Figure S1 (A)

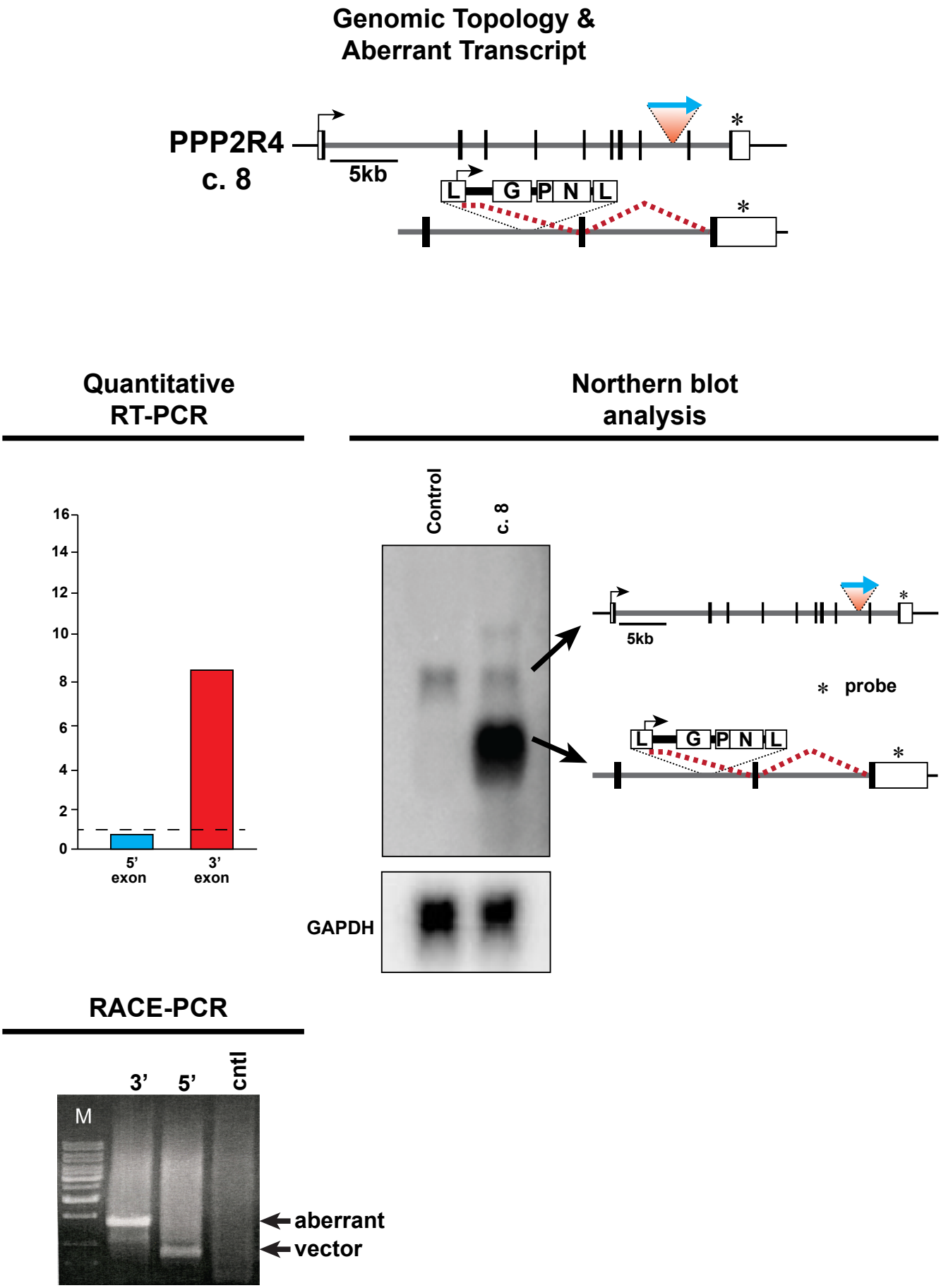
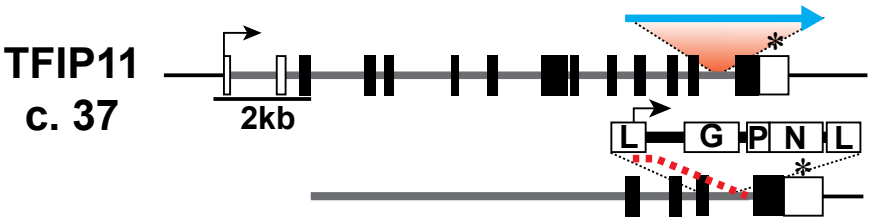

Genomic Topology &
Aberrant Transcript
PPP2R4
c. 8
5kb
L
G
P
N
L
*
Quantitative
RT-PCR
16
14
12
10
8
6
4
2
0
5'
exon
3'
exon
Northern blot
analysis
Control
c. 8
5kb
* probe
GAPDH
RACE-PCR
3'
5'
cntl
M
aberrant
vector

Figure S1 (B)

Genomic Topology & Aberrant Transcript



Quantitative RT-PCR

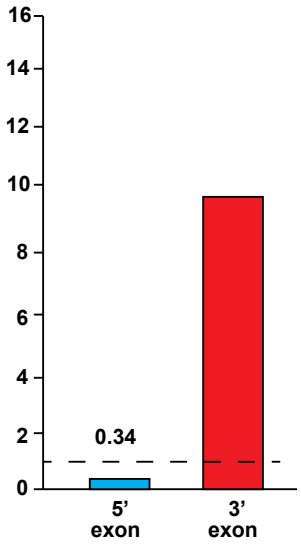


Figure S1 (C)

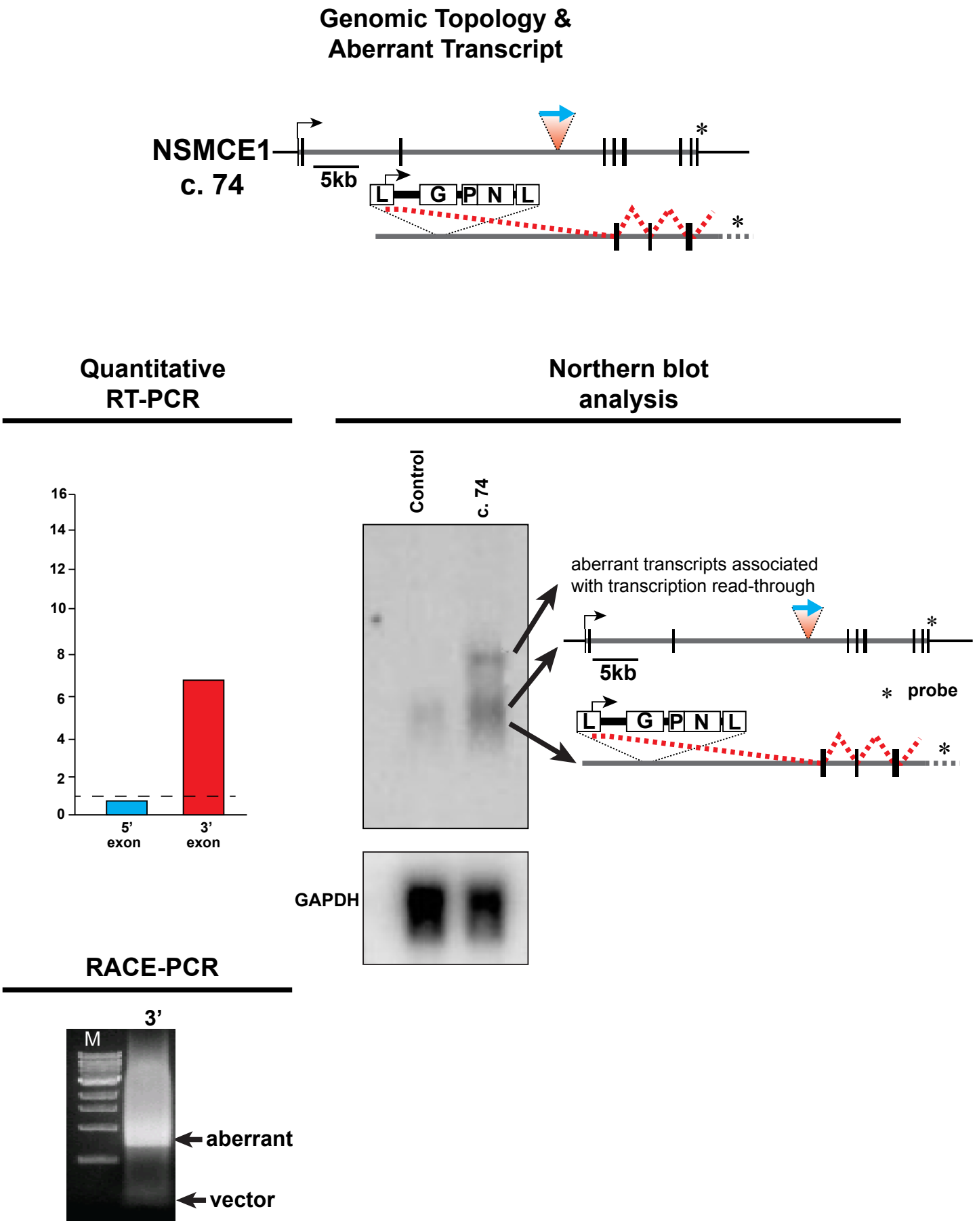

Genomic Topology &
Aberrant Transcript
NSMCE1
c. 74
5kb
L
G
P
N
L
*
*
Quantitative
RT-PCR
16
14
12
10
8
6
4
2
0
5'
exon
3'
exon
Northern blot
analysis
Control
c. 74
aberrant transcripts associated
with transcription read-through
5kb
* probe
L
G
P
N
L
*
*
GAPDH
RACE-PCR
3'
M
aberrant
vector

