

# DdCBE Mediates Efficient and Inheritable Modifications in Mouse Mitochondrial Genome

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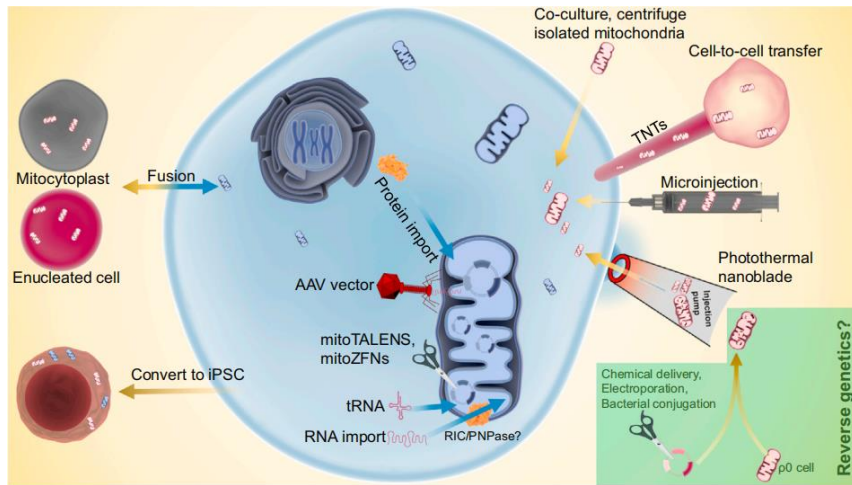
CAM-SU Genomic Resource Center  
Soochow University, China  
September 2021

# The Discovery of DdCBE

## Mitochondrial Genome Editing

- Double Membrane
- Multiple copies

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Patananan AN, *et al.* (2016) *Cell Metabolism*

## Article

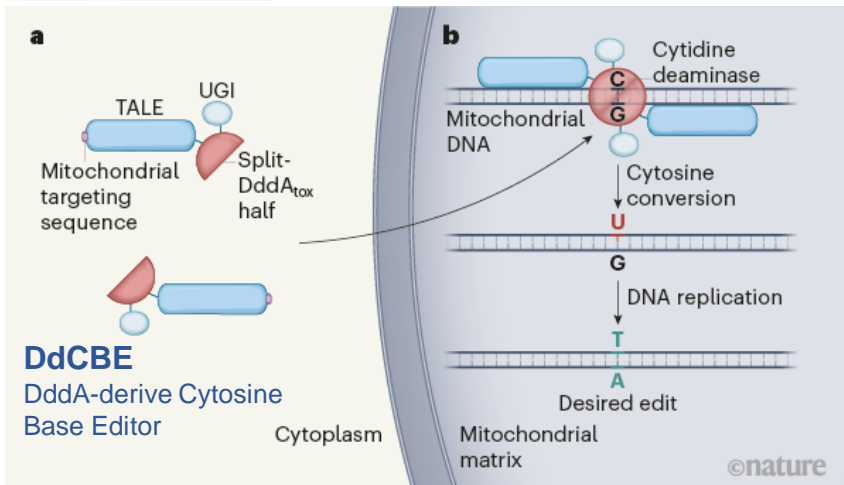
# A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing

<https://doi.org/10.1038/s41586-020-2477-4>

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Beverly Y. Mok<sup>1,2,3,11</sup>, Marcos H. de Moraes<sup>4,11</sup>, Jun Zeng<sup>5</sup>, Dustin E. Bosch<sup>4,5</sup>, Anna V. Kotrys<sup>8,9,10</sup>, Aditya Raguram<sup>1,2,3</sup>, FoSheng Hsu<sup>4</sup>, Matthew C. Radey<sup>4</sup>, S. Brook Peterson<sup>4</sup>, Vamsi K. Mootha<sup>8,9</sup>, Joseph D. Mougous<sup>4,6,7,8</sup> & David R. Liu<sup>12,3,10</sup>



