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(54) **GFP FUSION PROTEINS AND THEIR USE**

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(60) Provisional application No. 60/303,622, filed on Jul. 6, 2001.

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(52) **U.S. Cl.** **435/6.17**; 530/350; 435/69.7

(57) **ABSTRACT**

The present invention provides fusion proteins including a green fluorescent protein inserted into the internal amino acid sequence of a Gαs protein and further provides method of using the fusion protein construct to follow activation of a G-protein receptor by a candidate drug.

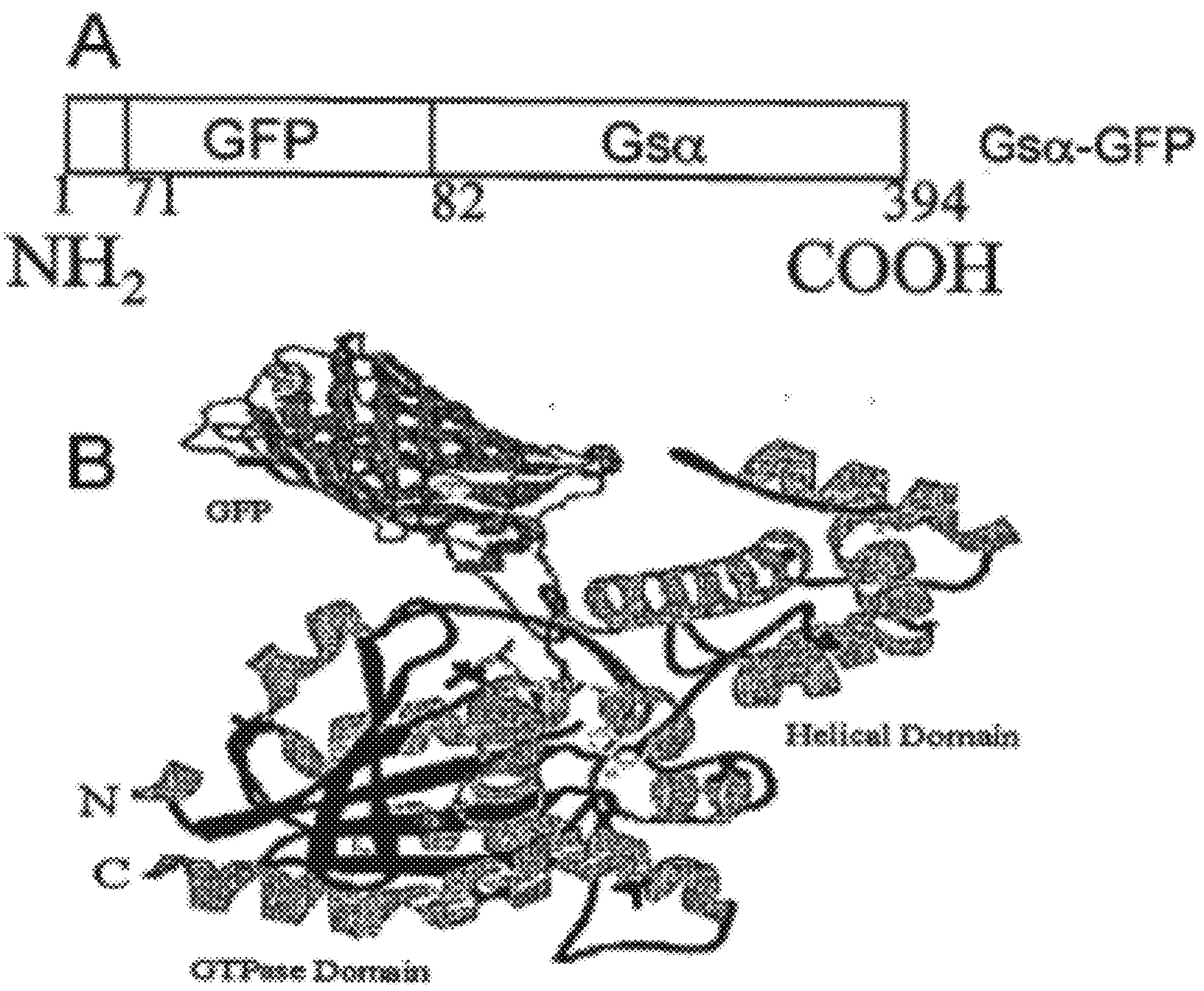

FIG. 1

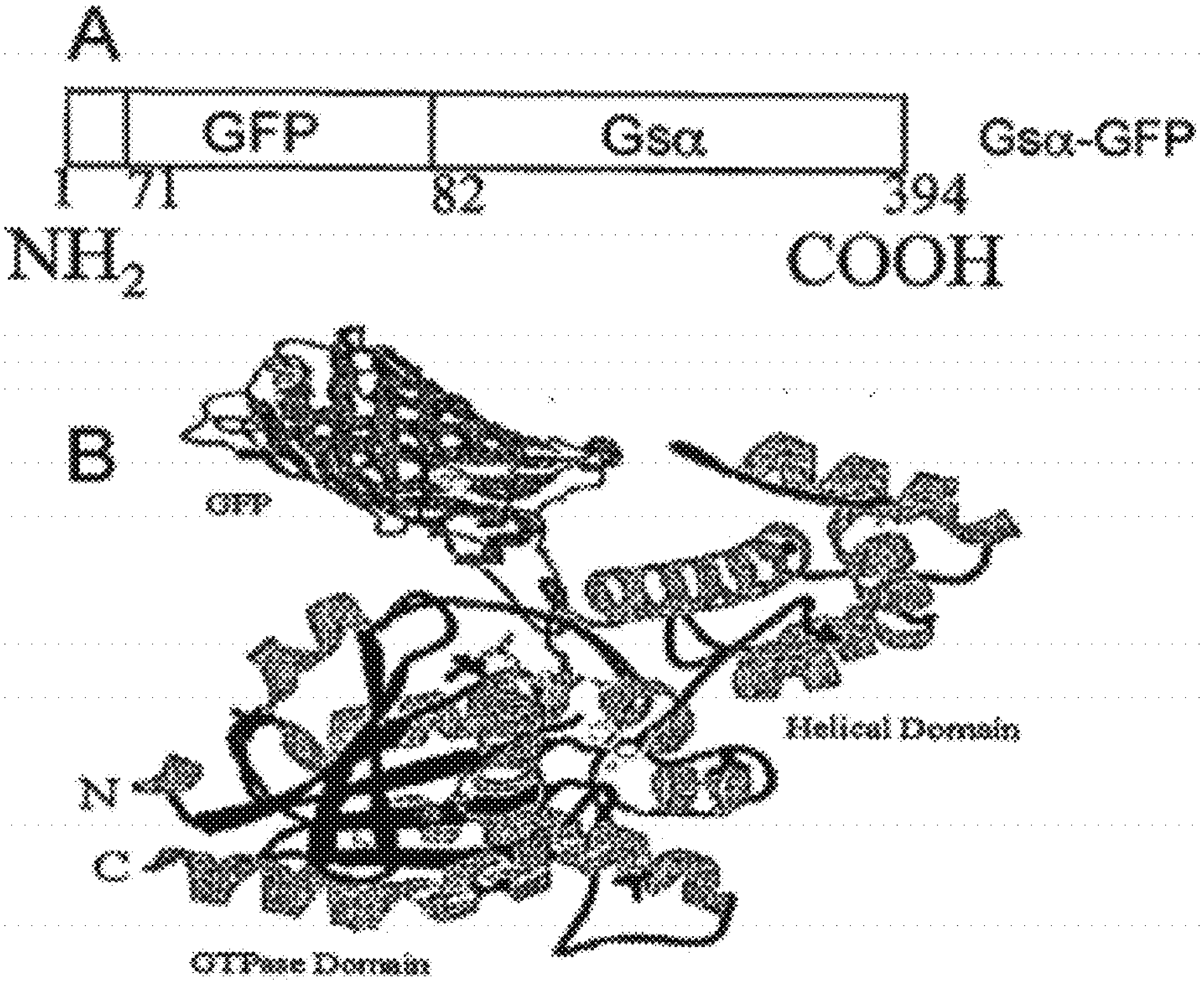
A
GFP
Gsα
Gsα-GFP
1
71
82
394
NH$_2$
COOH
B
GFP
Helical Domain
N
C
GTPase Domain

