Table S1. **Mutations in EB genes from different clones**

Hela EB1/2/3mut EB1 control

EB1

```
  gRNA: TGGAAAAGACTATGACCCTG
AACTATGATGGAAAAGACTATGACCCTGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAAA
 N  Y  D  G  K  D  Y  D  P  V  A  A  R  Q  G  Q  E  T  A  V  A  P  S  L  V  A  P  A  L  N  K
```

```
Clone #1  Insertion: C   EB1 126AA +21AA after frameshift
AACTATGATGGAAAAGACTATGACCCCTGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAA
 N  Y  D  G  K  D  Y  D  P  C  G  C  Q  T  R  S  R  N  C  S  G  S  F  P  C  C  S  S  S  E  Stop
```

```
Clone #1 Deletion:    C   EB1 125AA +20AA after frameshift
AACTATGATGGAAAAGACTATGACC-TGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAAA
 N  Y  D  G  K  D  Y  D   L  W  L  P  D  K  V  K  K  L  Q  W  L  L  P  L  L  L  Q  L  Stop
```

```
Clone #2 Deletion: TAGC   EB1 123AA +21AA after frameshift
AACTATGATGGAAAAGAC----TACCTGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAAA
 N  Y  D  G  K  D      Y  L  W  L  P  D  K  V  K  K  L  Q  W  L  L  P  L  L  L  Q  L  Stop
```

```
Clone #2 Deletion: C   EB1   125AA +20AA after frameshift
AACTATGATGGAAAAGACTATGACC-TGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAAA
 N  Y  D  G  K  D  Y  D   L  W  L  P  D  K  V  K  K  L  Q  W  L  L  P  L  L  L  Q  L  Stop
```

EB2

```
   gRNA:CCGGAAGCACACAGTGCGCG
ATTCCTTTCCGGAAGCACACAGTGCGCGGGGAGCGTTCCTACAG(intron)
 I  P  F  R  K  H  T  V  R  G  E  R  S  Y
```

```
Clone #1 Deletion: GCGGGGAGCG   EB2 34AA +15AA after frameshift
ATTCCTTTCCGGAAGCACACAGTGC----------TTCCTACAG(intron)
 I  P  F  R  K  H  T  V           L  P  T
```

```
Clone #1Deletion: CACACAGTGCGCGGGGAGCGTTCCTACAG   EB2 31AA +20AA after frameshift
ATTCCTTTCCGGAAG----------------------------(intron)
 I  P  F  R  K
```

```
Clone #2 Insertion: C   EB2 34AA +293 AA after frameshift
ATTCCTTTCCGGAAGCACACAGTGCCGCGGGGAGCGTTCCTACAG(intron)
 I  P  F  R  K  H  T  V  P  R  G  A  F  L  Q
```

```
Clone #2 Deletion: CAGTGCGCGGGGAGCGTTCCTA  EB2 32AA +13AA after frameshift
ATTCCTTTCCGGAAGCACA----------------------CAG(intron)
 I  P  F  R  K  H                        T
```

EB3

```
          gRNA: TGCACCTCAACTATACCAAG
TGGGTCAACGACTCCCTGCACCTCAACTATACCAAGATAGAACAGCTTTGTTCAG(intron)
 W  V  N  D  S  L  H  L  N  Y  T  K  I  E  Q  L  C  S
```

```
Clone #1 Deletion: GACTCCCTGCACCTCAAC   EB3 Deletion of 6AA in CH domain
TGGGTCAAC------------------TATACCAAGATAGAACAGCTTTGTTCAG(intron)
 W  V  N                    Y  T  K  I  E  Q  L  C  S
```

```
Clone #2 Insertion: A    EB3 28AA +59AA after frameshift
TGGGTCAACGACTCCCTGCAACCTCAACTATACCAAGATAGAACAGCTTTGTTCAG(intron)
 W  V  N  D  S  L  Q  P  Q  L  Y  Q  D  R  T  A  L  F
```

RPE EB1/3mut

EB1

```
  gRNA: TGGAAAAGACTATGACCCTG
AACTATGATGGAAAAGACTATGACCCTGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAAA
 N  Y  D  G  K  D  Y  D  P  V  A  A  R  Q  G  Q  E  T  A  V  A  P  S  L  V  A  P  A  L  N  K
Deletion: C EB1 125AA +20AA after frameshift
AACTATGATGGAAAAGACTATGACC-TGTGGCTGCCAGACAAGGTCAAGAAACTGCAGTGGCTCCTTCCCTTGTTGCTCCAGCTCTGAATAAA
 N  Y  D  G  K  D  Y  D   L  W  L  P  D  K  V  K  K  L  Q  W  L  L  P  L  L  L  Q  L  Stop
```

EB2

```
  gRNA: CCGGAAGCACACAGTGCGCG
ATTCCTTTCCGGAAGCACACAGTGCGCGGGGAGCGTTCCTACAG(intron)
 I  P  F  R  K  H  T  V  R  G  E  R  S  Y
Insertion: A   EB2 34AA +27AA after frameshift
ATTCCTTTCCGGAAGCACACAGTGCAGCGGGGAGCGTTCCTACAG(intron)
 I  P  F  R  K  H  T  V  Q  R  G  A  F  L  Q
Insertion: C   EB2 34AA +27AA after frameshift
ATTCCTTTCCGGAAGCACACAGTGCCGCGGGGAGCGTTCCTACAG(intron)
 I  P  F  R  K  H  T  V  P  R  G  A  F  L  Q
```

EB3

```
          gRNA: TGCACCTCAACTATACCAAG
TGGGTCAACGACTCCCTGCACCTCAACTATACCAAGATAGAACAGCTTTGTTCAG(intron)
 W  V  N  D  S  L  H  L  N  Y  T  K  I  E  Q  L  C  S
Insertion: A   EB3 28AA +59AA after frameshift
TGGGTCAACGACTCCCTGCAACCTCAACTATACCAAGATAGAACAGCTTTGTTCAG(intron)
 W  V  N  D  S  L  Q  P  Q  L  Y  Q  D  R  T  A  L  F
```

For each gene, the gRNA target sequence, the WT sequence, and its translation are shown in black, deletions are indicated by dashes, and insertions and amino acids resulting from frameshifts are shown in red. Parentheses indicate start of an intron. Note that in HeLa1/2/3mut clone 1, a small in-frame deletion is present in the EB3 CH domain, but no EB3 reactivity is detected in this clone with anti-EB3 rabbit polyclonal antibodies. All the different allelic variants of EB-encoding genes recovered from each clone are shown.