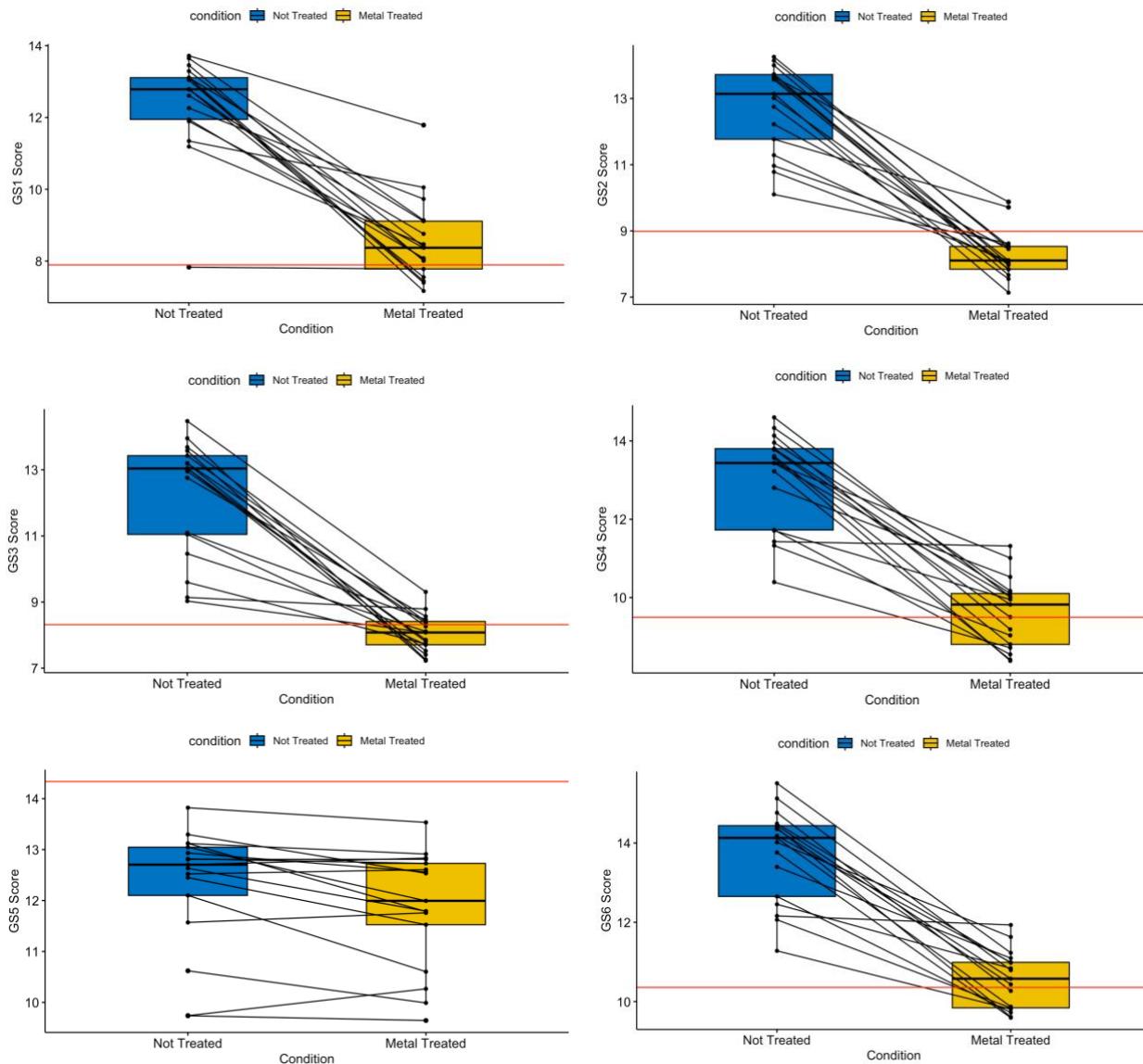
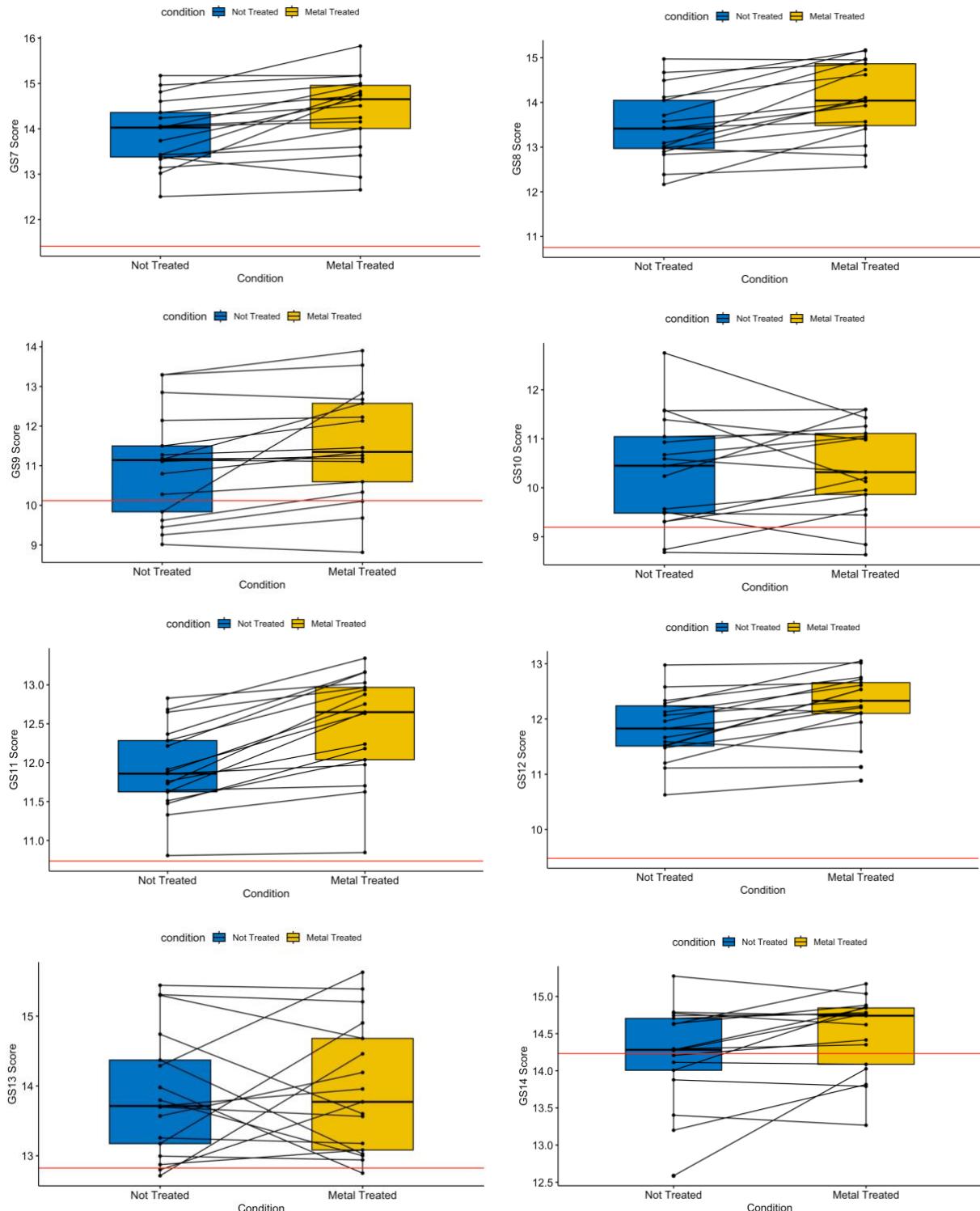
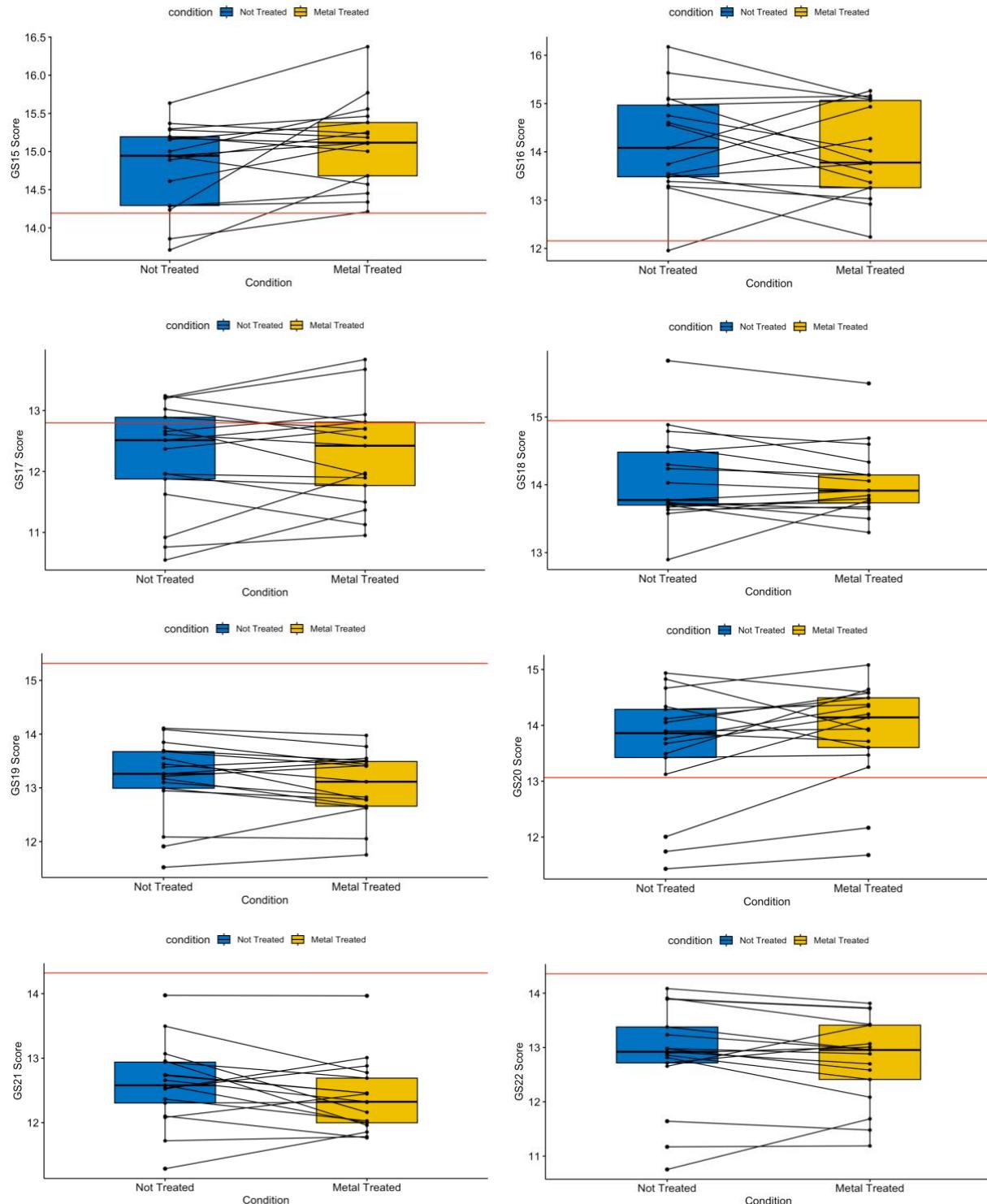


