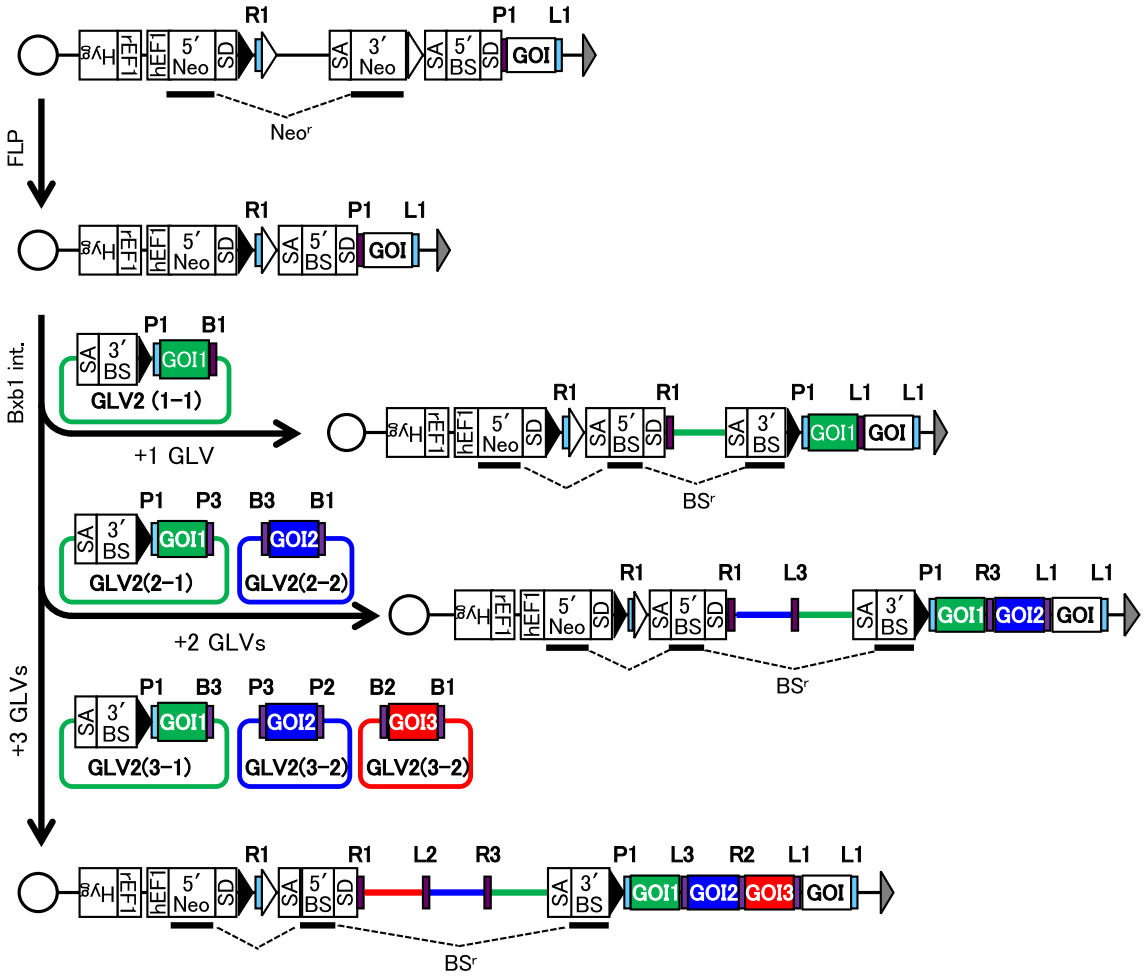
