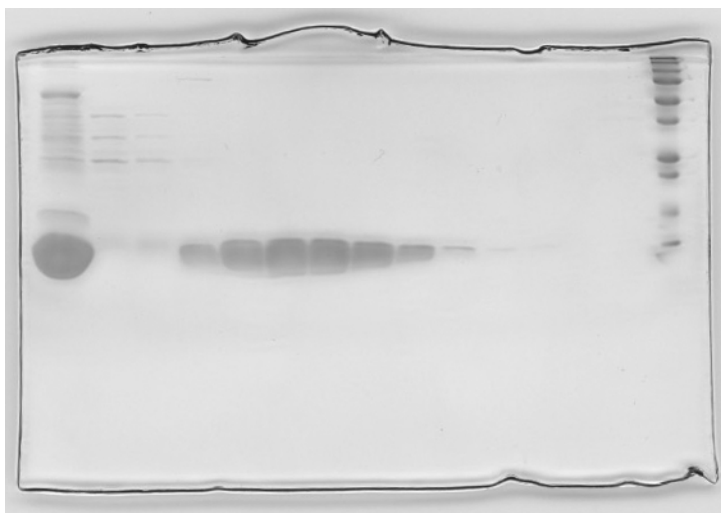
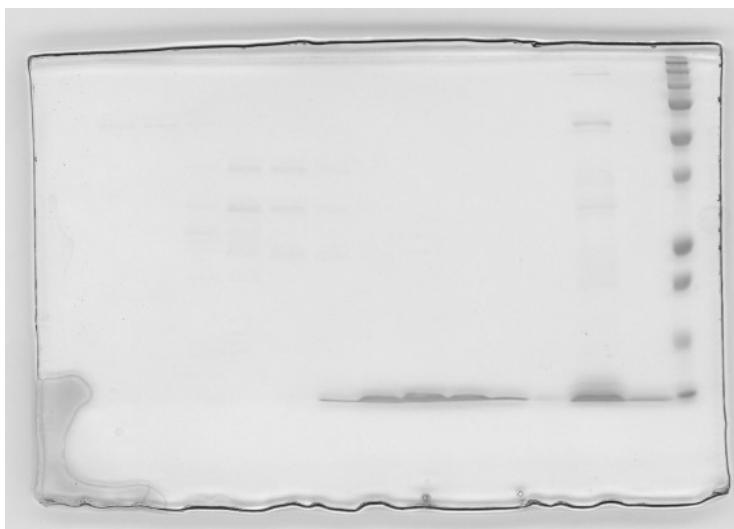


