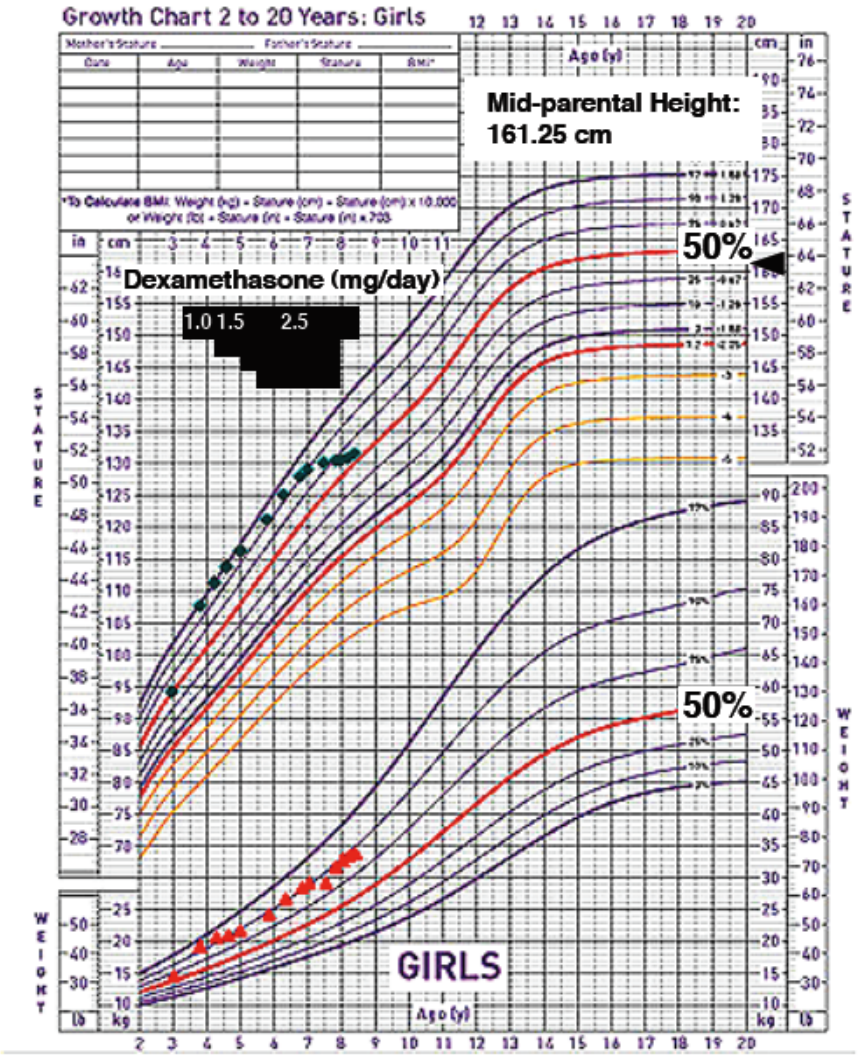
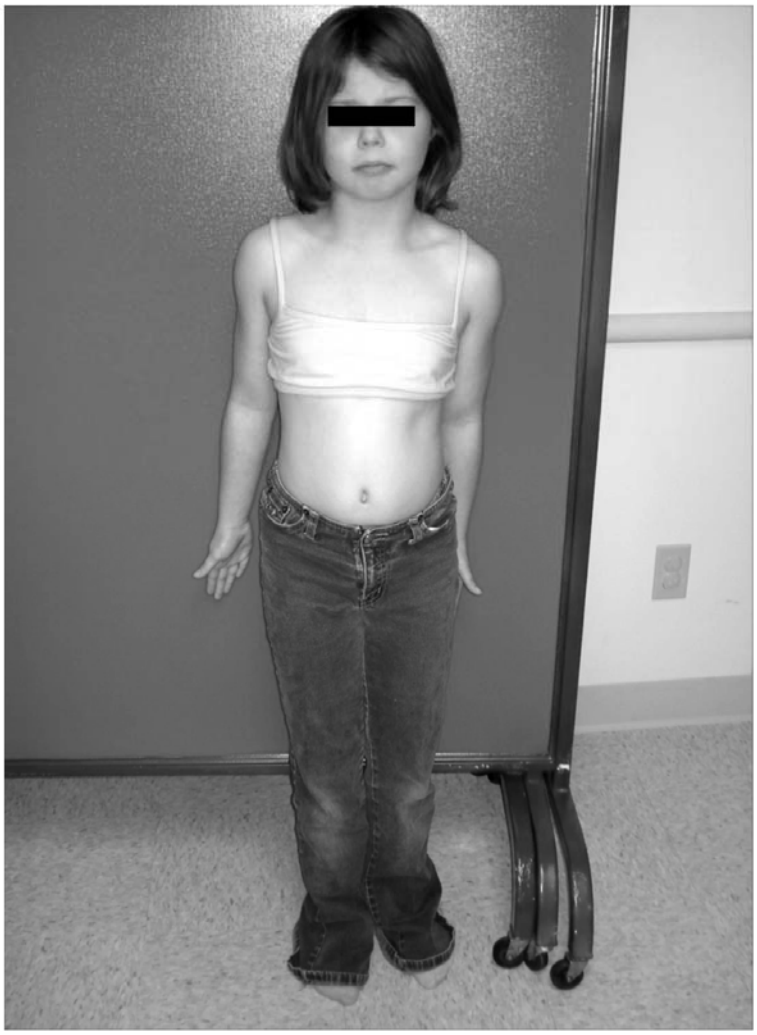
Supplemental Figures

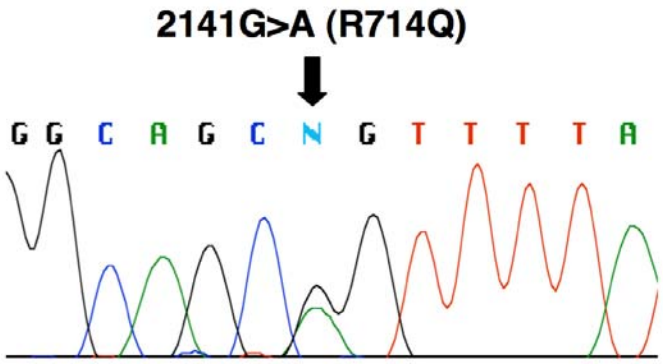
A



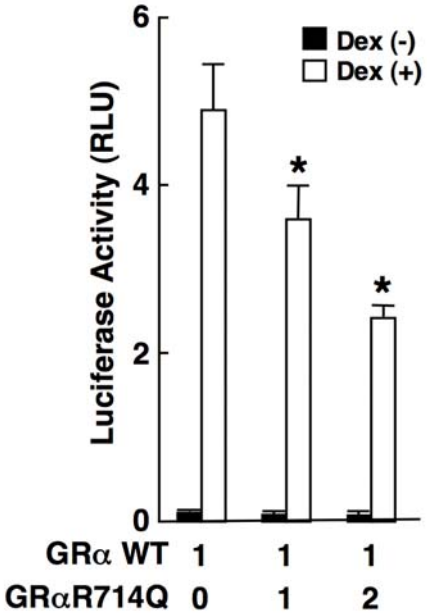
B



Supplemental Figure 1: The patient has been well treated with dexamethasone from age 2 to 8. A: Growth curves of the patient. Growth curves of patient's height and body weight are shown. Period/amounts of the dexamethasone used for the treatment is shown in the top. Red lines indicate 50% percentile of height and body weight. Patient's mid-parental height (161.25 cm) is shown with an arrowhead. B: Photo of the patient at 8 years old.



Supplemental Figure 2: The patient has a heterozygotic point mutation replacing guanine by adenine at the nucleotide 2141 of the glucocorticoid receptor gene. The mutation causes amino acid replacement from arginine to glutamine at amino acid position 714 of the hGRα protein. (Nucleotide number is counted from translation start site)



Supplemental Figure 3: hGRαR714Q has a dominant negative activity on the wild type GRα-induced transcriptional activity of a glucocorticoid-responsive promoter in HCT116 cells. HCT116 cells were transfected with indicated amounts of the wild type hGRα and/or hGRαR714Q-expressing plasmids together with pMMTV-luc and pGL4.73[*hRluc*/SV40], and were treated with 10^{-8} M of dexamethasone. pRSerbA^{-1} was used to keep the same amount of plasmids throughout the experiment. Bars represent mean ± S.E. values of the firefly luciferase activity normalized for the renilla luciferase activity in the absence or presence of 10^{-6} M of dexamethasone. The experiment was repeated 3 times and representative data are shown. hGRαR714Q suppressed dexamethasone-stimulated and wild type hGRα-induced transcriptional activity of the MMTV promoter in a dose-dependent fashion, indicating that hGRαR714Q acts as a dominant negative receptor for the wild type GRα on the latter's transactivation of the glucocorticoid-responsive genes. $p<0.01$, compared to the value obtained in the presence of wild type hGRα-expressing plasmid and dexamethasone.

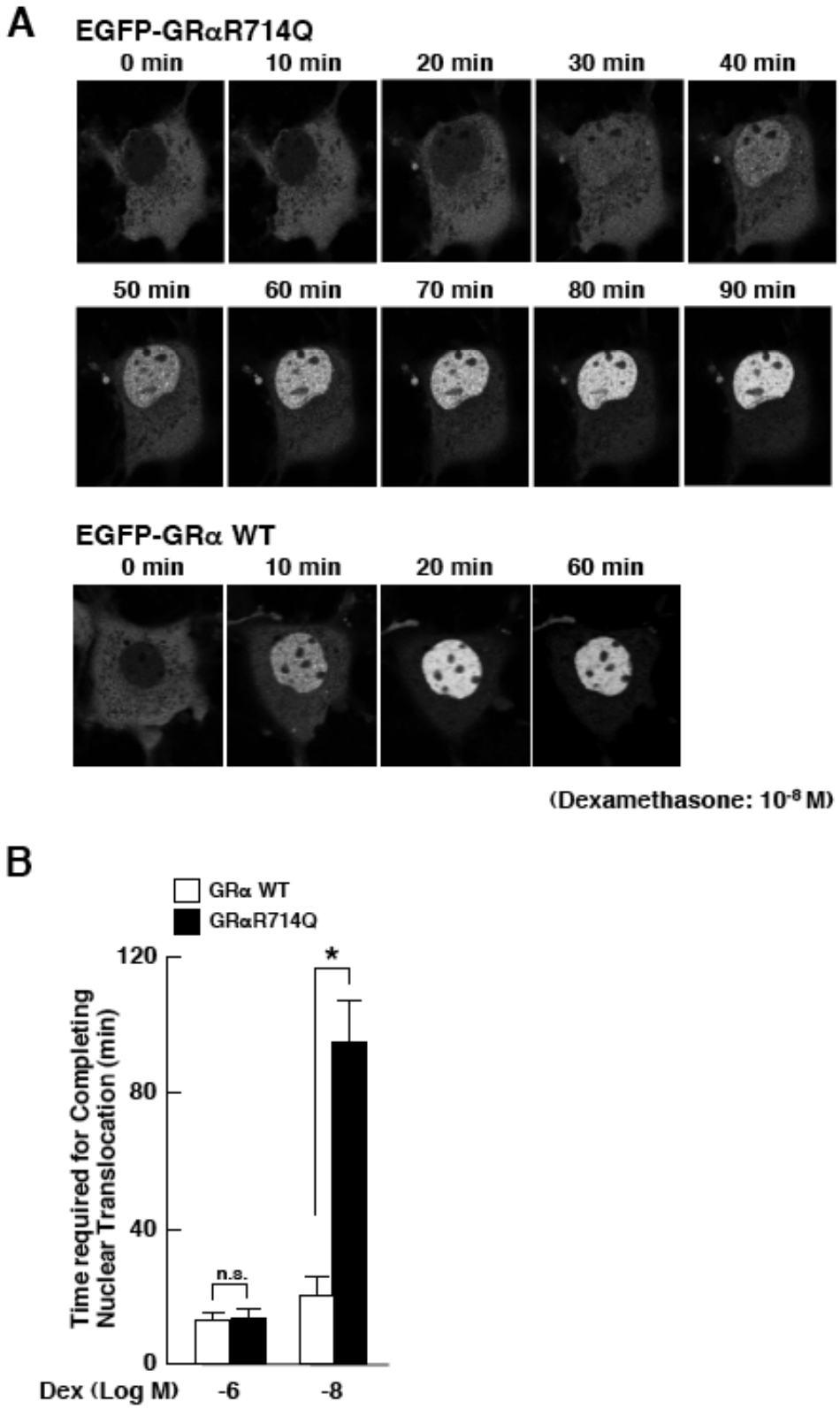
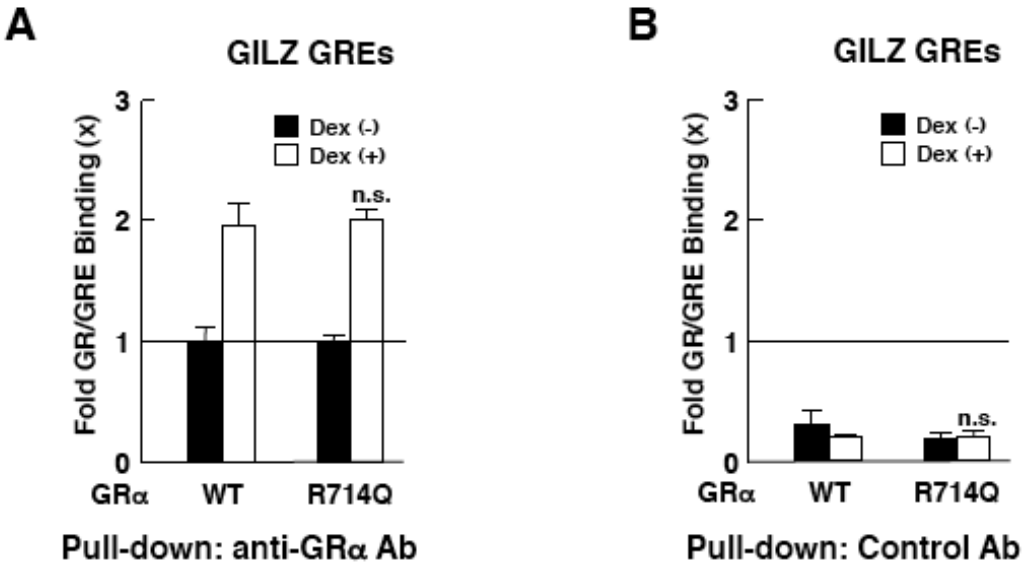
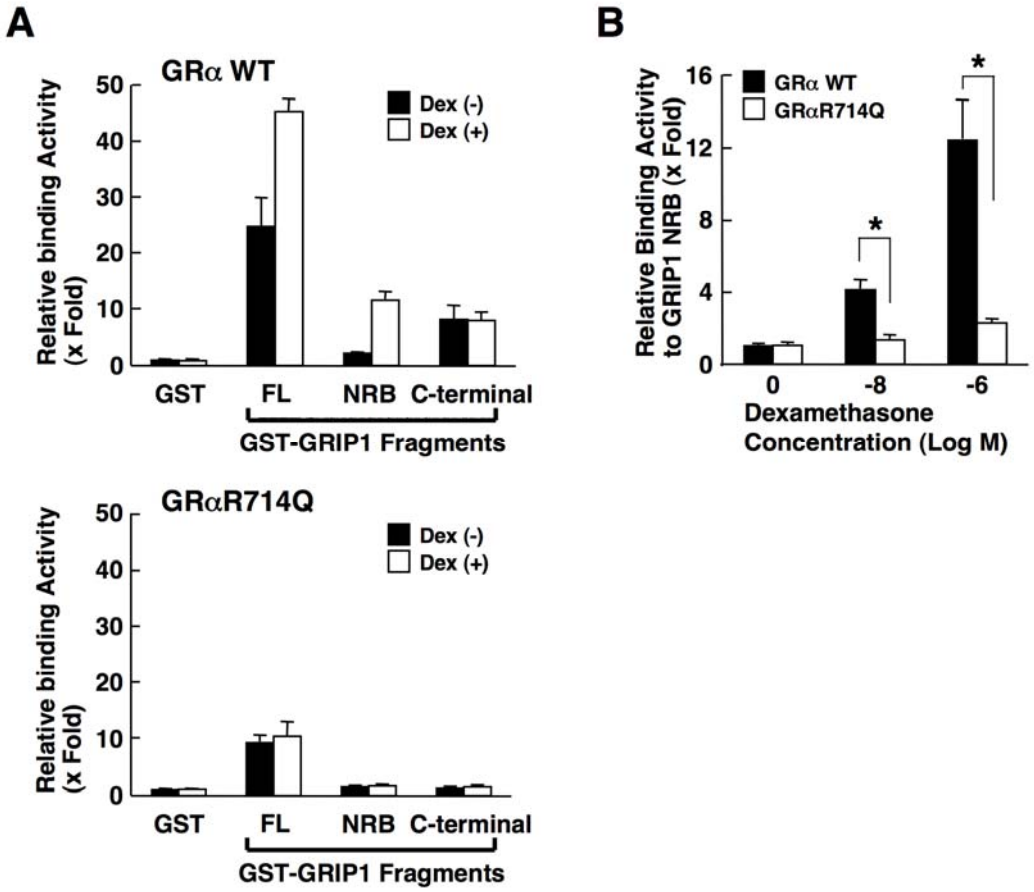


Figure S4: hGRαR714Q shows slower nuclear translocation than the wild type GRα in a lower, but not higher concentration of dexamethasone. HCT116 cells were transfected

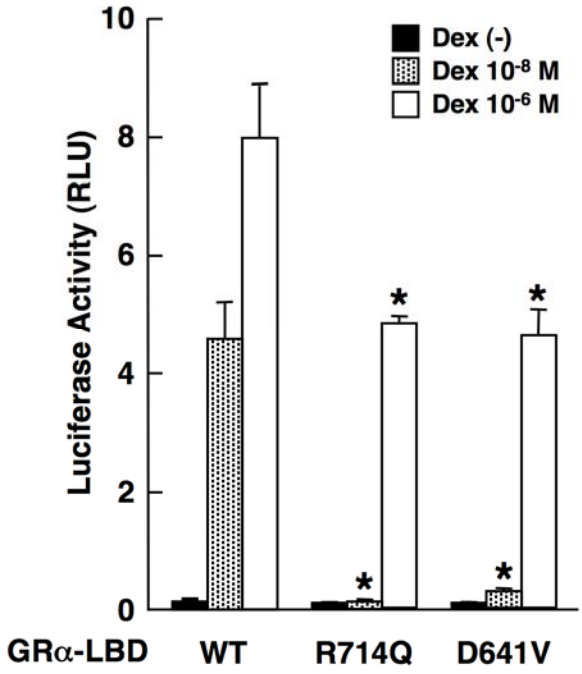
with plasmids expressing EGFP-fused wild type (WT) hGRα or hGRαR714Q and exposed to the concentrations of dexamethasone indicated. Panel A shows representative images of EGFP-hGRαR714Q (top panels) and EGFP- hGRα WT (bottom panels) subcellular localization before and after the incubation with 10^{-8} M of dexamethasone, while panel B indicates mean ± S.E. values of the time required for completing nuclear translocation of EGFP-hGRα WT or -hGRαR714Q in over 20 cells. The results suggest that the molecular machinery for nuclear translocation is intact in hGRαR714Q, while reduced affinity of this mutant receptor to dexamethasone may be responsible for its reduced nuclear translocation at a lower concentration of dexamethasone. n.s.: not significant, *: $p<0.01$, compared to the conditions indicated.



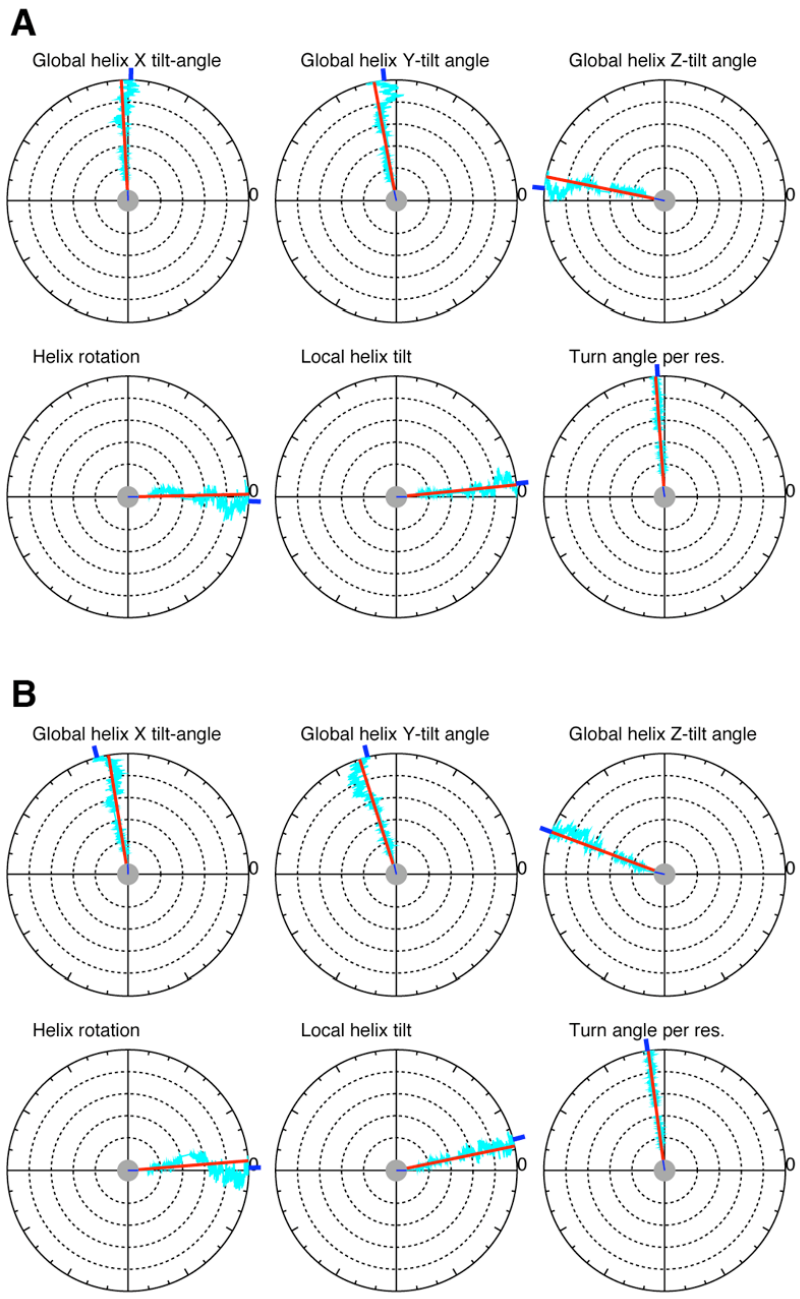
Supplemental Figure 5: hGRαR714Q has the GRE-binding activity similar to that of the wild type GRα in a ChIP assay, indicating that the GRE-binding activity of the mutant receptor is preserved. HCT116 cells were transfected with wild type (WT) hGRα- or hGRαR714Q-expressing plasmid and ChIP assays were performed by using anti-hGRα (A) or control (B) antibody. Bars represent mean ± S.E. values of fold binding of the receptors to GILZ GREs corrected for input in the absence or presence of 10^{-8} M or 10^{-6} M of dexamethasone. n.s.: not significant, compared to the value obtained in the presence of GRα WT and dexamethasone.



