Structural and biochemical characterization of 20β-hydroxysteroid dehydrogenase from *Bifidobacterium adolescentis* strain L2-32

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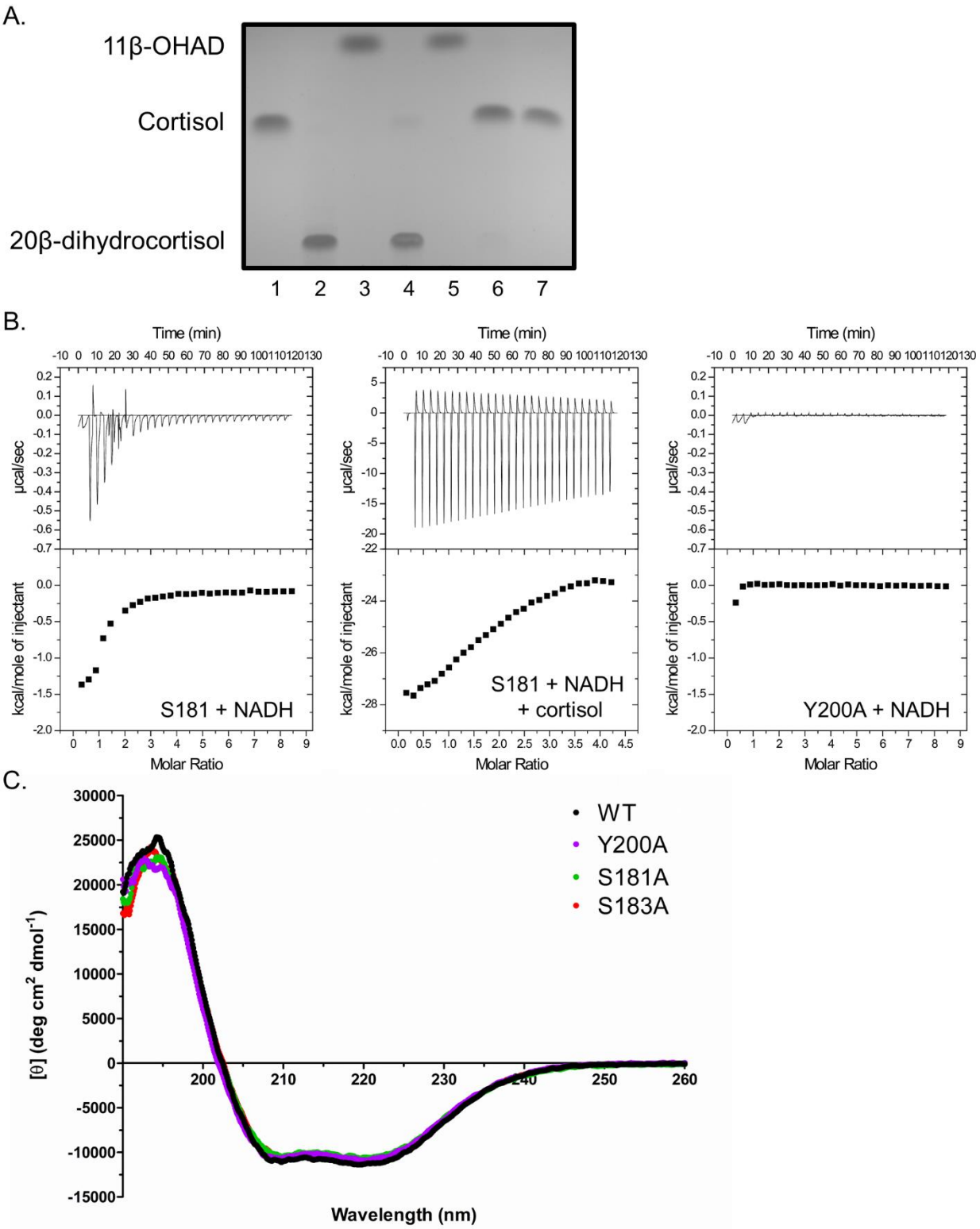

