Supplementary Information

Assembly and Validation of Versatile Transcription Activator-Like Effector Libraries

Yi Li[1,2], Kristina Ehrhardt[1,2], Michael Q. Zhang[2,3], and Leonidas Bleris[1,2,4]

[1]Bioengineering Department, The University of Texas at Dallas, 800 West Campbell Road, Richardson TX 75080 USA

[2]Center for Systems Biology, The University of Texas at Dallas, 800 West Campbell Road, Richardson TX 75080 USA

[3]Molecular and Cell Biology Department, The University of Texas at Dallas, 800 West Campbell Road, Richardson TX 75080 USA

[4]Electrical Engineering Department, The University of Texas at Dallas, 800 West Campbell Road, Richardson TX 75080 USA

[*]Corresponding author: Leonidas Bleris, The University of Texas at Dallas, NSERL 4.708, 800 West Campbell Road, Richardson TX 75080 USA

Email: bleris@utdallas.edu

Phone: 972-883-5785

Supplementary Methods

Library construction

A modified protocol based on the Golden Gate Assembly (Addgene, #1000000016) was used to construct the 11-mer TALE-VP16 library[1,2].

Part 1: Construction of a pFUS_A library containing all possible combinations of 10 tandem repeats (RVDs 1-10)

Step 1 (Day 1, 3 h)| The Golden Gate Assembling reactions were performed in strip PCR tubes (8 well, Applied Biosystems, #N801-0560). In a standard reaction, 37.5 ng of each of the 40 RVD building blocks (NN1 to NN10, NI1 to NI10, NG1 to NG10, and HD1 to HD10) were included. Then, 150 ng of pFUS_A plasmid, 1 µL of BsaI (New England Biolabs, #R0535S), 1 µL of BSA (3 mg/mL, New England Biolabs, #B9000S), 1 µL of T4 DNA Ligase (New England Biolabs, #M0202S), 3 µL of T4 DNA Ligase Reaction buffer (New England Biolabs, #B0202S) were added. Nuclease free water (Life Technologies, #10977) was then added to bring to 30 µL of total reaction volume.

Step 2 (Day 1, 10 min)| The mixture was mixed and briefly spun down.

Step 3 (Day 1-Day 2, 13 h)| The mixture was then incubated on a Thermal Cycler (Bio-Rad, #S1000). The thermal cycling conditions were 50 cycles of 5 min at 37°C and 10 min at 16°C, followed by 1 cycle of 5 min at 50°C, and 1 cycle of 5 min at 80°C. The number of digestion/ligation reaction cycles was increased to 50 to ensure the highest possible reaction efficiency.

Step 4 (Day 2, 1 hr)| Subsequently, the reaction mixture (30 µL) was treated with 1 µL of Plasmid-Safe ATP-Dependent DNase (Epicentre, #E3110K) and 1 µL of 10 mM ATP (New England Biolabs, #P0756S) at 37°C for 1 hour.

Step 5 (Day 2, 4 h)| 6 transformations were then performed. For each transformation, 5 µL of the reaction mixture was transformed into 100 µL of the XL10-Gold Ultracompetent cells (Agilent, #200314). The resulting 1 mL of cell suspension was then plated onto 2 10cm LB agar plates with Carbenicillin (100 µg/mL, Teknova, #C2113). In total, 12 plates were used and a lawn, which indicates the cells grew to full

confluency, was observed on all plates. This ensures that at least 1,048,576 independent clones for the pFUS_A library were collected.

Step 6 (Day 3, 6 h)| The cells were harvested after 16 hours and the plasmids were then prepared using the Qiagen Plasmid Midi Kit (Qiagen, #12143).

Part 2: Construction of a pFUS_B library containing all possible combinations of 1 tandem repeat (RVD 1)

Step 1 (Day 1, 3 h)| The Golden Gate Assembling reactions were performed in strip PCR tubes (8 well, Applied Biosystems, #N801-0560). In a standard reaction, 37.5 ng of each of the 4 RVD building blocks (NN1, NI1, NG1 and HD1) were included. Then, 150 ng of pFUS_B plasmid, 1 µL of BsaI (New England Biolabs, #R0535S), 1 µL of BSA (2 mg/mL, New England Biolabs, #B9000S), 1 µL of T4 DNA Ligase (New England Biolabs, #M0202S), 2 µL of T4 DNA Ligase Reaction buffer (New England Biolabs, #B0202S) were added. Nuclease free water (Life Technologies, #10977) was then added to bring to 20 µL of total reaction volume.

Step 2 (Day 1, 10 min)| The mixtures were mixed and briefly spun down.

Step 3 (Day 1-Day 2, 13 h)| The mixtures were then incubated on a Thermal Cycler (Bio-Rad, #S1000). The thermal cycling conditions were 50 cycles of 5 min at 37°C and 10 min at 16°C, followed by 1 cycle of 5 min at 50°C, and 1 cycle of 5 min at 80°C.

Step 4 (Day 2, 1 hr)| Subsequently, the reaction mixture (30 µL) was treated with 1 µL of Plasmid-Safe ATP-Dependent DNase (Epicentre, #E3110K) and 1 µL of 10 mM ATP (New England Biolabs, #P0756S) at 37°C for 1 hour.

Step 5 (Day 2, 4 h)| One transformation was then performed. 5 µL of the reaction mixture was transformed into 100 µL of the XL10-Gold Ultracompetent cells (Agilent, #200314). The resulting 1 mL of cell suspension was then plated onto 2 10cm LB agar plates with Carbenicillin (100 µg/mL, Teknova, #C2113). In total, 2 plates were used and a lawn, which indicates the cells grew to full confluency, was observed on both plates. This ensures that at least 4 independent clones for the pFUS_B library were collected.

Step 6 (Day 3, 6 h)| The cells were harvested after 16 hours and the plasmids were then prepared using the Qiagen Plasmid Midi Kit (Qiagen, #12143).

Part 3: Construction of a TALE-VP16 library containing all possible combinations of 11 tandem repeats (RVDs 1-11)

The pFUS_A (RVDs 1-10) and pFUS_B (RVD 1) plasmid libraries were conjoined.

Step 1 (Day 4, 3 h)| Specifically, in a standard 20 µL reaction, 150 ng of pFUS_A (RVDs 1-10) plasmid library, 150 of pFUS_B (RVD 1) plasmid library, 150 ng of pLR-HD vector (the last "half-repeat"), and 75 ng of PTAL1-VP16 were included. Then 1 µL of Esp3I (Thermo Scientific, #ER0452), 1 µL of T4 DNA Ligase (New England Biolabs, #M0202S), 2 µL of T4 DNA Ligase Reaction buffer (New England Biolabs, #B0202S) were added. Nuclease free water (Life Technologies, #10977) was then added up to 20 µL of total reaction volume. Four such reactions were set up.

Step 2 (Day 4, 10 min)| The mixtures were mixed and briefly spun down.

Step 3 (Day 4-Day 5, 13 h)| The mixtures were then incubated on a Thermal Cycler (Bio-Rad, #S1000). The thermal cycling conditions were 50 cycles of 5 min at 37°C and 10 min at 16°C, followed by 1 cycle of 15 min at 37°C, and 1 cycle of 5 min at 80°C. Similar to before, the number of digestion/ligation reaction cycles was increased to 50 to ensure the highest possible reaction efficiency.

Step 4 (Day 5, 1 hr)| The reaction mixtures were then treated with 1 µL of KpnI (New England Biolabs, #R0142S) at 37°C for 1 hour to linearize the unused PTAL1-VP16 plasmids.

Step 5 (Day 5, 4 h)| 20 transformations were then performed. For each transformation, 4 µL of the reaction mixture was transformed into 100 µL of the XL10-Gold Ultracompetent cells (Agilent, #200314). The resulting 1 mL of cell suspension was then plated onto 2 10cm LB agar plates with Carbenicillin (100 µg/mL, Teknova, #C2113). In total, 40 plates were used and a lawn, which indicates the cells grew to full confluency, was observed on all plates. This ensures that at least 4,194,304 independent clones for the 11-mer TALE-VP16 library were collected.

