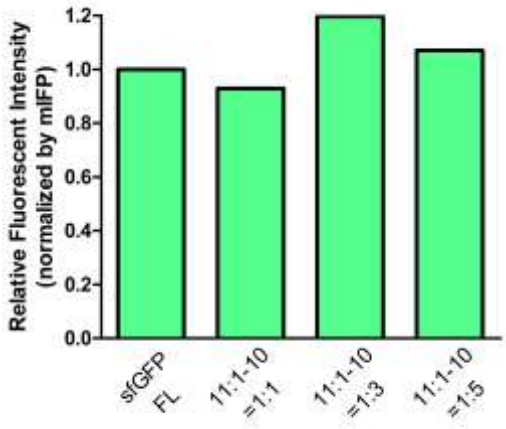
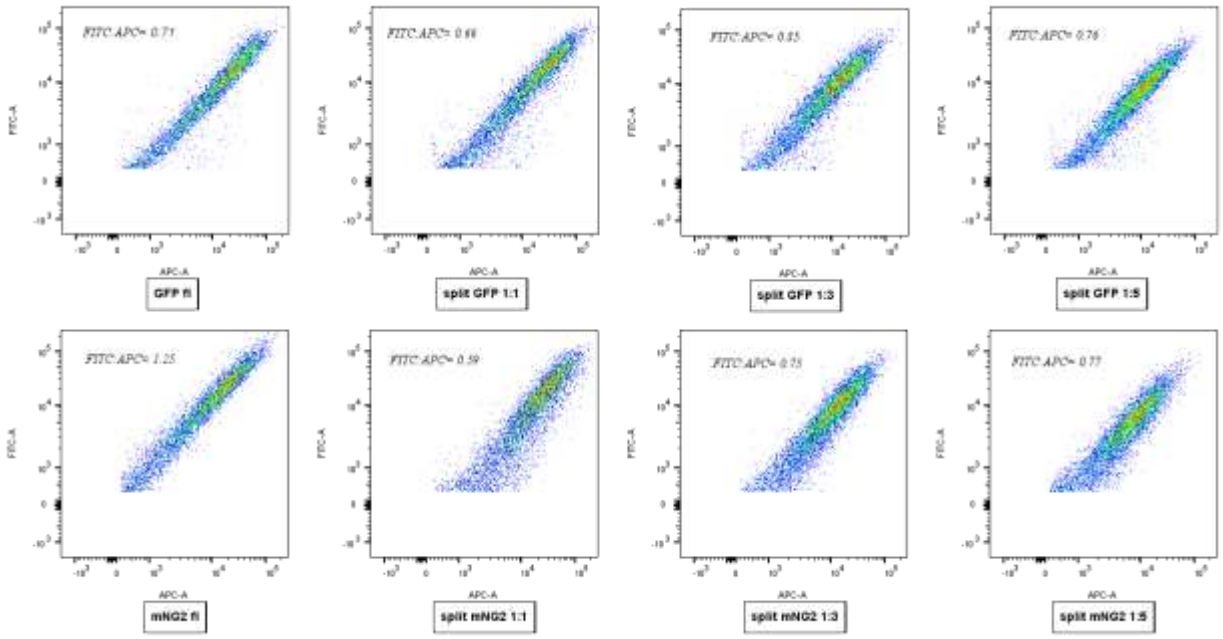
Description of Supplementary Files

File Name: Supplementary Information
Description: Supplementary Figures and Supplementary Tables

