CYTOKINES

IL-17 integrates multiple self-reinforcing, feed-forward mechanisms through the RNA binding protein Arid5a

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Interleukin-17A (IL-17A) not only stimulates immunity to fungal pathogens but also contributes to autoimmune pathology. IL-17 is only a modest activator of transcription in experimental tissue culture settings. However, IL-17 controls posttranscriptional events that enhance the expression of target mRNAs. Here, we showed that the RNA binding protein (RBP) Arid5a (AT-rich interactive domain-containing protein 5a) integrated multiple IL-17–driven signaling pathways through posttranscriptional control of mRNA. IL-17 induced expression of Arid5a, which was recruited to the adaptor TRAF2. Arid5a stabilized IL-17–induced cytokine transcripts by binding to their 3′ untranslated regions and also counteracted mRNA degradation mediated by the endoribonuclease MCPIP1 (Regnase-1). Arid5a inducibly associated with the eukaryotic translation initiation complex and facilitated the translation of the transcription factors (TFs) IκBζ (*Nfkbiz*) and C/EBPβ (*Cebpb*). These TFs in turn transactivated IL-17–dependent promoters. Together, these data indicated that Arid5a orchestrates a feed-forward amplification loop, which promoted IL-17 signaling by controlling mRNA stability and translation.

INTRODUCTION

Interleukin-17A (IL-17) is an inflammatory cytokine that is crucial for host defense against microbial pathogens, particularly fungi such as the opportunistic fungus *Candida albicans* (*1*). IL-17 also drives immunopathology in autoimmune and chronic inflammatory conditions (*2*). The successful clinical outcomes of drugs blocking IL-17 or its receptor for plaque psoriasis underscore the utility of targeting the IL-17 pathway for therapeutic benefit (*2*). Therefore, understanding the mechanisms by which IL-17 functions has important clinical implications.

IL-17 is produced by T helper 17 (T_H17) cells and innate lymphocytes (*3*) and signals through a dimeric receptor composed of IL-17 receptor A (IL-17RA) and IL-17RC (*4*). The IL-17R recruits nuclear factor κB (NF-κB) activator 1 (Act1), an adaptor and E3 ubiquitin ligase that is upstream of nearly all known IL-17–dependent activities. Act1 binds to TNF (tumor necrosis factor) receptor–associated factor 6 (TRAF6), which in turn activates the canonical NF-κB pathway and MAPK (mitogen-activated protein kinase) cascades. Together, these pathways drive de novo transcription of IL-17–induced signature genes encoding cytokines [IL-6 and G-CSF (granulocyte-colony stimulation factor)], antimicrobial proteins [lipocalin 2 (Lcn2) and β-defensins], and chemokines (*4*, *5*). Although activation of NF-κB is often considered the major IL-17 signaling event, IL-17 also induces other transcription factors (TFs) including CCAAT/enhancer binding proteins (C/EBPs) and the activator protein 1 (AP1) complex (*4*). Confirming their importance, DNA binding sites for C/EBP, NF-κB, and AP1 are enriched in the proximal promoter regions of IL-17 target genes (*6*).

IL-17 signaling in culture systems is typically modest compared to potent inflammatory stimuli such as TNFα (*2*, *7*). Nonetheless, the biological impact of IL-17 is profound because a deficiency in IL-17 signaling or T_H17 cells results in protection from autoimmunity [e.g., experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis] and increased susceptibility to pathogens (e.g., the fungus *C. albicans*) (*8*). One explanation for this paradox is that IL-17 synergizes potently with other inflammatory stimuli commonly found in an inflammatory environment. The molecular basis for synergy is not fully understood but is mediated, in part, through cooperative activation of IL-17–induced TFs that activate downstream genes (*9*, *10*). For example, IL-17 induces IκBζ (NF-κB inhibitor ζ, encoded by *Nfkbiz*) (*11*), an atypical member of the NF-κB family that facilitates transcription of several IL-17–dependent genes in cooperation with TNFα (*12*–*14*). Similarly, C/EBP family members are implicated in functional cooperativity between IL-17 and TNFα (*7*, *11*, *15*).

In addition, IL-17 promotes stabilization of mRNA (*16*). Many IL-17–induced transcripts have adenylate-uridylate–rich elements or other stability-determining sequences in the 3′ untranslated region (3′UTR) (*17*). IL-17 promotes mRNA stabilization of numerous genes, including *Il6*, C-X-C motif chemokine ligand 1 (*Cxcl1*), and *Cxcl5* among others (*16*). RNA binding proteins (RBPs) such as Hu-antigen R (HuR) and DEAD-box helicase 3 X-Linked (DDX3X) are recruited to the IL-17R/Act1 complex through the adaptors TRAF2 and TRAF5. HuR binds to and stabilizes *Cxcl1* and *Cxcl5* by competing for 3′UTR occupancy with the RNA decay factor splicing factor 2 (SF2) (*18*–*20*). Another IL-17 target gene that influences mRNA stability is the endoribonuclease MCP-1 (monocyte chemoattractant protein-1)–induced protein 1 [MCPIP1, also known as Regnase-1, encoded by the gene zinc finger CCCH-type containing 12A (*Zc3h12a*)] (*15*, *21*). IL-17 induces *Zc3h12a* expression and promotes its mRNA stability through DDX3X (*20*, *22*). MCPIP1 mediates 3′UTR-mediated mRNA decay of *Il6*, *Nfkbiz*, and other transcripts and thus is a feedback inhibitor of IL-17 signaling (*22*). Although these studies provided insights into the importance of mRNA control in the IL-17 pathway, the full extent to which IL-17 regulates mRNA remains incompletely understood.

Here, we showed that the RBP AT-rich interactive domain-containing protein 5A (Arid5a) promoted IL-17 signaling. IL-17 stimulation of target cells increased the abundance of Arid5a and triggered its recruitment to the adaptor TRAF2. Arid5a promoted the expression

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of several IL-17–dependent cytokine mRNA transcripts (*Il6*, *Cxcl1*, and *Cxcl5*) by binding to their 3′UTR sequences, enhancing mRNA stability, and counteracting the negative effects of MCPIP1. However, Arid5a also promoted expression of certain IL-17–dependent genes (e.g., *Lcn2*) without affecting mRNA stability. This observation led us to interrogate the impact of Arid5a on IL-17–induced TFs, specifically C/EBPβ and IκBζ. Arid5a stabilized mRNA encoding *Nfkbiz* but not *Cebpb*. Rather, Arid5a strongly enhanced IL-17–induced translation of C/EBPβ and IκBζ proteins, revealing its potential to control translational circuitry.

RESULTS

IL-17 increases Arid5a expression and promotes its association with TRAF2

To identify RBPs that might participate in IL-17 signaling cascades, we screened for IL-17–dependent genes encoding RBPs that were induced during a strongly IL-17–dependent immune response (*23*), specifically, oropharyngeal candidiasis (OPC). Expression of *Arid5a* was enhanced in the oral mucosa (tongue) of wild-type (WT) mice after oral *C. albicans* infection and was impaired in *Il17ra*$^{-/-}$ mice (Fig. 1A). This finding paralleled our observations with another RBP, *Zc3h12a* (encoding MCPIP1), which is induced by IL-17 in the tongue during OPC (*22*). Similarly, IL-17 stimulated the expression of *Arid5a* in the IL-17–responsive stromal cell line, ST2 (Fig. 1B) (*22*). IL-17–induced *Arid5a* expression was blocked by an NF-κB inhibitor, suggesting that its expression is NF-κB–dependent (fig. S1A).

Some IL-17–dependent pathways that lead to mRNA stabilization are initiated through Act1, TRAF2, and TRAF5 (*18*–*20*). To test the hypothesis that Arid5a uses similar proximal signaling intermediates, we transfected a Flag/Myc-Arid5a construct into human embryonic kidney–293T (HEK293T) cells [commercial antibodies (Abs) against endogenous Arid5a are ineffective for immunoprecipitation (IP)] and co-immunoprecipitated Arid5a and TRAF2. Arid5a did not co-immunoprecipitate with Act1 but did associate with TRAF2 (Fig. 1C and fig. S1B). To determine whether the TRAF2-Arid5a association is IL-17–dependent, we transfected ST2 cells with Flag/Myc-Arid5a, stimulated with IL-17 for 2 hours and lysates subjected to IP with Myc Abs. Endogenous TRAF2 co-immunoprecipitated with Arid5a, and the TRAF2-Arid5a interaction peaked at ~15 to 60 min after IL-17 treatment (Fig. 1D and fig. S2E). Together, these data show that IL-17 enhances Arid5a expression, which inducibly associates with TRAF2.

Arid5a augments the cellular response to IL-17

To ascertain whether Arid5a affects the response to IL-17, we transfected ST2 cells with pooled small interfering RNAs (siRNAs) against Arid5a (knockdown efficiency was typically 40 to 60%; fig. S1, C and D). Cells were treated with IL-17 for 3 hours, and expression of prototypical IL-17 target genes was assessed. Knockdown of Arid5a suppressed IL-17–mediated induction of *Il6* mRNA and secreted IL-6 protein (Fig. 2A and fig. S3A). Similar results were obtained for other canonical IL-17 target genes, including CXCL1 and CXCL5 (Fig. 2A and fig. S3A). However, not all IL-17–driven genes were detectably regulated by Arid5a, such as *Ccl20* and *Csf2* (Fig. 2A). Together, these data show that Arid5a is required for the expression of a subset of IL-17 target genes.

Because Arid5a associated with TRAF2 (Fig. 1, C and D), we postulated that TRAF2 participates in Arid5a-mediated increased expression of IL-17 target genes. ST2 cells were transfected with siRNAs against TRAF2 and/or Arid5a (knockdown efficiency was ~60 to 70% for Arid5a and ~70 to 80% for TRAF2; fig. S1E). Silencing of TRAF2 decreased IL-17–induced expression of *Il6*, whereas knockdown of TRAF2 in combination with Arid5a did not reduce expression of *Il6* compared to either alone (Fig. 2B). These data are consistent with a model in which Arid5a and TRAF2 act in the same pathway.

TRAF2 promotes mRNA stabilization in the IL-17 pathway through the RBP HuR (encoded by the *Elavl1* gene) (*19*). Double knockdown of Arid5a and HuR did not change or only marginally decreased IL-17–induced expression of *Il6* or *Cxcl1* compared to knockdown of Arid5a or HuR alone (fig. S2A). Knockdown efficiency was typically ~60% for Arid5a and >95% for HuR (fig. S2B). These results suggest that Arid5a and HuR are likely to function in the same pathway in IL-17 signal transduction. However, in contrast to Arid5a, IL-17 did not induce expression of HuR (*Elav1*) mRNA (fig. S2C).

In the IL-17 and other inflammatory signaling pathways, competition among RBPs helps determine the overall amount of target mRNA transcripts (*4*, *18*, *19*). IL-17 induces the endoribonuclease MCPIP1, which remains elevated during prolonged stimulation and binds to a similar RNA binding site as Arid5a (*22*, *24*). Because the net effect of IL-17 is to enhance expression of mRNAs that can be inhibited by MCPIP1, e.g., *Il6* or *Lcn2* (Fig. 2C), this paradox suggested that IL-17 augments the activity of RBPs that offset the negative effects of MCPIP1. To determine whether Arid5a might serve in this capacity, we knocked down Arid5a and MCPIP1 together (efficiency, ~80%; fig. S1F) and assessed *Il6* and *Lcn2* expression. As reported, MCPIP1 knockdown enhanced IL-17–induced expression of *Il6* and *Lcn2* mRNA (Fig. 2D) and IL-6 protein (fig. S3B), but knockdown of Arid5a partially offsets the inhibitory effect of MCPIP1 deficiency (Fig. 2D and fig. S3B). Knockdown of Arid5a also suppressed the IL-17–induced abundance of LCN2 protein (fig. S3A). Therefore, Arid5a counteracts the activity of MCPIP1 stimulated by IL-17.

IL-17 increases *Il6* expression both through acting on its proximal promoter and by stabilizing its mRNA transcript (*7*, *25*, *26*). However, we saw no impact of Arid5a on IL-6 promoter activation, as co-expression of Arid5a with a luciferase reporter driven by the mouse *Il6* promoter did not increase luciferase activity (fig. S1G). To determine whether Arid5a promoted IL-17–dependent stability of *Il6* mRNA, we transfected ST2 cells with siRNAs against Arid5a and primed with TNFα for 3 hours to induce *Il6* mRNA without activating IL-17 signaling. After the cells were washed and treated with actinomycin D to block further transcription, we assessed the half-life ($t_{½}$) of target transcripts over 90 min (longer treatments led to cell toxicity) in the presence or absence of IL-17. As previously reported, IL-17 reduced decay of *Il6* transcripts (*26*), but *Il6* stabilization was impaired upon Arid5a knockdown (Fig. 2E and fig. S8A). Arid5a similarly stabilized *Cxcl1* and *Cxcl5* transcripts (Fig. 2E and figs. S3C and S8B). When we cotransfected HEK293T cells with Arid5a and a luciferase reporter fused to the *Il6* 3′UTR (*27*), expression of Arid5a increased luciferase activity (Fig. 2F). Control expression of either Act1 enhanced *Il6* 3′UTR reporter activity, whereas MCPIP1 suppressed activity, as expected (*28*, *29*). Thus, these data suggest that Arid5a stabilizes IL-17 target mRNA transcripts encoding inflammatory cytokines and chemokines through interaction with transcript 3′UTRs.

Arid5a promotes translation of C/EBPβ and IκBζ

Unlike *Il6* transcripts, IL-17 does not alter the mRNA stability of *Lcn2* (*6*). Moreover, Arid5a knockdown did not increase the half-life of *Lcn2*

mRNA (fig. S3E). Even so, when Flag/Myc-Arid5a was coexpressed with a luciferase reporter driven by the mouse *Lcn2* promoter (*6*), luciferase activity was significantly increased (Fig. 3A). Because there are no apparent Arid5a recognition sites within the *Lcn2* promoter, these data raised the possibility that Arid5a may transactivate the *Lcn2* promoter indirectly through an IL-17–dependent TF. The *Lcn2* promoter contains a C/EBP binding element required for IL-17–dependent induction (*6*, *10*), and Arid5a failed to activate an *Lcn2* promoter with a C/EBP binding site mutation (Fig. 3A) (*6*). In response to IL-17, *Cebpb* expression increased two - to fourfold (Fig. 3B), consistent with prior observations (*7*, *30*–*32*) and raising the possibility that Arid5a increases *Lcn2* by enhancing C/EBPβ expression. However, Arid5a knockdown did not impair expression of IL-17–induced *Cebpb* mRNA (Fig. 3C). By Western blot, we confirmed that IL-17 increases the abundance of C/EBPβ protein isoforms known as LAP (liver-activated protein) and LIP (liver inhibitory protein), which are generated by alternative translation (*30*). We found that silencing of Arid5a strongly inhibited IL-17–induced expression of all C/EBPβ