Figure S1 (D)

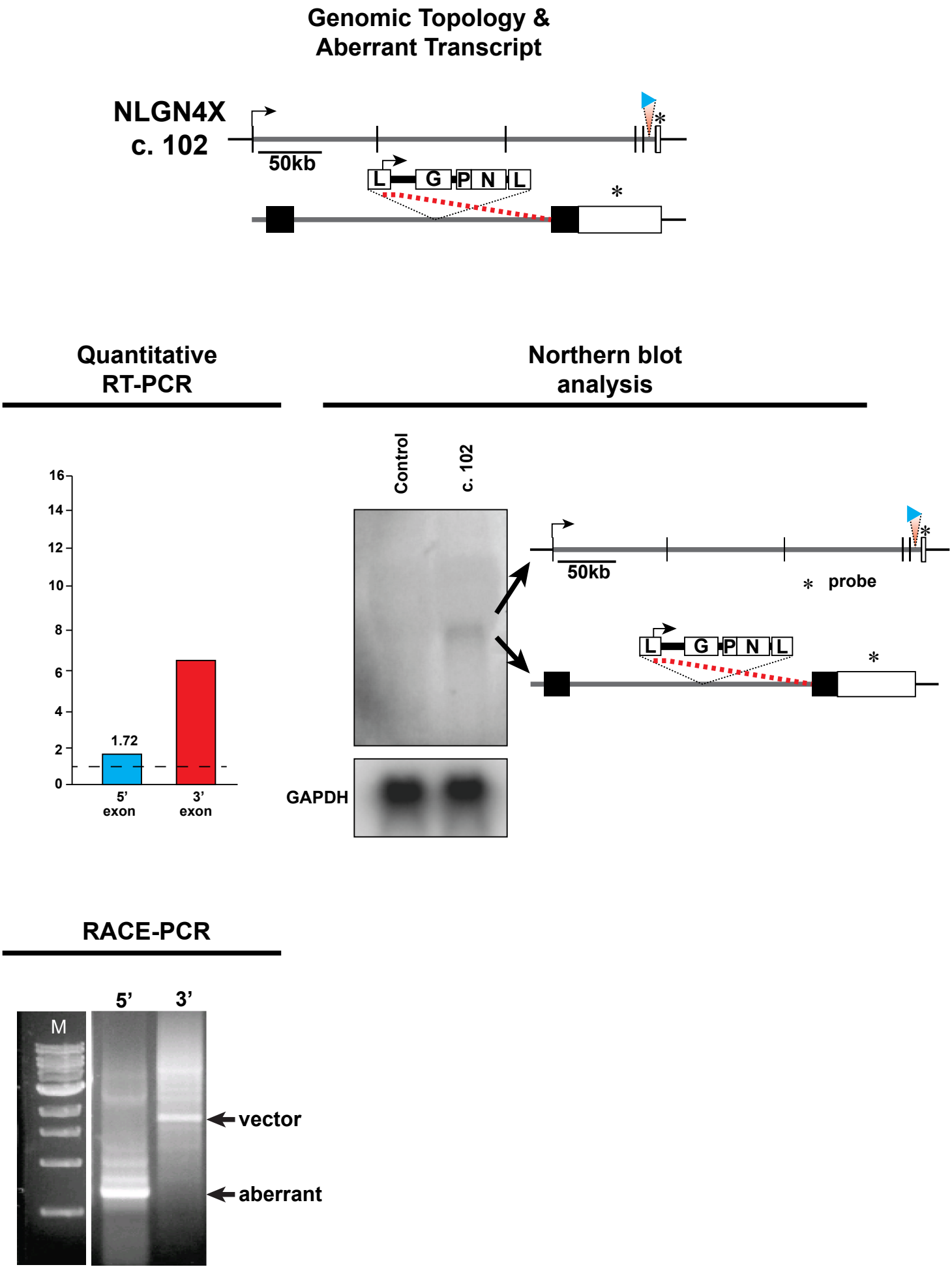

Genomic Topology &
Aberrant Transcript
NLGN4X
c. 102
50kb
L
G
P
N
L
*
Quantitative
RT-PCR
16
14
12
10
8
6
4
2
0
1.72
5'
exon
3'
exon
Northern blot
analysis
Control
c. 102
50kb
* probe
L
G
P
N
L
*
GAPDH
RACE-PCR
5'
3'
M
vector
aberrant

Figure S1 (E)

