Supplemental Figures

Figure S1

(**A**) HSD3B1 positive Leydig cell number in WT (n=3) and *Lhb*-/- mice injected with PBS (n=3), hCG (n=3) or osteocalcin (3ng/g/day) (n=3). The count of the HSD3B1 positive Leydig cells was performed on 30 sections for each conditions (Scale bar, 100µm). (**B**) Analysis of 2 week-old *Lhb*-/- male mice injected for one month with PBS (n=5), osteocalcin (3ng/g/day) (n=5) or hCG (Human Chorionic Gonadotrophin) (5UI twice a week) (n=5). Immunofluorescence, using anti-Cyp17, anti-3b-HSD or anti-Cyp11a antibodies as markers of mature Leydig cells. All of the analyses were performed on nonbreeder C57BL/6J mice. (**C**) Circulating LH levels in WT and Osteocalcin-/- mice untreated or treated with hCG (5UI twice a week) (n=7 for each groups).

Figure S2

(**A**) In situ hybridization of *Gprc6a* performed in testis (Scale bar, 200µm), pituitary gland (Scale bar, 500µm) and hypothalamus (Scale bar, 500µm) in WT and *Gprc6a*-/- mice. (**B**) qPCR analysis of *Gprc6a* expression level in WT and *Gprc6a*-/- pancreas, kidney, testis, hypothalamus and pituitary gland.

Figure S3

(**A-C**) qPCR analysis of (**A**) *Osteocalcin,* (**B**) *Gamma-glutamyl carboxylase* (*Ggcx*) and (**C**) *Vitamin K epoxide reductase complex, subunit 1 (Vkorc1)* in differentiated

osteoblasts treated for 4 hours with vehicle, 100UI, 10UI, 3UI, 1UI and 0.3UI of hCG for 4 hours.

Figure S4

(**A**) *Ctsk-Cre*;*DTA*$^{fl/+}$ mice developed a classical osteopetrosis phenotype characterized by very dense bone on X-ray images and an absence of incisors eruption due to a severe impairment in bone resorption. (**B**) Sperm counts, (**C**) seminal vesicle weight normalized to body weight (mg/g of BW), (**D**) circulating testosterone levels in *Ctsk-Cre*;*DTA*$^{fl/+}$ male mice injected with vehicle (n=5) or osteocalcin (3ng/g/day) (n=5). All analyses were performed on nonbreeder C57BL/6J mice.

Figure S5

(**A**) Measurement of the uncarboxylated (GLU-OCN), carboxylated (GLA13-OCN), total (Total-OCN) and undercarboxylated(GLU13-OCN) forms of osteocalcin in serum of WT (n=12), , *Opg*+/- (n=10), *Osteocalcin*+/- (n=3) and *Osteocalcin*+/-*; Opg*+/- (n=6) mice at 12-weeks of age. The data are represented in % versus WT serum.

Figure S6

(**A**) Schematic representation of the amino acid sequence containing the substitution. This region is highly conserved in several species and the amino acid mutated in the patients is

highlighted in red. (B) Family tree of the patient originating from Togo. (C) Family tree of the patient originating from Senegal. **O** indicates female and □ indicates male. The arrow designated the propositus who carries the F464Y mutation on the osteocalcin receptor *GPRC6A* and presents a severe oligospermia due to peripheral hypogonadism.

Figure S7

(**A**) Western blot analysis of MYC in HEK293T cells transfected with GPRC6A-pcDNA3 or GPRC6A mutant-pcDNA3. Beta-tubulin has been used as a loading control.

Figure S8

(**A-B**) Testis pictures of all of the models presented in this paper. (**A**) Testis of control, *Ctsk-Cre*;$DTA^{fl/+}$ and *Ctsk-Cre*;$DTA^{fl/+}$ injected with osteocalcin (3ng/g/ day during 1 month) versus WT male mice. (**B**) Testis of control, *Opg*-/- and *Opg*+/- mice. (**C**) Testis of control and $InsR_{osb}$+/-; *Osteocalcin*+/- mice. (**D**) Testis of control and $InsR_{osb}$+/-; *Gprc6a*+/- mice.

Supplemental Table 1

Table containing the values of the sperm count (million/ml) of the patient with cryptochidy.

