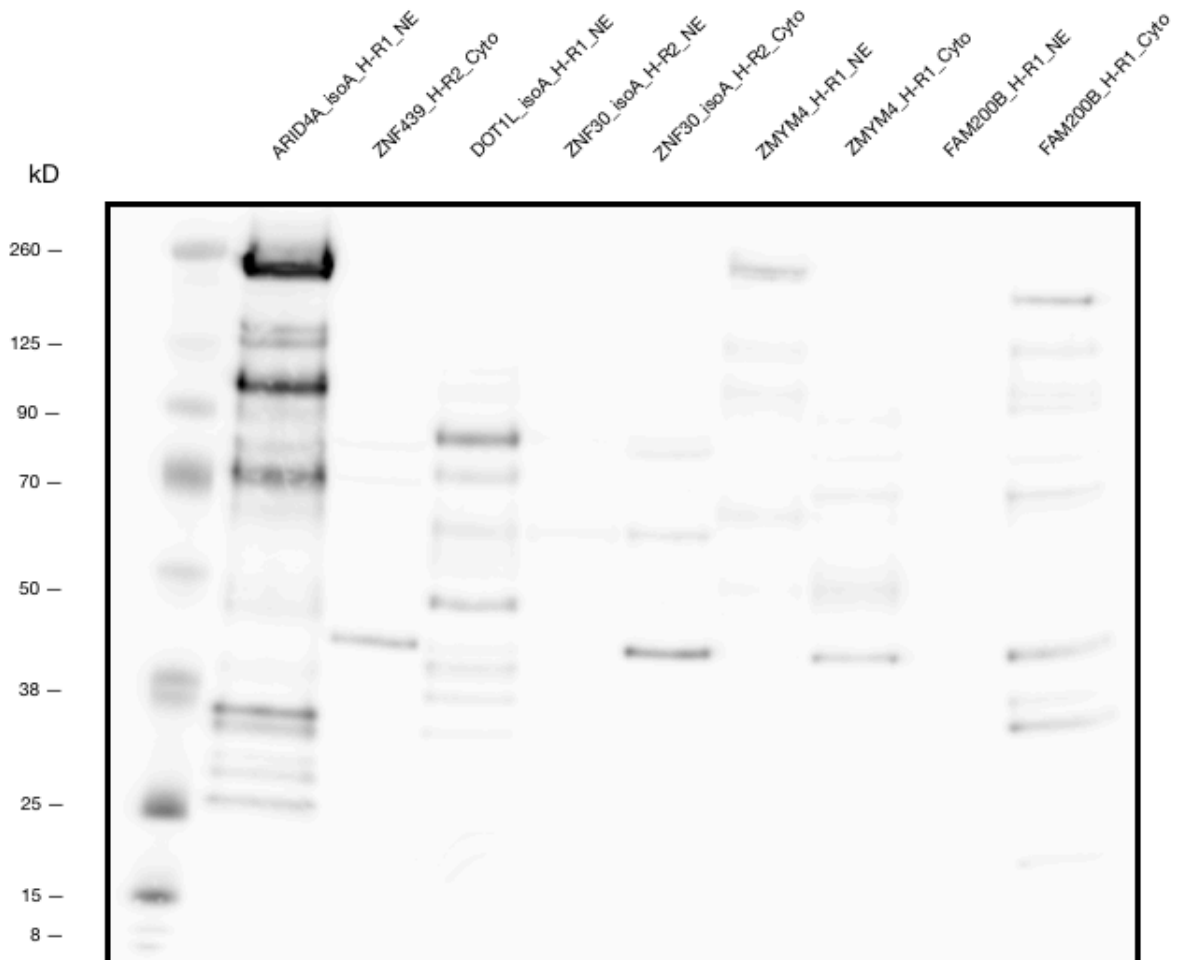


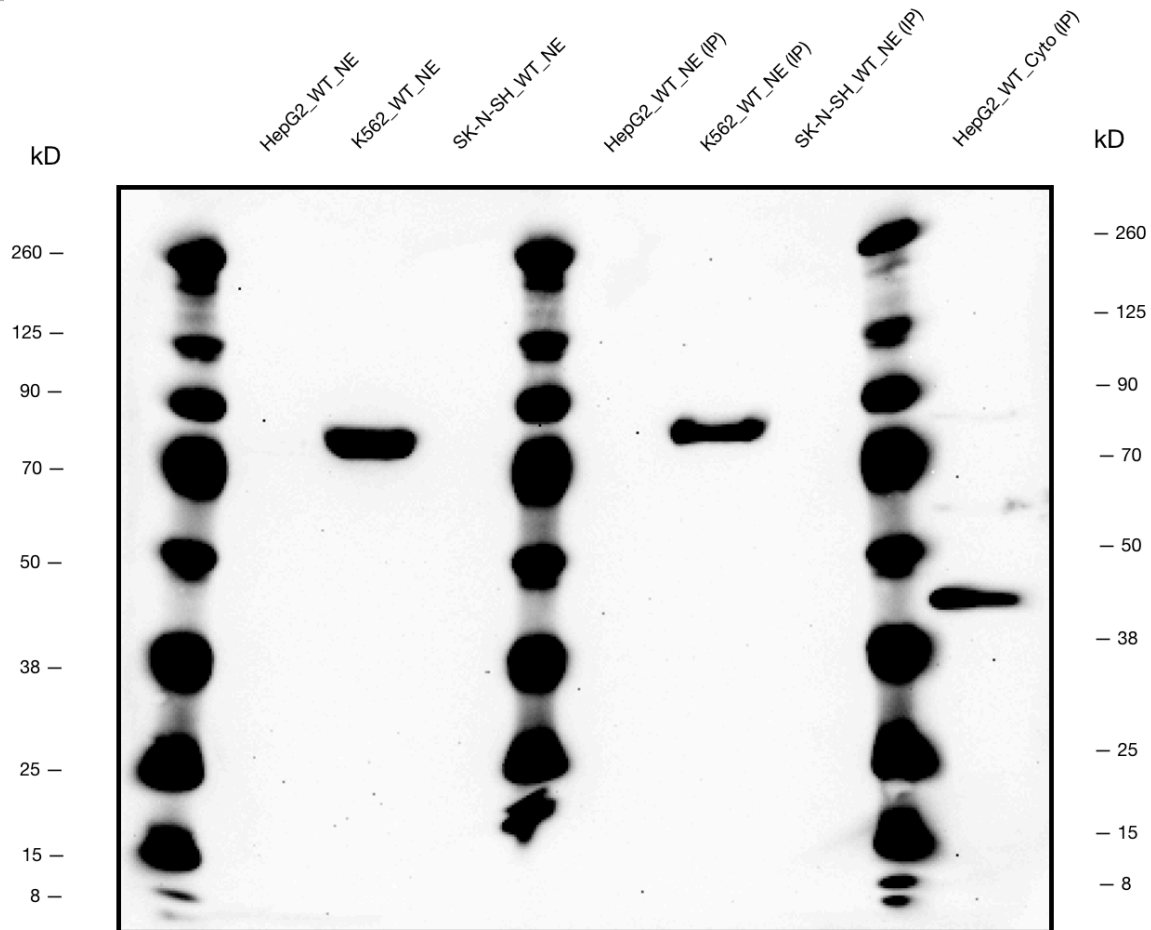
**ARID4A (*Homo sapiens*), ZNF439 (*Homo sapiens*), DOT1L (*Homo sapiens*), ZNF30 (*Homo sapiens*), ZNF30 (*Homo sapiens*), ZMYM4 (*Homo sapiens*), ZMYM4 (*Homo sapiens*), FAM200B (*Homo sapiens*), and FAM200B (*Homo sapiens*)**

**Method:**  
Western Blot Validation

**Caption:**  
Each FLAG-tagged sample was immunoprecipitated from its corresponding protein isolate (500 uL - nuclear; 1 mL - cytoplasmic) 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 five 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 HepG2 nuclear lysate (lane 6) and HepG2 cytoplasmic lysate (Lane 10).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-ARID4A_isoA_HepG2 rep 1 (nuclear extract)	146	Predicted size was 146 kDa. The observed size was 250 kDa, which is within 20% of an observed band of 220 kDa seen in <a href="https://www.novusbio.com/products/retinoblastoma-binding-protein-1-antibody_nbp1-26616">https://www.novusbio.com/products/retinoblastoma-binding-protein-1-antibody_nbp1-26616</a> . The bands below may be degradation products. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	FLAG-ZNF439_HepG2 rep 2 (cytoplasmic extract)	61	Single non-specific band seen in the negative control
4	FLAG-DOT1L_isoA_HepG2 rep 1 (nuclear extract)	168	Multiple bands below 20% of the expected size. PTMs: Phosphorylation
5	FLAG-ZNF30_isoA_HepG2 rep 2 (nuclear extract)	74	No visible banding
6	FLAG-ZNF30_isoA_HepG2 rep 2 (cytoplasmic extract)	74	Faint banding, with a darker non-specific band around 45 kDa
7	FLAG-ZMYM4_HepG2 rep 1 (nuclear extract)	176	Predicted size was 176 kDa. The observed size was 230 kDa, which is within 20% of an observed band of 220 kDa seen in <a href="https://www.novusbio.com/products/zmy4-antibody_nb100-94999">https://www.novusbio.com/products/zmy4-antibody_nb100-94999</a> . PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
8	FLAG-ZMYM4_HepG2 rep 1 (cytoplasmic extract)	176	Banding smaller than the expected size. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
9	FLAG-FAM200B_HepG2 rep 1 (nuclear extract)	79	No visible banding
10	FLAG-FAM200B_HepG2 rep 1 (cytoplasmic extract)	79	Multiple bands throughout the gel



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)	None	No visible banding
3	K562 Wild-Type (nuclear extract)	None	Single non-distinct band at around 80 kDa
4	SK-N-SH Wild-Type (nuclear extract)	None	No visible banding
5	Ladder	N/A	N/A
6	HepG2 Wild-Type (nuclear extract IP)	None	No visible banding
7	K562 Wild-Type (nuclear extract IP)	None	Single non-distinct band at around 80 kDa
8	SK-N-SH Wild-Type (nuclear extract)	None	No visible banding
9	Ladder	N/A	N/A
10	HepG2 Wild-Type (cytoplasmic extract IP)	None	Dark non-distinct band at around 45 kDa, and two lighter bands at about 60 kDa and 80 kDa

**Submitted by:**

Mark Mackiewicz and Michael Betti

**Lab:**

Richard Myers, HAIB

**Grant:**

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