

Cell Reports, Volume 8

Supplemental Information

A Role for RUNX3 in Inflammation-Induced Expression of *IL23A* in Gastric Epithelial Cells

Yit Teng Hor, Dominic Chih-Cheng Voon, Jason Kin Wai Koo, Huajing Wang, Wen Min Lau, Hassan Ashktorab, Shing Leng Chan, and Yoshiaki Ito

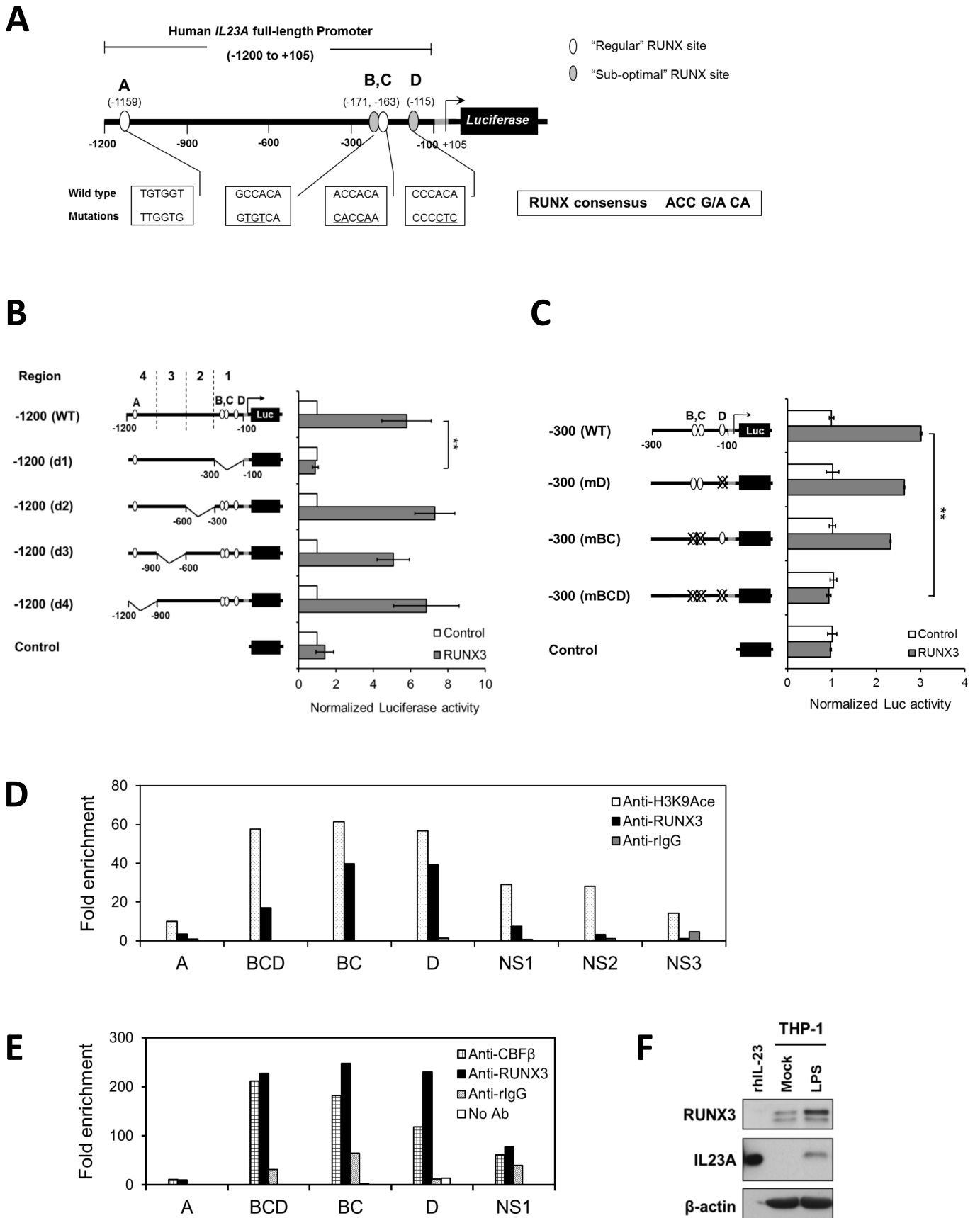


Figure S1. Functional RUNX binding sites in *IL23A* promoter. (Related to Figure 1)

