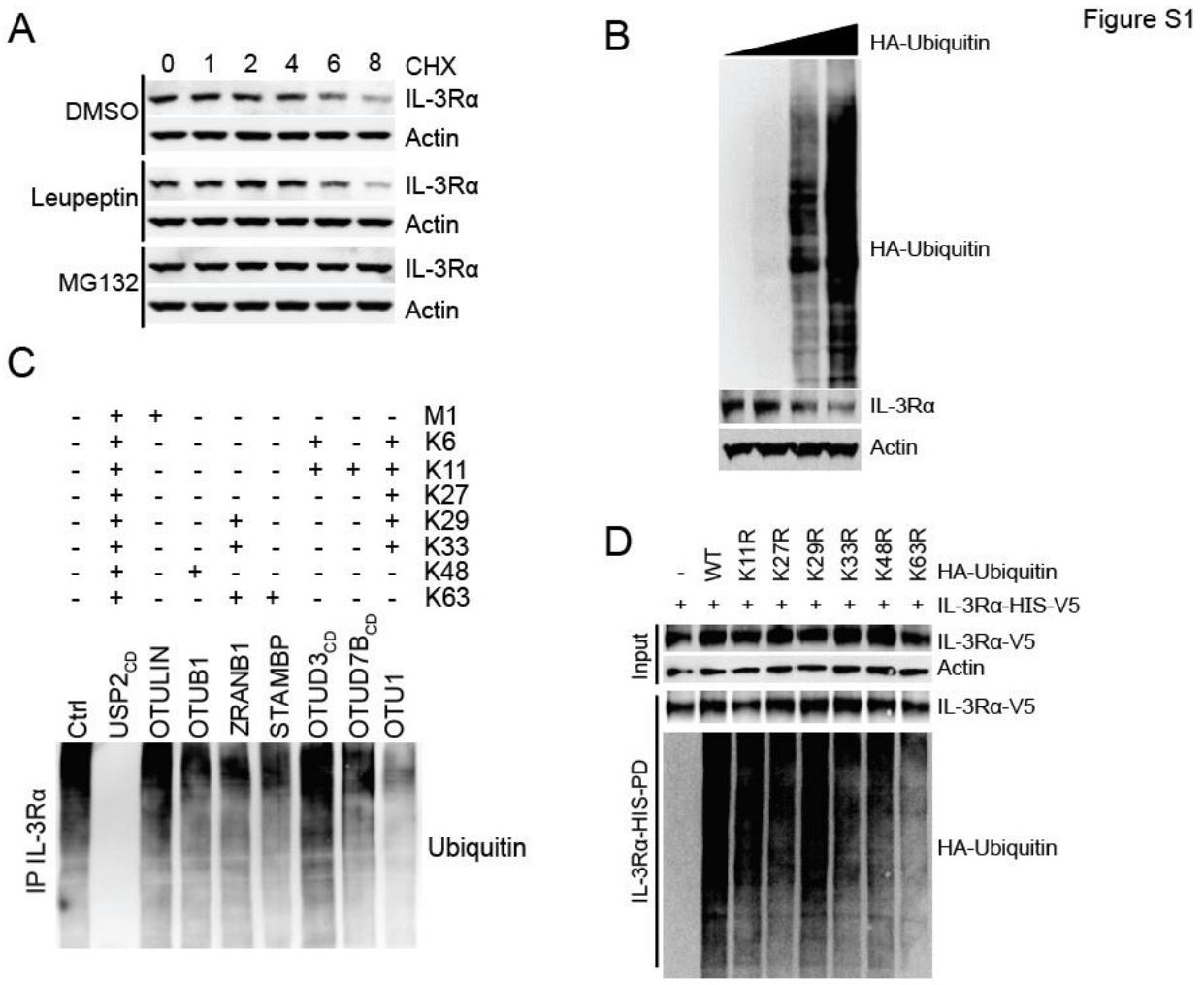


**Figure S1. IL-3Rα is ubiquitinated and assembles specific poly-ubiquitin linkage types.** (**A**) Western blot analysis of IL-3Rα abundance in lysates of RAW 264.7 cells treated with cycloheximide (CHX) with or without MG-132 or Leupeptin over time. Blots are representative of 2 independent experiments. (**B**) Western blot analysis of IL-3Rα abundance in lysates of MLE cells transfected with increasing amounts of a HA-Ubiquitin construct. Blots are representative of 2 independent experiments. (C) UbiCREST analysis of IL-3Rα immunoprecipitates from lysate of MLE cells after MG132 treatment. Blots are representative of 2 independent experiments. (D) Western blot analysis of IL-3Rα and Ubiquitin in IL-3Rα immunoprecipitates from lysate of MLE cells transfected with V5-IL-3Rα and HA-Ubiquitin K→R point mutants after MG132 treatment. Blots are representative of 2 independent experiments.

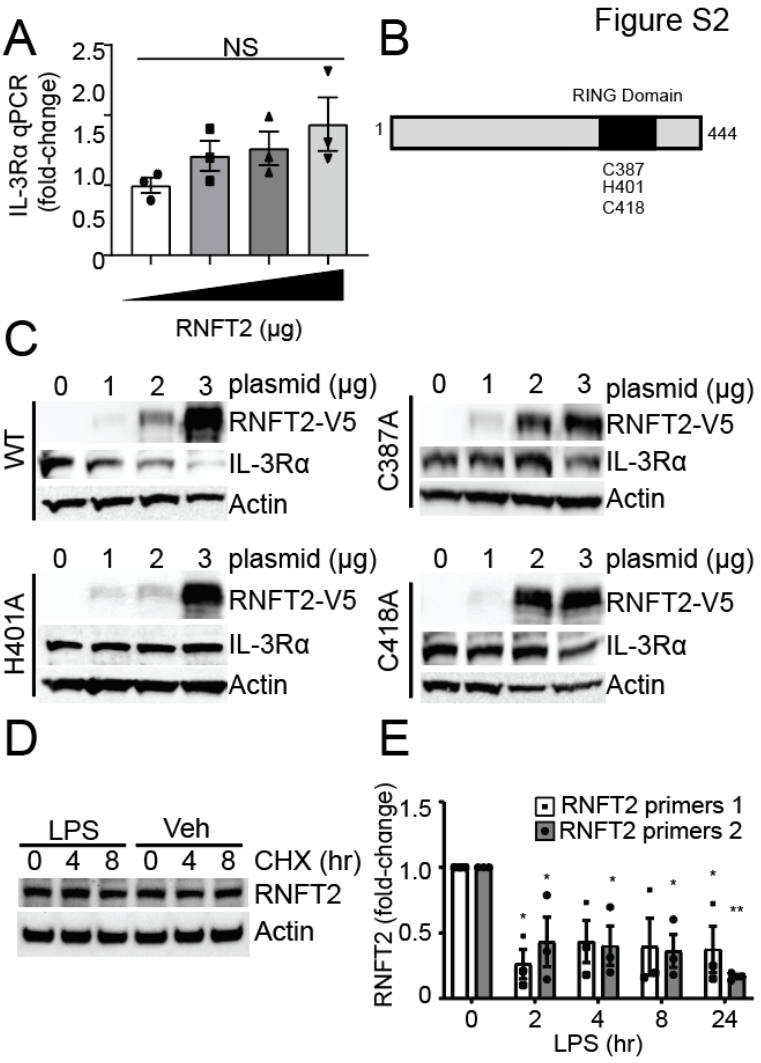


**Figure S2. RNFT2 is a RING Ubiquitin E3 ligase and is transcriptionally down-regulated by LPS (A)** RT-qPCR analysis of IL-3Rα mRNA expression in MLE cells transfected with increasing amounts of RNFT2. Data are pooled from 3 independent experiments. (**B**) Schematic of RNFT2 sequence and critical residues within its predicted RING domain. (**C**) Western blot analysis of IL-3Rα abundance in lysates of MLE cells transfected with increasing amounts of RNFT2 WT and RING mutant constructs. (**D)** Immunoblot analysis of MLE cells treated with CHX time course without or with LPS co-treatment. (**E**) RT-qPCR analysis of RNFT2 mRNA expression in MLE cells treated with a time course of LPS and represented as fold change relative to 0hr time point. Blots are representative of 2-3 independent

experiments. Data and means ± SEM of 3 independent experiments NS, P > 0.05, *P ＜0.05, **P ＜0.01; by one-way ANOVA with Dunnett's post hoc test (A, E).

