Multi-omic Profiling of Central Nervous System Leukemia Identifies mRNA Translation as a Therapeutic Target

Vanner and Dobson et al.

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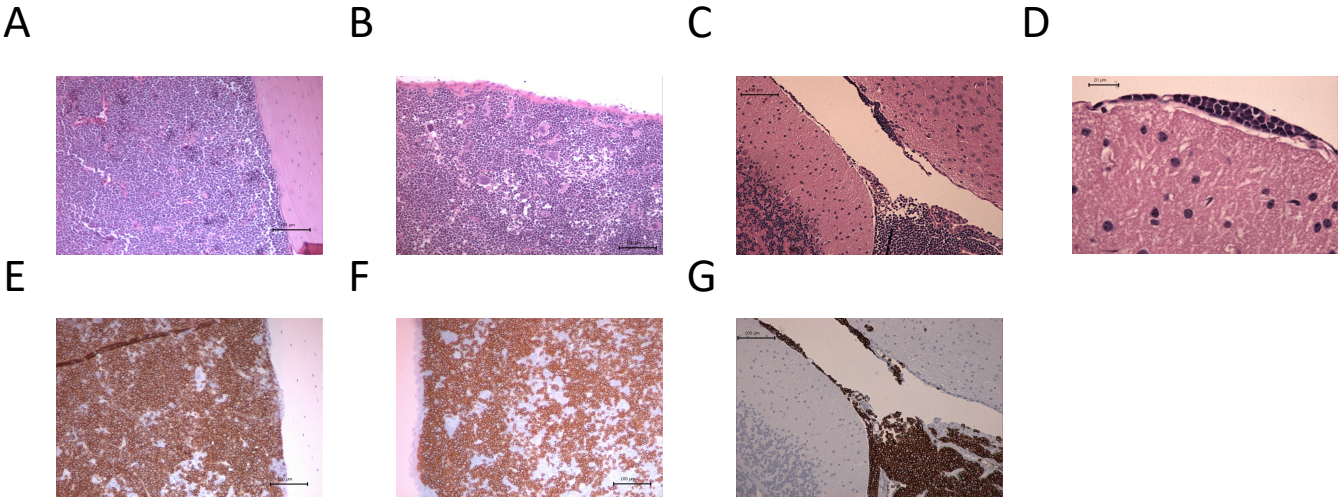
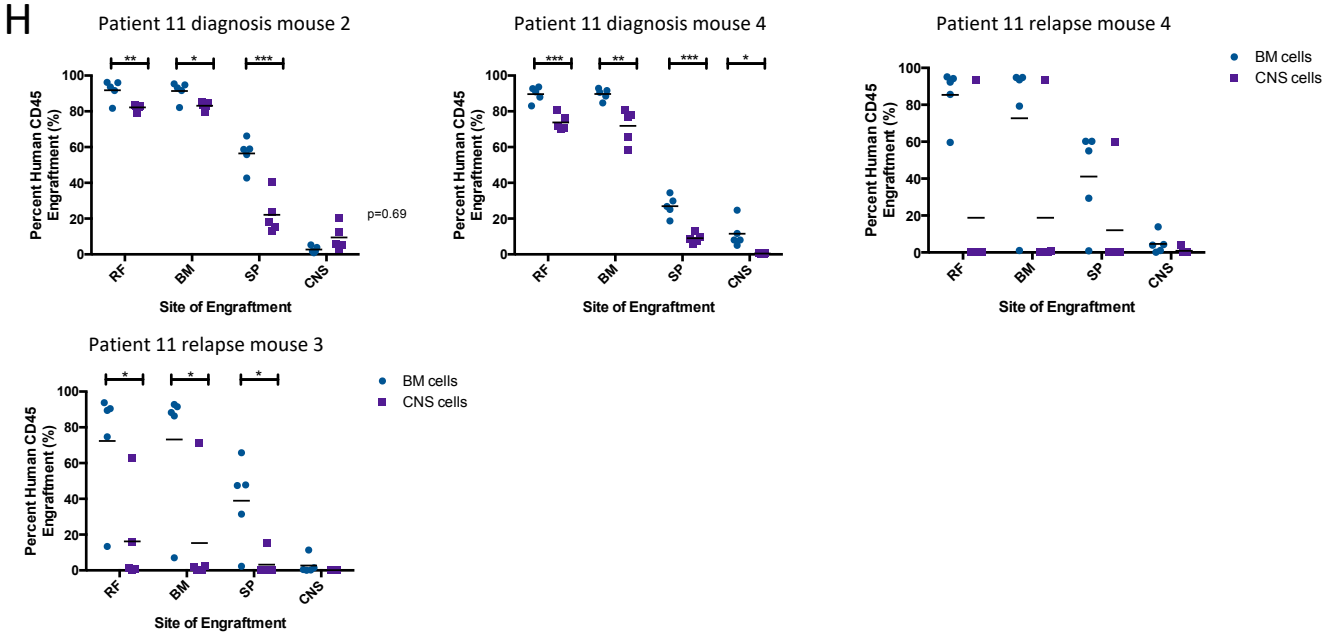
Supplementary Table S1: Clinical Characteristics of B-ALL Patient Samples.

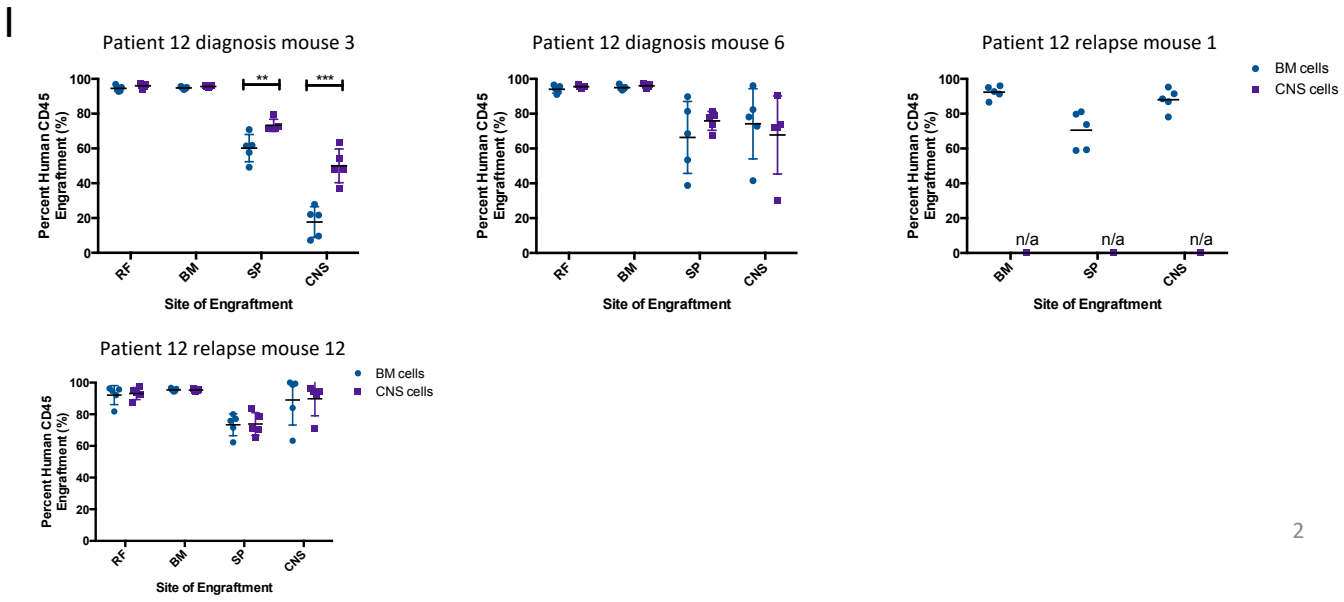
Supplementary Table S2: B-ALL Xenografts Used in Current Study.

Supplementary Table S3: Differentially abundant proteins as quantified by label-free liquid chromatography mass spectrometry in KMT2A-r B-ALL xenografts.

