Supplemental information

DNA breaks and chromatin structural changes enhance the transcription of Autoimmune Regulator target genes

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Running title: *AIRE expression and chromatin structure*

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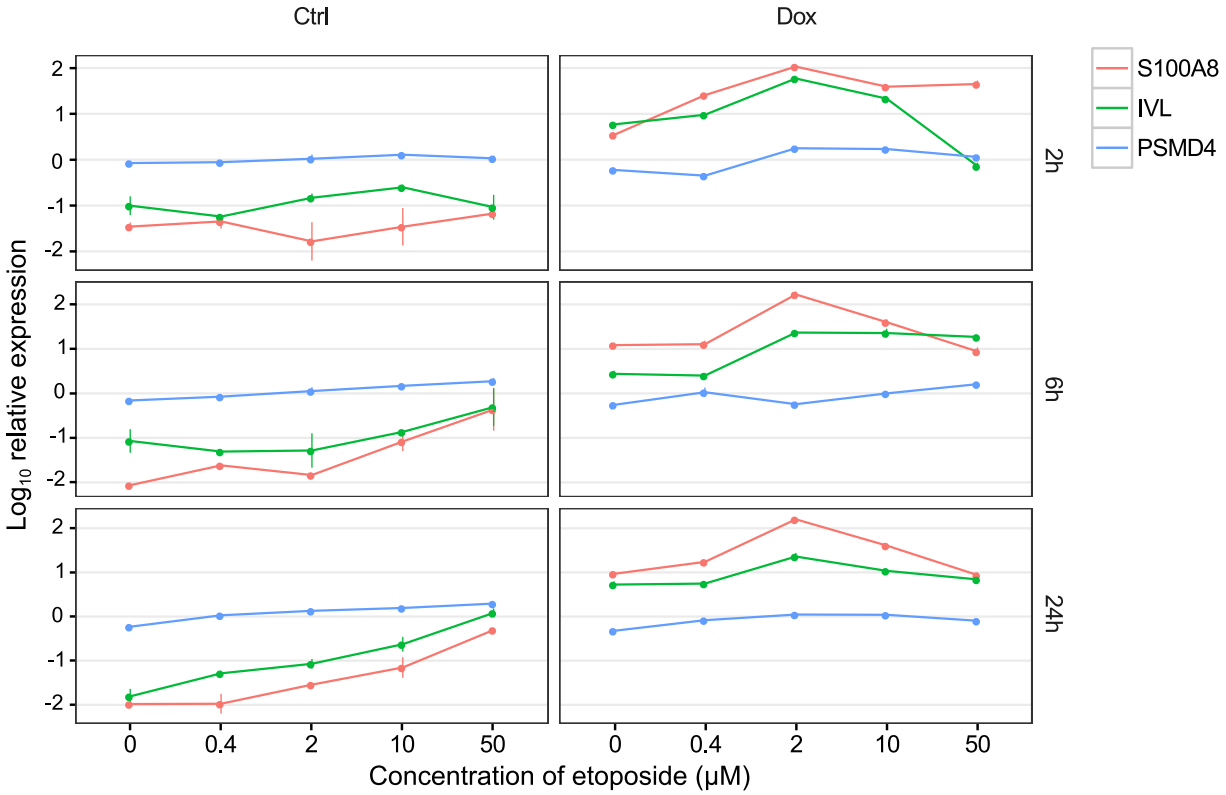
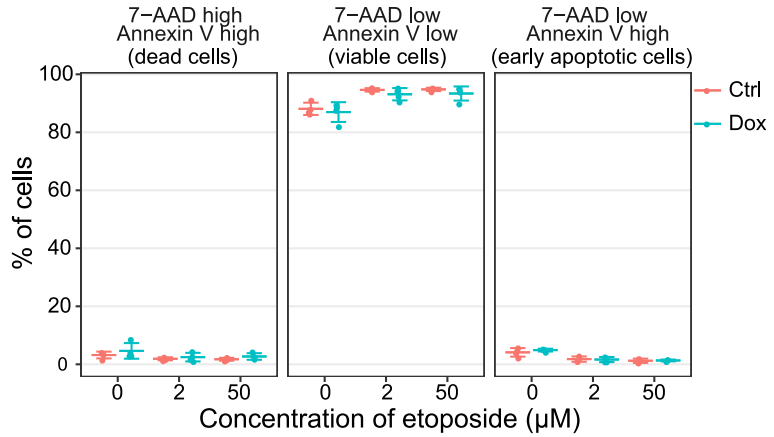
Supplemental Table 1. List of oligonucleotides used in the study.

Expression analysis	Oligo name	Sequences
S100A8	Hu_S100A8_F	CTCAGTATATCAGGAAAAAGGGTGCAGAC
	Hu_S100A8_R	CACGCCCATCTTTATCACCAGAATGAG
IVL	Hu_IVL_F	GCCTTACTGTGAGTCTGGTTGACA
	Hu_IVL_R	GGAGGAACAGTCTTGAGGAGCT
HBG2	Hu_HBG2_F	CATAAAGCACCTGGATGATCTC
	Hu_HBG2_R	CAGGAGCTTGAAGTTCTCAG
PDYN	Hu_PDYN1_F	TGCCTTTGTTCTATTTTTGCAGGT
	Hu_PDYN1_R	CAGCAATTCCTGCGGCTTG
DMBT1	Hu_DMBT1_F	AGAACCCAGCAAAATGGGGA
	Hu_DMBT1_R	TTGGGATCCACCCACCTGTA
KRT73	Hu_KRT73_F	GAGTGCAGGATGTCCGGAGAATA
	Hu_KRT73_R	TTGCTGAATCCAAAGCCAGCC
CEACAM5	Hu_CEA5_F	CCTGGATGTCCTCTATGGGC
	Hu_CEA5_R	TACTGCGGGGATGGGTTAGA
PSMD4	Hu_PSMD4_F	GAAGGTGAAAGAGACTCA
	Hu_PSMD4_R	GTCATACTGCTTAGGTCA
HPRT	Hu_HPRT_F	GACTTTGCTTTCCTTGGTCAGG
	Hu_HPRT_R	AGTCTGGCTTATATCCAACACTTCG
ChIP analysis		
S100A8 promoter	ch_SA81_F	TGTGCTGGGTCCCCAATGGC
	ch_SA81_R	GCTGCTTGGGGTCCCTCTGC
S100A8 control	ch_SA81_con_F	TGGCTTTGGTCTCGCCGTCTAAGTAA
	ch_SA81_con_R	TGGTGGGTTCAAGGTGCACTGTAGAT
IVL promoter	ch_IVL_F	CCAATCCTTTAGATATGGTACACAG
	ch_IVL_R	TCCCCAGGTCTCTGGTTCTT
IVL control	ch_IVL_con_F	TGTTTGTGTTGTGCAAGGCCGAGA
	ch_IVL_con_R	AGGAACATTTTGTCAGGCCAAGGCT
DMBT1 promoter	ch_DMBT1_F	AGGTTCACCGAGAGGGAAGT
	ch_DMBT1_R	GAACAATCTGGCTGTTGCCC
PSMD4 promoter	ch_PSMD4_F	GATAGTCCCGGGTTACCAC
	ch_PSMD4_R	TGTAGCTAAAGACAGACCCG
GAPDH promoter	ch_GAPDH_F	CCCGTCCTTGACTCCCTAGT
	ch_GAPDH_R	GGGGGAAGGGACTGAGATT
CEACAM CTCF site 9	ch_CTCF9_F	GGAGGTGGGTCGAGGTGATC
	ch_CTCF9_R	CAGAGGGCAGCAGAGTCC
CEACAM CTCF site 10	ch_CTCF10_F	GGCTGGGATTGTGGCAGTAA
	ch_CTCF10_R	ACTCTCCTGGCCCCTTTTTG
CEACAM CTCF site 11	ch_CTCF11_F	CGAGCTGAAACCTGGTAGCA
	ch_CTCF11_R	TGGTGGACAGGAGGGAAGTG
3C analysis		
CTCF site 10 forward	3c_anchor_site10_F	CTCCCCAAGCTCTAACAACCAA
CTCF site 10 reverse	3c_anchor_site10_R	CTCTTTGCACCTCAGTCCTCTC
CTCF site 1	3c_site1_F	GACTTAGAGGCTTCAGTCATCATCC
CTCF site 2	3c_site2_F	TAAGGAGCAAGGAGACCAGGAG
CTCF site 3	3c_site3_F	CTTCCCTTGGCCATTTCCCA
CTCF site 4	3c_site4_F	CAAATTCCCGTCCTCAAGCAATC
CTCF site 5	3c_site5_F	GAAATTAGCCTCACTGAGTCACTGT
CTCF site 6	3c_site5_R	GAGCTGGGAAATAACACTCACACTA
CTCF site 7	3c_site7_F	AGTTGGTAGGAGCGACTTTAGAAAT
CTCF site 8	3c_site8_F	CCTATGACCCTTAGCCTCTCTGAG
CTCF site 9	3c_site9_F	AGATTGTGGTCTTATGTCAGGTCAA
CTCF site 11	3c_site11_R	TGGTGGACAGGAGGGAAGTG
CTCF site 12	3c_site12_F	TTTGCTAAGGAAGTGGAGGTGGA
CTCF site 13	3c_site13_R	CTTGTGGAAACTCTGAGAACTGCAT
CTCF site 14	3c_site14_R	CGGAGAACTGCTTCACAATCTTTAA
CTCF site 15	3c_site15_R	AAAATGAAGCGACTTGTCCAGG
CTCF site 16	3c_site16_F	CACATATTCCCAACAACTCTGCAAG
CTCF site 17	3c_site17_F	TGGAAGTAACTGTCAGAGAGAGCT
CTCF site 18	3c_site18_F	AAAATATAGAAATATGGGGCCGGGC
CTCF site 19	3c_site19_F	CACTTATTCCCAACAACTCTGCAAG
CTCF site 20	3c_site20_F	ACAAACAAACTCAGGCTGTAAAGAC
CTCF site 21/22	3c_site21/22_F	GTGCAGCTAGATGGTCAGTCC
CTCF site 23	3c_site23_R	GCCAATTTAGATTTACCTGCCCC
CTCF site 24	3c_site24_R	AGTGTAAACAACGGTGCTTTTAACA

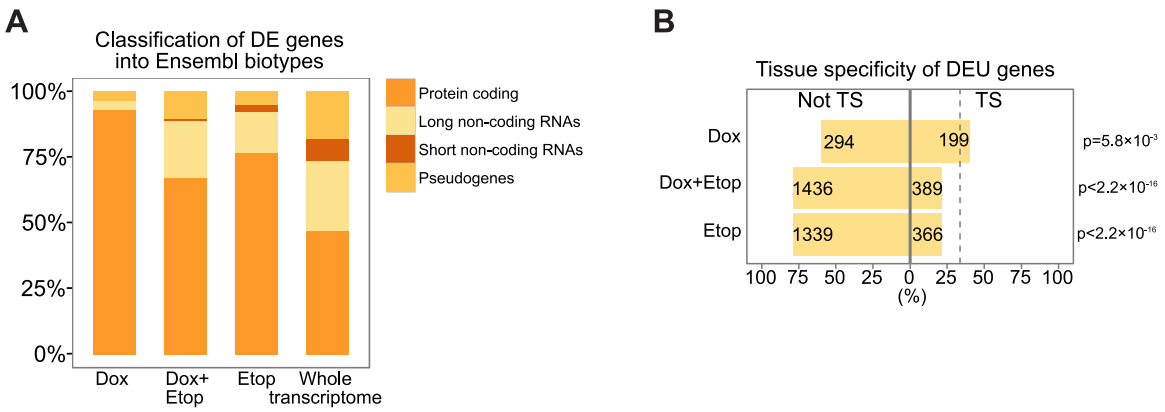
A



B



Supplemental Figure 1. The effect of etoposide treatment on AIRE target gene expression. A, the expression levels of the AIRE-dependent genes (S100A8 and IVL) and the AIRE-independent PSMD4 gene were analyzed after 2, 6 or 24 h incubation of uninduced (Ctrl) and induced (Dox) Aire-Tet cells with 0.4, 2, 10 and 50 μM etoposide concentrations. Log_{10}-tranformed data show the mean ± SD of two independent qPCR experiments. B, FACS analysis of apoptosis of uninduced (Ctrl) and induced (Dox) Aire-Tet cells treated with 0, 2 and 50 μM etoposide using FITC-conjugated Annexin V and 7-AAD as markers for early and late apoptosis, respectively. The individual data points together with their mean ± SD are from four independent experiments.



Supplemental Figure 2. Features of genes affected by the induction of AIRE, treatment with etoposide or AIRE/etoposide combination in AIRE-Tet cells. A, Differentially expressed genes classified by their biotype derived from Ensembl (release 75) annotations and compared to the whole AIRE-Tet transcriptome. C, Percentage of alternatively spliced genes that are classified as TS or broadly expressed in our experimental conditions. The dashed line marks the percentage of TS genes in the genome (34%), which was compared to the proportions of TS genes in each condition with the X^2-test.