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INTRODUCTION

The loss of elasticity is a characteristic feature of skin aging. Indeed, elastic fibers are poorly renewed and are degraded much quicker than synthesized. To compensate for this imbalance, a unique extract from the traditional ayurvedic plant *Murraya koenigii* was developed.



The efficacy of the *Murraya Koenigii* extract

1 Increase of dermal proteins synthesis involved in the maintenance and integrity of extracellular matrix

A proteomic study combined with bioinformatic analysis: Objective and comprehensive review of the statistically relevant biological activities.

Number of proteins whose expression is up/down regulated in fibroblasts		
Groups (n=3 experiments)	Number of deregulated proteins (cut off p-value ≤ 5E-02)	Proteins related to "extracellular matrix" term
<i>Murraya Koenigii</i> extract	92	31 (33.7%)

ECM structure

- ELN FBN1
- FBN2 LTBP2
- LAMC1 CO1A2
- CO8A1 FBLN1
- TBB4A

ECM formation

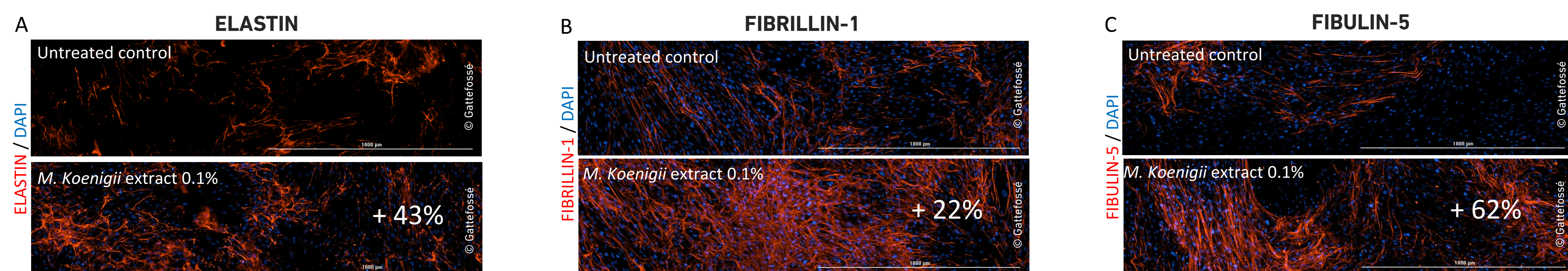
- POSTN
- LOX
- CNDP2

ECM maintenance and integrity

- IBP3 MK14 M4K4
- POSTN CERS2 TETN
- TRFL CCD80
- IBP5 GREM1 IBP4 LOX
- PZP ANT3 ELN FBN BGH3
- FBN2 ITIH5 TIMP3 AT1B1
- LTBP2 CO1A2

2 Increase of elastin and elastic fibers-associated proteins synthesis

Monolayer cultures of NHDF



3D bio-engineered skin model

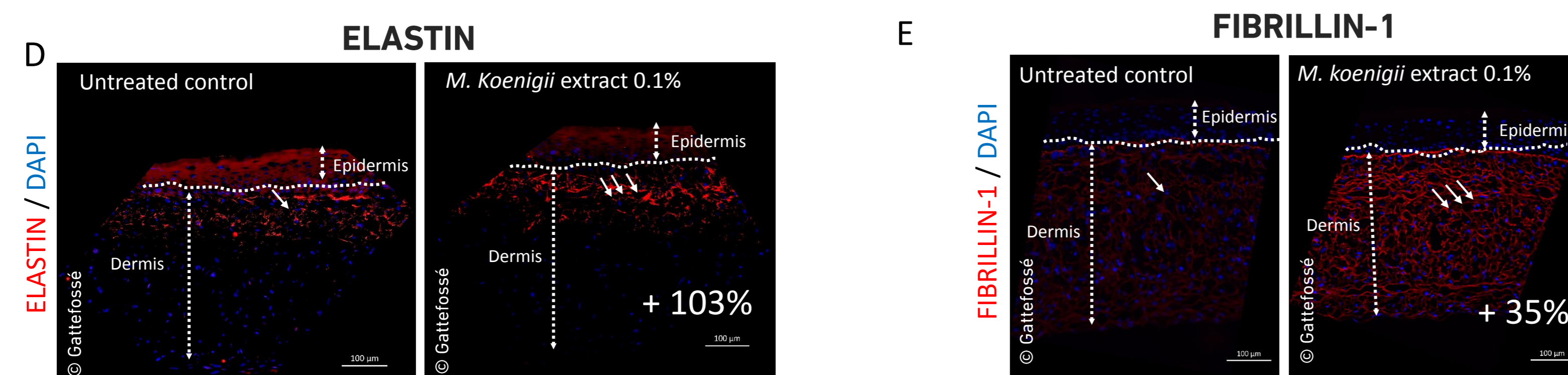


Figure 1: Immunofluorescent staining of elastin, fibrillin-1 and fibulin-5 deposits in Normal Human Dermal Fibroblasts (NHDF) monolayers cultures (A-C) treated for 48h with the *M. Koenigii* extract at 0.1%. Ratio of stained area/ROI vs untreated control. Scale bare: 100µm.

