SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE LEGENDS

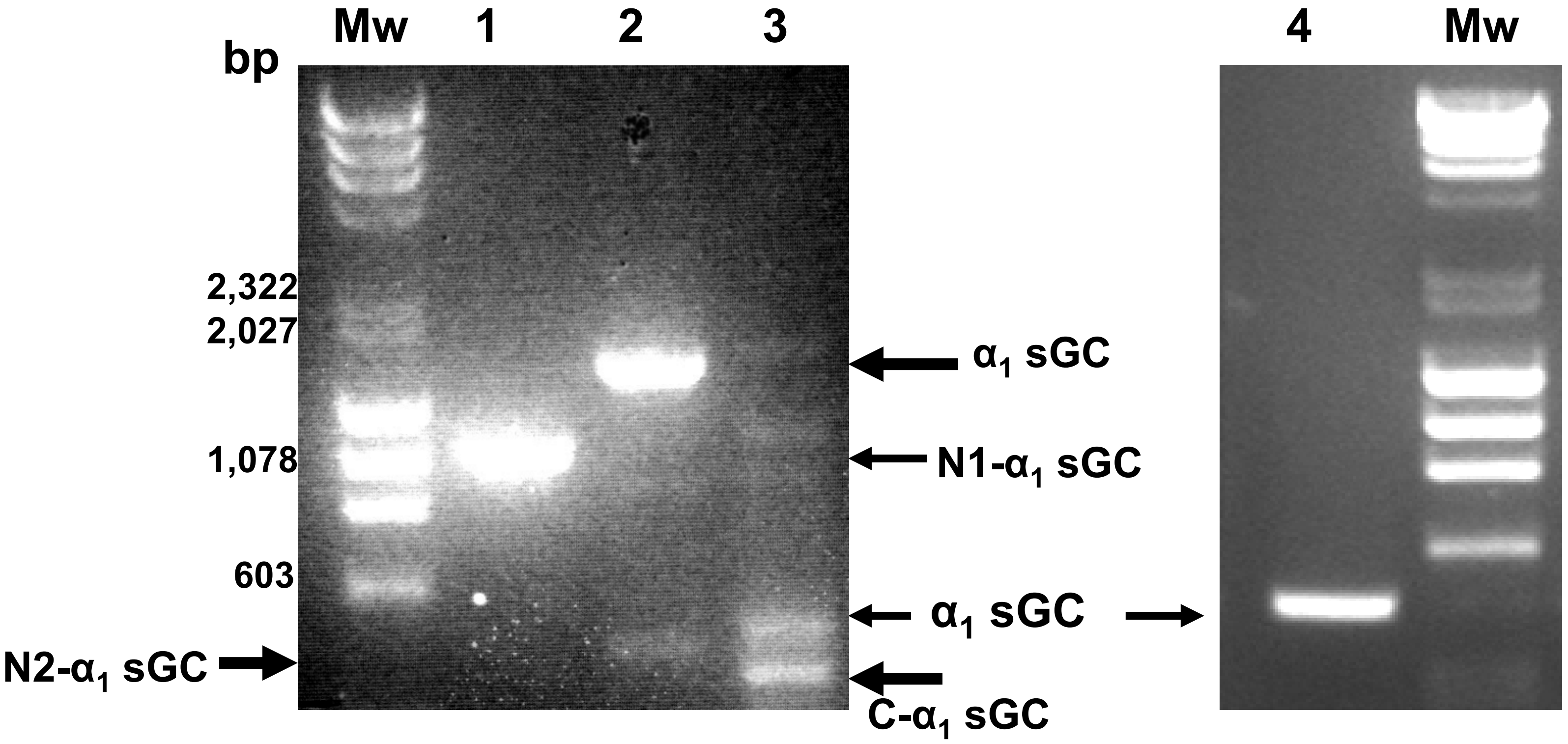
Figure **S1.** *Detection α_1 sGC splice forms in BE2 cells by RT-PCR.* **Lane 1 -** Primers PR1 and PR2 (Fig. 1A), specific for N1-α_1 sGC splice mRNA, amplify a 1100 bp fragment containing the coding sequence for N1-α_1 sGC splice form. **Lane 2 -** PR3 and PR4 primers, specific for sequences surrounding the start and stop codons of α_1 sGC mRNA, amplify a 427 bp fragment containing the coding sequence for N2- α_1 sGC (marked with arrow) and a 2 kb fragment containing the coding sequence of the full size α_1 sGC (top band). **Lane 3 -** Primers PR5 and PR6, amplify a 380 bp fragment from the full size sGC transcript and a 217 bp fragment containing a deletion specific for C-α_1 sGC splice mRNA (AK226125 accession number). **Lane 4 -** Primers PR7 and PR8, amplify a 468 bp fragment from the full size sGC transcript and a 330 bp fragment containing a deletion specific for C*-α_1 transcript (BX649180 accession number), absent in BE2 cells. Molecular weight marker is a mixture of Lambda DNA-Hind III and φX174 DNA-Hae III digests (New England Biolabs, USA).

