

## Supplemental Material

# Normoxic HIF-1 $\alpha$ Stabilization Caused by Local Inflammatory Factors and Its Consequences in Human Coronary Artery Endothelial Cells

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## 1. Supplementary methods

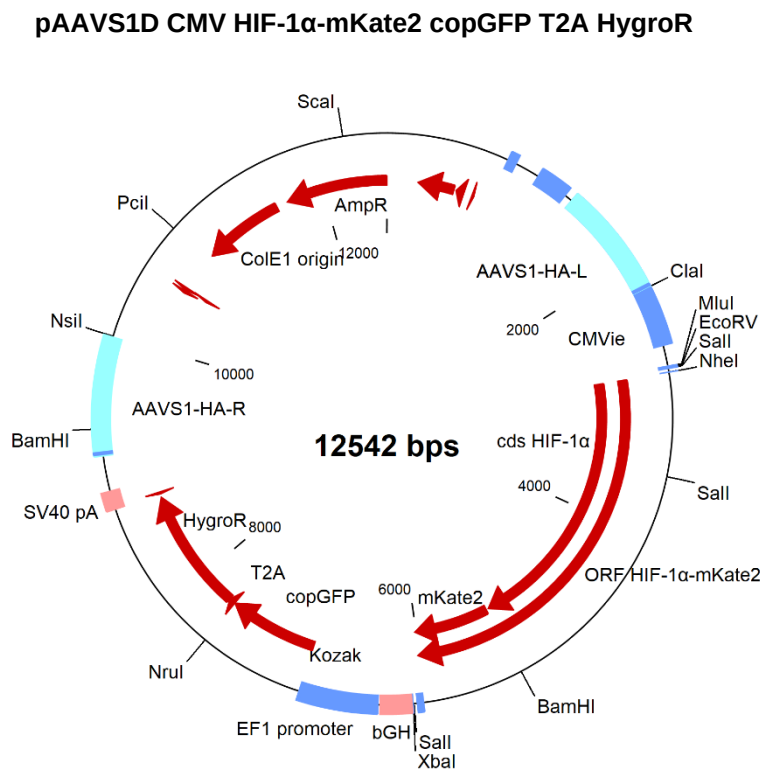
### 1.1 Preparation of the donor vector

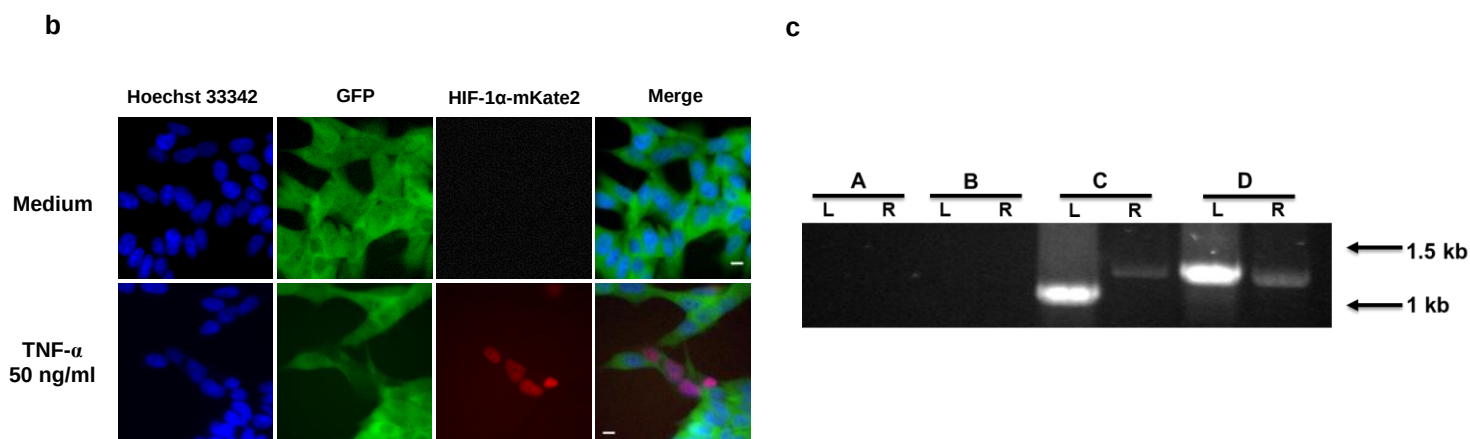
HIF-1 $\alpha$  cds excluding the stop codon was fused to mKate2 using pmKate2-N (Evrogen, Moscow, Russia) and further subcloned into the commercial AAVS1 TALE-Nuclease Kit donor vector (System Biosciences, Palo Alto, USA). The donor vector was additionally modified by exchange of the PGK promoter for a CMVie promoter and the exchange of puromycin resistance for hygromycin resistance. PCR fragments of the CMVie promoter and hygromycin resistance were created using pShuttle (Takara Bio, Kusatsu Japan) and pGL4.32 (Promega, Madison, USA) as templates and cloned via assembly cloning using an In-Fusion cloning kit (Takara Bio, Kusatsu Japan).

