Electronic Supplementary Information

Electricity-driven 7α-Hydroxylation of Steroid Catalyzed by Cytochrome P450 Monooxygenase in Engineered Yeast

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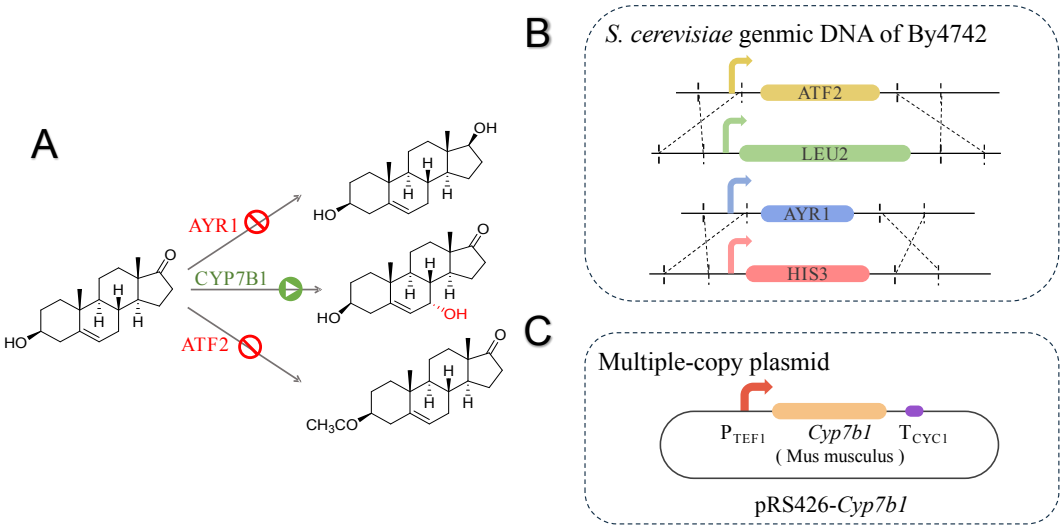

Fig. S1 Design and construction of *S. cerevisiae* 7α-OH-DHEA production strain (A) Metabolic engineering scheme for production of 7α-OH-DHEA. *S. cerevisiae* owns two genes *ayr1* and *atf2*, leading to undesirable byproducts accumulation. (B) A depiction of the double-deletion strategy for *ayr1* and *atf2* genes to gain *S. cerevisiae* Δ*ayr1*Δ*atf2*. (C) The recombinant plasmid that contains codon-optimized *Cyp7b1*gene.

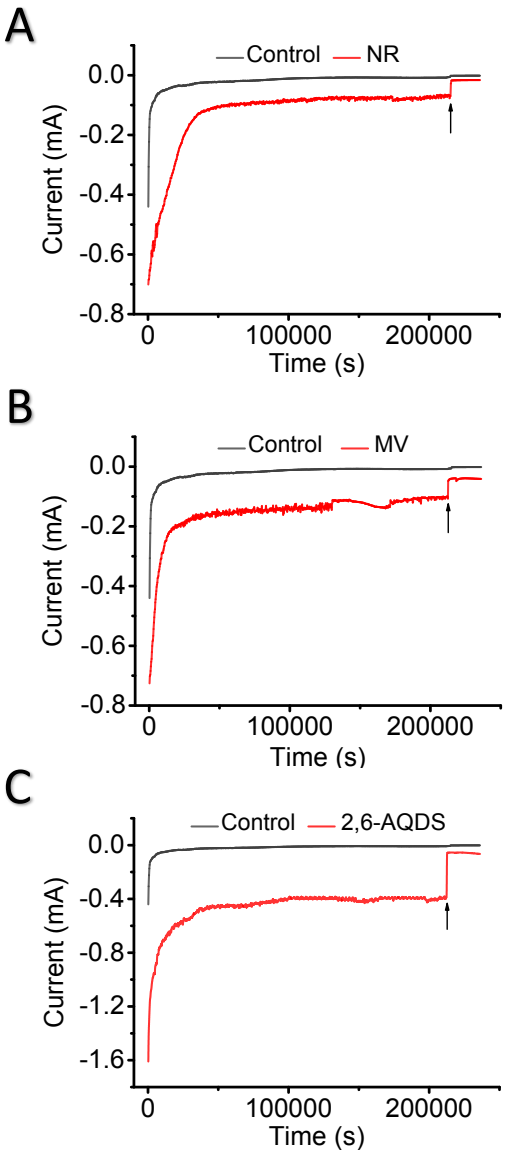

Fig. S2 The amperometry curves under the bioelectrocatalysis operation poised at -0.8 V (*vs.* Ag/AgCl) with the addition of electron shuttles and 200 mg/L DHEA (M-β-CDs:DHEA = 2:1). (A) The catholyte with 0.1 mM NR (neutral red). (B) The catholyte with 0.1 mM MV (methylviologen). (C) The catholyte with 0.1 mM 2,6-AQDS (anthraquinone-2,6-disulfonate). Control: no electron shuttles, and polarized at -0.8 V *vs.* Ag/AgCl; Arrow, the time point of stopping reactions. When the reaction stopped without air and agitation after 60 hours potentiostatic operation, the currents of the BES system and the control were not zero but decreased.

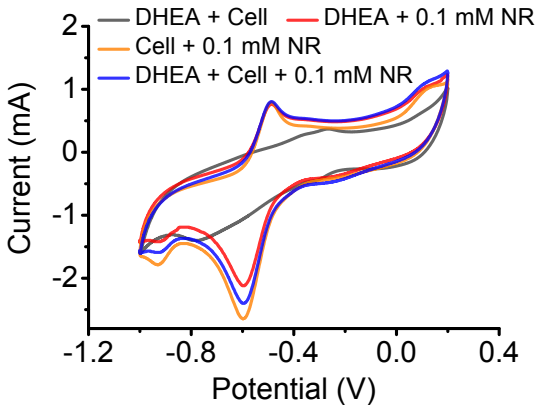

Fig. S3 The cyclic voltammograms (CV) of the catholyte with the presence and absence of NR, substrate DHEA and yeast cell. The scan rate was 50 mV/s and an Ag/AgCl electrode (3.5 M KCl) was used as the reference electrode. DHEA + Cell, the catholyte was added with 200 mg/L DHEA and yeast cells (OD_{600} = 1.5); DHEA + 0.1 mM NR, the catholyte was added with 200 mg/L DHEA and 0.1 mM NR; Cell + 0.1 mM NR, the catholyte was added with yeast cells of OD_{600} = 1.5 and 0.1 mM NR, except steroid substrate DHEA; DHEA + Cell + 0.1 mM NR, the catholyte was added with 200 mg/L DHEA, 0.1 mM NR as well as the same OD_{600} of cells. The redox potential of NR had a negligible shift with the addition of steroid substrate DHEA and cells.

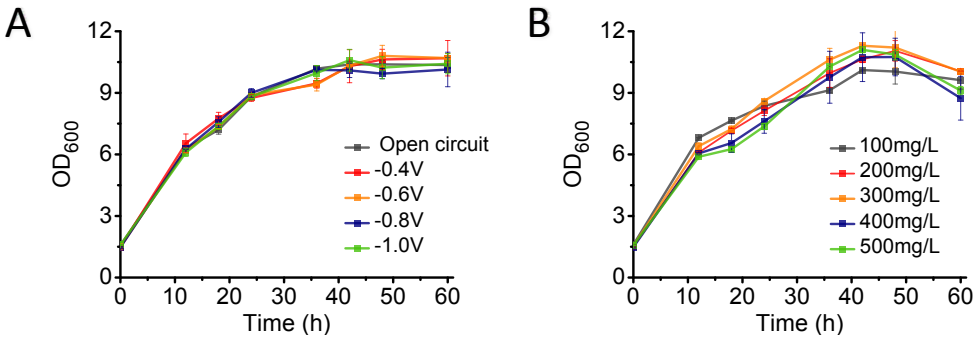

Fig. S4 The growth profiles of *S. cerevisiae* in the cathodic chamber of the bioelectrocatalysis system (BES). (A) The growth profiles of cells with the cathode poised at different potentials, -0.4 V, -0.6 V, -0.8 V, -1.0 V (*vs.* Ag/AgCl) as well as the open circuit. 0.1 mM NR was used as the electron shuttle and 200 mg/L DHEA was added (M-β-CDs:DHEA = 2:1). (B) The growth conditions of *S. cerevisiae* with different levels of DHEA from 100 mg/L to 500 mg/L (M-β-CDs:DHEA = 2:1) fed in the cathodic chamber. The cathode potential was poised at -0.6 (*vs.* Ag/AgCl) and the electron shuttle NR was added with a concentration of 0.1 mM.