FIG.2

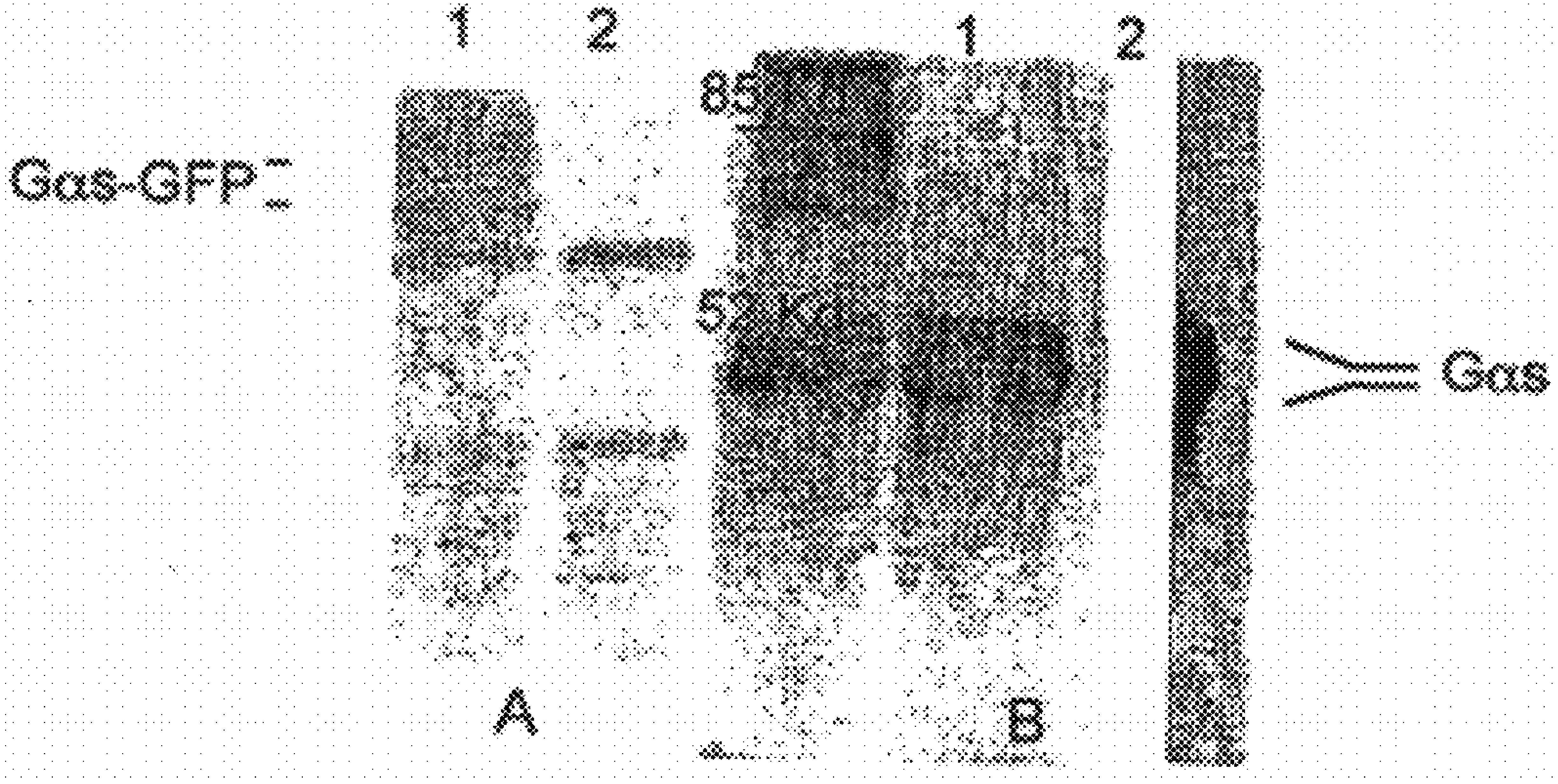

FIG.3

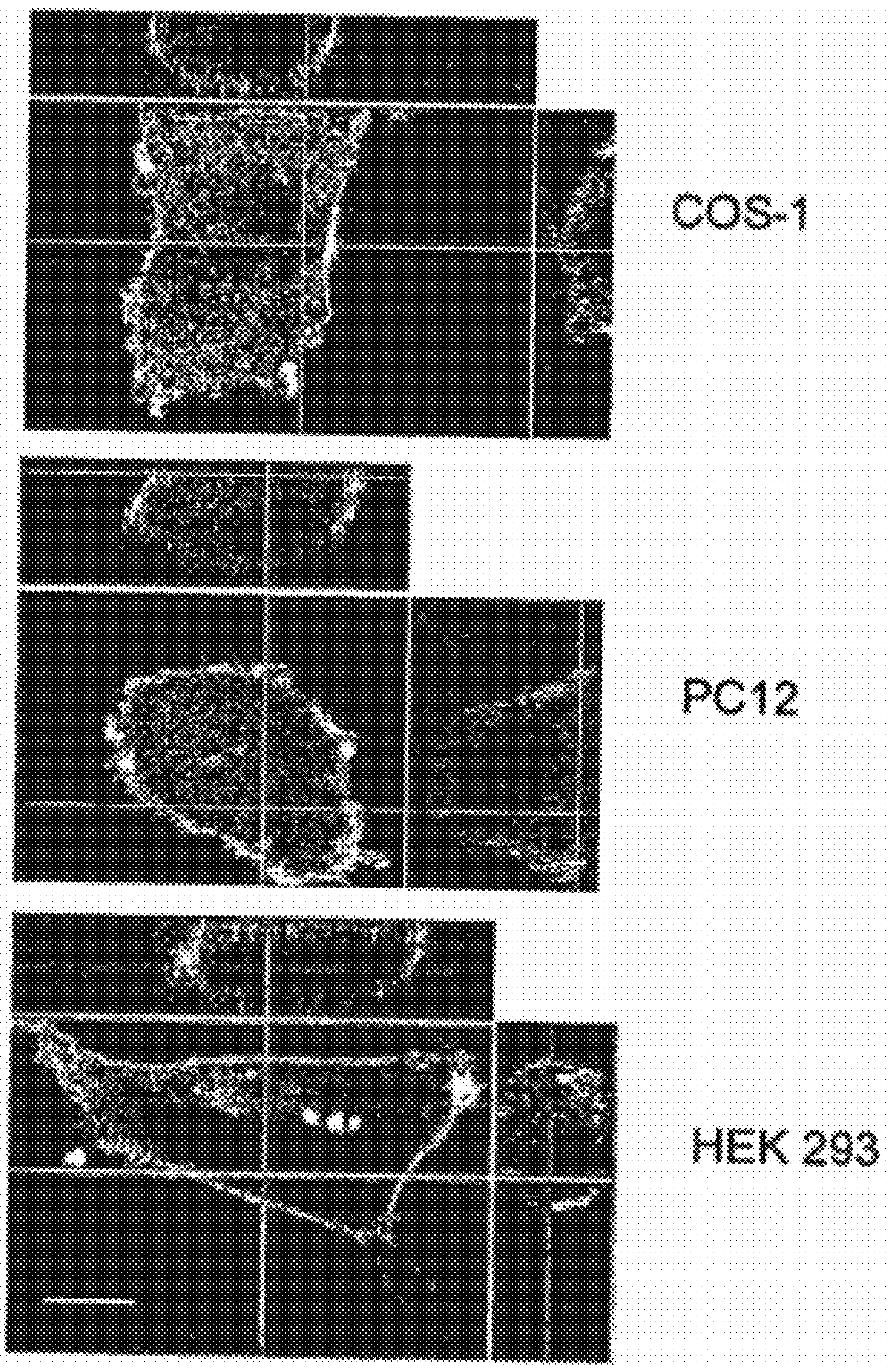

FIG.4

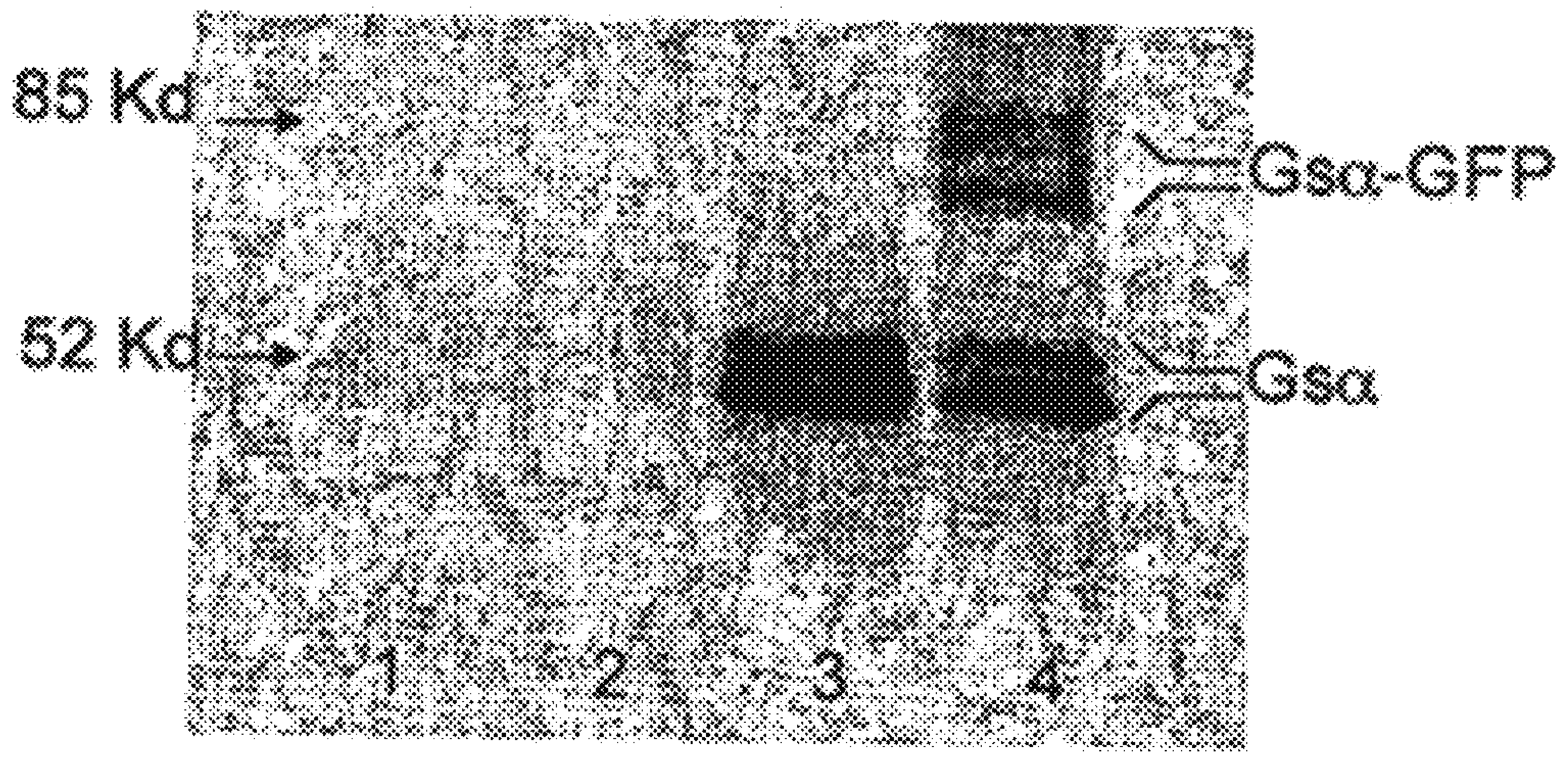

FIG.5

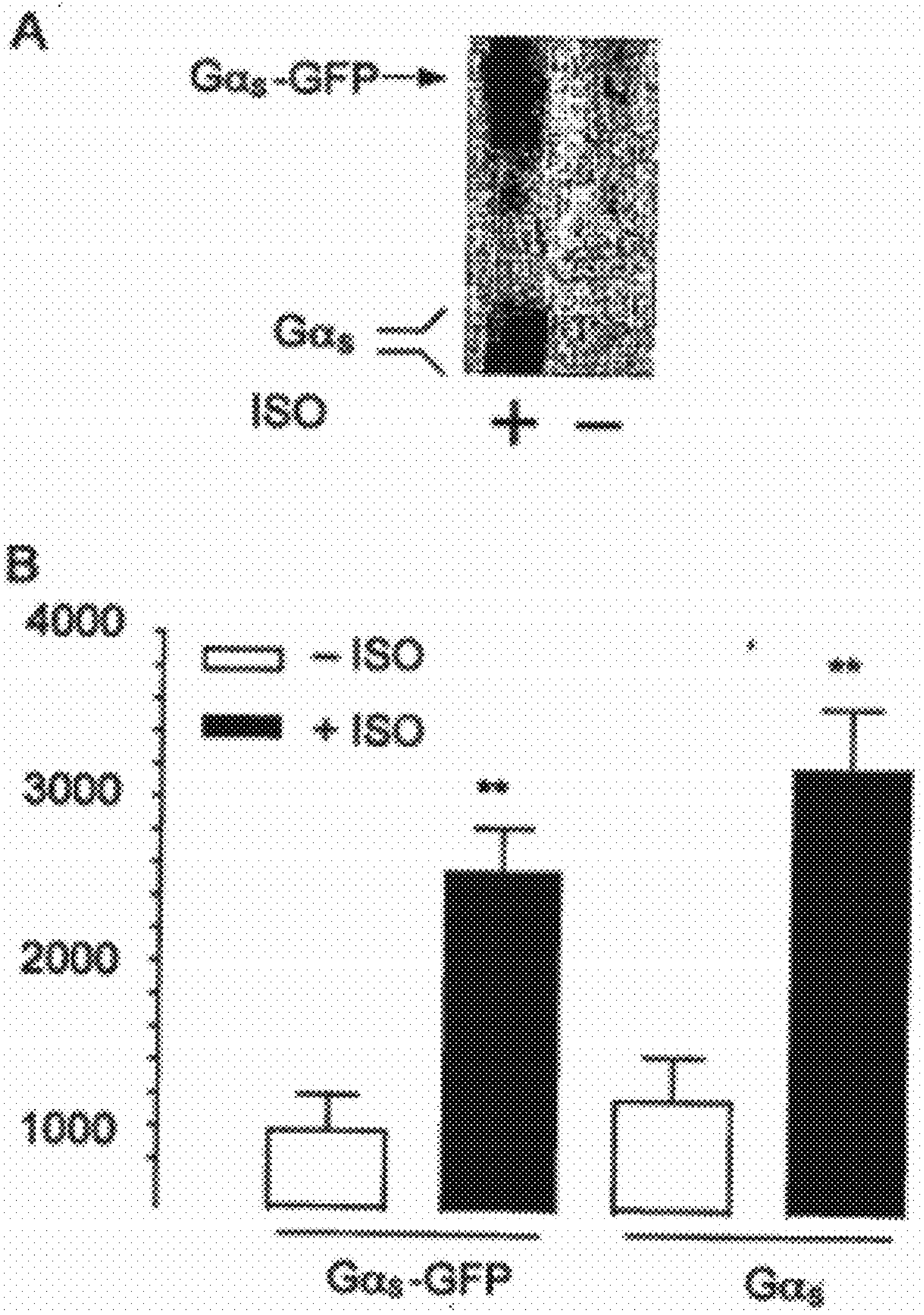
A
$G\alpha_s$-GFP
$G\alpha_s$
ISO
+
−
B
Densitometric Units
4000
3000
2000
1000
− ISO
+ ISO
**
**
$G\alpha_s$-GFP
$G\alpha_s$

