Cell Reports, Volume *25*

Supplemental Information

Microcephaly Modeling of Kinetochore Mutation Reveals a Brain-Specific Phenotype

Attya Omer Javed, Yun Li, Julien Muffat, Kuan-Chung Su, Malkiel A. Cohen, Tenzin Lungjangwa, Patrick Aubourg, Iain M. Cheeseman, and Rudolf Jaenisch

SUPPLEMENTAL FIGURES TITLES AND LEGENDS

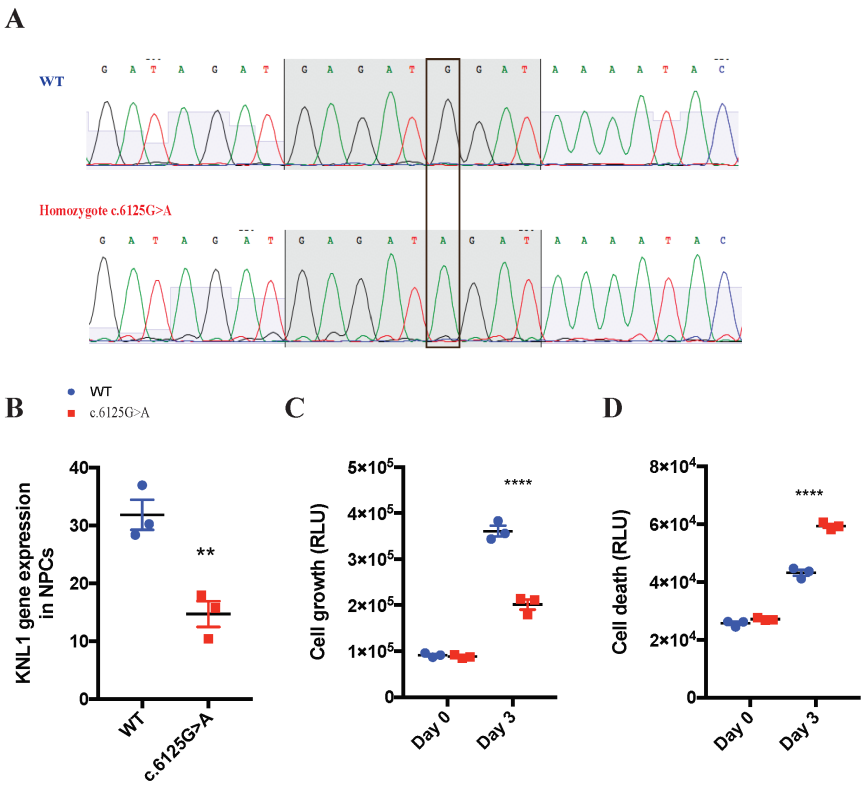

Figure S1: Reduced KNL1 expression, cell growth and increased cell death are detected in KNL1$^{c.6125G>A}$ neural progenitors, derived from an iPSC line, related to Figures 1 and 2

(A) Sanger sequencing on targeted iPSC clones, confirming a homozygous patient point mutation.

(B) Quantitative RT-PCR analysis of *KNL1* expression in neural progenitors, derived from an iPSC line, normalized to *GAPDH*. Welch's t test (two-tailed) was applied.

(C) ATP assay, represented in Relative Light Units, on a 2-D adherent neural progenitor culture, day 0 represent the plating day. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

(D) Apoptosis assay in WT and KNL1$^{c.6125G>A}$ neural progenitors, based on Caspase-3/7 activity. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

One non-targeted wild type clone, two wild-type clones and three patient mutation clones derived from the same CRISPR-Cas9 targeting are plotted in each graph. Results are mean +/- SEM. **p<0.01, ****p<0.0001.

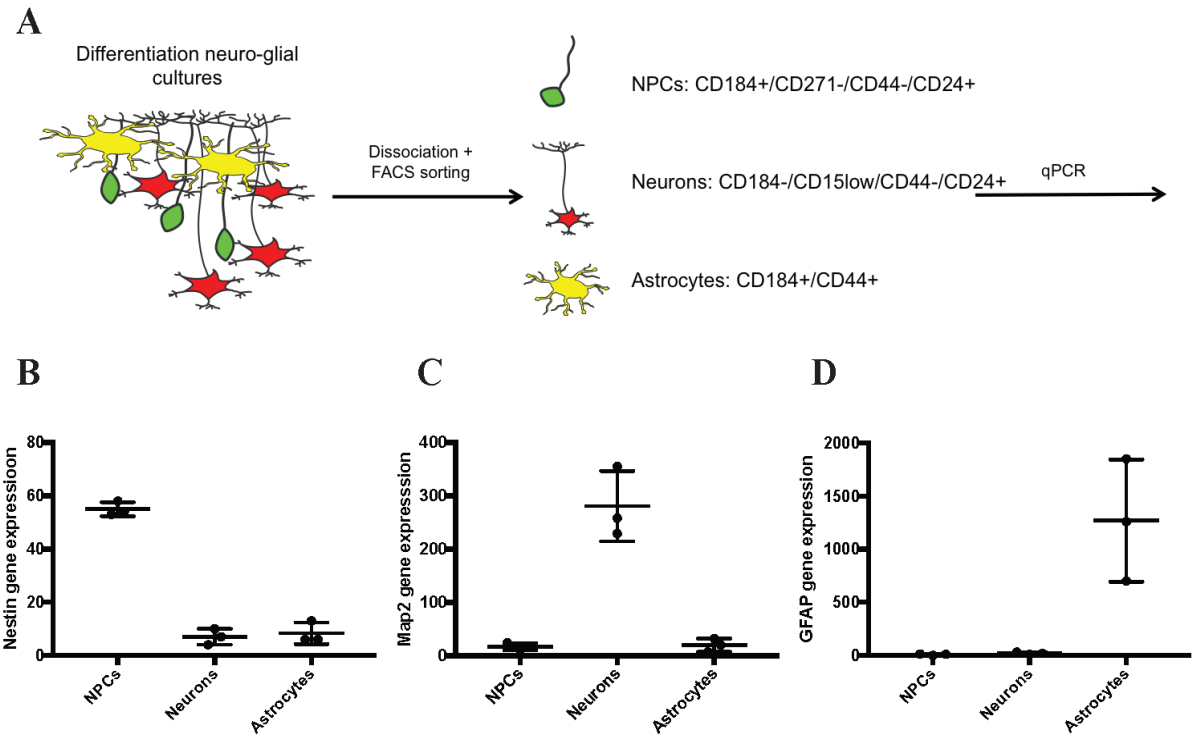

Figure S2: Characterization of neural progenitors, neurons and astrocytes cell type sorted using FACS, based on cell surface markers, related to Figure 4

(A) Cell surface marker used to isolate neural progenitors, neurons and astrocytes in neuro-glial 2D cultures

(B-D) Quantitative RT-PCR analysis of *NESTIN* (B), *MAP2* (C) and *GFAP* (D) expression, normalized to *GAPDH*, in the three populations identified as neural progenitors, neurons, astrocytes to confirm their identity.

One non-targeted wild type clone and two wild type clones derived from a CRISPR-Cas9 targeting are plotted in each graph. Results are mean +/- SEM.

