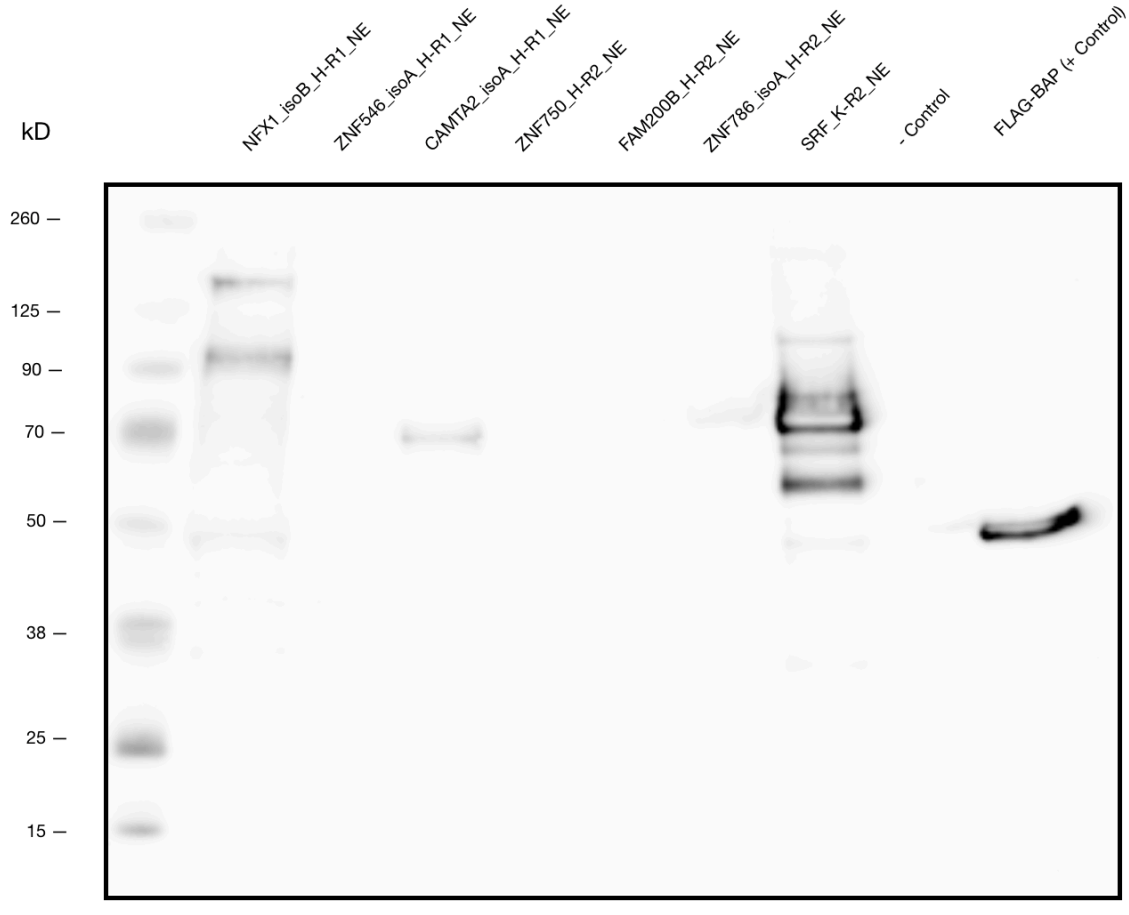
NFX1 (*Homo sapiens*), ZNF546 (*Homo sapiens*), CAMTA2 (*Homo sapiens*), ZNF750 (*Homo sapiens*), FAM200B (*Homo sapiens*), ZNF786 (*Homo sapiens*), and SRF (*Homo sapiens*)

