Supplemental Material to:

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Seasonal H3N2 influenza A virus fails to enhance
Staphylococcus aureus co-infection in a non-human
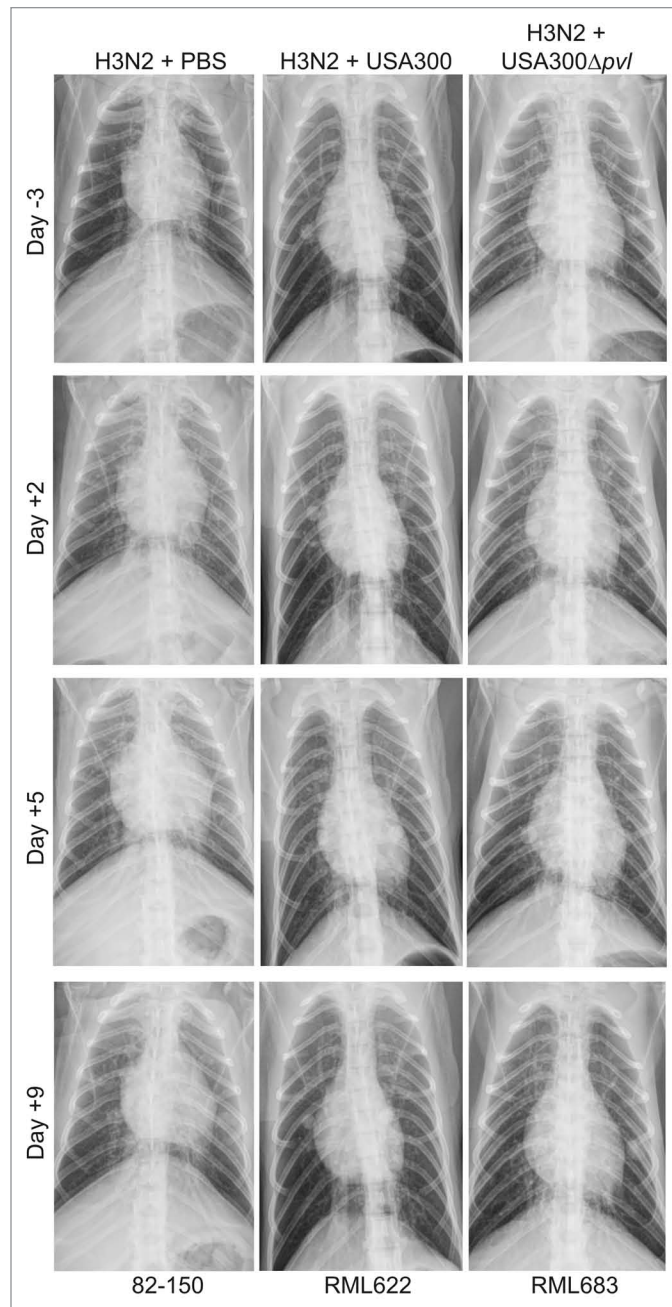primate respiratory tract infection model

Figure S1. X-ray imaging during the course of respiratory tract infection. Animals were anesthetized and X-ray imaging of the chest of all animals was performed as described in Methods. Shown is the full series of X-rays from one animal representative of each treatment group as indicated. X-rays taken 3 days prior to infection (D −3, row 1) were used as baseline (uninfected).
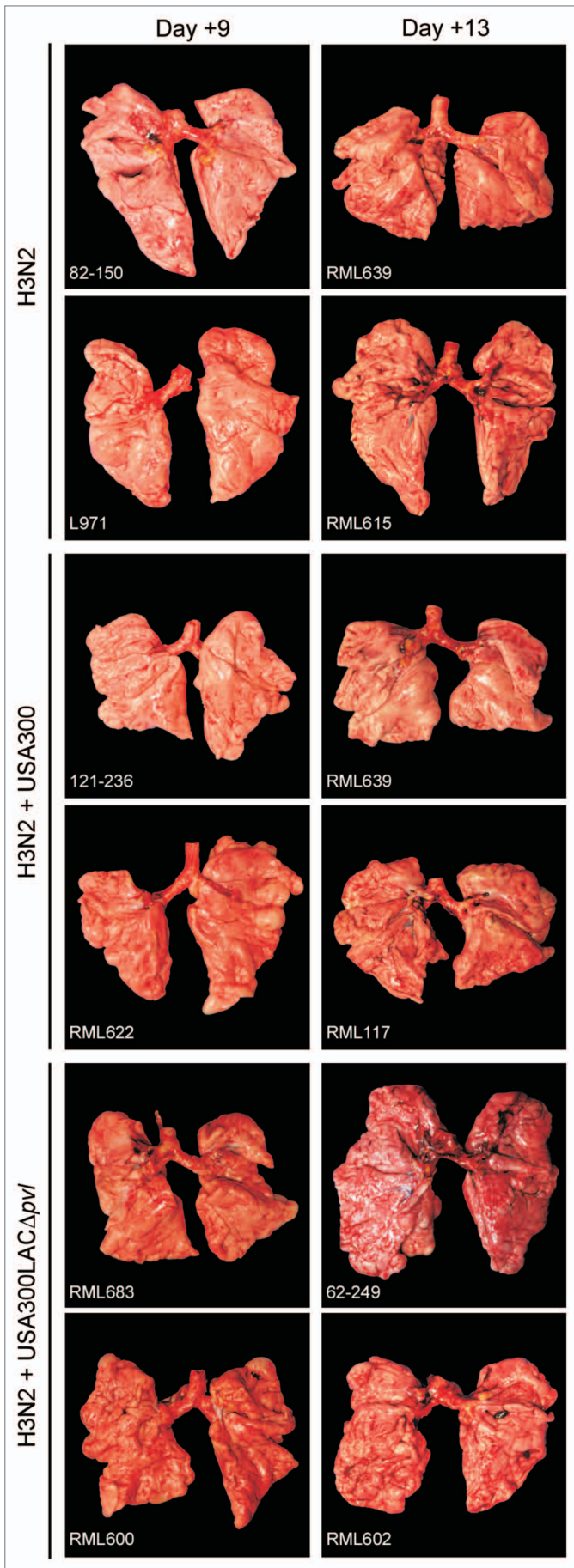
Figure S2. Gross lung pathology. Infected animals were euthanized 9 or 13 days after infection with seasonal H3N2 IAV as indicated. Intact lungs were resected from each animal and assessed for disease. There were no notable differences in pathology among the lungs from the treatment groups.