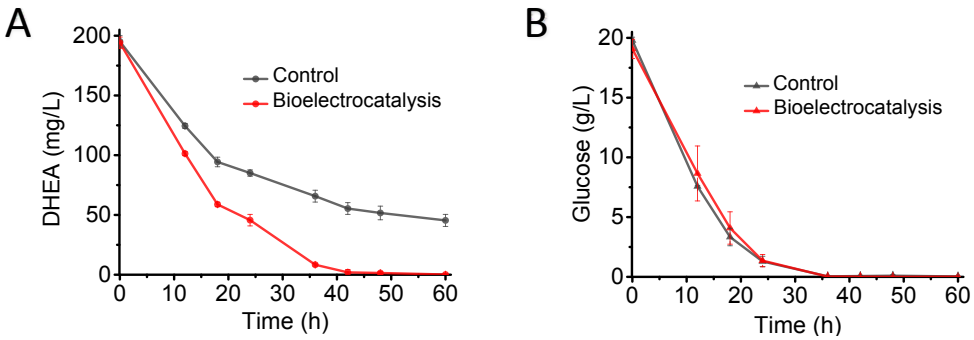

Fig. S5 The substrate DHEA and glucose consumption in the BES under the condition of the cathode potential poised at -0.6 V (*vs.* Ag/AgCl), 0.1 mM NR and 200 mg/L DHEA fed (M-β-CDs:DHEA = 2:1). (A) The DHEA consumption profile. The substrate DHEA was almost completely converted to 7α-OH-DHEA in the BES, while nearly half of DHEA cannot be transformed to 7α-OH-DHEA in the control group**.** (B) The glucose consumption profile. The carbon resource was nearly exhausted at ~24 h in both the BES and control. The control referred to the whole-cell catalysis process in the absence of EET pathway (i.e., no electron shuttle NR).

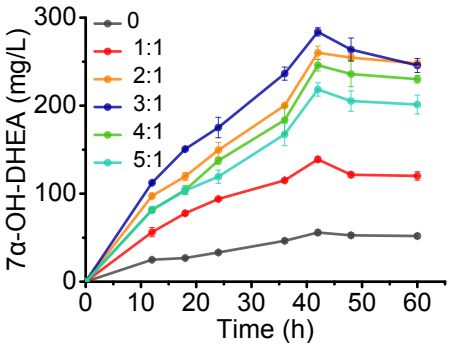

Fig. S6 The temporal profile of the 7α-OH-DHEA production in the BES poised at -0.6 V (*vs.* Ag/AgCl) with the addition of 0.1 mM NR, 400 mg/L DHEA under different molar ratios of M-β-CDs:DHEA = 0, 1:1, 2:1, 3:1, 4:1, 5:1.

