Supplementary Information

Site Specific Integration in CHO Cells Mediated by CRISPR/Cas9 and Homology-Directed DNA Repair Pathway

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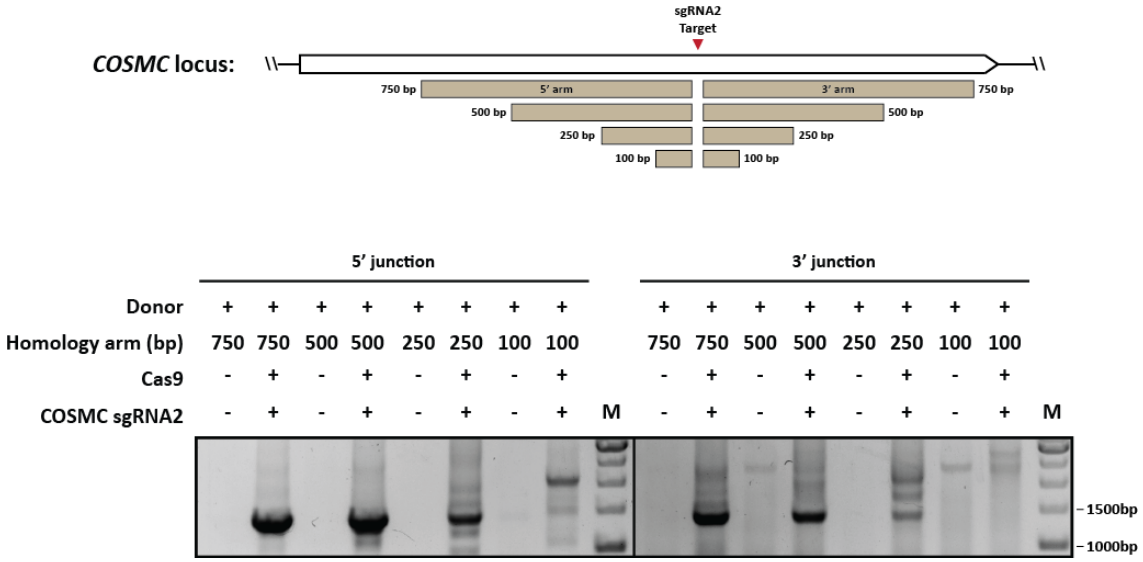
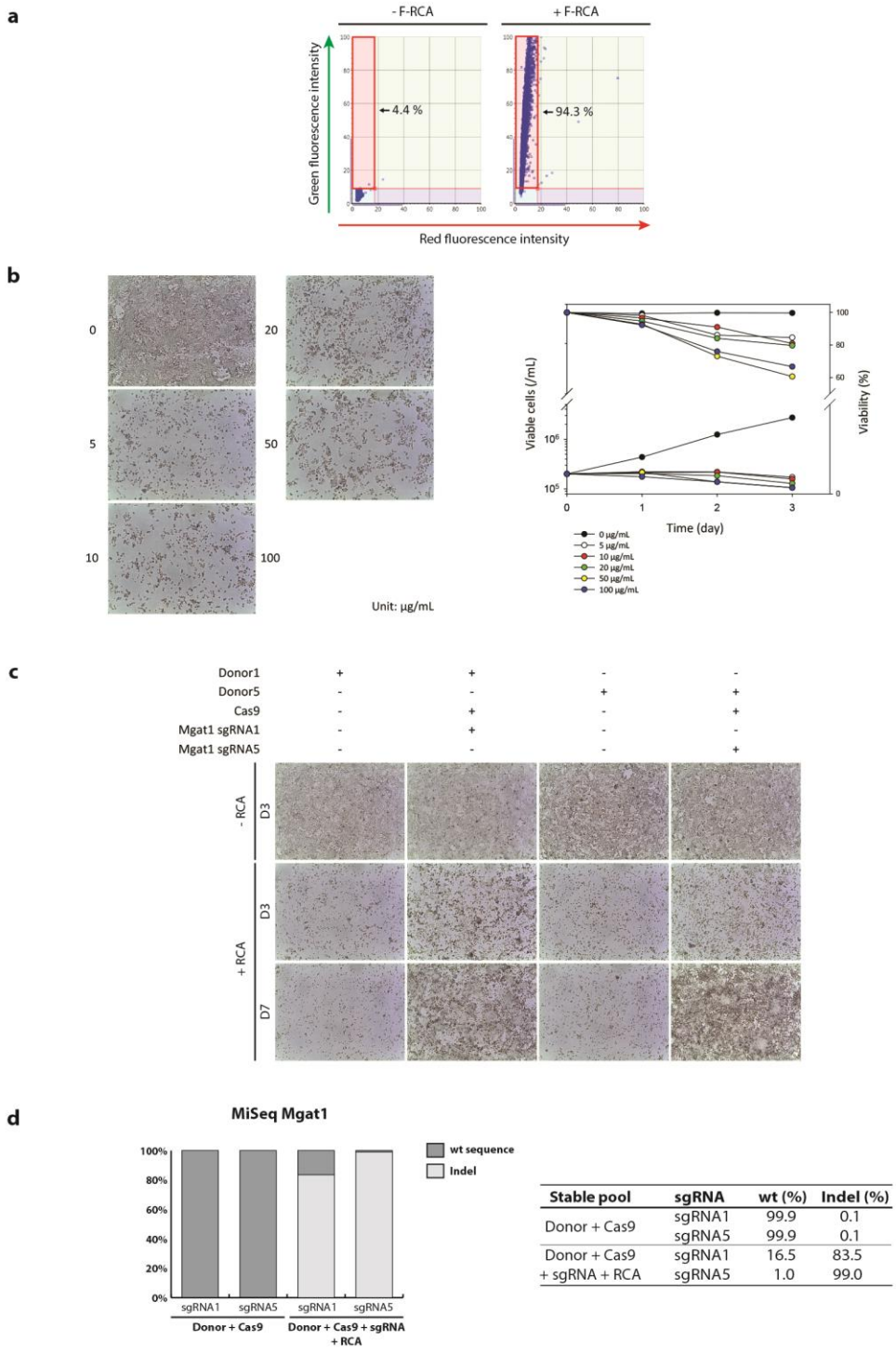
Figures

