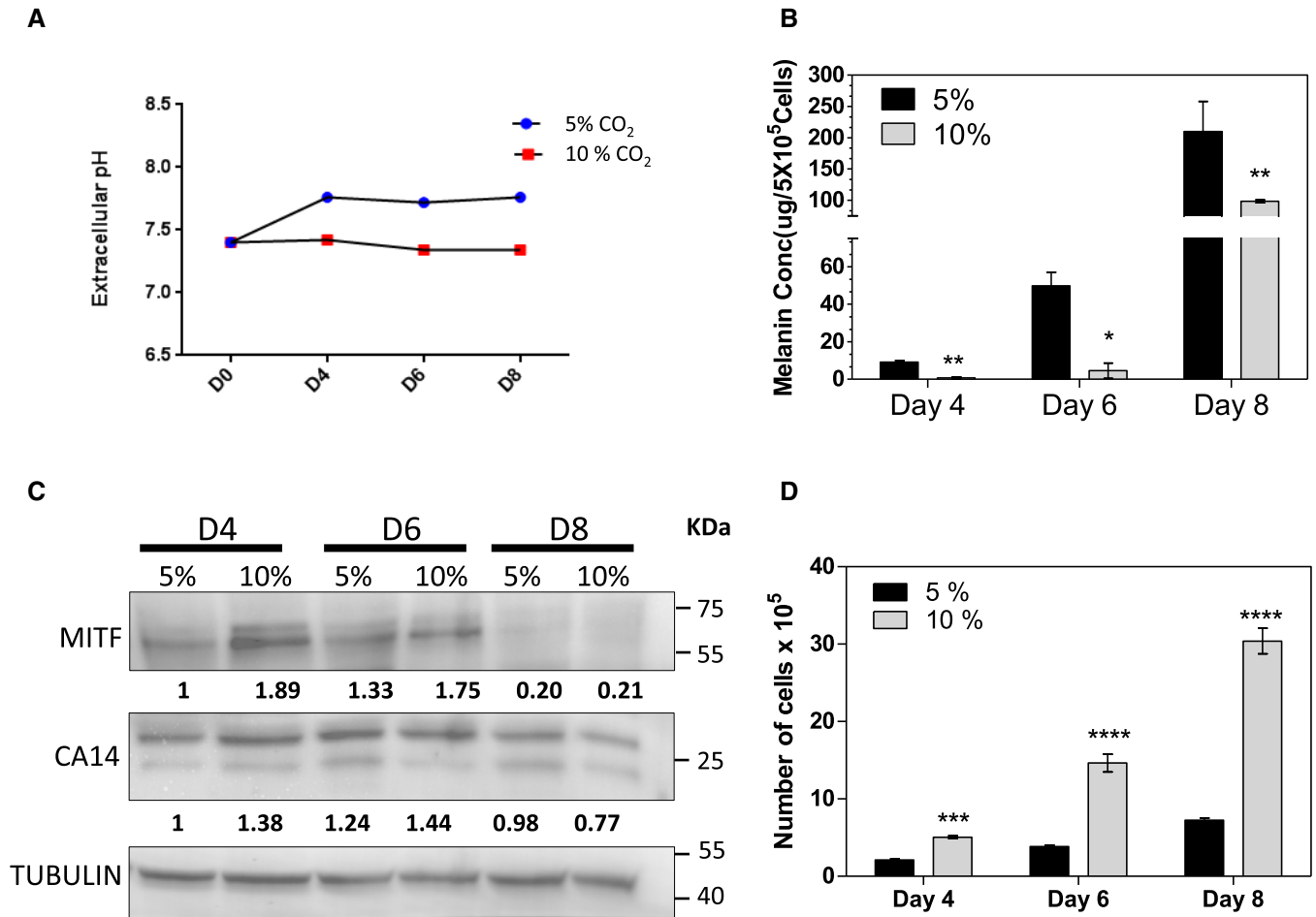# Expanded View Figures

Figure EV1. 10% CO₂ leads to extracellular acidification leading to decreased melanin content and increased proliferation.

A   Line graph showing extracellular pH_e at various days of pigmentation in 5% and 10% CO_2 cultured B16 cells.

B   Bar graph depicts the colorimetric melanin estimation of B16 melanoma cells cultured under 5% CO_2 and 10% CO_2 growth conditions, on indicated days of pigmentation using synthetic melanin as a standard. Bars represent mean ± SEM independent biological replicate, $n = 3$.

C   Western blot analysis of CA14 and MITF normalized to tubulin on days 4, 6, and 8 under 5 and 10% CO_2.

D   Alteration of cellular pH by the modulation of CO_2 results in changes in cell proliferation in B16 cells as measured by cell count. Bars indicate mean ± SEM across three independent experiments.

Data information: Student's *t*-test. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.
Source data are available online for this figure.

Figure EV2. CA14 is a putative pigmentation regulator associated with MITF.

A   Correlation analysis of the expression of the melanocyte maturation-associated genes identified from the two reversible pigmentation models with the expression of MITF in the Cancer genome atlas (TCGA) data of melanoma samples (SKCM). Pearson's correlation coefficient and *P*-value describing the chance of the correlation being random was calculated across 473 melanoma samples with the normalized gene expression using RNA sequencing data from TCGA and represented as a heat-map. Expression of CA14 has a very high correlation with that MITF, suggesting that it may be a target gene like others such as Tyr, Cdk2, and Mcoln3.

B   Western blot analysis of CA14 and MITF normalized to tubulin in primary human melanocytes upon 100 μM IBMX treatment.

C   Western blot analysis of CA14 and MITF normalized to tubulin in primary human melanocytes upon silencing of MITF using siRNA.

D   Luciferase reporter assay using *ca14* promoter in B16 cells normalized to renilla luciferase upon 100 μM IBMX treatment. Indicated are the four constructs tested, 3kB upstream region, or 1 kb each of proximal, mid-, and distal regions. Bars represent mean ± SEM across *n* = 3 biological replicates.

E   Chromatin immunoprecipitation studies with C5 MITF antibody followed by qPCR analysis of select regions of the *ca14* promoter. Circle represents percent input in IgG enrichment, and dark hexagons represent percent input in MITF ChIP. Two independent replicates are depicted.

F   Luciferase reporter assay using the *ca14* promoter in B16 cells followed by 100 μM IBMX treatment, normalized to renilla luciferase, upon mutation of E/M box sequence 1, 2, or both. Bars represent mean ± SEM across *n* = 3 biological replicates.

Data information: Student's *t*-test. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

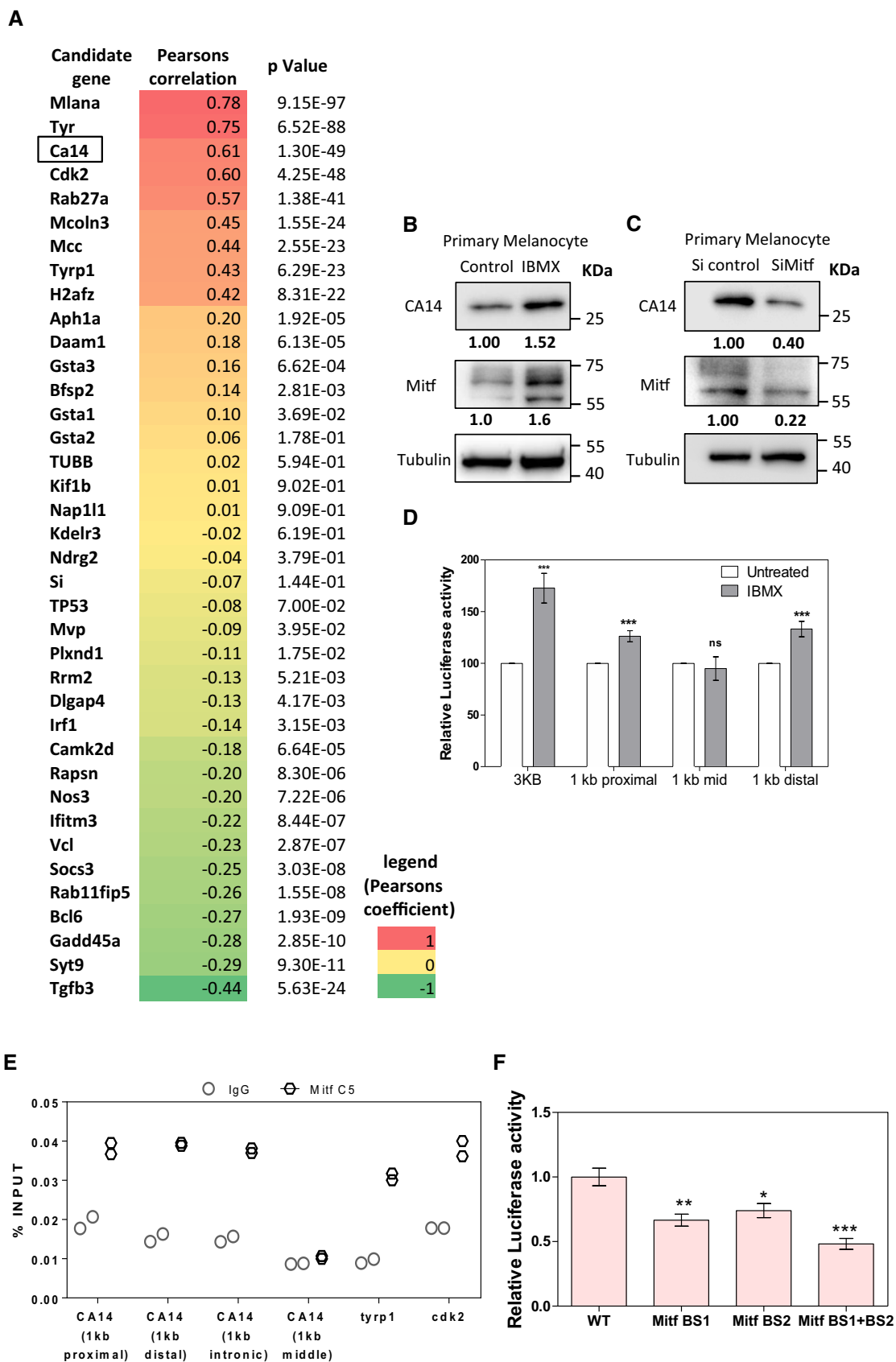Source data are available online for this figure.

A

Candidate gene	Pearsons correlation	p Value
Mlana	0.78	9.15E-97
Tyr	0.75	6.52E-88
Ca14	0.61	1.30E-49
Cdk2	0.60	4.25E-48
Rab27a	0.57	1.38E-41
Mcoln3	0.45	1.55E-24
Mcc	0.44	2.55E-23
Tyrp1	0.43	6.29E-23
H2afz	0.42	8.31E-22
Aph1a	0.20	1.92E-05
Daam1	0.18	6.13E-05
Gsta3	0.16	6.62E-04
Bfsp2	0.14	2.81E-03
Gsta1	0.10	3.69E-02
Gsta2	0.06	1.78E-01
TUBB	0.02	5.94E-01
Kif1b	0.01	9.02E-01
Nap1l1	0.01	9.09E-01
Kdelr3	-0.02	6.19E-01
Ndrg2	-0.04	3.79E-01
Si	-0.07	1.44E-01
TP53	-0.08	7.00E-02
Mvp	-0.09	3.95E-02
Plxnd1	-0.11	1.75E-02
Rrm2	-0.13	5.21E-03
Dlgap4	-0.13	4.17E-03
Irf1	-0.14	3.15E-03
Camk2d	-0.18	6.64E-05
Rapsn	-0.20	8.30E-06
Nos3	-0.20	7.22E-06
Ifitm3	-0.22	8.44E-07
Vcl	-0.23	2.87E-07
Socs3	-0.25	3.03E-08
Rab11fip5	-0.26	1.55E-08
Bcl6	-0.27	1.93E-09
Gadd45a	-0.28	2.85E-10
Syt9	-0.29	9.30E-11
Tgfb3	-0.44	5.63E-24

legend (Pearsons coefficient)

	1
	0
	-1

B

Primary Melanocyte

Control   IBMX   KDa

CA14   1.00   1.52   — 25

Mitf   1.0   1.6   — 75 — 55

Tubulin   — 55 — 40

C

Primary Melanocyte

Si control   SiMitf   KDa

CA14   1.00   0.40   — 25

Mitf   1.00   0.22   — 75 — 55

Tubulin   — 55 — 40

D

E

F

Figure EV2.