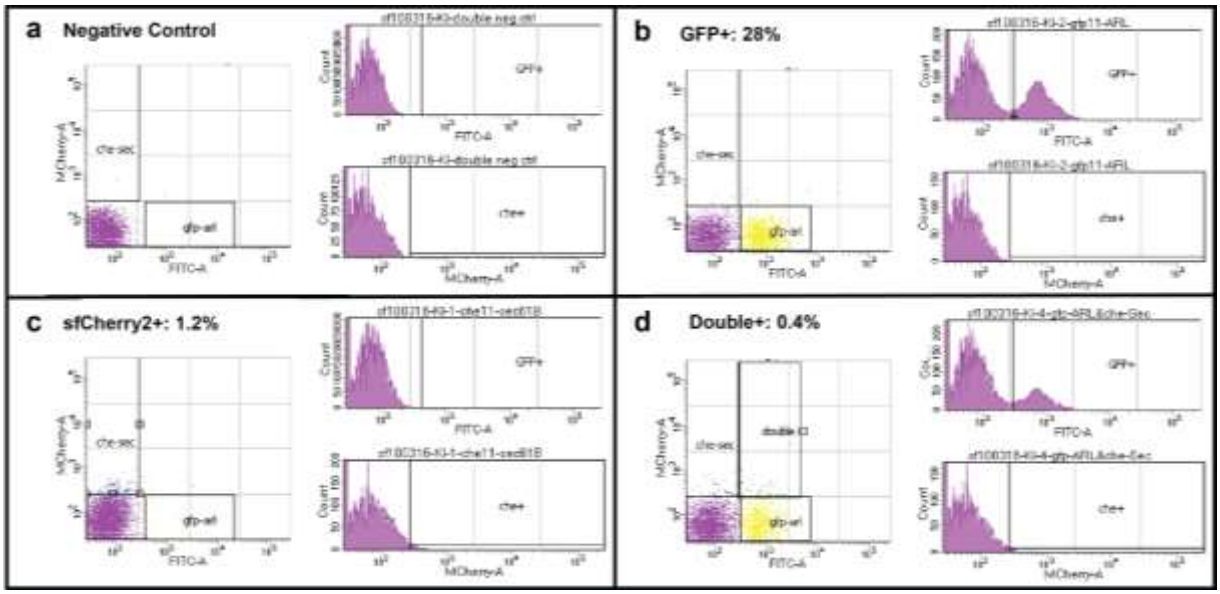
a



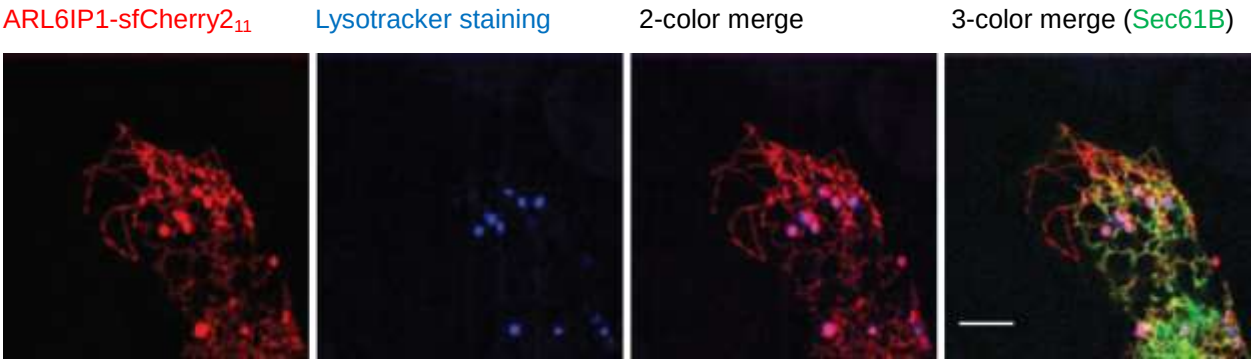
b



Supplementary Figure 1 (a) Whole cell fluorescence intensity of full length GFP and GFP_{11}-CLTA/GFP_{1-10}, measured by flow cytometry and normalized for expression level by mIFP fluorescence signal. Number of cells > 6000. **(b)** FACS raw data of bar graphs in Fig. 2e and Supplementary Fig. 1a. The X-axis is mIFP fluorescence intensity (ex=633 nm, em=710/50 nm) and the Y-axis is green fluorescence intensity (ex=488 nm, em=530/30 nm). The “FITC:APC” value is the population mean of green fluorescence normalized by mIFP fluorescence.



Supplementary Figure 2. FACS results of single knock-in or simultaneous double knock-in using GFP_{11} or/and $sfCherry2_{11}$. **(a)** Background fluorescence of HEK 293T cells stably expressing both GFP_{1-10} and $sfCherry2_{1-10}$. **(b)** Single knock-in of GFP_{11} into ARL6IP1 with an efficiency of ~28% (GFP postitive). **(c)** Single knock-in of $sfCherry2_{11}$ into Sec61B with an efficiency of ~1.2% (sfCherry2 postitive). **(d)** Simultaneous double knock-in of GFP_{11} and $sfCherry2_{11}$ into ARL6IP1 and Sec61B respectively, with an efficiency of ~0.4% (double postitive).



Supplementary Figure 3. Lysotracker live-cell staining visualize the colocalization of lysosomes and sfCherry2 puncta in the same ARL6IP1-sfCherry2$_{11}$/Sec61B-GFP$_{11}$ knock-in HEK 293T cell shown in Figures 6a and 6b. Scale bar: 5 μm.

Supplementary Table 1: Sequence of $mNG2_{1-10}$/$mNG2_{11}$, $sfCherry2_{1-10}$/$sfCherry2_{11}$, $PAsfCherry2_{1-10}$ and 32-residue spacer in the screening construct.