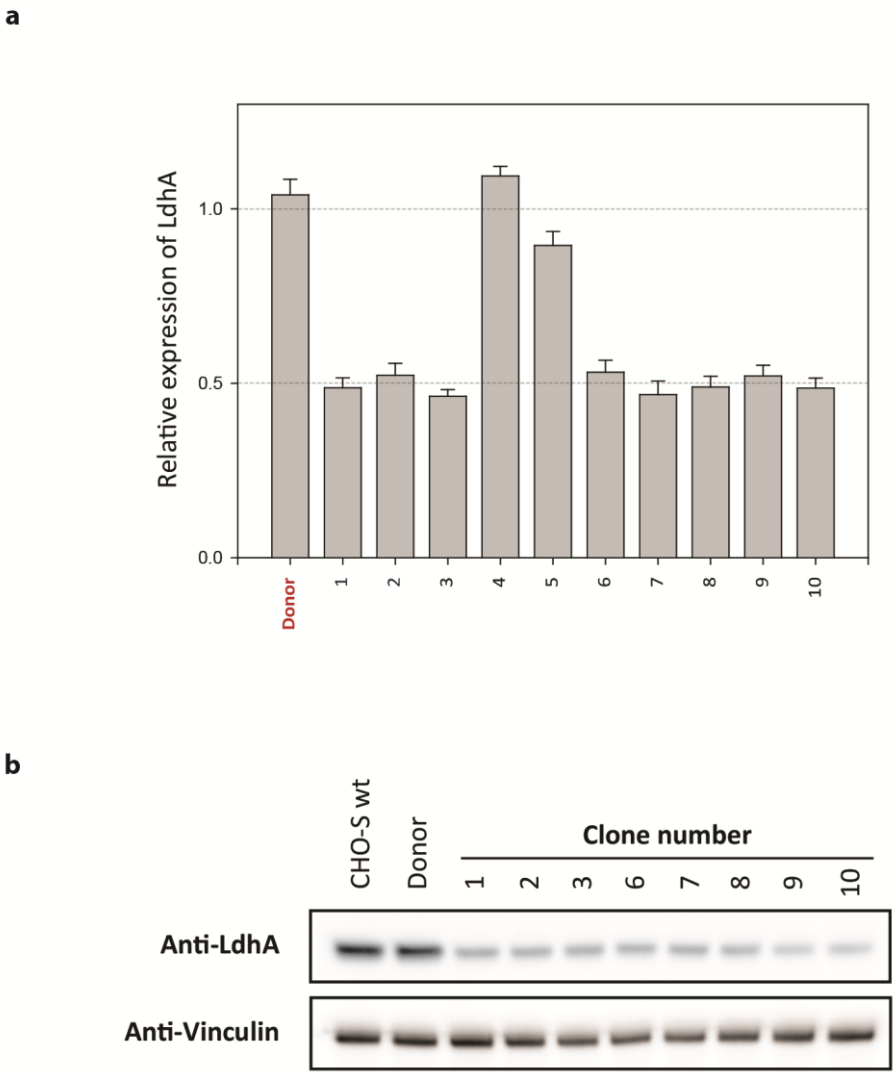


Supplementary Figure 1. Effect of the length of homology arms on targeted integration. Donor plasmids containing different length of 5' and 3' homology arms flanking *COSMC* sgRNA2 target site were designed. Genomic DNA extracted from stable pool of cells transfected with Cas9, sgRNA2, and each donor plasmid was used for 5'/3' junction PCR to investigate target specific knock-in.

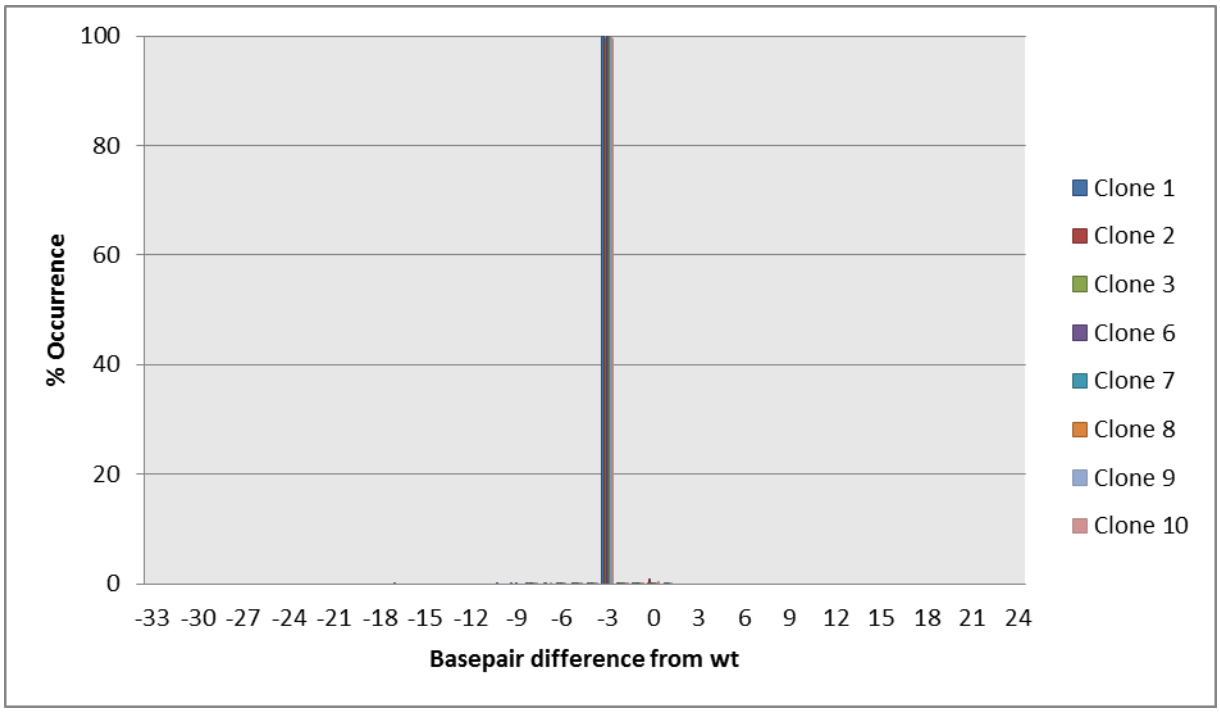


Stable pool	sgRNA	wt (%)	Indel (%)
Donor + Cas9	sgRNA1	99.9	0.1
	sgRNA5	99.9	0.1
Donor + Cas9 + sgRNA + RCA	sgRNA1	16.5	83.5
	sgRNA5	1.0	99.0

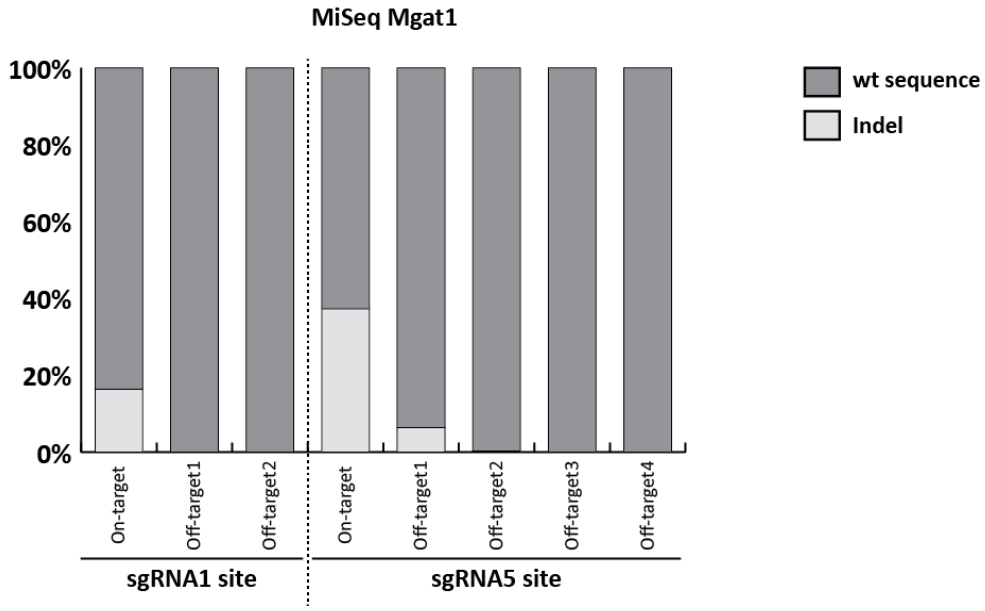
Supplementary Figure 2. RCA-I sensitivity analysis on wild type CHO-S cells and *Mgat1* knockout cells. (a) F-RCA staining of CHO-S cells, followed by quantification based on green fluorescent levels. (b) Effect of RCA-I treatment on CHO-S cells with regard to cell growth and viability. CHO-S cells were cultivated in the presence of various concentrations of RCA-I. Viable cell concentration and viability was subsequently measured on a daily basis, and representative microscopy images were acquired on day 3 (*left*). (c) Acquisition of RCA-I resistance occurred by CRISPR/Cas9 activity for the *Mgat1* locus. Stable pool of cells transfected with Cas9, sgRNA, and donor plasmid targeting *Mgat1* locus formed confluent in the presence of RCA-I. (d) Knockout of *Mgat1* locus in RCA-I resistant cells was confirmed by deep sequencing analysis of the *Mgat1* locus.



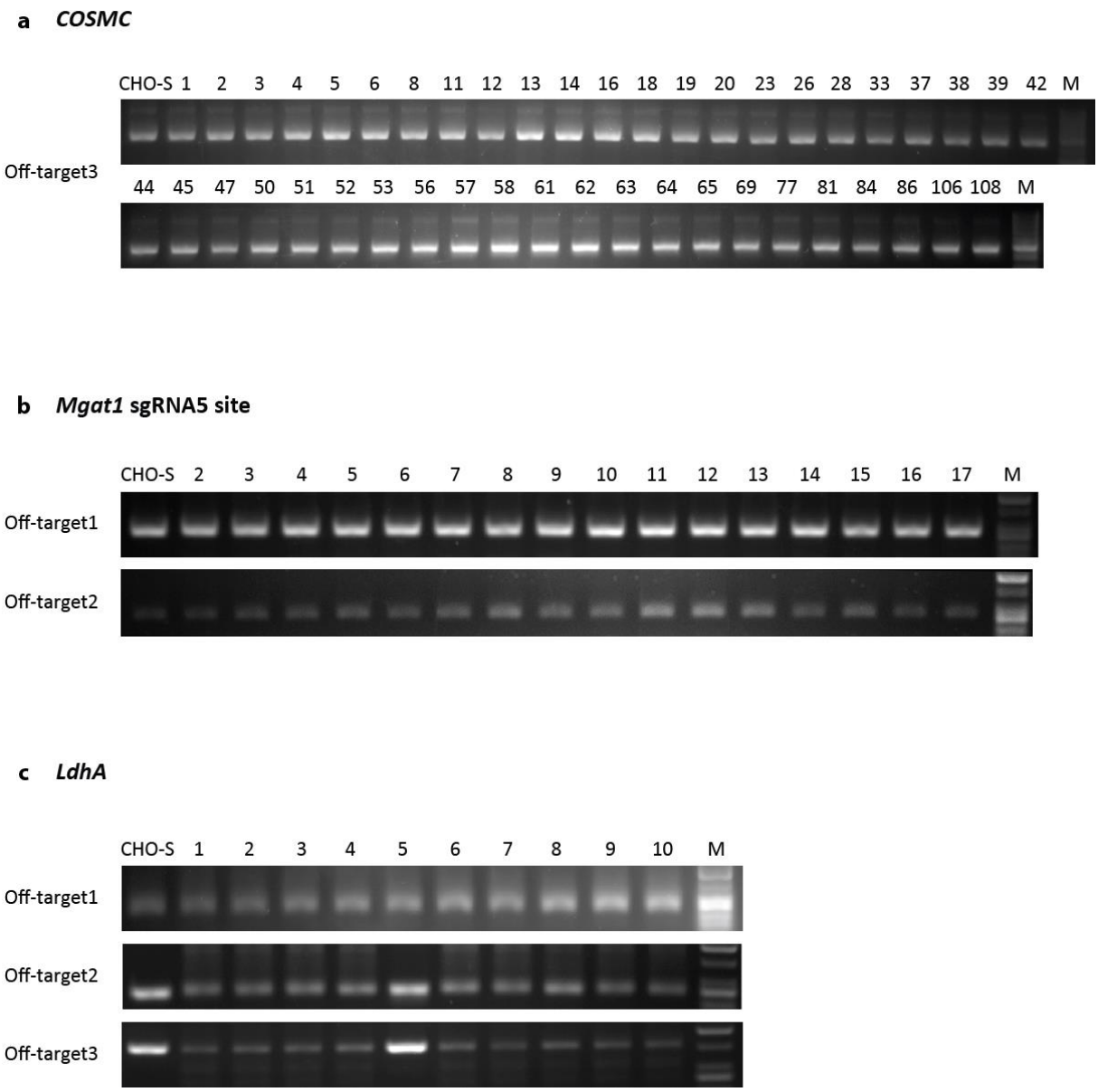
Supplementary Figure 3. Comparison of the relative LdhA expression level in *LdhA* targeted clonal cells. (a) Analysis of LdhA mRNA. Total RNAs from each individual clone were isolated using TRIzol® Reagent (Life Technologies), followed by DNase treatment to remove contaminating DNA (TURBO DNA-free™ DNase Treatment and Removal Reagents, Life Technologies). cDNAs were synthesized from 1 µg of total RNAs using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific). For each cDNA sample, the qRT-PCR was performed to quantify LdhA mRNA levels. All samples were normalized for the beta-actin (ACTB) level. The control used to estimate the relative expressions was the mRNA expression in CHO-S wild type cells, which was set at 1.0. Average values from three independent experiments ± SD are shown. (b) Analysis of LdhA protein. Cells were lysed in a fresh lysis buffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2.5 mM $MgCl_2$, 0.5% Triton X-100, supplemented with 1x Roche protease inhibitor cocktail). 10 µg of protein was separated on 4-12% Bis-Tris NuPAGE® gel (Life Technologies), followed by transfer to nitrocellulose membranes. Blots were probed with anti-LdhA (Cell Signaling Technology, Danvers, MA) or anti-Vinculin (Sigma-Aldrich; loading control) antibodies, and bands were then visualized using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare Life Sciences, Buckinghamshire, UK).



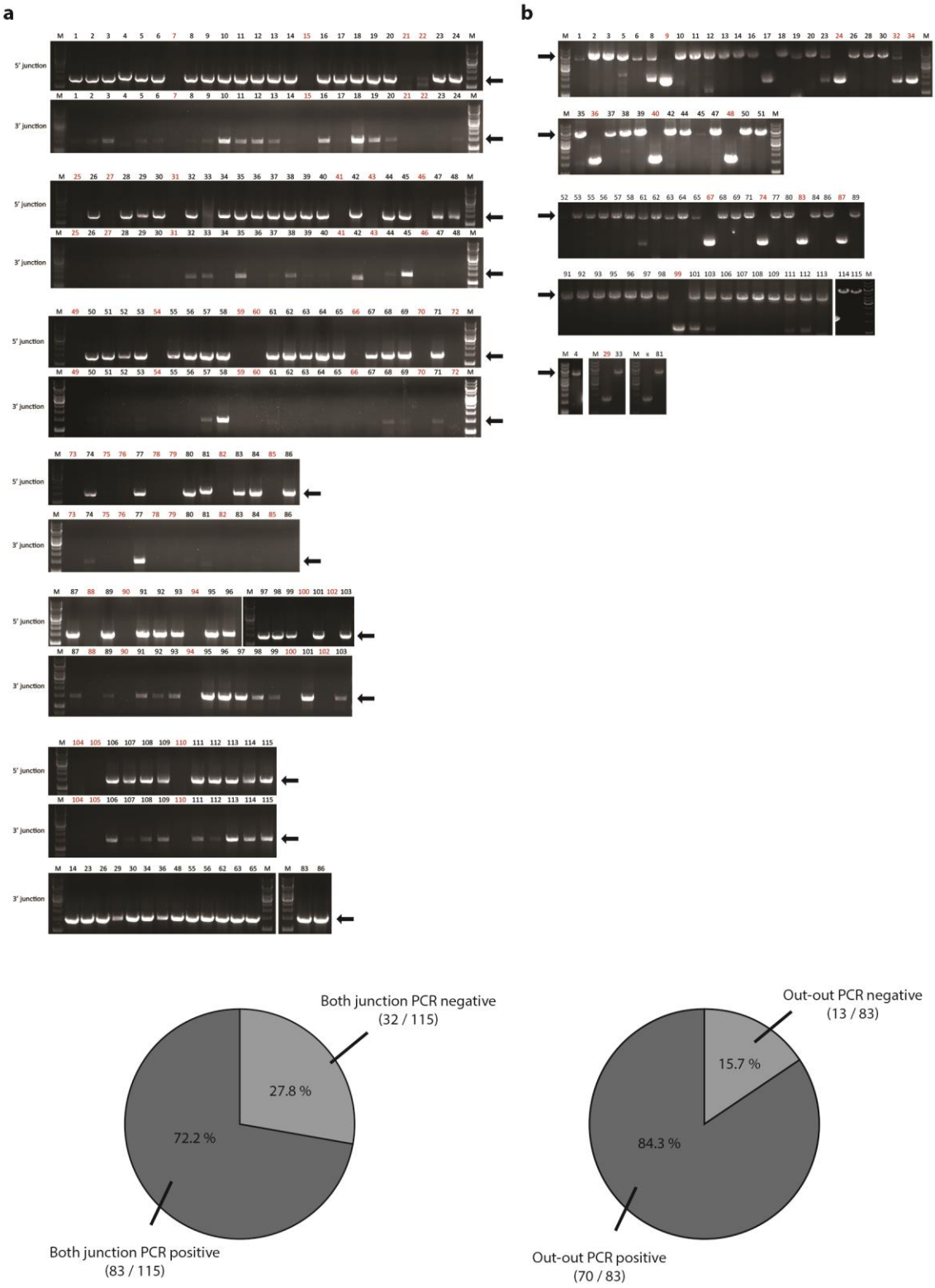
Supplementary Figure 4. Frequency distribution of indel sizes at the *LdhA* locus in *LdhA* targeted clonal cells. The regions spanning the integration site at the *LdhA* locus, sgRNA2 target site, were subjected to deep sequencing analysis using Miseq. The size distribution of indels ranging from -33 bp (33 bp deletion) to 24 bp (24 bp insertion) is shown.



Supplementary Figure 5. Indel frequencies at *Mgat1* on-target sites and off-target sites before RCA-I enrichment. Genomic DNA was extracted from two stable pool of cells transfected with Cas9, sgRNA, and donor plasmids, one for each sgRNA (sgRNA1 or sgRNA5). PCR amplicons, which span the *Mgat1* on-target sites and potential off-target sites as described in Fig. 4B and 5C, were subjected to deep sequencing analysis using Miseq.

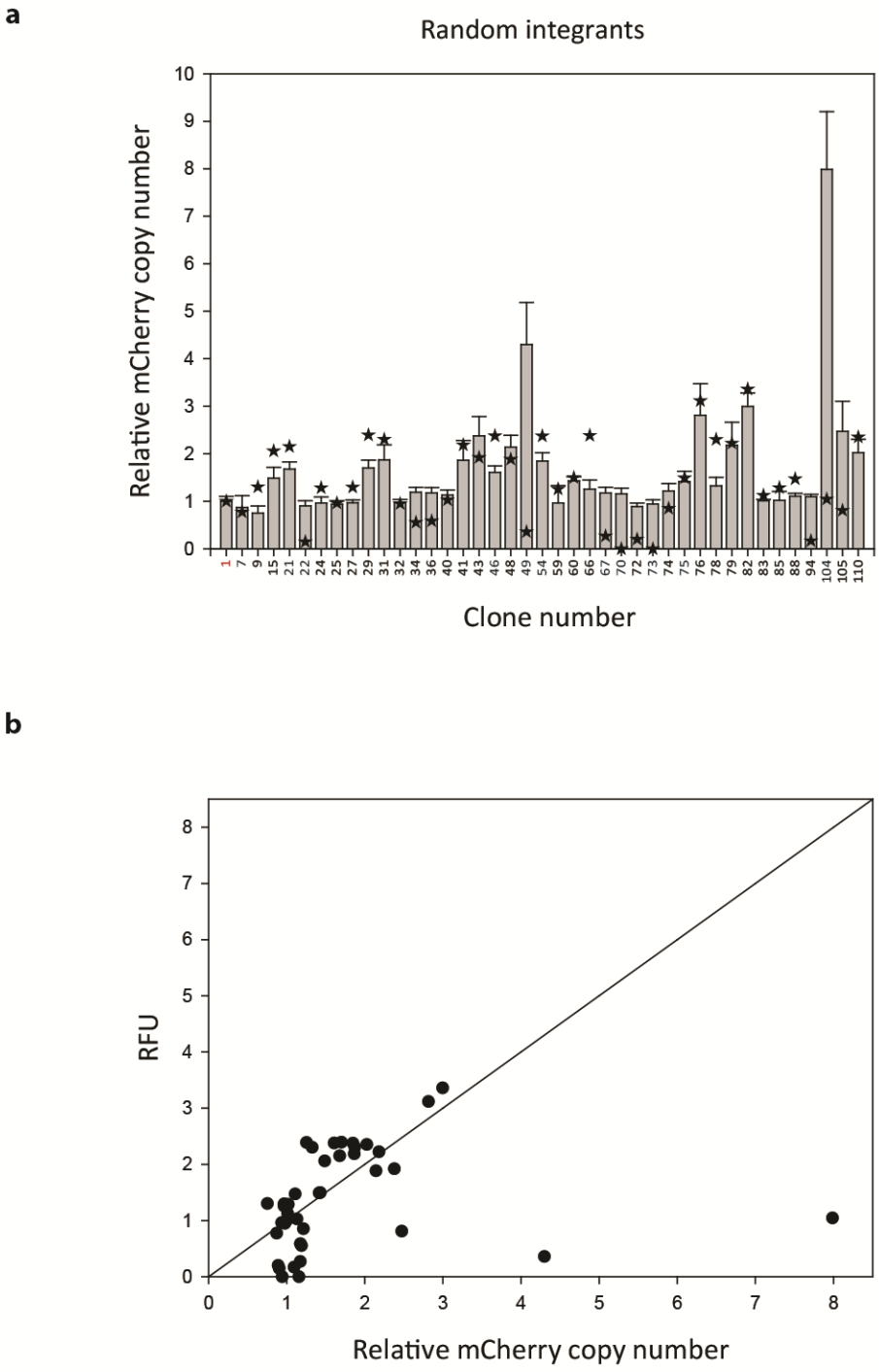


Supplementary Figure 6. PCR amplification of potential off-target sites. PCR was performed on genomic DNAs of each individual clone that were used for off-target analysis. PCR amplicons spanning off-target sites at any measurable frequency were generated, and amplicon size was compared with that from CHO-S wild type cells.

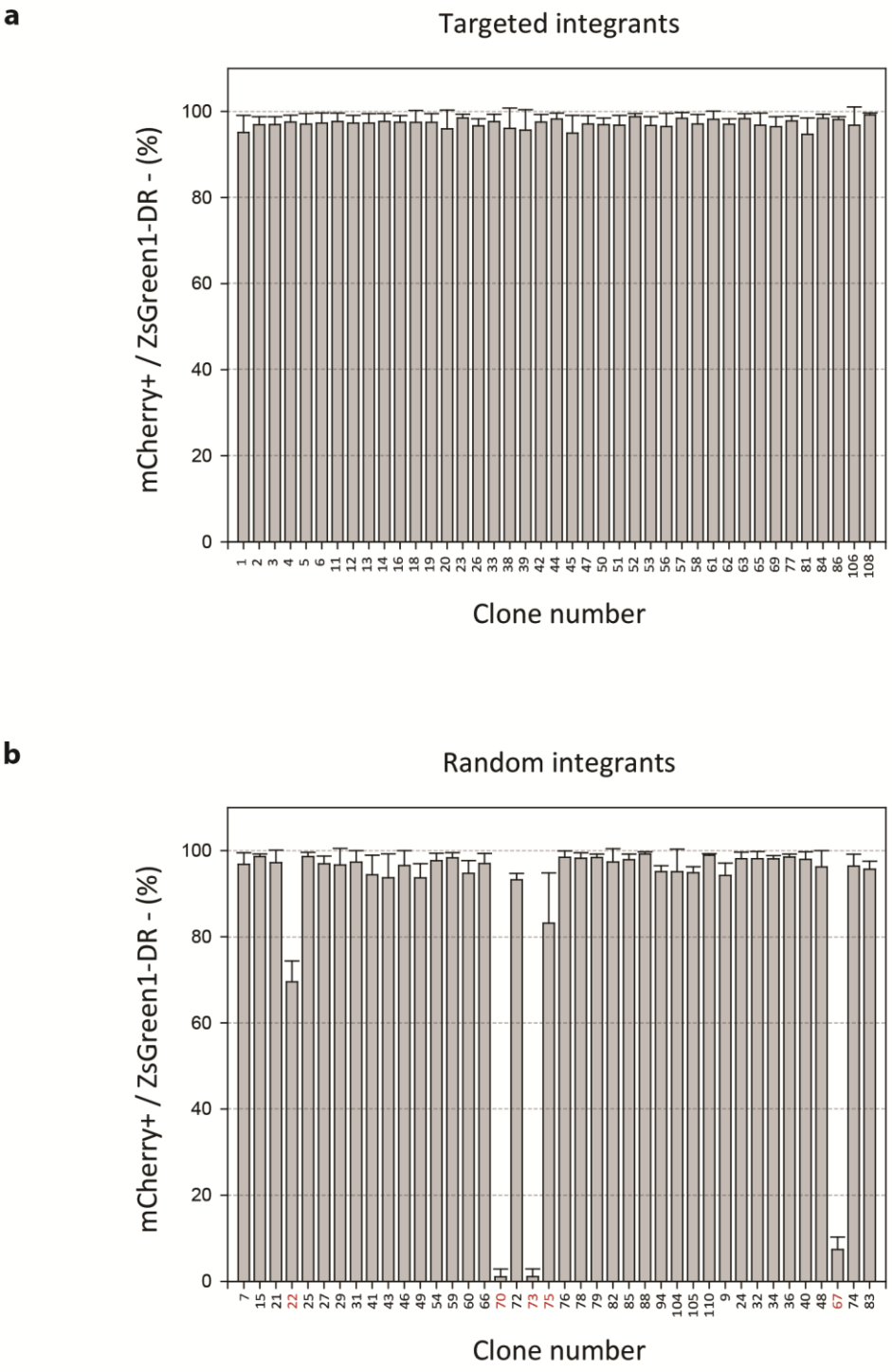


Supplementary Figure 7. Selection of targeted integrants at the *COSMC* locus. (a) 5'/3' junction PCR was performed on genomic extract from 115 clones. 83 of the clones (72.2%) were both junction PCR positive, which were further analyzed by (b) out-out PCR. Expected size of amplicons were indicated by arrows. Numerals indicated the number of each clone.

The number of PCR negative clones was shown in red. M, 1 kb DNA ladder. (*bottom*) Pie charts displaying the contribution of PCR positive and negative clones to a total.



Supplementary Figure 8. Relationship between the relative copy number of mCherry regions and the expression level of mCherry (RFU) in random integrants. (a) (Gray bar) relative mCherry copy number; (black star) RFU. Each measured value was normalized by that of the targeted integrant clone number 1 (*COSMC* locus; shown in red). (b) Scatter plot of RFU versus relative mCherry copy number. A diagonal line indicates a one to one correlation.



Supplementary Figure 9. Percentage of mCherry positive/ZsGreen1-DR negative cells in individual clone. mCherry positive/ZsGreen1-DR negative clonal cells in the population of either (a) targeted integrants (*COSMC* locus) or (b) random integrants were expanded prior to batch cultures and subsequently inoculated for batch cultures. On day 3, the percentage of mCherry positive/ZsGreen1-DR negative cells was measured by imaging cell cytometer. Clones with a decreased percentage were shown in red. The error bars represent the standard deviations calculated from the data obtained in triplicate experiments.

Tables

Supplementary Table 1. sgRNA genomic target sequences

Target gene	sgRNA name	GNNNNNNNNNNNNNNNNNNNNGG	Ref.
COSMC	sgRNA2_C	GAATATGTGAGTGTGGATGGAGG	Ronda et al. (2014)
MGAT1	sgRNA1	GCTCACACCCTTACGGCCAAAGG	In this study
	sgRNA2	GGACACGTCGACCCCTGAACTGG	In this study
	sgRNA3	GAGGGGGTCGCAGGCACACGGGG	In this study
	sgRNA4	GGGGCACAGCGATGTTACTCAGG	In this study
	sgRNA5	GTGGAGTTGGAGCGGCAGCGGGG	In this study
LDHA	sgRNA1	GAACAAGATTACGATTGTTGGGG	In this study
	sgRNA2	GCTGGGCACTGATACCGACAAGG	In this study
	sgRNA3	GGACTGTATTTCACAACGTTGGG	In this study
	sgRNA4	GCTCGATTCCGTTATCTGATGGG	In this study
	sgRNA5	GCTGTCATGGATGGGTCCTAGGG	In this study

Supplementary Table 2. Primer sequences

Primer name	Purpose	Sequence (5'-3')
sgRNA expression plasmid		
Mgat1_sgRNA1_fwd	Sense oligo for Mgat1_sgRNA1	GGAAAGGACGAAACACCGCTCACACCCTTACG GCCAAGTTTTAGAGCTAGAAAT
Mgat1_sgRNA1_rev	Antisense Oligo for Mgat1_sgRNA1	CTAAAACTTGGCCGTAAGGGTGTGAGCGGTGTT TCGTCCTTTCCACAAGATAT
Mgat1_sgRNA2_fwd	Sense oligo for Mgat1_sgRNA2	GGAAAGGACGAAACACCGGACACGTCGACCCC TGAACGTTTTAGAGCTAGAAAT
Mgat1_sgRNA2_rev	Antisense Oligo for Mgat1_sgRNA2	CTAAAACGTTCAGGGGTCGACGTGTCCGGTGTT TCGTCCTTTCCACAAGATAT
Mgat1_sgRNA3_fwd	Sense oligo for Mgat1_sgRNA3	GGAAAGGACGAAACACCGAGGGGGTCGCAGG CACACGGTTTTAGAGCTAGAAAT
Mgat1_sgRNA3_rev	Antisense Oligo for Mgat1_sgRNA3	CTAAAACCGTGTGCCTGCGACCCCCTCGGTGTT TCGTCCTTTCCACAAGATAT
Mgat1_sgRNA4_fwd	Sense oligo for Mgat1_sgRNA4	GGAAAGGACGAAACACCGGGGCACAGCGATGT TACTCGTTTTAGAGCTAGAAAT
Mgat1_sgRNA4_rev	Antisense Oligo for Mgat1_sgRNA4	CTAAAACGAGTAACATCGCTGTGCCCCGGTGTT TCGTCCTTTCCACAAGATAT
Mgat1_sgRNA5_fwd	Sense oligo for Mgat1_sgRNA5	GGAAAGGACGAAACACCGTGGAGTTGGAGCG GCAGCGGTTTTAGAGCTAGAAAT
Mgat1_sgRNA5_rev	Antisense Oligo for Mgat1_sgRNA5	CTAAAACCGCTGCCGCTCCAACTCCACGGTGTTT CGTCCTTTCCACAAGATAT
LdhA_sgRNA1_fwd	Sense oligo for LdhA_sgRNA1	GGAAAGGACGAAACACCGAACAAGATTACGAT TGTTGGTTTTAGAGCTAGAAAT
LdhA_sgRNA1_rev	Antisense Oligo for LdhA_sgRNA1	CTAAAACCAACAATCGTAATCTTGTTCGGTGTTT CGTCCTTTCCACAAGATAT
LdhA_sgRNA2_fwd	Sense oligo for Mgat1_sgRNA2	GGAAAGGACGAAACACCGCTGGGCACTGATAC CGACAGTTTTAGAGCTAGAAAT
LdhA_sgRNA2_rev	Antisense Oligo for LdhA_sgRNA2	CTAAAACTGTCGGTATCAGTGCCCAGCGGTGTT TCGTCCTTTCCACAAGATAT
LdhA_sgRNA3_fwd	Sense oligo for LdhA_sgRNA3	GGAAAGGACGAAACACCGGACTGTATTTCACA ACGTTGTTTTAGAGCTAGAAAT
LdhA_sgRNA3_rev	Antisense Oligo for LdhA_sgRNA3	CTAAAACAACGTTGTGAAATACAGTCCGGTGTT

		TCGTCCTTTCCACAAGATAT
LdhA_sgRNA4_fwd	Sense oligo for LdhA_sgRNA4	GGAAAGGACGAAACACCGCTCGATTCCGTTATC TGATGTTTTAGAGCTAGAAAT
LdhA_sgRNA4_rev	Antisense Oligo for LdhA_sgRNA4	CTAAAACATCAGATAACGGAATCGAGCGGTGTT TCGTCCTTTCCACAAGATAT
LdhA_sgRNA5_fwd	Sense oligo for LdhA_sgRNA5	GGAAAGGACGAAACACCGCTGTCATGGATGGG TCCTAGTTTTAGAGCTAGAAAT
LdhA_sgRNA5_rev	Antisense Oligo for LdhA_sgRNA5	CTAAAACTAGGACCCATCCATGACAGCGGTGTT TCGTCCTTTCCACAAGATAT
Donor plasmid		
COSMC 5' arm_fwd	USER PCR primer for COSMC donor plasmid (Homology arm)	AGTCGGTGUGTAATCCATGGAGGAGTTTCT
COSMC 5' arm_rev	USER PCR primer for COSMC donor plasmid (Homology arm)	ACGCTGCTUAAGGTCTCCAGATTTTACAGT
COSMC 3' arm_fwd	USER PCR primer for COSMC donor plasmid (Homology arm)	AGGTCTGAGUGATTGTCTTAAGCATAGAGTC
COSMC 3' arm_rev	USER PCR primer for COSMC donor plasmid (Homology arm)	AGCGACGUCCTCATTTGCATATATTTGAA
Mgat1 5' arm_fwd - set1	USER PCR primer for Mgat1 donor plasmid (Homology arm)	AGTCGGTGUTTGCAGCAAATCAGGGAGCAT
Mgat1 5' arm_rev - set1	USER PCR primer for Mgat1 donor plasmid (Homology arm)	ACGCTGCTUTCATCGTTCTTGAAATTTCTG
Mgat1 3' arm_fwd - set1	USER PCR primer for Mgat1 donor plasmid (Homology arm)	AGGTCTGAGUCATGGGCAGTTCTTTGATCAGC
Mgat1 3' arm_rev - set1	USER PCR primer for Mgat1 donor plasmid (Homology arm)	AGCGACGUAGGAGCCAGGTTAGGGTCAAC
Mgat1 5' arm_fwd - set2	USER PCR primer for Mgat1 donor plasmid (Homology arm)	AGTCGGTGUTCACTGTGTTTCCTTACTAAGT
Mgat1 5' arm_rev - set2	USER PCR primer for Mgat1 donor plasmid (Homology arm)	ACGCTGCTUCTCAGCGTCCTCAGCCAGG
Mgat1 3' arm_fwd - set2	USER PCR primer for Mgat1 donor plasmid (Homology arm)	AGGTCTGAGUGCTGTTGCAGCAAATCAGGGAG
Mgat1 3' arm_rev - set2	USER PCR primer for Mgat1 donor plasmid (Homology arm)	AGCGACGUCCCTTACGGCCAAAGGTCATCG
LdhA 5' arm_fwd	USER PCR primer for LdhA donor plasmid (Homology arm)	AGTCGGTGUTTCATTAATCTTGATGCTAGTG
LdhA 5' arm_rev	USER PCR primer for LdhA donor plasmid (Homology arm)	ACGCTGCTUTCTGGATTCAGATTCTTCAGGG
LdhA 3' arm_fwd	USER PCR primer for LdhA donor plasmid (Homology arm)	AGGTCTGAGUAGCAGTGGAATGAGGTTCACAA
LdhA 3' arm_rev	USER PCR primer for LdhA donor plasmid (Homology arm)	AGCGACGUAGCCAGGGCCACAGAGAAAAC
EF-1a_fwd	USER PCR primer for donor plasmid (EF-1a)	AAGCAGCGUGTGAGGCTCCGGTGCCC
EF-1a_rev	USER PCR primer for donor plasmid (EF-1a)	ATGACGTCUTCACGACACCTGAAATGGAA
kozak_mcherry_fwd	USER PCR primer for donor plasmid (mCherry-BGH pA)	AGACGTCAUCGCCACCATGGTGAGCAAGG
BGH pA_rev	USER PCR primer for donor plasmid (mCherry-BGH pA)	ATCGCACUCCATAGAGCCCACCGCATCC
Marker NeoR_fwd	USER PCR primer for donor plasmid (NeoR)	AGTGCGAUCTGTGGAATGTGTGTCAGTT
Marker NeoR_rev	USER PCR primer for donor plasmid (NeoR)	ACTCAGACCUCAGACATGATAAGATACATTG
CMV_fwd	USER PCR primer for donor plasmid (ZsGreen1)	ACGTCGCUGTTGACATTGATTATTGACT

BGH pA_rev2	USER PCR primer for donor plasmid (ZsGreen1)	ACGCAAGUCCATAGAGCCCACCGCATCC
pJ204 backbone_fwd	USER PCR primer for donor plasmid (Backbone)	ACTTGCGUAGTGAGTCGAATAAGGGCGACACAAA
pJ204 backbone_rev	USER PCR primer for donor plasmid (Backbone)	ACACCGACUGAGTCGAATAAGGGCGACACCCCA
5'/3' Junction PCR and Out-Out PCR		
COSMC genomic fwd [junction]	COSMC amplicon for 5' junction PCR	TGGTTTCTAGGCTAATGCTTTGA
COSMC genomic rev [junction]	COSMC amplicon for 3' junction PCR	CCTGCCCCCACAGAAAAGTA
Mgat1 genomic fwd [junction] – set1	Mgat1 amplicon for 5' junction PCR	TAGACTGGGGAAGTGAGC
Mgat1 genomic rev [junction] – set1	Mgat1 amplicon for 3' junction PCR	CAATAAAAGACGTCAGGAGGC
Mgat1 genomic fwd [junction] – set2	Mgat1 amplicon for 5' junction PCR	TCTGCTGTCTCTACTGCCCT
Mgat1 genomic rev [junction] – set2	Mgat1 amplicon for 3' junction PCR	CTATAGACACGGGCAAGGAA
LdhA genomic fwd [junction]	LdhA amplicon for 5' junction PCR	GGCTGCTGTTCAGAGGTCTT
LdhA genomic rev [junction]	LdhA amplicon for 3' junction PCR	TGAGGCTTACACACAGCACA
EF-1a rev [junction]	Amplicon for 5' junction PCR	ATCCTGGCCCGCATTTACAA
NeoR fwd [junction]	Amplicon for 3' junction PCR	CTGGACGAAGAGCATCAGGG
Deep sequencing		
COSMC sgRNA2_F_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCCACCTTGTTCAGGACACT
COSMC sgRNA2_R_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGATCCATCGCAGCCTTTCTAT
Mgat1 sgRNA1_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA1 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCAAGGCCTTCTGGGATGAC
Mgat1 sgRNA1_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA1 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAACTGCTGGTTCAGCTTG
Mgat1 sgRNA2_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA2 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCGGGTACAGTACACTAGCAGA
Mgat1 sgRNA2_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA2 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGGCAGGTGCTGCTAATTCCA
Mgat1 sgRNA3_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA3 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCAGCAAATCAGGGAGCAT
Mgat1 sgRNA3_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA3 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAGGGCCGATAGTGCAACAA
Mgat1 sgRNA4_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA4 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCCATCATTGTCAGCCGTC
Mgat1 sgRNA4_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA4 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAACTTGTTGAAGATCTGGCCC
Mgat1 sgRNA5_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCTGGACACGCCCAGC
Mgat1 sgRNA5_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCACGGTGGGCACTTT
LdhA sgRNA1_F_Nex	LdhA amplicon for MiSeq analysis (sgRNA1 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAGTCCAAGATGGCAACACTCAA
LdhA sgRNA1_R_Nex	LdhA amplicon for MiSeq analysis (sgRNA1 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCCAATGCTTGGGCTTGTG
LdhA sgRNA2_F_Nex	LdhA amplicon for MiSeq analysis (sgRNA2 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCTCTCCTGTGGAAACATTG

LdhA sgRNA2_R_Nex	LdhA amplicon for MiSeq analysis (sgRNA2 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGAGTTTCCATGCTGCCAATCACG
LdhA sgRNA3_F_Nex	LdhA amplicon for MiSeq analysis (sgRNA3 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GACAGCAAACTCCAAGCTGGT
LdhA sgRNA3_R_Nex	LdhA amplicon for MiSeq analysis (sgRNA3 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGCCAAGCCACGTAGGTCAAGA
LdhA sgRNA4_F_Nex	LdhA amplicon for MiSeq analysis (sgRNA4 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GCTTGACCTACGTGGCTTGGA
LdhA sgRNA4_R_Nex	LdhA amplicon for MiSeq analysis (sgRNA4 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGTGATACTAAAGACTTACCACTGGAG
LdhA sgRNA5_F_Nex	LdhA amplicon for MiSeq analysis (sgRNA5 site)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GTCTGCTCGATTCCGTTATCTGAT
LdhA sgRNA5_R_Nex	LdhA amplicon for MiSeq analysis (sgRNA5 site)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGTTCTTAACAGAGCCATCTTTCT
COSMC OT1_F_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target1)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GGTGTTCACAGCTGGGGCAT
COSMC OT1_R_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target1)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGAAAGGGCAGGGGACTGAAA
COSMC OT2_F_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target2)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GAGAGGGAGGGGATGCTAAGT
COSMC OT2_R_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target2)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGCCTTGGGTGTAAAGGCCCAT
COSMC OT3_F_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target3)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GATTCAGGCCTGTTTGAGCCA
COSMC OT3_R_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target3)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGGTAAAGGAGACCGCCATGT
COSMC OT4_F_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target4)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GAGACATTGTCCACCCACAGC
COSMC OT4_R_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target4)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGTGAGTGGAGGGTCTGGGAT
COSMC OT5_F_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target5)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GGGAAAATACTACGTTATCCTTTGCT
COSMC OT5_R_Nex	COSMC amplicon for MiSeq analysis (sgRNA2 site: off-target5)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGCGTGGAGGAATAGCCTCTGT
Mgat1_sg1-OT1_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA1 site: off-target1)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GTGGTGTAGATTGTCCCACACCC
Mgat1_sg1-OT1_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA1 site: off-target1)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGAAGACCTTTGGCTGGGATGTA
Mgat1_sg1-OT2_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA1 site: off-target2)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GGAAGCCATTGAGCTGCAAGTT
Mgat1_sg1-OT2_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA1 site: off-target2)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGCACTGAATGGGAAAAGGGG
Mgat1_sg5-OT1_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target1)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GTGCGTGCAGCGTCCT
Mgat1_sg5-OT1_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target1)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGCGGAGGGTGGCCTGCT
Mgat1_sg5-OT2_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target2)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GCTAAGGAAGAGCGTCAGATGGT
Mgat1_sg5-OT2_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target2)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGCGCCCTCGACTTTGACAGGA
Mgat1_sg5-OT3_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target3)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GTGTACAACATGACTAATGGCAGC
Mgat1_sg5-OT3_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target3)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGCCTACCCCCACCCCGTG
Mgat1_sg5-OT4_F_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target4)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GTTCATGGGCCTCATCCCCTA

Mgat1_sg5-OT4_R_Nex	Mgat1 amplicon for MiSeq analysis (sgRNA5 site: off-target4)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGGGCACATTGGACTCAGACA
	qRT-PCR	
EF-1a - mCherry junction fwd	mCherry amplicon for qRT-PCR [Amplicon size: 100 bp]	CCTCAGACAGTGGTTCAAAGT
EF-1a - mCherry junction rev	mCherry amplicon for qRT-PCR [Amplicon size: 100 bp]	GATGGCCATGTTATCCTCCTC
COSMC fwd	COSMC amplicon for qRT-PCR [Amplicon size: 144 bp]	GCAGCCTTTCTATCTAGGACAC
COSMC rev	COSMC amplicon for qRT-PCR [Amplicon size: 144 bp]	CCACCTTGTTCAGGACACTT
LdhA fwd	LdhA amplicon for qRT-PCR [Amplicon size: 94 bp]	GAGTGAATGTAGCTGGTGTCTC
LdhA rev	LdhA amplicon for qRT-PCR [Amplicon size: 94 bp]	ACCTGCTTGTGAACCTCATT
Mgat1-sg1 fwd	Mgat1 amplicon for qRT-PCR (sgRNA1 site) [Amplicon size: 113 bp]	GGCCTGTATTCGTCCAGAAA
Mgat1-sg1 rev	Mgat1 amplicon for qRT-PCR (sgRNA1 site) [Amplicon size: 113 bp]	CGAACTGCTGGTTCAGCTTG
Mgat1-sg5 fwd	Mgat1 amplicon for qRT-PCR (sgRNA5 site) [Amplicon size: 128 bp]	CTCACCCGTGAGGTGTTC
Mgat1-sg5 rev	Mgat1 amplicon for qRT-PCR (sgRNA5 site) [Amplicon size: 128 bp]	GCCACGGTGGGCACTTT
Vinculin fwd	Vinculin amplicon for qRT-PCR [Amplicon size: 73 bp]	GCTGGTTGCTAAGAGGGAGG
Vinculin rev	Vinculin amplicon for qRT-PCR [Amplicon size: 73 bp]	ATCAGAGGCAGCTTTCACGG
Beta-actin fwd	Beta-actin amplicon for qRT-PCR [Amplicon size: 202 bp]	TTTCCAGCCTTCCTTCCTGGGTGA
Beta-actin rev	Beta-actin amplicon for qRT-PCR [Amplicon size: 202 bp]	TGGCATAGAGGTCTTTGCGGATGT

Supplementary Table 3. Plasmids used in this study as PCR templates

Plasmid name	**Elements**	**Source**
pBudCE4.1	EF-1a	Life Technologies
pmCherry-N1	mCherry	Clontech
pcDNA3.1(+)	pCMV, BGH pA, pSV40- NeoR – SV40 pA	Life Technologies
pZsGreen1-DR	ZsGreen1-DR	Clontech
pU0020	AmpR, pUC19 replication origin	Lund et al., 2014