

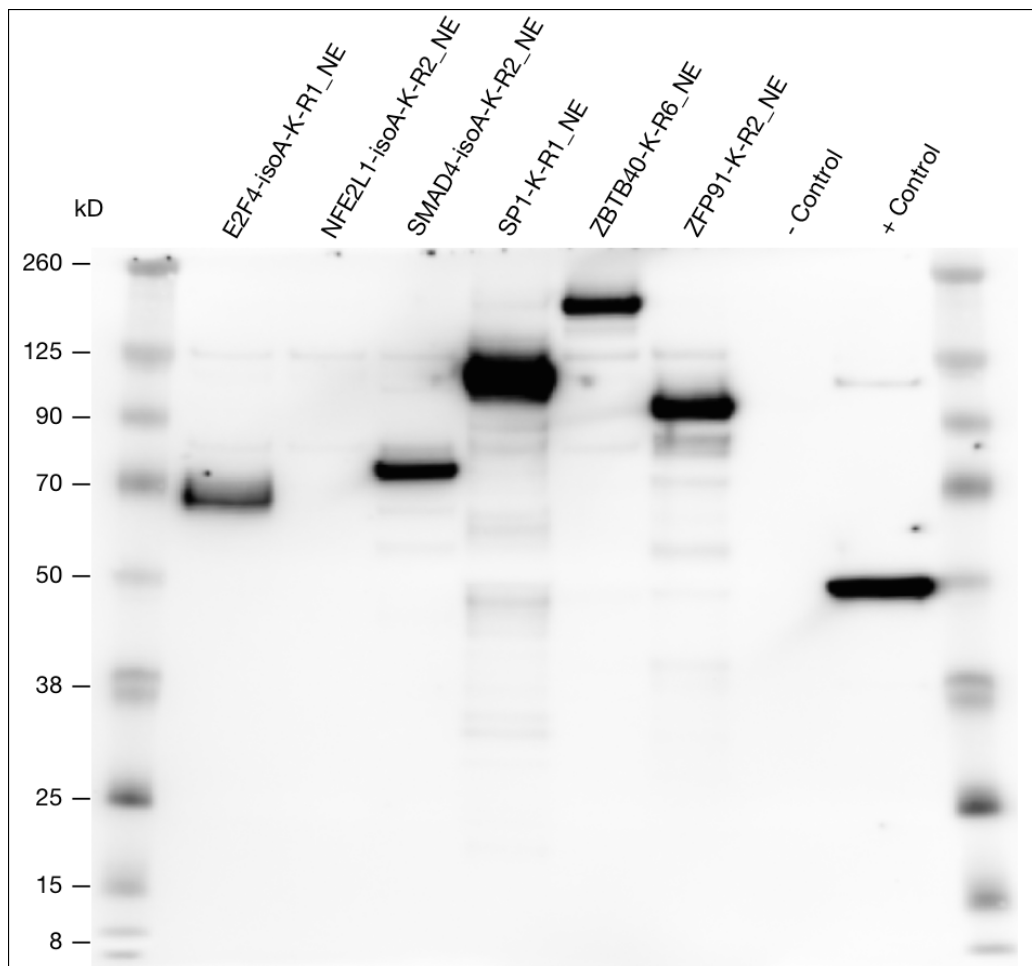
**E2F4 (*Homo sapiens*), NFE2L1 (*Homo sapiens*), SMAD4 (*Homo sapiens*), SP1 (*Homo sapiens*), ZBTB40 (*Homo sapiens*), and ZFP91 (*Homo sapiens*)**

**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-E2F4_isoA-K562 rep 1 (nuclear extract)	44	Predicted size was 44 kDa. The observed size was near 70 kDa, which is the same size as an observed band seen in <a href="https://www.ptglab.com/products/E2F4-Antibody-10923-1-AP.htm">https://www.ptglab.com/products/E2F4-Antibody-10923-1-AP.htm</a> PTMs: Ubiquitination, Phosphorylation, Acetylation.
3	FLAG-NFE2L1_isoA-K562 rep 2 (nuclear extract)	85	No distinct band.
4	FLAG-SMAD4_isoA-K562 rep 2 (nuclear extract)	60	Distinct band within 20% of the expected size.
5	FLAG-SP1-K562 rep 1 (nuclear extract)	81	Distinct band within 20% of the expected size.
6	FLAG-ZBTB40-K562 rep 6 (nuclear extract)	138	Distinct band within 20% of the expected size.
7	FLAG-ZFP91-K562 rep 2 (nuclear extract)	63	Predicted size was 63 kDa. The observed size was near 92 kDa, which is the same size as an observed band seen in <a href="https://www.fortislife.com/products/primary-antibodies/rabbit-anti-zfp91-antibody/BETHYL-A303-245">https://www.fortislife.com/products/primary-antibodies/rabbit-anti-zfp91-antibody/BETHYL-A303-245</a> PTMs: Acetylation, Methylation, Phosphorylation Ubiquitination.
8	Negative Control (Blank IP)	NA	PASS
9	Positive Control (FLAG BAP fusion protein)	NA	PASS
10	Ladder	N/A	N/A

**Submitted by:** Mark Mackiewicz and Amy Nesmith.

**Lab:** Richard Myers, HAIB

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