Journal of clinical and experimental hematopathology
Vol. 58 No.2, 87-94, 2018

Original article

Genome-wide siRNA screening in mouse bone marrow-derived macrophages revealed that knockdown of ribosomal proteins suppresses IL-10 and enhances TNF-α production

Yoshihiko Okamura,1) Naoyuki Makita,1) Yoshiyuki Hizukuri,1) Yasuhiro Hayashi1,2)

Macrophages play a central role in the immune response, and their diverse functions are attributed to the spectrum of their functional states. To elucidate molecules involved in modulating the balance between the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokine TNF-α, we conducted genome-wide siRNA screening. First, we established an siRNA screening system using mouse bone marrow-derived macrophages, which are a suitable model for studying functional states of macrophages *in vitro*. In the primary screen and the subsequent reproducibility assay, 112 siRNA pools demonstrated enhancement of IL-10 production and 497 siRNA pools suppressed IL-10 production. After a deconvolution assay for IL-10-up-regulating siRNA pools, 8 genes were identified as IL-10 repressors, including *Cnot1* and *Rc3h1*, components of the CCR4-NOT complex known to degrade cytokine mRNAs. On the other hand, siRNA pools targeting ribosomal proteins were frequently found among those that down-regulated IL-10 production and up-regulated TNF-α production. Four pools were assayed using deconvoluted siRNAs and identified as high-confidence hits. Thus, we found that the genome-wide knockdown of 19 ribosomal proteins resulted in decreased IL-10 and increased TNF-α production.

Keywords: macrophages; interleukin-10; genome-wide siRNA screen; ribosomal proteins

INTRODUCTION

Macrophages are involved in host defense through pathogen phagocytosis, antigen presentation, and production of pro-inflammatory cytokines. In addition, macrophages are involved in the resolution of inflammation via removal of apoptotic cells and production of several molecules, including interleukin-10 (IL-10), which has anti-inflammatory properties.[1, 2] Balance between pro- and anti-inflammatory cytokines is a characteristic difference among macrophage phenotypes.

IL-10 is an anti-inflammatory cytokine and its receptor is expressed in several types of immune cells. It is mainly produced by T helper cells, but also by other cells such as macrophages and dendritic cells. Recently, it was reported that IL-10 produced by macrophages, but not by T cells, suppresses gut inflammation in mice.[3, 4] The result that a certain enterobacterium promotes IL-10 production by macrophages suggests the significance of macrophage IL-10 production in the maintenance of gut immunity. Furthermore, IL-10 production from tumor-associated macrophages (TAMs), which are present in tumor tissues, is also significant. IL-10 in cerebrospinal fluid may be positively associated with the infiltration level of TAMs in primary central nervous system lymphoma.[5] In addition, IL-10 contributes to the proliferation of lymphoma via STAT3 activation,[6] which is related with PD-L1/2 expression by lymphoma cell lines.[7]

The mechanism of IL-10 production has been studied in macrophages as well as in other cells.[8] In macrophages, several studies have demonstrated that downstream pathways of pattern recognition receptors, particularly toll-like receptors (TLRs), regulate the production of TLR-induced cytokines, including IL-10.[9] Therefore, the regulation of TLRs or their downstream factors may change the production of all TLR-induced cytokines, but not the balance between the production of IL-10 and the pro-inflammatory cytokines. Several other molecules have been reported to affect the production of IL-10. Bcl3, a member of the IκB family, reportedly inhibits the transcription of IL-10 in macrophages.[10] Tristetraprolin (Zfp36) is an RNA-binding protein required

---

Received: December 4, 2017. Revised: February 15, 2018. Accepted: February 26, 2018. J-STAGE Advance Published: April 13, 2018
DOI:10.3960/jslrt.17036

1)Asubio Pharma Co., Ltd., Kobe, Japan.
Corresponding author: Yasuhiro Hayashi, Ph.D.
2)Present Address: Oncology Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan. E-mail: hayashi.yasuhiro.zu@daiichisankyo.co.jp

for the rapid degradation of mRNAs containing AU-rich elements, including IL-10.[11] Prostaglandin E_2 induces macrophage IL-10 production and the formation of regulatory-like macrophages via a salt-inducible kinase.[12] Furthermore, the Bcr-Abl and Src inhibitors dasatinib and bosutinib, respectively, elevate the production of IL-10 and suppress several pro-inflammatory cytokines in bone marrow-derived macrophages (BMDMs) by inhibiting salt-inducible kinase 2.[13] Macrophage polarization also plays a key role in IL-10 production. However, the molecules involved in IL-10 production and the balance between pro- and anti-inflammatory cytokines in macrophages have remained elusive.

To identify these molecules, we took a functional genomics approach in this study. In the last decade, this approach has been frequently adopted using RNAi screening with the emergence of small interfering RNA (siRNA) and short hairpin RNA (shRNA).[14, 15] In RNAi screening, different sizes of siRNA or shRNA libraries, from focused to genome-wide, are used for different purposes. The screen can be performed in an arrayed or pooled format. Generally, the arrayed format requires a greater amount of resources, whereas the pooled format limits the assay type. In the arrayed format, siRNA and shRNA can be used; however, an arrayed shRNA library is relatively expensive. RNAi screening has already been applied in studies on macrophages.[16] Ley S. *et al*. reported that targets whose knockdown induced co-suppression of IL-6/IL-10 expression in primary human monocyte-derived macrophages were identified from 8,495 shRNA constructs. In this study, however, we planned to identify modulators of the balance between pro- and anti-inflammatory cytokines using a whole-genome library. In particular, we first planned to measure IL-10 production and subsequently measure TNF-α production as representatives of anti- and pro-inflammatory cytokines, respectively. We chose the arrayed format because we measured concentrations of these cytokines in the supernatant. Furthermore, mouse BMDMs are a suitable *in vitro* model for understanding the mechanisms controlling functional states of macrophages because they can be obtained in large numbers and are suitable for large-scale screening.[17-19]

EXPERIMENTAL METHODS

Primary screening and the reproducibility assay were performed with the mouse siGENOME siRNA library (G-015005-01; Mouse Genome, GE Dharmacon, Lafayette, CO, USA). This library contains 19,061 siRNA pools (SMARTpools): each pool contains four distinct siRNAs against non-overlapping regions of the target gene mRNA. BMDMs were generated as described previously.[20] siRNAs were transfected into BMDMs at a final concentration of 45 nM using HiPerFect transfection reagent (Qiagen, Hilden, Germany) in a 384-well format. After 3 days, the cells were stimulated with 100 ng/mL of lipopolysaccharide (LPS) for 24 hr. Concentrations of IL-10 or TNF-α in the media were quantitated with the AlphaLISA mouse IL-10 immunoassay kit or AlphaLISA mouse TNF-α immunoassay kit (PerkinElmer, Waltham, MA, USA). Cell viability was quantitated by the CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI, USA). Please refer to the Supplemental Materials and Methods for detailed explanations of experimental methods. In the deconvolution assay, 4 single siRNAs comprising each siRNA pool were individually assayed as in the reproducibility assay described above. The details of single siRNAs used in Fig. 2 and 4 are shown in Supplemental Table 1.

RESULTS

Establishment of a high throughput siRNA assay in mouse BMDMs

To conduct an siRNA screen, it is important to determine the conditions under which sufficient knockdown is achieved. An additional factor in our study was that primary macrophages are known to be difficult to transfect.[21] Therefore, we examined several siRNA transfection conditions, and selected the HiPerFect transfection reagent because it exhibited the best knockdown effects without cell toxicity (Supplemental Table 2).

The timeline of the screening procedure is shown in Fig. 1A. As BMDMs did not produce cytokines without stimulus, we stimulated BMDMs with LPS. We planned to stimulate BMDMs with LPS after protein knockdown. The period of time required for sufficient protein knockdown after siRNA transfection differs among genes, but gene expression usually recovers 96 to 120 hr after siRNA transfection.[22] Therefore, we added LPS to cells 4 days after siRNA transfection, before the gene expression recovered. The concentration of IL-10 in BMDM supernatants and cell viability were measured one day after LPS stimulation.

To adjust for plate-to-plate variability, control siRNAs were needed. As mentioned in the introduction, it was reported that Bcl3 inhibits the transcription of *IL-10*[10] and that Zfp36 degrades *IL-10* mRNA.[11] The supernatants of BMDMs transfected with either Bcl3 or the Zfp36 siRNA pool and stimulated with LPS had high IL-10 concentrations. Therefore, we deconvoluted these pools and re-assayed. We chose Zfp36 siRNA (D-041045-04) as one of the controls because it was the strongest IL-10 inducer. Several non-targeting siRNAs were prepared by GE Dharmacon, and we chose non-targeting siRNA (D-001210-02) as an additional control because it exhibited a degree of IL-10 production similar with that of cells not transfected with siRNA.

Primary screening for siRNAs that up- or down-regulate IL-10

An overview of the screening cascade, and relationships with the following figures and tables are shown in Fig. 1B. We screened the GE Dharmacon mouse siGENOME siRNA library (genome-wide; 19,061 genes). The normalized percentage of control (NPC), whose formula is presented in Supplemental Materials and Methods, was used for candidate gene selection. We set NPC >20 as the criterion for

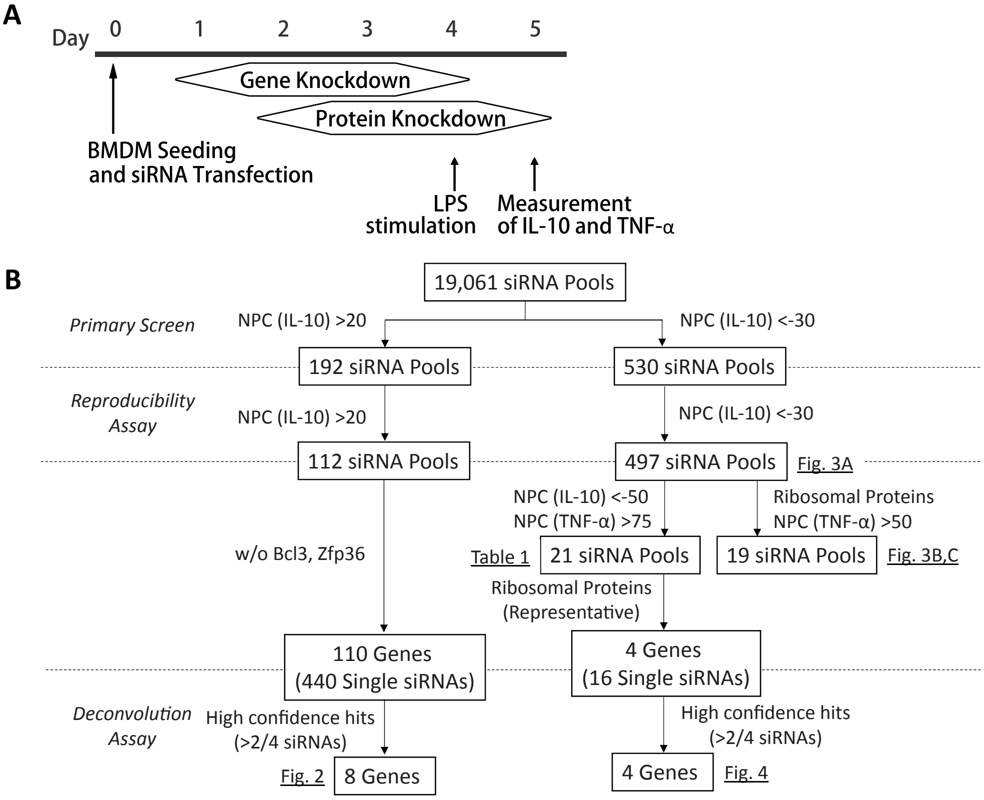


Fig. 1. Overview of screening. (***A***) Time sequence of experimental steps during the screening procedure. (***B***) Overview of screening cascade and relationships with the following figures and tables.

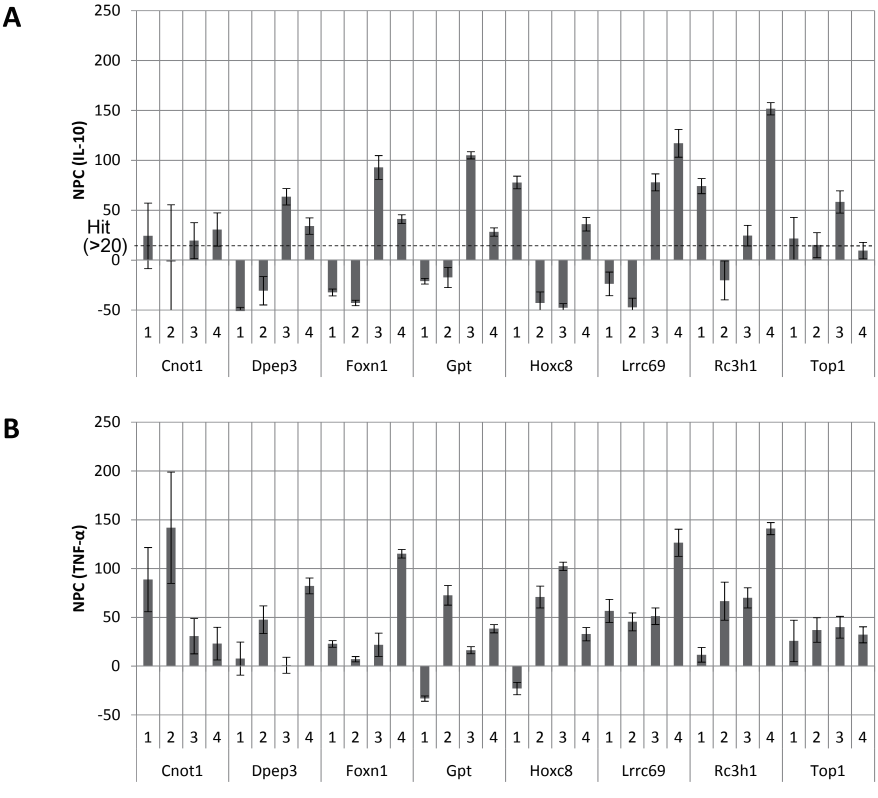


Fig. 2. Deconvolution assay for IL-10-up-regulating siRNAs. NPC (IL-10) (***A***) and NPC (TNF-α) (***B***) of each single siRNA are shown. Values are means ± SD (n=4).

IL-10-up-regulating hits because this criterion was roughly equal to the value of the average plus 3-times the standard deviation in "non-targeting siRNA". On the other hand, we set NPC <-30 as the criterion for IL-10-down-regulating hits because the number of IL-10-down-regulating siRNA pools was large, and we needed to narrow down the hit siRNA pools to a manageable size (Supplemental Fig. 1B). We disregarded siRNA pools that affected cell viability. The index of assay quality via this screen was acceptable (Supplemental Fig. 2).

Overall, 192 IL-10-up-regulating hits (NPC >20) and 530 IL-10-down-regulating hits (NPC <-30) were obtained (Fig. 1B), and they had no overt effects on cell viability. Indicative of a successful screen, we recovered Bcl3 and Zfp36 siRNA pools in up-regulating hits, and Tlr4 and Myd88 siRNA pools, which target the genes encoding the LPS receptor and its adaptor proteins, respectively, in down-regulating hits. Next, we assayed these 192 and 530 hits, among which 112 and 497 siRNA pools were reproducible, respectively (Fig. 1B). We again recovered Bcl3 and Zfp36 siRNA pools. We took 110 siRNA pools for further validation without Bcl3 and Zfp36 siRNA pools because their functions were known. In the reproducibility assay for IL-10-down-regulating hits, both IL-10 and TNF-α in the BMDM supernatants were measured at the same time; we focused on these data later.

Deconvolution assay for removal of off-target effect hits and evaluation of TNF-α production with IL-10-up-regulating siRNAs

The rate of off-target effects (OTEs) is generally high in RNAi screens.[23] OTEs were mainly derived from partial complementarity between seed regions (bases 2-8) of siRNAs and 3' untranslated regions (UTRs) of off-target genes. The deconvolution assay is the standard method when primary screens are performed on 3-4 siRNA pools/well. The siRNA pools scored with more than 2 siRNAs after deconvolution are generally regarded as high-confidence hits. Therefore, 110 up-regulating siRNA pools were deconvoluted to 440 single siRNAs, and these siRNAs were then assayed. In this screen, the cut-off value was set at NPC >20. Seventy-eight single siRNA hits were identified (Supplemental Table 3), and the number of siRNA pools scored with more than 2 siRNAs was 8 (Fig. 2A).

These 8 high-confidence hits included *Cnot1* (CCR4-NOT transcription complex subunit 1) and *Rc3h1* (RC3H1 ring finger and CCCH-type domains 1, Roquin-1), components of the CCR4-NOT complex, which binds to the stem loops of *ICOS* and *TNF-α* mRNAs and degrades them.[24] Our result suggested that the CCR4-NOT complex also binds and degrades *IL-10* mRNA.

In the deconvolution assay, both IL-10 and TNF-α in the BMDM supernatants were measured at the same time (Fig. 2B). For reference, cell viability data are shown in Supplemental Fig. 3A. Overall, no siRNAs significantly decreased TNF-α production. Therefore, we focused on other siRNAs that suppressed IL-10 production.

Evaluation of TNF-α production with IL-10-down-regulating siRNAs

As the number of IL-10-down-regulating siRNA pools was much larger than that of IL-10-up-regulating siRNA pools, it was difficult to carry out deconvolution assays for all of the down-regulating siRNA pools. Therefore, we analyzed the data for TNF-α production in 497 reproducible hits of IL-10-down-regulating siRNA pools in the reproducibility assay before performing deconvolution assays. We focused on inverse regulators of IL-10 and TNF-α from siRNA pools that met the criteria of NPC (IL-10) <-50 and NPC (TNF-α) >75 (Fig. 3A, Table 1). Interestingly, there were 7 siRNA pools targeting ribosomal proteins among the 21 siRNA pools listed in Table 1. Furthermore, the number of siRNA pools targeting ribosomal proteins among 497 reproducible hits in the IL-10-down-regulating siRNA pools in the reproducibility assay was 24. Among these 24 siRNA pools, only 1 siRNA pool *(Rps18)* caused low viability. The result of the 23 siRNA pools targeting ribosomal proteins is shown in Fig. 3B and C. Nineteen siRNA pools demonstrated relatively high TNF-α production (NPC (TNF-α) >50). For reference, there were 64 siRNA pools targeting ribosomal proteins included in the whole-genome library.

siRNA pools targeting *Rps3* have been reported as negative regulators of inflammatory signaling [25] and exhibited high TNF-α production in this study (Fig. 3C). We speculated that there were negative regulators of inflammatory signaling in ribosomal proteins other than Rps3. We performed a deconvolution assay for 4 representative siRNA pools *(Rpl4, Rpl11, Rpl35* and *Rps15a)* that caused high TNF-α production in siRNA pools targeting ribosomal proteins. Almost all of the single siRNAs demonstrated, not only low IL-10 production (Fig. 4A), but also high TNF-α production (Fig. 4B). In addition, these siRNAs did not exhibit cell toxicity (Supplemental Fig. 3B). Thus, these ribosomal proteins were high-confidence hits and their knockdown suppressed IL-10 production and enhanced TNF-α production in mouse BMDMs.

DISCUSSION

We carried out a genome-wide siRNA screen using BMDMs and picked up IL-10-up-regulating and down-regulating siRNA pools. To confirm whether these siRNA pools were high-confidence hits, deconvolution assays were conducted. In the deconvolution assay for IL-10-up-regulating siRNA pools, only 17.7% (78 out of 440) single siRNAs were active, and only 8 siRNA pools were identified as high-confidence hits, including siRNAs targeting 2 members of the CCR4-NOT complex, *Cnot1* and *Rc3h1*. In addition, siRNA pools targeting *Cnot3* and *Cnot10*, also members of the complex, had scores of 1 (Supplemental Table 3). In this context, these siRNAs were likely high-confidence hits. Therefore, we considered true hits to exist in siRNA pools that only scored 1. In contrast, 4 representative IL-10-down-regulating siRNA pools were assayed in the deconvolution

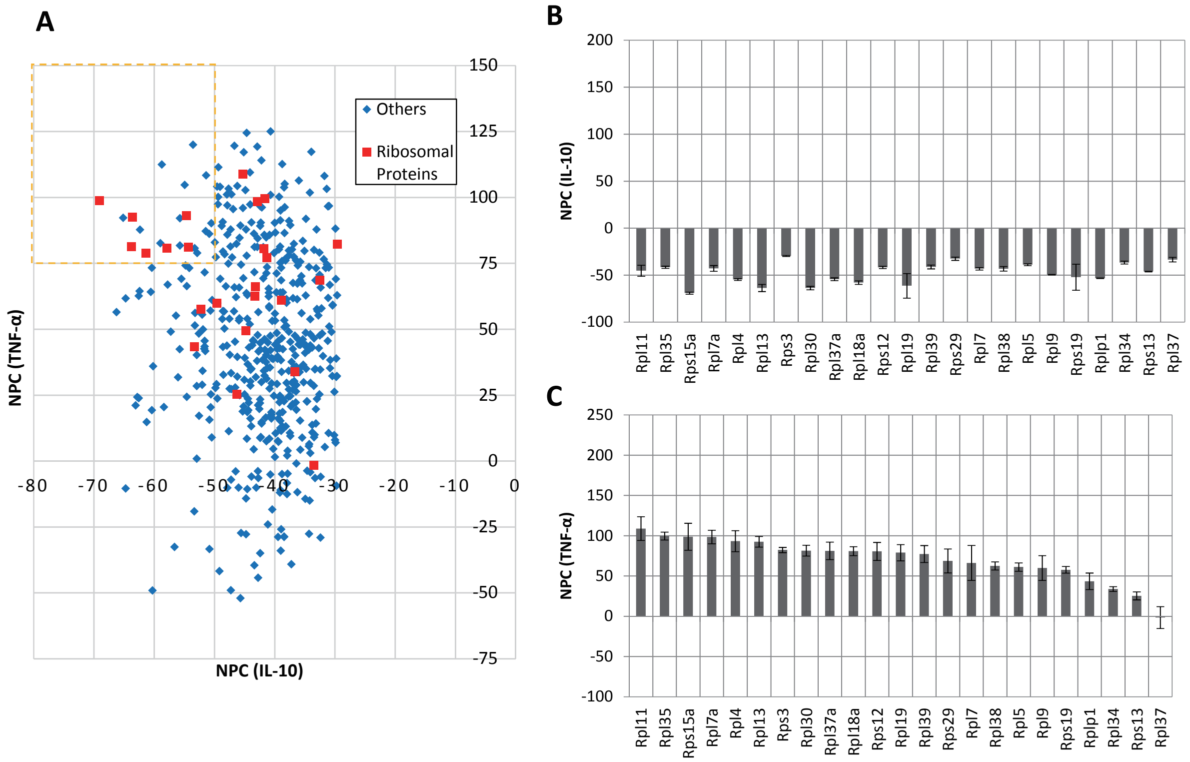


Fig. 3. IL-10 and TNF-α data of IL-10-down-regulating siRNA pools. (*A*) Relationships between IL-10 and TNF-α data of 497 reproducible hits in IL-10-down-regulating siRNA pools in the reproducibility assay. Each plot shows averages of quadruplicates. The data for siRNAs targeting ribosomal proteins are shown as red squares. siRNA pools within the orange dashed line met the criteria of NPC (IL-10) <-50 and NPC (TNF-α) >75. (*B*, *C*) The NPC (IL-10) (*B*) and NPC (TNF-α) (*C*) of siRNA pools targeting ribosomal proteins are shown in descending order of NPC (TNF-α). Values are means ± SD (n=4).

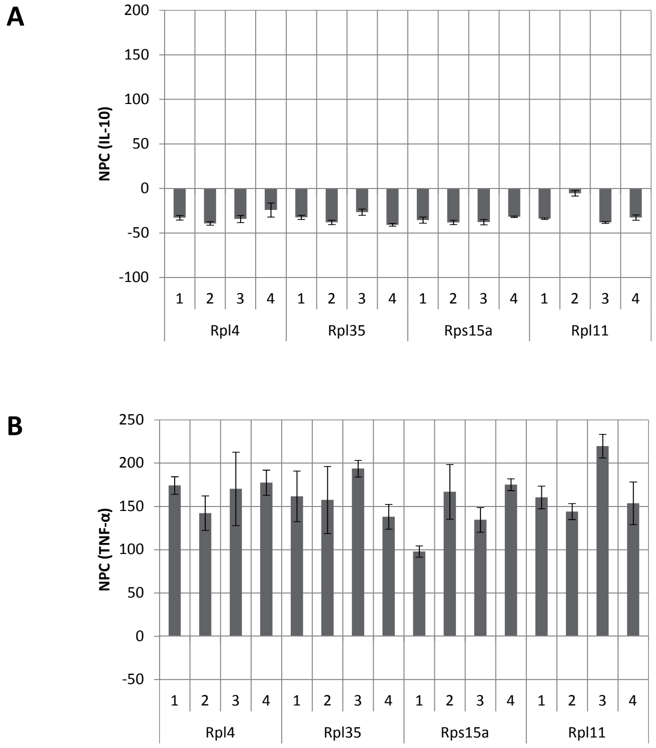


Fig. 4. Deconvolution assay for siRNA pools targeting 4 representative ribosomal proteins. The NPC (IL-10) (*A*) and NPC (TNF-α) (*B*) of each single siRNA are shown. Values are means ± SD (n=4).

format, and almost all of the single siRNAs were active (Fig. 4A). We speculated that this gap came not only from setting a stricter cut-off criterion for IL-10-down-regulating siRNAs than for IL-10-up-regulating siRNAs, but also from a higher Z' value between the "no LPS" and "non-targeting siRNA" groups than that between the "non-targeting siRNA" and "Zfp36 siRNA" groups (Supplemental Fig. 2C).

The deconvoluted single siRNAs from the 8 high-confidence hits of IL-10-up-regulating siRNA pools did not exhibit lower TNF-α production than "non-targeting siRNA". However, the siRNAs with relatively lower TNF-α production warrant further investigation as IL-10 inducers. For example, *Gpt* (glutamate-pyruvate transaminase), with a score of 2 (Fig. 2A), is an enzyme that catalyzes the reversible transamination of L-alanine and α-ketoglutarate to form pyruvate and L-glutamate.[26] Recent studies have found that feeding pyruvate into the TCA cycle and subsequent oxidative phosphorylation as a result of glucose metabolic change polarized macrophages towards the anti-inflammatory phenotype.[27, 28] Thus, the knockdown of Gpt may result in such metabolic change.

In this study, we focused on siRNAs targeting ribosomal proteins; however, other siRNAs that suppressed IL-10 and enhanced TNF-α production were also noteworthy. For example, *Pde1b* (phosphodiesterase 1B) was the target of siRNA that had low IL-10 production and the highest TNF-α

Table 1. The list of IL-10-down-regulating siRNA pools that met the criteria of NPC (IL-10) < 50 and NPC (TNF-α) >75 in reproducibility assays after the primary screen. The NPC (IL-10) and NPC (TNF-α) for each siRNA pool are shown. Cell viability of BMDMs transfected with siRNA pools relative to "non-targeting siRNA" is also shown. Values are means ± SD (n=4). The data are listed in descending order of NPC (TNF-α). Gene symbols of ribosomal proteins are underlined.

Duplex Catalog Number	Gene Targeted by siRNA pool	NPC (IL-10)	NPC (TNF-α)	Cell Viability ("non-targeting siRNA" = 100)
M-050497-01	*Zc3h13*	-53.6±14.8	120.0±5.5	105.3±2.7
M-051237-00	*1300002F13RIK*	-58.7±1.4	112.5±7.0	109.3±1.8
M-058402-01	*Slc34a2*	-51.4±2.5	108.4±14.3	119.1±9.1
M-048442-01	*Trim23*	-54.9±1.4	104.8±8.2	108.7±2.4
M-064970-01	*Rps15a*	-69.1±1.1	98.8±16.7	102.4±4.1
M-064743-01	*Rpl4*	-54.6±1.0	93.2±13.0	74.5±3.8
M-041275-01	*Rpl13*	-63.6±3.9	92.5±6.6	95.8±6.3
M-056497-01	*Prtg*	-65.1±1.9	92.3±12.5	107.6±2.4
M-051261-00	*Rnf110*	-55.7±1.3	92.1±10.1	117.3±0.4
M-040619-00	*Ulk2*	-50.5±3.0	90.3±0.7	97.5±2.2
M-064569-01	*Xpo1*	-62.3±2.2	87.8±14.5	76.8±3.7
M-051719-00	*Rab40c*	-51.4±3.5	87.3±13.7	75.4±3.4
M-051119-01	*Snip1*	-50.8±2.8	86.2±12.6	109.4±9.8
M-062442-00	*Ercc3*	-59.0±5.0	82.7±4.1	112.1±3.7
M-059360-01	*Mgrn1*	-55.7±1.4	81.8±11.6	94.2±1.4
M-055809-01	*Rpl30*	-63.8±1.8	81.4±6.7	83.6±5.3
M-062340-01	*Rpl37a*	-54.3±1.5	81.2±10.8	82.5±10.2
M-061716-01	*Rpl18a*	-57.9±1.9	80.9±5.6	108.0±1.8
M-068930-13	*Zfp551*	-53.3±4.2	80.8±4.3	102.8±3.7
M-063788-00	*Loxl1*	-52.8±1.9	78.9±10.8	117.5±0.6
M-046185-01	*Rpl19*	-61.4±13.0	78.9±10.1	82.4±4.7

production in this study (data not shown). *Pde1b2* is one of two *Pde1b* variants and is expressed in macrophages.[29] It is possible that Pde1b siRNA increases cAMP because Pde1b hydrolyzes cAMP and cGMP, and it was reported that Pde1b2 knockdown resulted in reduced cAMP levels in the monocyte cell line HL-60.[30] Elevating cAMP levels enhances IL-10 production and suppresses TNF-α production.[31] Therefore, our results regarding Pde1b siRNA may be a result of decreased levels of cAMP in BMDMs.

Ribosomal proteins compose ribosomes with ribosomal RNA and affect protein translation. The biogenesis of ribosomes is critical in controlling cell growth and proliferation. Therefore, dysregulation of this process may result in diseases such as cancer and metabolic disorders.[32] In addition, ribosomal proteins have extraribosomal functions distinct from ribosomal biogenesis. Extraribosomal functions are involved in tumorigenesis, immune signaling, and diseases in development.[25] Rps3 is a negative regulator of inflammatory signaling that acts by modulating NF-κB-targeting gene expression.[25] In this study, several siRNAs targeting ribosomal proteins demonstrated low IL-10 production and high TNF-α production (Fig. 4A, B). It was reported that 16 ribosomal proteins enhance p53 activity via MDM2 inhibition.[33] Among them, *Rpl5* and *Rpl11* were the targets of siRNAs that had low IL-10 production and high TNF-α production in this study. The role of p53 in inflammation as an NF-κB activity inhibitor has already been reported.[34] Macrophages from $p53^{-/-}$ mice produced greater amounts of pro-inflammatory cytokines in response to LPS than those from $p53^{+/+}$ mice.[35] Therefore, Rpl5 and Rpl11 siRNAs may inhibit p53 activity, followed by enhancement of the production of the pro-inflammatory cytokine TNF-α. In addition, it was reported that casein kinase 2 beta subunit (Csnk2b), a regulatory subunit of casein kinase 2, which was the target of the siRNA scoring 1 in the deconvolution assay of IL-10-up-regulating siRNAs that exhibited low TNF-α production in this study (Supplemental Table 3), binds and phosphorylates Rpl5.[36, 37] Phosphorylation of ribosomal proteins may also be involved in the regulation of cytokine balance. Other ribosomal proteins that were targets of siRNAs shown in Fig. 3B and C were identified; however, elucidating the mechanisms of these ribosomal proteins and their relationships with cytokine production in BMDMs requires further studies. Regarding the balance between production of IL-10 and TNF-α, it was reported that rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), reduced IL-10 expression and enhanced TNF-α expression induced by LPS in macrophages.[38] mTORC1 controls the synthesis of ribosomal proteins, which is one of the steps in ribosome biogenesis controlled by mTORC1.[39] Taken together with our data, the effects of rapamycin mentioned above may be due to inhibition of ribosome biogenesis.

In conclusion, we found that the knockdown of several ribosomal proteins resulted in low IL-10 production and high TNF-α production in BMDMs. This study may aid future research on diseases caused by dysfunction of ribosomal proteins.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1 Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol. 2010; 10 : 427-439.

2 Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. Front Immunol. 2014; 5 : 491.

3 Hayashi A, Sato T, Kamada N, *et al*. A single strain of Clostridium butyricum induces intestinal IL-10-producing macrophages to suppress acute experimental colitis in mice. Cell Host Microbe. 2013; 13 : 711-722.

4 Kanai T, Mikami Y, Hayashi A. A breakthrough in probiotics: Clostridium butyricum regulates gut homeostasis and anti-inflammatory response in inflammatory bowel disease. J Gastroenterol. 2015; 50 : 928-939.

5 Sasayama T, Tanaka K, Mizowaki T, *et al*. Tumor-Associated Macrophages Associate with Cerebrospinal Fluid Interleukin-10 and Survival in Primary Central Nervous System Lymphoma (PCNSL). Brain Pathol. 2016; 26 : 479-487.

6 Sawada L, Nagano Y, Hasegawa A, *et al*. IL-10-mediated signals act as a switch for lymphoproliferation in Human T-cell leukemia virus type-1 infection by activating the STAT3 and IRF4 pathways. PLoS Pathog. 2017; 13 : e1006597.

7 Ma C, Horlad H, Pan C, *et al*. Stat3 inhibitor abrogates the expression of PD-1 ligands on lymphoma cell lines. J Clin Exp Hematop. 2017; 57 : 21-25.

8 Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010; 10 : 170-181.

9 Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010; 11 : 373-384.

10 Riemann M, Endres R, Liptay S, Pfeffer K, Schmid RM. The IkappaB protein Bcl-3 negatively regulates transcription of the IL-10 gene in macrophages. J Immunol. 2005; 175 : 3560-3568.

11 Stoecklin G, Tenenbaum SA, Mayo T, *et al*. Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. J Biol Chem. 2008; 283 : 11689-11699.

12 MacKenzie KF, Clark K, Naqvi S, *et al*. PGE(2) induces macrophage IL-10 production and a regulatory-like phenotype via a protein kinase A-SIK-CRTC3 pathway. J Immunol. 2013; 190 : 565-577.

13 Ozanne J, Prescott AR, Clark K. The clinically approved drugs dasatinib and bosutinib induce anti-inflammatory macrophages by inhibiting the salt-inducible kinases. Biochem J. 2015; 465 : 271-279.

14 Mohr S, Bakal C, Perrimon N. Genomic screening with RNAi: results and challenges. Annu Rev Biochem. 2010; 79 : 37-64.

15 Mohr SE, Perrimon N. RNAi screening: new approaches, understandings, and organisms. Wiley Interdiscip Rev RNA. 2012; 3 : 145-158.

16 Ley S, Weigert A, Heriche JK, *et al*. RNAi screen in apoptotic cancer cell-stimulated human macrophages reveals co-regulation of IL-6/IL-10 expression. Immunobiology. 2013; 218 : 40-51.

17 Ying W, Cheruku PS, Bazer FW, Safe SH, Zhou B. Investigation of macrophage polarization using bone marrow derived macrophages. J Vis Exp. 2013; 76 : e50323.

18 Zhang X, Goncalves R, Mosser DM. The isolation and characterization of murine macrophages. Curr Protoc Immunol. 2008; 111 : 14.1.1-14.1.16.