FIG.6

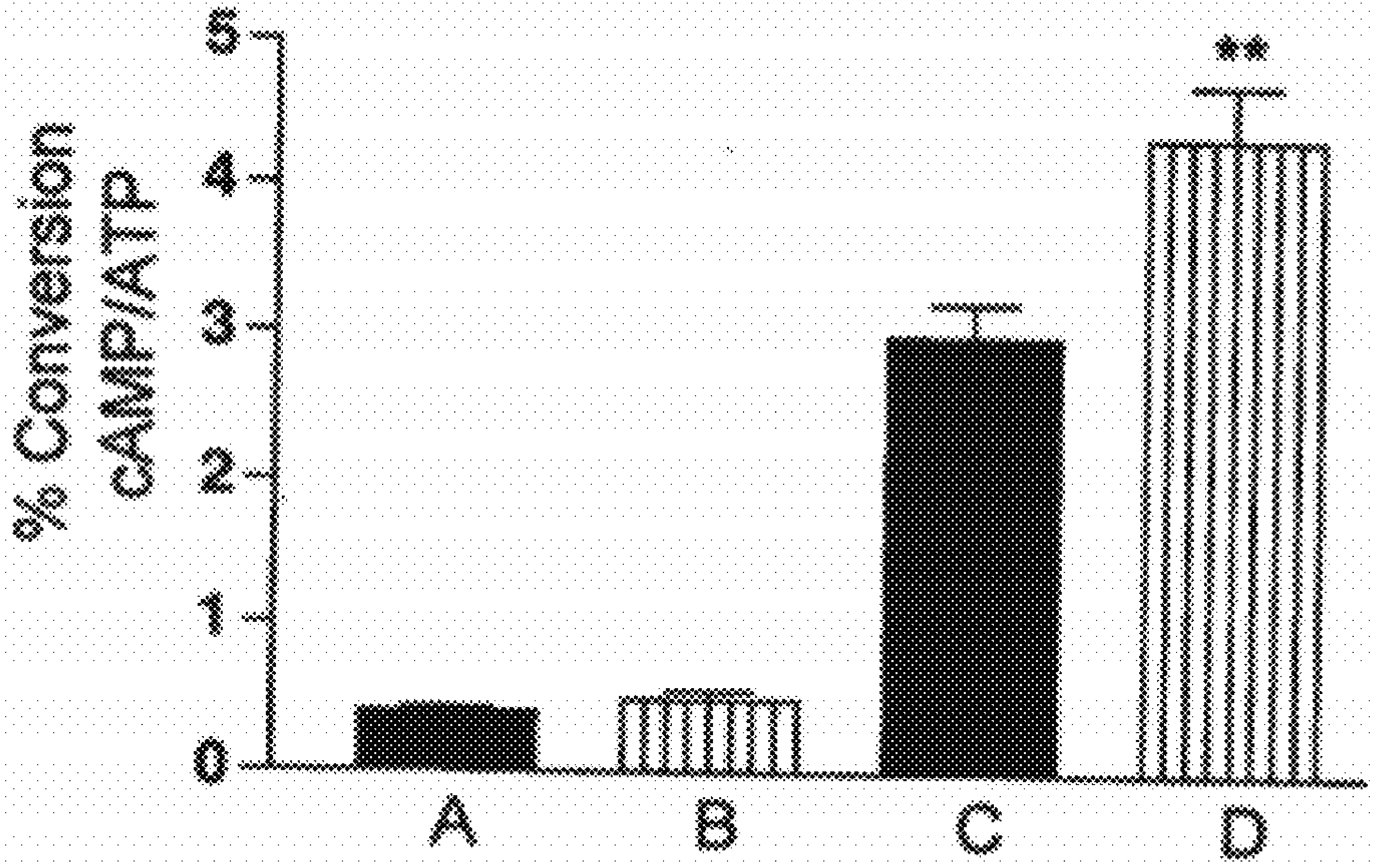

FIG.7

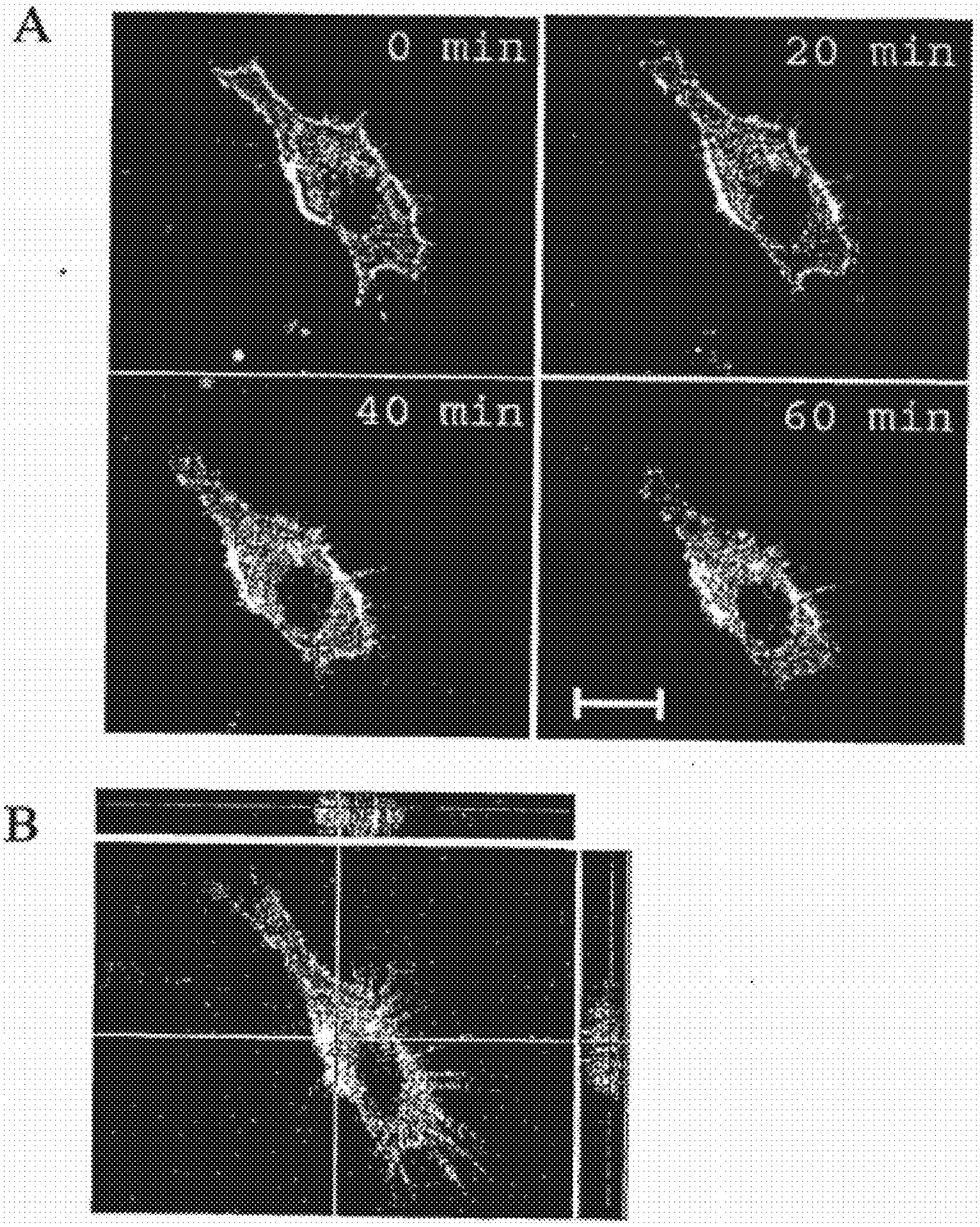

FIG.8

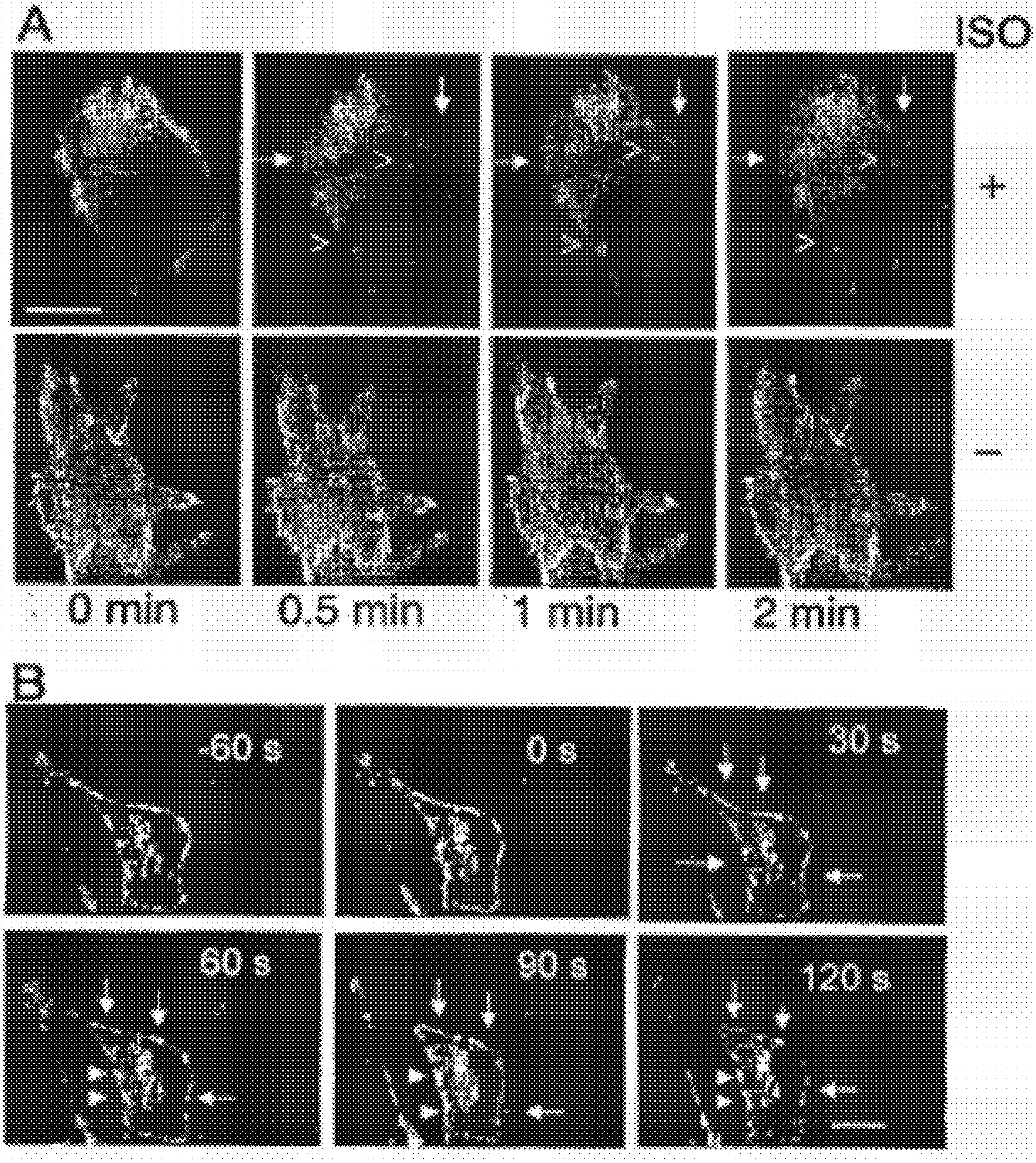

FIG.9A

AAGCTTGCCATGGGCTGCCTCGGCAACAGTAAGACCGAGGACCAGCGCAACGAGGAGAAGGCGCAGCGCGAGGCCAACAAAAAGATCGAG
TTCGAACGGTACCCGACGGAGCCGTTGTCATTCTGGCTCCTGGTCGCGTTGCTCCTCTTCCGCGTCGCGCTCCGGTTGTTTTTCTAGCTC
MetGlyCysLeuGlyAsnSerLysThrGluAspGlnArgAsnGluGluLysAlaGlnArgGluAlaAsnLysLysIleGlu

AAGCAGCTGCAGAAGGACAAGCAGGTCTACCGGGCCACGCACCGCCTGCTGCTGCTGGGTGCTGGAGAGTCTGGCAAAAGCACCATTGTG
TTCGTCGACGTCTTCCTGTTCGTCCAGATGGCCCGGTGCGTGGCGGACGACGACGACCCACGACCTCTCAGACCGTTTTCGTGGTAACAC
LysGlnLeuGlnLysAspLysGlnValTyrArgAlaThrHisArgLeuLeuLeuLeuGlyAlaGlyGluSerGlyLysSerThrIleVal

AAGCAGATGAGGATCCTGCATGTTAATGGGTTTAACGGAGAGGGCGGCGAAGAGGACGAATTCGCCACCATGGTGAGCAAGGGCGAGGAG
TTCGTCTACTCCTAGGACGTACAATTACCCAAATTGCCTCTCCCGCCGCTTCTCCTGCTTAAGCGGTGGTACCACTCGTTCCCGCTCCTC
LysGlnMetArgIleLeuHisValAsnGlyPheAsnGlyGluGlyGlyGluGluAspGluPheAlaThrMetValSerLysGlyGluGlu

CTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGAT
GACAAGTGGCCCCACCACGGGTAGGACCAGCTCGACCTGCCGCTGCATTTGCCGGTGTTCAAGTCGCACAGGCCGCTCCCGCTCCCGCTA
LeuPheThrGlyValValProIleLeuValGluLeuAspGlyAspValAsnGlyHisLysPheSerValSerGlyGluGlyGluGlyAsp

GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTAC
CGGTGGATGCCGTTCGACTGGGACTTCAAGTAGACGTGGTGGCCGTTCGACGGGCACGGGACCGGGTGGGAGCACTGGTGGGACTGGATG
AlaThrTyrGlyLysLeuThrLeuLysPheIleCysThrThrGlyLysLeuProValProTrpProThrLeuValThrThrLeuThrTyr

GGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGC
CCGCACGTCACGAAGTCGGCGATGGGGCTGGTGTACTTCGTCGTGCTGAAGAAGTTCAGGCGGTACGGGCTTCCGATGCAGGTCCTCGCG
GlyValGlnCysPheSerArgTyrProAspHisMetLysGlnHisAspPhePheLysSerAlaMetProGluGlyTyrValGlnGluArg

ACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAG
TGGTAGAAGAAGTTCCTGCTGCCGTTGATGTTCTGGGCGCGGCTCCACTTCAAGCTCCCGCTGTGGGACCACTTGGCGTAGCTCGACTTC
ThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLysPheGluGlyAspThrLeuValAsnArgIleGluLeuLys

FIG.9B

GGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAG
CCGTAGCTGAAGTTCCTCCTGCCGTTGTAGGACCCCGTGTTCGACCTCATGTTGATGTTGTCGGTGTTGCAGATATAGTACCGGCTGTTC
GlyIleAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleMetAlaAspLys

CAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACC
GTCTTCTTGCCGTAGTTCCACTTGAAGTTCTAGGCGGTGTTGTAGCTCCTGCCGTCGCACGTCGAGCGGCTGGTGATGGTCGTCTTGTGG
GlnLysAsnGlyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHisTyrGlnGlnAsnThr

CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGAT
GGGTAGCCGCTGCCGGGGCACGACGACGGGCTGTTGGTGATGGACTCGTGGGTCAGGCGGGACTCGTTTCTGGGGTTGCTCTTCGCGCTA
ProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuSerThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAsp

CACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCTCTAGAAACAGCGATGGTGAG
GTGTACCAGGACGACCTCAAGCACTGGCGGCGGCCCTAGTGAGAGCCGTACCTGCTCGACATGTTCAGGAGATCTTTGTCGCTACCACTC
HisMetValLeuLeuGluPheValThrAlaAlaGlyIleThrLeuGlyMetAspGluLeuTyrLysSerSerArgAsnSerAspGlyGlu

AAGGCCACCAAAGTGCAGGACATCAAAAACAACCTGAAGGAGGCCATTGAAACCATTGTGGCCGCCATGAGCAACCTGGTGCCCCCCGTG
TTCCGGTGGTTTCACGTCCTGTAGTTTTTGTTGGACTTCCTCCGGTAACTTTGGTAACACCGGCGGTACTCGTTGGACCACGGGGGGCAC
LysAlaThrLysValGlnAspIleLysAsnAsnLeuLysGluAlaIleGluThrIleValAlaAlaMetSerAsnLeuValProProVal

GAGCTGGCCAACCCTGAGAACCAGTTCAGAGTGGACTACATTCTGAGCGTGATGAACGTGCCAAACTTTGACTTCCCACCTGAATTCTAT
CTCGACCGGTTGGGACTCTTGGTCAAGTCTCACCTGATGTAAGACTCGCACTACTTGCACGGTTTGAAACTGAAGGGTGGACTTAAGATA
GluLeuAlaAsnProGluAsnGlnPheArgValAspTyrIleLeuSerValMetAsnValProAsnPheAspPheProProGluPheTyr

GAGCATGCCAAGGCTCTGTGGGAGGATGAGGGAGTTCGTGCCTGCTACGAGCGCTCCAACGAGTACCAGCTGATCGACTGTGCCCAGTAC
CTCGTACGGTTCCGAGACACCCTCCTACTCCCTCAAGCACGGACGATGCTCGCGAGGTTGCTCATGGTCGACTAGCTGACACGGGTCATG
GluHisAlaLysAlaLeuTrpGluAspGluGlyValArgAlaCysTyrGluArgSerAsnGluTyrGlnLeuIleAspCysAlaGlnTyr

TTCCTGGACAAGATTGATGTGATCAAGCAGGCCGACTACGTGCCAAGTGACCAGGACCTGCTTCGCTGCCGGGTCCTGACCTCTGGAATC
AAGGACCTGTTCTAACTACACTAGTTCGTCCGGCTGATGCACGGTTCACTGGTCCTGGACGAAGCGACGGCCCAGGACTGGAGACCTTAG
PheLeuAspLysIleAspValIleLysGlnAlaAspTyrValProSerAspGlnAspLeuLeuArgCysArgValLeuThrSerGlyIle

FIG.9C

TTTGAGACCAAGTTCCAGGTGGACAAAGTCAACTTCCACATGTTCGATGTGGGCGGCCAGCGCGATGAACGCCGCAAGTGGATCCAGTGC
AAACTCTGGTTCAAGGTCCACCTGTTTCAGTTGAAGGTGTACAAGCTACACCCGCCGGTCGCGCTACTTGCGGCGTTCACCTAGGTCACG
PheGluThrLysPheGlnValAspLysValAsnPheHisMetPheAspValGlyGlyGlnArgAspGluArgArgLysTrpIleGlnCys

TTCAATGATGTGACTGCCATCATCTTCGTGGTGGCCAGCAGCAGCTACAACATGGTCATCCGGGAGGACAACCAGACCAACCGTCTGCAG
AAGTTACTACACTGACGGTAGTAGAAGCACCACCGGTCGTCGTCGATGTTGTACCAGTAGGCCCTCCTGTTGGTCTGGTTGGCAGACGTC
PheAsnAspValThrAlaIleIlePheValValAlaSerSerSerTyrAsnMetValIleArgGluAspAsnGlnThrAsnArgLeuGln

GAGGCTCTGAACCTCTTCAAGAGCATCTGGAACAACAGATGGCTGCGTACCATCTCTGTGATCCTCTTCCTCAACAAGCAAGATCTGCTT
CTCCGAGACTTGGAGAAGTTCTCGTAGACCTTGTTGTCTACCGACGCATGGTAGAGACACTAGGAGAAGGAGTTGTTCGTTCTAGACGAA
GluAlaLeuAsnLeuPheLysSerIleTrpAsnAsnArgTrpLeuArgThrIleSerValIleLeuPheLeuAsnLysGlnAspLeuLeu

GCTGAGAAGGTCCTCGCTGGGAAATCGAAGATTGAGGACTACTTTCCAGAGTTCGCTCGCTACACCACTCCTGAGGATGCGACTCCCGAG
CGACTCTTCCAGGAGCGACCCTTTAGCTTCTAACTCCTGATGAAAGGTCTCAAGCGAGCGATGTGGTGAGGACTCCTACGCTGAGGGCTC
AlaGluLysValLeuAlaGlyLysSerLysIleGluAspTyrPheProGluPheAlaArgTyrThrThrProGluAspAlaThrProGlu

CCCGGAGAGGACCCACGCGTGACCCGGGCCAAGTACTTCATCCGGGATGAGTTTCTGAGAATCAGCACTGCTAGTGGAGATGGACGTCAC
GGGCCTCTCCTGGGTGCGCACTGGGCCCGGTTCATGAAGTAGGCCCTACTCAAAGACTCTTAGTCGTGACGATCACCTCTACCTGCAGTG
ProGlyGluAspProArgValThrArgAlaLysTyrPheIleArgAspGluPheLeuArgIleSerThrAlaSerGlyAspGlyArgHis

TACTGCTACCCTCACTTTACCTGCGCCGTGGACACTGAGAACATCCGCCGTGTCTTCAACGACTGCCGTGACATCATCCAGCGCATGCAT
ATGACGATGGGAGTGAAATGGACGCGGCACCTGTGACTCTTGTAGGCGGCACAGAAGTTGCTGACGGCACTGTAGTAGGTCGCGTACGTA
TyrCysTyrProHisPheThrCysAlaValAspThrGluAsnIleArgArgValPheAsnAspCysArgAspIleIleGlnArgMetHis

CTTCGCCAATACGAGCTGCTCTAAGAAGGGAAGGCCCAAATTTAATTCAGCCTTAAGCACAATTAATTAAGAGTGAAACGCAATCGTACA
GAAGCGGTTATGCTCGACGAGATTCTTCCCTTCCGGGTTTAAATTAAGTCGGAATTCGTGTTAATTAATTCTCACTTTGCGTTAGCATGT
LeuArgGlnTyrGluLeuLeuter

AGCAGTTGATCACCCACCATAGGGCATGATCAACACCGCAACCTTTCCCTTTTCTCCCCAGTGATTCTGAAAACCCCCTCTTCCCTTCAG
TCGTCAACTAGTGGGTGGTATCCCGTACTAGTTGTGGCGTTGGAAAGGGAAAAGAGGGGTCACTAAGACTTTTGGGGGAGAAGGGAAGTC

CTTGCTTAGATGTTCTCTAGA 2091
GAACGAATCTACAAGAGATCT

GFP FUSION PROTEINS AND THEIR USE

[0001] This application is a Continuation of co-pending U.S. patent application Ser. No. 10/482,980, filed Sep. 22, 2004, which is a national stage application of PCT/US02/21484 filed Jul. 3, 2002, which claims priority to U.S. Provisional Application No. 60/303,622 filed Jul. 6, 2001. The disclosures set forth in the above-referenced applications are incorporated herein by reference in their entireties.

[0002] This invention was made with government support under MH39595-10 and AG15482 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

[0003] The present invention relates a protein that is constructed by adding a green fluorescent protein designated GFP that is internal to the amino acid sequence of a G protein, in particular the Gαs protein. The resulting fusion protein is a non-radioactive marker used, for example, for high throughput screening of G protein-coupled receptor drug targets.

BACKGROUND OF THE INVENTION

[0004] A family of heterotrimeric nucleotide-binding proteins that bind to guanine (G proteins) transduces chemical and sensory signals across the plasma membrane by sequential interactions with receptor and second messenger-generating effectors. Because of the wide array of cellular processes that are mediated by G proteins, the study of G protein function and regulation is a significant area of research in the signal transduction field. There are reports containing suggestions of an important function for G protein at cellular locations other than the plasma membrane. Certain G proteins were detected at intracellular membranes, for example, the Golgi complex, whereas others associate with cytoskeletal structures, for example, microtubules and microfilaments. The mechanisms that govern the cellular destinations of G proteins and the relative proportions of G proteins that traffic to subcellular compartment are just beginning to be revealed.

