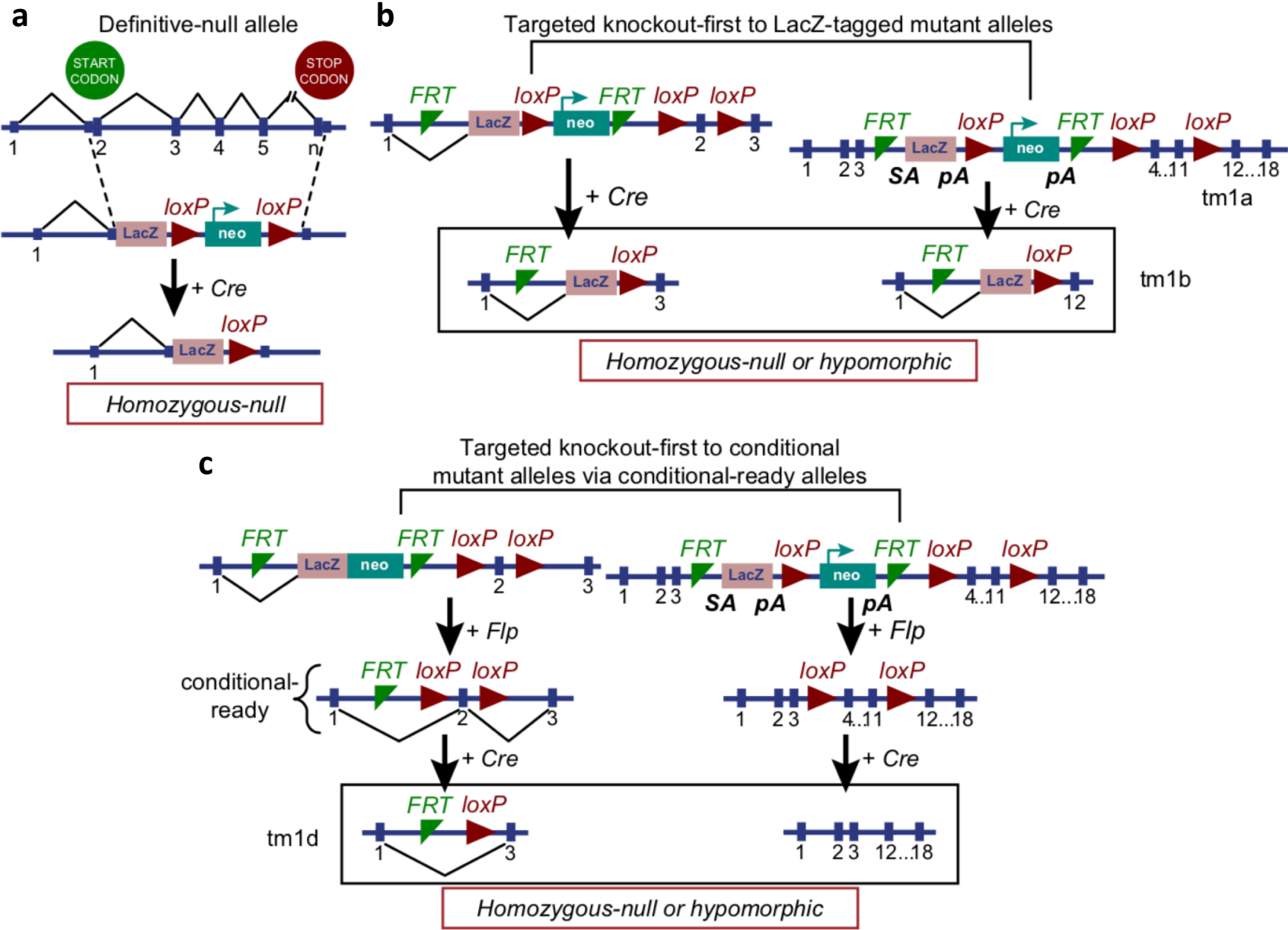


Supplementary Figure 1.

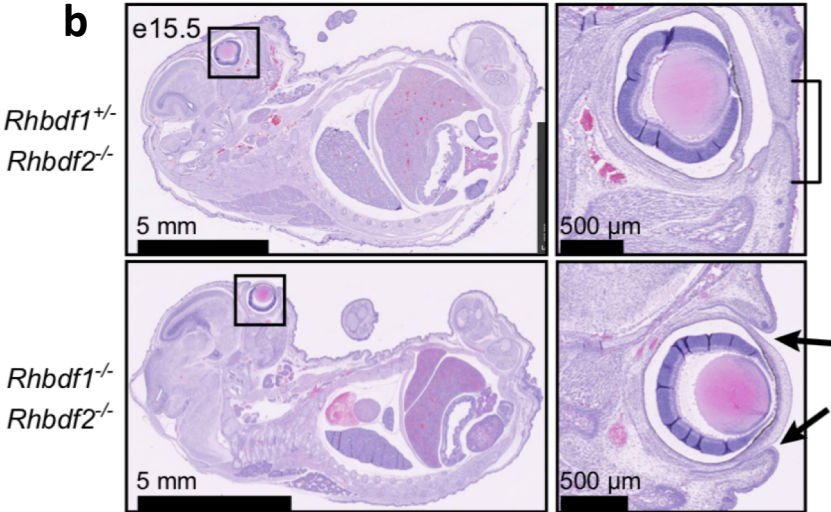


Supplementary Figure 2.

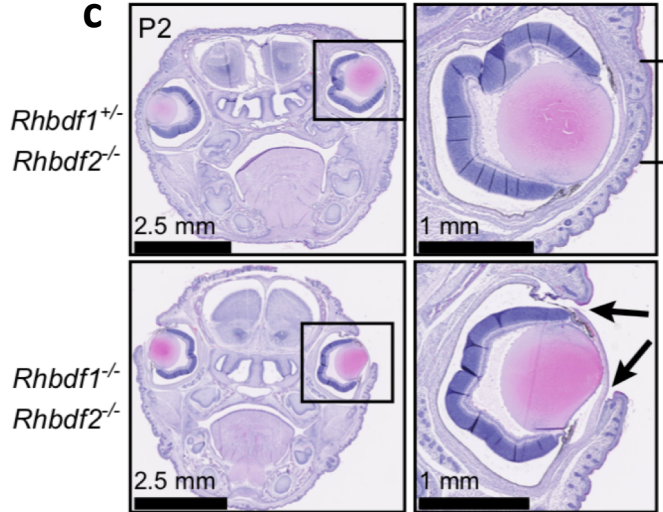
a

	<i>Rhbdf1</i> ^{+/+} <i>Rhbdf2</i> ^{-/-}	<i>Rhbdf1</i> ^{-/-} <i>Rhbdf2</i> ^{-/-}	<i>Rhbdf1</i> ^{-/-} <i>Rhbdf2</i> ^{-/-}
	Number (%) Expected 25%	Number (%) Expected 50%	Number (%) Expected 25%
e9.5	4 (29%)	8 (57%)	2 (14%)
e10.5	1 (4%)	18 (75%)	5 (21%)
e12.5	5 (42%)	6 (50%)	1 (8%)
e13.5	8 (40%)	9 (45%)	3 (15%)
e14.5	4 (44%)	4 (44%)	1 (11%)
e15.5	2 (25%)	1 (12.5%)	5 (62.5%)
e17.5	2 (33%)	2 (33%)	2 (33%)
e18.5	5 (33%)	9 (60%)	1 (7%)
P1-3	6 (23%)	16 (61%)	4 (15%)
>P4	9 (24%)	29 (76%)	0

