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Lucile Senicourt, Albane Le Maire, Frédéric Allemand, João Carvalho, Laura Guee, et al.. Structural insights into the cooperative interaction of the intrinsically disordered co-activator TIF2 with retinoic acid receptor heterodimer (RXR/RAR). 2020. hal-03047222v1

HAL Id: hal-03047222

<https://hal.science/hal-03047222v1>

Preprint submitted on 16 Dec 2020 (v1), last revised 9 Nov 2021 (v2)

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Structural insights into the cooperative interaction of the intrinsically disordered co-activator TIF2 with retinoic acid receptor heterodimer (RXR/RAR)

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Keywords

TIF2, Nuclear hormone Receptors, Intrinsically Disordered Protein, NMR, X-Ray crystallography

ABSTRACT

Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) form heterodimers that activate target gene transcription by recruiting co-activator complexes in response to ligand binding. The nuclear receptor (NR) co-activator TIF2 mediates this recruitment by interacting with the ligand-binding domain (LBD) of NRs through the nuclear receptor interaction domain (TIF2_{NRID}) containing three highly conserved α -helical LxxLL motifs (NR-boxes). The precise binding mode of this domain to RXR/RAR is not clear due to the disordered nature of TIF2. Here we present the structural characterization of TIF2_{NRID} by integrating several experimental (NMR, SAXS, CD, SEC-MALS) and computational data. Collectively, the data are in agreement with a largely disordered protein with partially structured regions, including the NR-boxes and their flanking regions, which are evolutionary conserved. NMR and X-ray crystallographic data on TIF2_{NRID} in complex with RXR/RAR reveal a cooperative binding of the three NR-boxes as well as an active role of their flanking regions in the interaction.

INTRODUCTION

The 48 human nuclear receptors (NRs) belong to a major family of ligand regulated-transcription factors that represent important drug targets for several diseases (cancer, diabetes, obesity, infertility) (Germain et al., 2006c; Khan and Lingrel, 2010). NRs are activated by small lipophilic ligands such as hormones, vitamins and dietary lipids to regulate diverse functions connected to homeostasis, reproduction, development and metabolism (Germain et al., 2006c; Mark et al., 2006). The retinoic acid receptor (RAR) and the retinoid X Receptor (RXR) form a heterodimer that binds to specific DNA sequences (called RAREs - Retinoic Acid Response Elements) of the targeted genes and regulate

gene transcription in response to retinoids. RXR/RAR heterodimer regulated genes are involved in crucial physiological processes, such as embryo development and organ homeostasis (Germain et al., 2006a, 2006b). The ligand-dependent transcriptional activity of the RXR/RAR heterodimer relies on the dynamical balance between binding and release of co-regulator proteins. Unliganded and inverse agonist bound heterodimers exert a repressive effect by interacting with co-repressors, such as N-CoR or SMRT, which act as molecular platforms to recruit proteins with a histone deacetylase activity that induces chromatin compaction and precludes gene transcription (Gronemeyer et al., 2004; Perissi et al., 2010). Conversely, binding of RAR agonists induces conformational changes in RAR that dissociate the co-repressor complex and subsequently, lead to the recruitment of members of the nuclear receptor coactivator 1-3 (NCOA1-3) families of proteins such as SRC1, TIF2 or RAC3, respectively. Those co-activators associate to histone acetylases to uncompact chromatin and trigger gene transcription (Dasgupta et al., 2014). Co-activators interact with the NR ligand binding domain (LBD) through a region named the nuclear receptor interaction domain (NRID) characterized by the presence of multiple conserved LxxLL motifs (the so-called NR-boxes) essential for the interaction (Heery et al., 1997; Le Douarin et al., 1996; le Maire and Bourguet, 2014; Plevin et al., 2005; Torchia et al., 1997; Voegel, 1998). Similar motifs (LxxI/HlxxI/L, called CoRNR-boxes) are identified in co-repressors (Hu and Lazar, 1999; Nagy et al., 1999; Perissi et al., 1999). Importantly, NRIDs are mainly intrinsically disordered regions with these pre-formed secondary structure elements corresponding to NR- and CoRNR-boxes (Cordeiro et al., 2019; de Vera et al., 2017; Devarakonda et al., 2011; Guillien et al., 2020).

The structural bases of the interactions between some co-regulator proteins and RAR have been revealed by crystallographic structures of RAR LBD in complex with peptides harbouring the interacting motifs (le Maire et al., 2010; Osz et al., 2012; Pogenberg et al., 2005; Sato et al., 2010). These structural studies demonstrate that the core of NR- and CoRNR-boxes adopt α -helical conformations and bind to the surface formed by residues from helices H3, H4 and H12 on the LBD of RAR. Importantly, the conformation of the LBD C-terminal helix (named helix 12 or H12) dictates the class of co-regulator recruited by NRs. Indeed, on the one hand, co-repressor binding to unliganded RAR is mediated by the formation of an extended β -strand (β 1) in CoRNR1 that forms an antiparallel β -sheet with RAR residues (S3), while H12 remains flexible in solution (Chrisman et al., 2018; Cordeiro et al., 2019; Kojetin and Burris, 2012; le Maire et al., 2010; Nagy, 2004; Nahoum et al., 2007). On the other hand, binding of agonist induces the transition of S3 to H11 and the folding of H12 in its active position that triggers co-repressor release and co-activator recruitment. It is worth noting that the LBD-interacting surface with NR-box overlaps the co-repressor one (le Maire et al., 2010). Despite this atomistic information available for interacting peptides bound to RAR, the mechanism by which RAR in the context of a heterodimer with RXR recognizes co-regulator fragments harbouring multiple boxes is still poorly understood. Moreover, the contribution of RXR in the recruitment of co-activators is still under debate. Some structural and functional studies argue in favour of a binding of the NR-boxes to both subunits (deck model) (Chandra et al., 2017, 2008; de Vera et al., 2017; Meng et al., 2017; Pogenberg et al., 2005), whereas other studies claim that the binding of the co-activator is exclusively to RAR (asymmetric model) (Osz et al., 2012; Rochel et al., 2011). On the side of co-

repressors, a complete structural study on the interaction of N-CoR NRID with RXR/RAR allowed us to show an equilibrium between a major population of asymmetric binding of the co-repressor to RXR/RAR and a minor population of doubly bound N-CoR in which both CoNR-boxes simultaneously interact with the heterodimer, accounting for the cooperativity of the interaction (Cordeiro et al., 2019). The binding mode of co-activators remains unclear due to the inherent disorder of NRIDs and the presence of several similar (or equivalent) motifs within this domain even if affinities toward RAR and RXR vary between the different NR-boxes (Pogenberg et al., 2005). The presence of a variable number of boxes in NRID of co-regulator proteins found in different eukaryotes might suggest that the disordered part acts as an entropic chain in order to have an efficient cooperative binding to heterodimeric NRs (Van Der Lee et al., 2014).

Recently, TIF2 NRID (residues 624 to 823) was assigned by Nuclear Magnetic Resonance (NMR) in order to study its interaction with RXR/PPAR heterodimer (de Vera et al., 2017). By combining NMR and hydrogen/deuterium exchange mass spectrometry, the authors showed that all three NR-boxes interact with the heterodimer. However, the atomistic details of this interaction could not be unveiled. Thus, in order to gain insights into the molecular mechanism of NR regulation, we have structurally characterised the human co-activator TIF2 NRID fragment (from residues 624 to 773, hereafter named TIF2_{NRID}) and its interaction with RXR/RAR heterodimer. This minimal fragment encompasses the three NR-boxes of the protein (Figure 1), and is still functional in terms of interaction with NRs (Leers et al., 1998; Voegel, 1998). To highlight its conformational preferences, we have used multiple NMR observables including backbone chemical shifts, ¹⁵N NMR relaxation parameters and ¹D_{NH} residual dipolar couplings (RDCs), in combination with other biophysical techniques and computational tools (Sibille and Bernado, 2012). Altogether, the results are consistent with a largely disordered protein containing several transiently structured elements. In addition to the three NR-boxes, supplementary upstream and/or downstream regions with conformational preferences are identified within TIF2_{NRID}. We show that these flanking regions play a relevant role in the interaction with the heterodimer. The crystallographic structures of RAR and RXR with an extended peptide including NR-box2 and the downstream flanking region unveil a mechanism of specificity in the interaction of co-activators with NRs. Finally, NMR interaction experiments show that the three TIF2_{NRID} NR-boxes are involved in the interaction through cooperative mechanism enabled by the inherent flexibility within the co-regulator. Our study provides new insights into the structural bases of the multifaceted transcriptional regulation mechanism of NRs by co-regulators.

RESULTS

TIF2_{NRID} is an intrinsically disordered protein with partially structured elements

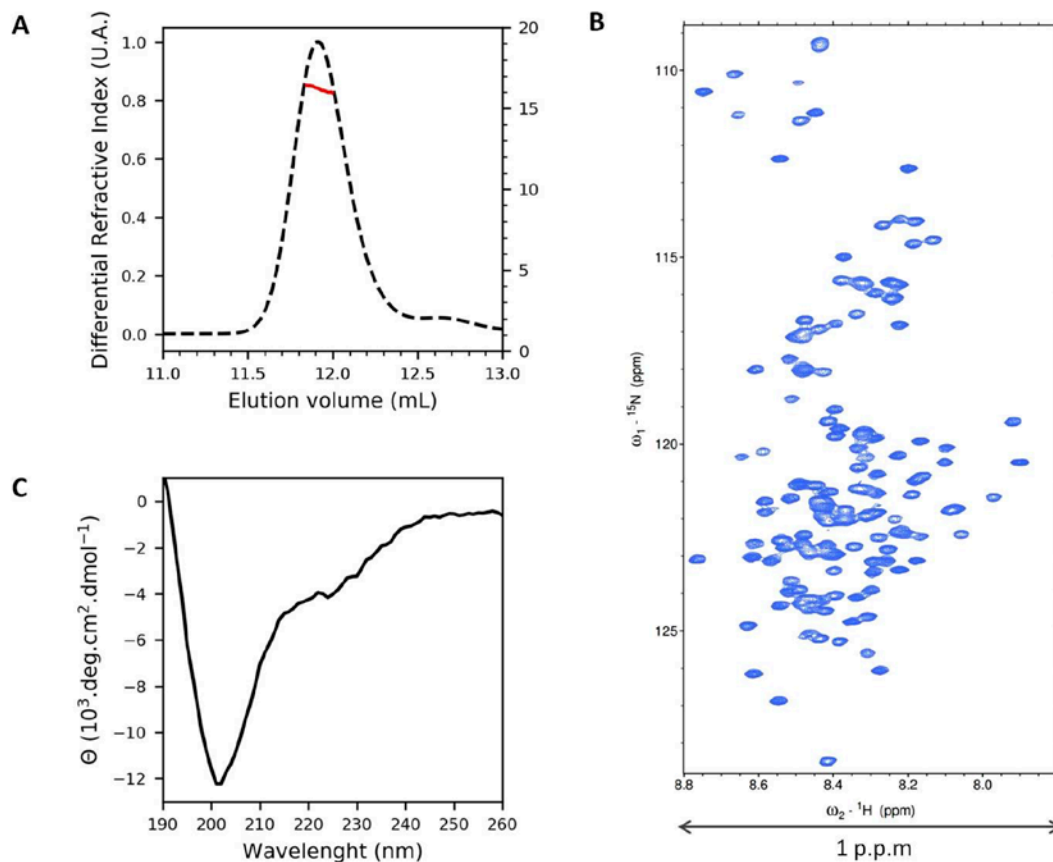
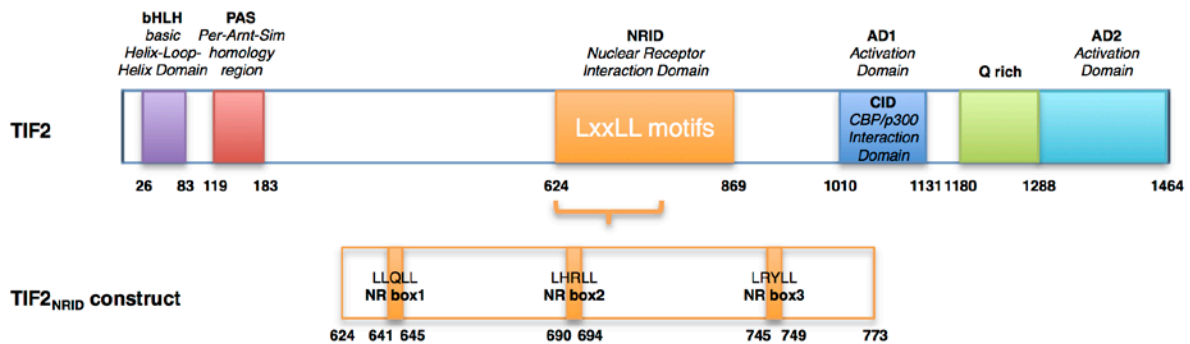


Figure 1. TIF2_{NRID} is monomeric, disordered and contains secondary structure elements in solution. Top panel) Schematic representation of the functional domains identified in TIF2. The TIF2 sequence contains several functional domains including a basic helix–loop–helix (bHLH) similarity domain; a Per-Arnt-Sim (PAS) homology domain; a nuclear receptor interacting domain (NRID); a glutamine-rich (Q rich) domain and two autonomous activation domains (AD): a CBP interaction domain (CID/AD1) to recruit the transcriptional co-activators CBP or p300, and the AD2 to recruit an arginine methyltransferase (CARM-1) (Chen et al., 2000; Teyssier et al., 2002; Voegel, 1998; Voegel et al., 1996). TIF2_{NRID} construct (residues 624 to 773) contains the three LxxLL (i.e. LLQLL, LHRLI and LRYLL) NR binding motifs (NR-boxes 1-3) to NRs. **A)** Size exclusion chromatogram of TIF2_{NRID} (dashed line and left axis), and molar mass derived from MALS (thick line and right axis) at room temperature in the NMR buffer. **B)** TIF2_{NRID} backbone ¹⁵N-HSQC of ¹⁵N/¹³C TIF2_{NRID}. **C)** CD spectrum of TIF2_{NRID} at 10°C in the NMR buffer.

We produced a fragment of the co-activator TIF2 spanning from residues Glu624 to residue Thr773. This construct encompasses the three nuclear receptor binding motifs, NR-box1 (from 641 to 645), NR-box2 (from 690 to 694) and NR-box3 (from 745 to 749) (Figure 1, top panel).

Biophysical characterisation of TIF2_{NRID}. The recombinant protein TIF2_{NRID} elutes from a SEC column as a single peak (Figure 1A) at a volume that corresponds to an apparent molar mass of 45 kDa according to the molecular weight standards. However, the mass derived from MALS analysis is 16.4 kDa ($\pm 0.5\%$), in agreement with the expected theoretical mass of 16.6 kDa, demonstrating that TIF2_{NRID} is monomeric in solution. The elution volume is smaller than that of a globular protein of the same weight, a feature that is typical of either a folded protein with an elongated shape or a disordered one (Uversky, 2012). The low backbone amide ¹H chemical shifts dispersion in the ¹⁵N-HSQC spectrum of TIF2_{NRID} is also a signature of the disordered nature of TIF2_{NRID} (Figure 1B). Finally, Far-UV CD spectrum of TIF2_{NRID} recorded at 283 K shows that the TIF2_{NRID} adopts a random coil conformation with some secondary structure contributions with a minimum shifted above 200 nm rather than at 198 nm. Furthermore, a shoulder around 220 nm, which is a typical signature of helical regions, is also observed (Figure 1C) (Woody, 1996, 1992). SAXS data were collected in order to probe the overall properties of TIF2_{NRID} in solution. The SAXS profile of TIF2_{NRID} (Figure S1A) and its Kratky representation (Figure S1B) with no clear maximum and a monotonic increase along the momentum transfer range, are typical of a disordered protein. Guinier's analysis of the initial part of the curve ($sR_g < 1.3$; where s is the momentum transfer and R_g is the radius of gyration) indicates that TIF2_{NRID} has an R_g of 37.4 ± 0.09 Å. This value is slightly larger than that expected for an IDP of 154 residues ($R_g^{RC} = 35.2$ Å) confirming that TIF2_{NRID} transiently adopts secondary structures that extend its overall shape (Bernadó and Blackledge, 2009; Jensen et al., 2009). Altogether, these data confirm that TIF2_{NRID} is essentially disordered although it contains low populations of structured elements.

Bioinformatics characterisation of TIF2_{NRID}. Consistently with experimental data presented above, bioinformatic analysis of TIF2_{NRID} sequence using various computational tools (Table S1) indicated that TIF2_{NRID} is characterized by a high intrinsic disorder propensity and also revealed some structural features. First, the amino acid composition of TIF2_{NRID} shows a low content (~ 22%) of order-promoting residues (W, F, Y, C, V, I and N), and a high content (~ 67%) of disorder-promoting ones (Q, G, K, S and P), which is a typical feature of IDPs (Figure S2A) (Dunker et al., 2001; Uversky, 2013, 2011). Also, according to the charge-hydropathy plot, TIF2_{NRID} lies on the boundary between globular and disordered proteins (Uversky et al., 2000), suggesting the presence of some structuration in the protein (Figure S2B). The degree of disorder for TIF2_{NRID} was predicted using several servers as shown in Figure 2A; all predictors indicated that TIF2_{NRID} is globally disordered except for regions encompassing the three NR-boxes (i.e. residues L641-L645, L690-L694 and L745-L749). In addition, secondary structure predictions highlighted the existence of helical secondary structure elements in the three NR-boxes, as expected from the literature (Chandra et al., 2017; Heery et al., 1997; le Maire et al., 2010; Nolte et al., 1998; Pogenberg et al., 2005), although with different boundaries (Figure 2B). Additional secondary structure elements were also predicted for NR-box2 and NR-box3 flanking regions (P700-A710 and E727-K739, respectively). Interestingly, regions predicted to have a higher tendency of being ordered/structured were found to be evolutionary conserved, according to the

GREMLIM sequence conservation prediction (Figure 2C). Overall, bioinformatics analyses converge in showing that TIF2_{NRID} is an IDP with partially structured regions, the three NR-boxes and their flanking regions, that in addition show evolutionary conservation. These characteristics suggest an important functional role for these newly identified regions.

The structural and dynamic characterization of TIF2_{NRID}, at the residue level by NMR highlights the importance NR-box flanking regions

In order to identify structural features at the residue level, the NMR study of TIF2_{NRID} in solution was performed (Figure S3) thanks to the almost complete peak assignment of the protein (93% of residues of its primary sequence: i.e. 133 of 143 expected NMR peaks of the 154 residues as the 10 prolines and the first residue G1 are not visible in an ¹⁵N-HSQC).

Secondary structure propensity and dynamic features of TIF2_{NRID}. Quantitative analysis of the secondary structure populations using the secondary structure propensity (SSP) algorithm (Marsh et al., 2006) and backbone secondary chemical shift exploration of TIF2_{NRID} shown in Figure 2D and Figure S4, respectively, are consistent with a mostly random coil polypeptide chain (Tamiola et al., 2010), and report multiple weakly populated segments containing secondary structures (see below). Further evidence for transient secondary structure elements came from ³J_{HNHA} scalar coupling that strongly correlate with backbone dihedral Φ angles (Vuister and Bax, 1993). These couplings (Figure S5) are consistent with an overall unstructured protein (65% of the residues) and smaller proportions of helical type (34%) and extended (1%) conformations. Regarding TIF2_{NRID} backbone dynamics (Kosol et al., 2013), the heteronuclear NOEs (Figure S6A) display the typical bell shape profile of an IDP with small but positive values (average of 0.21 +/- 0.02), with the exception of the highly flexible N- and C-termini, which present negative values. Moreover, ¹⁵N longitudinal (T₁) profile is relatively smooth along the sequence with an average value of 572 +/- 20 ms (Figure S6B), whereas more variability is observed for the transversal (T₂) relaxation times (Figure S6C) and, consequently, for T₁/T₂ ratios (Figure 2E), with observed average values of 219.2 ± 20 ms and 2.88 +/- 0.15, respectively. Interestingly, these observations indicate restricted mobility in regions identified as partially structured by chemical shifts and bioinformatics analyses. Concretely, the region G680-S697, which contains NR-box2, and more significantly the region E741-D750, which contains NR-box3, display helical propensities (< 15% and < 25%, respectively). For NR-box1, the helical tendency is shifted upstream of the LxxLL motif. Remarkably, the highest helix propensity appears for the flanking region (P700-A710) downstream of the NR-box2 (named α -helical flanking region, see below). Interestingly, residues in the three NR-boxes and the α -helical flanking region present large T₁/T₂ values suggesting chemical exchange phenomena due to conformational fluctuations on the μ s-ms time-scale. In addition, SSP analysis highlights extended structures at TIF2_{NRID} C-terminus (G758-T774) and upstream NR-box3 (E727-K739).

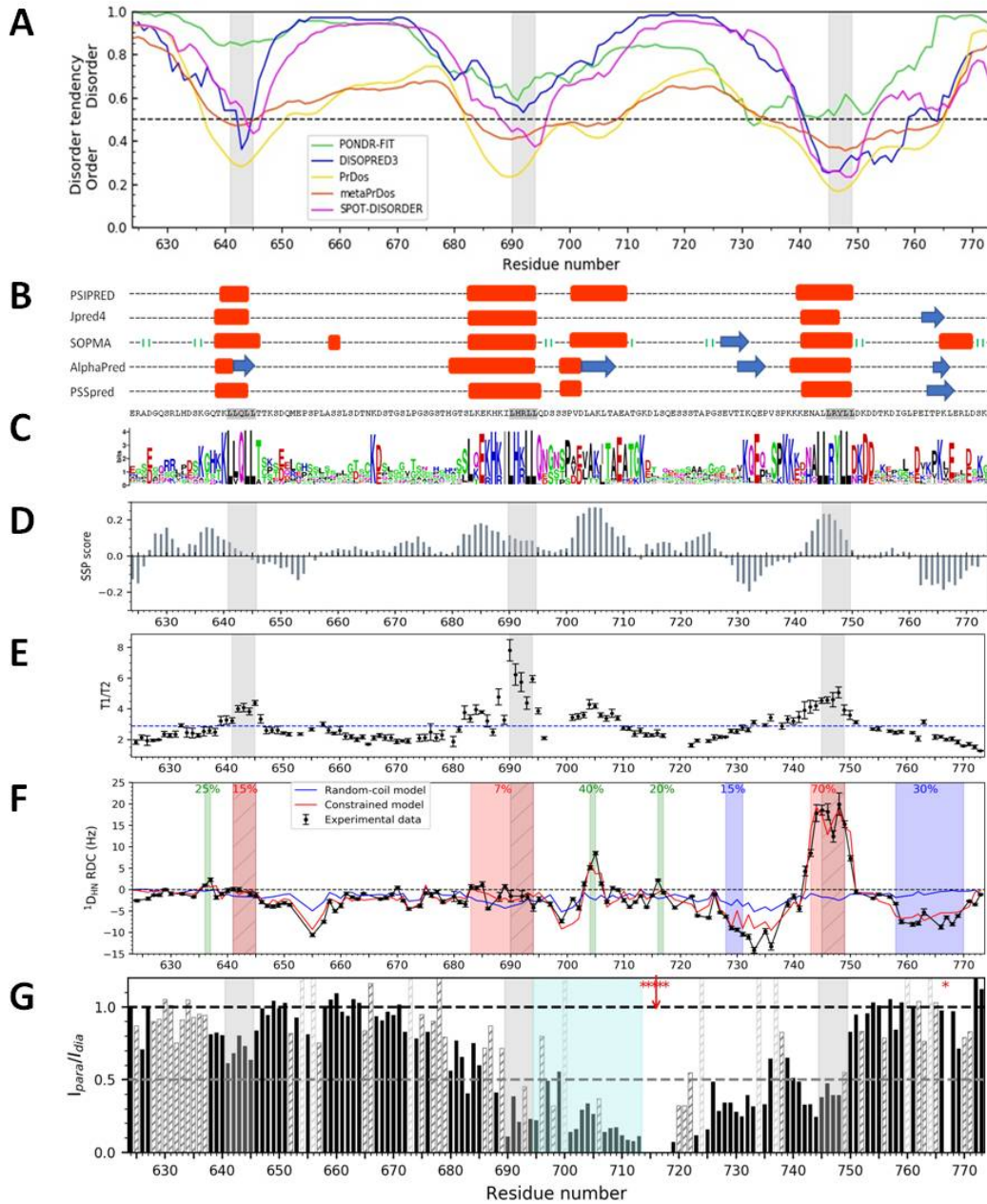


Figure 2. Bioinformatic predictions, secondary structure consensus, backbone dynamics, long-range contacts of TIF2_{NRID}. **A**) Disorder predictions from POND-R-FIT (green line), meta-PrDOs (blue line), DISOPRED3 (red line), PrDos (yellow line) and SPOT-DISORDER (magenta line) servers. Lower values indicate greater likelihood of being structured (scores < 0.5; dashed line). **B**) Secondary structure predictions from Psipred, Jpred4, SOPMA, AlphaPred and PSSpred servers. Predicted α -helices (red boxes), β -strands (blue arrows) and turns (green bars). **C**) Sequence logo conservation from GREMLIN analysis of a multiple sequence alignment of 86 sequences (Table S2). Letter height indicates the relative frequency and conservation in the alignment. **D**) Secondary structure propensity (SSP) obtained from experimental C^α and C^β chemical shifts (positive and negative scores for propensity to form, respectively α -helices and β -strands). **E**) ^{15}N relaxation times along the sequence: T_1 (longitudinal) over T_2 (transverse) (blue dashed line represents the average value). **F**) Back-calculated and experimental $^1\text{D}_{\text{HN}}$ RDCs of TIF2_{NRID}. Comparison of the experimental $^1\text{D}_{\text{HN}}$ RDCs measured in alcohol mixture (black line) with back-calculated values from a completely random-coil ensemble generated with FM (blue line) and from an ensemble including conformational preferences (red line) populated as indicated in the figure (regions shaded in red (helical structure), blue (extended structure) or green (α -turn)). **G**) PRE data of TIF2_{NRID} S716C mutant. Values were calculated from the ratio of intensity ($I_{\text{para}}/I_{\text{dia}}$) of ^{15}N -HSQC spectra measured for the paramagnetic (I_{para}) sample in the position indicated with a red star, and for the diamagnetic sample (I_{dia}). Dark grey and light grey-hashed bars correspond to overlapped peaks and prolines residues, respectively. Star labelled residues correspond to disappearing peaks. NR-boxes (LxxLL motifs; i.e. residues L641-L645, L690-L694 and L745-L749) are highlighted in grey or hashed (F).

Ensemble description. To further study the presence of transient structured regions in TIF2_{NRID}, we analysed ¹D_{HN} RDCs measured in two different media (alcohol mixture and filamentous Pf1). While ¹D_{HN} RDCs measured in alcohol mixture (Figure 2F, black line) are mainly negative, as normally observed in disordered proteins, segment-encompassing NR-box3 displays a large positive value. As we found the same features for RDCs measured in Pf1, which does not interact with the protein, we concluded that the helical propensity in the NR-box3 fragment was present in solution but was slightly amplified by the alcohol alignment medium, which displays very small perturbations in the chemical shifts (0.04-0.05 ppm) only in this specific region. To precisely localized transient conformations present in TIF2_{NRID}, we evaluated deviations of the experimental RDC profile from that of a fully disordered model computed from 100,000 conformations built with Flexible-Meccano (FM) (Bernado et al., 2005; Ozenne et al., 2012), (Figure 2F, blue line). Several regions presenting disagreement between experimental and back-calculated RDCs were identified: the NR-box3 (L745-L749) and its flanking regions (E727-K739), the C-terminal region (G758-T774) and, to a lesser extent, the central NR-box2 plus the subsequent α -helical flanking region (L683-L706). Most of these regions were predicted to adopt secondary structures according to bioinformatic and SSP analysis of TIF2_{NRID} (see above), and thus were used to build structurally biased ensembles of 100,000 conformers by imposing previously predicted/determined secondary structure populations. The quality of these ensembles was evaluated by comparing their back-calculated RDCs with the experimental ones (Figure 2F, red and black lines, respectively). The best agreement was found by imposing: 15%, 7% and 70% of α -helix in L642-L645 (NR-box1), L683-L694 (upstream extended NR-box2) and A743-L749 (upstream slightly extended NR-box3) segments, respectively; and 15% and 30% of β -strand in V728-K731 (up) and G758-D770 (downstream NR-box3) segments, respectively. Additionally, 25%, 40% and 20% α -turn torsion angles for residues K736-G737, A704-K705 (in the α -helical flanking region) and S716-Q717, respectively, were also imposed. Introduction of these secondary structure preferences resulted in a much better description of the entire chain. Furthermore, SAXS was used to characterize the conformational space sampled by TIF2_{NRID} in solution and to validate the structure ensemble derived from the RDC data. After adding side-chains to 2,000 conformers from the RDC-refined ensemble, the average theoretical SAXS profiles was calculated and compared with the experimental one (represented by black lines in Figure S1A). An excellent agreement between both curves was observed ($\chi^2 = 1.38$), which notably improved that obtained from a pure random coil model ($\chi^2 = 1.57$) (data not shown).

Intramolecular long-range contacts. Finally, the presence of long-range contacts in TIF2_{NRID} was explored using Paramagnetic Relaxation Enhancement (PRE) NMR experiments. As TIF2_{NRID} does not contain any native cysteine, Ser716, which lies just after the partially structured flanking region at the C-terminus of NR-box2 (α -helical flanking region), has been mutated to a cysteine (S716C) and subsequently labelled with a paramagnetic probe (nitroxide radical *MTSL*) to measure residue-specific PRE ratios (I_{para}/I_{dia}) (Figure 2G). Excluding residues directly adjacent to C716, the mapping shows a significant intensity reduction ($I_{para}/I_{dia} < 0.50$) for the region encompassing the NR-box 2 until NR-box 3, indicating the presence of extensive long-range contacts in the C-terminus of TIF2_{NRID}. Interestingly, PREs measured in the segment containing the NR-box2 and its downstream flanking

region (shaded in light blue) display a bell-shape with stronger PRE effects in both partially structured regions suggesting a transient interaction between those two helical regions (L690-L694, NR-box2 and P700-A710, α -helical flanking region) in the free state.

Cooperative interaction with RXR and RAR within TIF2_{NRID}

Ligand modulation of the TIF2 affinity for RAR/RXR. Firstly, we measured the affinity of TIF2_{NRID} for the heterodimer RXR/RAR in different liganded states (Figure 3 and Figure S7). In the absence of ligand, the affinity is low ($K_d = 1.34 \mu\text{M}$) but higher than the one measured with the equivalent fragment of the co-activator SRC1 (Pogenberg et al., 2005) ($K_d = 4.80 \mu\text{M}$), suggesting a preference of the heterodimer for the co-activator TIF2. The addition of RAR or RXR agonists (AM580 and CD3254, respectively) significantly increases the affinity of the liganded heterodimer for TIF2_{NRID}, although more efficiently for the RAR agonist. Similar measurements done in the presence of RAR and RXR antagonists (BMS614 and UVI3003, respectively) confirmed the predominant role of RAR in the interaction with the co-activator. In fact, the simultaneous addition of a RAR agonist (AM580) and RXR antagonist (UVI3003) still allows a strong interaction of the liganded heterodimer with TIF2_{NRID} ($K_d = 0.2 \mu\text{M}$). On the contrary, the affinity of the heterodimer for TIF2_{NRID} in the presence of a RAR antagonist (BMS614) and RXR agonist is nearly equal as for the unliganded state. Finally, a cooperativity in the interaction is observed in the presence of both agonists, suggesting that at least two interaction motifs of TIF2_{NRID} can be simultaneously involved in the interaction with the fully activated heterodimer.

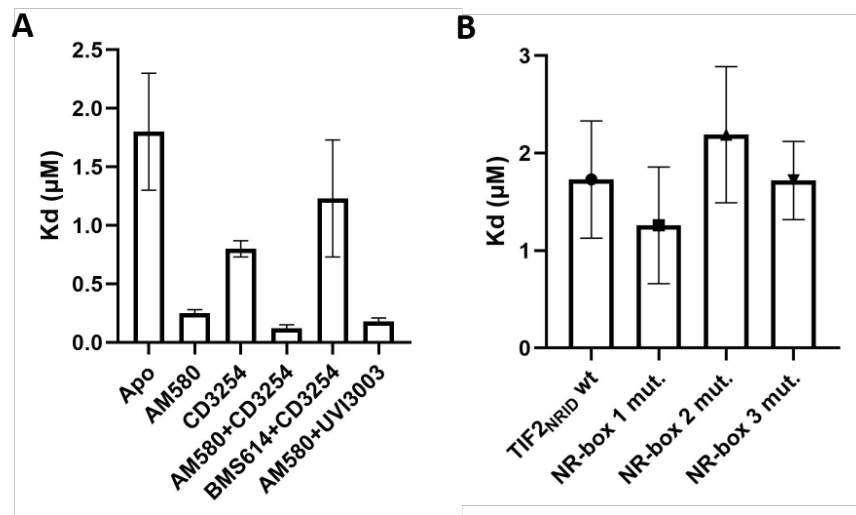


Figure 3: Recruitment of TIF2_{NRID} by the heterodimer RXR/RAR LBDs. **A)** Dissociation constants derived from titration of FITC-labelled TIF2_{NRID} by RXR/RAR in the absence of ligand (Apo) or in the presence of RAR agonist (AM580), RXR agonist (CD3254), or combination of ligands including RAR antagonist (BMS614) and RXR antagonist (UVI3003). **B)** Dissociation constants derived from titration of Alexa-labelled TIF2_{NRID} LXXAA mutants by RXR/RAR in the absence of ligand.

NMR exploration of the TIF2:RAR/RXR complex. To map TIF2_{NRID} regions affected by the interaction with the heterodimer RXR/RAR (Figure 4), ¹⁵N-TIF2_{NRID} was incubated with unlabelled RXR/RAR (RXR/RAR:TIF2_{NRID} molar ratio of 1.1:1) using concentrations around the K_d of the interaction measured by fluorescence anisotropy (Figure 3). Comparison of ¹⁵N-HSQC spectra of ¹⁵N-TIF2_{NRID}

with and without the apo RXR/RAR LBD heterodimer (Figure 4A) shows that addition of RXR/RAR LBD resulted in the attenuation or disappearance of several peaks, confirming the interaction of TIF2_{NRID} with the heterodimer. Although to a different extent, a clear decrease of peak intensity appears in the ¹⁵N-HSQC of TIF2_{NRID} upon binding to RXR/RAR for residues within the three NR-boxes and also for their flanking residues. More precisely, the decrease in intensity and thus interaction is localized around the NR-box1 (T639-K648) and NR-box3 that extends towards its C-terminal flanking region (K739-T763). Notably the NR-box2 binding region extends towards both flanking regions (spanning from G680 to E718), especially the downstream one (P700-A710, α -helical flanking region) (Figure 4B). Indeed, the strongest attenuation with peaks vanishing from the spectrum happens for residues within NR-box2 and its flanking regions, (Figure 4A), in accordance with higher affinity of RAR for NR-box2 of co-activator (Pogenberg et al., 2005). Note that these regions involved in the interaction were identified by NMR relaxation and secondary structure analysis as transiently structured with chemical exchange phenomena in unbound TIF2_{NRID}. These results clearly demonstrate that all three LxxLL motifs are involved in the interaction with the heterodimer, highlight the leading role of the NR-box2 and the participation of several flanking regions in the heterodimer recognition.

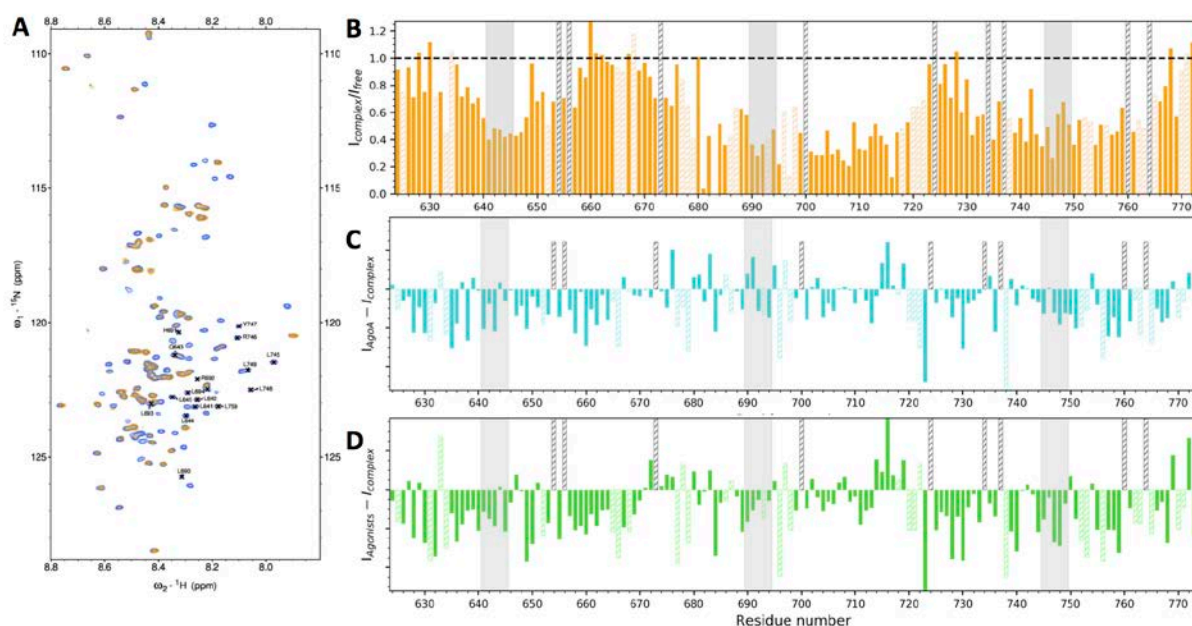


Figure 4. Interaction of TIF2_{NRID} with RXR/RAR LBDs and agonists effects. **A)** Superposition of ¹⁵N-HSQC spectra of 5 μM of ¹⁵N labelled TIF2_{NRID} in absence (blue) and in the presence (orange) of 6 μM of unlabelled RXR/RAR LBDs. Residues belonging to NR-boxes are indicated. **B)** Relative peak intensity ratios $I_{\text{Complex}}/I_{\text{TIF2NRID}}$ between the two spectra presented in A. **C)** and **D)** Peak intensity differences between ¹⁵N-HSQC spectra of RXR/RAR:TIF2_{NRID} complex (1.1:1) in the presence of 1.2 molar excess of RAR agonist AM580 (cyan, C); then followed by the addition of 1.2 molar excess of RXR agonist CD3254 (green, D). Bars highlighted in grey indicate NR-boxes. Dashed bars correspond to prolines (grey) or ambiguous and overlapped peaks (coloured).

To monitor the effect of ligands on the interaction, RAR and RXR agonists (AM580 and CD3254, respectively) were sequentially added to RXR/RAR:TIF2_{NRID} sample and changes in TIF2_{NRID} peak intensities in ¹⁵N-HSQC spectra were monitored (Figure 4C and 4D). As shown in Figure 4C, addition of RAR agonist induces a slight decrease of peak intensity relatively to the apo complex for residues belonging to NR-boxes1 and 3 but more significantly for their flanking residues. The strongest attenuation appears for NR-box3 flanking regions whereas NR-box2 interacting region seems not to

be further affected by addition of the ligand. We observed more accentuated changes in intensity after addition of the second ligand (RXR agonist) (Figure 4D). These data indicate that the presence of agonists into the two subunits of the heterodimer strengthens its interaction with TIF2_{NRID} by increasing the cooperativity of the interaction involving the different NR-boxes and their flanking regions. Therefore, NMR data are in agreement with affinity measurements (Figure 3A), showing that the presence of RAR agonist leads to a 9-fold decrease of the K_d value with respect to the value of the apo, and it is further increased, although moderately, in the additional presence of RXR agonist.

Binding mode of TIF2 to RXR/RAR heterodimer. To further understand the role of the three individual NR-boxes in the interaction of TIF2_{NRID}, NMR interaction experiments were performed with RXR/RAR of three TIF2_{NRID} LL/AA double mutants in which the LxxLL motifs of NR-boxes-1, -2 and -3 were individually mutated into LLQA₆₄₄A₆₄₅, LHRA₆₉₃A₆₉₄ and LLRYA₇₄₈A₇₄₉, respectively. Note that these mutations strongly diminish the interaction of the individual boxes with the heterodimer (Germain et al., 2002). The affinities of each TIF2_{NRID} mutant for the RXR/RAR heterodimer in the absence of ligand were measured by fluorescence anisotropy (Figure 3B and Figure S7). Interestingly, no significant changes with respect to the wild-type were observed, suggesting that the presence of only two NR-boxes in TIF2_{NRID} is sufficient for the interaction with the heterodimer. Similarly, NMR intensity profiles demonstrated that even if one interaction box is suppressed by the double mutation, the two other ones take over the interaction (Figure 5). For instance, NR-box1 mutant showed that while the mutated NR-box1 was not interacting with the heterodimer, the entire region encompassing NR-box2 and NR-box3 presents a strong decrease of peak intensities (Figure 5A). An identical behaviour was observed when mutating NR-box3 (Figure 5C). For NR-box2 mutant, NR-box1 takes over less clearly even if we still see a decrease in intensity that confirms its involvement (Figure 5B). This result is corroborated with fluorescence anisotropy measurement (Figure 3B) where we see that NR-box2 mutant loses more affinity for RXR/RAR than the two other NR-box mutants, which still contain the NR-box2. These results substantiate the contribution of the three NR-boxes in the interaction with RXR/RAR heterodimer with a dominant role of NR-box2.

Finally, in order to further disentangle the sequential interacting mode of the three NR-boxes and their flanking regions with the heterodimer, we performed NMR mapping interaction of TIF2_{NRID} and its three mutants with RAR and RXR separately (Figure S8). In the absence of agonist, the interacting profiles of TIF2_{NRID} WT and mutants are the same with RAR LBD alone, whereas they show no interaction with the RXR LBD. Addition of specific agonist towards RAR LBD domain increases the NR-box3 binding to RAR for WT or mutants containing the LxxLL motif in NR-box3, whereas RXR agonist towards RXR LBD domain increases the binding of TIF2_{NRID} WT in a non-specific manner, but no interaction is detected if one of the NR-box is suppressed by mutation. These results reinforce the idea that the specific and dominant interaction of NR-box2 towards RAR is necessary to properly drive the cooperativity of the interaction.

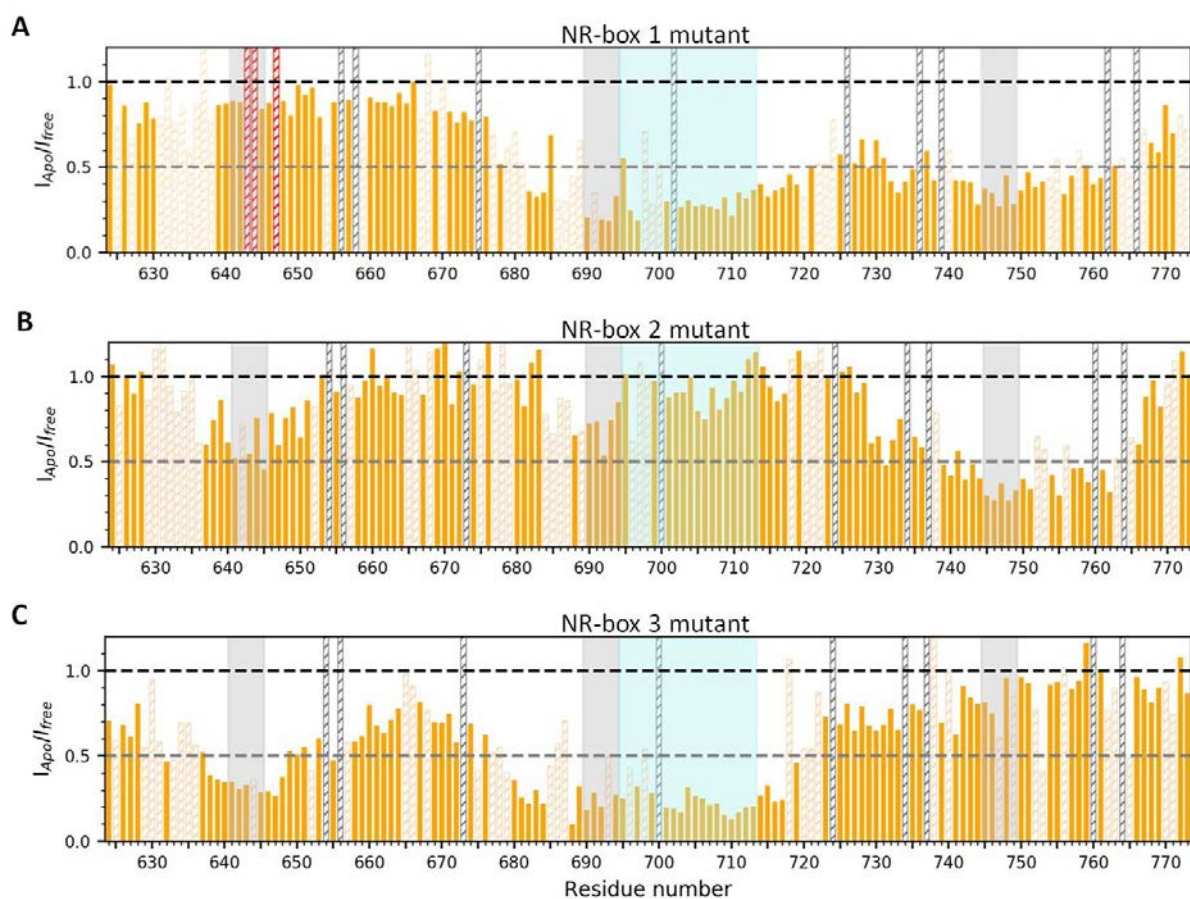


Figure 5. Interaction of individual TIF2_{NRID} NR-box mutants with unliganded RXR/RAR LBDs heterodimer. Relative peak intensity ratios of ¹⁵N-HSQC spectra of 5 μM of ¹⁵N labelled TIF2_{NRID}-NR-box mutant in absence (I_{free}) and in the presence (I_{Apo}) of 6 μM of unlabelled RXR/RAR LBDs. **A)** NR-box1 mutant. Red hashed bar correspond to non-assigned peaks, **B)** NR-box 2 mutant, **C)** NR-box3 mutant. Grey highlight boxes indicate NR-boxes. Hashed bars correspond to prolines (grey) or ambiguous and overlapped peaks (orange).

A new interaction motif of TIF2_{NRID} with RAR that could account for NR specificities in the different co-activator paralogues.

NMR experiments have highlighted the involvement of NR-boxes flanking regions of TIF2_{NRID} in its interaction with RXR/RAR heterodimer (Figure 4). Of particular interest, the region following NR-box2, spanning from residues 700 to 710, shows a high propensity to be ordered (Figure 2D) and a high sequence conservation (Figures 2A-C), suggesting a particular functional role. To evaluate the putative involvement of this sequence in the interaction with the receptors, we prepared crystals of both agonist-bound RAR and RXR LBDs in complex with a TIF2 peptide comprising the NR-box2 (NR2) and its C-terminal α-helical flanking region spanning from residue K686 to K713 (here after named Ext for extended part). Data collection and refinement statistics are summarized in Table S3. The 2.4 Å resolution crystal structure of RAR LBD in complex with AM580 and TIF2 NR2-Ext displays two complexes per asymmetric unit, both showing that the interaction of the LHRLI helix of NR-box2 (named α1) with the co-activator binding groove of the receptor is preserved and that the extension folds as a long α-helix (residues P700 to T711, named α2) making hydrophobic contacts with residues from both helix α1 and RAR (Figure 6A and Figure S9). In particular, L703, L706, T707, E709 and A710 lie on the same side of α2 and make hydrophobic interactions with I689, R692 and L693 of NR2

$\alpha 1$, and with T233, I237 and L409 from RAR. In addition, a weak electrostatic interaction (4.0 Å in length) between R692 ($\alpha 1$) and E709 ($\alpha 2$) may stabilise further the positioning of helix $\alpha 2$ (Figure 6B). Interestingly, using fluorescence anisotropy we were able to show that the TIF2 NR2-Ext peptide has a three-fold higher affinity for the agonist-bound RAR LBD compared to the shorter TIF2 NR2 peptide (Figure 6C), confirming the involvement of $\alpha 2$ in the interaction between TIF2 and RAR. Conversely, similar experiments with RXR suggested that $\alpha 2$ plays no or very little role in the interaction with this receptor as the short and longer versions of TIF2 peptides display very similar affinities (Figure 6C). Competition experiments of each unlabelled peptide against a fluorescently labelled SRC1 NR2 peptide allowed us to confirm that RAR interacts more avidly with TIF2 NR2-Ext whereas RXR shows no preference (Figure S10). Next, we solved the crystal structure of RXR LBD in complex with the TIF2 NR2-Ext peptide and the agonist LG100268 at a resolution of 2.8 Å (Figure 6D). The structure shows two molecules of liganded-RXR LBD per asymmetric unit, each bound to one TIF2 NR2-Ext peptide. Whereas in both complexes TIF2 NR2 ($\alpha 1$) interacts classically with RXR, the extension is either disordered (Figure 6D) or not visible (not shown), confirming that it is not involved in the interaction with RXR. The superposition of RAR and RXR LBD structures reveals that steric hindrance generated by the two large aromatic RXR residues F277 and F450 (corresponding to I237 and L409 in RAR) could account for the differential involvement of the extension in the interaction with the two receptors (Figure S11). These structural data further substantiate the transient nature of the helical folding of TIF2 NR2 $\alpha 2$, which adopts a helical conformation in complex with RAR but is fully disordered in complex with RXR.

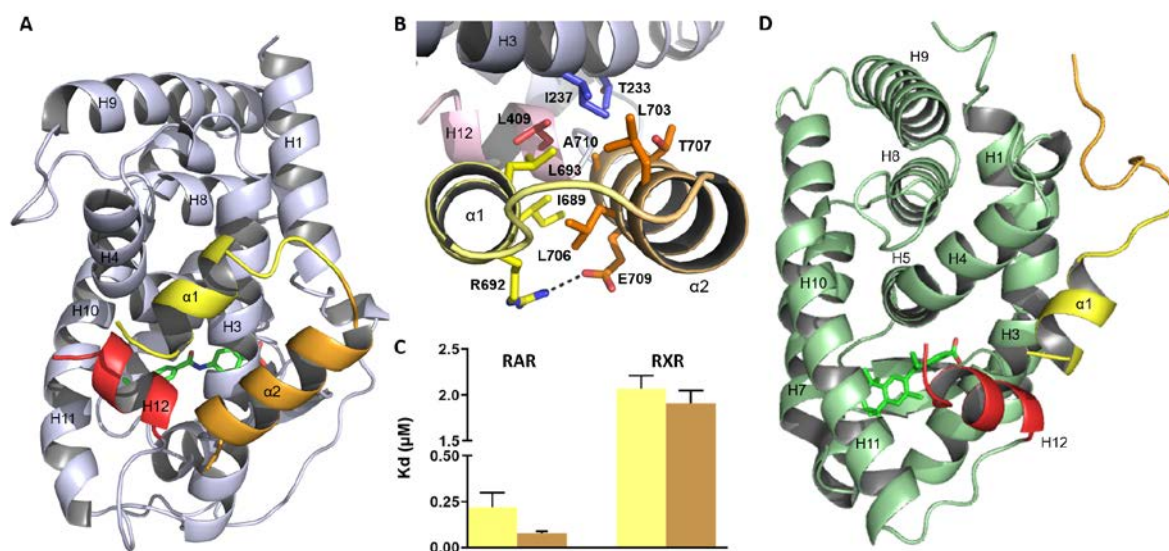


Figure 6. X-Ray crystallographic structures of the TIF2 NR2-Ext peptide in complex with RAR and RXR LBDs. **A)** Overall structure of RAR LBD in complex with the agonist AM580 and TIF2 NR2-Ext. The TIF2 NR2-Ext peptide is shown in yellow ($\alpha 1$) and orange ($\alpha 2$) cartoon and RAR is coloured in light blue with H12 coloured in red. **B)** Close-up view of the interaction surface of the TIF2 NR2-Ext peptide with RAR highlighting the hydrophobic contacts of L703, L706, T707 and A710 from helix $\alpha 2$ with L694, I689 from helix $\alpha 1$ or I237 in H3 and L409 in H12 from RAR. An electrostatic bond between E651 ($\alpha 2$) and R694 ($\alpha 1$) is also observed. **C)** Affinity constants (Kd in μM) deduced from fluorescence anisotropy experiments between either RAR or RXR LBDs and TIF2 NR2 or TIF2 NR2-Ext. **D)** Crystal structure of RXR LBD in complex with TIF2 NR2-Ext and the RXR agonist LG100268.

Having shown the structural properties and functional role of the N-terminal extension of NR2 in TIF2, we next examined whether this feature is unique to TIF2 (NCOA2) or conserved in other co-activator paralogues, namely SRC1 (NCOA1) and RAC3 (NCOA3). We first collected sequences for TIF2, SRC1 and RAC3 of different vertebrate species, from lampreys to humans (Table S4), aligned them and analysed their evolutionary relationships by phylogenetic tree reconstruction (Figure S12). The phylogenetic tree revealed that the vertebrate NCOA paralogues fall into two distinct groups: (i) SRC1 and (ii) TIF2 plus RAC3. Furthermore, within the TIF2 plus RAC3 group, the three representatives of the sea lamprey *Petromyzon marinus*, a jawless vertebrate, formed a separate branch at the base of the clade, which is indicative of an origin by lineage-specific gene duplication. To evaluate, whether the vertebrate NCOA genes have been under positive, neutral or negative selection, we used the full-length NCOA sequences to calculate the substitution rates at non-synonymous and synonymous sites. The resulting dN/dS ratio (or ω value) was well below 1 for all three NCOA paralogues (Figure S12), suggesting that SRC1, TIF2 and RAC3 are under negative (i.e. purifying) selection. We next focused our attention on the TIF2 NR2-Ext sequences and corresponding regions in SRC1 and RAC3. Sequence logos calculated for the three peptides revealed specific evolutionary signatures for each of the NCOA paralogues, with the SRC1 NR2-Ext peptide seemingly less conserved than the corresponding domains of TIF2 and RAC3, a result that was coherent with the phylogenetic tree analysis (Figure 7A and Figure S12). Similar to the results obtained for the full-length NCOA sequences, the dN/dS ratio analysis for the three NR2-Ext domains revealed, once again, a very strong negative (i.e. purifying) selection. However, when comparing the ω values for each of the three NR2-Ext paralogues, we noticed that the ω value for TIF2 was more than three times higher than those for SRC1 and RAC3 (Figure 7A). This suggests that, in evolutionary terms, TIF2 NR2-Ext is subjected to weaker purifying selection than the corresponding domains of its two paralogues, SRC1 and RAC3. Taken together, our sequence analyses are consistent with a conserved role for the NR2-Ext peptides in TIF2 and RAC3, but not in SRC1. To test this hypothesis, we solved the crystal structure of the RXR/RAR LBDs heterodimer bound to RAR and RXR specific agonists and to the SRC1 NR2-Ext peptide (Figure 7B). As expected, the structure shows that the peptide interacts classically with both receptors through the helical LxxLL motif ($\alpha 1$), while the rest of the sequence remains highly mobile with no interpretable electron density on both sides of the dimer. In line with these data, fluorescence anisotropy measurements revealed no noticeable difference in the affinities of SRC1 NR2 and SRC1 NR2-Ext peptides for RAR (Figure 7C). As a whole these data support the notion that NR box flanking regions may play different roles and may drive NR specificities in the different co-activator paralogues.

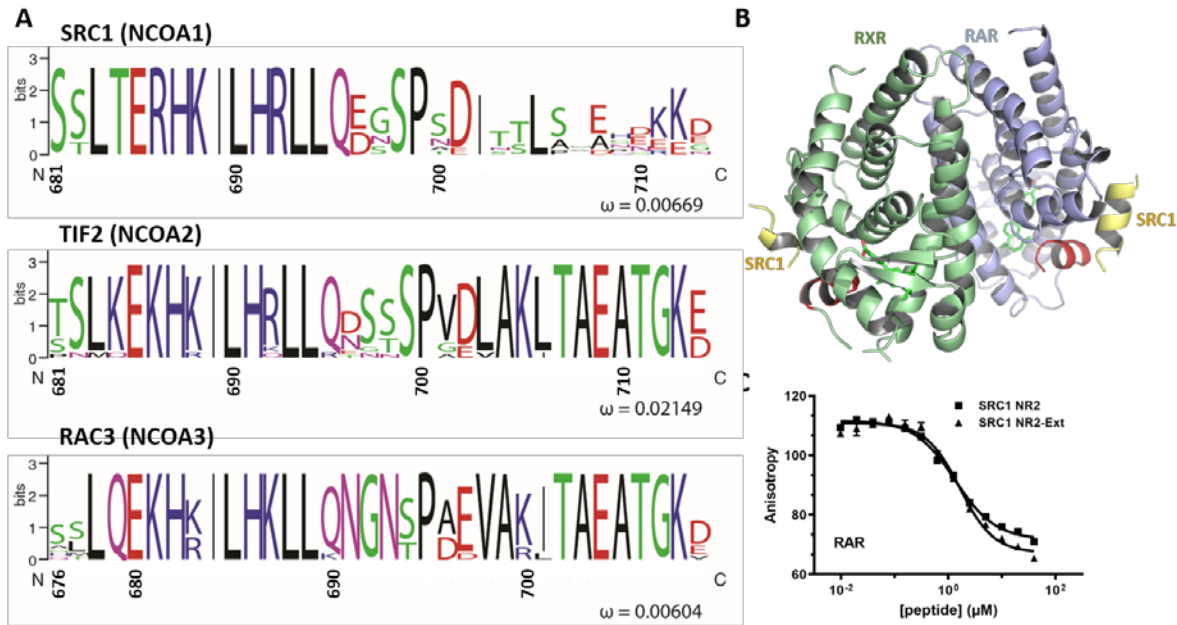


Figure 7: Interaction of SRC1 NR2-Ext peptide with RXR and RAR LBDs. A) Sequence logos for the NR2-Ext peptide sequence of nuclear receptor co-activator (NCOA) paralogues: SRC1 (NCOA1), TIF2 (NCOA2) and RAC3 (NCOA3). The ratio between non-synonymous and synonymous substitutions (ω) is shown for the NR2-Ext peptides of each of the three paralogues. **B)** Crystal structure of RXR/RAR LBD heterodimer in complex with the agonists AM580 and LG100268 and SRC1 NR2-Ext. The only part of SRC1 NR2-Ext peptide visible in the structure is shown in yellow ($\alpha 1$) cartoon, and RXR and RAR are coloured in light green and light blue, respectively, with H12 coloured in red. **C)** Competition curves at 0.5 μM of RAR LBDs of SRC1 NR2 by unlabeled SRC1 NR2 and SRC1 NR2-Ext peptides in the presence of AM580. The derived K_i is nearly the same for the two peptides.

DISCUSSION

Recruitment of co-activators is a critical step in ligand-dependent activation of gene transcription by NRs but little is known structurally about their interactions with NRs and their dynamic properties. In this study, we have first provided a complete structural characterization of the NRID of the co-activator TIF2 (TIF2_{NRID}). Combination of solution-state structural methods and biophysical experiments show that, as suggested by its amino acid sequence and in accordance with a previous study (de Vera et al., 2017), TIF2_{NRID} behaves as an intrinsically disordered protein (IDP) with multiple transiently structured elements. The large conformational heterogeneity of IDPs usually makes their detailed structural characterization challenging, since it requires an ensemble representation to appropriately describe averaged experimental observables (Click et al., 2010). The combination of different high-resolution NMR parameters extracted from TIF2_{NRID}, such as chemical shifts, RDCs and ¹⁵N-relaxation, as well as low resolution information provided by SAXS, together with computationally generated conformational ensembles allowed to precisely highlight functional regions with conformational preferences and rigidity that significantly deviate from pure random-coil. In addition to the three evolutionary conserved NR-boxes that adopt various degrees of pre-structured helices when free in solution (Figure 2), we have identified previously undescribed regions flanking both sides of NR-box2 and NR-box3 with preferential conformations and motional restriction. Notably, the upstream

(T681-I689) and downstream (V701-A710, $\alpha 2$) NR2 flanking regions present a significant degree of evolutionary conservation (Figure 2C) that could anticipate a functional role of these regions.

The interaction mode of the LxxLL motif with NRs is known for some time and has been described in many structural studies (Ahmad et al., 2003; Chandra et al., 2017, 2008; de Vera et al., 2017; Gampe et al., 2000; Hur et al., 2004; le Maire et al., 2010; Liu et al., 2006; Nolte et al., 1998; Pogenberg et al., 2005; Suino et al., 2004; Woody, 1996; Wright and Dyson, 2009). Moreover, several functional and biochemical analyses of the interaction of NRs with different mutated or chimeric LxxLL-containing peptides from TIF2 or SRC1 have suggested that residues immediately flanking the core motif could play important roles in the affinity and, in NR specificity (Chang et al., 1999; Darimont et al., 1998; He and Wilson, 2003; Heery et al., 1997; McInerney et al., 1998). In this line, our structural and biophysical interaction studies highlighted the participation of the pre-structured flanking regions in the interaction of TIF2 with the RXR/RAR LBD heterodimer and gave new insights into the role of these less well-characterized stretches in the specificity of the interaction between co-activators and NRs. More specifically, NMR data showed that the recruitment of TIF2_{NRID} by the RXR/RAR LBD heterodimer is preferentially driven by the interaction between RAR and NR-box2 and its downstream flanking region, $\alpha 2$ (Figure 4B and Figure S8). These data were fully confirmed by a crystallographic analysis revealing that the TIF2 NR2-Ext peptide adopts a helix-turn-helix conformation and that both helices $\alpha 1$ (LxxLL core motif) and $\alpha 2$ (C-terminal flanking region) are involved in the interaction with RAR (Figure 6). Interestingly, the PRE data also suggested that the two helical elements found in the crystallographic structure present transient contacts in the unbound form (Figure 2G), possibly reducing the entropic cost of the recognition event (Mohan et al., 2006; Pancsa and Fuxreiter, 2012). Further sequence, structural and interaction analyses revealed that, through its interaction with RAR, $\alpha 2$ confers higher affinity to the NR-box2 for this receptor, whereas it has no effect on the interaction with RXR. Furthermore, they also showed that the mechanism described here for TIF2, a member of the NCOA2 family of co-activators, is very likely to apply to members of the NCOA3 family, but not to the NCOA1 family members as shown in the present study with SRC1. As a whole, these results provide a structural basis supporting the notion that NR-box flanking regions are involved in NR recognition specificity.

The interplay of the different NR-boxes in the interaction with NR dimers is also a matter of debate. Two models of the interaction between co-activators and NR heterodimers have been previously proposed. In the first one, the so called “asymmetric model”, TIF2 would be recruited preferentially by RAR, most likely using NR-box2, which presents the highest affinity (Osz et al., 2012; Rochel et al., 2011). In the second one, the “deck model”, two NR-boxes can simultaneously recognise the two members of the heterodimer, providing a cooperative binding mechanism (Chandra et al., 2017, 2008; de Vera et al., 2017; Meng et al., 2017; Pogenberg et al., 2005). Our results unambiguously demonstrate that the three NR-boxes can interact with the heterodimer, in line with the interaction of TIF2 with RXR/PPAR (de Vera et al., 2017). Our data strongly suggest a main mechanism on which TIF2_{NRID} is initially recruited by the liganded heterodimer through the NR-box2 and its downstream flanking region ($\alpha 2$) into the hydrophobic groove generated on RAR. This anchoring point restricts the exploration of the conformational space facilitating interaction of the NR-box3 or NR-box1 with RXR.

We used TIF2_{NRID} LxxAA mutants to more precisely access to the role of each NR-box and its flanking regions. Our results suggest that two NR-boxes are sufficient to trigger the cooperative interaction with RXR/RAR. Moreover, we found that the mutation of the NR-box2 is more detrimental to the interaction with RXR/RAR than the mutation of the two other boxes, meaning that the combination of NR-box1 and 3 triggers less cooperative binding than any other combination with NR-box2. Differences in the affinities of the individual NR-boxes for RAR as well as in the length of the entropic chain between interacting NR-box pairs most likely account for this observation. Indeed, the sequence distance between two interacting sites in a disordered chain decreases the effective concentration and inevitably affects cooperativity (Zhou, 2003). This observation advocates for a binding mode encompassing two neighbouring NR-boxes and where the NR-box2 is still the primary driver of the interaction.

In summary, our study delineates the structural bases of the regulation of gene transcription by RAR and highlights the importance of disorder in this process. The presence of multiple NR interaction boxes in co-regulator proteins provides a strong cooperativity in the complex while enabling the capacity to transit from an active to an inactive state or vice versa. Although the mechanisms observed for TIF2 and RAR seem to be general in NRs, specific structural features in the LBDs and co-regulator boxes and associated flanking regions most probably define the efficiency and selectivity of gene transcription.

MATERIAL AND METHODS

TIF2_{NRID} expression, purification and preparation

The TIF2_{NRID} polypeptide studied here corresponds to the NRID fragment of the human TIF2 co-activator (Uniprot entry Q15596-1) ranging from residue 624 to residue 773. Four extra residues (GPHM) at the N-terminal are remaining from the His-tag cleavage site. TIF2_{NRID} recombinant protein is composed of 154 residues with a theoretical molecular weight of 16.6 kDa.

The DNA encoding human TIF2_{NRID}, optimized for bacterial expression (purchased to Integrated DNA Technologies) was cloned (InFusion cloning kit) into a pDB vector between the NdeI and XhoI restriction sites. pDB-TIF2_{NRID} construct codes for a protein containing a hexa histidine-tag and a HRV 3C cleavage site (Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro) at the N terminus, where a specific cleavage occurs between Gln and Gly. Uniformly ¹⁵N- or ¹³C/¹⁵N-labelled recombinant TIF2_{NRID} was expressed in *E. coli* BL21 (DE3), grown in 2 L of LB containing 50 µg/mL of kanamycin at 37°C, harvested at an OD_{600nm} of 3 and resuspended in 2 L of M9 minimal medium with isotopic enrichment (1 g/L of ¹⁵NH₄Cl and/or 3 g/L of ¹³C₆-glucose, Cambridge Isotope Laboratories). After 30-45 min, overexpression was induced with 0.5 mM IPTG for 4 h at 37°C. Cells were harvested and resuspended in 300 mM NaCl, 20 mM Tris pH 7.5, 2 mM DTT (buffer A), plus one tablet of Complete EDTA free protease inhibitor cocktail and sonicated on ice. Cells were pelleted by centrifugation at 18,000 x g at 4°C for 20 min to remove inclusion bodies. The supernatant was clarified by a 5 µm filter followed by a 0.45 µm filter and loaded on a cComplete His-Tag purification Column (Roche) with a column volume (CV) of 1 mL. The

column was washed and equilibrated with buffer A and the protein was eluted with a linear gradient using buffer A supplemented with 300 mM of imidazole in 25 CV. Fractions containing the protein (monitored by SDS-PAGE) were pooled, incubated with GST-tagged 3C protease (at a protease-to-target protein ratio of 1:100 (w/w), to cleave the His-tag), and at the same time dialyzed overnight in buffer A supplemented with 10 mM imidazole at 4°C. To remove proteases and not digested proteins, the pool was loaded in 0.5 mL GST resin mixed with 1 mL of cOmplete His-tag purification resin (Roche) stacked in a 12 mL polyprep column. Resin was washed and equilibrated with 3 CV of buffer A supplemented with 10 mM imidazole. The flow through was collected, concentrated and loaded in a HiLoad 16/60 Superdex 75 PG (GE Healthcare) equilibrated with 150 mM NaCl, 50 mM BisTris pH 6.8, and 0.5 mM EDTA (NMR buffer) at 1 mL/min. The protein eluted after 53 mL of run approximately. The purity of the sample was evaluated by SDS-PAGE analysis. The pure fractions containing proteins were pooled and concentrated by centrifugation using a Vivaspin™ protein concentrator with a 5 kDa cut-off. Protein concentration was determined by UV absorbance at 280 nm using the extinction coefficient calculated from the amino acid composition ($1,490 \text{ M}^{-1} \cdot \text{cm}^{-1}$) and by refractometry (0.1814 mL/g as refractive index according to the software SEDFIT (Schuck, 2000)). The yield of pure doubly labelled protein was around 2.5 mg per liter of bacterial culture. TIF2_{NRID} was stable in solution for months and resisted to un/freezing without detecting signs of degradation.

For paramagnetic relaxation enhancement experiments (PRE), single-cysteine mutant (S716C) of TIF2_{NRID} (corresponding gene purchased to Integrated DNA Technologies) was expressed and purified as the wild type. Paramagnetic spin labelling was realized by incubating the mutant with 20-fold molar excess of nitroxide spin label MTSL (Toronto Research Medical) solubilized in acetonitrile. To remove any trace of DTT, the protein solution was passed through a Zeba™ Spin desalting column (7K) equilibrated in NMR buffer pH 7.5 before labelling. After incubation at 4°C overnight, excess of free MTSL was removed by passage through a Zeba™ Spin desalting column (7K) equilibrated with NMR buffer pH 6.8. The yield of labelling close to 100% was estimated with DTNB assays (Ellman's reagent from ThermoFisher Scientific).

Preparation of LXXAA mutants of TIF2_{NRID}

The motifs in the three TIF2_{NRID} NR-boxes (LxxLL) were double mutated into LxxAA mutants. Each mutant was realized by PCR QuikChange™ Site-Directed Mutagenesis reactions from Agilent™. A pair of complementary oligonucleotides carrying the desired double mutation has been ordered from Integrated DNA Technologies™. The mutated motifs LLQA₆₄₄A₆₄₅, LHRA₆₉₃A₆₉₄ and LLRYA₇₄₈A₇₄₉, were respectively obtained for NR-boxes 1, 2 and 3. All the mutants were isolated and characterized by sequencing. These mutants were purified following the same protocol as wild-type TIF2_{NRID}.

RXR/RAR expression and purification

The RAR LBD studied here corresponds to a 246-residue long fragment (~ 27.8 kDa) of the RAR α protein (50.8 kDa full-length protein amino acid sequence as described in Uniprot entry P10276-1) coded by the human RARA gene. The RXR LBD studied here corresponds to a 241-residue long fragment (~ 26.8 kDa) of the RXR α protein (51.2 kDa full-length protein amino acid sequence as

described in Uniprot entry P28700-1) coded by the mouse RXRA gene. Consequently, the RXR/RAR LBD recombinant heterodimer has a theoretical molecular weight around 54.6 kDa.

The hexa histidine-tagged LBD of human RAR α LBD (residues 176-421 in a pDB-His 3C vector) and mouse RXR α LBD (residues 227-467 in a pET-3a vector) were expressed in *E. coli* BL21(DE3) and copurified. The heterodimer RXR/RAR was prepared as previously described in (Poggenberg et al., 2005) with some modifications. Briefly, after a nickel affinity column (5 mL His Trap FF, GE Lifescience), fractions containing RXR/RAR heterodimer were pooled with 3C to remove the His-tag and dialyzed overnight at 4°C against the NMR buffer. The sample was further purified using a Superdex 75 26/60 gel filtration column (Amersham Biosciences) equilibrated with NMR buffer. The peak fractions were analysed by SDS-PAGE and the fractions containing pure RXR/RAR heterodimer were pooled. Protein concentration was determined by UV absorbance at 280 nm using the extinction coefficient calculated from the amino acid composition ($28,045 \text{ M}^{-1} \cdot \text{cm}^{-1}$) and by refractometry (0.188 mL/g as refractive index according to the software SEDFIT (Schuck, 2000).

Ligands and peptides

All the ligands (AM580, CD3254, LG100268, BMS614 and UVI3003) were purchased from Tocris Bioscience. All compound stock solutions were prepared at 10 mM in DMSO. The peptides TIF2 NR2 (KHKILHRLLDSS and CTSLKEKHKILHRLLDSS), TIF2 NR2-Ext (KHKILHRLLDSSSPVDLAKLTAEATGK and CTSLKEKHKILHRLLDSSSPVDLAKLTAEATGK), FITC-SRC1 NR2 (FITC-LTERHKILHRLDQEGSP), SRC1 NR2 (RHKILHRLDQEGS) and SRC1 NR2-Ext (RHKILHRLDQEGSPDITTLVPEPKK) were purchased from EZbiolab.

Bioinformatics sequence analyses

Amino acid composition of TIF2_{NRID} was analysed using Composition Profiler Tool (Vacic et al., 2007) that detects protein amino acid composition bias by comparing to the average amino acid frequencies reference values of a representative set of folded proteins from the PDB (Berman et al., 2000). Uversky's plot (Charge-Hydrophathy - CH) was generated using PONDR (Romero et al., 2001). The CH border is described by $H=(C+1.151)/2.785$ (Uversky et al., 2000).

Predictions of disorder in the TIF2_{NRID} protein were computed using several tools: the metaPrDOS web server that integrates the results of eight different methods (PrDOS, DISOPRED2, DisEMBL, DISPROT, DISpro, IUPred, POODLE-S and DISOclus) (Ishida and Kinoshita, 2007); the meta-predictor PONDR-FIT that combines the results of six different methods (PONDR-VLXT, PONDR-VSL2, PONDR-VL3, FoldIndex, IUPred, and TopIDP) (Xue et al., 2010); PrDOS a structure-based method (Ishida and Kinoshita, 2008); SPOT-disorder based on a window-based neural network (SPINE-D) (Hanson et al., 2017); and finally DISOPRED3 that combines three machine learning models: support vector machine, neural network and nearest neighbour (Jones and Cozzetto, 2014).

Secondary structure predictions were obtained using different servers: PSIPRED v3.3 (Buchan et al., 2013); Jpred4 (Drozdetskiy et al., 2015) SOPMA (Geourjon and Deleage, 1995); PSSpred (Yan et al., 2013) and a α -turns predictor ALPHAPRED (Kaur and Raghava, 2004).

Sequence conservation was analysed using GREMLIN (Generative Regularized Models of proteins) software (Kamisetty et al., 2013). Multiple sequence alignment were generated using HHblits algorithm (Kamisetty et al., 2013; Remmert et al., 2011) (with an E-value cut-off of 10^{-10} and sequences having > 75% gaps are filtered out), which performed eight sequence search iterations, obtaining 86 sequences of TIF2_{NRID} homologs (Table S2).

SEC-MALS

Size-Exclusion Chromatography-Multi Angle Light Scattering (SEC-MALS) experiment was performed at 25°C using a Superdex 200 10/300 GL column (GE HealthCare) connected to a miniDAWN-TREOS light scattering detector and an Optilab T-rEX differential refractive index detector (Wyatt Technology, Santa Barbara, CA). The column was equilibrated with 0.1 μ m filtered NMR buffer and the SEC-MALS system was calibrated with a sample of Bovine Serum Albumin (BSA) at 1 mg/mL. A sample of 40 μ L of TIF2_{NRID} protein at 3.3 mg/mL (200 μ M) was injected at 0.5 mL/min. Data acquisition and analyses were performed using the ASTRA software (Wyatt). Based on measurement on BSA sample under the same or similar conditions, we have estimated an experimental error in molar mass around 5%.

Circular Dichroism spectroscopy (CD)

CD spectrum of a 1 mg/mL (60 μ M) sample of TIF2_{NRID} in NMR buffer was recorded on a Chirascan Plus spectropolarimeter using quartz cuvette (0.1 mm path length) at 10°C. Spectra were scanned from 185 to 250 nm with an increment of 0.2 nm, an integration time of 2 s. The signal from the scan of the buffer was subtracted from the corresponding sample scan and signal was converted to mean residue ellipticity [$\text{deg.cm}^2.\text{dmol}^{-1}.\text{res}^{-1}$] using a mean residue weight of 104 Da.

Steady-state fluorescence anisotropy

Effect of ligands on the recruitment of TIF2_{NRID} by the heterodimer RXR/RAR. TIF2_{NRID} was amine-labelled with a fluorescent probe (fluorescein isothiocyanate, FITC, Sigma) according to the manufacturer's instructions. Assays were performed using a CLARIOstar microplate reader (BMG Labtech) with the excitation wavelength set at 485 nm and emission measured at 530 nm. The buffer solution was 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 5 mM DTT and 10% (v/v) glycerol. Measurements were initiated at the highest concentration of protein (7 μ M) and the protein sample was diluted successively two-fold with the buffer solution. For each point of the titration curve, the protein sample was mixed with 10 nM of fluorescent TIF2_{NRID} and 21 μ M of ligand (three molar equivalents). Binding data were fitted using a sigmoidal dose-response model (GraphPad Prism, GraphPad Software). The reported data are the average of three independent experiments.

Effect of LXXAA mutations on the interaction of TIF2_{NRID} with the heterodimer RXR/RAR. Wild-type and the three LXXAA mutants of TIF2_{NRID} were amine-labelled with a fluorescent probe (Alexa 488 NHS Ester, ThermoFisher) according to the manufacturer's instructions. Measurement of binding affinities was performed using a Safire2 microplate reader (TECAN) with the excitation wavelength set at 470 nm and emission measured at 530 nm. The buffer solution was 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 5 mM DTT and 10% (v/v) glycerol. Measurements were initiated at the highest

concentration of protein (20 μM) and the protein sample was diluted successively two-fold with the buffer solution. For each point of the titration curve, the protein sample was mixed with 10 nM of fluorescent TIF2_{NRID} (wt or mutants). Binding data were fitted using a sigmoidal dose-response model (GraphPad Prism, GraphPad Software). The reported data are the average of three independent experiments.

Interaction of RAR LBD with TIF2 NR2 and TIF2 NR2-Ext peptides. The peptides were labelled with Alexa Fluor 488 C5 Maleimide (ThermoFisher) on the additional cysteine at the C-terminus and the labelled peptides were subsequently HPLC purified to separate them from free fluorophore. Measurement of binding affinities was performed using a Safire2 microplate reader (TECAN) with the excitation wavelength set at 470 nm and emission measured at 530 nm. The buffer solution was 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 5 mM DTT and 10% (v/v) glycerol. Measurements were initiated at the highest concentration of protein (RAR or RXR, 20 μM) and the protein sample was diluted successively two-fold with the buffer solution, in the presence of two-molar excess of RAR or RXR ligands (AM580 and CD3254, respectively). For each point of the titration curve, the protein sample was mixed with 4 nM of fluorescent TIF2 peptide. Binding data were fitted using a sigmoidal dose-response model (GraphPad Prism, GraphPad Software). The reported data are the average of three independent experiments.

Competition experiments. The protein (RAR at 0.5 μM or RXR at 5 μM) was mixed with a constant amount of FITC-SCR1 NR2 (4 nM) and two-molar excess of agonist ligands (AM580 and CD3254, respectively). Titrations were initiated at the highest concentration of unlabelled peptide (20 μM) and the peptide sample was diluted successively two-fold with the same buffer solution as mentioned above. Binding data were fitted using a One site competition model (GraphPad Prism, GraphPad software). The reported data are the average of three independent experiments.

NMR spectroscopy data collection and analysis

All NMR experiments were recorded at 283 K on a Bruker Avance III spectrometer equipped with a 5 mm triple (TCI) resonance z-gradient cryoprobe operating at 18.8 T (800 MHz ^1H Larmor frequency) and at 16.4 T (700 MHz ^1H Larmor frequency). Spectra were processed using TopSpin NMR (Bruker) and analysed using Sparky (Goddard and Kneller, 2008). ^1H chemical shifts were referenced directly and ^{13}C and ^{15}N indirectly (Wishart et al., 1995) with 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, methyl ^1H signal at 0.00 ppm).

Spectral assignment was done on a 350 μM of $^{15}\text{N}/^{13}\text{C}$ -TIF2_{NRID} sample in NMR buffer. All NMR samples were supplemented with 5-10% (v/v) D₂O. ^1H , ^{15}N , ^{13}C O, $^{13}\text{C}_\alpha$, $^{13}\text{C}_\beta$ were assigned using a two dimensional ^{15}N -HSQC, and a set of three dimensional HNCO, HN(CA)CO, HN(CO)CACB, HNCACB, HN(CO)CA, HNCA experiments. A partial automatic assignment of the backbone resonances was obtained using the program MARS (Jung and Zweckstetter, 2004) and then completed manually. Resulting backbone chemical shift data are available on the Biological Magnetic Resonance Bank (BMRB accession code 50477).

NMR carbon chemical shifts are highly sensitive to backbone conformations (Kragelj et al., 2013; Wishart and Sykes, 1994), $^{13}\text{C}_\alpha$ and $^{13}\text{C}_\beta$, and to a lower extent $^1\text{H}_\alpha$, ^{13}CO , ^{15}N and ^1HN secondary

chemical shifts report on the secondary structural preferences at residue level. Secondary chemical shift ($\Delta\delta$) values were calculated as the difference between the experimental chemical shifts and their amino-acid specific random-coil values. We used random coil chemical shifts from two different databases: one based on a set of penta-disordered peptides (Ac-QQXQQ-NH₂) (referred as “Poulsen”; Poulsen IDP/IUP random coil chemical shifts) (Kjaergaard et al., 2011) and the other one based on a set of IDPs (referred as “Potenci”) (Nielsen and Mulder, 2018). Both use nearest neighbour amino acid sequence corrections (Schwarzinger et al., 2001; Tamiola et al., 2010) and also include corrections for temperature and pH effects (Kjaergaard et al., 2011; Nielsen and Mulder, 2018). *Secondary structure propensity (SSP)* score was calculated using the webserver ncSPC (Tamiola and Mulder, 2012) using the C_α and C_β secondary chemical shifts. The program uses random coil reference data from ncIDP (Tamiola et al., 2010) and a algorithm for converting the measured data into secondary structure propensities (Marsh and Forman-Kay, 2012). For a given residue, a positive or negative SSP score indicate a propensity for helical or extended (β-strand) structures, respectively (with a score of 1 or -1 for a fully formed helical or β-strand structure).

Backbone amide ¹⁵N longitudinal (T₁) and transverse (T₂) relaxation times and heteronuclear ¹⁵N{¹H} NOEs are good indicators of local backbone mobility on the ps-ns timescale (Farrow et al., 1994; Konrat, 2014), and T₂ is in addition sensitive to exchange processes taking place on the μs to ms timescale. They were measured using the methods described in (Farrow et al., 1994) using a 200 μM ¹⁵N-labelled TIF2_{NRID} sample in the NMR buffer. For ¹⁵N T₁ and T₂, a series of eight ¹⁵N-HSQC spectra with relaxation delays of 10, 50, 100, 200, 400, 600, 800 and 1000 ms for T₁, and 16, 32, 64, 96, 160, 240, 480 and 640 ms for T₂ were recorded. T₁ and T₂ spectra were recorded with 8 scans and 2.5 s of recycle delay, 2048 direct complex points and 256 indirect complex points with an echo/antiecho acquisition scheme. The intensity of the backbone amide signals was fitted to a single exponential decay using Sparky (Lee et al., 2014). Signal overlap prevented the reliable measurement of the signal intensity for several residues, which were excluded from the analysis. ¹⁵N{¹H} NOE values were obtained by recording two sets of spectra in the presence and in the absence of a 5 s proton saturation period. Heteronuclear NOE spectra were acquired with 16 scans, 2048 direct complex points and 256 indirect complex points. ¹⁵N{¹H} NOEs were calculated from the ratio of intensities measured in the saturated (I) and unsaturated spectra (I₀).

Three-bond H_N-H_α J-coupling constants (³J_{HNHα}) were extracted from the 3D HNHA experiment (Vuister and Bax, 1993). ³J_{HNHα} values were obtained from intensity ratios of H^N- H^α cross-peaks (S_{cross}) and the corresponding HN-HN diagonal peaks (S_{diag}) using the equation:

$${}^3J_{\text{HNH}\alpha} = \frac{\arctan \sqrt{S_{\text{cross}}/S_{\text{diag}}}}{2\pi\zeta f}$$

where 2ζ, the total evolution time for the homonuclear ³J_{HNHα} coupling, was set to 26.1 ms. The empirical scaling factor f, required to correct for differential T₁ relaxation effects (Vuister and Bax, 1993), was set to 1 since the apparent effective T₁ of the H^α spin is long enough for the highly flexible TIF2_{NRID}, which has an apparent correlation time of approximately 3.4 ns, as estimated from the T₁/T₂ ratio (Kay et al., 1989). In all cases, errors in the measurement of intensities were estimated from the standard deviation of the experimental noise level.

Residual Dipolar Couplings (RDCs) are linked to the relative orientation of internuclear bond vectors with respect to the applied magnetic field so they are sensitive to both local and global conformations and are good indicator of presence of secondary structures in IDPs (Jensen et al., 2009; Salmon et al., 2012). Two different alignment media have been used to achieve a partial alignment in the magnetic field: alcohol mixture, containing C₈E₅ glycol (polyoxyethylene 5 Octyl Ether, C₁₈H₃₆O₆) and 1-octanol ($r = 0.87$) (Rückert and Otting, 2000), and filamentous phages Pf1 (Clare et al., 1998; Hansen et al., 1998; Zweckstetter and Bax, 2001) provided by ASLA biotech at an estimated concentration of 18 mg/mL previously dialyzed in the NMR buffer. The homogeneity of the partially aligned samples was confirmed by the well shaped ²H doublet from the solvent (Rückert and Otting, 2000). A ²H quadrupolar splitting of 36.6 Hz was observed for the alcohol mixture and 10 Hz for the Pf1 phages. Both anisotropic and isotropic samples contained 250 μM of TIF2_{NRID} in NMR buffer. RDC values (¹D_{HN}) were extracted from the differences between the ¹J_{HN}+¹D_{HN} couplings and the ¹J_{HN} scalar couplings measured using ¹⁵N-HSQC-DSSE (In Phase Anti Phase IPAP) (Cordier et al., 1999) on an anisotropic and isotropic sample, respectively (¹D_{HN} RDCs were corrected for the negative gyromagnetic ratio of ¹⁵N). Errors were estimated from the line-width and signal to noise ratio.

Ensembles of explicit models were generated for TIF2_{NRID} using Flexible-Meccano, which sequentially builds peptide planes based on amino acid specific conformational propensity and a simple volume exclusion term (Bernadó et al, 2005; Ozanne et al, 2012). The algorithm randomly selected torsion angle (φ, ψ) pairs from a database of amino-acid-specific conformations present in loop regions of high-resolution x-ray structures. To account for deviations from a random-coil description of TIF2_{NRID}, different structure ensembles were computed including user-defined local conformational propensities in different regions of the protein. Each ensemble comprised 100,000 conformers. ¹D_{HN} RDCs were calculated for each conformer of individual ensembles, and the per-residue averages over all conformers were computed and defined as back-calculated RDC values. Comparing the back-calculated and experimental values according to the equation assessed the quality of each ensemble:

$$\chi^2 = \frac{1}{N} \sum (RDC_{\text{exp}} - \kappa \cdot RDC_{\text{back_cal}})^2$$

where RDC_{exp} and RDC_{back_cal} are the experimental and back-calculated RDC values, respectively, and N is the number of analysed RDCs. All back-calculated RDC_{back_cal} values were scaled uniformly by a factor κ to account for the uncertainty in the degree of sample alignment.

Paramagnetic Relaxation Enhancement (PRE) experiments of the MTSL labelled TIF2_{NRID} S716C mutant were performed to obtain long-range contact information, since the unpaired electron of a nitroxyl radical MTSL causes line broadening of the NMR signals in a distance-dependent manner up to 20 Å (Battiste and Wagner, 2000). PRE measurements were realized using samples at a concentration of TIF2_{NRID} S716C mutant adjusted to 50 μM to minimize the effects of nonspecific intermolecular interactions. Reference diamagnetic samples were obtained after adding 5-fold molar excess of fresh ascorbic acid, with pH adjusted to 6.8, to the paramagnetic sample. PRE effects were determined from the peak intensity ratios ($I_{\text{para}}/I_{\text{dia}}$) between two ¹⁵N-HSQC spectra of paramagnetic and diamagnetic samples. For both states, spectra were recorded using a recycling delay of 2 s and the same NMR parameters.

Interaction mapping was performed using unlabelled RXR/RAR LBDs, and NMR ^{15}N -HSQCs were recorded for complex with ^{15}N -TIF2_{NRID}: RXR/RAR concentrations of 5 μM :6 μM (1:1.1). After this first experiment, AM580 RAR agonist was added to have a 1.2-fold molar excess of ligand. Subsequently, after the second experiment, CD3254 RXR agonist (1.2-fold molar excess) was added to the sample. All ligands were purchased in Tocris Bioscience and stock solutions were prepared at a concentration of 10^{-3} M in DMSO.

Small-angle X-ray Scattering (SAXS) measurement and analysis

Synchrotron radiation small-angle X-ray scattering (SAXS) data were acquired for TIF2_{NRID} at the P12 beamline of the European Molecular Biology Laboratory (EMBL) at the storage ring PETRA-III at the Deutsches Elektronen-Synchrotron (DESY), Hamburg (Roessle et al., 2007). Using a Pilatus 2M detector at a sample-to-detector distance of 3.1 m and a wavelength of 0.124 nm, a momentum transfer range of $0.026 < s < 5.04 \text{ nm}^{-1}$ was covered ($s = 4\pi \cdot \sin\theta/\lambda$, where 2θ is the scattering angle). SAXS data were acquired for a dilution series (1.7, 5.2 and 7.1 mg/mL) measured with the robotic sample changer at 10°C. Radial averaging, frame averaging and buffer subtraction were done using standard protocols (Franke et al., 2017). No concentration effects were observed between the three measured concentrations and curves from all measurements were merged to reduce interparticle interactions appearing at the beginning of the curve while preserving a good signal to noise ratio at the end and averaged curve was used for the subsequent structural analysis. The forward scattering intensity, $I(0)$, and the radius of gyration, R_g , were evaluated using Guinier's approximation (Guinier, 1939), assuming that at very small angles ($s < 1.3/R_g$), the intensity can be described as $I(s) = I(0)e^{-(sR_g)^2/3}$. The molecular mass of TIF2_{NRID} was evaluated by comparing its forward scattering with that of a reference solution of Bovine Serum Albumin (MW = 66 kDa).

Simulated SAXS curves from RDC-derived ensemble models were calculated using CRY SOL (Svergun et al., 1995) after addition of side chains using SCRWL (Canutescu et al., 2003) for each conformer. Then, the individual SAXS curves were averaged to obtain the ensemble scattering curve. Both curves were directly compared by scaling the theoretical curve using a momentum transfer range of $0.015 < s < 0.50 \text{ \AA}^{-1}$. Constant subtraction was used. A total of 2,000 conformers were used to calculate the average curve, which was sufficient to reach convergence.

Crystallization of the RAR α and RXR α LBDs in complex with TIF2 NR2-Ext peptide

Expression and purification of the human RAR α and RXR α LBDs as well as on the heterodimer RXR/RAR have already been described (le Maire et al., 2010; Nahoum et al., 2007; Pogenberg et al., 2005) The final buffers were 20 mM Tris-HCl pH 7.5, 150 mM NaCl, 10% Glycerol and 2 mM DTT for RAR and RXR/RAR LBDs and 20mM Tris-HCl pH 7.5, 150 mM NaCl, 10% Glycerol and 5 mM DTT for RXR LBD. Fractions containing the purified receptor were pooled, mixed with a threefold molar excess of ligand, AM580 or LG100268, for RAR or RXR, respectively, and a two-molar excess of the TIF2 NR2-Ext peptide and concentrated to 8 mg/ml. Similarly, the RXR/RAR heterodimer was mixed with threefold molar excess of both ligands (AM580 and LG100268) and a two-molar excess of the SCR1

NR2-Ext peptide and concentrated to 8 mg/ml. Crystals were obtained by vapor diffusion at 293 K. The well buffer contained 0.1 M Na HEPES, 0.2 M sodium acetate pH 7.5, 27% PEG 3350 for RXR complex, 0.2 M lithium chloride, 0.1 M MES pH 6.0, 20% PEG 6000 for RAR complex and 0.2 M Na acetate, 0.1 M Bis-Tris propane pH 7.0, 24% PEG 3350 for RXR/RAR complex. Crystals grew in a few days and were of space group P1, P2₁ and P4₃2₁2 for RXR, RAR and RXR/RAR complexes, respectively. For each complex, a single crystal was mounted from the mother liquor onto a cryoloop, soaked in the reservoir solution containing an additional 25% glycerol and frozen in liquid nitrogen.

Crystallographic data collection, processing and structure refinement

Diffraction data were collected at the BL13-XALOC beamline of the synchrotron ALBA (Barcelona, Spain) for RXR and RXR/RAR complexes at 2.8 and 2.55 Å resolution, respectively, and at the PXI beamline of the synchrotron SLS (Villigen, Switzerland) at 2.4 Å resolution, for RAR complex. Diffraction data were processed using XDS (Kabsch, 2010) and scaled with SCALA or Aimless from the CCP4 program suite (Winn et al., 2011). The structures were solved by using the previously reported structures 3E94 (le Maire et al., 2009), 3KMR (le Maire et al., 2010) and 1XDK (Pogenberg et al., 2005), for RXR, RAR and RXR/RAR complexes, respectively, from which the ligand was omitted. Initial F_o-F_c difference maps showed a strong signal for the ligand, which could be fitted accurately into the electron density. The structure was modelled with COOT (Emsley et al., 2010) and refined with REFMAC (Vagin et al., 2004) or with Phenix (phenix.refine) (Liebschner et al., 2019) using rigid body refinement, restrained refinement, and individual B-factor refinements. Data collection and refinement statistics are summarized in Table S3. Figures were prepared with PyMOL (<http://pymol.org/>).

