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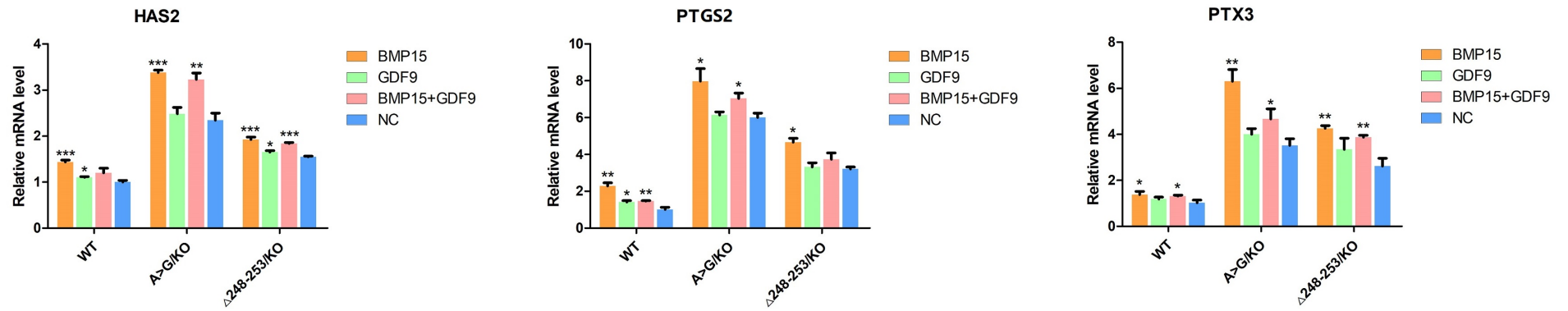
## Supplementary Material

### **Generation and characterisation of a COV434 cell clone carrying a monoallelic *FecB<sup>B</sup>* mutation introduced by CRISPR/Cas9**

