Supporting Information for **Kemp Eliminase Activity of Ketosteroid Isomerase**

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Figure S1

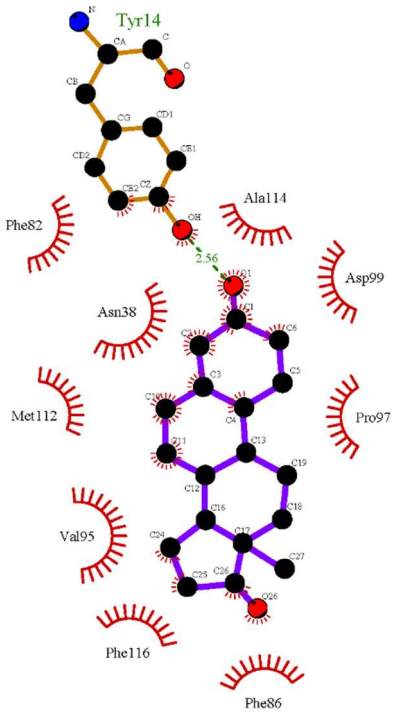

Figure S1. Residues in the active site of tKSI. This figure was generated with LigPlot^{+} (ref S1) using the KSI structure with PDB ID 1OHP.

Figure S2.

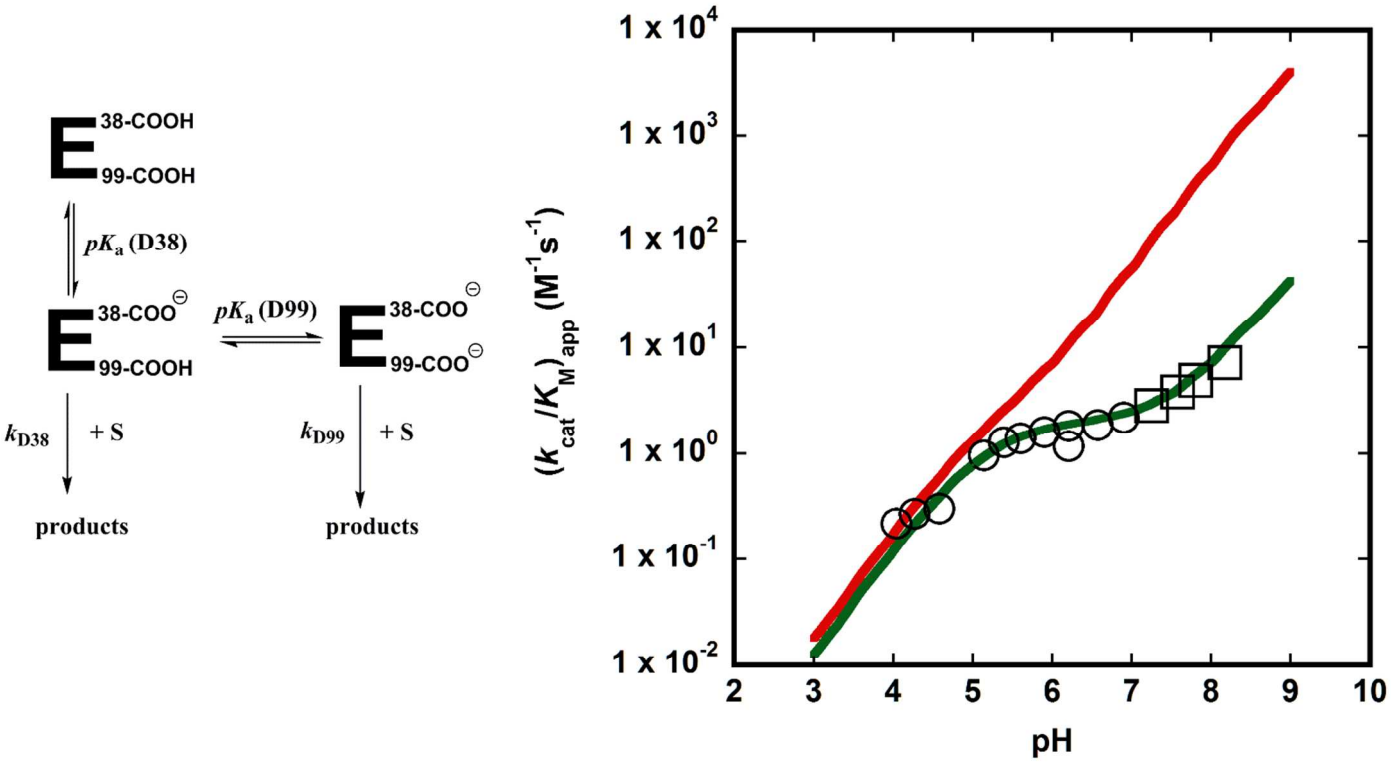

Figure S2. Simulation of the expected pH rate-profile in case both D38 and D99 can acts as the catalytic base in wt tKSI. The values of $(k_{cat}/K_M)_{app}$ were calculated using the equation, derived from the Scheme shown in Figure, $(k_{cat/}K_M)_{app} = \frac{k_{D38}\times 10^{-pka\,(D38)}}{10^{-pka\,(D38)}+\,10^{-pH}} + \frac{k_{D99}\times 10^{-pka\,(D99)}}{10^{-pka\,(D99)}+\,10^{-pH}}$, with pK_a (D38) = 4.6, pK_a (D38) = 9.5, k_{D38} = 2.5 $M^{-1}s^{-1}$ and k_{D99} = 17,000 $M^{-1}s^{-1}$ (red line) or 170 $M^{-1}s^{-1}$ (green line).

Figure S3.