### 1.2 Confirmation of stable genomic integration into the AAVS1 locus (junction PCR)

After transfection of the vector into HEK293 cells, stable genomic integration into the AAVS1 locus on chromosome 19 was confirmed by junction PCR. For this, genomic DNA (gDNA) was extracted from transfected HEK293 cells. The flanking regions 3' and 5' of the TALE nuclease cut site at the AAVS1 locus were amplified using two primer groups (right and left). Each primer group consisted of a primer for the original genomic sequence and a primer within the donor vector sequence. PCRs were carried out according to the manufacturer's protocols (System Biosciences, Palo Alto, USA). The PCR products could only be expected if the integration was successful (Supplementary Figure 1c).

a





**Supplementary Figure S1. Generation of the HIF-1 $\alpha$  biosensor.** **a)** Vector map used for the generation of HIF-1 $\alpha$  biosensor. **b)** Hoechst 33342 nuclear staining (blue), green fluorescent protein (GFP) produced by cells (green) and stabilized HIF-1 $\alpha$ -mKate2 in the nucleus (red). HIF-1 $\alpha$ -mKate2 expressing cells were incubated with the cell culture medium and with 50 ng/ml TNF- $\alpha$  for 12 h. TNF- $\alpha$  caused normoxic HIF-1 $\alpha$  stabilization (scale bar = 10  $\mu$ m). **c)** Junction PCR products. A: wt-HEK293 cells (negative control), B: HEK293-cells transfected with pAAVS1D-CMV-RFP-GFP (negative control), C: HEK293 cells transfected with pAAVS1D- CMV-RFP-GFP and pZT-AAVS1-L1/R1 (positive control), D: HEK293 cells transfected with pAAVS1D-HIF-1 $\alpha$ -mKate2-GFP and pZT-AAVS1-L1/R1. The PCR products can only be expected if the integration into AAVS1 is successful (C and D).

### 1.3 Statistical analysis

**Supplementary Table S1. Details of the statistical tests performed.**

| <b>Figure</b> | <b>Type of test</b>       | <b>Sample size</b> | <b><i>p</i> value</b>                                                                                                                                                                                                                                                                                                                                                                                      |
|---------------|---------------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1 b</b>    | Unpaired Student's t test | N = 4              | Medium vs. TNF- $\alpha$ = <b>0.0002</b><br>Medium vs. INF- $\gamma$ = <b>&lt;0.0001</b><br>Medium vs. IL-1 $\beta$ = <b>0.0009</b><br>Medium vs. IL-8 = <b>0.0104</b><br>Medium vs. M-CSF = <b>0.0058</b><br>Medium vs. IGF-I = <b>0.0014</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0001</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0015</b> |
| <b>2 b</b>    | Unpaired Student's t test | N = 3              | Medium vs. TNF- $\alpha$ = <b>0.0001</b><br>Medium vs. INF- $\gamma$ = <b>&lt;0.0001</b><br>Medium vs. IL-1 $\beta$ = <b>0.0005</b><br>Medium vs. IL-8 = <b>0.0070</b><br>Medium vs. M-CSF = <b>0.0002</b><br>Medium vs. IGF-I = <b>0.0041</b>                                                                                                                                                             |
| <b>2 c</b>    | Unpaired Student's t test | N = 3              | Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0173</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0205</b><br>Cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0362</b>                                                                                                                            |
| <b>3 c</b>    | Mann–Whitney test         | N $\leq$ 60        | Medium vs. TNF- $\alpha$ = <b>&lt;0.0001</b><br>Medium vs. INF- $\gamma$ = <b>&lt;0.0001</b><br>Medium vs. IL-1 $\beta$ = <b>&lt;0.0001</b><br>Medium vs. M-CSF = <b>&lt;0.0001</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>&lt;0.0001</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>&lt;0.0001</b>                                                    |
|               | Unpaired Student's t test | N $\leq$ 60        | Medium vs. IL-8 = <b>&lt;0.0001</b><br>Medium vs. IGF-I = <b>&lt;0.0001</b>                                                                                                                                                                                                                                                                                                                                |
| <b>4 a</b>    | Unpaired Student's t test | N = 2              | Medium vs. TNF- $\alpha$ = <b>0.0013</b><br>Medium vs. INF- $\gamma$ = <b>0.0930</b><br>Medium vs. IL-1 $\beta$ = <b>0.0149</b><br>Medium vs. IL-8 = <b>0.1120</b><br>Medium vs. M-CSF = <b>0.0120</b><br>Medium vs. IGF-I = <b>0.0372</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0307</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0046</b>     |

