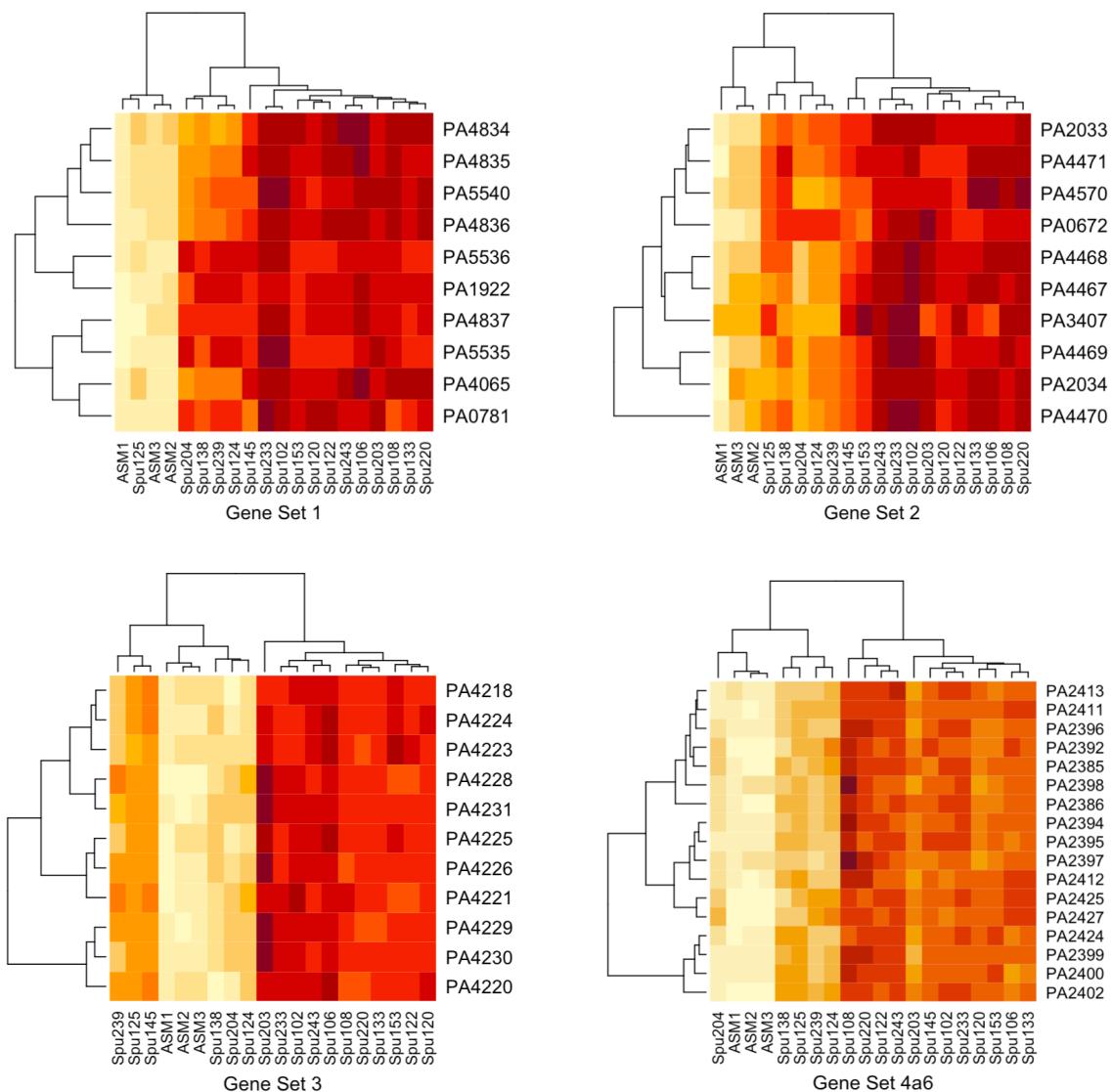
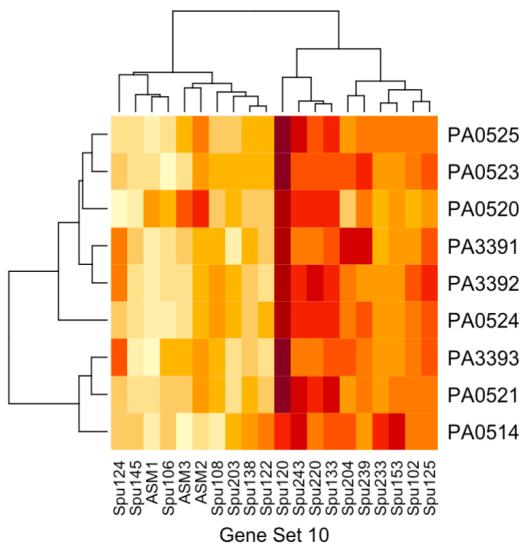
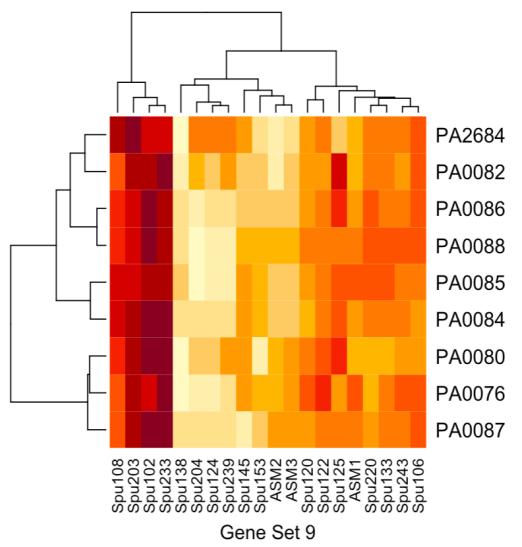
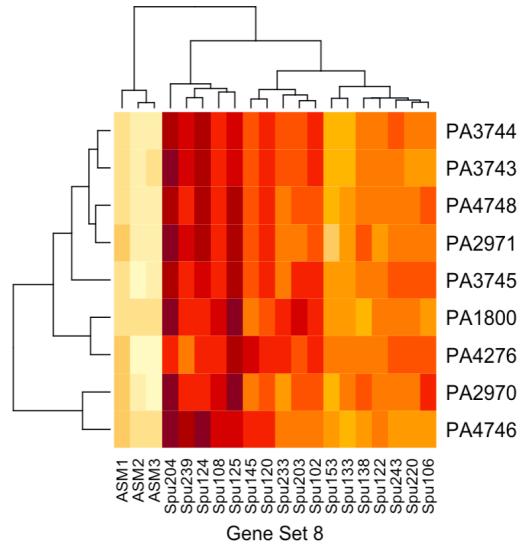
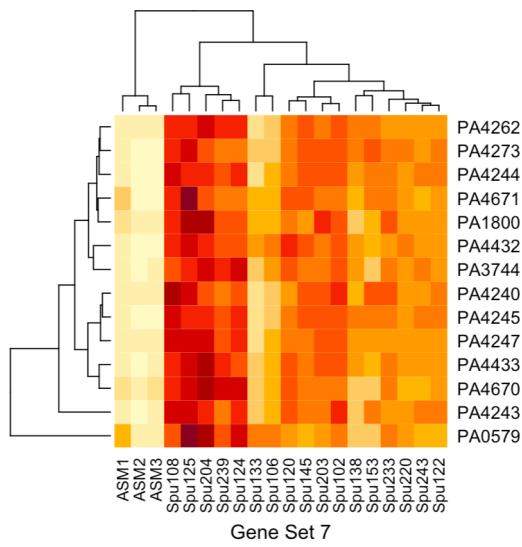
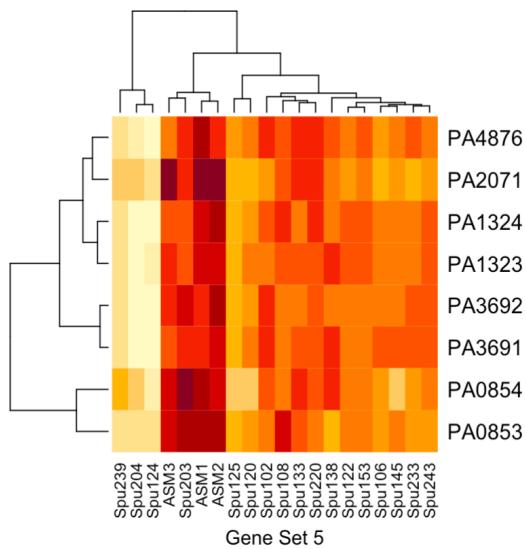


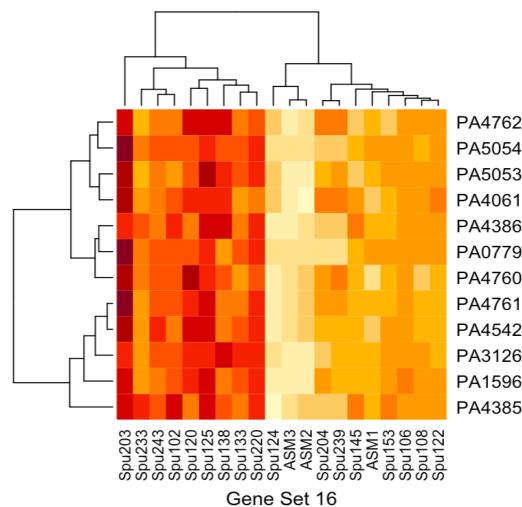
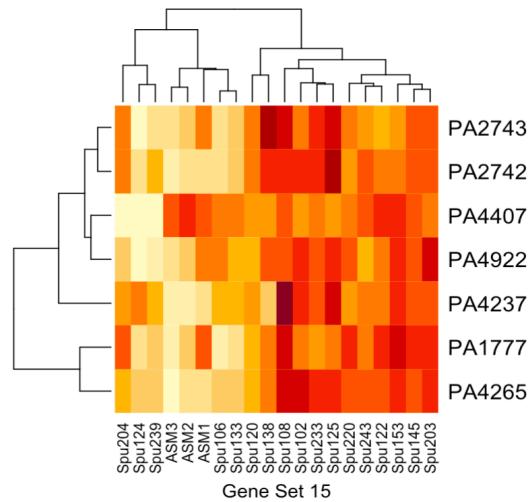
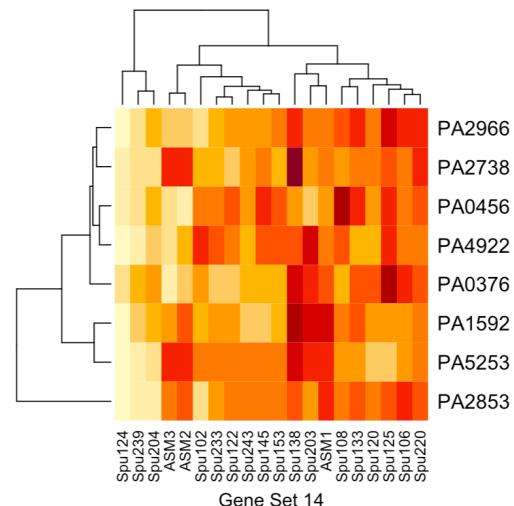
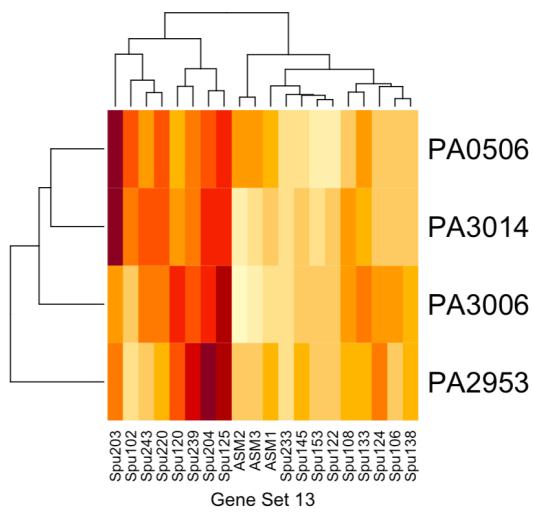
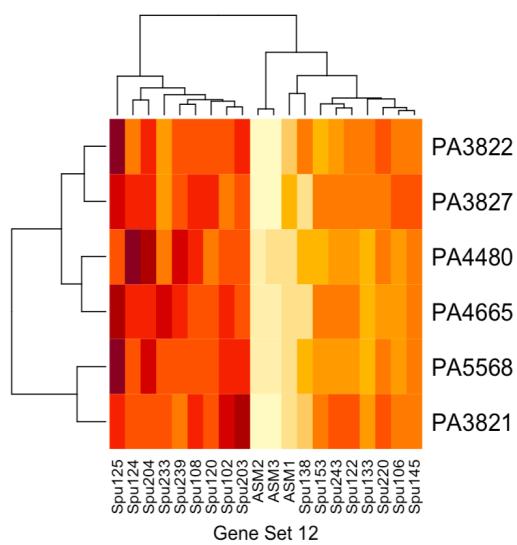
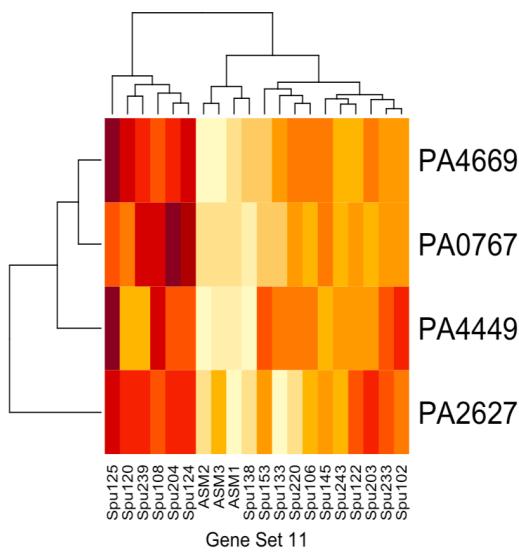
Supplementary Figures

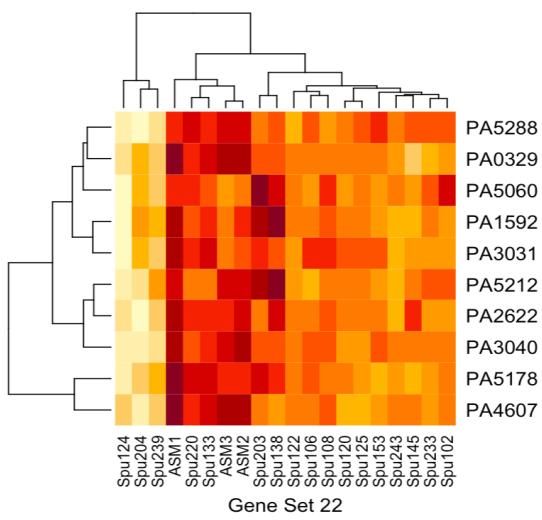
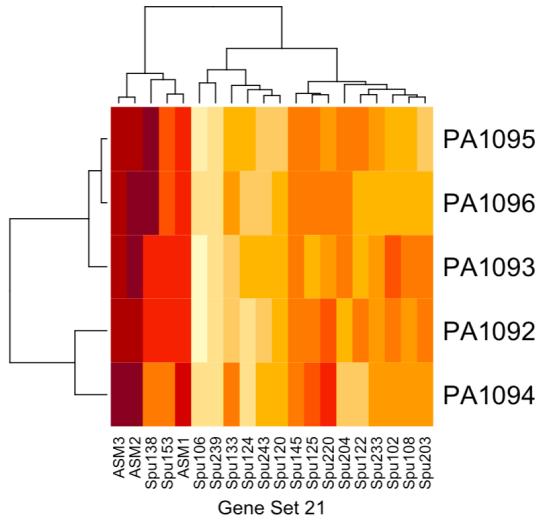
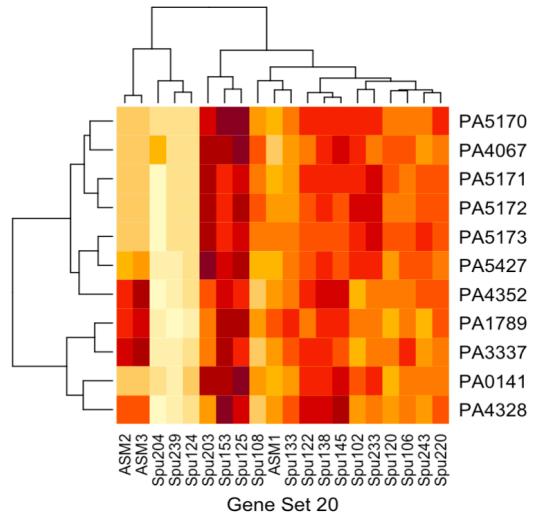
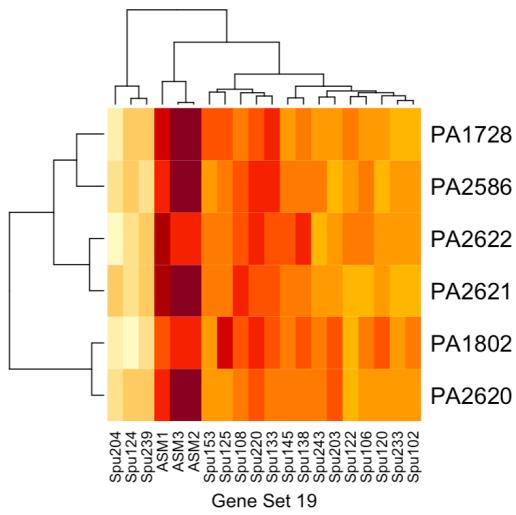
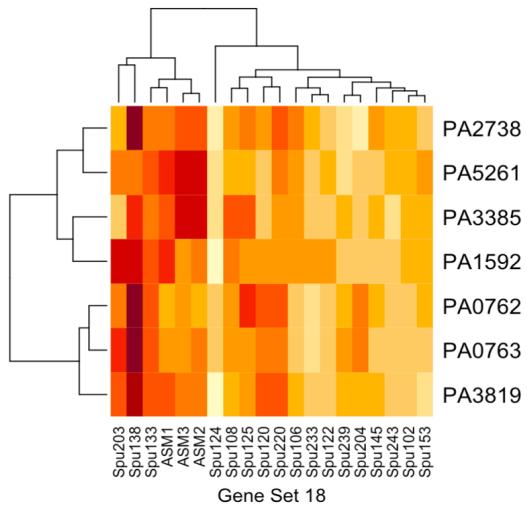
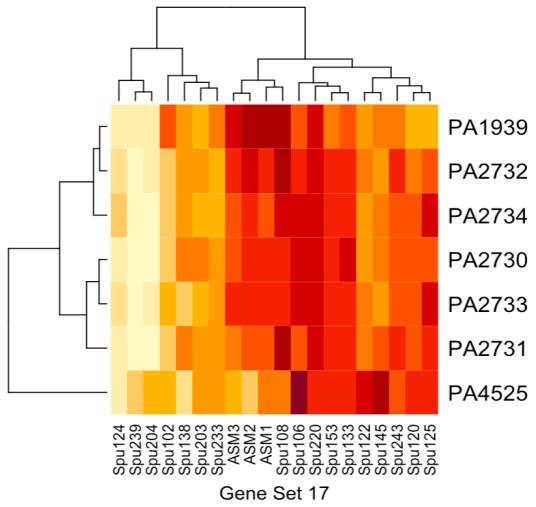
Figure S1. Heat maps demonstrating the expression of constituent genes in the 23 gene sets across the spike-in sputum (untreated and metal-treated) and ASM samples. In these panels, darker red boxes represent relatively higher gene expression, while lighter yellow boxes represent relatively lower expression. (A) The first set of heat maps compares the untreated sputum samples to the ASM samples. (B) The second set of heat maps compares the metal-treated sputum samples to the untreated sputum samples, and also includes the ASM samples.

Figure S1A. CF sputum samples (untreated) vs. ASM









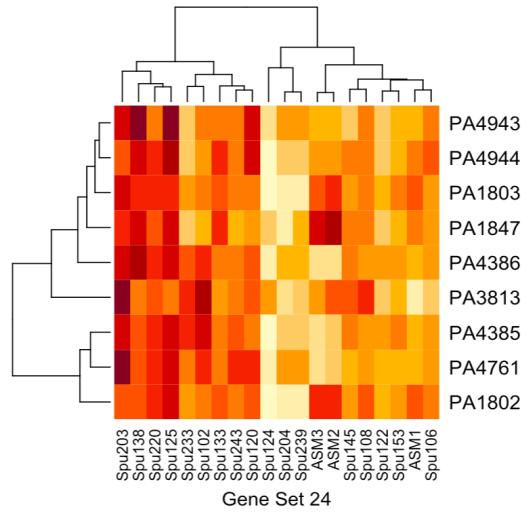
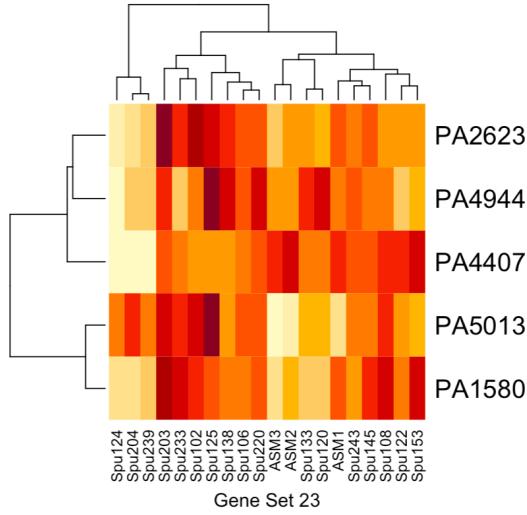
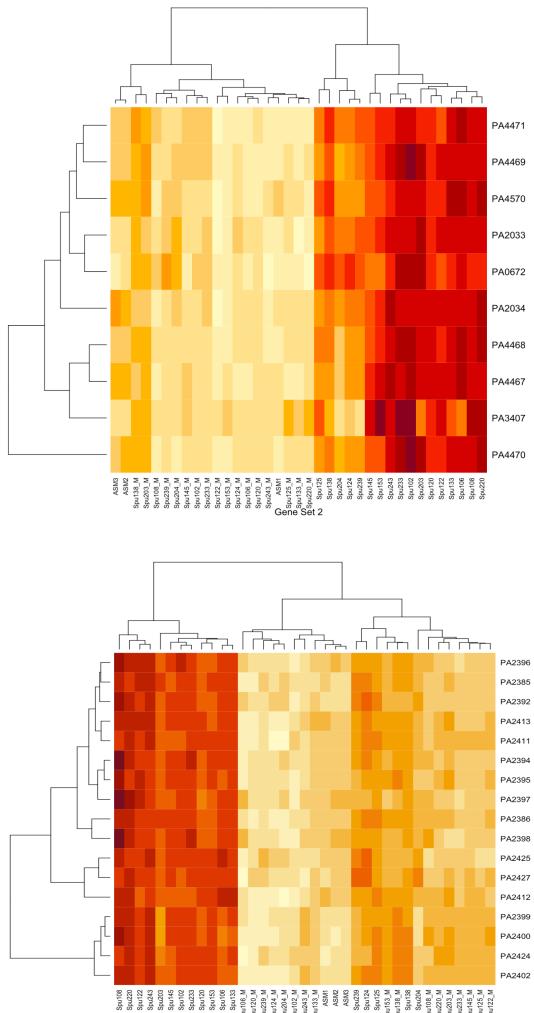
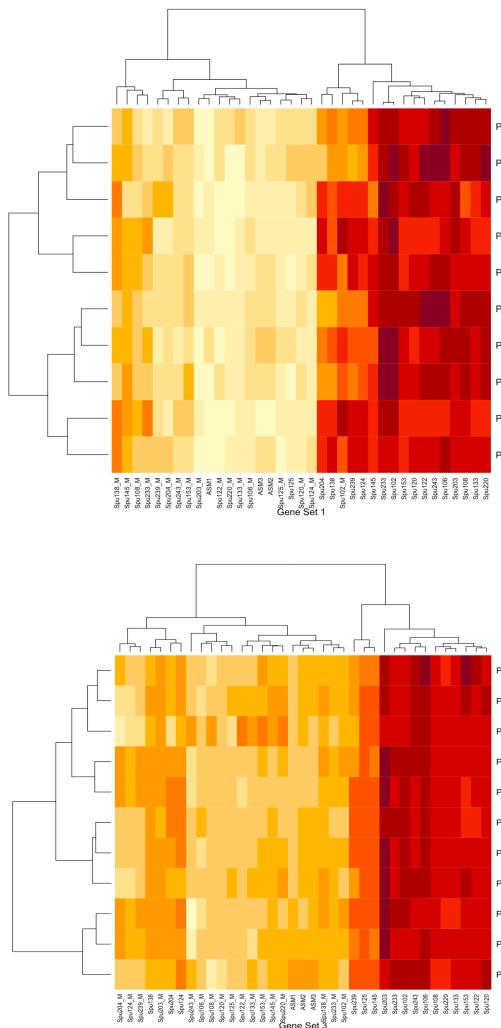
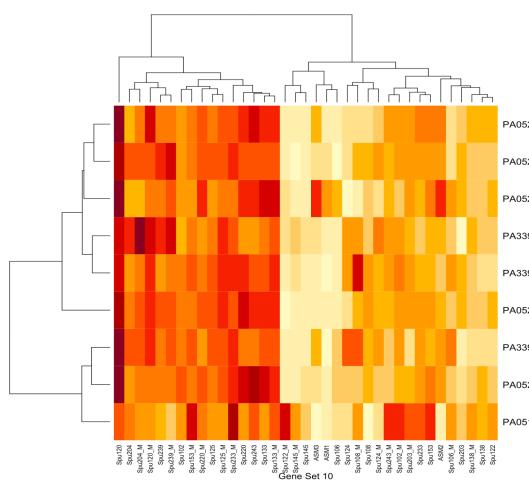
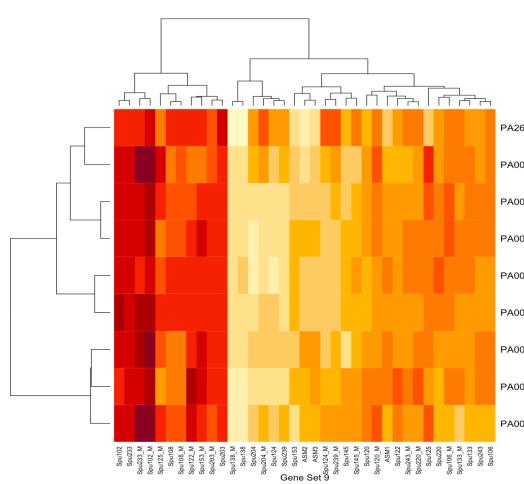
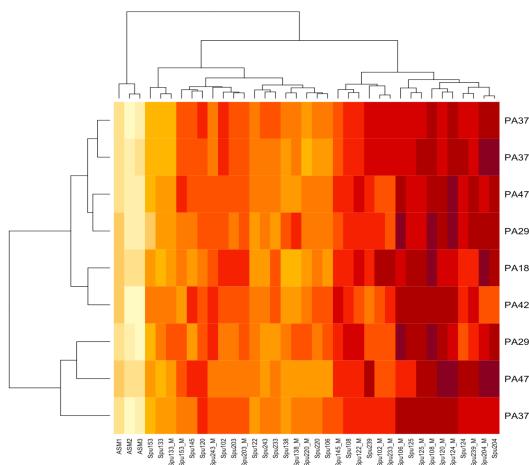
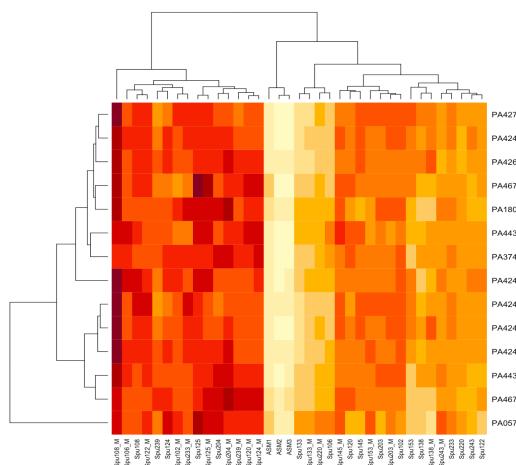
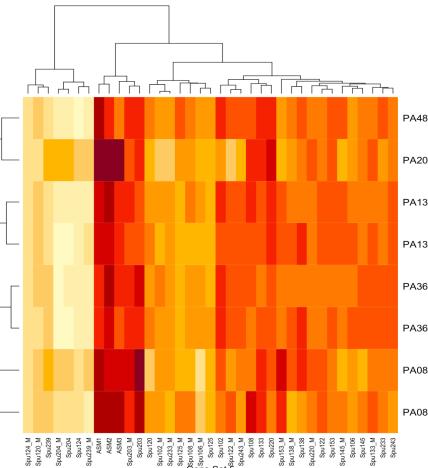
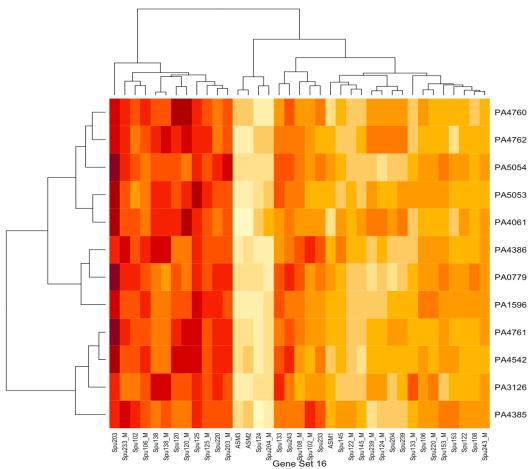
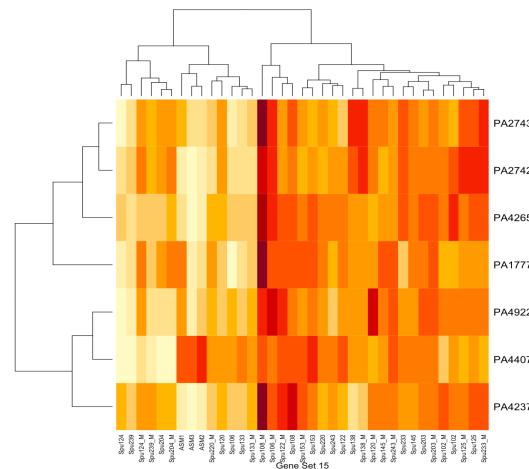
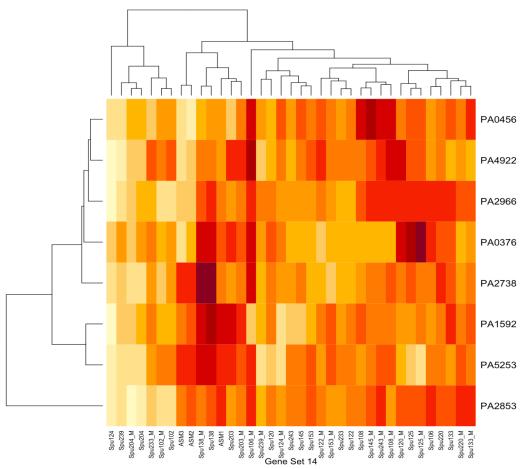
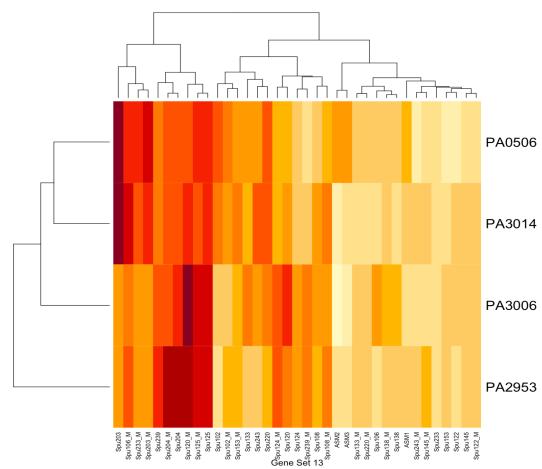
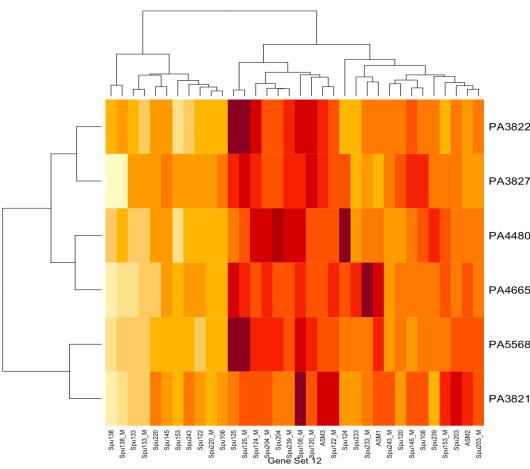
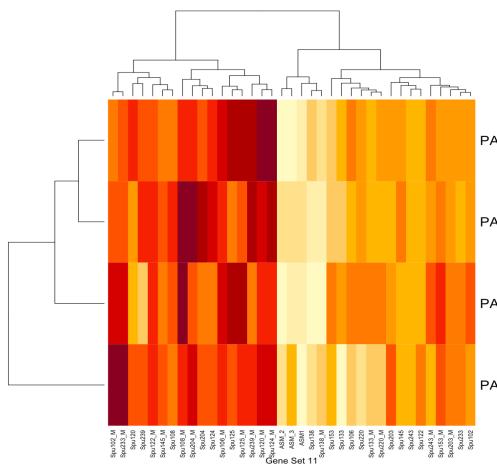
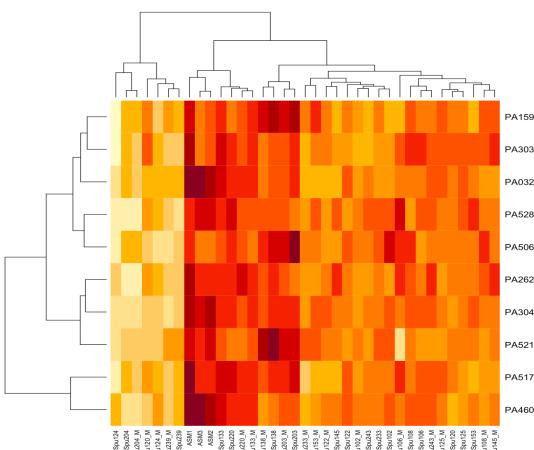
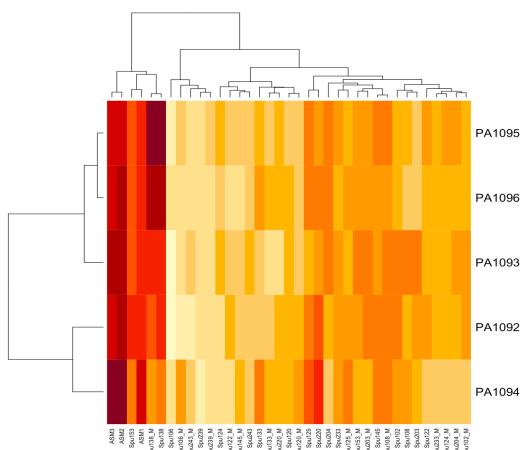
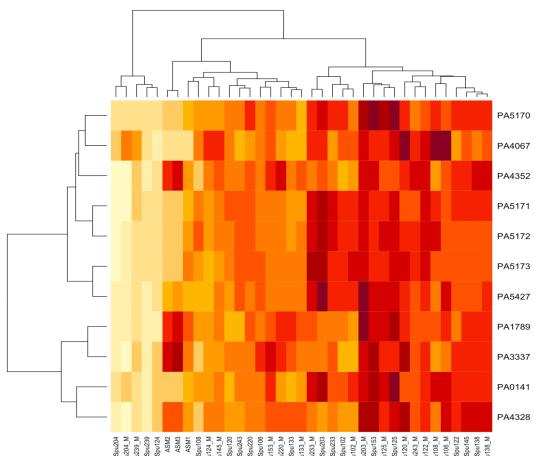
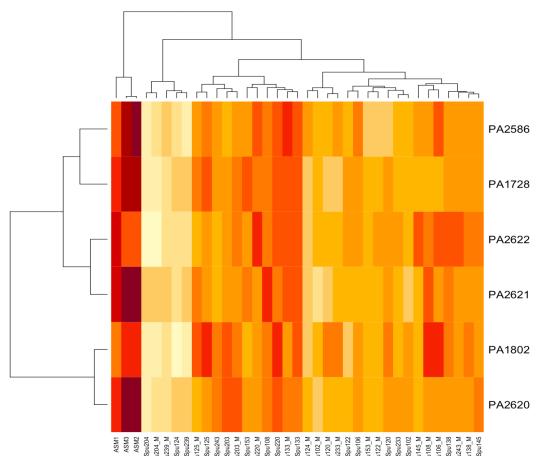
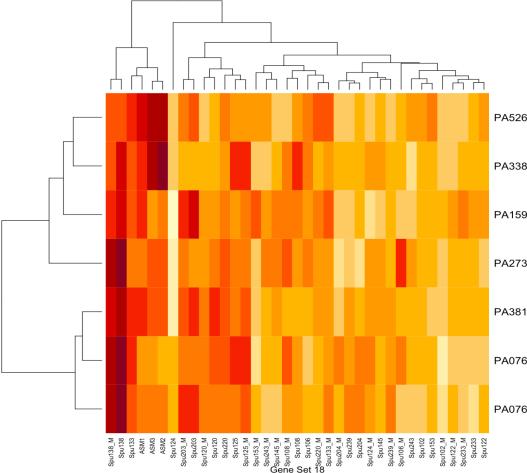
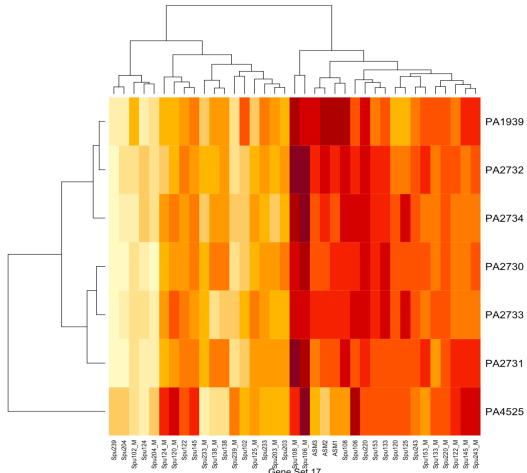


Figure S1B. CF sputum samples (untreated) vs. CF sputum samples (metal treated) and ASM









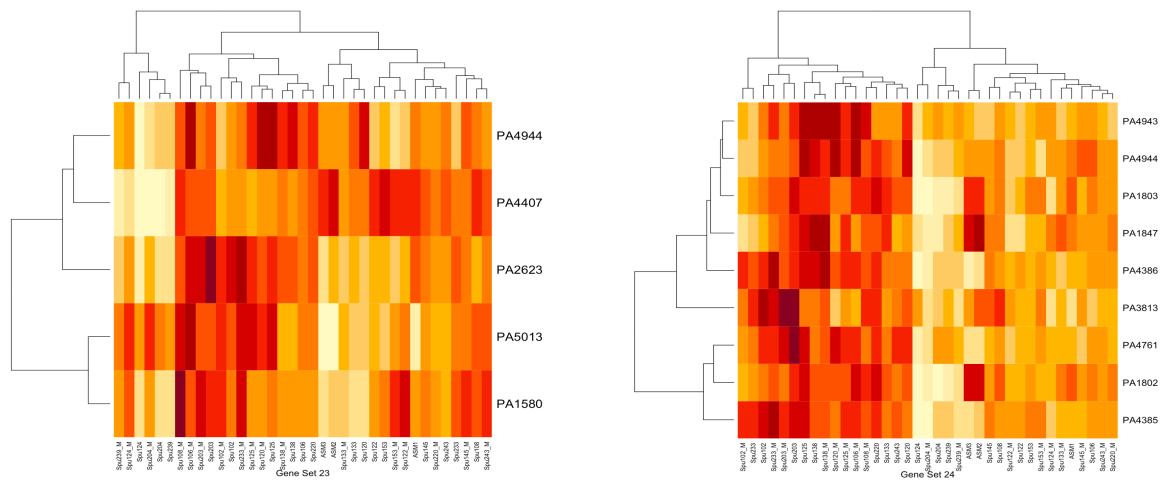
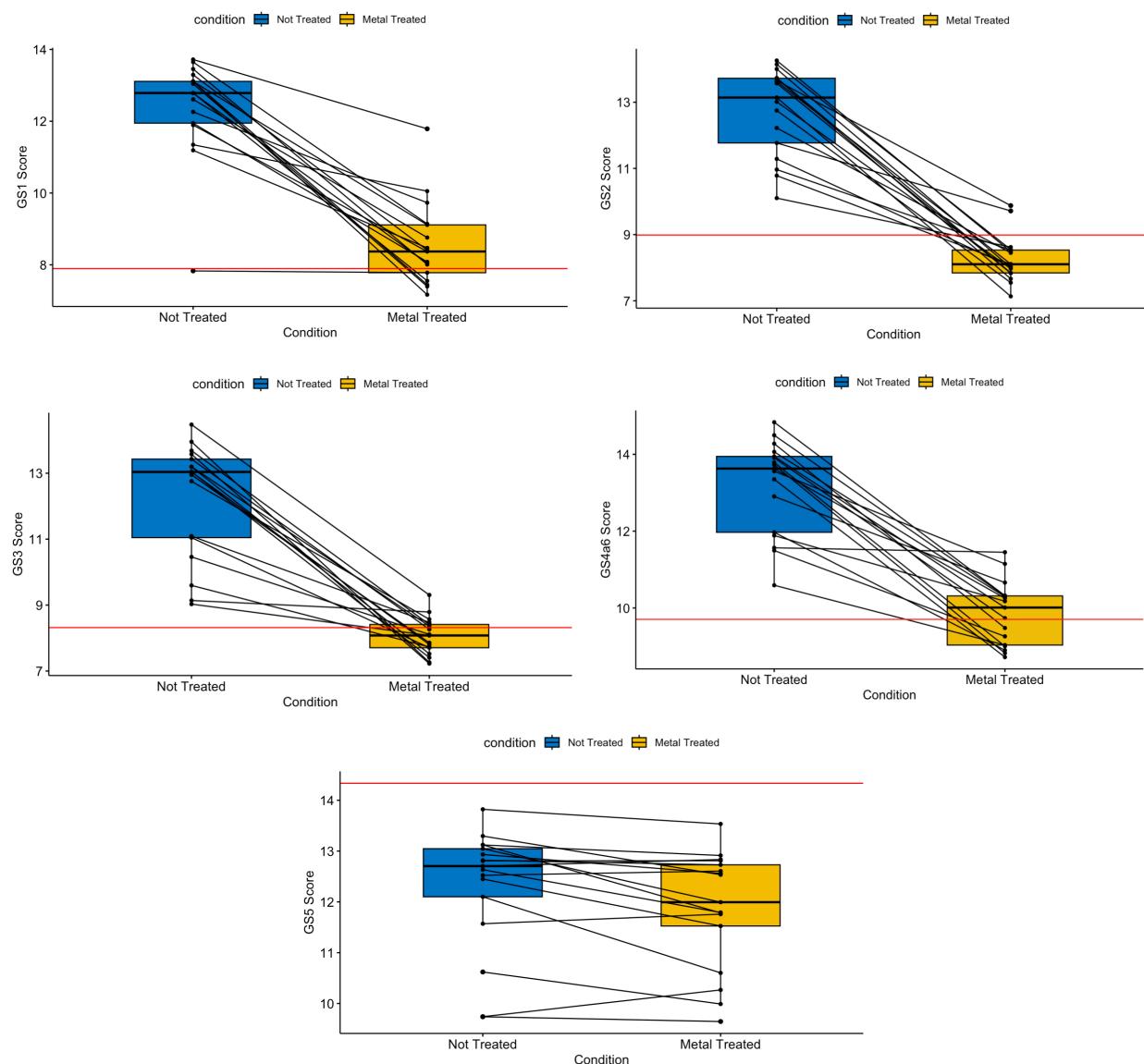
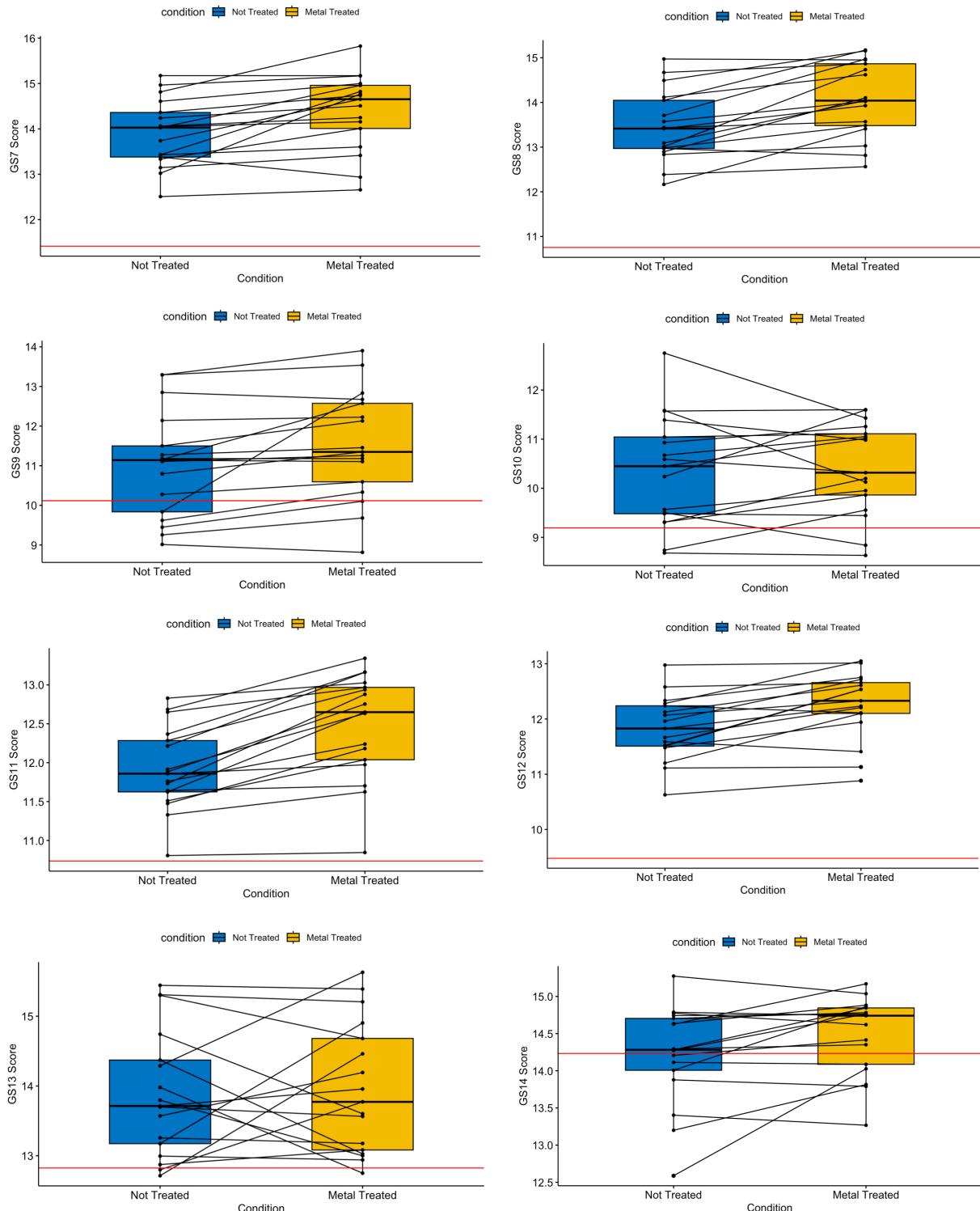
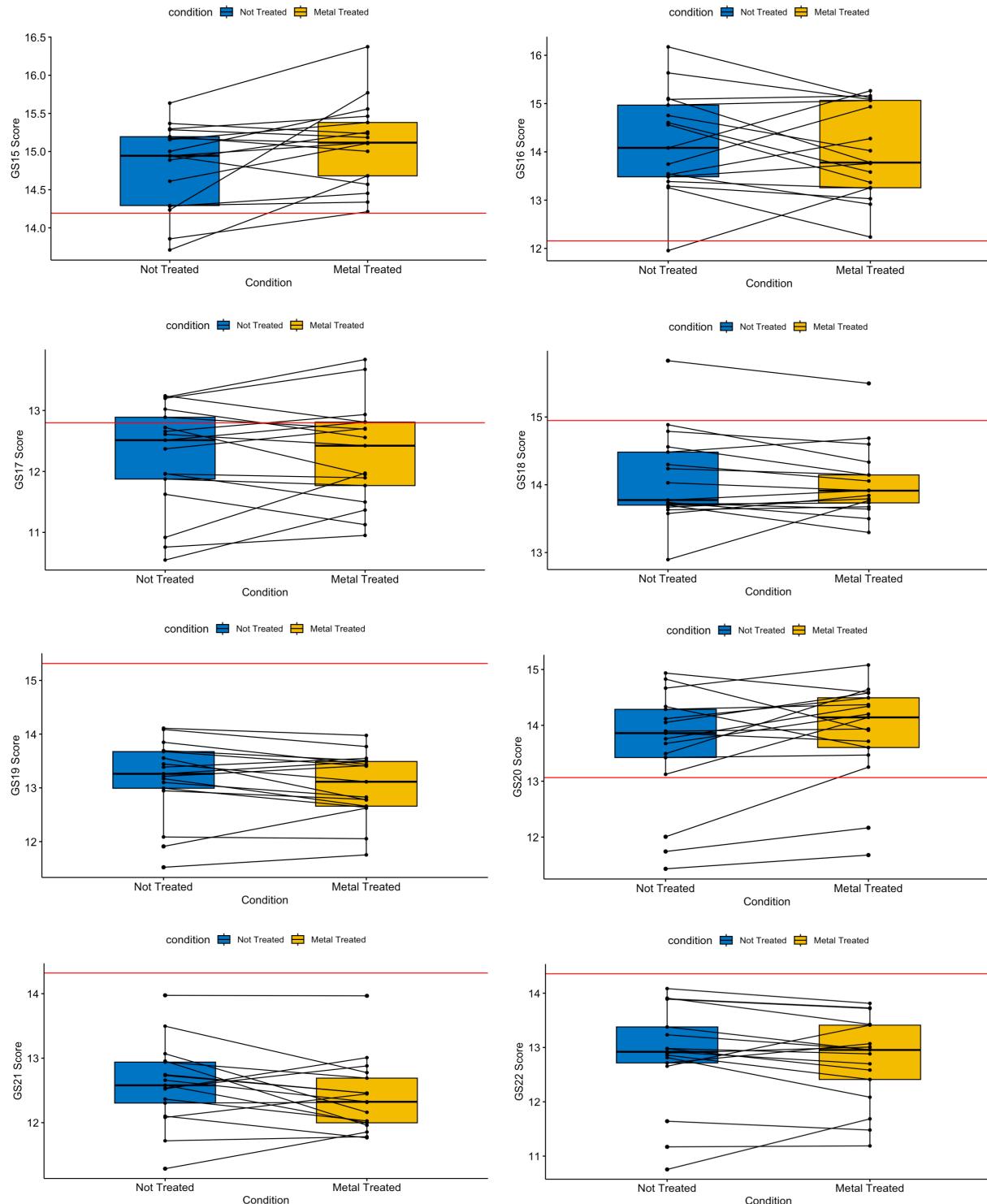


Figure S2. Response to metal exposure for all 23 ADAGE-constructed gene sets [Table 3]. The activity score (see results) was calculated for each gene set on a sample-by sample basis. (A) The blue boxplot represents the activity scores of CF sputum samples not treated with metals while the yellow boxplot represents the corresponding metal-treated samples. The red line indicates the median activity score for each gene set across the ASM samples. The corresponding volcano plots show (B) the differential expression of genes in the spike-in sputum samples in response to metal exposure, as well as (C) the differential expression of genes between spike-in sputum (untreated) and ASM samples.

Figure S2A. Activity scores of metal-treated (yellow) vs. non-treated (blue) sputum samples, with reference to the median activity score for ASM samples (red line)







