

“The 5th International Workshop on Regulatory Approaches for Agricultural Applications of Animal Biotechnologies” Virtual Workshop.

Development of genome edited chickens for egg component regulation and production of recombinant proteins

Aug. 20, 2024

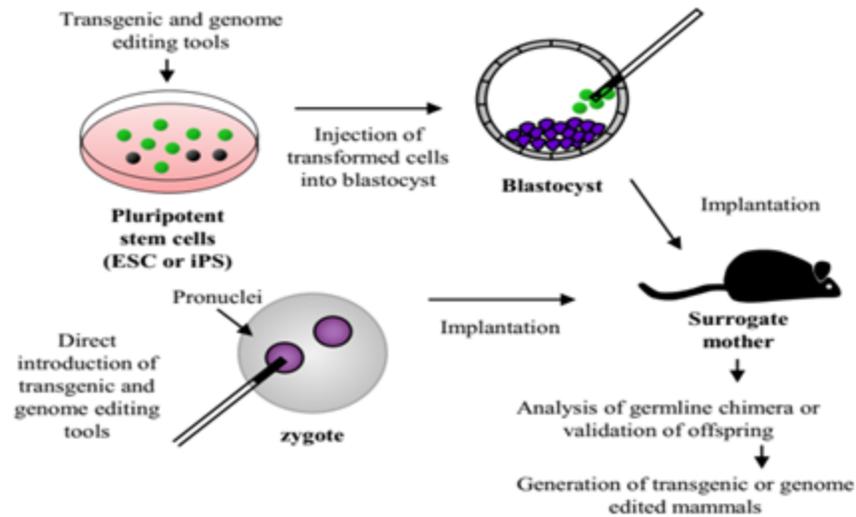
Jae Yong Han
Seoul National University



Development of transgenic and genome editing system in avian species



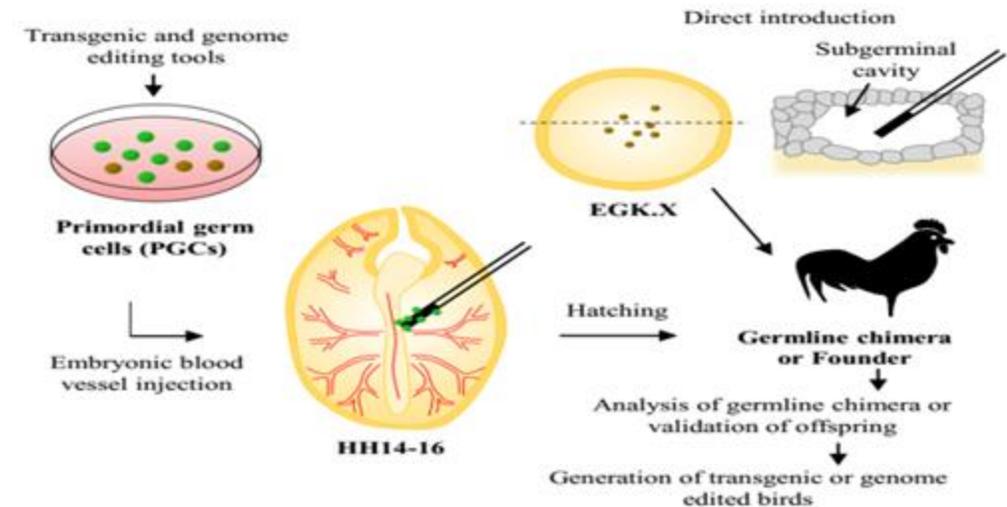
Mammalian gene editing



- ✓ Microinjection of gRNA and Cas9 into egg
- ✓ Stem cell mediated gene editing
- ✓ Possibility of somatic cell nuclear transfer(SCNT)



Avian gene editing



- ✓ Microinjection and SCNT not possible due to different developmental physiology
- ✓ Germ cell transplantation and Germline Chimera
- ✓ Germ cell-mediated gene editing

VS

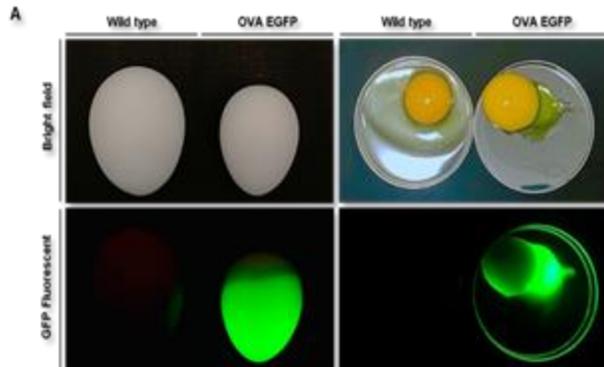


Significance of Primordial Germ Cells

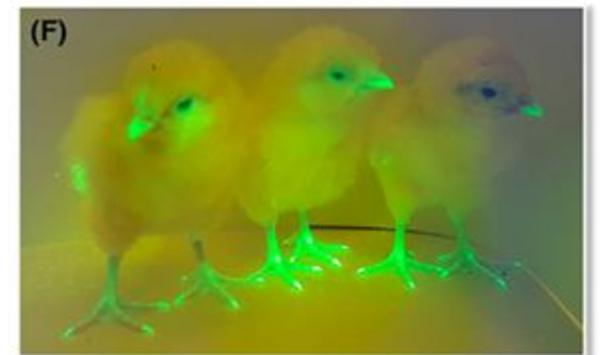
Evolution of biotechnology in avian species has been achieved by development of the avian germline transmission system using primordial germ cells (PGCs)



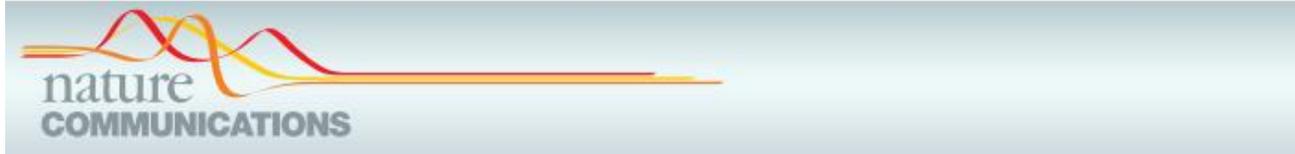
**Germline
Transmission**



- ✓ Precise genome editing
- ✓ Conservation of endangered bird species



Genome editing for germ cell sterilization



ARTICLE

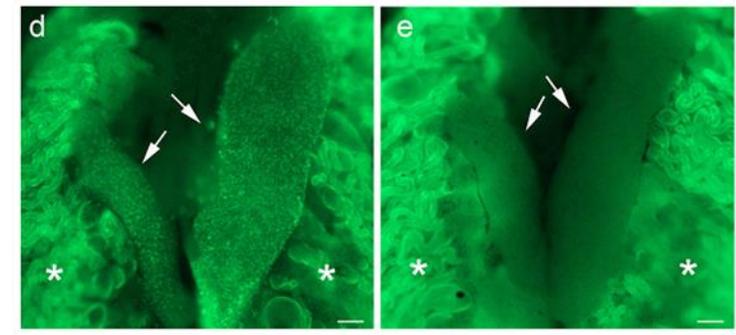
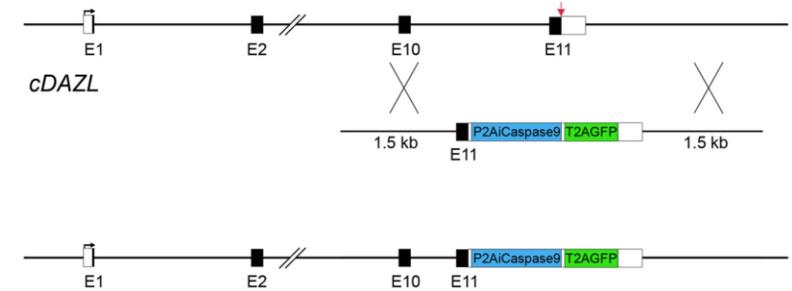
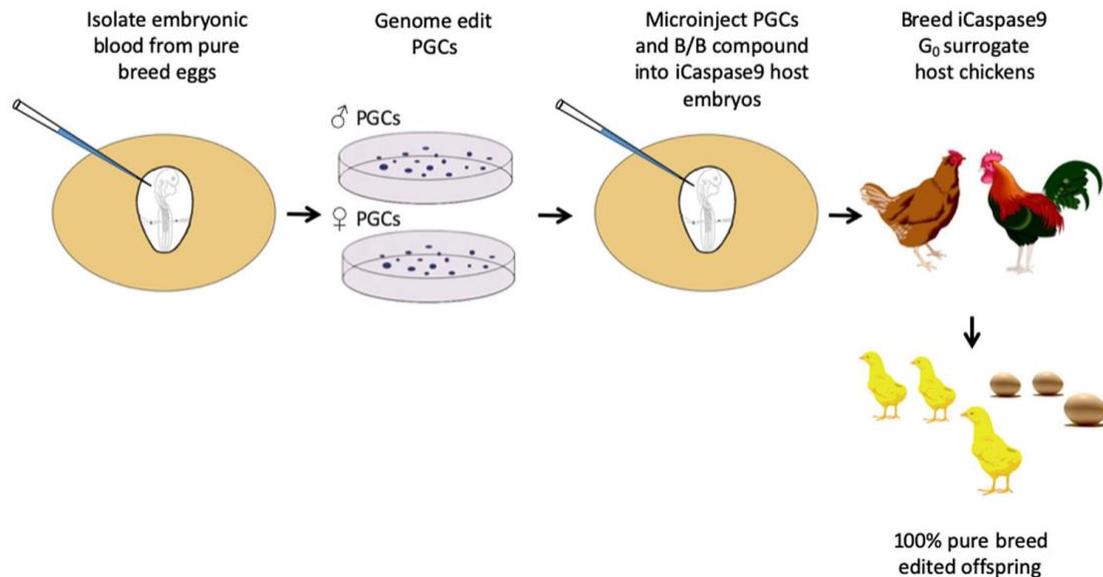
Check for updates

<https://doi.org/10.1038/s41467-020-20812-x>

OPEN

Direct allele introgression into pure chicken breeds using Sire Dam Surrogate (SDS) mating

Maeve Ballantyne^{1,2}, Mark Woodcock^{1,2}, Dadakhalandar Doddamani², Tuanjun Hu^{1,2}, Lorna Taylor², Rachel J. Hawken³ & Mike J. McGrew^{1,2}