|            |                           |       |                                                                                                                                                                                                                                                                                                                                                                                                            |
|------------|---------------------------|-------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>4 b</b> | Unpaired Student's t test | N = 3 | Medium vs. TNF- $\alpha$ = <b>0.0873</b><br>Medium vs. INF- $\gamma$ = <b>0.0042</b><br>Medium vs. IL-1 $\beta$ = <b>0.0012</b><br>Medium vs. IL-8 = <b>0.0046</b><br>Medium vs. M-CSF = <b>0.0360</b><br>Medium vs. IGF-I = <b>0.0005</b>                                                                                                                                                                 |
| <b>4 c</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0038</b><br>Medium vs. INF- $\gamma$ = <b>0.0004</b><br>Medium vs. IL-1 $\beta$ = <b>0.0008</b><br>Medium vs. IL-8 = <b>0.0011</b><br>Medium vs. M-CSF = <b>0.0078</b><br>Medium vs. IGF-I = <b>&lt;0.0001</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0079</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0095</b> |
| <b>4 d</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0062</b><br>Medium vs. INF- $\gamma$ = <b>0.0061</b><br>Medium vs. IL-1 $\beta$ = <b>0.0277</b><br>Medium vs. IL-8 = <b>0.0256</b><br>Medium vs. M-CSF = <b>0.0341</b><br>Medium vs. IGF-I = <b>0.0286</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0054</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0372</b>     |
| <b>4 e</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0147</b><br>Medium vs. INF- $\gamma$ = <b>0.1318</b><br>Medium vs. IL-1 $\beta$ = <b>0.0055</b><br>Medium vs. IL-8 = <b>0.0092</b><br>Medium vs. M-CSF = <b>0.0005</b><br>Medium vs. IGF-I = <b>0.0167</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0016</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.1022</b>     |
| <b>4 f</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0189</b><br>Medium vs. INF- $\gamma$ = <b>&lt;0.0001</b><br>Medium vs. IL-1 $\beta$ = <b>0.0365</b><br>Medium vs. IL-8 = <b>0.0066</b><br>Medium vs. M-CSF = <b>0.0067</b><br>Medium vs. IGF-I = <b>0.0018</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0474</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0069</b> |
| <b>4 g</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0425</b><br>Medium vs. INF- $\gamma$ = <b>0.0430</b><br>Medium vs. IL-1 $\beta$ = <b>0.0591</b><br>Medium vs. IL-8 = <b>0.0152</b><br>Medium vs. M-CSF = <b>&lt;0.0001</b><br>Medium vs. IGF-I = <b>0.0148</b>                                                                                                                                                             |

|            |                           |       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|------------|---------------------------|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            |                           |       | Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0750</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0154</b>                                                                                                                                                                                                                                                                                                                                     |
| <b>4 h</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0016</b><br>Medium vs. INF- $\gamma$ = <b>&lt;0.0001</b><br>Medium vs. IL-1 $\beta$ = <b>0.0028</b><br>Medium vs. IL-8 = <b>&lt;0.0001</b><br>Medium vs. M-CSF = <b>0.2572</b><br>Medium vs. IGF-I = <b>0.0942</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0051</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0123</b>                                                                               |
| <b>4 i</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>&lt;0.0001</b><br>Medium vs. INF- $\gamma$ = <b>&lt;0.0001</b><br>Medium vs. IL-1 $\beta$ = <b>0.0027</b><br>Medium vs. IL-8 = <b>0.0116</b><br>Medium vs. M-CSF = <b>0.0118</b><br>Medium vs. IGF-I = <b>0.0116</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0024</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0116</b>                                                                               |
| <b>4 j</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0010</b><br>Medium vs. INF- $\gamma$ = <b>0.0018</b><br>Medium vs. IL-1 $\beta$ = <b>0.0222</b><br>Medium vs. IL-8 = <b>0.0027</b><br>Medium vs. M-CSF = <b>0.0237</b><br>Medium vs. IGF-I = <b>&lt;0.0001</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0281</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0011</b>                                                                                   |
| <b>5 b</b> | Unpaired Student's t test | N = 2 | Medium vs. TNF- $\alpha$ = <b>0.0039</b><br>Medium vs. INF- $\gamma$ = <b>0.0169</b><br>Medium vs. IL-1 $\beta$ = <b>0.0070</b><br>Medium vs. IL-8 = <b>0.0010</b><br>Medium vs. M-CSF = <b>0.0047</b><br>Medium vs. IGF-I = <b>0.0085</b><br>Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0015</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0011</b><br>Medium vs. Hypoxia 12 h = <b>0.0040</b><br>Medium vs. Hypoxia 18 h = <b>0.0020</b> |
| <b>5 c</b> | Unpaired Student's t test | N = 3 | Medium vs. TNF- $\alpha$ = <b>0.0201</b><br>Medium vs. INF- $\gamma$ = <b>0.0010</b><br>Medium vs. IL-1 $\beta$ = <b>0.0384</b><br>Medium vs. IL-8 = <b>0.0197</b><br>Medium vs. M-CSF = <b>0.0212</b><br>Medium vs. IGF-I = <b>0.0451</b>                                                                                                                                                                                                                                                   |

|  |  |  |                                                                                                                                                                                                                                                |
|--|--|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  |  | Medium vs. cocktail 1 (TNF- $\alpha$ , INF- $\gamma$ and IL-1 $\beta$ ) = <b>0.0137</b><br>Medium vs. cocktail 2 (IL-8, M-CSF and IGF-I) = <b>0.0774</b><br>Medium vs. Hypoxia 12 h = <b>0.0119</b><br>Medium vs. Hypoxia 18 h = <b>0.0367</b> |
|--|--|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## 2. Supplementary videos