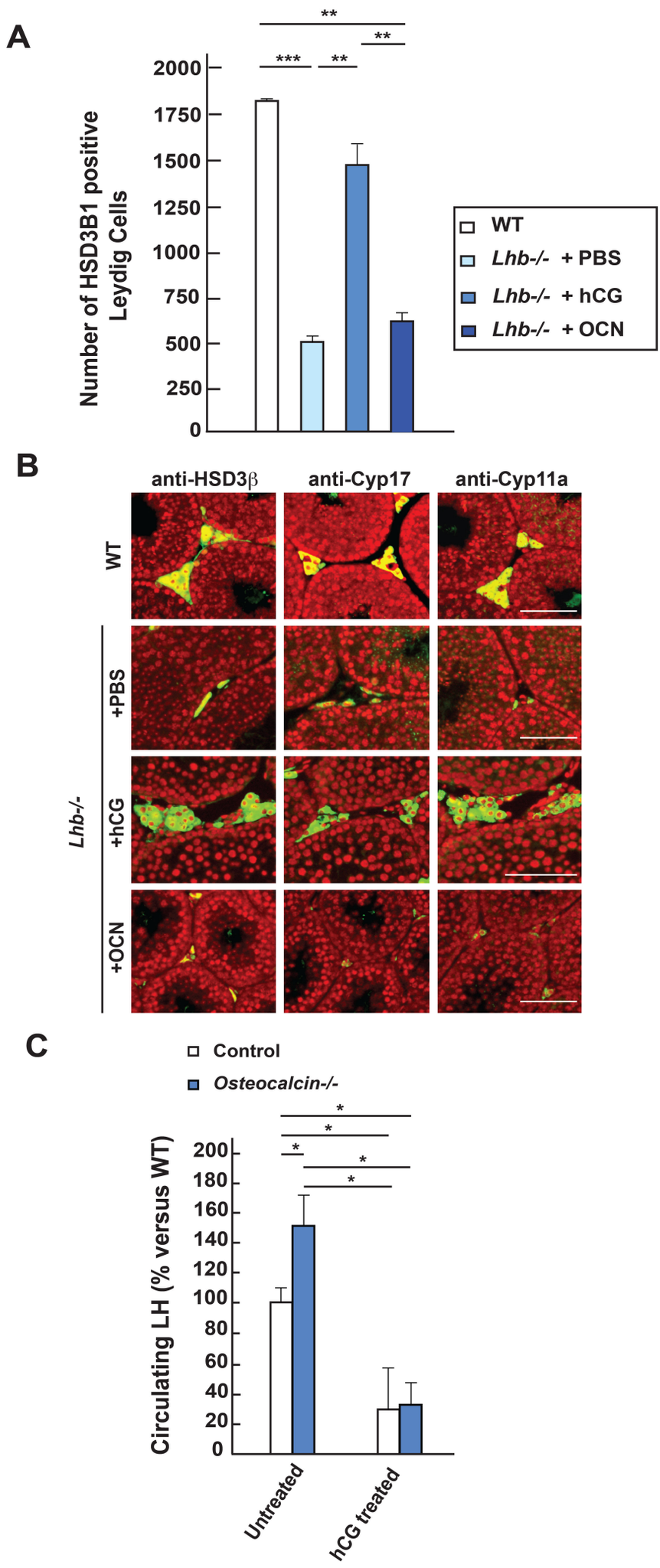

A
Number of HSD3B1 positive Leydig Cells
2000
1750
1500
1250
1000
750
500
250
0
**
***
**
**
WT
Lhb-/- + PBS
Lhb-/- + hCG
Lhb-/- + OCN
B
anti-HSD3β
anti-Cyp17
anti-Cyp11a
WT
Lhb-/-
+PBS
+hCG
+OCN
C
Control
Osteocalcin-/-
Circulating LH (% versus WT)
200
180
160
140
120
100
80
60
40
20
0
*
*
*
*
*
Untreated
hCG treated

