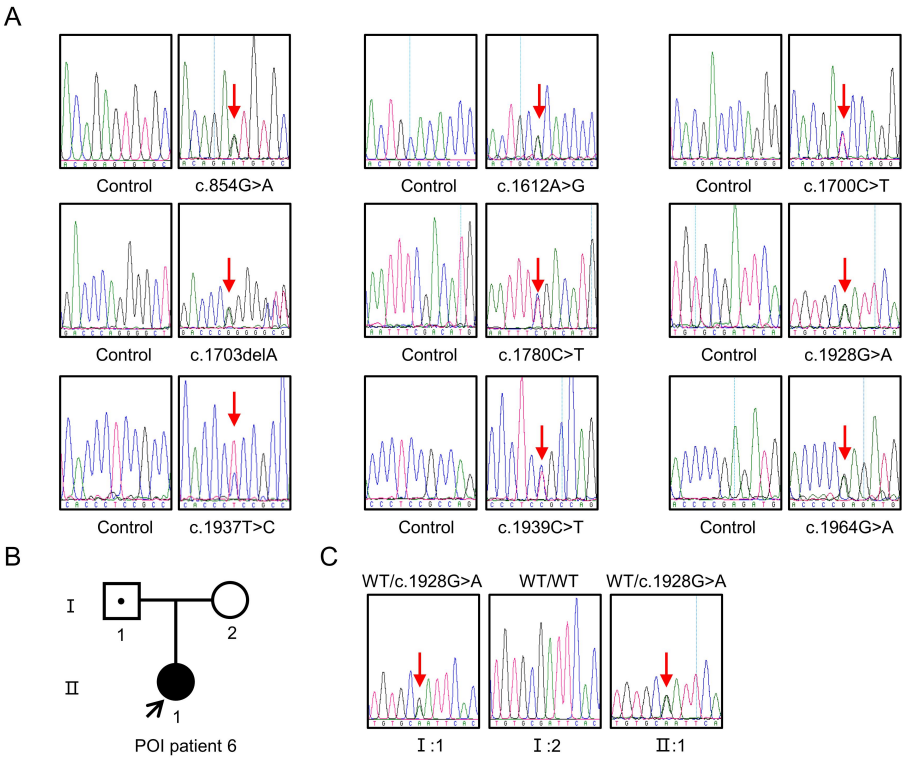
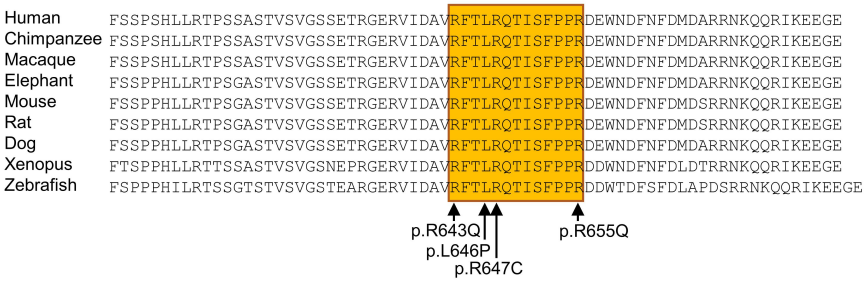
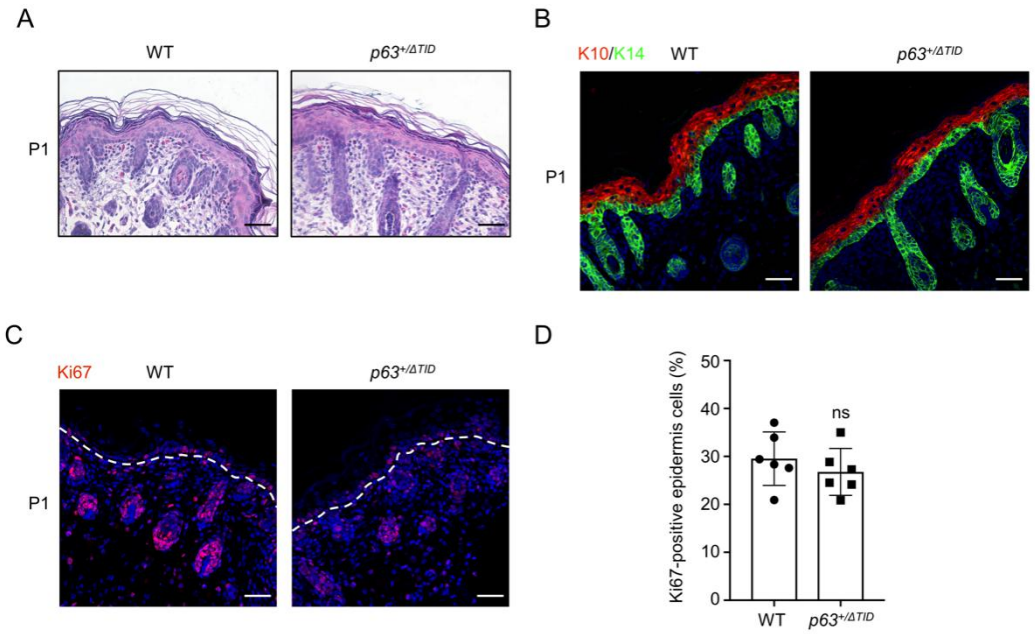


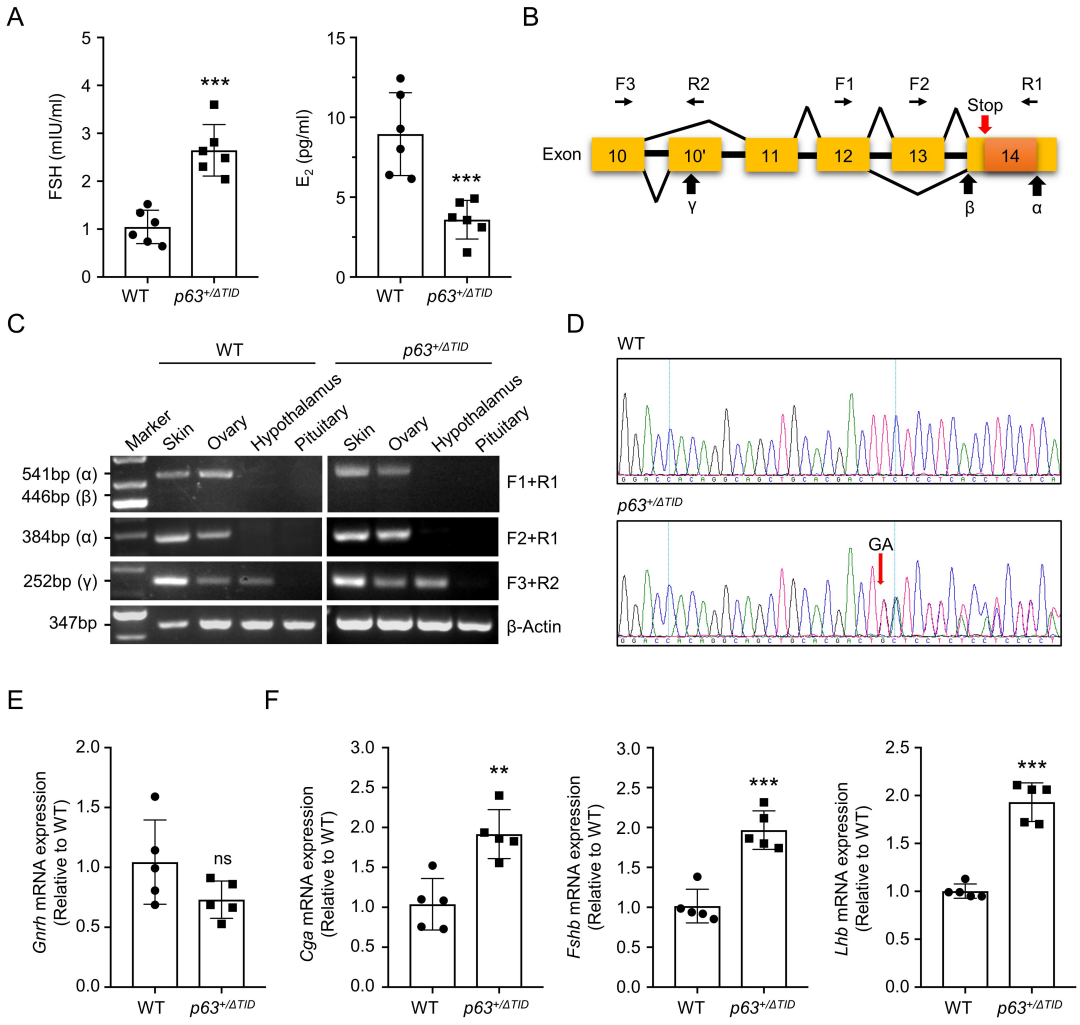

**Supplemental Figure 1. Validation of the *TP63* mutations in POI patients. (A)** Sequence chromatograms showing nine heterozygous variants in POI patients that were not present in the controls. NM_003722.5 was used as the reference sequence. **(B and C)** Sanger sequencing confirmed the presence of the c.1928G>A variant in POI patient 6 and her father, but not in her mother.




**Supplemental Figure 2. Conservation analysis of amino acids in the TID among various species.** The yellow frame indicates the core sequence of the TID. The positions of the four novel point mutations in the core sequence are shown.

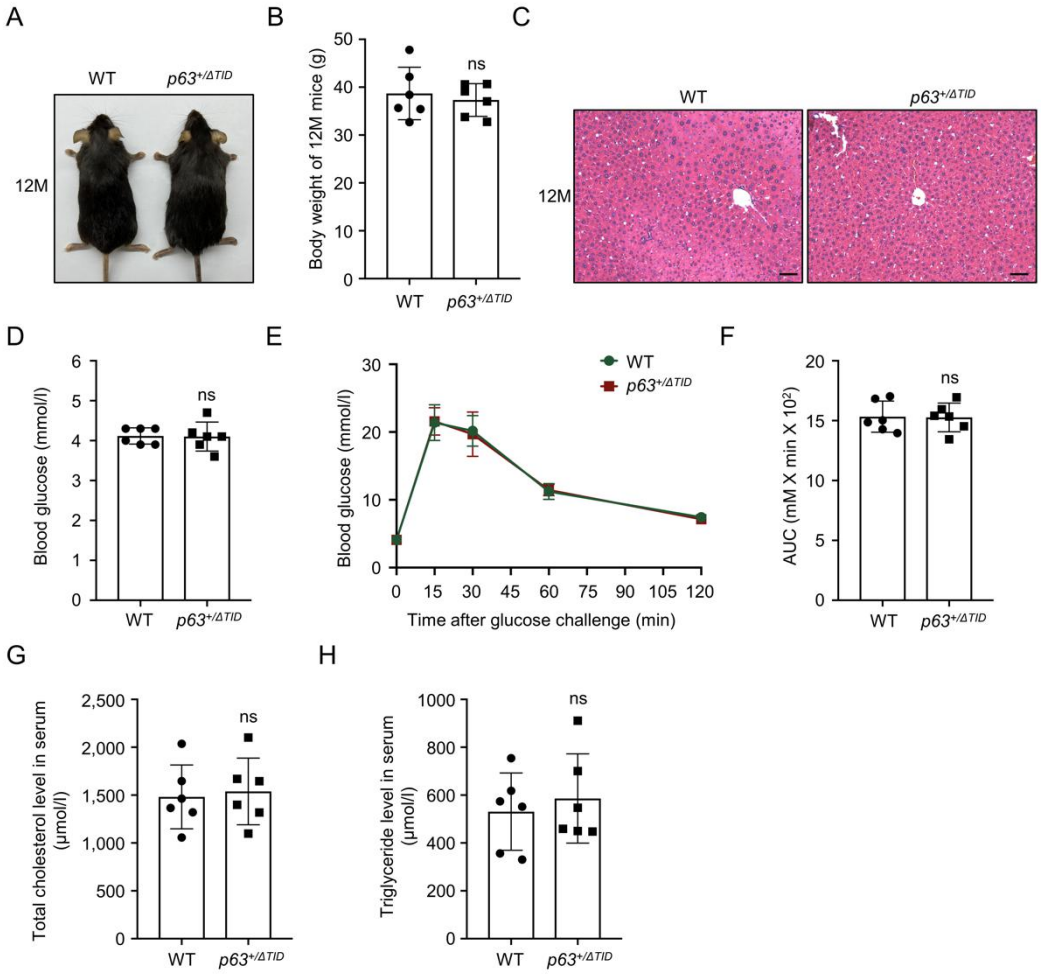



**Supplemental Figure 3. Epidermis characterization of *p63*$^{+/\Delta TID}$ mice. (A)** HE staining of dorsal skin sections of P1 WT and *p63*$^{+/\Delta TID}$ mice. Scale bar: 50 µM. **(B and C)** IF staining of K10 (red), K14 (green), and Ki67 (red) on dorsal skin sections of P1 WT and *p63*$^{+/\Delta TID}$ mice. Cell nuclei were counterstained with DAPI (blue). Scale bar: 50 µM. **(D)** Quantitative analysis of Ki67-positive cells in the epidermis. Data are shown as the mean ± SD, n = 6 for each genotype. Unpaired two-tailed Student's *t*-test was used for the comparison of the two groups. ns, not significant.




**Supplemental Figure 4. Expression of *p63* and functional analysis of the hypothalamus and pituitary in *p63*$^{+/\Delta TID}$ mice. (A)** Serum follicle-stimulating hormone (FSH) and Estradiol ($E_2$) levels were measured in 2M WT and *p63*$^{+/\Delta TID}$ mice (n = 6 for each genotype). **(B)** C-terminal structure of the *p63* gene. The primers F1, F2, F3, R1, and R2 are indicted by horizontal black arrows. The stop codons of *p63α*, *p63β*, and *p63γ* are indicted by vertical black arrows, and the introduced stop codon in *p63α* is indicated by the red arrow. **(C)** Semi-quantitative RT-PCR analysis of *p63* isoform expression in the P1 epidermis, P1 ovary, 2M hypothalamus, and 2M pituitary of WT and *p63*$^{+/\Delta TID}$ mice. The three isoforms amplified by the primers are indicated in parentheses. The *p63β* isoform with a predicted band size of 446 bp was not detected. **(D)** Sanger sequencing of the PCR products of F1+R1 primers obtained by amplifying the cDNA from P1 WT and *p63*$^{+/\Delta TID}$ ovaries. The