Progressive stages of dysmetabolism are associated with impaired biological features of human cardiac stromal cells mediated by the oxidative state and autophagy

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Supplementary Figures S1, S2

Supplementary Table S1

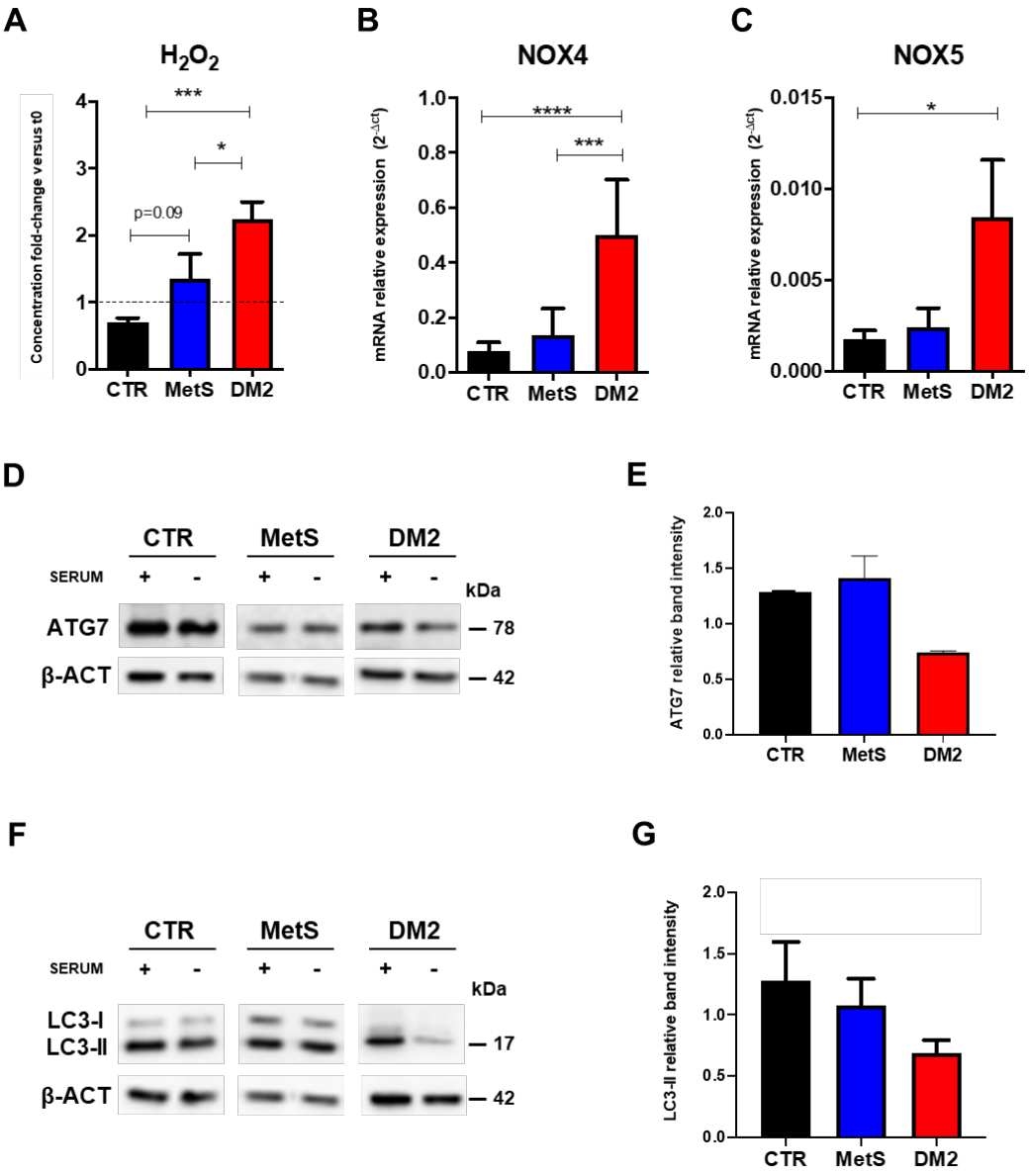

Figure S1. Oxidative state and autophagy modulation in CSCs after starvation. (A) Quantification of H_2O_2 concentration in conditioned media samples of CSCs from the different donor groups. Gene expression analysis by RT-qPCR of *NOX4* (B) and *NOX5* (C) in CSCs from different groups. N>=6 for each group. Representative western blot panels of ATG7 (D) LC3-II (F) protein levels in CSCs from different donor groups, and relative densitometric analysis (E-G) after 4 hours of starvation, normalized to each corresponding non-starved reference. N>=3 for each group. * P<0.05. *** P<0.001. **** P<0.0001.

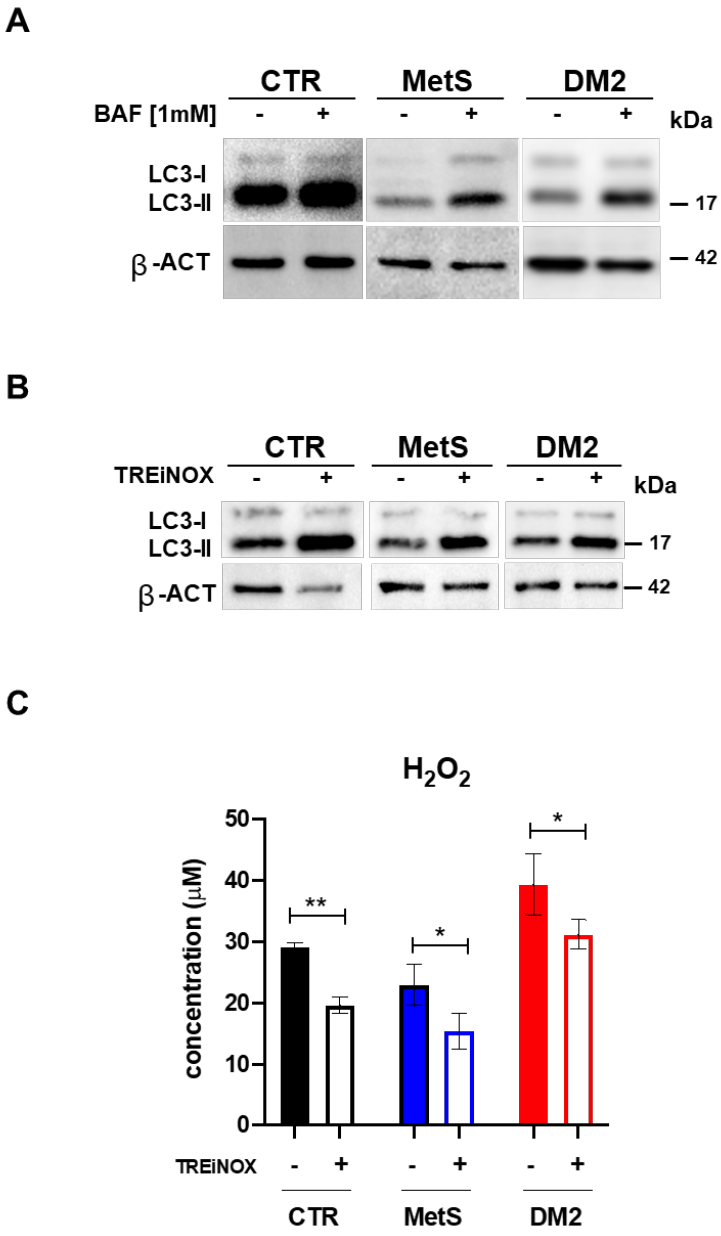

Figure S2. Validation of autophagy activation and TREiNOX treatment in CSCs. (A) Representative western blot panels showing LC3-II protein levels at the end of the serum starvation performed adding either DMSO or Bafilomycin A (BAF) to CTR, MetS or DM2-CSC cultures 2 hours before harvesting the cells. (B) Representative western blot panel showing LC3-II and LC3-I, with and without TREiNOX treatment, to test for boosted autophagosome formation rather than reduced lysosome activity. (C) Quantification of hydrogen peroxide release in conditioned media with or without TREiNOX treatment, in CTR, MetS or DM2-CSC cultures to check for the treatment efficacy in reducing oxidative stress. N=3 for each group. * P<0.05. ** P<0.01.

