Supplementary Material for:

Antidiabetic Phospholipid–Nuclear Receptor Complex Reveals the Mechanism for Phospholipid Driven Gene Regulation

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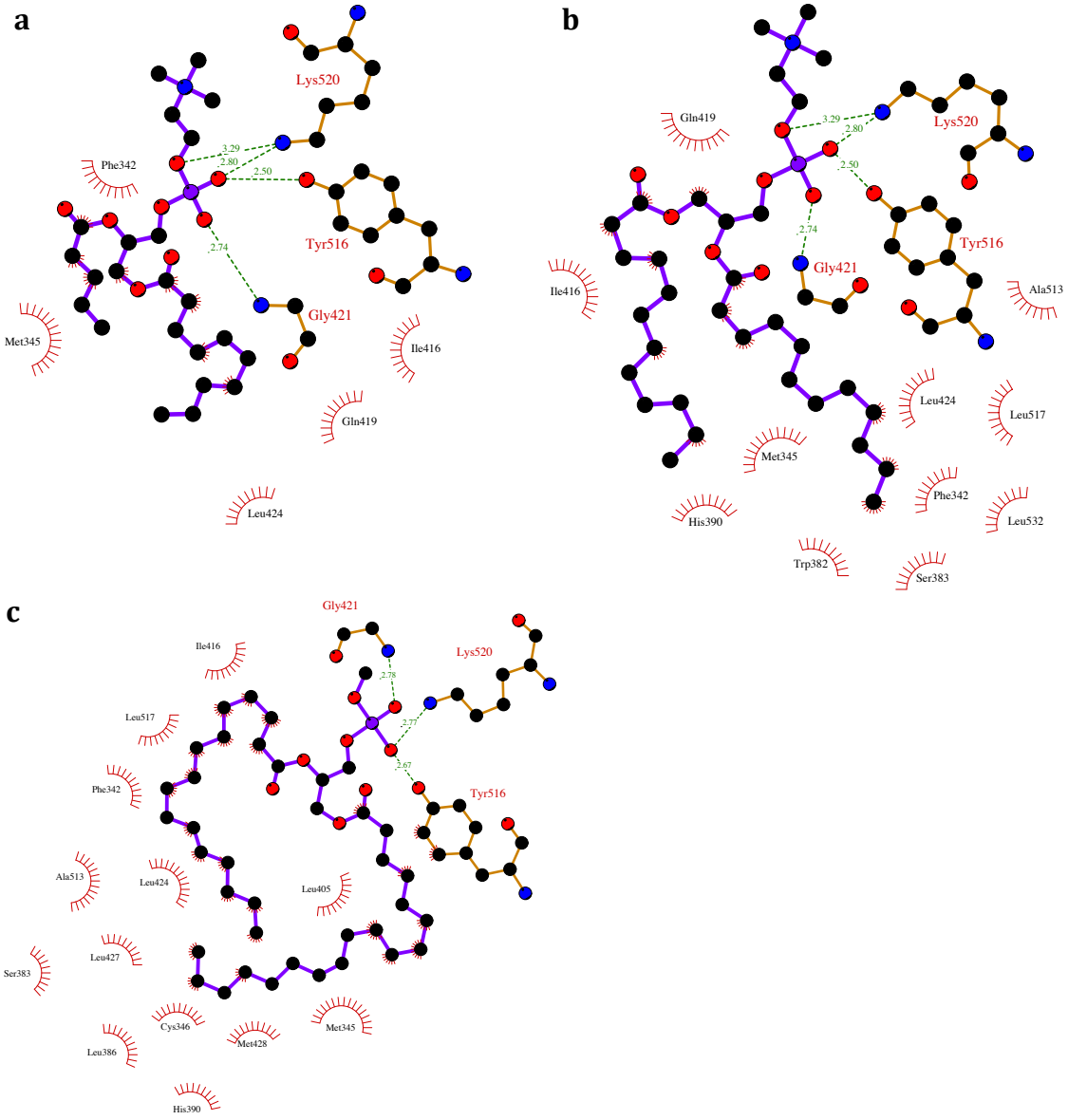
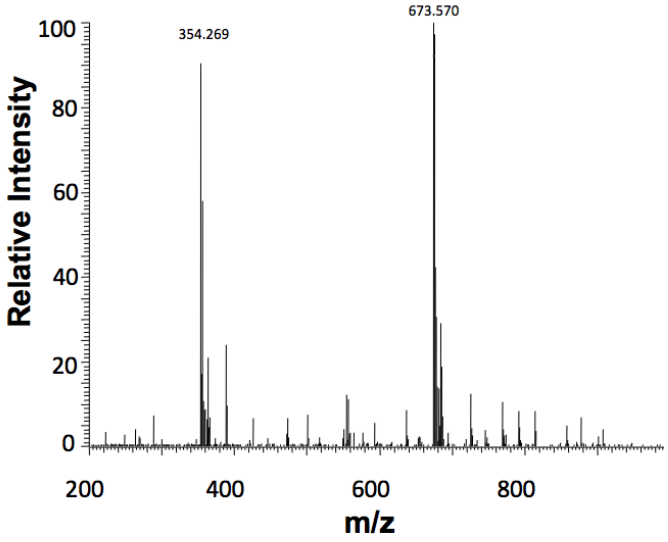
Supplementary Figures

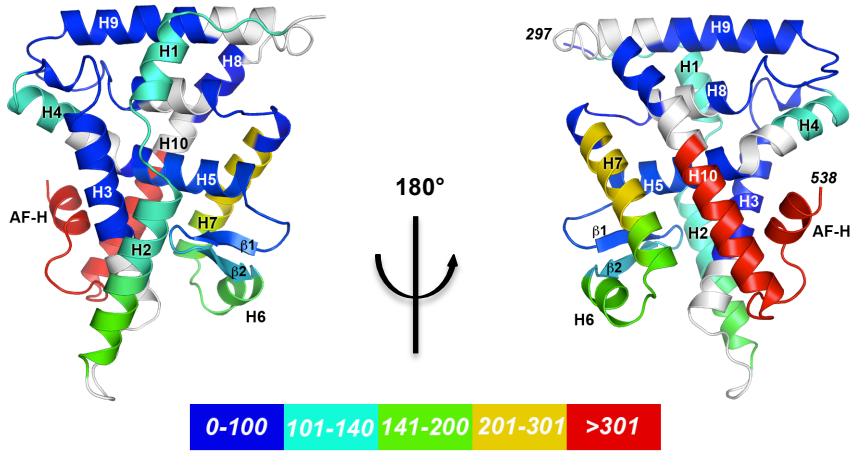


Supplementary Figure 1 Ligplot analysis of contacts between lipids and LRH-1. (**a**) Ligplot representation of contacts between LRH-1-DLPC showing only well ordered ligand atoms, (**b**) LRH-1-DLPC showing all ligand atoms, and (**c**) LRH-1-*E. coli* PL (PDB code 1YUC)[1]. The LRH-1-DLPC complex shows far fewer contacts between the acyl tails and the back for the LRH-1 ligand binding pocket vs. the LRH-1-E. coli PL complex. Figures were generated in Ligplot+ and LigEd[2].

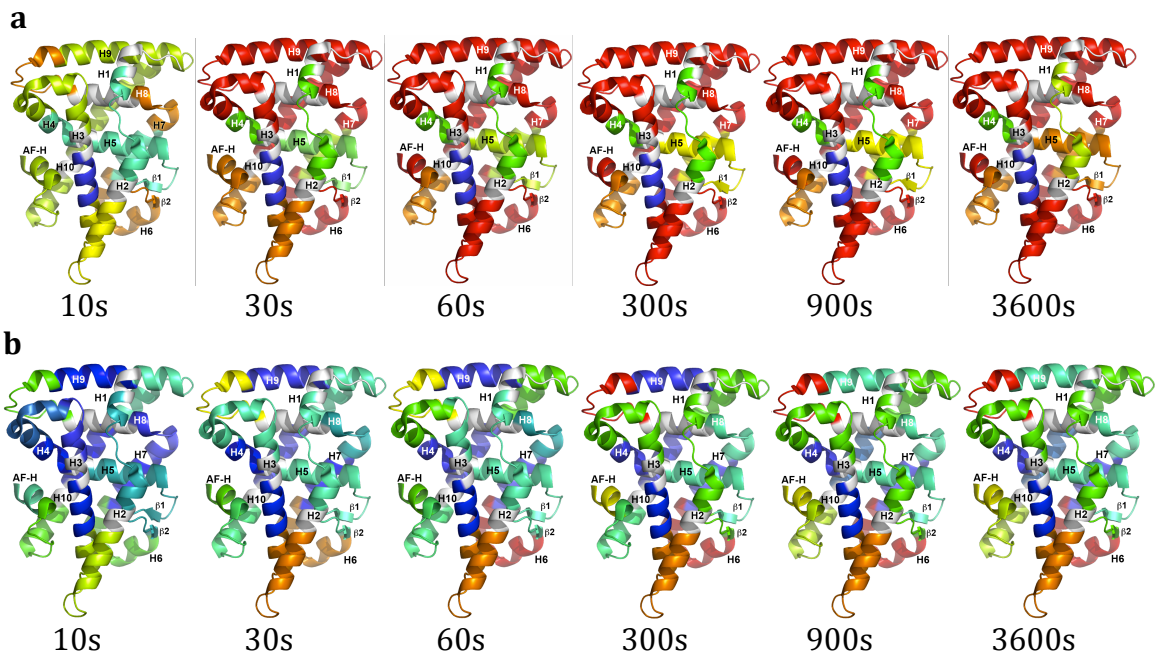
a



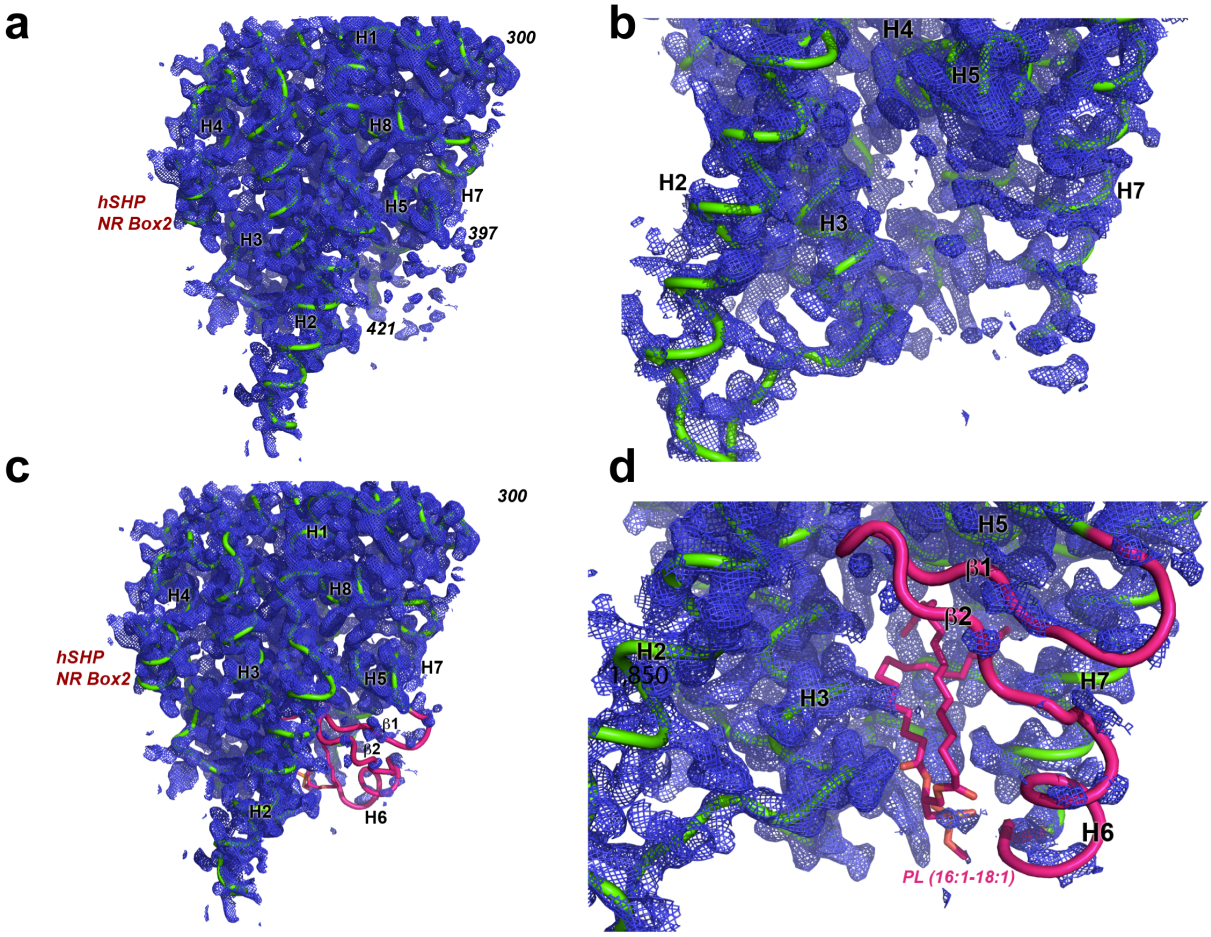
b



Supplementary Figure 2 Refolded LRH-1 is devoid of lipids and has increased sensitivity to proteolysis. (**a**) Negative ESI MS of apo LRH-1 showing no evidence for bound phospholipid. (**b**) Degree of protection from chymotrypsin proteolysis conferred by DLPC binding (Supplementary Table 2) mapped with color on DLPC-LRH-1 (a higher number is a higher degree of protection from proteolysis after DLPC binding). Residues that were not observed via MS are colored grey.



Supplementary Figure 3 Apo LRH-1 incorporates deuterium faster than native LRH-1. (**a**) Percent deuterium incorporation over time apo LRH-1 LBD and (**b**) native LRH-1 LBD mapped to PDB ID code 1YUC[1].



Supplementary Figure 4 Apo LRH-1 shows no evidence of a bound lipid or a portion of the ligand binding pocket. (**a-b**) $2F_o$-F_c electron density contoured at 1σ showing no evidence for either bound PL or for amino acids residues 397-421comprising the β1-H7 region of the ligand binding pocket. Apo LRH-1 is rendered as a cartoon loop with observed residues in green. (**c-d**) Identical views of apo LRH-1 showing the location of missing residues (magenta) that are well ordered in the LRH-1-DLPC complex. Attempts to model either PL or the missing apo LRH-1 residues resulted in clear negative F_o-F_c electron density (not shown).

Supplementary Tables

	Coregulator Peptide Recruitment									
	PGC-1α		**SRC1**		**TIF2**		**SHP**		**SMRT**	
Ligand	K_d (μM)	R^2	K_d (μM)	R^2	K_d (μM)	R^2	K_d (μM)	R^2	K_d (μM)	R^2
LRH-1 E. coli PLs	6.1 +/- 0.4	0.99	8.8 +/- 0.8	0.99	20.1 +/- 2.9	0.98	0.6 +/- 0.1	0.99	n.b.	~
Refolded/Apo	n.b.	~	8.0 +/- 0.9	0.97	2.3 +/- 0.4	0.95	7.36 +/- 0.4	0.99	0.050 +/- .003	0.98
LRH-1 DLPC	6.8 +/- 0.5	0.99	2.3 +/- 0.4	0.96	6.5 +/- 2.0	0.91	n.b.	~	n.b.	~

n.b. - no binding detected

Supplementary Table 1 K_d's of LRH-1 in various ligand states binding to coregulator peptides.

