

## Supplementary Material

### **Methylation matters: Binding of Ets-1 to the demethylated *Foxp3* gene contributes to the stabilization of Foxp3 expression in regulatory T cells**

Julia K. Polansky<sup>1,2,\*</sup>, Lisa Schreiber<sup>1,\*</sup>, Christoph Thelemann<sup>3</sup>, Leif Ludwig<sup>3</sup>, Melanie Krüger<sup>4</sup>,  
Ria Baumgrass<sup>4</sup>, Sascha Cording<sup>1</sup>, Stefan Floess<sup>1</sup>, Alf Hamann<sup>3</sup> and Jochen Huehn<sup>1</sup>

<sup>1</sup>Experimental Immunology, Helmholtz Centre for Infection Research, Inhoffenstr. 7, 38124  
Braunschweig, Germany

<sup>2</sup>current address: Immunobiology, Leibniz-Center for Medicine and Biosciences, Parkallee  
30, 23845 Borstel, Germany

<sup>3</sup>Experimental Rheumatology, Charité University Medicine Berlin and German Rheumatism  
Research Center, Charitéplatz 1, 10117 Berlin, Germany

<sup>4</sup>Signal Transduction, German Rheumatism Research Center, Charitéplatz 1, 10117 Berlin,  
Germany

Table 1: Primer sequences used for cloning of luciferase-reporter sequences. All primers were purchased from Operon. Introduced restriction sites are indicated in bold, introduced mutations are underlined. Primer sequences which were needed in the reverse complement orientation, too, are marked with an asterics, but only one sequence is depicted.

Name	Sequence 5' - 3'	used for cloning of
FoxPro_Asp718_for	GATCGGTACCATTACATGGCAGGCTTCAG	FoxPro-LUC
FoxPro_XhoI_rev	GATCCTCGAGCCAAAGTCTTACCTGGAGT	FoxPro-LUC
TSDR_Asp718_for	GATCGGTACCAAGTTGTCCCAGGAGAGC	TSDR-LUC
TSDR_XmaI_rev	GATCCCCGGGCATATGGCTGGACCATGG	TSDR-LUC
TSDR_A_SpeI_for	CATGACTAGTAAGTTGTCCCAGGAGAGC	TSDR-FoxPro-Luc A-FoxPro-LUC and all point mutations
TSDR_B_SpeI_for	GATCACTAGTAATGGAGCTCAGGAGGGA	B-FoxPro-LUC BCD-FoxPro-LUC
TSDR_C_SpeI_for	GATCACTAGTTAGCCAGATGGACGTCAC	C-FoxPro-LUC CD-FoxPro-LUC
TSDR_D_SpeI_for	GATCACTAGTGTCCCAGAAACAACCTCC	D-FoxPro-LUC
TSDR_A_EcoRI_rev	GATCGAATTCCTCCCTCCTGAGCTCCATT	A-FoxPro-LUC
TSDR_B_EcoRI_rev	GATCGAATTCGTGACGTCCATCTGGCTA	B-FoxPro-LUC
TSDR_C_EcoRI_rev	GATCGAATTCGGAGGTTGTTTCTGGGAC	C-FoxPro-LUC
TSDR_D_EcoRI_rev	GATCGAATTCATATGGCTGGACCATGG	TSDR-FoxPro-Luc BCD-FoxPro-LUC CD-FoxPro-LUC D-FoxPro-LUC and all point mutations
mutEBS1_for*	GACGTCACCTACCACAGACGCTAGCACCCACATC	TSDR-mutEBS1-FoxPro-LUC
mutEBS2_for*	GATGAAGCCCAATGCAGACGGCCGCCATGACGTC	TSDR-mutEBS2-FoxPro-LUC TSDR-mutEBS1/2-FoxPro-LUC
mutCBS1_for*	ATGGTAGCCAGATGGTGGTCACCTACCACATC	TSDR-mutCBS1-FoxPro-LUC
mutCBS2_for*	CATCCGGCCGCCATGIGTCAATGGCAGAAAA	TSDR-mutCBS2-FoxPro-LUC TSDR-mutCBS1/2-FoxPro-LUC
NFkB_G2T_for*	CACCCTACCTGIGCCTATCCGG	TSDR-NFkB_G2T-FoxPro-LUC
NFkB_T8G_for*	CTGGGCCTAGCCGGCTACAGG	TSDR-NFkB_T8G-FoxPro-LUC
TSDR_PstI_for	GATCCTGCAGAAGTTGTCCCAGGAGAGC	TSDR-Pro-LUC (pCpGL)
TSDR_SpeI_rev	GATCACTAGTCATATGGCTGGACCATGG	TSDR-Pro-LUC (pCpGL)
mCpG1-for*	GCATTCGTATGTATGAACTTTGCAAG	TSDR(mutCpG1)-FoxPro-LUC
mCpG2-for*	GAGATTCTAAAATCATTGGCTTTGAGA	TSDR(mutCpG2)-FoxPro-LUC
mCpG3-for*	GCTTTGAGAAATGATATAGTACAGTTCTGAG	TSDR(mutCpG3)-FoxPro-LUC
mCpG4-for*	CCAAGAAAGACAGAATTGATAGAACTTGGG	TSDR(mutCpG4)-FoxPro-LUC
mCpG5-for*	GCCAGATGGAAGTACCTACCAC	TSDR(mutCpG5)-FoxPro-LUC
mCpG6-for*	CCTACCACATCAGCTAGCACCCAC	TSDR(mutCpG6)-FoxPro-LUC
mCpG7-for*	CTGGGCCTATCIGGCTACAGG	TSDR(mutCpG7)-FoxPro-LUC
mCpG8-for*	GCCACTTCTGAGAACGAAACCTG	TSDR(mutCpG8)-FoxPro-LUC
mCpG9-for*	CCACTTCTCGGAAAGAAACCTGTGG	TSDR(mutCpG9)-FoxPro-LUC
mCpG10-for*	CTCCTTGTGCTGATGAAGCCCAATG	TSDR(mutCpG10)-FoxPro-LUC
mCpG11-for*	CAATGCATCCAGCCGCCA	TSDR(mutCpG11)-FoxPro-LUC
mCpG12-for*	CATCCGGCTGCCATGACG	TSDR(mutCpG12)-FoxPro-LUC
mCpG13-for*	CCGCCATGAGTTCAATGGCAG	TSDR(mutCpG13)-FoxPro-LUC
mCpG14-for*	CCAGATGTAGACCCAAATAGGAAAAC	TSDR(mutCpG14)-FoxPro-LUC
mCpG15-for*	CAAACAGGAAAGCCCAACAGAC	TSDR(mutCpG15)-FoxPro-LUC
TSDR_for	AAGTTGTCCCAGGAGAGC	all point mutations