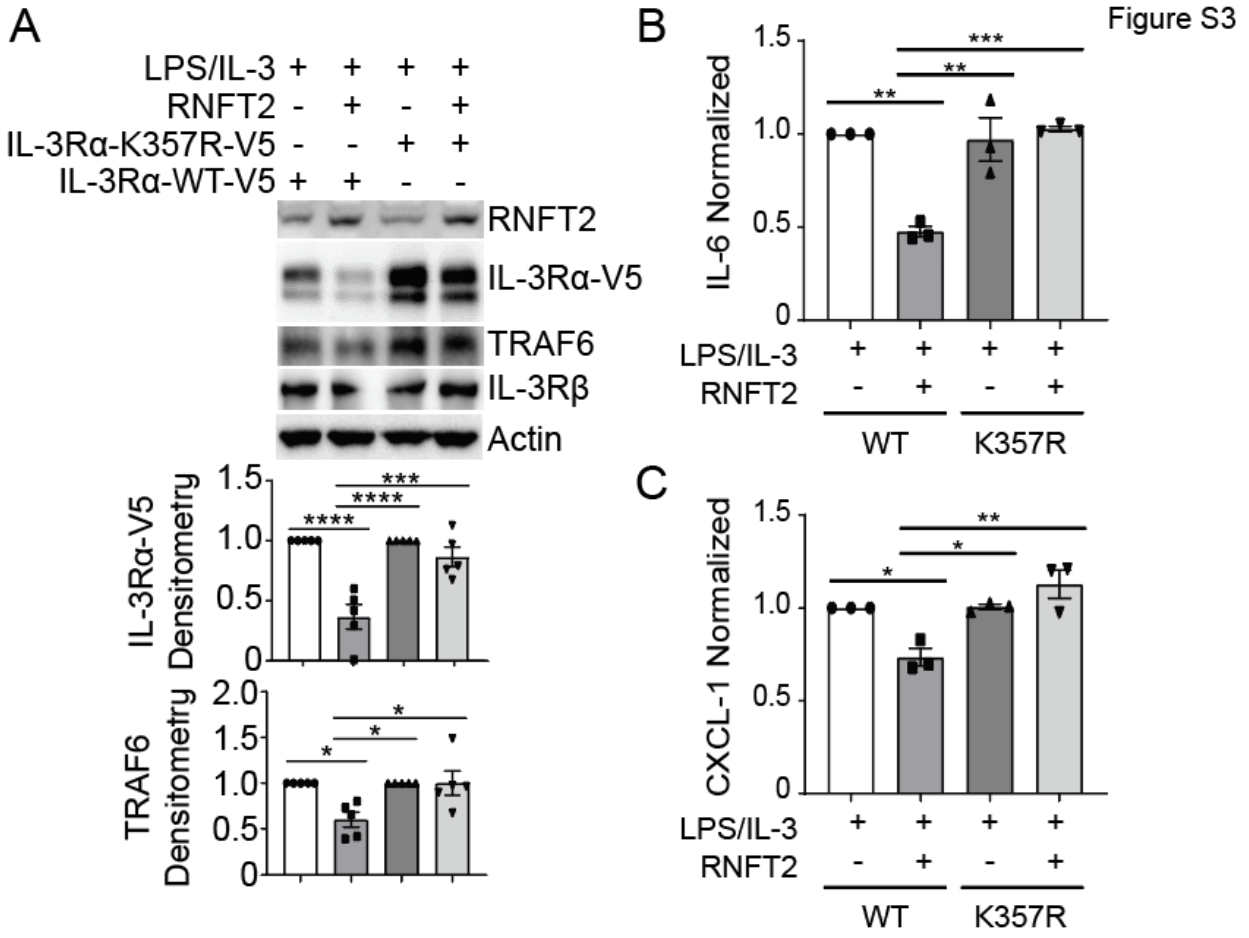


**Figure S3. RNFT2 effect on inflammation proceeds through IL-3Rα** (**A**) Immunoblot analysis of IL-3Rα, IL-3Rβ, and TRAF6 from MLE co-expressing WT or ubiquitin-resistant K357R mutant IL-3Rα and RNFT2 prior to LPS and IL-3 treatment. Data and means ± SEM of 5 independent experiments. (**B-C**) ELISA analysis of IL-6 and CXCL-1 abundances from MLE co-expressing WT or ubiquitin-resistant K357R mutant IL-3Rα and RNFT2 prior to LPS and IL-3 treatment. Data and means ± SEM of 3 independent experiments NS, P > 0.05, *P ＜0.05, **P ＜0.01, ***P ＜0.001, ****P ＜0.0001 by one-way ANOVA with Tukey's post hoc test (A-C).

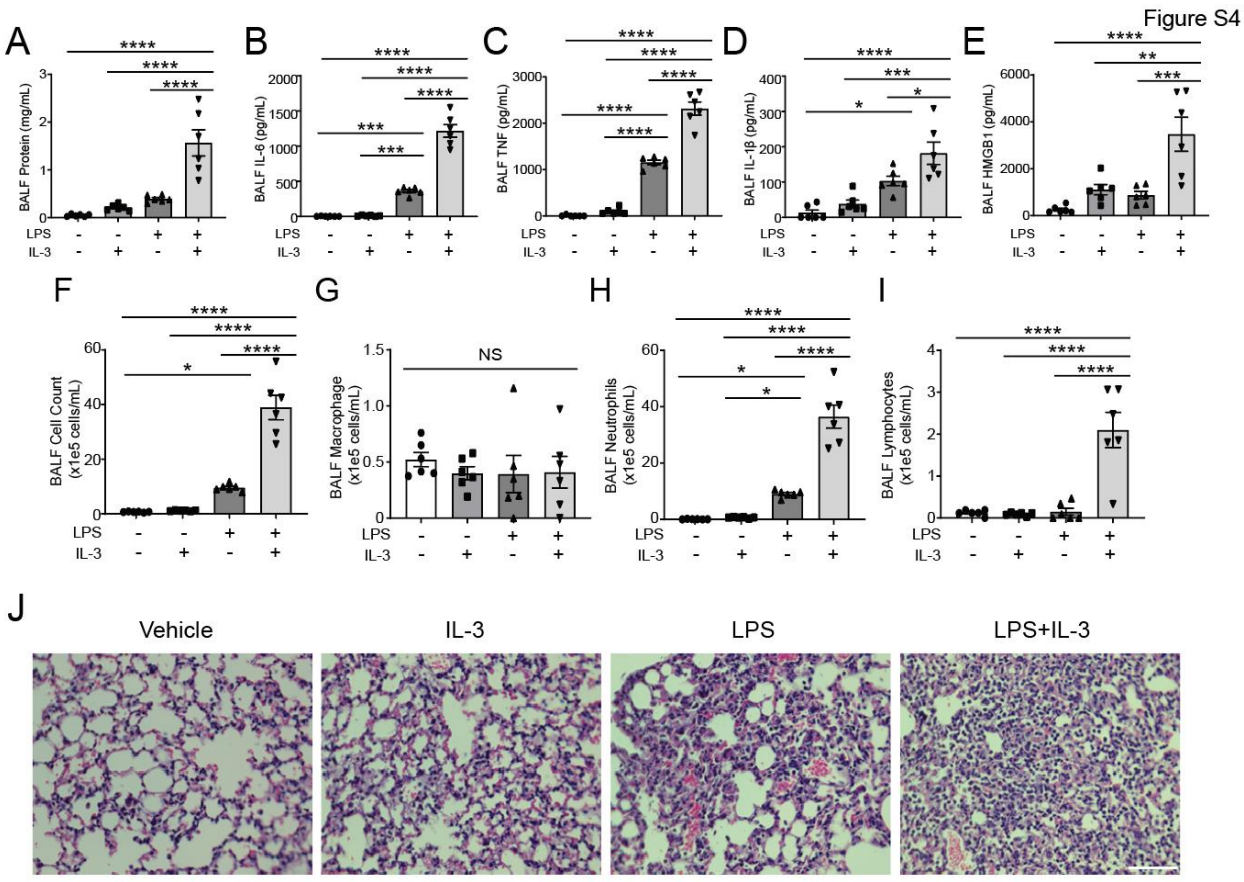


**Figure S4. IL-3 aggravates LPS-induced lung injury *in vivo* (A** to **E)** ELISA analysis of total protein (A), IL-6 (B), TNF (C), IL-1β (D) and HMGB1 (E) concentration in BAL fluid from mice intratracheally treated with LPS and PBS or recombinant IL-3, as indicated. (**F** to **I**) Number of total cells (F), macrophages (G), neutrophils (H), and lymphocytes (I) in BAL fluid from mice treated intratracheally with LPS and PBS or recombinant IL-3, as indicated. Data are means ± SEM of 3 mice per group from 2 independent experiments. (**J**) Histological analysis of lung samples from mice treated as indicated. Images are representative of all independent experiments. Scale bar, 100 μm. *P ＜0.05, **P ＜0.01, ***P ＜0.001, ****P ＜ 0.0001, by one-way ANOVA with Tukey's post hoc test (A-I).

