

# Supporting Information

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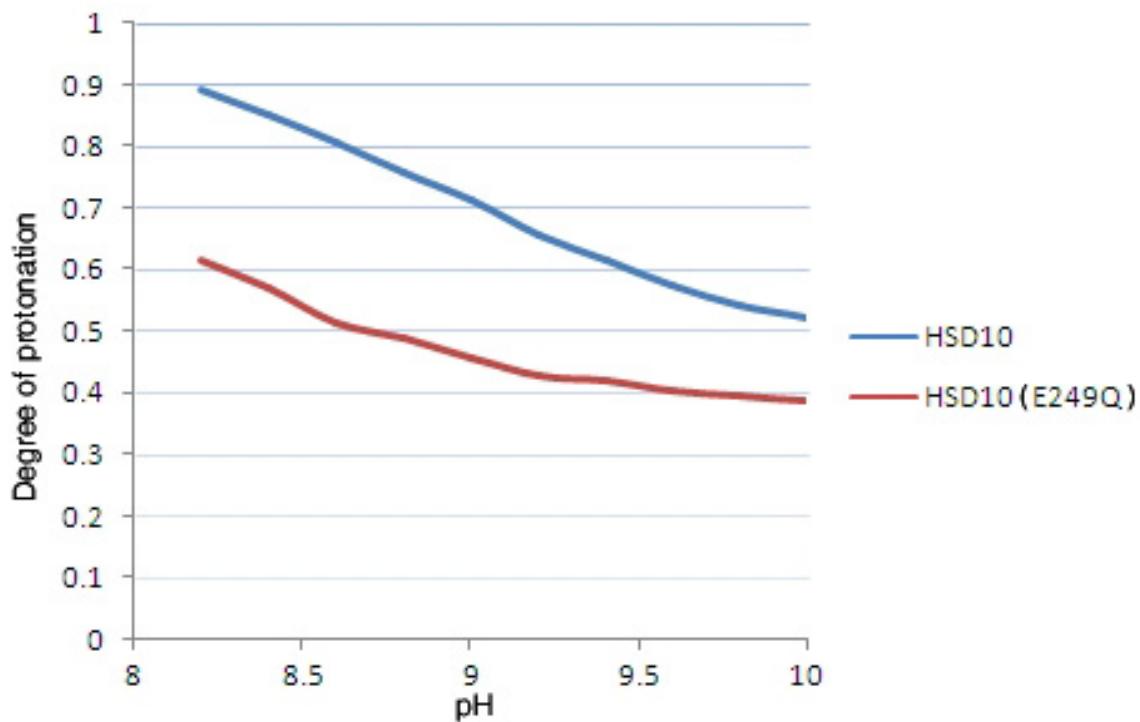


Fig. S1. A portion of the predicted titration curves for lysine 172 in HSD10 (blue line) and HSD10(E249Q) (redline).

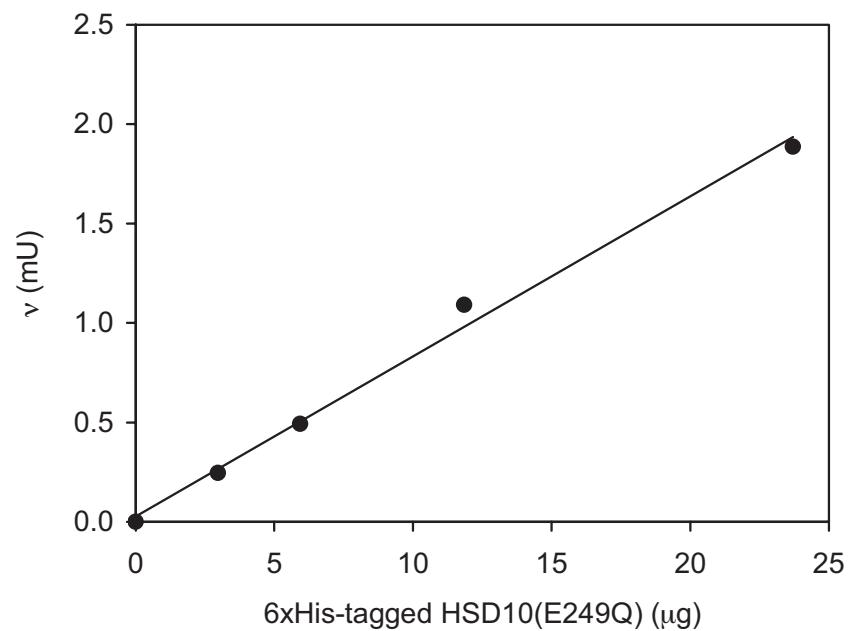
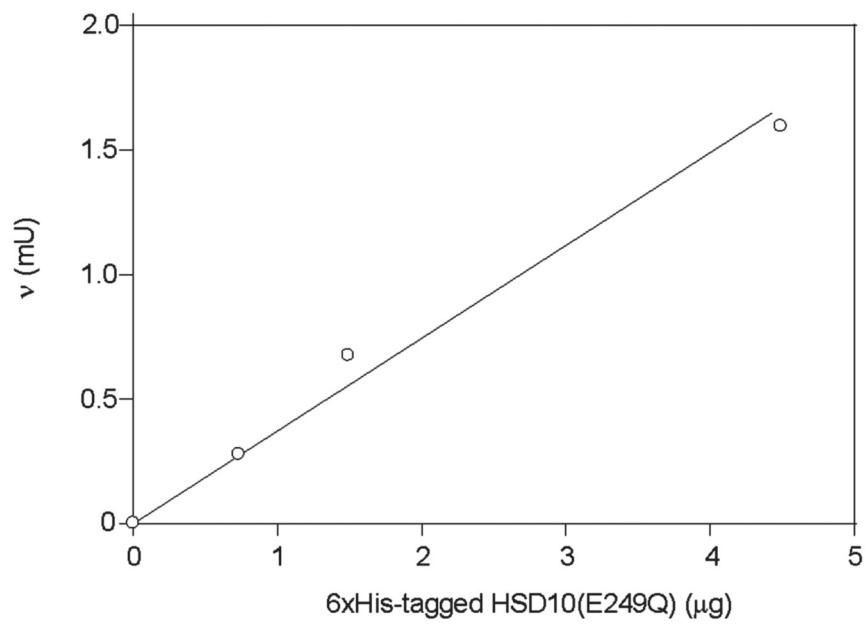


Fig. S2. Initial velocities of the oxidative reaction of allopregnanolone (51 μM) as a function of the amount of mutant enzyme 6×His-tagged HSD10(E249Q).



**Fig. S3.** Initial velocities of the dehydrogenation of 2-methyl-3-hydroxybutyryl-CoA (16  $\mu\text{M}$ ) as a function of the amount of mutant enzyme 6 $\times$ His-tagged HSD10(E249Q).

**Table S1. Nucleotide sequences of synthesized oligomers**

Name of oligomer	Nucleotide sequence (5' → 3')
PCR primer	
HSDF	CCATCAAGCTCACAGGTGGTTAGGAG
HSDR	GGCGTGTGGCAGATCGTATATCCTC
Sequencing primer	
HSDR1	TGTCCCAACGTAATCACGAGACTAGG
HSDR2	AGTCTCGGACCTCTTGTCCG
DHADFF1	TTCAAACCGGCACACCTA
DHADF2	TACTGTGACGGGTAACGA
DHADR2	TTGGAAGCAGAGTATGCAGC
HSDF1A	AGGTTGCAGTGAGCTGAGATCGGCCATTGC
HSDF3	GATCACGTGAGATCAGGAGTTC
HBHADPE3	CAGGTCAGAACAGCACAGCAGAGGCT
Mutagenic primer	
E776C	CCCATTCTCAATGGACAGGTATCCGGCTGG
E776G	CCAGCCGGATGACCTGTCATTGAGGAATGGG
R419T	CACCTTCAATGTGATCTGCCCTGGCTGGTG
R419A	CACCAGCCACCAGGCAGATCACATTGAAGGTG
6xHis insert	
6HISF	TATGCATCACCACCATACCATCACATGGCAGCAGCGTGTGGAGCGTGAAG
6HISR2	GCCCTTCACGCTCCGACACGCTGCTGCCATGTGATGGTGTGATGGTGTGCA