**Supplementary Figures**

# The transcriptional PPARβ/δ network in human macrophages defines a unique agonist-induced activation state

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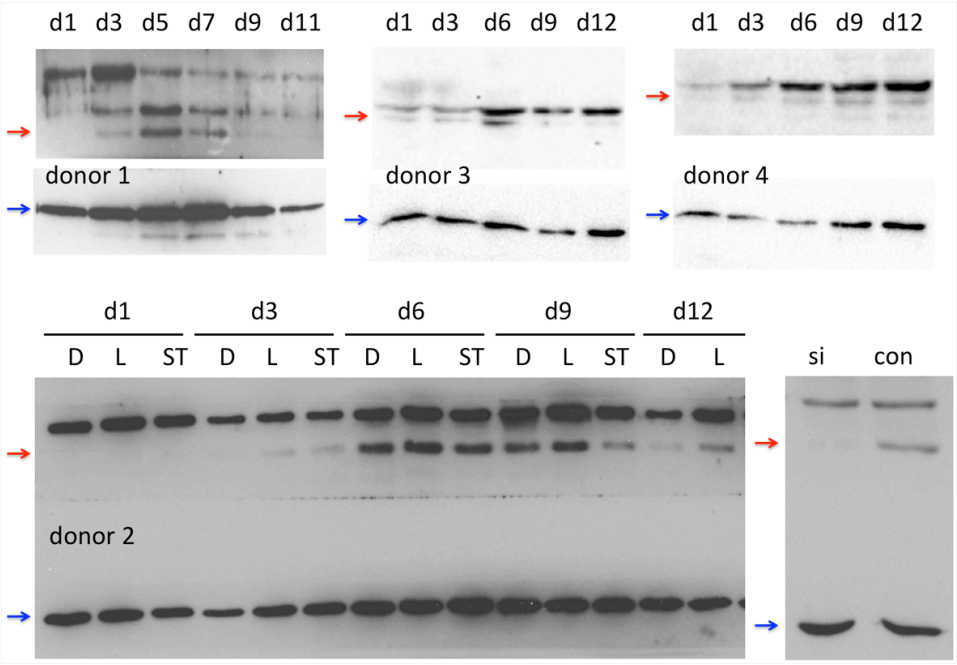


**Figure S1** – Immunoblot analysis of PPARβ/δ expression during MDM differentiation. The blot shows the data for cells from four different donors used for the quantification in Figure 1B. Red arrows: PPARβ/δ bands, identified by siRNA-mediated knockdown (bottom right blot). Blue arrows: lactate dehydrogenase (LDH; loading control). PPARβ/δ bands were quantified (ChemiDoc MP) and adjusted to LDH band intensities( Figure 1B). The immunoblot of donor 2 MDMs shows that the agonist L165,041 (L) and the inverse agonist ST247 (ST) have no effect on PPARβ/δ protein expression.

