SUPPORTING INFORMATION

Inflammatory and mitogenic signals drive interleukin 23 subunit alpha (IL23A) secretion independent of IL12B in intestinal epithelial cells

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LIST OF SUPPORTING INFORMATION

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Generation of lentivirus expression vectors and reporter gene constructs

The coding region of the p44 isoform of RUNX3 was previously cloned into the pBOBI lentivirus transfer vector, as described in detail in (54). Using a similar strategy, we amplified by PCR (Supp. Table S1) the coding region of isoform 1B of RUNX1 from pEF-Bos-RUNX1 (59) and introduced it between a 5'*Xba*I and a 3' *Xho*I site within the multiple cloning site of the pBOBI vector. The firefly reporter constructs containing the long (nt -1200 to +105) and minimal (nt -300 to +105) human *IL23* promoter regions and the mutation of the RUNX and NF-κB sites were previously described (14). Site-directed mutagenesis of the AP-1 site was performed using the QuikChange II kit (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer's instructions using oligonucleotides mutAP1_F1 and mutAP1_R1 (Supp. Table S1) for reporter constructs with wild type NF-κB site; and mutAP1_F2 and mutAP1_R2 (Supp. Table S1) and for reporter constructs bearing a mutant NF-κB site that is adjacent to the targeted AP-1 site.

Supplementary Table S1: Gene-specific oligonucleotide Taqman probes and primers used in quantitative RT-PCR. All sequences are oriented 5' to 3'.

TaqMan Probes		
Gene	**TaqMan ID**	
GAPDH	Hs99999905_m1	
IL23A	Hs00900829_g1	
IL12B	Hs99999037_m1	
EBI3	Hs01057148_m1	
SYBR primers		
Gene	**Forward**	**Reverse**
IL23A	AGCCGCCCGGGTCTT	TCCTTGAGCTGCTGCCTTTAG
IL12B	GGCTCCATGAAGGTGCTAC	GTTCAGCCTCAGAATGCAAAA
PCR Primers for generating biotinylated *IL23A* promoter fragment		
Forward	Biotin-ATTACACATGTGTCCCATCC	
Reverse	TGTAAGGCCCGCCCTTTATA	
Molecular cloning and site-directed mutagenesis		
RUNX1_F	AAAAAATCTAGAACCGCCATGCGTATCCCCGTAGATG	
RUNX1_R	AAAAAACTCGAGTCAGTAGGGCCTCCACAC	
mutAP1_F1	TCCCACCTGCTGACGCGTACCTGCTGGTAT	
mutAP1_R1	ATACCAGCAGGTACGCGTCAGCAGGTGGGA	
mutAP1_F2	CGTCACCTGCTGACGCGTACCTGCTGGTAT	
mutAP1_R2	ATACCAGCAGGTACGCGTC`AGCAGGTGACG	

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Activating effects of TNF-α and PMA are mediated via the proximal promoter in HCT 116 cells. The -1200 to +105 region of the *IL23A* promoter firefly reporter construct and its deletion variants were transiently transfected into HCT 116 cells and treated with TNF-α (50ng/ml) or PMA (1μM) for 8 h prior to luciferase assay. Normalized luciferase activities are expressed relative to the values of samples transfected with an empty Control vector. Data presented are as mean ±SEM from four replicates.

Figure S2: Excerpt from the USCS Genome Browser (GRCh37hg19; http://genome.ucsc.edu) showing the enrichment of genomic fragments within the proximal *IL23A* promoter in the indicated ChIP-Seq experiments, performed by the ENCODE project (31,32).

Figure S3: Ectopic RUNX1 more strongly increased basal IL23A expression in HCT 116. The image presented is from the same blot as that in Fig. 2G, captured over a longer exposure.

Figure S4: Exogenous RUNX1 and RUNX3 enhance the effects of PMA/TNF-α on IL23A induction. SW480 and SW620 CRC cells were transduced with Control, RUNX1 or RUNX3 lentiviruses for 48 h and treated with TNF-α (5ng/ml) or PMA (20ng/ml) for another 24 h. Cell lysates were subjected to Western blotting for the detection of IL23A, RUNX3, RUNX1 and GAPDH (loading control).

