HTLV-1 bZIP factor suppresses TDP1 expression through inhibition of NRF-1 in adult T-cell leukemia

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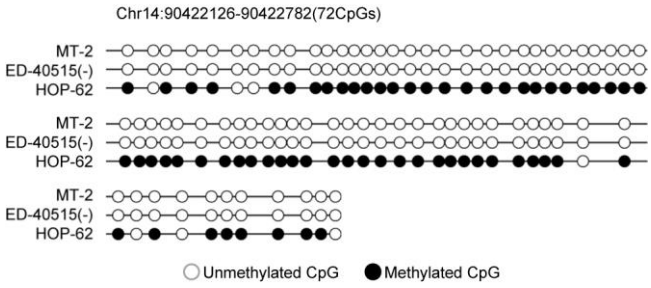
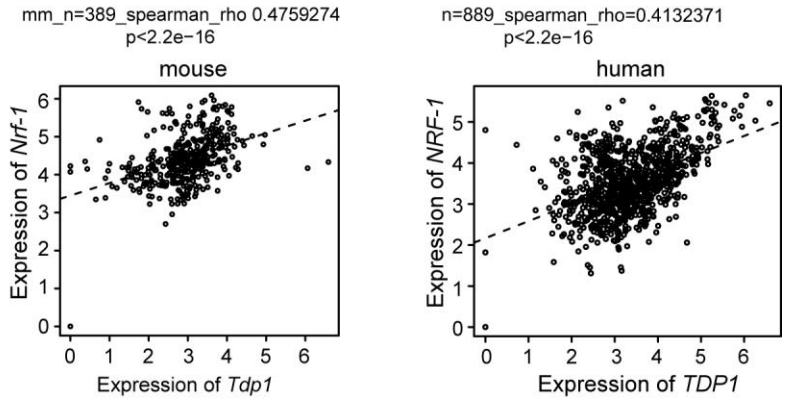
Supplementary Figure and Figure Legend

Supplementary Figure S1



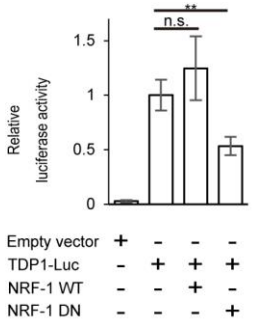
Supplementary Figure S1. DNA methylation in the promoter region of the *TDP1* gene in HTLV-1-infected cell lines ED-40515(-) and MT-2 and in the positive-control cell line (HOP-62). The chromosomal region (Chr14: 90422126-90422782) was sequenced. Non-methylated CpG dinucleotides are represented by white circles, and methyl-CpG dinucleotides by black circles.

Supplementary Figure S2



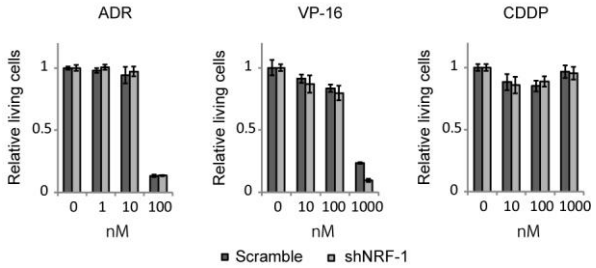
Supplementary Figure S2. Correlation between the gene expression levels of *TDP1*(*Tdp1*) and *NRF-1*(*Nrf-1*) across 889 human (right) and 389 mouse (left) samples was analyzed in the FANTOM5 Phase 1 data (mouse: Spearman rho 0.476, $P < 0.0001$; human: Spearman rho 0.413, $P < 0.0001$).

