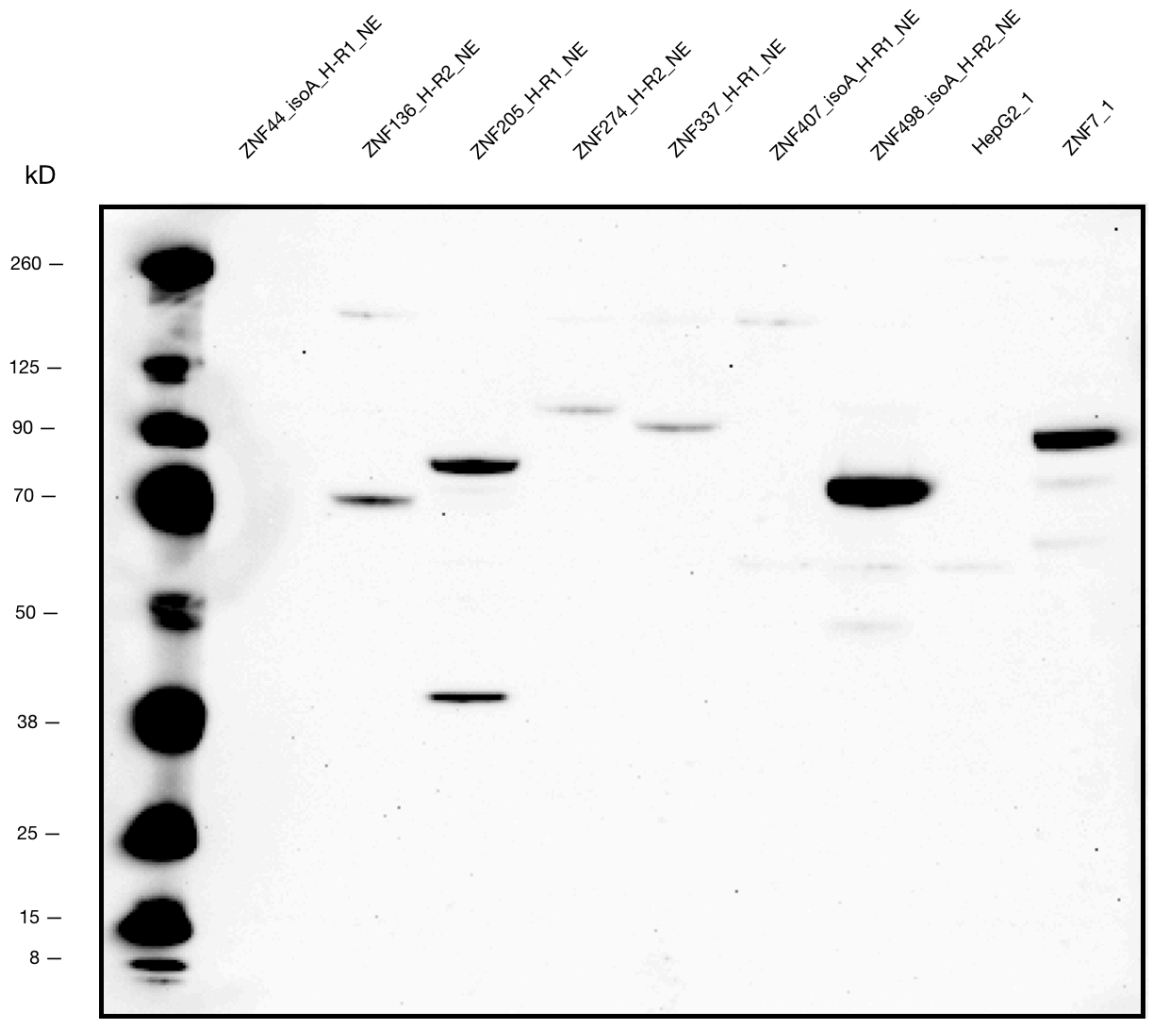
ZNF44 (*Homo sapiens*), ZNF136 (*Homo sapiens),* ZNF205 (*Homo sapiens),* ZNF274 (*Homo sapiens*), ZNF337 (*Homo sapiens)*, ZNF407 (*Homo sapiens*), and ZNF498 (*Homo sapiens*)

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	Ladder	N/A	N/A
2	FLAG-ZNF44_isoA_HepG2 rep 1 (nuclear extract)	80	No visible banding
3	FLAG-ZNF136_HepG2 rep 2 (nuclear extract)	66	Distinct banding within 20% of the expected. PTMs: Phosphorylation
4	FLAG-ZNF205_HepG2 rep 1 (nuclear extract)	64	Darker band at around 80 kDa and lighter band around 40 kDa. Found comparable western blot with banding regions at similar molecular weights, near 82 kDa and 50 kDa, respectively: https://www.proteinatlas.org/ENSG00000122386-ZNF205/antibody#western_blot. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-ZNF274_HepG2 rep 2 (nuclear extract)	77	Single distinct band near 100 kDa. Found comparable western blot with banding around 105 kDa, similar to the observed: https://www.novusbio.com/products/znf274-antibody_h00010782-d01p
6	FLAG-ZNF337_HepG2 rep 1 (nuclear extract)	90	Single distinct band within 20% of the expected. PTMs: Isopeptide bonding and Ubl conjugation
7	FLAG-ZNF407_isoA_HepG2 rep 1 (nuclear extract)	250	Very faint banding. PTMs: Phosphorylation
8	FLAG-ZNF498_isoA_HepG2 rep 2 (nuclear extract)	64	Dark band within 20% of expected. PTMs: Isopeptide bonding and Ubl conjugation
9	Wild-Type Hep G2 (nuclear extract) (negative control)	None	Single faint non-distinct band
10	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Dark band within 20% of expected

Submitted by:
Mark Mackiewicz and Michael Betti

Lab:
Richard Myers, HAIB

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