

# Supporting information for

## “Exploring ion suppression in mass spectrometry imaging of a heterogeneous tissue”

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Supporting information is provided including plots further detailing concentration dependent ion suppression in MALDI MSI, selection of cluster number, and mean spectra from all clusters for comparison of DESI and MALDI MSI.

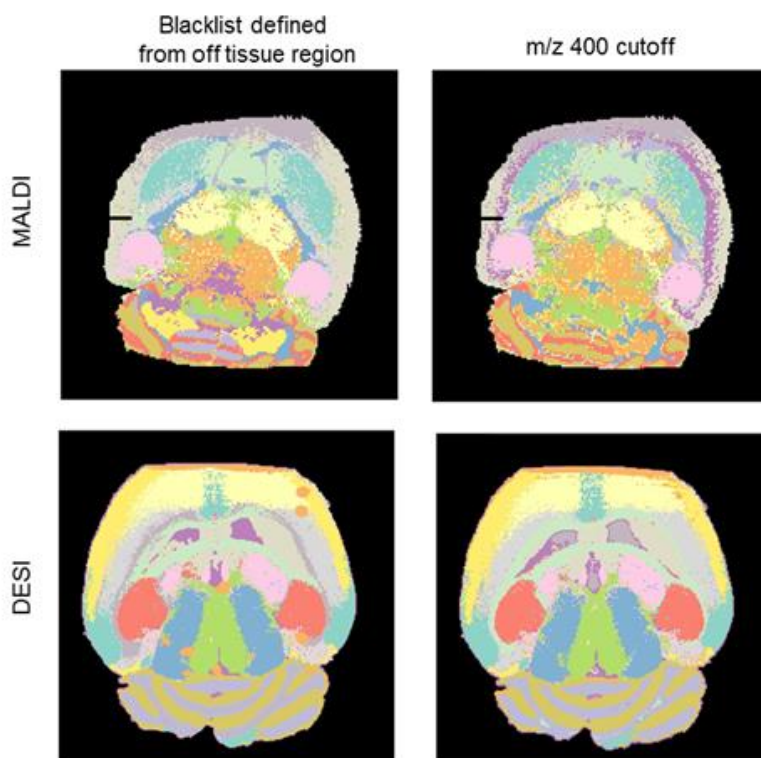


Figure S-1. Segmentation of MALDI (top) and DESI (bottom) MSI images shown in figure 2, using a blacklist of peaks from the off tissue region (left) or a  $m/z$  400 cutoff approach (right) to remove the influence of Olanzapine fragments and adducts on segmentation.

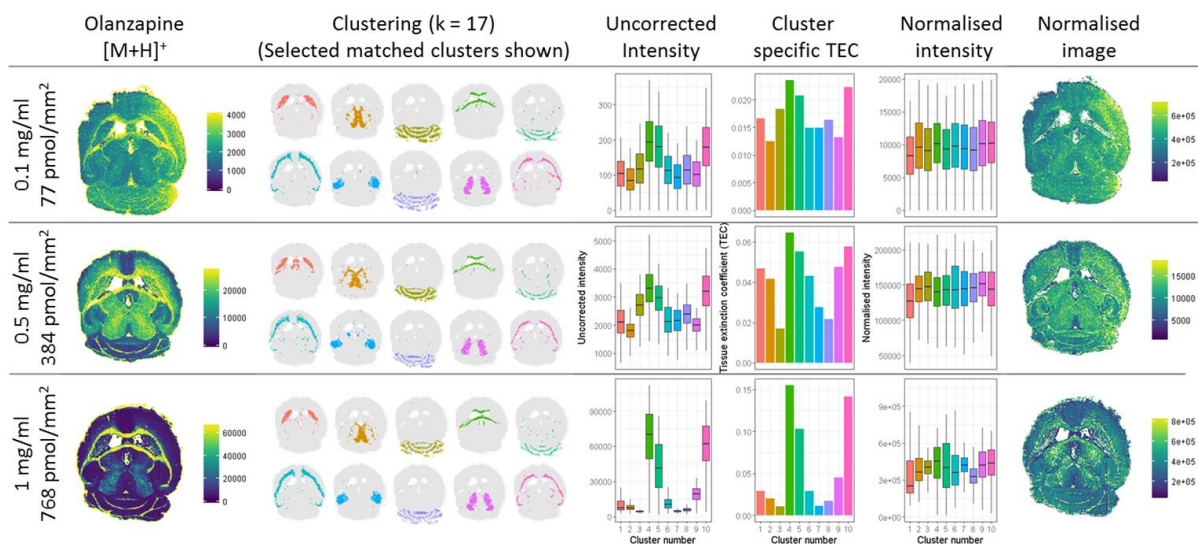


Figure S-2. Concentration dependent suppression. Olanzapine was sprayed at 0.1, 0.5 and 1 mg/l in 95:5 MeOH:Water onto serial horizontal cryosections of mouse brain before analysis by MALDI MSI using sprayed CHCA matrix. The tissue region of each image was segmented by graph cuts clustering into 17 clusters, of which 10 matching clusters were selected for all sections. TECs were calculated from each cluster and used to produce a TEC normalised image of the Olanzapine [M+H]<sup>+</sup> peak.

