

Supporting information for

Visualization of relative 11 β -hydroxylase (CYP11B1) activity in human cortisol-producing adrenocortical adenoma (CPA) using mass spectrometry imaging-based enzyme histochemistry

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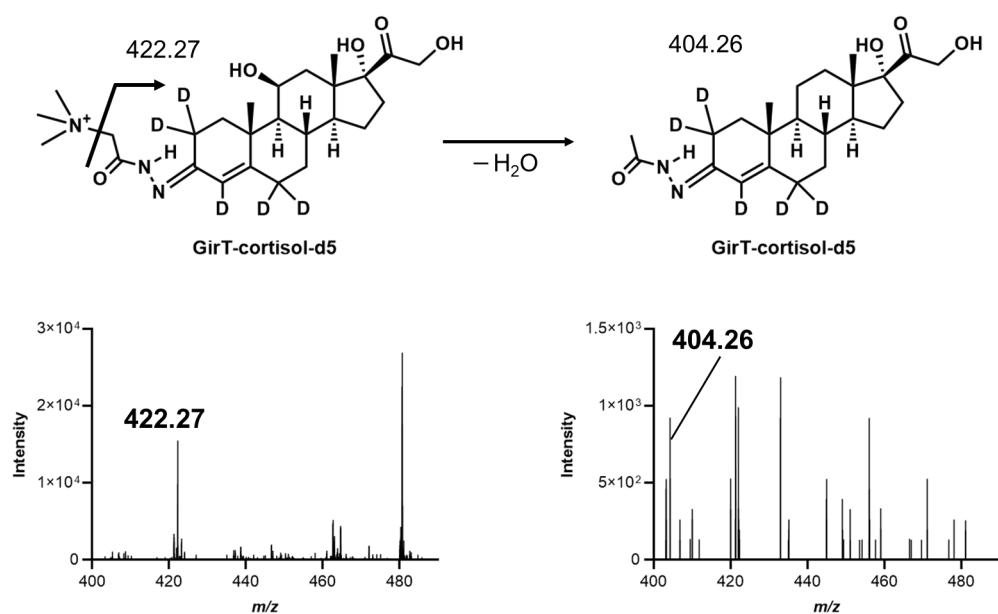
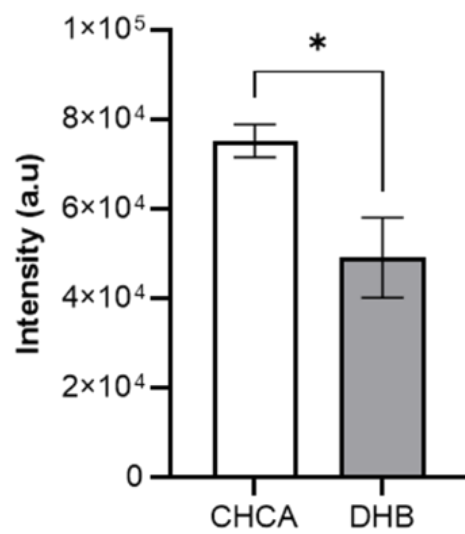


Figure S1. Product ion spectra obtained using tandem mass spectrometry (MS/MS) and third-stage fragmentation (MS³) of GirT-cortisol-d5 converted by CYP11B1 in adrenal gland sections. Structure of GirT-cortisol-d5 and its corresponding MS/MS spectrum (m/z 422.27; left). Structure of GirT-cortisol-d5 and its corresponding MS³ spectrum (m/z 404.26; right).



* $p < 0.05$

Figure S2. Comparison of detection sensitivity of cortisol-d5 (m/z 422.27) using α -cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) as the matrix. Experiments were conducted in triplicate for each group.

