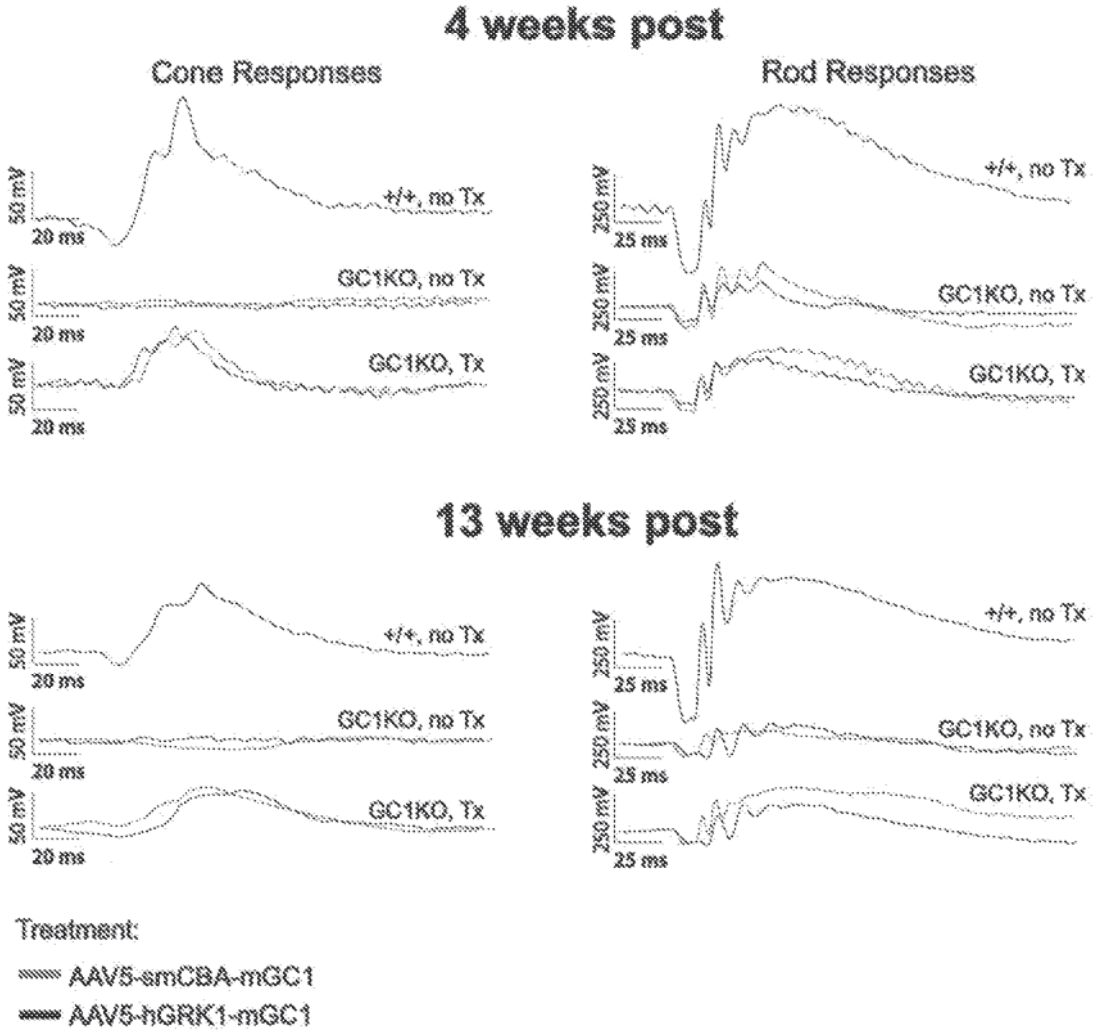

4 weeks post
Cone Responses
Rod Responses
50 mV
20 ms
250 mV
25 ms
+/+, no Tx
GC1KO, no Tx
GC1KO, Tx
13 weeks post
Treatment:
AAV5-smCBA-mGC1
AAV5-hGRK1-mGC1

FIG. 1

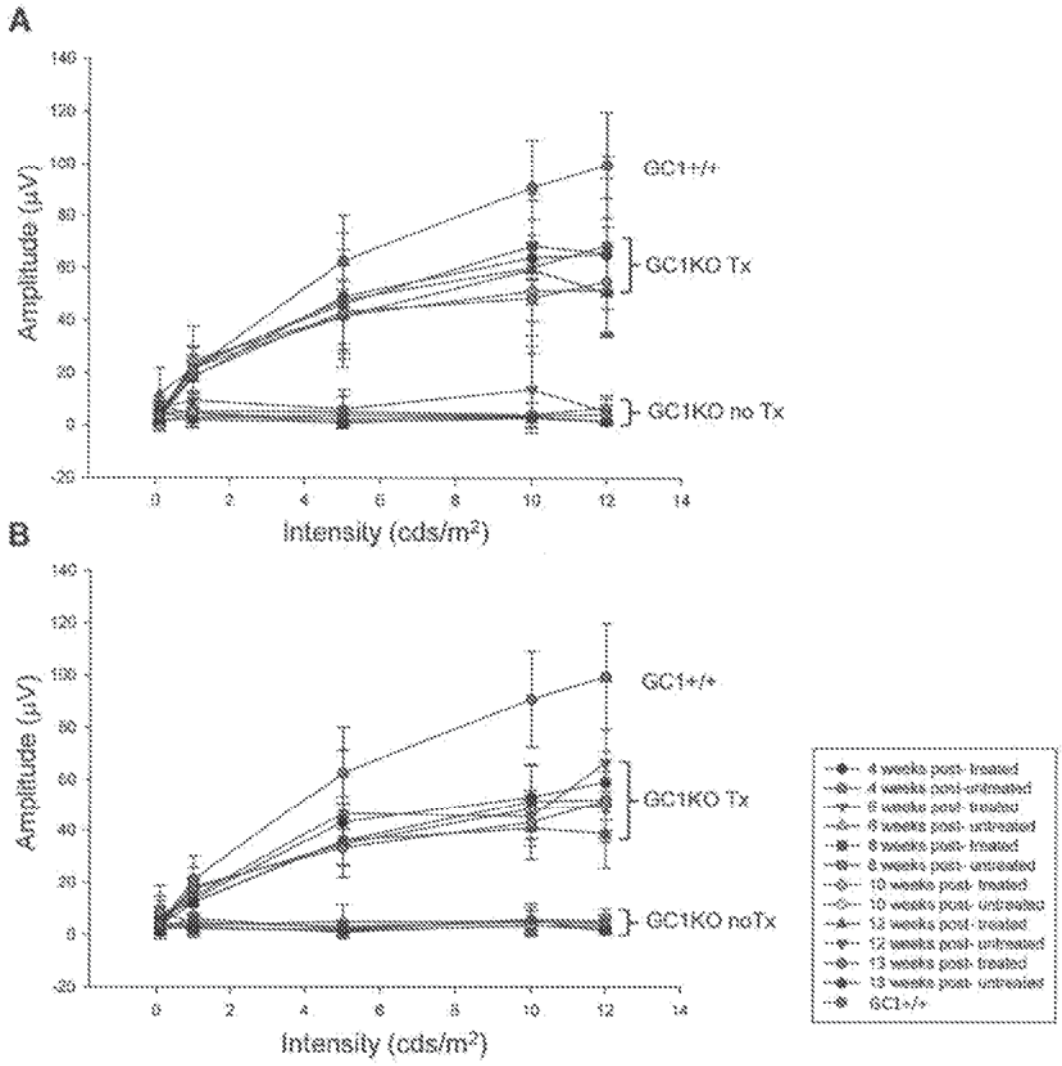

A
Amplitude (μV)
Intensity (cds/m²)
GC1+/+
GC1KO Tx
GC1KO no Tx
B
Amplitude (μV)
Intensity (cds/m²)
GC1+/+
GC1KO Tx
GC1KO noTx
4 weeks post- treated
4 weeks post- untreated
6 weeks post- treated
6 weeks post- untreated
8 weeks post- treated
8 weeks post- untreated
10 weeks post- treated
10 weeks post- untreated
12 weeks post- treated
12 weeks post- untreated
13 weeks post- treated
13 weeks post- untreated
GC1+/+

FIG. 2A and 2B

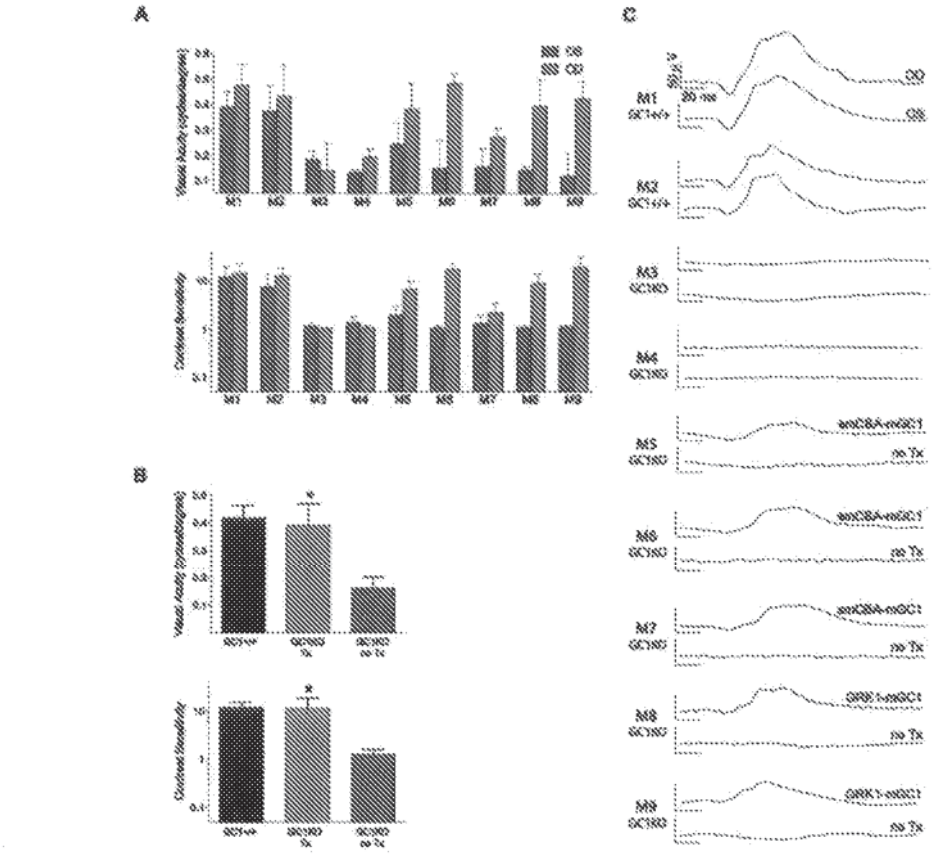


FIG. 3A, FIG. 3B, and FIG. 3C

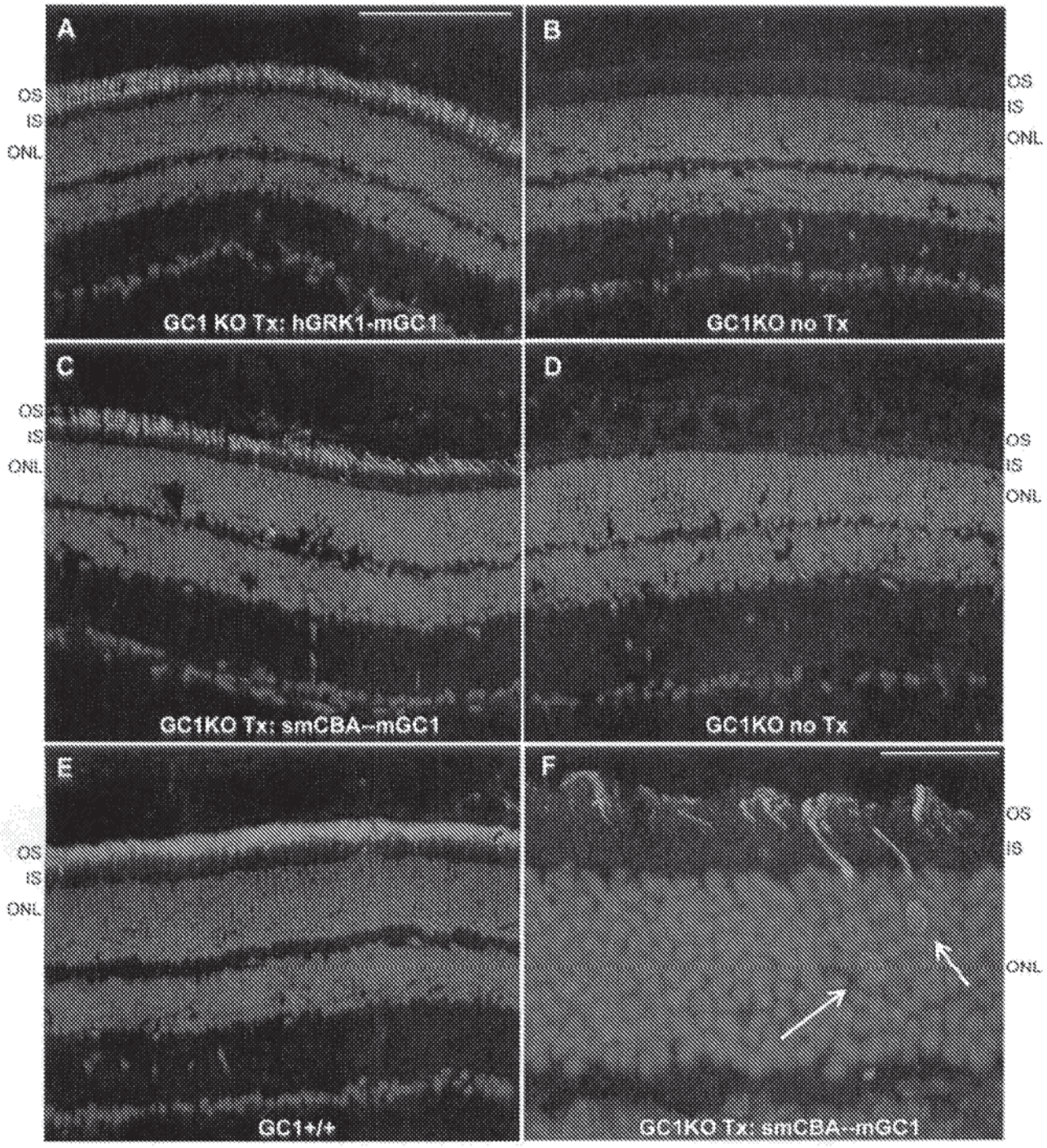


FIG. 4A, FIG. 4B, FIG. 4C, FIG. D, and FIG. F

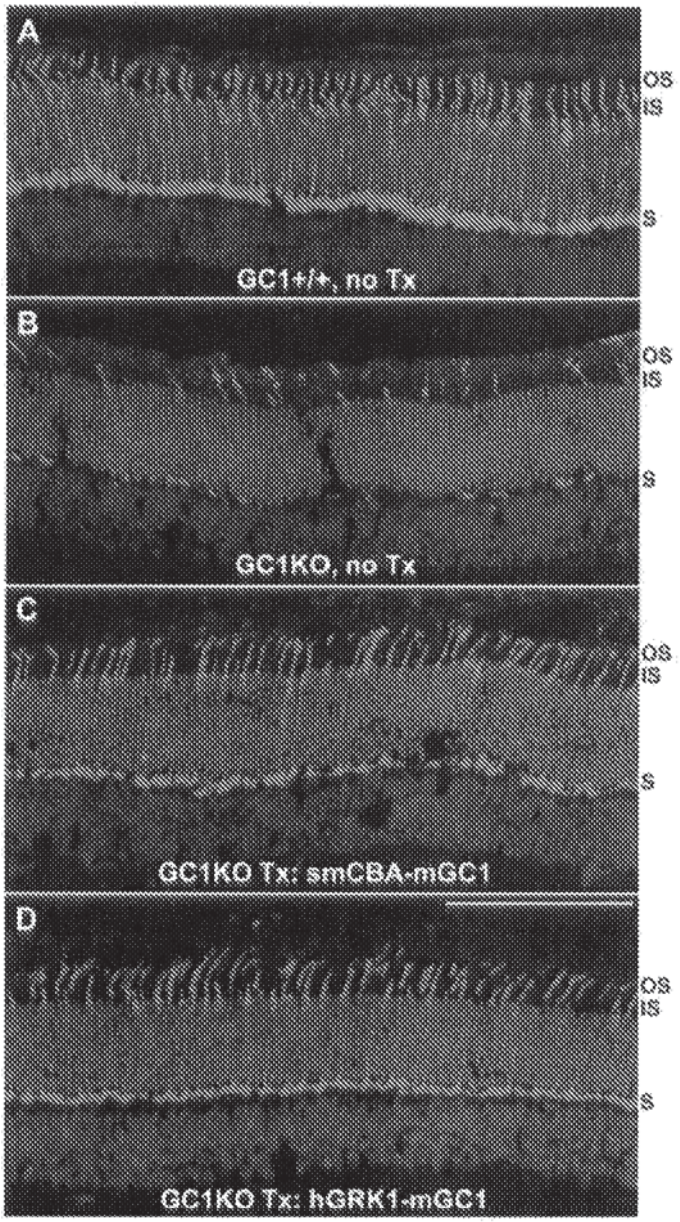


FIG. 5A, FIG. 5B, FIG. 5C and FIG. 5D

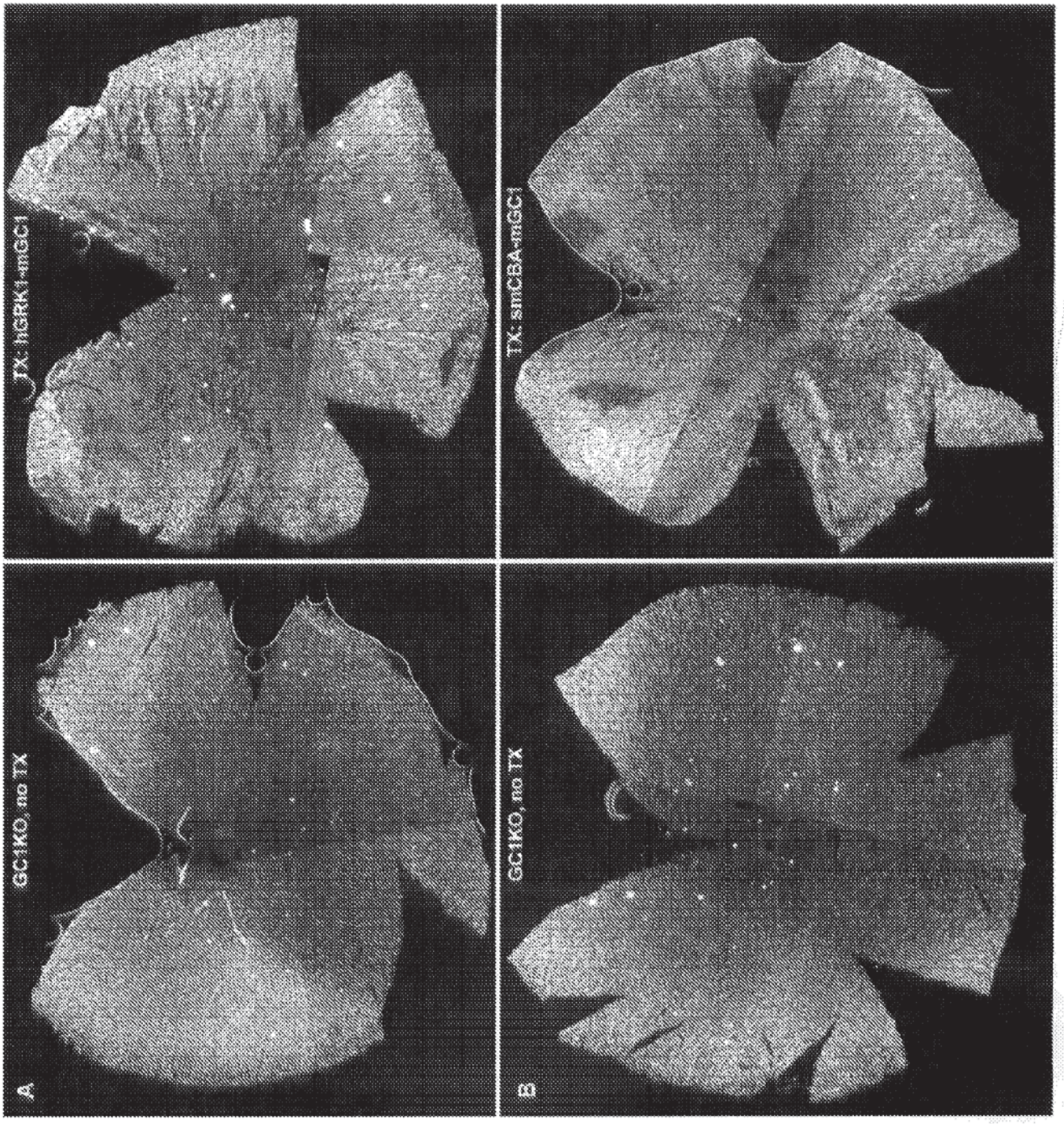


FIG. 6A, FIG. 6B, FIG. 6C and FIG. 6D

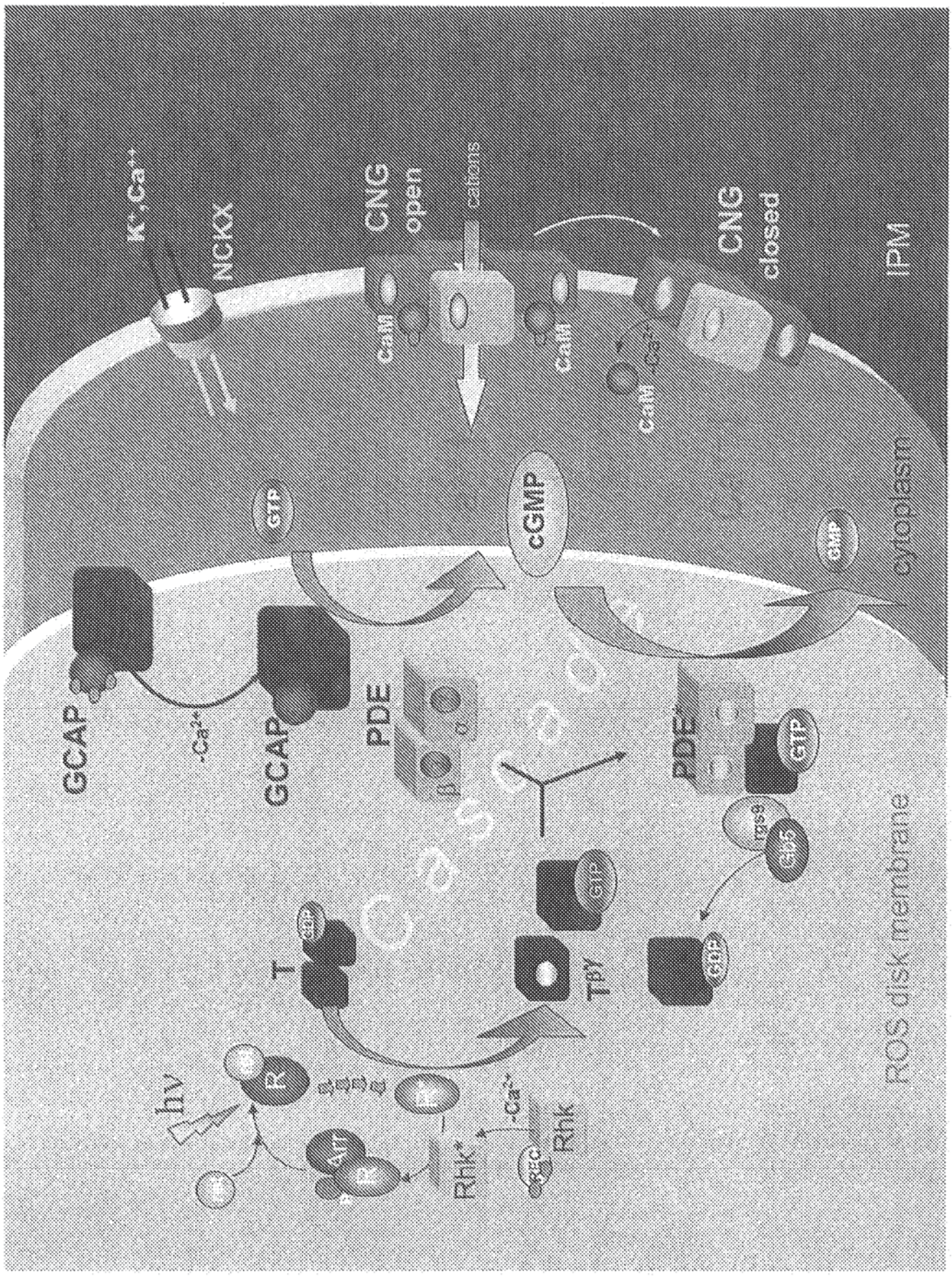

