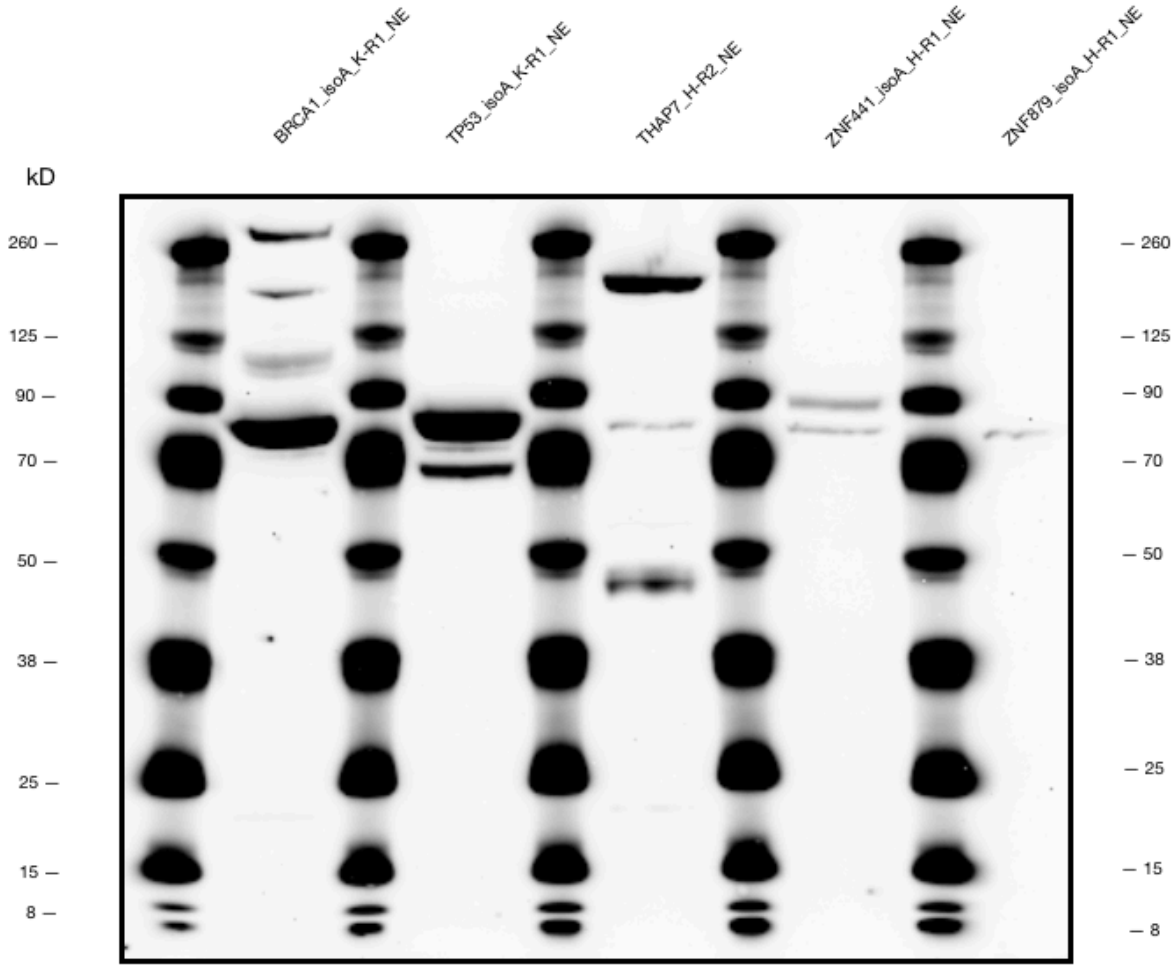
BRCA1 (*Homo sapiens*), TP53 (*Homo sapiens*), THAP7 (*Homo sapiens*), ZNF441 (*Homo sapiens*), and ZNF879 (*Homo sapiens*)

