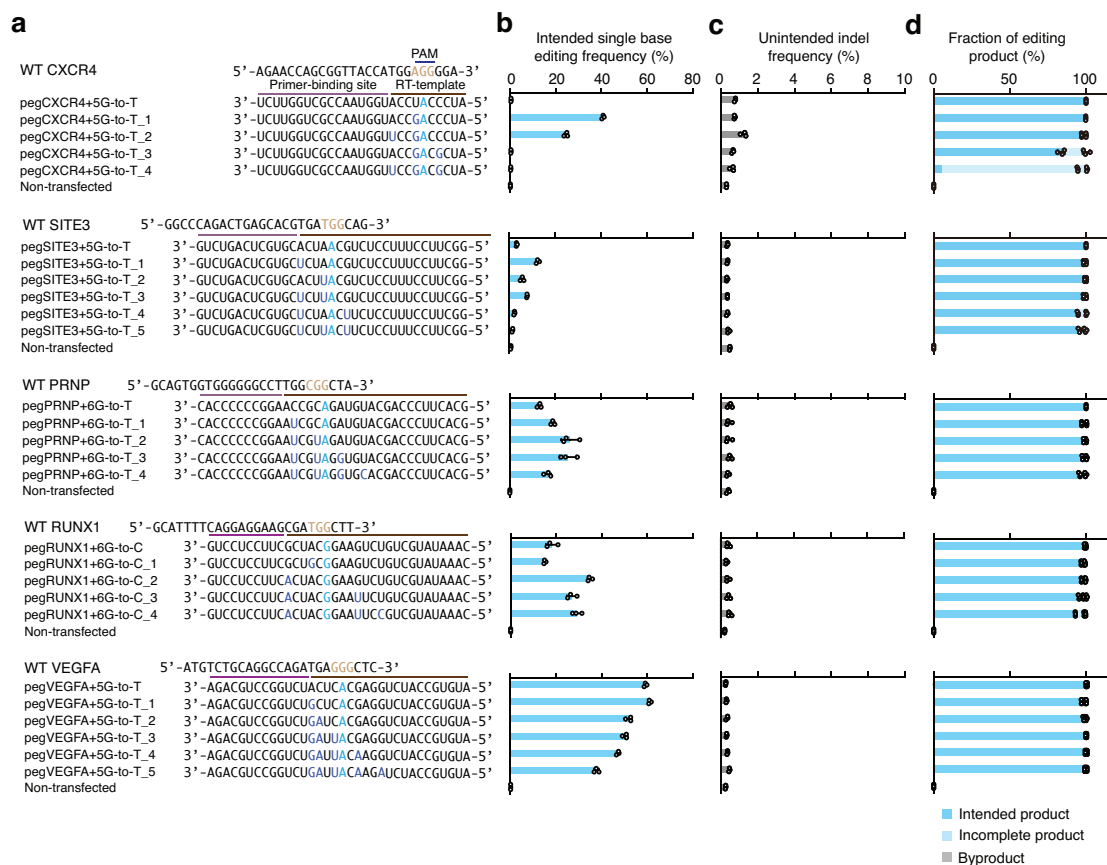


Supplementary figures and legends



Supplementary Fig. 1. pegRNA containing additional base substitutions induced higher editing efficiencies of single-base substitution at more target sites.

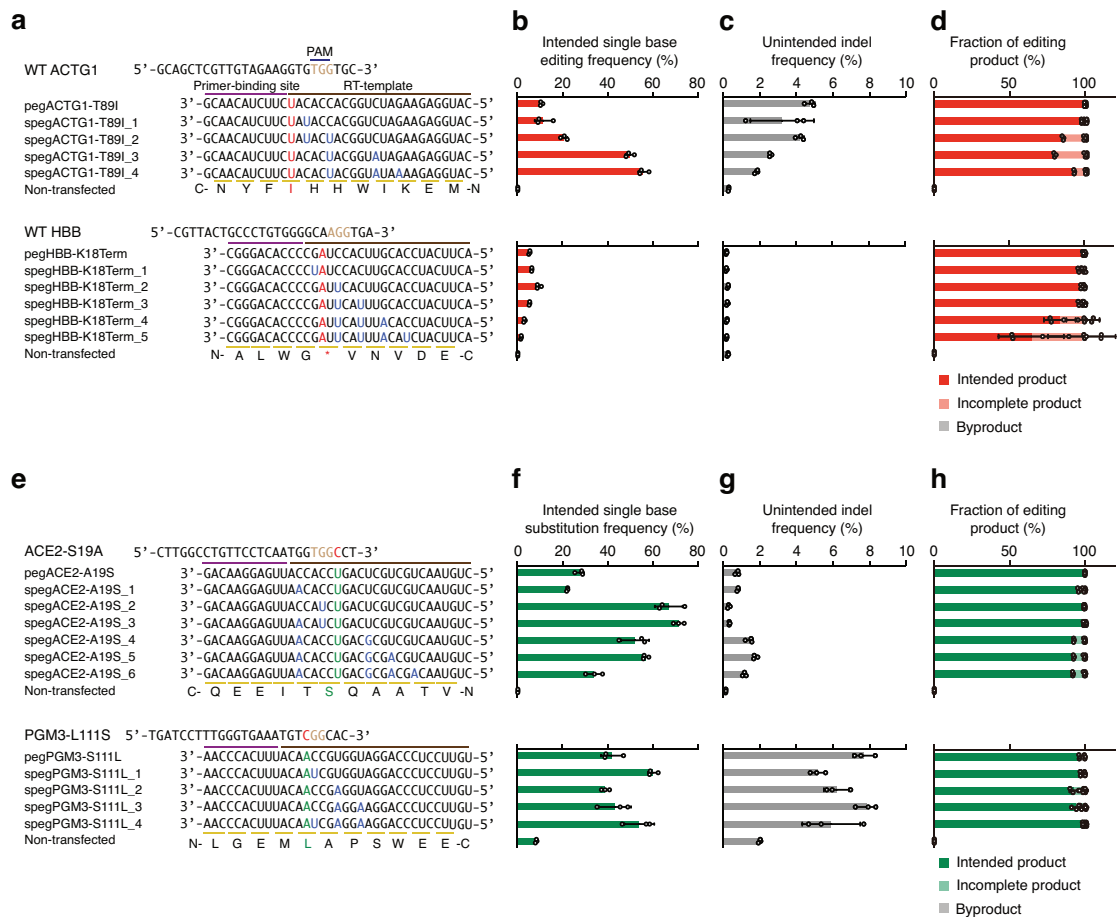
(a) Sequences of the PBS and RTT of pegRNAs and WT on-target genomic sites.

Intended single-base edits are in cyan, additional base substitutions are in blue and PAMs are in brown. (b-d) The intended single-base editing frequencies (b),

unintended indel frequencies (c) and fractions of editing product (d) were induced by the pegRNAs in (a) at the indicated target sites under the PE3 setting. Means \pm s.d.

are from three independent experiments. Source data are provided as a Source Data

file.



Supplementary Fig. 2. pegRNA containing SSMs induced higher editing

efficiencies to generate or repair pathogenic point mutations at more target sites.

(a) Sequences of the PBS and RTT of pegRNAs and spegRNAs for generating

pathogenic point mutations and WT on-target genomic sites. Pathogenic point

mutations are in red and SSMs are in blue. (b-d) The intended single-base editing

frequencies (b), unintended indel frequencies (c) and fractions of editing product (d)

were induced by the indicated pegRNAs and spegRNAs in (a). (e) Sequences of the

PBS and RTT of pegRNAs and spegRNAs for repairing pathogenic mutations and on-

target genomic sites with pre-installed mutations. Corrected bases are in green, pre-

installed point mutations are in red and SSMs are in blue. (f-h) The intended single-

base editing frequencies (**f**), unintended indel frequencies (**g**) and fractions of editing product (**h**) were induced by the indicated pegRNAs and spegRNAs in (**e**). (**b, c, d, f, g, h**) Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.

a

pegCXCR4+5G-toT-1
 WT AGAAC CAGCGGTTACCATGGAGGGAATCAGTGTAA
 Complete product AGAAC CAGCGGTTACCATGGAGGGAATCAGTGTAA (40.57%)
 Intended products AGAAC CAGCGGTTACCATGGAGGGAATCAGTGTAA (0.03%)
 Incomplete products AGAAC CAGCGGTTACCATGGAGGGAATCAGTGTAA (0.05%)

pegEMX1+4G-toC-2
 WT CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG
 Complete product CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (27.50%)
 CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.01%)
 CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.01%)
 Intended products CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.06%)
 CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.15%)
 CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.06%)
 Incomplete products CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.01%)
 CCGAGCAGAAGAAGAAAGGCTCCCATCACATCAACCGGTGGCG (0.12%)

pegSITE3+5G-toT-1
 WT CAGACTGAGCAGTGTGGCAGAGGAAAGGAAAGCCCTGCTTCC
 Complete product CAGACTGAGCAGTGTGGCAGAGGAAAGGAAAGCCCTGCTTCC (11.89%)
 Intended products CAGACTGAGCAGTGTGGCAGAGGAAAGGAAAGCCCTGCTTCC (0.08%)
 Incomplete products CAGACTGAGCAGTGTGGCAGAGGAAAGGAAAGCCCTGCTTCC (0.12%)

pegPRNP+6G-toT-2
 WT GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC
 Complete product GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (24.83%)
 GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (0.03%)
 Intended products GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (0.02%)
 GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (0.10%)
 GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (0.17%)
 Incomplete products GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (0.03%)
 GTGGGGGCTTGGCGCTACATGCTGGGAAGTGC (0.02%)

pegRUNX1+6G-toC-2
 WT CAGGAGGAAGGATGGCTCAGACAGCATATTTG
 Complete product CAGGAGGAAGGATGGCTCAGACAGCATATTTG (24.33%)
 Intended products CAGGAGGAAGGATGGCTCAGACAGCATATTTG (3.91%)
 Incomplete products CAGGAGGAAGGATGGCTCAGACAGCATATTTG (0.52%)

pegVEGFA+5G-toT-1
 WT TCTGCAGGCCAGATGAGGCTCCAGATGGCAGAT
 Complete product TCTGCAGGCCAGATGAGGCTCCAGATGGCAGAT (57.40%)
 Intended products TCTGCAGGCCAGATGAGGCTCCAGATGGCAGAT (1.31%)
 Incomplete products TCTGCAGGCCAGATGAGGCTCCAGATGGCAGAT (0.47%)

b

pegACTG1-T891-4
 WT CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA
 Complete product CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (45.06%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.75%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (5.24%)
 Intended products CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.38%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.13%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.03%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.57%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.02%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.03%)
 Incomplete products CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.03%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.01%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (0.76%)
 CGTTGTAGAAGTGTGGTCCAGATCTTCTCATGTCGTCCCA (2.61%)

pegCFTR-L23M-3
 WT GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC
 Complete product GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC (16.61%)
 GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC (0.02%)
 Intended products GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC (0.56%)
 GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC (0.04%)
 Incomplete products GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC (0.02%)
 GATACAGACAGCGCTGGAAATGTGACAGATATACCAATCCC (4.01%)

pegHBB-K18Term-2
 WT GCCCTGTGGGGCAAGTGAACCTGGATGAAGTTGGTGGTG
 Complete product GCCCTGTGGGGCAAGTGAACCTGGATGAAGTTGGTGGTG (9.01%)
 Intended products GCCCTGTGGGGCAAGTGAACCTGGATGAAGTTGGTGGTG (0.03%)
 Incomplete products GCCCTGTGGGGCAAGTGAACCTGGATGAAGTTGGTGGTG (0.06%)

c

pegACE2-A19S-3
 WT CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG
 Complete product CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG (70.91%)
 CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG (0.21%)
 Intended products CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG (0.05%)
 CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG (0.37%)
 Incomplete products CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG (0.06%)
 CTGTTCTCAATTGTGGCCTGAGCAGCAGTTACAGCAACAAGG (0.02%)

pegHBB-V7E-2
 WT CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG
 Complete product CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (39.22%)
 CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (0.11%)
 Intended products CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (0.01%)
 CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (0.05%)
 Incomplete products CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (0.01%)
 CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (0.04%)
 CACCTGACTCCTGTGAGAAAGTCTGCCGTACTGCCCTGTGGG (0.01%)

pegPGM3-S111L-1
 WT TTGGGTGAAATGTGGCACCATCTGGGAGGAACATGCCACCT
 Complete product TTGGGTGAAATGTGGCACCATCTGGGAGGAACATGCCACCT (46.39%)
 Intended products TTGGGTGAAATGTGGCACCATCTGGGAGGAACATGCCACCT (6.80%)
 Incomplete products TTGGGTGAAATGTGGCACCATCTGGGAGGAACATGCCACCT (0.09%)

Supplementary Fig. 3. Incomplete products induced by the pegRNAs containing additional base substitutions at on-target sites.

(a) The frequencies of complete products (with intended single-base editing and all additional base substitutions), intended products (with intended single-base editing but not all additional base substitutions) and incomplete products (with additional base substitutions but no intended single-base editing) induced by the optimized

pegRNAs with additional base substitutions in Fig. **1a** and Supplementary Fig. **1a. (b)**

The frequencies of complete products (with intended single-base editing and all SSMs), intended products (with intended single-base editing but not all SSMs) and incomplete products (with SSMs but no intended single-base editing) induced by the optimized spegRNAs in Fig. **1a** and Supplementary Fig. **2a. (c)** The frequencies of complete products (with intended single-base editing and all SSMs), intended products (with intended single-base editing but not all SSMs) and incomplete products (with SSMs but no intended single-base editing) induced by the optimized spegRNAs in Fig. **1a** and Supplementary Fig. **2e**. The frequencies are means from three biologically independent experiments.

a

■ RT-template ■ Scaffold insertion

pegCXCR4+5G-to-T	
WT	AGAACACCGGTTACCATGGAGGGATCAGTGTAA
Complete product	AGAACACCGGTTACCATGGAGGGATCAGTGTAA (0.0329%)
pegCXCR4+5G-to-T_1	
WT	AGAACACCGGTTACCATGGAGGGATCAGTGTAA
Complete product	AGAACACCGGTTACCATGGAGGGATCAGTGTAA (40.57%)
Byproducts	AGAACACCGGTTACCATGGAGGGATCAGTGTAA (0.0213%)
	AGAACACCGGTTACCATGGAGGGATCAGTGTAA (0.0137%)
	AGAACACCGGTTACCATGGAGGGATCAGTGTAA (0.0001%)
pegEMX1+4G-to-C	
WT	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG
Complete product	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (2.56%)
Byproducts	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (0.0812%)
pegEMX1+4G-to-C_2	
WT	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG
Complete product	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (27.50%)
Byproducts	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (0.905%)
	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (0.0284%)
	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (0.0028%)
	CCGAGCAGAAGAAGAGGCTCCCATCACATCAACCGTGGCG (0.0002%)
pegSITE3+5G-to-T	
WT	CAGACTGACGACGTGAGCCAGAGAAAGAAAGCCCTGCTTCC
Complete product	CAGACTGACGACGTGAGCCAGAGAAAGAAAGCCCTGCTTCC (3.20%)
Byproducts	CAGACTGACGACGTGAGCCAGAGAAAGAAAGCCCTGCTTCC (0.00017%)
pegSITE3+5G-to-T_1	
WT	CAGACTGACGACGTGAGCCAGAGAAAGAAAGCCCTGCTTCC
Complete product	CAGACTGACGACGTGAGCCAGAGAAAGAAAGCCCTGCTTCC (11.69%)
Byproducts	CAGACTGACGACGTGAGCCAGAGAAAGAAAGCCCTGCTTCC (0.001%)
pegPRNP+6G-to-T	
WT	GTGGGGGCGCTTGGCGCTACATGCTGGGAAGTGCATGAGCA
Complete product	GTGGGGGCGCTTGGCGCTACATGCTGGGAAGTGCATGAGCA (11.73%)
Byproducts	GTGGGGGCGCTTGGCGCTACATGCTGGGAAGTGCATGAGCA (0.0028%)
pegPRNP+6G-to-T_2	
WT	GTGGGGGCGCTTGGCGCTACATGCTGGGAAGTGCATGAGCA
Complete product	GTGGGGGCGCTTGGCGCTACATGCTGGGAAGTGCATGAGCA (24.83%)
Byproducts	GTGGGGGCGCTTGGCGCTACATGCTGGGAAGTGCATGAGCA (0.0027%)
pegRUNX1+6G-to-C	
WT	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT
Complete product	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (16.65%)
Byproducts	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (0.1107%)
pegRUNX1+6G-to-C_2	
WT	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT
Complete product	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (14.91%)
Byproducts	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (0.2096%)
	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (0.0472%)
	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (0.0027%)
	CAGAGGAAGCGATGCTTCAGACAGCATATTGAGTCATTT (0.0024%)
pegVEGFA+5G-to-T	
WT	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG
Complete product	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (58.13%)
Byproducts	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0219%)
	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0045%)
pegVEGFA+5G-to-T_1	
WT	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG
Complete product	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (57.40%)
Byproducts	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0208%)
	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0004%)
	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0003%)
	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0001%)
	TCTGAGCCAGATGAGGCTCCAGATGGACATTTGTAGAG (0.0001%)

b

pegACTG1-T89I	
WT	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA
Complete product	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (8.43%)
Byproducts	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0546%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0034%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0003%)
spegACTG1-T89I_4	
WT	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA
Complete product	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (45.06%)
Byproducts	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0455%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0022%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0072%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0059%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0044%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0029%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0013%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.001%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0007%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0008%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0002%)
	CGTTGTAGAAGGTGGTGCAGATCTTCTCCATGCTGCCA (0.0002%)
pegCFTR-L23M	
WT	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC
Complete product	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (2.24%)
Byproducts	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (0.104%)
spegCFTR-L23M_3	
WT	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC
Complete product	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (16.61%)
Byproducts	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (3.798%)
	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (0.1479%)
	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (0.0128%)
	GATACAGACAGCGCCGTAATTTGACACATATACCAATCCC (0.003%)
pegHBB-K18Term	
WT	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT
Complete product	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT (5.01%)
Byproducts	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT (0.198%)
	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT (0.0069%)
spegHBB-K18Term_2	
WT	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT
Complete product	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT (9.01%)
Byproducts	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT (0.0498%)
	GCCCTGTGGGGCAGTGAACGTGGATGAAGTTGGTGGT (0.0005%)
pegHBB-V7E	
WT	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG
Complete product	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (12.20%)
Byproducts	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.1258%)
	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.0014%)
spegHBB-V7E_2	
WT	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG
Complete product	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (39.22%)
Byproducts	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.086%)
	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.0008%)
	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.0003%)
	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.0002%)
	CACCTGACTCTGTGGAGAGTCTGCCGTTACTGCCCTGTGG (0.0002%)