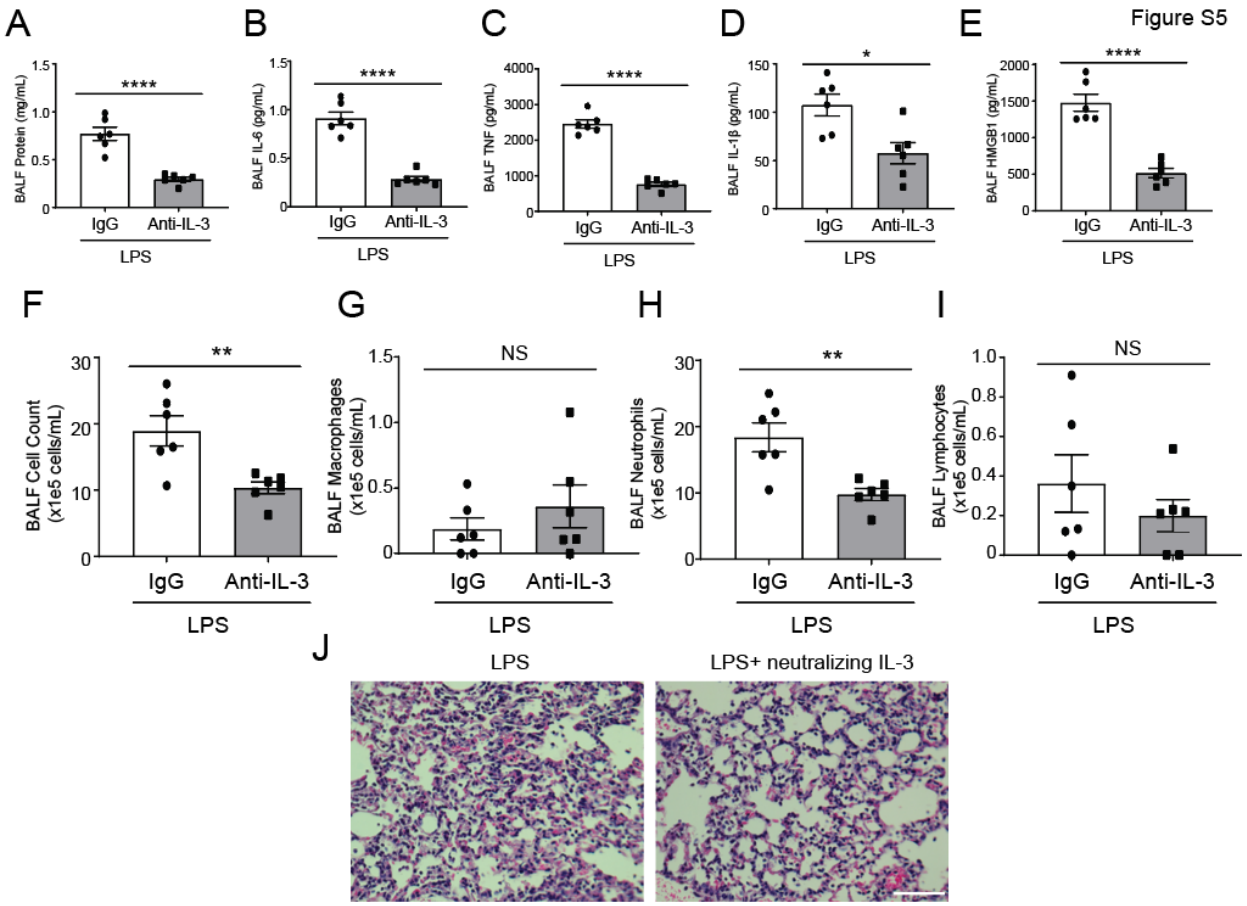


**Figure S5. IL-3 blocking antibody attenuates LPS-induced lung injury. (A** to **E**) ELISA analysis of total protein (A), IL-6 (B), TNF (C), IL-1β (D) and HMGB1 (E) concentration in BAL fluid from intratracheally treated with LPS and control or neutralizing IL-3 antibody, as indicated. Mice were euthanized after 18h, and lungs were lavaged with saline, and BALF were measured from mice. (**F** to **I**) Number of total cells (F), macrophages (G), neutrophils (H), and lymphocytes (I) in BAL fluid of mice treated intratracheally with LPS and control or neutralizing IL-3 antibody, as indicated. (**J**) Histological analysis of lung samples from mice treated as indicated. Images are representative of all independent experiments. Scale bar, 100 μm. Data are means ± SEM of 3 mice per group from 2 independent experiments. NS, $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, by Student's t-test (A-I).

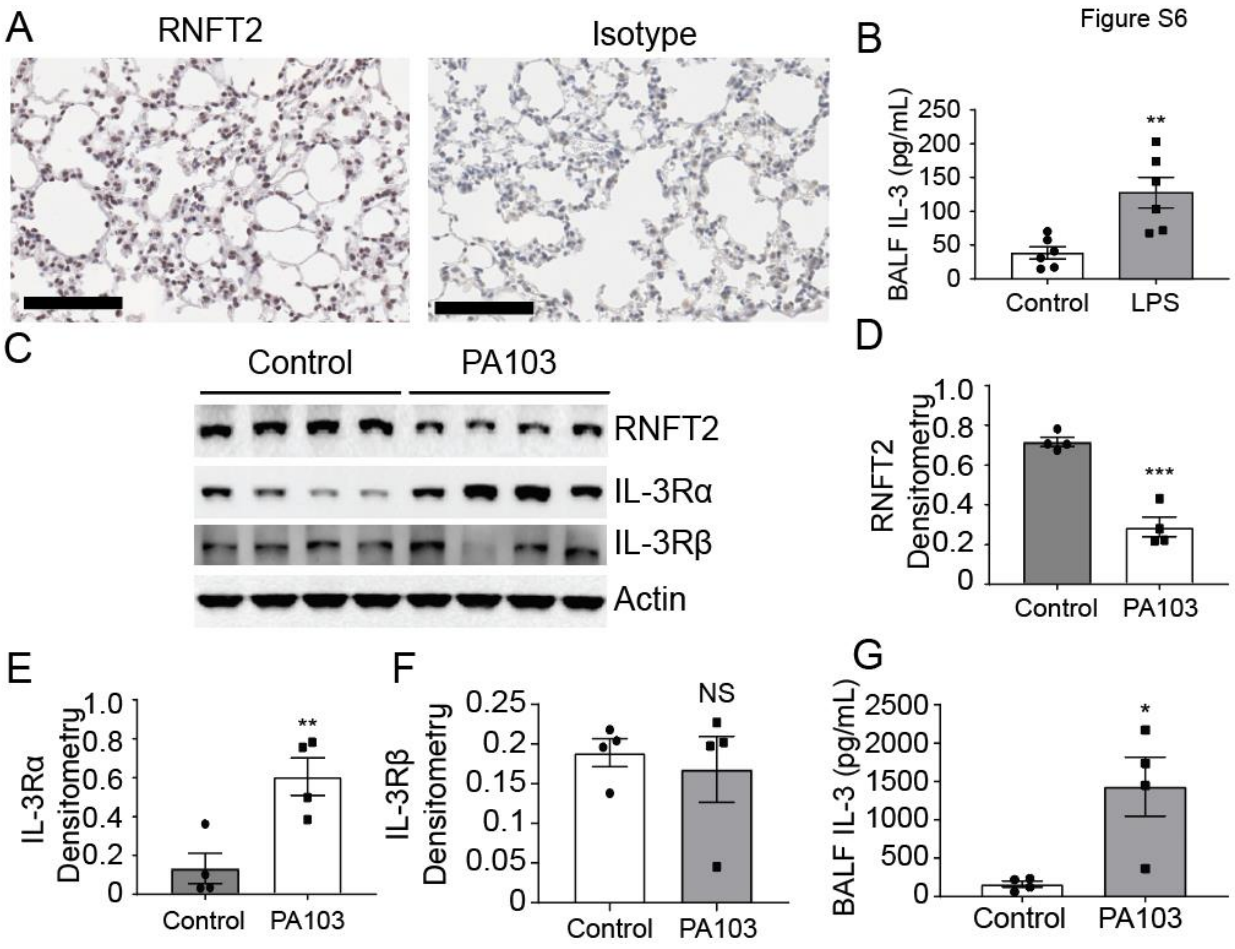


**Figure S6. Bacterial infection influences Rnft2:Il3ra protein levels (A)** Immunohistochemical staining of Rnft2 in murine lung, scale bar = 100μm, Isotype control staining is also shown **(B)**. ELISA analysis of Il3 in mouse BAL fluid treated intratracheally with LPS and PBS as indicated. Data are means ± SEM of 3 mice per group from 2 independent experiments. **(C-F).** Immunoblot analysis of mouse lung homogenate from Control or PA103-treated mice. (C). Protein densitometry analysis of RNFT2 (D), IL-3Rα (E), and IL-Rβ(F), from data in (C). **(G)**. ELISA analysis of Il3 in mouse BAL fluid treated intratracheally with PA103 and PBS as indicated. Data are means ± SEM of 3 mice per group from 2 independent experiments. NS, $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, by Student's t-test (B, D, E-G).

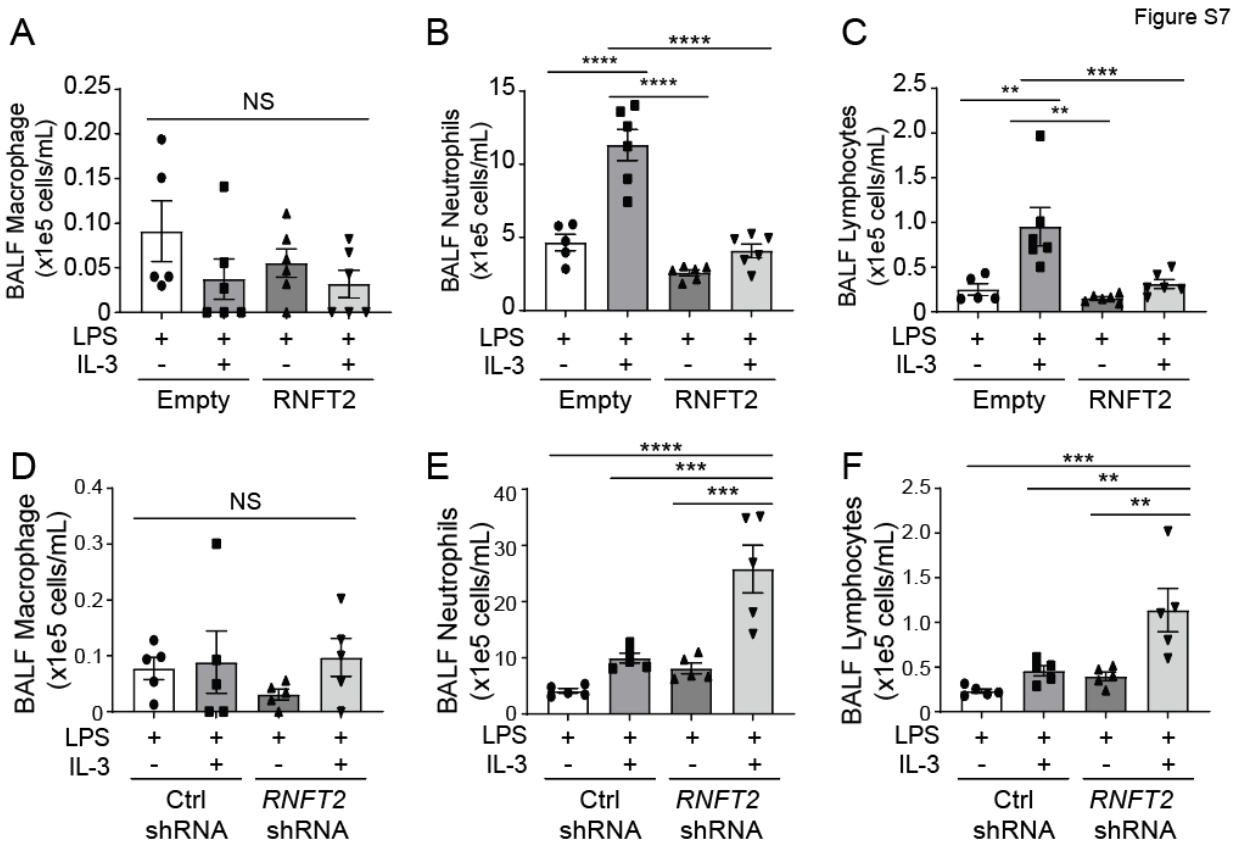


**Figure S7. RNFT2 regulates neutrophils counts in lung inflammation and injury.** (A to C) Total number of cells (A), neutrophils (B), and lymphocytes (C) in BAL fluid from mice that received Lenti-Empty or Lenti-RNFT2 intratracheally before treatment with LPS with rIL-3, as indicated. Mice were euthanized after 18h, and lungs were lavaged with saline, and lavage cells were then processed for Wright-Giemsa stain; lavage macrophages, neutrophils, and lymphocytes were counted and graphed (A-C). (D to F) Total number of macrophages (D), neutrophils (B), and lymphocytes (C) in BAL fluid from mice that received Lenti-control shRNA or Lenti-RNFT2 shRNA intratracheally before treatment with LPS and rIL-3, as indicated. All data and means ± SEM of 2-3 mice per group are from 2 independent experiments. *P ＜0.05, **P ＜0.01, and ***P ＜0.001 by one-way ANOVA with Tukey's post hoc test (A-F).

