
Supplementary information

Antisense oligonucleotide therapeutic approach for Timothy syndrome

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Antisense oligonucleotide therapeutic approach for Timothy syndrome

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Supplementary Table 1. Primer sequences used in this study.

Supplementary Table 2. PCR cycling conditions.

Supplementary Table 3. HEK cells transfection scheme used for minigene splicing reporters.

Supplementary Table 4. HEK cells transfection scheme used for studying the role of PTBP1 on exon 8A and exon 8 splicing.

Supplementary Table 5. HEK cells transfection scheme used for GCaMP imaging shown in **Extended Data Fig. 8**.

Supplementary Figure 1

Gating strategy used for the flow cytometry data presented in **Fig. 2**

hCO not treated (**a**) and treated with Cy5-ASO (**b**) were dissociated and stained with the neuronal cell surface protein CD90.

Supplementary Figure 2

Full Western blot images for the data shown in **Extended Data Fig. 10b**

Supplementary Figure 3 Plasmid map and sequence of the WT minigene splicing reporter.

Supplementary Figure 4 Plasmid map and sequence of the TS minigene splicing reporter.

Supplementary Figure 5 Plasmid map and sequence of over expression plasmids of human WT *CACNA1C*.

Supplementary Figure 6 Plasmid map and sequence of over expression plasmids of human *CACNA1C* containing TS mutation.

Supplementary Notes

Source code in R for analyzing the GCaMP imaging shown **Extended Data Fig. 8**

Supplementary Table 1

Name	Sequence	Purpose
NGS.exon7-9.S.2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTacaacttgccttcgccatg	NGS
NGS.exon7-9.AS.2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTgtcttcggcctgagtgatcc	
mini.NGS.S.2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTatgaagttggtggtgaggcc	
mini.NGS.AS.2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTaggagtggacagatcccaaa	
GAPDH.S	CATGAGAAGTATGACAACAGCCT	qPCR-human
GAPDH.AS	AGTCCTTCCACGATACCAAAGT	
qPCR.exon8.S	ACGCTATGGGCTATGAGTTACC	
qPCR.exon8.AS	GGCCTTCTCCCTCTCTTTG	
qPCR.exon8A.S	TTTGACAACCTTGCCTTCGC	
qPCR.exon8A.AS	TCCCTTCCTACGGCATCATT	qPCR-rat
Rat.Gapdh.S	CAACTCCCTCAAGATTGTCAGCAA	
Rat.Gapdh.AS	GGCATGGACTGTGGTCATGA	
Rat.Exon8.S.1	ACGCTATGGGCTATGAGTTGCC	
Rat.Exon8.AS.1	GGCTTTCTCCCTCTCTTTG	
Rat.Exon8a.S	TTGACAACCTTCGCCTTCGC	
Rat.Exon8a.AS.1	TCCCTTCCTACGGCATCATT	Cloning of minisplicing reporter
1.Minigene.AS.bgiii	TGCCCAGATCTCTCCTCCATTGTCCACCACC	
1.Minigene.S.apa	TGATAGGGCCCGCGGGCCGAGGTCCCCTTCG	RFLP
CA.RT.S2	GATGACCCTTCCCCTTGTGC	
CA.RT.AS2	CATCCATGCCTTCGTCCTCA	Minisplicing reporter RT-PCR
PCR.mini.S.1	AGAAGTCTGCCGTTACTGCC	
PCR.mini.AS.3	AACCTCTGGGTCCAAGGGTA	Off-target assessment
DGKK.S	AGAAGAGATGAACACCCAGGGCAA	
DGKK.AS	GCAGGTTTGGCAAGGAGATGGTTT	
TMEM105.S	TGGCAGCAGGGATAACAG	
TMEM105.AS	TGAGCAACAGAGCAAGACT	
Cav1.3-F	CTTCGACAACGTCCTCTCTGCT	
Cav1.3-R	GCCGATGTTCTCTCCATTGAG	
USP28.S	ACTCAGACTATTGAACAGATGTACTGC	
USP28.AS	CTGCATGCAAGCGATAAGG	

