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Supplementary Materials for

Exposure to isocyanates predicts atopic dermatitis prevalence and disrupts therapeutic pathways in commensal bacteria

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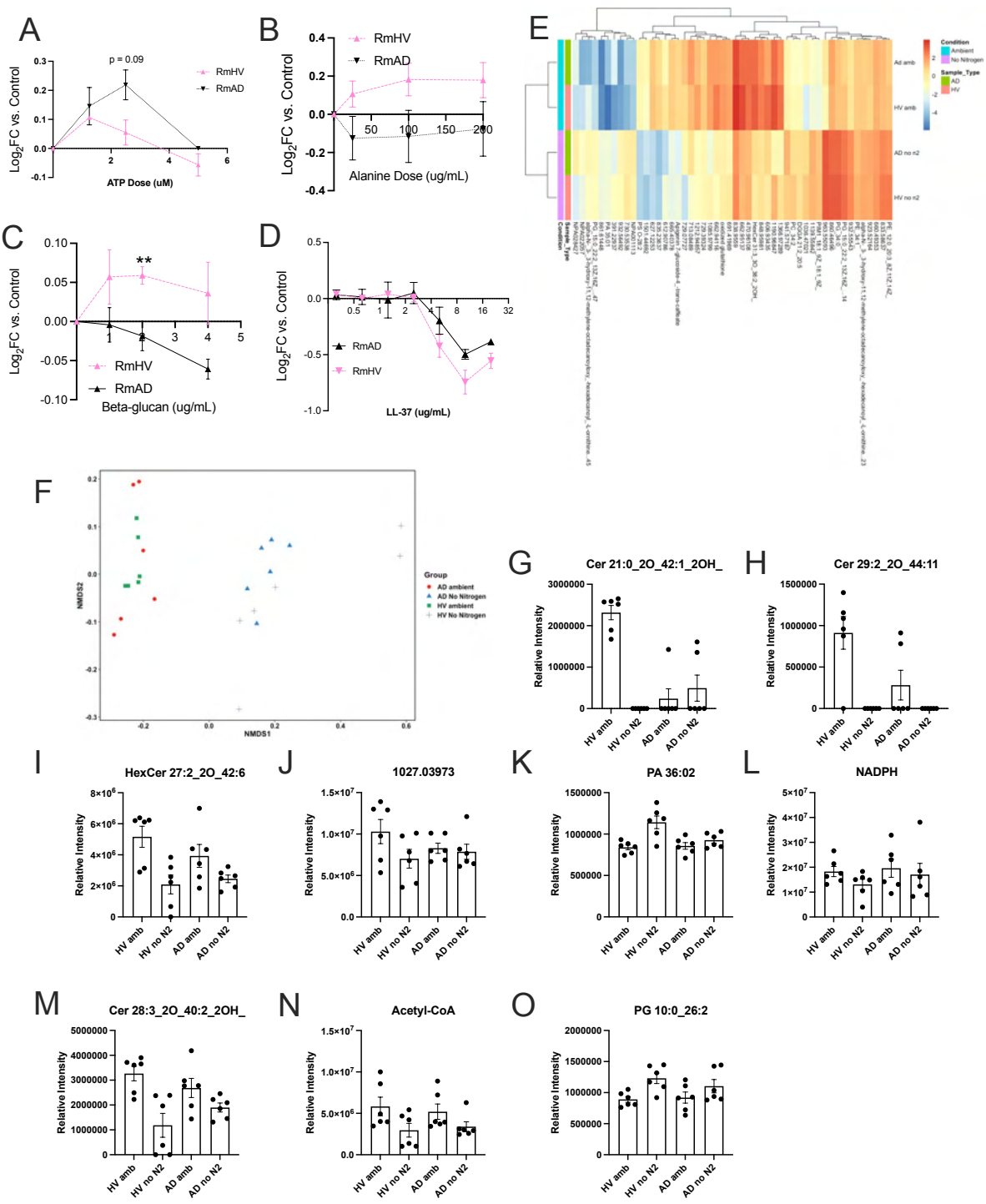
Figs. S1 to S10