Supplementary Figure S3



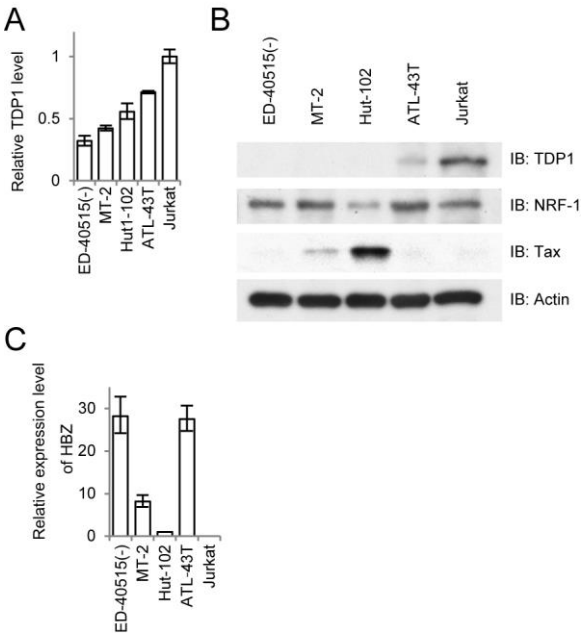
Supplementary Figure S3. Effects of NRF-1 and its mutants on the luciferase activity of the *TDP1* promoter. 5 μg of TDP1-Luc (-126/+193) was transfected by electroporation into 1×10^7 Jurkat T cells with or without vectors expressing wild-type (WT) (1.5 □g) or dominant-negative mutant (DN) (0.5 □g) of NRF-1. Relative luciferase activity was calculated by comparison with the basal luciferase activity of TDP1-Luc. Data shown are the mean ± SD (n = 3).

Supplementary Figure S4



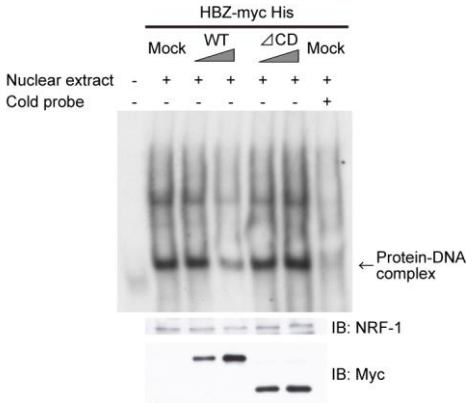
Supplementary Figure S4. Viability of shNRF-1 transfected Jurkat T cells after 48 h treatment with the indicated dose of adriamycin (ADR), etoposide (VP-16) and cisplatin (CDDP). MTS values of treated cells relative to untreated cells are shown. Data shown are the mean ± SD (n = 3).

Supplementary Figure S5



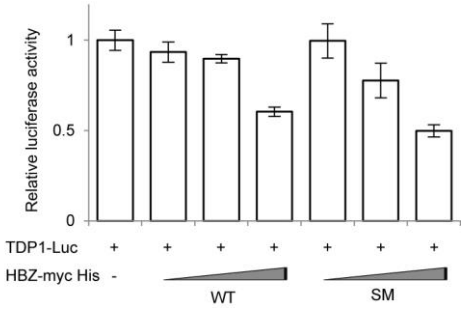
Supplementary Figure S5. Comparison of *TDP1* gene expression in HTLV-1-infected cell lines. (A) Comparison of *TDP1* mRNA expression in HTLV-1-infected cell lines by real-time PCR. Data shown are the mean ± SD (n = 3). (B) Comparison of TDP1, NRF-1, and Tax protein expression in HTLV-1-infected cell lines by immunoblotting. (C) Comparison of *HBZ* mRNA expression in HTLV-1-infected cell lines by real-time PCR. Data shown are the mean ± SD (n = 3).

Supplementary Figure S6



Supplementary Figure S6. HBZ reduces NRF-1 binding to the NRF-1 binding motif in the TDP1 promoter through the central domain (CD). A gel-shift assay was performed to analyze protein-DNA interactions. HEK293T cells were transfected with increasing amounts of HBZ wild-type (WT) and HBZ-ΔCD. Expression of the NRF-1 and HBZ (WT and ΔCD) proteins contained in the nuclear extracts were analyzed by immunoblotting. The positions of the protein-DNA complexes are indicated by the arrow.

Supplementary Figure S7



Supplementary Figure S7. Effects of *HBZ* and its mutants on *TDP1* promoter activity. Luciferase reporter (TDP1-Luc) was transfected into HEK293T cells with or without vectors expressing wild-type (WT) or mutated *HBZ* (SM) (0.05, 0.1, or 0.2 μg). Relative luciferase activities were calculated in comparison with the basal luciferase activity of TDP1-Luc. Data shown are the mean ± SD (n = 3).