

Effects of Small Molecule Compounds on Longevity, Senescence and Wound Healing

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Abstract

Organismal aging causes decline in a wide range of processes in the cell, resulting in diverse diseases and disabilities in old age. Five Alarm Bio are developing small molecule compounds that boost the ability of the cell to defend itself against the damage that accumulates with age, and hence be candidate treatments for age-associated disease. Here we present initial results of this programme. Starting from a low potency 'probe' molecule, we synthesised a custom library of compounds and screened them for pathway activation, and for functional modulation of two *in vitro* models of aging. We show that our compounds can defer replicative senescence in cultured primary cells, and restore the migratory phenotype in dermal fibroblasts and keratinocytes. This work could be completed in under 18 months thanks to flexible, ready-to-go facilities at LiveL@bs, and both the screen and the migration assay were performed on high-end instrumentation that FAB could only access by being part of the Babraham LiveL@bs community. Our success illustrates the power of strong science deployed in the LiveL@bs environment.

About LiveL@bs

LiveL@bs provides a flexible, co-working lab space for early-stage life science companies (up to 8 people). Located within an established Campus with access to state-of-the-art facilities and the opportunity to be part of one of the best ecosystems in Europe. Fully serviced and equipped labs ideal for molecular biology and cell culture work are available. Share, learn and network with other co-founders and CEOs and benefit from the 'LiveL@bs community'. Short-term memberships (as little as 3 months initial term) are offered for maximum flexibility.



Societal Impact of Aging

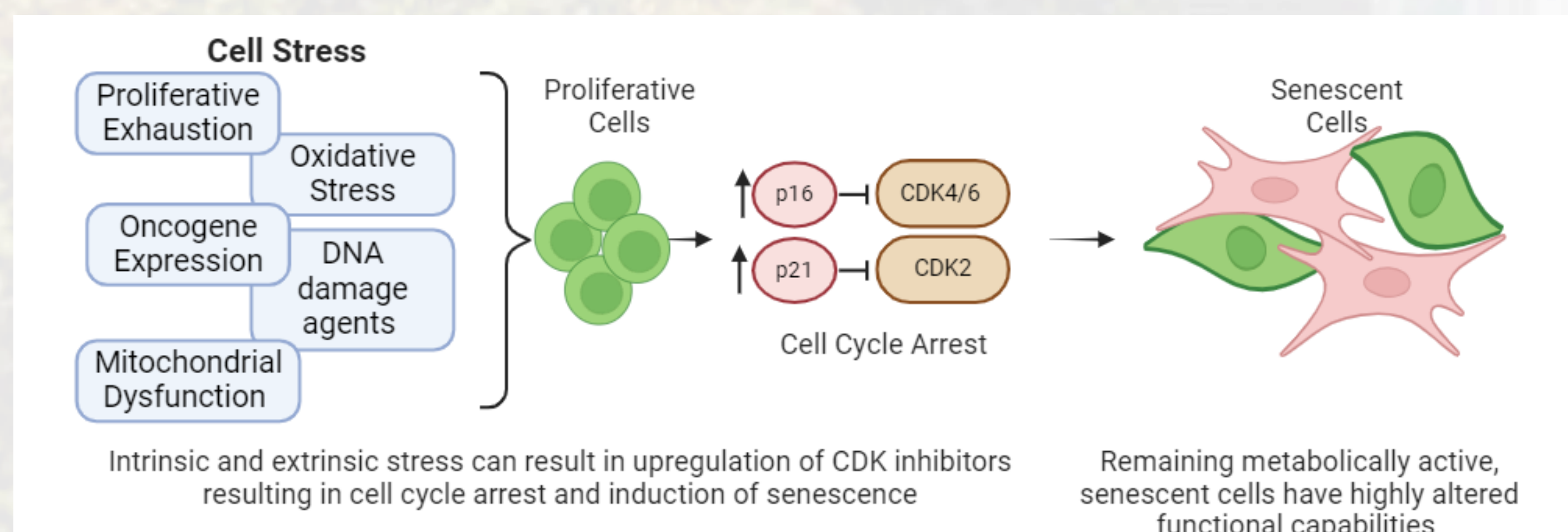
Aging is the number one health issue of our time and the proportion of the world's population over 60 years will nearly double from 12% to 22% by 2050. An increase of 2.2 years life expectancy will yield \$7 trillion of health saving over 50 years.



Who Are Five Alarm Bio?

