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LC-MS Orbitrap-based metabolomics using a novel hybrid zwitterionic hydrophilic interaction liquid chromatography and rigorous metabolite identification reveals doxorubicin-induced metabolic perturbations in breast cancer cells

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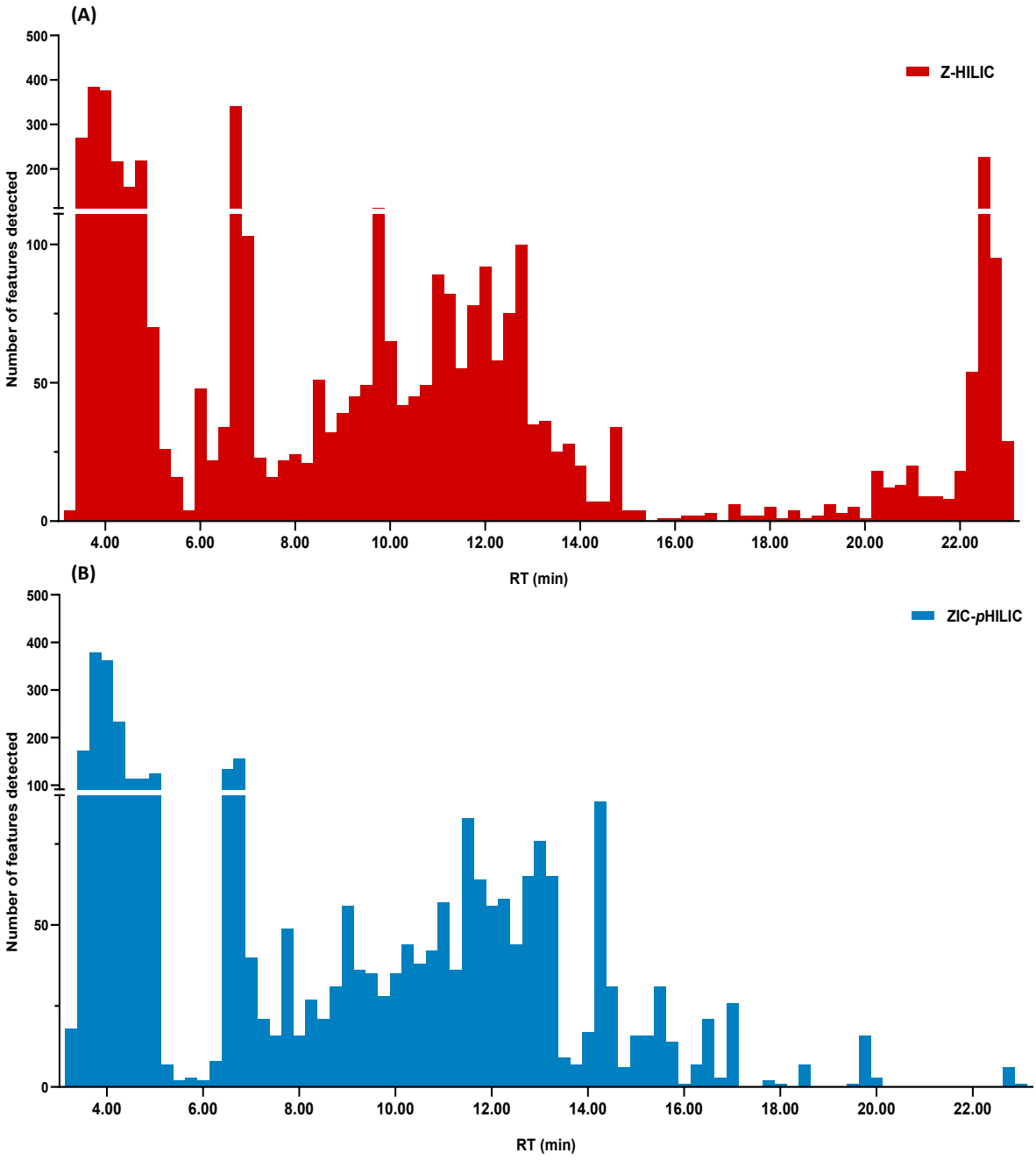

Fig. S1 Retention time distribution of the detected features in the spiked cell extracts analyzed with (A) Z-HILIC and (B) ZIC-*p*HILIC LC-MS for metabolic profiling.

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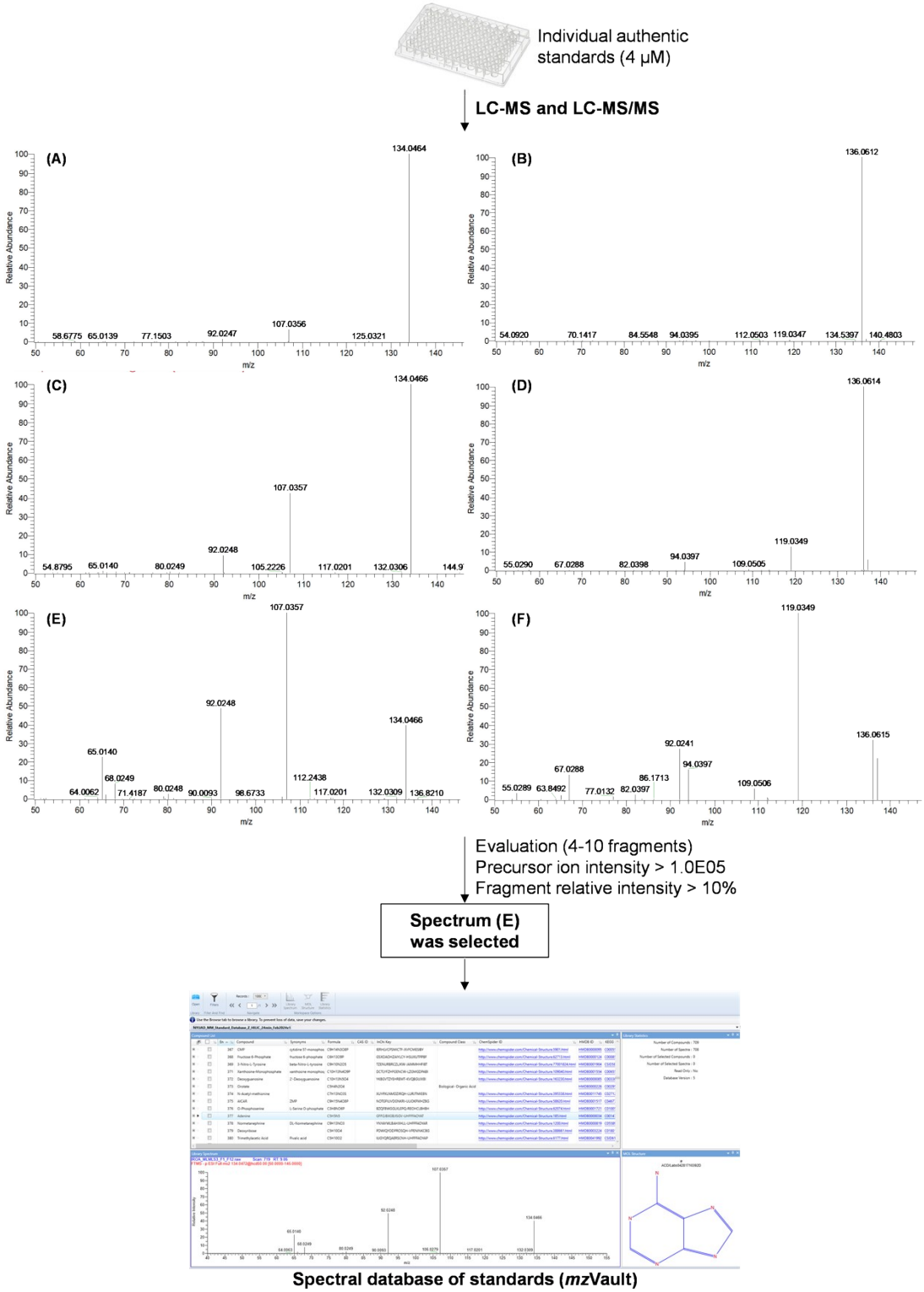

Fig. S2 Selection of MS/MS spectra for the curation of standard database. Adenine was selected as an example to illustrate the process of evaluating and selecting standard spectra for the spectral database using *mz*Valut. Spectra (A/B), (C/D) and (E/F) are MS/MS spectra of adenosine in (ESI-/ESI+) at CE 20, 30 and 50 eV, respectively.

