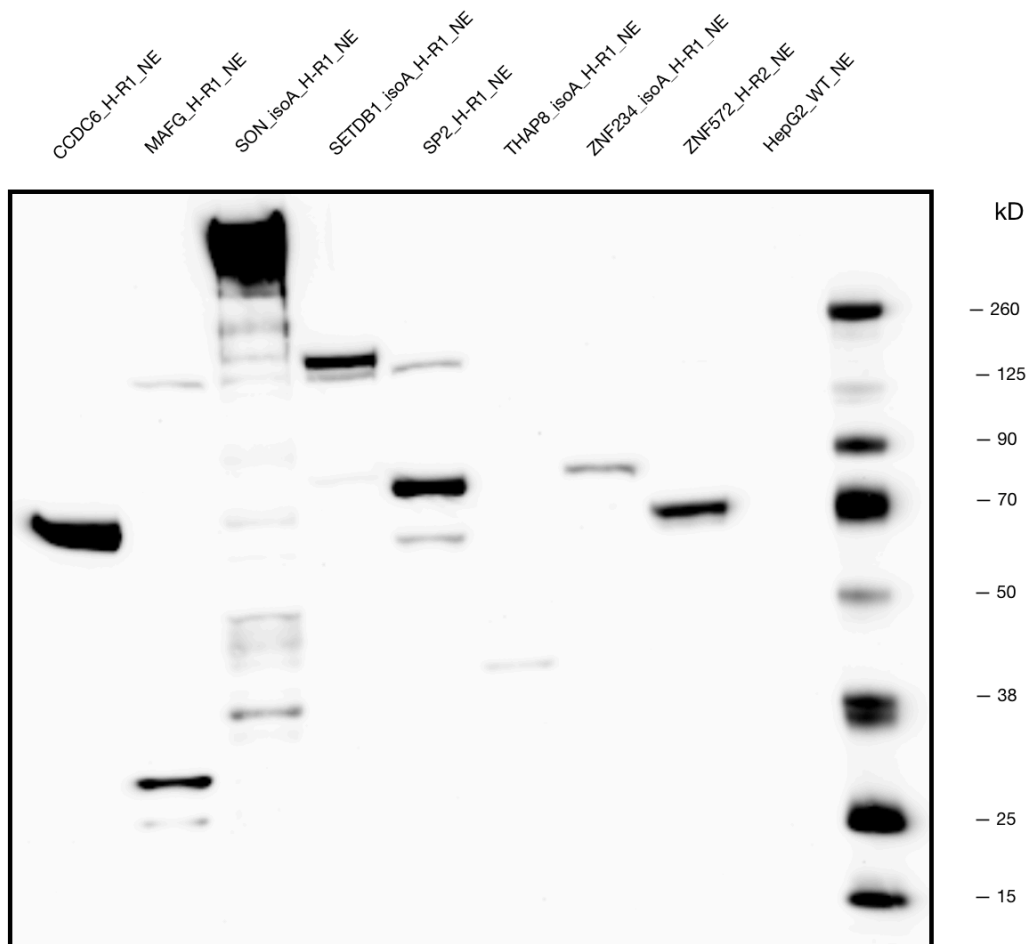


CCDC6 (*Homo sapiens*), MAFG (*Homo sapiens*), SON (*Homo sapiens*), SETDB1 (*Homo sapiens*), SP2 (*Homo sapiens*), THAP8 (*Homo sapiens*), ZNF234 (*Homo sapiens*), and ZNF572 (*Homo sapiens*)

Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (238 mcg - CCDC6, 235 mcg - MAFG, 183 kDa - SON, 245 kDa - SETDB1, 217 kDa - SP2, 214 kDa - THAP8, 281 kDa - ZNF234, 296 kDa - ZNF572, and 50 kDa - 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-CCDC6_HepG2 rep 1 (nuclear extract) | 56 | Dark band around 67 kDa, within 20% of the expected size. PTMs: Acetylation, Methylation, and Phosphorylation |
| 2 | FLAG-MAFG_HepG2 rep 1 (nuclear extract) | 21 | Distinct band at about 30 kDa, beyond 20% of the expected size. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation |
| 3 | FLAG-SON_isoA_HepG2 rep 1 (nuclear extract) | 267 | Predicted size was 267 kDa. The observed size was 350 kDa, which is within 20% of an observed band of 300 kDa seen in https://www.proteinatlas.org/ENSG00000159140-SON/antibody#western_blot . PTMs: Acetylation, Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation |
| 4 | FLAG-SETDB1_isoA_HepG2 rep 1 (nuclear extract) | 146 | Dark band within 20% of the expected size. PTMs: Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation |
| 5 | FLAG-SP2_HepG2 rep 1 (nuclear extract) | 68 | Dark band within 20% of the expected size. PTMs: Phosphorylation |
| 6 | FLAG-THAP8_isoA_HepG2 rep 1 (nuclear extract) | 33 | Predicted size was 33 kDa. The observed size was 43 kDa, which is within 20% of an observed band of 40 kDa seen in https://www.thermofisher.com/antibody/product/THAP8-Antibody-Polyclonal/PA5-48531 |
| 7 | FLAG-ZNF234_isoA_HepG2 rep 1 (nuclear extract) | 84 | Single distinct band within 20% of the expected size |
| 8 | FLAG-ZNF572_HepG2 rep 2 (nuclear extract) | 64 | Single dark band within 20% of the expected size. PTMs: Acetylation, Isopeptide bonding and Ubl conjugation |
| 9 | Wild-Type Hep G2 (nuclear extract) (negative control) | None | No visible banding |
| 10 | Ladder | N/A | N/A |

Submitted by:
Mark Mackiewicz and Michael Betti

Lab:
Richard Myers, HAIB

Grant:
UM1 HG009411

Download: