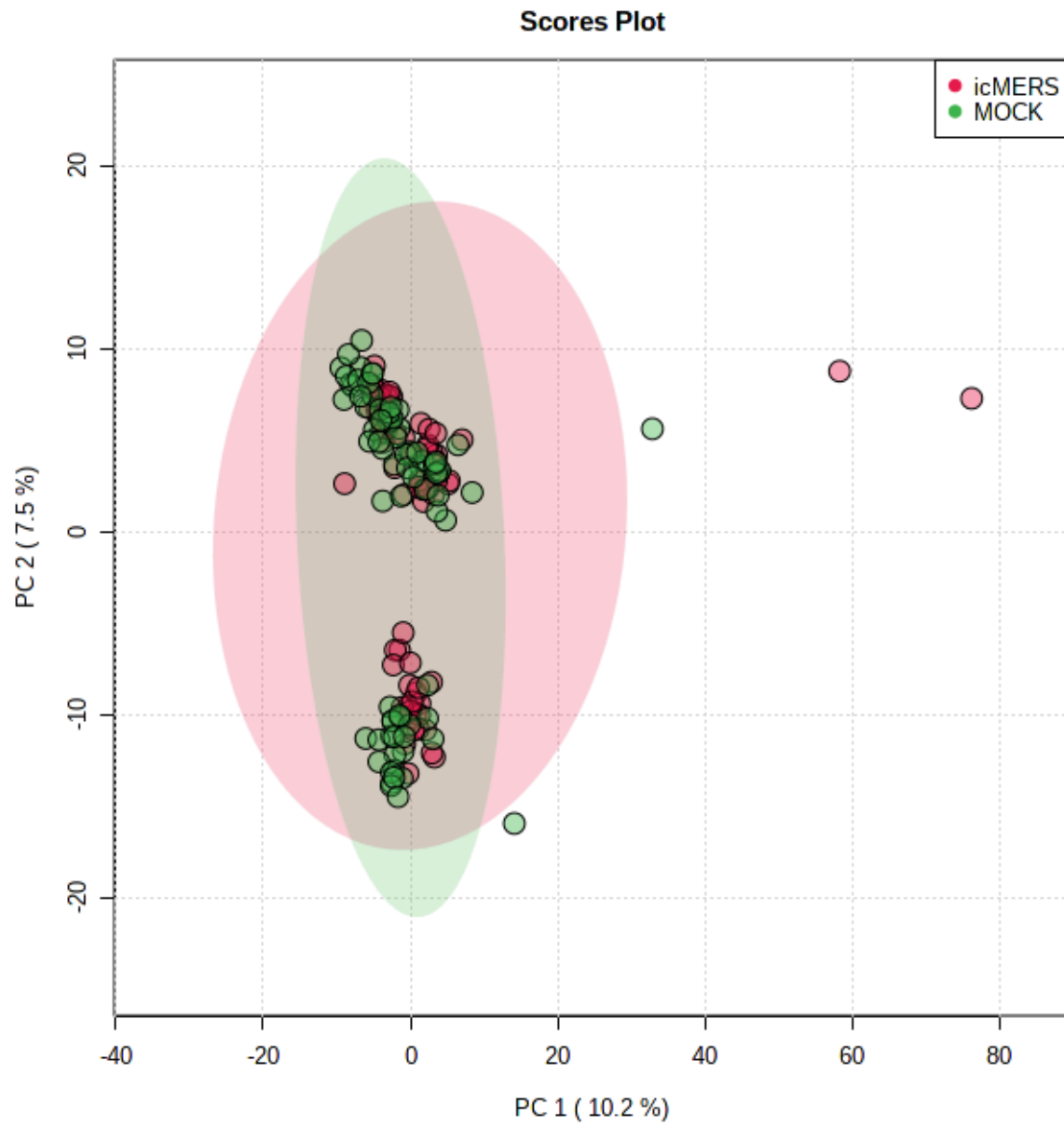
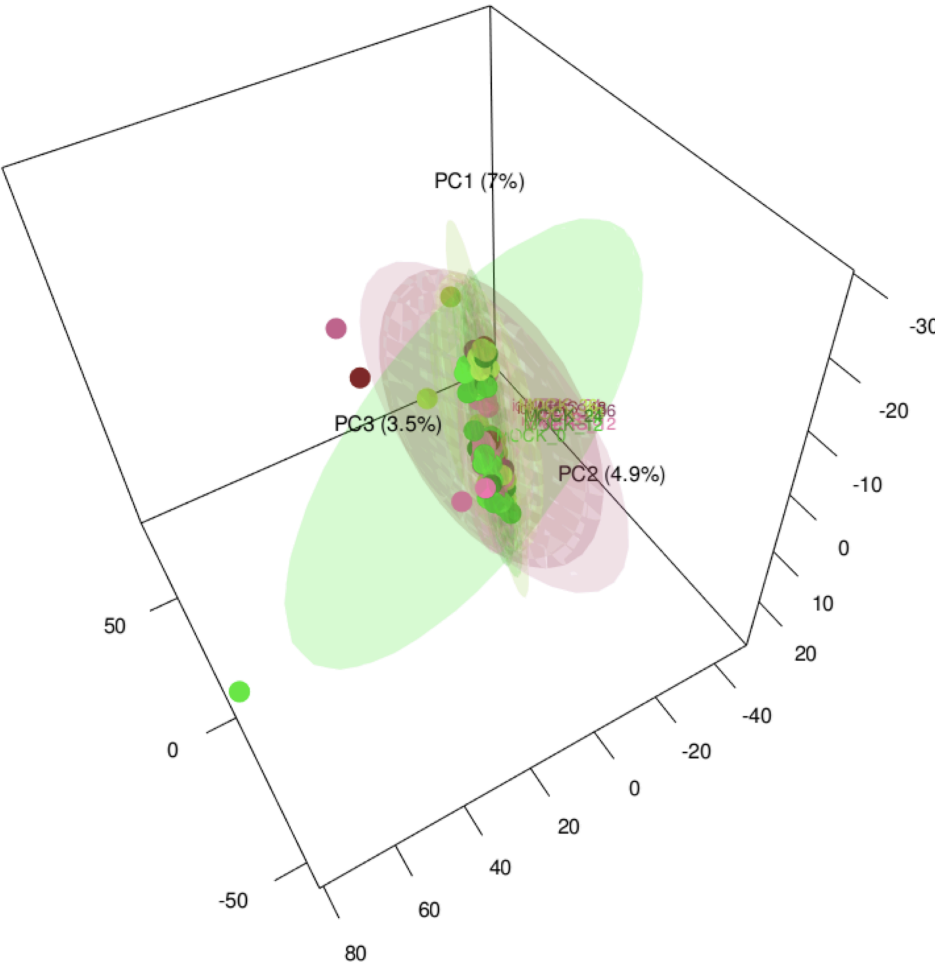


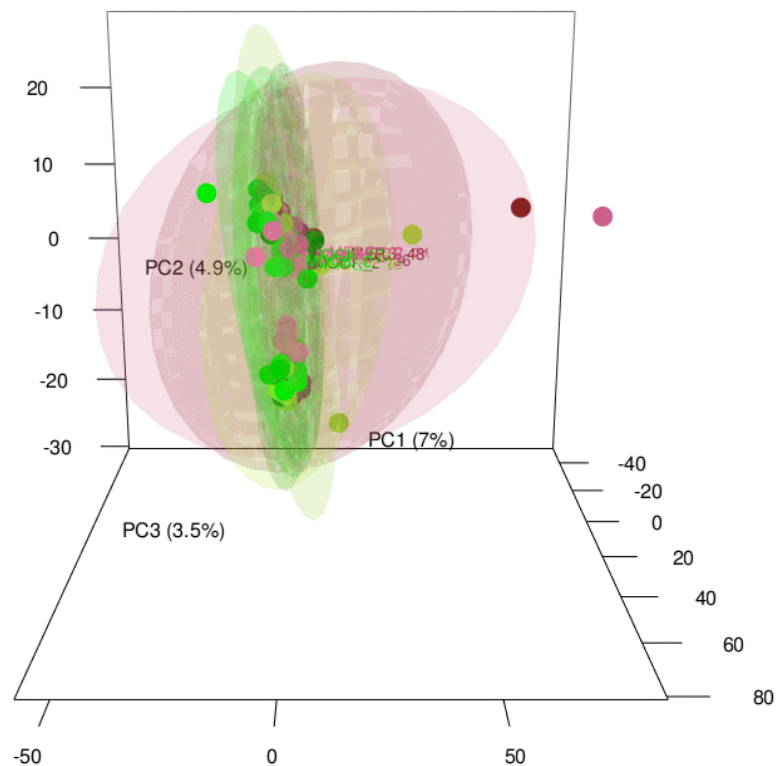
All combined
PCA



PCA-All combined with ellipses (3D)



This is an animation of the plot for analysing better the differences in 3 dimensions.



