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Supplemental Information

Telomere Dysfunction Induces Sirtuin Repression that Drives Telomere-Dependent Disease

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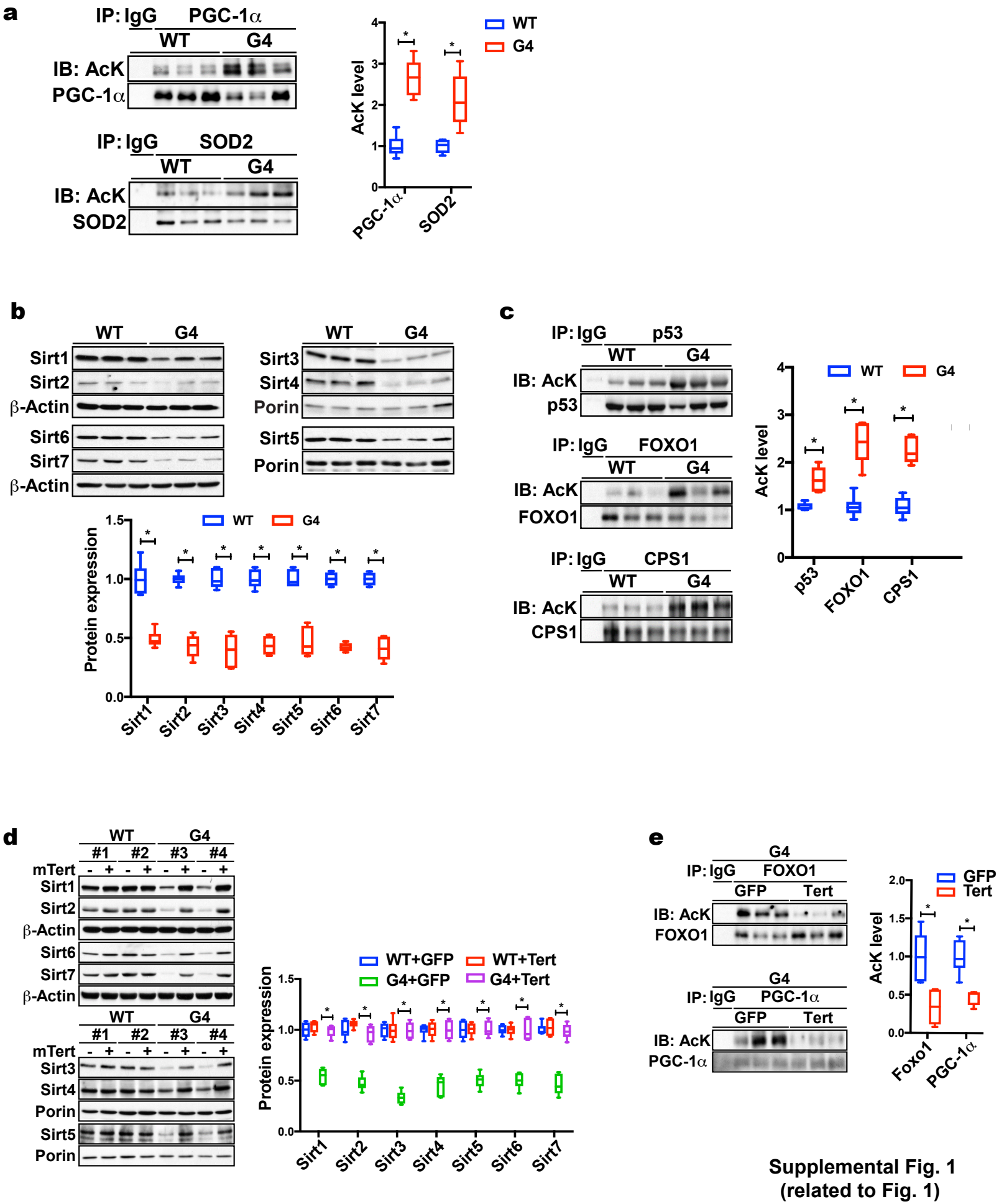
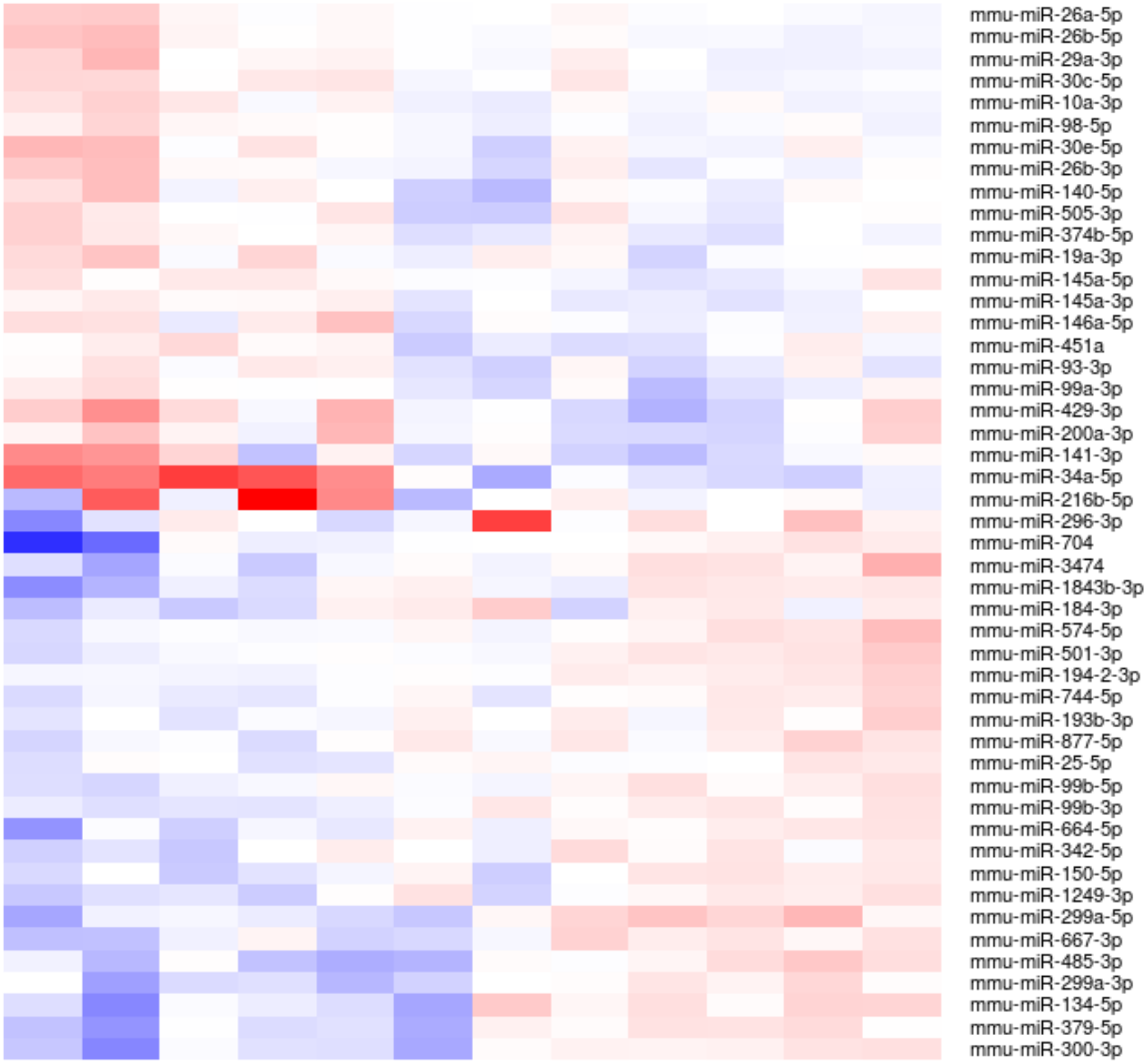


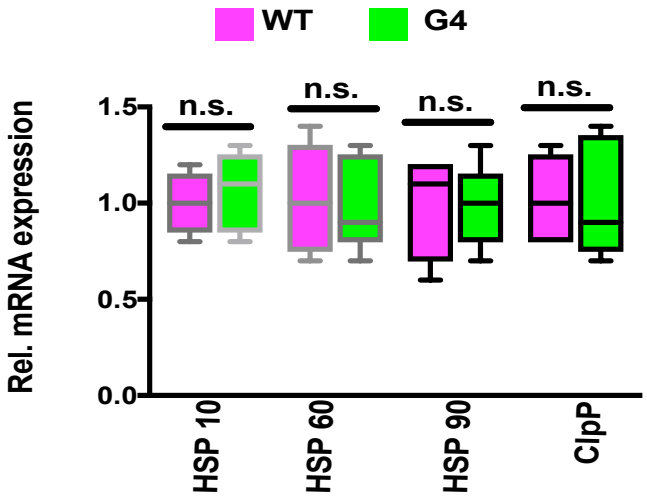
Supplemental Fig. 1
(related to Fig. 1)

a



Supplemental Fig. 2
(related to Fig. 4)

a



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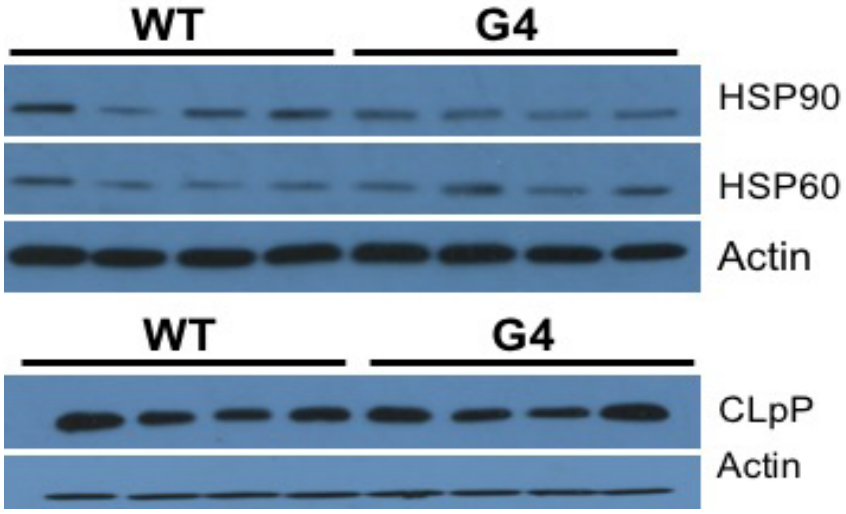
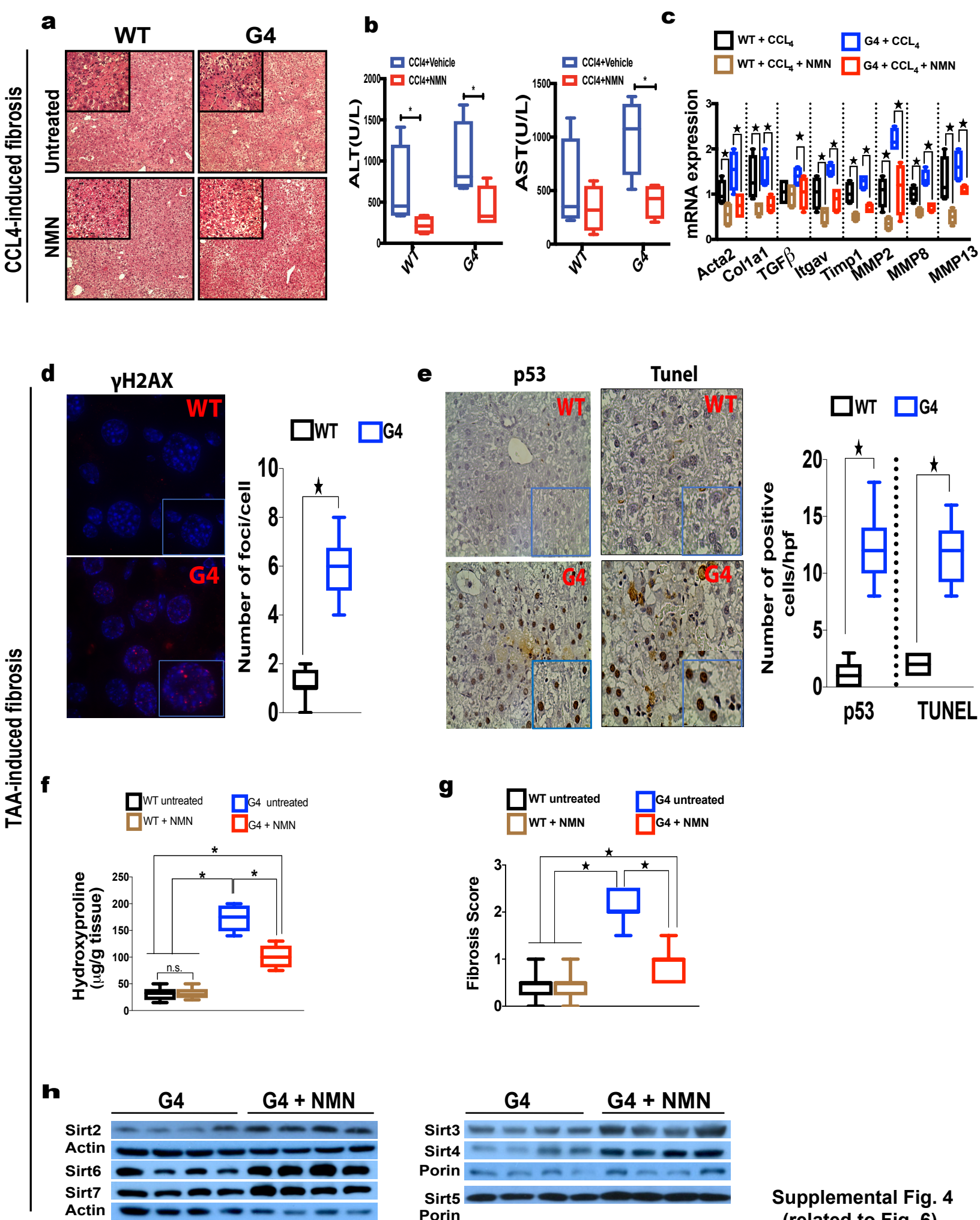
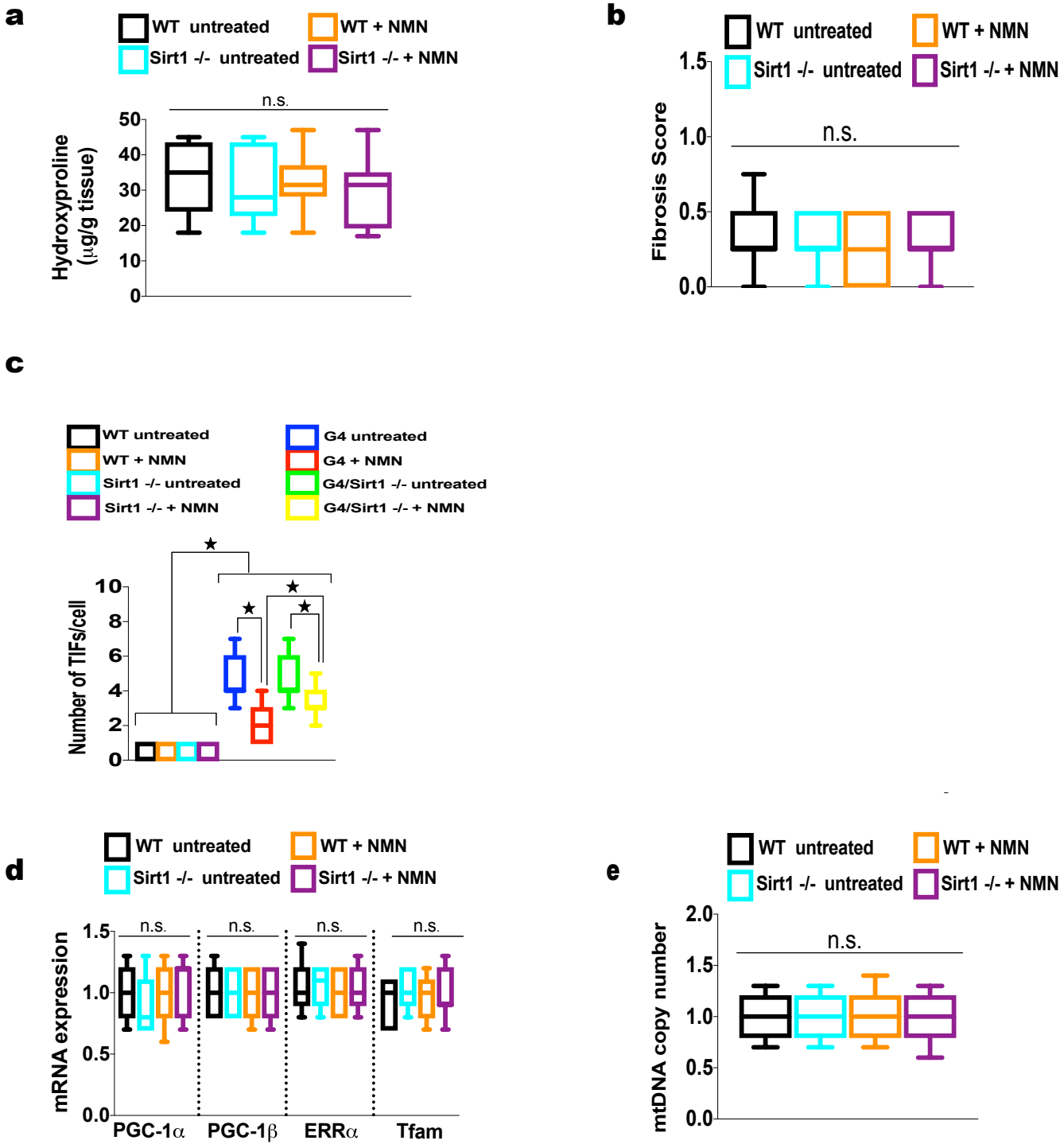


Supplemental Fig. 3
(related to Fig. 5)



Supplemental Fig. 4
(related to Fig. 6)



Supplemental Fig. 5
(related to Fig. 7)

Supplemental Fig. 1 (related to Fig. 1) Telomere dysfunction induces sirtuin repression in MEFs and liver tissue while telomerase reactivation reverses these changes (a) IP-western: Pgc-1α and Sod2 are hyperacetylated in G4 liver compared to WT liver tissue (shown are 3 per group; n= 9 per group; $p < 0.05$); (b) Western blot analysis demonstrates that sirtuins are repressed in a cell-autonomous manner in G4 MEFs (3 independent cell lines/group; $p < 0.05$); (c) Combined IP-western blot analysis reveals hyperacetylation of several sirtuin targets (p53, Foxo1, Cps1) in G4 MEFs (3 independent MEF lines/group; $p < 0.05$); (d) Telomerase reactivation in two WT (#1; #2) and two G4 (#3; #4) MEF cell lines increases sirtuin protein abundance in G4 MEFs without affecting sirtuin levels in WT MEFs; (e) Telomerase reactivation reverses hyperacetylation of PGC-1α and FOXO1 in three G4 MEF cell lines; Results are quantified by densitometry and expressed as mean ± s.e.m.; t-test was used to determine statistical significance with $p < 0.05$ considered as significant, as indicated by (*).

Supplemental Fig. 2 (related to Fig. 4) P53 regulates distinct set of miRNAs in G4 mice (a) Heat map of differentially regulated miRNAs in G4/p53 +/+ (n= 5) and G4/p53 -/- (n= 6) liver tissue as determined by RNA sequencing; (b) RT-qPCR-based analysis of p53-dependent miRNAs in liver tissue of WT and G4 mice (n= 8 per group). Results are expressed as mean ± s.e.m.; t-test was used to determine statistical significance with $p < 0.05$ considered as significant, as indicated by (*).

Supplemental Fig. 3 (related to Fig. 5) Telomere dysfunction is not associated with activation of mtUPR response in the liver (a) RT-qPCR analysis of WT or G4 liver tissue does not show changes in expression levels of mtUPR markers heat shock protein HSP 10, 60, 90 and protease ClpP (n= 4 per group; t-test was used to determine statistical significance); (b) Western blot analysis of mtUPR markers does not show any difference between WT and G4 mice (n= 4 per group)

Supplemental Fig. 4 (related to Fig. 6) (a) Representative H&E staining of liver section derived from WT and G4 mice shows that NMN treated mice have less necrosis (see insert for blow-up of necrotic areas; n= 12 per group); (b) Liver transaminase (ALT, AST) levels in peripheral blood are decreased in mice treated with NMN indicative of decreased liver cell damage (n= 12 per group); (c) RT-qPCR analysis demonstrates decreased expression of fibrosis-associated genes in mice treated with NMN (n= 12 per group); (d, e) γH_2AX, p53 and Tunel staining show minimal effect of TAA in WT mice compared to G4 mice; graphs on the right show quantification of γH_2AX foci per cell and number of p53 or Tunel positive cells per high-power field; (n = 8 per group; for γH_2AX determination a total of 50 cells per mouse were scored from 5 randomly chosen liver sections and for p53 and Tunel the number of positive cells were determined from 5 random section and counted per high-power field per mouse); (f) Hydroxyproline determination shows minimal collagen accumulation in TAA-treated WT mice compared to G4 mice, which was significantly reduced with NMN treatment (n= 8 mice per group); (g) Quantification of fibrosis shows TAA induces minimal fibrosis in WT mice while G4 mice develop marked fibrosis, which is significantly improved with NMN treatment (n= 8 mice per group); (h) Western blot analysis of G4 mice subjected to TAA shows significant increase of sirtuins in mice treated with NMN (shown are 4 mice of 8 total analyzed). Results are expressed as mean ± s.e.m.; t-test was used to determine statistical significance with $p < 0.05$ considered as significant, as indicated by (*).

Supplemental Fig. 5 (related to Fig. 7) NMN has no effect in TAA- treated wild type mice with preserved telomeres (a, b) TAA treatment has little effect on fibrosis development in WT mice after 4 week of treatment as indicated by (a) low hydroxyproline and (b) fibrosis score, which is not altered by NMN treatment; (c) number of telomere-induced foci (TIF's) per cell is reduced with NMN treatment in G4 mice and this is blunted in the absence of Sirt1; WT mice have very few TIFs and this is not impacted by NMN; (d-f) NMN does not affect (d) mitochondrial biogenesis factors, (e) mtDNA copy number and (f) complex I and IV activity (in mice with preserved telomeres, irrespective of Sirt1 status; (n= 8 per group). Results are expressed as mean ± s.e.m.; t-test was used to determine statistical significance with $p < 0.05$ considered as significant, as indicated by (*).