YMTHE, Volume *29*

Supplemental information

Adenine base editing reduces misfolded protein accumulation and toxicity in alpha-1 antitrypsin deficient patient iPSC-hepatocytes

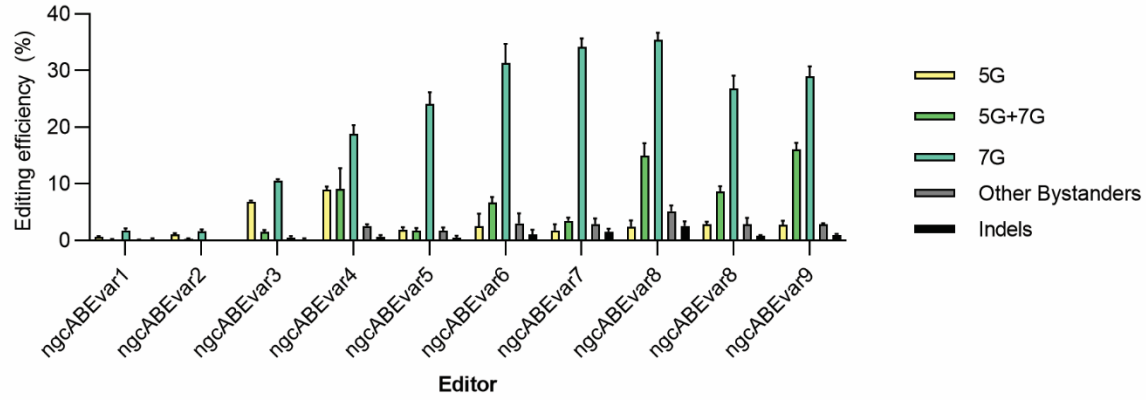
Rhiannon B. Werder, Joseph E. Kaserman, Michael S. Packer, Jonathan Lindstrom-Vautrin, Carlos Villacorta-Martin, Lauren E. Young, Yvonne Aratyn-Schaus, Francine Gregoire, and Andrew A. Wilson

Supplemental Figure 1

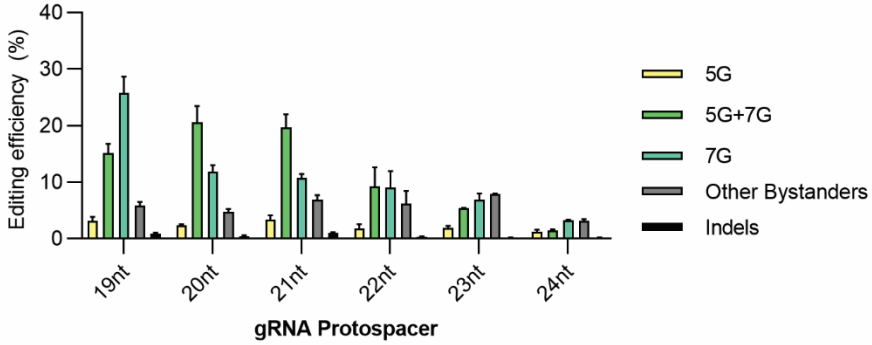
A

Code name	TadA	TadA variant	PAM variant	Template
ngcABEvar1	Dual	TadA_7.10	MQKSER	pMSP640
ngcABEvar2	Dual	TadA_7.10	Cas9-NG	pMSP641
ngcABEvar3	Dual	TadA_7.10	MQKFRAER	pMSP642
ngcABEvar4	Dual	TadA_7.10+V82S	MQKFRAER	pMSP643
ngcABEvar5	Mono	TadA_7.10+V82S	MQKFRAER	pMSP510
ngcABEvar6	Mono	TadA_7.10+I76Y+V82S	MQKFRAER	pPMSP591
ngcABEvar7	Mono	TadA_7.10+V82T	MQKFRAER	pMSP592
ngcABEvar8	Mono	TadA_7.10+I76Y+V82T	MQKFRAER	pMSP623
ngcABEvar9	Mono	TadA_7.10+I76Y+V82T+Y147T+Q154S	MQKFRAER	pMSP661

B



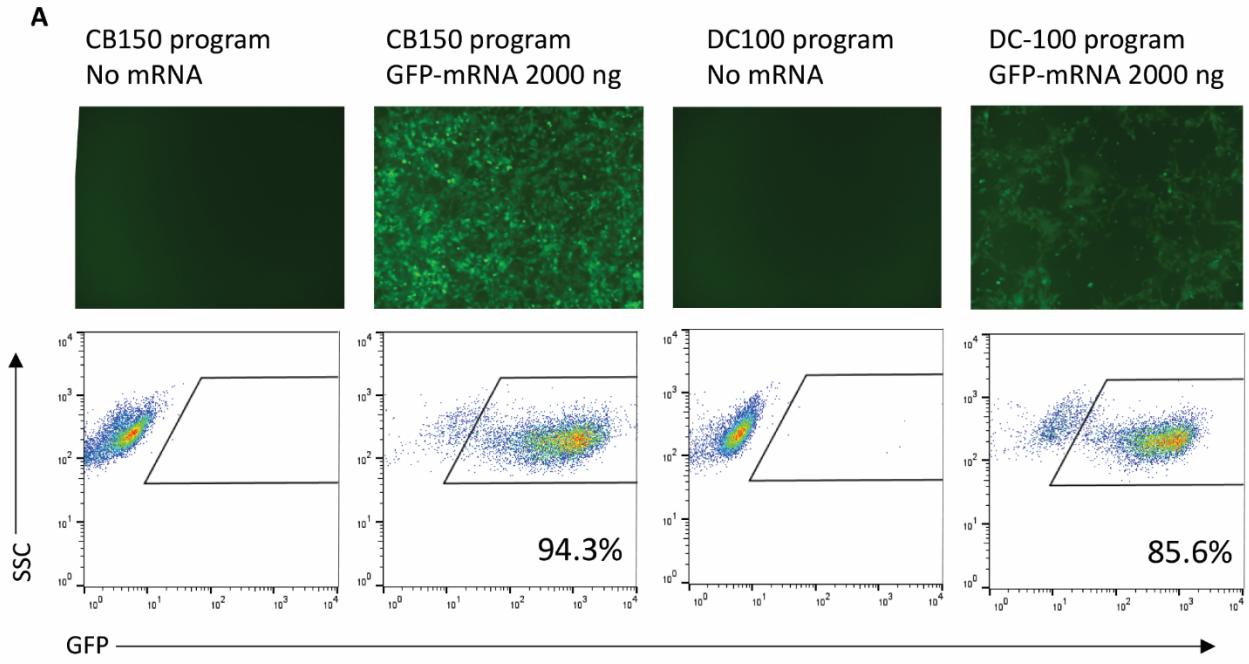
C

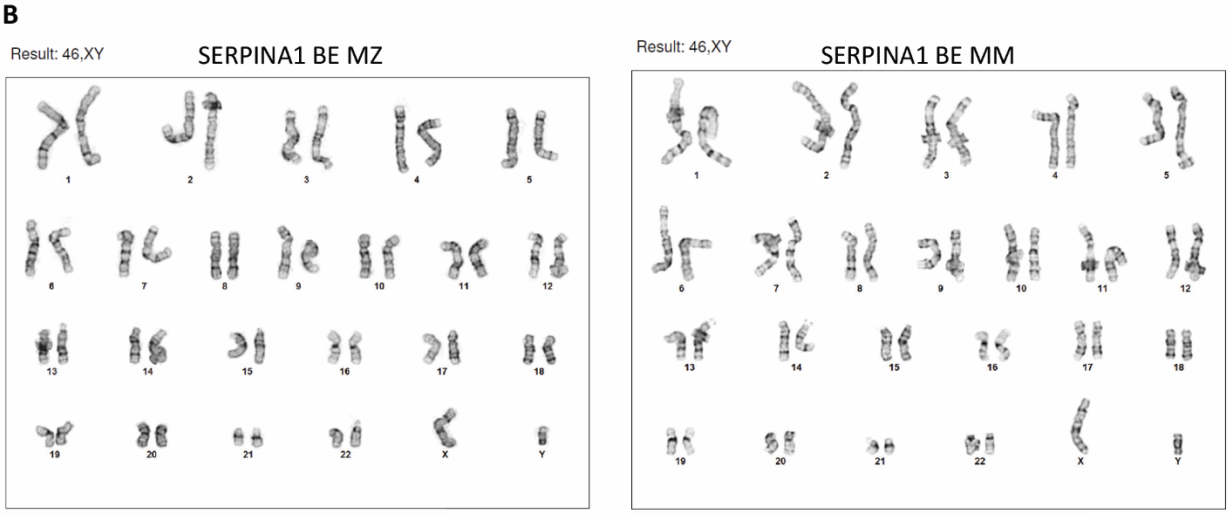


s

Figure S1. (A) Details of ngcABEvar1-9. (B) Editing efficiency in patient-derived PiZ homozygous fibroblasts. (C) Editing efficiency with gRNAs of differing protospacer length.

Supplemental Figure 2





C

sample	# somatic variants	# A->G	fraction A->G
PiZZ1_MZ	428	57	0.133
PiZZ1_MM	341	45	0.132
PiZZ1_ZZ_P34	68	16	0.235

Figure S2. Relates to Figure 1. (A) Delivery of GFP-mRNA to iPSCs with nucleofector program CB150 or DC100. GFP fluorescence was determined using a Keyence microscope (top) and by flow cytometry (bottom). (B) Karyotype for MZ and MM corrected iPSCs. (C) Additional analysis of WGS using the LoFreq tool to determine somatic mutations and overlayed with potential off-target sites (determined with Cas-OFFinder). MZ and MM samples were compared to passage matched ZZ cells. Earlier passage ZZ cells (P34) were also assessed.

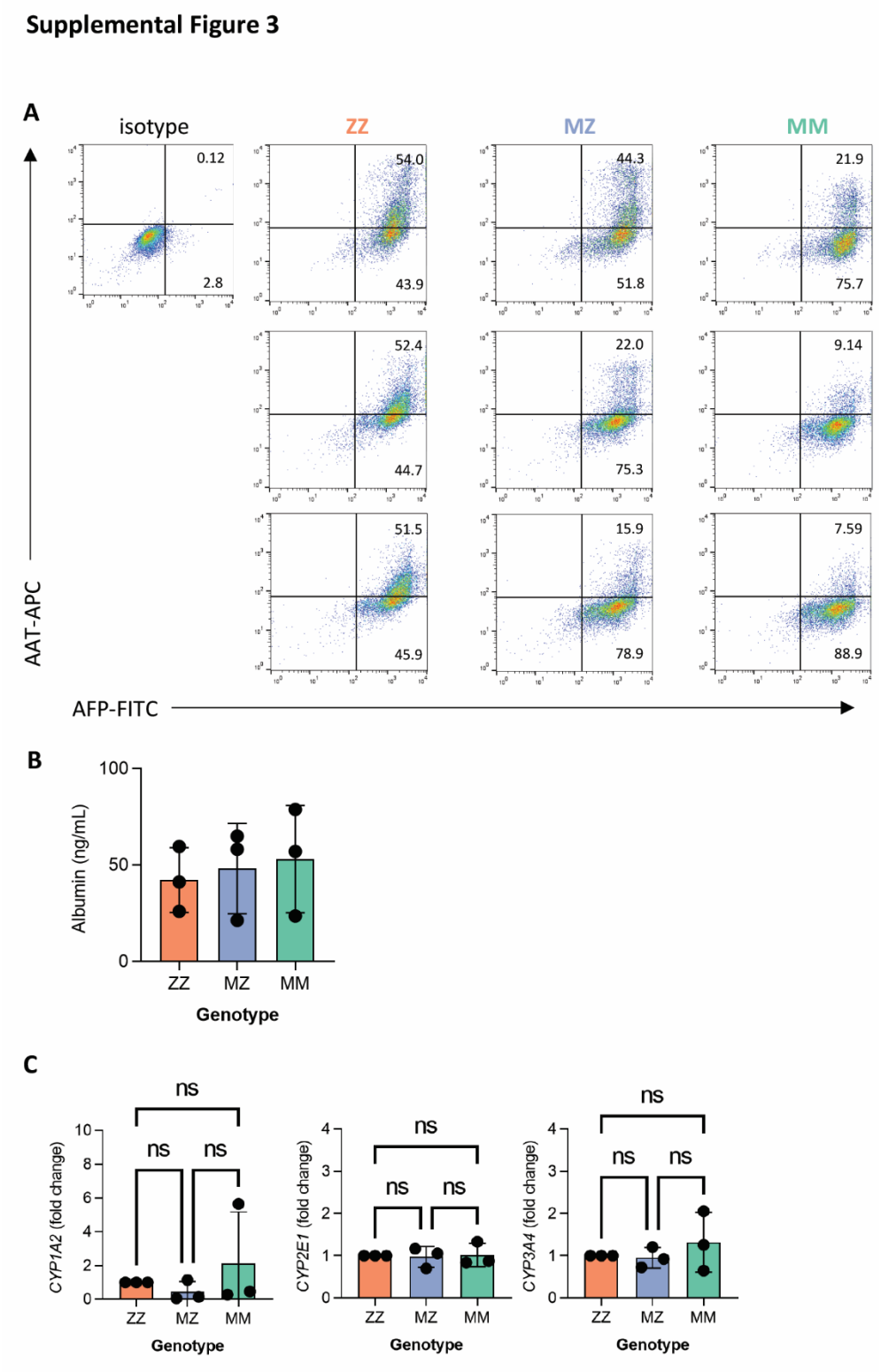


Figure S3. Relates to Figure 2. (A) Flow cytometry plots for AFP and AAT for three independent differentiations. (B) Albumin secretion. (C) *CYP1A2*, *CYP2E1* and *CYP3A4* expression.

Supplemental Figure 4

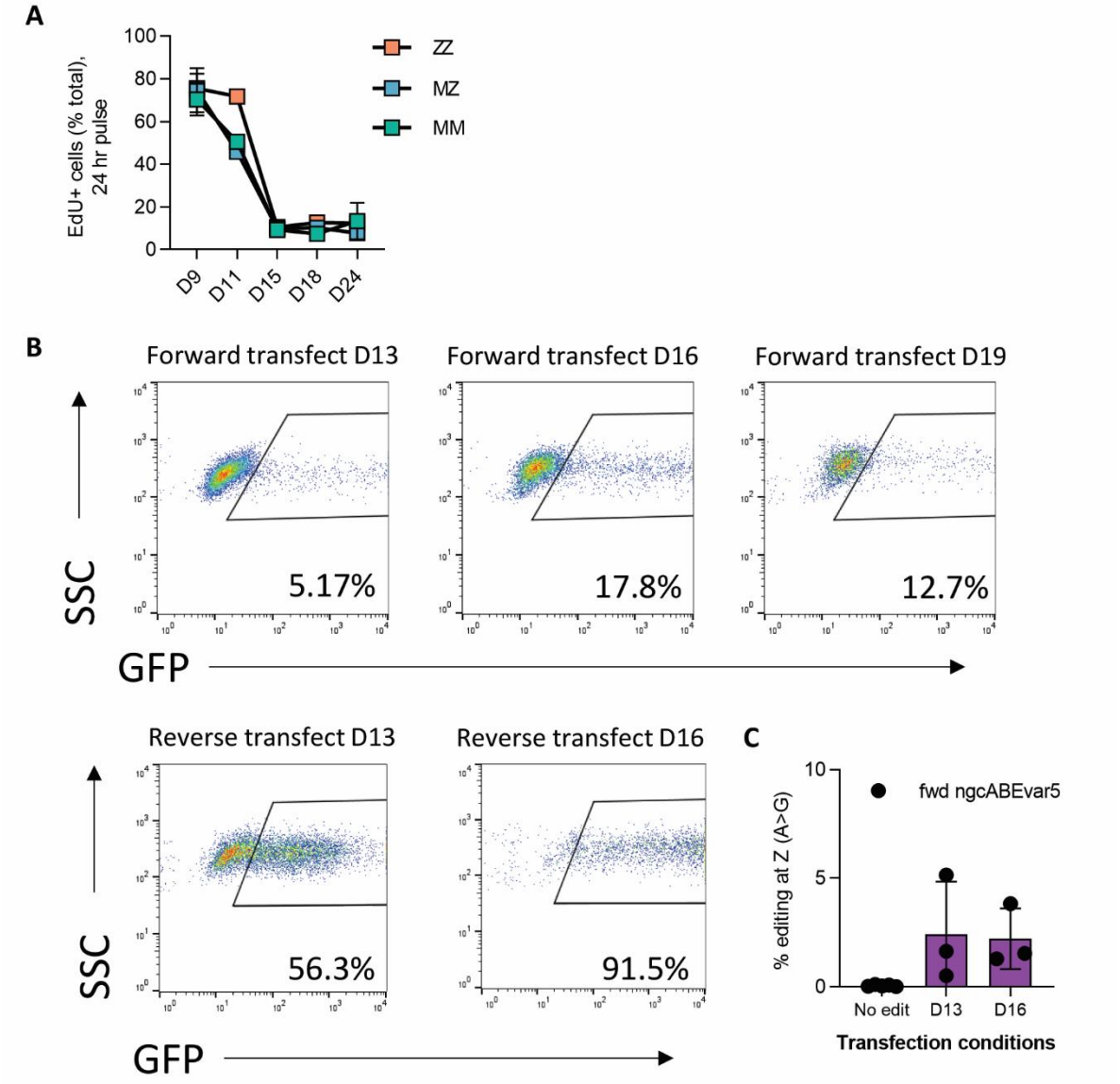


Figure S4. Relates to Figure 3 (A) EdU incorporation across the differentiation protocol in ZZ, MZ or MM cells. (B) Delivery of GFP-mRNA to iPSCs undergoing directed differentiation to hepatocytes. Cells were forward or reverse transfected on day 13, 16 or 19 of the differentiation protocol. (C) iHeps were forward transfected with ngcABEvar5 base-editor on D13 or D16 of the directed differentiation protocol. Editing efficiency was assessed by Next Generation Sequencing (NGS) to quantify the correction of the Z mutation from A>G.

Supplemental Figure 5

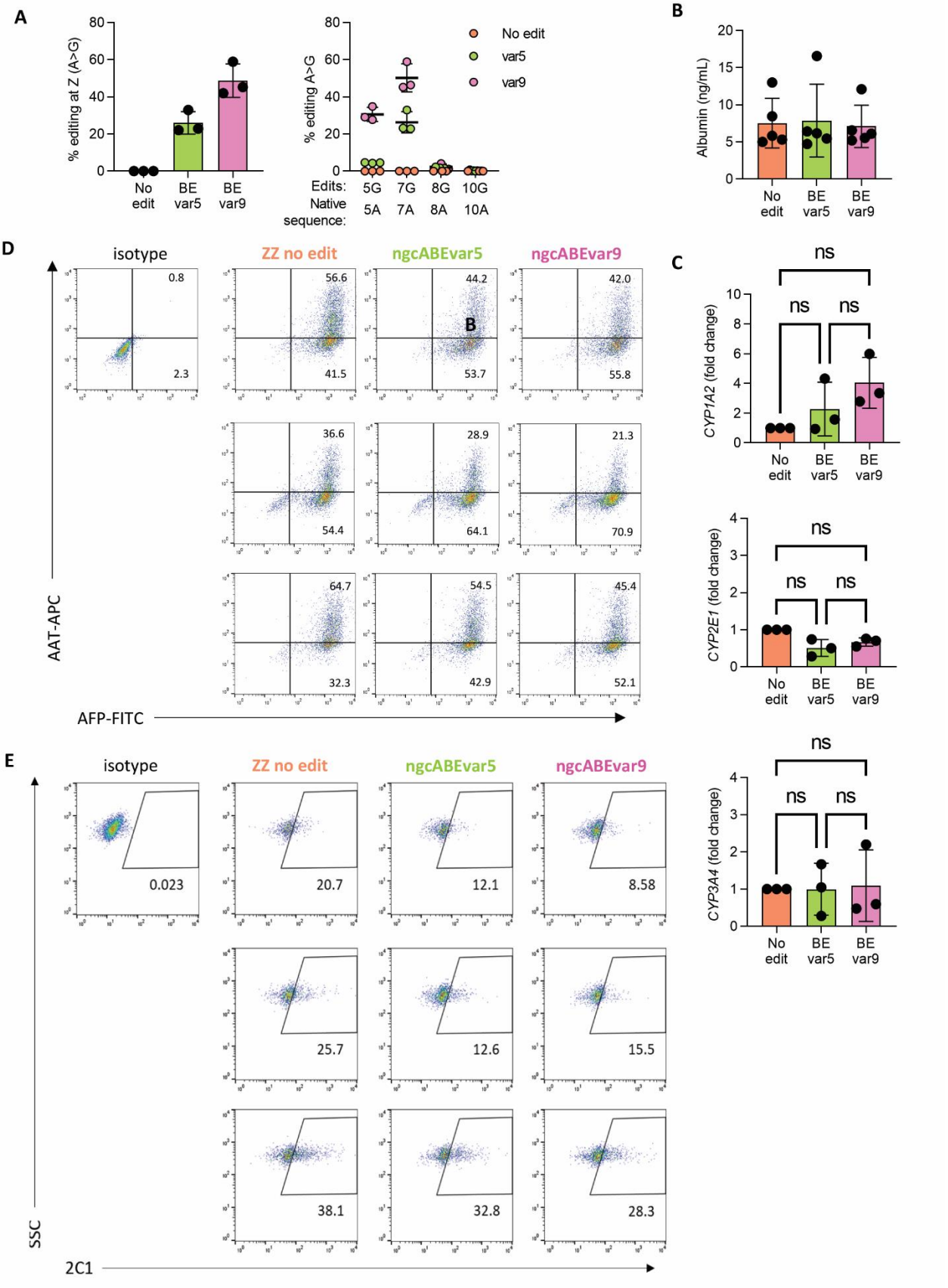


Figure S5. Relates to Figure 4. (A) Editing efficiency and bystander editing was assessed by Next Generation Sequencing to quantify the correction of the Z mutation from A>G. (B) Albumin secretion. (C) *CYP1A2*, *CYP2E1* and *CYP3A4* expression. (D) Flow cytometry plots for AFP and AAT for three independent differentiations. (E) Flow cytometry plots for 2C1 for three independent differentiations.

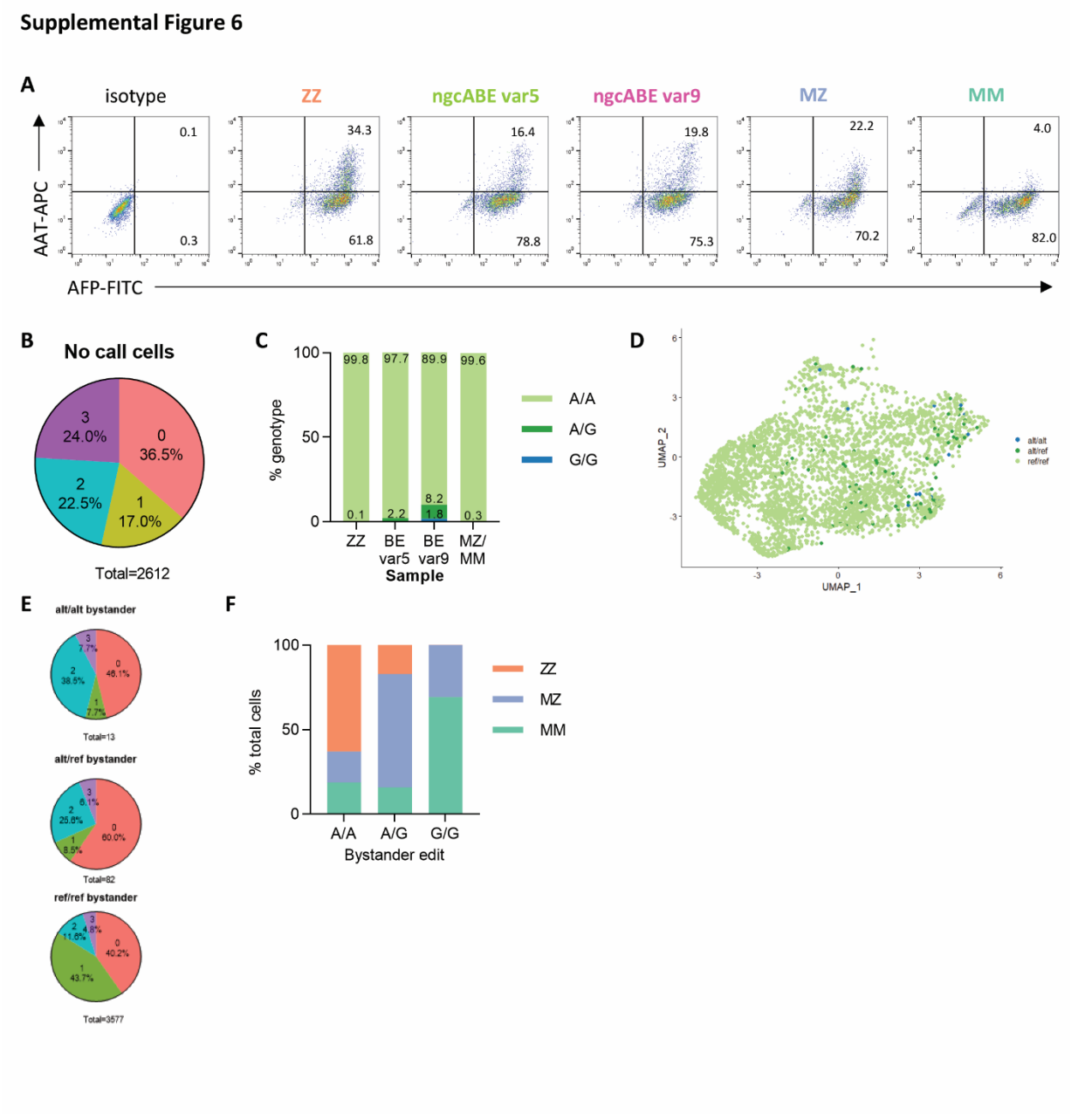


Figure S6. (A) Flow cytometry plots for AFP and AAT in cells that were used for single-cell RNA sequencing. (B) Distribution of cells across clusters without a genotype assigned by Vartrix. These cells were excluded for subsequent analyses. (C) Genotype of cells with bystander (D341G) edits. (D) UMAP projection of cells with bystander D341G edits. (E) Distribution of cells with bystander D341G edits across clusters. (F) Distribution of cells with bystander D341G edits by Z mutation status (ZZ, MZ or MM).

Supplemental Figure 7

CLUSTER 0	pval	fdr
GO_TRANSLATIONAL_INITIATION	2.60E-65	1.90E-61
GO_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	7.30E-63	2.80E-59
GO_PROTEIN_LOCALIZATION_TO_ENDOPLASMIC_RETICULUM	1.10E-59	2.00E-56
GO_ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_ENDOPLASMIC_RETICULUM	1.30E-59	2.00E-56
GO_NUCLEAR_TRANSCRIBED_MRNA_CATABOLIC_PROCESS_NONSENSE_MEDIATED_DECAY	1.30E-59	2.00E-56
GO_VIRAL_GENE_EXPRESSION	6.10E-51	7.70E-48
GO_NUCLEAR_TRANSCRIBED_MRNA_CATABOLIC_PROCESS	9.70E-51	1.00E-47
GO_PROTEIN_TARGETING_TO_MEMBRANE	3.40E-48	3.20E-45
GO_PEPTIDE_BIOSYNTHETIC_PROCESS	6.10E-48	5.10E-45
GO_RNA_CATABOLIC_PROCESS	5.10E-46	3.80E-43
GO_AMIDE_BIOSYNTHETIC_PROCESS	2.10E-44	1.40E-41
GO_PEPTIDE_METABOLIC_PROCESS	2.60E-42	1.60E-39
GO_ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_MEMBRANE	1.10E-41	6.60E-39
GO_CELLULAR_MACROMOLECULE_CATABOLIC_PROCESS	3.10E-41	1.60E-38
GO_CELLULAR_NITROGEN_COMPOUND_CATABOLIC_PROCESS	1.10E-40	5.80E-38
GO_INTERSPECIES_INTERACTION_BETWEEN_ORGANISMS	3.20E-40	1.50E-37
GO_MACROMOLECULE_CATABOLIC_PROCESS	1.10E-38	5.10E-36
GO_ORGANIC_CYCLIC_COMPOUND_CATABOLIC_PROCESS	1.70E-37	6.90E-35
GO_ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_ORGANELLE	1.90E-37	7.40E-35
GO_CELLULAR_AMIDE_METABOLIC_PROCESS	7.90E-37	3.00E-34

CLUSTER 1	pval	fdr
GO_PROTEIN_LIPID_COMPLEX_SUBUNIT_ORGANIZATION	2.00E-15	1.50E-11
GO_PROTEIN_LIPID_COMPLEX_ASSEMBLY	5.60E-15	1.70E-11
GO_OXIDATION_REDUCTION_PROCESS	7.00E-15	1.70E-11
GO_SMALL_MOLECULE_METABOLIC_PROCESS	6.90E-14	1.30E-10
GO_RESPONSE_TO_INORGANIC_SUBSTANCE	6.40E-13	9.70E-10
GO_PROTEIN_CONTAINING_COMPLEX_REMODELING	2.00E-12	2.60E-09
GO_DRUG_METABOLIC_PROCESS	4.00E-12	4.30E-09
GO_REGULATION_OF_PLASMA_LIPOPROTEIN_PARTICLE_LEVELS	5.80E-12	5.00E-09
GO_RESPONSE_TO_TOXIC_SUBSTANCE	6.00E-12	5.00E-09
GO_CHYLOMICRON_ASSEMBLY	7.10E-12	5.40E-09
GO_ION_TRANSPORT	1.10E-11	7.60E-09
GO_ATP_METABOLIC_PROCESS	1.50E-11	9.60E-09
GO_HIGH_DENSITY_LIPOPROTEIN_PARTICLE_REMODELING	1.70E-11	1.00E-08
GO_RESPONSE_TO_ABIOTIC_STIMULUS	4.40E-11	2.30E-08
GO_PHOSPHOLIPID_EFFLUX	4.60E-11	2.30E-08
GO_OXIDATIVE_PHOSPHORYLATION	7.60E-11	3.50E-08
GO_SECRETION	7.80E-11	3.50E-08
GO_GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY	1.10E-10	4.30E-08
GO_NEGATIVE_REGULATION_OF_HYDROLASE_ACTIVITY	1.10E-10	4.30E-08
GO_PLATELET_DEGRANULATION	1.40E-10	5.30E-08

CLUSTER 2	pval	fdr
GO_ACTIN_FILAMENT_BASED_PROCESS	2.80E-47	2.10E-43
GO_CYTOSKELETON_ORGANIZATION	2.10E-44	7.70E-41
GO_BIOLOGICAL_ADHESION	1.50E-37	3.80E-34
GO_CELLULAR_COMPONENT_MORPHOGENESIS	1.90E-36	3.50E-33
GO_SUPRAMOLECULAR_FIBER_ORGANIZATION	7.00E-36	1.10E-32
GO_LOCOMOTION	3.60E-34	4.60E-31
GO_CELL_MOTILITY	1.30E-30	1.40E-27
GO_CELL_PROJECTION_ORGANIZATION	6.30E-30	5.90E-27
GO_ACTIN_FILAMENT_ORGANIZATION	1.40E-29	1.20E-26
GO_REGULATION_OF_ANATOMICAL_STRUCTURE_MORPHOGENESIS	1.60E-28	1.20E-25
GO_EXOCYTOSIS	4.80E-27	3.30E-24
GO_ENZYME_LINKED_RECEPTOR_PROTEIN_SIGNALING_PATHWAY	6.60E-27	4.10E-24
GO_NEGATIVE_REGULATION_OF_RESPONSE_TO_STIMULUS	1.10E-25	6.20E-23
GO_REGULATION_OF_CELLULAR_COMPONENT_MOVEMENT	1.00E-24	5.50E-22
GO_CELL_ACTIVATION	1.10E-24	5.50E-22
GO_NEGATIVE_REGULATION_OF_SIGNALING	2.80E-24	1.20E-21
GO_REGULATION_OF_ACTIN_FILAMENT_BASED_PROCESS	2.80E-24	1.20E-21
GO_SECRETION	2.90E-24	1.20E-21
GO_REGULATION_OF_CELL_DEATH	5.80E-24	2.30E-21
GO_POSITIVE_REGULATION_OF_CELLULAR_COMPONENT_ORGANIZATION	6.00E-24	2.30E-21

CLUSTER 3	pval	fdr
GO_RESPONSE_TO_ENDOPLASMIC_RETICULUM_STRESS	6.50E-30	4.90E-26
GO_SECRETION	2.70E-29	1.00E-25
GO_EXTRACELLULAR_STRUCTURE_ORGANIZATION	1.90E-28	4.70E-25
GO_INTRACELLULAR_TRANSPORT	3.50E-26	6.60E-23
GO_CELLULAR_MACROMOLECULE_LOCALIZATION	1.30E-25	1.90E-22
GO_EXOCYTOSIS	1.50E-25	1.90E-22
GO_CELL_ACTIVATION	1.30E-22	1.40E-19
GO_RESPONSE_TO_TOPOLOGICALLY_INCORRECT_PROTEIN	1.70E-21	1.60E-18
GO_MYELOID_LEUKOCYTE_ACTIVATION	5.50E-20	4.60E-17
GO_ENDOPLASMIC_RETICULUM_TO_GOLGI_VESICLE_MEDIATED_TRANSPORT	3.60E-19	2.70E-16
GO_MYELOID_LEUKOCYTE_MEDIATED_IMMUNITY	4.70E-19	3.20E-16
GO_BIOLOGICAL_ADHESION	2.00E-18	1.20E-15
GO_PROTEOLYSIS	4.50E-18	2.60E-15
GO_CELL_ACTIVATION_INVOLVED_IN_IMMUNE_RESPONSE	5.20E-18	2.80E-15
GO_CELLULAR_RESPONSE_TO_TOPOLOGICALLY_INCORRECT_PROTEIN	6.30E-18	3.10E-15
GO_RESPONSE_TO_NITROGEN_COMPOUND	8.20E-18	3.90E-15
GO_CELL_MOTILITY	1.50E-17	6.50E-15
GO_RESPONSE_TO_WOUNDING	1.60E-17	6.70E-15
GO_ENDOPLASMIC_RETICULUM_UNFOLDED_PROTEIN_RESPONSE	1.80E-17	7.00E-15
GO_CELL_JUNCTION_ORGANIZATION	2.70E-17	1.00E-14

Figure S7. Gene-set enrichment analysis (GSEA) for each cluster.

Supplemental Figure 8

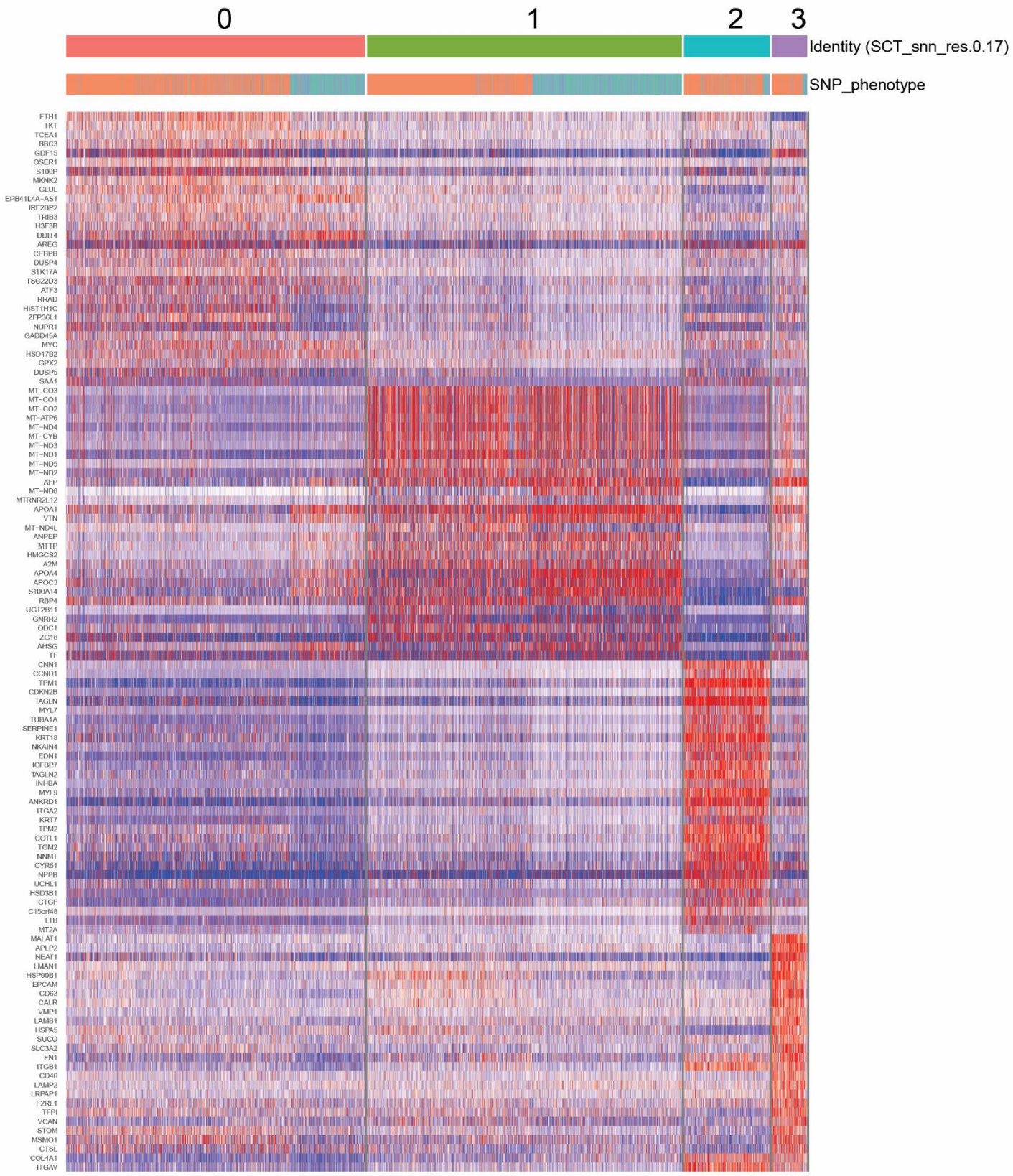


Figure S8. Top 30 differentially expressed genes across clusters.

Supplemental Table 1. Relates to Figure 1. Next generation sequencing of base-edited iPSC clones.

Supplemental Table 2. Relates to Figure 3. Next generation sequencing of base-edited iPSC-derived hepatocytes.

Supplemental Table 3. Relates to Figure 4. Next generation sequencing of base-edited iPSC-derived hepatocytes.

Supplemental Table 4. Relates to Figure 5. Next generation sequencing of base-edited iPSC-derived hepatocytes.

Supplemental Table 5. Primer sequences used in this study.

Supplemental Table 6. ngcABE mRNA template sequences used in this study.

Supplemental Table 7. gRNA sequences used in this study.

Supplemental Table 8. Whole genome sequencing statistical output from VarScan and Mutect2 of passage matched samples (ZZ, ZZ cells that were nucleofected without editors, MZ and MM) compared to earlier passage ZZ cells (the same passage used to commence base-editing).