ROS disk membrane
cytoplasm
IPM
hν
R
R*
GRK
ATP
ADP
R
Rhk*
Rhk
-Ca2+
Gαc
T
GDP
GTP
Tβγ
GCAP
GCAP
-Ca2+
PDE
α
β
γ
cGMP
GTP
GMP
PDE*
RGS9
Gβ5
CNG
open
CNG
closed
CaM
Ca2+
cations
K+,Ca++
NCKX

FIG. 7

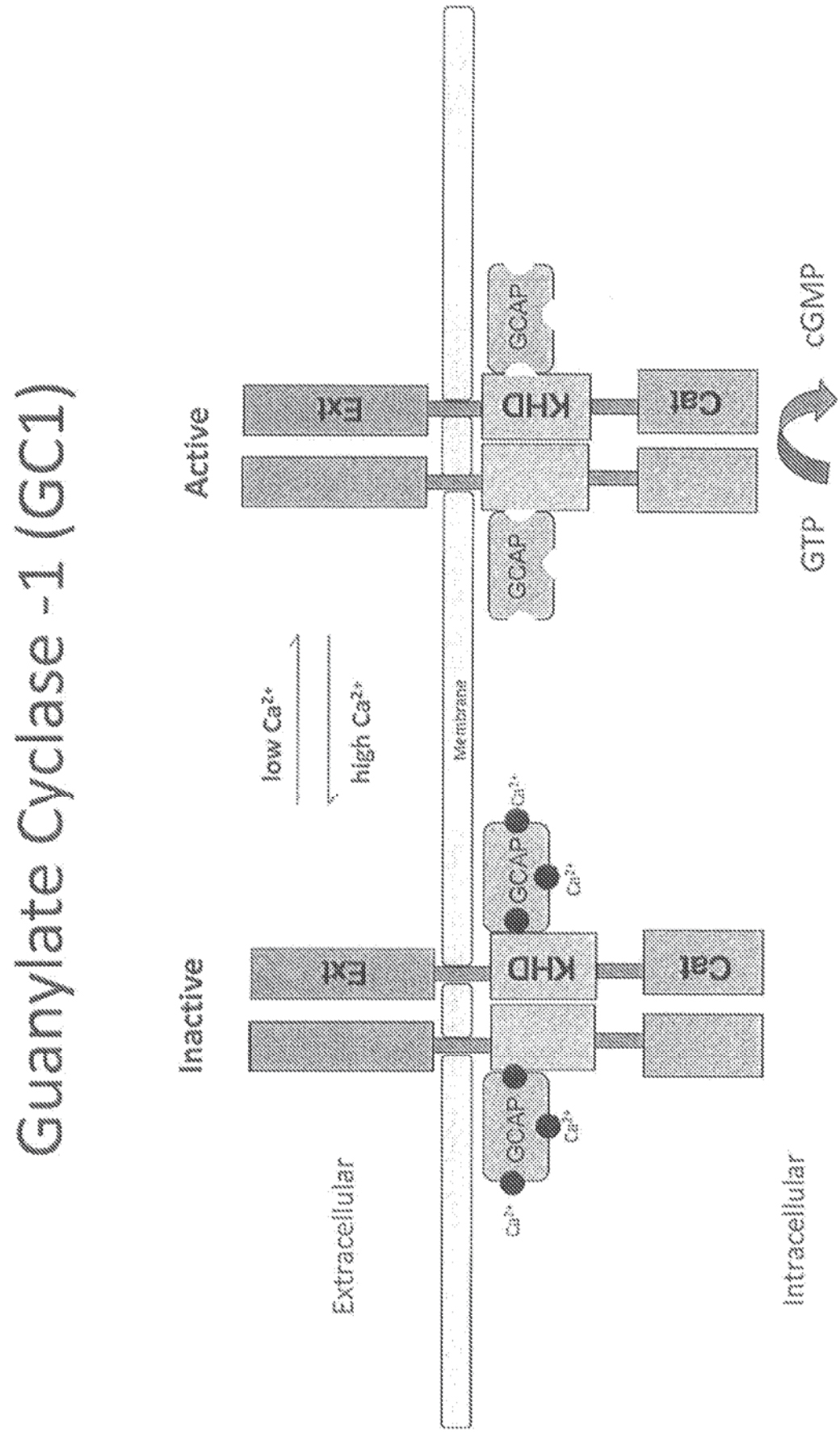

Guanylate Cyclase -1 (GC1)
Active
Inactive
Ext
KHD
Cat
GCAP
low Ca2+
high Ca2+
Membrana
Extracellular
Intracellular
GTP
cGMP
Ca2+

FIG. 8

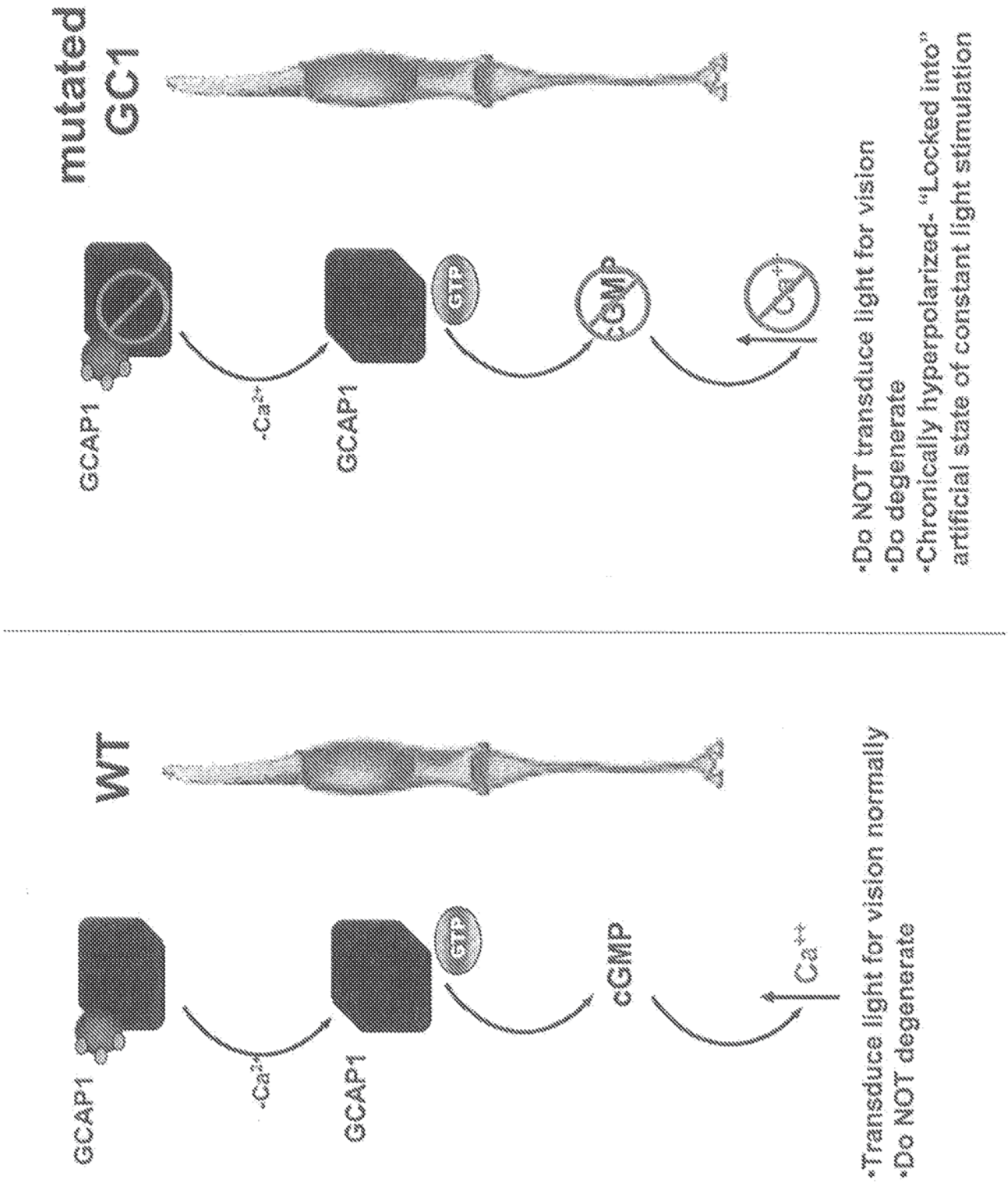

WT
GCAP1
Ca^{2+}
GCAP1
GTP
cGMP
Ca^{++}
•Transduce light for vision normally
•Do NOT degenerate
mutated GC1
GCAP1
Ca^{2+}
GCAP1
GTP
cGMP
Ca^{++}
•Do NOT transduce light for vision
•Do degenerate
•Chronically hyperpolarized- "Locked into" artificial state of constant light stimulation

FIG. 9
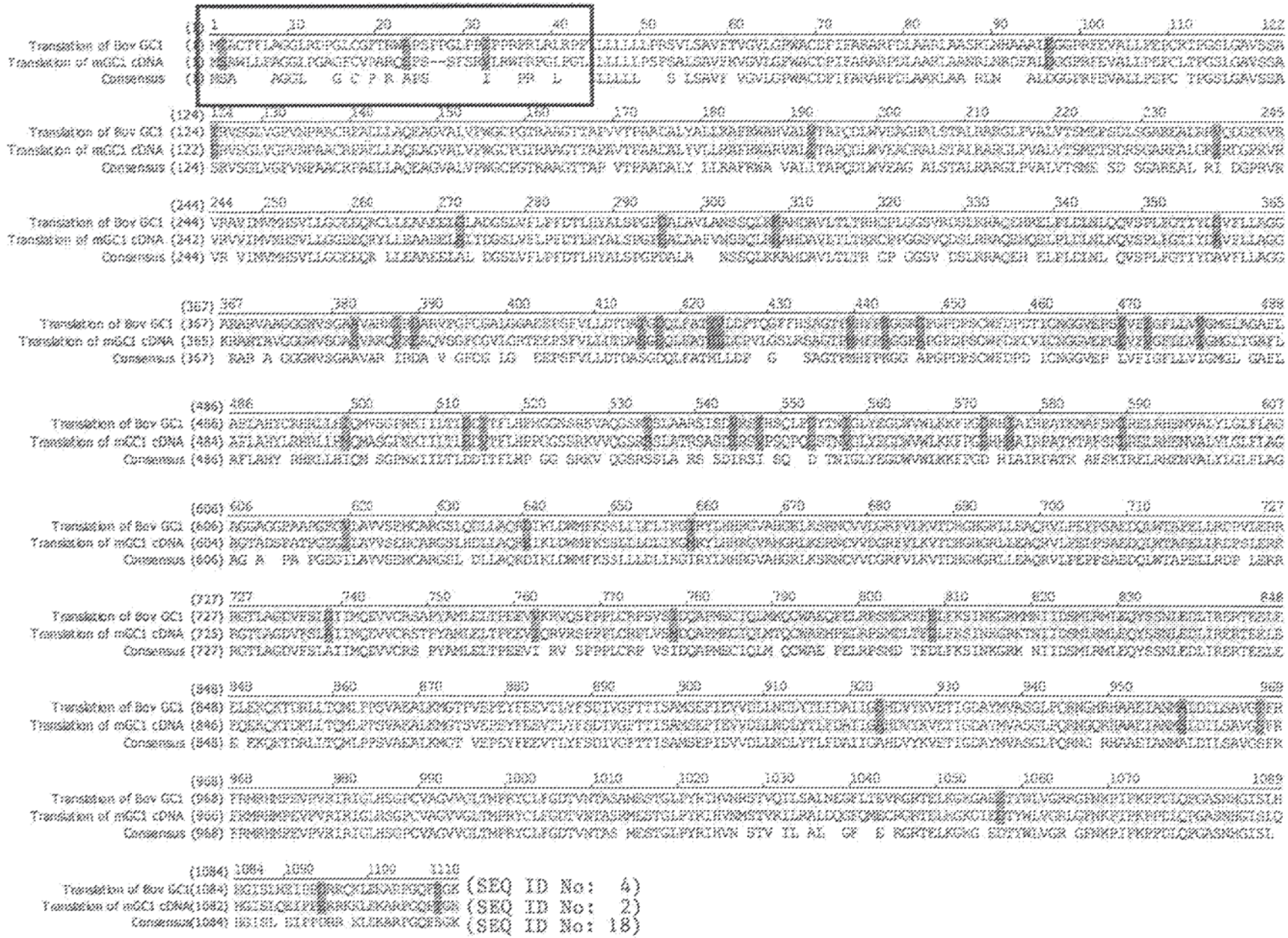

Translation of Bov GC1
Translation of mGC1 cDNA
Consensus
(SEQ ID No: 4)
(SEQ ID No: 2)
(SEQ ID No: 18)

