**Toll-like receptors, environmental caging and lung dysbiosis**

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**Fig.S1 | PCA and ordination of experimental murine lung samples and isolation and procedural control specimens.** Ordination demonstrates that when data clustered by murine lung or procedural control status is significantly different by PERMANOVA supporting significantly different community compositions in these grouped specimens



**Fig.S2 | Shannon diversity indices and rarefraction curves of murine lung specimens and isolation and procedural control specimens.** S2) Shannon diversity indices were significantly higher overall in murine lung specimens compared to controls specimens. S3) Rarefraction curves demonstrate that microbial communities are more even in murine lung specimens that in isolation and procedural control specimens.



**Fig. S3 | Ranked relative abundance of lung microbial communities in isolation and procedural control specimens and murine lung specimens.** The final plot is a ranked abundance plot of isolation and procedural control specimens ranked in order of the most abundant OTUs in the murine lung specimens.



**Fig. S4 | Bacterial burden in co-housed and randomized experimental mice.** Co-housed and randomized wild type mice (blue) display a trend towards reduced bacterial burden on randomization. There was no significant difference sin bacterial burden in TLR2-/- (light brown), TLR4-/- (orange) and TLR5-/- (green) co-housed and randomized mice. (non=co-housed, ran=randomized) (unpaired t test or Mann Whitney test where applicable)



**Fig.S5. Bacterial burden of lung microbiota across groups compared to wild type.** **A)** Co-housed experimental mice, bacterial burden when compared to wild type is significantly different in TLR5-/- mice only. **B)** Bacterial burden in randomized mice shows increased bacterial burden in TLR5-/- mice compared to wild type. (unpaired t test or Mann Whitney test where appropriate, \*P<0.05)

**Supplemental methods**

**16S reads and controlling for contamination in low biomass experiments**

We had a total of 77 murine lung specimens (sequenced in duplicate) and 17 isolation and procedural controls. These control specimens included DNA extracted from bead tubes, AE buffer, instrument blades, H20 and plate water. The total number of 16S rRNA reads isolated was 4,519,862 with a mean of 48,084 per specimen (S.E.M 16,495 - range 13-972,584). Ranked relative abundance analysis of isolation and procedural control specimens compared to the ranked relative abundance of murine lung specimens did demonstrate a significant abundance of both OTU0001 and OTU0003 in both murine lung specimens and controls. However, the removal of these OTUs from the analysis did not alter our observations or conclusions. Hence, all biological specimens and OTUs are included in the analysis