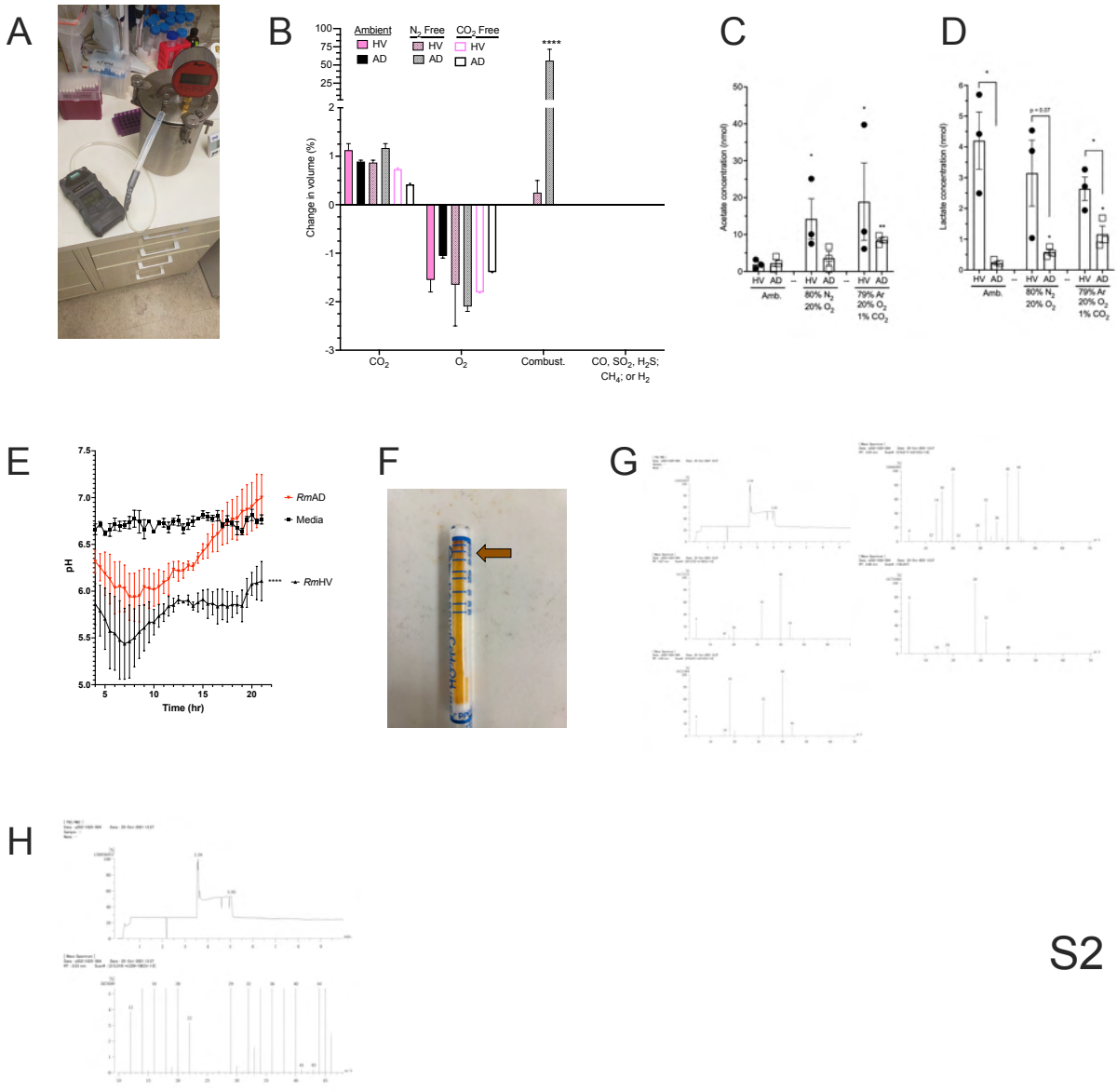
Supplementary Materials

Supplemental Figures S1-S10

S1

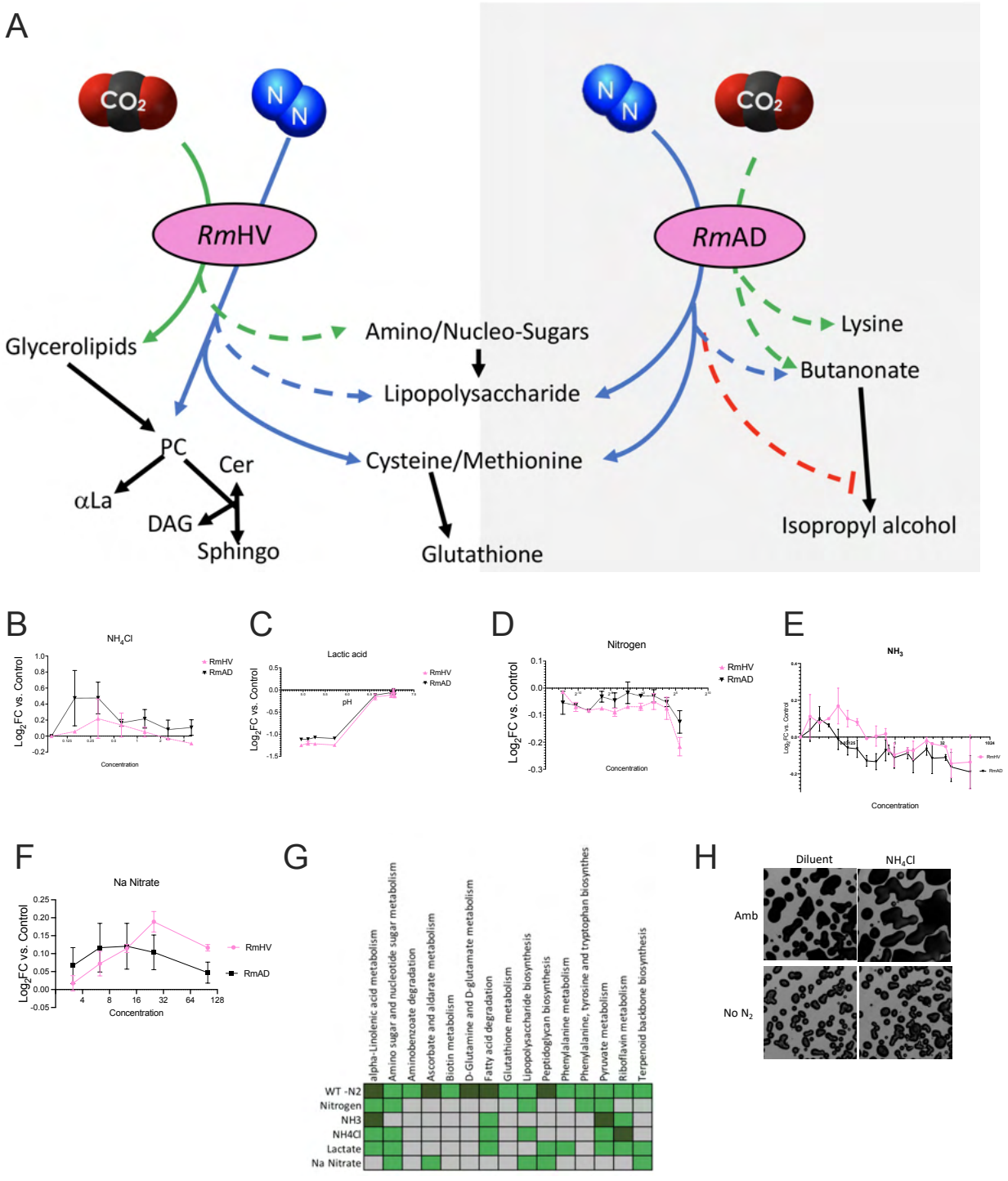


Supplemental Figure 1. Health associated isolates of *R. mucosa* differ in response to citrate and urea cycle challenges. (A-D) Log2 fold chance (FC) in absorbance (OD600) at 22 hours for 3 isolates of *Roseomonas* from healthy volunteers (*Rm*HV) and 3 isolates of *Roseomonas* from patients with AD (*Rm*AD) cultured with media supplemented with ATP (A), alanine (B), beta-glucan from *Candida* spp. (C), or the antimicrobial peptide LL-37 (E). Three isolates of *Roseomonas* from healthy volunteers (*Rm*HV) and 3 isolates of *Roseomonas* from patients with AD (*Rm*AD) were grown in ambient or N2 deprivation conditions. (E-F) Heatmap for differentially impacted metabolites (E) and NMDS similarity plots (F). (G-O) Intensity level for indicated annotated metabolites. Data represent three or more independent experiments and expressed as mean $\pm$ SEM (D-I).

