

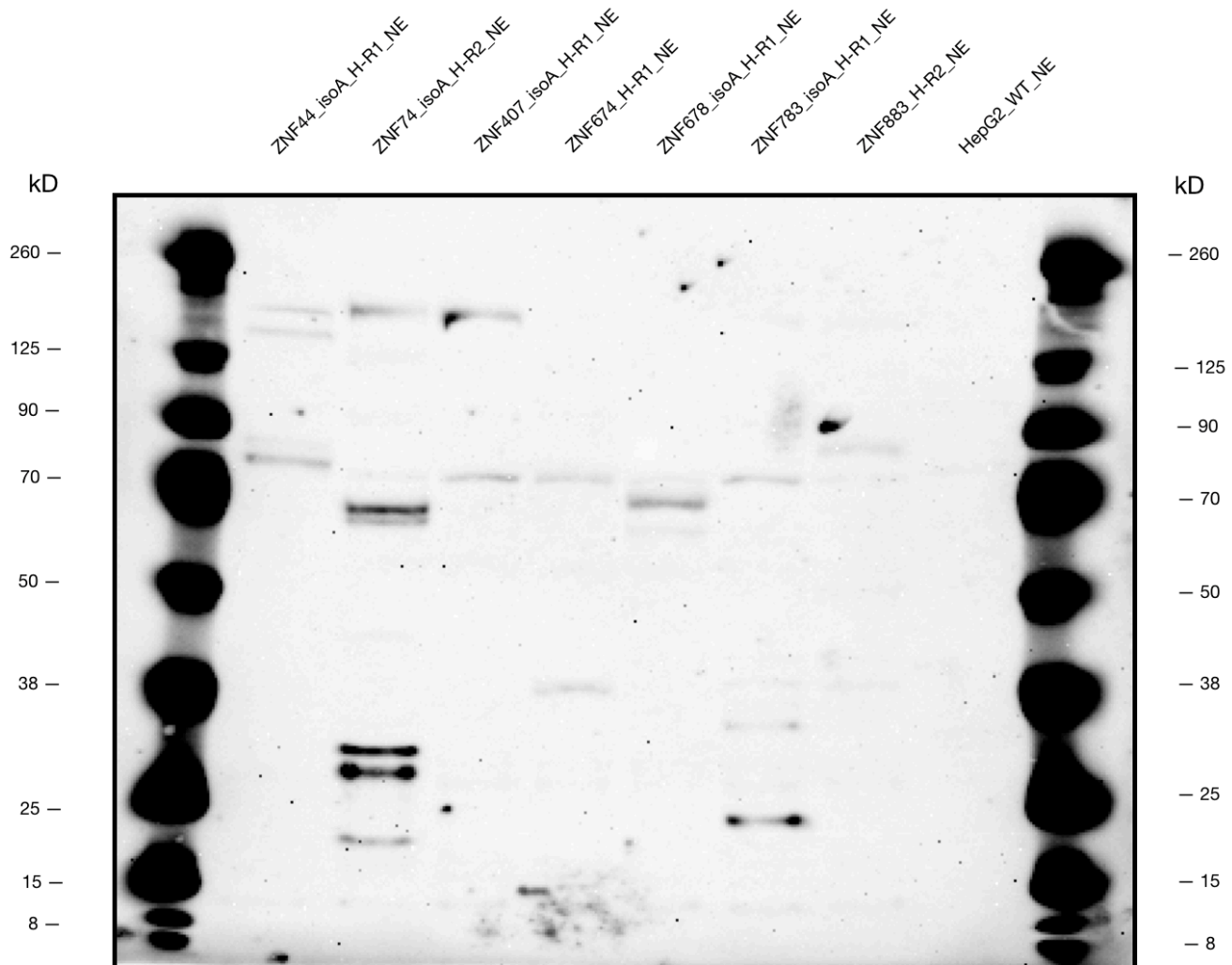
ZNF44 (*Homo sapiens*), ZNF74 (*Homo sapiens*), ZNF407 (*Homo sapiens*), ZNF674 (*Homo sapiens*), ZNF678 (*Homo sapiens*), ZNF783 (*Homo sapiens*), and ZNF883 (*Homo sapiens*)

Method:

Western Blot Validation

Caption:

Each nuclear protein isolate (252 mcg - ZNF44; 300 mcg - ZNF74, ZNF678, and ZNF883; 271 mcg - ZNF407; 235 mcg - ZNF674; 178 mcg - ZNF783; 50 mcg - HepG2 Wild-Type) 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. 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).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-ZNF44_isoA_HepG2 rep 1 (nuclear extract)	80	Three bands of similar distinctness at different molecular weights
3	FLAG-ZNF74_isoA_HepG2 rep 2 (nuclear extract)	75	Multiple bands, with the most distinct ones far from the expected. PTMs: Phosphorylation
4	FLAG-ZNF407_isoA_HepG2 rep 1 (nuclear extract)	250	Distinct band around 200 kDa. Found comparable western blot with banding near 210 kDa, within 20% of the observed: https://www.lsbio.com/antibodies/znf407-antibody-aa951-1000-biotin-wb-western-ls-c438350/451056
5	FLAG-ZNF674_HepG2 rep 1 (nuclear extract)	70	Distinct band near 38 kDa, beyond 20% but within the region of the expected
6	FLAG-ZNF678_isoA_HepG2 rep 1 (nuclear extract)	64	Distinct band within 20% of the expected
7	FLAG-ZNF783_isoA_HepG2 rep 1 (nuclear extract)	34	Distinct band near 20% of the expected. PTMs: Acetylation
8	FLAG-ZNF883_HepG2 rep 2 (nuclear extract)	47	Distinct banding around 80 kDa. Found comparable western blot with banding near 72 kDa, within 20% of the observed: https://www.antibodies-online.cn/antibody/2838303/anti-Zinc+Finger+Protein+883+ZNF883+AA+129-157,+N-Term+antibody/
9	Wild-Type HepG2 (nuclear extract) (negative control)	None	No visible banding
10	Ladder	N/A	N/A

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