# Senotherapeutic Nanoformulation for the Treatment of Vascular Ageing

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## BACKGROUND

Ageing is the primary driver of cardiovascular diseases (CVDs), largely mediated by cellular senescence. This state of irreversible cell cycle arrest is characterised by ↑ p16/p21, ↑ SA-β-Gal activity, alongside ↓ Ki67 proliferation. These cells also develop a pro-inflammatory secretory phenotype (SASP) and exhibit impaired mitophagy [1].

In the vasculature, endothelial cell (EC) senescence initiates CVDs. Current senotherapies, including senolytics (which eliminate senescent cells) and senomorphics (which suppress SASP), have important limitations because they broadly target multiple senescent cell types without selectively targeting senescent endothelial cells [2]. Moreover, the identification of novel signaling pathways capable of rejuvenating endothelial cells is essential for the prevention of CVDs.

## OBJECTIVE

Develop a **novel senotherapeutic nanoformulation (NF) to rejuvenate the senescent endothelium**, overcoming current limitations of other senotherapeutics.

## MAIN FINDINGS

High-throughput screening of 2047 NFs identified **NF-1** as lead candidate that uniquely attenuated senescence phenotype in irradiated endothelial cells (iECs), senescent model of radiation-induced DNA damage (Fig.1). Specifically, NF-1 significantly ↓ p16 (80%) and ↓ p21(0.85-fold) levels, while restoring Ki67 expression (40% ↑ ), effectively rejuvenating iECs (Fig.2). It also ↓ SASP factors both *in vitro* (Fig.2d) and *in vivo* (Fig.6d) and restores mitochondrial fitness (20% ↑ of mitochondrial events – Fig.3). It shows high EC-specificity, with NF-1 endogenous expression ↓ in iECs (40%) and ↑ in iSMCs (2-fold), with no change in iFibs (Fig.4). It was identified the pathway as a primary target of NF-1 in iECs (Fig.5). NF-1 ↑ mice body weight, transitioned 100% of mice from frail/pre-frail to non-frail status, and efficacy was confirmed by a ↓ 25% of the target pathway metabolite (Fig.6).

This is the first attempt to use this type of NF as a therapeutic agent to improve ECs characteristics associated with senescence, overcoming the limitations of traditional therapies.

### Acknowledgements:

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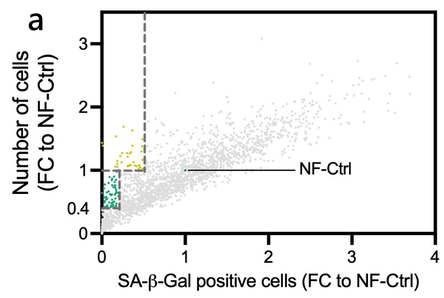
### References:

[1] Huang *et al.*, Nature Reviews Nephrology, 2022; [2] Zhang *et al.*, npj Aging, 2026.