Step 6 (Day 6, 6 h)| The cells were harvested after 16 hours and the plasmids were then prepared using the Qiagen Plasmid Midi Kit (Qiagen, #12143).

Step 7 (Day 7)| The libraries were then subjected to standard Sanger sequencing using the forward primer (P23, 5'-CGTCGCTGTCACGTATCAGCACATAATC-3') and the reverse primer (P24, 5'-GCGTACGCTGGGTGGGAGTGGCAC-3'). The sequencing profiles were then analyzed using FinchTV (Geospiza).

Step 8 (Day 7, 2 h)| The pFUS_A (RVDs 1-10), pFUS_B (RVD 1), and the final 11-mer TALE-VP16 libraries were also subjected to 0.7% agarose gel electrophoresis along with the original pFUS_A, pFUS_B and PTAL1-VP16 mother vectors. The electrophoresis image showed that the libraries were free of noticeable contamination from their respective mother vectors (**Supplementary Fig. 11**, from left to right: 2-Log DNA ladder (New England Biolabs, #N3200S), pFUS_A (RVDs 1-10), pFUS_A, pFUS_B (RVD 1), pFUS_B, 2-Log DNA ladder, 11-mer TALE-VP16 library, and PTAL1-VP16).

Part 4: Construction of a biased TALE-VP16 library for the p53-responsive element

Similar procedures (Steps 1-3) were used to prepare for the p53-responsive element-targeting TALE-VP16 library, which targets 5'-RRRCWWGYYYN-3'. For each position of the Golden Gate Assembly reactions, the amounts of the 4 building blocks were adjusted according to the desired nucleotide target. Specifically, 37.5 ng of each of the four building blocks were mixed when the nucleotide target is N. 75 ng of HD and NG were mixed when the target is Y. 75 ng of NI and NG were mixed when the target is W. 75 ng of NI and NN were mixed when the target is R. For the final assembling reactions, at least 1,024 independent clones were harvested for preparation of the plasmid libraries. All libraries were then subjected to standard Sanger sequencing using the forward primer (P23, 5'-CGTCGCTGTCACGTATCAGCACATAATC-3') and the reverse primer (P24, 5'-GCGTACGCTGGGTGGGAGTGGCAC-3'). The sequencing profiles were then analyzed using FinchTV (Geospiza).

Yeast one-hybrid assay

The Matchmaker Gold Yeast One-Hybrid Screening System was purchased from Clontech (# 630491). The TALE-VP16 library-based yeast one-hybrid assay was performed according to the manufacturer's protocol (Clontech, #PT4087-1). To prepare for the bait yeast strain, the bait plasmids were linearized using BstBI (New England Biolabs, #R0519S) and then transformed into Y1HGold yeast cells using Yeastmaker Yeast Transformation System 2 (Clontech, #630439), closely following the manufacturer's protocol (Clontech, #PT1172-1). The yeast bait stable clones were then selected by SD/-Ura agar medium (Clontech, # 630315). The 11-mer TALE-VP16 cDNA library was PCR amplified using the 11-mer TALE-VP16 plasmid library as the template and primers P27 and P28 (**Supplementary Table 1**). The VP16 domain was fused with the TALE DBD (DNA binding domain) in addition to the GAL4 activation domain within the pGADT7 destination plasmid to further enhance the transactivation activities. The 11-mer TALE-VP16 cDNA library was transformed into the bait yeast cells using the Yeastmaker Yeast Transformation System 2. In each experiment, ~1-1.2 x 10^6 individual clones were plated onto 50 150-mm agar plates. Briefly, the library-scale transformation protocol was used, which resulted in 15 mL of the transformation solution in 0.9% (w/v) NaCl solution. To calculate the number of total individual clones, 10 µL of the transformation solution was diluted into 990 µL of the 0.9% (w/v) NaCl solution. Subsequently, 100 µL of this diluent was plated onto one 10cm SD/-Leu agar plate. In a representative experiment, 72 clones were recovered from this control plate, which indicates that the total individual clone number was: 72 x (15 mL/1 µL) = 1.08 x 10^6. The positive clones were then selected by using SD/-Leu agar medium (Clontech, #630311) containing either 100 ng/ml Aureobasidin A (Clontech, #630466) or 0.5 µg/ml cycloheximide (Sigma-Aldrich, #C7698-1G). Subsequently, the positive clones were cultured in SD/-Leu broth (Clontech, #630310) and the pGADT7-TALE-VP16 plasmids were then prepared using the PrepEase Yeast Plasmid Isolation Kit (Affymetrix, #79220). The plasmids were then rescued into XL10-Gold Ultracompetent cells (Agilent, #200314) and prepared using the Qiagen Plasmid Mini Kit (Qiagen, #12123).

Yeast one-hybrid assay false-positives: To investigate the false-positive issue we used TALE-NT 2.0 to determine all possible target sites within the pAbAi backbone for the TALE fusions isolated from our experiments (13 for SCN9A, and 4 for miR-34b). Of the 68 possible TALE RVDs (the original 11-mer plus one additional RVD being NI, NN, NG or HD), only 9 of them are predicted to have one additional target within the pAbAi backbone (**Supplementary Table 4**). Importantly, all these targets are significantly far from the translation start site (ATG) of the Aba resistance gene (larger than 1 kb) and naturally have no/minimal effect on its expression activity (the average length of promoters in *s. cerevisiae* is ~455bp[3]).

Quantitative reverse transcription-PCR

In mammalian cells, 48 hours post transfection, total RNA was extracted using the RNeasy Mini Kit (Qiagen, #74104). First strand synthesis was performed using the QuantiTect Reverse Transcription Kit (Qiagen, #205311). Quantitative PCR was performed using the KAPA SYBR FAST Universal qPCR Kit (KAPA Biosystems, #KK4601, KAPA Biosystems). GAPDH was used for normalization. The forward primer for GAPDH was 5'-AATCCCATCACCATCTTCCA-3', and the reverse primer for GAPDH was 5'-TGGACTCCACGACGTACTCA-3'. The forward primer for SCN9A was 5'-CGTGGACAAACACTTGATGG-3', and the reverse primer for SCN9A was 5'-GTCACTGTGAGGCTGGGATT-3'. The forward primer for VP16 was 5'-CTCCACTTAGACGGCGAGGA-3', and the reverse primer for VP16 was 5'-GAAGTCGGCCGTATCCAGAG-3'.The thermal cycling conditions were 3 min at 95°C followed by 40 cycles of denaturation for 15 s at 95°C and annealing for 30 s at 60°C. The relative expression levels of SCN9A were determined by $2^{-\Delta\Delta Ct}$ analysis. The values of fold-change are reported as mean with standard deviation.

In yeast, the yeast transformants containing pGADT7-TALE-A8-VP16, pGADT7-TALE-A35-VP16 or pGADT7 were grown in SD-Leu broth (Clontech, #630310) until reaching stationary phase. Total RNA was extracted using the MasterPure Yeast Purification Kit (Epicentre, #MPY80200). First

strand synthesis was performed using the QuantiTect Reverse Transcription Kit (Qiagen, #205311). Quantitative PCR was performed using the KAPA SYBR FAST Universal qPCR Kit (KAPA Biosystems, #KK4601). Yeast ACT1 was used for normalization. The forward primer for ACT1 was 5'-TTGGCCGGTAGAGATTTGACTGAC-3', and the reverse primer for ACT1 was 5'-AGCGGTTTGCATTTCTTGTTCG-3'. The forward primer for PDR3 was 5'-ATACTGCCGAACGGAGAAGA-3', and the reverse primer for PDR3 was 5'-CTGAAATCCTTCGGCAAGAG-3'. The forward primer for PDR5 was 5'-TGACGCTTTTGCATCAGTTC-3', and the reverse primer for PDR5 was 5'-GAGAAAACCGCGACAATGTT-3'. The thermal cycling conditions were 3 min at 95°C followed by 40 cycles of denaturation for 15 s at 95°C and annealing for 30 s at 60°C. The relative expression levels of PDR3 and PDR5 were determined by $2^{-\Delta\Delta Ct}$ analysis. The values of fold-change are reported as mean with standard deviation.

In mammalian cells, 48 hours post transfection, total RNA was extracted using the miRNeasy Micro Kit (Qiagen, #217084). First strand synthesis was performed using the miScript II RT Kit with HiSpec Buffer (Qiagen, #218161). Quantitative PCR was performed using the miScript SYBR Green PCR Kit (Qiagen, #218073). RNU6 was used for normalization. The miScript Primer Assays for Hs_RNU6 (Qiagen, # MS00033740) and Hs_miR-34b (Qiagen, #MS00008190) were used. The thermal cycling conditions were 15 min at 95°C followed by 40 cycles of denaturation for 15 s at 94°C, annealing for 30 s at 55°C, and extension for 30 s at 70°C. The relative expression levels of miR-34b were determined by $2^{-\Delta\Delta Ct}$ analysis. The values of fold-change are reported as mean with standard deviation.

Molecular cloning

All restriction enzymes were purchased from New England Biolabs. All other reagents, unless specified, were purchased from Sigma-Aldrich.

pAbAi-SCN9A: Total DNA was isolated from HEK293 cells using the DNeasy Blood & Tissue Kit (Qiagen, #69504). Part of the 5'-UTR and ORF of the human SCN9A gene was amplified by using the HEK293 cells' total DNA as the template and primers P1 and P2, and then cloned into pAbAi (Clontech, #630491) using HindIII and XhoI.

pAbAi-SCN9A(ΔATACAGGATGA): A mutagenesis PCR reaction was performed using pAbAi-SCN9A as the template and primers P3 and P4. The PCR product was digested with SacII and then ligated to produce pAbAi-SCN9A(ΔATACAGGATGA).

pAbAi-SCN9A(ΔTATGTGAGGAGCTGA): A mutagenesis PCR reaction was performed using pAbAi-SCN9A as the template and primers P5 and P6. The PCR product was digested with SacII and then ligated to produce pAbAi-SCN9A(ΔTATGTGAGGAGCTGA).

pAbAi-SCN9A(ΔGTTGCCTCCCCC): A mutagenesis PCR reaction was performed using pAbAi-SCN9A as the template and primers P7 and P8. The PCR product was digested with SacII and then ligated to produce pAbAi-SCN9A(ΔGTTGCCTCCCCC).

PEF-1-SCN9A-T10/T17/T9/T110/T34-VP16: SCN9A-TALET10/T17/T9/T110/T34-VP16 were PCR amplified from pGADT7-SCN9A-T10/T17/T9/T110/T34-VP16 using primers P9 and P10, and then were cloned into PEF-1 using EcoRI.

PEF-1-SCN9A-T10/T17/T9/T110/T34-KRAB: The KRAB domain was extracted from PEF-1-TALETRE#3-KRAB using XbaI [14]. PEF-1-SCN9A-T10/T17/T9/T110/T34-VP16 were digested with XbaI and dephosphorylated with CIP (Calf Intestinal Alkaline Phosphatase, New England Biolabs, #M0290S), and then ligated with the KRAB domain to produce PEF-1-SCN9A-T10/T17/T9/T110/T34-KRAB.

pAbAi-miR-34b/c: Total DNA was isolated from HEK293 cells using the DNeasy Blood & Tissue Kit (Qiagen, #69504). Part of the promoter of human miR-34b/c gene was amplified by using the HEK293

cells' total DNA as the template and primers P11 and P12, and then cloned into pAbAi using HindIII and XhoI.

pAbAi-miR-34b/c(ΔTTTCTAGGTAT): A mutagenesis PCR reaction was performed using pAbAi-miR-34b/c as the template and primers P13 and P14. The PCR product was digested with SacII and then ligated to produce pAbAi-miR-34b/c(ΔTTTCTAGGTAT).

PEF-1-miR-34b/c-TALE-M1-VP16: miR-34b/c-TALE-M1-VP16 was PCR amplified from pGADT7-miR-34b/c-TALE-M1-VP16 using primers P15 and P16, and then was cloned into PEF-1 using EcoRI and KpnI.

pAbAi-mKATE2: MKATE2 was PCR amplified from PCMV-mKATE2 (Evrogen, #FP181) using primers P17 and P18. pAbAi was PCR amplified using primers P25 and P26. These two PCR products were digested with NheI and NotI and then ligated to produce pAbAi-mKATE2.

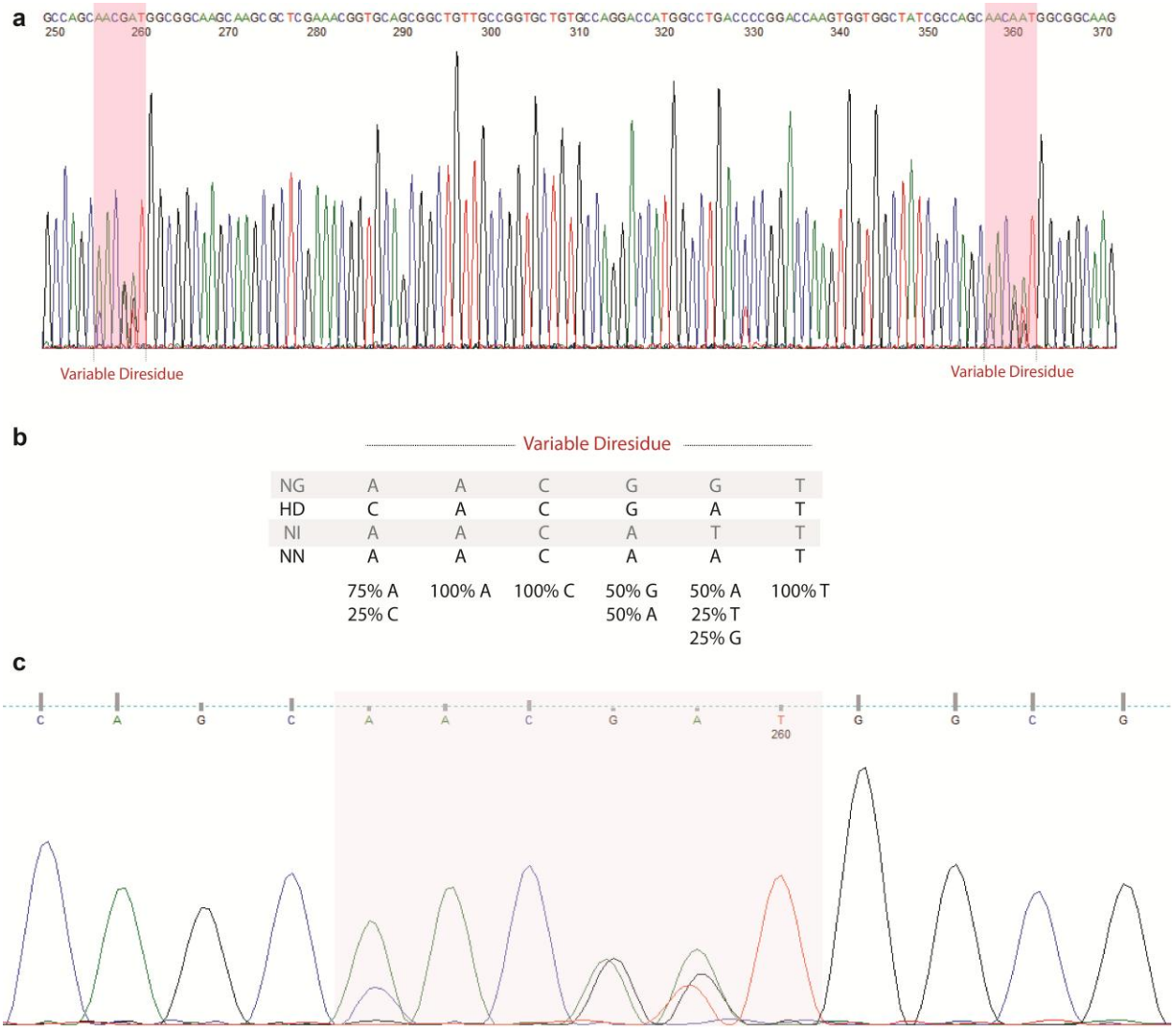
pAbAi-4XA35/PDR5-mKATE2: 4XA35/PDR5-mKATE2 was PCR amplified from pAbAi-mKATE2 using primers P19 and P20, and then cloned into pAbAi-mKATE2 using HindIII and NheI.

pAbAi-4XA35/PDR3-mKATE2: 4XA35/PDR3-mKATE2 was PCR amplified from pAbAi-mKATE2 using primers P21 and P20, and then cloned into pAbAi-mKATE2 using HindIII and NheI.