Lung Scoring Protocol

For each monkey, there are 7 slides.

L1 = left upper lobe
L2 = left middle lobe
L3 = left lower lobe
L4 = right upper lobe
L5 = right middle lobe (site of instillation)
L6 = right lower lobe (medial)
L7 = right lower lobe (lateral-basal)

Since the left lungs were processed fresh without prior formalin inflation, detailed assessment of microscopic features is difficult to perform. In comparison, the right lung was insufflated with formalin at necropsy to give optimal histology. As such, histological scoring will focus on the right lung (slides L4-L7).

The right lung was insufflated with formalin, fixed for 2 weeks, and cut. For the upper and middle lobe (slides L4 and L5, respectively), the pleura was removed by sharp dissection and the entirety of the parenchyma was processed as a single block. For the lower lobe, the pleura was removed by sharp dissection and the lung was bisected (blade cut from superior/cephalad to inferior/caudal) to create medial and lateral portions which were also entirely submitted (slides L6 and L7, respectively). Although some minor trimming of corners and margins was required to fit each tissue into one cassette, for all practical purposes, each section represents the entirety of the lobe and there is no selection bias between specimens.

The following scoring system was devised to:
1. maximize the quantitative assessment of histological features to compare pathology differences in lobes, strain treatment groups, and/or time points
2. maintain intra-observer and inter-observer reproducibility
3. be easily understood by the scientific community

Each lung slide from each animal will be graded independently for the three most prominent features, perivascular lymphocyte cuffing, interstitial pneumonia, and acute pneumonia score (see below).

Scoring Scales

Perivascular lymphocyte cuffing
Scored according to proportion of vessels with peri-vascular cuffs, one score per slide

0 = none
0.25 = fewer than 25% of vessels have lymphocyte cuffs
0.5 = 26-50% of vessels have lymphocyte cuffs
0.75 = 51-75% of vessels have lymphocyte cuffs
1.0 = 76-100% of vessels have lymphocyte cuffs

Interstitial pneumonia
Scored according to severity and volume of pneumonia;
Proportion of surface area in each slide with corresponding severity;
When possible, proportions should be expressed in quartiles (factors of 25%)

0 = none
1 = mildly increased cellularity in the alveolar walls (trace pneumonia)
2 = moderately increased cellularity causing alveolar wall thickening (mild pneumonia)
3 = markedly increased cellularity with consolidation (bad pneumonia, but not the worst case)
4 = dense consolidation due to alveolar wall disease (bad pneumonia, far end of the spectrum)

Acute pneumonia
Scored according to severity and volume of pneumonia
Proportion of surface area in each slide with corresponding severity;
When possible, proportions should be expressed in quartiles (factors of 25%)

0 = none
1 = mildly increased PMNs and/or necrotic debris in the air space (trace pneumonia)
2 = moderately increased cellularity in the air space (mild pneumonia)
3 = markedly increased cellularity with consolidation (bad pneumonia, but not the worst case)
4 = dense consolidation with obliteration of airspace (bad pneumonia, far end of the spectrum)
MRSA Lower Respiratory Tract Histology Data Sheet

Pathologist: _____   Date of Examination _____

Animal_____Slide _____

Perivascular Cuffing: (record one score of 0–1.0 for the entire slide): _____

| Interstitial Pneumonia: | (record the % surface area for each intensity):
(estimate surface area in quartiles (factors of 25%) | 0_____%
1_____%
2_____%
3_____%
4_____% |

| Interstitial Pneumonia: | (record the % surface area for each intensity):
(estimate surface area in quartiles (factors of 25%) | 0_____%
1_____%
2_____%
3_____%
4_____% |

Animal_____Slide _____

Perivascular Cuffing: (record one score of 0-1.0 for the entire slide): _____

| Interstitial Pneumonia: | (record the % surface area for each intensity):
(estimate surface area in quartiles (factors of 25%) | 0_____%
1_____%
2_____%
3_____%
4_____% |

| Interstitial Pneumonia: | (record the % surface area for each intensity):
(estimate surface area in quartiles (factors of 25%) | 0_____%
1_____%
2_____%
3_____%
4_____% |

Animal_____ Slide _____

Perivascular Cuffing: (record one score of 0-1.0 for the entire slide): _____

| Interstitial Pneumonia: | (record the % surface area for each intensity):
(estimate surface area in quartiles (factors of 25%) | 0_____%
1_____%
2_____%
3_____%
4_____% |

| Interstitial Pneumonia: | (record the % surface area for each intensity):
(estimate surface area in quartiles (factors of 25%) | 0_____%
1_____%
2_____%
3_____%
4_____% |