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1 **Quaternary GR structure highlights allosteric interdomain communication**

2

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17

18 **Abstract**

19 The glucocorticoid receptor (GR) is a ligand activated transcription factor that binds DNA and
20 assembles context dependent coregulator complexes to regulate gene transcription. GR
21 agonists are widely prescribed to patients with inflammatory and autoimmune diseases. Here,
22 we determine the first high resolution, multi-domain structures of GR in complex with ligand,
23 DNA and a coregulator peptide. The structures reveal how the receptor forms an asymmetric
24 dimer on the DNA and provide a detailed view of the domain interactions within and across
25 the two monomers. Hydrogen deuterium exchange and DNA binding experiments

26 demonstrate that ligand dependent structural changes are communicated across the different
27 domains in the full-length receptor. Based on the structural analysis, we design mutant
28 receptor constructs and functional assays to validate the signaling pathways identified in the
29 structures. This study demonstrates how GR forms a distinct architecture on DNA and how
30 signal transmission can be modulated by the ligand pharmacophore. The results provide a
31 platform where we can build a new level of understanding for how receptor modifications can
32 drive disease progression and offer key insight for future drug design.

33

34 **Introduction**

35 The glucocorticoid receptor (GR) is a ubiquitously expressed nuclear hormone receptor.
36 Together with the androgen, progesterone and mineralocorticoid receptors, GR forms the
37 keto-steroid receptor subfamily which evolved from an estrogen receptor (ER) like ancestor in
38 vertebrates¹. GR is activated through endocrine signaling by the glucocorticoid hormone
39 cortisol, and is of fundamental importance for development, skeletal growth, behavior,
40 glucose homeostasis and inflammation.

41

42 GR comprises a large, disordered N-terminal domain, a DNA-binding domain (DBD) and a
43 ligand-binding domain (LBD). In the apo state, GR primarily resides in a chaperone complex
44 in the cytoplasm. Ligand activation triggers nuclear translocation and receptor binding to
45 specific DNA sequences. On the DNA, GR recruits co-regulators and other transcription
46 factors in a context dependent manner to ultimately repress or activate transcription of a large
47 set of target genes². As such, GR provides a plastic scaffold which integrates signaling input
48 from the ligand pharmacophore, the sequence of the DNA and post-translational

49 modifications and the outcome of the signaling event is governed by the identity of the
50 assembled co-regulators.

51

52 Signaling efficiency is dependent on comprehensive allosteric networks that enable
53 communication in between distinct functional sites across the receptor domains. GR typically
54 binds as a dimer to the canonical GR binding sequence (GBS), which is an imperfect
55 palindromic hexameric DNA sequence repeat, separated by a 3-nucleotide spacer³. However,
56 GR can also bind as a monomer to a canonical half-site⁴ or as discrete monomers on opposite
57 sides of the DNA to an inverted repeat sequence⁵. This adds a layer of combinatorial
58 flexibility and contributes to signaling diversity, but also suggests that the protein interfaces
59 must be able to adapt to different boundary conditions.

60

61 To date, structural studies have focused on the individual domains within the receptor. Studies
62 of the DBD in complex with DNA have demonstrated how the nucleotide sequence drives
63 structural rearrangement within the DBD dimer⁶. The structures of the LBD in complex with a
64 wide array of ligands have highlighted the plasticity of the ligand binding pocket⁷. However, a
65 full understanding of the allosteric transmission in between the ligand and DNA binding
66 events and effects on co-regulator recruitment requires structural information from a multi-
67 domain construct.

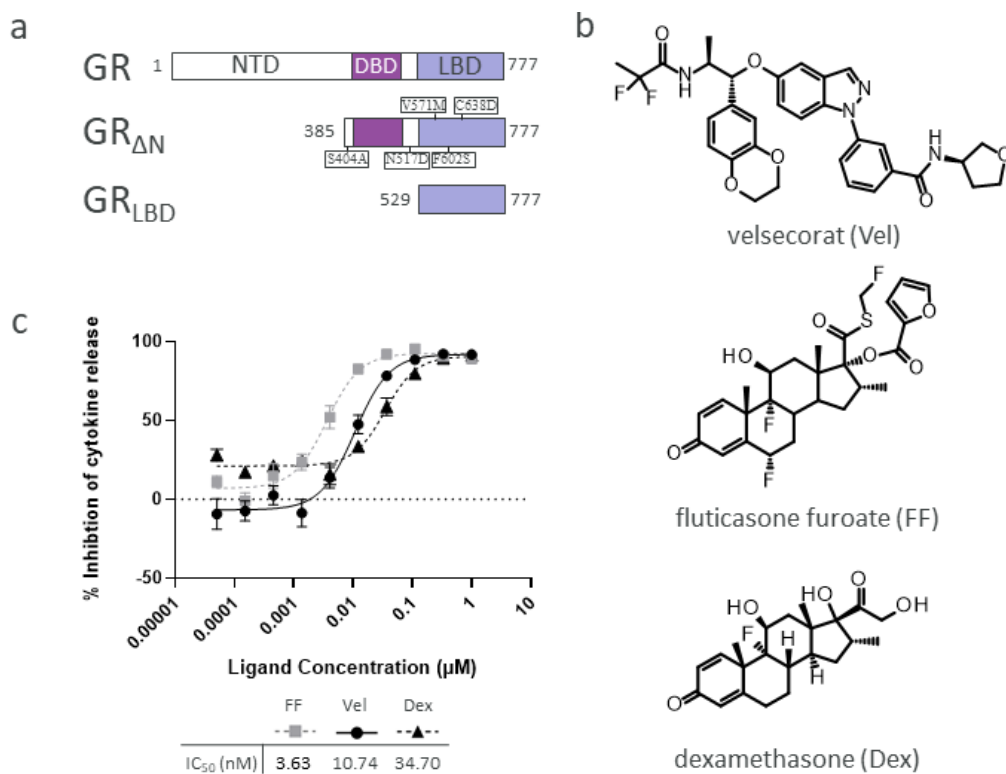
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69 Here we present the crystal structures of a GR construct (residues 385-777, encompassing the
70 DBD and LDB) in complex with the agonists velsecorat⁸ and fluticasone furoate⁹, a natural
71 GBS and a co-regulator peptide. These are the first high-resolution multidomain structures of
72 a steroid receptor and reveal how the receptor forms a unique architecture on the DNA.

73

74 Results

75 To determine the multidomain structure of GR, we expressed and purified several GR
76 constructs (Fig. 1a) in the presence of the strong agonists velsecorat (Vel), fluticasone furoate
77 (FF), or dexamethasone (Dex) (Fig. 1b). All three ligands exhibit strong anti-inflammatory
78 responses, inhibiting lipopolysaccharide (LPS) induced tumor necrosis factor α (TNF α)
79 secretion in human whole blood with an IC₅₀ of 10.74 nM, 3.63 nM and 34.70 nM,
80 respectively (Fig. 1c), and have demonstrated clinical efficacy in a range of disease
81 indications¹⁰⁻¹².



82

83 **Figure 1 | GR protein constructs and agonists used in this study. a,** GR protein constructs

84 used in this study. The GR_{ΔN} construct includes five stabilizing mutants highlighted in the

85 schematic. **b,** The chemical structure of the GR agonists velsecorat, fluticasone furoate and

86 dexamethasone. **c,** Velsecorat, fluticasone furoate and dexamethasone inhibit LPS induced

87 TNF α production in human whole blood. Data points are mean \pm s.e.m. of six replicates from
88 three donors.

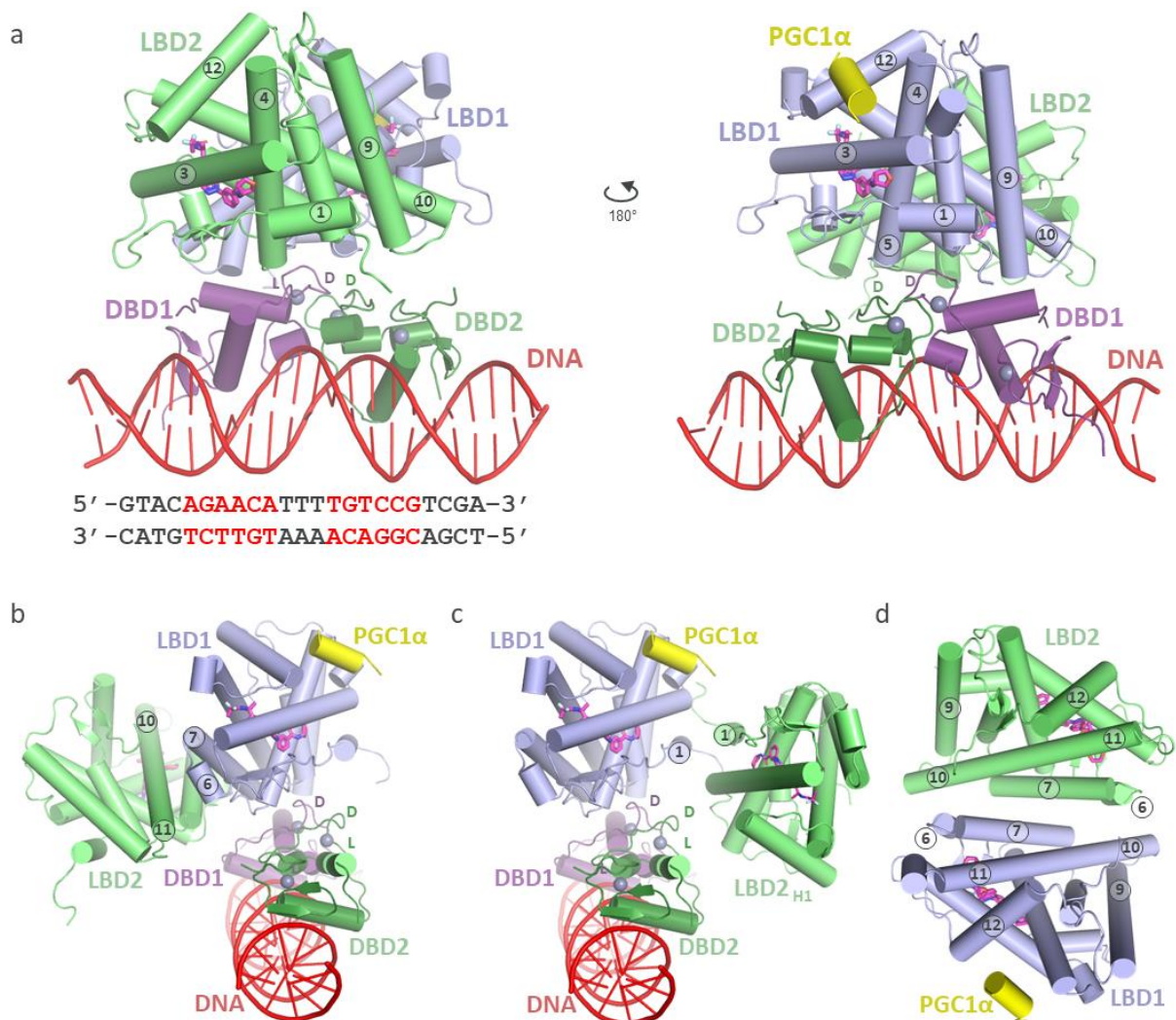
89

90 The purified receptors were then mixed with dsDNA and coregulator peptide to obtain
91 quaternary complexes (Extended Data Fig. 1). The GR $_{\Delta N}$ contains 5 stabilizing mutants (Fig.
92 1a) and yielded diffracting crystals with both velsecorat and fluticasone furoate. The highest
93 resolution structure (2.5 Å, Extended Data Table 1) was obtained from the GR $_{\Delta N}$ construct in
94 complex with velsecorat [GR $_{\Delta N}$ (Vel)], a GBS from the serum and glucocorticoid-regulated
95 kinase-1 (*SGK-1*) promoter, and a peptide (residues 134-154) derived from the co-regulator
96 peroxisome proliferator-activated receptor γ coactivator 1- α (PGC1 α).

97

98 The structure reveals that the LBD dimer is asymmetrically placed on top of the center of the
99 DBD dimer resulting in a shifted tetrahedral architecture on the DNA (Fig. 2). In the crystal
100 lattice, there are two alternative LBD dimer interfaces; one consisting of two separate regions
101 spanning the N-terminal end of H10/11 and H6-H7 (Fig. 2a, b, d), and one primarily mediated
102 by H1 (Fig. 2c). We could not detect any electron density for the linker residues (residues
103 489-525) in between the LBD and DBD in any of our structures, which is likely due to the
104 flexible nature of this region¹³. However, the H10/11 interface partially overlaps with the
105 canonical ER dimer interface¹⁴ and exhibits a larger total buried surface area of 904.4 Å²
106 (compared to 710.2 for the H1 dimer interface). We propose that this is the primary LBD
107 dimer for GR activation on the SGK-1 GBS. The LBD dimer is tilted relative to the DBD
108 dimer (Fig. 2a, b), which positions the LBD N-termini in proximity of the DBD C-termini.
109 Based on distances, number of missing residues and space constraints between the C-terminus
110 of the DBD domains and the N-terminus of LBD domains, we suggest that DBD1 is

111 connected to LBD1 and DBD2 to LBD2, but the alternative connectivity cannot be fully
 112 excluded.



113

114 **Figure 2 | Structure of GR_{ΔN}(Vel) in complex with SGK and PGC1_{α134-154}.**

115 **a, b**, High resolution crystal structure of GR_{ΔN}(Vel) in complex with dsDNA SGK (red) and
 116 the coactivator peptide PGC1_{α134-154} (yellow). Velsecorat is shown as a stick model (magenta)
 117 and Zn atoms as spheres (gray). GR LBD helix numbering is annotated within the circles. The
 118 DBD dimerization loops and lever arms are marked D and L, respectively. The nucleotide
 119 sequence of the SGK GBS is highlighted with the two half-sites in red. **c**, The alternative
 120 LBD dimer (LBD1: LBD2_{H1}) in the crystallographic lattice in which the LBD-LBD interface
 121 is mediated by H1 interactions. **d**, Top view of the head-to-tail LBD dimer interface as shown
 122 in **a** and **b**.

123

124 The asymmetric placement of the LBD dimer on the DBD dimerization loops (D-loops)
125 results in LBD1 interacting with the D-loops of both DBD1 and DBD2 with a combined
126 buried surface area of 731 Å², while LBD2 only contacts the lever arm of DBD1 with a buried
127 surface area of 145.2 Å² (Fig. 2a, b). This arrangement of the LBD dimer on the DBD dimer
128 agrees with NMR studies of DBD complexes with multiple GBSs which, combined with
129 functional cell assay data, suggested that both the D-loop and the lever arm could be involved
130 in inter-domain communication^{3,6}. In addition, NMR studies of WT GR LBD revealed an
131 allosteric pathway from the ligand binding pocket to the N-terminal end of helix 1¹⁵. In our
132 structure the LBD1 H1 is in direct contact with the DBD D-loops, and likely forms a
133 communication network that links the DNA sequence to the ligand pharmacophore.

134

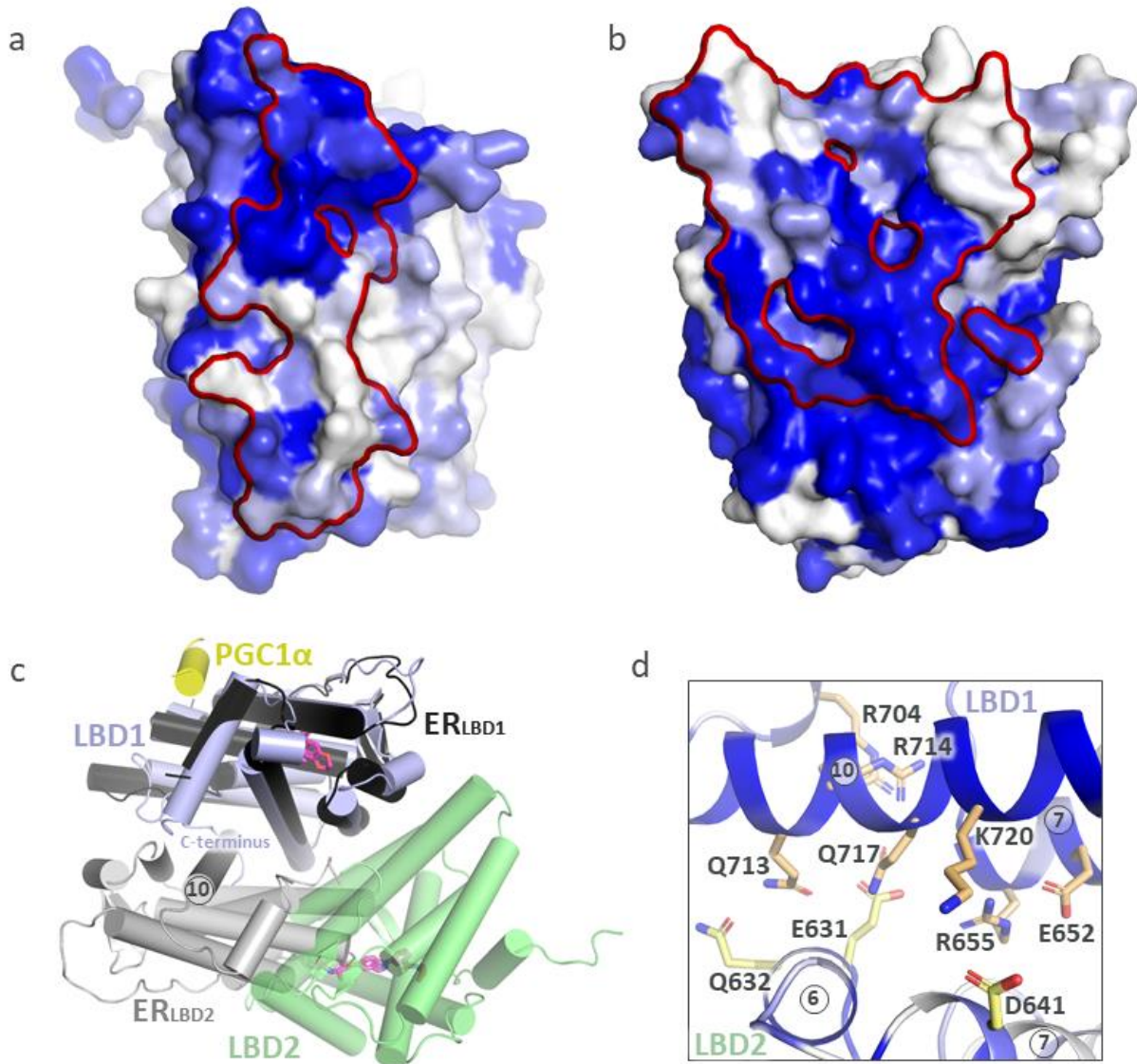
135 In the keto-steroid receptors, a conserved C-terminal extension (residues 767-777) prevents
136 formation of the stable head-to-head LBD dimer observed for ER¹⁶. In contrast, the
137 GR_{ΔN}(Vel) LBDs form a unique head-to-tail dimer (Fig. 2c), which has not been observed in
138 any GR LBD structure to date¹⁷. The LBD construct alone remains predominantly monomeric
139 in solution¹⁸ and formation of a relevant LBD dimer likely requires DNA binding (Extended
140 Data Fig. 1).

141

142 To study the degree of sequence conservation of the LBD interface residues, we collected 34
143 GR and 34 reference ER sequences from vertebrates with a similar degree of sequence
144 diversity (see Materials and Methods and Extended Data Fig. 3 and 4). We then mapped the
145 mean pairwise column identity of each residue onto the corresponding LBD structure surface
146 (ER PDB: 3ERD) with the dimerization interface outlined in red (Fig. 3a, b). The ER LBD
147 forms a stable dimer in solution with a total buried surface area of 1581.5 Å². The ER dimer

148 interface and the conservation pattern are strikingly different from the ones observed in GR.
149 However, when superimposing the ER LBD dimer onto the GR LBD dimer (Fig. 3c), the GR
150 dimer interface has merely shifted to an overlapping position due to the GR LBD1 C-terminus
151 shielding the original ER interface. In addition, both interfaces exhibit a similar degree of
152 total conservation, with an average mean pairwise column identity of 0.70 and 0.72 for GR
153 and ER, respectively. A detailed investigation of the GR LBD dimerization interface reveals a
154 cluster of conserved polar residues forming a network of interactions across the two domains
155 (Fig. 3d). Analysis of other key interfaces suggests that the LBD regions facing the DBD
156 dimer (Extended Data Fig. 5a) and the coregulator binding site (AF-2, Extended Data Fig. 5b)
157 are highly conserved.

158



159

160 **Figure 3 a**, Degree of conservation of GR and **b**, ER. LBD residues colored according to
 161 column identity between 0.4 (white) and 1.0 (blue) with LBD dimerization interfaces outlined
 162 in red (using a 5Å distance cutoff). **c**, The ER LBD dimer (in black and gray) superposed on
 163 the GR LBD dimer presented in this study (in blue and green). The structures have been
 164 overlaid using GR LBD1 as reference. **d**, Highly conserved polar residues in the GR LBD
 165 dimer interface.

166

167 The dimer we observe is distinct from the ones reported in published structures of the GR
 168 LBD alone. The dimer interface proposed in the first GR LBD publication includes the loop

169 in between H1 and H3, the antiparallel β -sheet region and the C-terminal end of H5¹⁹. In our
170 structure, this area is directed towards the DBD-DNA for both LBD1 and LBD2, preventing
171 formation of this interface in the crystal lattice. A recent evaluation of all GR LBD structures
172 to date¹⁷ highlighted that H9 forms significant crystal contacts either in a parallel or anti-
173 parallel fashion in many of the LBD structures. In the quaternary structure we present here,
174 this interaction is obstructed by dsDNA from crystallographic neighbors in the lattice. The
175 most prevalent potential dimer interaction in the GR LBD structures, however, is the option
176 mediated by H1¹⁷. This interface is also present in our lattice (Fig. 2c). It has been proposed
177 that GR may form transient higher-order oligomeric states^{20,21}. It is plausible that the H1
178 interaction (or even H9 interactions) may mediate formation of tetramers or even higher-order
179 states (Extended Data Fig. 6), bringing distal GBS together in a folded assembly.

180

181 The best characterized co-regulator interaction surface is the activation function 2 (AF-2), at
182 the intersect of LBD helices 3, 4 and 12 (H3, H4 and H12), where the canonical co-activator
183 LxxLL peptide sequence motif binds upon agonist activation. The LBD arrangement
184 presented here, aligns both AF-2 surfaces on the outside of the tetrahedron, accessible for co-
185 regulator binding. However, while both LBDs have H12 arranged in an active position, only
186 the LBD1 AF-2 site is occupied by the PGC1 α peptide (Fig. 2a, b). Crystal packing analysis
187 suggests that the LBD2 AF-2 is in close proximity to DBD1 of a crystallographic neighbor
188 which likely precludes peptide binding.

189

190 The DBDs form a head-to-head dimer arrangement in the multidomain structure, which
191 recapitulates the structure of the isolated GR DBDs on the same GBS (PDB: 3G9O) with a C α
192 root mean square deviation (r.m.s.d.) of 0.876 (138 atoms) (Extended Data Fig. 2a). To
193 validate the LBD structures in the complex, we determined the structure of the isolated,

194 monomeric wild type (WT) GR LBD in complex with velsecorat [GR_{LBD}(Vel)] and
195 PGC1 α ₁₃₄₋₁₅₄ (Extended Data Table 1). The GR _{Δ N}(Vel) LBD1 and LBD2 both exhibit the
196 typical 3 layered α -helical sandwich fold and overlay on the GR_{LBD}(Vel) structure with a C α
197 r.m.s.d. of 0.480 and 0.549 for LBD1 (248 atoms) and LBD2 (248 atoms), respectively
198 (Extended Data Fig. 2b, c), confirming that the LBD V571M, F602S and C628D mutants of
199 the GR _{Δ N} construct have minimal impact on the GR LBD conformation. The additional
200 S404A and N517D mutants of the GR _{Δ N} construct are located in unstructured regions adjacent
201 to the DBDs and are unlikely to affect the structure.

