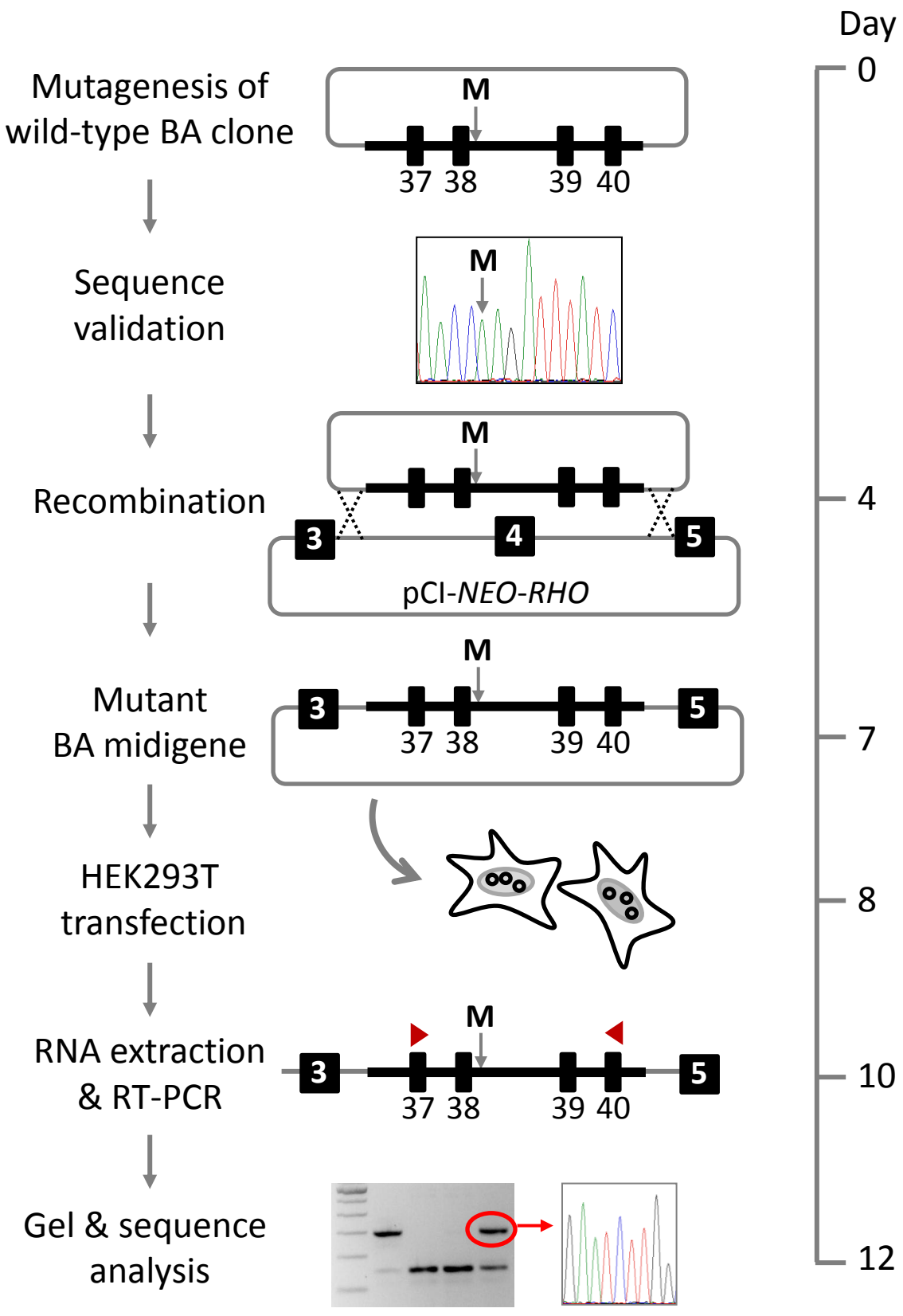
Supplemental Figure S1

Supplemental Figure S1. Schematic representation of the midigene assay workflow. The main steps are shown on the time line scale at the right side. To assess the effect of any selected non canonical splice site variant indicated as “M”, site-directed mutagenesis was performed on the respective wild-type BA clone to generate the mutant construct. After sequence validation, the mutant clone was recombined with the home made expression vector pCI-NEO-*RHO* and the resulting construct transfected in HEK293T cells. Forty-eight hours post-transfection, cells were harvested following total RNA extraction and subjected to RT-PCR analysis using *ABCA4* primers, indicated as red triangles. The RT-PCR products were analyzed using agarose gel electrophoresis and validated by Sanger sequencing.


Day
0
4
7
8
10
12
Mutagenesis of wild-type BA clone
M
37 38 39 40
Sequence validation
M
Recombination
M
3
4
5
pCI-NEO-RHO
Mutant BA midigene
M
3
5
37 38 39 40
HEK293T transfection
RNA extraction & RT-PCR
M
3
5
37 38 39 40
Gel & sequence analysis