

Supporting Information

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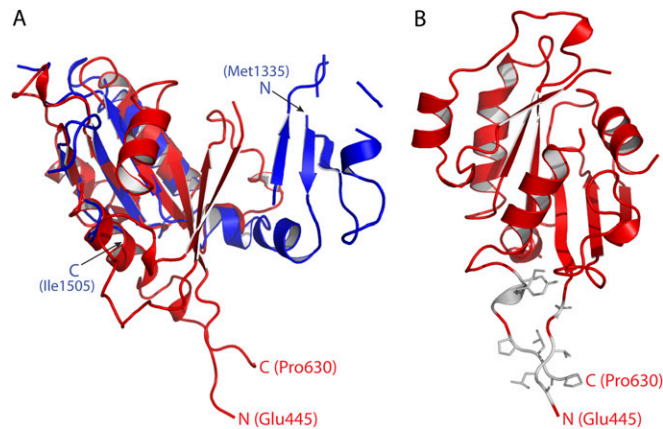


Fig. S1. Structures of the Ras of complex proteins (Roc) domain. (A) Cartoon representation of human (blue) and *Chlorobium tepidum* (red) superimposed. N and C termini are marked with fond colors matching each structure. This shows the N and C termini of human Roc is far apart, whereas the termini of *C. tepidum* are close together. (B) Cartoon representation of Roc domain of *C. tepidum* showing a hydrophobic patch near the termini. Hydrophobic residues are shown as gray sticks.

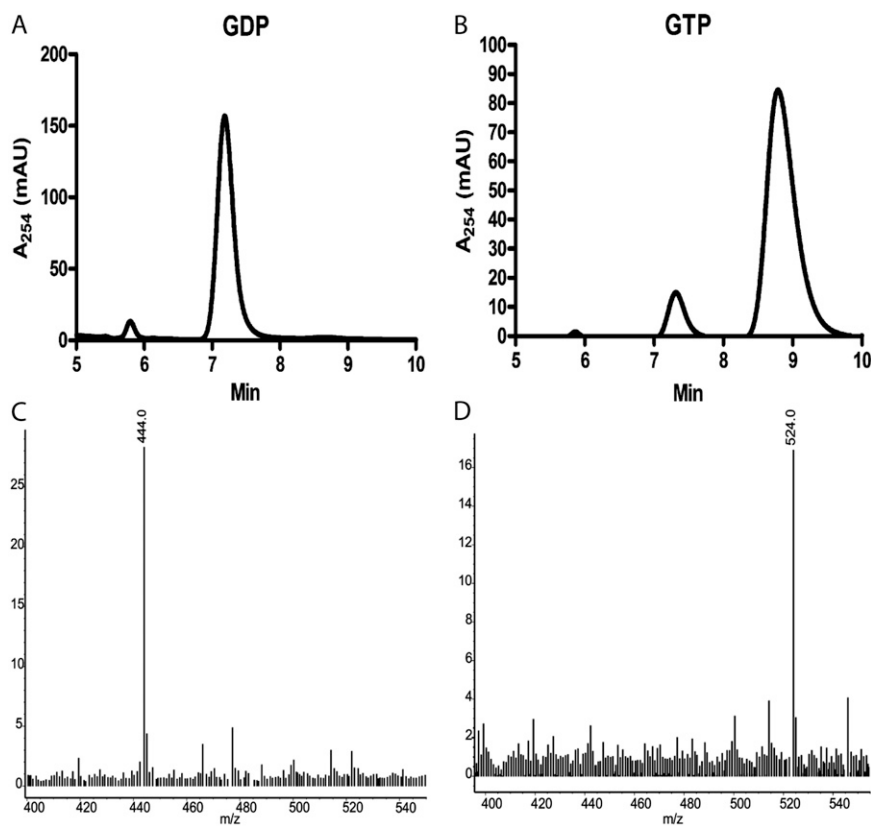


Fig. S2. Liquid chromatography–tandem MS (LC/MS/MS). (A and B) Hydrophilic interaction liquid chromatography (HILIC) HPLC profile of control GDP (A) and GTP (B) showing retention time at 7.2 and 8.9 min, respectively. This control experiment helped us find the peaks in our reaction mixture that corresponding to each nucleotide. (C and D) Electrospray mass spectrometry in tandem with HILIC HPLC confirmed the identity of Roc GTPase product GDP (C) and the remaining substrate GTP (D) in each reaction presented in Fig. 4A.

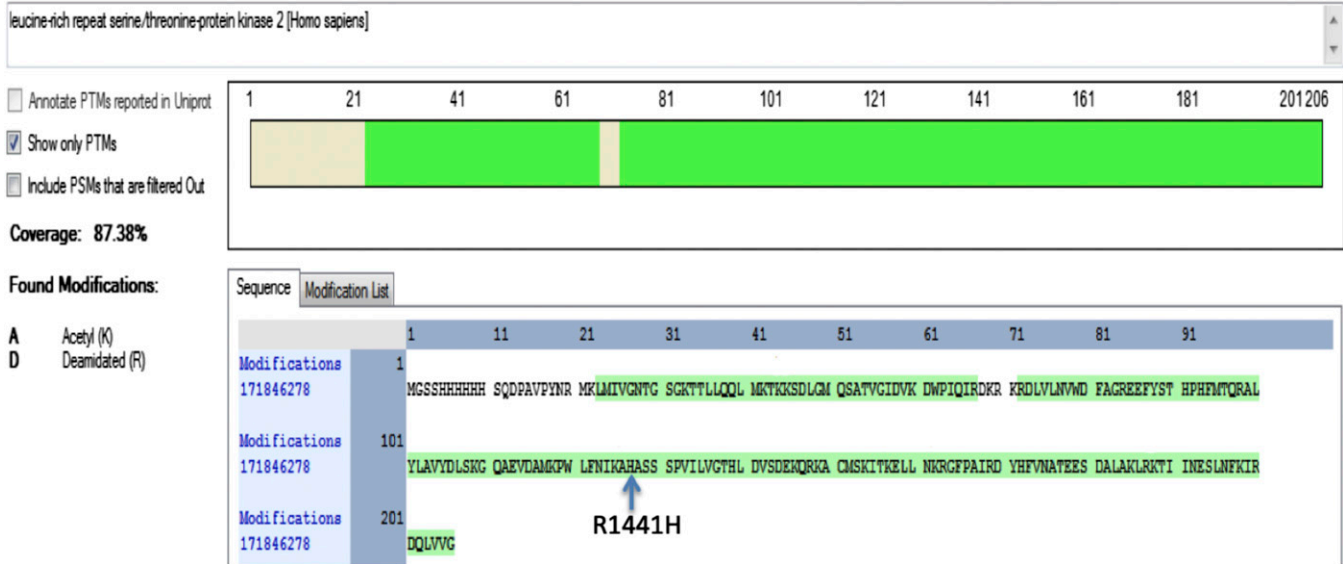


Fig. S3. LC/MS/MS-based protein sequencing confirming successful substitution of residue lysine at 1441 for histidine.