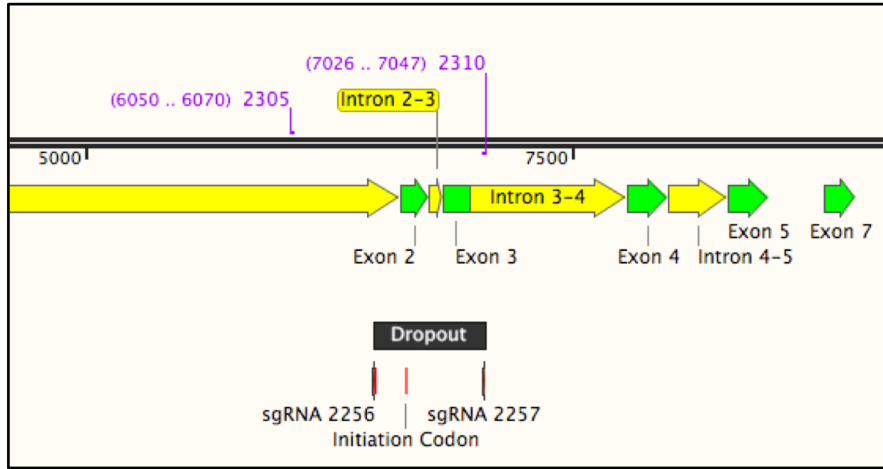
b



c



Supplementary Figure 3



2256 sgRNA GGAGGATCTGTGGAGTTC

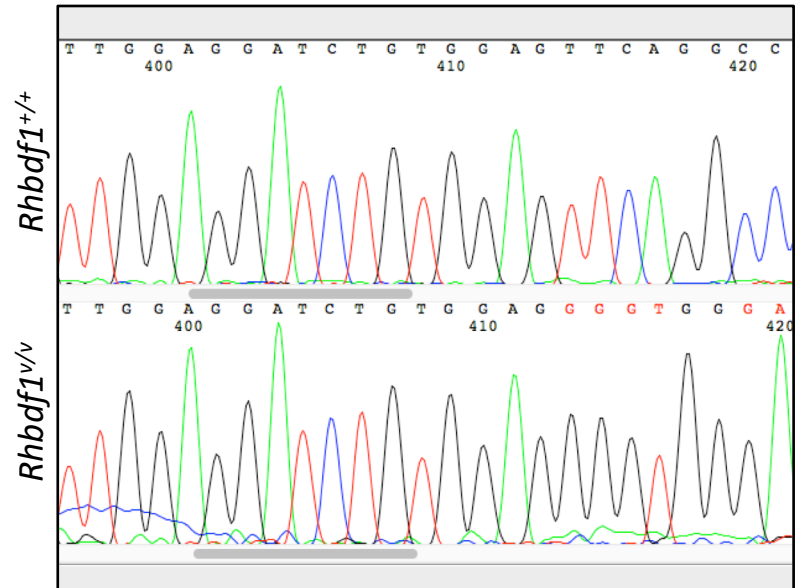
2257 sgRNA GAATCCCTGTTAGGTACC

2305 Forward AAGGAGAGTCCTCTGCATACT

2310 Reverse ACCTAACAGGGATTCTGTATGC

Phusion High-Fidelity PCR

CYCLE STEP	CYCLES	TEMP	TIME
Initial denaturation	1	98°C	30 seconds
Denaturation	30	98°C	10 seconds
Annealing		72°C	30 seconds
Extension		72°C	1 minute
Final extension	1	72°C	5 minutes
Hold	1	4°C	∞

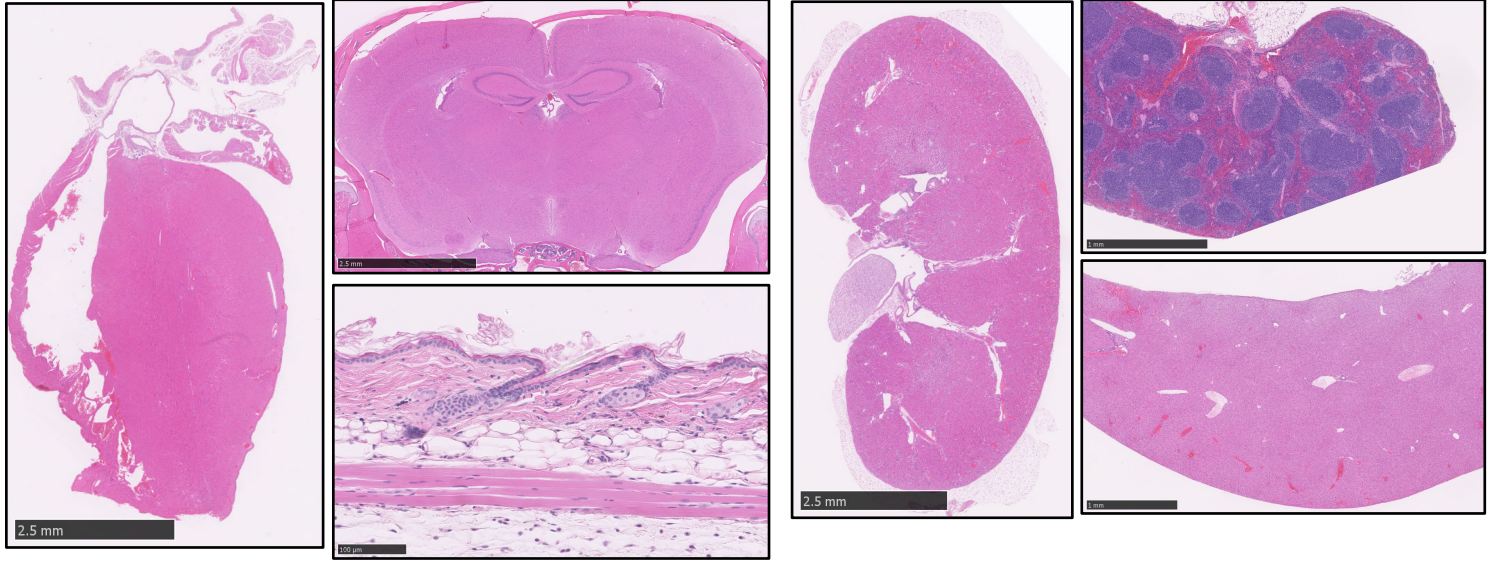


Wildtype sequence TTGTTGGAGGATCTGTGGAGTTCAGGCCAGAGCATGCCA

Mutant sequence TTGTTGGAGGATCTGTGGAG-GGGTGGGAATGGGGCAAAGG

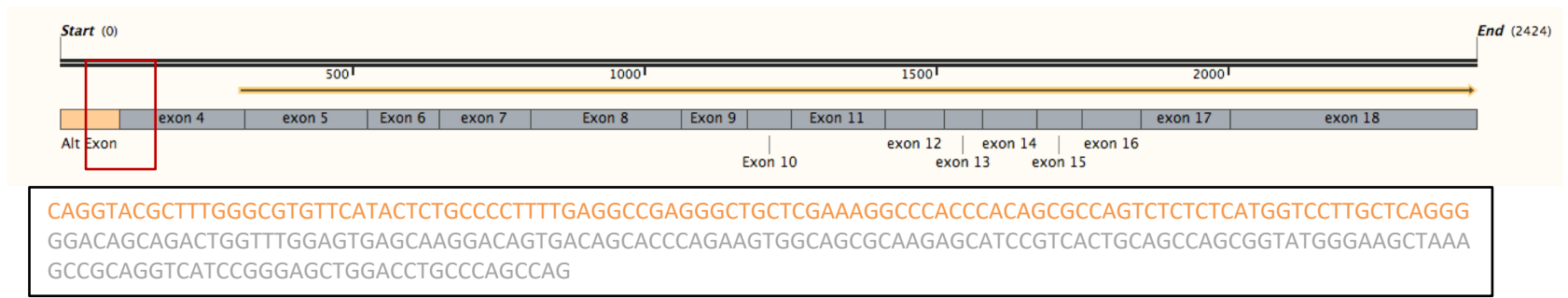
Supplementary Figure 4

a



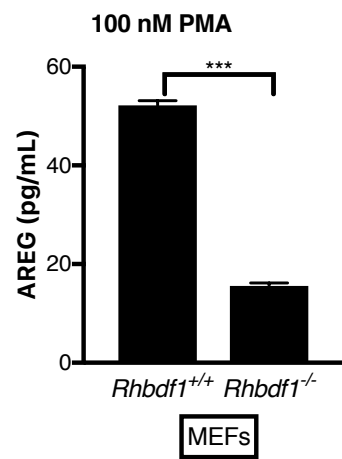
b

Splice variants of *Rhbf1*^{v/v}

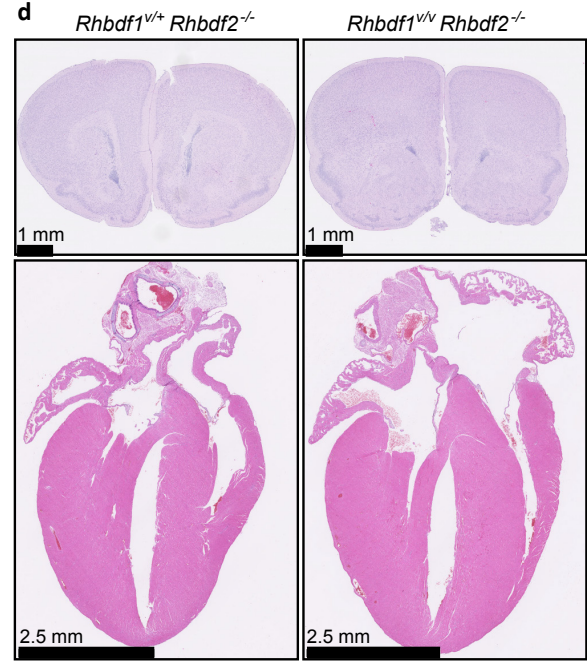
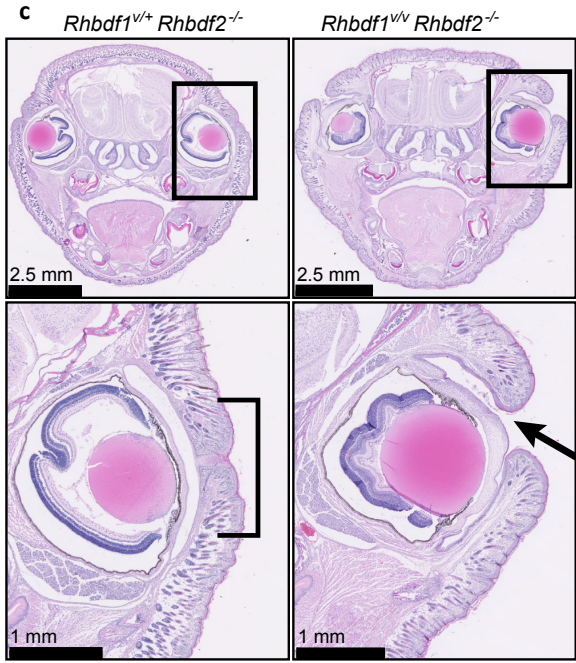
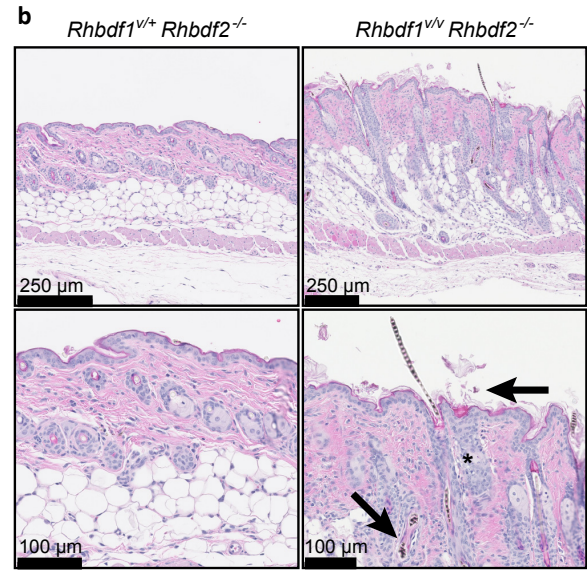
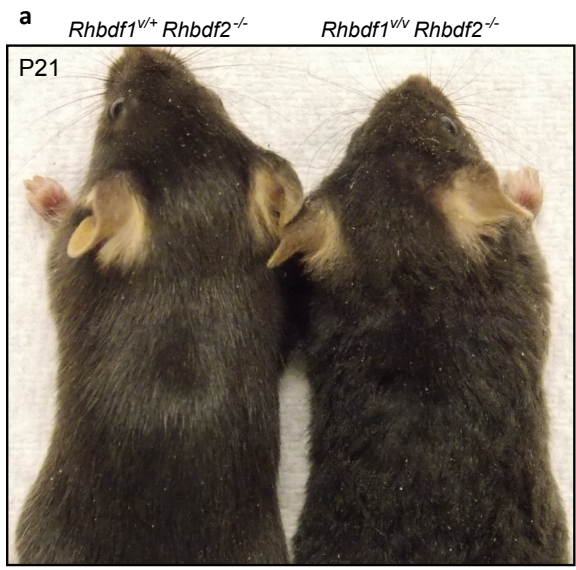


Transcript variant	NCBI Reference Sequence
X1	XM_011243658.2
X2	XM_006514494.4
X3	XM_006514495.4
X4	XM_006514496.3
X9	XR_872202.2
X14	XR_001779873.2

