ENCODE Antibody Validation Documentation
Transcription factor: YY1 (GeneID 7528)

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Transcription factor: YY1 (GeneID 7528; ~60 kDa)

Antibody: YY1 (C-20), Santa Cruz Biotechnology (sc-281)
Rabbit polyclonal, epitope mapping at the C-terminus of YY1 of human origin
Web: http://www.scbt.com/datasheet-281-yy1-c-20-antibody.html

Validation 1: Immunoblot Analysis

For an antibody to meet ENCODE validation standards, a single band of the predicted size, or a band of no less than half the total signal, must be detected in a lane on a Western blot.

a. Vendor immunoblot analysis

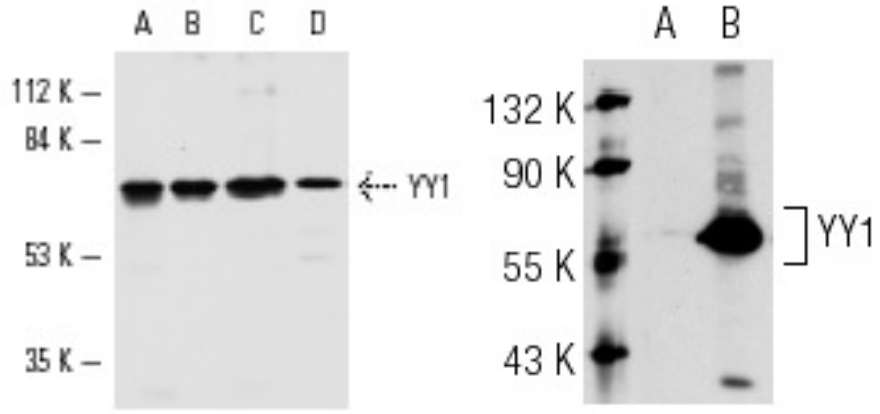

Figure Legend: Left: Western blot analysis of YY1 expression in HeLa (A) A-431 (B) Y79 (C) and NIH/3T3 (D) whole cell lysates. Right: Western blot analysis of YY1 expression in non-transfected sc-117752 (A) and mouse YY1 transfected sc-124689 (B) 293T whole cell lysates.

b. Myers Lab immunoblot analysis

Western blot protocol 1

Whole cell lysates were immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. After separation, the samples were transferred to a nitrocellulose membrane using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

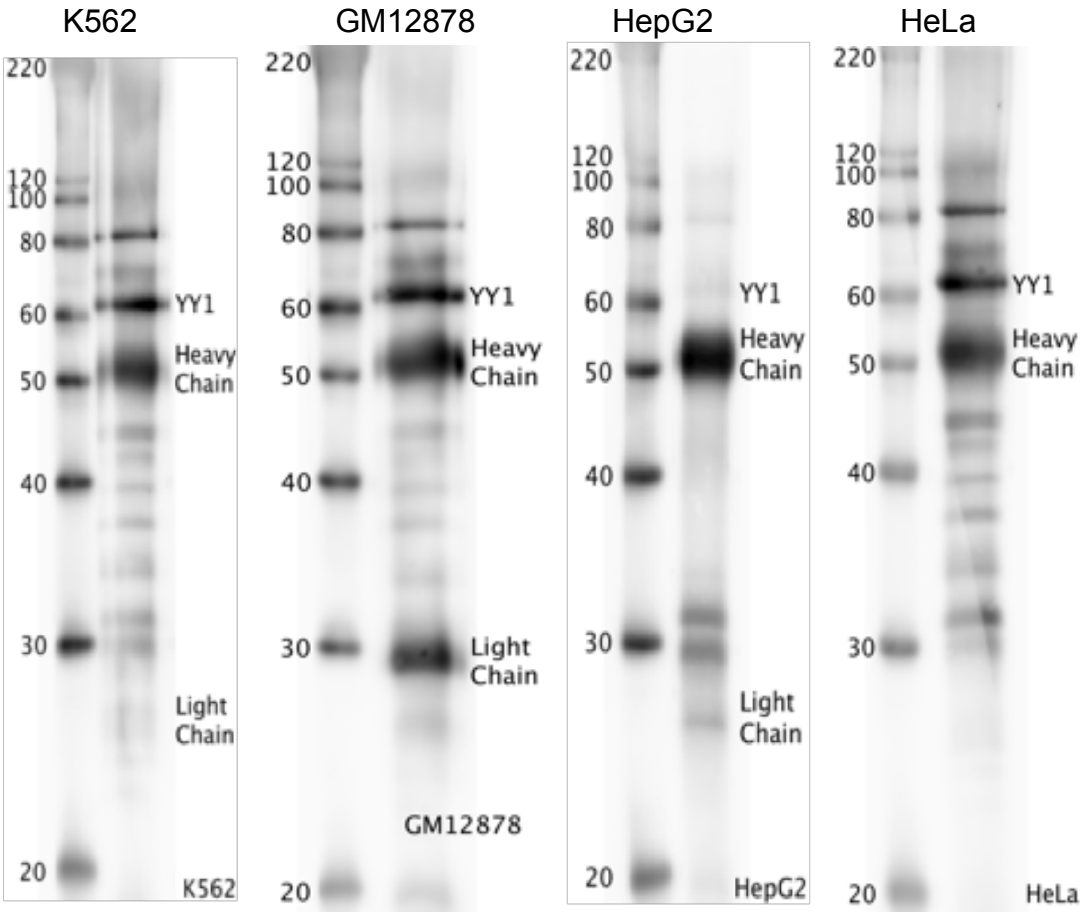

Figure Legend: YY1 immunoblots: IP-western with sc-281 YY1 antibody in whole cell lysates of K562, GM12878, HepG2, and HeLa cultured cells. Heavy and light chains of IgG are indicated. YY1 band is indicated at ~60 kDa.

Western blot protocol 2

Whole cell lysates were immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. After separation, the samples were transferred to a nitrocellulose membrane with an Invitrogen iBlot system. Blotting with primary (same as that used for IP) and secondary HRP-conjugated antibodies was performed on an Invitrogen BenchPro 4100 system. Visualization was achieved using SuperSignal West Femto solution (Thermo Scientific).

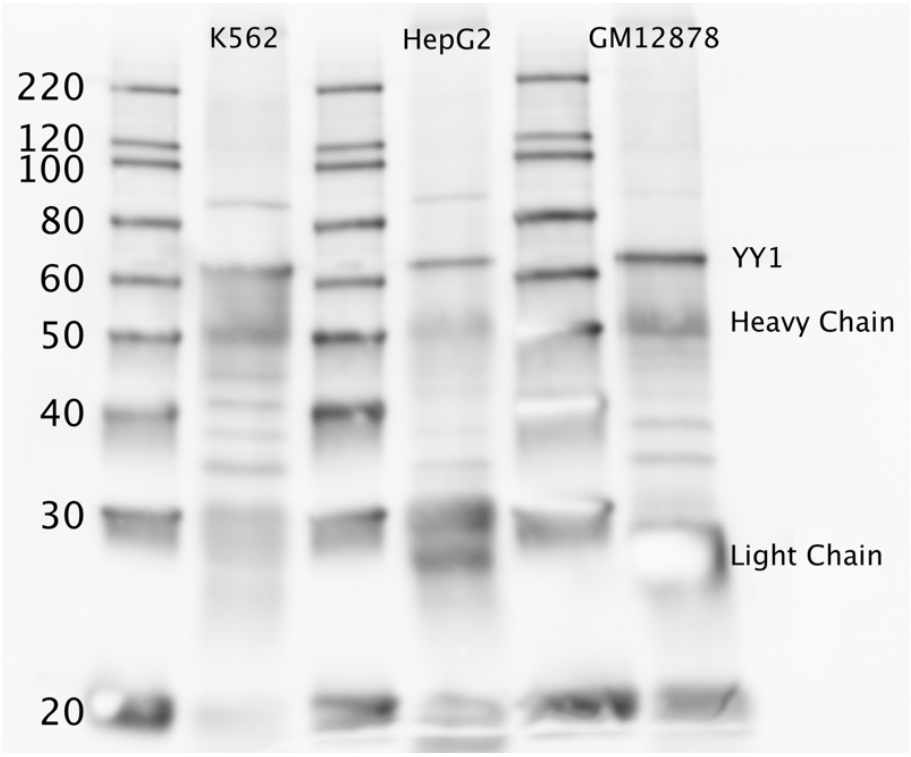

Figure Legend: YY1 immunoblots: IP-western with sc-281 YY1 antibody in whole cell lysates of K562, HepG2, and GM12878. Heavy and light chains of IgG are indicated. YY1 band is indicated at ~60 kDa.

Validation 2: In progress