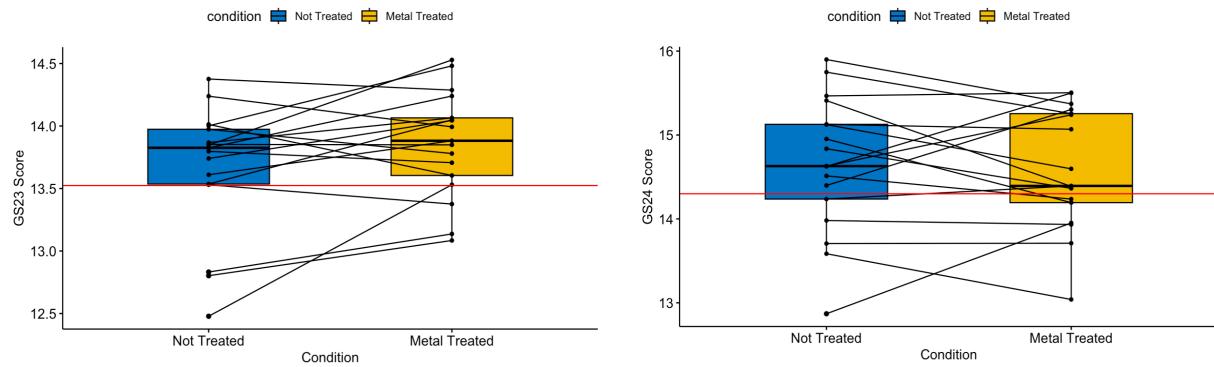
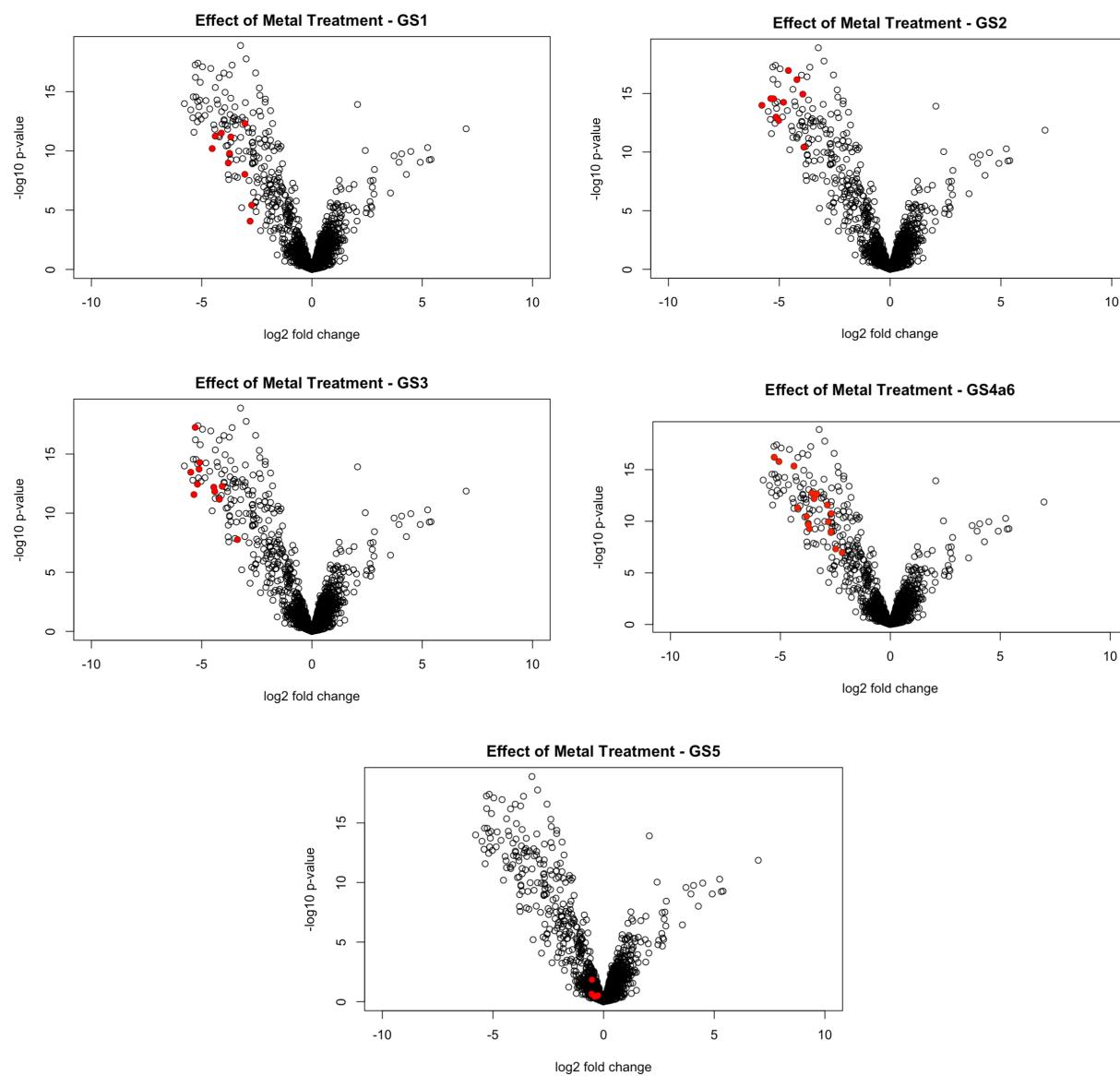
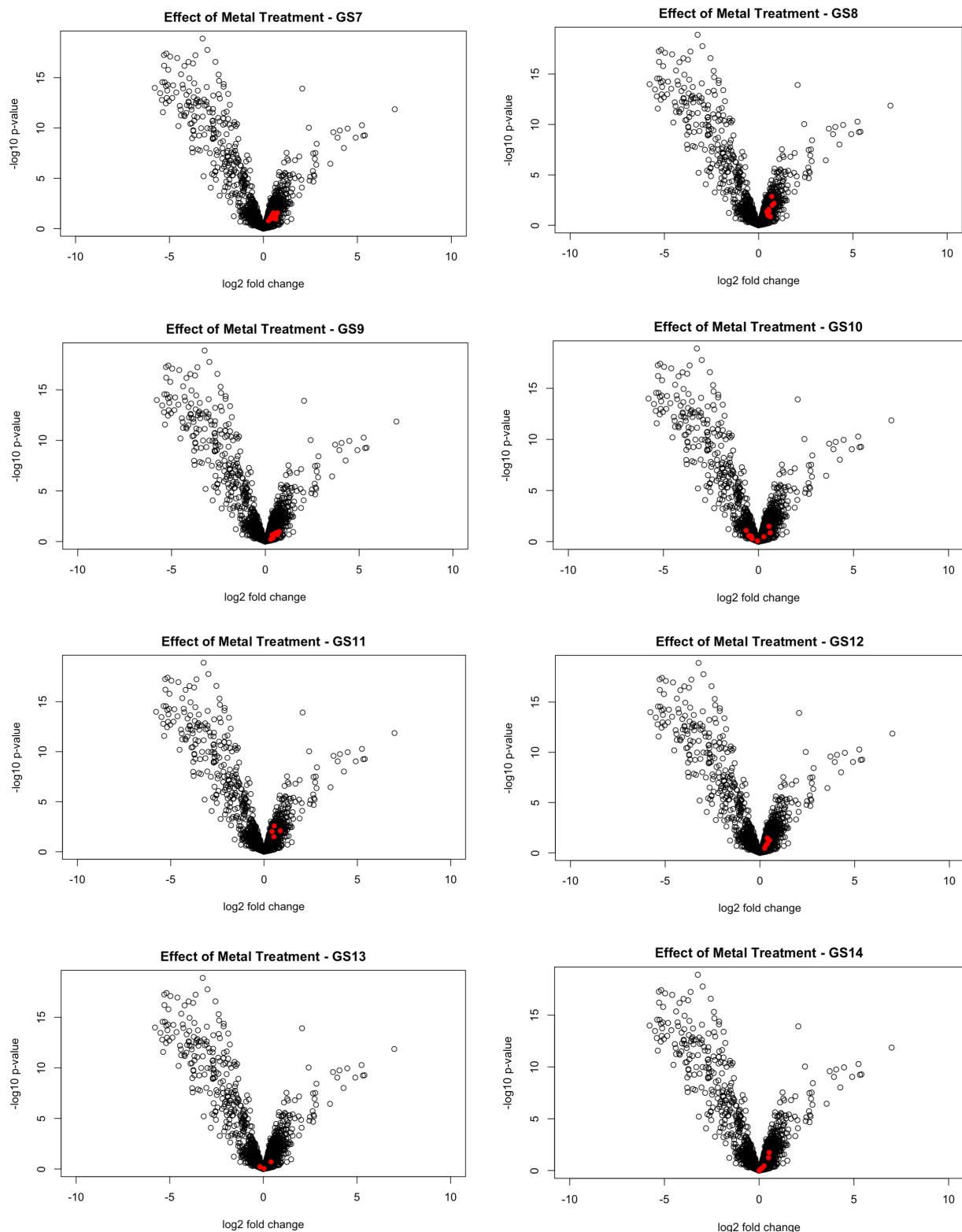
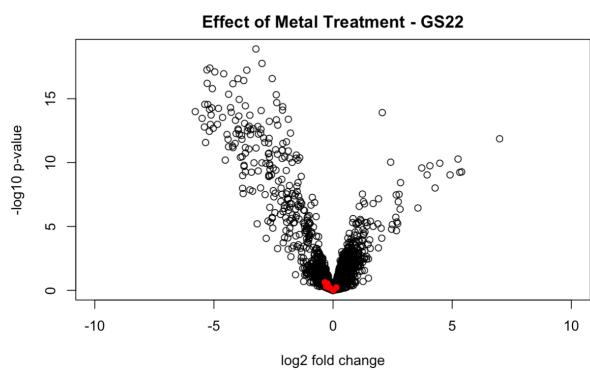
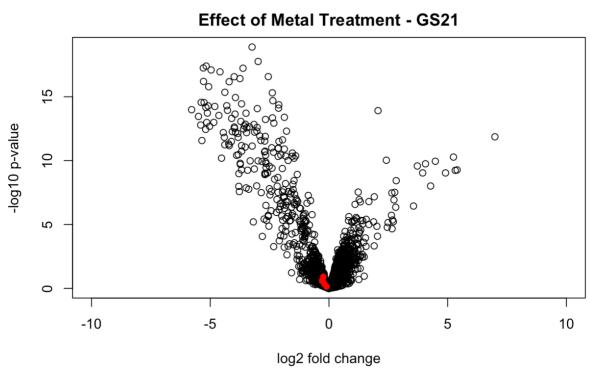
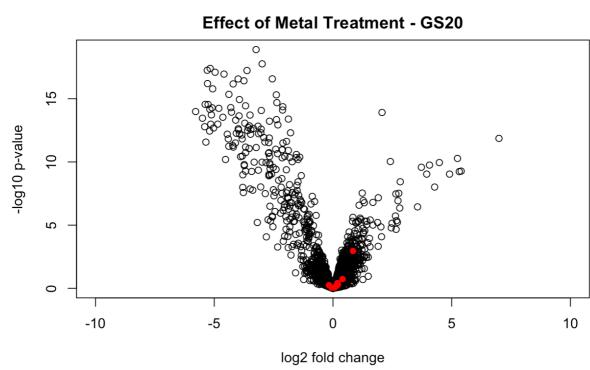
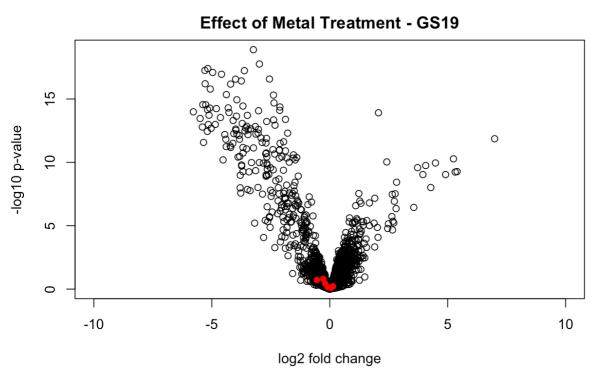
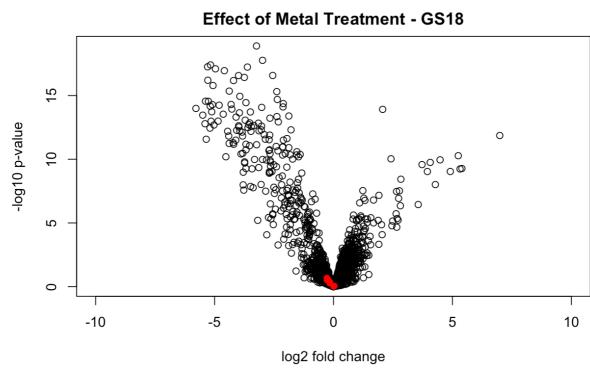
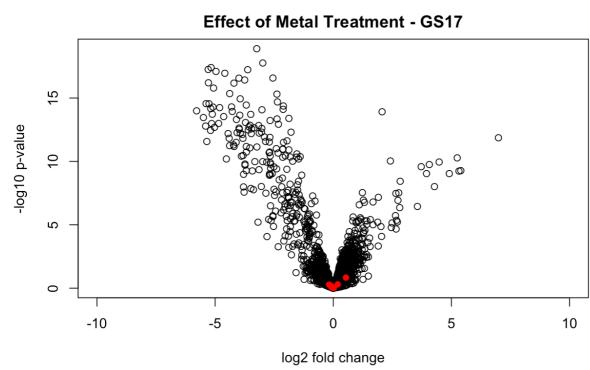
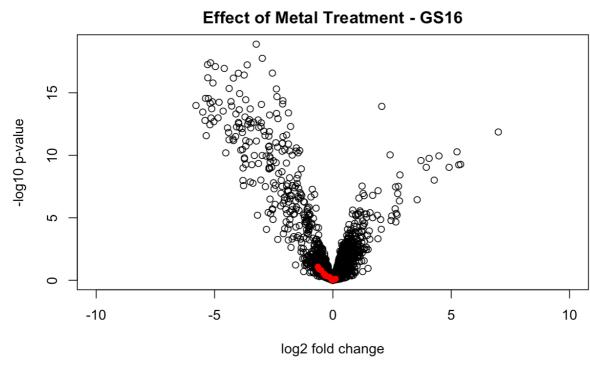
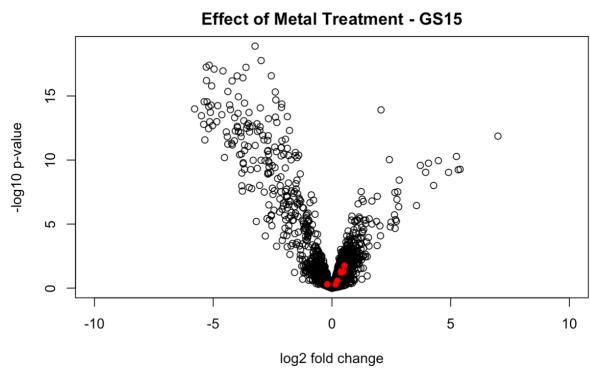


Figure S2B. Volcano plots demonstrating differential expression of genes in the spike-in sputum samples in response to metal exposure







