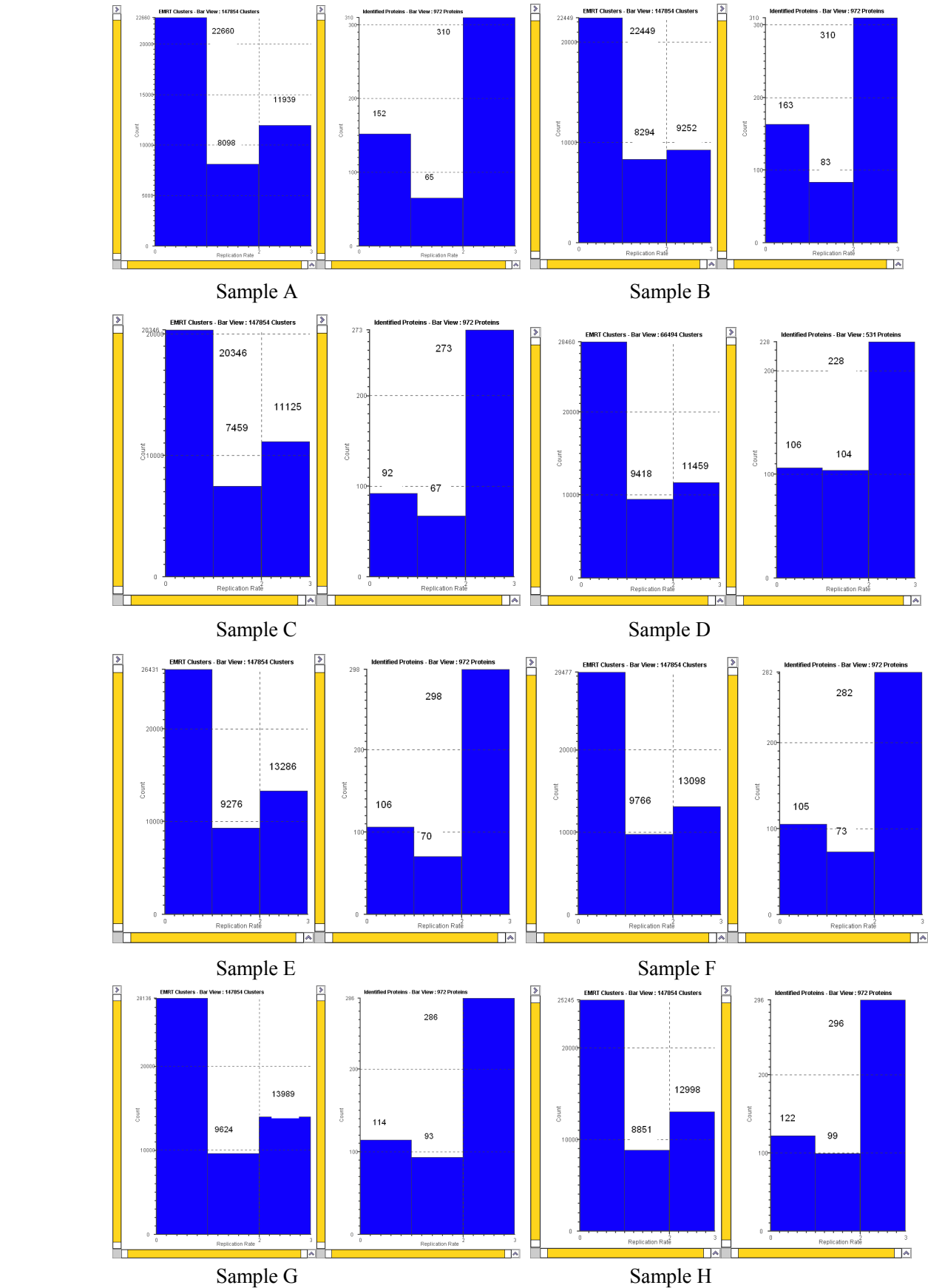


Supplemental materials

Supplemental Fig. 1: Replication rate

This assessment is to see how many ions there are in common between analytical replicates. Below are the replication rate plots extracted from PLGS for the three samples. An ion with a replication rate of 1 appears in only one of the three replicates in its sample, similarly a rate of 3 indicates that a specified ion appears in all of the three replicates. In this figure, the left pannel indicates EMRT clusters and the right pannel indicate identified proteins.

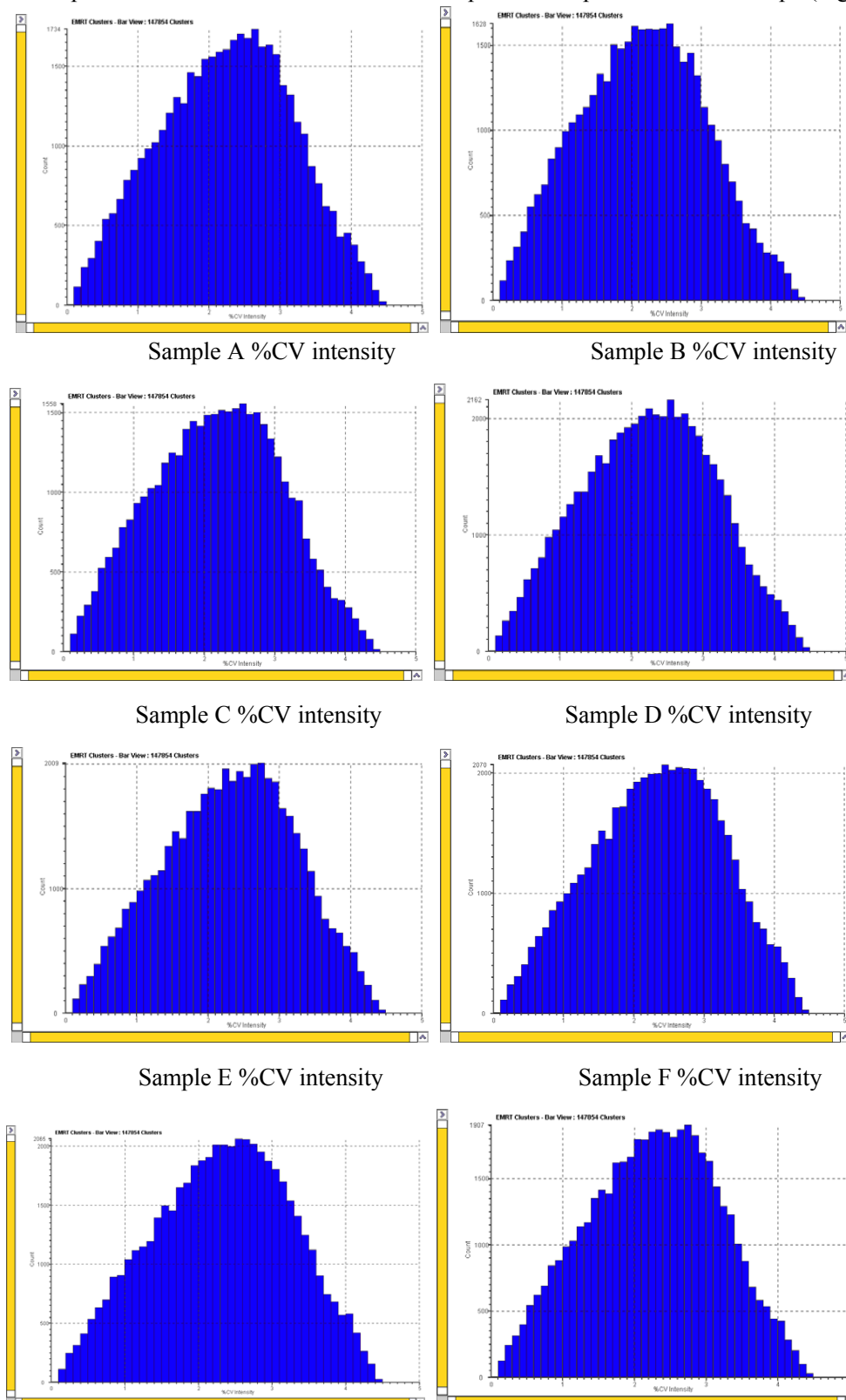


Supplemental Fig.1 A: 4day after operation, model group; B: 4day after operation, sham group; C:14day after operation, model group; D: 14th day after operation, sham group; E: 28day after operation, model group; F: 28day after operation, sham group; G: 45day after operation, model group; H: 45day after operation, sham group.

All eight samples show high rep rate 1 counts, indicating a lot of “noise” in the sample. This is normal for highly complex samples, and indicates very low abundance ions detected by the algorithm that do not replicate. Ideally this plot should be “U” shaped with most of the ions being in rep rate 1 or 3. All eight samples show very good plots. Especially, the identified proteins in replicates 2 and 3 account for about 70%, which are very good replication rates.

Supplemental Fig.2: Intensity reproducibility

This looks at how reproducible the ion intensities are from replicate to replicate within a sample(Fig.2).