Figure **S2.** *Detection C-α_1 sGC splice forms in normal human tissues by RT-PCR.* ***A***. Primers PR5 and PR6 were used to detect amplicons specific for the α_1 and C-α_1 transcripts in the following human tissues: 1, Esophagus; 2, Heart; 3, Kidney; 4, Liver; 5, Lung; 6, Adipose; 7, Bladder; 8, Brain; 9, Cervix; 10, Colon; 11, Testes; 12, Spleen; 13, Thyroid; 14, Thymus; 15, Trachea; 16, Ovary; 17, Placenta; 18, Prostate; 19, Small intestine; 20, Skeletal Muscle. **B**. The bands indicated by arrows obtained from the tissues 1, 2, 14 and 18 were excised from the gel and sequenced to confirm the identity of the amplicons.

Figure **S3.** *Detection C*-α_1 sGC splice forms in normal human tissues by RT-PCR.* ***A***. Primers PR7 and PR8 were used to detect amplicons specific for the α_1 and C*-α_1 transcripts in the following human tissues: 1, Esophagus; 2, Heart; 3, Kidney; 4, Liver; 5, Lung; 6, Adipose; 7, Bladder; 8, Brain; 9, Cervix; 10, Colon; 11, Testes; 12, Spleen; 13, Thyroid; 14, Thymus; 15, Trachea; 16, Ovary; 17, Placenta; 18, Prostate; 19, Small intestine; 20, Skeletal Muscle. **B**. The bands indicated by arrows obtained from the tissues 4, 5, 6, 12, 15, 18 were excised from the gel and sequenced to confirm the identity of the amplicons.

Figure **S4**. *Co-expression of N1-α_1 sGC inhibits α_1/β_1 sGC activity in Sf9 cells.* Rate of cGMP production in response to DEA-NO (200 μM), BAY 41-2272 (2 μM) or both treatments in lysates of Sf9 cell expressing α_1/β_1 sGC and different amounts of N1-α_1 protein. Data from three independent experiments are presented as mean ± SD.

Figure **S5.** ***Ni-agarose pull-down of α_1/β_1 and N1-α_1/β_1 proteins from the Sf9 lysate.*** Sf9 lysates expressing α_1 and 6His-tagged β_1 subunits or flagged N1-α_1 and 6His-tagged β_1 were loaded on 1 ml HisTrap column (GE Healthscience), washed with 10 mM and eluted with 150 mM imidazole.



bp
Mw
1
2
3
4
Mw
2,322
2,027
1,078
603
α1 sGC
N1-α1 sGC
α1 sGC
C-α1 sGC
N2-α1 sGC

