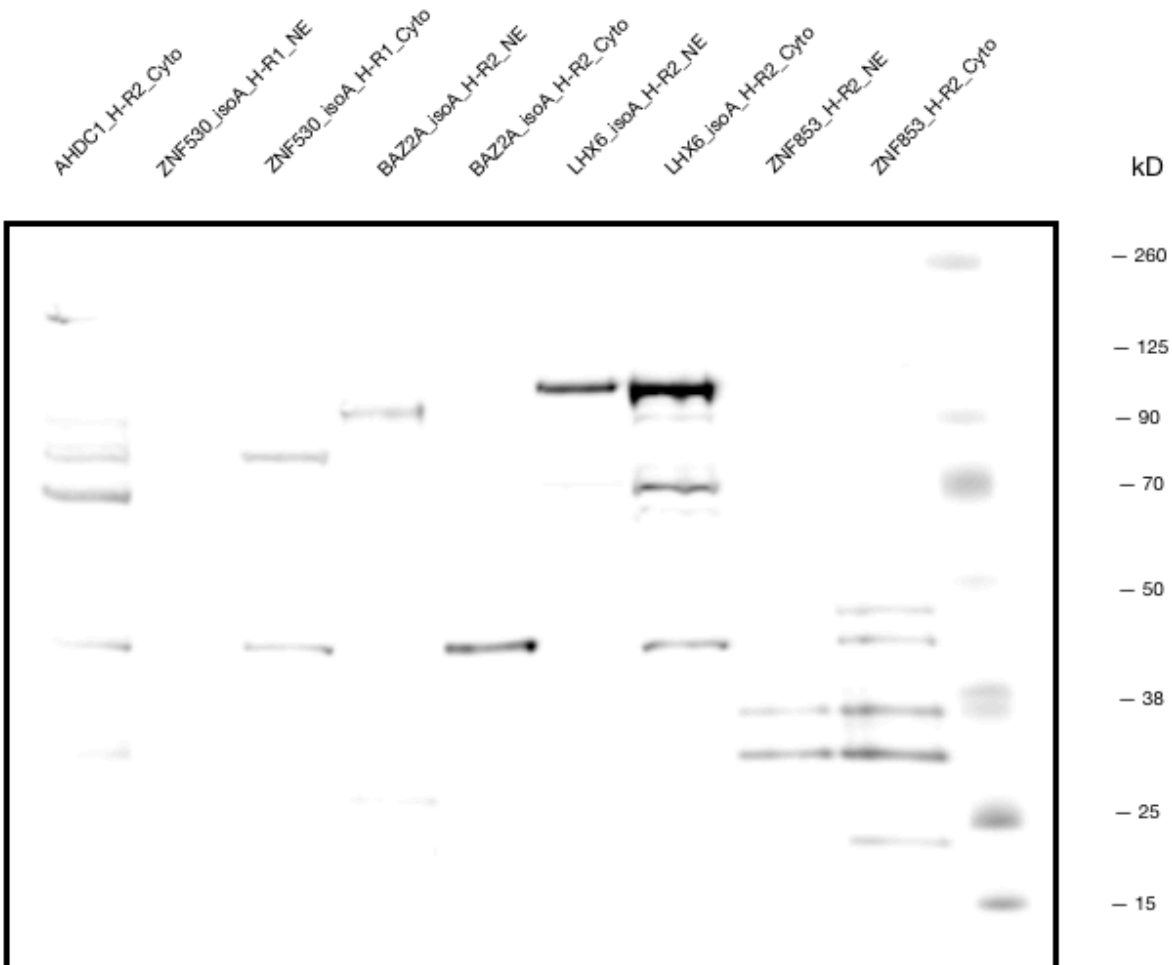


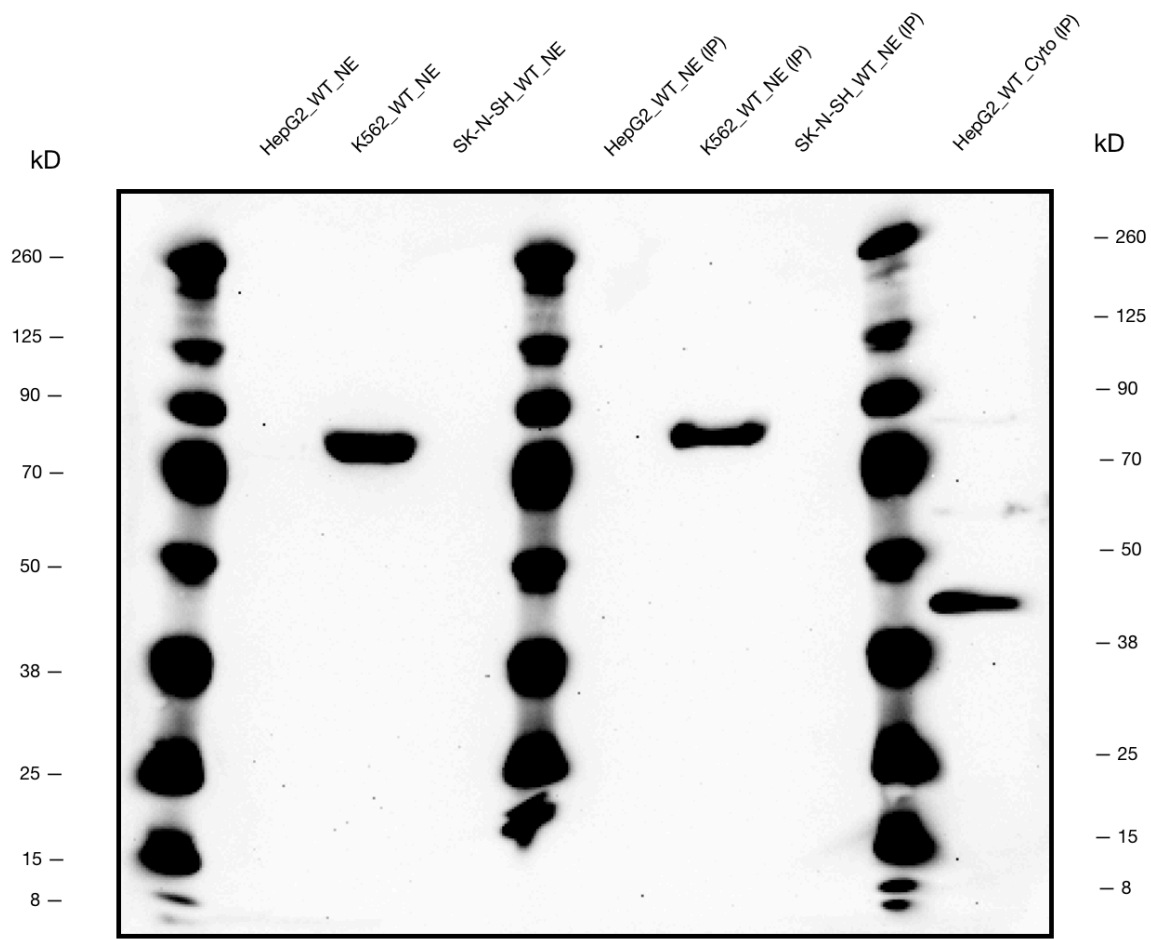
AHDC1 (*Homo sapiens*), ZNF530 (*Homo sapiens*), ZNF530 (*Homo sapiens*), BAZ2A (*Homo sapiens*), BAZ2A (*Homo sapiens*), LHX6 (*Homo sapiens*), LHX6 (*Homo sapiens*), ZNF853 (*Homo sapiens*), and ZNF853 (*Homo sapiens*)

Method:
Western Blot Validation

Caption:
Each FLAG-tagged sample was immunoprecipitated from its corresponding protein isolate (500 uL - nuclear; 1 mL - cytoplasmic) 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).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	FLAG-AHDC1_HepG2 rep 2 (cytoplasmic extract)	171	Predicted size was 171 kDa. The observed size was 65 kDa, which is within 20% of an observed band of 52 kDa seen in https://www.usbio.net/antibodies/243166/AHDC1-AT-hook-DNA-Binding-Motif-Containing-1-CL23945-DJ159A193-RP1159A191 . Band at 45 kDa is a non-specific band seen in the negative control. The faint banding above likely corresponds with another one of the isoforms that shares the same tagged stop codon. PTMs: Acetylation, Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation
2	FLAG-ZNF530_isoA_HepG2 rep 1 (nuclear extract)	72	No visible banding
3	FLAG-ZNF530_isoA_HepG2 rep 1 (cytoplasmic extract)	72	Distinct band within 20% of the expected size. Band at 45 kDa is a non-specific band seen in the negative control.
4	FLAG-BAZ2A_isoA_HepG2 rep 2 (nuclear extract)	214	Distinct band, but smaller than 20% of the expected size. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-BAZ2A_isoA_HepG2 rep 2 (cytoplasmic extract)	214	Non-specific band seen in the negative control. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
6	FLAG-LHX6_isoA_HepG2 rep 2 (nuclear extract)	43	Single band larger than 20% of the predicted size
7	FLAG-LHX6_isoA_HepG2 rep 2 (cytoplasmic extract)	43	Dark band beyond 20% of the predicted size
8	FLAG-ZNF853_HepG2 rep 2 (nuclear extract)	78	Banding smaller than the expected size
9	FLAG-ZNF853_HepG2 rep 2 (cytoplasmic extract)	78	Banding smaller than the expected size
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	Dark non-distinct band at around 45 kDa, and two lighter bands at about 60 kDa and 80 kDa

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Grant:

UM1 HG009411

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