

Chemoprophylaxis with spermidine protects the liver endothelium and ameliorates chronic liver injury in mice

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SUPPLEMENTARY FIGURES AND TABLES

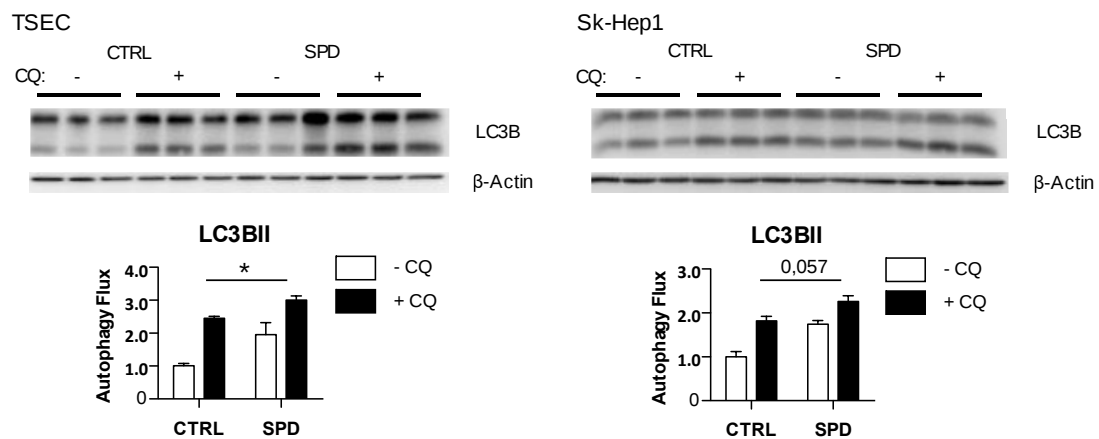
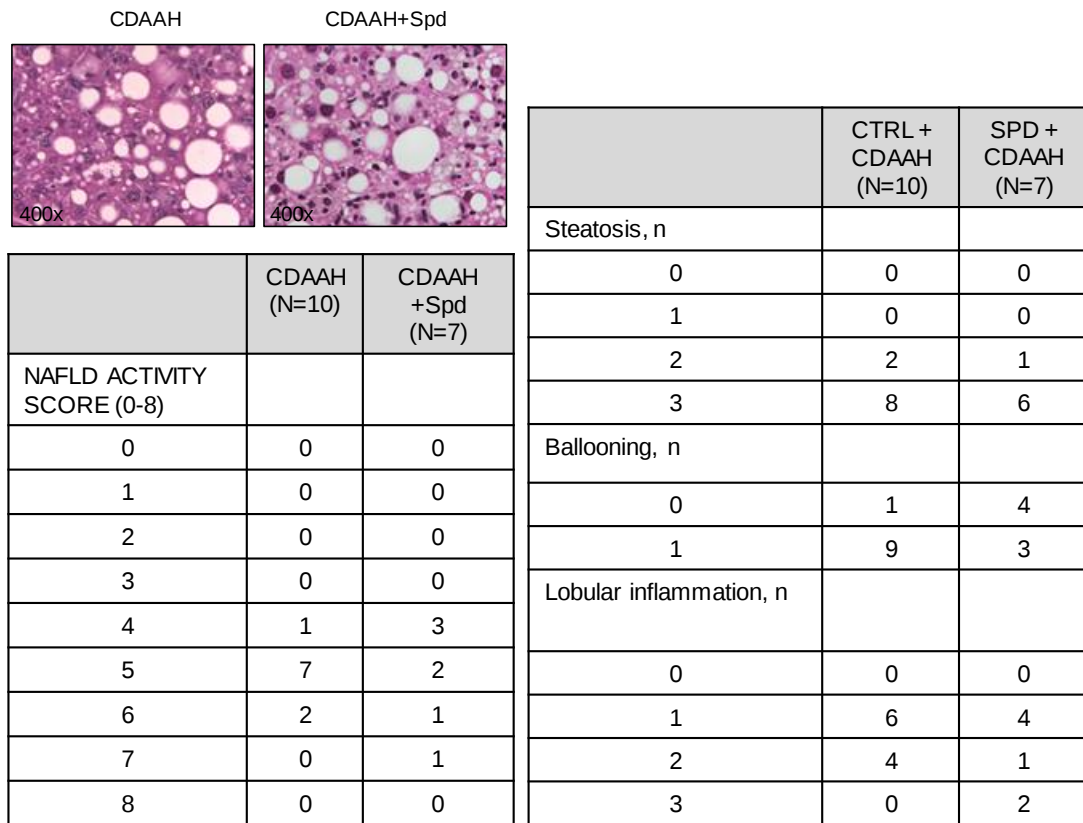
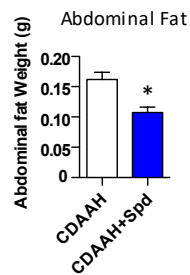


Figure S1. SPD treatment increase autophagy levels in TSEC and Sk-Hep1 cells. TSEC and SK-HEP1 cells were seeded in 6-well plates in triplicates. Next day cells were treated with SPD with or without 20 μ M CQ for 24 h. Cells were then collected and analyzed by western blot against LC3B antibody. LC3BII band was quantified and shown in the graphs below. β -Actin was used as loading control. One representative of two independent experiments is shown. Results are the mean $\pm$ SEM. * $P < 0.05$ of cells treated with CQ compared to their non-treated controls. Student's t-test.

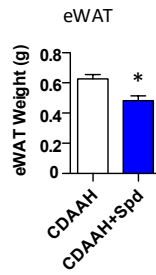
S2A



S2B



S2C



S2D

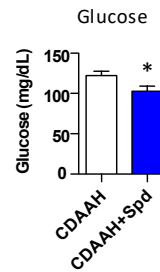


Figure S2. SPD does not modify hepatic steatosis but causes slight improvement in the altered metabolic phenotype in the CDAAH mouse model. A. Representative images and histological liver analysis of steatosis, ballooning and lobular inflammation showing a slight decrease of ballooning without changes in NAFLD ACTIVITY SCORE. B-C. Weight of the abdominal and epididymal adipose tissue, respectively, of mice treated with CDAAH diet with or without SPD supplementation. D Plasma glucose levels in CDAAH mice with or without SPD supplementation. Results are the mean $\pm$ SEM. * $P < 0.05$ compared to the correspondent control. Exact Fisher test in A and Student's t-test in B-D.

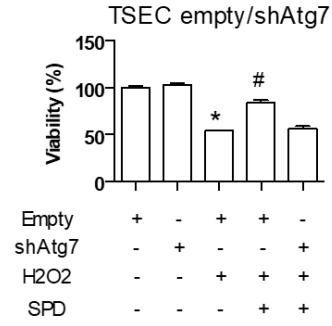


Figure S3. Deficient autophagy impairs SPD protection against oxidative stress in vitro. TSEC infected with an shRNA against Atg7 or with an empty control plasmid were seeded in a 96-well plate in triplicates. Next day cells were pre-treated with SPD for 24 h. Medium was then changed and H2O2 added for another 24 h before adding the MTS solution. One representative of two independent experiments is shown. Results are the mean $\pm$ SEM. * $P < 0.05$ compared to the empty control plasmid. # $P < 0.05$ compared to H2O2 treatment. Student's t-test.