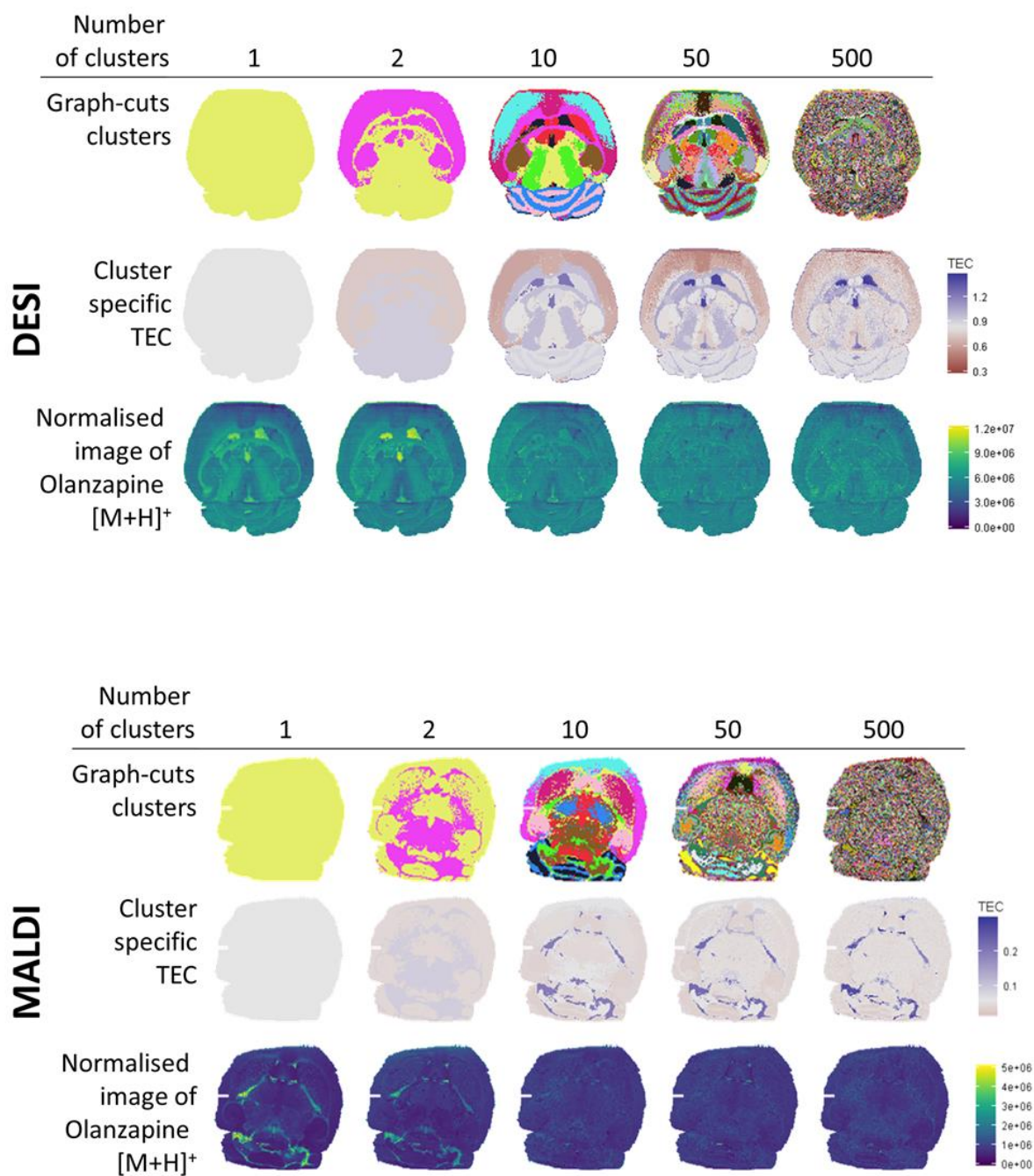
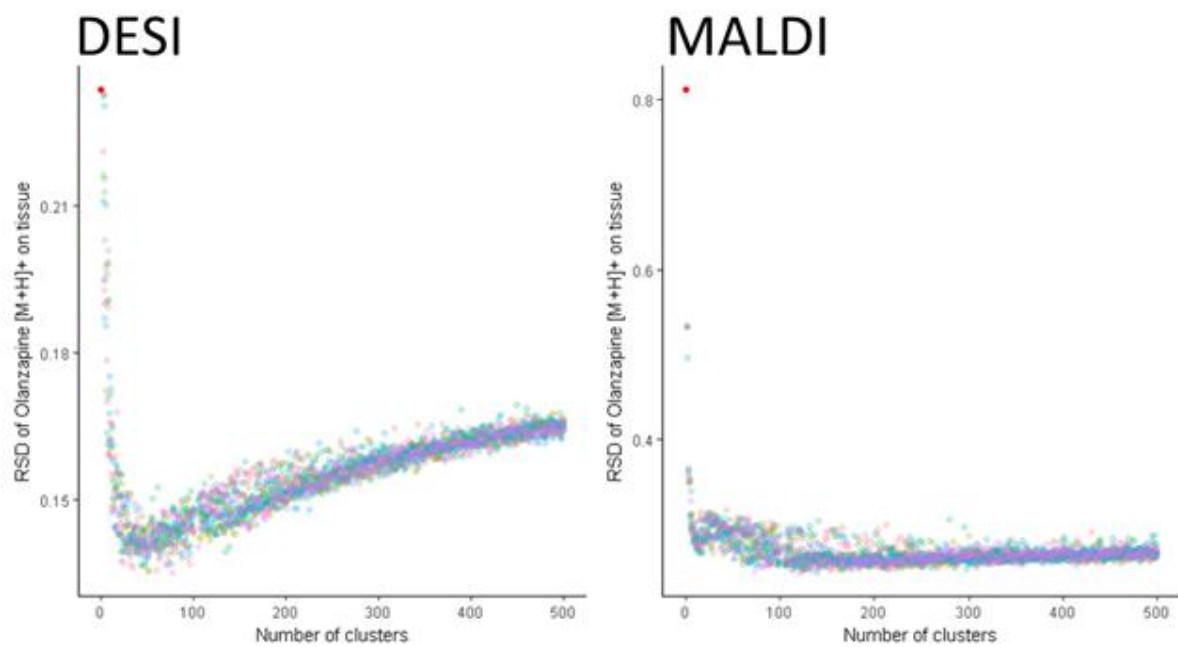


Figure S-3. Influence of cluster number on TEC based normalisation



\* Red dot represents RSD of Olanzapine intensity with  $k = 1$ . Colours represent 5 clustering replicates.