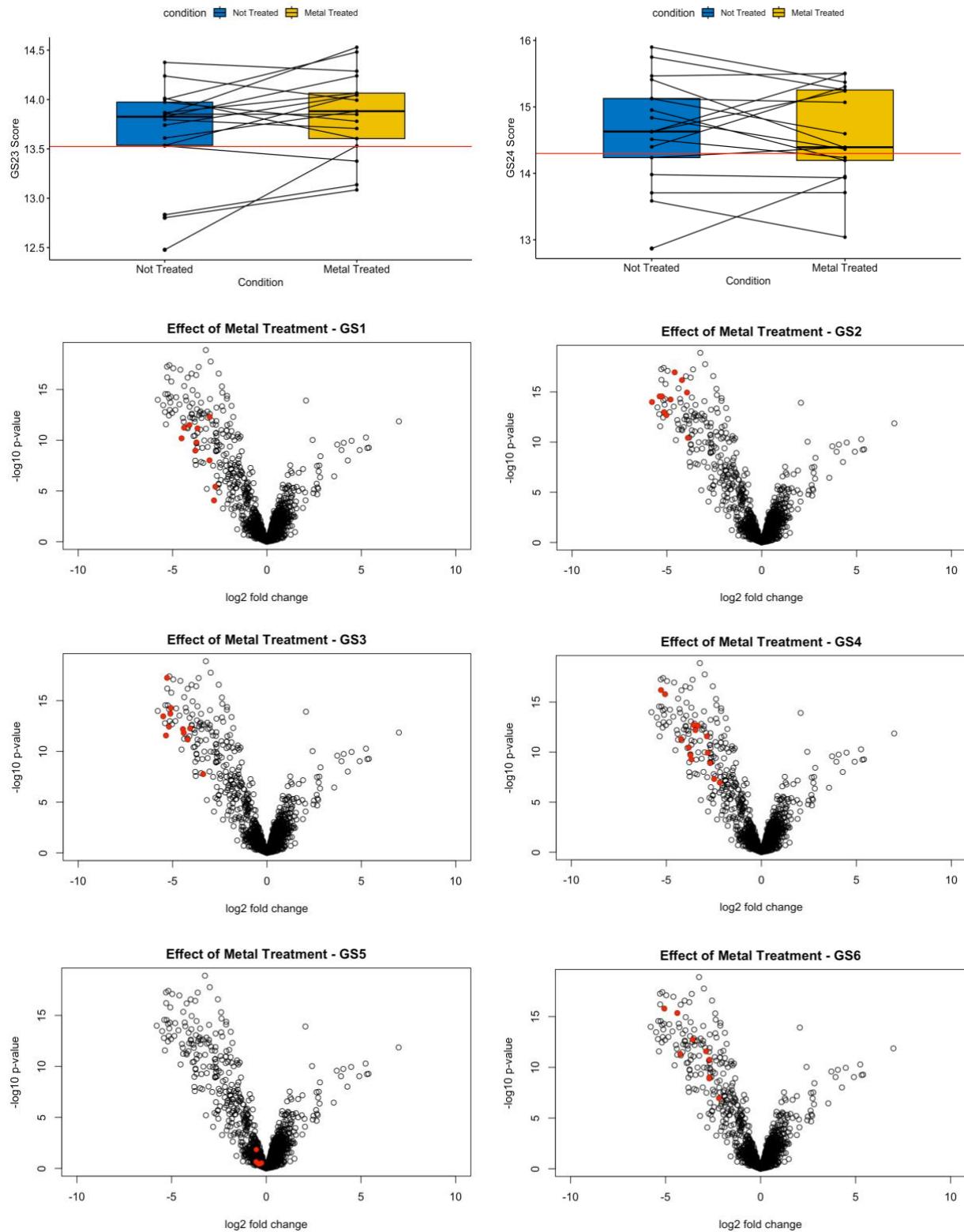
Supplementary Figures

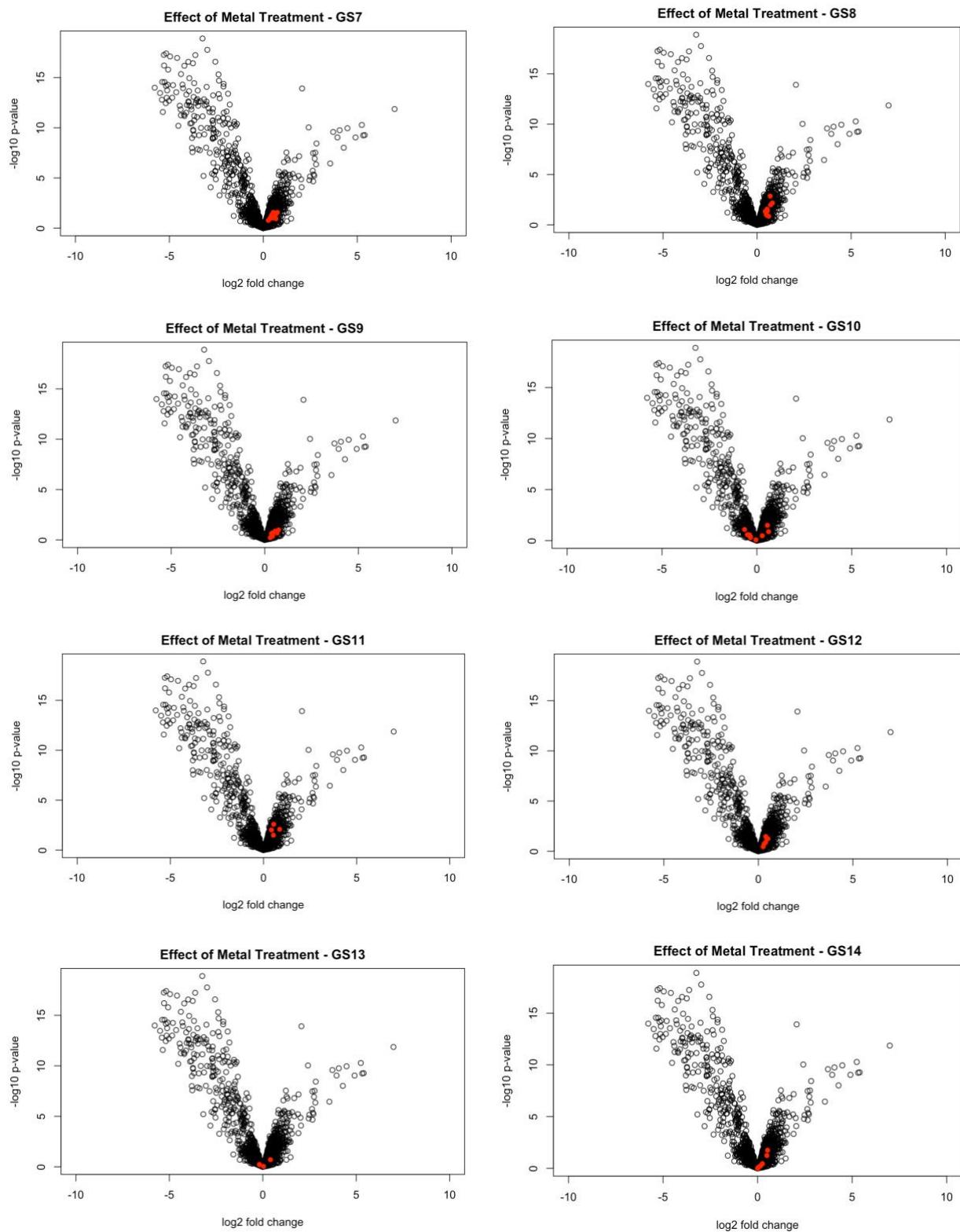
Figure S1. Response to metal exposure for all 24 ADAGE-constructed gene sets [Table 3]. The activity score (see results) was calculated for each gene set on a sample-by sample basis. The blue boxplot represents the activity scores of CF sputum samples not treated with metals while the yellow boxplot 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 the differential expression of genes in the spike-in sputum samples in response to metal exposure, as well as the differential expression of genes between spike-in sputum (untreated) and ASM samples.

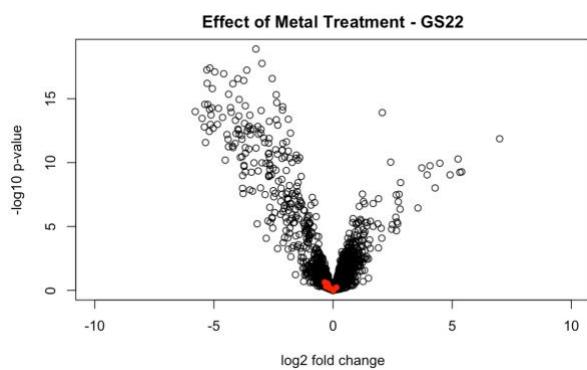
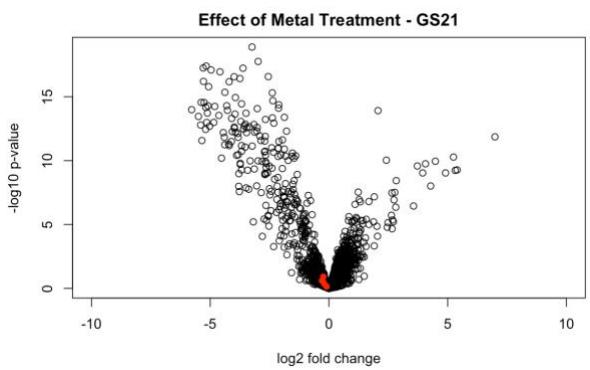
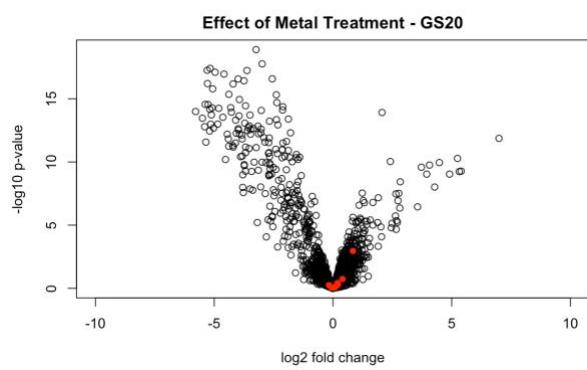
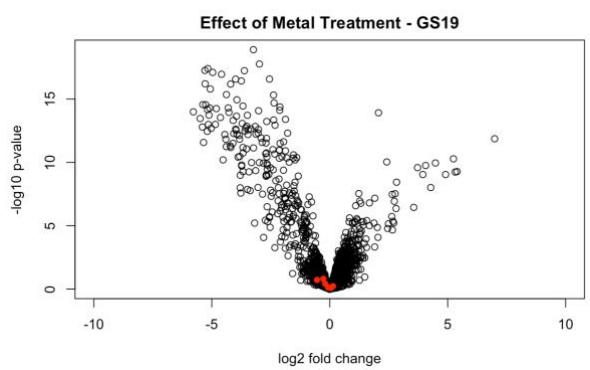
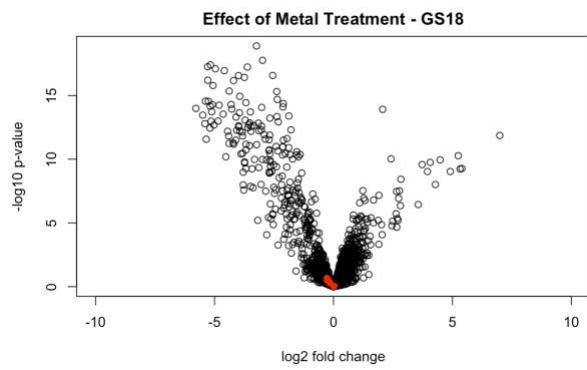
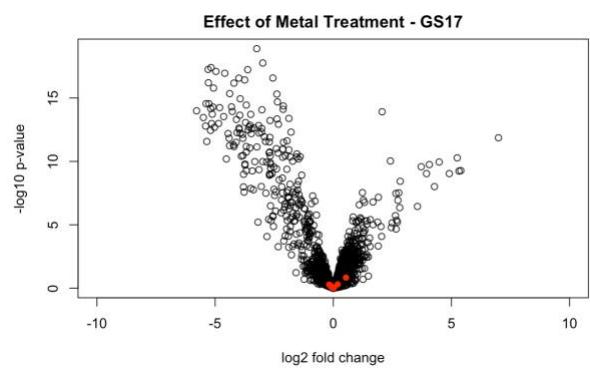
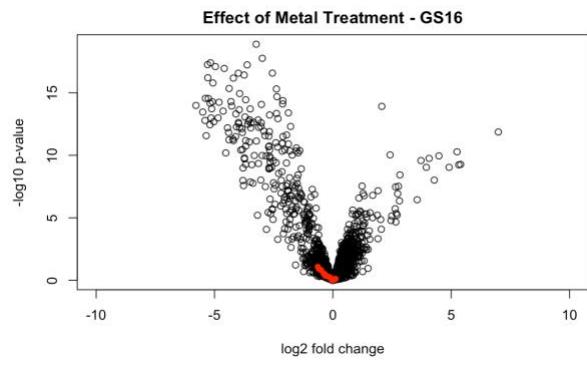
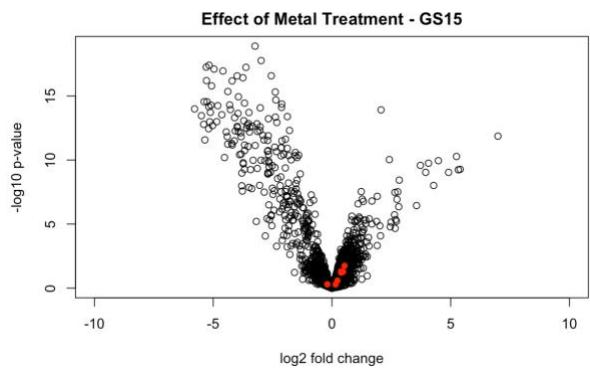


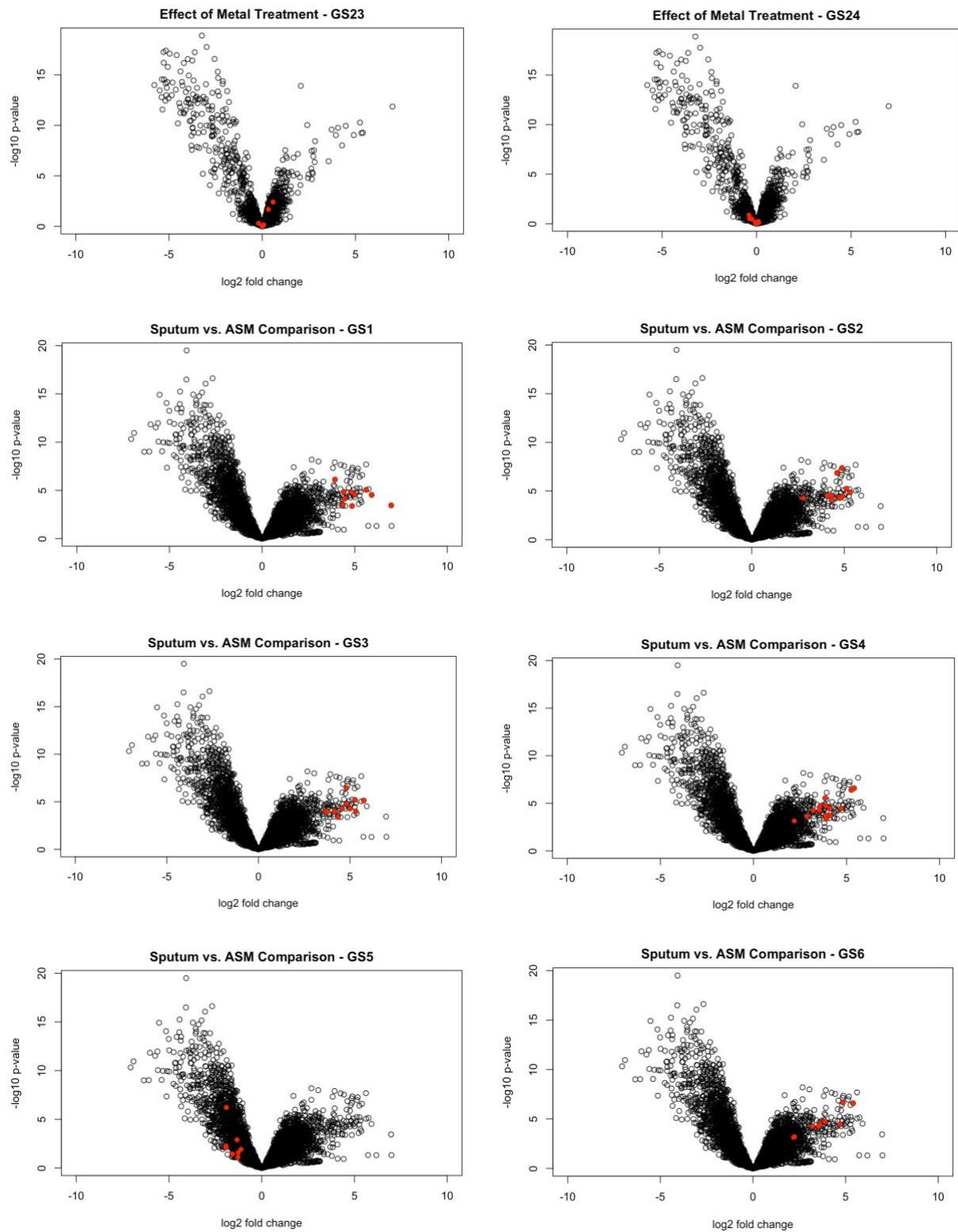


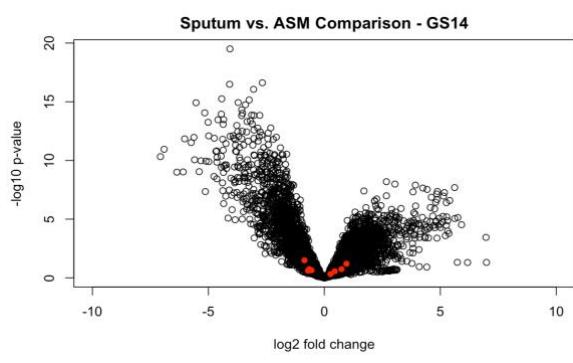
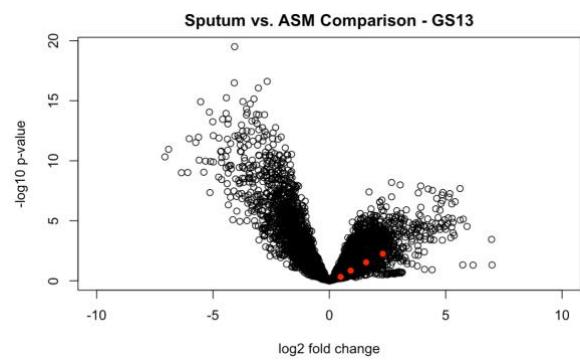
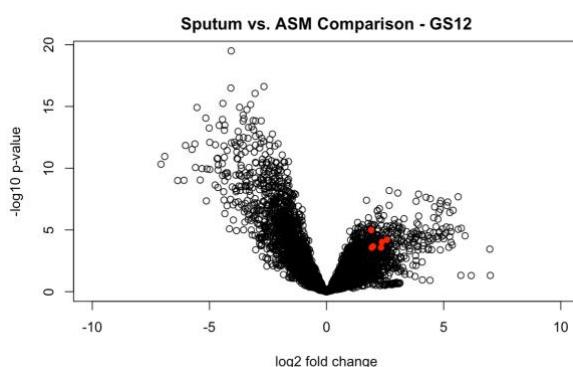
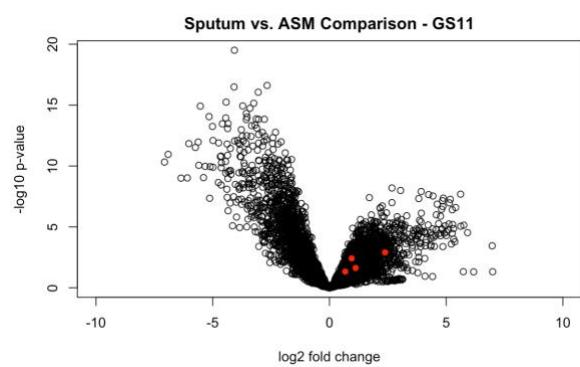
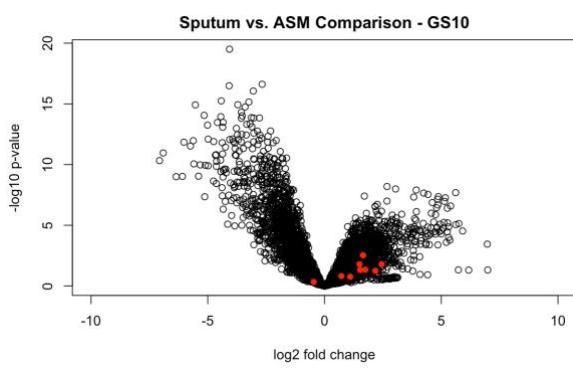
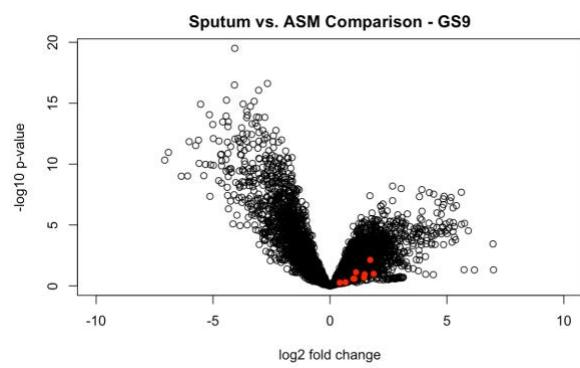
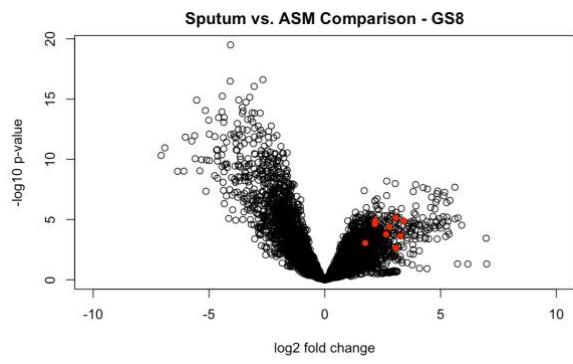
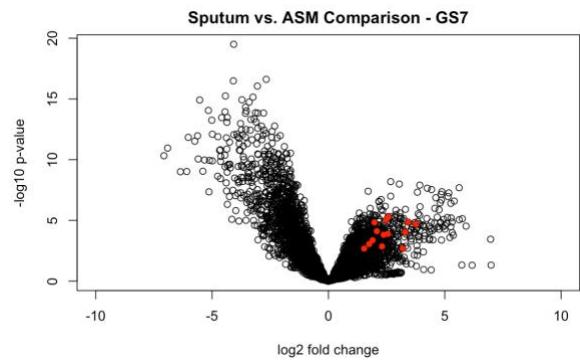


