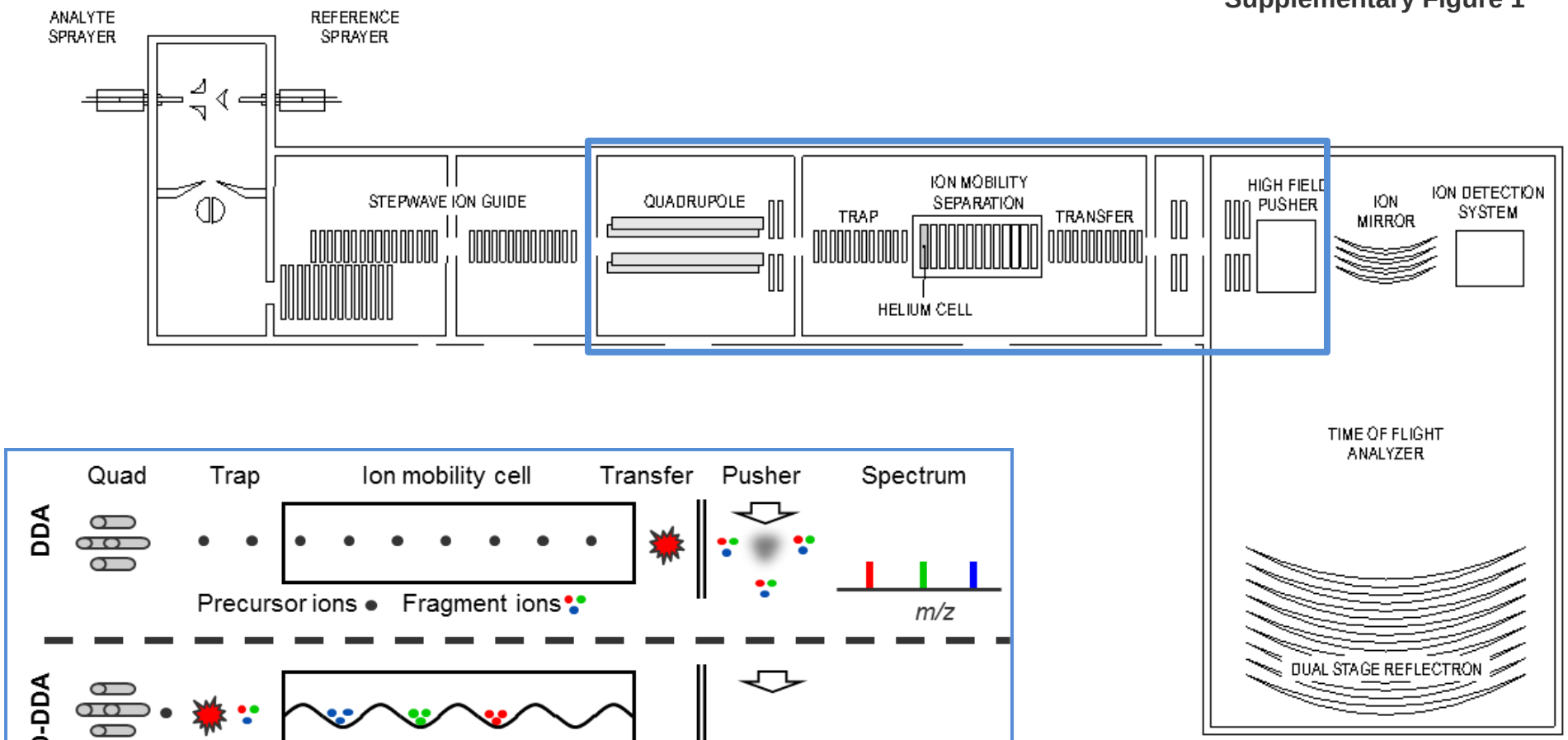
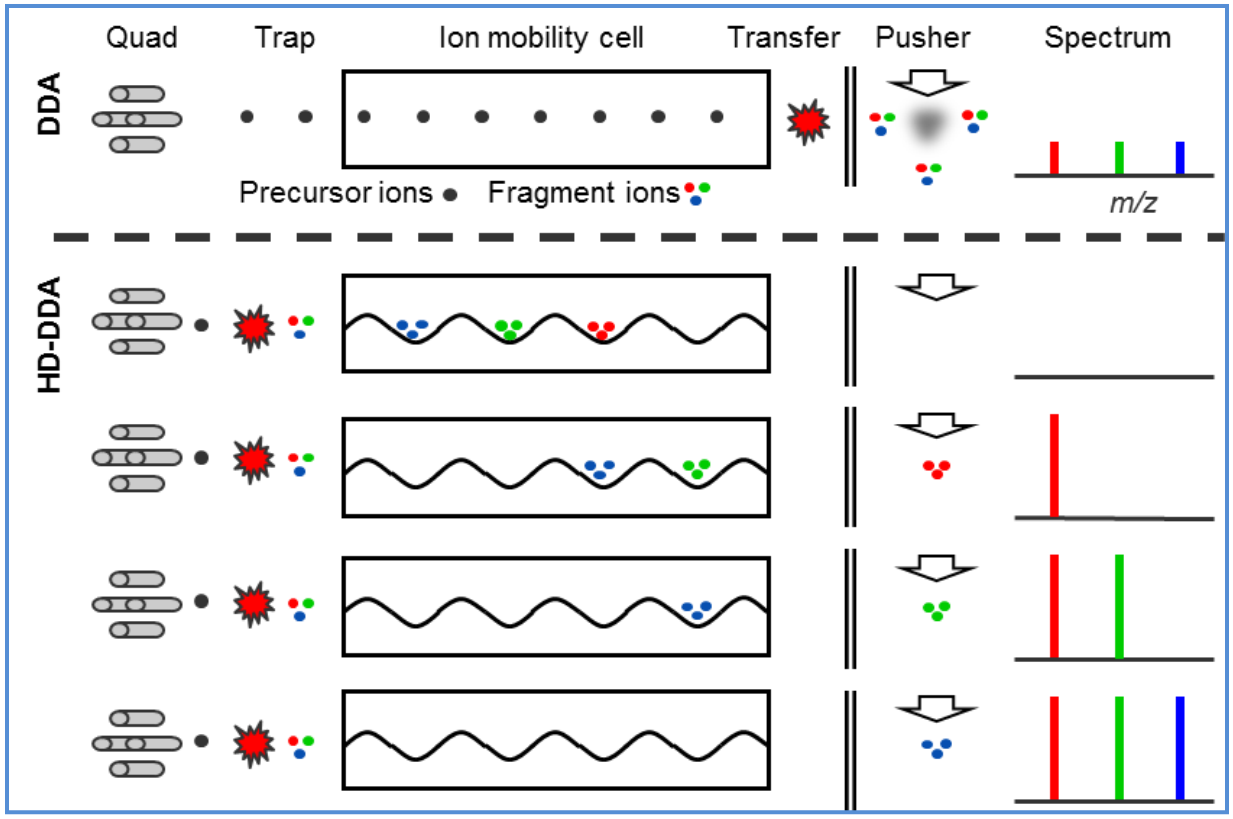
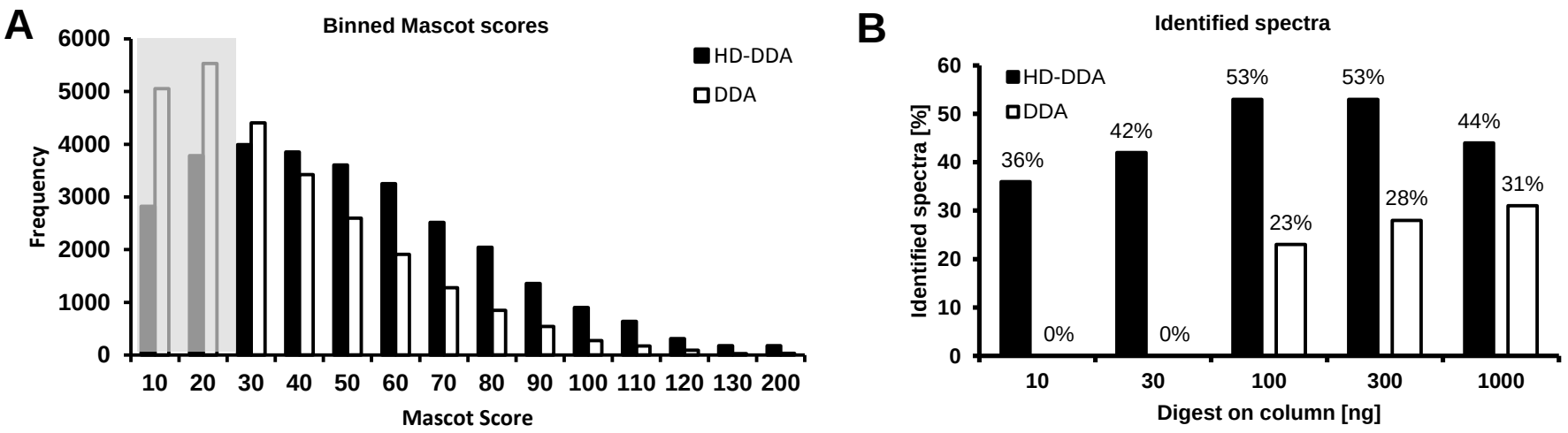
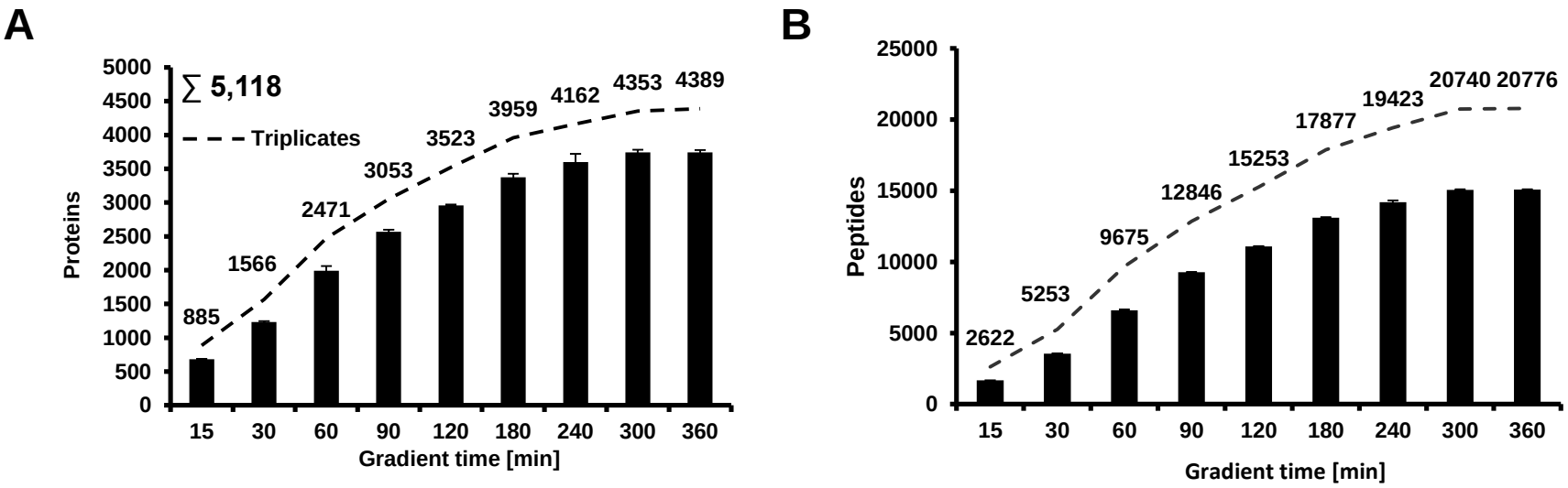


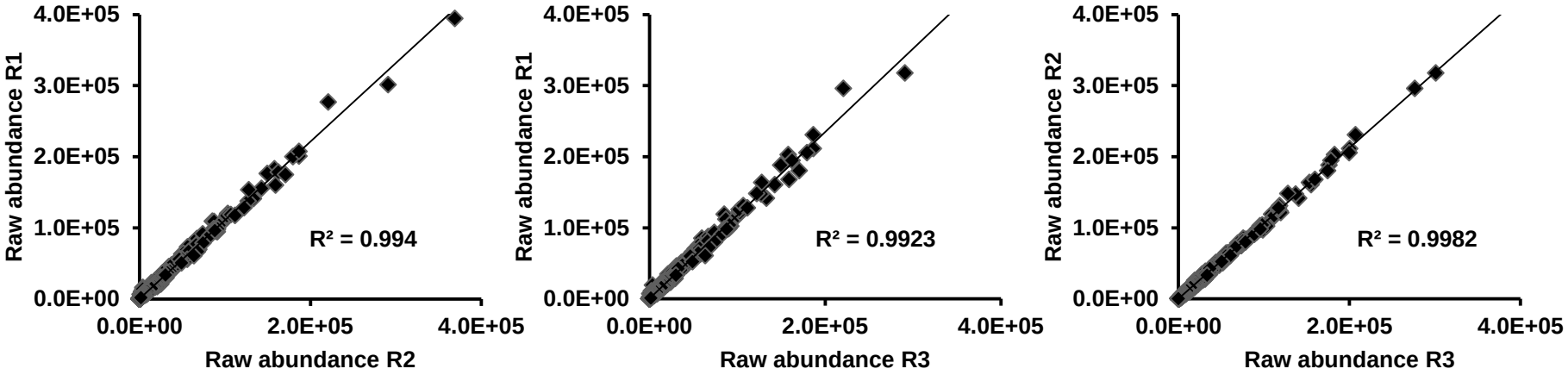

**Supplementary Fig. 1** | Top: Schematic representation of the qTOF instrument architecture used in this study. Highlighted in blue is the region between the quadrupole and the pusher including the travelling wave ion mobility device. Left: schematic representation of tandem mass spectrometry using classical DDA or the new HD-DDA method.



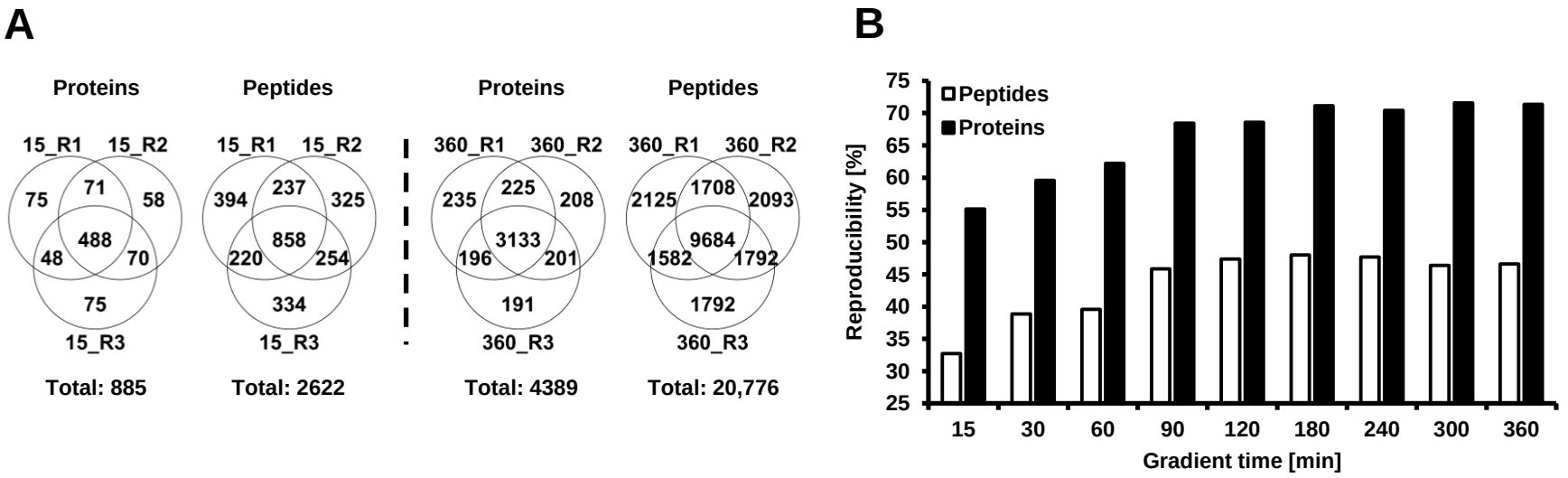

**Supplementary Fig. 2** | Comparison of DDA and HD-DDA at the level of peptide identification score (here Mascot ion score) and the productivity of tandem MS measured by the fraction of MS/MS spectra leading to a successful identification. (**A**) Binned Mascot score distribution obtained from the measurement of 1.000 ng of HeLa digest on column on a 60-min LC gradient for DDA (white bars) and HD-DDA (black bars). Only "rank 1" peptide spectrum matches (PSMs) were used in this plot. The grey zone indicates PSMs below the Mascot identity threshold. (**B**) Comparison of the fraction of successfully identified MS/MS spectra from DDA (white bars) and HD-DDA (black bars) measurements across a dilution series of a HeLa digest from 10 – 1.000 ng digest on column and analyzed by a 60-min LC gradient.



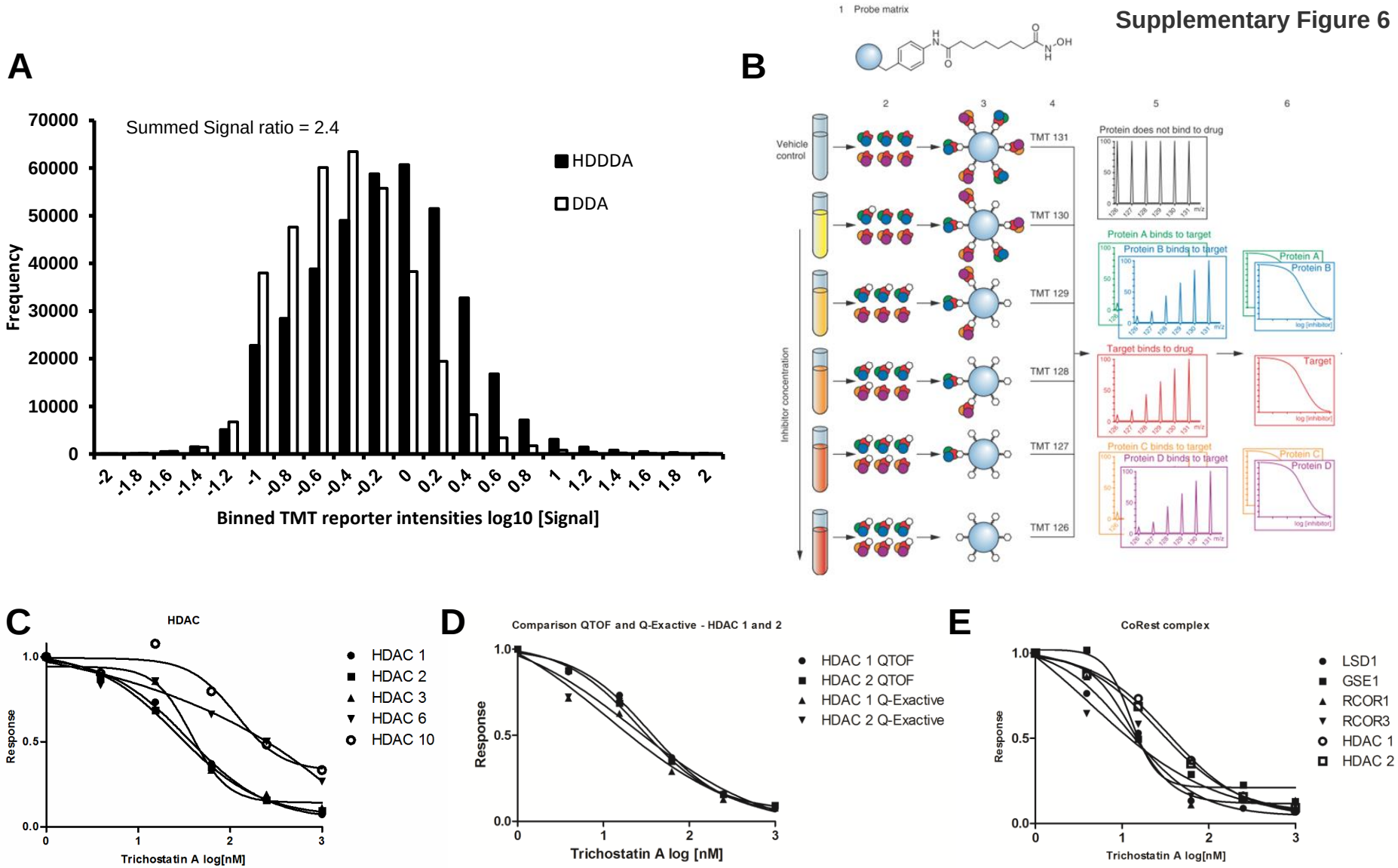

**Supplementary Fig. 3** | Analysis of triplicate measurements of 1.000 ng HeLa digest using different LC gradient times. (**A**) Number of identified proteins as a function of LC gradient time. (**B**) Same as (a) but for identified peptides. The numbers above the bars represent the sum of the triplicate identifications.




**Supplementary Fig. 4** |. Reproducibility of peptide intensities (expressed as Progenesis raw abundances) between replicate analysis (denoted R1, R2, R3). The data shown represents pair-wise comparisons of three 60' LC-MS/MS experiments using 1 μg HeLa digest on column each.




**Supplementary Fig. 5** | Reproducibility analysis of triplicate measurements of 1.000 ng HeLa digest using different LC gradient times . (**A**) Analysis of the reproducibility/recall of protein and peptide identification for 15' and 360' LC gradients. (**B**) Same as in (A) but for all gradient times. It is apparent that the recall rate increases with longer gradients but does not substantially improve beyond 90' gradient time.




**Supplementary Fig. 6** | Chemoproteomic characterization of the HDAC inhibitor Trichostatin A using a SAHA matrix and a TMT quantification read out. (**A**) Extracted (log10) TMT intensity from a sample analyzed by standard DDA (white bars) and HD-DDA (black bars). (**B**) Schematic overview over the biochemical workflow (**C**) Inhibition profiles of all identified HDACs in response to Trichostatin A. (**D**) Comparison of the results obtained by HD-DDA at the TUM author site vs. the results obtained by Q-Excactive analysis at the Cellzome author site. (**E**) Members of the CoRest complex show similar dose dependent reduction of bead binding elicited by Trichostatin A.