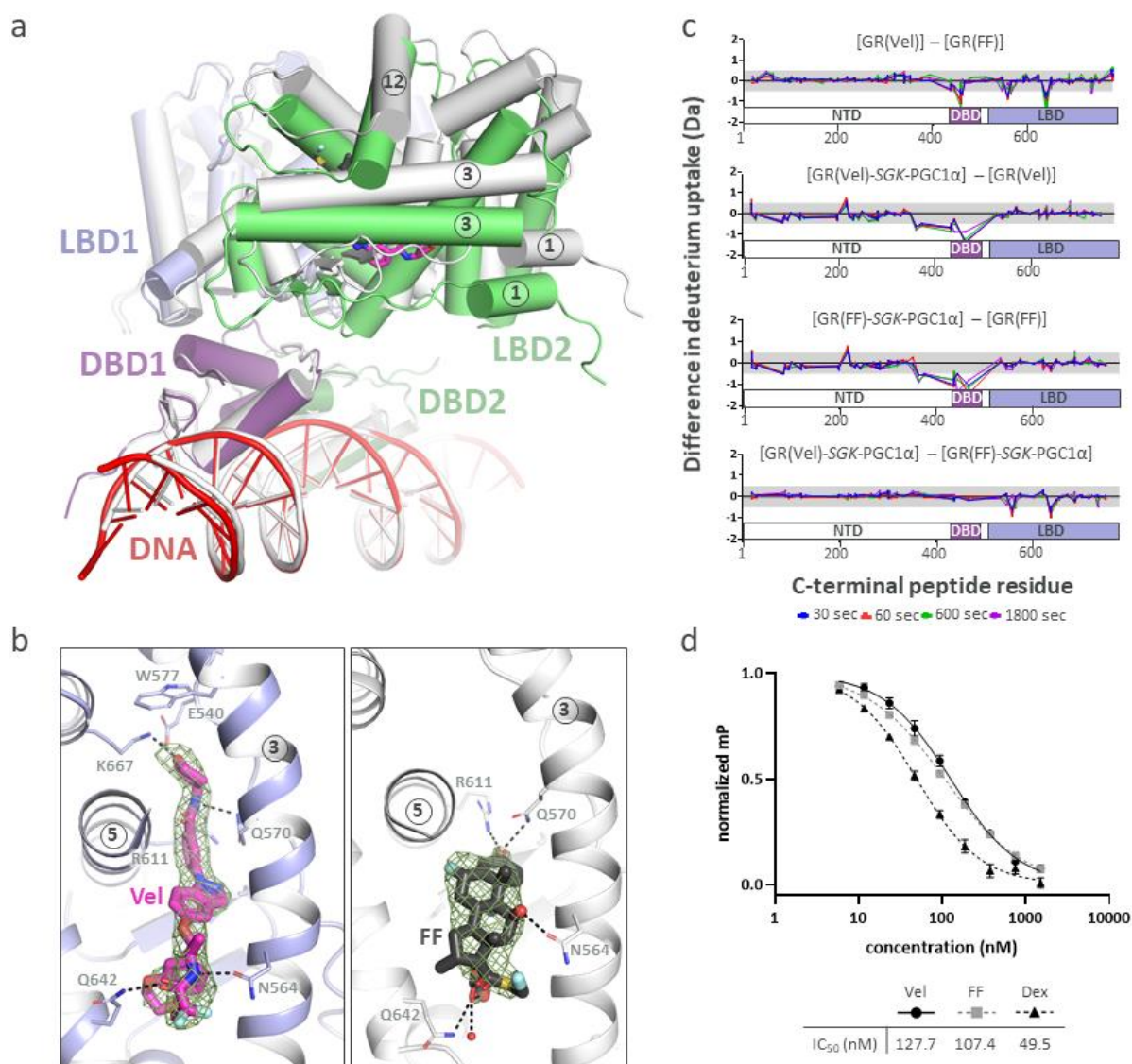
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203 In contrast to the electron microscopy structures of multi-domain nuclear hormone
204 receptors^{22,23}, the X-ray structures²⁴⁻²⁷ generally display a more compact arrangement with
205 significant contacts in between the LBDs and DBDs. However, the domain arrangement
206 among the X-ray structures varies dramatically and the structural overlay reveals that the GR
207 organization is unique (Extended Data Fig. 7). The previous X-ray structures all bind to direct
208 repeat DNA response elements with the DBDs arranged in a head-to-tail fashion in the
209 quaternary complex. As the nucleotide linker length in between the binding sequences vary,
210 the DBDs are placed on different sides along the DNA duplex. In addition, as for the other
211 keto-steroid receptors, the isolated GR LBD is monomeric in solution, while the receptors
212 with known multi-domain structures form stable head-to-head LBD-LBD dimers that are
213 conserved in the multi-domain structures. Together, this presents distinct boundary conditions
214 for positioning the LBDs in the quaternary complex. However, despite the differences in the
215 domain arrangement, it is interesting to note that as observed for the previous structures²⁴⁻²⁷,
216 the LBD1 H9-H10 loop is part of the interface with the DBD on the 5' DNA binding
217 sequence (DBD1).

218

219 The receptor transcriptional regulation is highly ligand dependent^{28,29}. To investigate potential
 220 ligand impact on the domain arrangement, we determined the structure of GR_{ΔN} in complex
 221 with the steroid fluticasone furoate, the GBS from *SGK-1* and the PGC1 α coregulator peptide
 222 [(GR_{ΔN}(FF)-*SGK*-PGC1 α), Extended Data Table S1]. GR_{ΔN}(FF)-*SGK*-PGC1 α crystallizes in
 223 the same space group as GR_{ΔN}(Vel)-*SGK*-PGC1 α , and the structures of the individual
 224 domains are very similar with a C α r.m.s.d. of 1.004 and 0.852 for LBD1 and LBD2 (248
 225 atoms), respectively, and 0.340 for the DBDs (142 atoms, Fig. 4a).

226



227

228 **Figure 4 | Ligand specific structural rearrangements.**

229 **a**, Overlay of the structures of GR_{ΔN}-*SGK*-PGC1 α complexes in the presence of velsecorat (in
230 color) and FF (in white). The structures have been overlaid using the 5' GBS binding motif as
231 reference. GR LBD helix numbering is annotated within the circles. **b**, Ligand binding site
232 with velsecorat (magenta) and FF (dark gray). $|Fo|-|Fc|$ densities (in green) were calculated
233 before models were refined with ligands and are contoured at 3σ . **c**, HDX difference plots
234 showing deuterium uptake difference [complex A] – [complex B] for all peptides captured
235 from the full-length receptor. Negative values indicate that peptides are protected in A relative
236 to B and vice versa. **d**, Competition binding experiments monitoring exchange of 6-FAM-
237 *SGK* dsDNA in complex with GR(ligand) with unlabeled *SGK* dsDNA using fluorescence
238 polarization. Data points are mean \pm s.e.m. of three replicates from one experiment.

239

240 Velsecorat and fluticasone furoate both bind in the same central ligand binding pocket
241 forming an interaction with N564, which is important for stabilizing H12 in the active
242 position (Fig. 4b)³⁰. As for GR_{ΔN}(Vel), H12 is positioned in the active conformation in both
243 GR_{ΔN}(FF) LBDs, but the PGC1 α peptide is only observed at the AF-2 site for LBD1.
244 Opposite N564, both compounds form an interaction with Q642, but the position of the side-
245 chain is different due to the less bulky, non-steroidal scaffold, of velsecorat. The most notable
246 distinction, however, is beyond the intersect in between H3 and H5. While the 3-keto moiety
247 of the steroid interacts with Q572 and R611, velsecorat rearranges these residues and extends
248 into a novel pocket beneath W577, at the intersect in between H1, H3 and H5.

249

250 While the domain arrangement is conserved in the quaternary complexes, the position of
251 LBD2 is slightly shifted in the GR_{ΔN}(FF)-*SGK*-PGC1 α structure (Fig. 4a). This is likely
252 because fluticasone furoate has a bulkier substituent at the 17 α position and pushes on the H6-
253 H7 region in LBD1 (Extended Data Fig. 8). This region is part of the LBD-LBD dimer

254 interface and consequently shifts the position of the H10/11 in the other monomer. The H6-
255 H7 region is known to be flexible and may be part of the ligand entry mechanism³¹. While
256 fluticasone furoate also makes an analogous push on the H6-H7 region in LBD2, LBD1 is less
257 likely to move since the H10/11 region interacts with DBD1. As a consequence, the
258 GR_{ΔN}(FF)-*SGK*-PGC1 α LBD2 position is shifted, and the total buried surface areas are
259 reduced to 704.5 Å² at the LBD dimer interface and to 16.6 Å² in between LBD2 and DBD1.

260

261 To investigate how these structural changes translate to receptor modulation in solution, we
262 expressed the full-length receptor with velsecorat, fluticasone furoate or dexamethasone and
263 performed hydrogen/deuterium exchange mass spectrometry (HDX-MS). The deuterium
264 uptake over time correlates with solvent accessibility and protein dynamics³². Comparing
265 GR(Vel) to GR(FF) (Fig. 4c, top panel [GR(Vel)]-[GR(FF)], and Extended Data Fig. 9), the
266 data confirms that velsecorat protects the protein near helix 1 where the ligand extends into
267 the novel pocket (residues 536-565) and the region near H6 and H7 where we observed the
268 rearrangements in the GR_{ΔN}(FF) structure (residues 621-647). Interestingly, the data revealed
269 that velsecorat and fluticasone furoate also impact the deuterium uptake level in a region of
270 the DBD differently, suggesting ligand specific communication across the two domains, even
271 in absence of DNA.

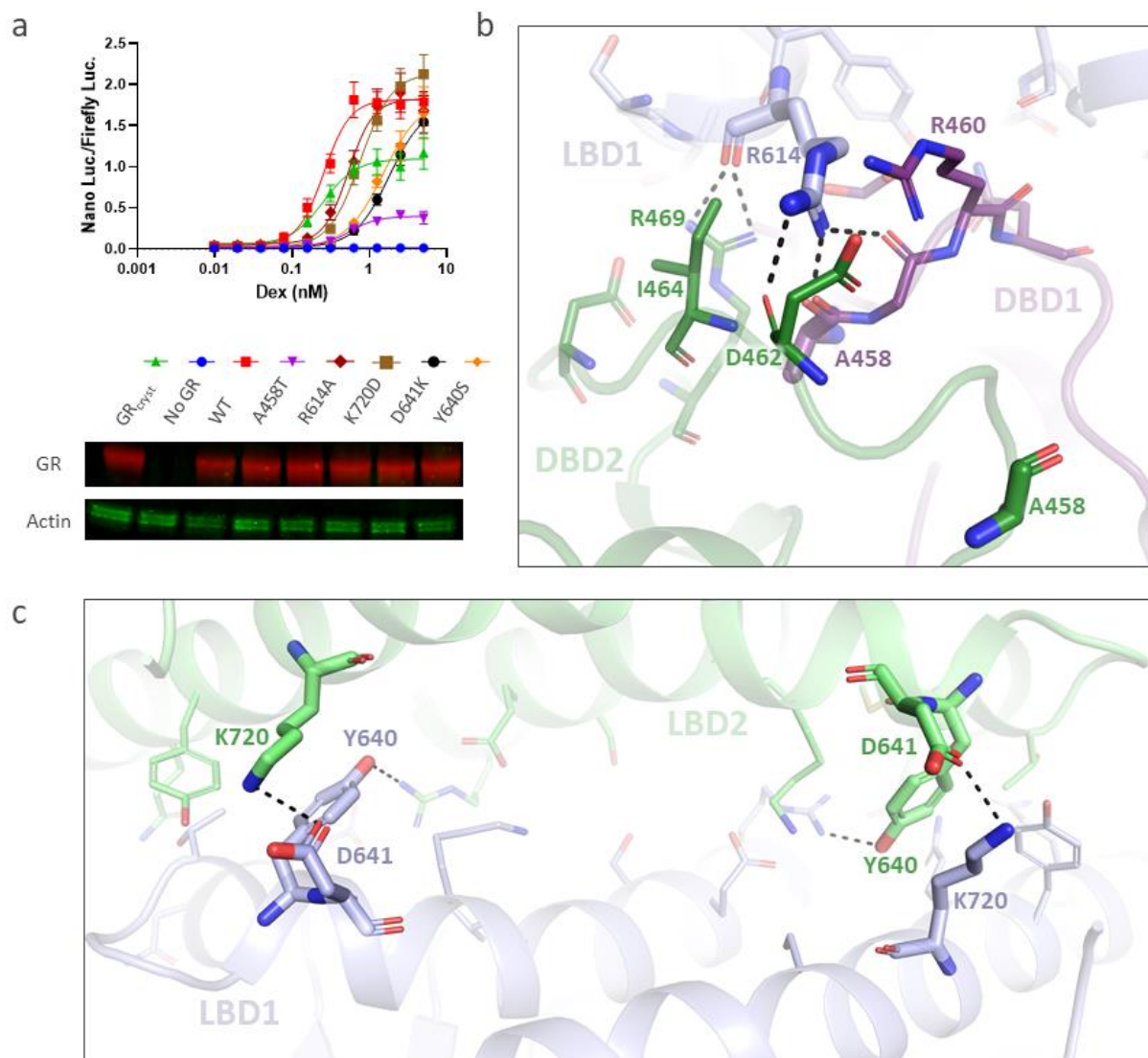
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273 When analyzing the complex of GR with *SGK* GBS and the PGC1 α peptide with HDX-MS,
274 we obtained a lower peptide coverage than for the GR protein alone (Extended Data Fig. 10).
275 A large section of the DBD (residues 424-467), including the N-terminal α -helix 1, the
276 dimerization loop (D-loop) and the lever arm, is protected from HDX in the GR(Vel)-*SGK*-
277 PGC1 α and GR(FF)-*SGK*-PGC1 α complexes compared to the isolated GR(Vel) and GR(FF)
278 samples, confirming DNA binding (Fig. 4c, second and third panel from top). However, when

279 comparing the HDX-MS data from the GR(Vel)-*SGK*-PGC1 α and the GR(FF)-*SGK*-PGC1 α
280 complexes, we did not detect differences in the DBD domain (Fig. 4c, bottom panel). This
281 may be due to DNA binding itself being such a dominant stabilization process engaging many
282 residues at the interface that it obscures potential ligand specific rearrangements across the
283 two domains. The HDX difference data for GR(Dex) supports that dexamethasone is of the
284 same steroid chemotype as fluticasone furoate with no significant signal in the LBD
285 (Extended Data Fig. 11, top panel). The GR(Dex) data also confirms ligand specific
286 communication across the LBD-DBD domains in absence of DNA.

