

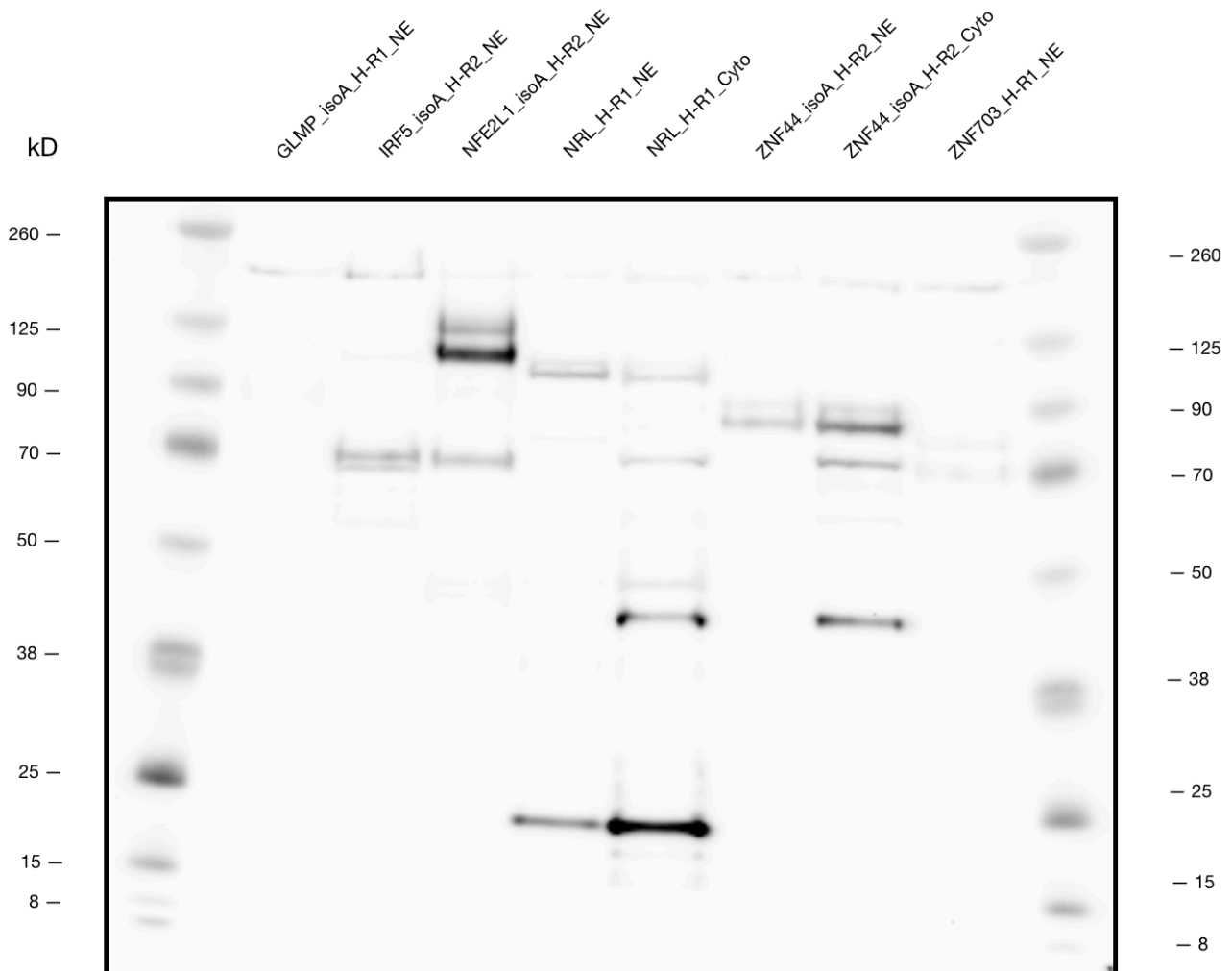
**GLMP (*Homo sapiens*), IRF5 (*Homo sapiens*), NFE2L1 (*Homo sapiens*), NRL (*Homo sapiens*), ZNF44 (*Homo sapiens*), and ZNF703 (*Homo sapiens*)**

**Method:**

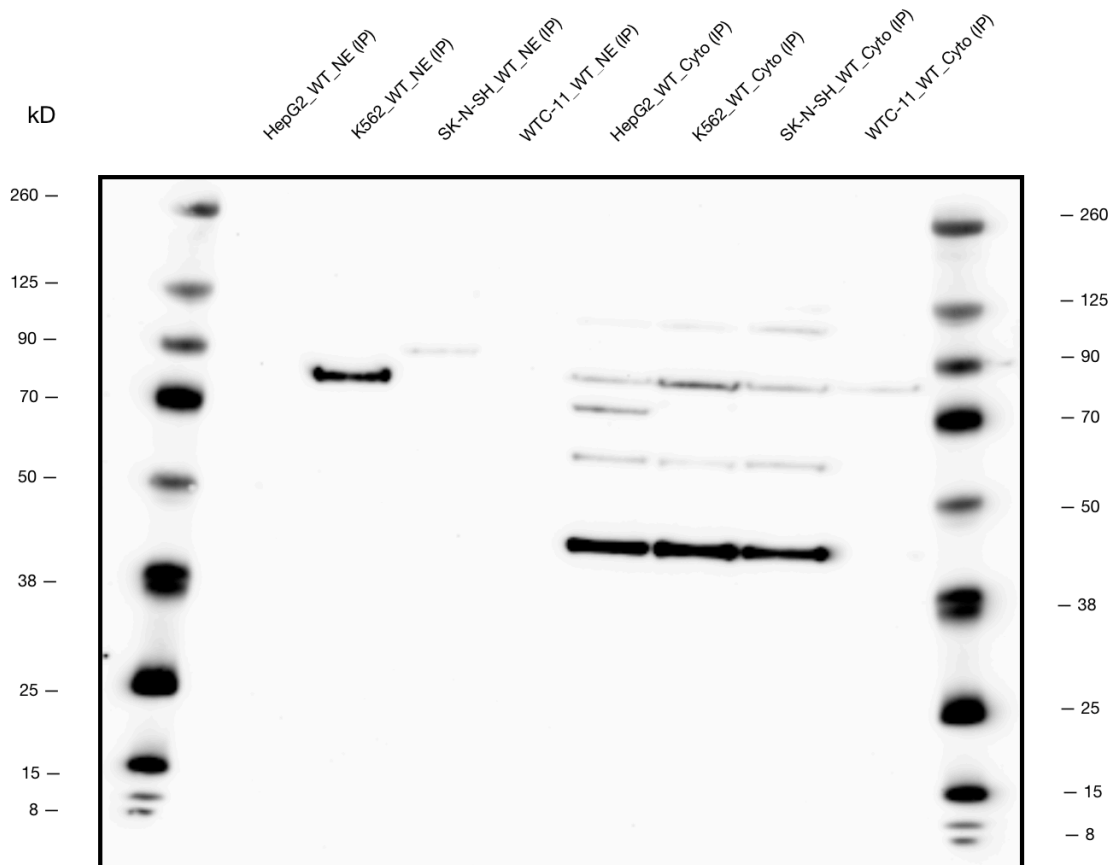
Western Blot Validation

**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 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 2) and HepG2 cytoplasmic lysate (Lane 6).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-GLMP_isoA_HepG2 rep 1 (nuclear extract)	47	Single faint non-specific band. PTMs: Glycosylation
3	FLAG-IRF5_isoA_HepG2 rep 2 (nuclear extract)	59	Distinct banding within 20% of the predicted size. PTMs: Isopeptide bonding and Ubl conjugation
4	FLAG-NFE2L1_isoA_HepG2 rep 2 (nuclear extract)	88	Predicted size was 88 kDa. The observed size was 110 kDa, which is within 20% of an observed band of 95 kDa seen in <a href="https://www.thermofisher.com/antibody/product/NFE2L1-Antibody-Polyclonal/PA5-66577">https://www.thermofisher.com/antibody/product/NFE2L1-Antibody-Polyclonal/PA5-66577</a> . PTMs: Glycosylation, Phosphorylation, and Ubl conjugation
5	FLAG-NRL_HepG2 rep 1 (nuclear extract)	29	Predicted size was 29 kDa. The observed size was 21 kDa, which is within 20% of an observed band of 25 kDa seen in <a href="https://www.bosterbio.com/anti-nrl-antibody-a00505-boster.html">https://www.bosterbio.com/anti-nrl-antibody-a00505-boster.html</a> . The larger band near 100 kDa likely corresponds with one of the larger is-forms sharing the same tagged stop codon. PTMs: Isopeptide bonding and Ubl conjugation
6	FLAG-NRL_HepG2 rep 1 (cytoplasmic extract)	29	Predicted size was 29 kDa. The observed size was 21 kDa, which is within 20% of an observed band of 25 kDa seen in <a href="https://www.bosterbio.com/anti-nrl-antibody-a00505-boster.html">https://www.bosterbio.com/anti-nrl-antibody-a00505-boster.html</a> . Bands near 70 and 45 kDa are non-specific bands seen in the HepG2 cytoplasmic negative control. Some of the other larger banding may correspond with the larger isoforms sharing the same tagged stop codon. PTMs: Isopeptide bonding and Ubl conjugation
7	FLAG-ZNF44_isoA_HepG2 rep 2 (nuclear extract)	80	Distinct banding within 20% of the predicted size
8	FLAG-ZNF44_isoA_HepG2 rep 2 (cytoplasmic extract)	80	Distinct banding within 20% of the predicted size. The bands below at 70 kDa, 60 kDa, and 45 kDa are non-specific bands seen in the HepG2 cytoplasmic negative control
9	FLAG-ZNF703_HepG2 rep 1 (nuclear extract)	61	Predicted size was 61 kDa. The observed size was 78 kDa, which is within 20% of an observed band of 65 kDa seen in <a href="https://www.novusbio.com/products/znf703-antibody_nbp2-21043">https://www.novusbio.com/products/znf703-antibody_nbp2-21043</a> . PTMs: Acetylation, Methylation, and Phosphorylation
9	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

**Submitted by:**

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**Lab:**

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**Grant:**

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