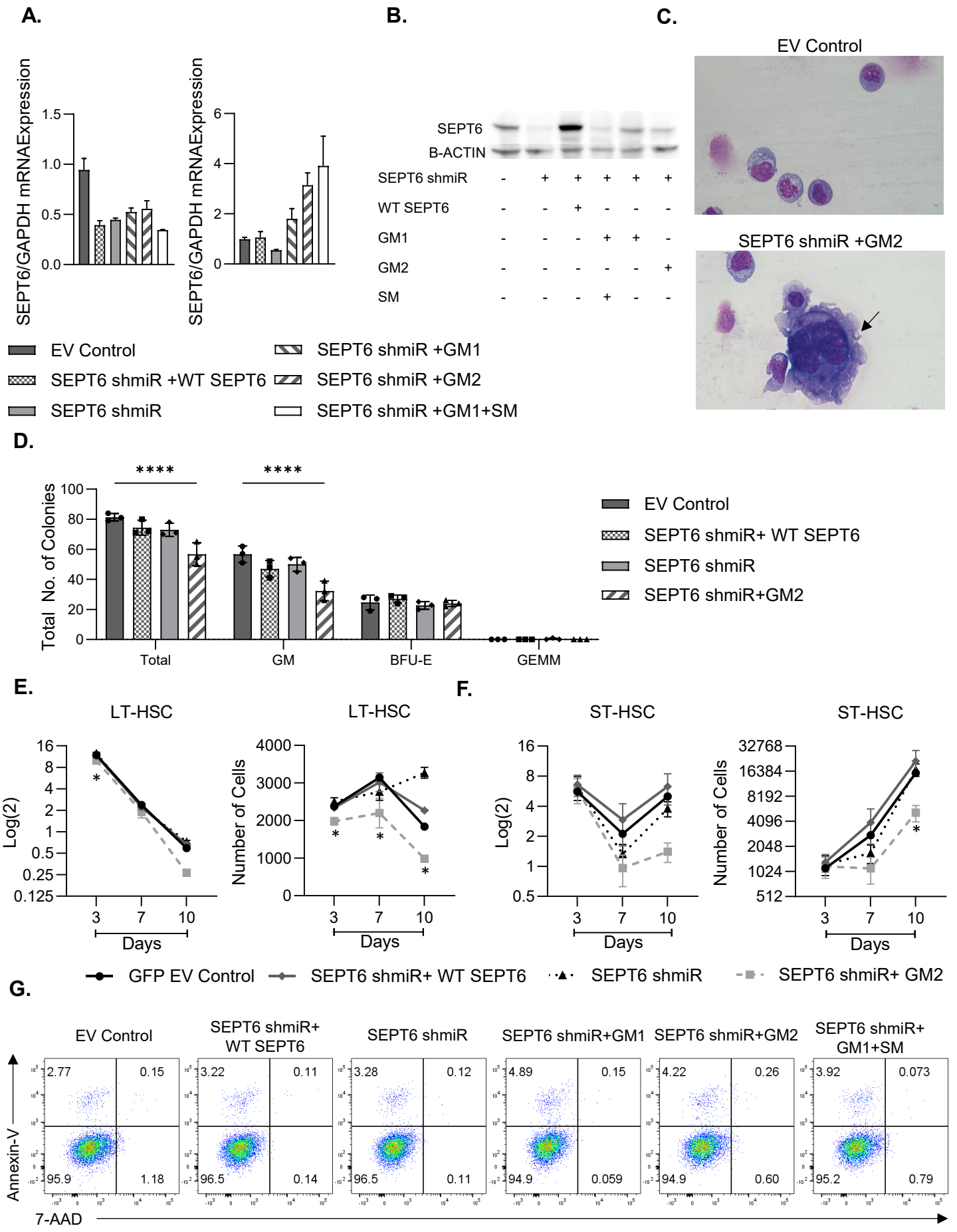
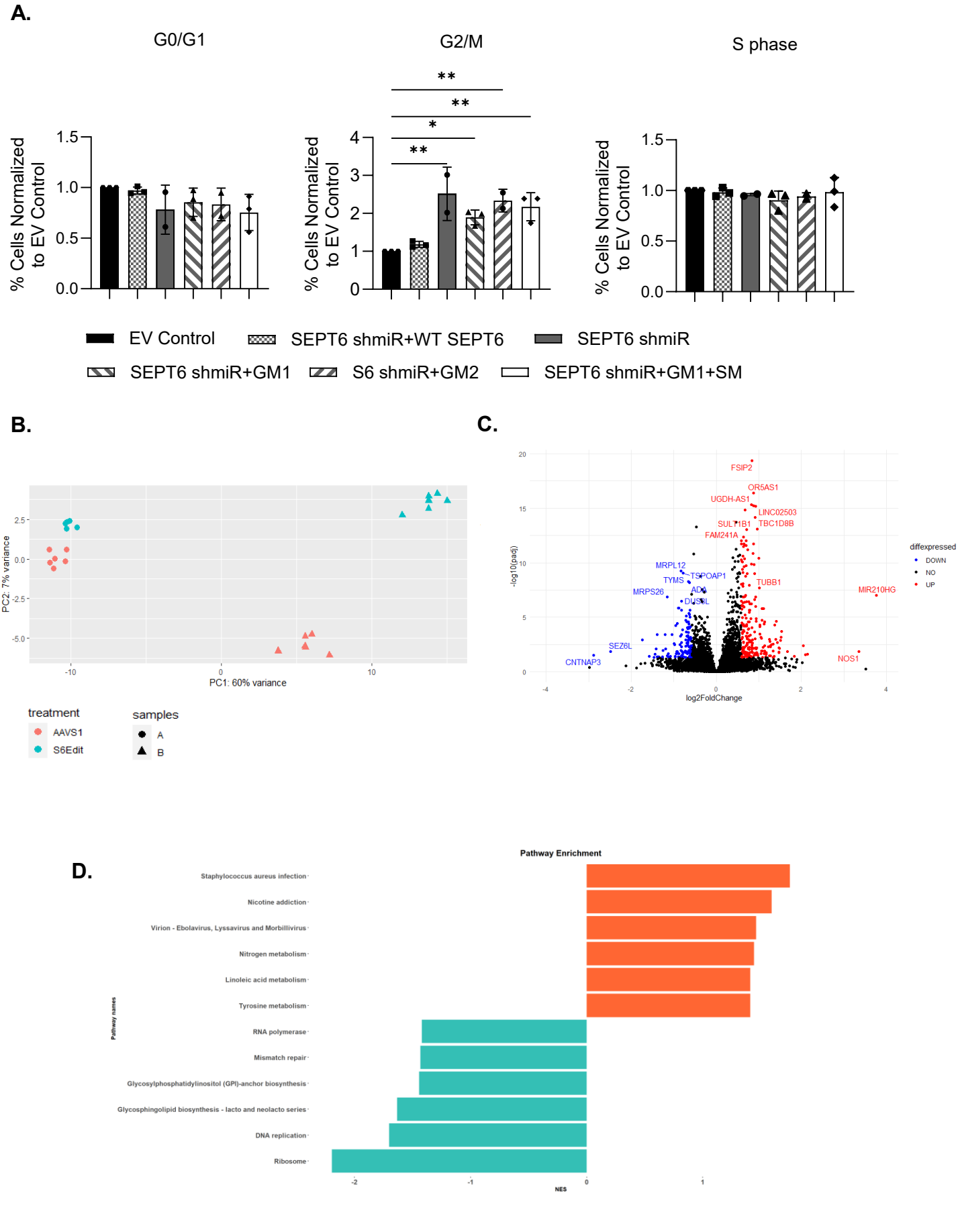
**Supplemental Figure 1**



