Overexpression of enhancer of zeste homolog 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as a target for epigenetic therapy

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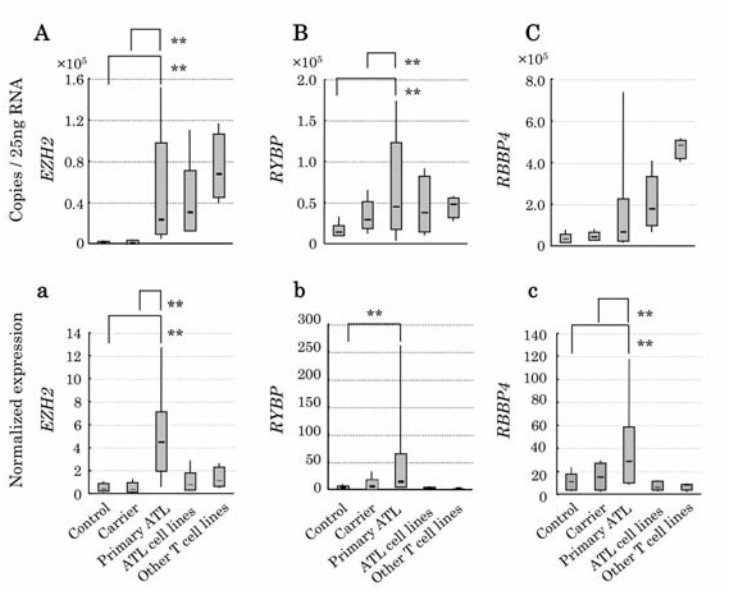
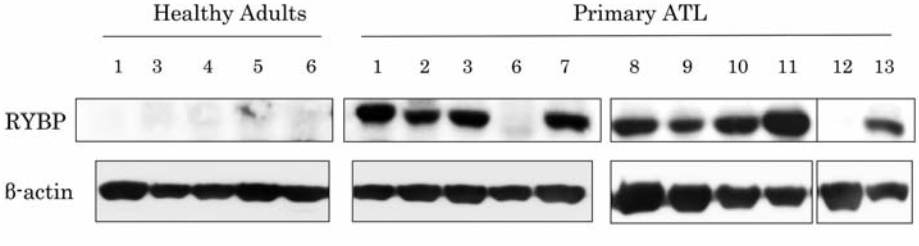
Online Supplementary Table S1. PCR primers and probes for PcG protein genes.

Gene name (Accession n.)		Primer and probe sequence	Product size (bp)
BMI1 (NM_005180)	F	5'-GCCTACATTTATTCCTGGAGAAG-3'	135
	R	5'-CCCAGAGTCACTTTCCAGTT-3'	
	P	5'-FAM-TTGTCAGTCCATCTCTCTGGTGACTGATCT-TAMRA-3'	
YY1 (NM_003403)	F	5'-CAACAAGAAGTGGGAGCAG-3'	143
	R	5'-GAGGTGAGTTCTCTCCAATGAT-3'	
	P	5'-FAM-CTCGGTCACCATGTGGTCCTCAGATGA-TAMRA-3'	
RYBP (NM_012234)	F	5'-CTGACATTCTGAAAGATCCTCC-3'	143
	R	5'-AGTTACTGCCAACTGCTGTG-3'	
	P	5'-FAM-TGCAAATGCTACAACAAAGACCAGCGA-TAMRA-3'	
RBBP4 (NM_05610)	F	5'-ATGCCCCAGAACCCTTGT-3'	132
	R	5'-ATGTCCACGGAGACGCAA-3'	
	P	5'-FAM-CTCCTTCCAGTGATGTTCTTGTCTTTGACT-TAMRA-3'	
EED (NM_152991)	F	5'-GAATATCCAGACGGACACTC-3'	126
	R	5'-AGAGAATGATCCATACCACAG-3'	
	P	5'-FAM-ATAATCAGCACTTAGAACTTCATCTCTGTGCC-TAMRA-3'	
EZH2 (NM_152998)	F	5'-GATGTGGATACTCCTCCAAG-3'	149
	R	5'-GAACTGTCACAAGGCTGC-3'	
	P	5'-FAM-ACGGCTCCTCTAACCATGTTTACAACTATCA-TAMRA-3'	
PBGD (NM_000190)	F	5'-AACCAGCTCCCTGCGAAGA-3'	134
	R	5'-CCAGGATGATGGCACTGAACT-3'	
	P	5'-FAM-ACTCCTGAACTCCAGATGCGGGAACT-TAMRA-3'	