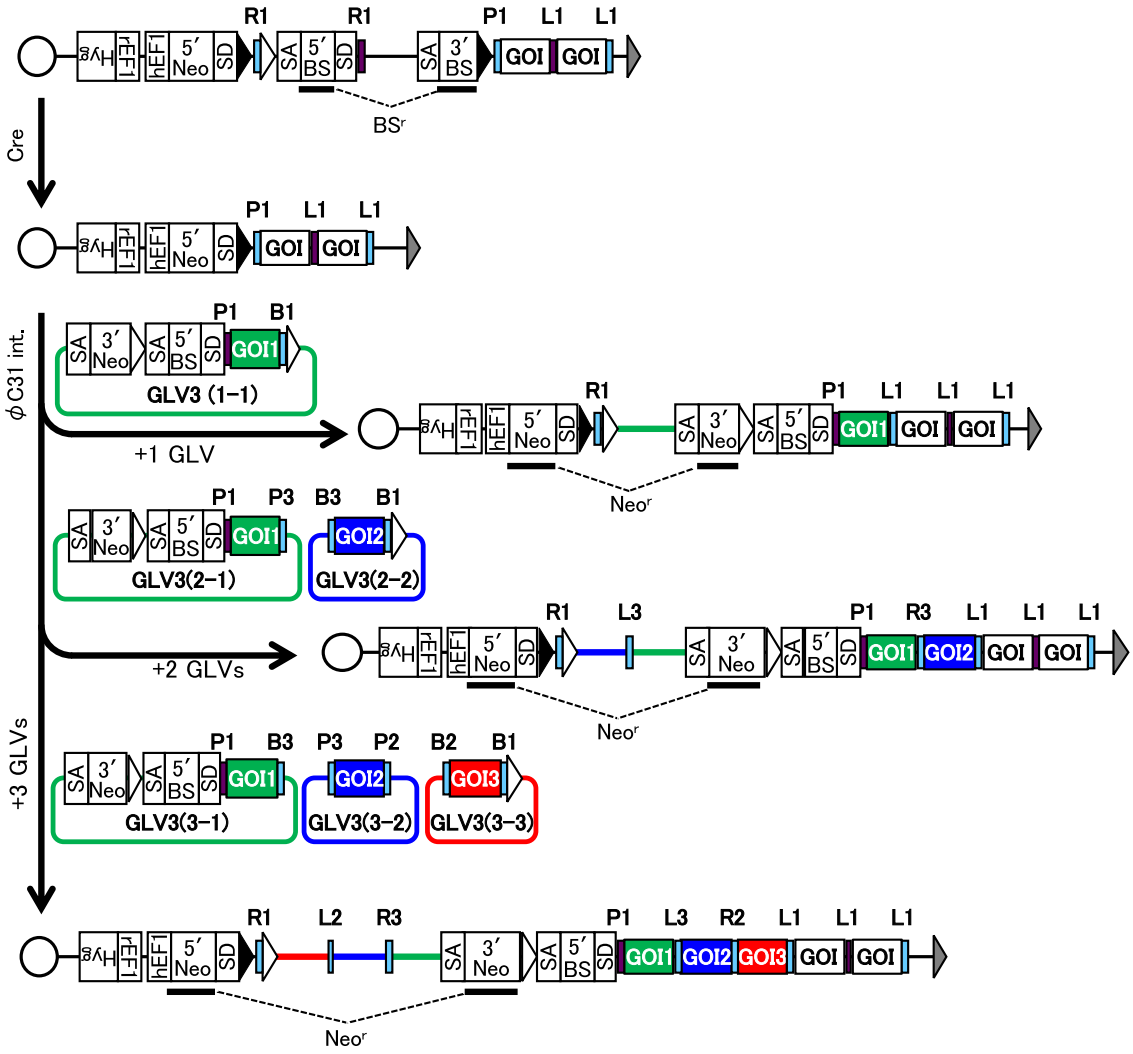
Table S1 Primer sequences.

FAMAC construction (left side validation)	5'-TGACAGAGAGCTTCCTCCTGCCTCTGTA-3'
	5'-CACTTGGAGAGACTTCATCTGCGCTATC-3'
FAMAC construction (right side validation)	5'-GAATGTGGCTTCTTCGTAAGAGATGCAG-3'
	5'-CACTCTTTACCCCTCACCGCTAACCTTG-3'
FAMAC transfer to CHO-K1	5'-CCGATTCTCAGCATGTAAGCTCTCAGTC-3'
	5'-TCTGCATCTCTTACGAAGAAGCCACATT-3'
DT40 contamination detection	5'-TTCCCCATGAAGTGCAGCAA-3'
	5'-AAACCACCGTGCAGTGCAGA-3'
GLV1s loading	5'-CCAACTCCAGGACGGAGTCAGT-3'
	5'-TCATGCTCCTGTGTGGAAAGATC-3'
GLV2 loading	5'-AACATCACCGTGACATGCAG-3'
	5'-AGGCCAGGCTGGATGTGGCT-3'
GLV3s loading	5'-CTGCTGCCCGACAACCACTA-3'
	5'-ACAGTCGAGGCTGATCAGCG-3'
Junction PCR for FAMAC-GLV2	5'-TGCCAGGCCAGGATCTGCTG-3'
	5'-GCTCTTTCAATGAGGGTGGATTC-3'
Sequencing primer for the junction PCR amplicon	5'-TGAAGGATCATAGGGACTGC-3'
gRNA targeting endogenous Rtcb	5'-GCAGAAAGCCACCAACACCT-3'
Rtcb target site amplification	5'-ACTCAGTAACCATAGCTACTGAC-3'
	5'-TGCCATAAAGGTTCACAAATGC-3'
Sequencing primer for the Rtcb target site amplicon	5'-CTTGTCATGTATGCAACTACTG-3'
qRT-PCR primers for Xbp1s	5'-TGCTGAGTCCGCAGCAGGTG-3'
	5'-GACAGGGTCCAACTTGTCCA-3'
qRT-PCR primers for Gapdh	5'-AATGTGTCCGTCGTGGATCTGA-3'
	5'-GATGCCTGCTTCACCACCTTCT-3'



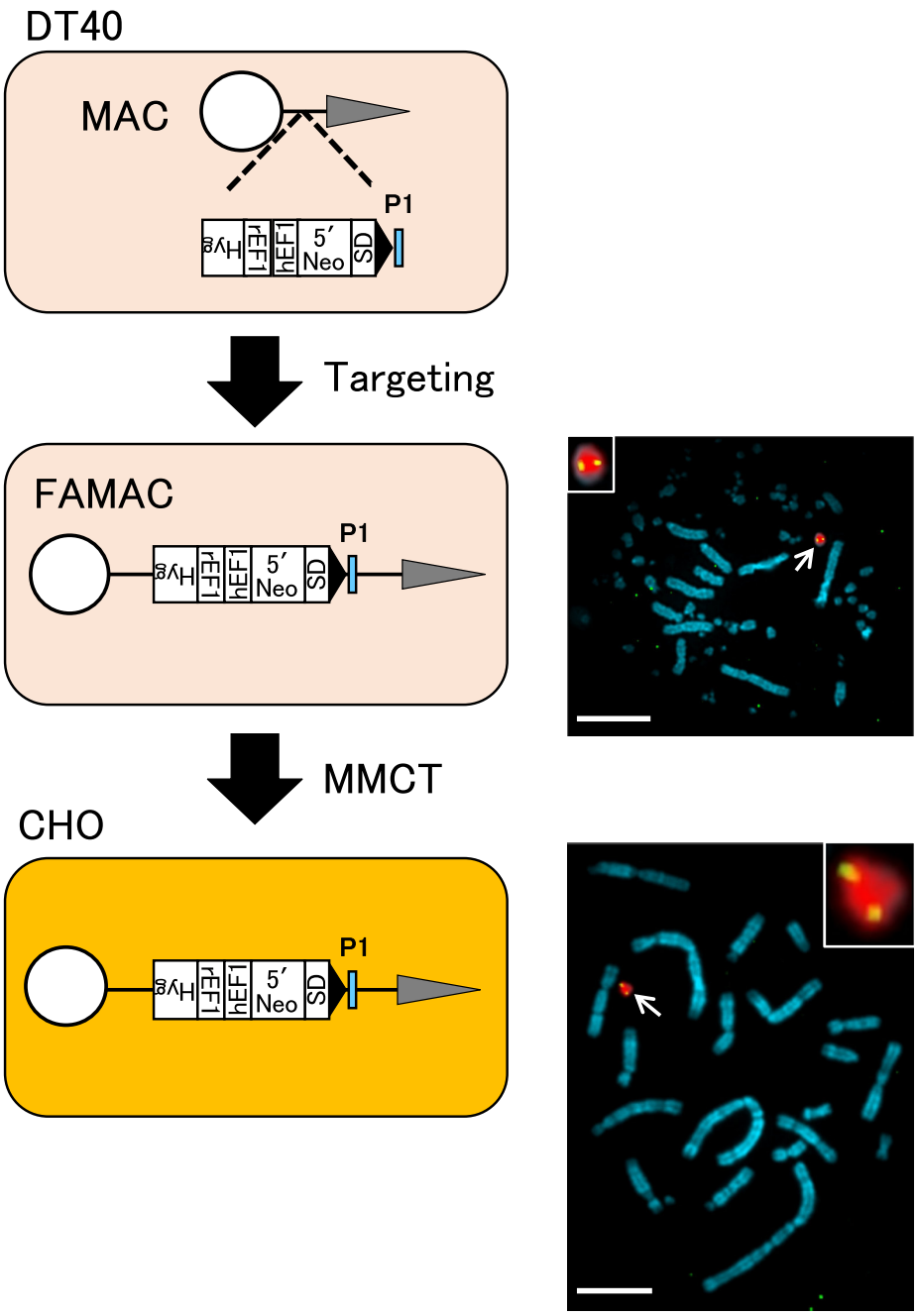
Supplementary Figure 1

Supplementary Fig. 1 Step II gene loading. Step II gene-loading was performed using Flp recombinase and Bxb1 integrase. Flp recombinase removes vector backbone sequences of the already integrated GLV1(s) and the 3′ portion of the neo-resistance gene unit, while Bxb1 integrase connects and loads GLV2(s) onto the FAMAC. Up to three GLVs could be introduced simultaneously to the FAMAC using GLV2s, which contain various combinations of Bxb1 att sequences using Bxb1 integrase. GLV2(1-1) is for single GLV loading; GLV2(2-1) and GLV2(2-2) are for two-GLV loadings; and GLV2(3-1), GLV2(3-2), and GLV2(3-3) are for three-GLV loadings. The BS-resistance gene is reconstituted upon GLV2(s) loading.



