eLIFE
elifesciences.org

Figures and figure supplements

A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics

Nicole A Rapicavoli, et al.

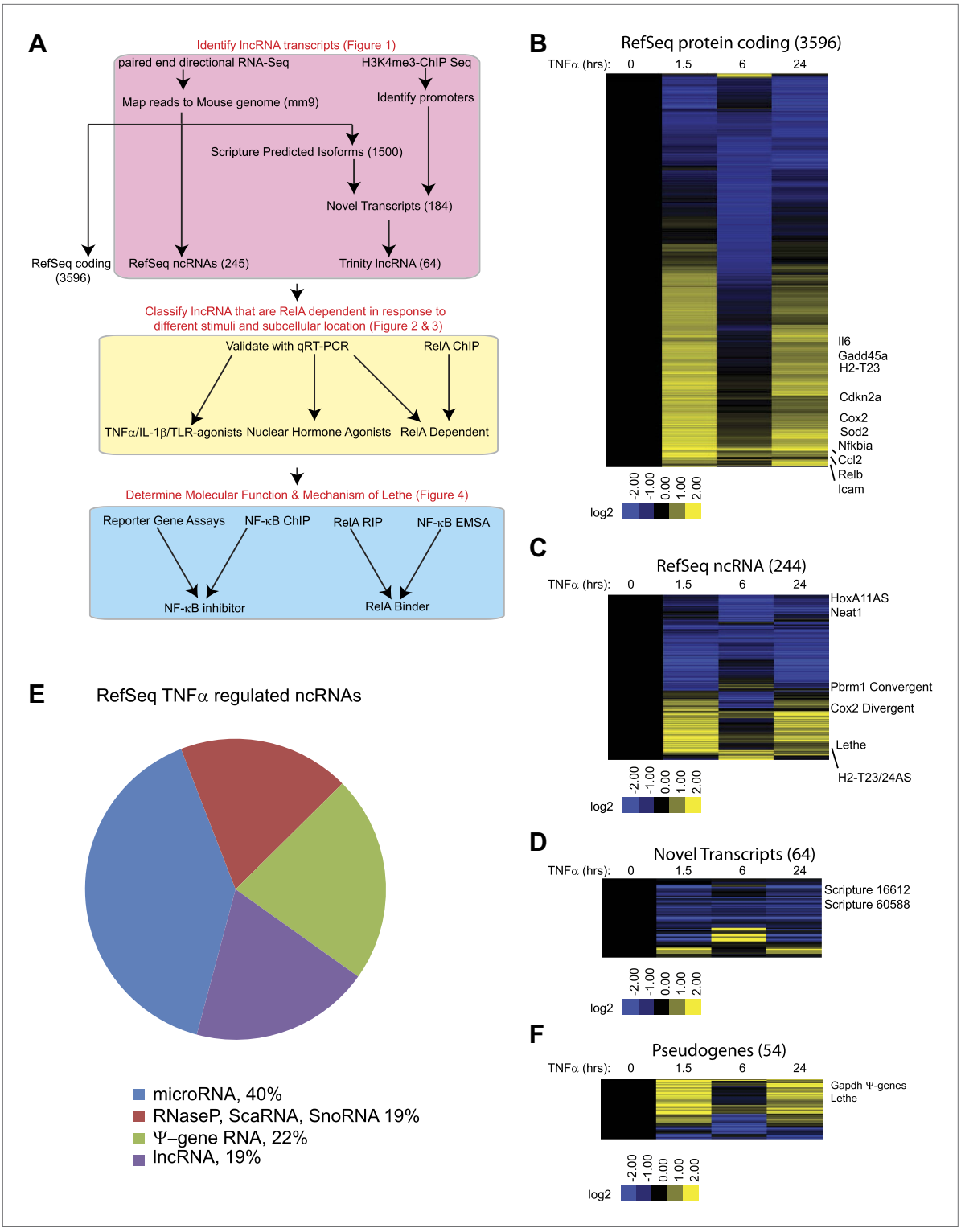

Figure 1. TNFα regulates the transcription of many coding and noncoding genes. (**A**) Workflow for strategy for discovery of NF-κB regulated lncRNAs. (**B**) 3596 RefSeq protein coding genes are regulated by TNFα. Values are normalized to the 0 hr time point. (**C**) 244 RefSeq ncRNAs are regulated by TNFα. (**D**) 64 de novo lncRNAs are regulated by TNFα. (**E**) The fraction of all RefSeq ncRNAs for each class of transcript. (**F**) 54 pseudogene lncRNAs are regulated by TNFα.

DOI: 10.7554/eLife.00762.003

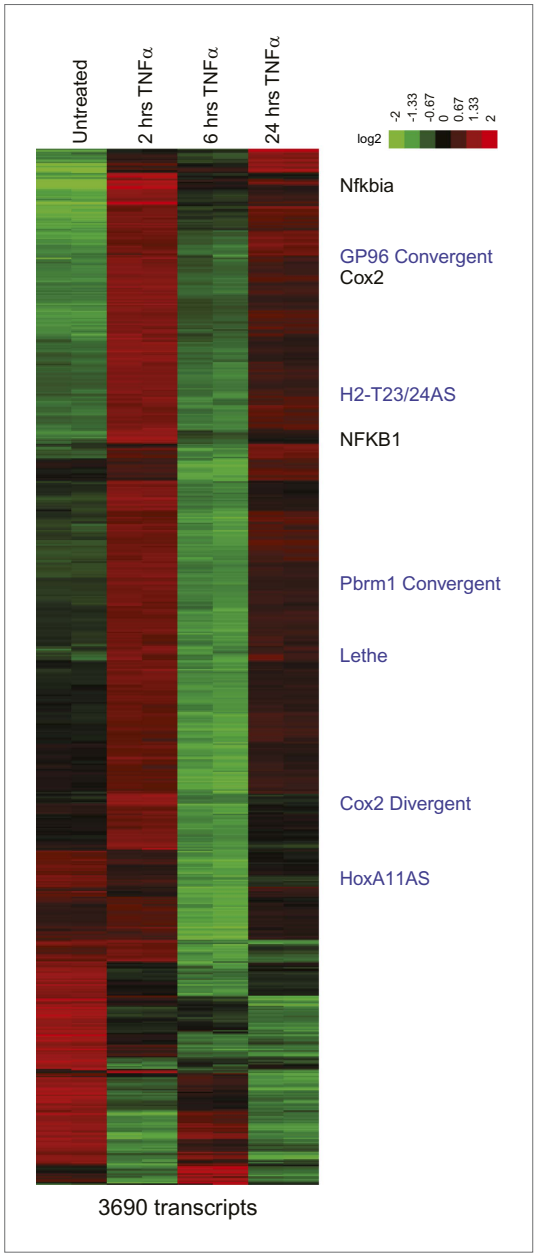

Figure 1—figure supplement 1. Heatmap of RefSeq genes. Mean centered heatmap of RefSeq protein coding genes and RefSeq lncRNAs.
DOI: 10.7554/eLife.00762.004

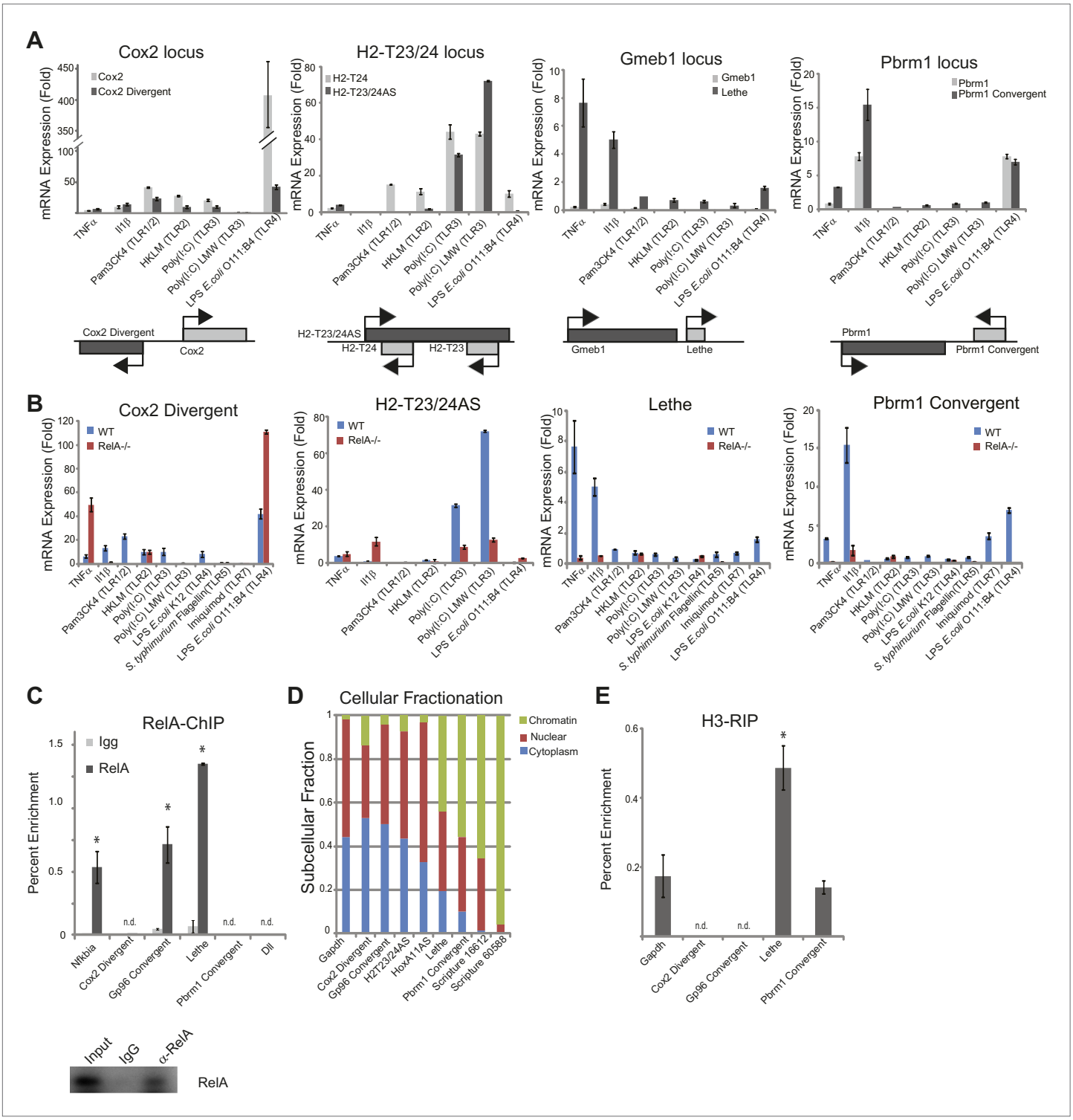

Figure 2. LncRNAs distinguish between different stimuli and are regulated by NF-κB. (**A**) Validation of lncRNAs expression alongside the closest protein coding gene under a variety of different stimuli by qRT-PCR. Genomic organization is shown below. MEFs were treated with 20 ng/ml TNFα for 0 and 6 hr. Quantitative Taqman real time RT-PCR of the indicated RNAs is normalized to Actin levels (mean ± SD). (**B**) LncRNAs are regulated by RelA. qRT-PCR in WT and *RelA*−/− littermate cells under a variety of different stimuli. MEFs were treated with 20 ng/ml TNFα for 0 and 6 hr. Quantitative Taqman real time RT-PCR of the indicated RNAs is normalized to Actin levels (mean ± SD). (**C**) Endogenous RelA is recruited to the promoters of lncRNAs. MEFs were treated with 20 ng/ml TNFα for 0 and 15 min. ChIP with α-RelA antibodies was performed and RelA percent enrichment relative to input is shown (mean ± SD, *Nkfbia*, p<0.0518; *Gp96 Convergent*, p<0.007; *Lethe*, p<0.002). (**D**) LncRNAs are found throughout the cell. Cellular fractionation was performed