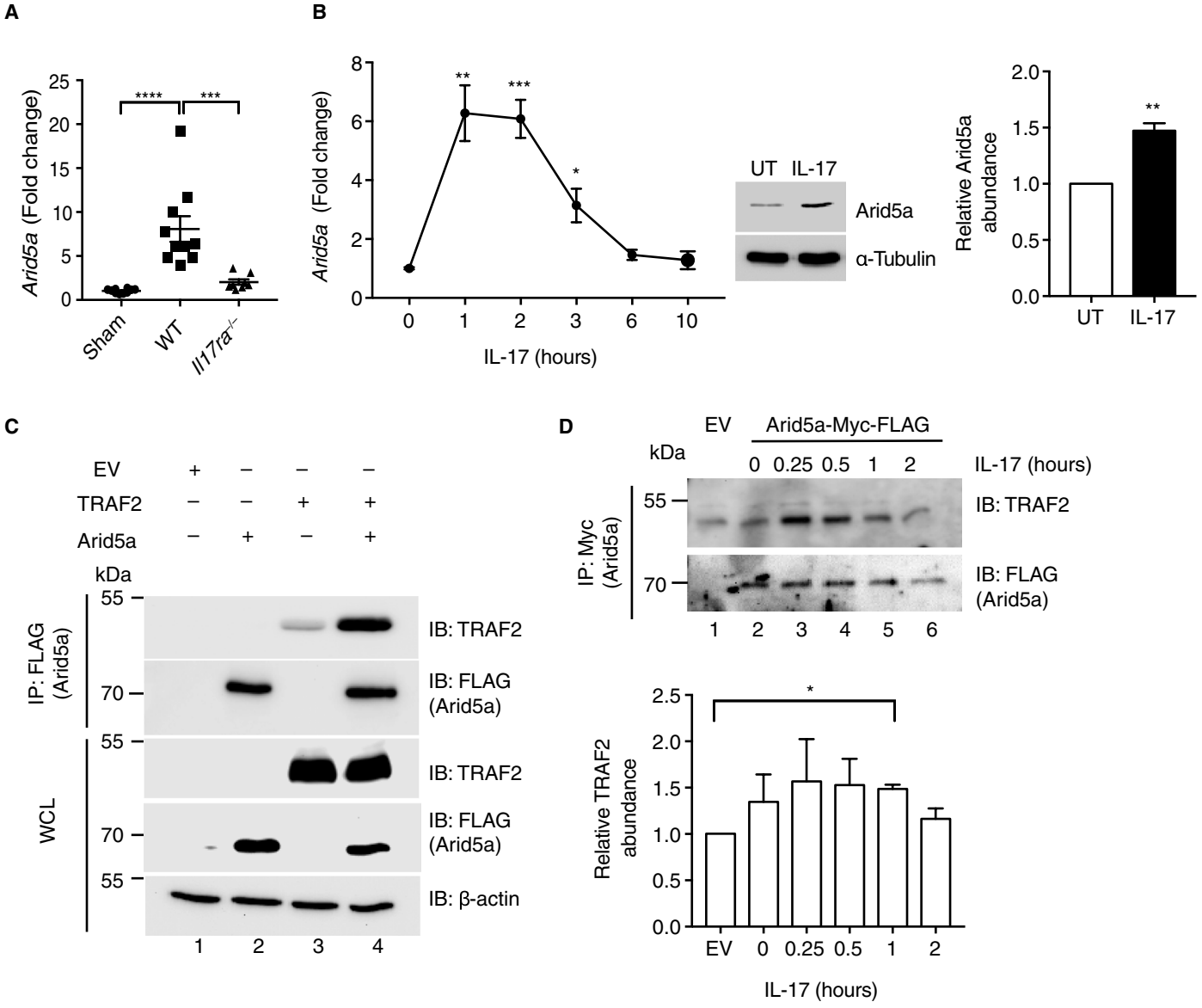

Fig. 1. IL-17 increases the abundance of Arid5a, which inducibly associates with TRAF2. (**A**) Quantitative reverse transcription PCR (qRT-PCR) analysis of *Arid5a* mRNA expression in the tongue tissue of WT or *Il17ra*$^{-/-}$ mice at 24 hours after oral exposure to phosphate-buffered saline (sham) or *C. albicans* (CAF2-1). Fold change data are means ± SEM of at least eight mice per group from two independent experiments. (**B**) Left: qRT-PCR analysis of *Arid5a* mRNA expression in ST2 cells treated with IL-17 for the indicated times. Fold change data are means ± SEM from three independent experiments. Right: Western blot analysis of Arid5a on lysates from ST2 cells treated with IL-17A for 4 hours. Blots are representative of three independent experiments. Quantified band intensity values are means ± SEM from all experiments. UT, untreated. (**C**) Co-immunoprecipitation analysis of Arid5a interaction with TRAF2 in lysates from HEK293T cells transfected with empty vector (EV), Flag/Myc-Arid5a, or TRAF2 and immunoprecipitated for Flag. Blots are representative of two independent experiments (fig. S1B). WCL, whole cell lysate. (**D**) CoIP analysis of Arid5a interaction with TRAF2 in lysates from ST2 cells transfected with Flag/Myc-Arid5a, treated with IL-17 for the indicated times and immunoprecipitated with Ab against Myc. Blots are representative of three independent experiments (fig. S2E). Quantified band intensity values are means ± SEM from all experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001 by analysis of variance (ANOVA) with post hoc Tukey's test (A), Dunnett's test (B, left), or paired Student's *t* test (B, right).

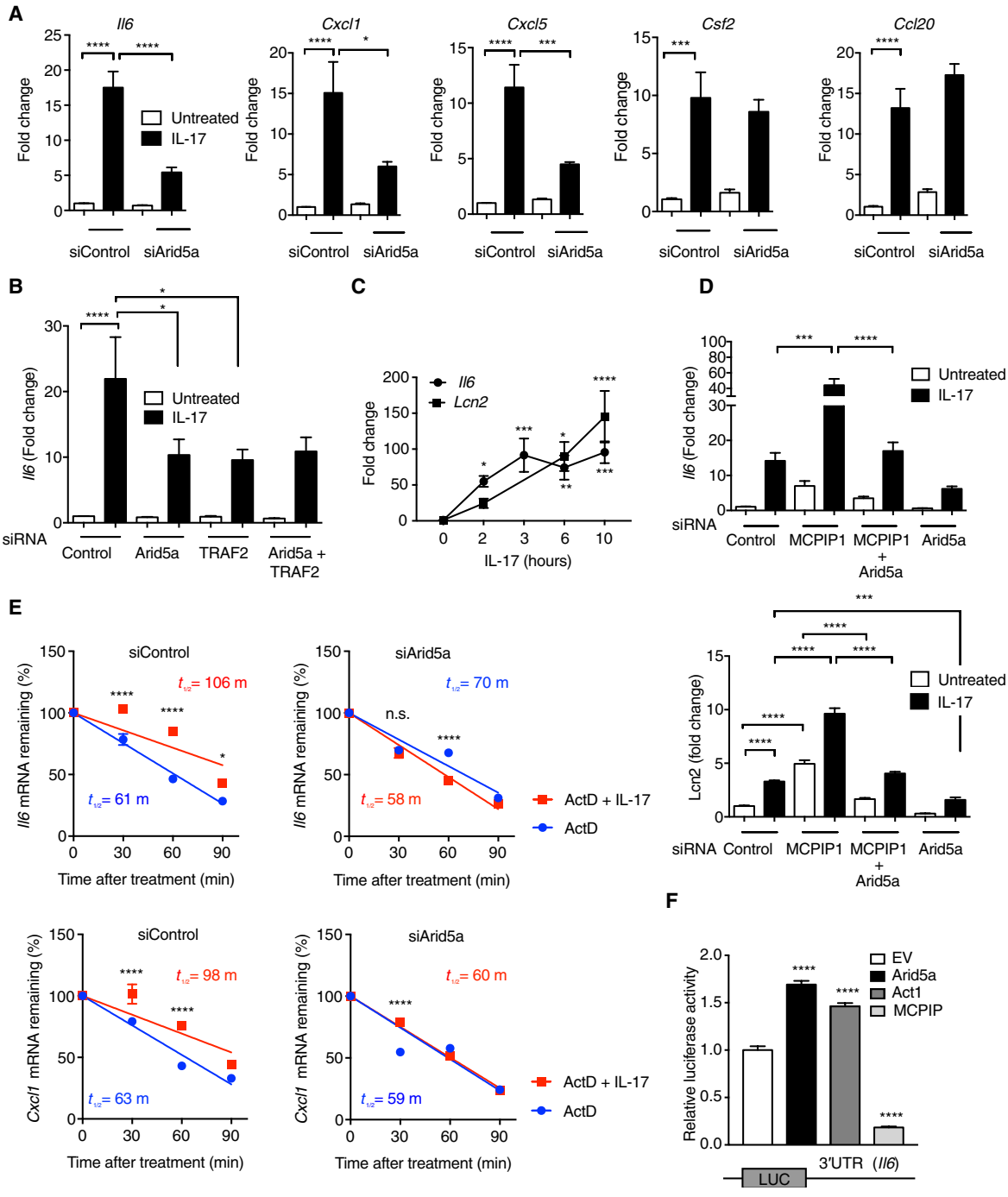

Fig. 2. Arid5a promotes cellular responses to IL-17. (**A**) qRT-PCR analysis of the indicated mRNAs in ST2 cells transfected with pooled siRNAs targeting Arid5a (siArid5a) or scrambled control (siControl) and treated with IL-17 for 3 hours. Fold change data are means ± SEM from three independent experiments. (**B**) qRT-PCR analysis of *Il6* mRNA expression in ST2 cells transfected with pooled siRNAs targeting Arid5a, TRAF2, or scrambled control and treated with IL-17 for 3 hours. Fold change data are means ± SEM from three independent experiments. (**C**) qRT-PCR analysis of *Il6* or *Lcn2* mRNA expression in ST2 cells treated with IL-17 for the indicated times. Fold change data are means ± SEM from three independent experiments. (**D**) qRT-PCR analysis of *Il6* or *Lcn2* in ST2 cells transfected with pooled siRNAs targeting Arid5a ± MCPIP1 or scrambled control and treated with IL-17 for 3 hours. Fold change data are means ± SEM from three independent experiments. (**E**) qRT-PCR analysis of *Il6* or *Cxcl1* mRNA in ST2 cells transfected with pooled siRNAs targeting Arid5a or scrambled control, pretreated with TNFα for 3 hours, and then treated with actinomycin D (ActD) and IL-17 for the indicated times. Remaining mRNAs compared to time = 0 data are means ± SEM representative of three independent experiments. (**F**) Luciferase (LUC) assay of *Il6* 3′UTR activity in HEK293T cells at 24 hours after transfection with a luciferase reporter and EV, Flag/Myc-Arid5a, Act1-Myc, or MCPIP1 and analyzed after 24 hours. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ by ANOVA with post hoc Tukey's test (A, B, D, and F) or Dunnett's test (C); half-lives ($t_{½}$) were determined using equations that defined decay kinetics as shown by colored lines in the graph, as described (*42*) (E). n.s., not significant.

protein isoforms (Fig. 3D). However, TRAF2 was dispensable for IL-17–induced expression of C/EBPβ (fig. S2D). Furthermore, neither IL-17 stimulation nor Arid5a knockdown affected the mRNA stability of *Cebpb* transcripts (fig. S3D). Thus, Arid5a appears to promote C/EBPβ protein abundance but not mRNA expression.