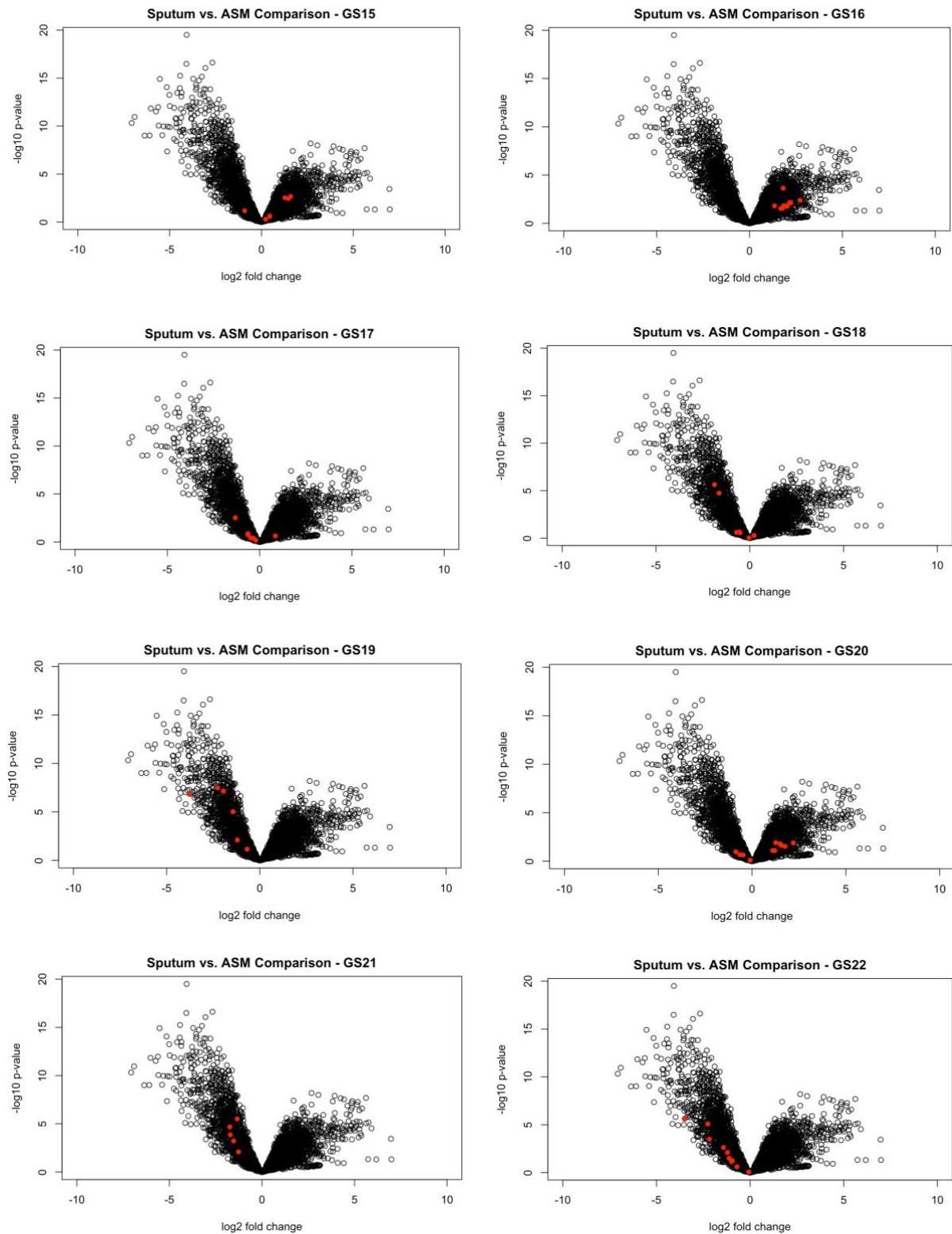












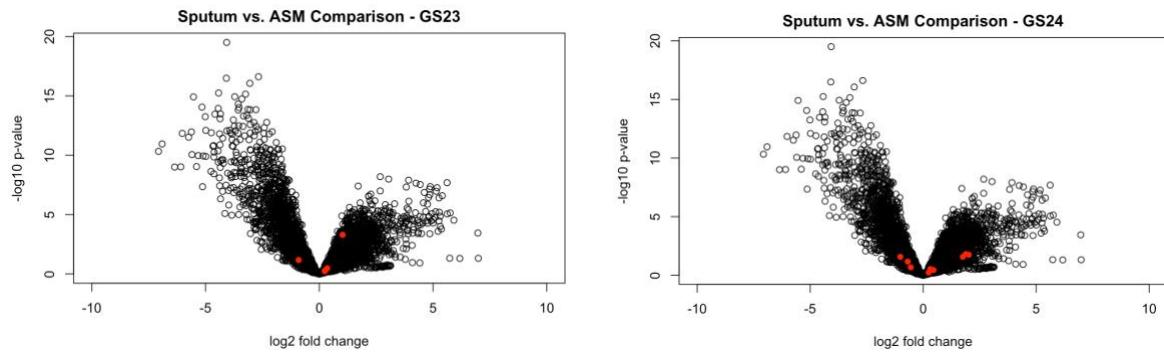


Figure S2. GO terms that are significantly activated or repressed by the addition of the metal mixture to the spike-in sputum samples. This comparison involves just the 17 untreated spike-in samples and the 17 corresponding metal-treated samples. As noted in the manuscript, each donor has a corresponding treated and untreated sample, as noted in the manuscript.

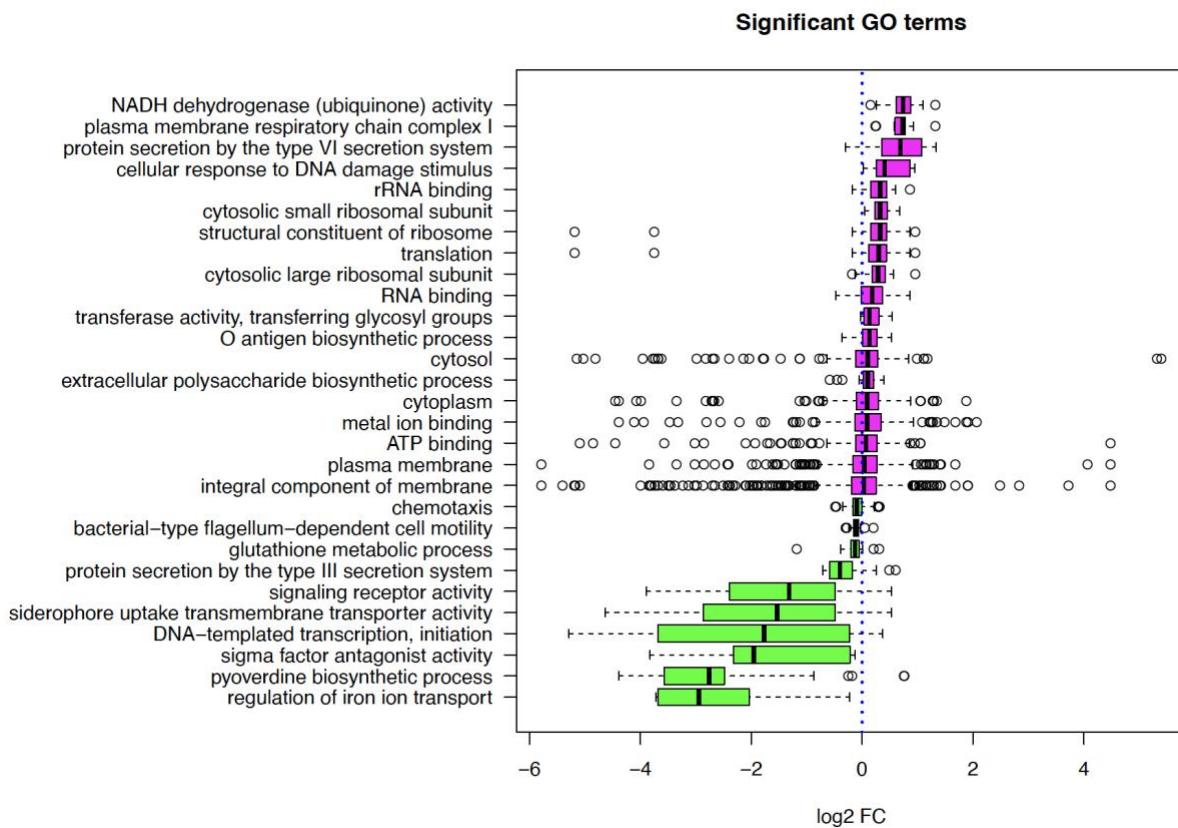
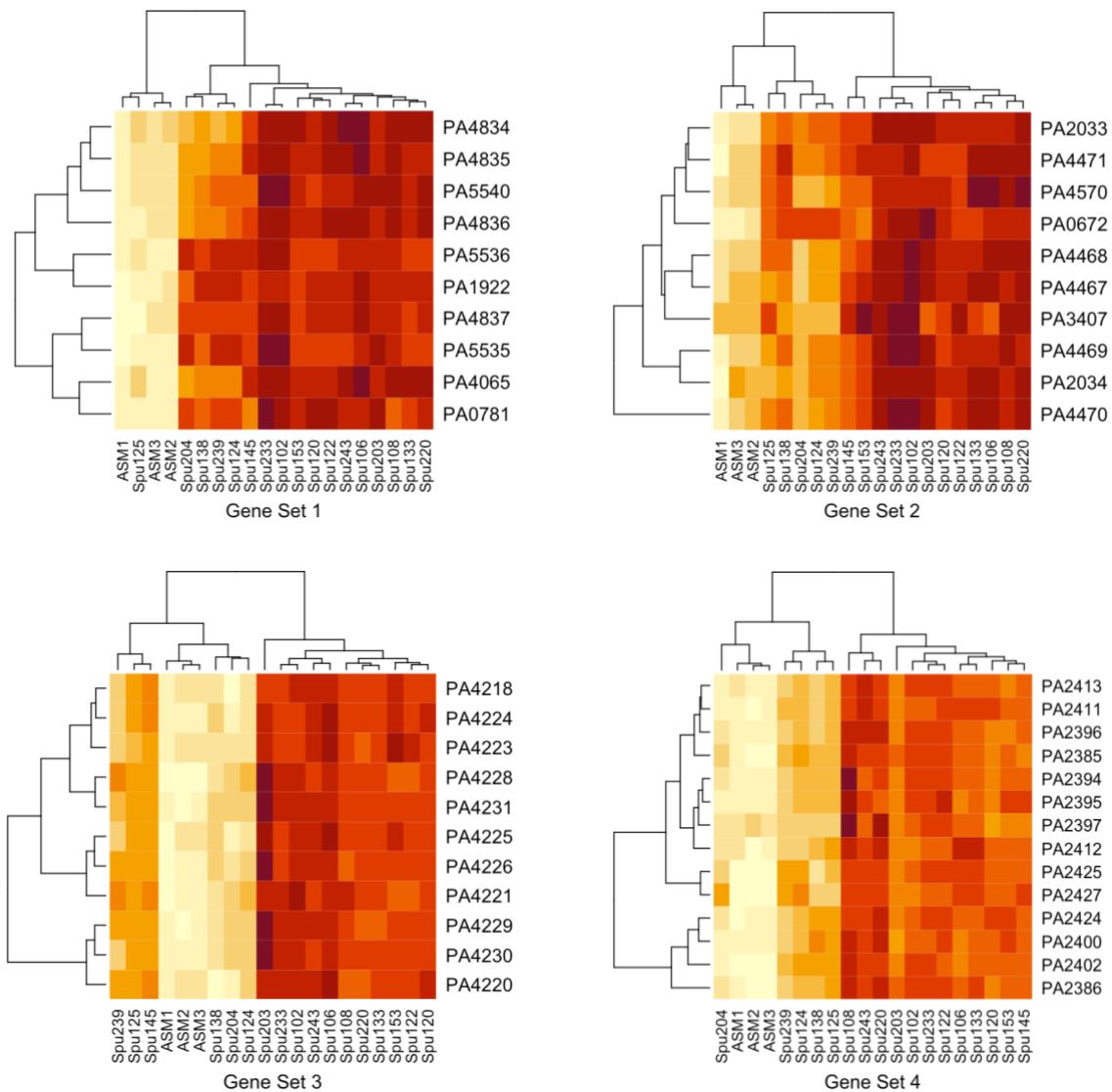
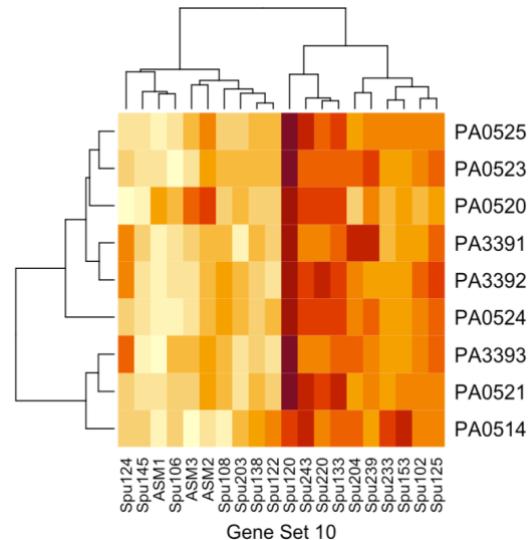
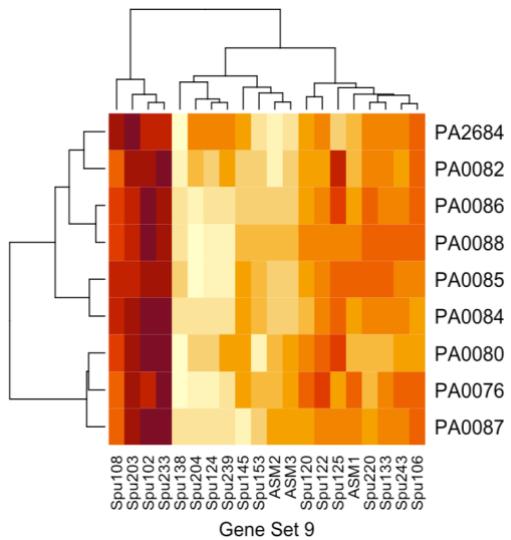
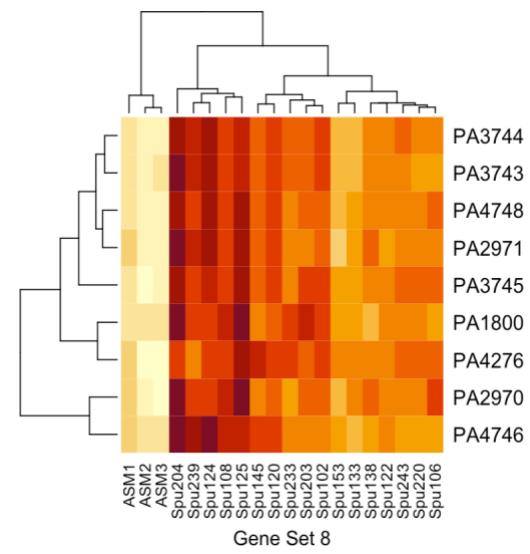
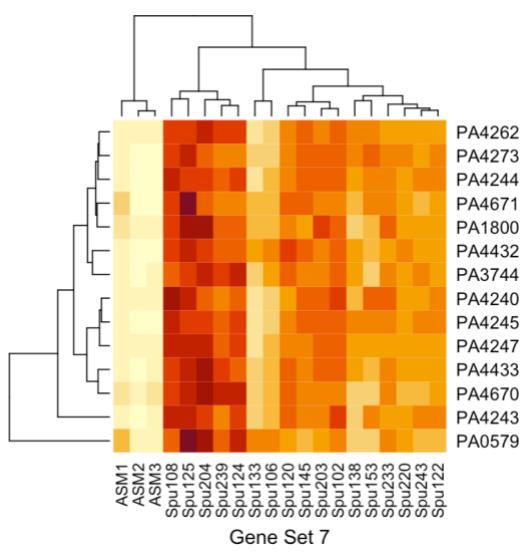
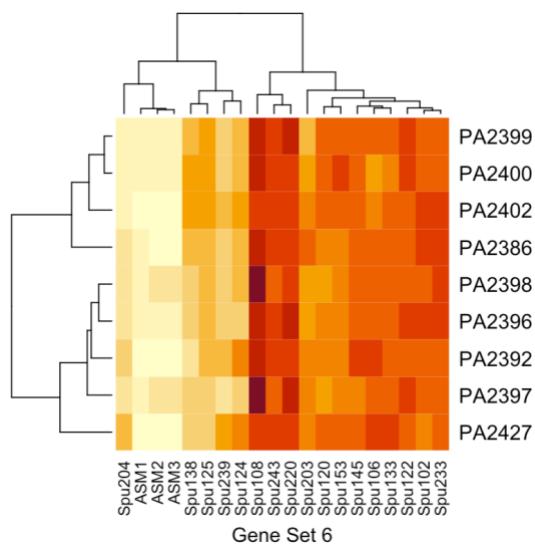
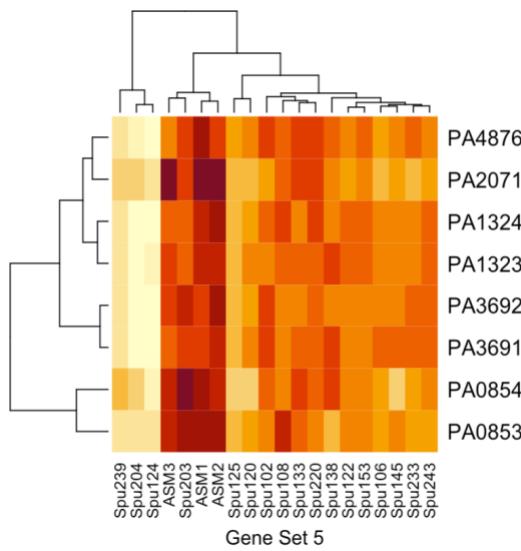
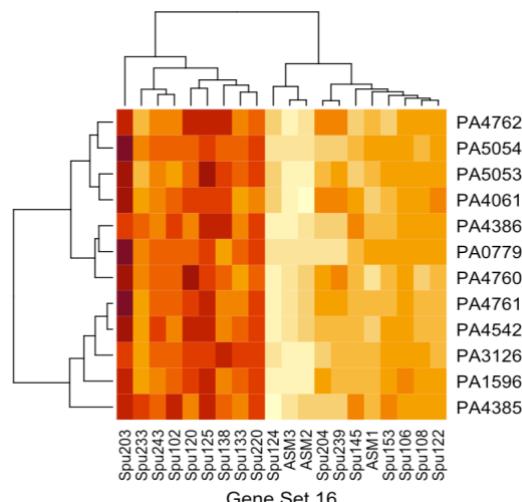
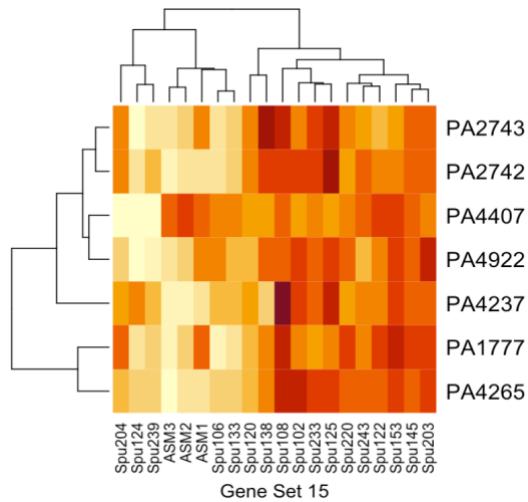
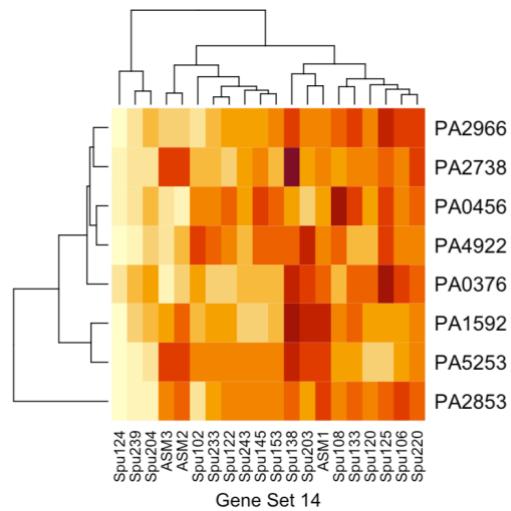
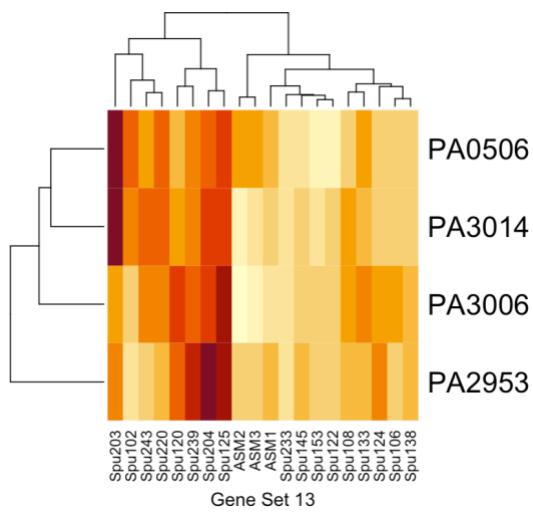
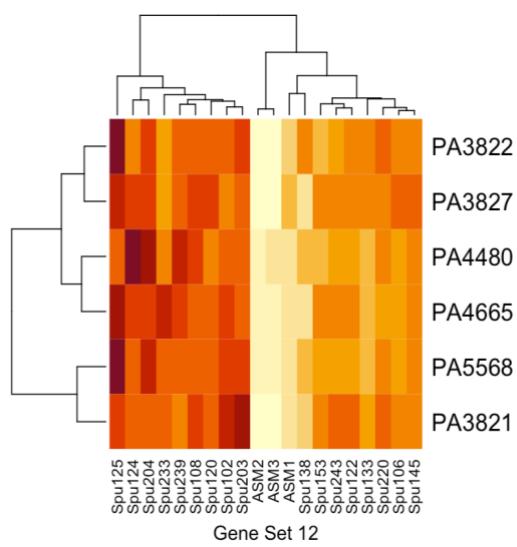
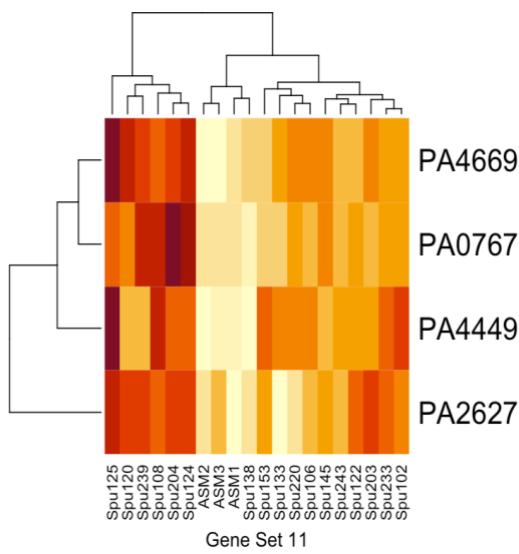
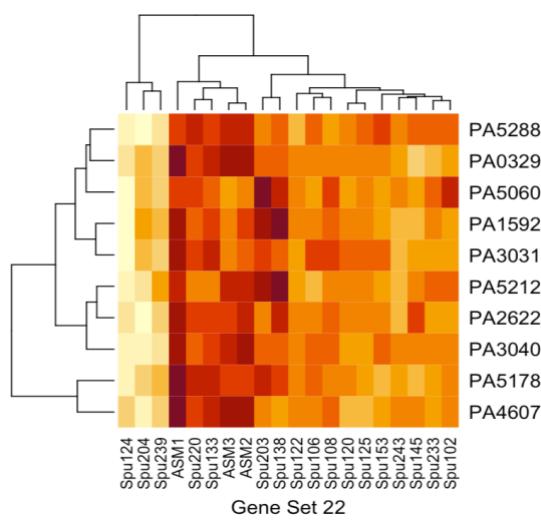
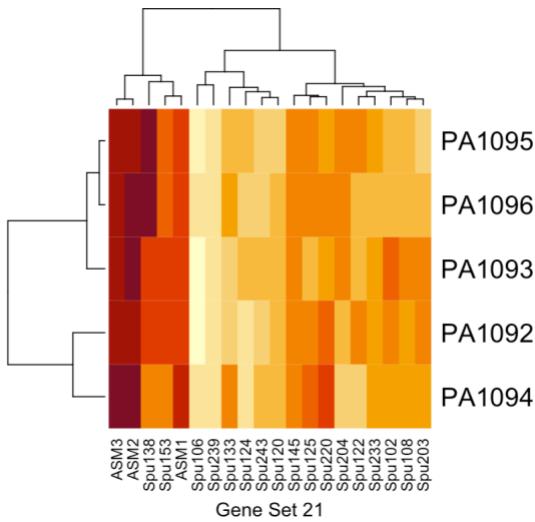
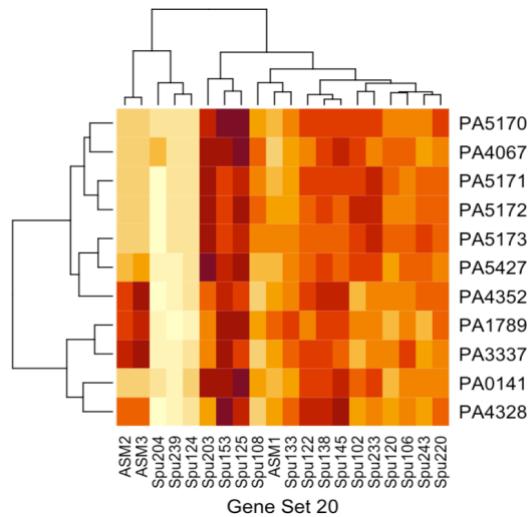
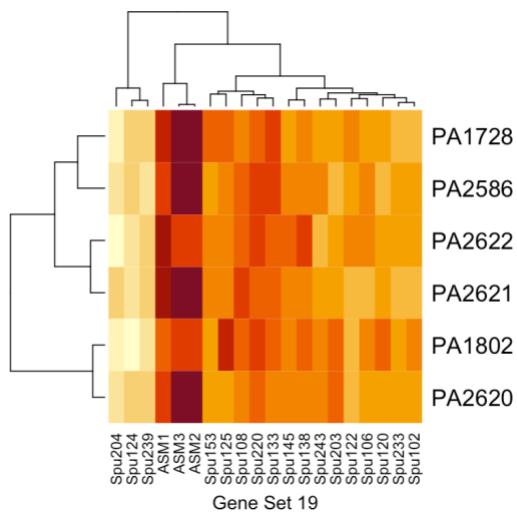
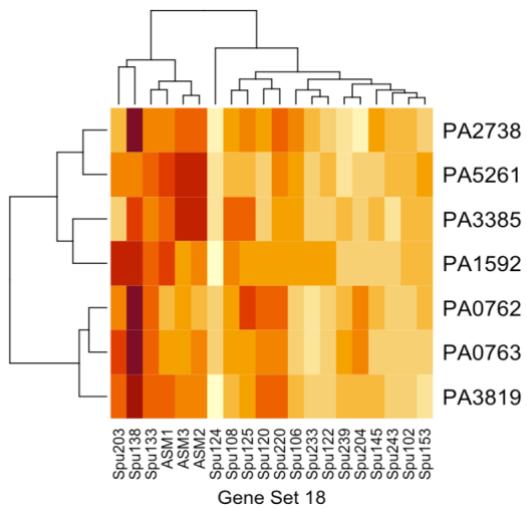
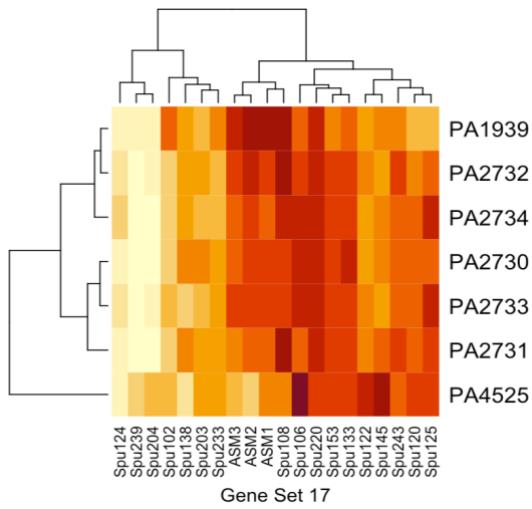