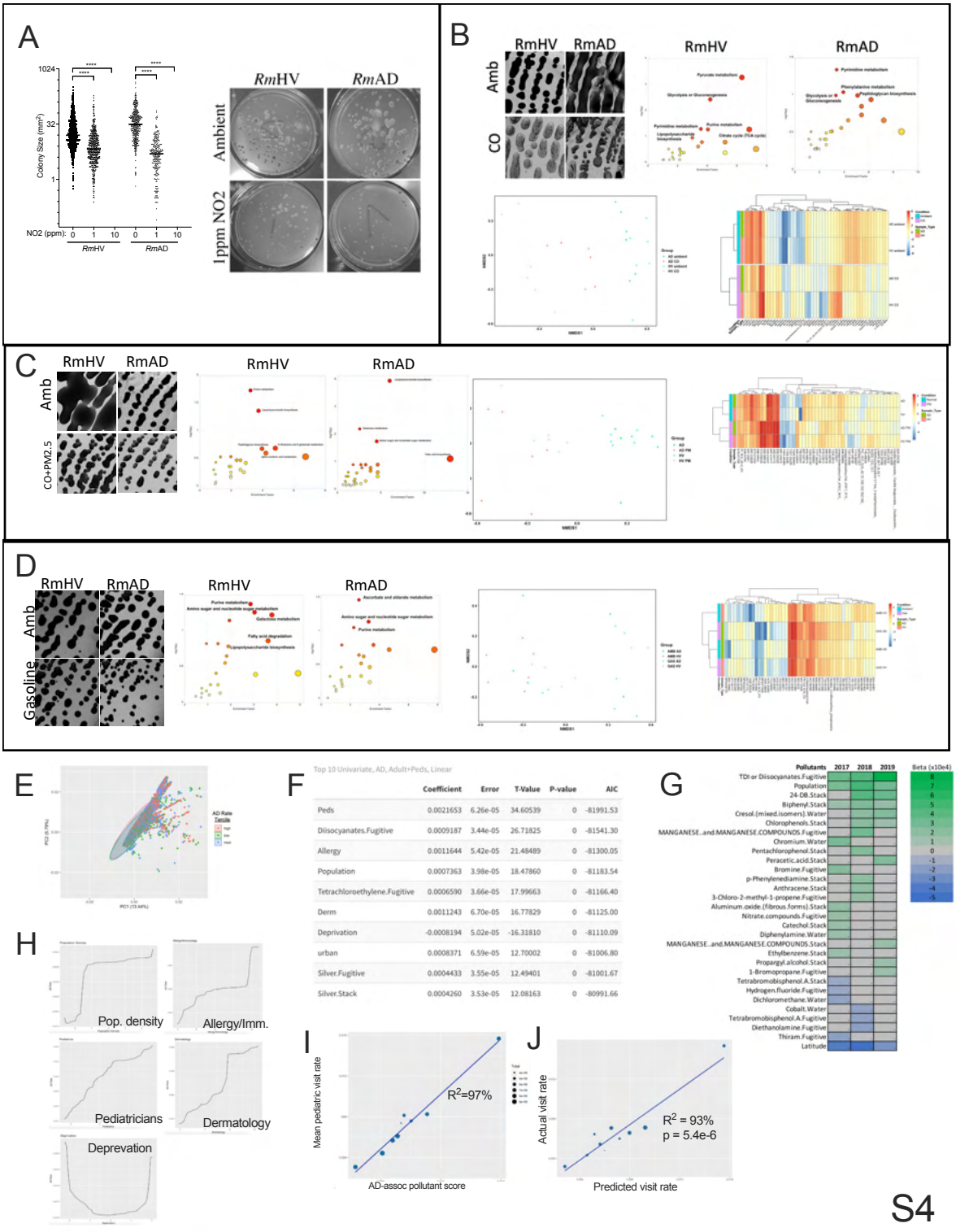


S2

Supplemental Figure 2. N_2 deprivation in AD associated *Roseomonas* generates fermentation of isopropyl alcohol. (A) Image of experimental set up where gas monitor was connected to outlet port of culture container. (B) Measurements of indicated gases for 3 *Rm*HV isolates versus 3 *Rm*AD isolates in indicated conditions. Values are % of total volume except for combustible gas (Combust.) which is indicated as % lower explosive limit (LEL). (C-D) Enzymatic assessment of acetate and lactic acid was measured from a loopful of agar growth after 48 hours of culture for 3 *Rm*HV isolates versus 3 *Rm*AD in indicated conditions. (E) pH over time for cultures with media alone or with *Rm*HV or *Rm*AD isolates. (F) Image of results from gas detection tube designed to assess ethanol and isopropyl alcohol, brown arrow indicates area of color change indicating positive results. (G-H) Gas chromatography tracings of peaks from *Rm*AD isolates grown in N_2 deprivation, demonstrating peaks consistent with (but not confirmatory for) isopropyl alcohol but peaks inconsistent with ethanol. Data shown are representative of two or more independent experiments. * = $P < 0.05$.