These data suggested that Arid5a may increase C/EBPβ translation. Therefore, we used RNA-immunoprecipitation (RIP) assays to determine whether Arid5a affects the occupancy of *Cebpb* within the eukaryotic eIF4F translation initiation complex (Fig. 3E) (*33*). Accordingly, ST2 cells were transfected with Arid5a siRNA and treated with IL-17, and lysates were immunoprecipitated with Abs against eIF4G, a scaffolding subunit of eIF4F associated with mRNA undergoing active translation (*34*). We found that *Cebpb* transcripts were enriched in the eIF4G IP fraction after IL-17 stimulation, consistent with increased

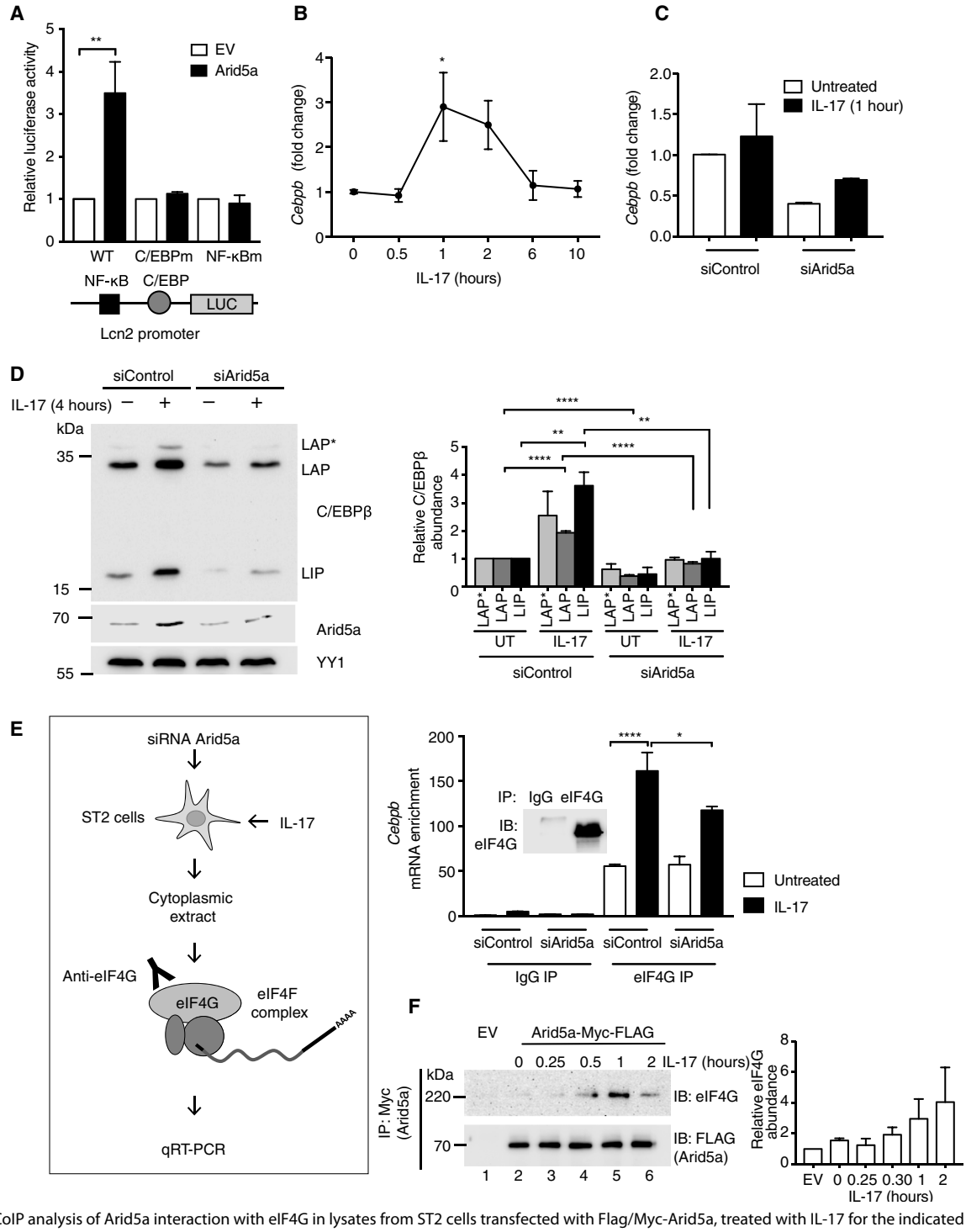

Fig. 3. Arid5a mediates translation of C/EBPβ mRNA. (**A**) Luciferase assay of *Lcn2* proximal promoter activity in HEK293T cells transfected with a luciferase reporter together with EV or Flag/Myc-Arid5a and analyzed after 24 hours. Fold change data are means ± SEM from three to five independent experiments. (**B**) qRT-PCR analysis of *Cebpb* mRNA expression in ST2 cells treated with IL-17 for the indicated times. Fold change data are means ± SEM from three independent experiments. (**C**) qRT-PCR analysis of *Cebpb* mRNA expression in ST2 cells transfected with pooled siRNAs targeting Arid5a or scrambled control and treated with IL-17 for 1 hour. Fold change data are means ± SEM from three independent experiments. (**D**) Western blot analysis of C/EBPβ isoforms (LAP*, LAP, and LIP) in nuclear extracts from ST2 cells transfected with siRNAs targeting Arid5a or control siRNA and treated with IL-17A for 4 hours. Blots are representative of three independent experiments. Quantified band intensity values are means ± SEM from all experiments. (**E**) RIP assay (left) of *Cebpb* mRNA amount by qRT-PCR analysis on immunoglobulin G (IgG) or eIF4G immunoprecipitates from cytoplasmic extracts of ST2 cells after transfection with siRNAs targeting Arid5a or control siRNA and treatment with IL-17 for 3 hours. Inset: Western blot analysis of eIF4G in cytoplasmic fractions immunoprecipitated with IgG or eIF4G. Data are fold change means ± SEM representative of three independent experiments. (**F**) CoIP analysis of Arid5a interaction with eIF4G in lysates from ST2 cells transfected with Flag/Myc-Arid5a, treated with IL-17 for the indicated times, and immunoprecipitated with Ab against eIF4G. Blots are representative of three independent experiments (fig. S2F). Quantified band intensity values are means ± SEM from all experiments. **P* < 0.05, ***P* < 0.01, and *****P* < 0.0001 by ANOVA with post hoc Tukey's test (A and C to F) or Dunnett's test (B).

translation (Fig. 3E and fig. S7A). Similarly, *Il6* and *Cxcl1* transcripts were also enriched, which was expected because their corresponding proteins are increased in abundance in conditioned media after IL-17 stimulation (figs. S5A and S7C). Moreover, Arid5a knockdown impaired enrichment of *Cebpb*, *Il6*, and *Cxcl1* transcripts in the eIF4F complex, supporting a role for Arid5a in promoting translation of C/EBPβ and other target proteins. Total *Cebpb* mRNAs in the cytoplasmic lysate used as the input for RIP were not affected by Arid5a knockdown (fig. S5B). To determine whether Arid5a itself interacts with the translation initiation complex, we transfected ST2 cells with Flag/Myc-Arid5a and stimulated these cells with IL-17 for 2 hours. When lysates were immunoprecipitated with Myc Abs, we

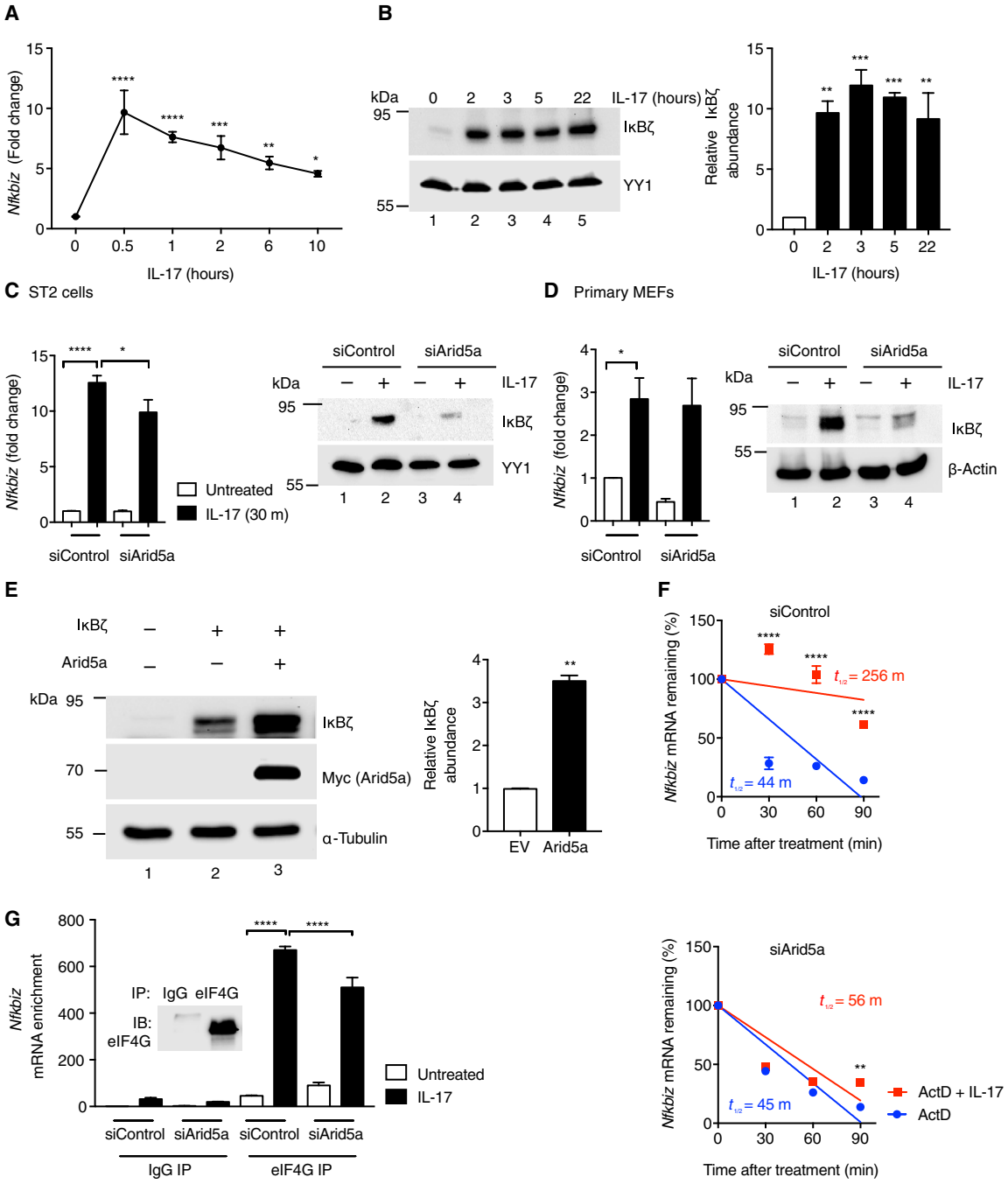

Fig. 4. Arid5a mediates translation of IκBζ. (**A**) qRT-PCR analysis of *Nfkbiz* mRNA expression in ST2 cells treated with IL-17 for the indicated times. Fold change data are means ± SEM from three independent experiments. (**B**) Western blot analysis of IκBζ in nuclear extracts from ST2 cells treated with IL-17A for indicated times. Blots are representative of three independent experiments. Quantified band intensity values are means ± SEM from all experiments. (**C**) Left: qRT-PCR analysis of *Nfkbiz* mRNA expression in ST2 cells transfected with pooled siRNAs targeting Arid5a or scrambled control and treated with IL-17 for 30 min. Fold change data are means ± SEM from two independent experiments. Right: Western blot analysis of IκBζ in nuclear extracts from ST2 cells transfected with siRNAs targeting Arid5a or control siRNA and treated with IL-17A for 4 hours. Blots are representative of four independent experiments. (**D**) Left: qRT-PCR analysis of *Nfkbiz* mRNA expression in primary MEFs transfected with pooled siRNAs targeting Arid5a or scrambled control and treated with IL-17 for 24 hours. Fold change data are means ± SEM from four independent experiments. Right: Western blot analysis of IκBζ in whole-cell lysates from primary MEFs transfected with siRNAs targeting Arid5a or control siRNA and treated with IL-17A for 24 hours. Blots are representative of three independent experiments. (**E**) Western blot analysis of IκBζ and Myc-tagged Arid5a in lysates from HEK293T cells transfected with EV, IκBζ, or Flag/Myc-Arid5a. Blots are representative of three independent experiments. Quantified band intensity values are means ± SEM from all experiments. (**F**) qRT-PCR analysis of *Nfkbiz* mRNA expression in ST2 cells transfected with pooled siRNAs targeting Arid5a or scrambled control that were pretreated with TNFα for 3 hours and then treated with actinomycin D and IL-17 for the indicated times. Remaining mRNAs compared to time = 0 data are means ± SEM representative of two independent experiments. (**G**) RIP assay of *Nfkbiz* mRNA amount by qRT-PCR analysis on IgG or eIF4G immunoprecipitates from cytoplasmic extracts of ST2 cells after transfection with siRNAs targeting Arid5a or control siRNA and treatment with IL-17 for 3 hours. Data are fold change means ± SEM representative of three independent experiments. Inset: Western blot analysis of eIF4G in cytoplasmic fractions immunoprecipitated with IgG or eIF4G. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001 by ANOVA with post hoc Tukey's test or Dunnett's test (A and B); half-lives ($t_{½}$) were assessed as described (*42*) (E).

found that endogenous eIF4G co-immunoprecipitated with Arid5a in an IL-17–dependent manner (Fig. 3F and fig. S2F). These data indicate that Arid5a promotes IL-17–induced translation of C/EBPβ by interacting with eIF4F translation initiation complex.

The NF-κB family also mediates responses to IL-17, and the *Lcn2* promoter contains an NF-κB site required for IL-17 activation. This site interacts with IκBζ (encoded by *Nfkbiz*), a noncanonical member of the NF-κB family that is induced by IL-17 and cooperates with

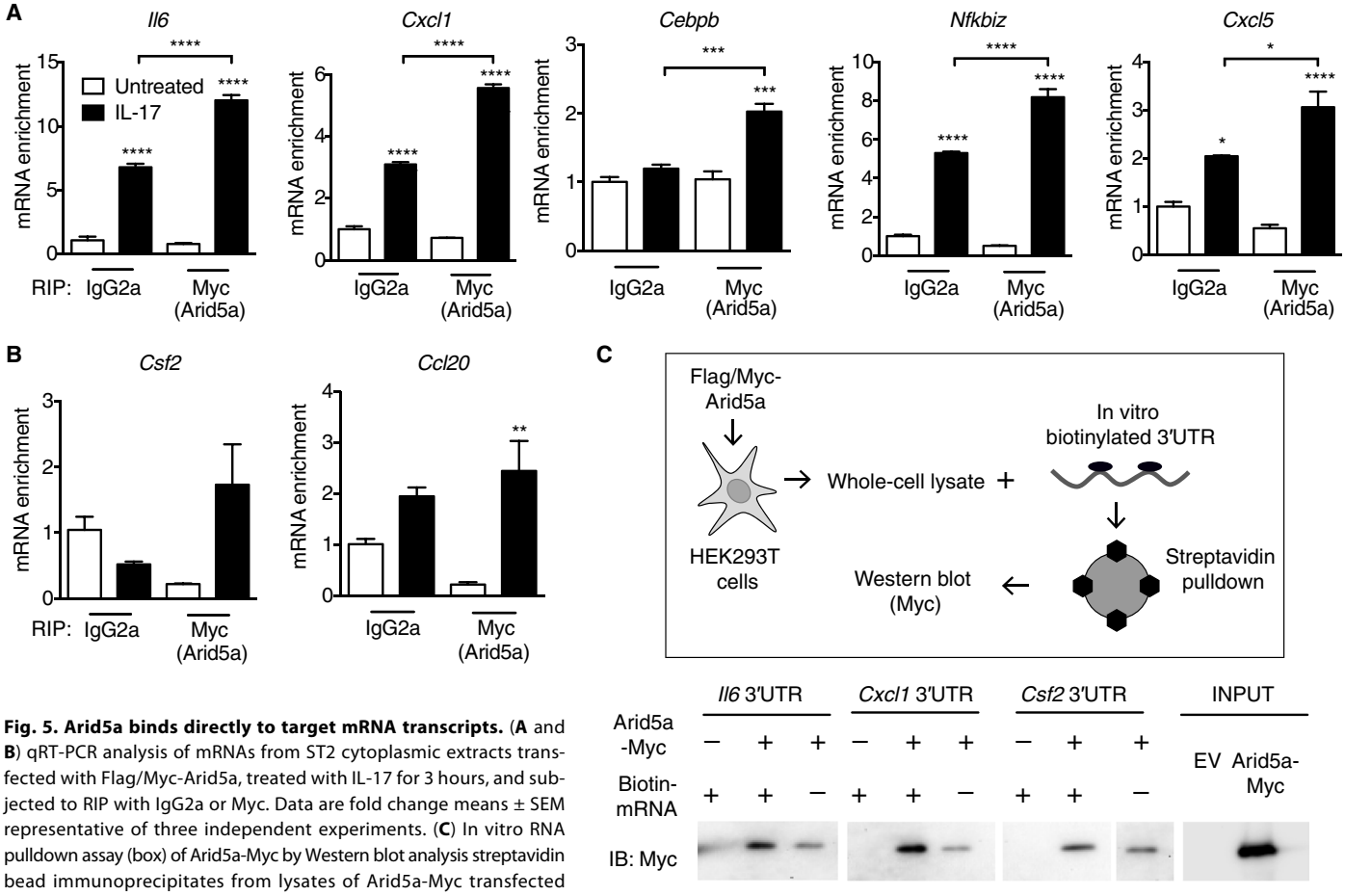

Fig. 5. Arid5a binds directly to target mRNA transcripts. (**A** and **B**) qRT-PCR analysis of mRNAs from ST2 cytoplasmic extracts transfected with Flag/Myc-Arid5a, treated with IL-17 for 3 hours, and subjected to RIP with IgG2a or Myc. Data are fold change means ± SEM representative of three independent experiments. (**C**) In vitro RNA pulldown assay (box) of Arid5a-Myc by Western blot analysis streptavidin bead immunoprecipitates from lysates of Arid5a-Myc transfected HEK293T cells incubated with the indicated in vitro–generated, biotinylated mRNAs. Data are derived from the same blot and are representative of three independent experiments for *Il6* and two independent experiments for *Cxcl1* and *Csf2*. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001 by ANOVA with post hoc Tukey's test.