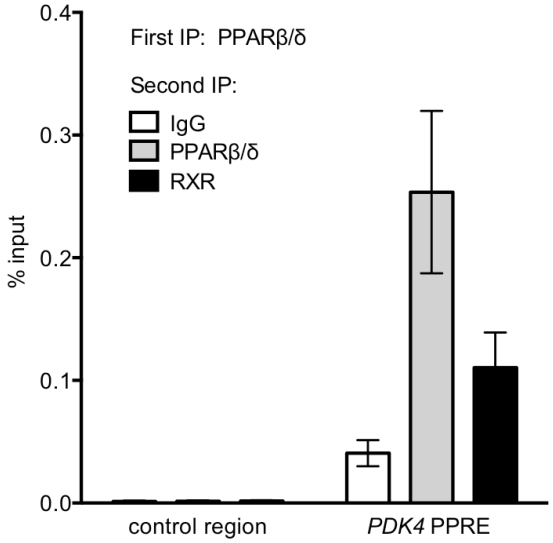


**Figure S2** – Re-ChIP analysis of PPARβ/δ-RXR complexes at the *PDK4* enhancer in MDMs. After differentiation for 7 days, MDMs (sample size = 5) were fixed with formaldehyde, and sequential ChIP (ChIP re-ChIP) was carried out with sc-7197 anti-PPARβ/δ (Santa Cruz), sc-553 anti-RXR (Santa Cruz) or rabbit IgG I5006 (Sigma-Aldrich). For re-ChIP, chromatin complexes were eluted with 50 µl of 1X TE containing 2% SDS, 15 mM DTT and protease inhibitors for 30 min at at 37 °C with agitation. After centrifugation, the supernatant was diluted 30X with dilution buffer (50 mM Tris pH 8.0, 200 mM NaCl, 5 mM EDTA, 0.5% NP40). The second round of IPs was carried out as described in the Methods section. DNA was analyzed by qPCR with primers for the *PDK4* upstream enhancer and an irrelevant control region as in Figure 1D. The control primers had the following sequences: AAGGGATTTCCCCAGCAG (forward); GAAATAGCAGGGACCTCGTG (reverse).

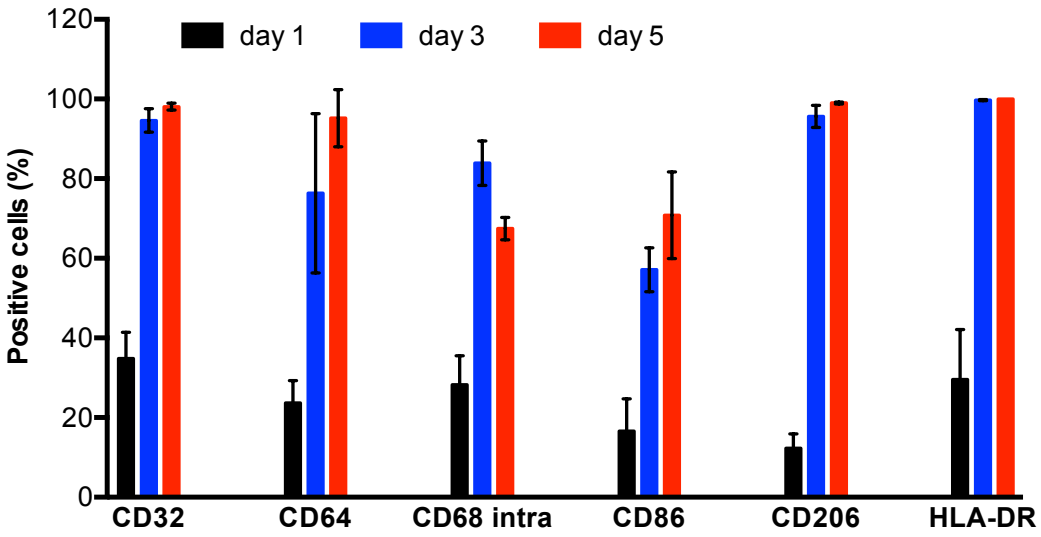


**Figure S3** – Expression of the macrophage surface markers CD32, CD64, CD86, CD206 and HLA-DR and intracellular CD68 on differentiating MDMs (biological replicates with cells from 3 different donors; experimental setup as in Figure 1). Staining and FACS analysis were performed as described in Materials and Methods.