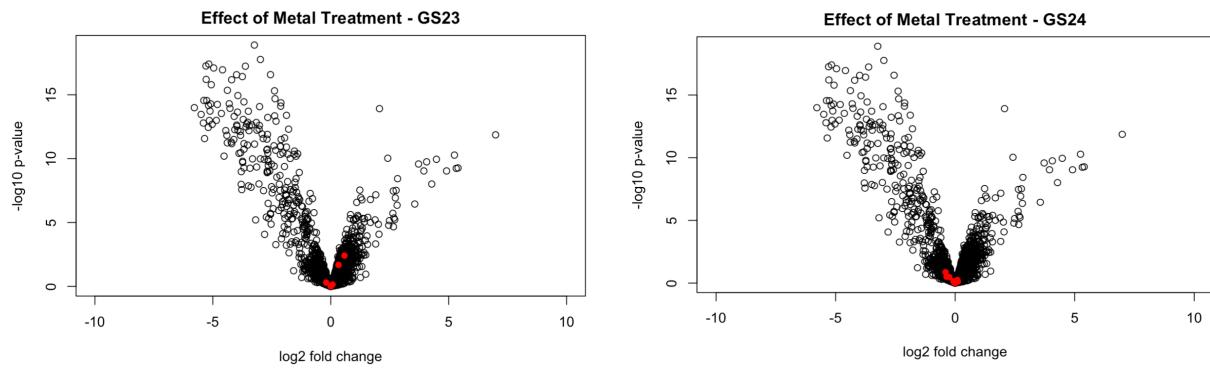
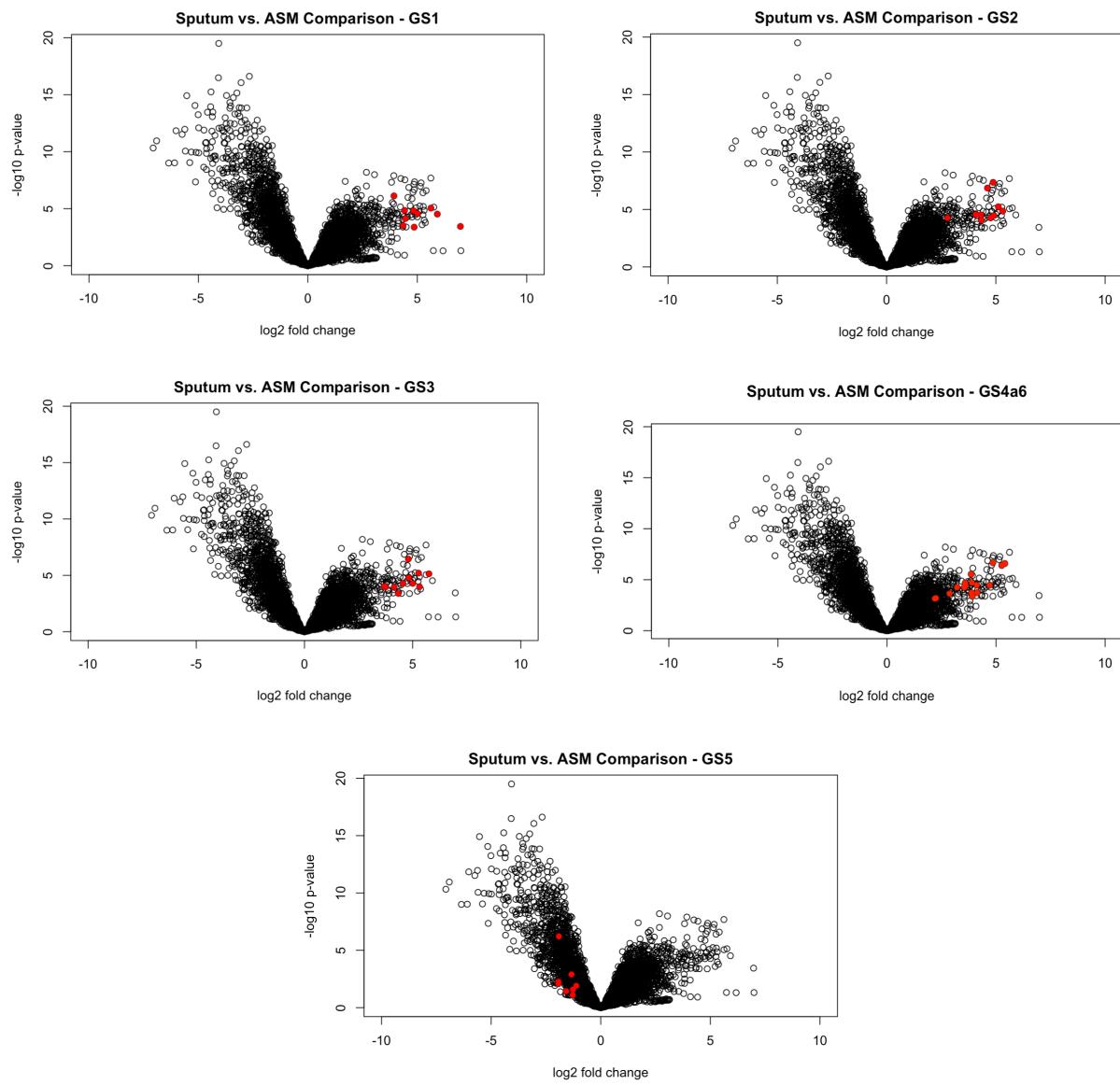
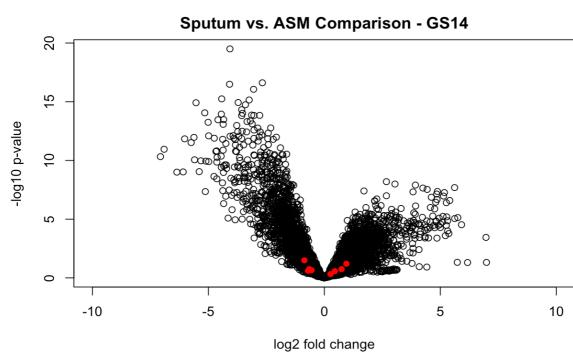
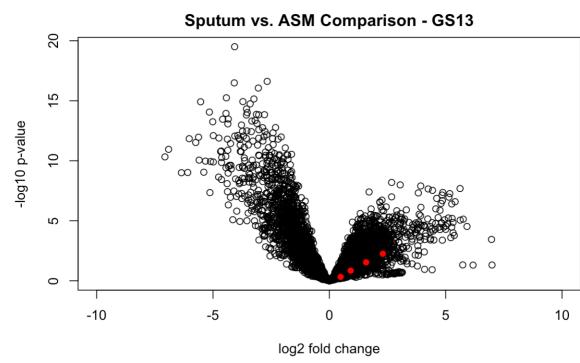
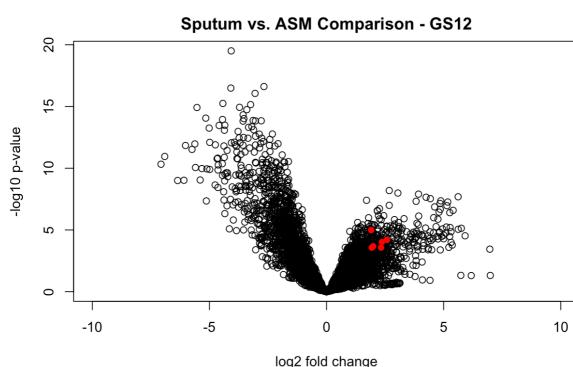
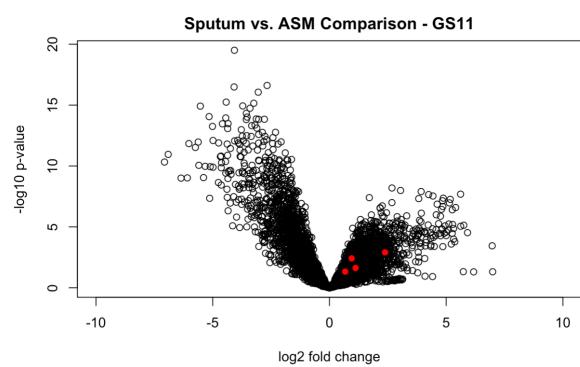
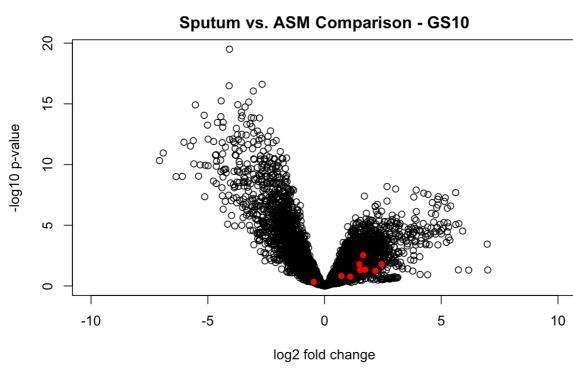
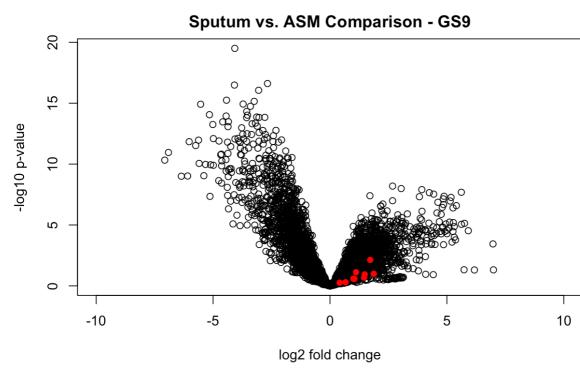
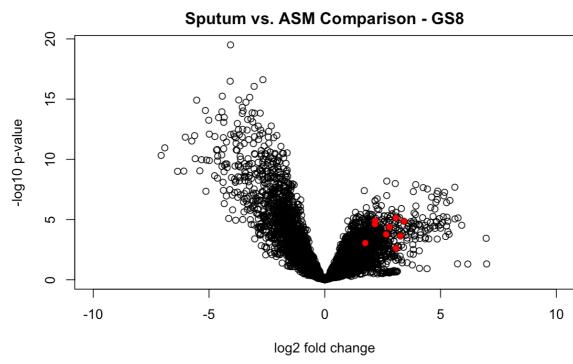
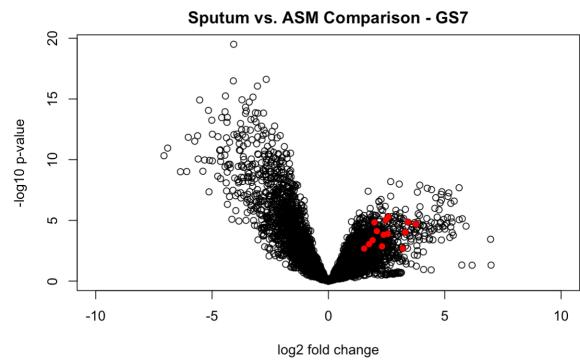
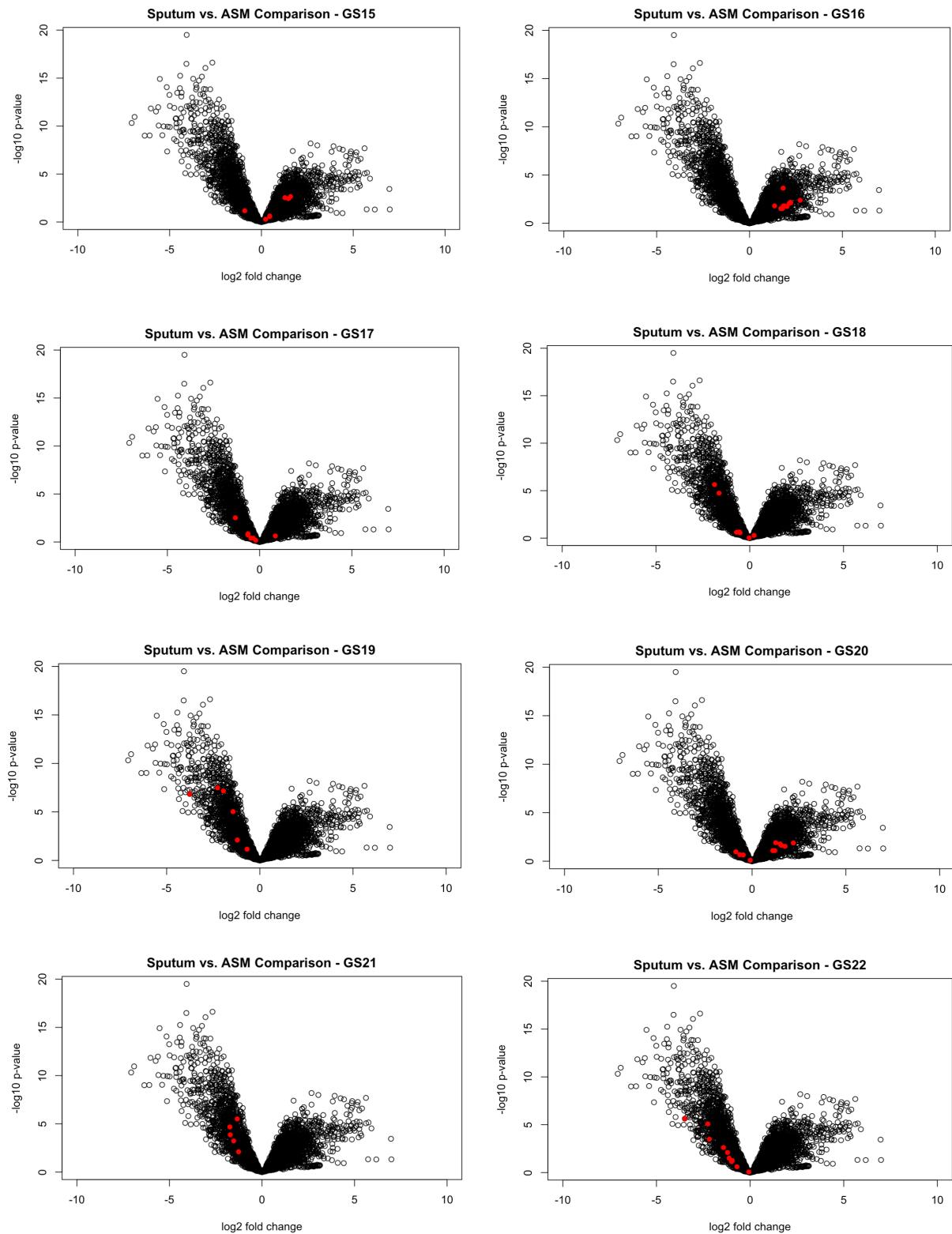


Figure S2C. Volcano plots demonstrating differential expression of genes in the spike-in sputum samples (untreated) compared to ASM







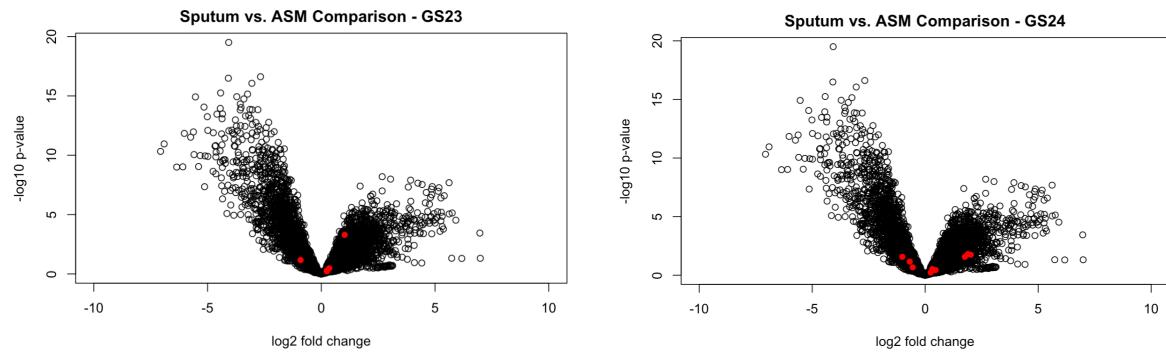


Figure S3. GO terms that are significantly activated or repressed by the addition of the metal mixture to the spike-in sputum samples. This comparison involves just the 17 untreated spike-in samples and the 17 corresponding metal-treated samples. As noted in the manuscript, each donor has a corresponding treated and untreated sample, as noted in the manuscript.

