iTOL. Ultrafast bootstrap values based on 1,000 replications are indicated at branch points. The scale bar denotes 0.1 substitutions per amino acid position. HFam, homologous family; SeqID, sequence identity.

[0017] **Fig. 2** shows the results of screening 21 identified fatty acid hydratases for fatty acid-hydroxylating activities. Fig. 2(a) shows the classification of both identified and control fatty acid hydratases into three different subtypes based on the profile of hydroxy fatty acids produced from oleic acid (OA) and linoleic acid (LA) in Wilfarin OA-7075. Fig. 2(b) shows a scatter plot illustrating the total conversion of hydroxy fatty acids from oleic acid and linoleic acid by the 21 identified fatty acid hydratases and the two control fatty acid hydratases. The graph shows the mean percentage conversion ± standard deviations of two independent replicates after 48 hours of incubation at 40°C. Fig. 2(c) shows a bar graph illustrating the percentage conversion of oleic acid into 10-hydroxystearic acid (10-HSA), and linoleic acid into (12Z)-10-hydroxyoctadec-12-enoic acid (10-HOE), (9Z)-13-hydroxyoctadec-9-enoic acid (13-HOE) and 10,13-dihydroxystearic acid (10,13-dHSA) by the 21 identified fatty acid hydratases and the two control fatty acid hydratases. Bars show the mean percentage conversion ± standard deviations of two independent replicates after 24 hours of incubation at 40°C. For fatty acid abbreviations, refer to Table 2.

[0018] **Fig. 3** shows schematic illustrations of the hydroxylation reactions of unsaturated fatty acids catalysed by fatty acid hydratases. Fig. 3 (a-e) show hydroxylation of monounsaturated fatty acids. Fig. 3 (f-i) show hydroxylation of polyunsaturated fatty acids. For fatty acid abbreviations, refer to Table 2.

[0019] **Fig. 4** shows the results of characterising the regioselectivity, substrate selectivity, and product scopes of 17 identified fatty acid hydratases. The 17 identified fatty acid hydratases, representing the top performers of each subtype, were selected for characterisation with a panel of nine different unsaturated fatty acid substrates, alongside the two control fatty acid hydratases (indicated with an asterisk). The heat map shows the mean percentage conversion ± standard deviation of three independent replicates (light grey, no conversion; dark grey, 100% conversion) after 24 hours of incubation at 40°C. For fatty acid abbreviations, refer to Table 2.

[0020] **Fig. 5** shows the results of using dual-protein systems for poly-hydroxy fatty acid production. Fig. 5(a) is a schematic illustration of the dual-protein system, consisting of an identified fatty acid hydratase of the *cis*-Δ9 subtype and an identified fatty acid hydratase of the *cis*-Δ12 subtype. The protein pair was thought to work simultaneously or sequentially to convert a polyunsaturated fatty acid first into mono-hydroxy fatty acid intermediates and then into the di-hydroxy fatty acid product. Fig. 5 (b-e) are bar charts showing the efficiency of the dual-protein systems in generating di-hydroxy fatty acids from (Fig. 5b) linoleic acid (LA), (Fig. 5c) arachidonic acid (ARA), (Fig. 5d) α-linolenic acid (ALA), and (Fig. 5e) γ-linolenic acid (GLA) as compared to those produced by the top two identified fatty acid hydratases of the *cis*-Δ9/12 subtype. The bar charts show the mean percentage conversion ± standard deviation of three independent replicates after 24 hours of incubation at 40°C. The numerical values above the bars indicate the mean percentage conversion of di-hydroxy fatty acids. For fatty acid abbreviations, refer to Table 2.

[0021] **Fig. 6** shows a phylogenetic tree based on protein sequences illustrating the relationship between the 21 identified fatty acid hydratases (FAHs), the two control fatty acid hydratases, and the 55