

## Supplementary Files

### A new 3-ketosteroid- $\Delta^1$ -dehydrogenase with high activity and broad substrate scope for efficient transformation of hydrocortisone at high substrate concentration

**Table S1** PCR primer sequences used in this study

| Primer Name              | Nucleotide sequence (5'-3') <sup>(a)</sup> |
|--------------------------|--|
| <i>SatkstD</i> - forward | ATGGCGATCTGGGACGACGAGTG                    |
| <i>SatkstD</i> - reverse | TCAGCGGGTGAGCATGTCCAGC                     |
| <i>NvkstD</i> - forward  | ATGACCTGGGATAATTCATACGACGTCATAGTGG         |
| <i>NvkstD</i> - reverse  | TCAGGATGCGGTGGCGTCCGC                      |

(a) Primers were designed with NdeI and HindIII restriction sites to clone the *kstD* genes into the plasmids pET21a(+).

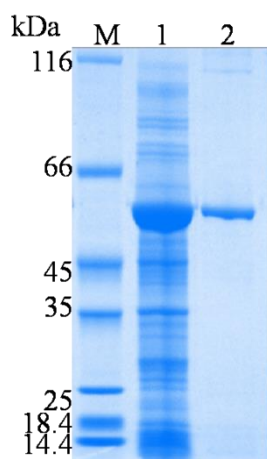
**Table S2** Organic solvent screening for the dehydrogenation of hydrocortisone catalyzed by

*Escherichia. coli* cells overexpressing the *PrkstD*

| Organic solvent    | Log P |
|--------------------|-------|
| Dimethyl sulfoxide | -1.30 |
| 1,4-dioxane        | -1.10 |
| Dimethyl formamide | -1.00 |
| Methanol           | -0.76 |
| Ethanol            | -0.24 |
| Isopropyl alcohol  | 0.33  |
| Tetrahydrofuran    | 0.49  |

**Table S3** Purification of recombinant PrKstD

| Purification step | Activity (U) | Specific activity (U/mg) | Purification ( <i>n</i> -fold) | Yield (%) |
|-------------------|--------------|--------------------------|--------------------------------|-----------|
| Cell extract      | 18695        | 26                       | 1.0                            | 100       |
| Ni-NTA            | 16342        | 188                      | 7.2                            | 87        |

**Figure S1** Protein purity of PrKstD by Ni<sup>2+</sup> column. M, protein marker (kDa); 1, cell extract supernatant; 2, purified PrKstD from the elution step.**Table S4** Kinetic parameters of PrKstD for the oxidation of hydrocortisone with PMS/DCPIP

| Electron acceptor | <i>K<sub>m</sub></i> (μM) | <i>k<sub>cat</sub></i> (s <sup>-1</sup> ) | <i>k<sub>cat</sub></i> / <i>K<sub>m</sub></i><br>s <sup>-1</sup> μM <sup>-1</sup> | <i>K<sub>i</sub></i> (mM) |
|-------------------|---------------------------|---|---|---------------------------|
| PMS/DCPIP         | 24.0±10                   | 207.7±27                                  | 8.7   | 2.2±0.8                   |

Enzyme activities were measured in a spectrophotometric assay at 600 nm in 50 mM potassium phosphate buffer pH 8.0, 0.5 mM hydrocortisone, 0.15 mM DCPIP, 0.02 μg PrKstD, 0-4 mM PMS.



**Figure S2** The sequence alignment of known KstD enzymes. SQ1 KstD from *Rhodococcus erythropolis* SQ1 (PDB entry 4C3Y). The FAD-binding domain is boxed in red. Active site residues essential for its activity in *R. erythropolis* SQ1 are indicated by red asterisks.