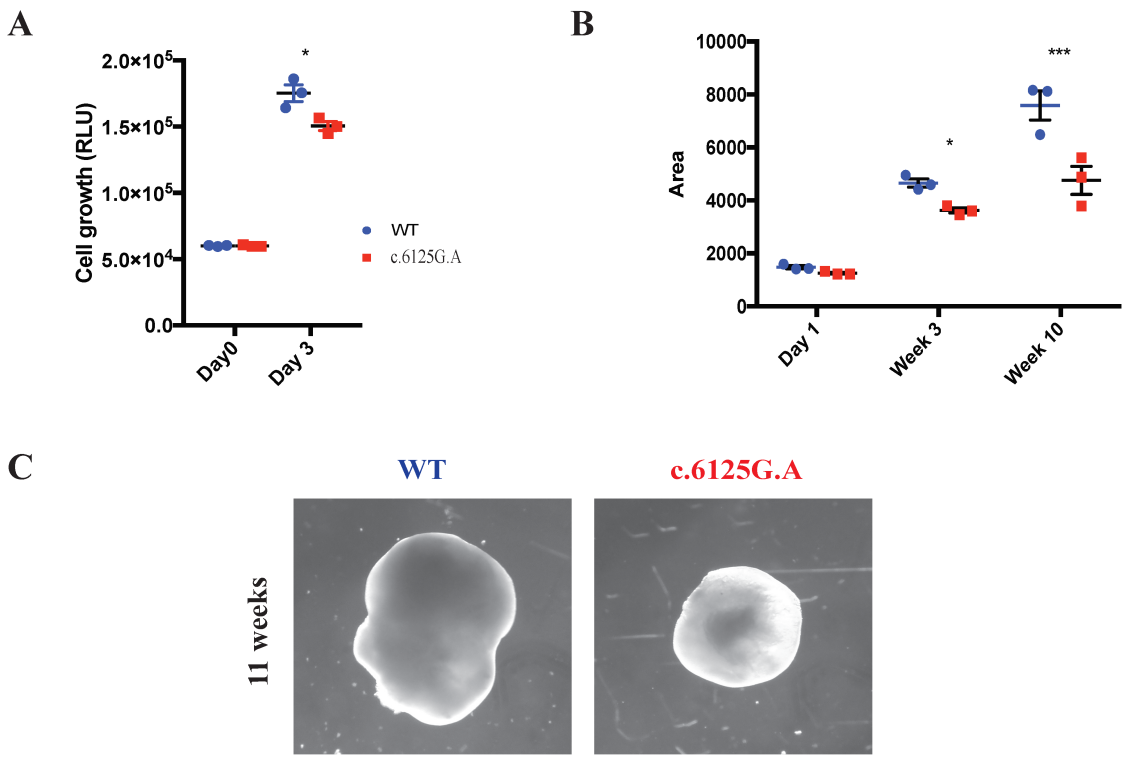

Figure S3: Altered cell growth in KNL1$^{c.6125G>A}$ hESCs, leading to smaller cerebral organoids, related to Figure 5

(A) ATP assay on a 2-D adherent hESC culture. A statistical ANOVA analysis was performed followed by a post hoc group comparisons using Bonferroni test.

(B) Area measurement of WT and KNL1$^{c.6125G>A}$ cerebral organoids in a time course of 10 weeks. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

(C) WT and KNL1$^{c.6125G>A}$ cerebral organoids KNL1 organoids at 11 weeks.

One non-targeted wild type clone, two wild type clones and three patient mutation clones derived from the same CRISPR-Cas9 targeting are plotted in each graph. Results are mean +/- SEM. *p<0.05, ***p<0.001.