Figure S1. Active site mutant thin layer chromatography (TLC) isothermal titration calorimetry (ITC) and circular dichroism (CD). (A) Wild-type and active site mutant 20β-HSDH overnight reaction products separated by TLC. (1) Cortisol standard, (2) 20β-dihydrocortisol standard, (3) 11β-hydroxyandrostenedione standard, (4) WT + NADH + cortisol, (5) WT + NADH + 11β-OHAD, (6) S181A + NADH + cortisol, (7) Y200A + NADH + cortisol. (B) Ligand binding order of S181A and Y200A studied

by ITC. Left panel is 2 mM NADH binding to S181A, middle panel is 1 mM cortisol binding to S181A with 2 mM NADH, right panel is 2 mM NADH binding to Y200A. (C) CD spectra of purified recombinant 20β-HSDH and its active site mutants.

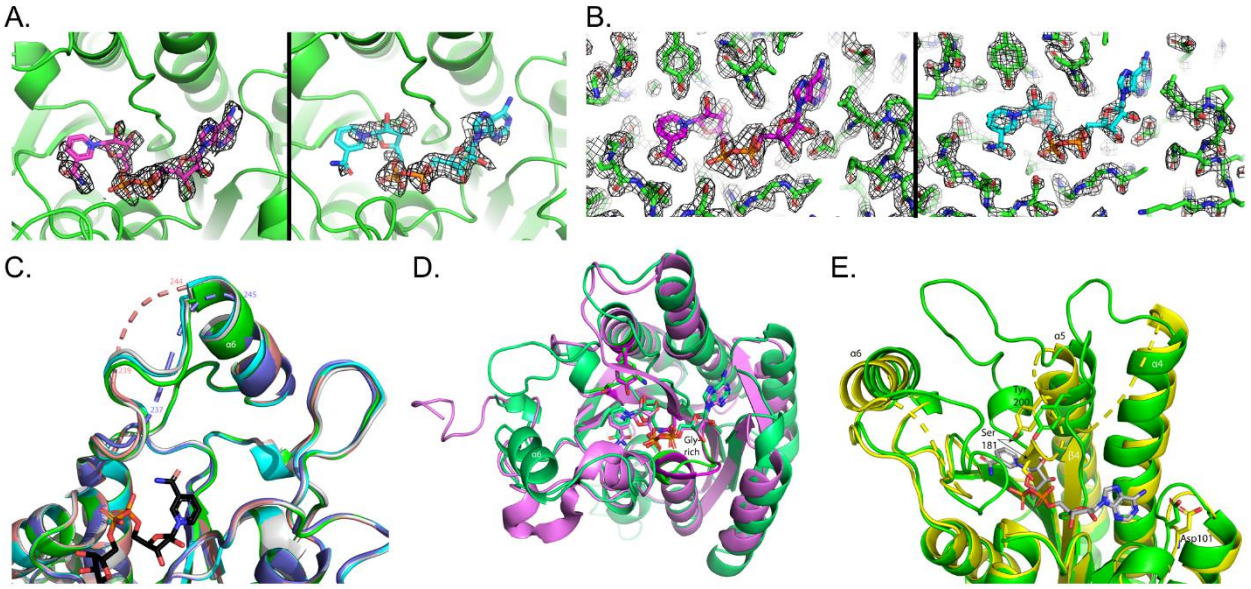

Figure S2. Structural analysis of 20β-HSDH. (A) Fo-Fc omit map of NADH from Chain A and E shown at 2 sigma. (B) 2Fo-Fc map of NADH and surrounding backbone chains from Chain A and E shown at 2 sigma. (C) Alignment of 5 of the monomers from the NADH-bound structure showing the flexibility of loop 235-245 near where cortisol is predicted to bind. The 3 monomers not shown look similar to those shown. (D) Comparison of 20β-HSDH (green) to 3α, 20β-HSDH from *Streptomyces hydrogenans (pink)* The Gly-rich region is highlighted in brighter colors near the adenine of NADH and the side chains of the residues making up the catalytic tetrad are shown. Ser181 of the catalytic tetrad has been mutated to an alanine in 20β-HSDH. (E) Superposition of apo (yellow) and S181A mutant holo structure with NADH bound (green).

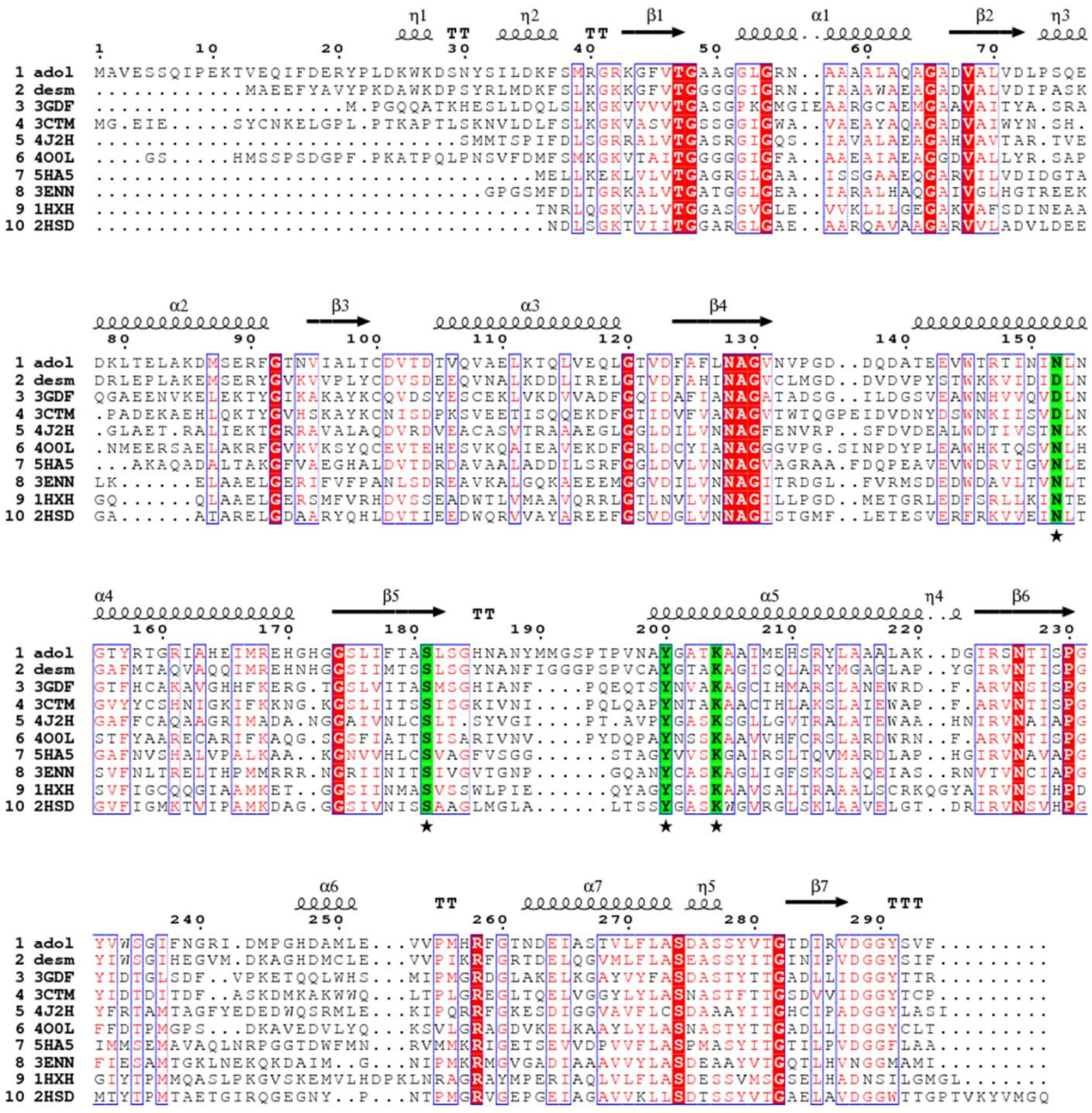

Figure S3. Sequence alignment of 20β-HSDH found in *B. adolescentis* and other structurally similar SDR members. Red highlights indicate identical residues, green highlights and stars indicate active site residues, blue boxes indicate conserved residues. Secondary structural elements of apo-20β-HSDH are displayed on the top of the alignment. The abbreviations of protein names are as follows: adol, 20β-HSDH from *Bifidobacterium adolescentis* strain L2-32; desm, 20β-HSDH from *Butyricicoccus desmolans* ATCC 43058; remaining labels correspond to PDB IDs. The sequences were aligned with Clustal Omega (1.2.4) and secondary structure rendered by ESPript 3.0 web server.

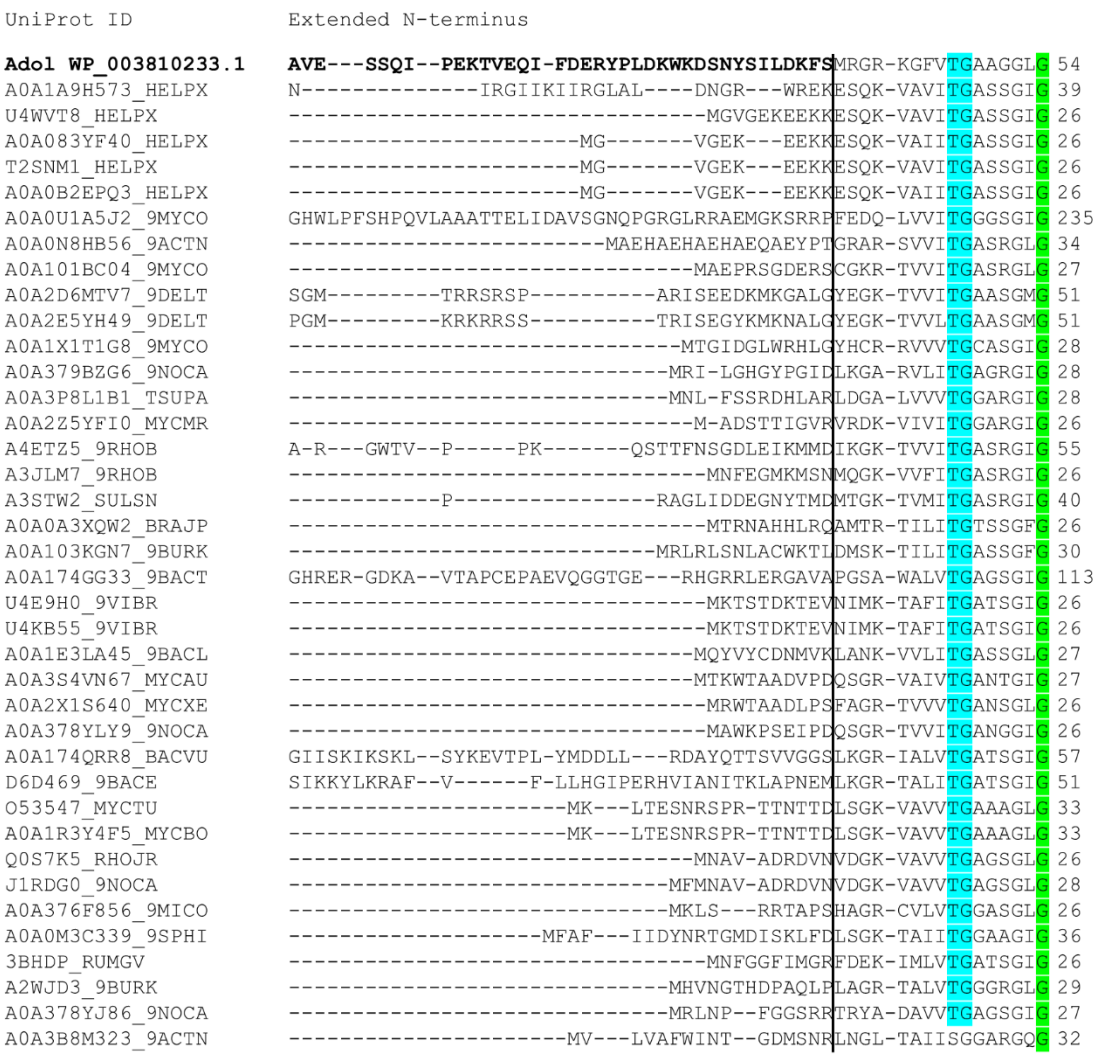

Figure S4. SDR family subset with extended N-terminus multiple sequence alignment. A Clustal Omega (1.2.4) alignment was performed with 555 sequences labeled as SDR family, hydroxysteroid dehydrogenase, and of bacterial origin from the UniProt database. This excerpt from the total sequence alignment includes 39 sequences with a ≥10 amino acid residue extended N-terminus. Conserved residues are highlighted, bolded residues indicate the *B. adolescentis* 20β-HSDH extended N-terminus, and the line indicates the beginning of the *B. adolescentis* 38-residue extended N-terminus.

