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Supplementary Materials for

Noncoding RNA Gas5 Is a Growth Arrest– and Starvation-Associated Repressor of the Glucocorticoid Receptor

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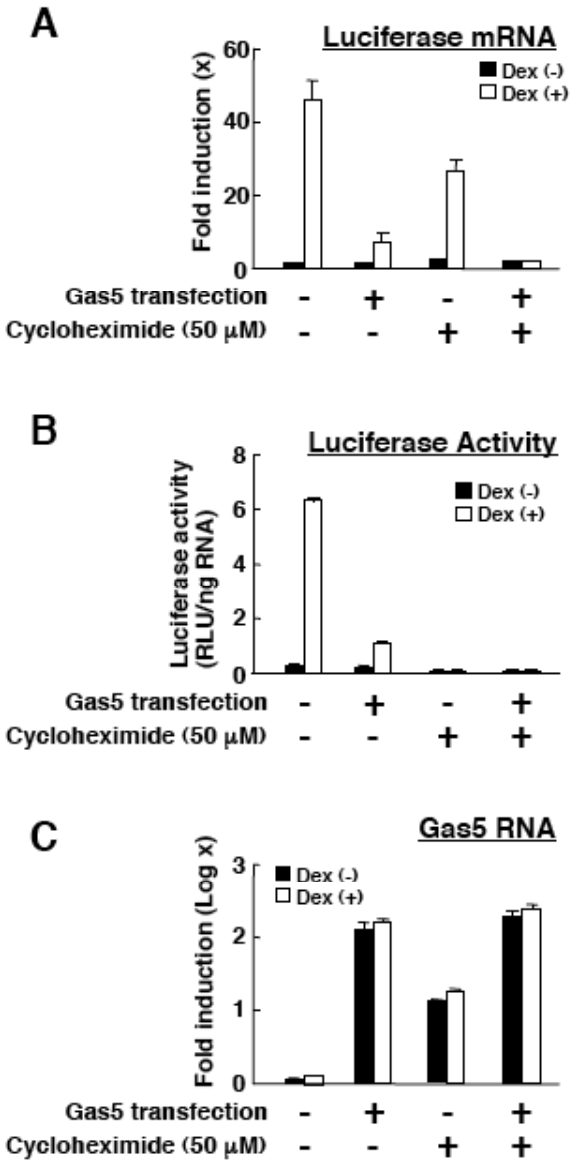

fig. S1. Translation into protein is not required for Gas5 to repress GR-induced transcriptional activity in HeLa cells. HeLa cells were pretreated with 50 μM of cycloheximide and subsequently transfected with Gas5-expressing plasmid together with pMMTV-Luc and pSV40-β-Gal. The amounts of RNAs encoding luciferase and Gas5 were measured with SYBR Green real-time PCR, and luciferase activity assays were performed with the same samples remaining after the total RNA purification. Bars represent mean +/- SEM of *luciferase* mRNA (**A**), luciferase activity (**B**), and Gas5 RNA (**C**) in the absence or presence of 10^{-6} M of dexamethasone (n=3). As seen in panel **B**, this concentration of cycloheximide completely abolished luciferase activity from the transfected MMTV reporter construct, indicating that protein synthesis was fully inhibited. In contrast to luciferase activity, the mRNA for luciferase was detected both in the presence and absence of cycloheximide, as seen in panel **A**. Gas5 RNA repressed GR-induced expression of the luciferase mRNA both in the presence and absence of cycloheximide (see panel **A**), indicating that Gas5 repressed GR-induced transcriptional activity as a ncRNA rather than as a translated protein(s).