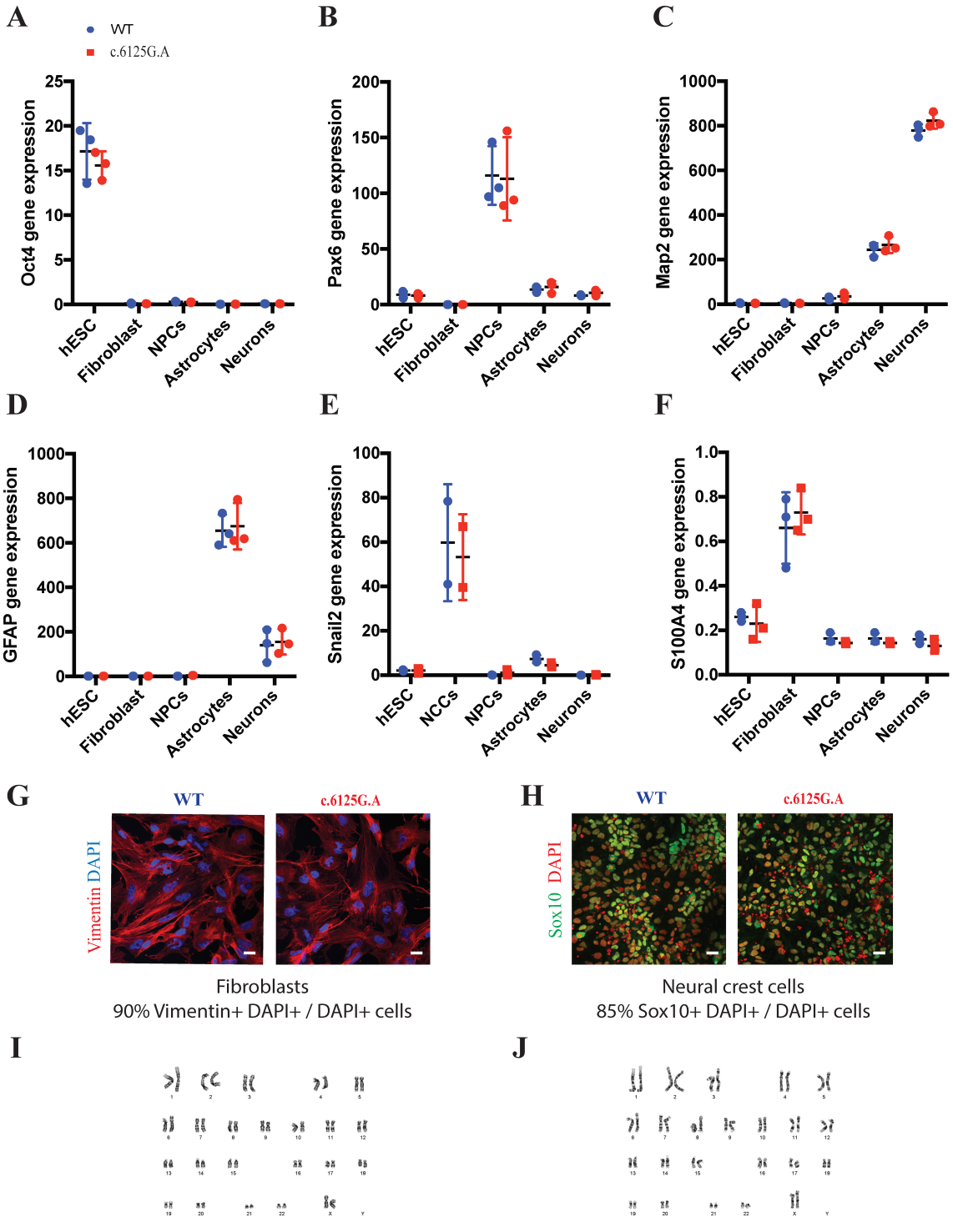
A
WT
c.6125G.A
Oct4 gene expression
hESC
Fibroblast
NPCs
Astrocytes
Neurons
B
Pax6 gene expression
C
Map2 gene expression
D
GFAP gene expression
E
Snail2 gene expression
NCCs
F
S100A4 gene expression
G
WT
c.6125G.A
Vimentin DAPI
Fibroblasts
90% Vimentin+ DAPI+ / DAPI+ cells
H
Sox10 DAPI
Neural crest cells
85% Sox10+ DAPI+ / DAPI+ cells
I
J

Figure S4: Characterization of neural progenitors, neural crest cells, neurons, astrocytes and fibroblasts cell type using immunostaining and quantitative RT-PCR, related to Figure 7

(A-E) Cell type analysis using hESC marker *OCT4* (A), neural progenitors marker *PAX6* (B); neuronal marker *MAP2* (C); glial marker *GFAP* (D); neural crest marker *SNAIL2* (E) and fibroblast marker *S100A4* (F) using quantitative RT-PCR in WT and KNL1$^{c.6125G>A}$ neural progenitors, neural crest cells, neurons, astrocytes and fibroblasts.

(G) Immunostaining using antibodies against Vimentin (red) on WT and KNL1$^{c.6125G>A}$ fibroblast at 1-month post-differentiation (Scale bar 25 μm).

(H) Immunostaining using antibodies against SOX10 (green) on WT and KNL1$^{c.6125G>A}$ neural crest cells at 20 days post-differentiation (Scale bar 25 μm).

(I) Karyotype analysis of KNL1$^{c.6125G>A}$ fibroblasts.

(J) Karyotype analysis of KNL1$^{c.6125G>A}$ neural crest cells.

One non-targeted wild type clone, two wild type clones and three patient mutation clones derived from the same CRISPR-Cas9 targeting are plotted in each graph. Results are mean +/- SEM.

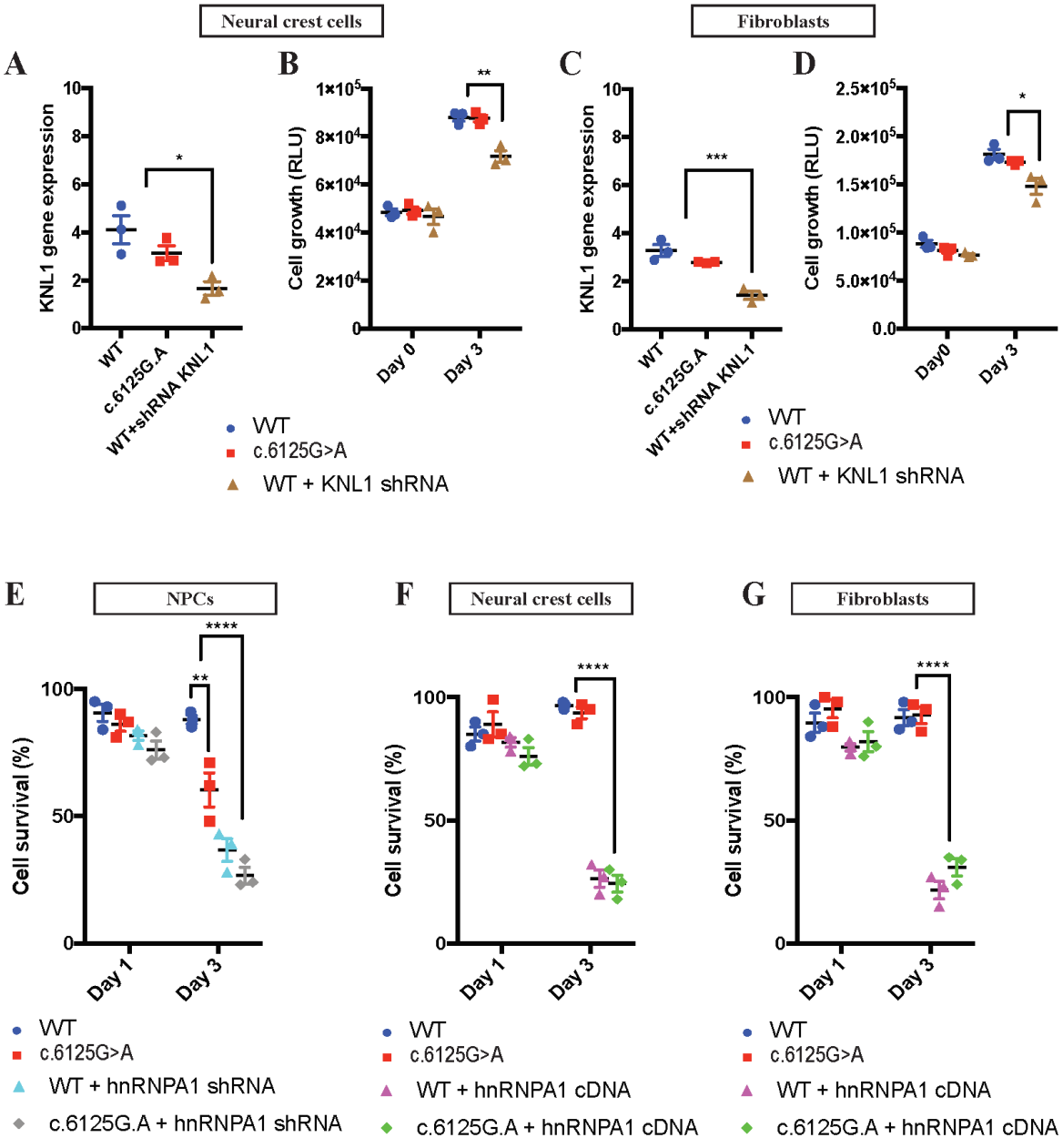
Neural crest cells
Fibroblasts
A
B
C
D
KNL1 gene expression
Cell growth (RLU)
WT
c.6125G.A
WT+shRNA KNL1
Day 0
Day 3
Day0
WT
c.6125G>A
WT + KNL1 shRNA
E
F
G
NPCs
Cell survival (%)
Day 1
Day 3
WT + hnRNPA1 shRNA
c.6125G.A + hnRNPA1 shRNA
WT + hnRNPA1 cDNA
c.6125G.A + hnRNPA1 cDNA