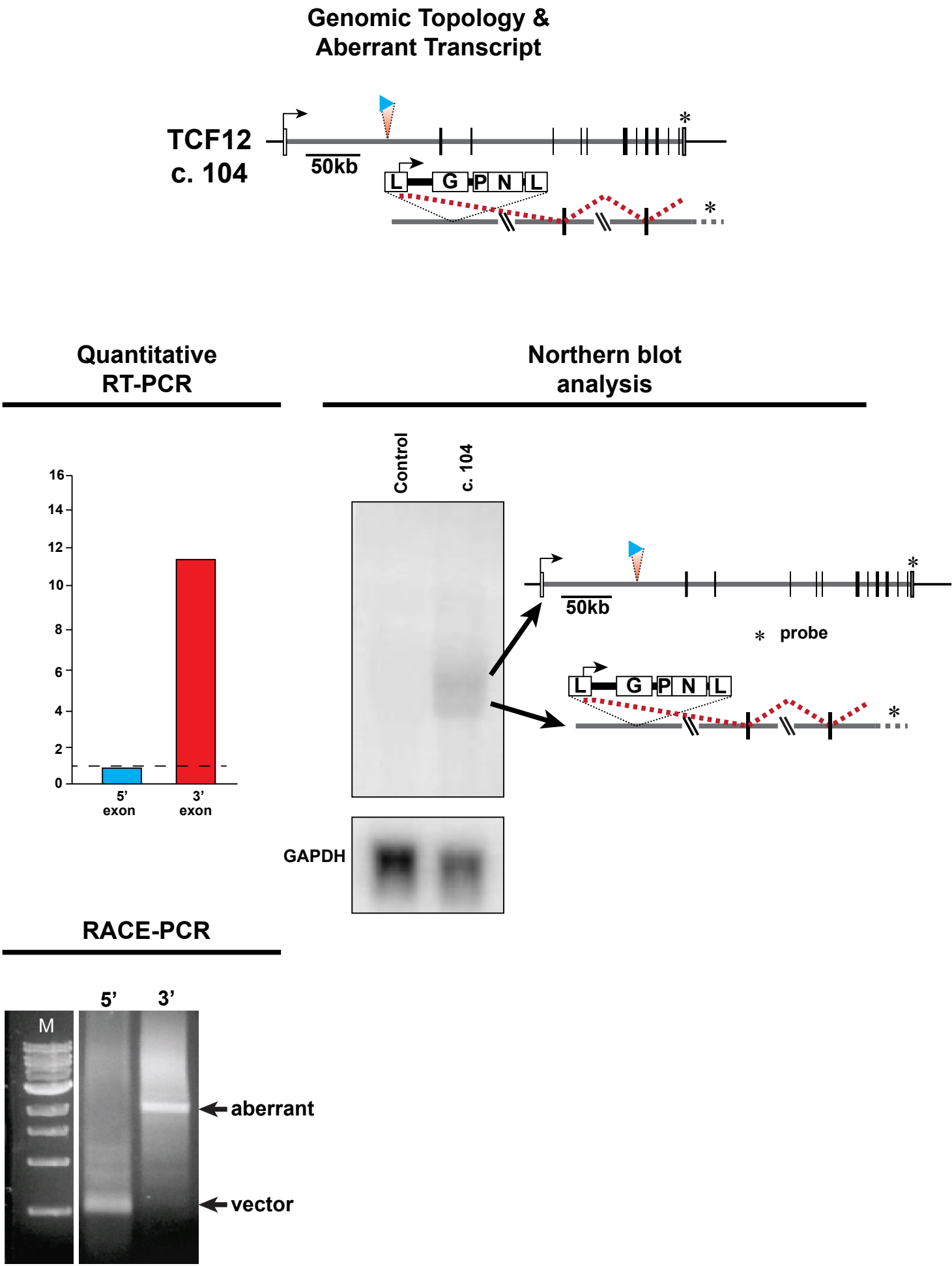

Genomic Topology &
Aberrant Transcript
TCF12
c. 104
50kb
L
G
P
N
L
*
Quantitative
RT-PCR
16
14
12
10
8
6
4
2
0
5'
exon
3'
exon
Northern blot
analysis
Control
c. 104
50kb
* probe
GAPDH
RACE-PCR
5'
3'
M
aberrant
vector

Figure S1 (F)

