

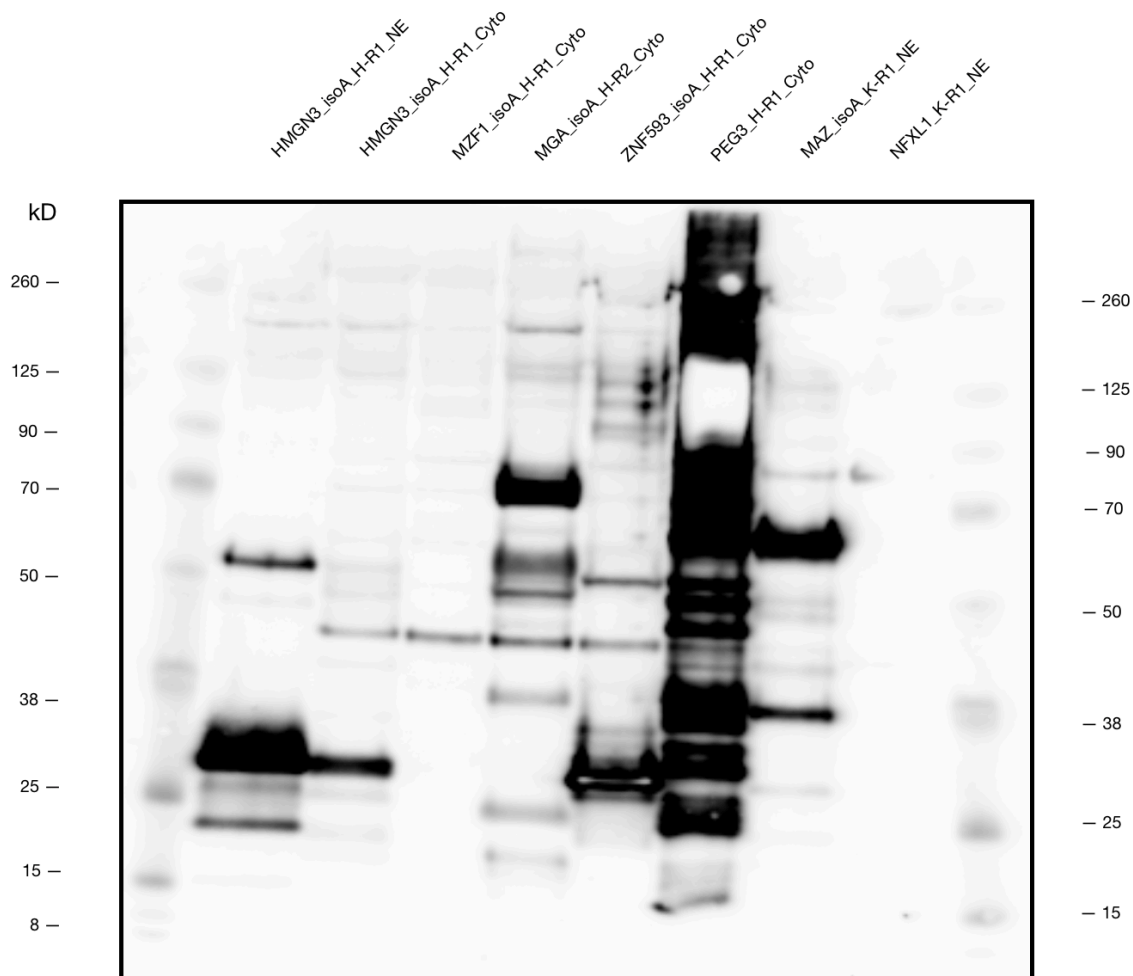
HMGN3 (*Homo sapiens*), MZF1 (*Homo sapiens*), MGA (*Homo sapiens*), ZNF593 (*Homo sapiens*), PEG3 (*Homo sapiens*), MAZ (*Homo sapiens*), and NFXL1 (*Homo sapiens*)

Method:

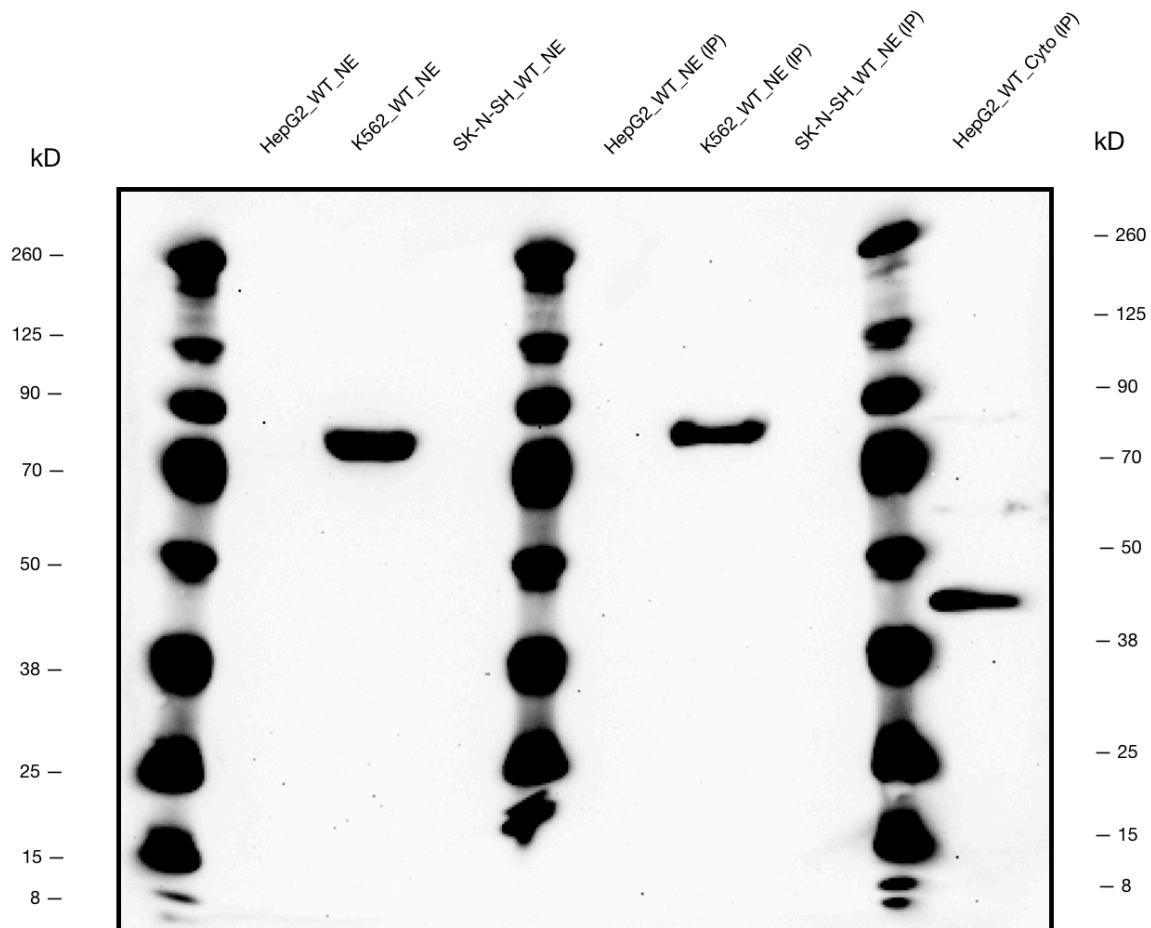
Western Blot Validation

Caption:

Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear 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 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 HepG2 nuclear lysate (Lane 6), K562 nuclear lysate (Lane 7), and HepG2 cytoplasmic lysate (lane 10).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-HMGN3_isoA_HepG2 rep 1 (nuclear extract)	14	Predicted size was 14 kDa. The observed size was 28 kDa, which is within 20% of an observed band of 26 kDa seen in https://us.acris-antibodies.com/proteins-growth-factors/hmgn3-trip7-tp313403.htm . Banding at larger and smaller sizes may correspond to alternate isoforms sharing the same stop codon. PTMs: Phosphorylation
3	FLAG-HMGN3_isoA_HepG2 rep 1 (cytoplasmic extract)	14	Predicted size was 14 kDa. The observed size was 28 kDa, which is within 20% of an observed band of 26 kDa seen in https://us.acris-antibodies.com/proteins-growth-factors/hmgn3-trip7-tp313403.htm . PTMs: Phosphorylation
4	FLAG-MZF1_isoA_HepG2 rep 1 (cytoplasmic extract)	85	Faint non-distinct banding. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
5	FLAG-MGA_isoA_HepG2 rep 2 (cytoplasmic extract)	339	Dark band significantly smaller than the expected size. PTMs: Disulfide bonding, Glycosylation, and Sulfation
6	FLAG-ZNF593_isoA_HepG2 rep 1 (cytoplasmic extract)	18	Predicted size was 18 kDa. The observed size was 26 kDa, which is within 20% of an observed band of 22 kDa seen in https://www.usbio.net/antibodies/044298/ZNF593-ID-ZNF593-Zinc-finger-protein-593-Zinc-finger-protein-T86
7	FLAG-PEG3_HepG2 rep 1 (cytoplasmic extract)	184	Dark, overexposed banding throughout the lane
8	FLAG-MAZ_isoA_K562 rep 1 (nuclear extract)	52	Dark band within 20% of the expected size. Less intense band around 38 kDa could correspond to the smaller isoform which shares the same stop codon. PTMs: Phosphorylation
9	FLAG-NFXL1_K562 rep 1 (cytoplasmic extract)	27	Faint partial banding. PTMs: 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)	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	Single non-distinct band at around 45 kDa

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