PERMANOVA ANALYSIS

- Phenotype (icMERS and MOCK)
999 permutations
 $R^2 = 0.036$
 $F = 5.4727$
 $P = 0.005$
- Pheno-Time (icMERS_0, icMERS_12, icMERS_24, icMERS_36, icMERS48, MOCK_0, MOCK_12, MOCK_24, MOCK_36, MOCK_48)
999 permutations

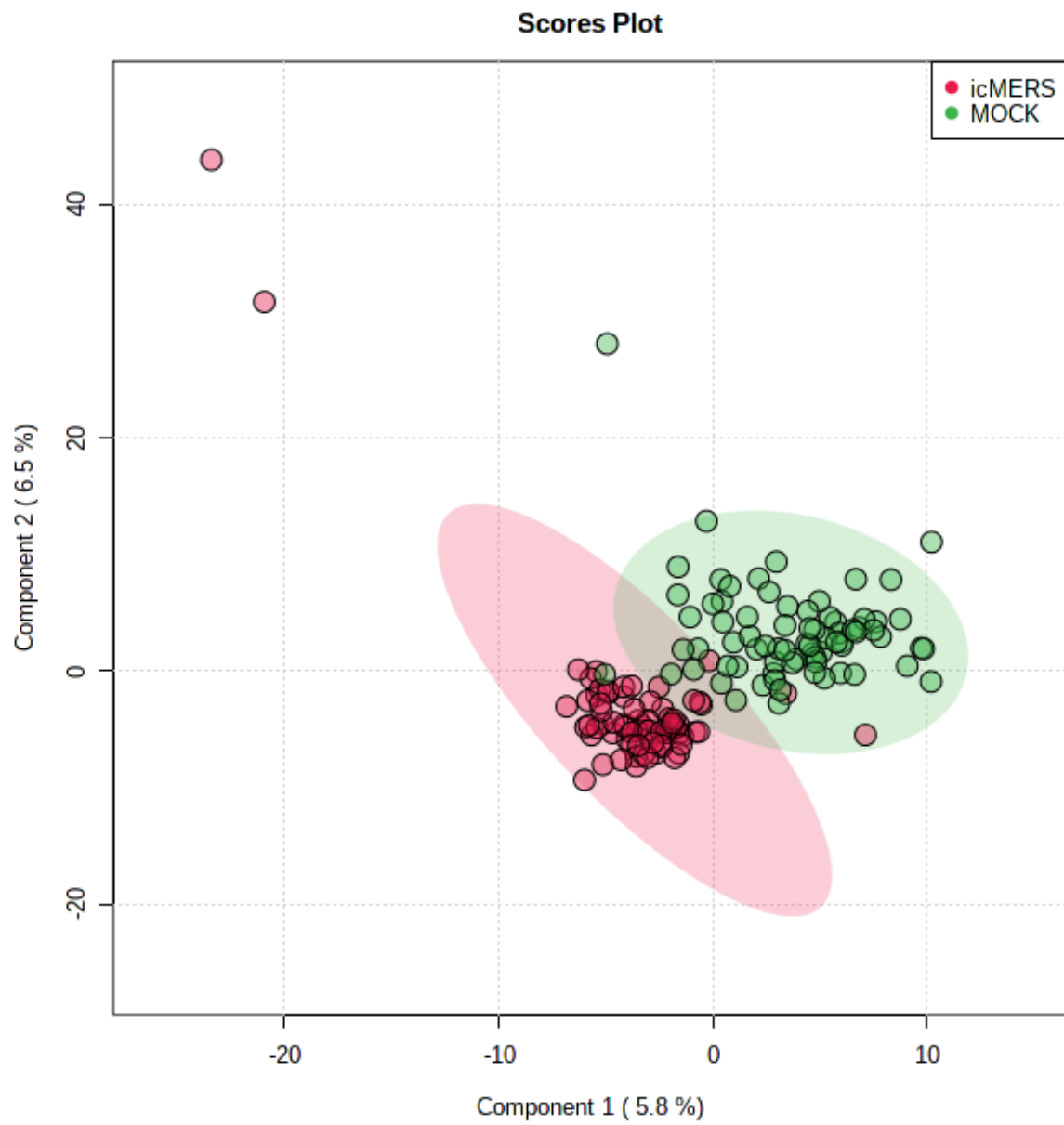
$R^2 = 0.380$

$F = 9.5412$

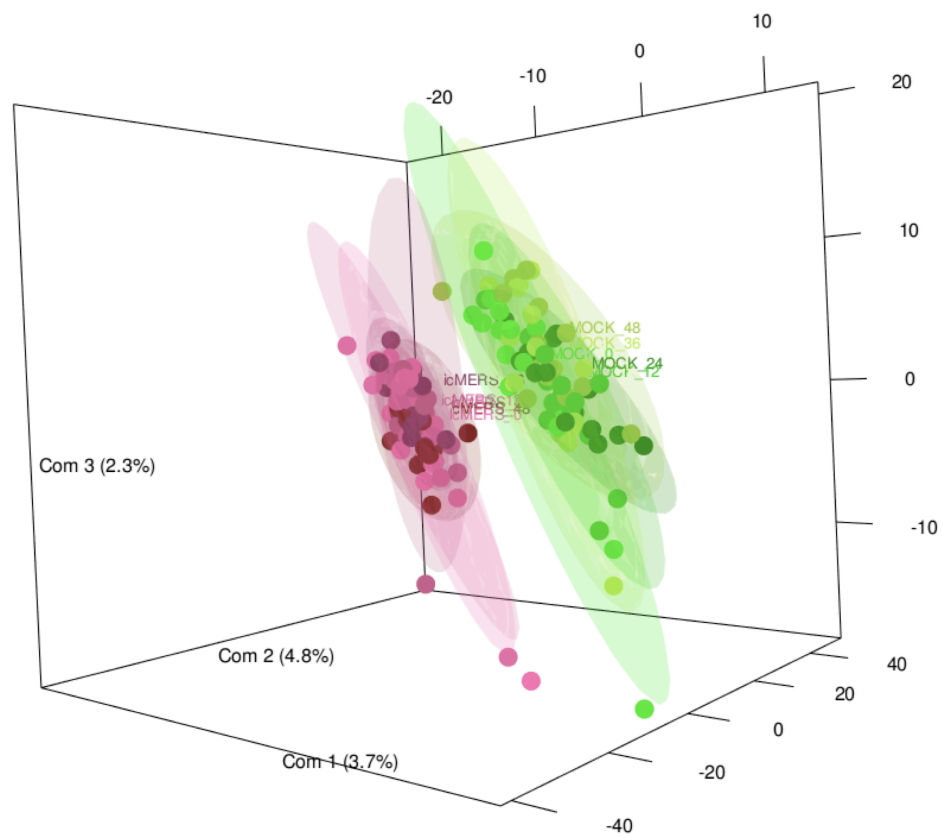
$P = 0.001$

- MHAЕ (1, 2, 3)
999 permutations
 $R^2 = 0.064$
 $F = 10.056$
 $P = 0.001$
- Time (0, 12, 24, 36, 48)
 $R^2 = 0.160$
 $F = 28.164$
 $P = 0.001$