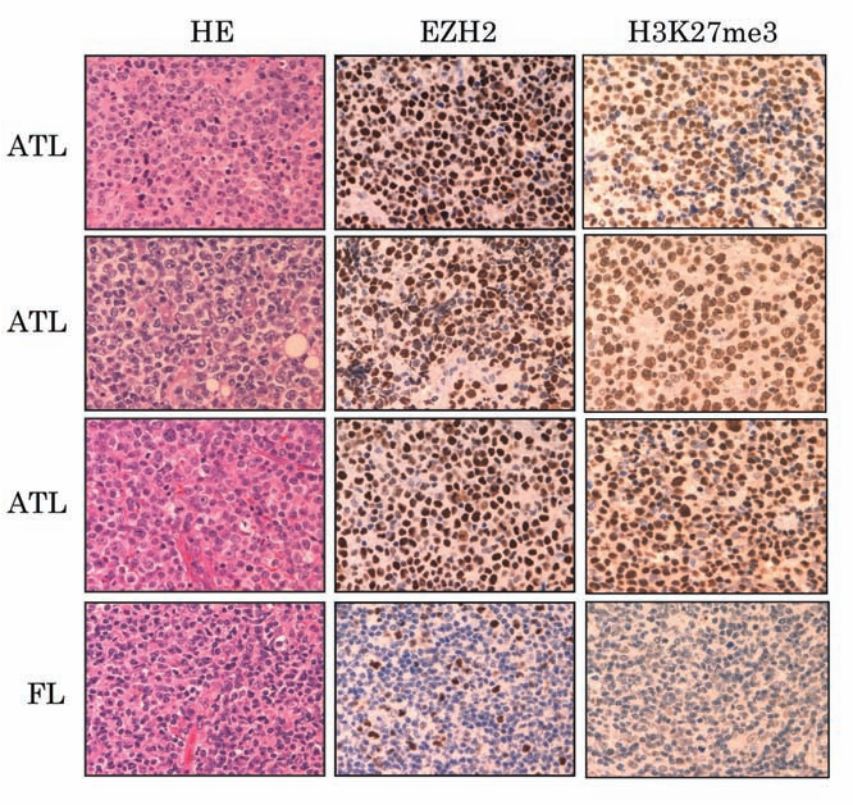
F: forward primer; R: reverse primer; P: TaqMan probe.



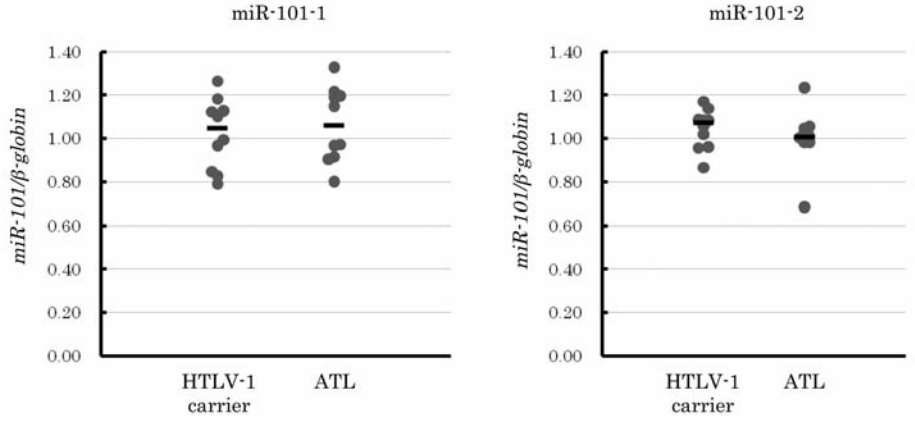
Online Supplementary Figure S1. Quantitative real-time RT-PCR for PcG genes. (A-F, a-f) Expressions of PcG protein genes *EZH2* (A, a), *RYBP* (B, b), *RBBP4* (C, c), *BMI1* (D, d), *YY1* (E, e), and *EED* (F, f) were compared among healthy adults (Control), HTLV-1 carriers (Carrier), ATL patients (Primary ATL), ATL cell lines, and non-ATL T-cell lines. Capital letters (A-F) indicate absolute copy number per 25 ng of total RNA, and small letters (a-f) indicate normalized expression. ATL cells showed significantly higher levels of *EZH2* and *RYBP* transcripts than the cells from healthy adults and HTLV-1 carriers, in terms of both absolute copy number and normalized expression (A, a, B, b, Mann-Whitney's U test). *RBBP4* transcript was significantly increased in ATL cells only in terms of normalized expression (C, c, Mann-Whitney's U test). There was no difference in *BMI1*, *YY1*, and *EED* expression levels among these groups (D, d, E, e, F, f). $**P<0.01$.



