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Supplemental information

Cas9 protein delivery non-integrating lentiviral vectors for gene correction in sickle cell disease

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Supplementary table

Supplementary table 1. Guide RNA target sequence

Guide RNA	Target sequence
GFP	GGGCACGGGCAGCTTGCCGG
BG1	CTTGCCCCACAGGGCAGTAA
BG2	TCCACATGCCCAGTTTCTAT
BG3	ACCAATAGAAACTGGGCATG
BG4	CCACGTTCACCTTGCCCCAC
BG5	AAGGTGAACGTGGATGAAGT
BG6	TCCCACCCTTAGGCTGCTGG
BG7	GCAACCTCAAACAGACACCA
BG8	GTGAACGTGGATGAAGTTGG
BG9	GAAGTTGGTGGTGAGGCCCT
BG10	GTTACTGCCCTGTGGGGCA
BG11	GTAACGGCAGACTTCTCCTC
SG11	GTAACGGCAGACTTCTCCAC

Supplementary table 2. Statistical analysis among all groups (Tukey's HSD test)

Figure 1b

No mutation vector titers	None	CypA-Cas9 2mg	CypA-Cas9 4mg	CypA-Cas9 8mg	Cas9-CypA 2mg	Cas9-CypA 4mg	Cas9-CypA 8mg
None	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CypA-Cas9 2mg	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CypA-Cas9 4mg	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CypA-Cas9 8mg	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cas9-CypA 2mg	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cas9-CypA 4mg	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cas9-CypA 8mg	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Mutation vector titers	None	CypA-Cas9 2mg	CypA-Cas9 4mg	CypA-Cas9 8mg	Cas9-CypA 2mg	Cas9-CypA 4mg	Cas9-CypA 8mg
None	n.s.	n.s.	n.s.	<0.05	<0.05	n.s.	<0.05
CypA-Cas9 2mg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CypA-Cas9 4mg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CypA-Cas9 8mg	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cas9-CypA 2mg	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cas9-CypA 4mg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cas9-CypA 8mg	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Figure 1d

GFP-YFP-	Cas9 integration	Integrating gRNA with CypA-Cas9	Integrating gRNA with Cas9-CypA	Non-integrating gRNA with CypA-Cas9	Non-integrating gRNA with Cas9-CypA	No Cas9	No transduction

Cas9 integration	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Integrating gRNA with CypA-Cas9	<0.01	n.s.	n.s.	n.s.	n.s.	<0.01	<0.01
Integrating gRNA with Cas9-CypA	<0.01	n.s.	n.s.	n.s.	<0.05	<0.01	<0.01
Non-integrating gRNA with CypA-Cas9	<0.01	n.s.	n.s.	n.s.	n.s.	<0.01	<0.01
Non-integrating gRNA with Cas9-CypA	<0.01	n.s.	<0.05	n.s.	n.s.	<0.01	<0.01
No Cas9	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

GFP+YFP+	Cas9 integration	Integrating gRNA with CypA-Cas9	Integrating gRNA with Cas9-CypA	Non-integrating gRNA with CypA-Cas9	Non-integrating gRNA with Cas9-CypA	No Cas9	No transduction
Cas9 integration	n.s.	<0.01	<0.01	<0.01	<0.01	n.s.	<0.05
Integrating gRNA with CypA-Cas9	<0.01	n.s.	n.s.	n.s.	n.s.	<0.01	<0.01
Integrating gRNA with Cas9-CypA	<0.01	n.s.	n.s.	n.s.	n.s.	<0.01	<0.01
Non-integrating gRNA with CypA-Cas9	<0.01	n.s.	n.s.	n.s.	n.s.	<0.01	<0.01
Non-integrating gRNA with Cas9-CypA	<0.01	n.s.	n.s.	n.s.	n.s.	<0.01	<0.01
No Cas9	n.s.	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01
No transduction	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.

GFP-YFP+	Cas9 integration	Integrating gRNA with CypA-Cas9	Integrating gRNA with Cas9-CypA	Non-integrating gRNA with CypA-Cas9	Non-integrating gRNA with Cas9-CypA	No Cas9	No transduction
Cas9 integration	n.s.	<0.05	n.s.	n.s.	<0.01	<0.01	<0.01
Integrating gRNA with CypA-Cas9	<0.05	n.s.	n.s.	<0.01	<0.01	<0.01	<0.01
Integrating gRNA with Cas9-CypA	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01	<0.01
Non-integrating gRNA with CypA-Cas9	n.s.	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01
Non-integrating gRNA with Cas9-CypA	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01
No Cas9	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

Figure 1e

GFP-YFP-	CypA-Cas9	Cas9-CypA	Cas9	No Cas9	No transduction
CypA-Cas9	n.s.	n.s.	n.s.	<0.01	<0.01
Cas9-CypA	n.s.	n.s.	n.s.	<0.01	<0.01
Cas9	n.s.	n.s.	n.s.	<0.01	<0.01
No Cas9	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	n.s.	n.s.

GFP+YFP+	CypA-Cas9	Cas9-CypA	Cas9	No Cas9	No transduction
CypA-Cas9	n.s.	<0.05	n.s.	<0.01	<0.01
Cas9-CypA	<0.05	n.s.	n.s.	<0.01	<0.01
Cas9	n.s.	n.s.	n.s.	<0.01	<0.01
No Cas9	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	n.s.	n.s.

GFP-YFP+	CypA-Cas9	Cas9-CypA	Cas9	No Cas9	No transduction
CypA-Cas9	n.s.	<0.05	<0.05	<0.01	<0.01
Cas9-CypA	<0.05	n.s.	n.s.	<0.01	<0.01
Cas9	<0.05	n.s.	n.s.	<0.01	<0.01
No Cas9	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	n.s.	n.s.

Figure 1h

GFP-YFP-	Integrating Cas9	Donor vector with Cas9 protein	gRNA vector with Cas9 protein	No gRNA	No Cas9	No transduction
Integrating Cas9	n.s.	<0.01	n.s.	<0.01	<0.01	<0.01
Donor vector with Cas9 protein	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01
gRNA vector with Cas9 protein	n.s.	<0.01	n.s.	<0.01	<0.01	<0.01
No gRNA	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.
No Cas9	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.

GFP+YFP+	Integrating Cas9	Donor vector with Cas9 protein	gRNA vector with Cas9 protein	No gRNA	No Cas9	No transduction
Integrating Cas9	n.s.	<0.05	n.s.	n.s.	n.s.	<0.01
Donor vector with Cas9 protein	<0.05	n.s.	<0.05	n.s.	<0.01	<0.01
gRNA vector with Cas9 protein	n.s.	<0.05	n.s.	n.s.	n.s.	<0.01
No gRNA	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
No Cas9	n.s.	<0.01	n.s.	n.s.	n.s.	<0.01

No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.

GFP-YFP+	Integrating Cas9	Donor vector with Cas9 protein	gRNA vector with Cas9 protein	No gRNA	No Cas9	No transduction
Integrating Cas9	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01
Donor vector with Cas9 protein	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.
gRNA vector with Cas9 protein	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01
No gRNA	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.
No Cas9	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.
No transduction	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.

Figure 2b

Titers	All-in-one	2xgRNA	4xgRNA	6xgRNA	9xgRNA
All-in-one	n.s.	n.s.	<0.01	<0.01	<0.05
2xgRNA	n.s.	n.s.	<0.05	<0.05	n.s.
4xgRNA	<0.01	<0.05	n.s.	n.s.	n.s.
6xgRNA	<0.01	<0.05	n.s.	n.s.	n.s.
9xgRNA	<0.05	n.s.	n.s.	n.s.	n.s.

Figure 2c

GFP-YFP-	All-in-one	2xgRNA	4xgRNA	6xgRNA	9xgRNA	Donor only	No transduction
All-in-one	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2xgRNA	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01
4xgRNA	<0.01	<0.01	n.s.	n.s.	<0.01	<0.01	<0.01
6xgRNA	<0.01	<0.01	n.s.	n.s.	<0.01	<0.01	<0.01
9xgRNA	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01
Donor only	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

GFP+YFP+	All-in-one	2xgRNA	4xgRNA	6xgRNA	9xgRNA	Donor only	No transduction
All-in-one	n.s.	<0.05	<0.05	<0.05	<0.01	<0.05	<0.01
2xgRNA	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
4xgRNA	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
6xgRNA	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
9xgRNA	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Donor only	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No transduction	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

GFP-YFP+	All-in-one	2xgRNA	4xgRNA	6xgRNA	9xgRNA	Donor only	No transduction
All-in-one	n.s.	n.s.	<0.05	n.s.	n.s.	<0.01	<0.01
2xgRNA	n.s.	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.
4xgRNA	<0.05	<0.01	n.s.	n.s.	<0.01	<0.01	<0.01
6xgRNA	n.s.	<0.01	n.s.	n.s.	<0.01	<0.01	<0.01
9xgRNA	n.s.	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.
Donor only	<0.01	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.
No transduction	<0.01	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.

Figure 2d

GFP-YFP-	MOI 5	MOI 10	MOI 25	MOI 50	4xgRNA	Donor only	No transduction
MOI 5	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
MOI 10	<0.01	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01
MOI 25	<0.01	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01
MOI 50	<0.01	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01
4xgRNA	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01

Donor only	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

GFP+YFP+	MOI 5	MOI 10	MOI 25	MOI 50	4xgRNA	Donor only	No transduction
MOI 5	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MOI 10	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MOI 25	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
MOI 50	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05
4xgRNA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Donor only	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No transduction	n.s.	n.s.	<0.01	<0.05	n.s.	n.s.	n.s.