Sample G %CV intensity

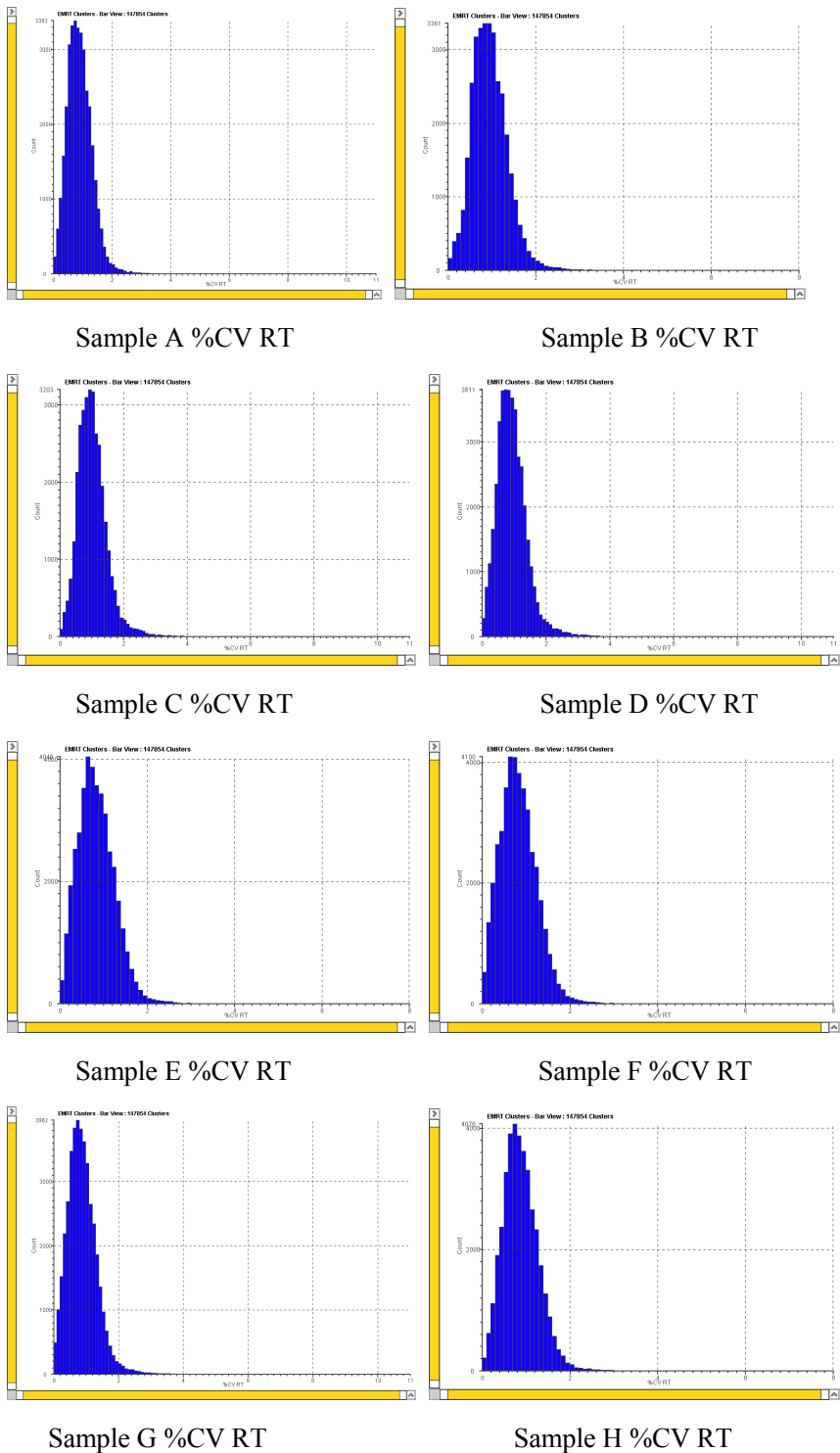
Sample H %CV intensity

Supplemental Fig.2: A: 4day after operation, model group; B: 4day after operation, sham group; C: 14 day after operation, model group; D: 14day after operation, sham group; E: 28day after operation, model group; F: 28day after operation, sham group; G: 45day after operation, model group; H: 45day after operation, sham group.

The intensity of the ions is very reproducible, typically at about 2-3% CV, well within the specification of the system. This means that observed fold changes based on ion intensities are valid.

Supplemental Fig.3:Retention time (RT) reproducibility

This is checked to ensure the nanoACQUITY is providing reproducible chromatography. This is important so ions can be paired across runs(Fig.3).