Supplementary Figure 1







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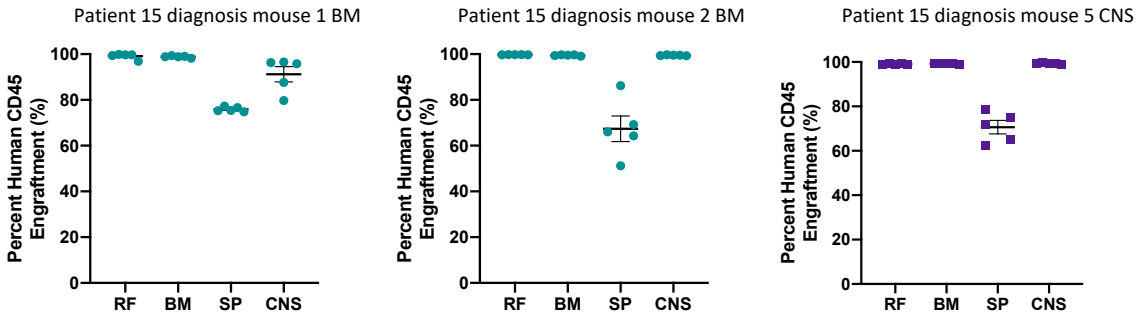
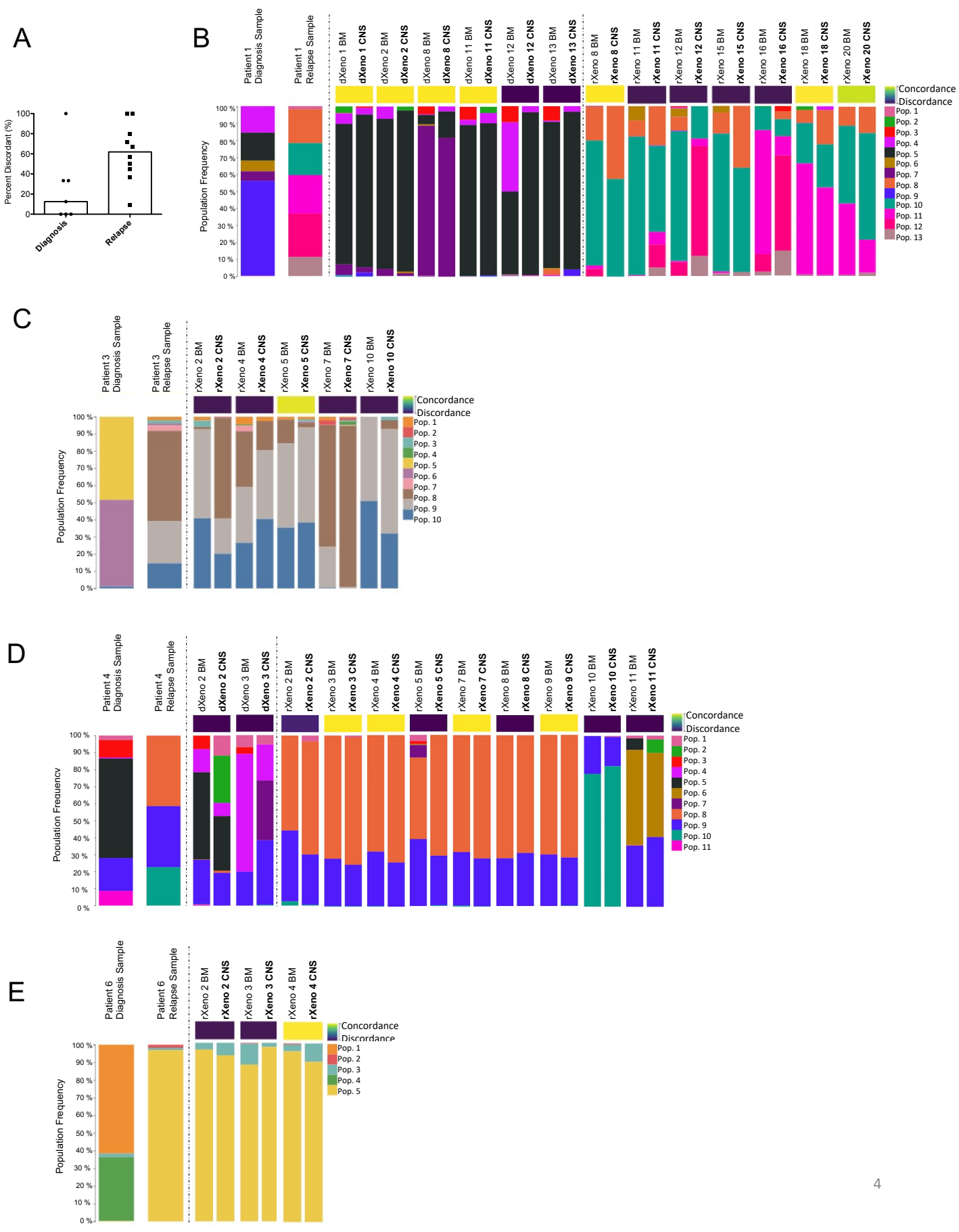
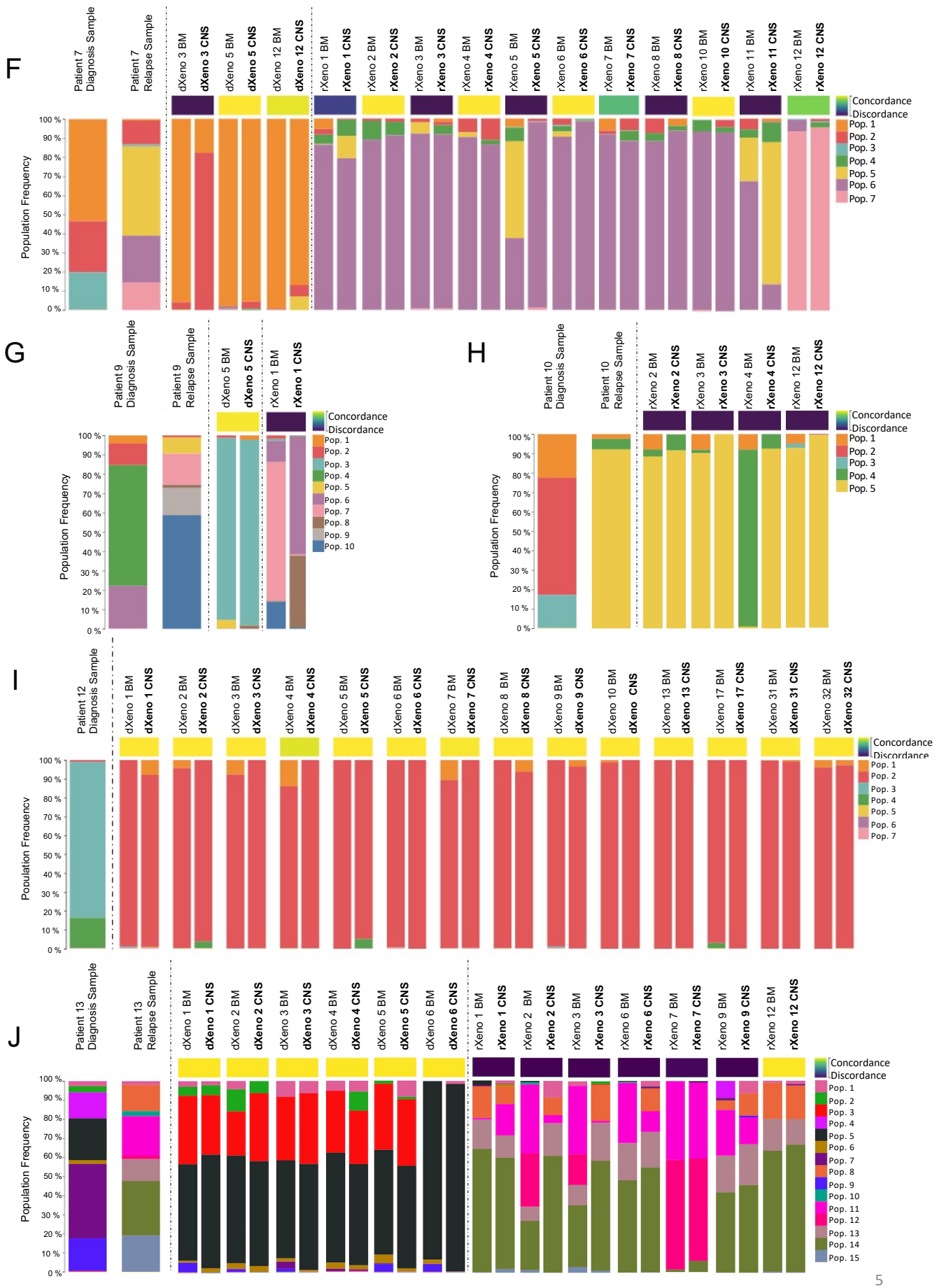


Supplementary Figure 1. Immunohistochemistry and secondary transplantation of B-ALL xenografts. Representative hematoxylin and eosin stained sections of bone marrow (A), spleen (B), and CNS (C and D) from patient 12 B-ALL xenograft with matched anti-human CD45 immunoperoxidase staining for bone marrow (E), spleen (F), and CNS (G). Scale bar represents 100 µm. BM (green) or CNS (purple) cells of primary xenografts from patient 11 (H) 12 (I), or 15 (J) were transplanted into secondary mice by intrafemoral injection, then mice were sacrificed 12 weeks post-transplantation, upon displaying symptoms, or upon emergence of blasts in peripheral blood (J) and the percentage of human leukemic blasts in each tissue was quantified by flow cytometry. Donor mice and or tissue are indicated in graph titles (cells injected: 1 x 10^5 per mouse in H,I; 2 x 10^5 per mouse in J).

Supplementary Figure 2




F
Patient 7 Diagnosis Sample
Patient 7 Relapse Sample
dXeno 3 BM
dXeno 3 CNS
dXeno 5 BM
dXeno 5 CNS
dXeno 12 BM
dXeno 12 CNS
rXeno 1 BM
rXeno 1 CNS
rXeno 2 BM
rXeno 2 CNS
rXeno 3 BM
rXeno 3 CNS
rXeno 4 BM
rXeno 4 CNS
rXeno 5 BM
rXeno 5 CNS
rXeno 6 BM
rXeno 6 CNS
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rXeno 7 CNS
rXeno 8 BM
rXeno 8 CNS
rXeno 10 BM
rXeno 10 CNS
rXeno 11 BM
rXeno 11 CNS
rXeno 12 BM
rXeno 12 CNS
Concordance
Discordance
Pop. 1
Pop. 2
Pop. 3
Pop. 4
Pop. 5
Pop. 6
Pop. 7
Population Frequency
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
G
Patient 9 Diagnosis Sample
Patient 9 Relapse Sample
dXeno 5 BM
dXeno 5 CNS
rXeno 1 BM
rXeno 1 CNS
Concordance
Discordance
Pop. 1
Pop. 2
Pop. 3
Pop. 4
Pop. 5
Pop. 6
Pop. 7
Pop. 8
Pop. 9
Pop. 10
Population Frequency
H
Patient 10 Diagnosis Sample
Patient 10 Relapse Sample
rXeno 2 BM
rXeno 2 CNS
rXeno 3 BM
rXeno 3 CNS
rXeno 4 BM
rXeno 4 CNS
rXeno 12 BM
rXeno 12 CNS
Concordance
Discordance
Pop. 1
Pop. 2
Pop. 3
Pop. 4
Pop. 5
Population Frequency
I
Patient 12 Diagnosis Sample
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dXeno 1 CNS
dXeno 2 BM
dXeno 2 CNS
dXeno 3 BM
dXeno 3 CNS
dXeno 4 BM
dXeno 4 CNS
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Concordance
Discordance
Pop. 1
Pop. 2
Pop. 3
Pop. 4
Pop. 5
Pop. 6
Pop. 7
Population Frequency
J
Patient 13 Diagnosis Sample
Patient 13 Relapse Sample
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dXeno 1 CNS
dXeno 2 BM
dXeno 2 CNS
dXeno 3 BM
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Concordance
Discordance
Pop. 1
Pop. 2
Pop. 3
Pop. 4
Pop. 5
Pop. 6
Pop. 7
Pop. 8
Pop. 9
Pop. 10
Pop. 11
Pop. 12
Pop. 13
Pop. 14
Pop. 15
Population Frequency

Supplementary Figure 2. Mutation population frequencies in B-ALL BM and CNS xenografts (A) Percentage of discordant xenografts per patient sample (diagnosis samples - patient 1,4,7,9, 11, 12 and 13; relapse - patient 1, 3, 4, 6, 7, 9, 10, 11, 12 and 13). (B-J) Mutational population frequencies of matched BM and CNS samples analyzed using Pairtree from patient 1 (B) (diagnosis, n=6 xenografts; relapse n=7 xenografts); patient 3 (C) (relapse, n=5 xenografts); patient 5 (D) (diagnosis, n=2 xenografts; relapse n=8 xenografts); patient 6 (E) (relapse, n=3 xenografts); patient 7 (F) (diagnosis, n=3 xenografts; relapse, n=11 xenografts); patient 9 (G) (diagnosis, n= 1 xenograft; relapse, n=1 xenograft); patient 10 (H) (relapse, n=4 xenografts); patient 12 diagnosis (I) (n=14 xenografts); patient 13 (J) (diagnosis, n= 6 xenografts; relapse, n=7 xenografts).

Supplementary Figure 3

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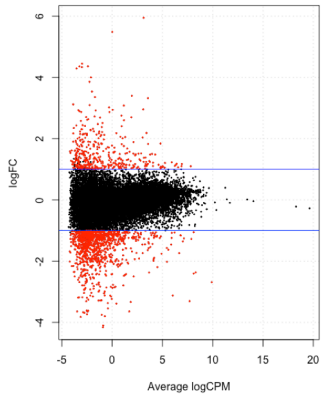

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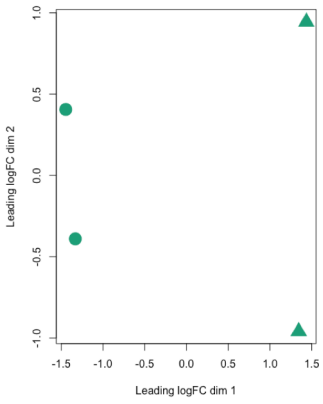

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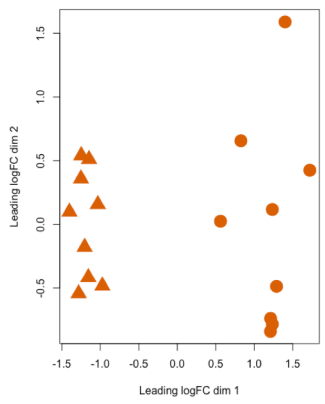

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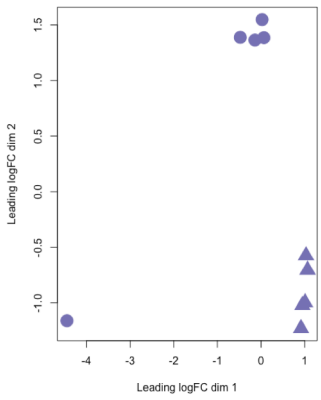

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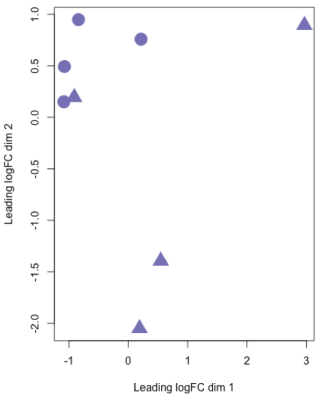

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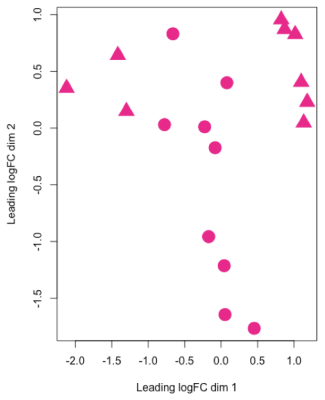

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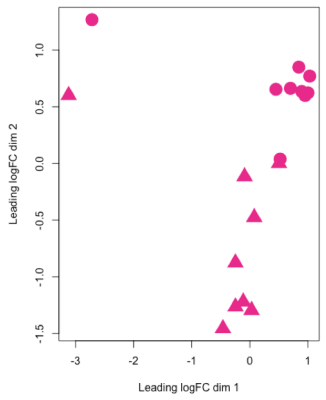

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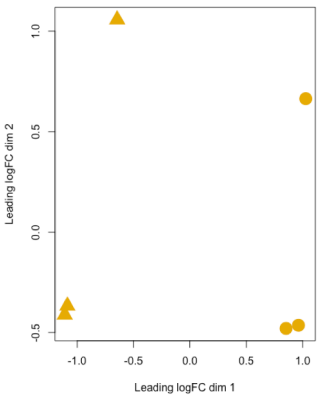
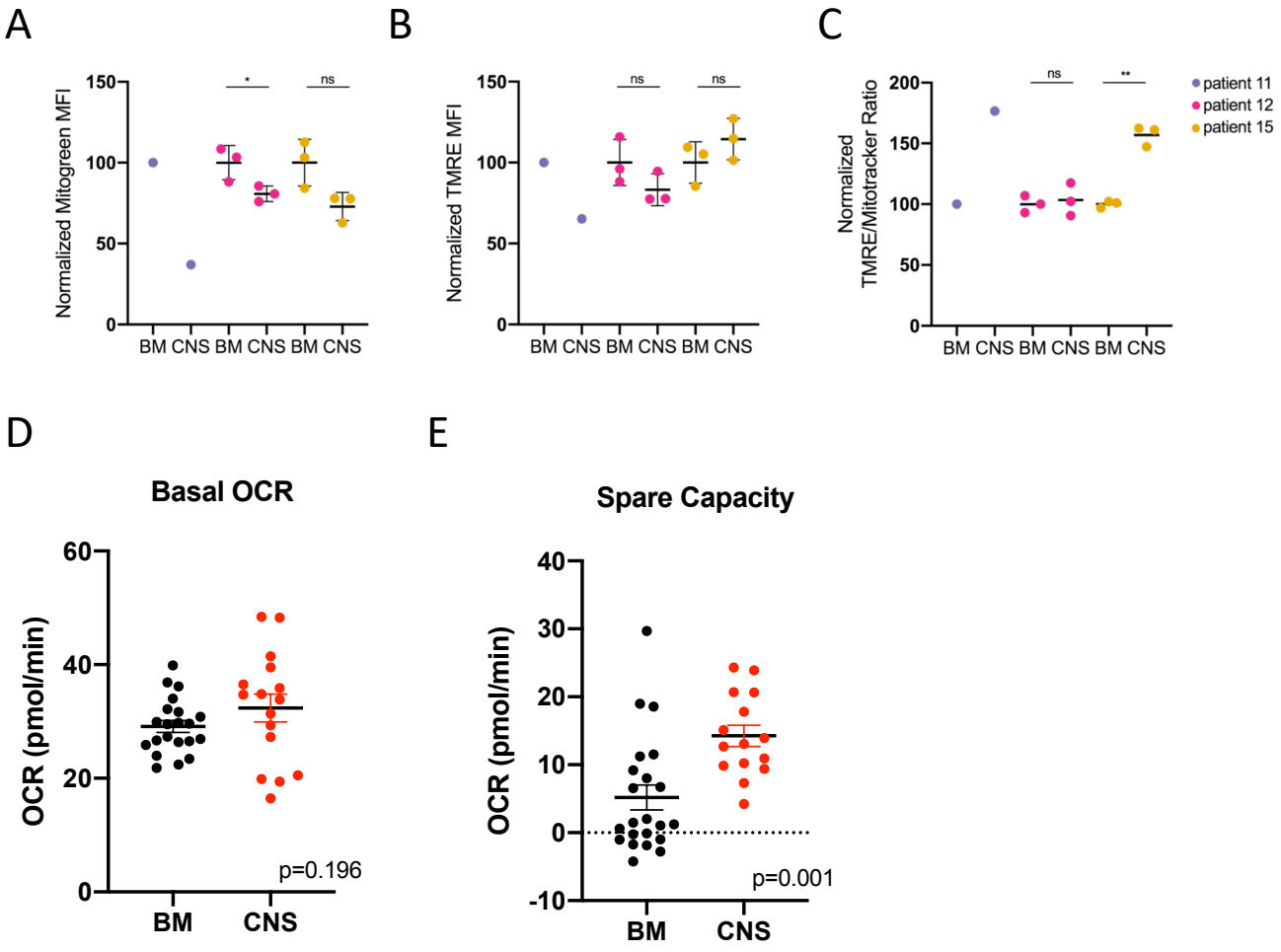


Supplementary Figure 3. Genes differentially expressed between B-ALL BM and CNS xenografts. (A) Differential gene expression using edgeR comparing n =43 BM (patient 6 n=2, patient 7 n=10, patient 11 n=9, patient 12 n=18, patient 13 n=1, and patient 15 n=3) and n=43 matched CNS blasts (patient 6 n=2, patient 7 n=10, patient 11 n=9, patient 12 n=18, patient 13 n=1, and patient 15 n=3) identified 381 CNS-upregulated and 1064 BM-upregulated genes with fold change >= 2 (blue lines) and $p < 0.05$ corrected for multiple-hypothesis testing (Each dot represents one gene with significantly differentially expressed genes in red). Multidimensional scaling of RNAseq gene counts from matched BM (cells from bilateral femurs and tibias) (circles) and CNS (triangles) xenografts derived from patient 6 relapse (B) (n=2 xenografts), patient 7 relapse (C) (n=9 xenografts), patient 11 diagnosis (D) (n=5 xenografts), patient 11 relapse (E) (n=4 xenografts), patient 12 diagnosis (F) (n=9 xenografts), patient 12 relapse (G) (n=9 xenografts) and patient 15 diagnosis (H) (n=3 xenografts).

Supplementary Figure 4



Supplementary Figure 4. Metabolic activity in BM and CNS of B-ALL xenografts. (A) Mitochondrial mass measured by mean fluorescence intensity (MFI) of Mitotracker Green, (B) Mitochondrial membrane potential represented by MFI of TMRE, (C) Mitochondrial membrane potential per unit mass with ratio of TMRE MFI/Mitotracker Green MFI in BM and CNS xenografts (n=1 patient 11, n=3 patient 12, n=3 patient 15, ns = not significant and * = $p < 0.05$ Student's two-sided unpaired t-test). Basal oxygen consumption rate (OCR) (D) and mitochondrial spare capacity (E) were quantified using the Seahorse XF analyzer in KMT2A-rearranged diagnosis BM (n=8 mice, n=22 replicate wells) and CNS (n=6 mice, n=15 replicate wells) blasts. Bars represent mean ± standard error. P values derived from Student's two-sided unpaired t-test.

