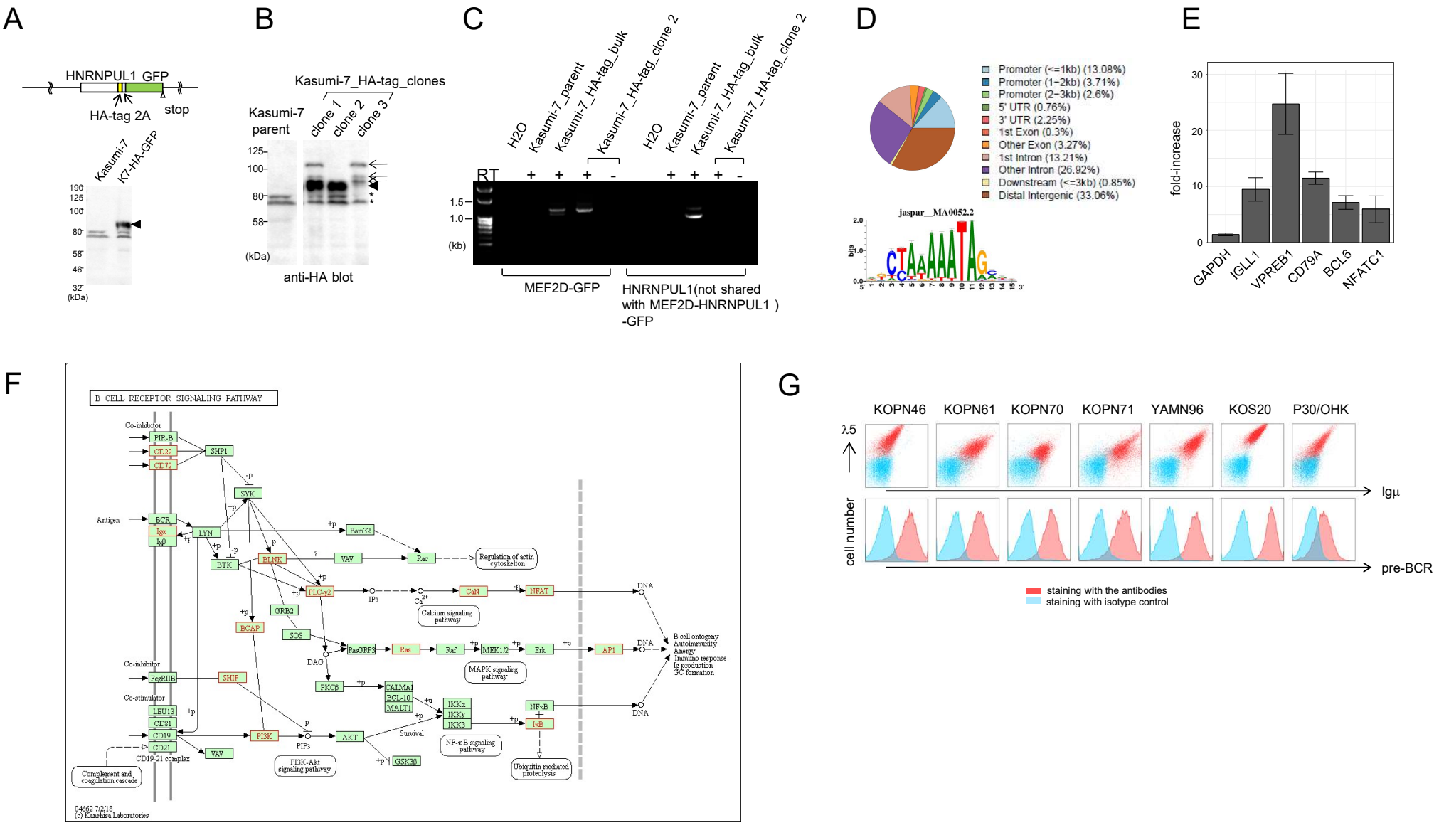
Figure S1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HA-tag 2A
stop
Kasumi-7
K7-HA-GFP
190
125
100
80
58
46
32
(kDa)
B
Kasumi-7_HA-tag_clones
Kasumi-7 parent
clone 1
clone 2
clone 3
125
100
80
58
(kDa)
anti-HA blot
C
H2O
Kasumi-7_parent
Kasumi-7_HA-tag_bulk
Kasumi-7_HA-tag_clone 2
RT
1.5
1.0
(kb)
MEF2D-GFP
HNRNPUL1(not shared with MEF2D-HNRNPUL1) -GFP
D
Promoter (<=1kb) (13.08%)
Promoter (1−2kb) (3.71%)
Promoter (2−3kb) (2.6%)
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3' UTR (2.25%)
1st Exon (0.3%)
Other Exon (3.27%)
1st Intron (13.21%)
Other Intron (26.92%)
Downstream (<=3kb) (0.85%)
Distal Intergenic (33.06%)
jaspar__MA0052.2
bits
E
fold-increase
30
20
10
0
GAPDH
IGLL1
VPREB1
CD79A
BCL6
NFATC1
F
B CELL RECEPTOR SIGNALING PATHWAY
Co-inhibitor
PIR-B
CD22
CD72
SHP1
SYK
Antigen
BCR
Igα
Igβ
LYN
Bam32
BTK
BLNK
VAV
Rac
Regulation of actin cytoskeleton
PLC-γ2
IP3
Ca++
CaN
NFAT
DNA
Calcium signaling pathway
GRB2
BCAP
SOS
RasGRP3
Ras
Raf
MEK1/2
Erk
AP1
DNA
B cell ontogeny
Autoimmunity
Anergy
Immuno response
Ig production
GC formation
DAG
MAPK signaling pathway
Co-inhibitor
FcgRIIB
SHIP
PKCβ
CALMA1
BCL-10
MALT1
IKKα
IKKγ
IKKβ
NFκB
IκB
DNA
Co-stimulator
LEU13
CD81
CD19
CD21
CD19-21 complex
VAV
PI3K
PIP3
AKT
Survival
GSK3β
NF-κB signaling pathway
Ubiquitin mediated proteolysis
PI3K-Akt signaling pathway
Complement and coagulation cascade
04662 7/2/18
(c) Kanehisa Laboratories
G
KOPN46
KOPN61
KOPN70
KOPN71
YAMN96
KOS20
P30/OHK
λ5
Igμ
cell number
pre-BCR
staining with the antibodies
staining with isotype control