Supplementary Table 2

Purpose	PCR cycling condition	Polymerase
NGS	98°C 2min	Phire Tissue Direct PCR Master Mix
	98°C 7s, 65°C 7s, 72°C 20s for 40 cycles	
	72°C 1min	
Cloning of minigene Splicing reporter	95°C 2min	GoTaq Long PCR Master Mix
	92°C 30s, 65°C 1min for 35 cycles	
	72°C 10min	
RFLP	98°C 2min	Phire Tissue Direct PCR Master Mix
	98°C 7s, 66°C 7s, 72°C 20s for 40 cycles	
	72°C 1min	
Minisplicing reporter RT-PCR	98°C 2min	Phire Tissue Direct PCR Master Mix
	98°C 7s, 68°C 7s, 72°C 20s for 40 cycles	
	72°C 1min	

Supplementary Table 3

	24-well plate	
HEK 293T Cells	30,000-75,000 cells/well (1.65 μ l PEI per well, medium replaced at 24 h post transfection)	
Plasmids	pDup.8-8a ^{WT}	pDup.8-8a ^{TS}
DNA (ng)		600
	600	

Supplementary Table 4

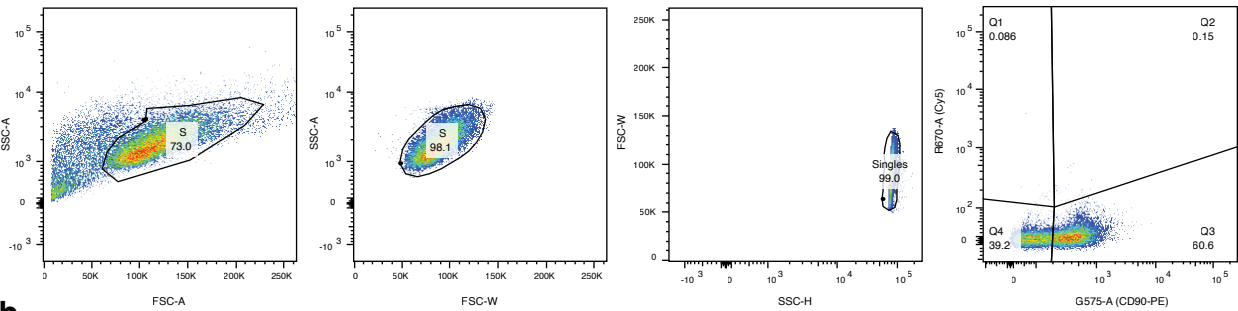
	24-well plate		
HEK 293T cells	30,000-75,000 cells/well (1.65 μ l PEI per well, medium replaced at 24 h post transfection)		
	pDup.8-8a ^{WT}	pDup.8-8a ^{TS}	pPTBP1
Plasmids DNA (ng)	300		
	300		300
		300	
		300	300

Supplementary Table 5

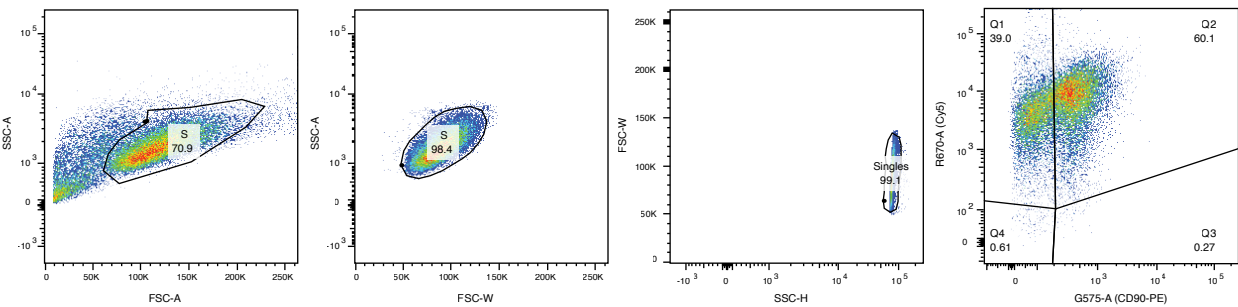
	24-well plate				
HEK 293T cells	30,000-75,000 cells/well (1.65 μ l PEI per well, medium replaced at 24 h post transfection)				
	pLV.CAG.CACNA1C	pLV.CAG.CACNA1C.TS	pCMV.GCaMP6	p β 1b	p α 2 δ
Plasmids DNA (ng)	400	0	100	200	200
	390	10	100	200	200
	380	20	100	200	200
	370	30	100	200	200
	360	40	100	200	200
	350	50	100	200	200
	340	60	100	200	200
	330	70	100	200	200
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	0	400	100	200	200