287

288 To further explore how the ligand pharmacophore impacts the DNA binding event, we used
289 fluorescence polarization (FP) to analyze the stability of the GR(ligand) in complex with
290 fluorescently labelled 6-FAM-*SGK* dsDNA, by adding increasing concentrations of
291 competing unlabeled *SGK* and determined the IC₅₀. As the cytokine release inhibition assay
292 highlighted (Fig. 1c), velsecorat and fluticasone furoate are the stronger GR agonists and they
293 also lead to a 2-fold increase in the stability of the GR-*SGK* complex in comparison to
294 dexamethasone, reflected by the higher concentration of unlabeled *SGK* required to exchange
295 the labelled DNA (Fig. 3d). Altogether, the HDX-MS and FP data provide biophysical
296 support for inter-domain communication that could form the basis for ligand driven functional
297 differentiation.



298

299

300 **Figure 5 | Domain interfaces in the GR_{AN}(Vel) complex. a**, MMTV reporter assay using
 301 GR_{cryst}(S404A, N517D, V571M, F602S, C638D), GR_{WT}, GR(A458T), GR(R614A),
 302 GR(K720D), GR(D641K) and GR(Y640S). Data points are mean \pm s.e.m. of eight replicates
 303 from four experiments. The corresponding western blots showing GR and actin protein
 304 expression levels in the bottom panel. **b**, Interface of LBD1 with DBD1 and DBD2 and **c**,
 305 LBD1-LBD2 interface in GR(Vel)-SGK-PGC1 α . The residues mutated in the transcriptional
 306 activation studies (**a**) are highlighted in bold.

307

308 To explore the relevance of the receptor interfaces for the signaling complex, we designed FL
309 GR constructs with mutants located in the domain junctions. We transfected the GR
310 constructs and a luciferase reporter under the control of the mouse mammary tumor virus
311 (MMTV) promoter into COS7 cells, which lack endogenous GR, to monitor receptor
312 activation in response to increasing concentrations of dexamethasone. All GR protein
313 constructs express well (Fig. 5a, bottom panels). WT GR activates the luciferase reporter in
314 response to dexamethasone with an EC₅₀ of 257 pM (Fig. 5a, top graph). The GR construct
315 containing the crystallization mutations, GR_{cryst}, exhibits a similar EC₅₀ of 252 pM confirming
316 that mutants in the GR_{ΔN} construct have limited effect on signaling potency. However, the
317 maximal signaling efficacy is reduced. The crystallization mutant construct is designed to
318 stabilize the receptor in a specific state. It is conceivable that the signaling event requires
319 structural dynamics that the mutations in GR_{cryst} interfere with. The control mutant A458T,
320 which is located in the DBD D-loops (Fig. 5b) causing a clash in the DBD-DBD interface³³,
321 lowers the EC₅₀ to 555 pM accompanied by a reduction of the maximal signaling efficacy. In
322 LBD1, R614 engages in H-bonds with residues in the D-loops of both DBD1 (G459, A458)
323 and DBD2 (D462, R469) and exhibits hydrophobic contacts with I464 of DBD2 and R460 of
324 DBD1 (Fig. 5b). In keeping with this central position, the R614A mutant shifts the signaling
325 EC₅₀ to 533 pM. At the LBD1-LBD2 interface, we designed the Y640S, D641K and K720D
326 mutant constructs (Fig. 5c), yielding a change in the signaling potency to an EC₅₀ of 1.50 nM,
327 1.79 nM and 762 pM, respectively. In humans, the D641V mutant has been reported to be
328 associated with glucocorticoid resistance³⁴. Altogether, the functional data presented here
329 confirm that the strategically placed mutants impair receptor signaling. However, a full
330 characterization of the receptor interfaces requires a comprehensive functional study with
331 mutants distributed across all receptor surfaces.

332

333 70 years ago Phillip Hench administered the hormone cortisone to patients with rheumatoid
334 arthritis with a transformative outcome³⁵. After the isolation of human and rat receptors^{36,37}
335 and identification of the GR cDNA^{38,39}, GR remains one of the most intensely studied nuclear
336 receptors. GR is distinct among the steroid receptors in that it binds a wider range of DNA
337 elements, either as a dimer or as a monomer⁴⁰. As such, the individual receptor domains are
338 required to adapt to different oligomeric arrangements. The structures of GR_{ΔN} binding to the
339 canonical GBS reveal a distinct domain arrangement. The keto-steroid receptor specific C-
340 terminal extension prevents the formation of the strong LBD dimer observed in ER¹⁶. Instead,
341 the GR LBD dimerization surface is shifted to an adjacent overlapping position, and the LBDs
342 form a unique head-to-tail dimer with a relatively small interface. We propose that this may
343 be a consequence of the requirement to signal in different contexts, as a strong LBD dimer
344 would potentially shift the signaling balance. The detailed quaternary arrangement presented
345 here provides a structural context for disease mutations and the information on the domain
346 interfaces will provide critical insights for future drug design. Future studies of multidomain
347 GR constructs in complex with DNA half-sites, inverted repeat binding elements and different
348 coregulators will further build the understanding of how the signaling flexibility correlates
349 with different structural arrangements.

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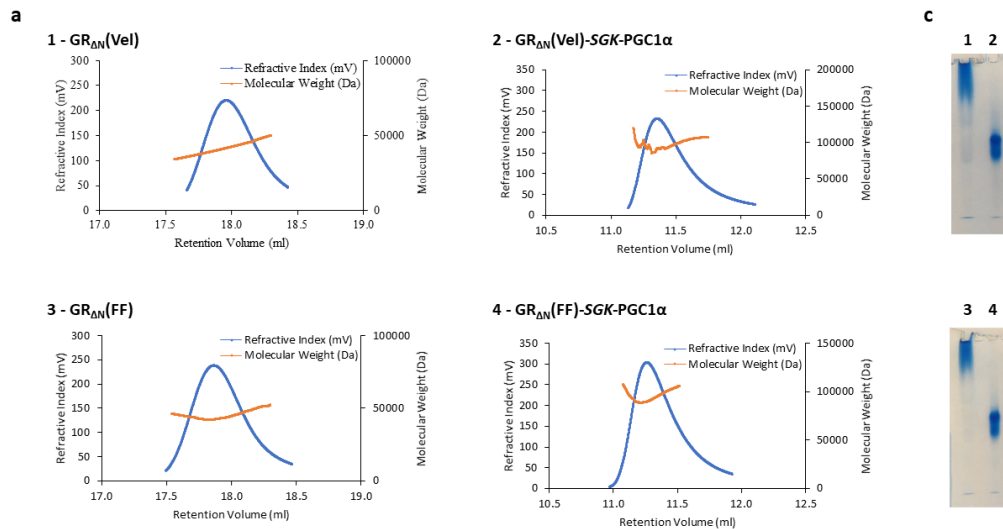
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b

Sample No	Sample	Protein	Peptide	dsDNA	Ligand	MALS MW (kDa)	calculated MW (kDa)
1	GR _{ΔN} (Vel)	GR ₃₈₅₋₇₇₇ [S404A, N517D, V571M, F602S, C638D]			velsecorot	43.14	44.5
2	GR _{ΔN} (Vel)-SGK-PGC1α	GR ₃₈₅₋₇₇₇ [S404A, N517D, V571M, F602S, C638D]	PGC1α	SGK23blunt	velsecorot	98.14	107.5
3	GR _{ΔN} (FF)	GR ₃₈₅₋₇₇₇ [S404A, N517D, V571M, F602S, C638D]			fluticasone furoate	45.15	44.5
4	GR _{ΔN} (FF)-SGK-PGC1α	GR ₃₈₅₋₇₇₇ [S404A, N517D, V571M, F602S, C638D]	PGC1α	SGK23overhang	fluticasone furoate	98.47	107.5

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477 **Extended Data Figure 1 | SEC-MALS of GR_{ΔN}(Vel), GR_{ΔN}(FF), GR_{ΔN}(Vel)-SGK-PGC1α**
 478 **and GR_{ΔN}(FF)-SGK-PGC1α.** **a**, The monomeric GR proteins and dimeric GR complexes
 479 eluted as single peaks. **b**, The experimentally determined and expected molecular weights,
 480 GR proteins and GR complexes separated on a native PAGE.

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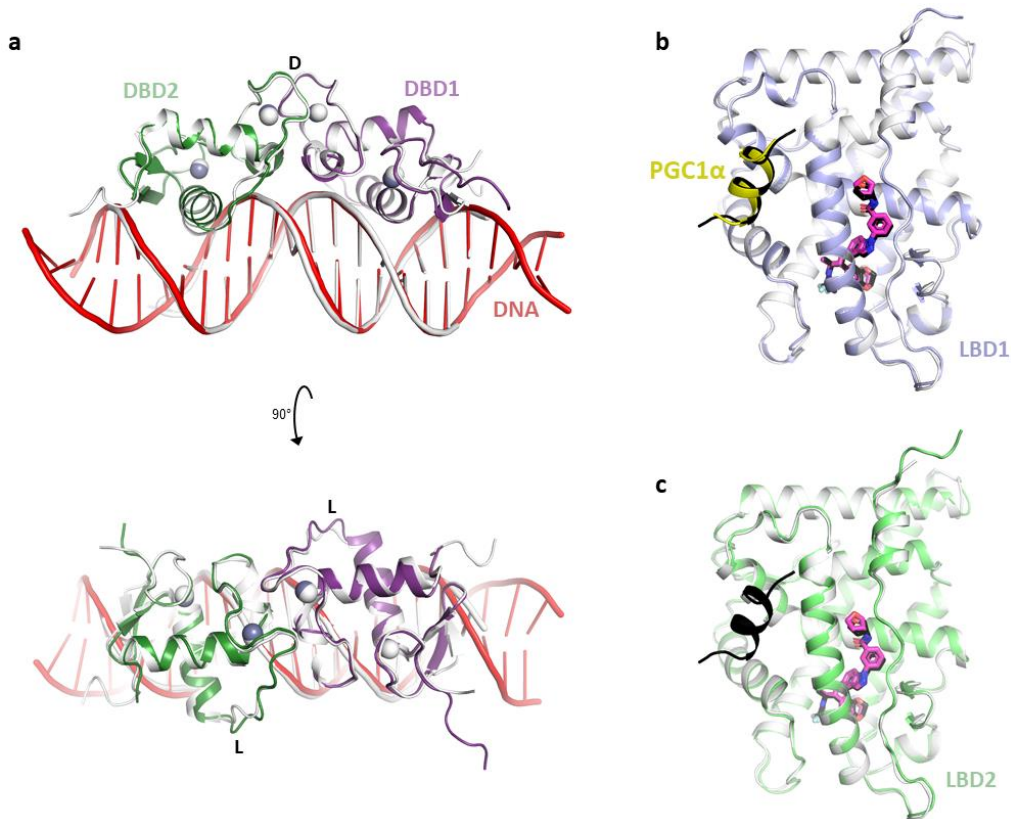
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486**Extended Data Table 1 | Crystallographic data collection and refinement statistics.**

Name	GR _{AN} (Vel)-SGK-PGC1 α	GR _{LBD} (Vel)-PGC1 α	GR _{AN} (FF)-SGK-PGC1 α
GR construct	GR α ₃₈₅₋₇₇₇ [S404A, N517D, V571M, F602S, C638D]	GR α ₅₂₉₋₇₇₇ [WT]	GR α ₃₈₅₋₇₇₇ [S404A, N517D, V571M, F602S, C638D]
Ligand	Velsecorat	Velsecorat	fluticasone furoate
peptide	PGC1 α ₁₃₄₋₁₅₄	PGC1 α ₁₃₄₋₁₅₄	PGC1 α ₁₃₄₋₁₅₄
DNA	SGK23blunt	N/A	SGK23overhang
Data collection			
Space group	P212121	P21	P212121
a, b, c (Å)	80.21, 122.72, 130.45	42.34, 73.43, 43.93	79.67, 119.72, 135.53
α , β , γ (°)	90.0, 90.0, 90.0	90.0, 105.86, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	0.96550	0.97242	0.96545
Resolution (Å)	89.39-2.5 (2.87-2.5)	73.43-2.2 (2.27-2.2)	68.7-2.7 (3.0-2.7)
Rmerge	0.082 (0.792)	0.124 (0.928)	0.067 (1.035)
Rpim	0.036 (0.373)	0.081 (0.466)	0.029 (0.425)
I/ σ (I)	12.6 (1.9)	6.6 (1.7)	16.3 (1.6)
CC1/2 (%)	0.999 (0.744)	0.995 (0.709)	0.999 (0.706)
Completeness spherical (%)	51.0 (7.6)		70.2 (13.8)
Completeness ellipsoidal (%)	93.4 (76.1)		93.9 (63.5)
Completeness		99.0 (88.1)	
Multiplicity (%)	6.3 (5.4)	3.3 (3.1)	6.5 (6.9)
Refinement			
Resolution (Å)	89.39-2.5	42.26-2.2	68.7-2.7
Total reflections	45176	42980	36333
Unique reflections	23070	13075	24963
Rwork/Rfree (%)	0.202/0.263	0.226/0.281	0.216/0.254
No. of non-hydrogen atoms			
Protein	5299	2168	5262
Nucleic Acid	939	-	896
Ligands	116	44	90
Water	64	46	30
Average B-factor (Å ²)	88.0	50.0	103.0
Wilson B-factor (Å ²)	66.0	27.7	76.1
RMS deviations			
Bond lengths (Å)	0.008	0.008	0.008
Bond angles (°)	0.92	0.97	0.92
Ramachandran			
Most favored (%)	94	98	94
Outliers (%)	0	0	1
Clashscore	5	2	6
PDB code	7PRW	7PRX	7PRV