| Category | Age | Sex | FiO2 | Primary ARDS Risk Factor | IL-3 | TNFα | CCL4 | IL1β | IL8 |
|---|---|---|---|---|---|---|---|---|---|
| No Lung Injury | 46.0 | Male | 40 | | 34.6 | 14.0 | 29.5 | 2.0 | 23.7 |
| No Lung Injury | 68.6 | Male | 40 | | 40.4 | 22.7 | 19.6 | 2.5 | 26.3 |
| No Lung Injury | 73.6 | Male | 40 | | 41.0 | 22.7 | 56.5 | 3.0 | 38.5 |
| No Lung Injury | 71.3 | Male | 40 | | 22.8 | 9.3 | 60.6 | 1.5 | 21.3 |
| ARFA | 29.2 | Male | 100 | Aspiration | 156.6 | 26.8 | 39.2 | 3.1 | 33.0 |
| ARFA | 62.0 | Male | 40 | Non-pulmonary sepsis | 80.0 | 23.9 | 183.3 | 3.1 | 77.6 |
| ARFA | 33.1 | Male | 50 | Pneumonia | 42.3 | 15.2 | 42.2 | 2.5 | 24.8 |
| ARFA | 63.9 | Male | 40 | Non-pulmonary sepsis | 62.2 | 5.7 | 64.8 | 1.8 | 15.3 |
| ARFA | 58.3 | Male | 40 | Pneumonia | 32.3 | 39.3 | 47.9 | 3.7 | 34.0 |
| ARFA | 60.3 | Female | 40 | Pneumonia | 57.0 | 12.2 | 65.2 | 2.0 | 21.3 |
| ARFA | 59.4 | Male | 60 | Non-pulmonary sepsis | 38.7 | 18.7 | 193.7 | 2.6 | 115.0 |
| ARFA | 66.2 | Female | 50 | Non-pulmonary sepsis | 171.9 | 26.8 | 189.0 | 3.4 | 78.2 |
| ARFA | 45.2 | Male | 60 | Aspiration | 35.9 | 0.0 | 113.3 | 0.5 | 26.8 |
| ARFA | 42.6 | Female | 40 | Non-pulmonary sepsis | 110.1 | 25.1 | 129.7 | 3.6 | 117.4 |
| ARFA | 67.1 | Female | 50 | Non-pulmonary sepsis | 113.7 | 0.0 | 108.7 | 1.2 | 38.3 |
| ARFA | 57.5 | Male | 40 | Aspiration | 63.8 | 0.0 | 129.7 | 0.1 | 32.7 |
| ARFA | 32.1 | Female | 40 | Pneumonia | 124.8 | 65.2 | 76.9 | 6.6 | 90.9 |
| ARFA | 25.2 | Male | 40 | Pneumonia | 37.0 | 0.0 | 45.1 | 1.2 | 14.6 |
| ARFA | 60.6 | Male | 60 | Pneumonia | 309.1 | 90.2 | 252.1 | 9.1 | 107.7 |
| ARFA | 66.5 | Female | 50 | Blood Transfusion | 44.8 | 0.0 | 77.8 | 1.9 | 40.1 |

**Supplemental Table 1**: Demographic and Clinical Characteristics of subjects for which IL-3 and other pro-inflammatory cytokines were obtained in the University of Pittsburgh Acute Lung Injury Registry. FiO2 = Fraction of inspired oxygen; ARDS = Acute Respiratory Distress Syndrome

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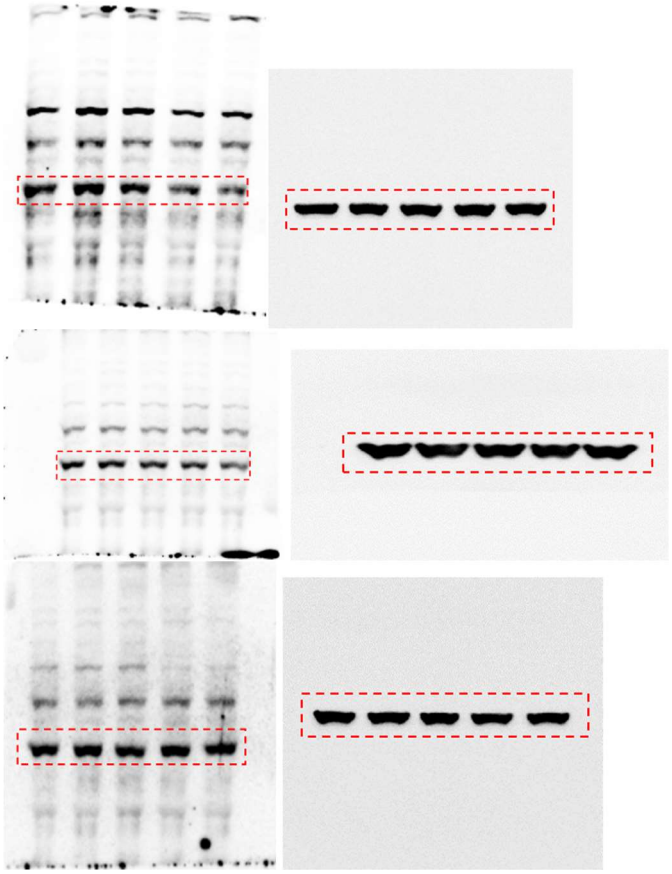

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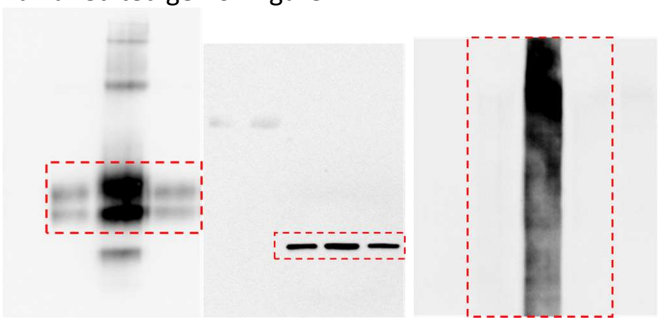

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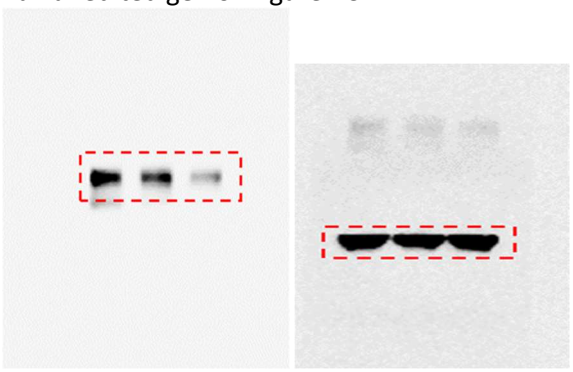

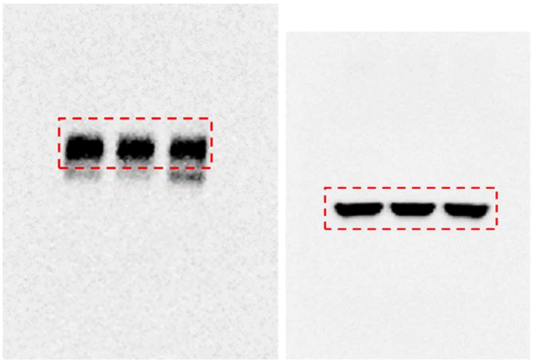

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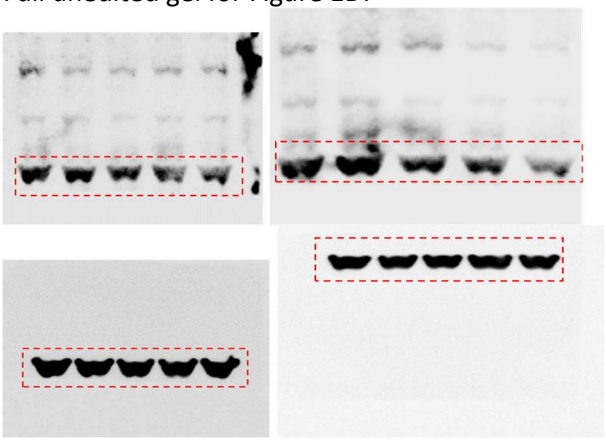

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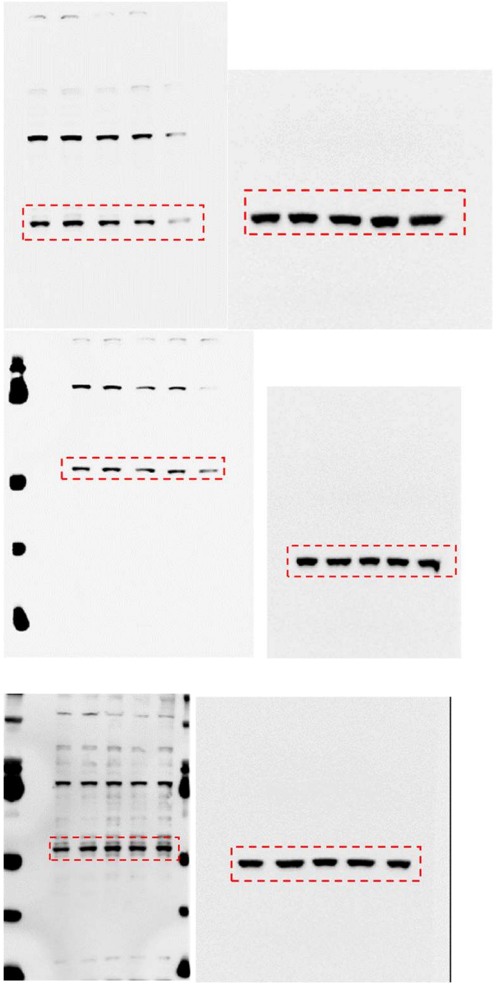

