

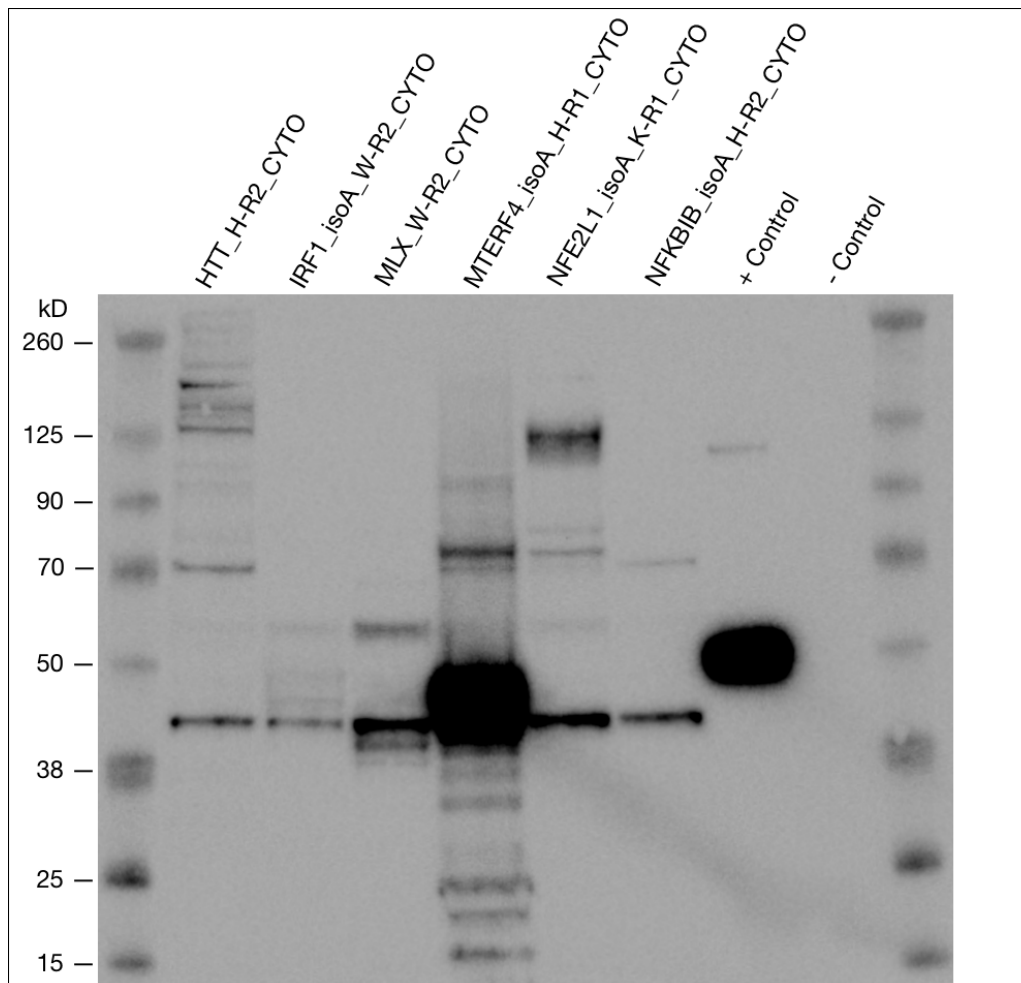
HTT (*Homo sapiens*), IRF1 (*Homo sapiens*), MLX (*Homo sapiens*), MTERF4 (*Homo sapiens*), NFE2L1 (*Homo sapiens*), and NFKBIB (*Homo sapiens*)

Method:

Western Blot Validation

Caption:

Each FLAG-tagged sample was immunoprecipitated from its corresponding nuclear/cyto 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. A blank IP was included as a negative control, and an immunoprecipitated FLAG-BAP fusion protein provided in the kit as a positive control. 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).



Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-HTT-HepG2 rep 2 (CYTO extract)	348	N/A
3	FLAG-IRF1_isoA-WTC11 rep 2 (CYTO extract)	37	No distinct band within 20% of the expected size.
4	FLAG-MLX-WTC11 rep 2 (CYTO extract)	34	Distinct band within 20% of the expected size.
5	FLAG-MTERF4_isoA-HepG2 rep 1 (CYTO extract)	44	Distinct band within 20% of the expected size.
6	FLAG-NFE2L1_isoA-K562 rep 1 (CYTO extract)	85	Distinct band above 20% of the expected size.
7	FLAG-NFKBIB_isoA-HepG2 rep 2 (CYTO extract)	38	Distinct above 20% of the expected size which is the same size observed in: https://www.fishersci.no/shop/products/human-nfkbib-full-length-orf-np-002494-2-1-a-a-356-a-a-recombinant-protein-gst-tag-at-n-terminal-abnova-2/16180718
8	Positive Control (FLAG BAP fusion protein)	NA	PASS
9	Negative Control (Blank IP)	NA	PASS
10	Ladder	N/A	N/A

Submitted by: Mark Mackiewicz and Amy Nesmith.

Lab: Richard Myers,
HAIB

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