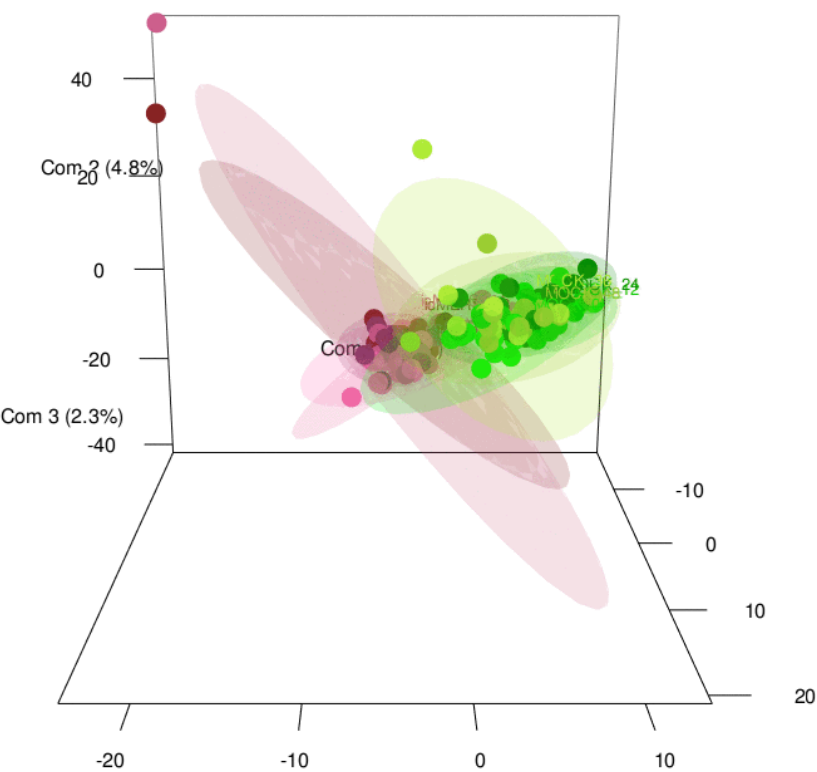
PLS-DA

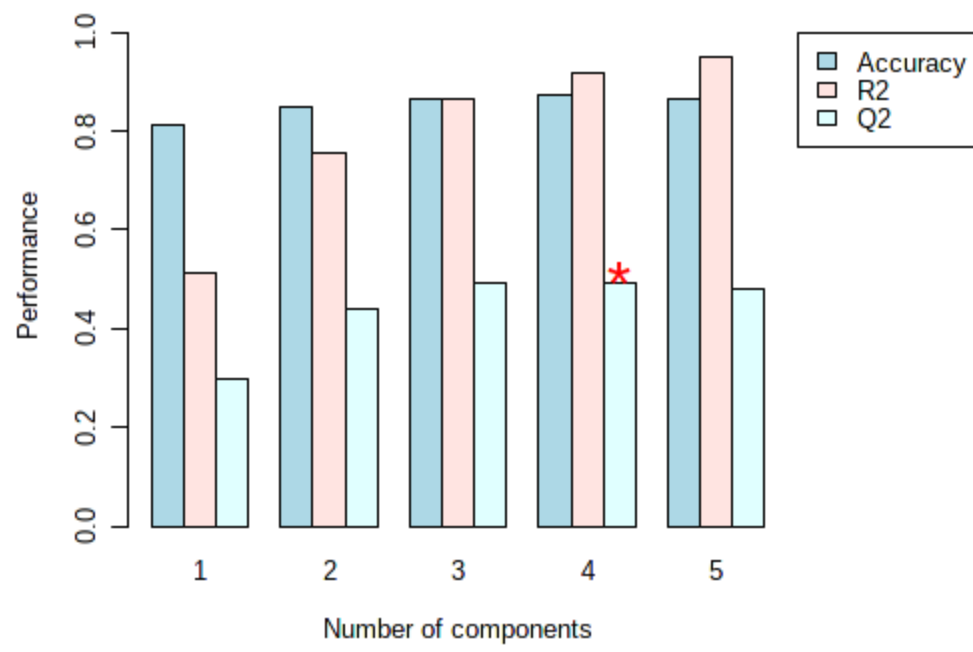


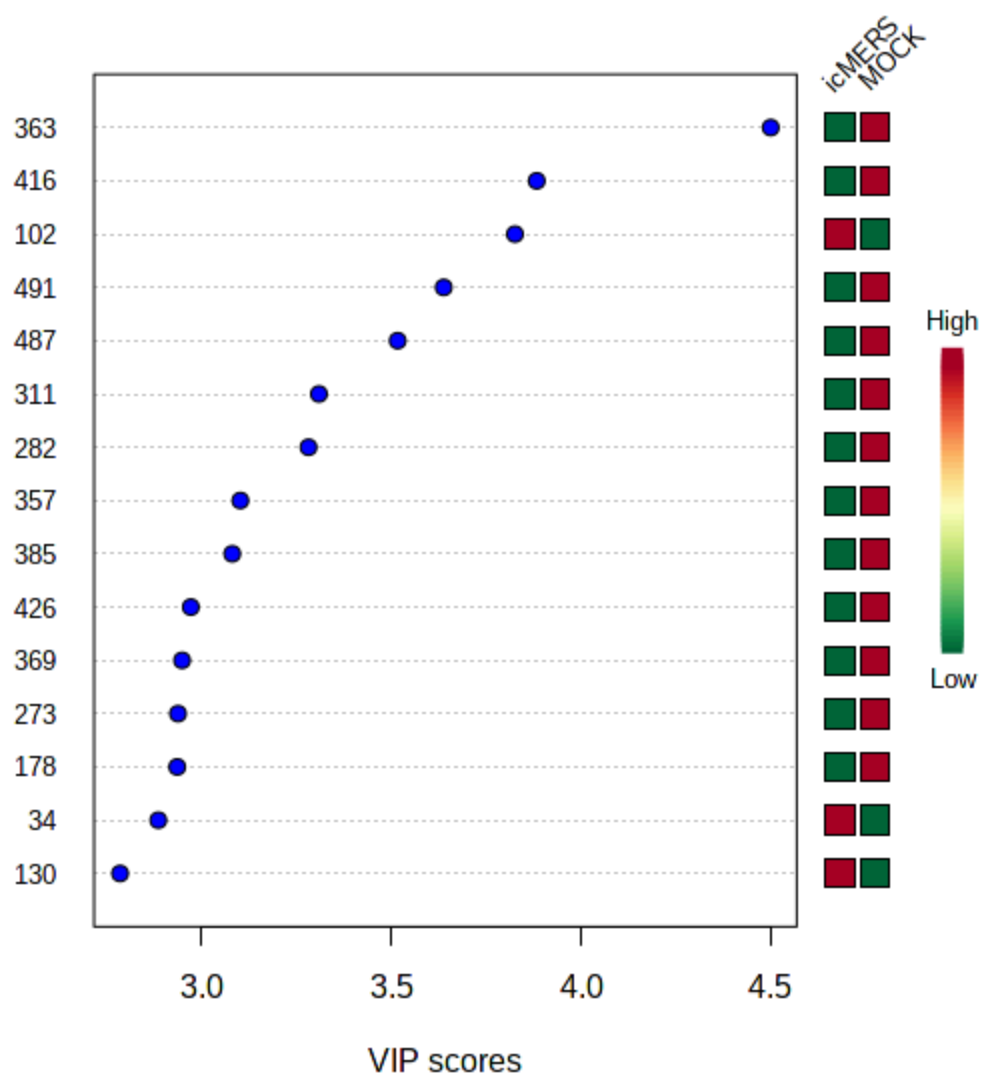
PLSDA-All combined with ellipses (3D)



