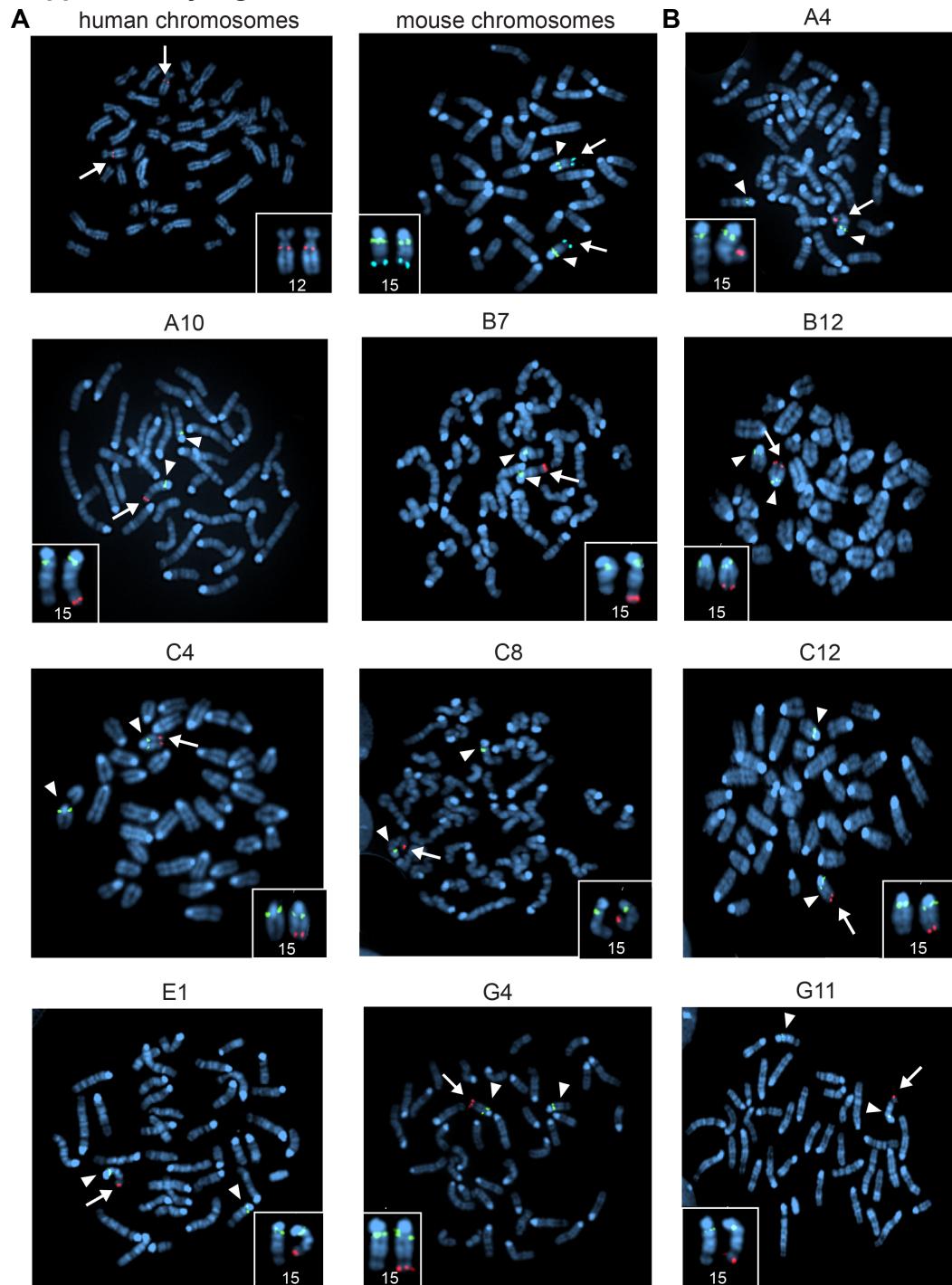


Supporting Information

The contribution of homology arms to nuclease-assisted genome engineering

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Supplementary Figure 1



Supplementary Figure 1. FISH analysis

A) FISH probes were tested on human fibroblast chromosomes (left panel) and mouse E14Tg2a wt cells (right panel) to determine the specificity of the probes. Left panel: Human KMT2D BAC probe (red; arrow) showed strong signals on both pairs of the human chromosome 12 at band 12q13 (where the human KMT2D is located), whereas human KMT2D gave no signals on mouse chromosomes above background (not shown). Right panel: Mouse Kmt2d BAC probe (blue; arrow) showed strong signals on both pairs of mouse chromosome 15 at band 15qF1 (where the mouse Kmt2d gene is located) but no signals on human chromosomes above background (not shown). A probe against mouse 15qA1 (green; arrowhead) served as an internal control and a means to identify chromosome 15.

B) Representative FISH metaphase images selected for the positive KMT2D targeted clones named above each panel. As in A), the control 15qA1 probe is green with arrowhead; the human KMT2D probe is red with arrow. In all cases, only one chromosome 15 pair is labelled red.

Supplementary Figure 2

mKmt2d locus

CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA wt
gRNA-1

CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA G3
CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA G4
CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA G11
CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA F10
CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA F11
CCC AGT TTG CTG ACA GCT TTT GGC TCC TCA GG GGT AGC CCT TTA F12

Supplementary Figure 2. Absence of indels on the other Kmt2d allele

Sequence alignment of mouse Kmt2d locus to check integrity of the other Kmt2d allele using specific primer pair (a + d) to amplify wt allele only. gRNA-1 target site and PAM (blue) are highlighted.

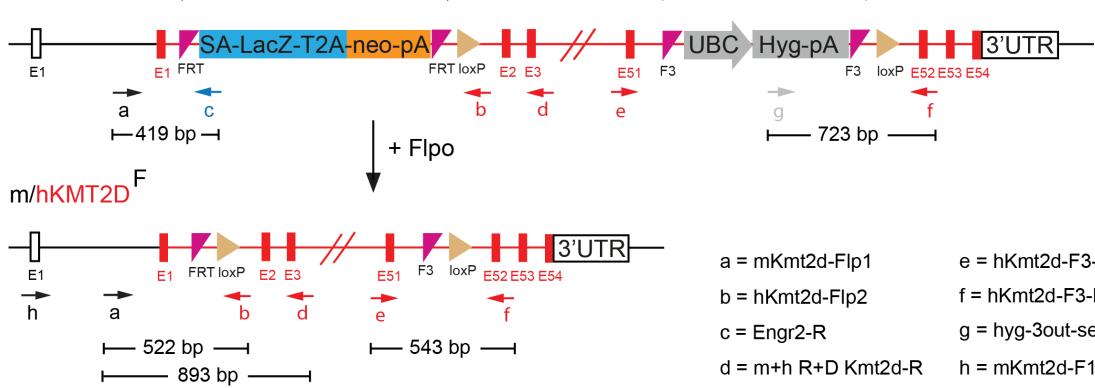
Supplementary Figure 3

A

mKmt2d



m/hKMT2D



B

	Flp-neo	Flp-neo complete	Flp-Hyg	Flp-Hyg complete	Flp-neo Flp-Hyg complete	Flp-neo; Flp-Hyg complete wt-allele + a+d
Clone Nr.	a+b	a+c	e+f	g+f		
F11	97% (35/36)	86% (31/36)	97% (35/36)	38% (14/36)	30% (11/36)	30% (11/36)
G4	97% (35/36)	91% (33/36)	100% (36/36)	36% (13/36)	36% (13/36)	36% (13/36)

Complete recombination is absence of PCR-product with (a+c) and (g+f) primer combinations

Supplementary Figure 3. Flp recombinase cassette removal

A) Wild-type (mKmt2d), targeted (m/h KMT2D), and Flp recombined Kmt2d (m/h KMT2D^F) alleles were amplified with the depicted primer pairs to define recombination of the two FRT flanked selection cassettes.

B) Frequency of Flp recombination.

Supplementary Table 1. LOA screening summary

After neomycin selection of ES cells lipofected with the indicated quantities of the mouse/human hybrid BACs with or without gRNA 1/2 (500ng each) and Cas9 expression plasmids, cells were grown and surviving colony numbers are shown as colonies picked. These were tested for sensitivity to hygromycin (because correct colonies should be hygromycin resistance) as a first indication of correct targeting. Because most colonies were hygromycin resistant, this was not a good indicator of homologous recombination. Colonies positive by the loss-of-allele assay (LOA) indicated that only in the presence of Cas9/gRNA-1/2 targeting had been successful.

