

## Preclinical evaluations of the effects a foot-care formula on restoration and recovery of skin barrier integrity

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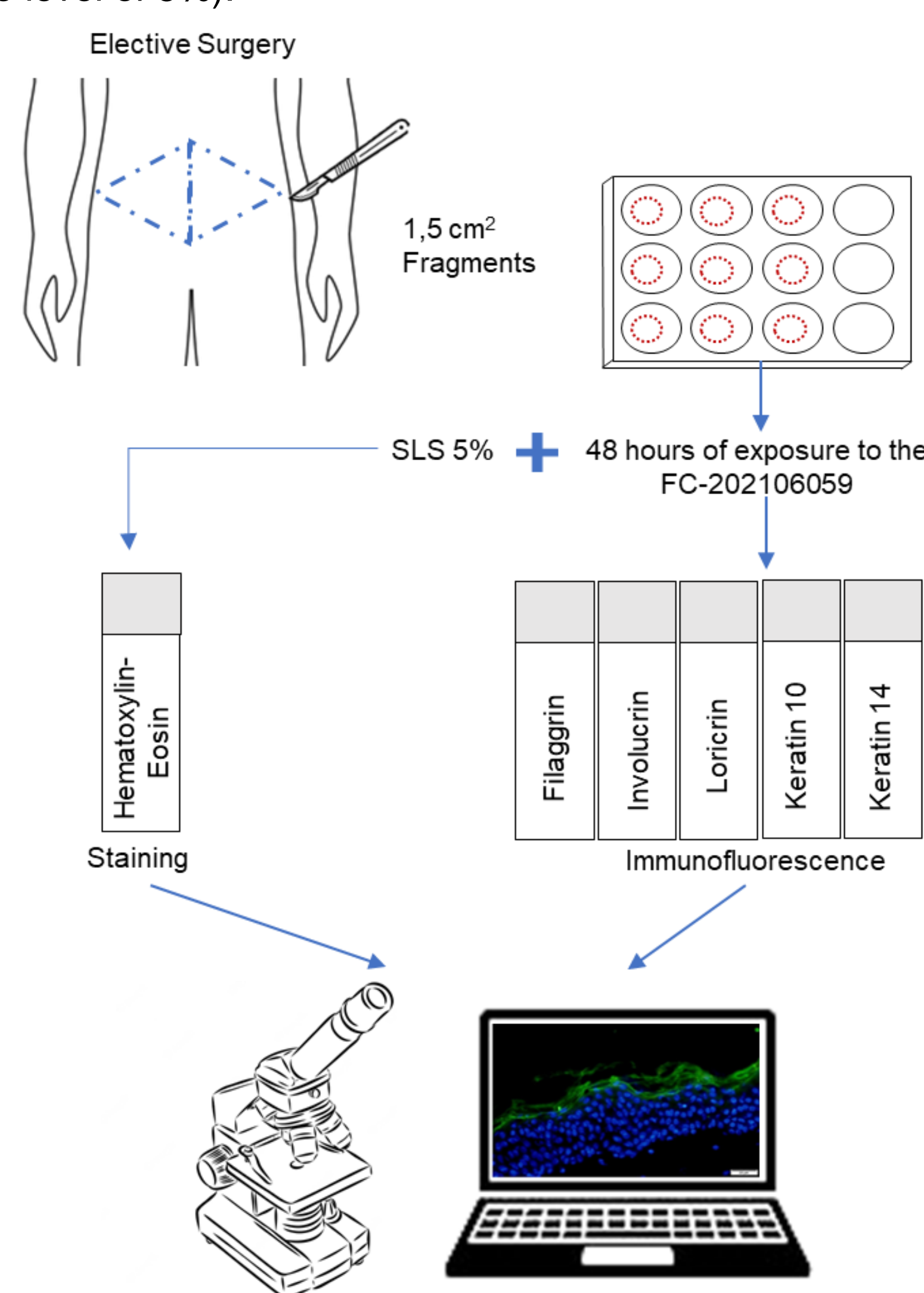
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### Introduction

The skin barrier relies on key proteins like filaggrin, involucrin, loricrin and keratins for stratum corneum formation, improve cell stability and create a protective shield that ensures skin resilience against external threats. Dysfunction in these proteins can compromise the skin barrier, leading to various dermatological issues. The purpose for this study was to evaluate the preclinical effects of a foot-care product 202106059 (FC202106059) on the production of biological markers involved in skin barrier integrity and epidermal repair through immunofluorescence analysis in skin explants.