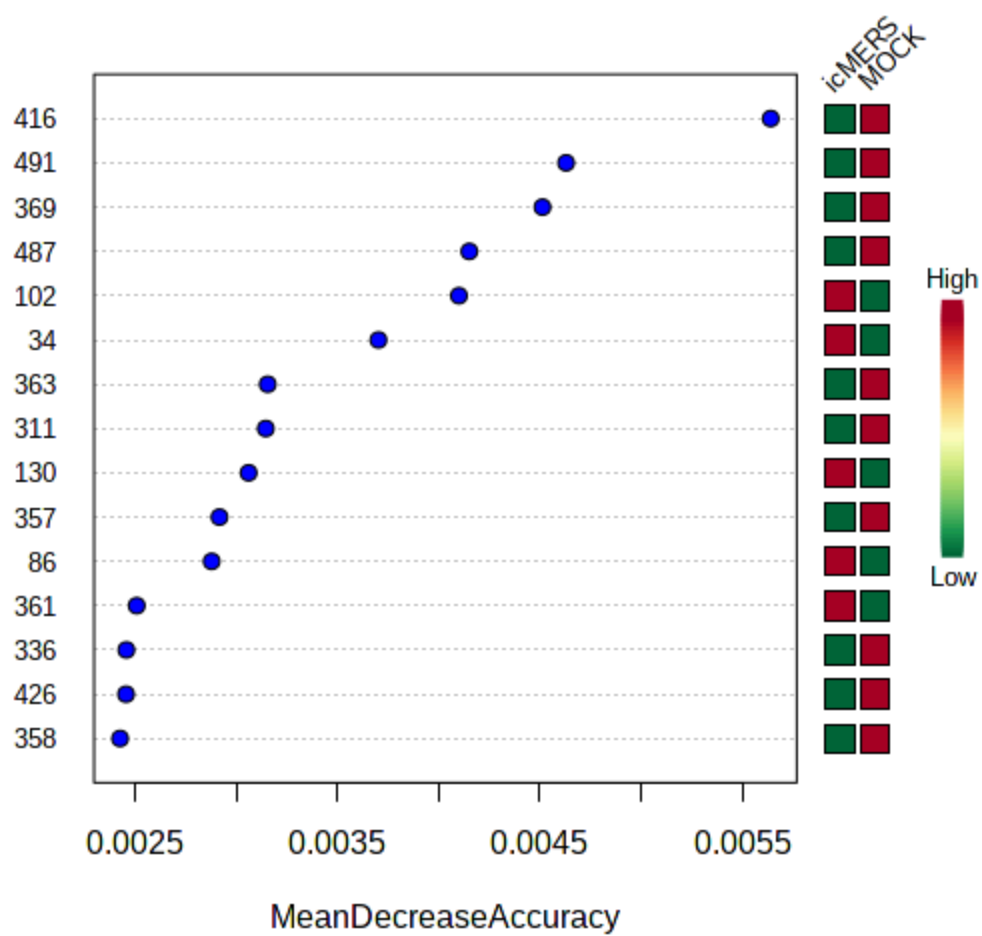
This is an animation of the plot for analysing better the differences in 3 dimensions.



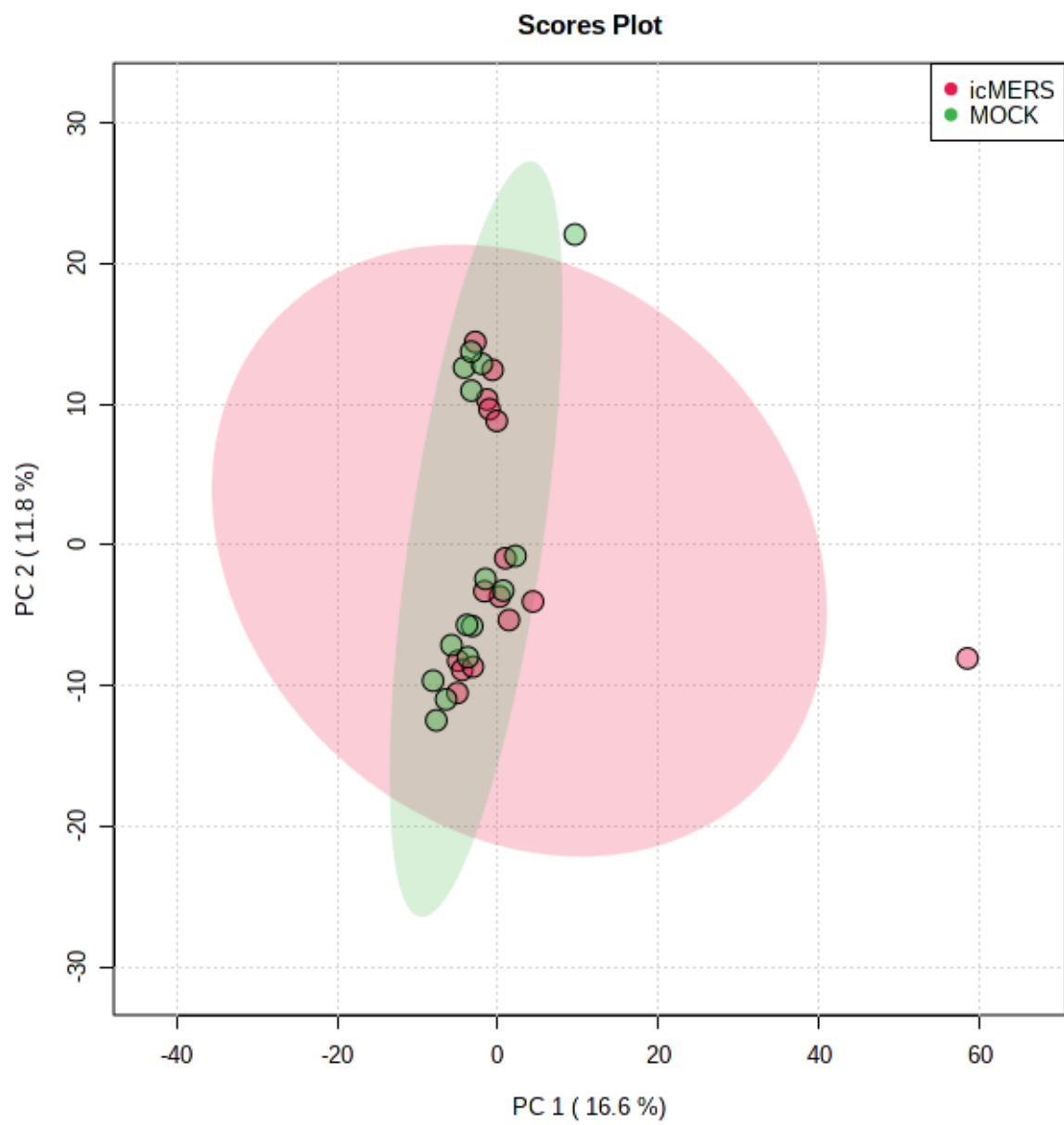




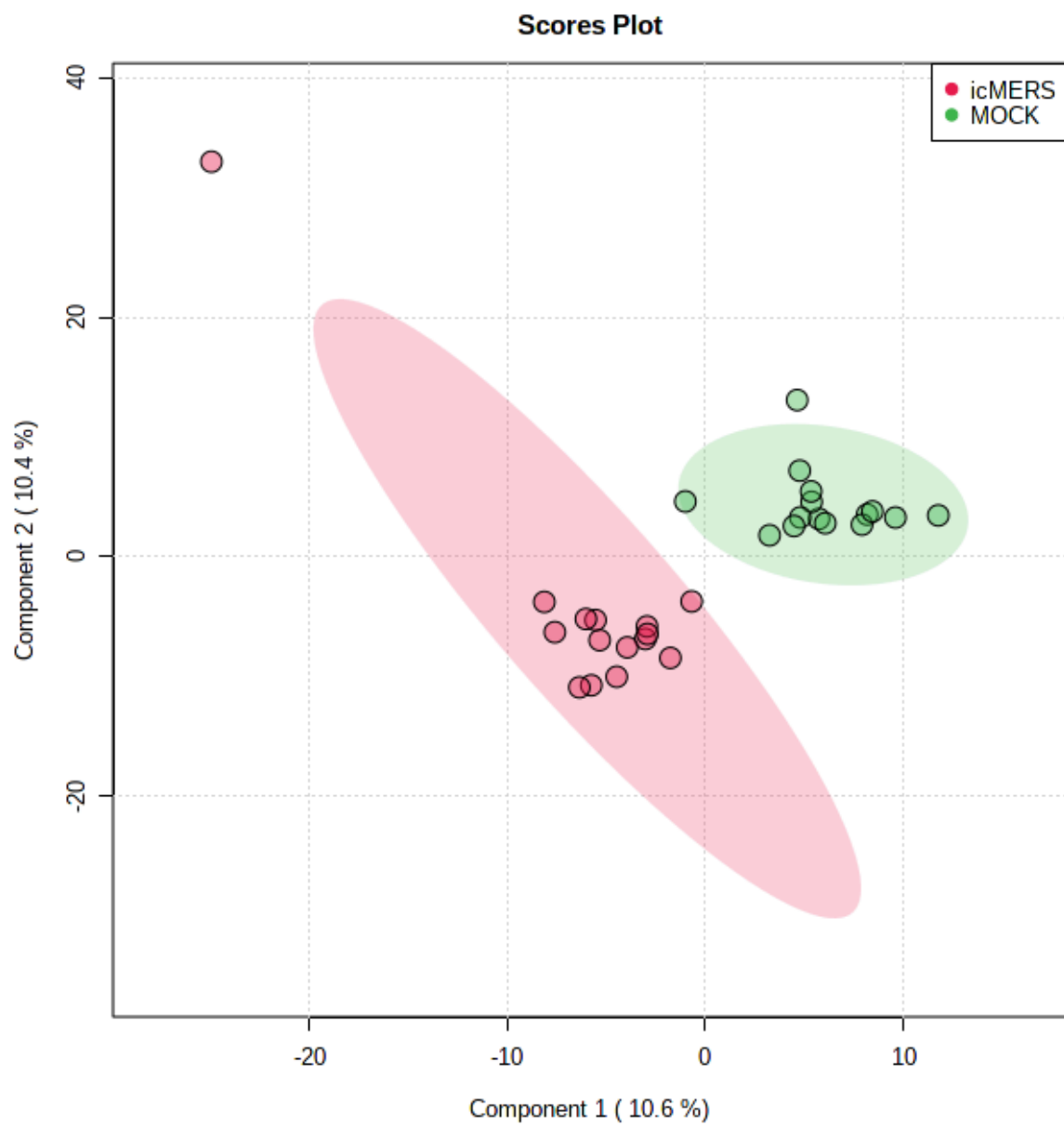
Random Forest OOB 0.107

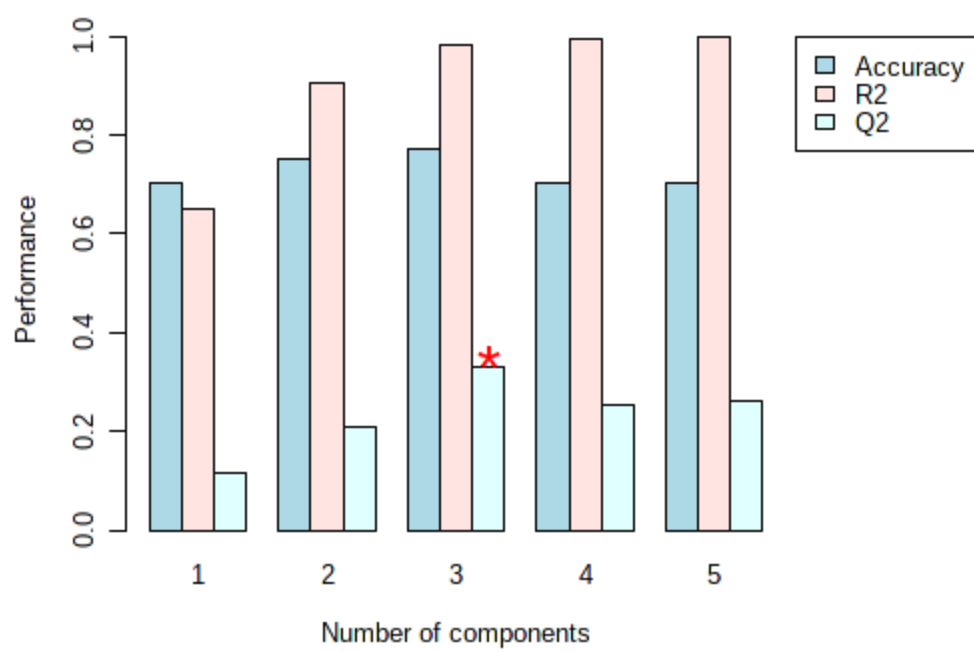


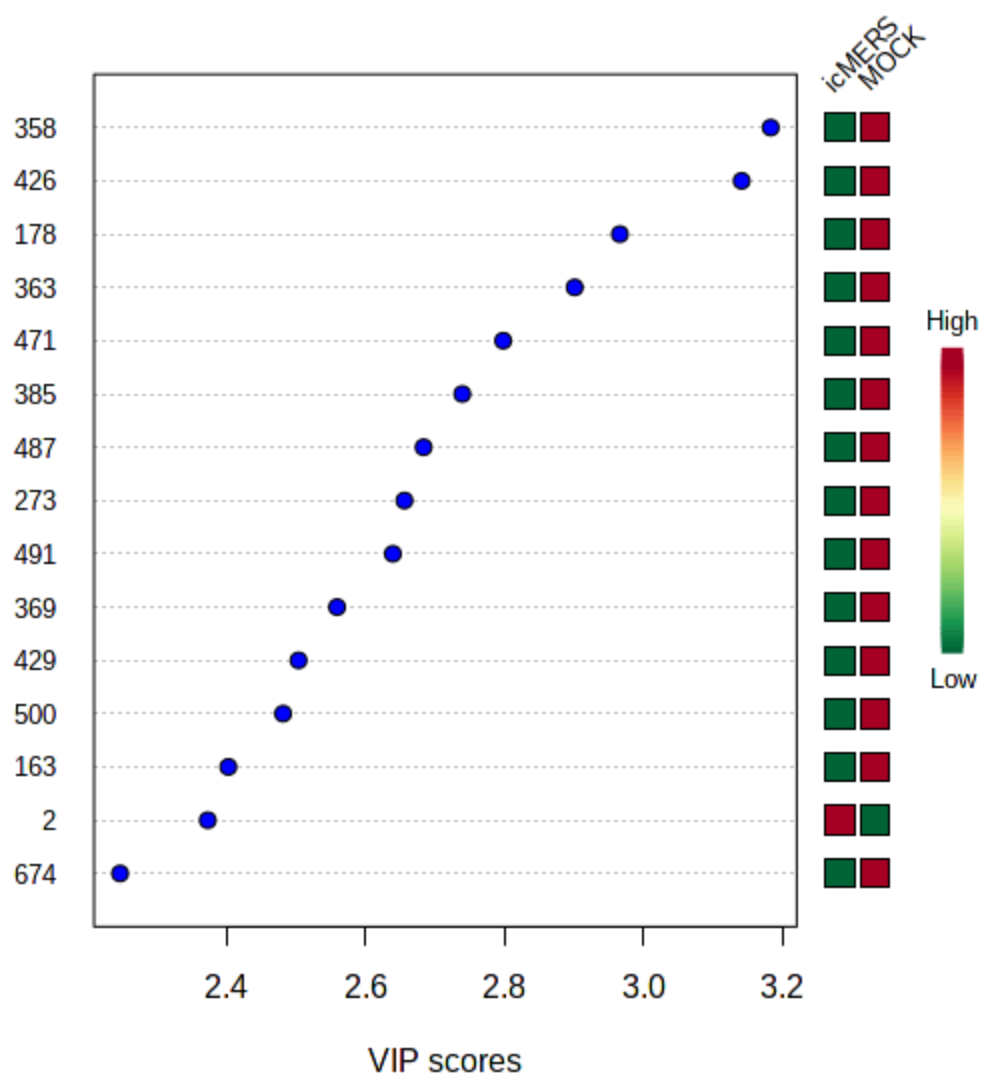
48 hours
PCA



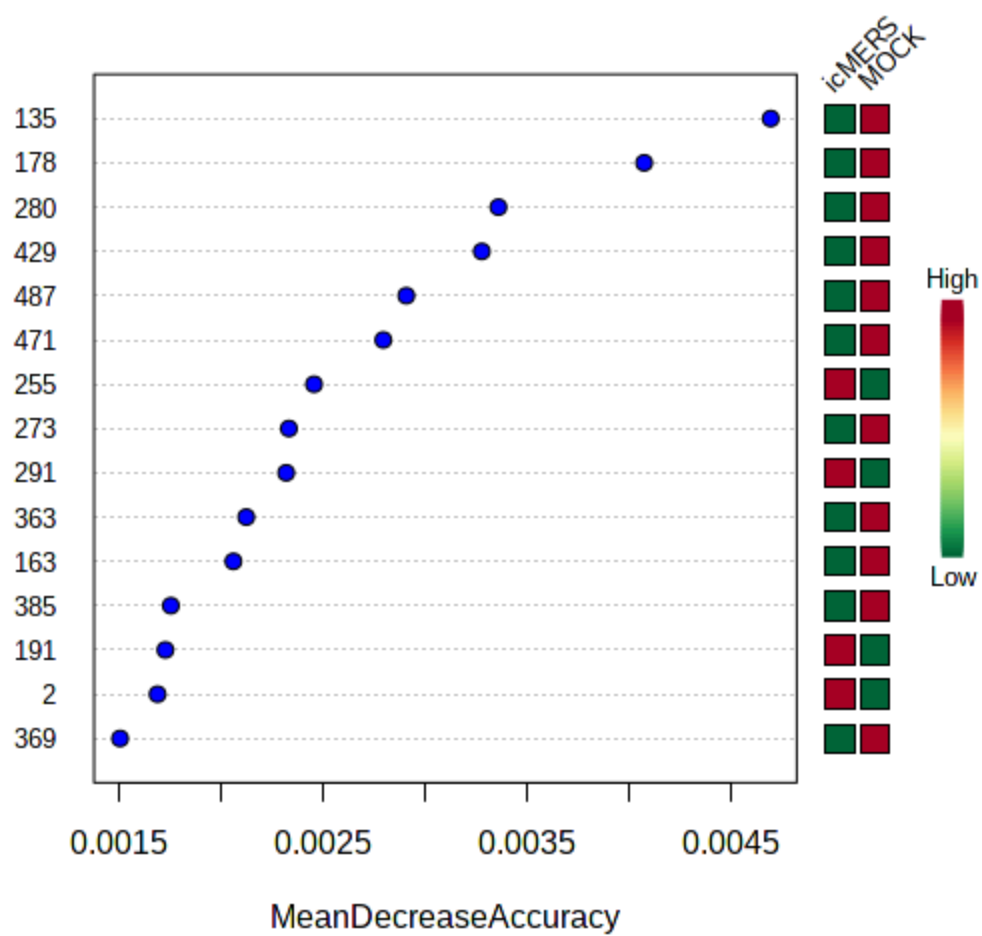
PLSDA







Random forest OOB 0.267



Features with high Component 1 value - MinPairCos 0.7

Annotation	Cluster index	Component 1	-Log (p-value)	Log2(FC)	MeanDecreaseAccuracy
Maltose MEOX1 TMS	487	3.5162	8.0471	-1.8872	0.0044119
Cellobiose meox2 TMS	491	3.6377	8.6332	-2.318	0.0035826
N-Acetyl-D-glucosamine, tetrakis(trimethylsilyl) ether, methyloxime (syn)	357	3.1016	6.253	-2.1639	0.0035523
Silanol, trimethyl-, phosphate (3:1)	102	3.8254	x	x	0.0035547
L-Alanine	34	2.8853	x	x	0.00245
Uridine	385	3.0802	6.1686	-1.1466	0.0011811

Features from MeanDecreaseAccuracy graph - MinPairCos 0.7

Annotation	Cluster index	Comp. 1	-Log (p-value)	Log2(FC)	MeanDecreaseAccuracy	Response icMERS	Response MOCK
x	416	x	x	x	x		
<u>Cellobiose meox2 TMS</u>	<u>491</u>	<u>3.6377</u>	<u>8.6332</u>	<u>-2.318</u>	<u>0.0035826</u>	Low	High
x	369	x	x	x	x		
<u>Maltose MEOX1 TMS</u>	<u>487</u>	<u>3.5162</u>	<u>8.0471</u>	<u>-1.8872</u>	<u>0.0044119</u>	Low	High
<u>Silanol, trimethyl-, phosphate (3:1)</u>	<u>102</u>	<u>3.8254</u>	x	x	<u>0.0035547</u>	High	Low
<u>L-Alanine</u>	<u>34</u>	<u>2.8853</u>	x	x	<u>0.00245</u>	High	Low
x	363	x	x	x	x		
x	311	x	x	x	x		
x	130	x	x	x	x		
<u>N-Acetyl-D-glucosamine, tetrakis(trimethylsilyl) ether, methyloxime (syn)</u>	<u>357</u>	<u>3.1016</u>	<u>6.253</u>	<u>-2.1639</u>	<u>0.0035523</u>	Low	High
x	86	x	x	x	x		
Ribose-5-phosphate TMS	361	2.0846	x	x	0.0022977	High	Low
7-(Trimethylsilyl)-2,6-bis[(trimethylsilyl)oxy]-7H-purine	336	1.3102	1.3854	-7.0566	0.0024315	Low	High
x	426	x	x	x	x		
