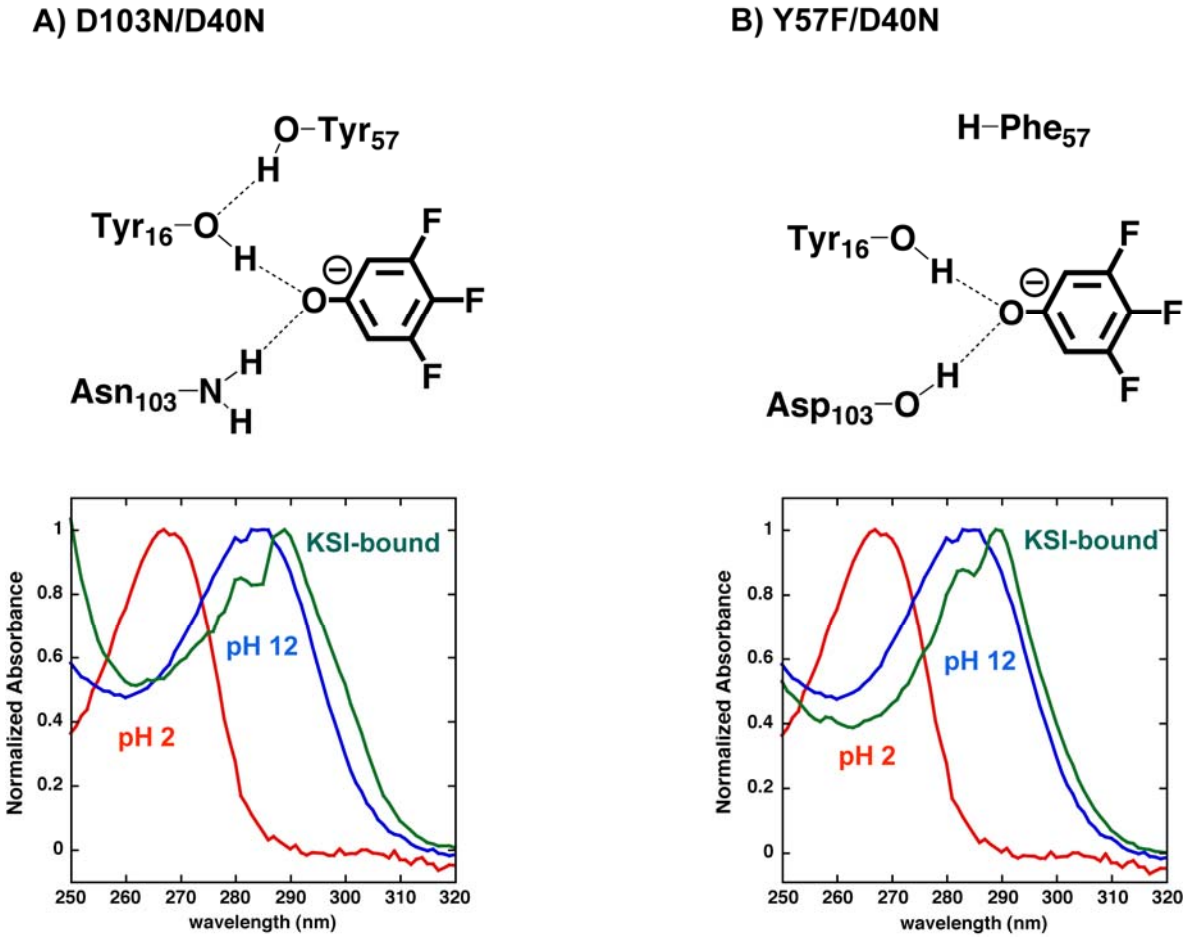
SUPPORTING INFORMATION

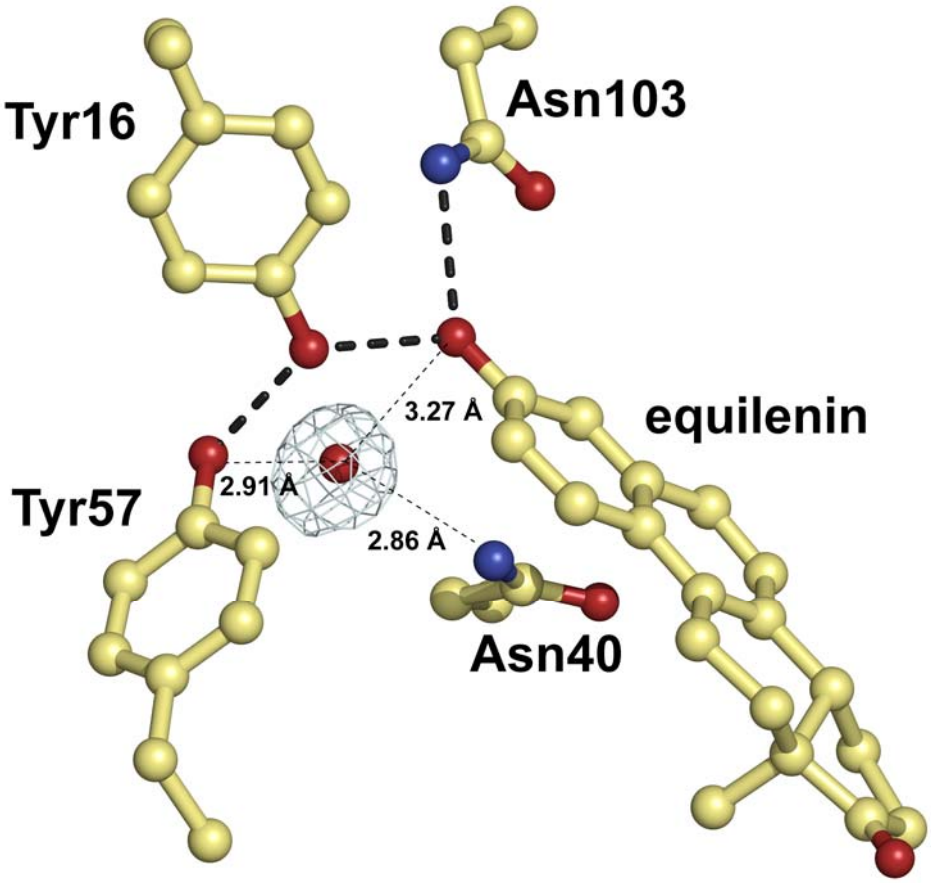
Hydrogen Bond Coupling in the Ketosteroid Isomerase Active Site

Figure S1: Determination of Ionization State of Phenols Bound to tKSI Mutants



Absorbance spectra of 50 μM 3,4,5-F_3-phenol (pK_a 8.2) measured in 10 mM HCl (red), 10 mM NaOH (blue), and bound to 300 μM (**A**) tKSI$^{D103N/D40N}$ and (**B**) tKSI$^{Y57F/D40N}$ in 40 mM potassium phosphate, pH 7.2, (green) were consistent with binding as ionized phenolates. Concentrations of enzyme and phenol were sufficient to give >95% bound phenol (data not shown).

Figure S2: Structural Model of the Ordered Water Molecule Observed in the Oxyanion Hole of the pKSI$^{D103N/D40N}$•Equilenin Complex



Sigma-A weighted $2F_o - F_c$ electron density is shown for the observed water molecule (contoured at 1.5 σ).

Assignment of Downfield Peaks Observed in Spectra of tKSI$^{D103N/D40N}$ and tKSI$^{Y57F/D40N}$ Bound to 3,4-dinitrophenol (Fig. 2).

D103N

a. A single far-downfield peak at 15.1 ppm is observed in the tKSI$^{D103N/D40N}$•3,4-dinitrophenolate spectrum, as expected for the single short 2.5 Å Y16•phenolate hydrogen bond that remains upon lengthening the D103•phenolate hydrogen bond from the D103N mutation. A lengthening of the residue-103•oxyanion hydrogen bond to 2.9 Å in D103N is observed in the 1.3 Å resolution X-ray structure with bound equilenin described in the text and in a 1.8 Å resolution X-ray structure with bound phenol (unpublished results).

b. The chemical shift of the single peak observed in tKSI$^{D103N/D40N}$•phenolate complexes becomes progressively deshielded as the pK_a of the bound phenolate increases, as expected for the short hydrogen bond donated by Y16 to the oxyanion of the bound phenolate (unpublished results).

On the basis of these observations, we assign the single far-downfield peak observed in the tKSI$^{D103N/D40N}$•3,4-dinitrophenolate spectrum to the Y16•phenolate hydrogen bond.

Y57F

As depicted in Figure 1, Y57 donates a hydrogen bond to the side-chain hydroxyl oxygen of Y16 in the unmodified oxyanion hole. The Y57F mutation ablates this hydrogen bond to Y16, but leaves intact both short hydrogen bonds donated by Y16 and D103 to the ligand oxyanion (see PDB entry 1GS3).

a. Two downfield NMR peaks at 11.7 ppm and 14.7 ppm are observed in the spectrum of tKSI$^{Y57F/D40N}$•3,4-dinitrophenolate, as expected for the two hydrogen bonds donated by Y16 and D103 to the phenolate oxygen.

b. Both downfield peaks observed in tKSI$^{Y57F/D40N}$•phenolate complexes become progressively deshielded as the pK_a of the bound phenolate increases, as expected for the hydrogen bonds donated by D103 and Y16 to the phenolate oxygen (unpublished results).

On the basis of these observations, we assign the two downfield peaks (colored red) in the tKSI$^{Y57F/D40N}$•3,4-dinitrophenolate spectrum in Fig. 2 to the hydrogen-bonded protons of Y16 and D103. Further assignment of the 11.7 ppm peak to Y16 and the 14.7 ppm peak to D103 is suggested (but not proven) by the following considerations:

Ablation of the Y57•Y16 hydrogen bond from the Y57F mutation is expected to weaken the hydrogen bond donating ability (i.e. raise the pK_a) of Y16. Thus, the simplest expectation is that the Y57F mutation would lengthen the Y16•oxyanion hydrogen bond and result in an upfield shift of its associated NMR resonance. Any detected change in the D103 hydrogen bond NMR peak position from the Y57F mutation is expected to be small compared to the effect on the Y16 hydrogen bond, as there is no direct interaction between D103 and Y57. Thus, the large upfield shift of the 11.7 ppm peak in the Y57F mutant, relative to the small downfield shift of the 14.7 ppm peak, suggests that the 11.7 ppm peak arises from Y16 while the 14.7 ppm peak is from D103. This assignment is also consistent with the "anti-cooperative" coupling detected in the H/D substitution studies described in the text in which lengthening of one oxyanion hole hydrogen bond is accompanied by shortening of the other.