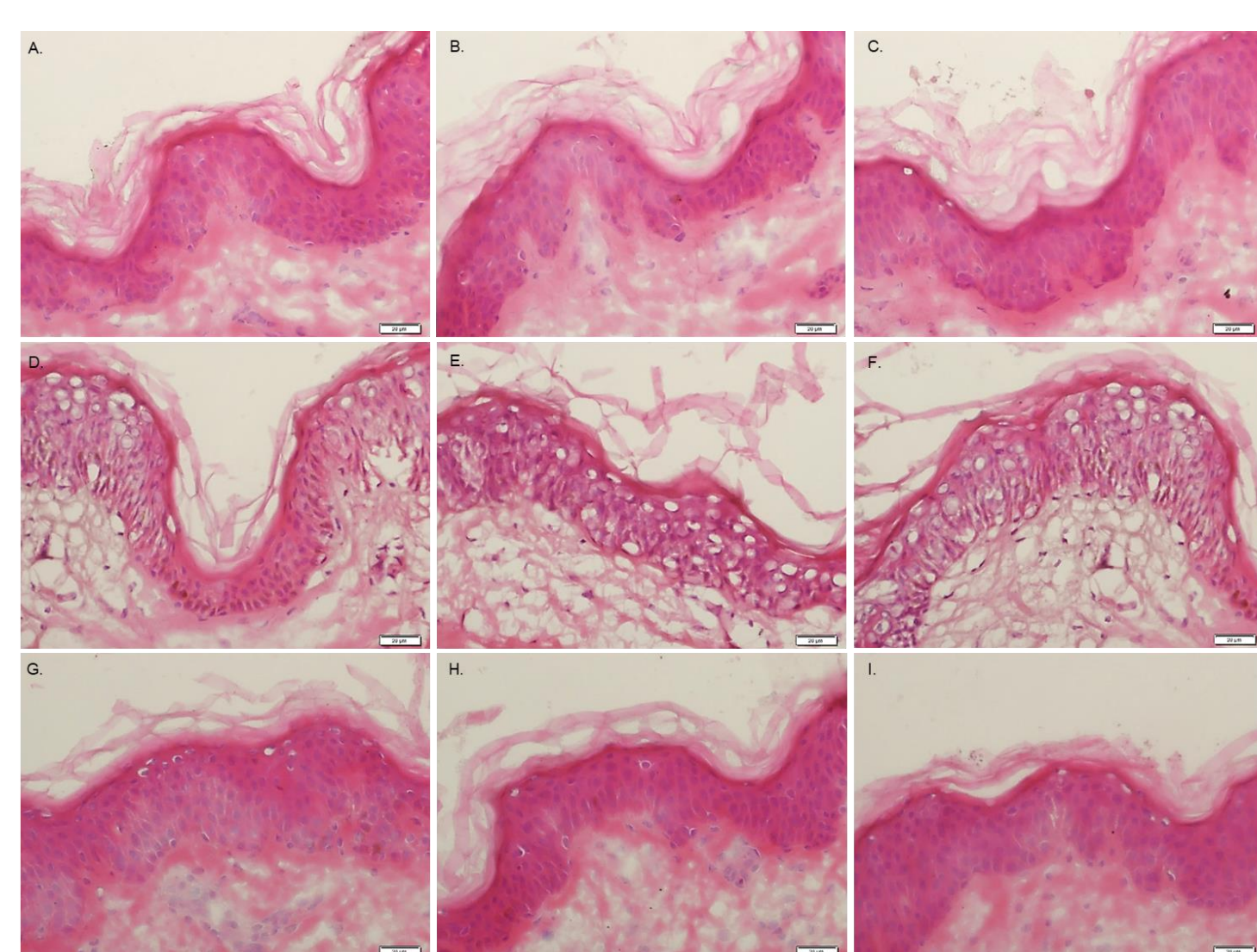
### Materials & Methods

FC-202106059 was composed by skin conditioners such as Cyclopentasiloxane, Butyrospermum parkii butter, Ricinus communis seed oil, Bisabolol, Retinyl Palmitate and Arachis hypogaea oil and antioxidants such as Tocopheryl acetate and Tocopherol. We subjected ex-vivo skin fragments, from elective plastic surgery, to barrier disruption with sodium lauryl sulfate (SLS) and treated with FC-202106059 for 48 hours for histological evaluation of epidermal repair (Hematoxylin-Eosin staining) and semiquantification of filaggrin, involucrin, loricrin, keratin 10 (K10) and keratin 14 (K14), using the immunofluorescence technique. Fluorescence intensity was quantified using ImageJ software. ANOVA and Bonferroni post-test were applied (significance level of 5%).



### Results & Discussion

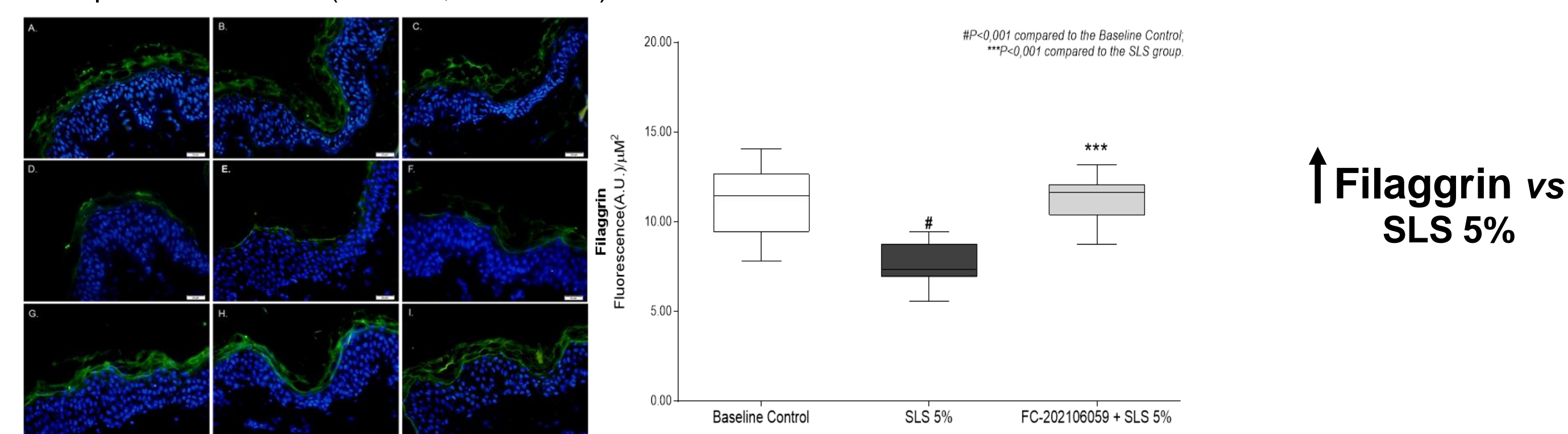
**Figure 1** - Histological evaluation with Hematoxylin-Eosin staining of epidermal repair in cultured human skin subjected to barrier disruption with 5% SLS.



**A-C** - Histological section of ex vivo skin without treatment (Baseline Control). **D-F** - Histological section of ex vivo skin after barrier disruption with SLS. **G-I** - Ex vivo histological section of skin after barrier disruption with SLS and treatment with the evaluated product FC-202106059. The reference bar corresponds to 20 µm.