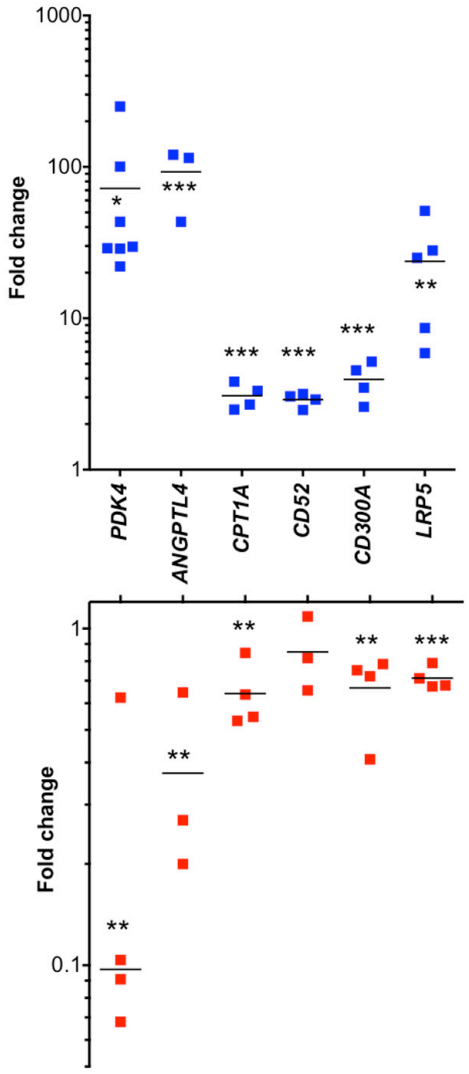


**Figure S4** – RT-qPCR analysis of target gene regulation by L165,041 (agonist; blue dots) and ST247 (inverse agonist; red dots). Each dot represents a biological replicate with cells from a different donor (n = 4-9). Horizontal lines indicate the median. *P<0.05; **P<0.01; ***P<0.001 by t-test relative to DMSO-treated cells

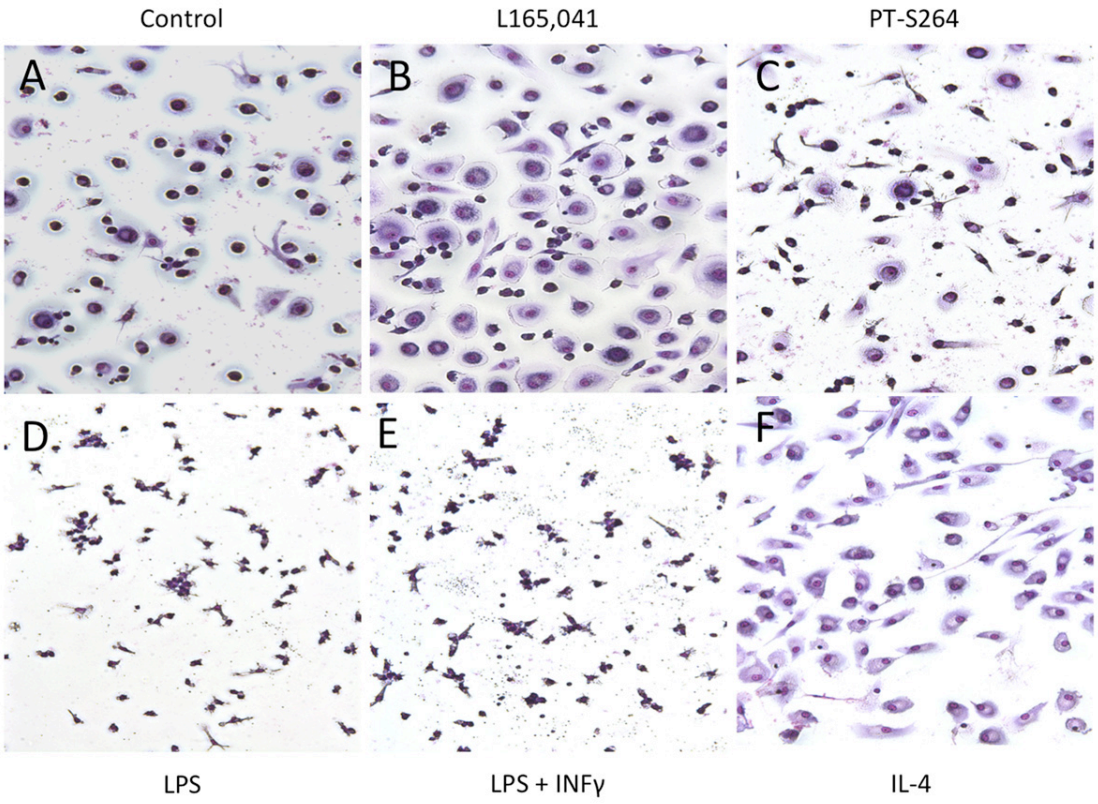


**Figure S5** – Effects of PPARβ/δ ligands on the morphology of human MDMs. Human monocytes were differentiated in R10 medium for 6 d in the presence of the indicated additives. Cells were stained with Giemsa dye after treatment with **(A)** DMSO (solvent control), **(B)** L165,041 (agonist), **(C)** PT-S264 (inverse agonist), **(D**) LPS ("M1" macrophages), **(E)** ILPS+FNγ ("M1" macrophages) and **(F)** IL-4 ("M2" macrophages).

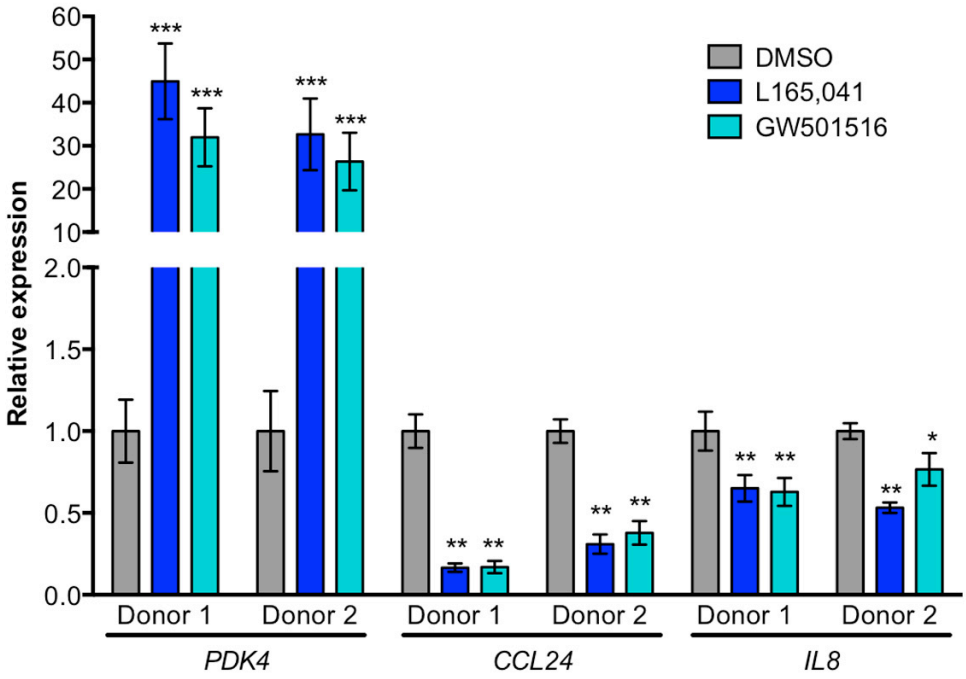


**Figure S6** – RT-qPCR analysis of target gene regulation by GW501516 in MDMs compared to L165,041. The data represent 3 experiments performed with cells from two different donors; error bars show the standard deviation. *P<0.05; **P<0.01; ***P<0.001 by t-test relative to DMSO-treated cells.

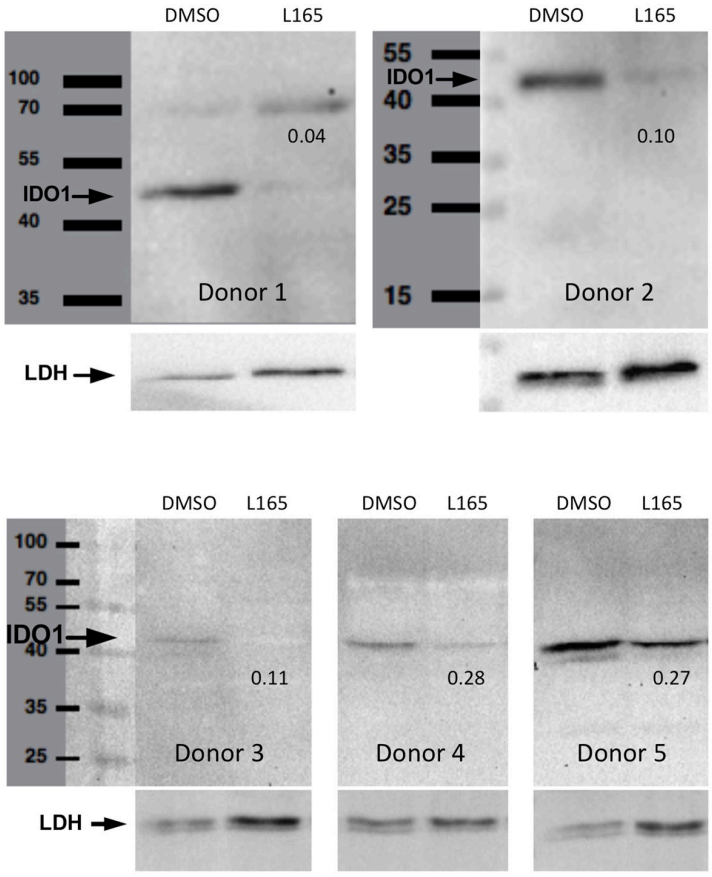


**Figure S7** – Immunoblot analysis of IDO-1 expression in MDMs treated with DMSO or L165,041. The blot shows the data for cell from five different donors (as in Figure 7C). L165: L165,041, LDH: lactate dehydrogenase (loading control). IDO-1 bands were quantified (ChemiDoc MP) and adjusted to LDH band intensities. Expression values (relative to DMSO) are shown below the IDO-1 bands.

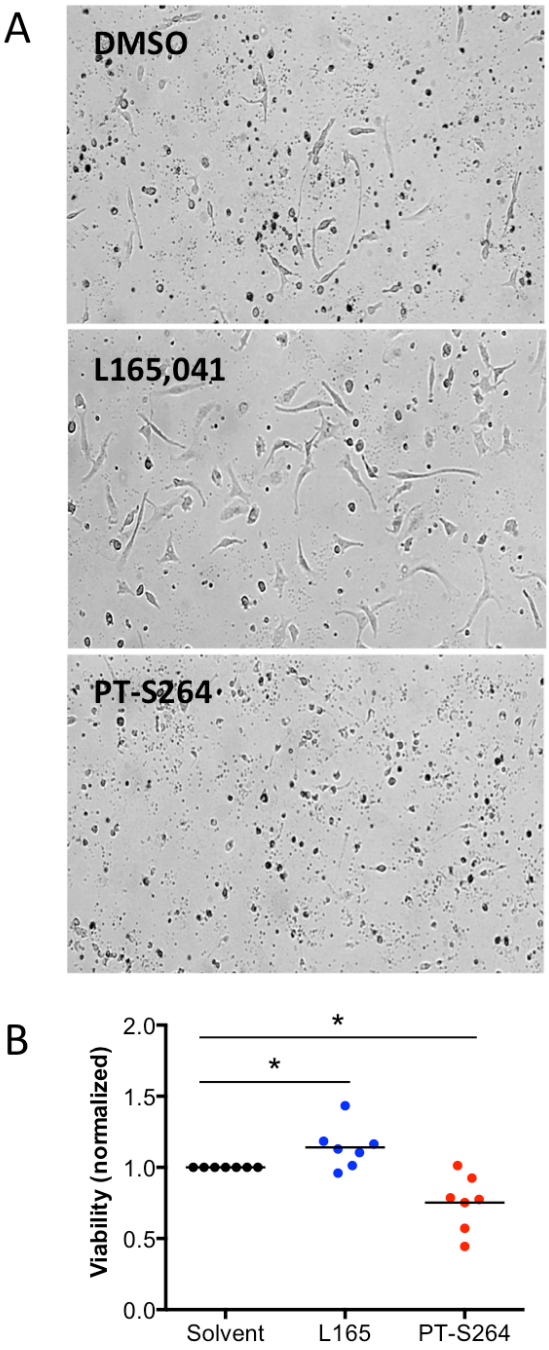


**Figure S8** – Survival of MDMs exposed to hypoxia and L165,041 or PT-S264 for 4 d. (A) Photomicrographs of MDMs subjected to hypoxia for 2 days beginning on day 7 of differentiation in the presence of the indicated ligands or solvent. (B) Viability of adherent cells was determined by MTT assay with MDM from 7 different donors as follows: After the incubation period, cells were treated with 1.2 mM MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Aldrich) for four hours at 37 °C followed by a three-hour lysis in 0.005 mM HCl and 5% SDS (final concentration) at the same temperature. Measurements were performed with a SpectraMax 340 (MWGbiotech) at 570 nm.

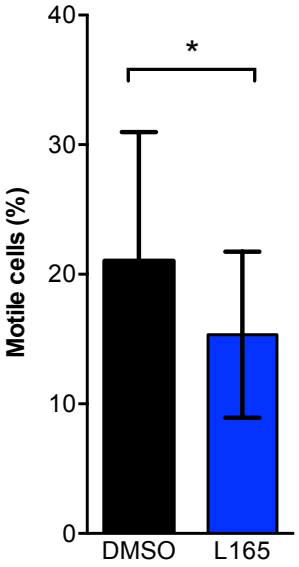


**Figure S9** – Quantification of the MDM motility by time-lapse video microscopy. Cells on day 6 of differentiation (R0 medium) were treated with DMSO or 1 µM L165,041 for 2 h and images were captured by life cell video microscopy for 30 min at the same conditions as in regular cell culture incubator (37 °C, 5% $CO_2$). Recording was carried out with an Axiovert microscope (Zeiss) equipped with a 10x differential interference contrast (DIC) objecticve and a $CO_2$ incubator. Images were captured every 5 min. Cells were tracked with the Image J / Fiji Plugin "Particle Tracker 2D/3D" with a chosen radius of 11, a cutoff of 0,0 a percentile between 1-2, a link range of 5, a displacement of 60 and Brownian dynamics (Sbalzarini and Koumoutsakos, 2005). The data shown are derived from 5 independent experiments, tracking 910 cells in total. Statistical significance was determined by paired t test.

## Reference

Sbalzarini, I.F. and Koumoutsakos, P. (2005) Feature point tracking and trajectory analysis for video imaging in cell biology. *J Struct Biol*, **151**, 182-195.

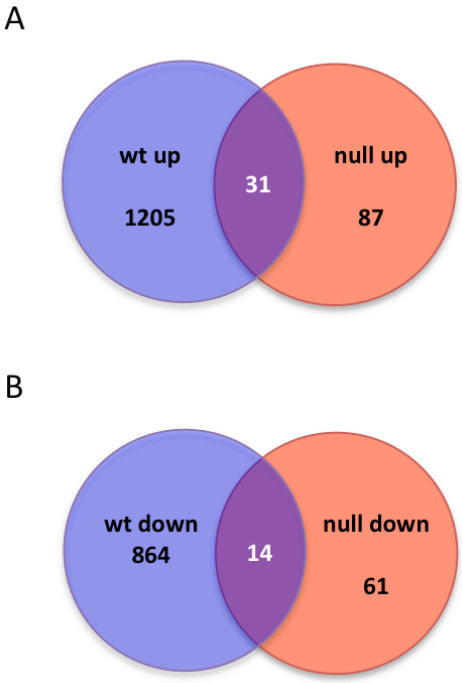


**Figure S10** – Specificity of GW501516. Mouse bone marrow cells from wt and *Ppard* null mice were cultured for 2 days in GM-CSF, yielding a mixed population of granulocytes and immature monocytic cells at different stages of differentiation, as described in ref. 36. These cells were treated for 1 day with 1 μM GW501516 or solvent (DMSO) in the presence of GM-CSF. RNA was analyzed by microarrays as published (36). The Venn diagrams show the number of genes induced (A) or down-regulated (B) by the ligand in either wt (blue) or null (red) mice (fold change >1.5).