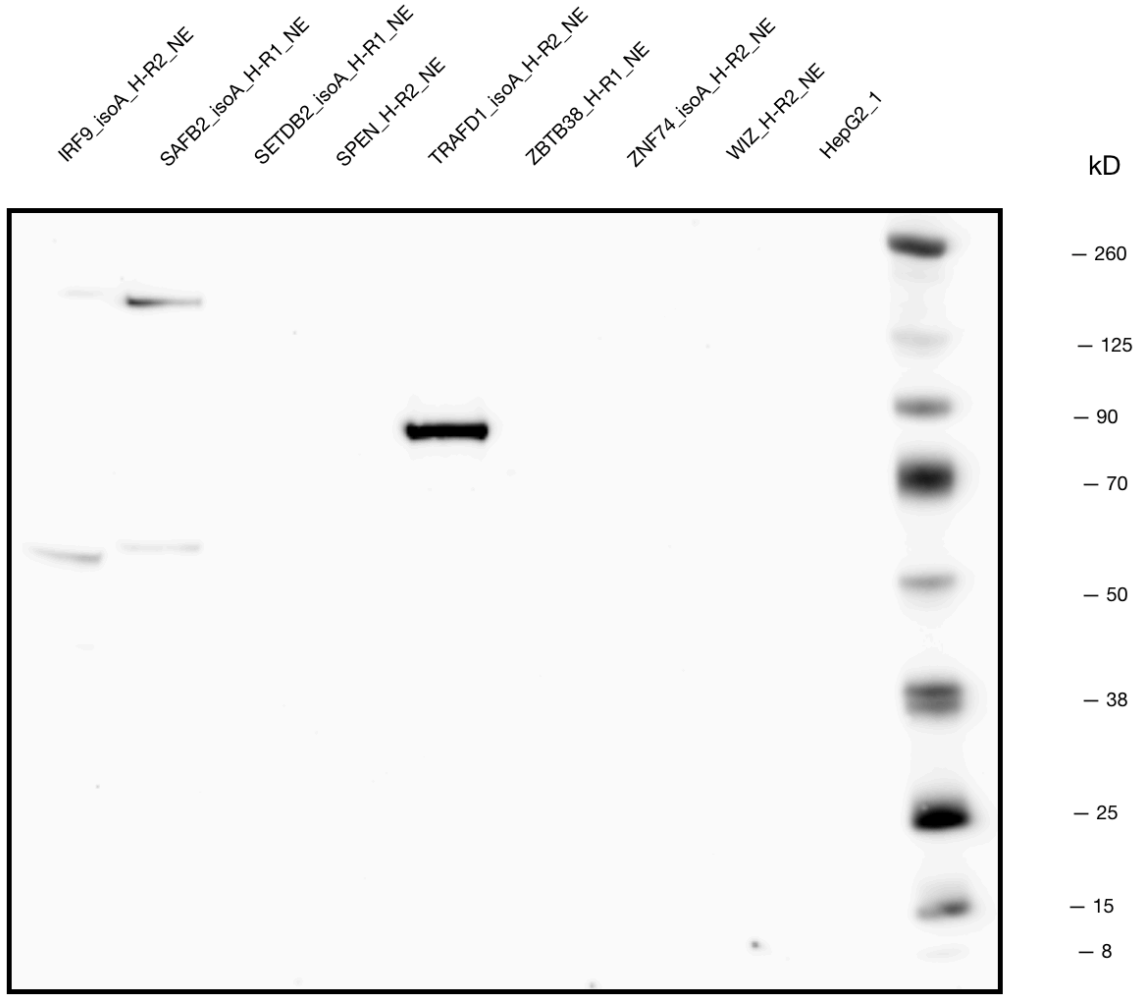
IRF9 (*Homo sapiens*), SAFB2 (*Homo sapiens),* SETDB2 (*Homo sapiens),* SPEN (*Homo sapiens*), TRAFD1 (*Homo sapiens)*, ZBTB38 (*Homo sapiens*), ZNF74 (*Homo* sapiens), and WIZ (*Homo sapiens*)

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. 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	FLAG-IRF9_isoA_HepG2 rep 2 (nuclear extract)	47	Distinct band within 20% of the expected. PTMs: Phosphorylation
2	FLAG-SAFB2_isoA_HepG2 rep 1 (nuclear extract)	110	Predicted size was 110 kDa. The observed size was 200 kDa, which is within 20% of an observed band of 170 kDa seen in https://www.novusbio.com/products/scaffold-attachment-factor-b2-antibody_nb100-58791. The faint band below at 60 kDa could be due to the presence of a degradation product. PTMs: Acetylation, Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation
3	FLAG-SETDB2_isoA_HepG2 rep 1 (nuclear extract)	85	No visible banding
4	FLAG-SPEN_HepG2 rep 2 (nuclear extract)	405	No visible banding. PTMs: Methylation and Phosphorylation
5	FLAG-TRAFD1_isoA_HepG2 rep 2 (nuclear extract)	68	Single dark band near 85 kDa. Found comparable western blot with banding near 82 kDa, within 20% of the observed: https://www.ptglab.com/products/TRAFD1-Antibody-27741-1-AP.htm. PTMs: Acetylation and Phosphorylation
6	FLAG-ZBTB38_HepG2 rep 1 (nuclear extract)	137	No visible banding. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF74_isoA_HepG2 rep 2 (nuclear extract)	75	No visible banding. PTMs: Isopeptide bonding and Ubl conjugation
8	FLAG-WIZ_HepG2 rep 2 (nuclear extract)	182	No visible banding. PTMs: Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation
9	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
10	Ladder	N/A	N/A

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