Cell Reports, Volume *39*

Supplemental information

Mapping *cis*-regulatory elements in human neurons links psychiatric disease heritability and activity-regulated transcriptional programs

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Figure S1. Membrane depolarization and gene expression in human Glu and GABA neurons (Related to Figure 1).

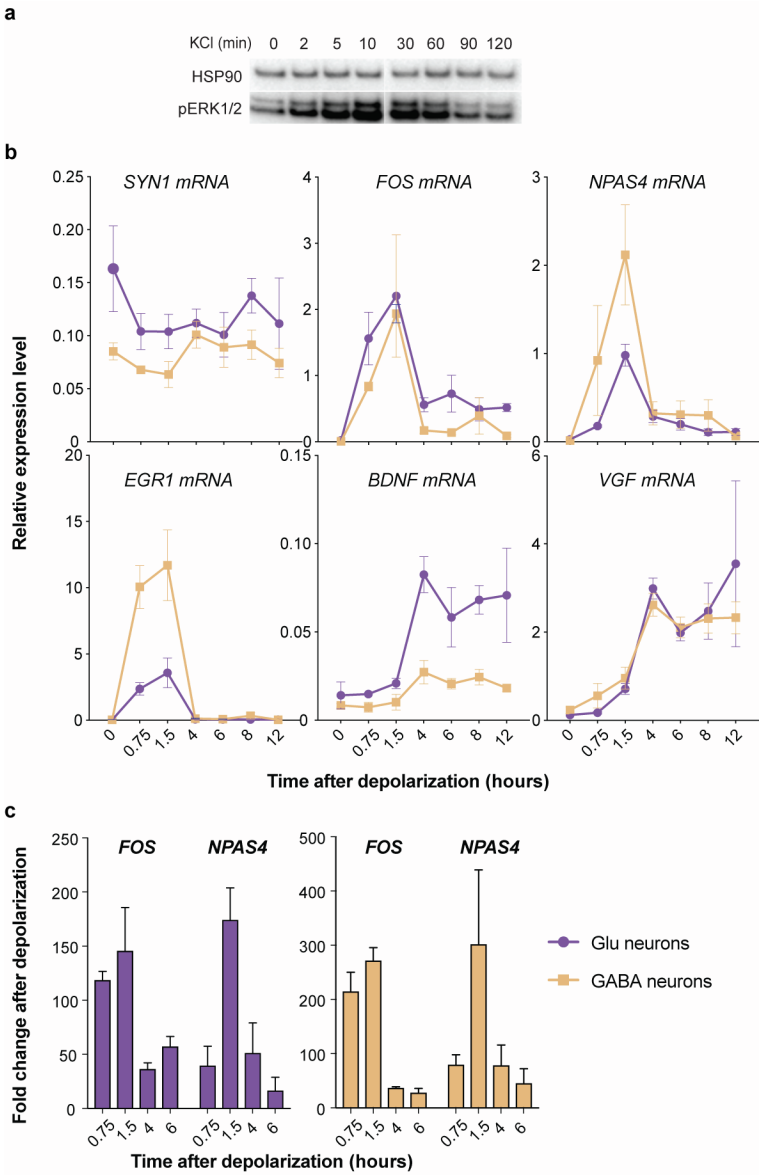

Figure S1. (**a**) Immunoblot analyses of proteins extracted from human neuron cultures before and after membrane depolarization at the indicated time showed the MAPK/ERK activation kinetics. HSP90 was used as the loading control. Images are representative of more than three independent experiments. (**b, c**) Normalized gene expression values (**b**) and fold changes of mRNA levels of selected genes in the human Glu and GABA neurons at indicated time following membrane depolarization as measured by qPCR. Membrane depolarization induces the expression of prototypical ERGs and LRGs in both neuronal subtypes. The expression level of *SYN1*, a non-activity induced gene, did not change after membrane depolarization. Data are represented as mean ± s.e.m., n = 3 biological replicates.

Figure S2. Membrane depolarization-induced changes in the transcriptome of human Glu and GABA neurons (Related to Figure 2).