[0005] G proteins are reported to couple the receptors for hormones or neurotransmitters to intracellular effectors such as adenylyl cyclase or phospholipase C. Twenty forms of the α-subunit of G proteins were identified and each is involved in the conveyance of multiple hormonal neurotransmitter signals from the outside of the cell to the effects that those hormones and neurotransmitters have on the inside of the cell.

[0006] G proteins may leave the membrane in response to neurotransmitter or hormone signals, but this has been very difficult to prove.

[0007] GFP, an autofluorescent protein of 238 amino acids, is a reporter molecule useful to monitor gene and protein expression and to observe the dynamics of protein movements within the living cell. Fusing GFP to another protein of interest allows time-course studies to be performed on living samples in real time. Accounts of GFP fusion proteins include receptors, secretory proteins, cytoskeleton proteins and signaling molecules. Presently, GFP fusion proteins are constructed by generating an expression construct that contains GFP fused in frame to either the N-amino or C-carboxyl terminus of the protein of interest. However, this attachment may alter the function of the protein fused with GFP consequently may not give results reflective of the natural state.

SUMMARY OF THE INVENTION

[0008] Fusion of a GFP protein at either NH_2 or COOH ends of Gαs protein subunits is not acceptable because the NH_2 region is important for association with Gαs protein βγ subunits, and the COOH terminal is required for interaction with receptors. Consequently, a biologically active Gαs-GFP that incorporated GFP at some other positions of the molecule was developed. Suitable regions for insertion of a GFP sequence are those regions that are free of interactions with receptors or effectors.

[0009] A fusion protein was constructed by inserting an amino acid sequence of a green fluorescent protein designated GFP, into the interior of an amino acid sequence of a G-protein, in particular the Gαs protein. Although, green fluorescent proteins have been inserted at either end of G-proteins, a method was needed to insert GFP into the internal amino acid sequence of a G-protein without altering the biological activity of the protein.

[0010] Green fluorescent protein (GFP) was inserted within the internal amino acid sequence of Gαs to generate a Gαs-GFP fusion protein. The fusion protein maintained a bright green fluorescence and was also identified by antibodies against Gαs or GFP, respectively. The cellular distribution of Gαs-GFP was similar to that of endogenous Gαs. Gαs-GFP was tightly coupled to the β adrenergic receptor to activate the Gαs effector, adenylyl cyclase. Activation of Gαs-GFP by cholera toxin caused a gradual displacement of Gαs-GFP from the plasma membrane throughout the cytoplasm in living cells. Unlike the slow release of Gαs-GFP induced by cholera toxin, the β adrenergic agonist isoproterenol caused a rapid partial release of Gαs-GFP into the cytoplasm. At 1 min after treatment with isoproterenol, the extent of this Gαs-GFP release from plasma membrane was maximal. Translocation of Gαs-GFP induced by isoproterenol suggested that the internalization of Gαs might play a role in signal transduction by interacting with effector molecules and cytoskeletal elements at multiple cellular sites.

[0011] Uses for the Gαs-GFP fusion construct of the present invention include:

[0012] 1. G proteins from the intracellular plasma membrane move in response to activation by an antagonist. Following the activation of a G protein and discovering the time course for that activation. The occupancy of a receptor by an agonist is only the first step in a signaling cascade. The intracellular processes might be activated at different rates or, at specific areas within a cell. Gαs-GFP is useful because it can be followed in real time as events take place without disrupting natural progress of events.

[0013] 2. Tracking protein functions in living cells.

[0014] 3. As a non-radioactive marker for high throughput screening of G-proteins coupled receptor drug targets, following the course of activation of a putative receptor or a putative ligand. For example, if a drug company has a candidate that it believes activates G protein coupled receptors in a functional sense, the Gαs-GFP fusion construct is useful as a high throughput screen, because a change in fluorescence in response to the application of an agonist is detectable. Conversely, the activity of an antagonist is visualized by adding it in 96 well plates, and screening significant numbers of samples on a fluorimeter to determine which compounds block the expected fluorescence change. Gαs-GFP could be used in combination with a fluorescent receptor such as that developed by the Biosignal Corporation in Montreal. To do this, cells are transfected with fluorescent receptors and Gαs-GFP. A ligand which activated the receptor in such a way that the G protein was also activated should decrease the fluorescence of GFP induced by the emitted light from the receptor (fluorescence resonance energy transfer-FRET). Thus, a number of candidate compounds may be screened for receptor and G protein activation by conducting these assays in e.g. 96 well plates.

[0015] 4. The use of green fluorescent protein (GFP) in the study of cellular signaling allows not only the observation of G protein trafficking, but the opportunity to study the dynamics of G proteins in real time as well as their function.

[0016] Other molecules may be modified in the same way, for example the other of the 20 G protein α subunits. Insertion sites for GFP are determined by an analysis of the sequence. None of the Gαs can be modified by adding GFP to either the amino or carboxy terminus because their function would be destroyed. Putting the GFP in the internal regions does not harm the effects of the protein, but rather bestows on its new properties. Several other signaling molecules may be suitable candidates for the fusion proteins of the present invention.

[0017] 5. Gαs-GFP is modified in such a way that it will couple to other receptors. Modification of amino acids near the carboxy terminal generates a fluorescent Γα that is capable of coupling to receptors which normally couple to Gαi, Gαo or Gαq (Conklin, et al., 1996). This will allow the same fluorescent G protein to assess potency and efficacy of putative agonists and agonists for a large number of G protein coupled receptors.

[0018] The 5 C terminal residues of Gαs are QYELL (SEQ ID NO: 3). They are replaced with DCGLF (SEQ ID NO: 4) for Gi1 or Gi2, with ECGLY (SEQ ID NO: 5) for Gi3, with RCGLY (SEQ ID NO: 6) for Go, and with EYNLV (SEQ ID NO: 7) for Gq.

[0019] COS1 or HEK293 cells are suitable because they are easy to transfect. These or comparable cells are co-transfected with GFP-Gαs (either in its native form or engineered to couple to a receptor which normally couples to Gi or Gq) and the desired receptor. Putative agonists are screened by monitoring loss of fluorescence from the membrane. High-throughput fluorescence monitoring instruments that are known to those of skill in the art are used for this purpose. Putative antagonists are screened by assessing their ability to block the effects of known receptor agonists to evoke this phenomenon.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. **1** shows Gαs fusion protein cDNA construction. (A) shows a schematic of Gαs-GFP. Gαs-GFP defines the fusion protein in which GFP was inserted within the NH_2-terminal domain of the long Gαs. (B) presents a model of Gαs-GFP. The structure of GFP is shaded. The Gαs subunit structure is that of Gαs-GTPγS.

[0021] FIG. **2** shows expression of the Gαs-GFP fusion in COS-1 cells. COS-1 cells were lysed 24 h after transiently transfecting with Gαs-GFP (3 μg DNA). 30 μg protein was loaded, separated by SDS-PAGE gel, detected with polyclonal antibody against Gαs (panel B) or monoclonal antibody against GFP (Panel A), as indicated. Lane 1 represents a lysate from cells transfected with Gαs-GFP, Lane 2 is the lysate from control cells.

[0022] FIG. **3** shows Gαs-GFP is associated with the plasma membrane in transfected cells. 24 h post-transfection, cells were observed by confocal microscopy at 37° C. A computer-generated cross section of a typical cell is displayed on the top (x-z plane) and on the right (y-z plane). Each image shown is representative of at least 20 cells subjected to a z-scan analysis. Similar results were obtained with COS-1, PC12, and HEK 293 cells.

[0023] FIG. **4** shows subcellular distribution of Gαs-GFP in COS-1 cells. Particulate and soluble fractions were isolated from cells transfected with Gαs-GFP constructs 24 h post transfection as described herein. 20 μg protein was loaded, separated by SDS-PAGE gel and detected with a polyclonal antibody against the C-terminal peptide of Gαs. Lanes 1 and 2 represent the soluble portion from the control cells or cells transfected with Gαs-GFP, respectively. Lanes 3 and 4 indicate the particulate fraction from control cells or cells transfected with Gαs-GFP, respectively.

[0024] FIG. **5** shows Gαs-GFP binding to AAGTP. COS-1 cells were co-transfected with cDNA encoding Gαs-GFP (1 μg) and β-adrenergic receptor (4 μg). (A) Shows cell membranes prepared 24 h post-transfection and incubated with ^{32}P AAGTP in the presence and absence of isoproterenol (as indicated). Proteins were resolved by SDS-PAGE and autoradiography. Results shown are from one of four similar experiments. (B) Presents densitometric analysis of Gαs-GFP binding to AAGTP. Densitometric analysis of four independent experiments were carried out and displayed in densitometric units. [Shown is the mean±Standard error, n=4, ** indicates significant difference from control treated without ISO (P<0.01].

[0025] FIG. **6** shows Gαs-GFP activates adenylyl cyclase. Cells were transfected with GFP (control) or Gαs-GFP, respectively and assayed for cAMP formation in the presence or absence of isoproterenol (ISO: 50 μM) as indicated. (A) control cells in the absence of ISO. (B) Gαs-GFP transfected cells in the absence of ISO. C. control cells with ISO. D. Gαs-GFP transfected cells treated with ISO. The values shown are mean±standard error of nine samples from three experiments. Identical levels of Gαs-GFP in each group were determined by western blotting. ** indicates significant difference from control cells treated without ISO; (P<0.01).