19 Marim FM, Silveira TN, Lima DS, Jr., Zamboni DS. A method for generation of bone marrow-derived macrophages from cryopreserved mouse bone marrow cells. PLoS One. 2010; 5 : e15263.

20 Makita N, Hizukuri Y, Yamashiro K, Murakawa M, Hayashi Y. IL-10 enhances the phenotype of M2 macrophages induced by IL-4 and confers the ability to increase eosinophil migration. Int Immunol. 2015; 27 : 131-141.

21 Zhang X, Edwards JP, Mosser DM. The expression of exogenous genes in macrophages: obstacles and opportunities. Methods Mol Biol. 2009; 531 : 123-143.

22 Mocellin S, Provenzano M. RNA interference: learning gene knock-down from cell physiology. J Transl Med. 2004; 2 : 39.

23 Schultz N, Marenstein DR, De Angelis DA, *et al*. Off-target effects dominate a large-scale RNAi screen for modulators of the TGF-beta pathway and reveal microRNA regulation of TGFBR2. Silence. 2011; 2 : 3.

24 Leppek K, Schott J, Reitter S, *et al*. Roquin promotes constitutive mRNA decay via a conserved class of stem-loop recognition motifs. Cell. 2013; 153 : 869-881.

25 Zhou X, Liao WJ, Liao JM, Liao P, Lu H. Ribosomal proteins: functions beyond the ribosome. J Mol Cell Biol. 2015; 7 : 92-104.

26 Adeva M, Gonzalez-Lucan M, Seco M, Donapetry C. Enzymes involved in l-lactate metabolism in humans. Mitochondrion. 2013; 13 : 615-629.

27 Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res. 2015; 25 : 771-784.

28 Haschemi A, Kosma P, Gille L, *et al*. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. Cell Metab. 2012; 15 : 813-826.

29 Bender AT, Ostenson CL, Wang EH, Beavo JA. Selective up-

regulation of PDE1B2 upon monocyte-to-macrophage differentiation. Proc Natl Acad Sci U S A. 2005; 102 : 497-502.

30 Bender AT, Beavo JA. PDE1B2 regulates cGMP and a subset of the phenotypic characteristics acquired upon macrophage differentiation from a monocyte. Proc Natl Acad Sci U S A. 2006; 103 : 460-465.

31 Eigler A, Siegmund B, Emmerich U, *et al*. Anti-inflammatory activities of cAMP-elevating agents: enhancement of IL-10 synthesis and concurrent suppression of TNF production. J Leukoc Biol. 1998; 63 : 101-107.

32 Wang W, Nag S, Zhang X, *et al*. Ribosomal proteins and human diseases: pathogenesis, molecular mechanisms, and therapeutic implications. Med Res Rev. 2015; 35 : 225-285.

33 Narla A, Ebert BL. Ribosomopathies: human disorders of ribosome dysfunction. Blood. 2010; 115 : 3196-3205.

34 Shao J, Fujiwara T, Kadowaki Y, *et al*. Overexpression of the wild-type p53 gene inhibits NF-kappaB activity and synergizes with aspirin to induce apoptosis in human colon cancer cells. Oncogene. 2000; 19 : 726-736.

35 Liu G, Park YJ, Tsuruta Y, Lorne E, Abraham E. p53 Attenuates lipopolysaccharide-induced NF-kappaB activation and acute lung injury. J Immunol. 2009; 182 : 5063-5071.

36 Kim JM, Cha JY, Marshak DR, Bae YS. Interaction of the beta subunit of casein kinase II with the ribosomal protein L5. Biochem Biophys Res Commun. 1996; 226 : 180-186.

37 Park JW, Bae YS. Phosphorylation of ribosomal protein L5 by protein kinase CKII decreases its 5S rRNA binding activity. Biochem Biophys Res Commun. 1999; 263 : 475-481.

38 Baker AK, Wang R, Mackman N, Luyendyk JP. Rapamycin enhances LPS induction of tissue factor and tumor necrosis factor-alpha expression in macrophages by reducing IL-10 expression. Mol Immunol. 2009; 46 : 2249-2255.

39 Iadevaia V, Liu R, Proud CG. mTORC1 signaling controls multiple steps in ribosome biogenesis. Semin Cell Dev Biol. 2014; 36 : 113-120.

Supplemental Materials and Methods

Mice.

C57BL/6J mice were purchased from Charles River Laboratories Japan (Yokohama, Japan). All experiments were approved by the Ethics Committee for Animal Experiments of Asubio Pharma Co., Ltd. (Approval Number: AEK-12-216).

Preparation of BMDMs.

Bone marrow cells from hind legs of 6-8-week-old male C57BL/6J mice were washed in RPMI 1640 medium, centrifuged, and the cell pellet was resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, 10 µg/mL of streptomycin, and 90 ng/mL of mouse M-CSF (R&D Systems, Minneapolis, MN, USA). After 3 days of incubation at 37°C in a 5% CO_2 atmosphere in plastic tissue culture dishes, the cells were given fresh media containing M-CSF. On day 7 of culture, the dishes were washed, and adherent cells were collected by scraping. Cells were cryopreserved with CELLBANKER 1 (Nippon Zenyaku Kogyo Co. Ltd., Tokyo, Japan).

Selection of transfection reagents.

The non-targeting siRNA pool, Adgre1 siRNA pool, or Itgam siRNA pool (D-001206-13, M-043323-01, and M-046775-01, respectively; siGENOME, GE Dharmacon, Lafayette, CO, USA) were dispensed (500 nM, 2 µL) onto white ViewPlate-384 TC plates (PerkinElmer, Waltham, MA, USA). Transfection reagents (Supplemental Table 2) were diluted with Opti-MEM (Thermofisher Scientific, Waltham, MA, USA), and 20 µL of the reagents were added to the wells containing the siRNAs. Cryopreserved BMDMs were dissolved in DMEM (Nacalai tesque, Kyoto, Japan) supplemented with 0.1% heat-inactivated fetal bovine serum (Nichirei Biosciences, Tokyo, Japan). The BMDMs were seeded (1.5×10^4 cells, 10 µL/well) and incubated for 48 hr at 37°C in a 5% CO_2 atmosphere. Total RNA was isolated using the RNeasy micro kit (Qiagen, Hilden, Germany). cDNA was synthesized from total RNA using the QuantiTect cDNA Reverse Transcript Kit (Qiagen). Quantitative PCR was performed using the TaqMan Gene Expression Master Mix (Thermofisher Scientific), PrimeTime qPCR Assays (Integrated DNA Technologies, Coralville, IA, USA) consisting of gene-specific TaqMan primers and probes, and the 7900HT Fast Real-Time PCR System (Thermofisher Scientific). Expression was normalized to that of β-actin, and expression levels were analyzed by the $2^{-\Delta\Delta}$Ct method. Cell viability was measured using the CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI, USA), where the cell number generally correlates with RLU.

Primary genome-wide siRNA screen and reproducibility assay.

The siRNA screen was performed using the mouse siGENOME siRNA library (G-015005-01; Mouse Genome, GE Dharmacon) in singlicate. This library contains 19,061 siRNA pools (SMARTpools): each pool contains four distinct siRNAs against non-overlapping regions of the target gene mRNA. This siRNA library was diluted to 500 nM with the siRNA buffer (GE Dharmacon) and 2 µL of the diluted siRNAs were dispensed onto white ViewPlate-384 TC plates (PerkinElmer). Cryopreserved BMDMs were thawed and suspended in DMEM (Nacalai tesque) supplemented with 0.1% heat-inactivated fetal bovine serum (Nichirei Biosciences). HiPerFect transfection reagent (Qiagen) was diluted 25-times with Opti-MEM (Thermofisher Scientific). Ten microliters of the diluted HiPerFect transfection reagent was aliquoted into each well containing the siRNA using mini-Gene LD-01 (Biotec, Tokyo, Japan). After a 10-min incubation, BMDMs were plated using mini-Gene LD-01 (1.5×10^4 cells, 10 µL/well). The plates were briefly spun at 1,000 rpm and placed at 37°C in a 5% CO_2 atmosphere. At this point, the siRNA concentration was 45 nM. The next day, 10 µL of DMEM supplemented with 31.8% (final dilution being 10%) heat-inactivated fetal bovine serum was added using mini-Gene LD-01. After 3 days, the cells were stimulated with 100 ng/mL of lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA) for 24 hr.

Several control wells were set in each plate. The “non-targeting siRNA” cells were transfected with non-targeting siRNA (D-001210-02; GE Dharmacon) and stimulated with LPS (16 wells/plate). The “Zfp36 siRNA” cells were transfected with mouse Zfp36 siRNA (D-041045-04; GE Dharmacon) and stimulated with LPS (8 wells/plate). The “no siRNA” cells were treated with the transfection reagent and LPS – identical to the “non-targeting siRNA” cells, but without siRNA (4 wells/plate). The “no LPS” cells were transfected with non-targeting siRNA (D-001210-02; GE Dharmacon), but were not stimulated with LPS (14 wells/plate). Concentrations of these siRNAs were the same as those of the siRNA libraries described above. The plate layouts are shown in Supplemental Fig. 1A.