**Video S1:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells incubated with cell culture medium (control group).

**Video S2:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml TNF- $\alpha$ .

**Video S3:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml INF- $\gamma$ .

**Video S4:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml IL-1 $\beta$ .

**Video S5:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml IL-8.

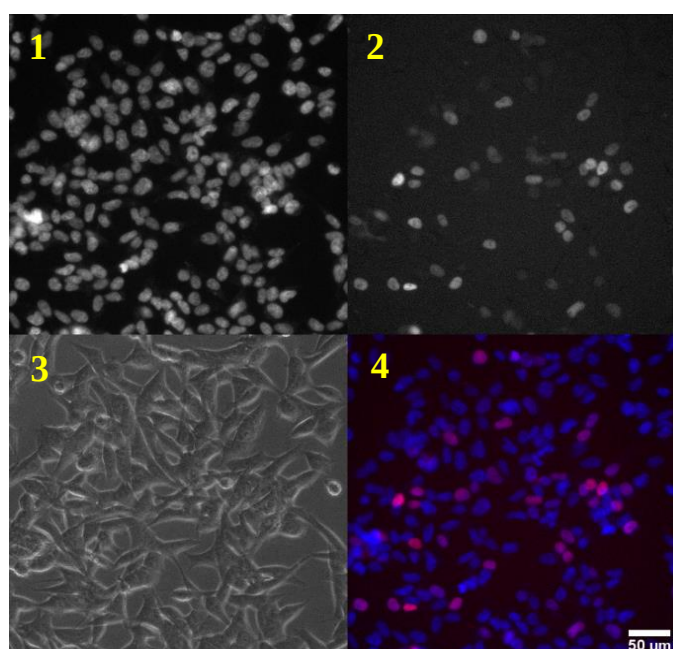
**Video S6:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml M-CSF.

**Video S7:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml IGF-I.

**Video S8:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml cocktail 1 (TNF- $\alpha$ , INF- $\gamma$  and IL-1 $\beta$ ).

**Video S9:** Live cell imaging video of HIF-1 $\alpha$ -mKate2-expressing cells treated with 50 ng/ml cocktail 2 (IL-8, M-CSF and IGF-I).

All videos consist of four parts (Supplementary Figure 2).



**Supplementary Figure S2. Illustration of a selected frame of live cell imaging video.** 1: Hoechst 33342 nuclear staining, 2: HIF-1-mKate2 signal, 3: phase contrast, 4: merged Hoechst 33342 and mKate2 signals in the nucleus. MFs caused normoxic HIF-1 $\alpha$  stabilization in a time-dependent manner (videos S2-S9).

