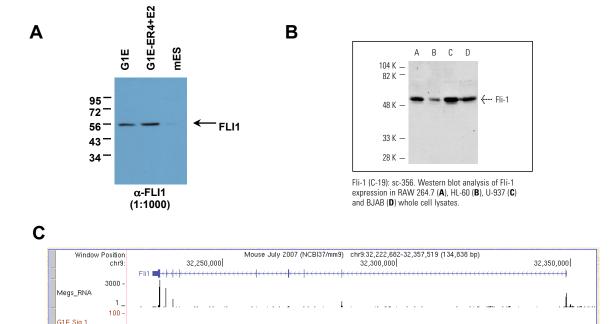
Hardison Lab @ Penn State University FLI1 (GeneID 2313) antibody validation



Validation of FLI1 (Friend leukemia virus integration 1) antibody Santa Cruz Biotechnology, Inc., FLI1 (C-19), cat# sc-356.

100

0 _ 100 -

G1E-ER4 Sig 1

ES-Bruce4 E0 Sg 1

A. Western blot analysis of FLI1 expression in various mouse cell lines.

Whole cell extracts prepared from G1E, G1E-ER4+E2, and mouse E14 ES cells were separated by SDS-PAGE and transferred to PVDF using standard immunoblotting methods. FLI1 expression was detected using rabbit anti-FLI1 (C-19; sc-356, 1:1000) followed by incubation with anti-rabbit secondary antibody (1:5000) and detected by ECL Plus (Amersham Biosciences). The FLI1 antibody detected a protein band of the expected size (MW = 51 kDa) in erythroid cell lines G1E and G1E-ER4+E2 cells. Trace amounts of FLI1 expression was also detected in the mouse E14 ES cell line, consistent with the low expression level of Fli1 in two different mouse embryonic stem cell lines in the MOE430 Gene Atlas Data set (Lattin et al., 2008) on BioGPS (Wu et al., 2009; http://biogps.org/#goto=genereport&id=2313).

Lattin JE, Schroder K, Su Al, Walker JR, Zhang J, Wiltshire T, Saijo K, Glass CK, Hume DA, Kellie S, Sweet MJ (2008) Expression analysis of G Protein-Coupled Receptors in mouse macrophages. *Immunome Res.* 4:5.

Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW 3rd, Su AI (2009) BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol.* **10**(11):R130.

B. Western blot image from Santa Cruz Biotechnology datasheet for FLI1 (C-19): sc-356.

C. RNA-seq analysis of Fli1 expression levels in various mouse cell lines and primary megakaryocytes.

RNA-seq expression data indicate that Fli1 is expressed at low levels in G1E and G1E-ER4+E2 cell lines and at high levels in primary megakaryocytes from fetal liver (Hardison lab, mouse ENCODE). By comparison, a very low level of expression Fli1 expression is detected in mouse ES Bruce4 cell line (Ren lab, mouse ENCODE), consistent with the western blot results shown in **A**, and supporting the specificity of the antibody.