Table 2: IRD700-labeled and unlabeled oligonucleotide sequences used for EMSA. Underlined bases indicate mutations. mC represents a methylated cytosine.

Name	Sequence 5' - 3'
EBS-1_for	IRD700-ACGTCACCTACCACATCCGCTAGCACCCACATC
EBS-1_rev	IRD700-GATGTGGGTGCTAGCGGATGTGGTAGGTGACGT
EBS-2_for	IRD700-ATGAAGCCCAATGCATCCGGCCGCCA
EBS-2_rev	IRD700-TGGCGGCCGGATGCATTGGGCTTCAT
mutEBS-1_for	IRD700-ACGTCACCTACCACAG <u>AC</u> GCTAGCACCCACATC
mutEBS-1_rev	IRD700-GATGTGGGTGCTAGCG <u>ICT</u> GTGGTAGGTGACGT
mutEBS-2_for	IRD700-ATGAAGCCCAATGCAG <u>AC</u> GGCCGCCA
mutEBS-2_rev	IRD700-TGGCGGCCG <u>ICT</u> GCATTGGGCTTCAT
methEBS-1_for	IRD700-ACGTCACCTACCACATCmCGCTAGCACCCACATC
methEBS-1_rev	IRD700-GATGTGGGTGCTAGmCGGATGTGGTAGGTGACGT
methEBS-2_for	IRD700-ATGAAGCCCAATGCATCmCGGCCGCCA
methEBS-2_rev	IRD700-TGGCGGCmCGGATGCATTGGGCTTCAT
Ets1-ctrl.1_for	CCAGAGGAGGAAGTGTAGGAGCAGGT
Ets1-ctrl.1_rev	ACCTGCTCCTACACTTCCTCCTCTGG
Ets1-ctrl.2_for	GATCTCGAGCCGGAAGTTCGA
Ets1-ctrl.2_rev	TCGAACTTCCGGCTCGAGATC
CREB_TSDR_for	GGCCGCCATGACGTCAATGGCAG
CREB_TSDR_rev	CTGCCATTGACGTCATGGCGGCC