Table S1. Sequences of the primers used for RT-qPCR.

Primer name	**Sequence**
GATA4 Forward	GTTTTTTCCCCTTTGATTTTTGATC
GATA4 Reverse	AACGACGGCAACAACGATAAT
ACTA2 Forward	ATGAAGATCCTGACTGAGCG
ACTA2 Reverse	GCAGTGGCCATCTCATTTTC
THY1 Forward	CAGCGGAAGACCCCAGT
THY1 Reverse	CGTTAGGCTGGTCACCTTCT
NKX2-5 Forward	GGTGGAGCTGGAGAAGACAGA
NKX2-5 Reverse	CGCCGCTCCAGTTCATAG
POU5F1 Forward	GATCAGCCACATCGCCCAG
POU5F1 Reverse	TCCCCCTGAGAAAGGAGACC
COL1A1 Forward	CACACGTCTCGGTCATGGTA
COL1A1 Reverse	AAGAGGAAGGCCAAGTCGAG
COL3A1 Forward	CATGCCCTACTGGTCCTCAG
COL3A1 Reverse	ATAGCCTGCGAGTCCTCCTA
MMP1 Forward	ACAACTGCCAAATGGGCTTG
MMP1 Reverse	TGTCCTTGGGGTATCCGTGT
NOX4 Forward	AACCAAGGGCCAGAGTATCA
NOX4 Reverse	GGATAAGGCTGCAGTTGAGG
NOX5 Forward	CATCGATGTGTGTGCACGGC
NOX5 Reverse	ATCCGGGTCAATGGAGCCAC
HPRT1 Forward	TCCTCCTCCTGAGCAGTCA
HPRT1 Reverse	ACCCTTTCCAAATCCTCAGC
TNNI3 Forward	GGACAAGGTGGATGAAGAGA
TNNI3 Reverse	AGGGTGGGCCGCTTAAACT
SNAI1 Forward	CTTCTCTAGGCCCTGGCTG
SNAI1 Reverse	CATCTGAGTGGGTCTGGAGG
ADRB2 Forward	AGGCAGCTCCAGAAGATTGA
ADRB2 Reverse	GTCTTGAGGGCTTTGTGCTC
KIT Forward	GATGGATGGATGGTGGAGAC
KIT Reverse	GGGATTTTCTCTGCGTTCTG

CDH5 Forward	CAACGGAACAGAAACATCCC
CDH5 Reverse	CTGCTGCTGCCACTGCT
MYH1 Forward	CAGAAGAAGAAGATGGATGC
MYH1 Reverse	CGCTGGTGTCCTGCTCCT
SNAI2 Forward	TCGGACCCACACATTACCTT
SNAI2 Reverse	GCAGTGAGGGCAAGAAAAAG
CTNNB1 Forward	AGGTCTGAGGAGCAGCTTCA
CTNNB1 Reverse	ATTGTCCACGCTGGATTTTC
KDR Forward	AAAGGGTGGAGGTGACTGAG
KDR Reverse	CGGTAGAAGCACTTGTAGGC
GJA1 Forward	AGGAGTTCAATCACTTGGCG
GJA1 Reverse	GAGTTTGCCTAAGGCGCTC
CDH2 Forward	GTGCATGAAGGACAGCCTCT
CDH2 Reverse	AGCTTCTCACGGCATACACC
TGFBR2 Forward	AGAAGTCGGAATATAACACCAGC
TGFBR2 Reverse	TCACACAGGCAGCAGGTTAG
VIM Forward	ACCCACTCAAAAAGGACACTTC
VIM Reverse	GGTCATCGTGATGCTGACAA