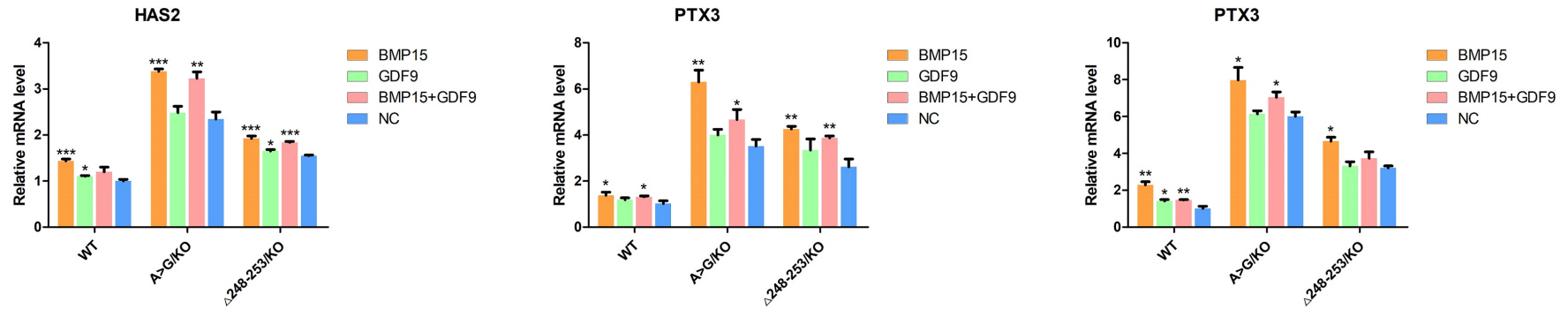
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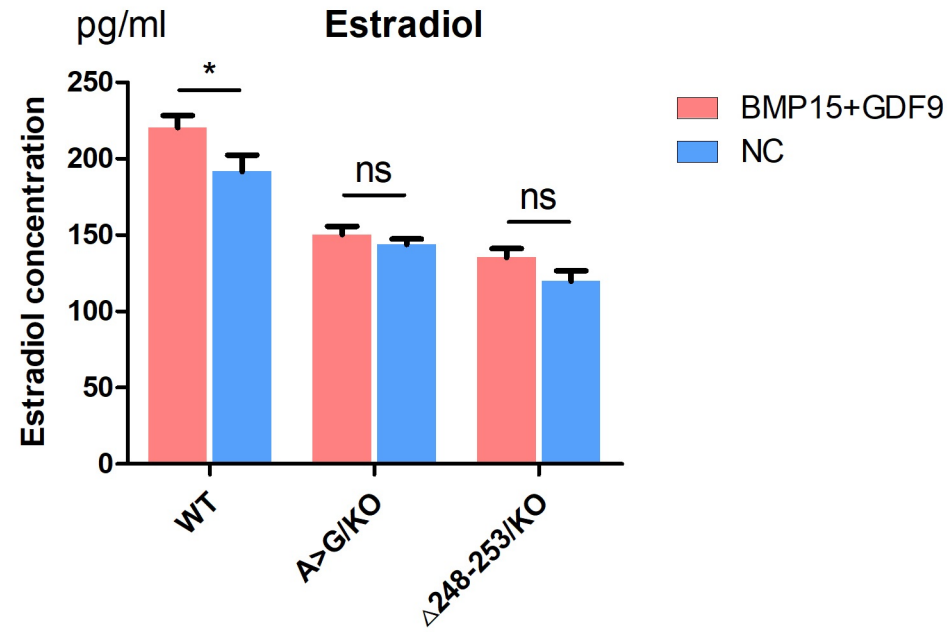
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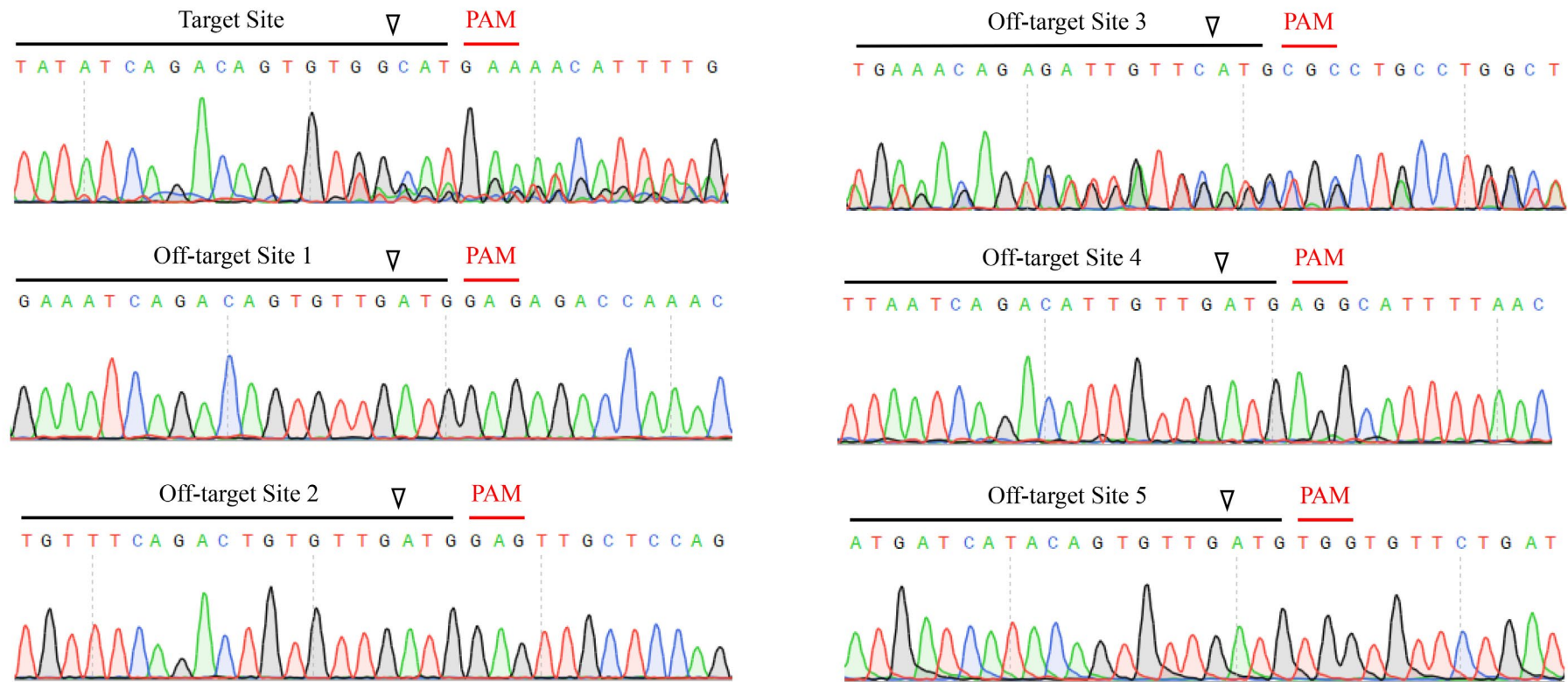
**Fig. S1.** Schematic diagram of the technical route.