NF-κB p50 (*6*, *10*, *15*). Arid5a did not activate a luciferase construct driven by the *Lcn2* promoter mutated at its NF-κB binding site (Fig. 3A), which suggested that Arid5a may enhance expression of IκBζ. We found that IL-17 enhanced expression of IκBζ both at mRNA and protein levels (Fig. 4, A and B), consistent with prior reports (*15*, *22*, *35*, *36*). Arid5a silencing only modestly inhibited IL-17–induced mRNA expression of *Nfkbiz* in ST2 cells and had no effect in primary mouse embryonic fibroblasts (MEFs; Fig. 4, C and D). In contrast to *Cebpb* transcripts, Arid5a knockdown impaired IL-17–induced stabilization of *Nfkbiz* mRNA (Fig. 4F and fig. S8C). However, Arid5a knockdown reduced IL-17–induced IκBζ protein abundance in both ST2 cells and MEFs (Fig. 4, C and D, and fig. S4, A and B). Consistently, we found that coexpression of Arid5a with IκBζ in HEK293T cells increased IκBζ abundance (Fig. 4E), and TRAF2 was not required for IL-17–induced expression of IκBζ (fig. S2D). As with *Cebpb* transcripts, *Nfkbiz* mRNA was less abundant in eIF4G RIP fractions after Arid5a knockdown, which suggested that Arid5a promotes *Nfkbiz* translation (Fig. 4G and figs. S4B and S7B). Thus, our results showed that Arid5a enhanced both mRNA stability and translation of IκBζ. Collectively, these data suggest that Arid5a indirectly stimulates target genes that are activated by the IL-17–inducible TFs C/EBPβ and IκBζ.

Arid5a binds to the 3′UTR of IL-17–induced mRNAs

To determine whether Arid5a binds directly to target mRNAs, ST2 cells were transfected with Flag/Myc-Arid5a, stimulated with IL-17, and nuclear and cytoplasmic fractions of cell lysates were subjected to RIP with Abs against Myc. We found that the association of *Il6*, *Cxcl1*, *Cxcl5*, *Nfkbiz*, and *Cebpb* transcripts with Arid5a was increased in cytoplasmic fractions after IL-17 stimulation (Fig. 5A and fig. S6). These transcripts were not enriched in control or nuclear fractions (fig. S5, C to H), consistent with the fact that posttranscriptional control of mRNA takes place in the cytoplasm (*17*). In contrast, *Ccl20 and Csf2* transcripts were associated with Arid5a, which agrees with our earlier results that Arid5a knockdown does not affect IL-17–dependent expression of these genes (Fig. 5B and fig. S6; see also Fig. 2A). Together, our data suggest that Arid5a associates specifically with IL-17–induced, cytoplasmically localized mRNA transcripts.

To verify that Arid5a binds directly to the 3′UTRs of target transcripts, we used an in vitro RNA pulldown assay. Biotinylated transcripts encoding the 3′UTR sequences of *Il6*, *Cxcl1*, and *Csf2* were generated in vitro and incubated with recombinant Flag/Myc-Arid5a derived from transfected HEK293T cells. Biotinylated transcripts were isolated with streptavidin-conjugated beads and precipitates subjected to immunoblotting with Myc Abs (Fig. 5C). Consistent with the earlier RIP data, Arid5a was

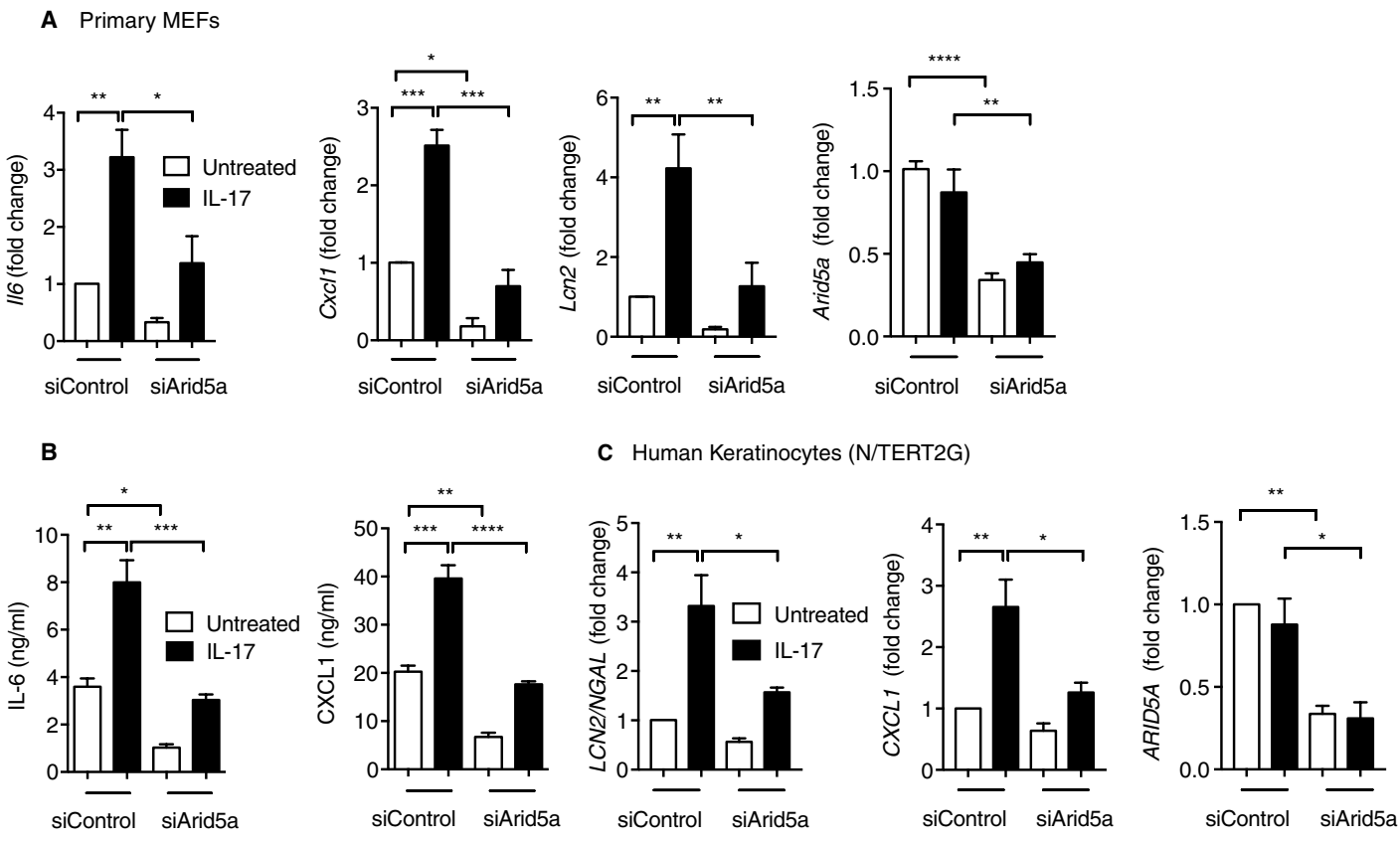

Fig. 6. Arid5a promotes responses to IL-17 in primary MEFs and human KCs. (**A**) qRT-PCR analysis of primary MEFs transfected with pooled siRNAs targeting Arid5a or scrambled control and treated with IL-17 for 24 hours. Fold change data are means ± SEM from three independent experiments. (**B**) Enzyme-linked immunosorbent assay (ELISA) analysis of conditioned supernatants from MEFs transfected with pooled siRNAs targeting Arid5a or scrambled control and treated with IL-17 for 24 hours. Fold change data are means ± SEM representative of two independent experiments. (**C**) qRT-PCR analysis of human KC (N/TERT2G) cells transfected with pooled siRNAs targeting Arid5a or scrambled control and treated with IL-17 for 5 hours. Fold change data are means ± SEM from three independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001 by ANOVA with post hoc Tukey's test.

detected in fractions corresponding to the *Il6* and *Cxcl1* 3′UTR sequences but not with the *Csf2* 3′UTR (Fig. 5C). Thus, Arid5a stabilizes IL-17–induced target mRNAs by binding directly to the 3′UTR.

Arid5a promotes expression of IL-17 target genes in primary MEFs and human keratinocytes

Although ST2 cells are generally a good reflection of IL-17–dependent events in other cell types, we also assessed Arid5a function by siRNA knockdown in primary murine MEFs and N/TERT2G-immortalized human keratinocytes (KCs) (*37*). N/TERT2G cells maintain normal KC differentiation patterns and are considered to be a good representation of primary human KCs (*38*). In MEF cells, Arid5a deficiency also decreased IL-17–induced expression of *Il6*, *Cxcl1*, and *Lcn2* mRNA and IL-6 and CXCL1 protein abundance (Fig. 6, A and B). Similarly, silencing of Arid5a in N/TERT2G cells impaired expression of human *LCN2* and *CXCL1* stimulated by IL-17 (Fig. 6C). Thus, Arid5a promotes IL-17 responses in multiple cells, including primary murine and human cell types.