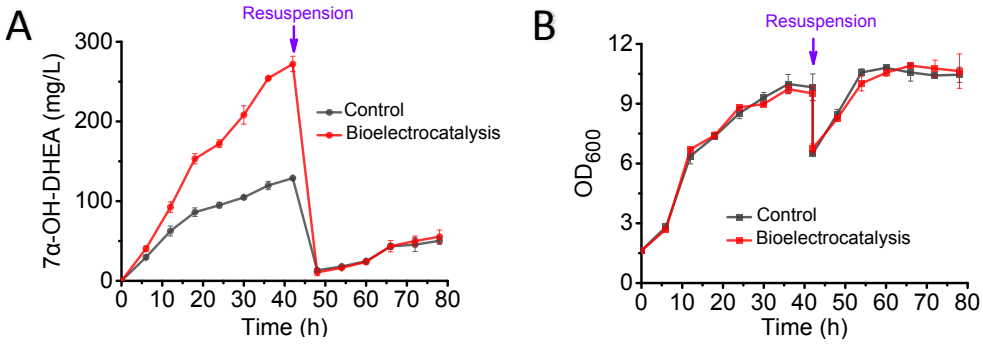

Fig. S7 The effect of the fresh cell-mediated and the recycled cell-mediated BES on the 7α-hydroxylation under the condition of 0.1 mM NR, poised at -0.6 V (*vs.* Ag/AgCl), 400 mg/L DHEA (M-β-CDs:DHEA = 3:1). Control, the whole-cell catalysis process in the absence of EET pathway (i.e., no electron shuttle NR). (A) The enhancement of the 7α-OH-DHEA yield by the fresh cells-mediated BES during 0~42 hours, and the 7α-OH-DHEA yield in the recycled cells-mediated BES (42~78 h). (B) The cell growth (OD_{600}) profiles during the fresh cells-mediated phase (0~42 h) and the recycled cells-mediated phase (42~78 h) of the BES and the control system. Violet arrow referred to the time point at which we collected the cathodic cells, washed cell pellets with phosphate buffer and resuspended in fresh catholyte (400 mg/L DHEA, M-β-CDs:DHEA = 3:1, 0.1 mM NR and polarized at -0.6 V *vs.* Ag/AgCl).

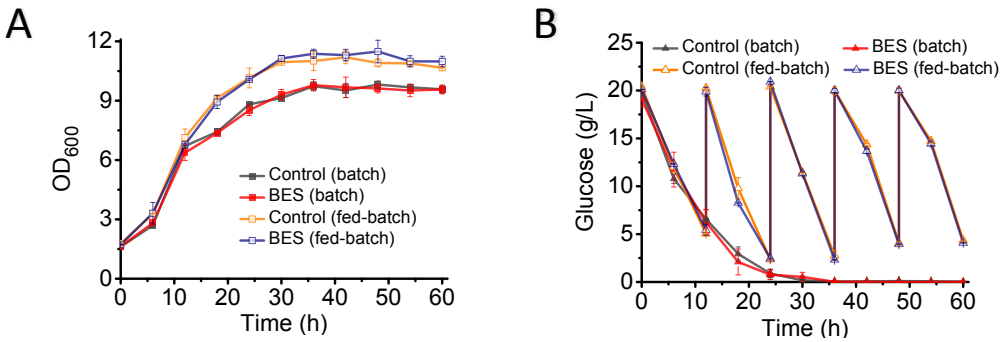

Fig. S8 The cell growth (OD_{600}) and glucose consumption profiles in the batch/fed-batch BES systems poised at -0.6 V (*vs.* Ag/AgCl), 0.1 mM NR, 400 mg/L DHEA substrate (M-β-CDs:DHEA = 3:1). (A) The growth profiles (OD_{600}) of *S. cerevisiae* in BES (batch/fed-batch) and the control groups (batch/fed-batch). (B) The glucose consumption and replenishment profiles in the BES (batch/fed-batch) and the control group (batch/fed-batch). Glucose was replenished to 20 g/L every 12 hours.