The relative concentration of IL-10 was measured using the AlphaLISA mouse IL-10 immunoassay kit (PerkinElmer). EDR-384SII (Biotec) was used for liquid handling. Briefly, 4 µL of culture supernatants was transferred into assay plates (white ProxiPlate-384 Plus plates; PerkinElmer). Four microliters of the mixture containing acceptor beads and anti-IL-10 antibodies were added into the assay plates and incubated for 1 hr at room temperature. The assay plates were incubated in the dark at room temperature for 30 min, followed by the addition of 4 µL of the solution containing donor beads. The assay plates were read using an EnVision plate reader (PerkinElmer). The obtained signal data were normalized to remove plate-to-plate variation. The normalized percentage of control (NPC) was calculated using the following formula:

$$\text{NPC}=\frac{(\textit{each value - average of "non - targeting siRNA"})}{(\textit{average of "Zfp36 siRNA" - average of "non-targeting siRNA"})}\times 100$$

Cell viability was also measured using the CellTiter-Glo luminescent cell viability assay (Promega), and calculated as

Supplemental Table 1. Details of single siRNAs used in deconvolution assays.

Gene Symbol	#	Duplex Catalog Number	siRNA Sequence
Cnot1	1	D-167425-01	CAAUAAGGUUCUCGGUAUA
	2	D-167425-02	GAGGAUGACAAUCGAGAAA
	3	D-167425-03	CCGACUUACUGCUGCGAUA
	4	D-167425-04	UGUUAGAGGCUUACGUUAA
Rc3h1	1	D-044230-01	GAACAUGACUCCCAGAUAG
	2	D-044230-02	UCUCAUAUGCAGUCUAUUA
	3	D-044230-03	GAAUUUGAACCGACUAAGA
	4	D-044230-04	GUACGGUAGUGCAUGGAUU
Top1	1	D-047567-01	GCACUGUACUUCAUUGAUA
	2	D-047567-02	CGAAUAUACUACUAAGGAA
	3	D-047567-03	GUAGCUACAUUCUUUGCAA
	4	D-047567-04	UAGCAAAGACGCAAAGGUU
Dpep3	1	D-046022-01	GAAUAUUCCUGAUGACAUA
	2	D-046022-02	GAUCUGAGUUCAUUGGGAU
	3	D-046022-03	GCAGAAAGCGCCACAAAAU
	4	D-046022-04	GCUAACGUGUCCACUGUAG
Foxn1	1	D-046191-01	GAACAAGCAUGCUAACUUC
	2	D-046191-02	GGACUGACCUGGAUGCUAU
	3	D-046191-03	GCAGAGGCUUGGUGCAAUA
	4	D-046191-04	GCACGAUACUCUACUGCCA
Gpt	1	D-041549-01	GAACGCAUCUUGCAGGCAU
	2	D-041549-02	GAGGUUAUCCGUGCCAAUA
	3	D-041549-03	GAGGAUGUGGCGCAAUAUA
	4	D-041549-04	CUGGGUGCCUAUAGCAUUA
Hoxc8	1	D-043646-01	GCGCAGCGGUCGACAAACU
	2	D-043646-02	GAACCGGCCUAUUACGACU
	3	D-043646-03	GAAGCCAUGCGCUGGUGUA
	4	D-043646-04	AGGCGAGCGUGGUGCAAUA
Lrrc69	1	D-050166-01	GCUGAAAGAUUAUUGGUAA
	2	D-050166-02	ACACUAAGAUCCUGACUUU
	3	D-050166-03	GCAAGAGGAUUACCAAGAU
	4	D-050166-04	ACUUAAGCCUCAACCGUAA
Rpl4	1	D-064743-01	GUUCAAAGCUCCCAUUCGA
	2	D-064743-02	CAGGGUGCCUUUGGAAAUA
	3	D-064743-03	CUGCUUCCCUCAAGAGUAA
	4	D-064743-04	GUCUAAAGGUCAUCGUAUU
Rpl35	1	D-048193-01	GAGCGGCUGUAUCCUCUGC
	2	D-048193-02	GCAAGUAUGCAGUCAAGGC
	3	D-048193-03	UCGCAAGUCUAUCGCCCGA
	4	D-048193-04	GCGUCCAAGCUCUCCAAGA
Rps15a	1	D-064970-01	CGGAUACAUUGGUGAAUUU
	2	D-064970-02	AGUCAUCGUUCGGUUCUUA
	3	D-064970-03	GGAAGAUUGUUGUGAACCU
	4	D-064970-04	GCCCUAGAUUUGAUGUUCA
Rpl11	1	D-064528-01	GCAGAAGUACGAUGGAAUC
	2	D-064528-02	GAGUAUGAGUUGCGGAAAA
	3	D-064528-03	AAACACAGAAUCAGCAAGG
	4	D-064528-04	GGAUCUACGGCCUGGACUU

the ratio of RLU of each siRNA pool-treated well to the average RLU of “non-targeting siRNA”. Briefly, part of the supernatant in culture plates was discarded, and 10 µL of the supernatant was left. After equilibration to room temperature, 5 µL of CellTiter-Glo substrate was added into the culture plates. The culture plates were read using an EnVision plate reader (PerkinElmer).

For reproducibility confirmation, hit siRNA pools were selected from the siRNA library and assayed as described above, but in quadruplicate and with additional measurement of TNF-α using the AlphaLISA mouse TNF-α immunoassay kit (PerkinElmer).

Supplemental Table 2. Optimization of siRNA transfection.

Reagent Name	Company Name	Cat. No.	Times Diluted with Opti-MEM	Relative Gene Expression (*Adgre1*)	Relative Gene Expression (*Itgam*)	Viability (non-targeting siRNA)
Oligofectamine	Thermofisher Scientific	*12252011*	100	0.58	0.88	0.69
			300	1.20	1.41	1.14
siPORT Amine	Thermofisher Scientific	*AM4502*	44	0.05	0.20	0.64
			133	0.38	0.68	1.04
GenMute	SignaGen	*SL100568*	33	0.14	0.32	0.43
			100	0.66	0.97	0.98
Lullaby	OZ Biosciences	*LL70500*	17	0.49	0.55	1.09
			50	0.55	0.81	1.03
X-tremeGENE siRNA	Roche	*4476093001*	27	0.35	0.76	0.49
			80	0.87	0.72	1.12
RNAiMAX	Thermofisher Scientific	*13778030*	44	0.07	0.36	0.51
			133	0.19	0.67	1.17
HiPerFect	Qiagen	*301705*	13	0.12	0.59	0.17
			25	0.12	0.15	0.83
INTERFERin	PolyPlus	*409-01*	50	0.13	0.52	0.75
			100	0.15	0.41	0.97
Promofectin-Macrophage	PromoKine	*PK-CT-2000-MAC*	33	0.25	0.50	0.52
			67	0.85	0.93	0.99
GeneSilencer	Genlatis	*T500020*	27	0.60	0.73	0.85
			40	0.71	0.88	1.28
Dharmafect4	GE Dharmacon	*T-2004*	67	0.08	0.22	0.70
			100	0.07	0.21	0.83
TransIT-siQUEST	MirusBio	*MIR2114*	73	0.03	0.15	1.10
			220	0.26	0.46	0.97
TransIT-TKO	MirusBio	*MIR2154*	44	0.08	0.17	1.13
			132	0.88	1.03	0.99

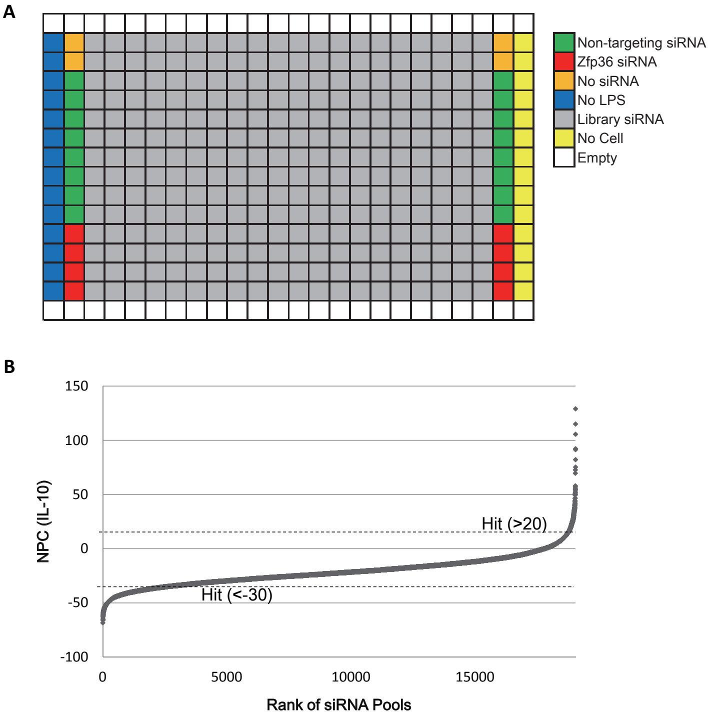
To achieve the best knockdown conditions, we examined 13 transfection reagents with Adgre1 (F4/80) and Itgam (Mac-1) siRNAs (siGENOME; GE Dharmacon), as they are common macrophage markers. In this examination, we used the reverse transfection method, adding cells to pre-plated transfection reagent–siRNA complexes. Thirteen transfection reagents were examined at more than 2 concentrations; the results at 2 concentrations are shown. Expression levels of *Adgre1* and *Itgam* in Adgre1 and Itgam siRNA-transfected BMDMs relative to those in non-targeting siRNA pool-transfected BMDMs are shown, respectively. Simultaneously, the cell viability of non-targeting siRNA pool-transfected BMDMs relative to that of non-transfected BMDMs was calculated using the CellTiter-Glo luminescent luciferase assay. Values are means (n = 2). We chose conditions in which the knockdown efficiency for both *Adgre1* and *Itgam* mRNA was >80%, with concomitant high cell viability (>80%). Values marked with red met these criteria. Among these transfection reagents, HiPerFect, TransIT-siQUEST, and TransIT-TKO induced sufficient mRNA knockdown (>80%) with concomitant high cell viability (>80%) at a certain concentration. Further examination revealed that the appearance of BMDMs transfected with TransIT-TKO was erroneous and the TNF-α concentration in the supernatant of BMDMs transfected with TransIT-siQUEST was low. Thus, we chose the HiPerFect transfection reagent for the siRNA screen.

