Figure 1

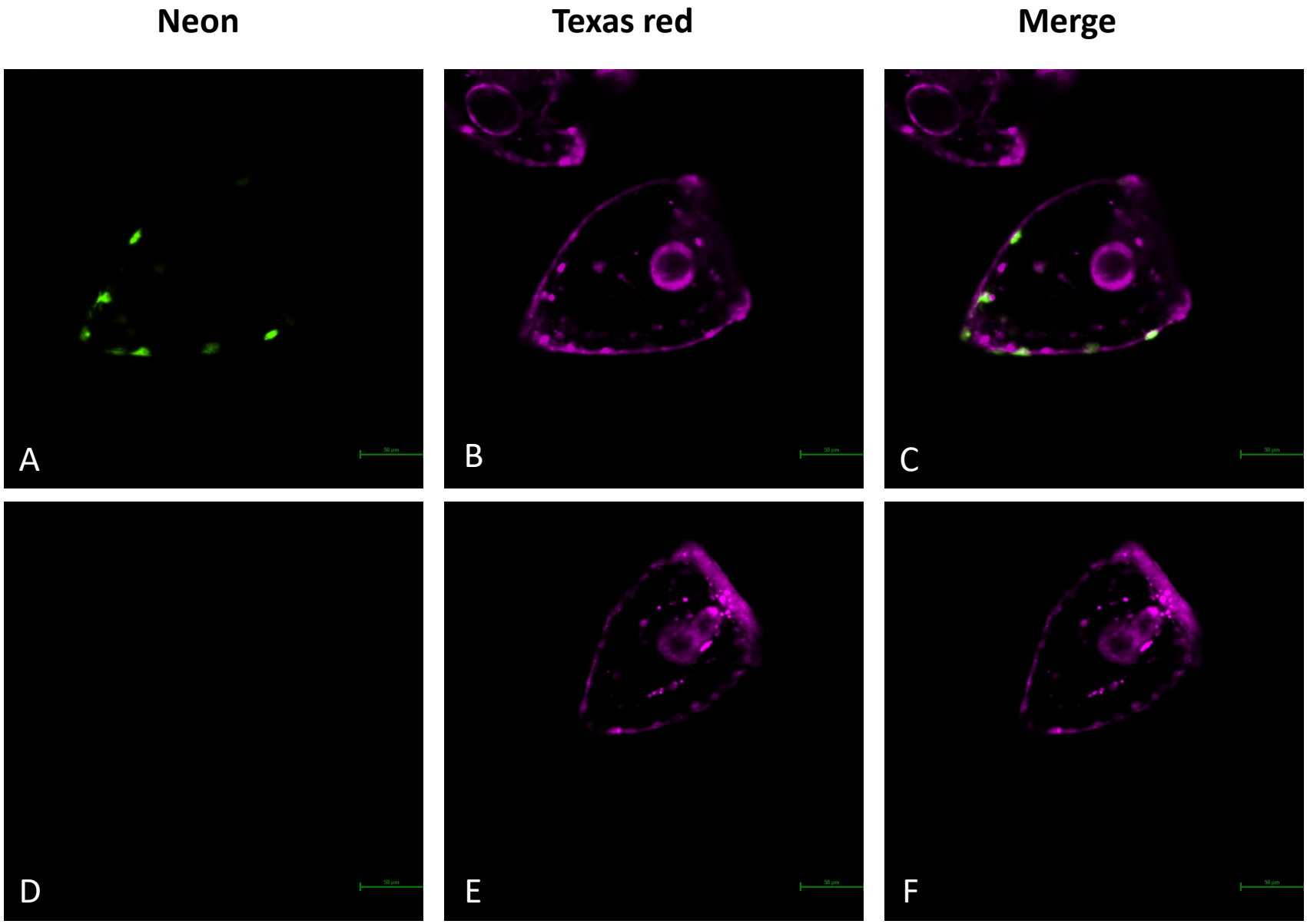
