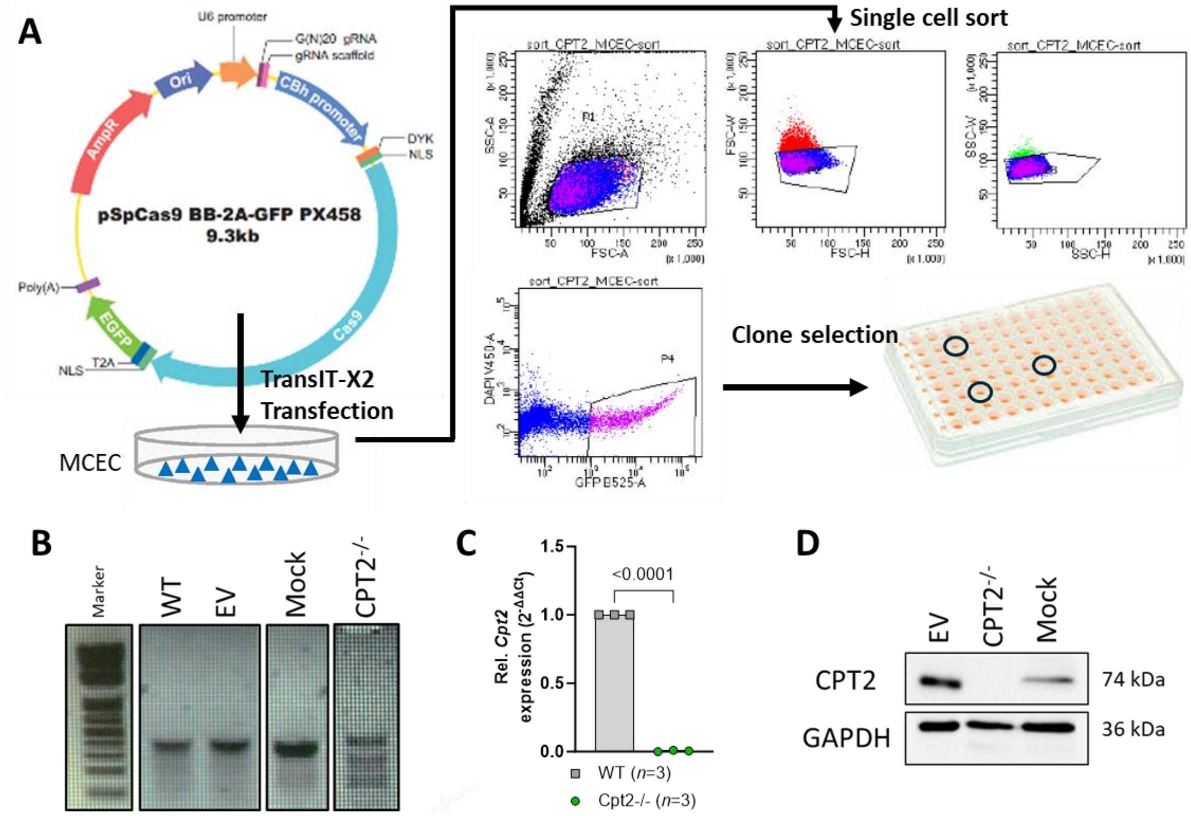
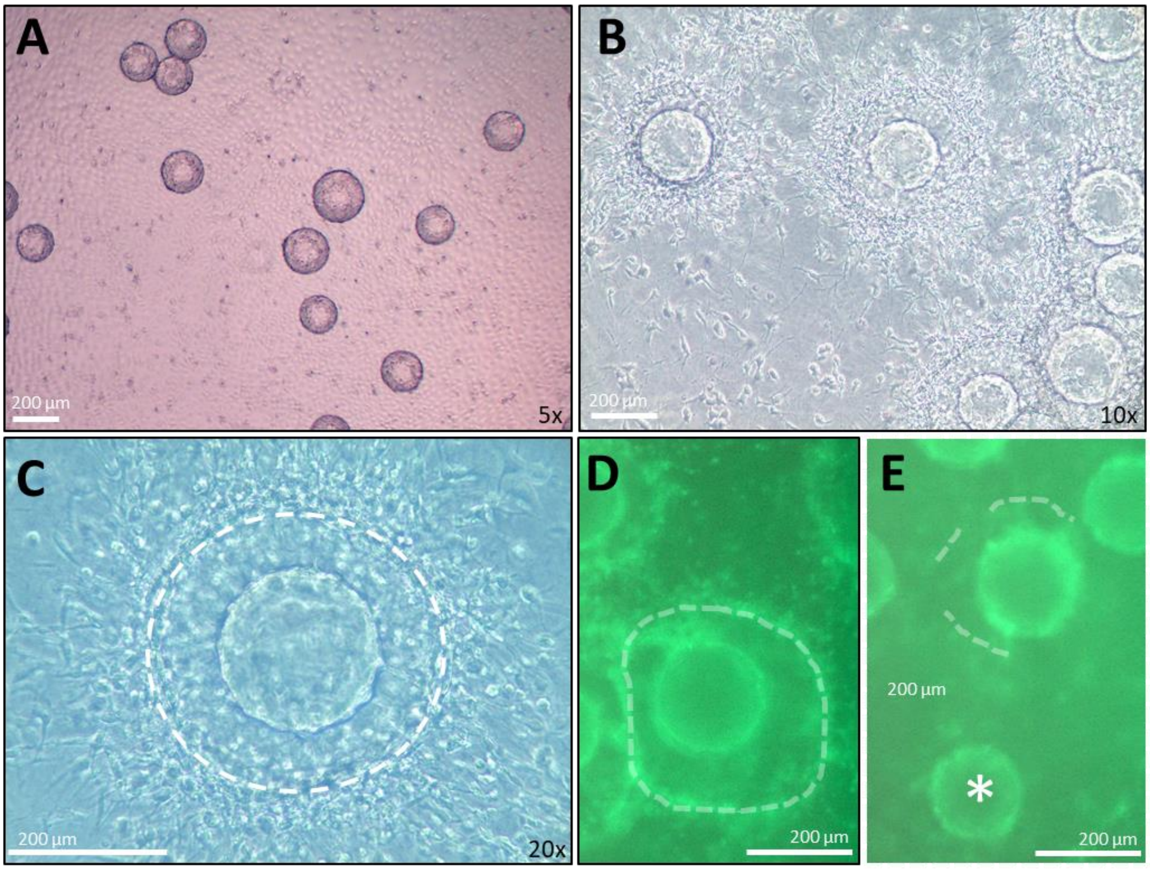
Supplementary Figures

