

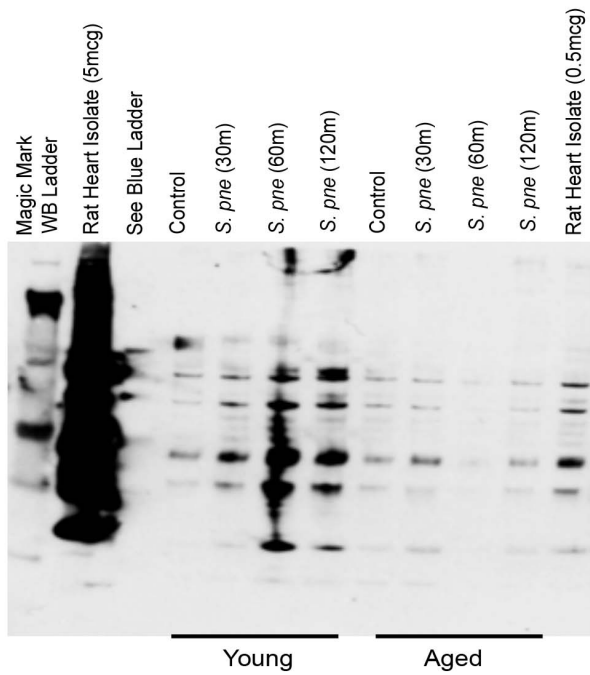
Supplemental Information:

**Mitochondrial Dysfunction in Aged Macrophages and Lung
during Primary *Streptococcus pneumoniae* Infection is Improved
with Pirfenidone**

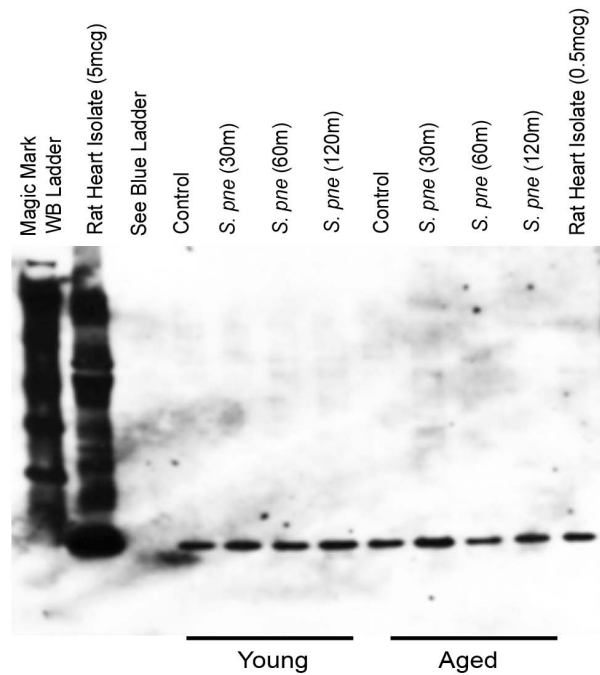
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Seon Yun¹, Kristen T. Schiffer¹, and Heather W. Stout-Delgado¹

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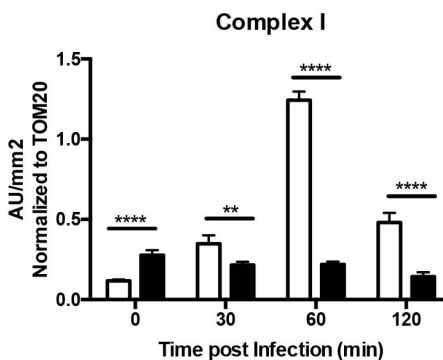
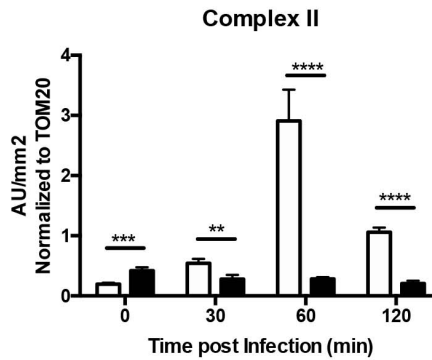
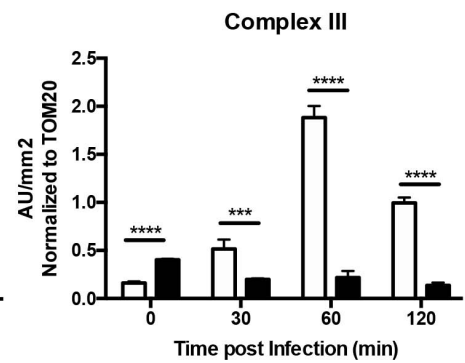
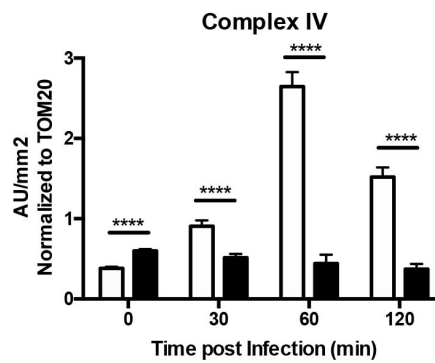
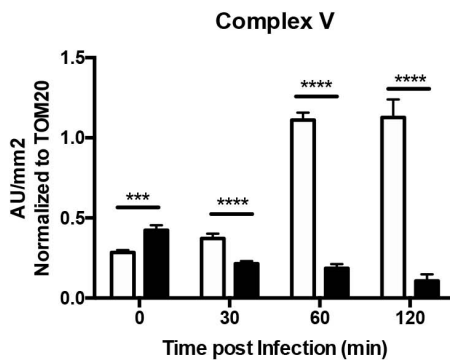
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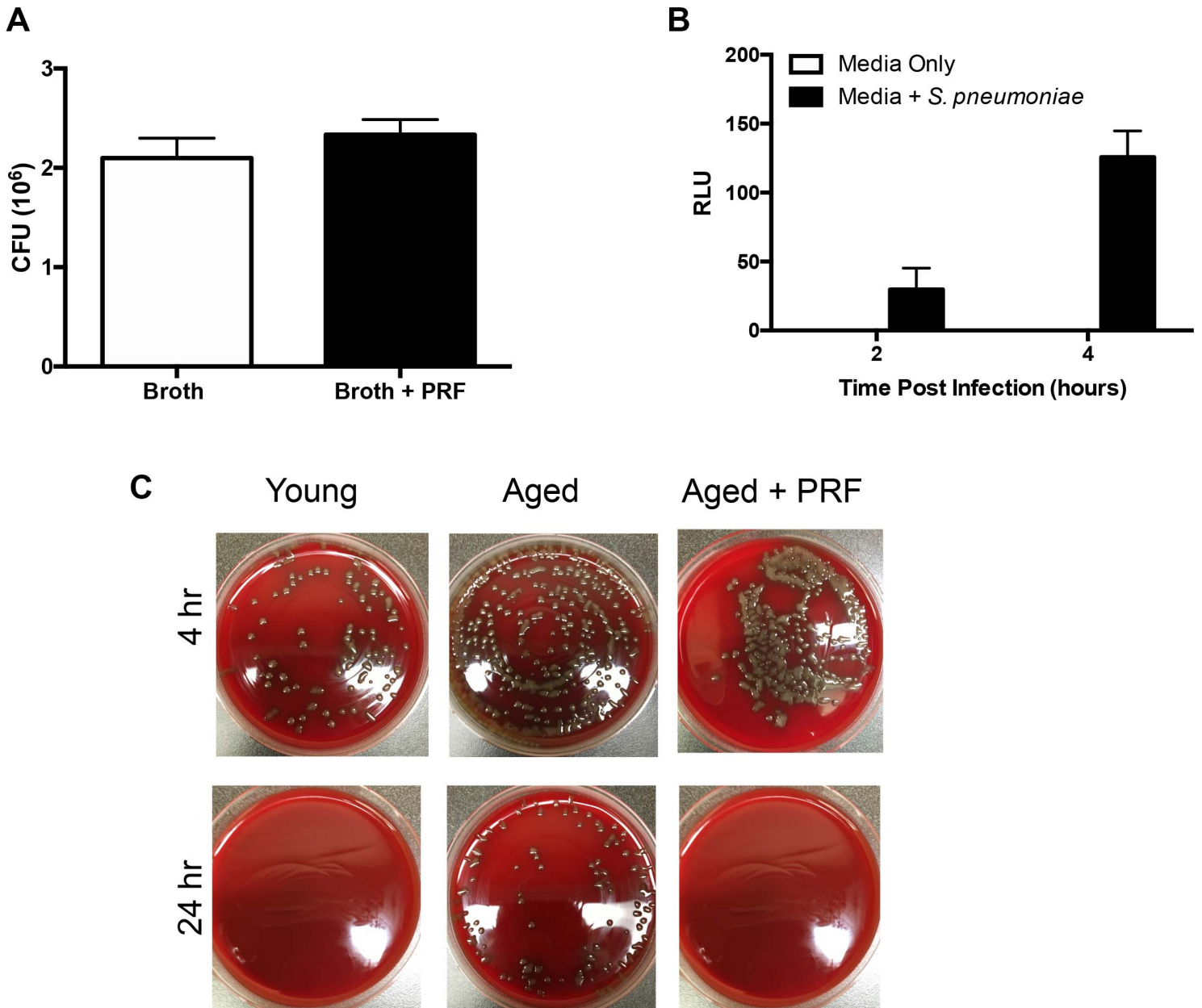
Uncropped OXPPOS Western Blot



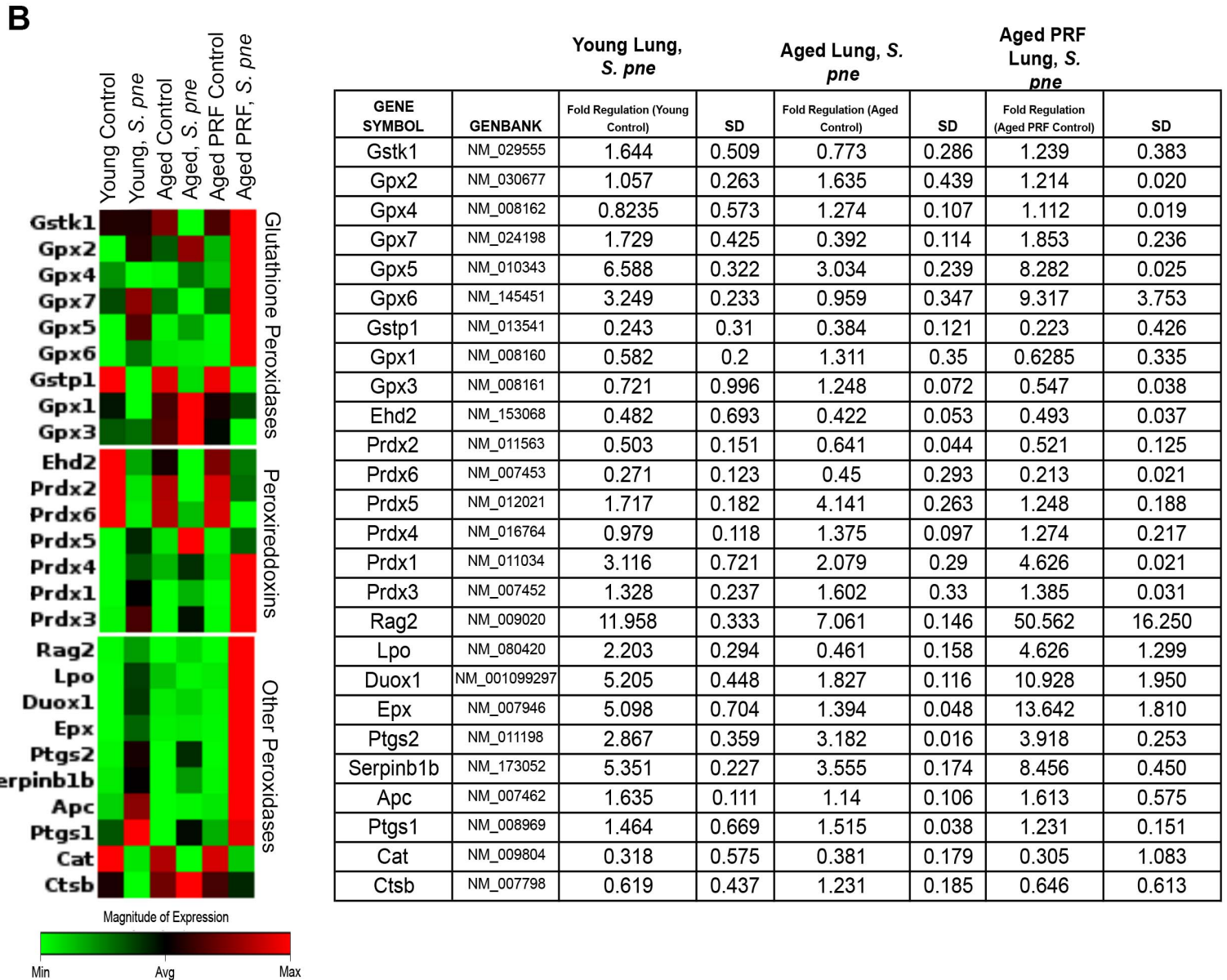
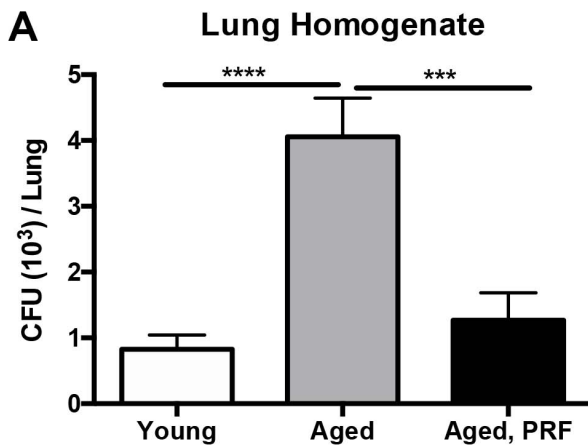
Uncropped TOM20 Western Blot



Supplemental Figure 1: Young and aged macrophages were cultured with *S. pneumoniae* (MOI=25). Mitochondria were isolated from young and aged macrophages at select time points post infection and protein expression was assessed using Total OXPPOS antibody cocktail. The blot was stripped and re-probed for TOM20 expression to confirm equivalent protein input between lanes. Full-length gels are included in Supplemental Figure 1 for comparison. Densitometry analysis for these gels is also included. Similar results were obtained from at least three independent experiments.



Supplemental Figure 2: *S. pneumoniae* was grown overnight on 10% sheep blood agar plates overnight. (A) An inoculating loop of bacteria was cultured in Todd Hewitt broth containing 2% yeast extract in the absence or presence of pirfenidone (500 μ g/ml) for 4 hours. Bacterial cultures were centrifuged for 15,000 x g for 1 minute and serial dilutions in broth were performed prior to plating. (B) Non-cellular H₂O₂ quantification of media alone or media containing *S. pneumoniae*. (C) Young, aged, and pirfenidone (PRF) pre-treated aged macrophages were cultured with *S. pneumoniae*. Bacterial growth was assessed at 4 and 24 hours post culture. Similar results were obtained from at least two independent experiments.



Supplemental Figure 3: Young and aged untreated or pretreated with pirfenidone (PRF, 280mg/kg) mice were infected with *S. pneumoniae* (1x10⁶ CFU). Lung tissue was collected at 24 hours post infection. (A) Serial dilutions of lung homogenates were plated on blood agar plates and CFU was quantified. (B) RNA was isolated and gene expression was assessed using Mouse Oxidative Stress PAMM-065ZA PCR arrays.