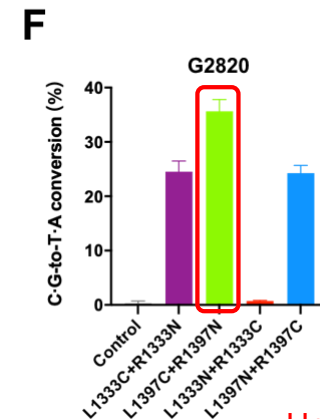
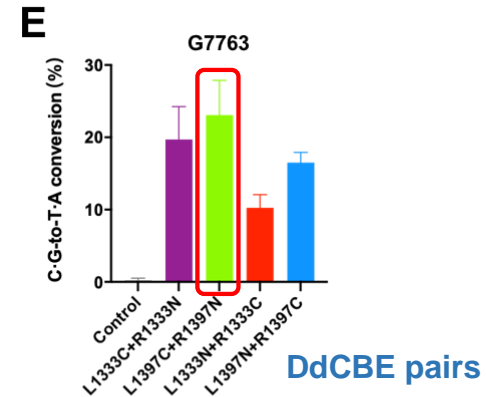
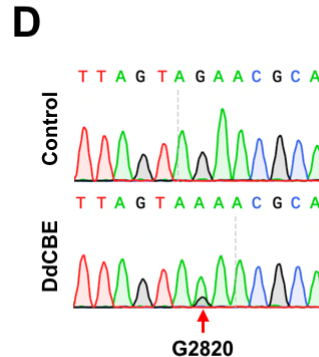
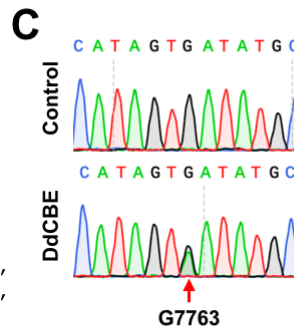
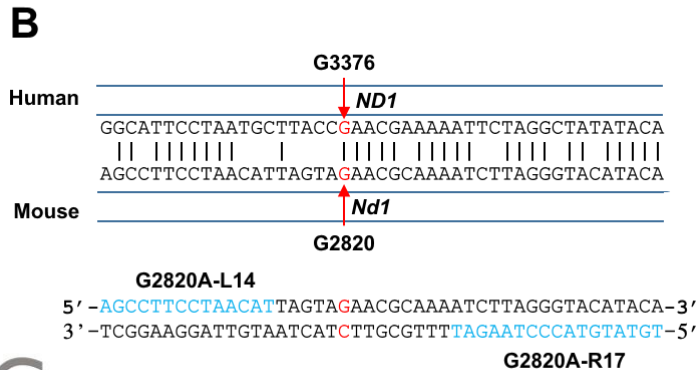
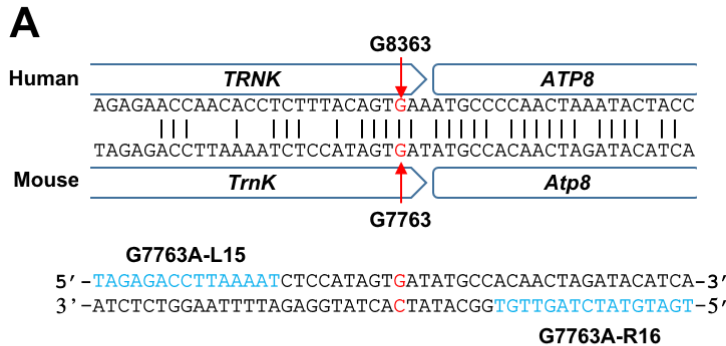
Beverly YM, *et al.* (2020) *Nature*

# Target Selection for DdCBE Editing

Human mtDNA Mutation	Locus	Category	Pathogenic Phenotypes	Mouse Targeting
<b>G8363A</b>	MT-TK	tRNA	MICM+DEAF / MERRF / Autism / Leigh Syndrome / Ataxia + Lipomas	G7763A
<b>G3376A</b>	MT-ND1	Coding	LHON MELAS overlap	G2820A
<b>G13513A</b>	MT-ND5	Coding	Leigh Disease / MELAS / LHON-MELAS Overlap Syndrome / negative association w Carotid Atherosclerosis	G12918A
<b>G8340A</b>	MT-TK	tRNA	Myopathy / Exercise Intolerance / Eye disease + SNHL	G7741A

