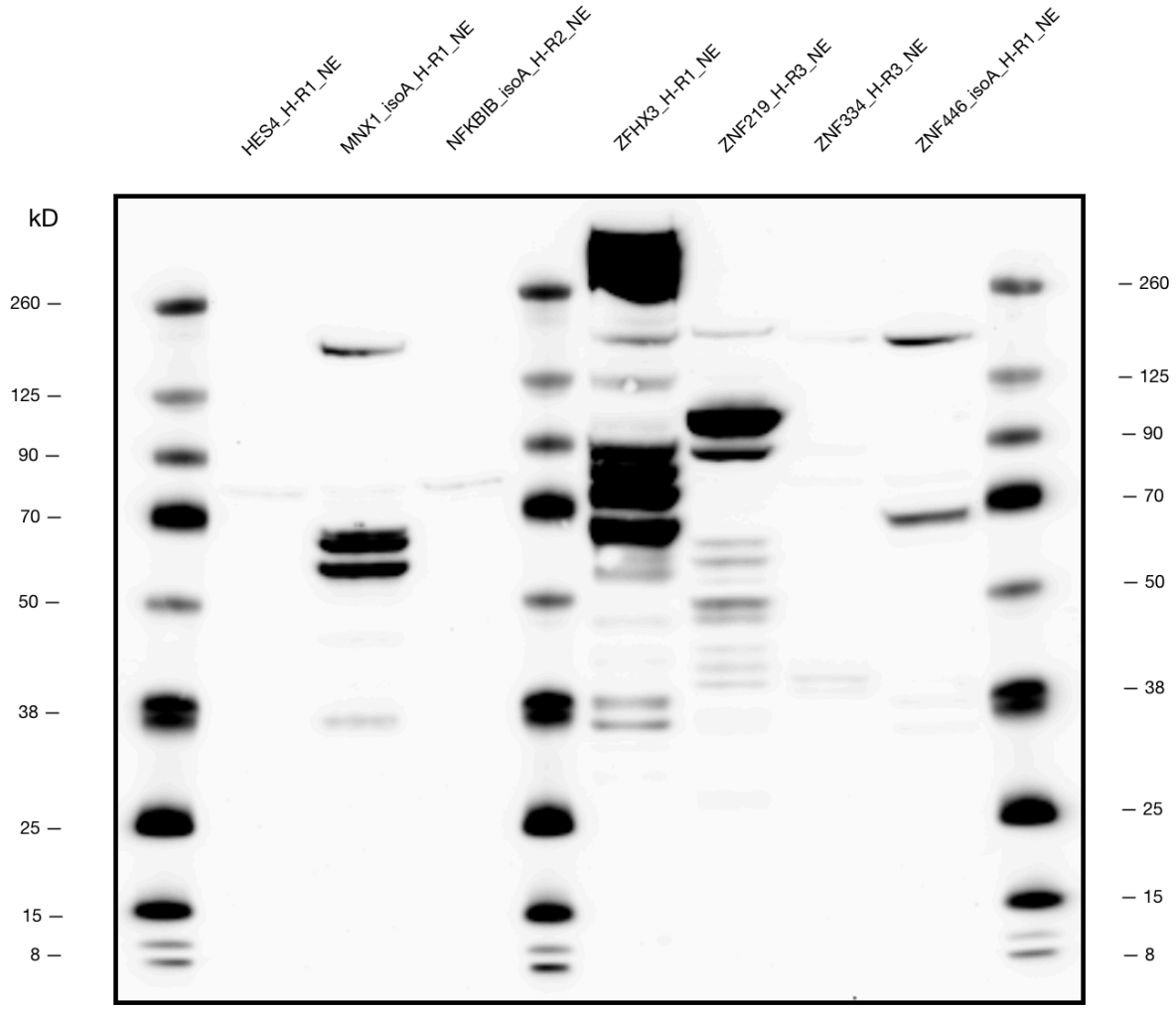
HES4 (*Homo sapiens*), MNX1 (*Homo sapiens*), NFKBIB (*Homo sapiens*), ZFHX3 (*Homo sapiens*), ZNF219 (*Homo sapiens*), ZNF334 (*Homo sapiens*), and ZNF446 (*Homo* sapiens)