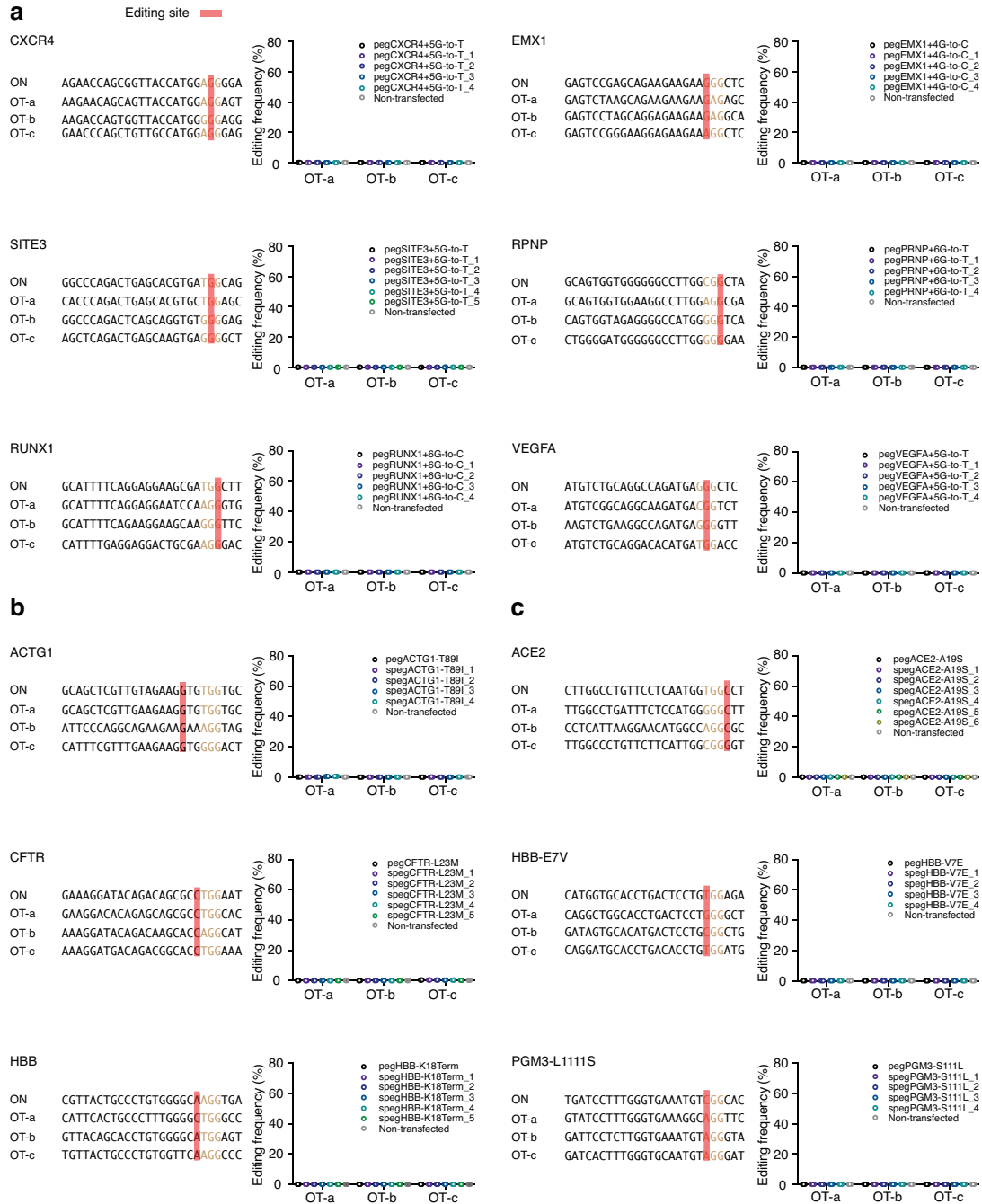
c

pegACE2-A19S	
WT	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG
Complete product	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (25.28%)
Byproducts	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (0.0802%)
	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (0.0093%)
spegACE2-A19S_3	
WT	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG
Complete product	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (70.91%)
Byproducts	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (0.0833%)
	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (0.0099%)
	CTGTTCTCAATGTTGACTGAGCAGCAGTTACAGCAACAGG (0.0021%)
pegPGM3-S111L	
WT	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT
Complete product	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (27.57%)
Byproducts	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (1.728%)
	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (0.044%)
spegPGM3-S111L_1	
WT	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT
Complete product	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (46.39%)
Byproducts	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (1.495%)
	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (0.1751%)
	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (0.1724%)
	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (0.0049%)
	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (0.0014%)
	TGGGTGAAATGTTGGCACCCTCTGGGAGGAACATGCCACCT (0.0011%)

Supplementary Fig. 4. Byproducts induced by the pegRNAs containing additional base substitutions at on-target sites.

(a) The frequencies of complete products and byproducts (with pegRNA scaffold incorporation) induced by regular pegRNAs and the optimized pegRNAs containing

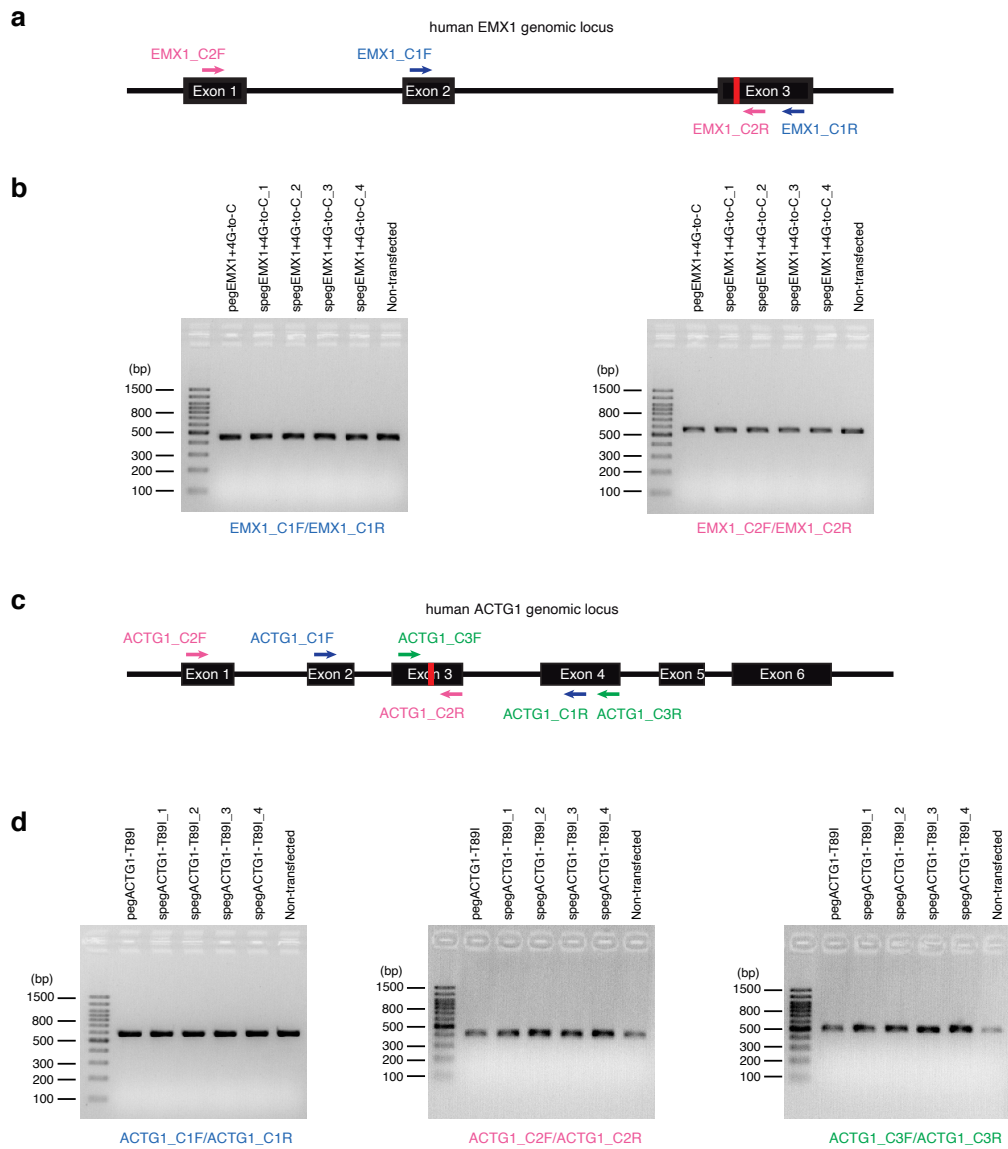
additional base substitutions in Fig. **1a** and Supplementary Fig. **1a**. **(b)** The frequencies of complete products and byproducts induced by regular pegRNAs and the optimized spegRNAs in Fig. **1a** and Supplementary Fig. **2a**. **(c)** The frequencies of complete products and byproducts induced by regular pegRNAs and the optimized spegRNAs in Fig. **1a** and Supplementary Fig. **2e**. The frequencies are means from three biologically independent experiments.



Supplementary Fig. 5. Off-target editing induced by the pegRNAs containing additional base substitutions.

(a) The sequences of on- and off-target sites for the pegRNAs in Fig. 1a and Supplementary Fig. 1a are shown. The base substitution frequencies were determined at the indicated off-target sites. (b) The sequences of on- and off-target sites for the

pegRNAs in Fig. **1a** and Supplementary Fig. **2a** are shown. The base substitution frequencies were determined at the indicated off-target sites. (c) The sequences of on- and off-target sites for the pegRNAs in Fig. **1a** and Supplementary Fig. **2e** are shown. The base substitution frequencies were determined at the indicated off-target sites. Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.

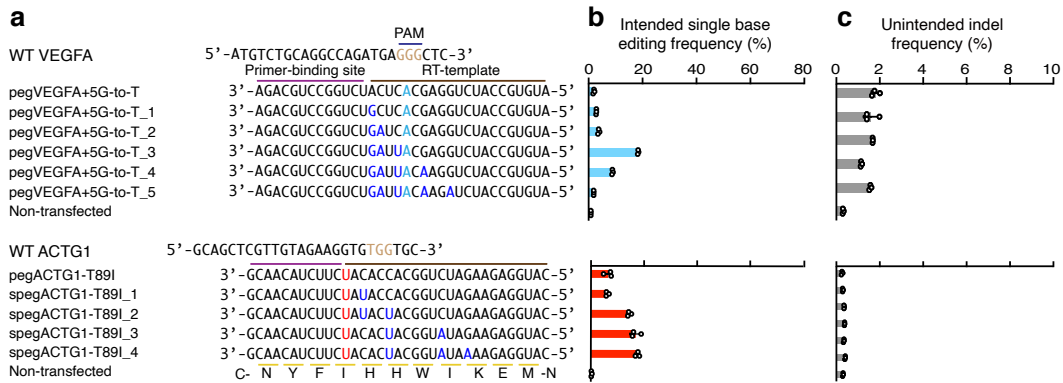


Supplementary Fig. 6. Splicing patterns of the exons edited by the pegRNAs containing additional base substitutions.

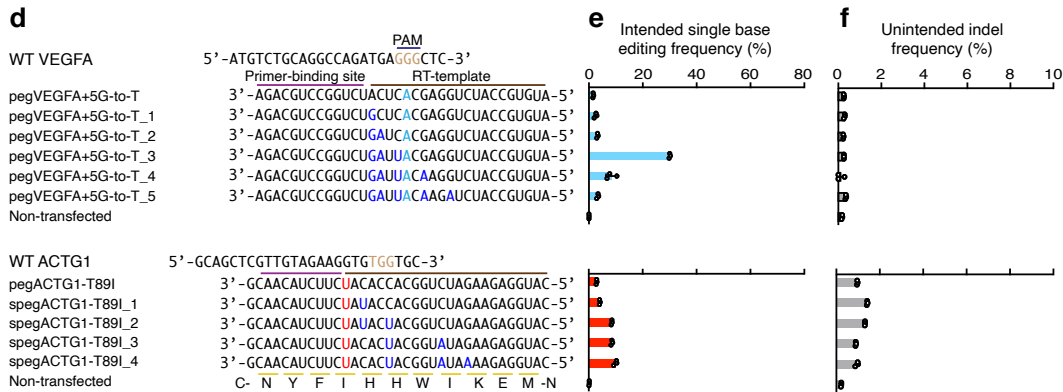
(a) Schematic diagrams illustrating the position of PCR primers to detect the splicing pattern of the edited exon 3 of *EMX1*. (b) RT-PCR results showed that no aberrant splicing of *EMX1* exon 3 was triggered by the indicated pegRNAs. (c) Schematic diagrams illustrating the position of PCR primers to detect the splicing pattern of the edited exon 3 of *ACGT1*. (d) RT-PCR results showed that no aberrant splicing of

ACGTI exon 3 was triggered by the indicated pegRNAs. Agarose gel data are representative of three independent experiments. Uncropped gels for **(b)** and **(d)** are provided.

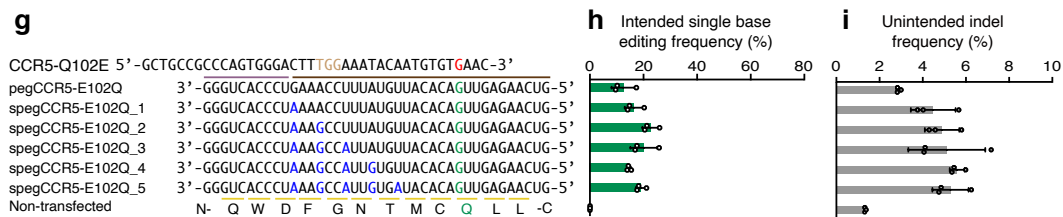
U2OS



HeLa



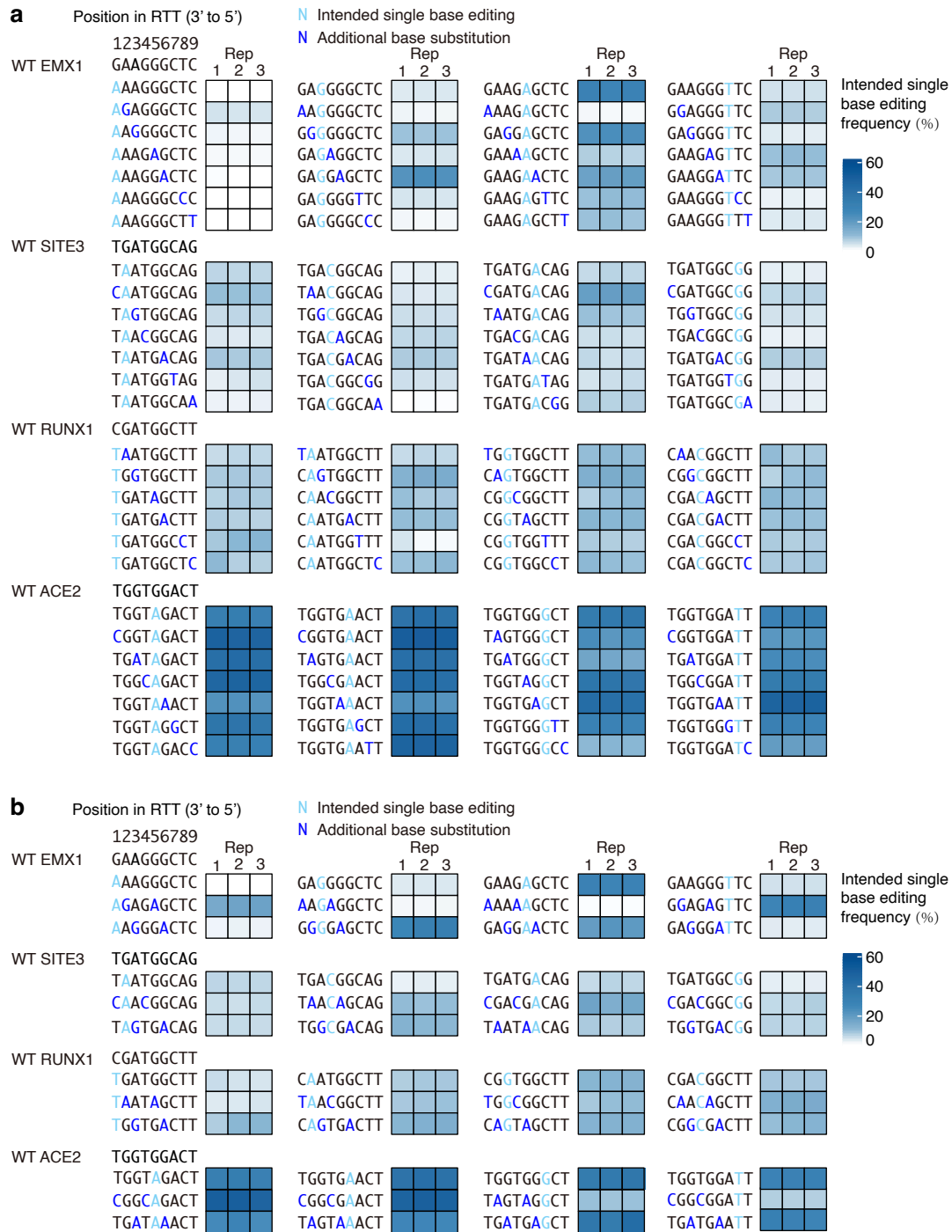
HeLa_mutation correction



Supplementary Fig. 7. The pegRNA containing additional base substitutions or SSMs induced higher single-base editing efficiencies in different cells.

(a) Sequences of the PBS and RTT of pegRNAs and spegRNAs and WT on-target genomic site in U2OS cells. Intended single-base edits are in cyan, pathogenic point mutations are in red and additional base substitutions and SSMs are in blue. (b, c) The

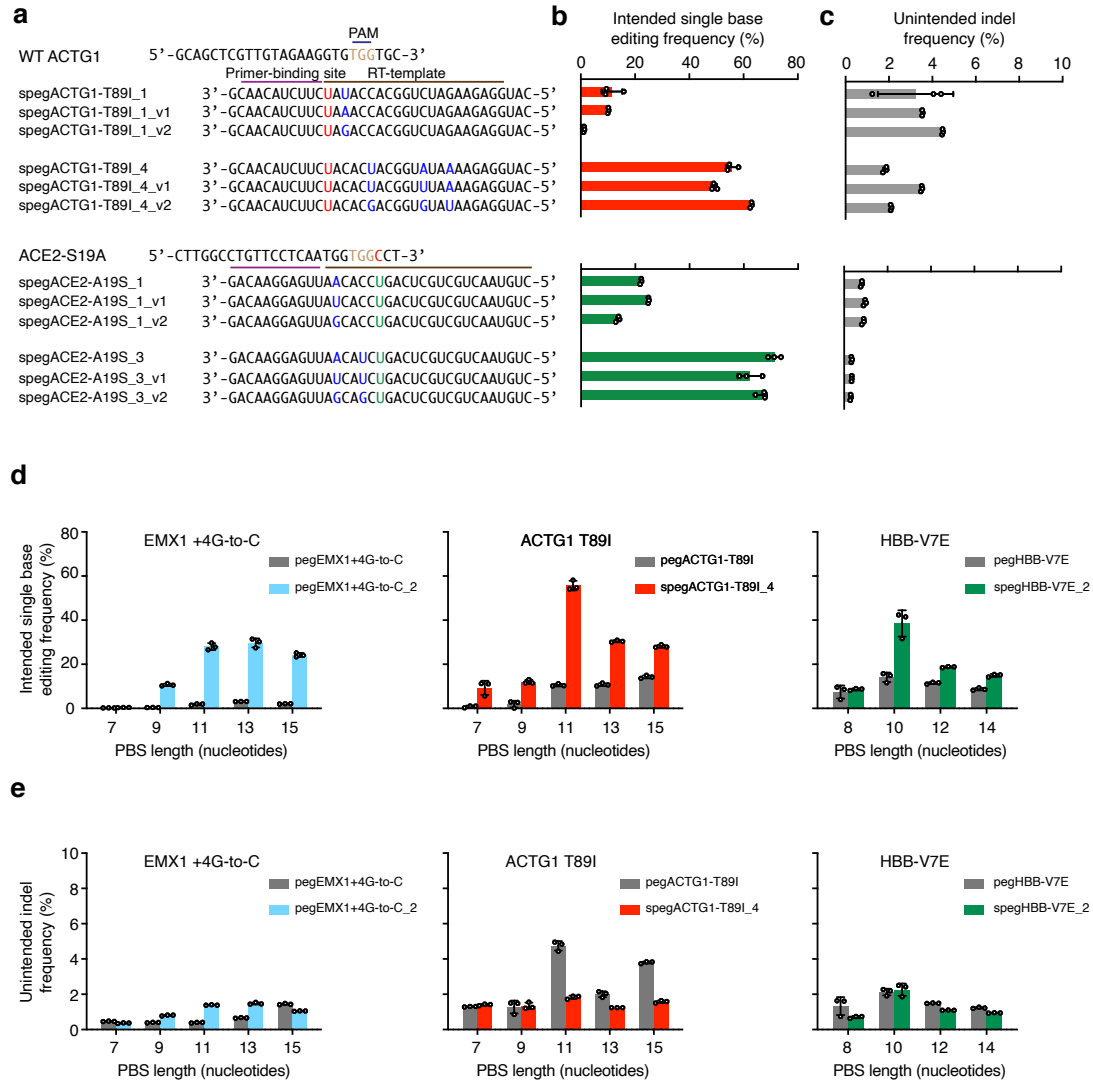
intended single-base editing frequencies (**b**) and unintended indel frequencies (**c**) were induced by the indicated pegRNAs and spegRNAs in (**a**) in U2OS cells. (**d**) Sequences of the PBS and RTT of pegRNAs and spegRNAs and WT on-target genomic site in HeLa cells. Intended single-base edits are in cyan, pathogenic point mutations are in red and additional base substitutions and SSMs are in blue. (**e, f**) The intended single-base editing frequencies (**e**) and unintended indel frequencies (**f**) were induced by the indicated pegRNAs and spegRNAs in (**d**) in HeLa cells. (**g**) Sequences of the PBS and RTT of spegRNAs and on-target genomic site in the HeLa cells with a pre-installed point mutation. Corrected bases are in green, pre-installed point mutations are in red and SSMs are in blue. (**h, i**) The intended single-base editing frequencies (**h**) and unintended indel frequencies (**i**) were induced by the indicated pegRNAs in (**g**) in the HeLa cells with the pre-installed point mutation. (**b, c, e, f, h, i**) Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.



Supplementary Fig. 8. Effect of additional base substitution position in RTT on editing efficiency.

(a) Heatmaps show the intended single-base editing frequencies induced by the pegRNAs containing one additional base substitution at the indicated positions in

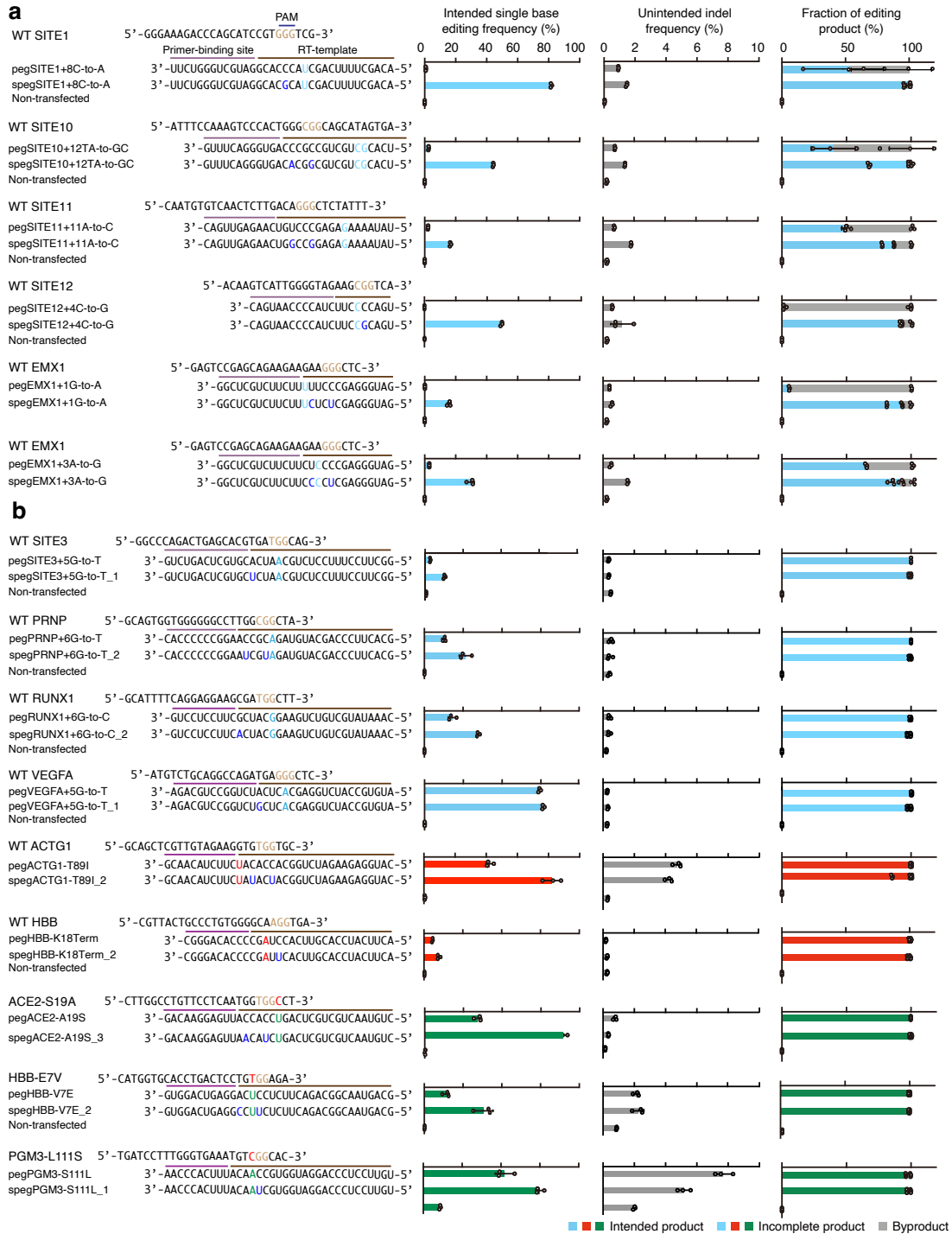
RTT. **(b)** Heatmaps show the intended single-base editing frequencies induced by the pegRNAs containing two additional base substitution at the indicated positions in RTT. Source data are provided as a Source Data file.



Supplementary Fig. 9. Effects of the mutation type and PBS length of spegRNA on editing efficiency.

(a) Sequences of PBS and RTT of the spegRNAs with different kind of SSMs (*e.g.*, transition and transversion). Pathogenic point mutations are in red and SSMs are in blue. (b, c) The intended single-base editing frequencies (b) and unintended indel frequencies (c) were induced by the spegRNAs in (a). (d, e) The intended single-base editing frequencies (d) and unintended indel frequencies (e) induced by the spegRNAs with different length of PBS at the indicated target sites. (b, c, d, e) Means

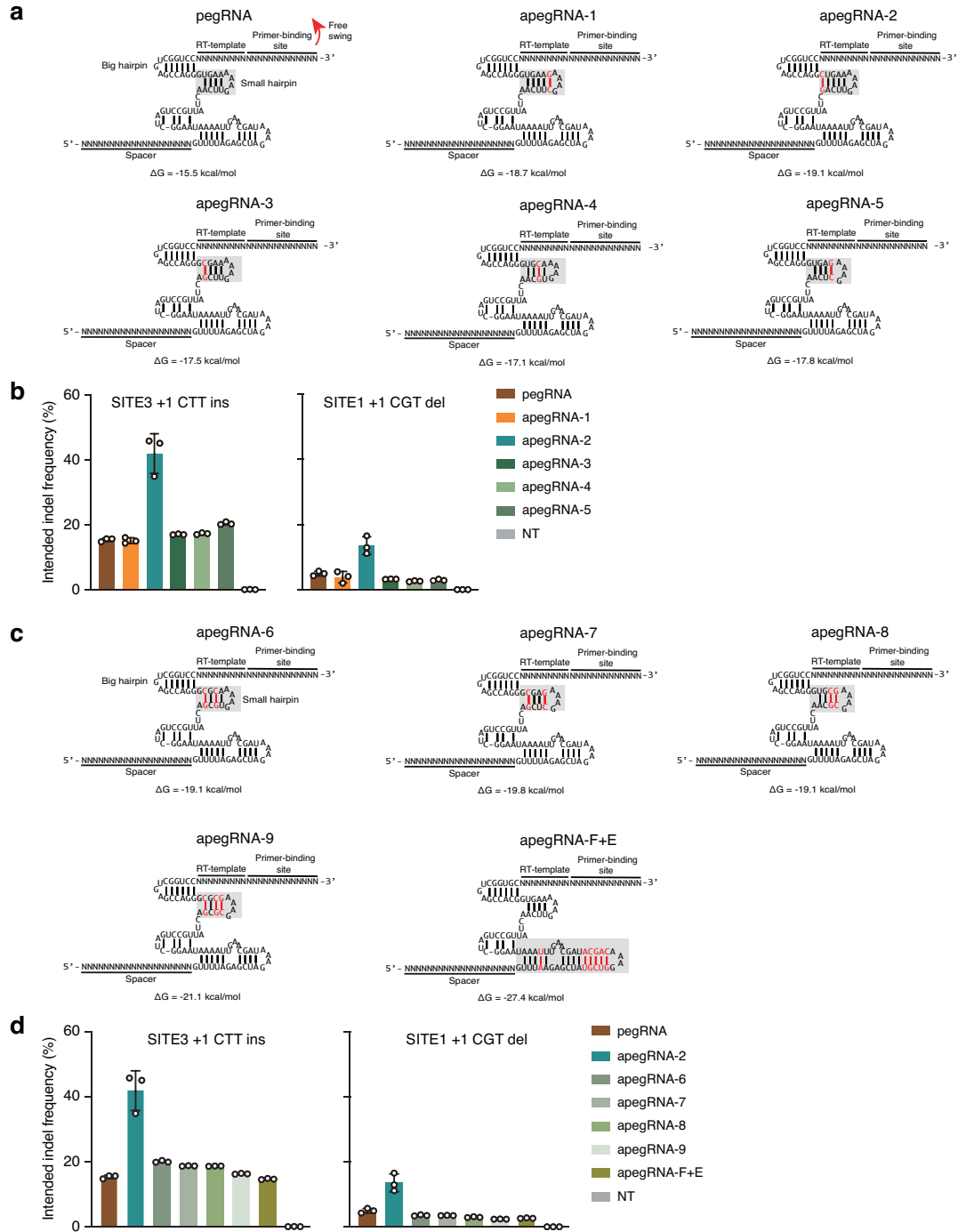
\pm s.d. are from three independent experiments. Source data are provided as a Source Data file.



Supplementary Fig. 10. Comparison of the editing efficiencies by pegRNA and spegRNA at more target sites.

(a) The intended single-base editing frequencies, unintended indel frequencies and fractions of editing product were induced by the indicated pegRNAs and spegRNAs

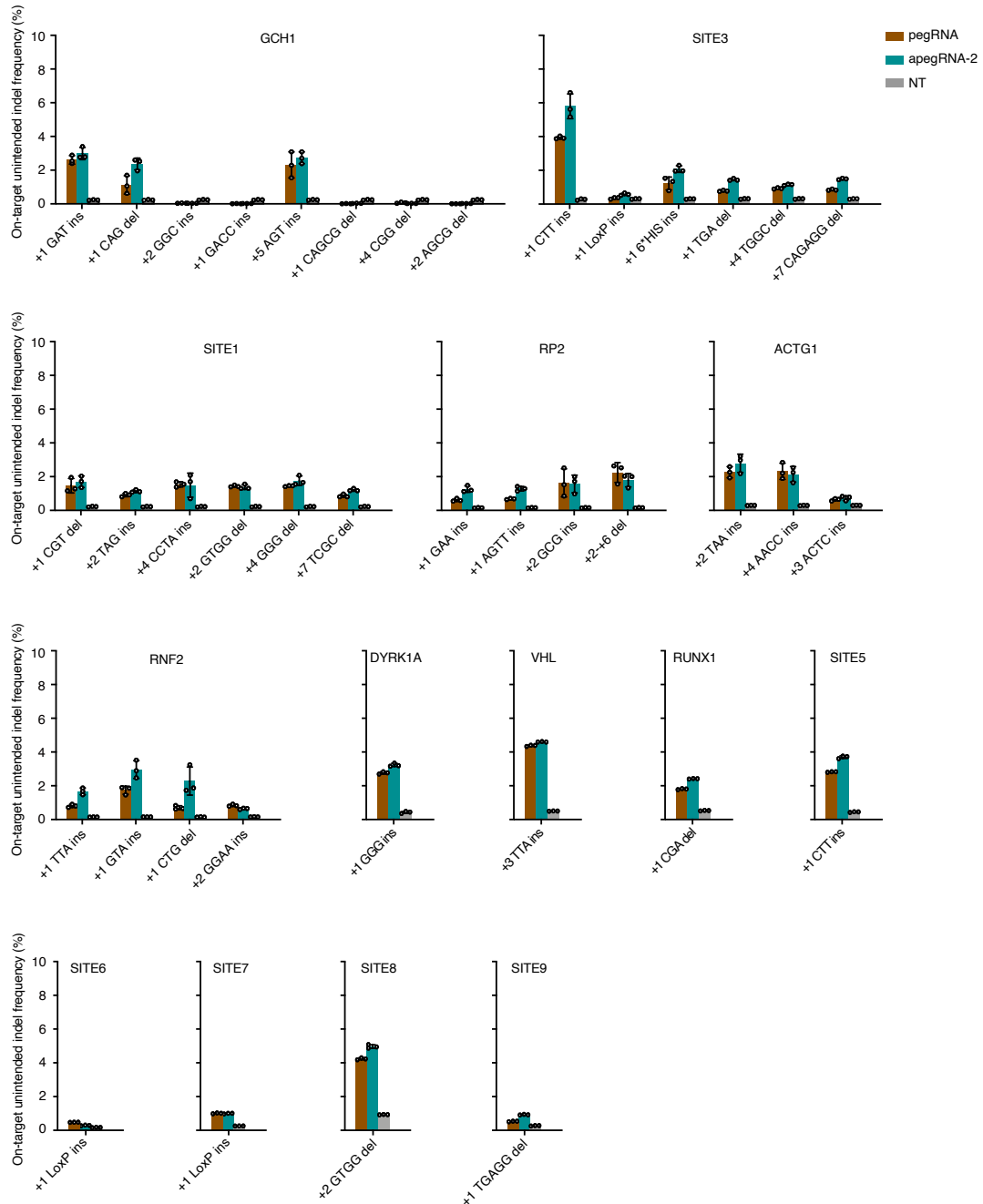
at newly tested target sites. **(b)** Reanalyzing the intended single-base editing frequencies, unintended indel frequencies and fractions of editing product that were induced by the indicated pegRNAs and spegRNAs at previously tested target sites. Means \pm s.d. are from three independent experiments. The data are from Fig. **1a** and Supplementary Fig. **1** and **2. (a, b)**. Source data are provided as a Source Data file.



Supplementary Fig. 11. Effect of pegRNA scaffold structure on editing efficiency.

(a) Schematic diagrams illustrating the predicted secondary structures of a regular pegRNA and the apegRNAs with one A/U-to-G/C base pair change in the small hairpin. (b) Intended indel frequencies were induced by the pegRNAs in (a) under the

PE3 setting. **(c)** Schematic diagrams illustrating the predicted secondary structures of the apegRNAs with two A/U-to-G/C base pair change in the small hairpin or with a previously published structure¹⁷. **(d)** Intended indel frequencies were induced by apegRNA-2 and the pegRNAs in **(c)** under the PE3 setting. **(b, d)** Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.



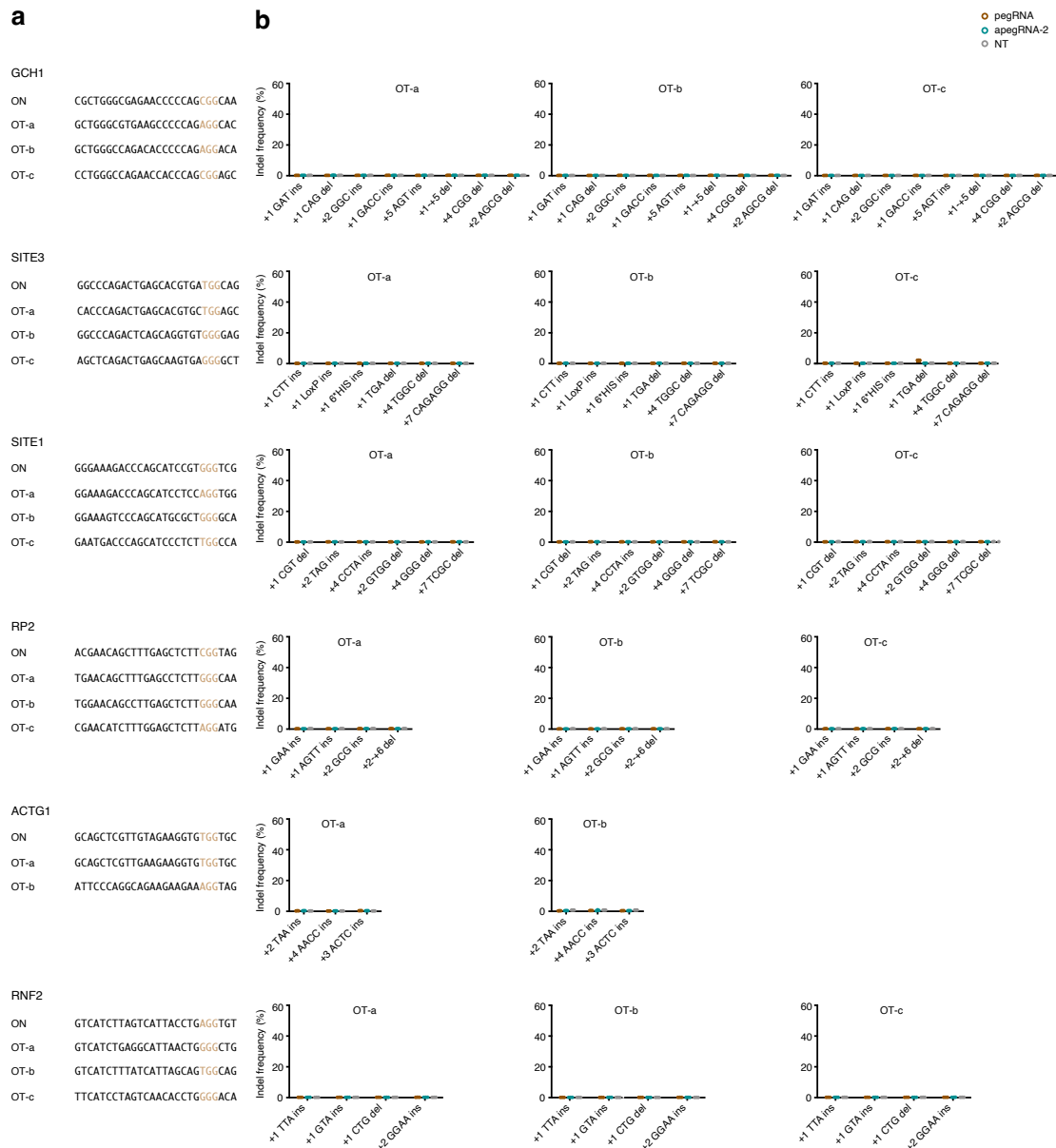
Supplementary Fig. 12. Unintended indels induced by regular pegRNA and apegRNA-2 at on-target sites.

On-target unintended indel frequencies induced by the regular pegRNA and apegRNA-2 at the indicated sites under PE3 setting. Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.

GCH1 (+1 GAT ins)		RT template scaffold insertion	
WT	GGCCGAGAACC---CAGCCGCAAGGGCTGCTCAAGA	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CAT CAGCCGCAAGGGCTGCTCAAGA	29.72%	32.53%
Byproducts	GGCCGAGAACC CA T CAGCCGCAAGGGCTGCTCAAGA 0.2800% 0.2470% GGCCGAGAACC CC AT CAGCCGCAAGGGCT GA AAAGA 0 0.0170%		
GCH1 (+1 CAG del)			
WT	GGCCGAGAACC AG CGCAAGGGCTGCTCAAGA	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGA	3.16%	16.80%
Byproducts	GGCCGAGAACC CC CGCAAGGGCT GC TCAAGA 0.1070% 0.0200%		
GCH1 (+2 GGC ins)			
WT	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGCCCT	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CCCG CGCAAGGGCTGCTCAAGCCCT	17.65%	29.32%
Byproducts	GGCCGAGAACC CCCGG CGCAAGGGCTGCTCAAGCCCT 0.2600% 0.0959%		
GCH1 (+1 GACC ins)			
WT	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGCCCT	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CCCGAC CGCAAGGGCTGCTCAAGCCCT	10.73%	27.87%
Byproducts	GGCCGAGAACC CCCGACG CGCAAGGGCTGCTCAAGCCCT 0.0253% 0.0968%		
GCH1 (+5 GAT ins)			
WT	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGCCCT	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CCCGACG CGCAAGGGCTGCTCAAGCCCT	3.11%	8.12%
Byproducts	GGCCGAGAACC CCCGACG CGCAAGGGCTGCTCAAGCCCT 0 0.0724%		
GCH1 (+1 CAGCG del)			
WT	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGCCCT	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CCCG CGCAAGGGCTGCTCAAGCCCT	8.65%	21.11%
Byproducts	GGCCGAGAACC CCCG CGCAAGGGCTGCTCAAGCCCT 0.4615% 0.7205%		
GCH1 (+4 CGG del)			
WT	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGCCCT	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CCCG CGCAAGGGCTGCTCAAGCCCT	7.33%	29.77%
Byproducts	GGCCGAGAACC CCCGG CGCAAGGGCTGCTCAAGCCCT 0.0329% 0.0802% GGCCGAGAACC CCCG CGCAAGGGCTGCTCAAGCCCT 0 0.0094%		
GCH1 (+2 AGCG del)			
WT	GGCCGAGAACC CC CGCAAGGGCTGCTCAAGCCCT	pegRNA	apegRNA-2
Complete product	GGCCGAGAACC CCCG CGCAAGGGCTGCTCAAGCCCT	9.07%	26.36%
Byproducts	GGCCGAGAACC CCCGG CGCAAGGGCTGCTCAAGCCCT 0.0171% 0.2711%		
SITE1 (+2 TAG ins)			
WT	AAGACCAGC ATCC ---GTGGCTGCTGAAAAGCTGGGAAATCGGCT	pegRNA	apegRNA-2
Complete product	AAGACCAGC ATCCG AGTGGCTGCTGAAAAGCTGGGAAATCGGCT	1.07%	6.23%
Byproducts	AAGACCAGC ATCC AGTGGCTGCTGAAAAGCTGGGAAATCGGCT 0 0.0037% AAGACCAGC ATCC AGTGGCTGCTGAAAAGCTGGGAAATCGGCT 0.0069% 0		
SITE1 (+2 GTGG del)			
WT	AAGACCAGC ATCC GTGGCTGCTGAAAAGCTGGGAAATCGGCT	pegRNA	apegRNA-2
Complete product	AAGACCAGC ATCC GTGGCTGCTGAAAAGCTGGGAAATCGGCT	20.1%	18.49%
Byproducts	AAGACCAGC ATCC GTGGCTGCTGAAAAGCTGGGAAATCGGCT 0.0140% 0.0078%		
SITE1 (+4 GGG del)			
WT	AAGACCAGC ATCC GTGGCTGCTGAAAAGCTGGGAAATCGGCT	pegRNA	apegRNA-2
Complete product	AAGACCAGC ATCCG GTGGCTGCTGAAAAGCTGGGAAATCGGCT	15.55%	22.59%
Byproducts	AAGACCAGC ATCCG GTGGCTGCTGAAAAGCTGGGAAATCGGCT 0.0022% 0.0160%		
SITE1 (+7 TCGC del)			
WT	AAGACCAGC ATCC GTGGCTGCTGAAAAGCTGGGAAATCGGCT	pegRNA	apegRNA-2
Complete product	AAGACCAGC ATCCG GTGGCTGCTGAAAAGCTGGGAAATCGGCT	2.29%	12.41%
Byproducts	AAGACCAGC ATCCG GTGGCTGCTGAAAAGCTGGGAAATCGGCT 0.0040% 0.0114%		
SITE3 (+1 TGA del)			
WT	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	pegRNA	apegRNA-2
Complete product	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	9.17%	26.58%
Byproducts	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT 0.0140% 0.0167%		
SITE3 (+4 TGGC del)			
WT	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	pegRNA	apegRNA-2
Complete product	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	8.89%	22.85%
Byproducts	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT 0.0032% 0.0567%		
SITE3 (+7 CAGAGG del)			
WT	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	pegRNA	apegRNA-2
Complete product	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	8.11%	22.85%
Byproducts	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT 0.0133% 0.0262%		
SITE3 (+1 6'HIS ins)			
WT	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	pegRNA	apegRNA-2
Complete product	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	12.50%	25.11%
Byproducts	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT 0.0200% 0.0001%		
SITE3 (+1 LoxP ins)			
WT	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	pegRNA	apegRNA-2
Complete product	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT	3.25%	15.69%
Byproducts	CAGACTGAGC ACTGA TGCAGAGGAAGAACCTCTCTCCAGAGGGCT 0 0.0100%		
RP2 (+1 GAA ins)			
WT	ACAGCTTTGAGCT---CTCGTAGTAGGTATAGGAACAT	pegRNA	apegRNA-2
Complete product	ACAGCTTTGAGCT GA CTCGTAGTAGGTATAGGAACAT	5.25%	13.74%
Byproducts	ACAGCTTTGAGCT GA CTCGTAGTAGGTATAGGAACAT 0.2970% 0.6030%		
RP2 (+1 AGTT ins)			
WT	ACAGCTTTGAGCT---CTCGTAGTAGGTATAGGAACAT	pegRNA	apegRNA-2
Complete product	ACAGCTTTGAGCT AGTT CTCGTAGTAGGTATAGGAACAT	5.75%	11.54%
Byproducts	ACAGCTTTGAGCT AGTT CTCGTAGTAGGTATAGGAACAT 0.3430% 1.0500%		
RP2 (+2 GCG ins)			
WT	ACAGCTTTGAGCT---CTCGTAGTAGGTATAGGAACAT	pegRNA	apegRNA-2
Complete product	ACAGCTTTGAGCT CGG CTCGTAGTAGGTATAGGAACAT	2.60%	5.84%
Byproducts	ACAGCTTTGAGCT CGG CTCGTAGTAGGTATAGGAACAT 0.0005% 0.0009%		
RP2 (+2 TTCGG del)			
WT	ACAGCTTTGAGCTCTCGTAGTAGGTATAGGAACAT	pegRNA	apegRNA-2
Complete product	ACAGCTTTGAGCTCTCGTAGTAGGTATAGGAACAT	0.29%	1.67%
Byproducts	ACAGCTTTGAGCTCTCGTAGTAGGTATAGGAACAT 0.0009% 0.0003%		
ACTG1 (+2 TAA ins)			
WT	CTCGTTGAGAAGG---TGGCGCCAGACTTCTCCATGCTGCC	pegRNA	apegRNA-2
Complete product	CTCGTTGAGAAGG TA TGGCGCCAGACTTCTCCATGCTGCC	7.62%	14.18%
Byproducts	CTCGTTGAGAAGG TA TGGCGCCAGACTTCTCCATGCTGCC 0.0170% 0.0130%		
ACTG1 (+4 AAC ins)			
WT	CTCGTTGAGAAGG---TGGCGCCAGACTTCTCCATGCTGCC	pegRNA	apegRNA-2
Complete product	CTCGTTGAGAAGG GA CTGGCGCCAGACTTCTCCATGCTGCC	6.84%	12.59%
Byproducts	CTCGTTGAGAAGG GA CTGGCGCCAGACTTCTCCATGCTGCC 0.0030% 0.0100%		
ACTG1 (+3 ACTC ins)			
WT	CTCGTTGAGAAGG---TGGCGCCAGACTTCTCCATGCTGCC	pegRNA	apegRNA-2
Complete product	CTCGTTGAGAAGG ACTC TGGCGCCAGACTTCTCCATGCTGCC	3.68%	4.32%
Byproducts	CTCGTTGAGAAGG ACTC TGGCGCCAGACTTCTCCATGCTGCC 0.0020% 0.0025% CTCGTTGAGAAGG ACTC TGGCGCCAGACTTCTCCATGCTGCC 0 0		
RNF2 (+1 TTA ins)			
WT	TCTTAGTCATTAC---CTGAGGTTGTTGTTAACTCATATA	pegRNA	apegRNA-2
Complete product	TCTTAGTCATTAC TA CTGAGGTTGTTGTTAACTCATATA	9.50%	24.23%
Byproducts	TCTTAGTCATTAC TA CTGAGGTTGTTGTTAACTCATATA 0.0470% 0.0300% TCTTAGTCATTAC TA CTGAGGTTGTTGTTAACTCATATA 0 0.0300%		
RNF2 (+1 GTA ins)			
WT	TCTTAGTCATTAC---CTGAGGTTGTTGTTAACTCATATA	pegRNA	apegRNA-2
Complete product	TCTTAGTCATTAC TA CTGAGGTTGTTGTTAACTCATATA	16.46%	24.23%
Byproducts	TCTTAGTCATTAC TA CTGAGGTTGTTGTTAACTCATATA 0.4824% 0.1965%		
RNF2 (+1 CTD del)			
WT	TCTTAGTCATTAC TA CTGAGGTTGTTGTTAACTCATATA	pegRNA	apegRNA-2
Complete product	TCTTAGTCATTAC---AGGTTGTTGTTAACTCATATA	6.01%	19.48%
Byproducts	TCTTAGTCATTAC---AGGTTGTTGTTAACTCATATA 0.0900% 0.2470% TCTTAGTCATTAC---AGGTTGTTGTTAACTCATATA 0 0		
RNF2 (+2 GGAA ins)			
WT	TCTTAGTCATTAC---TGAGGTTGTTGTTAACTCATATA	pegRNA	apegRNA-2
Complete product	TCTTAGTCATTAC GA AGGTTGTTGTTAACTCATATA	8.99%	7.99%
Byproducts	TCTTAGTCATTAC GA AGGTTGTTGTTAACTCATATA 0.0778% 0.0550% TCTTAGTCATTAC GA AGGTTGTTGTTAACTCATATA 0.0718% 0		
VHL (+3 TTA ins)			
WT	GAGGGAATCCCGG---AGGCGGAGAACTGGACGAG	pegRNA	apegRNA-2
Complete product	GAGGGAATCCCGG TA AGGCGGAGAACTGGACGAG	21.71%	30.82%
Byproducts	GAGGGAATCCCGG TA AGGCGGAGAACTGGACGAG 0.0484% 0.1098%		
RUNX1 (+1 CGA del)			
WT	TTTCAGGAGGAAG CGA TGCTCAGACAGCATATT	pegRNA	apegRNA-2
Complete product	TTTCAGGAGGAAG---TGCCTCAGACAGCATATT	11.60%	16.04%
Byproducts	TTTCAGGAGGAAG---TGCCTCAGACAGCATATT 0.0368% 0.1635%		
SITES (+1 CTT ins)			
WT	CAGACTGAGCAGC---TGCAGAGCCAGAAATGCAGAG	pegRNA	apegRNA-2
Complete product	CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG	16.61%	23.66%
Byproducts	CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG 0.0532% 0.0957% CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG 0 0.0188% CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG 0 0.0043%		
SITE8 (+2 GTGG del)			
WT	CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG	pegRNA	apegRNA-2
Complete product	CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG	11.14%	14.38%
Byproducts	CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG 0.26% 0.3854% CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG 0.0131% 0.0141%		
SITE9 (+1-+5 TGAGG del)			
WT	CAGACTGAGCAGC CTT TGCAGAGCCAGAAATGCAGAG	pegRNA	apegRNA-2
Complete product	CAGACTGAGCAGC---GCTCAGCCTCCATGGAG	1.27%	4.65%
Byproducts	CAGACTGAGCAGC---GCTCAGCCTCCATGGAG 0.0002% 0.0079% CAGACTGAGCAGC---GCTCAGCCTCCATGGAG 0 0.0137%		

Supplementary Fig. 13. Byproducts induced by regular pegRNA and apegRNA-2 at on-target sites.

The frequencies of complete products and byproducts (with pegRNA scaffold incorporation) induced by the regular pegRNA and apegRNA-2 in Fig. **2b**. The frequencies are means from three biologically independent experiments.

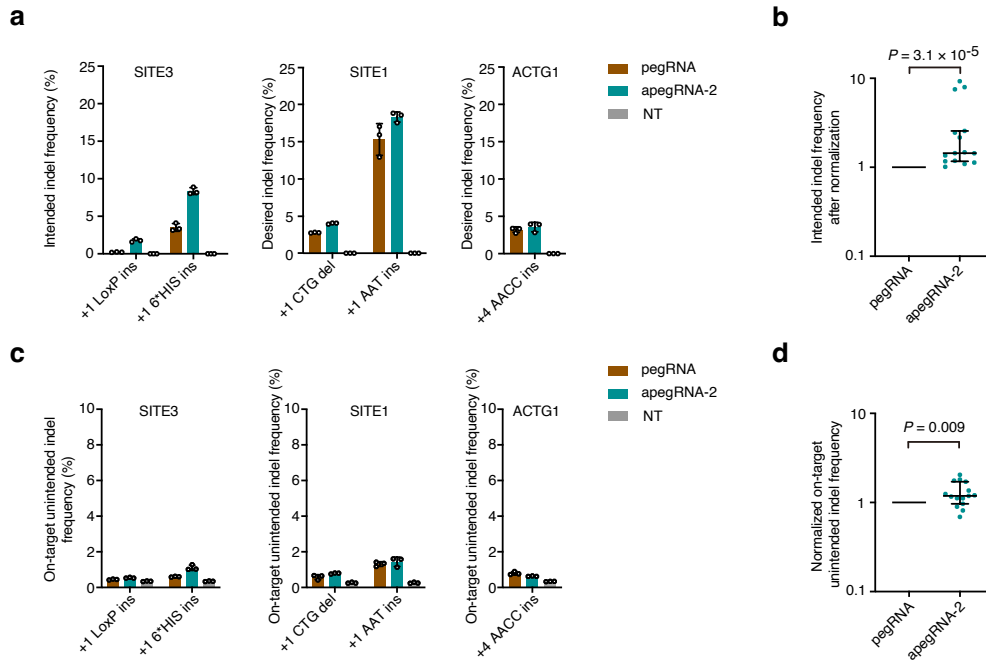


Supplementary Fig. 14. Off-target editing induced by regular pegRNA and apegRNA-2.

(a) The sequences of on- and off-target sites for the pegRNAs in (b). (b) The indel frequencies were determined at the predicted off-target sites. 293FT cells were either treated with the regular pegRNA, apegRNA-2 or left non-transfected (NT). Means \pm

s.d. are from three independent experiments. Source data are provided as a Source Data file.