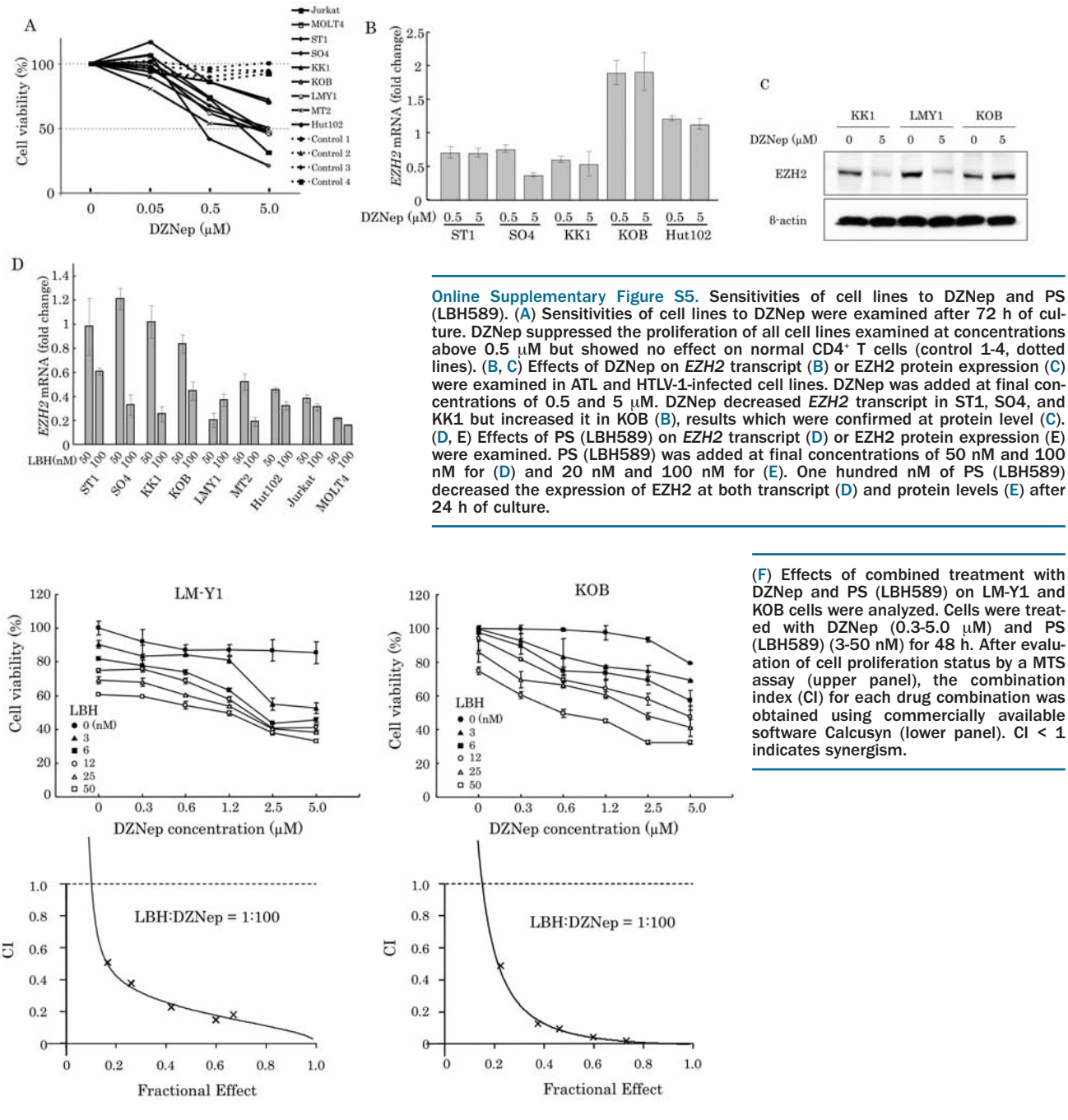
Online Supplementary Figure S2. RYBP protein expression. Western blot analysis for RYBP protein was performed on primary ATL cells and cells from healthy adults. Most primary ATL samples showed a clear band for RYBP. In contrast, cells from healthy adults lacked the band.



Online Supplementary Figure S3. Immunostaining for EZH2 and H3K27me3 in lymph nodes. Lymph nodes from patients with lymphoma-type ATL and follicular lymphoma (FL) were stained for EZH2 and H3K27me3. Representative results of 3 ATL lymph nodes and 1 FL lymph node are shown. ATL lymph nodes were all strongly positive for both EZH2 and H3K27me3 without exception in their cell nuclear staining (brown color). In contrast, FL lymph nodes were sparsely positive for EZH2 and mostly negative for H3K27me3. HE: hematoxylin-eosin stain. EZH2 and H3K27me3: immunostaining, Nikon Eclipse 80i, magnification ×200.

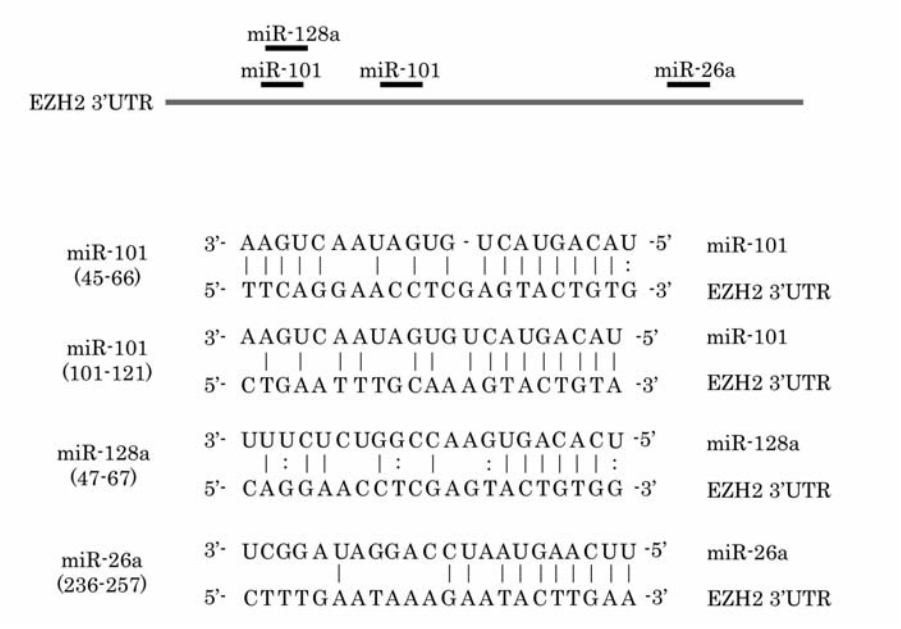


Online Supplementary Figure S4. Quantitative genomic PCR for miR-101. PCR was performed in two loci, miR-101-1 (chromosome 1p31) and miR-101-2 (chromosome 9p24), in 10 primary ATL samples and cells from 10 HTLV-1 carriers as a control. Both loci were preserved in ATL cells, refuting the possibility that downregulation of miR-101 is caused by genomic loss of the gene.