Supplementary Figure 1

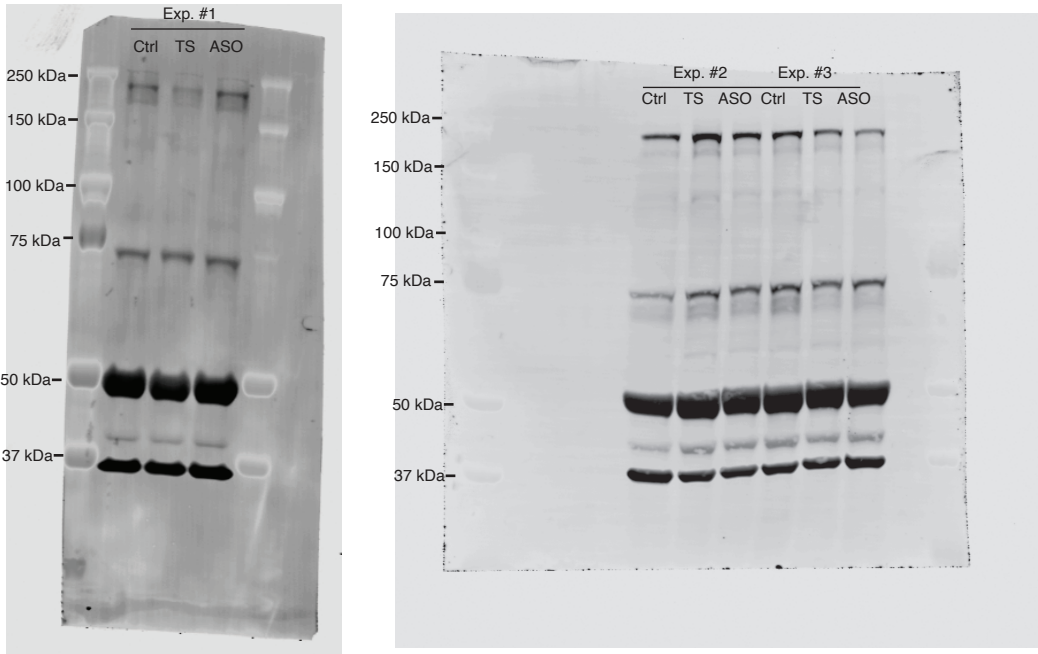
a Mock



b Cy5-ASO.14

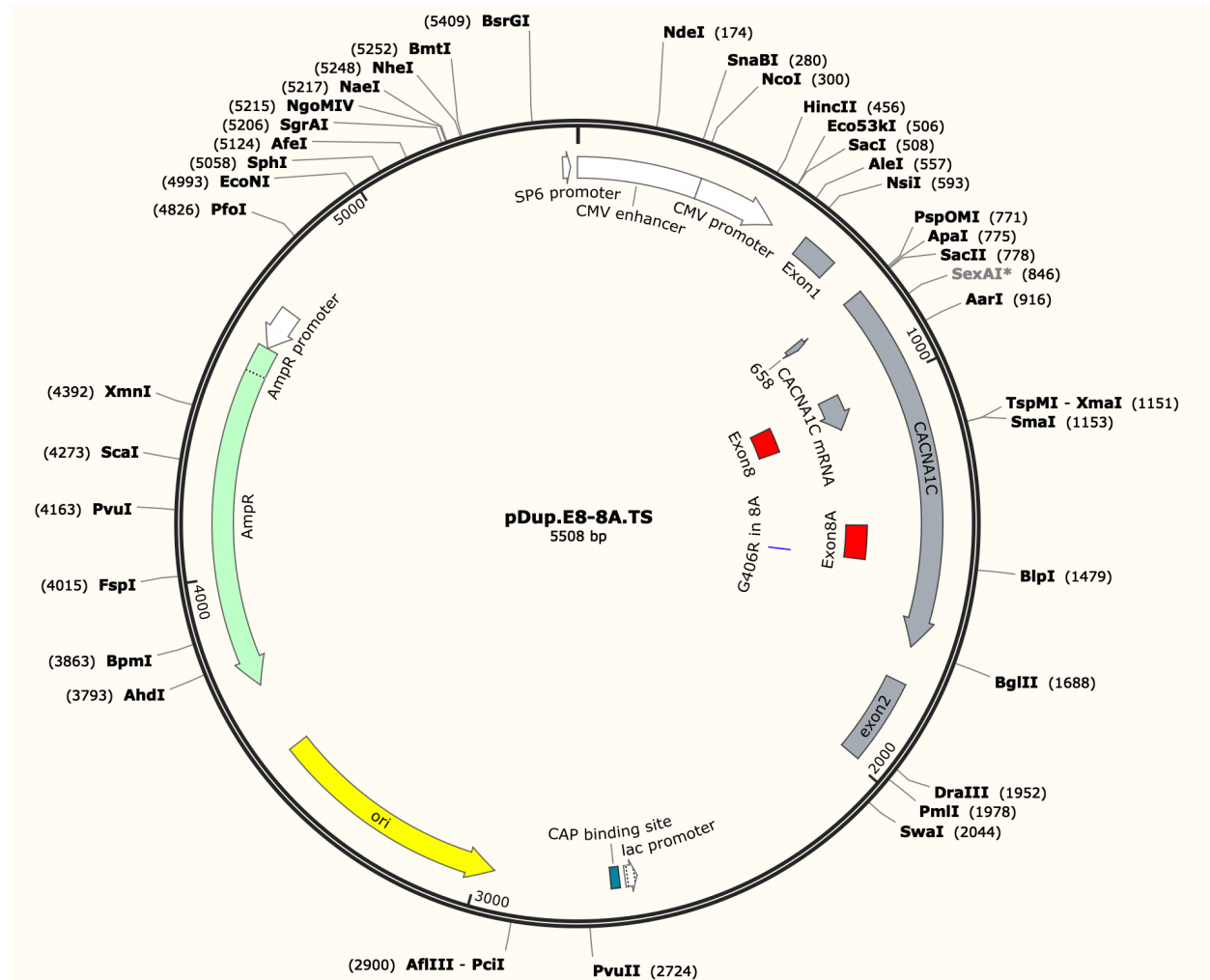


