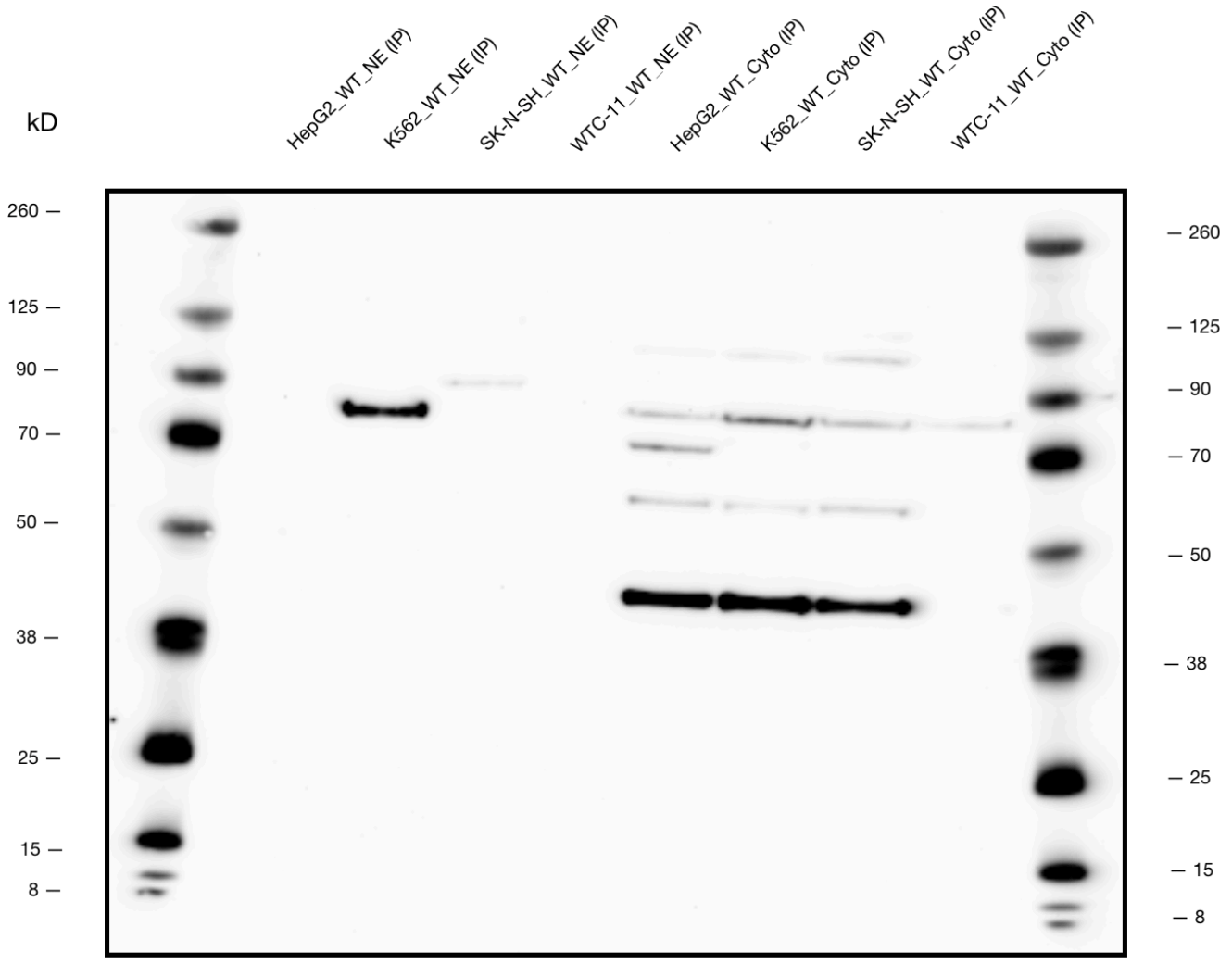
PRDM15 Control (*Homo sapiens*), PRDM15 Variant (*Homo sapiens*), ZNF221 (*Homo sapiens*), ZNF605 (*Homo sapiens*), and MYBL1 (*Homo sapiens*)

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 HepG2 nuclear lysate (Lane 2).

Monoclonal ANTI-FLAG M2-Peroxidase (HRP) antibody produced in mouse

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	FLAG-PRDM15_ctrl_mut_HepG2 rep 2 (nuclear extract)	172	Distinct band within 20% of the predicted size. The banding below could be due to potential degradation products. PTMs: Isopeptide bonding and Ubl conjugation
2	Ladder	N/A	N/A
3	FLAG-PRDM15_var_HepG2 rep 2 (nuclear extract)	172	Distinct band within 20% of the predicted size. The banding below could be due to potential degradation products. PTMs: Isopeptide bonding and Ubl conjugation
4	Ladder	N/A	N/A
5	FLAG-ZNF221_HepG2 rep 1 (nuclear extract)	74	No visible banding
6	Ladder	N/A	N/A
7	FLAG-ZNF605_HepG2 rep 1 (nuclear extract)	77	Single distinct band within 20% of the predicted size. PTMs: Isopeptide bonding and Ubl conjugation
8	Ladder	N/A	N/A
9	FLAG-MYBL1_HepG2 rep 1 (nuclear extract)	89	Dark band within 20% of the predicted size. The fainter banding below could be due to potential degradation products. PTMs: Acetylation, Isopeptide bonding, and Ubl conjugation
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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