Supplemental Material for

Inverse solvent isotope effects arising from substrate triggering in the factor inhibiting HIF (FIH-1)

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1. Ionic Strength Effect on FIH
2. Solvent Viscosity Effect

1. Ionic Strength Effect on FIH

Steady-state assays varying CTAD were performed in 50 mM HEPES pH 7.00. Initial rates were measured as described in the text, using buffers with ionic strengths ranging from 25 mM to 150 mM.

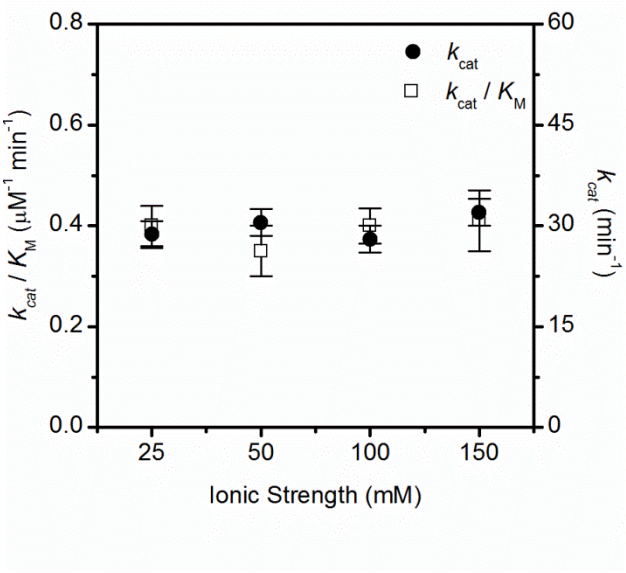

Figure S1. Effect of ionic strength on the kinetic parameters, k_{cat} (●), $k_{cat}/K_{M(CTAD)}$(□) of FIH. FIH (0.5 μM), ascorbate (2 mM), αKG (500 μM), $FeSO_4$ (50 μM) and CTAD (0-250 μM) were in 50mM HEPES pH 7.00. Ionic strength was adjusted using NaCl.

2. **Solvent Viscosity Effect**

The solvent viscosity effect was measured on k_{cat}, $k_{cat}/K_{M(CTAD)}$ and $k_{cat}/K_{M(\alpha KG)}$, as described in the text.

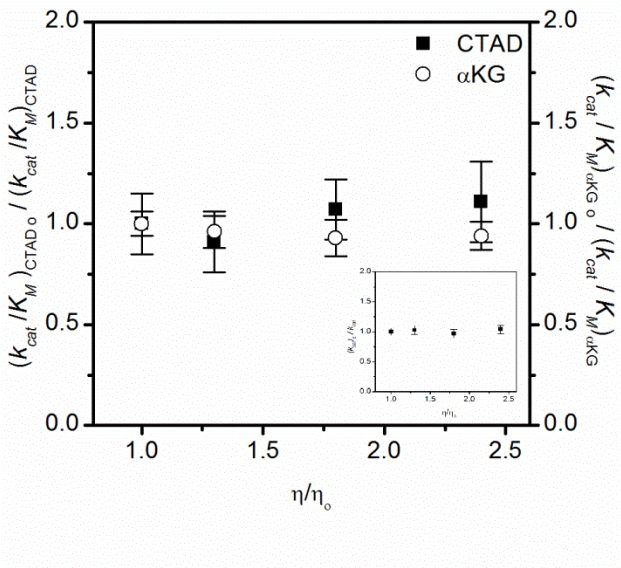

Figure S2. Microviscosity (sucrose) activity profile of FIH. CTAD was the varied substrate, k_{cat}/K_{MCTAD}(■) and αKG as the varied substrate, $k_{cat}/K_{M(\alpha KG)}$(○). FIH (0.5 μM), ascorbate (2 mM), αKG (500 μM or 5-200 μM), $FeSO_4$ (50 μM) and CTAD (90 μM or 0-250 μM) were in 50mM HEPES pH 7.00. Inset: Effect of microviscosity on k_{cat}. FIH (0.5 μM), ascorbate (2 mM), αKG (500 μM), $FeSO_4$ (50 μM) and CTAD (0-250 μM) were in 50mM HEPES pH 7.00