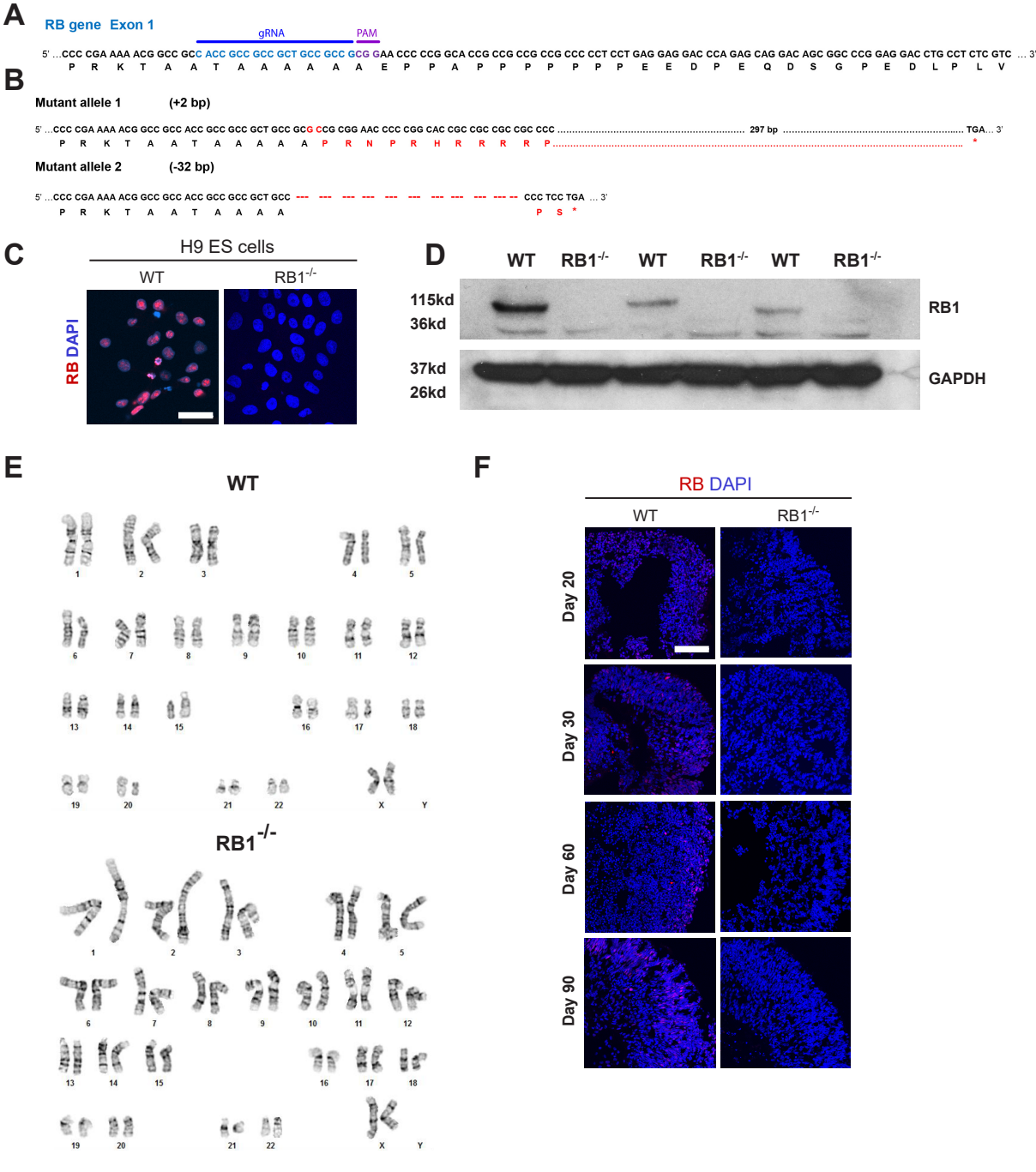
A
RB gene Exon 1
gRNA
PAM
5' ...CCC CGA AAA ACG GCC GCC ACC GCC GCC GCT GCC GCC GCG GAA CCC CCG GCA CCG CCG CCG CCG CCC CCT CCT GAG GAG GAC CCA GAG CAG GAC AGC GGC CCG GAG GAC CTG CCT CTC GTC ... 3'
P R K T A A T A A A A A A E P P A P P P P P P P E E D P E Q D S G P E D L P L V
B
Mutant allele 1 (+2 bp)
5' ...CCC CGA AAA ACG GCC GCC ACC GCC GCC GCT GCC GCG CCG CGG AAC CCC CGG CAC CGC CGC CGC CGC CCC ..... 297 bp ..... TGA... 3'
P R K T A A T A A A A A P R N P R H R R R R P ..... *
Mutant allele 2 (-32 bp)
5' ...CCC CGA AAA ACG GCC GCC ACC GCC GCC GCT GCC --- --- --- --- --- --- --- --- --- --- CCC TCC TGA ... 3'
P R K T A A T A A A A P S *
C
H9 ES cells
WT
RB1-/-
RB DAPI
D
WT
RB1-/-
WT
RB1-/-
WT
RB1-/-
115kd
36kd
RB1
37kd
26kd
GAPDH
E
WT
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
RB1-/-
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
F
RB DAPI
WT
RB1-/-
Day 20
Day 30
Day 60
Day 90