

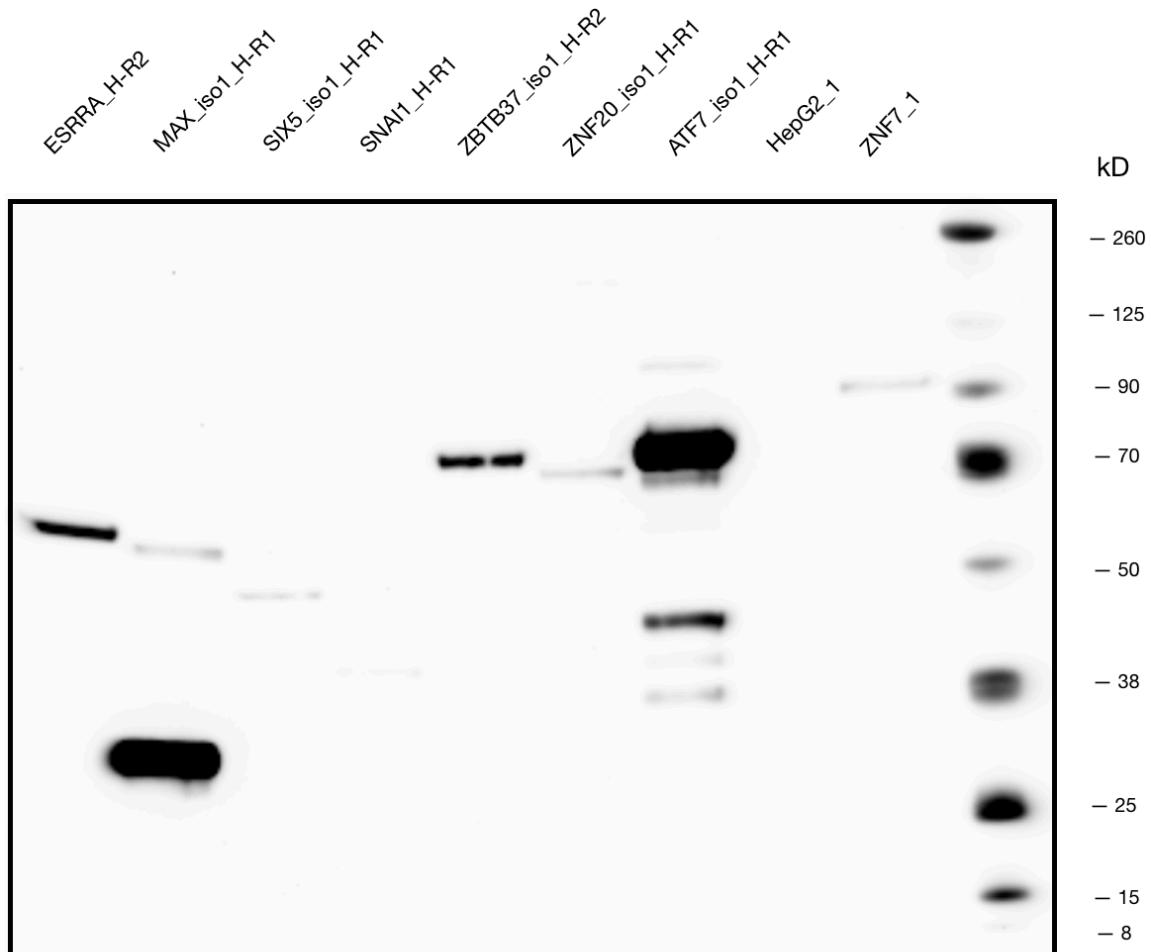
ESRRA (*Homo sapiens*), MAX (*Homo sapiens*), SIX5 (*Homo sapiens*), SNAI1 (*Homo sapiens*), ZBTB37 (*Homo sapiens*), ZNF20 (*Homo sapiens*), and ATF7 (*Homo sapiens*)

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).



Monoclonal ANTI-FLAG M2-Peroxidase (HRP) antibody produced in mouse

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	FLAG-ESRRA_HepG2 rep2 (nuclear extract)	49	Single band within 20% expected. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
2	FLAG-MAX_iso1_HepG2 rep 1 (nuclear extract)	21	Distinct band around 30 kDa. Found similar western blot with band at 35 kDa: https://www.abcam.com/max-antibody-73c5a-ab53570.html . PTMs: Acetylation and Phosphorylation
3	FLAG-SIX5_iso1_HepG2 rep1 (nuclear extract)	78	Single distinct band, but may deviate further than 20% from expected. PTMs: Phosphorylation
4	FLAG-SNAI1_HepG2 rep 1 (nuclear extract)	32	Single faint band within 20% expected. PTMs: ADP-ribosylation, Glycosylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-ZBTB37_iso1_HepG2 rep 2 (nuclear extract)	59	Single distinct band near 20% expected
6	FLAG-ZNF20_iso1_HepG2 rep1 (nuclear extract)	65	Distinct band within 20% expected
7	FLAG-ATF7_iso1_HepG2 rep1 (nuclear extract)	56	Distinct band near 75 kDa. Found similar western blot with band around 83 kD: https://www.proteinatlas.org/ENSG00000170653-ATF7/antibody#western_blot . PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
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

Submitted by:

Mark Mackiewicz and Michael Betti

Lab:

Richard Myers, HAIB

Grant:

UM1 HG009411

Download: