Supplemental Table 1. CRISPR/Cas9 targeting guide RNAs, oligonucleotides for homologous recombination, and PCR primers

TMD patient 8	
gRNA (5'-3')	CACTTATGTTTGGCTGGCGACGG
PCR primers	F: GAGACTTCAGCATGCAAACC R: AAACTGCCATTCCAATAGTC
TMD patient 145	
gRNA (5'-3')	CGCCAGCATTCCCCAAACCAGG & CACCGTCTACGTTGAGTTTTGG
PCR primers to detect *DYRK1A* large deletion	F: TCATGGTTCATCCTACCCGT R: GTTTTCCTAGCCCGCTGTAGAC
qPCR primers to detect *DYRK1A* copy number	F: CCAAACCTTCCGTGACCCAG R: TAGAATCGTCTCCCTGGCCC
qPCR primers to detect *DYRK1A* large deletion	F: CGTCGCCAGCCAAACATAAG R: TCAGGCATCACCTGGTTAGT

Supplemental Table 2. *GATA1* and *DYRK1A* characterization of induced pluripotent stem cell clones

TMD patient	***GATA1***	***DYRK1A***	
TMD patient 8 (T21)	wtGATA1	-/-/-	Clone 1
		-/-/-	Clone 2
	GATA1 g.4652G>T (GATA1s)	-/-/-	Clone 1
		-/-/-	Clone 2
		-/-/-	Clone 3
TMD patient 8 (Euploid)	GATA1 g.4652G>T (GATA1s)	-/-	Clone 1
TMD patient 145	GATA1 c.3_4insG (GATA1s)	-/-/-	Clone 1
		-/-/-	Clone 2

Supplemental Table 3. Flow cytometry antibodies

Antibody	Manufacturer	Catalog #	Dilution
SSEA-3 Alexa Fluor 488	BioLegend Clone MC-631	330306	1:200
SSEA-4 Alexa Fluor 647	BioLegend Clone MC-813-70	330408	1:200
Tra-1-60 Alexa Fluor 488	BioLegend Clone TRA-160-R	330614	1:40
Tra-1-81 Alexa Fluor 647	BioLegend Clone TRA-1-81	330706	1:40
KDR (CD309) Alexa Fluor 647	BioLegend Clone 7D4-6	359910	1:100
CD31 FITC	BD Biosciences Clone WM59	555445	1:20
CD34 APC	BioLegend Clone 561	561209	1:100
CD43 FITC	BD Biosciences Clone 1G10	555475	1:20
CD41a PE	BD Biosciences Clone HIP8	555467	1:20
CD41a PE/Cy7	BioLegend Clone HIP8	303718	1:400
CD42a FITC	BD Biosciences Clone ALMA.16	558818	1:20
CD42b APC	BD Biosciences Clone HIP1	551061	1:40
CD42b FITC	BD Biosciences Clone HIP1	555472	1:40
CD235a APC	BD Biosciences Clone GA-R2	551336	1:5000
CD71 FITC	BD Biosciences Clone M-A712	555536	1:20
CD45 PE	BD Biosciences Clone HI30	555483	1:20

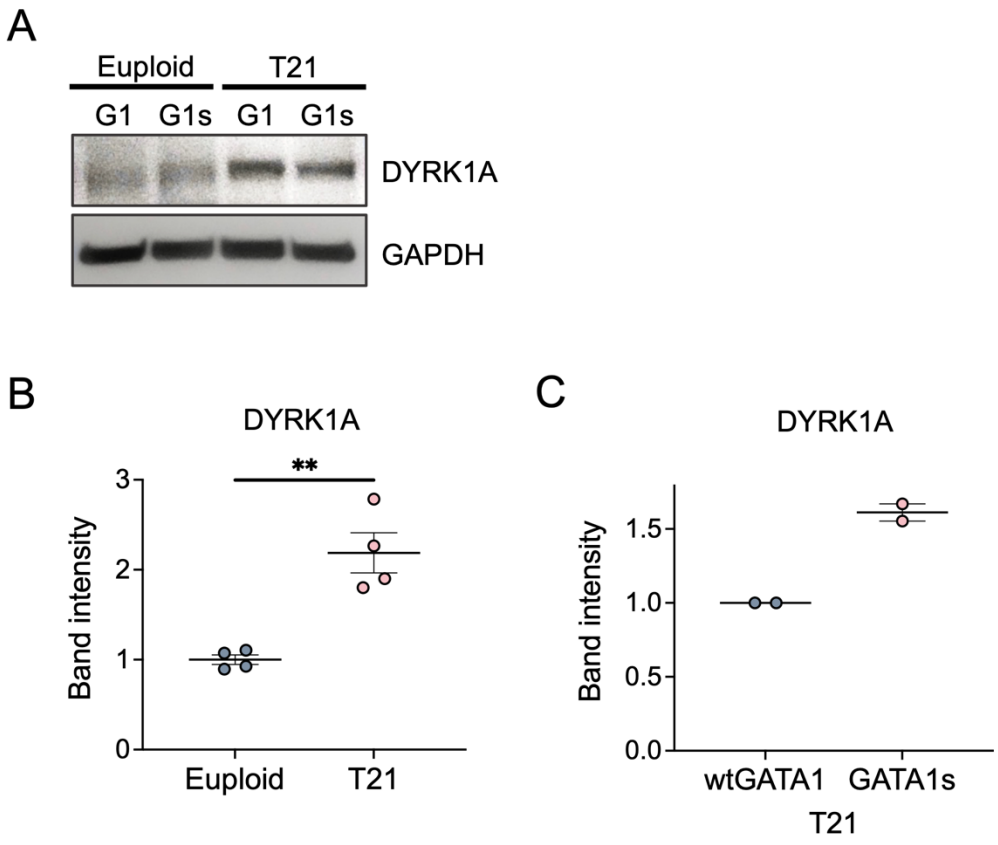
CD18 APC	BD Biosciences Clone 6.7	551060	1:20
PAC-1 FITC	BD Biosciences Clone PAC-1	340507	1:20
Ki67 APC	BioLegend Clone Ki-67	350514	1:100
Annexin V FITC	BD Biosciences	556420	1:20
7-AAD	BioLegend	420404	1:200
FxCycle Violet	Invitrogen	F10347	1:1000
DyeCycle Violet	Invitrogen	V35003	1:1000

Supplemental Table 4. Real-time qPCR primers (5'-3')

hGAPDH	F: GTCAGTGGTGGACCTGACCT R: TGAGCTTGACAAAGTGGTCG
hCyclin D1	F: TCTACACCGACAACTCCATCCG R: TCTGGCATTTTGGAGAGGAAGTG
hCyclin D2	F: GAGAAGCTGTCTCTGATCCGCA R: CTTCCAGTTGCGATCATCGACG
hGATA2	F: CAGCAAGGCTCGTTCCTGTTCA R: ATGAGTGGTCGGTTCTGCCCAT
hCyclin A2	F: AACCATTGGTCCCTCTTGATTAT R: TCCTCATGGTAGTCTGGTACTT
hCDC6	F: GGAGATGTTCGCAAAGCACTGG R: GGAATCAGAGGCTCAGAAGGTG
hCDC25A	F: TCTGGACAGCTCCTCTCGTCAT R: ACTTCCAGGTGGAGACTCCTCT
hE2F8	F: GAGGCTCAAAGAGGGCAAGCAT R: ATGAGCACTGCGTGAGAGGGAT
hMCM2	F: TGCCAGCATTGCTCCTTCCATC R: AAACTGCGACTTCGCTGTGCCA
hCHK1	F: GTGTCAGAGTCTCCCAGTGGAT R: GTTCTGGCTGAGAACTGGAGTAC
hPF4	F: CCCACTGCCCAACTGATAG R: GCAAATGCACACACGTAGG
hVWF	F: TCCCCTGTCTCATCGGA R: GCACTCCAGGTCATAGTTCTG
hGP9 (CD42a)	F: TACCTGCCGCGCCCTGGAAAC R: CACGGACTGAAGGCTGTTGTTG
hSELP (CD62P)	F: TCCGCTGCATTGACTCTGGACA R: CTGAAACGCTCTCAAGGATGGAG

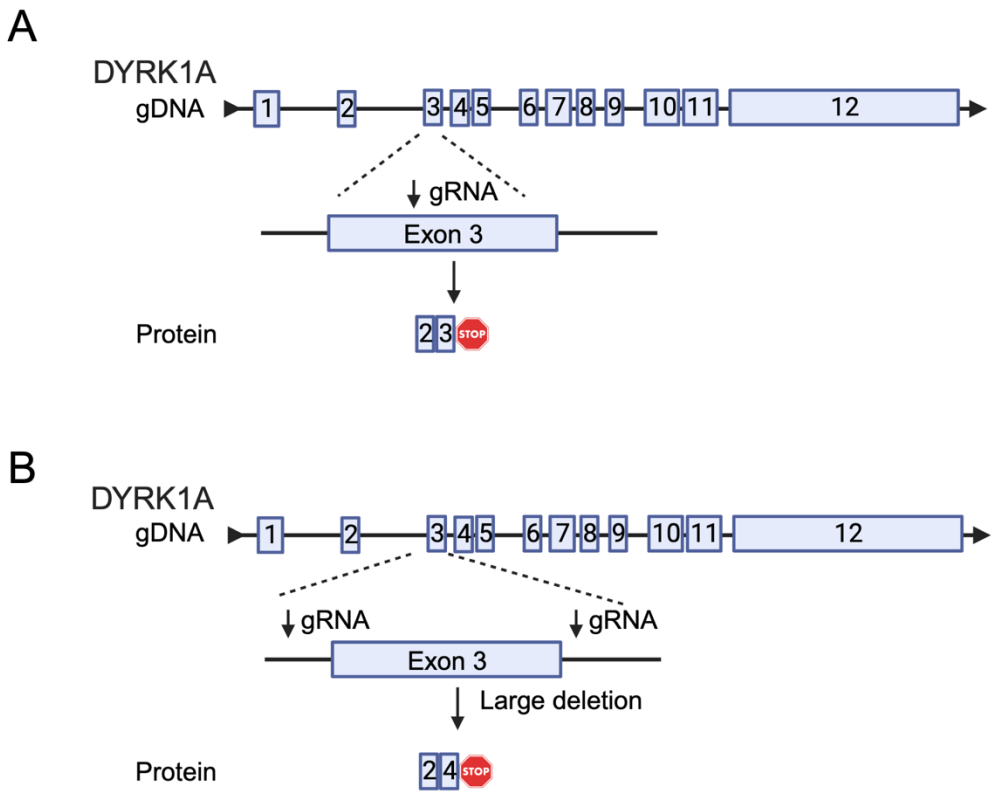
Supplemental Table 5. Western blot antibodies

	Antibody	Manufacturer	Catalog #	Dilution
Primary antibodies	DYRK1A	Cell Signaling Technology	8765	1:750
		Abcam	ab65220	1:600
	Cyclin D1	Cell Signaling Technology	55506	1:1000
	Cyclin D2	Cell Signaling Technology	3741	1:1000
	Cyclin D3	Cell Signaling Technology	2936	1:2000
	Phospho-Cyclin D2 (Thr280)	Aviva Systems Biology	OAAJ06410	1:500
	Phospho-Cyclin D3 (Thr283)	Cell Signaling Technology	53966	1:1000
	Cyclin A2	Cell Signaling Technology	67955	1:1000
	Cyclin B1	Cell Signaling Technology	4138	1:1000
	CDK1	Cell Signaling Technology	9116	1:1000
	β-actin	Abcam	ab6276	1:20000
	Hypophospho-Rb	BD Biosciences	554164	1:500
	Phospho-Rb (Ser780)	Cell Signaling Technology	8180	1:1000
	Phospho-Rb (Ser807/811)	Cell Signaling Technology	9308	1:1000
	Rb	BD Biosciences	554136	1:500
	GATA1	Cell Signaling Technology	4589	1:1000
	GATA2	Abcam	ab109241	1:1000
	CDK4	Cell Signaling Technology	12790	1:1000
	GAPDH	Abcam	ab181602	1:10000
Secondary antibodies	Mouse IgG (HRP-conjugated)	Invitrogen	31430	1:20000
	Rabbit IgG (HRP-conjugated)	Cell Signaling Technology	7074	1:10000



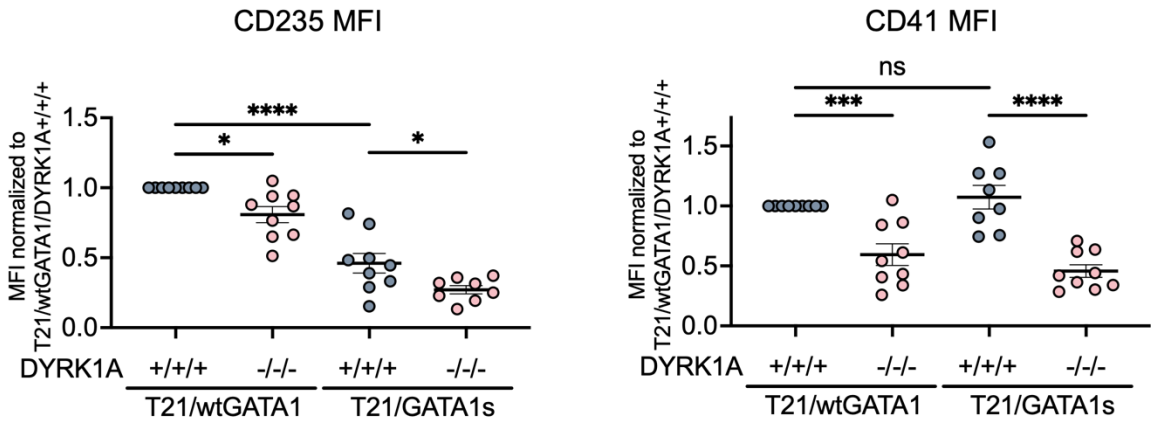
Supplemental figure 1. DYRK1A is overexpressed in human trisomy 21 cells.

(A) Representative Western blot for DYRK1A expression in isogenic undifferentiated iPSCs from euploid or T21 iPSCs, with wild-type GATA1 (G1) or GATA1short (G1s). DYRK1A expression quantified by Western blot band intensity for **(B)** Euploid and T21 iPSC and **(C)** day 6 T21/wtGATA1 and T21/GATA1s megakaryocytes. Bands normalized to housekeeping gene. n = 4 and 2 independent experiments per genotype for **(B)** and **(C)**, respectively. Data represent the mean ± SEM. Statistical significance was determined by 2-tailed, unpaired t-test. $^{**}p \leq 0.01$.

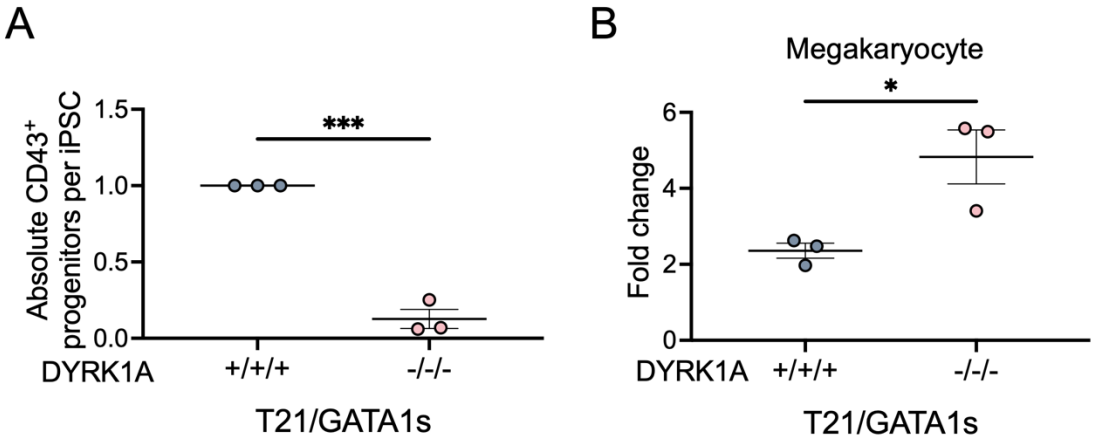


Supplemental figure 2. CRISPR/Cas9 targeting of *DYRK1A* in T21 iPSCs.

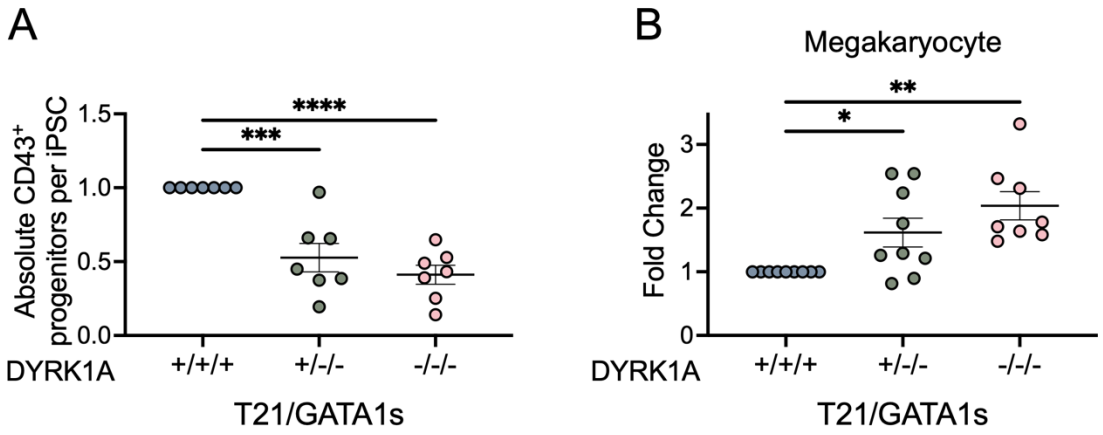
CRISPR/Cas9 targeting strategy for *DYRK1A* knockout in T21/wtGATA1 and T21/GATA1s iPSCs. gRNAs were designed to **(A)** target exon 3 of human *DYRK1A* to introduce an insertion-deletion in patient 1, or **(B)** target introns 2 and 3 of human *DYRK1A* to generate a large deletion that includes exon 3 in patient 2.



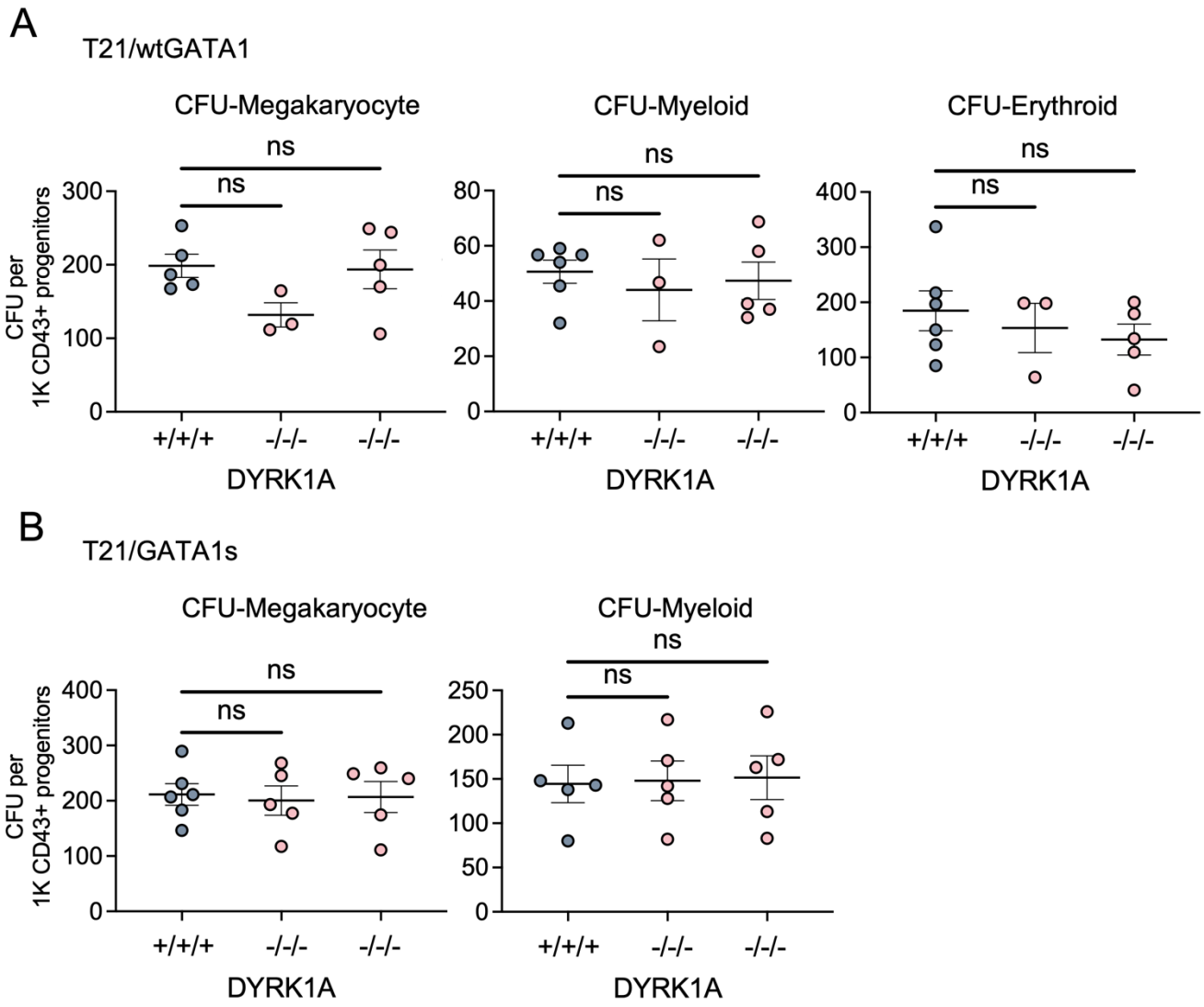
Supplemental figure 3. DYRK1A loss shows aberrant hematopoietic progenitors regardless of GATA1 status. Mean fluorescence intensity (MFI) for CD41 and CD235 for day 7 hematopoietic progenitors gated on $CD43^{+}41^{+}235^{+}$ and normalized to MFI for T21/wtGATA1/$DYRK1A^{+/+/+}$. *n* = 8-9 independent experiments per genotype. Data represent the mean ± SEM. Statistical significance was determined by ordinary one-way ANOVA. ns, non-significant, *$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$.



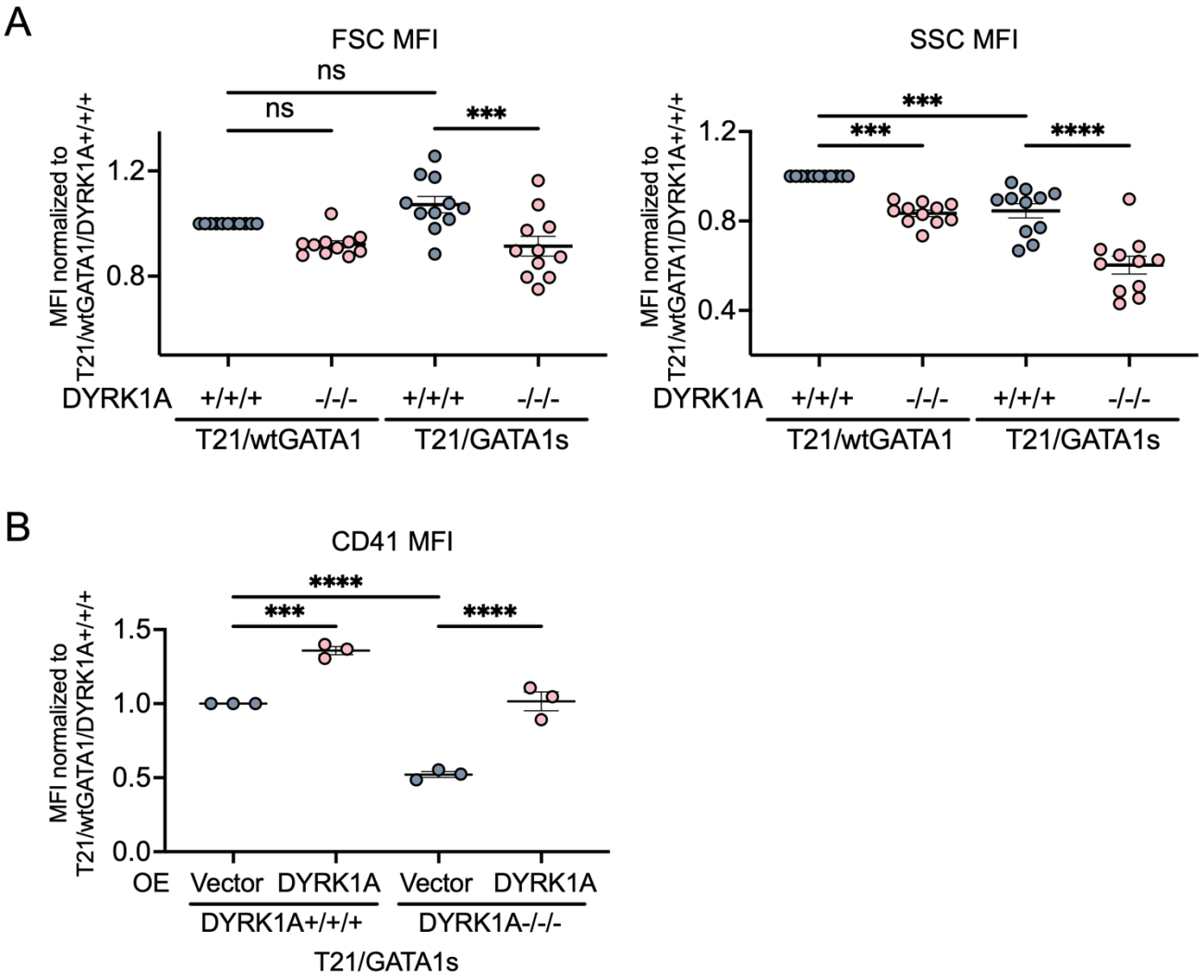
Supplemental figure 4. Hematopoietic phenotype of independently generated iPSC clones from a distinct patient with T21/GATA1s. (A) Absolute CD43+ progenitor number generated per iPSC undergoing differentiation. **(B)** Fold change of $CD41^{+}42^{+}$ megakaryocytes differentiated in liquid culture for 6 days from T21/GATA1s iPSC-derived CD43+ progenitors with DYRK1A wild-type (+/+/+) or knockout (-/-/-). *n* =3 independent experiments per clone. Data represent the mean ± SEM. Statistical significance was determined by 2-tailed, unpaired *t*-test. *$p \leq 0.05$, ***$p \leq 0.001$.



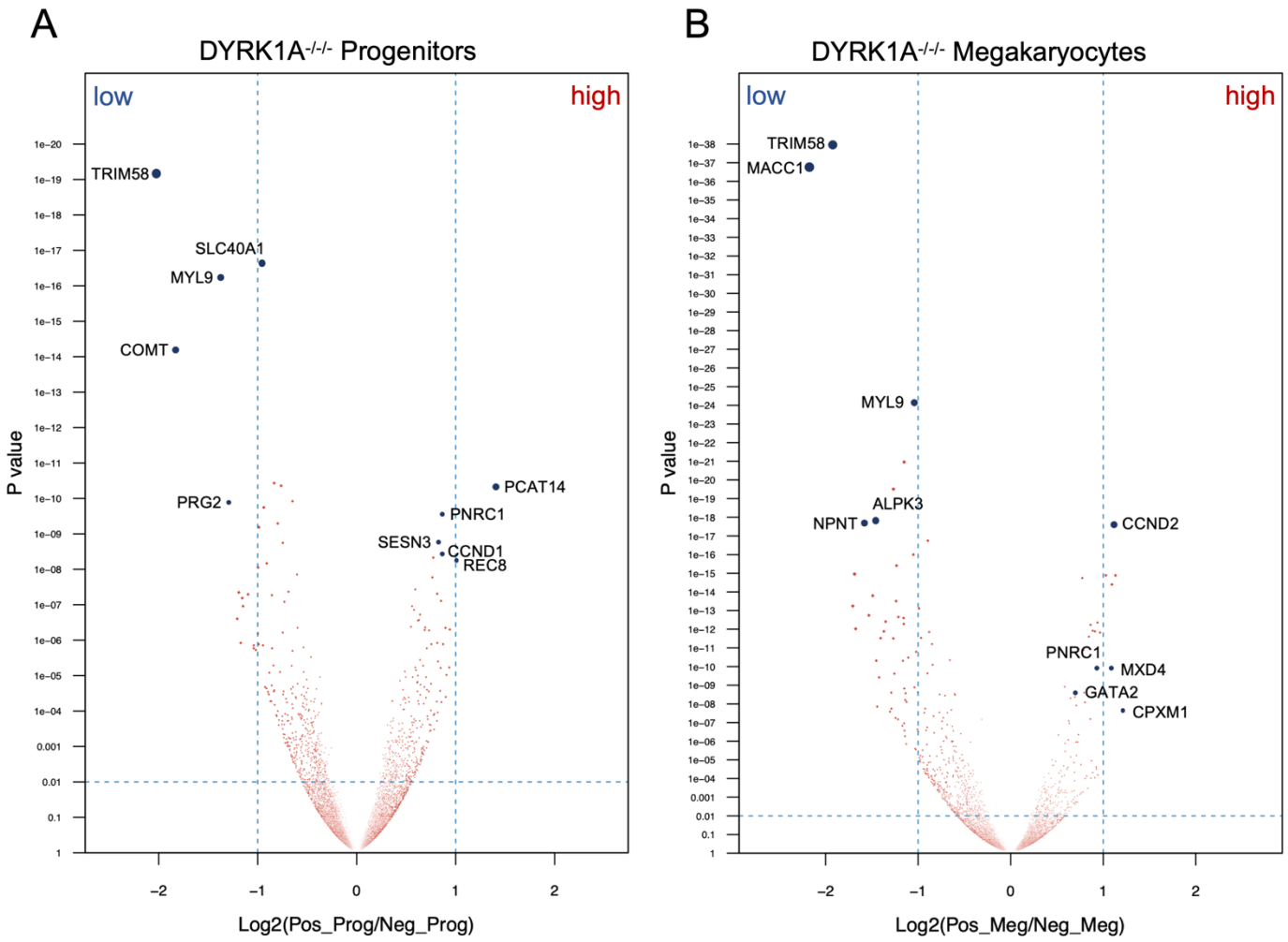
Supplemental figure 5. DYRK1A knockdown showed intermediate phenotype compared to DYRK1A knockout. (A) Absolute CD43+ progenitor yield on day 7 of EB differentiation, normalized to starting DYRK1A$^{+/+/+}$ iPSC number. **(B)** Fold change from T21/GATA1s iPSC-derived CD43+ hematopoietic progenitors with DYRK1A wild-type, knockdown or knockout, differentiated in liquid culture for 6 days to support megakaryocytes (CD41^{+}42^{+}), normalized to starting DYRK1A$^{+/+/+}$ CD43+ progenitors. Each column represents an individual iPSC clone. *n* = 7-9 independent experiments per clone. Data represent the mean ± SEM. Statistical significance was determined by ordinary one-way ANOVA. *$p \leqslant 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.



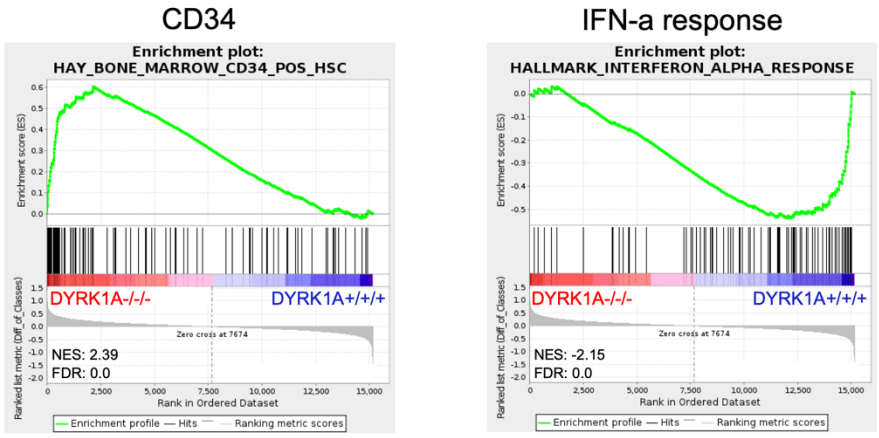
Supplemental figure 6. DYRK1A knockout does not affect hematopoietic colony formation. Colony-forming unit (CFU) assays were performed on $CD43^{+}$ progenitors derived from **(A)** T21/wtGATA1 and **(B)** T21/GATA1s iPSCs with DYRK1A wild-type (+/+/+) or knockout (-/-/-). Each column represents an individual iPSC clone. *n* = 3-6 independent experiments per clone. Data represent the mean ± SEM. Statistical significance was determined by ordinary one-way ANOVA. ns, non-significant.



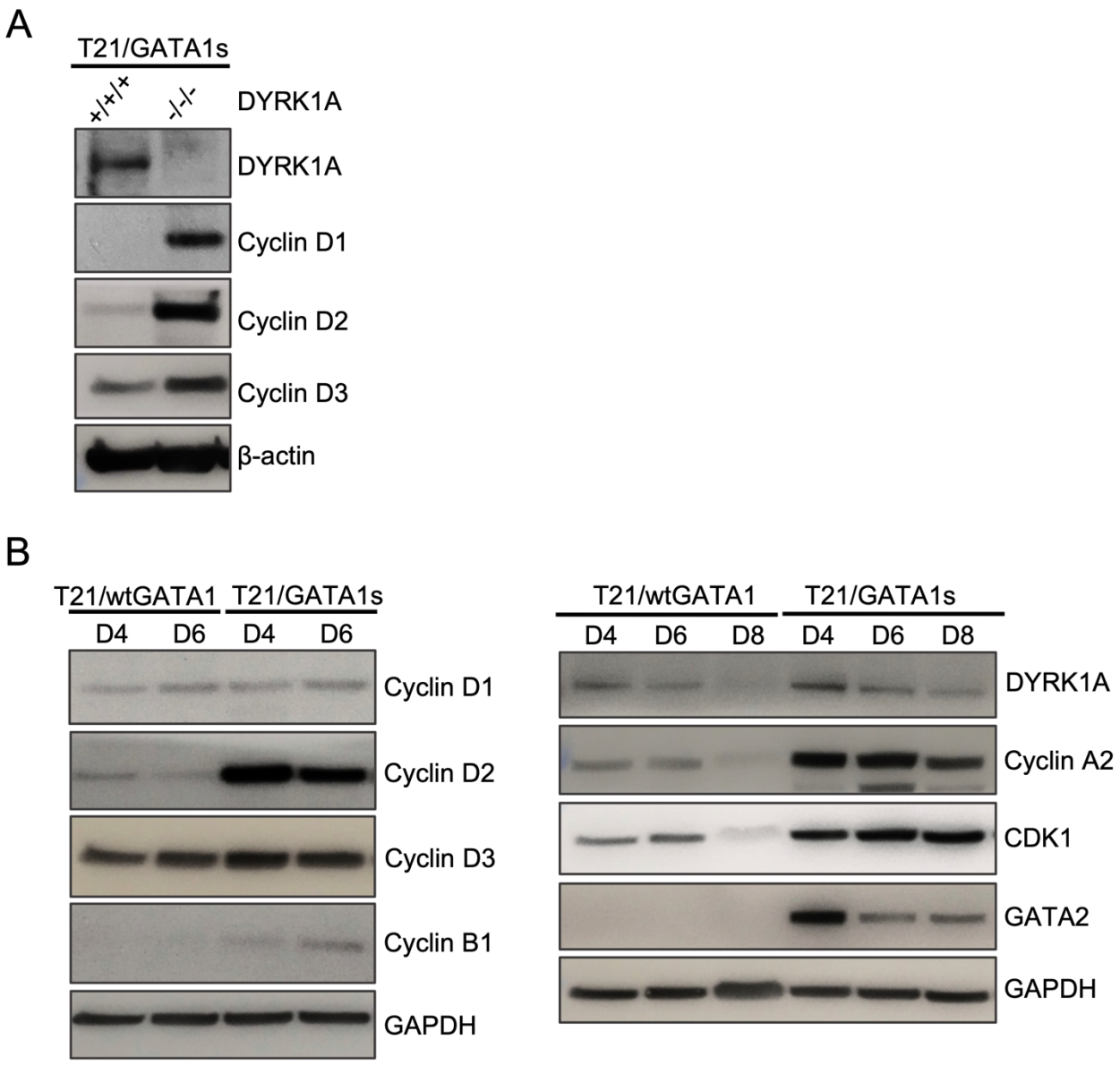
Supplemental figure 7. DYRK1A knockout impairs megakaryocyte maturation in T21/GATA1s cells. (A) Forward scatter (FSC) and side scatter (SSC) for day 5 to 6 $CD41^{+}42b^{+}$ megakaryocytes. *n* = 11 independent experiments per clone. **(B)** CD41 mean fluorescence intensity (MFI) of T21/GATA1s $DYRK1A^{+/+/+}$ and $DYRK1A^{-/-/-}$ megakaryocytes assayed on day 5 to 6 of lineage-specific liquid culture after lentiviral overexpression (OE) of DYRK1A compared to vector alone. *n* = 3 independent experiments. Data represent the mean ± SEM. Statistical significance was determined by ordinary one-way ANOVA. ns, non-significant, ***$p \leq 0.001$, ****$p \leq 0.0001$.



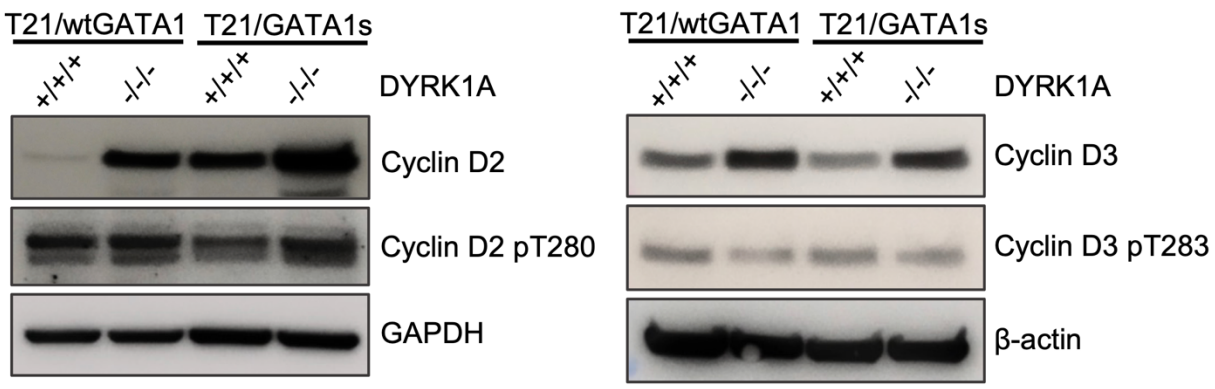
Supplemental figure 8. Volcano plots showing differential gene expression for T21/GATA1s/DYRK1A$^{-/-/-}$ compared with T21/GATA1s/DYRK1A$^{+/+/+}$ in sorted progenitors **(A)** and day 4 megakaryocytes **(B)**. Each dot represents a gene, top 5 up and down-regulated genes in DYRK1A$^{-/-/-}$ compared to DYRK1A$^{+/+/+}$ are labeled in blue.



Supplemental figure 9. RNA-seq analysis shows enhanced CD34 cell properties and decreased interferon signaling in T21/GATA1s/DYRK1a$^{-/-/-}$ progenitors. Selected gene set enrichment analysis (GSEA) plots of RNA-sequencing data from sorted T21/GATA1s/DYRK1A$^{+/+/+}$ and DYRK1A$^{-/-/-}$ CD43^{+}CD41^{+}235^{+} hematopoietic progenitors.



Supplemental figure 10. Cell cycle protein expression in iPSC-derived megakaryocytes from independently generated iPSC clones from a distinct patient with T21/GATA1s. Representative western blot analysis of **(A)** D-type cyclins in T21/GATA1s megakaryocytes with DYRK1A wild-type (+/+/+) or knockout (-/-/-) on day 6 of liquid culture and **(B)** cell cycle proteins in isogenic T21/wtGATA1 and T21/GATA1s iPSC-derived megakaryocytes on days 4, 6, and 8 of lineage-specific liquid culture.



Supplemental figure 11. DYRK1A knockout decreases Cyclin D2 and Cyclin D3 phosphorylation level.

Representative Western blot analysis of day 4 T21/wtGATA1 or T21/GATA1s megakaryocytes with DYRK1A wild-type (+/+/+) or knockout (-/-/-) for phosphorylated and total Cyclin D2 and Cyclin D3.