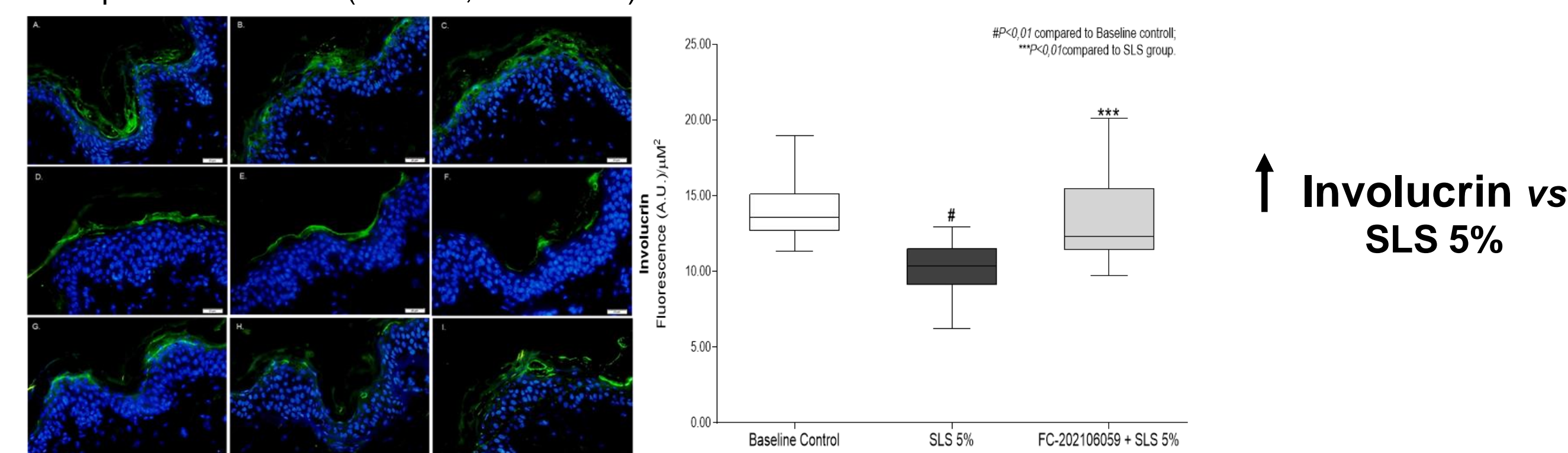
**Regeneration mainly in the epidermal region observed by the increase in cohesion between keratinocytes.**

**Figure 2** - Histological evaluation and semi-quantification of fluorescence intensity (Arbitrary Units - A.U.) of filaggrin synthesis in human skin culture subjected to barrier disruption with SLS 5%. Data represent the mean  $\pm$  range of values of 12 experimental areas (ANOVA, Bonferroni).



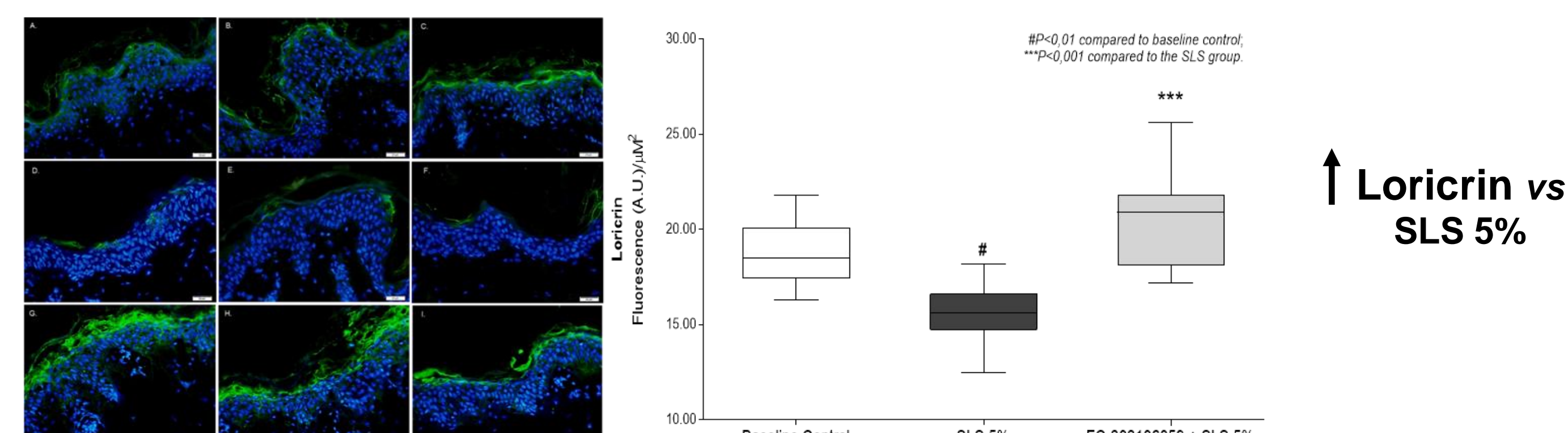
**A-C** - Histological section of ex vivo skin without treatment (Baseline Control). **D-F** - Histological section of ex vivo skin after barrier disruption with SLS. **G-I** - Ex vivo histological section of skin after barrier disruption with SLS and treatment with the evaluated product FC-202106059. Filaggrin is marked in green and the blue marking represents the cell nucleus (DNA). Reference bar corresponds to 20 µm.