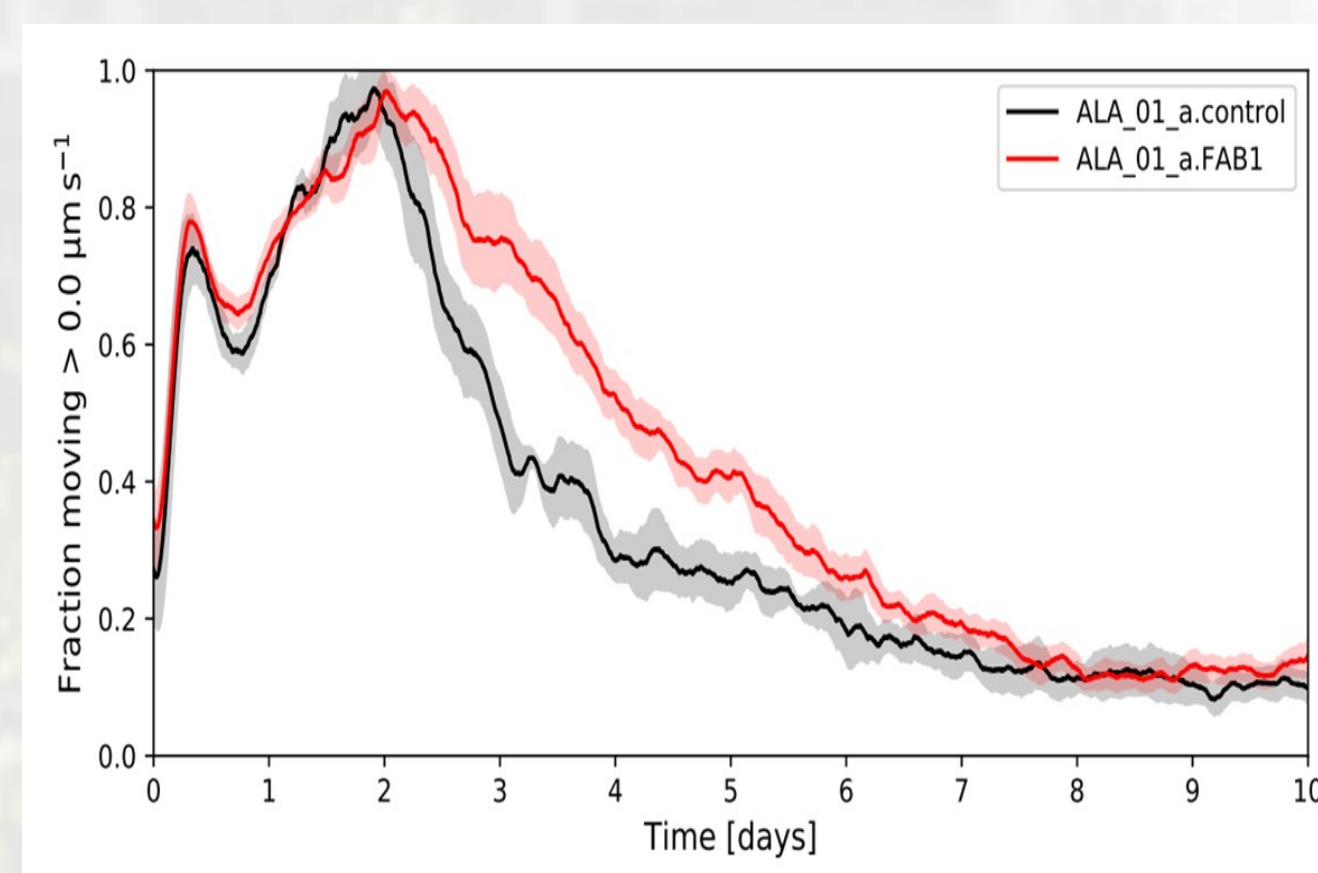
Five Alarm Bio is developing a small molecule approach to boost the body's defence to ageing, based on a new understanding of how the chemical damage associated with age accumulates in cells. Five Alarm Bio plans to deploy our discoveries to develop safe and effective treatments for a range of age-related disease and disabilities. Founded in 2016 by three experienced entrepreneurs, Five Alarm Bio is based near Cambridge, UK, and is carrying out research at the LiveL@bs facility on the Babraham Campus

