OAS1 and OAS3 negatively regulate the expression of chemokines and interferon-responsive genes in human macrophages

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

rRNA cleavage assay

Cells were transfected with 1 μg/ml poly(I:C) (Invivogen) using Lipofectamine 2000 (Life Technologies/ThermoFisher Scientific) according to the manufacturer's protocol. The cells were harvested 6 h after transfection and lysed with TRIzol to isolate RNA for analysis by Bioanalyzer (Agilent Technologies) and agarose gel electrophoresis.

Immunoblotting

For immunoblot analyses, cells were lysed for 15 min at 4°C in radioimmunoprecipitation assay (RIPA) lysis buffer [100 mM Tris-HCl (pH 8.0), 50 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1% Triton X-100, 50 mM β-glycerophosphate, 50 mM NaF, 0.1 mM Na_3VO_4, and 0.5% sodium deoxycholate] with protease inhibitor cocktail (Roche).

Lysates were centrifuged at 1,300 × g for 15 min at 4°C to remove nuclei. Then, proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were developed with Amersham enhanced chemiluminescence (ECL) reagents, followed by detection of the signal using the ImageQuant LAS 4000 system (GE Healthcare). The anti-OAS1 (HPA003657) and anti-OAS3 (HPA041253) antibodies were obtained from Atlas Antibodies, and the anti-RNaseL (#27281) and anti-GAPDH antibodies (#5174) were purchased from Cell Signaling Technology. All antibodies were used at a 1:1,000 dilution for immunoblot analysis.

mRNA sequencing and data analysis

RNA from cell lysates was purified using the RNeasy mini kit (Qiagen) with DNase digestion, and converted to cDNA according to the Illumina TruSeq Stranded mRNA Sample Preparation Guide. mRNA-seq was performed on the Illumina HiSeq 2500 platform at Macrogen in Korea. The Tuxedo tools were used for processing RNA-seq data, as described in Trapnell et al. {Trapnell, 2012 #118}. The RNA-seq data were mapped to the genome (mm10) using TopHat (version 2.0.10), which includes Bowtie as a short-read aligner. Mapped reads (alignments) were assembled on the genome by Cufflinks (version 2.1.1). Cufflinks calculates the relative abundance of transcripts. When assembling transcripts, the NCBI mRNA reference sequence collection (RefSeq) was used. Subsequently, the sample set was normalized using Cuffnorm (version 2.2.1) to minimize inter-sample variation and to compare fragments per kilobase of transcript per million mapped reads of all samples from different experimental conditions. The GEO accession number for the RNA sequencing data reported in this paper is GSE113363. Furthermore, innateDB (www.innatedb.com) was used for gene ontology analysis. The gene lists used in ontology analysis were annotated by RefSeq ID. Hypergeometric analysis was selected as the ontology analysis algorithm, and the Benjamini–Hochberg method

with P-value correction was used for statistical analysis.

mRNA quantification by quantitative RT-PCR

Total RNA was extracted with TRIzol Reagent (Invitrogen/ThermoFisher Scientific) according to the manufacturer's protocol. cDNA was synthesized using Superscript II Reverse Transcriptase (Invitrogen/ThermoFisher Scientific) with oligo-dT primers. The expression of individual genes was measured by real-time PCR using a Bio-Rad CFX, and quantitatively normalized to the expression of the housekeeping gene G*APDH* by the change-in-cycling-threshold ($\Delta\Delta C_T$) method. Primers used are listed in **Supplementary Table 2**.

ELISAs

CCL2, CCL8, and CXCL10 secretion in culture supernatants was measured using enzyme-linked immunosorbent assay (ELISA) kits (Biolegend).

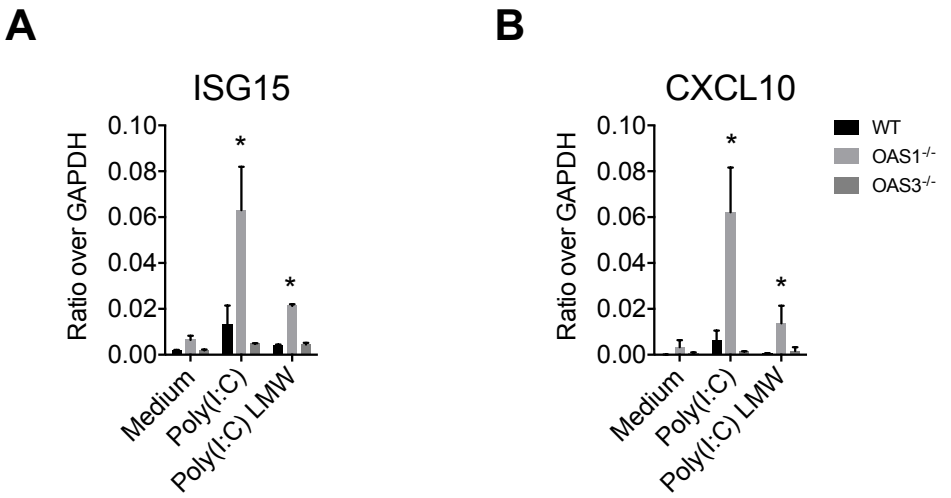
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. The expression of ISG15 and CXCL10 is increased in OAS1$^{-/-}$ THP-1 cells treated extracellularly with poly(I:C). WT, OAS1$^{-/-}$, and OAS3$^{-/-}$ dTHP-1 cells were cultured for 16 h in the absence or presence of poly(I:C) (10 μg/ml), and the mRNA levels of (A) ISG15 and (B) CXCL10 were quantified by qRT-PCR.

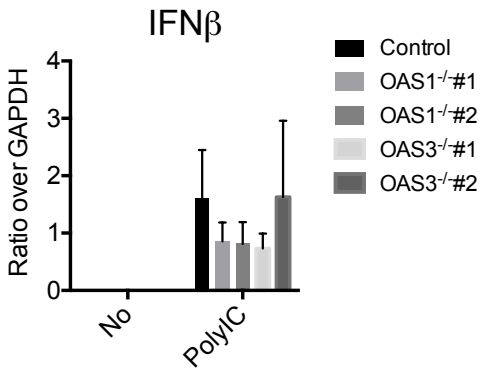
Supplementary Figure 2. The induction of IFN-β in response to intracellular poly(I:C) is no significant differences in WT, OAS1$^{-/-}$, and OAS3$^{-/-}$ dTHP-1 cells. WT, OAS1$^{-/-}$, and OAS3$^{-/-}$ dTHP-1 cells were transfected with 1 μg/ml poly(I:C) for 6 h, and the mRNA levels of IFN-β were quantified by qRT-PCR.

Supplementary Figure 3. OAS3 is not required for type I interferon-induced chemokine expression in THP-1 cells. (A) WT, OAS1$^{-/-}$, and OAS3$^{-/-}$ dTHP-1 cells were treated with the indicated doses of (A-C) IFN-α or (D-F) IFN-β for 16 h, and production of (A, D) CCL2, (B, E) CCL8, and (C, F) CXCL10 was measured by ELISA.

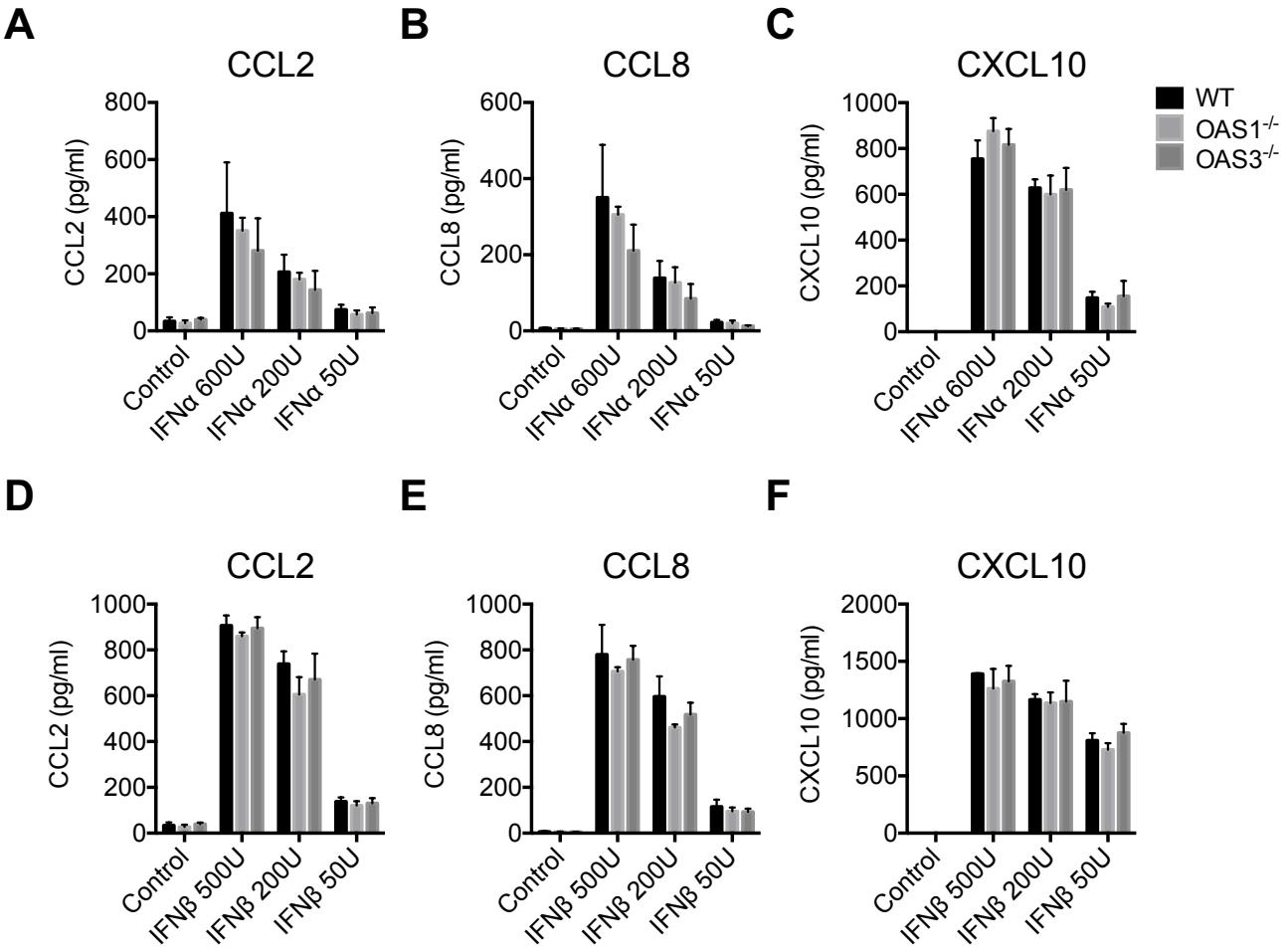
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Table 1