Figure S-4 Determining optimal number of clusters

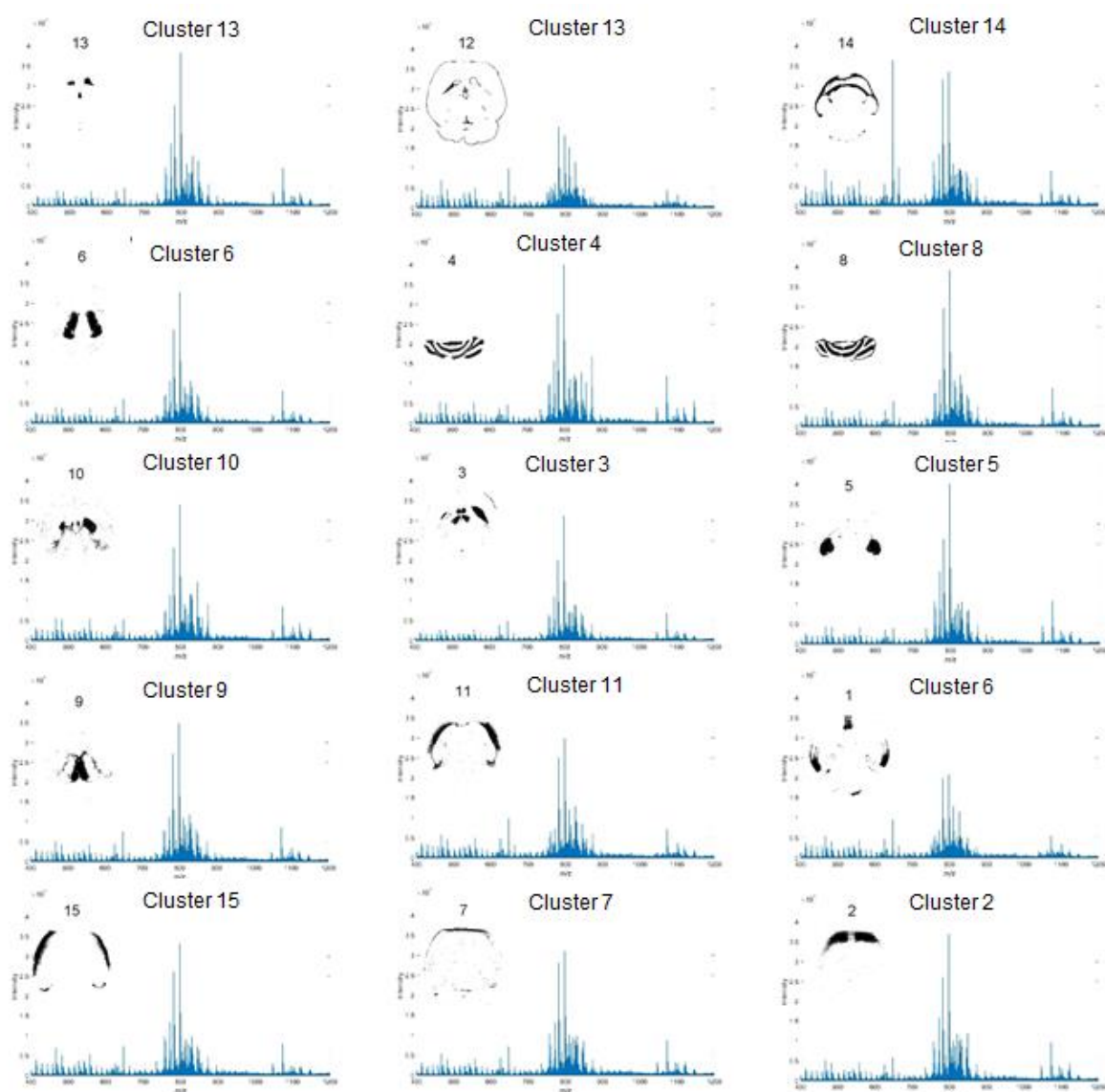


Figure S-5. All DESI clusters and mean spectra from  $m/z$  400-1200 for data shown in figure 5, ranked by TEC (left-to right, top-to-bottom).