Figure EV3. **Mitf regulates pH via CA14, and pigmentation changes associated with CA14 are observed in primary human melanocytes.**

A   Levels of *ca14* mRNA measured by qRT PCR analysis across different days of pigmentation. Bars indicate mean $\pm$ SEM across three independent experiments.

B pH_i upon activation of MITF using 1 µM α-MSH in B16 cells. Data from 50 cells across two independent experiments. The solid symbol indicates mean, and error bars indicate SEM.

C pH_i upon MITF overexpression and knockdown using siRNA in B16 cells. Data from 50 cells across two independent experiments. The solid symbol indicates mean, and error bars indicate SEM.

D   Western blot analysis of CA14 normalized to tubulin upon silencing of Ca14 using siRNA.

E   Western blot analysis of CA14 normalized to tubulin upon silencing of Ca14 using two different sequence independent shRNAs.

F   Western blot analysis of CA14 normalized to tubulin upon overexpression of Ca14.

G   Fractionation of melanosomes from B16 cells followed by Western blot analysis depicting the levels of CA14 and Dct to assess localization to melanosomes.

H   Immunofluorescence study of CA14 in NHEM (normal human primary melanocytes) counter-stained with DAPI, upon culturing of cells under two different media conditions as indicated. Scale bars: 10 µm.

I   Western blot analysis of CA14 in NHEM cells grown under the two conditions. (bottom) Cell pellets and their melanin intensity quantitation. Bars represent mean $\pm$ SEM across $n = 3$ biological replicates.

Data information: Student's *t*-test. ***$P \leq 0.001$, ****$P \leq 0.0001$.
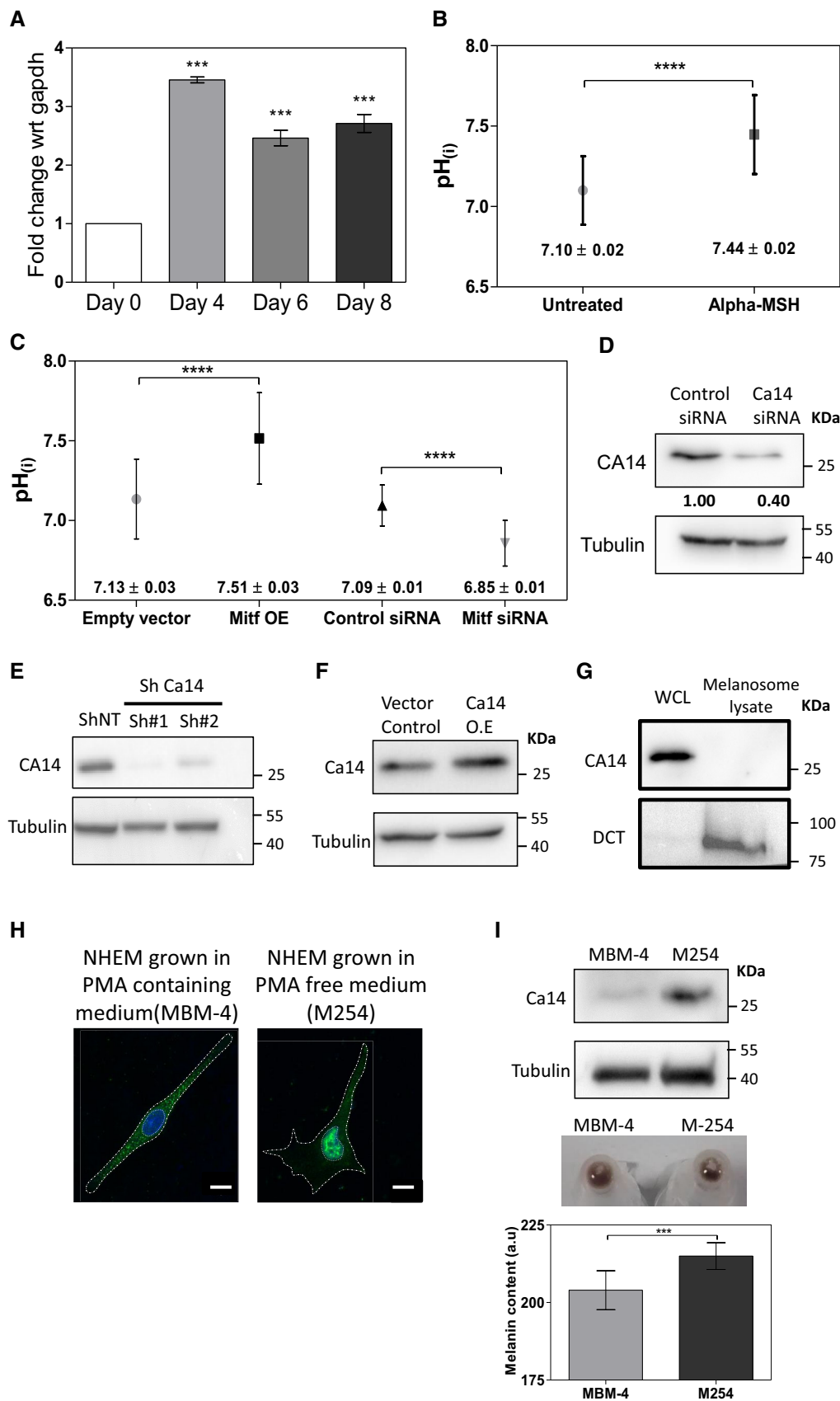
Source data are available online for this figure.

