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Supplemental Information

Establishment of SLC15A1/PEPT1-Knockout Human-Induced Pluripotent Stem Cell Line for Intestinal Drug Absorption Studies

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Figure S1

Clone number	1st allele	2nd allele	PEPT1 knockout
No. 1 (used in this study)	EF1α-PuroR-pA cassette knock-in	29 bp deletion	✓
No. 2	EF1α-PuroR-pA cassette knock-in	120 bp insertion	✓
No. 3	EF1α-PuroR-pA cassette knock-in	53 bp deletion	✓
No. 4	EF1α-PuroR-pA cassette knock-in	2 bp deletion	✓
No. 5	EF1α-PuroR-pA cassette knock-in	9 bp deletion	✓

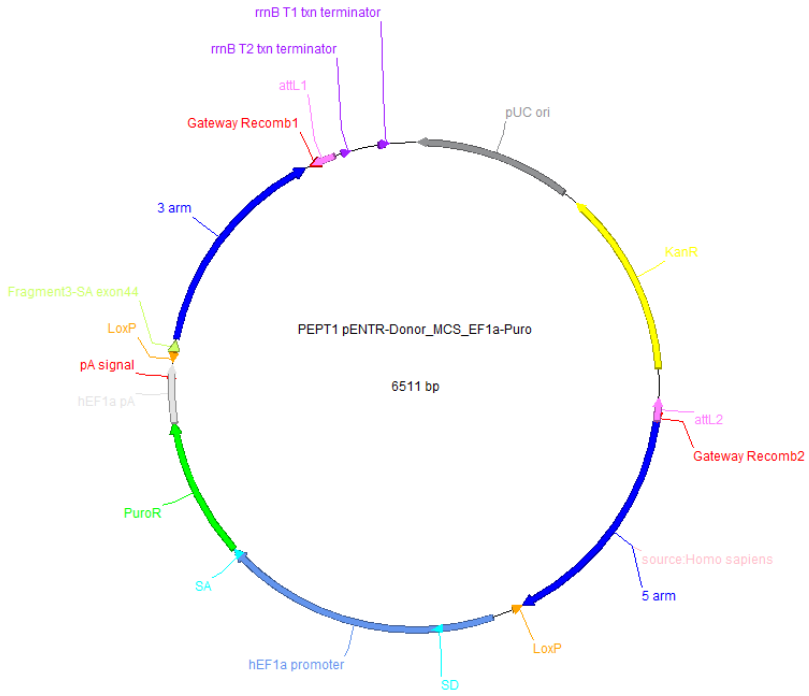
Supplemental figure 1　Establishment efficiency of PEPT1-KO iPS-IEC

Genotyping and sequencing analyses were performed in five Puro-resistant iPS cell clones (No.1-No5).

Supplemental Materials and Methods

Donor plasmids (PEPT1)

To knock-in the EF1α-PuroR-pA (elongation factor 1 alpha promoter followed by puromycin-resistance gene and polyadenylation sequence) cassette into the *PEPT1* locus, a donor template plasmid was generated. The donor template plasmid was generated by conjugating the following four fragments: two homology arms (PEPT1: 0.93 kb for the 3' arm and 1.00 kb for the 5' arm), an EF1α-PuroR-pA cassette and linearized backbone plasmids (pENTR donor plasmids). The backbone plasmids were the kind gift of Dr. Akitsu Hotta (Center for iPS Cell Research and Application, Kyoto University). In the all experiments, we used the PEPT1 donor plasmid which have two homology arms. Full sequences of PEPT1 donor plasmid are shown below.



PEPT1 donor plasmid:

AACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGG
CGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGA
GGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTC
GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGG
GAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTC
GCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCC
GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC
ACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG

GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCC
AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTA
GCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAA
GATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGGGGCCCAATCTGAATAA
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AATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGG
AGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCC
GACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGT
GAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCT
TTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACC
AAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAA
GGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAAC
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TGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCA
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TTCCCAAGGCAGTCTGGAGCGCTAGGTCTTGAAAGGAGTGGGAATTGGCTCCGGTGCCC
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TGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGT
TCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGC
CCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAG
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ATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGA
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TTATTTGATGCCTGGCAGTTCCCTACTCTCGCTTAGTAGTTAGACGTCCCCGAGATCCATG
CTAGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAG

CRISPR/Cas9 plasmid

The plasmids expressing hSpCas9 and sgRNA were generated by ligating double-stranded oligos into the *Bbs*I site of pX330 (Addgene, no 42230, http://www.addgene.org/42230/). The sgRNA sequences are shown below.

PEPT1 gRNA : cagagccatgttaactgtgt

Genotyping primers and sequencing primers

The sequences of genotyping primers are shown below.

PEPT1 F: 5'- agggaagaggctcagtatctacttc -3'

PEPT1 R: 5'- caagacagtaataattcttattcact -3'

The sequences of sequencing primer are shown below.

PEPT1: 5'- cccattgcaagtgtaaaatgca -3'