Myo-Inositol, 6TMS derivative	358	1.8178	x	x	8.5806E-4	Low	High

Features from VIP Scores graph - MinPairCos 0.7

Annotation	Cluster index	Component 1	-Log (p-value)	Log2(FC)	MeanDecreaseAccuracy	Response MERS	Response MOCK
x	363	x	x	x	x		
x	416	x	x	x	x		
Silanol, trimethyl-, phosphate (3:1)	102	3.8254	x	x	0.0035547	High	Low
Cellobiose meox2 TMS	491	3.6377	8.6332	-2.318	0.0035826	Low	High
Maltose MEOX1 TMS	487	3.5162	8.0471	-1.8872	0.0044119	Low	High
x	311	x	x	x	x		
x	282	x	x	x	x		
N-Acetyl-D-glucosamine, tetrakis(trimethylsilyl) ether, methyloxime (syn)	357	3.1016	6.253	-2.1639	0.0035523	Low	High
Uridine	385	3.0802	6.1686	-1.1466	0.0011811	Low	High
x	426	x	x	x	x		
x	369	x	x	x	x		
x	273	x	x	x	x		
x	178	x	x	x	x		
L-Alanine	34	2.8853	x	x	0.00245	High	Low
x	130	x	x	x	x		

Conclusions:

- Some important features without p-value and FC.
- Sugars and alanine are up in MERS infected cells.
- Fosfate and uridine are low in MERS infected cells.

→ Changing MinPairCos to 0.5.

Features with high Component 1 value - MinPairCos 0.5

Compound	Cluster index	Component 1	-Log (p-value)	Log2(FC)	MeanDecrease Accuracy
D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime	363	4.4997	13.845	-1.8965	0.0059811
Silanol, trimethyl-, phosphate (3:1)	102	3.8254	9.5989	0.54883	0.0035547
Cellobiose meox2 TMS	491	3.6377	8.6472	-2.3514	0.0035826
Maltose MEOX1 TMS	487	3.5162	8.0393	-1.8912	0.0044119
4-hydroxylphenyllactic acid	311	3.3088	7.1437	-0.84757	0.0018511
3-(1-benzothiophen-2-yl)-3-methyl-1-isobenzofuranone	282	3.2813	6.9837	-0.70656	0.0015153
Uridine	385	3.0802	6.1793	-1.1481	0.0011811
TMS-derivative of 2-d-glucosw-methyloxime	369	2.948	5.7028	-3.3025	0.0023303
4,5-dimethyl-N-prop-2-enyl-2-trimethylsilyl-3-thiophenecarboxamide	178	2.9352	5.5786	-0.52258	0.0033426

(Now there is no Alanine...)