Senescence and Longevity



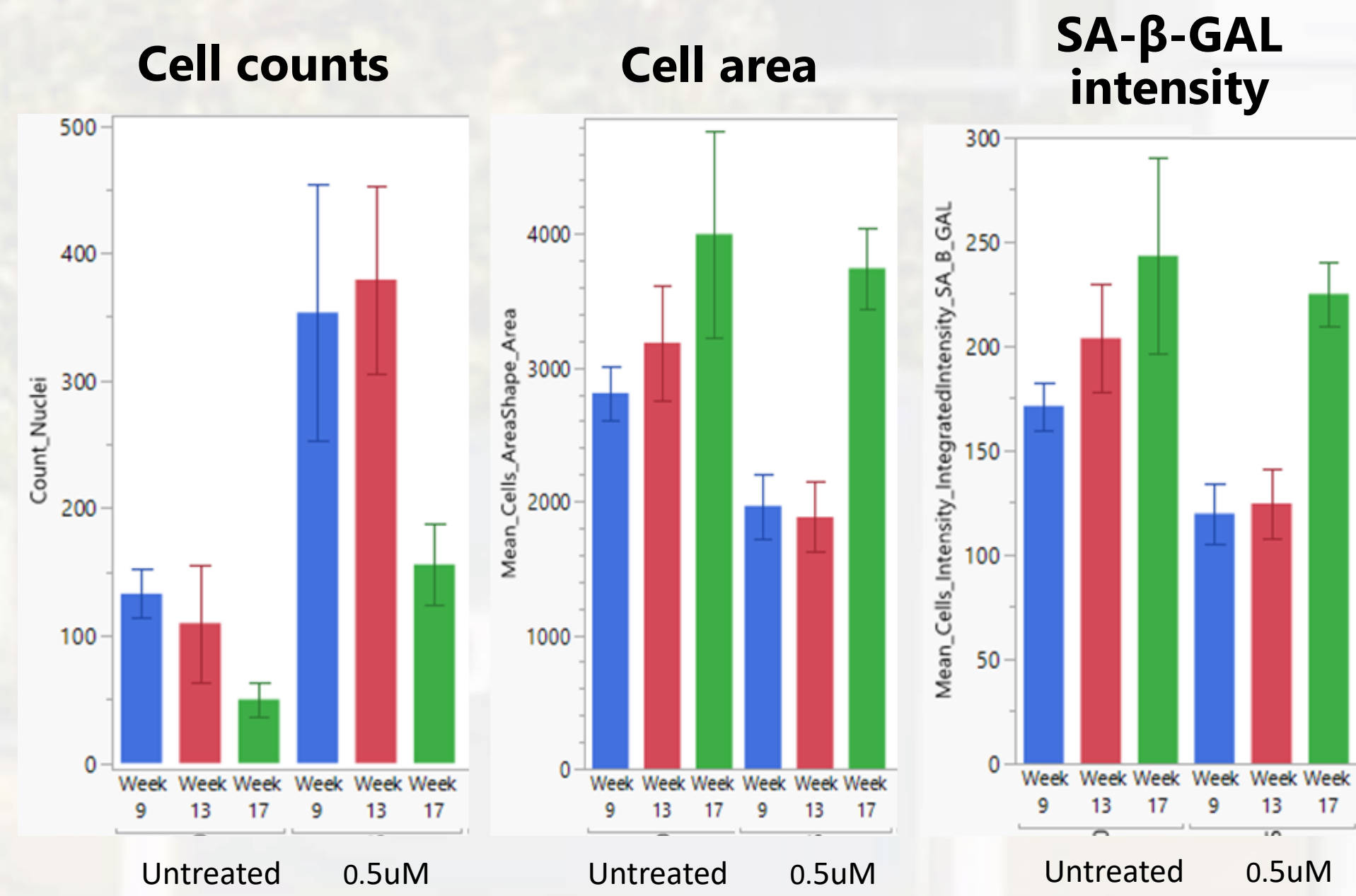
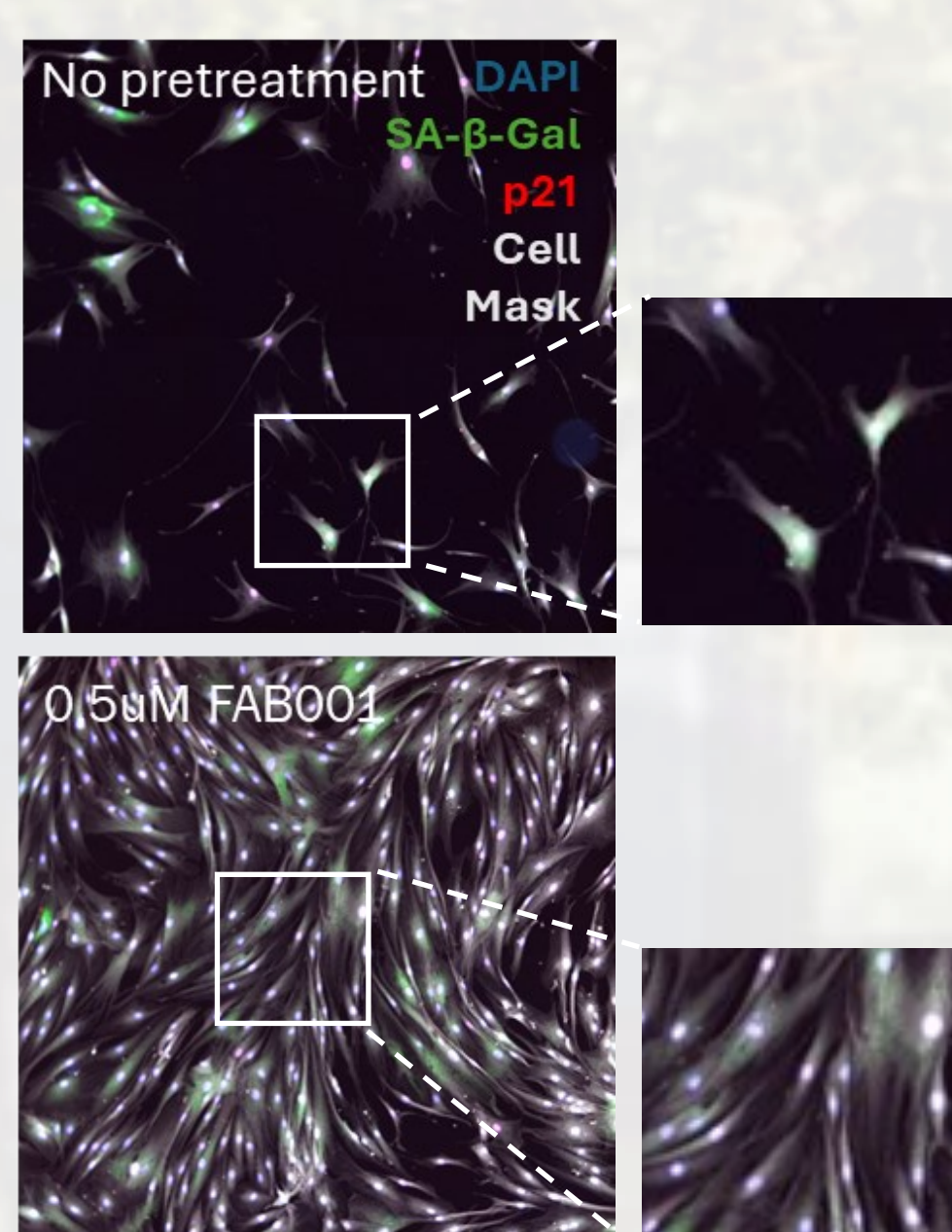
Probe Compound FAB1 Enhances *Caenorhabditis elegans* Longevity

