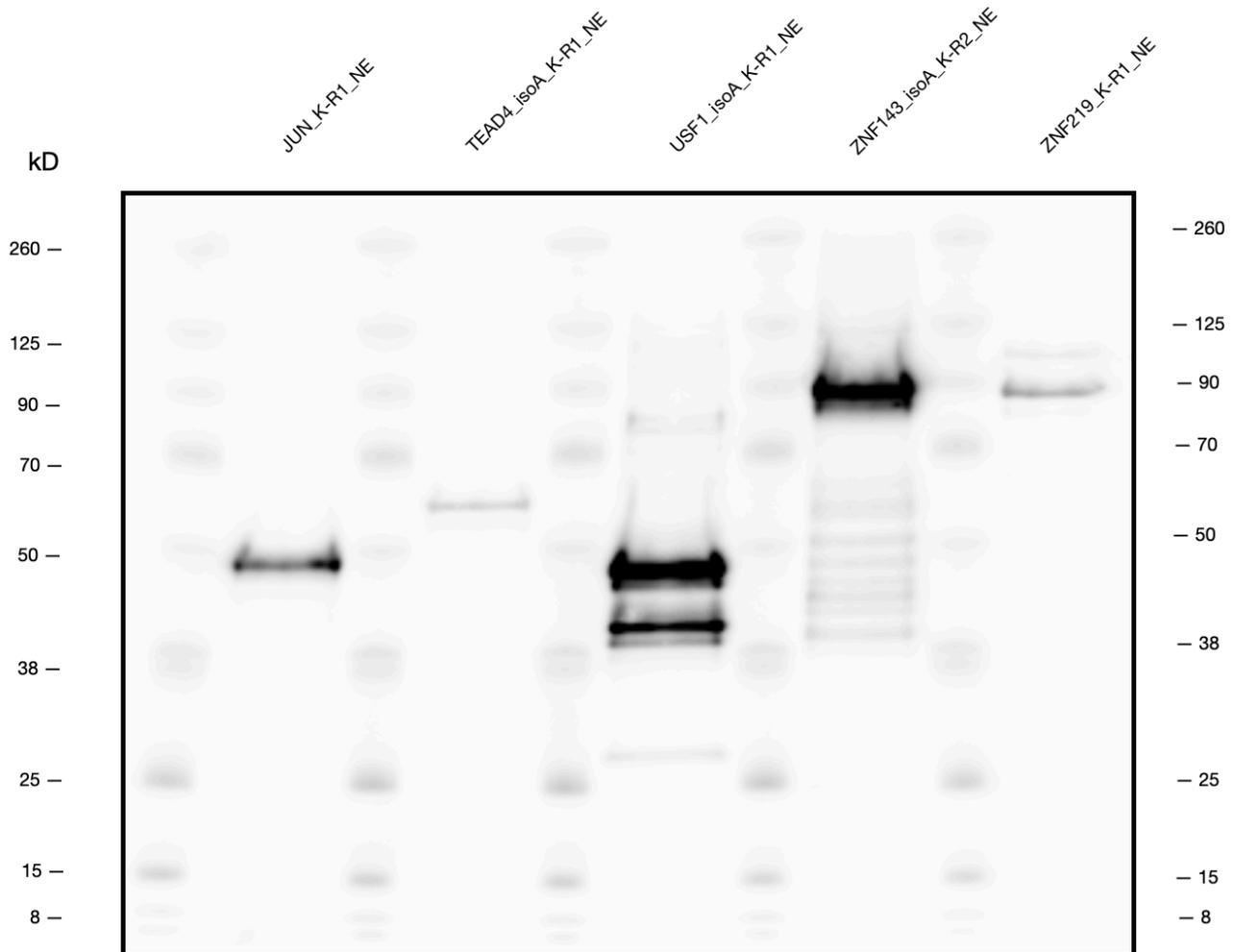


**JUN (*Homo sapiens*), TEAD4 (*Homo sapiens*), USF1 (*Homo sapiens*), ZNF143 (*Homo sapiens*), and ZNF219 (*Homo sapiens*)**