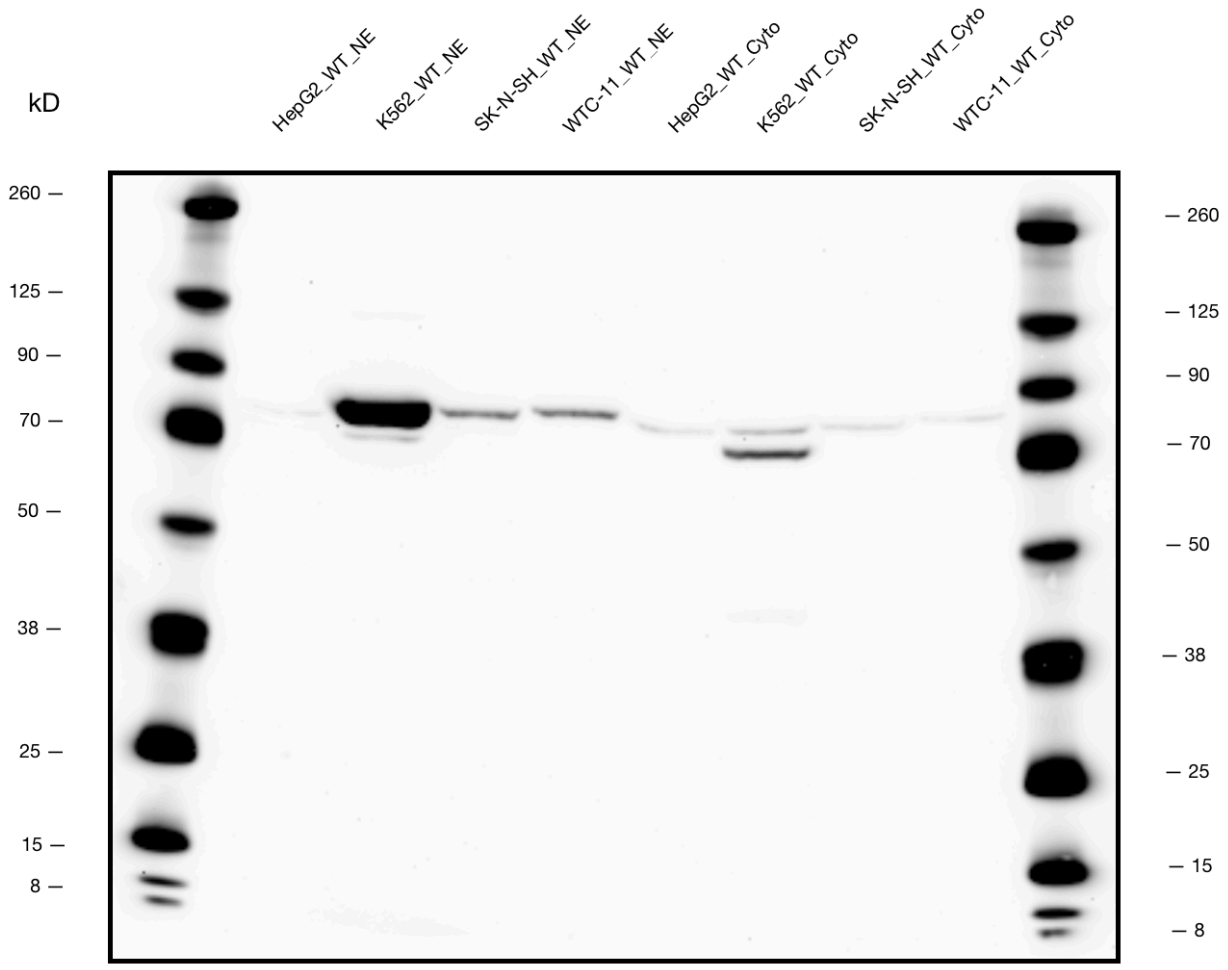
Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (95 mcg - BRCA1, 78 mcg - TP53, 129 mcg - THAP7, 162 mcg - ZNF441, and 157 mcg - ZNF879) 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 five 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 controls prepared with HepG2 nuclear lysate (Lane 2) and K562 nuclear lysate (Lane 3).



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-BRCA1_isoA_K562 rep 1 (nuclear extract)	211	Predicted size was 211 kDa. The observed sizes were 280 kDa, 230 kDa, and 115 kDa, which are within 20% of observed bands of 290 kDa, 220 kDa, and 130 kDa seen in https://www.abcam.com/brca1-antibody-ab238983.html. The dark band around 80 kDa is a non-distinct band seen in the K562 negative control. PTMs: Acetylation, Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	Ladder	N/A	N/A
4	FLAG-TP53_isoA_K562 rep 1 (nuclear extract)	47	Predicted size was 47 kDa. The observed size was 67 kDa, which is within 20% of an observed band of 55 kDa seen in https://www.cellsignal.com/products/primary-antibodies/phospho-p53-antibody-sampler-kit/9919. PTMs: Acetylation, Glycosylation, Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation
5	Ladder	N/A	N/A
6	FLAG-THAP7_HepG2 rep 2 (nuclear extract)	37	Most distinct band far from the expected size. PTMs: Phosphorylation
7	Ladder	N/A	N/A
8	FLAG-ZNF441_isoA_HepG2 rep 1 (nuclear extract)	83	Two bands within 20% of the expected size. The lower band at 80 kDa corresponds to the faint non-specific band seen in the negative control
9	Ladder	N/A	N/A
10	FLAG-ZNF879_isoA_HepG2 rep 1 (nuclear extract)	68	Single faint non-specific band seen in the negative control



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	Very faint band around 75 kDa
3	K562 Wild-Type (nuclear extract)	None	Dark band near 80 kDa
4	SK-N-SH Wild-Type (nuclear extract)	None	Band near 80 kDa
5	WTC-11 Wild-Type (nuclear extract)	None	Band near 80 kDa
6	HepG2 Wild-Type (cytoplasmic extract)	None	Faint band around 80 kDa
7	K562 Wild-Type (cytoplasmic extract)	None	Faint band around 80 kDa, with a darker band below at 70 kDa
8	SK-N-SH Wild-Type (cytoplasmic extract)	None	Faint band around 80 kDa
9	WTC-11 Wild-Type (cytoplasmic extract)	None	Faint band around 80 kDa
10	Ladder	N/A	N/A

Submitted by:
Mark Mackiewicz and Michael Betti

Lab:
Richard Myers, HAIB

Grant:
UM1 HG009411

Download: