

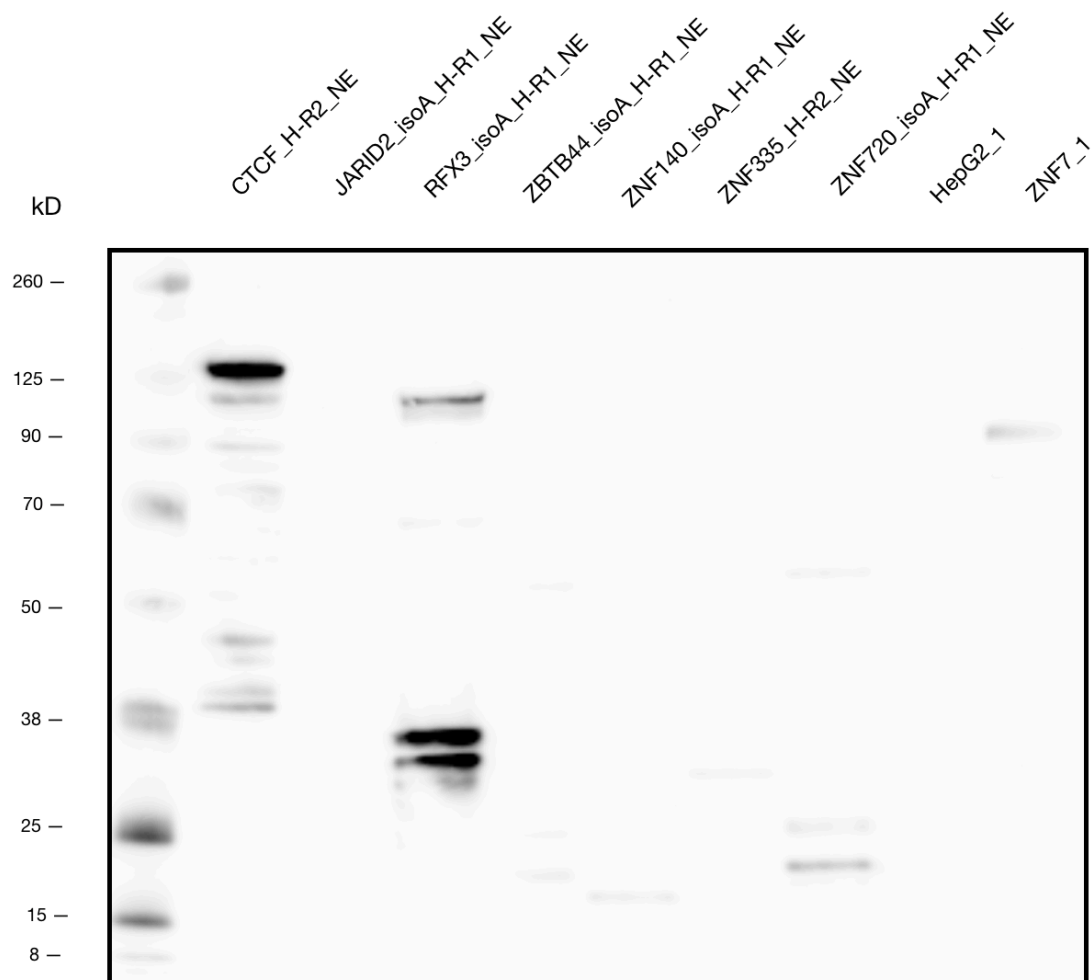
CTCF (*Homo sapiens*), JARID2 (*Homo sapiens*), RFX3 (*Homo sapiens*), ZBTB44 (*Homo sapiens*), ZNF140 (*Homo sapiens*), ZNF335 (*Homo sapiens*), and ZNF720 (*Homo sapiens*)

Method:

Western Blot Validation

Caption:

Each nuclear protein isolate (25 mcg - CTCF; 50 mcg - JARID2, RFX3, ZBTB44, ZNF140, ZNF225, and ZNF720) 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).



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-CTCF_HepG2 rep 2 (nuclear extract)	86	Half of the original standardized sample (25 mcg of total protein) was loaded to reduce background banding. Distinct band around 140 kDa. Found similar western blot with band at approximately 123 kDa: https://www.abcam.com/ctcf-antibody-chip-grade-ab70303.html . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	FLAG-JARID2_isoA_HepG2 rep 1 (nuclear extract)	142	No visible banding. PTMs: Acetylation and Phosphorylation
4	FLAG-RFX3_isoA_HepG2 rep 1 (nuclear extract)	87	Three distinct regions of banding. Found comparable western blot with bands near 84, 79, and 45 kDa: https://www.proteinatlas.org/ENSG00000080298-RFX3/antibody#western_blot
5	FLAG-ZBTB44_isoA_HepG2 rep 1 (nuclear extract)	67	Partial faint banding visible around 20 kDa, far from the expected. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
6	FLAG-ZNF140_isoA_HepG2 rep 1 (nuclear extract)	56	Single band far from 20% expected
7	FLAG-ZNF335_HepG2 rep 2 (nuclear extract)	148	Single band far from 20% expected. PTMs: Phosphorylation, Isopeptide bonding, and Ubl conjugation
8	FLAG-ZNF720_isoA_HepG2 rep 1 (nuclear extract)	17	Distinct band within 20% expected
9	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
10	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Distinct band within 20% expected

Submitted by:

Mark Mackiewicz and Michael Betti

Lab:

Richard Myers, HAIB

Grant:

UM1 HG009411

Download:

