

PPAR α were determined by chemical-mediated fluorescence energy transfer assays using the AlphaScreen Technology from Packard BioScience³⁰. The experiments were conducted with 5 nM PPAR α LBD of biotinylated peptide containing individual motifs (Fig. 3a), following the manufacturer's instructions for the hexahistidine detection kit in a buffer containing 50 mM MOPS, pH 7.4, 50 mM NaF, 0.05 mM CHAPS, 0.1 mg ml⁻¹ bovine serum albumin, and 10 mM dithiothreitol (DTT). The binding signals were detected with the increasing concentrations of GW6471, and the results from four repeated experiments were normalized as a percentage of the binding in the absence of GW6471.

The effects of GW6471 on the affinity of the SMRT or N-CoR peptides with purified PPAR α LBD were determined by fluorescence polarization in a buffer containing 10 mM HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate-20, 5 mM DTT and 2.5% DMSO. Varied concentration of PPAR α LBD in the presence or absence of 40 μ M GW6471 were incubated at room temperature with 10 nM of a fluorescein-labelled peptide of N-CoR2 or SMRT2 (Fig. 3a). The fluorescence polarization values for each concentration of receptor were determined using a BMG PolarStar Galaxy fluorescence reader with 485 nm excitation and 520 nm emission filters. The apparent dissociation constant (K_d) values were determined by the binding curves derived from a nonlinear least-squares-fit of the data for a simple 1:1 interaction.

Mutational analysis of the SMRT co-repressor motif interaction with the PPAR α and TR β LBDs was also performed by fluorescence polarization. To determine the importance of each amino acid in the SMRT motif for binding to nuclear receptors, SMRT peptides with alanine substitution at each position were added to inhibit the binding of 1 μ M TR β LBD or 2 μ M PPAR α to the fluorescent N-CoR2 peptide. For the PPAR α experiments we added 10 μ M GW6471. The inhibition curves were constructed and IC₅₀ values were determined by nonlinear least-squares-fit of the data to a simple 1:1 interaction.

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1. Horlein, A. J. *et al.* Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**, 397–404 (1995).
2. Chen, J. D. & Evans, R. M. A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**, 454–457 (1995).
3. Nagy, L. *et al.* Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89**, 373–380 (1997).
4. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L. & Ayer, D. E. Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell* **89**, 341–347 (1997).
5. Laherty, C. D. *et al.* Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell* **89**, 349–356 (1997).
6. Hong, S. H., David, G., Wong, C. W., Dejean, A. & Privalsky, M. L. SMRT corepressor interacts with PLZF and with the PML-retinoic acid receptor alpha (RAR α) and PLZF-RAR α oncoproteins associated with acute promyelocytic leukemia. *Proc. Natl Acad. Sci. USA* **94**, 9028–9033 (1997).
7. Grignani, F. *et al.* Fusion proteins of the retinoic acid receptor- α recruit histone deacetylase in promyelocytic leukaemia. *Nature* **391**, 815–881 (1998).
8. Yoh, S. M., Chatterjee, V. K. & Privalsky, M. L. Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T3 receptors and transcriptional corepressors. *Mol. Endocrinol.* **11**, 470–480 (1997).
9. Jackson, T. A. *et al.* The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. *Mol. Endocrinol.* **11**, 693–705 (1997).
10. Issemann, I. & Green, S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**, 645–650 (1990).
11. Xu, H. E. *et al.* Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. *Proc. Natl Acad. Sci. USA* **98**, 13919–13924 (2001).
12. Onate, S. A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**, 1354–1357 (1995).
13. Shiau, A. K. *et al.* The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95**, 927–937 (1998).
14. Darimont, B. D. *et al.* Structure and specificity of nuclear receptor-coactivator interactions. *Genes Dev.* **12**, 3343–3356 (1998).
15. Gampe, R. T. Jr *et al.* Asymmetry in the PPAR γ /RXR α crystal structure reveals the molecular basis of heterodimerization among nuclear receptors. *Mol. Cell* **5**, 545–555 (2000).
16. Nolte, R. T. *et al.* Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor- γ . *Nature* **395**, 137–143 (1998).
17. Heery, D. M., Kalkhoven, E., Hoare, S. & Parker, M. G. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387**, 733–736 (1997).
18. Yang, W., Rachez, C. & Freedman, L. P. Discrete roles for peroxisome proliferator-activated receptor gamma and retinoid X receptor in recruiting nuclear receptor coactivators. *Mol. Cell Biol.* **20**, 8008–8017 (2000).
19. Nagy, L. *et al.* Mechanism of corepressor binding and release from nuclear hormone receptors. *Genes Dev.* **13**, 3209–3216 (1999).
20. Hu, X. & Lazar, M. A. The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. *Nature* **402**, 93–96 (1999).
21. Perissi, V. *et al.* Molecular determinants of nuclear receptor-co-repressor interaction. *Genes Dev.* **13**, 3198–3208 (1999).
22. Zhou, G. *et al.* Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. *Mol. Endocrinol.* **12**, 1594–1604 (1998).
23. Xu, H. E. *et al.* Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell* **3**, 397–403 (1999).
24. Otwinowski, Z. & Minor, W. in *Macromolecular Crystallography* (eds Carter, J. C. W. & Sweet, R. M.) 307–326 (Academic, New York, 1997).
25. Navaza, J., Gover, S. & Wolf, W. in *Molecular Replacement: Proceedings of the CCP4 Study Weekend* (ed. Dodson, E. J.) 87–90 (SERC, Daresbury, 1992).
26. Cowtan, K. in *Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography* **31**, 34–38 (1994).
27. Nolte, R. T., Conlin, R. M., Harrison, S. C. & Brown, R. S. Differing roles for zinc fingers in DNA

recognition: structure of a six-finger transcription factor IIIA complex. *Proc. Natl Acad. Sci. USA* **95**, 2938–2943 (1998).

28. Brunger, A. T. *et al.* Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr. D* **54**, 905–921 (1998).
29. Oberfield, J. L. *et al.* A peroxisome proliferator-activated receptor gamma ligand inhibits adipocyte differentiation. *Proc. Natl Acad. Sci. USA* **96**, 6102–6106 (1999).
30. Ullman, E. F. *et al.* Luminescent oxygen channeling immunoassay: measurement of particle binding kinetics by chemiluminescence. *Proc. Natl Acad. Sci. USA* **91**, 5426–5430 (1994).

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Competing interests statement

The authors declare that they have no competing financial interests.

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(e-mail: ex11957@gsk.com). The Protein Data Bank code for the PPAR α /GW6471/SMRT complex and the PPAR α /GW409544/SRC-1 complex is 1KQQ and 1K7L, respectively.

correction

Autonomic healing of polymer composites

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In this Letter, the middle infrared spectrum in Fig. 3b, corresponding to an authentic sample of poly(DCPD) prepared with Grubbs' catalyst and DCPD monomer, was a duplicate of the top spectrum owing to a formatting error. The corrected spectra are shown below. □

