

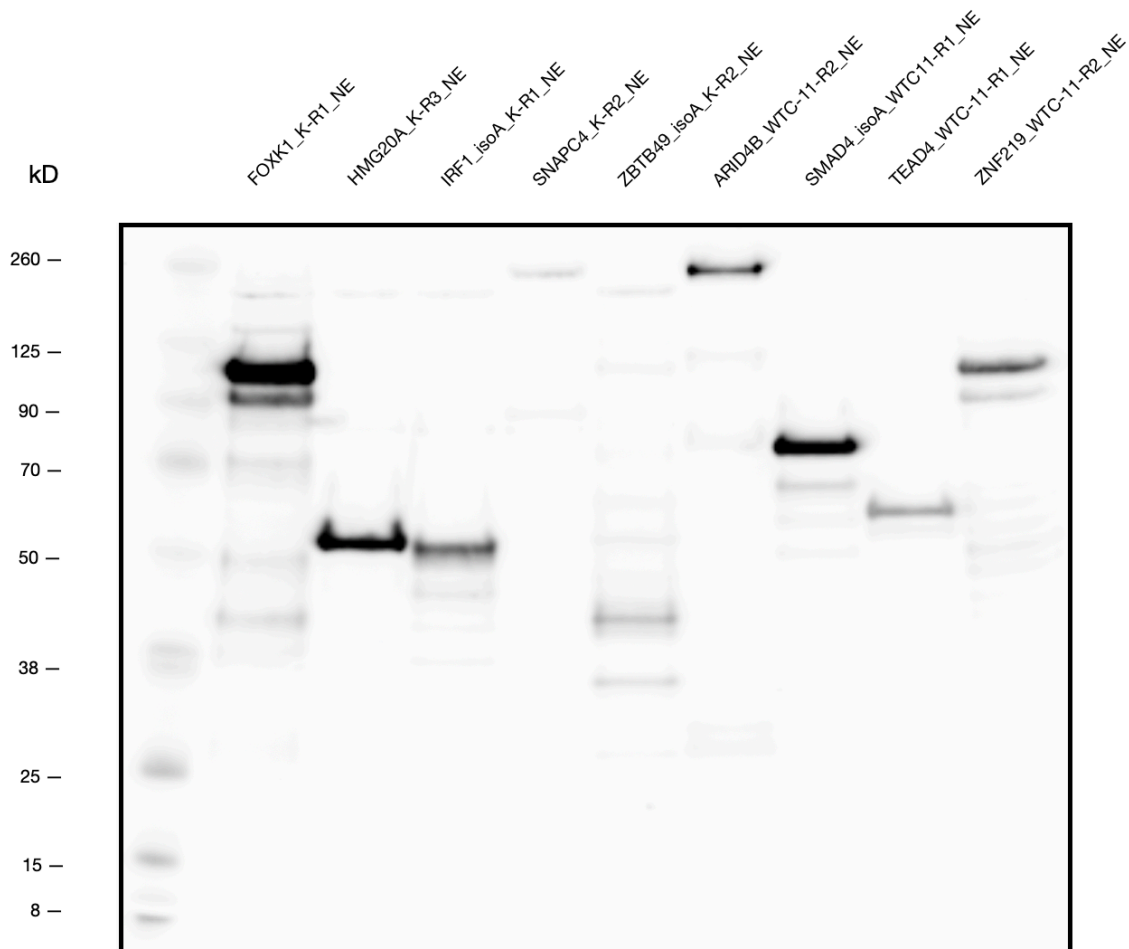
**FOXK1 (*Homo sapiens*), HMG20A (*Homo sapiens*), IRF1 (*Homo sapiens*), SNAPC4 (*Homo sapiens*), ZBTB49 (*Homo sapiens*), ARID4B (*Homo sapiens*), SMAD4 (*Homo sapiens*), TEAD4 (*Homo sapiens*), and ZNF219 (*Homo sapiens*)**

**Method:**

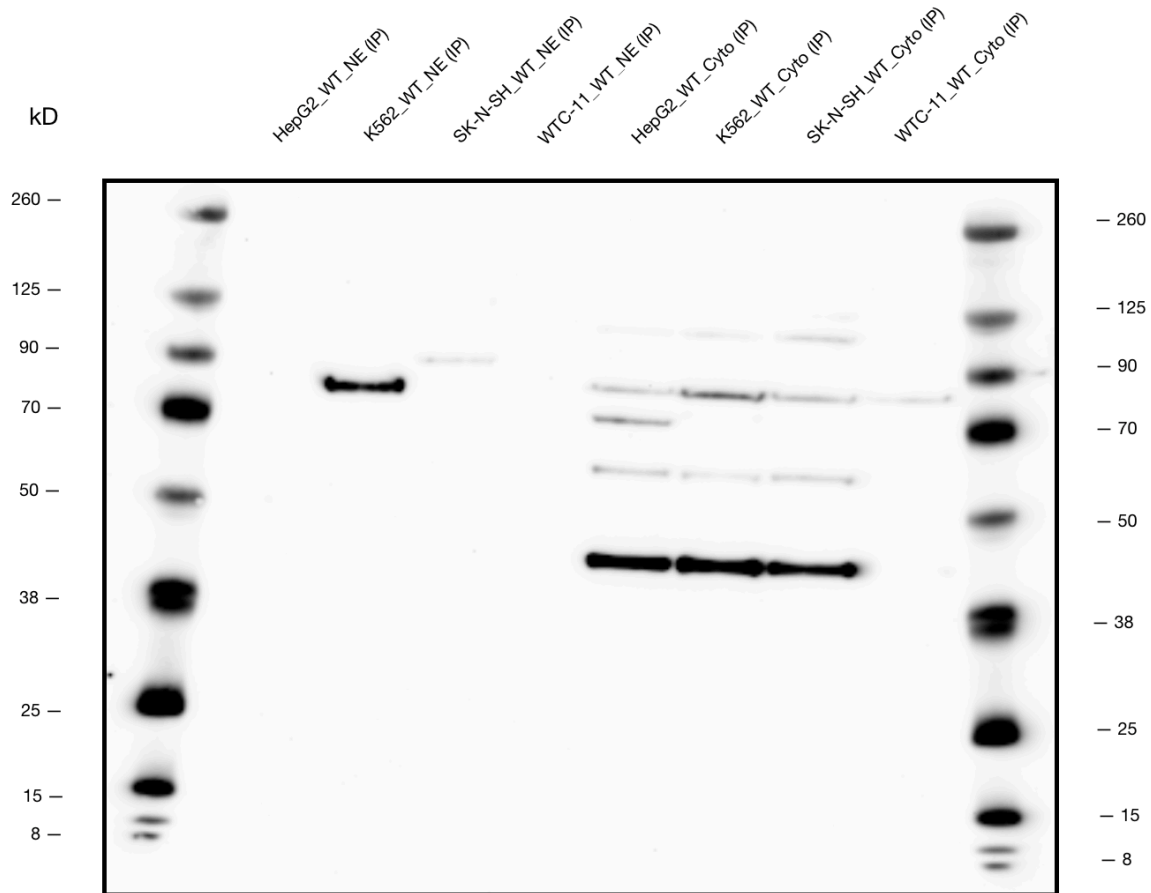
Western Blot Validation

**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. 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). The second western blot image depicts negative control IPs prepared with K562 nuclear lysate (Lane 3) and WTC-11 nuclear lysate (Lane 5).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-FOXK1_K562 rep 1 (nuclear extract)	78	Predicted size was 78 kDa. The observed size was 110 kDa, which is within 20% of observed band of 100 kDa seen in <a href="https://www.proteinatlas.org/ENSG00000164916-FOXK1/antibody#western_blot">https://www.proteinatlas.org/ENSG00000164916-FOXK1/antibody#western_blot</a> . The fainter banding below could be due to potential degradation products. PTMs: Acetylation, Methylation, and Phosphorylation
3	FLAG-HMG20A_K562 rep 3 (nuclear extract)	43	Single dark band within 20% of the predicted size. PTMs: Phosphorylation
4	FLAG-IRF1_isoA_K562 rep 1 (nuclear extract)	40	Predicted size was 40 kDa. The observed size was 50 kDa, which is within 20% of an observed band of 49 kDa seen in <a href="https://www.cellsignal.com/products/primary-antibodies/irf-1-d5e4-xp-rabbit-mab/8478">https://www.cellsignal.com/products/primary-antibodies/irf-1-d5e4-xp-rabbit-mab/8478</a> . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-SNAPC4_K562 rep 2 (nuclear extract)	162	Distinct band, but larger than 20% of the predicted size. PTMs: Phosphorylation
6	FLAG-ZBTB49_isoA_K562 rep 2 (nuclear extract)	88	Predicted size was 88 kDa. The observed size was 44 kDa, which is within 20% of an observed band of 55 kDa seen in <a href="https://www.thermofisher.com/antibody/product/ZBTB49-Antibody-Polyclonal/PA5-55081">https://www.thermofisher.com/antibody/product/ZBTB49-Antibody-Polyclonal/PA5-55081</a> . The band below could be due to a potential degradation product
7	FLAG-ARID4B_WTC-11 rep 2 (nuclear extract)	151	Predicted size was 151 kDa. The observed size was 250 kDa, which is within 20% of an observed band of 230 kDa seen in <a href="https://www.novusbio.com/products/brca1-antibody_nbp1-26619">https://www.novusbio.com/products/brca1-antibody_nbp1-26619</a> . PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
8	FLAG-SMAD4_isoA_WTC11 rep 1 (nuclear extract)	63	Dark band within 20% of the predicted size. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
9	FLAG-TEAD4_WTC-11 rep 1 (nuclear extract)	51	Single distinct band within 20% of the predicted size
10	FLAG-ZNF219_WTC-11 rep 2 (nuclear extract)	80	Predicted size was 80 kDa. The observed size was 110 kDa, which is within 20% of an observed band of 115 kDa seen in <a href="https://www.thermofisher.com/antibody/product/ZNF219-Antibody-Polyclonal/PA5-21120">https://www.thermofisher.com/antibody/product/ZNF219-Antibody-Polyclonal/PA5-21120</a> . PTMs: Phosphorylation



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	HepG2 Wild-Type (nuclear extract IP)	None	No visible banding
3	K562 Wild-Type (nuclear extract IP)	None	Dark band near 80 kDa
4	SK-N-SH Wild-Type (nuclear extract IP)	None	Band near 90 kDa
5	WTC-11 Wild-Type (nuclear extract IP)	None	No visible banding
6	HepG2 Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, 70 kDa, and 60 kDa. Dark band at 45 kDa
7	K562 Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, and 60 kDa. Dark band at 45 kDa
8	SK-N-SH Wild-Type (cytoplasmic extract IP)	None	Faint bands at 110 kDa, 85 kDa, and 60 kDa. Dark band at 45 kDa
9	WTC-11 Wild-Type (cytoplasmic extract IP)	None	Faint band at 85 kDa
10	Ladder	N/A	N/A

**Submitted by:**

Mark Mackiewicz and Michael Betti

**Lab:**

Richard Myers, HAIB

**Grant:**

UM1 HG009411

**Download:**