Fig. S1

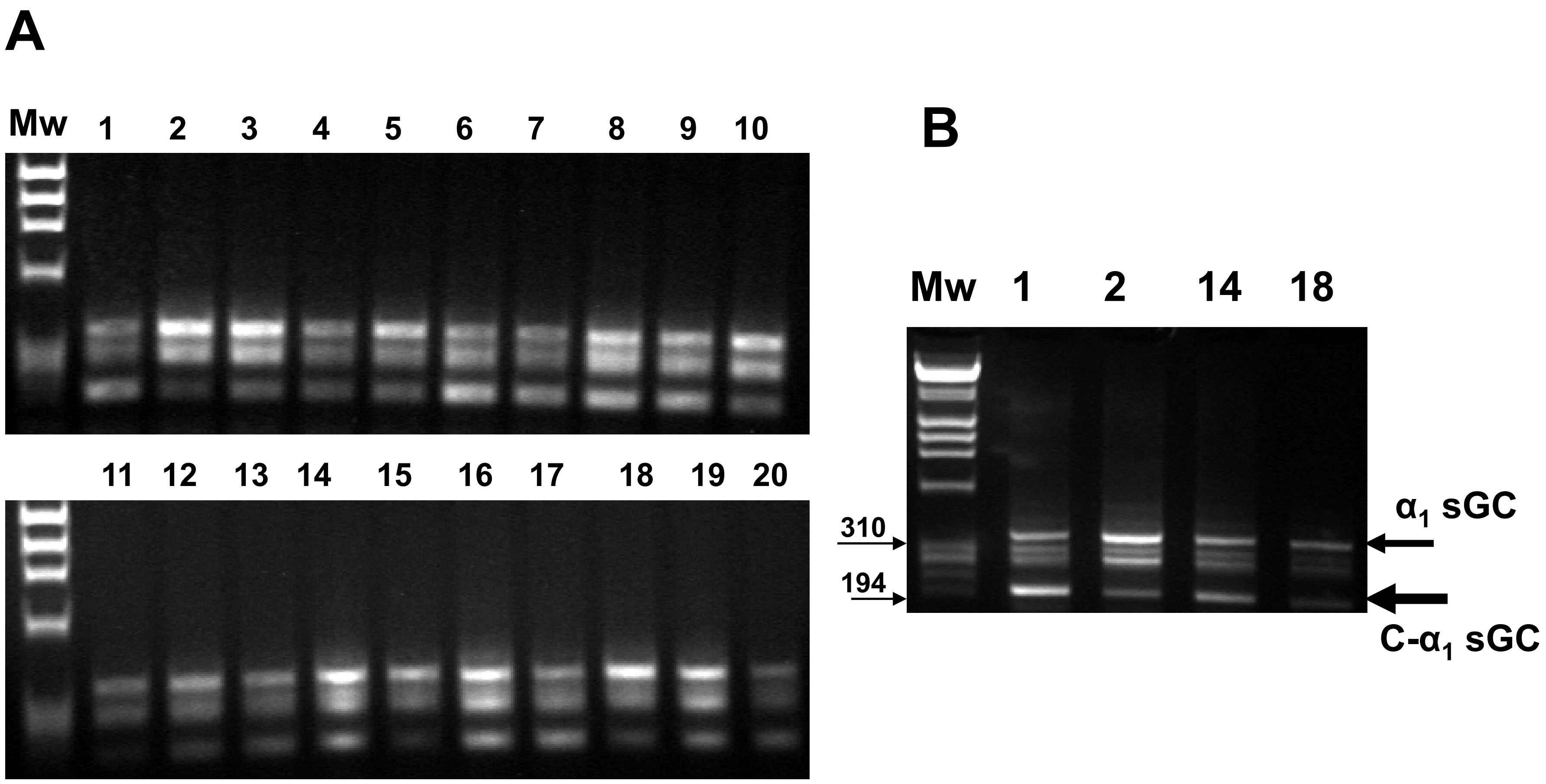

A
Mw
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
B
Mw
1
2
14
18
310
194
α1 sGC
C-α1 sGC

Fig. S2

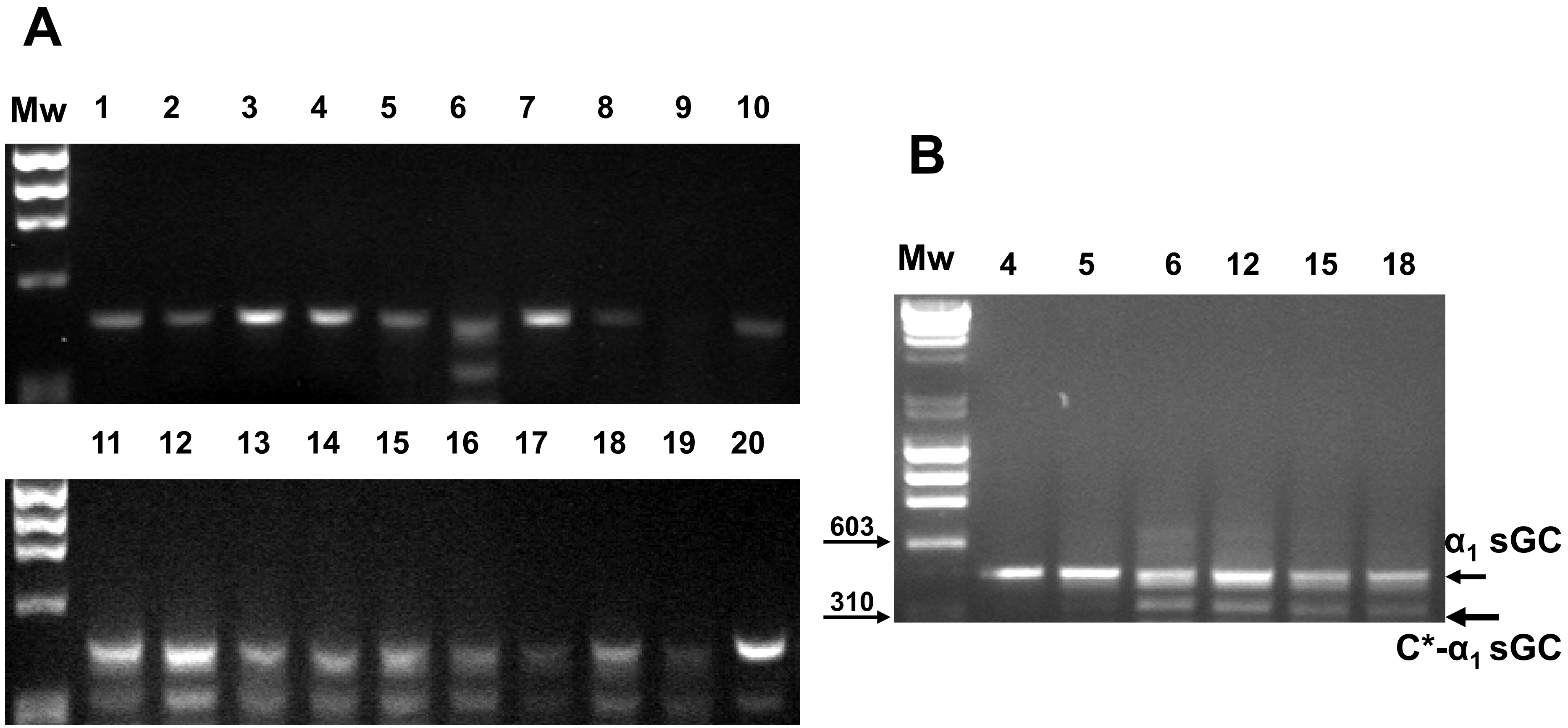