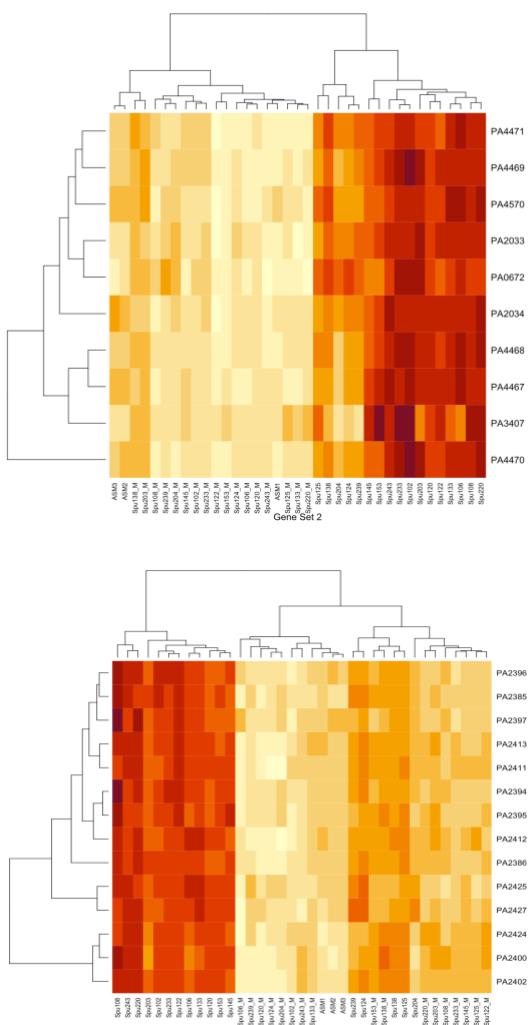
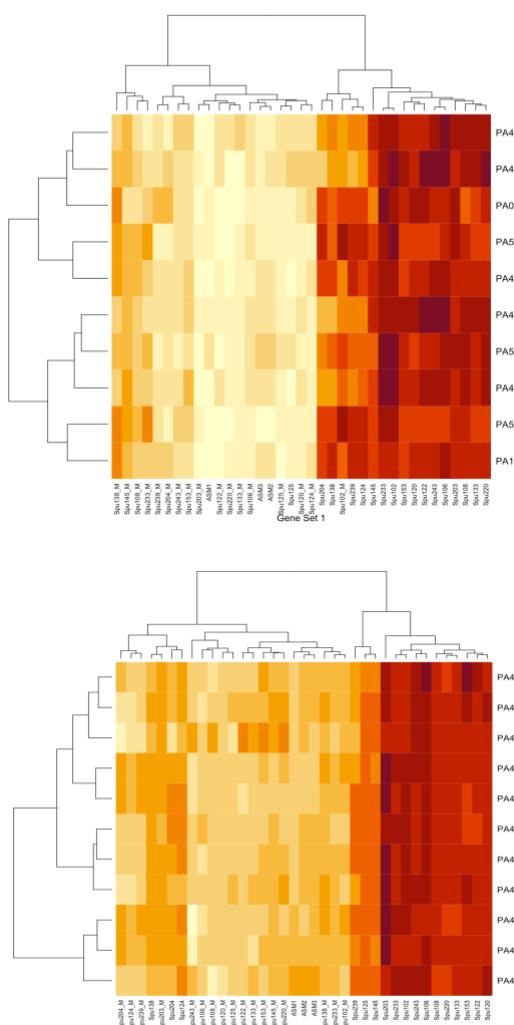
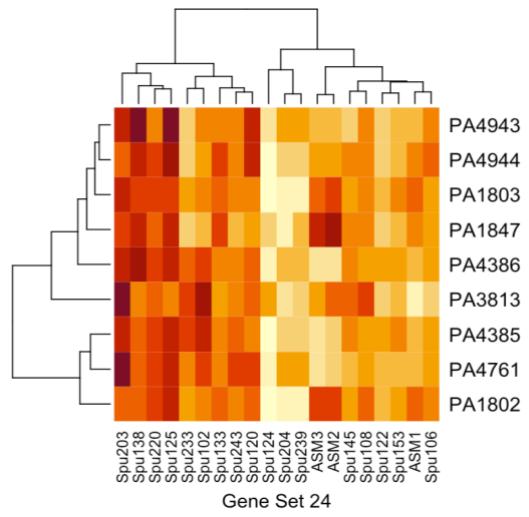
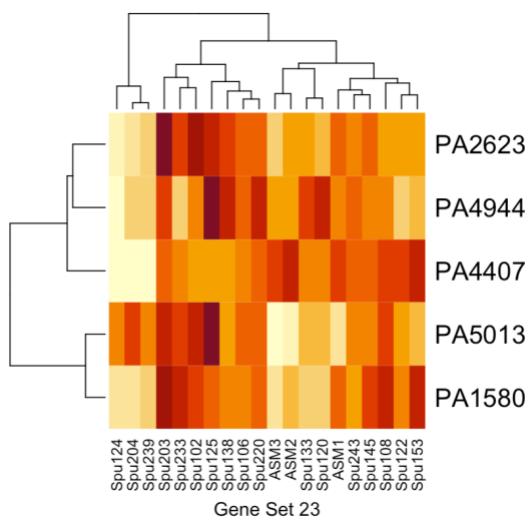
Figure S3. Heat maps demonstrating the expression of constituent genes in the 24 gene sets across the spike-in sputum (untreated and metal-treated) and ASM samples. In these panels, darker red boxes represent relatively higher gene expression, while lighter yellow boxes represent relatively lower expression. The first set of heat maps compares the untreated sputum samples to the ASM samples. The second set of heat maps compares the metal-treated sputum samples to the untreated sputum samples, and also includes the ASM samples.

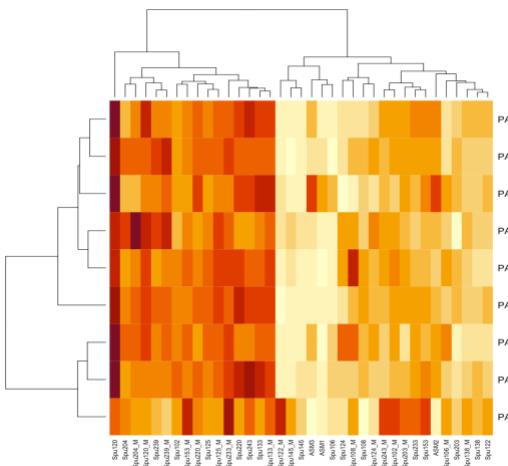
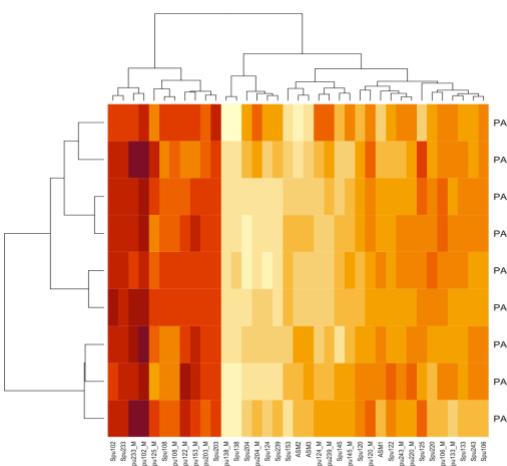
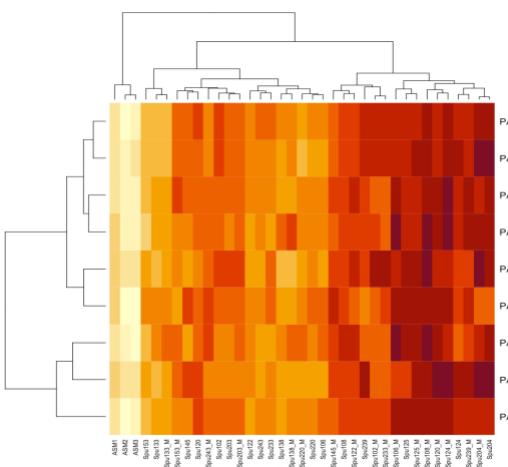
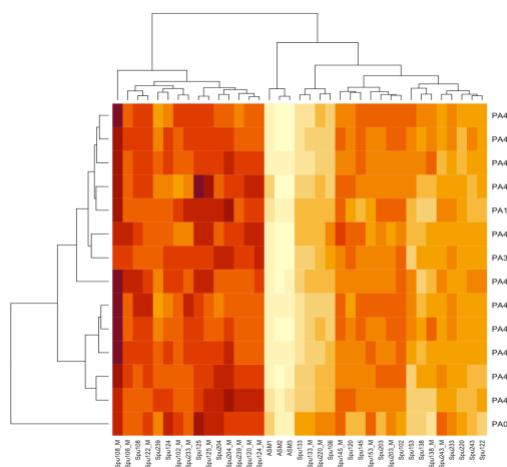
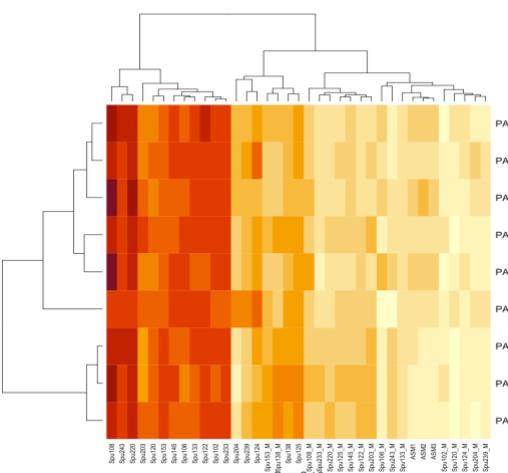
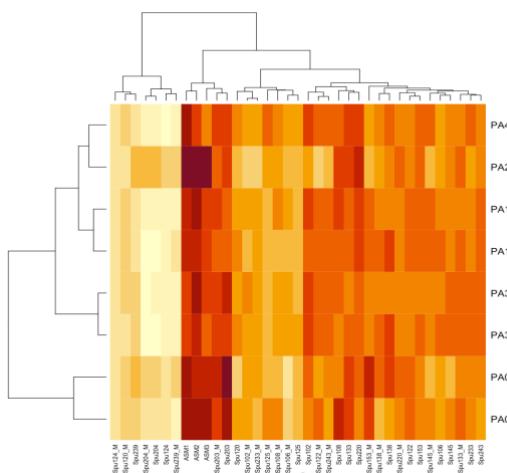


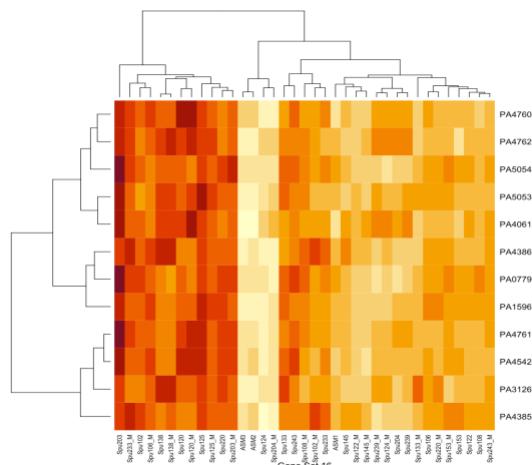
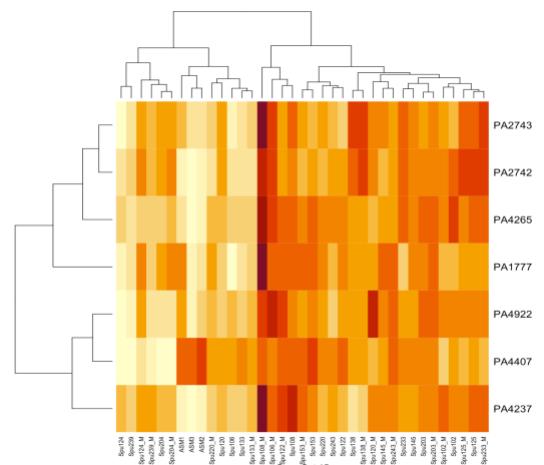
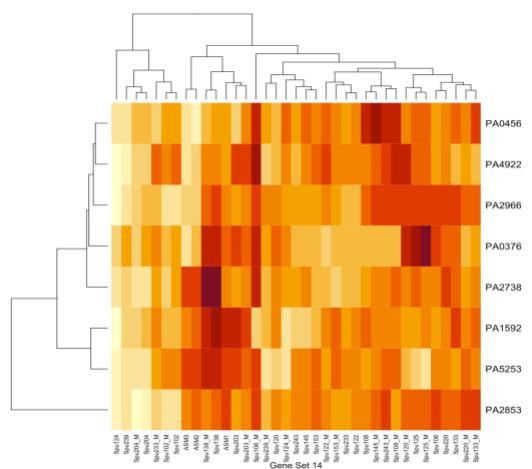
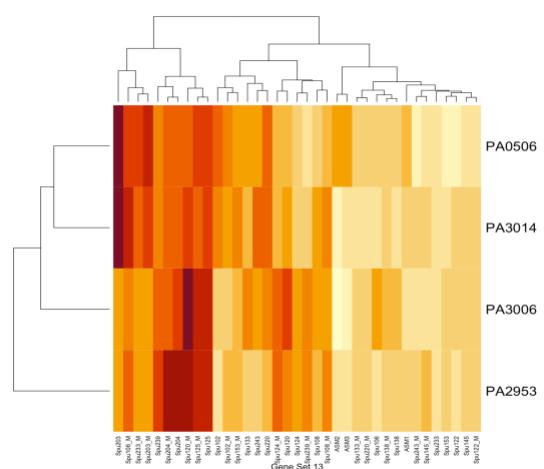
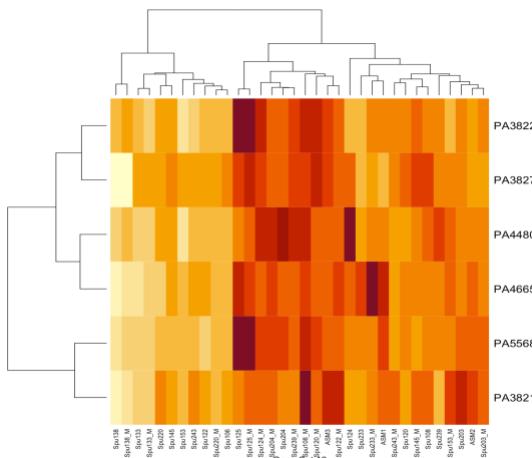
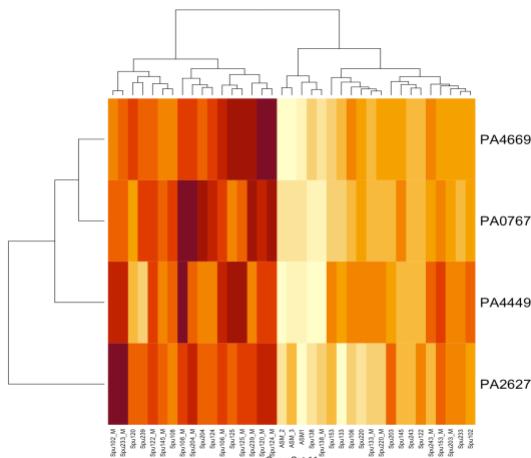


