Table S1. *Tal1/Lmo2* tumors are functionally heterogeneous

	Disease Latency and Penetrance					
Dilution	**8129**	**2695**	**1002**	**2697**	**2692**	**1450**
10^6	4/4 (26)	N.D.	8/8 (10)	N.D.	N.D.	N.D.
10^5	3/3 (29)	4/4 (32)	8/8 (13)	2/2 (38)	4/4 (45)	3/3 (25)
10^4	3/4 (41)	3/4 (42)	8/8 (18)	1/3 (69)	2/4 (70)	N.D.
10^3	1/4 (53)	2/4 (61)	7/8 (26)	0/3 (181)	0/4 (175)	2/3 (40)
10^2	N.D.	N.D.	2/8 (35)	N.D.	N.D.	0/3 (257)
L-IC frequency	1:30,735	1:4,579	1:2,209	1:10,725	1:16,085	1:4,962
% DN3	1.04	11.08	29.29	4.12	67.61	5.47

( ) number in parentheses indicates latency in days; N.D. not determined

Table S2. Notch1 inhibition reduces leukemia-initiating cell activity

	Disease Latency and Penetrance									
	1007		1426		1928		2544		3304	
Dilution	Vehicle	GSI	Vehicle	GSI	Vehicle	GSI	Vehicle	GSI	Vehicle	GSI
10^5	3/3 (17)	3/3 (20)	3/3 (31)	0/3 (181)	4/4 (17)	3/4 (29)	4/4 (28)	0/4 (72)	4/4 (22)	4/4 (30)
10^4	2/3 (21)	2/3 (29)	4/4 (51)	0/4 (181)	3/4 (24)	3/4 (38)	3/4 (39)	0/4 (72)	4/4 (25)	1/4 (38)
10^3	1/3 (31)	0/3 (145)	0/3 (181)	0/3 (181)	0/3 (145)	0/3 (145)	1/4 (17)*	0/4 (72)	4/4 (31)	0/4 (72)

( ) number in parentheses indicates latency in days; * Mouse died of causes unrelated to leukemia

Figure S1. Purity of sorted *TNR/Tal1/Lmo2* preleukemic thymi and validation that GFP+ populations exhibit increased Notch1 target gene expression
(A) Preleukemic cells from *TNR/Tal1/Lmo2* thymi were isolated and stained with lineage-specific, CD25, and CD44 antibodies. Cells were gated on lineage-negative then analyzed based on the indicated populations and for GFP expression. Population purity was examined post-sorting and is indicated above the resulting population. *c-Myc* (B) or *Deltex1* (C) expression in the indicated populations were analyzed by real time quantitative PCR. Samples were analyzed in triplicate, but performed on one sorted thymi since results are consistent with published data.[1]

Figure S2. Purity of sorted tumor populations transplanted into syngeneic recipients
Leukemic cells from *Tal1/Lmo2* mice were isolated and stained with CD4, CD8 antibodies; lineage negative cells were then stained with CD25 and CD44 antibodies. Cells were sorted based on the indicated populations and population purity was examined post-sorting. The DP population is shown in the upper two panels and the DN3 population in the lower two panels. Purity is indicated above the resulting post-sort populations.

Figure S3. GSI administration to leukemic mice results in decreased Notch1 target gene expression
Mice transplanted with primary mouse leukemic cells were treated with vehicle or GSI and *c-Myc* (A) or *Deltex1* (B) expression analyzed by real time quantitative PCR. *c-Myc* (C) and *Deltex1* (D) mRNA levels were also monitored at the time of disease recurrence. Copy number was normalized to *β*-actin using the *ΔΔ*CT method.

Figure S4. Transplanted mice treated with GSI continued to gain weight throughout the treatment period
Recipient mice transplanted with mouse T-ALLs 1928, 1426, and 1007 were treated with vehicle or 150mg/kg GSI (MRK-003) using an intermittent dosing regimen.[2] Vehicle mice were given an equal volume of methylcellulose by oral gavage. The animals were weighed once per week, with GSI administered immediately following transplant and at 1 and 2 weeks post-transplant.