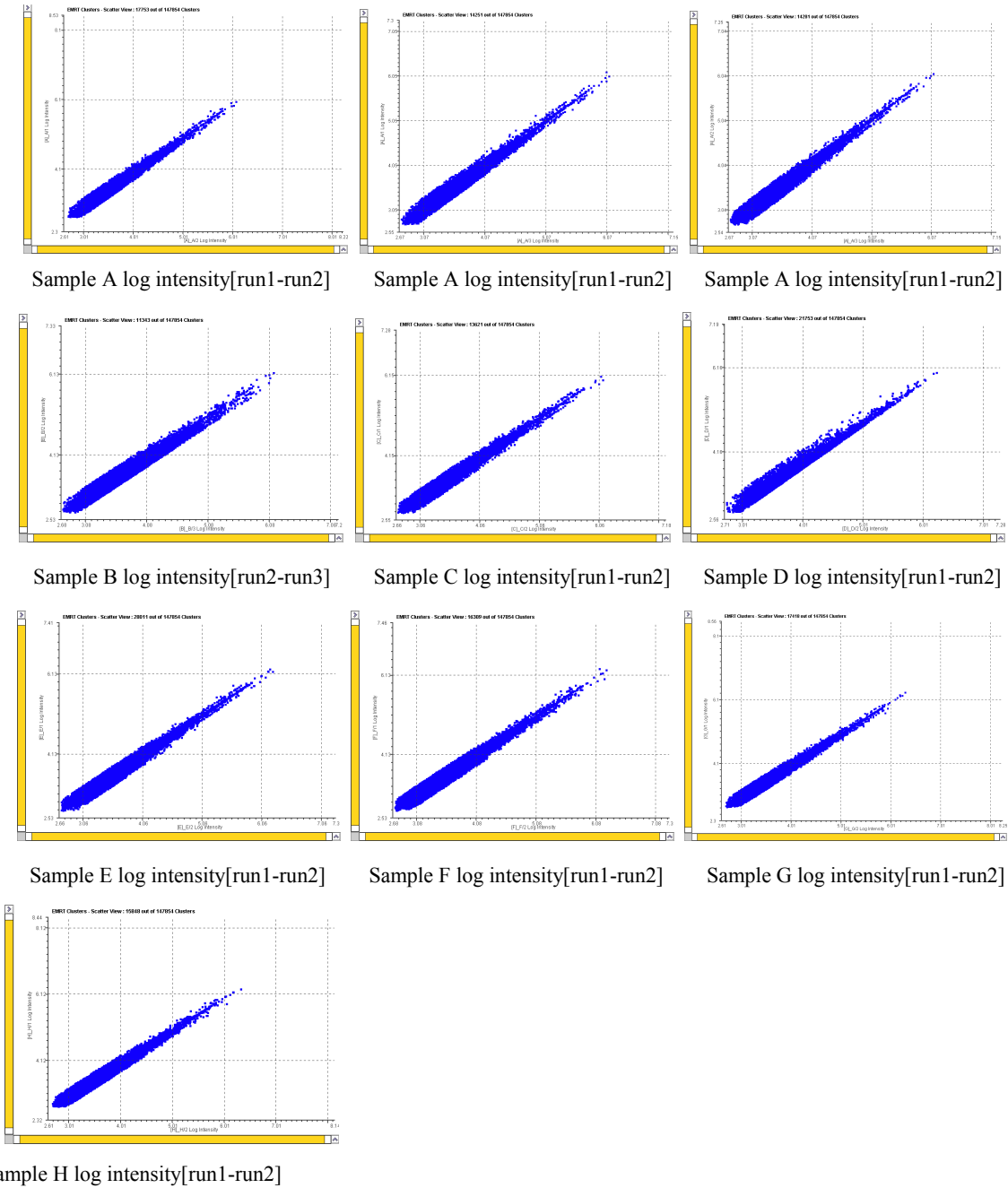


Supplemental Fig.3: A: 4day after operation, model group; B: 4day after operation, sham group; C:14day after operation, model group; D: 14day after operation, sham group; E: 28day after operation, model group; F: 28day after operation, sham group; G: 45day after operation, model group; H: 45day after operation, sham group

All samples show results typically around 0.8% CV, which is very good reproducibility.

Supplemental Fig.4:Log Log plots

These are plots where two replicates from the same sample are compared against each other. The log intensity of an ion in one replicate is plotted against the log intensity of the ion in another replicate. If the data is reproducible, a cluster of ions on the line $x=y$ should be seen(Fig.4).



Supplemental Fig.4: A: 4day after operation, model group; B: 4day after operation, sham group; C:14day after operation, model group; D: 14day after operation, sham group; E: 28day after operation, model group; F: 28day after operation, sham group; G: 45day after operation, model group; H: 45day after operation, sham group.

The plots look good for all samples. There is minimal scatter in the data, and the intensities appear to be reproducible over about 3.5 orders.