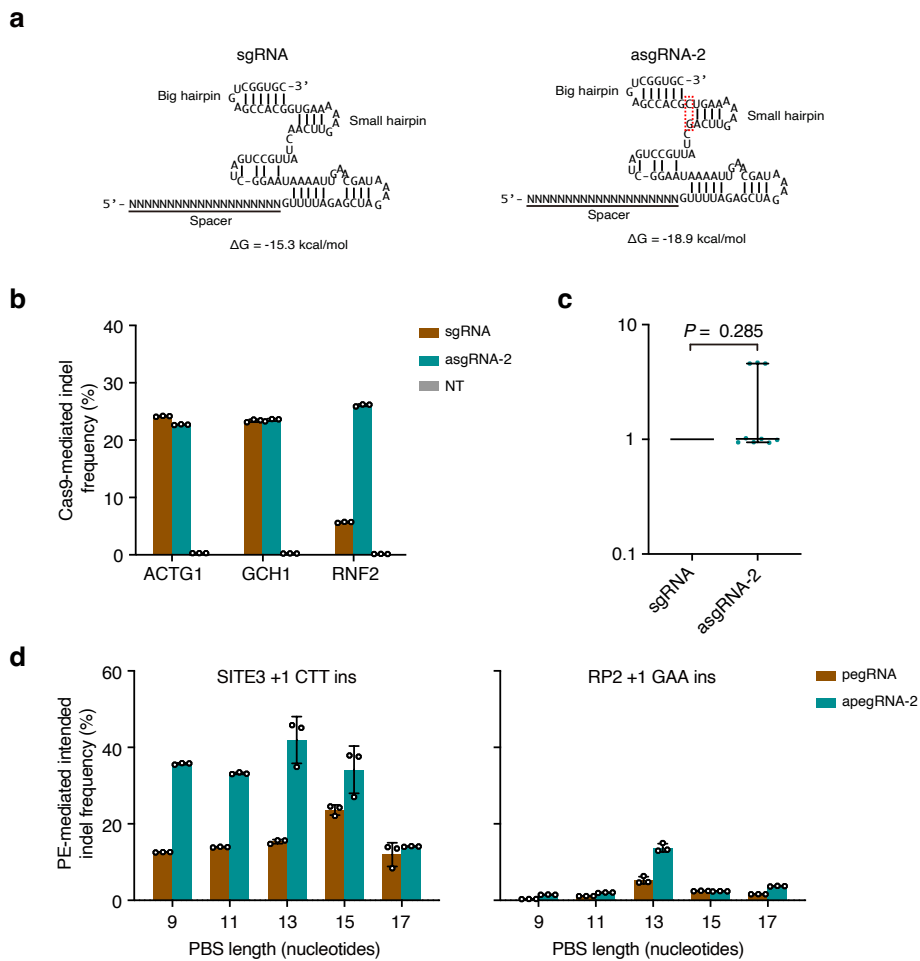
U2OS



Supplementary Fig. 15. apegRNA-2 induced higher intended indel efficiencies than regular pegRNA in U2OS cells.

(a) On-target intended indel editing frequencies induced by the regular pegRNA and apegRNA-2 at the indicated sites in U2OS cells. (b) Statistical analysis of normalized editing frequencies, setting the frequencies induced by regular pegRNAs as 1. $n = 15$ editing from three independent experiments shown in (a). (c) On-target unintended indel frequencies induced by the regular pegRNA and apegRNA-2 at the indicated sites in U2OS cells. (d) Statistical analysis of normalized indel frequencies, setting the frequencies induced by regular pegRNAs as 1. $n = 15$ editing from three independent experiments shown in (c). (a, c) Means \pm s.d. are from three independent

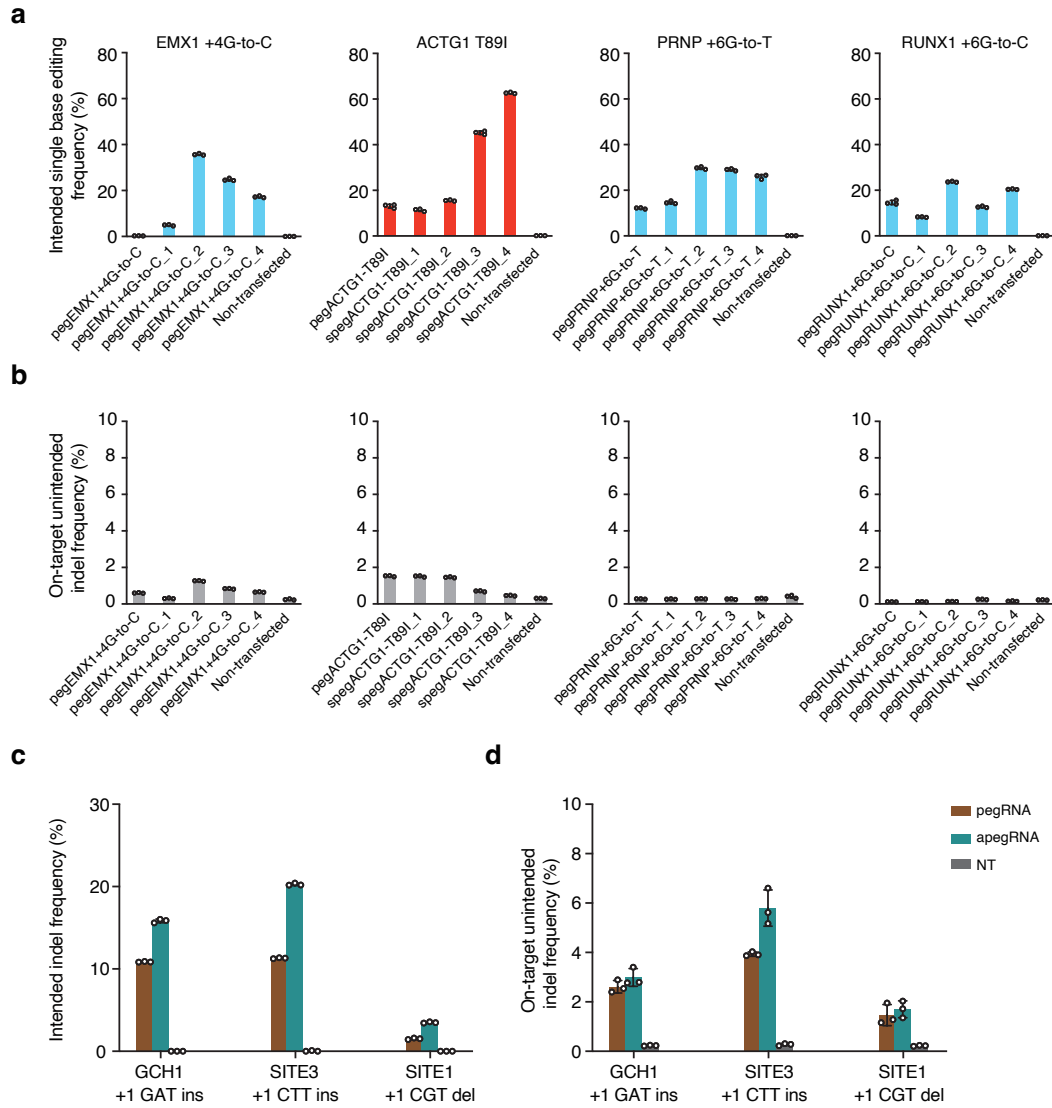
experiments. **(b, d)** P value, Wilcoxon one-tailed signed-rank test. The median and interquartile range (IQR) are shown. Source data are provided as a Source Data file.



Supplementary Fig. 16. The effect of sgRNA scaffold structure on Cas9-mediated gene editing efficiency and the effect of apegRNA-2 PBS length on PE3-mediated prime editing efficiency.

(a) Schematic diagrams illustrating the predicted secondary structures of the regular sgRNA and asgRNA-2, which contains the same small hairpin as apegRNA-2. (b) On-target indel frequencies were induced by the co-expression of Cas9 nuclease and the regular sgRNA or asgRNA-2. (c) Statistical analysis of normalized indel frequencies, setting the frequencies induced by the regular sgRNA as 1. n = 9 editing from three independent experiments shown in (b). *P* value, Wilcoxon one-tailed

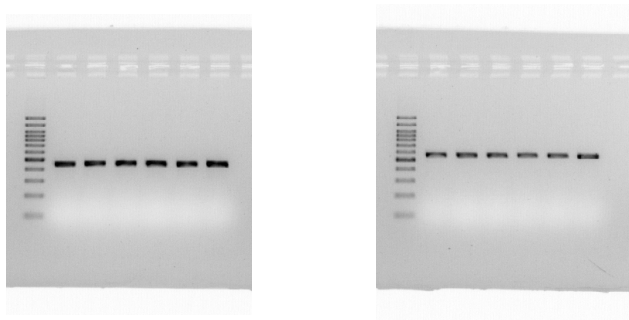
signed-rank test. The median and interquartile range (IQR) are shown. **(d)** The intended indel editing frequencies induced by the regular pegRNA and apegRNA-2 with different length of PBS at the indicated target sites. **(b, d)** Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.



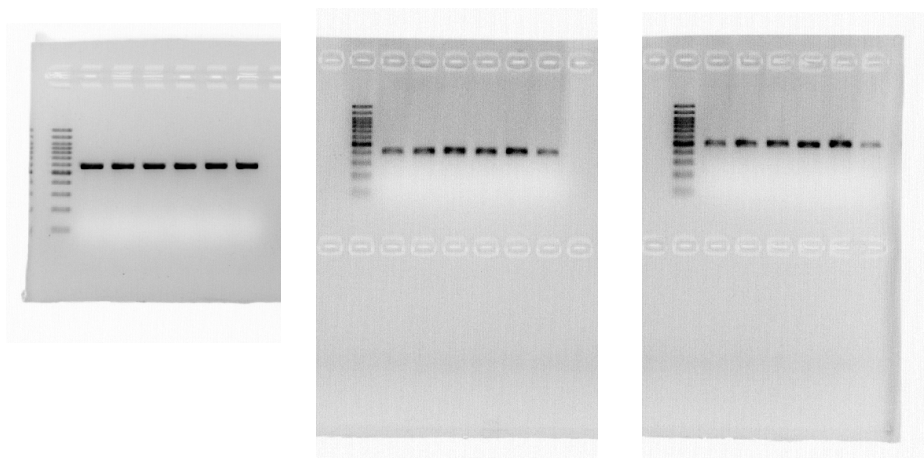
Supplementary Fig. 17. Efficacy of spgRNA and apegRNA in PE2 system.

(a, b) The intended single-base editing frequencies (a) and unintended indel frequencies (b) were induced by the regular pegRNA and the pegRNAs containing additional base substitutions under the PE2 setting. (c, d) Intended indel editing frequencies (c) and unintended indel frequencies (d) induced by the regular pegRNA and apegRNA at the indicated target sites under the PE2 setting. Means \pm s.d. are from three independent experiments. Source data are provided as a Source Data file.

Uncropped gels for Supplementary Fig. 6b



Uncropped gels for Supplementary Fig. 6d



Uncropped gels Supplementary Fig. 6.