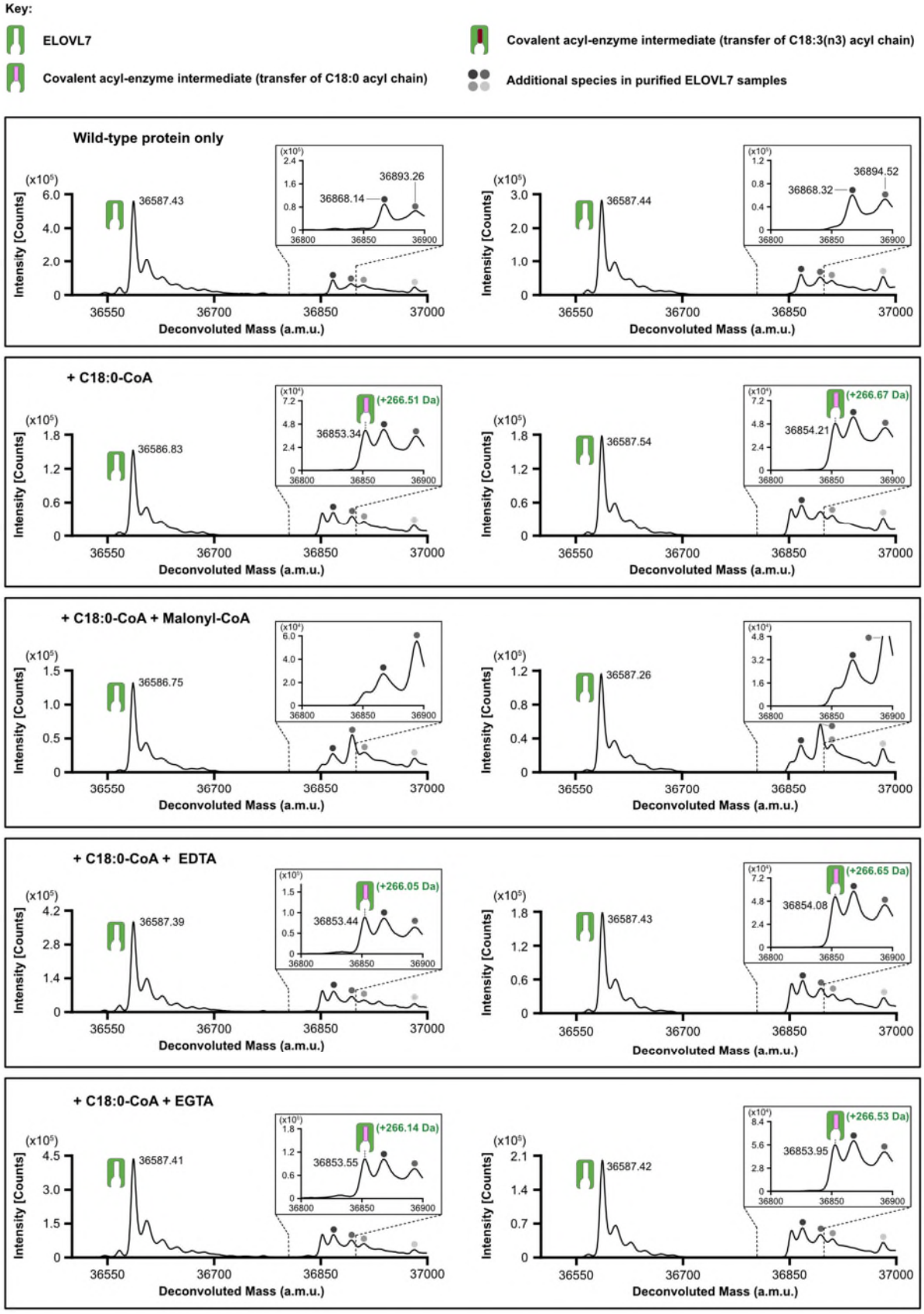
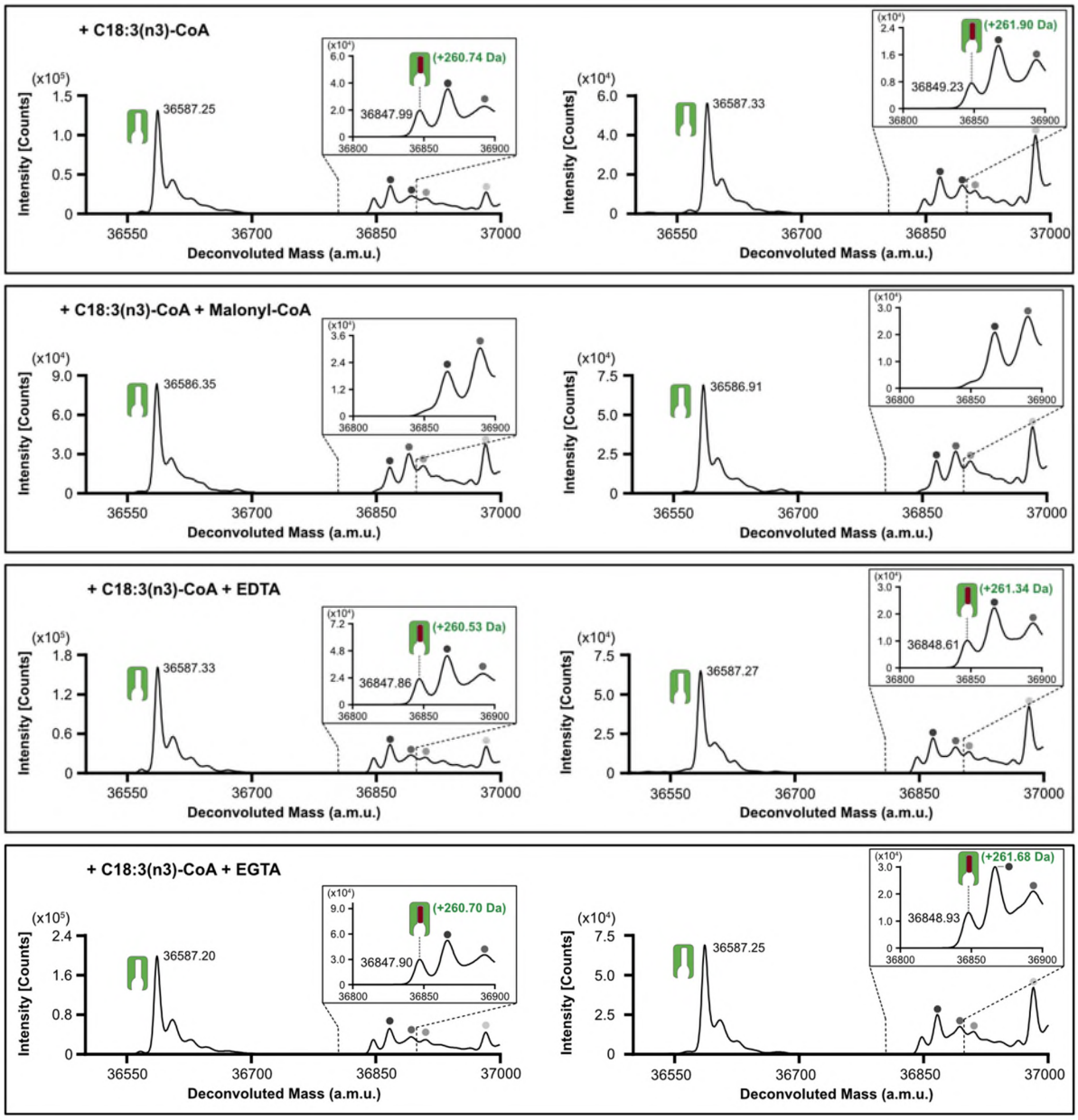
# Supplementary Information File

## The structural basis of fatty acid elongation by the ELOVL elongases

**Authors**

Laiyin Nie, Ashley C. W. Pike, Tomas C. Pascoa, Simon R. Bushell, Andrew Quigley, Gian Filippo Ruda, Amy Chu, Victoria Cole, David Speedman, Tiago Moreira, Leela Shrestha, Shubhashish M.M. Mukhopadhyay, Nicola A. Burgess-Brown, James D. Love, Paul E. Brennan, Elisabeth P. Carpenter.

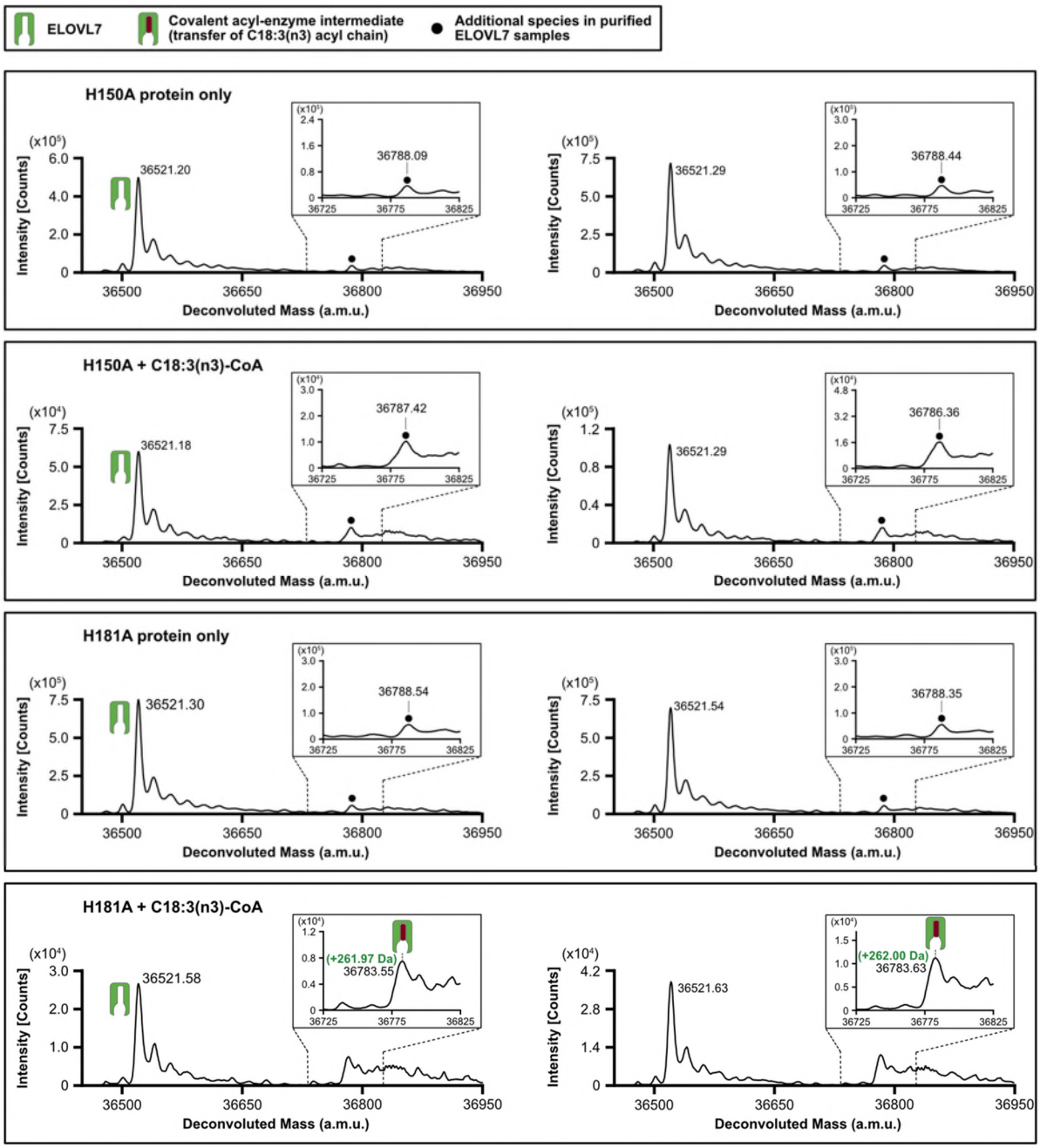

Key:
ELOVL7
Covalent acyl-enzyme intermediate (transfer of C18:0 acyl chain)
Covalent acyl-enzyme intermediate (transfer of C18:3(n3) acyl chain)
Additional species in purified ELOVL7 samples
Wild-type protein only
36587.43
36893.26
36868.14
36587.44
36894.52
36868.32
+ C18:0-CoA
36586.83
36853.34
(+266.51 Da)
36587.54
36854.21
(+266.67 Da)
+ C18:0-CoA + Malonyl-CoA
36586.75
36587.26
+ C18:0-CoA + EDTA
36587.39
36853.44
(+266.05 Da)
36587.43
36854.08
(+266.65 Da)
+ C18:0-CoA + EGTA
36587.41
36853.55
(+266.14 Da)
36587.42
36853.95
(+266.53 Da)
Intensity [Counts]
Deconvoluted Mass (a.m.u.)




**Supplementary Fig. 1 Identification of a covalent acyl-enzyme intermediate of ELOVL7**

Replicate intact mass spectrometry data for experiments presented in Extended Data Figure 6. Purified, tagged, wild-type ELOVL7 was incubated for 2h at 37°C in the presence and absence of known substrates and metal-chelating agents prior to LC-ESI-MS intact mass analysis. Deconvoluted intact mass spectra are shown side-by-side for two independent experiments. Spectra are shown for ELOVL7 incubated in the absence of substrates**,** with 100µM C18:0-CoA (expected mass addition for acyl intermediate upon reaction with C18:0-CoA: +266.47 Da), with 100µM C18:0-CoA and 100µM malonyl-CoA (no covalent acyl-enzyme

intermediate accumulation), with 100μM C18:0-CoA in the presence of either 1mM EDTA or 1mM EGTA. Identical experiments were performed with a different acyl-CoA substrate (100μM C18:3(n3)-CoA). Expected mass addition for acyl intermediate upon reaction with C18:3(n3)-CoA: +260.42 Da. The inset panel is a zoomed view of the region that encompasses the mass range where the acyl covalent intermediate would be present.




**Supplementary Fig. 2 Covalent acyl-enzyme intermediate is formed upon substrate reaction at His150.**

Replicate intact mass data for experiments presented in Extended Data Figure 7. LC-ESI-MS intact mass analysis of H150A and H181A mutant proteins are shown after incubation with either no substrate or an acyl-CoA (100µM C18:3(n3) CoA) at 37ºC for 2h. Deconvoluted

intact mass spectra are shown side-by-side for two independent experiments. The inset panel is a zoomed view of the region that encompasses the mass range where the acyl covalent intermediate would be present. Expected mass shift upon reaction with C18:3(n3) CoA: +260.42 Da.