FIG. 10

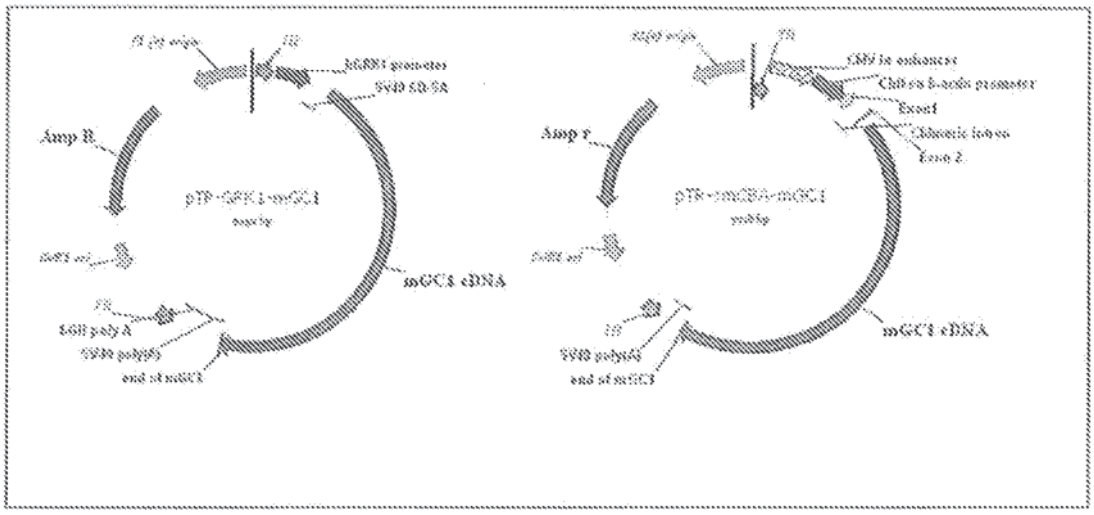


FIG. 11

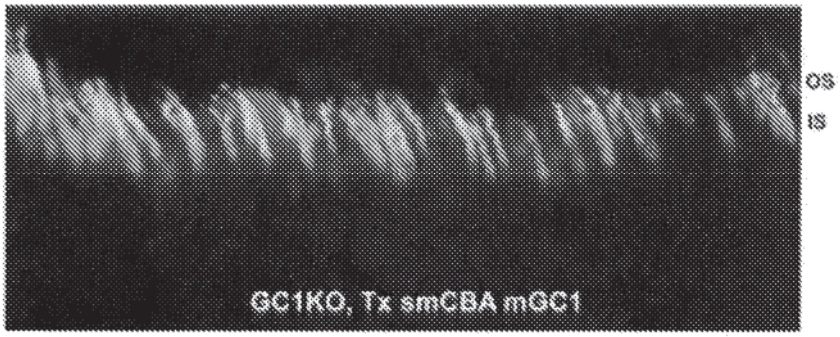


FIG. 12

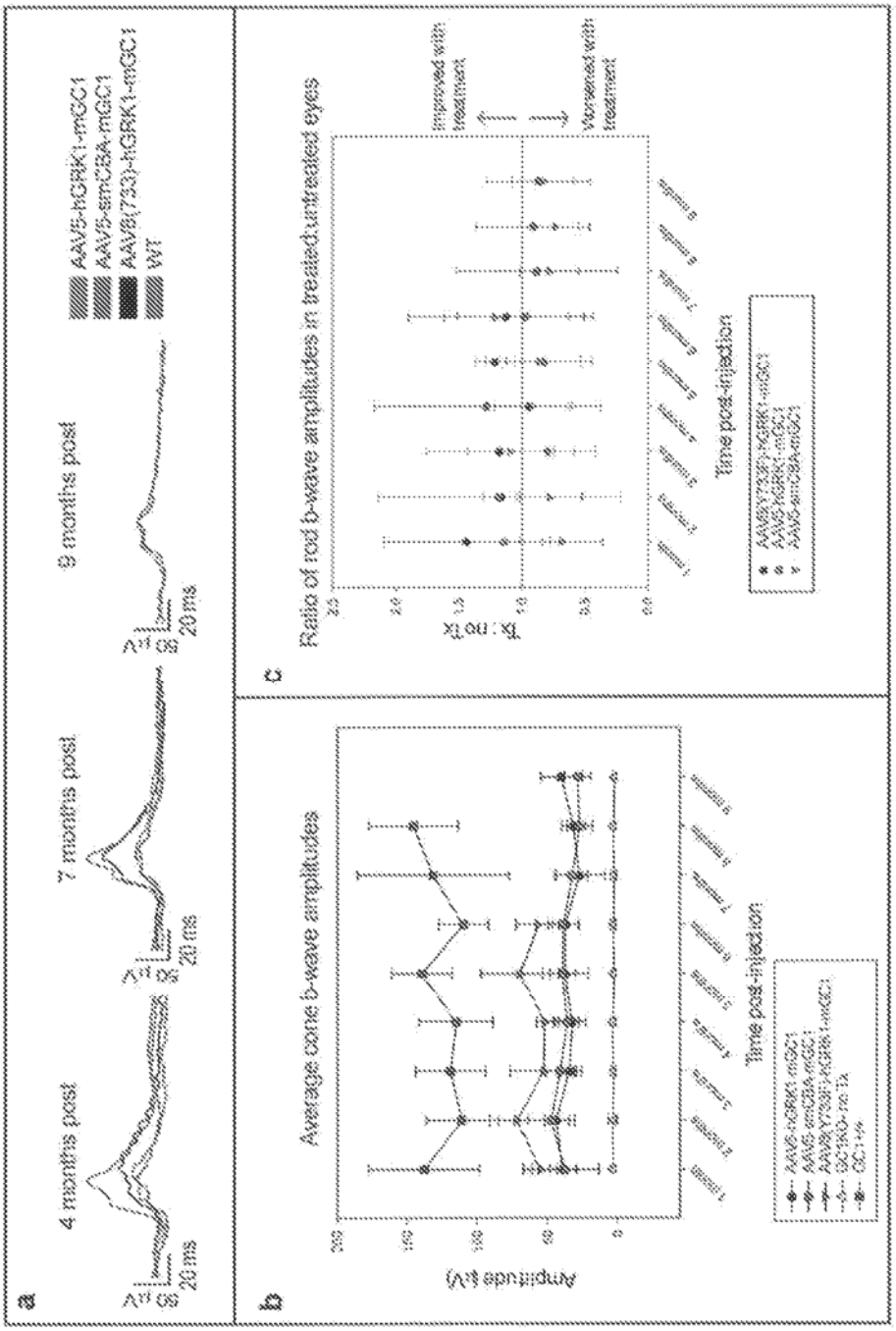


FIG. 13A, FIG. 13B, and FIG. 13C

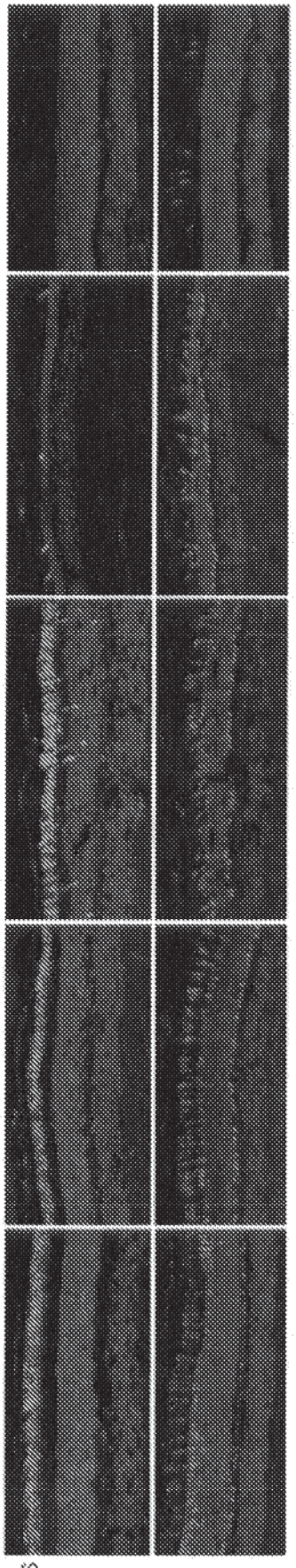

GC1+/+
AAV8(733)-hGRK1-mGC1
AAV8-smCBA-mGC1
AAV8-hGRK1-mGC1
GC1KO- no Tx
OS
INL
OS
INL

FIG. 14

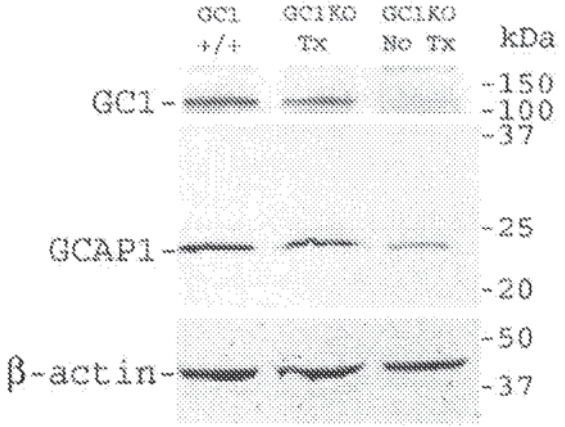

GC1 +/+
GC1KO Tx
GC1KO No Tx
kDa
GC1
GCAP1
β-actin
150
100
37
25
20
50
37

FIG. 15

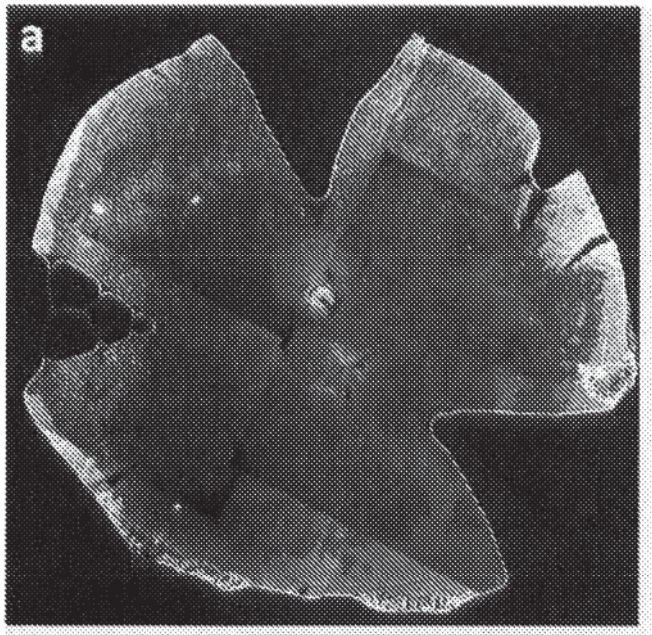


FIG. 16A

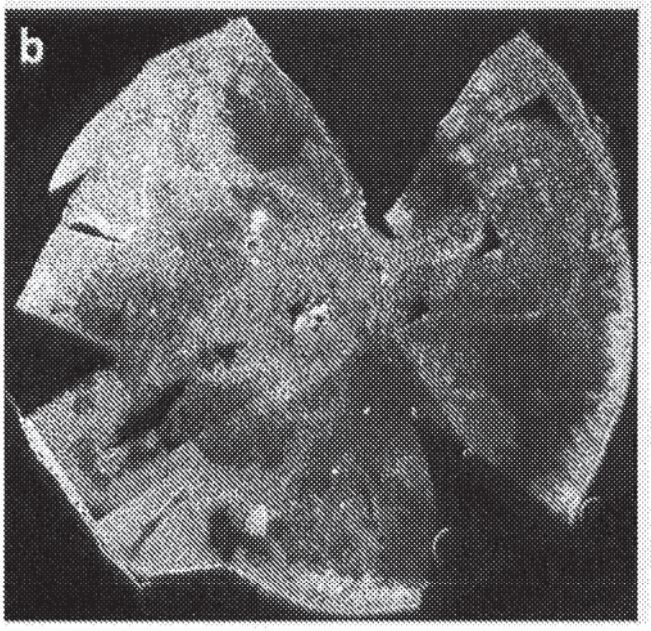


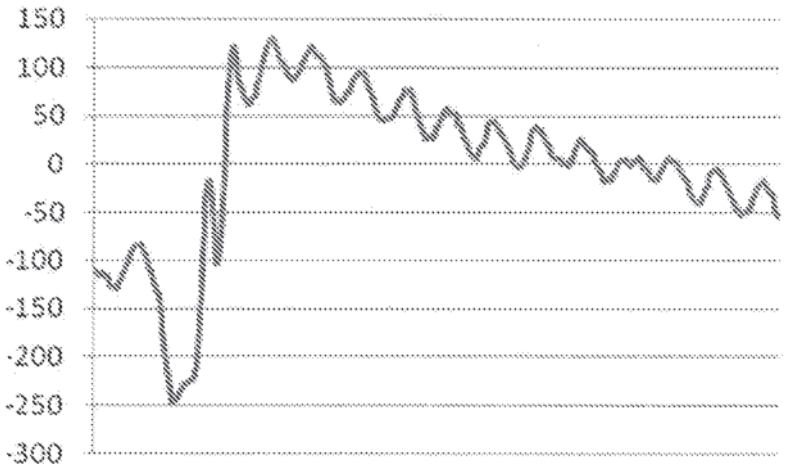
FIG. 16B

Detection of rAAV vector sequences by PCR in GC1KO tissue samples

	M1a	M2b	M3b	M4c	M5c	M6c	M7c	M8c	M9b	M10c	M11b	M12c