Data from **MITOMAP**

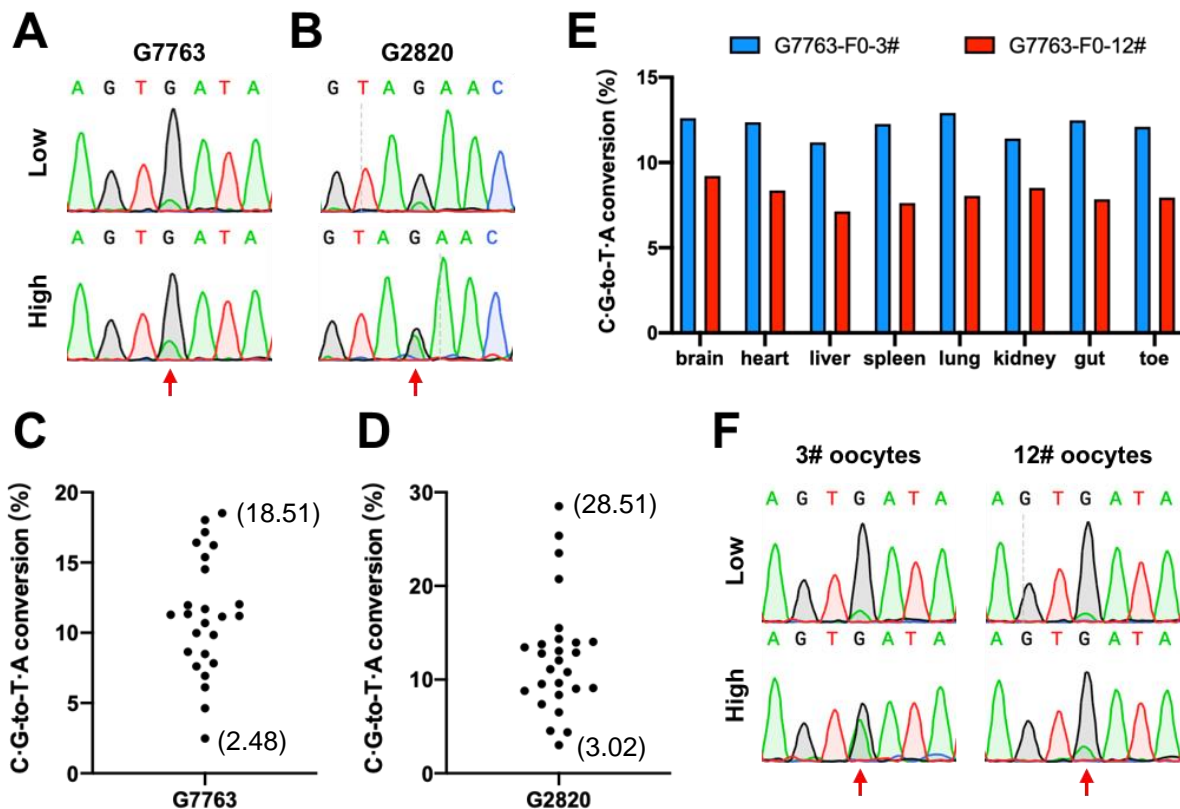
# DdCBE-mediated mtDNA Editing *in vitro*