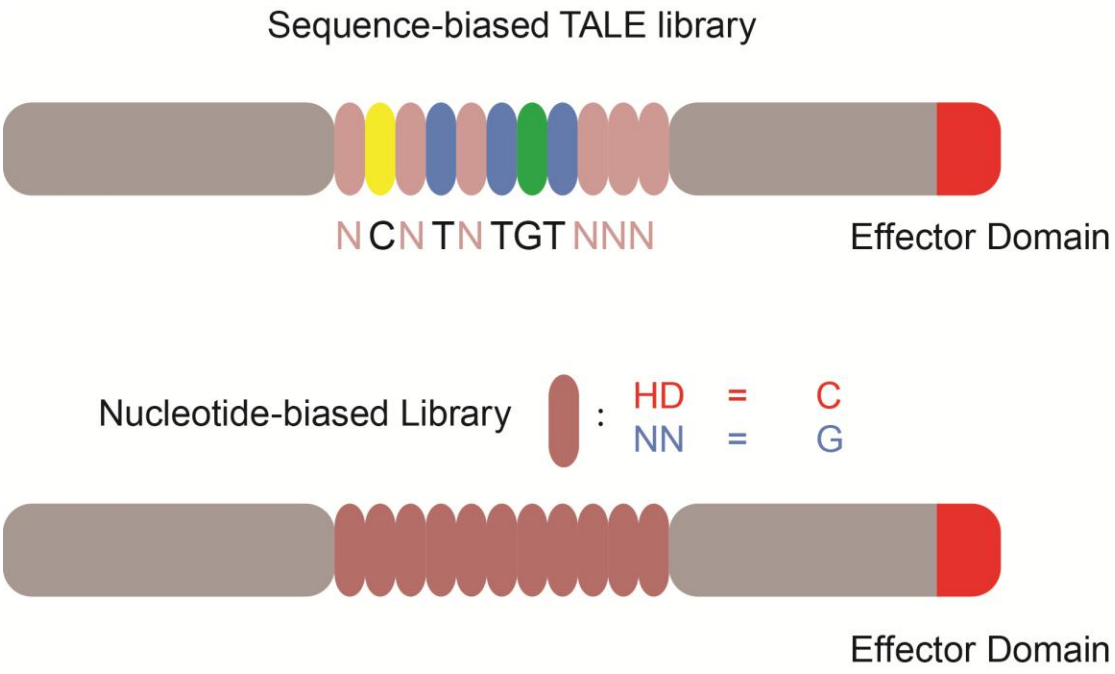
pAbAi-4XA8/PDR3-mKATE2: 4XA8/PDR3-mKATE2 was PCR amplified from pAbAi-mKATE2 using primers P22 and P20, and then cloned into pAbAi-mKATE2 using HindIII and NheI.

AAV-SCN9A-T34: The SCN9A-T34 TALE-VP16 cDNA transcript was PCR amplified from PEF-1-SCN9A-T34-VP16 using primers P31 and P32, and then cloned into AAV-MCS using ClaI and HindIII.

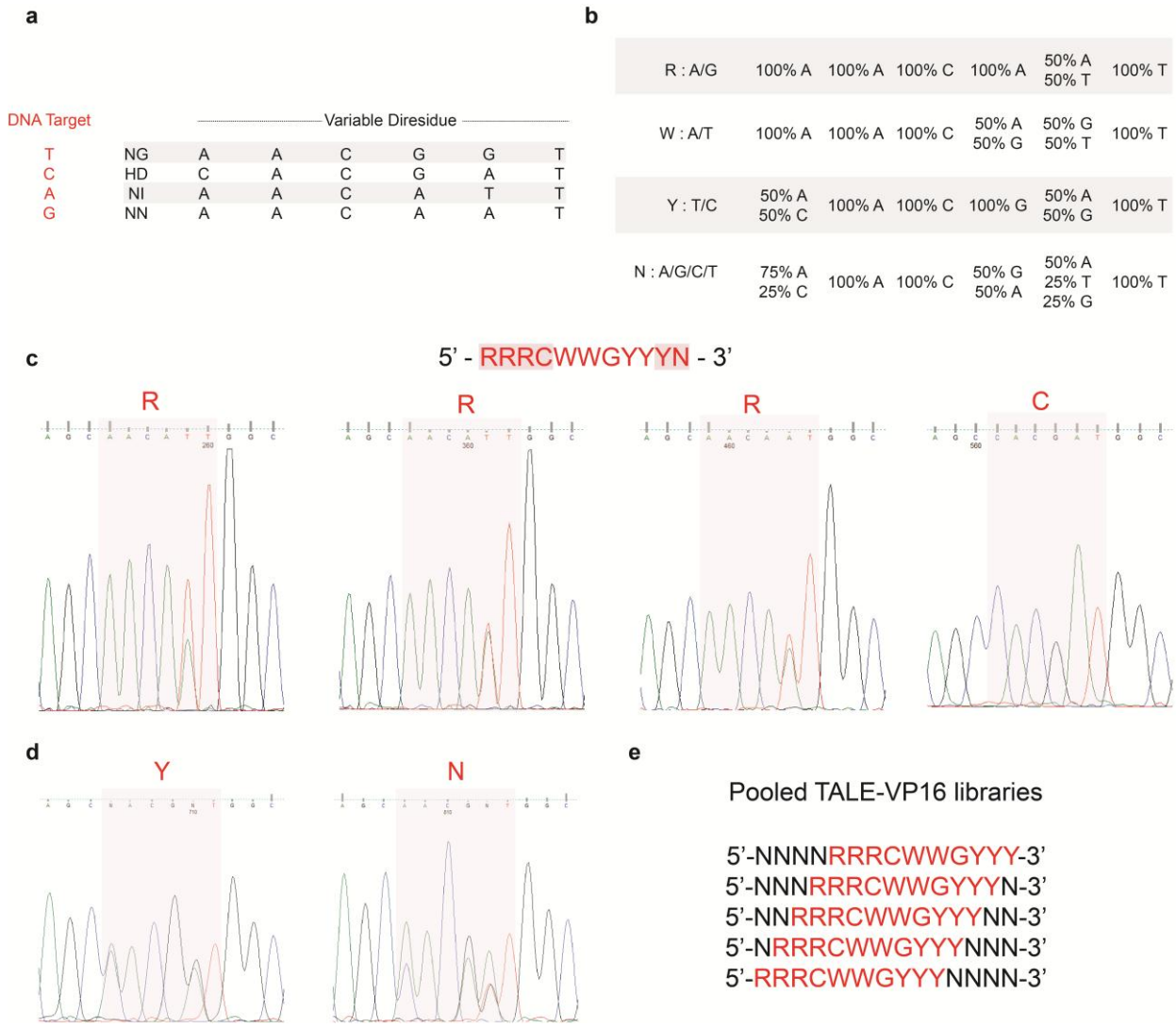
Supplementary Figures



Supplementary Figure 1: Sanger sequencing profiles of the 11-mer TALE library. (a) The 11-mer TALE library was subjected to Sanger sequencing using the forward primer (P23, 5'-CGTCGCTGTCACGTATCAGCACATAATC-3') and the reverse primer (P24, 5'-GCGTACGCTGGGTGGGAGTGGCAC-3'). There are 6-nucleotide long repeats (RVDs), spaced by 102 nucleotides, showing "noisy" signals. (b) Expected nucleotide compositions of the RVD domain of our TALE library. (c) Observed nucleotide compositions of the RVD domain of our TALE library, which closely tracks the predicted composition.



