

# Plasticity of Naturally Occurring Regulatory T Cells in Allergic Airway Disease is Modulated by the Transcriptional Activity of *Il-6*

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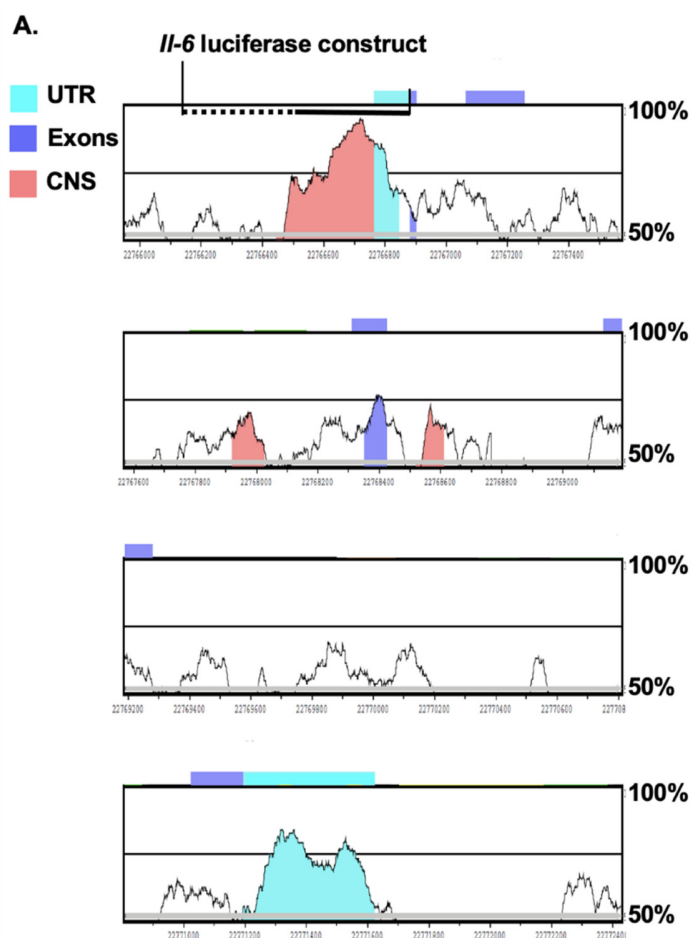
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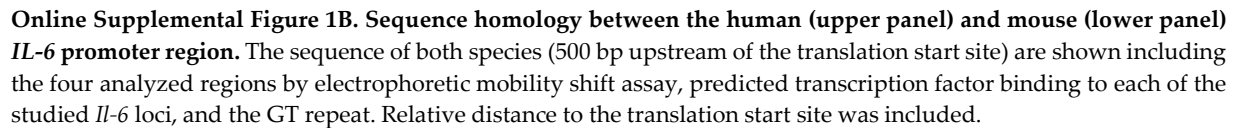


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**Online Supplemental Figure 1A. The promoter regions of the human *Il-6* and mouse *Il-6* promoter share high homology.** Percentage of alignment between the human *Il-6* and mouse *Il-6* gene using the VISTA genome browser software. Location of the luciferase construct (dotted and solid line) including the region of sequence homology in humans and mice as shown in Online Supplemental Figure 1B (solid line) are

**B.**



-1139 AGACTTGAGC ATTGGAGGGG TTATTTCAGAG TGAGACGTAC CACCTTCAGA TTCAAATCCT GTCATCCAGT AGAAGGGAGC TTCAACACACA AGCTAGCTAA  
TCTGAACTCG TAACCTCCCC AATAAGTCTC ACTCTGCATG GTGGAAGTCT AAGTTTAGGA CAGTAGGTCA TCTTCCCTCG AAGTTTGTGT TCGATCGATT  
luciferase construct 5' start

-1039 GATACAATGA GGTCTTCTTT CGATATCTTT ATCTTCCATA TACCATGAAT CAAAGAACT TCAACAACAT GAGGACTGCA ACAGACCTTC AAGCCTCCTT  
CTATGTTACT CCAGGAAGAA GGTATAGAAA TAGAAGGTAT ATGGTACTTA GTTCTTTGA AGTTGTTGA CTCCTGACGT TGTCTGGAAG TTCGGAGGAA

-939 GCATGACCTG GAAATGTTTT GGGGTGTCTT GGCAGCAGTG GGATCAGCAC TAACAGATAA GGGCAACTCT CACAGAGACT AAAGGTCTTA ACTAAGAAGA  
CGTACTGGAC CTTTACAAAA CCCACAGGA CCGTCGTCAC CCTAGTCGTG ATTGTCTATT CCCGTTGAGA GTGTCTCTGA TTTCCAGAAAT TGATTCTTCT

-839 TAGCCAAGAG ACCACTGGGG AGAATGCAGA GAATAGGCTT GGACTTGGAA GCCAAGATTG CTTGACAACA GACAGAAGAT ATTTCTGTAC TTCACCCACT  
ATCGGTTCTC TGGTGACCCC TCTTACGTCT CTTATCCGAA CCTGAACCTT CGGTCTTAAC GAACTGTTGT CTGTCTTCTA TAAAGACATG AAGTGGGTGA

-739 TTACCCACCT GGCAACTCCT GGAACAACCT GCACAAAATT TGGAGGTGAA CAAACCATTG GAAACAACCTG GTCCTGACAA GACACAGGAA AAACAAGCAA  
AATGGGTGGA CCGTTGAGGA CTTTGTGTA CGTGTGTTAA ACCTCCACTT GTTGGTAAT CTTGTTGAC CAGGACTGTT CTGTCTCCTT TTTGTTCTGT

