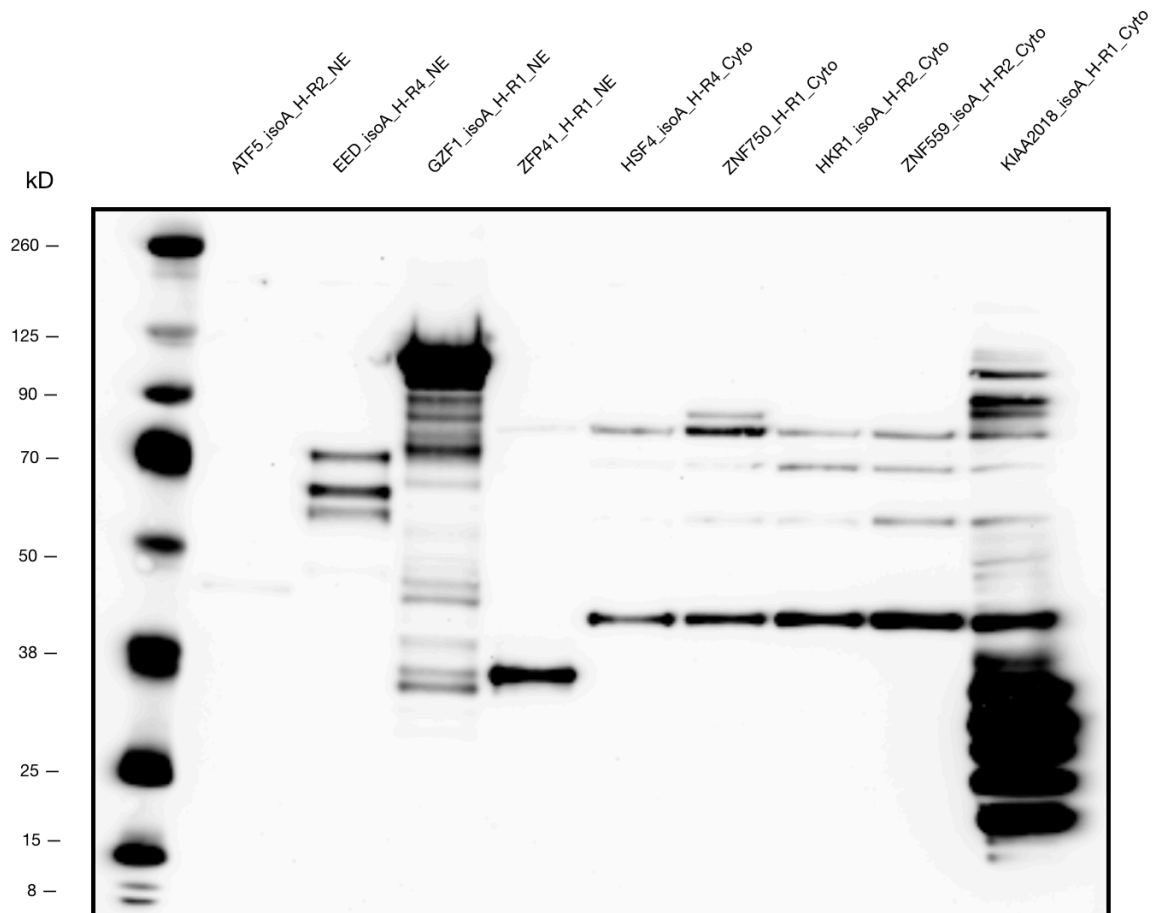


ATF5 (*Homo sapiens*), EED (*Homo sapiens*), GZF1 (*Homo sapiens*), ZFP41 (*Homo sapiens*), HSF4 (*Homo sapiens*), ZNF750 (*Homo sapiens*), HKR1 (*Homo sapiens*), ZNF559 (*Homo sapiens*), and KIAA2018 (*Homo sapiens*)