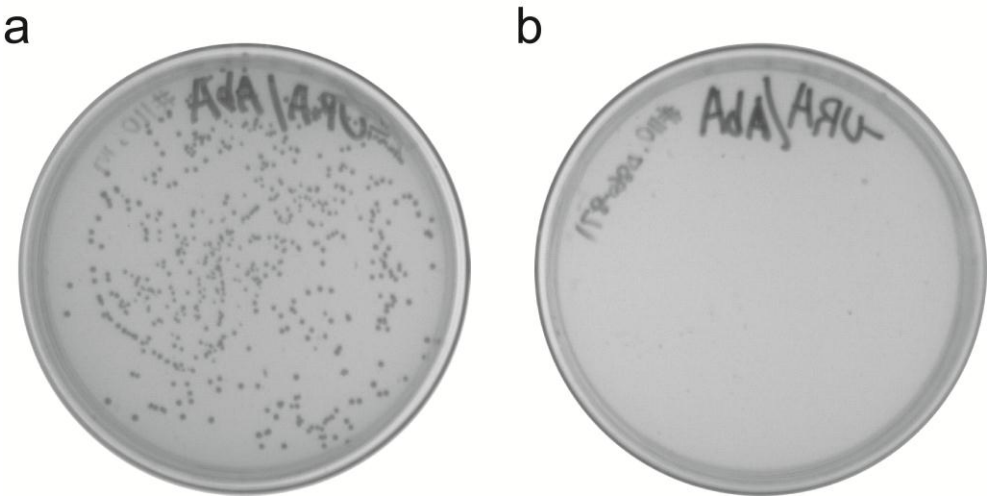
Supplementary Figure 2: Schematic illustration of sequence or nucleotide-biased TALE libraries. A sequence-biased TALE library has constrained nucleotides, while a nucleotide-biased library could be GC-rich.



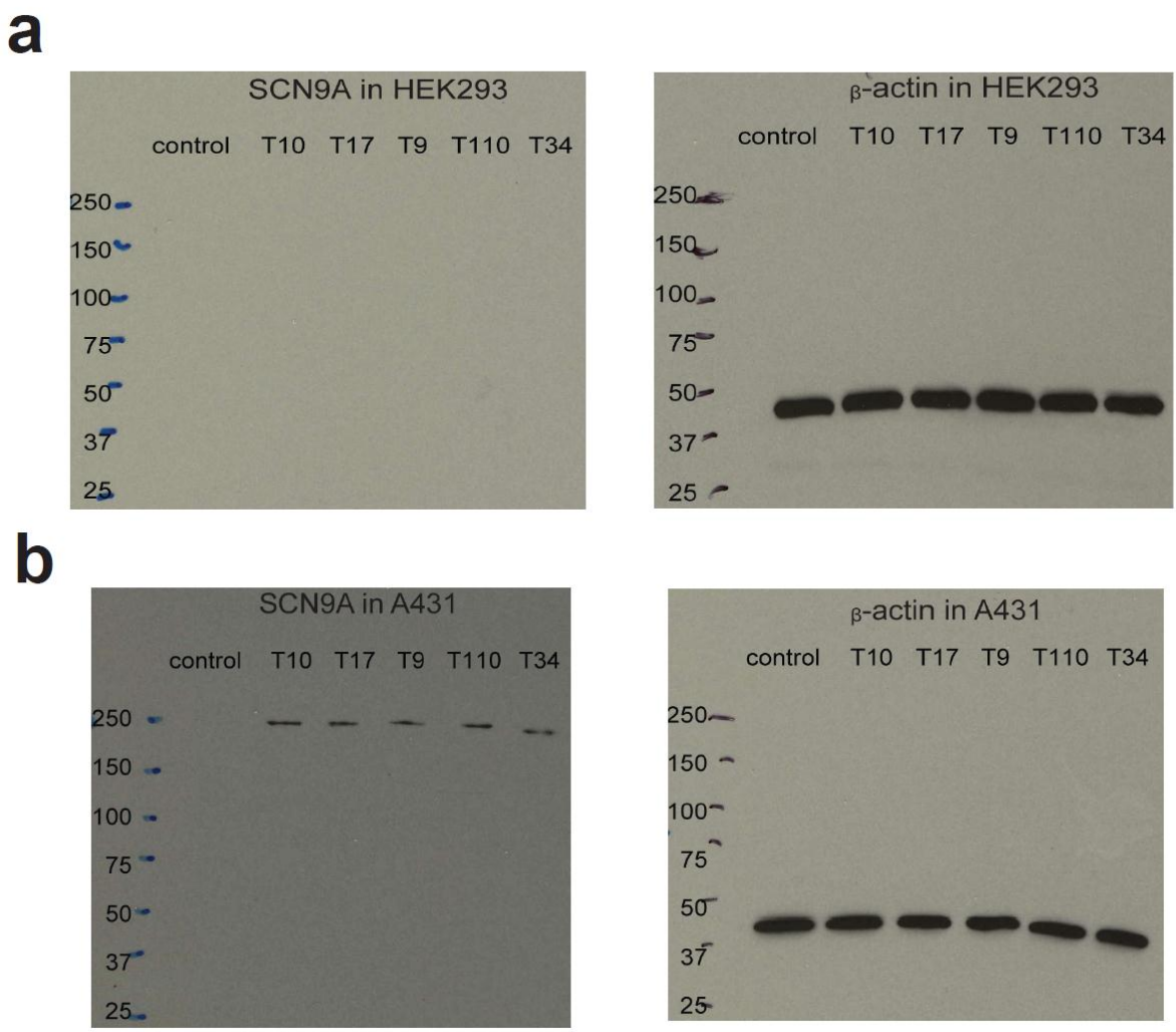
Supplementary Figure 3: Sanger sequencing profiles of the p53-biased 11-mer TALE library. (a) The nucleotide compositions of the variable diresidues (RVDs) for four target nucleotides (T, C, A, G). (b) The expected nucleotide compositions of the RVD domains for various target nucleotides (R, W, Y, N). (c) Observed nucleotide compositions of the first four RVD domains of the p53-biased 11-mer TALE library using the forward primer (P23), which closely tracks the predicted composition. (d) Observed nucleotide compositions of the last two RVD domains of the p53-biased 11-mer TALE library using the reverse primer (P24), which closely tracks the predicted composition. (e) Since TALE binding sites are

preferentially preceded by a T, five 14-mer TALE-VP16 libraries which target 5'-NNNNRRRCWWGYYY-3', 5'-NNNRRRCWWGYYYN-3', 5'-NNRRRCWWGYYYNN-3', 5'-NRRRCWWGYYYNNN-3', and 5'-RRRCWWGYYYNNNN-3' can be prepared separately. Pooling these five libraries is predicted to cover at least $1-(0.75)^5 = 75\%$ of all possible 14-mer DNA target sequences which contain a p53-responsive element and are preceded by a T.

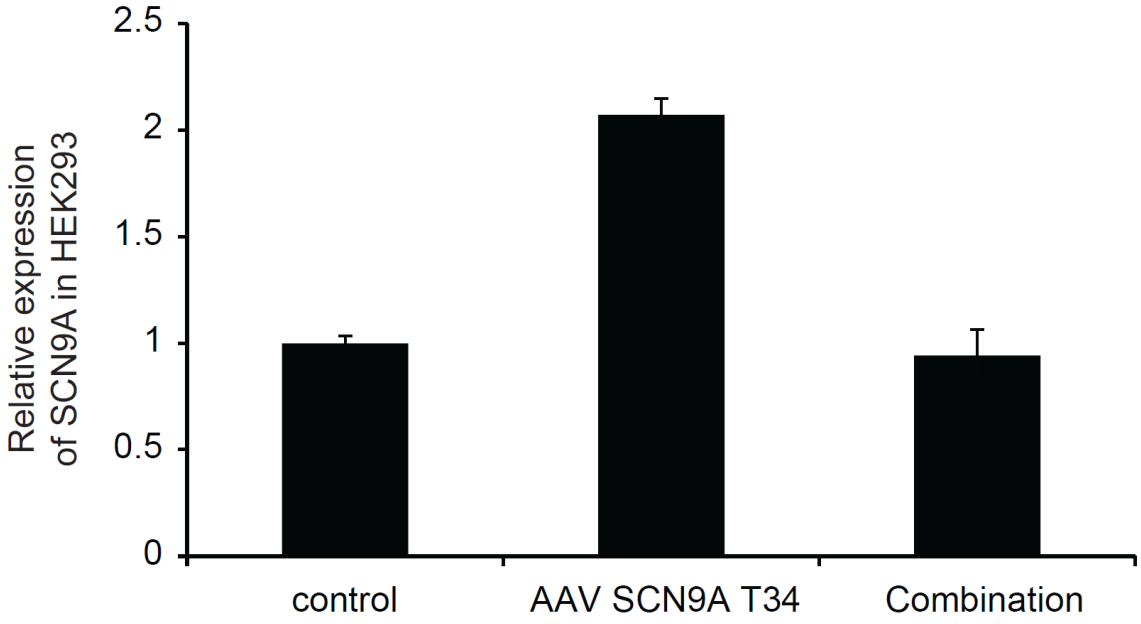
Supplementary Figure 4: Secondary confirmation of the positive yeast clones for the SCN9A bait. The positive clones isolated from the yeast one-hybrid screening were re-streaked onto –Leu plates containing 100 nM Aureobasidin A. After 3 days, the TALE-VP16 positive clones (#109 and #110) showed robust growth, while the control (0, yeast cells which are transformed with empty prey vector pGADT7) failed to grow.