**Supplemental Figure 1: Functional effect of the GM2:SEPT6 c.1282T>A germline mutation:** Using the backbone described in **Figure 2A**, human CD34+ cells were transduced and cultured in a serum-free medium with appropriate cytokines. 5 days post transduction, (A) qPCR was used to determine levels of native *SEPT6* knockdown using primers targeting the 3' untranslated region (left). Primers targeting the exons were used to determine the expression levels of mutated *SEPT6* cDNA (right). N=3 (B) Western blot of K562 cells after lentiviral transduction. Actin is used as a loading control. (C) Sections of transduced CD34+ cells were obtained 7 days post transduction to observe for morphological changes (100X magnification). N=3 biological replicates with similar results. (D) Transduced cells were plated into methylcellulose for progenitor assays 2 days post lentiviral transduction. Colonies were counted 14 days after plating. N=Three independent experiments performed in triplicate, ****$p<0.0001$. (E-F) A time point analysis of LT-HSC (E) and ST-HSC (F) similar to **Figure 2H-I** where cells were immunophenotypically identified through flow cytometry based on their expression of CD34, CD45RA, CD90, EPCR and ITGA3. Bars represent mean +/- SEM, N=3, *$p<0.05$. (G) Apoptosis assays using Annexin-V and 7-AAD were performed. The percentage of single and double positive cells is shown in each quadrant. Upper left quadrant: Early apoptotic cells, Upper right quadrant: Late Apoptotic cells. N=3.

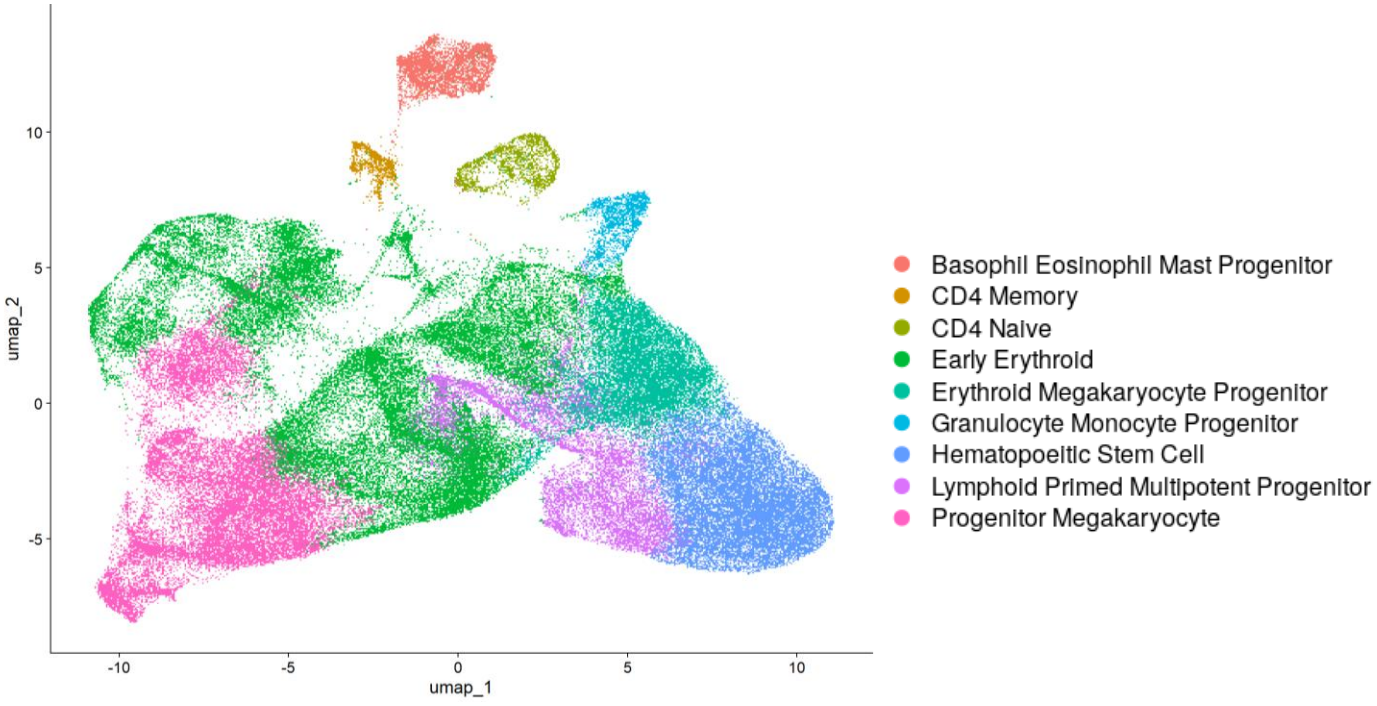
**Supplemental Figure 2**



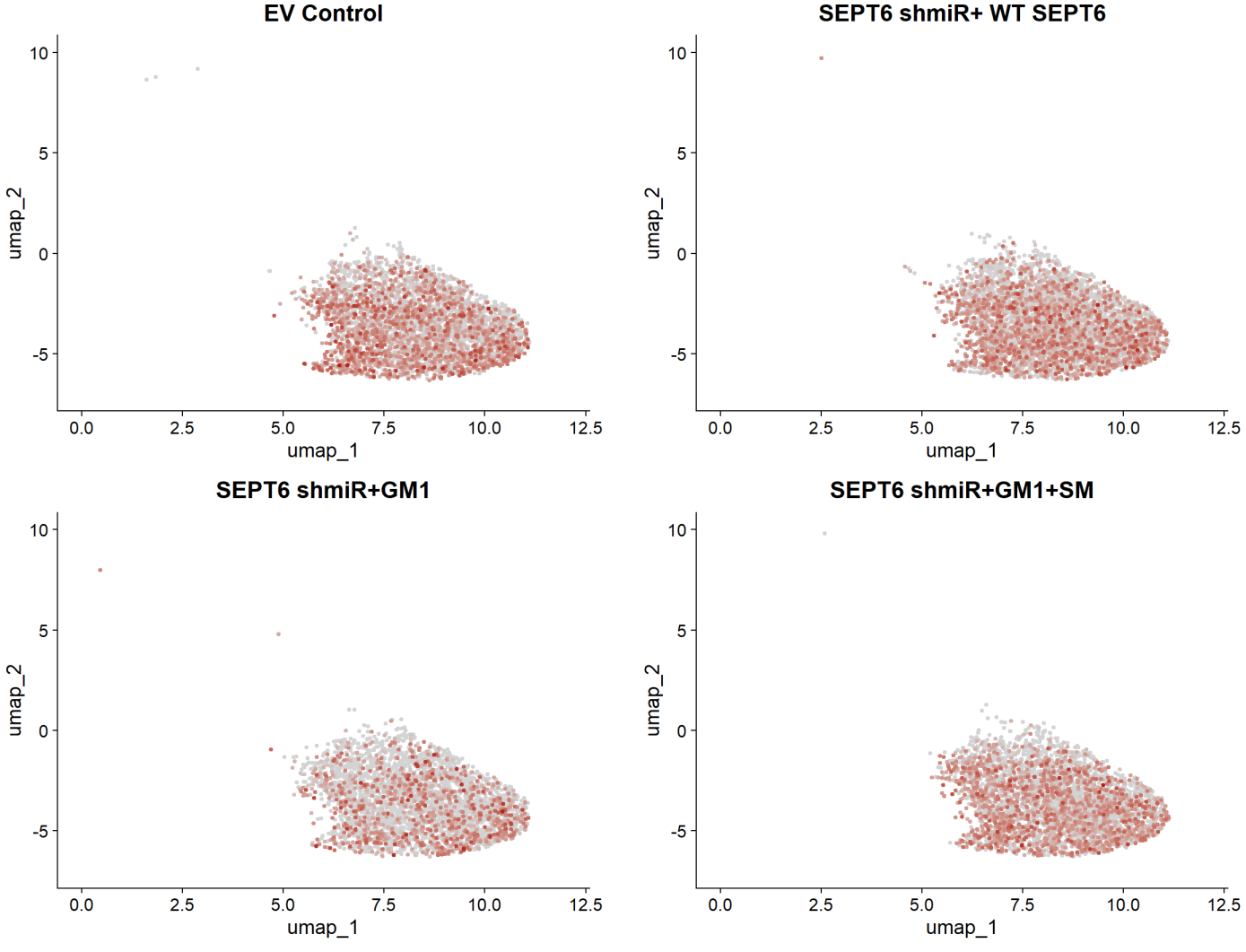
**Supplemental Figure 2: Bulk mRNA sequencing of *SEPT6* edited CD34+ cells show amplification of ribosome and DNA replication pathways.** (A) Cell cycle assays were performed 5 days post culture on LVV transduced CD34+ cells. BrDU staining and flow analysis was used to identify the percentage of cells in the different phases of cell cycle normalized to EV Control. N=3, *p<0.05, **p<0.005. (B-D) SmartSeq2 mRNA sequencing platform was utilized to sequence 1000 *AAVS1* Control or *SEPT6* GM1 edited CD34+ human cells. (B) PCA plots. (C) Volcano plots depicting the genes that were down and upregulated at p<0.05. (D) KEGG pathway enrichment analysis of upregulated and downregulated genes.

**Supplemental Figure 3**

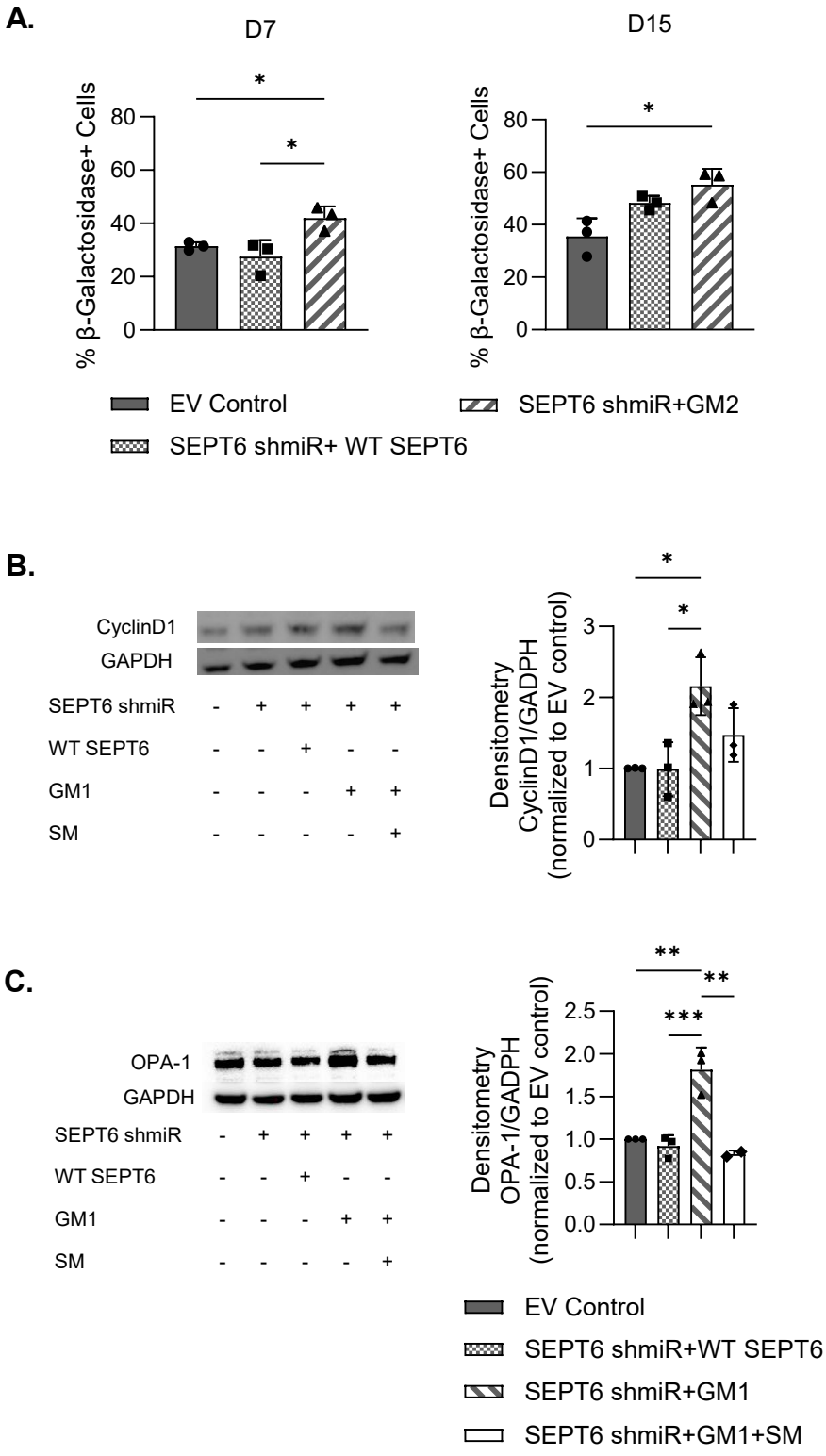
**A.**




**B.**



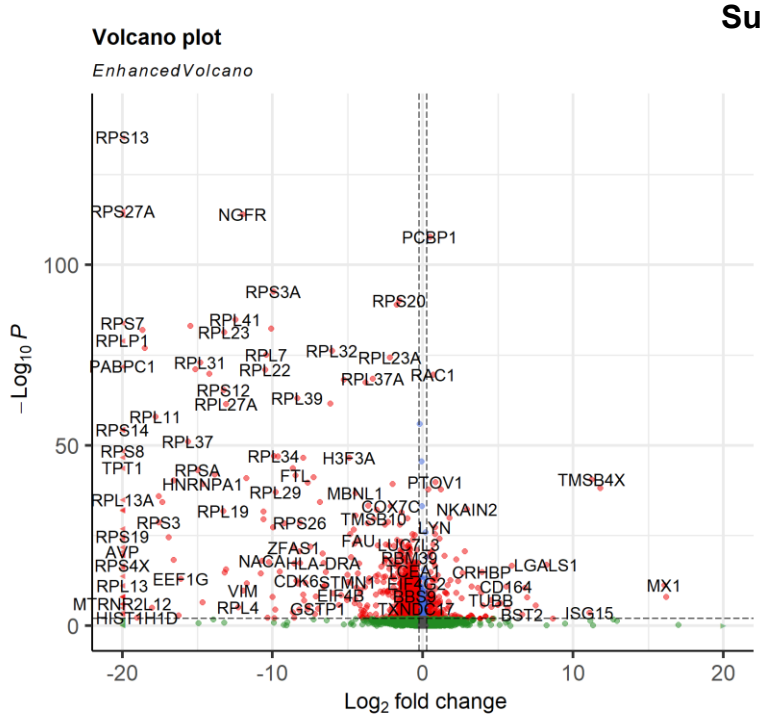
**Supplemental Figure 3: The downregulation of *MIF* gene expression in GM1 HSC.** (A) Uniform manifold approximation and projection (UMAP) of transduced cultured cells subjected to single cell mRNA sequencing. Clusters were annotated based on the expression of the top 25 marker genes. (B) Annotation of *MIF* gene expression in the HSC subset.



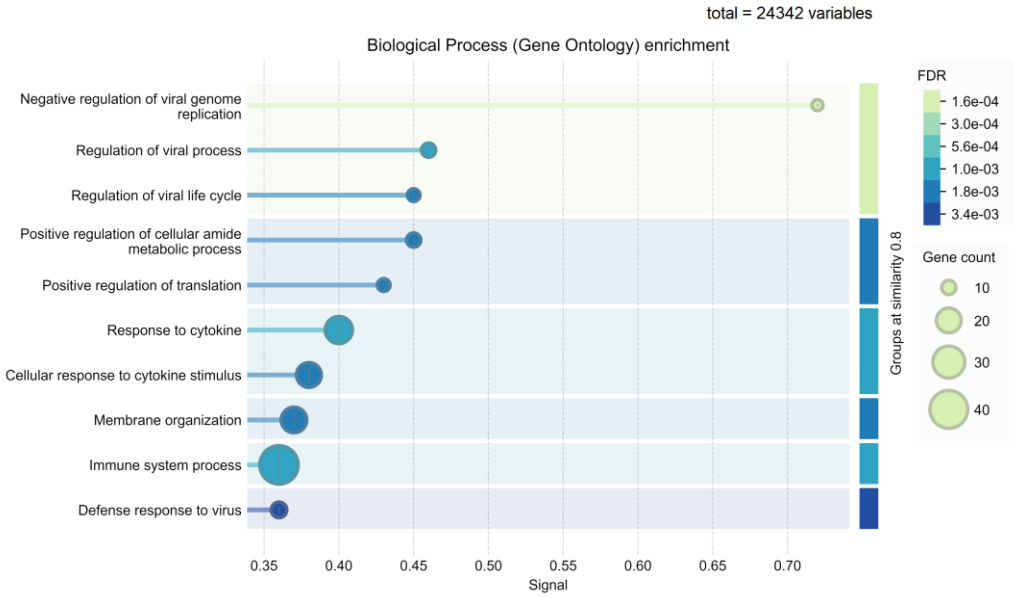

**Supplemental Figure 4:** (A) A β-gal assay was performed to determine the percentage of senescent cells in human CD34+ cells on day 7 (left panel) and day 15 (right panel) post lentivirus transduction. N=3. Horizontal lines represent mean +/- SD, *p<0.05. (B) Western Blot analysis of Cyclin D1. N=3. (C) Western Blot analysis of OPA-1. N=3, * p<0.05, ** p<0.005. *** p<0.0005.