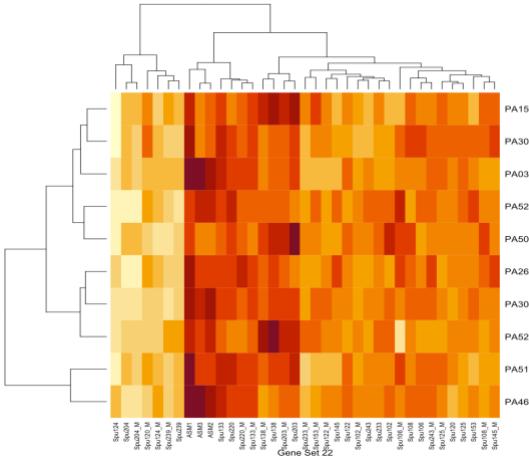
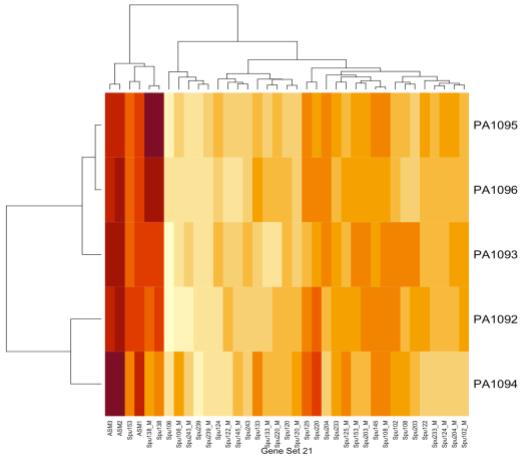
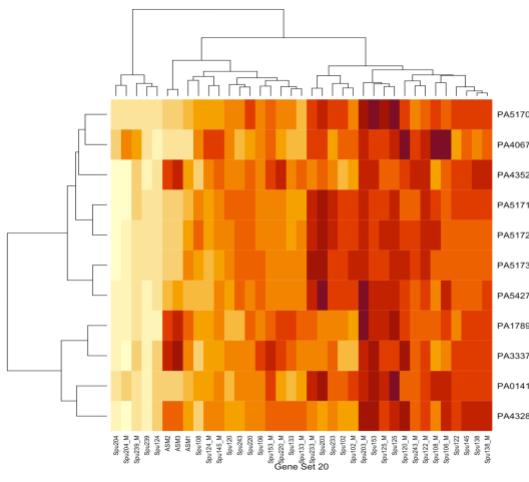
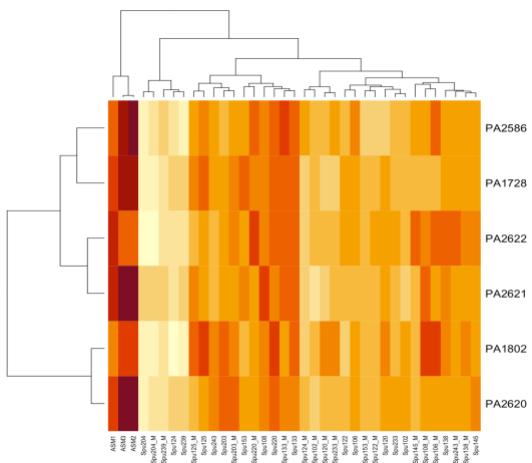
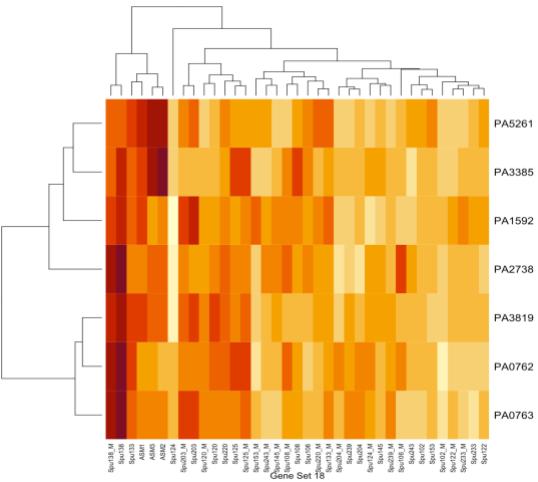
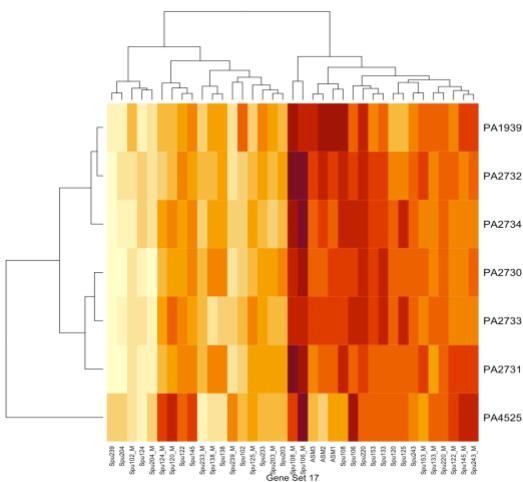












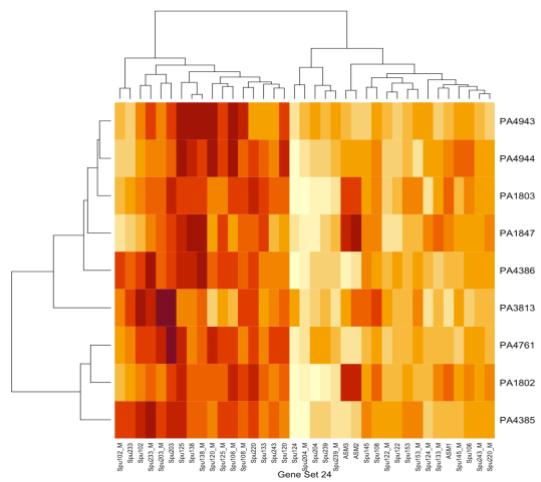
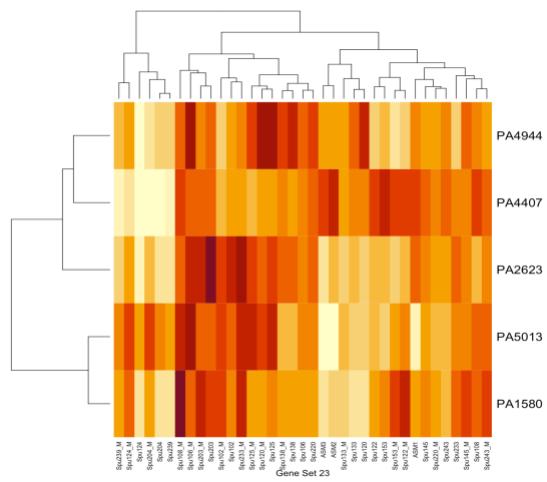
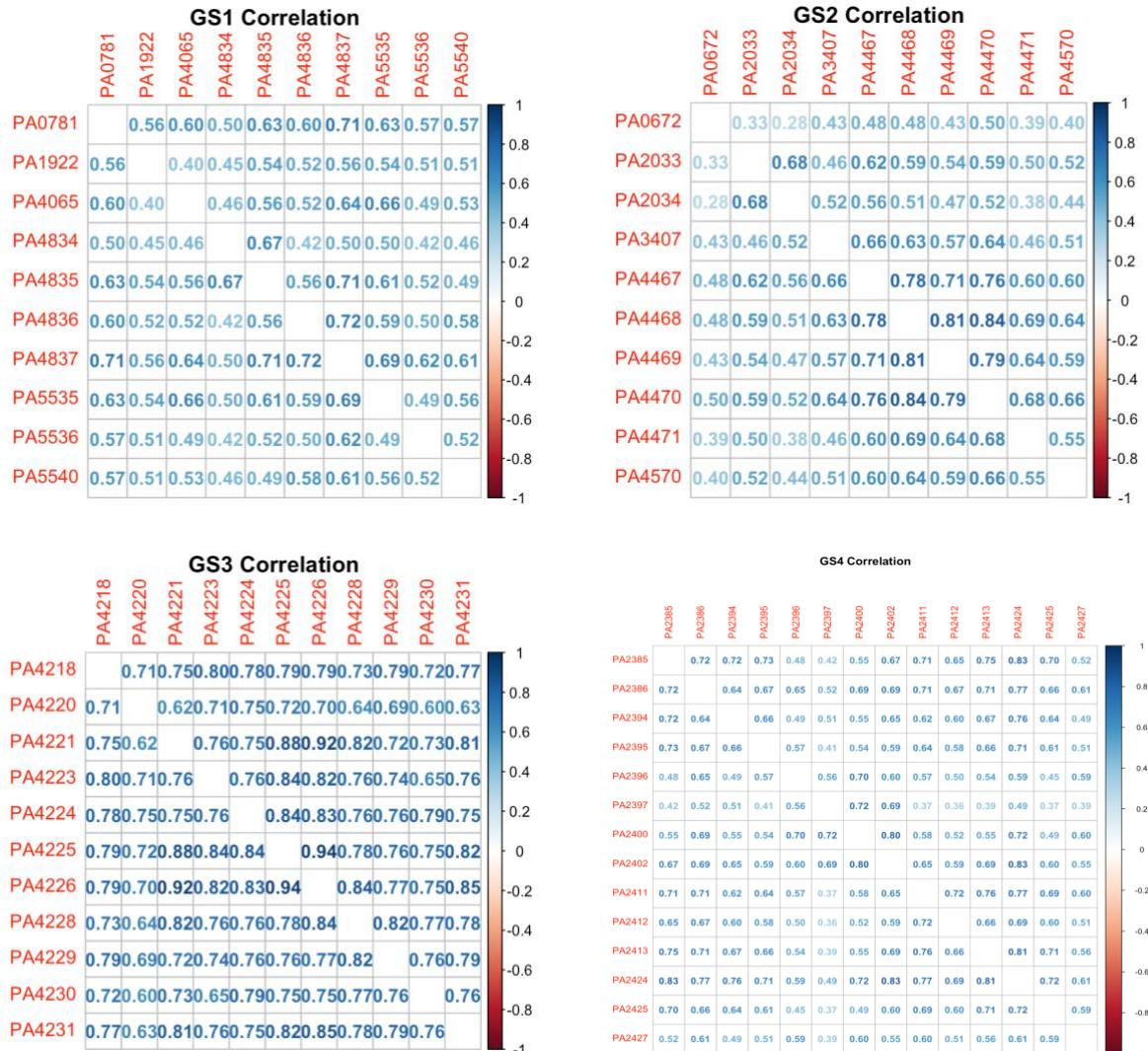
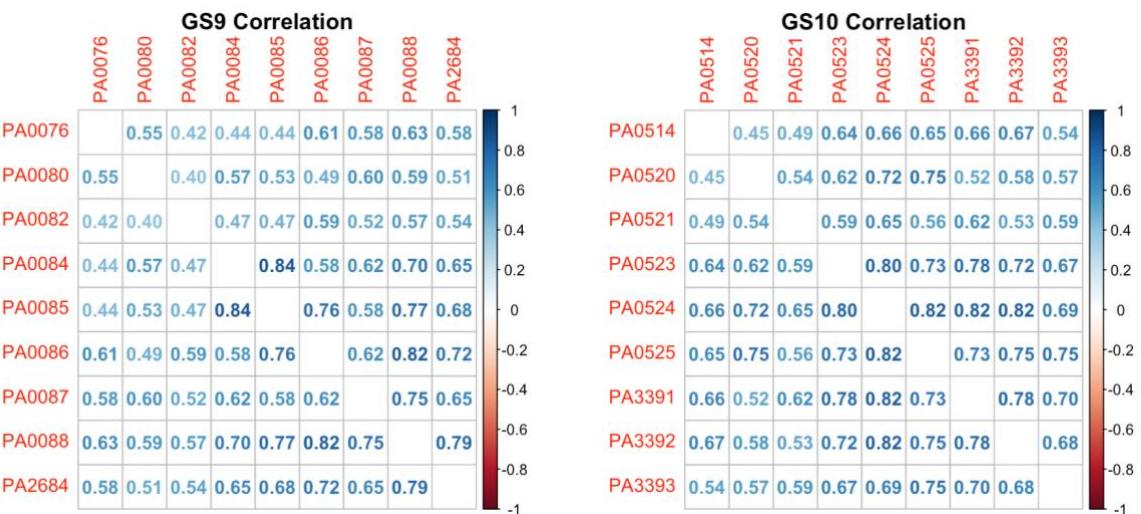
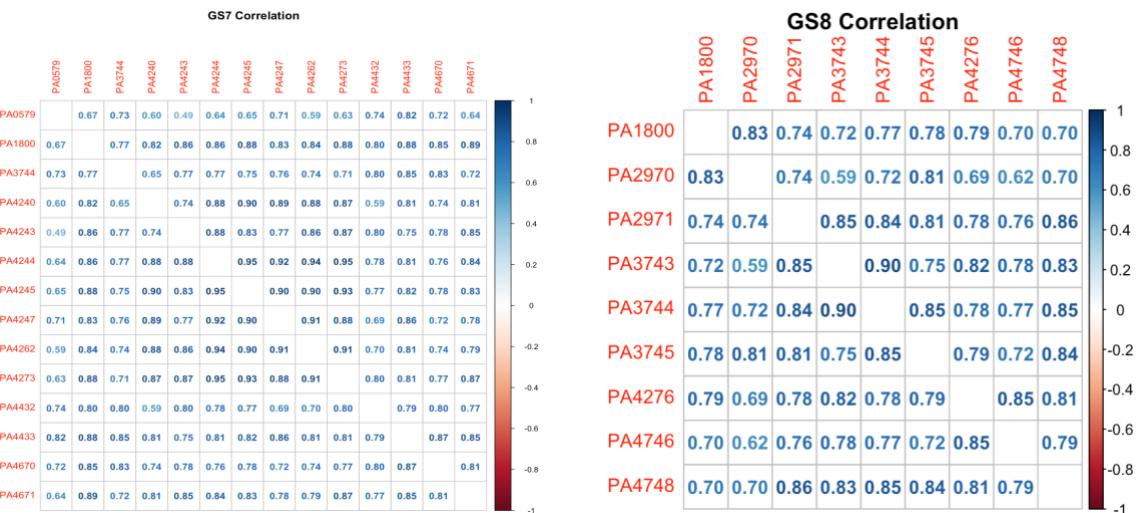
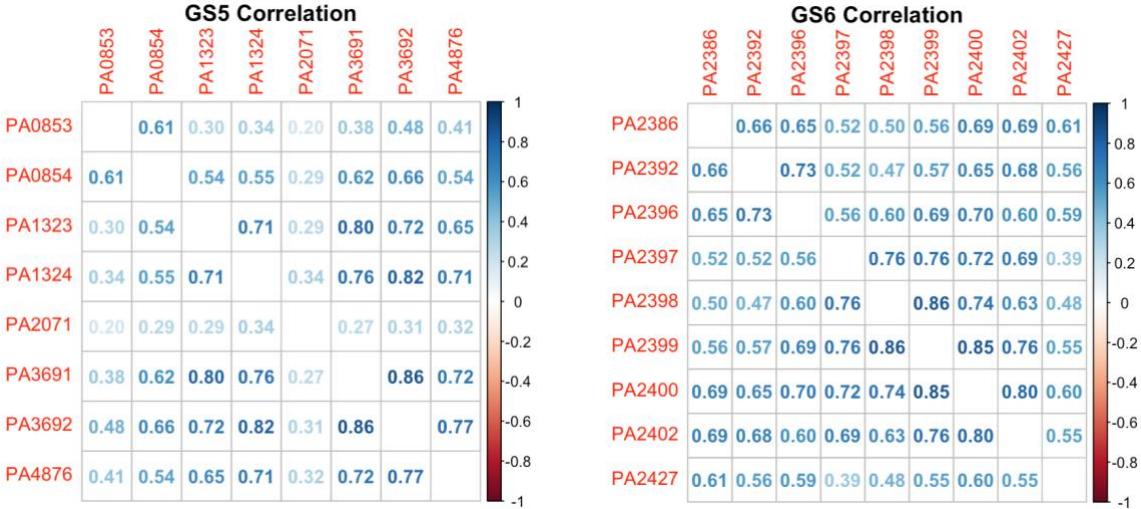
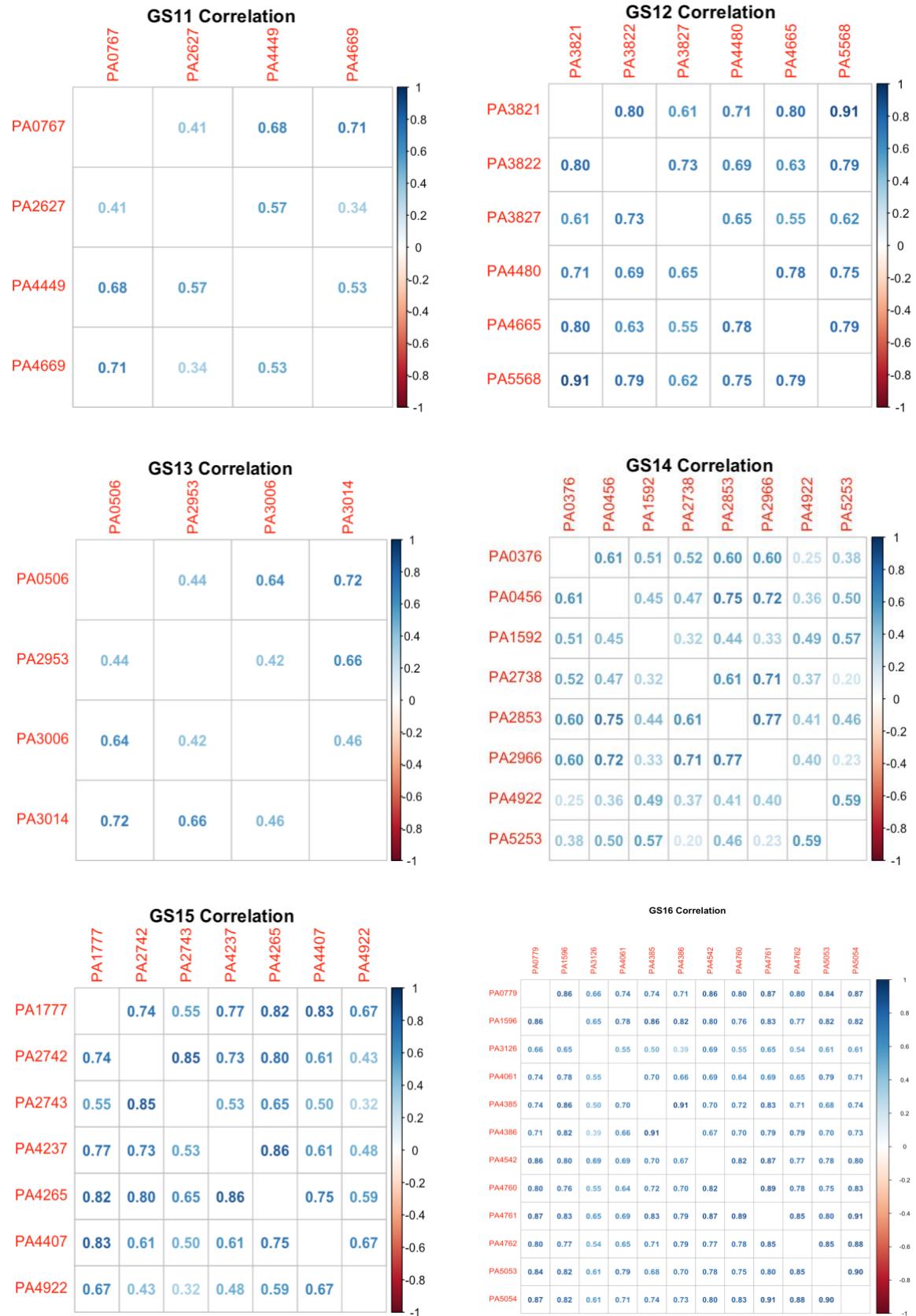
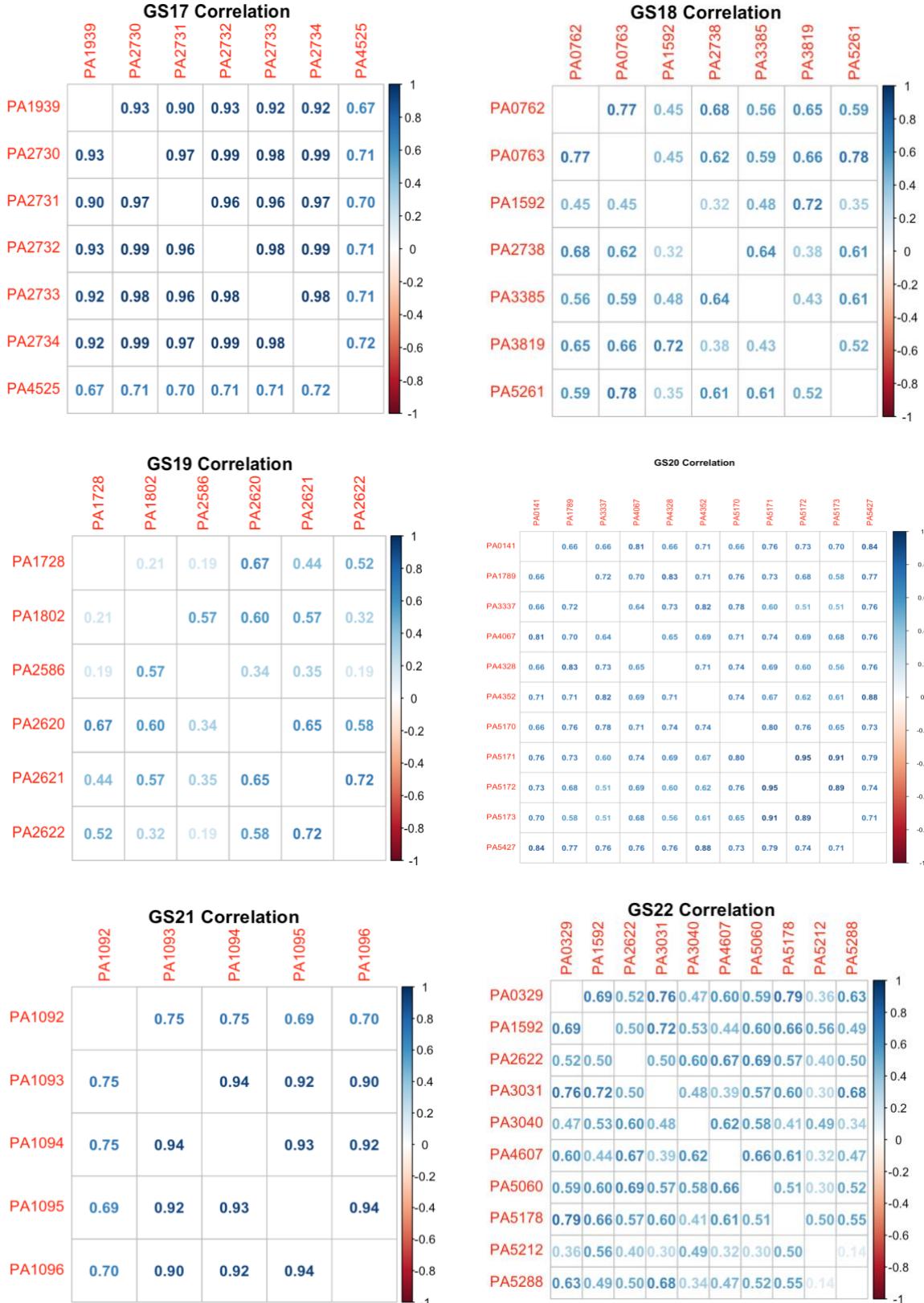


Figure S4. ADAGE-constructed gene sets were screened against an independent compendium of 890 PAO1 gene expression samples to check whether the constituent genes in each gene set remained correlated. All constituent genes in each gene set remain positively correlated, though some correlations are more moderate than others. The level of correlation is demonstrated in the correlation plots presented below.









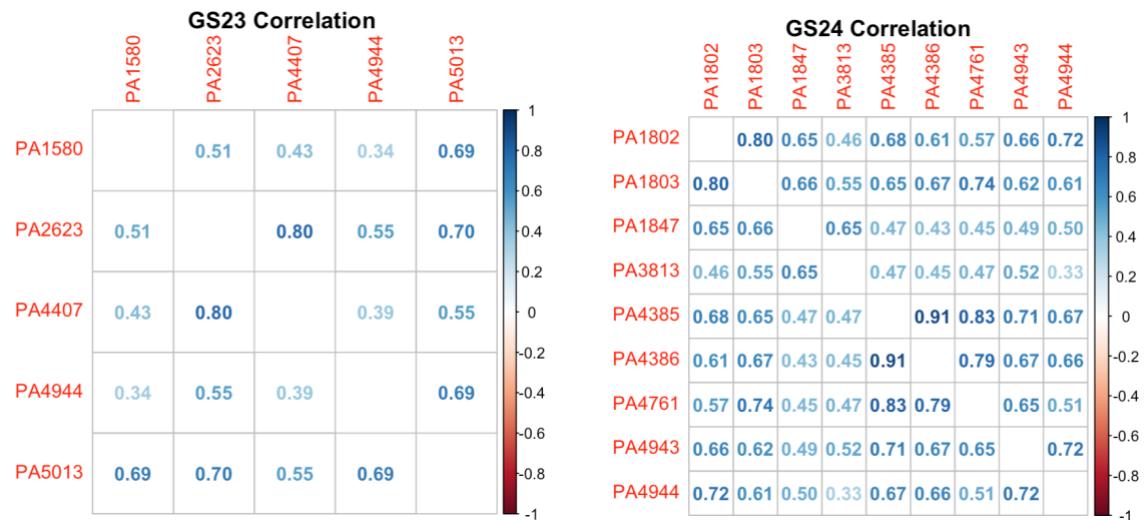
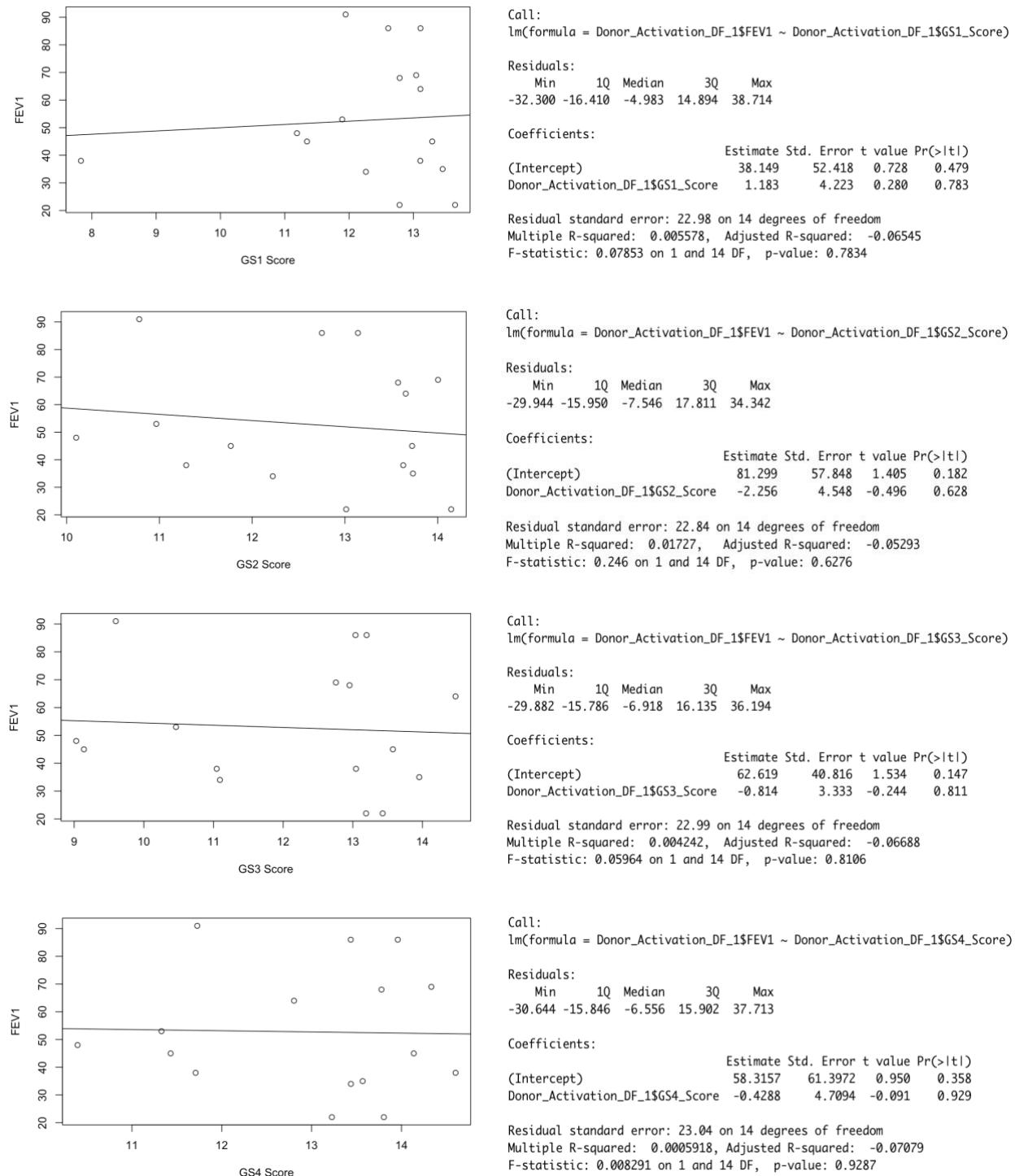
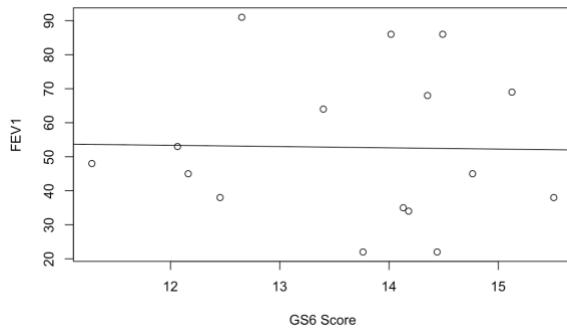


Figure S5. Linear regression analysis demonstrates the association between donor FEV1 and the average expression of metal acquisition gene sets.





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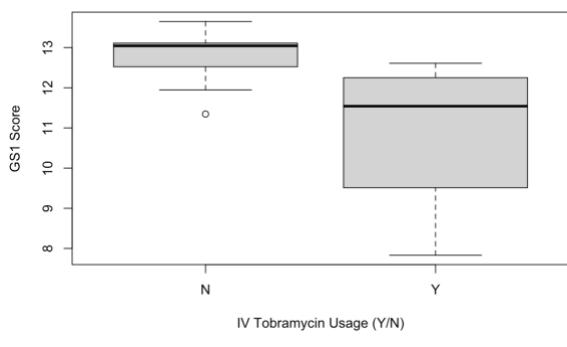
Call:
lm(formula = Donor_Activation_DF_1$FEV1 ~ Donor_Activation_DF_1$GS6_Score)

Residuals:
    Min      1Q   Median      3Q     Max 
-30.717 -15.800  -6.494  15.827 37.867 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)    
(Intercept) 57.8736   67.1378   0.862   0.403    
Donor_Activation_DF_1$GS6_Score -0.3747   4.8919  -0.077   0.940    
                                                        
Residual standard error: 23.04 on 14 degrees of freedom
Multiple R-squared:  0.0004189, Adjusted R-squared:  -0.07098 
F-statistic: 0.005867 on 1 and 14 DF, p-value: 0.94

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Figure S6. 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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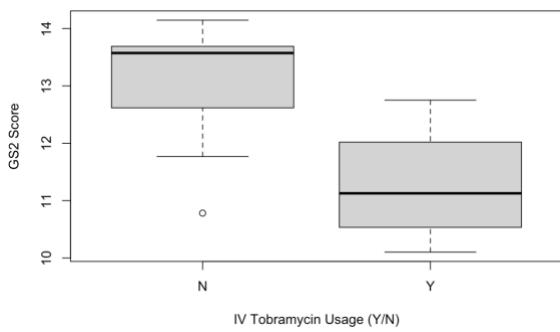
Call:
lm(formula = Donor_Activation_DF_2$GS1_Score ~
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.))

Residuals:
    Min      1Q   Median      3Q     Max 
-3.0499 -0.2438  0.3091  0.4349  1.7285 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)    
(Intercept) 12.7668   0.3532 36.144 1.98e-14 ***
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.)Y -1.8862   0.6840  -2.758  0.0163 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.171 on 13 degrees of freedom
Multiple R-squared:  0.3691, Adjusted R-squared:  0.3205 
F-statistic: 7.605 on 1 and 13 DF, p-value: 0.0163

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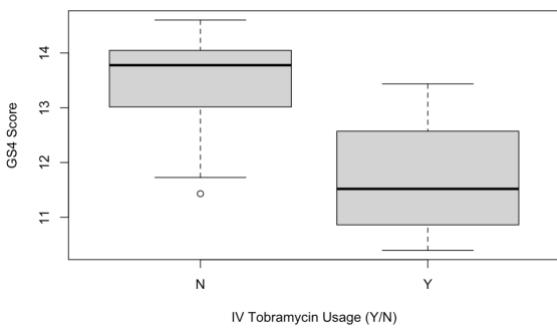
Call:
lm(formula = Donor_Activation_DF_2$GS2_Score ~
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.))

Residuals:
    Min      1Q   Median      3Q     Max 
-2.27731 -0.57422  0.08062  0.62991  1.47443 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)    
(Intercept) 13.0600   0.3202 40.785 4.17e-15 ***
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.)Y -1.7826   0.6201  -2.875  0.013 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.062 on 13 degrees of freedom
Multiple R-squared:  0.3886, Adjusted R-squared:  0.3416 
F-statistic: 8.264 on 1 and 13 DF, p-value: 0.01302

```



```

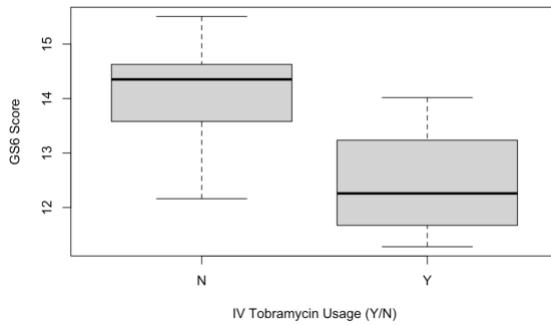
Call:
lm(formula = Donor_Activation_DF_2$GS4_Score ~
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.))

Residuals:
    Min      1Q   Median      3Q     Max 
-1.95444 -0.48439  0.05209  0.66283  1.71861 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)    
(Intercept) 13.3852   0.3281 40.800 4.15e-15 ***
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.)Y -1.6682   0.6353  -2.626  0.021 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.088 on 13 degrees of freedom
Multiple R-squared:  0.3466, Adjusted R-squared:  0.2963 
F-statistic: 6.895 on 1 and 13 DF, p-value: 0.02095

```



```

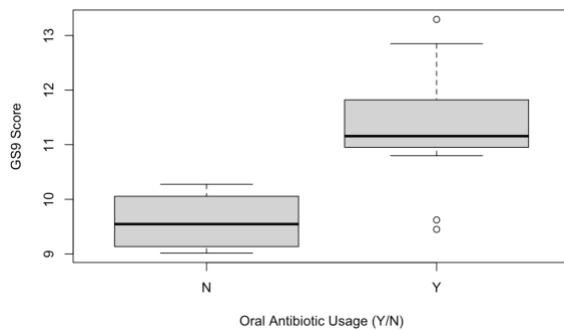
Call:
lm(formula = Donor_Activation_DF_2$GS6_Score ~
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.))

Residuals:
    Min     1Q   Median     3Q    Max 
-1.9139 -0.5334  0.1034  0.5519  1.5638 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 14.0757   0.3156 44.606 1.31e-15 ***
as.factor(Donor_Activation_DF_2$IV.Tobramycin..Y.N.)Y -1.6218   0.6111 -2.654  0.01986 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.047 on 13 degrees of freedom
Multiple R-squared:  0.3514, Adjusted R-squared:  0.3015 
F-statistic: 7.044 on 1 and 13 DF, p-value: 0.01986

```



```

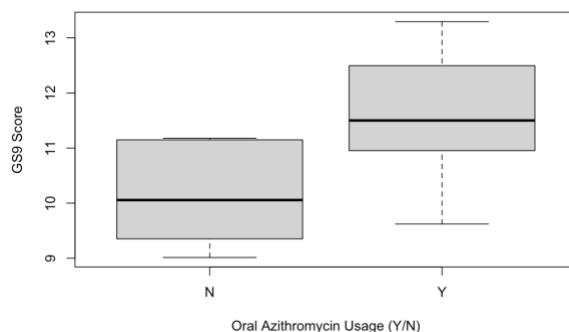
Call:
lm(formula = Donor_Activation_DF_2$GS9_Score ~ as.factor(Donor_Activation_DF_2$Oral.Abx..Y.N.))

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

```



```

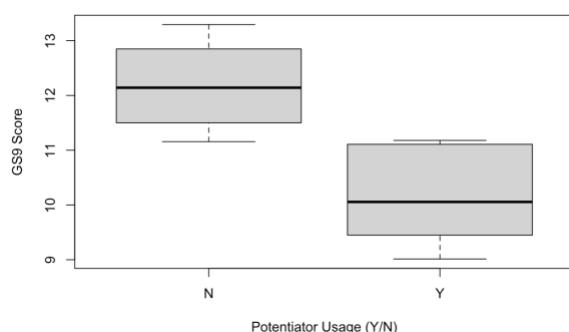
Call:
lm(formula = Donor_Activation_DF_2$GS9_Score ~
as.factor(Donor_Activation_DF_2$Oral.Azithromycin..Y.N.))

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 *  
---
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.))

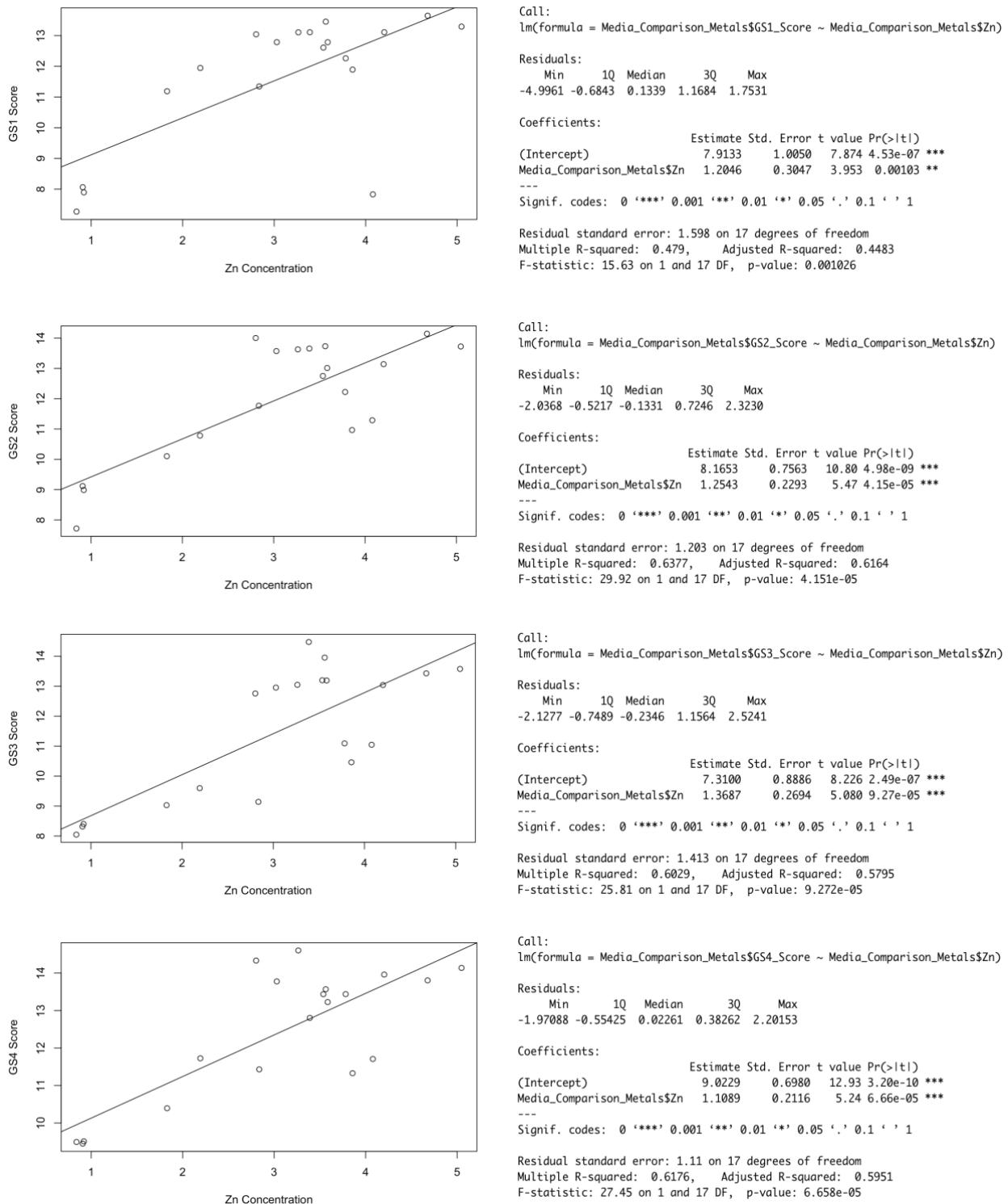
Residuals:
    Min     1Q   Median     3Q    Max 
-1.15397 -0.70322 -0.04668  0.80066  1.10522 

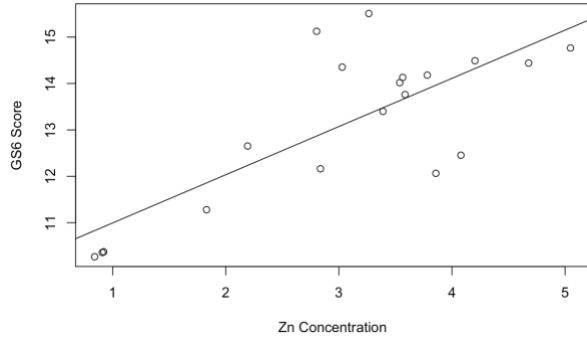
Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 12.1886   0.3833 31.799 1.03e-13 ***
as.factor(Donor_Activation_DF_2$Potentiator.Therapy..Y.N.)Y -2.0210   0.4694 -4.305 0.000855 *** 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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 S7. Linear regression analysis demonstrates the association between metal concentration and the average expression of metal acquisition gene sets.





```

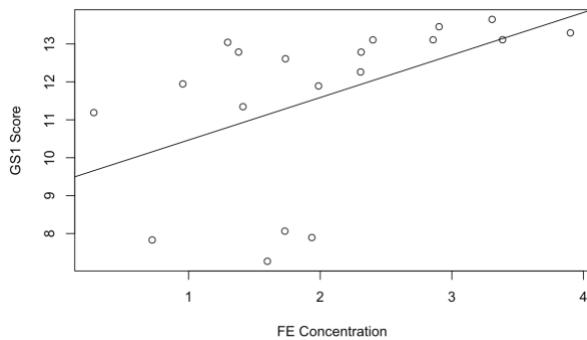
Call:
lm(formula = Media_Comparison_Metals$GS6_Score ~ Media_Comparison_Metals$Zn)

Residuals:
    Min      1Q  Median      3Q     Max 
-1.89823 -0.55314 -0.07863  0.40223  2.25697 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  9.9575   0.6946 14.34 6.35e-11 ***
Media_Comparison_Metals$Zn  1.0383   0.2106  4.93 0.000127 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.105 on 17 degrees of freedom
Multiple R-squared:  0.5884, Adjusted R-squared:  0.5642 
F-statistic: 24.3 on 1 and 17 DF,  p-value: 0.0001269

```



```

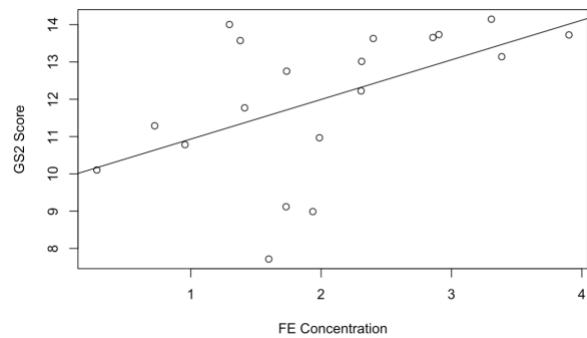
Call:
lm(formula = Media_Comparison_Metals$GS1_Score ~ Media_Comparison_Metals$Fe)

Residuals:
    Min      1Q  Median      3Q     Max 
-3.8692 -0.2305  0.5589  1.1934  2.2431 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  9.3445   1.0570  8.840 9.13e-08 ***
Media_Comparison_Metals$Fe  1.1220   0.4753  2.361  0.0304 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.921 on 17 degrees of freedom
Multiple R-squared:  0.2469, Adjusted R-squared:  0.2026 
F-statistic: 5.573 on 1 and 17 DF,  p-value: 0.03045

```



```

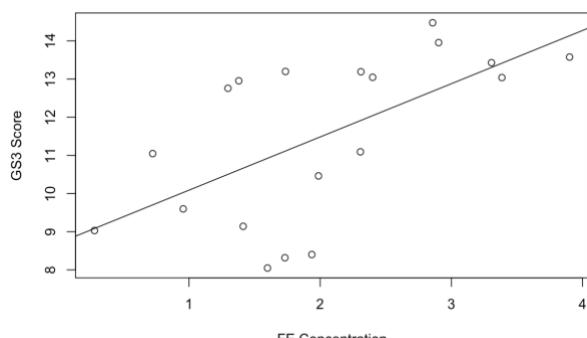
Call:
lm(formula = Media_Comparison_Metals$GS2_Score ~ Media_Comparison_Metals$Fe)

Residuals:
    Min      1Q  Median      3Q     Max 
-3.8520 -0.3082  0.3991  0.7713  2.7568 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  9.8686   0.9378 10.52 7.29e-09 ***
Media_Comparison_Metals$Fe  1.0629   0.4217  2.52   0.022 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.705 on 17 degrees of freedom
Multiple R-squared:  0.272, Adjusted R-squared:  0.2292 
F-statistic: 6.353 on 1 and 17 DF,  p-value: 0.022

```



```

Call:
lm(formula = Media_Comparison_Metals$GS3_Score ~ Media_Comparison_Metals$Fe)

Residuals:
    Min      1Q  Median      3Q     Max 
-2.99267 -0.91103 -0.05395  1.30878  2.33509 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  8.6937   0.9776  8.893 8.4e-08 ***
Media_Comparison_Metals$Fe  1.3948   0.4396  3.173  0.00556 ** 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.777 on 17 degrees of freedom
Multiple R-squared:  0.3719, Adjusted R-squared:  0.335 
F-statistic: 10.07 on 1 and 17 DF,  p-value: 0.005562

```

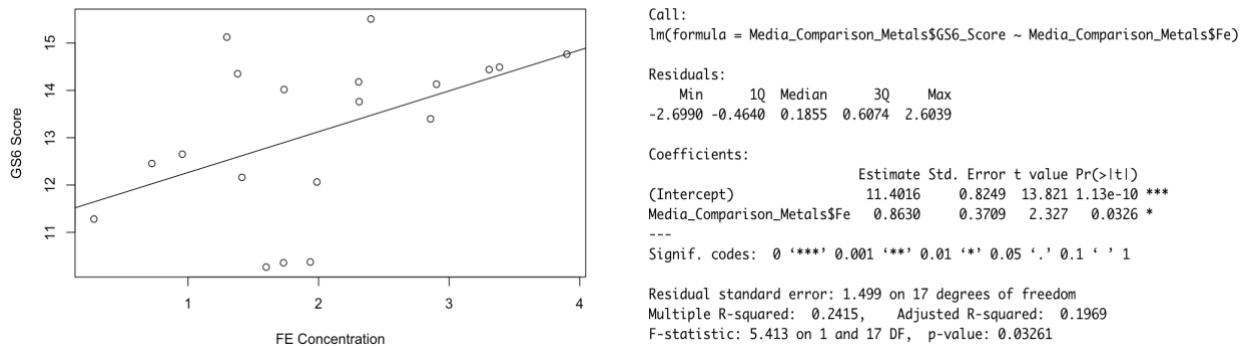


Figure S8. Composition of RNA sequencing reads in the spike-in and ASM samples. Reads were mapped to various species including *P. aeruginosa*, other common CF pathogens, and the human genome.