Supplementary Figure 5: Confirmation of the binding between the isolated TALE-VP16 fusion and its predicted target sequence within the SCN9A bait sequence. The isolated clone pGADT7-SCN9A-TALE#110-VP16 was predicted to target 5'-GTTGCCTCCCC-3' within the SCN9A bait sequence. The full-length bait sequence (pAbAi-SCN9A) or a bait with a deletion of the predicted target site (pAbAi-SCN9A(ΔGTTGCCTCCCCC)) was stably integrated into yeast cells. pGADT7-SCN9A-TALE#110-VP16 was then transformed into either cell line. Only cells which contained the intact bait sequence (panel a) survived the 100 nM Aureobasidin A selection.

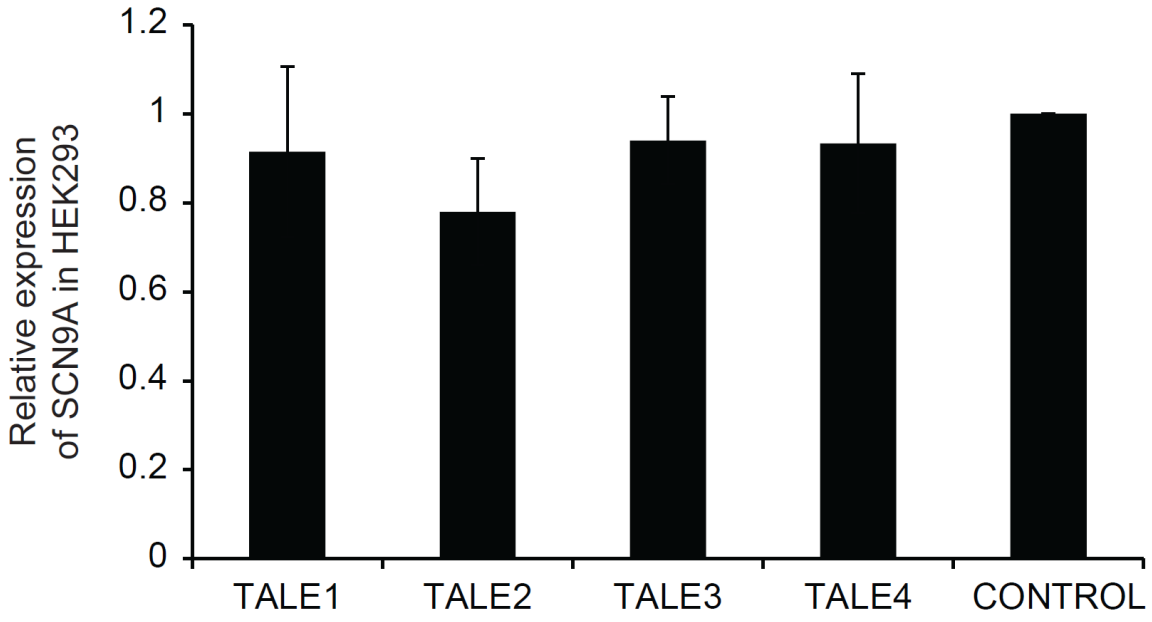


Supplementary Figure 6: The isolated TALE-VP16 fusions induced the overexpression of SCN9A protein in A431 cells, but not in HEK293 cells. 500,000 of A431 or HEK293 cells were seeded on each well of 6-well cell culture plates. After 16 hours, the cells were transiently transfected with 2 µg of each isolated TALE-VP16 constructs. The empty PEF-1 plasmid was used as the control. 72 hours later, the cells were lysed in RIPA buffer with protease inhibitors. For Western blot, 15 µg of whole cell lysates were used per lane. panel a: Western blot for HEK293 cells; panel b: Western blot for A431 cells. The TALE-VP16 fusions induced the overexpression of SCN9A protein in A431 cells, but not in HEK293 cells.



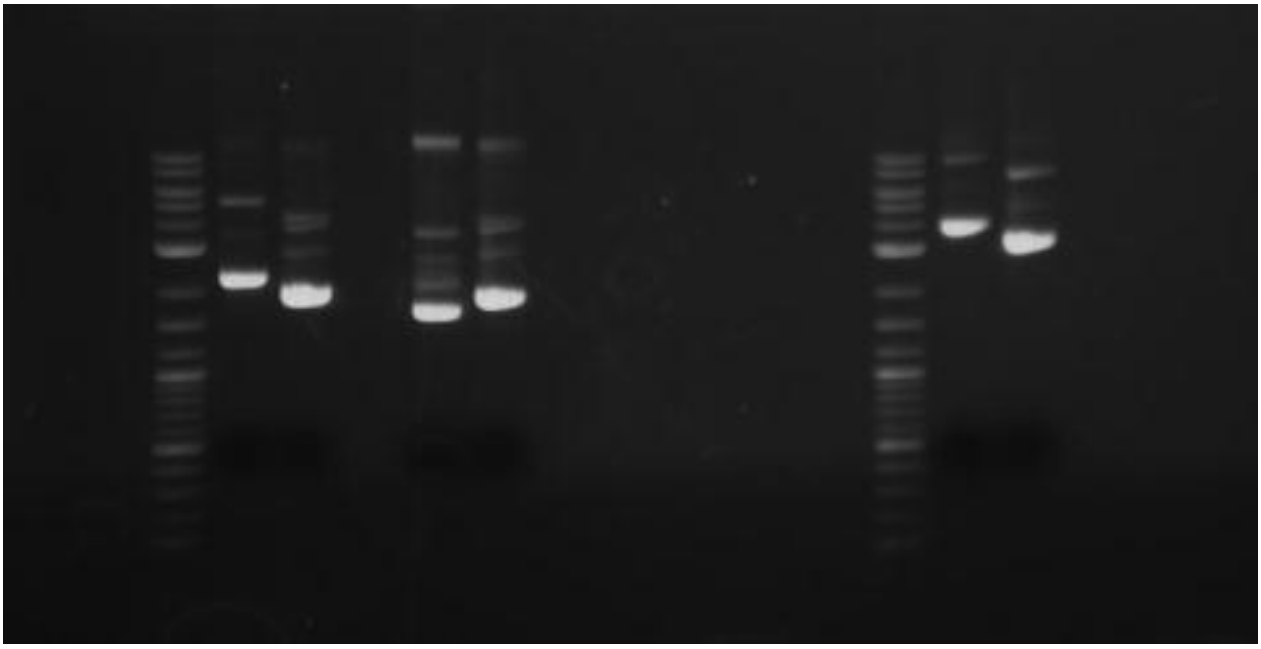
Supplementary Figure 7: The effects of SCN9A T34 AAV viral stock and the combinations of 4 TALE fusions designed by TALE-NT2.0 in HEK293 cells. Four TALE-VP16 fusions were designed according to TALE-NT2.0 and cloned into the PEF-1 vector. A combination of 250 ng of each TALE fusion construct was transiently transfected into HEK293 cells. An empty vector (PEF-1) was used as the control. In addition, the TALE-VP16 T34 cDNA was cloned into AAV-MCS vector and the corresponding AAV viral vectors were prepared. Subsequently, HEK293 cells were infected with this SCN9A T34 AAV viral stock at a MOI of 400. 48 hours later, the mRNA levels of SCN9A were determined by quantitative RT-PCR. The T34 AAV viral stock induced the overexpression of the SCN9A gene by 2 fold. In contrast, the combination of the four SCN9A bait-targeting TALE-VP16 fusions designed by TALE-NT2.0 failed to induce its overexpression.