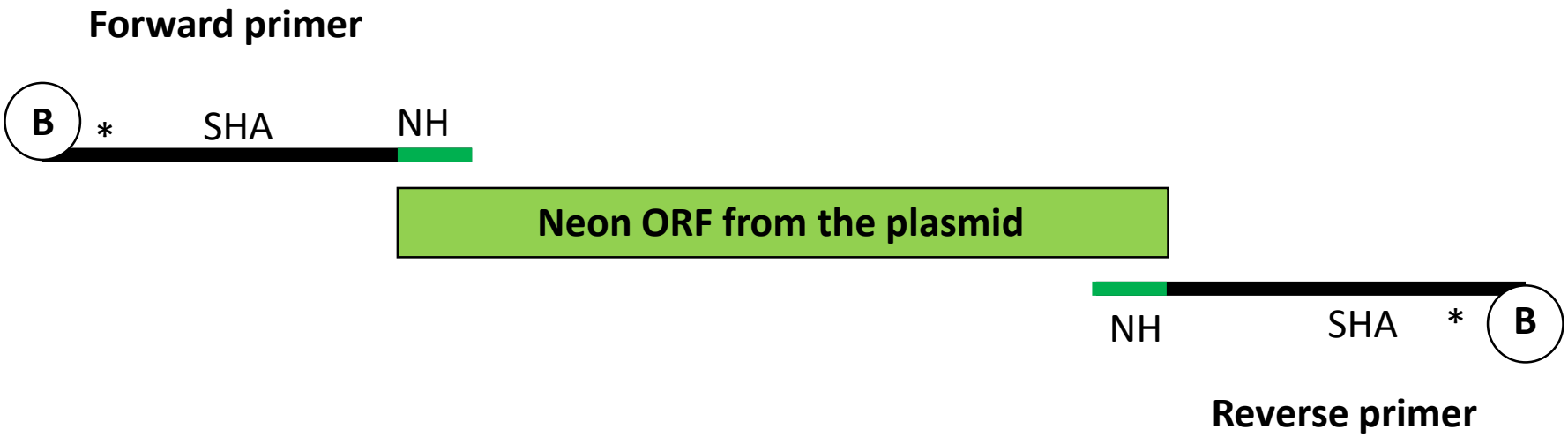