487 Values in parentheses are for the highest resolution shell.

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492 **Extended Data Figure 2 | GR_{AN}(Vel)-SGK-PGC1 α domains overlaid on structures of the**493 **isolated domains. a**, DBD1 (purple), DBD2 (green) and dsDNA (red) overlaid on the

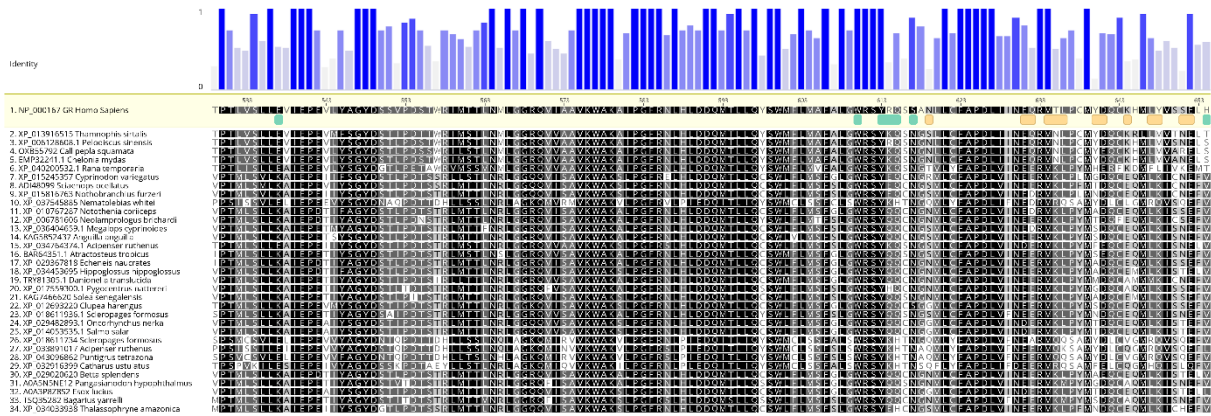
494 structure of the DBD dimer alone on the same GBS (PDB: 3G9O, all in white). Zn atoms are

495 denoted as grey and white spheres, respectively. **b**, LBD1 (blue) with velsecorat (magenta)496 and coactivator peptide PGC1 α ₁₃₄₋₁₅₄ (yellow) overlaid on the structure of GR_{LBD} (white) in497 complex with velsecorat (black) and coactivator peptide PGC1 α ₁₃₄₋₁₅₄ (black). **c**, LBD2498 (green) with velsecorat (magenta) overlaid on the structure of GR_{LBD} (white) in complex499 with velsecorat (black) and coactivator peptide PGC1 α ₁₃₄₋₁₅₄ (black).

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504 **Extended Data Figure 3 | Sequence conservation of the GR LBD.** Alignment of a set of

505 diverse GR related vertebrate sequences with a pairwise identity of 37-84%. The mean

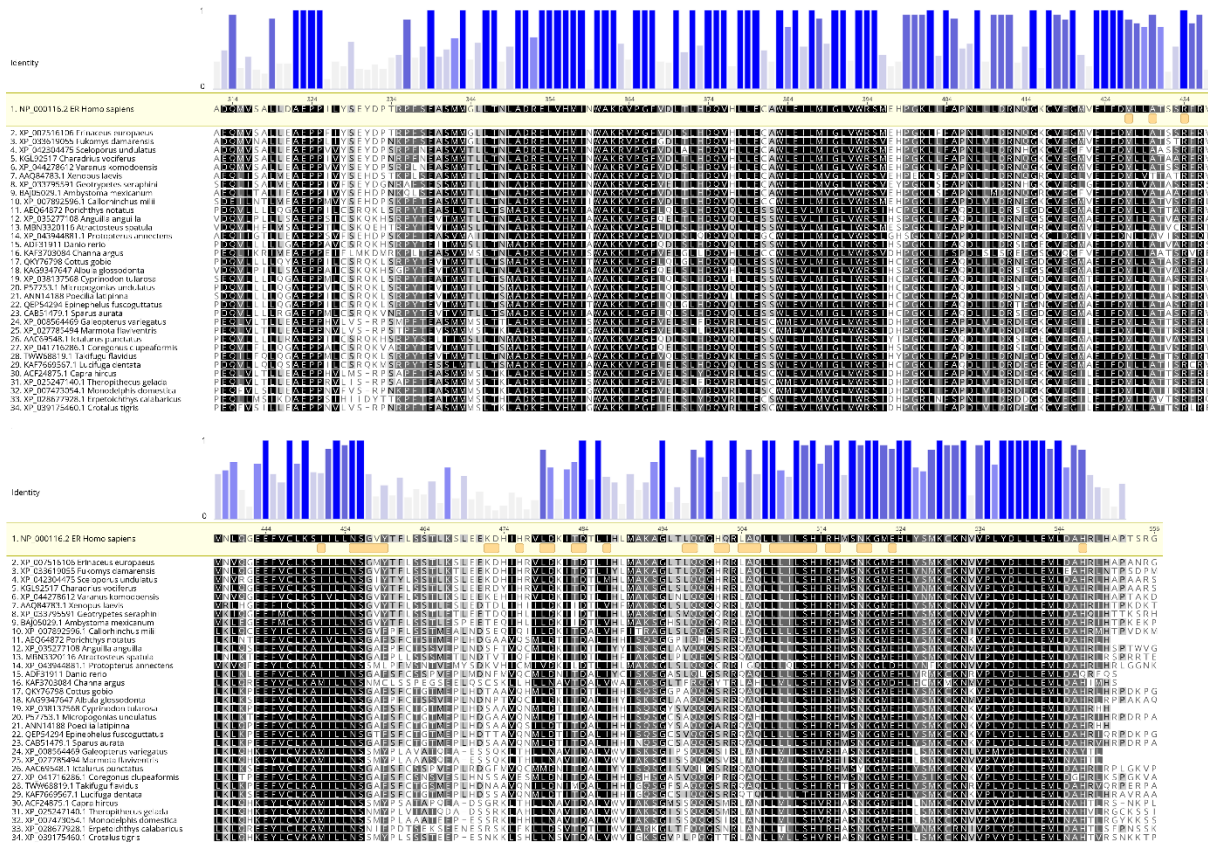
506 pairwise column identity of each residue as calculated by Geneious Prime is shown as bars in

507 the top graph. The residues involved in the LBD:LBD and LBD:DBD interfaces are indicated

508 by orange and green boxes, respectively.

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Extended Data Figure 4 | Sequence conservation of the ER LBD. a, Alignment of a set of

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diverse ER related vertebrate sequences with a pairwise identity of 38-90%. The mean

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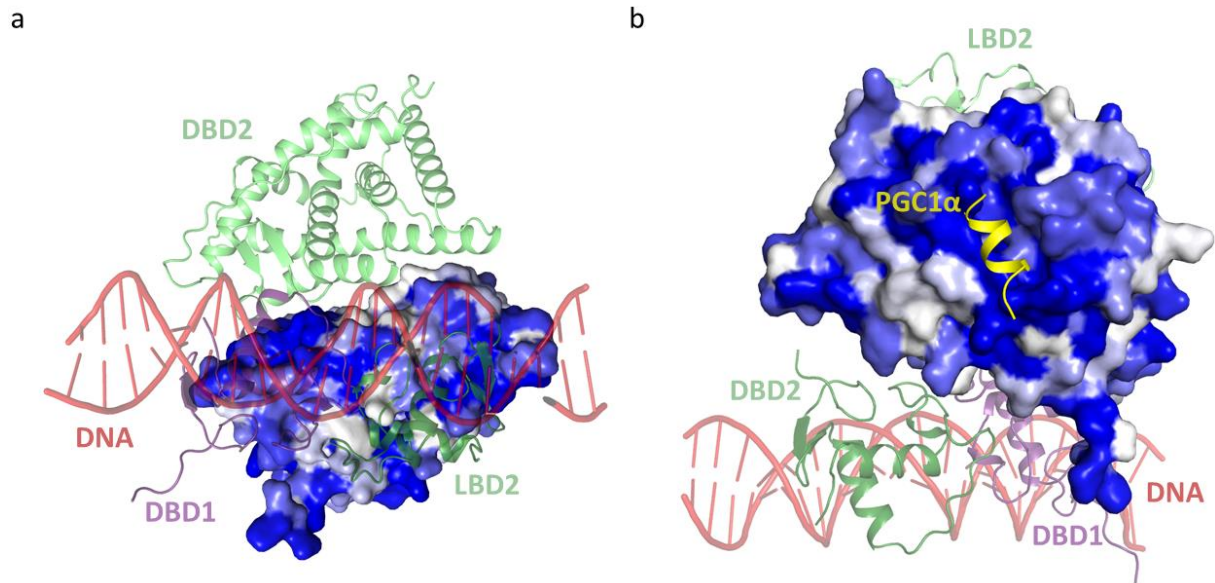
pairwise column identity of each residue as calculated by Geneious Prime is shown as bars in

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the top graph. LBD dimer interface residues are highlighted with orange boxes.

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519 **Extended Data Fig. 5 | Sequence conservation of key interfaces in the GR_{ΔN}(Vel)-SGK-**

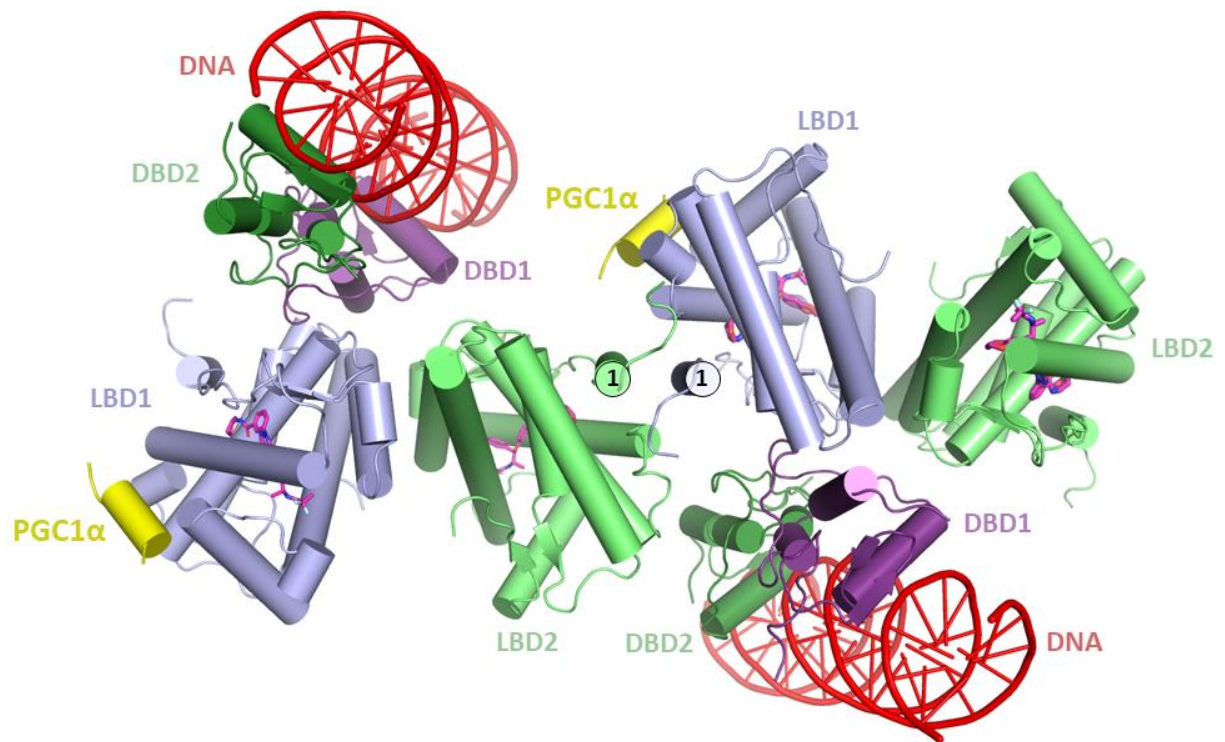
520 **PGC1α complex.** Conservation of GR LBD1 residues colored according to column identity

521 between 0.4 (white) and 1.0 (blue) and shown as cartoon highlighting the LBD1 interface

522 with **a**, the DBD and DNA and **b**, the PGC1α peptide (yellow).