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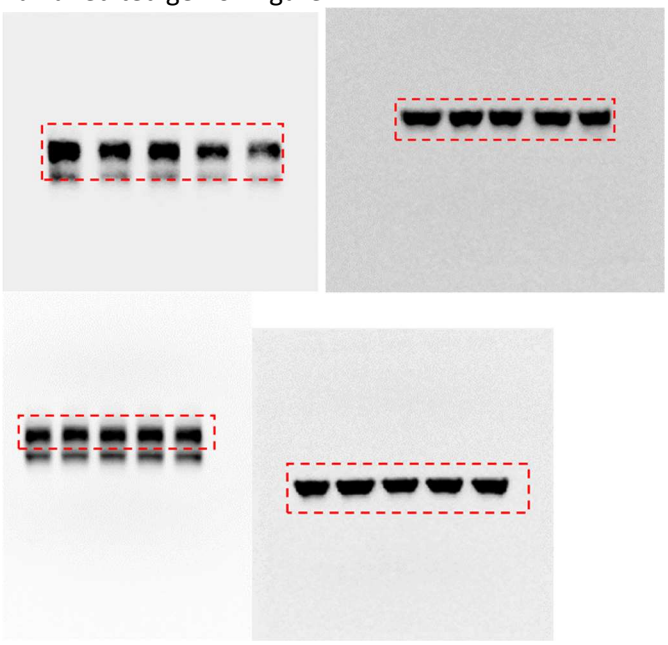

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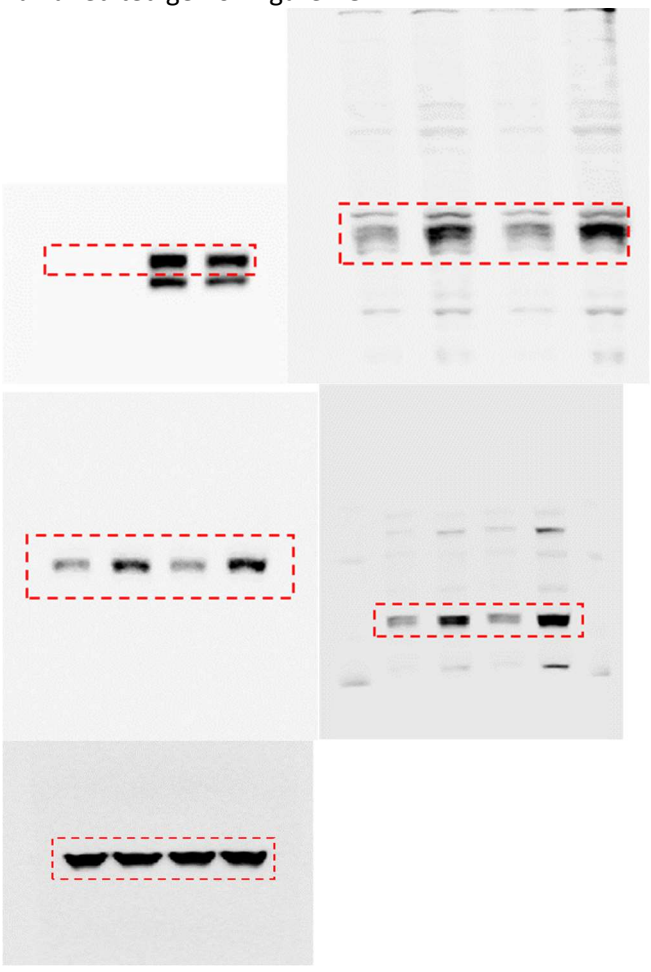

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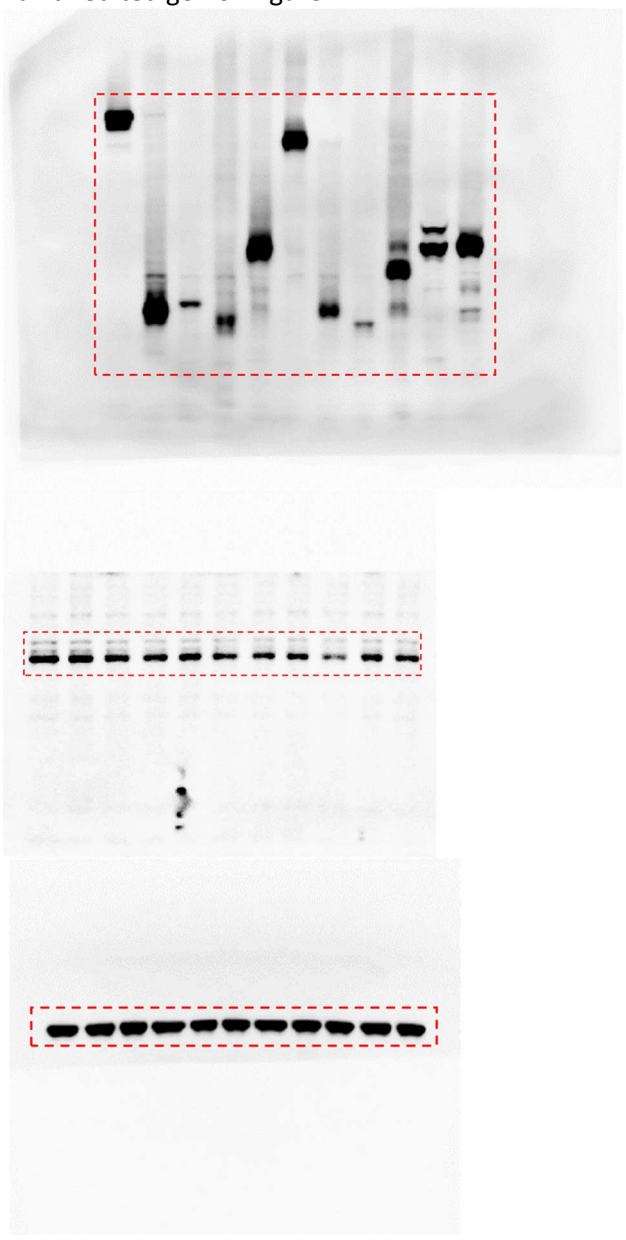

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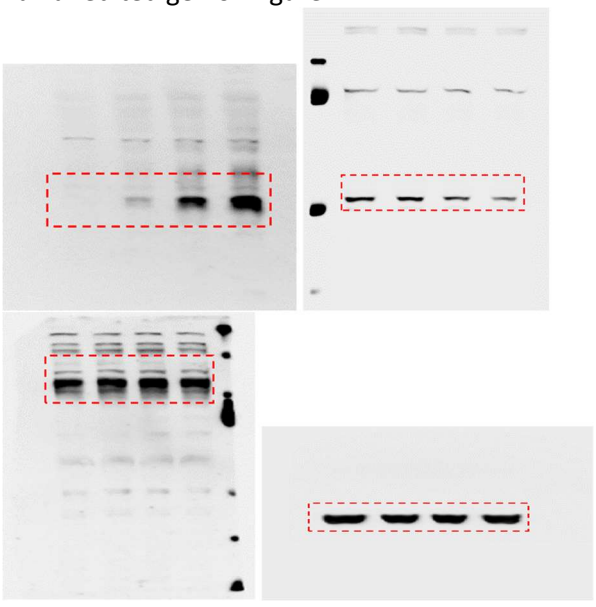

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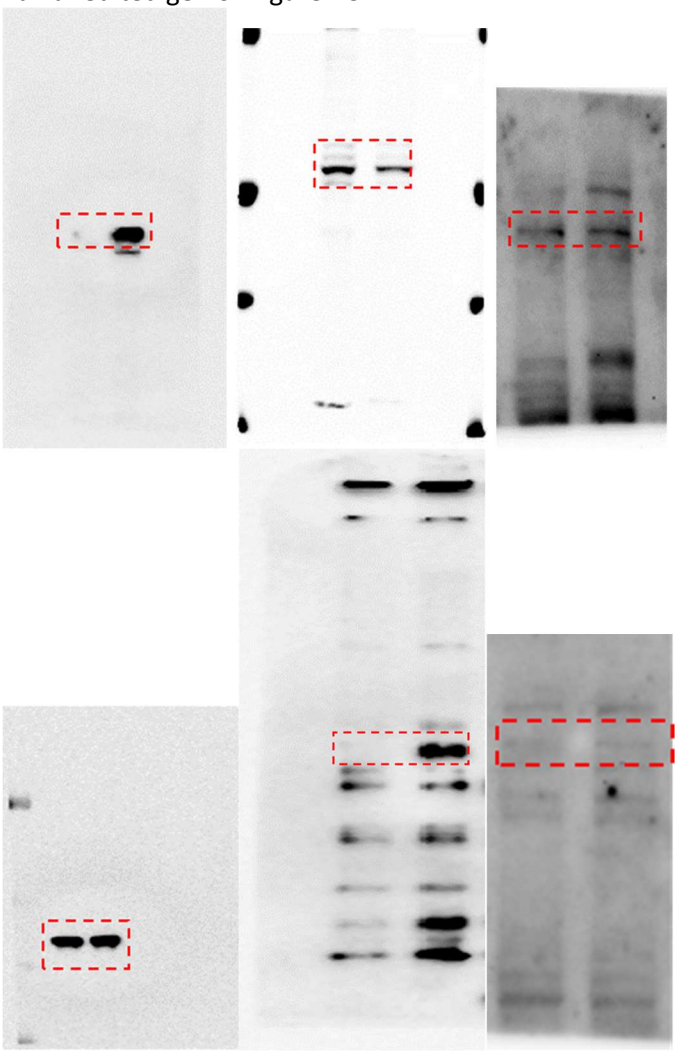