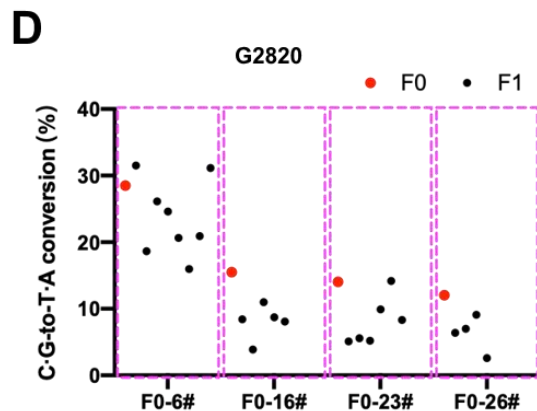
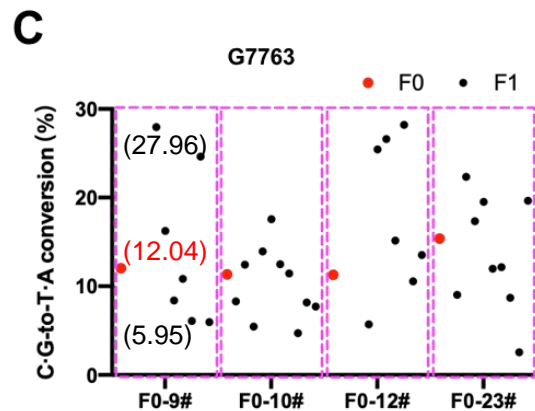
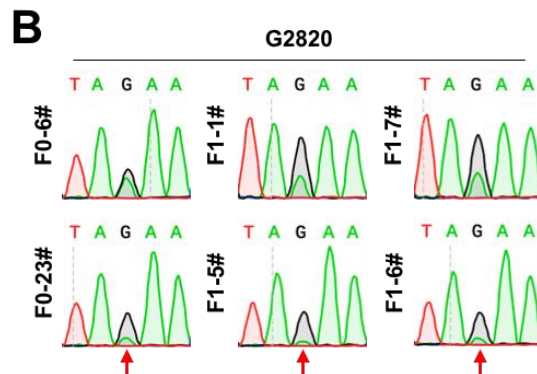
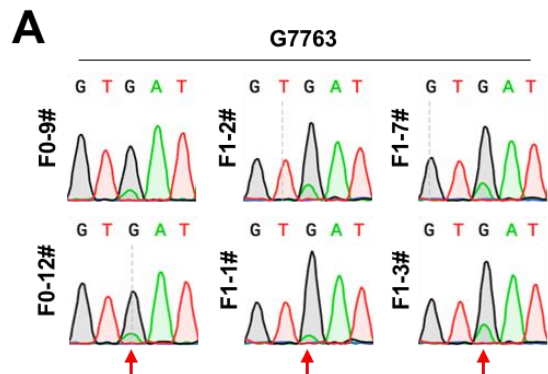
# DdCBE mRNA Injection Summary

Site	Time of injection	Concentration of DdCBE mRNAs (ng/μL)	Number of transplanted embryos	Total pups (rate)	Live pups	Number of edited pups (rate)	Base Conversion Rate		
m.G7763A	1-cell embryo	25	107	51 (0.48)	51	0 (0)	0		
		100	50	14 (0.28)	80	14	25	5 (0.35)	2.48%-16.42%
		150	48	22 (0.46)		22		5 (0.23)	8.49%-15.38%
		200	141	33 (0.23)		32		13 (0.41)	5.76%-18.51%
	2-cell embryo	150	57	5 (0.09)		4		1 (0.25)	11.20%
		200	21	8 (0.38)		8		1 (0.125)	6.94%
m.G2820A	1-cell embryo	150	52	29 (0.56)	66	28	27	16 (0.57)	3.02%-14.33%
		200	25	13 (0.52)		12		3 (0.25)	6.51%-7.38%
	2-cell embryo	150	49	20 (0.51)		18		6 (0.33)	4.54%-23.50%
		200	26	8 (0.31)		8		2 (0.25)	25.36%-28.51%