A

B

C

D

E

F

G

H

NHEM grown in PMA containing medium(MBM-4)

NHEM grown in PMA free medium (M254)
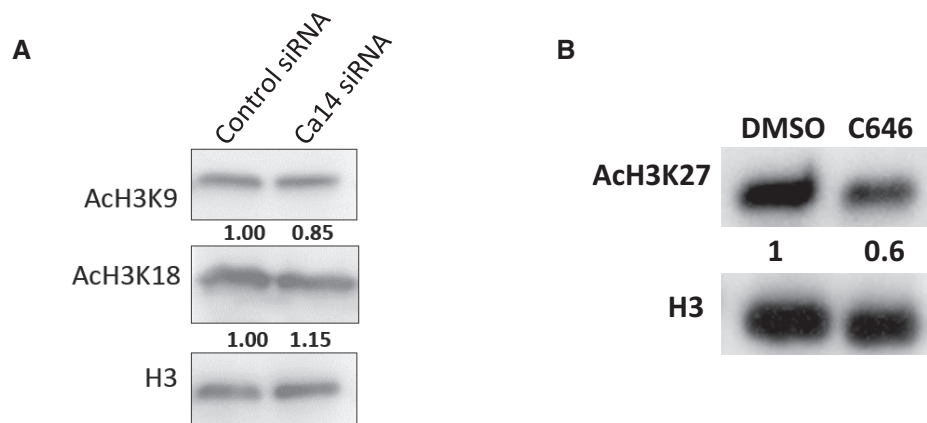
I

Figure EV3.

A

B

Figure EV4. Histone acetylation changes upon *ca14* silencing and C646 treatment.

A Western blot analysis of control and *ca14* siRNA-treated cells for various acetylation modifications on histone H3. Quantitation with respect to to control cells is depicted below the blot images.