-639 TATGCAACAT TACTGTCTGT TGTCCAGGTT GGGTGCTGGG GGTGGGAGAG GGAGTGTGTG TCTTTGTATG ATCTGAAAA ACTCAGGTCA GAACATCTGT  
ATACGTTGTA ATGACAGACA ACAGGTCCAA CCCACGACCC CCACCTCTC COTCACACAC AGAACATAC TAGACTTTTT TGAGTCCAGT CTTGTAGACA  
GT-repeat

-539 AGATCCTTAC AGACATACAA AAGAATCCTA GCCTCTTATT CATGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTA TGTGTGTGTC  
TCTAGGAATG TCTGTATGTT TTCTTAGGAT CGGAGATAAA GTACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAT ACACACACAG

II-6\_4 (predicted: EBOX)

-439 GTCTGTCATG CGCGCGTGCC TCGGTTTAAA TAACATCAGC TTTAGCTTCT CTTTCTCCTT ATAAACATT GTGAATTCA GTTTCTTTTC CCATCAGAC  
CAGACAGTAC CGCGCGCAGG ACGCAAATTT ATTGTAGTCG AATCGAAGA GAAAGAGGAA TATTTTGTAA CACTTAAAGT CAAAAGAAAG GGTAGTTCTG

II-6\_3 (predicted: AP1)

-339 ATGCTCAAGT GCTGAGTCAC TTTTAAAGAA AAAAAAGAAG AGTGCTCATG CTTCTTAGGG CTAGCCTCAA GGATGACTTA AGCACAATTT CCCCTTCCTA  
TACGAGTTCA CGACTCAGT AAAATTTCTT TTTTCTTTC TCACGAGTAC GAAGAATCCC GATCGGAGTT CCTACTGAAT TCGTGTGAAA GGGGAAGGAT

II-6\_2 (predicted: C/EBP)

-239 GTTGTGATTC TTTCGATGCT AAACGACGTC ACATTGTGCA ATCTTAATAA GGTTCCTAAT CAGCCCCACC CACTCTGGCC CCACCCCCAC CCTCCACAA  
CAACACTAAG AAAGCTACGA TTGCTGCAG TGTAAACAGT TAGAATTATT CCAAAGGTTA GTCGGGGTGG GTGAGACCGG GGTGGGGGTG GGAGGTTGTT

transcription start site

II-6\_1 (predicted: NF-κB)

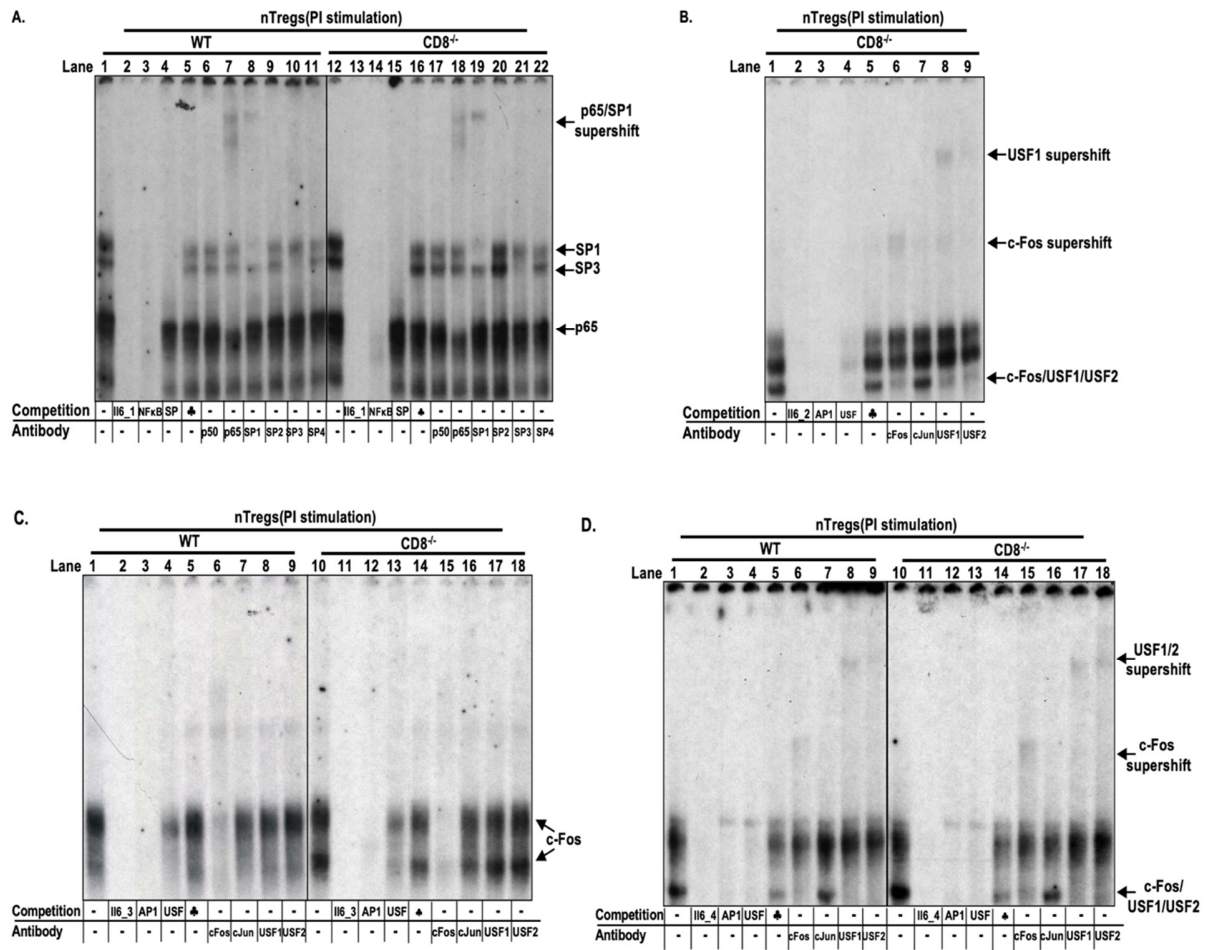
-139 AGATTTTAT CAAATGTGGG ATTTTCCCAT GAGTCTCAAA ATTAGAGAGT TGACTCCTAA TAAATATGAG ACTGGGGATG TCTGTAGCTC ATTCTGCTCT  
TCTAAAAATA GTTTACACCC TAAAGGGTA CTCAGAGTTT TAATCTCTCA ACTGAGGATT ATTTATACTC TGACCCCTAC AGACATCGAG TAAGACGAGA

