

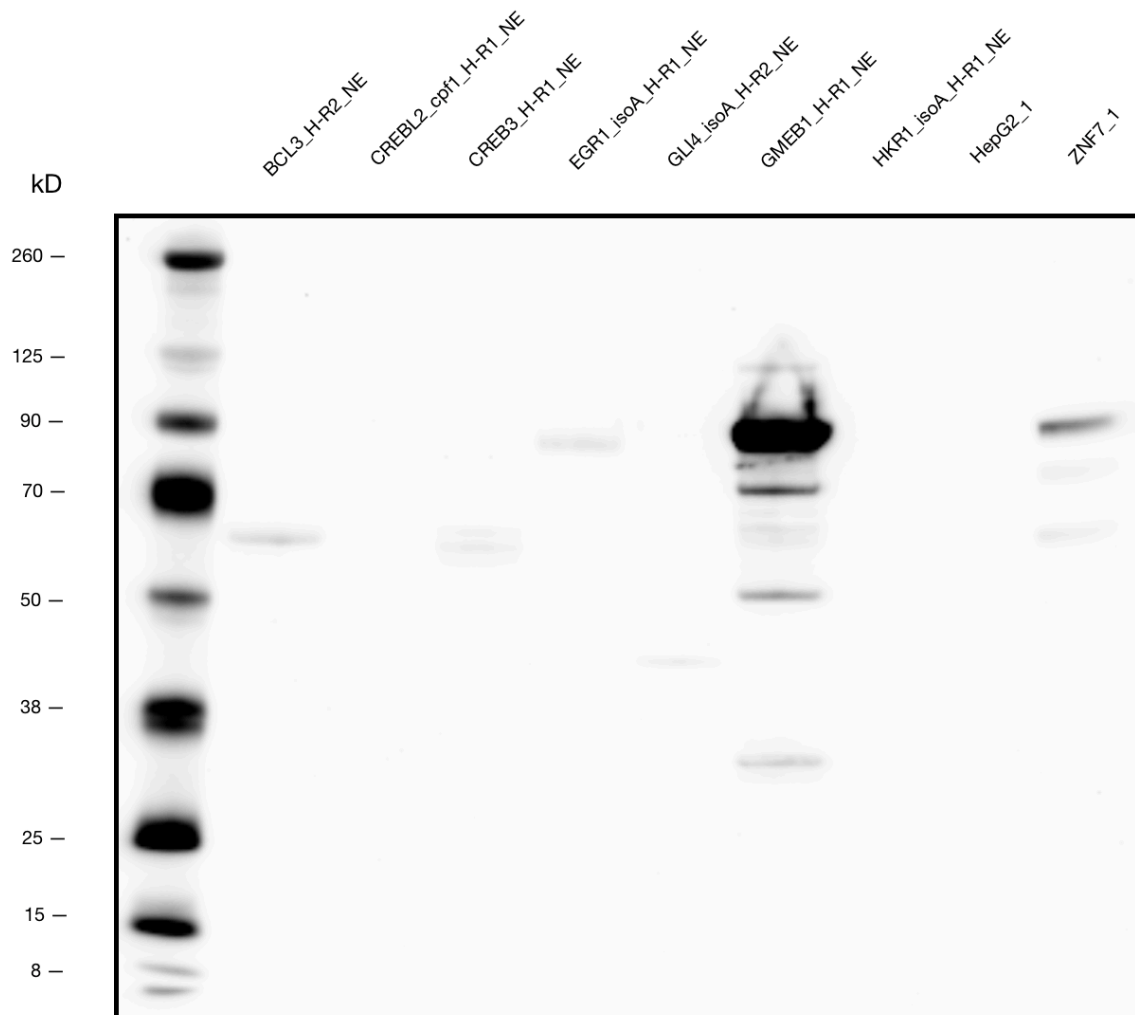
BCL3 (*Homo sapiens*), CREBL2 (*Homo sapiens*), CREB3 (*Homo sapiens*), EGR1 (*Homo sapiens*), GLI4 (*Homo sapiens*), GMEB1 (*Homo sapiens*), and HKR1 (*Homo sapiens*)

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	Ladder	N/A	N/A
2	FLAG-BCL3_HepG2 rep 2 (nuclear extract)	51	Single distinct band within 20% expected. PTMs: Phosphorylation and Ubl conjugation
3	FLAG-CREBL2_cpf1_HepG2 rep 1 (nuclear extract)	17	No visible banding. PTMs: Phosphorylation
4	FLAG-CREB3_HepG2 rep 1 (nuclear extract)	44	Two close bands near 20% from expected. PTMs: Glycosylation
5	FLAG-EGR1_isoA_HepG2 rep 1 (nuclear extract)	61	Single distinct band near 80 kDa. Found comparable western blot with banding at the same molecular weight as the observed: https://www.abcam.com/egr1-antibody-epr50142-ab133695.html#description_images_1
6	FLAG-GLI4_isoA_HepG2 rep 2 (nuclear extract)	44	Single distinct band near the expected. PTMs: Phosphorylation
7	FLAG-GMEB1_HepG2 rep 1 (nuclear extract)	66	Dark band near 85 kDa. Found comparable western blot with banding around 90 kDa, within 20% from the observed: https://www.proteinatlas.org/ENSG00000162419-GMEB1/antibody#western_blot
8	FLAG-HKR1_isoA_HepG2 rep 1 (nuclear extract)	78	No visible banding
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

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Lab:

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