Figure 2. Continued on next page

Figure 2. Continued

and fraction found in the chromatin, nucleus and cytoplasm is shown. MEFs were treated with 20 ng/ml TNFα for 6 hr. Quantitative Taqman real time RT-PCR of the indicated RNAs is shown (mean ± SD is shown). (**E**) LncRNAs are found on the chromatin. MEFs were treated with 20 ng/ml TNFα for 6 hr. RNA-IP with α-H3 antibodies was performed. RNA was isolated and Quantitative Taqman real time RT-PCR of the indicated RNAs is shown (mean ± SD, Lethe $p<0.004$).

DOI: 10.7554/eLife.00762.005

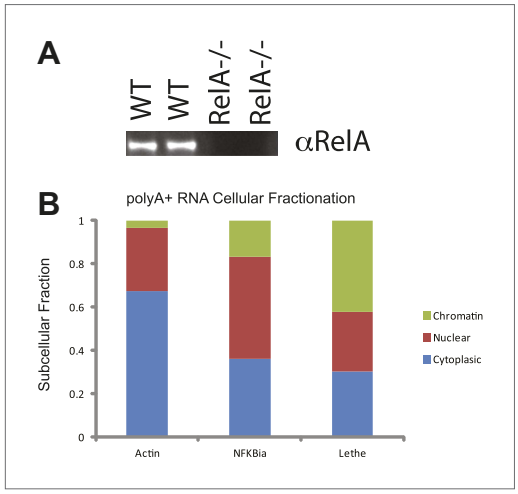

Figure 2—figure supplement 1. (**A**) Western analysis of RelA protein levels. (**B**) PolyA+ Lethe is found on the chromatin. Immunoblot of wildtype and RelA−/− MEFs. Cellular fractionation was performed, total RNA was purified and polyA+ selection was performed. The fraction polyA+ RNA found in the chromatin, nucleus and cytoplasm is shown. MEFs were treated with 20 ng/ml TNFα for 6 hr. Quantitative Taqman real time RT-PCR of the indicated RNAs is shown (mean ± SD is shown).

DOI: 10.7554/eLife.00762.006

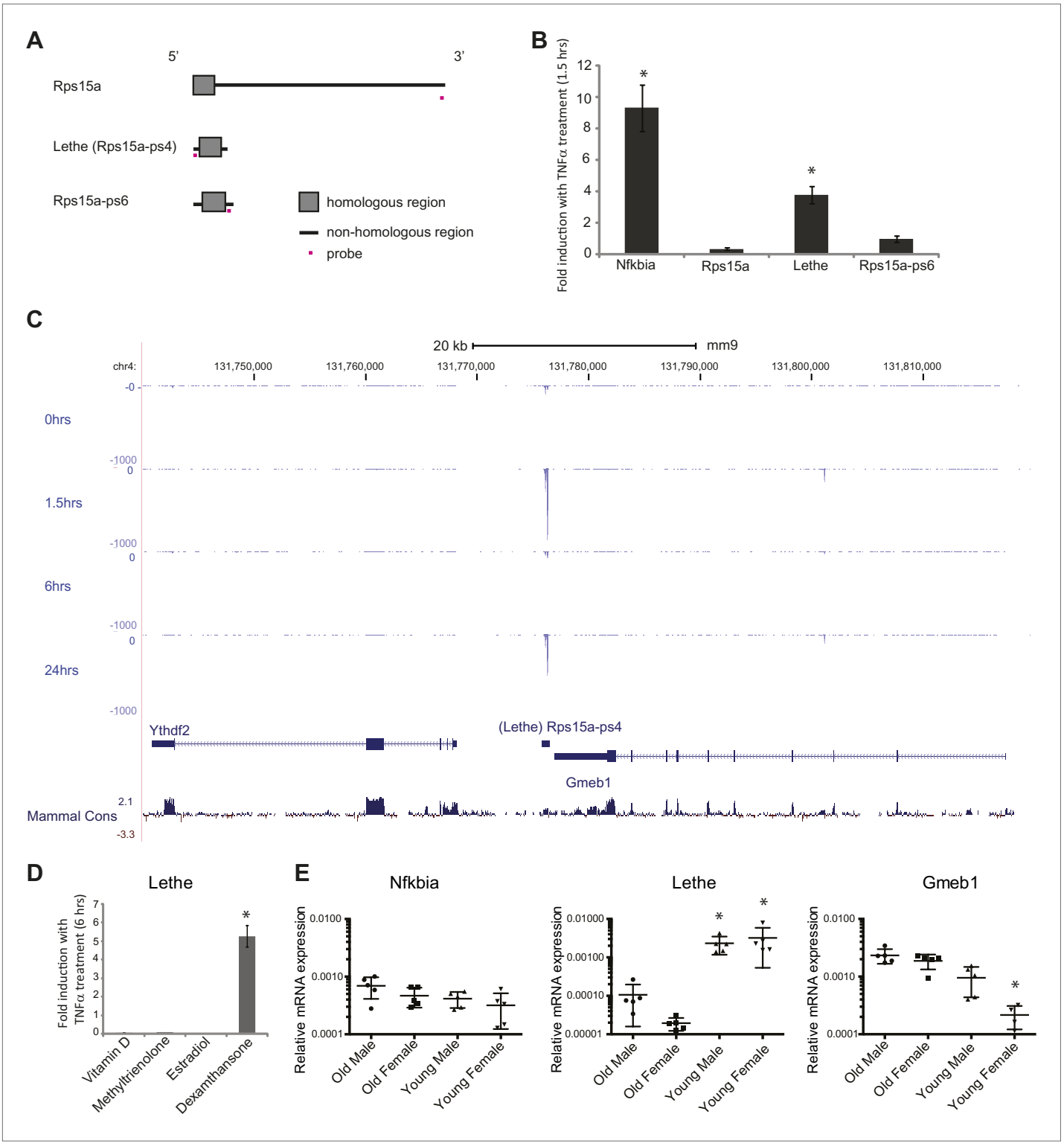

Figure 3. Lethe is a pseudogene lncRNAs that is regulated by NF-κB, Glucocorticoid Receptor and in aging. (**A**) Gene structure, homology and Taqman probe design of *Rps15a* and pseudogene family members. (**B**) Lethe is induced by TNFα, but other family members are not. MEFs were treated with 20 ng/ml TNFα for 0 and 1.5 hr. Quantitative Taqman real time RT-PCR of the indicated RNAs is shown normalized to Actin levels (mean ± SD, p<0.012). (**C**) Genomic organization of Lethe with RNA-Seq data at time 0, 1.5, 6 and 24 hr post TNFα treatment. Lethe is located on mouse chromosome 4 between *Gmeb1* and *Ythd2*. *Gmeb1* and *Ythd2* are not induced by TNFα stimulation. (**D**) Lethe is induced by dexamethasone treatment, but not other nuclear hormone receptor agonists. MEFs were treated with either 10 nM vitamin D, 100 nM methyltrienolone, 100 nM estradiol, or 1 μM

Figure 3. Continued on next page

Figure 3. Continued

dexamethasone for 0 and 6 hr. Quantitative Taqman real time RT-PCR of the indicated RNAs is shown normalized to Actin levels (mean ± SD, $p<0.003$). (**E**) Lethe is down-regulated in aged mice. Lethe is expressed in young spleen from male and female mice. Five mice were used for each sex and time point. Quantitative Taqman real time RT-PCR of the indicated RNAs is shown normalized to Actin levels (mean ± SD, Lethe $p<0.001$, Gmeb1 $p<0.003$). ANOVA analysis was performed to determine significance.

DOI: 10.7554/eLife.00762.008

```
CLUSTAL 2.1 multiple sequence alignment


Lethe           ----------------AGG--TAAACA---CCCAGAGCCCAGTTTGTCCAAGGGACCCCA 39
Rps15a-6        TCTGCTGGTGAAACAGAGGCGTAGATAGGTTTAAAAGCCTAGGTAGTTTAA--AACTCCA 58
                               ***  ** * *      * **** ** * **  **   ** ***

Lethe           G----GGGA------------------------CTCTTTCCTC------------TTCAA- 59
Rps15a-6        GATATGGGATGAGTGATGGTTAAGAGCACTGGCTGTTTCTCCACAAGACCCAGGTTCAAT 118
                *    ****                       ** ****  *            *****

Lethe           ----AGC----------CTGTTC-------TCTGTCCC--------------TCTGTTCA 84
Rps15a-6        TCCCAGCACATACATGGCTGCTCACACCTGTCTGTTCCAGGAGATCTAATGCTCTCTTTT 178
                    ***          *** **       ***** **              *** **

Lethe           GGACTGAGGAGG-------------ACAT------CAGAAA---------------------- 106
Rps15a-6        GGCCTCTGGGGGCATCAAGCATGCACATGGTGTACAGAAATATATGGTGGCAAAACATTC 238
                ** **  ** **             ****      ******

Lethe           --------GAACTCAGAATCCGCAC---TCTTTCTGCCATCTTCCCTCGCCGCCACCATG 155
Rps15a-6        ATGCATATAAAATTAAAACCAAAACAACCCTTTCTGCCATCTTCCTTCGCCGCCACCATG 298
                         ** * * ** *   **    *************** **************

Lethe           GTGCGAATGAATGTTCTGGCGGATGCTCTCAGGAGCATCAACAACGCTGAGAAGAGAGGC 215
Rps15a-6        GTGTGAATGAATGTTCTGGCGGATGCTCTCAAGAGCATCAACAACGCTGAGAAGAGAGGC 358
                *** ************************** ****************************

Lethe           AAACGCCAGGTCCTCATCAGGCCATGTTCTAAAGTCATCGTTCGGTTCTTAACCGTGATG 275
Rps15a-6        AAACGCCAGGTCCTCATCAGGCCATGTTCTAAAGTCATCGTTCGGTTCTTAACTGTGATG 418
                ************************************************** ******

Lethe           GTGAAGCACGGGTACATTGGTGAATTCGAGATCATTGATGATCACAGAGCTGGGAAGATT 335
Rps15a-6        ATGAAGCACGGATACATTGGTGAATTCGAGATCATTGATGGTCACAGAGCTGGGAAGATT 478
                 ********** ***************************** ******************

Lethe           GTTGTGAACCTCACAGGAAGGTTGAACAAGTGTGGCGTTATAAGCCCTAGATTTGATGTT 395
Rps15a-6        GTTGTGAACCTCACAGGAAGGTTGAACAAGTGTGGCGTTATAAGCCCTAGATTTGATGTT 538
                ************************************************************

Lethe           CAACTCAAAGACCTAGAGAAATGGCAGAACAACCTGCTCCCTTCACGGCAGTTTGGCTTC 455
Rps15a-6        CAACTCAAAGACCTAGAGAAATGGCAGAACAACCTGCTCCCTTCACGTCAGTTTGGCCTC 598
                ********************************************* ********* **

Lethe           ATTGTGCTGACAACCTAGGCTGGCATCATGAACCATGAAGAGGCAAGACGAAAACATACA 515
Rps15a-6        ATTGTGCTGACAACCTCGGCTGGCATTATGGACCATGAAGAGGCAAGACGAAAACATACA 658
                *************** ********* *** ****************************

Lethe           GGAGGGAAAATCCTGGGATTCCTTTTTAAAATGTAAAGCATAAATAAAAAGCCTTCGTGG 575
Rps15a-6        GGAGGGAAAATCCTGGGATTCTTTTTTTAAATGTAAAGCATAAATAAAAAGCCTTTGTGG 718
                ******************** ***** ************************** ****

Lethe           ACTGTGAAAAAAAAAAAAAAAAAGAACTCAGAATCCTTGATCTACACTCCTCTTCTGCATC 635
Rps15a-6        ACTGTGAAAAAAAAAAAAAAA-----CCAAAACAACCAAACAAAACCTCC---CCAAACA 770
                ********************      ** **      * * * **  *    *   *

Lethe           ATCTTTTCAGCTCCTAAAAAGCCATAGTTTGATTCTCATGCTCTGCCACA-TTAGTAACT 694
Rps15a-6        AACCCCCCCGAAACCAAGAACACGCAACCCAG-----ATACCCAGATGTAGTCTATAAT- 824
                * *    * *   * ** **  *  *           ** * * *    * *   ***

Lethe           TCC 697
Rps15a-6        ---
```

Figure 3—figure supplement 1. Alignment of Lethe with Rps15a-ps6. ClustalW2 alignment was performed on Lethe (Rps15a-ps4) and Rps15a-ps6.

DOI: 10.7554/eLife.00762.009

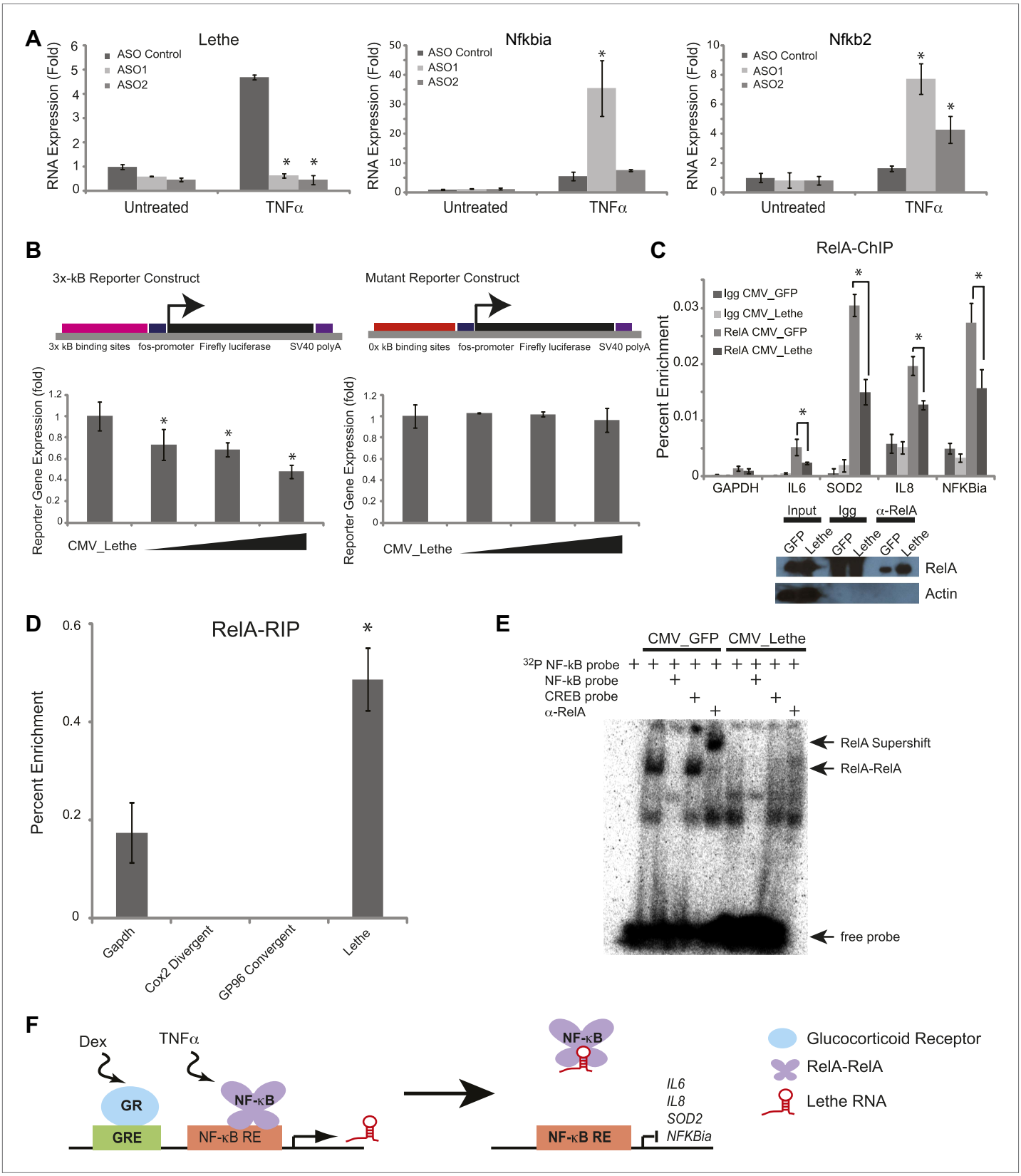

Figure 4. Lethe Binds to RelA and inhibits RelA occupancy of DNA. (**A**) Increased expression of NF-κB regulated genes in Lethe knockdown cells. Quantitative Taqman real time RT-PCR of the indicated RNAs is shown normalized to Actin levels (mean ± SD, p<0.05 is shown) (**B**) Lethe inhibits TNFα

Figure 4. Continued on next page

Figure 4. Continued

induced reporter gene expression. RLU of 3x-κB reporter activity and mutant reporter activity (mean ± SD, $p<0.05$ is shown) in CMV_Lethe transfected 293T cells. Reporter constructs are diagrammed above. (**C**) Endogenous RelA recruitment to the promoters of target genes is reduced in the presence of Lethe. 