C. elegans were exposed either 5 mM FAB1 or vehicle and the fraction of worms moving determined by video microscopy and image analysis. This was used to indicate the longevity of the worms. FAB clearly improves the motility of *C. elegans*, especially between 2 and 7 days. Notably, FAB1 is not toxic to the worms at 5 mM



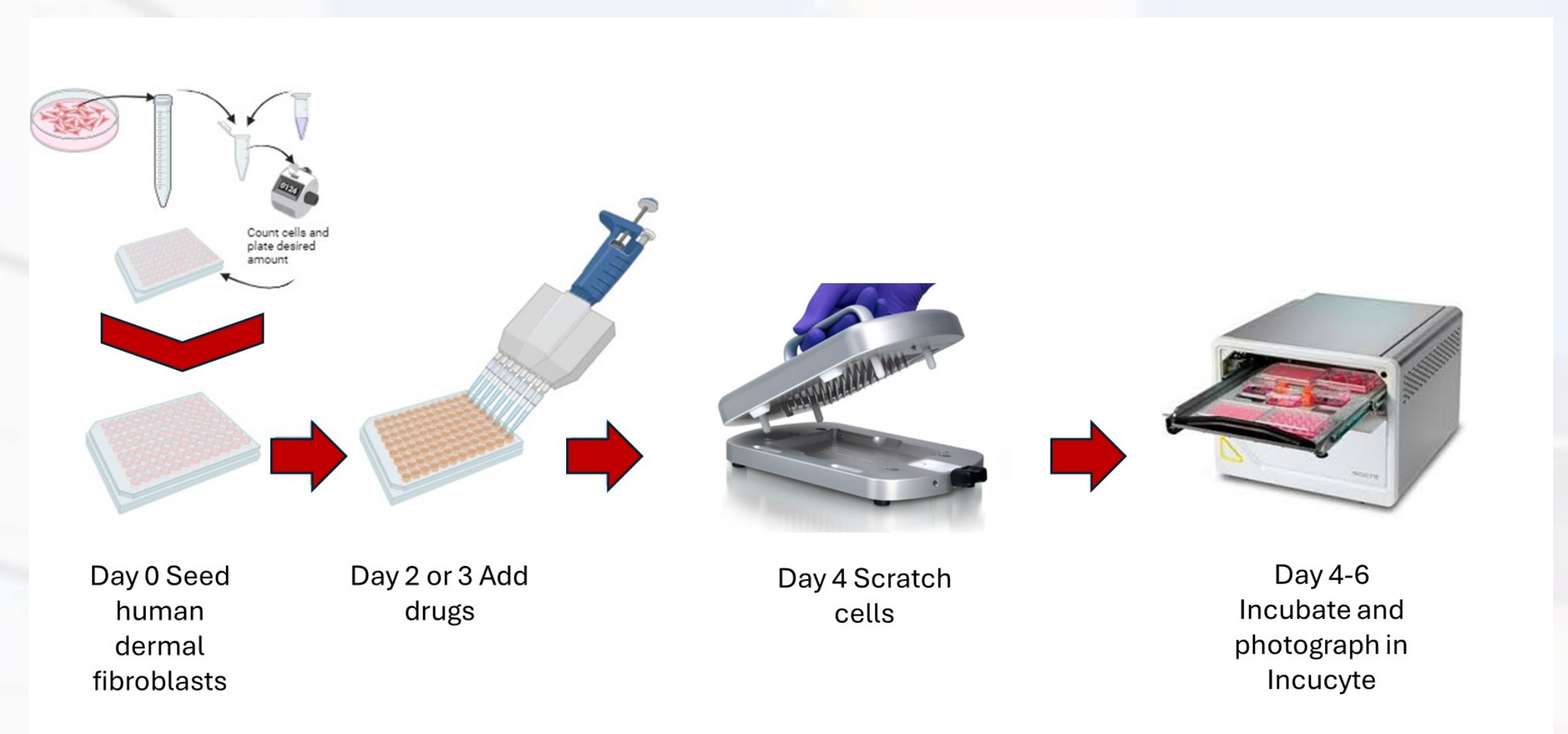
FAB1 Reduces Senescence Marker Expression

