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Biophysical characterization of dualENH. A synthetic gene encoding the dualENH sequence was constructed with a C-terminal $(His)_6$-tag, cloned into an expression plasmid, and transformed into *Escherichia coli* cells. Expression of dualENH at 37 °C produced >10 mg of soluble protein per liter of *E. coli* culture. Far-UV circular dichroism (CD) spectroscopy showed that dualENH is a fully refoldable, entirely α-helical protein (Extended Data Fig. 2a). The CD curve for dualENH is very similar to that of wild-type ENH, suggesting a high degree of structural similarity. Thermal denaturation monitored by CD showed that dualENH has a melting temperature (T_m) of 59 °C (Extended Data Fig. 2b), which makes it more stable than wild-type ENH (T_m = 49 °C).

Size-exclusion chromatography and analytical ultracentrifugation were used to determine the oligomeric state of dualENH. Different initial concentrations of dualENH were run over a Superdex 75 size-exclusion column. As the protein concentration was reduced, the peak elution volume gradually moved from an earlier position (~14 ml) to a later position (~19 ml) (Extended Data Fig. 3a), indicating that dualENH is present in different oligomeric states at different concentrations. To determine its oligomeric state explicitly, a sedimentation velocity experiment was run,

which showed that the principal dualENH species present at 40 μM is a homodimer, with a very small amount of higher-order oligomers (Extended Data Fig. 3b).

We used fluorescence polarization to determine whether dualENH binds dsDNA probes strongly and specifically. The polarization will increase if a dsDNA probe is bound by dualENH due to a reduction in the tumbling rate of the larger complex. We used a fluorescein-labeled dsDNA probe containing the ENH binding motif TAATTA (probe-1) that had previously been used in wild-type ENH binding studies[25]. The polarization of a 25 nM solution of probe-1 increased from its intrinsic value of ~140 mP to a plateau of 210 mP as the concentration of dualENH was increased from 0 nM to 100 nM (Extended Data Fig. 3d). The same experiment run with wild-type ENH showed a very similar trend and polarization values (Extended Data Fig. 4a), indicating that dualENH and wild-type ENH have similar binding affinities to probe-1. To test the binding specificity, we designed probe-2, which is identical to probe-1 except for a single mutation to its binding motif (TA[C]TTA). Compared to probe-1, a weaker response was observed between probe-2 and dualENH: polarization increased to only 176 mP at 100 nM dualENH and did not plateau until the concentration of dualENH reached 10 μM (Extended Data Fig. 3d). An additional probe that had previously been used as a negative control[25] for wild-type ENH binding was also tested. As expected, neither dualENH nor wild-type

ENH showed observable binding to this probe at 100 nM of protein (Extended Data Fig. 4b). Together, these data show that dualENH binds strongly and specifically to the wild-type ENH binding motif, TAATTA.

We next sought to confirm that each dualENH homodimer could bind two dsDNA fragments as illustrated in Fig. 1c. Using a Förster resonance energy transfer (FRET)-based experiment, a 15-nt dsDNA sequence $(TAA)_5$ was labeled with Cy3 or Cy5 dye to serve as a FRET donor or acceptor, respectively. We mixed the two labeled $(TAA)_5$ probes with dualENH and observed a strong FRET signal as shown in Extended Data Fig. 3e, indicating that the two pieces of dsDNA were brought within Förster distance by dualENH. To ensure that the spectral change was not caused by interactions between dualENH and the fluorophores, we performed two control experiments with Cy3-(TAA)5 or Cy5-(TAA)5 with dualENH. Extended Data Fig. 3f shows that The Cy3-(TAA)5 or Cy5-(TAA)5 emission intensity slightly increased and decreased, respectively, after adding dualENH. This change is the opposite direction of the FRET signal, further emphasizing the strong FRET signal observed in Extended Data Fig. 3e. These experiments indicate that the DNA-binding domain and the homodimerization domain of dualENH are structurally independent, and that they can function cooperatively.