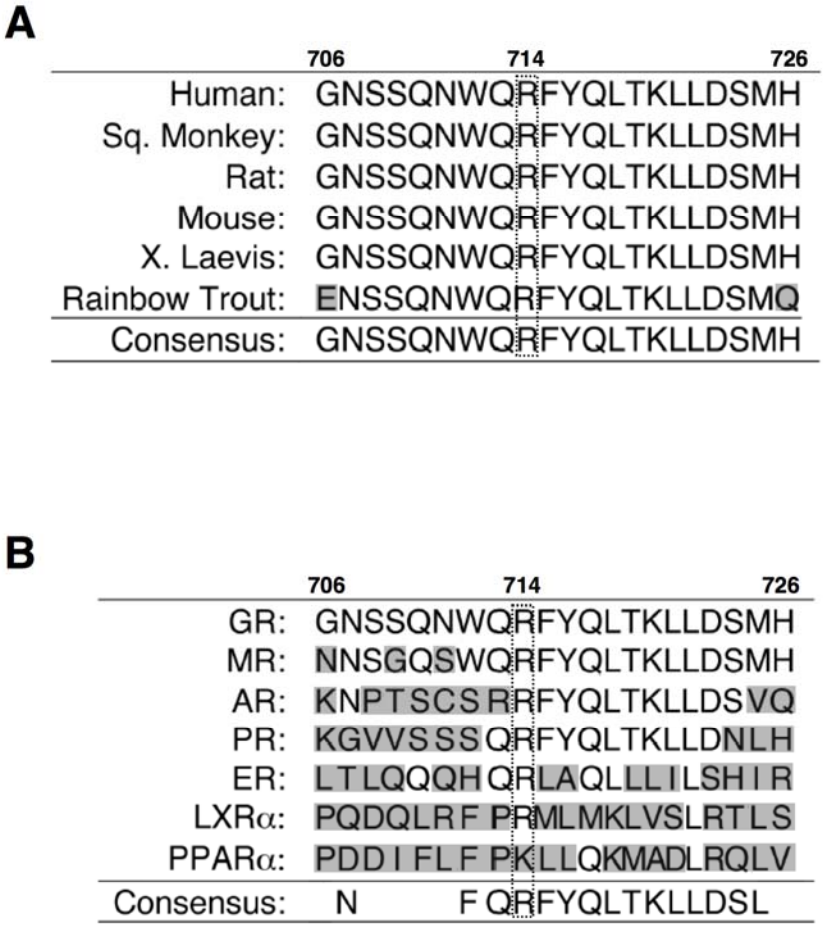
Supplemental Figure 6: hGRαR714Q has reduced binding activity to GRIP1 *in vitro*. **A:** hGRαR714Q has reduced binding activity to various portions of GRIP1 in a GST pull-down assay. Band intensity of ^{35}S-labeled hGRαs shown in Figure 1C, left panel was recorded and relative binding activity was calculated by correcting with intensity signal of input. Fold binding activity was further calculated by dividing relative binding activity of each point with that of baseline (GST in the absence of dexamethasone). Mean ± S.E. values of relative binding activity obtained from 3 independent experiments are shown. $p<0.01$, compared to the condition indicated. **B:** hGRαR714Q has reduced binding activity to the NRB domain of GRIP1 in a GST pull-down assay. Band intensity of ^{35}S-labeled hGRαs shown in Figure 1C, left panel was recorded and relative binding activity was calculated by correcting with intensity signal of input. Fold binding activity was further calculated by dividing relative binding activity of each point with that of baseline (in the absence of dexamethasone of corresponding hGRα protein binding). Mean ± S.E. values of relative binding activity obtained from 3 independent experiments are shown. $p<0.01$, compared to the condition indicated.



Supplemental Figure 7: hGRαR714Q has AF2 with reduced transactivation activity, which is pronounced in a lower concentration of dexamethasone, in HCT116 cells. HCT116 cells were transfected with pM-GRα-LBD, -GRαR714Q-LBD or -GRαD641V-LBD together with pGLA4-E1B-TK-Luc and pGL4.73[*hRluc*/SV40], and were treated with indicated concentrations of dexamethasone. Bars represent mean ± S.E. values of the firefly luciferase activity normalized for the renilla luciferase activity in the absence or presence of 10^{-8} M or 10^{-6} M of dexamethasone. GAL4-DBD-fused hGRαR714Q LBD demonstrated almost no transactivation activity at 10^{-8} M of dexamethasone. In contrast, the fusion protein induced much stronger transactivation activity at 10^{-6} M of dexamethasone, although it did not reached to the level of transactivation exerted by the wild type hGRα LBD. The alteration observed in the transactivation activity of hGRαR714Q LBD was similar to that of the hGRαD641V LBD, a previously reported pathologic GR mutant causing generalized glucocorticoid resistance syndrome (1). These results suggest that AF2 of hGRαR714Q LBD has defective transactivation activity. $p<0.01$, compared to the value obtained in the presence of wild-type GRα-expressing plasmid and the same concentrations of dexamethasone.



Supplemental Figure 8: Position of the helix of the LXXLL coactivator motif shifts more on hGRαR714Q LBD than on hGRα LBD over the course of the simulation. A comparison of the position of the helix of the LXXLL coactivator motif indicates a shift over the course of the simulation from the hGRα LBD (**A**) to the hGRαR714Q LBD (**B**). The light blue trace plots the position of the helix by components of position and other descriptors. The red lines indicate the best linear fit to the data. This shift is significant and suggests a differential binding affinity for the LXXLL motif between the native and the mutant structures.



Supplemental Figure 9: Arginine (R) located at amino acid 714 of the hGRα is preserved in GRs of different species and is shared among human steroid receptors. **A:** Arginine (R) 714 of the hGRα is persevered in the GRs of other species listed. GR amino acid sequences of the squeal monkey, rat, mouse, xenopus laevis and rainbow trout corresponding to the amino acids from 706 to 726 of the human GRα are assembled. The sequence of the hGRα is shown in the top while the consensus sequence of these species is shown in the bottom. Amino acids mismatching to those of the human GRα are shown in grey boxes. **B:** Arginine (R) 714 of the hGRα is shared with other human steroid receptors and LXRα. Amino acid sequences of the human mineralocorticoid receptor (MR), androgen receptor (AR), progesterone receptor A (PR), estrogen receptor α (ER), liver X receptor α (LXR) and peroxisome proliferators-activated receptor α (PPARα) corresponding to the amino acids from 706 to 726 of the hGRα are assembled. The sequence of the hGRα is shown in the top, while the consensus sequence of all receptors is shown in the bottom. Amino acids mismatching to those of the hGRα are shown in grey boxes. Note that PPARα has lysine (K), which is a basic amino acid similarly to arginine (R), at the position of the preserved arginine residue.