# SUPPLEMENTARY DATA

## Modulation of hypochlorous acid (HOCl) induced damage to vascular smooth muscle cells by thiocyanate and selenium analogues.

**Konstantina Flouda[1], Bente Gammelgaard[2], Michael J. Davies[1], Clare L. Hawkins[1*]**

[1]*Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3B, Copenhagen, DK-2200, Denmark*

[2]*Department of Pharmacy, University of Copenhagen, Universitetsparken 2, Copenhagen, DK-2100, Denmark*

**Keywords:** Myeloperoxidase; thiocyanate; selenocyanate; selenomethionine; inflammation; atherosclerosis.

---

* To whom correspondence should be addressed: Prof Clare Hawkins, Department of Biomedical Sciences, University of Copenhagen, Panum, Blegdamsvej 3B, Copenhagen N, DK-2200, Denmark. Telephone: +45 35 33 70 05. Email: clare.hawkins@sund.ku.dk

**Table S1.** HCASMC donor characteristics

| *Donor* | *Gender* | *Race* | *Age* | *Cell viability* | *Doubling time (h)* | *Population doubling* | *Source* |
|---|---|---|---|---|---|---|---|
| ***1596*** | male | caucasian | 53 | 96.20% | 40.2 | 15 | Non-plaque region of diseased artery |
| ***1522*** | male | black | 54 | 90.90% | 41.5 | >16 | Normal human coronary artery |
| ***1559*** | male | black | 22 | 97.40% | 44.5 | >16 | Normal human coronary artery |

**Table S2.** Housekeeping and target gene primer sequences

| Gene | | Forward Primer | Reverse Primer |
|---|---|---|---|
| **Reference** | 18s | GAGGATGAGGTGGAACGTGT | TCTTCAGTCGCTCCAGGTCT |
| | B2M | AGATGAGTATGCCTGCCGTG | GCGGCATCTTCAAACCTCCA |
| **Inflammatory** | VCAM-1 | ATGCCTGGGAAGATGGTCG | GACGGAGTCACCAATCTGAGC |
| | ICAM-1 | GGCTGGAGCTGTTTGAGAAC | ACTGTGGGGTTCAACCTCTG |
| | MCP-1 | AGCCACCTTCATTCCCCAAG | TTGGGTTTGCTTGTCCAGGT |
| | IL- 6 | CCAGAGCTGTGCAGATGAGT | AGCTGCGCAGAATGAGATGA |
| | Egr-1 | CGCCCACCATGGACAACTAC | AGGAAAAGACTCTGCGGTCAG |
| | MMP2 | GCTACGATGGAGGCGCTAAT | TCAGGTATTGCACTGCCAACT |
| | MMP9 | TTCAGGGAGACGCCCATTTC | TGGGTGTAGAGTCTCTCGCT |
| | AP-1 | GCTGCTCTGGGAAGTGAGTT | TTTCTCTAAGAGCGCACGCA |
| **Phenotypic** | SMC α actin | AGGGAAGGTCCTAACAGCCC | AGGATTCCCGTCTTAGTCCC |
| | OPN | AGGCATCACCTGTGCCATAC | GGCCACAGCATCTGGGTATT |
| | Calponin | CTGACTCCCGAGTACCCAGA | TGCCATGCAGGGAGAGGG |
| | S100A4 | TCTTGGTTTGATCCTGACTGCT | ACTTGTCACCCTCTTTGCCC |
| | Cx43 | TCTTCATGCTGGTGGTGTCC | ACCACTGGTCGCATGGTAAG |
| | Type 1 Collagen | ACAAGGCATTCGTGGCGATA | ACCATGGTGACCAGCGATAC |
| | Runx2 | CCGGAATGCCTCTGCTGTTA | TGTCTGTGCCTTCTGGGTTC |

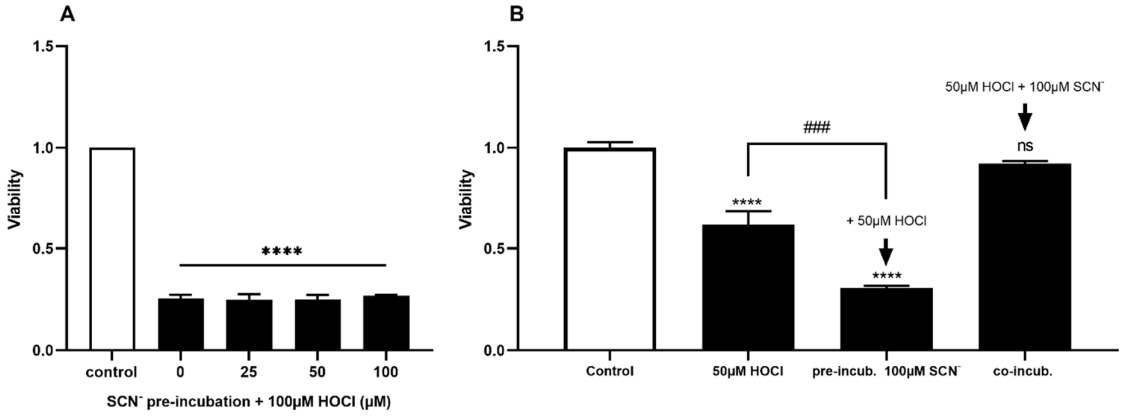


**Figure S1. Pre-treatment of HCASMC with $SCN^-$ and exposure to HOCl.** HCASMC were pre-treated in HBSS for 15 min (A) with increasing concentrations of $SCN^-$, before washing and exposure to 100 µM HOCl for a further 15 min, or (B) with 100 µM $SCN^-$ before washing and addition of 50 µM HOCl for 15 min. Changes in viability are expressed as a fold change compared to control cells as determined by metabolic activity using MTS assays. **** indicates a significant ($p < 0.0001$) difference compared to the non-treated control, ### indicates a significant ($p < 0.001$) difference comparing with and without pre-incubation with $SCN^-$, by one-way ANOVA with Dunnett's post hoc testing.

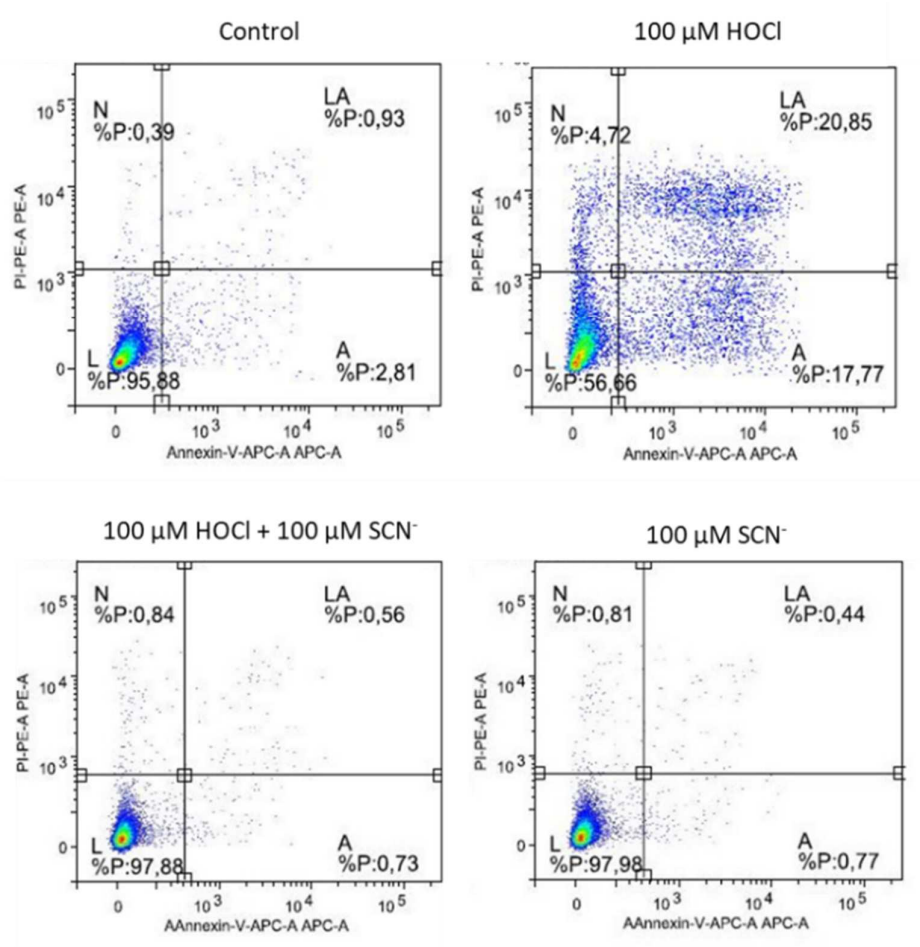


**Figure S2. $SCN^-$ prevents HOCl-induced cell death by necrosis / apoptosis.** HCASMC were exposed to either HBSS, 100 µM HOCl, 100 µM HOCl in the presence of 100 µM $SCN^-$, or 100 µM $SCN^-$, for 15 min. Data show representative flow cytometry dot plots, after the treatment, following by washing, re-incubation in growth medium for 24 h and staining with Annexin V/PI. Lower left quadrant shows live cells (L, A-/PI-), upper left quadrant shows necrotic cells (N, A-/PI+), lower right quadrant shows early apoptotic cells (A, A+/PI-), upper right quadrant shows late apoptotic/necrotic cells (LA, A+/PI+).

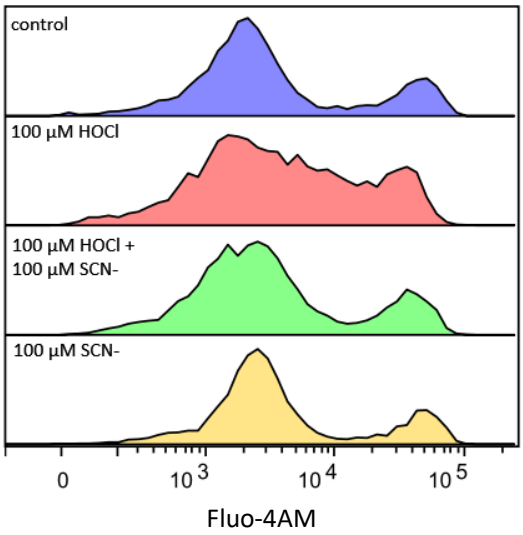


**Figure S3. Representative histograms for $Ca^{2+}$ accumulation.** Cells were exposed to 100 µM HOCl, 100 µM HOCl in the presence of 100 µM $SCN^-$, or 100 µM $SCN^-$ alone, for 15 min. Data show representative histograms of $Ca^{2+}$ accumulation after treatment and staining with Fluo-4AM and analysis by flow cytometry.

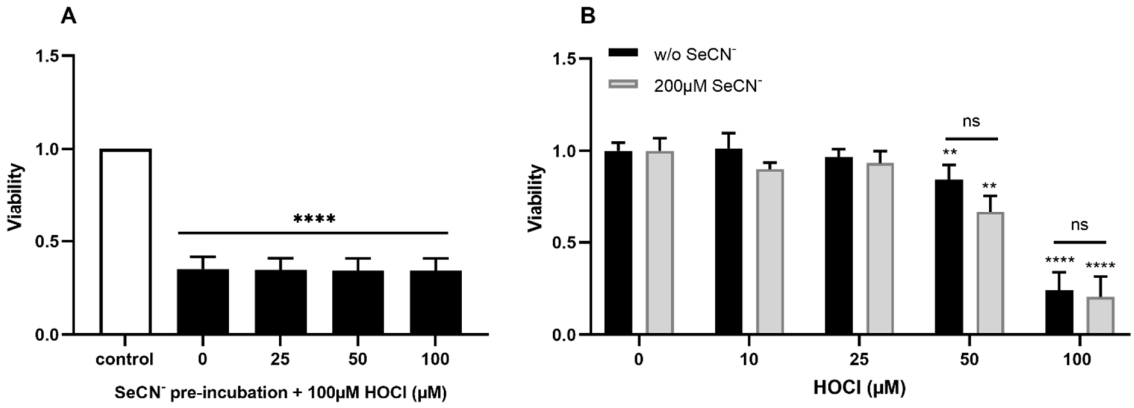


**Figure S4. Pre-treatment of HCASMC with $SeCN^-$ and exposure to HOCl.** HCASMC were pre-treated with: (A) increasing concentrations of $SeCN^-$ in HBSS for 15 min before washing, addition of 100 µM HOCl and further incubation for 15 min, and (B) with 200 µM $SeCN^-$ in cell medium (grey bars) or without (black bars) for 24 h before washing, addition of increasing concentrations of HOCl and incubation for 15 min. Changes in viability are expressed as a fold change compared to non-treated cells determined by measurement of metabolic activity using an MTS assay. ** and **** indicate a significant ($p < 0.01$ and $p < 0.0001$) difference compared to the non-treated control, by one- or two-way ANOVA with Dunnett's post hoc testing.

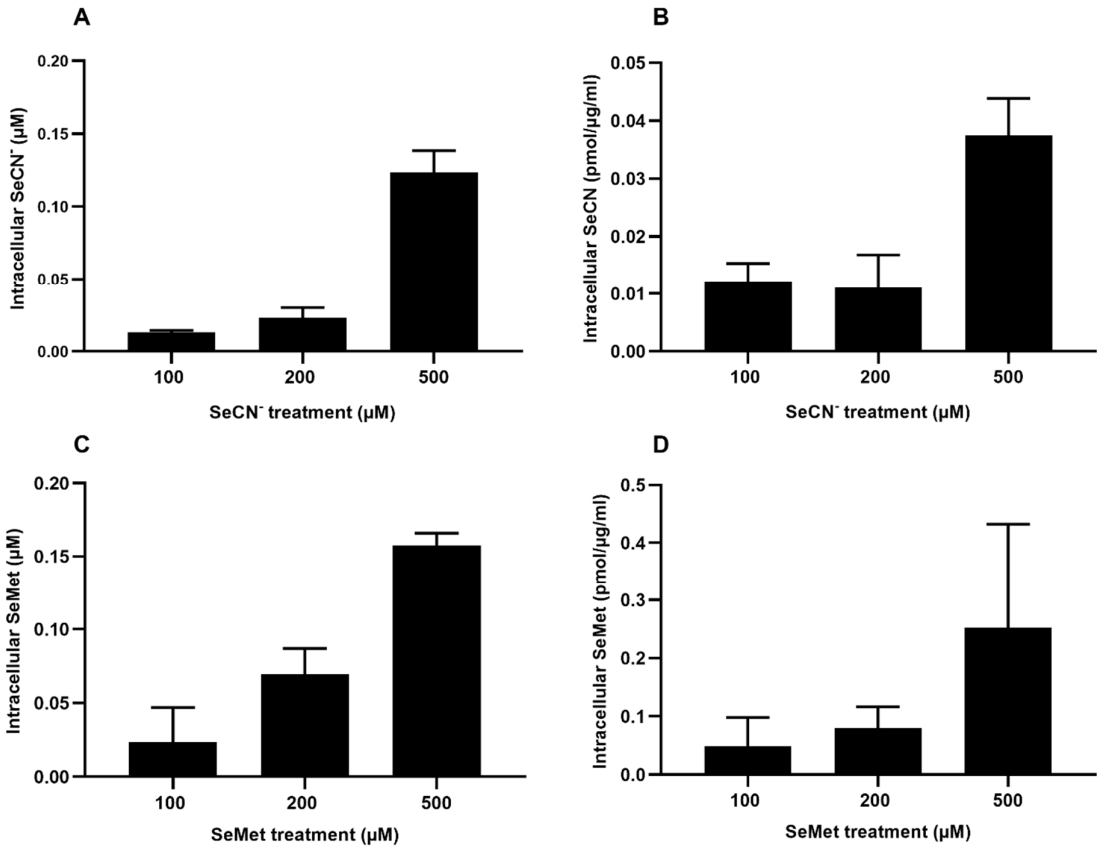


**Figure S5. Intracellular SeCN and SeMet levels.** HCASMC were exposed to $SeCN^-$ or SeMet for 15 min. After exposure the cells were lysed, and the lysates analyzed by HPLC-ICP-MS for the presence of the selenium species. (A) and (B) show $SeCN^-$ concentration detected in the HCASMC lysates, and (C) and (D) the SeMet concentration detected in the HCASMC lysates. Panels (A) and (C) show the absolute concentrations detected, whereas panels (B) and (D) show the data normalized to the cell protein concentration. Data represent means of $\leq$ 2 individual donors ± SEM.

