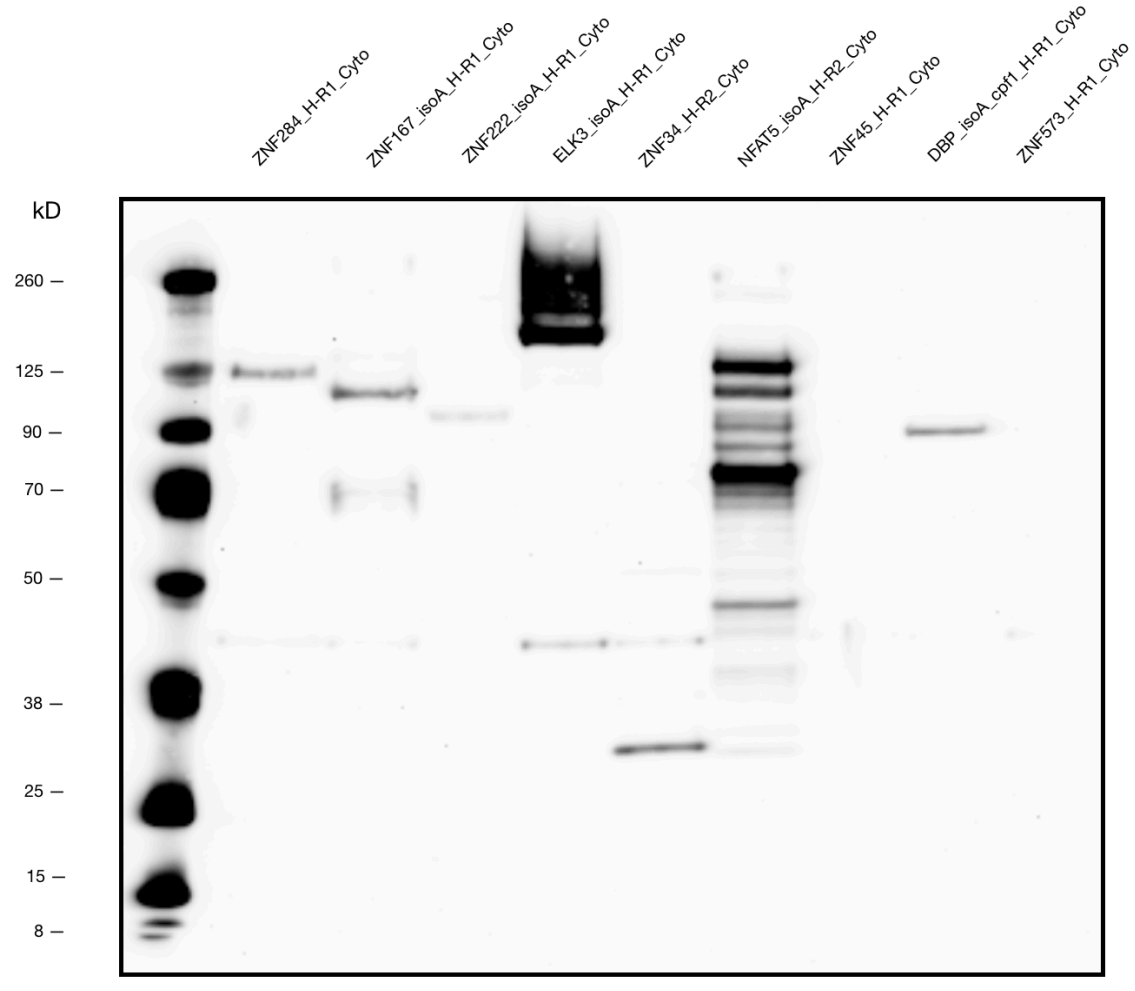
ZNF284 (*Homo sapiens*), ZNF167 (*Homo sapiens*), ZNF222 (*Homo sapiens*), ELK3 (*Homo sapiens*), ZNF34 (*Homo sapiens*), NFAT5 (*Homo sapiens*), ZNF45 (*Homo sapiens*), DBP (*Homo sapiens*), and ZNF573 (*Homo sapiens*)

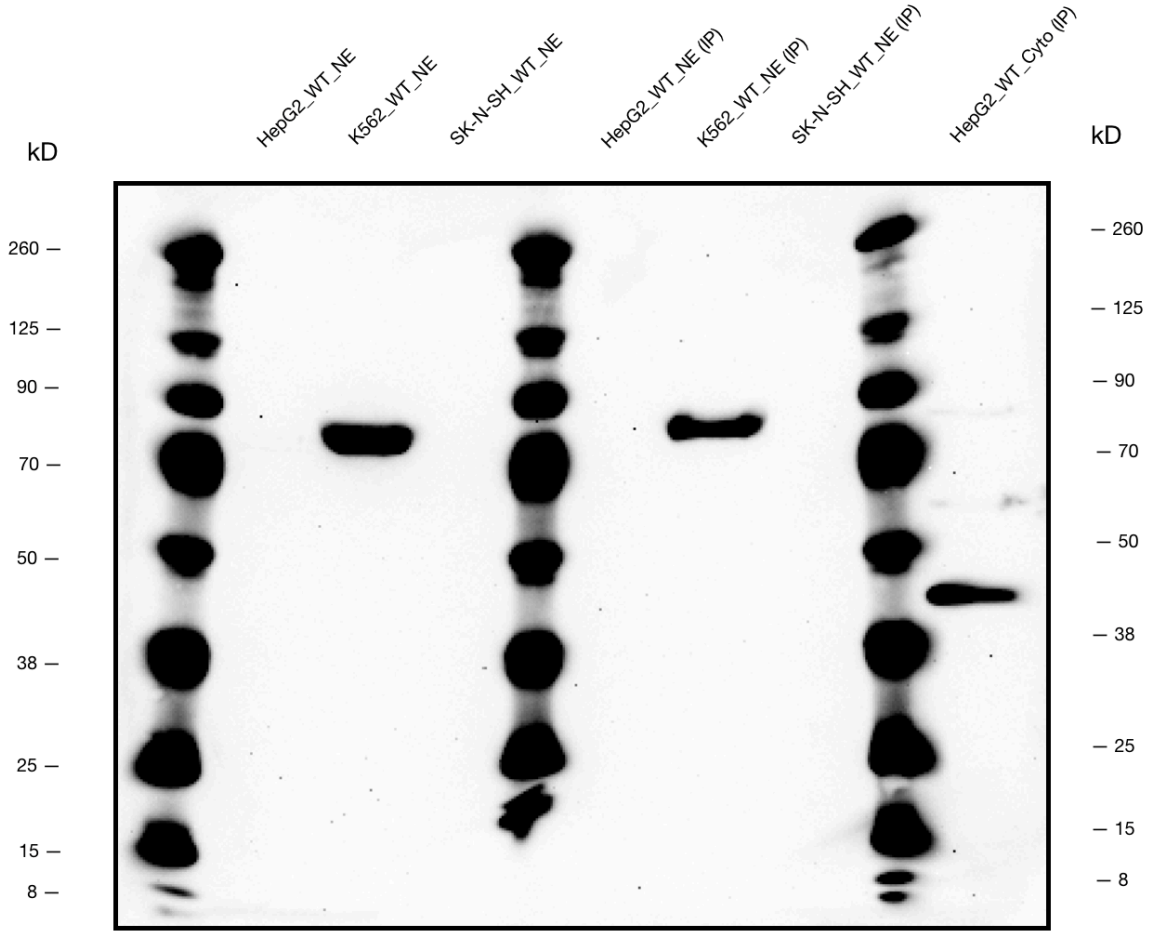
Method:
Western Blot Validation

Caption:
Each FLAG-tagged sample was immunoprecipitated from its corresponding protein isolate (1 mL) 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 HepG2 cytoplasmic lysate (Lane 10).



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-ZNF284_HepG2 rep 1 (cytoplasmic extract)	72	Single distinct band beyond 20% of the expected size
3	FLAG-ZNF167_isoA_HepG2 rep 1 (cytoplasmic extract)	88	Distinct band within 20% of the expected size. PTMs: Isopeptide bonding and Ubl conjugation
4	FLAG-ZNF222_isoA_H-R1 (cytoplasmic extract)	55	Predicted size was 55 kDa. The observed size was 87 kDa, which is within 20% of an observed band of 73 kDa seen in https://www.fishersci.com/shop/products/znf222-protein/89968079
5	FLAG-ELK3_isoA_HepG2 rep 1 (cytoplasmic extract)	47	Dark band beyond 20% of the expected size. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
6	FLAG-ZNF34_HepG2 rep 2 (cytoplasmic extract)	67	Predicted size was 67 kDa. The observed size was 31 kDa, which is within 20% of an observed band of 37 kDa seen in https://www.usbio.net/antibodies/135696/ZNF34-Zinc-Finger-Protein-34-Zinc-Finger-Protein-KOX32-KOX32. PTMs: Phosphorylation
7	FLAG-NFAT5_isoA_HepG2 rep 2 (cytoplasmic extract)	169	Predicted size was 169 kDa. The observed size was 70 kDa, which is within 20% of an observed band of 65 kDa seen in https://www.avivasysbio.com/nfat5-antibody-middle-region-arp37465-p050.html. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
8	FLAG-ZNF45_HepG2 rep 1 (cytoplasmic extract)	81	No visible banding. PTMs: Isopeptide bonding and Ubl conjugation
9	FLAG-DBP_isoA_cpf1_HepG2 rep 1 (cytoplasmic extract)	37	Predicted size was 37 kDa. The observed size was 80 kDa, which is within 20% of an observed band of 85 kDa seen in http://www.cloud-clone.com/products/PAB810Mu03.html
10	FLAG-ZNF573_HepG2 rep 1 (cytoplasmic extract)	81	No visible banding



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)	None	No visible banding
3	K562 Wild-Type (nuclear extract)	None	Single non-distinct band at around 80 kDa
4	SK-N-SH Wild-Type (nuclear extract)	None	No visible banding
5	Ladder	N/A	N/A
6	HepG2 Wild-Type (nuclear extract IP)	None	No visible banding
7	K562 Wild-Type (nuclear extract IP)	None	Single non-distinct band at around 80 kDa
8	SK-N-SH Wild-Type (nuclear extract)	None	No visible banding
9	Ladder	N/A	N/A
10	HepG2 Wild-Type (cytoplasmic extract IP)	None	Dark non-distinct band at around 45 kDa, and two lighter bands at about 60 kDa and 80 kDa

Submitted by:
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