

Supplementary Materials for

Ameliorating the hallmarks of cellular senescence in skeletal muscle myogenic progenitors in vitro and in vivo

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Figs. S1 to S6

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S8

Supplementary Figure 1

	Aujusteu p-values written inside the cens			
EZH2	0	0.480	0.108	0.019
HIST1H1E	1.270e-024	0.895	0.569	
HIST1H2AB	2.920e-022			0.006
HIST1H2AG	1.550e-033	0.480	0.401	0.005
HIST1H2AH	6.670e-025			0.006
HIST1H2AI	2.580e-028		0.070	0.001
HIST1H2AJ	1.910e-016	0.721	0.654	0.044
HIST1H2AL	4.740e-018	0.806	0.487	0.018
HIST1H2AM	4.340e-013	0.890	0.861	0.061
HIST1H2BB	2.180e-033		0.044	2.310e-005
HIST1H2BD	5.350e-045	0.886	0.242	0.049
HIST1H2BF	9.090e-016	0.370	0.198	0.008
HIST1H2BJ	7.880e-035	0.828	0.562	0.016
HIST1H2BL	1.500e-025	0.811		
HIST1H2BO	5.220e-025			0.006
HIST1H3B	5.810e-026	0.462	0.289	0.007
HIST1H3C	9.610e-025	0.371		0.001
HIST1H3H	3.860e-026		0.232	0.001
HIST1H3I	2.110e-023		0.070	0.015
HIST1H3J	3.560e-031	0.361		0.002
HIST1H4A	1.590e-022	0.478	0.486	0.006
HIST1H4B	2.420e-023	0.579	0.669	
HIST1H4J	9.880e-022	0.927	0.619	0.047
HIST2H2AB	6.730e-019	0.592	0.562	0.036
HIST2H2BB	2.770e-018	0.704		0.023
HIST2H2BF	4.030e-032	0.727	0.390	0.019
HIST2H3A	4.200e-027		0.013	9.140e-005
HIST2H3C	6.110e-021	0.549	0.107	0.004
HIST2H3D	2.850e-023	0.418	0.094	0.006
HIST2H4A	2.470e-025	0.806	0.533	
HIST2H4B	5.460e-033	0.632	0.244	0.004
Log2 Fold Chang	s.	s	S	s.
Log2 Fold Challg	S SA	VS	SV	SV
	\prec	N5	110	115
1 2 3 4	5	\mathbf{S}	SN	SN

Adjusted p-values written inside the cells

Supplementary Figure 1: Heat map of mRNA expression level of histone proteins and

EZH2 histone methyl transferase. The color represents the Log2 fold increase and the number

in each box represents the adjusted p-value.



Supplementary Figure 2: Dox had no effect on heterochromatin marks and DNA damage response. Control S cells (without NANOG transgene) were treated with Dox at 1µg/ml concentration for 15 days. A) Dox treatment did not change the levels of heterochromatin mark (H3K9me3) in control senescent cells. B) Dox treatment did not change the levels of DNA damage marker (γ H2AX) in control senescent cells. \$ signs denote P<0.05 as compared to all other samples.



Supplementary Figure 3: Restoration of proteostasis by NANOG in senescent cells. (A-E) GSEA analysis suggests that (A, B) NANOG expression for 5-15 days in senescent cells leads to significant enrichment of proteasome compartments as well as ubiquitin mediated proteolysis pathway by NANOG; (C, D) NANOG initially downregulates ribosome and peptide elongation pathway but eventually increases the activity of these pathways in senescent cells; (E) NANOG expression restores protein folding processes in senescent cells. (F) Immunostaining of Y, S, SN15, and SNR myoblasts for NANOG (red), nuclei (blue) and F-Actin (green). (G) Quantification of the cellular cytoplasmic area; data shown as mean \pm 95% CI for >200 cells per condition and mean \pm STD for three donors. (H) Western blotting analysis of LC3 protein upon starvation+chloroquine (SQ) treatment for 1.5hr and quantification of the increase in LC3-II after SQ treatment as a metric of autophagosome formation. (I) GSEA analysis suggests no statistically significant difference in the transcriptional regulation of autophagy pathway. (J-K) Western blotting quantification of autophagy proteins. \$ denotes p<0.05 as compared to all other samples. # denotes p<0.05 as compared to S.



Supplementary Figure 4: (A&B) Enrichment plots for Oxidative Phosphorylation pathway as well as TCA Cycle and Respiratory Electron Transport pathway comparing SN15 vs S.





Supplementary Figure 5: Dox had no effect on mitochondrial function. Control senescent cells (without NANOG transgene) were treated with Dox at the concentration of 1μ g/ml for 15 days (S+Dox). A&B) Assessing mitochondrial membrane potential by staining for Mitotracker (green) and TMRM (red) dyes and quantifying the fluorescence intensity. C&D) Measurement of Oxygen Consumption Rate (OCR) in a Mitostress test to assess the mitochondrial respiration capacity. # signs denote P<0.05 as compared to S.

Supplementary Figure 6



Supplementary Figure 6: Immunostaining of LAKI and LAKIN muscle cross-sections for NANOG (green) and Pax7 (red) near the Atridox injection site. The arrows point to the Pax7+ Nanog+ cells. The white square is magnified on the right.