red arrow indicates the site of GA insertion. **(E and F)** Quantitative RT-PCR analyses of the *Gnrh* gene in

the hypothalamus and the *Cga, Fshb*, and *Lhb* genes in the pituitary of 2M WT and $p63^{+/\Delta TID}$ mice. *Gapdh* was used as the internal control. n = 5 for each phenotype. In panel A, E, and F, data are presented as the mean ± SD, and differences between the groups were analyzed for statistical significance by the unpaired two-tailed Student's *t*-test. $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant.




**Supplemental Figure 5. Metabolic phenotypes of WT and *p63*$^{+/\Delta TID}$ mice. (A)** Gross morphology of 12M WT and *p63*$^{+/\Delta TID}$ females. **(B)** No significant differences were observed in body weight between 12M WT and *p63*$^{+/\Delta TID}$ mice. n = 6 per group. **(C)** HE staining of liver sections of 12M WT and *p63*$^{+/\Delta TID}$ mice. Scale bar: 50 μM. **(D and E)** Fasting blood glucose levels and results of the glucose tolerance test performed in 2M WT and *p63*$^{+/\Delta TID}$ mice fasted for 16 h. Each point on the graph indicates the level of glucose in the blood. Blood glucose levels were detected at 15 min, 30 min, and 60 min in the glucose tolerance test. n = 6 per group. **(F)** Areas under the curves (AUC) calculated from mice in the glucose tolerance test. n = 6 for each genotype. **(G and H)** Serum total cholesterol and triglyceride levels in 2M WT and *p63*$^{+/\Delta TID}$ mice. n = 6 for each genotype. In panel B, D, and F-H, data are presented as the mean ± SD, and differences between the groups were analyzed for statistical significance by the unpaired two-tailed Student's *t*-test. ns, not significant.

