Deregulation of tumor angiogenesis and blockade of tumor growth in PPARβ deficient mice

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Supplemental Figures

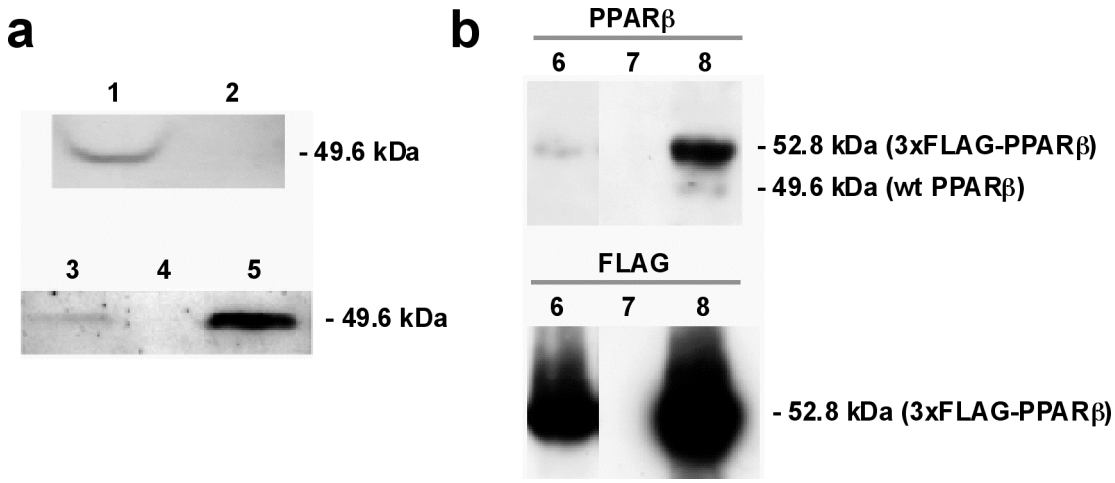

Figure S1: Analysis of PPARβ expression in cells from *Pparb*$^{+/+}$ or *Pparb*$^{-/-}$ mice and in retrovirally transduced null fibroblasts. Equal loading was verified by Ponceau S Red staining of the membrane (not shown). The 49.6 kDa and 52.8 kDa bands representing endogenous PPARβ and the exogenous 3xFLAG-tagged protein are indicated.

(**a**) Immunoblot analysis of PPARβ expression in fibroblasts (lanes 1 and 2) and aortic ECs (lanes 3-5) established from *Pparb*$^{+/+}$ (lanes 1 and 3) or *Pparb*$^{-/-}$ mice (lanes 2 and 4) using an antibody directed against the N-terminus of PPARβ (SC1987, Santa Cruz). For comparison, cells transiently transfected with a CMV-*PPARb* vector are shown (lane 5).

(**b**) Immunoblot analysis of null fibroblasts transduced with a retrovirus expressing FLAG-tagged PPARβ (3Fb1 cells; lane 6) and control cells harboring the empty retroviral vector (Lpcx cells; lane 7) using antibodies directed against PPARβ (as in panel a) or FLAG-tag. For comparison, cells transiently transfected with a CMV promoter driven 3xFLAG-*Pparb* vector are shown (lane 8).

The data show that null cells lack any detectable PPARβ protein, whereas PPARβ can be readily visualized in 3Fb1 cells.

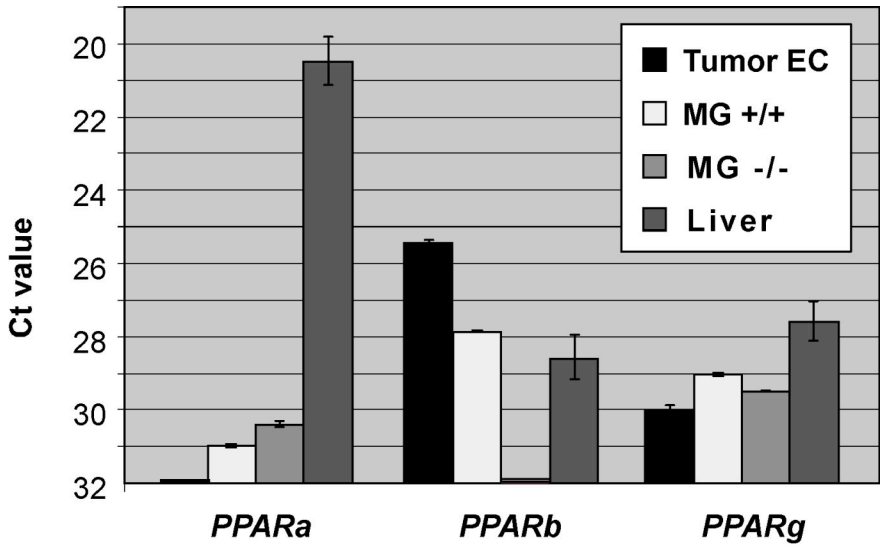

Figure S2: Differential mRNA expression of different *Ppar* subtypes in mouse endothelial cells isolated from a subcutaneous tumor, in matrigel-invading cells from *Pparb*$^{+/+}$ and *Pparb*$^{-/-}$ mice, and in *Pparb*$^{+/+}$ mouse liver, determined by qPCR. Values represent Ct values (averages of triplicates ± S.D) normalized to *Arp0*.