Chimeric BAC	gRNA-1/2 / Cas9	Colonies picked	Hyg sensitive	LOA positive
HA 4/7 kb				
500 ng	-	11	1	0/10
1000 ng	-	7	0	0/7
2000 ng	-	3	0	0/3
500 ng	+	21	1	0/20
1000 ng	+	23	0	3/23
2000 ng	+	25	2	5/23
HA 4/50 kb				
500 ng	-	6	0	0/6
1000 ng	-	9	0	0/9
2000 ng	-	10	1	0/9
500 ng	+	17	0	6/17
1000 ng	+	24	1	2/23
2000 ng	+	19	3	2/16
HA 50/7 kb				
500 ng	-	8	1	0/7
1000 ng	-	10	1	0/9
2000 ng	-	10	1	0/9
500 ng	+	23	0	0/23
1000 ng	+	21	4	4/17
2000 ng	+	24	2	2/22

Supplementary Table 2	List of Oligonucleotides	Sequence 5' to 3'
Name		Sequence 5' to 3'
Construction of chimeric BAC		
mKmt2d-zeo-A (Step 2 mouse)	GAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCG C	TAAGGAGGTCCATGGTATGCCAAGTTGACCAGTGC
mKmt2d-zeo-B (Step 2 mouse)	TACCGTTCTTCAATCTAACGATGCCAAGGGTAGCCTAAAGCTCAGCCT CTCCTCGGCCACGA	CCTCGCTACCTAGGACCGTTAGTTATCAGTCCTG
mKmt2d-genta-A (Step 4 mouse)	TCCCCGAGAGGGCCCTGCCAGTCGGAGAGAGGG TGATGATGG	ATGGACAGCCAGAAGCTGGCTGGTAGGATAAAGATGGTACCGGCCTGG
mKmt2d-genta-B (Step 4 mouse)	GGGAATTCCCCTGCCTGGTAGCCTCAAAGCTTC GGTCGAT	TTAGTTCATCCATTCCGACAATTCCAGGCTCCACAGTTAGGTGGCGGTACTTG
hKmt2d-spec-A (Step 2 human)	TGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAA ACGAACAAATTAATAAAGTG	TGTCCATCCCTCTCCGACTGGCAGGGCCCTCTGGGGAGACCTGTTGATGCCAAAAGATAACCTGTATTAAATTATAATT
hKmt2d-spec-B (Step 2 human)	TTTAATCTGTTATTAAATAGTTATAG TAT TCC CCA GAA CAT CAG GTT	AGGTCGGAAATGGATGAACTAAGAACGCTTGAGGCTACCAGGCAGGGAAATTCCCCCATTCCGCCTCTTAATTAAATTAAATT
h-ccdB-amp-A (Step 4 human)	TGTGCGCTGTCGACGGTACCCCTATAGTCGAGGGACCTATTGTTATTCTAAATACATTCA	GTCACAGGAGAGCAGGAGGAAAGCTGTTGGTGGGAGAAGGGAAAGGCAGGTACGCAACACGACTCGACCTCTATTCTGGTGA
MII4-Hyg-A (Step 6 human)	GTCACAGGAGAGCAGGAGGAAAGCTGTTGGTGGGAGAAGGGAAAGGCAGGTACGCAACACGACTCGACCTCTATTCTGGTGA TAGCAC	AGGTAGGAGACTCAGGCAGTGGGGCTTTGTCATGACTTCATTCTTGAGAATCAGGCATGTTCTGCGTAGTGTCACTCAT
MII4-Hyg-B (Step 6 human)	AGGTAGGAGACTCAGGCAGTGGGGCTTTGTCATGACTTCATTCTTGAGAATCAGGCATGTTCTGCGTAGTGTCACTCAT CAGA	ATTATCCAAAACCTGAGTGGATCAGAGTGTGATATTCAACTGTTGTCCTGTCATCAGGAGACGACGACGAACA
MII4-neo-A (Step 10)	ATGAGCTAAGTCTATGAGCAGATAAGTAATGGAACAATTGTTCTTGGCGTGTTCGAGCATGTTCTGCGTAGTGTCACTCAT CC	ATTATCCAAAACCTGAGTGGATCAGAGTGTGATATTCAACTGTTGTCCTGTCATCAGGAGACGACGACGAACA