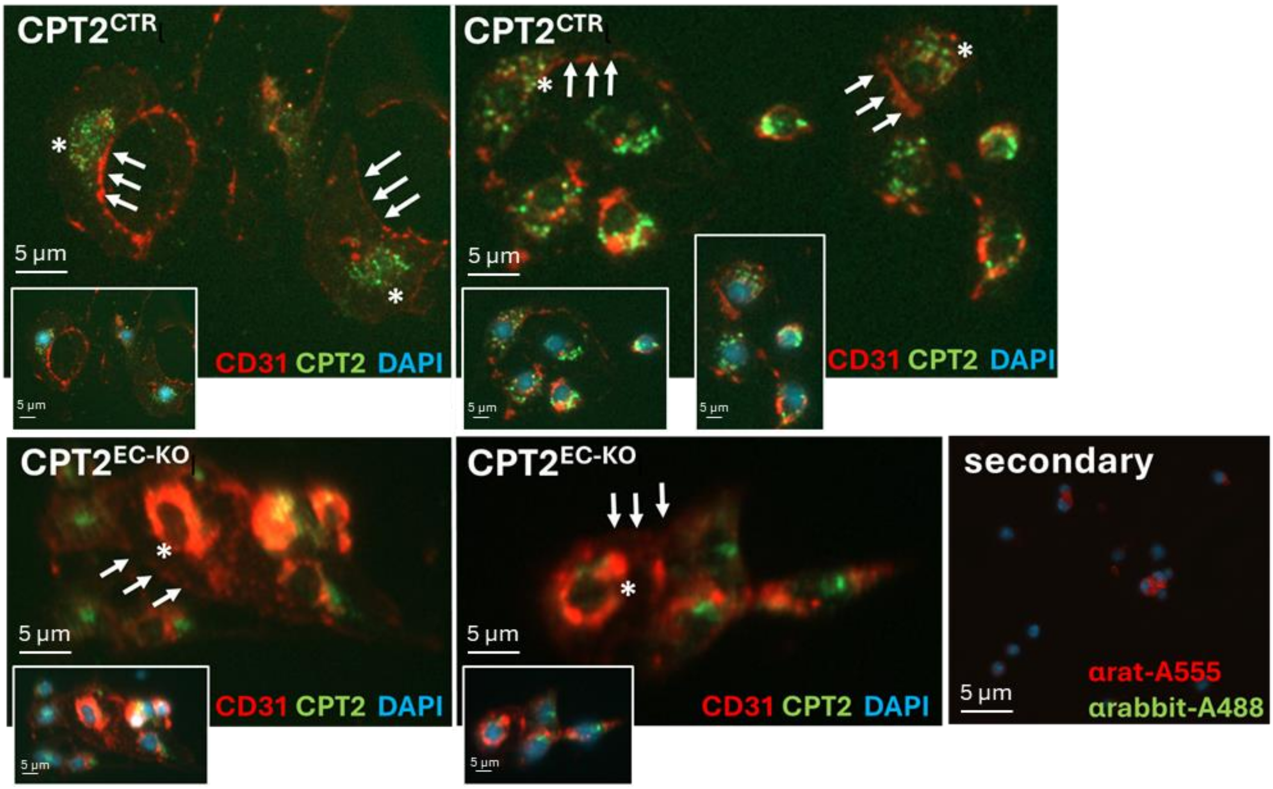


Supplementary Fig. 1. Confirmation of CRISPR-Cas9-induced *Cpt2* deletion in MCEC.
(**A**) Schematic overview of Cpt2-/- MCEC production. MCEC were transfected with Cpt2-specific sgRNA-PX458 Cas9-GFP vector via TransIT-X2® Dynamic Delivery System. GFP cassette allows a FACS-based single cell sorting of positively transfected cells in the 96-well format. Sorted cell clones were grown to confluency and tested for genetic *Cpt2* deletion. (**B**) T7 Endonuclease I assay of isolated DNA from Cpt2-/- MCEC and control MCEC (WT, EV, Mock). Cpt2-/- shows cleaving of mismatched DNA introduced by CRISPR-Cas9. (**C**) qPCRbased analysis of *Cpt2* mRNA expression in Cpt2-/- MCEC and control WT MCEC. *n*=3 technical replicates. Statistical analysis with unpaired Student´s test. (**D**) Representative western blot of Cpt2-/- MCEC and control MCEC (EV, Mock). Cas9 (Caspase 9), Cpt2 (carnitine palmitoyltransferase II), EV (empty vector), GFP (green fluorescent protein), MCEC (murine cardiac endothelial cells), WT (wildtype).



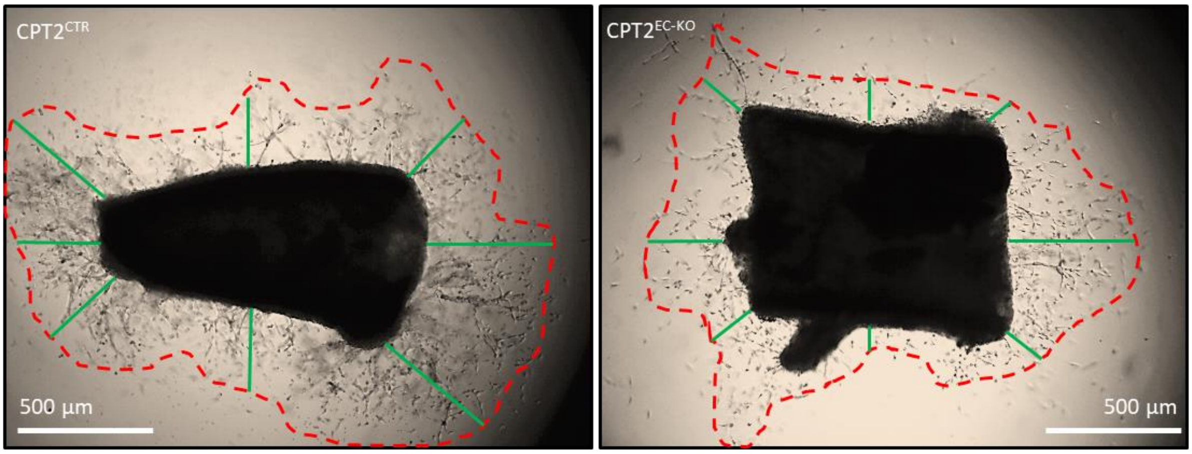
Supplementary Fig. 2. 3D Fibrin Sprouting Assay in Cpt2-/- MCEC.