Supplementary Figure 8: Secondary confirmation of the positive yeast clones for the human miRNA-34b/c bait. The positive clones isolated from the yeast one-hybrid screening were re-streaked onto –Leu plates containing 100 nM Aureobasidin A. After 4 days, the TALE-VP16 positive clones (M16 and M17) showed robust growth, while the control (Wild-type, yeast cells which are transformed with empty prey vector pGADT7) failed to grow.



Supplementary Figure 9: The SCN9A bait-targeting TALEs designed by TALE-NT2.0 failed to induce the overexpression of the SCN9A gene in HEK293 cells. Four TALE-VP16 fusions were designed according to TALE-NT2.0 and cloned into the PEF-1 vector. 1 µg of each TALE fusion construct was transiently transfected into HEK293 cells. 48 hours later, the mRNA levels of SCN9A were determined by quantitative RT-PCR. An empty vector (PEF-1) was used as the control. All 4 TALEs designed according to TALE-NT2.0 failed to induce the overexpression of SCN9A in HEK293 cells.

Supplementary Figure 10: Secondary confirmation of the positive yeast clones conferring cycloheximide resistance. 18 positive clones were isolated from the cycloheximide resistance screening. The positive clones were re-streaked onto –Leu plates containing 0.5 µg/ml of cycloheximide. After 3 days, the TALE-VP16 positive clones (A11 and A12) showed robust growth, while the control (Wild-type, yeast cells which are transformed with empty prey vector pGADT7) failed to grow.



Supplementary Figure 11: Gel electrophoresis of 11-mer TALE-VP16 plasmid libraries in the PTAL1-VP16 plasmid. 600 ng of the pFUS_A (RVDs 1-10), pFUS_B (RVD 1), and the final 11-mer TALE-VP16 libraries (in the PTAL1-VP16 plasmid) were subjected to 0.7% agarose gel electrophoresis along with the original pFUS_A, pFUS_B and PTAL1-VP16 mother vectors. The electrophoresis image shows that the libraries were free of noticeable contamination from their respective mother vectors. From left to right: 2-Log DNA ladder (New England Biolabs, #N3200S), pFUS_A (RVDs 1-10), pFUS_A, pFUS_B (RVD 1), pFUS_B; 2-Log DNA ladder, 11-mer TALE-VP16 library, and PTAL1-VP16.

Supplementary Tables

Supplementary Table 1: Primers used in this study.

Primer ID	Primer sequence (5'->3')	Application
P1	CAGTACAAGCTTTCTGATGGGTCTGGGAAAAGTAAAT	forward primer for SCN9A
P2	CAGTACCTCGAGTGGGTTCCTTTGATTTTCTTTCAGCAA	reverse primer for SCN9A
P3	CAGTACCCGCGGAAAGATGGCAATGTTGCCTCCCCCA	forward primer for pAbAi-SCN9A(ΔATACAGGATGA)
P4	CAGTACCCGCGGATTTTAATTCCTCTTCAGCTCCTCAC	reverse primer for pAbAi-SCN9A(ΔATACAGGATGA)
P5	CAGTACCCGCGGATTAAAATATACAGGATGAAAAGATGG	forward primer for pAbAi-SCN9A(ΔTATGTGAGGAGCTGA)
P6	CAGTACCCGCGGAGAGGCCTGGATGGAAACAAAGAAAT	reverse primer for pAbAi-SCN9A(ΔTATGTGAGGAGCTGA)
P7	CAGTACCCGCGGAGGACCTCAGAGCTTTGTCCATTTC	forward primer for pAbAi-SCN9A(ΔGTTGCCTCCCCC)
P8	CAGTACCCGCGGATTGCCATCTTTTCATCCTGTATA	reverse primer for pAbAi-SCN9A(ΔGTTGCCTCCCCC)
P9	CAGTACGAATTCGTGCACCATGGATCCCATTCGTCCGCG	forward primer for PEF-1 cloning
P10	CAGTACGAATTCTCACTGAGGCAATAGCATATGTCTAGA	reverse primer for PEF-1 cloning
P11	CAGTACAAGCTTCATTGTCCATCTCACCAACTAGACC	forward primer for miR-34b/c
P12	CAGTACCTCGAGGCATATTTACTCATAGGAAATATAA	reverse primer for miR-34b/c
P13	CAGTACCCGCGGACTATCAAGAAGTTTAATAATCTGA	forward primer for pAbAi-miR-34b/c(ΔTTTCTAGGTAT)
P14	CAGTACCCGCGGCTAGTTTATATGTTGAAGCAAGACT	reverse primer for pAbAi-miR-34b/c(ΔTTTCTAGGTAT)
P15	CAGTACGAATTCCCGTGCACCATGGATCCCATTCGTCCGCG	forward primer for PEF-1 cloning
P16	CAGTACGGTACCCTGAGGCAATAGCATATGTCTAGA	reverse primer for PEF-1 cloning
P17	CAGTCCGCTAGCGCTACCGGTCGCCACCATGGTGAGC	forward primer for pAbAi-mKATE2
P18	CAGTCCGCGGCCGCTTAATCTGAGTCCGGAACCTCCTCCACC	reverse primer for pAbAi-mKATE2
P19	CAGTACAAGCTTCAATACAAACAAGGCCTCCAATACAAACAAGGCCTCCAATACAA ACAAGGCCTCCAATACAAACAAGGCCTCGAATTCGAGCTCGGTACCCGGGGAT	forward primer for pAbAi-4XA35/PDR5-mKATE2
P20	CAGTACGCTAGCTATTAATTTAGTGTGTGTA	reverse primer for pAbAi-4XA35/PDR5-mKATE2
P21	CAGTACAAGCTTGCTTTCAGGTAAAGCCATGCTTTCAGGTAAAGCCATGCTTTCAGG TAAAGCCATGCTTTCAGGTAAAGCCATGAATTCGAGCTCGGTACCCGGGGAT	forward primer for pAbAi-4XA35/PDR3-mKATE2
P22	CAGTACAAGCTTAACTGCATTTCACTTTTCAACTGCATTTCACTTTTCAACTGCATTTC ACTTTTCAACTGCATTTCACTTTTCGAATTCGAGCTCGGTACCCGGGGAT	forward primer for pAbAi-4XA8/PDR3-mKATE2
P23	CGTCGCTGTCACGTATCAGCACATAATC	forward primer for Sanger sequencing
P24	GCGTACGCTGGGTGGGAGTGGCAC	reverse primer for Sanger sequencing
P25	CAGTCCGCGGCCGCGGAAATCCATTATGTACTATTTAAAAA	forward primer for pAbAi-mKATE2
P26	CAGTCCGCTAGCTATTAATTTAGTGTGTGTATTTGTGTTTGC	reverse primer for pAbAi-mKATE2
P27	AAGCAGTGGTATCAACGCAGAGTGGCCATTATGGCCGTGCACCATGGATCCCATTC GTCCGCG	forward primer for TALE-VP16 cDNA library
P28	TCTAGAGGCCGAGGCGGCCGACATGCTGAGGCAATAGCATATGTCTAGA	reverse primer for TALE-VP16 cDNA library
P29	CTCCACTTAGACGGCGAGGA	forward primer for VP-16
P30	GAAGTCGGCCGTATCCAGAG	reverse primer for VP-16
P31	TCGGCAATCGATCCACCATGGATCCCATTCGTCCGCGCAGGCC	forward primer for AAV-SCN9A-T34 cloning
P32	TCGGCAAAGCTTTCACTGAGGCAATAGCATATGTCTA	reverse primer for AAV-SCN9A-T34 cloning

Supplementary Table 2: The bait sequences for human SCN9A and miR-34b/c in the yeast one-hybrid assays.