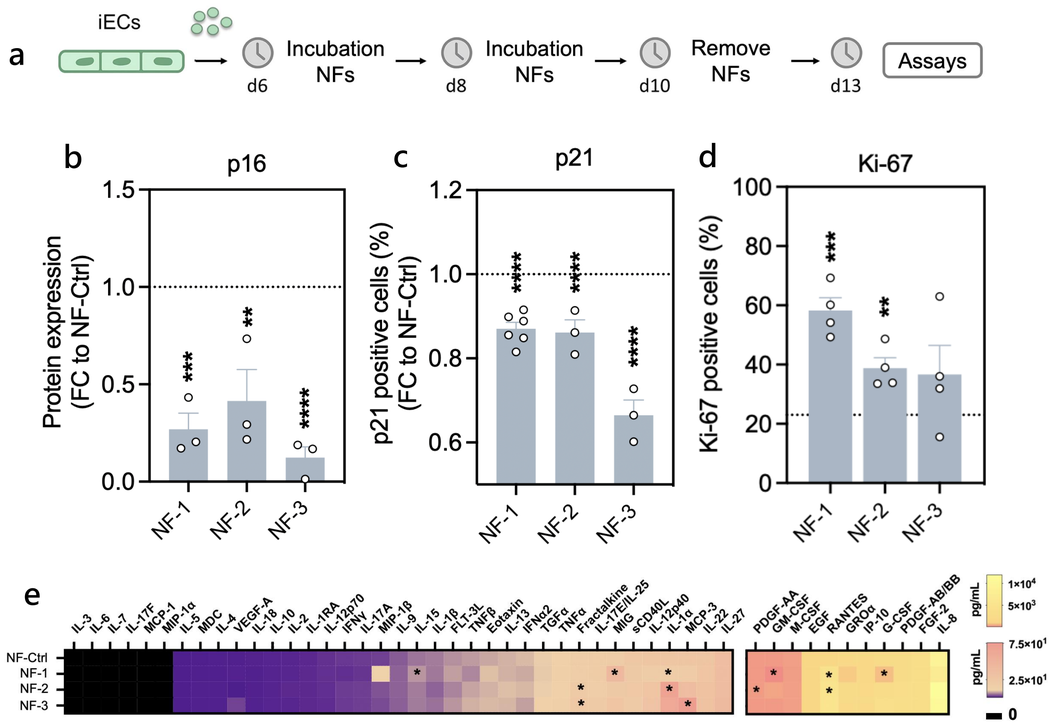
## RESULTS

### 1 High-Throughput Identification of Senotherapeutic NFs



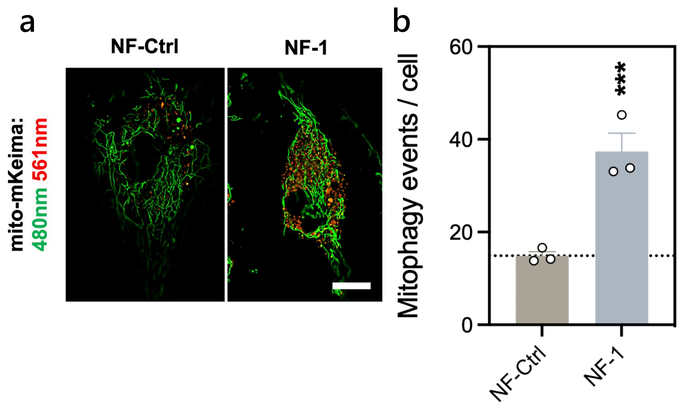

(a) Screening hits: group 1 (yellow), 36 NFs ( ↑ cell count, 50% reduction in SA-β-Gal); group 2 (green), 48 NFs (40% viability, 80% reduction in SA-β-Gal). Data normalised as fold change (FC) to NF-Ctrl in iECs.

### 2 Selection of The Lead Senotherapeutic NF



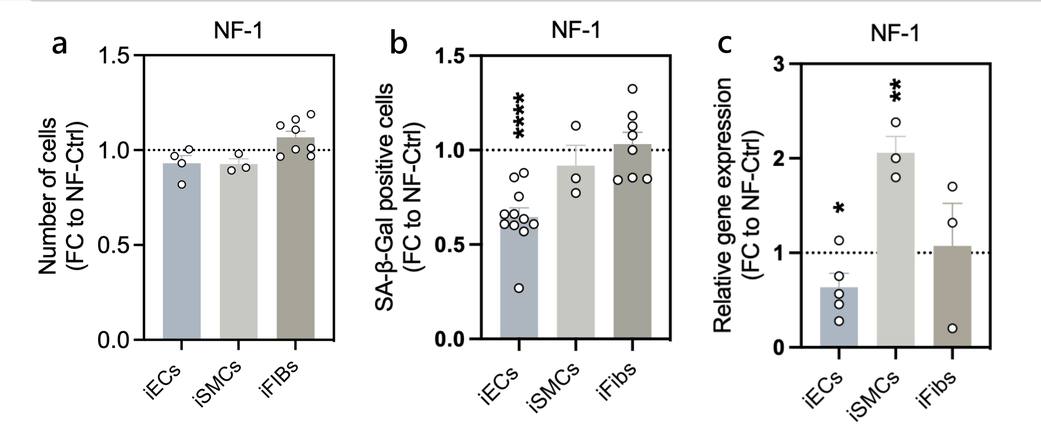

(a) Experimental design. (b) p16 expression (cell cycle arrest) via WB (GAPDH control). (c, d) ICC quantification of p21 (cell cycle arrest) and Ki67 (cell proliferation). (e) Heatmap of 48 SASP factors (cytokines/chemokines) in medium via multiplex analysis. All data normalised to NF-Ctrl in iECs.