293T expressing CMV_GFP or CMV_Lethe were treated with 20 ng/ml TNFα for 15 min. ChIP with α-RelA antibodies was performed and RelA percent enrichment relative to input is shown (mean ± SD; *Il6*, $p<0.033$; *Sod2*, $p<0.001$; *Il8*, $p<0.003$; *Nfkbia*, $p<0.015$). (**D**) Lethe binds to RelA. MEFs were treated with 20 ng/ml TNFα for 6 hr. RNA-IP with α-RelA antibodies was performed. RNA was isolated and Quantitative Taqman real time RT-PCR of the indicated RNAs is shown (mean ± SD, $p<0.020$). (**E**) Lethe expression blocks DNA binding of the RelA homodimer to its target. NF-κB EMSA of GFP or Lethe transfected 293T nuclear extracts treated with 20 ng/ml TNFα for 15 min. Extracts were pretreated with unlabeled NF-κB (specific) or CREB (nonspecific competitor), or α-RelA antibodies for 15 min prior to incubation with probe. (**F**) Model for Lethe regulation of gene expression. Upon addition of TNFα or dexamthasone, Lethe is transcribed. Lethe can then bind to RelA–RelA homodimers and block binding to other NF-κB response elements, inhibiting NF-κB.

DOI: 10.7554/eLife.00762.010