

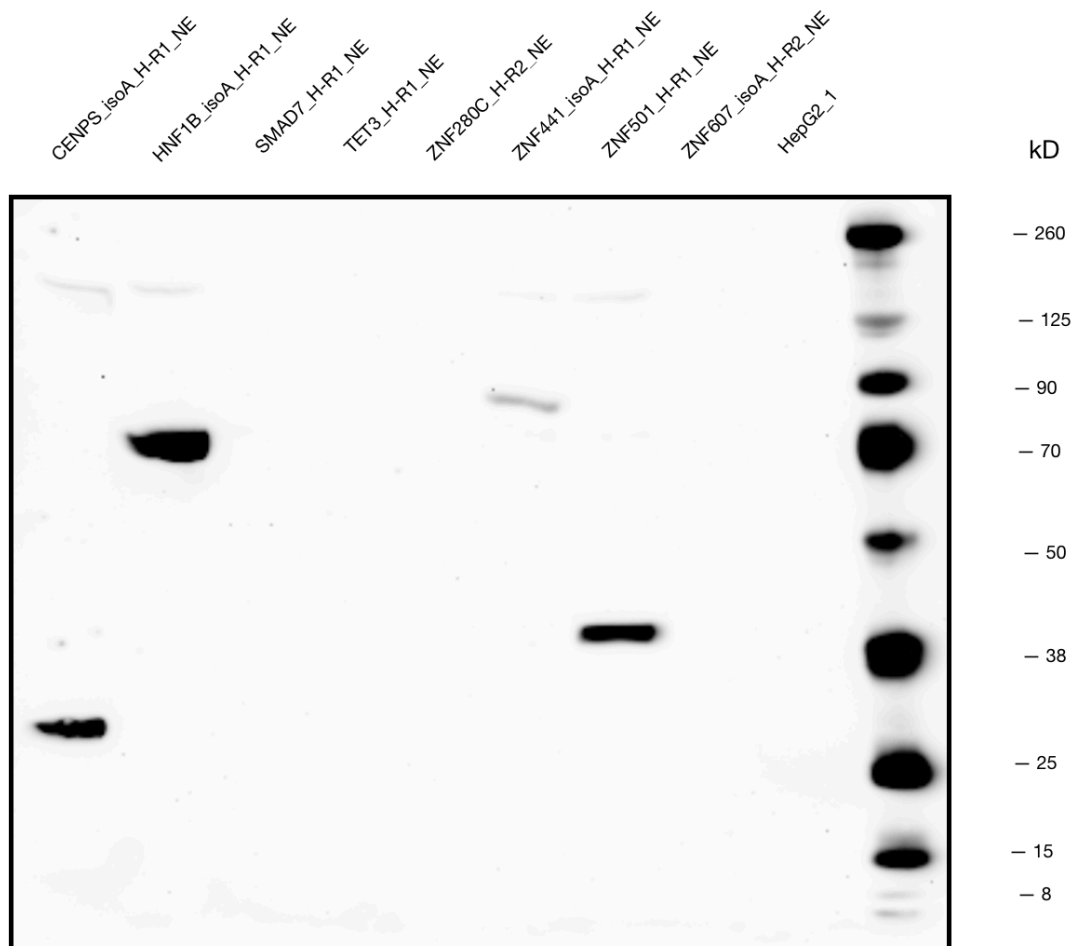
CENPS (*Homo sapiens*), HNF1B (*Homo sapiens*), SMAD7 (*Homo sapiens*), TET3 (*Homo sapiens*), ZNF280C (*Homo sapiens*), ZNF441 (*Homo sapiens*), ZNF501 (*Homo sapiens*), and ZNF607 (*Homo sapiens*)

Method:

Western Blot Validation

Caption:

Each nuclear protein isolate (236 mcg - CENPS, 230 mcg - HNF1B, 248 mcg - SMAD7, 298 mcg - TET3, 281 mcg - ZNF280C, 246 mcg - ZNF441, 242 mcg - ZNF501, 227 mcg - ZNF607, and 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	FLAG-CENPS_isoA_HepG2 rep 1 (nuclear extract)	19	Distinct band near 30 kDa, beyond 20% of the expected size. PTMs: Acetylation
2	FLAG-HNF1B_isoA_HepG2 rep 1 (nuclear extract)	64	Dark band within 20% of the expected size. PTMs: Phosphorylation
3	FLAG-SMAD7_HepG2 rep 1 (nuclear extract)	49	No visible banding. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
4	FLAG-TET3_HepG2 rep 1 (nuclear extract)	182	No visible banding. PTMs: Isopeptide bonding and Ubl conjugation
5	FLAG-ZNF280C_HepG2 rep 2 (nuclear extract)	86	No visible banding. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
6	FLAG-ZNF441_isoA_HepG2 rep 1 (nuclear extract)	83	Single distinct band within 20% of the expected size
7	FLAG-ZNF501_HepG2 rep 1 (nuclear extract)	34	Dark band within 20% expected size
8	FLAG-ZNF607_isoA_HepG2 rep 2 (nuclear extract)	84	No visible banding
9	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
10	Ladder	N/A	N/A

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Grant:

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