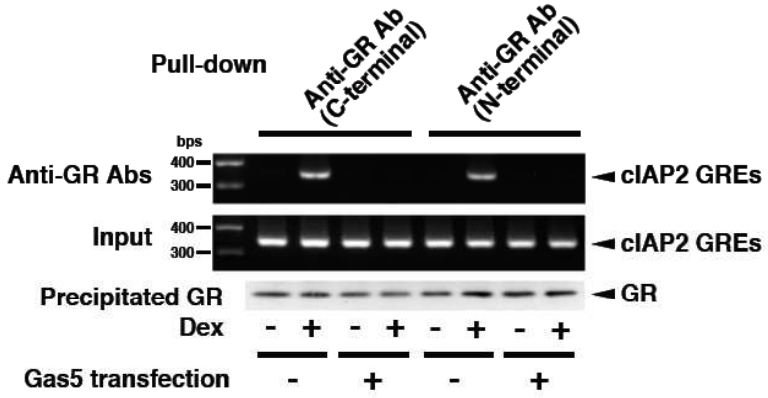

fig. S2. Gas5 suppresses association of GR to *cIAP2* GREs. The effect of Gas5 on the interaction of GR with cIAP2 GREs was examined in ChIP with two GR antibodies, which respectively recognized GR ligand-binding domain (LBD) or N-terminal domain (NTD). HeLa cells were transfected with Gas5-expressing plasmid, treated with 10^{-6} M of dexamethasone, and ChIP assays were performed with the indicated GR antibody, which recognized GR LBD (C-terminal) or the GR N-terminal domain (N-terminal). The *cIAP2* promoter fragment that contains tandem GREs was amplified by regular PCR with adjusted cycles using a specific primer pair. Results of the Western blot demonstrating precipitated GR are shown in the bottom gel.

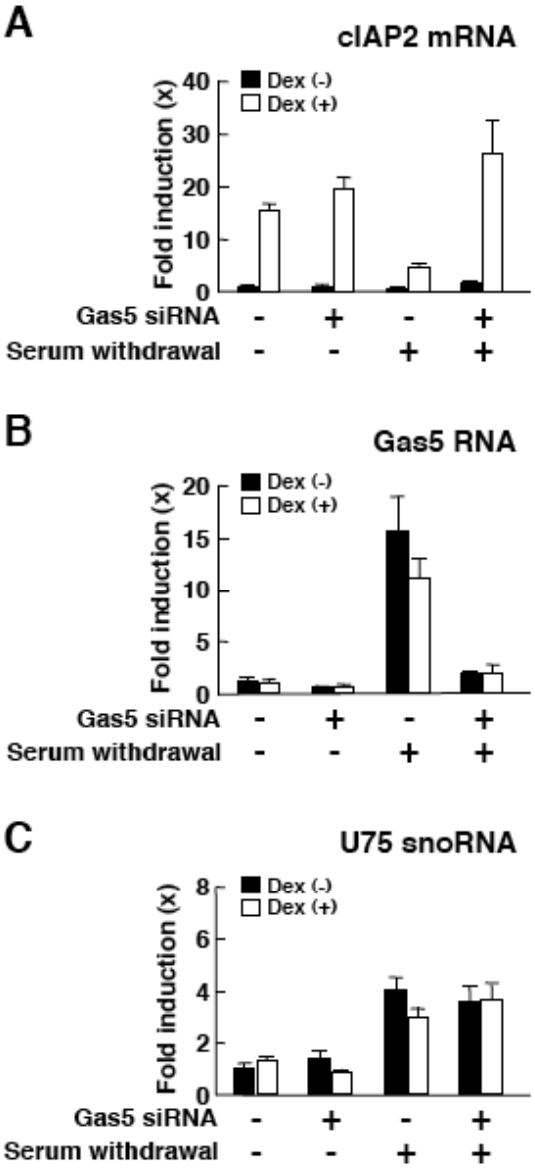

fig. S3. **Gas5 expression correlates with suppression of GR-induced *cIAP2* mRNA expression, whereas that of U75 does not.** HeLa cells were transfected with Gas5 or control siRNA and were cultured in serum-free medium for 72 hours. Expression of *cIAP2* mRNA (**A**), Gas5 RNA (**B**), and U75 snoRNA (**C**) were examined with SYBR Green real-time PCR. Bars represent the mean +/- SEM values of fold mRNA/RNA expression compared to the baseline (in the presence of control siRNA and in the absence of serum withdrawal and dexamethasone) (n=3).