Figure S1

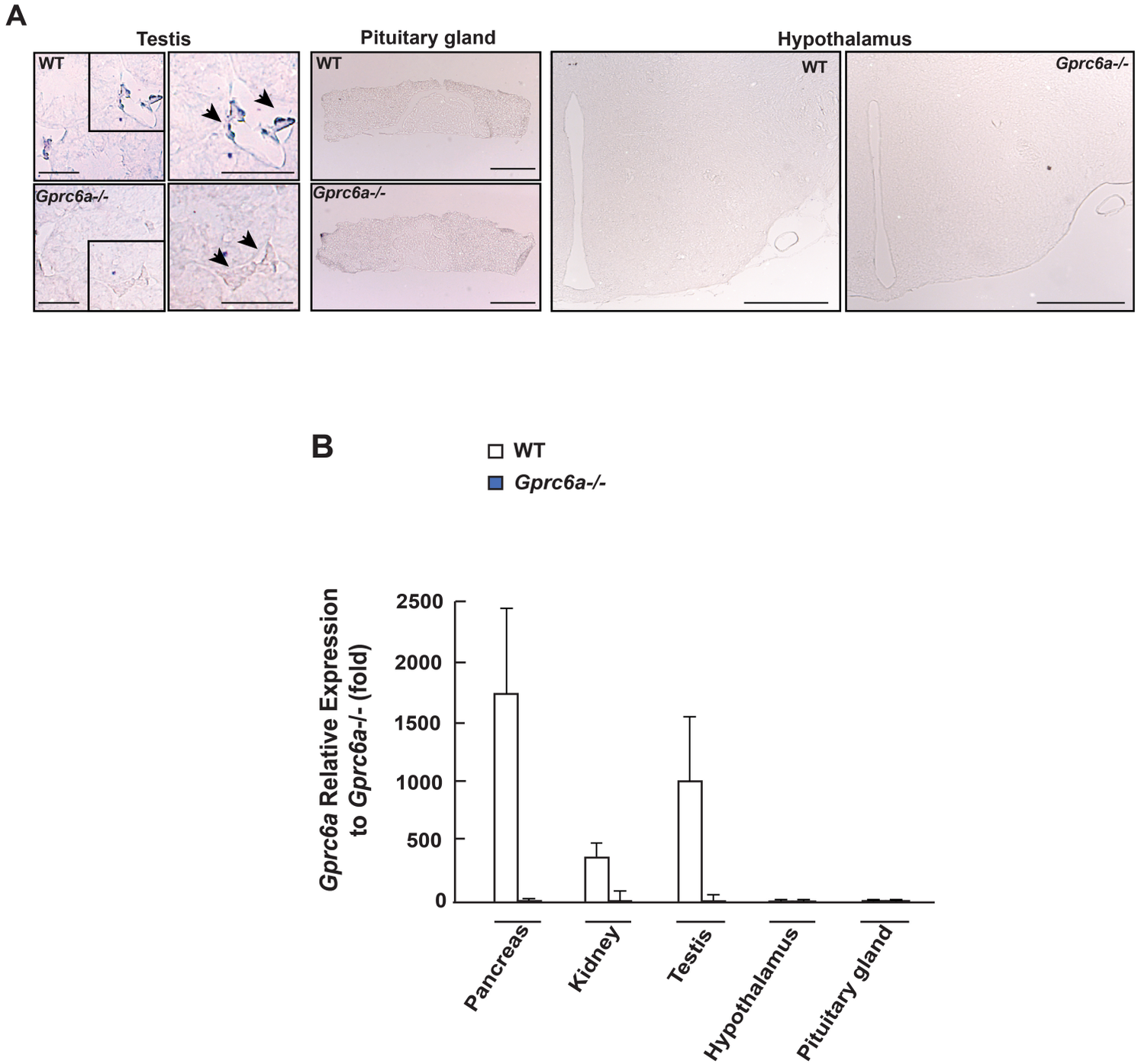

A
Testis
Pituitary gland
Hypothalamus
WT
Gprc6a-/-
WT
Gprc6a-/-
WT
Gprc6a-/-
B
WT
Gprc6a-/-
Gprc6a Relative Expression to Gprc6a-/- (fold)
2500
2000
1500
1000
500
0
Pancreas
Kidney
Testis
Hypothalamus
Pituitary gland