Supplemental Table 3. The results of 78 IL-10-up-regulating single siRNAs that met the criteria of NPC (IL-10) >20 in the deconvolution assay.

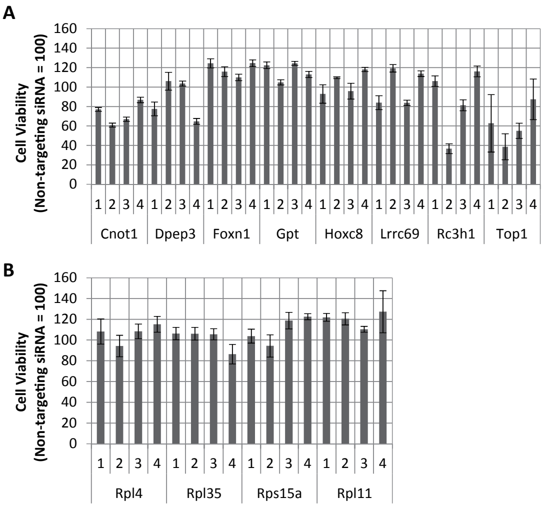
Duplex Catalog Number	siRNA Sequence	Gene Targeted by Single siRNA	NPC (IL-10)	NPC (TNF-α)	Cell Viability (non-targeting siRNA = 100)
D-062645-02	GGACAGCGUUCACUGCACA	*Lbx2*	185.7± 7.2	125.1± 7.7	116.6± 3.6
D-052462-04	UCGUUUUAUAUGUGCAAUAG	*Vmn1r200*	152.9±16.5	-1.8± 5.0	80.5± 7.9
D-044230-04	GUACGGUAGUGCAUGGAUU	*Rc3h1*	151.7± 4.9	141.0± 6.2	116.1± 5.6
D-042356-04	GGAAGUUAAUCGGGUUGGA	*Cog1*	146.6±17.1	-79.8± 6.2	108.8± 6.7
D-054082-02	GAUGAGGAGGGCCAAGAUA	*D2Wsu81e*	146.0±24.4	22.2±13.2	117.8± 6.2
D-060444-04	GGAACUACGCUUGCAAUAG	*Bub3*	128.4±14.2	-8.6± 9.6	64.9± 2.8
D-050319-03	GCGGAAAAGUGUGCAAUAA	*Cd244*	120.6±11.7	2.9± 7.2	76.2± 2.9
D-050166-04	ACUUAAGCCUCAACCGUAA	*Lrrc69*	117.0± 0.5	126.5±13.9	113.7± 2.9
D-041549-03	GAGGAUGUGGCGCAAUAUA	*Gpt*	105.1±16.9	16.4± 3.6	124.4± 2.0
D-040614-02	CCAGCUAACUCCCAAGAUA	*Mast2*	99.0±10.2	-1.4± 5.4	128.2± 3.3
D-066189-10	GAACACGAAUCCCAGCUCG	*Hoxc11*	96.9±20.3	28.4± 9.5	89.7±12.9
D-044848-02	CGAUGAAGCUCCAGCCAAA	*Ercc8*	95.5± 5.0	94.1± 7.3	93.9± 4.7
D-046191-03	GCAGAGGCUUGGUGCAAUA	*Foxn1*	92.8±18.3	21.9±11.9	109.9± 3.4
D-056518-02	AGAACGAGACUGGCAAUAA	*Cnot10*	91.6±12.1	-22.5± 6.9	95.4± 9.3
D-043721-04	CGAACCACCCGAGCAAUAA	*Mbd2*	90.9± 7.1	22.0± 9.5	84.6± 9.3
D-040194-03	GCAGGGUAGUUGAGCCAAA	*Hnrnpa2b1*	78.4±16.4	27.0± 4.2	110.2± 7.8
D-050166-03	GCAAGAGGAUUACCAAGAU	*Lrrc69*	77.9± 8.5	51.2± 8.5	83.7± 2.8
D-043646-01	GCGCAGCGGUCGACAAACU	*Hoxc8*	77.8± 5.8	-23.0± 6.3	92.9± 9.5
D-160340-12	GCCGCAAGGGGUAGAGGAA	*Rhox3h*	75.3± 9.8	-31.9± 3.6	102.4±10.0
D-050031-17	CCGCAGUCUGAGGGCCAAA	*C2cd4d*	74.9±14.2	97.6± 9.4	101.5±10.8
D-044230-01	GAACAUGACUCCCAGAUAG	*Rc3h1*	74.1±16.9	11.6± 7.5	106.1± 5.5
D-062583-04	CUAAGUGGCCUGACCGUAU	*Ppp6c*	70.0±11.4	97.4±10.6	116.2± 3.6
D-062857-02	GCACAGAAUUAUAGCCAAU	*Tinag*	64.8± 6.2	63.3± 5.0	106.8± 5.9
D-040137-03	GAAUGAACCUGAAAGCAAA	*Mark2*	64.4± 4.2	36.9± 7.5	120.9± 4.8
D-046022-03	GCAGAAAGCGCCACAAAAU	*Dpep3*	63.5±11.0	1.0± 8.2	103.6± 2.6
D-054441-02	GAUUGAAGUUCUACAAGAU	*Sulf1*	61.6±14.6	-12.5± 3.6	81.4± 7.8
D-040645-02	GGAAGCAGGUCACCAAGAU	*Trhr*	61.3±36.1	0.8±11.6	73.3±20.2
D-062997-04	CGUUAGCUCUGGAGGAUAU	*Atg10*	59.2± 2.6	42.6± 6.4	145.5± 2.4
D-041944-03	GUUCAUCCGCGGAGCCAAA	*Hpca*	58.5±13.1	71.7± 7.0	110.4± 3.5
D-047567-03	GUAGCUACAUUCUUUGCAA	*Top1*	58.3± 8.7	39.9±11.1	55.0± 7.8
D-065241-02	AGACUCAGCAGCGCAAUAA	*Opa3*	55.7±18.1	-9.0± 7.6	63.0± 4.3
D-063661-03	GGUAUUCGCUUUGAUCCGG	*Rbpms2*	55.4±11.5	49.8±13.2	101.3± 1.7
D-041546-02	GGAUGAGGGUUCAGCCAGA	*Smug1*	49.7± 5.8	-7.0± 2.6	127.1± 2.0
D-049417-04	GCAGGGAGACUUUGGCUAC	*Csnk2b*	49.5± 3.3	-34.4± 6.1	118.0± 5.6
D-043338-04	GCUCAUGUAAGGUGCAAUU	*Phex*	48.9± 6.3	18.7± 5.0	129.4± 5.2
D-049687-01	GGACAACCCUCAUCUGCAA	*Cyp2d40*	48.2± 9.3	94.8± 2.8	115.0± 4.3
D-066783-13	GGACAUUGUUCCACUGCAU	*Cyp2d12*	45.0± 9.7	57.5± 3.5	113.5± 4.3
D-047529-02	GGAAAUGGCACCUGGAAUG	*Eif4enif1*	43.6±10.7	86.0± 6.4	90.8± 3.3
D-042714-01	UAACCCGGGUGGCCAAAUU	*Pctp*	43.4±13.2	54.4± 6.2	109.0± 1.4
D-050694-04	GGACCAGCUCAGAGAGGGA	*Il27*	42.3±16.2	8.2± 7.2	111.2± 6.1