MII4-neo-B (Step 10)	ATTATCCAAAACCTGAGTGGATCAGAGTGTGATATTCAACTGTTGTCCTGTCATCAGGAGACGACGACGAACA GAG	TGCCCCGACGTTAACCGGGCTGCATCCGATGCAAGTGTGCGCTGCGAC TTACCAATGCTTAATCAGTGAGGC
BAC-amp-3 (Step 12)	ACACAAAAATAAACAGGCAGAGAGGGCTATCTGGACATGTTCCAACTCC TTTAATCTGTTATTAAATAGTTATAG	ACACAAAAATAAACAGGCAGAGAGGGCTATCTGGACATGTTCCAACTCC CCTCGCTACCTAGGACCGTTAGTTATTATAATT
MII4-Spec-4kb (Step 12)	GACTGATAGTTTCAAGCCCAGAGAAAAGAGAGAATTAGTAAC CATTCAAATATG	TATAACGGTCTAAGGTAGCGAGGTTGTTATTCTAAATA
MII4-amp-7kb (Step 12)	CTACACATGTCGTAAGCTAGGAATTCTCCACATAGTACTAAC ATTCAAATATG	TATAACGGTCTAAGGTAGCGAGGTTGTTATTCTAAATA
MII4-amp-50kb (Step 12)	TTGTGTATTAAATAAGCCATTATAGTCATTCCCTGCTAATACAGTATACCGGTGATCGGGCGCTTCCGCTTCGCTCA ACAATTACACAGAGATAGTCTGGAACTTGTAACTATCTGGCTTAAGTCGATCGACCGGTACATTCAAATATGTTATCCGCTCATG	TTGTGTATTAAATAAGCCATTATAGTCATTCCCTGCTAATACAGTATACCGGTGATCGGGCGCTTCCGCTTCGCTCA ACAATTACACAGAGATAGTCTGGAACTTGTAACTATCTGGCTTAAGTCGATCGACCGGTACATTCAAATATGTTATCCGCTCATG
HPRT	CATGGCAACTTTAAATGTAAATTAGGAGTCAAACCTCAAGTGTGCTTGGCGTCACTGGCGCTTCCGCTTCGCTCA TTTGTAATTAAGAGATAGGACTCTAACATCCCAAACCTTGATAGTCTACGATCGACCGGTACATTCAAATATGTTATCCGCTCATG	CATGGCAACTTTAAATGTAAATTAGGAGTCAAACCTCAAGTGTGCTTGGCGTCACTGGCGCTTCCGCTTCGCTCA TTTGTAATTAAGAGATAGGACTCTAACATCCCAAACCTTGATAGTCTACGATCGACCGGTACATTCAAATATGTTATCCGCTCATG
pBR322-200bp-A	TGTAACAATTGCTGTTCAGCATGTTACTTATATCAAATTCTGAGTACCGGTGATCGGGCGCTTCCGCTTCGCTCA ATGATCTTGAGTGTGAGAGCTACCCCTGGATAGTCAGGGAGAAATTGTCGATCGACCGGTACATTCAAATATGTTATCCGCTCATG	TGTAACAATTGCTGTTCAGCATGTTACTTATATCAAATTCTGAGTACCGGTGATCGGGCGCTTCCGCTTCGCTCA ATGATCTTGAGTGTGAGAGCTACCCCTGGATAGTCAGGGAGAAATTGTCGATCGACCGGTACATTCAAATATGTTATCCGCTCATG
pBR322-200bp-B	TTAAACTAGCTTTATAAAATCGTTAAAAGATCAAAGTATCTAAAGTACCGGTGATCGGGCGCTTCCGCTTCGCTCA GAAATACAAACCAACCAATGGTGGCACACACCTATAATTCCATCATGTGACGATCGACCGGTACATTCAAATATGTTATCCGCTCATG	TTAAACTAGCTTTATAAAATCGTTAAAAGATCAAAGTATCTAAAGTACCGGTGATCGGGCGCTTCCGCTTCGCTCA GAAATACAAACCAACCAATGGTGGCACACACCTATAATTCCATCATGTGACGATCGACCGGTACATTCAAATATGTTATCCGCTCATG
pBR322-500bp-A		
pBR322-500bp-B		
pBR322-1kb-A		
pBR322-1kb-B		
pBR322-2kb-A		
pBR322-2kb-B		

pBR322-5kb-A	GAAGAGGGTGTCAAGATCTCATTACGGATGGTTGAGCCACCATGTGACCGGTCGATGGCGCTTCCGCTTCGCTCA
pBR322-5kb-B	TTTCAAACACACACA ACTGATTAATGACACAAAGAAAAGA ACTGTACAA CGATCGACCGG TACATTCAA ATATGTATCCG CTCATG
pBR322-10kb-A	CCAGAAGAGGGTGTCAAGATCTCATTACGGATGGTTGAGCCACCATGTGACCGGTCGATGGCGCTTCCGCTTCGCTCA
pBR322-10kb-B	TTTCAAACACACACA ACTGATTAATGACACAAAGAAAAGA ACTGTACAA CGATCGACCGG TACATTCAA ATATGTATCCG CTCATG
HPRT ssOligo donor	AGGATAATAATTGACACTGGTAAAACA ATGC CAA ACTTTGCTTCCCTGGTAA GCAGTACAGCCCC AAATGGTTAAGGTTGCAAGGTA TGTATGCCACTT
gRNA cloning	
gRNA-HPRT ssOligo	CGATTCTGGCTTATATCTTG GAAAGGACGAA ACACCGT GGGGCTGT ACTGCTTA ACC CGTT TAGAGCT AGAAATAGCAAGT TAAAAATAAGGCTAGTCGG
gRNA-HPRT* ssOligo	CGATTCTGGCTTATATCTTG GAAAGGACGAA ACACCGT GGGGCTGT ACTG TACCA CGTT AGAGCT AGAAATAGCAAGT TAAAAATAAGGCTAGTCGG
gRNA-1 ssOligo	CGATTCTGGCTTATATCTTG GAAAGGACGAA ACACCG GTGACAGC GCTTGG CTCCTCG TTAGAGCT AGAAATAGCAAG TAAAAATAAGGCTAGTCGG
gRNA-2 ssOligo	CGATTCTGGCTTATATCTTG GAAAGGACGAA ACACCG GATAACGG CTTAAGG TAGCGG TTAGAGCT AGAAATAGCAAG TAAAAATAAGGCTAGTCGG
LOA assay	
mLOA-a	GGA CCA GGG ACA ACA GAC AT
mLOA-b	CTC CCC CAA CAG TGG TAC AG
Nxt2-A	GCGAGGTTCGTTTGTC
Nxt2-B	GCTGGAAATGGTCTGCTCCA
Southern probes	
MII4-5'probe-fwd-2	GATGGATCCACCGGTGCTCTTTGTGTC
MII4-5'probe-rev-2	CATCGAATTCTAAAGAGG CACACCC AAG
MII4-3'-probe-fwd	GATGGATCCAAAGAGAGAATTAGTCTCCT
MII4-3'-probe-rev	CATCGAATTCCCTGAGCTTAAGGTTGATGTC
Genotyping	
a = mKmt2d-Flp1	CTGGGGAAGCTTAGTGGACATA
b = hKmt2d-Flp2	CACCA GACGC ATGAGATT ATCC
c = Engr2-R	CCTTC CTC TACATAG TTGGCA
d = m+h R+D Kmt2d-R	CAGTT ACAGAG AGCACA ACGCC
e = hKmt2d-F3-F	TCT ACGAAG AGCAGG TATGAGC
f = hKmt2d-F3-R	CTAA ATCC CTC TTCTCGTCA
g = hyg-3out-seq	TTCGATGATGC CAGCTTGGCG
h = mKmt2d-F-1	ATGAGG ATTCTGG ATCGCCC
i = mKmt2d-E3-R	GACCT CCCC GATACACAGAA
j = hKmt2d-RNA-1-R	GAGAC CTCT CCCCACATGT GGGT
k = hKmt2d-RNA-2-R	AAGC CTGG ACTCCC CAGAA ACTAA