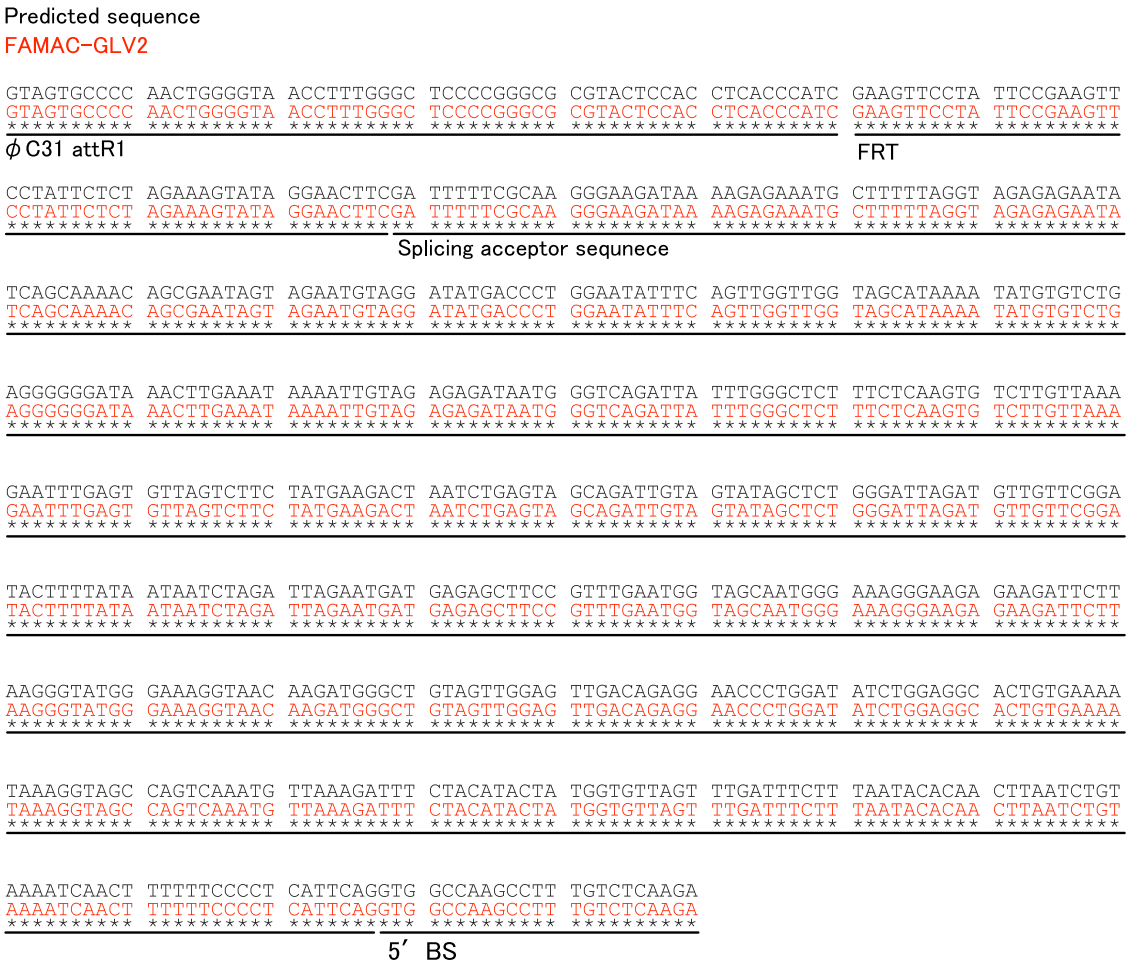
Supplementary Figure 2

Supplementary Fig. 2 Step III gene loading. Step III gene-loading was performed using Cre recombinase and φC31 integrase. Cre recombinase removes vector backbone sequences of the already integrated GLV2(s), the BS-resistance gene unit, and FRT while φC31 integrase connects and loads GLV3(s) onto the FAMAC. Up to three GLVs could be introduced simultaneously to the FAMAC using GLV3s, which contain various combinations of φC31 att sequences using φC31 integrase. GLV3(1-1) is for single GLV loading; GLV3(2-1) and GLV3(2-2) are for two-GLV loadings; and GLV3(3-1), GLV3(3-2), and GLV3(3-3) are for three-GLV loadings. The neo-resistance gene is reconstituted upon GLV3(s) loading.



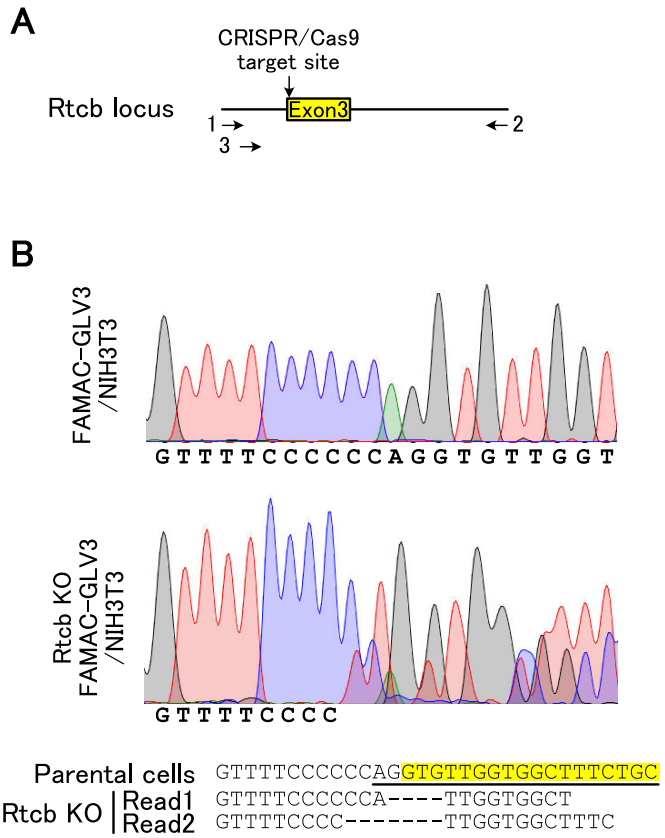
Supplementary Figure 3

Supplementary Fig. 3 Construction of FAMAC. The gene-loading pad for the FA system was introduced to MAC via homologous recombination in DT40 cells to generate FAMAC. FAMAC was then transferred to CHO cells for gene loading. FAMAC in DT40 and CHO cells was verified by FISH. The samples were counterstained with DAPI to visualize chromosomes. Insets show high magnification images of FAMAC (arrows). Red, mouse Cot-1; Green, gene-loading pad sequence. Scale bars, 10 μm.



Supplementary Figure 4

Supplementary Fig. 4 Junction sequence of FAMAC-GLV2. The junctional sequence of the 5′ Neo and 5′ BS was analyzed. Predicted sequence is shown in black. Sequence data obtained from FAMAC-GLV2 is shown in red. Sequence of each element is underlined. Asterisks indicate matched sequences.



Supplementary Figure 5

Supplementary Fig. 5 Enzymatic activity of RTCB protein expressed from FAMAC-GLV3. (A) Primers used to analyze disruption of endogenous Rtcb in FAMAC-GLV3/NIH3T3 cells. Primer 1 and 2 were used to amplify genomic sequence encompassing CRISPR/Cas9 target site. Primer 3 is a primer for sequencing the amplicon. Note that these primers are specific for endogenous Rtcb gene. (B) Sequencing chromatograms and corresponding sequences of parental FAMAC-GLV3/NIH3T3 cells and an Rtcb KO FAMAC-GLV3/NIH3T3 clone. Two distinct indels were identified from the Rtcb KO clone (Read1 and Read2). Note that the splicing acceptor site of exon 3 was disrupted in both sequences. Underline denotes CRISPR/Cas9 target sequence. The exon 3 sequence is highlighted in yellow. (C) Xbp1 splicing in endogenous Rtcb-disrupted FAMAC-GLV3/NIH3T3 cells was analyzed by qRT-PCR. Cells were treated with 10 μg/mL tunicamycin to induce ER-stress. Statistical significance was assessed by two-way ANOVA followed by Tukey's HSD post-hoc test (n=3; ***, $P<0.001$).

Fig. 3A

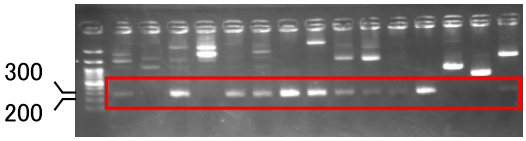


Fig. 3B

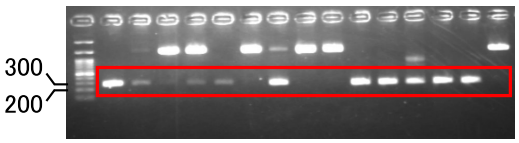


Fig. 3D

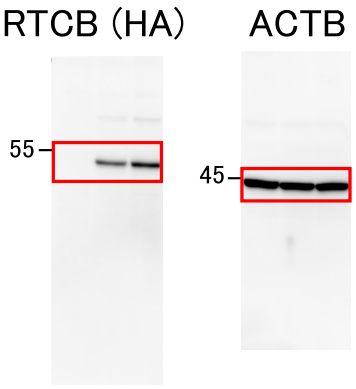


Fig. 4A

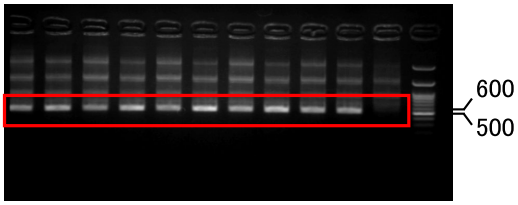


Fig. 4B

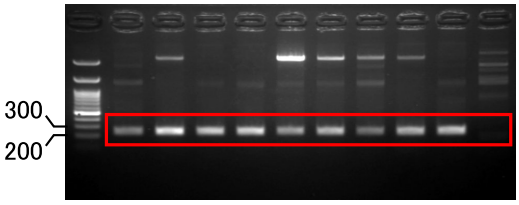
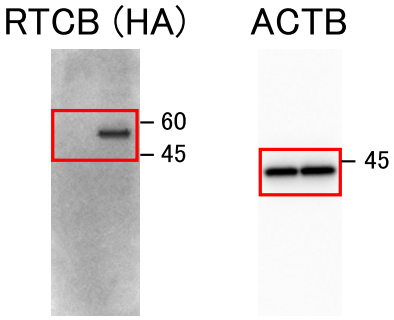


Fig. 5D



Supplementary Figure 6

Supplementary Fig. 6 Uncropped images of gels and blots. Red boxes indicate the cropped region shown in the main figure.