Supplementary Figure 5

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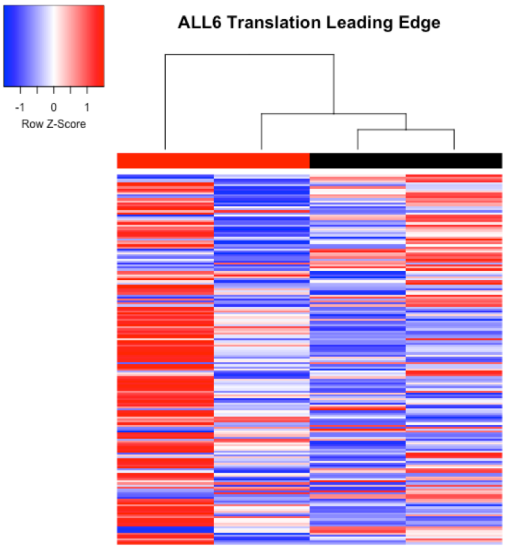

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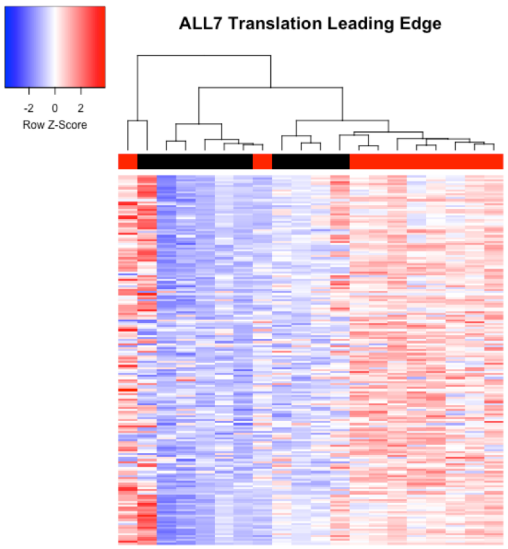

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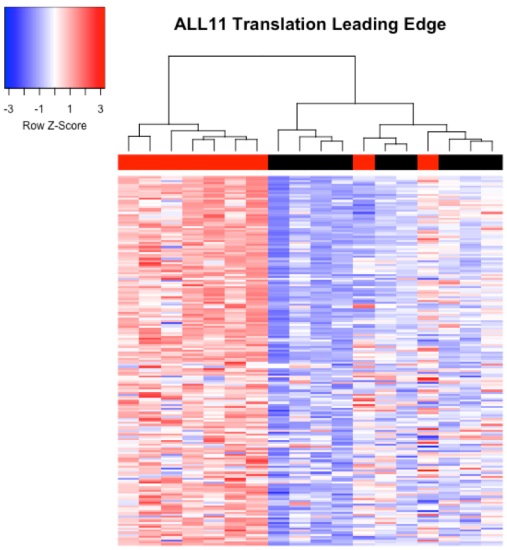

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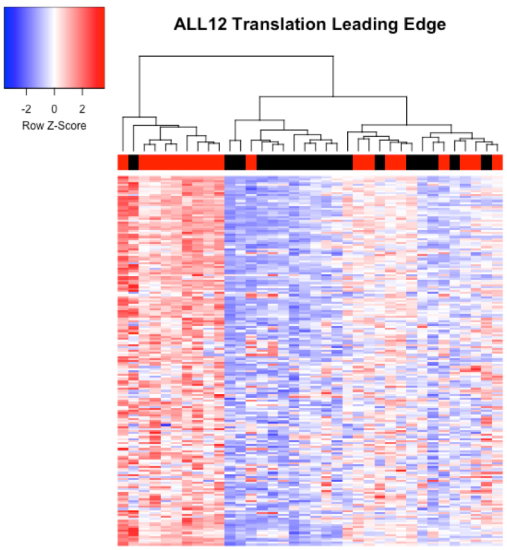

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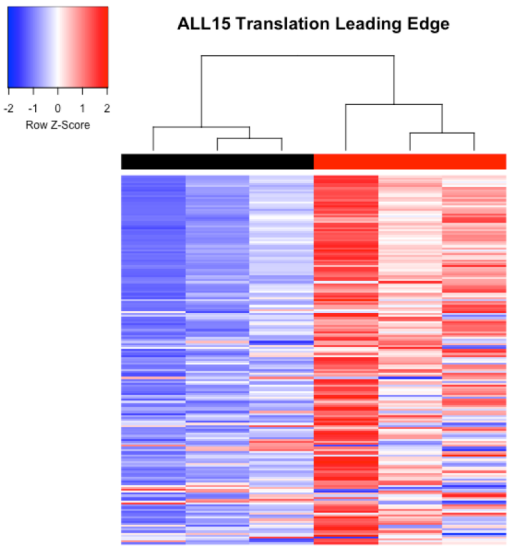

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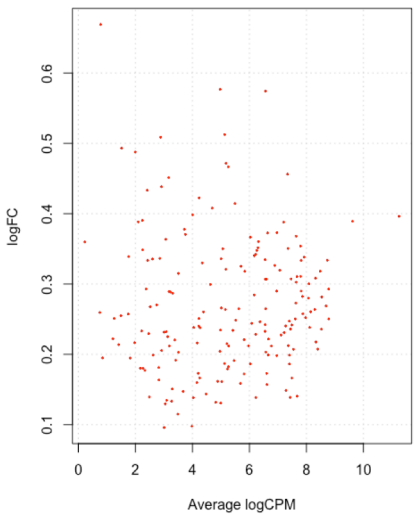

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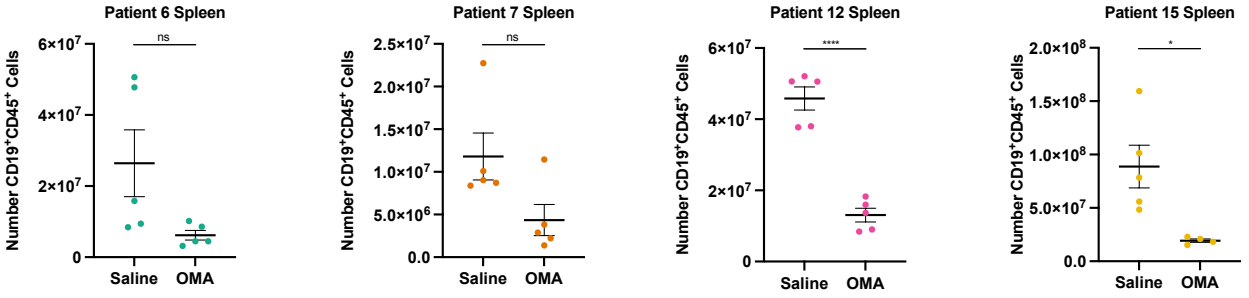