**Supplemental Table 1. Clinical characteristics of POI patients with mutations in *TP63*.**

| Patient ID | Phenotype | Menarche age (yr) | Amenorrhea age (yr) | Age at diagnosis (yr) | FSH (IU/l) | $E_2$ (pg/ml) | Left ovary (cm*cm) | Right ovary (cm*cm) |
|---|---|---|---|---|---|---|---|---|
| 1 | SA | 13 | 16 | 28 | 88.88 | 20 | Invisible | Invisible |
| 2 | SA | 15 | 29 | 38 | 88.27 | <5 | 1.5*0.7 | 1.6*1 |
| 3 | SA | 13 | 19 | 28 | 67.3 | <5 | 1.4*0.7 | 1.4*1 |
| 4 | PA | - | - | 32 | 34.51 | 11.5 | Invisible | 1.3*0.5 |
| 5 | PA | - | - | 27 | 48.73 | <5 | Invisible | Invisible |
| 6 | PA | - | - | 24 | 54.6 | <5 | 1.1*0.5 | 1.4*0.7 |
| 7 | PA | - | - | 23 | 70.91 | <5 | Invisible | Invisible |
| 8, 9, 10 | SA | 13; 19; 16 | 25; 19; 16 | 27; 28; 30 | 55.77; 78.32; 26.45 | <5; 20; <5 | 1.5*0.8; Invisible; 1.4*1.1 | 1.4*0.6; Invisible; 1.7*0.8 |
| 11 | SA | 13 | 13 | 28 | 67.16 | <5 | 1.7*0.7 | 1.7*0.6 |

$E_2$, estradiol; FSH, follicle-stimulating hormone; POI, premature ovarian insufficiency; PA, primary amenorrhea; SA, secondary amenorrhea; yr, years; -, not available.

**Supplemental Table 2. Comparison of amino acid sequences in different C-terminal knockout strategies.**

| Name | Sequences | Auto-activation in oocyte | Reference |
|---|---|---|---|
| Cα | VRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKQTSMQSQ SSYGNSSPPLNKMNSMNKLPSVSQLINPQQRNALTPTTMPEGMGANIPMMGT HMPMAGDMNGLSPTQALPPPLSMPSTSHCTPPPPYPTDCSIVSFLARLGCSSCL DYFTTQGLTTIYQIEHYSMDDLASLKIPEQFRHAIWKGILDHRQLHDFSSPPHLL RTPSGASTVSVGSSETRGERVIDAVRFTLRQTISFPPRDEWNDFNFDMDSRRNK QQRIKEEGE* | - | Lena AM, et al. Nat Commun, 2021; Suh EK, et al. Nature, 2006 |
| CαΔTID | VRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKQTSMQSQ SSYGNSSPPLNKMNSMNKLPSVSQLINPQQRNALTPTTMPEGMGANIPMMGT HMPMAGDMNGLSPTQALPPPLSMPSTSHCTPPPPYPTDCSIVSFLARLGCSSCL DYFTTQGLTTIYQIEHYSMDDLASLKIPEQFRHAIWKGILDHRQLHD* | + | This study |
| Cβ | VRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKQTSMQSQ SSYGNSSPPLNKMNSMNKLPSVSQLINPQQRNALTPTTMPEGMGANIPMMGT HMPMAGDMNGLSPTQALPPPLSMPSTSHCTPPPPYPTDCSIVRIWQV* | + | Lena AM, et al. Nat Commun, 2021 |
| Cα’ | VRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKQTSMQSQ SSYGNSSPPLNKMNSMNKLPSVSQLINPQQRNALTPTTIPDGMGANIS* | - | Suzuki D, et al. Development, 2015 |
| Cγ | VRGRETYEMLLKIKESLELMQYLPQHTIETYRQQQQQQHQHLLQKHLLSACF RNELVEPRGEAPTQSDVFFRHSNPPNHSVYP* | - | Lena AM, et al. Nat Commun, 2021 |

Green, SAM domain; Red, TID domain; Blue, essential for activation in oocytes (EAO) domain; *, stop codon; Underline, sequences of Cβ and Cγ different from Cα; +/-, auto-activation/inactivation in oocyte.