Representative brightfield microscopy images of endothelial sprouting from microcarrier beads in fibrin matrix in 5x (**A**), 10x (**B**) and 20x objective (**C**). MCEC form ring-shaped sprouting around beads (white dashed circle). Representative pictures of quantification of FITC-Lectin stained ring-shaped sprouting of WT (**D**) and Cpt2-/- (**E**) MCEC. White dashed lines represent quantified pixel area of intact ring sprouting. WT sprouting shows a complete ring integrity around beads, whereas Cpt2-/- sprouting is less and interrupted, including beads where no ring sprouting appeared (white asterisk).



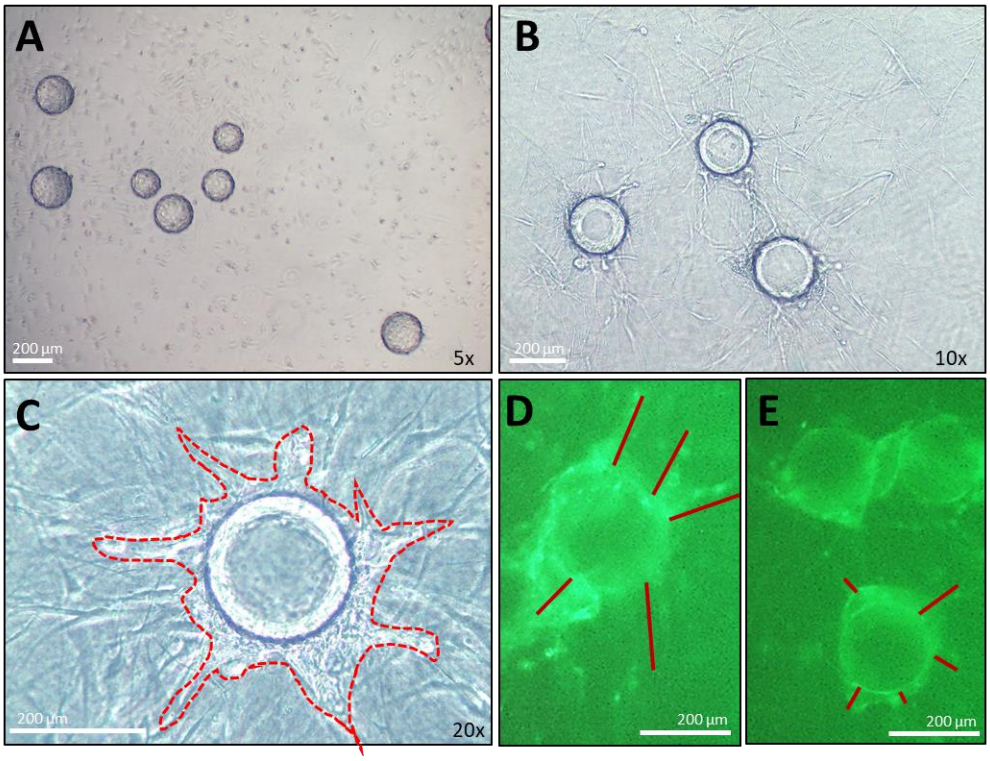
Supplementary Fig. 3. Confirmation of EC-specific Cpt2 knockout via immune fluorescence staining of isolated ECs.

Representative pictures of isolated liver ECs from CPT2$^{EC\text{-}KO}$ and CPT2^{CTR} mice stained against endothelial CD31 (red) and CPT2 (green). Nuclei were counterstained with DAPI (blue). White arrows indicate staining of endothelial marker CD31 at the surface of the cell membrane. White asterisk indicates green dotted CPT2 staining around nucleus in CPT2^{CTR} mice, whereas it is absent in CPT2$^{EC\text{-}KO}$ mice.



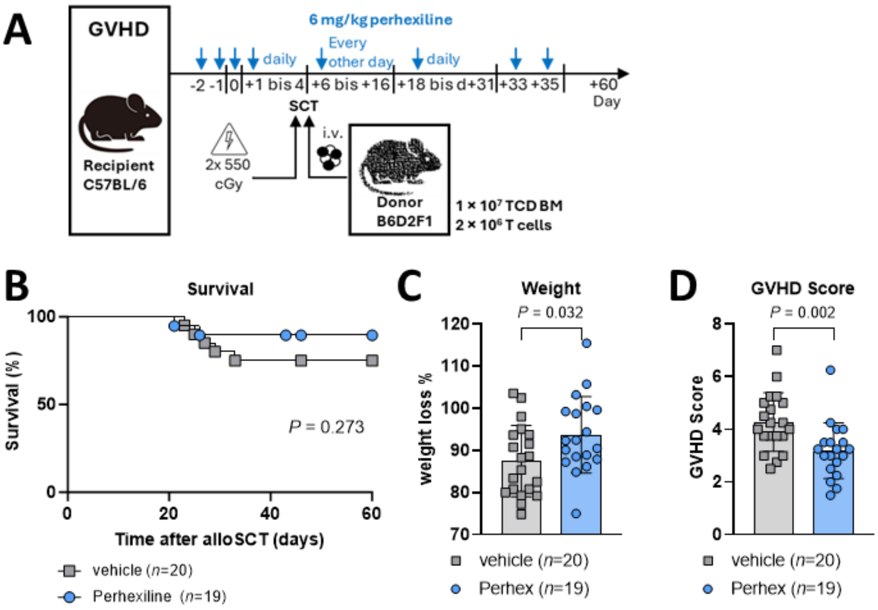
Supplementary Fig. 4. Aortic sprouting assay in CPT2$^{EC\text{-}KO}$ GVHD mice.

Representative brightfield pictures of Matrigel embedded thoracic aorta pieces of CPT2$^{EC\text{-}KO}$ and CPT2^{CTR} mice. Endothelial sprouting was assessed by quantifying the sprout (green line) perimeter (dashed red line) around each aorta piece.



