

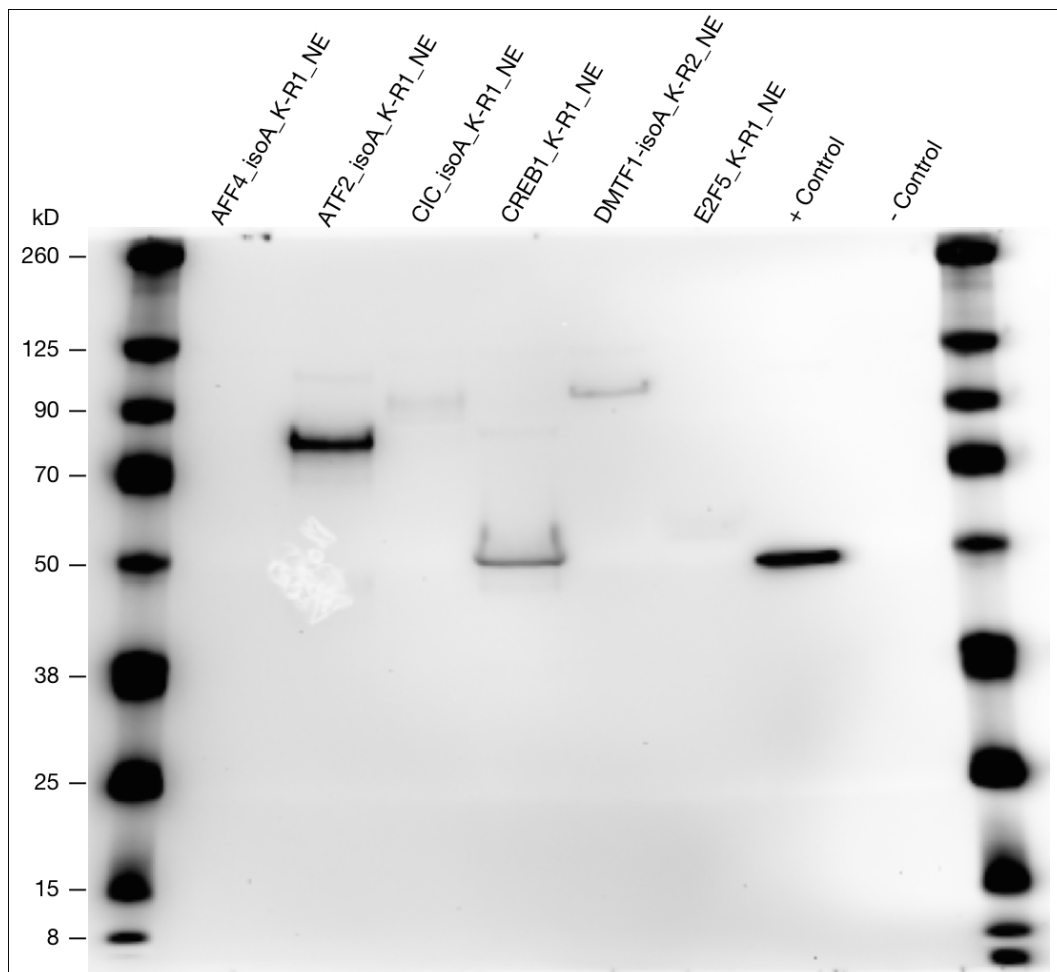
AFF4 (*Homo sapiens*), ATF2 (*Homo sapiens*), CIC (*Homo sapiens*), CREB1 (*Homo sapiens*), DMTF1 (*Homo sapiens*), and E2F5 (*Homo sapiens*)

Method:

Western Blot Validation

Caption:

Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear protein isolate (500 uL) using the FLAG Immunoprecipitation Kit (Sigma-Aldrich; cat# FLAGIPT1). The final elution step was performed by suspending the sample-bound resin in NuPage Sample Reducing Agent 10X and NuPage LDS Sample Buffer 4X (Thermo Fisher Scientific) and heating for 3 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 blank IP was included as a negative control, and an immunoprecipitated FLAG-BAP fusion protein provided in the kit 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).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-AFF4_isoA-K562 rep 1 (nuclear extract)	127	No distinct band.
3	FLAG-ATF2_isoA-K562 rep 1 (nuclear extract)	54	Predicted size was 54 kDa. The observed band near 80 kDa, is within 20% of the observed bands seen in https://www.scbt.com/p/atf-2-antibody-f2br-1 PTMs: Acetylation, Phosphoprotein.
4	FLAG-CIC_isoA-K562 rep 1 (nuclear extract)	164	Distinct band below 20% of the expected size.
5	FLAG-CREB1-K562 rep 1 (nuclear extract)	35	Distinct band within 20% of that observed in https://www.scbt.com/p/creb-1-antibody-d-12?qclid=Cj0KCQjwmuiTBhDoARIsAPiv6L_I81kOaZMPCi7hGGNhyp1dFbDyHiDtRrV_UOW80mkzLhlzC877-EMaAqJ-EALw_wcB .
6	FLAG-DMTF1_isoA-K562 rep 2 (nuclear extract)	84	Distinct band within 20% of the expected size.
7	FLAG-E2F5-K562 rep 1 (nuclear extract)	38	Distinct band within 20% of the expected size.
8	Positive Control (FLAG BAP fusion protein)	NA	PASS
9	Negative Control (Blank IP)	NA	PASS
10	Ladder	N/A	N/A

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Grant: UM1

HG009411

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