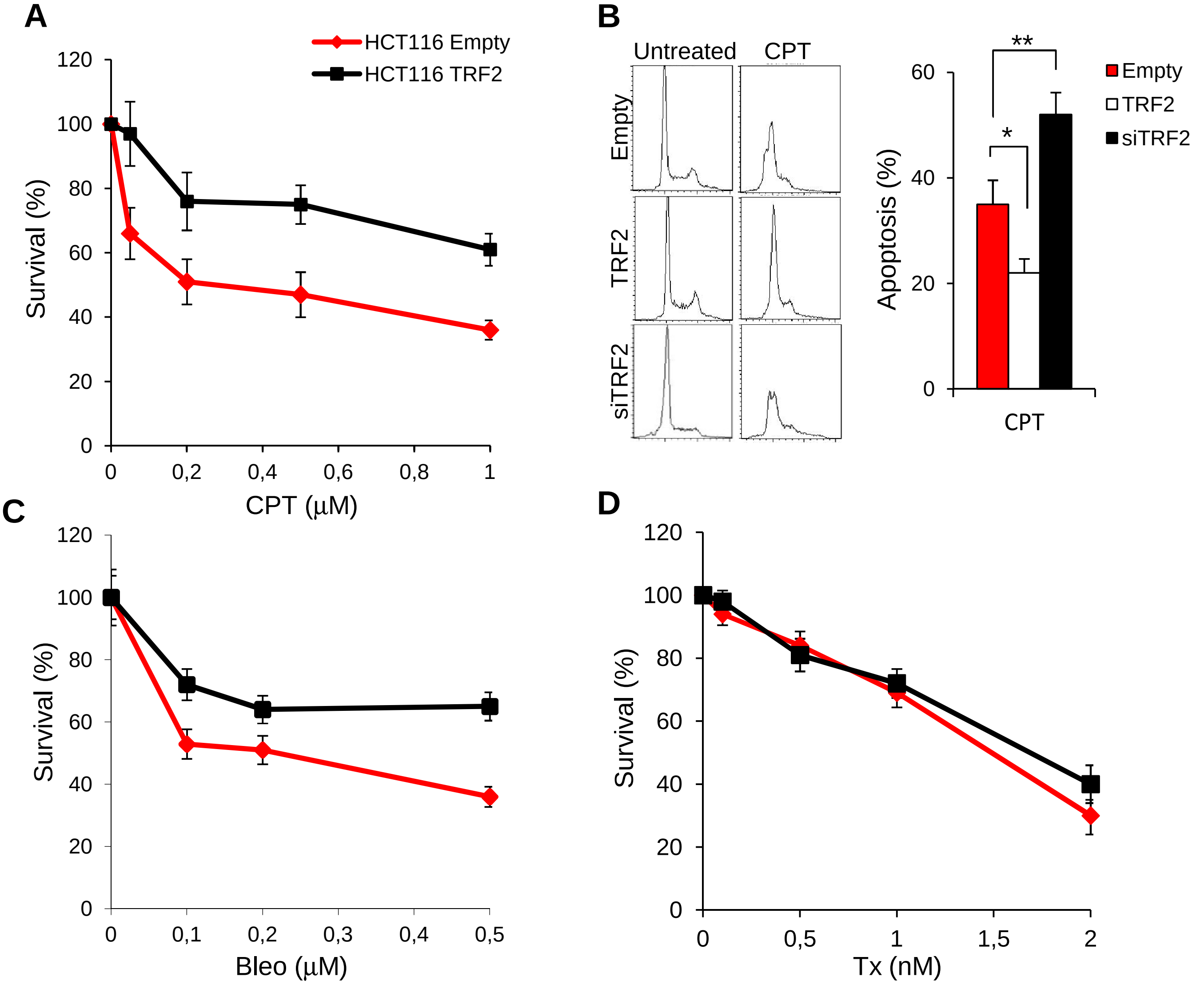
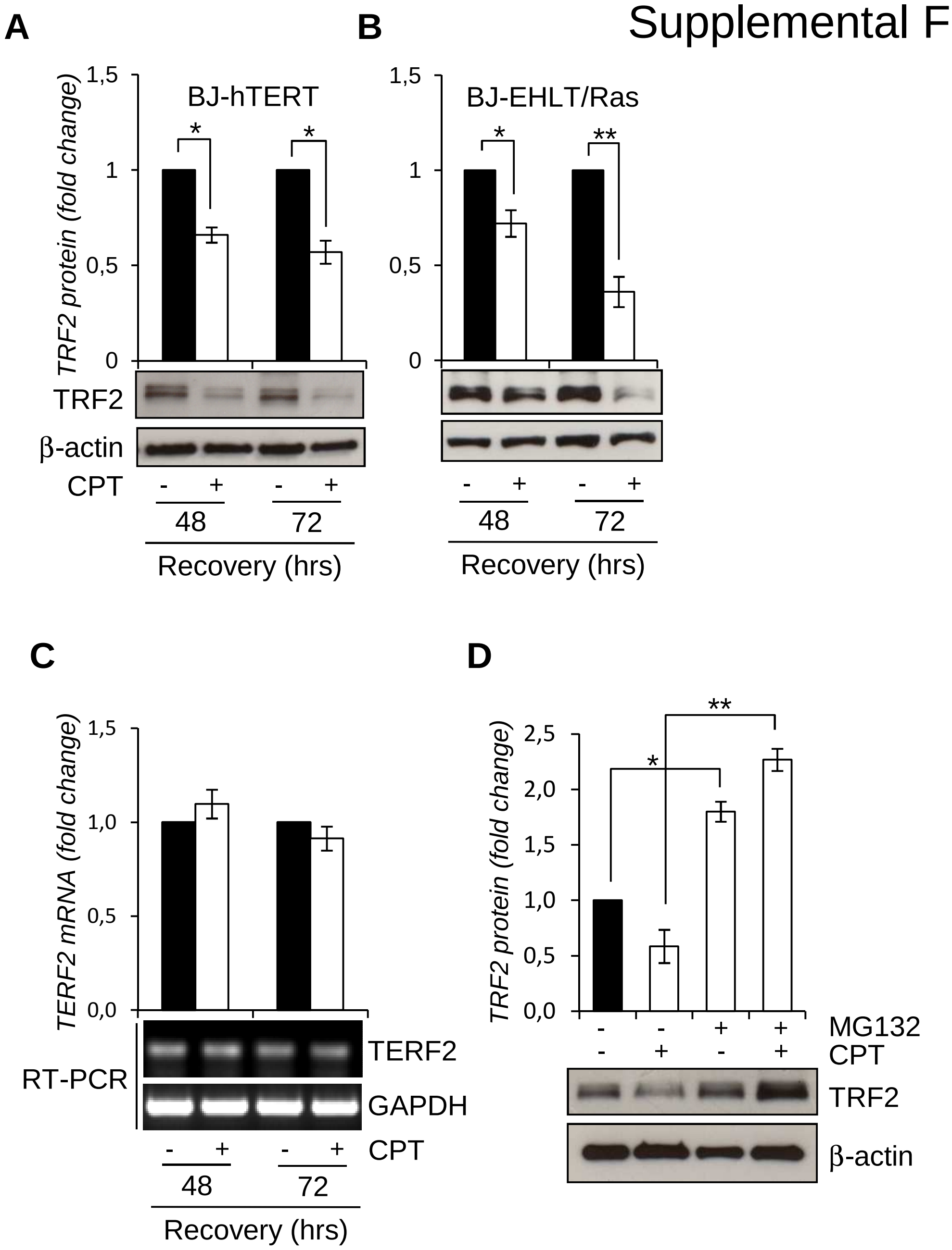
Supplemental Figure 1



Supplemental Figure 1. TRF2 overexpression confers resistance to CPT and Bleo exposure. A) Survival curves of HCT116 cells infected with Myc-TRF2 or Empty retroviral vectors exposed for 2 hrs to the indicated doses of Camptothecin (CPT). B) Cell cycle analysis with PI staining of HCT116 cells transfected with the indicated constructs or with the siRNA for TRF2 untreated or treated with 0.2 µM CPT. y axis, cell numbers; x axis, DNA content. The percentages of apoptosis reported in the bar graph were calculated by the ModFit LT software. C-D) Survival curves of HCT116 cell lines exposed to the indicated doses of Bleomicin (Bleo; C) and Taxol (Tx; D). The data represent the mean of three independent experiments ± SD.

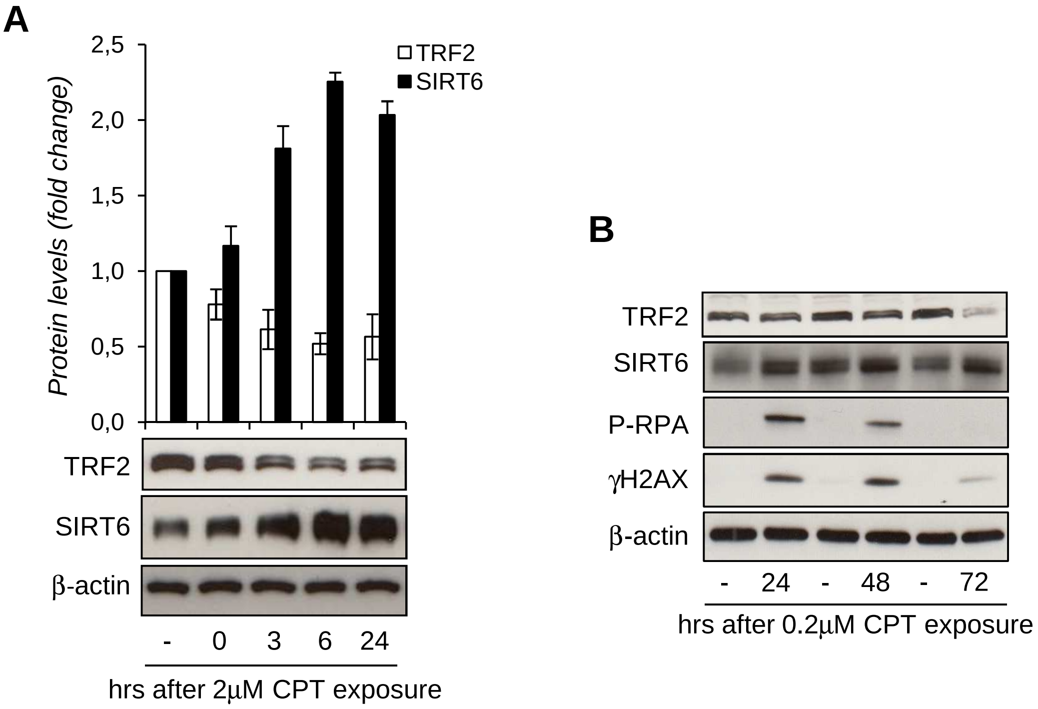
Supplemental Figure 2



Supplemental Figure 2. TRF2 is degraded upon CPT treatment through a proteasome-mediated p53-independent mechanism.

