

	T (°C)	2-Me (mM)	[XRCC4] ( $\mu$ M)	[XLF] ( $\mu$ M)	Ratio	N	$K_a$ (M <sup>-1</sup> )	$\Delta H^0$ (kcal/mol)	$K_d$ ( $\mu$ M)	$-\Delta G^0$ (kcal/mol)	$-T\Delta S^0$ (kcal/mol)	Thermogram shown in	Source data	$\langle K_d \rangle$ ( $\mu$ M)	$\langle \Delta H^0 \rangle$ (kcal/mol)	$\langle -\Delta G^0 \rangle$ (kcal/mol)	$\langle -T\Delta S^0 \rangle$ (kcal/mol)
			ITC cell	ITC syringe													
XRCC4-wt XLF-wt	10	10	17	168	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Fig. 5 – Sup. 1A	A				
	25	10	17	168	10	0.97	536000	-1.7	1.8	-7.8	-6.1	--	B	3.2 ± 0.8	-2.4 ± 0.4	-7.5 ± 0.2	-5.2 ± 0.5
	25	10	33	330	10	0.91	306000	-2.9	3.2	-7.5	-4.6	Fig. 5 – Sup. 1B	C				
	25	10	25	330	13	0.84	222000	-2.6	4.5	-7.3	-4.7	--	D				
	37	0	23	300	13	0.85	273000	-6.0	3.7	-7.7	-1.7	--	E	2.4 ± 0.7	-7.1 ± 1	-8.0 ± 0.2	-1.0 ± 0.8
	37	0	23	300	13	0.89	278000	-4.9	3.6	-7.7	-2.9	--	F				
	37	0	12	154	13	0.92	848000	-8.6	1.2	-8.4	0.2	Fig. 5A	G				
	37	0	12	154	13	0.84	760000	-8.8	1.3	-8.3	0.4	--	H				
XRCC4-Ala XLF-Ala	25	10	25	330	13	1.21	300000	-1.7	3.3	-7.5	-5.8	Fig. 5 – Sup. 1C	I				
	37	0	25	325	13	1.07	637000	-3.1	1.6	-8.2	-5.1	--	J	2.3 ± 0.4	-5.8 ± 1.4	-8.0 ± 0.1	-2.2 ± 1.4
	37	0	25	325	13	1	343000	-3.7	2.9	-7.8	-4.1	--	K				
	37	0	19	250	13	0.92	345000	-8.4	2.9	-7.8	0.6	--	L				
	37	0	19	250	13	0.92	582000	-8.1	1.7	-8.2	-0.1	Fig. 5B	M				
XRCC4-Asp XLF-Asp	25	10	35	350	10	1.27	672000	-0.9	1.5	-7.9	-7.0	--	N				
	25	10	25	330	13	0.82	263000	-1.2	3.8	-7.4	-6.2	Fig. 5 – Sup. 1D	O	2.4 ± 0.4	-3.7 ± 0.8	-8.0 ± 0.1	-4.3 ± 0.7
	37	0	32	416	13	1.06	289000	-2.4	3.4	-7.8	-5.4	--	P				
	37	0	32	416	13	0.95	425000	-2.3	2.4	-8.0	-5.7	--	Q				
	37	0	19	250	13	0.88	566000	-4.7	1.8	-8.2	-3.5	Fig. 5C	R				
	37	0	17	220	13	0.85	538000	-5.5	1.8	-8.1	-2.6	--	S				

2-Me stands for 2-Mercaptoethanol

Ratio indicates the titrant over analyte concentration ([XLF]/[XRCC4])

Source data specifies the column corresponding to each thermogram in Table 1 – source data 1

Last four columns report mean values  $\pm$  Standard Error of the Mean (SEM) from 4 experimental runs at 37 °C (grey cells) and 3 runs at 25 °C (white cells)

n.d. denotes no detectable heat transfer (at 10 °C)