Human dermal fibroblasts were treated with 0.5 μ M FAB1 for up to 17 weeks then examined by immunofluorescence microscopy. Analysis revealed that treatment reduced the expression of senescence markers such as SA- β -galactosidase and p21. Morphological parameters associated with senescence such as increased cell area were also notably decreased.

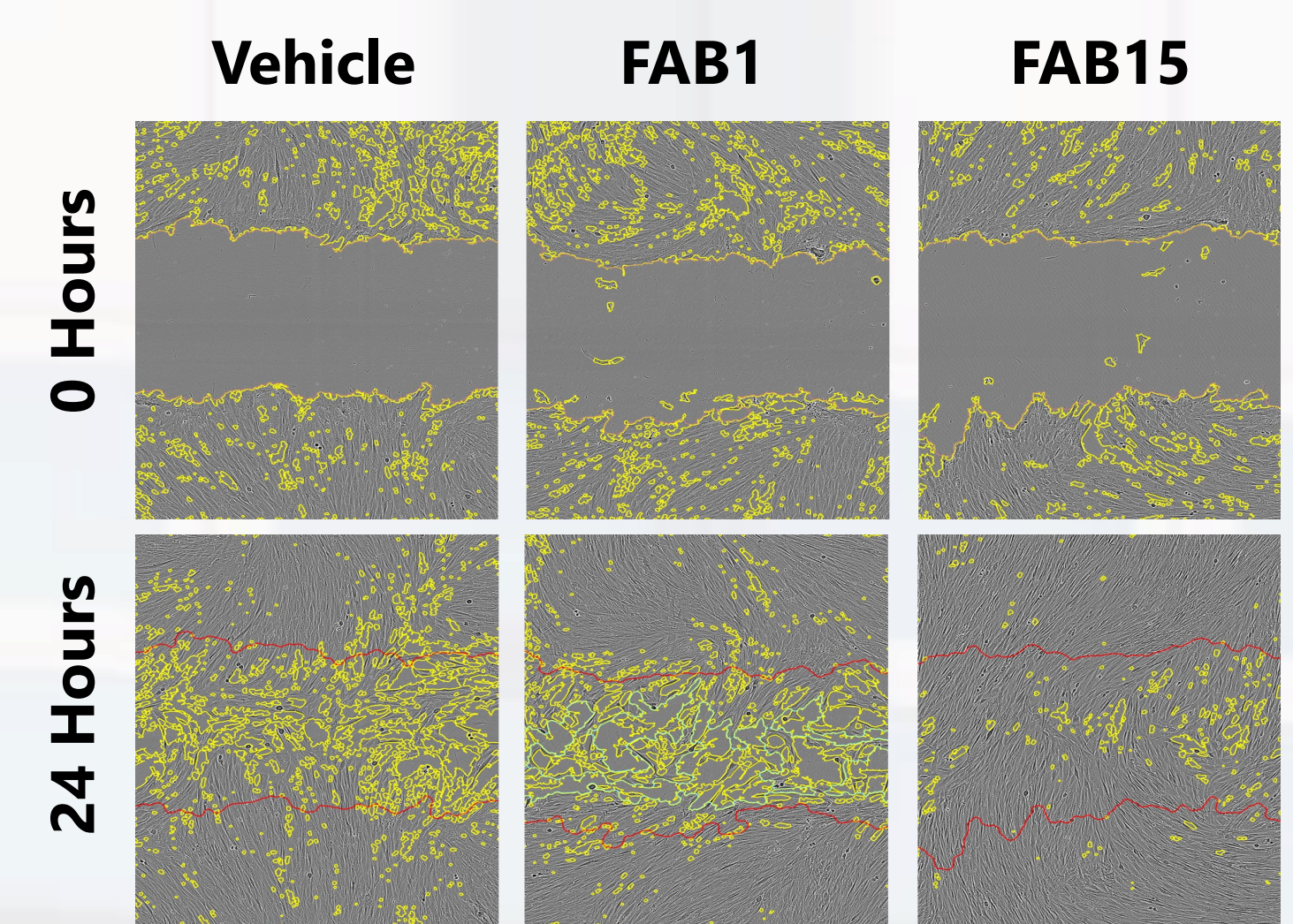
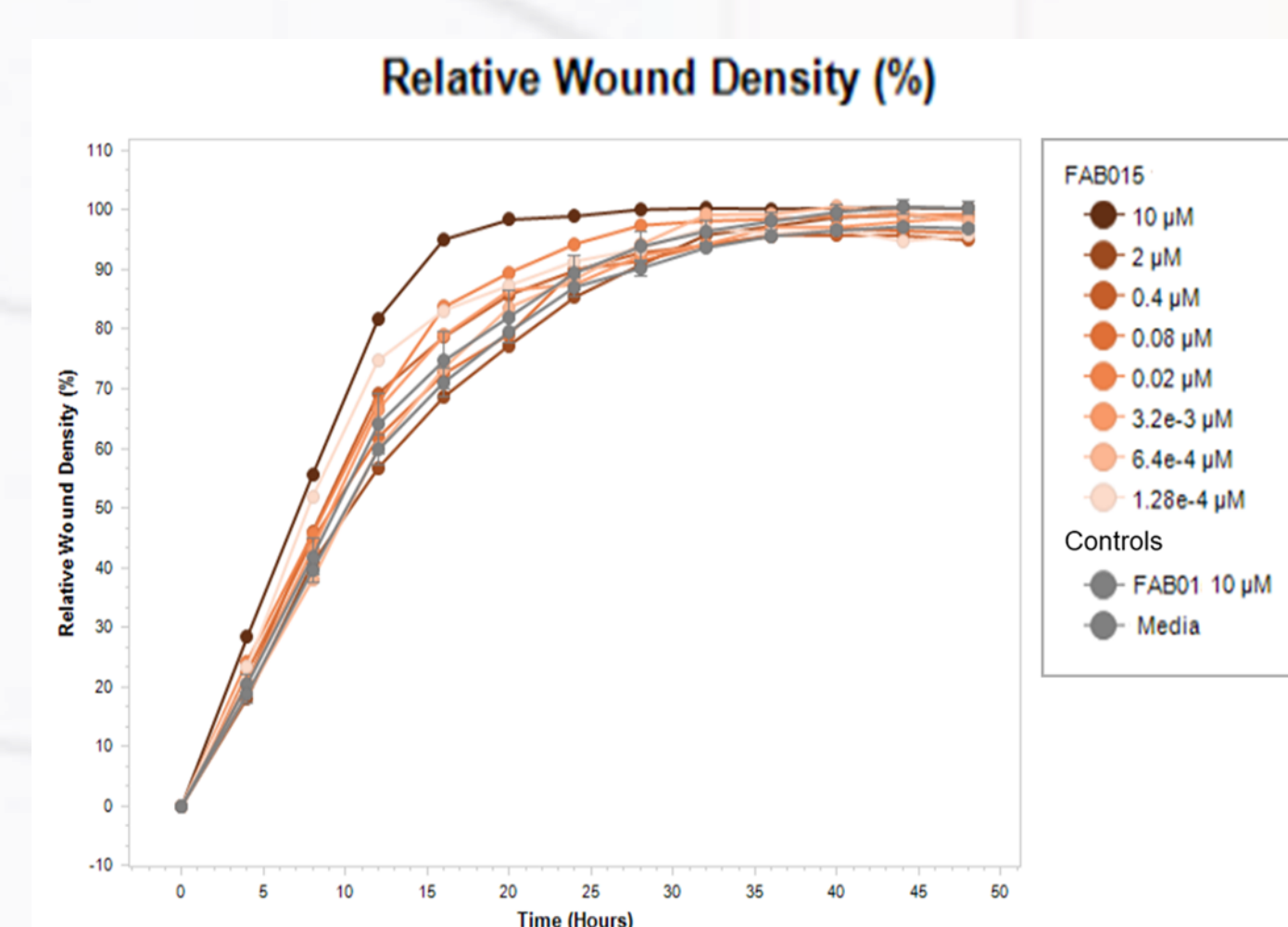


Novel Compound FAB15 Promotes Wound Healing *in vitro*

Following a high-throughput phenotypic screen performed on an ImageExpress HT.ai high content imager at the Babraham Institute, a smaller panel of compounds was chosen for further analysis by an *in vitro* wound healing scratch assay utilizing the Incucyte imager at the Institute.

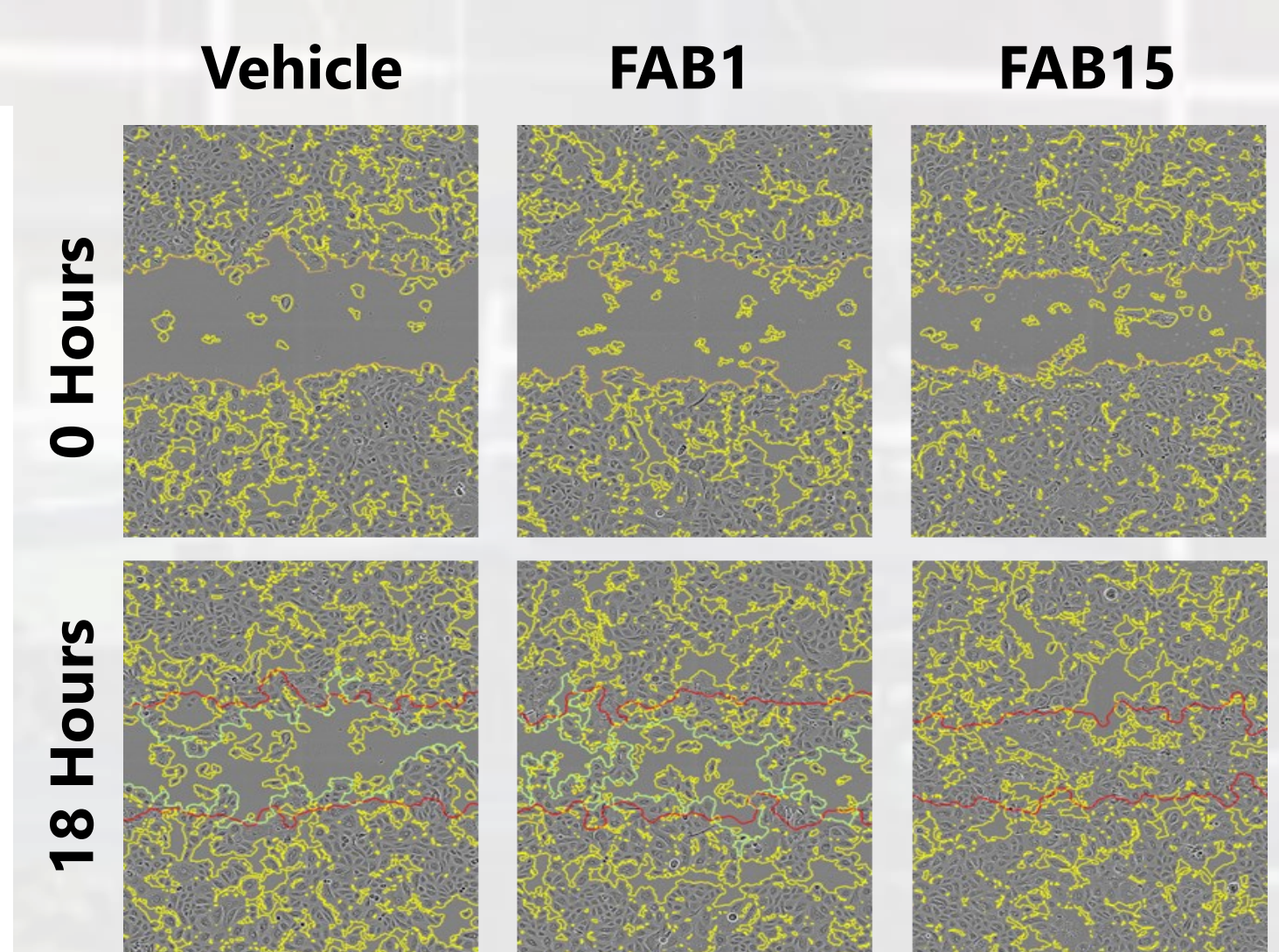
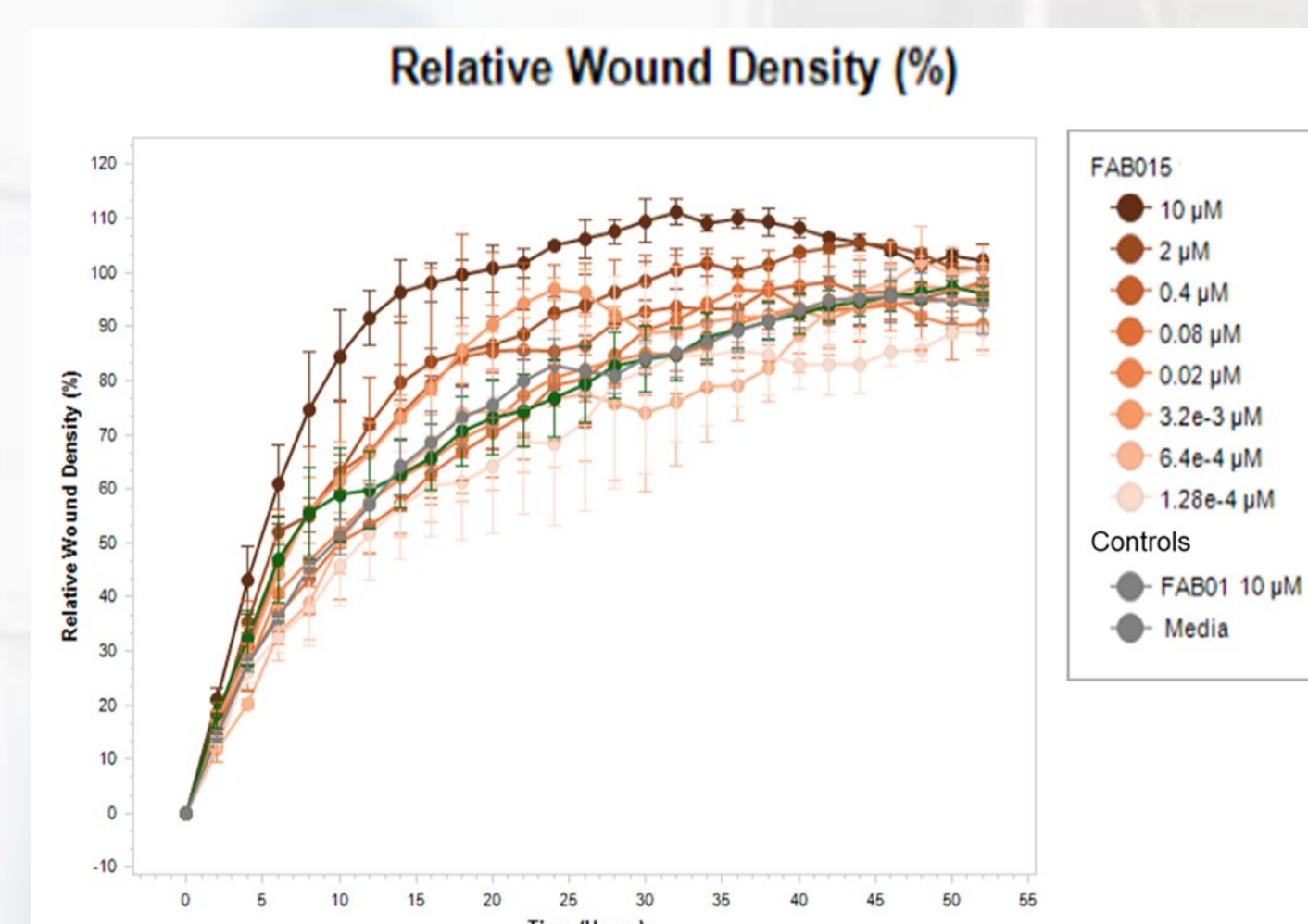


Human Dermal Fibroblasts



FAB15 greatly reduced the time to close 95% of the wound from 44 hours to 24 hours.

Human Keratinocytes



Similarly FAB15 notably improved wound closure in human keratinocyte cells.

Conclusion

Our early work showed that a small probe molecule improved longevity in a *C. elegans* model and reduces senescence marker expression in an *in vitro* replicative senescence assay. Subsequently, we have developed a novel compound that promotes wound healing in both human dermal fibroblasts and keratinocytes. This work was achieved by using the excellent facilities available to Five Alarm Bio through membership of LiveL@bs and the Babraham Campus.

Acknowledgments

Five Alarm Bio would like to thank Hanneke Okkenhaug for her help with the high throughput imaging performed at the Babraham Institute, and also Simon Walker for advice and assistance in use of the Incucyte and data analysis. We would also like to acknowledge Ruth Campbell of the LiveL@bs team for laboratory management and support. Magnitude Bioscience performed the *C. elegans* assays. Screening compounds were synthesized by O2h.