Gene ontology analysis from mRNA sequencing

4 fold increased by intracellular Poly(I:C) in OAS3$^{-/-}$ cells

GO Term ID	GO Term Name	Proportion	GO Term ORA P-Value
GO:0060337	type I interferon signaling pathway	30%	<1.0E-5
GO:0051607	defense response to virus	16%	<1.0E-5
GO:0008009	chemokine activity	24%	<1.0E-5
GO:0019221	cytokine-mediated signaling pathway	10%	<1.0E-5
GO:0006935	chemotaxis	10%	<1.0E-5

4 fold decreased by intracellular Poly(I:C) in OAS3$^{-/-}$ cells

GO Term ID	GO Term Name	Proportion	GO Term ORA P-Value
GO:0071560	cellular response to transforming growth factor beta sti mulus	8%	0.00037
GO:0006998	nuclear envelope organization	18%	0.00068
GO:1902230	negative regulation of intrinsic apoptotic signaling pathw ay in response to DNA damage	14%	0.00112
GO:0043488	regulation of mRNA stability	14%	0.00112
GO:0045333	cellular respiration	11%	0.00187

Supplementary Table 2

Primers for qRT-PCR

Gene	Sequence(Forward) 5'-3'	Sequence(Reverse) 5'-3'	GeneBank_ID
TNFa	GGAGAAGGGTGACCGACTCA	CTGCCCAGACTCGGCAA	NM_000594
IL-1b	TTTGAGTCTGCCCAGTTCCC	TCAGTTATATCCTGGCCGCC	NM_000576
CCL2	CCTTCATTCCCCAAGGGCTC	GGTTTGCTTGTCCAGGTGGT	NM_002982
CCL5	TCCAACCCAGCAGTCGTCT	TTGGCGGTTCTTTCGGGTG	NM_002985
CCL8	GTTTCTGCAGCGCTTCTGTG	ATCTCTCCTTGGGGTCAGCA	NM_005623
CXCL9	CAATTTGCCCCAAGCCCTTC	TCACATCTGCTGAATCTGGGT	NM_002416
CXCL10	TGGCATTCAAGGAGTACCTC	TTGTAGCAATGATCTCAACACG	NM_001565
ISG15	AGGCAGCGAACTCATCTTTG	CCAGCATCTTCACCGTCAG	NM_005101
OASL	AAAAGAGAGGCCCATCATCCTG	AAGGGTTCACGATGAGGTTGAA	NM_003733
GBP1	CTTTGTTCAGAAGCTACAAGACC	CTTTCTTTGCATTTCCTGCAAC	NM_002053
GBP3	ACTGGTGGCGAATCCAGAAG	ACTGTGGAGCCCAGAGAGAA	NM_018284
GBP4	GGTGGCCATTGTAGGGCTAT	AGAAGGACCAGGGTGTGGTT	NM_052941
GBP5	CCGCTGCATACAAATCAGGC	AGGTTGCGTAATGGCAGACA	NM_052942
IFIT1	CTGCAGAACGGCTGCCTAAT	GGCTTCCTCATTCTGGCCTT	NM_001548
IFNb	AGGACAGGATGAACTTTGAC	TGATAGACATTAGCCAGGAG	NM_002176
GAPDH	CCATGTTCGTCATGGGTGTG	GGTGCTAAGCAGTTGGTGGTG	NM_002046

CRISPR-Cas9 gRNA

Gene	gRNA #	Sequence(Forward) 5'-3'	Sequence(Reverse) 5'-3'
hOAS1	guide RNA1	CACCGCCAAGACCGTCCGAAATCCC	AAACGGGATTTCGGACGGTCTTGGC
	guide RNA2	CACCGTCAGTACGAAGCTGAGCGCA	AAACTGCGCTCAGCTTCGTACTGAC
hOAS3	guide RNA1	CACCGAATTGCTGACCATCTTCGCC	AAACGGCGAAGATGGTCAGCAATTC
	guide RNA2	CACCGCGAAGATGGTCAGCAATTCC	AAACGGAATTGCTGACCATCTTCGC
RNaseL	guide RNA1	CACCGGCAGGTGGCATTTACCGTCA	AAACTGACGGTAAATGCCACCTGCC