Figure S2

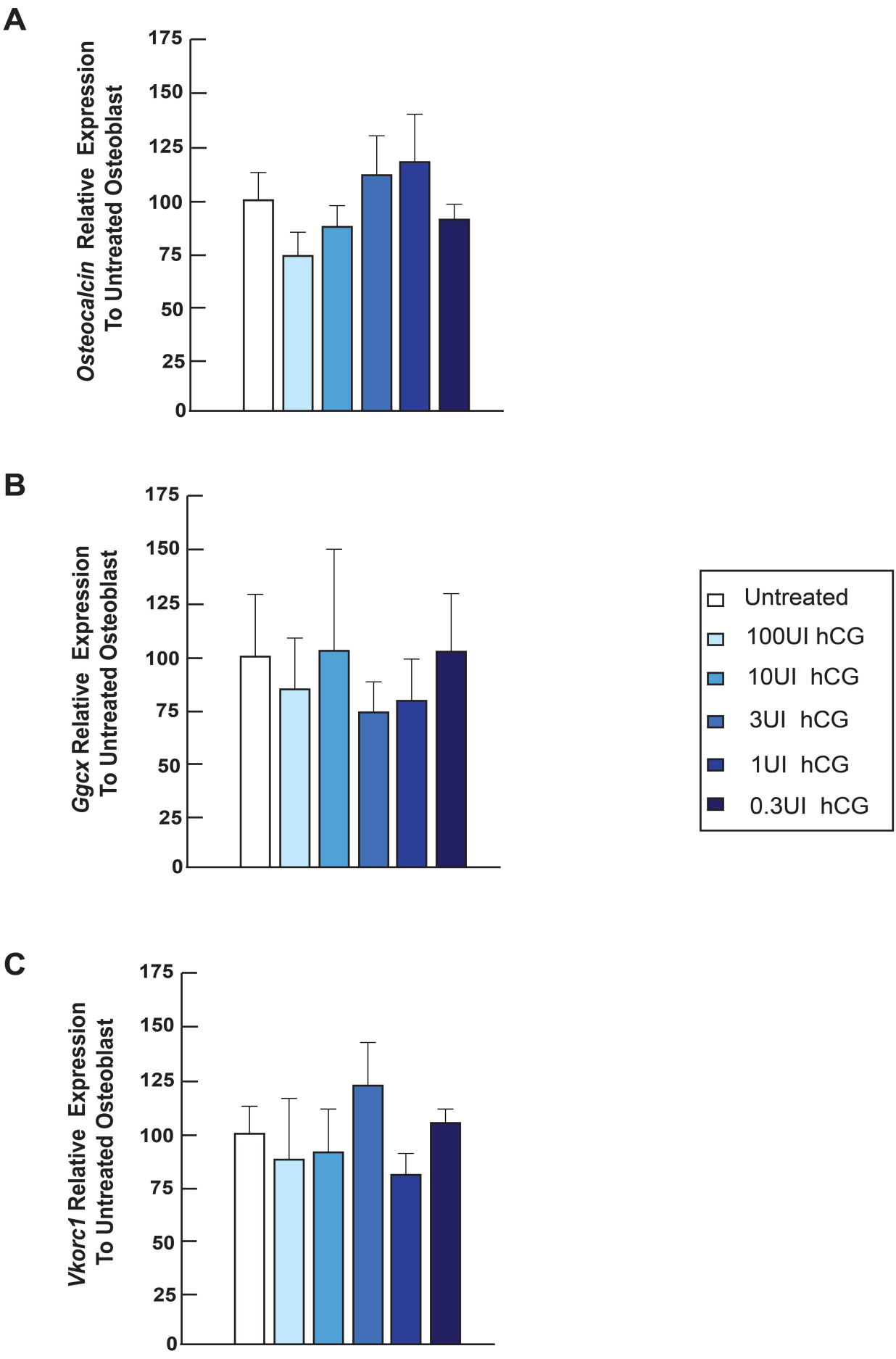

A
Osteocalcin Relative Expression
To Untreated Osteoblast
175
150
125
100
75
50
25
0
B
Ggcx Relative Expression
To Untreated Osteoblast
175
150
125
100
75
50
25
0
Untreated
100UI hCG
10UI hCG
3UI hCG
1UI hCG
0.3UI hCG
C
Vkorc1 Relative Expression
To Untreated Osteoblast
175
150
125
100
75
50
25
0