translation start site

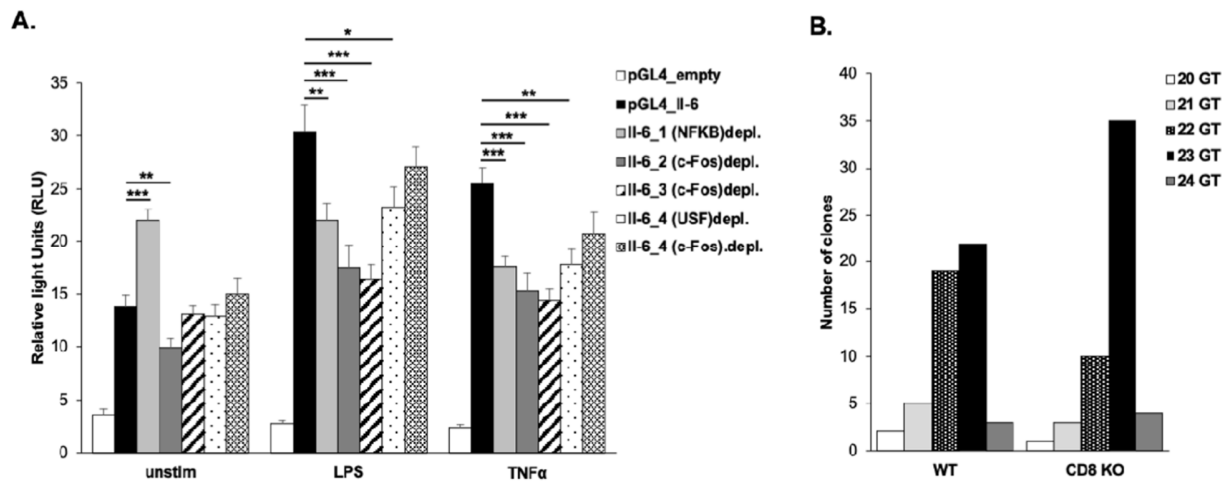
-39 GGAGCCACCC AAGAACGATA GTCAATTCCA GAAACCGCTT TGAAGTTCCT CTCTGCAAGT AAGTGAAGGC AGTTCCTTGC CCTCTGGCGG AGCTATTGAG  
CCTCGGGTGG TTCTTCTAT CAGTTAAGGT CTTTGGCGAT ACTTCAAGGA GAGACGTTCA TTCACTTCCG TCAAGGAACG GGAGACCGCC TCGATACTC

luciferase construct 3' end

**Online Supplemental Figure 2. Sequence of the mouse *Il-6* promoter region.** The four analyzed regions by electrophoretic mobility shift assay and the GT repeat are underlined. The location of the cloned *Il-6* promoter region is depicted. Transcription and translation start site of *Il-6* are marked. Relative distance to the translation start site is shown.



**Online Supplemental Figure 3. Distinct affinity of transcription factor binding to promoter regions within the *Il-6* gene in nTregs from wild-type (WT) and CD8<sup>-/-</sup> mice.** A-D. EMSA analyses of four different *Il-6* promoter loci upstream of the transcription start site of the *Il-6* gene were performed including A. *Il-6*\_1, B. *Il-6*\_2, C. *Il-6*\_3, and D. *Il-6*\_4 using nuclear extract isolated from nTregs of WT or CD8<sup>-/-</sup> (KO) mice (5 µg) stimulated with PMA/ionomycin (50 ng/ml/1 µM) for 3 hours. Competitors (100-fold molar excess) and supershift antibodies (4 µg) for each experiment are noted below the respective lanes. ♦: competition experiment using an unrelated oligo.



**Online Supplemental Figure 4. Four defined regions are essential for transcription factor binding and a novel variable length STR is found in the *Il-6* promoter** **A.** NIH3T3 cells were transiently transfected with the empty pGL4 luciferase vector (pGL4 empty) or with different reporter constructs of the *Il-6* promoter (1,138 bp). In the construct *Il-6*\_1 (NF-κB)depl., the NF-κB binding site as identified by EMSA within *Il-6*\_1 was depleted by site-directed mutagenesis. Correspondingly, the c-Fos binding site within *Il-6*\_2 [*Il-6*\_2(c-Fos)depl.], the c-Fos binding site within *Il-6*\_3 [*Il-6*\_3(c-Fos)depl.], the USF binding site within *Il-6*\_4 [*Il-6*\_4(USF)depl.], and the c-Fos binding site within *Il-6*\_4 [*Il-6*\_4(c-Fos)depl.] were mutated (4-5 independent experiments, 2 technical replicates). Cells were left in medium or stimulated with 10 ng/ml LPS or 0.5 ng/ml TNFα. Luciferase activity was normalized for transfection efficiency using the control plasmid pRL-TK. The relative luciferase activity is presented in relative light units (RLU). Paired, two tailed students t-test was performed. \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001. **B.** *Il-6* luciferase promoter constructs were generated using DNA isolated from wild-type (WT) or CD8<sup>-/-</sup> (CD8 KO) nTregs. To determine the distribution of a GT short tandem repeat in nTregs of both strains, a total of 104 clones (WT=51, CD8<sup>-/-</sup>=53) were sequenced. Distribution of number of clones in nTregs from each strain containing 20-24 GT repeats are depicted.