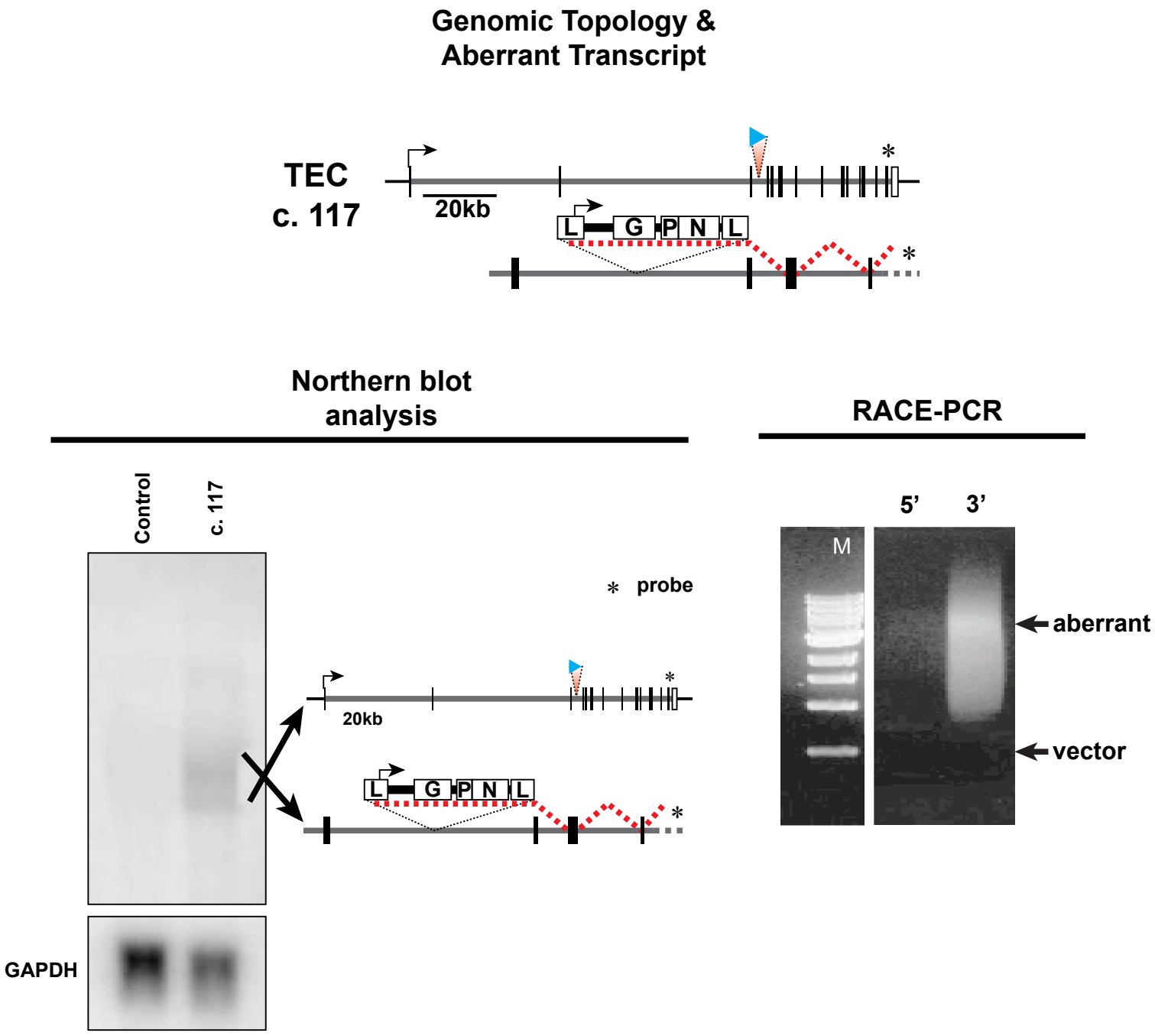

Genomic Topology &
Aberrant Transcript
TEC
c. 117
20kb
L
G
P
N
L
*
Northern blot
analysis
RACE-PCR
Control
c. 117
* probe
20kb
GAPDH
5'
3'
M
aberrant
vector

Figure S1 (G)

