

**Structural basis for the simultaneous recognition of NEMO and acceptor ubiquitin by the
HOIP NZF1 domain**

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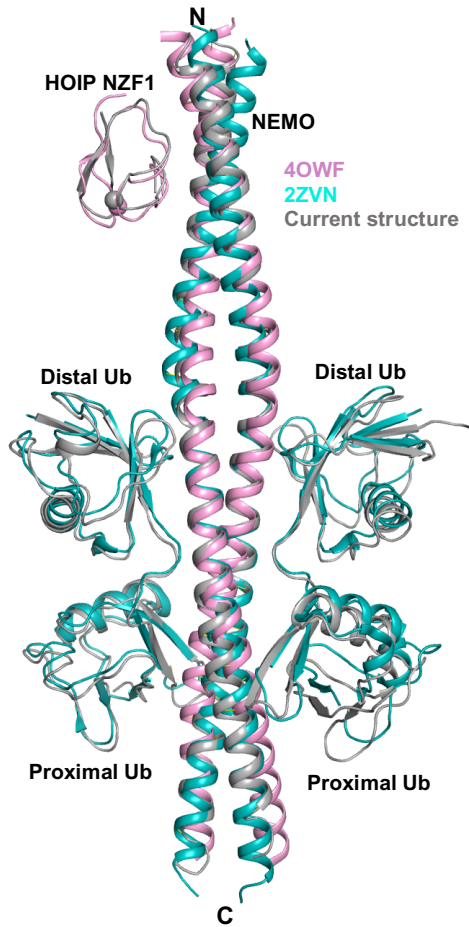
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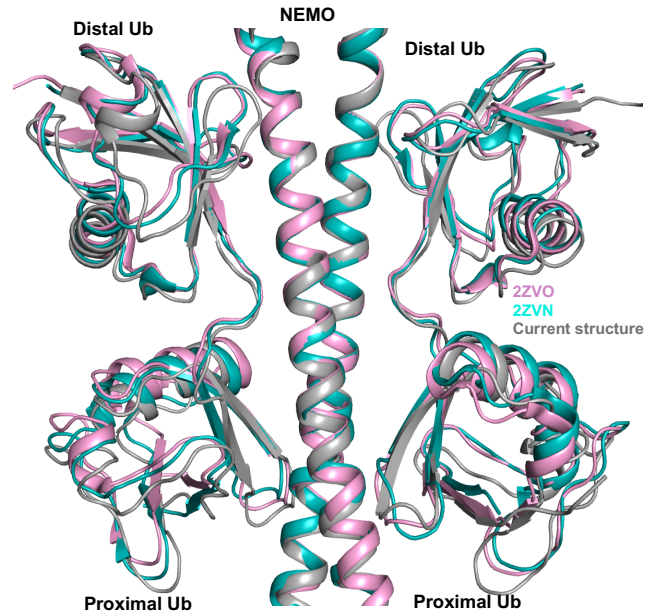
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Supplementary Figure S1

(A)



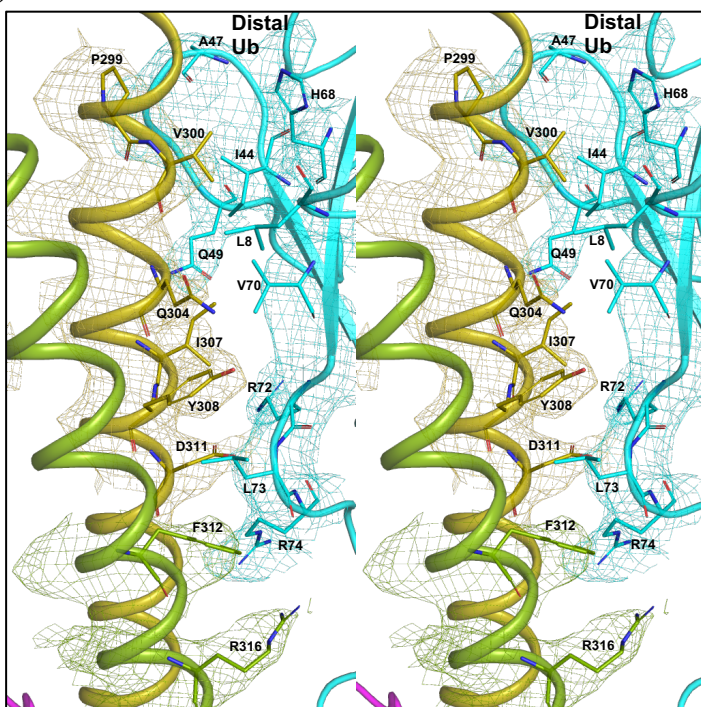
(B)



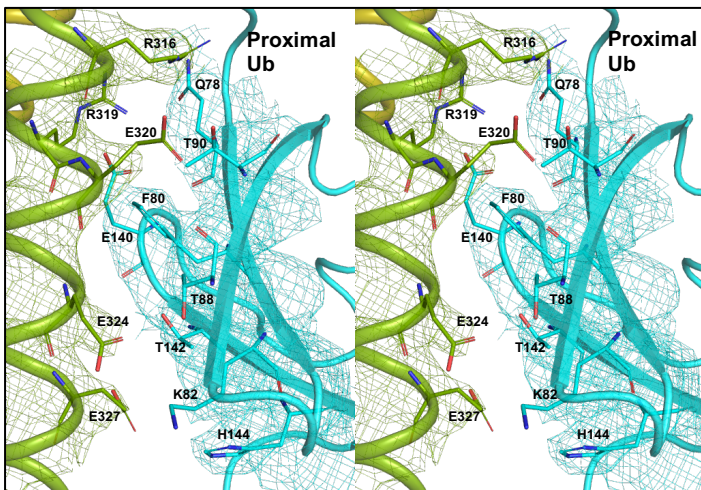
Supplementary Figure S1. NEMO in the heteropentameric structure adopts the same binding mode for HOIP-NZF1 and linear diubiquitins as in the heterotrimeric and heterotetrameric structures. (A) Superimposition of the C α atoms of NEMO CoZi in the heteropentameric crystal structure (Gray) on NEMO in complex structures containing HOIP NZF1 (PDB ID: 4OWF, pink) and diubiquitins (PDB ID: 2ZVN, cyan). (B) Superimposition of the C α atoms of UBAN in the heteropentameric structure (Gray) on the UBAN in crystal structures containing linear diubiquitins (2ZVO and 2ZVN).

Supplementary Figure S2

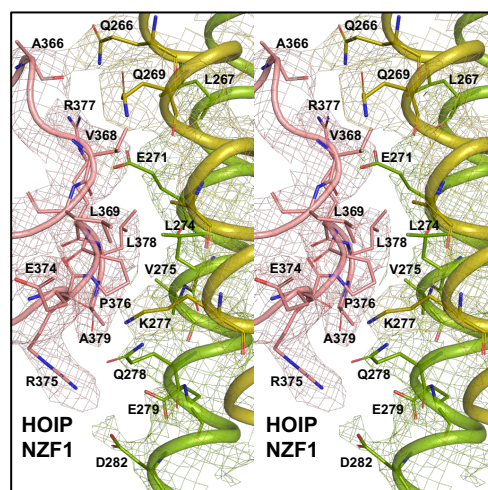
(A)



(B)

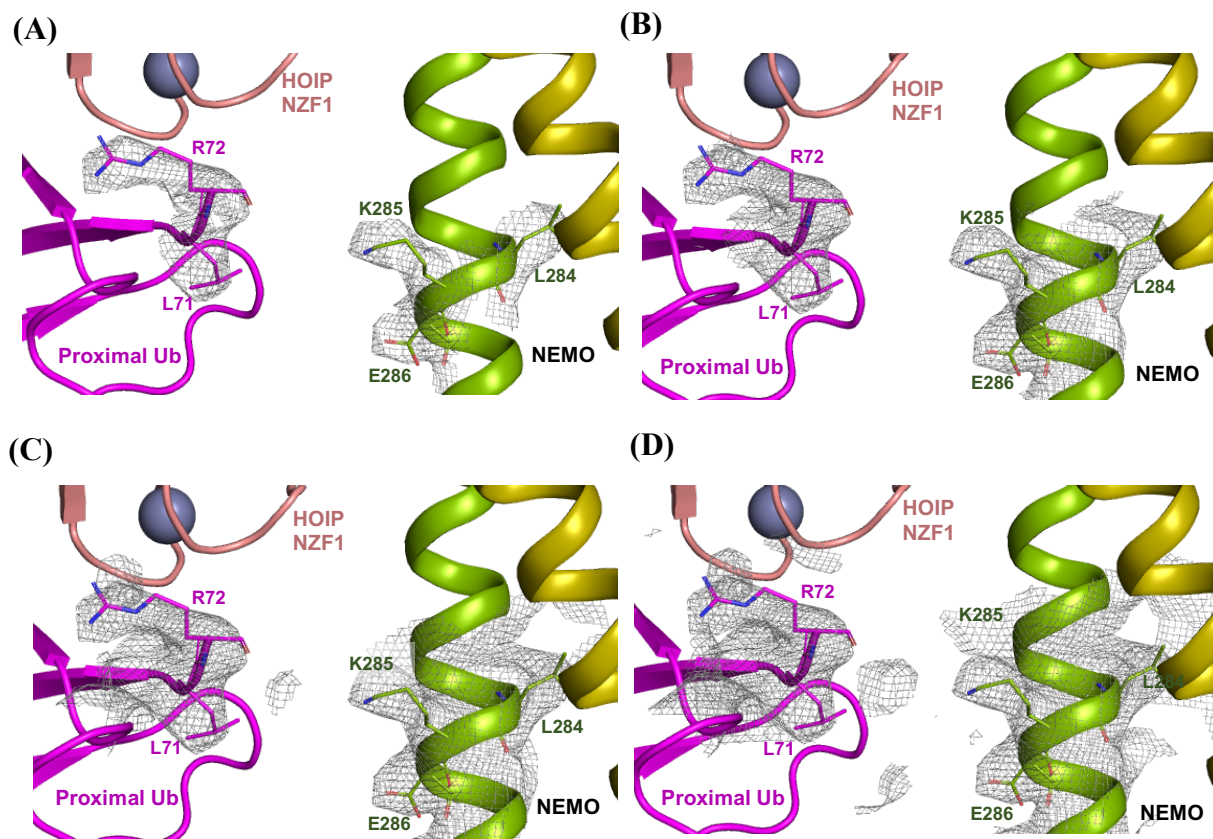


(C)



Supplementary Figure S2. Stereo-view of interactions of NEMO with distal (A) and proximal (B) ubiquitin in a linear diubiquitin chain, and NEMO with HOIP NZF1 (C). The final $2F_o - F_c$ electron density map is contoured at 1.0σ .

Supplementary Figure S3



Supplementary Figure S3. The C-terminal tail of proximal ubiquitin, including residues L73, R74, G75, and G76, is not visible in the electron density map. (A) to (D) represent the 2Fo - Fc electron density map contoured at 1.0 σ , within 1.6 \AA , 3.0 \AA , 4.0 \AA , and 5 \AA of the L71-R72 of proximal ubiquitin and L284-E286 of NEMO, respectively.