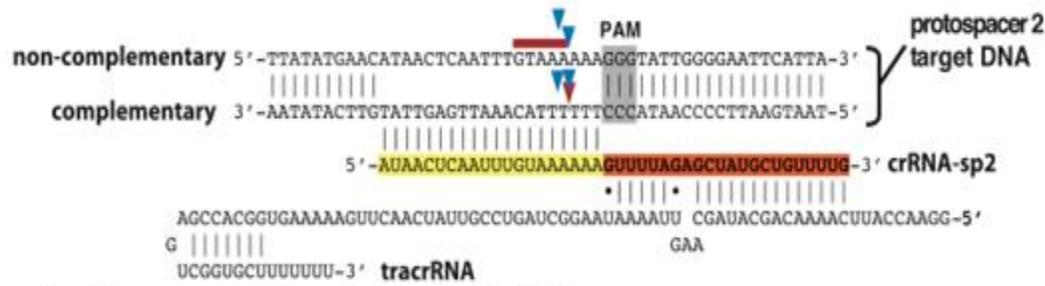


CRISPR/Cas9 system: genetic scissors

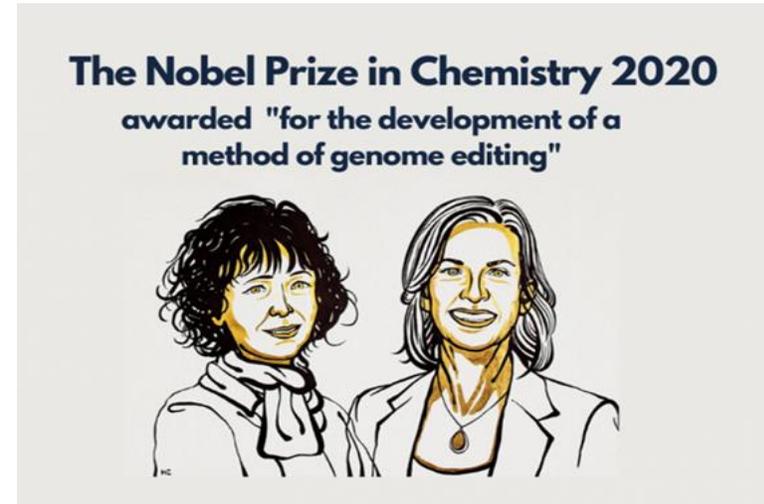
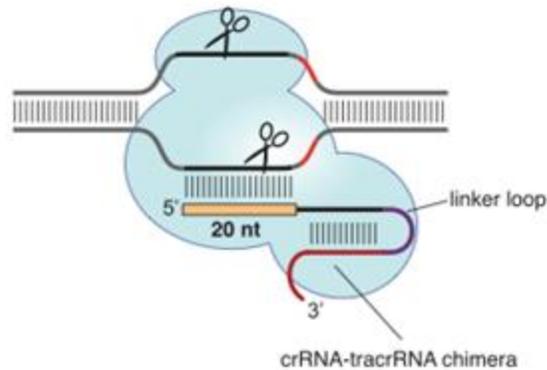
Science

A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

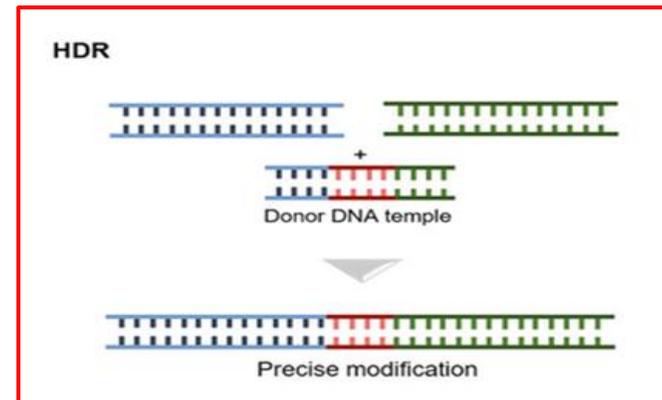
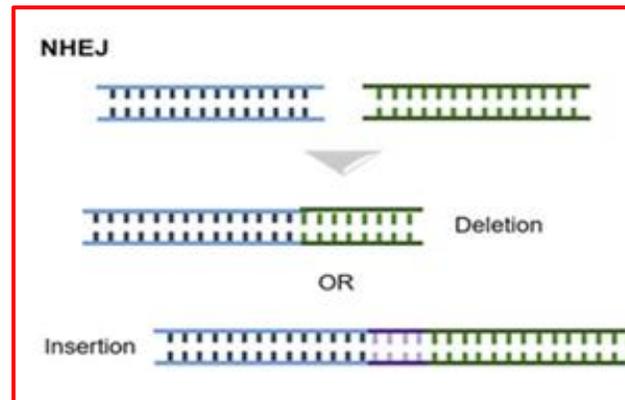
Martin Jinek,^{1,2*} Krzysztof Chylinski,^{3,4*} Ines Fonfara,⁴ Michael Hauer,^{2,†} Jennifer A. Doudna,^{1,2,5,6‡} Emmanuelle Charpentier^{4‡}



Cas9 programmed by single chimeric RNA



- *Derived from bacterial immune system*
- *Cas9 + sgRNA induce double strand breaks*



Egg components modulation by PGC genome editing



- Ovalbumin
- Ovotransferrin
- Ovomuroid

- Recombinant Proteins, Peptides

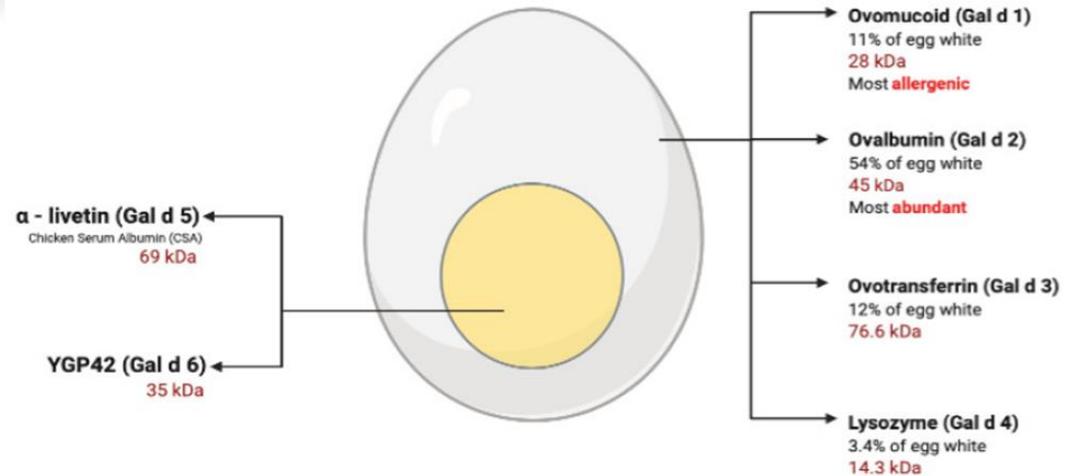
Applications of Genome editing

- ✓ Egg components modification
- ✓ Production of recombinant protein in **Egg White** or **Egg Yolk**

Major albumen proteins in a 60 g egg¹

Constituent	Weight (g)	Fraction of total protein (%)
Ovalbumin	2.20	54.0
Ovotransferrin	0.50	12.0
Ovomucoid	0.50	12.0
Lysozyme	0.15	3.4

(Lillico et al., 2005, Drug Discov Today)



Minor allergens in egg yolk

Major allergens in egg white

(Dona and Suphioglu, 2020, International journal of molecular sciences)



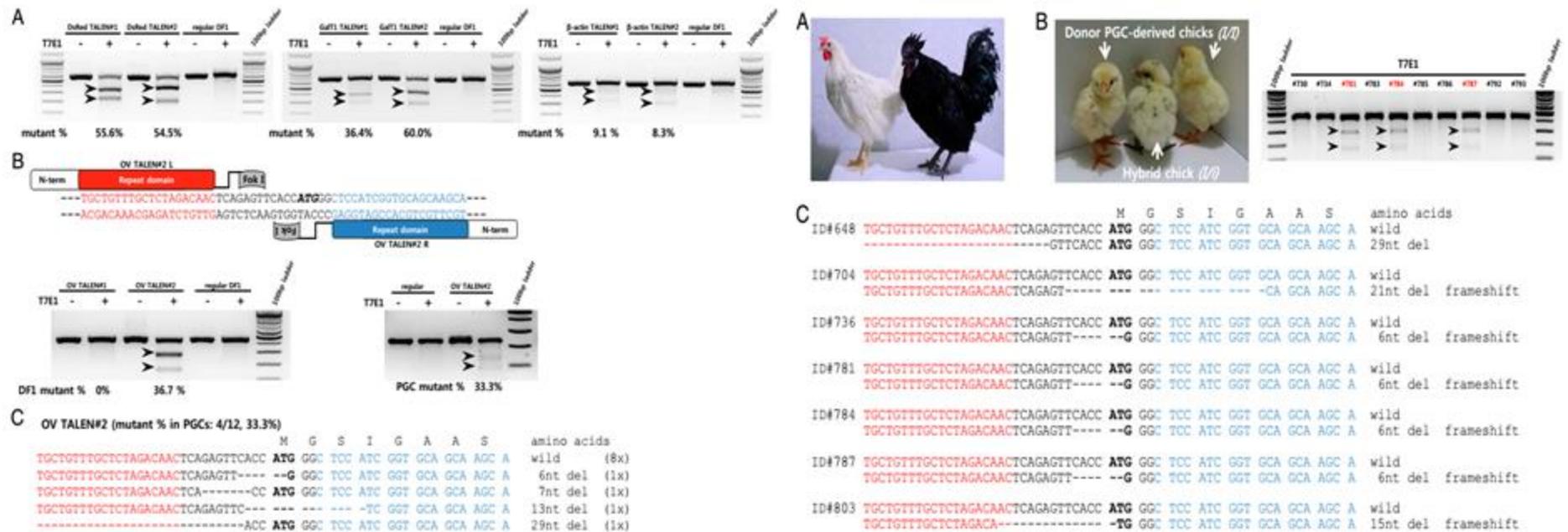
Production of *OV* targeted genome edited chickens

PNAS

Targeted gene knockout in chickens mediated by TALENs

Tae Sub Park^{a,b}, Hong Jo Lee^a, Ki Hyun Kim^a, Jin-Soo Kim^c, and Jae Yong Han^{a,1}

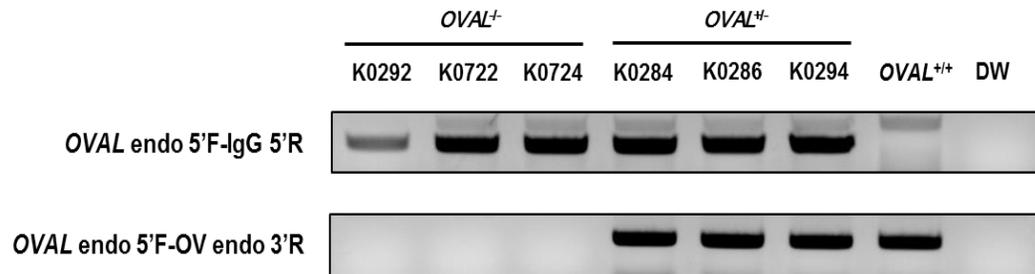
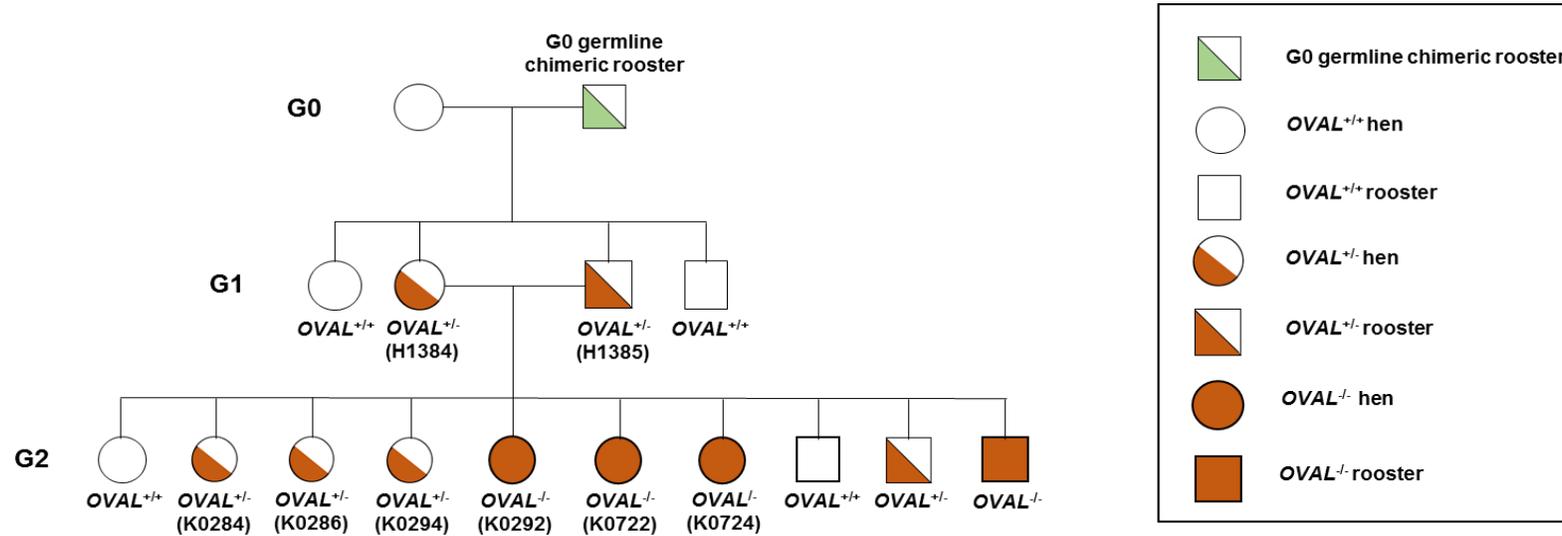
^aDepartment of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea; ^bInstitute of Green-Bio Science and Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 232-916, Korea; and ^cDepartment of Chemistry, College of Natural Science, Seoul National University, Seoul 151-921, Korea



✓ Knock-out of **OV** gene using **TALEN** in cultured PGC



Production of *OVAL* targeted genome edited chickens (CRISPR/Cas)



5' junction sequencing

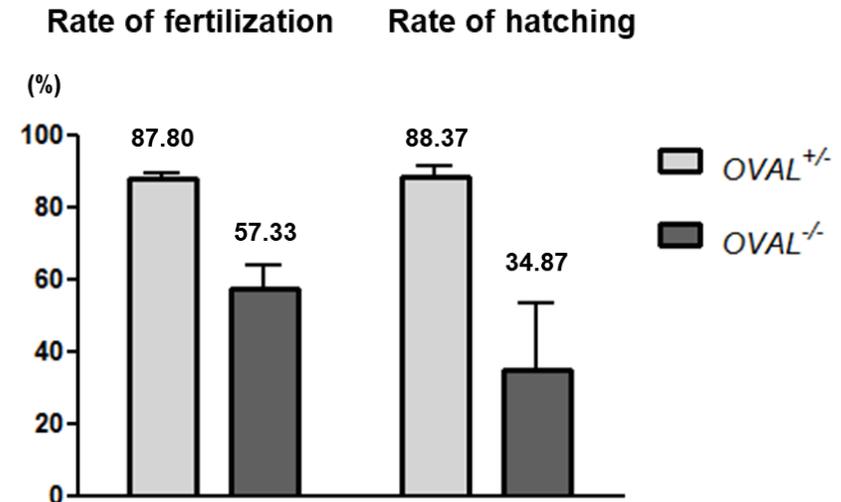
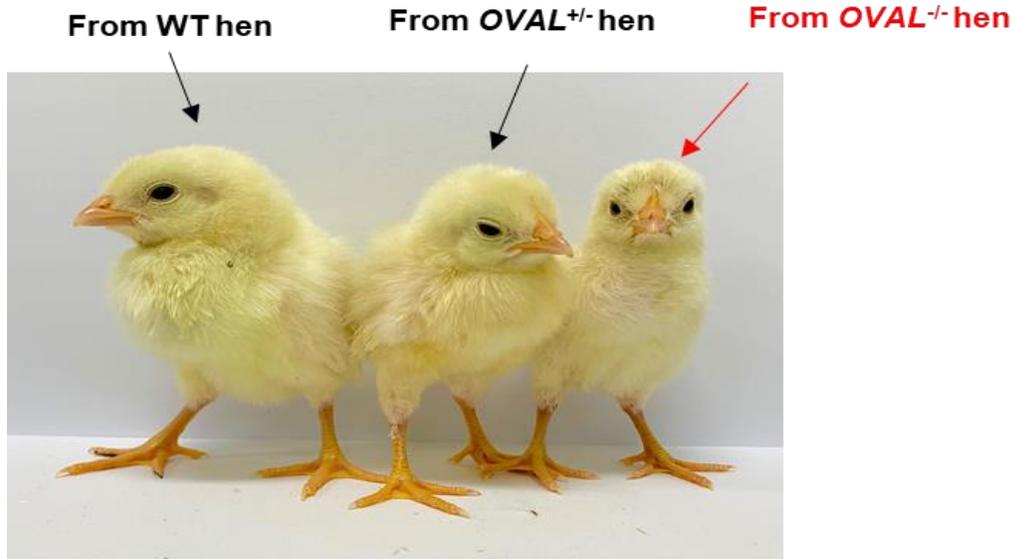
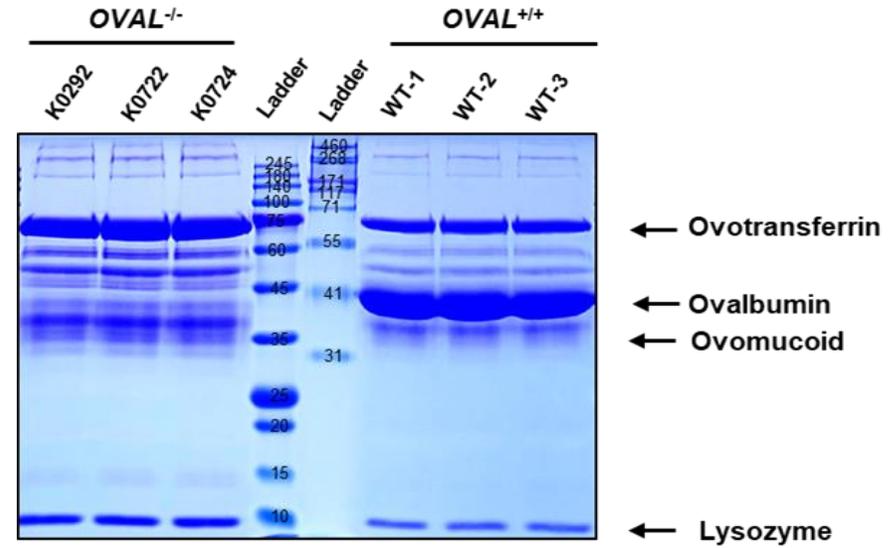
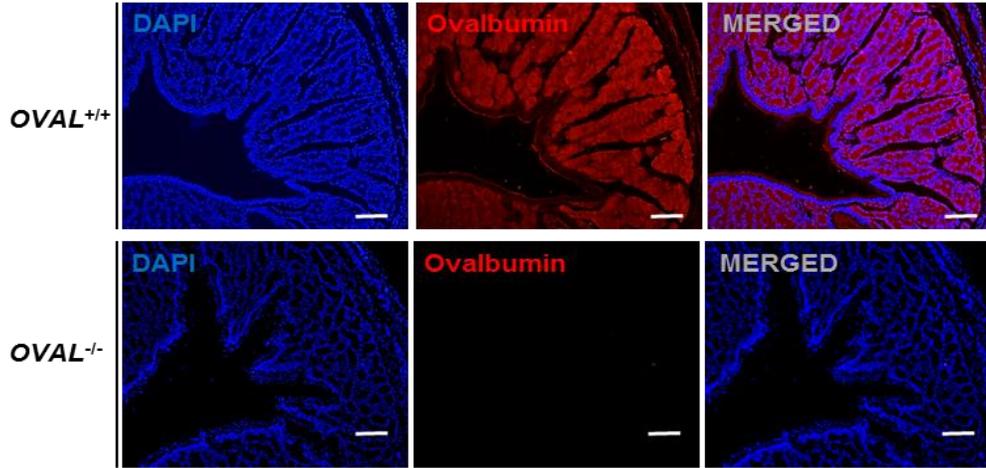
	<i>OVAL</i> genome sequence	PAM + gRNA	Vector sequence
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H1384	CTGTTGTAGCCTACTATAGAGTACCCTGCATGGTACTATGTACAGCATTCCATCCTTACATTTTCACCTGTTCTGCTGTTTGTCTAGACAACCTCAGAGTTCACC		
H1385	CTGTTGTAGCCTACTATAGAGTACCCTGCATGGTACTATGTACAGCATTCCATCCTTACATTTTCACCTGTTCTGCTGTTTGTCTAGACAACCTCAGAGTTCACC		

3' junction sequencing

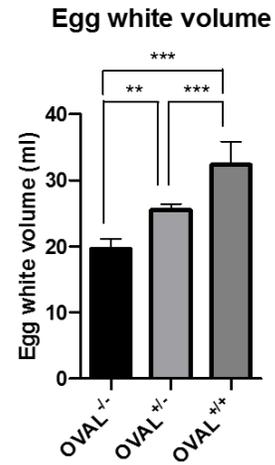
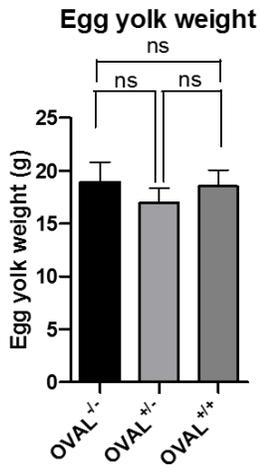
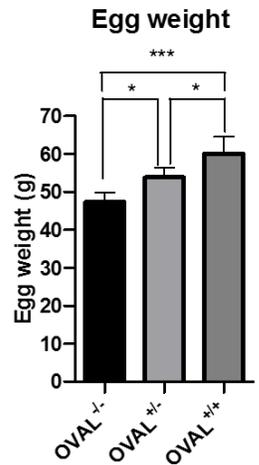
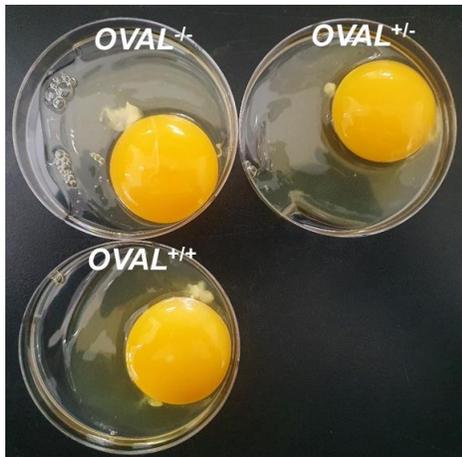
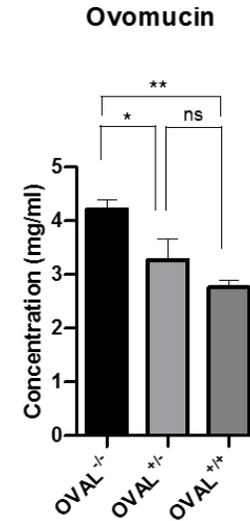
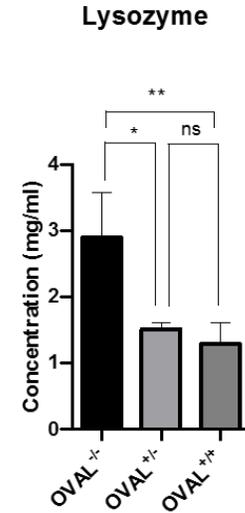
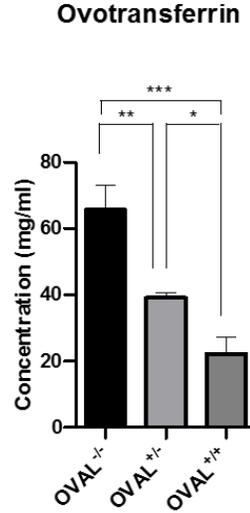
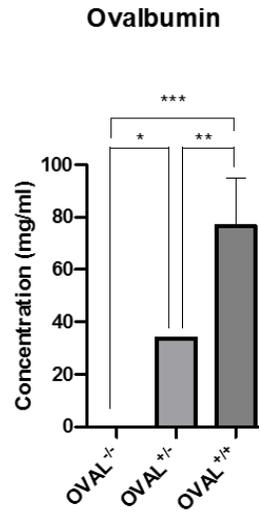
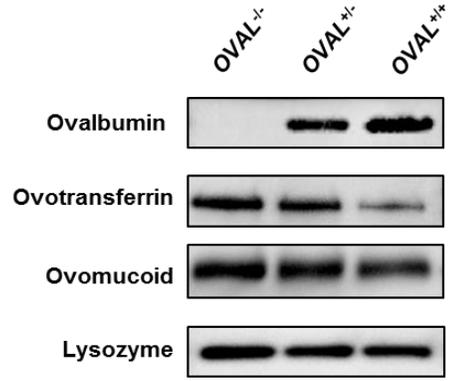
	Vector sequence	PAM + gRNA	<i>OVAL</i> genome sequence
Reference	GCCATGGCGCCGCGGGAATTCGATTCTACCATAGAGTACCCTGCATGGTACTATGTACAGCTGGTACTATGTACAGCATTCCATCCTTACATTTTCACCTGT		
H1384	GCCATGGCGCCGCGGGAATTCGATTCTACCATAGAGTACCCTGCATGGTACTATGTACAGCTGGTACTATGTACAGCATTCCATCCTTACATTTTCACCTGT		
H1385	GCCATGGCGCCGCGGGAATTCGATTCTACCATAGAGTACCCTGCATGGTACTATGTACAGCTGGTACTATGTACAGCATTCCATCCTTACATTTTCACCTGT		