DISCUSSION

IL-17 signaling dominantly occurs in epithelial and mesenchymal cell types (*39*), and thus, IL-17 functions as a bridge between the immune system and inflamed tissue. The signal transduction mechanisms downstream of IL-17 and related cytokines are still incompletely understood. Here, we identified the RNA binding protein Arid5a as a driver of cellular responses to IL-17 in mouse mesenchymal cells and in human KCs. Arid5a bound directly to the 3′UTR of multiple target mRNAs and stabilized IL-17–induced mRNA transcripts encoding IL-6 and CXC chemokines. These activities are similar to those described for Arid5a in the Toll-like receptor (TLR4) signaling pathway (*24*, *40*). In addition, we observed that Arid5a facilitated the translation of two IL-17–dependent TFs, C/EBPβ and IκBζ, which allowed for increased expression of genes reliant on these TFs such as *Lcn2* (Fig. 7). Thus, in these capacities, Arid5a promotes IL-17–driven immunity.

The IL-17 family is a distinct subclass of cytokines. Accumulating evidence indicates that the mechanisms by which IL-17 mediates mRNA stabilization are not the same as better-studied pathways (*25*, *26*, *41*–*43*). For example, IL-17 does not stabilize target mRNAs through the commonly used RBP tristetraprolin and or the adaptor TRAF6 (*19*, *43*–*45*). Rather, RBPs such as HuR, DDX3X, and Act1 serve to stabilize IL-17–dependent transcripts in a pathway initiated by TRAF2 and TRAF5 (*19*, *20*, *28*). Typically, the RNA-stabilizing activity of these RBPs is offset by destabilizing RBPs, e.g., SF2 (*18*, *19*), which allows for rapid changes in the accumulation of inflammatory mRNAs in response to cues from

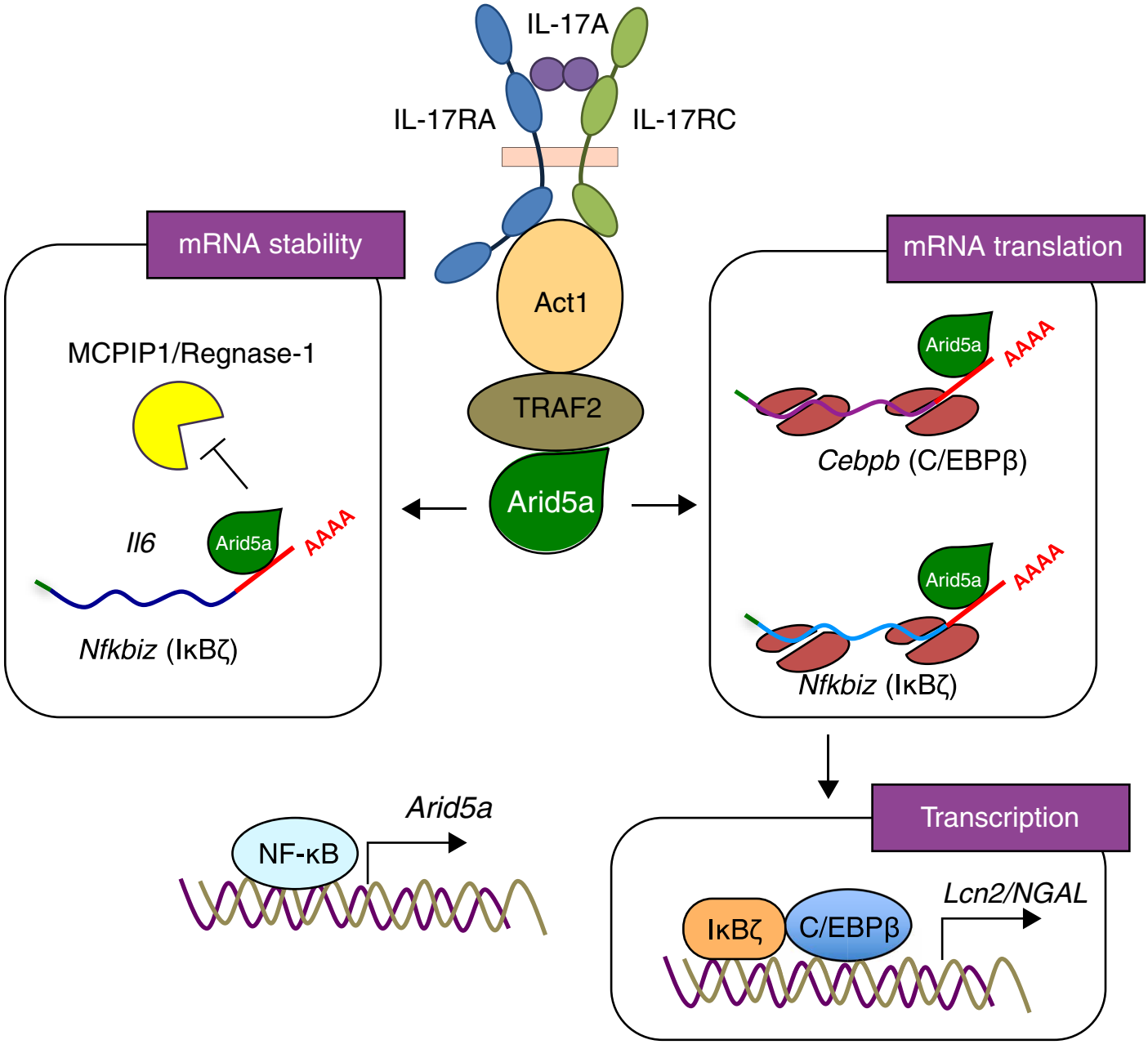

Fig. 7. Model of Arid5a in the IL-17R signaling pathway. Upon IL-17 stimulation, Arid5a expression is increased, and this RBP is recruited to TRAF2. Arid5a promotes the mRNA stability of multiple genes, including cytokines and chemokines, and the TF *Nfkbiz* (IκBζ). In addition, Arid5a enhances the translation of *Nfkbiz* and *Cebpb*, TFs that in turn regulate downstream genes such as *Lcn2*, which are not intrinsically unstable. Thus, Arid5a is a central player in the posttranscriptional IL-17 signaling cascade.

IL-17 or other stimuli. In an analogous manner, Arid5a function appeared to be offset by the endoribonuclease, MCPIP1 (Regnase-1), which is also induced by IL-17 and which degrades many of the same IL-17–dependent mRNA transcripts (*21*, *22*). In the setting of lipopolysaccharide signaling in macrophages, Arid5a and MCPIP1 bind to an overlapping RNA binding sequence on the *Il6* 3′UTR (*24*, *46*). Nonetheless, in the IL-17 pathway, not all the targets of Arid5a and MCPIP1 were identical (e.g., *Ccl20*, which was strongly affected by MCPIP1 but not detectably altered by Arid5a). Thus, Arid5a and MCPIP1 appear to be opposing players in an RBP-mediated signaling cascade triggered by IL-17.

There is considerable evidence linking Arid5a to IL-17–induced pathways in vivo. We saw that Arid5a was induced in an IL-17–dependent manner in the oral mucosa during *C. albicans* infections, although a contribution of Arid5a to fungal host defense has not been directly demonstrated. Arid5a$^{-/-}$ mice are resistant to EAE (*24*), an IL-17–dependent model of autoimmunity (*47*). Mice lacking either IL-17A or Arid5a show similar resistance to bleomycin-induced lung injury (*48*–*50*). In addition, Arid5a is linked to T_H17 cell differentiation through stabilization of *Il6*, *Stat3*, and *Ox40* in antigen-presenting cells or T cells (*24*, *40*, *51*, *52*) and thus may enhance IL-17–dependent

responses by virtue of increasing IL-17 expression in the inflamed environment.

IL-17 acts almost exclusively on nonhematopoietic cells (*39*), so factors such as Arid5a and MCPIP1 that target both T_H17 cells and IL-17 signaling could potentially function in one or both contexts (*21*, *22*). In this regard, the cell type(s) where Arid5a functions in EAE or bleomycin injury have not been determined; however, in a model of IL-17–driven psoriasis, MCPIP1 acts entirely within the nonhematopoietic compartment (*53*). More studies will be needed to delineate the specific cell types in which Arid5a (or MCPIP1) contributes to autoimmune responses.

