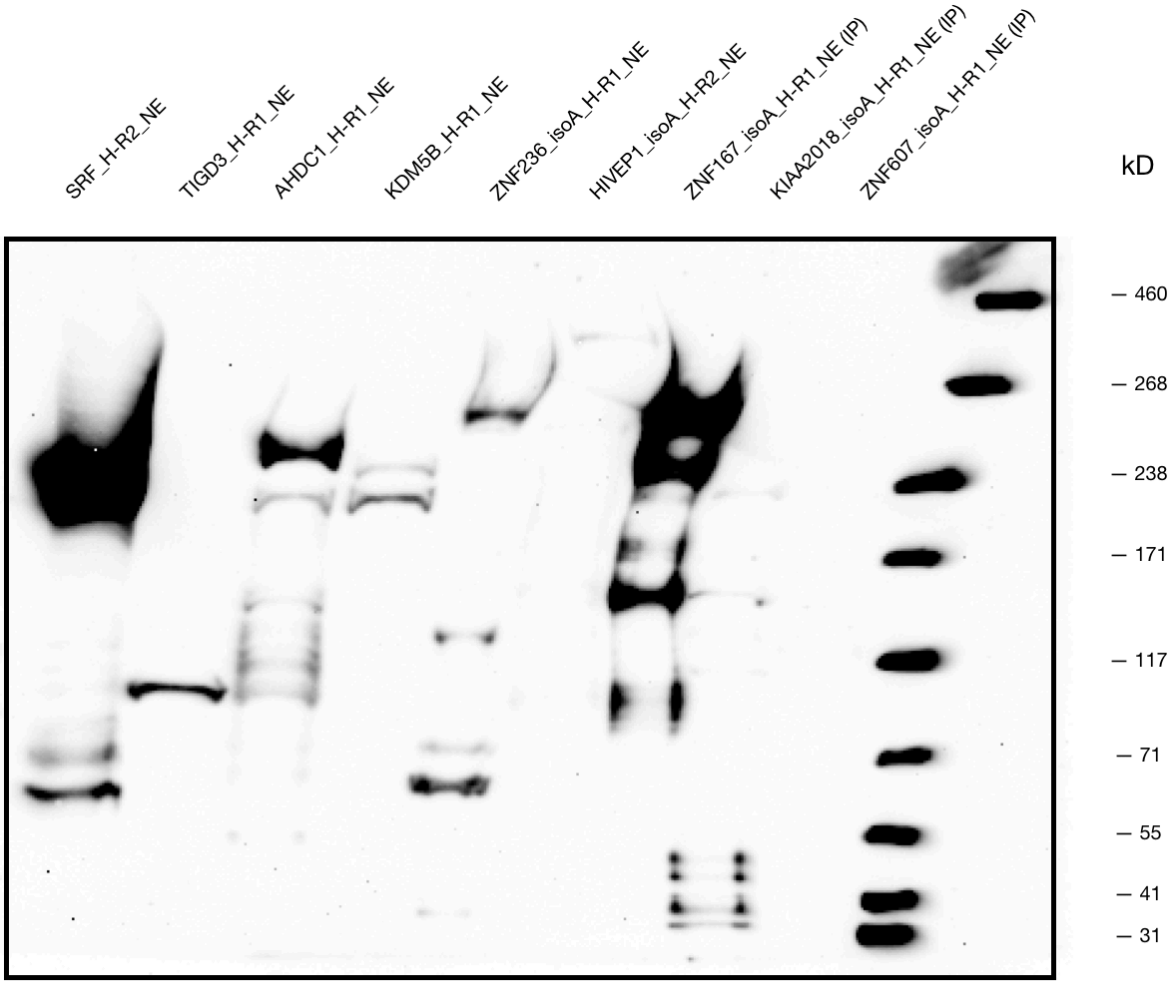
SRF (*Homo sapiens*), TIGD3 (*Homo sapiens*), AHDC1 (*Homo sapiens*), KDM5B (*Homo sapiens*), ZNF236 (*Homo sapiens*), HIVEP1 (*Homo sapiens*), ZNF167 (*Homo sapiens*), KIAA2018 (*Homo* sapiens), and ZNF607 (*Homo sapiens*)