**Figure 3** - Histological evaluation and semi-quantification of fluorescence intensity (Arbitrary Units - A.U.) of involucrin synthesis in human skin culture subjected to barrier disruption with SLS 5%. Data represent the mean  $\pm$  range of values of 12 experimental areas (ANOVA, Bonferroni).



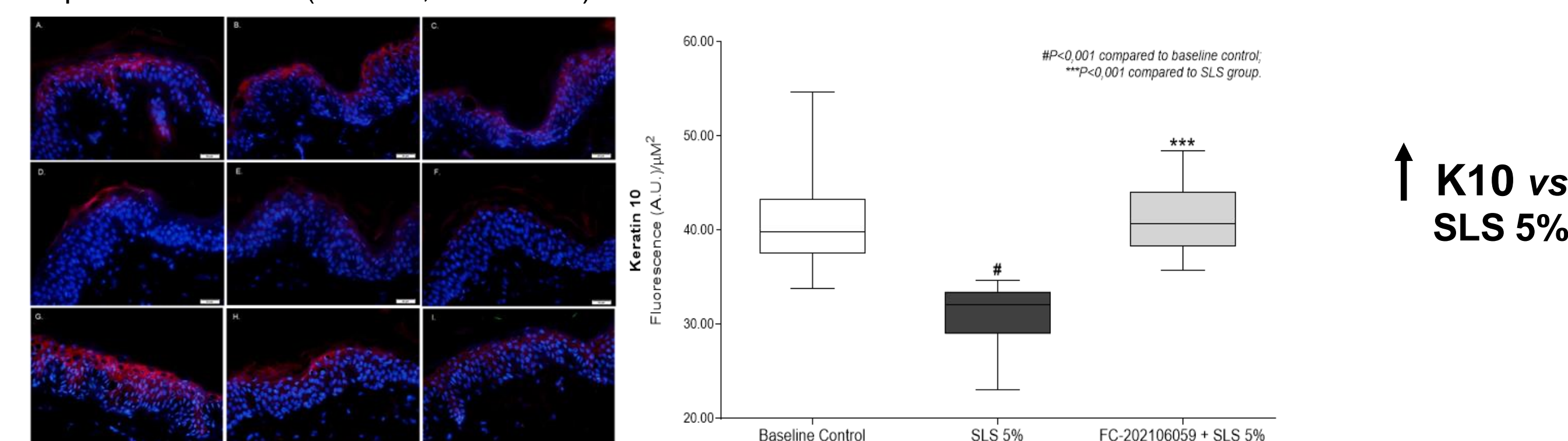
**A-C** - Histological section of ex vivo skin without treatment (Baseline Control). **D-F** - Histological section of ex vivo skin after barrier disruption with SLS. **G-I** - Ex vivo histological section of skin after barrier disruption with SLS and treatment with the evaluated product FC-202106059. Involucrin is marked in green and the blue marking represents the cell nucleus (DNA). Reference bar corresponds to 20 µm.

**Figure 4** - Histological evaluation and semi-quantification of fluorescence intensity (Arbitrary Units - A.U.) of loricrin synthesis in human skin culture subjected to barrier disruption with SLS 5%. Data represent the mean  $\pm$  range of values of 12 experimental areas (ANOVA, Bonferroni).



