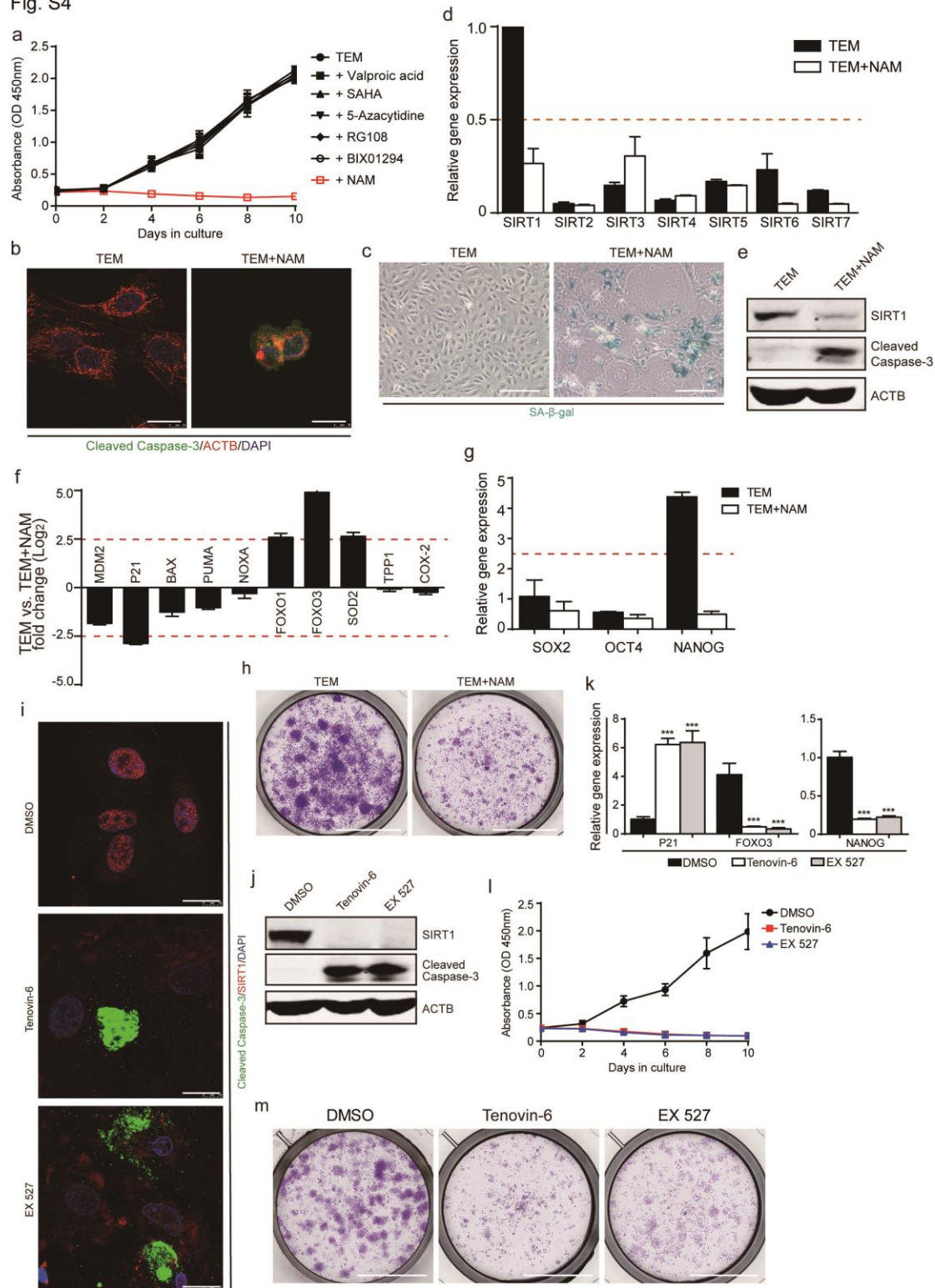


Fig. S4



Supplementary information, fig. S4 Inhibition of SIRT1 signaling prevents the conversion, related to Fig. 2. (a) CCK-8 analyses of HepLPCs in TEM with epigenetic modifiers. 2 mM Valproic acid and 5 mM SAHA, HDAC inhibitors. 2 mM 5-Azacytidine and 0.04 μ M RG108, DNA methylation inhibitors. 1 μ M BIX01294, H3K9 methyltransferase family inhibitor. 10 mM NAM, nicotinamide, sirtuin deacetylase family inhibitor. Error bars represent s.d.; n = 5 technical replicates from one donor. (b) Immunofluorescence images of Cleaved Caspase-3 and ACTB in PHCs cultured in TEM without (left) or with (right) NAM at day 4. Scale bars, 20 μ m. (c) PHCs cultured in TEM with NAM show sign of cell senescence (SA- β -gal staining) as compared to that in TEM at day 6. Scale bars, 100 μ m. (d) QPCR analyses for the expression of sirtuin family genes of PHCs in TEM or TEM with NAM at day 4. Error bars represent s.d.; n = 3 donors. (e) Western blot analysis of SIRT1 and Cleaved Caspase-3 expression in TEM or TEM with NAM at day 4. ACTB as the loading control. (f and g) QPCR analyses for the expression of SIRT1-targeted genes (f) and SIRT1-related stemness genes (g) of PHCs in TEM or TEM with NAM at day 4. Error bars represent s.d.; n = 3 donors. (h) Light microscopy images demonstrating a reduction in clone formation in TEM with NAM versus TEM by crystal violet staining. Scale bars, 10mm. (i) Immunofluorescence images of SIRT1 and Cleaved Caspase-3 in PHCs in TEM, TEM with 5 μ M Tenovin-6 and TEM with 50 μ M EX 527 at day 4. Scale bars, 20 μ m. (j) Western blot analysis of SIRT1 and Cleaved Caspase-3 expression in TEM, TEM with Tenovin-6 and TEM with EX 527 at day 4. ACTB as the loading control. (k) QPCR analyses for the expression of P21, FOXO3 and NANOG in TEM, TEM with Tenovin-6 and TEM with EX 527 at day 4.

Error bars represent s.d.; n = 3 donors (one-way ANOVA with Dunnett correction for multiple comparisons, *** $p < 0.001$). (l) CCK-8 analyses demonstrating suppression of cell proliferation in the presence of Tenovin-6 or EX 527 at day 10. Error bars represent s.d.; n = 5 technical replicates from one donor. (m) Light microscopy images show a reduction in clone formation following inhibition of SIRT1 with Tenovin-6 or EX 527 by crystal violet staining. Scale bars, 10mm.