

Supplementary Information

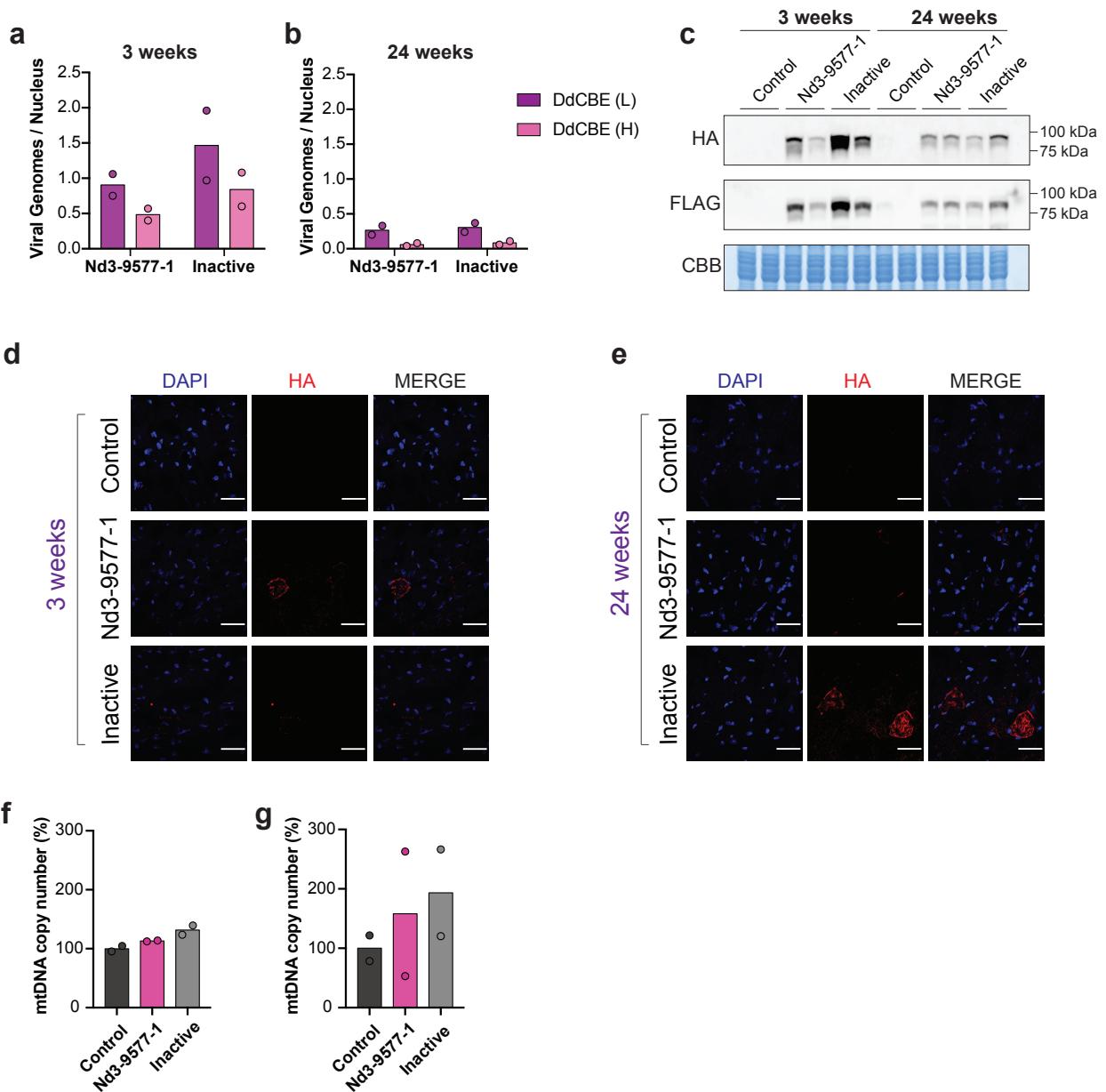
In vivo mitochondrial base editing via adeno-associated viral delivery to mouse post-mitotic tissue

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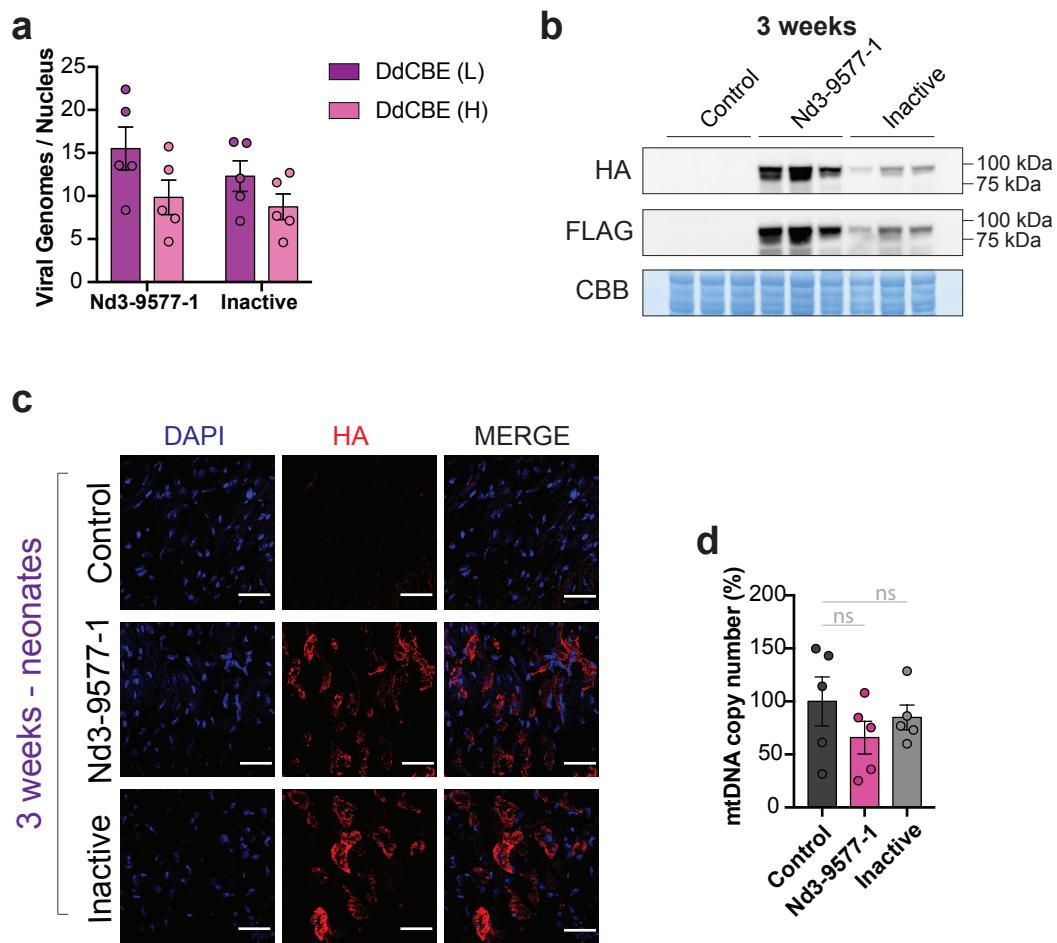
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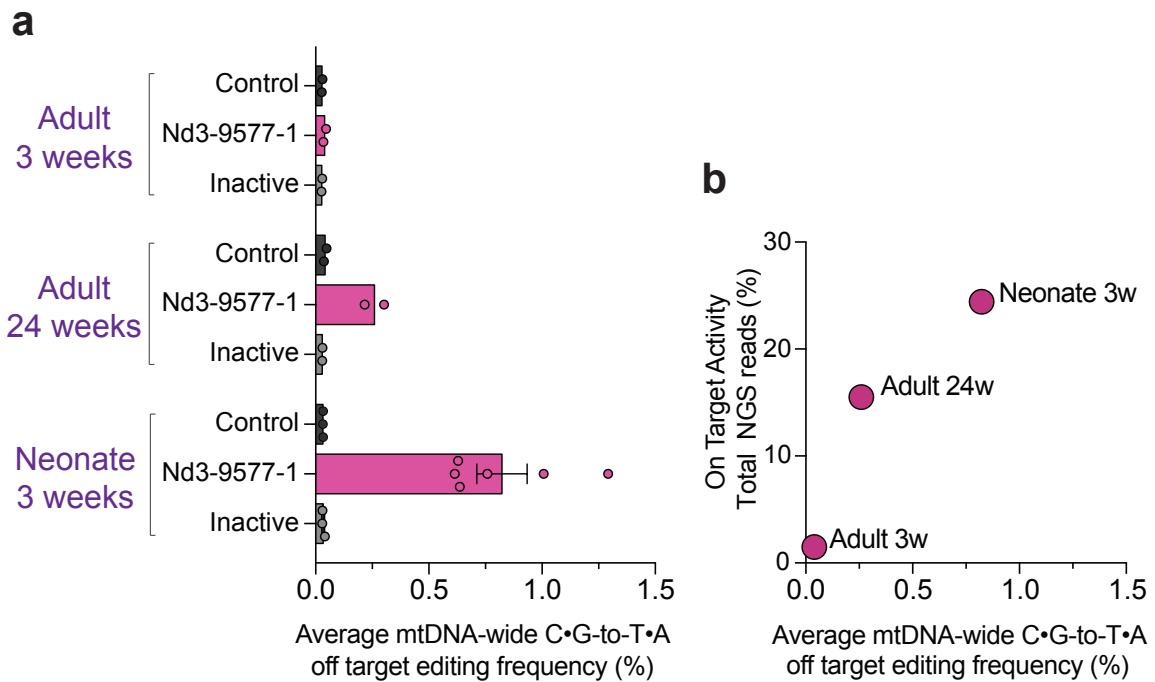
Supplementary Fig. 1 | Characterization of mouse hearts upon adult-delivery of AAV-DdCBEs.

a,b. Real-Time qPCR quantification of AAV-DdCBE (L) and AAV-DdCBE (H) viral genomes per nucleus in hearts of adult-injected mice at 3 weeks (a) or 24 weeks (b) post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair. Bars represent the mean ($n=2$). Source data are provided as a Source Data file. **c.** Western blot analysis of the levels of HA-tagged DdCBE (L) and Flag-tagged DdCBE (H) in hearts of adult-injected mice at 3 weeks or 24 weeks post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair and vehicle injected mice (control). Coomassie Brilliant Blue (CBB) was used as loading control. Images representative of $n=2$ independent biological replicates. Each lane represents a sample from an individual mouse. **d,e.** Immunohistochemistry analysis of the HA-tagged DdCBE (L) in hearts of adult-injected mice at 3 weeks (d) or 24 weeks (e) post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair and vehicle injected mice (control). Scale bars: 30 μ m. **f,g.** Real-Time qPCR relative quantification of mtDNA copy number in hearts of adult-injected mice at 3 weeks (f) or 24 weeks (g) post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair. Bars represent the mean ($n=2$). Source data are provided as a Source Data file.



Supplementary Fig. 2 | Characterization of mouse hearts upon neonatal-delivery of AAV-DdCBEs.

a. Real-Time qPCR quantification of AAV-DdCBE (L) and AAV-DdCBE (H) viral genomes per nucleus in hearts of neonate-injected mice at 3 weeks post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair. Bars represent the mean and error bars represent \pm SEM ($n = 5$). Source data are provided as a Source Data file. **b.** Western blot analysis of the levels of HA-tagged DdCBE (L) and Flag-tagged DdCBE (H) in hearts of neonate-injected mice at 3 weeks post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair and vehicle injected mice (control). Coomassie Brilliant Blue (CBB) was used as loading control. Images representative of $n=3$ independent biological replicates. **c.** Immunohistochemistry analysis of the HA-tagged DdCBE (L) in hearts neonate-injected mice at 3 weeks post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair and vehicle injected mice (control). Scale bars: 30 μ m. **d.** Real-Time qPCR relative quantification of mtDNA copy number in hearts of neonate-injected mice at 3 weeks post-injection of either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair. Bars represent the mean and error bars represent \pm SEM ($n = 5$). Ordinary one-way ANOVA with Dunnett's test: not significant - ns (Control vs Nd3-9577, P-value = 0.3142; Control vs Inactive, P-value = 0.7703). Source data are provided as a Source Data file.



Supplementary Fig. 3 | Assessment of mtDNA-wide off-target activity *in vivo* upon delivery of AAV-DdCBEs.

a. NGS analysis of the average mtDNA-wide C•G to T•A off-target editing frequency by AAV-DdCBEs in adult mouse heart at 3 weeks or 24 weeks post-injection and in neonatal mouse hearts at 3 weeks post-injection. Each mouse was injected with either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair or vehicle (control). Bars represent the mean and error bars represent \pm SEM ($n=2$ for all experiments in adults; $n=3$ for vehicle and inactive base editor controls in neonates; $n=6$ for neonates injected with DdCBE-Nd3-9577-1 AAV). Source data are provided as a Source Data file. **b.** Correlation between on-target activity and average mtDNA-wide C•G to T•A off-target editing frequency by the catalytically active DdCBE-Nd3-9577-1 AAV pair in adult mouse heart at 3 weeks or 24 weeks post-injection and in neonatal mouse hearts at 3 weeks post-injection. Source data are provided as a Source Data file.

a MT-*Nd3* on-target:

L-strand 5' **TCAGAAAAAGCAAATC**CATATG AATGCG G ATT**C₁₇G** ACCCTACAAGCTCTGCA
H-strand 3' AGTCTTTTCGTTAGGTATA**C₆**TTACGC₁₂₁₃TAA**G C₁₈T**GGGATGTTGAGACGT

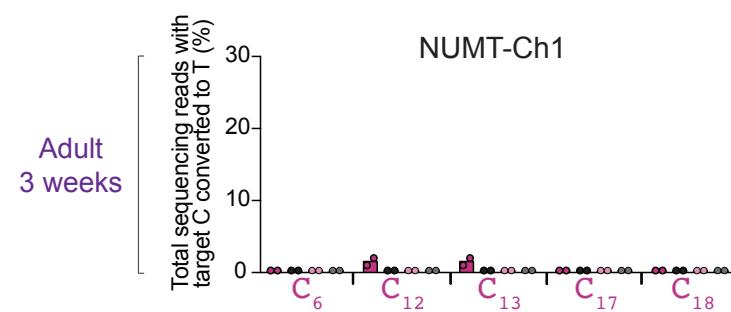
Nuclear MT-*Nd3* pseudogene chromosome 1 (NUMT-Ch1):

Locus Ident. [%] **TCAGAAAAAGCAAATC**CATATG AATGCG G ATT**C₁₇G** ACCCTACAAGCTCTGCA
Ch. 1 100 AGTCTTTTCGTTAGGTATA**C₆**TTACGC₁₂₁₃TAA**G C₁₈T**GGGATGTTGAGACGT

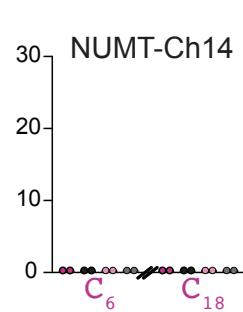
Nuclear MT-*Nd3* pseudogene chromosome 14 (NUMT-Ch14):

Locus Ident. [%] **TCAGAAAAAGCAAATC**CATATG AATGCA **A C G T T T G** ACCCCACAAGCTCCGTA
Ch. 14 86 AGTCTTTTCGTTAGGTATA**C₆**TTACGT **G T A A A C₁₈T**GGGGTGTTCGAGGCAT

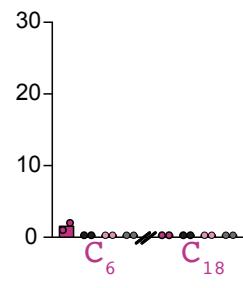
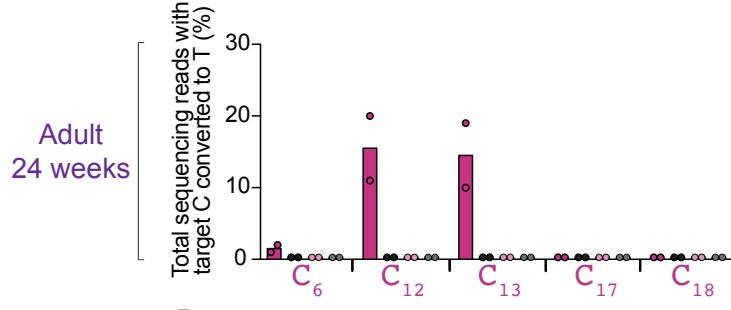
b



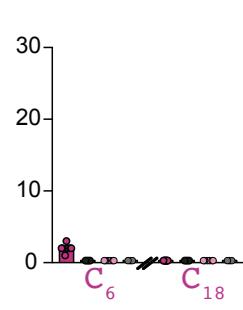
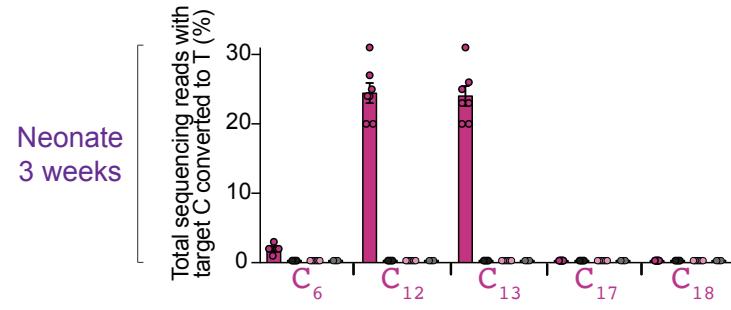
e



c



d



■ mtDNA (Nd3-9577-1) ■ nDNA (Control) ■ nDNA(Nd3-9577-1) ■ nDNA (Inactive)