Production of *OVAL* targeted genome edited chickens



Production of *OVAL* targeted genome edited chickens



Targeted nucleotide substitution (Base editing)

nature
LETTER

doi:10.1038/nature

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

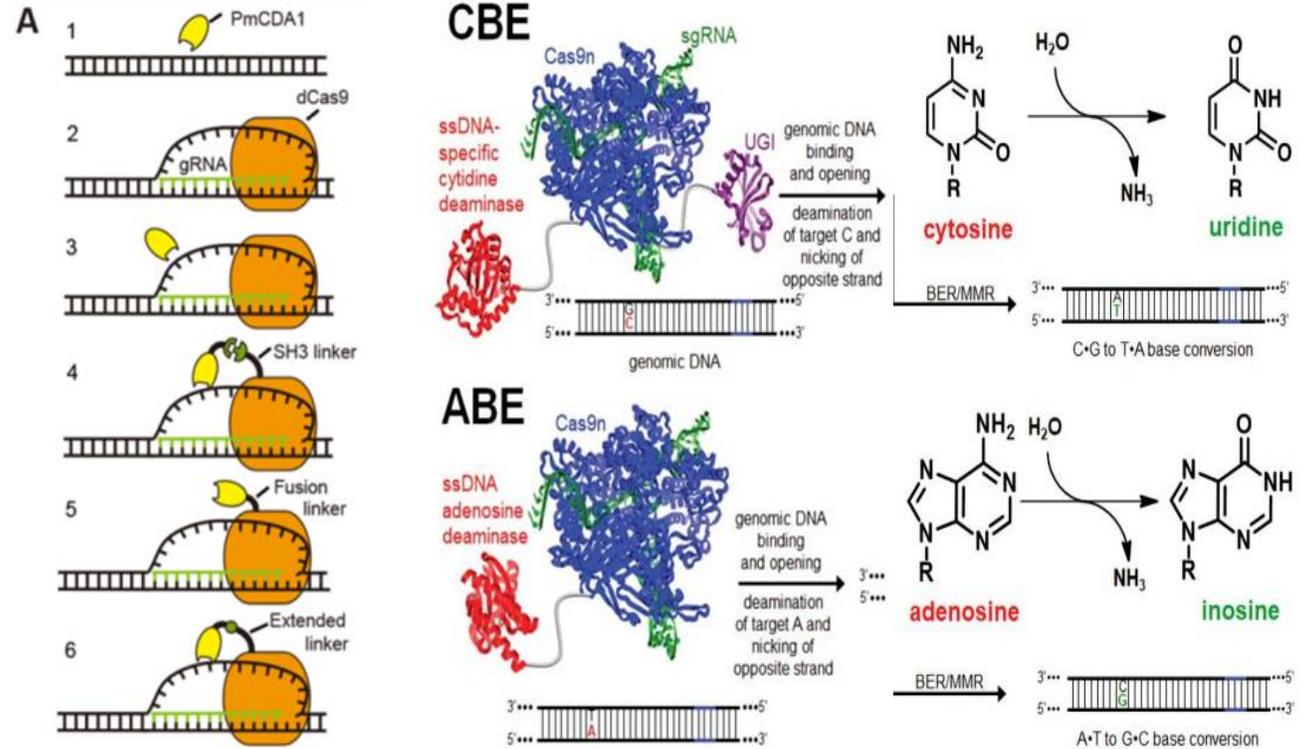
Alexis C. Komor^{1,2}, Yongjoo B. Kim^{1,2}, Michael S. Packer^{1,2}, John A. Zuris^{1,2} & David R. Liu^{1,2}

ARTICLE

doi:10.1038/nature24644

Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

Nicole M. Gaudelli^{1,2,3}, Alexis C. Komor^{1,2,3†}, Holly A. Rees^{1,2,3}, Michael S. Packer^{1,2,3†}, Ahmed H. Badran^{1,2,3}, David I. Bryson^{1,2,3†} & David R. Liu^{1,2,3}



- ✓ dCas9 is a mutated Cas9 protein that does not have nuclease activity.
- ✓ Cytosine base editor (CBE) converts a C/G base pair into a T/A base pair, and adenosine base editor (ABE) converts an A/T base pair into a G/C base pair.



Ovotransferrin (TF) KO-Applying base editing

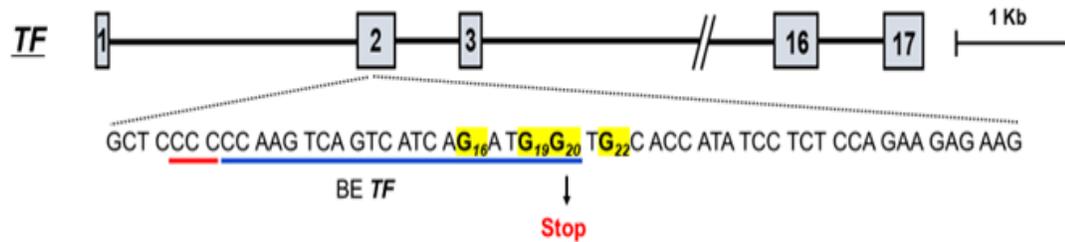
RESEARCH ARTICLE

FASEB JOURNAL

Highly elevated base excision repair pathway in primordial germ cells causes low base editing activity in chickens

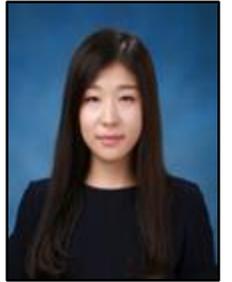
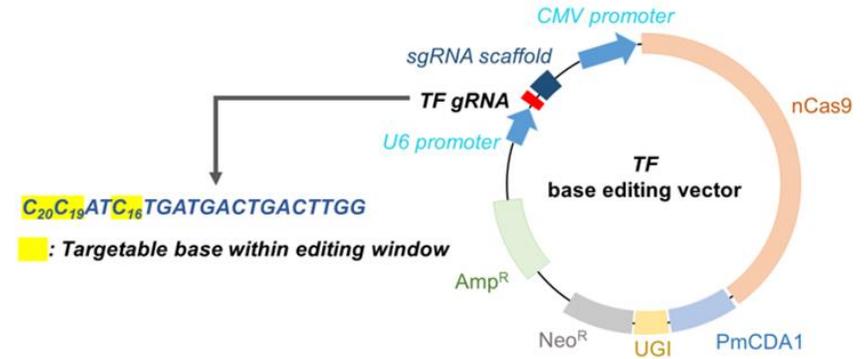
Kyung Youn Lee¹ | Hong Jo Lee¹ | Hee Jung Choi¹ | Soo Taek Han¹ | Kyu Hyuk Lee¹ |
 Kyung Je Park¹ | Jin Se Park¹ | Kyung Min Jung¹ | Young Min Kim¹ | Ho Jae Han² |
 Jae Yong Han¹

Induction of premature stop codon in exon2 of TF gene

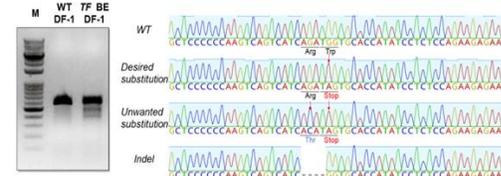


Expected Base editing
 C CCC AAG TCA GTC ATC AG₁₆A TA₁₉A₂₀
 C CCC AAG TCA GTC ATC AG₁₆A TA₁₉G₂₀

✓ The efficiency of the desired nucleotide substitution was 80.0% (12/15) in DF-1 cells, 28.0% (7/25) in PGCs



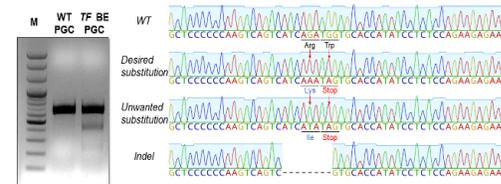
C DF-1 cells



D

Reference	Frequency	Nucleotide Change	Amino Acid Change
GCT CCC CCC AAG TCA GTC ATC AG ₁₆ A TG ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(16x)	(WT)	
GCT CCC CCC AAG TCA GTC ATC AG ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(5x)	(G19, G to A)	(W28 stop)
GCT CCC CCC AAG TCA GTC ATC AG ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(G19, G to A' / G20, G to A)	(W28 stop)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(3x)	(G16, G to A' / G19, G to A)	(R27K, W28 stop)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(G16, G to A' / G19, G to A)	(R27K, W28 stop)
GCT CCC CCC AAG TCA GTC ATC AC ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(G16, G to C' / G19, G to A)	(R27T, W28 stop)
GCT CCC CCC AAG TCA GTC ATC AG16A TG19G20 TG22C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(-4 bp)	(Frameshift mutation)

E PGCs

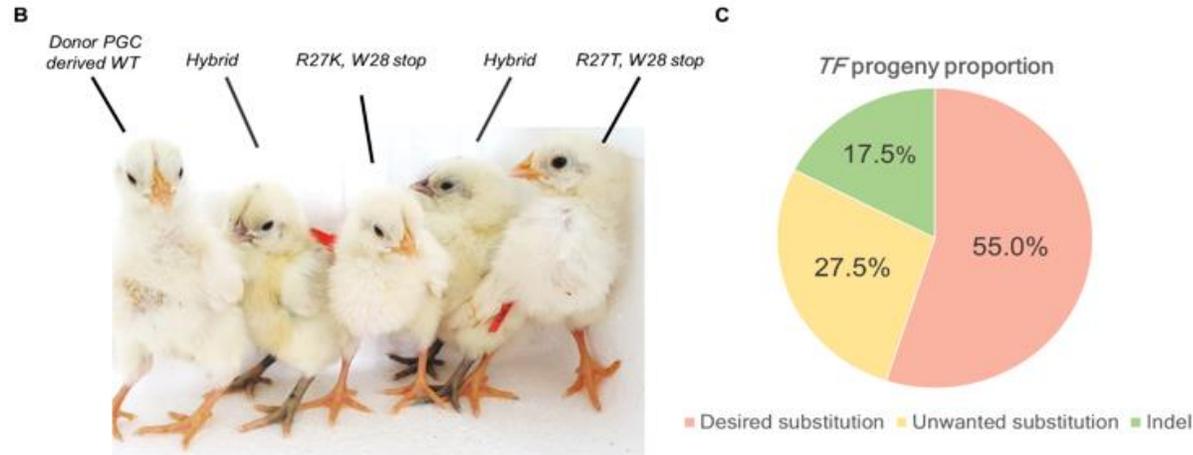
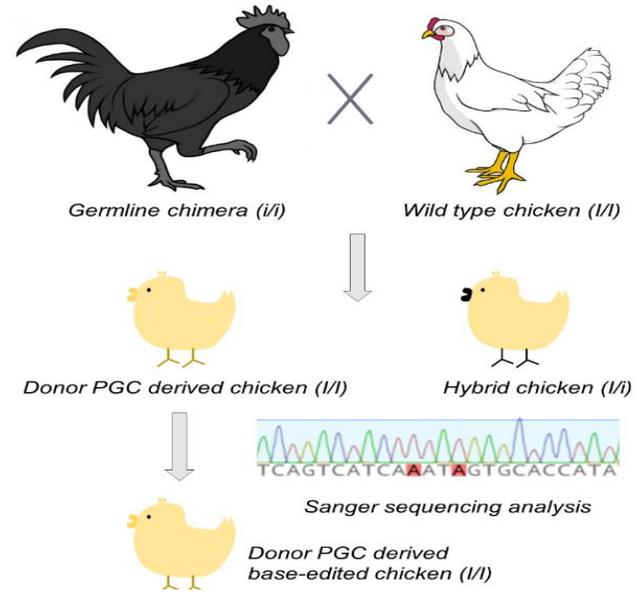


F

Reference	Frequency	Nucleotide Change	Amino Acid Change
GCT CCC CCC AAG TCA GTC ATC AG ₁₆ A TG ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(WT)	
GCT CCC CCC AAG TCA GTC ATC AG ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(G19, G to A)	(W28 stop)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(G16, G to A' / G19, G to A)	(R27K, W28 stop)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(G16, G to A' / G19, G to A)	(R27K, W28 stop)
GCT CCC CCC AAG TCA GTC ATC AG ₁₆ A TG ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(G19, G to C)	(W28 S)
GCT CCC CCC AAG TCA GTC ATC AT ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(2x)	(G16, G to T' / G19, G to A)	(R27L, W28 stop)
GCT CCC CCC AAG TCA GTC ATC AT ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(G16, G to T' / G19, G to T)	(R27L, W28L)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TG ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(G16, G to A' / G20, G to C)	(R27K, W28C)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TA ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(G16, G to A' / G19, G to A / G20, G to C)	(R27K, W28Y)
GCT CCC CCC AAG TCA GTC AG16A TG19G20 TG22C ACC ATA TCC TCT CCA GAA GAG AAG	(5x)	(-8 bp)	(Frameshift mutation)
GCT CCC CCC AAG TCA GTC AG16A TG19G20 TG22C ACC ATA TCC TCT CCA GAA GAG AAG	(1x)	(-7 bp)	(Frameshift mutation)
GCT CCC CCC AAG TCA GTC ATC AA ₁₆ A TG ₁₉ G ₂₀ TG ₂₂ C ACC ATA TCC TCT CCA GAA GAG AAG	(5x)	(G16, G to A' / 3bp)	(R27K, W28 del)



Ovotransferrin (TF) KO-Applying base editing



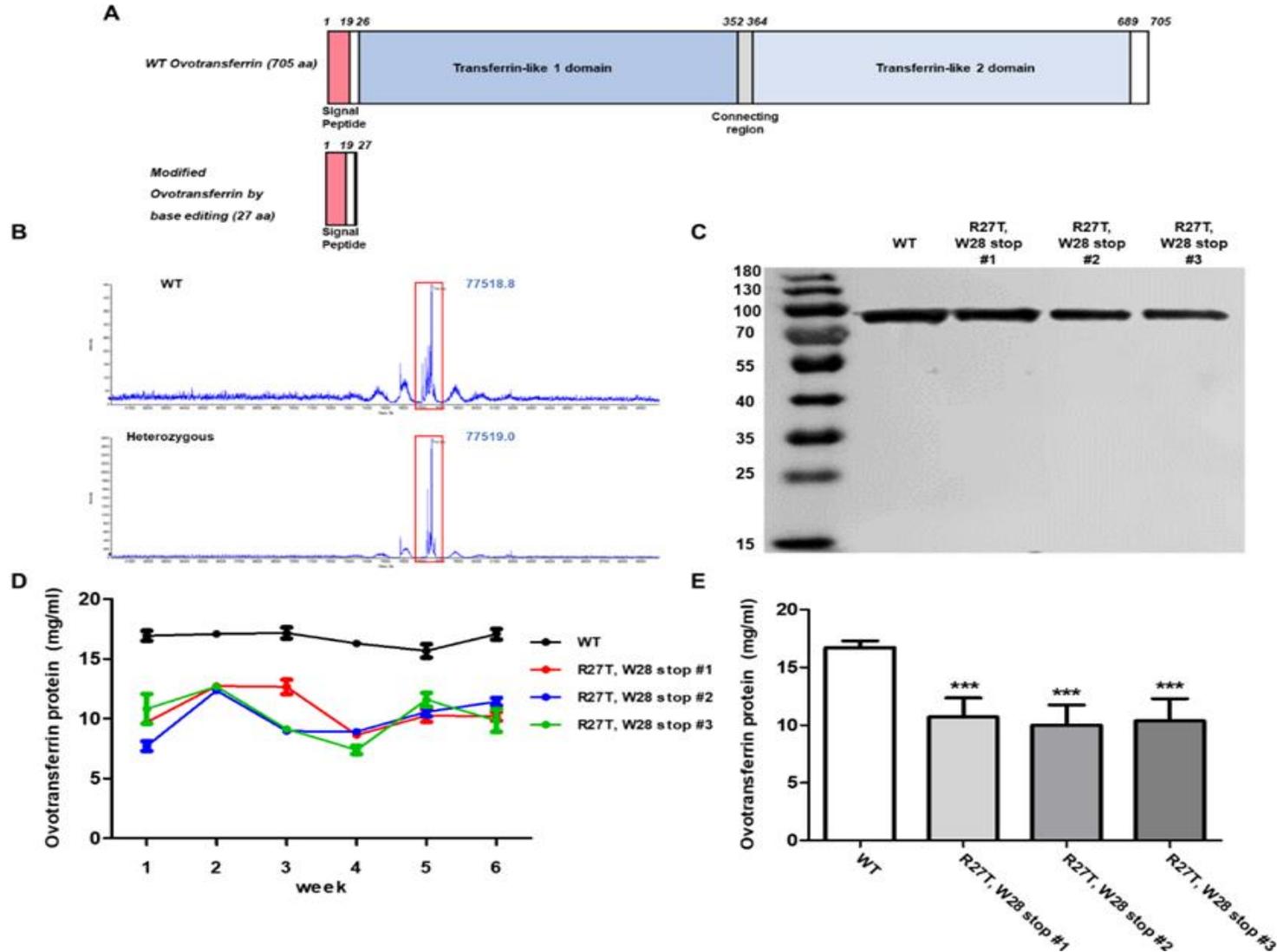
D

	A	P	P	K	S	V	I	R	W	C	T	I	S	S	P	E	E	K
Reference	GCT	CCC	CCC	AAG	TCA	GTC	ATC	AG ₁₉ A	TG ₂₀ G	TG ₂₂ C	ACC	ATA	TCC	TCT	CCA	GAA	GAG	AAG
Frequency																		
Nucleotide Change																		
Amino Acid Change																		
Desired Substitution																		
Unwanted substitution																		
Indel																		

- ✓ 55.0% (44/80): the desired nucleotide substitution, 27.5% (22/80): unwanted nucleotide substitutions, 17.5% (14/80): deletion mutations.
- ✓ The efficiency of nucleotide substitution within the targeting window was highest at **G in the 19th position (G19)** and lowest at G20; no base editing occurred at G22



