# Supplementary file. 1

To investigate the cytoprotecting potential of NF-hAM from induced oxidative stress, HSF cells were exposed to H2O2 treatment at a final concentration of 300  $\mu$ M for 12 hours, followed by 48 hours of NF-hAM treatment. Then, we studied their survival by MTT assay (Fig. 1) and resistance to apoptosis induction (Fig. 1). Our analysis demonstrated a significantly higher proliferation rate in the NF-hAM group as compared to H<sub>2</sub>O<sub>2</sub>-treated cells (Fig. 1).

#### Protective effect on HSF

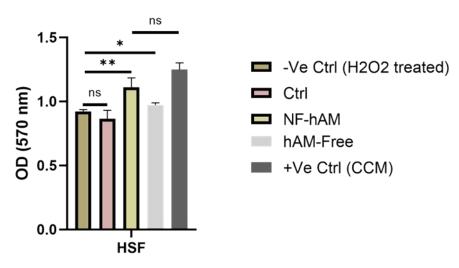


Fig. 1 NF-hAM treatment protects HSF from H2O2 cytotoxicity. MTT assay to investigate HSF proliferative capacity upon  $H_2O_2$  treatment, followed by NF-hAM treatments.

Table 1: Primer sequence used in RT-qPCR analysis.

| Gene<br>(protein) | Primer  | Sequence (5' to 3')    |
|-------------------|---------|------------------------|
|                   |         |                        |
| β-actin           | Forward | CACCATTGGCAATGAGCGGTTC |
|                   | Reverse | AGGTCTTTGCGGATGTCCACGT |
| ID 1              | Forward | GCTGTTACTCACGCCTCAA    |
|                   | Reverse | CAACTGAAGGTCCCTGATGTAG |
| ID2               | Forward | CAAGAAGGTGAGCAAGATGGA  |
|                   | Reverse | GGTGATGCAGGCTGACAATA   |
| VEGFA             | Forward | ATCTGCATGGTGATGTTGGA   |
|                   | Reverse | GGGCAGAATCATCACGAAGT   |
| PDGF              | Forward | GATACTTTGCGCGCACACAC   |
|                   | Reverse | GGTTTTCTCTTTGCAGCGAGG  |
| Ki-67             | Forward | ACGAGACGCCTGGTTACTATC  |

|          | Reverse | GCTCATCAATAACAGACCCATTTAC |
|----------|---------|---------------------------|
| PCNA     | Forward | GGCCGAAGATAACGCGGATAC     |
|          | Reverse | GGCATATACGTGCAAATTCACCA   |
| Snail    | Forward | ACCACTATGCCGCGCTCTT       |
|          | Reverse | GGTCGTAGGGCTGCTGGAA       |
| Vimentin | Forward | TGTCCAAATCGATGTGGATGTTTC  |
|          | Reverse | TTGTACCATTCTTCTGCCTCCTG   |

#### Raw data

| 1 | Table format: |       | Group A |       |       | Group B |       |       | Group C     |       |      | Group D |      |  |
|---|---------------|-------|---------|-------|-------|---------|-------|-------|-------------|-------|------|---------|------|--|
|   | Grouped       |       | Ctrl    |       |       | NF-hAM  |       | N     | NF-free hAM |       | hAM  |         |      |  |
| _ | ×             | A:1   | A:2     | A:3   | B:1   | B:2     | B:3   | C:1   | C:2         | C:3   | D:1  | D:2     | D:3  |  |
| 1 | BMSCs         | 0.591 | 0.581   | 0.585 | 0.615 | 0.606   | 0.610 | 0.585 | 0.575       | 0.580 | 0.59 | 0.60    | 0.59 |  |
| 2 | HSF           | 0.456 | 0.496   | 0.476 | 0.504 | 0.512   | 0.508 | 0.450 | 0.470       | 0.464 | 0.48 | 0.49    | 0.48 |  |
| 3 | AMSCs         | 0.540 | 0.586   | 0.594 | 0.764 | 0.775   | 0.700 | 0.610 | 0.661       | 0.670 | 0.69 | 0.68    | 0.69 |  |
| 4 | Title         |       |         |       |       |         |       |       |             |       |      |         |      |  |
| 5 | Title         |       |         |       |       |         |       |       |             |       |      |         |      |  |

Fig.2 A. raw data for MTT analysis of BMSCs, AMSCs, and HSF under different experimental conditions

| able format: | format: Group A  |   |   |  | Group B  |  | Group C   |   |   |
|--------------|------------------|---|---|--|--|--|---|---|---|
| Grouped      |                  | Ctrl  |   | NF-hAM   |  |  | NF-free hAM   |   |   |
| ×            | A:1              | A:2   | A:3   | B:1  | B:2  | B:3  | C:1   | C:2   | C:3   |
| G0-G1        | 59.800           | 61.200  | 63.300  | 61.500   | 62.500   | 62.900   | 57.400  | 56.800  | 57.600  |
| S            | 6.680            | 6.370   | 6.180   | 13.500   | 12.200   | 13.100   | 10.400  | 10.900  | 8.430   |
| G2-M         | 13.100           | 14.400  | 15.500  | 13.200   | 12.400   | 12.500   | 13.500  | 10.900  | 10.400  |
|              | Grouped  GO-G1 S | Grouped    X A:1     G0-G1   59.800     S   6.680 | Grouped Ctrl  A:1 A:2  G0-G1 59.800 61.200  S 6.680 6.370 | Grouped Ctrl  A:1 A:2 A:3  G0-G1 59.800 61.200 63.300  S 6.680 6.370 6.180 | Grouped Ctrl  A:1 A:2 A:3 B:1  G0-G1 59.800 61.200 63.300 61.500  S 6.680 6.370 6.180 13.500 | Grouped Ctrl NF-hAM   X A:1 A:2 A:3 B:1 B:2  G0-G1 59.800 61.200 63.300 61.500 62.500  S 6.680 6.370 6.180 13.500 12.200 | Grouped Ctrl NF-hAM  A:1 A:2 A:3 B:1 B:2 B:3  G0-G1 59.800 61.200 63.300 61.500 62.500 62.900  S 6.680 6.370 6.180 13.500 12.200 13.100 | Grouped Ctrl NF-hAM N  A:1 A:2 A:3 B:1 B:2 B:3 C:1  G0-G1 59.800 61.200 63.300 61.500 62.500 62.900 57.400  S 6.680 6.370 6.180 13.500 12.200 13.100 10.400 | Grouped Ctrl NF-hAM NF-free hAM  A:1 A:2 A:3 B:1 B:2 B:3 C:1 C:2  G0-G1 59.800 61.200 63.300 61.500 62.500 62.900 57.400 56.800  S 6.680 6.370 6.180 13.500 12.200 13.100 10.400 10.900 |

Fig. 2C raw data for Bar graphs indicating BMSC population percentages in different cell cycle phases

|   | Table format:    |       | Group A |       |        | Group B |       | Group C     |       |       |
|---|------------------|-------|---------|-------|--------|---------|-------|-------------|-------|-------|
|   | Grouped          | Ctrl  |         |       | NF-hAM |         |       | NF-free hAM |       |       |
| 4 | ×                | A:1   | A:2     | A:3   | B:1    | B:2     | B:3   | C:1         | C:2   | C:3   |
| 1 | Annexin V + PI - | 1.370 | 1.570   | 1.510 | 0.310  | 0.350   | 0.330 | 0.370       | 0.550 | 0.370 |

|   | Table format:    |        | Group A |        |        | Group B |        | Group C     |            |        |
|---|------------------|--------|---------|--------|--------|---------|--------|-------------|------------|--------|
|   | Grouped          |        | Ctrl    |        | NF-hAM |         |        | NF-free hAM |            |        |
| 4 | ×                | A:1    | A:2     | A:3    | B:1    | B:2     | B:3    | C:1         | C:2        | C:3    |
| 1 | Annexin V + PI + | 3.300  | 3.370   | 3.100  | 1.560  | 1.720   | 1.570  | 1.860       | 1.740      | 1.880  |
|   | Table format:    |        | Group A |        |        | Group B |        |             | Group C    |        |
|   | Grouped          |        | Ctrl    |        |        | NF-hAM  |        | N           | F-free hAM |        |
| 4 | ×                | A:1    | A:2     | A:3    | B:1    | B:2     | B:3    | C:1         | C:2        | C:3    |
| 1 | Annexin V - PI + | 17,100 | 17,100  | 17.400 | 15.100 | 14.500  | 15.200 | 11.300      | 10.800     | 10.800 |

Fig.3B, C, and D: apoptosis analysis

| Tab | ole format: | X      |       | Group A |       |             | Group B |       |  |
|-----|-------------|--------|-------|---------|-------|-------------|---------|-------|--|
|     | XY          | BMSCs  |       | NF-hAM  |       | NF-free hAM |         |       |  |
|     | ×           | X A:Y1 |       | A:Y2    | A:Y3  | B:Y1        | B:Y2    | B:Y3  |  |
| 1   | Ctrl        |        | 0.138 | 0.135   | 0.150 | 0.138       | 0.135   | 0.136 |  |
| 2   | 5 μg/ml     |        | 0.167 | 0.165   | 0.166 | 0.156       | 0.151   | 0.154 |  |
| 3   | 50 μg/ml    |        | 0.173 | 0.166   | 0.165 | 0.160       | 0.148   | 0.155 |  |

Fig. 4B MTT assay for BMSCs treated with NF-hAM for 24 hours

| ole format: | X     |       | Group A |       |             | Group B |       |  |  |
|-------------|-------|-------|---------|-------|-------------|---------|-------|--|--|
| XY          | BMSCs |       | NF-hAM  |       | NF-free hAM |         |       |  |  |
| ×           | X     | A:Y1  | A:Y2    | A:Y3  | B:Y1        | B:Y2    | B:Y3  |  |  |
| Ctrl        |       | 0.366 | 0.365   | 0.369 | 0.366       | 0.365   | 0.369 |  |  |
| 5 μg/ml     |       | 0.405 | 0.406   | 0.405 | 0.376       | 0.365   | 0.366 |  |  |
| 50 μg/ml    |       | 0.408 | 0.409   | 0.410 | 0.359       | 0.357   | 0.358 |  |  |

Fig. 4D MTT assay for BMSCs treated with NF-hAM for 48 hours

| Tab | ole format: | Х     |       | Group A |           | Group B<br>NF-free hAM |       |       |  |  |
|-----|-------------|-------|-------|---------|-----------|------------------------|-------|-------|--|--|
|     | XY          | BMSCs |       | NF-hAM  |           |                        |       |       |  |  |
| -   | ×           | Х     | A:Y1  | A:Y2    | A:Y3 B:Y1 | B:Y1                   | B:Y2  | B:Y3  |  |  |
| 1   | Ctrl        |       | 0.388 | 0.385   | 0.382     | 0.388                  | 0.385 | 0.382 |  |  |
| 2   | 5 μg/ml     |       | 0.412 | 0.416   | 0.414     | 0.384                  | 0.388 | 0.385 |  |  |
| 3   | 50 μg/ml    |       | 0.423 | 0.426   | 0.425     | 0.383                  | 0.397 | 0.390 |  |  |

Fig. 4F MTT assay for BMSCs treated with NF-hAM for 72 hours

| Tab | ole format: | Х   |       | Group A  |       | Group B<br>48 hours |       |       |  |  |
|-----|-------------|-----|-------|----------|-------|---------------------|-------|-------|--|--|
|     | XY          | HSF |       | 24 hours |       |                     |       |       |  |  |
|     | ×           | X   | A:Y1  | A:Y2     | A:Y3  | B:Y1                | B:Y2  | B:Y3  |  |  |
| 1   | Ctrl        |     | 0.263 | 0.257    | 0.251 | 0.376               | 0.369 | 0.373 |  |  |
| 2   | 5 μg/ml     |     | 0.264 | 0.271    | 0.268 | 0.401               | 0.416 | 0.405 |  |  |
| 3   | 10 μg/ml    |     | 0.271 | 0.272    | 0.272 | 0.416               | 0.400 | 0.410 |  |  |
| 4   | 50 μg/ml    |     | 0.275 | 0.277    | 0.280 | 0.436               | 0.415 | 0.425 |  |  |
| 5   | Title       |     |       |          |       |                     |       |       |  |  |

Fig. 4I MTT assay for HSF treated with NF-hAM for 24 and 48 hours

| Table format:           | Group A<br>Ctrl |       |       |        | Group B |       | Group C     |       |       |
|-------------------------|-----------------|-------|-------|--------|---------|-------|-------------|-------|-------|
| Grouped                 |                 |       |       | NF-hAM |         |       | NF-free hAM |       |       |
| ×                       | A:1             | A:2   | A:3   | B:1    | B:2     | B:3   | C:1         | C:2   | C:3   |
| 1 Glucose concentration | 0.682           | 0.679 | 0.655 | 0.142  | 0.139   | 0.148 | 0.258       | 0.256 | 0.295 |
| 0                       |                 |       |       |        |         |       |             |       |       |

Fig. 6A BMSCs glucose consumption

|     | Table format:      | Group A<br>Ctrl |       |       | Group B<br>NF-hAM |       |       | Group C<br>NF-free hAM |       |       |
|-----|--------------------|-----------------|-------|-------|-------------------|-------|-------|------------------------|-------|-------|
|     | Grouped            |                 |       |       |                   |       |       |                        |       |       |
| - 4 | ×                  | A:1             | A:2   | A:3   | B:1               | B:2   | B:3   | C:1                    | C:2   | C:3   |
| 1   | Lactate production | 0.501           | 0.504 | 0.505 | 0.556             | 0.534 | 0.547 | 0.510                  | 0.510 | 0.508 |

## Fig. 6B BMSCs lactate production

| Table format:     | Group A<br>Ctrl |       |       |        | Group B |       | Group C  |       |       |
|-------------------|-----------------|-------|-------|--------|---------|-------|----------|-------|-------|
| Grouped           |                 |       |       | NF-hAM |         |       | hAM-Free |       |       |
| ×                 | A:1             | A:2   | A:3   | B:1    | B:2     | B:3   | C:1      | C:2   | C:3   |
| 1 H2O2 production | 0.123           | 0.125 | 0.131 | 0.084  | 0.082   | 0.077 | 0.076    | 0.082 | 0.078 |

Fig. 6C HSF H2O2 production

| Table format: <b>Grouped</b> |                       | Group A<br>Ctrl |       |         |        | Group B |       | Group C  |       |       |  |
|------------------------------|-----------------------|-----------------|-------|---------|--------|---------|-------|----------|-------|-------|--|
|                              |                       |                 |       |         | NF-hAM |         |       | hAM-Free |       |       |  |
| - 4                          | ×                     | A:1             | A:2   | A:3     | B:1    | B:2     | B:3   | C:1      | C:2   | C:3   |  |
| 1                            | Glucose concentration | 1.443           | 1.462 | 1.363   | 1.117  | 0.955   | 1.181 | 1.308    | 1.083 | 1.206 |  |
| _                            |                       |                 |       | 1000000 |        |         |       |          |       |       |  |

Fig. 6D HSF glucose consumption

|   | Table format:      |       | Group A |       |        | Group B |       | Group C |       |       |  |
|---|--------------------|-------|---------|-------|--------|---------|-------|---------|-------|-------|--|
|   | Grouped            | Ctrl  |         |       | NF-hAM |         |       |         |       |       |  |
| 4 | ×                  | A:1   | A:2     | A:3   | B:1    | B:2     | B:3   | C:1     | C:2   | C:3   |  |
|   | Lactate production | 0.450 | 0.470   | 0.422 | 0.460  | 0.476   | 0.500 | 0.425   | 0.422 | 0.384 |  |

### Fig. 6E HSF lactate production

| J   | Table format:         | Group A Ctrl |       |       |        | Group B |       | Group C  |       |       |  |
|-----|-----------------------|--------------|-------|-------|--------|---------|-------|----------|-------|-------|--|
|     | Grouped               |              |       |       | NF-hAM |         |       | hAM-Free |       |       |  |
| - 4 | X                     | A:1          | A:2   | A:3   | B:1    | B:2     | B:3   | C:1      | C:2   | C:3   |  |
| 1   | Glucose concentration | 2.295        | 2.249 | 2.413 | 1.847  | 1.944   | 2.049 | 2.444    | 2.192 | 2.270 |  |

Fig. 6F AMSCs glucose consumption

|   | Table format:      | Group A |       |       |        | Group B |       | Group C<br>hAM-Free |       |       |
|---|--------------------|---------|-------|-------|--------|---------|-------|---------------------|-------|-------|
|   | Grouped            |         | Ctrl  |       | NF-hAM |         |       |                     |       |       |
|   | ×                  | A:1     | A:2   | A:3   | B:1    | B:2     | B:3   | C:1                 | C:2   | C:3   |
| 1 | Lactate production | 1.286   | 1.412 | 1.446 | 2.056  | 2.060   | 2.182 | 1.499               | 1.152 | 1.236 |
| 0 |                    |         |       |       |        |         |       |                     |       |       |

Fig. 6G AMSCs lactate production