Our data revealed a role for Arid5a in increasing expression of the TFs C/EBPβ and IκBζ, which play vital roles in IL-17–driven disease. For example, *Arid5a*$^{-/-}$, *Cebpb*$^{-/-}$, and *Nfkbiz*$^{-/-}$ mice are all refractory to EAE (*24*, *54*, *55*), and *Nfkbiz*$^{-/-}$ mice are also resistant to imiquimod-induced inflammation, a model representing psoriasis-like IL-17–dependent skin inflammation (*35*). Once induced, C/EBPβ and IκBζ promote expression of genes encoded by mRNAs that are not themselves intrinsically unstable, such as the canonical IL-17 target gene *Lcn2* (*6*). IL-17 enhanced *Cebpb* and *Nfkbiz* mRNA, albeit modestly (*7*, *56*), whereas concomitant protein expression of both TFs was profoundly increased. However, the mechanisms underlying translational control of these proteins have been elusive (*30*, *32*, *36*, *57*). We observed that Arid5a associated with the translation initiation complex and that Arid5a knockdown impaired translation of both C/EBPβ and IκBζ. Although TRAF2 inducibly associated with Arid5a and is implicated in promoting HuR-induced stabilization in the IL-17 pathway (*19*, *28*), TRAF2 was dispensable for promoting translation of these TFs. Consistently, TRAF2 is not found in translationally active polysomes after IL-17 stimulation (*19*). Accordingly, Arid5a appears to direct mRNA translation and mRNA stabilization by differing mechanisms. Arid5a is highly expressed in the nucleus, but TLR4 signaling induces its translocation to the cytoplasm (*58*). Although it is not known whether IL-17 similarly alters Arid5a subcellular localization, Arid5a occupancy with its target mRNAs occurred only in cytoplasmic, not in nuclear, extracts. Collectively, these findings show that, by inducing expression of these key TFs, Arid5a amplifies the IL-17–driven signaling program.

Blocking IL-17 is remarkably effective in treating psoriasis and is under evaluation for other autoimmune conditions (*59*, *60*). Hence, defining the molecular basis of IL-17 signal transduction may inform therapeutic strategies for diseases where IL-17 is implicated (*61*). Exploiting RNA is particularly attractive, given the potential for exquisite specificity in targeting otherwise "undruggable" molecules. There are emerging therapeutic approaches directed at RNA or RBPs (*62*). For example, oligonucleotide "aptamers" representing an Act1 recognition site in the *Cxcl1* 3′UTR were effective in preclinical models of autoimmunity (*28*). Arid5a was reported to be a target of the antipsychotic drug chlorpromazine (*51*), suggesting a possible option for diseases involving IL-17. Hence, gaining a mechanistic understanding of how RNA expression is regulated could lead to rational design of new therapies.

MATERIALS AND METHODS

Cell culture

ST2, HEK293T, and MEFs were cultured in α-minimum essential medium (α-MEM; Sigma-Aldrich, St. Louis MO) with L-glutamine, antibiotics, and 10% fetal bovine serum (*11*). N/TERT2G cells were cultured in KC serum-free media (Gibco) supplemented with bovine pituitary extract (30 μg/ml), recombinant human epidermal growth factor (0.2 ng/ml), $CaCl_2$, and antibiotics. Murine IL-17, human IL-17, and murine TNFα (PeproTech, Rocky Hill, NJ) were used at 200, 100, and 10 ng/ml, respectively. The IκB kinase inhibitor VII (EMD Millipore, Burlington, MA) and actinomycin D (Sigma-Aldrich, St. Louis, MO) were used at 10 μM and 5 μg/ml, respectively.

Mice

Oral candidiasis was performed by sublingual inoculation of *C. albicans* (CAF2-1) for 75 min under anesthesia (*23*). RNA was prepared from tongue after dissociation on a GentleMACS (Miltenyi Biotec, Cambridge, MA) with M tubes. Mice were age-matched on the C57BL/6 background, and both sexes were used. WT mice were from the Jackson Laboratory (Bar Harbor, ME). *Il17ra*$^{-/-}$ mice were from Amgen. Protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and adhered to guidelines in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH).

siRNA, plasmids, and qPCR

ON-TARGETplus SMARTpool siRNAs targeting Arid5a, HuR, TRAF2, and MCPIP1 were from Dharmacon (Lafayette, CO). For RNA silencing, ST2 cells and MEFs were seeded overnight in antibiotic-free α-MEM, and N/TERT2G cells were seeded in KC serum-free media (Gibco) with supplements. Transfection was performed 18 hours later with 50 nM siRNAs with DharmaFECT Reagent 1. Culture media were replaced after 24 hours, and IL-17 was administered 24 hours later. Flag/Myc-Arid5a was from OriGene (Rockville, MD). All other constructs were described (*6*, *22*, *27*, *63*). Transfections of ST2 cells and MEFS were performed with FuGENE HD (Promega, Madison, WI) or $CaPO_4$ (HEK293T cells). RNA was prepared with RNeasy kits (Qiagen, Valencia, CA). Complementary DNA was synthesized by SuperScript III First Strand Kits (Thermo Fisher Scientific, Waltham MA). Quantitative real-time PCR (qPCR) was performed with the SYBR Green FastMix (Quanta BioSciences, Beverly, MA) and analyzed on an ABI 7300 real-time instrument. Primers were from QuantiTect Primer Assays (Qiagen).

ELISA, Abs, IP, and luciferase assays

ELISA kits were from eBioscience (Thermo Fisher Scientific). Abs used for Western blots and coIP were Arid5a, FLAG (Sigma-Aldrich), β-actin horseradish peroxidase (HRP), α-tubulin HRP (Abcam, Cambridge, UK), IκBζ, Myc (Cell Signaling, Beverly, MA), C/EBPβ (BioLegend, San Diego, CA), IκBα, MCPIP1, TRAF2, Act1, and YY1 (Santa Cruz Biotechnology, Santa Cruz, CA). Protein G or protein A beads (Roche) were used to pull down Abs during coIP. Luciferase assays used the Dual-Luciferase Reporter Assay System (Promega) (*6*).

RIP and biotinylated RNA pulldown

Cytoplasmic extracts were isolated with polysome lysis buffer for Myc RIP [100 mM KCl, 5 mM $MgCl_2$, 10 mM Hepes (pH 7.0), 0.5% NP-40, and 1 mM dithiothreitol (DTT)] with RNAse Out (100 U/ml; Invitrogen) or CHAPS lysis buffer for eIF4G RIP [0.3% CHAPS, 40 mM Hepes (pH 7.5), 120 mM NaCl, 1 mM EDTA, 10 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 50 mM NaF, 1.5 mM sodium orthovanadate, and 1 mM DTT], supplemented with protease inhibitor cocktail (Sigma-Aldrich). Nuclear extracts were isolated

as described (*64*). Lysates were precleared with isotype control Abs (Abcam) and protein G agarose beads (Roche) or protein G–conjugated magnetic dynabeads for eIF4G RIP (Life Technologies) and immunoprecipitated with Myc- or eIF4G Abs (Cell Signaling) (*34*). Immunoprecipitates were incubated with protein G beads or protein G–conjugated magnetic dynabeads for eIF4G RIP. Total RNA was extracted with acid phenol. For biotinylation, 3′UTR motifs from *Il6*, *Cxcl1*, and *Csf2* (GeneArt, Thermo Fisher Scientific) were subcloned in pCR2.1. RNA was synthesized with biotinylated cytidine 5′-triphosphate (Enzo Life Sciences, Farmingdale, NY) in the TranscriptAid T7 High Yield Transcription Kit (Thermo Fisher Scientific) and mixed with lysates from HEK293T cells transfected with Flag-MycArid5a, followed by streptavidin Dynabeads M-280 (Thermo Fisher Scientific). Fractions were isolated by magnetic separation.

Statistics

ANOVA with post hoc Tukey's analysis or Student's *t* test was used to assess statistical significance, with $P < 0.05$ considered significant.

SUPPLEMENTARY MATERIALS

www.sciencesignaling.org/cgi/content/full/11/551/eaat4617/DC1
Fig. S1. Arid5a interactions within the IL-17 signaling pathway.
Fig. S2. Role of TRAF2 and HuR in Arid5a-mediated IL-17 response.
Fig. S3. Arid5a promotes IL-17–induced mRNA stability.
Fig. S4. Arid5a promotes IL-17–induced IκBζ expression.
Fig. S5. Arid5a promotes IL-17 responses through distinct mechanisms.
Fig. S6. Arid5a binds directly to target mRNA transcripts.
Fig. S7. Arid5a promotes translation of IL-17–induced mRNAs.
Fig. S8. Arid5a promotes mRNA stability of IL-17–induced genes.

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IL-17 integrates multiple self-reinforcing, feed-forward mechanisms through the RNA binding protein Arid5a

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Stabilizing the IL-17 response

The inflammatory cytokine IL-17 can stimulate both antifungal host defense and autoimmunity by promoting the stability of target mRNAs (see the Focus by Puel and Casanova). Amatya *et al*. found that IL-17 increased the abundance of the RNA binding protein Arid5a in mouse cells. Loss of Arid5a decreased the cellular response to IL-17 by reducing the mRNA stability of a selection of IL-17–stimulated transcripts. For others, Arid5a instead interacted with the translation initiation factor eIF4G to augment their translation. Thus, Arid5a uses multiple posttranscriptional mechanisms to enhance IL-17 signaling.