D-048732-03	UGACGGUGCUGCGCAAGAA	*Edf1*	41.3± 9.9	76.7± 7.5	93.7± 6.3
D-056012-03	GCACUACUCUGCAAGCAAG	*Eci2*	41.1± 4.6	5.5± 4.9	76.9± 6.6
D-046191-04	GCACGAUACUCUACUGCCA	*Foxn1*	41.1± 4.9	115.2± 4.4	124.5± 3.4
D-045692-02	GGGAAGGCCUUUAGUUUGA	*Zfp397*	41.1± 9.8	0.8±15.1	136.0±20.4
D-042262-04	GGCCGACGCCAAAGAGGAA	*Olig1*	38.3± 3.2	-5.9± 4.8	132.0± 8.2
D-045887-04	GUACAGAACAGGCACAUCA	*Spata31*	38.2±10.9	129.6±54.5	79.2± 6.4
D-063560-03	CGGAGAGGCAUCAGUUUCA	*Spryd4*	37.8± 5.7	-43.9± 2.6	116.2± 5.0
D-043646-04	AGGCGAGCGUGGUGCAAUA	*Hoxc8*	36.0± 8.4	32.8± 6.8	118.1± 2.2
D-051817-02	GCGAUGCUGUGAAGUACAA	*C8b*	35.3± 3.9	47.3± 6.3	92.3± 6.3
D-059782-02	GAAGCGAGGCGGCACAUCA	*Zhx2*	35.3± 4.1	98.4±10.1	99.9± 1.9
D-042946-02	CGGAACAGCUGAAUGCACA	*Hic1*	35.2±10.1	30.2± 7.4	124.8± 2.4
D-162305-09	GCAGAAAUGUACAGCCAAA	*Vmn2r54*	35.0± 2.2	76.3±14.0	118.8± 5.7
D-046022-04	GCUAACGUGUCCACUGUAG	*Dpep3*	34.1±11.0	82.3± 8.2	64.7± 3.0
D-044233-04	GGUCAAUGAACCAGCCAAA	*Ccna1*	33.4± 1.4	77.8±10.4	105.9± 5.7
D-047681-04	UCGCAGACAUAUUUGGGUU	*Vmn1r15*	32.0± 8.0	53.1±12.9	112.9± 4.4
D-046224-04	GGUAAUGCUUAAGGCAAUU	*2700060E02Rik*	31.3± 7.2	3.8± 8.9	113.6± 9.6
D-167425-04	UGUUAGAGGCUUACGUUAA	*Cnot1*	30.7± 6.7	23.1±16.7	86.7± 2.8
D-043951-17	GCAUAGGUGGACAGCCGAA	*Tnfrsf9*	30.6±11.1	30.7±17.2	105.8±11.5
D-056406-02	GCAACGAAGUGGCCAAAUU	*Pogz*	29.7± 9.3	59.6± 8.3	87.4±13.2
D-061424-04	GCUACGAGGAGCAGCCGAA	*Tmem231*	29.4± 7.7	47.7±12.2	121.4± 0.4
D-057458-01	GGACAAAUAUGCAGCCGAA	*Socs4*	29.2± 3.2	-2.5±11.4	105.5±11.7
D-056640-06	CUAAGCAGACCGCUCGCAA	*Hist1h3c*	28.6± 7.8	55.0± 9.5	112.8± 2.0
D-041549-04	CUGGGUGCCUAUAGCAUUA	*Gpt*	28.2±14.3	38.4± 4.2	112.9± 3.1
D-041723-01	GAGACCAGCUCGAGCAAGU	*Kcnb1*	26.9±10.1	75.1±14.9	109.1± 1.3
D-045984-01	GGAAAGAGCUACUGGGUUA	*Grm8*	26.9±21.7	85.0± 4.0	111.6± 2.4
D-052632-04	CAACCAGUCUAGCAGUACA	*Cnot3*	26.5± 5.7	77.0±14.0	71.6± 2.6
D-054929-02	GCGGCAACCUCAUGUACAA	*Slc32a1*	26.1± 2.9	104.1±17.4	89.4± 1.5
D-062968-01	GCUCGGAUGUUCAGCACAA	*Snx8*	25.9±11.3	-26.5±11.5	138.9± 1.4
D-050533-03	CCGACAAACUGAGGAAUAU	*Nadsyn1*	25.6± 2.9	35.6± 4.3	87.2± 6.3
D-058486-01	GAAGACCGCGUUCUGCAGA	*Ccnd2*	24.7± 6.9	62.3±17.2	122.2± 2.8
D-044230-03	GAAUUUGAACCGACUAAGA	*Rc3h1*	24.6± 3.8	70.1±10.3	81.2± 5.6
D-167425-01	CAAUAAGGUUCUCGGUAUA	*Cnot1*	24.3± 8.3	88.8±32.8	77.0± 1.9
D-062675-03	UCAACCGGCUGUACAAAGA	*Rnf34*	24.3±17.6	-30.7±15.6	109.8±13.5
D-063793-03	GAGAAGAAGAGCCGUUUCA	*Aurkb*	23.0± 6.2	43.3± 8.6	80.0± 4.5
D-047567-01	GCACUGUACUUCAUUGAUA	*Top1*	21.5±36.0	25.8±21.2	62.7±29.5
D-047979-03	GCUACCACACGCUGCAAGA	*Nrtn*	21.4±10.5	1.8± 2.1	121.9±13.6
D-061601-02	GGAAGGAGGGUAUGAUGCA	*Dazap2*	20.3±16.3	77.0± 6.5	79.0± 8.9
D-167425-03	CCGACUUACUGCUGCGAUA	*Cnot1*	19.5± 6.2	30.8±18.1	66.9± 2.2

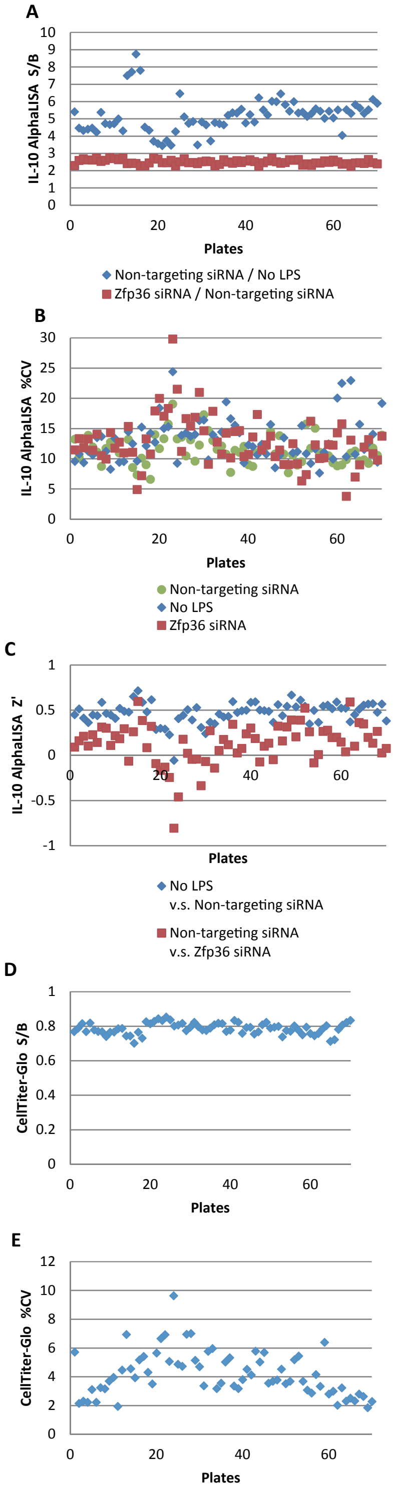
In the deconvolution assay, IL-10-up-regulating siRNA pools were deconvoluted to 440 single siRNAs, and these siRNAs were then assayed. The results for 78 single siRNAs that met the criteria of NPC (IL-10) >20 are shown. The NPC (IL-10) and NPC (TNF-α) of each single siRNA are shown in descending order of NPC (IL-10). Cell viability of BMDMs transfected with each single siRNA relative to “non-targeting siRNA” is also shown. Values are means ± SD (n=4).



Supplemental Fig. 1. Primary screen.
(***A***) Plate layout. (***B***) All data from the genome-wide siRNA screen are shown in order of NPC (IL-10).



Supplemental Fig. 3. Cell viability of BMDMs transfected with each single siRNA from IL-10-up-regulating siRNA pools (***A***), and each single siRNA from siRNA pools targeting 4 ribosomal proteins (***B***) relative to "non-targeting siRNA" in the deconvolution assay. Values are means ± SD (n=4).



Supplemental Fig. 2. Indicators of assay quality in the primary screen. In total, 70 plates were assayed in the primary screen, and S/B, %CV, and Z' factor were calculated for each plate. (***A***) S/B calculated from averaged AlphaLISA IL-10 signals for controls. (***B***) %CV of IL-10 AlphaLISA signals for controls. (***C***) Z' factor calculated from IL-10 AlphaLISA signals for controls. (***D***) The ratio of the average of CellTiter-Glo signals in "non-targeting siRNA" to "no siRNA". (***E***) %CV of CellTiter-Glo signals for "non-targeting siRNA". The Z' value between "no LPS" and "non-targeting siRNA" for IL-10-down-regulating siRNAs was approximately 0.5, whereas for IL-10-up-regulating siRNAs between "non-targeting siRNA" and "Zfp36 siRNA", it was lower than 0.5 and, in some plates, lower than 0. A Z' factor >0.5 is the general criterion in a compound screen. However, a lower Z' factor may be acceptable in RNAi screens.