## Resources Table

**Table S2. Antibodies**

| Target antigen                     | Vendor or Source          | Catalog #   | Working concentration  | Persistent ID/URL                                                                                                                                                                                                                                                                                                                                                                                                       |
|------------------------------------|---------------------------|-------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HIF-1 $\alpha$                     | BD Biosciences            | 610959      | IB: 1:250<br>IF: 1:100 | <a href="https://www.bdbiosciences.com/en-eu/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-human-hif-1.610958">https://www.bdbiosciences.com/en-eu/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-human-hif-1.610958</a>                                                                                                 |
| VEGF                               | Santa Cruz Biotechnology  | sc-7269     | IB: 1:500              | <a href="https://www.scbt.com/p/vegf-antibody-c-1?gclid=EAlaIQobChMI7LabmsPc-QIV0oxoCR1hsQ8tEAAYA SAAEgl8FD_BwE">https://www.scbt.com/p/vegf-antibody-c-1?gclid=EAlaIQobChMI7LabmsPc-QIV0oxoCR1hsQ8tEAAYA SAAEgl8FD_BwE</a>                                                                                                                                                                                             |
| $\beta$ -actin                     | Sigma–Aldrich             | A2228       | IB: 1:1000             | <a href="https://www.sigmaaldrich.com/DE/en/substance/monoclonalantibactinantibodyproducedinmouse1234598765?gclid=Cj0KCQjw39uYBhCLARIsAD_SzMRiv5vzbVC8hVkyyscey5mIdE36TmZqfxs6rBzbHxuHT3Rvd7C8hwaAs_WEALw_wcB">https://www.sigmaaldrich.com/DE/en/substance/monoclonalantibactinantibodyproducedinmouse1234598765?gclid=Cj0KCQjw39uYBhCLARIsAD_SzMRiv5vzbVC8hVkyyscey5mIdE36TmZqfxs6rBzbHxuHT3Rvd7C8hwaAs_WEALw_wcB</a> |
| VE-Cadherin                        | Cell Signaling Technology | #2158       | IF: 1:200              | <a href="https://www.cellsignal.com/products/primary-antibodies/ve-cadherin-antibody/2158?site-search-type=Products&amp;N=4294956287&amp;Ntt=%232158&amp;fromPage=plp&amp;_requestid=2094312">https://www.cellsignal.com/products/primary-antibodies/ve-cadherin-antibody/2158?site-search-type=Products&amp;N=4294956287&amp;Ntt=%232158&amp;fromPage=plp&amp;_requestid=2094312</a>                                   |
| ZO-1                               | Cell Signaling Technology | #13663      | IF: 1:400              | <a href="https://www.cellsignal.com/products/primary-antibodies/zo-1-d6l1e-rabbit-mab/13663?site-search-type=Products&amp;N=4294956287&amp;Ntt=%2313663&amp;fromPage=plp&amp;_requestid=2094442">https://www.cellsignal.com/products/primary-antibodies/zo-1-d6l1e-rabbit-mab/13663?site-search-type=Products&amp;N=4294956287&amp;Ntt=%2313663&amp;fromPage=plp&amp;_requestid=2094442</a>                             |
| Goat IgG anti-Mouse IgG (H+L)-HRPO | Dianova GmbH              | 115-035-003 | IB: 1:5000             | <a href="https://www.dianova.com/en/shop/115-035-003-goat-igg-anti-mouse-igg-hl-hrpo-minx-none/">https://www.dianova.com/en/shop/115-035-003-goat-igg-anti-mouse-igg-hl-hrpo-minx-none/</a>                                                                                                                                                                                                                             |

|                                        |                                      |         |           |                                                                                                                                                                                                                                                                                     |
|----------------------------------------|--------------------------------------|---------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Goat anti-Mouse IgG, Alexa Fluor™ 594  | Invitrogen, Thermo Fisher Scientific | A-11005 | IF: 1:200 | <a href="https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11005">https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11005</a>                 |
| Goat anti-Rabbit IgG, Alexa Fluor™ 594 | Invitrogen, Thermo Fisher Scientific | A-11037 | IF: 1:200 | <a href="https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11037">https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11037</a> |
| Goat anti-Rabbit IgG, Alexa Fluor™ 488 | Invitrogen, Thermo Fisher Scientific | A32731  | IF: 1:200 | <a href="https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32731">https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32731</a>   |

**Table S3. Cultured cells**

| Name                                                 | Vendor or Source            | Sex (F, M, or unknown) | Persistent ID/URL                                                                                                                                                                 |
|------------------------------------------------------|-----------------------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Immortalized Human Coronary Artery Endothelial Cells | Applied Biological Material | F                      | <a href="https://www.abmgood.com/immortalized-human-coronary-artery-endothelial-cells.html">https://www.abmgood.com/immortalized-human-coronary-artery-endothelial-cells.html</a> |
| HEK293                                               | DSMZ                        | unknown                | <a href="https://www.dsmz.de/collection/catalogue/details/culture/ACC-305">https://www.dsmz.de/collection/catalogue/details/culture/ACC-305</a>                                   |

**Table S4. Other**

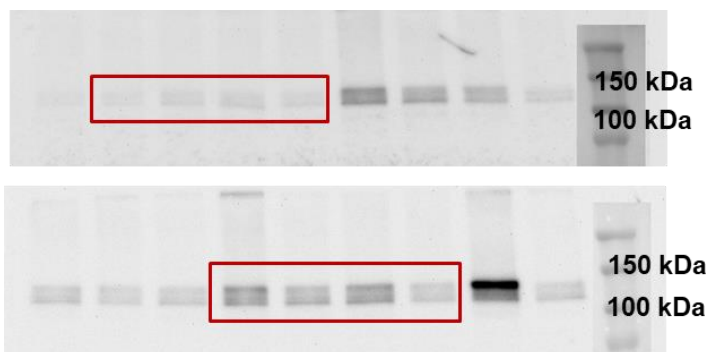
| Description                               | Source/Repository | Persistent ID/URL                                                                                                                                                                                                       |
|-------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AAVS1 TALE nuclease kit                   | SBI               | <a href="https://www.systembio.com/products/crispr-cas9-systems/aavs1-safe-harbor-targeting?cat=155">https://www.systembio.com/products/crispr-cas9-systems/aavs1-safe-harbor-targeting?cat=155</a>                     |
| Bio-Plex Pro Human Cytokine 27-plex Assay | Bio-Rad           | <a href="https://www.bio-rad.com/de-de/sku/M500KCAF0Y-bio-plex-pro-human-cytokine-27-plex-assay?ID=M500KCAF0Y">https://www.bio-rad.com/de-de/sku/M500KCAF0Y-bio-plex-pro-human-cytokine-27-plex-assay?ID=M500KCAF0Y</a> |
| Human IGF-I                               | Peptotech         | <a href="https://www.peptotech.com/en/recombinant-human-igf-i">https://www.peptotech.com/en/recombinant-human-igf-i</a>                                                                                                 |
| Human IL-1β                               | Miltenyi Biotec   | <a href="https://www.miltenyibiotec.com/DE-en/products/human-il-1b.html#gref">https://www.miltenyibiotec.com/DE-en/products/human-il-1b.html#gref</a>                                                                   |
| Human IL-8                                | Miltenyi Biotec   | <a href="https://www.miltenyibiotec.com/DE-en/products/human-il-8.html#research-grade:10-ug">https://www.miltenyibiotec.com/DE-en/products/human-il-8.html#research-grade:10-ug</a>                                     |