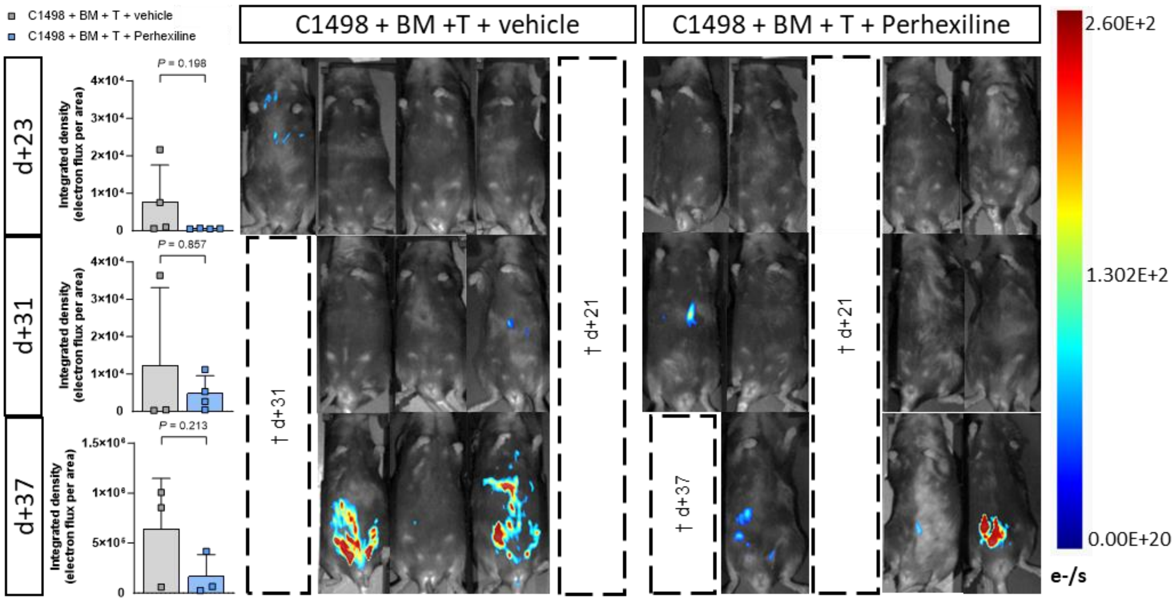
Supplementary Fig. 5. 3D Fibrin Sprouting Assay in perhexiline-treated HUVEC.

Representative brightfield microscopy images of endothelial sprouting from microcarrier beads in fibrin matrix in 5x (**A**), 10x (**B**) and 20x objective (**C**). HUVECs form radial sprouts emerging from the beads, with the extent of outgrowth varying according to sprouting capacity (dashed red line). Representative pictures of quantification of FITC-Lectin stained sprouts of vehicle- (**D**) and perhexiline-treated (**E**) HUVEC. Length of 5 sprouts per bead were calculated in pixels (red lines).



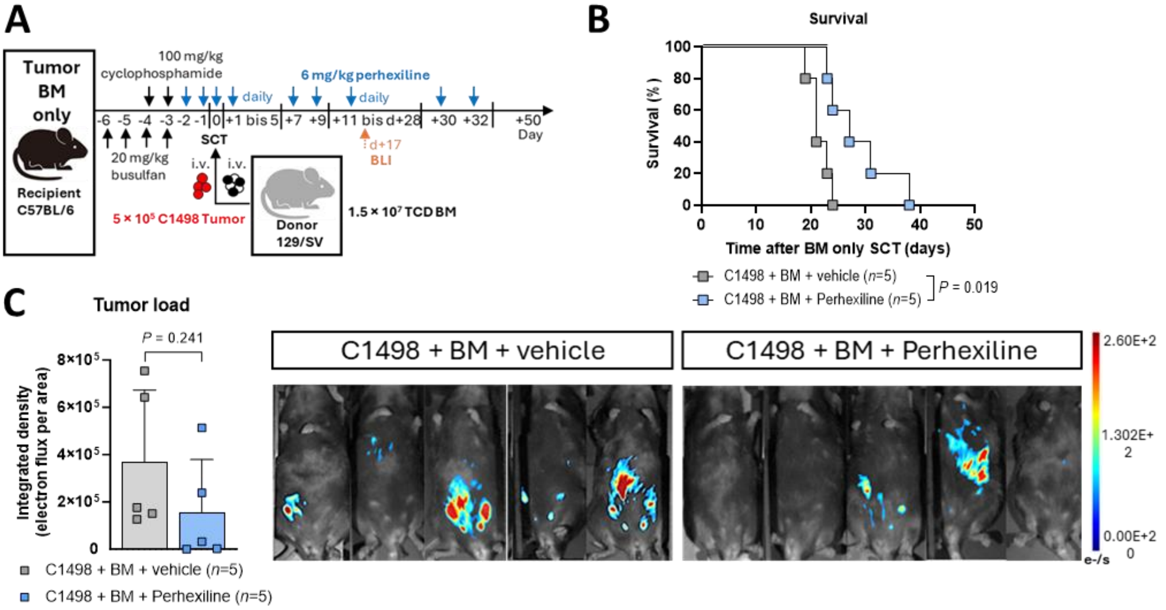
Supplementary Fig. 6. Perhexiline treatment in haploident alloSCT GVHD model.

(**A**) Schematic illustration of murine haploident alloSCT GVHD model B6D2F1 into C57BL/6 with perhexiline treatment. (**B**) Percent survival. *n* =19 or 20 mice per group, analyzed by Mantel-Cox test. Weight loss (**C**) and clinical GVHD score (**D**) during established GVHD after alloSCT. *n* =19 or 20 mice per group. Statistical analysis by unpaired Student's t test and by Mann-Whitney U test, respectively. Error bars indicate mean +SD. allo (allogeneic), BM (bone marrow), GVHD (graft-versus-host disease), SCT (stem cell therapy).