Supplementary Figure 2



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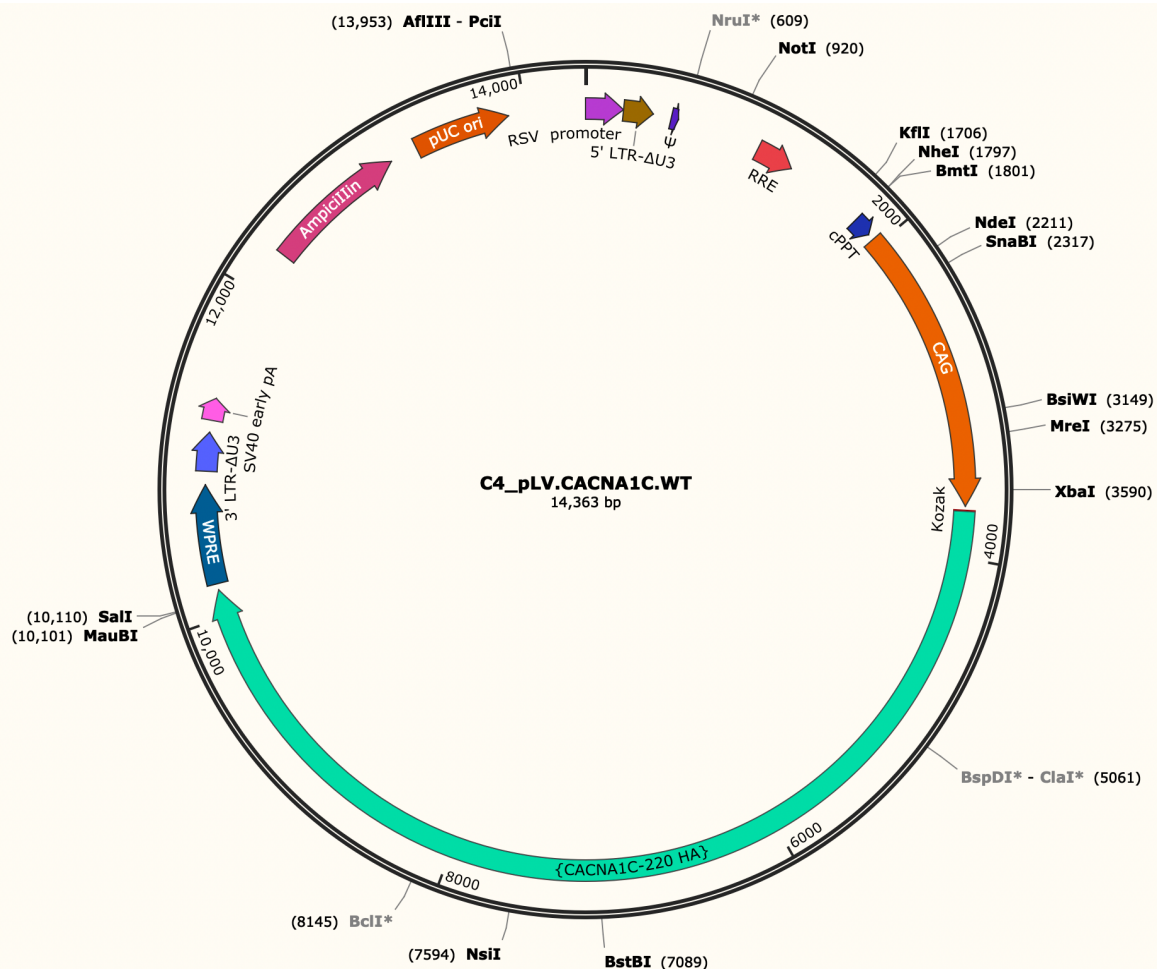
Supplementary Figure 4