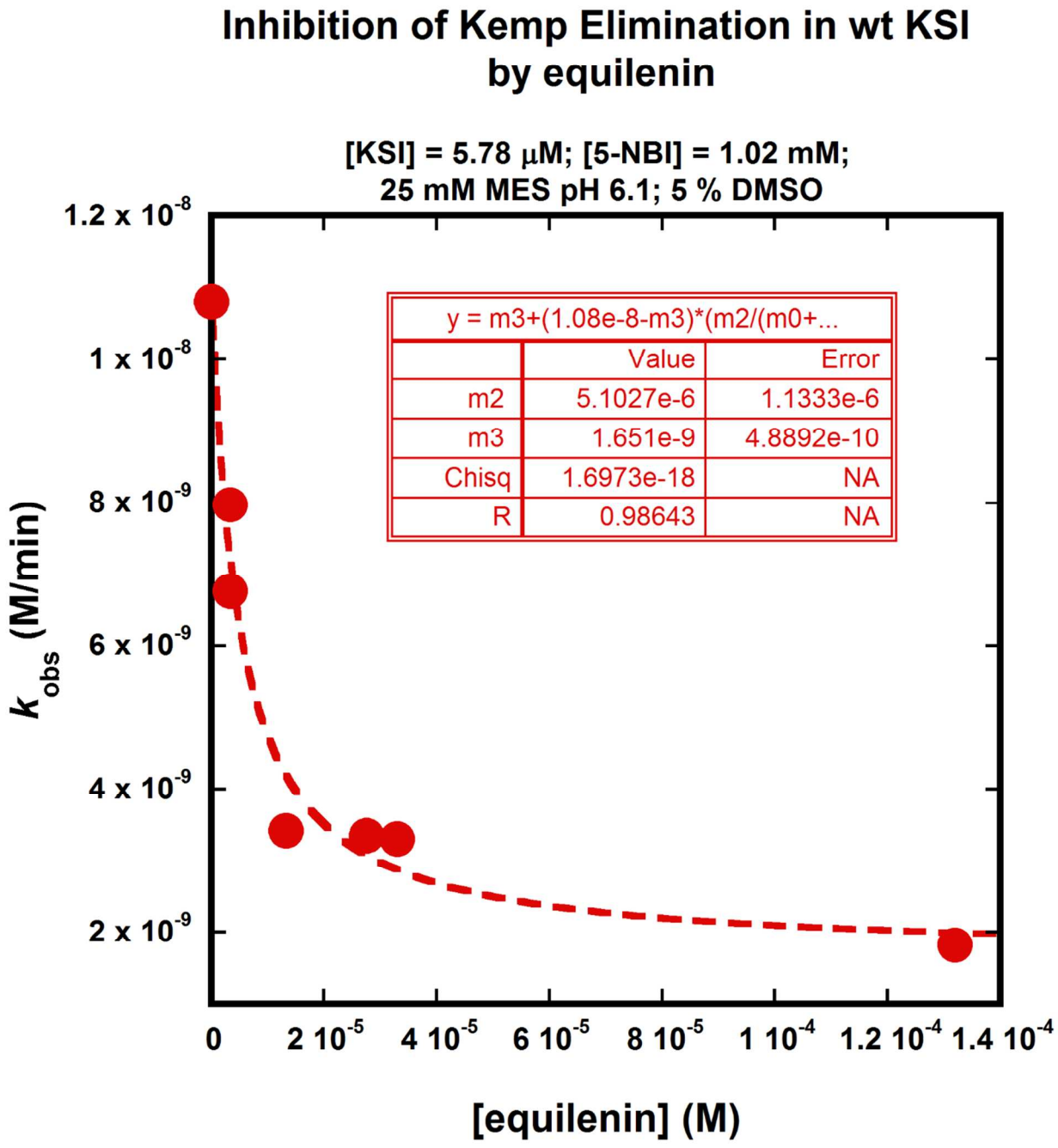

Figure S3. Inhibition of wt tKSI by equilenin. Experimental details are in the figure.