|                     |             |                                                                                                                                                                                                                         |
|---------------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Human IFN- $\gamma$ | Peprtech    | <a href="https://www.peprtech.com/en/recombinant-human-ifn-2-2">https://www.peprtech.com/en/recombinant-human-ifn-2-2</a>                                                                                               |
| Human M-CSF         | Peprtech    | <a href="https://www.peprtech.com/en/recombinant-human-m-csf">https://www.peprtech.com/en/recombinant-human-m-csf</a>                                                                                                   |
| Human TNF- $\alpha$ | R&D Systems | <a href="https://www.rndsystems.com/products/recombinant-human-tnf-alpha-hek293-expressed-protein-cf_10291-ta">https://www.rndsystems.com/products/recombinant-human-tnf-alpha-hek293-expressed-protein-cf_10291-ta</a> |

## Original blots used for analysis and graphic representation

Figure S3a:

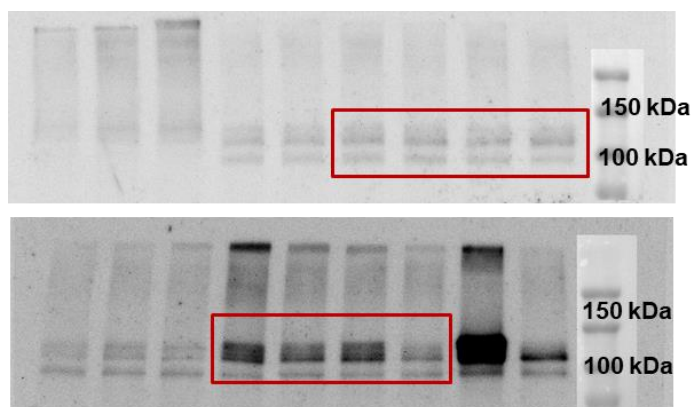
HIF-1 $\alpha$ , 6 h



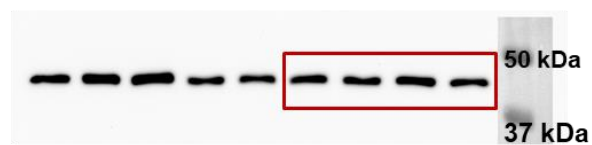
$\beta$ -actin, 6 h



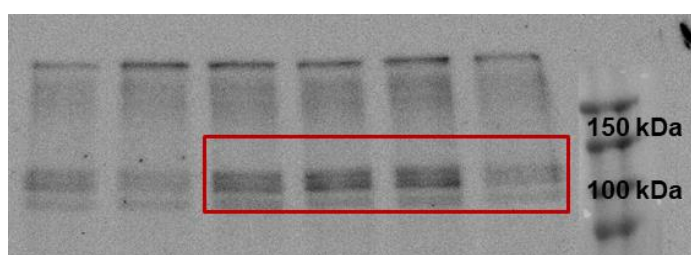
HIF-1 $\alpha$ , 12 h



$\beta$ -actin, 12 h

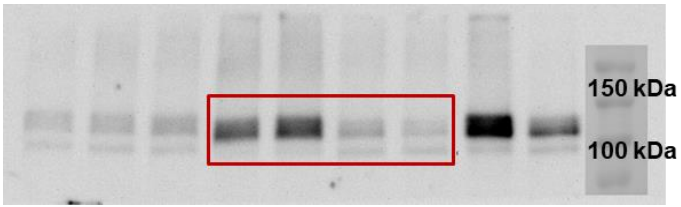


HIF-1 $\alpha$ , 18 h

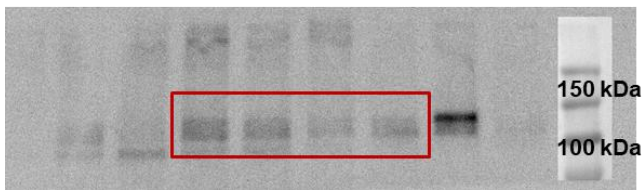
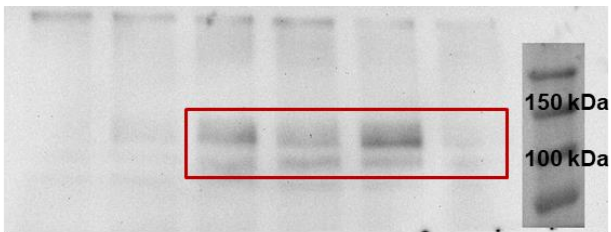