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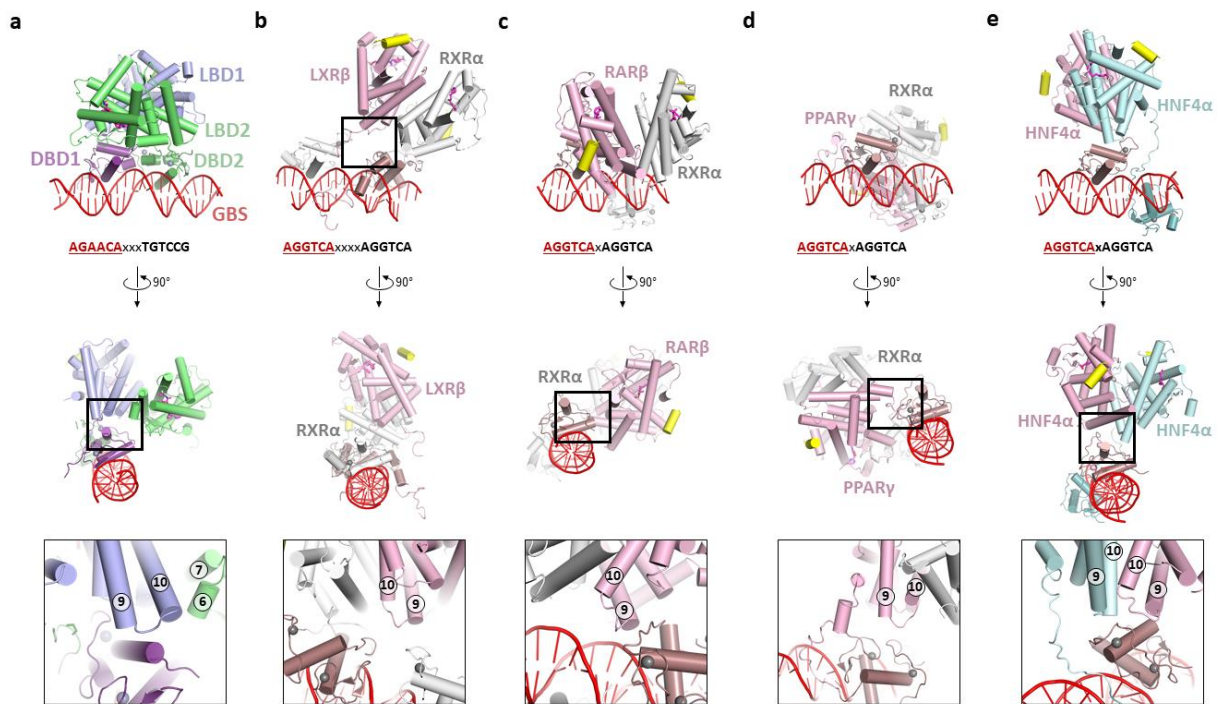
527 **Extended Data Fig. 6 | Structure of GR Δ N(Vel) in complex with SGK and PGC1 α ₁₃₄₋₁₅₄.**

528 The structure of GR Δ N(Vel)-SGK-PGC1 α and a crystallographic neighbor highlighting

529 putative tetramer formation through the H1 interface.

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Extended Data Figure 7 | The domain organization of the multidomain nuclear receptor

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X-ray structures on DNA. a, GR_{ΔN}(Vel)-SGK-PGC1 α . b, LXR β -RXR α c, RAR β -RXR α . d,

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PPAR γ -RXR α . e, HNF-4 α . The sequence of the DNA binding motif is indicated below the

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DNA duplex with x denoting nucleotide spacer. The structures have been overlaid using the

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5' DNA binding sequence as reference (highlighted in red) and are all shown from the same

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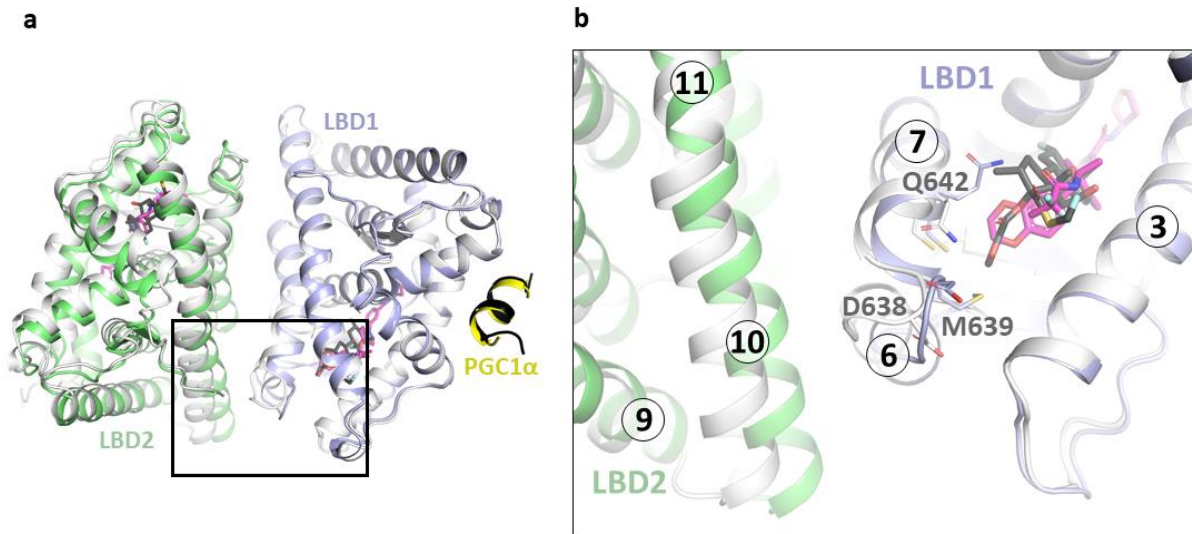
angle. The different coregulator peptides are shown in yellow and the ligands in magenta.

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Extended Data Figure 8 | Fluticasone furoate rearranges the region where H6 and H7

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meet. a, Overlay of the GR Δ N(FF)-SGK-PGC1 α LBD1 (white) with PGC1 α peptide in black

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on the GR Δ N(Vel)-SGK-PGC1 α LBD1 (blue) with PGC1 α peptide in yellow. Fluticasone

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furoate and velsecorat are shown in black and magenta, respectively. **b**, Fluticasone furoate

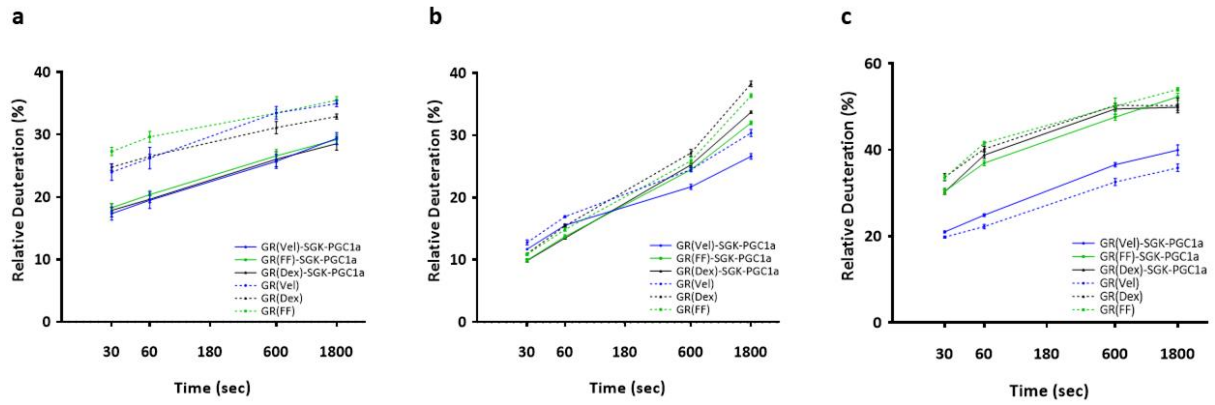
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repositions Q642 and pushes on D638 and M639, rearranging the H6-H7 loop. Helix

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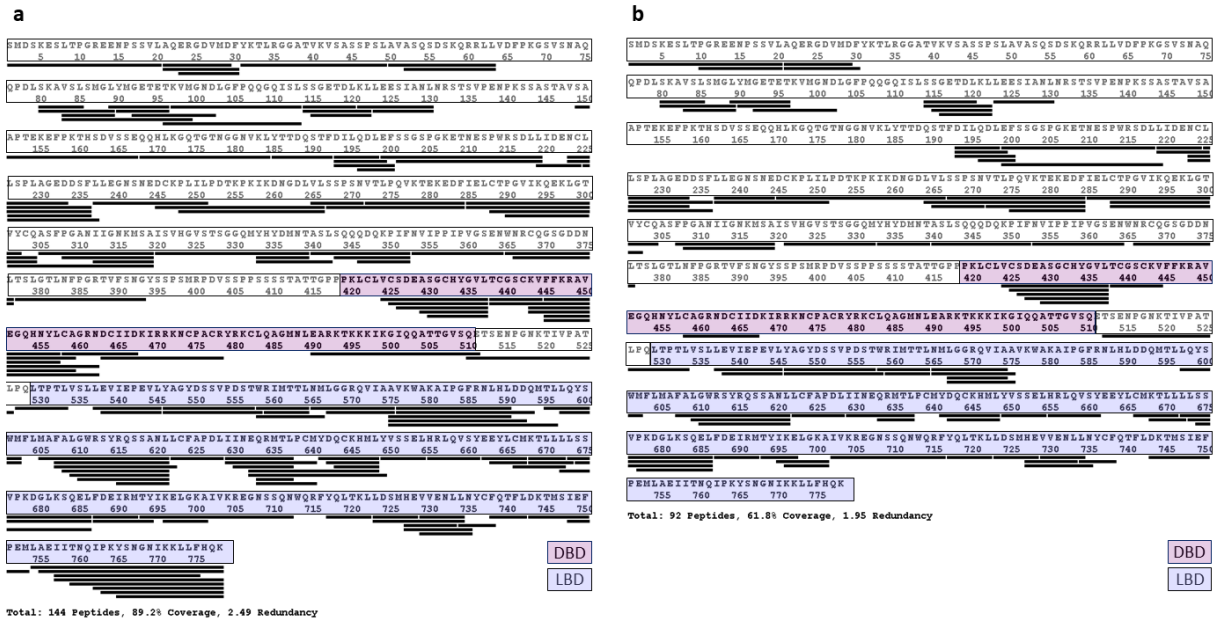
numbering is annotated within the circles.

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 552 **Extended Data Figure 9 | Relative deuteration of specific peptides in GR(Vel)-SGK-**
 553 **PGC1α, GR(FF)-SGK-PGC1α, GR(Dex)-SGK-PGC1α, GR(Vel), GR(FF) and GR(Dex)**
 554 **complexes. a, peptide 425-436. b, peptide 536-544. c, peptide 628-636.**

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567 **Extended Data Figure 10 | Protein coverage of GR and GR-SGK-PGC1 α in HDX-MS. a,**

568 peptides used for HDX-MS analysis of GR. A coverage of 89.2% of the sequence was

569 achieved. **b**, peptides used for HDX-MS analysis of GR-SGK-PGC1 α complexes. A coverage

570 of 61.8% of the sequence was achieved.

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573 **Extended Data Table 2 | Relative Uptake and Relative Uptake Error as identified with**

574 **the DynamX 3.0 software (Waters) for all peptides and used for HDX analysis of GR in**

575 **the presence of the ligands dexamethasone GR(Dex), fluticasone furoate GR(FF) and**

576 **velsecrat GR(Vel).**



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579 **Extended Data Table 3 | Relative Uptake and Relative Uptake Error as identified with**

580 **the DynamX 3.0 software (Waters) for all peptides and used for HDX analysis of**

581 **GR(Dex), GR(FF), GR(Vel) and the complexes GR(Vel)-SGK-PGC1 α , GR(FF)-SGK-**

582 **PGC1 α and GR(Dex)-SGK-PGC1 α .** Vel=velsecorat, FF=fluticasone furoate,
583 Dex=dexamethasone.



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586 **Extended Data Table 4 | Relative Uptake Difference as identified with the DynamX 3.0**
587 **software (Waters) and p-values for all peptides used for HDX analysis of [GR(Vel)]**
588 **minus [GR(FF)].** Significant p-values are highlighted in grey. Vel=velsecorat,
589 FF=fluticasone furoate.



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592 **Extended Data Table 5 | Relative Uptake Difference as identified with the DynamX 3.0**
593 **software (Waters) and p-values for all peptides used for HDX analysis of [GR(Vel)-SGK-**
594 **PGC1 α] minus [GR(FF)-SGK-PGC1 α], [GR(Vel)-SGK-PGC1 α] minus [GR(Vel)] and**
595 **[GR(FF)-SGK-PGC1 α] minus [GR(FF)].** Significant p-values are highlighted in grey.
596 Vel=velsecorat, FF=fluticasone furoate.



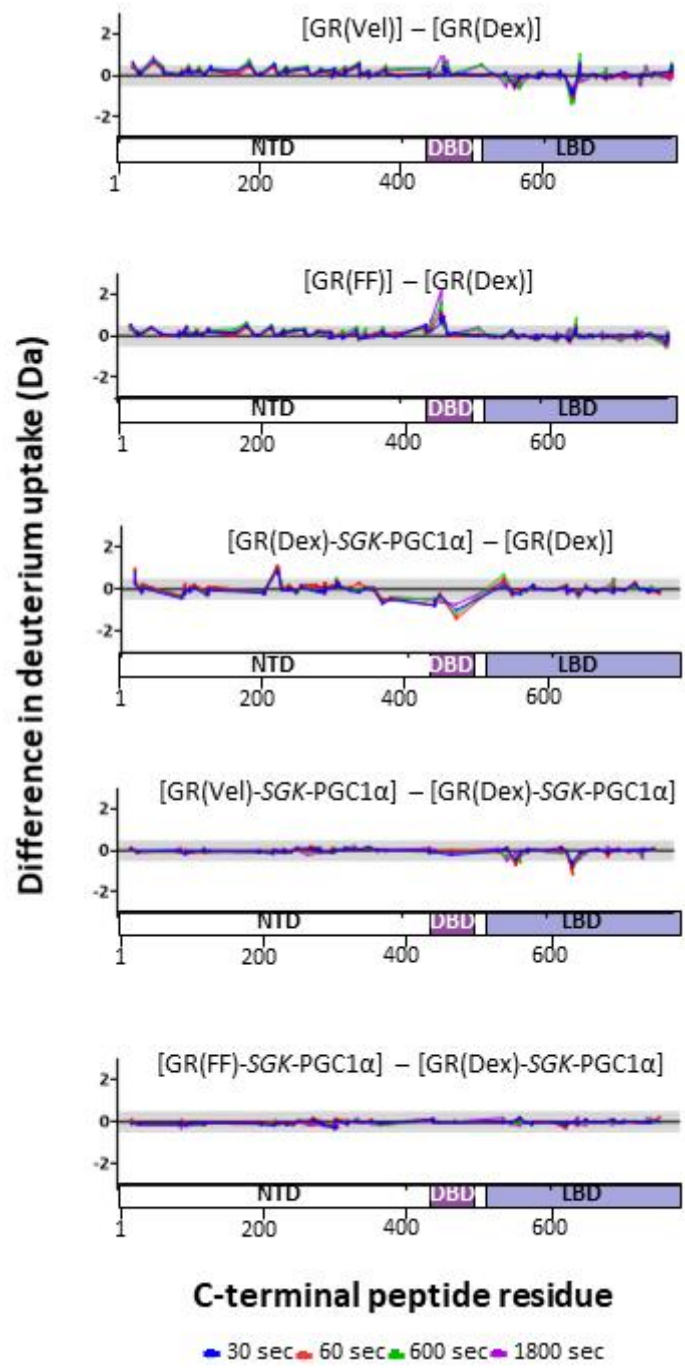
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602 **Extended Data Figure 11 | HDX difference plots showing deuterium uptake difference**

603 **between [complex A] – [complex B] for all peptides.** Negative value indicate that peptides

604 are protected in A relative to B and vice versa.

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606 **Extended Data Table 6 | Relative Uptake Difference as identified with the DynamX 3.0**
607 **software (Waters) and p-values for all peptides used for HDX analysis of [GR(Vel)]**
608 **minus [GR(Dex)] and [GR(FF)] minus [GR(Dex)].** Significant p-values are highlighted in
609 grey. Vel=velsecorat, FF=fluticasone furoate, Dex=dexamethasone.



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612 **Extended Data Table 7 | Relative Uptake Difference as identified with the DynamX 3.0**
613 **software (Waters) and p-values for all peptides used for HDX analysis of [GR(Dex)-**
614 **SGK-PGC1 α] minus [GR(Dex)], [GR(Vel)-SGK-PGC1 α] minus [GR(Dex)-SGK-PGC1 α]**
615 **and [GR(FF)-SGK-PGC1 α] minus [GR(Dex)-SGK-PGC1 α].** Significant p-values are
616 highlighted in grey. Vel=velsecorat, FF=fluticasone furoate, Dex=dexamethasone.



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617

618 **Materials and Methods**

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620 **Protein expression and purification**

621 Optimized DNA sequences of GR (human GR α_{1-777} [WT]) and GR Δ_N (human GR $\alpha_{385-777}$
622 [S404A, N517D, V571M, F602S, C638D]) with N-terminal 6xHN-tag followed by a TEV
623 site were cloned into pFastBacTM1 and recombinant baculoviruses generated using the Bac-to-
624 Bac[®] system (invitrogen). S404 is a known phosphorylation site¹ and the mutations N517D,
625 V571M, F602S and C638D stabilize the GR LBD²⁻⁴. *Spodoptera frugiperda* insect cells
626 (SF21) were cultured at 27°C in GibcoTM Sf-900TM II SFM medium and infected with
627 baculovirus at a density of 2.5-3.0 x 10⁶ mL⁻¹. 24 h after transfection 10 μ M ligand in DMSO
628 (velsecorat, fluticasone furoate or dexamethasone) was added to the cultures and 48 h after
629 transfection the cells were harvested. The cells were resuspended in 20 mM Hepes pH 7.0, 10
630 % (v/v) glycerol, 1 mM TCEP, 10 μ M ligand, cOmpleteTM EDTA-free protease inhibitor
631 cocktail (Roche, 1 tablet per 50 ml of solution) and flash frozen in liquid nitrogen. After
632 thawing the NaCl concentration was adjusted to 0.5 M by adding adequate amounts of a stock
633 solution containing 4.5 M NaCl, 20 mM Hepes pH 7.0, 10 % (v/v) glycerol, 1 mM TCEP, 10
634 μ M ligand, the solution incubated for 15 Min on ice and the supernatant cleared by
635 centrifuging at 30.000 g for 45 Min. The supernatant was incubated with WorkBeadsTM 40
636 Ni-NTA (Bio-Works) for 2 hours at 4°C, the beads were washed by gravity flow with buffer
637 containing 20 mM HEPES pH 7.0, 500 mM NaCl, 10 % glycerol, 1 mM TCEP, 10 μ M
638 ligand, 25 mM Imidazole and 5 mM ATP and the protein eluted with increasing Imidazole
639 concentrations. The elution buffer was exchanged on a desalting column to 20 mM HEPES
640 pH 7.0, 500 mM NaCl, 10 % glycerol, 1 mM TCEP, 10 μ M ligand before the HN-tag was
641 cleaved off by TEV digestion overnight at 4°C. The proteins were further purified by size

642 exclusion and the peak fractions pooled, concentrated to 1-3 mg/mL, flash frozen in liquid N₂
643 and stored at -80°C.
644 The wildtype GR_{LBD}(Vel) (human GR $\alpha_{529-777}$ [WT] with velsecorat) was cloned into the
645 pET24a vector (Novagen) featuring an N-terminal His6-tag and a TEV protease cleavage site.
646 The expression vector was transformed into *E. coli* BL21(DE3) STAR, followed by expression
647 in PASM-5052 autoinduction medium. 50 μ M velsecorat was added after the cell culture
648 reached an OD of 0.6 followed by expression over 48 hours at 16 °C. All purification buffers
649 were degassed and contained 2 mM TCEP and 20 μ M velsecorat. The harvested cells were
650 resuspended in lysis buffer (50 mM Tris pH 8, 10% glycerol, 1% CHAPS) supplemented by
651 protease inhibitors (Complete, Roche) and DNase. Cells were lysed by sonication. The cleared
652 lysate was applied to a nickel affinity column equilibrated with wash buffer (50 mM Tris pH
653 8.0, 10% glycerol, 1% CHAPS, 60 mM NaCl) and eluted by a 300 mM imidazole gradient. The
654 purification tag was removed by TEV protease cleavage while dialyzing against a 50 mM Tris
655 buffer at pH 9.0 overnight followed by a second Nickel affinity step to collect the cleaved target
656 protein in the flowthrough. Remaining impurities were removed by size exclusion using 50 mM
657 Tris buffer at pH 9.0, 2 mM TCEP, 20 μ M velsecorat as running buffer. The purified protein in
658 50 mM Tris buffer pH 9.0, 2 mM TCEP, 10 μ M velsecorat was flash frozen in liquid N₂ and
659 stored at -80°C.

660

661 **GR α complex formation with DNA and peptide**

662 Peptides of human peroxisome proliferator-activated receptor γ coactivator 1- α (PGC1 α_{134-}
663 ₁₅₄: PPQEAEEPSLLKLLLLAPANT) dissolved in water and dsDNA (see below) from the
664 GR DNA response element from the serum and glucocorticoid-regulated kinase-1 (*SGK-1*)
665 were used for complex formation with GR α . The purified ligand bound GR α proteins were
666 mixed with dsDNA (1 : 0.6 ratio of LBD monomer to ds DNA) and peptide (1 : 1.3 ratio of

667 LBD monomer to peptide) and dialyzed overnight into 20 mM MOPS pH 7.0, 150 mM NaCl,
 668 3mM MgCl₂, 0.75 % glycerol, 1 mM TCEP, 10 μM ligand for crystallization or SEC-MALS.

669

670 DNA oligonucleotides (Sigma Aldrich) used for *SGK* dsDNA formation:

<i>SGK</i> dsDNA	Oligo 1	Oligo 2
<i>SGK23overhang</i>	<i>SGK23oF</i> : 5'-TACAGAACATTTTGTCCGTCGAC-3'	<i>SGK23oR</i> : 5'-TCGACGGACAAAATGTTCTGTAC-3'
<i>SGK23blunt</i>	<i>SGK23bF</i> : 5'-GTACAGAACATTTTGTCCGTCGA-3'	<i>SGK23bR</i> : 5'-TCGACGGACAAAATGTTCTGTAC-3'
<i>SGK24blunt</i>	<i>SGK24F</i> : 5'-GTACAGAACATTTTGTCCGTCGAC-3'	<i>SGK24R</i> : 5'-GTCGACGGACAAAATGTTCTGTAC-3'
FAM- <i>SGK24</i>	6-FAM- <i>SGK24F</i> : 5'-(6-FAM)-GTACAGAACATTTTGTCCGTCGAC-3'	<i>SGK24R</i> : 5'-GTCGACGGACAAAATGTTCTGTAC-3'

671

672 **Crystallization and crystal structure determination**

673 The complex GR_{ΔN}(FF)-*SGK*-PGC1α (human GR_{α385-777} [S404A, N517D, V571M, F602S,
 674 C638D] with the agonist fluticasone furoate, the dsDNA *SGK23overhang* and the peptide
 675 PGC1α₁₃₄₋₁₅₄) was concentrated to approx. 10 mg/mL and crystallization optimized in hanging
 676 drops using multiple rounds of seeding. Drops were set up with a 1 : 1 ration of protein
 677 complex solution to well solution (8 % PEG3350, 0.1 M Bis-Tris-Propane pH 6.5, 8 % 2,2,2-
 678 Trifluoroethanol, 0.1 M Guanidine HCl, 0.3 M Hexanediol). The complex GR_{ΔN}(Vel)-*SGK*-
 679 PGC1α with the agonist velsecorat, *SGK23blunt* and the peptide PGC1α was concentrated to
 680 approx. 10 mg/mL and crystallization optimized in hanging drops using multiple rounds of
 681 seeding. Drops were set up with a 1 : 1 ratio of protein complex to well solution (8.6 %
 682 PEG3350, 0.1 M Bis-Tris-Propane pH 6.5, 2 % 2,2,2-Trifluoroethanol, 0.1 M Guanidine HCl,

683 0.3M 1,6-Hexanediol). 30 % ethylene glycol or 30 % glycerol in well solution was used as
684 cryoprotectant and crystals were flash frozen in liquid nitrogen for data collection. Data was
685 collected with a Pilatus3 x 2M detector at the automated beamline ID30A-1/MASSIF-1 at the
686 European Synchrotron Radiation Facility (ESRF).

687 A twofold molar excess of PGC1 α ₁₃₄₋₁₅₄ was added to GR_{LBD}(Vel) (human GR α ₅₂₉₋₇₇₇ [WT],
688 co-expressed with velsecorat) and the protein concentrated to 10 mg/mL. Crystals were grown
689 at 20 °C in sitting drops using a 1 : 1 (100 nL + 100 nL) ratio of protein and well solution (18
690 % PEG8000, 2 % 2-Propanol, 0.1 M Sodium acetate, 0.1 M HEPES pH7.5). Crystals were
691 cryo-protected in well solution supplemented with 20 % glycerol and flash frozen in liquid
692 nitrogen. Data was collected with a Pilatus 6M detector at the beamline ID23-1 at the ESRF.

693 All datasets were integrated and scaled with autoPROC (Global Phasing)⁵ and initial models
694 obtained performing molecular replacement with the known structures 4P6W and 3G9O using
695 PHASER⁶. The models were improved by iterative rounds of refinement using BUSTER
696 (Global Phasing)⁷ and manual model building in COOT⁸. The PyMOL Molecular Graphics
697 System, Version 2.1.0, Schrödinger, LLC, was used to generate figures.