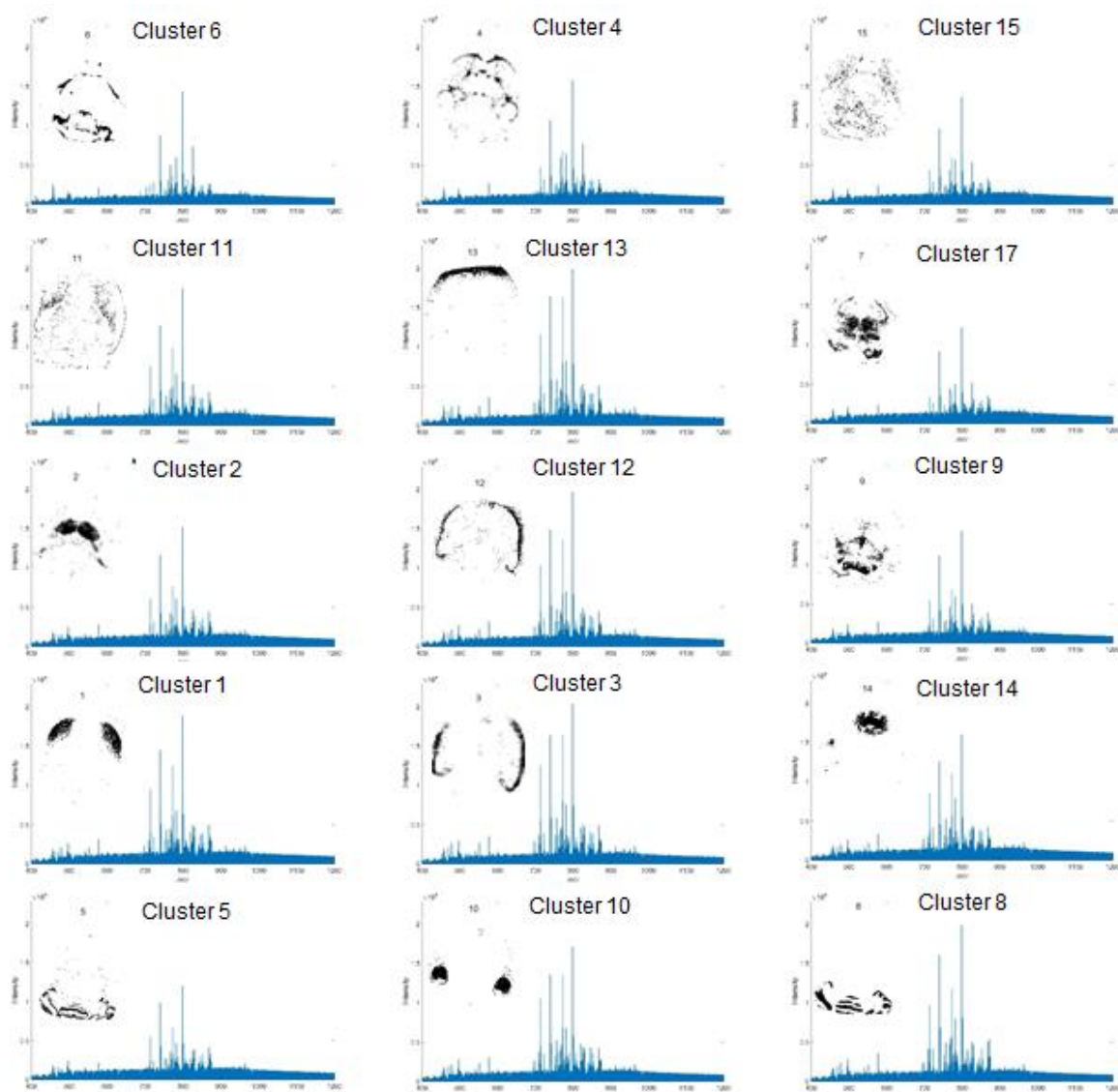


Figure S-6. All MALDI clusters and mean spectra from  $m/z$  400-1200 for data shown in figure 5, ranked by TEC (left-to right, top-to-bottom).