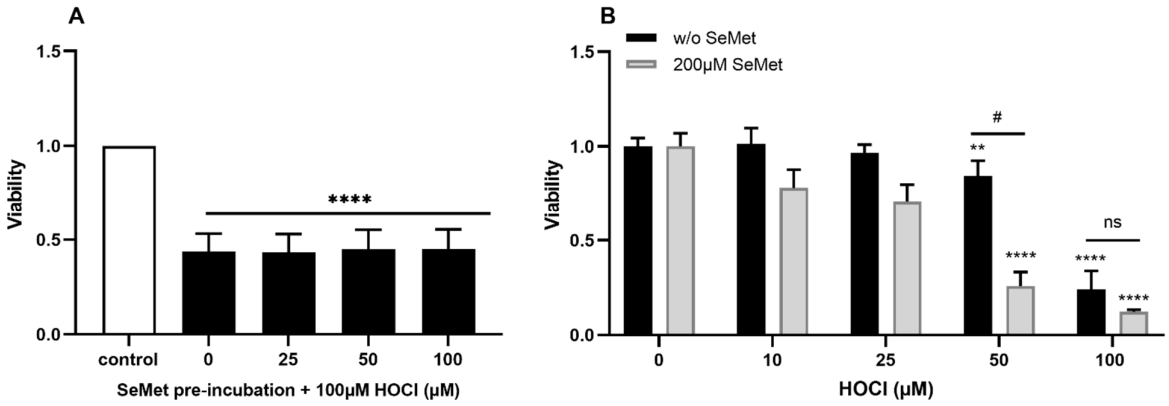


**Figure S6. Effects of pre-treatment of HCASMC with SeMet and subsequent exposure to HOCl on cell viability as determined by metabolic activity measurements.** HCASMC were pre-treated (A) with HBSS and increasing concentrations of SeMet for 15 min before washing, addition of 100 µM HOCl and further incubation for 15 min, and (B) with 200 µM SeMet in cell medium (grey bars) or without (black bars) for 24 h before washing, addition of increasing concentrations of HOCl, and incubation for 15 min. Changes in viability are expressed as a fold change compared to non-treated cells as determined by assay of metabolic activity using MTS assays. ** and **** indicate a significant ($p < 0.01$ and $p < 0.0001$) difference compared to the non-treated control, by one- or two-way ANOVA with Dunnett's post hoc testing. # indicates a significant ($p < 0.05$) difference between the with and without (w/o) SeMet treatment by two-way ANOVA with Dunnett's post hoc testing.