Figure S5: Treatment with inhibitors has no significant effect on cell viability. SW620 cells were plated and cultured on a 96-well plate overnight. Cells were treated with DMSO (control) trametinib (10 nM), BAY 11-7082 (10 μM) or in combination for the indicated time and harvested for MTT cell viability assay (supplier) in accordance to the accompanying instructions.

Figure S6: Treatment with additional MEK1/2 inhibitors attenuate PMA induction of *IL23A*. SW620 cells were pre-treated with MEK inhibitors trametinib (10 nM), PD98059 (10 μM) or PD0325901 (1 μM) for 2 h. For comparison, cells were also treated with ROCK inhibitor Y-27632 (10 μM). The pre-treated cells were then treated with DMSO or PMA (50 ng/ml) for an additional 12 h and harvested for quantitative RT-PCR analysis. Student's *t*-test was performed and statistical significance are indicated as follows: *, $p<0.05$; **, $p<0.01$; n.s., not significant.

Figure S7: (A) SW620 cells were transiently transfected with a series of reporter constructs containing the wild type (WT) minimal *IL23A* promoter (-300 to +105) or mutant variants, in which AP-1 (mA), NF-κB (mK) and three functional RUNX sites (mR) are mutated in combinations as indicated. Transfected cells were rested for 24h post-transfection and treated with DMSO (Control) or trametinib (10ng/ml). After 24 h of treatments, the cells were harvested for luciferase assays. Normalized luciferase values are expressed relative to DMSO-treated WT values and presented as mean ± SEM (n=4).

Figure S8: Relative expression levels of IL-12 family member *EBI3* in selected cancer cell lines. Normalized RNA-Seq data derived from cancer cell lines of hematopoietic, gastric and colorectal (large intestine) lineages were extracted from the CCLE database (https://portals.broadinstitute.org/ccle) and charted against copy number.

Figure S9: *EBI3* expression is highly restricted in human CRC lines. *EBI3* mRNA levels were evaluated by qRT-PCR using a specific Taqman hydrolysis probe. *EBI3* expression is normalized against that of

GAPDH and compared with that of AGS human gastric epithelial cells and THP-1 human monocytes. The corresponding Ct values of every sample (with a cut-off limit of 34 cycles) are presented in grey bars.

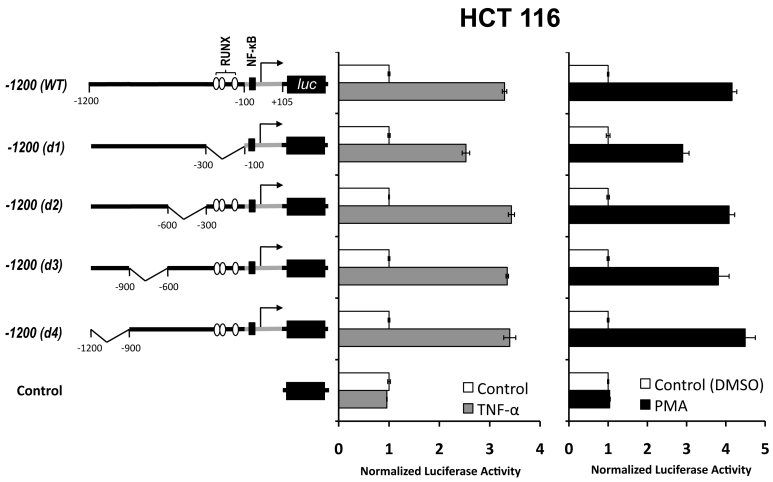
Figure S10: *EBI3* expression in resting and PMA-activated CRC cells. The basal and PMA-induced *EBI3* mRNA levels of SW620, SW480, HCT 116 and COLO 205 lines were measured by Taqman qRT-PCR. Cells were treated with PMA (1 μM) for 10 h. JVM3 human lymphoblasts were used as a positive control. Expression of *EBI3* in PMA-induced SW620 is estimated to be >130,000 times lower than that of *IL23A*.

Figure S11: ELISA measurement of secreted IL23A by activated CRC cells and THP-1 human monocytes. SW620 and COLO 201 cells were treated with DMSO (-PMA) or PMA (100ng/ml) for 36 h before the supernatants were collected for ELISA. THP-1 monocytes were treated with DMSO (Mock) or PMA (100ng/ml) for 48h to promote their differentiation into secretory M2-like macrophages. As positive controls, culture supernatants were collected from undifferentiated or differentiated THP-1 cells that were treated with PBS or lipopolysaccharide (LPS; 1μg/ml) for 24 h.