Supplementary Figure 5



Supplementary Figure 6



a

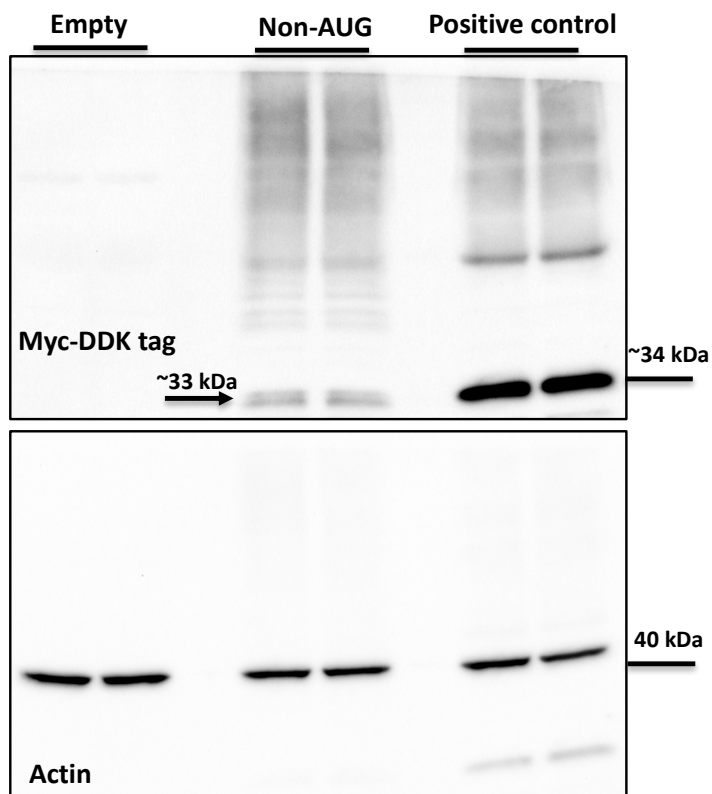
Transcript: Mouse *Rhbdf1*-206 ENSMUST00000143988

R_PCCIGTKGRCEITSREYCDFMRGYFHEEATLCSQVHCMDDVCGLLPFLNPMTVLRDLEKLAGWHRIAIYLLSGITGNLASAIFLPYRAEIVGPAGSQFGILACLFVELFQ
SWQILARPWRAFFKLLAVVLFLEAFGLLPWIDNFAHISGFVSGFLFSFAFLPYISFGKFDLYRKRCQIIIFQVVFLGLLAGLVVLFYFYPVRCWECEFLTICIPFTDKFCEKYELD
AQLH*

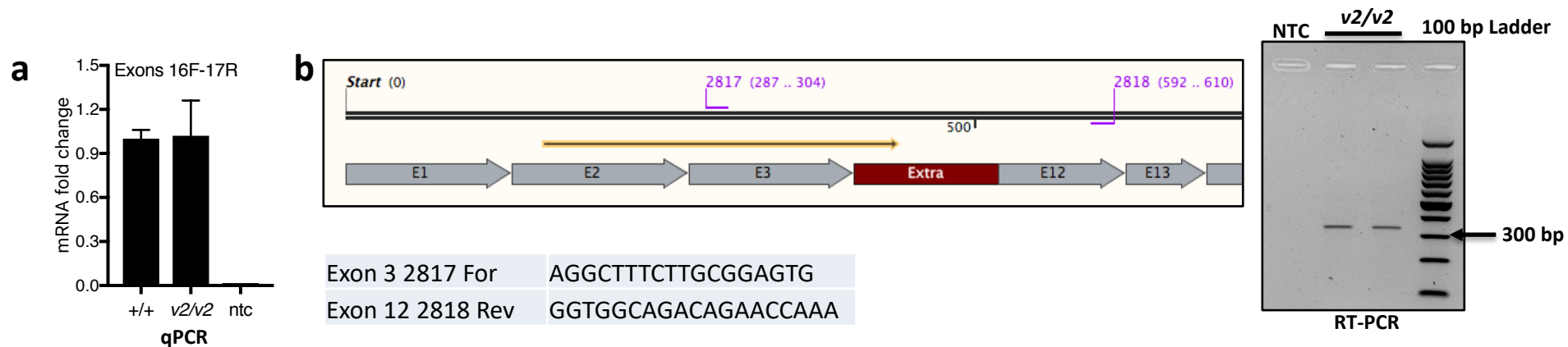
Transcript: Human *RHBDF1*-205 ENST00000448893

R_PCCIGTKGRCEITSREYCDFMRGYFHEEATLCSQVHCMDDVCGLLPFLNPMTVLRDLEKLAGWHRIAIYLLSGVTGNLASAIFLPYRAEIVGPAGSQFGILACLFVELFQ
SWQILARPWRAFFKLLAVVLFLEFTFGLLPWIDNFAHISGFISGLFLSFAFLPYISFGKFDLYRKRCQIIIFQVVFLGLLAGLVVLFYVYPVRCWECEFLTICIPFTDKFCEKYELDA
QLH*

b



Supplementary Figure 8

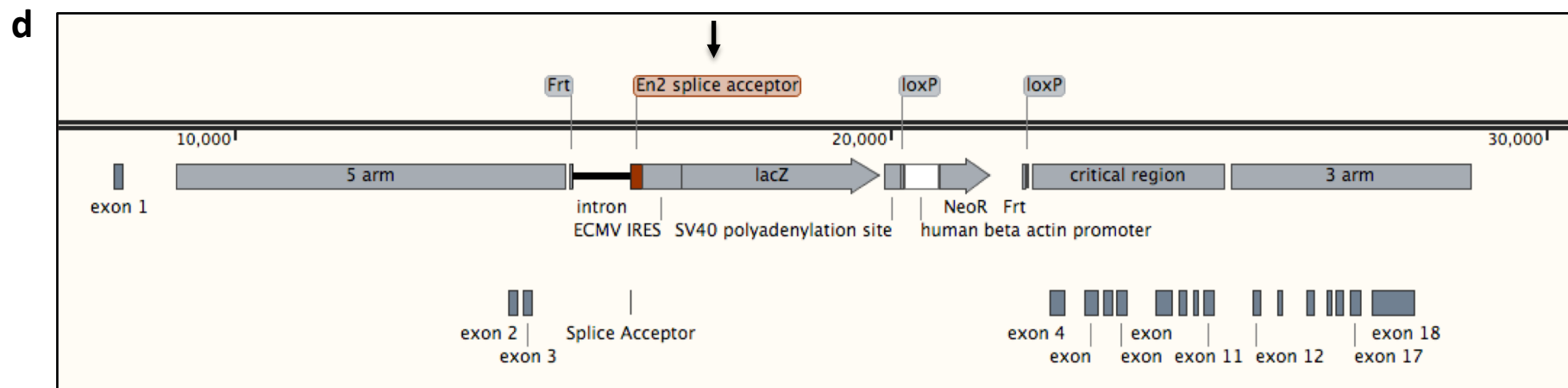


c

AGGCTTTCTTGCGGAGTGTGAGTATGCCAGCTGAGACAGCCCGGGTCCCATCACCCCACCATGAGCCTCGCCGGCTGGTGCT
 GCAGCGTCAGACATCCATCACACAGACCATCCGCAG**GTCCCAGGTCCCGAAAACCAAAGAAGAACCCTAACAAAGAGG**
ACAAGCGGCCTCGCACAGCCTTCACTGCTGAGCAGCTCCAGAGGCTCAAGGCTGAGTTTCAGACCAACAGTCCACGTTGTC
 GGTGTGGGTGAAGTGGCCCGTTCATCCTAGTGCCCCAGACCTTGCAGGCAACAAGAGACAG**TTTGTTCTGTCTGCCACC**