Figure S3

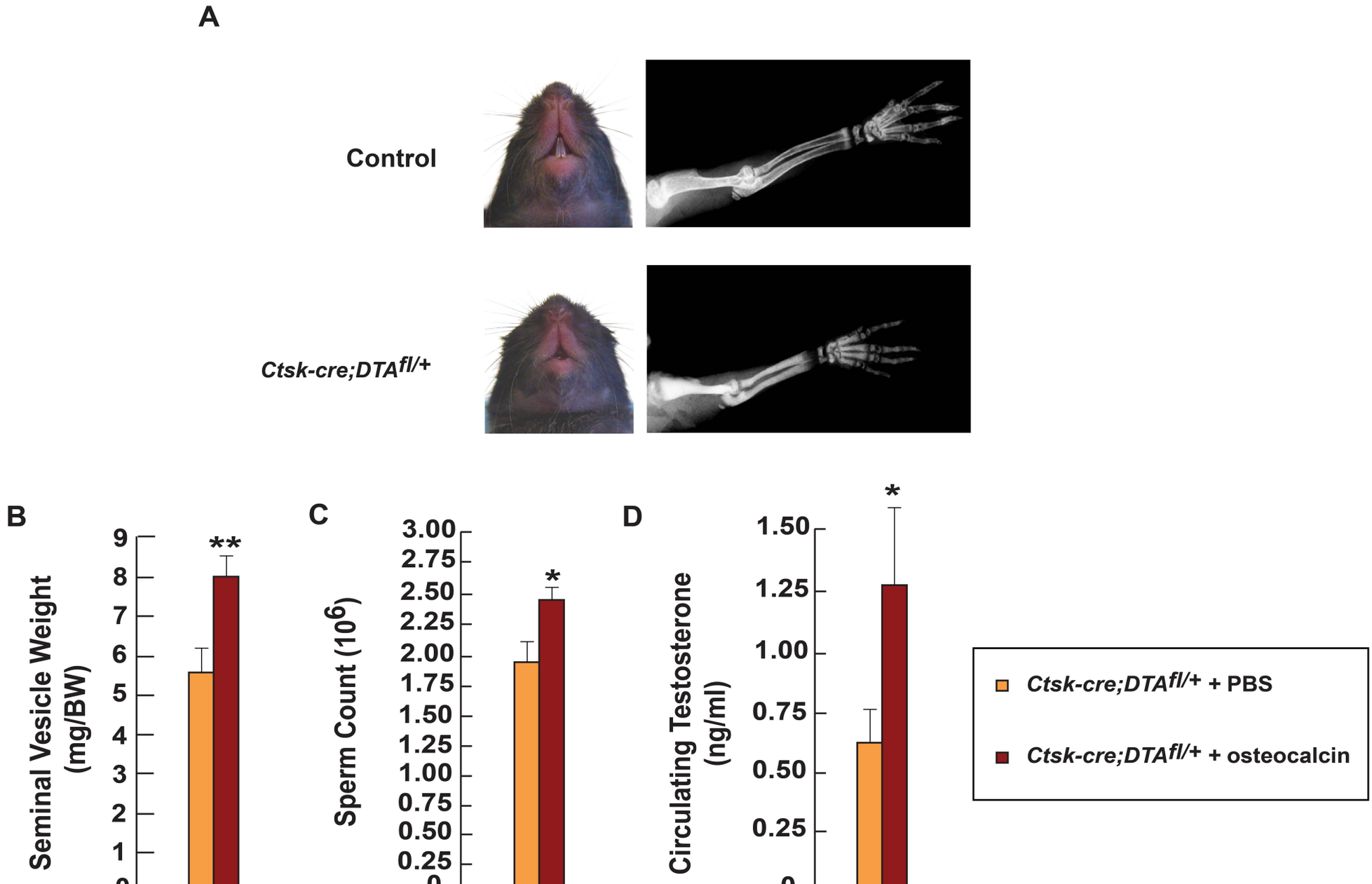

A
Control
Ctsk-cre;DTA^fl/+
B
Seminal Vesicle Weight (mg/BW)
0
1
2
3
4
5
6
7
8
9
**
C
Sperm Count (10^6)
0
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
*
D
Circulating Testosterone (ng/ml)
0
0.25
0.50
0.75
1.00
1.25
1.50
*
Ctsk-cre;DTA^fl/+ + PBS
Ctsk-cre;DTA^fl/+ + osteocalcin