Figure S12: Treatment with inhibitors has no effect on endogenous RUNX3 expression. HCT 116 and SW620 cells were pre-treated with DMSO (control) trametinib (10 nM), BAY 11-7082 (10 μM) or in combination for 2 h. Subsequently, cells were treated with TNF-α (20 ng/ml) and PMA (50 ng/ml) for 12 h and harvested for Western blotting measurements.

Supplementary Figures

S1



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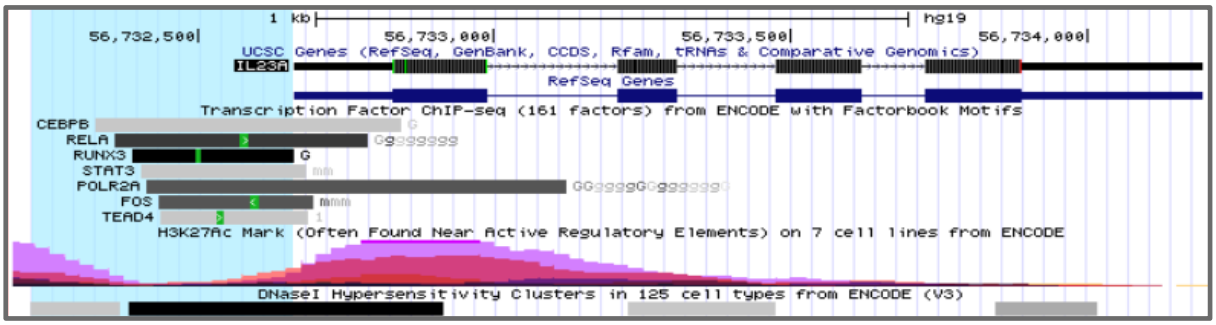

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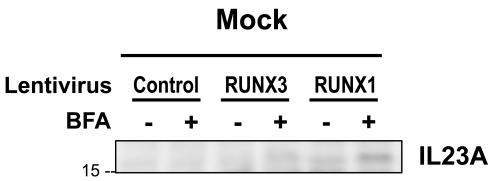

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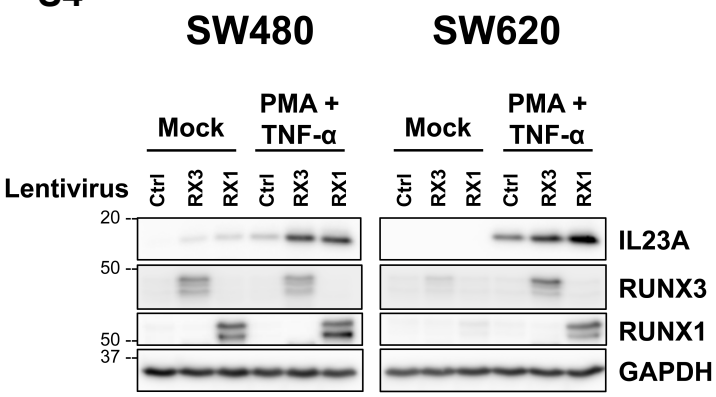

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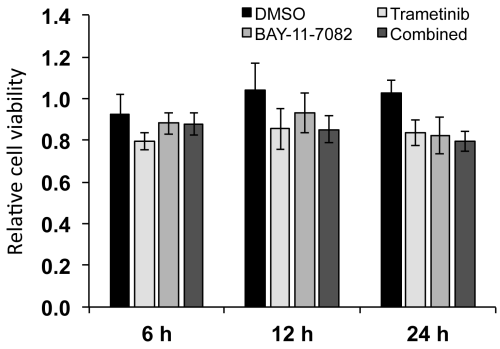

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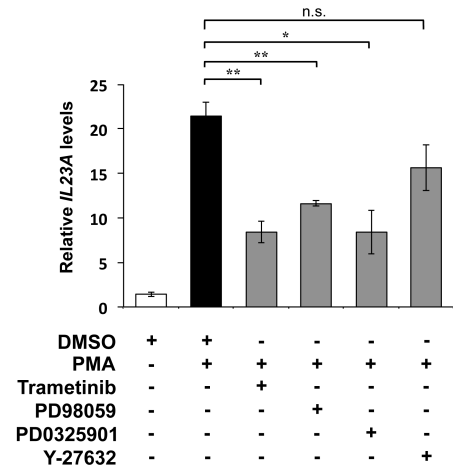