Bold: Primers 2817/2818

Extra: *En2* splice acceptor sequence from the targeted KO-first cassette

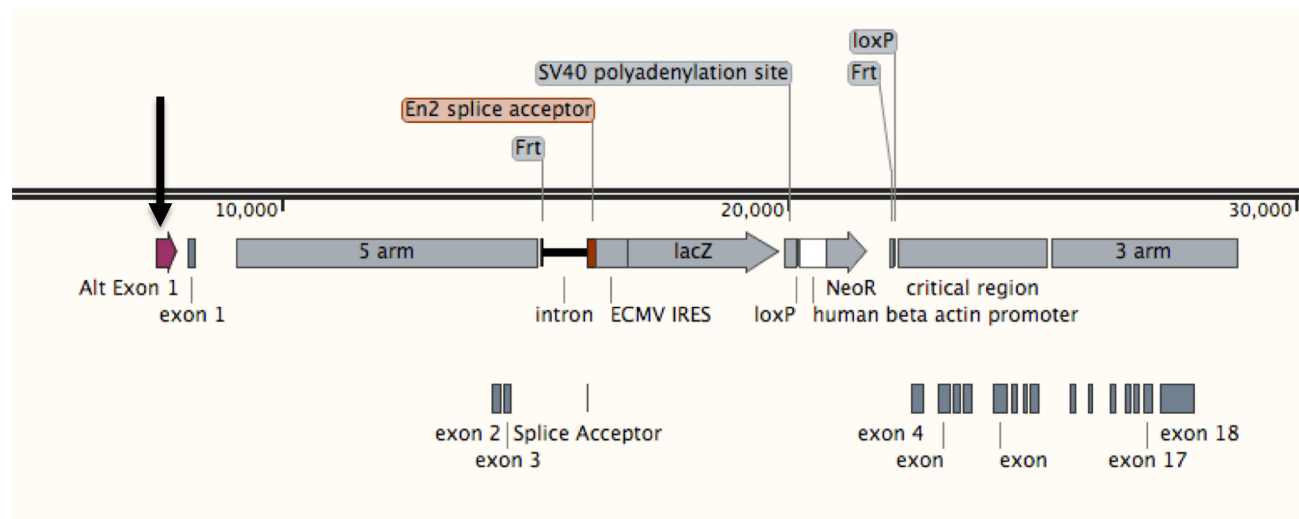


e

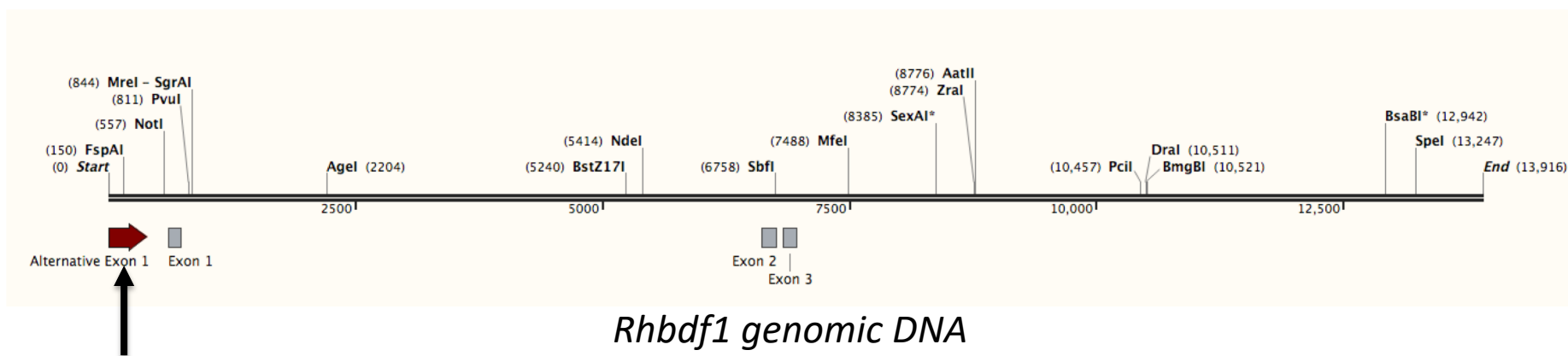
Putative 10.8 kDa truncated protein sequence:
 MSEARRDSTSSLQRKKPPWLKLDIPAAVPPAAEEPSFLQPLRRQAFILRSVSMPTARVPSPHHEPRRLVLQRQTSITQTIRRSQVPKTKEEP*

a

Rhbd1 targeted KO-first allele



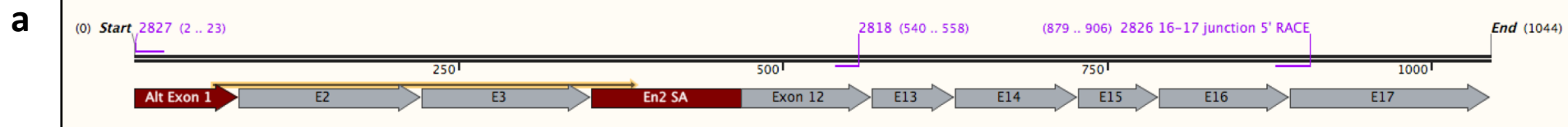
b



Alternative exon 1 sequence:

```
tgtacctgctgggaaagaagtaggggaaggggtaattgctgagctgcggtcctgtgaaatggtgtatcagtgtatgaacgtgagtgtgcacactataaatgtgtagggtagag
agtgtgagcccatggaagtgcattttgaataggtgtgcgcacgtgtctgtgtaaacaggtgcgagaggcctgaatgcatgaatgtttgtagaaagatcaggcgctgtgcacagg
acaagtgagagcttcgggacctcagtgatgtgccagtgctgccttcggaaggggcttcaatctgccttttaggtccctccacgccaccctcttgcctttggaggtccccttctc
tctccaagcacaggggcagcacaagatgaaggagtatggaaaggcgagtcca
```


Supplementary Figure 10

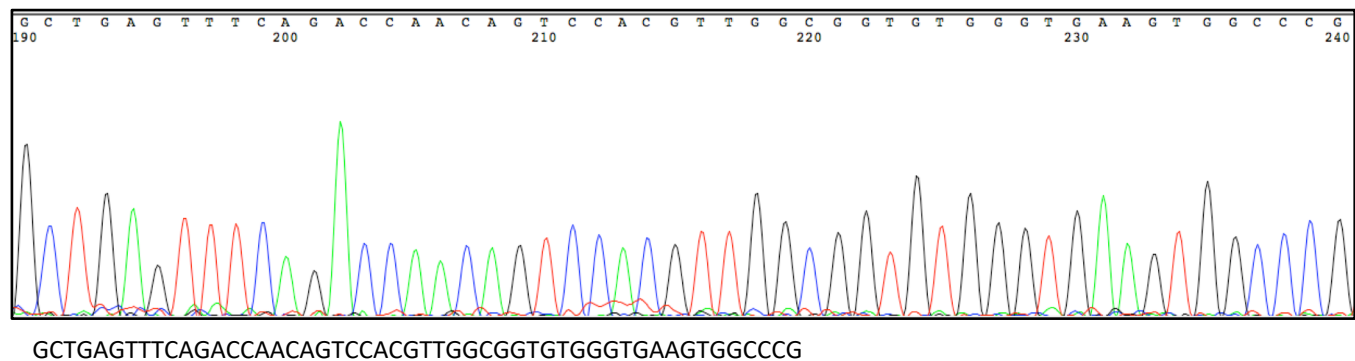
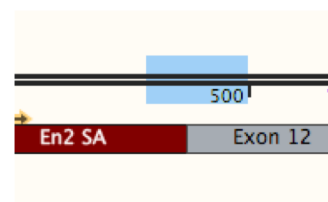
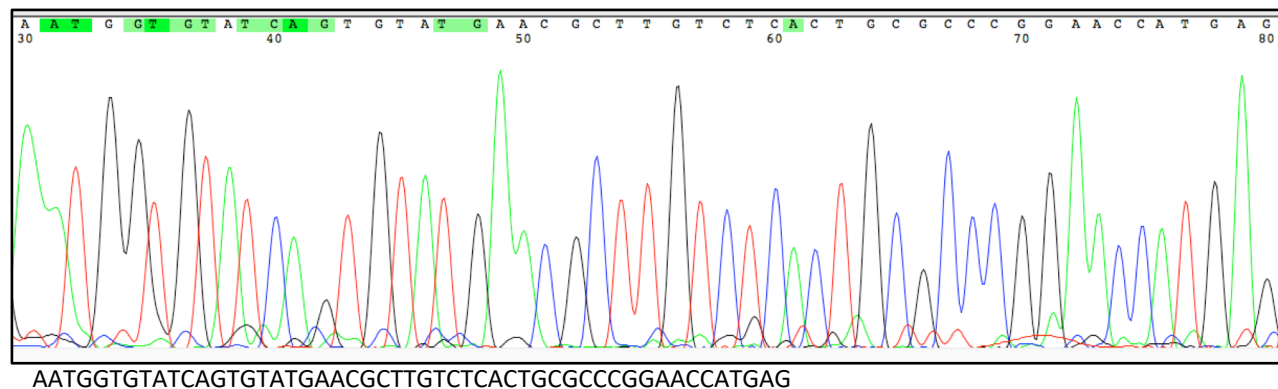
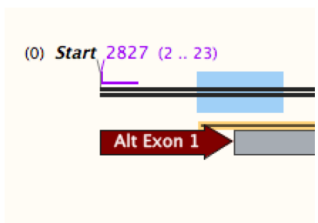


Putative 12.3 kDa truncated protein sequence:

MVYQCMNACLTAPGMTSEARRDSTSSLQRKKPPWLKLDIPAAVPPAAEEPSFLQPLRRQAFILRSVSMPAETARVPSPHHEPRLVLQRQTSITQTIRRSQVPKT
KEEP*

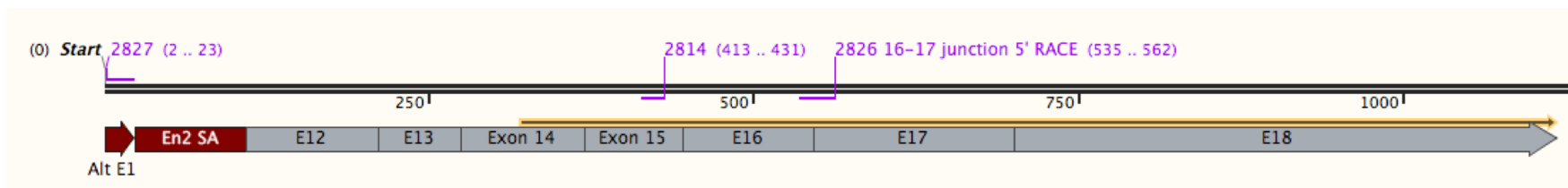
b

2827 F	GTACCTGCTGGGAAAGAAGTAG
2818 R	GGTGGCAGACAGAACCAAA
2826 5' RACE	GATTACCCAAGCTT CAAGCAGTGCAGGATCCCAGCGTGCAGG



Supplementary Figure 11

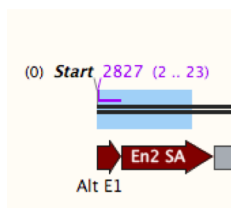
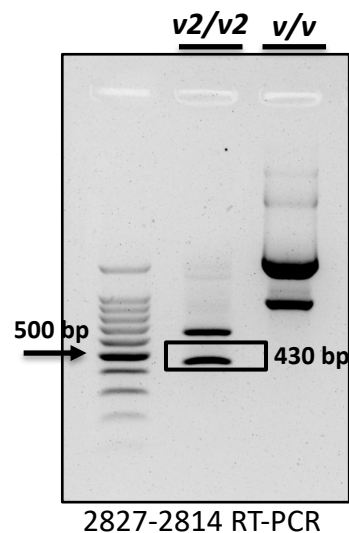
a



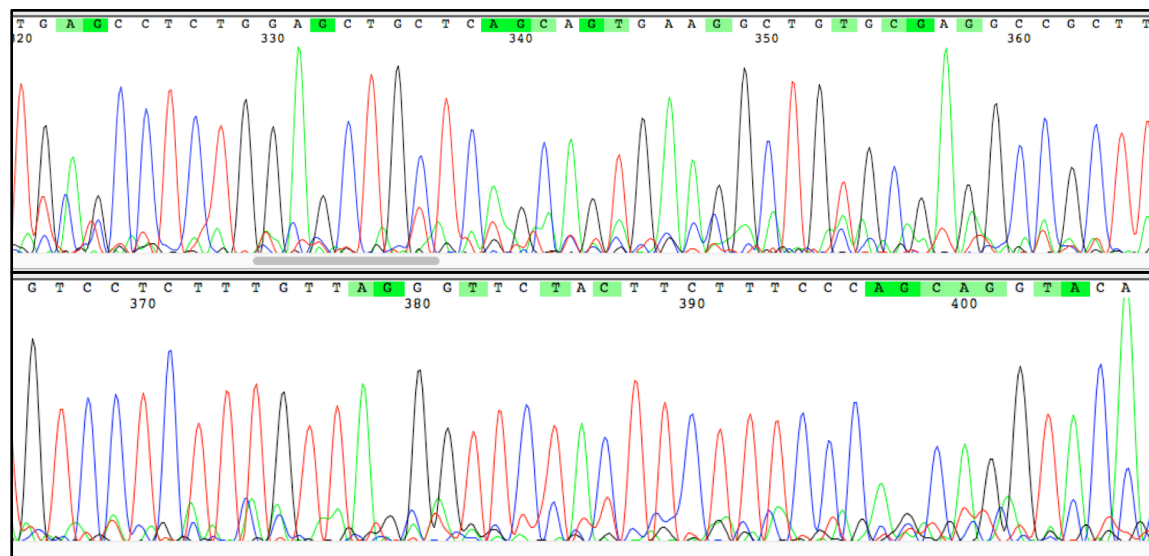
Variant 1; 32.77 kDa

MDCVITGRPCCIGTKGRCEITSREYCDFMRGYFHEEATLCSQVHCMDVDCGLLPFLNPEVPDQFYRLWLSLFLHAGILHCLVSVCFQMTVLRDLEKLAGWHRIAIIYLLSG
ITGNLASAIFLPYRAEVPAGSQFGILACLFVELFQSWQILARPWRAFFKLLAVVLFLEFAFGLLPWIDNFAHISGFVSGFLFSFAFLPYISFGKFDLYRKRCQIIIFQVVFLGLLA
GLVVLFFYFYPVRCEWCEFLTCIPFTDKFCEKYELDAQLH(EQKLISEEDLDYKDDDDK)

b

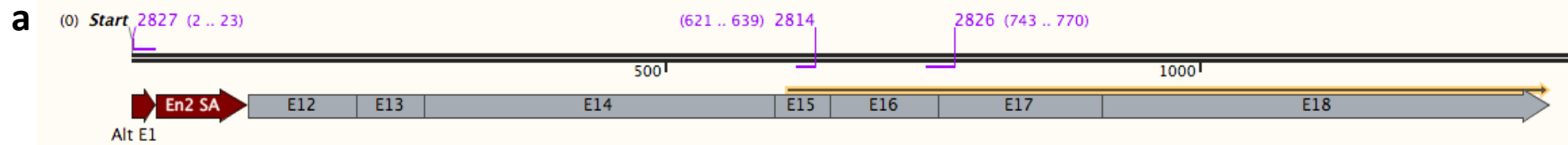


TGAGCCTCTGGAGCTGCTCAGCAGTGAAGGCTGTGCGAGGCCGCTT
GTCCTCTTTGTTAGGGTTCTACTTCTTTCCAGCAGGTACA



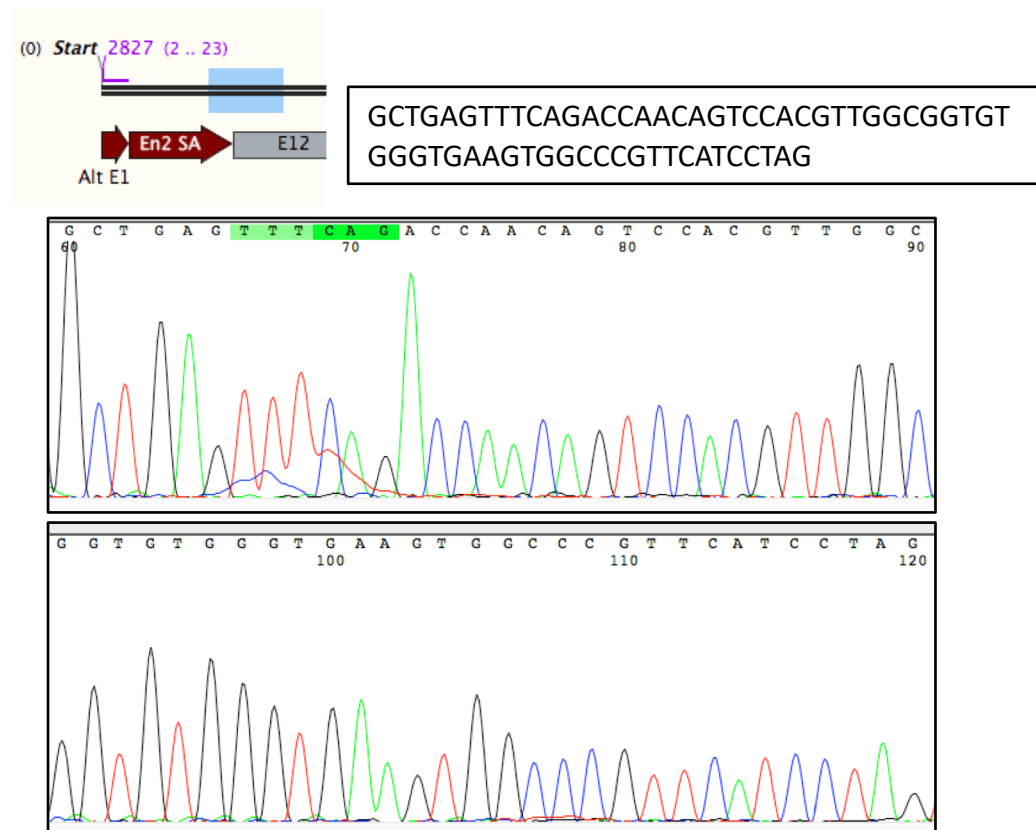
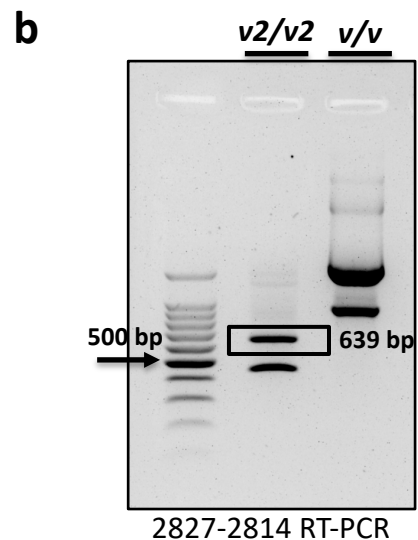
Taq polymerase PCR			
CYCLE STEP	CYCLES	TEMP	TIME
Initial denaturation	1	95°C	2 minutes
Denaturation	30	95°C	15 seconds
Annealing		52°C	30 seconds
Extension		68°C	2 minute
Final extension	1	72°C	5 minutes
Hold	1	4°C	∞