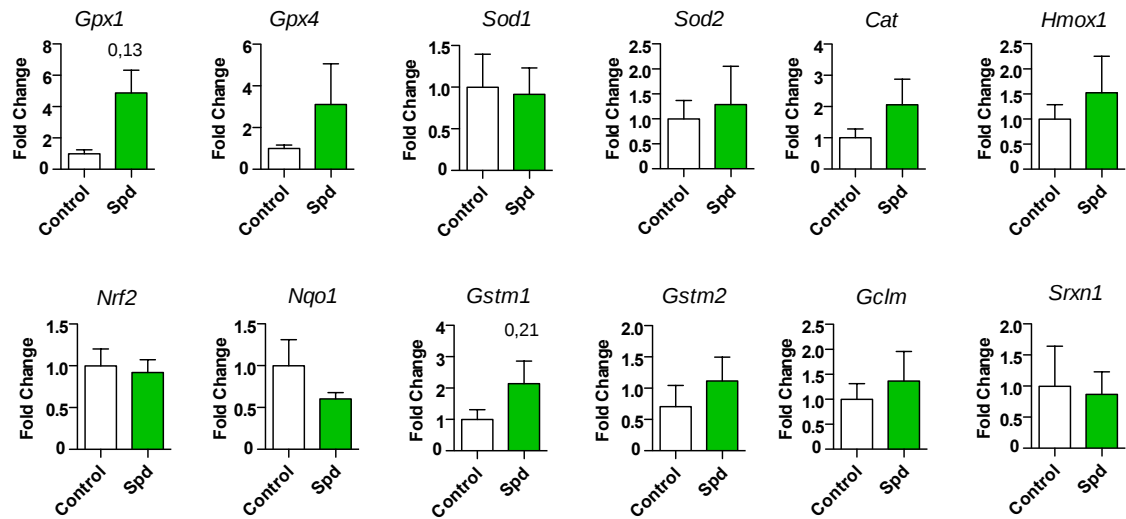
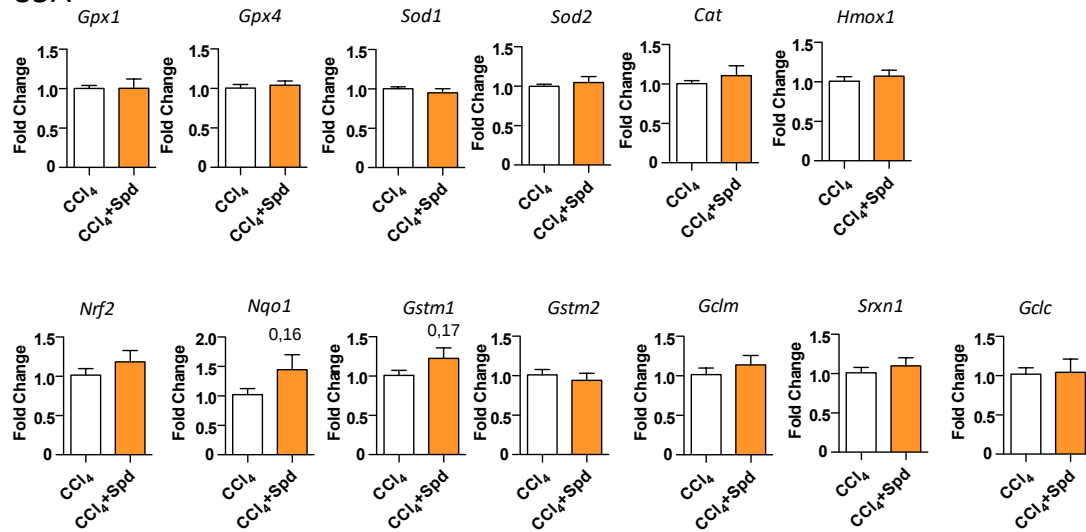


Figure S4. SPD treatment tends to increase classical and NRF2-mediated antioxidant enzyme systems. TSEC were plated in 6-well plates in triplicates. Next day, cells were treated with 10 μ M SPD for 24 h and then collected for RNA isolation. Graphs represent the results obtained by qRT-PCR for each indicated gene in controls versus SPD-treated TSEC. Gapdh was used as housekeeping gene. Results are the mean $\pm$ SEM. Student's t-test.

S5A



S5B

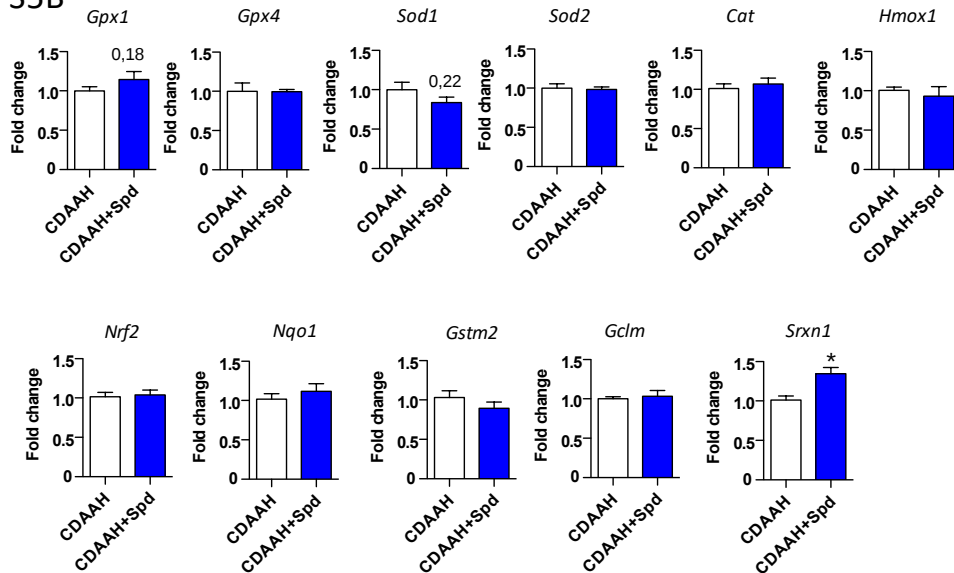
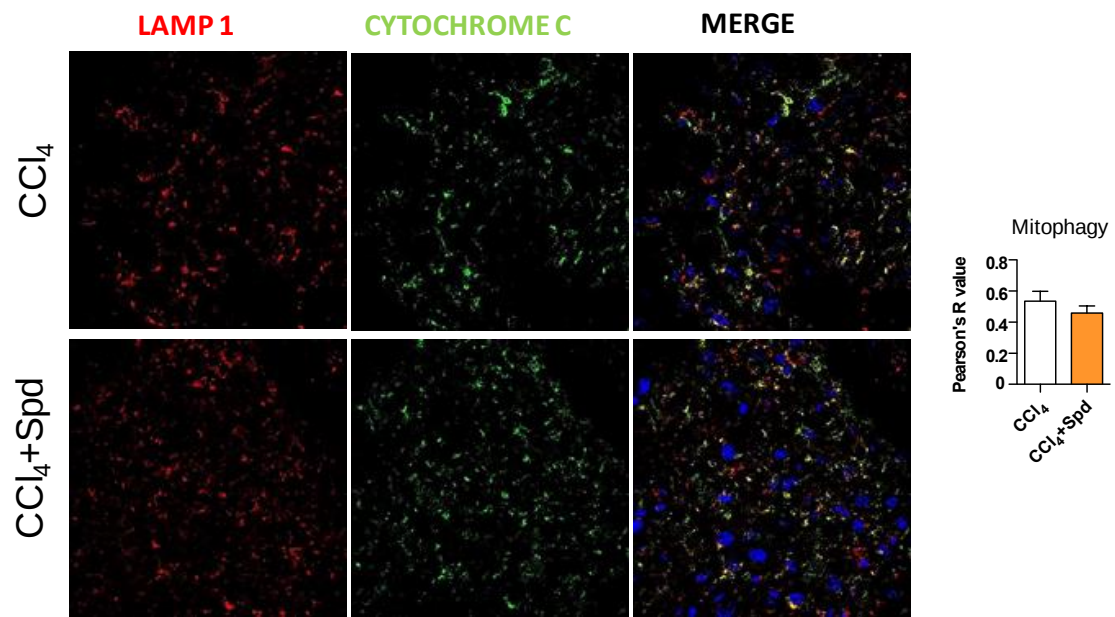


Figure S5. SPD treatment did not show increased hepatic expression of antioxidant enzymes. A-B. qRT-PCR quantification of classical and NRF2-mediated antioxidant enzymes in total liver tissue of mice treated with CCl₄ or the CDAAH diet with or without SPD supplementation. Gapdh was used as housekeeping gene. Results are the mean $\pm$ SEM. Student's t-test.

