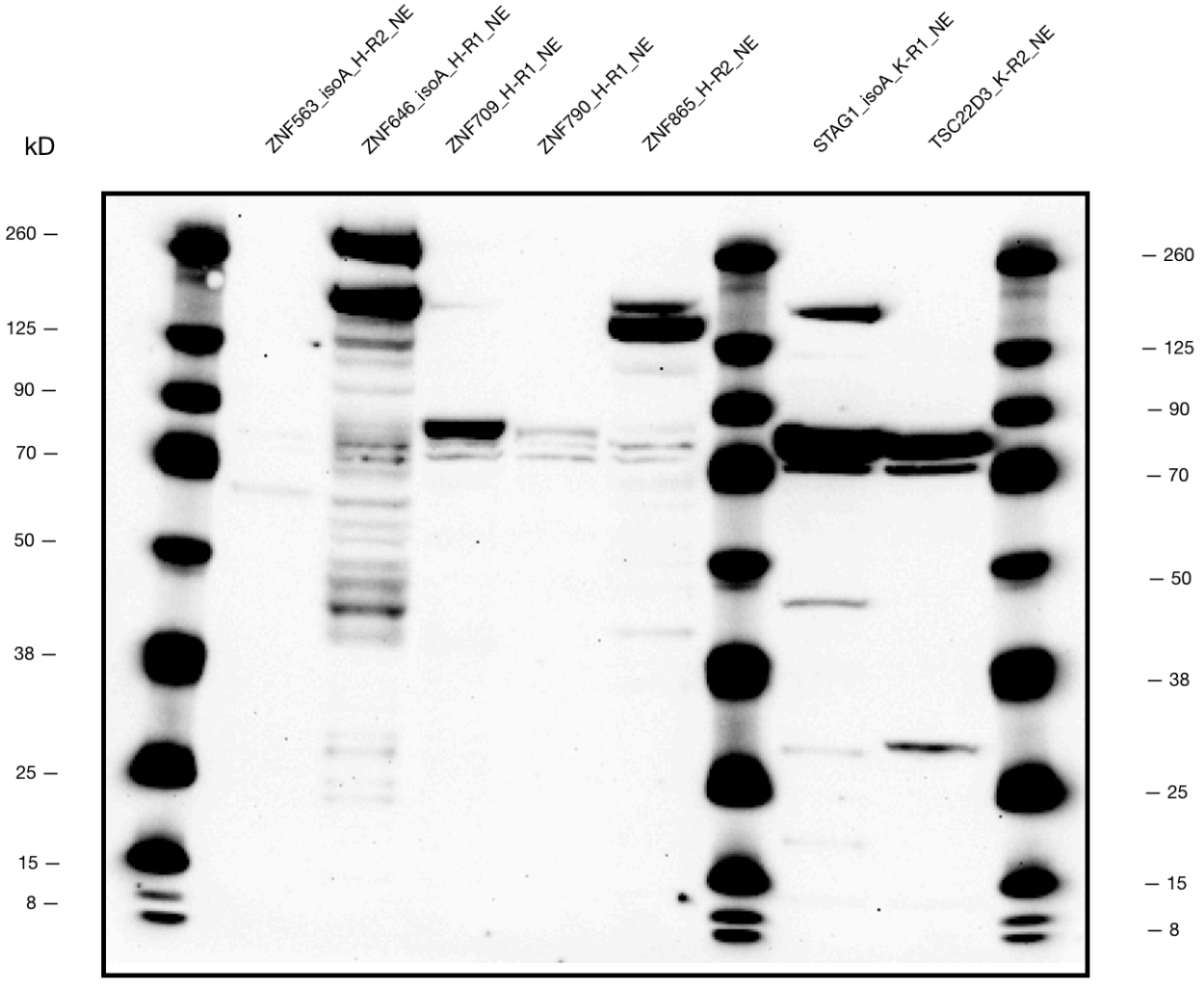
ZNF563 (*Homo sapiens*), ZNF646 (*Homo sapiens*), ZNF709 (*Homo sapiens*), ZNF790 (*Homo sapiens*), ZNF865 (*Homo sapiens*), STAG1 (*Homo sapiens*), and TSC22D3 (*Homo* sapiens)

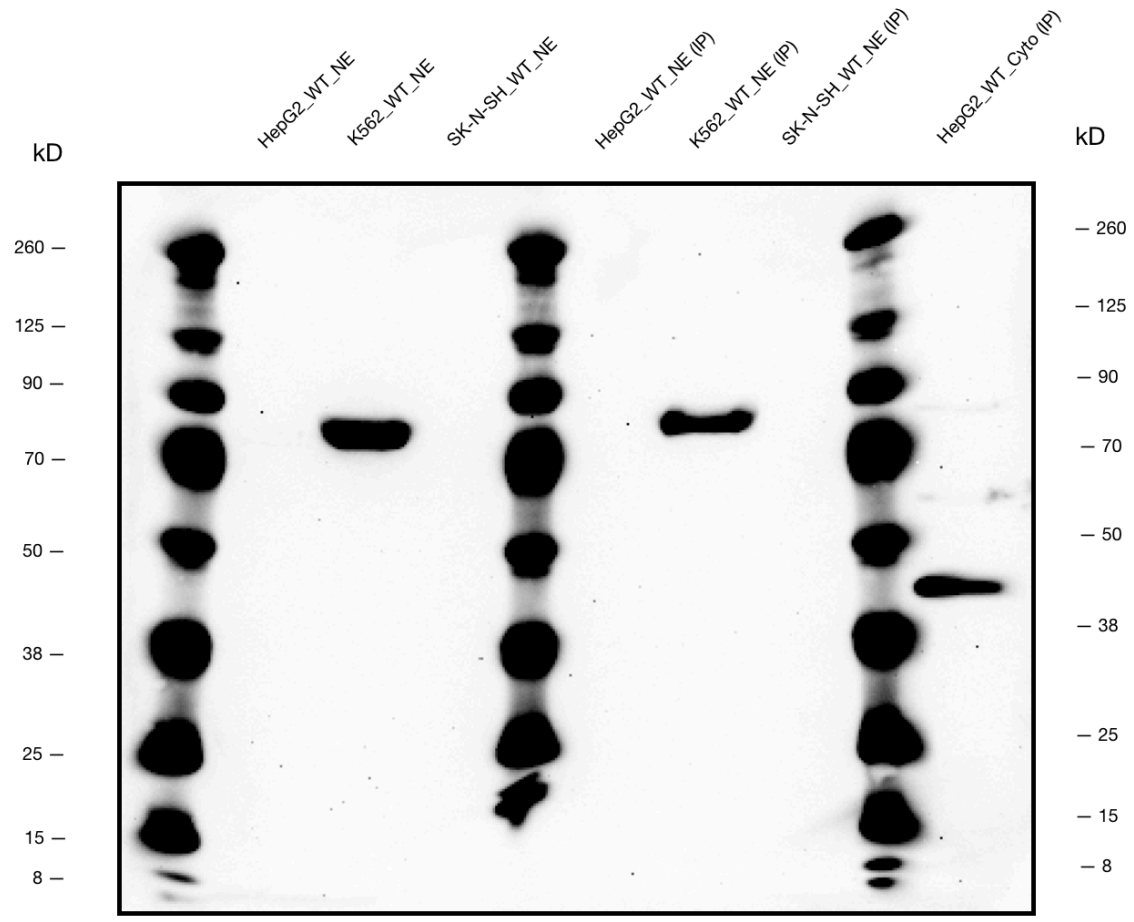
Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (71 mcg - ZNF563; 255 mcg - ZNF646; 234 mcg - ZNF709; 129 mcg - ZNF790; 244 mcg - ZNF865; 103 mcg - STAG1; and 108 mcg - TSC22D3) was standardized in a solution containing a volume of 2% Halt Protease and Phosphatase Inhibitor Single-Use Cocktail Mixture (Thermo Fisher Scientific), NuPage Sample Reducing Agent 10X, and NuPage LDS Sample Buffer 4X (Thermo Fisher Scientific). After heating the solution for 15 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 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-ZNF563_isoA_HepG2 rep 2 (nuclear extract)	58	Distinct band within 20% of the expected size
3	FLAG-ZNF646_isoA_HepG2 rep 1 (nuclear extract)	204	Predicted size was 204 kDa. The observed sizes were 200 kDa and 260 kDa, which are both within 20% of an observed band of 250 kDa seen in https://www.proteinatlas.org/ENSG00000167395-ZNF646/antibody#western_blot. The presence of two dark bands is likely due to multiple isoforms sharing the tagged stop codon. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
4	FLAG-ZNF709_HepG2 rep 1 (nuclear extract)	78	Dark band within 20% of the expected size
5	FLAG-ZNF790_HepG2 rep 1 (nuclear extract)	78	Distinct band within 20% of the expected size
6	FLAG-ZNF865_HepG2 rep 2 (nuclear extract)	114	Dark band within 20% of the expected size. PTMs: Isopeptide bonding and Ubl conjugation
7	Ladder	N/A	N/A
8	FLAG-STAG1_isoA_K562 rep 1 (nuclear extract)	147	Distinct band within 20% of the expected size. Darker banding below is non-distinct and can be seen in the negative control. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
9	FLAG-TSC22D3_K562 rep 2 (nuclear extract)	18	Predicted size was 18 kDa. The observed size was 30 kDa, which is within 20% of an observed band of 32 kDa seen in https://www.lsbio.com/antibodies/anti-tsc22d3-antibody-gilz-antibody-n-terminus-elisa-if-immunofluorescence-ihc-wb-western-ihc-plus-ls-b10077/241011. Darker banding above is non-distinct and can be seen in the negative control. 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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Grant:
UM1 HG009411

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