

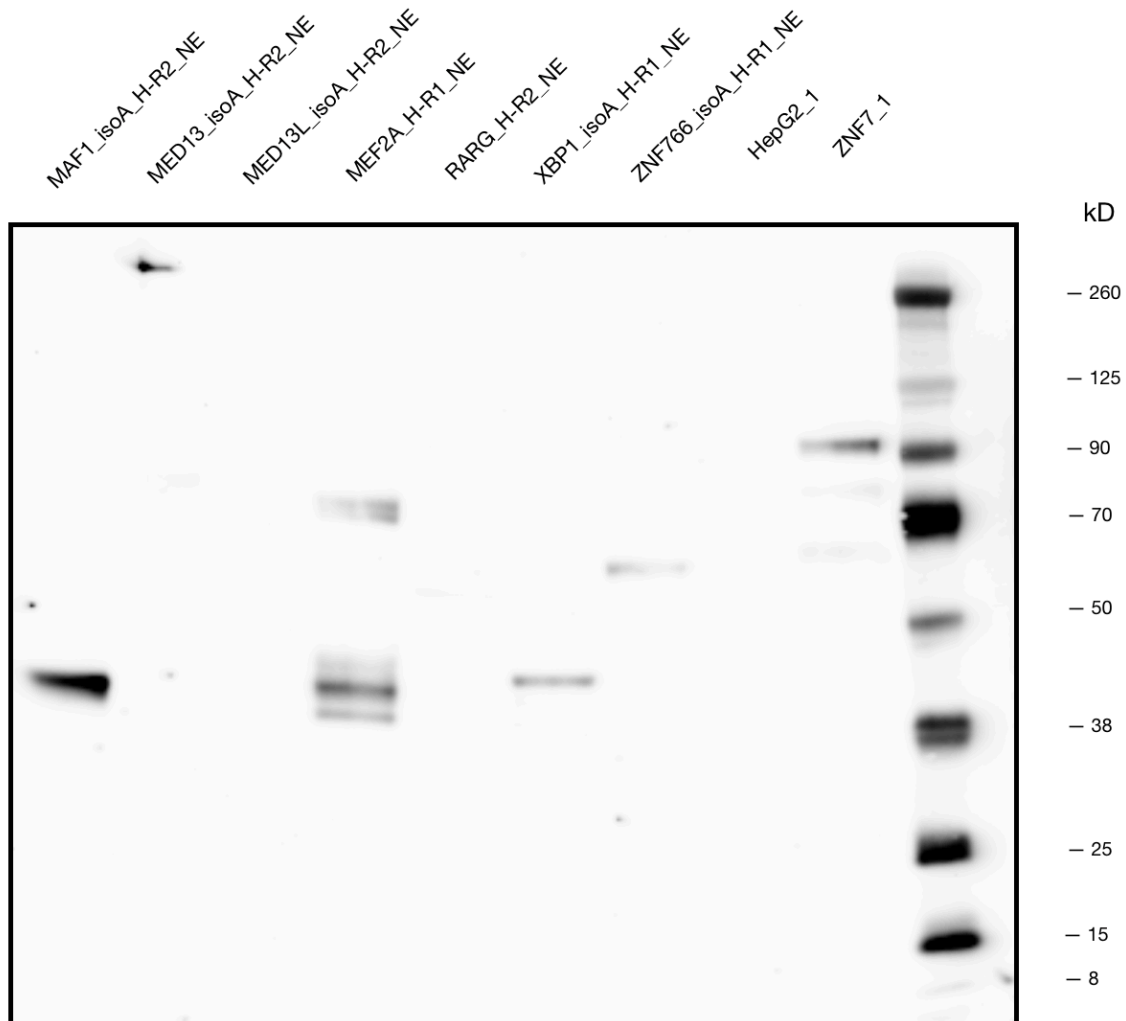
MAF1 (*Homo sapiens*), MED13 (*Homo sapiens*), MED13L (*Homo sapiens*), MEF2A (*Homo sapiens*), RARG (*Homo sapiens*), XBP1 (*Homo sapiens*), and ZNF766 (*Homo sapiens*)

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).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	FLAG-MAF1_isoA_HepG2 rep 2 (nuclear extract)	32	Single dark band at about 42 kDa. Found comparable western blot with banding at 35 kDa, 20% from the observed molecular weight. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
2	FLAG-MED13_isoA_HepG2 rep 2 (nuclear extract)	242	Single partial band near 300 kDa. Found comparable western blot with banding at a similar molecular weight: https://www.abcam.com/med13-antibody-ab76923.html#description_images_1 . PTMs: Phosphorylation
3	FLAG-MED13L_isoA_HepG2 rep 2 (nuclear extract)	246	No visible banding
4	FLAG-MEF2A_HepG2 rep 1 (nuclear extract)	58	Distinct band around 42 kDa. Found comparable western blot with banding around 43 kDa: https://www.novusbio.com/products/mef2a-antibody-2f9h2_nbp2-37298 . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-RARG_HepG2 rep 2 (nuclear extract)	53	No visible banding
6	FLAG-XBP1_isoA_HepG2 rep 1 (nuclear extract)	32	Single band at 44 kDa. Found comparable western blot with banding at the same molecular weight: https://www.abcam.com/xbp1-antibody-ab37152.html . PTMs: Acetylation, Cleavage on a pair of basic residues, Phosphorylation, and Ubl conjugation
7	FLAG-ZNF766_isoA_HepG2 rep 1 (nuclear extract)	58	Single band within 20% expected
8	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
9	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Distinct band within 20% expected
10	Ladder	N/A	N/A

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