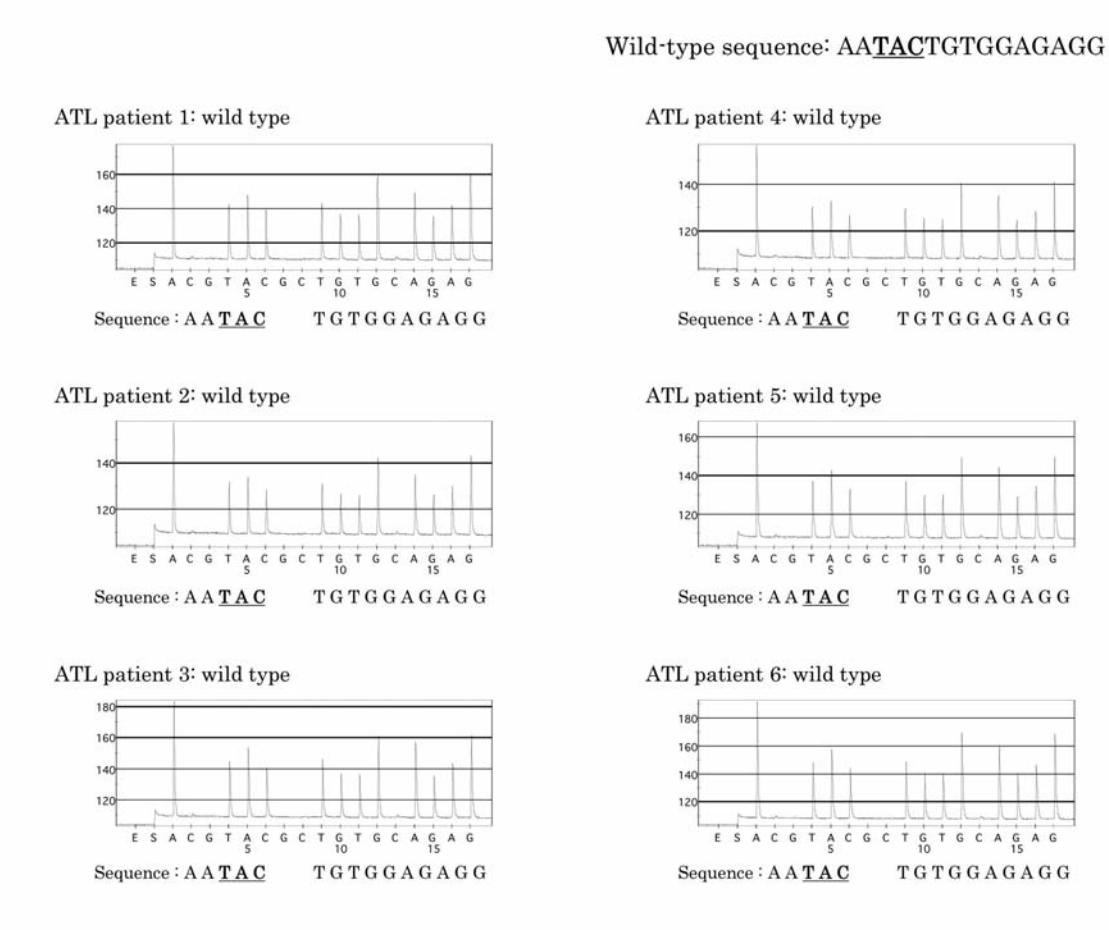


Online Supplementary Figure S5. Sensitivities of cell lines to DZNep and PS (LBH589). (A) Sensitivities of cell lines to DZNep were examined after 72 h of culture. DZNep suppressed the proliferation of all cell lines examined at concentrations above 0.5 μM but showed no effect on normal $CD4^+$ T cells (control 1-4, dotted lines). (B, C) Effects of DZNep on *EZH2* transcript (B) or EZH2 protein expression (C) were examined in ATL and HTLV-1-infected cell lines. DZNep was added at final concentrations of 0.5 and 5 μM. DZNep decreased *EZH2* transcript in ST1, SO4, and KK1 but increased it in KOB (B), results which were confirmed at protein level (C). (D, E) Effects of PS (LBH589) on *EZH2* transcript (D) or EZH2 protein expression (E) were examined. PS (LBH589) was added at final concentrations of 50 nM and 100 nM for (D) and 20 nM and 100 nM for (E). One hundred nM of PS (LBH589) decreased the expression of EZH2 at both transcript (D) and protein levels (E) after 24 h of culture.

(F) Effects of combined treatment with DZNep and PS (LBH589) on LM-Y1 and KOB cells were analyzed. Cells were treated with DZNep (0.3-5.0 μM) and PS (LBH589) (3-50 nM) for 48 h. After evaluation of cell proliferation status by a MTS assay (upper panel), the combination index (CI) for each drug combination was obtained using commercially available software Calcusyn (lower panel). CI < 1 indicates synergism.



Online Supplementary Figure S6. Analysis of 3'-UTR sequence of EZH2 to predict potential target sites for miRNA. In addition to the target sites for miR-101 and miR-26a, there is also a potential target site for miR-128a in the 3'-UTR of EZH2 near one of the miR-101 target sites.



Online Supplementary Figure S7. Sequence analysis of EZH2. Pyrosequence analysis of EZH2 Try641 was performed in 10 ATL patients and 10 HTLV-1 carriers. Pyrograms of 6 ATL patients are shown. There were no mutations in the examined samples.