Ovotransferrin (TF) KO-Applying base editing

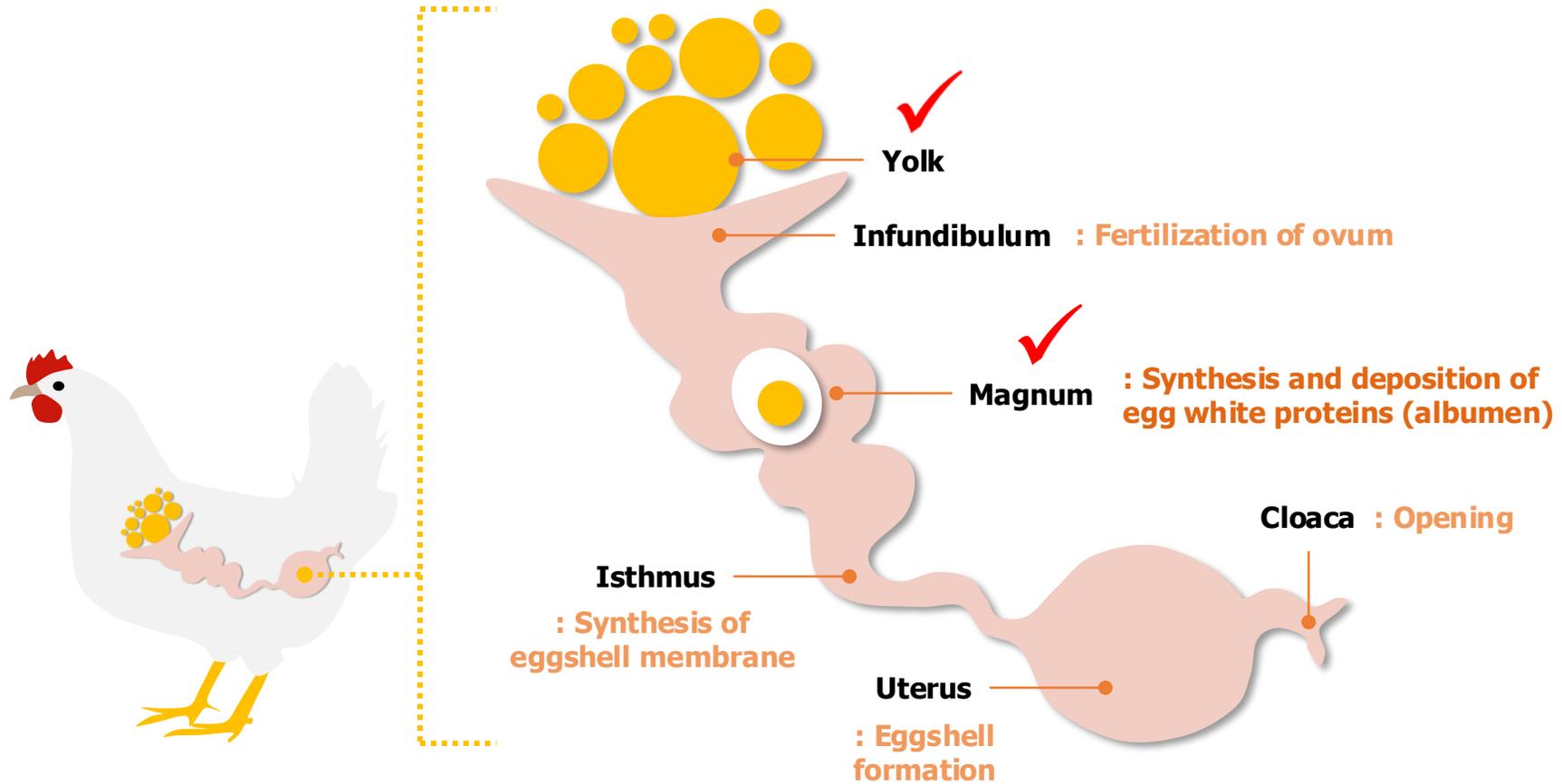


✓ TF null mutant: embryonic lethality between HH stages 16 and 46

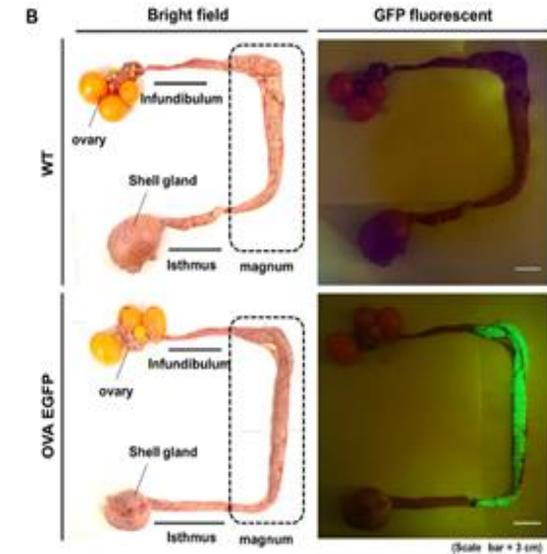
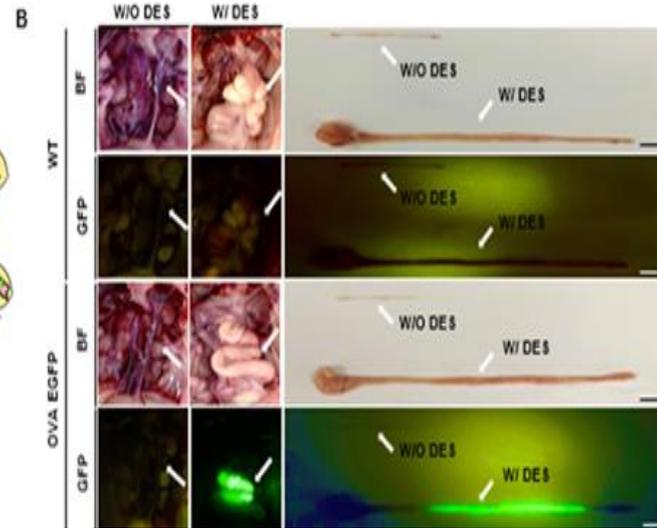
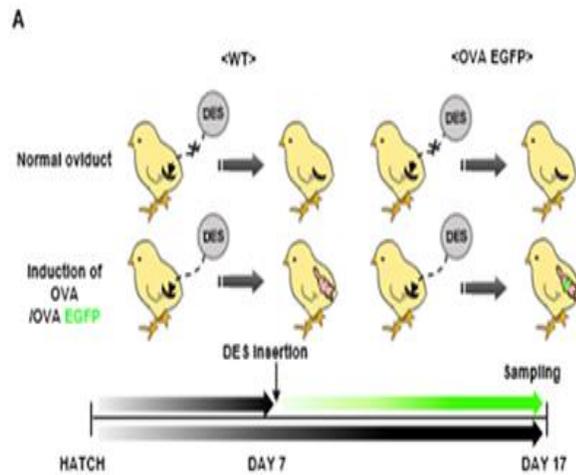
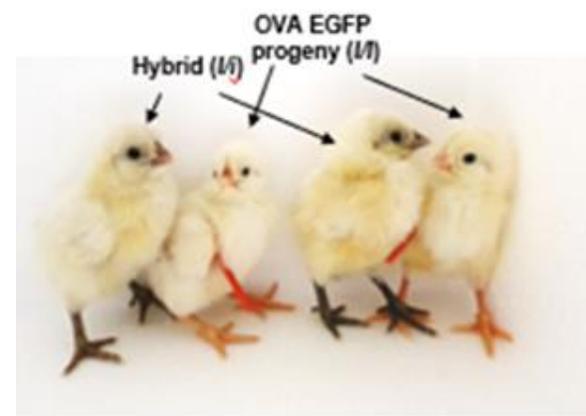
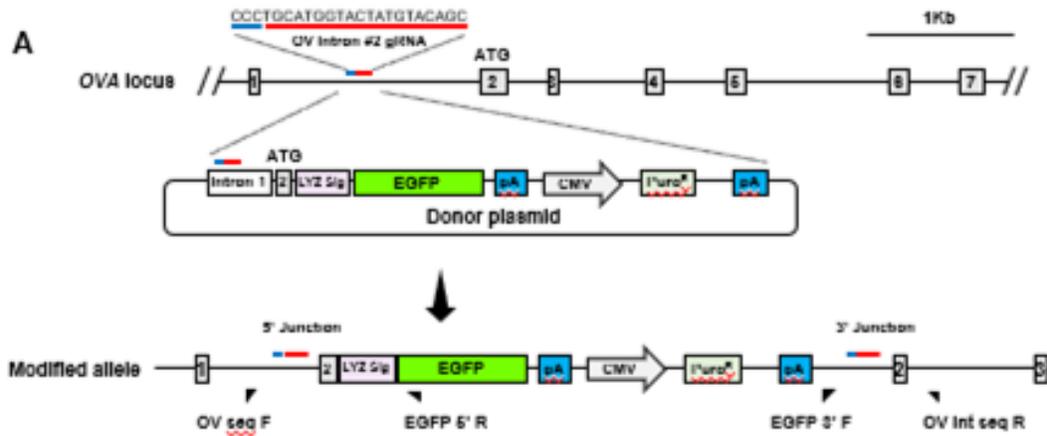
✓ TF concentration of the KO heterozygotes (10.70 mg/ml) was ~60% of WT (16.70mg/ml)



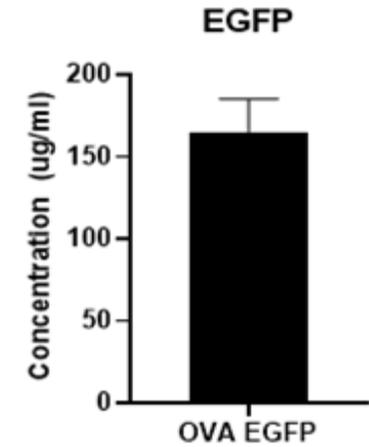
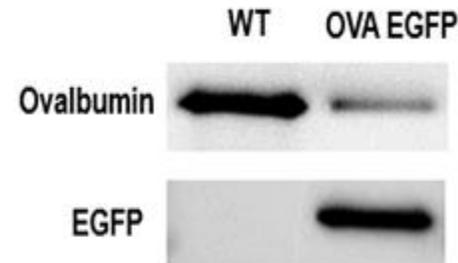
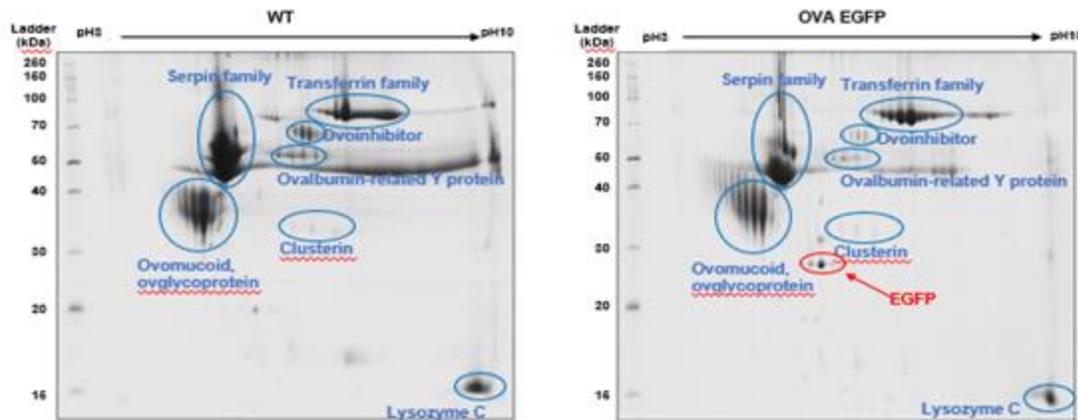
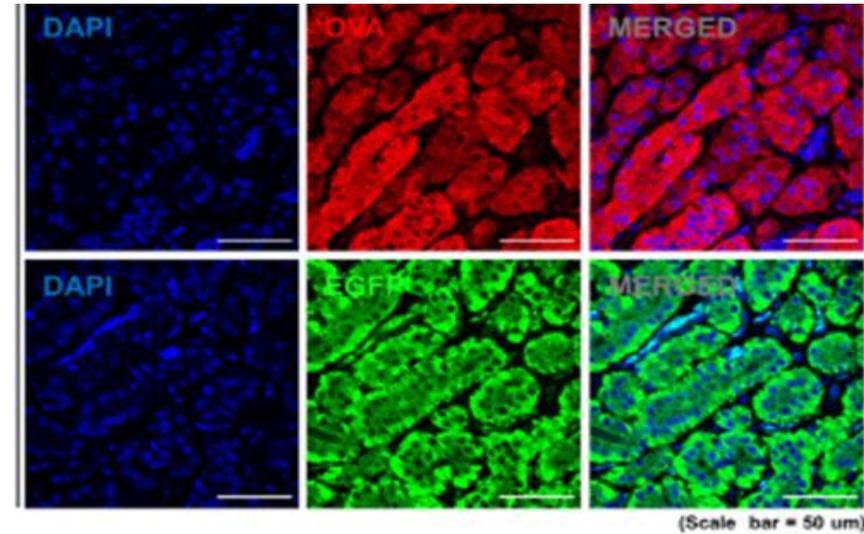
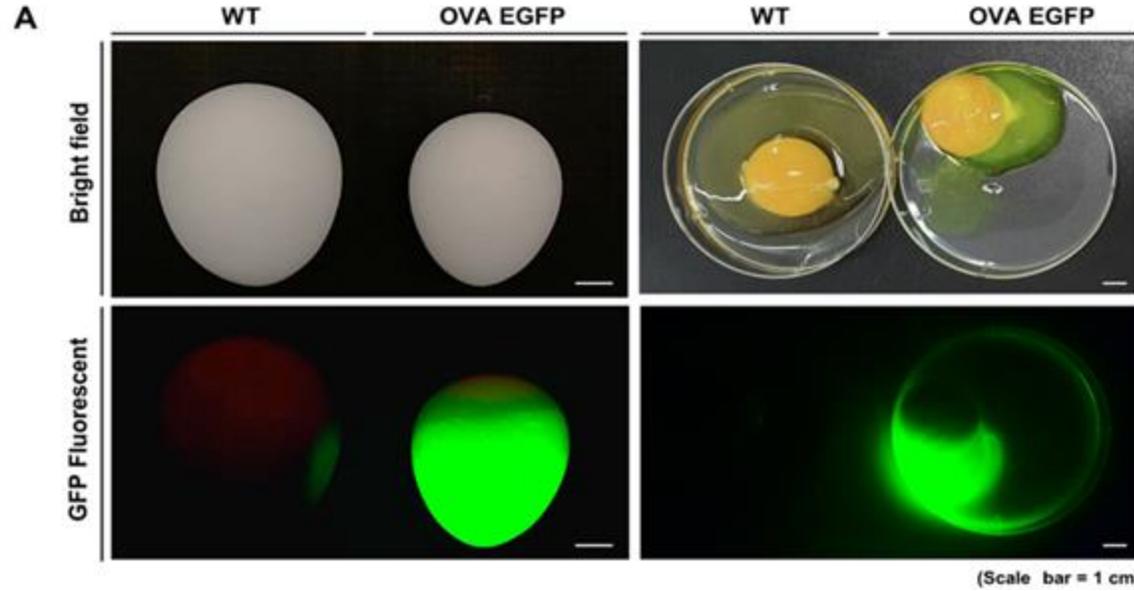
Production of “recombinant proteins” in egg white and yolk