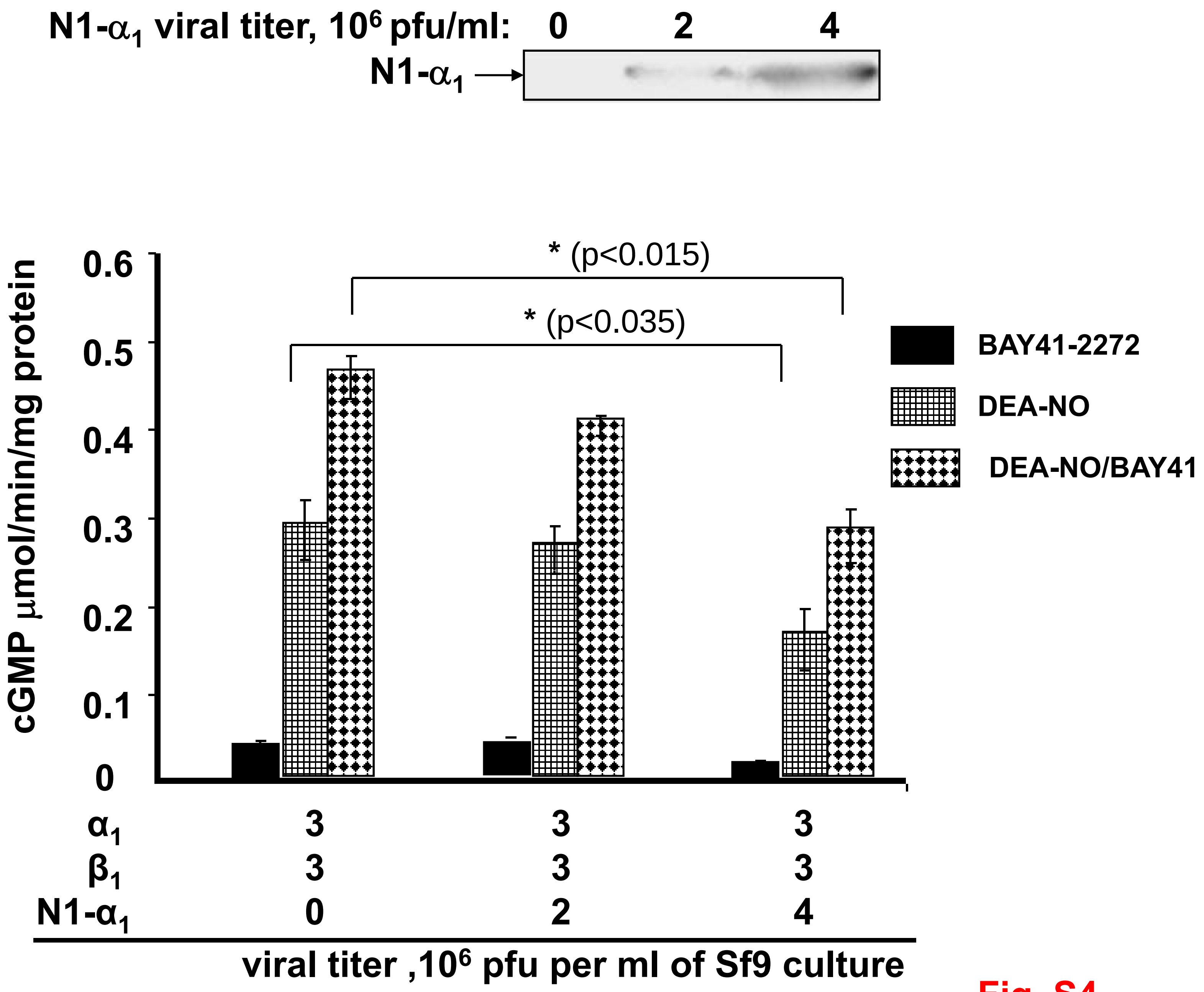

