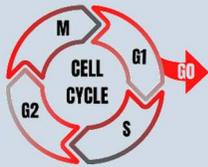


Cellular senescence is a permanent state of cell cycle arrest of aged cells, characterized by the irreversible stop of the proliferative potential of the cells. This replicative senescence state is associated with an increased lysosomal content, which is often detected as high β -galactosidase activity (SA- β -gal, for Senescence-Associated β -galactosidase). Because accumulation of senescent cells in tissues possibly promotes aged-related diseases, **senolytic** ingredients are required to specifically eliminate senescent cells without affecting the proliferative ones.



In function of the similarities with replicative senescence, StratiCELL has developed Stress-Induced Premature Senescence (or SIPS) *in vitro* models to evaluate senolytic ingredients. The models use normal human dermal fibroblasts (NHDF) treated with a unique H₂O₂ subcytotoxic stress in order to induce SIPS.

- In the first model, the capacity of senolytic ingredients is measured by SA- β -gal activity under a colorimetric assay. Data are normalized to the total number of cells in order to discriminate effect on senescent *versus* proliferative cells.
- In the second model (proposed for screening of ingredients), the ability of ingredients to induce cell death in SIPS fibroblasts and in proliferative fibroblasts is compared.

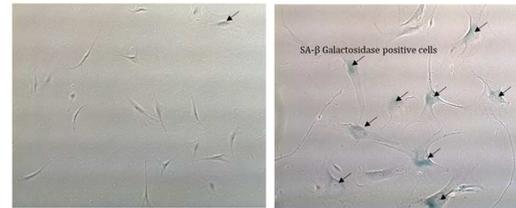
In both cases, the senolytic potential of the ingredient is compared to a positive senolytic reference molecule for full objectivation.

SKIN MODELS:

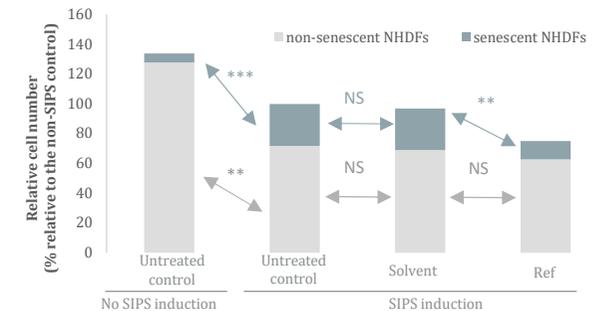
- **NHDF-SIPS:** Normal Human Dermal Fibroblast treated with a chemical premature senescence inducer, at a sub-cytotoxic dose.
- **Positive reference (Ref) available for full objectivation**

ENDPOINTS:

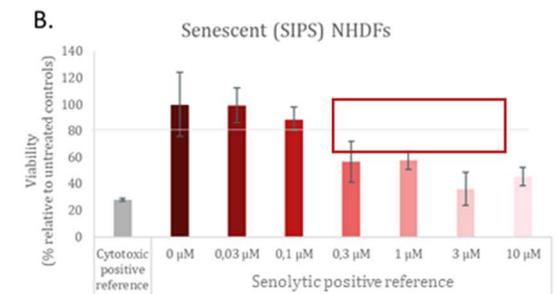
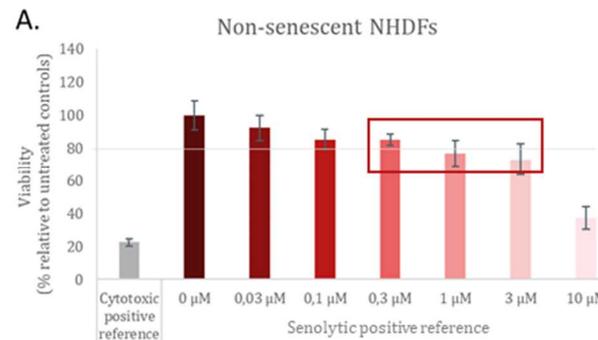
- **Senescence Associated β -Galactosidase** staining and counting
- Cytotoxicity measurement by **cytotoxicity assay**



SA- β galactosidase staining of NHDF non-SIPS (left) or SIPS-induced (right). The increase of the SA- β -galactosidase activity is detected by the use of X-gal, the chromogenic substrate of β -galactosidase (blue staining, indicated by the arrows).



Proportions of senescent (SA- β -galactosidase positive, blue bar) and non-senescent (SA- β -galactosidase negative fibroblasts, grey bar) NHDFs under SIPS induction or not, in the absence (untreated control or solvent) or presence of a senolytic-positive reference (Ref). A statistical analysis (student t-test) was performed in order to compare the proportions of senescent and non-senescent cells to their respective controls (bleu and grey arrows); *: $p < 0,05$ - **: $p < 0,01$ - ***: $p < 0,001$.



Cytotoxicity measurement of the senolytic positive reference at 6 concentrations on non-SIPS proliferative (A) or SIPS (B) NHDFs. Cell viability is measured after 2 days of treatment with the different concentrations ($n=4$), compared to respective negative controls, i.e. untreated control for cytotoxic positive reference and solvent for the reference treated conditions.