**Supplemental Table 1: *In silico* transcription factor (TF) binding analyses of the *Il-6* promoter region including 600 bp upstream of the translation start site.** Relative distance upstream of the translation start site and the chromosomal strand (+ or -) of the predicted binding of the TF are depicted. Capital letters in the sequence indicate the core sequence of a predicted TF binding matrix with the highest degree of conservation. Letters marked in red reflect the conserved sequences (>60%) between the TF matrix and *Il-6*. Marked in bold are predicted TF binding regions further evaluated by electrophoretic mobility shift assay.

TF	TF family	Name of TF family	Relative distance to TLSS (bp)		Strand	Core similarity	Matrix similarity	Sequence
<i>BTEB3</i>	<i>KLFS</i>	Krueppel like transcription factors	-595	-579	+	1.000	0.939	ggagag <b>GGAG</b> gtgtgt
<i>GAGA</i>	<i>GABF</i>	GA-boxes	-598	-574	+	1.000	0.81	gtggg <b>AGAG</b> ggagtgtgtccttg
<i>TIEG</i>	<i>SP1F</i>	GC-Box factors SP1/GC	-591	-577	+	0.750	0.879	ag <b>GGAG</b> gtgtgtct
<i>TAXCREB</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-591	-571	-	0.750	0.713	atacaa <b>AGAC</b> acactccct
<i>GATA3</i>	<i>GATA</i>	GATA binding factors	-575	-563	-	1.000	0.932	ttc <b>AGAT</b> cataca
<i>AREB6</i>	<i>ZFHX</i>	Two-handed zinc finger homeodomain transcription factors	-559	-547	-	1.000	0.945	ttctg <b>ACCT</b> gagt
<i>REV-ERBA</i>	<i>RORA</i>	v-ERB and RAR-related orphan receptor alpha	-563	-541	+	1.000	0.895	aaaaactcag <b>GTC</b> Agaacatctg
<i>FHXB</i>	<i>FKHD</i>	Fork head domain factors	-556	-540	+	0.818	0.853	caggtc <b>AGAA</b> catctgt
<i>TAL1ALPHA47</i>	<i>HAND</i>	Twist subfamily of class B bHLH transcription factors	-554	-534	-	1.000	0.952	ggatcta <b>CAGAt</b> ttctgacc
<i>NGN_NEUROD</i>	<i>NEUR</i>	NeuroD, Beta2, HLH domain	-550	-538	+	1.000	0.985	agaa <b>CATC</b> gttag
<i>RP58</i>	<i>RP58</i>	RP58 (ZFP238) zinc finger protein	-550	-538	+	1.000	0.954	agaa <b>CATC</b> gttag
<i>PRE</i>	<i>GREF</i>	Glucocorticoid responsive and related elements	-552	-534	-	1.000	0.851	ggatctacaga <b>TGT</b> ctga
<i>POU3F3</i>	<i>OCT1</i>	Octamer binding protein	-510	-494	-	0.784	0.823	cacat <b>GAAT</b> aaggagct
<i>PIT1</i>	<i>PIT1</i>	GHF-1 pituitary specific pou domain transcription factor	-509	-495	+	1.000	0.93	gcctc <b>TAT</b> tcatgt
<i>PAX4_PD</i>	<i>PAX6</i>	PAX-4/PAX-6 paired domain binding sites	-436	-418	-	1.000	0.919	cag <b>GCA</b> Cgcgcgatgaca
<i>ZF5</i>	<i>ZF5F</i>	ZF5 POZ domain zinc finger	-432	-422	+	1.000	0.967	atg <b>cCGC</b> Cgtg
<i>HELT</i>	<i>HESF</i>	Vertebrate homologues of enhancer of split complex	-432	-418	-	1.000	0.959	cagg <b>CACG</b> cgcgat
<i>AHRARNT</i>	<i>AHRR</i>	AHR-amt heterodimers and AHR-related factors	-436	-412	+	1.000	0.952	tgtcatgcgc <b>CCGT</b> gcctgcgttta
<b>MYCMAX</b>	<b>EBOX</b>	<b>E-box binding factors</b>	<b>-430</b>	<b>-418</b>	<b>-</b>	<b>1.000</b>	<b>0.921</b>	<b>caggcaCGCGcgc</b>
<i>OTP</i>	<i>CART</i>	Cart-1 (cartilage homeoprotein 1)	-423	-403	-	0.751	0.841	gatgtt <b>ATT</b> Taaacgcaggca

<i>E4BP4</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-421	-401	-	0.758	0.852	ctgatgttatTTAAacgcagg
<i>TEF_HLF</i>	<i>PARF</i>	PAR/bZIP family	-419	-403	+	0.784	0.82	tcggtTTAAataacatc
<i>E4BP4</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-420	-400	+	0.769	0.803	ctgcgtttaaATAAcatcagc
<i>LHX3</i>	<i>LHXF</i>	Lim homeodomain factors	-421	-399	-	0.751	0.812	agctgatgttATTTaaacgcagg
<i>TEF</i>	<i>PARF</i>	PAR/bZIP family	-418	-402	-	0.773	0.888	tgatgttatTTAAacgc
<i>MTBF</i>	<i>HMTB</i>	Human muscle-specific Mt binding site	-413	-405	-	1.000	0.902	tgttATTTa
<i>PROPI</i>	<i>CART</i>	Cart-1 (cartilage homeoprotein 1)	-418	-398	+	0.753	0.844	gcgtttAAATaacatcagctt
<i>CREB</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-416	-396	-	0.862	0.863	taaagcTGATgttatttaaac
<i>BARBIE</i>	<i>BARB</i>	Barbiturate-inducible element box from pro-eukaryotic genes	-407	-393	-	1.000	0.91	agctAAAGctgatgt
<i>EVI1</i>	<i>EVI1</i>	EVI1-myeloid transforming protein	-403	-387	-	0.750	0.841	aaagagAAGCtaaagctg
<i>IRF7</i>	<i>IRFF</i>	Interferon regulatory factors	-401	-381	-	1.000	0.892	aggaGAAAgagaagctaaagc
<i>LTATA</i>	<i>VTBP</i>	Vertebrate TATA binding protein factor	-390	-374	-	1.000	0.84	tttTATAaggagaaaga
<i>HOXD13</i>	<i>ABDB</i>	Abdominal-B type homeodomain transcription factors	-386	-370	+	1.000	0.919	tctcctaTAAAcatt
<i>CDX2</i>	<i>CDXF</i>	Vertebrate caudal related homeodomain protein	-386	-368	-	1.000	0.852	acaatgtTTTAtaaggaga
<i>PBX_HOXA9</i>	<i>HOXC</i>	HOX - PBX complexes	-384	-368	-	0.750	0.849	acaTGTTttataagga
<i>VTATA</i>	<i>VTBP</i>	Vertebrate TATA binding protein factor	-383	-367	+	1.000	0.941	cctaTAAAcattgtg
<i>FHXB</i>	<i>FKHD</i>	Fork head domain factors	-378	-362	-	0.909	0.855	aaattcACAAtgttita
<i>FAST1</i>	<i>FAST</i>	FAST-1 SMAD interacting proteins	-375	-359	+	1.000	0.884	aacatTGTGaattcag
<i>IRF1</i>	<i>IRFF</i>	Interferon regulatory factors	-371	-351	-	1.000	0.901	aaagaaaactGAAAttcaca
<i>HMG1Y</i>	<i>SORY</i>	SOX/SRY-sex/testis determinig and related HMG box factors	-372	-350	+	1.000	0.93	attgtgAATTcagttttcttc
<i>DINR</i>	<i>INRE</i>	Core promoter initiator elements	-364	-354	+	1.000	0.965	ttTCAGtttc
<i>RBPJK</i>	<i>RBPF</i>	RBPJ - kappa	-357	-343	-	1.000	0.946	ttgaTGGGaaagaaa
<i>PAX1</i>	<i>PAX1</i>	PAX-1 binding sites	-355	-337	+	0.750	0.689	tCTTTcccatcaagacatg
<i>OCT3_4</i>	<i>OCT1</i>	Octamer binding protein	-342	-326	+	1.000	0.824	gacATGCtcaagtgtg
<i>P53</i>	<i>P53F</i>	p53 tumor suppressor	-344	-322	-	1.000	0.935	gactcagcacttgagCATGtctt
<i>DEC2</i>	<i>HESF</i>	Vertebrate homologues of enhancer of split complex	-339	-325	-	0.871	0.972	tcagcaCTTGagcat
<i>NKX25</i>	<i>NKXH</i>	NKX homeodomain factors	-339	-321	+	1.000	1	atgctcAAGTgctgagtca

<i>TCF11MAFG</i>	<i>AP1R</i>	MAF and AP1 related factors	-335	-315	-	1.000	0.957	taaaag <b>TGAC</b> tcagcactga
<b>AP1</b>	<b>AP1F</b>	<b>AP1, Activating protein 1</b>	<b>-329</b>	<b>-319</b>	<b>+</b>	<b>1.000</b>	<b>0.968</b>	<b>gctgAGTCact</b>
<b>AP1</b>	<b>AP1F</b>	<b>AP1, Activating protein 1</b>	<b>-329</b>	<b>-319</b>	<b>-</b>	<b>0.971</b>	<b>0.974</b>	<b>agTGACtcagc</b>
<i>NFE2</i>	<i>AP1R</i>	MAF and AP1 related factors	-333	-313	+	1.000	1	aagt <b>gCTGA</b> gtcacttttaa
<i>VTATA</i>	<i>VTBP</i>	Vertebrate TATA binding protein factor	-326	-310	-	1.000	0.927	ttct <b>TAA</b> Aagtgactc
<i>BARBIE</i>	<i>BARB</i>	Barbiturate-inducible element box from pro+eukaryotic genes	-310	-296	+	1.000	0.888	aaaa <b>AAAG</b> aagagtg
<i>EVI1.07</i>	<i>EVI1</i>	EVI1-myeloid transforming protein	-310	-294	+	1.000	0.916	aaaaa <b>AAGA</b> aagtgct
<i>PAX2</i>	<i>PAX8</i>	PAX-2/5/8 binding sites	-297	-283	+	0.923	0.94	tg <b>CTCA</b> tgcttctta
<i>HMX2</i>	<i>HOMF</i>	Homeodomain transcription factors	-272	-254	+	1.000	0.916	caaggatga <b>CTTA</b> agcaca
<i>CREB2</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-272	-252	+	1.000	0.906	caagga <b>TGAC</b> ttagcacact
<i>ATATA</i>	<i>VTBP</i>	Vertebrate TATA binding protein factor	-269	-253	-	1.000	0.812	gtgt <b>gctTAAG</b> tcaccc
<i>HMX2</i>	<i>HOMF</i>	Homeodomain transcription factors	-267	-249	-	1.000	0.896	gaaagtgtg <b>CTTA</b> agtcac
<i>MYT1</i>	<i>MYT1</i>	MYT1 C2HC zinc finger protein	-260	-248	-	1.000	0.799	gga <b>AAGT</b> gtgctt
<i>BLIMP1</i>	<i>PRDF</i>	Positive regulatory domain I binding factor	-261	-243	-	1.000	0.838	gaaggga <b>GAAA</b> gtgtgctta
<i>IRF7</i>	<i>IRFF</i>	Interferon regulatory factors	-259	-239	-	0.821	0.905	ctag <b>GAA</b> Ggggaaagtgtgct
<i>MZF1</i>	<i>MZF1</i>	Myeloid zinc finger 1 factors	-254	-244	-	1.000	1	aa <b>GGGG</b> aaagt
<i>GABP</i>	<i>ETSF</i>	Human and murine ETS1 factors	-254	-234	-	1.000	0.868	cacaacta <b>GGA</b> Aggggaaagt
<i>IRF4</i>	<i>IRFF</i>	Interferon regulatory factors	-236	-216	-	1.000	0.942	gtttagcatc <b>GAAA</b> gaatcac
<i>PAX2</i>	<i>PAX2</i>	PAX-2 binding sites	-236	-214	+	1.000	0.788	gtgattcttcgatgct <b>AAAC</b> Gga
<i>CREB1</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-223	-203	-	1.000	0.974	acaatg <b>TGAC</b> gtcggttagca
<i>E4F</i>	<i>E4FF</i>	Ubiquitous GLI - Krueppel like zinc finger involved in cell cycle regulation	-217	-205	+	1.000	0.893	<b>acgACGT</b> cacatt
<i>CREB</i>	<i>CREB</i>	cAMP-responsive element binding proteins	-220	-200	-	1.000	0.902	tgcaaatg <b>TGAC</b> gtcggtta
<i>SOX9</i>	<i>SORY</i>	SOX/SRY-sex/testis determinig and related HMG box factors	-218	-196	-	1.000	0.944	agattgc <b>ACAA</b> tgtgacgtcggt
<b>CEBPB</b>	<b>CEBP</b>	<b>Ccaat/Enhancer Binding Protein</b>	<b>-210</b>	<b>-196</b>	<b>+</b>	<b>1.000</b>	<b>0.989</b>	<b>cacattgtGCAAtct</b>



<i>HLF</i>	<i>PARF</i>	PAR/bZIP family	-211	-195	+	0.836	0.869	tcacattgt <b>GCAA</b> tctt
<i>HHEX</i>	<i>HOMF</i>	Homeodomain transcription factors	-206	-188	+	1.000	0.953	ttgtgcaatct <b>TAATA</b> aagg
<i>BRN3</i>	<i>BRNF</i>	Brn POU domain factors	-202	-184	+	1.000	0.84	gcaatct <b>TAATA</b> aaggttc
<i>VAX2</i>	<i>HBOX</i>	Homeobox transcription factors	-202	-184	-	1.000	0.852	gaaacct <b>ATTA</b> agattgc
<i>BARX2</i>	<i>HOMF</i>	Homeodomain transcription factors	-202	-184	+	1.000	0.952	gcaatct <b>TAATA</b> aaggttc
<i>NKX61</i>	<i>NKX6</i>	NK6 homeobox transcription factors	-200	-186	+	1.000	0.915	aatc <b>TTAA</b> aaggtt
<i>BRN5</i>	<i>BRN5</i>	Brn-5 POU domain factors	-203	-181	-	1.000	0.922	ttggaaacct <b>ATTA</b> agattgca
<i>OCT1</i>	<i>OCT1</i>	Octamer binding protein	-200	-184	-	1.000	0.861	gaaacct <b>ATTA</b> agatt
<i>NKX25</i>	<i>NKXH</i>	NKX homeodomain factors	-200	-182	+	1.000	0.899	aatct <b>TAATA</b> aaggtttcca
<i>NFAT5</i>	<i>NFAT</i>	Nuclear factor of activated T-cells	-198	-180	-	1.000	0.888	att <b>GGAA</b> acctattaaga
<i>BRN2</i>	<i>BRNF</i>	Brn POU domain factors	-195	-177	-	0.900	0.879	ctG <b>ATT</b> Tggaaacctatta
<i>NFY</i>	<i>CAAT</i>	CCAAT binding factors	-188	-174	+	1.000	0.951	gttt <b>CCA</b> Atcagccc
<i>CDP</i>	<i>CLOX</i>	CLOX and CLOX homology (CDP) factors	-189	-171	+	1.000	0.969	ggtttc <b>CAAT</b> cagccccac
<i>INSM1</i>	<i>INSM</i>	Insulinoma associated factors	-180	-168	-	1.000	0.907	tgggt <b>GGGG</b> ctga
<i>BKLF</i>	<i>KLFS</i>	Krueppel like transcription factors	-182	-166	-	1.000	0.99	agt <b>GGGT</b> ggggctgatt
<i>MAZR</i>	<i>MAZF</i>	Myc associated zinc fingers	-179	-167	-	1.000	0.895	gtgggt <b>GGGG</b> ctg
<i>GC</i>	<i>SP1F</i>	GC-Box factors SP1/GC	-180	-166	-	0.872	0.942	agt <b>GGT</b> Ggggctga
<i>WT1</i>	<i>EGRF</i>	EGR/nerve growth factor induced protein C & related factors	-179	-163	-	1.000	0.932	cagag <b>TGGG</b> tggggctg
<i>GLI3</i>	<i>GLIF</i>	GLI zinc finger family	-178	-164	+	1.000	0.881	agcc <b>CCAC</b> ccactct
<i>NGFIC</i>	<i>EGRF</i>	EGR/nerve growth factor induced protein C & related factors	-177	-161	-	0.770	0.833	gcca <b>GAGT</b> gggtggggc
<i>PLAG1</i>	<i>PLAG</i>	Pleomorphic adenoma gene	-176	-156	-	0.833	0.881	<b>GTGG</b> ggccagagt <b>gg</b> tgggg
<i>BKLF</i>	<i>KLFS</i>	Krueppel like transcription factors	-167	-151	-	1.000	0.998	tgg <b>GGGT</b> ggggccagag
<i>MAZR</i>	<i>MAZF</i>	Myc associated zinc fingers	-164	-152	-	1.000	0.949	gggggt <b>GGG</b> cca
<i>GC</i>	<i>SP1F</i>	GC-Box factors SP1/GC	-165	-151	-	0.872	0.945	tggg <b>GGT</b> Ggggccag
<i>GKLF</i>	<i>KLFS</i>	Krueppel like transcription factors	-165	-149	-	1.000	0.984	ggtgggg <b>GTGG</b> ggccag
<i>PLAG1</i>	<i>PLAG</i>	Pleomorphic adenoma gene	-166	-146	-	1.000	0.885	<b>GAGG</b> gtgggggtggggccaga
<i>ZBP89</i>	<i>ZBPF</i>	Zinc binding protein factors	-166	-144	+	1.000	0.976	tctggcccca <b>CCCC</b> accctcca
<i>WT1</i>	<i>EGRF</i>	EGR/nerve growth factor induced protein C & related factors	-162	-146	-	1.000	0.998	gaggg <b>TGGG</b> gtggggc

