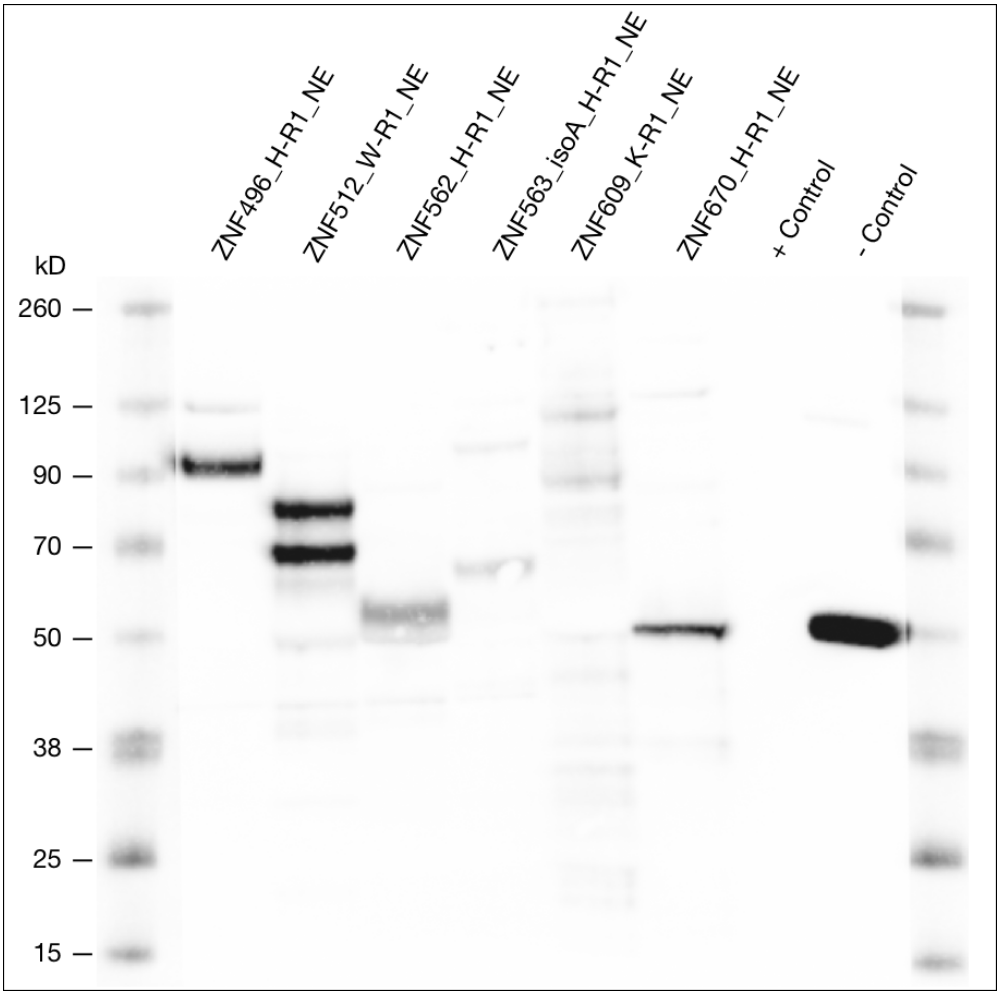
ZNF496 (*Homo sapiens*), ZNF512 (*Homo sapiens*), ZNF562 (*Homo sapiens*), ZNF563 (*Homo sapiens*), ZNF609 (*Homo sapiens*), and ZNF670 (*Homo sapiens*)

Method:
Western Blot Validation

Caption:
Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear/cyto protein isolate (500 uL) using the FLAG Immunoprecipitation Kit (Sigma-Aldrich; cat# FLAGIPT1). The final elution step was performed by suspending the sample-bound resin in NuPage Sample Reducing Agent 10X and NuPage LDS Sample Buffer 4X (Thermo Fisher Scientific) and heating for 3 minutes at 90C. Followed by cooling on ice, the protein samples were loaded onto a NuPage 4-12% Bis-Tris gel (Thermo Fisher Scientific) and separated using a PowerEase 90W system (Thermo Fisher Scientific) running at 150 V for 1 hour. A blank IP was included as a negative control, and an immunoprecipitated FLAG-BAP fusion protein provided in the kit as a positive control. The protein bands were transferred to a nitrocellulose membrane using the Invitrogen iBlot 2 System (Thermo Fisher Scientific), and blocked overnight at 4C in 5% milk solution with gentle rocking. The membrane was treated with a 1:5000 dilution of monoclonal M2-Peroxidase-conjugated ANTI-FLAG antibody (diluted in 5% BSA solution) (Sigma-Aldrich; cat# A8592) for 1 hour. Following four 5-minute washes with 1X TBST, visualization was attained with the Super Signal West Femto solution kit (Thermo Fisher Scientific) and a MyECL Imager (Thermo Fisher Scientific).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-ZNF496-HepG2 rep 1 (nuclear extract)	67	Distinct band within 20% of the expected size.
3	FLAG-ZNF512-WTC11 rep 1 (nuclear extract)	65	Distinct band near 68 kDa and 75 which is the same size observed in https://www.sigmaaldrich.com/US/en/product/sigma/av30060 PTMs: Isopeptide bond, Ubl conjugation
4	FLAG-ZNF562-HepG2 rep 1 (nuclear extract)	49	Distinct band within 20% of the expected size.
5	FLAG-ZNF563_isoA-HepG2 rep 1 (nuclear extract)	55	Distinct band within 20% of the expected size.
6	FLAG-ZNF609-K562 rep 1 (nuclear extract)	151	No distinct bands within correct size.
7	FLAG-ZNF670-HepG2 rep 1 (nuclear extract)	45	Distinct band within 20% of the expected size.
8	Negative Control (Blank IP)	NA	PASS
9	Positive Control (FLAG BAP fusion protein)	NA	PASS
10	Ladder	N/A	N/A

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