Figure S4

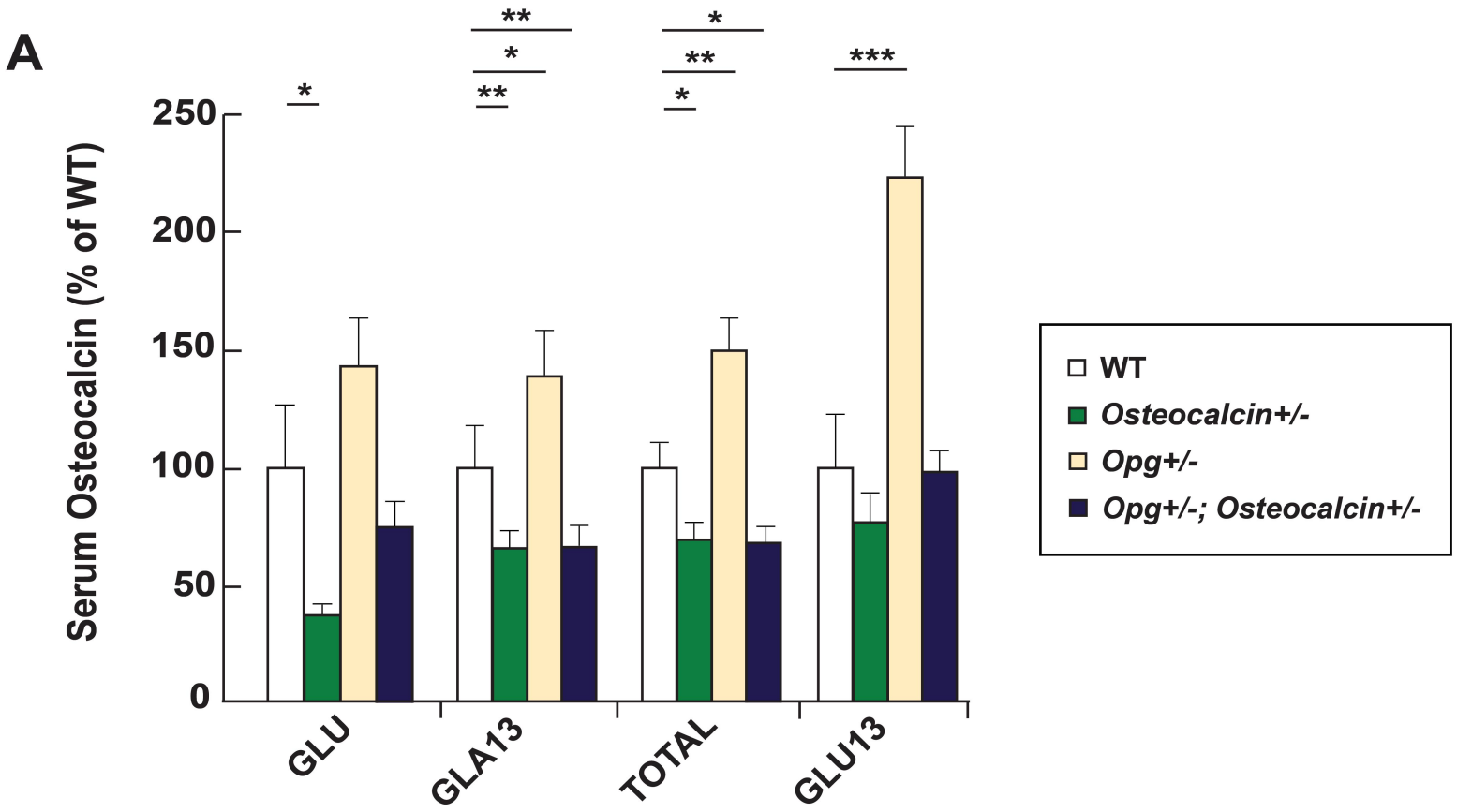
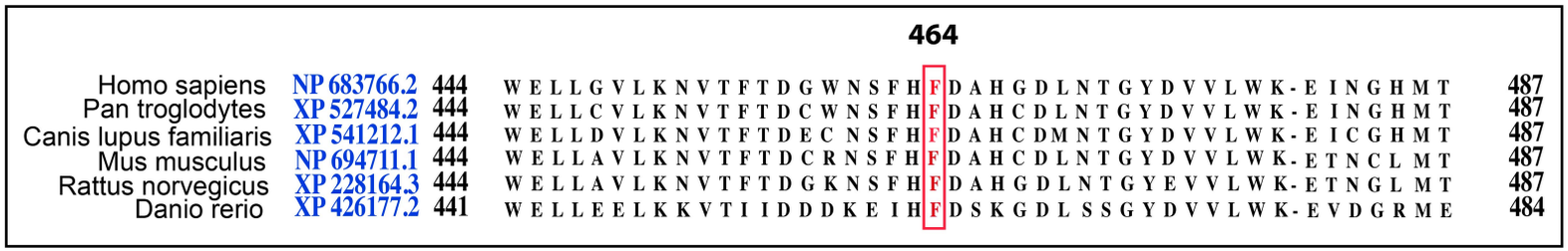
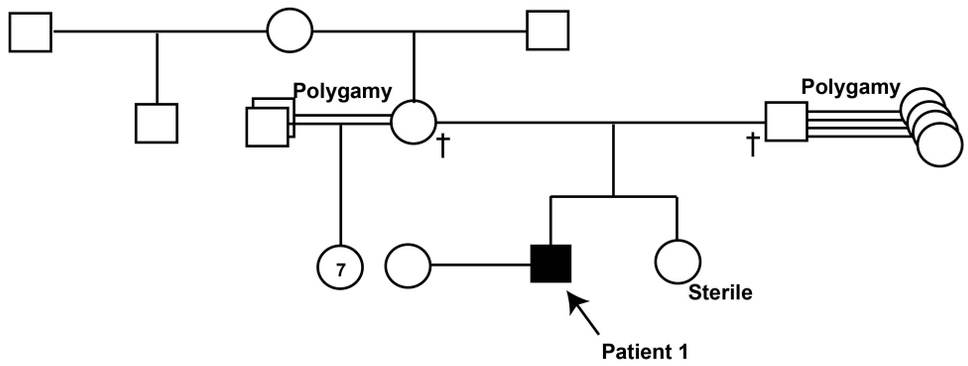

