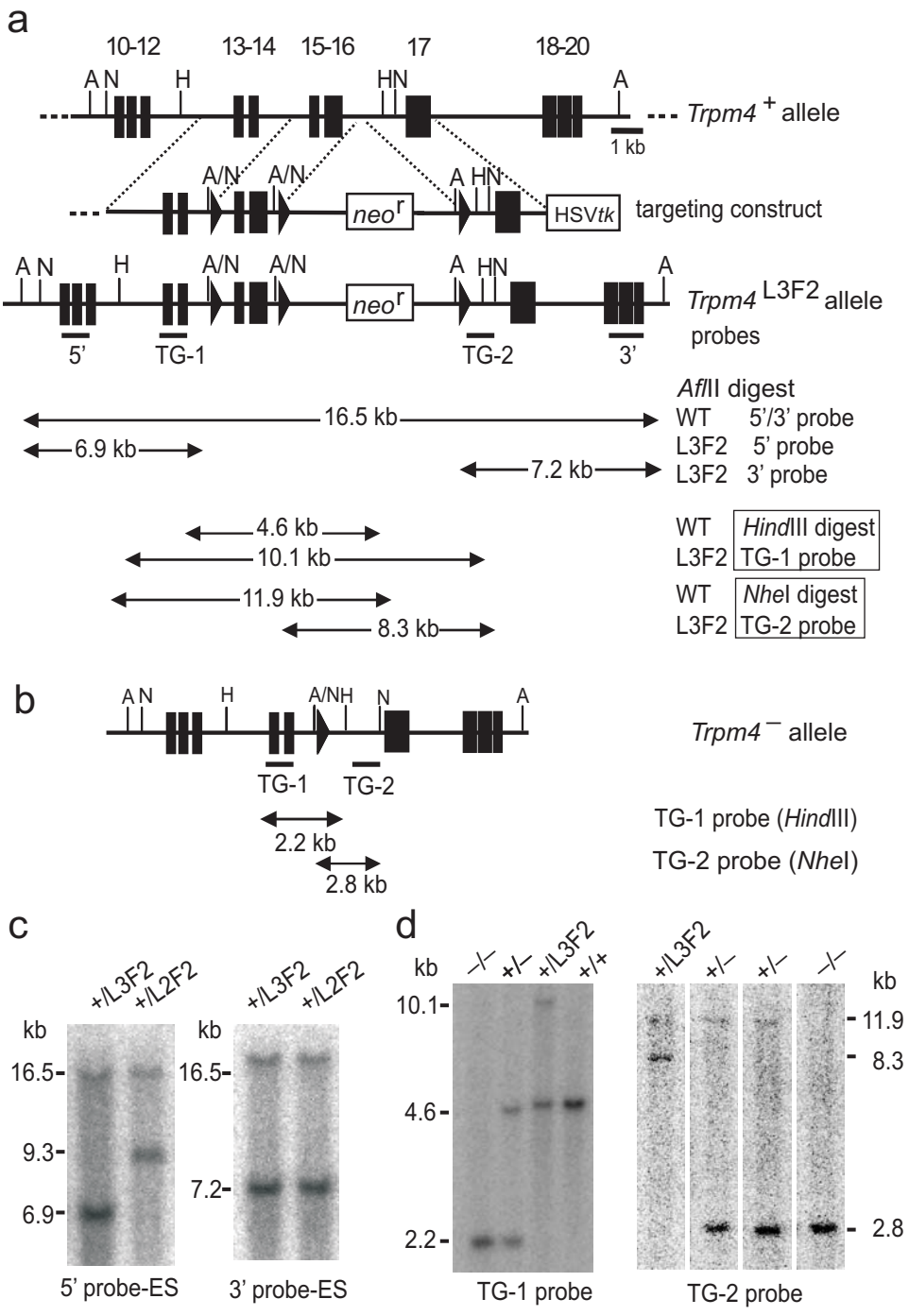
a
10-12
13-14
15-16
17
18-20
A N
H
HN
A
Trpm4 + allele
1 kb
A/N
A/N
A HN
neor
HSVtk
targeting construct
Trpm4 L3F2 allele
probes
5'
TG-1
TG-2
3'
AflII digest
16.5 kb
6.9 kb
7.2 kb
WT 5'/3' probe
L3F2 5' probe
L3F2 3' probe
4.6 kb
10.1 kb
11.9 kb
8.3 kb
WT HindIII digest
L3F2 TG-1 probe
WT NheI digest
L3F2 TG-2 probe
b
A/NH
N
Trpm4 − allele
2.2 kb
2.8 kb
TG-1 probe (HindIII)
TG-2 probe (NheI)
c
+/L3F2
+/L2F2
kb
16.5
9.3
6.9
7.2
5' probe-ES
3' probe-ES
d
−/−
+/−
+/+
10.1
4.6
2.2
11.9
8.3
2.8
TG-1 probe
TG-2 probe

Supplementary Figure 1. Targeted disruption of the *Trpm4* gene. We used the Cre-loxP strategy to excise exons 15 and 16, which encode the first transmembrane-spanning domain of TRPM4. (**a**) The wild type *Trpm4* allele, targeting construct and recombinant *Trpm4*^{L3F2} allele. Translated exons (not in scale) are shown as filled boxes. The *Trpm4*^{L3F2} allele contains one loxP site (filled triangles) upstream, and a loxP-flanked PGK-*neo*^{r} cassette downstream, of exon 16. H, *Hind*III; A, *Afl*II; N, *Nhe*I. Probes and sizes of genomic DNA fragments as expected by Southern blots are indicated. (**b**) Cre-mediated conversion of the *Trpm4*^{L3F2} allele to the *Trpm4*$^{-}$ allele in mice. (**c**) Identification of the recombinant *Trpm4*^{L3F2} and *Trpm4*^{L2F2} alleles in ES cells by Southern blot analysis (*Afl*II digest) using 5' and a 3' probes placed external to the targeted sequence. The *Trpm4*^{L2F2} allele may arise upon incomplete homologous recombination of the targeting construct and lacks the first loxP site located between exon 14 and 15 in the *Trpm4*^{L3F2} allele. (**d**) Cre-mediated generation of the *Trpm4*$^{-}$ allele in mice resulted in the conversion of the 10.1kb fragment of the *Trpm4*^{L3F2} allele to a 2.2kb fragment (TG-1 probe, *Hind*III digest) and the conversion of the 11.9kb fragment of the *Trpm4*^{L3F2} allele to a 2.8kb fragment (TG-2 probe, *Nhe*I digest).