

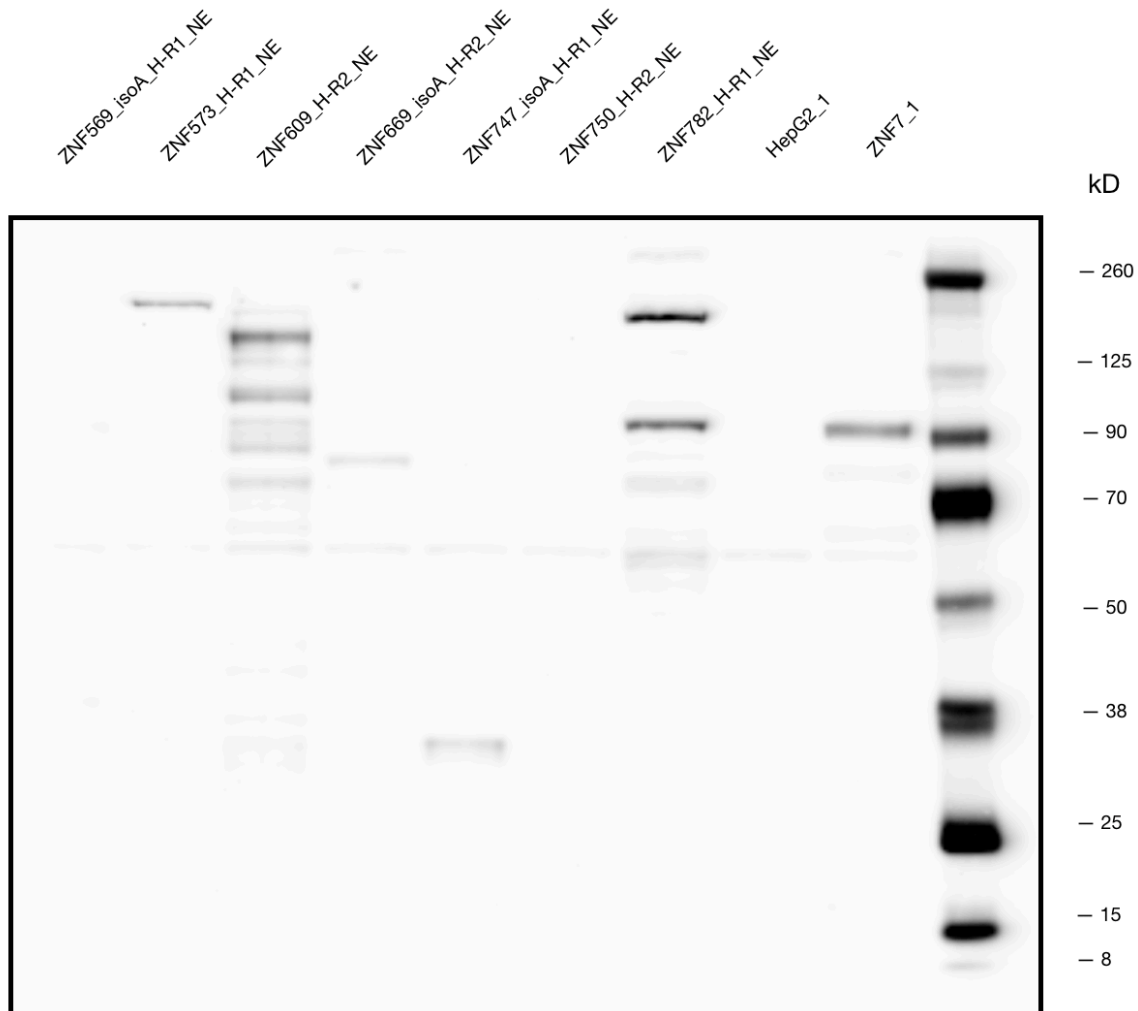
ZNF569 (*Homo sapiens*), ZNF573 (*Homo sapiens*), ZNF609 (*Homo sapiens*), ZNF669 (*Homo sapiens*), ZNF747 (*Homo sapiens*), ZNF750 (*Homo sapiens*), and ZNF782 (*Homo sapiens*)

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-ZNF569_isoA_HepG2 rep 1 (nuclear extract)	83	No visible banding
2	FLAG-ZNF573_HepG2 rep 1 (nuclear extract)	81	Single band at around 170 kDa, far from 20% of expected
3	FLAG-ZNF609_HepG2 rep 2 (nuclear extract)	154	Band near expected, but not drastically distinct from other band slightly below it. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
4	FLAG-ZNF669_isoA_HepG2 rep 2 (nuclear extract)	56	Single band near 85 kDa, beyond 20% from the expected
5	FLAG-ZNF747_isoA_HepG2 rep 1 (nuclear extract)	24	Single distinct band around 33 kDa, slightly beyond 20% of the expected
6	FLAG-ZNF750_HepG2 rep 2 (nuclear extract)	80	No visible banding
7	FLAG-ZNF782_HepG2 rep 1 (nuclear extract)	84	Two distinct bands near 95 kDa and 203 kDa. Found comparable western blot with bands around 82 kDa and 190 kDa, within 20% of each of the respective observed bands
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

Submitted by:

Mark Mackiewicz and Michael Betti

Lab:

Richard Myers, HAIB

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