Immunofluorescent staining of elastin and fibrillin-1 deposits in 3D bio-engineered skin model (D-E) treated with the *M. Koenigii* extract at 0.1%. Four weeks of dermis reconstruction, including 2 weeks of treatment and three weeks of epidermis, including 2 weeks of treatment. Ratio of stained area/ROI vs untreated control. Scale bar: 100µm.

3 Protection of elastic fibers degradation:

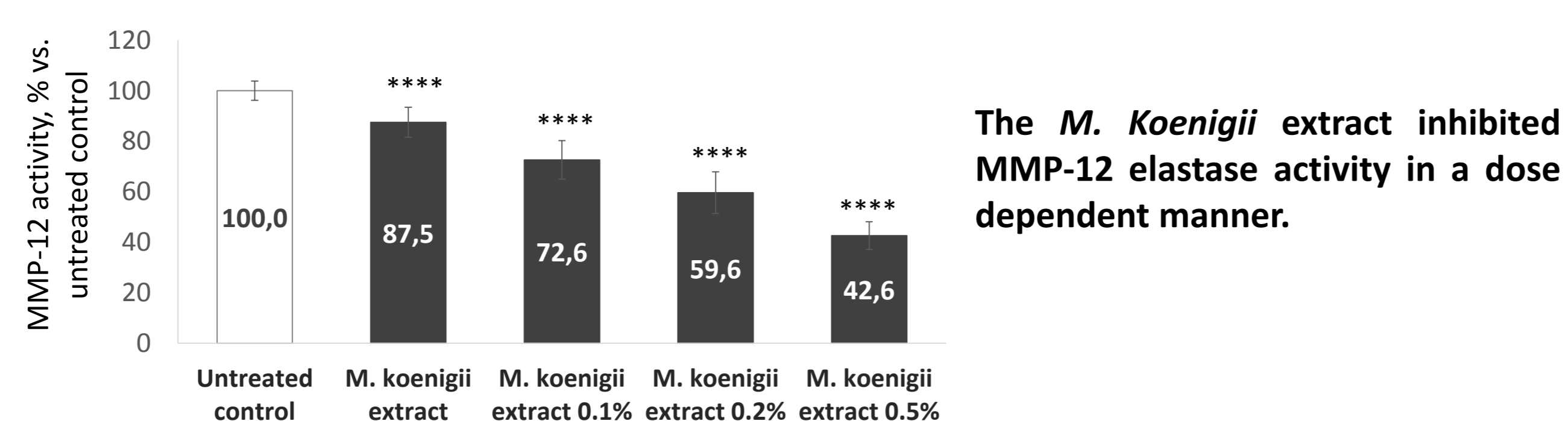
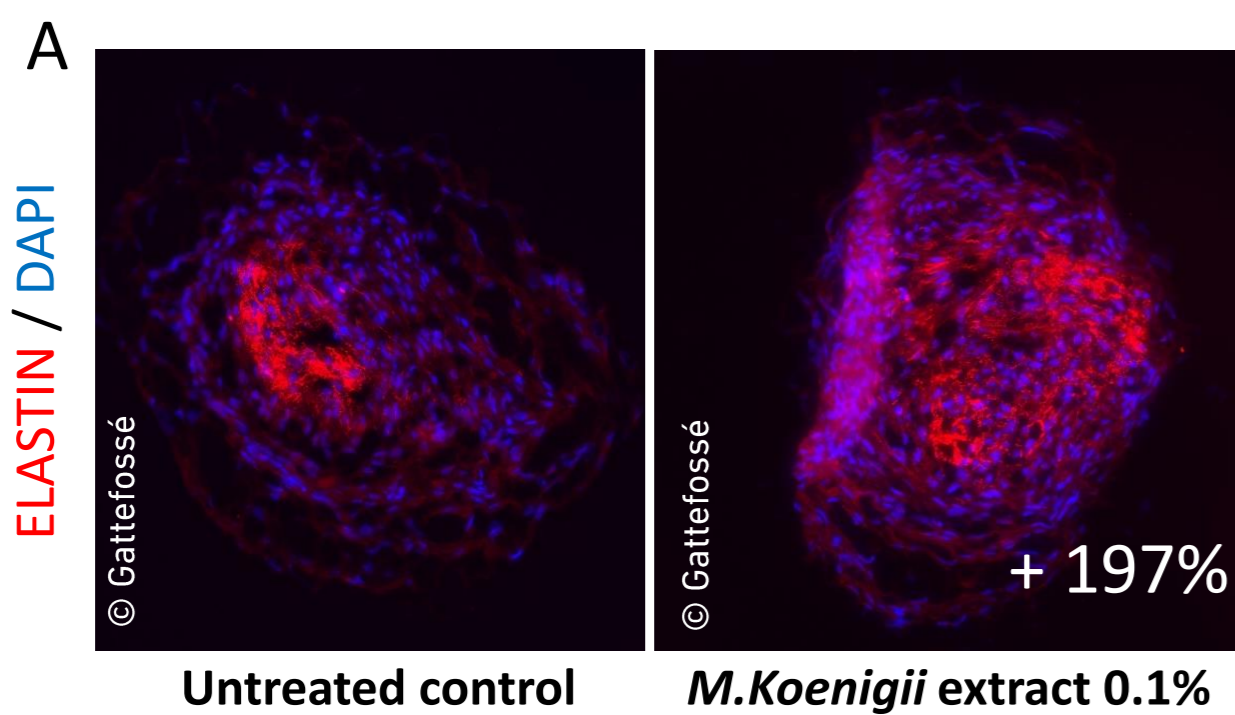