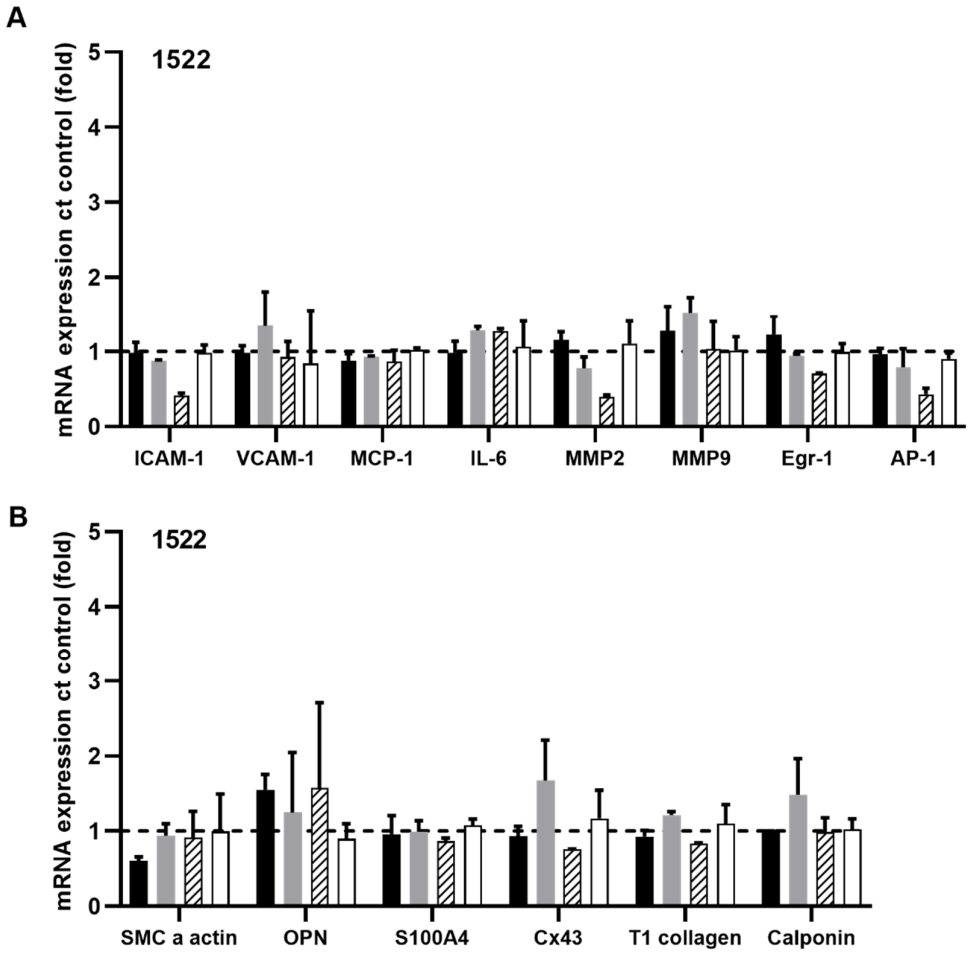


**Figure S7. Effect of HOCl and HOCl co-treatments with $SCN^-$, $SeCN^-$ and SeMet on gene expression – donor 1522.** HCASMC were exposed to 50 µM HOCl (black bars) with addition of 100 µM $SCN^-$ (grey bars) or $SeCN^-$ (dashed bars) or SeMet (white bars) for 15 min, before re-incubation in cell media for 24 h. Graphs show the mRNA expression of a range of (A) inflammatory and (B) phenotypic genes, analyzed by qPCR and normalized to the housekeeping genes 18S and B2M. Data show the results from the donor 1522. Results represent the mean ± SD of 3 replicates and are expressed as a change compared to (ct) the non-treated control.

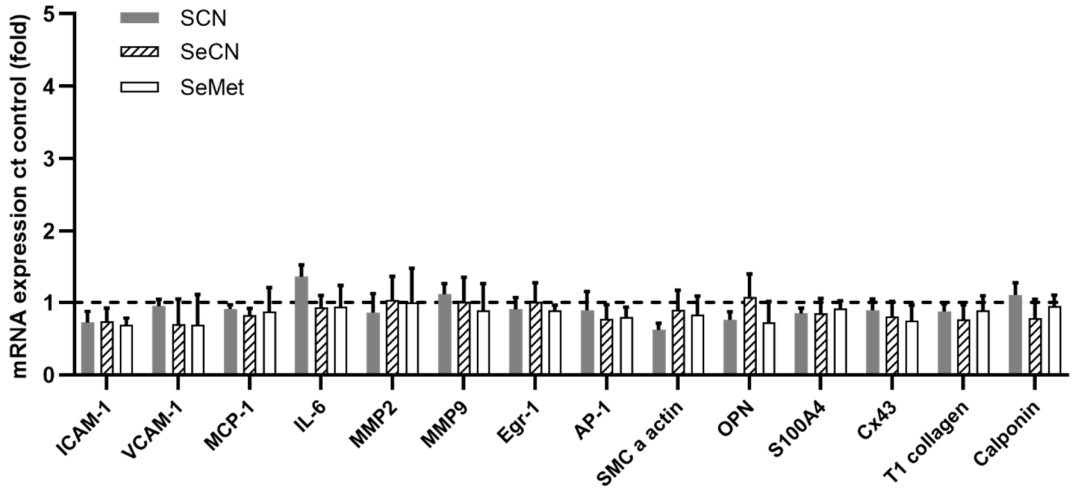


**Figure S8. Effect of $SCN^-$, $SeCN^-$ and SeMet on gene expression.** HCASMC were exposed to 100 µM $SCN^-$ (grey bars) or $SeCN^-$ (dashed bars) or SeMet (white bars) for 15 min, before re-incubation in cell media for 24 h. Graphs show the mRNA expression of a range of inflammatory and phenotypic genes, analyzed by qPCR and normalized to the housekeeping genes 18S and B2M. Results represent the mean ± SEM of 3 donors and are expressed as a change compared to (ct) the non-treated control.

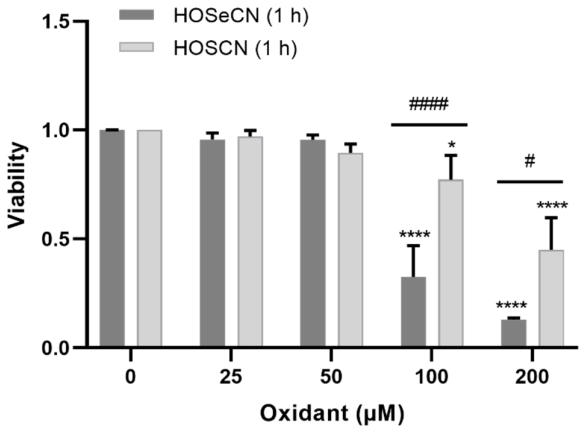


**Figure S9. Comparison of viability in HCASMC on exposure to HOSeCN or HOSCN for 1 h.** The metabolic activity was determined by MTS assay directly after the treatment. Data represent mean ± SEM from 2 individual donors (1596, 1559). * $p < 0.05$ and **** $p < 0.0001$ indicate a significant decrease compared to the non-treated control cells; # $p < 0.05$ and #### $p < 0.0001$ indicate a significant difference between the two oxidants, by two-way ANOVA with Dunnett's multiple comparison post hoc test.