

Intrapulmonary administration of purified NEIL2 abrogates NF- κ B-mediated inflammation

Nisha Tapryal¹, Shandy Shahabi², Anirban Chakraborty¹, Koa Hosoki^{1,3}, Maki Wakamiya⁴, Gobinda Sarkar^{5,6}, Gulshan Sharma¹, Victor J. Cardenas¹, Istvan Boldogh⁷, Sanjiv Sur^{1,3}, Gourisankar Ghosh², and Tapas K. Hazra^{1*}

List of Supporting Material

1. Table S1
2. Table S2
3. Figure S1-S7

Table S1. List of primers used in qPCR assays.

Exon Primers	Sequence
mCxcl1-exon-For	5' CCTATCGCCAATGAGCTGC 3'
mCxcl1-exon-Rev	5' ACTTGGGGACACCTTTTAGC 3'
mCxcl2-exon-For	5' CACCAACCACCAGGCTACA 3'
mCxcl2-exon-Rev	5' GGATGATTTTCTGAACCAGGGG 3'
mCxcl10-exon-For	5' GATCCCTCTCGCAAGGACG 3'
mCxcl10-exon-Rev	5' TCAACACGTGGGCAGGATAG 3'
mIl6-exon-For	5' GTCCTTCCTACCCCAATTTCCA 3'
mIl6-exon-Rev	5' AGGTTTGCCGAGTAGATCTCAA 3'
mTnf α -exon-For	5' AAATGGTTCGTACAGCAGCG 3'
mTnf α -exon-Rev	5' GCACGGAAGTAGTCTCCACG 3'
mIL1 β -exon-For	5' CATCAGCACCTCACAAGCAG 3'
mIL1 β -exon-Rev	5' TAAGTGGTTGCCCATCAGAGG 3'
mGapdh-exon-For	5' GTCACCAGGGCTGCCATTTG 3'
mGapdh-exon-Rev	5' GATGTTAGTGGGGTCTCGCT 3'
Promoter Primers	Sequences
mCxcl1-Pro-For	5' ATCCTTGGGAGTGGAGCAAG 3'
mCxcl1-Pro-Rev	5' GGAGTCTGGAGTGCTGGAAC 3'
mCxcl2-Pro-For	5' AGCGCAGACATCACTTCCTT 3'
mCxcl2-Pro-Rev	5' TGAAGTGTGGCTGGAGTCTG 3'
mCxcl10-Pro-For	5' GCTCACGCTTTGGAAAGTGAA 3'
mCxcl10-Pro-Rev	5' ATGTCTCTCAGCGGTGGATG 3'
mIl6-Pro-For	5' CCCACCCTCCAACAAAGATT 3'
mIl6-Pro-Rev	5' CAGAGAGGAACTTCATAGCGGT 3'
mTNF α -Pro-For	5' TCTCAAGCTGCTCTGCCTTC 3'
mTNF α -Pro-Rev	5' GACCATGCCTGTGTCTATTTCC 3'

Table S2. List of the mouse DNA oligonucleotides used in EMSA analysis. For clarity, the NF- κ B motif sequences are shown in red and bases marked in blue represent the alterations in sequence to generate mutation.

DNA Oligos	Label	Duplex Sequence
mCxcl1-WT	C1-WT (Cxcl1)	<p style="text-align: center;">NF-κB Motif</p> 5' GAAACACCCTGTACTCCGGGAATTTCCCTGGCCCGGAGCTCTG 3' 3' ATTTGTGGGACATGAGGCCCTTAAAGGGACCGGGCCTCGAGAC 5'
mCxcl1-Mutant	C1-M	5' GAAACACCCTGTACTCCGAAAAGGTCCCTGGCCCGGAGCTCTG 3' 3' CTTTGTGGGACATGAGGCTTTTCCAGGGACCGGGCCTCGAGAC 5'
mCxcl2-WT	C2-WT (Cxcl2)	<p style="text-align: center;">NF-κB Motif</p> 5' GGACCCTGAGCTCAGGGAATTTCCCTGGTCCCCGGGCT 3' 3' CCTGGGACTCGAGTCCCTTAAAGGGACCAGGGGCCCGA 5'
mCxcl2-Mutant	C2-M	5' GGACCCTGAGCTCAGAAAAGGTCCCTGGTCCACGGGCT 3' 3' CCTGGGACTCGAGTCTTTTCCAGGGACCAGGTGCCCGA 5'
mIl6-WT	IL6-WT (Il6)	<p style="text-align: center;">NF-κB Motif</p> 5' TTTTATCAAATGTGGGATTTTCCCATGAGTCTCAAAA 3' 3' AAAAAATAGTTTACACCCTAAAAGGGTACTCAGAGTTTT 5'
mIl6-Mutant	IL6-M	5' TTGCTATTAAATGTGTTCTGTTTGTACAGAGTCTCAAAA 3' 3' AACGATAATTTACACAAGCAAAACATGTCTCAGAGTTTT 5'
mTnf α -WT	Tnf α	<p style="text-align: center;">NF-κB Motif</p> 5' AAGAACTCAAACAGGGGGCTTTCCCTCCTCAATATCAT 3' 3' TTCTTGAGTTTGTCCTCCGAAAGGGAGGAGTTATAGTA 5'