Supplementary Fig. 4 | Assessment of nuclear DNA off-target activity *in vivo* upon delivery of AAV-DdCBEs.

a. Schematic of the DdCBE-Nd3-9577-1 on-target region within the *MT-Nd3* gene and related regions in nuclear DNA bearing greatest homology: nuclear MT-*Nd3* pseudogene located on chromosome 1 (NUMT-Ch1) and nuclear MT-*Nd3* pseudogene located on chromosome 14 (NUMT-Ch14). Locus and percentage of sequence identity with the canonical site in mtDNA (Ident.) are shown for each nuclear pseudogene. TALE binding sites are presented in blue and targeted Cs are shown in pink. Bases in red indicate divergence from the target sequence. Arrows indicate the mtDNA targeted Cs conserved in the nuclear pseudogenes. **b,c,d.** NGS analysis of nuclear DNA off-target editing frequency of Cs within the nuclear MT-*Nd3* pseudogene 1 (NUMT-Ch1) by AAV-DdCBEs in adult mouse heart at 3 weeks (b) or

24 weeks (c) post-injection and in neonatal mouse hearts at 3 weeks (d) post-injection. Each mouse was injected with either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair or vehicle (control). The on-target mtDNA editing frequency is shown for each C for comparison. Bars represent the mean and error bars represent \pm SEM (n=2 for all experiments in adults; n=3 for vehicle and inactive base editor controls in neonates; n=6 for neonates injected with DdCBE-Nd3-9577-1 AAV). Source data are provided as a Source Data file. **e,f,g.** NGS analysis of nuclear DNA off-target editing frequency of Cs within the nuclear MT-Nd3 pseudogene 2 (NUMT-Ch14) by AAV-DdCBEs in adult mouse heart at 3 weeks (e) or 24 weeks (f) post-injection and in neonatal mouse hearts at 3 weeks (g) post-injection. Each mouse was injected with either the catalytically active or inactive DdCBE-Nd3-9577-1 AAV pair or vehicle (control). The on-target mtDNA editing frequency is shown for each C for comparison. Bars represent the mean and error bars represent \pm SEM (n=2 for all experiments in adults; n=3 for vehicle and inactive base editor controls in neonates; n=6 for neonates injected with DdCBE-Nd3-9577-1 AAV). Source data are provided as a Source Data file.

Supplementary Sequences 1 | Amino acid sequences of DdCBEs-Nd3-9577 used in the cell screen.

The halves used to target the L-strand - **DdCBE (L)** – in the cell screen have the general architecture of (from N- to C-terminus):

SOD2 MTS – **3xHA** – **TALE** – 2aa linker – **DddA_{tox}** split – 4aa linker – **UGI** – **T2A** – **GFP***

Targeting: 5'-TCAGAAAAAGCAAATC-3'

DdCBE (L) - Nd3-9577 - G1333-N:

MLSRAVCGTSRQLAPVLGYLGSRKHSLPAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQKEKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHDGKQALETVQRLLPVLCQAHLGLTPAQ
VVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHL
GLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHL
VLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHL
VQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNG
GKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPAQVV
AIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHL
TPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHL
CQAHLGLTPEQVVAIASHDGGRPALESIVAQLSRPDPAALTLNDHLVALACLGGRPALDAV
KKGLPHAPALIKRTNRRIPERTSHRVAGSGSYALGPYQISAPQLPAYNGQTVGTFYYVND
GGLESKVFSSGGSGGSNLSDIIEKETGKQLVIQESILMPEEEVEEVIGNKPESDILVHTAYD
ESTDENVMILLTSDAPEYKPWALVIQDSNGENIKMLRSGSGEGRGSLLTCGDVEEN/T2A_c
leavage_site/PGPRLEMVSKGGEELFTGVVPILVELGDGVNGHKFSVSGEGEGDATYGKLTLK
FICTTGKLPVPWP TLVTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY
KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNNSHNVYIMADKQKNGIKVNFKIRH
NIEDGSVQLADHYQQNTPIGDPVLLPDNHYLSTQSALKDPNEKRDHMVLFVTAAGITL
GMDELYK*

DdCBE (L) - Nd3-9577 - G1333-C:

MLSRAVCGTSRQLAPVLGYLGSRKHSLPAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQKEKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHDGKQALETVQRLLPVLCQAHLGLTPAQ
VVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHL
GLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHL
VLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHL
VQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNG
GKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPAQVV
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TPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHL
CQAHLGLTPEQVVAIASHDGGRPALESIVAQLSRPDPAALTLNDHLVALACLGGRPALDAV
KKGLPHAPALIKRTNRRIPERTSHRVAGSPTPYPNYANAGHVEGQSAFLMRDNGISEGLVF
HNNPEGTCGFCVNMTETLLPENAKMTVVPPEGAIPVKRGATGETKVFTGNSNSPKSPTKG
GCSGGSTNLSDIIEKETGKQLVIQESILMPEEEVEEVIGNKPESDILVHTAYDESTDENVMILLT
SDAPEYKPWALVIQDSNGENIKMLRSGSGEGRGSLLTCGDVEEN/T2A_cleavage_site/PG

PRLEMVSKGEELFTGVVPILVELGDVNGHKFSVSGEGEGDATYGKLTGKFICTTGKLPVP
WPTLVTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE
DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA
DHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*

DdCBE (L) - Nd3-9577 - G1337-N:

MLSRAVCGRQLAPVGLGSRQKHSLPAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQEIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPV
VLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALET
VQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNN
GKQALETVQRLLPVLCQAHGLTPEQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPDQVVA
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CQAHGLTPEQQVVAIASHDGGRPALESIVAQLSRPDPAALTNDHLVALACLGGRPALDAV
KKGLPHAPALIKRTNRRIPERTSHRVAGSGSYALGPYQISAPQLPAYNGQTGTFYYVND
GGLESKVFSGGPTPYPNYANAGHVEGQSAFMRDNGISEGLVFHNNEGTGFCVNMT
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TAYDESTDENVMLLTSDAPEYKPWLVIQDSNGENKIKMLRSGSGEGRGSLLTCGDVEEN/
T2A_cleavage_site/PGPRLEMVSKGEELFTGVVPILVELGDVNGHKFSVSGEGEGDATYG
KLTKFICTTGKLPVPWPTLVTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGLTGVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKV
NFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFV
TAAGITLGMDELYK*

DdCBE (L) - Nd3-9577 - G1337-C:

MLSRAVCGRQLAPVGLGSRQKHSLPAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQEIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPV
VLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALET
VQRLLPVLCQAHGLTPEQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNN
GKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPDQVVA
AIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGL
TPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPV
CQAHGLTPEQQVVAIASHDGGRPALESIVAQLSRPDPAALTNDHLVALACLGGRPALDAV
KKGLPHAPALIKRTNRRIPERTSHRVAGSAIPVKRGATGETKVFTGNSNSPKSPTKGCGSG
GSNLSDIIIEKETGKQLVIQESILMLPEEEVIGNKPESDILVHTAYDESTDENVMLLTSDAP
EYKPWLVIQDSNGENKIKMLRSGSGEGRGSLLTCGDVEEN/T2A_cleavage_site/PGPRLE
MVSKEELFTGVVPILVELGDVNGHKFSVSGEGEGDATYGKLTGKFICTTGKLPVPWPTL
VTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGLTGV
NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*

The halves used to target the H-strand - DdCBE (H) – in the cell screen have the general architecture of (from N- to C-terminus):

SOD2 MTS – 3xHA – TALE – 2aa linker – DddA_{tox} split – 4aa linker – UGI – E2A – RFP*

Targeting: 5'-TGCAGAGCTTGTAGG-3'

DdCBE (H) - Nd3-9577 - G1333-N:

MLSRAVCCTSRLAPVLGYLGSRQKHSPLAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGSQQQQEIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVIAISNNGKQALETVQRLLPVLCQAHLGLTPEQ
VVAIASHDGGKQALETVQRLLPVLCQAHLGLTPAQVVIAISNIGGKQALETVQRLLPVLCQAHL
GLTPDQVVAIASNNGKQALETVQRLLPVLCQAHLGLTPEQVVIAISNIGGKQALETVQRLLP
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PVLCQAHLGLTPEQVVIAISNNGGPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALD
AVKKGLPHAPALIKRTNRIPERTSHRVAGSGSYALGPYQISAPQLPAYNGQTVGTFYYVN
DAGGLESKVFSSGGSGGSTNLSDIIEKETGKQLVIQESILMPEEEVIGNKPESDILVHTA
YDESTDENVMLLTSDAPEYKPWALVIQDSNGENIKMLRSGQCTNYALLKLAGDVESNPG/E2A_cleavage_site/PGGGVSKGEELIKENMHMKLYMEGTVNHHFKCTSEGEKPYEGTQ
TMRIKVVEGGPLPFADILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGV
LTATQDTSLQDGCLIYNVKIRGVNFPNSGPVMQKKTLGWEANTEMLYPADGGLEGRSDMA
LKVGGGLICNFKTTYSKKPAKNLKMPGVYYYVDHRLERIKEADKETYVEQHEVAVARYC
DLPSKLGHKLNKLNPPDESGPGCMSCKCVLS*