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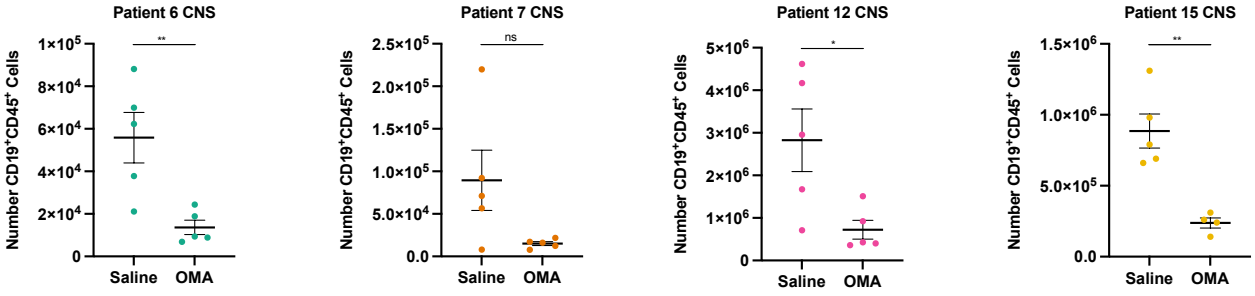
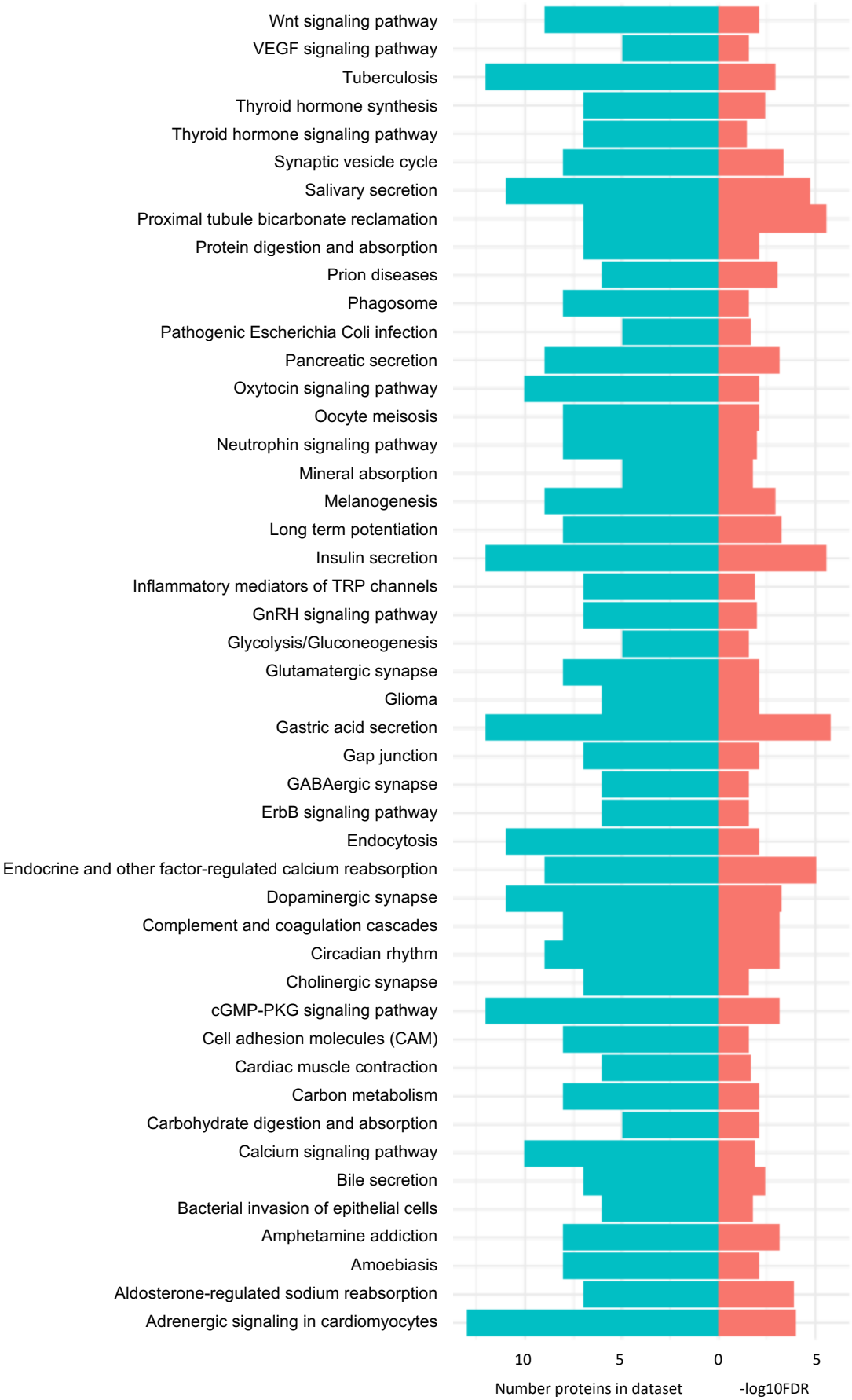
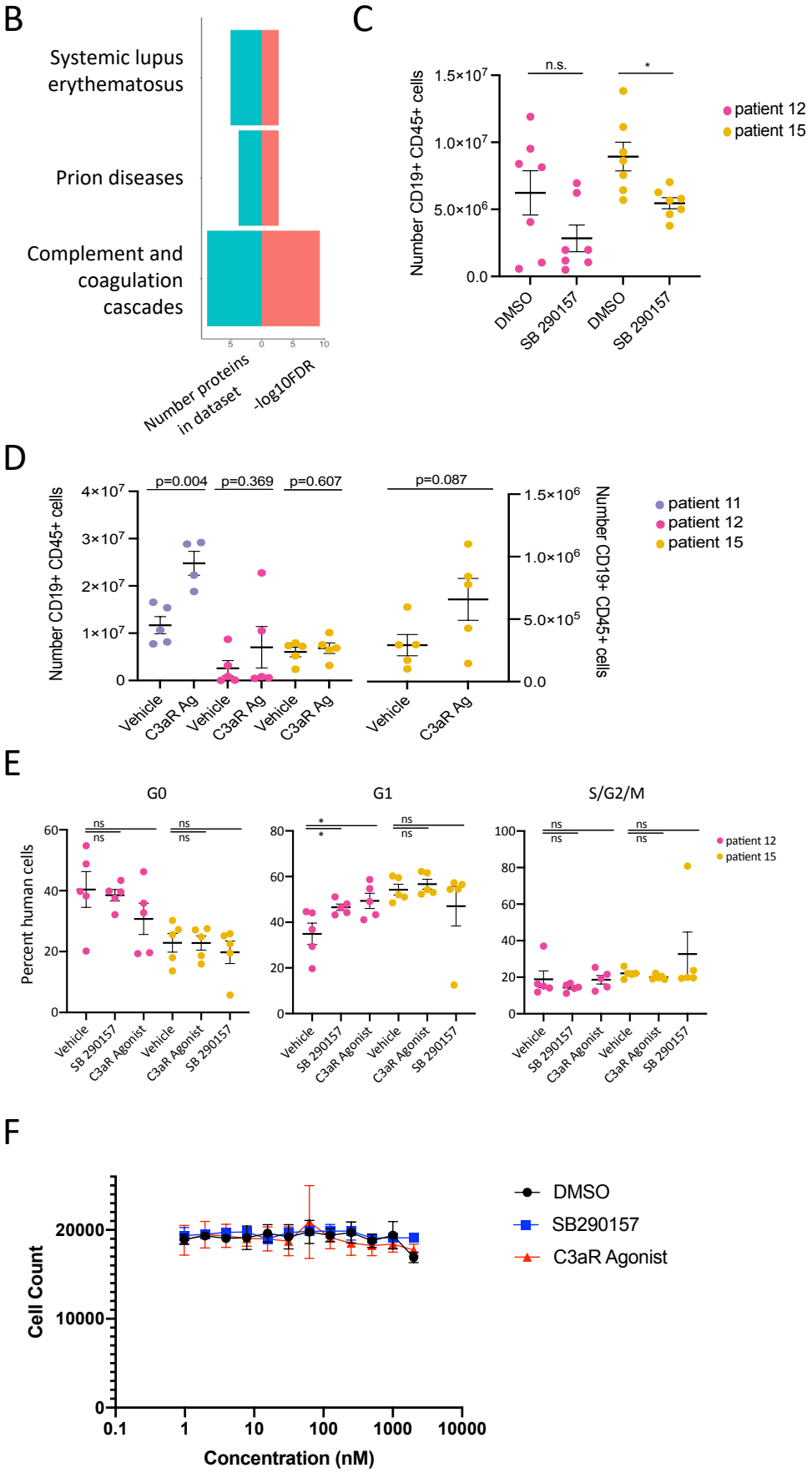


