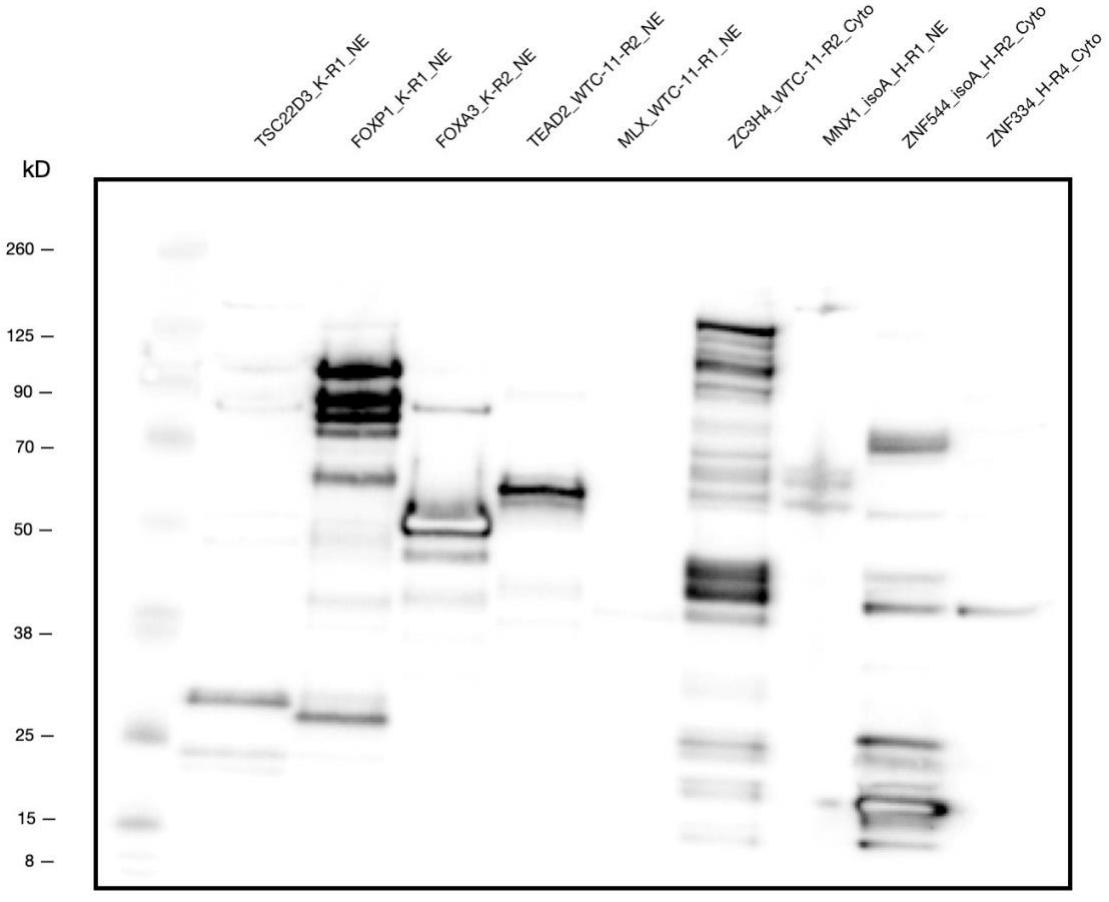
TSC22D3 (*Homo sapiens*), FOXP1 (*Homo sapiens*), FOXA3 (*Homo sapiens*), TEAD2 (*Homo sapiens*), MLX (*Homo sapiens*), ZC3H4 (*Homo sapiens*), MNX1 (*Homo sapiens*), ZNF544 (*Homo sapiens*), and ZNF334 (*Homo sapiens*)

Method:
Western Blot Validation

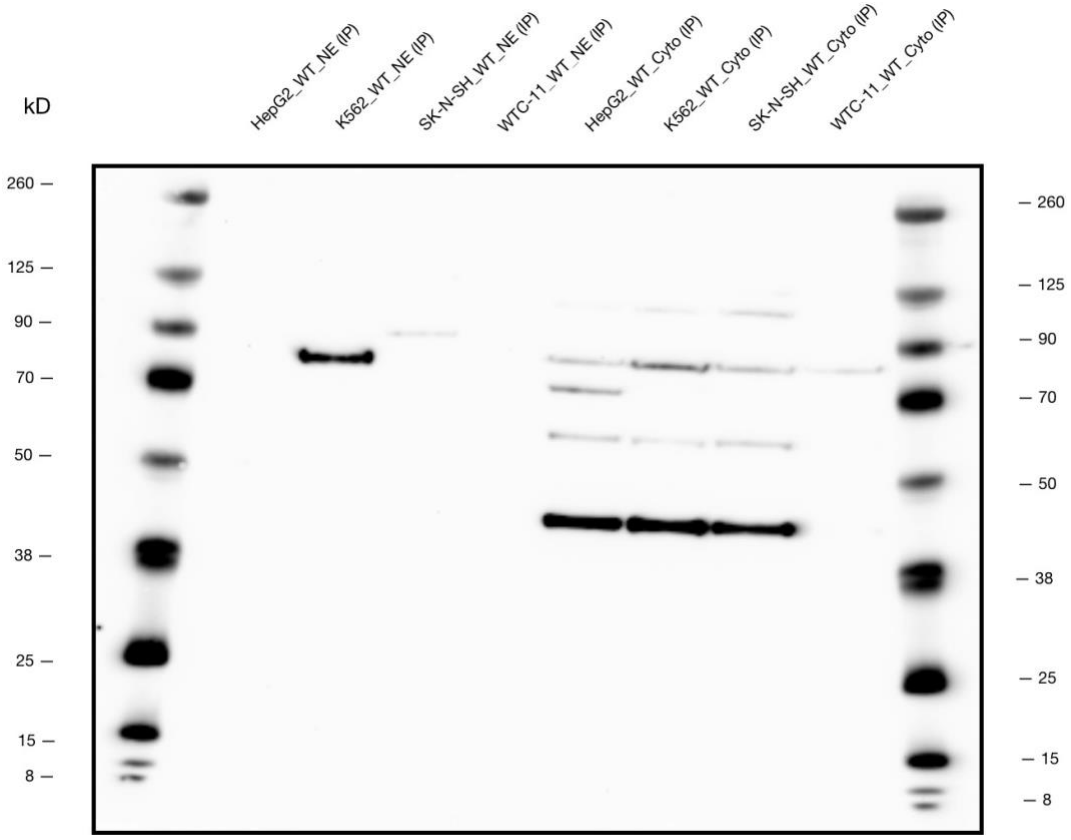
Caption:
Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear protein isolate (500 uL - nuclear and 1 mL - cytoplasmic) using the FLAG Immunoprecipitation Kit (Sigma-Aldrich; cat# FLAGIPT1). The final elution step was performed by suspending the sample-bound resin in NuPage Sample 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 M2Peroxidase-conjugated ANTI-FLAG antibody (diluted in 5% BSA solution) (SigmaAldrich; 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 2), HepG2 cytoplasmic lysate (Lane 6), K562 nuclear lysate (Lane 3), and WTC-11 nuclear lysate (Lane 5).

Lane	Loaded Sample	Expected Band Size (kDa)	Comments



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

1	Ladder	N/A	N/A
2	FLAG-TSC22D3_K562 rep 1 (nuclear extract)	18	Predicted size was 18 kDa. The observed size was 30 kDa, which is within 20% of observed band of 31 kDa seen in https://www.lsbio.com/antibodies/ihc-plus-tsc22d3antibody-gilz-antibody-elisa-if-immunofluorescence-ihc-wb-western-lsb10077/241011. The fainter band below could be a potential degradation product. PTMs: Phosphorylation
3	FLAG-FOXP1_K562 rep 1 (nuclear extract)	78	Predicted size was 78 kDa. The observed sizes were 100 kDa and 85 kDa, which are within 20% of observed bands of 95 kDa and 87 kDa seen in https://www.bosterbio.com/anti-foxp1-rabbit-monoclonal-antibody-m00723-boster.html. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
4	FLAG-FOXA3_K562 rep 2 (nuclear extract)	40	Predicted size was 40 kDa. The observed sizes were 52 kDa and 48 kDa, which are within 20% of an observed band of 50 kDa seen in https://www.lsbio.com/antibodies/foxa1-foxa2-foxa3-antibody-acetylation-site-of-k264-253-211.-elisa-wb-western-ls-c380575/392676. The band above at 80 kDa is a non-specific band seen in the K562 control, and the band below near 43 kDa could be a degradation product
5	FLAG-TEAD2_WTC-11 rep 2 (nuclear extract)	52	Single dark band within 20% of the predicted size
6	FLAG-MLX_WTC-11 rep 1 (nuclear extract)	36	Single faint band within 20% of the predicted size. PTMs: Phosphorylation
7	FLAG-ZC3H4_WTC-11 rep 2 (cytoplasmic extract)	143	Dark band within 20% of the predicted size, with degradation banding below. The dark band near 43 kDa is a non-specific band seen in the HepG2 cytoplasmic control. PTMs: Methylation and Phosphorylation
8	FLAG-MNX1_isoA_HepG2 rep 1 (nuclear extract)	44	Predicted size was 44 kDa. The observed sizes were 65 kDa and 60 kDa, which are within 20% of a band of 65 kDa seen in https://www.scbt.com/p/hb9-antibody-f-5. PTMs: Acetylation and Phosphorylation
9	FLAG-ZNF544_isoA_HepG2 rep 2 (cytoplasmic extract)	85	Distinct band within 20% of the predicted size, with non-specific banding and potential degradation products below. PTMs: Isopeptide bonding and Ubl conjugation
10	FLAG-ZNF334_HepG2 rep 4 (cytoplasmic extract)	83	Single non-specific band seen in the HepG2 cytoplasmic control



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 IP)	None	No visible banding
3	K562 Wild-Type (nuclear extract IP)	None	Dark band near 80 kDa
4	SK-N-SH Wild-Type (nuclear extract IP)	None	Band near 90 kDa
5	WTC-11 Wild-Type (nuclear extract IP)	None	No visible banding
6	HepG2 Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, 70 kDa, and 60 kDa. Dark band at 45 kDa
7	K562 Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, and 60 kDa. Dark band at 45 kDa
8	SK-N-SH Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, and 60 kDa. Dark band at 45 kDa
9	WTC-11 Wild-Type (cytoplasmic extract IP)	None	Faint band at 85 kDa
10	Ladder	N/A	N/A

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