**Supplemental Table 3. Primers used in this study.**

| Primer | Sequence (5'-3') | Product |
|---|---|---|
| **Genotyping *p63*$^{+/\Delta TID}$ mice** | | |
| Forward | CATTTAAGCCAAAACACCAGAGAGT | 387bp |
| Reverse | ATTCTCCTTCCTCTTTGATACGCT | |
| **Genotyping *p63*$^{+/R647C}$ mice** | | |
| Forward | TGAGGCCAGTGGAGAACAAG | 582bp |
| Reverse | GCCTCCTAATTCTCCGTCCC | |
| **qPCR** | | |
| *Noxa*-Forward | GTTCGCAGCTCAACTCAGGA | 72bp |
| *Noxa*-Reverse | ACCACAGTTATGTCCGGTGC | |
| *Puma*-Forward | CAGCACTTAGAGTCGCCCG | 196bp |
| *Puma*-Reverse | GTGAGGGTCGGTGTCGATG | |
| *Gapdh*-Forward | AGGTCGGTGTGAACGGATTTG | 123bp |
| *Gapdh*-Reverse | TGTAGACCATGTAGTTGAGGTCA | |
| *Gnrh*-Forward | GGGAAAGAGAAACACTGAACA | 98bp |
| *Cnrh*-Reverse | GGACAGTACATTCGAAGTGCT | |
| *Cga*-Forward | CTGTTGCTTCTCCAGGGCATA | 66bp |
| *Cga*-Reverse | TTCTTTGGAACCAGCATTGTCTT | |
| *Fshb* -Forward | GGAGAGCAATCTGCTGCCATA | 77bp |
| *Fshb*-Reverse | GCAGAAACGGCACTCTTCCT | |
| *Lhb* -Forward | TGGCCGCAGAGAATGAGTTC | 84bp |
| *Lhb*-Reverse | CTCGGACCATGCTAGGACAGTAG | |
| **Semi-quantitative RT-PCR** | | |
| F1-Forward | GACTCAGCCCTACCCAAGCTCTC | F1+R1 (α/β): |
| R1-Reverse | TGTAGGGGCTGGGAGGTGGAAG | 541bp/446bp |
| F2-Forward | GACCACCATCTATCAGATTGAGC | F2+R1 (α): |
| R1-Reverse | TGTAGGGGCTGGGAGGTGGAAG | 384bp |
| F3-Forward | GTGAGAGGTCGTGAGACGTAC | F3+R2 (γ): |

| | | |
|---|---|---|
| R2-Reverse | CTATGGGTACACGGAGTGGTT | 252bp |
| β-Actin-Forward | TGTCCCTGTATGCCTCTGGTCG | 347bp |
| β-Actin-Reverse | GAACCGCTCGTTGCCAATAGTG | |
| **Human mutations** | | |
| Forward (c.854) | TTTTGCCACCAACATCCTGT | 387bp |
| Reverse (c.854) | CTGAAGGGAAAGCATGTGGAG | |
| Forward (c.1612) | TGTAGGCTGTTTGAAGGGGT | 653bp |
| Reverse (c.1612) | GAGTAAGTGAAGGCGAGGGA | |
| Forward (c.1700, 1703) | TGTCACCAGTAATCTCCAGACC | 543bp |
| Reverse (c.1700, 1703) | CTCCTCTTTCCCACCTTGAGA | |
| Forward (c.1780, 1928, 1937, 1939, 1964) | GTTCTACACAGGCAGGAAAGAC | 697bp |
| Reverse (c.1780, 1928, 1937, 1939, 1964) | GCCAGAATCAGAATTAGATGCCA | |