$\beta$ -actin, 18 h





**HIF-1 $\alpha$ , 24 h**



**β-actin, 24 h**

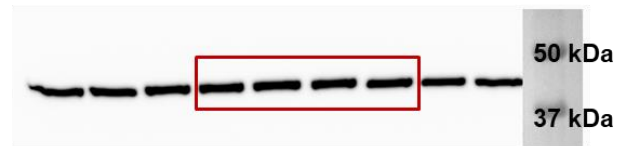


Figure S3b:

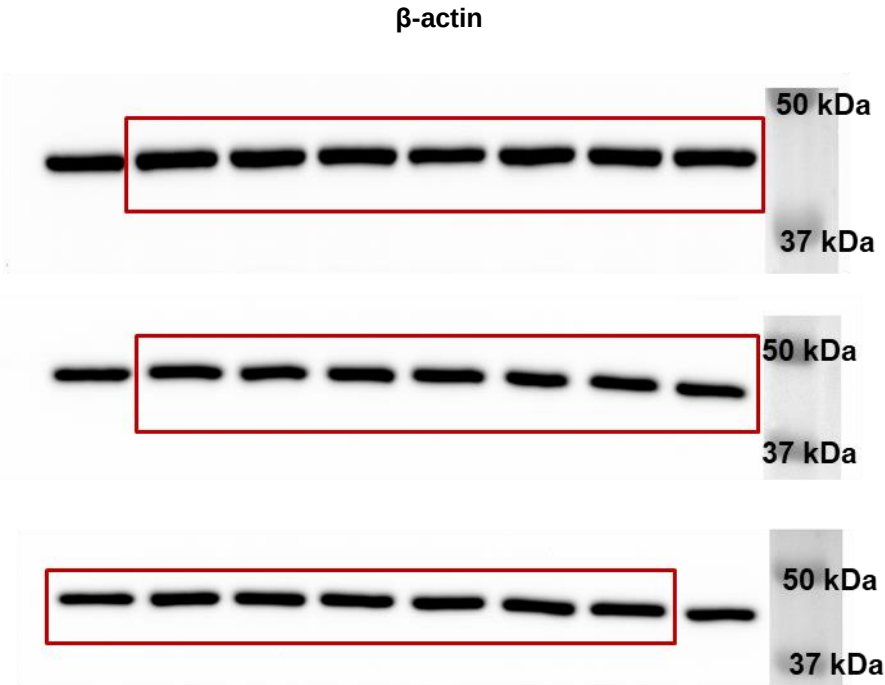
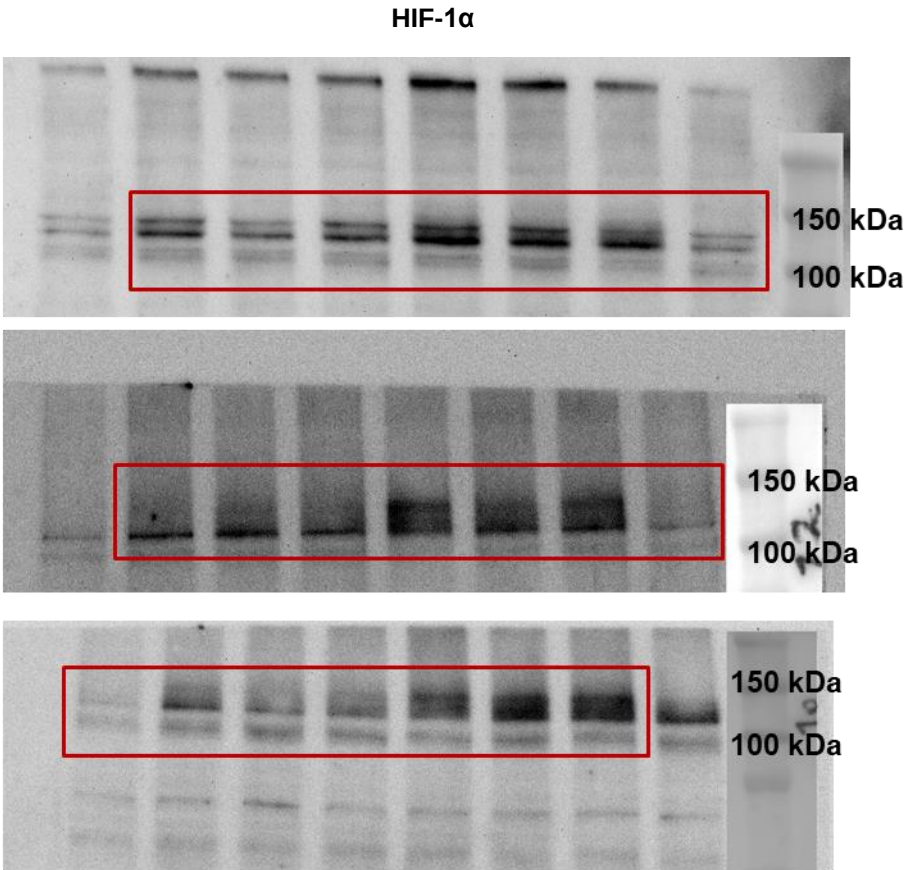