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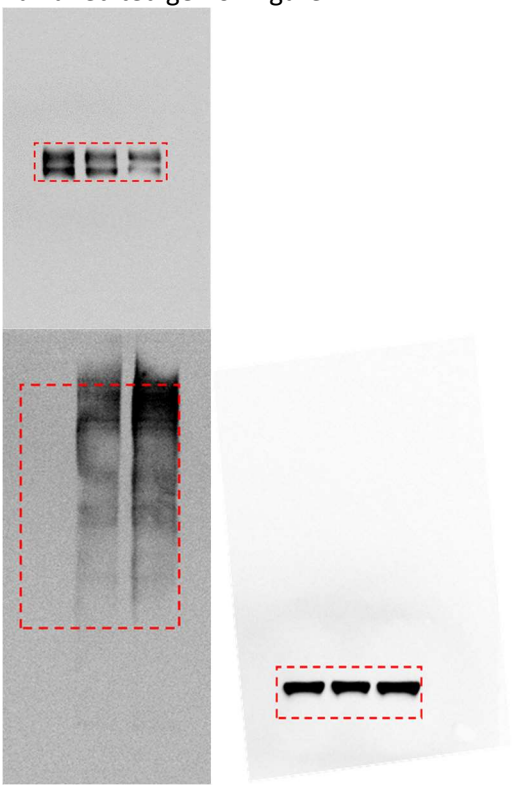

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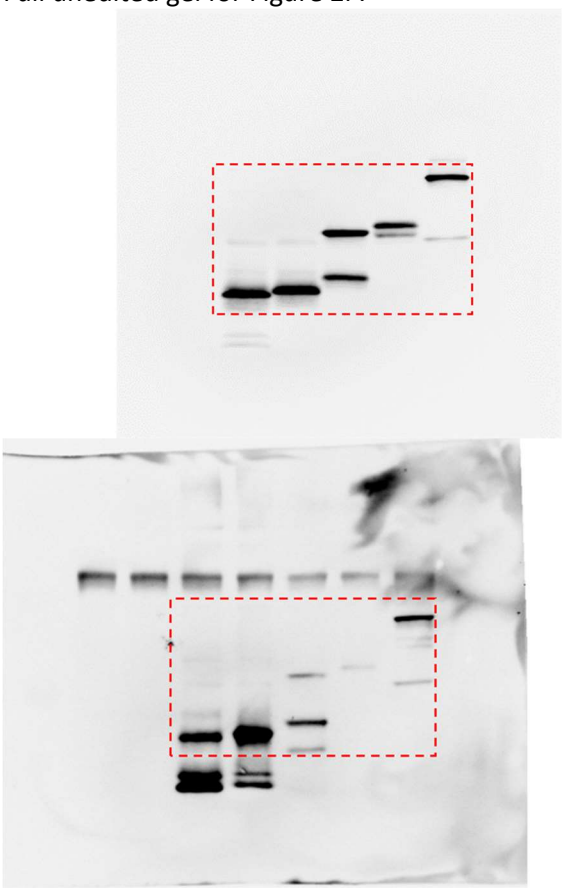

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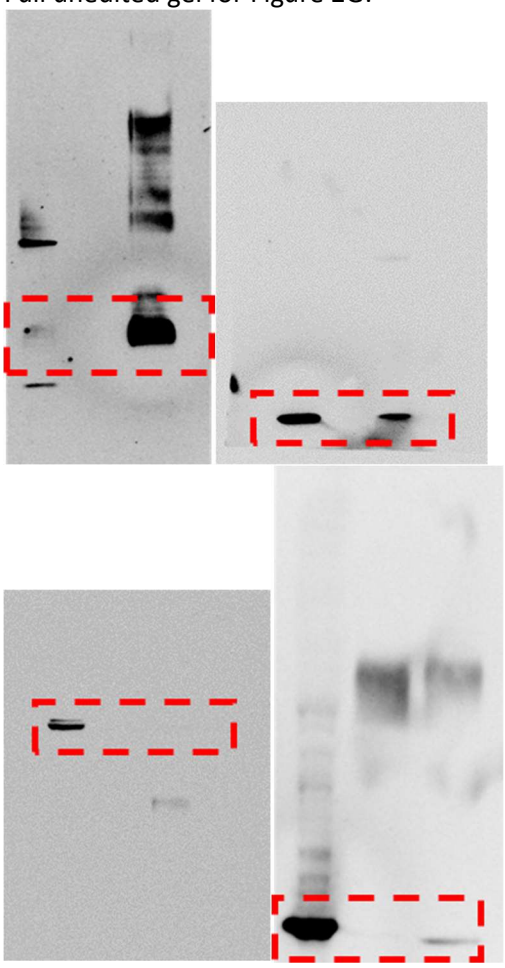

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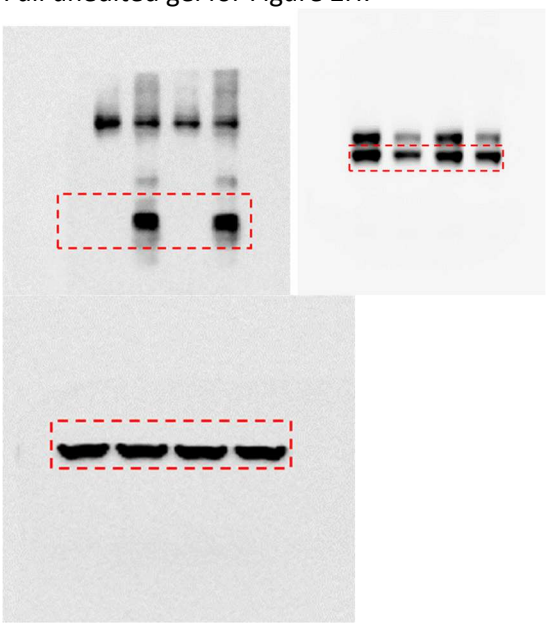

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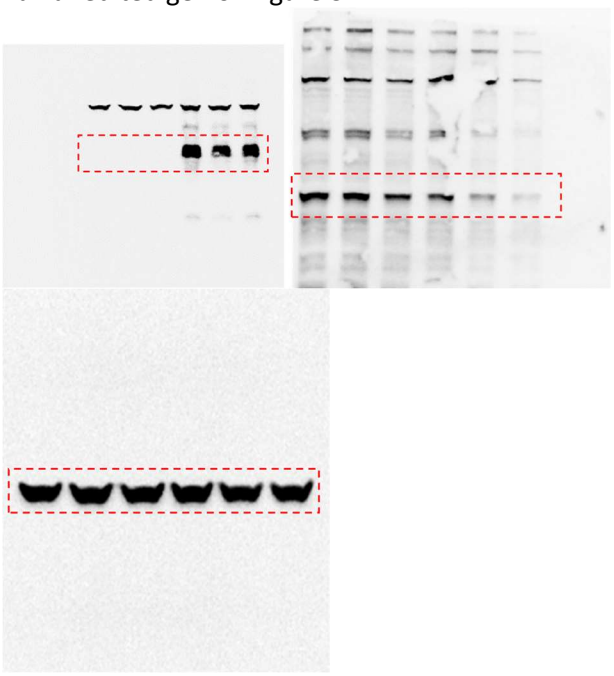

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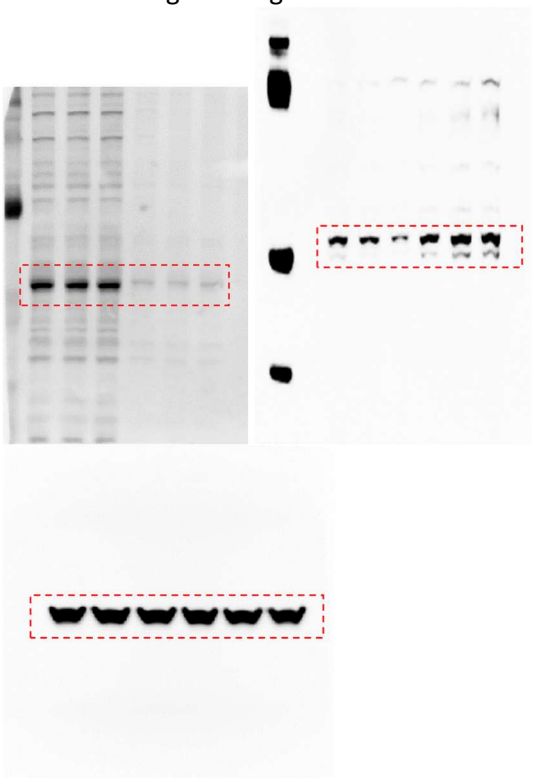

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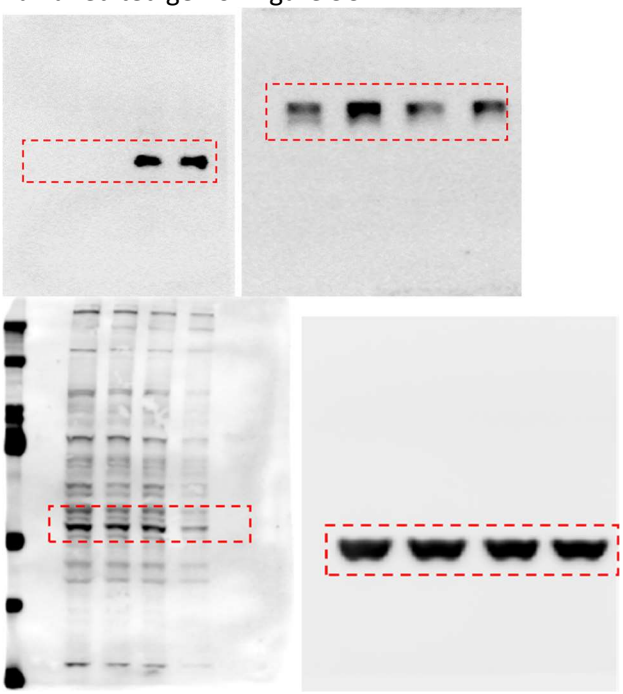

