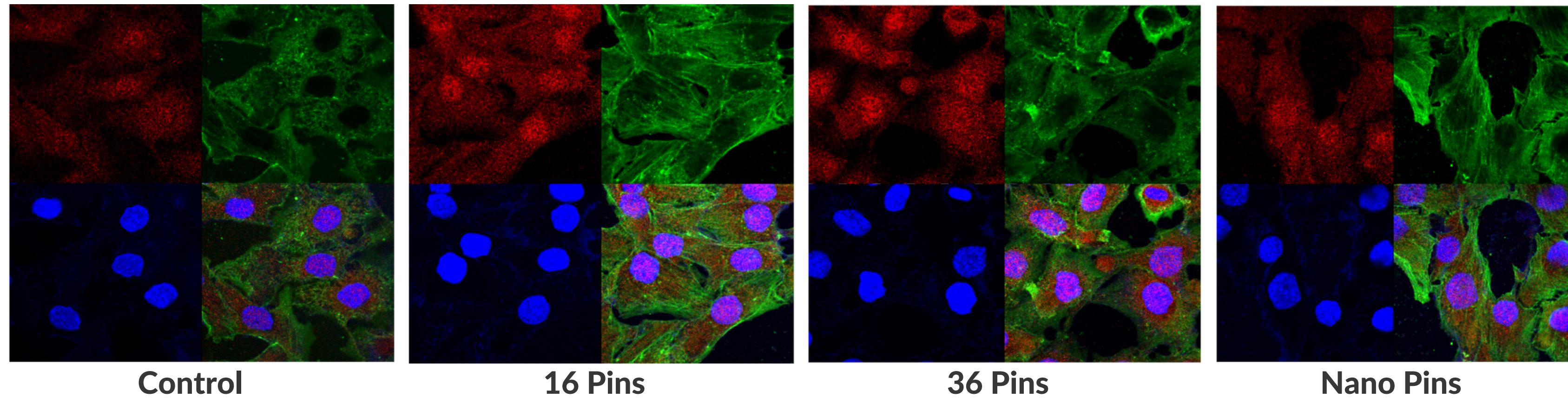


Fig. 6: Confocal microscopy images of HaCaT cells with ATG-12 (red). A time course of 4 hours showed increased nuclear translocation of ATG-12 with 16 and 36 pin treatment.

Results

RNA Sequencing

Using 500 features

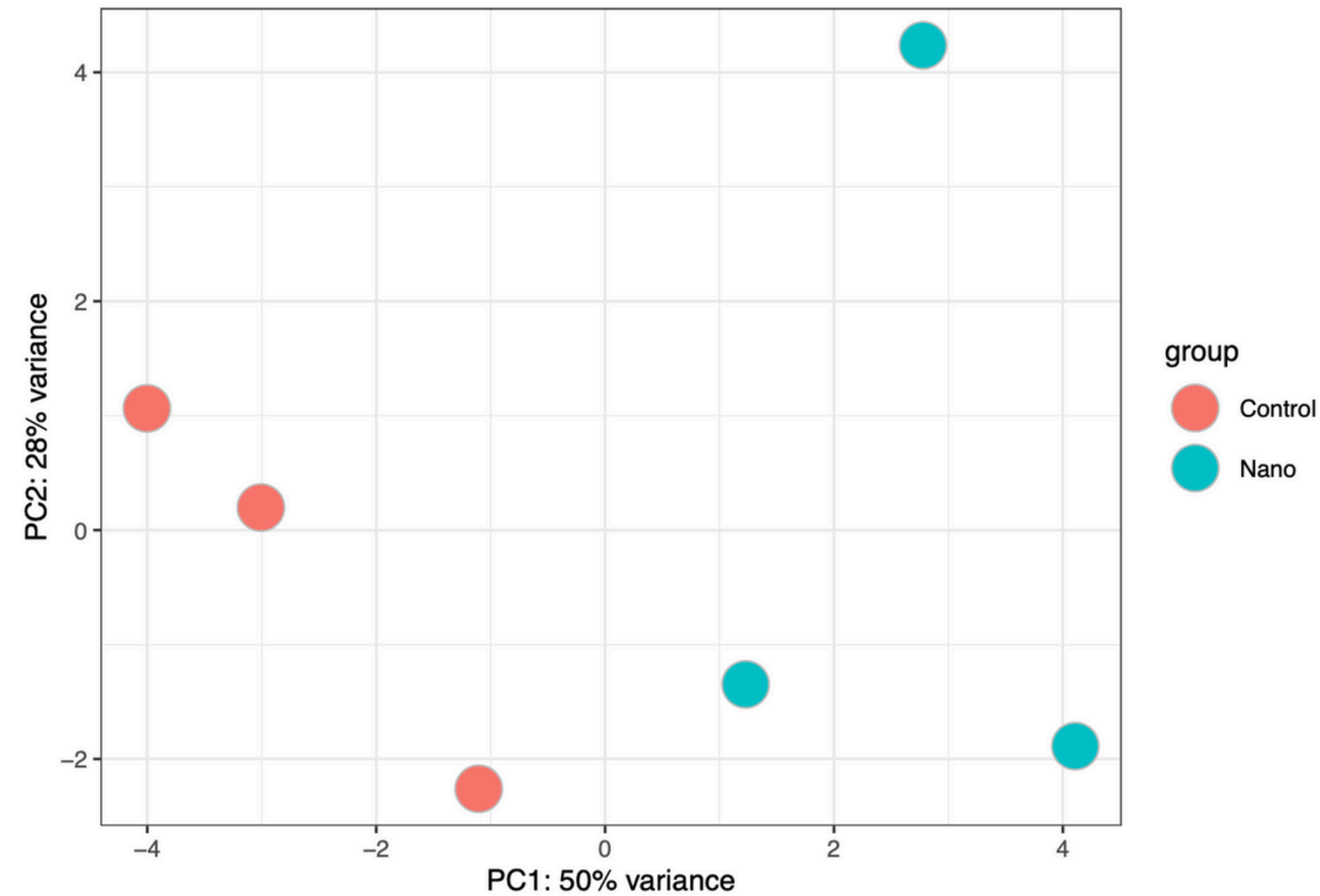


Fig. 7: Principal component analysis of control and nano samples of HaCaT cells. The separation shows sequence differentials.

Volcano plot: Nano vs. Control

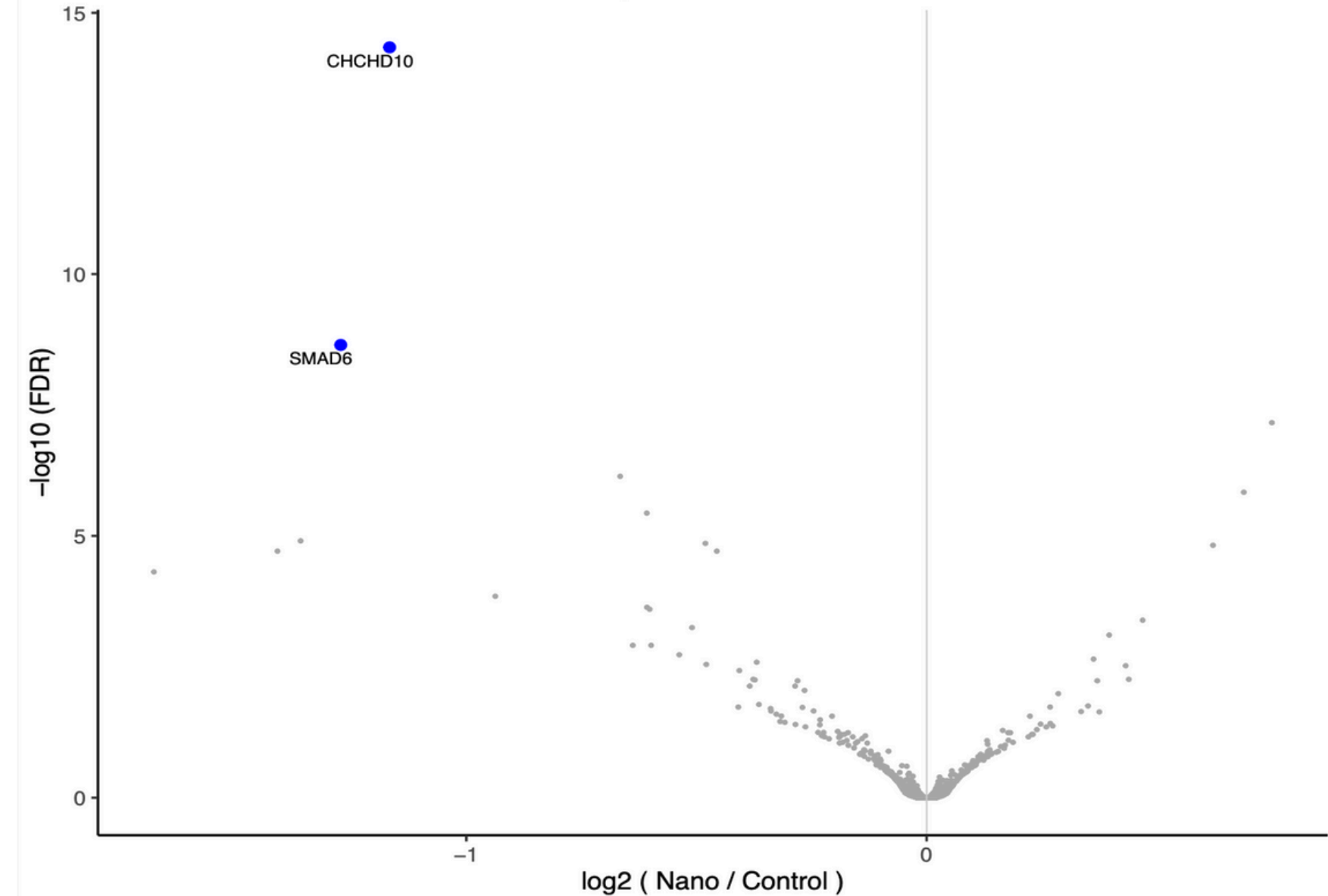


Fig. 8: Volcano plot to visualize differentially expressed genes. SMAD6 and CHCHD10, two genes associated with growth and mitochondrial function.

- Altered cytoskeleton structure
- Induced nuclear translocation of autophagy related proteins ATG-5 and ATG-12
- Increased phosphorylation of Histone-3
- Downregulation of growth related genes
- SMAD6 and CHCHD10
- Further research will delineate cell signaling pathways affected by microneedles

Conclusion

Thank You!

**A special thank you to our PI, Dr. Yinsheng Wan,
our fellow lab peers, Amy Goggin, and the entire
Center for Engaged Learning.**

Questions?