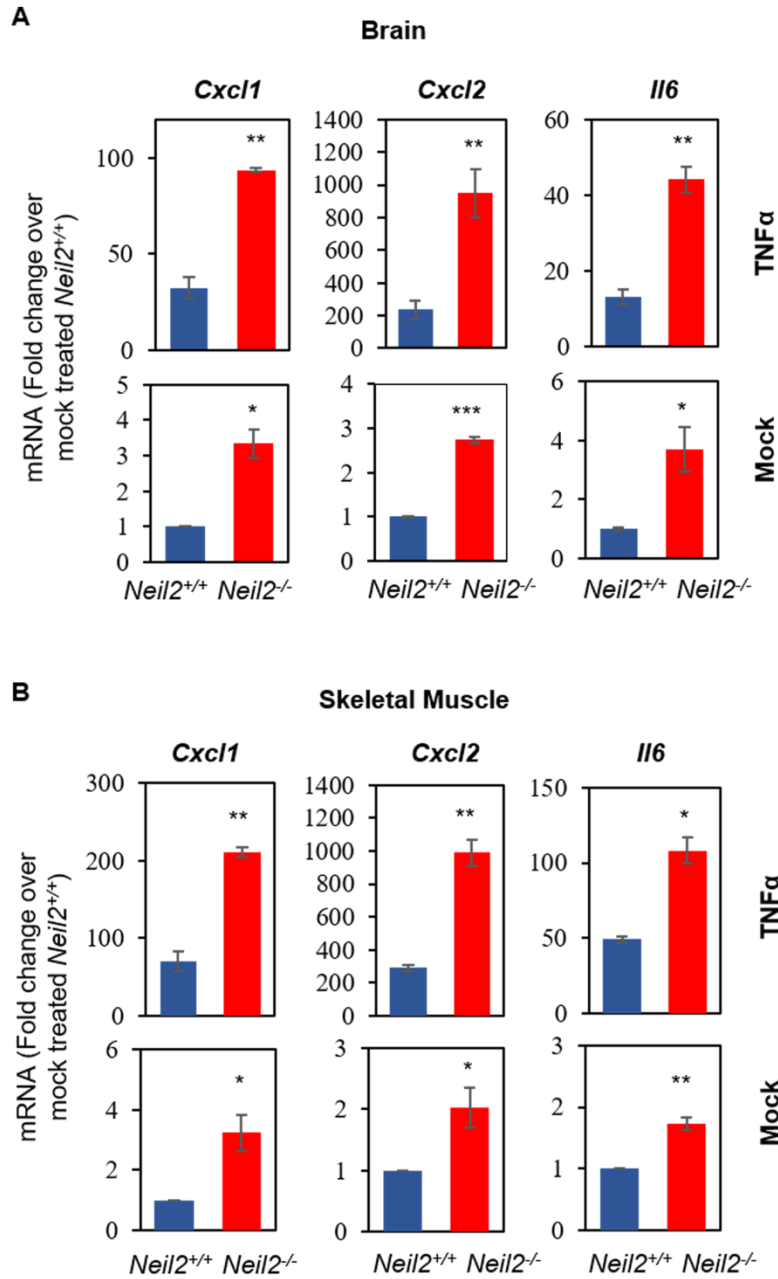


Figure. S1. Systemic inflammation in brain and skeletal muscle of *Neil2*^{-/-} vs. *Neil2*^{+/+} mice.

(A and B) Real-Time quantitative reverse transcription polymerase chain reaction analysis with total RNA isolated from brain (A) and skeletal muscles (B) of mock- (lower panel) or TNF α -treated (upper panel) (intraperitoneal) *Neil2*^{+/+} or *Neil2*^{-/-} mice relative to mock-treated *Neil2*^{+/+} groups. Results are normalized to *Gapdh*. Error bars represent \pm standard deviation from the mean. n=2 independent experiments from samples prepared by pooling tissues from n=3 mice per group. *= P <0.05; **= P <0.01; ***= P <0.005 vs. *Neil2*^{+/+} groups.

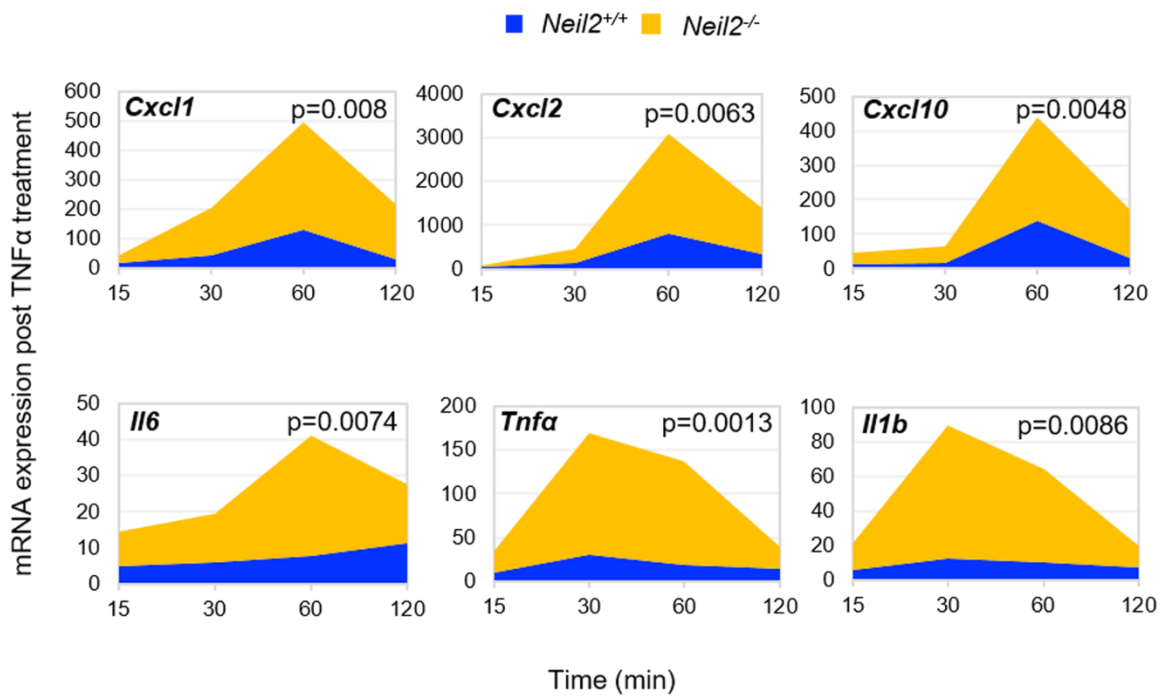


Figure. S2. TNF α -induced inflammatory gene expression in the lungs of *Neil2*^{-/-} vs. *Neil2*^{+/+} mice. Area plot displays mRNA expression of *Cxcl1*, *Cxcl2*, *Cxcl10*, *Il6*, *Tnfa* and *Il1 β* in *Neil2*^{+/+} or *Neil2*^{-/-} mice lung at 15-, 30-, 60- and 120-min post TNF α intranasal treatment as analyzed by Real-Time quantitative reverse transcription polymerase chain reaction analysis. Results are normalized to *Gapdh* expression and mock-treated *Neil2*^{+/+} groups; n=2 independent experiments, with data collected from n=4 mice per group; p-values for *Neil2*^{-/-} vs. *Neil2*^{+/+} groups are shown.

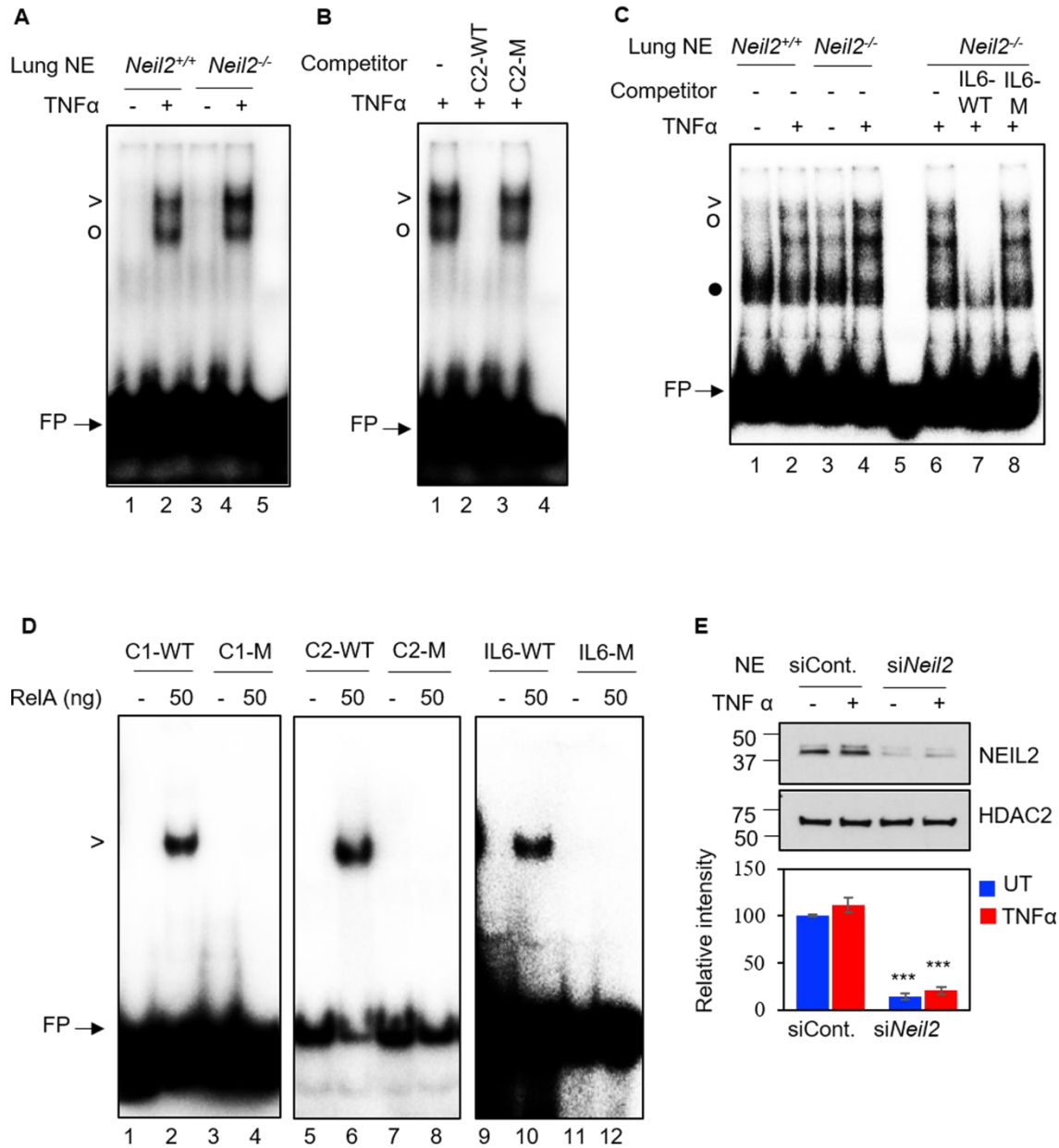


Figure. S3. Electrophoretic mobility-shift assays (EMSAs) of NF-κB's binding to its cognate motif derived from target gene promoters.

(A-C) EMSA using nuclear extracts (NEs) from mock- or TNFα-treated intranasally *Neil2*^{+/+} or *Neil2*^{-/-} male mice lung (pooled, n=3) with ³²P-labeled probe containing wild-type κB-motif from mouse *Cxcl2* (C2-WT, A and B) and *Il6*- promoter (IL6-WT, C); competition analysis in B, lanes 1-3, and in C, lanes 6-8 with 100-fold molar excess of wild-type or mutant competitor as indicated. (D) EMSAs of recombinant RelA protein complex using ³²P-labeled wild-type C1-WT, C2-WT and IL6-WT or mutant C1-M, C2-M and IL6-M NF-κB-motifs; Lanes 1, 3, 5, 7, 9 and 11 show 'probe only' lanes. (E)

Immunoblot of NEIL2 in nuclear extracts (NEs) from untreated (-) or TNF α -treated (+) MLE12 cells transfected with control siRNA (*siCont.*) or NEIL2-specific siRNA (*siNeil2*). HDAC2 was used as loading control; Quantification shows NEIL2 band intensities normalized to HDAC2 and represented relative to untreated *siCont.* group arbitrarily taken as 100. Representative data images from three independent experiments are shown. > and \circ denote RelA-DNA complexes; \bullet denotes nonspecific complex; FP represents free probe. Error bars represent \pm standard deviation from the mean. n=3 independent experiments. ***= $P < 0.005$ vs. *siCont.* groups.