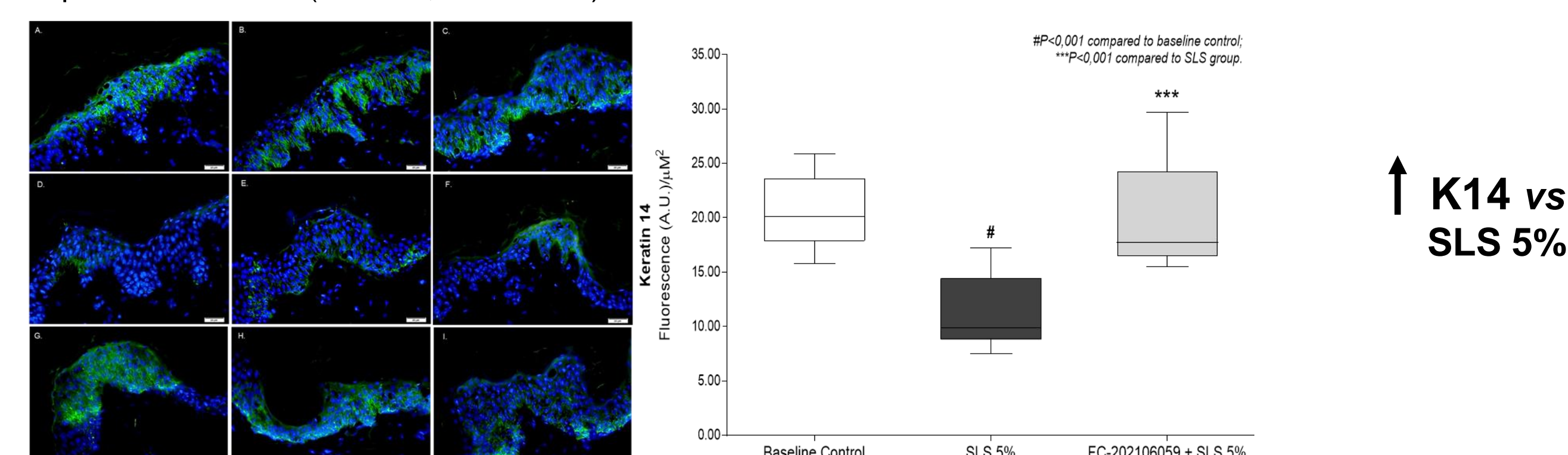
**A-C** - Histological section of ex vivo skin without treatment (Baseline Control). **D-F** - Histological section of ex vivo skin after barrier disruption with SLS. **G-I** - Ex vivo histological section of skin after barrier disruption with SLS and treatment with the evaluated product FC-202106059. Loricrin is marked in green and the blue marking represents the cell nucleus (DNA). Reference bar corresponds to 20 µm.

**Figure 5** - Histological evaluation and semi-quantification of fluorescence intensity (Arbitrary Units - A.U.) of K10 synthesis in human skin culture subjected to barrier disruption with SLS 5%. Data represent the mean  $\pm$  range of values of 12 experimental areas (ANOVA, Bonferroni).



**A-C** - Histological section of ex vivo skin without treatment (Baseline Control). **D-F** - Histological section of ex vivo skin after barrier disruption with SLS. **G-I** - Ex vivo histological section of skin after barrier disruption with SLS and treatment with the evaluated product FC-202106059. K10 is marked in red and the blue marking represents the cell nucleus (DNA). Reference bar corresponds to 20 µm.

**Figure 6** - Histological evaluation and semi-quantification of fluorescence intensity (Arbitrary Units - A.U.) of K14 synthesis in human skin culture subjected to barrier disruption with SLS 5%. Data represent the mean  $\pm$  range of values of 12 experimental areas (ANOVA, Bonferroni).



**A-C** - Histological section of ex vivo skin without treatment (Baseline Control). **D-F** - Histological section of ex vivo skin after barrier disruption with SLS. **G-I** - Ex vivo histological section of skin after barrier disruption with SLS and treatment with the evaluated product FC-202106059. K14 is marked in green and the blue marking represents the cell nucleus (DNA). Reference bar corresponds to 20 µm.

### Conclusions

We concluded that the FC-202106059 positively influenced tissue repair by boosting the synthesis of essential proteins such as filaggrin, involucrin, loricrin, K10, and K14. This increase promoted skin hydration and facilitated barrier recovery, especially after stress exposure. The results underscore FC-202106059's role in restoring and fortifying the skin barrier, preventing water loss, and protecting against external threats, thereby supporting overall skin health and integrity.

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