[0026] FIG. **7** demonstrates cholera toxin treatment translocates Gαs-GFP in living PC12 cells. (A) 24 h post-transfection with Gαs-GFP, media was replaced as described in Methods and living cells were viewed by confocal microscopy at 37° C. Cells were initially imaged (0 min), cholera toxin (3 μg/ml) was added and cells were observed for 1 h. Bar=10 μm. (B) computer-generated cross section of the whole cell after completion of the one hour, is displayed on the top (x-z plane) and on the right (y-z plane). Results shown are from one of four comparable experiments. Observation of other cell lines (COS-1 and HEK 293) showed similar results for response to cholera toxin.

[0027] FIG. **8** shows isoproterenol-stimulated rapid internalization of Gαs-GFP in living COS cells. Cells were transfected with Gαs-GFP and observed 24 h later at 37° C. with confocal or digital fluorescent microscopy. (A) cells were treated with or without isoproterenol (20 μM), and images were captured every 5 seconds (A video scan; showed COS-1 cell treatment with ISO for 2 min. and; shown control COS-1 cell for 2 min). Arrows indicate areas where membrane-bound Gαs-GFP released from plasma membrane significantly. Clusters of Gαs-GFP form subjacent to the plasma membrane (indicates by open arrowhead). (B) Observation of Gαs-GFP release from plasma membrane using confocal microscopy. Arrows display regions where Gαs-GFP released from plasma membrane significantly. The arrowheads indicate the sites where the Gαs-GFP was inserted after the 2 minutes time point. Bar=10 mm. These results are typical of 40 of 58 cells observed during the course of 15 experiments. Approximately 70% of the cells showed internalized Gαs-GFP in response to isoproterenol [ISO]. Thirty percent did not show a significant response to this agonist.

[0028] FIG. **9**A-**9**C are cDNA (nucleotide) (SEQ ID NO: 1) and its complement and amino acid sequences (SEQ ID NO: 2) of the Gαs-GFP. The letters in a box indicate the start codon for Gαs-GFP. The circled letters form the stop codon for Gαs-GFP. A, G, T and C are abbreviations of Adenine, Guanine, Thymine and Cytosine, respectively.

DETAILED DESCRIPTION OF THE INVENTION

[0029] Construction of Gαs-GFP

[0030] Full length cDNAs encoding Gαs were excised from the PcDNA-1 vector by digesting with Sam I and Xba I restriction enzymes. The full length EGFP cDNA was obtained by PCR from the PEGFP-N3 using appropriate primers (sense 5' GGAATTCATGAGCAAGGGCGAGGAACTG-3' (SEQ ID NO: 8); antisense 5'-GCTCTAGACGACTTGTACAGCTCGT-3') (SEQ ID NO: 9) and adding restriction sites to its cDNA (EcoR I at the initiation codon and Xba I at end of cDNA). To insert the EGFP within the sequence of Gαs, the first fragment of Gαs (from 1 to 71 amino acids) was amplified by PCR with restriction sites for Kap 1 at initiation codon and EcoR I at end of the fragment. The cDNA of the fragment was cloned into PcDNA3 vector by the Kap 1 and EcoR 1 restriction sites using primers (sense 5'GGGTACCATGGGCTGCCTCGGCAACA-3' (SEQ ID NO: 10); antisense 5'-GGAATTCGTCCTCTTCGCCGCCCTTCT-3') (SEQ ID NO: 11). Modified 7 EGFP cDNA was spliced into the first fragment of Gαs by EcoR 1 and Xba 1 restriction sites on PcDNA3 to get the fusion cDNA sequence of the first fragment of Gαs and EGFP. The second fragment of Gαs (from 82 to 394 amino acids) was also obtained using PCR with appropriate primers. The sense primer contained a part of a sequence overlapping with the 3' end of EGFP (5'-CAGAGCTGGACAAGTCCAACAGCGATGGTGAGAA-3') (SEQ ID NO: 12). The anti-sense primer contained an additional Xba 1 restriction site (5'-GCTCTAGACGACTTGTACAGCTCGT-3') (SEQ ID NO: 9) The presenion cDNA fragment described above was amplified by PCR. The Gαs-GFP fusion fragment and the second fragment of Gαs were also linked using PCR strategy. The full length Gαs-GFP was cloned into PcDNA3 at Kap 1 and Xba 1 restriction sites. All DNA manipulations, including ligations, PCR, bacterial transformation were carried out using procedures disclosed herein. Plasmid purification was done using "plasmid purification kit" following the manufacture instruction (QIAGEN).

Ligation Protocol

[0031] 1. In a 1.5 ml microfuge tube, cut 10 mL expression vector with the desired restricted enzyme in a total volume of 20 mL for 2 h at 31° C.
2. Loading the sample into 1% agarose gel, run the gel applying a voltage of 100 V. Run the gel long enough to resolve the fragments of interest.
3. Turn off the power supply and remove the gel from the apparatus.
4. Using "Gel Extraction Kit" (QIAGEN), purify fragments from gel.
5. In 0.5 ml microfuge tube, mix the fragments of vector (0.03 mg) and relevant inserts, add 5 mL 4' ligation buffer (GIBCOBRL), incubating in a total volume of 20 mL at 14° C. overnight with 0.1 units T_4 ligase.
6. Take out 10 mL to transformation.

Polymerase Chain Reaction

[0032] 1. In 0.5 ml thin wall tube mix the following ingredients.

10' buffer (GIBCOBRL)	5 μL
$MgCl_2$ (GIBCOBRL)	5 μL
primer 1	1 μL
prime r 2	1 μL
template DNA	0.5 μL
4 dNTP mix (GIBCOBRL)	10 μL
H_2O	26.5 μL
Taq polymerase (GIBCOBRL)	1 μL
	50 mL

2. Spin down one time for 15 seconds and put one-drop mineral oil in tube.
3. Turn on the automated thermal cycler.
4. First denature simples 2 min at 94° C., then run program for 35 cycles.

[0033] Denature 90 seconds

[0034] Anneal 50 seconds at 58° C.

[0035] Extend 1 min at 72° C.

When cycles finish, 7 min perform extra-extend at 72° C.
5. Run gel and purify the DNA with "PCP Purification Kit" (QIAGEN).

Transformation Protocol

[0036] 1. Add 5 ml of LB medium (10 g tryptone, 5 g yeast extract, 10 g NaCl in 1 L H_2O) to sterile 10 ml tube.
2. Scrape HB 101 bacterial cells (one colony) from stock plate with loop. Transfer cells to medium and shake bacterial cells off loop. Put the tube in shaking incubator at 31° C. for 12 h.
3. Spin down bacterial cells at 2000×g for 3 min at room temperature.
4. Gently resuspend pellet of bacterial cells in 1 ml 50 mM $CaCl^2$, incubate for 40 min on ice.
5. Spin down again at 2000×g for 3 min at 4° C. Resuspend pellet of bacterial cells in 100 ml 50 mM $CaCl^2$.
6. In 1.5 ml sterile microfuge tube, add 10 mL ligated plasmid vector, then mix it with 100 mL competent bacterial cells.
7. Incubate the mixture on ice for 20 min and then transfer tube to 42° C. for heat shock for 30 seconds.
8. Take the mixture, and add to plate (with antibiotic), agar side top incubating at 37° C. overnight.

[0037] Three Gαs-GFP fusion constructs were made and expressed in COS-1 cells. In the Gαs-NGFP expression vector, in which the GFP was spliced to the N-amino terminus of Gαs sequence, the fusion protein could not associate with the plasma membrane of cells (see FIG. **1**, FIG. **2**A). The attachment of palmitate at Cys-3 of Gαs is required both for its membrane association and for its ability to mediate hormonal stimulation of adenylyl cyclase. A sequence motif that serves as a predictor for a subset of palmitoylated proteins is Met-Gly-Cys at the amino terminus of a protein. This motif found in the Gi and Gαs subfamily of G-protein subunits and other proteins such as receptor tyrosine kinases. The GFP connected with the amino terminus of Gαs may affect the palmitoylation of Cys-3. A GFP tagged COOH terminal of Gαs,

Gαs-CGFP was also constructed. Although this attached to the membrane, it did not respond to hormone activation.

[0038] Gαs exists as a short and a long splice variant. Compared with short Gαs, long Gαs contains an additional 15 amino acids inserted at position 72 of the polypeptide chain, and there is an exchange of glutamate for apartate at position 71. Although there has been some indication that subtle differences between short Gαs and long Gαs exist, the general function of the two forms is similar. No substantial difference in the function of the two forms has been detected. Furthermore, the yeast Gαs, GPA1, has an "extra loop" in this region as well. Levis et al. (1992) modified the long Gαs form at a site (residues 77-81) within the 15 amino acid insert to confer upon it recognition by an antibody directed against a well-defined peptide of the influenza hemaglutinin (HA). Addition of the HA epitope did not alter the ability of wild type Gαs to mediate hormonal stimulation of adenylyl cyclase or to attach to cell membranes. Given the possibility that this region was "inert", a Gαs-GFP2 fusion protein was constructed by replacing the residues (72-81) within the long Gαs with a GFP sequence (see FIG. **1**). A western blot of membrane and cytosolic fractions (FIG. **2**B), probed with an anti-Gαs polyclonal or anti-GFP monoclonal antibody, shows that Gαs-GFP2 is expressed in COS-1 cells with a distribution comparable to that of intrinsic Gαs. These results indicate that the GFP in the Gαs-GFP2 should not alter the attachment of Gαs to membranes. In addition, the fluorescence of GFP in Gαs-GFP2 is visual and stable with UV irradiation.