Figure 2



Primers to make the donor DNA:

B: Biotin

* : phosphorothioate bond of the first 5 bases to prevent degradation by nucleases

SHA: Short homology arm from the targeted gene (30 to 40 bp)

NH: neon homology (18 to 21 bp)

Fig.S1

Cas9	DNA donor	Strand	Homology arms	phenotype	knock-in (microscope)
mRNA	Plasmid	double	1kb	100% albinos	inconsistent
IDT 2uM	PCR PKS 20 ng/ul	double	40 nt	Died at blastula	X
IDT 2uM	PCR PKS 1 ng/ul	double	40 nt	100% albinos	0%
IDT 2uM	PCR biotin PKS 1 ng/ul	double	40 nt	100% albinos	0%
mRNA	PCR biotin PKS 1 ng/ul	double	40 nt	100% albinos	0%
mRNA	PCR biotin PKS 10 ng/ul (23nM)	double	40 nt	100% albinos	2%
IDT 2uM	PCR biotin PKS 10 ng/ul (23nM)	double	40 nt	100% albinos	2%
mRNA	PCR biotin PKS (23nM)	double	200 nt	100% albinos	0.5%
mRNA	Megamer PKS (23 nM)	single	40 nt	100% albinos	0%

Fig.S2

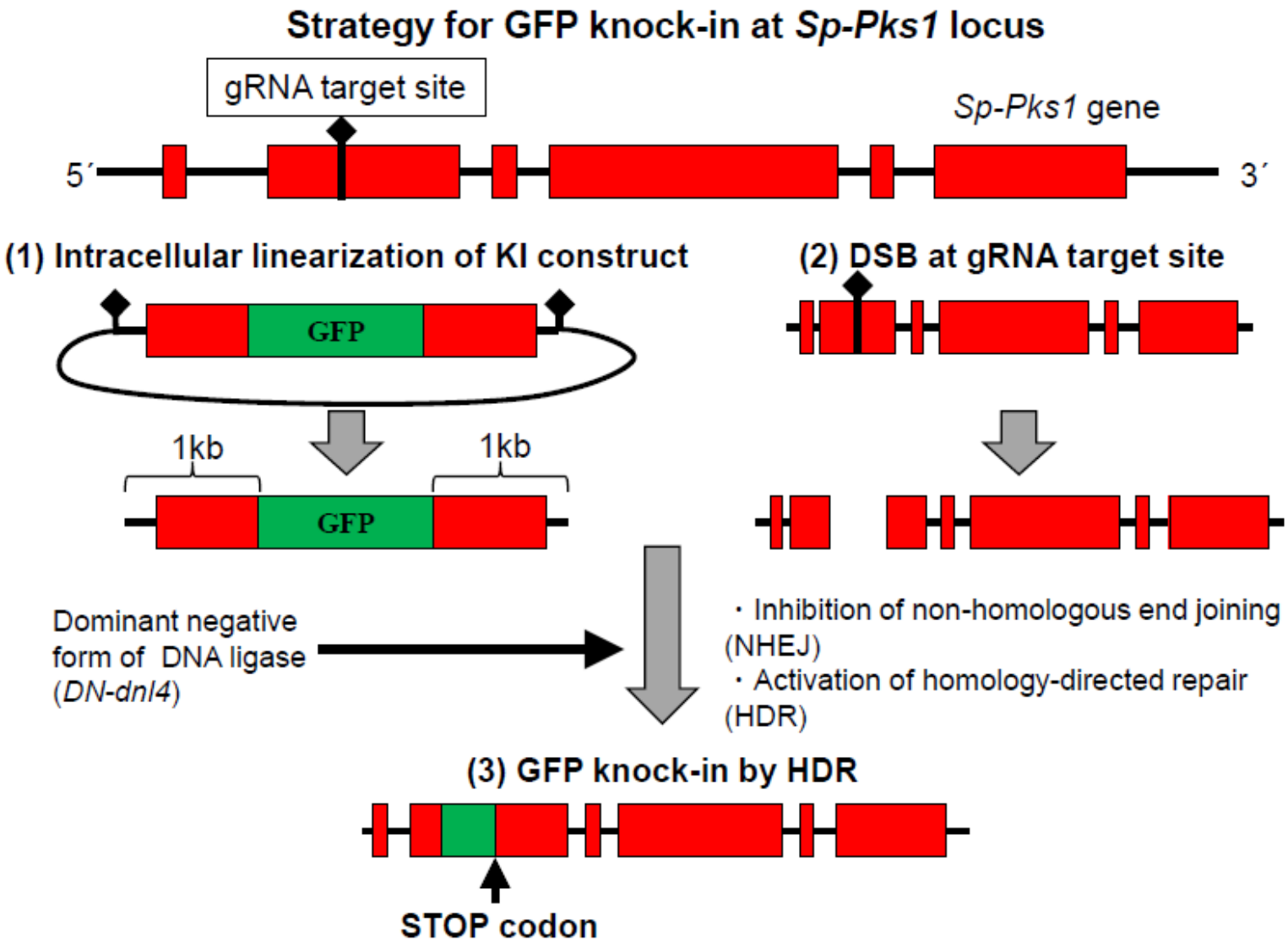

Strategy for GFP knock-in at *Sp-Pks1* locus
gRNA target site
Sp-Pks1 gene
5´
3´
(1) Intracellular linearization of KI construct
GFP
1kb
1kb
GFP
(2) DSB at gRNA target site
Dominant negative form of DNA ligase (*DN-dnl4*)
・Inhibition of non-homologous end joining (NHEJ)
・Activation of homology-directed repair (HDR)
(3) GFP knock-in by HDR
STOP codon

Fig.S3

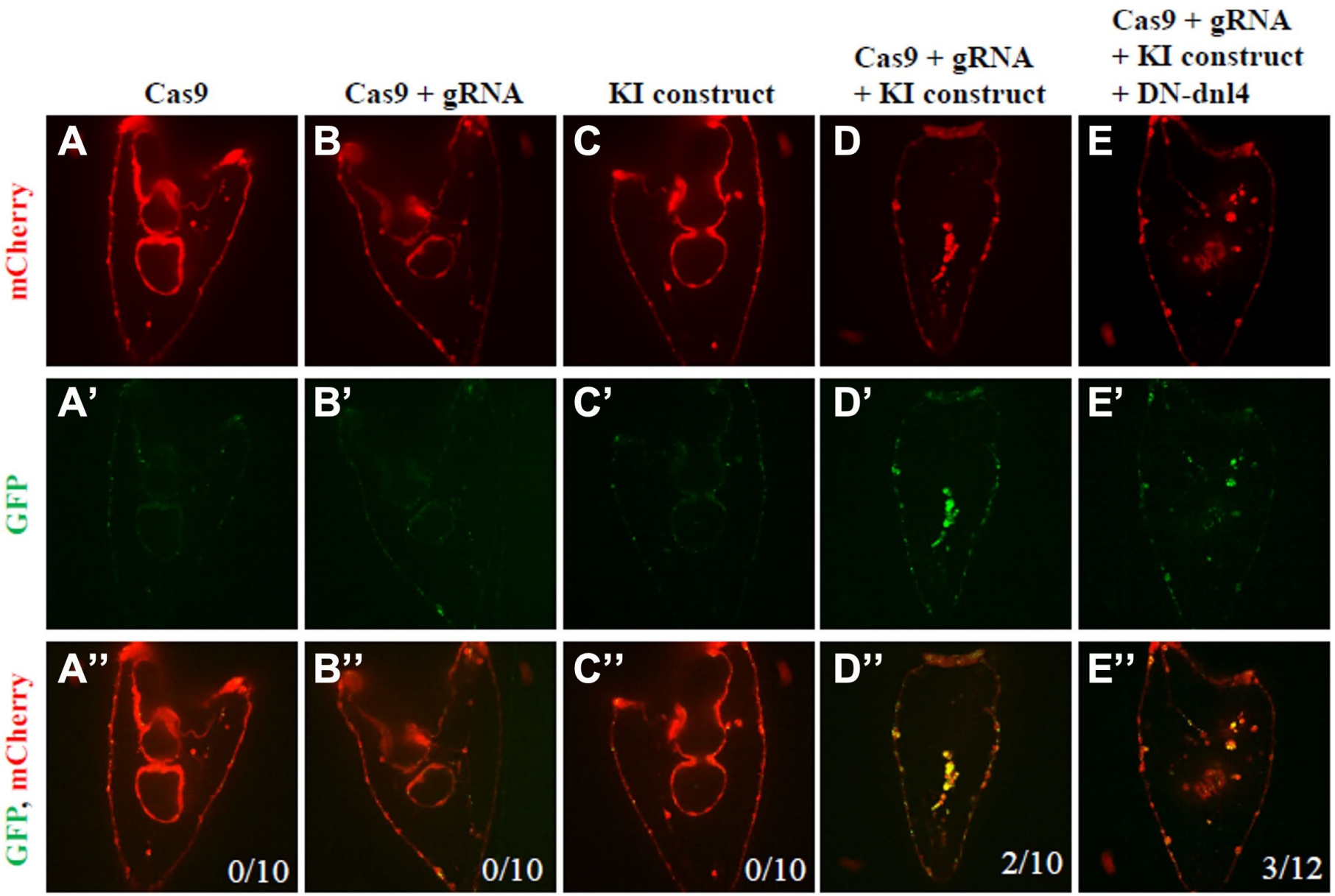

Cas9
Cas9 + gRNA
KI construct
Cas9 + gRNA + KI construct
Cas9 + gRNA + KI construct + DN-dnl4
mCherry
GFP
GFP, mCherry
A
B
C
D
E
A'
B'
C'
D'
E'
A''
B''
C''
D''
E''
0/10
0/10
0/10
2/10
3/12

Fig.S4

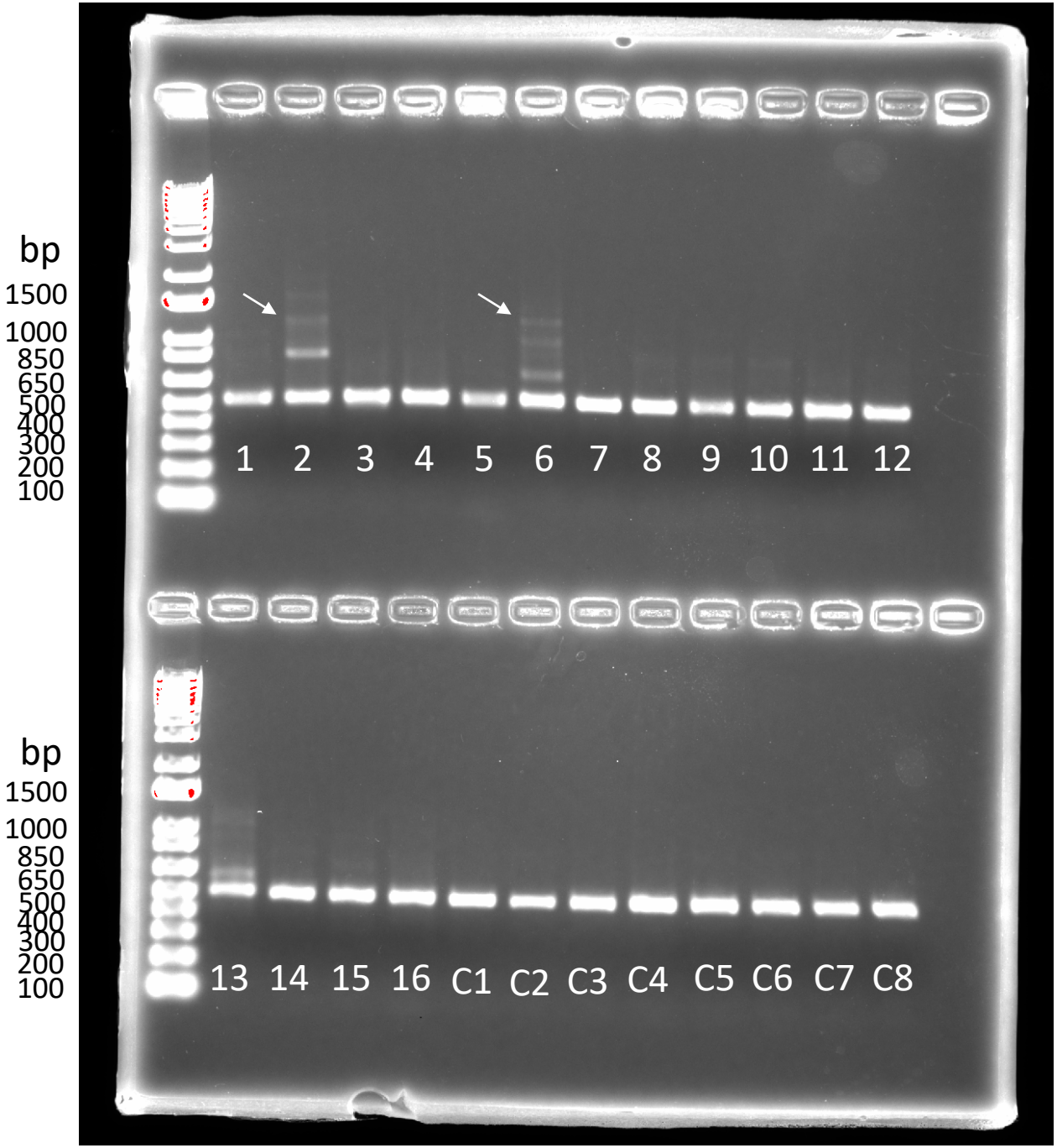

bp
1500
1000
850
650
500
400
300
200
100
1 2 3 4 5 6 7 8 9 10 11 12
bp
1500
1000
850
650
500
400
300
200
100
13 14 15 16 C1 C2 C3 C4 C5 C6 C7 C8

Fig.S5

GACATCACAGTCGGGTTCAGCGCCCTTGGTGTTCTCTCACCAGATGGCCGTTGCAGTCCCTTCT
CAAGTACCGCCAATGGCTACGTCCGCAGTGAAGGATGGGGCGCCATTGTGAGCAAGGGCGAGGA
GGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATCTTTGGCTCCATCAACGGTGTG
GACTTTGACATGGTGGGTCAGGGCACCGGCAATCCAAATGATGGTTATGAGGAGTTAAACCTGA
AGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTT
CCATCAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCC
GGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCT
ACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGA
CGGTCCTGTGATGACCAACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCC
AACGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGCAAGCGCTACC
GGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACCA
GCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAG
TGGCAAAAGGCCTTTACCGATGTGATGGGCATGGACGAGCTGTACAAGGTCCTCAAACCCCTCA
GCCAGGCTCTTGCTGACAATGACCACATCTATACTGTCATACGCGGTAGCGCCATCGCAGCCAA
TGGGTTAGCCAACAGTTTGACCATGCCATCTCCTCCTGCTCAGGAGTACGTCATGAAGGAAGCC
TATGAGAAGTTCGGTGTATCCATGTCCGATGTGCACTATGTCGGAGCTCACGGCACCGGAACCA
TGGTTGGTGATCCTCTGGAAGCAGAGGCCATCTCCAGGGCATTCAACCGCACCAAAGACAACCC
TCTCAAGATTGGATCCGTAAAGAGCAACTTTGGACATACCGAGGTTGCTGCCGGAGTGACAGCT
GCCATCAAAGTCGCCTTGATGATGGAGAACCGTACCATCCCACCAACCATCAATTTCGTCTCTT
CAAACCCCCACATCGACCCTGAAGAGATG

Fig.S6

Sp PKS1 control:

DITVGFSALGVLSPDGRCSPFSSTANGYVRSEGWGAIVLKPLSQALADNDHIYTVIRGSAIAANGLANS
LTMPSPPAQEYVMKEAYEKFGVSMSDVHYVEAHGTGTMVGDPLEAEAISRAFNRTKDNPLKIGSVKS
NFGHTEVAAGVTAAIKVALMMENRTIPPTINFVSSNPHIDPEEM

Sp PKS1 neon knock in:

DITVGFSALGVLSPDGRCSPFSSTANGYVRSEGWGAIVSKGEEDNMASLPATHELHIFGSINGVDFDM
VGQGTGNPNDGYEELNLKSTKGDLQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVH
RTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKTIISTF
KWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHSKTELNFKEWQKAFTDVMG
MDELYKVLKPLSQALADNDHIYTVIRGSAIAANGLANSLTMPSPPAQEYVMKEAYEKFGVSMSDVHY
VGAHGTGTMVGDPLEAEAISRAFNRTKDNPLKIGSVKSNFGHTEVAAGVTAAIKVALMMENRTIPPTI
NFVSSNPHIDPEEM