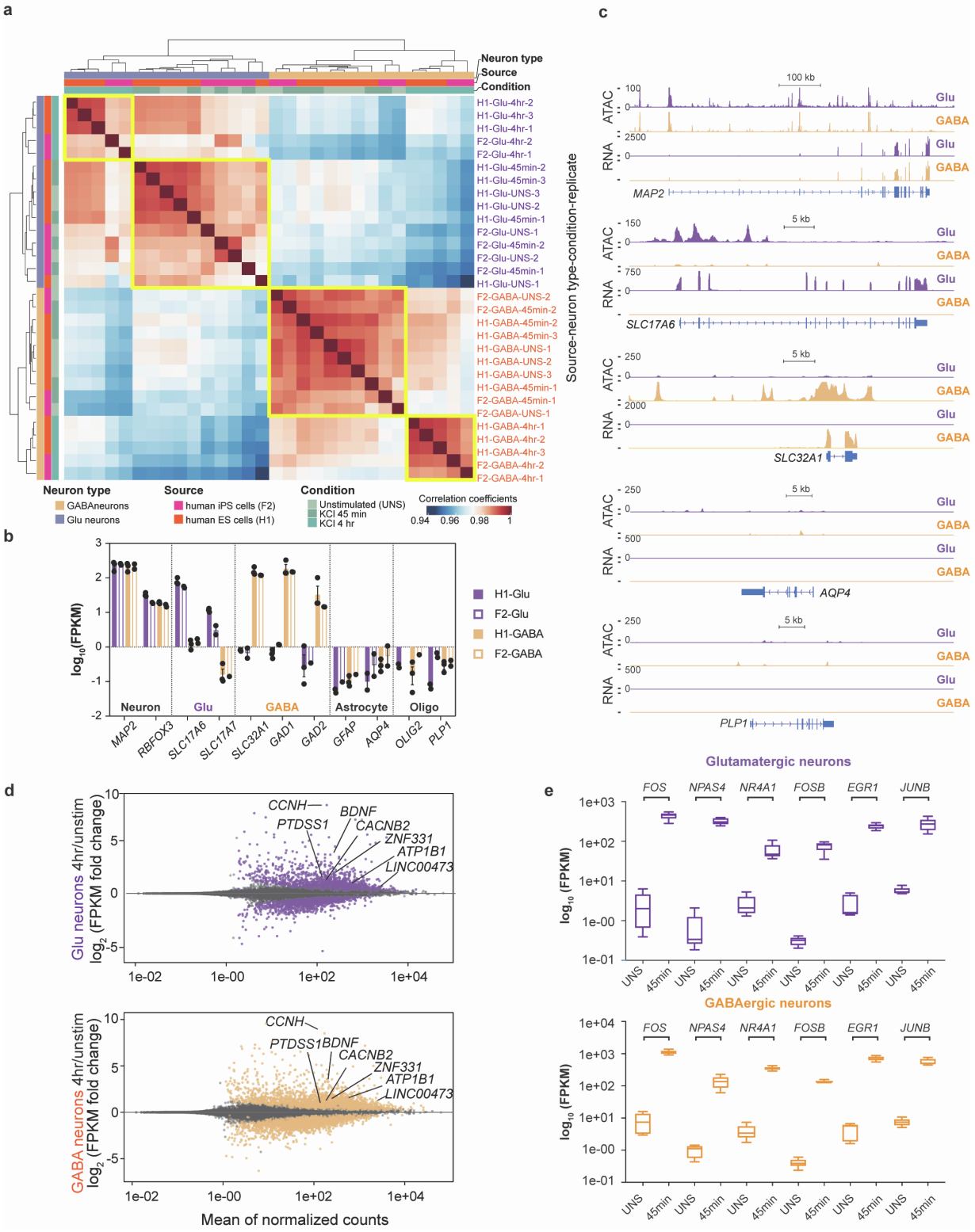

Figure S2. (**a**) The correlation plot shows the relationship of transcriptome profiles among neuronal subtypes and activity states. Sample-to-sample distance matrix with hierarchical clustering was calculated using rlog-transformed read counts of 42,315 transcripts. (**b**) mRNA expression level of pan-neuronal genes (*MAP2*, *RBFOX3*), synaptic genes specific to Glu neurons (*SLC17A6*, *SLC17A6*) or GABA neurons (*SLC32A1*), GABA-specific genes (*GAD1*, *GAD2*), astrocyte marker genes (*GFAP*, *AQP4*) and oligodendroglia-lineage gene (*OLIG2, PLP1*) measured by RNA-seq in H1 and F2 derived neurons. Each gene expression value is provided in the bar plot (± s.e.). Each dot represents an independent sample. (**c**) UCSC Genome browser tracks for the marker gene loci in (**b**), indicating chromatin accessibility (ATAC) and gene expression (RNA). Robust expression and chromatin accessibility of genes associated with the neuronal identity and subtype specificity, but not glial lineage, is observed in Glu and GABA neurons. (**d**) mRNA expression level changes measured by RNA-seq of H1 and F2 derived neurons at 4 h after membrane depolarization, compared to the expression level in unstimulated cultures represented by MA-plot. Genes with a significantly different gene expression level and fold-change magnitude above the threshold after depolarization are marked purple for Glu neurons and orange for GABA neurons. (**e**) Box and whisker plot displaying fold changes in the mRNA levels of different ERG TFs after membrane depolarization of Glu and GABA neurons as measured by RNAseq.

Figure S3. Transcriptional dynamics in Glu and GABA neurons upon membrane depolarization (Related to Figure 2).

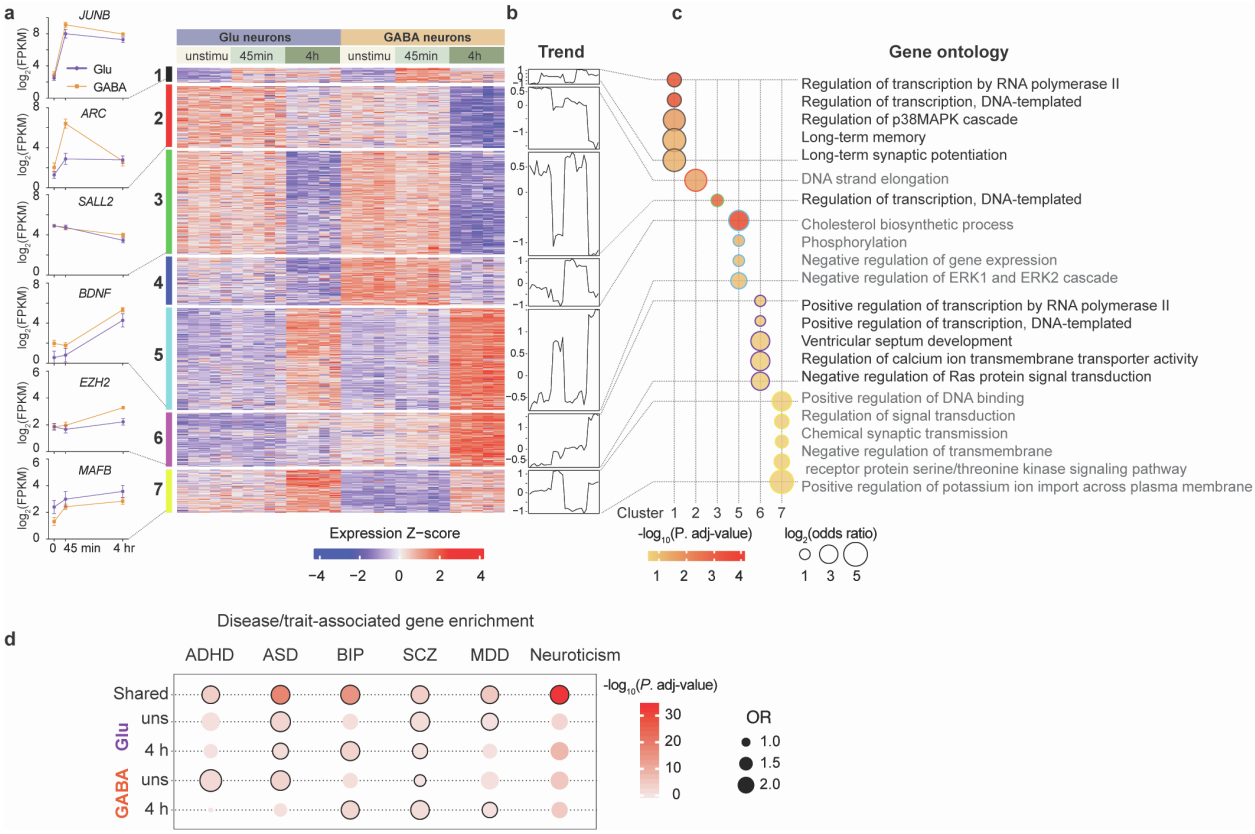

Figure S3. (**a**) Partitioning around medoids clustering-of-expression dynamics using genewise-scaled Z-scores of 2,711 DE-genes (5 samples/condition; k=7 clusters). Numbers indicate clusters. Left line plots indicate expression levels for signature genes selected for the indicated cluster. (**b**) The trend line plots indicate the average gene expression level of each sample in the corresponding cluster. (**c**) Gene Ontology enrichment analysis for clusters shown in (**a, b**). Each ribbon shows clusters with their respective significant gene groups using Enrichr. Up to five significant terms per cluster are shown (*P*-values adjusted with the Benjamini-Hochberg method for correction). No significant enrichment was observed for Cluster 4. Circle sizes indicate enrichment of ontology genes in each cluster. Names on the right y-axis indicate common ontology names. (**d**) Dot plot shows the neuropsychiatric disorder or brain trait-associated gene enrichment analysis results. The dot size is based on the odds ratio. The color shows the significance of enrichment for common neuronal genes, cell-type-specific genes, or activity-regulated genes displayed as -log10 (adjusted *P*-value). Dots with black circles indicate an adjusted *P*-value less than 0.05.

Figure S4. Disease heritability enrichment in H3K27ac regions (Related to Figure 5).