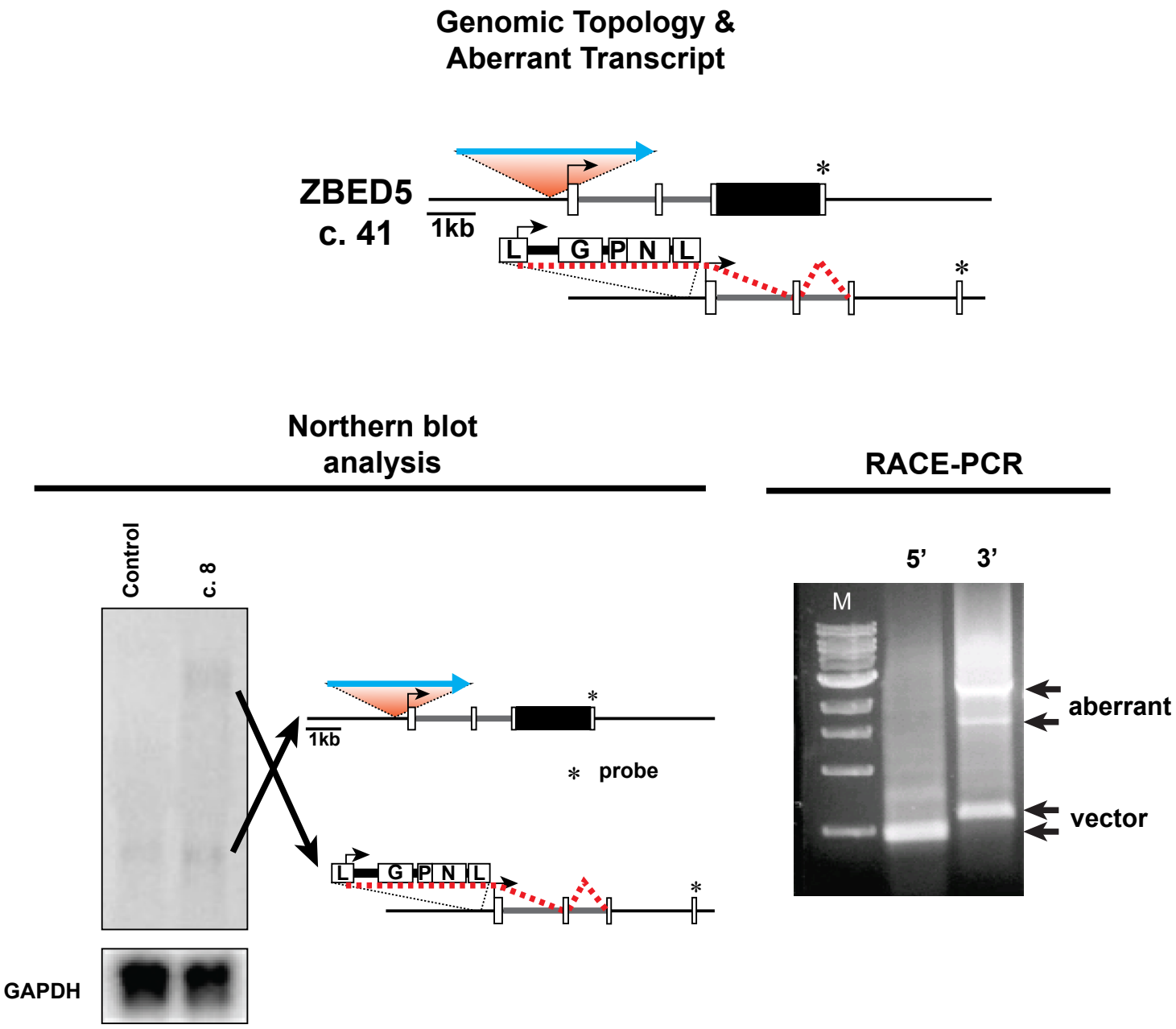

Genomic Topology &
Aberrant Transcript
ZBED5
c. 41
1kb
L
G
P
N
L
*
*
Northern blot
analysis
RACE-PCR
Control
c. 8
1kb
*
* probe
L
G
P
N
L
*
GAPDH
5'
3'
M
aberrant
vector

Figure S1 (H)

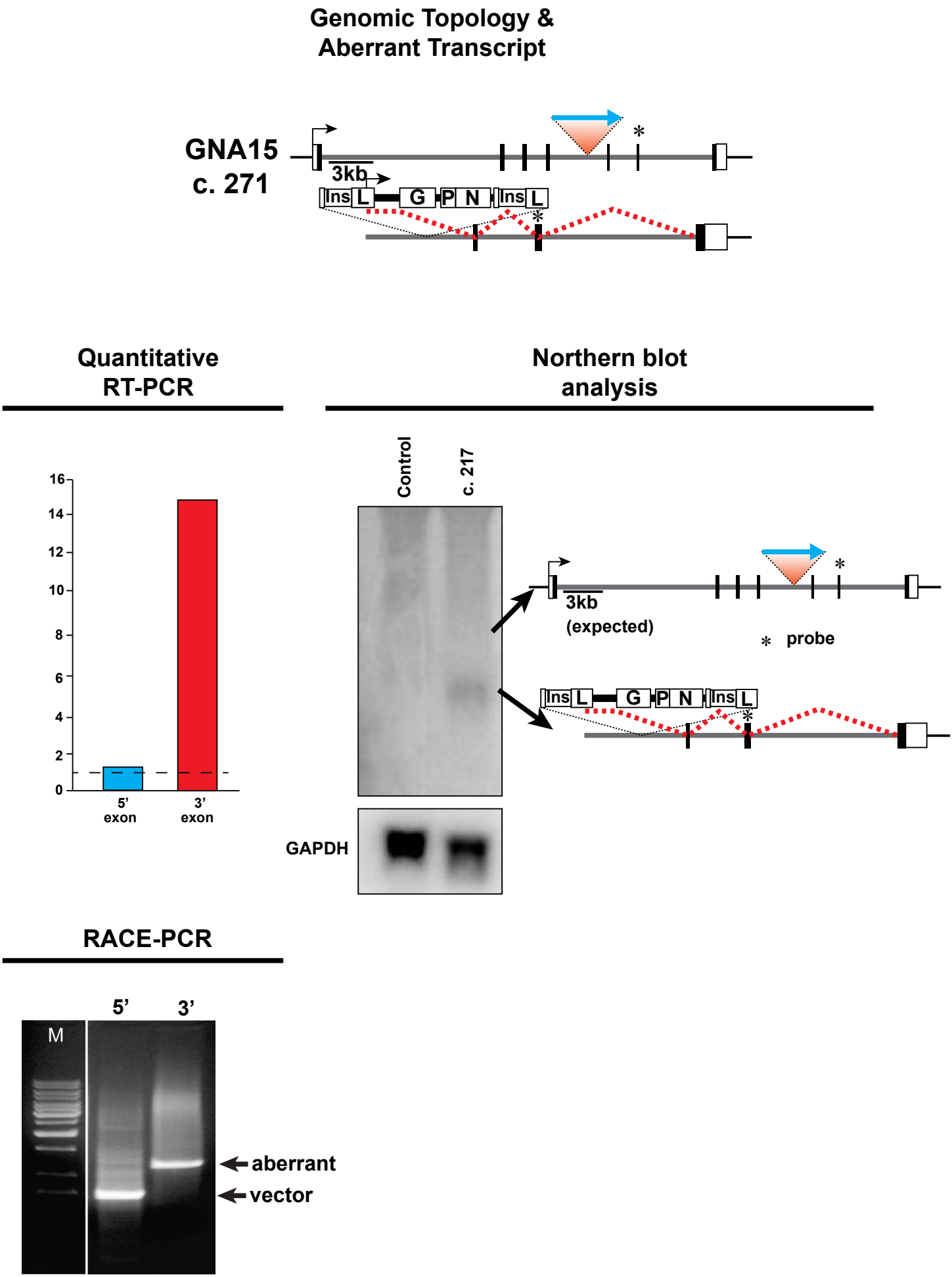
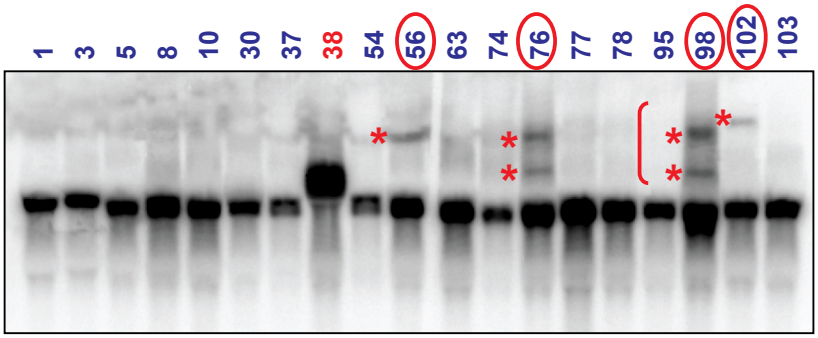
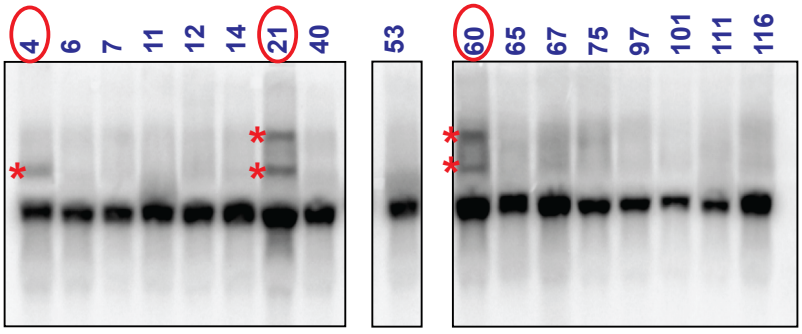
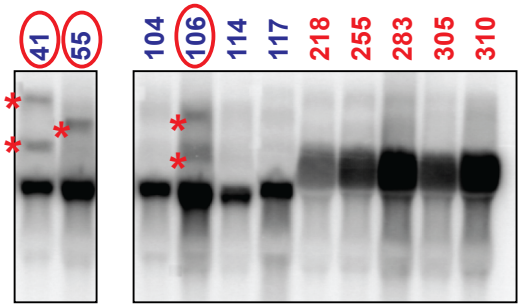
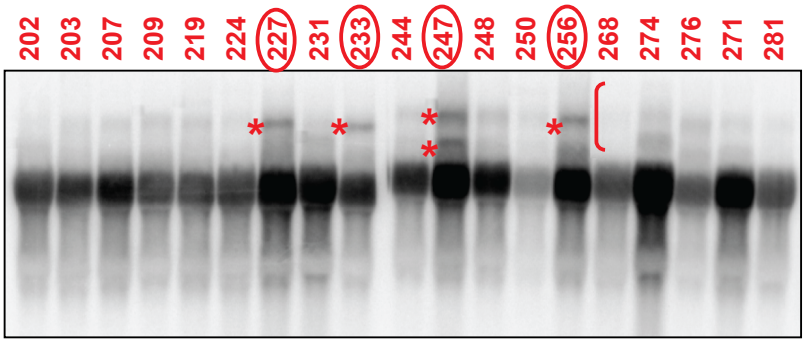

Genomic Topology &
Aberrant Transcript
GNA15
c. 271
3kb
Ins L G P N Ins L
*
Quantitative
RT-PCR
16
14
12
10
8
6
4
2
0
5'
exon
3'
exon
Northern blot
analysis
Control
c. 217
3kb
(expected)
* probe
Ins L G P N Ins L
GAPDH
RACE-PCR
5'
3'
M
aberrant
vector

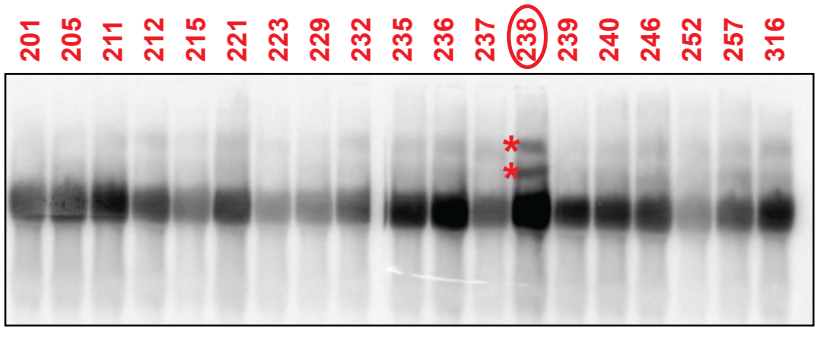
Figure S2

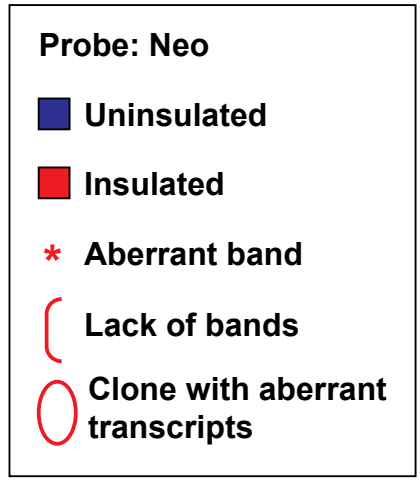

1
3
5
8
10
30
37
38
54
56
63
74
76
77
78
95
98
102
103


4
6
7
11
12
14
21
40
53
60
65
67
75
97
101
111
116


41
55
104
106
114
117
218
255
283
305
310


202
203
207
209
219
224
227
231
233
244
247
248
250
256
268
274
276
271
281


201
205
211
212
215
221
223
229
232
235
236
237
238
239
240
246
252
257
316


Probe: Neo
Uninsulated
Insulated
Aberrant band
Lack of bands
Clone with aberrant transcripts

Supplementary Figure Legends

Figure S1 Analysis of aberrant vector transcription in association with dysregulated cellular genes. Data are presented in support of the aberrant vector transcription associated with dysregulated cellular genes diagrammed in Figure1. Data is presented for (A)the PPP2R4 gene in HT1080 clone 8, (B)the TFIP11 gene in HT1080 clone 37, (C) the NSMCE1 gene in HT1080 clone 74, (D)the NLGN4X gene in HT1080 clone 102, (E) the TCF12 gene in HT1080 clone 104, (F)the TEC gene in HT1080 clone 117, (G)the ZBED5 gene in HT1080 clone 41, and (H)the GNA15 gene in HT1080 clone 271. For each gene/clone combination, the genomic topology and aberrant transcript is shown (derived from Figure 1), along with one or more sources of supporting data, including: quantitative real-time RT-PCR for cellular gene expression 5' and 3' of the VIS; Northern blotting; and RACE-PCR. For the quantitative real-time RT-PCR, the relative expression of cellular gene exons located 5' and 3' of the VIS was assess using RNA from the specific HT1080 cell clones. Signal intensities were normalized to a loading control (the housekeeping gene GAPDH), and are reported as fold-expression versus equivalent signals from untransduced HT1080 cells used as a control. For Northern analysis, blots were prepared using RNA from the specific HT1080 cell clones anduntransduced HT1080 cells as a negative control (control). Each blot was hybridizedwith a probe specific for the dysregulated cellular gene, and imaged by phosphoimager. The specific probes were derived from the same gene segments included in the expression microarrays used to first identify the dysregulated cellular genes. The blots were subsequently stripped and rehybridized with a probe for the housekeeping gene GAPDH as a loading control. For RACE-PCR analysis, RNA was prepared from the specific HT1080 cell clones and untransduced HT1080 cells as a negative control (cntl.). 5' RACE was performed using an anchor primer located in the ψpackaging sight, while 3' RACE was performed using a primer immediately proximal to the 3' LTR (as for Figure 3b). Expected vector and unexpected aberrant extension products are indicated.

Figure S2 Detection of aberrant vector transcripts by Northern blot analysis of HT1080 clones containing multiple VISs. Northern blots were prepared using RNA from a total of 85 HT1080 cell clones containing multiple copies of the uninsulated vector MGPN2 (clones indicated in red type face, n=41) and this same vector flanked with the cHS4 insulator (blue type face, n=44). These were hybridized with a probe for Neo. The presence of aberrant transcripts (or lack of expected transcripts) are indicated in red. See Table 2 for a summary of results.