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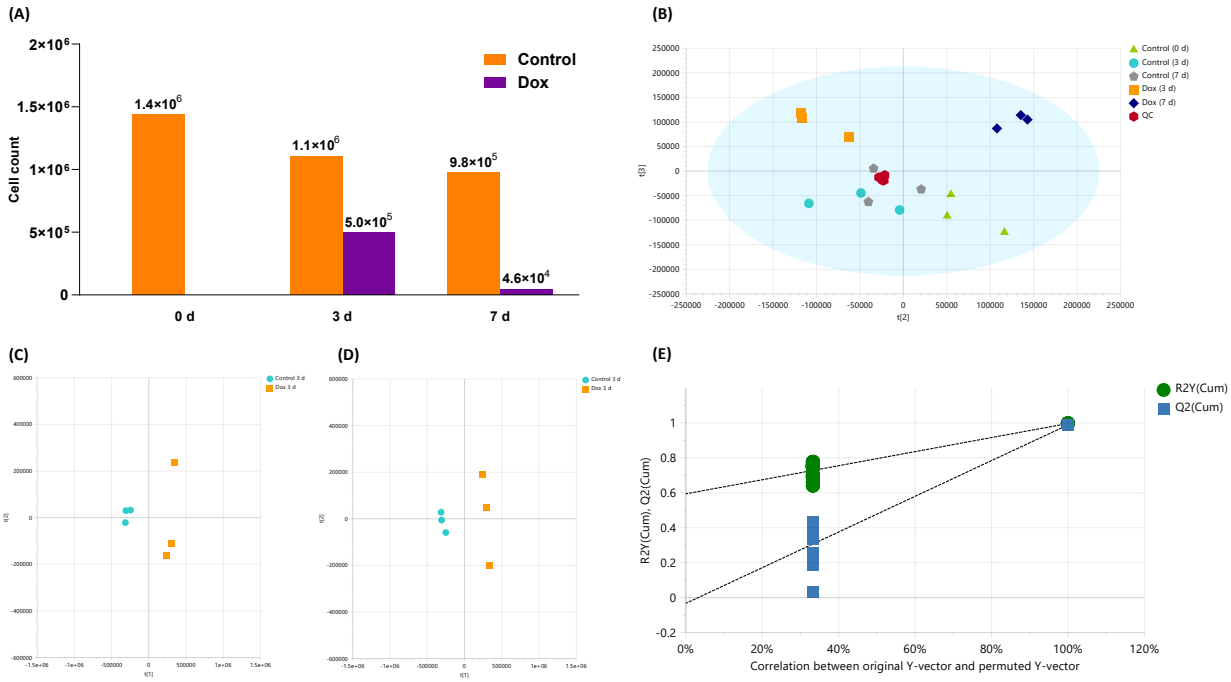

Fig. S3 Metabolic profiling of Hs578T cells exposed to Dox compared to controls analyzed with LC-MS. (A) Cell count of the samples in the study used for normalization. (B) PCA score plot of the metabolic profiles of all samples without normalization to check the analytical performance of the LC-MS. (C) and (D) are comparative PCA and PLS-DA score plots, respectively, of the metabolic profiles of Control 3 d and Dox 3 d; Cross validation values were $R^2X = 0.905$, $Q^2 = 0.764$ for PCA and $R^2X = 0.898$, $R^2Y = 0.996$, $Q^2 = 0.988$ for PLS-DA. (E) Permutation test used for the validation of the of the PLS-DA for significant features selection.