<i>RREB1</i>	<i>RREB</i>	Ras-responsive element binding protein	-161	-147	+	1.000	0.876	cCCCAccccacct
<i>BKLF</i>	<i>KLFS</i>	Krueppel like transcription factors	-161	-145	-	1.000	0.974	ggaGGGTgggggtgggg
<i>GC</i>	<i>SP1F</i>	GC-Box factors SP1/GC	-159	-145	-	0.872	0.893	ggagGGTGgggggtgg
<i>ZNF219</i>	<i>ZBPF</i>	Zinc binding protein factors	-163	-141	+	1.000	0.941	ggccccCCCCcaccctcaaca
<i>SPZ1</i>	<i>SPZ1</i>	Testis-specific bHLH-Zip transcription factors	-154	-144	-	1.000	0.958	tGGAagggtggg
<i>LEF1</i>	<i>LEFF</i>	LEF1/TCF	-149	-133	+	1.000	0.876	cctccaaCAAagatttt
<i>GFI1</i>	<i>GFI1</i>	Growth factor independence transcriptional repressor	-146	-132	-	1.000	0.905	aaaAATCttgttgg
<i>SOX2</i>	<i>SORY</i>	SOX/SRY-sex/testis determinig and related HMG box factors	-150	-128	+	1.000	0.929	ccctccaACAAagatttttatca
<i>CDX2</i>	<i>CDXF</i>	Vertebrate caudal related homeodomain protein	-141	-123	+	1.000	0.862	aaagattTTTAatcaatgt
<i>GATA</i>	<i>GATA</i>	GATA binding factors	-137	-125	-	1.000	0.936	atttGATAaaaat
<i>SMARCA3</i>	<i>RUSH</i>	SWI/SNF related nucleophosphoproteins with a RING finger DNA binding motif	-131	-121	-	0.960	0.965	ccACATttgat
<i>NFAT5</i>	<i>NFAT</i>	Nuclear factor of activated T-cells	-128	-110	-	1.000	0.904	atgGGAAaatccacattt
<b><i>NFκB p65</i></b>	<b><i>NFKB</i></b>	<b>Nuclear factor kappa B/c-rel</b>	<b>-124</b>	<b>-112</b>	<b>+</b>	<b>1.000</b>	<b>0.982</b>	<b>gtgggattTTCCc</b>
<b><i>NFκB</i></b>	<b><i>NFKB</i></b>	<b>Nuclear factor kappa B/c-rel</b>	<b>-122</b>	<b>-110</b>	<b>-</b>	<b>1.000</b>	<b>0.891</b>	<b>atGGGAaaatccc</b>
<i>IK1</i>	<i>IKRS</i>	Ikaros zinc finger family	-120	-108	-	1.000	0.941	tcatGGGAaaatc
<i>RBPJK</i>	<i>RBPF</i>	RBPJ - kappa	-121	-107	-	1.000	0.965	ctcaTGGGaaatcc
<i>OLF1</i>	<i>NOLF</i>	Neuron-specific-olfactory factor	-121	-99	+	1.000	0.894	ggatttTCCCatgagtctcaaaa
<i>PHOX2</i>	<i>CART</i>	Cart-1 (cartilage homeoprotein 1)	-111	-91	-	1.000	0.882	ctctcTAATtttgagactcat
<i>MSX</i>	<i>HOMF</i>	Homeodomain transcription factors	-107	-89	-	1.000	0.989	aactctcTAATtttgagac
<i>GSH1</i>	<i>HBOX</i>	Homeobox transcription factors	-104	-86	-	1.000	0.881	gtcaactctcTAATttga
<i>MYT1L</i>	<i>MYT1</i>	MYT1 C2HC zinc finger protein	-96	-84	+	1.000	0.945	agagAGTTgactc
<i>HOXC13</i>	<i>ABDB</i>	Abdominal-B type homeodomain transcription factors	-88	-72	+	1.000	0.949	gactcctaaTAAAtatg
<i>BRN3</i>	<i>BRNF</i>	Brn POU domain factors	-88	-70	-	0.759	0.844	ctcatatTTATtaggagtc
<i>PHOX2</i>	<i>CART</i>	Cart-1 (cartilage homeoprotein 1)	-87	-67	+	1.000	0.881	actccTAATaaatatgagact