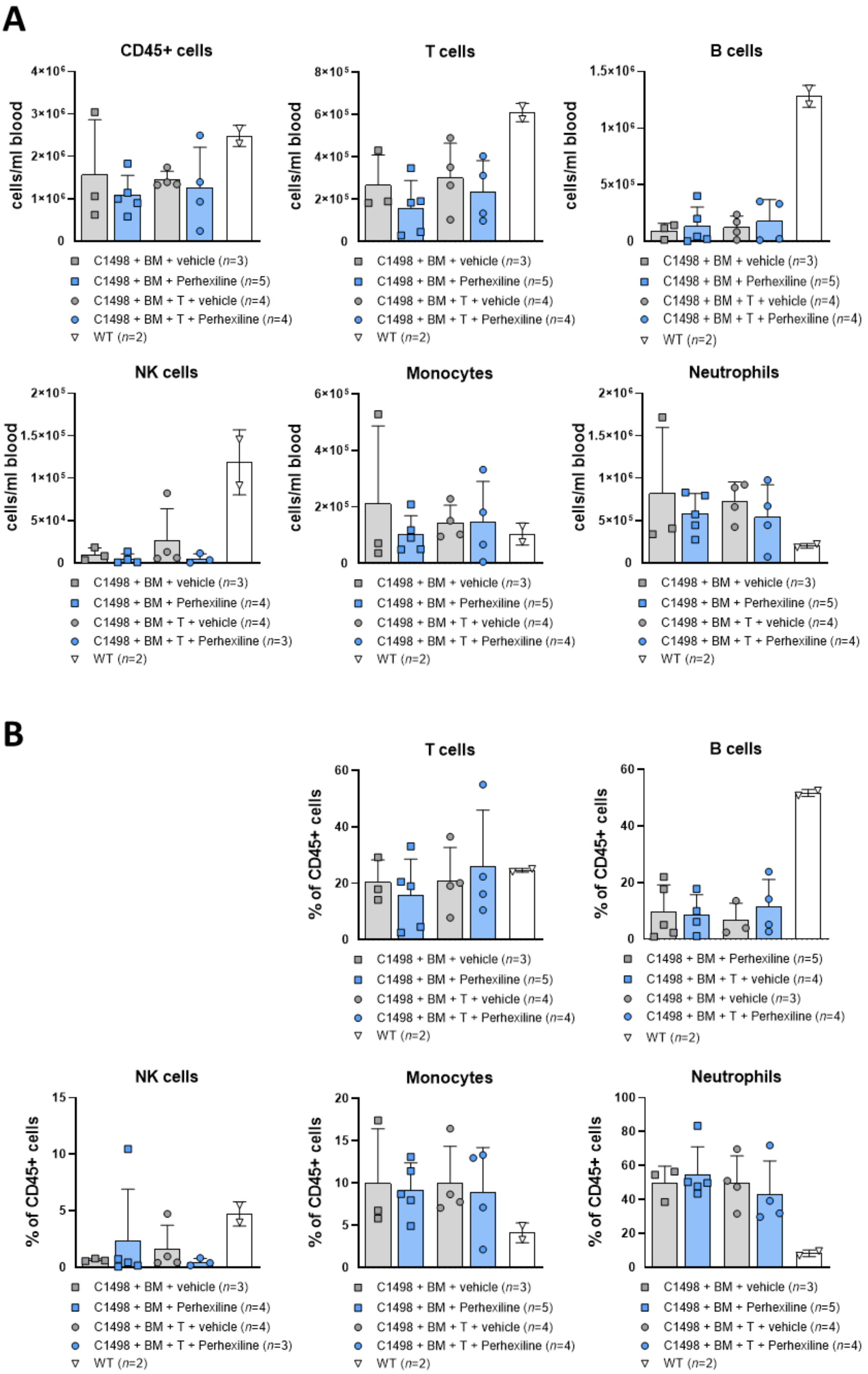


Supplementary Fig. 7. GVL effect remains intact after alloSCT with perhexiline treatment at later timepoints.

Quantification of tumor load 23, 31 and 37 days after alloSCT and tumor induction (acute myeloid leukemia cells C1498) of perhexiline and vehicle treated mice via bioluminescence imaging (BLI). Representative BLI pictures. *n* =4 (d+23), 3 or 4 (d+31), 3 (d+37) mice per group. Statistical analysis by unpaired Student´s t test. Error bars indicate mean +SD. allo (allogeneic), BM (bone marrow), GVL (graft-versus-leukemia), SCT (stem cell therapy).



Supplementary Fig. 8. Perhexiline reduces tumor growth in BM-transplanted mice.
(A) Schematic illustration of Bone marrow only transplantation model 129/SV into C57BL/6 with tumor induction (acute myeloid leukemia cells C1498) and perhexiline treatment. (**B**) Percent survival. *n* =5 mice per group, analyzed by Mantel-Cox test. (**C**) Quantification of tumor load 17 days after BM only SCT and tumor induction via bioluminescence imaging (BLI). Representative BLI pictures. *n* =5 mice per group. Statistical analysis by unpaired Student´s t Test. Error bars indicate mean +SD. BM (bone marrow) SCT (stem cell therapy).



Supplementary Fig. 9. Perhexiline does not have a significant effect on immune reconstitution during GVL activity. Blood was analyzed from mice of murine minor mismatch alloSCT GVL model 129/SV into C57BL/6 with tumor induction (acute myeloid leukemia cells C1498) and perhexiline treatment at time of sacrifice. Absolute cell numbers of immune cells per ml blood (**A**) and percentage of CD45+ cells (**B**). All immune cells were identified as CD45+. Within the

CD45+ population neutrophils were identified as Ly6G+, monocytes as Ly6G-CD115+, B cells as CD19+B220+, NK cells as NK1.1+TCRb- and T cells as NK1.1-TCRbeta+. *n* =2-5 mice per group. Error bars indicate mean +SD. allo (allogeneic), BM (bone marrow), GVL (graft-versus-leukemia), NK (natural killer), SCT (stem cell therapy), WT (wildtype).

Supplementary Figure 10: Plasmid vector pSpCas9(BB)-2A-GFP (PX458). Vector designed by Ran et al. [doi: 10.1038/nprot.2013.143], graphics taken from https://www.addgene.org/48138/.