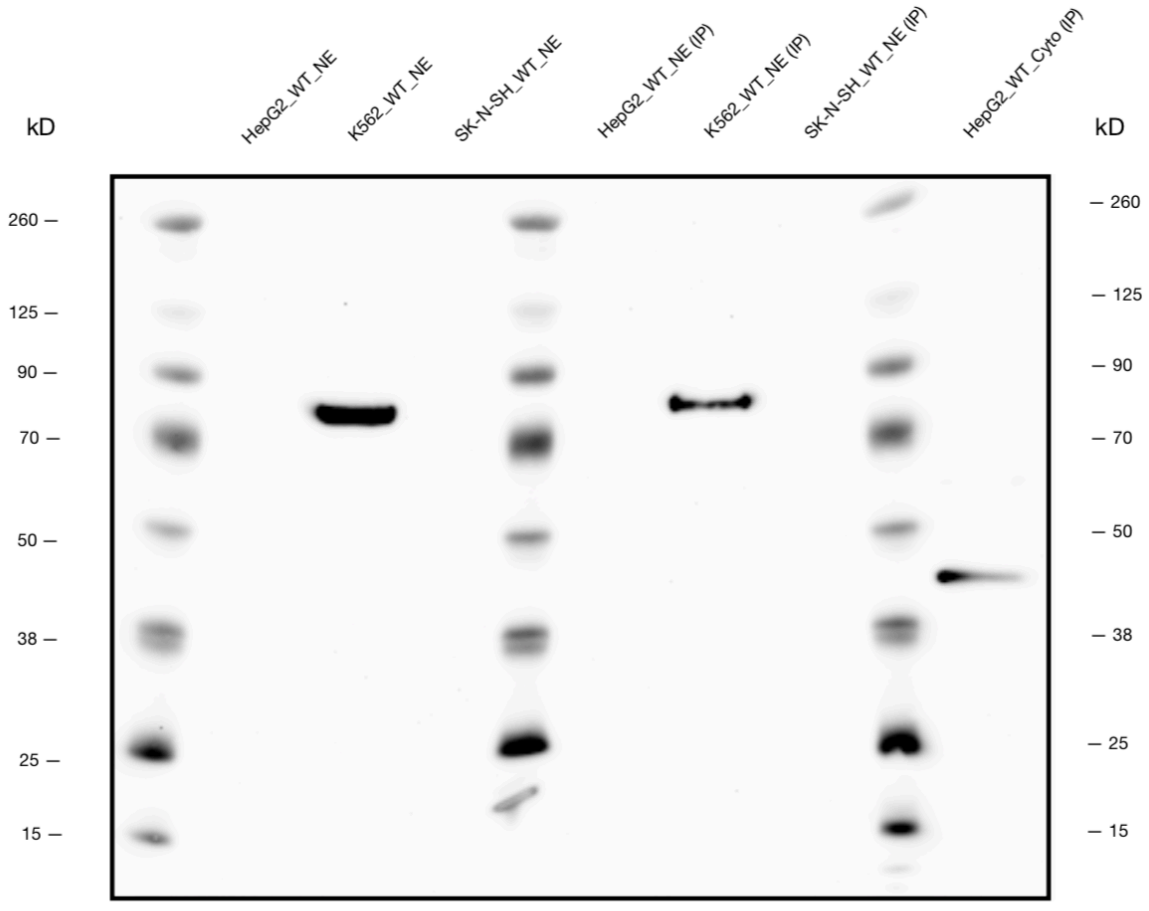
Method:
Western Blot Validation

Caption:
Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear protein isolate (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. 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 blank IP was included as a negative control, and an immunoprecipitated FLAG-BAP fusion protein provided in the kit 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). The second western blot image depicts negative control IPs prepared with HepG2 nuclear lysate (Lane 6) and K562 nuclear lysate (Lane 7).



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-NFX1_isoB_HepG2 rep 1 (nuclear extract)	127	Distinct band around 100 kDa. Found comparable western image with banding around 120 kDa, within 20% of the observed band: https://www.proteinatlas.org/ENSG00000086102-NFX1/antibody#western_blot. PTMs: Phosphorylation and Ubl conjugation
3	FLAG-ZNF546_isoA_HepG2 rep 1 (nuclear extract)	101	No visible banding. PTMs: Acetylation, Isopeptide bonding and Ubl conjugation
4	FLAG-CAMTA2_isoA_HepG2 rep 1 (nuclear extract)	135	Single distinct band further than 20% from the expected size
5	FLAG-ZNF750_HepG2 rep 2 (nuclear extract)	80	No visible banding
6	FLAG-FAM200B_HepG2 rep 2 (nuclear extract)	79	No visible banding
7	FLAG-ZNF786_isoA_HepG2 rep 2 (nuclear extract)	93	Single faint band around 70 kDa. Found comparable western image with banding around 72 kDa, within 20% of the observed band: https://www.proteinatlas.org/ENSG00000197362-ZNF786/antibody#western_blot
8	FLAG-SRF_K562 rep 2 (nuclear extract)	55	Distinct band near the expected size. PTMs: Glycosylation and Phosphorylation
9	FLAG-BAP (Positive Control)	49	Dark band around 50 kDa
10	Negative Control (Blank IP)	None	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	Single non-distinct band at around 45 kDa

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