### Figure 5—Source Data 1. Binding affinities of Top6B mutants for different length duplexes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Enzyme construct | Duplex length | | | |
|  | 30bp | 40bp | 60bp | 70bp | |
| Wildtype  Kd,app(nM) | 84±6 | 49 ±3 | 38±2 | 62±4 | |
| KGRRAAA  Kd,app(nM) | 65±4 | 67 ±4 | 99±2 | 124±6 | |
| KGRREEE  Kd,app(nM) | 82±7 | 75 ±4 | 119±3 | 193±4 | |
| Stalk/WKxYAAA  Kd,app(nM) | N/A\* | N/A\* | N/A\* | N/A\* | |
| Stalk/WKxYEEE  Kd,app(nM) | N/A\* | N/A\* | N/A\* | N/A\* | |
| H2THAAA  Kd,app(nM) | 94±4 | 58 ±3 | 167±9 | 180±12 | |
| H2THEEE  Kd,app(nM) | 94±5 | 45 ±2 | 109±3 | 103±3 | |
| h\*\* | 1.3±0.1 | 1.6±0.1 | 1.8±0.1 | 1.7±0.1 | |

\*Kd,app not determined due to inconsistent or poorly defined ΔFAmax values compared to wildtype.

\*\*Hill coefficient fit for data better represented by a cooperative binding model.

\*\*\*Standard errors in fit parameters are reported.