**Fig. S2.** Evaluation of the cumulus expansion-related genes (*HAS2*, *PTGS2*, and *PTX3*) in the same cell strain under different treatments, BMP15, GDF9, and BMP15+GDF9. NC indicates the cells given no treatment.



**Fig. S3.** Evaluation of estradiol secretion in the same cell strain under different treatments, BMP15, GDF9, and BMP15+GDF9. NC indicates the cells given no treatment.



**Fig. S4.** Chromatogram of Sanger sequencing of potential off-target sites. The protospacer adjacent motif (PAM) is labeled in red. The Cas9 cleavage site on each target site is indicated by an arrow head.

**Table S1. Sequences of sgRNA oligos**

Oligo	Sequence (5' to 3')
sgRNA-top	CACCGTATATCAGACAGTGTTGATG
sgRNA-bottom	AAACCATCAACACTGTCTGATATAC

**Table S2. Sequences of primers used for T7E1 assay**

Oligo	Sequence (5' to 3')	Amplicon size (bp)
T7E1-F	TTATCGTTCCTGGTCTG	974
T7E1-R	CTCGCATTTCCTGTTTCT	

**Table S3. Information of antibodies used in this study**

Antibodies used for Western Blot (WB) were diluted in tris buffered saline (TBS) solution containing 3% bovine serum albumin (BSA) (w/v), for Flow Cytometric

Analysis(F) were diluted in PBS solution

Antibody	Category	Application	Brands	Cat No.	Dilution Rate(v/v)
BMPR1B	Primary Antibody	F	abcam	ab175385	1:250
P-Smad1/5	Primary Antibody	WB	CST	9516S	1:1000
P-Smad2	Primary Antibody	WB	CST	3108S	1:1000
P-Smad3	Primary Antibody	WB	CST	9520S	1:1000
HSD3B2	Primary Antibody	WB	abcam	ab154385	1:500
GAPDH	Primary Antibody	WB	proteintech	10494-1-AP	1:3000
Beta Tubulin	Primary Antibody	WB	abcam	ab179513	1:2500
Anti-Rabbit IgG (Goat)	Secondary Antibody	WB	Pekin Elmer	NEF812001EA	1:5000
Anti-rabbit IgG (H+L), F(ab') <sub>2</sub> Fragment	Secondary Antibody	F	CST	4412	1:750



**Table S4. Sequences of primers used for qPCR**

Oligo	Sequence (5' to 3')	Amplicon size (bp)	Accession ID of target gene
<i>BMPR1B</i> -F	CTTTTGCGAAGTGCAGGAAAAT	130	NM_001256792
<i>BMPR1B</i> -R	TGTTGACTGAGTCTTCTGGACAA		
<i>Has2</i> -F	CTCTTTTGGACTGTATGGTGCC	205	NM_005328
<i>Has2</i> -R	AGGGTAGGTTAGCCTTTTCACA		
<i>Ptgs2</i> -F	CTGGCGCTCAGCCATACAG	94	NM_000963
<i>Ptgs2</i> -R	CGCACTTATACTGGTCAAATCCC		
<i>Ptx3</i> -F	AGGCTTGAGTCTTTTAGTGCC	94	NM_002852
<i>Ptx3</i> -R	ATGGATTCCCTCTTTGTGCCATAG		
<i>β-actin</i> -F	CATGTACGTTGCTATCCAGGC	250	NM_001101
<i>β-actin</i> -R	CTCCTTAATGTCACGCACGAT		

**Table S5. Details of the potential off-target sites**

Name	Sequence (5' to 3')	PAM	Score	Chromosome	Strand	Position
OTS-1	GAAATCAGACAGTGTTGATG	GAG	5.383333	chr13	-	95506683
OTS-2	TGTTTCAGACTGTGTTGATG	GAG	1.650235	chr13	+	74972914
OTS-3	TGTAAGAGACAGTGTTGATG	CGG	1.514493	chr16	-	85010610
OTS-4	TTAATCAGACATTGTTGATG	AGG	1.49872	chr12	+	130836020
OTS-5	ATGATCATACAGTGTTGATG	TGG	1.457521	chr11	+	99225661