Supplementary Figure 5. Transcriptional upregulation and therapeutic targeting of mRNA translation in CNS blasts. Unsupervised hierarchical clustering of normalized gene counts from the leading edge genes of the CNS-enriched Reactome Translation geneset in (A) patient 6 (n= 2 mice), (B) patient 7 (n= 10 mice), (C) patient 11 (n= 9 mice), (D) patient 12 (n=18 mice), and (E) patient 15 (n= 3 mice) matched BM and CNS xenografts. (F) RNA-seq results displaying normalized gene counts per million in CNS blasts versus BM blasts of leading edge genes from CNS-enriched Reactome 'Translation' geneset in (A). logFC = log2fold change in CNS relative to BM, each dot represents one gene with red dots denoting differential expression with $p < 0.05$ after correction for multiple-hypothesis testing. Human leukemic cell counts for Spleen (G) and CNS (H) xenografts from saline and OMA treated mice as in Figure 4B and 4E. Bars represent mean ± standard error, p values derived from two-sided unpaired student's t-test (D-G) with * representing $p < 0.05$, ** $p<0.01$, **** $p \leq 0.0001$, ns = not significant.

Supplementary Figure 6

A




B
Systemic lupus erythematosus
Prion diseases
Complement and coagulation cascades
Number proteins in dataset
-log10FDR
C
Number CD19+ CD45+ cells
n.s.
*
patient 12
patient 15
DMSO
SB 290157
D
Number CD19+ CD45+ cells
p=0.004
p=0.369
p=0.607
p=0.087
patient 11
patient 12
patient 15
Vehicle
C3aR Ag
E
G0
G1
S/G2/M
Percent human cells
ns
patient 12
patient 15
Vehicle
SB 290157
C3aR Agonist
F
Cell Count
Concentration (nM)
DMSO
SB290157
C3aR Agonist

Supplementary Figure 6. Proteomic analysis and targeting complement signaling in B-ALL xenografts and in vitro cultures. STRING analysis of KEGG pathways upregulated in CNS proteome (A) and amongst the secreted ligands detected in CNS (B). Green bars represent the number of proteins in the pathway detected by mass spectrometry, pink bars represent the –log10 False Discovery Rate (FDR). Pathways are identified on the y axis. (C) Human leukemic cell counts for CNS xenografts from DMSO and SB 290157 treated mice as in Figure 5D and 5E. Bars represent mean ± standard error, p values derived from two-sided unpaired student's t-test. with * representing $p < 0.05$, ns = not significant. (D) Human leukemic cell counts for CNS xenografts from DMSO and C3a receptor agonist treated mice as in Figure 5F and 5G. Bars represent mean ± standard error, p values derived from two-sided unpaired student's t-test. with * representing $p < 0.05$, ** $p < 0.01$, **** $p \leq 0.0001$, ns = not significant. (E) Cell cycle analysis of human CD45+ CNS xenografts from mice treated with vehicle, SB 290157, or C3aR agonist as in Figure 5H (G0= Hoechst 2n, Ki-67-, G1= Hoechst 2n, Ki-67+, S/G2/M= Hoechst 2n-4n, Ki-67+). * = $p < 0.05$, ns= not significant, derived from student's unpaired-t test. Error bars represent mean ± standard error. (F) Number of viable RS4;11 cells per well 72 hours after treating 5000 cells with DMSO vehicle or a dilution series of SB 290157 or C3a receptor agonist (C3aR Agonist). Each point represents the mean ± standard error. Data from one of three representative experiments is shown.