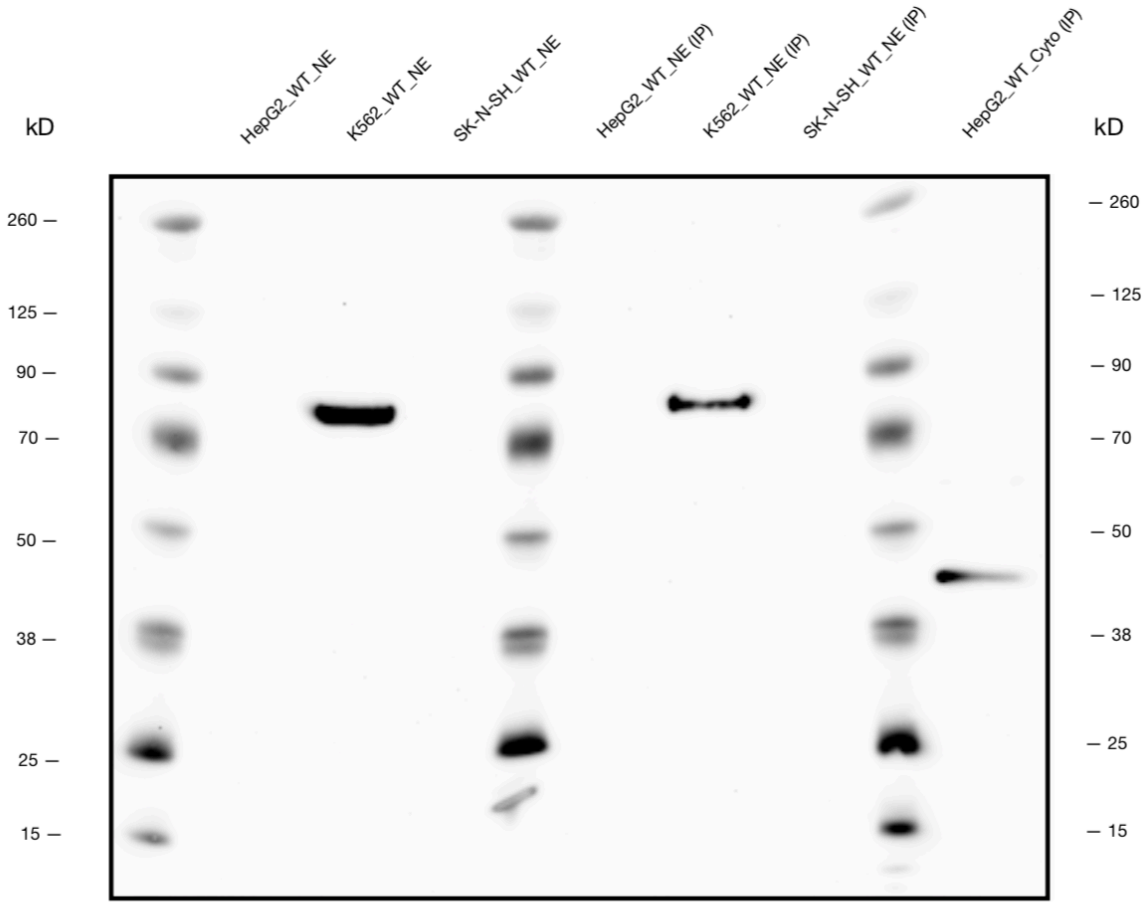
Method:
Western Blot Validation

Caption:
ZNF167, KIAA2018, and ZNF607 were immunoprecipitated from their corresponding nuclear protein isolates (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. For the remaining samples, each nuclear protein isolate (300 mcg - SRF and ZNF236; 212 mcg - TIGD3; 237 mcg - AHDC1; 225 mcg - KDM5B; and 190 mcg - HIVEP1) 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 3-8% Tris-Acetate 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 the negative controls, an untagged HepG2 lysate (lane 2) and an IP prepared with HepG2 nuclear lysate (Lane 6).



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

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	FLAG-SRF_HepG2 rep 2 (nuclear extract)	55	Dark band far beyond the expected size. PTMs: Glycosylation and Phosphorylation
2	FLAG-TIGD3_HepG2 rep 1 (nuclear extract)	55	Single dark band around 100 kDa. Found comparable western image with banding around the same size as the observed band: https://www.novusbio.com/products/tigd3-antibody_nbp1-70725?utm_source=biocompare&utm_medium=referral&utm_campaign=NBP1-70725&utm_content=editorial&utm_term=primaryantibodies
3	FLAG-AHDC1_HepG2 rep 1 (nuclear extract)	171	Dark band slightly beyond 20% of the expected size. PTMs: Acetylation, Isopeptide bonding, Methylation, Phosphorylation, Ubl conjugation
4	FLAG-KDM5B_HepG2 rep 1 (nuclear extract)	179	Distinct band around 220 kDa. Found comparable western image with banding near the same molecular weight as the observed band: https://www.proteinatlas.org/ENSG00000117139-KDM5B/antibody#western_blot. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-ZNF236_isoA_HepG2 rep 1 (nuclear extract)	207	Distinct band slightly beyond 20% of the expected size
6	FLAG-HIVEP1_isoA_HepG2 rep 2 (nuclear extract)	300	Single faint band within 20% of the expected size. PTMs: Phosphorylation
7	FLAG-ZNF167_isoA_HepG2 rep 1 (IP) (nuclear extract)	88	Distinct bands beyond 20% of the expected size. PTMs: Isopeptide bonding and Ubl conjugation
8	FLAG-KIAA2018_isoA_HepG2 rep 1 (IP) (nuclear extract)	245	Faint band within 20% of the expected size
9	FLAG-ZNF607_isoA_HepG2 rep 1 (IP) (nuclear extract)	84	No visible banding
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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