**Table S6. Sequences of primers used for off-target analysis**

Oligo	Sequence (5' to 3')	Amplicon size (bp)
OTS1-F	ACATGGGATGGACTGGTGGGA	787
OTS1-R	GGGTGATGTGGGATTCAAAC	
OTS2-F	AGTGGCCAGAACGTCACAAA	916
OTS2-R	AGCCAATTTGGGGCTCAACT	
OTS3-F	GTTGCCTGAAAGGGCATCTG	457
OTS3-R	GTACCAGGCCACAAAGTGACTA	
OTS4-F	CATGCAAACAATTCCCACAG	962
OTS4-R	CGTGAAATGGGGACAGTGGTA	
OTS5-F	GGTTACCGCTGGAAAGGCTA	966
OTS5-R	GGGATCCTAAAGACCCTCACG	

**Table S7. Sequence of ssODN oligo**

Oligo	Sequence (5' to 3')
ssODN	GAAGCCAGCTGGTTCAGAGAGACAGAAATATATCGGACAGTGTTG ATGCGCCATGAAAACATTTTGGGTGAGTAAAAGTCTGCATAGCTA

**Table S8. Genotype and translational prediction of COV434 single cell derived clones**

ID	Genotype	Size of indels	Description	Protein translation	Premature stop codon
1	Biallelic	18bp deletion.	2 <sup>nd</sup> -19 <sup>th</sup> nt before PAM	BMPR1B <sup>△248-253</sup>	NO
		10bp deletion	1 <sup>st</sup> -10 <sup>th</sup> nt before PAM	KO	YES
2	Biallelic	4bp deletion.	5 <sup>th</sup> -8 <sup>th</sup> nt before PAM	KO	YES
		3bp substitution	HDR	HDR	NO
3	Biallelic	3bp substitution	HDR	HDR	NO
		1bp deletion	4 <sup>th</sup> nt before PAM	KO	YES
4	WT	WT	WT	WT	NO
		WT	WT	WT	NO
5	WT	WT	WT	WT	NO
		WT	WT	WT	NO

6	Biallelic	3bp deletion	3 <sup>rd</sup> -5 <sup>th</sup> nt before PAM	BMPR1B <sup>Δ253</sup>	NO
		2bp insertion	1 <sup>st</sup> -2 <sup>nd</sup> nt before PAM	KO	YES
7	WT	WT	WT	WT	NO
		WT	WT	WT	NO
8	Biallelic	1bp insertion	1 <sup>st</sup> nt before PAM	KO	YES
		1bp deletion	2 <sup>nd</sup> nt before PAM	KO	YES
9	Homozygous	1bp deletion	2 <sup>nd</sup> nt before PAM	KO	YES
		1bp deletion	2 <sup>nd</sup> nt before PAM	KO	YES
10	Biallelic	5bp deletion	5 <sup>th</sup> -6 <sup>th</sup> and 8 <sup>th</sup> -10 <sup>th</sup> nt before PAM	KO	YES
		1bp deletion	1 <sup>st</sup> nt before PAM	KO	YES
11	WT	WT	WT	WT	NO
		WT	WT	WT	NO

12	WT	WT	WT	WT	NO
		WT	WT	WT	NO
13	Biallelic	1bp deletion	4 <sup>th</sup> nt before PAM	KO	YES
		1bp deletion	2 <sup>nd</sup> nt before PAM	KO	YES
14	WT	WT	WT	WT	NO
		WT	WT	WT	NO
15	Biallelic	1 bp insertion	3 <sup>rd</sup> nt before PAM	KO	YES
		2bp insertion	4 <sup>th</sup> nt before PAM	KO	YES
16	WT	WT	WT	WT	NO
		WT	WT	WT	NO
17	Biallelic	6bp deletion	5 <sup>th</sup> -10 <sup>th</sup> nt before PAM	BMPR1B <sup>Δ251-252</sup>	NO
		4bp deletion	6 <sup>th</sup> -9 <sup>th</sup> nt before PAM	KO	YES

18	WT	WT	WT	WT	NO
		WT	WT	WT	NO
19	WT	WT	WT	WT	NO
		WT	WT	WT	NO

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