

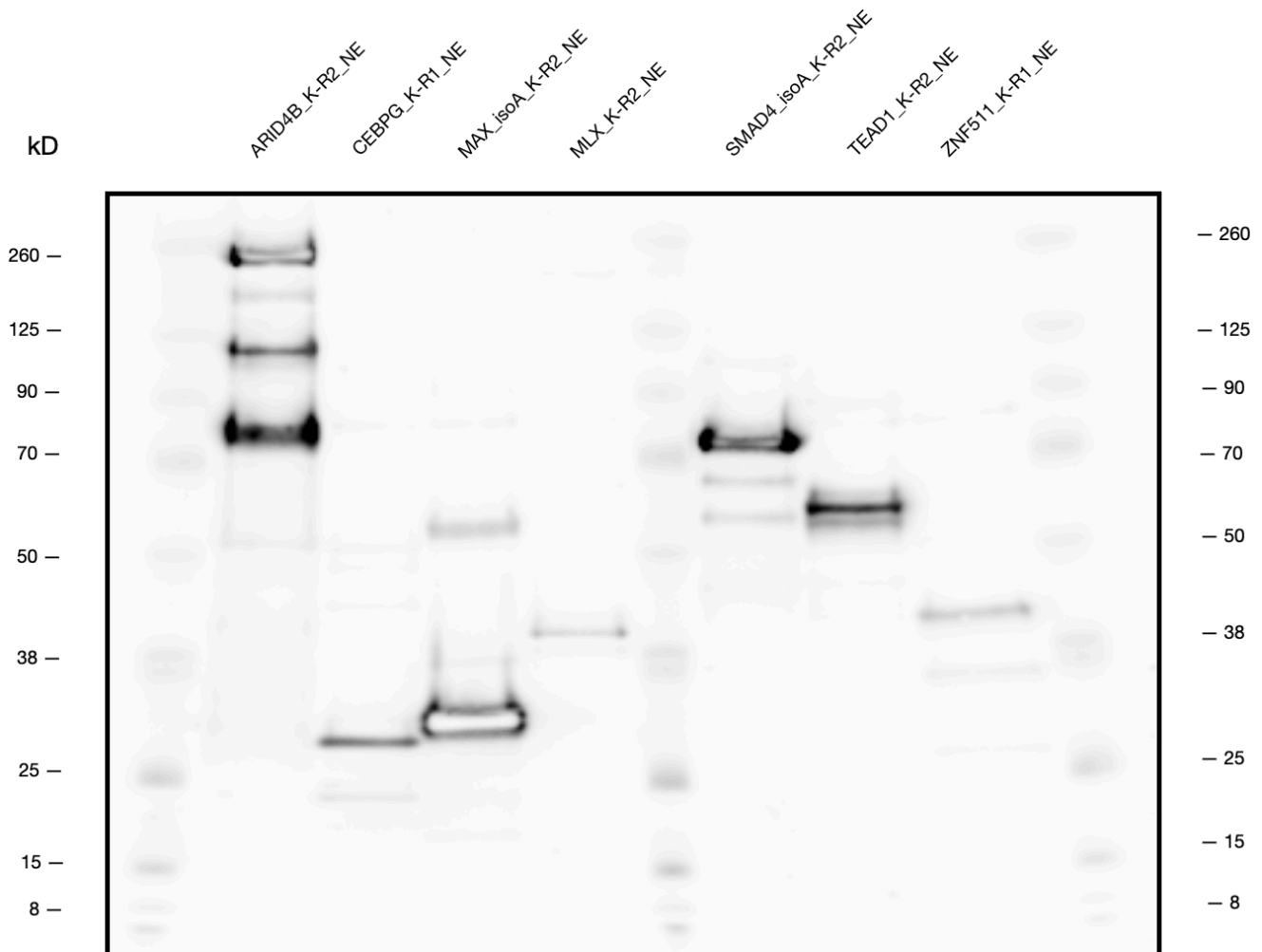
ARID4B (*Homo sapiens*), CEBPG (*Homo sapiens*), MAX (*Homo sapiens*), MLX (*Homo sapiens*), SMAD4 (*Homo sapiens*), TEAD1 (*Homo sapiens*), and ZNF511 (*Homo sapiens*)

Method:

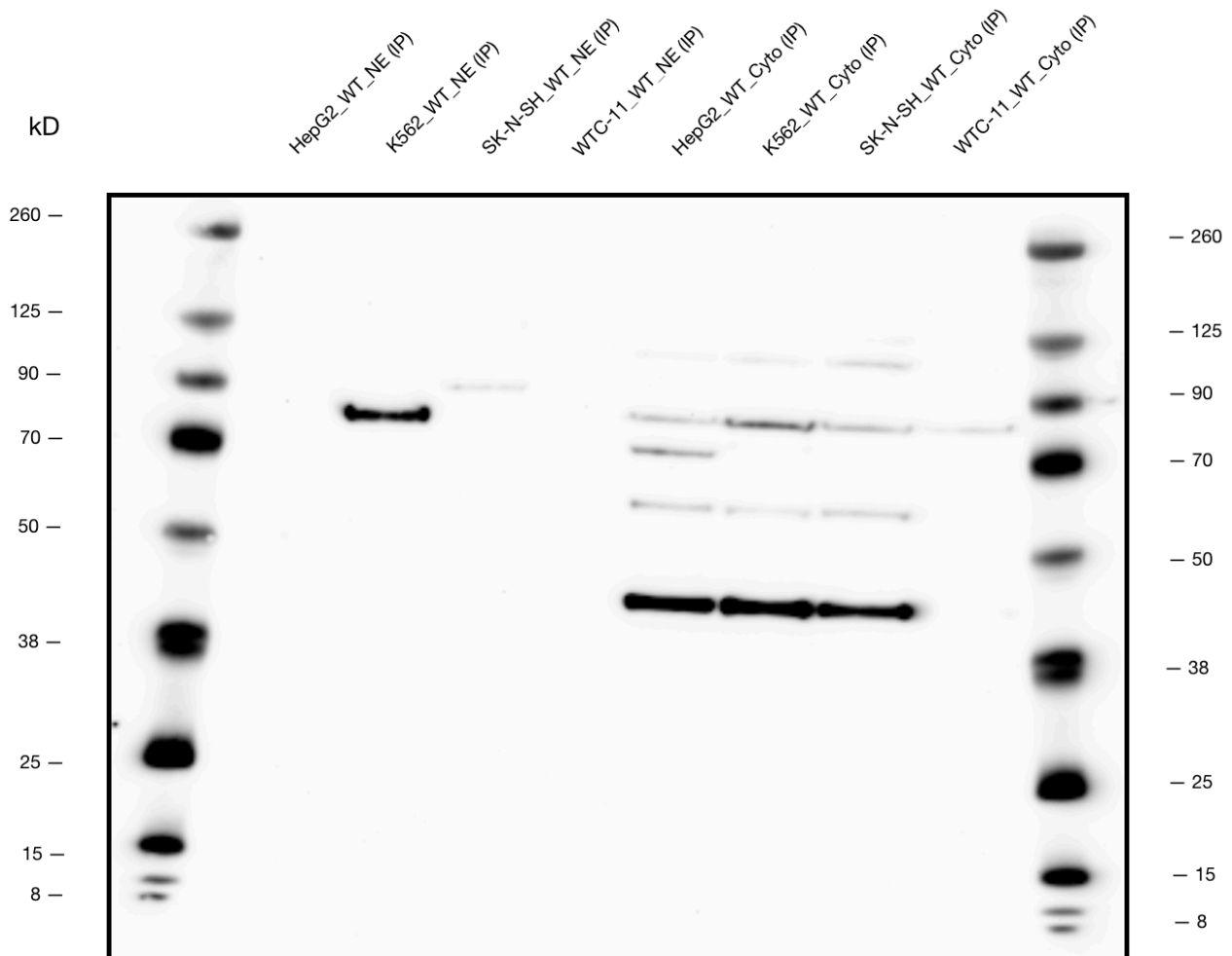
Western Blot Validation

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 a negative control IP prepared with K562 nuclear lysate (Lane 3).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-ARID4B_K562 rep 2 (nuclear extract)	151	Predicted size was 151 kDa. The observed sizes were 240 kDa, 120 kDa, and 80 kDa, which are within 20% of observed bands of 200 kDa, 150 kDa, and 100 kDa seen in https://www.ptglab.com/Products/ARID4B-Antibody-24499-1-AP.htm . PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	FLAG-CEBPG_K562 rep 1 (nuclear extract)	19	Predicted size was 19 kDa. The observed size was 30 kDa, which is within 20% of an observed band of 28 kDa seen in https://www.prosci-inc.com/cebpg-antibody-55-523.html . PTMs: isopeptide bonding and Ubl conjugation
4	FLAG-MAX_isoA_K562 rep 2 (nuclear extract)	21	Predicted size was 21 kDa. The observed sizes were 54 kDa and 30 kDa, which are within 20% of observed bands of 45 kDa and 24 kDa seen in https://www.ptglab.com/Products/MAX-Antibody-10426-1-AP.htm#validation . PTMs: Acetylation and Phosphorylation
5	FLAG-MLX_K562 rep 2 (nuclear extract)	36	Single distinct band within 20% of the predicted size. PTMs: Phosphorylation
6	Ladder	N/A	N/A
7	FLAG-SMAD4_isoA_K562 rep 2 (nuclear extract)	63	Distinct band within 20% of the predicted size. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
8	FLAG-TEAD1_K562 rep 2 (nuclear extract)	51	Distinct band within 20% of the predicted size. PTMs: Acetylation and Phosphorylation
9	FLAG-ZNF511_K562 rep 1 (nuclear extract)	31	Predicted size was 31 kDa. The observed size was 42 kDa, which is within 20% of an observed band of 36 kDa seen in https://www.novusbio.com/products/znf511-antibody_nbp1-80279 . PTMs: Acetylation and Phosphorylation
10	Ladder	N/A	N/A



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

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