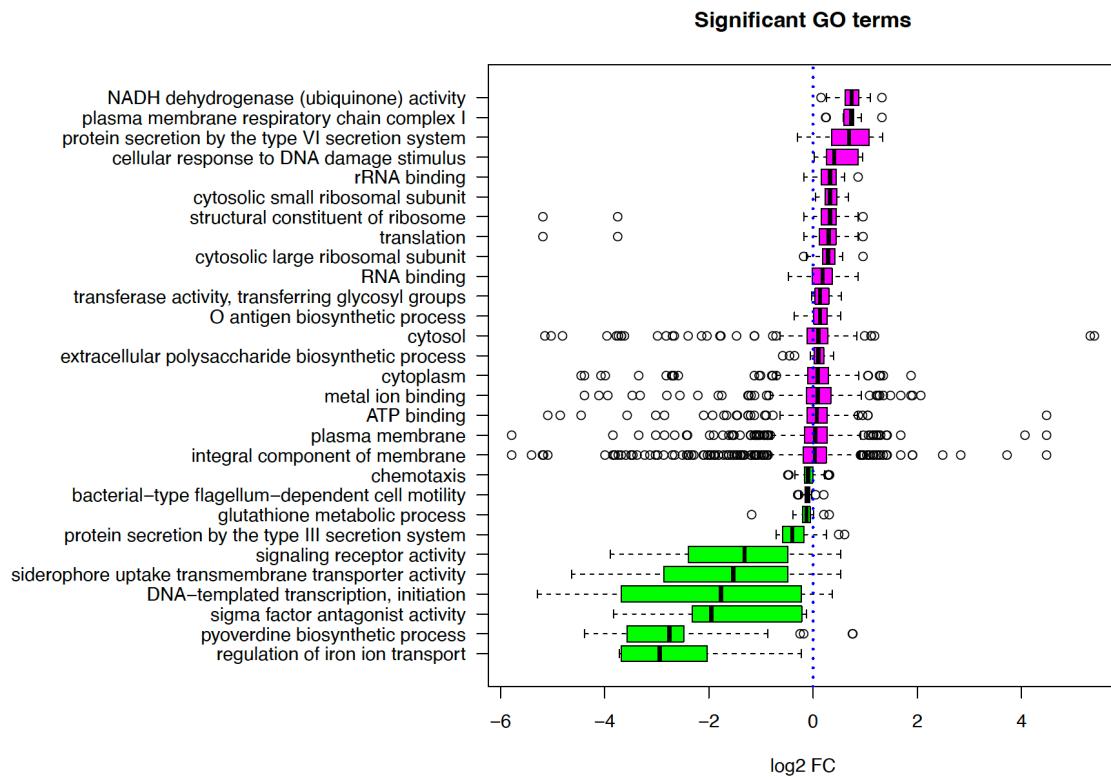


Figure S4. Linear regression analysis demonstrates the association between donor FEV1 and the average expression of metal acquisition gene sets.

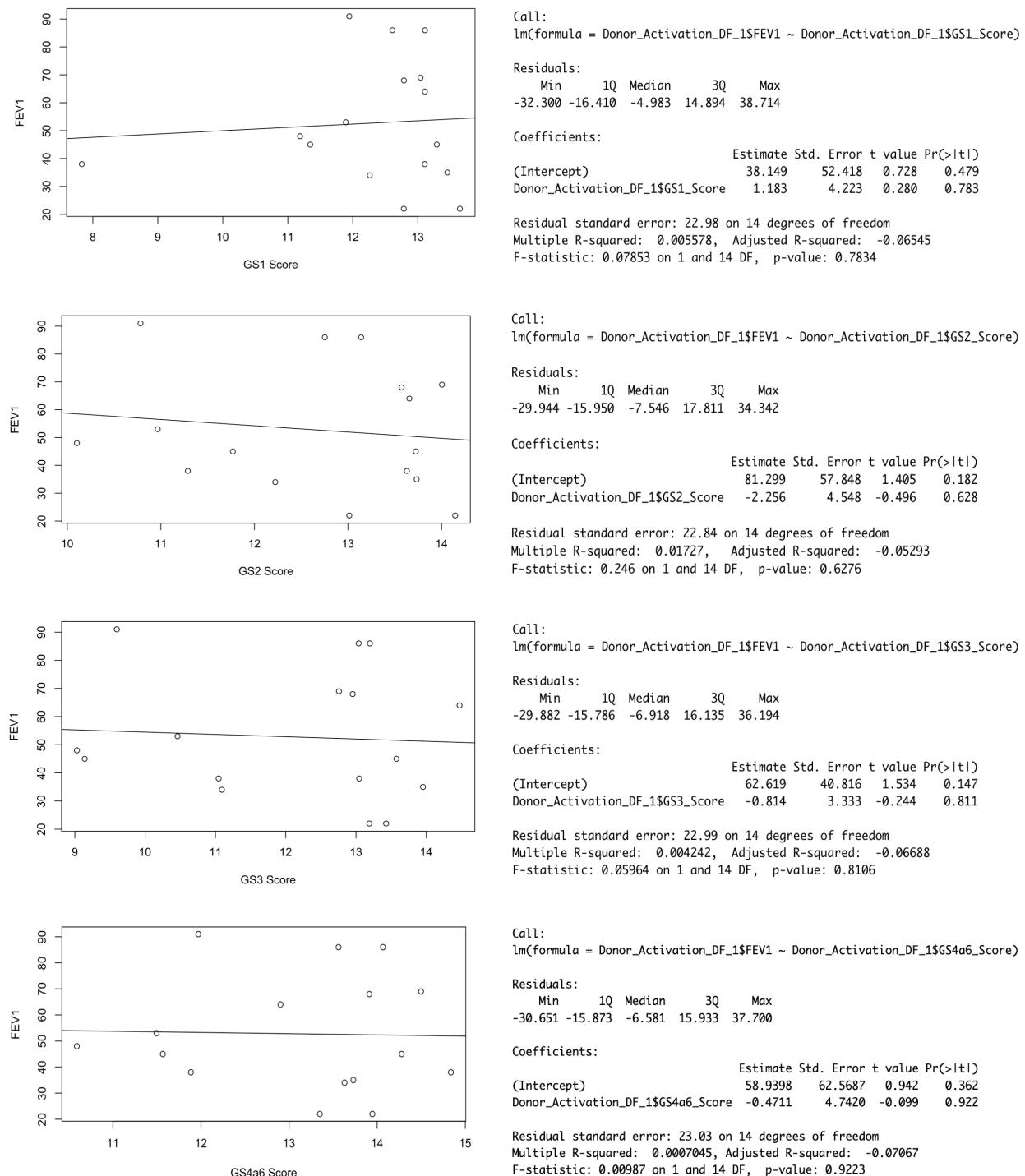
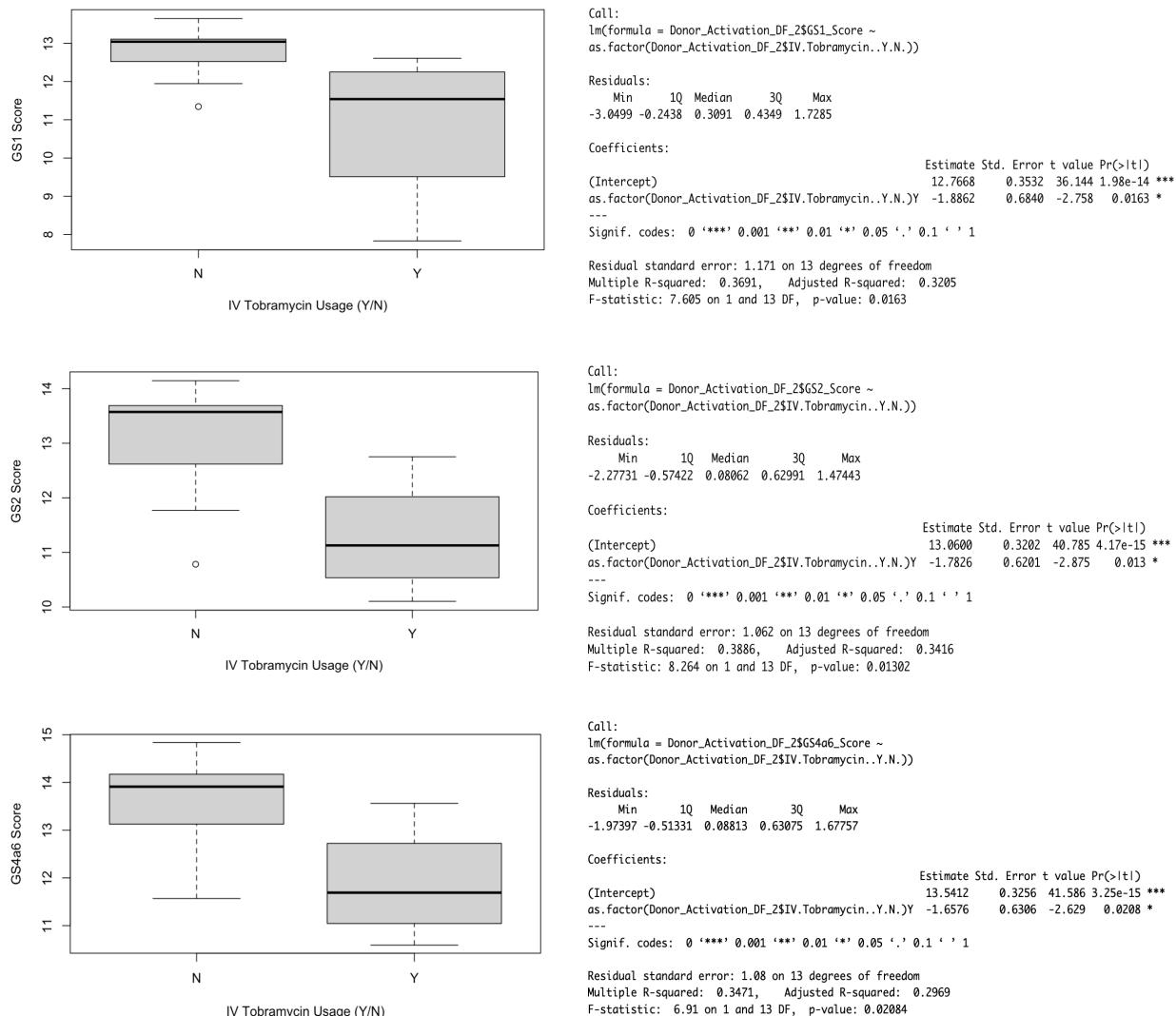
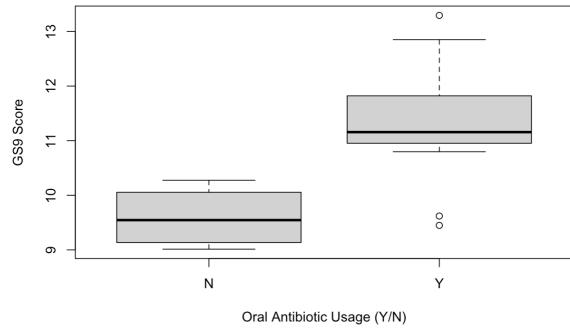


Figure S5. Linear regression analysis demonstrates the association between drug usage and average expression of gene set 9 (type VI secretion) as well as metal acquisition gene sets.





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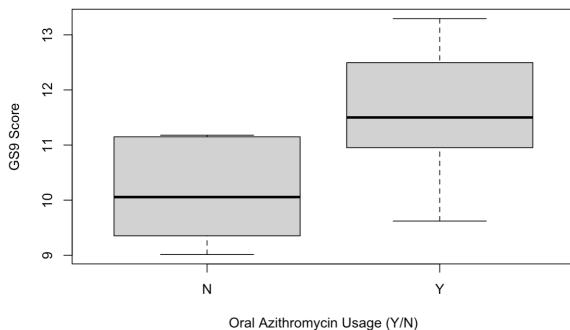
Call:
lm(formula = Donor_Activation_DF_2$GS9_Score ~ as.factor(Donor_Activation_DF_2$Oral.Abx..Y.N.))

Residuals:
    Min      1Q  Median      3Q     Max 
-1.8453 -0.4183 -0.1372  0.4607 1.9994 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 9.5952   0.5316 18.050 1.38e-10 ***
as.factor(Donor_Activation_DF_2$Oral.Abx..Y.N.)Y 1.6992   0.6208  2.737  0.0169 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.063 on 13 degrees of freedom
Multiple R-squared:  0.3656, Adjusted R-squared:  0.3168 
F-statistic: 7.492 on 1 and 13 DF,  p-value: 0.01695

```



```

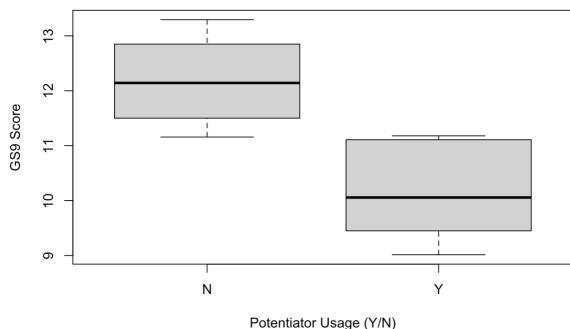
Call:
lm(formula = Donor_Activation_DF_2$GS9_Score ~
as.factor(Donor_Activation_DF_2$Oral.Azithromycin..Y.N.))