GFP-YFP+	MOI 5	MOI 10	MOI 25	MOI 50	4xgRNA	Donor only	No transduction
MOI 5	n.s.	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05
MOI 10	<0.05	n.s.	<0.01	<0.01	n.s.	<0.01	<0.01
MOI 25	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01
MOI 50	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01
4xgRNA	<0.01	n.s.	<0.01	<0.01	n.s.	<0.01	<0.01
Donor only	<0.05	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.05	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

Figure 3b

β-globin /YFP intensity	Integrating						Non-integrating						No transduction
	BG1	BG3	BG4	BG5	BG8	SG11	BG1	BG3	BG4	BG5	BG8	SG11	

Integrating	BG1	n.s.	<0.01	n.s.	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01
	BG3	<0.01	n.s.	<0.01	n.s.	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	BG4	n.s.	<0.01	n.s.	<0.01	<0.05	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	BG5	<0.01	n.s.	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	BG8	<0.01	n.s.	<0.05	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	SG11	n.s.	<0.01	n.s.	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Non-integrating	BG1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01	n.s.
	BG3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.	<0.05	<0.01	<0.01	<0.05
	BG4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01	n.s.
	BG5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	n.s.	<0.05	n.s.	<0.01
	BG8	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	n.s.	n.s.	<0.01
	SG11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.	n.s.	<0.01
No transduction		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	<0.05	n.s.	<0.01	<0.01	<0.01	n.s.

Figure 3f

HDR at the DNA level	gRNA vector and donor vector	gRNA-donor all-in-one	Donor-gRNA all-in-one	Donor only	No transduction

gRNA vector and donor vector	n.s.	n.s.	n.s.	<0.05	<0.05
gRNA-donor all-in-one	n.s.	n.s.	n.s.	n.s.	n.s.
Donor-gRNA all-in-one	n.s.	n.s.	n.s.	<0.05	<0.05
Donor only	<0.05	n.s.	<0.05	n.s.	n.s.
No transduction	<0.05	n.s.	<0.05	n.s.	n.s.

Indel at the DNA level	gRNA vector and donor vector	gRNA-donor all-in-one	Donor-gRNA all-in-one	Donor only	No transduction
gRNA vector and donor vector	n.s.	n.s.	n.s.	<0.05	<0.05
gRNA-donor all-in-one	n.s.	n.s.	n.s.	<0.05	<0.05
Donor-gRNA all-in-one	n.s.	n.s.	n.s.	<0.01	<0.01
Donor only	<0.05	<0.05	<0.01	n.s.	n.s.
No transduction	<0.05	<0.05	<0.01	n.s.	n.s.

Figure 3h

Gene correction at the protein level	gRNA vector and donor vector	gRNA-donor all-in-one	Donor-gRNA all-in-one	Donor only	No transduction
gRNA vector and donor vector	n.s.	n.s.	n.s.	<0.01	<0.01
gRNA-donor all-in-one	n.s.	n.s.	n.s.	<0.05	<0.05
Donor-gRNA all-in-one	n.s.	n.s.	n.s.	<0.01	<0.01
Donor only	<0.01	<0.05	<0.01	n.s.	n.s.
No transduction	<0.01	<0.05	<0.01	n.s.	n.s.

Figure 3i

Cell counts	gRNA vector and donor vector	gRNA-donor all-in-one	Donor-gRNA all-in-one	Donor only	No transduction
gRNA vector and donor vector	n.s.	n.s.	n.s.	n.s.	n.s.
gRNA-donor all-in-one	n.s.	n.s.	n.s.	n.s.	n.s.
Donor-gRNA all-in-one	n.s.	n.s.	n.s.	n.s.	n.s.
Donor only	n.s.	n.s.	n.s.	n.s.	n.s.
No transduction	n.s.	n.s.	n.s.	n.s.	n.s.

Supplementary figure 1c

%GFP	Cas9 integration	CypA-Cas9	Cas9-CypA	No Cas9	No transduction
Cas9 integration	n.s.	<0.01	<0.05	<0.01	<0.01
CypA-Cas9	<0.01	n.s.	n.s.	<0.01	<0.01
Cas9-CypA	<0.05	n.s.	n.s.	<0.01	<0.01
No Cas9	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	n.s.	n.s.

Supplementary figure 2b

GFP-YFP-	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9	No transduction
One time transduction	n.s.	n.s.	<0.01	<0.01	<0.01
Two time transduction	n.s.	n.s.	<0.01	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	n.s.	n.s.

Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.	n.s.
No transduction	<0.01	<0.01	n.s.	n.s.	n.s.

GFP+YFP+	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9	No transduction
One time transduction	n.s.	n.s.	<0.05	<0.01	n.s.
Two time transduction	n.s.	n.s.	<0.05	<0.01	n.s.
One time transduction without Cas9	<0.05	<0.05	n.s.	n.s.	<0.01
Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.	<0.01
No transduction	n.s.	n.s.	<0.01	<0.01	n.s.

GFP-YFP+	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9	No transduction
One time transduction	n.s.	n.s.	<0.01	<0.01	<0.01
Two time transduction	n.s.	n.s.	<0.01	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	n.s.	n.s.
Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.	<0.01

No transduction	<0.01	<0.01	n.s.	<0.01	n.s.

Supplementary figure 2c

Day 3	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9
One time transduction	n.s.	n.s.	<0.01	<0.05
Two time transduction	n.s.	n.s.	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	<0.05
Two time transduction without Cas9	<0.05	<0.01	<0.05	n.s.

Day 6	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9
One time transduction	n.s.	n.s.	<0.01	<0.01
Two time transduction	n.s.	n.s.	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	n.s.
Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.

Day 8	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9
One time transduction	n.s.	<0.01	<0.01	n.s.
Two time transduction	<0.01	n.s.	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	<0.01
Two time transduction without Cas9	n.s.	<0.01	<0.01	n.s.

Day 10	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9
One time transduction	n.s.	n.s.	<0.01	<0.01
Two time transduction	n.s.	n.s.	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	n.s.
Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.

Day 13	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9
One time transduction	n.s.	n.s.	<0.01	<0.01

Two time transduction	n.s.	n.s.	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	n.s.
Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.

Day 15	One time transduction	Two time transduction	One time transduction without Cas9	Two time transduction without Cas9
One time transduction	n.s.	n.s.	<0.01	<0.01
Two time transduction	n.s.	n.s.	<0.01	<0.01
One time transduction without Cas9	<0.01	<0.01	n.s.	n.s.
Two time transduction without Cas9	<0.01	<0.01	n.s.	n.s.

Supplementary figure 4a

GFP-YFP-	All-in-one	Donor only	No transduction
All-in-one	n.s.	<0.01	<0.01
Donor only	<0.01	n.s.	n.s.
No transduction	<0.01	n.s.	n.s.

GFP+YFP+	All-in-one	Donor only	No transduction
All-in-one	n.s.	<0.01	<0.01
Donor only	<0.01	n.s.	n.s.
No transduction	<0.01	n.s.	n.s.

GFP-YFP+	All-in-one	Donor only	No transduction
All-in-one	n.s.	<0.01	<0.01
Donor only	<0.01	n.s.	<0.05
No transduction	<0.01	<0.05	n.s.

Supplementary figure 4b

GFP VCN	All-in-one	Donor only	No transduction
All-in-one	n.s.	<0.05	<0.05
Donor only	<0.05	n.s.	n.s.
No transduction	<0.05	n.s.	n.s.

YFP VCN	All-in-one	Donor only	No transduction
All-in-one	n.s.	<0.01	<0.01
Donor only	<0.01	n.s.	n.s.
No transduction	<0.01	n.s.	n.s.

Supplementary figure 4c

LTR VCN	All-in-one	Donor only	No transduction
All-in-one	n.s.	n.s.	n.s.
Donor only	n.s.	n.s.	n.s.
No transduction	n.s.	n.s.	n.s.

PS VCN	All-in-one	Donor only	No transduction
All-in-one	n.s.	<0.05	n.s.
Donor only	<0.05	n.s.	n.s.
No transduction	n.s.	n.s.	n.s.

Supplementary figure 5

GFP-YFP-	A promoter for YFP	No promoter for YFP	Donor only	No transduction
A promoter for YFP	n.s.	<0.01	<0.01	<0.01

No promoter for YFP	<0.01	n.s.	<0.01	<0.01
Donor only	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	n.s.	n.s.

GFP+YFP+	A promoter for YFP	No promoter for YFP	Donor only	No transduction
A promoter for YFP	n.s.	<0.01	<0.01	<0.01
No promoter for YFP	<0.01	n.s.	n.s.	n.s.
Donor only	<0.01	n.s.	n.s.	n.s.
No transduction	<0.01	n.s.	n.s.	n.s.

GFP-YFP+	A promoter for YFP	No promoter for YFP	Donor only	No transduction
A promoter for YFP	n.s.	<0.01	<0.01	<0.01
No promoter for YFP	<0.01	n.s.	<0.05	<0.05
Donor only	<0.01	<0.05	n.s.	n.s.
No transduction	<0.01	<0.05	n.s.	n.s.

Supplementary figure 6

Titers	All-in-one	9xgRNA	No Cas9
All-in-one	n.s.	<0.01	<0.05
9xgRNA	<0.01	n.s.	n.s.
No Cas9	<0.05	n.s.	n.s.

Supplementary figure 7

Cas9 amounts	All-in-one	4xgRNA	No gRNA	No Cas9
All-in-one	n.s.	<0.01	<0.05	<0.01
4xgRNA	<0.01	n.s.	<0.01	<0.01
No gRNA	<0.05	<0.01	n.s.	<0.01
No Cas9	<0.01	<0.01	<0.01	n.s.

Supplementary figure 9c

%GFP	gRNA1	gRNA2	gRNA3	gRNA4	gRNA5	gRNA6	gRNA7	gRNA8	gRNA9	gRNA10	gRNA11	Donor only
gRNA 1	n.s.	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
gRNA 2	<0.05	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
gRNA 3	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
gRNA 4	<0.01	<0.01	<0.01	n.s.	n.s.	<0.05	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01
gRNA 5	<0.01	<0.01	<0.01	n.s.	n.s.	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01
gRNA 6	<0.01	<0.01	<0.01	<0.05	<0.01	n.s.	<0.01	n.s.	<0.05	<0.01	n.s.	<0.01
gRNA 7	<0.01	<0.01	<0.01	n.s.	n.s.	<0.01	n.s.	<0.01	<0.01	<0.01	<0.01	<0.01
gRNA 8	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	n.s.	n.s.	<0.01	n.s.	<0.01
gRNA 9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	n.s.	n.s.	<0.01	n.s.	<0.01
gRNA 10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.		<0.01
gRNA 11	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	n.s.	n.s.	<0.01	n.s.	<0.01
Donor only	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.

Supplementary figure 10

βs-globin	Integrating	All-in-one	4xgRNA	No transduction
Integrating	n.s.	<0.01	<0.01	<0.01
All-in-one	<0.01	n.s.	<0.01	<0.01

4xgRNA	<0.01	<0.01	n.s.	<0.01
No transduction	<0.01	<0.01	<0.01	n.s.

DNA break	Integrating	All-in-one	4xgRNA	No transduction
Integrating	n.s.	<0.01	<0.01	<0.01
All-in-one	<0.01	n.s.	<0.01	<0.01
4xgRNA	<0.01	<0.01	n.s.	<0.01
No transduction	<0.01	<0.01	<0.01	n.s.

Supplementary figure 12a

β-globin	0.3kb	0.4kb	0.7kb	1.7kb	2.0kb	gRNA only	No transduction
0.3kb	n.s.	n.s.	n.s.	<0.05	<0.01	<0.01	<0.01
0.4kb	n.s.	n.s.	n.s.	<0.01	<0.01	<0.01	<0.01
0.7kb	n.s.	n.s.	n.s.	<0.05	<0.01	<0.01	<0.01
1.7kb	<0.05	<0.01	<0.05	n.s.	<0.01	<0.01	<0.01
2.0kb	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01
gRNA only	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.

Indel	0.3kb	0.4kb	0.7kb	1.7kb	2.0kb	gRNA only	No transduction
0.3kb	n.s.	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01
0.4kb	<0.01	n.s.	n.s.	<0.01	n.s.	<0.01	<0.01
0.7kb	<0.01	n.s.	n.s.	<0.05	n.s.	<0.01	<0.01
1.7kb	n.s.	<0.01	<0.05	n.s.	<0.05	<0.01	<0.01
2.0kb	<0.01	n.s.	n.s.	<0.05	n.s.	<0.01	<0.01
gRNA only	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01
No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.

βs-globin	0.3kb	0.4kb	0.7kb	1.7kb	2.0kb	gRNA only	No transduction
0.3kb	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
0.4kb	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
0.7kb	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01

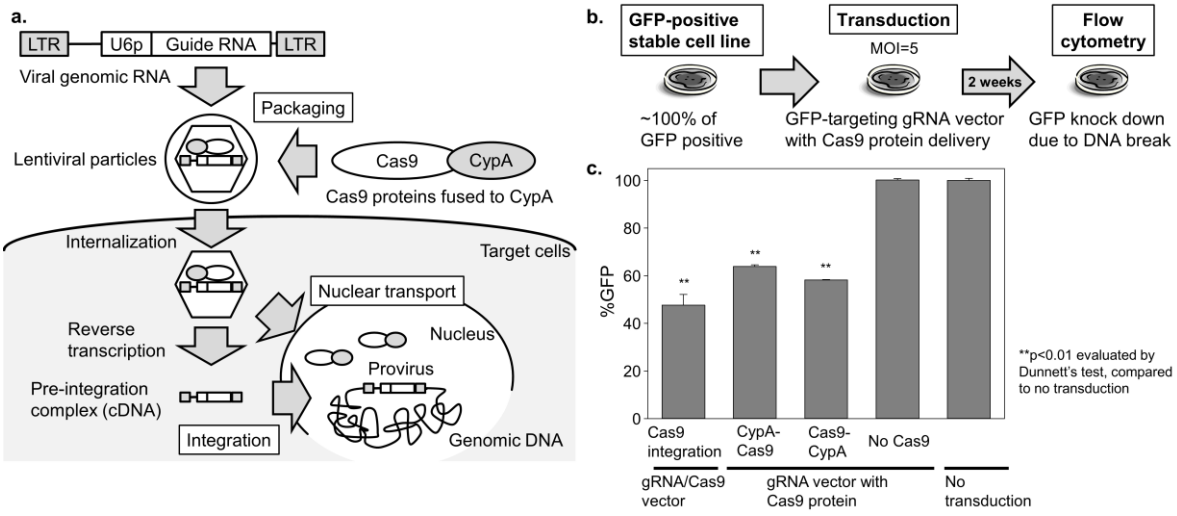
1.7kb	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
2.0kb	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
gRNA only	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01
No transduction	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.

Supplementary figure 13

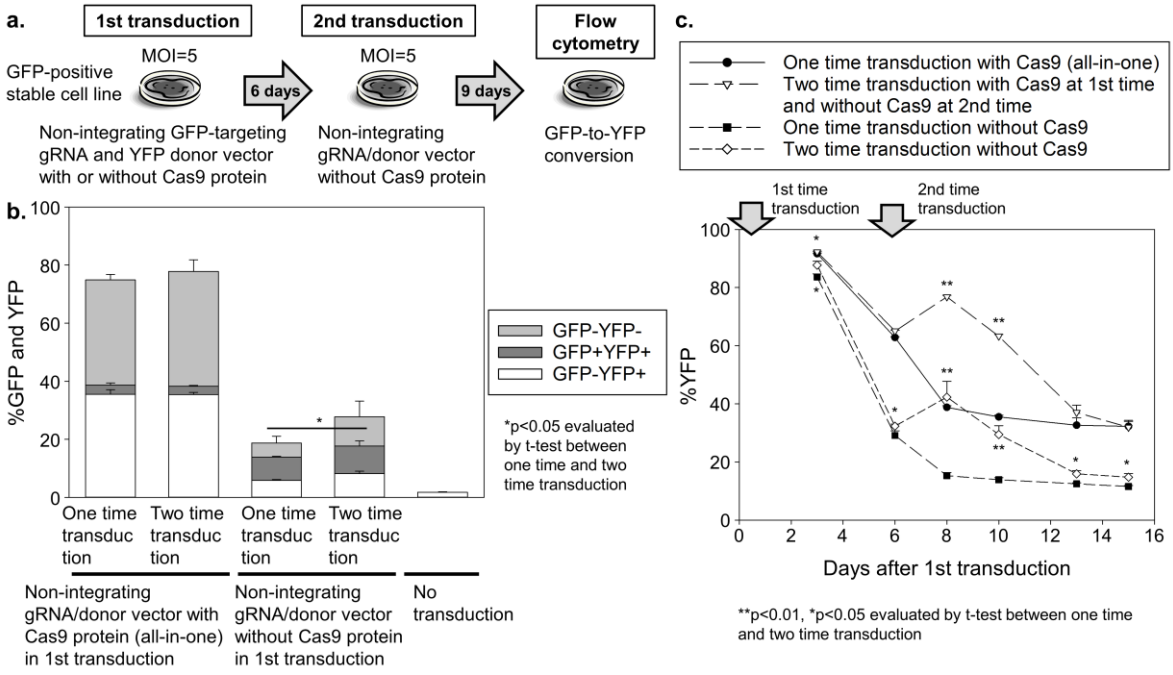
Off-target editing	gRNA vector and donor vector	gRNA-donor all-in-one	Donor-gRNA all-in-one	Donor only	No transduction
gRNA vector and donor vector	n.s.	n.s.	n.s.	n.s.	n.s.
gRNA-donor all-in-one	n.s.	n.s.	n.s.	n.s.	n.s.
Donor-gRNA all-in-one	n.s.	n.s.	n.s.	n.s.	n.s.
Donor only	n.s.	n.s.	n.s.	n.s.	n.s.
No transduction	n.s.	n.s.	n.s.	n.s.	n.s.

Supplementary figures

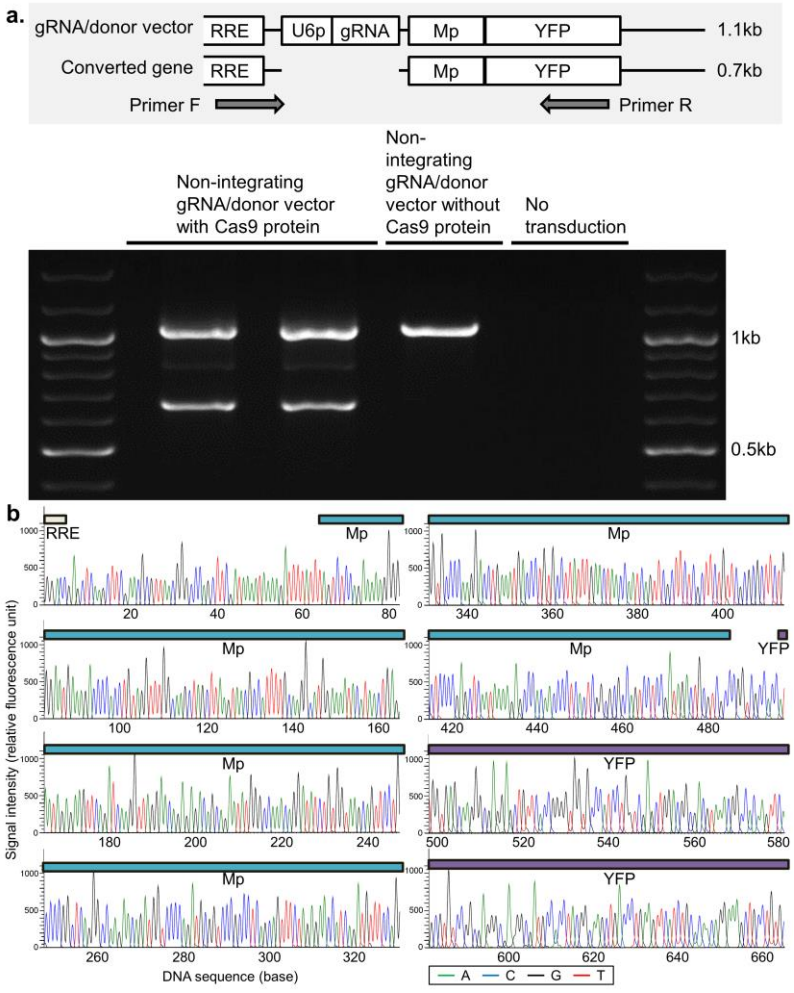
Supplementary figure 1. Genome editing with Cas9 protein delivery lentiviral vectors encoding guide RNA. (a) Hypothetical model for delivery of Cas9 protein via Cas9-CypA fusion proteins with guide RNA-encoding lentiviral vectors for genome editing. (b) A GFP knock-down model using a GFP-positive HEL cell line transduced with lentiviral vectors encoding GFP-targeting guide RNA with CypA-Cas9 or Cas9-CypA protein delivery. (c) GFP DNA breakage, evaluated by %GFP in flow cytometry 14 days post-transduction. Values: mean ± standard error. The experiments were performed in triplicate.



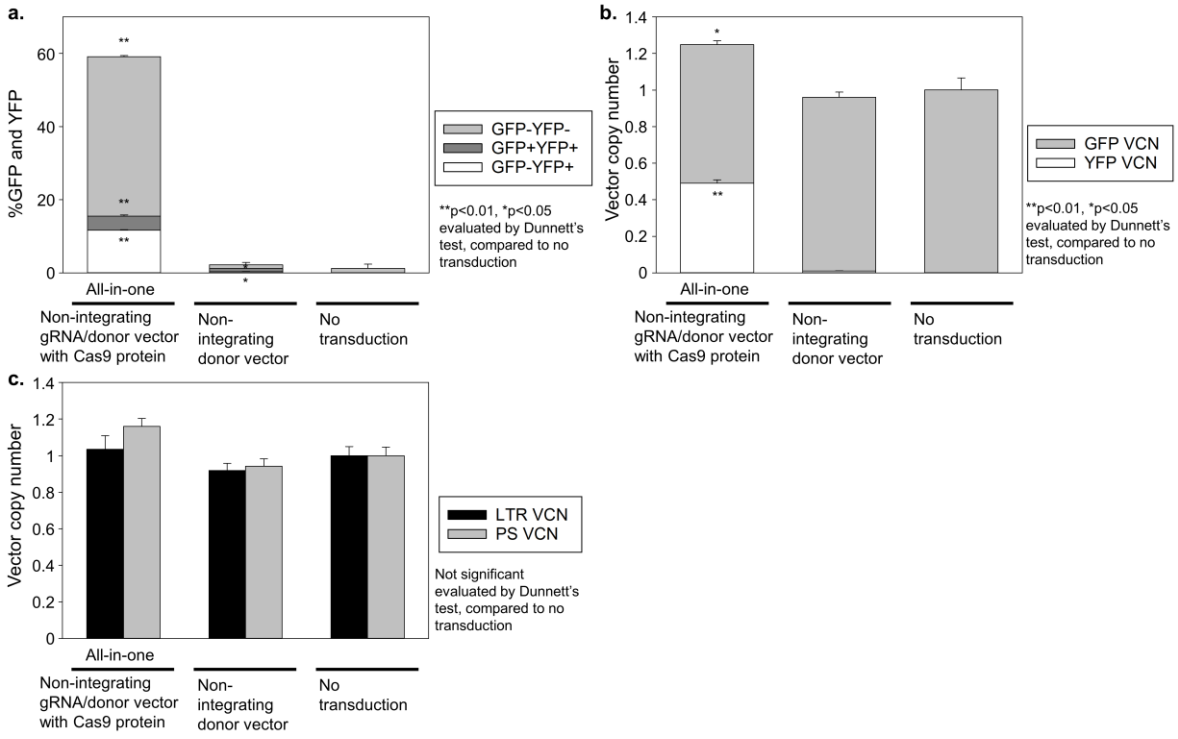
Supplementary figure 2. Transient nuclease function is observed with Cas9 protein delivery guide RNA/donor DNA NILV. (a) Transduction of a GFP+ HEL cell line with all-in-one NILVs encoding GFP-targeting guide RNA and YFP donor DNA with Cas9 protein delivery at MOI 5, followed by second transduction (6 days later) with guide RNA/donor DNA NILV without Cas9 protein at MOI 5. (b) GFP DNA breakage and GFP-to-YFP conversion, evaluated by flow cytometry 15 days post-1st transduction. (c) Time-series %YFP after 1st (and 2nd) transduction with NILVs. Values: mean ± standard error. The experiments were performed in triplicate.



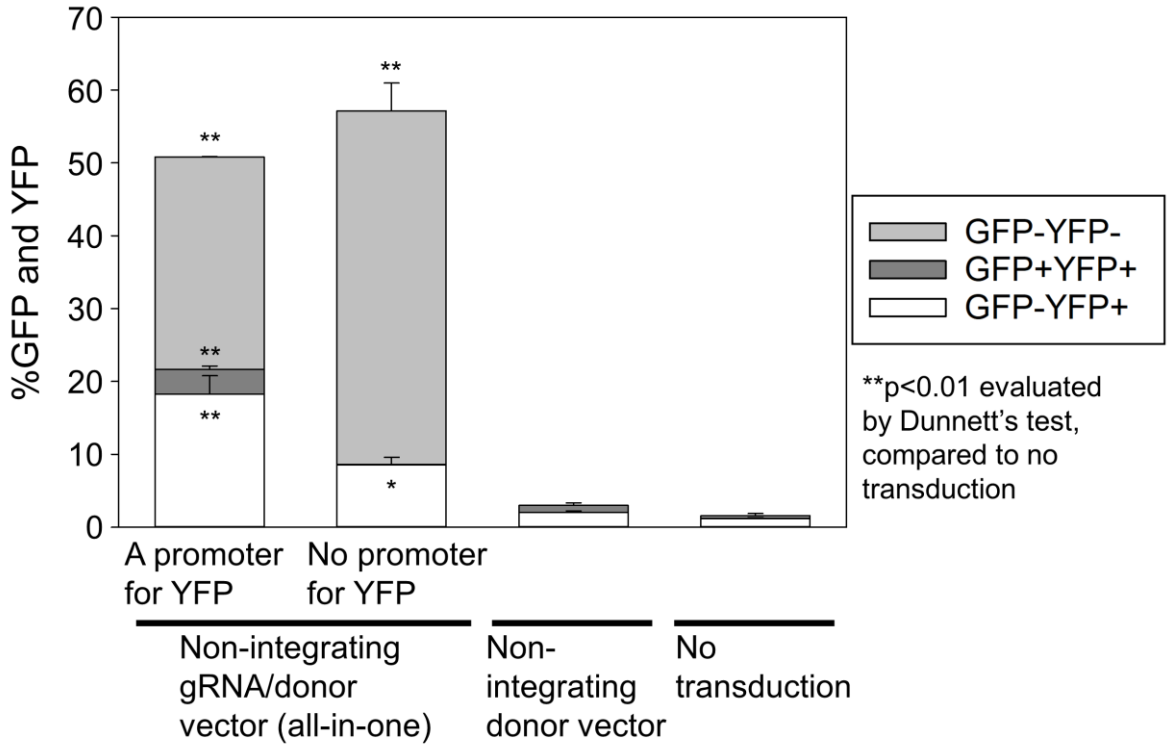
Supplementary figure 3. GFP-to-YFP gene conversion is detected by polymerase chain reaction (PCR). (a) GFP-to-YFP gene conversion using NILVs encoding GFP-targeting guide RNA and YFP donor DNA with Cas9 protein delivery, evaluated by PCR (0.7kb) as compared to YFP donor DNA vector (1.1kb) 15 days post-transduction in a GFP+ HEL cell line. (b) DNA sequencing of 0.7kb PCR fragments for GFP-to-YFP gene conversion. RRE: rev response element. The experiments were performed in single run.



