Supplemental material

Supplemental figure, Fig. S1 *p. 2*
Supplemental figure, Fig. S2 *p. 3*
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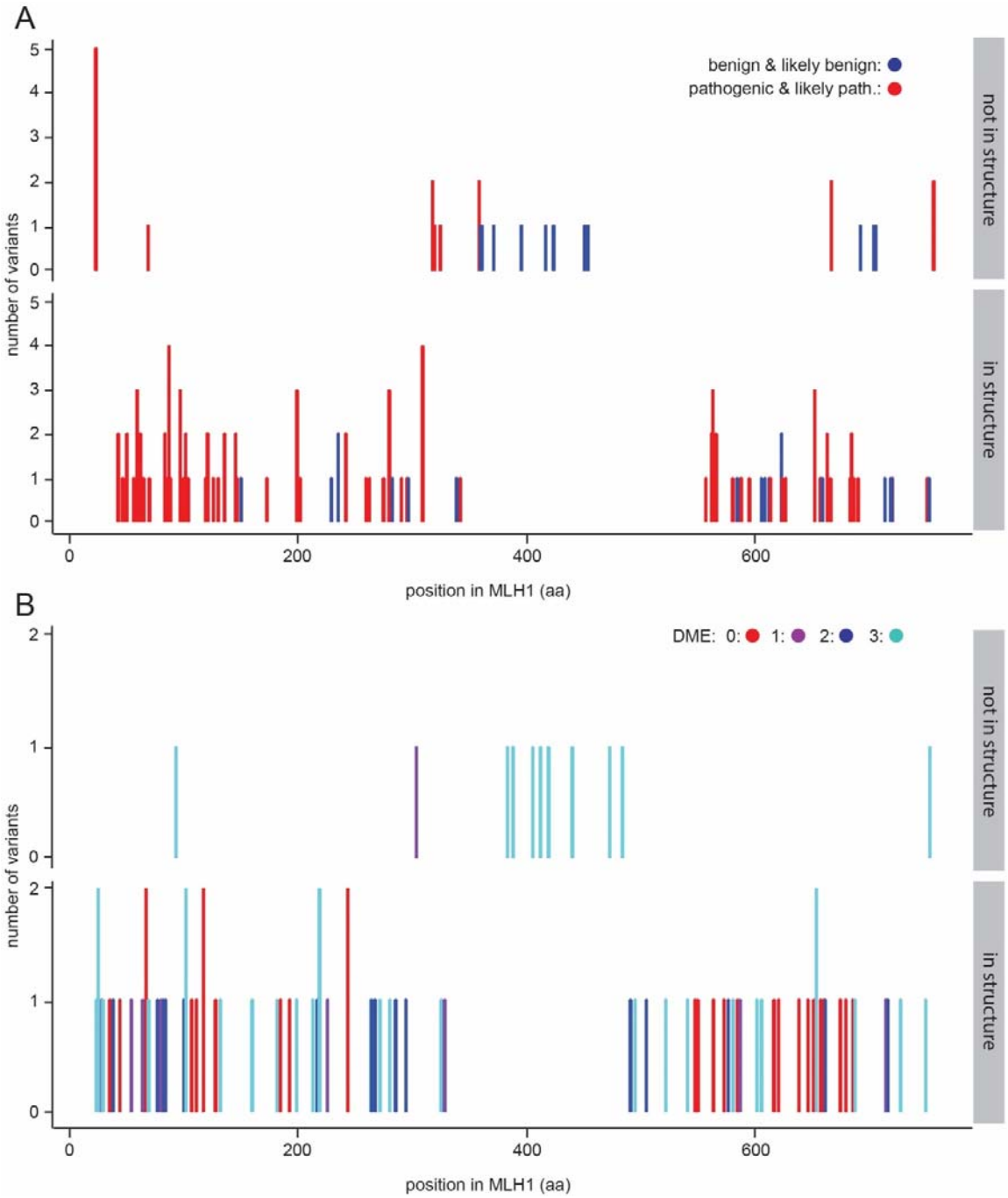

Figure S1 – *Variants within the central disordered region.* (A) ClinVar benign and likely benign variants (blue) are found throughout the protein, while pathogenic and likely pathogenic variants (red) are enriched in the structured domains. (B) The variants previously tested for function (Takahashi et al., 2007) are distributed throughout the MLH1 protein. Similar to the observations from ClinVar, functional variants (DME score 3) are seen throughout the protein, while loss-of-function in multiple assays (DME scores <2) are enriched in the structured domains.