A
Serum Osteocalcin (% of WT)
250
200
150
100
50
0
*
**
*
**
*
**
*
***
GLU
GLA13
TOTAL
GLU13
WT
Osteocalcin+/-
Opg+/-
Opg+/-; Osteocalcin+/-

Figure S5

A



B



C

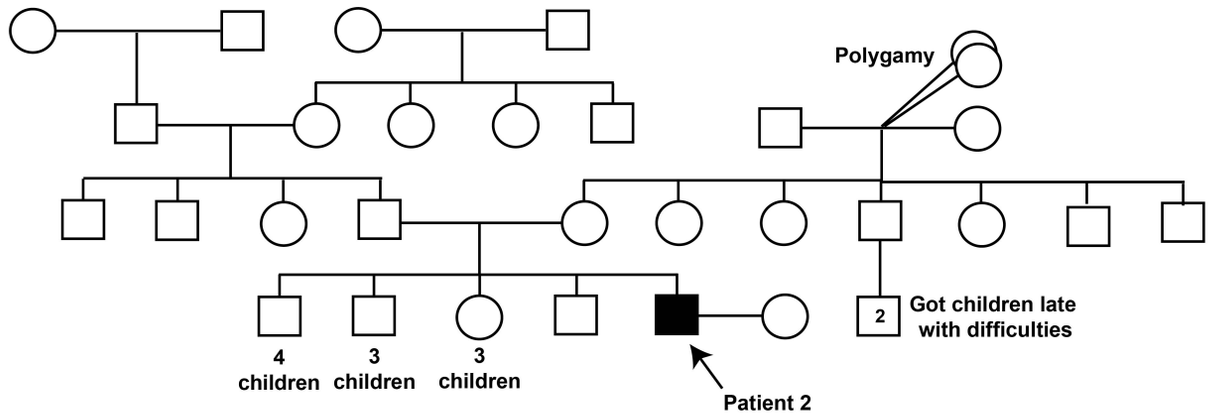


Figure S6

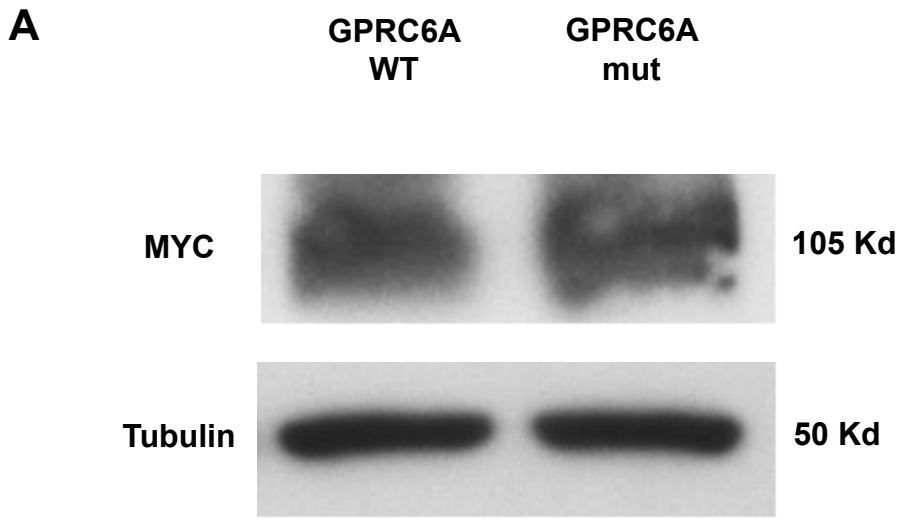


Figure S7

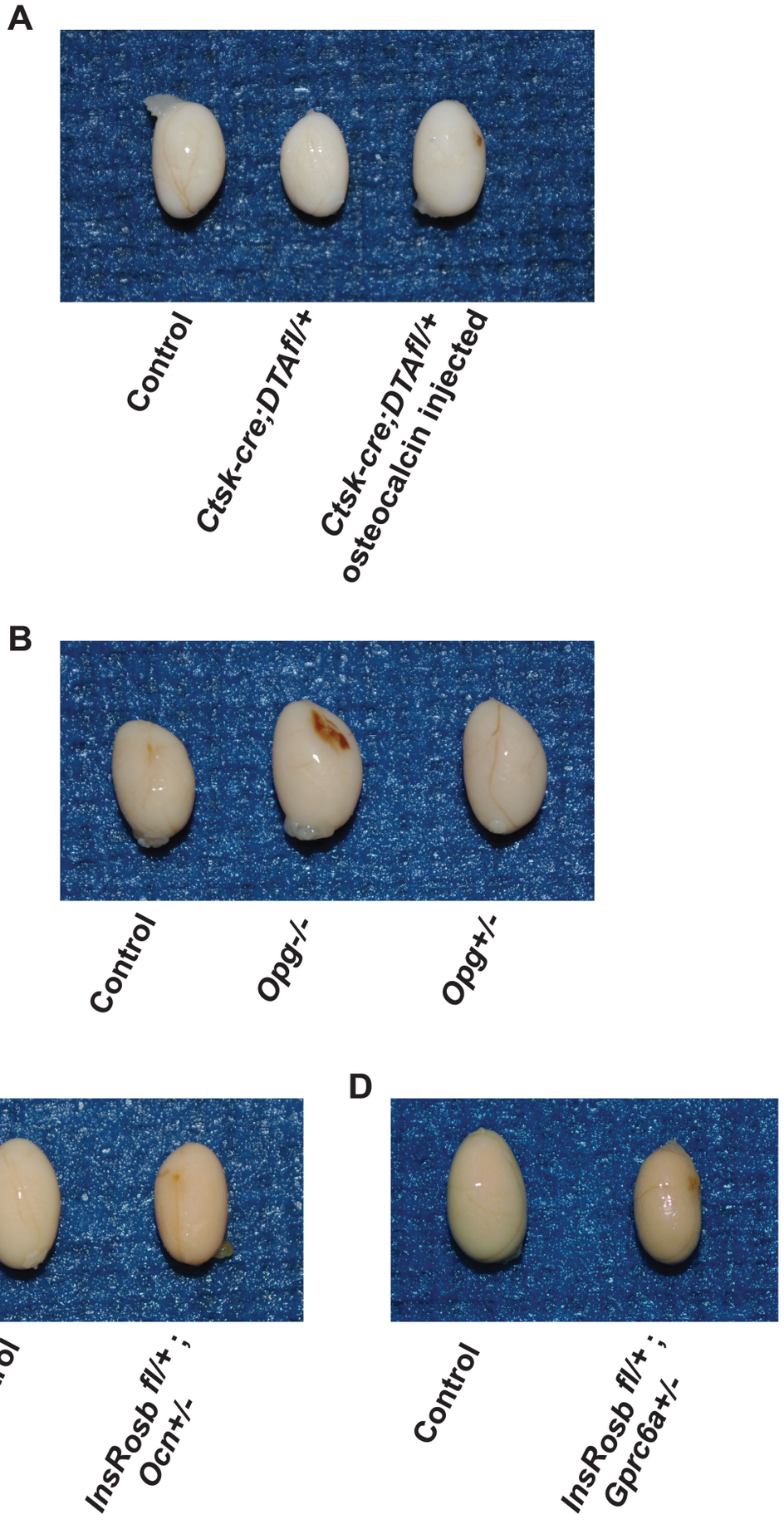

A
Control
Ctsk-cre;DTAfl/+
Ctsk-cre;DTAfl/+
osteocalcin injected
B
Control
Opg-/-
Opg+/-
C
Control
InsRosb fl/+ ;
Ocn+/-
D
Control
InsRosb fl/+ ;
Gprc6a+/-

Figure S8

	Date of birth	Sperm count (million/ml)	
Patient 1	09/01/1964	12.0	
Patient 2	01/11/1977	51.0	
Patient 3	07/05/1975	2.7	
Patient 4	03/17/1982	8.5	
Patient 5	08/18/1981	34.0	
Patient 6	02/02/1971	20.0	
Patient 7	05/26/1984	4.6	
Patient 8	02/06/1973	18.6	
Patient 9	03/17/1982	8.8	
		17.8	Mean

Supplemental Table 1