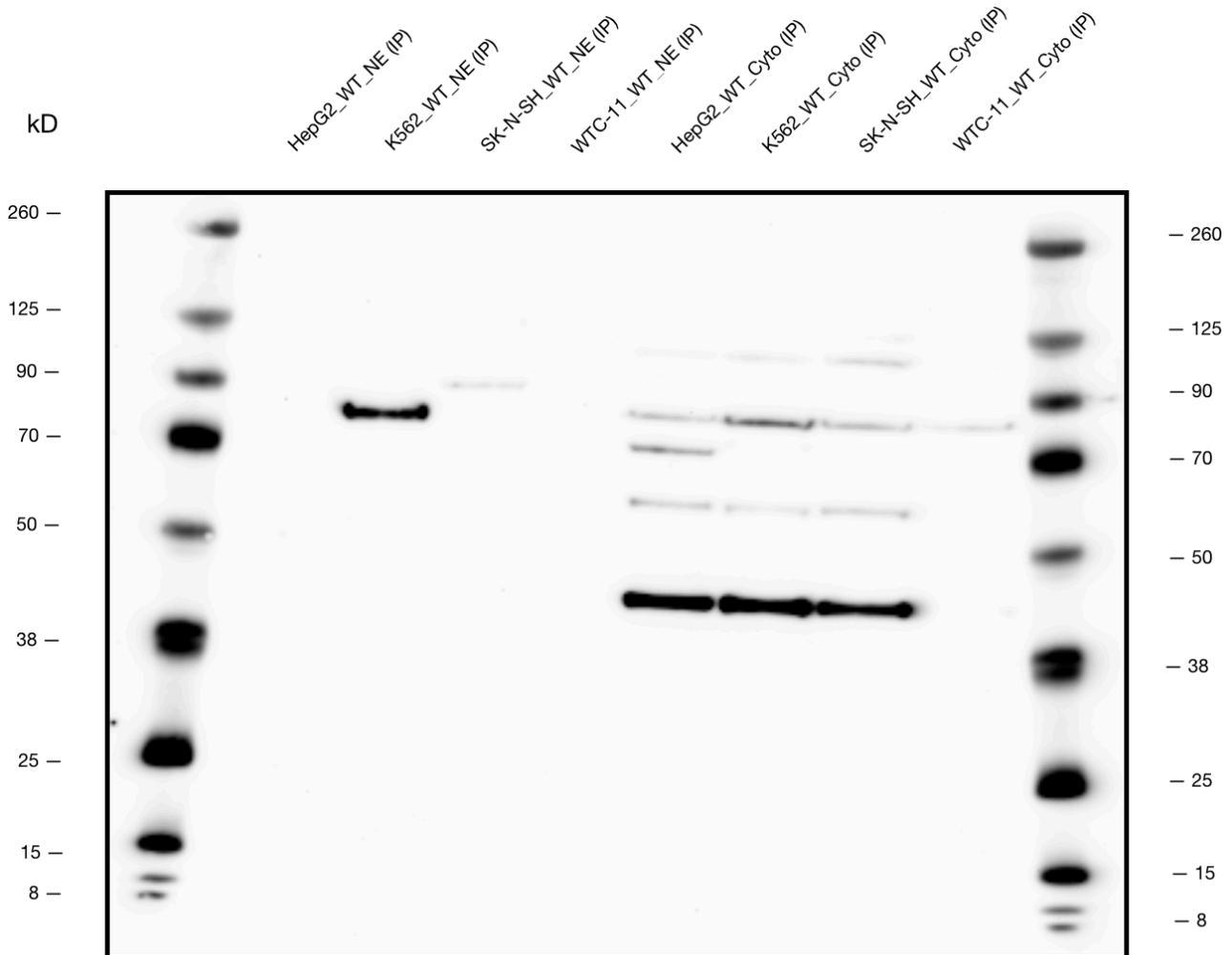
**Method:**  
Western Blot Validation

**Caption:**  
Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear 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. 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). The second western blot image depicts a negative control IP prepared with K562 nuclear lysate (Lane 3).



Monoclonal ANTI-FLAG M2-Peroxidase (HRP) antibody produced in mouse

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-JUN_K562 rep 1 (nuclear extract)	39	Predicted size was 39 kDa. The observed size was 49 kDa, which is within 20% of an observed band of 45 kDa seen in <a href="https://www.novusbio.com/products/c-jun-antibody-320831_mab2670">https://www.novusbio.com/products/c-jun-antibody-320831_mab2670</a> . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	Ladder	N/A	N/A
4	FLAG-TEAD4_isoA_K562 rep 1 (nuclear extract)	51	Single distinct band within 20% of the predicted size
5	Ladder	N/A	N/A
6	FLAG-USF1_isoA_K562 rep 1 (nuclear extract)	37	Predicted size was 37 kDa. The observed sizes were 48 kDa and 40 kDa, which are within 20% of observed bands of 44 kDa and 38 kDa seen in <a href="https://www.ptglab.com/Products/USF1-Antibody-67114-1-ig.htm">https://www.ptglab.com/Products/USF1-Antibody-67114-1-ig.htm</a> . PTMs: Isopeptide bonding and Ubl conjugation
7	Ladder	N/A	N/A
8	FLAG-ZNF143_isoA_K562 rep 2 (nuclear extract)	72	Predicted size was 72 kDa. The observed size was 89 kDa, which is within 20% of an observed band of 82 kDa seen in <a href="https://www.antibodies-online.com/antibody/563441/anti-Zinc+Finger+Protein+143+ZNF143+AA+1-626+antibody/">https://www.antibodies-online.com/antibody/563441/anti-Zinc+Finger+Protein+143+ZNF143+AA+1-626+antibody/</a> . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
9	Ladder	N/A	N/A
10	FLAG-ZNF219_K562 rep 1_NE (nuclear extract)	80	Distinct band within 20% of the predicted size. PTMs: Phosphorylation



Monoclonal ANTI-FLAG M2-Peroxidase (HRP) antibody produced in mouse

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	HepG2 Wild-Type (nuclear extract IP)	None	No visible banding
3	K562 Wild-Type (nuclear extract IP)	None	Dark band near 80 kDa
4	SK-N-SH Wild-Type (nuclear extract IP)	None	Band near 90 kDa
5	WTC-11 Wild-Type (nuclear extract IP)	None	No visible banding
6	HepG2 Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, 70 kDa, and 60 kDa. Dark band at 45 kDa
7	K562 Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, and 60 kDa. Dark band at 45 kDa
8	SK-N-SH Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, and 60 kDa. Dark band at 45 kDa
9	WTC-11 Wild-Type (cytoplasmic extract IP)	None	Faint band at 85 kDa
10	Ladder	N/A	N/A

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Richard Myers, HAIB

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UM1 HG009411

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