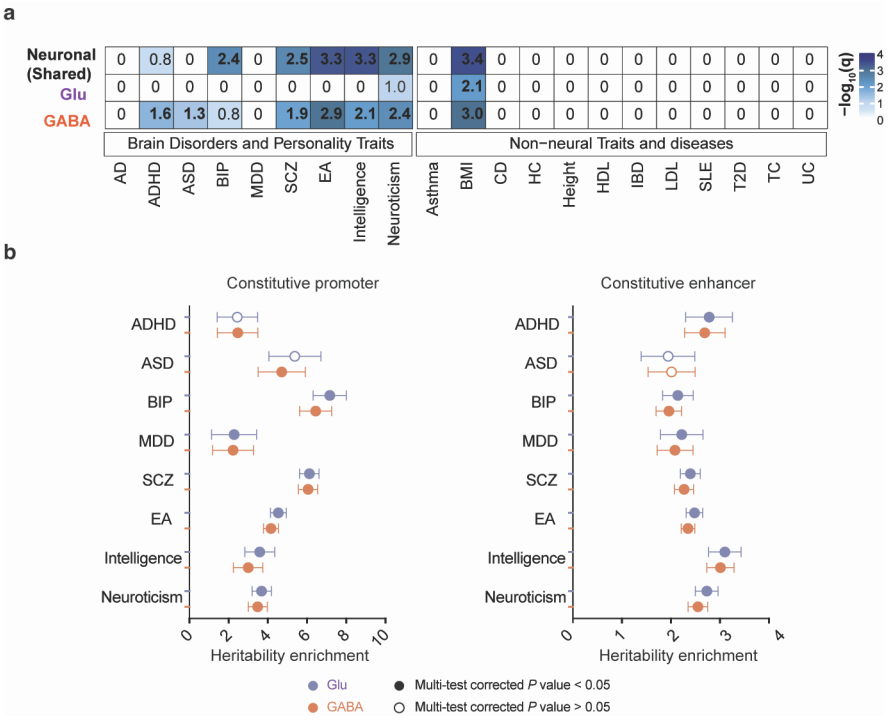

Figure S4. (**a**) Heatmap of LDSC analysis for genetic variants associated with brain disorders and behavior traits displayed as -log$_{10}$(q) value for significance of enrichment for shared or cell-type-specific H3K27ac regions in Glu and GABA neurons. Bold text indicates a q-value less than 0.05. The top 17,362 peaks from Glu and GABA neuron datasets were used for the analysis. (**b**) Heritability enrichment of constitutive promoter regions and enhancer regions in Glu and GABA neurons across neuropsychiatric disorders and behavioral traits. Each heritability enrichment value is provided in mean ± s.e. Filled circle indicates a multitest corrected *P*-value less than 0.05.

Figure S5. Genes linked by ABC-Max to psychiatric diseases via activity-responsive enhancers (Related to Figure 6).

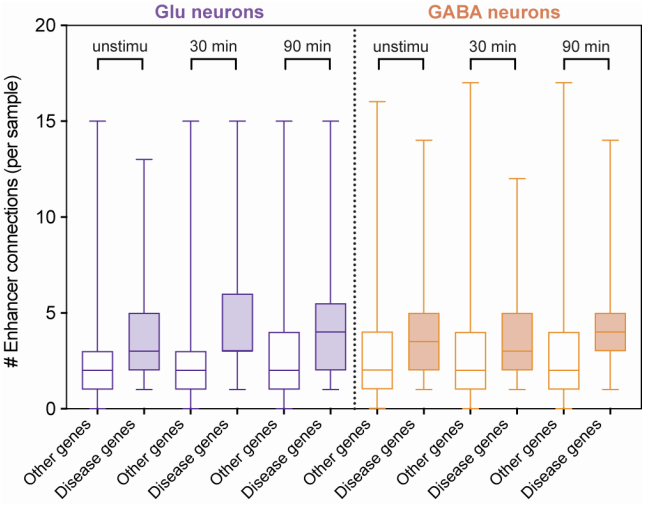

Figure S5. Non-activity responsive genes linked to disease via SNPs in activity-inducible enhancers have more ABC-enhancers in the samples where gene expression is detected. Box and whisker plot shows the number of ABC enhancer-gene connections in each condition (min to the max; median).

Table S7. Oligonucleotide sequences. Related to Figures 2&S1.

Table S7A. Quantitative PCR primers.

Gene ID	Primer forward	Primer reverse
TUBB3	GCAACTACGTGGGCGACT	CGAGGCACGTACTTGTGAGA
FOS	AAAGGAGAATCCGAAGGGAAAG	GTTGGTCTGTCTCCGCTTG
NPAS4	TGGGTTTACTGATGAGTTGCAT	TTCCCCTCCACTTCCATCTT
SYN1	GACGGAAGGGATCACATCAT	CTGGTGGTCACCAATGAGC
EGR1	TCGGACATGACAGCAACCT	TTTCCCCTTTCCCTTTAGCA
BDNF	GAGTGGCCATCCCAAGGT	CTTCAGAGGCCTTCGTTTTG
VGF	CTCATTGAGCTGTCCACCAA	GGAGGGGCGTTCTTCTTC

Table S7B. ATAC-seq library preparation primers.

PCR primer name	Sequence
v2_Ad1_No-MX	TCGTCGGCAGCGTCAGATGTGTAT
Custom Barcodes (index i7):	**Adapter**
v2_Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.13_TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.14_CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.15_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.16_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.17_GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.18_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.19_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.20_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.21_CGCGGACA	CAAGCAGAAGACGGCATACGAGATTGTCCGCGGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.22_TTCCATAT	CAAGCAGAAGACGGCATACGAGATATATGGAAGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.23_AATTCGTT	CAAGCAGAAGACGGCATACGAGATAACGAATTGTCTCGTGGGCTCGGAGATGTG
v2_Ad2.24_GGCGTCGA	CAAGCAGAAGACGGCATACGAGATTCGACGCCGTCTCGTGGGCTCGGAGATGTG