# DdCBE-mediated mtDNA Editing *in vivo*

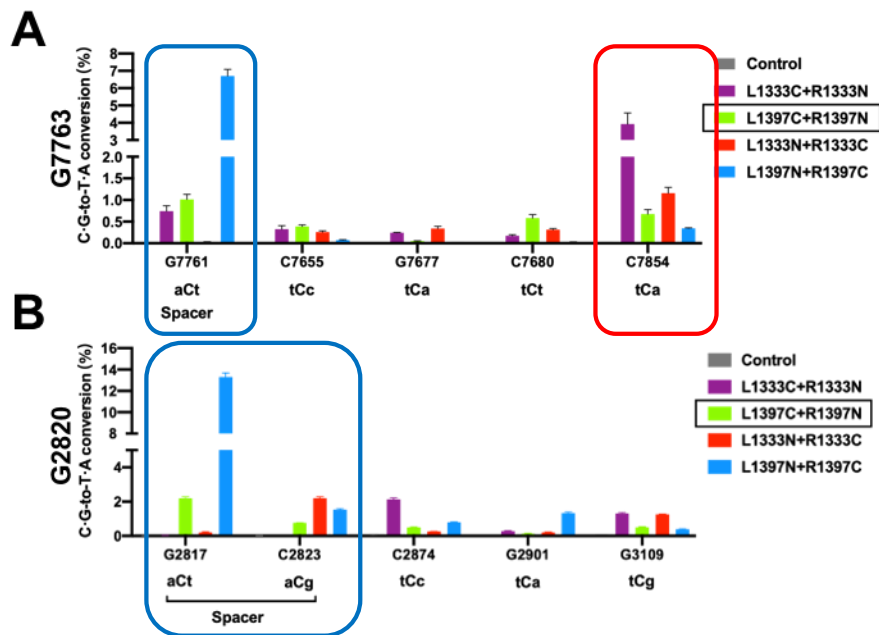


# Germline Transmission of mtDNA Mutation

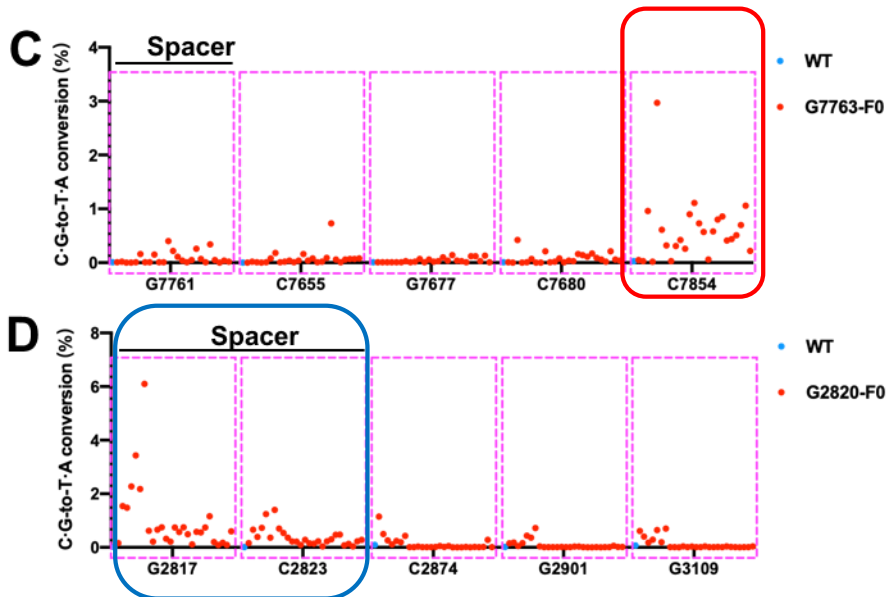


# Off-target Analysis for DdCBE

## N2A Cell



## F0 Founder

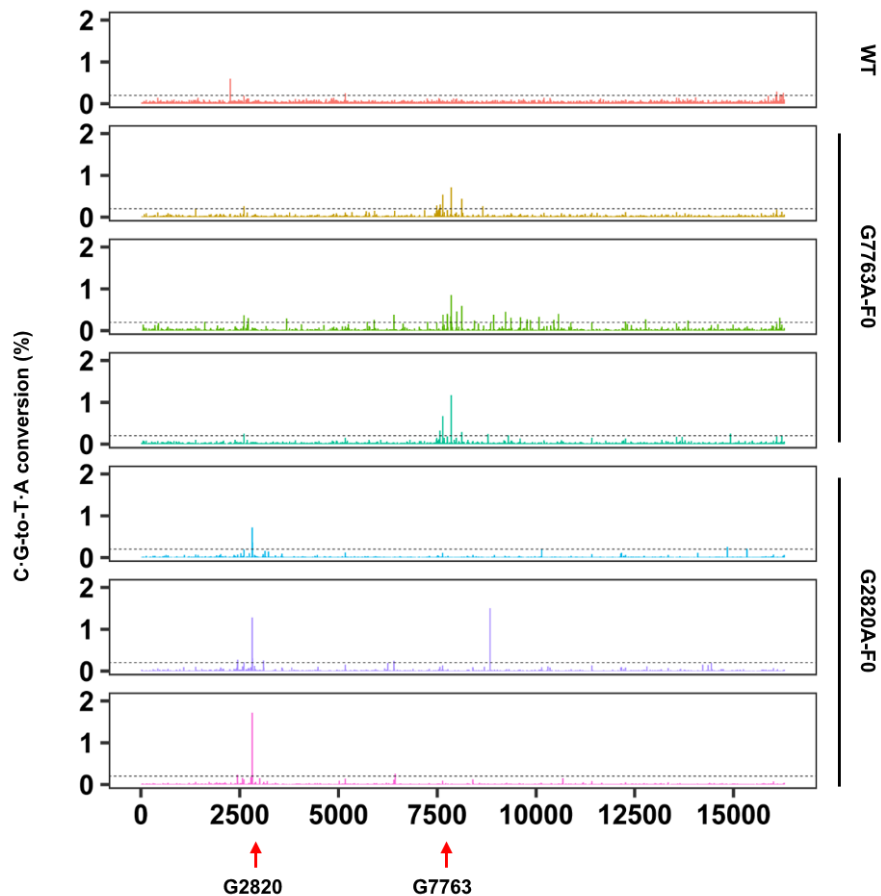




