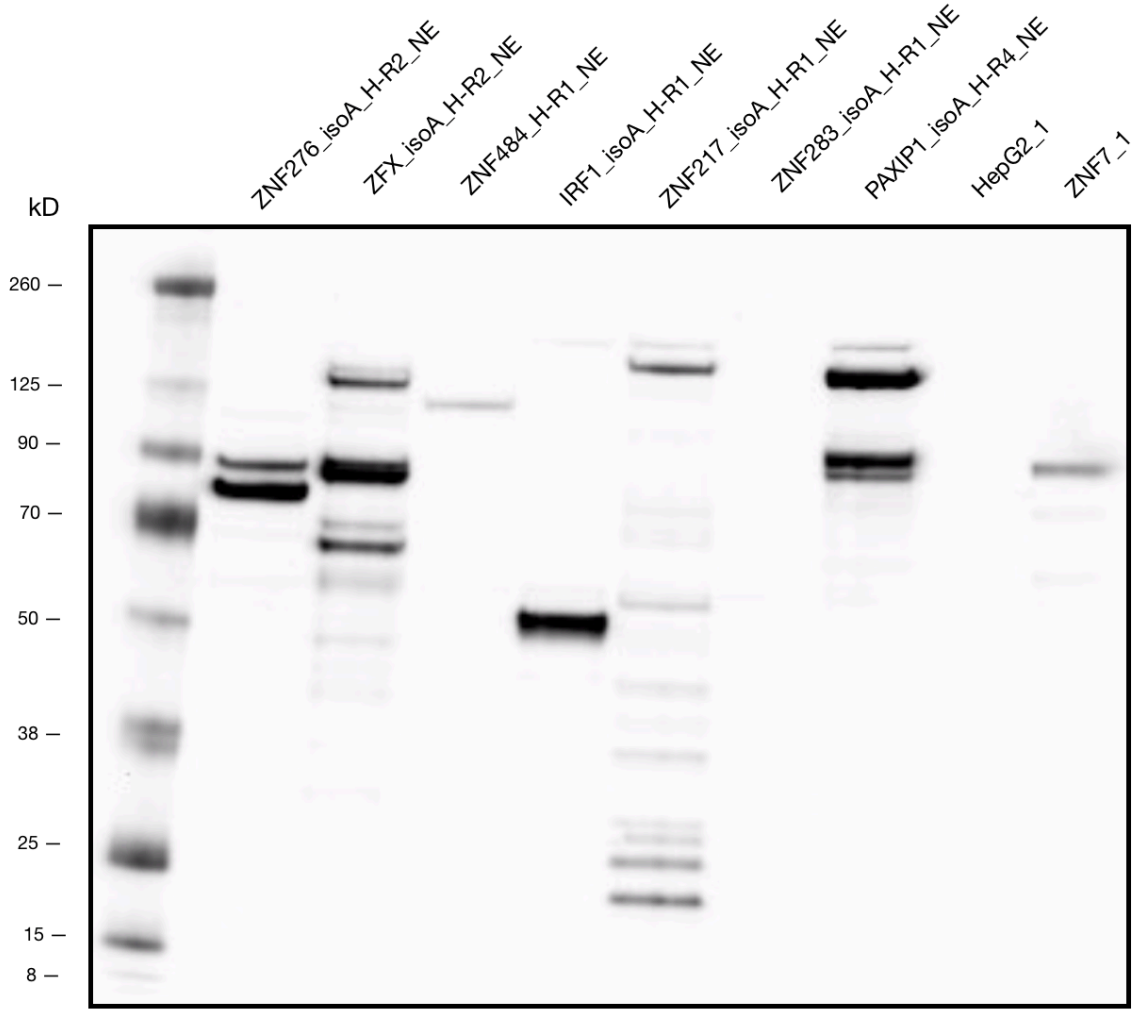
ZNF276 (*Homo sapiens*), ZFX (*Homo sapiens),* ZNF484 (*Homo sapiens),* IRF1 (*Homo sapiens*), ZNF217 (*Homo sapiens)*, ZNF283 (*Homo sapiens*), and PAXIP1 (*Homo sapiens*)

Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (50 mcg) 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. A HepG2 untagged nuclear isolate was included as a negative control, and a ZNF7-tagged nuclear isolate 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).



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-ZNF276_isoA_HepG2 rep2 (nuclear extract)	70	Distinct band within 20% expected.
3	FLAG-ZFX_isoA_HepG2 rep2 (nuclear extract)	94	Distinct band within 20% expected. PTMs: Phosphorylation
4	FLAG-ZNF484_HepG2 rep1 (nuclear extract)	101	Single band within 20% expected. PTMs: Isopeptide bonding and Ubl conjugation
5	FLAG-IRF1_isoA_HepG2 rep1 (nuclear extract)	40	Found similar western blot with band at 48 kDa: https://www.abcam.com/irf1-antibody-epr18301-ab186384.html#description_images_7. PTMs: Acetylation, Isopeptide bonding, phosphorylation, and Ubl conjugation
6	FLAG-ZNF217_isoA_HepG2 rep1 (nuclear extract)	118	Many bands of similar distinctness. PTMs: Isoeptide bonding, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF283_isoA_HepG2 rep1 (nuclear extract)	81	No visible banding. PTMs: Isopeptide bonding and Ubl conjugation
8	FLAG-PAXIP1_isoA_HepG2 rep4 (nuclear extract)	124	Found similar western blot with 2 distinct bands at different molecular weights: https://www.proteinatlas.org/ENSG00000157212-PAXIP1/antibody#western_blot. PTMs: Phosphorylation
9	Wild-Type HepG2 (nuclear extract) (negative control)	None	No visible banding
10	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Distinct band within 20% expected

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