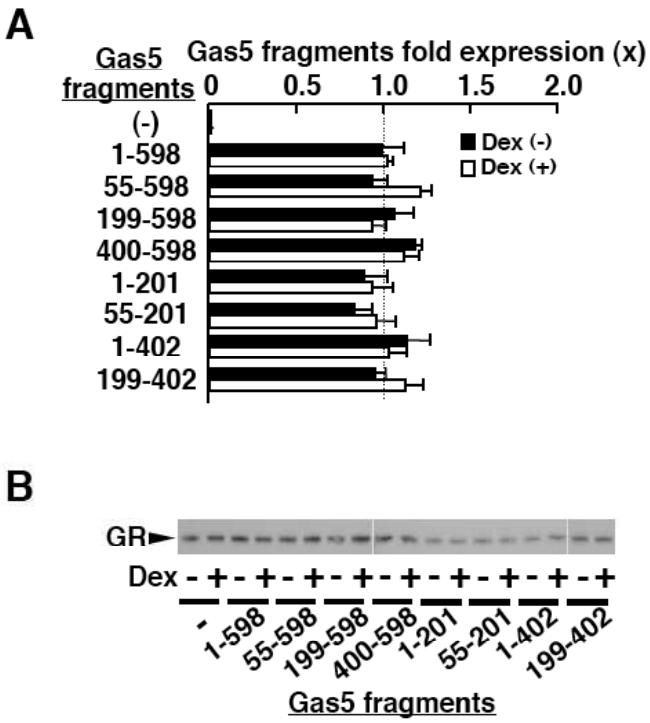

fig. S4. Cells have similar amounts of the Gas5 fragments and cells expressing these fragments have similar amounts of GR. (**A**) Exogenously expressed Gas5 fragments are all similarly expressed in HeLa cells. Total RNA was isolated from aliquots of HeLa cells used in fig. 8A and the abundance of the exogenously expressed Gas5 fragments was measured with SYBR Green real-time PCR using the primer pairs employed in fig. 8A. (**B**) The abundance of GR is similar in the cells transfected to express the Gas5 fragments with and without dexamethasone treatment. Aliquots of HeLa cells used in fig. 8A were lysed and GR was detected by Western blotting.

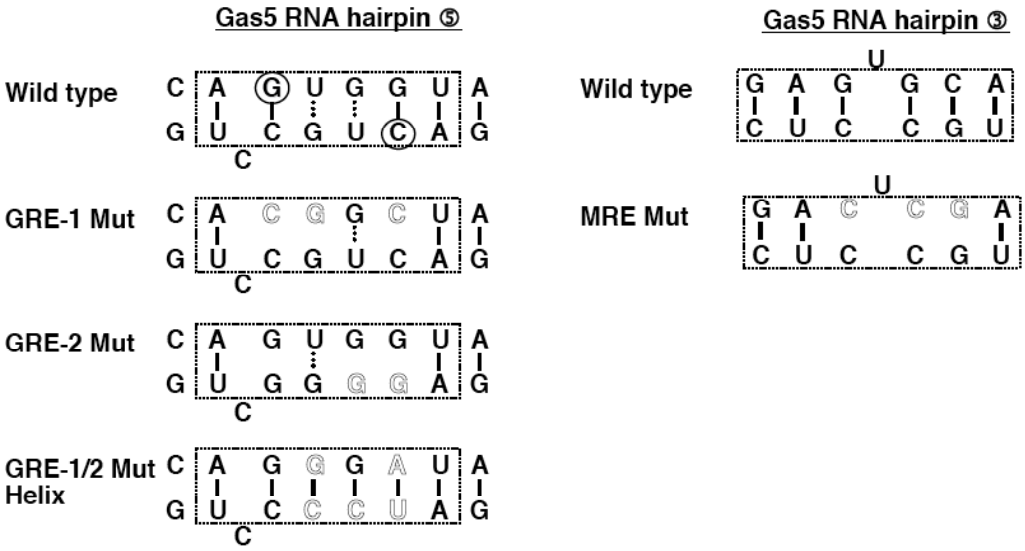

fig. S5. Sequences of wild-type Gas5 and Gas5 mutants used in the analyses. Replaced nucleotides of GRE- or MRE-like sequences in hairpins #5 and #3 are shown with outlined fonts. GRE-1 Mut, GRE-2 Mut, and MRE Mut do not form double helical structures, whereas GRE-1/2 Mut Helix does. Broken-lined rectangles illustrate GRE- and MRE-like Gas5 dsRNAs in hairpin #5 and #3, respectively. Solid lines indicate Watson-Click base-pairing and broken lines designate Wobble base-pairing. Circled G and C indicate the conserved G540 and C554 in the wild type Gas5 hairpin #5.

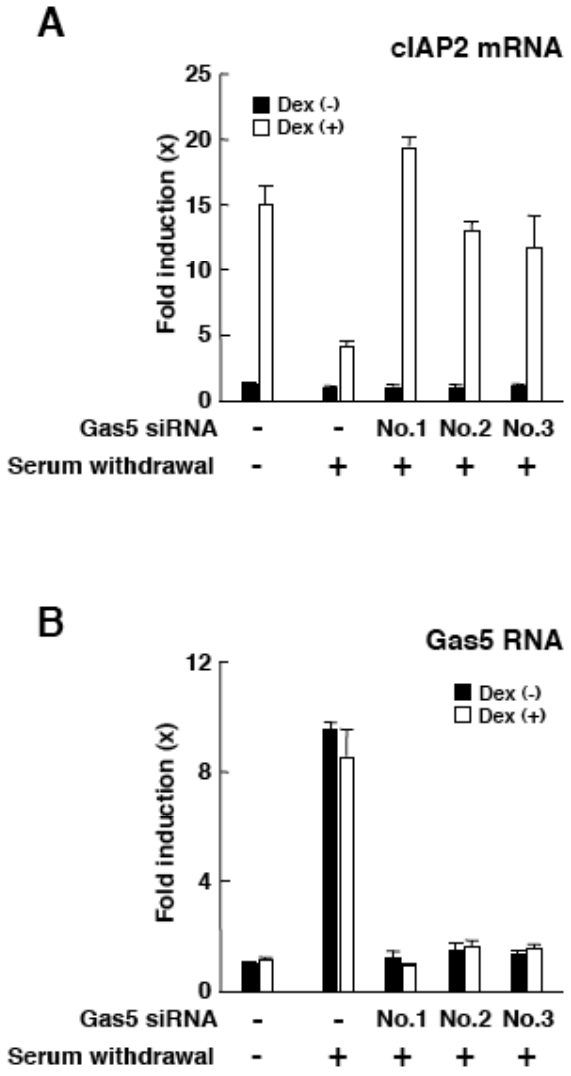

fig. S6. Repression of Gas5 accumulation and activity with siRNA in serum-starved cells. Three Gas5 siRNAs efficiently suppress Gas5 RNA accumulation and reverse Gas5-mediated suppression of GR-stimulated *cIAP2* mRNA expression in HeLa cells cultured in serum-free medium. HeLa cells were transfected with three Gas5 siRNAs indicated and cultured in serum-free medium for 48 hours. Cells were further incubated with 10^{-6} M of dexamethasone or vehicle for 24 hours. (**A**) *cIAP2* mRNA and (**B**) Gas5 RNA were measured with SYBR Green real-time PCR. Bars represent mean ± S.E. values of cIAP2 mRNA and Gas5 RNA in the absence or presence of 10^{-6} M of dexamethasone. Sequences of Gas5 siRNAs used:

No.1 Gas5 siRNA: 5’-CUUGCCUGGACCAGCUUAAdTdT-3’
No.2 Gas5 siRNA: 5’-GGGCAGACCUGUUAUCCUAdTdT-3’
No.3 Gas5 siRNA: 5’-GGAUGACUUGCUUGGGUAAdTdT-3’

No. 1 Gas5 siRNA was used for experiments described in the main text.

Table S1: Copy numbers of Gas5 in HeLa cells cultured in the presence and absence of dexamethasone. HeLa cells were maintained in medium supplemented with or without 10% FBS for 72 hours, and were subsequently cultured for 1 hour in the presence or absence of 10^{-6} M dexamethasone. Cells were harvested and total RNA was purified. Cell numbers were also counted using aliquots of cell suspensions. The abundance of Gas5 RNA was measured with SYBR Green real-time PCR using the primer pair shown in Table 1 (main text), with its standard curve obtained by dilution of synthetic Gas5 RNA. Using the value "210067.2" as the molecular weight of Gas5 (1-651) (Gas5 1-598 + poly A tail: GenBank Accession # NR_002578), copy numbers of Gas5 were calculated. The results indicate that a portion of Gas5 translocated into the nucleus in response to dexamethasone both in nutrition-rich and starved conditions.

Gas5 copy number (molecule/cell)

	HeLa cells cultured with FBS		HeLa cells cultured without FBS	
Dex (10^{-6} M)	-	+	-	+
Cytoplasm	6812.5 ± 911.13	4086.0 ± 1311.29	73102.3 ± 18377.59	66149.0 ± 9965.11
Nucleus	691.1 ± 53.72	2423.0 ± 306.59	2265.3 ± 197.16	4250.0 ± 471.50
Total	7503.1 ± 859.44	6509.0 ± 1615.44	75367.6 ± 18301.40	70399.0 ± 9784.87

Dex: dexamethasone, FBS: fetal bovine serum