Supplemental Figure S6 C **ELAVL1 knockout RN2c** RN2c GAL LSC DOWN BROWN MYELOID CELL **DAPI** DEVELOPMENT **DAPI Enrichment Score** NES 1.71 **NES 1.62** FDR 0.002 FDR 0.028 **Primary AML** В LinbcCML MLL-AF9 37 kDa D Down G3BP1 51 G3BP1 Elavl1 15 Tmem250-ps Bound Tuba8 1392 Ptp4a3 137 Dazap1 161 Dist Up Gfi1b Tspan31 E RN2c Мус Gpr56 GO_MRNA_SPLICE_SITE_ SELECTION Nup210 0.8 IRF7 expression (relative to ACTB) MYC expression (relative to ACTB) NRF1 expression (relative to ACTB) Tgfb1 FDR q-val: 0.194 ES Rpn1 H2afy 0.0 Spn DMSO MS444 DMSO MS444 DMSO MS444 F G Н Skipped Exons Reg. RNA splicing Bound Not Bound MAPK cascad 0.75 mRNA processing 0.50 Chromatin modification K-S test p=2.2e-16 0.25 RNA splicing Reg. of Transcription, DNA-dependent 0.00 0.0 0.1 0.2 % of genes in GO category/ -1.0 0.0 RNA-seq Rank Score IncDifference Level expressed genes in GO category phospholipid dephosphorylation **ELAVL1** knockdown primary AML • GAL LSC DOWN BROWN MYELOID CELL DEVELOPMENT myc transcriptio, validated targets migration chemotaxis transforming **Enrichment Score** regulation growth beta NES 1.21 NES 1.48 mediated FDR 0.106 FDR 0.080 import ••• neutrophil anslation amide hematopoletic lymphoid organ biosynthetic mediated immunity substance signal extracellula autophagy autophagosom organization homeostasis cellular metal sterol rhodopsin stimulus ligand binding mechanical • extrinsic transcribed epithelium triglyceride apoptotic death receptors mirnas involvement sepsis nthi process differen developmental • Border colour Concordant with BeatAML Discordant with BeatAML Not in BeatAML

Supplemental Figure S6: Characterization of ELAVL1 targets and global influence on the transcriptional landscape. (A) GSEA plots showing LSC and myeloid signature enrichments in the Elavl1-knockout RN2c transcriptome. (B) Western blot of Elavl1 in murine Lin-, bcCML and MLL-AF9 cells. (C) Immunofluorescent microscopy of ELAVL1, nuclear marker (DAPI) and cytoplasmic marker (G3BP1). Scale bars of 20μM are shown. (D) Summary of intersection of transcripts bound (grey) and differentially expressed (up=red, down=blue, p. adj <0.05) upon Elavl1-deletion. (E) qPCR of ELAVL1 targets in RN2c 24-72h post-treatment with 2.2uM MS-444 or vehicle control (DMSO). (F) Expression distribution of bound and not bound transcripts in Elavl1-knockout RN2c cells. (G) GO term enrichment analysis for transcripts both bound and differentially expressed (p adj <0.05) upon Elavl1deletion. (H) Volcano plot of differential exon skipping in ELAVL1 knockout RN2c cells, >15% inclusion and exclusion at FDR<0.1 indicated in red and green respectively. (I) GSEA plots of LSC and myeloid signature enrichments in AML. (J) Enrichment map of pathways significantly (FDR<0.1) changed in human AML following ELAVL1 knockdown with borders demarcating concordance/discordance with enrichments from below-median expressors of ELAVL1 from the BeatAML data set.