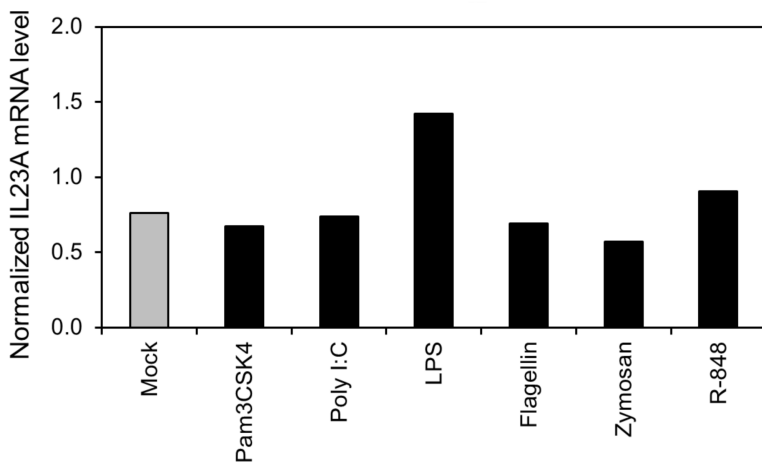


Figure S2. TLR ligands have minor effects on IL23A expression in SNU16 cells. (Related to Figure 2)

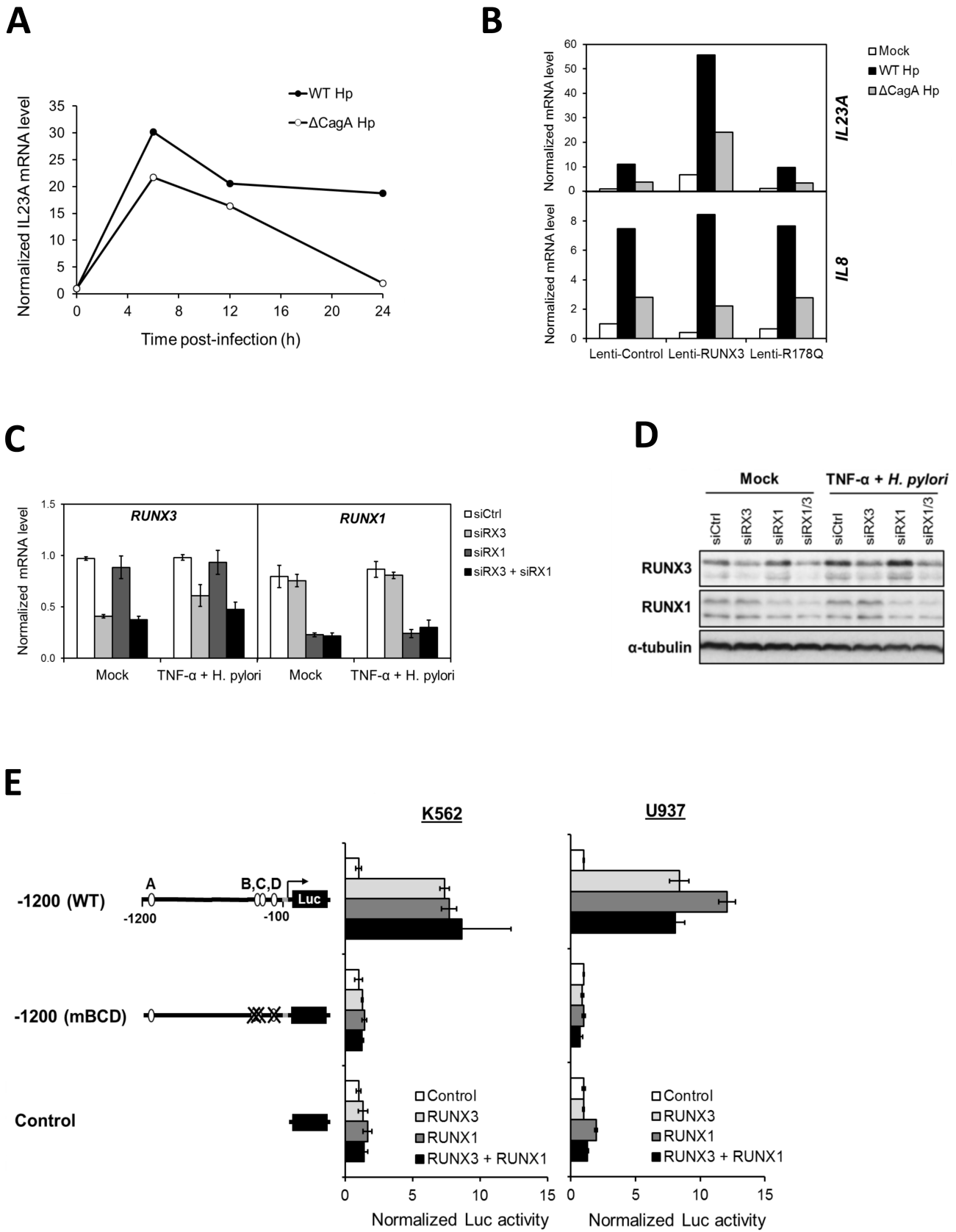


Figure S3. Contribution of RUNX3 and RUNX1 in the regulation of IL23A. (Related to Figure 3)

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Functional RUNX binding sites in *IL23A* promoter. (Related to Figure 1)

(A) Schematic diagram representing the upstream promoter region (-1200 to +105) of *IL23A* gene cloned into a firefly luciferase reporter construct. The four putative RUNX sites identified using Transcriptional Element Search System program were classified into regular RUNX sites (sites A and C) and sub-optimal RUNX sites (sites B and D) based on the RUNX consensus sequence. (B-C) RUNX3 transactivates *IL23A* via three RUNX sites in proximal region of the promoter. A series of (B) individual deletions and (C) single or compound mutations of the *IL23A*-1200 reporter constructs were transiently transfected into KATOIII cells together with either Control or RUNX3 expression vector as described for Fig. 1C. The normalized luciferase activities of each construct are expressed relative to those of their Control sample (means \pm SEM; n=3). (D-E) Specific binding of RUNX3 on *IL23A* is enriched in regions containing three functional RUNX binding sites. Chromatin of HFE-145 cells transduced with Lenti-RUNX3 (D) and LPS-induced THP-1 cells (E) were cross-linked and immunoprecipitated as described for Fig. 1D. Enrichment of chromatin fragments were quantitated using real-time PCR and expressed relative to rIgG control of region A. (F) Whole cell lysates from LPS-stimulated or non-stimulated THP-1 cells were analyzed by Western blotting for the protein expression of *IL23A*, RUNX3 and β -actin (loading control).

Figure S2. TLR ligands have minor effects on *IL23A* expression in SNU16 cells. (Related

to Figure 2) RUNX1- and RUNX3-positive SNU16 cells were used for screening the effects

of TLR ligands on *IL23A* expression. Cells were treated with the corresponding TLR ligands for 6h as described for Fig. 2B. The normalized levels of *IL23A* were measured by qRT-PCR and expressed relative to Mock sample values.

Figure S3. Contribution of RUNX3 and RUNX1 in the regulation of IL23A. (Related to

Figure 3) (A) Time-dependent effects of WT and CagA-defective isogenic mutant (Δ CagA)

strains of *H. pylori* on *IL23A* expression. AGS cells were infected with either WT or Δ CagA strains of *H. pylori* at MOI 100 for 0h, 6h, 12h and 24h prior to the assessment of *IL23A* transcripts by qRT-PCR. The normalized *IL23A* mRNA levels are expressed relative to the values of uninfected controls. (B) RUNX3 specifically enhanced *H. pylori*-induced *IL23A* but

not *IL8* in gastric epithelial cells. AGS cells transduced with the indicated lentiviruses for 48h were treated with either WT or Δ CagA *H. pylori* for 6h prior to the measurement of *IL23A* and *IL8* transcripts by qRT-PCR. Normalized values are expressed relative to those of untreated Lenti-Control samples. (C-D) The degree of RNAi knockdown of RUNX3 and

RUNX1 in HFE-145. (C) HFE-145 cells were transiently transfected with corresponding siRNAs as described for Fig. 3F. Normalized mRNA levels of *RUNX3* and *RUNX1* were

measured by qRT-PCR and expressed as a ratio to the basal values of untreated siCtrl sample (Mock) (means \pm SEM; n=3). (D) Measurement of RUNX1, RUNX3 and α -tubulin

expression by Western blotting following RNAi knockdown. (E) RUNX proteins

transactivate *IL23A* promoter in monocytes. Monocytic cell lines K562 and U937 were transfected with wild type or mutant *IL23A*-1200 reporter constructs together with Control, RUNX1 or RUNX3 expression vectors as indicated. Normalized luciferase activities

of each construct are presented relative to the values of corresponding control samples (means \pm SEM; n=3).