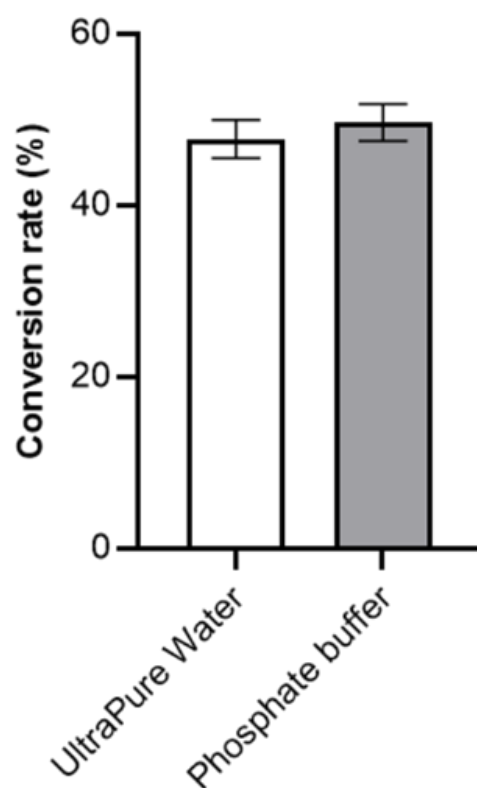


Figure S3. Comparison of detection sensitivity of cortisol-d5 (m/z 422.27) using ultrapure water and 0.05 M phosphate buffer (pH 7.0) as the solvent for the substrate solution. Experiments were conducted in triplicate for each group.

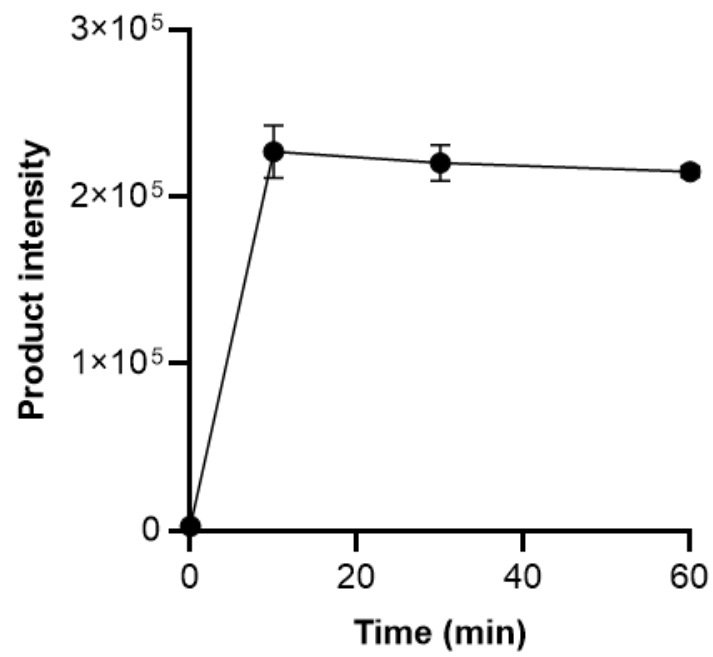


Figure S4. Comparison of substrate ionic strength (NADPH, 10 mM) at different incubation times. All experiments were repeated three times using serial sections of CPA tissues.

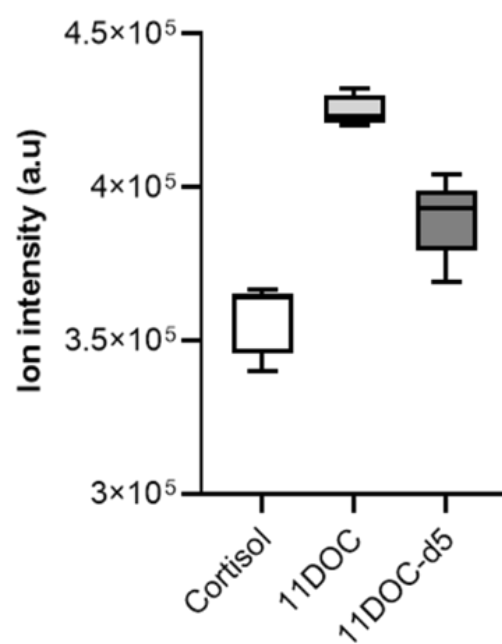


Figure S5. Ion intensities of cortisol, 11DOC, and 11DOC-d5 used to estimate the ionization efficiency ratio of cortisol-d5 to 11DOC-d5 using a three-step method. Experiments were conducted five times for each group.