[0039] Based on the α-carbon model of the α-subunit of the retinal G-protein transducin, the sequence within which the 15 amino acid insert is localized in the long Gαs serves as a linker between the ras-like domain and the α-helical domain. The guanine nucleotide-binding site is embedded between these two domains. Thus, the change in this linker sequence might be expected to diminish the ability of binding to guanine nucleotides of Gαs. To study this, COS-1 cells were co-transfected with Gαs-GFP2 and β-adrenergic receptor cDNA. COS1 membranes were incubated with the photoaffinity GTP analog ^{32}P AAGTP as in the presence and absence of a beta adrenergic agonist. Labeling of membranes from the transfected COS-1 cells was accomplished by incubating with 0.1 mM [^{32}P] AAGTP for 5 min at 23° C., followed by treatment with isoproterenol (ISO) for 3 min. Gαs-GFP2 in COS-1 bound [^{32}P] AAGTP in response to ISO (FIG. **3**). This result dramatically and unexpectedly demonstrated that the insertion of GFP into the linker sequence between two domains of Gαs does not disrupt agonist-induced guanine nucleotide exchange.

[0040] Cholera toxin activates Gαs by directly ADP ribosylating arginine 201 of Gαs and inhibiting the intrinsic GTPase. Thus, cholera toxin locks Gαs in the activated state. After, cholera toxin-activated was no longer observed at the plasma membrane, but instead it was distributed throughout the cytoplasm. Increased solubility of Gαs may correlate with activation-induced depalmitoylation of Gαs, but it is not absolutely clear that the removal of the lipid group is necessary for cytosolic translocation. FIG. **4** shows that the Gαs-GFP on the cellular membrane is internalized gradually subsequent to treatment of cells with cholera toxin. Cholera toxin activation of Gαs-GFP also provides further evidence that the fusion protein is capable of normal pysiological function.

[0041] The physiologic consequences of β-adrenergic receptor activation of Gαs were observed by examining the response of Gαs-GFP cos1 cells to isoproterenol. The rapid translocation of Gαs from membrane to cytoplasm was clearly delineated.

[0042] To determine whether Gαs-GFP was fully physiologically active, tests were performed to see if the fusion protein was capable of activating adenylyl cyclase. By measurement of cAMP accumulation in COS-1 cells transfected in different conditions, the overexpression of Gαs-GFP was found not to alter the base level of cAMP in cells. Isoproterenol treated cells showed the cAMP production in Gαs-GFP cells to be significantly higher than cells transfected with GFP-vector alone (FIG. **6**).

[0043] Thus, assay of subcellular distribution and signaling function shows in vitro and in vivo that the GFP insertion into the Gαs amino acid sequence does not substantially affect normal function of Gαs. The study indicates a new approach to constructing GFP fusion protein and the study of G protein molecular signaling transduction in cells.

DOCUMENTS CITED

[0044] Conklin, B. R., Herzmark, P., Ishida, S., Voyno-Yasenetskaya, T. A., Sun, Y., Farfel, Z. and Bourne, H. R. (1996) Carboxyl-terminal mutations of Gq alpha and Gs alpha that alter the fidelity of receptor activation. *Mol. Pharmacol.* 50: 885-890.

[0045] Hugges, T. E., Zhang, H., Logothetis, D. E., Berlot, C. H. (2001) Visualization of a functional Gaq-green fluorescent protein fusion in living cells. *J. Biol. Chem.* 276: 4227-4235.

[0046] Kallal, L. and Benovic, J. L. (2000) Using green fluorescent proteins to study G-protein receptor localization and trafficking. *TiPS* 21: 175-180.

[0047] Levis, M. J. and Bourne, H. R. (1992) Activation of a subunit of Gαs in intact cells alters its abundance, rate of degradation, and membrane avidity. *J. Cell Bio.* 5:1297-1300.

[0048] Sunahara, R. K., Tesmer J. J. G., Gilman, A. G. and Sprang S. R. (1997) Crystal structure of the adenylyl cyclase activator Gas. *Science* 278: 1943-1947.

SEQUENCE LISTING

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Glu Glu Lys Ala Gln Arg Glu Ala Asn Lys Lys Ile Glu Lys Gln Leu
15                  20                  25                  30

cag aag gac aag cag gtc tac cgg gcc acg cac cgc ctg ctg ctg ctg      147
Gln Lys Asp Lys Gln Val Tyr Arg Ala Thr His Arg Leu Leu Leu Leu
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ggt gct gga gag tct ggc aaa agc acc att gtg aag cag atg agg atc      195
Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Arg Ile
            50                  55                  60

cta cat gtt aat ggg ttt aac gga gag ggc ggc gaa gag gac gaa ttc      243
Leu His Val Asn Gly Phe Asn Gly Glu Gly Gly Glu Glu Asp Glu Phe
        65                  70                  75

gcc acc atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc      291
Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
    80                  85                  90

atc ctg gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg      339
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
95                  100                 105                 110

tcc ggc gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag      387
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
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ttc atc tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg      435
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
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acc acc ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac      483
Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
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gcc gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg      627
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
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aag ggc atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg      675
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gag tac aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag      723
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
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aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac      771
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    240                 245                 250

ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc      819
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
255                 260                 265                 270

gac ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc      867
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Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
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gag ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac       963
Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr
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335                 340                 345                 350

agc aac ctg gtg ccc ccc gtg gag ctg gcc aac cct gag aac cag ttc      1107
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aga gtg gac tac att ctg agc gtg atg aac gtg cca aac ttt gac ttc      1155
Arg Val Asp Tyr Ile Leu Ser Val Met Asn Val Pro Asn Phe Asp Phe
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cca cct gaa ttc tat gag cat gcc aag gct ctg tgg gag gat gag gga      1203
Pro Pro Glu Phe Tyr Glu His Ala Lys Ala Leu Trp Glu Asp Glu Gly
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gtt cgt gcc tgc tac gag cgc tcc aac gag tac cag ctg atc gac tgt      1251
Val Arg Ala Cys Tyr Glu Arg Ser Asn Glu Tyr Gln Leu Ile Asp Cys
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gcc cag tac ttc ctg gac aag att gat gtg atc aag cag gcc gac tac      1299
Ala Gln Tyr Phe Leu Asp Lys Ile Asp Val Ile Lys Gln Ala Asp Tyr
415                 420                 425                 430

gtg cca agt gac cag gac ctg ctt cgc tgc cgc gtc ctg acc tct gga      1347
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atc ttt gag acc aag ttc cag gtg gac aaa gtc aac ttc cac atg ttc      1395
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gat gtg ggc ggc cag cgc gat gaa cgc cgc aag tgg atc cag tgc ttc      1443
Asp Val Gly Gly Gln Arg Asp Glu Arg Arg Lys Trp Ile Gln Cys Phe
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gtg atc ctc ttc ctc aac aag caa gat ctg ctt gct gag aag gtc ctc      1635
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            530                 535                 540

gct ggg aaa tcg aag att gag gac tac ttt cca gag ttc gct cgc tac      1683
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acc act cct gag gat gcg act ccc gag ccc gga gag gac cca cgc gtg      1731
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gtg gac act gag aac atc cgc cgt gtc ttc aac gac tgc cgt gac atc     1875
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gcagttgatc acccaccata gggcatgatc aacaccgcaa cctttccctt ttctccccag   2041

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Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Arg Ile Leu His
    50                  55                  60

Val Asn Gly Phe Asn Gly Glu Gly Gly Glu Glu Asp Glu Phe Ala Thr
65                  70                  75                  80

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            100                 105                 110

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        115                 120                 125

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
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Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
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Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
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Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        275                 280                 285

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    290                 295                 300

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ser
305                 310                 315                 320

Ser Arg Asn Ser Asp Gly Glu Lys Ala Thr Lys Val Gln Asp Ile Lys
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Asn Asn Leu Lys Glu Ala Ile Glu Thr Ile Val Ala Ala Met Ser Asn
            340                 345                 350

Leu Val Pro Pro Val Glu Leu Ala Asn Pro Glu Asn Gln Phe Arg Val
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Ala Cys Tyr Glu Arg Ser Asn Glu Tyr Gln Leu Ile Asp Cys Ala Gln
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Tyr Phe Leu Asp Lys Ile Asp Val Ile Lys Gln Ala Asp Tyr Val Pro
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Ser Asp Gln Asp Leu Leu Arg Cys Arg Val Leu Thr Ser Gly Ile Phe
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Glu Thr Lys Phe Gln Val Asp Lys Val Asn Phe His Met Phe Asp Val
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Gly Gly Gln Arg Asp Glu Arg Arg Lys Trp Ile Gln Cys Phe Asn Asp
465                 470                 475                 480

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545                 550                 555                 560

Pro Glu Asp Ala Thr Pro Glu Pro Gly Glu Asp Pro Arg Val Thr Arg
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Gly Asp Gly Arg His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp
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I claim:

1. A fusion protein comprising a green fluorescent protein inserted into the internal amino acid sequence of a Gαs protein.

2. The fusion protein of claim **1**, wherein the insertion is at regions that are free of interactions with receptors or effectors.

3. The fusion protein of claim **1** modified for specific receptors by replacing amino acid residues at the C terminal end of Gαs.

4. A method for making a fusion protein, said method comprising:

(a) obtaining a molecule having an amino acid sequence of a green fluorescent protein; and

(b) inserting the molecule into the interior of a molecule having an amino acid sequence of a G-protein.

5. The method of claim **4** wherein the fusion protein has the amino acid sequence as in SEQ ID NO: 2.

6. The method of claim **4**, wherein the G-protein is the Gαs protein.

7. A method to follow an activation of a G-protein receptor by a candidate drug said method comprising:

(a) obtaining a G-protein green fluorescent fusion protein;

(b) monitoring fluorescence of the fusion protein in response to the candidate drug; and

(c) inferring from a change in fluorescence whether the drug is an agonist or antagonist.

8. Use of the fusion protein of claim **1** to follow the activation of a G-protein receptor.

9. Use of the fusion protein of claim **1** to track protein functions in living cells.

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