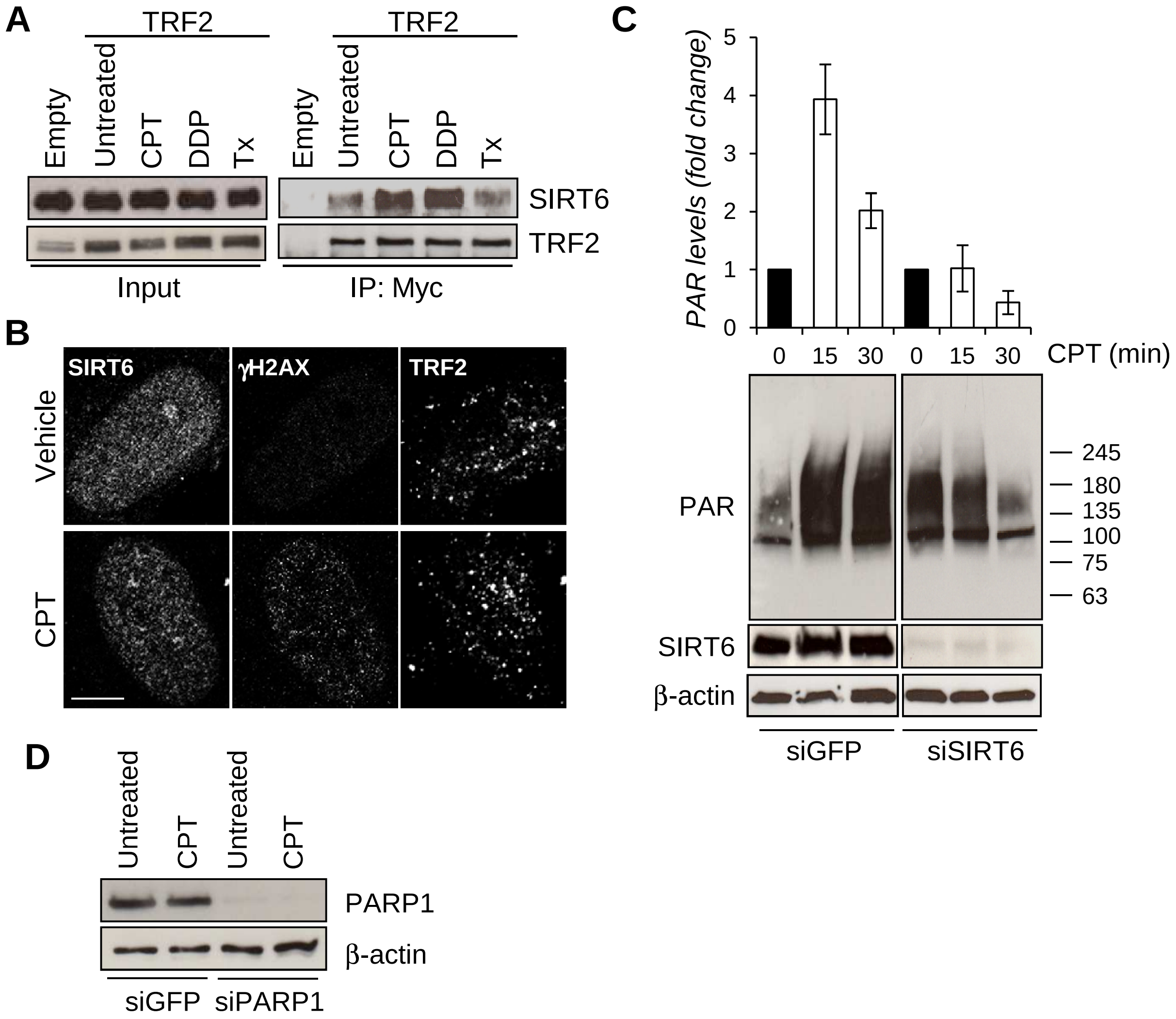
A-B) Expression levels of TRF2 were examined by western blot in BJ-hTERT (A) and BJ-EHLT/Ras (B) human fibroblasts treated with 0.2 µM CPT for 2 hours and then processed for immunoblotting after 48 and 72 hours by the end of treatment. C) RT–PCR analysis of *TERF2* mRNA with the picture on the lower panel showing the amplification products run on agarose gel in HCT116 cells treated as the fibroblasts in A-B. D) HCT116 cells were exposed to 2 µM CPT for 2 hours; at the end of treatment the medium was replaced in presence or not of the proteasome inhibitor MG132 (10 µM) and after 6 hours the cells were processed for western blot analysis of the indicated proteins. All histograms show the TRF2 expression levels expressed as fold changes in treated versus untreated samples, after b-actin normalization. The mean values of three independent experiments are reported. Bars indicate means ± SD. * = $p<0.05$; ** = $p<0.01$.