Figure S4

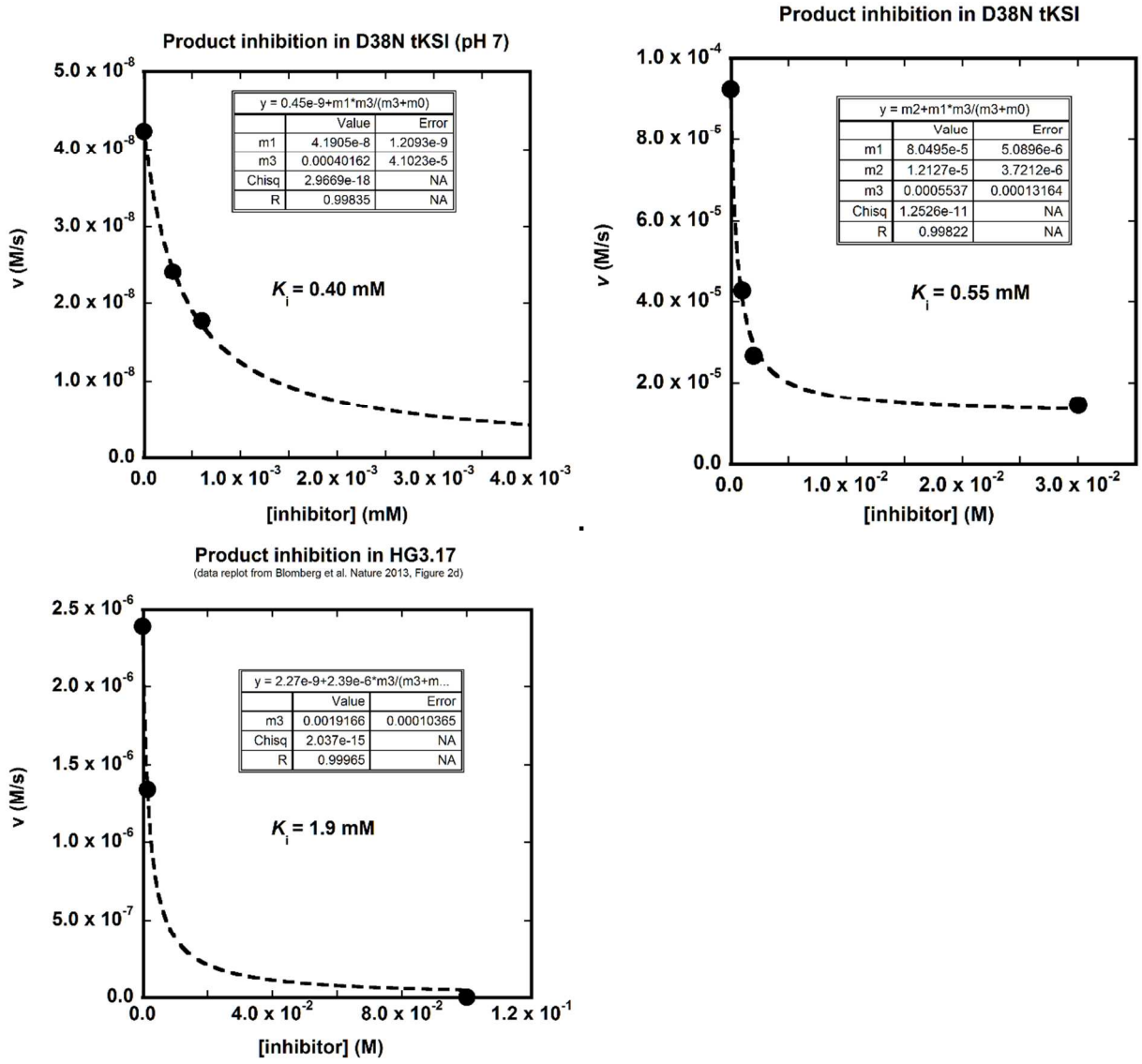

Figure S4. Product inhibition of (a) D38N tKSI (pH 7.0); (b) D38N tKSI (pH 9.3); (c) HG3.17 (pH 7.0). Data points in panel c are derived from Figure 2d of **reference 6.** Points were fit to the equation $v = v_{in} + v_{max} \times \frac{K_I}{K_I+[inhibitor]}$ where v_{in} represents the velocity at full inhibition (measured in the absence of enzyme), v_{max} the velocity in absence of the inhibitor, and K_I the inhibition constant.

FIGURE S5.

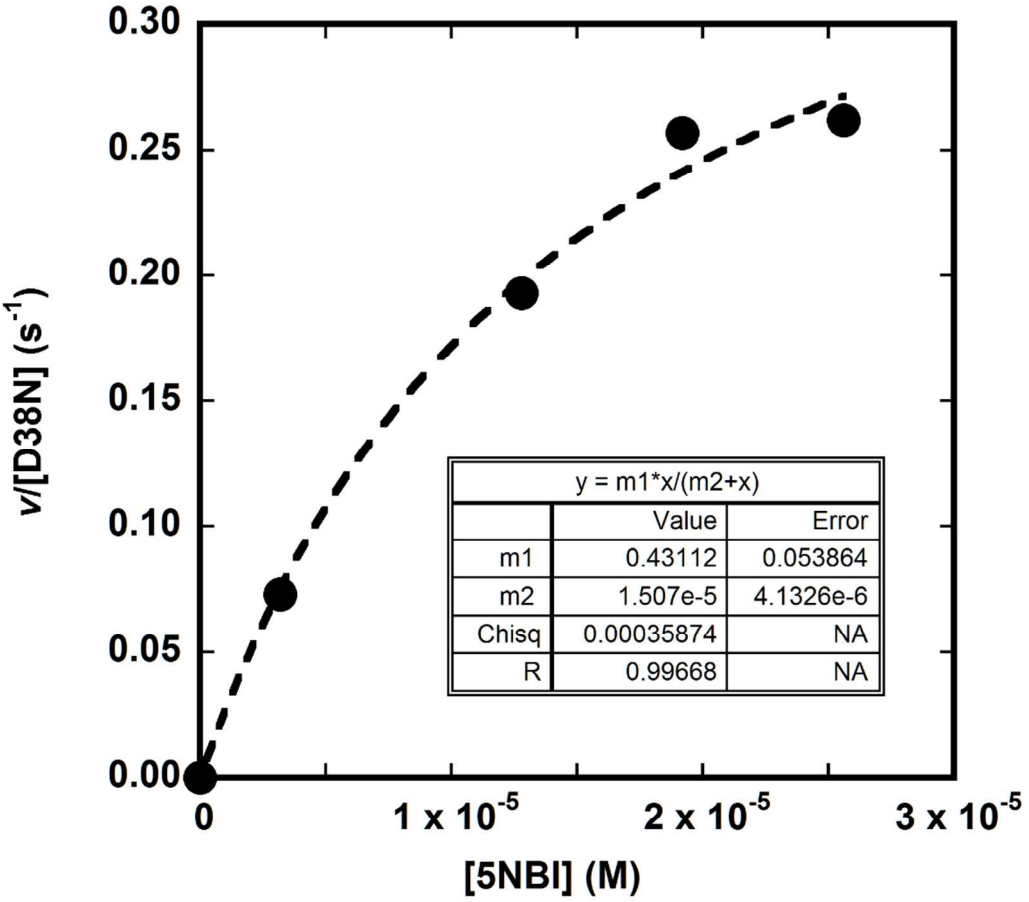

Figure S5. Reactions in the presence of 0.39 μM enzyme, pH 7.5, and different concentrations of 5NBI. Points were fit to the Michaelis-Menten equation to determine k_{cat}, K_M, and k_{cat}/K_M.

REFERENCES

[S1] Laskowski, R. A., and Swindells, M. B. (2011) LigPlot+: multiple ligand-protein interaction diagrams for drug discovery, *J. Chem. Inf. Model. 51*, 2778-2786.