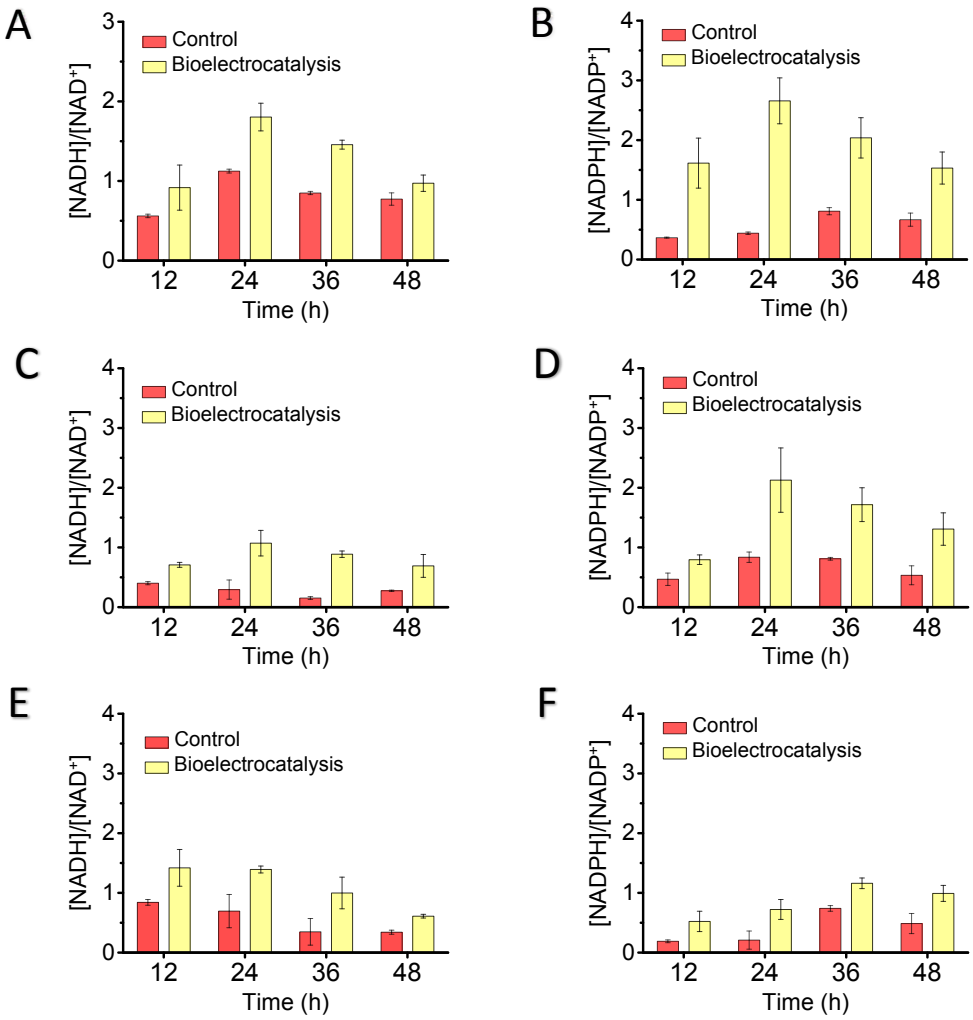

Fig. S9 The intracellular NAD(P)H/NAD(P)$^+$ ratio of the BES poised at -0.6 V (*vs.* Ag/AgCl) and 200 mg/L DHEA fed (M-β-CDs:DHEA = 2:1). Control: the whole-cell catalysis process in the absence of EET pathway (no NR and poised at -0.6 V vs. Ag/AgCl). (A) The temporal profile of the NADH/NAD$^+$ ratio in the *S. cerevisiae* Δ*atf2*Δ*ayr1*/*Cyp7b1* strain. (B) The temporal profile of the NADPH/NADP$^+$ ratio in the *S. cerevisiae* Δ*atf2*Δ*ayr1*/*Cyp7b1* strain. (C) The temporal profile of the NADH/NAD$^+$ ratio in the *S. cerevisiae* Δ*atf2*Δ*ayr1*Δ*ncp1*/*Cyp7b1* strain. (D) The temporal profile of the NADPH/NADP$^+$ ratio in the *S. cerevisiae* Δ*atf2*Δ*ayr1*Δ*ncp1*/*Cyp7b1* strain. (E) The temporal profile of the NADH/NAD$^+$ ratio in the *S. cerevisiae* Δ*atf2*Δ*ayr1*Δ*cbr1*/*Cyp7b1* strain. (F) The temporal profile of the NADPH/NADP$^+$ ratio of the *S. cerevisiae* Δ*atf2*Δ*ayr1*Δ*cbr1*/*Cyp7b1* strain.

Table S1 Primer sequences

Primers	Sequences
F1	AATTACAAAATGAACGCCACAGAAACTG
R1	GATGCGGCCCTTACAATTTTCTTCTTC
F2	CCC**AAGCTT**GCCGTACCACTTCAAAAC (Hind III)
R2	CGTTCATTTTGTAATTAAAACTTAG
F3	GAAAATTGTAAGGGCCGCATCATGTAATTAG
R3	CGC**GGATCC**GCAAATTAAAGCCTTCGAG (BamH I)

Table S2 Gene, promoter and terminator sequences

Promoter	**TEF1**

GCCGTACCACTTCAAAACACCCAAGCACAGCATACTAAATTTCCCCTCTTTCTTCCTCTAGGGTGTCGTTAATTACCCGTACTAAAGGTTTGGA
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Gene	***Cyp7b1*(codon-optimized)**

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Terminator	**CYC1**

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