Supplementary Methods

CRISPR-Cas9-Cpt2-sgRNA vector generation

To generate a *Cpt2* knockout (KO) in murine cardiac endothelial cells (MCEC), the CRISPR-Cas9 system was employed. Single guide RNAs (sgRNAs) of 25 nucleotides in length were designed using the online CRISPOR tool (https://crispor.gi.ucsc.edu, UC Santa Cruz) [doi: 10.1093/nar/gky354]. For the *Cpt2* locus, two sgRNAs were selected based on predicted specificity, efficiency and minimized off-target potential. The corresponding oligonucleotides were synthesized by BioTeZ GmbH (Supplementary Table 5). sgRNAs were cloned into the pSpCas9(BB)-2A-GFP (PX458) vector (Addgene, USA) [doi: 10.1038/nprot.2013.143], which encodes the *S. pyogenes* Cas9 endonuclease, a green fluorescent protein (GFP) reporter and the sgRNA scaffold (Supplementary Figure 10). For cloning, 10 µg of PX458 was digested with 30U of the restriction enzyme BbsI (New England Biolabs, USA) in NEB2.1 buffer for 2 hrs at 37 °C. Complete digestion of the plasmid backbone was verified by electrophoresis on a 1 % agarose gel alongside an undigested control. The linearized plasmid was gel-purified using the Innuprep Gel Extraction Kit (Analytik Jena, Germany) and DNA concentration and purity were assessed with NanoDrop 1000. Complementary sgRNA oligonucleotides were annealed by incubating 10 µl of 10x T4 DNA ligase buffer (New England Biolabs), 10 µl each of forward and reverse 100 µM sgRNA oligonucleotides, and 70 µl nuclease-free water at 95 °C for 15 min, followed by cooling to room temperature. The annealed oligonucleotides were ligated into the BbsI-linearized PX458 plasmid backbone. Ligation reaction contained 100 ng digested PX458, 2 ng annealed oligonucleotides, 2 µl of 10x T4 DNA ligase buffer, and 400U T4 DNA ligase (New England Biolabs) and was incubated at 17 °C overnight.

CRISPR-Cas9-Cpt2-sgRNA vector transformation and multiplication

Up to 100 ng of ligated PX458-Cpt2-sgRNA plasmids were transformed into E. coli NEB 10-beta competent cells (New England Biolabs) following the manufacturer's high-efficiency protocol. Transformed cells were plated on LB-agar plates (LB medium: MP Biomedicals, Germany; agar-agar: Carl Roth, Germany) supplemented with 100 µg/ml ampicillin (Sigma-Aldrich, USA) and incubated overnight at 37 °C in a bacterial incubator. The next day, individual colonies were picked with sterile pipette tips, streaked onto fresh LB-ampicillin plates for retransformation, and incubated overnight at 37 °C. In parallel, colonies were screened by PCR for the presence of the PX458-Cpt2-sgRNA construct (Supplementary Table 6). Successfully transformed colonies yielded a band of approximately 390 bp on a 1.5 % agarose gel. Two positive colonies were selected for sequencing. Colonies were inoculated from the retransformation plates into 2 ml LB-ampicillin medium in 15 ml culture tubes (Greiner Bio-One, Germany) and incubated overnight at 37 °C in a bacterial incubator. Plasmid DNA was extracted using the NucleoSpin Plasmid Kit (Macherey-Nagel, Germany) and concentrations were determined with NanoDrop 1000. 200 ng DNA was submitted for Sanger sequencing (Eurofins Genomics, Berlin) using the Fwd_U6 primer (5' TAA AAT GGA CTA TCA TAT GCT TAC C 3'), which binds the vector backbone before the sgRNA integration site. Resulting sequences were aligned against the PX458 reference sequence and analyzed with ApE Plasmid Editor v1.0.55. Colonies confirmed to contain correctly ligated PX458-Cpt2-sgRNA constructs were cultured in 200 ml LB-ampicillin medium overnight at 37 °C. Plasmid DNA was purified using the NucleoBond Xtra Maxi Kit (Macherey-Nagel) and DNA purity and integrity were assessed by NanoDrop 1000.

Transfection of MCEC with CRISPR-Cas9-Cpt2-sgRNA vector

MCEC were transfected with either Cpt2-sgRNA-PX458 or PX458 empty vector (control) using the TransIT-X2® Dynamic Delivery System (Mirus Bio, USA). MCEC were seeded into 6 cm cell culture dishes and cultured to approximately 80% confluence. The culture medium was then replaced with Opti-MEM™ I Reduced Serum Medium (Thermo Fisher Scientific, USA) and transfection was performed for 24 hrs according to the manufacturer’s instructions, using a DNA-to-reagent ratio of 1:2 (µg:µl).

Selection of Cpt2-/- MCEC

Cells were detached from culture dishes following a 2 min incubation with Accutase (Innovative Cell Technologies, USA). Cell sorting was performed by the BIH Cytometry Core Facility on a BD FACSAriaII SORP (BD Biosciences, USA), configured with 4 lasers (violet, blue, yellow-green, red), using an 85 µm nozzle. The instrument was calibrated following the manufacturer's recommendations. Viable GFP-positive cells were sorted as single cells and deposited individually into 0.1% gelatin-coated 96-well plates pre-filled with conditioned MCEC medium. Plates were incubated for up to two weeks until single-cell–derived colonies expanded sufficiently for transfer to 12-well plates and subsequent analysis. Cpt2 knockout was validated by T7 Endonuclease I assay to detect CRISPR-Cas9–induced mismatched DNA cleavage, by RT-qPCR to assess downregulation of *Cpt2* mRNA and by Western blot to confirm reduced CPT2 protein expression. For functional assays, MCEC transfected with Cpt2-sgRNA-PX458 were used, either displaying a Cpt2 knockout (Cpt2-/-) or not (Mock). Additional controls included MCEC transfected with the empty PX458 vector (EV) and wild-type MCEC (WT).

T7 Endonuclease I Assay

Genome editing efficiency was assessed using a T7 Endonuclease I assay (NEB, M0302S). Genomic DNA was isolated from single cell clone-derived colonies carrying Cpt2-sgRNA-PX458 and empty vector constructs using the DNeasy Blood & Tissue Kit (Qiagen, Netherlands). A 369 bp genomic fragment spanning the sgRNA target site, with the protospacer adjacent motif (PAM) located approximately in the center, was amplified by PCR (Supplementary Table 7). PCR products were loaded on 1.5 % agarose gels and purified using the Innuprep Gel Extraction Kit (Analytik Jena). For heteroduplex formation, 200 ng purified PCR product was diluted in nuclease-free water to a final volume of 9 µl, supplemented with 1 µl buffer B2 (NEB) and subjected to a rehybridization program on a thermocycler (95 °C for 5 min, ramp down to 85 °C at 2 °C/s, 85 °C for 1 min, ramp down to 25 °C at 0.1 °C/s, hold at 25 °C). The rehybridized products (10 µl) were digested for 30 min at 37 °C with 0.5 µl T7 Endonuclease I in 0.5 µl Buffer B2 and 4 µl nuclease-free water. Digested PCR products were resolved on a 2 % agarose gel. Successful CRISPR-Cas9 editing was indicated by cleavage of the PCR fragment into smaller bands in sgRNA-containing samples, whereas empty vector controls remained uncut.

RNA isolation & Quantitative real-time PCR (RT-qPCR)

RNA was extracted from MCEC pellets using the RNeasy Mini Kit (Qiagen) following manufacturer´s instructions. RNA concentration and purity were assessed with NanoDrop 1000. cDNA was synthesized from 1 µg RNA using the QuantiTect Reverse Transcription Kit (Qiagen) and diluted to a final concentration of 12.5 ng/µl in nuclease-free water and stored at -20°C until use. For TaqMan assays, 2µl cDNA was amplified on a DNA Engine Opticon (BioRad) using TaqMan™ Gene Expression Master Mix (Thermo Fisher Scientific) with primers and probes labeled with 6-carboxyfluorescein (FAM) (BioTez GmbH, Germany, Supplementary Table 4). Cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 49 cycles of 95°C for 10 s and 60°C for 1 min. Data was analyzed with the Opticon Monitor 3.1 analysis software (BioRad) and comparative 2-ΔΔCT method with normalization to *Gapdh*. The binary logarithmic expression fold change of Cpt2-/- ECs was compared to wildtype controls.

Western Blot

MCEC pellets were lysed for 30 min at 4°C in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitors (1 mM/ml NaF, 10 µg/ml leupeptin, 2 µg/ml aprotinin, 0.1 mM PMSF). Protein concentration was determined by Pierce™ BCA assay (Thermo Fisher Scientific). Equal amounts of protein (40 µg) were denatured with 4x Laemmli buffer + 10% β-mercaptoethanol + 5% DTT at 95°C for 5 min and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in 1x SDS-PAGE running buffer (2.5 mM Tris pH 8.0 + 19 mM glycine + 0.1% SDS + ddH$_2$O). A 12% separating and 5% stacking gel for SDS-PAGE was cast using the Mini-PROTEAN® Tetra Handcast System. The separating gel (30% Acrylamide Mix, 1.5 M Tris pH 8.8, 10% SDS, 15% APS, 0.4% TEMED in H$_2$0) was polymerized for ≥30 min at RT, followed by a stacking gel with 0.5 M Tris pH 6.8 polymerized on top for 30 min. SDS-PAGE was run at 80 V for 10 min followed by 120 V for 90 min to separate proteins. 250 kDA PageRuler Plus Prestained Protein Ladder (Thermo Fisher Scientific) was used. Proteins were transferred to methanol-activated PVDF membranes (Bio-Rad, USA) using the Power Blotter semi-dry transfer system with Pierce™ 1-Step Transfer Buffer (Thermo Fisher Scientific) under size-adjusted default settings. Successful transfer was verified by staining the membrane with 1x Ponceau solution (Sigma-Aldrich) for 10 min. Membranes were washed twice in ddH$_2$O, blocked 1 hr at RT (5% milk or BSA in PBST) and incubated with primary antibodies (1:100 anti-Cpt2, clone EPR13626, ab181114 by Abcam, rabbit and 1:2000 anti-GAPDH, polyclonal, G9545 by Sigma-Aldrich, rabbit) overnight at 4°C. Anti-rabbit HRP-conjugated secondary antibody (1:2000, clone 6B9G9, Biolegend) was incubated for 1 hr at RT. Bands were detected with ECL Prime Western Blot detection reagent (GE Healthcare) using a ChemiDoc MP imaging system with Image Lab 5.2.1 software (Bio-Rad). Membranes were UV-imaged in "chemi sensitive" mode with up to 1000 s exposure, acquiring images every 60 s until clear signals appeared.

Flow cytometry

Cryopreserved blood samples were thawed at 4 °C for 1 hr, washed with 2ml FACS buffer and centrifuged at 500 x g for 5 min. Cells were resuspended in blocking buffer (PBS +2% BSA +2% normal mouse/human/rabbit/rat serum) and stained with fluorochrome-conjugated antibodies (Supplementary Table 2) for 20 min at RT. After washing, cells were resuspended in 200 µl staining buffer. Directly before analysis, 10.000 Precision count beads (Biolegend) were added to each sample to allow quantification of absolute cell numbers. Samples were acquired on a Cytek® Aurora Cytometer equipped with 3 lasers and 38 detectors and running Spectroflo® v3.0.3. Instrument QC was performed daily according to the manufacturer´s instructions. Samples were acquired on low to medium speed. Data analysis was performed with FCS Express 7.18.0020 (Denovo Software, USA). First, all immune cells were identified as CD45+. Within the CD45+ population neutrophils were identified as Ly6G+, monocytes as Ly6G-CD115+, B cells as CD19+B220+, NK cells as NK1.1+TCRb- and T cells as NK1.1-TCRbeta+.

Supplementary Tables

Supplementary Table 1. Genotyping: primer sequences and PCR programs

Cpt2 loxP				
Primer				
Forward			5′ GCT GGC TTA GGA GAT TCT TAA CTT CC 3′	
Reverse			5′ AGC TCA GGT GGC AGA AAT GAT ACC 3′	
PCR Program			**Mastermix**	
95°C	10 min		12,5 µl	REDTaq® ReadyMix™ (Sigma-Aldrich)
95°C	1 min	35 cycles	0,3 µl	F-Primer 50 µM
60°C	1 min		0,3 µl	R-Primer 50 µM
72°C	1 min		6,9 µl	dH$_2$0
72°C	10 min		2 µl	DNA 100 ng
4°C	∞			
VE-Cadherin-Cre				
Primer				
Forward			5' GCG GTC TGG CAG TAA AAA CTA TC 3'	
Reverse			5' GTG AAA CAG CAT TGC TGT CAC TT 3'	
Forward internal			5' CTA GGC CAC AGA ATT GAA AGA TCT 3'	
Reverse internal			5' GTA GGT GGA AAT TCT AGC ATC ATC C 3'	
PCR Program			**Mastermix**	
95°C	3 min		12,5 µl	REDTaq® ReadyMix™ (Sigma-Aldrich)
95°C	30 s	35 cycles	0,3 µl	F-Primer 50 µM
51,7°C	1 min		0,3 µl	R-Primer 50 µM
72°C	1 min		0,3 µl	Internal F-Primer 50 µM
72°C	2 min		0,3 µl	Internal R-Primer 50 µM
4°C	∞		6,3 µl	dH$_2$0
			2 µl	DNA 100 ng

Supplementary Table 2. Antibodies used for flow cytometry

Epitope	Fluorophore	Clone	Dilution	Catalogue nr.	Company
CD31	PE	MEC13.3	1:100	553373	BD Biosciences
CD31	APC-Cy7	MEC13.3	1:200	102439	Biolegend
CD146	FITC	P1H12	1:50	562229	BD Biosciences
CD146	AF647	ME-9F1	1:200	562230	BD Biosciences
CD3	APC	BM10-37	1:100	553066	BD Biosciences
CD45	AF532	30-F11	1:400	58-0451-82	Thermo Fisher Scientific
CD19	PE-Cy5	6D5	1:400	115510	Biolegend
B220	APC-Fire810	RA3-6B2	1:400	103277	Biolegend
Ly6C/Ly6G	AF700	RB6-8C5	1:400	56-5931-80	Thermo Fisher Scientific
Ly6G	BV421	1A8	1:400	127628	Biolegend
TCRbeta	BV750	H57-597	1:400	747006	BD Biosciences

Supplementary Table 3. Antibodies used for immunofluorescence staining

Primary Antibodies					
Epitope	**Host**	**Clone**	**Dilution**	**Catalogue nr.**	**Company**
CD31	rat	MEC13.3	1:100	553370	BD Biosciences
CD4	rat	H129.19	1:500	550278	BD Biosciences
CD8a	rat	53-6.7	1:500	553027	BD Biosciences
Cpt2	rabbit	EPR13626	1:50	ab181114	Abcam
Caspase 3	rabbit	Asp175	1:250	9661	Cell Signaling
Secondary Antibodies					
Host	**Reactivity**	**Fluorophore**	**Dilution**	**Catalogue nr.**	**Company**
donkey	rat	AF488	1:1000	A21208	Thermo Fisher Scientific
donkey	rat	AF594	1:1000	A21209	Thermo Fisher Scientific
donkey	rabbit	AF488	1:1000	A21206	Thermo Fisher Scientific

Supplementary Table 4. TaqMan primers used for qPCR

Cpt2	
Forward Primer	5'-GGGCGAGCTTCAGCATATGA-3'
Reverse Primer	5'-GGCCCATCGCTGCTTCTT-3'
Probe	FAM-5'-CTGAGTGCTCCAAATACCATGGCC-3'-TAMRA
Gapdh	
Forward Primer	5'-GGCAAATTCAACGGCACAGT-3'
Reverse Primer	5'-AGATGGTGATGGGCTTCCC-3'
Probe	FAM-5'-AGGCCGAGAATGGGAAGCTTGTCATC-3'-TAMRA

Supplementary Table 5. sgRNAs for *Cpt2* locus

Gene sequence Exon 1 (296 bp)	**Sequence guide**	**Specificity Score**	**Predicted Efficiency**		**Out of frame Score**	**Off targets 0-1-2-3-4**	**PAM**
			Doench ´16	**Mor.-Mateos**			
ATGATGCCGCGCCTGCTGTTGCGTGACTGGCCC CGGTGCCCCTCGCTTGTCCTGGGAGCCCCTAGT CGGCCCTTAAGTGCTGTCTCGGGGCCCGCCGAG TATCTGCAGCACAGCATCGTACCCACCATGCAC TACCAGGACAGCCTGCCCAG	rev rev	97	64	45	78	27 0-0-0-1-26	GGG
Sequence sgRNA 5´-3´							
Cpt2-sgRNA F	CACCGAGCACTTAAGGGCCGACTAG						
Cpt2 sgRNA R	AAACCTAGTCGGCCCTTAAGTGCTC						

Supplementary Table 6. Transformation check PCR PX458-Cpt2-sgRNA. Primer Fwd-Cpt2 binds on integrated Cpt2-sgRNA, primer Rev_Cbh binds on PX458 vector backbone, primer Fwd_U6 binds on vector backbone before sgRNA integration side and is used for positive control.

Transformation check				
Primer				
Fwd_Cpt2			5' CAC CGA GCA CTT AAG GGC CGA CTA G 3'	
Fwd_U6			5' TAA AAT GGA CTA TCA TAT GCT TAC C 3'	
Rev_Cbh			5' GAA AGT CCC TAT TGG CGT TAC 3'	
PCR Program			**Mastermix**	
94°C	3 min		2 µl	Taq standard buffer (New England Biolabs)
94°C	45 s	32 cycles	0,25 µl	Taq Polymerase (New England Biolabs)
55°C	45 s		0,5 µl	10 mM dNTP Mix (New England Biolabs)
72°C	45 s		0,4 µl	Fwd Primer 50 µM
72°C	7 min		0,4 µl	Rev Primer 50 µM
4°C	∞		16,45 µl	ddH$_2$0
				Picked colony

Supplementary Table 7. PCR for T7 Endonuklease I Assay

Cpt2-T7-PCR with 369 bp product				
Primer				
Fwd_Cpt2_T7			5' TCC AAG CTG GGG AAT GTG AC 3'	
Rev_Cpt2_T7			5' GCG GAG TTG AAC TGG ACG TT 3'	
PCR Program			**Mastermix**	
94°C	5 min		12,5 µl	REDTaq® ReadyMix™ (Sigma-Aldrich)
94°C	45 s	35 cycles	0,3 µl	F-Primer 50 µM
55°C	45 s		0,3 µl	R-Primer 50 µM
72°C	30 s		6,9 µl	dH$_2$0
72°C	10 min		2 µl	DNA 100 ng
4°C	∞			