Features from MeanDecreaseAccuracy graph - MinPairCos 0.5

Annotation	Cluster index	Comp . 1	-Log (p-value)	Log2(F C)	MeanDecrease Accuracy	Response icMERS	Response MOCK
x	416	x	x	x	x		
<u>Cellobiose meox2 TMS</u>	<u>491</u>	<u>3.6377</u>	<u>8.6332</u>	<u>-2.318</u>	<u>0.0035826</u>	Low	High
<u>TMS-derivative of 2-d-glucosw-methyloxime</u>	<u>369</u>	<u>2.948</u>	<u>5.7028</u>	<u>-3.3025</u>	<u>0.0023303</u>	Low	High
<u>Maltose MEOX1 TMS</u>	<u>487</u>	<u>3.5162</u>	<u>8.0471</u>	<u>-1.8872</u>	<u>0.0044119</u>	Low	High
<u>Silanol, trimethyl-, phosphate (3:1)</u>	<u>102</u>	<u>3.8254</u>	<u>9.5989</u>	<u>0.54883</u>	<u>0.0035547</u>	High	Low
x	34	x	x	x	x		
<u>D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime</u>	<u>363</u>	<u>4.4997</u>	<u>13.845</u>	<u>-1.8965</u>	<u>0.0059811</u>	Low	High
<u>4-hydroxyphenyllactic acid</u>	<u>311</u>	<u>3.3088</u>	<u>7.1437</u>	<u>-0.84757</u>	<u>0.0018511</u>	Low	High
x	130	x	x	x	x		
x	357	x	x	x	x		
x	86	x	x	x	x		
Ribose-5-phosphate TMS	361	2.0846	3.0044	0.35324	0.0022977	High	Low
7-(Trimethylsilyl)-2,6-bis[(trimethylsilyl)oxy]-7H-purine	336	1.3102	1.3854	-7.0566	0.0024315	Low	High
x	426	x	x	x	x		
Myo-Inositol, 6TMS derivative	358	1.8178	2.362	-0.20714	8.5806E-4	Low	High

Features from VIP Scores graph - MinPairCos 0.5

Annotation	Cluster index	Component 1	-Log (p-value)	Log2(F C)	MeanDecreaseAccuracy	Response icMERS	Response MOCK
<u>D-Glucose</u> <u>2,3,4,5,6-pentakis-O-</u> <u>(trimethylsilyl)-</u> <u>O-methyloxime</u>	<u>363</u>	<u>4.4997</u>	<u>13.84</u> <u>5</u>	<u>-1.8965</u>	<u>0.0059811</u>	Low	High
x	416	x	x	x	x		
<u>Silanol, trimethyl-</u> <u>phosphate (3:1)</u>	<u>102</u>	<u>3.8254</u>	<u>9.598</u> <u>9</u>	<u>0.54883</u>	<u>0.0035547</u>	High	Low
<u>Cellobiose</u> meox2 <u>TMS</u>	<u>491</u>	<u>3.6377</u>	<u>8.633</u> <u>2</u>	<u>-2.318</u>	<u>0.0035826</u>	Low	High
<u>Maltose</u> MEOX1 <u>TMS</u>	<u>487</u>	<u>3.5162</u>	<u>8.047</u> <u>1</u>	<u>-1.8872</u>	<u>0.0044119</u>	Low	High
<u>4-hydroxyphenyllactic</u> <u>acid</u>	<u>311</u>	<u>3.3088</u>	<u>7.143</u> <u>7</u>	<u>-0.8475</u> <u>7</u>	<u>0.0018511</u>	Low	High
<u>3-(1-benzothiophen-</u> <u>2-yl)-3-methyl-1-isob</u> <u>enzofuranone</u>	<u>282</u>	<u>3.2813</u>	<u>6.983</u> <u>7</u>	<u>-0.7065</u> <u>6</u>	<u>0.0015153</u>	Low	High
x	357	x	x	x	x		
<u>Uridine</u>	<u>385</u>	<u>3.0802</u>	<u>6.168</u> <u>6</u>	<u>-1.1466</u>	<u>0.0011811</u>	Low	High
x	426	x	x	x	x		
<u>TMS-derivative of</u> <u>2-d-glucosw-methylo</u> <u>xime</u>	<u>369</u>	<u>2.948</u>	<u>5.702</u> <u>8</u>	<u>-3.3025</u>	<u>0.0023303</u>	Low	High
x	273	x	x	x	x		
<u>4,5-dimethyl-N-prop-</u> <u>2-enyl-2-trimethylsilyl</u> <u>-3-thiophenecarboxa</u> <u>mide</u>	<u>178</u>	<u>2.9352</u>	<u>5.578</u> <u>6</u>	<u>-0.5225</u> <u>8</u>	<u>0.0033426</u>	Low	High
x	34	x	x	x	x		
x	130	x	x	x	x		

Conclusions:

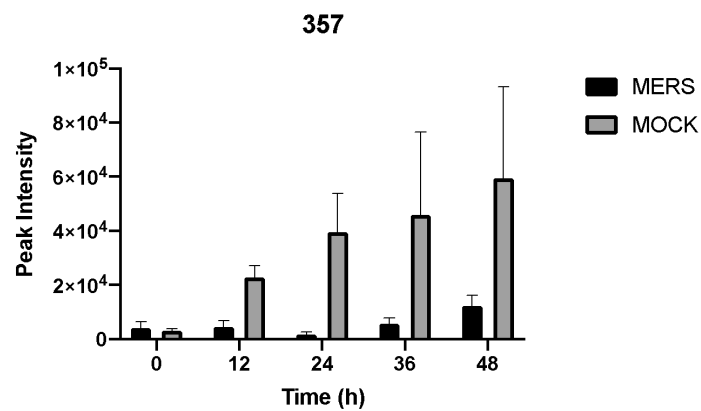
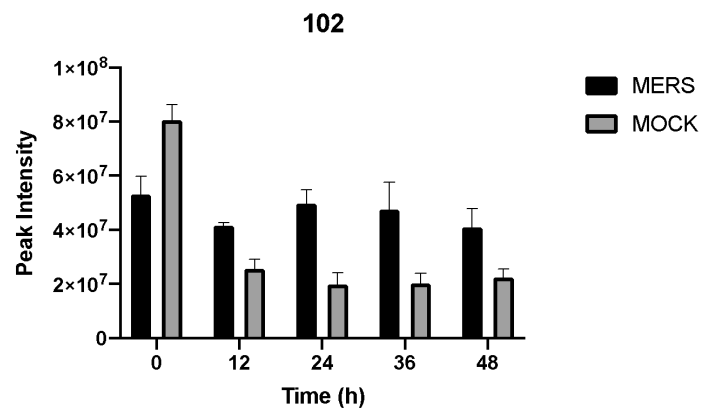
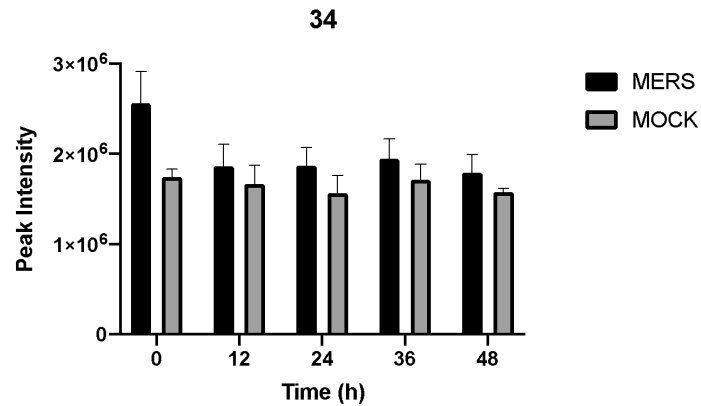
- All relevant features present p-value and FC.
- Now we can see more annotated features from the RandomForest graphs.
- Alanine is not present in Cytoscape anymore.
- Basically, we can get to the same general statements as before:
 - Sugars are up in MERS infected cells.
 - Fosfate and uridine are low in MERS infected cells

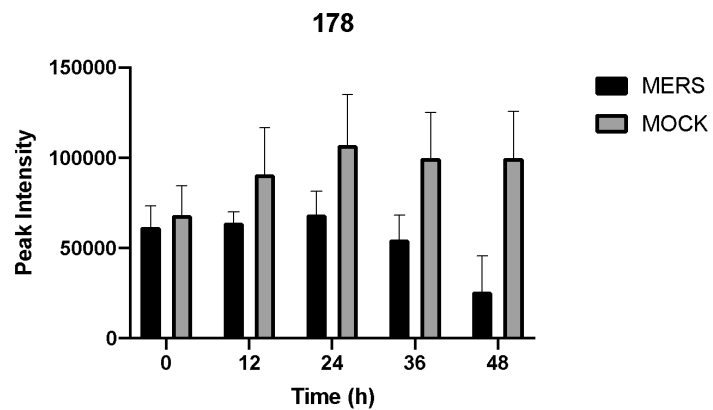
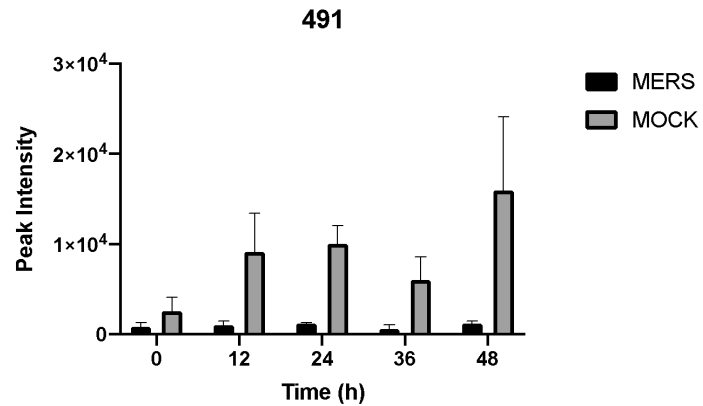
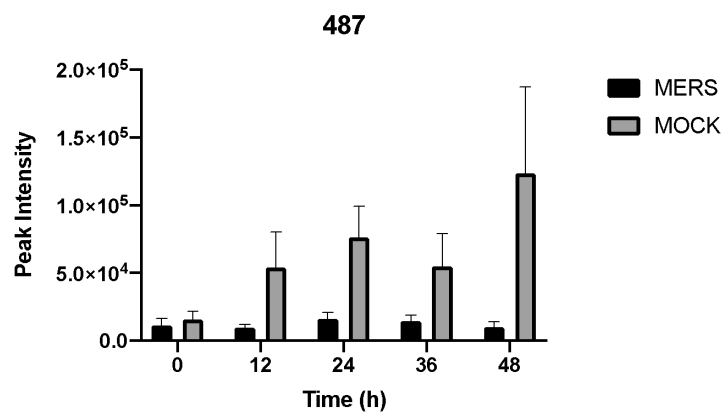
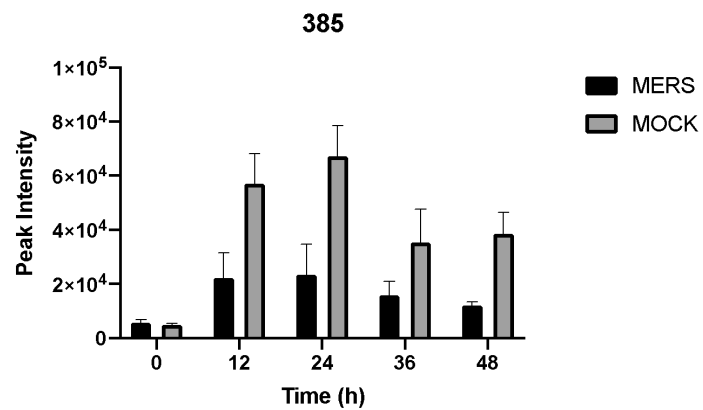
Check annotations...

Compound	Cluster index	Balance Score	Cosine Score
D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime	363	39.00	0.74
Silanol, trimethyl-, phosphate (3:1)	102	100.00	0.95
Cellobiose meox2 TMS	491	25.00	0.81
Maltose MEOX1 TMS	487	100.00	0.96
4-hydroxyphenyllactic acid	311	30.00	0.66
3-(1-benzothiophen-2-yl)- 3-methyl-1-isobenzofuran one	282	77.00	0.87
Uridine	385	79.00	0.75
TMS-derivative of 2-d-glucosw-methyloxime	369	22.00	0.77
4,5-dimethyl-N-prop-2-en yl-2-trimethylsilyl-3-thioph enecarboxamide	178	60.00	0.65

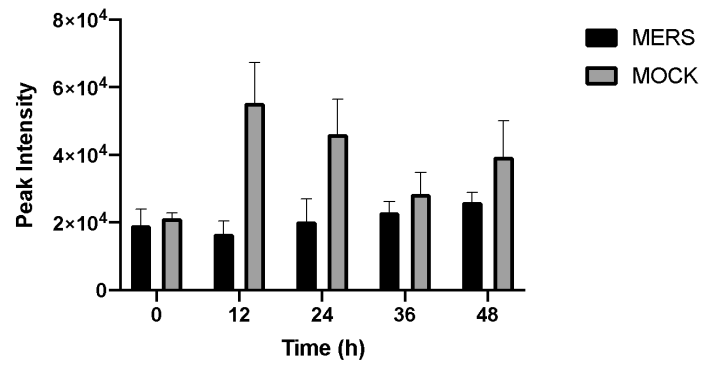
GC-MS data preliminary results

Features selected by p-value between MERS and MOCK across different time points of infection. Graphics show the average with SEM of three independent experiments, with at least three replicates each.

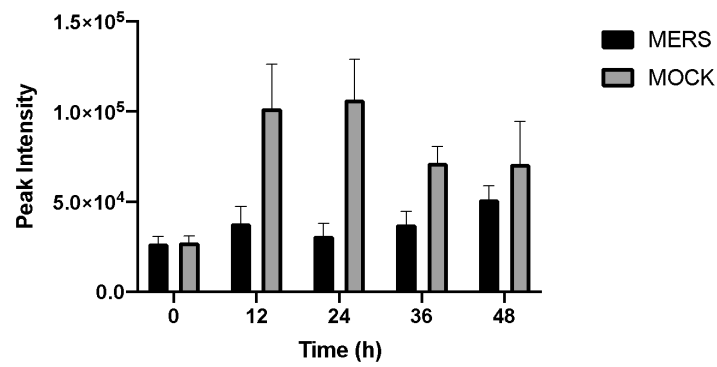




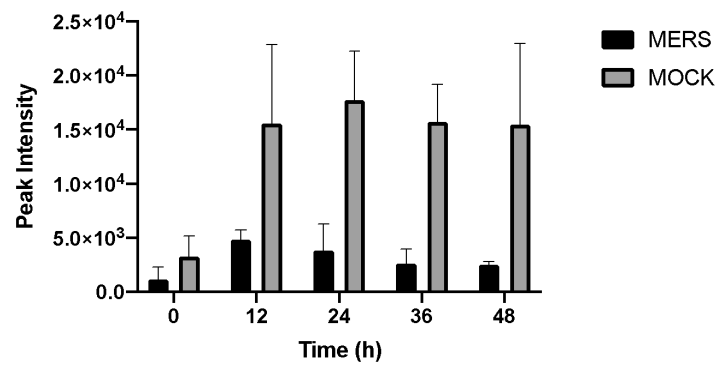
282



311



363



369