AA number	AA sequence	Protection Factor
299-323	SSPASIPHLILELLKCEPDEPQVQAKIM	119
324-325	AY	137
326	L	147
327-333	QQEQANR	155
334-347	SKHEKLSTFGLMCK	ND
348-354	MADQTLF	14
355-369	SIVEWARSSIFFREL	33
370-377	KVDDQMKL	115
378-382	LQNCW	ND
383-385	SEL	1
386-405	LILDHIYRQVVHGKEGSIF	47
406	L	63
407-413	VTGQQVDY	89
414-424	SIIASQAGATL	153
425-427	NNL	211
428	M	239
429-438	SHAQELVAKL	282
439-446	RSLQFDQR	8
447-452	EFVCLK	ND
453-481	FLVLFSLDVKNLENFQLVEGVQEQVNAAL	34
482-495	LDYTMCNYPQQTEK	ND
496-502	FGQLLLR	15
503-507	LPEIR	ND
508-539	AISMQAEEYLYYKHLNGDVPYNNLLIEMLHAK	439
540-541	RA	ND

Supplementary Table 2 Proteolysis protection data showing spectral count differences for trypsin fragments.

Supplementary Methods

Proteolytic Protection Assay

DLPC or apo LRH-1 (11.25 μg**)** was digested with 80 ηg of chymotrypsin (Protea Biosciences, Inc.) for 5 min at room temperature. The reaction was quenched with the addition of acetic acid and boiled for 5 minutes. The entire reaction was resolved by SDS-PAGE and stained by Coomassie blue. Gel regions below undigested intact protein were excised and were subjected to in-gel trypsin digestion. The digested peptides were analyzed by reverse-phase liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) as previously described[3]. Briefly, peptide mixtures were loaded onto a C_{18} column (75 μm i.d., 30 cm long, 3 μm resin from Michrom Bioresources, Inc., Auburn, CA) and eluted over a 12-35% gradient (Buffer A: 0.1% Formic Acid, 0.005% heptafluorobutyric acid, and 5% AcN; Buffer B: 0.1% formic acid, 0.005% heptafluorobutyric acid, and 95% AcN). Eluates were monitored in a MS survey scan followed by ten data-dependent MS/MS scans on an LTQ-Orbitrap ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The LTQ was used to acquire MS/MS spectra (2 m/z isolation width, 35% collision energy, 5,000 AGC target, 300 ms maximum ion time). The Orbitrap was used to collect MS scans (300-1600 m z^{-1}, 1,000,000 AGC target, 750 ms maximum ion time, resolution 60,000). The acquired MS/MS spectra were searched against a concatenated target-decoy *E.coli* database (UNIPROT January 23, 2011) that included the LRH-1 sequence using the SEQUEST Sorcerer algorithm (version 2.0, SAGE-N)[4]. Searching parameters included: partially tryptic restriction, parent ion mass tolerance (± 10 ppm), product ion tolerance (± 0.5 m z^{-1}), and dynamic modifications for oxidized Met (+15.9949 Da). The peptides were classified by charge state and trypticity (fully and partial) and filtered dynamically by increasing XCorr and ΔCn values to reduce the protein false discovery rate to less than 5%. The MS/MS spectra of matched LRH-1 peptides were manually inspected. Trypsin digest sites were removed manually and spectral counts per peptide were used to determine the relative amount of each chymotrypsin proteolysis fragment between DLPC and apo RLH-1. The protection factor reported in Supplementary Fig. 5 and Supplementary Table S2 is the result of subtracting spectral counts for chymotryptic proteolysis fragments observed in the LRH-1-DLPC complex from the same chymotryptic proteolysis fragments generated from the apo protein. A higher protection factor indicates less chymotrypsin cleavage events upon DLPC binding.

Supplementary References

1. Ortlund, E.A. et al. Modulation of human nuclear receptor LRH-1 activity by phospholipids and SHP. *Nat Struct Mol Biol* **12**, 357-63 (2005).
2. Laskowski, R.A. & Swindells, M.B. LigPlot+: Multiple Ligand-Protein Interaction Diagrams for Drug Discovery. *J Chem Inf Model* **51**, 2778-86 (2011).
3. Xu, P., Duong, D.M. & Peng, J. Systematical optimization of reverse-phase chromatography for shotgun proteomics. *J Proteome Res* **8**, 3944-50 (2009).
4. Eng, J., McCormack, A.L. & Yates, J.R., 3rd. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* **5**, 976-989 (1994).