

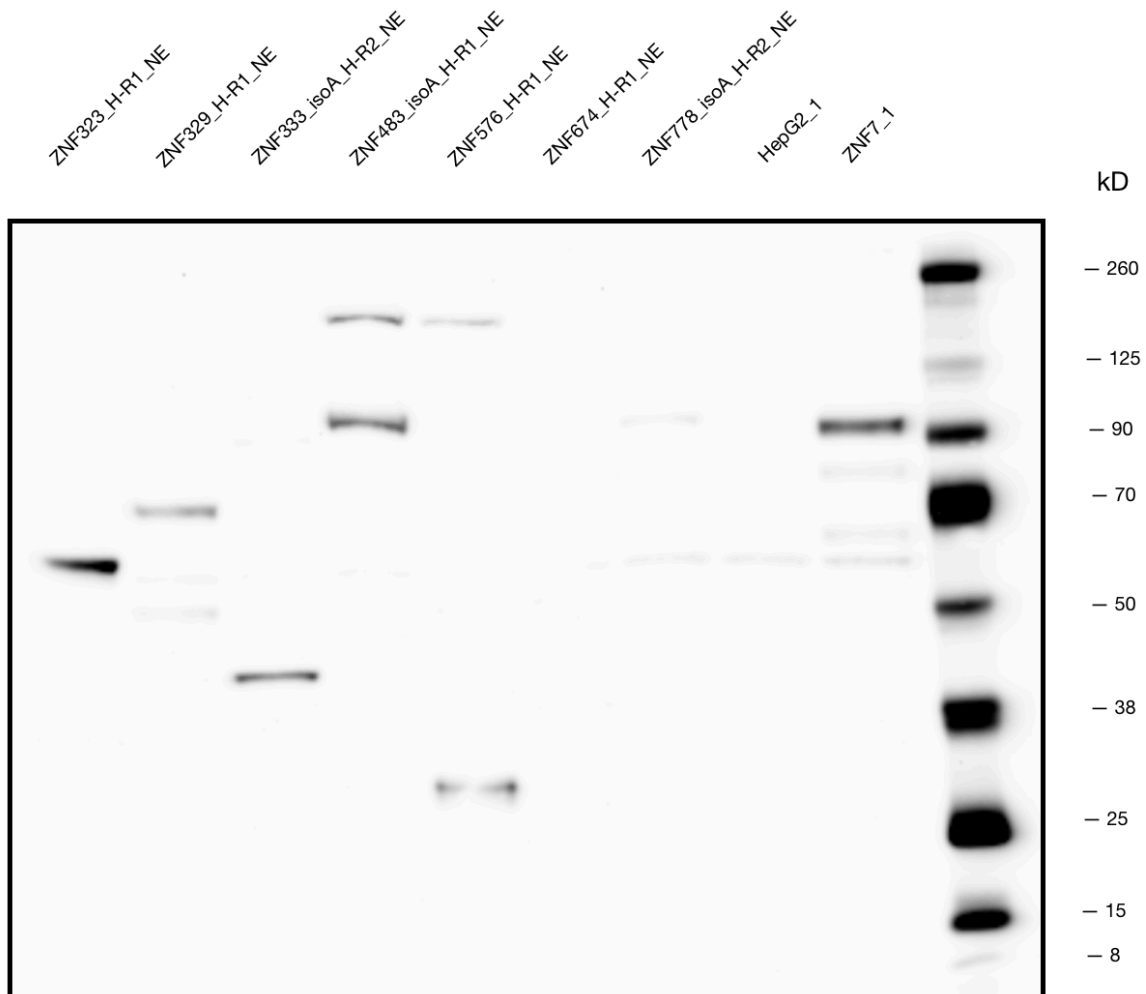
ZNF323 (*Homo sapiens*), ZNF329 (*Homo sapiens*), ZNF333 (*Homo sapiens*), ZNF483 (*Homo sapiens*), ZNF576 (*Homo sapiens*), ZNF674 (*Homo sapiens*), and ZNF778 (*Homo sapiens*)

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-ZNF323_HepG2 rep 1 (nuclear extract)	50	Distinct band within 20% expected. PTMs: Isopeptide bonding and Ubl conjugation
2	FLAG-ZNF329_HepG2 rep 1 (nuclear extract)	65	Distinct band within 20% expected. PTMs: Phosphorylation
3	FLAG-ZNF333_isoA_HepG2 rep 2 (nuclear extract)	79	Single distinct band near 40 kDa. Found comparable western blot with banding around 37 kDa, within 20% from the observed: https://www.antibodies-online.com/antibody/529693/anti-Zinc+Finger+Protein+333+ZNF333+AA+1-303+antibody/?utm_source=partner&utm_medium=biocompare&utm_campaign=non_sponsored&utm_content=primary&utm_term=ABIN529693
4	FLAG-ZNF483_isoA_HepG2 rep 1 (nuclear extract)	88	Distinct band within 20% expected. Second band also present without about half the strength as the primary band
5	FLAG-ZNF576_HepG2 rep 1 (nuclear extract)	22	Distinct band around 29 kDa, very close to 20% of the expected
6	FLAG-ZNF674_HepG2 rep 1 (nuclear extract)	70	No visible banding
7	FLAG-ZNF778_isoA_HepG2 rep 2 (nuclear extract)	85	Distinct band within 20% expected
8	Wild-Type Hep G2 (nuclear extract) (negative control)	None	Single faint non-specific band
9	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Distinct band within 20% expected
10	Ladder	N/A	N/A

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