Construction of stable mouse artificial chromosome from native mouse chromosome 10 for generation of transchromosomic mice

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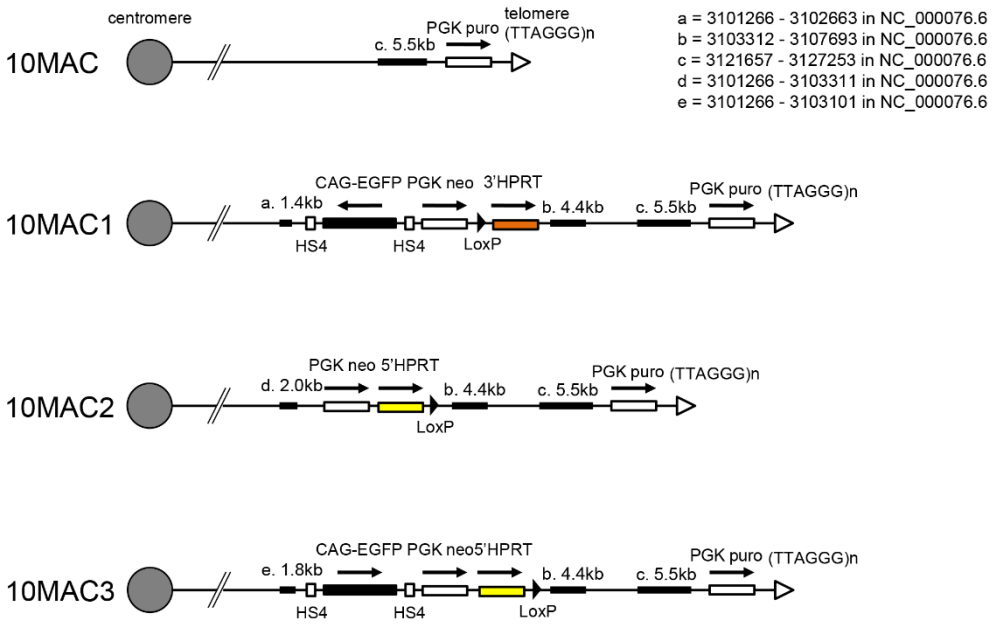
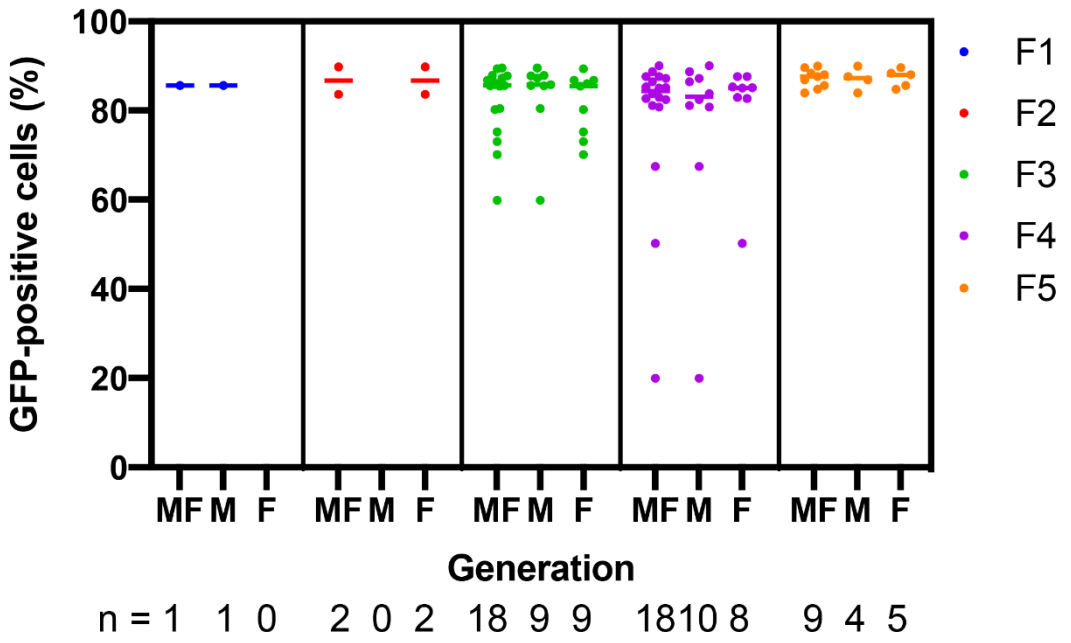


Supplementary Figure S1. Detailed maps of the MACs

(a-e) Homologous region on mChr.10 for gene targeting. EGFP-NeoR-3'HPRT, NeoR-5'HPRT-loxP and EGFP-NeoR-5'HPRT-loxP were inserted to generate 10MAC1, 10MAC2 and 10MAC3, respectively. The targeting vectors are described in Figs. 2 and 3.