B Western blot analysis of acetylated H3K27 levels with respect to total H3 levels upon 10 μM C646 treatment.
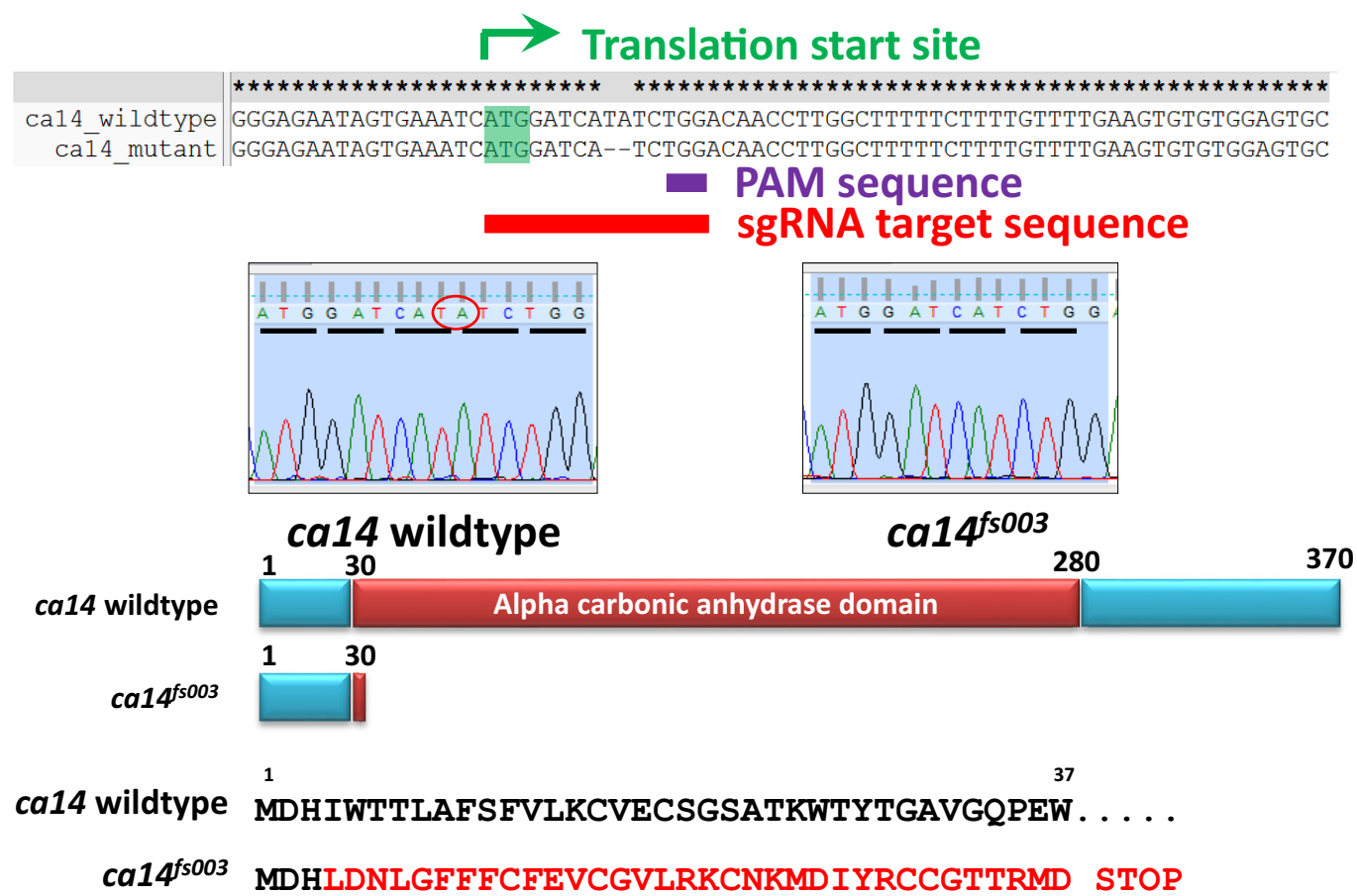
Source data are available online for this figure.

Translation start site

```
                                 **********************   ***************************************************
ca14_wildtype | GGGAGAATAGTGAAATCATGGATCATATCTGGACAACCTTGGCTTTTTCTTTTGTTTTGAAGTGTGTGGAGTGC
   ca14_mutant | GGGAGAATAGTGAAATCATGGATCA--TCTGGACAACCTTGGCTTTTTCTTTTGTTTTGAAGTGTGTGGAGTGC
```

■ **PAM sequence**

▬▬▬▬▬▬▬▬ **sgRNA target sequence**

ca14 wildtype   *ca14*fs003

ca14 wildtype
1   30   280   370
[ ] [Alpha carbonic anhydrase domain] [   ]

*ca14*fs003
1   30
[ ][]

ca14 wildtype
1   37
MDHIWTTLAFSFVLKCVECSGSATKWTYTGAVGQPEW.....

*ca14*fs003
MDHLDNLGFFFCFEVCGVLRKCNKMDIYRCCGTTRMD STOP

Figure EV5.   Schematic of zebrafish gene ca14 depicting that CRISPR target region and the observed mutation in *ca14*fs003.

(Top) DNA sequence alignment of *ca14* and *ca14*fs003 mutants. Depicted are the PAM sequence and the region of the crispr guide RNA (sgRNA). Below is the chromatogram depicting the deletion. (Bottom) Diagrammatic representation of the mutant gene and the amino acid sequence encoded by *ca14*fs003.