**Table S1 | Summary of the free energies of the pseudo-active and active Cas9 complex systems with (+) and without (-) Mg2+ bound at the HNH domain catalytic interface estimated by the end-point MM-GBSA approach (kcal/mol)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **+ Mg2+** | **- Mg2+** | **ΔΔG** |
| **ΔG(pseudo-active)** | -64538.9 (6.7) | -63947.6 (6.7) | -591.3 |
| **ΔG(active)** | -64513.5 (4.8) | -63954.8 (4.8) | -558.7 |
| **ΔΔG** | -25.4 |  |  |

Values are Mean (SEM) calculated from a sample of 2,000 structures.