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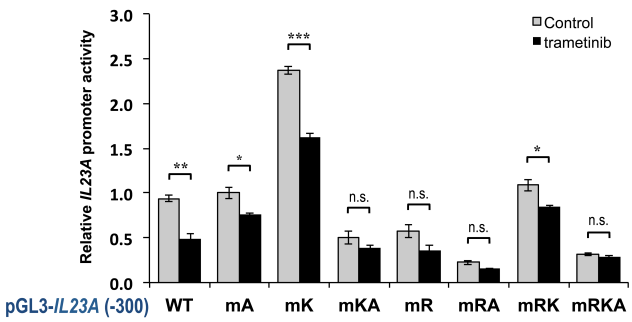

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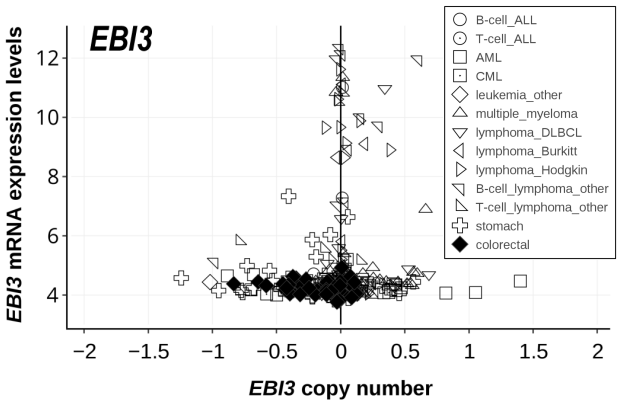

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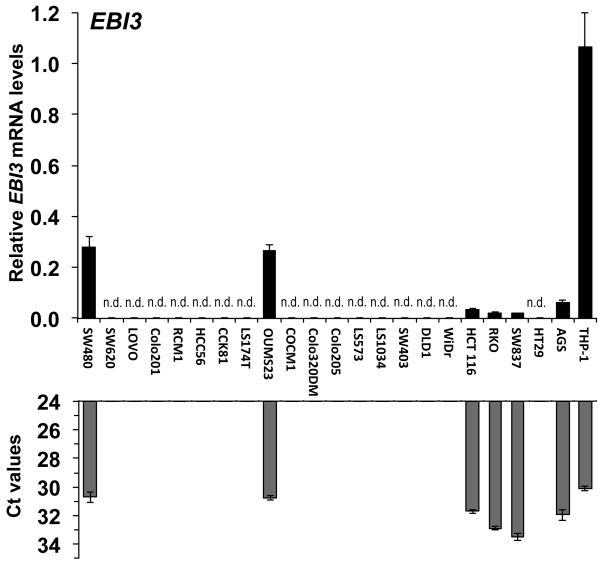

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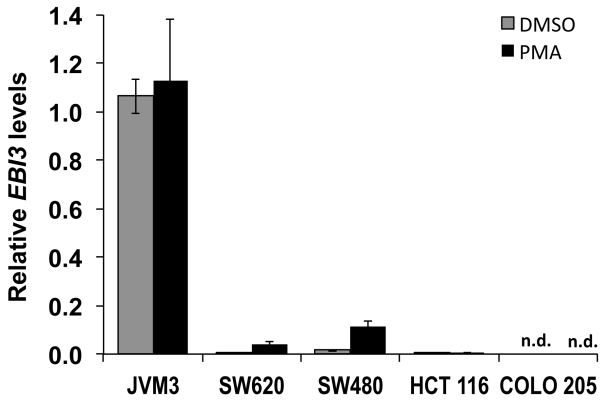

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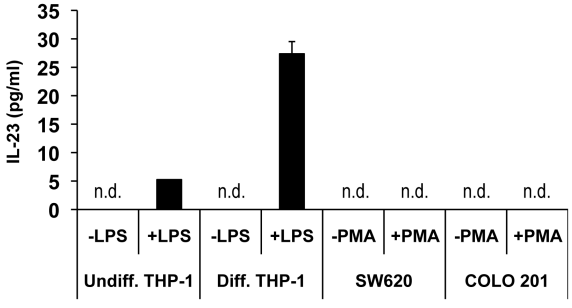

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