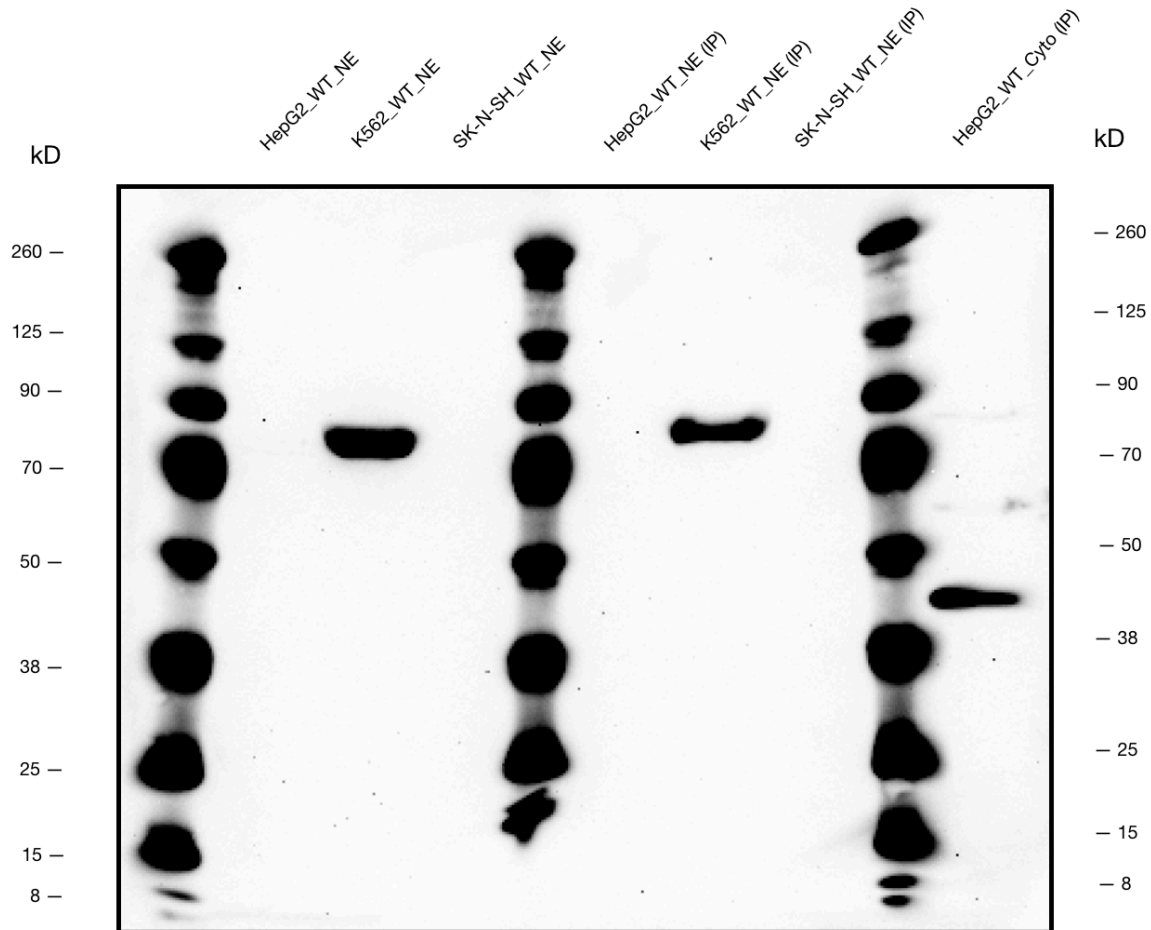
Method:
Western Blot Validation

Caption:
Each FLAG-tagged sample was immunoprecipitated from its corresponding protein isolate (nuclear - 500 μ L, cytoplasmic - 1 mL) 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. 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 five 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 HepG2 cytoplasmic lysate (Lane 10).



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-ATF5_isoA_HepG2 rep 2 (nuclear extract)	34	Single distinct band around 47 kDa. Found comparable western image with banding around 46 kDa, within 20% of the observed band: https://www.proteinatlas.org/ENSG00000169136-ATF5/antibody#western_blot . PTMs: Acetylation, Phosphorylation, and Ubl conjugation
3	FLAG-EED_isoA_HepG2 rep 4 (nuclear extract)	53	Distinct band within 20% of the expected size. Other bands correspond with additional isoforms sharing the same stop codon
4	FLAG-GZF1_isoA_HepG2 rep 1 (nuclear extract)	83	Dark band around 110 kDa. Found comparable western image with banding around 95 kDa, within 20% of the observed band: https://www.ibl-japan.co.jp/en/search/product/detail/id=3806 . PTMs: Phosphorylation
5	FLAG-ZFP41_HepG2 rep 1 (nuclear extract)	26	Distinct band around 36 kDa. Found comparable western image with banding around 40 kDa, within 20% of the observed band: https://www.biorbyt.com/zfp41-antibody-orb325607.html
6	FLAG-HSF4_isoA_HepG2 rep 4 (cytoplasmic extract)	56	No visible banding besides non-distinct bands. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF750_HepG2 rep 1 (cytoplasmic extract)	80	Distinct band within 20% of the expected size
8	FLAG-HKR1_isoA_HepG2 rep 2 (cytoplasmic extract)	78	No visible banding besides non-distinct bands
9	FLAG-ZNF559_isoA_HepG2 rep 2 (cytoplasmic extract)	65	No visible banding besides non-distinct bands
10	FLAG-KIAA2018_isoA_HepG2 rep 1 (cytoplasmic extract)	243	Many bands far from the expected size



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	Dark non-distinct band at around 45 kDa, and two lighter bands at about 60 kDa and 80 kDa

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Grant:

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