DdCBE (H) - Nd3-9577 - G1333-C:

MLSRAVCCTSRLAPVLGYLGSRQKHSPLAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGSQQQQEIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVIAISNNGKQALETVQRLLPVLCQAHLGLTPEQ
VVAIASHDGGKQALETVQRLLPVLCQAHLGLTPAQVVIAISNIGGKQALETVQRLLPVLCQAHL
GLTPDQVVAIASNNGKQALETVQRLLPVLCQAHLGLTPEQVVIAISNIGGKQALETVQRLLP
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AVKKGLPHAPALIKRTNRIPERTSHRVAGSPTPYPNYANAGHVEGQSAFLMRDNGISEGL
VFHNNPEGTCGFCVNMTETLLPENAKMTVVPPEGAIPVKRGATGETKVFTGNNSNPKSPT
KGCGSGGSTNLSDIIEKETGKQLVIQESILMPEEEVIGNKPESDILVHTAYDESTDENVM
LLTSDAPEYKPWALVIQDSNGENIKMLRSGQCTNYALLKLAGDVESNPG/E2A_cleavage_site/PGGGVSKGEELIKENMHMKLYMEGTVNHHFKCTSEGEKPYEGTQ
TMRIKVVEGGPLPFADILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGV
LTATQDTSLQDGCLIYNVKIRGVNFPNSGPVMQKKTLGWEANTEMLYPADGGLEGRSDMA
LKVGGGLICNFKTTYSKKPAKNLKMPGVYYYVDHRLERIKEADKETYVEQHEVAVARYC
DLPSKLGHKLNKLNPPDESGPGCMSCKCVLS*

DdCBE (H) - Nd3-9577 - G1337-N:

MLSRAVCCTSRLAPVLGYLGSRQKHSLPAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQEIKPKVRSTVAQHHEALVGHGFTAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVIAISNNGKQALETVQRLLPVLCQAHLTPEQ
VVAIASHDGGKQALETVQRLLPVLCQAHLTPAQVVAIASNIGGKQALETVQRLLPVLCQA
GLTPDQVVAIASNNGKQALETVQRLLPVLCQAHLTPEQVVAIASNIGGKQALETVQRLLP
VLCQAHLTPDQVVAIASNNGKQALETVQRLLPVLCQAHLTPEQVVAIASNIGGKQALE
TVQRLLPVLCQAHLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNG
GGKQALETVQRLLPVLCQAHLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQV
VVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNIGGKQALETVQRLLPVLCQA
GLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNNGGKQALETVQRLL
PVLCQAHLTPEQVVAIASNNGGPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALD
AVKKGLPHAPALIKRTNRRIPERTSHRVA**GSGSYALGPYQISAPQLPAYNGQTGTFYYVN**
DAGGLESKVFSSGGPTPYPNYANAGHVEGQSAFMRDNGISEGLVFHNPEGTCGFCVN
MTETLLPENAKMTVVPPEGSGGS**TNLSDIIEKETGKQLVIQESILMLPEEEVIGNKPESDIL**
VHTAYDESTDENVMLLTSAPEYKPWALVIQDSNGENKIKML**RSGQCTNYALLKLAGDVES**
NPG/E2A_cleavage_site/PGGG**VSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEKPYE**
GTQTMRIKVVEGGPLPFADILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTYYED
GGVLTATQDTSLQDGCLIYNVKIRGVNFPNSGPVMQKKTLGWEANTEMLYPADGGLEGRS
DMALKLVGGGHЛИCNFKTTYSKPKAKNLKMPGVYYVDHRLERIKEADKETYVEQHEVAVA
RYCDLPSKLGHKLNKNPPDESGPGCMSCKCVLS*

DdCBE (H) - Nd3-9577 - G1337-C:

MLSRAVCCTSRLAPVLGYLGSRQKHSLPAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQEIKPKVRSTVAQHHEALVGHGFTAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVIAISNNGKQALETVQRLLPVLCQAHLTPEQ
VVAIASHDGGKQALETVQRLLPVLCQAHLTPAQVVAIASNIGGKQALETVQRLLPVLCQA
GLTPDQVVAIASNNGKQALETVQRLLPVLCQAHLTPEQVVAIASNIGGKQALETVQRLLP
VLCQAHLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNIGGKQALE
TVQRLLPVLCQAHLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNG
GGKQALETVQRLLPVLCQAHLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQV
VVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNIGGKQALETVQRLLPVLCQA
GLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHLTPEQVVAIASNNGGKQALETVQRLL
PVLCQAHLTPEQVVAIASNNGGPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALD
AVKKGLPHAPALIKRTNRRIPERTSHRVA**GS**AIPVKRGATGETKVFTGNSNSPKSPTKGCG
SGGS**TNLSDIIEKETGKQLVIQESILMLPEEEVIGNKPESDIL**VHTAYDESTDENVMLLSD
APEYKPWALVIQDSNGENKIKML**RSGQCTNYALLKLAGDVES**NPG/E2A_cleavage_site/PG
GG**VSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEKPYEGTQTMRIKVVEGGPLPFAD**
DILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTYYEDGGVLTATQDTSLQDGCL
NVKIRGVNFPNSGPVMQKKTLGWEANTEMLYPADGGLEGRSDMALKLVGGGHЛИCNFKTT
YRSKPKAKNLKMPGVYYVDHRLERIKEADKETYVEQHEVAVARYCDLPSKLGHKLNKNPP
DESGPGCMSCKCVLS*

Supplementary Sequences 2 | Amino acid sequences of DdCBEs-Nd3-9577 used in the AAV experiments.

The halves used to target the L-strand - **DdCBE (L)** – in the AAV experiments are similar to the cell screen, except that the T2A self-cleavage peptide and the fluorescent marker GFP were substituted by a stop codon at the end of UGI, resulting in the architecture of (from N- to C-terminus):

SOD2 MTS – 3xHA – TALE – 2aa linker – DddA_{tox} G1333-C – 4aa linker – UGI*

Targeting: 5'-TCAGAAAAAGCAAATC-3'

AAV- DdCBE (L) - Nd3-9577 - G1333-C:

**MLSRAVCGTSRQLAPVLGYLGSRKHSPLAGYPYDVPDYAGYPYDVPDYAGYPYDVPDY
AIHGVPMDLRTLGYSQQQKEKIKPKVRSTVAQHHEALVGHGFTAHIVALSQHPAALGTV
AVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDTGQLLKIAKRG
GVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHDGKQALETVQRLLPVLCQAHLGLTPAQ
VVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALETVQRLLPVLCQA
GLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLP
VLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALE
VQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNN
GKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPAQVV
AIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHL
TPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALETVQRLLPV
CQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASNNGGKQALE
KKGLPHAPALIKRTNRRIPERTSHRVAGSPTPYPNYANAGHVEGQSALFMRDNGISEGLVF
HNNPEGTCGFVNMTETLLPENAKMTVVPPEGAIPVKRGATGETKVFTGNSNSPKSPTKG
GCSGGSTNLSDIIKETGKQLVIQESILMPEEEVEEIGNKPESDILVHTAYDESTDENVMILLT
SDAPEYKPWALVIQDSNGENKIKML***

The halves used to target the H-strand - **DdCBE (H)** – in the AAV experiments are similar to the cell screen, except that the E2A self-cleavage peptide and the fluorescent marker RFP were substituted by a stop codon at the end of UGI and the 3xHA tag substituted by a 3xFLAG tag, resulting in the architecture of (from N- to C-terminus):

SOD2 MTS – 3xFLAG – TALE – 2aa linker – DddA_{tox} G1333-N – 4aa linker – UGI*

Targeting: 5'-TGCAGAGCTTGTAGG-3'

AAV- DdCBE (H) - Nd3-9577 - G1333-N:

**MLSRAVCGTSRQLAPVLGYLGSRKHSPLAGDYKDHDGDYKDHDIDYKDDDDKIHGVPMV
DLRTLGYSQQQKEKIKPKVRSTVAQHHEALVGHGFTAHIVALSQHPAALGTVAVKYQDMI
AALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPGTLDTGQLLKIAKRGGVTAVEAV
HAWRNALTGAPLNLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHLGLTPDQVVAIASHDG
GKQALETVQRLLPVLCQAHLGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHLGLTPDQV**

AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGL
TPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPV
LCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALE
VQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNG
GKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV
AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNNGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPH
APALIKRTNRRIPERTSHRVAGSGSYALGPYQISAPQLPAYNGQTVGTFYYVNDAGGLESK
VFSSGGSGGSNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDEN
VMLLTSDAPEYKPWLVIQDSNGENKIKML*