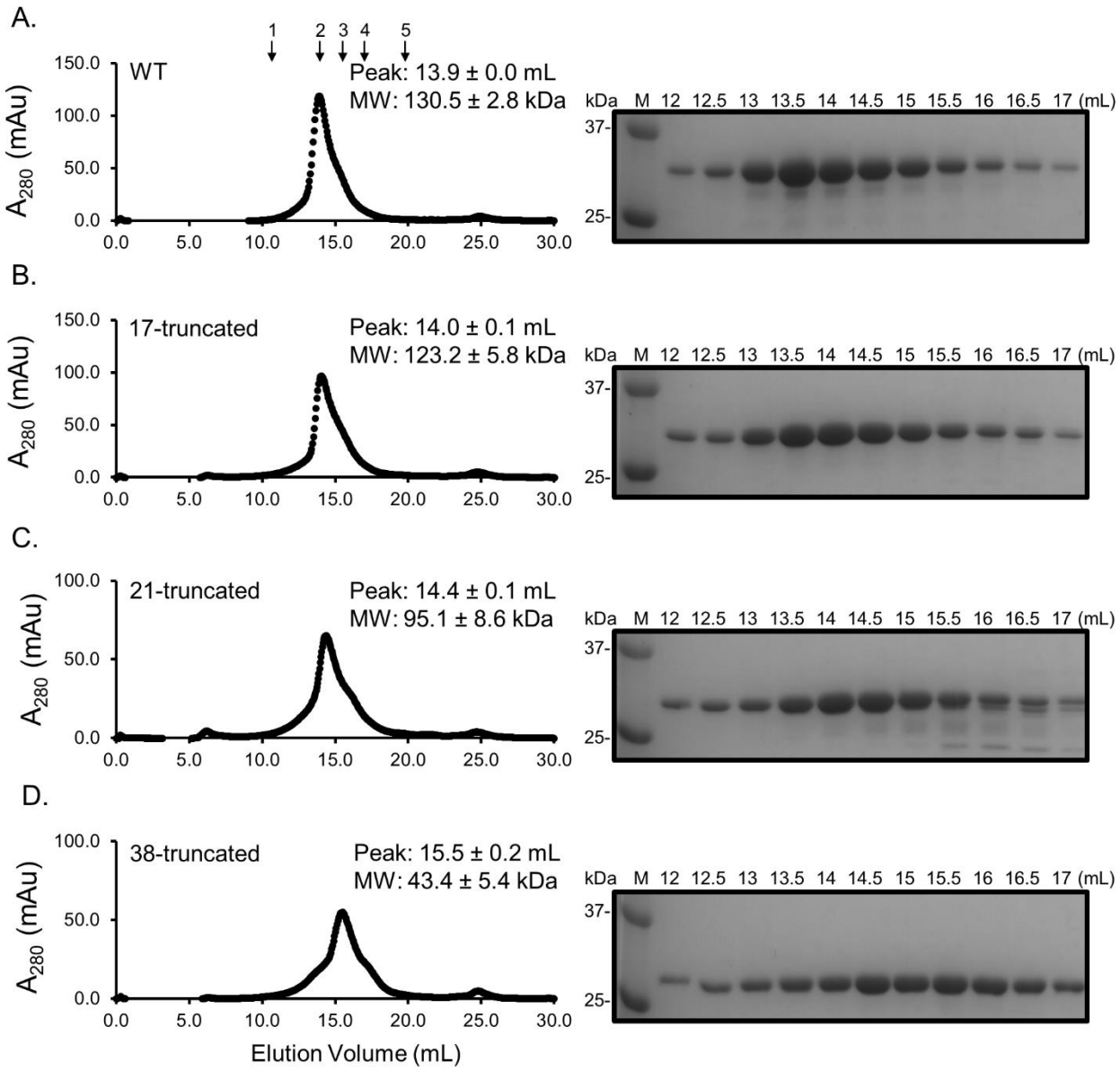

Figure S5. Gel filtration chromatography of 20β-HSDH truncation mutants. Native molecular weight estimates were based on elution time of 10 mg/ml WT and truncated 20β-HSDH from a Superose 6 10/300 GL analytical column. Fractions were collected and analyzed on SDS-PAGE for visualization. (A) WT, (B) 17-truncated, (C) 21-truncated, (D) 38-truncated. Numbers indicate elution time of standard proteins (1) thyroglobulin, (2) γ-globulin, (3) ovalbumin, (4) myoglobin, (5) vitamin B_{12}.

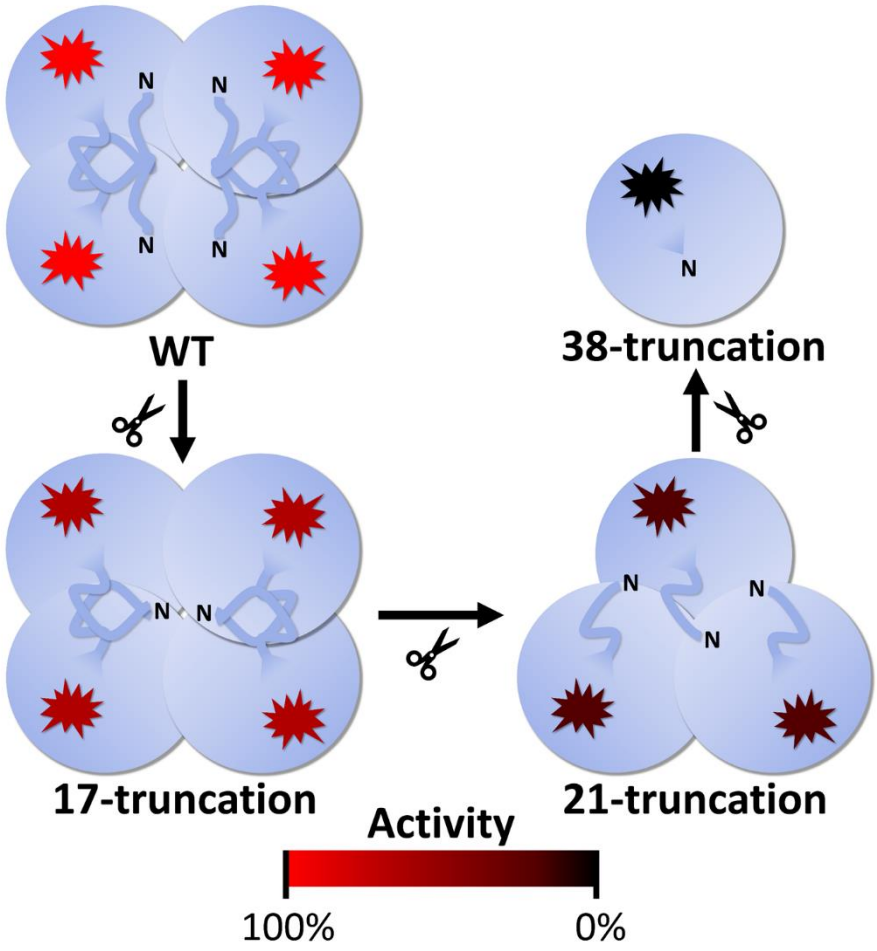

Figure S6. Model of 20β-HSDH N-terminal truncation effects. Oligomerization is shown by the number of blue circles, or subunits, and enzymatic activity is depicted by the color of the explosion shape. WT 20β-HSDH has 100% relative activity and is likely tetrameric. When the first 17 residues are truncated, the protein exhibits 62% activity, but remains a tetramer. When 21 residues are truncated, the protein loses quaternary structure and has only 2% activity. The full 38-residue truncation results in no activity and a mixed dimeric and monomeric form.