1. Mono-Ub variants purification:

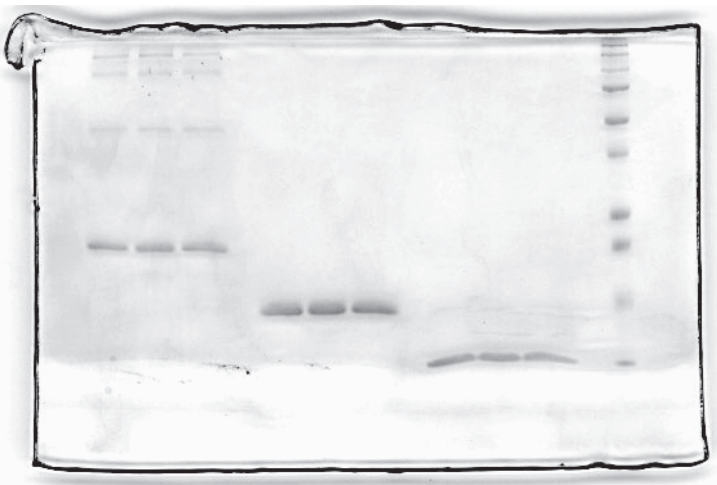
(A) Ub-WT mutant, S200, gel filtration



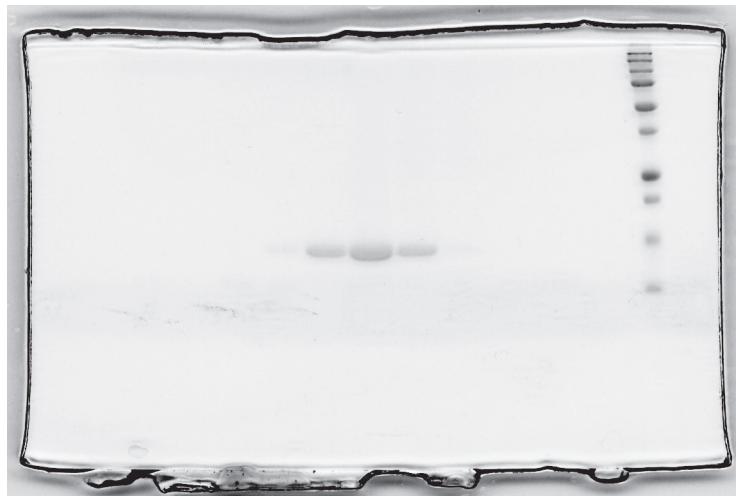
(B) I44A mutant, S200, gel filtration



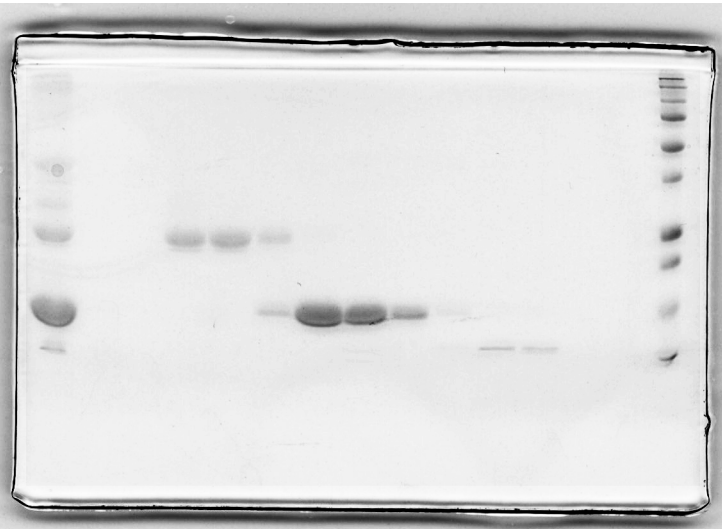
2. K63 linked di-Ub purification:



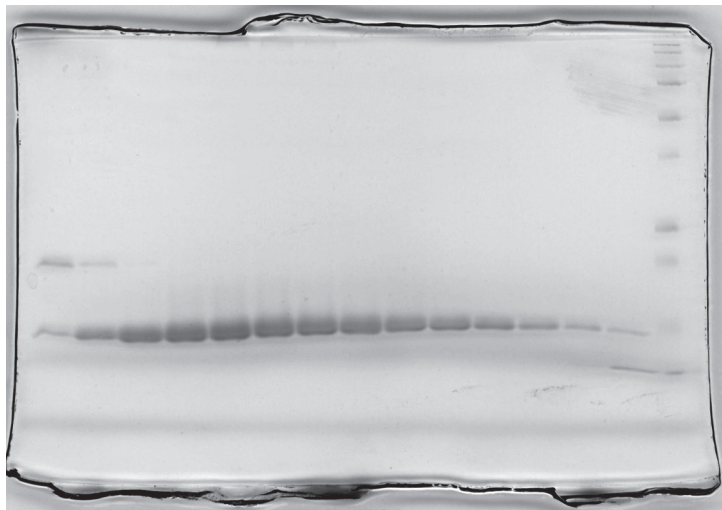
3. M1 linked di-Ub purification:



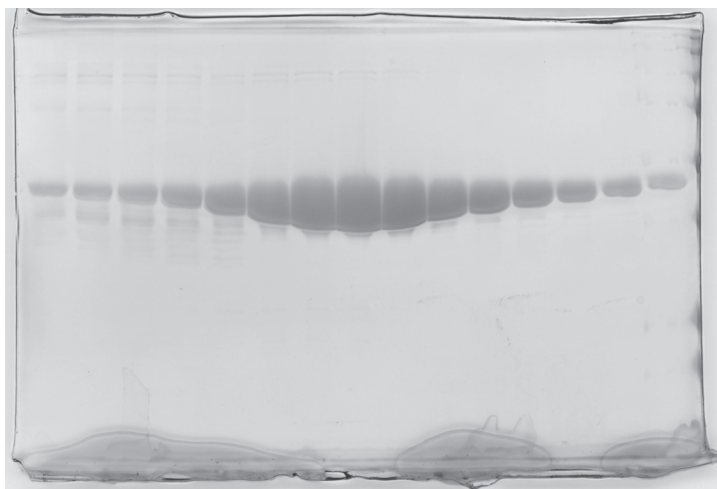
4. K48 linked di-Ub purification:



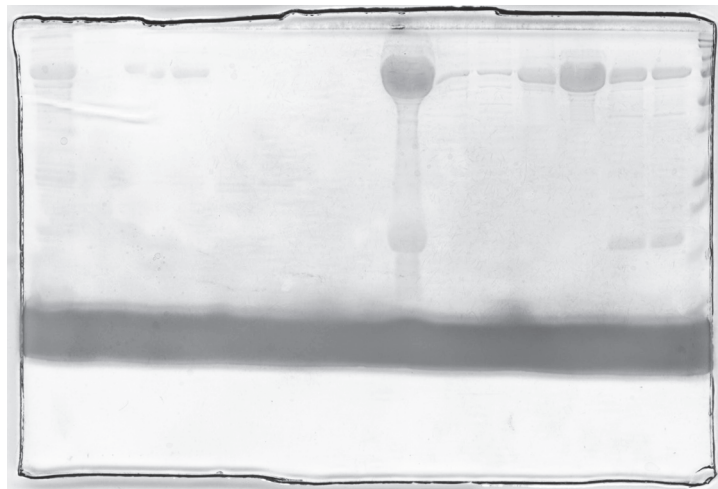
5. K63 linked di-Ub (Ub^{WT}-Ub^{I44A}) purification:



6. Rsp5 HECT domain purification:



7. Rsp5 ww1-HECT domain purification:



8. PY1-2(Art1) and PY1-2(*art1*^{ΔPY}) purification:

(A) PY1-2(Art1), S200, gel filtration

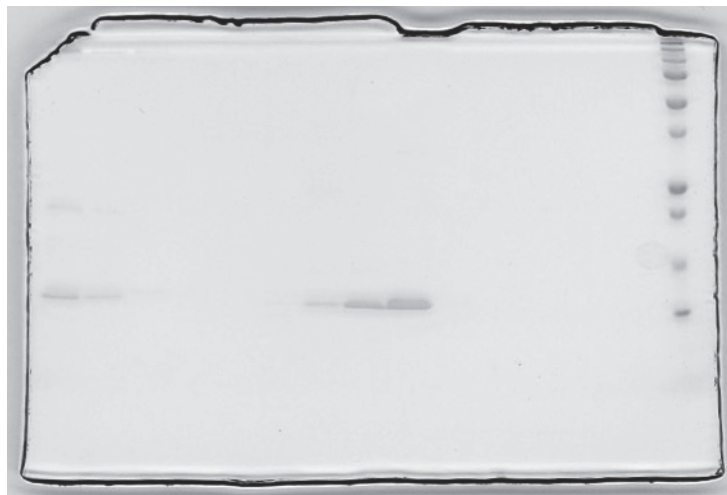


(B) PY1-2(*art1*^{ΔPY}), S200, gel filtration

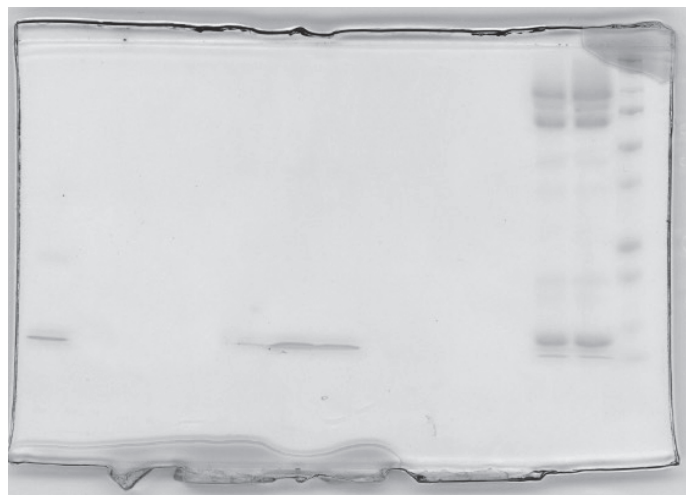


9. PY1-3(Art5) and PY1-3(*art5*^{ΔPY}) purification:

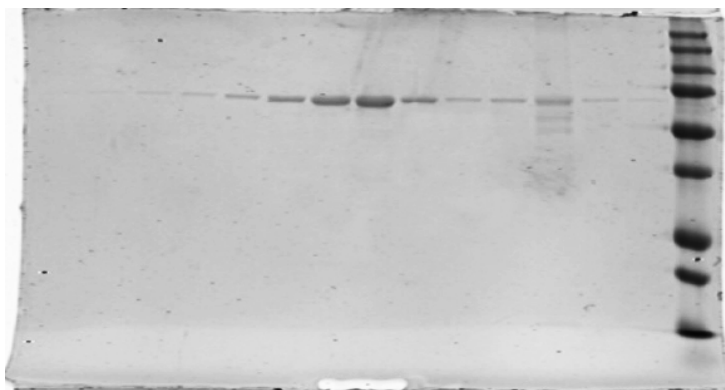
(A) PY1-3(Art5), S200, gel filtration



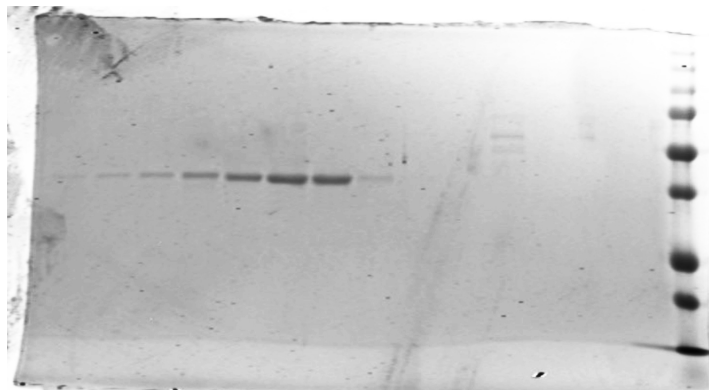
(B) PY1-3(*art5*^{ΔPY}), S200, gel filtration



10. Pub1 purification:

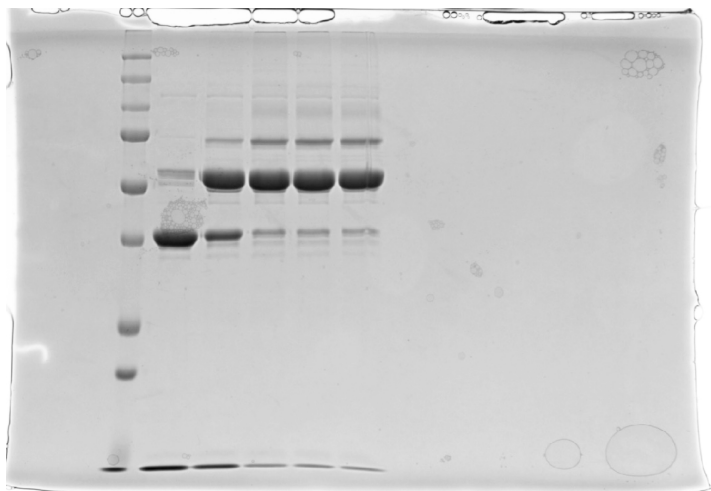


11. Any1 purification:



12. Any1-diUb purification.

(A) Synthesis of Any1-diUb



(B) Any1-diUb purification by S200 gel filtration.