### 3 Senotherapeutic NF-1 Rescue Mitochondrial Health



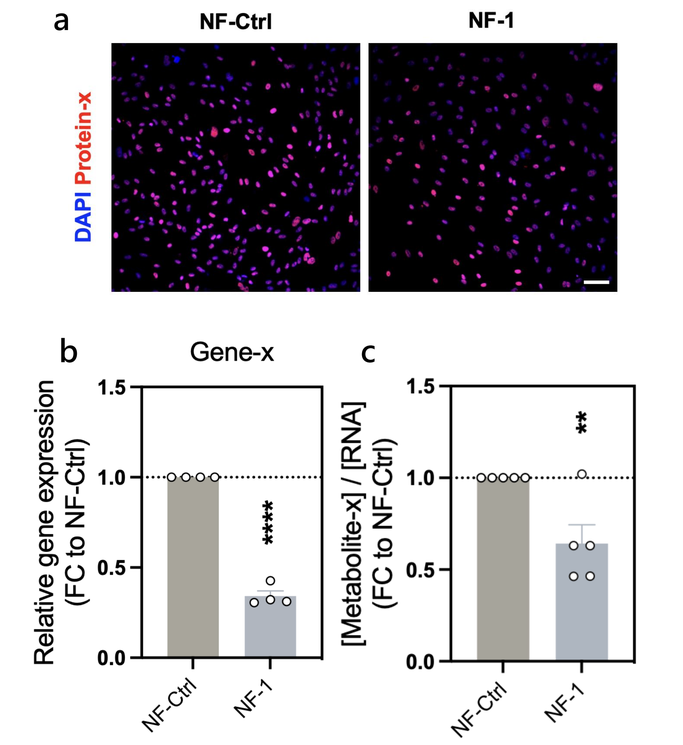

(a) Mitophagy activity after NF-1 incubation. (b) Mitophagy kinetics (total events/cell). Data normalised to NF-Ctrl. Scale bar: 10 µm.