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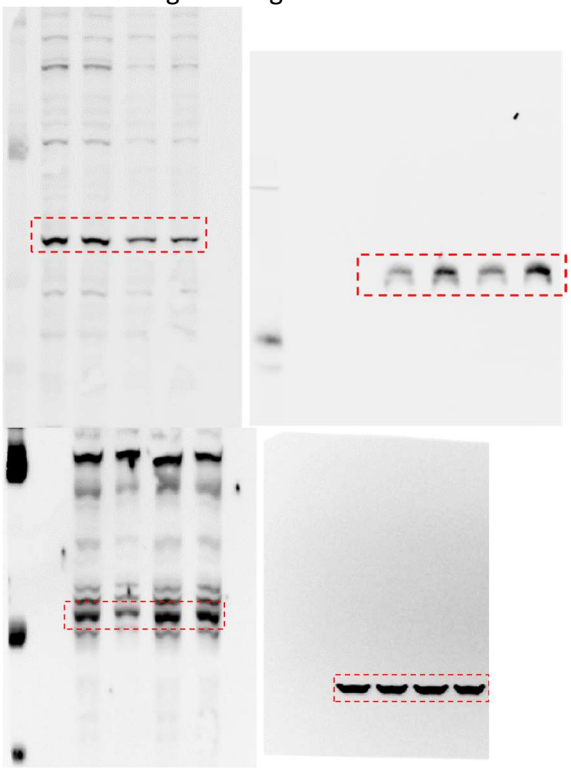

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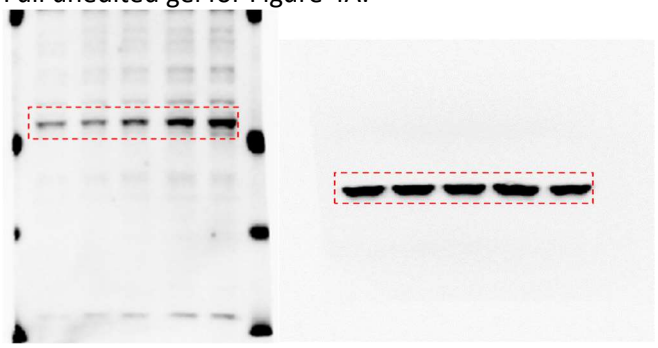

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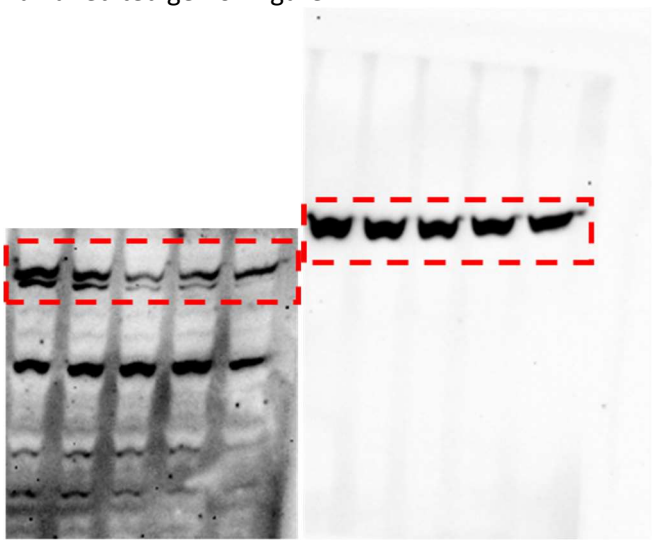

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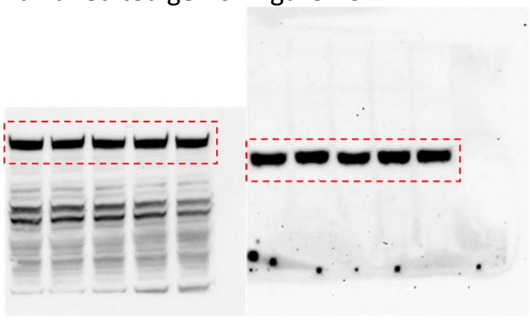

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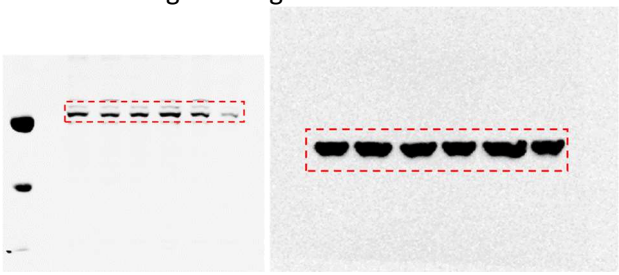

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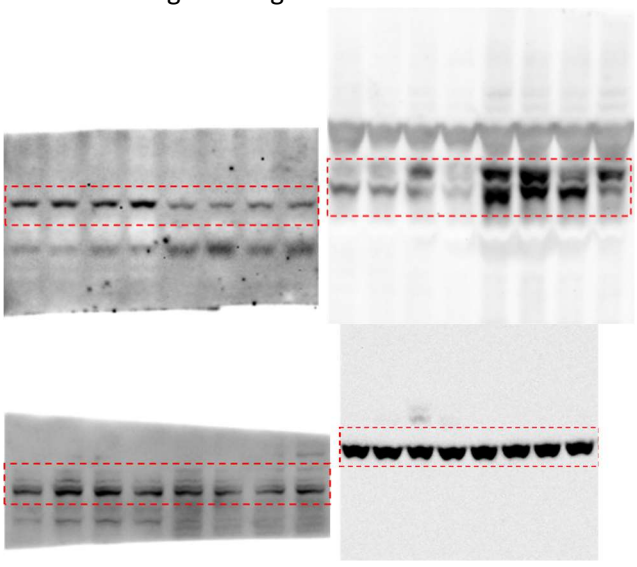

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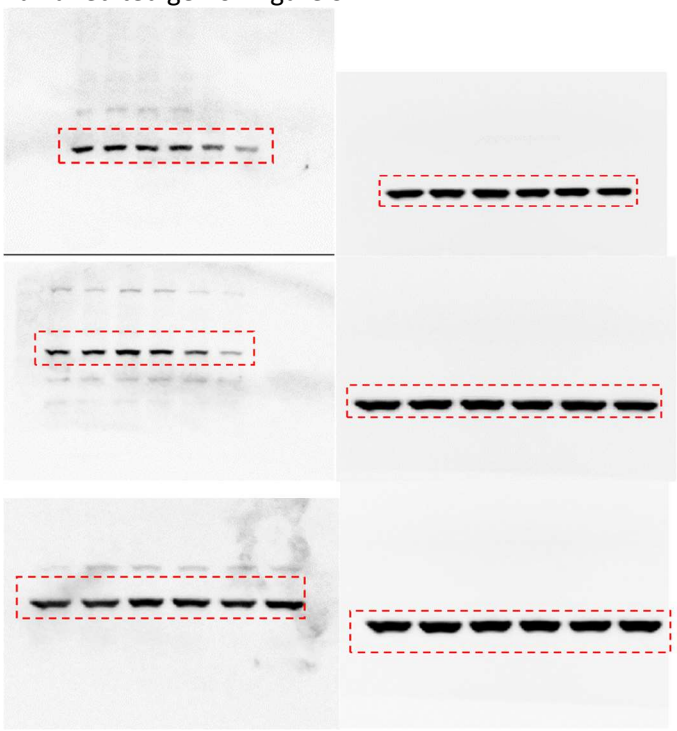

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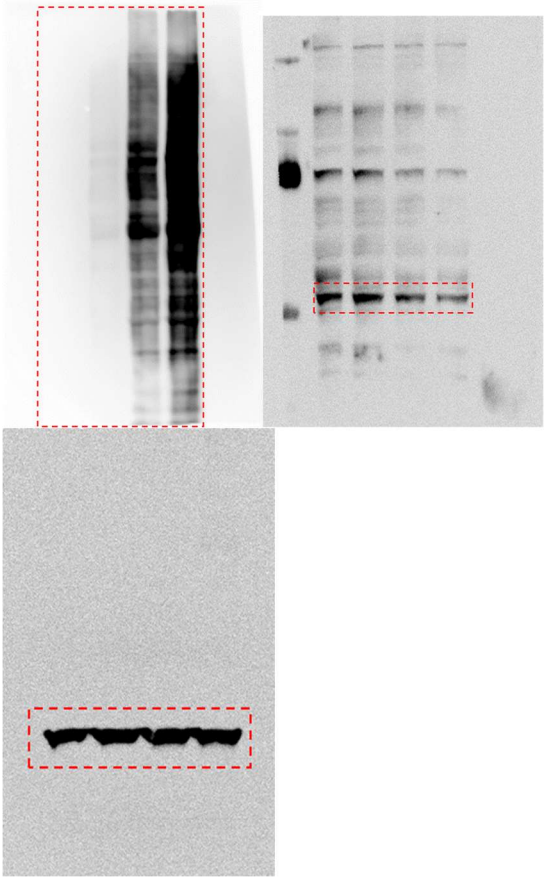

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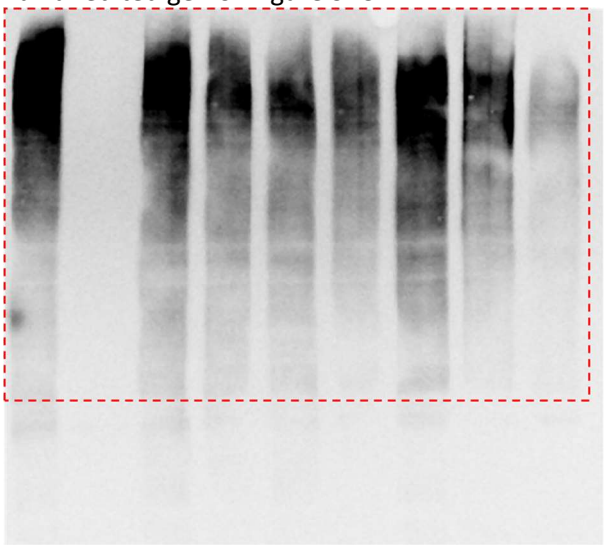

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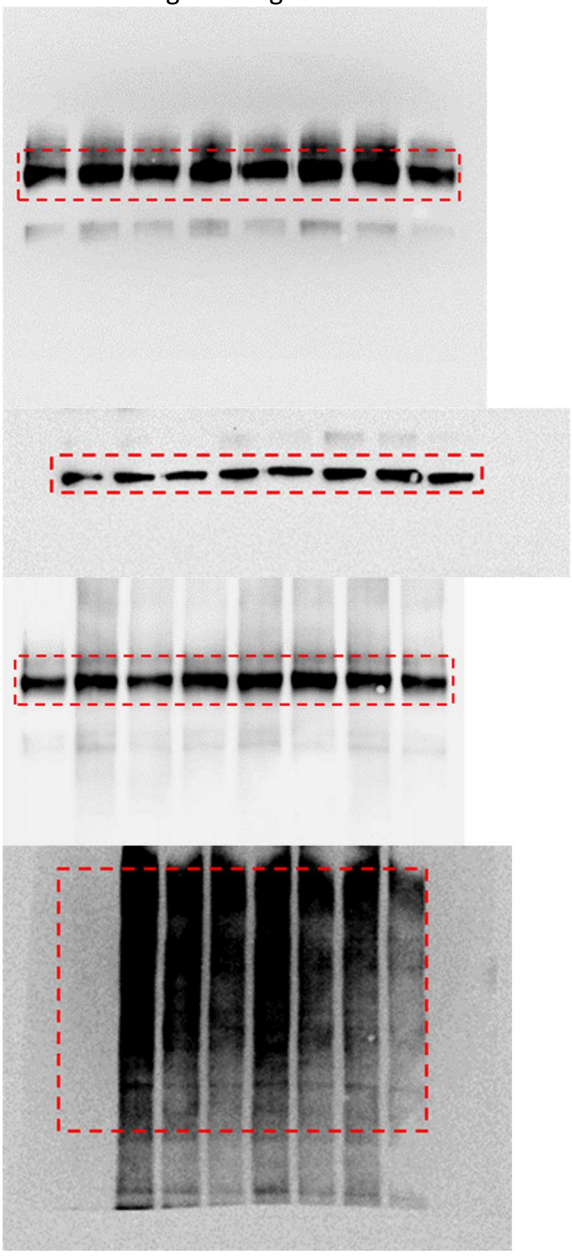

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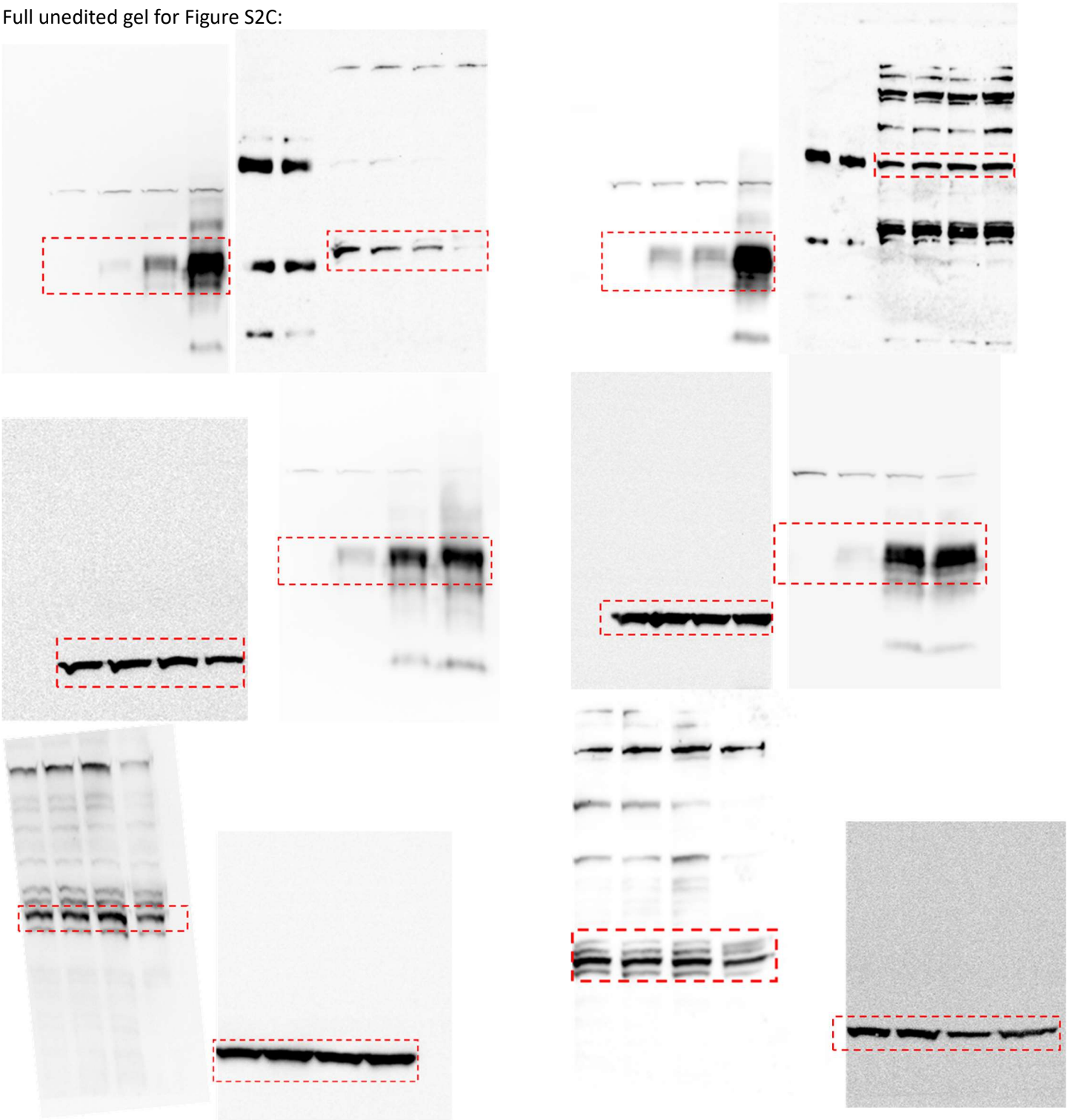

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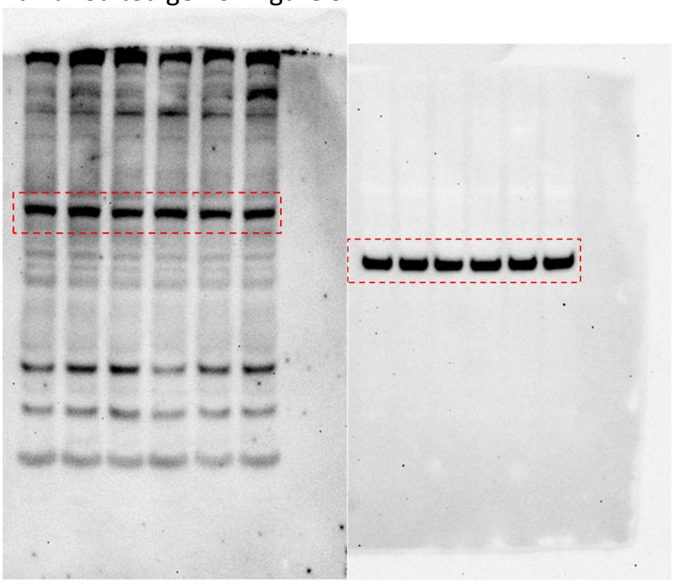

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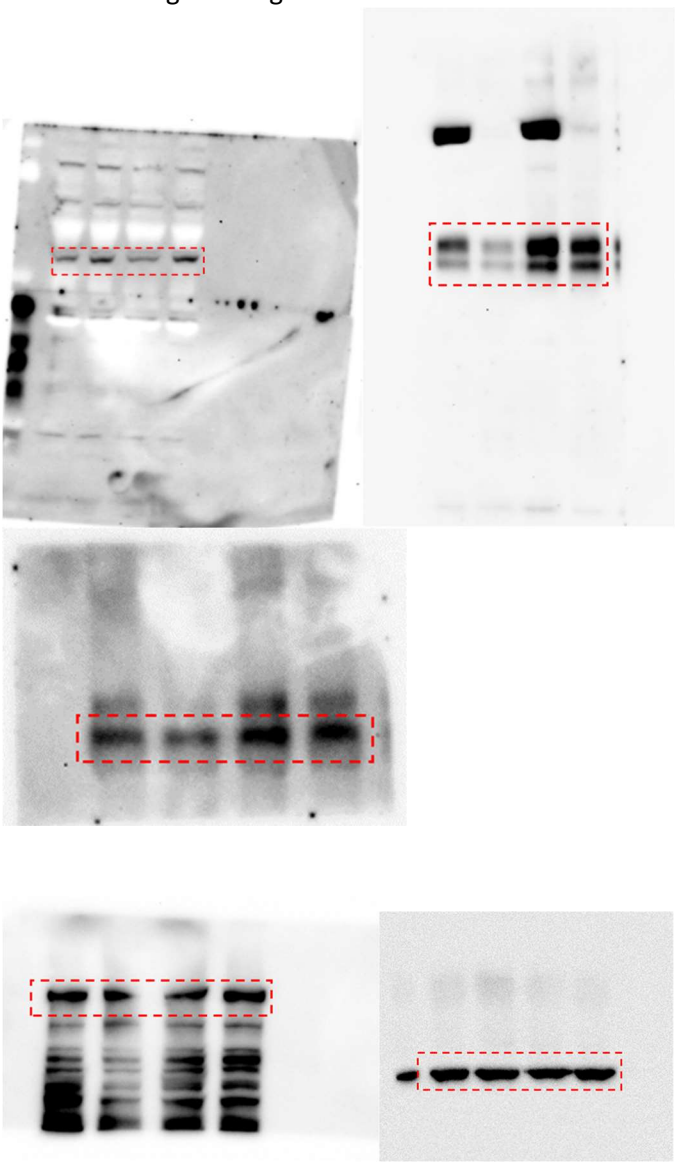

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