Phylogenetic analysis, signatures of selection and sequence logos

Full-length nucleotide sequences of NCOA family members were recovered from publicly available sequence databases. Accession numbers are provided in Table S4. The transcripts were translated into amino acid sequences and an initial amino acid alignment was performed using MAFFT (Kato and Standley, 2013), which was followed by automated refinement using Noisy (Dress et al., 2008) and final manual curation. The phylogenetic tree was calculated based on the alignment of 34 sequences using 1,562 amino acid positions and allowing gaps (Figure S12). Phylogenetic relationships were assessed using the Maximum Likelihood (ML) method as implemented in RAxML (Stamatakis, 2014). The ML tree was calculated applying a JTT matrix (Jones et al., 1992) for all 5 classes of amino acid profiles and a discrete Gamma distribution (+G) to model evolutionary rate differences among sites with empirical base frequencies (+F). The robustness of each node of the resulting tree was assessed by rapid bootstrap analyses (with 1,000 pseudoreplicates). The tree was visualized and midpoint rooted using FigTree (Rambaut, A. FigTree v1.3.1. 2010; Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. <http://tree.bio.ed.ac.uk/software/figtree/>), with branch lengths indicating the number of substitutions per site.

The amino acid alignment was further used as guide to generate a codon-based alignment for additional analyses. To detect signatures of selection, the ratios between non-synonymous and synonymous substitutions (ω) were estimated for each paralogous lineage (NCOA1, NCOA2 and

NCOA3, excluding the *Petromyzon marinus* sequences) using the branch-site model analysis of the CodeML program in PAML (Yang, 2007). A similar analysis was carried out for the NR2-Ext peptide sequences of NCOA1, NCOA2 and NCOA3. To highlight evolutionary conserved amino acid sites, a sequence logo was calculated for the NR2-Ext region of each of the three NCOA paralogs using WebLogo (Crooks et al., 2004).

ACCESSION NUMBERS

NMR backbone chemical shift data are available on the Biological Magnetic Resonance Bank (BMRB accession code 50477).

Atomic coordinates and structure factors for the reported crystal structures are currently being deposited to the Protein Data bank.

SUPPLEMENTARY DATA

FUNDING

This work was supported by the Labex EpiGenMed (ANR-10-LABX-12-01) awarded to N.S. and P.B. N.S. acknowledges the support of the ANR GPCteR (ANR-17-CE11-0022-01). A.I.M. acknowledges the support from the CNPq (Programa Universal 420416/2016-1). The CBS is a member of the French Infrastructure for Integrated Structural Biology (FRISBI) supported by the French National Research Agency (ANR-10-INSB-05). J.E.C. is financed by a FRM fellowship (SPF20170938703).

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SUPPLEMENTARY DATA

Structural insights into the cooperative interaction of the intrinsically disordered co-activator TIF2 with retinoic acid receptor heterodimer (RXR/RAR)

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Web server	Web server link
MetaPr-DOS	http://prdos.hgc.jp/cgi-bin/meta/top.cgi
PONDR-FIT	http://disorder.compbio.iupui.edu/pondr-fit.php
PrDOS	http://prdos.hgc.jp
DISOPRED3	http://bioinf.cs.ucl.ac.uk/psipred/
SPOT-Disorder	https://sparks-lab.org/server/spot-disorder/
PSIPRED v3.3	http://bioinf.cs.ucl.ac.uk/psipred
Jpred	http://www.compbio.dundee.ac.uk/jpred/
SOPMA	https://npsa-prabi.ibcp.fr/NPSA/npsa_sopma.html
ALPHAPRED	https://zhanglab.ccmb.med.umich.edu/PSSpred/
PSSpred	https://zhanglab.ccmb.med.umich.edu/PSSpred/
GREMLIM	http://gremlin.bakerlab.org/
CAPITO	http://capito.nmr.leibniz-fli.de/
Poulsen IDP/IUP random coil chemical shifts	https://spin.niddk.nih.gov/bax/nmrserver/Poulsen_rc_CS/
POTENCI	http://nmr.chem.rug.nl/potenci/

Table S1. Disorder and secondary structure prediction web servers used in this study.

>TIF2_{NRID}
ERADGGQSRLLHDSKGQTKLLQLLTTKSDQMEPSSLASSLSDTNKSDTSGSLPGSGSTHGTSLSKEKHKILHRLQLDSSSPVDLAKLTAEATGKDLSESSTTA
PGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERLDSKT
>tr|V8PAR9|V8PAR9_OPHHA Nuclear receptor coactivator 2 (Fragment) OS=Ophiophagus hannah GN=ncoa2 PE=4 SV=1
ERPDPGQNRLLHDGKSQTKLLQLLTTKSDQMEPSSLANTMGDINKDSMGSLSGSAHAGTSLKEKHKILHRLQLDSSSPIDLAKLTAEATGKELNQETSSTA
PGSEITCKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERLDSKT
>tr|I3M749|I3M749 ICTTR Uncharacterized protein OS=Ictidomys tridecemlineatus GN=NCOA1 PE=4 SV=1
RLSDGDSK---YSQSHKLVQLLTTTAEQ----QLRHADIDTSKEVLSC-TGTCPSHSSLTERHKILHRLQLQEGS-PSDITLTSVEPDKKDSASTSVSVQ
GNSNIKLELDTSKKKESKHQLLRYLLDKDEKDLRSTPNVKVKEK-DQM
>tr|A0A099YS76|A0A099YS76_TINGU Nuclear receptor coactivator 2 (Fragment) OS=Tinamus guttatus GN=N309_12388 PE=4 SV=1
ERPDPGQSKLHDGKSQTKLLQLLTTKSDQMEPSSLTMDVSKESTGGLAGSGSAHAGTSLKEKHKILHRLQLDSSSPVDLAKLTAEATGKELNQESSSTA
PGSEVTVKQEPASPKNNNALLRYLLDKDDTKDIGLPEITPKLERLDSKT
>tr|H3BD88|H3BD88_LATCH Uncharacterized protein OS=Latimeria chalumnae GN=NCOA2 PE=4 SV=1
ERTDGGQSRLLQVKGKVTLLQLLTTKSDQMEPSSLSSSTGDP-KDSIGLSLGSAAAPGSSSLKEKHKILHRLQLQESSSPGDLAKLTAEATGKELIQESNSMT
SSSEVPIKQEPGSLKRKENALLRYLLDKEDH----SPDLNPKLERMDSKT
>tr|A0A060W6B9|A0A060W6B9_ONCMY Uncharacterized protein OS=Oncorhynchus mykiss GN=GSONMT00067538001 PE=4 SV=1
EREDGGEQRELLNSKHTKLLQLLTKSEHMEPC--PHGGGDPNKDPMGMG-PGGHNNHSSSLKEKHKILHRLQLNSTSPVELAKLTAEATGKDLGQDQGGPA
SVAEMATKQEPISPKKKDNALLRYLLDKDDNTMQ---EKG-----IKM
>tr|V9K9Q7|V9K9Q7_CALMI Nuclear receptor coactivator 2 (Fragment) OS=Callorhynchus milii PE=2 SV=1
ERTDGGQSRLLQVKGKVTLLQLLTTKSDQMEPSSLSSSTGDP-KDSIGLSLGSAAAPGSSSLKEKHKILHRLQLDST-SVDLAKLTAEATGKEPNQDTGSSV
SGADATVKKQEQMSPKKKENALLRYLLDKDDTS---MQDIKPKLERMDSKV
>tr|U6DCF3|U6DCF3_NEOVI Nuclear receptor coactivator 3 (Fragment) OS=Neovison vison GN=NCOA3 PE=2 SV=1
EGSENRQRP-LESKHKLLQLLTCSSDDRGHSSLTNSPLDSSKSSINVPSTSNMHGSLLEKQHRILHKLQLDGNNSPADVAKITAEATGKDTSTST---AS
CVEGVS-VKQEQMSPKKKENALLRYLLDRDDPSDLSKELQPKVEGVGDKL
>tr|G3Q193|G3Q193_GORGO Uncharacterized protein (Fragment) OS=Gorilla gorilla gorilla PE=4 SV=1
EGAENQRGP-LESKHKLLQLLTCSSDDRGHSSLTNSPLDSSKSSVSVSTSNMHGSLLEKQHRILHKLQLQNGNSPAEVAKITAETGKDTSSI---TS
CGDGNVVKQEQMSPKKKENALLRYLLDRDDPSDALSKEQLQPKVEGVGDKM
>tr|G3Q022|G3Q022_GASAC Uncharacterized protein OS=Gasterosteus aculeatus GN=NCOA2 (2 of 2) PE=4 SV=1
ETGDAPNCLLNTKGHTKLLQLLTKSEADPCSSP---DC---KDQPGG---SGHNNHPTSLKEKHKILHKLQLQNGASPVELAKLTAEATGKDPAGPESAGA
SLGELFVKQEPGSPKK-DNALLRYLLDRDDNGVP---DKAIKAEVGD---
>tr|H2SV36|H2SV36_TAKRU Uncharacterized protein OS=Takifugu rubripes PE=4 SV=1
EDSVKAPLSSASQGNRPSQFHDGSGSES--SNSIHSL-----PQCPASHSTLTERHKILHRLQLDSSPNSSA--NNEEQNPAAIEKKEPP
A-MT---SPIP-----DHQLRFLLDTEKDLGLDQSIKRAKVEKRTSI-
>tr|F6YMZ9|F6YMZ9_CALJA Uncharacterized protein OS=Callithrix jacchus GN=NCOA3 PE=4 SV=1
EGSENRQRP-LESKHKLLQLLTCSSDDRGHSSLT---L---NCKESSVSTSSMHG---SLLQEKHRILHKLQLQNGNSPAEVAKITAETGKDTST---STS
CGDGNVVKQEQMSPKKKENALLRYLLDRDDPS---SKELQPKVEGVGDKM
>tr|A0A0Q3XHH9|A0A0Q3XHH9_ALLMI Nuclear receptor coactivator 3 isoform A OS=Alligator mississippiensis GN=NCOA3 PE=4 SV=1
RLSEGDAKG-AQATSHKLVQLLASEAQQLRHA-----TSCDPLSCAGSTCPSSHSLTERHKILHRLQLQEGSPS-DITLTSMEHDKKENV--PSTTQ
IPGTPEIKLEPELKKKDDHQLLRYLLDKDEKEL--LDDVVKVKEQTEQM-
>tr|V9K9I1|V9K9I1_CALMI Nuclear receptor coactivator 3 (Fragment) OS=Callorhynchus milii PE=2 SV=1
DNPPEGQRRFHENKNTKLLQLLTSPPDDMGLTSLTSL---GIKDSSGGITAAAGLHGSSVQEKHKILHKLQLQNGNSPAEVAQLTAEATGKESQDSS---T
AVGEAVIKQEQMSPKKKSNLLRYLLDKDDPKDPAINDIKPKIEGMDGK-
>tr|H3ADQ3|H3ADQ3_LATCH Uncharacterized protein OS=Latimeria chalumnae GN=NCOA3 PE=4 SV=1
EGTDNQRGASESGHKLLQLLTSPEDRGHSSS---L---NSKEPFTSVTSNIHTTSSSLQEKHKILHKLQLQNGNSPAEVAKITAETGKDTLHESPSAT
SGEVI-VKQEQMSPKNCRALLKYLDDKASK---LKDIPKIEGLDNK-
>tr|H2TG6|H2TG6_TAKRU Uncharacterized protein OS=Takifugu rubripes PE=4 SV=1
PGGESGRRVPDSKTHKLLQLLTSPTDDLVPNSHL--T--EAKDGAPAVTAAGQSTSQLQEKHKILHKLQLQNGNTPDEVARITAEATGKCATDSAAQPT
--RGTDLQEQMSPKKNALLHYLLNKDDSKG-G-GDIKPKLDELEGR-
>tr|W5JYV6|W5JYV6_ASTMX Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
DSGDLQNRLLPDNKGHTKLLQLLTKTEPIESTSP---GC---KDQSGGPGGASGNHATSLSKEKHKILHRLQLNSTSPVDLAKLTAEATGKEMCQD---SA
NVPDLVPKQEPVSPKK-DNALLRYLLDKDDNMMK---DKGVKMEPEGEI--
>tr|G3PR38|G3PR38_GASAC Uncharacterized protein (Fragment) OS=Gasterosteus aculeatus GN=NCOA3 (2 of 2) PE=4 SV=1
ASSEPSRRLPDSKGHKLLQLLTSPEELGMGGSGGPS-----GS--MSSAHTGSLQEKHKILHKLQLQNGNTPDEVAKITAETGKVTLLGGQEGEG
PGLVAEPKQEQSPKETHALLHYLLNKDDSKEP--VDVKPKLEELEGK-
>tr|A0A060YHM7|A0A060YHM7_ONCMY Uncharacterized protein OS=Oncorhynchus mykiss GN=GSONMT00014698001 PE=4 SV=1
ASSEPTRRVPDSKGHKLLQLLTSPEELGLGGTGSPTS-----LASAHTASLQEKHKILHKLQLQNSNTPDEVAKITAETGKVTLLGGQEV-G
AGGVTEPKQEQHSPKETHALLHYLLNKDDTKEP--VDVKPKPEELEGK-
>tr|M4A742|M4A742_XIPMA Uncharacterized protein OS=Xiphophorus maculatus PE=4 SV=1
DSHDAHRSRVDNKGNTKLLQLLTKPELETPLSP---GG---KDQA-GAGAVGNTHATSLSKEKHKILHKLQLQNSTSPVDLAKLTAEATGKELGQDQSQAS
SGAEITPKQEPSPKK-DNALLRYLLDKDDTVMK---DKVSKLEPGEV--
>tr|F7ECC3|F7ECC3_ORNAN Uncharacterized protein OS=Ornithorhynchus anatinus GN=NCOA1 PE=4 SV=1
HR-RPEGDGKPGQGHRPQQLPANERRPLPADADPGC-----GACPSHSSLTERHKILHRLQLQEGSPDIGSPPPEPER-----RDSGPGP
A-NR---AEQEGTRKKDDHQLLRYLLDKNEK----LDDVEVNLERAEPG-
>tr|F1SDJ9|F1SDJ9_PIG Nuclear receptor coactivator 1 OS=Sus scrofa GN=NCOA1 PE=4 SV=2
RLSDGDNKYS--QTSKLVQLLTTAEQQLRHA-----TSCKEVLSCTGGSCPSHSSLTERHKILHRLQLQEGSPS-DITLTSVEPDKKDSAST---Q
VPGNSGIKLELSDKEDHQLLRYLLDKDEKDL--LDDVVKVKEQEQM-
>tr|G3VKH3|G3VKH3_SARHA Uncharacterized protein OS=Sarcophilus harrisi GN=NCOA1 PE=4 SV=1
KLSEGENKYS--QTSKLVQLLATAEQQLRHT-----TSGKDALSCSNGTSPSSHSLTERHKILHRLQLQEGSPS-DITLTSVEHDKKDNSS---Q
VPGNSNIKLELSDKEDHQLLRYLLDKDEKDL--LDDVVKVKEQEQM-
>sp|P70365|NCOA1_MOUSE Nuclear receptor coactivator 1 OS=Mus musculus GN=Ncoa1 PE=1 SV=2
RLSEGDSKYS--QTSKLVQLLTTAEQQLRHA-----TSCKDLVLSCTGGTSPSSHSLTERHKILHRLQLQEGSPS-DITLTSVEPEKDSVPAST--Q

SQGSASIKLELDAKKKEDHQLLRLLDKDEKDL--LDDVKVKVEKKEQM-

>tr|U3K874|U3K874_FICAL Uncharacterized protein OS=Ficedula albicollis GN=NCOA1 PE=4 SV=1
-STEGSDAKPCQATSHRLVQLLASAEQQLRHH-----DTSSKDSLACAGNSCPSSHSLTERHKILHRLQLQEGSPS-DVPLAPEQDKKENPGGNS---
-----SAGAAEEKKEDHQLLRLLDKDEKDL--LDDVKVKVEKKEGAA-

>tr|S4RC77|S4RC77_PETMA Uncharacterized protein (Fragment) OS=Petromyzon marinus PE=4 SV=1
GDPDEPSRASDSKHTQLLQLLTNGGELVSTTSAA---S---KEALFGITLYSGSTNVKKERHKILHKLQDSSPTEEPGLTSPATATAAASTPS-P
GGGG--GGDGDGKKKE-DHVLRLYLLDKDEKEMA---EAGGAASPSQA-

>tr|M7BVH7|M7BVH7_CHEMY Nuclear receptor coactivator 2 (Fragment) OS=Chelonia mydas GN=UY3_01538 PE=4 SV=1
ERPDPGNRLHDGKSQTKLLQLLTTKSDQMEPSPLNSMGMGDANKDSTGGLSGSGSAHGTSLEKHKILHRLQLQDSSSPVDLAKLTAEATGKELNQESNSTA
PGSEVTVKQEPVSPKKENALLRYLLDKDDTKDIGLPDVTPLKLERLDSK-

>tr|H3BEV9|H3BEV9_LATCH Uncharacterized protein OS=Latimeria chalumnae GN=NCOA1 PE=4 SV=1
QLTEGESKAMQALGNTKLVQLLATAEQQLKHA-----GTNAKEFLSCVGTCPCLSHSSLTERHKILHRLQLQEGSPS-DITTLSELEQEKKEAGLCSGV-L
GSANSETSQELEPKKEDHQLLRLLDKDEMGL--LDDVKVKSEKSGEE-

>tr|F6UXS6|F6UXS6_CHICK Uncharacterized protein OS=Gallus gallus GN=NCOA1 PE=4 SV=1
RLTEGESKC-SQATSHKLVQLLASAEQQLRHA-----TSCKDSLACAGSTCPSSHSLTERHKILHRLQLQEGSPS-DITTLAMEHDKKDNV--PNPTQ
LPGTQDIKLESDMKKDDHQLLRLLDKDEKEL--LDDVKVKVEKTEQM-

>tr|AOA091SCD5|AOA091SCD5_NESNO Nuclear receptor coactivator 1 (Fragment) OS=Nestor notabilis GN=N333_09803 PE=4 SV=1
RLSEGDSC-SQATSHKLVQLLATAEQQLRHA-----TSCKDSLVCAGSTCPSSHSLTERHKILHRLQLQEGSPS-DLTSLAMEHEKKETA--SNPTQ
LPGNPDIKLEPDLKDDHQLLRLLDKDEKEL--LDDVKVKVEKTEQM-

>tr|Q4SNL2|Q4SNL2_TETNG Chromosome 15 SCAF14542, whole genome shotgun sequence (Fragment) OS=Tetraodon nigroviridis
GN=GSTENG00015249001 PE=4 SV=1
DLGDAQNHLPNAKGHTKLLQLLT-KADPSDPCSPS--DF---KDQLGVIGAGHNNNPTSLKEKHILHRLQLTSTSPVELAKLTAEATGKEPTGPETAGA
ALTELCIKQEPESPKK-DNALLRYLLDRDDNSIP---DKAIKMESD--

>tr|H2SIV8|H2SIV8_TAKRU Uncharacterized protein (Fragment) OS=Takifugu rubripes GN=NCOA2 (1 of 2) PE=4 SV=1
ELGDAPSHLLNAKGHTKLLQLLT-KVETSDPCSPS--DL---KEQLGEIAAGHNNNPTSLKEKHILHRLQLTSTSPVELAKLTAEATGKDPGPESAGA
ALSELCTKQEPGSPKK-DNALLRYLLDRDDNGIL---DKAIKMESD--

>tr|AOA087Y055|AOA087Y055_POEFO Uncharacterized protein OS=Poecilia formosa GN=NCOA3 (2 of 2) PE=4 SV=2
AGADPPRRLLPDSKGGHKKLLQLTSPTEELGISGSGGGPGLDSKEATGGMTSASAHYTGSLQEKHKILHKLQNGNTPDEVAKITAEATGKVTGGQEGEG
PGLIADPKQEQHSPKETHALLHYLLNKDDSKSEK--ADVKKVEDLEGK-

>tr|H2M9K5|H2M9K5_ORYLA Uncharacterized protein OS=Oryzias latipes GN=NCOA3 (1 of 2) PE=4 SV=1
TNSEQRRLPDSKGGHKKLLQLTSPTEELGIGSGGPRGLDCKDASGGMT--QAAGQGSLEKHKILHKLQNGNTPDEVAKITAEATGKVSLLGGQESE-
----ADSKQEQHSPKETHALLHYLLNKDDSKSEK--VDVKKLELEGK-

>tr|I3KF93|I3KF93_ORENI Uncharacterized protein OS=Oreochromis niloticus GN=LOC100700776 PE=4 SV=1
EDSVKAPLSSASQGNPRLNQLLDSGAESNNSNSIHS-----PQCPASHSSLTERHKILHRLQLDSSPNDAST--NSEEGKTEVEIKKEPAP
A-LG---AGPPKSDSREDHQLLRLLGTDEKDLDDLTQTVRIKVEKPKI-

>tr|M4ATN1|M4ATN1_XIPMA Uncharacterized protein OS=Xiphophorus maculatus PE=4 SV=1
EDSNKAPLSPASPGNPRNLNQLLDSGAESNNSNSIHS-----PQCPASHSSLTERHKILHRLQLDSSPNDASTAAAEADGKNEVEIKKEPVP
A-LS---TASHKNSREDHQLLRLLDTDEKDLGDLQTVRVKVEKRASG-

>tr|K7FK43|K7FK43_PELSI Uncharacterized protein OS=Pelodiscus sinensis GN=NCOA1 PE=4 SV=1
RLSEGDTC-SQATSHKLVQLLATAEQQLRHA-----TSCKDPDLSCTGACPSHSSLTERHKILHRLQLQEGSPS-DITTLMEQDKKDTA-----AQ
IPGGPEIKLEPELKKEDHQLLRLLDKDEKDL--LDDVKVKVEKTEQM-

>tr|W5KHP0|W5KHP0_ASTMX Uncharacterized protein OS=Astyanax mexicanus PE=4 SV=1
PGADSTLRGSDAKGNKLLQLTSPPEELSLGAGNPVTGMDSKPEGVCMTMGHG-MASHSVQKHKILHKLQNSNTPDDVARITAEATGKVLGSEHP-A
DGSGPEVKQEQQSPK--THVLLHYLLKDDSKDSCMGEGKPLDELEGK-

>tr|F1QMV7|F1QMV7_DANRE Uncharacterized protein OS=Danio rerio GN=ncoa3 PE=1 SV=1
AGGEVNRVDPDGKGGHKKLLQLTSPTEEIGGTATPVTS-DPKETGSGMMPSPGHSTHTLQEKHKILHKLQNGNTPDDVARITAEATGKSSLSSEHP-A
EGRNLELKQEQHSPKEGPQALLHYLLNKDDSKPEGATEVVKPKLELEAR-

>tr|S4RR69|S4RR69_PETMA Uncharacterized protein OS=Petromyzon marinus PE=4 SV=1
GEQQGEDDADKNQQTQTKMLQLLT-GAEQNGHCIGQ--LRSGDGRVLSGSGAMNSAHAASLTERHKILHKLQDGGSPDLSKLTGRPCASSLLTG--TA
GGLKQDGSLSPPQRKQE-DHALLRSLDKKEEKEMP---GKFSSPLHQ---

>tr|F7BUW8|F7BUW8_XENTR Uncharacterized protein OS=Xenopus tropicalis GN=ncoa1 PE=4 SV=1
RTVEGETKPLATSSNKLVLQALATAEQQLRD-----TNCRDPLTCHVGTCPSSHSLTERHKILHRLQLQEGSPS-DISSLIDHEKKTGSANN--T
PGGPPEVKMESEDKKDDHQLLRLLDKDEKEL--LEDVKVKVEK-EQV-

>tr|V8PDG4|V8PDG4_OPHHA Nuclear receptor coactivator 1 (Fragment) OS=Ophiophagus hannah GN=NCOA1 PE=4 SV=1
RLSEGDSC-LQATSHKLVQLLASAEQQLRHV-----TSCKEPLSCTGACPSHSSLTERHKILHRLQLQEGSPS-DIATLSMEHDKKDMGGTATQ
APGTPDIRLEADMKKKDDHQLLRLLDQDDKEL--LEDVKVK-ENTEQL-

>tr|H9G4A6|H9G4A6_ANOCA Uncharacterized protein OS=Anolis carolinensis GN=NCOA1 PE=4 SV=2
RLSEGDSC-LQATSQKLVQLLATAEQQLRHA-----TSCKEPLSCTGACPSHSSLTERHKILHRLQLQEGSPS-DINSLAMEHDKKDGAVAGNTAQ
GPGTPDMRLEADMKKKEDHQLLRLLDKDEKEL--LDDVKVK-ENPEQL-

>tr|M3ZWE1|M3ZWE1_XIPMA Uncharacterized protein OS=Xiphophorus maculatus GN=NCOA2 (1 of 2) PE=4 SV=1
DLADAANHLTTKGTKLLQLLT-KLEPSDPSPS--DC---KDQMC---AGHNNQTTSLKEKHILHRLQLNSTSPVELAKLTAEATGKDPGPESAGA
ALSELCTKQEPGSPKK-DNALLRYLLDRDDNGIL---DKAIKMEPSD--

>tr|I3KCD5|I3KCD5_ORENI Uncharacterized protein OS=Oreochromis niloticus GN=NCOA2 (2 of 2) PE=4 SV=1
DLSDAPNQLLNAKGHTKLLQLLT-KLEPSDPCSPS--DC---KDQLGG--VGHNNHSTSLKEKHILHRLQLNSTSPVELAKLTAEATGKDPGPESAGT
ALGELCTKQEPGSPKK-DNALLRYLLDRDDNSIL---DKAIKIEPGE---

>tr|H2LMJ5|H2LMJ5_ORYLA Uncharacterized protein OS=Oryzias latipes GN=NCOA2 (1 of 2) PE=4 SV=1
HLGDGNSRHLNKGHTKLLQLLT-KLEHSDSCSSP--DC---KDQLGGVALGHSNQSTSLKEKHILHRLQLNSTSPVELAKLTAEATGKDPVGSSESAGA
ALGELCTKQEPGSPKK-DNALLRYLLDRDDNGIL---DKEIKMEPAD---

>tr|H2LVN1|H2LVN1_ORYLA Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
---KDESHIQENKCHTKLLQLLT-KPEPLETPLSP--GC---KDQA-GSGTGSTHATSLKEKHILHRLQLNSTSPVDLAKLTAKATGKELGQDTTQAA
SGAEITPKQEPSPKK-DNALLRYLLDKDDTVMK---DKVPKLEPGEV--

>tr|G3NEF1|G3NEF1_GASAC Uncharacterized protein OS=Gasterosteus aculeatus PE=4 SV=1
---DSHSRLHDNKGHTKLLQLLT-KPEPLEMPLSP--GG---KDQA-GAGAGVNTATHSLKDKHKILHQLLQNTSPVDLAKLTAEATGKELGQDPTQAA
SGAELSLKQEPSPKK-DNALLRYLLDKDDTVGK--DKVPKLEPGEV--

>tr|H3CRY5|H3CRY5_TETNG Uncharacterized protein OS=Tetraodon nigroviridis PE=4 SV=1
---DAHSRLHD-KSHTKLLQLLT-KPEPLEMPLSP--GG---KDQP-GSGNGGNAHATSLKEKHILHQLLQNTSPVDLAKLTAEATGKELNQE--QAA
AG-EVTPKQEPSPKK-ENALLRYLLDKDDTVMK---DKVPKLEPGEV--

>tr|W5M971|W5M971_LEPOC Uncharacterized protein OS=Lepisosteus oculatus PE=4 SV=1
RTPDPQSRHLHDSKGTLLQLLTKEQMEPSSPLAGGDPNSKDSMGGGAGQAAGHGTSLSKEKHILHRLQLNSSSPVDLAKLTAEATGKDLSEQEGNGAA
PELGAAIKQEPSPKKDNALLRYLLDKDDPV---IQDKSIKLEPGEV--

>tr|E7F5J8|E7F5J8_DANRE Nuclear receptor coactivator 2 OS=Danio rerio GN=ncoa2 PE=4 SV=2
DGSDDPQSRHLRDNKSHKLLQLLT-KTEPIESTSPP--GC---KDGAGNGGGNGSHATSLKEKHILHRLQLNSSSPVDLAKLTAEATGKELCQD---AA
GVPELAIKQEPVSPKK-DNALLRYLLDKDDNVLK---GKGKMEPGEI--

>tr|B7ZSK8|B7ZSK8_XENLA Uncharacterized protein OS=Xenopus laevis PE=2 SV=1
DKTEGQSRLLDNKGQKLLKLLTIKSEPMPSALPNTLGD MNKDSLHAFMSASAHTSLREKHILHRLQLDSSSPVDLAKLTAEATGKELSQESNSTG
PGSEVTIKQEPVSPKKKEHALLRYLLDKDDTKD-NVADITPKLERADNK-

>tr|AOA0N8K0X0|AOA0N8K0X0_9TELE Nuclear receptor coactivator 2-like OS=Scleropages formosus GN=Z043_107609 PE=4 SV=1
DCSDAQGRLLHNDKSGHTKLLQLLXXKPESELPSPV--VC---KDPMSGSGQATGAHASSLKEKHILHRLQLNNTSPVDLAKLTAEATGKDVQCDGPP--
GVGELVPKQEPVSPKK-DNALLRYLLDKDDSQ----EKTIKMEPGEV--

>tr|AOA0F8BAX3|AOA0F8BAX3_LARCR Nuclear receptor coactivator 2 OS=Larimichthys crocea GN=EH28_04440 PE=4 SV=1
---DAHNRHLHDGKSHKLLQLLT-KPEPLETPLSP--GG---KDQA-GTGTGNTHATSLKEKHILHQLLQNTSPVDLAKLTAEATGKELEQA--QAA
SGGEIAPKQEPSPKK-DNALLRYLLDKDDTVMK---DKVPKLEPGEV--

>tr|AOA0P7WHA2|AOA0P7WHA2_9TELE Nuclear receptor coactivator 3-like (Fragment) OS=Scleropages formosus GN=Z043_118838 PE=4
SV=1
TSNEPHRRVPDGGKHKLLQLLTSPEELSGVTSPTGTESKEPIGCVTLASAACTASLQEKHILHKLQNGNTPDEVAKITAEATGKSLMGQEPGAP
PSGTADVKEQEQSPKRENHALLHYLLNKD-DSKDR--GSKPKLDEMEGK-

>tr|W5M8Q6|W5M8Q6_LEPOC Uncharacterized protein OS=Lepisosteus oculatus PE=4 SV=1
GGAEPQRRLSDSKGNKLLQLLTSPTDGLMV--AV-STLEPKPAGCVTSSAHHASLQEKHILHKLQNGNSPDEVAKITAEATGKETSHEAGAG
GSGVDPKQEQSP--KTHALLHYLLNND-PKEPA--DIKPKLEEGK-

>tr|M7BJ79|M7BJ79_CHEMY Nuclear receptor coactivator 3 OS=Chelonia mydas GN=UY3_06977 PE=4 SV=1
EGSESQRGPNESKGHKLLQLLTCSSDERGHSTLL--L--GCKESSTNVTNSNVHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKDTYHDSNAP
CVEGT-IKQEQSPKKNALLRYLLDKDDIK---SKEMKPKVDGLDNK-

>sp|O57539|NCOA3_XENLA Nuclear receptor coactivator 3 OS=Xenopus laevis GN=ncoa3 PE=1 SV=1
EGSESQRSQAESKGHKLLQLLTCFTEERGQSLMS--M--DCKDSS-NVTSNLHG--SMLQEKHILHKLQNGNSPAEVAKITAEATGKDVVFQETVSAP
CTEAT-VKREQLSPKKNALLRHLDDKDDWK---AKDIKPKVEHMDIK-

>tr|G1KSL3|G1KSL3_ANOCA Uncharacterized protein OS=Anolis carolinensis GN=NCOA3 PE=4 SV=2
EGSD-QRGAESKGHKLLQLLTCSSDERGHSTLS--L--TCKDSSTNAT-----NMQEKHILHKLRLNGNSPAEVAKITAEATGKDTYHDS--VS
CGEGM-VKQEQMSPKKNALLRYLLDKDDAK---SKDIKPKIECLDSK-

>tr|V8P9E0|V8P9E0_OPHHA Nuclear receptor coactivator 3 (Fragment) OS=Ophiophagus hannah GN=ncoa3 PE=4 SV=1
EGAD-QRGAESKGHKLLQLLTCSSSEERGHPTLS--L--NCKESSNAT-----NMQEKHILHKLRLNGNSPAEVAKITAEATGKDPFHDSN--VS
CGE-V-VKQEQMSPKKNALLRYLLDKDDGK---LKDVPKPIETLDTK-

>tr|G1M274|G1M274_MELGA Uncharacterized protein OS=Meleagris gallopavo GN=NCOA3 PE=4 SV=2
DAPESQRGQSEKGHKLLQLLTCSSDERGHSTAS--L--NCKESSTNVTNSNVHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKLVNIDTNSRS
SMRPG-FE-----DTRRCIQRFLCHNDGQSWNSWQCNSKRQN---Q-

>tr|AOA0Q3XJ83|AOA0Q3XJ83_ALLMI Nuclear receptor coactivator 2 isoform A OS=Alligator mississippiensis GN=NCOA2 PE=4 SV=1
EGSESQRGSPSEKGHKLLQLLTCSSSEERGHATLS--L--NCKESSTNVTNSNMHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKDTYHESSNTP
CGEGT-VKQEQSPKKNALLRYLLDKDDIK---SKDIKPKVESLDNK-

>tr|HOZFD8|HOZFD8_TAEGU Uncharacterized protein (Fragment) OS=Taeniopygia guttata GN=NCOA3 PE=4 SV=1
EASESQRGSPSEKGHKLLQLLTCSSDERGHSTAS--L--NCKESSTSVTNSNMHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKDTYHDASNTS
CGEGT-IKQEQSPKKNALLRYLLDKDDIK---SKELPKVEGVDNK-

>tr|G3WT03|G3WT03_SARHA Uncharacterized protein (Fragment) OS=Sarcophilus harrisi GN=NCOA3 PE=4 SV=1
EGPENQRGPPESKGHKLLQLLTCSSSEERGHSTLT--L--SCKDASSVTNSNMHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKDT-----TT
SGEGL-VKQEQSPKKNALLRYLLDRDDPN---SKDIKPKVEGGDTK-

>tr|G1S034|G1S034_RABIT Uncharacterized protein OS=Oryctolagus cuniculus GN=NCOA3 PE=4 SV=2
EGPENQRGPLESKGHKLLQLLTCSSDRGHSSLT--L--SCKDSSISVTNSNMHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKDT-----SAS
CGEGS-VKQEQSPKKNALLRYLLDRDDPS---AKELQPHLV--ESK-

>tr|H3D4Y3|H3D4Y3_TETNG Uncharacterized protein OS=Tetraodon nigroviridis GN=NCOA3 (2 of 2) PE=4 SV=1
AGSEPRRRVPSKGHKLLQLLTSPEELGGMGGGSPAK-----GGM-LASAHYTSLSQEKHILHKLQNGNTPDEVAKITAEATGKVTLLGGQEGES
APGMPETKQEQHSPKKEPHALLHYLLNKDSSKEA--ADVPKLEEGK-

>tr|E6ZFT1|E6ZFT1_DICLA Ncoa3 protein OS=Dicentrarchus labrax GN=NCOA3 PE=4 SV=1
AGGESNRRVPD--SHKLLQLLTPADELVPNHT--T--GAKDGTAGVTATGHLSQSLQEKHILHKLQNGNTPDEVARITAEATGKSSLDGAPPA
--RGSESKQEQHSPKKEPHALLHYLLNKDSSKE-G-GDIKPKVEDLEGR-

>tr|G3P6N9|G3P6N9_GASAC Uncharacterized protein OS=Gasterosteus aculeatus PE=4 SV=1
AGGESNRRVADTKSHKLLQLLSPTEDELVPNHP--T--EAKDGTAGVTGTGPTLSQSLQEKHILHKLQNGNTPDEVARITAEATGKSSLDGAPPV
--RGSESKQEQSPKKEPHALLHYLLNKDSSKE-S-ADVPKQEELEGR-

>tr|I3KWU7|I3KWU7_ORENI Uncharacterized protein OS=Oreochromis niloticus PE=4 SV=1
GAGESNRRVPDKCHKLLQLLTSPTDELVPSNHT--T--ESKDATAGVTSTGNVNNQSLQEKHILHKLQNGNTPDEVARITAEATGKSSLDGAPPG
--RGSESKQEQHSPKKEPNALLHYLLNKDSSKE-V-GEIKPK--DLGK-

>tr|F7DNP6|F7DNP6_ORNAN Uncharacterized protein OS=Ornithorhynchus anatinus GN=NCOA3 PE=4 SV=2
EGAEPHPRGPPESKGHKLLQLLTCSSDRGHSSALT--L--GCKDAAGAA-SNVHG--SLLQEKHILHKLQNGNSPAEVAKITAEATGKDTGN---GAP
CGDGP-VKQEQSPKKNALLRYLLDRDDPT---SKEINPKVEGADNK-

>tr|H2SDV3|H2SDV3_TAKRU Uncharacterized protein OS=Takifugu rubripes GN=NCOA3 (1 of 2) PE=4 SV=1

ASSEPQRRVPDSKGHKLLQLLTSPTTEELGIGGSGAPN-----SSDSLSSAHYTGSLQEKKHILHKLQNGNTPDEVAKITAEATGKVTLSQEGEA
 GPGMTETKQEQHSPKKETHALLHYLLNKDDSKEP--ADVPKMEELD GK-
 >tr|A0A087XMW8|A0A087XMW8_POEFO Uncharacterized protein OS=Poecilia formosa PE=4 SV=2
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 CGKGPEPKQEHSPPKPRLLQYLLNKDDSK E-G-GDVKPKLEDLDRR-
 >tr|H2MF27|H2MF27_ORYLA Uncharacterized protein OS=Oryzias latipes PE=4 SV=1
 GGGEANRRVPD-KCNKLLQLLTSPTDDLVP SNHP--I--DSKDG S----SSGHFSPQSLQEKKHILHKLQNGNTPDEVAQITLLATGKSSLSDSAAPAE
 --KGSETKQEHSPPKESHALLHYLLNKDDSK E-G-GDMKPKLEDLDRG-
 >tr|A0A060X6A4|A0A060X6A4_ONCMY Uncharacterized protein OS=Oncorhynchus mykiss GN=GSONMT00047328001 PE=4 SV=1
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 GARGAEMKQEHSPPKETHALLHYLLNKDDSEGAR-RDVKPKLDDLEGR-
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 GARGAEVKQELHSPKETHALLHYLLKDDSK EAR-GDVQPKGRGAQGA-
 >tr|A0A0N8K239|A0A0N8K239_9TELE Nuclear receptor coactivator 3-like (Fragment) OS=Scleropages formosus GN=Z043_103978 PE=4
 SV=1
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 GTGNGTTKQEQHSPKKERNALLHYLLNKDDSK EP--DMKSKMEELETK-
 >tr|Q45L5|Q45L5_TETNG Chromosome 9 SCAF14729, whole genome shotgun sequence (Fragment) OS=Tetraodon nigroviridis
 GN=GSTENG00023674001 PE=4 SV=1
 AGSEPQRRVPDSKGHKLLQLLTSPTTEELG MGGGSHHRLGQQGPRRGYD---LLW---RQPRSGVSRRL-----AGVGPLHLGLAAGKAQDPPQAPEH
 GERMPEPKQEHSPPKETHALLHYLLNKDDSK EA--ADVKPKLEEELEK-
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 TMAEMATKQESISPKKDNALLRYLLDKDDNTM---QEKGIKMEPG----
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 -----RQRCDNKGHTKLLQLLTKSQPLDTP LSP--NGGWDPKDPSSGLGAGPGGHATSLKDKHILHRLQNSTSPVDLAKLTAETGRELGEQGGCGG
 TDGNLTPKQEPLSPKKNALLRYLLMDKEDSGMKARQGGQVKTQKQDS--
 >sp|Q9EPU2|NCOA3_RAT Nuclear receptor coactivator 3 (Fragment) OS=Rattus norvegicus GN=Ncoa3 PE=2 SV=1
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 G-GEVSXQEQLSPPKKNALLRYLLDRDDPSDVLAKELQPQADGGDSKL
 >tr|G5E7N1|G5E7N1_MELGA Uncharacterized protein (Fragment) OS=Meleagris gallopavo GN=NCOA3 PE=4 SV=1
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 S-MRPGF-----EDTRRCIQRFLCHNDGQSWSNKRH-----YHEVS
 >tr|A0A094NMR1|A0A094NMR1_9AVES Nuclear receptor coactivator 3 (Fragment) OS=Podiceps cristatus GN=N338_07241 PE=4 SV=1
 EAPESQRGQSEKGHKLLQLLTCSSDERGHSTASSPLDSNCKESSTNVTSSSNMHGSL LQEKRILHKLQNGNSPAEVAKITAEATGKDTYNSTA---T
 C-GEGTVKQEQLSPKKNALLRYLLDKDDV KDP LSKELPKVEAVDNKM
 >tr|L5JY56|L5JY56_PTEAL Nuclear receptor coactivator 3 OS=Pteropus alecto GN=PAL_GLEAN10024440 PE=4 SV=1
 EGSENRGRPLENKGHKLLQLLTCSSDRGHSSLTSP LDTCKDSSNSVTSTSNMHGSL LQEKRILHKLQNGNSPAEVAKITAEATGKDTSSST---S
 C-VEGSVKQEQLSPKKNALLRYLLDRDDPSDTLSKELQPKVEGVDKAM
 >tr|Q4SR81|Q4SR81_TETNG Chromosome 11 SCAF14528, whole genome shotgun sequence OS=Tetraodon nigroviridis
 GN=GSTENG00014033001 PE=4 SV=1
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 GTRGTELKQEQLSPKKNALLHYLLNKDDSK EGS--DIKPKLEDELA--
 >tr|G5BGW1|G5BGW1_HETGA Nuclear receptor coactivator 3 OS=Heterocephalus glaber GN=GW7_06025 PE=4 SV=1
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 C-GQGTVKQEQLSPKKNALLRYLLDRNDPSDTLCKELQPKVEGMDNKM
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 SV=1
 -----GNSKLNQPLDNGGVG--AP-----R--DPKNTSTKSTPQCPASHSTLTERHKILHRLQDSSPA-EGS-----NGKESEIKKEPAP
 ATANP--NGPPSTPQ--DHQLLRFLD TDEKDLGDLQTVRVKTEKRAS--

Table S2. Related to **Figure 2C**. List of sequences used for GREMLIM analysis.

	RAR LBD / TIF2 NR2-Ext	RXR LBD / TIF2 NR2-Ext	RXR/RAR LBD / SCR1 NR2-Ext
Data Collection			
Resolution range	46.96-2.4 (2.486-2.4)	48.3-2.8 (2.9-2.8)	48.43-2.55 (2.64-2.55)
Space group	P 1 21 1	C 1 2 1	P 43 21 2
Unit cell	53.694 47.573 120.999 90 96.169 90	179.212 64.667 48.51 90 95.285 90	108.283 108.283 99.786 90 90 90
Total reflections	53519 (5190)	40714 (4446)	39832 (3920)
Unique reflections	22707 (2250)	13144 (1330)	19916 (1960)
Multiplicity	2.4 (2.3)	3.1 (3.3)	2.0 (2.0)
Completeness (%)	93.85 (94.13)	95.30 (98.01)	99.95 (100.00)
Mean I/sigma(I)	9.35 (3.03)	16.93 (10.08)	10.83 (1.37)
Wilson B-factor	39.56	30.12	60.09
R-merge	0.05802 (0.2506)	0.05557 (0.1355)	0.03226 (0.5087)
R-meas	0.07238 (0.3164)	0.06764 (0.1624)	0.04562 (0.7195)
R-pim	0.04253 (0.1903)	0.03804 (0.0885)	0.03226 (0.5087)
CC1/2	0.997 (0.923)	0.997 (0.984)	0.999 (0.651)
CC*	0.999 (0.98)	0.999 (0.996)	1 (0.888)
Refinement			
Reflections used in refinement	22683 (2247)	13137 (1330)	19914 (1960)
Reflections used for R-free	1080 (111)	629 (66)	1995 (200)
R-work	0.1854 (0.2511)	0.2741 (0.3183)	0.2050 (0.2936)
R-free	0.2403 (0.2857)	0.3398 (0.4553)	0.2624 (0.3569)
CC(work)	0.961 (0.886)	0.914 (0.830)	0.971 (0.753)
CC(free)	0.897 (0.749)	0.790 (0.705)	0.954 (0.622)
Non-hydrogen atoms	4228	3759	3795
macromolecules	4035	3652	3723
ligands	64	54	59
solvent	129	53	13
Protein residues	518	463	475
RMS(bonds)	0.009	0.011	0.002
RMS(angles)	0.83	1.51	0.49
Ramachandran favored (%)	96.06	93.57	96.98
Ramachandran allowed (%)	3.74	6.21	3.02
Ramachandran outliers (%)	0.20	0.22	0.00
Rotamer outliers (%)	2.01	4.29	0.00
Clashscore	6.28	12.36	6.69
Average B-factor	53.17	9.46	75.71
macromolecules	53.37	9.13	76.00
ligands	41.29	20.67	61.36
solvent	52.89	20.63	57.23
Number of TLS groups	25	3	13

Statistics for the highest-resolution shell are shown in parentheses.

Table S3: Crystallographic data collection and refinement statistics.