Supplemental Figure 3



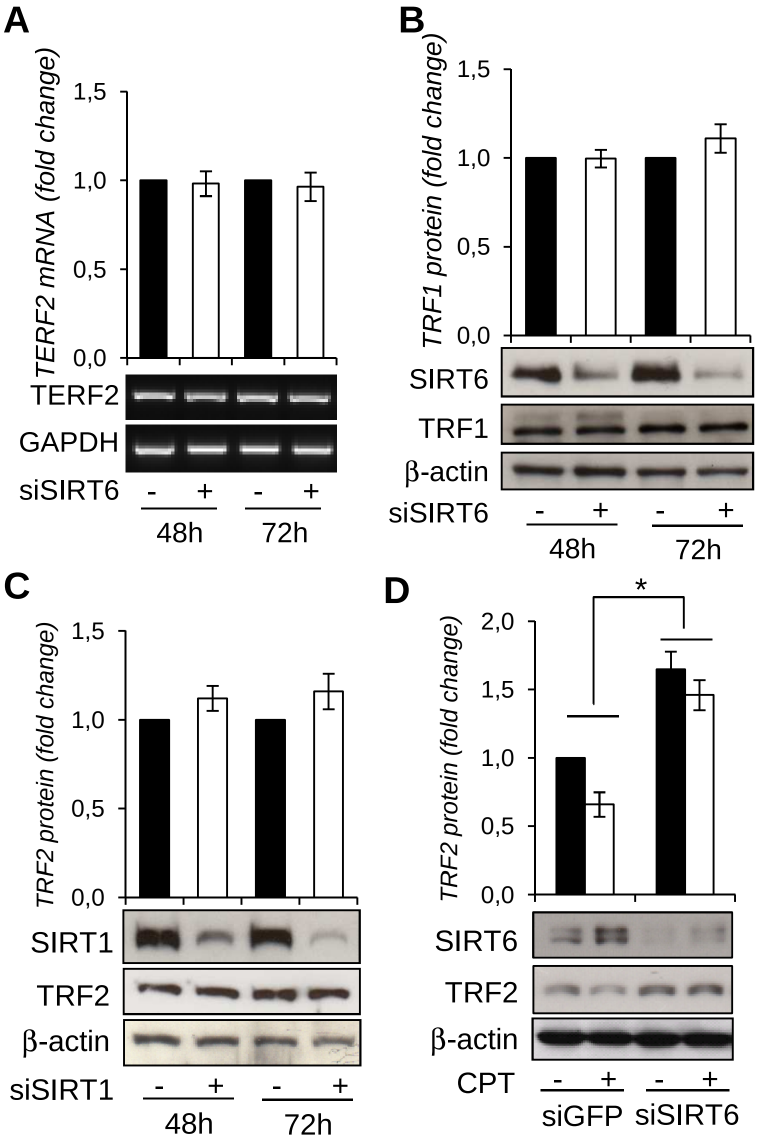
Supplemental Figure 3. TRF2 is degraded upon CPT treatment while SIRT6 is increased. Expression levels of indicated proteins were examined by western blot in HCT116 cells treated with 2μM (A) or 0.2μM (B) CPT for 2 hours and then processed at the indicated times by the end of treatment. In (A) the histograms show the TRF2 and SIRT6 expression levels reported as fold changes in treated versus untreated samples, after β-actin normalization. The mean values of three independent experiments are reported. Bars indicate means ± SD.

Supplemental Figure 4



Supplemental Figure 4. TRF2 and SIRT6 interaction is increased by DNA damage in a PARP-dependent manner. A) Nuclear extracts of HCT116 cells infected with Myc-TRF2 or Empty retroviral vectors were immunoprecipitated with an anti-Myc antibody. The IP was conducted on cells untreated or exposed to the indicated drugs as reported in the Methods section. Input and immunoprecipitates were then analyzed by WB for the expression of the indicated proteins. B) Representative confocal IF images of an untreated or 2 μM CPT exposed sample of BJ-hTERT fibroblast. The single staining for TRF2, SIRT6 and γH2AX relative to the experiment showed in Figure 3D are reported. Scale bar 10 μm. C) Western blot analysis of PAR polimer formation and SIRT6 levels in siGFP- or siSIRT6-transfected HCT116 cells untreated or exposed to 2 μM CPT for 15 or 30 minutes. PAR levels were expressed in the histograms as fold changes in untreated versus CPT treated samples, after β-actin normalization. D) WB analysis of PARP1 levels in siGFP- or siPARP1-transfected BJ-hTERT cells in untreated or CPT-treated samples relative to the experiment showed in Figure 3B-C.

Supplemental Figure 5



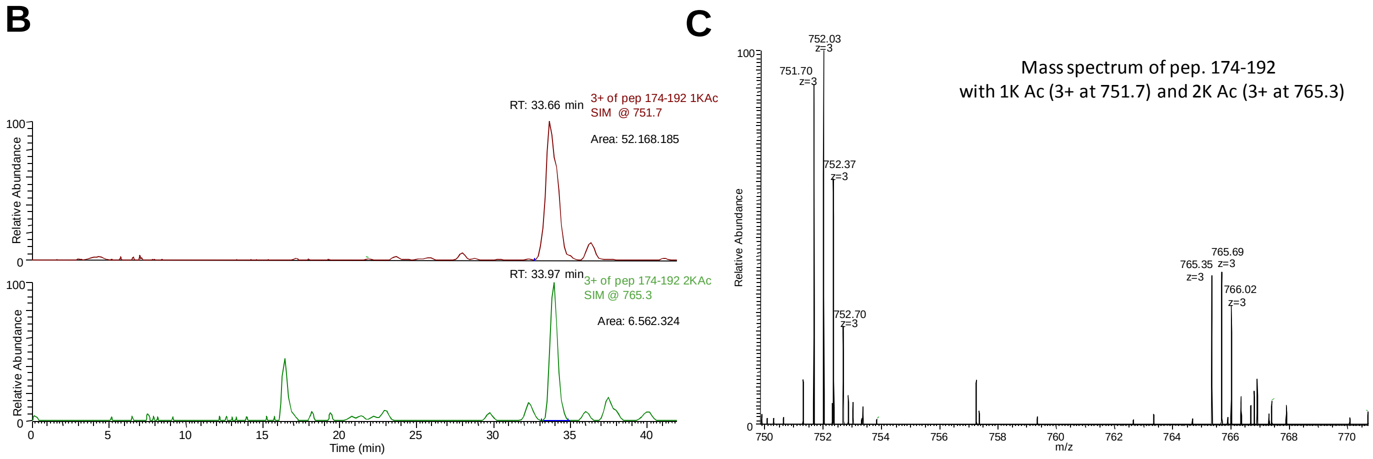
Supplemental Figure 5. SIRT6 affects TRF2 protein stability

A) RT-PCR analysis of *TERF2* mRNA in siGFP- or siSIRT6-transfected HCT116 cells after 48 and 72 hours of siRNA delivery. *TERF2* mRNA expression levels were expressed in the histograms (upper panel) as fold changes in siSIRT6 versus siGFP samples, after GAPDH normalization. Picture on the lower panel shows the RT-PCR amplification products run on agarose gel. B) Western blot analysis of TRF1 and SIRT6 levels in siGFP- or siSIRT6-transfected HCT116 cells after 48 and 72 hours of siRNA delivery (bottom panel). TRF1 expression levels were expressed in the histograms (upper panel) as fold changes in siSIRT6 versus siGFP samples, after b-actin normalization. C) Western blot analysis of TRF2 and SIRT1 levels in siGFP- or siSIRT1-transfected HCT116 cells after 48 and 72 hours of siRNA delivery (bottom panel). TRF2 expression levels were expressed in the histograms (upper panel) as fold changes in siSIRT1 versus siGFP samples, after β-actin normalization. D) Western blot analysis of TRF2 and SIRT6 levels in siGFP- or siSIRT6-transfected HeLa cells after 6 hours by the end of 2 μM CPT exposure (bottom panel). TRF2 expression levels were expressed in the histograms (upper panel) as fold changes in treated versus untreated samples, after β-actin normalization. All histograms show the mean values of three independent experiments. Bars indicate means ± SD. *= $p<0.05$.

Supplemental Figure 6

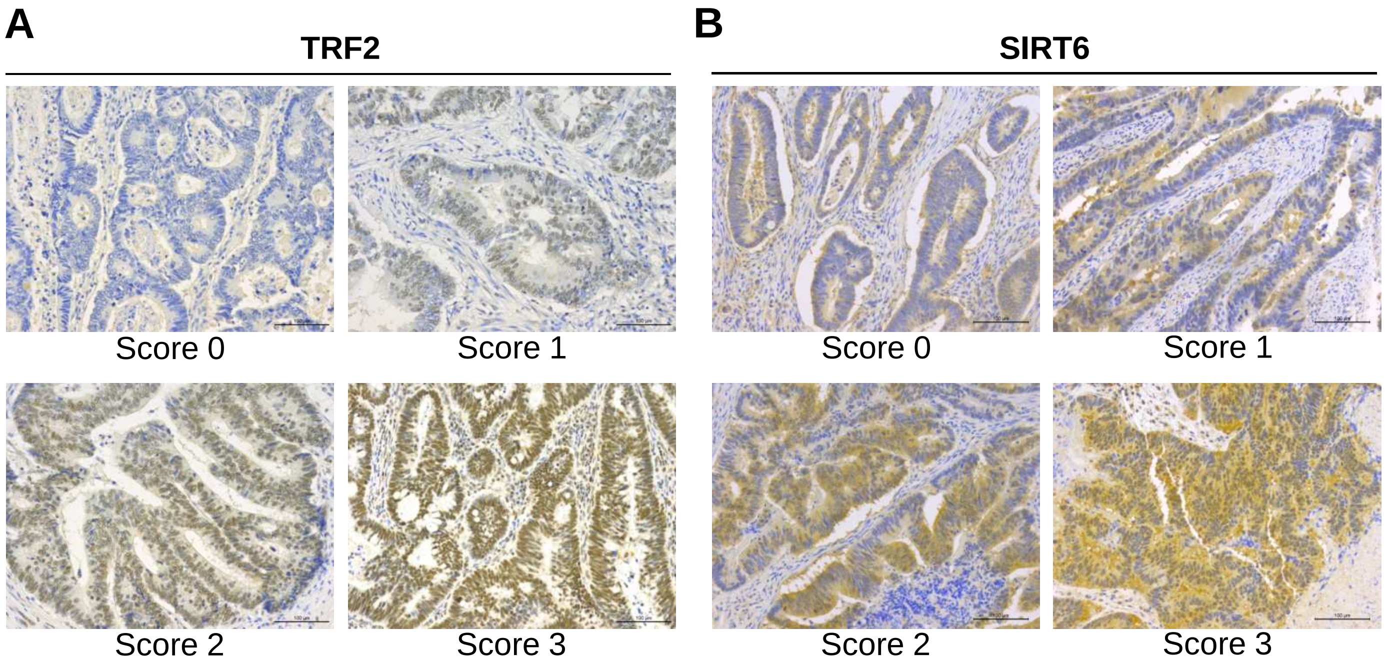
A

Accession	Score	Mass	Matches	Match(sig)	Sequences	Seq(sig)	Description
TERF2_HUMAN	2297	59728	128	91	21	16	Telomeric repeat-binding factor 2
EHD4_HUMAN	516	61365	28	18	12	9	EH domain-containing protein 4
GNL3_HUMAN	500	62468	29	24	12	12	Guanine nucleotide-binding protein-like 3
RL1D1_HUMAN	337	55167	17	13	9	7	Ribosomal L1 domain-containing protein 1
TCPD_HUMAN	289	58401	16	13	7	7	T-complex protein 1 subunit delta
TCPG_HUMAN	246	61066	12	6	7	3	T-complex protein 1 subunit gamma
RBM39_HUMAN	173	59628	8	7	3	3	RNA-binding protein 39
CPNE8_HUMAN	172	63638	19	11	9	4	Copine-8
SGPL1_HUMAN	160	64053	3	3	2	2	Sphingosine-1-phosphate lyase 1
IF2B3_HUMAN	144	64008	7	5	4	2	Insulin-like growth factor 2 mRNA-binding protein 3
RCC2_HUMAN	140	56790	10	7	7	5	Protein RCC2
PSMD3_HUMAN	132	61054	7	4	4	2	26S proteasome non-ATPase regulatory subunit 3
HNRPL_HUMAN	127	64720	3	2	2	1	Heterogeneous nuclear ribonucleoprotein L
KPYM_HUMAN	126	58470	21	8	9	6	Pyruvate kinase PKM
EHD1_HUMAN	115	60646	8	4	4	2	EH domain-containing protein 1
DJC11_HUMAN	111	63524	7	4	5	3	DnaJ homolog subfamily C member 11
NOP58_HUMAN	108	60054	9	5	6	4	Nucleolar protein 58
TCPZ_HUMAN	104	58444	9	6	4	4	T-complex protein 1 subunit zeta
CPNE3_HUMAN	102	60947	6	5	3	2	Copine-3



Supplemental Figure 6. ***In vivo*** **TRF2 acetylation pattern investigated by proteomic analysis.