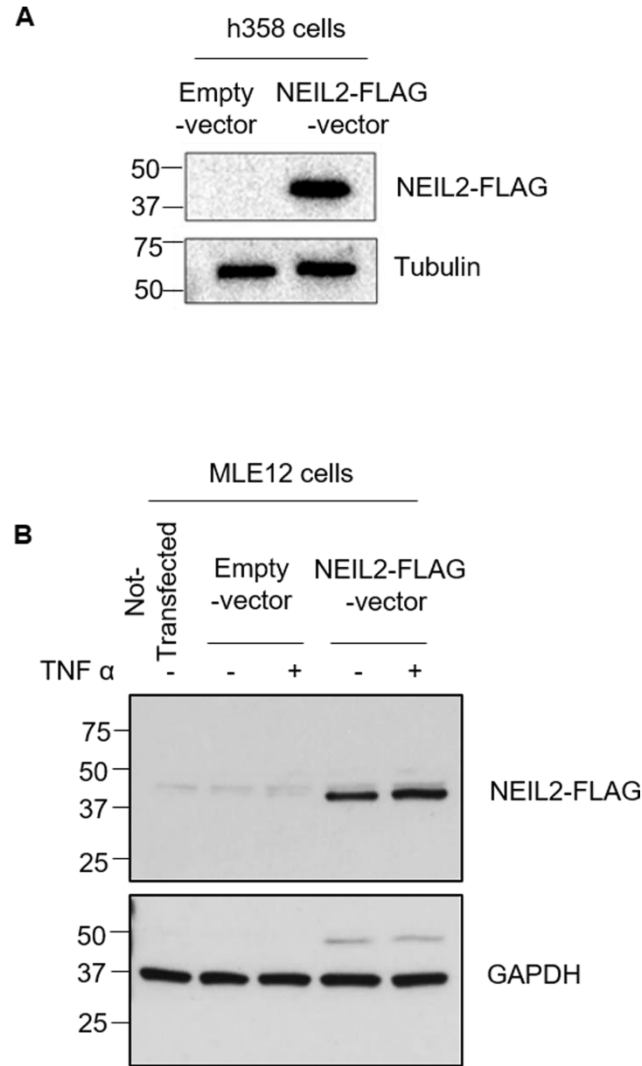


Figure. S4. NEIL2 overexpression in human and mouse cell lines.

(A) Immunoblot of cell extracts from h358 cells expressing control (Empty)- or NEIL2-FLAG-vectors, using anti -FLAG or -Tubulin antibodies. (B) Immunoblot of cell extracts from untreated (-) or TNF α -treated (+) MLE12 cells transfected with control or NEIL2-FLAG vectors, using anti -FLAG or -GAPDH antibodies. Un-transfected/untreated MLE12 cell extract was loaded as negative control. Representative data images from two independent experiments are shown.

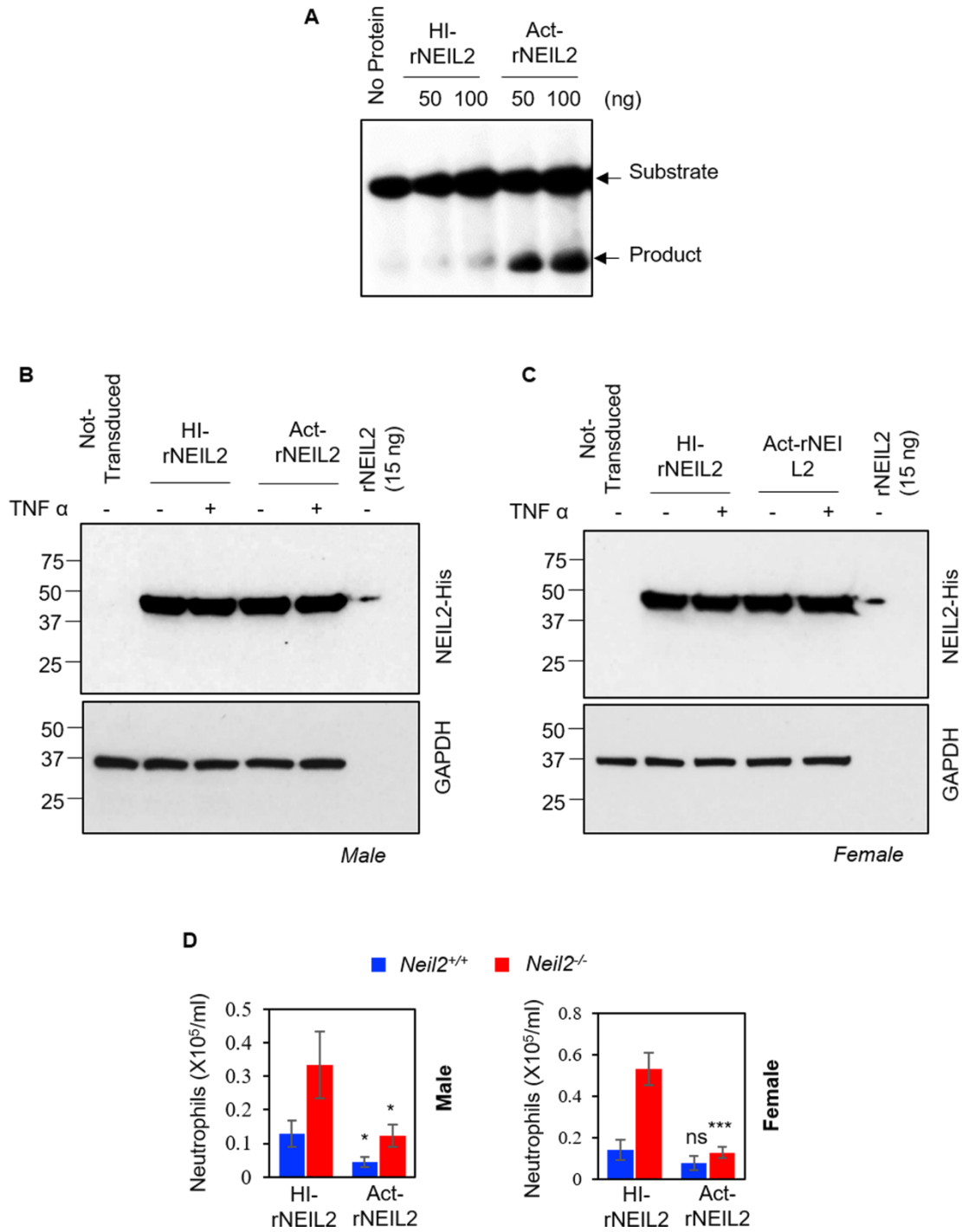


Figure. S5. Intrapulmonary rNEIL2 delivery in $Neil2^{+/+}$ or $Neil2^{-/-}$ male and female mice.

(A) Analysis of glycosylase activity of HI-rNEIL2 or Act-rNEIL2 with ^{32}P -labeled 5-hydroxyuracil DNA-bubble substrate (51-mer). The substrate and the cleaved 25-mer product are indicated by arrows. (B and C) Immunoblots of whole cell extracts from $-/+TNF\alpha$ treated $Neil2^{+/+}$ male (B) or female (C) mice lung,

72 h post-transduction with HI-rNEIL2 or Act-rNEIL2 using anti-His and GAPDH antibodies. Act-rNEIL2 protein (15 ng) was loaded as positive control. **(D)** Neutrophil counts post 16 h mock-challenge in broncho-alveolar lavage fluid of *Neil2*^{+/+}/*Neil2*^{-/-} male and female mice lung transduced with HI-rNEIL2 or Act-rNEIL2. Error bars represent \pm standard deviation from the mean (n=3). ns= not significant; *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.005$ vs. HI-rNEIL2 transduced group.

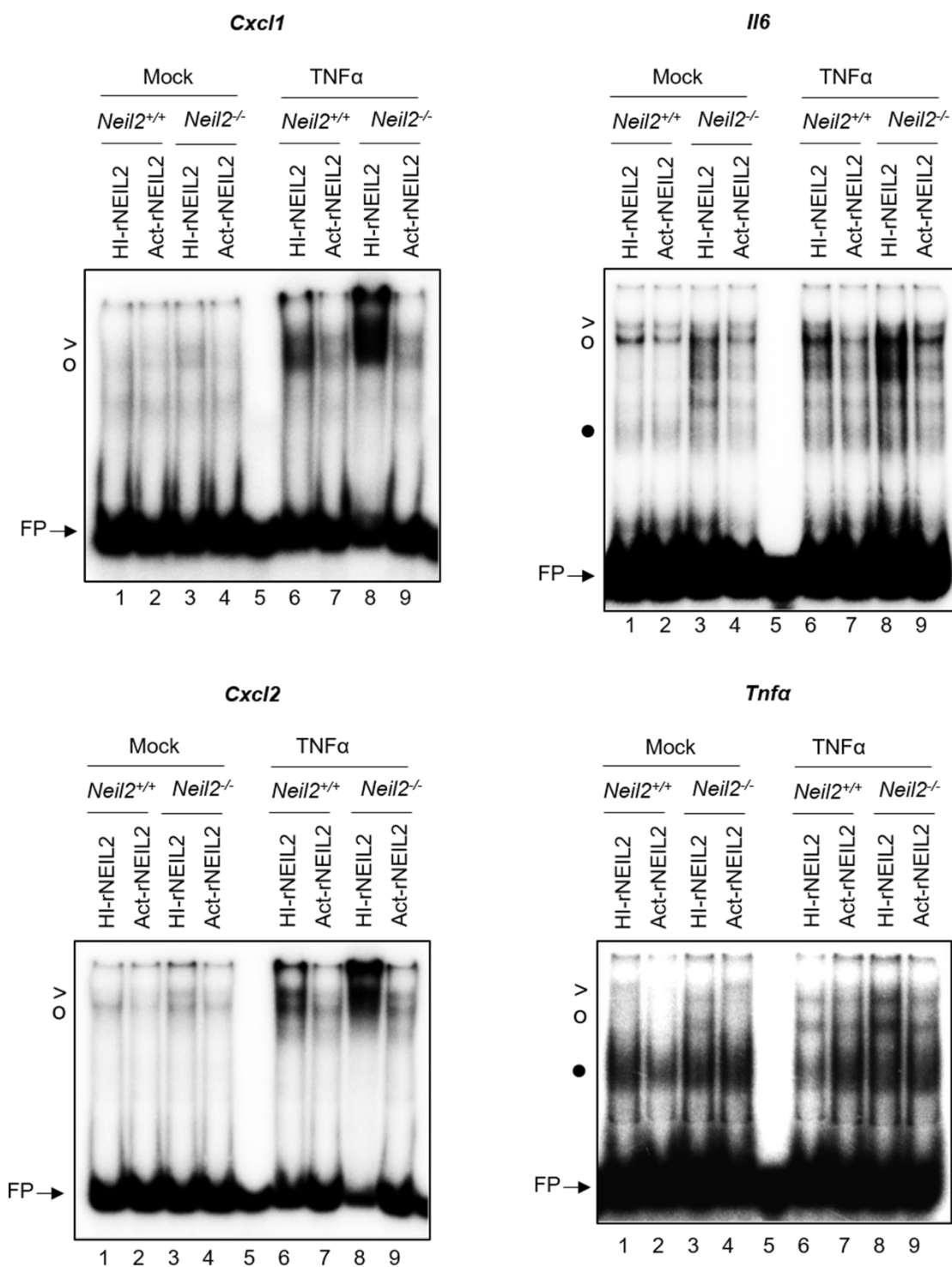


Figure. S6. Electrophoretic mobility-shift assays (EMSA) with lung nuclear extract from *Neil2*^{+/+} or *Neil2*^{-/-} male mice.

Full size EMSA images for Figure 5E are displayed here. EMSAs of NF-κB binding using nuclear extracts pooled from *Neil2*^{+/+}/*Neil2*^{-/-} male mice lung (n=3), transduced with HI-rNEIL2 or Act-rNEIL2 prior to

mock/TNF α -exposure, with radiolabeled probes derived from *Cxcl1*, *Cxcl2*, *IL6* and *Tnfa* promoters as indicated on the top; Representatives of three independent gels are shown; > and ^o denote RelA-DNA complexes; ● denotes nonspecific complex; FP represents free probe. lane 5: probe only.

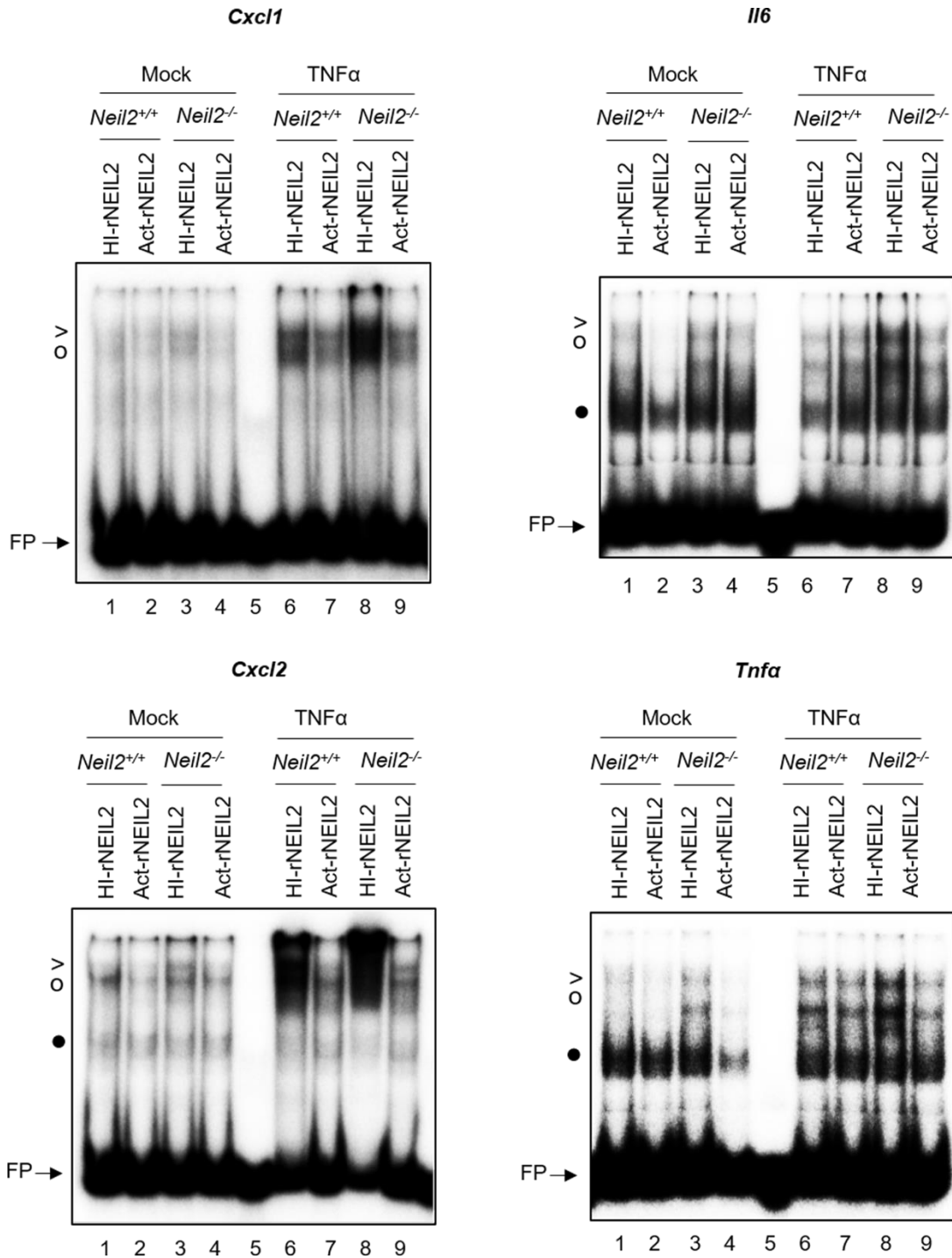


Figure. S7. Electrophoretic mobility-shift assays (EMSAs) with lung nuclear extract from *Neil2*^{+/+} or *Neil2*^{-/-} female mice.

EMSA of NF-κB binding using nuclear extracts pooled from *Neil2*^{+/+}/*Neil2*^{-/-} female mice lung (n=3), transduced with HI-rNEIL2 or Act-rNEIL2 prior to mock/TNFα-exposure, with radiolabeled probes

derived from *Cxcl1*, *Cxcl2*, *IL6* and *Tnfa* promoters as indicated on the top; Representatives of three independent gels are shown; > and ^o denote RelA-DNA complexes; ● denotes nonspecific complex; FP represents free probe. lane 5: probe only.