698 Detailed statistics of data collection and refinement can be found in Supplementary Table 1.

699 All coordinates and structure factors have been deposited in the Protein Data Bank (PDB)
700 under the accession codes 7PRV, 7PRW and 7PRX.

701 PDBePISA⁹ has been used for interface analysis and C α root mean square deviation (r.m.s.d.)
702 values calculated with lsqkab¹⁰.

703

704 **Analysis of the sequence conservation of GR**

705 A set of GR-like vertebrate sequences were gathered through iterative Blast searches¹¹,
706 starting with human GR (NP_000167.1). Sequences with an identity above 50% to the query
707 were aligned with Geneious Prime v. 2021.1.1 using MUSCLE¹². All entries with an identity

708 >90% to any other entry in the set were purged, while the least similar sequence to the query
709 was used for subsequent searches. This resulted in a diverse alignment of the GR family with
710 a pairwise sequence identity of 37-84% between all sequences (Extended Data Fig. 3). A set
711 of ER-like vertebrate sequences were gathered by a similar approach, starting with human ER
712 (NP_000116.2) (Extended Data Fig. 4) and purged to match the diversity of the GR set. The
713 mean pairwise column identity of each residue was calculated by Geneious, plotted by
714 Spotfire Analyst 11.4 (Extended Data Figure 3 and 4) and subsequently color mapped onto
715 the GR and ER LBD structures (Fig. 2d, e and Extended Data Fig. 5)

716

717 **Binding assay to dsDNA in competition mode**

718 The affinity of GR (human WT GR α_{1-777}) in the presence of different ligands to fluorescein
719 (6-FAM) labelled dsDNA FAM-*SGK24* was analyzed using fluorescence polarization in
720 competition mode.

721 150 nM GR complexed with the ligands dexamethasone [GR(Dex)], velsecorat [GR(Vel)] or
722 fluticasone furoate [GR(FF)] was mixed with 10 nM FAM-*SGK24* in HBSP buffer (10 mM
723 HEPES pH 7.4, 150 mM NaCl, 0.005 % Tween-20®). This preformed complex was mixed
724 with a dilution series (1.5 μ , 750 nM, 375 nM, 187.5 nM, 93.75 nM, 46.88 nM, 23.44 nM,
725 11.7 nM, 5.9 nM and 0 nM) of competing unlabeled dsDNA *SGK24*blunt. Fluorescence
726 polarization was measured using a PHERAStar microplate reader (BMG LABTECH) with the
727 FP Module 485-520/520. IC50 value was determined by simultaneous fitting all curve data
728 (three curve replicates from one experiment) using a four parameter non-linear curve fit in
729 GraphPad Prism 9.

730

731 **Hydrogen/deuterium exchange mass spectrometry (HDX-MS)**

732 The HDX experiments were carried out using a HDX Manager (Waters) equipped with a CTC
733 PAL sample handling robot (LEAP Technologies). GR (human GR α_{1-777} [WT]) in the
734 presence of the ligand dexamethasone [GR(Dex)], velsecorat [GR(Vel)] or fluticasone furoate
735 [GR(FF)] and also the complexes GR(Dex)-*SGK*-PGC1 α , GR(FF)-*SGK*-PGC1 α and
736 GR(Vel)-*SGK*-PGC1 α (GR with the dsDNA *SGK24*blunt and PGC1 $\alpha_{134-154}$) were dialyzed
737 into the HDX compatible buffer 20 mM HEPES pH 7.0, 200 mM NaCl, 2.5 % glycerol, 1
738 mM TCEP, 20 μ M ligand and the exchange reactions performed with a CTC PAL sample
739 handling robot (LEAP Technologies). The samples were labelled for 0 s, 30 s, 60 s, 180 s, 600
740 s and 1800 s by incubating 3 μ l protein (GR at 1 mg/mL or GR complexes at 0.6 mg/mL) with
741 57 μ l of D₂O buffer (20 mM HEPES pD 7.4, 200 mM NaCl, 2.5 % glycerol, 1 mM TCEP) at
742 22 °C. To stop the exchange reaction Quench (3M Urea, 0.1 % TFA, pH2.5) was added 1 : 1
743 at 4 °C and the mixture was injected onto an online pepsin digestion system with a Waters
744 Enzymate™ BEH Pepsin Column (2.1 x 30 mm, 5 μ m) in 0.1 % formic acid in water at 150
745 μ L/min for 3 min at 0.1 °C. The peptides were trapped/desalted for 3 min on a Waters
746 ACQUITY UPLC BEH C18 VanGuard Pre-Column (2.1 \times 5 mm, 1.7 μ m) and separated on a
747 using a C18 reverse phase column (ACQUITY UPLC BEH C18 Column, 1.7 μ m,
748 2.1 \times 100 mm, Waters) by running a linear gradient from 5 - 40 % solvent B (solvent A: 0.1 %
749 formic acid in water; solvent B: 0.1 % formic acid in acetonitrile) over 9 min. The peptides
750 were analyzed on a Waters Synapt G2-Si mass spectrometer, identified using MSE
751 fragmentation. Mass spectrometry experiments acquired over a mass range from 50 to
752 2000 m/z using an electrospray ionization source operated at a temperature of 200 °C and a
753 spray voltage of 4.5 kV. All reactions were carried out in triplicates and no correction was
754 made for back exchange, therefore all results are reported as relative deuterium exchange
755 levels.

756 Peptides were identified using the ProteinLynx Global Server 3.0.2 (PLGS, Waters) and
757 deuterium incorporation was analyzed in DynamX 3.0 (Waters). Tables including all peptides
758 with sequences, monoisotopic mass, retention time and mean deuterium uptake is supplied as
759 Extended Data Tables 2 and 3 following guidelines by¹³. Relative deuterium levels were
760 calculated for each peptide by subtracting the average mass of the deuterium-labeled GR with
761 ligand 1 from that of the deuterium-labeled GR with ligand 2. Statistical significance was
762 determined in t tests as p values < 0.05 and listed in Extended Data Tables 4, 5, 6 and 7.
763 Visualization was done using GraphPad Prism 9.

764

765 **Size exclusion chromatography multi angle light scattering (SEC-MALS)**

766 A Cytiva Superdex® 200 Increase 10/300 GL column on an OMNISEC RESOLVE/REVEAL
767 System (Malvern Panalytical) was used to analyze the absolute molecular weight (MW) of
768 GR_{ΔN}(FF) and GR_{ΔN}(Vel) (human GR_{α385-777} [S404A, N517D, V571M, F602S, C638D] with
769 the agonist fluticasone furoate or velsecorat) in the buffer 20 mM HEPES pH 7.0, 250 mM
770 NaCl, 2.5 % glycerol, 1 mM TCEP, 10 μM ligand at a flow rate of 0.4 mL/min.

771 A Cytiva Superose® 6 Increase 10/300 GL column on an OMNISEC RESOLVE/REVEAL
772 System (Malvern Panalytical) was used to analyze the absolute molecular weight (MW) of the
773 complexes GR_{ΔN}(FF)-SGK-PGC1 α and GR_{ΔN}(Vel)-SGK-PGC1 α (human GR_{α385-777} [S404A,
774 N517D, V571M, F602S, C638D] with the agonist fluticasone furoate or velsecorat, the
775 dsDNA SGK23overhang or SGK23blunt respectively and PGC1 α ₁₃₄₋₁₅₄) in the buffer 20 mM
776 MOPS pH 7.0, 150 mM NaCl, 3 mM MgCl₂, 0.75 % glycerol, 1 mM TCEP, 10 μM ligand at
777 a flow rate of 0.4 mL/min.

778 The software OmniSEC5.12 was used to analyze the data with the 3 detector method and to
779 determine the molecular weights of the proteins and protein-DNA-peptide complexes.

780 Analyzed proteins and protein complexes were also separated on a native PAGE
781 (NuPAGE™ 3 to 8%, Tris-Acetate, Invitrogen).

782

783 **Inhibition of cytokine (TNF- α) release in whole blood**

784 Whole blood from human with sodium heparin as anticoagulant was used as an anti-
785 inflammatory model to test for steroid like activity (TNF- α inhibition) of GR agonists. A
786 dilution series (1 μ M, 0.33 μ M, 0.11 μ M, 0.037 μ M, 0.012 μ M, 0.0041 μ M, 0.0013 μ M,
787 0.00045 μ M) of velsecorat, fluticasone furoate or dexamethasone in DMSO was analyzed. 1
788 μ l of GR agonist was mixed with 190 μ L of whole blood and incubated for 45 min at 37°C in
789 96-well plates. 10 μ l LPS, Lipopolysaccharide from E.coli serotype O127:B8 2mg/ml in PBS
790 (SigmaAldrich), was added per well and the plates were incubated overnight at 37°C. The
791 cells were spun down at 1800 rpm for 10 min at 4°C and the culture supernatants stored at -
792 80°C until analysis. The culture supernatants were diluted and added to ELISA plates (R&D
793 Systems) and TNF- α was measured according to the manufacturer's instructions. The raw
794 data in pg/ml was normalized to % inhibition. The IC50 value was determined by
795 simultaneous fitting all curve data (six curve replicates from three donors) using a four
796 parameter non-linear curve fit in GraphPad Prism 9.

797

798 **Transactivation assay**

799 A reporter vector for GR activity was constructed by cloning the glucocorticoid receptor
800 responsive MMTV promoter upstream of a codon optimized (GeneArt, ThermoFisher
801 Scientific) NanoLuc® Luciferase reporter to replace the CMV promoter in a pcDNA3.1
802 backbone. A plasmid containing a Firefly luciferase driven by the PGK promoter (#E5011,
803 Promega) was co-transfected and used for normalization.

804 GR_{WT} (human GR α_{1-777} [WT]) and 6 different GR mutants (GR_{cryst}(S404A, N517D, V571M,
805 F602S, C638D), GR(A458T), GR(R614A), GR(K720D), GR(D641K) and GR(Y640S) were
806 synthesized, codon optimized (GeneArt, ThermoFisher Scientific) and cloned under the
807 control of a CMV promoter into pcDNA3.1.

808 For transactivation assays COS-7 cells (ECACC 87021302, ¹⁴) were transfected using
809 Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions and seeded into
810 384-well OptiPlates (PerkinElmer) at 5000 cells / well and seeded into 6 well plates (620,000
811 cells /well) for subsequent immunoblotting. Following 24 hours of incubation the 384-well
812 plates were treated with dexamethasone at the indicated concentrations. Reporter gene
813 activation of NanoLuc® and Firefly Luciferase was read out 24 hours later using Nano-Glo®
814 Dual-Luciferase® Reporter Assay System (Promega) according to the manufacturer's
815 instructions. GraphPad Prism 9 was used for data analysis and graph generation. The EC50
816 value was determined by simultaneous fitting all curve data (eight curve replicates from four
817 experiments) using a four parameter non-linear curve fit in GraphPad Prism 9.

818 To verify the expression of the different GR mutants in COS-7 cells, immunoblots of the
819 transfected cells were carried out using a rabbit anti-GR-antibody (Cell Signaling, #3660) and
820 a mouse anti-actin antibody (Invitrogen, # MA5-11869). Detection was performed using
821 corresponding secondary antibodies (LI-COR #926-68071; #926-32210). Densitometry
822 quantification was performed using Image Studio Software (LI-COR)(LICOR).

823

824

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826

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866

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877

878

879 **Author contributions**

880 S.P. purified the proteins and crystallized the GR_{ΔN} complexes. C.K. purified the wildtype
881 GR_{LBD} protein. L.W. crystallized the GR_{LBD}. S.P. and K.E. solved the structures and wrote the
882 manuscript with input from all authors. S.P. and C.J. performed HDX experiments and
883 analyzed the data. S.P. and A.G. performed fluorescence polarization assays. S.P., M.C. and
884 E.G. performed SEC-MALS and analyzed the data. P.J. analyzed the sequence conservation
885 of GR and ER. B.C., D.Ö., L.F.R., S.P., K.E., I.D., S.D designed and cloned constructs and
886 performed cell assays. B.B., B.P.K. and I.B.M. helped to conceive and conceptualize the
887 study and interpret structural data.

888

889 **Competing interests**

890 Sandra Postel, Lisa Wissler, Carina A. Johansson, Anders Gunnarsson, Euan Gordon, Barry
891 Collins, Christian Köhler, Marie Castaldo, David Öling, Patrik Johansson, Linda Fröderberg
892 Roth, Ian Dainty, Stephen Delaney and Karl Edman were employed by AstraZeneca at the
893 time of the study. The authors declare no competing financial interest.

894

895 **Data availability**

896 The X-ray data and coordinates for the GR_{ΔN}(Vel)-SGK-PGC1 α , GR_{LBD}(Vel)-PGC1 α and
897 GR_{ΔN}(FF)-SGK-PGC1 α structures are deposited in the PDB (7PRW, 7PRX and 7PRV,
898 respectively). The data will be released upon publication.

899

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