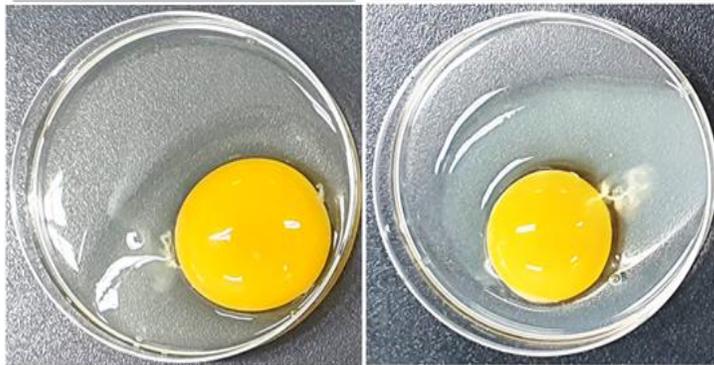
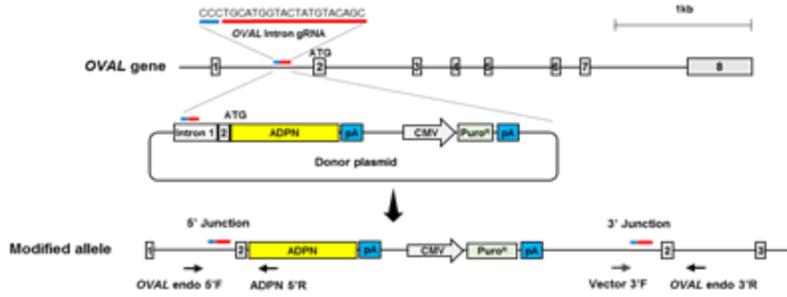
Production of “recombinant proteins” in egg white



Production of “recombinant proteins” in egg white

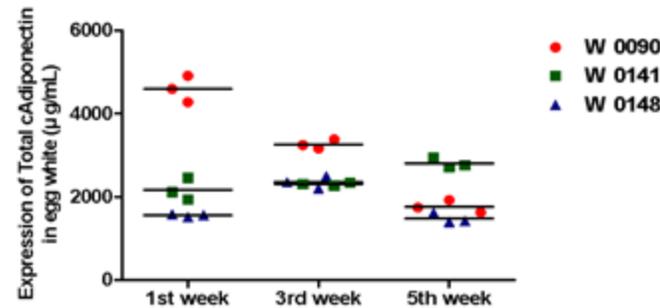
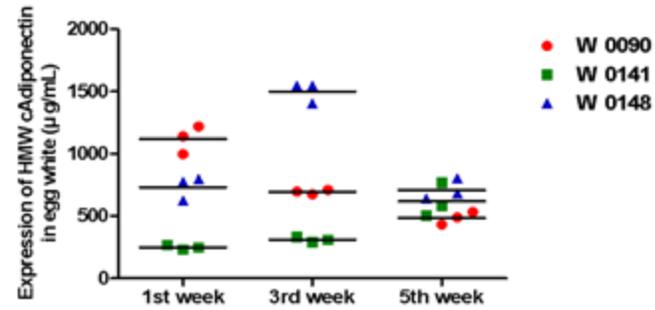


Production of “recombinant proteins” in egg white

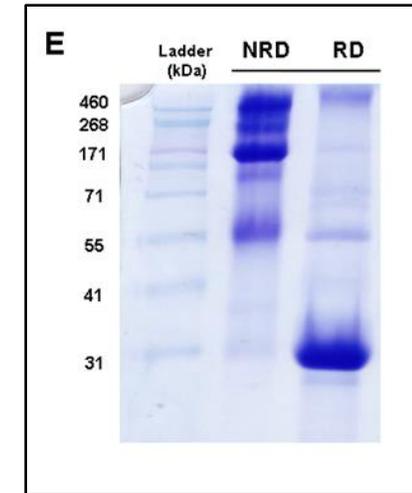


WT

EW with Adiponectin



***1.47 – 4.59 mg/ml for total Adiponectin
30-90mg/egg**



- ✓ Hitrap Q hp column and Superdex G75 analytical size exclusion chromatography.
- ✓ Most egg white derived human ADPN: form a hexamer (≥ 150 kDa) or HMW (≥ 360 kDa or more).



Production of “recombinant proteins” in egg white



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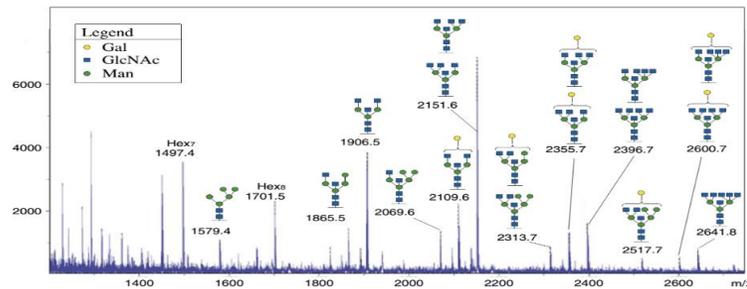


The transgenic chicken derived anti-CD20 monoclonal antibodies exhibits greater anti-cancer therapeutic potential with enhanced Fc effector functions

Young Min Kim^a, Jin Se Park^a, Sang Kyung Kim^a, Kyung Min Jung^a, Young Sun Hwang^a, Mookyung Han^a, Hong Jo Lee^a, Hee Won Seo^b, Jeong-Yong Suh^a, Beom Ku Han^c, Jae Yong Han^{a,d,*}

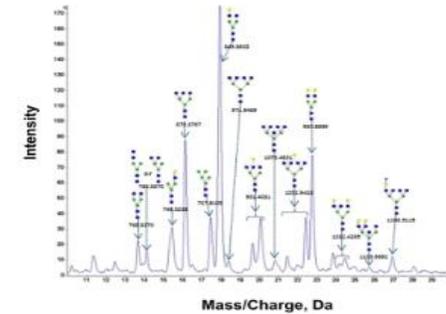
N-glycosylation of ovomucin from hen egg white

Marina Offengenden · Messele A. Fentabil · Jianping Wu

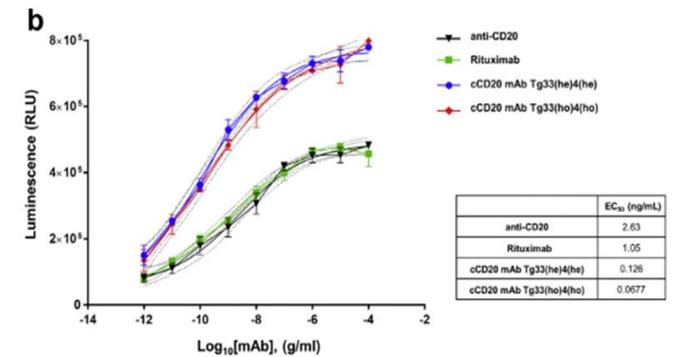
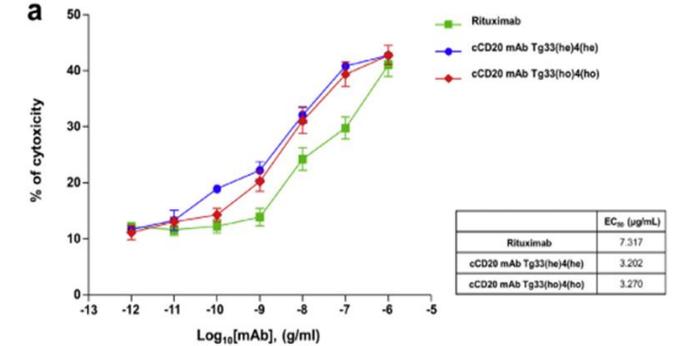


(Offengenden et al., 2011, *Glycoconj J*)

- ✓ The N-glycosylation pattern of egg white proteins is predominantly consist of **terminal mannose** and N-acetylglucosamine with **absence of core fucosylation** (afucosylation)



Peak	Monomer structure	DB	MS	Y	Ratio	glycan symbols
1		1000.0000	368.0000	2	2.7	Galactose
2		1000.0000	368.0000	2	1.3	Galactose
3		1460.0000	368.0000	2	1.7	Galactose
4		1730.0000	470.0000	2	10.2	Galactose
5		1440.0000	368.0000	2	0.3	Galactose
6		1000.0000	368.0000	2	20.4	Galactose
7		1000.0000	368.0000	2	0.6	Galactose
8		1000.0000	368.0000	2	0.1	Galactose
9		2100.0000	368.0000	2	1.0	Galactose
10		2100.0000	368.0000	2	2.0	Galactose
11		1000.0000	368.0000	2	10.4	Galactose
12		2000.0000	368.0000	2	1.0	Galactose
13		2000.0000	368.0000	2	0.5	Galactose
14		2300.0000	470.0000	2	1.0	Galactose

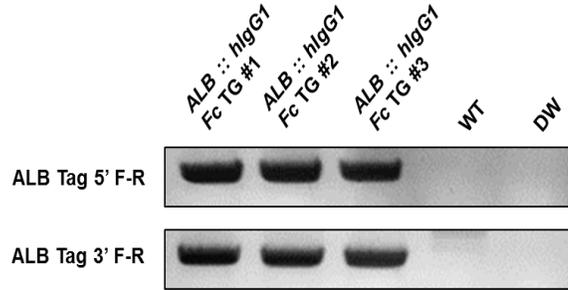
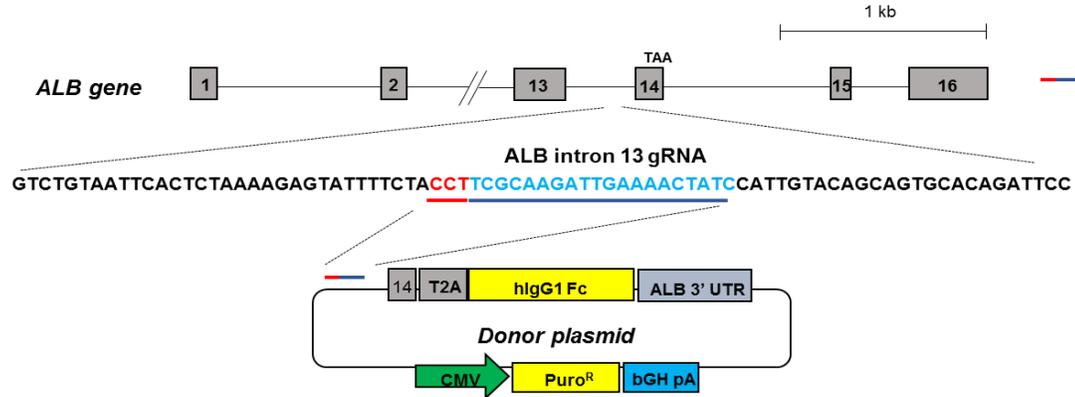


- ✓ CD20 mAb from EW: 14 N-glycan patterns with **high-mannose, afucosylation** and terminal galactosylation
- ✓ Afucosylated CD20 mAb exhibited **8- to 16- fold increase of antibody dependent cell cytotoxicity (ADCC) effect** compared to commercial Rituximab.