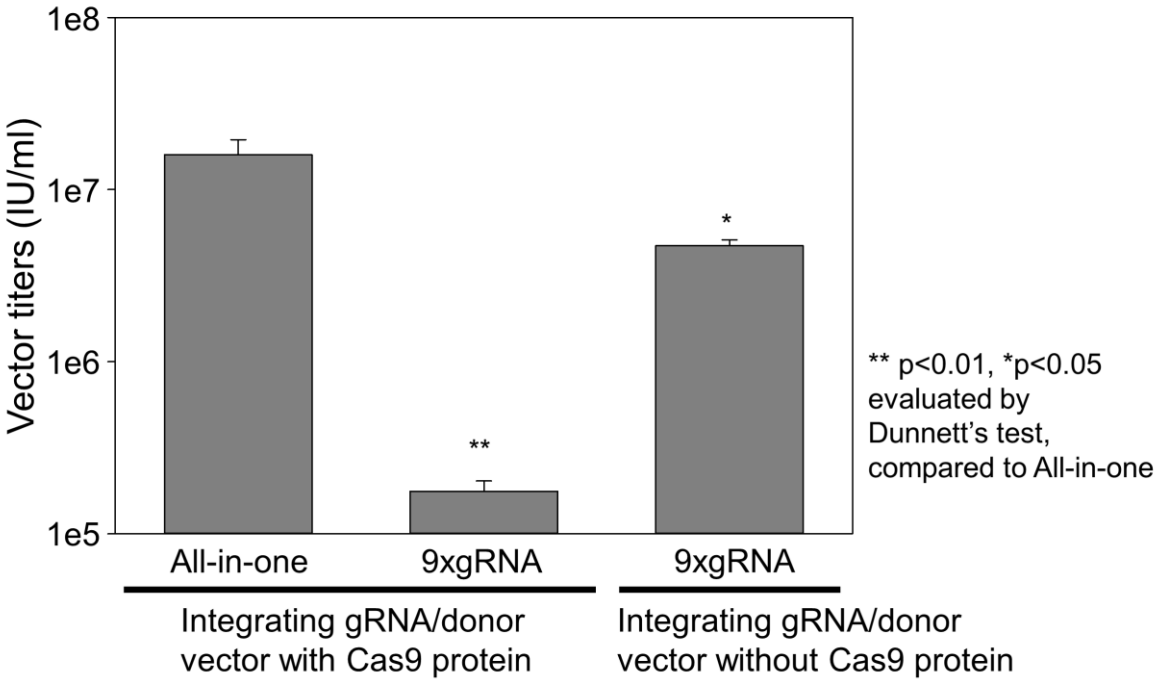
Supplementary figure 4. Comparison of GFP-to-YFP gene conversion between DNA and protein levels with Cas9 protein delivery NILVs. (a) GFP-to-YFP gene conversion using NILVs encoding GFP-targeting guide RNA and YFP donor DNA with Cas9 protein delivery (all-in-one) at the protein level, evaluated by flow cytometry 14 days post-transduction in a GFP+ HEL cell line. (b) GFP-to-YFP gene conversion at the DNA level using the same samples, evaluated by GFP- and YFP-specific vector copy number (VCN) in qPCR. (c) LTR- and PS-based VCNs in the same samples, evaluated by qPCR. Values: mean ± standard error. The experiments were performed in triplicate. LTR: long terminal repeat, PS: packaging signal.



Supplementary figure 5. GFP-to-YFP gene conversion occurs with Cas9 protein delivery guide RNA/donor DNA NILV without a promoter for YFP expression. GFP-to-YFP gene conversion 11 days post-transduction in a GFP+ HEL cell line using NILVs encoding GFP-targeting guide RNA and YFP donor DNA with Cas9 protein delivery (all-in-one vector) with or without a promoter (Mp) for YFP expression. Values: mean ± standard error. The experiments were performed in triplicate.

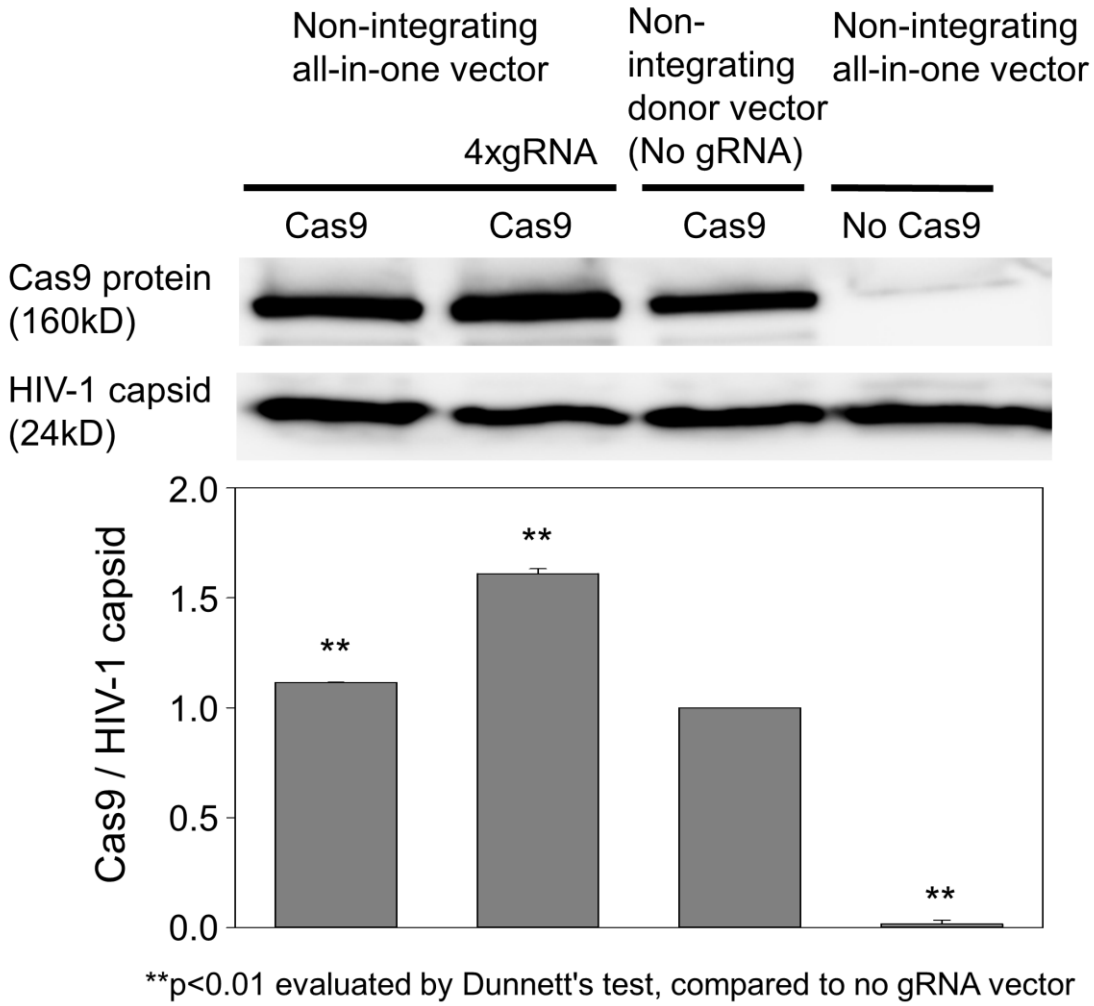


Supplementary figure 6. Lentiviral tiers for multiple guide RNA sequences with or without Cas9 protein. Integrating lentiviral vector titers for guide RNA/donor DNA (all-in-one) including 9x pseudo guide RNA with or without Cas9 protein, evaluated by YFP expression in Hela cells. Values: mean ± standard error. The experiments were performed in triplicate.



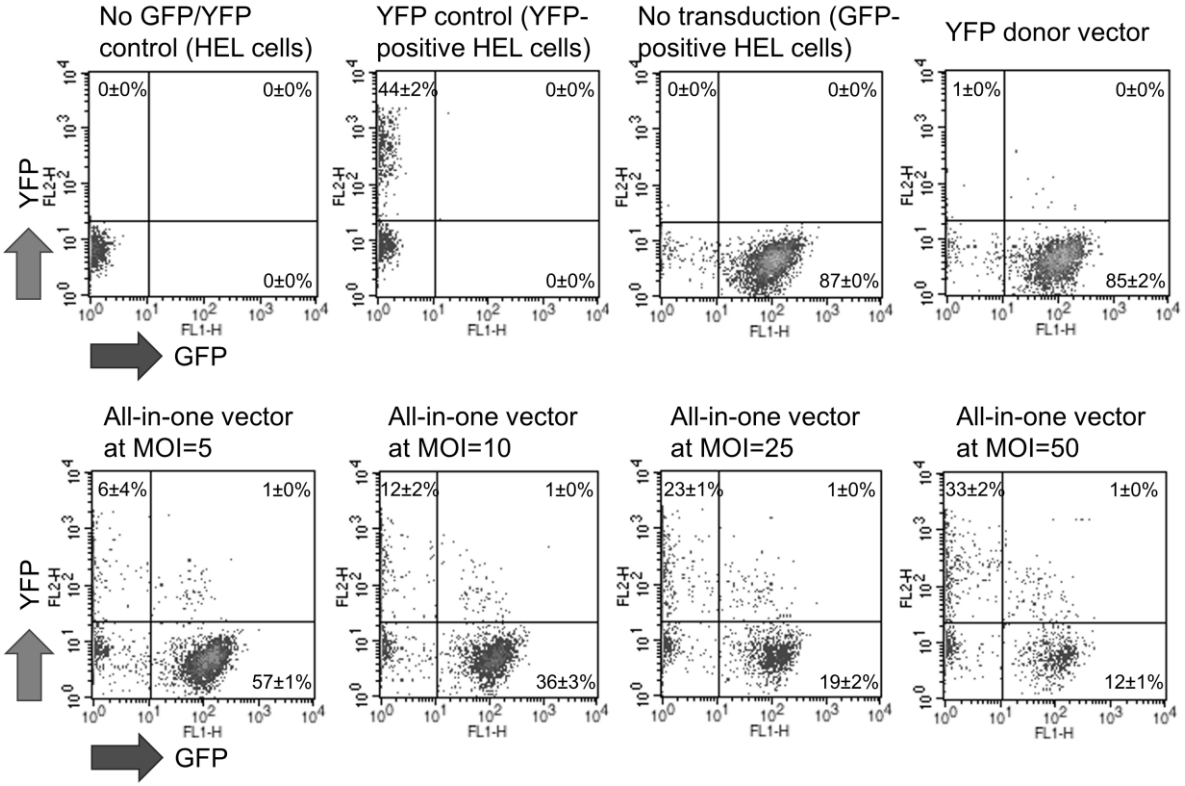
Supplementary figure 7. Detection of Cas9 protein in the pellets of lentiviral vectors.

NILVs encoding GFP-targeting guide RNA and YFP donor DNA (all-in-one), all-in-one including 4x pseudo guide RNA (4xgRNA), and donor DNA without guide RNA were prepared with a Cas9-expressing plasmid as well as without the Cas9 plasmid (only for all-in-one vector). Using Western blot analysis, Cas9 protein and HIV-1 capsid were detected in the vector pellets. The experiments were performed in duplicate.

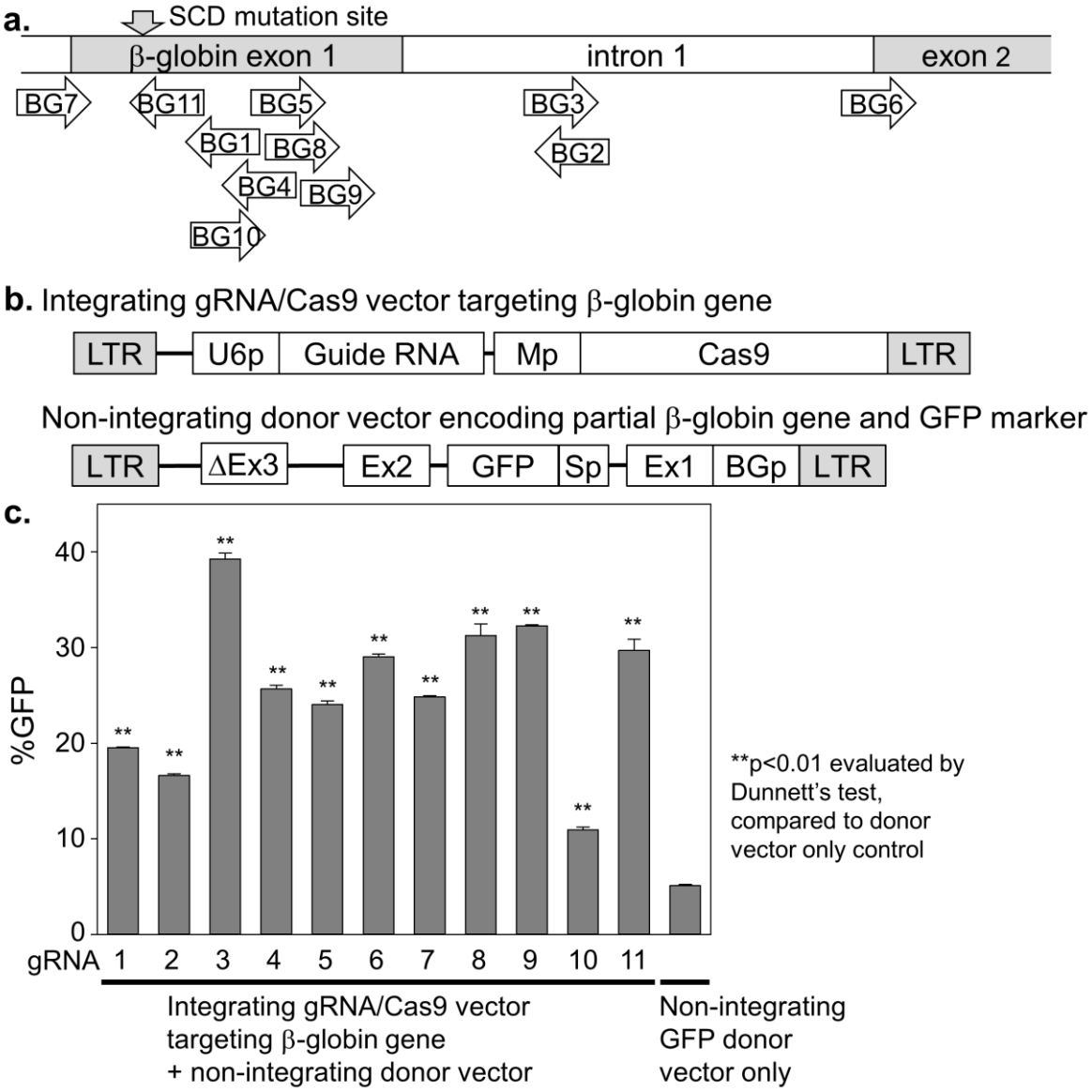


Supplementary figure 8. Flow cytometry panels to evaluate GFP-to-YFP gene conversion.

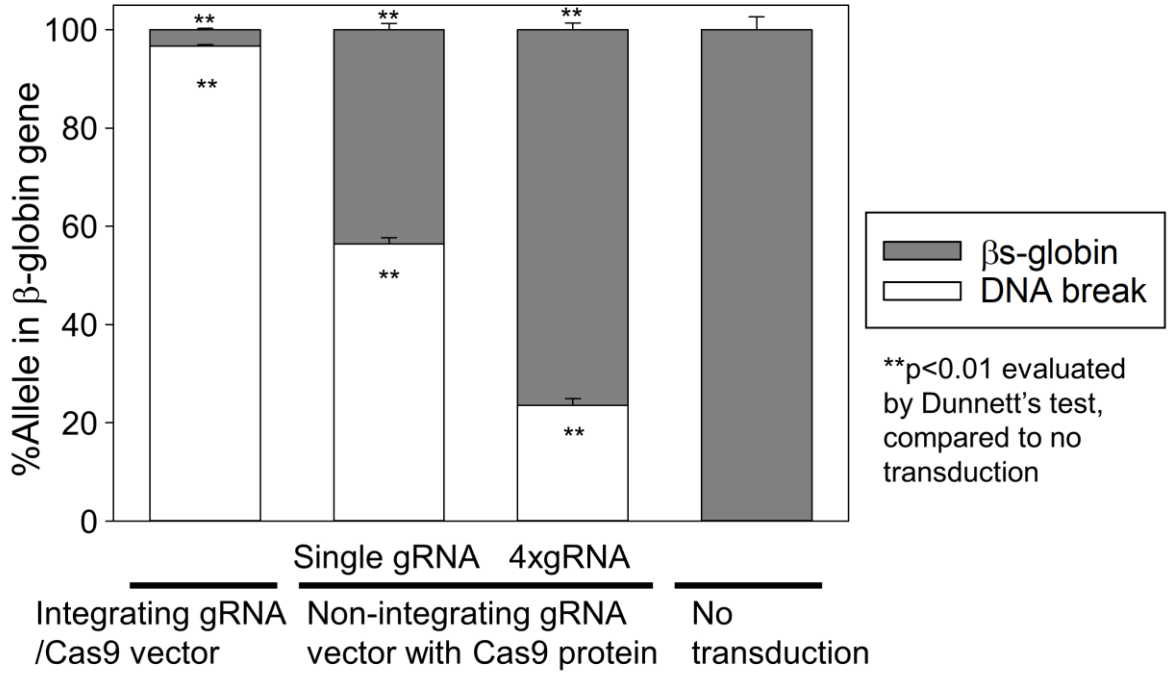
Flow cytometry panels to evaluate GFP-to-YFP gene conversion at escalating MOIs (5, 10, 25, and 50) of guide RNA/donor DNA (all-in-one) NILV with Cas9 protein delivery 14 days post-transduction in a GFP+ HEL cell line. HEL cells: human erythroleukemia cells. Values: mean ± standard error. The experiments were performed in triplicate.



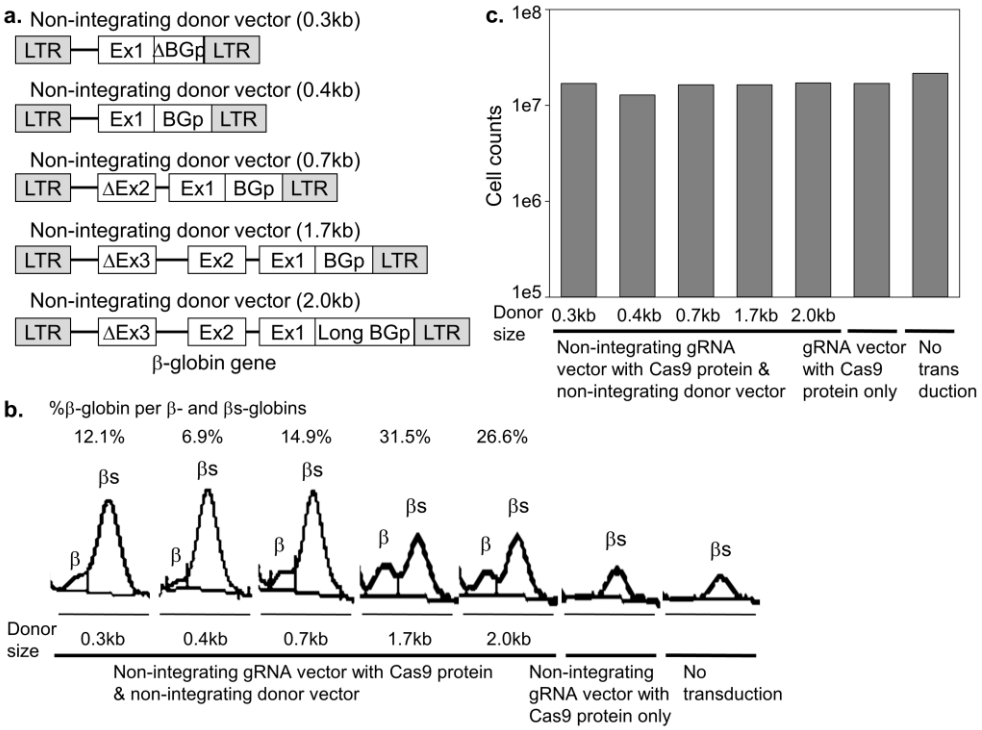
Supplementary figure 9. Screening of guide RNAs for β-globin gene editing in a GFP recombination model. (a) Design for several guide RNAs (BG1-11) targeting the β-globin gene. (b) Design of integrating guide RNA/Cas9 vectors targeting the β-globin gene as well as a donor DNA NILV encoding both partial β-globin gene and GFP marker gene. The donor vector doesn't express GFP due to the absence of a polyadenylation signal, while GFP expression is allowed by gene conversion at the endogenous β-globin gene. (c) GFP expression in K562 cells transduced with various guide RNA/Cas9 vectors and β-globin/GFP donor vector, evaluated by flow cytometry 7-8 days post-transduction. Sp: spleen focus forming virus promoter. Values: mean ± standard error. The experiments were performed in triplicate.


a.
SCD mutation site
β-globin exon 1
intron 1
exon 2
BG7
BG11
BG5
BG1
BG8
BG4
BG9
BG10
BG3
BG2
BG6
b. Integrating gRNA/Cas9 vector targeting β-globin gene
LTR
U6p
Guide RNA
Mp
Cas9
LTR
Non-integrating donor vector encoding partial β-globin gene and GFP marker
LTR
ΔEx3
Ex2
GFP
Sp
Ex1
BGp
LTR
c.
%GFP
0
10
20
30
40
gRNA 1 2 3 4 5 6 7 8 9 10 11
**p<0.01 evaluated by Dunnett's test, compared to donor vector only control
Integrating gRNA/Cas9 vector targeting β-globin gene + non-integrating donor vector
Non-integrating GFP donor vector only

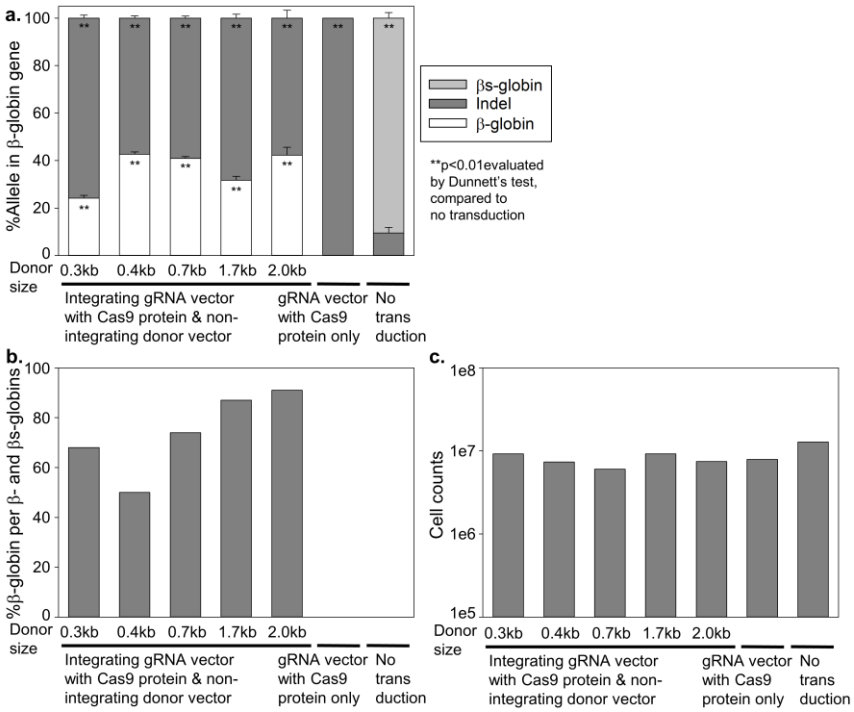
Supplementary figure 10. Efficient βs-globin DNA breakage with guide RNA NILVs with Cas9 protein delivery. The DNA break ratios in the endogenous βs-globin gene 8 days post-transduction in sickle HUDEP-2 cells, evaluated by quantitative polymerase chain reaction (qPCR). Values: mean ± standard error. The experiments were performed in triplicate.



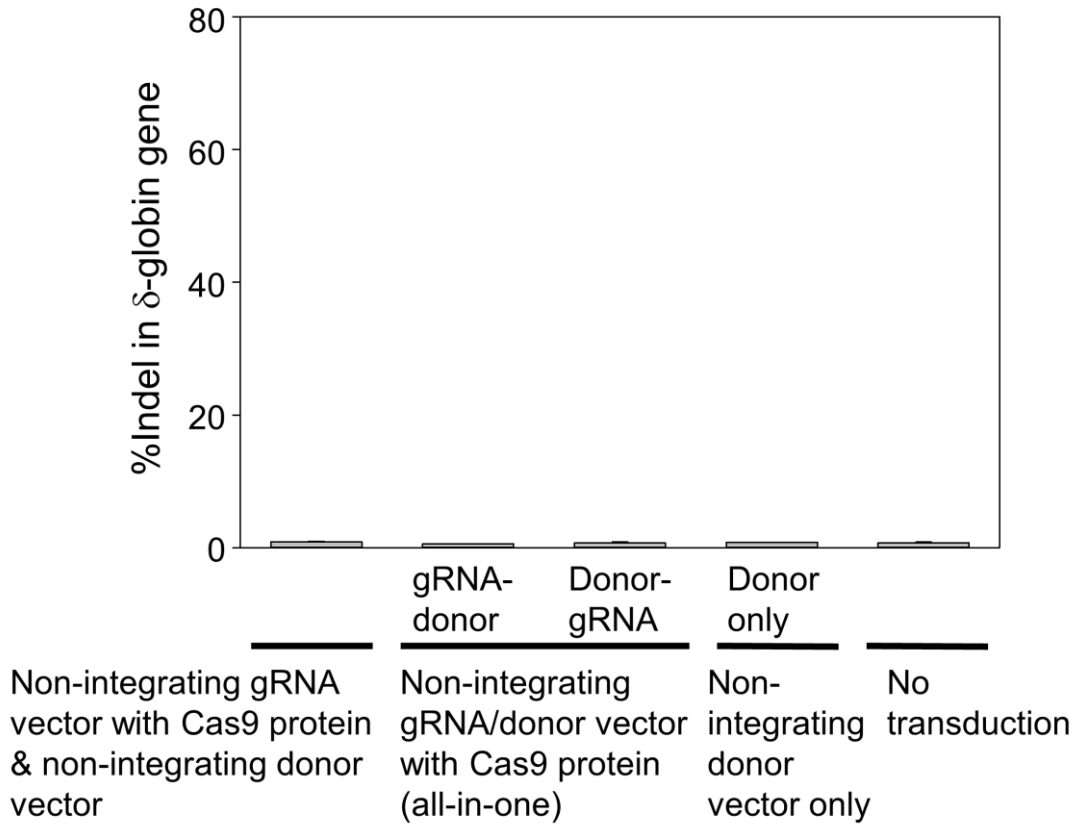
Supplementary figure 11. Various sizes of β-globin donor DNA NILVs for SCD gene correction. (a) Design for β-globin donor DNA NILVs encoding various sizes of normal β-globin gene (0.3kb, 0.4kb, 0.7kb, 1.7kb, and 2.0kb) for SCD gene correction. (b) βs-to-β-globin gene correction at the protein level (RP-HPLC) 19 days after erythroid differentiation in immortalized erythroid cells including the SCD mutation (sickle HUDEP-2 cells), which were transduced with a SG11 guide RNA vector with Cas9 protein delivery and each donor vector at MOI 25. (c) Cell counts in gene-corrected cells 19 days after erythroid differentiation. ΔEx2: 5'-side partial β-globin exon 2, ΔBGp: 3'-side β-globin promoter. All experiments were performed in a single run.



Supplementary figure 12. SCD gene correction with a Cas9 protein delivery integrating SG11 guide RNA vector along with β-globin donor DNA NILVs. (a) βs-to-β-globin gene correction at the DNA level (qPCR) 19 days after erythroid differentiation in immortalized erythroid cells including the SCD mutation (sickle HUDEP-2 cells), which were transduced with a Cas9 protein delivery integrating SG11 guide RNA vector and various sizes of β-globin donor NILVs (0.3kb, 0.4kb, 0.7kb, 1.7kb, and 2.0kb) at MOI 25. (b) βs-to-β-globin gene correction at the protein level (RP-HPLC) in gene-corrected immortalized erythroid cells. (c) Cell counts in gene-corrected cells following erythroid differentiation. Values: mean ± standard error. All experiments were performed in a single run, except Supplementary figure 12a (in triplicate).

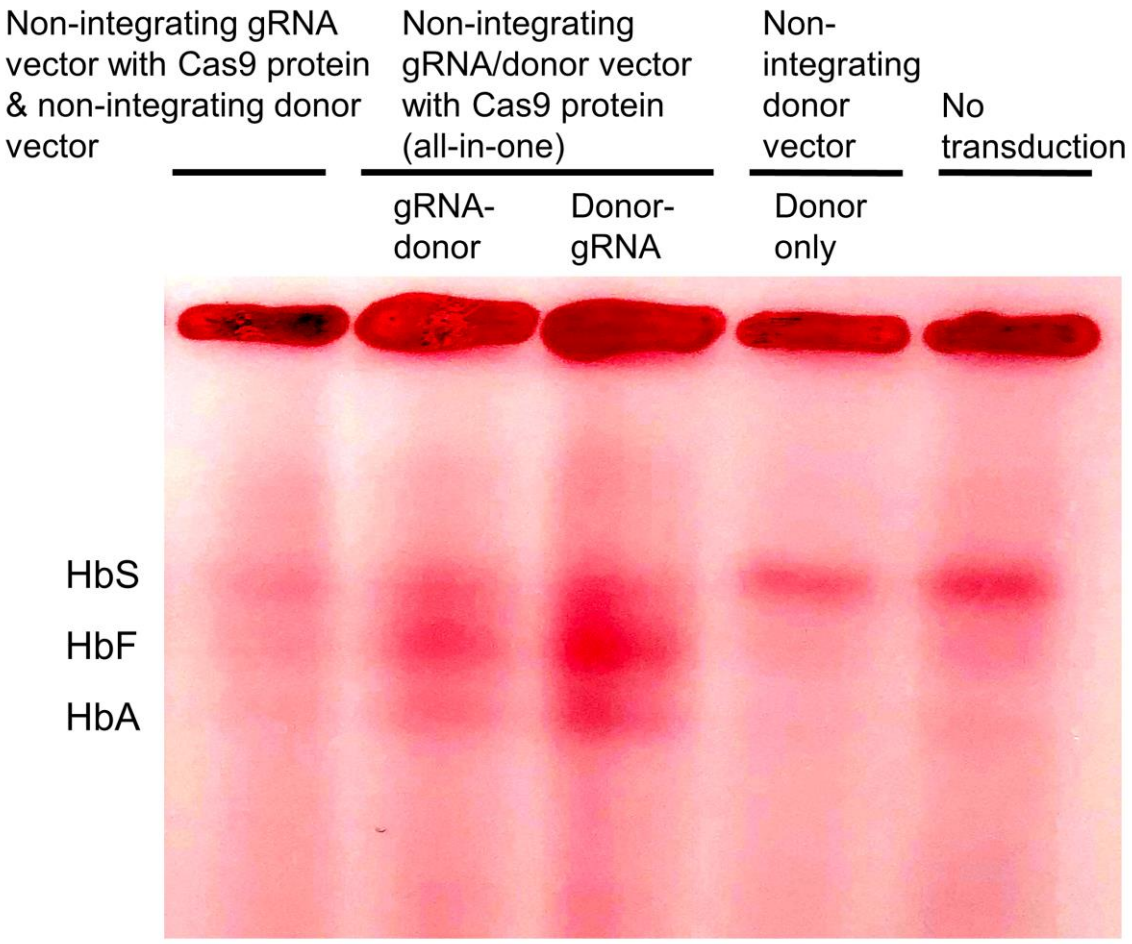


Supplementary figure 13. Undetectable off-target δ-globin gene editing in βs-to-β-globin gene correction with Cas9 protein delivery NILVs. Off-target δ-globin gene editing at the DNA level 7-8 days after erythroid differentiation in gene-corrected sickle HUDEP-2 cells, evaluated by targeted deep sequencing (the same samples in Figure 3f). Values: mean ± standard error. This experiment was performed in 1-4 times.

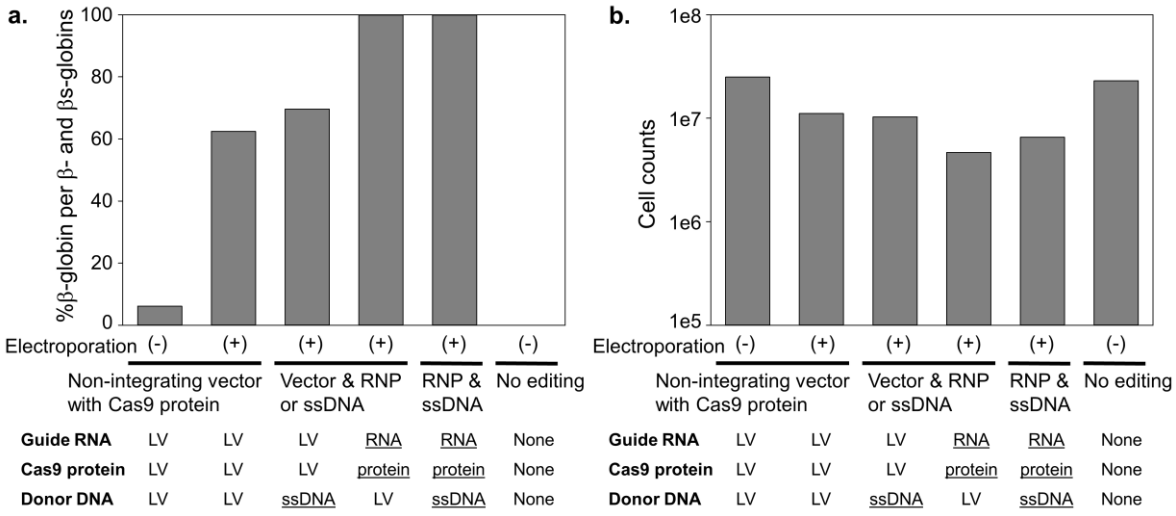


Not significant evaluated by Dunnett's test, compared to no transduction

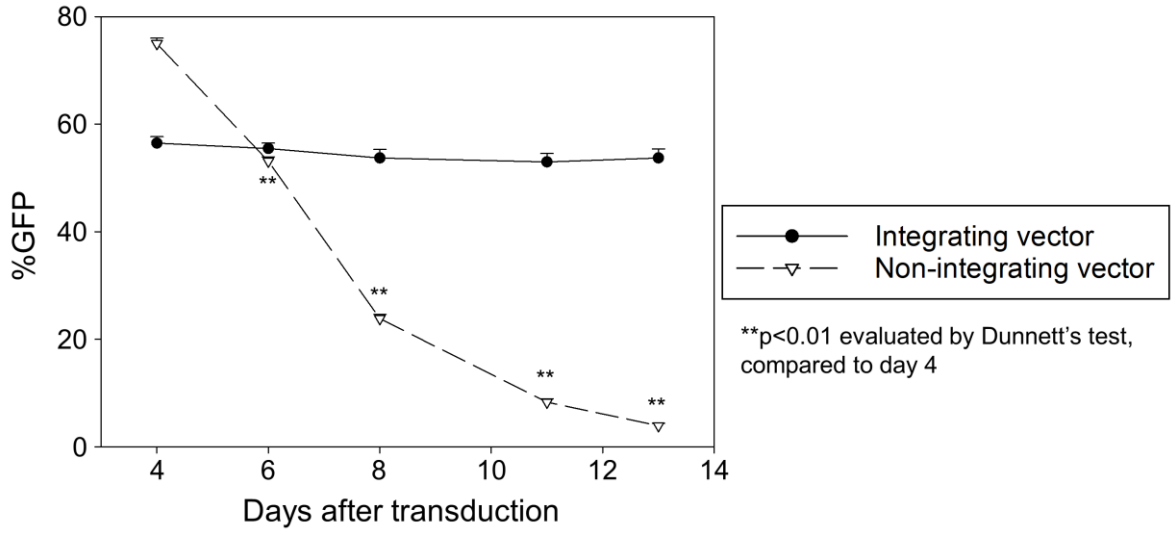
Supplementary figure 14. Efficient βs-to-β-globin gene correction with Cas9 protein delivery NILVs. βs-to-β-globin gene correction at the protein level (HbS-to-HbA) 7 days after erythroid differentiation in an immortalized erythroid cell line including the SCD mutation (sickle HUDEP-2 cells) with Cas9 protein delivery NILV transduction, evaluated by hemoglobin electrophoresis. HbS: sickle hemoglobin, HbF: fetal hemoglobin, HbA: adult hemoglobin. The experiments were performed in a single run.



Supplementary figure 15. Comparison of βs-to-β-globin gene correction between lentiviral Cas9 protein delivery and electroporation-mediated delivery. (a-b) Comparison of βs-to-β-globin gene correction (a) and cell counts (b) 19 days after erythroid differentiation in an immortalized erythroid cell line including the SCD mutation (sickle HUDEP-2 cells) among (1) Cas9 protein delivery SG11 guide RNA NILV and β-globin donor DNA NILV (0.4kb) at MOI 25 without electroporation, (2) the same guide RNA vector and donor vector transduction followed by electroporation at day 1, (3) Cas9 protein delivery guide RNA NILV and β-globin donor single strand DNA (ssDNA) (0.1kb) with electroporation, (4) SG11 guide RNA-Cas9 ribonucleoprotein (RNP) with electroporation and donor NILV, and (5) SG11 RNP and β-globin donor ssDNA with electroporation. The experiments were performed in a single run.



Supplementary figure 16. Transgene expression from integrating and NILVs. %GFP in K562 cells 2 weeks after transduction with either GFP integrating vector or GFP NILV (under the control of Mp) at MOI 0.5. Values: mean ± standard error. The experiments were performed in triplicate.



Supplementary figure 17. Comparison between GFP target vector, YFP donor vector, and guide RNA/YFP donor vector. Design for GFP target vector, YFP donor DNA vector, and guide RNA/YFP donor DNA vector. A single copy of vector genome (3.6kb) is integrated into a GFP-positive HEL cell line which was used in GFP-to-YFP gene conversion. The YFP donor DNA vector encodes the same vector backbone and 14-base difference within YFP gene, compared to the GFP targeted vector. The GFP-targeting guide RNA/YFP donor DNA vector includes 14-base difference within YFP gene and 390-base insertion of guide RNA expression cassette, compared to the GFP target vector. ψ: packaging signal, cPPT: central polypurine tract, RRE: Rev response element.