S6A



S6B

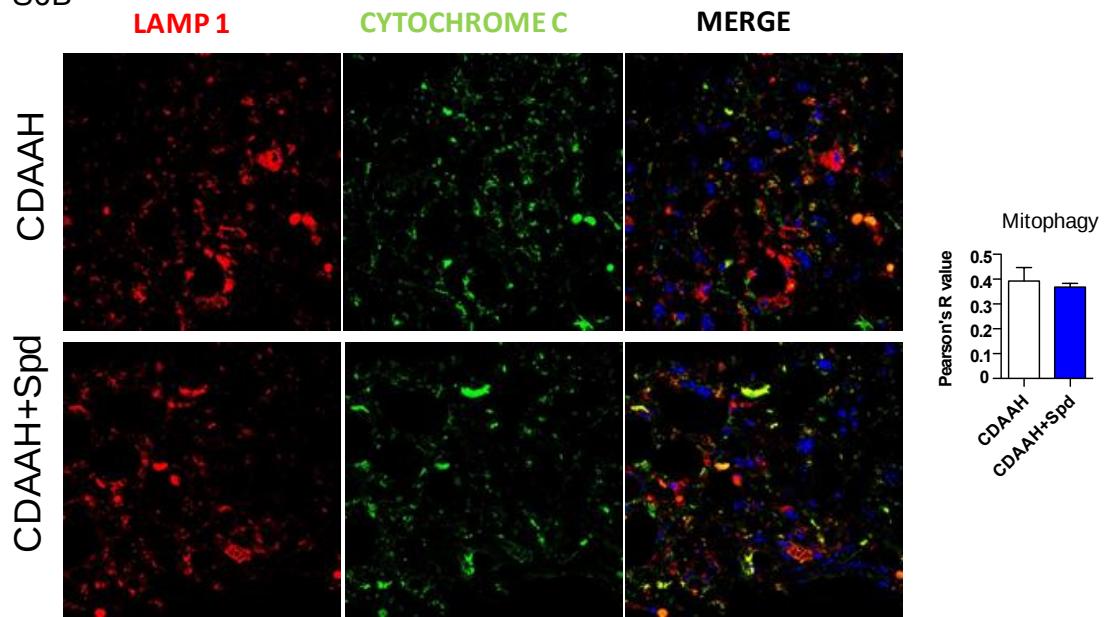


Figure S6. SPD treatment did not show changes in mitophagy activity. A-B. Co-immunofluorescence of LAMP1 and Cytochrome c in FFPE liver sections of mice treated with either CCl₄ or the CDAAH diet with or without SPD supplementation. Pearson's correlation between both signals was calculated and shown in the adjacent graphs. Results are the mean $\pm$ SEM. Student's t-test.

Supplementary Table S1

<i>Gpx1</i>	Gpx1_Fw	TCGGTTTCCCGTGCAATCA
	Gpx1_Rv	GTCGGACGTACTTGAGGGAA
<i>Gpx4</i>	Gpx4_Fw	TTACGAATCCTGGCCTTCCC
	Gpx4_Rv	TAGCCGGCTGCAAACCTCC
<i>Sod1</i>	Sod1_Fw	CTCACTCTCAGGAGAGCATTCC
	Sod1_Rv	TTCCACCTTTGCCCAAGTCA
<i>Sod2</i>	Sod2_Fw	AAGGAGCAAGGTCGCTTACA
	Sod2_Rv	AATCCCCAGCAGCGGAATAA
<i>Cat</i>	Cat_Fw	GGGATCTTGTGGGAAACAACAC
	Cat_Rv	CTGTGGGTTTCTCTTCTGGCTA
<i>Nrf2</i>	Nrf2_Fw	AGTGGATCCGCCAGCTAC
	Nrf2_Rv	CTCTGCCAAAAGCTGCATACA
<i>Srxn1</i>	Srxn1_Fw	GCACAACGTACCAATCGCC
	Srxn1_Rv	CAGGGTCCGCCAGGATCG
<i>Nqo1</i>	Nqo1_Fw	TCTCTGGCCGATTGAGAGTG
	Nqo1_Rv	CCAGACGGTTTCCAGACGTT
<i>Gclc</i>	Gclc_Fw	CTGCTGTCCCAAGGCTCG
	Gclc_Rv	TGTACTCCACCTCGTCACCC
<i>Gclm</i>	Gclm_Fw	TGGGCACAGGTAAAACCCAA
	Gclm_Rv	CTGGGCTTCAATGTCAGGGA
<i>Gstm1</i>	Gstm1_Fw	CCGTGCAGACATTGTGGAGA
	Gstm1_Rv	CTGCTTCTCAAAGTCAGGGTTG
<i>Gstm2</i>	Gstm2_Fw	CAGCCCTGACTTTGAGAAAAAGA
	Gstm2_Rv	GACCTTGTTCCCTGCAAACCA
<i>Gapdh</i>	Gapdh_Fw	AGACGGCCGCATCTTCTT
	Gapdh_Rv	TTCACACCGACCTTCACCAT
<i>Actb</i>	Actb_FW	CCCTAAGGCCAACCGTGAAA
	Actb_RV	CAGCCTGGATGGCTACGTAC