(Kim et al., *Biomaterials*, 2018)

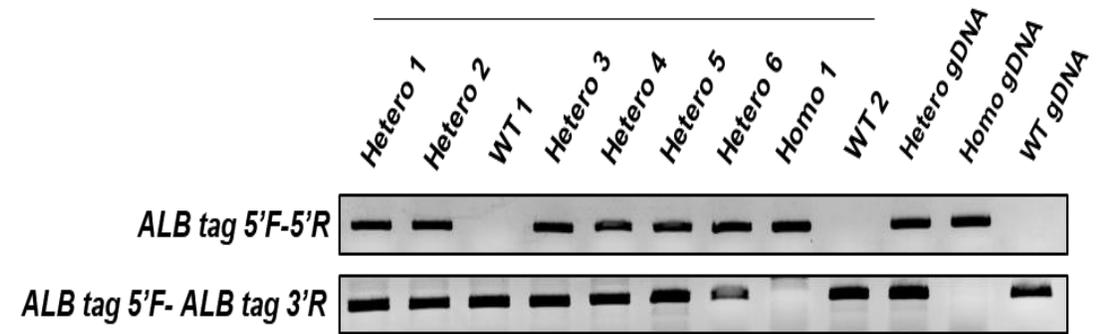


Egg Yolk: Production of recombinant proteins

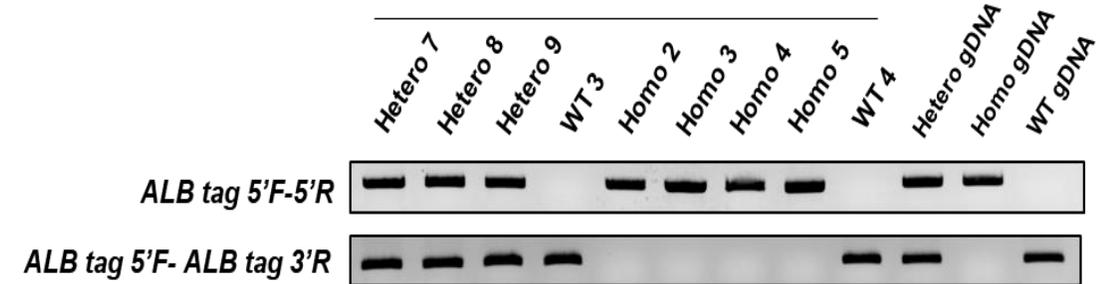


	Genomic DNA sequence	Vector sequence	Indels
5' Junction	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	
ALB::hlgG1 Fc TG #1	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	+1bp
ALB::hlgG1 Fc TG #2	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	+1bp
ALB::hlgG1 Fc TG #3	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	GTCTGTAATTCACCTCTAAAAGAGTATTTTCTACCTTCGCAAGATTGAAAACATCCATTGTACAGCAGTGCACAGATTCC	+1bp

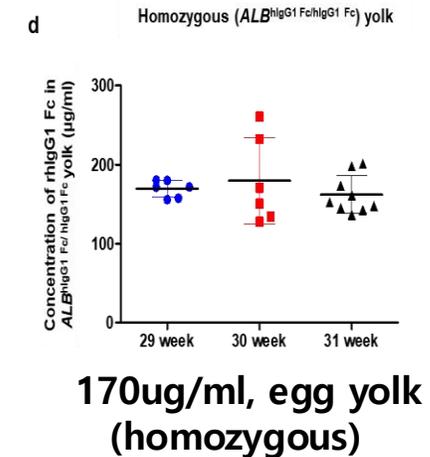
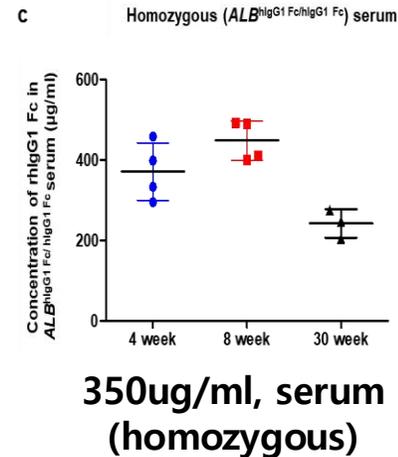
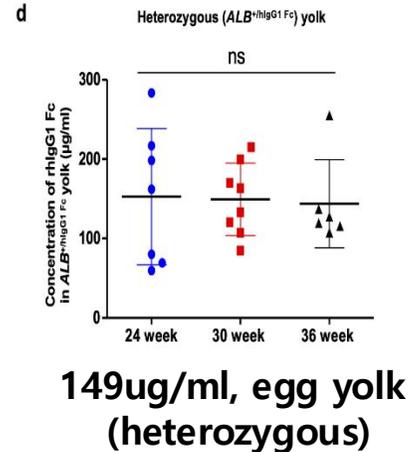
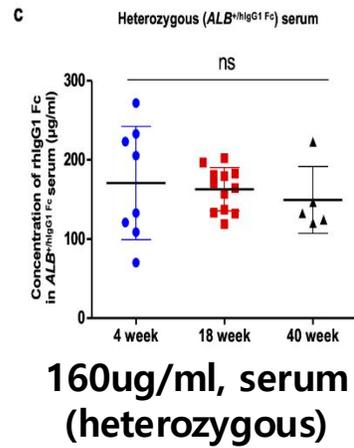
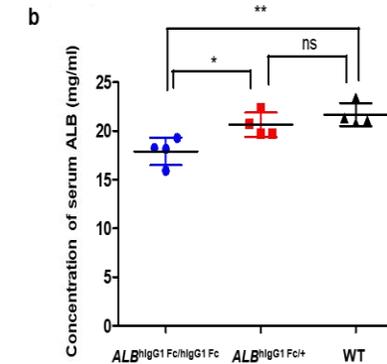
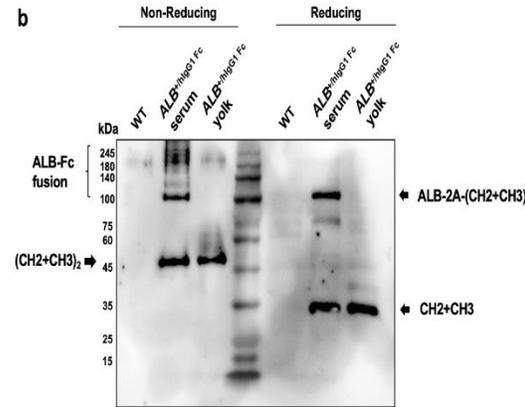
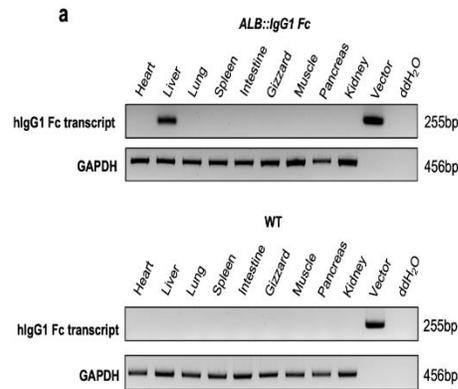
G3 progeny



G3 progeny



Egg Yolk: Production of recombinant proteins



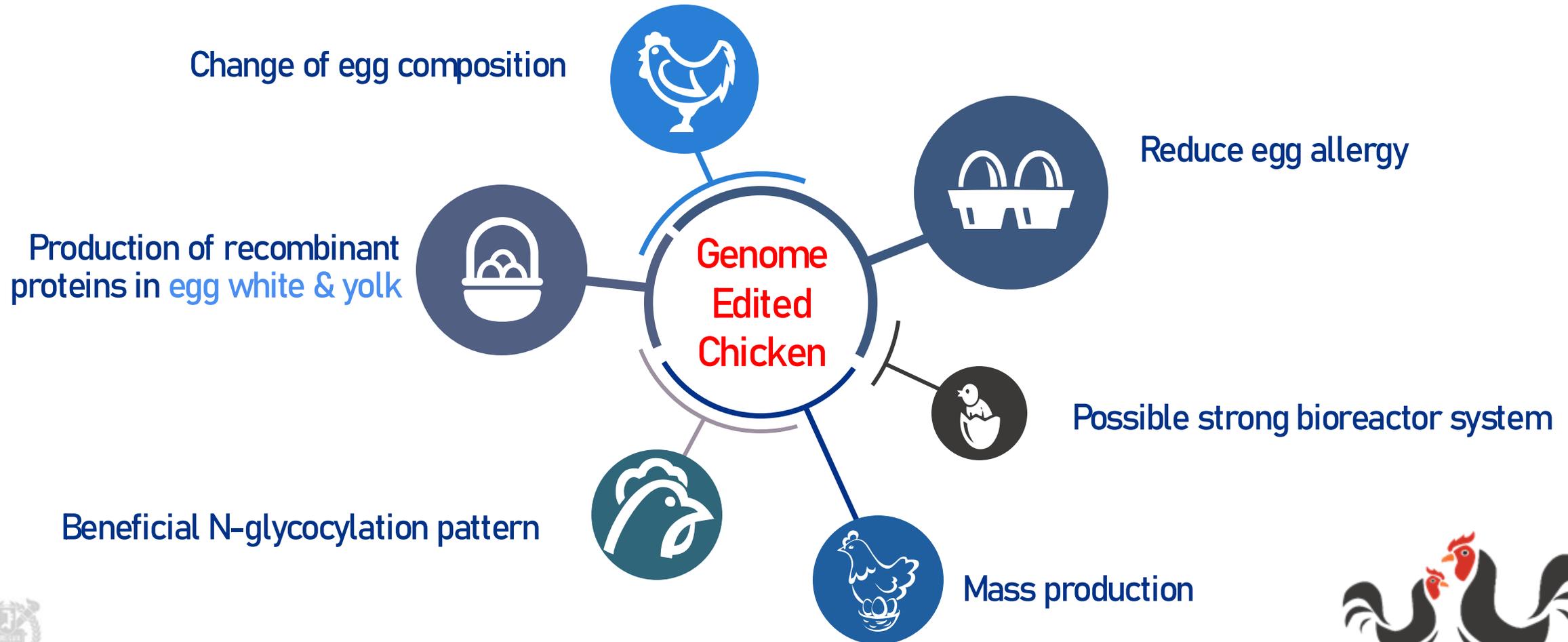
(3-5mg/egg)

✓ Accumulation of hlgG1 Fc in serum and egg yolks was verified by ELISA and western blot.



Conclusion

“Genome edited chicken: egg component regulation and production of recombinant proteins in egg”



Acknowledgement



Collaboration groups



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Mr. Seung Je Woo

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Ms. Jiwon Kim

Mr. Ki Hyeon Kim

Ms. Jinlee Kim

Mr. Chanyoung Kwon

Ms. Yun Ji Shin

Ms. Yu Jin Hong

Ms. Jin Mi Kim



THANK YOU