Figure S5. Mouse T-ALLs examined in the GSI treatment study express high levels of intracellular Notch1, harbor insertions/deletions that result in PEST truncation and express aberrant 3′ transcripts
(A) Mouse T-ALL cell lysates were examined for intracellular Notch1 protein levels by immunoblotting with Notch1 Val1744 (Cell Signaling, cat #2421S). Jurkat is a human T-ALL cell line that expresses full length ICN1; FVB is wild-type thymus; 2906 is a mouse tumor with truncated ICN1; 1007, 1426, 1928, 2544, and 3304 are the mouse *Tal1/Lmo2* T-ALLs examined in Figure 6. (B) DNA was isolated from the mouse T-ALLs and amplified with primers specific to exon 34 of the *Notch1* gene. PCR product was cloned into the TOPO TA cloning vector for sequencing using the universal M13 primers. Mutations were then analyzed using MacVector software. (C) Ratiometric *Notch1* quantitative RT-PCR analysis of mouse *Tal1/Lmo2* T-ALLs. The relative amounts of transcripts containing 5′ (exons 1 and 2) and 3' (exons 30 and 31) *Notch1* sequences were determined for primary tumors (pri) and transplanted tumors (trans) as described in [3]. Each determination was made in triplicate.

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2. Cullion K, Draheim KM, Hermance N, et al. Targeting the Notch1 and mTOR pathways in a mouse T-ALL model. *Blood*. 2009;113:6172–6181.

3. Ashworth TD, Pear WS, Chiang MY, et al. Deletion-based mechanisms of Notch1 activation in T-ALL: key roles for RAG recombinase and a conserved internal translational start site in Notch1. *Blood*. 2010;116:5455–5464.

Figure S1

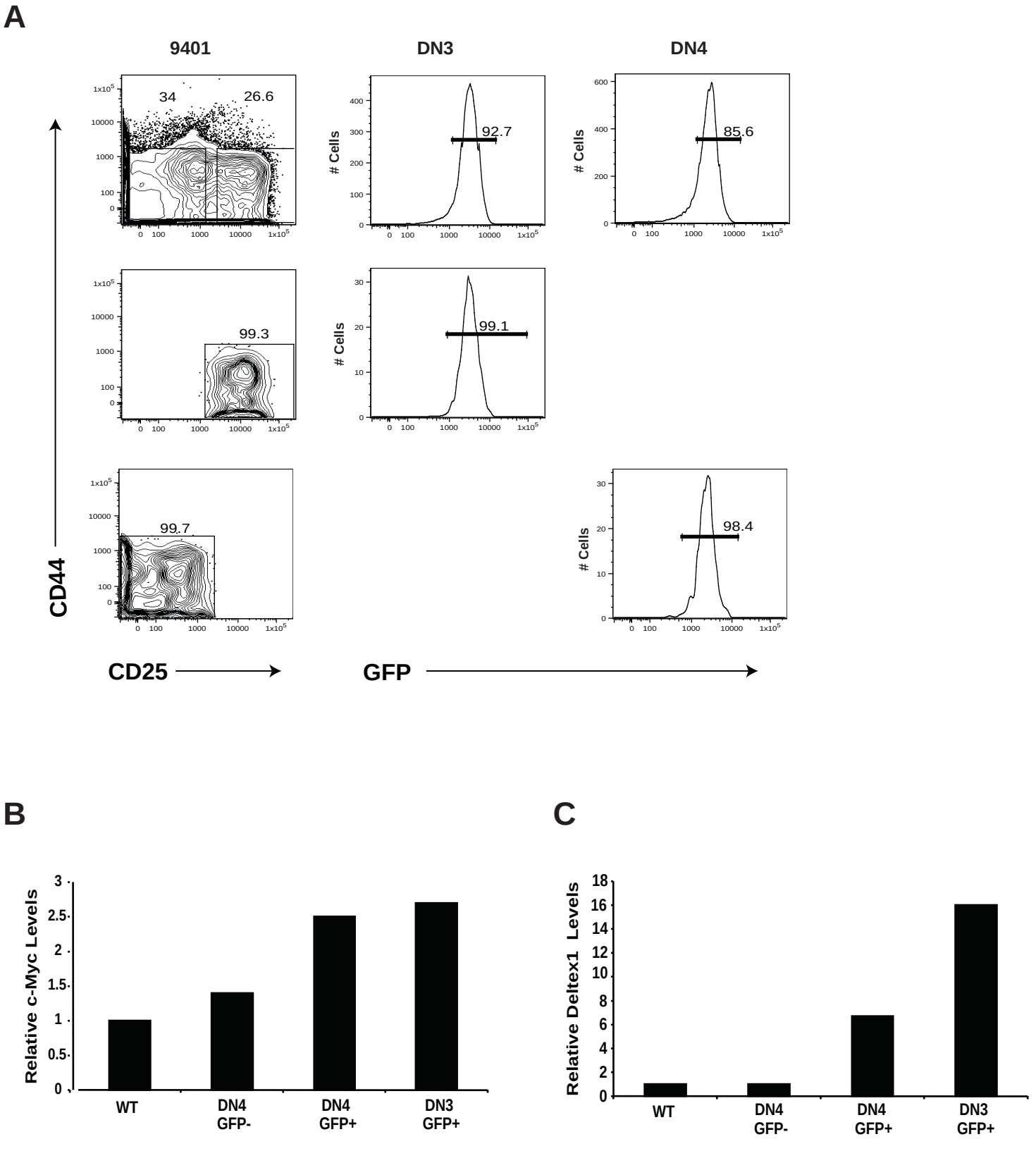
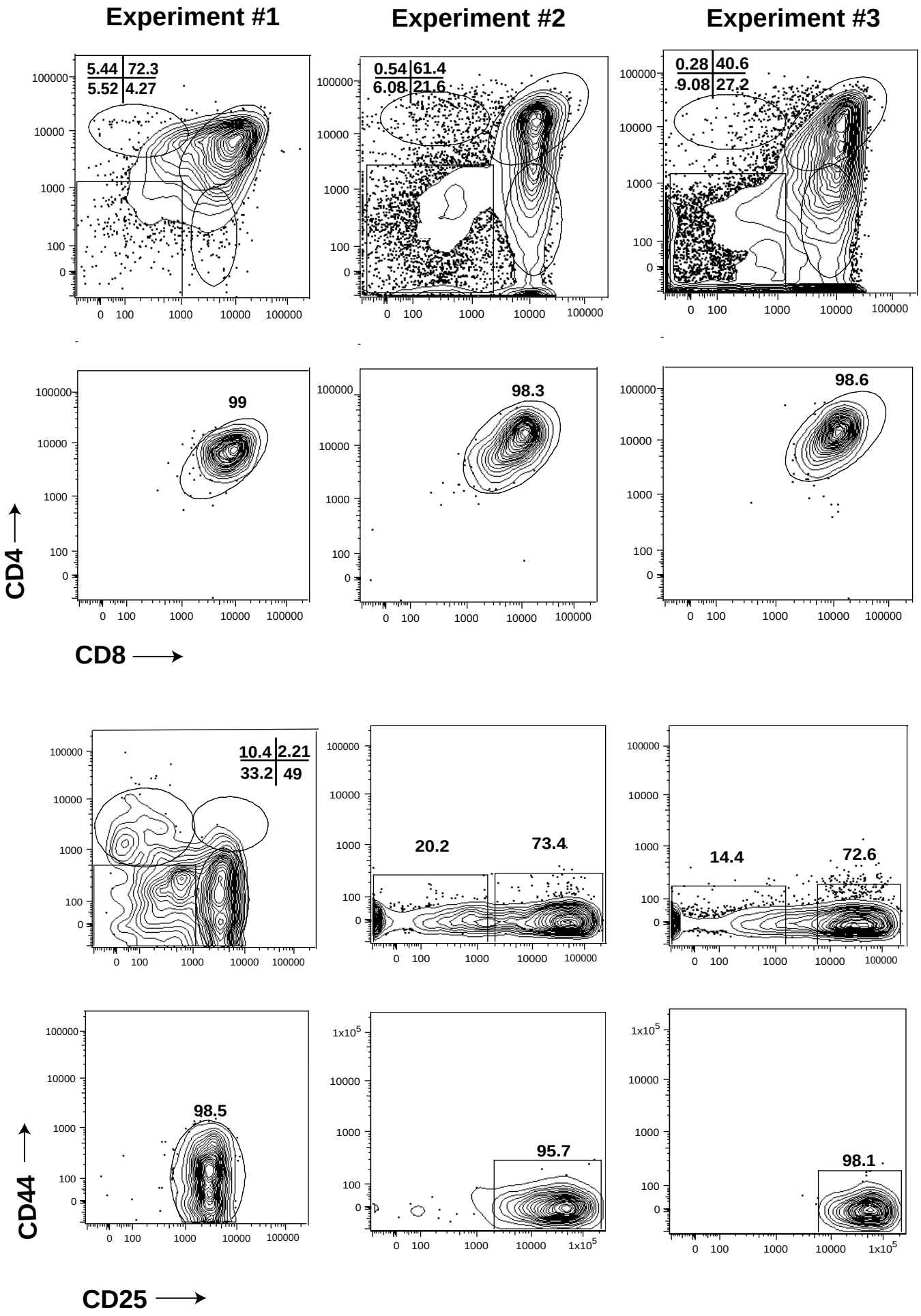

A
9401
DN3
DN4
34
26.6
92.7
85.6
99.3
99.1
99.7
98.4
Cells
CD44
CD25
GFP
B
Relative c-Myc Levels
WT
DN4 GFP-
DN4 GFP+
DN3 GFP+
C
Relative Deltex1 Levels
WT
DN4 GFP-
DN4 GFP+
DN3 GFP+

Figure S2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Experiment #2
Experiment #3
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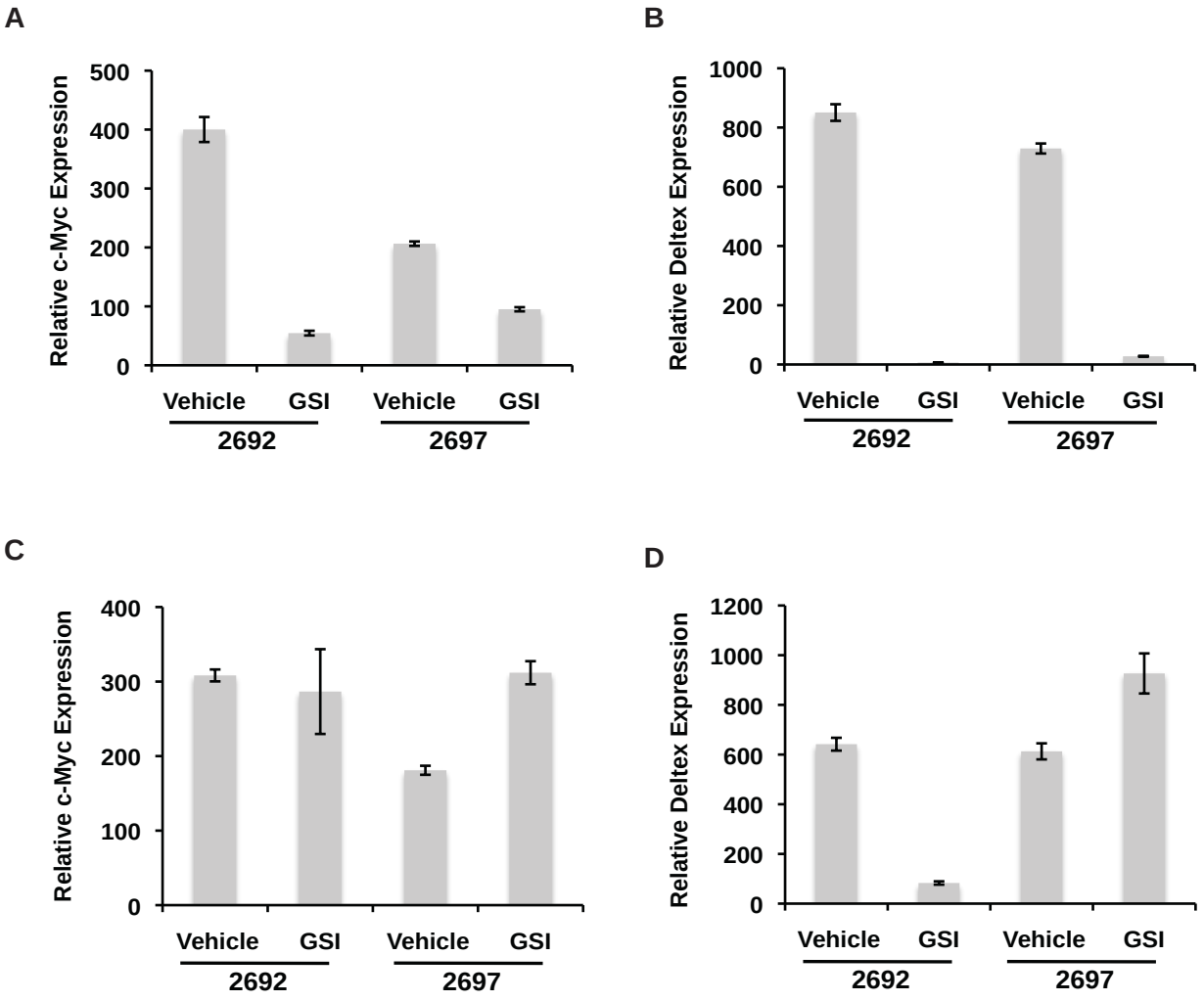
5.52 4.27
0.54 61.4
6.08 21.6
0.28 40.6
9.08 27.2
99
98.3
98.6
CD4
CD8
10.4 2.21
33.2 49
20.2
73.4
14.4
72.6
98.5
95.7
98.1
CD44
CD25

Figure S3


A
Relative c-Myc Expression
500
400
300
200
100
0
Vehicle
GSI
Vehicle
GSI
2692
2697
B
Relative Deltex Expression
1000
800
600
400
200
0
Vehicle
GSI
Vehicle
GSI
2692
2697
C
Relative c-Myc Expression
400
300
200
100
0
Vehicle
GSI
Vehicle
GSI
2692
2697
D
Relative Deltex Expression
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1000
800
600
400
200
0
Vehicle
GSI
Vehicle
GSI
2692
2697

Figure S4

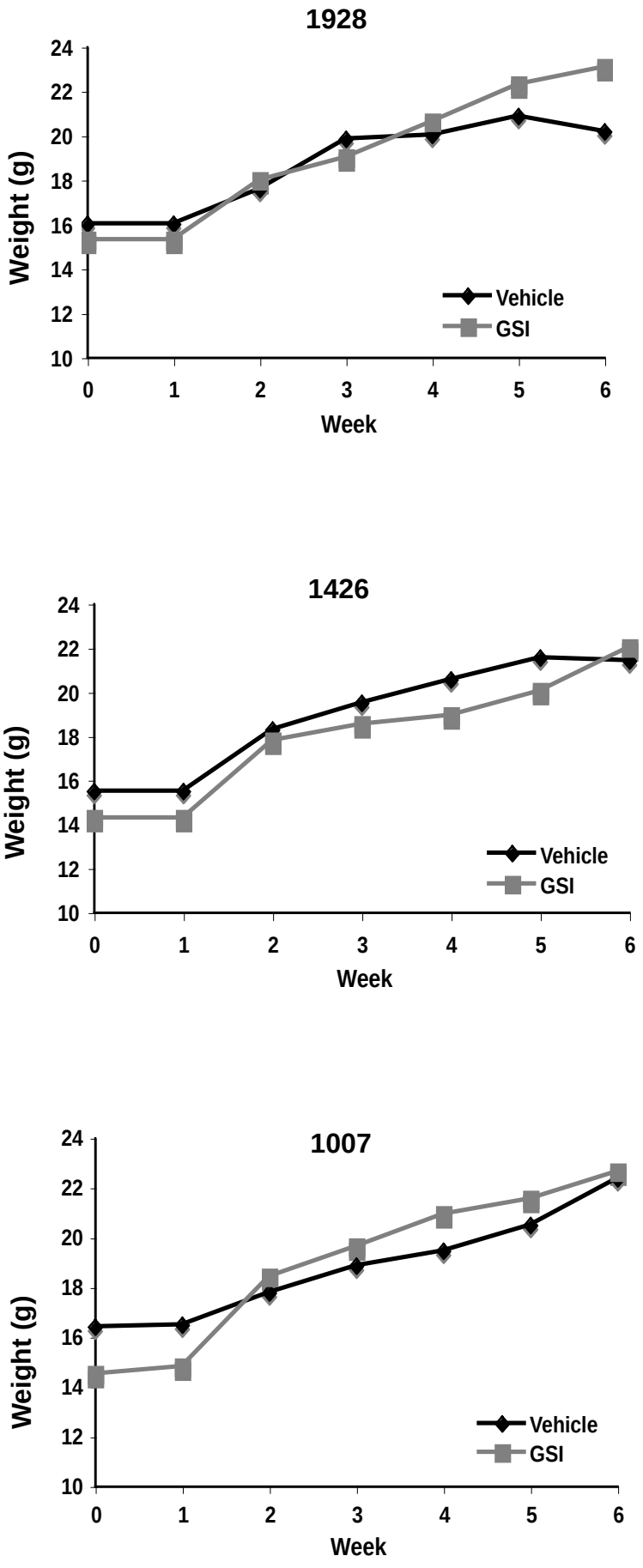
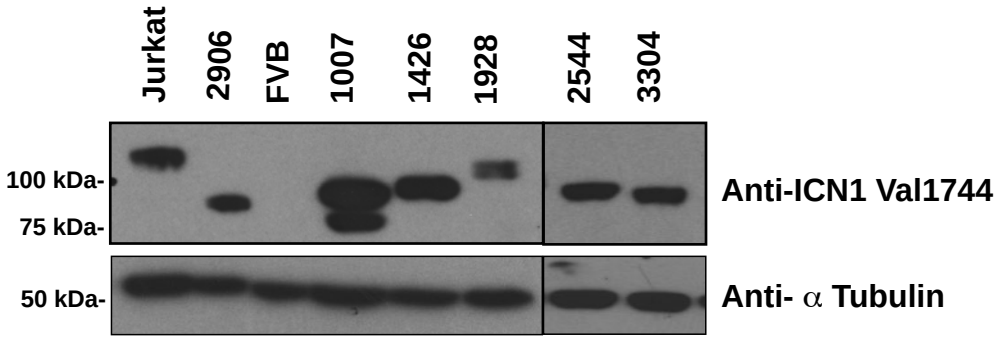

1928
Weight (g)
24
22
20
18
16
14
12
10
0
1
2
3
4
5
6
Week
Vehicle
GSI
1426
Weight (g)
24
22
20
18
16
14
12
10
0
1
2
3
4
5
6
Week
Vehicle
GSI
1007
Weight (g)
24
22
20
18
16
14
12
10
0
1
2
3
4
5
6
Week
Vehicle
GSI

Figure S5

A



B

Tumor #	Genotype	Nucleic Acid Change	Amino Acid Change
1007	*Tal1/Lmo2*	7215 ins TTTT	2406 T->F
1426	*Tal1/Lmo2*	7161 C->G, ins G	2387 T-> R
1928	*Tal1/Lmo2*	7131 del G	2377 D->I
2544	*Tal1/Lmo2*	7162 del G, ins CCCTC	2388 A->P
3304	*Tal1/Lmo2*	7273 del G, ins AA	2425 G->K

C