

## SUPPLEMENTARY MATERIAL

**Table A.** Sequence of the synthetic oligonucleotides used for site-directed mutagenesis of cDNA of the arabidopsis 3 $\beta$ HSD/D gene.

Strain	Sense oligonucleotide sequence (5' – 3')
D39V	CACGTTTCGTATCGCCGTTTTAGCTCCTGCGATAGTG
D70V	CAATACGTCCCCGCTGTTCTTCGGAACAAAACACTCAAG
D70A	CAATACGTCCCCGCTGCTCTTCGGAACAAAACACTCAAG
T129V	GAGGCTAATCTATGTAAGTTCTCCGAGTGTTGTGTTTGACGGG
S131A	GAGGCTAATCTATACAAGTGCTCCGAGTGTTGTGTTTGACGG
S133A	GGCTAATCTATACAAGTTCTCCGGCTGTTGTGTTTGACGGGGTC
S133T	CTATACAAGTTCTCCGACTGTTGTGTTTGACGGGGTC
S133Y	GGCTAATCTATACAAGTTCTCCGTAATGTTGTGTTTGACGGGGTC
Y159F	CCACCTAAGCATAAATGATTCATTTTCAGCTACTAAAGCTGAAGG
K163I	GATTCATATTCAGCTACTATAGCTGAAGGGGAAGCTTTGATTTTG
R318I	CCAGTGCTAACACCTTCTATTGTTAGGCTACTCTCTTGC
R326I	GGCTACTCTCTTGCAACATAACATTTGATTCTTCAAAGCAAGG
$\Delta$ [288-303]	CAAGTATAAAGATACCTGCAGGACCGTATGGGATGAAAGTAC

## LEGEND OF FIGURE A

Examples of double-reciprocal plots for recombinant *At*3 $\beta$ HSD/D activities by microsomes of *S.cerevisiae erg26* null mutant expressing wild-type *At*3 $\beta$ HSD/D and various active mutant enzymes. Assays were conducted as described in the Materials and Methods. Straight lines were fitted by using a computer-assisted least-squares method.

**Panel A :** *4 $\alpha$ -carboxy-cholest-7-en-3 $\beta$ -ol* [2] reaction by microsomes expressing wild-type *At*3 $\beta$ HSD/D (■), and mutants R318I (○), S131A (●), and S133A (▲) enzymes.

**Panel B :** *4 $\alpha$ -carboxy-4 $\beta$ -methyl-cholest-8,24-dien-3 $\beta$ -ol* [3] reaction by microsomes expressing wild-type *At*3 $\beta$ HSD/D (●), and mutant S133A (○) enzymes.

**Panel C :** Reaction by microsomes expressing mutant S133T with *4 $\alpha$ -carboxy-cholest-7-en-3 $\beta$ -ol* [2] (■) and *4 $\alpha$ -carboxy-4 $\beta$ -methyl-cholest-8,24-dien-3 $\beta$ -ol* [3] (●).

Figure A

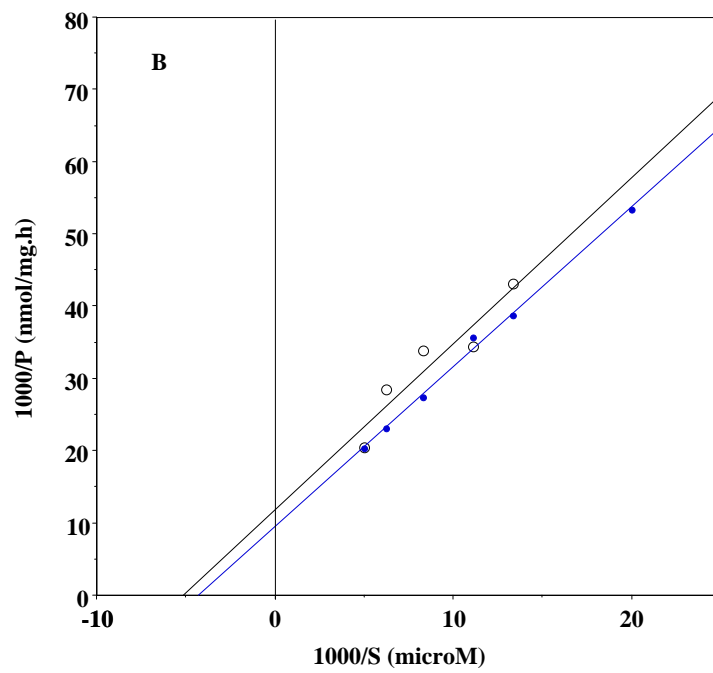
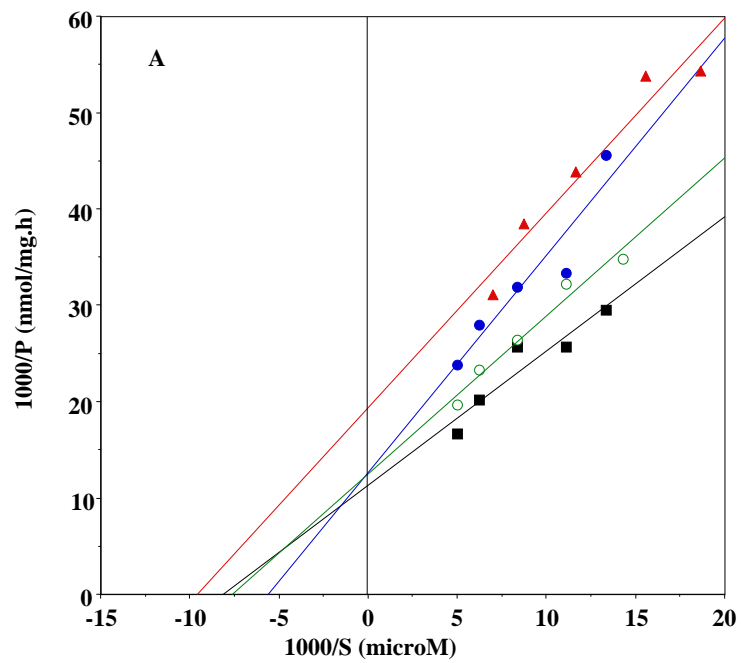


Figure A