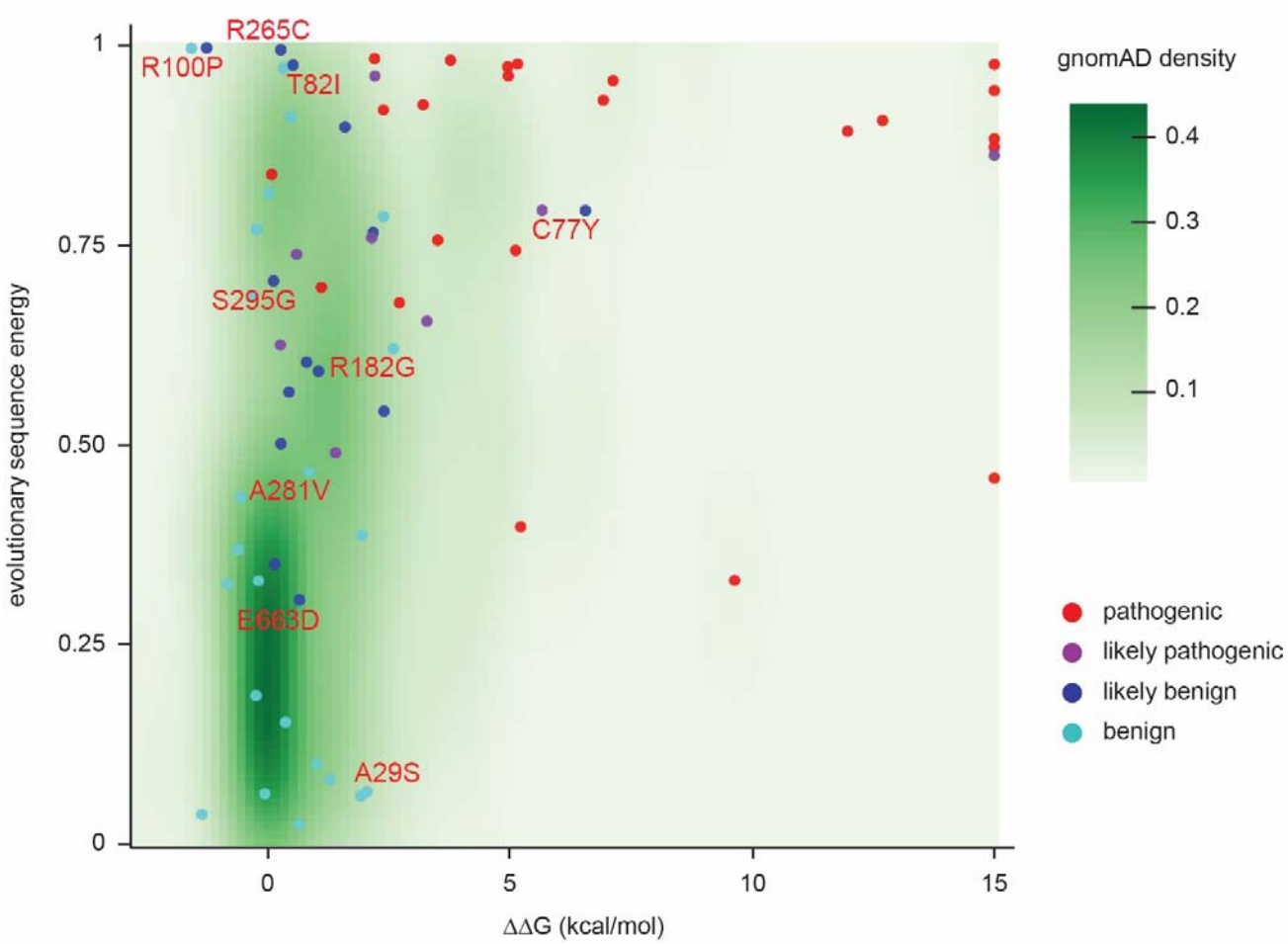

Figure S2 – *Landscape of ClinVar MLH1 variant tolerance*. Landscape of variant tolerance by combination of changes in protein stability (x axis) and evolutionary sequence energies (y axis), such that the upper right corner indicates most likely detrimental variants, while those in the lower left corner are predicted stable and observed in MLH1 homologs. The green background density illustrates the distribution of all variants listed in gnomAD. Outliers are discussed in the main text.

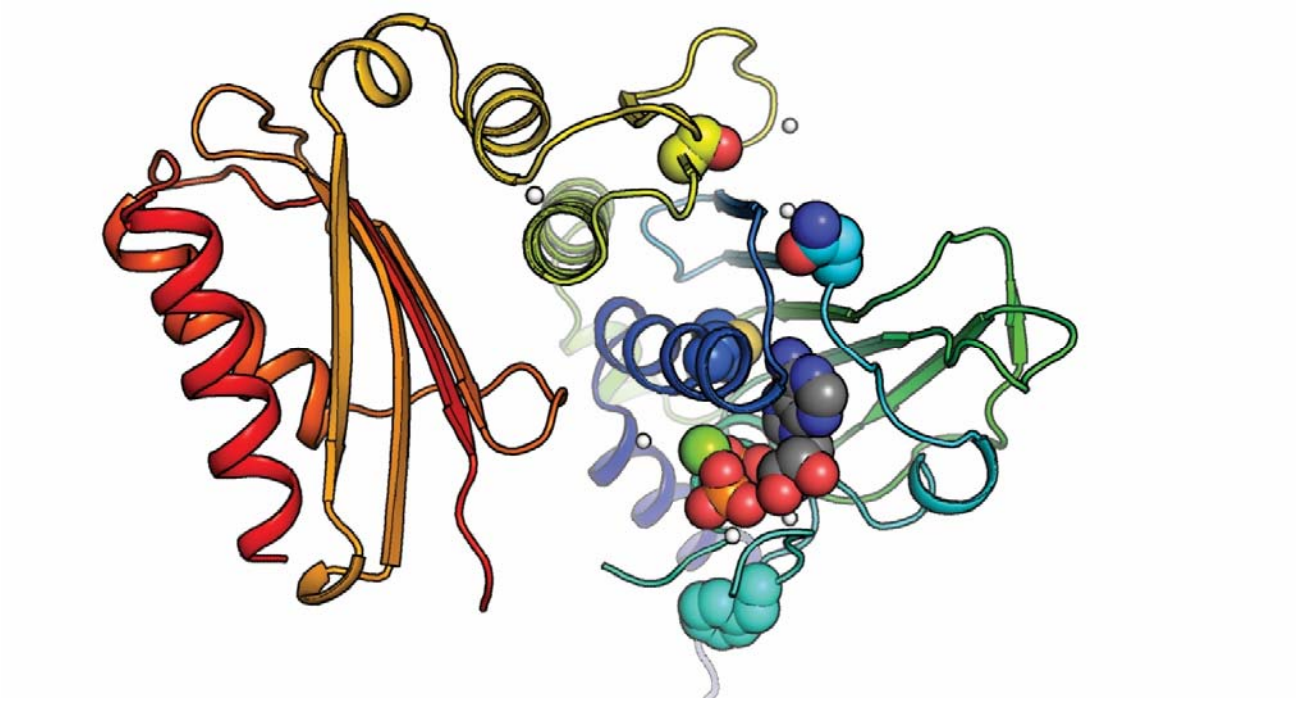

Figure S3 – *Positioning of selected variants near the active site in the N-terminal domain*. The MLH1 structure (PDB: 4P7A) (Wu et al., 2015) with highlighted stable loss-of-function MLH1 variants (M35R, N64S, F80V, S193P) as spheres, as well as ADP. The domain is colored in a rainbow color scheme, with blue at the N-terminus and red at the C-terminal end of the N-terminal domain (MLH1 sequence position ~300); sidechain and ligand oxygen atoms are red, nitrogens blue, ligand carbons gray. Three of the stable loss-of-function positions (M35R, N64S, F80V) are very close to the ligand. Variation at these sites may thus interfere with ligand binding, which could explain why they lead to loss of function despite wild-type-like cellular protein levels.

Supplemental references

Takahashi, M., Shimodaira, H., Andreutti-Zaugg, C., Iggo, R., Kolodner, R.D., and Ishioka, C. (2007). Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays. Cancer Res. *67*, 4595-4604.

Wu, H., Zeng, H., Lam, R., Tempel, W., Kerr, I.D., and Min, J. (2015). Structure of the human MLH1 N-terminus: implications for predisposition to Lynch syndrome. Acta Crystallogr. F. Struct. Biol. Commun. *71*, 981-985.