

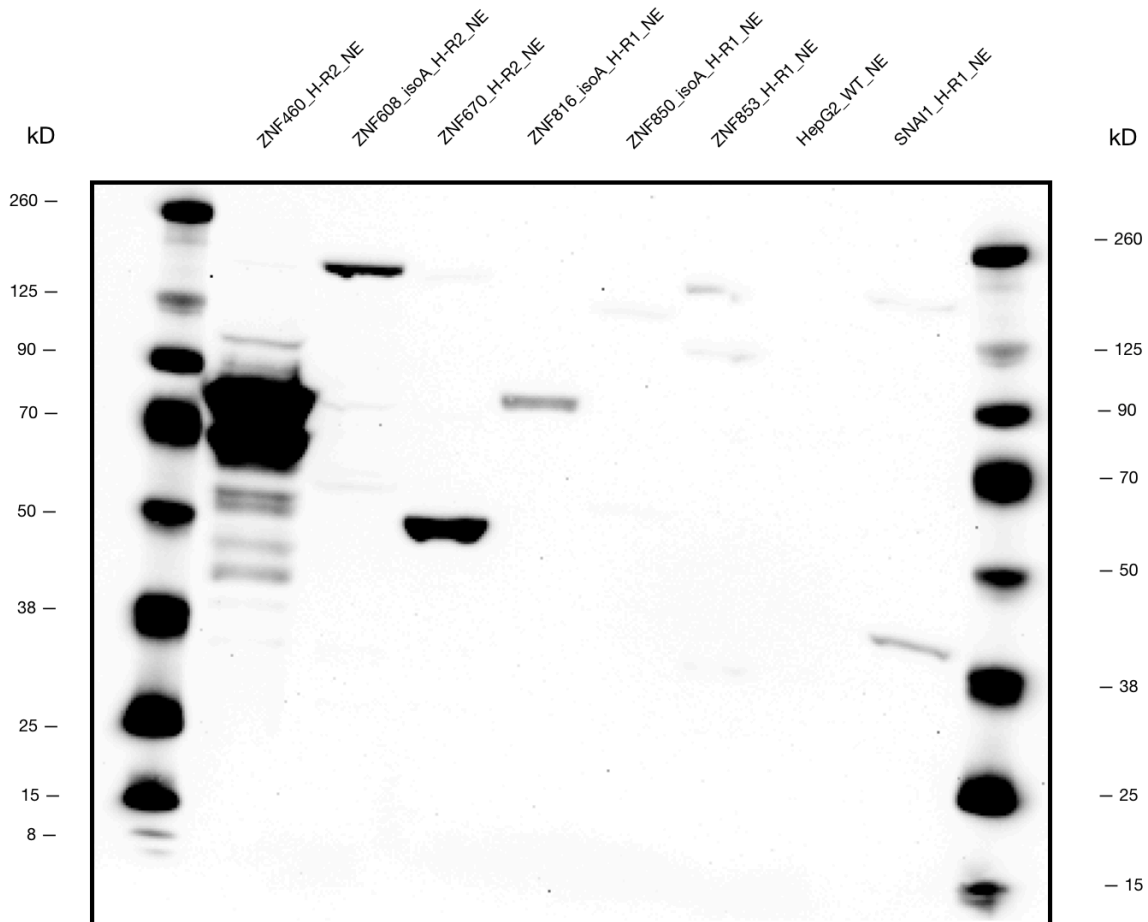
**ZNF460 (*Homo sapiens*), ZNF608 (*Homo sapiens*), ZNF670 (*Homo sapiens*), ZNF816 (*Homo sapiens*), ZNF850 (*Homo sapiens*), and ZNF853 (*Homo sapiens*)**

**Method:**

Western Blot Validation

**Caption:**

Each nuclear protein isolate (169 mcg - ZNF460; 226 mcg - ZNF608; 300 mcg - ZNF670; 203 mcg - ZNF816; 250 mcg - ZNF850; 257 mcg - ZNF813; 101 mcg - HepG2 Wild-Type; 291 mcg - SNAI1) 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 an SNAI1-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).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-ZNF460_HepG2 rep 2 (nuclear extract)	67	Two dark bands within 20% of the expected size. PTMs: Isopeptide bonding and Ubl conjugation
3	FLAG-ZNF608_isoA_HepG2 rep 2 (nuclear extract)	165	Dark band within 20% of the expected size. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
4	FLAG-ZNF670_HepG2 rep 2 (nuclear extract)	48	Dark band within 20% of the expected size. PTMs: Phosphorylation
5	FLAG-ZNF816_isoA_HepG2 rep 1 (nuclear extract)	79	Single distinct band within 20% of the expected size
6	FLAG-ZNF850_isoA_HepG2 rep 1 (nuclear extract)	128	Single faint band within 20% of the expected size. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF853_HepG2 rep 1 (nuclear extract)	78	Multiple bands beyond 20% from the expected size
8	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
9	FLAG-SNAI1_HepG2 rep 1 (nuclear extract) (positive control)	40	Distinct band within 20% of the expected size. PTMs: ADP-ribosylation, Glycosylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
10	Ladder	N/A	N/A

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