Residuals:
    Min      1Q  Median      3Q     Max 
-1.9954 -0.7664 -0.1153  0.9858 1.6778 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 10.1634   0.3834 26.509 1.06e-12 ***
as.factor(Donor_Activation_DF_2$Oral.Azithromycin..Y.N.)Y 1.4526   0.5612  2.588  0.0225 *  
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.084 on 13 degrees of freedom
Multiple R-squared:  0.3401, Adjusted R-squared:  0.2893 
F-statistic: 6.699 on 1 and 13 DF,  p-value: 0.0225

```



```

Call:
lm(formula = Donor_Activation_DF_2$GS9_Score ~
as.factor(Donor_Activation_DF_2$Potentiator.Therapy..Y.N.))

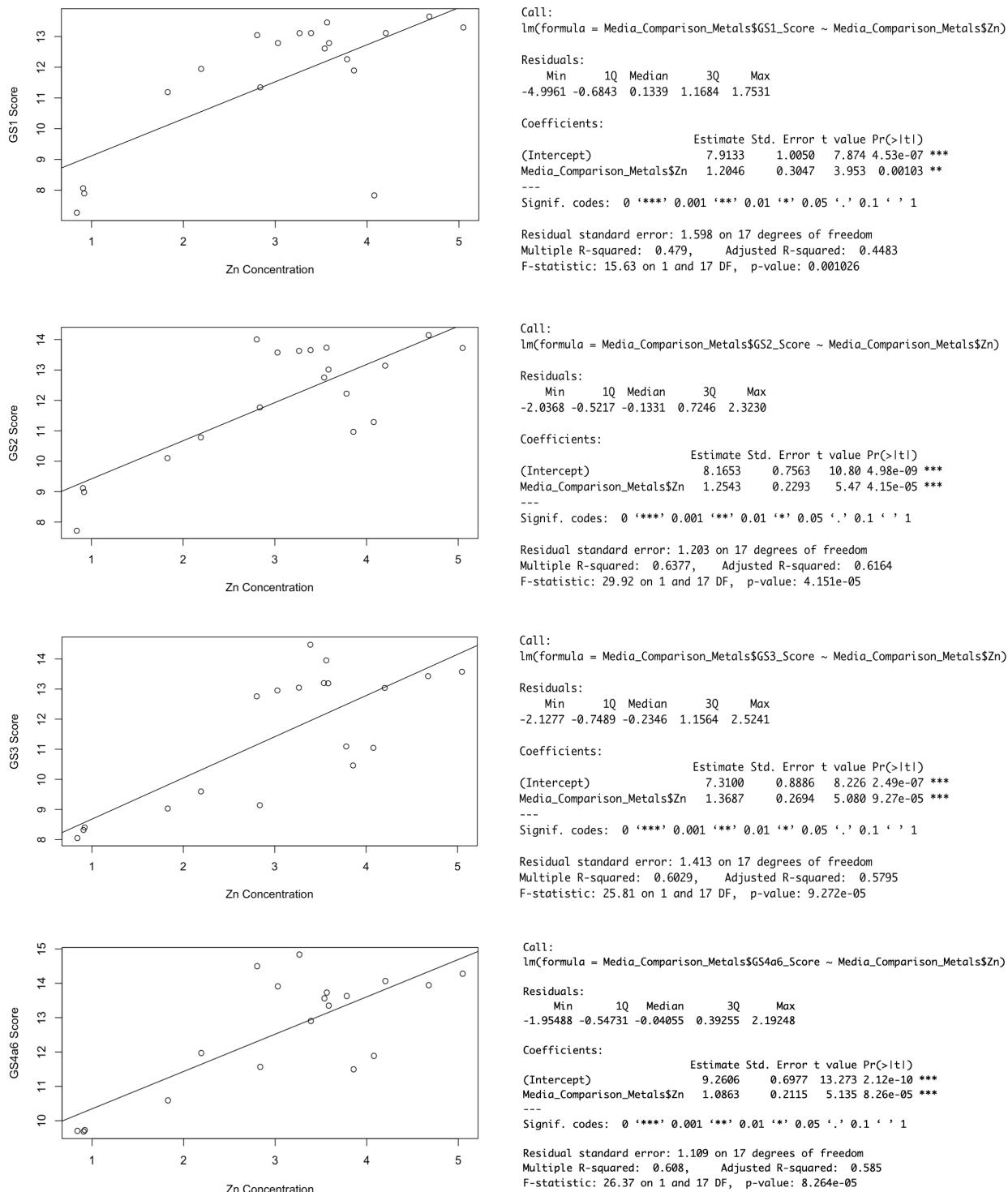
Residuals:
    Min      1Q  Median      3Q     Max 
-1.15397 -0.70322 -0.04668  0.80066 1.10522 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 12.1886   0.3833 31.799 1.03e-13 ***
as.factor(Donor_Activation_DF_2$Potentiator.Therapy..Y.N.)Y -2.0210   0.4694  -4.305  0.000855 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.8571 on 13 degrees of freedom
Multiple R-squared:  0.5877, Adjusted R-squared:  0.556 
F-statistic: 18.53 on 1 and 13 DF,  p-value: 0.0008552

```

Figure S6. Linear regression analysis demonstrates the association between metal concentration and the average expression of metal acquisition gene sets.



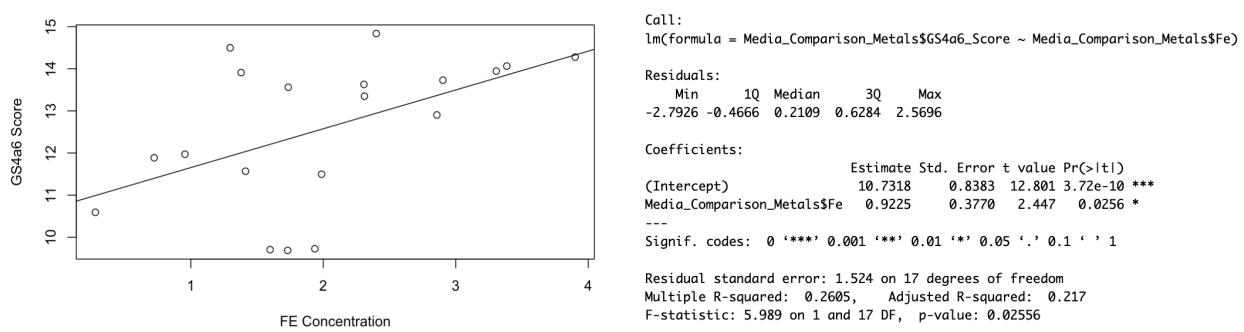
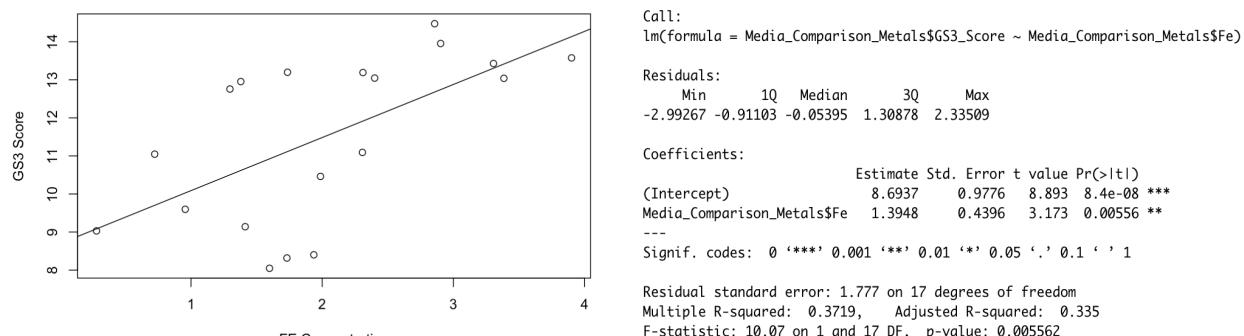
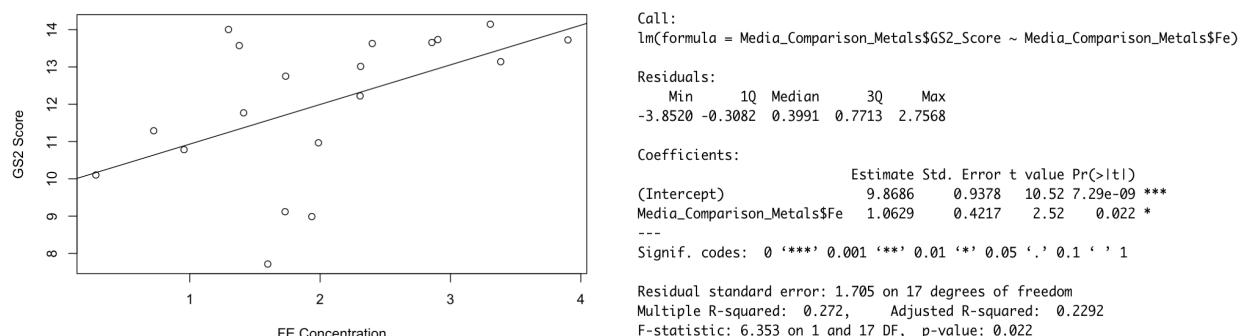
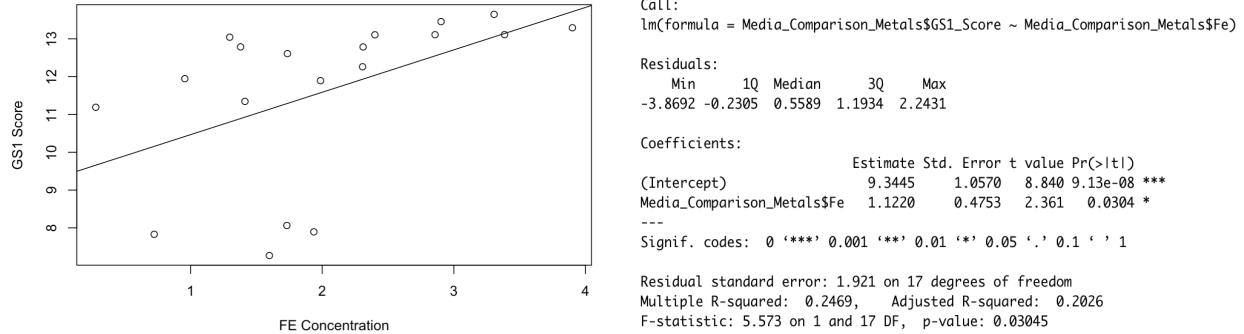


Figure S7. Composition of RNA sequencing reads in the spike-in and ASM samples. Reads were mapped to various species including *P. aeruginosa*, other common CF pathogens, and the human genome.

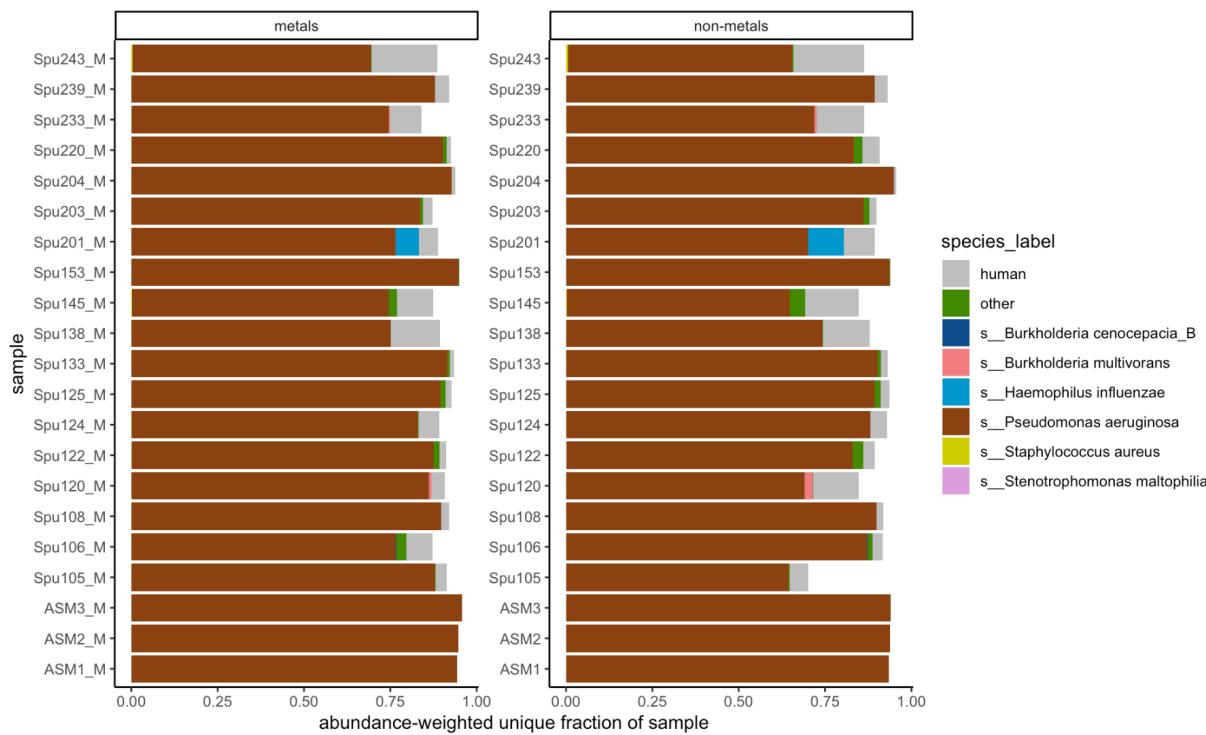


Figure S8. ADAGE-constructed gene sets were screened against an independent compendium of 890 PAO1 gene expression samples to check whether the constituent genes in each gene set remained correlated. All constituent genes in each gene set remain positively correlated, though some correlations are more moderate than others. The level of correlation is demonstrated in the correlation plots presented below.

