Supporting Information

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SI Materials and Methods

***C. elegans* Genetics.** The known Notch pathway alleles used in this study were LG I, *sup-17(n1258ts)* (1, 2); LG III, *sel-8(sa54)* (1, 3), *lin-12(n302)* and *lin-12(n676)* (4, 5), *glp-1(ar202ts)* (6) *glp-1 (e2141)* (7) and *glp-1(bn18ts)* (8); LG X, *adm-4* (9). Additional information about these alleles, as well as the incidental mutations *dpy-8(e130)*, *dpy-20(e1282)* and *unc-119(ed3)*, can also be found in Wormbase (www.wormbase.org).

***C. elegans* RNAi.** Individual bacterial strains producing double-stranded RNA (dsRNA) targeting the 21 identified tetraspanin genes were obtained (10) or created. Starved, synchronized, L1-stage *glp-1(ar202)* hermaphrodites were placed on dsRNA-expressing bacteria, and plates were shifted to 25 °C for three days. The production of progeny was evidence of suppression.

Plasmids Used to Assess Rescue of *tsp-12(ok239)*. Plasmid p809 was generated by amplifying the genomic region IV: 10149012 to IV: 10151919, containing the *tsp-12* 5′ flanking sequence, coding region, and *tsp-12* 3′ UTR. A PCR was performed using primers 5′-ATAAGAATGCGGCCGCCTCGAGGTGAAATTTTAGTTTTAGATTTGAAATTTATGATTTCATTTGGAC-3′ and 5′-CTGAATCGAATAACTGTTATTTGGAAGATTTAAAGTG-3′ with N2 genomic DNA as a template (Table 1). This PCR product was cloned into pCR-XL-TOP (Invitrogen). p809 at 198 ng/μL was bombarded into *unc-119(ed3)* worms along with pDP#MM016b [*C. elegans unc-119(+)*] (11) at 87 ng/μL to generate *arIs119* and *arIs120*.

As a negative control for potential transgene marker effects, we used plasmid p816, which is a transcriptional fusion containing approximately 1.9 kb of the *tsp-12* 5′ flanking region fused to *2Xnls::yfp* and the *tsp-12* 3′ UTR (details available upon request). p816 at 68 ng/mL was used to generate *arIs135* and *arIs136* using the same bombardment technique as above by cobombardment with pDP#MM016b, each at 68 ng/mL.

Cells and Cell Culture. HeLa cells were grown in DMEM supplemented with 10% FBS, 100 U/mL penicillin G, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere under 5% CO_2.

HeLa Cell RNAi. siRNA targeting human TSPAN5,TSPAN33, CD9, CD81 and the "AllStars" control siRNA were purchased from Qiagen (catalog numbers SI0415665, SI04249532, SI03650318, SI02777187, and SI02777236).

Quantitative Real-Time PCR. Total RNA from HeLa cells was extracted with RNAqueous kit (Ambion) following the manufacturer's instructions (Figs. 1 and 2 and Fig. S1). cDNA was generated with the ThermoScript RT-PCR system (Invitrogen) and analyzed by quantitative real-time PCR using SYBRGreen RT-PCR Core Reagents kit and the 7300 Real-Time PCR System, both from Applied Biosystems. Relative expression levels were based on *GAPDH* levels as reference control. Primer sequences are given here.

GAPDH Fw: 5′ GAA GGT GAA GGT CGG AGT 3′
GAPDH Rv: 5′ GAA GAT GGT GAT GGG ATT TC 3′
TSPAN5 Fw: 5′ ACCCAGTTTGGCTCTTCCTT 3′
TSPAN5 Rv: 5′ CAATGTCATCCCGATATGCTC 3′
TSPAN33 Fw: 5′ CCTCACCGCTGTGTTCCT 3′
TSPAN33 Rv: CTGGCCAAAATCAATGAGGT 3′
TSPAN33 Rv: CTGGCCAAAATCAATGAGGT 3′
CD9 FW: 5′ TTGGTGATATTCGCCATTGA 3′
CD9 RV: 5′ GCATAGTGGATGGCTTTCAG 3′
CD81 FW: 5′ TGACCACCTCAGTGCTCAAG 3′
CD81 RV: 5′ ATGATCACAGCGACCACGAT 3′

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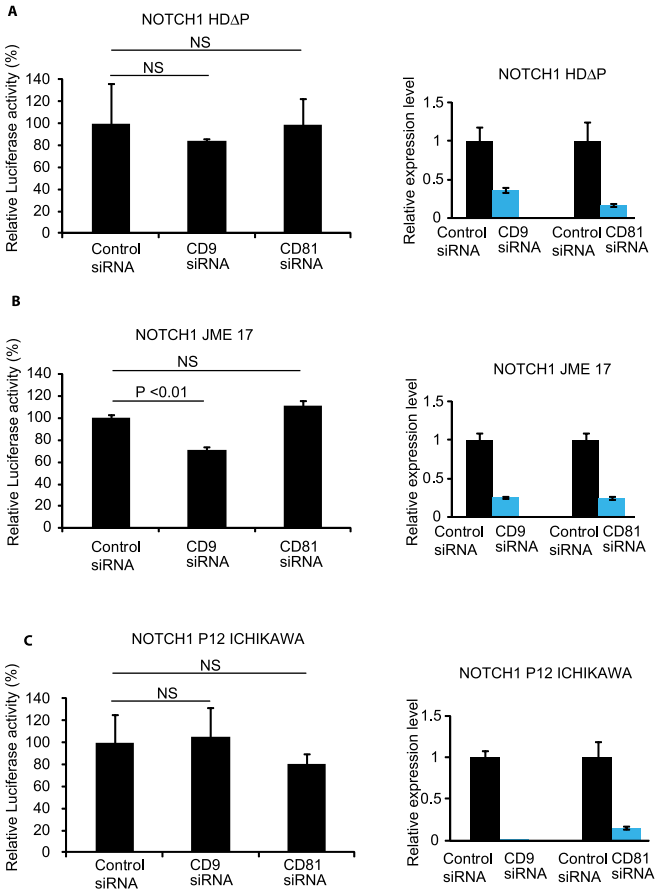

Fig. S1. Assessing the effect of CD9 or CD81 RNAi on the activity of missense mutant forms associated with T-ALL. One of two experiments, each performed with triplicate samples, is shown. (*A*–*C*) NOTCH/RBPJ luciferase reporter assays performed in HeLa cells expressing the *NOTCH1* L1601P-ΔPEST allele (NOTCH1 HD-ΔP), the *NOTCH1* Jurkat JME allele (NOTCH1 JME17), and the NOTCH1 P12 ICHIKAWA allele, in the presence of a control inactive siRNA or a siRNA targeting CD9 or CD81. NOTCH/RBPJ reporter activity after normalization with respect to the Renilla luciferase transfection control is shown as relative levels compared to those present in control siRNA transfected cells. Quantitative RT-PCR analysis of CD9 and CD81 (blue bars) demonstrating reduced levels of expression upon siRNA knockdown compared to siRNA control (black bars) is shown on the *Right*. Details are as described in *Materials and Methods and in SI Materials and Methods*. A significant decrease in reporter activity of the *NOTCH1* Jurkat JME17 allele in the presence of CD9 knockdown was not observed in a repeat experiment. Furthermore, as in the initial experiment shown, no decrease in reporter activity for the NOTCH1 P12 ICHIKAWA or NOTCH1 HD-ΔP was observed in the repeat experiment.