Supplementary Figure 12

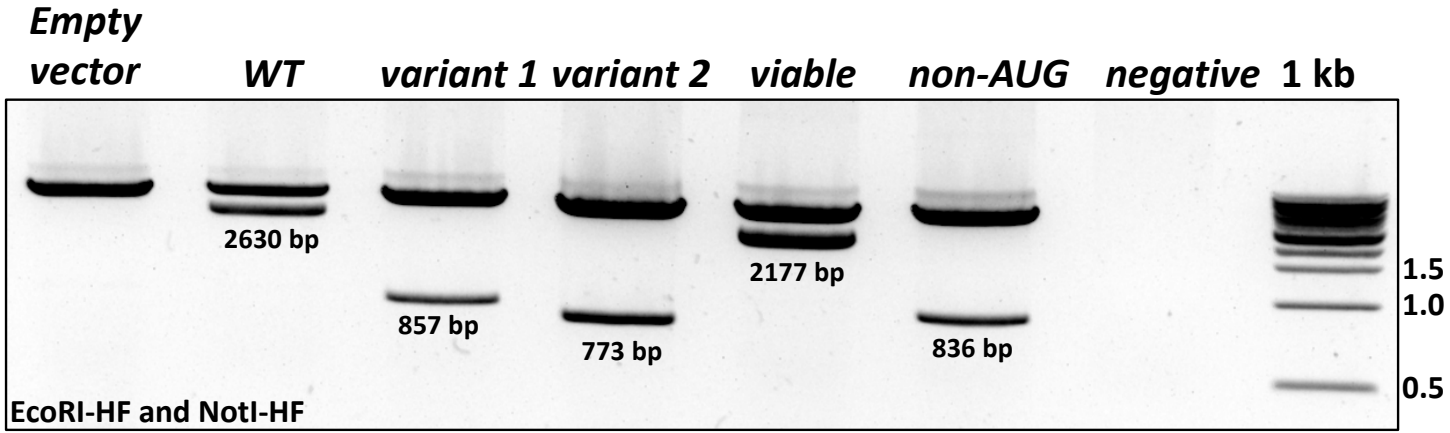


Variant 2; 29.6 kDa

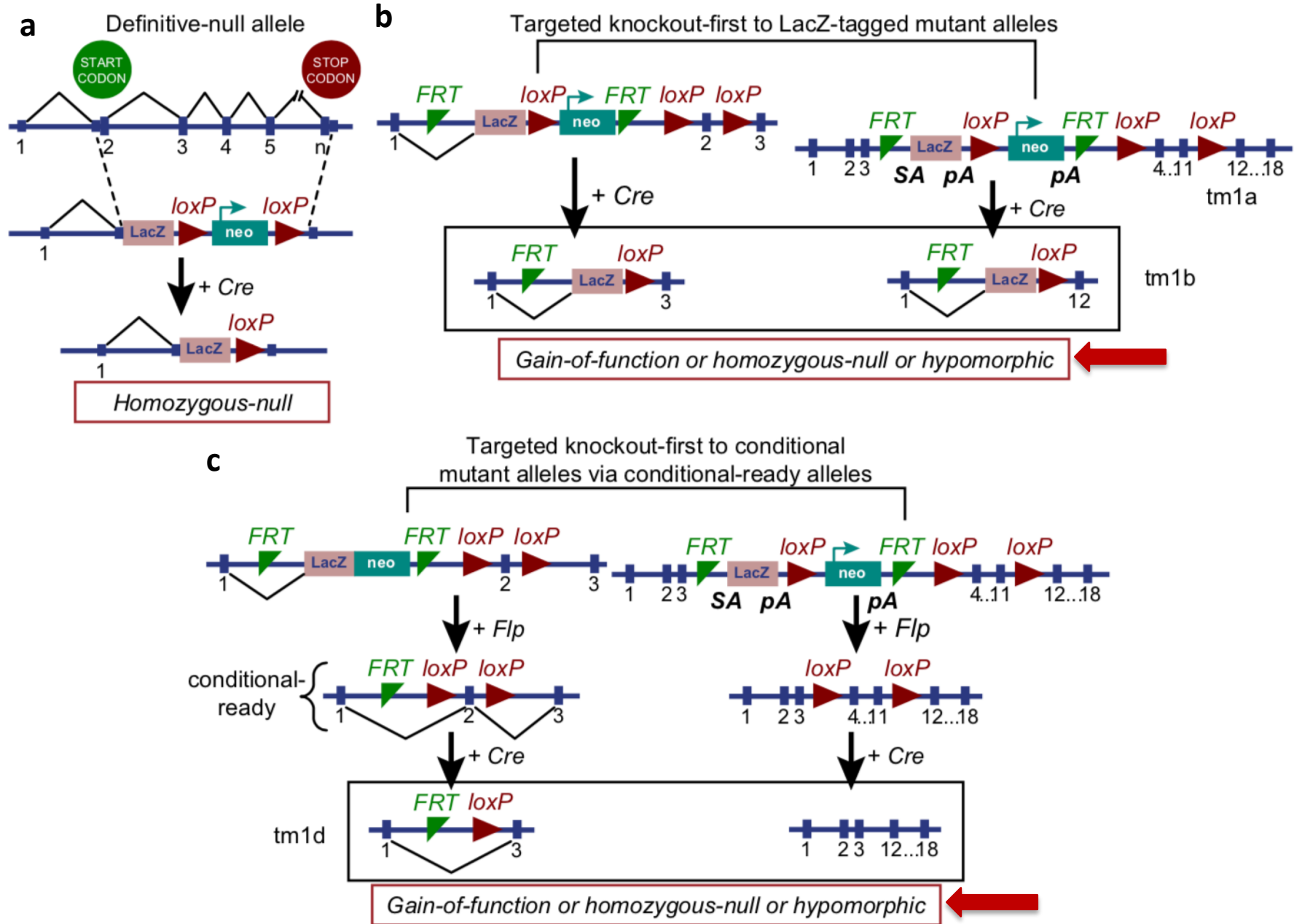
MRGYFHEEATLCSQVHCMDDVCGLLPFLNPEVDPDQFYRLWLSLFLHAGILHCLVSVCFQMTVLRDLEKLAGWHRIAIYLLSGITGNLASAIFLPYRAEVGPAG
 SQFGILACLFVELFQSWQILARPWRAFFKLLAVVLFLEAFGLLPWIDNFAHISGFVSGFLSFAFLPYISFGKFDLYRKRCQIIIFQVVFLGLLAGLVVLFYFYPVRC
 WCEFLTICIPFTDKFCEKYELDAQLH(EQKLISEEDLDYKDDDDK)



Supplementary Figure 13



Supplementary Figure 14



a

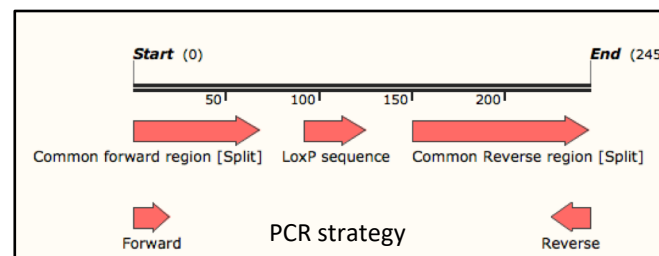
B6N(Cg)-*Rhbd1*^{tm1.1(KOMP)Vlcr/J}

Wt For	GCTCAGGTTTCAACCGACTC
Wt Rev	CAGGGAAGTCCACCATGTCT
Mt For	CGCTCCCAGTCCATTATTG
Mt Rev	CGGTCGCTACCATTACCACT

Taq polymerase PCR			
CYCLE STEP	CYCLES	TEMP	TIME
Initial denaturation	1	95°C	2 minutes
Denaturation	30	95°C	15 seconds
Annealing		62.5°C	30 seconds
Extension		68°C	2 minute
Final extension	1	72°C	5 minutes
Hold	1	4°C	∞

b

Rhbd2 KOMP allele



Forward	AGTAGCATCTCCCCGTGGAT
Reverse	AACCAATGGACCATGGACCC

Taq polymerase PCR			
CYCLE STEP	CYCLES	TEMP	TIME
Initial denaturation	1	95°C	2 minutes
Denaturation	30	95°C	15 seconds
Annealing		55°C	30 seconds
Extension		68°C	2 minute
Final extension	1	72°C	5 minutes
Hold	1	4°C	∞