**Supplemental Figure 5**

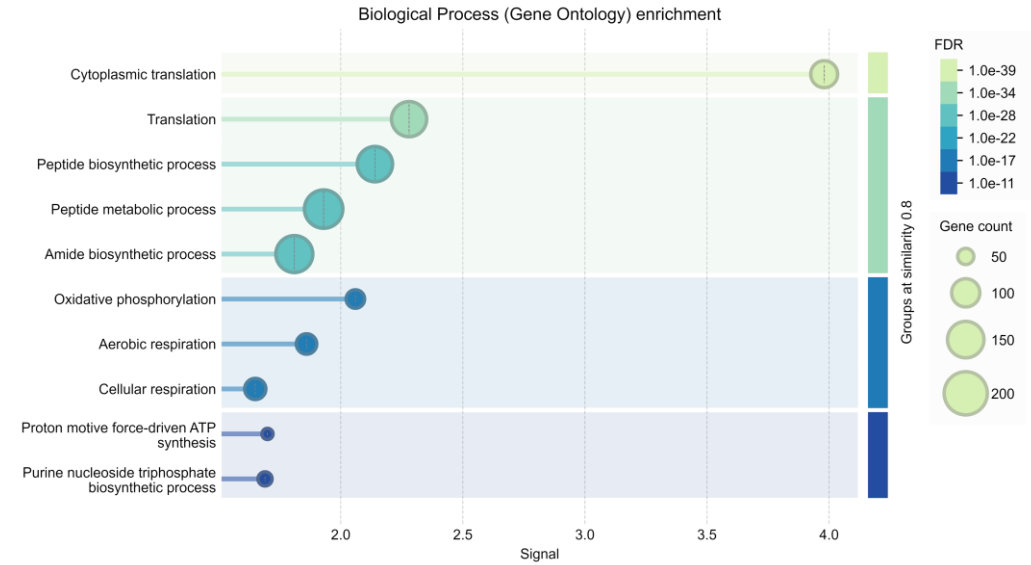
**A.**




**B.**




**C.**



**Supplemental Figure 5: Transcriptional profiling of human CD34+ cells transduced by the SEPT6 double mutant.** (A-C) Human CD34+ cells were transduced and flow sorted into GFP+CD34+CD45RA- cells 5 days post lentiviral transduction. Sorted cells were subjected to single cell mRNA sequencing analysis. (A) Volcano plots were plotted to determine the differential gene expression of SEPT6 shmiR+GM1+SM vs EV control and SEPT6 shmiR+ WT SEPT6. Threshold was set at 0.3/-0.3 log2 fold change, N=2 biological donors, $p<0.05$. (B) Upregulated and (C) downregulated biological process were identified and plotted through gene ontology analysis.

**Supplemental Table 3: List of antibodies used.**

| Antibody | Fluorophore | Clone |
|---|---|---|
| Antibodies used in Xenotransplantation analysis | | |
| CD10 | Pe Cy7 | HI10a |
| CD33 | Percp Cy5.5 | WM53 |
| CD34 | APC Cy7 | 581 |
| CD38 | BV711 | HIT2 |
| CD45RA | BV605 | HI100 |
| CD49f | Pacific Blue | GoH3 |
| CD90 | AF700 | 5E10 |
| CD123 | APC | 6H6 |
| CD133 | PE Dazzle | S16015F |
| CD45 | BV510 | HI30 |
| CD19 | BV750 | HIB19 |
| CD135 | PE | BV10A4H2 |
| CD3 | BV785 | OKT3 |
| CD14 | BV650 | M5E2 |
| Antibodies used to isolate LT-HSC and ST-HSC | | |
| CD34 | APC Cy7 | 581 |
| CD45RA | Pacific Blue | HI100 |
| ITGA3 | APC | ASC-1 |
| EPCR | PE | RCR-401 |
| CD90 | PE Cy7 | 5E10 |

**Supplemental Table 4: List of primers used**

| Gene | Primers |
|---|---|
| SEPT6 (Exon primers) | FP:5'-GTGTCCAGCTCCAGTCTAATAC-3' |
| | RP5'-GGCTTGTAGCTGTCCTCTTT-3' |
| SEPT6 (Untranslated region primers) | FP: 5'-GTGCAGTAGCTCCCGTTG-3' |
| | RP: 5'- GACAGTTCGGCAACCTTCA-3' |
| Cyclin D1 | FP: 5'-GCTGCGAAGTGGAAACCATC-3' |
| | RP: 5'-CCTCCTTCTGCACACATTTGAA-3' |
| Cyclin E1 | FP: 5'-ACTCAACGTGCAAGCCTCG-3' |
| | RP: 5'-GCTCAAGAAAGTGCTGATCCC-3' |