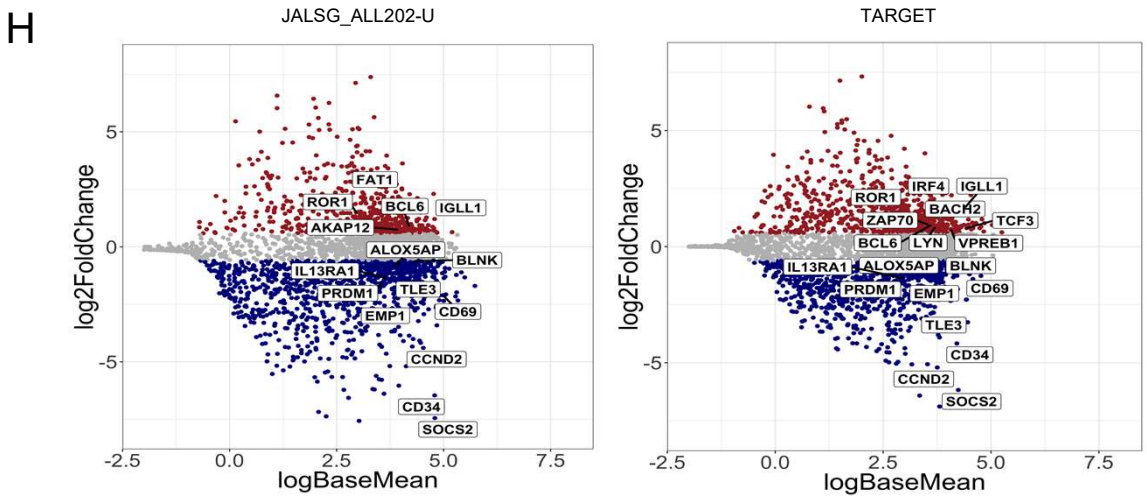


Figure S1. Generation of genome-edited Kasumi-7 cells, characterization of MEF2D-fusion-bound regions, analysis of pre-BCR expression in MEF2D-ALL cells, and analysis of pre-BCR-associated gene expression in clinical samples

(A) Schematic drawing of the 3'-end of the MEF2D-HNRNPUL1 fusion gene coding sequence in genome-edited Kasumi-7 (K7-HA-GFP) cells (upper panel). The 3'-end HA-tag enables identification of the fusion protein using an anti-HA antibody (lower panel). GFP, which is expressed from the 2A sequence, enables fluorescent identification of the tagged cells.

(B) Representative clones generated by genome-editing. Three independent clones were subjected to a western blot analysis with an HA-specific antibody. In Clone 1, both the MEF2D-HNRNPUL1 fusion (arrowhead) and non-rearranged HNRNPUL1 allele (arrows) were HA-tagged. In Clone 2, only the MEF2D-HNRNPUL1 fusion was HA-tagged. In clone 3, only the non-rearranged HNRNPUL1 allele was HA-tagged. Clone 2 was used throughout the remainder of the study. *: Non-specific bands.

(C) RT-PCR analysis demonstrating that in clone 2 from B, the MEF2D-HNRNPUL1 fusion was HA-tagged, whereas the non-rearranged HNRNPUL1 allele was not tagged. Kasumi-7 parent, bulk genome-edited, and K7-HA-GFP clone 2 cells were subjected to an RT-PCR analysis to determine the expression of transcripts encoding MEF2D and GFP, as well as HNRNPUL1 and GFP. For the latter, primer sets designed to amplify the transcript encompassing non-rearranged HNRNPUL1 and GFP were used.

(D) Proportion of genomic regions occupied by the MEF2D-HNRNPUL1 fusion protein in K7-HA-GFP cells (upper panel). Schematic drawing of the most enriched motif in the MEF2D-HNRNPUL1-bound regions (lower panel).

(E) ChIP-qPCR analysis of MEF2D-HNRNPUL1 occupancy in the genomic regions near the transcription start sites of the indicated genes in K7-HA-GFP cells. The fold-increases in DNA precipitated by an anti-HA antibody were compared with the DNA precipitated by normal IgG and are shown as means ± standard deviations (n=3).

(F) KEGG B cell receptor signaling pathway. Genes assigned to super-enhancers associated with MEF2D-HNRNPUL1 occupancy in K7-HA-GFP cells are shown in red.

(G) Pre-BCR expression in the indicated MEF2D-ALL cell lines was detected using dual-staining for Igμ and λ5 (upper panel) and single-staining for the pre-BCR complex (lower panel).

(H) Expression of genes associated with pre-BCR-positive versus -negative BCP-ALL in two clinical cohorts. MEF2D-ALL and non-MEF2D-ALL samples were compared (PBX1-rearranged samples were excluded), and the x-axis and y-axis represent the mean expression levels and log2-fold-change values (MEF2D-ALL/non-MEF2D-ALL) calculated using DESeq2, respectively.

Figure S2

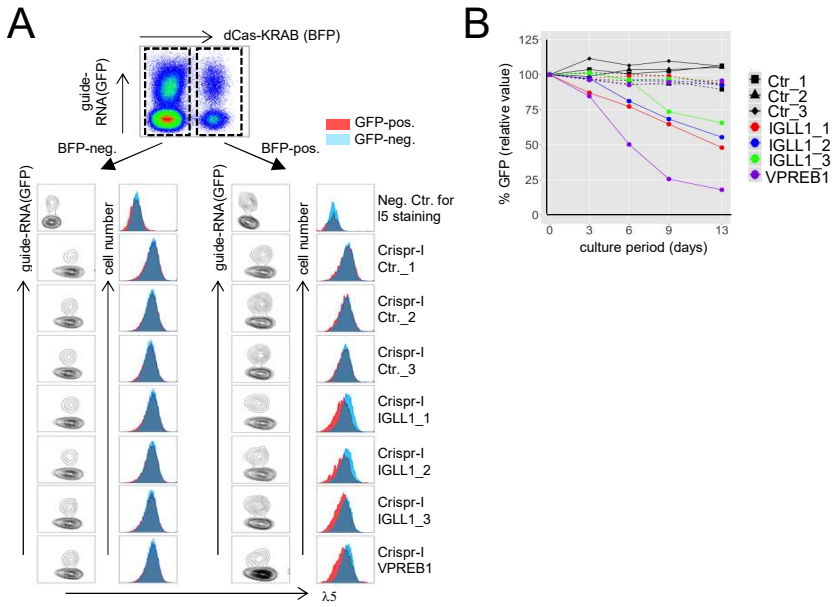


Figure S2. Crispr-l-mediated hindrance of enhances, involving MEF2D-HNRNPUL1-bound regions near TSSs of IGLL1 and VPREB1 genes, reduces pre-BCR expression and attenuates Kasumi-7 cell growth.

(A) Kasumi-7 cells were infected with lentiviruses engineered to co-express dCas-KRAB and BFP, as well as lentiviruses engineered to co-express guide-RNA and GFP. The cells were then subjected to flow cytometry to determine the expression of BFP, GFP, and pre-BCR. Pre-BCR expression was not altered in BFP-negative cell fractions, regardless of GFP expression. In BFP-positive cell fractions, pre-BCR expression was attenuated only in GFP-positive cells infected with the guide-RNA lentivirus that targeted enhancers of IGLL1 and VPREB1. Infection with a lentivirus encoding control guide RNA had no effects.

(B) The %GFP values in BFP+ cells are presented as relative values over the indicated culture period. Impedance of the IGLL1 and VPREB1 enhancers led to impaired cell growth.

Figure S3

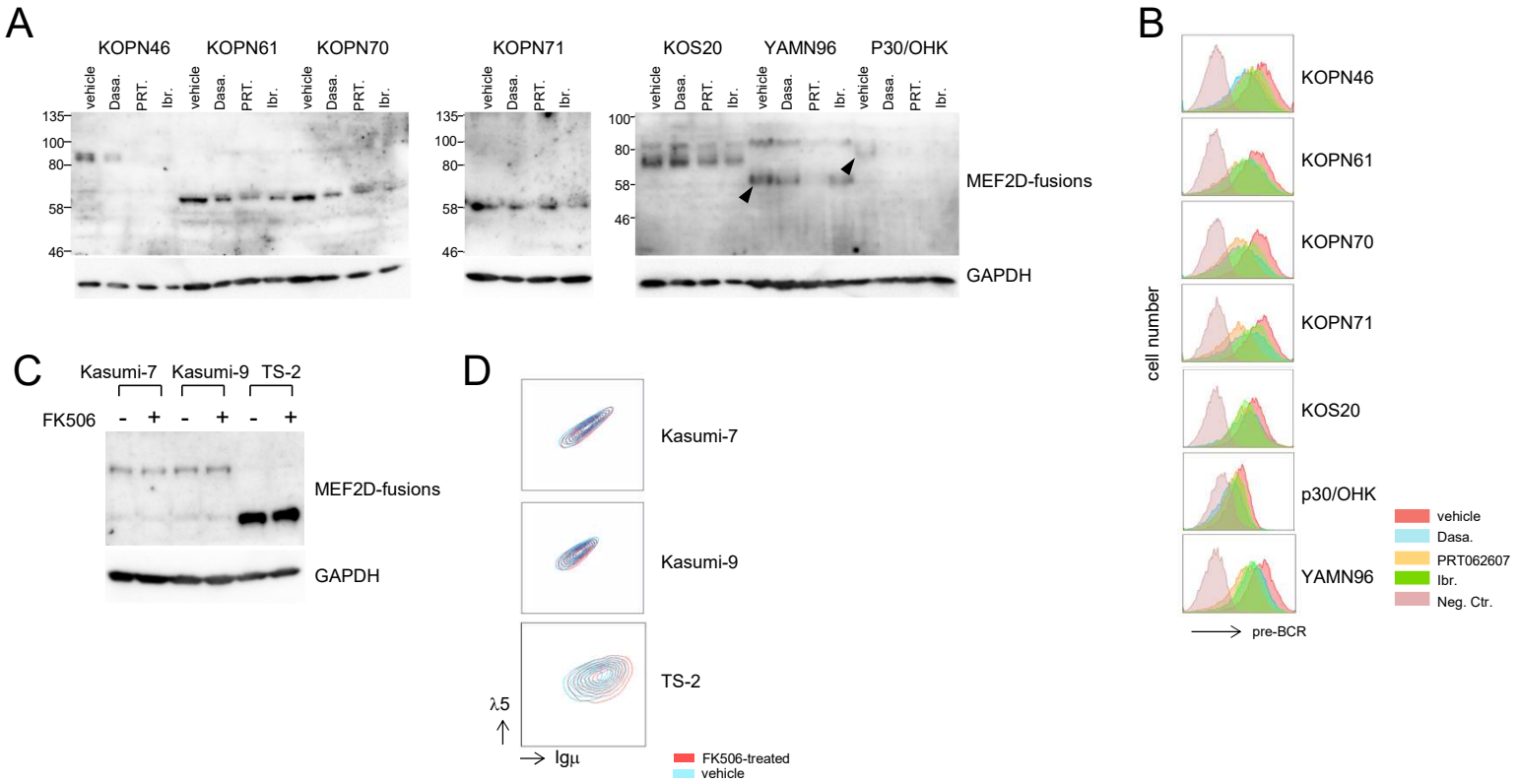


Figure S3. Effects of drug on the expression of MEF2D-fusion protein and pre-BCR

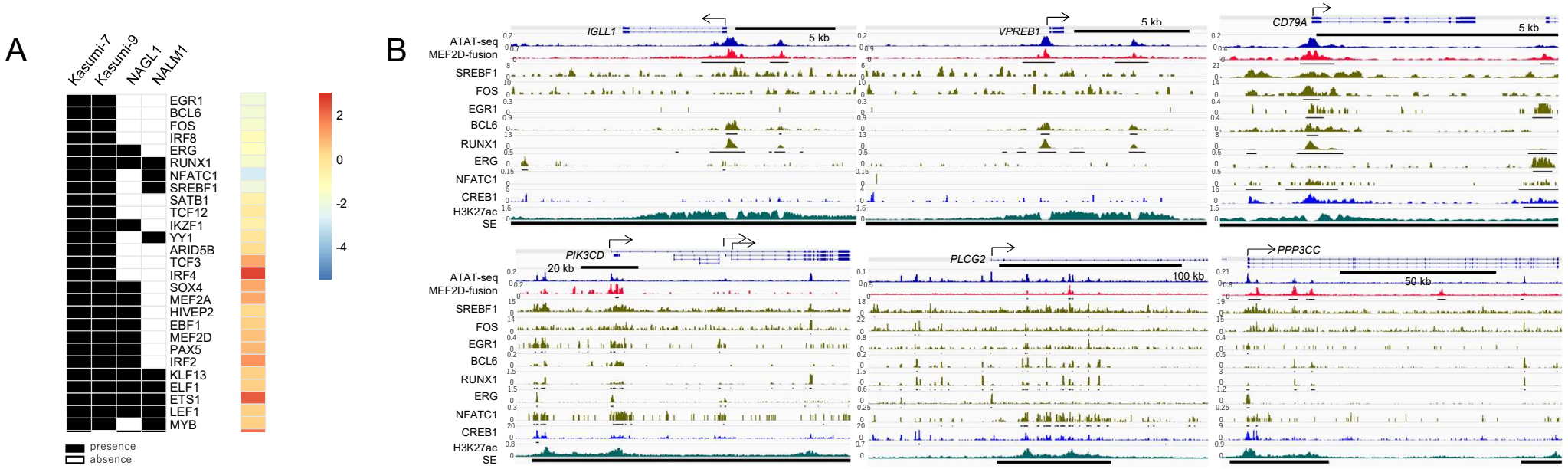
(A) Expression of the MEF2D-fusion protein in the indicated MEF2D-ALL cell lines after treatment with dasatinib, PRT062607, and ibrutinib as in Figure 3F.

(B) Expression of pre-BCR on the indicated MEF2D-ALL cell lines after treatment with dasatinib, PRT062607, and ibrutinib as in Figure 3G.

(C) Insensitivity of MEF2D-fusion protein expression to FK506 treatment (100 nM, 3 days) in MEF2D-ALL Kasumi-7, Kasumi-9, and TS-2 cells.

(D) Insensitivity of pre-BCR expression to FK506 treatment (100 nM, 3 days) in Kasumi-7, Kasumi-9, and TS-2 cells.

Figure S4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NALM1
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FOS
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RUNX1
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SREBF1
SATB1
TCF12
IKZF1
YY1
ARID5B
TCF3
IRF4
SOX4
MEF2A
HIVEP2
EBF1
MEF2D
PAX5
IRF2
KLF13
ELF1
ETS1
LEF1
MYB
2
0
-2
-4
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absence
B
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MEF2D-fusion
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ERG
NFATC1
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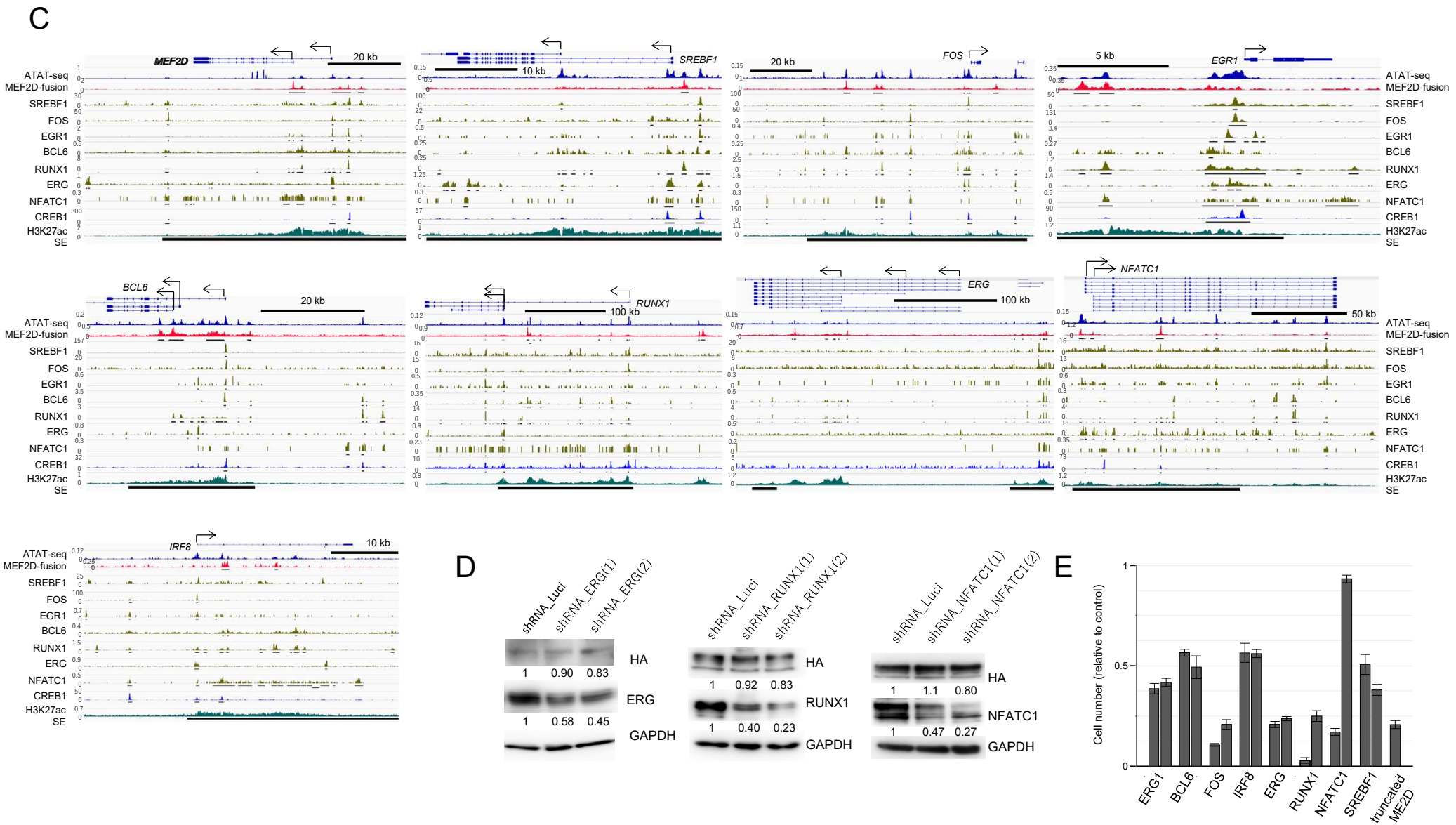
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CREB1
H3K27ac
SE
D
shRNA_Luci
shRNA_ERG(1)
shRNA_ERG(2)
HA
ERG
GAPDH
1 0.90 0.83
1 0.58 0.45
shRNA_RUNX1(1)
shRNA_RUNX1(2)
RUNX1
1 0.92 0.83
1 0.40 0.23
shRNA_NFATC1(1)
shRNA_NFATC1(2)
NFATC1
1 1.1 0.80
1 0.47 0.27
E
Cell number (relative to control)
1
0.5
0
ERG1
BCL6
FOS
IRF8
ERG
RUNX1
NFATC1
SREBF1
truncated ME2D

Figure S4. CRC constituent candidates and occupancy of the selected TFs near TSSs of genes; and influence of the knockdown of the candidates on MEF2D-fusion expression and Kasumi-7 cell growth

(A) Presence and absence of candidate CRC constituents, common to the Kasumi-7 and -9 cell lines, in the indicated cell lines. Changes in expression of the indicated candidate genes (log2 fold-change) in K7-HA-GFP cells, following the knockdown of MEF2D-HNRNPUL1 as in Figure 2E, are presented as a heatmap.

(B) Occupancy of MEF2D-HNRNPUL1 and ATAC-seq and H3K27ac ChIP-seq signals detected around the transcription start sites (TSSs) of IGLL1, VPREB1, CD79A, PIK3CD, PLCG2, and PPP3CC in K7-HA-GFP cells. SREBF1, FOS, EGR1, NFATC1 and CREB ChIP-seq signals in GM12878 B lymphoid cells and BCL6 (pre-B lymphocytes, GSM1438986), RUNX1 (pre-B lymphocytes, GSM1032075), and ERG (CD34+ immature blood cells, GSM585604) ChIP-seq signals are also shown. ChIP peaks and super-enhancers (SEs) are indicated by black lines.

(C) Occupancy of MEF2D-HNRNPUL1 and ATAC-seq and H3K27ac ChIP-seq signals detected around the TSSs of MEF2D, SREBF1, FOS, EGR1, BCL6, RUNX1, ERG, NFATC1, and IRF8 in K7-HA-GFP cells. SREBF1, FOS, EGR1, BCL6, RUNX1, ERG, NFATC1, and CREB ChIP-seq signals are also shown as in (B). ChIP peaks and SEs are indicated by black lines.

(D) MEF2D-HNRNPUL1 protein expression exhibited low sensitivity to the knockdown of ERG, RUNX1, and NFATC1. Relative expression levels are presented as in Fig. 4B.

(E) Effects of the knockdown of indicated genes on K7-HA-GFP cell growth over a 3-days culture period. Relative cell counts were calculated via comparison with the counts of control shRNA-infected cells (mean $\pm$ standard deviation; n=3).

Figure S5

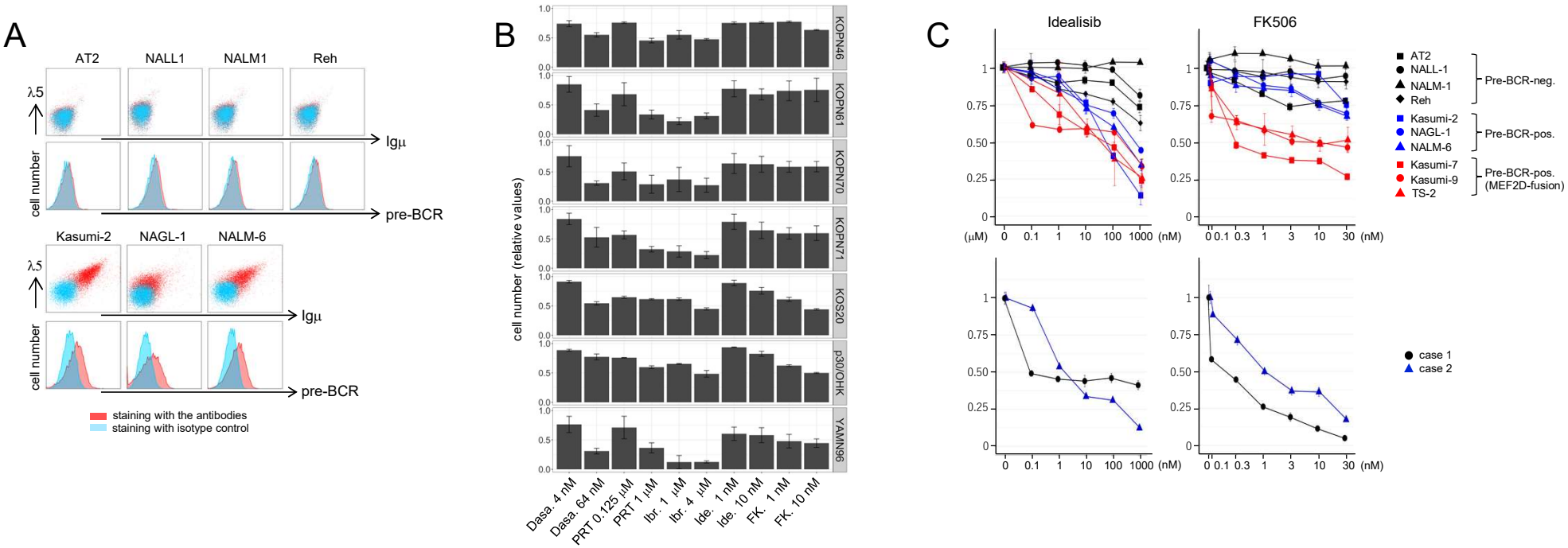


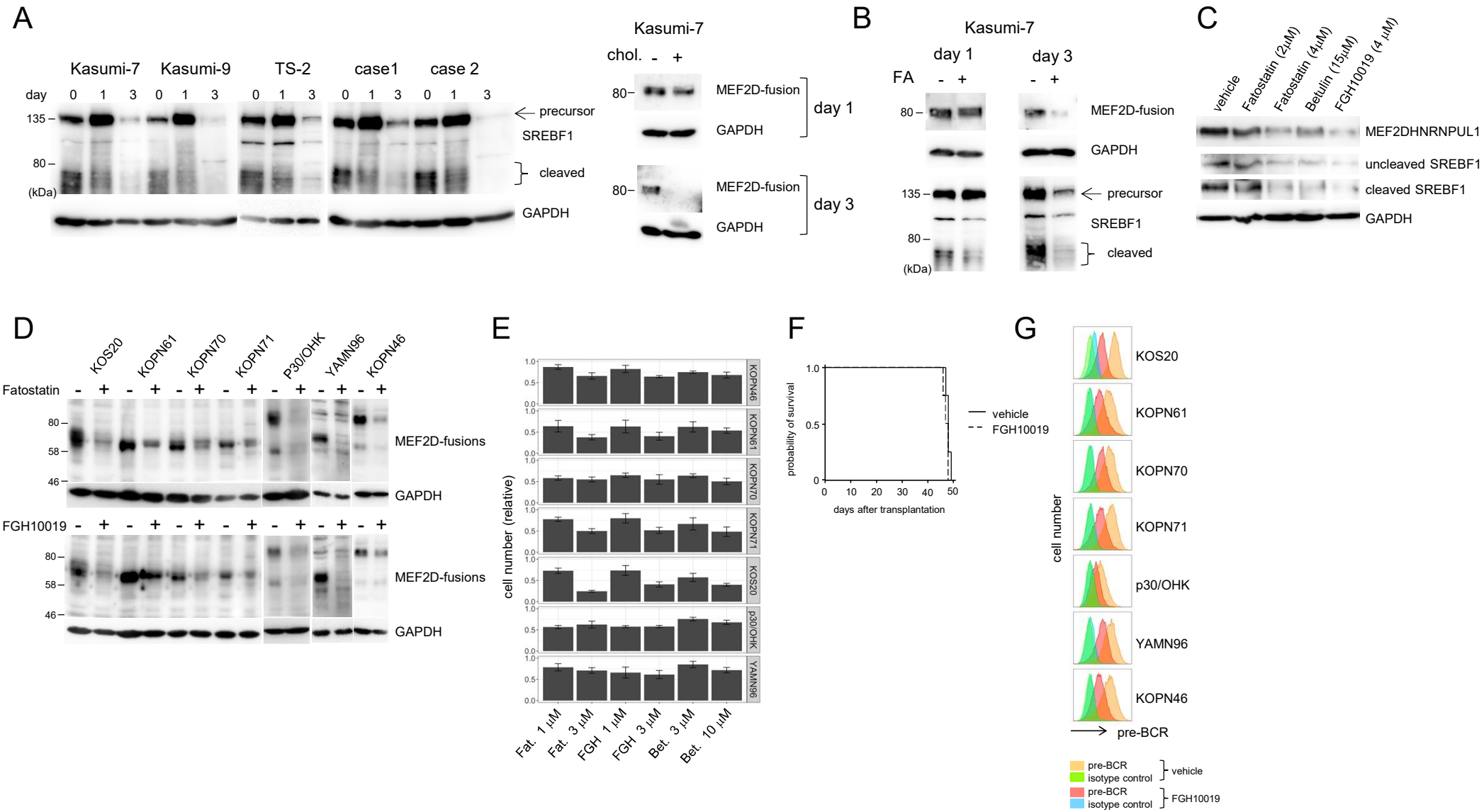
Figure S5. Pre-BCR expression of cells and the sensitivity of MEF2D-ALL cells to drugs

(A) Expression of pre-BCR in the indicated non-MEF2D-ALL cell lines as in Fig. 1D. AT2, NALL1, NALM1, and Reh cells were pre-BCR-negative, while Kasumi-2, NAGL1, and NALM-6 cells were pre-BCR-positive.

(B) The sensitivity of the indicated MEF2D-ALL cell lines in response to the indicated concentrations of pre-BCR signaling inhibitors was tested. Cell growth is presented relative to that of the vehicle control over a 3-day culture period (means ± SD; n=3). Dasa: dasatinib, PRT: PRT062607, Ibr: ibrutinib, ide: idelalisib, FK: FK506.

(C) The sensitivity of 10 cell lines (upper panel: four pre-BCR-negative ALL, three pre-BCR-positive non-MEF2D-ALL, and three MEF2D-ALL) and two clinical MEF2D-ALL primary cell lines (lower panel) for the indicated inhibitors was tested by analyzing growth inhibition. Cells were treated with varying concentrations of the inhibitors as indicated for 3 days (n=3). Cell growth is presented relative to that of vehicle-treated control cells [means ± standard deviations (SD)].

Figure S6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Kasumi-9
TS-2
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case 2
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(kDa)
precursor
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cleaved
GAPDH
Kasumi-7
chol. - +
80
MEF2D-fusion
GAPDH
day 1
day 3
B
Kasumi-7
day 1
day 3
FA - + - +
MEF2D-fusion
GAPDH
precursor
SREBF1
cleaved
C
vehicle
Fatostatin (2μM)
Fatostatin (4μM)
Betulin (15μM)
FGH10019 (4 μM)
MEF2DHNRNPUL1
uncleaved SREBF1
cleaved SREBF1
GAPDH
D
KOS20
KOPN61
KOPN70
KOPN71
P30/OHK
YAMN96
KOPN46
Fatostatin - + - + - + - + - + - + - +
80
58
46
MEF2D-fusions
GAPDH
FGH10019 - + - + - + - + - + - + - +
E
KOPN46
KOPN61
KOPN70
KOPN71
KOS20
p30/OHK
YAMN96
cell number (relative)
Fat. 1 μM
Fat. 3 μM
FGH 1 μM
FGH 3 μM
Bet. 3 μM
Bet. 10 μM
F
probability of survival
days after transplantation
vehicle
FGH10019
G
cell number
KOS20
KOPN61
KOPN70
KOPN71
p30/OHK
YAMN96
KOPN46
pre-BCR
pre-BCR
isotype control
vehicle
pre-BCR
isotype control
FGH10019

Figure S6. Effects of lipids and inhibitors of SREBF1 activation on the expression of MEF2D-fusion protein

(A, B) Incubation of MEF2D-ALL cells with cholesterol or fatty acids inhibits SREBF1 activation and diminishes SREBF1 protein expression. (A) Incubation with cholesterol (10 μg/ml cholesterol and 2 μg/ml 25-hydroxycholesterol) for 1 day reduced the cleavage of SREBF1, leading to a concomitant increase in the level of uncleaved SREBF1 protein. However, the levels of both cleaved and uncleaved SREBF1 were reduced after a 3-day incubation period (left). The decrease in the MEF2D-fusion protein level was marginal during a 1-day incubation (right) but became apparent over a 3-day period. (B) Supplementation of Kasumi-7 cells with fatty acid (0.1% fatty acid supplement in culture) inhibited the activation of SREBF1 but affected the expression of MEF2D-fusion protein only marginally after 1 day. However, the expression of both SREBF1 and MEF2D-fusion proteins was reduced after a 3-day incubation.

(C) Treatment of Kasumi-7 cells with the indicated drugs reduced cleaved and uncleaved SREBF1 as well as the expression of MEF2D-HNRNPUL1. Cells were treated for 3 days with the indicated concentrations of drugs and subjected to western blotting with antibodies specific for MED2D and SREBF1. GAPDH was analyzed as a loading control.

(D) Treatment of the indicated MEF2D-ALL cells with fatostatin and FGH10019 (3 μM, +) or vehicle (-) for 3 days reduced the expression of MEF2D-fusion protein.

(E) Effects of fatostatin (Fat), FGH10019 (FGH), and betulin (Bet) at the indicated concentrations on the growth of the indicated cells over a 3-day culture period (n=3). The values were calculated relative to the control condition (mean ± standard deviation).

(F) FGH10019 treatment (25 mg/kg/day, 6 days per week) did not affect the survival durations of mice transplanted with non-MEF2D-ALL Reh cells (n=8).

(G) Treatment with FGH10019 (3 μM, 3 days) reduced the expression of pre-BCR on the indicated MEF2D-ALL cells in culture.