Figure S3c:

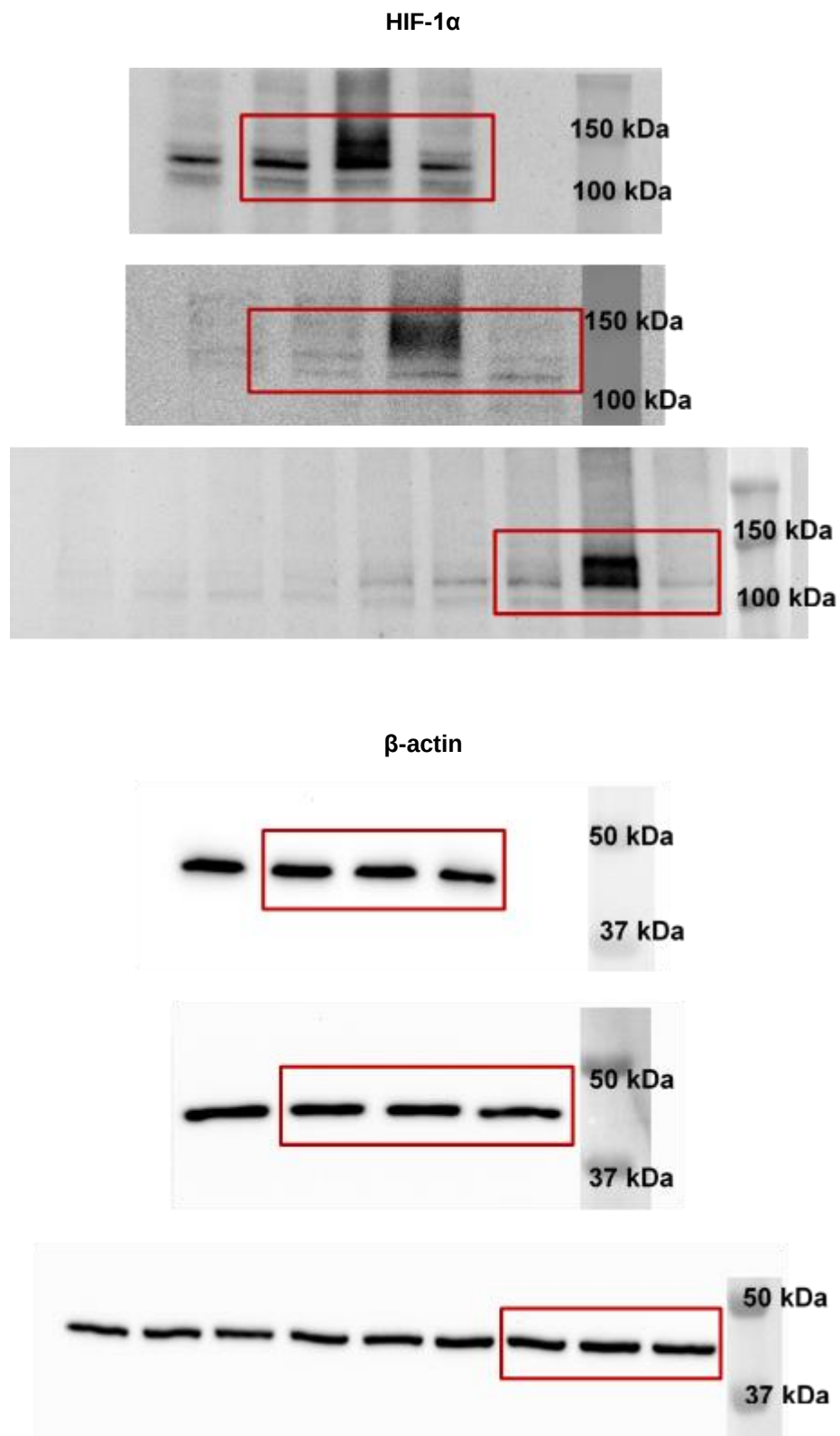


Figure S4b:

