

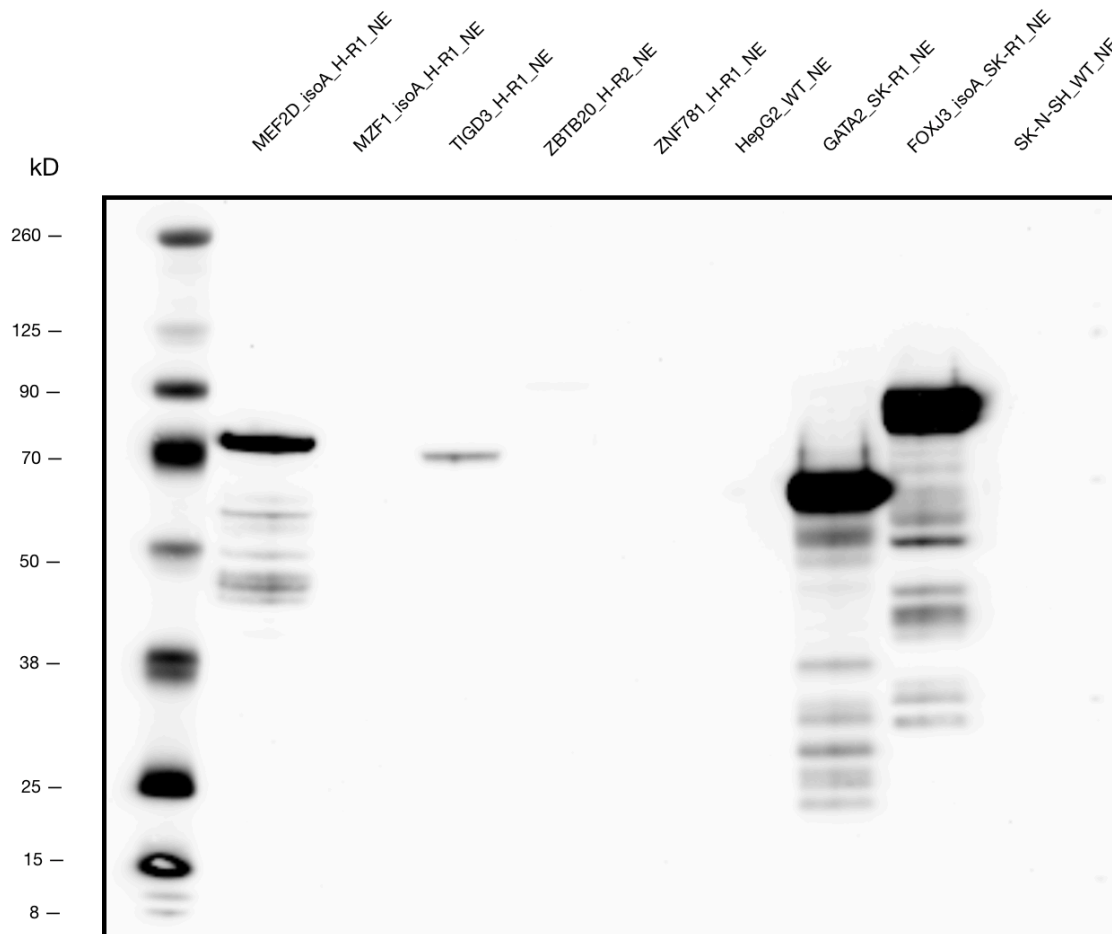
MEF2D (*Homo sapiens*), MZF1 (*Homo sapiens*), TIGD3 (*Homo sapiens*), ZBTB20 (*Homo sapiens*), ZNF781 (*Homo sapiens*), GATA2 (*Homo sapiens*), and FOXJ3 (*Homo sapiens*)

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. HepG2 and SK-N-SH untagged nuclear isolates were included as negative controls. 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	Ladder	N/A	N/A
2	FLAG-MEF2D_isoA_HepG2 rep 1 (nuclear extract)	59	Dark band near 72 kDa. Found comparable western blot with banding around 70 kDa, near the observed: https://www.abcam.com/mef2d-antibody-ab246884.html . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	FLAG-MZF1_isoA_HepG2 rep 1 (nuclear extract)	85	No visible banding. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
4	FLAG-TIGD3_HepG2 rep 1 (nuclear extract)	55	Single distinct band around 69 kDa, just beyond 20% of the expected
5	FLAG-ZBTB20_HepG2 rep 2 (nuclear extract)	84	Extremely faint band near 90 kDa, within 20% of the expected. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
6	FLAG-ZNF781_HepG2 rep 1 (nuclear extract)	45	No visible banding
7	Wild-Type HepG2 (nuclear extract) (negative control)	None	No visible banding
8	FLAG-GATA2_SK-N-SH rep 1 (nuclear extract)	54	Strong banding around 65 kDa. Found comparable western blot with banding around 62 kDa, near the observed: https://www.proteinatlas.org/ENSG00000179348-GATA2/antibody . PTMs: Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation
9	FLAG-FOXJ3_isoA_SK-N-SH rep 1 (nuclear extract)	72	Strong banding within 20% of the expected. PTMs: Phosphorylation
10	Wild-Type SK-N-SH (nuclear extract) (negative control)	None	No visible banding

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