A
Mw
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
B
Mw
4
5
6
12
15
18
603
310
α_1 sGC
C*-α_1 sGC

Fig. S3



N1-α1 viral titer, 10⁶ pfu/ml: 0 2 4
N1-α1
cGMP μmol/min/mg protein
0.6
0.5
0.4
0.3
0.2
0.1
0
* (p<0.015)
* (p<0.035)
BAY41-2272
DEA-NO
DEA-NO/BAY41
α1 3 3 3
β1 3 3 3
N1-α1 0 2 4
viral titer ,10⁶ pfu per ml of Sf9 culture

Fig. S4

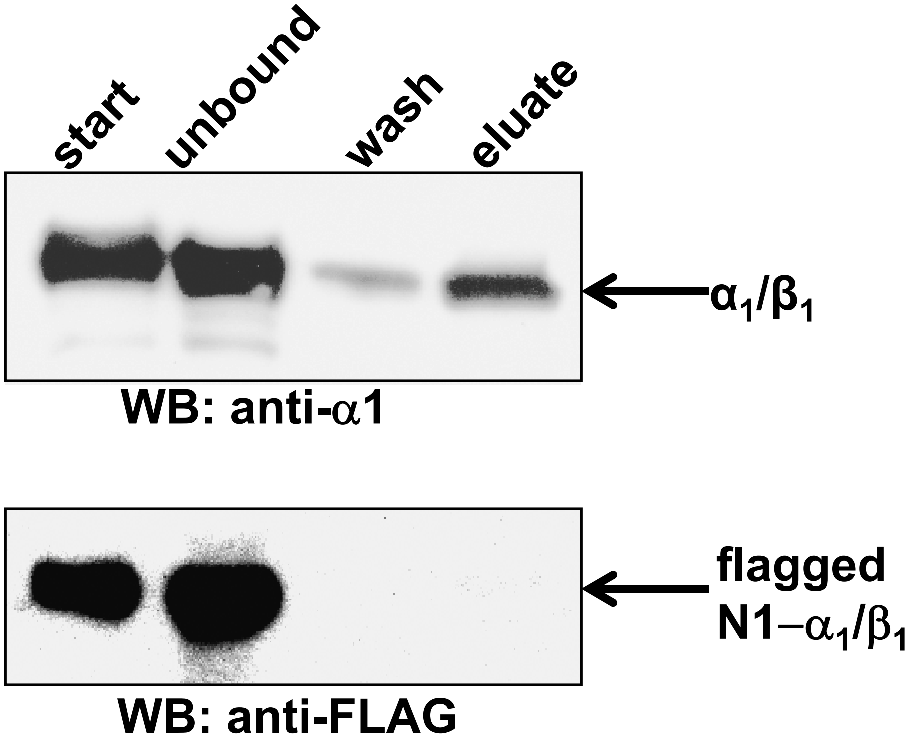

start
unbound
wash
eluate
α_1/β_1
WB: anti-α1
flagged
N1–α_1/β_1
WB: anti-FLAG

Fig. S5

Table S1. Diversity of human α_1 sGC mRNAs in NCBI database

Accession N	Predicted size of the transcript	Human tissue source	Encodes
BX647200	4.4 kb	salivary gland	full size protein
AK096588	2.6 kb	brain	full size protein
U58855	2.6 kb	kidney	full size protein
Y15723	3.1 kb	brain	full size protein
BC028384	2.7 kb	brain, hypothalamus	full size protein
X66534	3.0 kb	brain, frontal lobe	full size protein
BC095395	2.6 kb	brain, hippocampus	full size protein
BC012627	1.2 kb	prostate	330 aa C-term. deletion (N1-α_1)
CR618242	1.5 kb	neuroblastoma	564 aa C-term. deletion (N2-α_1)
CR614534	1.5 kb	placenta	564 aa C-term. deletion (N2-α_1)
BX649180	4.3 kb	small intestine	240 aa N-term. deletion (C*-α_1)
AK226125	4.3 kb	brain	240 aa N-term. deletion (C-α_1)

Table S2. Sequence variations in α_1 sGC splice forms.

A. Alternative 3’ end of exon 7 in N1-α_1 sGC transcript. Additional sequence and amino acids are marked in bold. Numeration is according CR618242 sequence.

S S R V **R K T** *

1400 tcttcaagg**gtaaggaaaacataatactatcttgaatatgaaagctatttcat**

AtttaagagcaagaaacaaaagggtaaaaatatatgcatcacttcaaaTgtt

tgataaaacatatgtaaaacaatggtaaaagaat 1540

B. Alternative 5’end of intron 7 in N2-α_1 sGC transcript. New peptide sequence and premature stop site are shown. Numeration is according BC012627 sequence.

G D H W R C L L C S W G I T Q R E *

576 ggagaccattggcgatgcctattgtgtagctgggggattacacaaagagagtga **630**

Table S3. Q-PCR assays.

	α_1 sGC	N1-α_1 sGC	N2-α_1 sGC
Accession number	Y15723	CR818242	BC012627
5' Primer	2043+ATGCACTGTACACTCGCTTCG	1358+AGTTTGTTGTACGAGTGAGG	539+TTGAAAAAACAATTGCAGAGC
3' Primer	2122-ACAATAGGCATCGCCAATGG	1434-AAGATAGTATTATGTTTTCCTTACC	606-GCTACACAATAGGCATCGC
Probe sequence	2099-CCACCTTGTAGACATCCAGCTCTCCACA	1405-AAGATTTTTTCACAGAGTTGTCCCA	582-CCACCTGCTGCAACTG
Amplicon	77 bases	79 bases	72 bases
Lowest limit for quantification	190 molecules	180 molecules	200 molecules
PCR efficiency	97%	95%	93%

Table S4. Relative numbers of N1- and N2-α_1 sGC transcripts by Q-PCR detection in normal human tissues (normalized to 36b4 levels).

Tissue	Full size α1	N1-type	N2-type
Esophagus	70926.99	62.0	688.0
Heart	349145.8	2350.4	2015.5
Kidney	261295.6	5604.5	3502.8
Liver	58203.7	774.4	612.3
Lung	206587.0	7219	1786.0
Adipose	228441.1	3235.7	2161.9
Bladder	148411.6	0	1775.9
Brain	1125930.0	4651.9	9875.5
Cervix	226653.4	3186.2	2203.1
Colon	211391.3	771.8	2199.0
Testes	42990.1	0	205.0
Spleen	158613.1	2763.0	1943.5
Thyroid	69515.2	0	889.8
Thymus	138170.2	2600.5	1206.0
Trachea	71643.6	1413.7	885.1
Ovary	539106.4	8871.9	7304.3
Placenta	212767.3	0	623.3
Prostate	568469.1	19679.4	4619.9
Small intestine	138709.6	1162.5	1419.6
Skeletal muscle	26445.7	0	399.1