# Off-target Analysis for DdCBE (Cont'd)

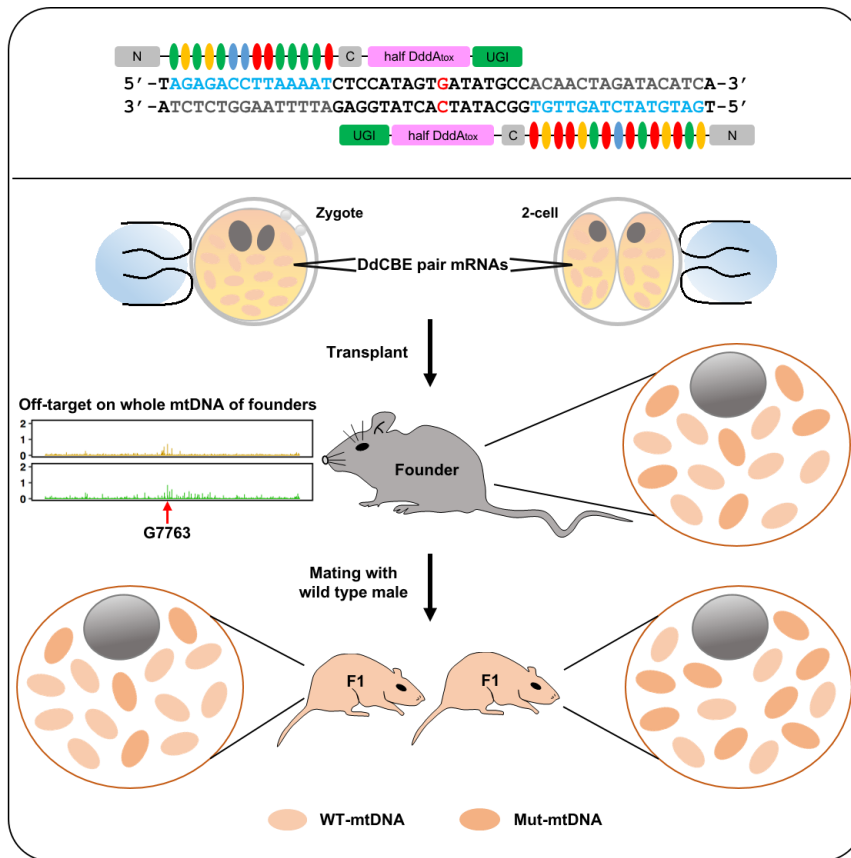


Whole  
mtDNA  
sequencing



Unpublished Data

# Work Summary



## Application

- Model generation
  - for G-C-to-A-T pathogenic mutations
- Therapeutic treatment
  - for A-T-to-G-C pathogenic mutations

Other BE for mtDNA?