	AAV8(733)-hGRK1	AAV8(733)-hGRK1	AAV5-smCBA	AAV5-smCBA	AAV5-smCBA	AAV5-smCBA	AAV5-smCBA	AAV5-smCBA	AAV5-hGRK1	AAV5-hGRK1	no Tx	no Tx
Optic Nerve R	na	na	+160	+1000	+970	+1890	na	+395	+3140	+1695	-	-
Optic Nerve L	+632	+25,885	-	na	na	na	na	na	-	na	na	na
Brain R	-	-	-	-	-	-	-	-	-	-	-	-
Brain L	-	-	-	-	-	-	-	-	+252	-	-	-

M, Animal; R, right; L, left; Tx, treatment; -, no PCR amplification; +, PCR amplification of vector sequence (replicated value shown is average, copy number per mg of DNA; na, not available (not determinable because of an unacceptable spike-in)
a 4 months post injection
b 7 months post injection
c 10 months post injection

FIG. 17



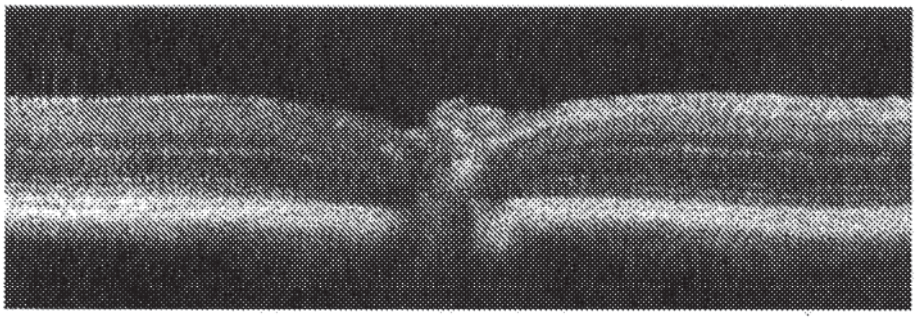
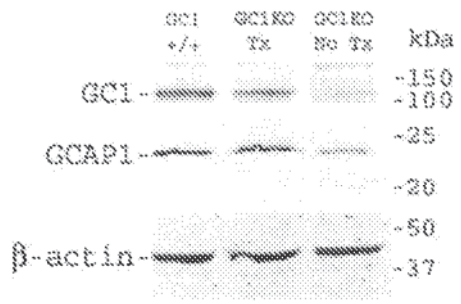


FIG. 18A

A



B

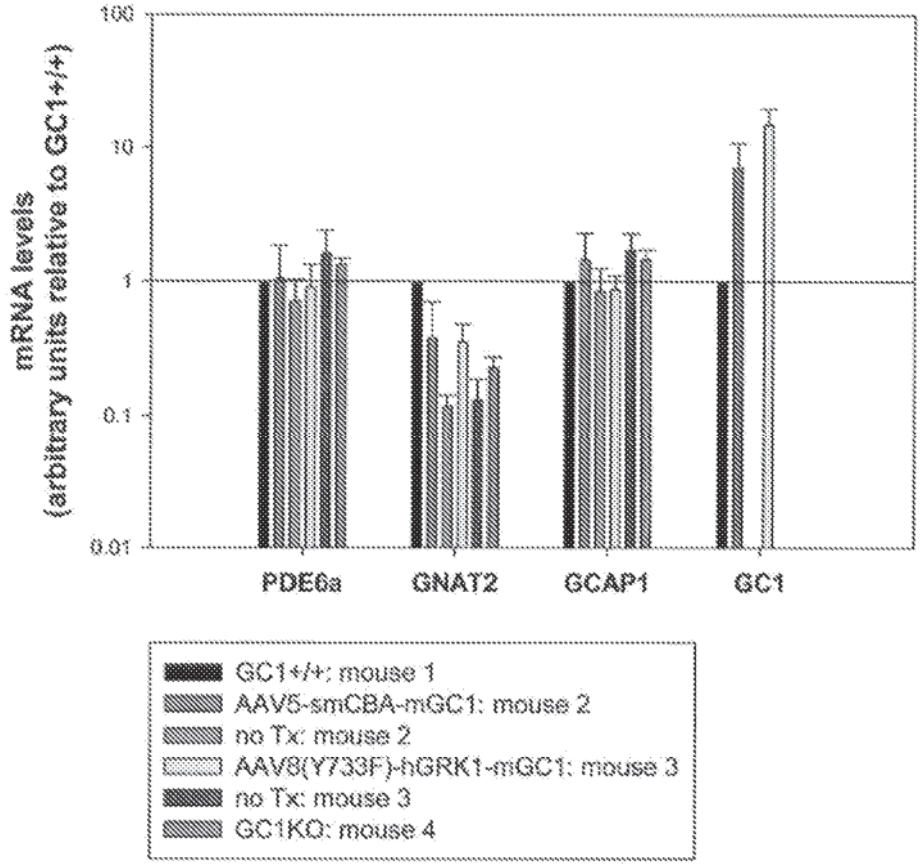
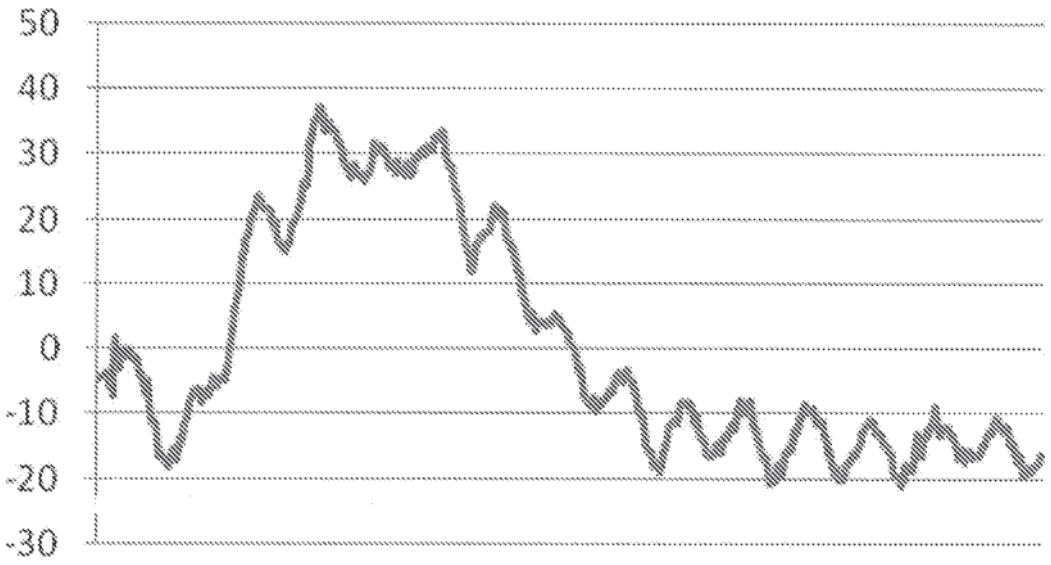