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Supplementary Figure 5

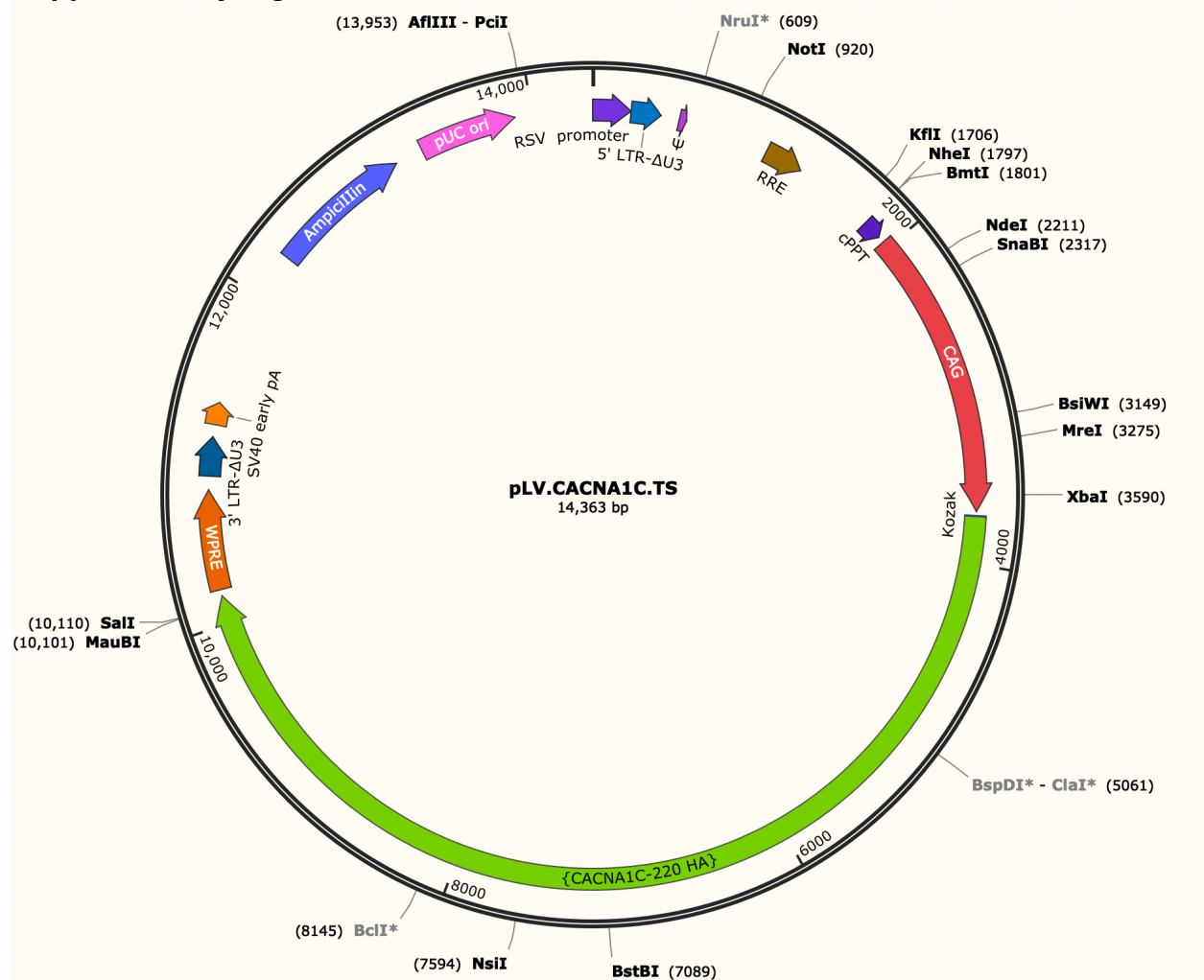


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Supplementary Figure 6



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GATTACGCCAAGCGCGCAATTAACCTCACTAAAGGGAACAAAAGCTGGAGCTGCAAGCTT

Supplementary Notes

```
---# This code normalizes the mean gray values to the baseline, removes low respondents & technical noise
```

```
---# and calculates the residual calcium
```

```
# Assuming you have the 'readxl' package installed  
library(readxl)
```

```
# Specify the path to your Excel file  
excel_file <- "/path/to/folder/sample1.xlsx"
```

```
# Read the data from the Excel sheet  
data <- read_excel(excel_file)
```

```
# Calculate the average baseline value of each cell  
cell_averages <- apply(data[1:30, ], 2, mean)
```

```
# Normalize the data based on the average value of each cell  
data <- sweep(data, 2, cell_averages, FUN = "/")
```

```
# Calculate the mean of the baseline period  
baseline_means <- apply(data[1:30, ], 2, mean)
```

```
# Calculate the threshold for response amplitude  
thresholds_min <- baseline_means * 1  
thresholds_max <- baseline_means * 20
```

```
# Identify cells with response amplitudes within the threshold range  
selected_cells <- apply(data[31:nrow(data), ], 2, function(cell) {  
  response_amplitude <- max(cell, na.rm = TRUE) - baseline_means  
  response_amplitude >= thresholds_min & response_amplitude <= thresholds_max  
})
```

```
# Convert the data to numeric format  
data <- as.data.frame(apply(data, 2, as.numeric))
```

```
# Filter out cells that have response amplitudes less than the threshold  
filtered_data <- data[, apply(selected_cells, 2, any), drop = FALSE]
```

```
# Save filtered and normalized data  
write.csv(filtered_data, "/path/to/folder/filt.csv")
```

```
# Calculating the residual calcium
```

```
# Assuming your data is stored in a variable called 'data'  
# where each column represents a cell and each row represents a timepoint
```

```
# Calculate the number of timepoints  
num_timepoints <- nrow(filtered_data)
```

```
# Initialize vectors to store the results  
peaks <- vector("numeric", ncol(filtered_data))
```

```

values_A <- vector("numeric", ncol(filtered_data))
values_B <- vector("numeric", ncol(filtered_data))
values_C <- vector("numeric", ncol(filtered_data))
ups <- vector("numeric", ncol(filtered_data))
downs <- vector("numeric", ncol(filtered_data))
residuals <- vector("numeric", ncol(filtered_data))

# Loop over each cell
for (i in 1:ncol(filtered_data)) {
  # Find the index of the peak value within frame 10 and 40
  peak_index <- which.max(filtered_data[10:40, i]) + 10

  # Check if peak_index is within valid range
  if (peak_index >= 21 && peak_index <= num_timepoints - 200) {
    # Extract values A, B, and C
    value_A <- filtered_data[peak_index - 20, i]
    value_B <- filtered_data[peak_index, i]
    value_C <- filtered_data[peak_index + 200, i]

    # Calculate 'up' and 'down'
    up <- value_C - value_A
    down <- value_B - value_A

    # Calculate 'residual'
    residual <- up / down

    # Store the results
    peaks[i] <- peak_index
    values_A[i] <- value_A
    values_B[i] <- value_B
    values_C[i] <- value_C
    ups[i] <- up
    downs[i] <- down
    residuals[i] <- residual
  }
}

# Create a data frame with the results
result <- data.frame(Cell = 1:ncol(filtered_data),
  PeakIndex = peaks,
  ValueA = values_A,
  ValueB = values_B,
  ValueC = values_C,
  Up = ups,
  Down = downs,
  Residual = residuals)

# Filter out cells with NA residuals or residuals smaller than -5 or larger than 5
filtered_result <- result[!is.na(result$Residual) & result$Residual > -5 & result$Residual < 5 &
result$Residual != 0, ]

print(filtered_result)

```

```
write.csv(filtered_result, "/path/to/folder/residuals.csv")
```