Gene Target	Bait sequence (5'->3')
human SCN9A	TCTGATGGGTCTGGGAAAAGTAAATTGAAAATCTTCTGGAAAGGATTCACCATTCTAGATGTTATTAGAACATTAATGATTTGTGGG AGGAGGGCAATATACAAACATTAATAGGAAATTGGAATGAGTTGATTCCAACTCTCATGGATGACTATGAGAGGTTTAAGACTTCA GTGATGGAAAGAACTGCAGATGTGGTGGAAATAGCAAGAGAATTAGAATTAAGAGATGGAGCCTAAAATTGTGACTGAATTGCTG CCATCTCGCGGTAACTTGAATGGATGAGGAGTTGCTTCTGATTGATAAGCAAAGAAAGTGGTTTCGTGAGATGGAATCTTCTCCTGG TCAAGATGTTATGAACATTGTTGAAGTGACAGCAGAGGATTTAGAATATTACATAAGTTTAGTTGGTAAAACAGCATCAGGCTGGA GAGTGGTGCTACCAAAGGCATTATCATAAAGACTTTTAAAAGAGGAAAGTTGAGTTGAATTGCACCAAAGGTAAATATAGACGAA AAGAAGGGGAATTTAGGAAAATTTACTCAGAAAATTGTCATTTTTTACTTTAAAGCTATGAAGCGTAAACTTCAAATTTCTGAGTAT AAAGATGGTTAGAAGGCACGAAAGATAAGTCCCGCCCATTGCCTGACACATAGTAATCCCTTAACAAGCTGATAATTCTAGAACAT GTTACCTTTTGTAGTTGAAACTAAAAATCTGTTTATGTTGTTATTATTAGTTTTTAATGGGCCTTTCTTGGCAGGCAAATAGTTAAGTC TTTATTTCTTTGTTTCCATCCAGGCCTCTTATGTGAGGAGCTGAAGAGGAATTAAAATATACAGGATGAAAAGATGGCAATGTTGCC TCCCCCAGGACCTCAGAGCTTTGTCCATTTCACAAAACAGTCTCTTGCCCTCATTGAACAACGCATTGCTGAAAGAAAATCAAAGGA ACCCA
human miR-34b/c	CATTGTCCATCTCACCAACTAGACCACATAAATCCTTAGAGATTGTTCTCTAAAGATGGAAAATATGCAACCTGTTTGCTCCTACTGA AGCCATACCTAAGAGCTCCGGCAACTTTAATTATAGAGACTATTTTGTTAGACTGCTTATGATTTACCTGAAAGATAAATTAACATTT TATACTGAAAGCAACAGTATTGATTAATCAATAAATATAAACAAGAAGCTAATTAAGAATCTGGTGCACTCTTACTAATGAGAGTA CAATGAAATGAAACTAGAAATATGGAATTATAAAAACCGCTGGCAGTTCATTTTAGCTCAAAAGAAAATCTTCTCCCATAAATAGA TTCAATTATTTTTAGTTCTTCAAAGGCACTGAATTGACTTCAATTT***AGTCTTGCTTCAACATATAAACTAGTTT****CTAGGTATACTATC AAGAAGTTTAATAATCTGACATTTATTGATCTATAAACCAAGATATGGTTTTATATTTCCTATGAGTAAATATGC

*: p53 responsive element: p53RE

Supplementary Table 3: The RVD sequences for isolated TALE-VP16 clones.

human SCN9A clone ID	**RVD sequence**	**gene target sequence (5'->3')**	**DNA strand**	**the lowest possible score**
T42	NG HD NI NG HD HD NN NN NG NI NG	TCATCCTGTAT	minus	5.79 (addition of NI)
T10	NG HD NN NG HD HD NG NI NG NI NG	TCATCCTGTAT	minus	6.91 (addition of NI)
T72	NG HD NG NG HD HD NG NN NG NI NG	TCATCCTGTAT	minus	5.31 (addition of NI)
T54	NG HD NN NG HD HD NG NN NG NN NG	TCATCCTGTAT	minus	4.91 (addition of NI)
T17	NG NI NG NN NG NN NI NN NN NG NN	TATGTGAGGAG	plus	7.77 (addition of HD)
T49	NG NI NG NN NG NI NI NN NN NI NN	TATGTGAGGAG	plus	8.52 (addition of HD)
T33	NG HD NN NN HD NG HD HD NG HD HD	TCAGCTCCTCA	minus	6.09 (addition of HD)
T9	NG HD NI NN HD NG HD HD NG HD HD	TCAGCTCCTCA	minus	5.24 (addition of HD)
T110	NN NG NG NN HD NI NG HD HD HD HD	GTTGCCTCCCC	plus	5.63 (addition of HD)
T28	NN NG NG NN HD HD NG NN HD HD HD	GTTGCCTCCCC	plus	5.30 (addition of HD)
T66	NN NG NG NN HD NG NG HD HD HD NI	GTTGCCTCCCC	plus	7.76 (addition of HD)
T91	NN NG NG NN HD NN NG HD HD HD HD	GTTGCCTCCCC	plus	5.30 (addition of HD)
T34	NN NN NN NN NN NI NN NN HD NI NI	GGGGGAGGCAA	minus	6.65 (addition of HD)
human miR-34b/c clone ID	**RVD sequence**	**gene target sequence (5'->3')**	**DNA strand**	**the lowest possible score**
M1	NI NG NN HD HD NG NN NN NI NN NN	ATACCTAGAAA	minus	6.32 (addition of HD)
M17	NI NG NN HD HD NG NI NN NI NN NI	ATACCTAGAAA	minus	4.60 (addition of HD)
M13	NG HD NN NG NI NI NG HD NG NN NN	TAATAATCTGA	plus	7.14 (addition of HD)
M16	NN HD NG HD NG NG NI NN NN NN NN	ACTCATAGGAA	minus	8.25 (addition of NI)
cycloheximide resistance clone ID	**RVD sequence**	**gene target sequence (5'->3')**	**DNA strand**	**the lowest possible score**
A7	NG NI NG HD NG NG HD NG NG NG HD	n.d.	n.d.	n.d.
A8	NN HD NN NG NG NG NI NG HD NG NG	GCATTTCACTT (PDR3)	plus	8.33 (addition of NG)
A11	NN NG NG NN HD NG HD HD NG NN NN	n.d.	n.d.	n.d.
A17	NN HD NI HD HD HD HD NG NN NN NG	n.d.	n.d.	n.d.
A35	NN HD NI NN NN NG NN NI NN NN HD	TCAGGTAAAGC (PDR3)	plus	8.51 (addition of HD)
A35	NN HD NI NN NN NG NN NI NN NN HD	ACAAACAAGGC (PDR5)	plus	8.88 (addition of HD)
human SCN9A designed by TALE-NT2.0 clone ID	**RVD sequence**	**gene target sequence (5'->3')**	**DNA strand**	
TALE1	NI NG NI HD NI NI NI HD NI NG NG	ATACAAACATT	plus	
TALE2	NI HD HD NI NI NI NN NN HD NI NG	ACCAAAGGCAT	plus	
TALE3	NI HD NG HD NI NN NI NI NI NI NG	ACTCAGAAAAT	plus	
TALE4	HD HD NI NN NN HD HD NG HD NG NG	CCAGGCCTCTT	plus	
*n.d. : not determined				

References

1. Maxwell, K. L. *et al.* Protein folding: defining a "standard" set of experimental conditions and a preliminary kinetic data set of two-state proteins. *Protein Sci* 14, 602-616 (2005).

2. Rouillard, J., Zuker, M. & Gulari, E. OligoArray 2.0: design of oligonucleotide probes for DNA microarrays using a thermodynamic approach. *Nucl. Acids Res.* 31, 3057-3062 (2003).

3. Kristiansson, E., Thorsen, M., Tamás, M. J. & Nerman, O. Evolutionary Forces Act on Promoter Length: Identification of Enriched Cis-Regulatory Elements. *Molecular Biology and Evolution* 26, 1299-1307 (2009).