FIG. 20A and FIG. 20B



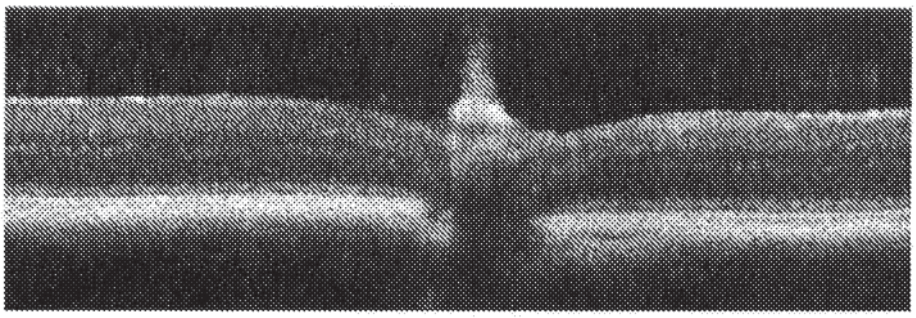


FIG. 18B

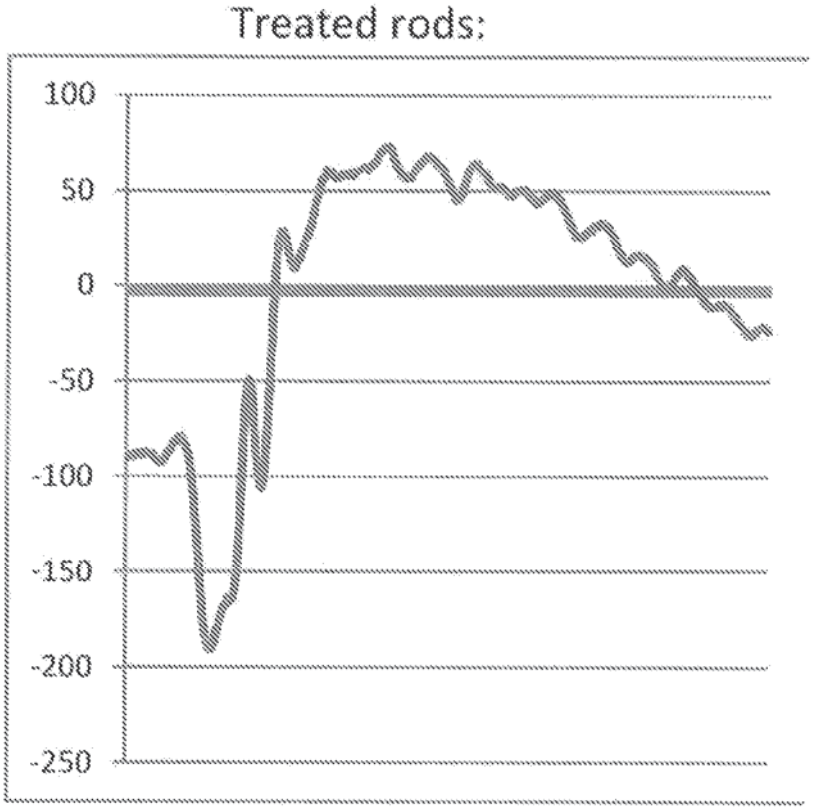


FIG. 19A

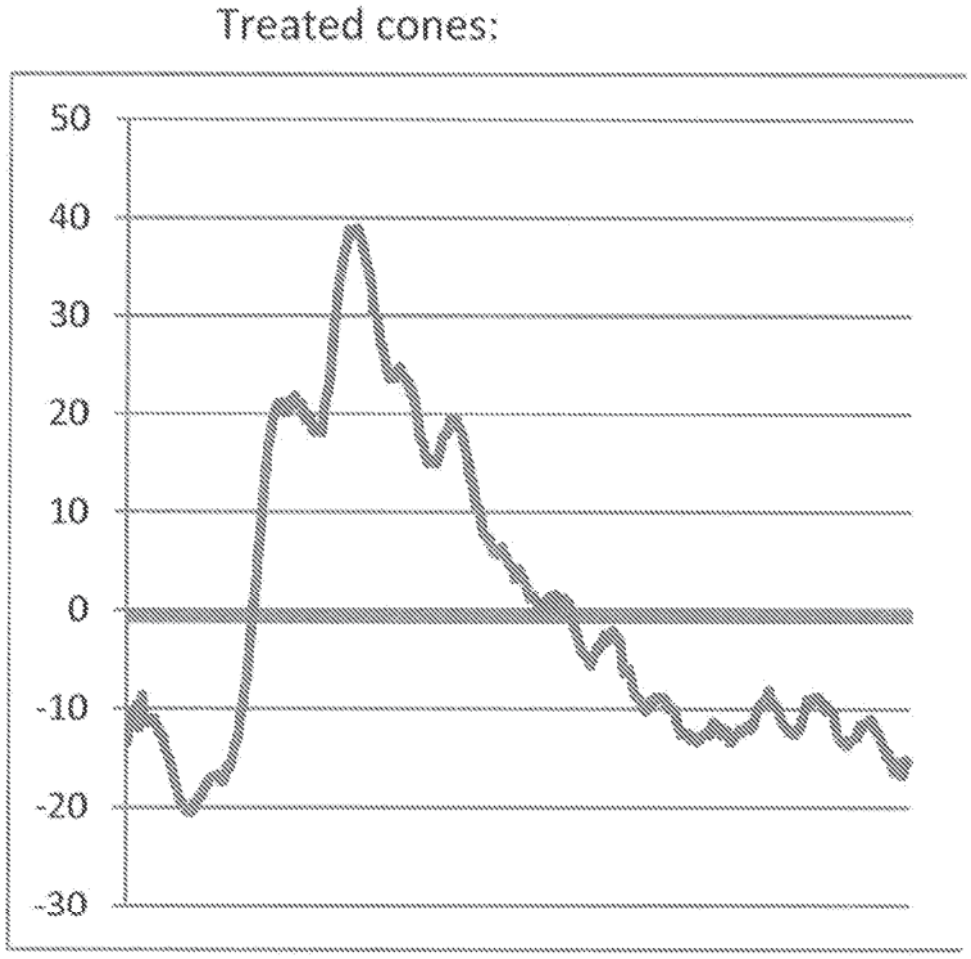


FIG. 19B

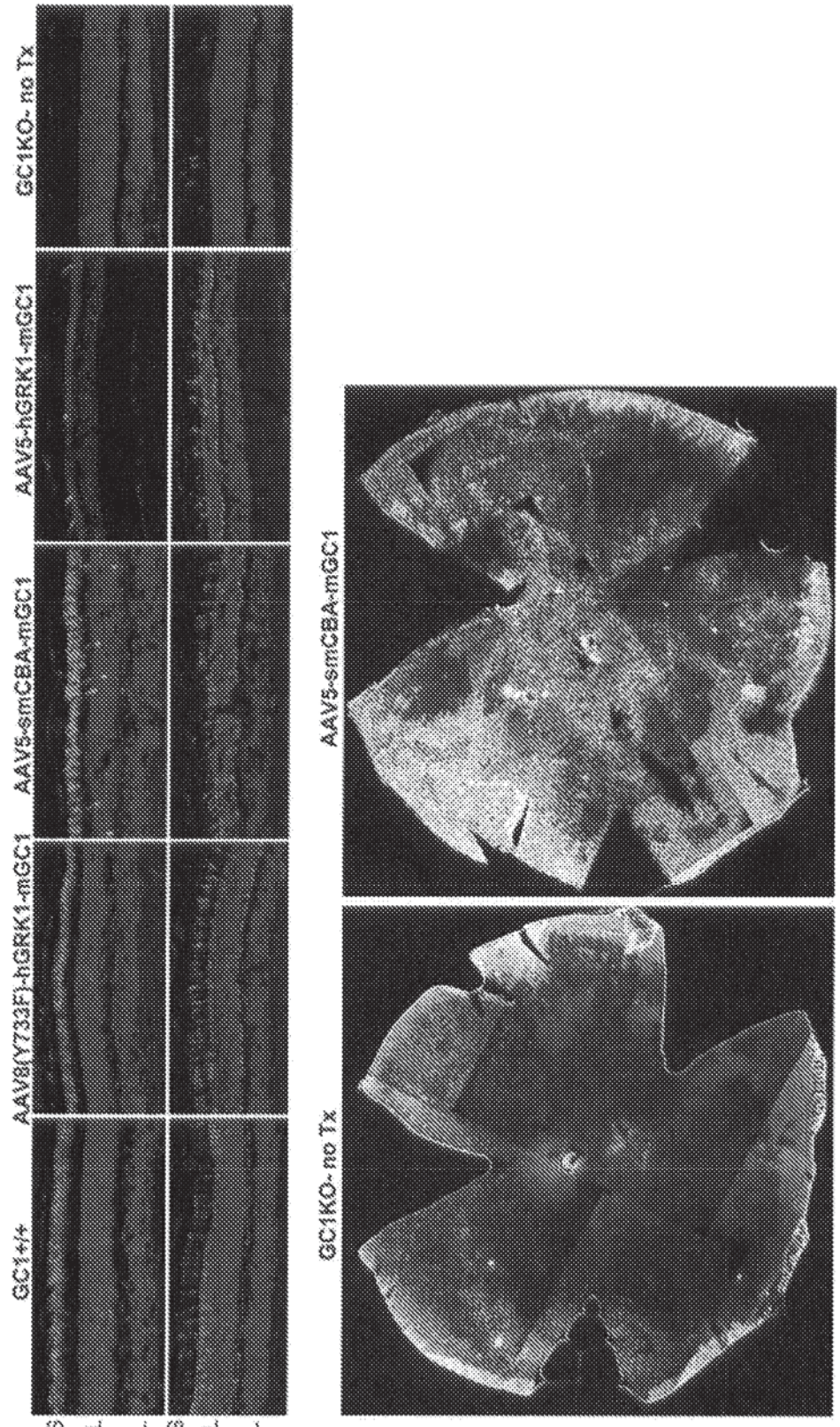


FIG. 21A and FIG. 21B