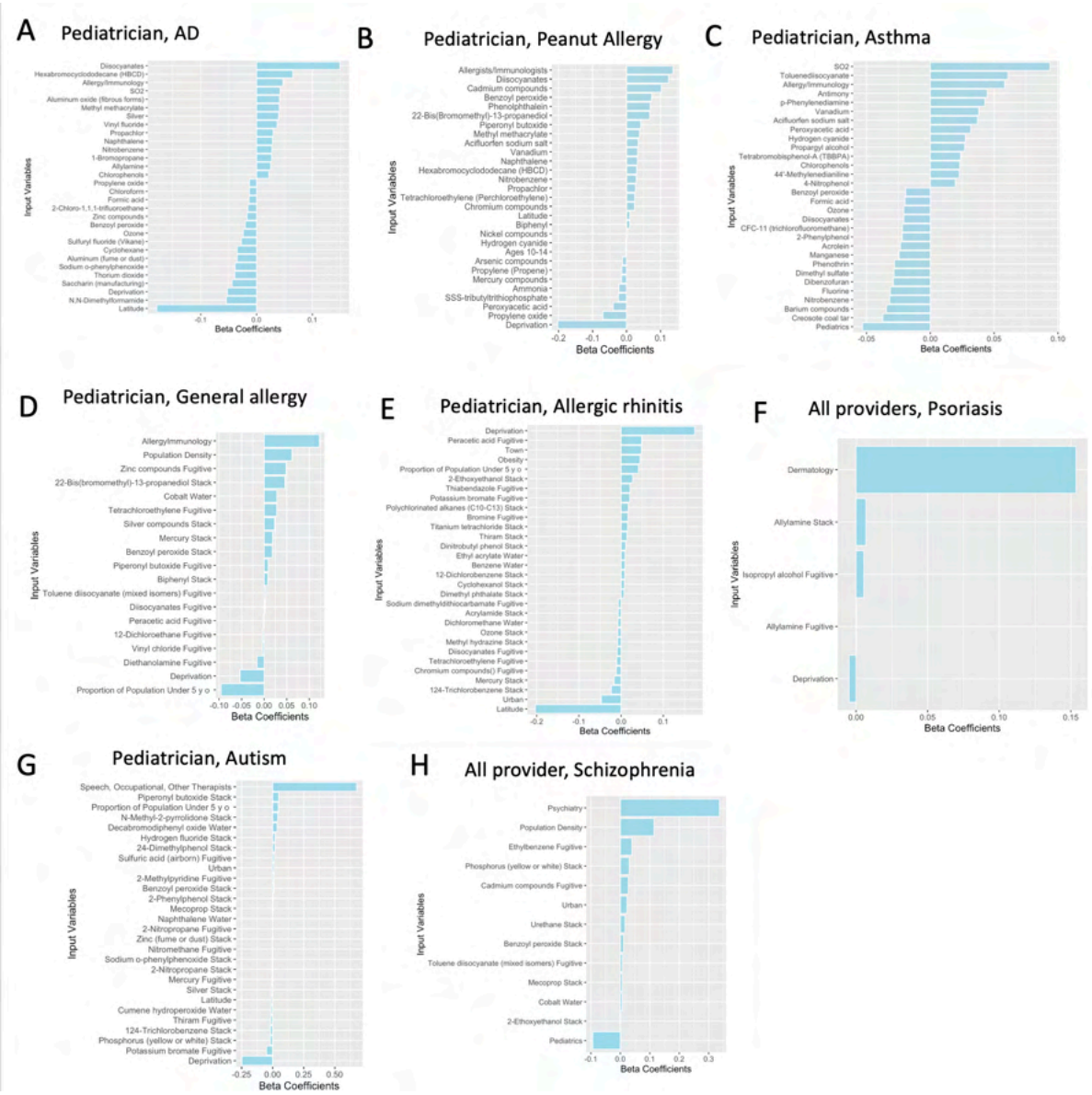


Supplemental Figure 3. Isolates of *R. mucosa* are not differentially impacted by broth supplementation of nitrogen-based pollutants. (A) Summary figure of atmospheric fixation differences between isolates of *Roseomonas* from healthy volunteers (*Rm*HV) or from patients with atopic dermatitis (*Rm*AD). Solid lines indicate metabolite pathways was identified by flux analysis; dotted line indicates pathway was impacted by gas deprivation. Green indicated CO_2, blue (or red inhibitory indicators) indicates N_2. (B-F) Log2 fold chance (FC) for 3 isolates of *Rm*HV and 3 isolates of *Rm*AD cultured with media supplemented with NH_4Cl (B), lactic acid (C), Nitrogen basic (D), NH_3 (E), or sodium nitrate (F) versus respective diluents. (G) Summary of pathways impacted by indicated challenges as measured by MetaboAnalyst along with impact on total annotated ceramide containing compounds for *Rm*HV; green indicates the pathway was altered, dark green indicates the pathway was significantly altered, grey indicates the pathways was not altered. (H) Representative image for *Rm*HV isolates grown in ambient or N_2 deprivation conditions after supplementation of culture media with NH_4Cl. Data represent three or more independent experiments.

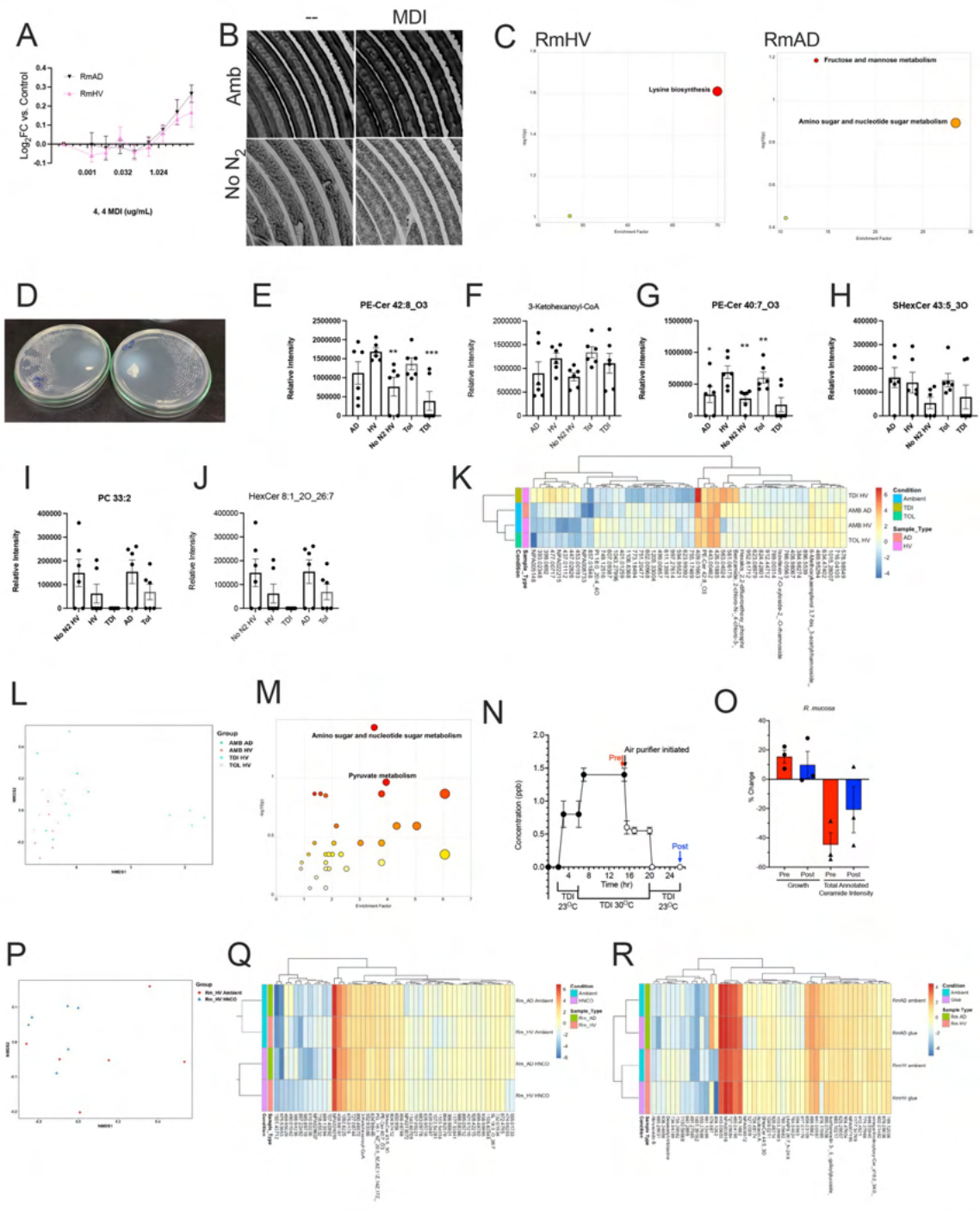


Supplemental Figure 4. Health associated isolates of *R. mucosa* are partially impacted by atmospheric pollutants. (A) Colony size for each dot representing one colony in plate and representative image for three isolates of *Roseomonas* from healthy volunteers (*Rm*HV) and 3 isolates of *Roseomonas* from patients with AD (*Rm*AD) cultured with indicated concentrations of NO_2. (B-D) Representative plate image, MetaboAnalyst pathway analysis, NMDS similarity plot, and heat map of most common metabolites for *Rm*HV and *Rm*AD cultured in presence of 1% carbon monoxide (CO; B), CO and particulate matter (PM; C), or gasoline placed in lid of agar culture plate (D). (E) PCA of all input variables separating zip codes into high, low, and middle terciles for pediatrician AD visit rate. (F) AIC derivation analysis for TRI versus AD by zip code for 2019. (G) Beta values ($x10^4$) for top 30 identified linear associations by lasso for available years 2017-2019 for impact of Toxic Release Inventory (TRI) pollutants versus rates of atopic dermatitis (AD) by US zip code. (H) Partial dependence plots for 2019 rates versus 2014-2019 aggregate exposure data for

indicated variables. (I) Aggregate AD-associated pollutant scores were generated from lasso for each zip code, summating the total score from each pollutant multiplied by its beta correlation value. Average score per decile versus pediatric AD visit rate is shown. (J) Aggregate scores for 80% of the data set were used to predict AD visit rate scores for the remaining 20% for each decile. Size of circle represents number of visits, R^2 weighted by visit also shown. Data represent two or more independent experiments (A-D) or single point analysis of aggregate data (E-J).