Figure S5: Cell growth defects were mimicked in fibroblasts and neural crest cells expressing KNL1 shRNA, related to Figure 7

(A) Quantitative RT-PCR analysis of *KNL1* expression on WT, KNL1$^{c.6125G>A}$, WT shRNA neural crest cells, normalized to *GAPDH*.

(B) ATP assay, represented in Relative Light Units, on WT, KNL1$^{c.6125G>A}$, WT shRNA neural crest cells, day 0 represent the plating day. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

(C) Quantitative RT-PCR analysis of *KNL1* expression on WT, KNL1$^{c.6125G>A}$, WT shRNA fibroblasts, normalized to *GAPDH*.

(D) ATP assay, represented in Relative Light Units, on WT, KNL1$^{c.6125G>A}$, WT shRNA fibroblasts, day 0 represent the plating day. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

(E) Cell survival analysis, based on blue trypan, of WT, KNL1$^{c.6125G>A}$, WT expressing *HNRNPA1* shRNA and KNL1$^{c.6125G>A}$ expressing *HNRNPA1* shRNA neural progenitors, day 1 represent the post- infection day. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

(F) Cell survival analysis, based on blue trypan, of WT, KNL1$^{c.6125G>A}$, WT expressing *HNRNPA1* cDNA and KNL1$^{c.6125G>A}$ expressing *HNRNPA1* cDNA neural crest cells, day 1 represent the post- infection day. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

(G) Cell survival analysis, based on blue trypan, of WT, KNL1$^{c.6125G>A}$, WT expressing *HNRNPA1* cDNA and KNL1$^{c.6125G>A}$ expressing *HNRNPA1* cDNA fibroblasts, day 1 represent the post- infection day. ANOVA was performed followed by a post-hoc group comparisons using Bonferroni test.

One non-targeted wild type clone, two wild type clones and three patient mutation clones derived from the same CRISPR-Cas9 targeting are plotted in each graph. For fibroblast and neural crest, infection and transfection have been repeated in triplicates. Results are mean +/- SEM. *$p<0.05$, ***$p<0.001$.