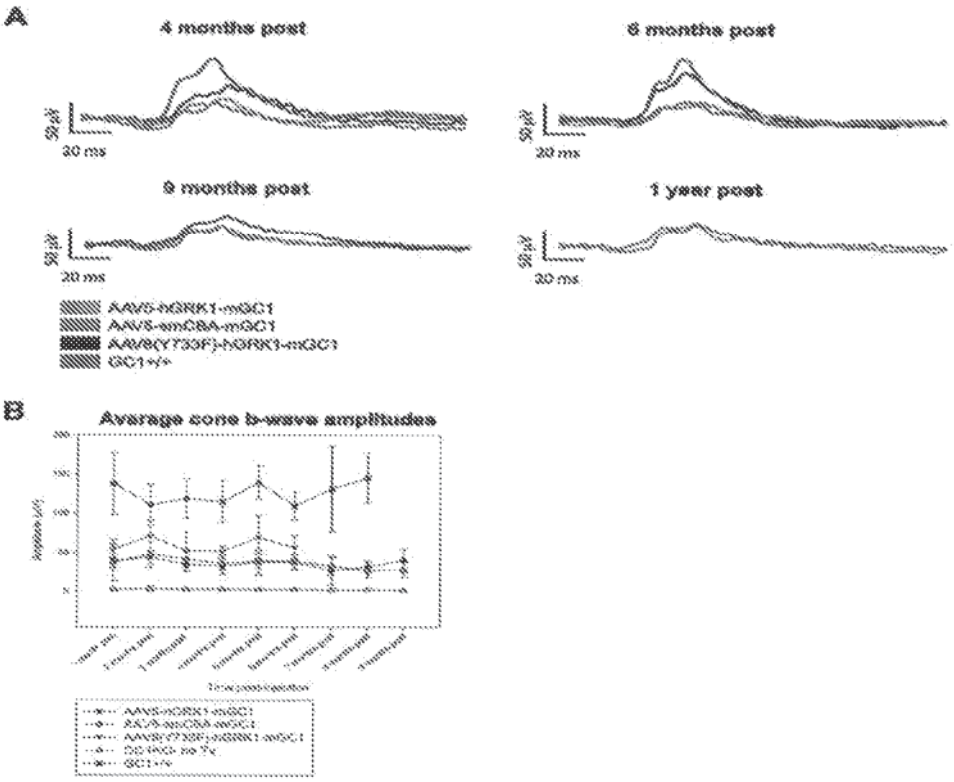


FIG. 22A and FIG. 22B

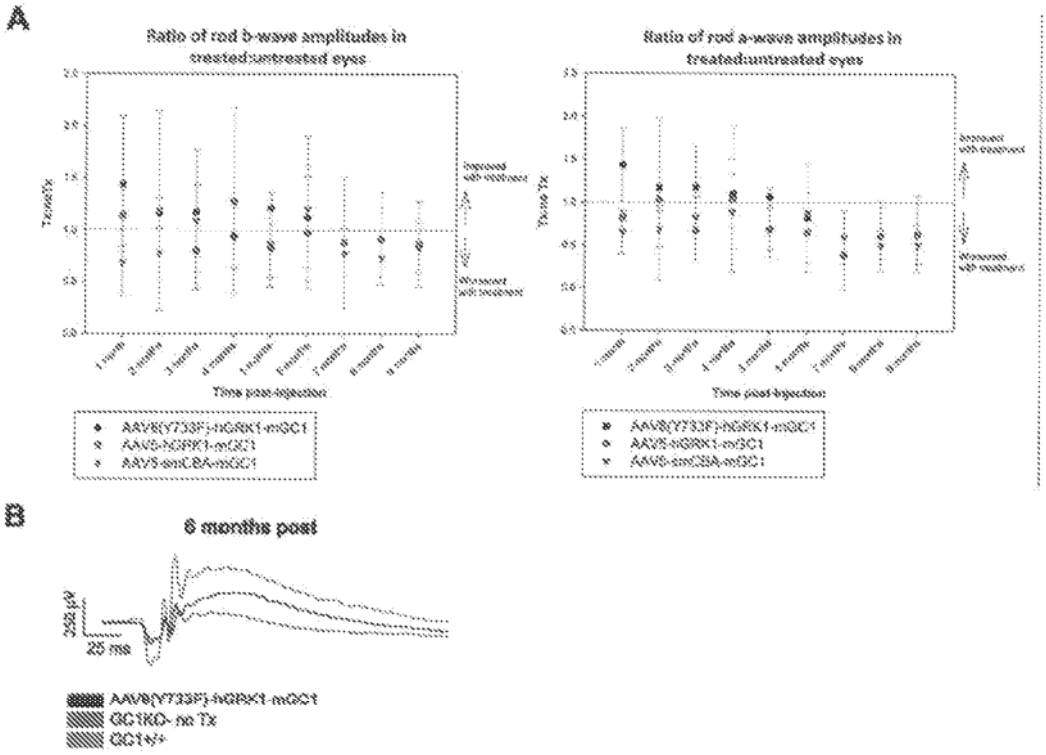


FIG. 23A and FIG. 23B

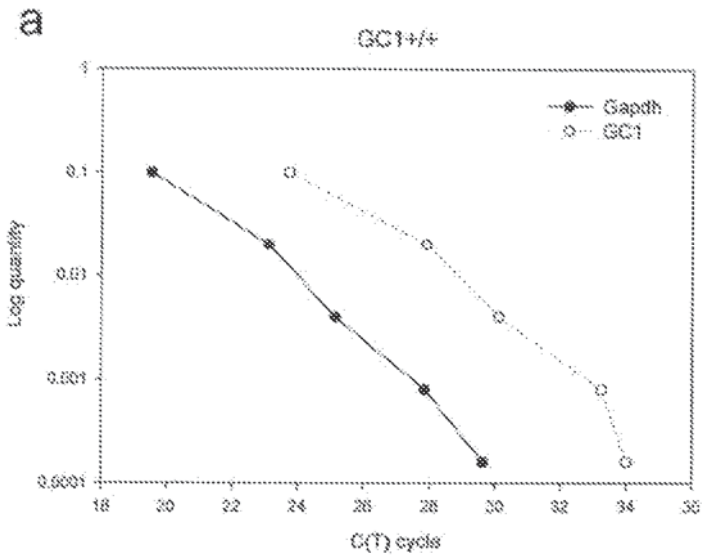


FIG. 24A

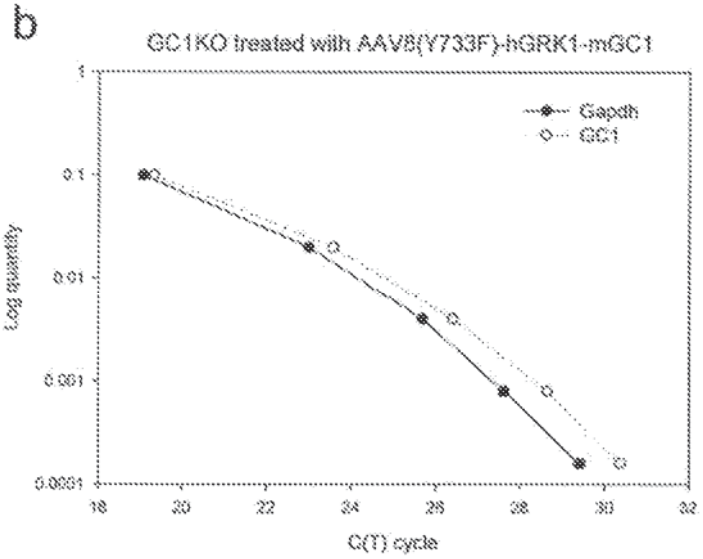


FIG. 24B