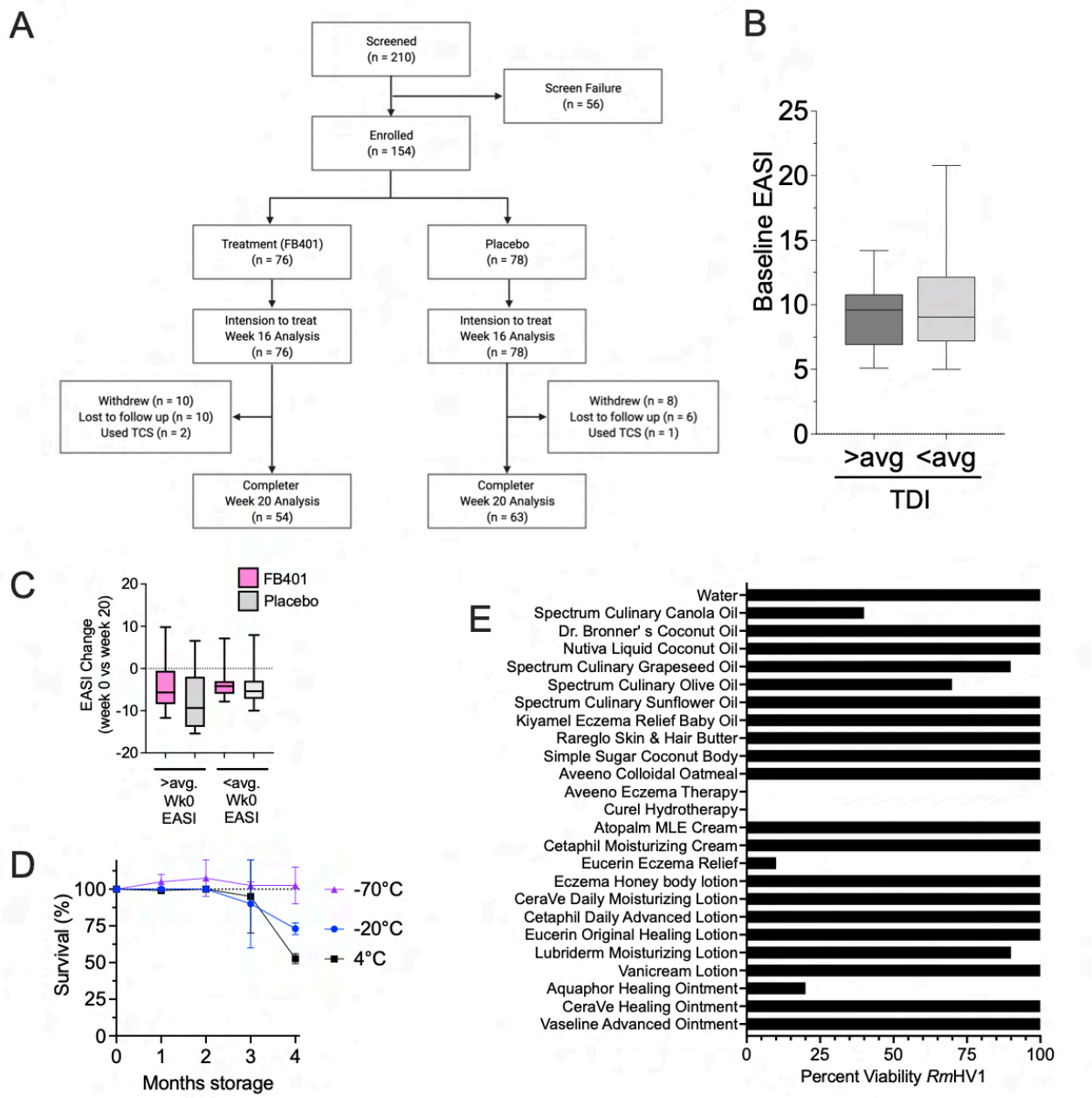


Supplemental Figure 5. Diisocyanates associated with related atopic diseases of children.
(A) Lasso linear regression plots with 1 standard error (1SE) restrictions for top associated variables for 2019 pediatrician visits for AD versus the Toxic Release Inventory (TRI) cumulative exposures for 2014-2019. (B-C) 1SE lasso regression plots for 2019 indicated visit type versus the Risk-Screening Environmental Indicators (RSEI) database. (D-H) 1SE lasso linear regression plots with for indicated visits versus TRI cumulative exposures for 2014-2019.

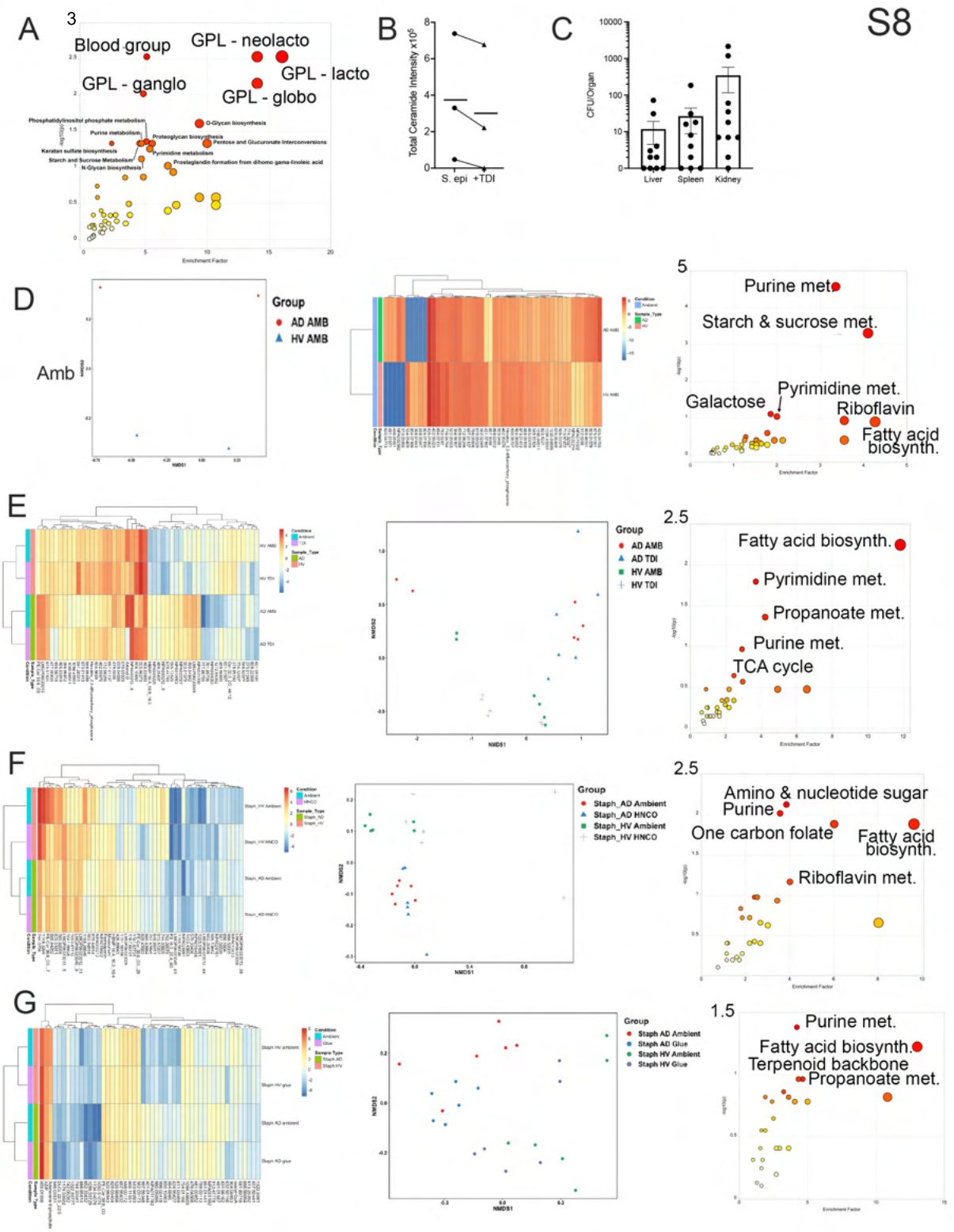


Supplemental Figure 6. Health associated isolates of *R. mucosa* are partially impacted by isocyanates. (A) Log2 fold chance (FC) at 22 hours for 3 isolates of *Roseomonas* from healthy volunteers (*Rm*HV) and 3 isolates of *Roseomonas* from patients with AD (*Rm*AD) cultured with media supplemented with 4,4 MDI. (B) Representative images for *Rm*HV in the presence of absence of MDI in ambient conditions or in N_2 deprivation. (C) MetaboAnalyst pathway analysis for *Rm*HV and *Rm*AD for growth in ambient conditions with and without MDI. (D) Representative image showing zone of inhibition created by 1mcg of TDI on lid of agar plate (white spot). (E-J) Total intensity for indicated metabolites annotated by MALDI on *Rm*AD in ambient conditions or *Rm*HV in ambient, N_2 deprivation (No N_2), or in ambient conditions with toluene or TDI on agar plate lid. (K) Heatmap, or NMDS similarity plot (L) for *R. mucosa* cultured with either toluene (TOL) or TDI on the lid. (M) MetaboAnalyst pathway analysis for metabolites impacted in *Rm*AD by culture with TDI in the lid of agar dish versus 1mcg of toluene. (N) TDI was placed into $52m^3$

size room and left at either 23^{O}C or 30^{O}C as indicated. Filtration performed using commercially available air filter during time points indicated and resultant air concentration measurements shown. (O) Percent change in growth and total annotated ceramide intensity for *Rm*HV exposed to the air from the TDI filled room either pre- or post-filtration. (P) NMDS similarity plot for *Rm*HV cultured with HNCO in agar lid. (Q-R) Heat maps for top metabolites for *Rm*HV and *Rm*AD with HNCO (Q) or polyurethane glue (R) in the agar plate lid. Data represent two or more independent experiments.

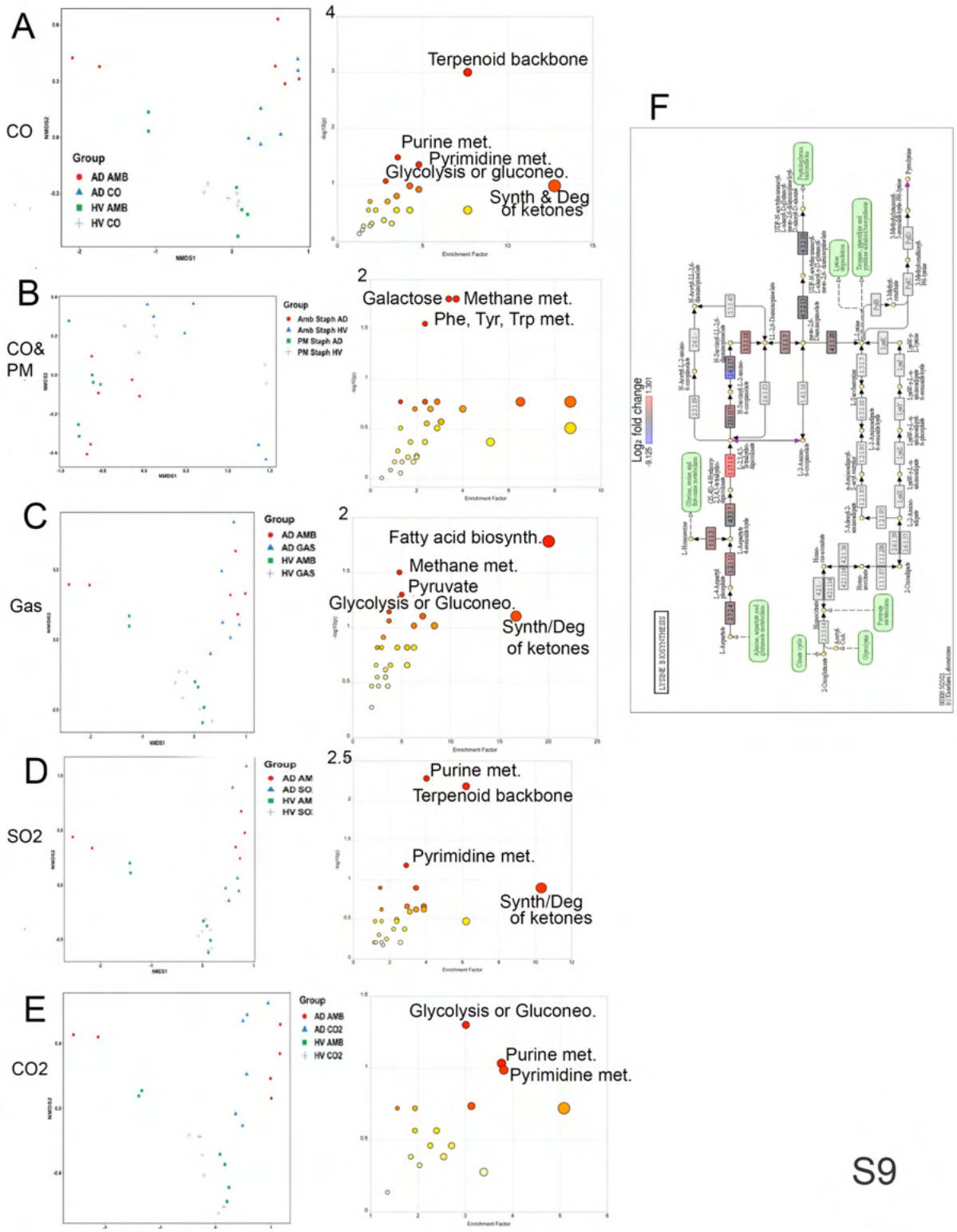


Supplemental Figure 7. Clinical trial design and probiotic derivation. (A) CONSORT Flow diagram for clinical trial; TCS = topical corticosteroid use between weeks 12 and 20 during the trial. (B) Post-hoc stratification of the completer population results by study sites' historic TDI exposure as above the mean (>avg) or below for baseline EASI. (C) EASI change between week 0 (Wk0) and week 20 post-hoc stratified by above or below average baseline Wk0 EASI score. (D) Stability of CFU count for *Rm*HV1 stored in capsule format and reconstituted in duplicate each month for 4 months in water. (E) A lawn of RmHV1 was plated on agar before exposure to 10mm coverslip disc containing the indicated topical product was placed in the center. Percent viability calculated by percent of growth coverage in the 1cm area outside of the coverslip.

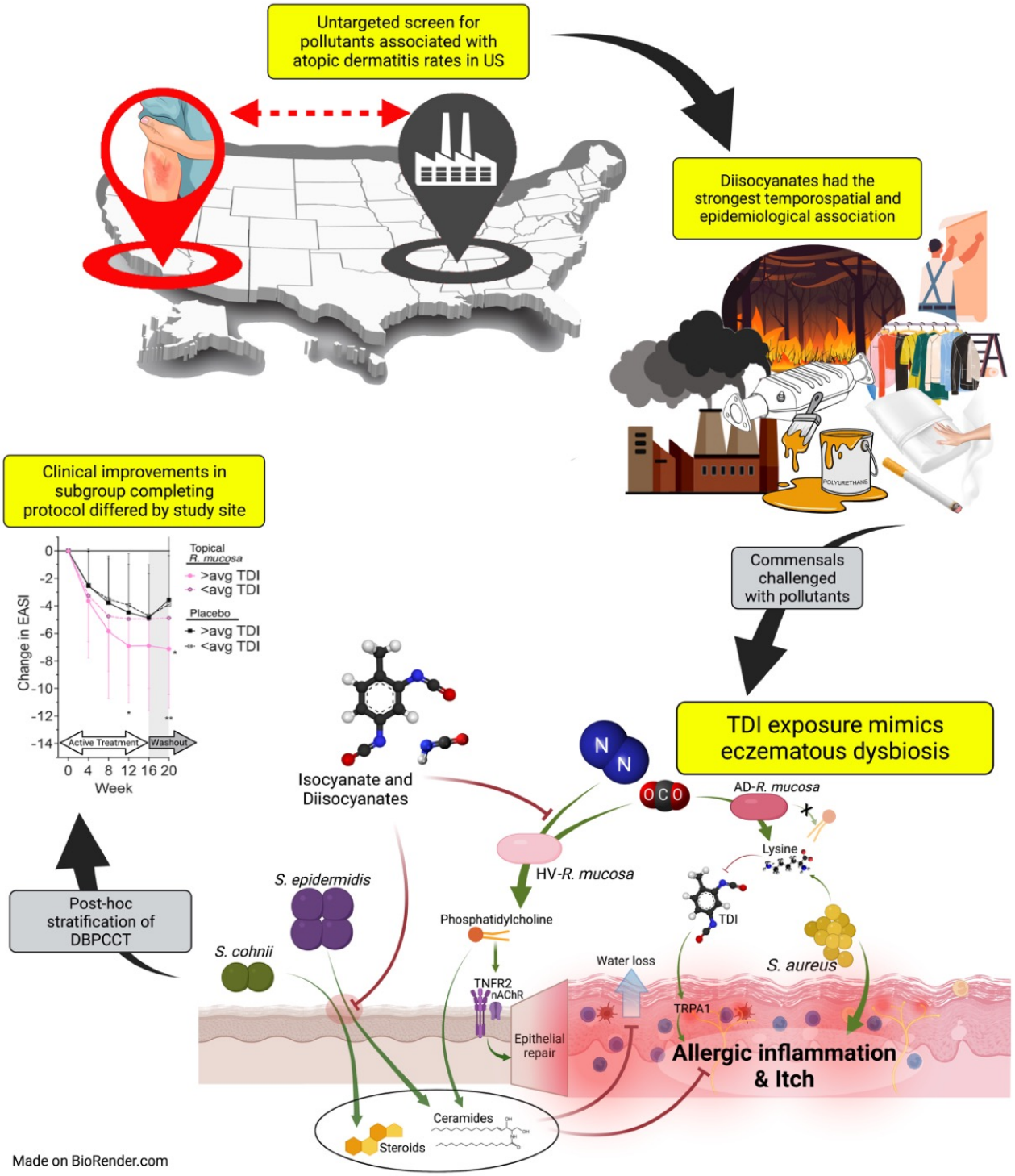


Supplemental Figure 8. Health associated isolates of *Staphylococcus* spp. are impacted by isocyanate exposure. (A) Pathway analysis for differentiating metabolites, and total annotated ceramide metabolites (B) from the culture supernatants of primary human keratinocytes incubated with three isolates of *Staphylococcus epidermidis* from healthy volunteers (HV) which had been either grown under ambient conditions or challenged with TDI prior to co-culture. (C) Colony count from organs at 72 hours of mice injected intravenously with 10e4 colonies of *S. cohnii*. (D-G) NMDS similarity plot, heat map, and MetaboAnalyst pathway analysis for 3 isolates of HV *Staphylococcus* spp. and 3 isolates of AD-associated *S. aureus* compared under ambient conditions (D), or compared under challenge with TDI (E), HNCO

(F), and polyurethane glue (G) in lid of agar dish. Data represent two or more independent experiments. ** = $p < 0.01$, *** = $p < 0.001$.



Supplemental Figure 9. Health associated isolates of *Staphylococcus* spp. are partially impacted by atmospheric pollutants. (A-E) 3 isolates of *Staph* HV and 3 isolates of *Staph* AD were cultured in ambient conditions or challenged with indicated atmospheric pollutant. NMDS similarity plot for both groups and MetaboAnalyst pathway analysis for *Staph* HD challenged in carbon monoxide (CO; A), CO and particulate matter rom burning wood (PM; B), gasoline placed in lid of agar culture plate (C), 2ppm of SO_2 (SOx; D), or 2% CO_2 (E) are shown. (F) Transcriptomics Log_2 fold change values for analysis of *Rm*AD (red, upregulated) versus *Rm*HV (blue) for the lysine biosynthesis pathway. Data represent two or more independent experiments. ** = $p < 0.01$, **** = $p < 0.0001$ as determined by ANOVA.



Supplemental Figure 10. Overall summary of study findings. Summary of stages of presented work as outlined in the publication.