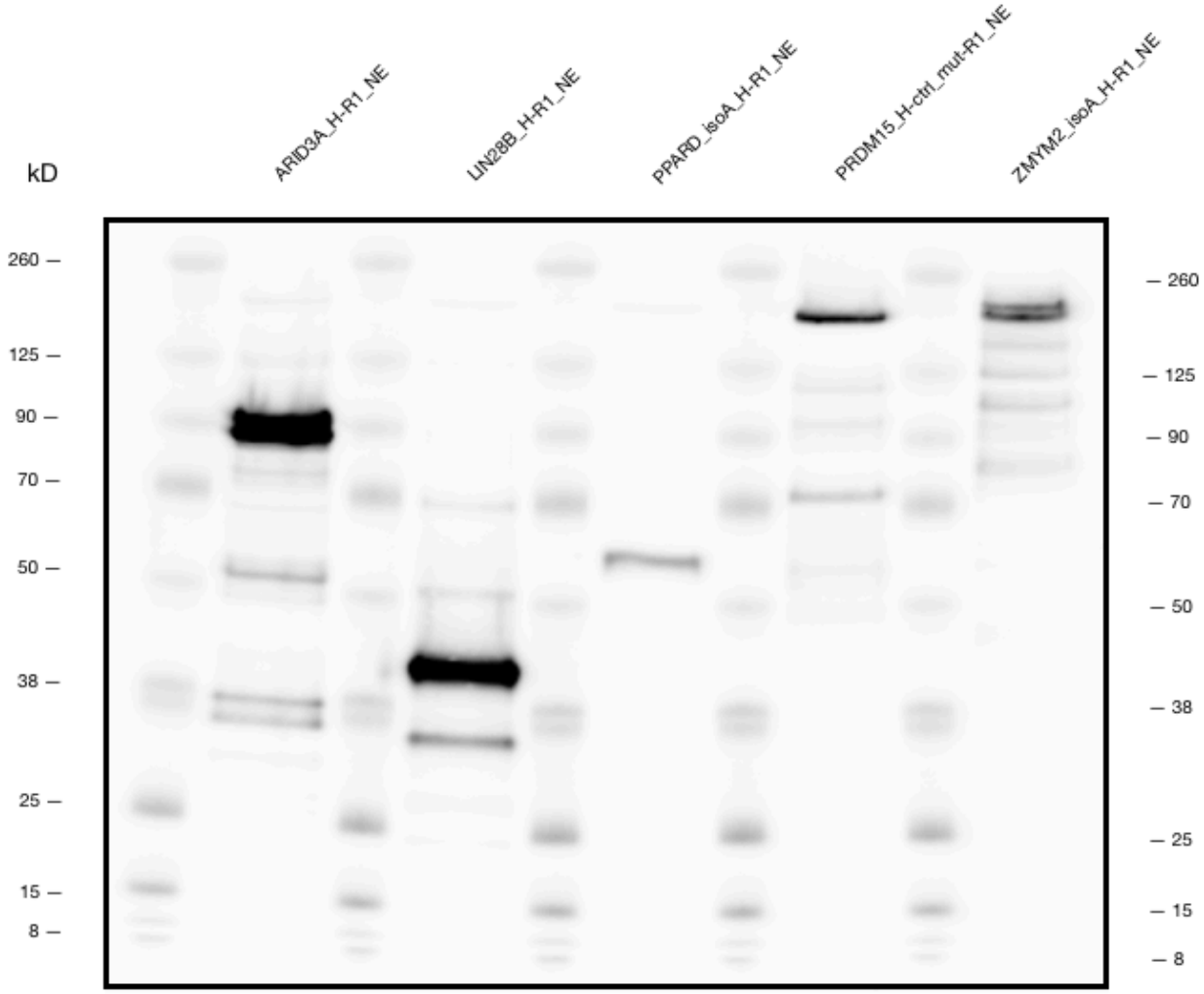
## ARID3A (*Homo sapiens*), LIN28B (*Homo sapiens*), PPARD (*Homo sapiens*), PRDM15 (*Homo sapiens*), and ZMYM2 (*Homo sapiens*)

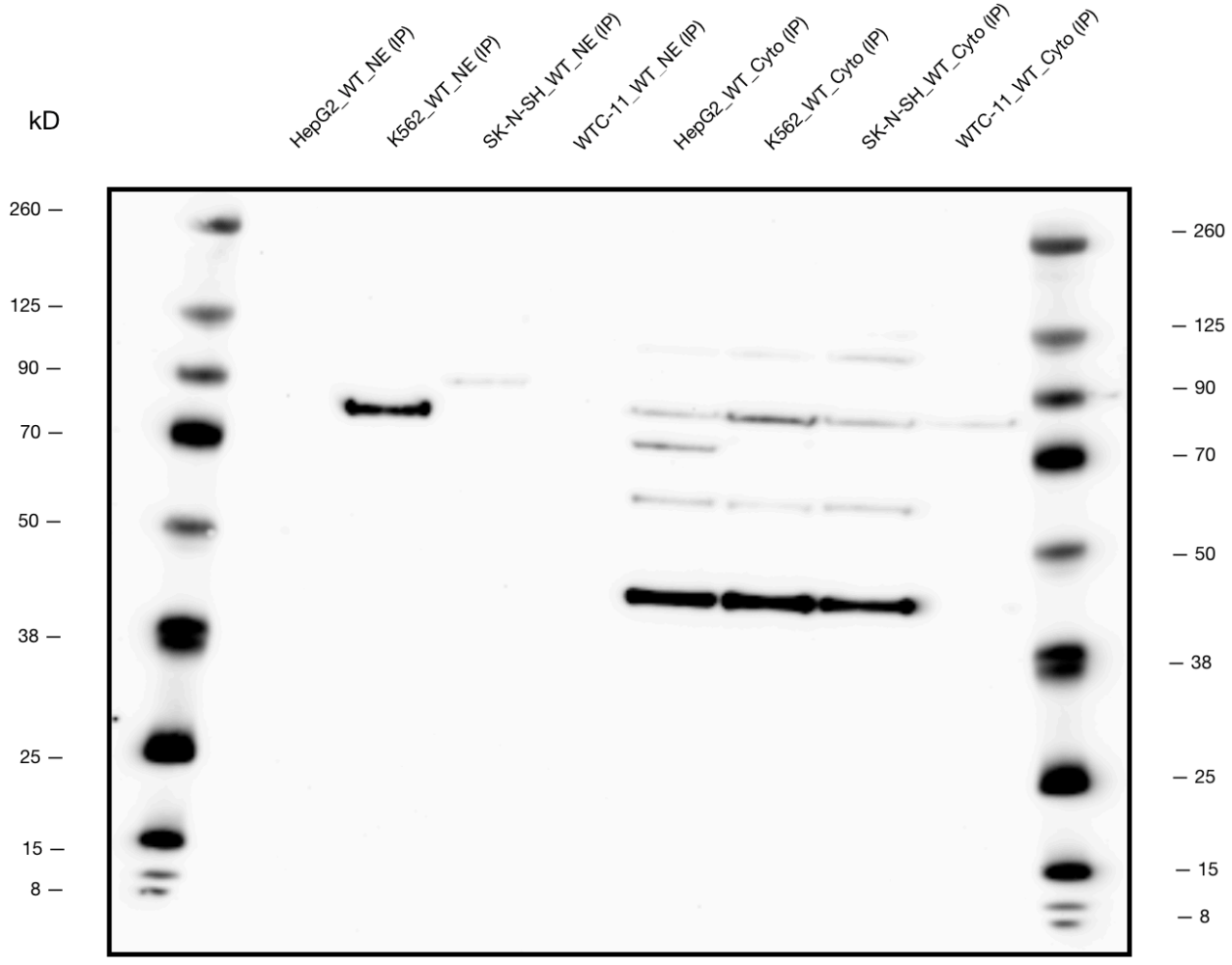
**Method:**
Western Blot Validation

**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 | Ladder | N/A | N/A |
| 2 | FLAG-ARID3A_HepG2 rep 1 (nuclear extract) | 66 | Predicted size was 66 kDa. The observed size was 90 kDa, which is approximately the same size as a band seen in https://www.mybiosource.com/polyclonal-human-mouse-rat-antibody/arid3a/9128587. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation |
| 3 | Ladder | N/A | N/A |
| 4 | FLAG-LIN28B_HepG2 rep 1 (nuclear extract) | 30 | Predicted size was 30 kDa. The observed size was 42 kDa, which is within 20% of an observed band of 40 kDa seen in https://www.abcam.com/lin28b-antibody-ab264333.html#description_images_1. PTMs: Phosphorylation |
| 5 | Ladder | N/A | N/A |
| 6 | FLAG-PPARD_isoA_HepG2 rep 1 (nuclear extract) | 53 | Single distinct band within 20% of the predicted size |
| 7 | Ladder | N/A | N/A |
| 8 | FLAG-PRDM15_HepG2-ctrl_mut rep 1 (nuclear extract) | 172 | Dark band within 20% of the predicted size, with faint degradation bands below. PTMs: Isopeptide bonding and Ubl conjugation |
| 9 | Ladder | N/A | N/A |
| 10 | FLAG-ZMYM2_isoA_HepG2 rep 1 (nuclear extract) | 158 | Predicted size was 158 kDa. The observed bands were both around 220 kDa, which is within 20% of the observed bands of 200 kDa seen in https://www.thermofisher.com/antibody/product/ZMYM2-Antibody-Polyclonal/PA5-28265. The faint banding below is likely due to degradation products. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation |




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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