Figure 2: MMP-12 elastase activity in supernatants from Normal Human Dermal Fibroblasts (NHDF) treated for 48h with the *M. Koenigii* extract. Non parametric statistical analyses were performed with the two way-ANOVA test followed by Bonferroni's multiple comparisons test. The level of significance is given by the p-value: **** p≤0,0001.

4 Structural quality of elastic fibers neo-synthesized within a 3D scaffold-free spheroid microtissue

Immunofluorescence images



To control both the structural and functional quality of elastic fibers neo-synthesized, Gattefosse developed a 3D scaffold-free spheroid microtissue (Lorion et al., JID, 2019).

M. Koenigii extract at 0.1% increased the synthesis and *in situ* deposition of elastin within the spheroid microtissue.

Second Harmonic Generation images

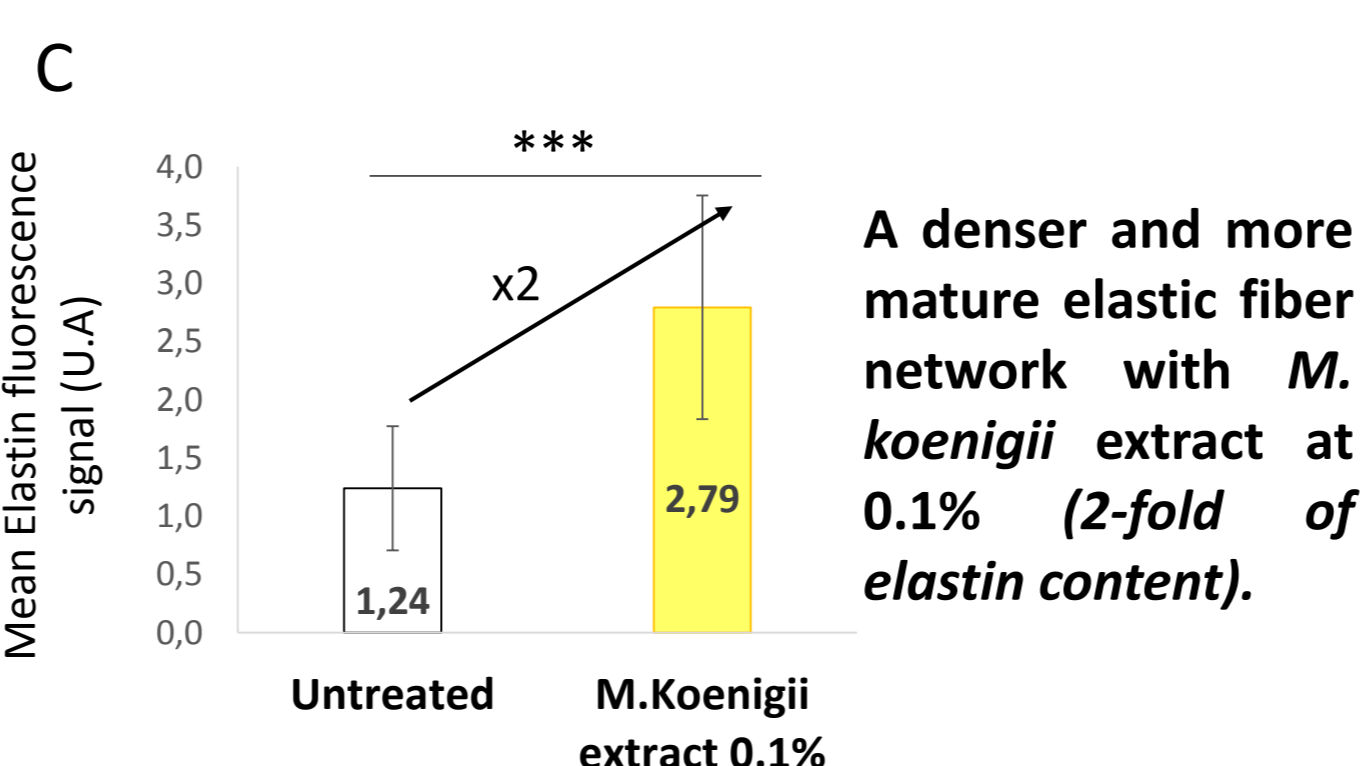
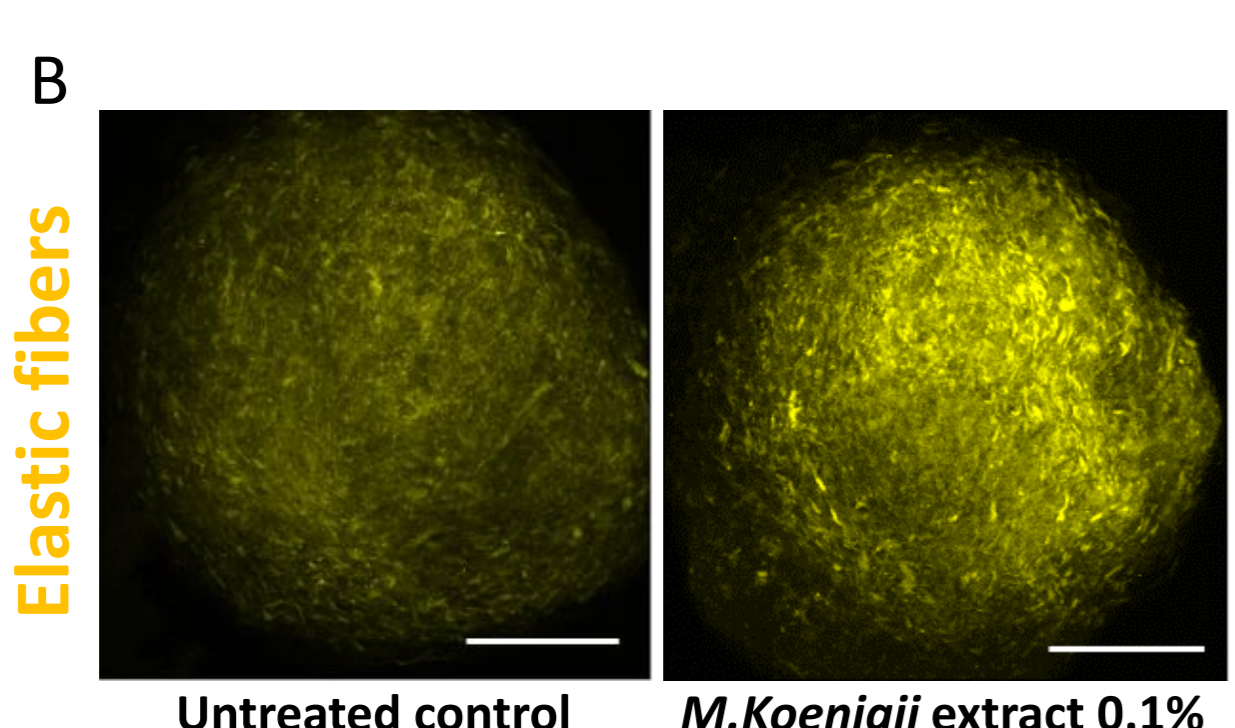


Figure 3: Spheroid microtissues generated from dermal fibroblasts and treated with *Murraya Koenigii* extract at 0.1% for 4 days (from day 8 to day 12 of culture) A. Immunofluorescent staining of elastin deposits. Scale bar: B. Z projection images of elastin deposits realized by Second Harmonic Generation Microscopy. Scale bar: 100µm. C. Analyses of the SHG images, statistical analyses were performed with the Wilcoxon test: p-value: *** p≤0,001.

5 Functional quality of elastic fibers neo-synthesized

Atomic Force Microscopy on treated spheroid microtissues

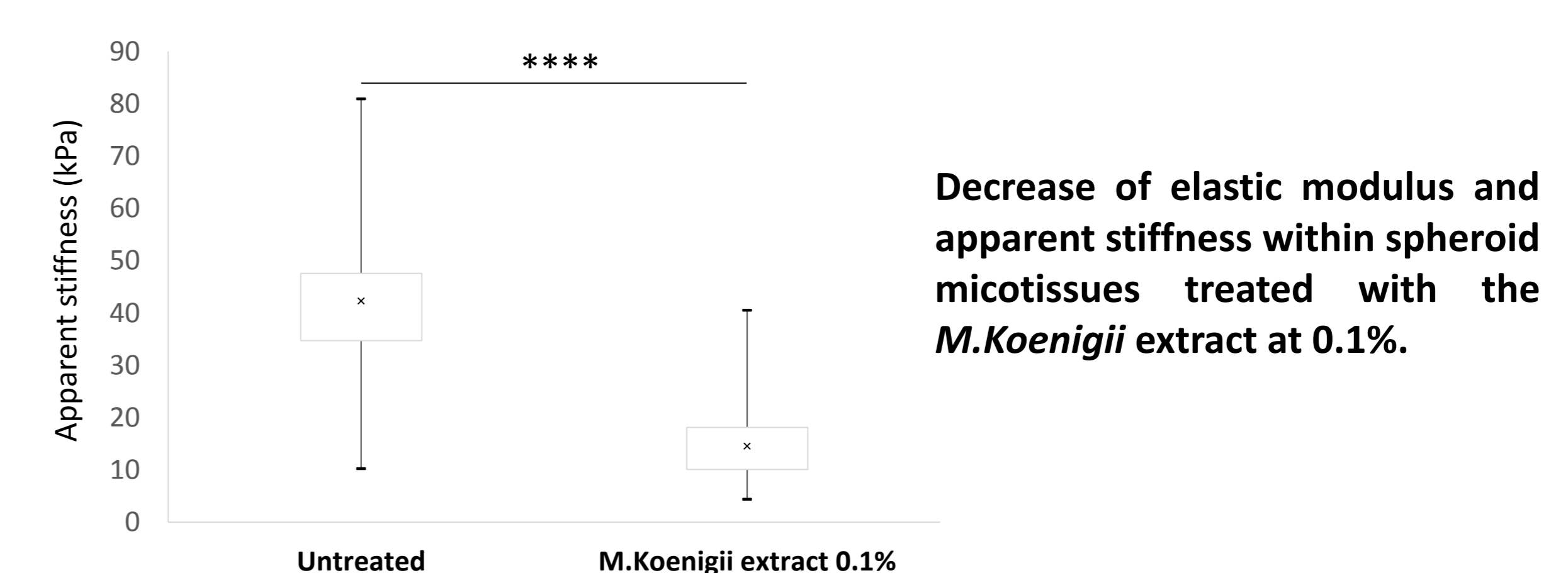


Figure 4: Distribution of apparent stiffness (kPa) extracted from spheroid microtissues generated from normal human dermal fibroblasts (NHDF) and treated with *M. Koenigii* extract at 0.1% for 4 days (from day 8 to day 12 of the culture). Non parametric statistical analyses were performed with the unpaired t test with Welch's correction. The level of significance is given by the p-value: **** p-values≤0,0001.

Immunofluorescence and AFM on treated spheroid microtissues sections

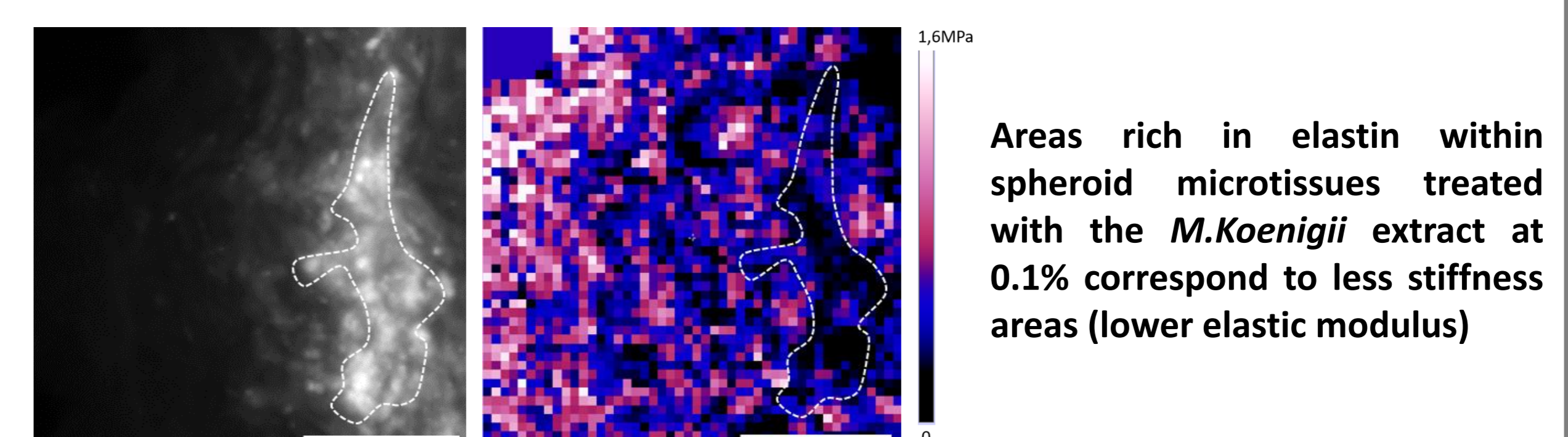


Figure 5: Immunofluorescence imaging of elastin deposits within dermis microtissues treated with *M. Koenigii* extract at 0.1% for 4 days (from day 8 to day 12 of the culture). Each image is associated with its mechanical properties map (apparent stiffness is represented in kPa). Areas rich in elastin are identified by dots and correspond to soft areas. Scale bar: 30µm.

CONCLUSION

To conclude, the *M. koenigii* extract displays undeniable elasticity boosting properties, highlighted at molecular, cellular and tissue levels, and appears as a powerful anti-aging ingredient.