

Supplementary Information

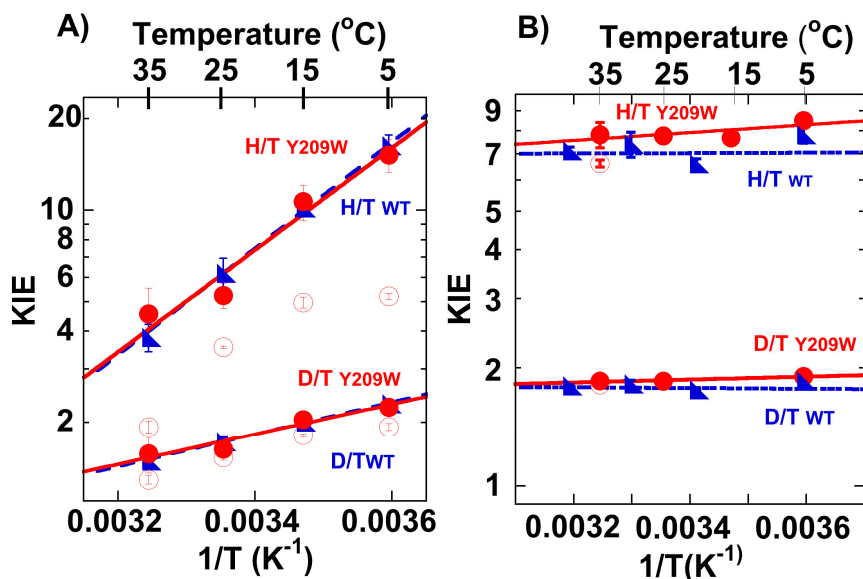


Figure S1. Arrhenius plots of the primary KIEs on the proton abstraction (A) catalyzed by WT [16] (blue) and Y209W (red) TSase and the hydride transfer (B) catalyzed by the WT [25] (blue) and Y209W [15] (red) TSase. The lines represent the least-squares nonlinear regression of the intrinsic KIEs to Equation (5) in the main text. The empty circles represent the observed KIEs, and filled circles represent the KIE_{intS}. (B) is adapted from [15] with permission from ACS.

Analysis of Products in the Proton Abstraction Step

We used an Agilent Technologies model 1100 HPLC system with a Supelco Discovery[®] C18 reverse phase column.

HPLC mobile phase solutions: A = 20 mM TEAA buffer (pH = 5.0); B = Acetonitrile.

Table S1. HPLC mobile phase gradient (flow rate 0.8 mL/min).

Time (min)	% A	% B
0	100	0
5	99.5	0.5
20	99	1
25	90	10
30	50	50
35	50	50

Table S2. KIE on the proton transfer catalyzed by Y209W.

Temperature (°C)	Observed KIE		Intrinsic KIE	
	H/T KIE	D/T KIE	H/T KIE	D/T KIE
5	5.21 ± 0.13	1.93 ± 0.05	15.19 ± 1.86	2.24 ± 0.13
15	4.97 ± 0.21	1.81 ± 0.02	10.67 ± 1.43	2.04 ± 0.1
25	3.54 ± 0.05	1.53 ± 0.03	5.24 ± 0.48	1.63 ± 0.04
35	1.93 ± 0.09	1.29 ± 0.04	4.56 ± 0.99	1.57 ± 0.21