** A) Proteins identified in the gel band at 60 kDa by nano-LC-MSMS coupled to MASCOT bioinformatic analysis are shown. The Score represents the identification reliability; the Matches and the Match(sig) give the number of MS/MS spectra matched to a particular protein (the matched value can be misleading if the same peptide has been scanned multiple times), while the Sequences and Pep(sig) represent the number of amino acid sequences identified by experimental evidence. Data were filtered for the correct MW. B) Samples of LCMSMS traces relative to the pep. 174-192 +1KAc (red) and +2KAc (green); peak areas are also reported. C) Triply charged ions of both peptides.

Supplemental Figure 7



Supplemental Figure 7. TRF2 and SIRT6 expression scores in IHC analysis of CRCs
Representative immunohistochemical staining of colon carcinomas showing TRF2 (A) and SIRT6 (B) expression score 0; 1+; 2+, 3+ respectively. Magnification 20X. Scale bar 100 µm.

Supplemental Table 1.

Biopathological characteristics of colon rectal cancer patients

Total number of pts	**185**
Tumor size	
1-2	21 (11%)
3	122 (66%)
4	42 (23%)
Node	
Negative	86 (46%)
Positive	99 (54%)
Grading	
1-2	153 (83%)
3	32 (17%)
Stage	
I-II	73 (39%)
III	68 (37%)
IV	44 (24%)
TRF2 expression	
0	35 (19%)
1+	28 (15%)
2+	69 (37%)
3+	53 (29%)
SIRT6 expression	
0	36 (19%)
1+	63 (34%)
2+	81 (44%)

Supplemental Table 2. Correlation between TRF2 and SIRT6 expression by IHC in stage I-IV colo- rectal cancer patients

	TRF2 IHC score		
SIRT6 IHC score	**0/1+ (Low)**	**2+/3+ (High)**	**TOT**
0/1+ (Low)	14 (14%)	85 (86%)	99 (54%)
2+/3+ (High)	49 (57%)	37 (43%)	86 (46%)
TOT	63 (34%)	122 (66%)	**185**

P= <0.0001