<i>BRN5</i>	<i>BRN5</i>	Brn-5 POU domain factors	-60	-38	+	0.751	0.844	gtctgtagctcATTCtgctctgg
<i>PAX6</i>	<i>PAX6</i>	PAX-4/PAX-6 paired domain binding sites	-47	-29	-	1.000	0.886	tgggtgggctCCAGagcaga
<i>HOMER</i>	<i>HZIP</i>	Homeodomain-leucine zipper transcription factors	-30	-16	-	1.000	0.839	tgactATCGttcttg
<i>TH1E47</i>	<i>HAND</i>	Twist subfamily of class B bHLH transcription factors	-21	-1	+	1.000	0.958	tagtcaattCCAGaaaccgct
<i>STAT</i>	<i>STAT</i>	Signal transducer and activator of transcription	-20	-2	-	1.000	0.896	gcggtttctGGAAttgact

**Supplemental Table 2:** Single-stranded oligonucleotides used for electrophoretic mobility shift assays (EMSA).

Oligonucleotide name	Oligonucleotide sequence (5'3')
Il-6_1_fwd	GATTTTATCAAATGTGGGATTTTCCCATGAGT
Il-6_1_rev	ACTCATGGGAAAATCCCACATTTGATAAAAAATC
Il-6_2_fwd	CTTTCGATGCTAAACGACGTCACATTGTGCAATCTT
Il-6_2_rev	AAGATTGCACAATGTGACGTCGTTTAGCATCGAAAG
Il-6_3_fwd	ATGCTCAAGTGCTGAGTCACTTTTAAAGAAAAA
Il-6_3_rev	TTTTTCTTTAAAAGTGACTCAGCACTTGAGCAT
Il-6_4_fwd	TGTGTCGTCTGTCATGCGCGCGTGCCTGCGTTTAA
IL6_4_rev	TTAAACGCAGGCACGCGCGCATGACAGACGACACA
USF consensus sequence_fwd	CACCCGGTCACGTGGCCTACACC
USF consensus sequence_rev	GGTGTAGGCCACGTGACCGGGTG
SP consensus sequence_fwd	ATTCGATCGGGGCGGGGCGAGC
SP consensus sequence_rev	GCTCGCCCCGCCCCGATCGAAT
NF-κB consensus site fwd	TCAGAGGGGACTTTCCGAGAGGCG
NF-κB consensus site rev	CGCCTCTCGGAAAGTCCCCTCTGA
AP1 consensus sequence_fwd	TGAGTCATGAGTCATGAGTCA
AP1 consensus sequence_rev	TGACTCATGACTCATGACTCA

**Supplemental Table 3:** Sequence of primers used to amplify the *Il-6* promoter region for generating luciferase reporter constructs, to verify the correctness of the sequence, and to perform site-directed mutagenesis. Underlined basepairs represent mutated region of a potential transcription factor in the *Il-6* promoter region used for site-directed mutagenesis.

Primer name	Primer sequence (5'3')	Application
Il-6 promoter_fwd	CTAACTGGCCGGTACCAgacttgagcattggaggggttattc *	PCR to amplify <i>Il-6</i> promoter
Il-6 promoter_rev	GGCCAGATCTTGATATCCTCGAGagcgggttctggaattgactatcG *	PCR to amplify <i>Il-6</i> promoter
RVprimer3	CTAGCAAAATAGGCTGTCCC	Sequencing of <i>Il-6</i> promoter construct
pGL4.10_rev	CGGTAAAGGCGATGGTGC	Sequencing of <i>Il-6</i> promoter construct
Il-6_1_Mut	GATTTTATCAAATGTGGGAT <u>G</u> TTCCCATGAGTCT	Site-directed mutagenesis of NF-κB in Il-6_1
Il-6_2_Mut	CTTTCGATGCTAAACGAT <u>A</u> TCACATTGTGCAATC	Site-directed mutagenesis of cFos in Il-6_2
Il-6_3_Mut	CCATCAAGACATGCTCAAGTGCTG <u>C</u> TTCACTTTTAAAG	Site-directed mutagenesis of cFos in Il-6_3
Il-6_4_Mut (USF)	GTGTCGTCTGTCATGCGCGT <u>A</u> TGCCTGCGTTTAAATAAC	Site-directed mutagenesis of USF in Il-6_4
Il-6_4_Mut (cFos)	GTGTGTCGTCTT <u>A</u> CATGCGCGCGTGCCTGC	Site-directed mutagenesis of cFos in Il-6_4

\* Capital letters indicate homologous overlap between the empty pGL4.10 reporter vector the *Il-6* promoter region designed to build the seamless clone using the Gibson Assembly Cloning kit according to manufacturer's protocol (Thermofisher).