Supplementary Figure S2. Retention rate of 10MAC1 in lymphocytes of Tc mice through generations

Retention rate of 10MAC1 in lymphocytes from peripheral blood of Tc mouse strain was determined as percentage by FCM analysis monitoring GFP-positive cells through generations (F1-5). M: male. F: Female.

Supplementary Table S1. Primer sequences for genomic PCR analyses

Gene, vector or aim	Primer name (forward)	Forward primer (5'-3')	Primer name (reverse)	Reverse primer (5'-3')	Product size
pBS-TEL/puro_10MAC	AscI_m10T F2	TCGAGGCGCGCCAGCCTTCTAGGGAACAGGAGATGTTCAA	BamHI_m10T R3	TCGAGGATCCGCCTTGAGTGGGGTTCTAGTCATCTTTC	5.6 kb
Telomere truncation	m10 F6	AACTACCCAGTTCTGCATTTGGTGTGAG	Puro I	GAGCTGCAAGAACTCTTCCTCACG	7.0 kb
Gm8155	Gm8155 F	ACCCCTCGAACCCCTATTGC	Gm8155 R	CACGCCATCGGTGATGGATA	192 bp
lyd	lyd F	TGGGATGACCCCCACTTCTTT	lyd R	TTTTGGCCTCTTGCCCCATA	192 bp
Plekhg1	Plekhg1 F	TGGATGGGTTTCAATGCCACT	Plekhg1 R	GGCATTCTCCCCTGTTGTGG	159 bp
p10MAC1, 10MAC1 construction	KpnI_m10 LA F	TCGAGGTACCTCTAAGTCAGGGAAAGATCCCCTTCTTG	XhoI_m10 LA R	TCGACTCGAGGACCATGAAGATGGTCCAACTAAAGCAA	2.0 kb
p10MAC1	SalI_m10 RA F	TCGAGTCGACCACTGCTCTTTCTTTAGTTACATGCAGCCC	NotI_m10 RA R	TCGAGCGGCCGCATTCTTGCCAAGCTACTCTTCCGAGCTA	4.4 kb
10MAC1 construction	m10 F1	TGAGAAATACCGAATGGCAGAGAAACAC	EGFP-F	CCTGAAGTTCATCTGCACCA	5.0 kb
10MAC1 construction	kj neo	CATCGCCTTCTATCGCCTTCTTGACG	m10 R2	GAGAGGAGGGAAGCTTGATGAGAAAATG	7.0 kb
p10MAC2, p10MAC3	NotI_m10 LA F	TCGAGCGGCCGCTCTAAGTCAGGGAAAGATCCCCTTCTTG	SalI_m10 LA R	TCGAGTCGACGACCATGAAGATGGTCCAACTAAAGCAA	2.0 kb
p10MAC2, p10MAC3	ClaI_m10 RA F	TCGAATCGATCACTGCTCTTTCTTTAGTTACATGCAGCCC	ClaI_m10 RA R	TCGAATCGATATTCTTGCCAAGCTACTCTTCCGAGCTA	4.4 kb
10MAC2 construction	m10 F1	TGAGAAATACCGAATGGCAGAGAAACAC	10MAC R1	CTCTTCAGCAATATCACGGGTAGCCAAC	4.6 kb
10MAC2 construction	10MAC F1	TGCTTGCATTGTATGTCTGGCTATTCTG	m10 R2	GAGAGGAGGGAAGCTTGATGAGAAAATG	4.9 kb
10MAC3 construction	m10 F1	TGAGAAATACCGAATGGCAGAGAAACAC	EGFP-R	TGCTCAGGTAGTGGTTGTCG	7.0 kb
10MAC3 construction	10MAC F1	TGCTTGCATTGTATGTCTGGCTATTCTG	m10 R2	GAGAGGAGGGAAGCTTGATGAGAAAATG	4.9 kb
CENPH	CENPH Fw	TTCCCCATGAAGTGCAGCAA	CENPH Rv	AAACCACCGTGCAGTGCAGA	250 bp
Furin	Furin-F	ACTCAGAGATCCACTGCACCAGGATCCAAGGGAGG	Furin-R	CCGCTCGAGCGGCTACACCACAGACACCATTGTTGGCTACTGCTGCC	272 bp
Cre/loxP system	TRANS L1	TGGAGGCCATAAACAAGAAGAC	TRANS R1	CCCCTTGACCCAGAAATTCCA	408 bp

Supplementary Table S2. Confirmation of gene loading by PCR

	TRANS L1/R1	X3.1IEGFPI AnJ F1/AnJ_lox161R	FISH analysis
CHO 10MAC1 X6.1	24/24 (100%)	N.D.	N.D.
CHO 10MAC2 X3.1-I-EGFP-I	23/23 (100%)	18/21 (85.7%)	3/6 (50%)
CHO 10MAC3 X3.1-I-tdTomato-I	24/24 (100%)	23/24 (95.8%)	1/5 (20%)