	DNA Sequence
$mNG2_{1-10}$	ATGGTGAGCAAGGGTGAGGAGGATAACATGGCCTCTCTCCCAGCGACTCATGAGTTACA CATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGTACCGGCAATC CAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCC CCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACG GGATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGATACCAAGTCCATCGCACA ATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGC CACATCAAAGGAGAGGCCCAGGTGATGGGGACTGGTTTCCCTGCTGACGGTCCTGTGA TGACCAACACGCTGACCGCTGCGGACTGGTGCATGTCGAAGAAGACTTACCCCAACGA CAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGTAAATGGCAAACGCTACCG GAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGA ACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCATG
$mNG2_{11}$	ACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATATGATG
$sfCherry2_{1-10}$	ATGGAGGAGGACAACATGGCCATCATCAAGGAGTTCATGAGATTCAAGGTGCACATGGA GGGCAGCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCACCCCTA CGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGC CTGGGACATCCTGAGCCCCCAGTTCATGTACGGCAGCAAGGCCTACGTGAAGCACCCC GCCGACATCCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCACCTGGGAGAGAG TGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACAGCAGCCTGCAGG ACGGCCAGTTCATCTACAAGGTGAAGCTGCTGGGCATCAACTTCCCCAGCGACGGCCC CGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCAGCACCGAGAGAATGTACCCCGAG GACGGCGCCCTGAAGGGCGAGATCAACCAGAGACTGAAGCTGAAGGACGGCGGCCAC TACGACGCCGAGGTGAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGC GCCTACAACGTGGACATCAAGCTGGACATCACCAGCCACAACGAGGAC
$sfCherry2_{11}$	TACACCATCGTGGAGCAGTACGAGAGAGCCGAGGCCAGACACAGCACC
$PAsfCherry2_{1-10}$	ATGGAGGAGGATAACATGGCCATCATTAAGGAGTTCATGCGCTTCAAGGTGCACATGGAG GGGTCCGTGAACGGCCACGTGTTCGAGATCGAGGGCGAGGGCGAGGGCCACCCCTAC GAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCACC TGGGACATCCTGTCCCCTCAATTCATGTACGGCTCCAATGCCTACGTGAAGCACCCCGC CGACATCCCCGACTACTTTAAGCTGTCCTTCCCCGAGGGCTTCACCTGGGAGCGCGTGA TGAAATTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACG GTCAGTTCATCTACAAGGTGAAGCTGCTGGGCATCAACTTCCCCTCCGACGGCCCCGTA ATGCAGAAGAAGACCATGGGCTGGGAGGCCCTCACCGAGCGGATGTACCCCGAGGACG GCGCCCTGAAGGGCGAGGTCAACCCGAGAGTGAAGCTGAAGGACGGCGGCCACTACG ACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTA CAACGTCGACCGCAAGTTGGACATCACCTCACACAACGAGGAC
32-residue spacer	GACGTTGGTGGTGGCGGATCAGAAGGAGGCGGTAGCGGGGGCCCTGGTTCGGGAGGG GAAGGTTCTGCTGGGGGAGGGAGCGCTGGCGGGGGGTCT

Supplementary Table 2: sgRNA sequence

Target Gene	Target Term	Sequence of DNA oligo for sgRNA synthesis
SEC61B	N	TAATACGACTCACTATAGGCTTGTCTCCCTCTACAGCCGTTTAAGAGCTATGCTGGAA
LMNA	N	TAATACGACTCACTATAGGCCATGGAGACCCCGTCCCAGGTTTAAGAGCTATGCTGGAA
CLTA	N	TAATACGACTCACTATAGGGCCATGGCGGGCAACTGAAGTTTAAGAGCTATGCTGGAA
ARL6IP1	N	TAATACGACTCACTATAGGATCCCCGAGACGATGGCGGGTTTAAGAGCTATGCTGGAA
RAB11A	N	TAATACGACTCACTATAGGGTAGTCGTACTCGTCGTCGGTTTAAGAGCTATGCTGGAA
SPTLC1	C	TAATACGACTCACTATAGGGACTCTGCCTAGAGCAGGAGTTTAAGAGCTATGCTGGAA