### 4 NF-1 as a Targeted Senotherapeutic With Endothelial Specificity



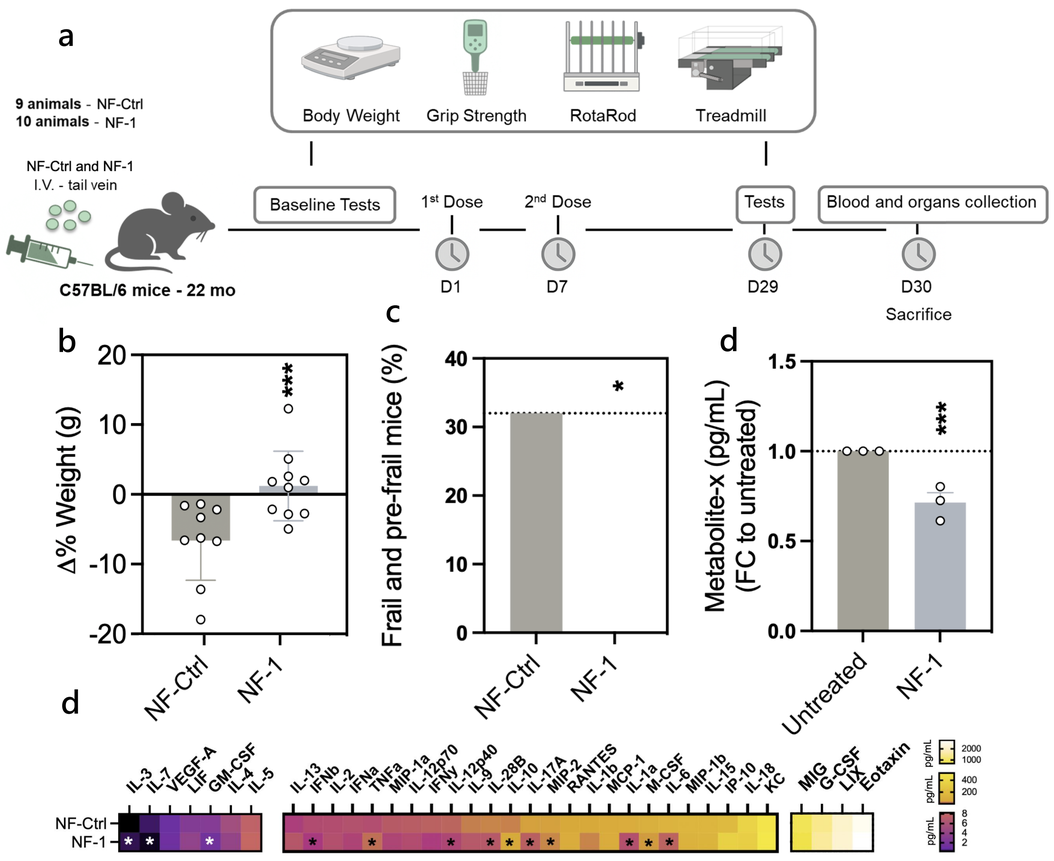

(a, b) Total cell count and SA-β-Gal positive cells in iECs, iSMCs and iFibs treated with NF-1. (c) Endogenous NF-1 expression in iECs, iSMCs, and iFibs (day 6 post-irradiation). Assays via RT-qPCR and normalised to U6 gene and respective NF-Ctrls.

### 5 Validation of RNAseq Identified NF-1 Pathway Target in iECs




(a) Representative images of protein-x (key from lead pathway) in iECs incubated with NF-1. (b) RT-qPCR validation of the lead pathway gene (normalised to HPRT1). (c) ELISA of key pathway metabolite in conditioned medium; concentrations (pg/mL) normalised to total RNA. Data normalised to NF-Ctrl. Scale bar: 100 µm.

### 6 NF-1 Reverses Frailty in Aged Mice With Attenuation of The Target Pathway




(a) Experimental design. (b) Body weight change in NF-1 treated animals. (c) Frailty phenotype distribution (frail vs. pre-frail) based on physical performance. (d) Plasma ELISA of key pathway metabolite. (e) Multiplex heatmap of 36 SASP factors (cytokines/chemokines) in aged mouse plasma. Data normalised to respective Ctrl.

### Methods:

Cell Lines and Senescence: cell lines used were human coronary artery endothelial cells (ECs), human umbilical artery smooth muscle cells (SMCs) and human skin fibroblasts AGO8468 (Fibs); senescence was induced via γ-irradiation (eg, iECs);

NFs Treatment *in vitro*: on day 6 and day 8 post-irradiation in cell medium, concluded on day 10, and all assays conducted on day 13;

Senescent Characterisation: SA-β-Gal - 10 µM C12FDG fluorescence; protein/gene expression - western blot (WB, Azure Biosystems), immunocytochemistry (ICC, IN Cell 2200), and real-time quantitative polymerase chain reaction (RT-qPCR, CFX96); SASP - Luminex™ 200 system;

Mitophagy: live-cell imaging of mito-mKeima (ratio of 561 nm/480 nm excitation);

*in vivo*: aged male C57BL/6 mice (22 months old -mo) received NF-1 (n=10) or NF-Ctrl (n=9) via tail vein (days 1 and 7), physical tests (grip, rota-rod, treadmill) were conducted on days 0 and 29, and sacrifice on day 30;

Statistical Analysis: non-parametric t-test was employed, comparing treatment with control (GraphPad Prism software). Significant results when $p<0.05$. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. Data are shown as mean ± SEM.