Scientific name	Common name	Gene name	Transcript accession number
<i>Anolis carolinensis</i>	Green anole	NCOA1	ENSACAT0000000794
<i>Anolis carolinensis</i>	Green anole	NCOA2	ENSACAT00000015190
<i>Anolis carolinensis</i>	Green anole	NCOA3	ENSACAT00000016493
<i>Danio rerio</i>	Zebrafish	NCOA1	ENSDART00000045948
<i>Danio rerio</i>	Zebrafish	NCOA2	ENSDART00000124740
<i>Danio rerio</i>	Zebrafish	NCOA3	ENSDART00000110621
<i>Gallus gallus</i>	Chicken	NCOA1	ENSGALT00000048236
<i>Gallus gallus</i>	Chicken	NCOA2	ENSGALT00000074784
<i>Gallus gallus</i>	Chicken	NCOA3	ENSGALT00000107895
<i>Homo sapiens</i>	Human	NCOA1	ENST00000406961
<i>Homo sapiens</i>	Human	NCOA2	ENST00000452400
<i>Homo sapiens</i>	Human	NCOA3	ENST00000371998
<i>Latimeria chalumnae</i>	Coelacanth	NCOA1	ENSLACT00000020570
<i>Latimeria chalumnae</i>	Coelacanth	NCOA2	ENSLACT00000019997
<i>Latimeria chalumnae</i>	Coelacanth	NCOA3	ENSLACT00000007840
<i>Lepisosteus oculatus</i>	Spotted gar	NCOA1	ENSLOCT00000019602
<i>Lepisosteus oculatus</i>	Spotted gar	NCOA2	ENSLOCT00000004920
<i>Lepisosteus oculatus</i>	Spotted gar	NCOA3	ENSLOCT00000004783
<i>Leucoraja erinacea</i>	Little skate	NCOA2	Contig13237+18810+1084
<i>Leucoraja erinacea</i>	Little skate	NCOA3	Contig11843+18314+13582
<i>Mus musculus</i>	House mouse	NCOA1	ENSMUST00000220434
<i>Mus musculus</i>	House mouse	NCOA2	ENSMUST00000006037
<i>Mus musculus</i>	House mouse	NCOA3	ENSMUST00000088095
<i>Petromyzon marinus</i>	Sea lamprey	NCOA2/3a	ENSPMAT00000002822
<i>Petromyzon marinus</i>	Sea lamprey	NCOA2/3b	ENSPMAT00000008126
<i>Petromyzon marinus</i>	Sea lamprey	NCOA2/3c	ENSPMAT00000007739
<i>Takifugu rubripes</i>	Torafugu	NCOA1	ENSTRUT00000016343
<i>Takifugu rubripes</i>	Torafugu	NCOA2a	ENSTRUT00000074474
<i>Takifugu rubripes</i>	Torafugu	NCOA2b	ENSTRUT00000074705
<i>Takifugu rubripes</i>	Torafugu	NCOA3a	ENSTRUT00000074888
<i>Takifugu rubripes</i>	Torafugu	NCOA3b	ENSTRUT00000024003
<i>Xenopus tropicalis</i>	Western clawed frog	NCOA1	ENSXETT00000096656
<i>Xenopus tropicalis</i>	Western clawed frog	NCOA2	ENSXETT00000034772
<i>Xenopus tropicalis</i>	Western clawed frog	NCOA3	ENSXETT00000073641

Table S4: List of accession numbers of full-length nucleotide sequences of NCOA family members recovered from publicly available sequence databases. NCOA1, NCOA2 and NCOA3 correspond to SCR1, TIF2 and RAC3, respectively.

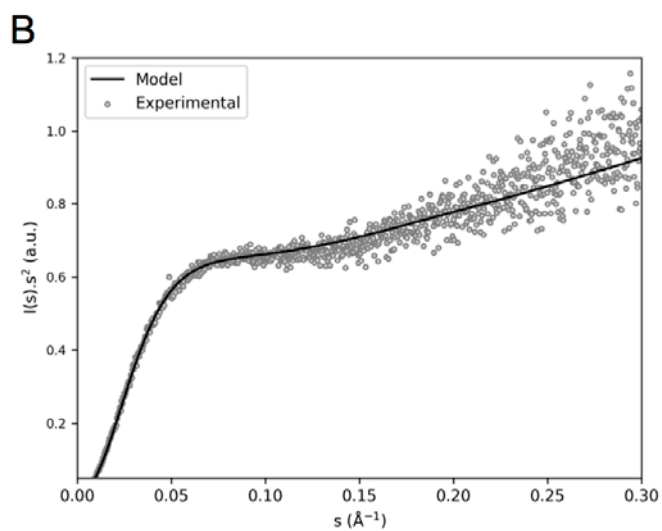
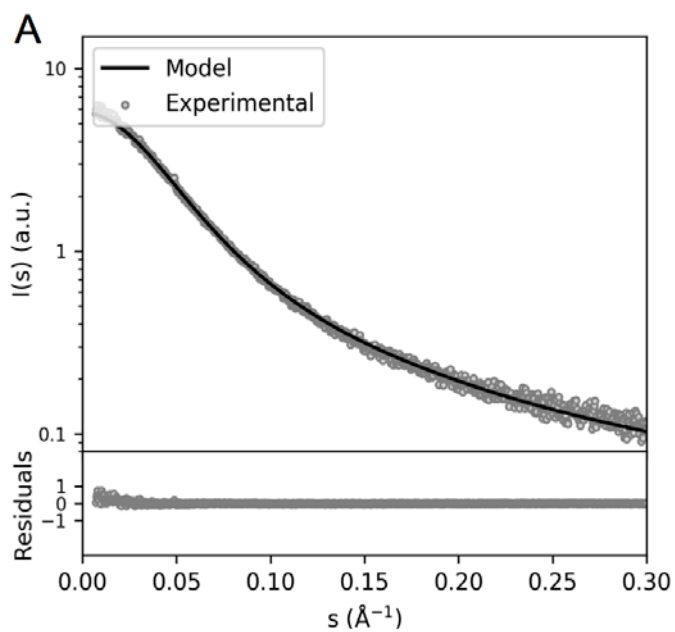


Figure S1. SAXS data and analysis of TIF2_{NRID}. **A)** Semi-logarithmic representation of the SAXS intensity versus momentum transfer s (open circles) and the averaged back-calculated curve derived from the final ensemble (2,000 conformers) that best explains the experimental RDCs (black curve) ($\chi^2 = 1.38$). The bottom panel shows the residual of the fitting to the experiment. **B)** Kratky representation of the data show in A.

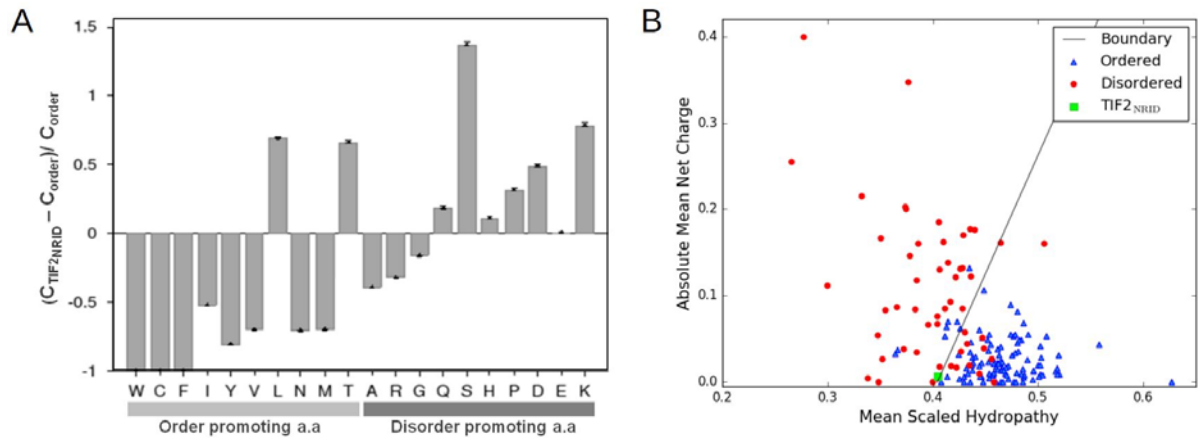


Figure S2. Related to **Figure 1.** **A)** Amino acid compositional analysis from Composition profiler server (<http://www.cprofler.org/>). Amino acids are divided into order-promoting (hydrophobic and uncharged amino acids) and disorder-promoting (polar amino acids) categories. TIF2_{NRID} is compared to the reference value of the average amino acid frequencies of the PDB database. Positive bars correspond to residues found more in TIF2_{NRID} than in ordered proteins, whereas negative bars show residues that are depleted in TIF2_{NRID}. **B)** Uversky plot (mean scaled hydropathy vs mean net charge) as a predictor of disordered protein structure. A set of 54 completely disordered proteins, and 105 completely ordered proteins are shown as red circles and blue triangles, respectively. The solid line represents a boundary separating ordered and disordered proteins and is empirically defined by the equation: $Hydropathy = (net\ charge + 1.151) / 2.785$. The green square corresponds to TIF2_{NRID}. Plot generated using PONDR (<http://www.pondr.com/>).

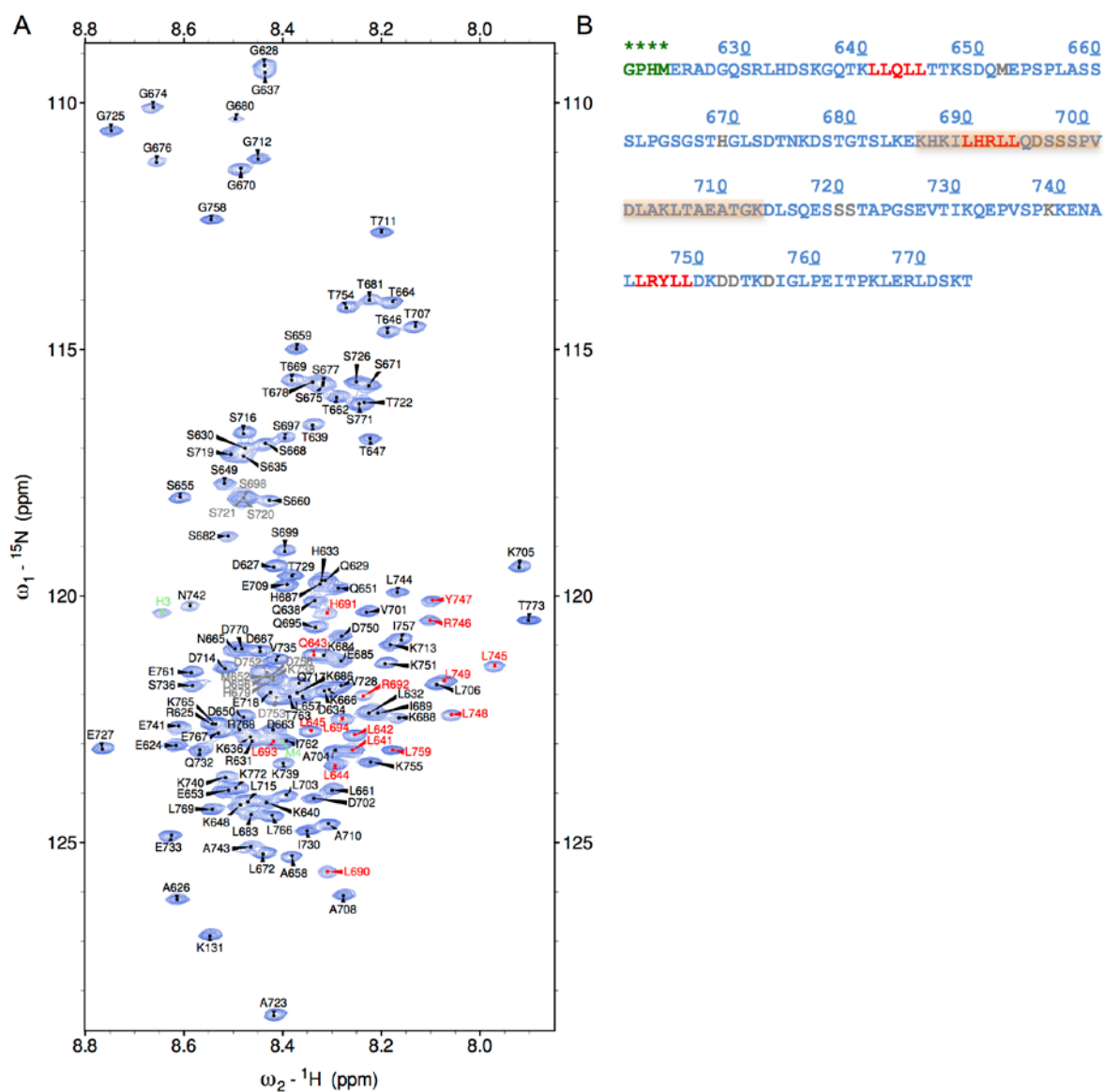


Figure S3. Related to **Figure 2.** **A)** Residue-specific assignment of the ^1H - ^{15}N HSQC NMR spectrum of $350 \mu\text{M}$ $^{15}\text{N}/^{13}\text{C}$ -TIF2_{NRID} at pH 6.8 and 10°C . Assigned cross-peaks are labeled with one-letter amino acid type and sequence number with respect to the full-length TIF2 protein. Some residues could not be unambiguously assigned due to the presence of overlapping cross-peaks in 3D spectra (S698, S720 and S721, H679, D696, S696, E718, S719, D752, D753 and D756), (BMRB accession code 50477). **B)** TIF2_{NRID} protein sequence. Tentatively assigned residues are indicated in grey, residues from the 3C cleavage site are indicated in green and residues belonging to NR boxes are indicated in red. TIF2 NR2-Ext peptide crystalized with RAR, from residue K686 to K713, is highlighted in orange.

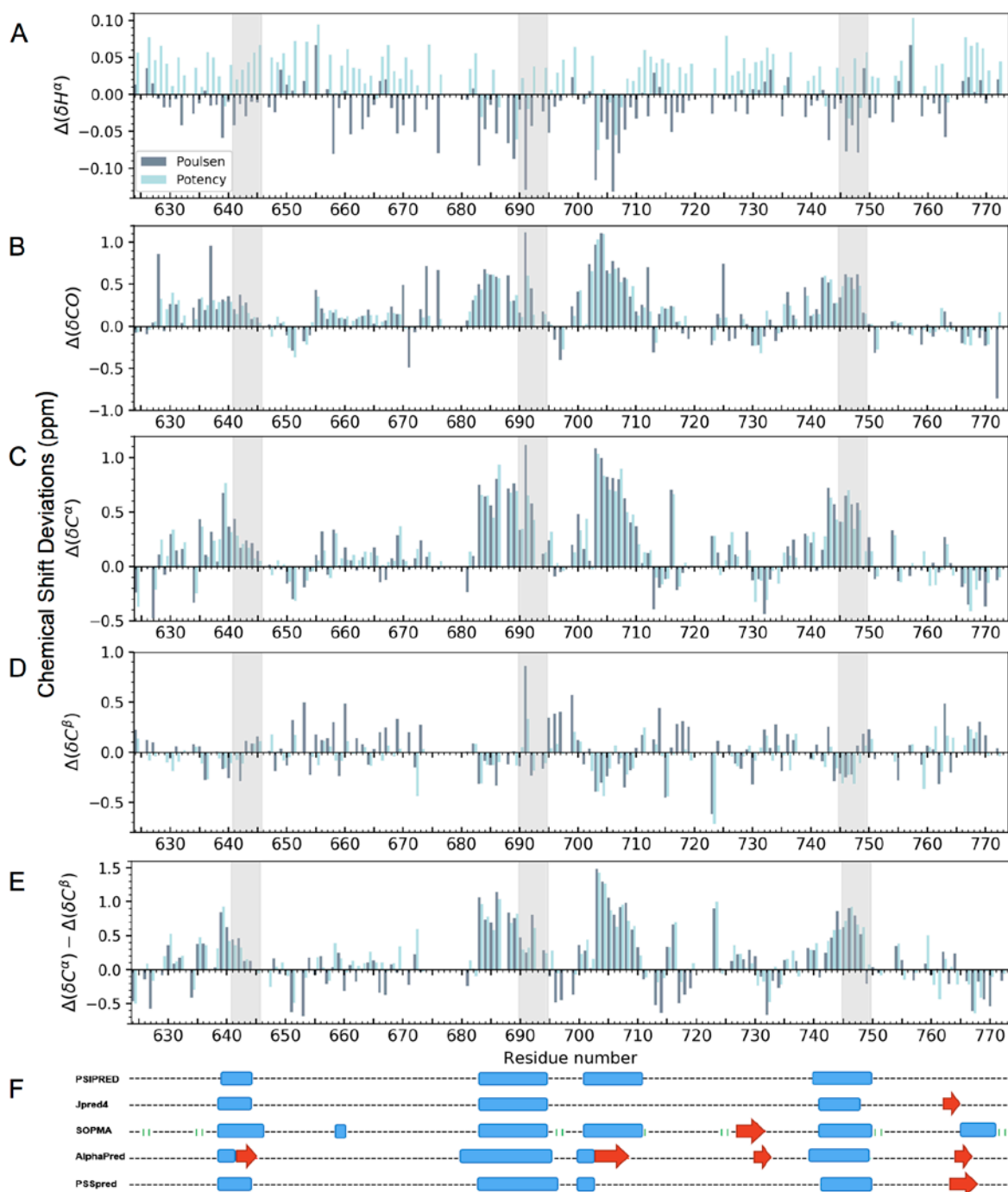


Figure S4. Related to **Figure 2D**. **Secondary structure of TIF2_{NRID} from backbone secondary chemical shift analysis per residue** **A)** H^α **B)** CO **C)** C^α and **D)** C^β chemical shift deviations from their random coil values obtained from the web servers: Poulsen IDP/IUP random coil chemical shifts (grey) and POTENCI (turquoise). **E)** Difference between C^α and C^β secondary chemical shifts. A positive (negative) deviation of $^{13}C_\alpha$ and CO chemical shifts from their corresponding random coil values is an indication of α -helical (β -sheet) structure and vice versa for $^{13}C_\beta$, $^1H_\alpha$, ^{15}N and 1HN . For H_α nuclei, a large difference between values from the two databases is observed as already mentioned before by both authors (Kjaergaard et al., 2011; Kjaergaard and Poulsen, 2011; Nielsen and Mulder, 2018) **F)** Secondary structure predictions from **Figure 2B** were added for an easier interpretation of the data. Grey boxes indicate NR Boxes (LxxLL motifs). Prolines, superimposed and ambiguous assigned residues were excluded from the analysis.

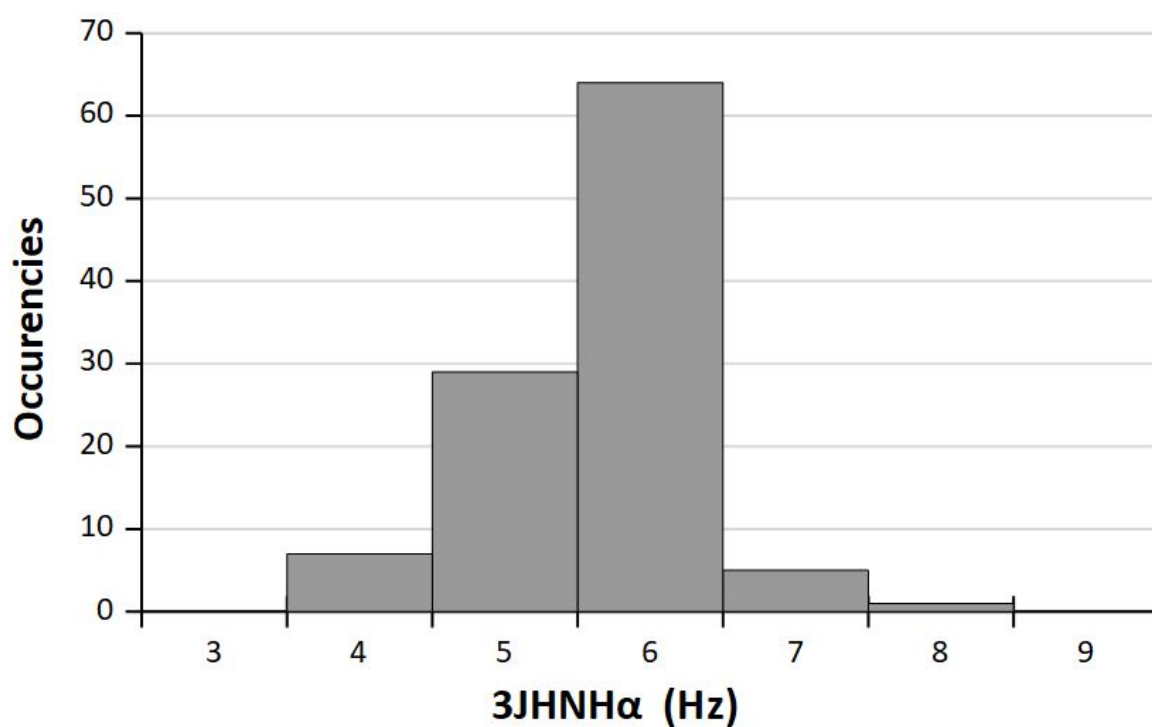


Figure S5. Histograms of the homonuclear $^3J_{\text{HNH}\alpha}$ coupling constants (in hertz) measured for TIF2_{NRID}. Values of 6-7 Hz indicate fast conformational averaging that is characteristic of random coil, while values below or above indicate helical or β -strand propensity, respectively. 65% of the measured values are in the range of 6-8 Hz.

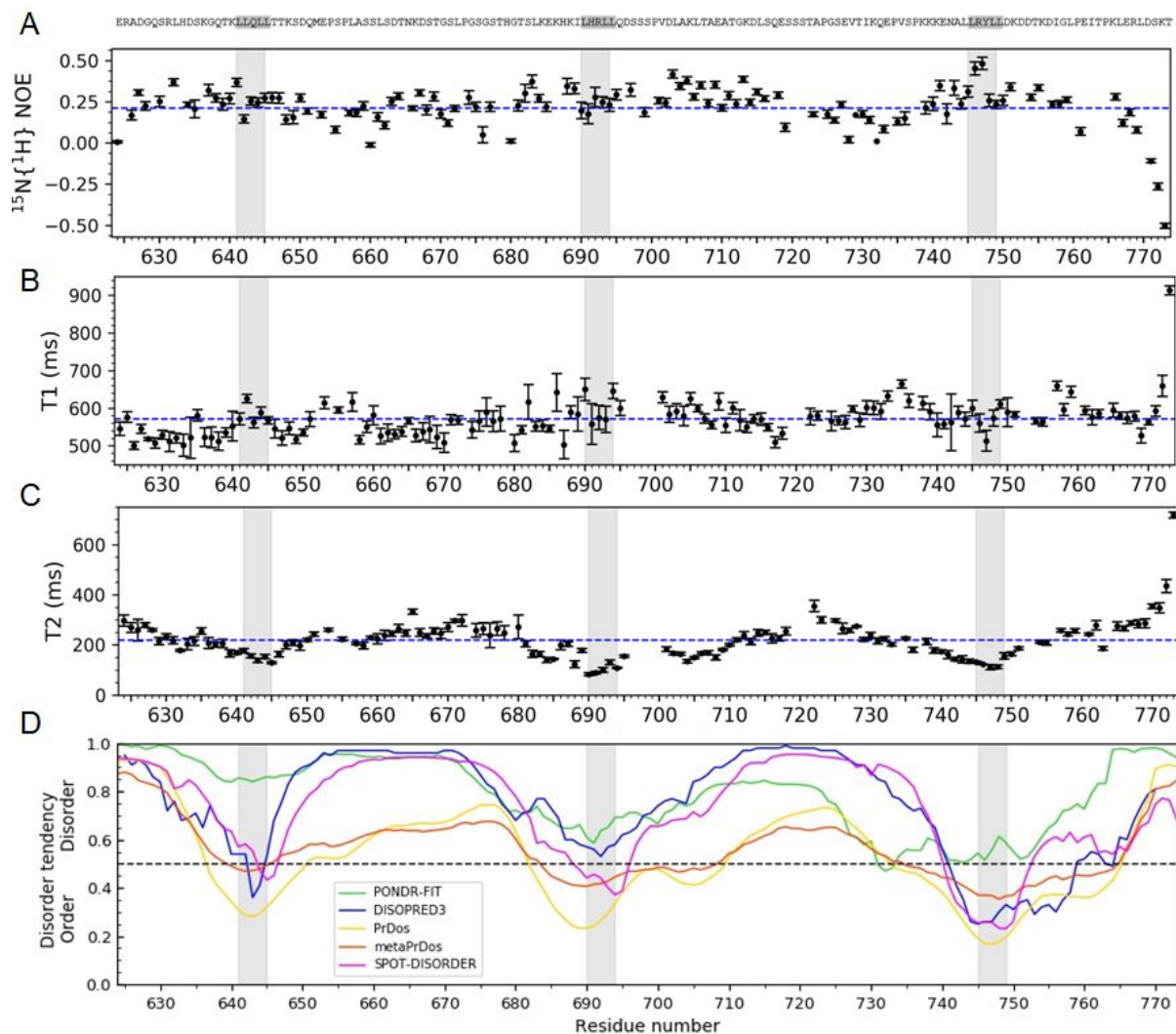


Figure S6. Related to **Figure 2E**. **Backbone dynamics of TIF2_{NRID}** per residue **A)** heteronuclear ^1H - ^{15}N NOE, **B)** ^{15}N - T_1 longitudinal, **C)** ^{15}N - T_2 transverse relaxation times and. Blue dashed lines represent the average values. All experiments were recorded at 10°C and at pH 6.8 with a 700 MHz spectrometer using 200 μM of protein in the NMR buffer. Disorder predictions **D)** from Figure 2 were added for easier interpretation of the data. Boxes highlighted in grey indicate NR Boxes (LxxLL motifs). Values and errors were calculated using Sparky. Proline, superimposed and ambiguous assigned residues were excluded from the analysis.