Supplementary Table 3: Oligo-nucleotide donor DNA sequence

Target Gene-FP$_{11}$ Tag	DNA sequence
LMNA-mNG2$_{11}$	TCCTTCGACCCGAGCCCCGCGCCCTTTCCGGGACCCCTGCCCCGC GGGCAGCGCTGCCAACCTGCCGGCCATGACCGAGCTCAACTTCAAG GAGTGGCAAAAGGCCTTTACCGATATGATGGGAGGTGGCATGGAGAC CCCGTCCCAGCGGCGCGCCACCCGCAGCGGGGCGCAGGCCAGCT CCACTCCGCTGTCGCCCA
CLTA-mNG2$_{11}$	CGGGCGTGGTGTCGGTGGGTCGGTTGGTTTTTGTCTCACCGTTGGT GTCCGTGCCGTTCAGTTGCCCGCCATGACCGAGCTCAACTTCAAGG AGTGGCAAAAGGCCTTTACCGATATGATGGGAGGTGGCATGGCTGAG CTGGATCCGTTCGGCGCCCCTGCCGGCGCCCCTGGCGGTCCCGCG CTGGGGAACGGAGTGG
RAB11A-mNG2$_{11}$	TGCAGCGACGCCCCCTGGTCCCACAGATACCACTGCTGCTCCCGCCCTTTC GCTCCTCGGCCGCGCAATGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGC CTTTACCGATATGATGGGTTCTGGCGGCGGCACCCGCGACGACGAGTACGA CTACCTCTTTAAAGGTGAGGCCATGGGCTCTCGCACTCTACACAGTC
SPTLC1-mNG2$_{11}$	GAACAAACAGAGGAAGAACTGGAGAGAGCTGCGTCCACCATCAAGGAGGTA GCCCAGGCAGTTCTGCTCGGTGGCTCTGGCACCGAGCTCAACTTCAAGGAG TGGCAAAAGGCCTTTACCGATATGATGTAGGCAGAGTCCCGGGACCATGGCC TCCTGCCACACAACACGCAGAGAGGACTCAAGACTCCCGCTGGCCA
LMNA-sfCherry2$_{11}$	TCCTTCGACCCGAGCCCCGCGCCCTTTCCGGGACCCCTGCCCCGC GGGCAGCGCTGCCAACCTGCCGGCCATGTACACCATCGTGGAGCAG TACGAGAGAGCCGAGGCCAGACACAGCACCGGTGGCGGCGAGACC CCGTCCCAGCGGCGCGCCACCCGCAGCGGGGCGCAGGCCAGCTC CACTCCGCTGTCGCCCACCC
ARL6IP1-sfCherry2$_{11}$	GCGGGTTTCGGTTGGAGGACTCGTTGGGGAGGTGGCCTGCGCTTGT AGAGACTGCATCCCCGAGACGATGTACACCATCGTGGAGCAGTACGA GAGAGCCGAGGCCAGACACAGCACCGGTGGCGGCGCGGAGGGAG ATAATCGCAGCACCAACCTGCTGGTGAGTCCTGGCTGCCTGTCCCCC GGGAGCCGAGCGA
SEC61B- sfCherry2$_{11}$	GTGTCTAGGCCGGGGTTCTGGGGCAGGCCTGCCGCGCTCACCCGT CTGTCTGCTTGTCTCCCTCTACAGTACACCATCGTGGAGCAGTACGA GAGAGCCGAGGCCAGACACAGCACCGGTGGCGGCCCTGGTCCGAC CCCCAGTGGCACTAACGTGGGATCCTCAGGGCGCTCTCCCAGCAAA GCAGTGGCCGCCCGGGC
SEC61B-GFP$_{11}$	GTGTCTAGGCCGGGGTTCTGGGGCAGGCCTGCCGCGCTCACCCGT CTGTCTGCTTGTCTCCCTCTACAGCGTGACCACATGGTCCTTCATGA GTATGTAAATGCTGCTGGGATTACAGGTGGCGGCCCTGGTCCGACCC CCAGTGGCACTAACGTGGGATCCTCAGGGCGCTCTCCCAGCAAAGC AGTGGCCGCCCGGGC

sfCherry2$_{11}$-GFP$_{11}$-CLTA	AGCTGATAATACGACTCACTATAGGGCTCTGCAACACCGCCTAGACC GACCGGATACACGGGTAGGGCTTCCGCTTTACCCGTCTCCCTCCTGG CGCTTGTCCTCCTCTCCCAGTCGGCACCACAGCGGTGGCTGCCGGG CGTGGTGTCGGTGGGTCGGTTGGTTTTTGTCTCACCGTTGGTGTCC GTGCCGTTCAGTTGCCCGCCATGTACACCATCGTGGAGCAGTACGAG AGAGCCGAGGCCAGACACAGCACCGGTGGCTCTGGAAGTTCAGGT GGAGGCTCGCGTGACCACATGGTCCTTCATGAGTATGTAAATGCTGC TGGGATTACAGGAGGCGGTATGGCTGAGCTGGATCCGTTCGGCGCC CCTGCCGGCGCCCCTGGCGGTCCCGCGCTGGGGAACGGAGTGGC CGGCGCCGGCGAAGAAGACCCGGCTGCGGCCTTCTTGGCGCAGCA AGAGAGCGAGATTGCGGGCATCGAGAACGACGAGGCCT

mNG2$_{11}$ Sequence
sfCherry2$_{11}$ sequence
GFP$_{11}$ sequence
Linker sequence
Coding region sequence