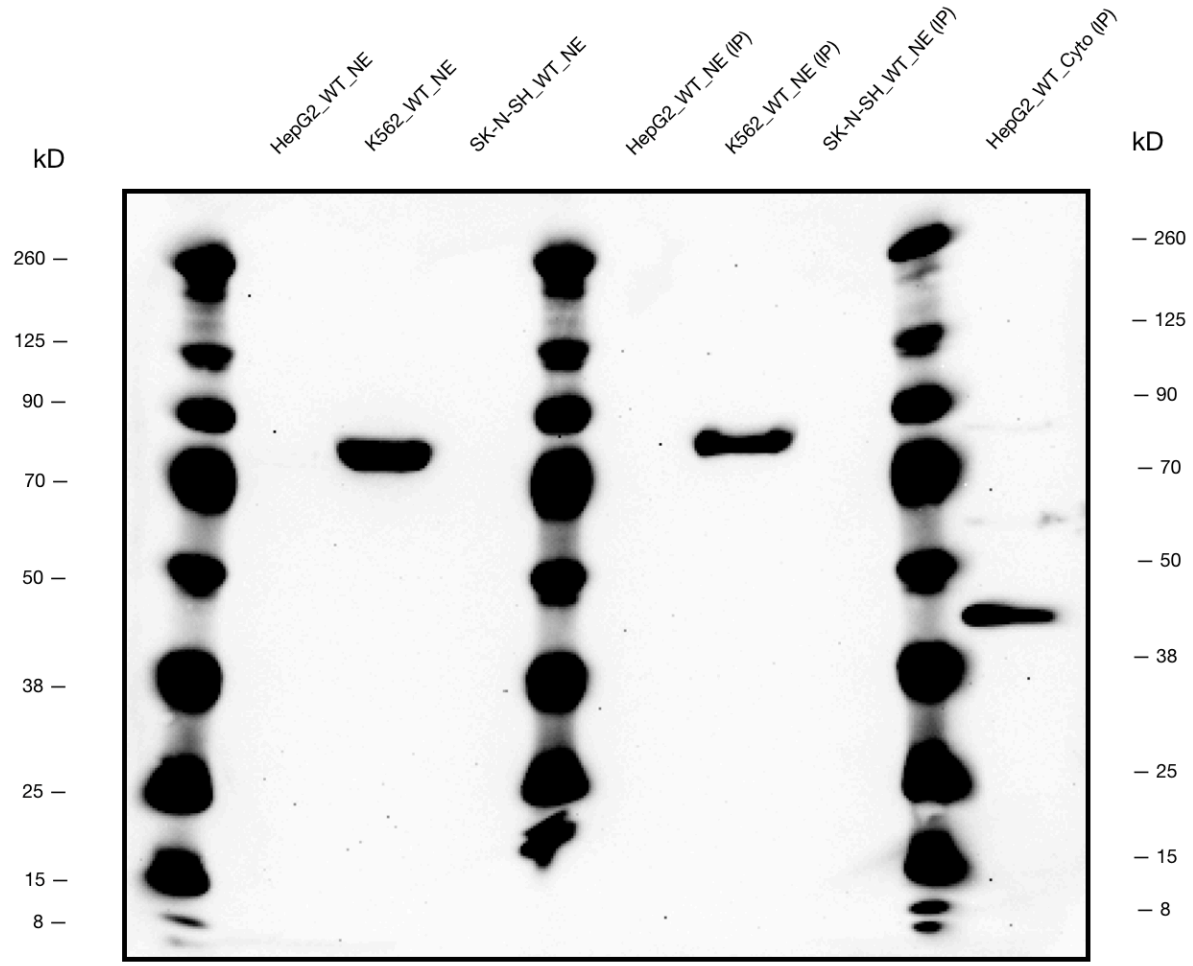
Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (300 mcg - HES4; 213 mcg - MNX1; 196 mcg - NFKBIB; 217 mcg - ZFHX3; 168 mcg - ZNF219; 293 mcg - ZNF334; and 294 mcg - ZNF446) was standardized in a solution containing a volume of 2% Halt Protease and Phosphatase Inhibitor Single-Use Cocktail Mixture (Thermo Fisher Scientific), NuPage Sample Reducing Agent 10X, and NuPage LDS Sample Buffer 4X (Thermo Fisher Scientific). After heating the solution for 15 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 prepared with HepG2 nuclear lysate (Lane 2).



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-HES4_HepG2 rep 1 (nuclear extract)	27	Faint band larger than 20% of the expected size
3	FLAG-MNX1_isoA_HepG2 rep 1 (nuclear extract)	44	Predicted size was 44 kDa. The observed sizes were 65 kDa and 60 kDa, which are within 20% of an observed band of 52 kDa seen in https://www.novusbio.com/products/mnx1-hlxb9-antibody_nbp1-71939. The presence of multiple bands likely corresponds with the multiple isoforms sharing the same tagged stop codon. PTMs: Acetylation and Phosphorylation
4	FLAG-NFKBIB_isoA_HepG2 rep 2 (nuclear extract)	41	Single non-distinct band. PTMs: Phosphorylation
5	Ladder	N/A	N/A
6	FLAG-ZFHX3_HepG2 rep 1 (nuclear extract)	407	Overexposed banding. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF219_HepG2 rep 3 (nuclear extract)	80	Predicted size was 80 kDa. The observed size was 110 kDa, which is the same size as an observed band seen in https://www.labome.com/product/LifeSpan-Biosciences/LS-C144345.html. PTMs: Phosphorylation
8	FLAG-ZNF334_HepG2 rep 3 (nuclear extract)	83	Distinct band far below 20% of the expected size
9	FLAG-ZNF446_isoA_HepG2 rep 1 (nuclear extract)	52	Predicted size was 52 kDa. The observed size was 68 kDa, which is the same size as an observed band seen in https://www.novusbio.com/primary-antibodies/znf446. Larger band may be non-distinct, as it is present in other lanes. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
10	Ladder	N/A	N/A



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	Single non-distinct band at around 45 kDa

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