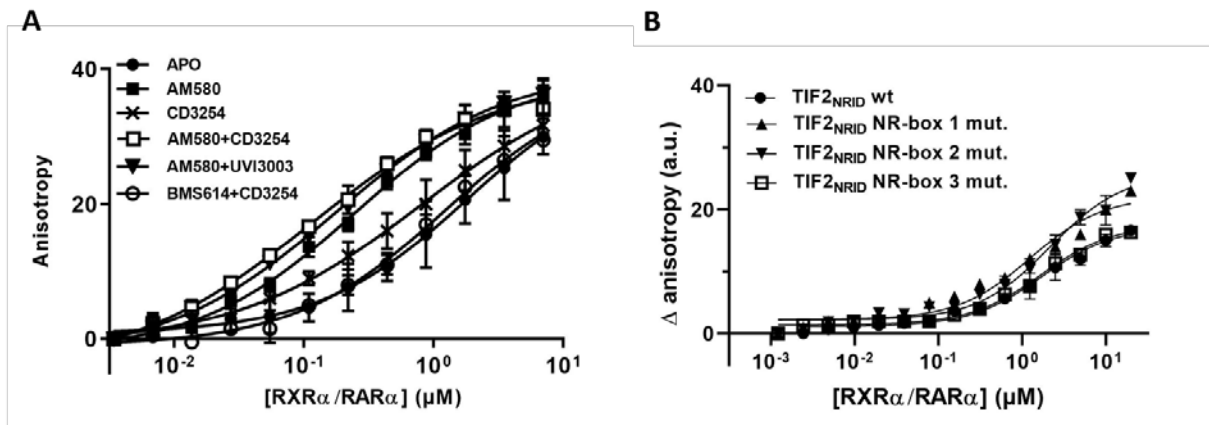


Figure S7 related to **Figure 3**. **A)** Titration curves of FITC-labelled TIF2_{NRID} by RXR/RAR in the absence of ligand (Apo) or in the presence of an excess of RAR agonist (AM580), RXR agonist (CD3254), or combination of ligands including RAR antagonist (BMS614) and RXR antagonist (UVI3003). **B)** Titration of Alexa-labelled TIF2_{NRID} wt or mutant for each NR-box by RXR/RAR in the absence of ligand.

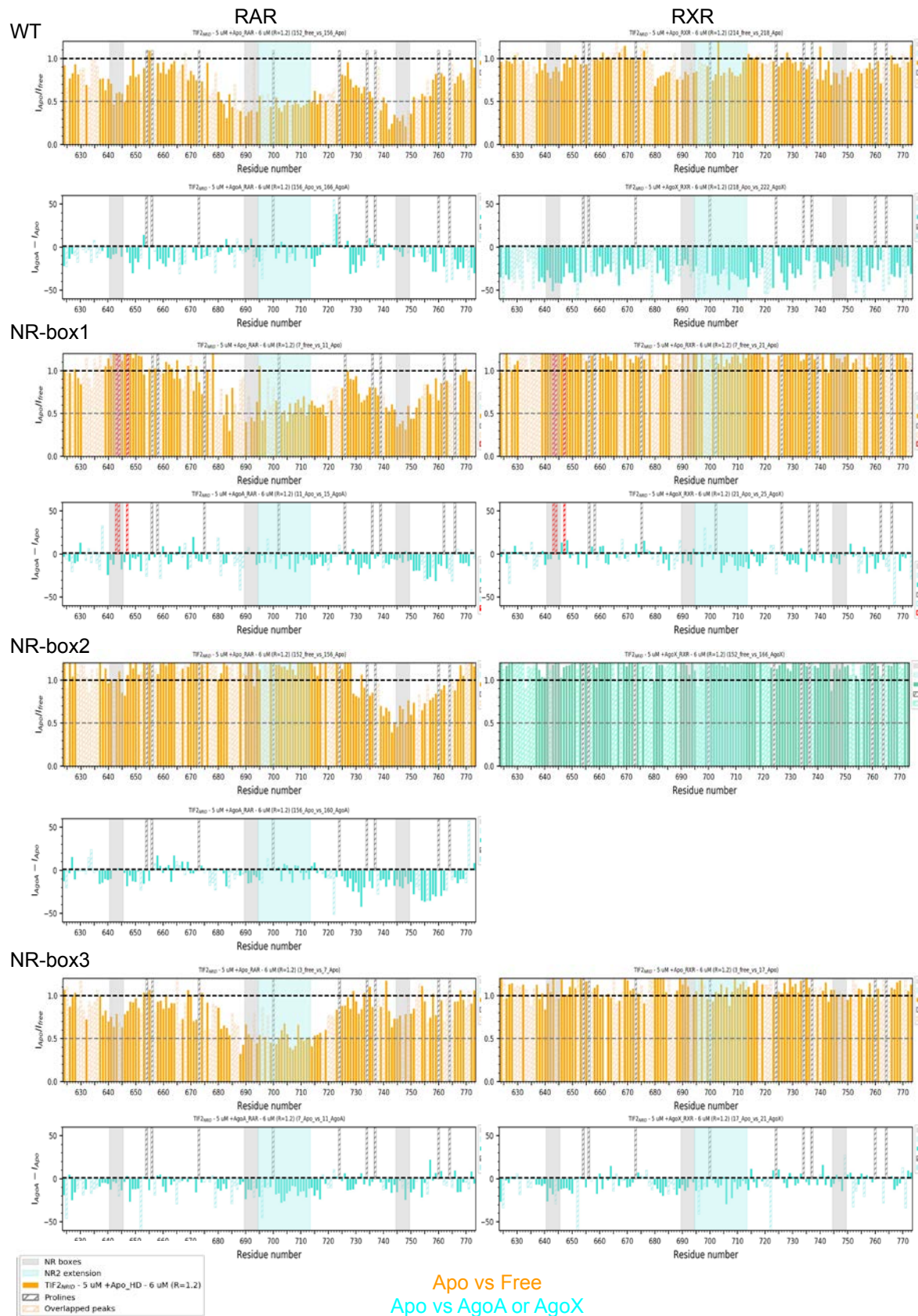


Figure S8 related to **Figure 4** and **5**. Interaction of WT and LLxxAA mutated TIF2_{NRID} with apo and liganded RXR or RAR LBDs. Relative peak intensity ratios $I_{Complex}/I_{TIF2NRID}$ and differential intensity $I_{liganded\ complex} - I_{apo\ complex}$ for **A)** WT TIF2_{NRID} **B)** NR-box1 mutant **C)** NR-box 2 mutant **C)** NR-box3 mutant in complex with RAR (left column) or RXR (right column). Grey highlighted boxes indicate NR-boxes. Hashed bars correspond to prolines or ambiguous and overlapped peaks.

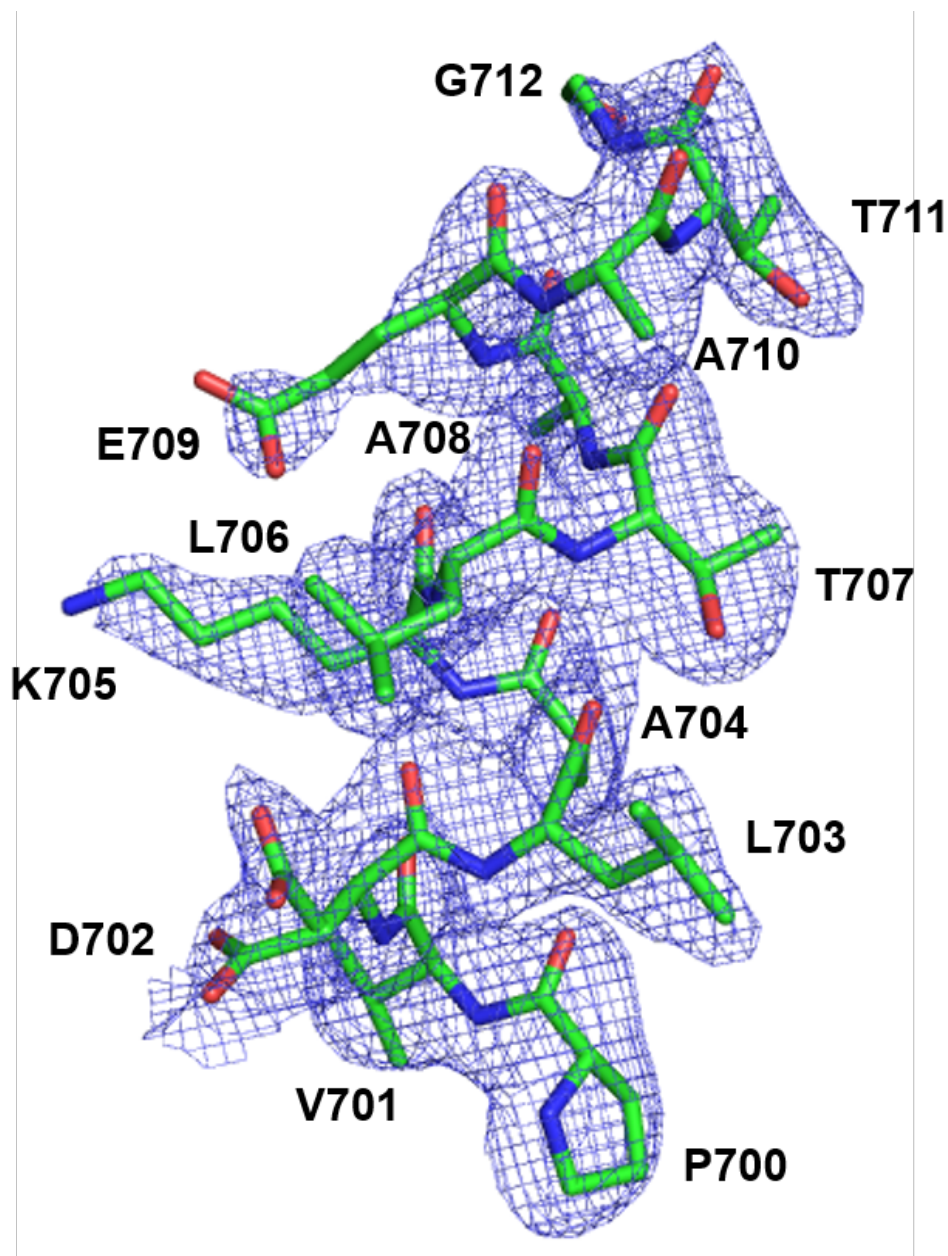


Figure S9, related to **Figure 6**. Helix $\alpha 2$ of TIF2 NR2-Ext peptide modelled into the difference density of the RAR LBD/TIF2 NR2-Ext crystal structure. Shown is an unbiased omit Polder map contoured at 2.5σ , with model bias reduction and exclusion of solvent density.

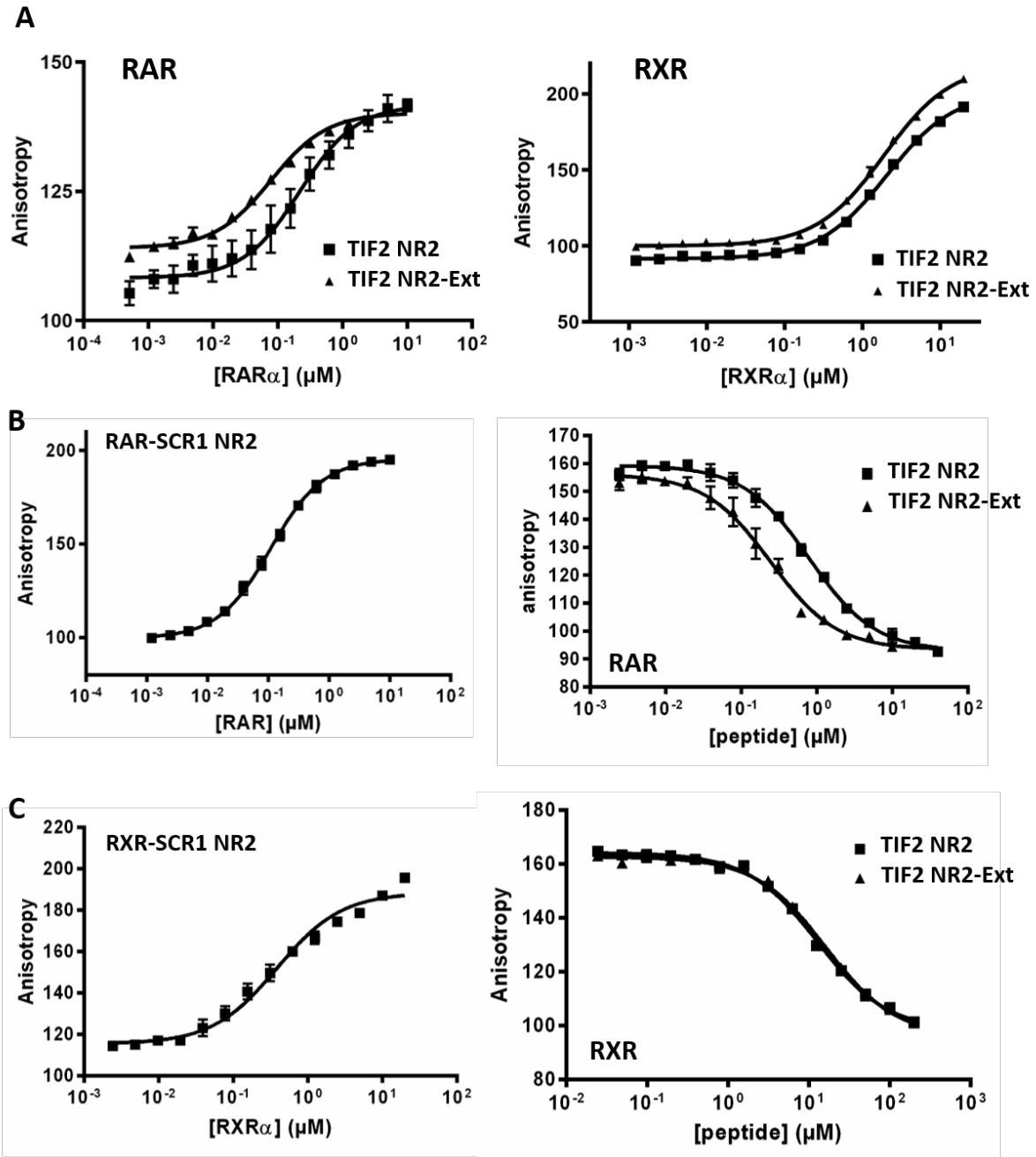


Figure S10 related to **Figure 6**. **A**) Titration curves of Alexa-labeled TIF2 NR2 and TIF2 NR2-Ext by RAR and RXR LBDs in the presence of AM580 (RAR agonist) and CD3254 (RXR agonist), respectively. **B**) Direct titration of FITC-labeled SCR1 NR2 peptide by RAR LBD in the presence of the RAR agonist AM580 (left) and competition curves at 0.5 μM of RAR LBD of SCR1 NR2 by unlabeled TIF2 NR2 and TIF2 NR2-Ext peptides (right) in the presence of AM580. **C**) Direct titration of FITC-labeled SCR1 NR2 peptide by RXR LBD in the presence of the RXR agonist LG100268 (left) and competition curves at 5 μM of RXR LBD of SCR1 NR2 by unlabeled TIF2 NR2 and TIF2 NR2-Ext peptides (right) in the presence of LG100268.

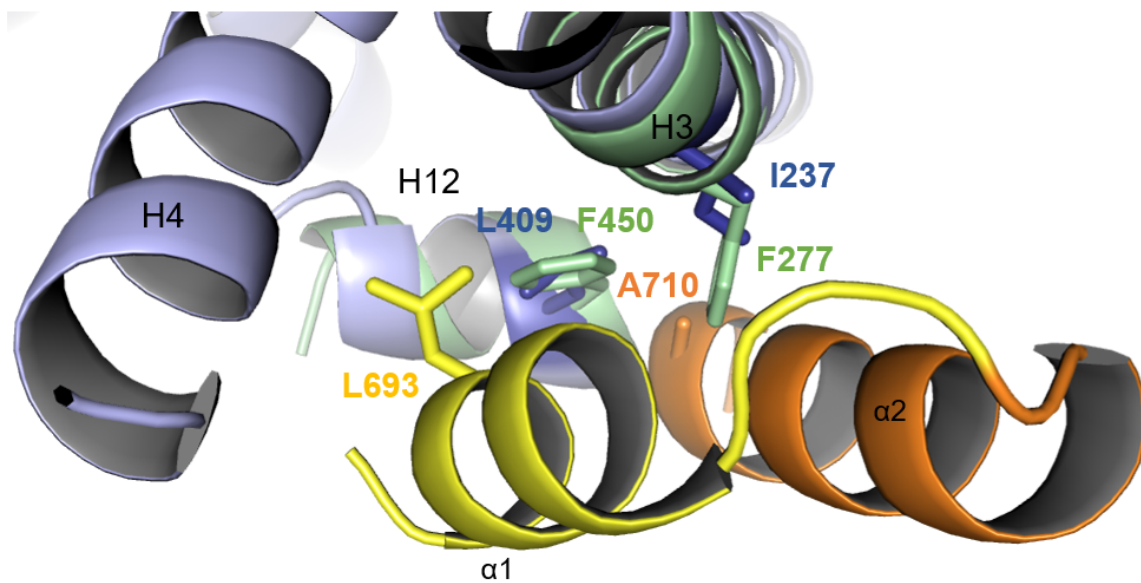


Figure S11 related to **Figure 6**. Zoom-in of the superposition of RAR LBD-TIF2 NR2-Ext structure (blue cartoon for RAR and yellow and orange cartoon for the peptide) and of RXR LBD-TIF2 NR2-Ext structure (green cartoon for RXR) to show the presence of two large phenylalanine residues (F277 and F450) at the place of the RAR residues I237 and L409 that most likely prevent the interaction between RXR and helix $\alpha 2$ of the TIF2 peptide.

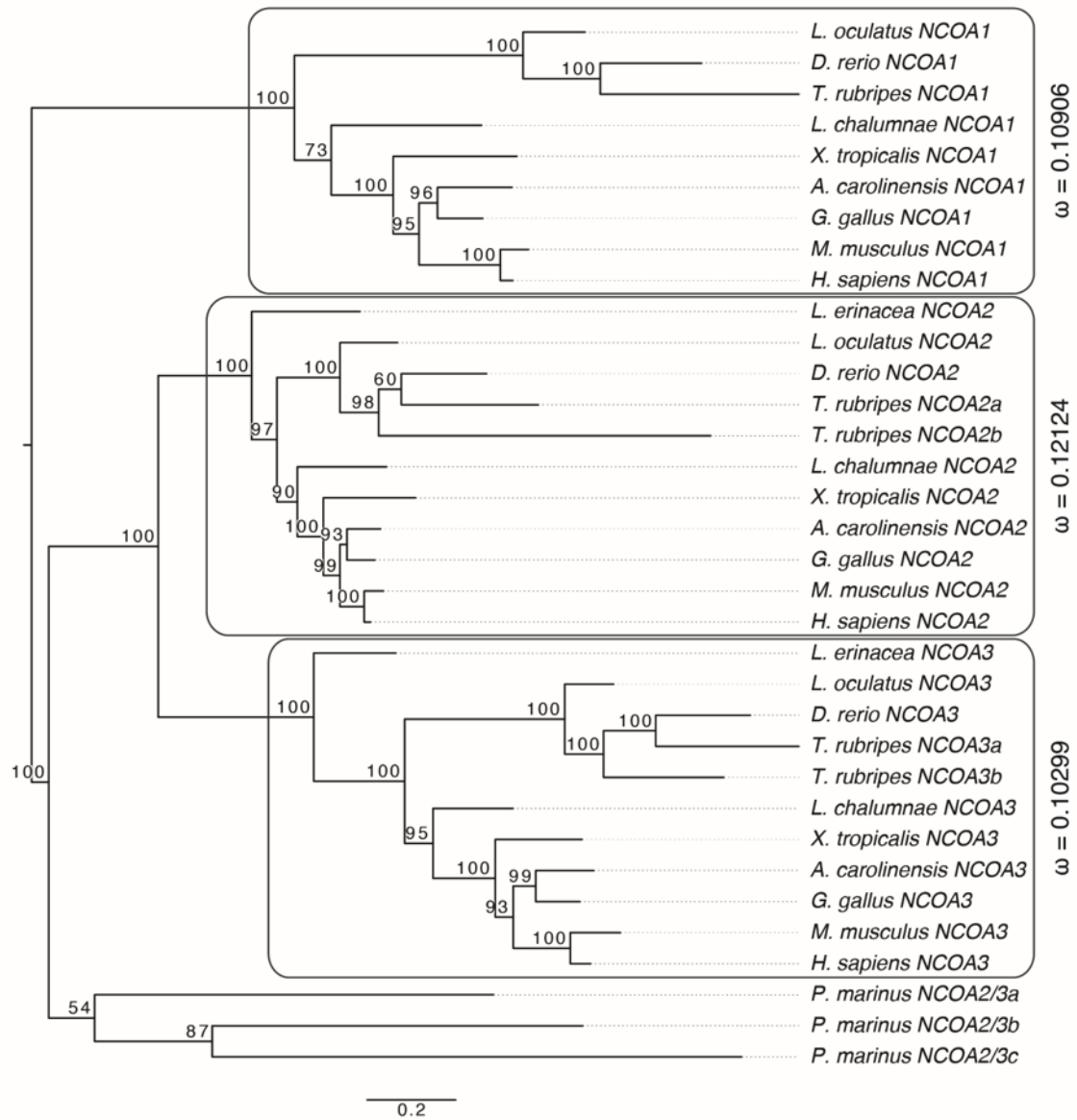


Figure S12 related to **Figure 7. Phylogenetic analysis of the vertebrate nuclear receptor co-activator (NCOA) family.** Maximum Likelihood (ML) tree of vertebrate NCOA proteins with branch support (Bootstrap percentages) indicated at each node. The tree is midpoint rooted and branch lengths correspond to sequence substitution rates. The ratio between non-synonymous and synonymous substitutions (ω) is shown for each of the three paralogous lineages (NCOA1 (SRC1), NCOA2 (TIF2) and NCOA3 (RAC3)).