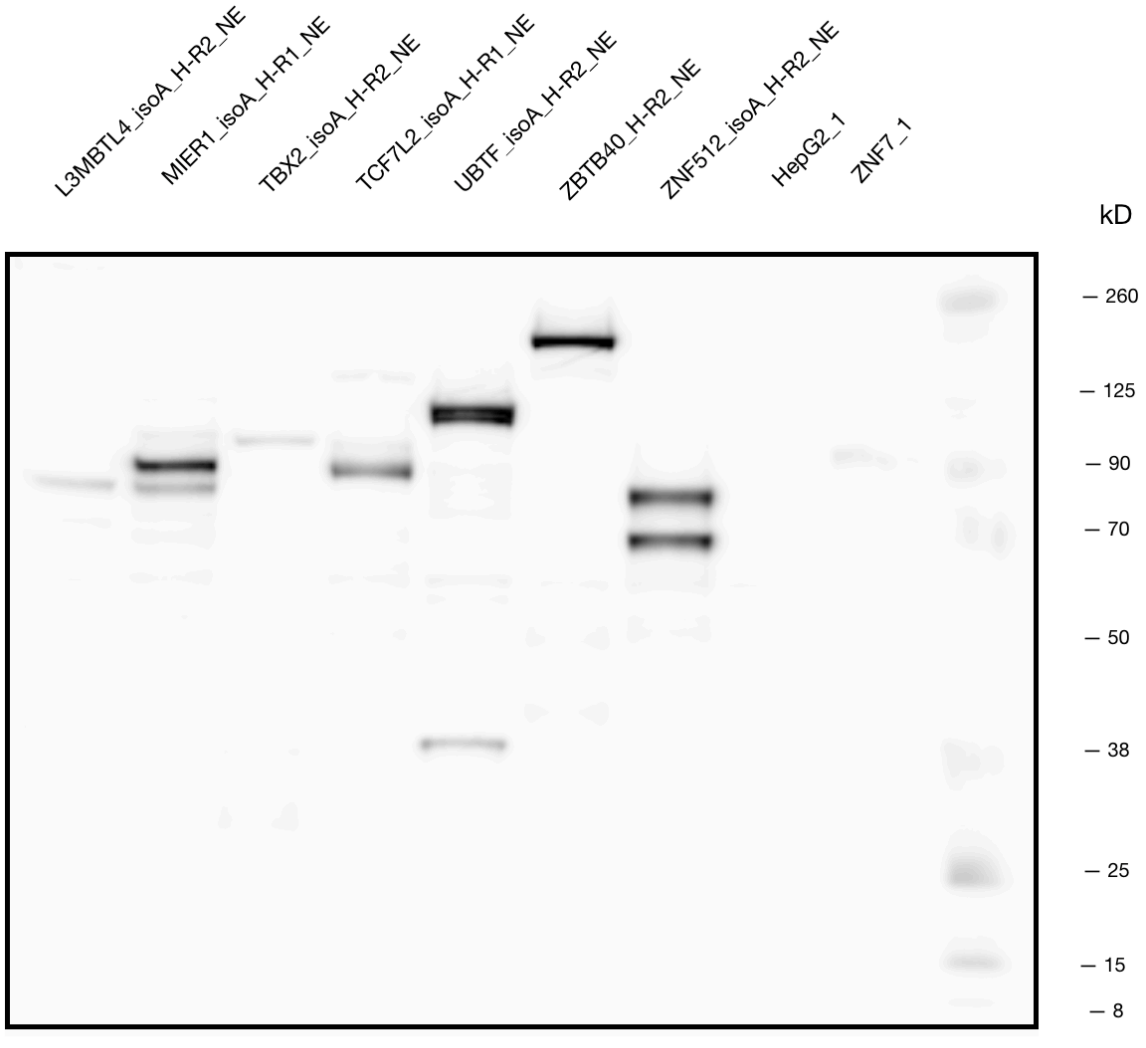
L3MBTL4 (*Homo sapiens*), MIER1 (*Homo sapiens),* TBX2 (*Homo sapiens),* TCF7L2 (*Homo sapiens*), UBTF (*Homo sapiens)*, ZBTB40 (*Homo sapiens*), and ZNF512 (*Homo sapiens*)

Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (50 mcg) 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 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 HepG2 untagged nuclear isolate was included as a negative control, and a ZNF7-tagged nuclear isolate 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).



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

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	FLAG-L3MBTL4_isoA_HepG2 rep 2 (nuclear extract)	74	Single band within 20% expected
2	FLAG-MIER1_isoA_HepG2 rep 1 (nuclear extract)	61	Distinct band near 95 kDa. Found comparable western blot with banding around 81 kDa, within 20% from the observed: https://www.ptglab.com/Products/MIER1-Antibody-11452-1-AP.htm. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	FLAG-TBX2_isoA_HepG2 rep 2 (nuclear extract)	78	Single band near 100 kDa. Found comparable western blot with banding around 81 kDa, within 20% from the observed: https://www.scbt.com/scbt/product/tbx2-antibody-d-3. PTMs: Phosphorylation
4	FLAG-TCF7L2_isoA_HepG2 rep 1 (nuclear extract)	71	Distinct band near 90 kDa. Found comparable western blot with banding around 81 kDa, within 20% from the observed: https://www.avivasysbio.com/tcf7l2-antibody-middle-region-arp33414-p050.html. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-UBTF_isoA_HepG2 rep 2 (nuclear extract)	92	Distinct band around 112 kDa. Found comparable western blot with banding near 96 kDa, within 20% of the observed: https://www.proteinatlas.org/ENSG00000108312-UBTF/antibody#western_blot. PTMs: Acetylation and Phosphorylation
6	FLAG-ZBTB40_HepG2 rep 2 (nuclear extract)	141	Single band around 173 kDa. Found comparable western blot with banding near 175 kDa, similar to the observed: https://www.abcam.com/zbtb40-antibody-c-terminal-ab190185.html#description_images_1 . PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF512_isoA_HepG2 rep 2 (nuclear extract)	68	Two distinct bands, each within 20% of the expected molecular weight. PTMs: Isopeptide bonding and Ubl conjugation
8	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
9	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Distinct band within 20% expected
10	Ladder	N/A	N/A

Submitted by:
Mark Mackiewicz and Michael Betti

Lab:
Richard Myers, HAIB

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