SUPPLEMENTAL TABLES

Table S1. Oligonucleotide primers and the positions of targeted regions for ChIP assay.

Targeted site	Amplified regions	Forward Primer	Reverse Primer
RUNX site A	-1211 to -1111	TTCTCAGAAGAAAAGGGTCAACCT	GGTCCCAGAACAATTTCGAAGA
RUNX site BC	-235 to -145	CCCCTTCTATAGCTATTTTCGATTCCT	GGGAAATGAGTAGTGTGGTTGCT
RUNX site D	-139 to -60	ACTGAGGCTGCATACCTGGG	AGCAGGTGACTCACAGCAGG
RUNX site BCD	-190 to -119	TCCCATCCCAGGCCTCTAG	CCCAGGTATGCAGCCTCAGT
Non-specific 1	-708 to -617	GAGGAGTCAGGTGGTTCTTAGTCAA	GGTCTTATTGCTCCCCATTTCA
Non-specific 2	-2040 to -1975	GTAGTTCCTGCCACGCAACA	TGCAGGAGTTGGGTAGGAAGA
Non-specific 3	-3169 to -3073	ATTGGCCCTCTCACTCACTGTAG	GGTGGCCAGTGCTGTAGTTA

Table S2. Gene-specific oligonucleotide primers and TaqMan probes used in quantitative RT-PCR.

SYBR Green Primers		
Gene	Forward	Reverse
<i>IL12A</i>	GCAGGCCCTGAATTTCAACA	CATGAAGAAGTATGCAGAGCTTGATT
<i>IL27A</i>	ATCTCACCTGCCAGGAGTGAA	TGAAGCGTGGTGGAGATGAAG
<i>EBI3</i>	GGGGAAGTGAAGTACTGGAG	AGTCGGTCATCTGAGGTTGC
<i>IL8</i>	TCACTGTGTGTAACATGACTTCCA	TGGCAAACTGCACCTTCC
<i>EpCAM</i>	GTGATAGCAGTTGTTGCTGG	TATGCATCTCACCCATCTCC
<i>CD45</i>	TCCATATATTAGCATTAGTCC	GAGCATGCAAAATTGAAAACC
<i>IL23A</i>	AGCCGCCCGGGTCTT	TCCTTGAGCTGCTGCCTTTAG
<i>IL12B</i>	GGCTCCATGAAGGTGCTAC	GTCAGCCTCAGAATGCAAAA
TaqMan Probes		
Gene	TaqMan ID	
<i>GAPDH</i>	Hs99999905_m1	
<i>IL23A</i>	Hs00900829_g1	
<i>IL12B</i>	Hs99999037_m1	
<i>RUNX3</i>	Hs00231709_m1	
<i>RUNX1</i>	Hs00231079_m1	

SUPPLEMENTAL EXPERIMENTAL PROCEDURE

Cloning of IL23A promoter

Human IL23A promoter sequence (Genebank accession no NM_001265) was obtained from UCSC Genome Bioinformatics (<http://www.genome.ucsc.edu/>). The promoter containing the genomic fragment -1200 to +105 of the *IL23A* gene was amplified by PCR from genomic DNA of KATOIII cells and cloned into the pGL3-basic vector (Promega, Madison, USA). Putative RUNX and NF- κ B binding sites were identified using the Transcriptional Element Search System program (www.cbil.upenn.edu/tess/) (Heinemeyer et al., 1998). Site-directed mutageneses of identified RUNX and NF- κ B binding sites were performed using the QuikChange II kit (Agilent Technologies, Santa Clara, CA), according to manufacturer's instructions.

SUPPLEMENTAL REFERENCE

Heinemeyer, T., E. Wingender, I. Reuter, H. Hermjakob, A. E. Kel, O. V. Kel, E. V. Ignatieva, E. A. Ananko, O. A. Podkolodnaya, F. A. Kolpakov, N. L. Podkolodny, and N. A. Kolchanov. 1998. Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. *Nucleic acids research* 26:362-367.