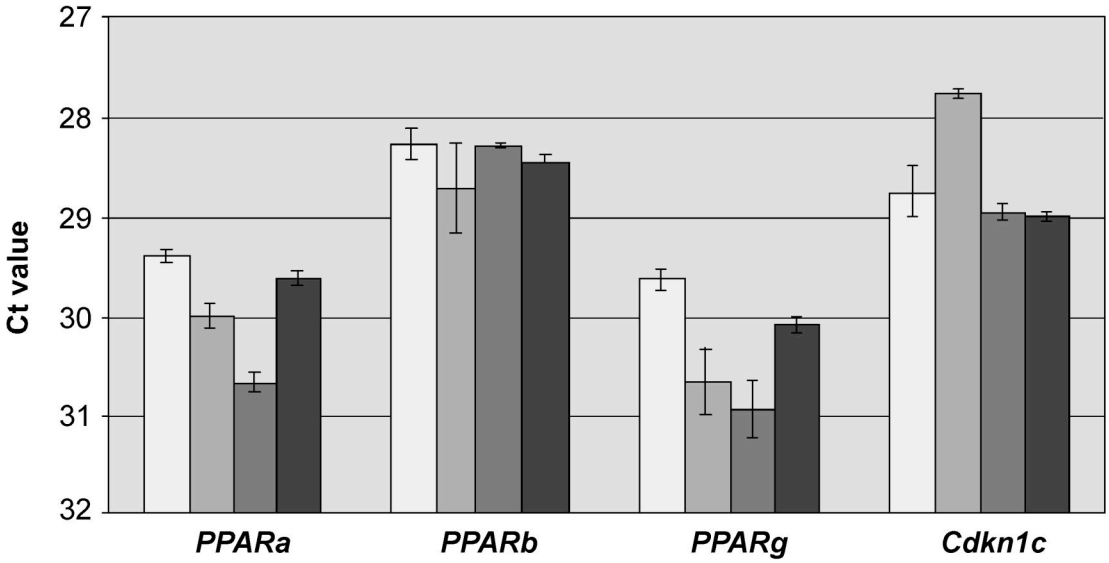

Figure S3: Differential mRNA expression of different *Ppar* subtypes and *Cdkn1c* in CD34^{+} endothelial cells isolated from human non-small cell lung carcinomas, determined by qPCR. Values represent Ct values (averages of triplicates ± S.D) normalized to *L27*.

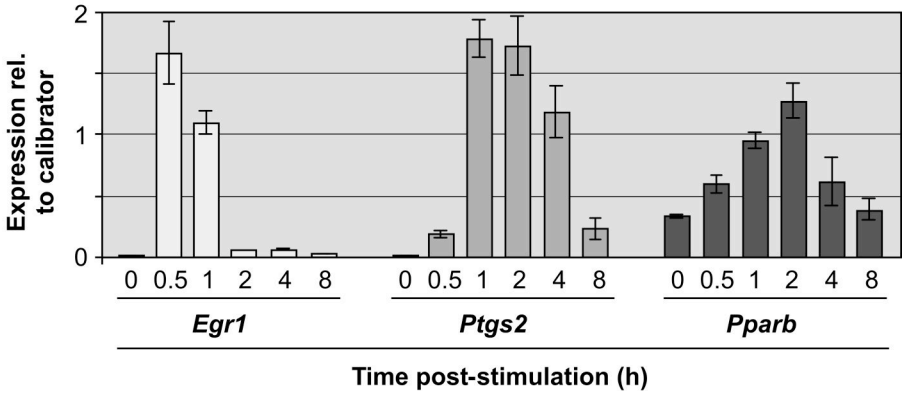

Figure S4: Induction of *Pparb* mRNA by angiogenic growth factors in an established mouse aortic EC line. Cells were deprived of growth factors for 5 hrs by incubation in EGM medium lacking FBS, FGF-2, VEGF, IGF-1 and EGF. Cells were then stimulated with complete EGM-2 medium plus additional FGF-2 (100 ng/ml human recombinant FGF-2, Biomol) for the indicated times. RNA levels were quantified by qPCR. Pooled cultured aortic ECs were used as calibrator and *Arp0* as normalizer. Relative expression values represent averages of triplicates ± S.D. *Egr-1* and *Ptgs2* were included as genes known to be induced by angiogenic growth factors (Abe and Sato, 2001).

Reference

Abe, M. and Sato, Y. (2001) cDNA microarray analysis of the gene expression profile of VEGF-activated human umbilical vein endothelial cells. *Angiogenesis*, **4**, 289-298.

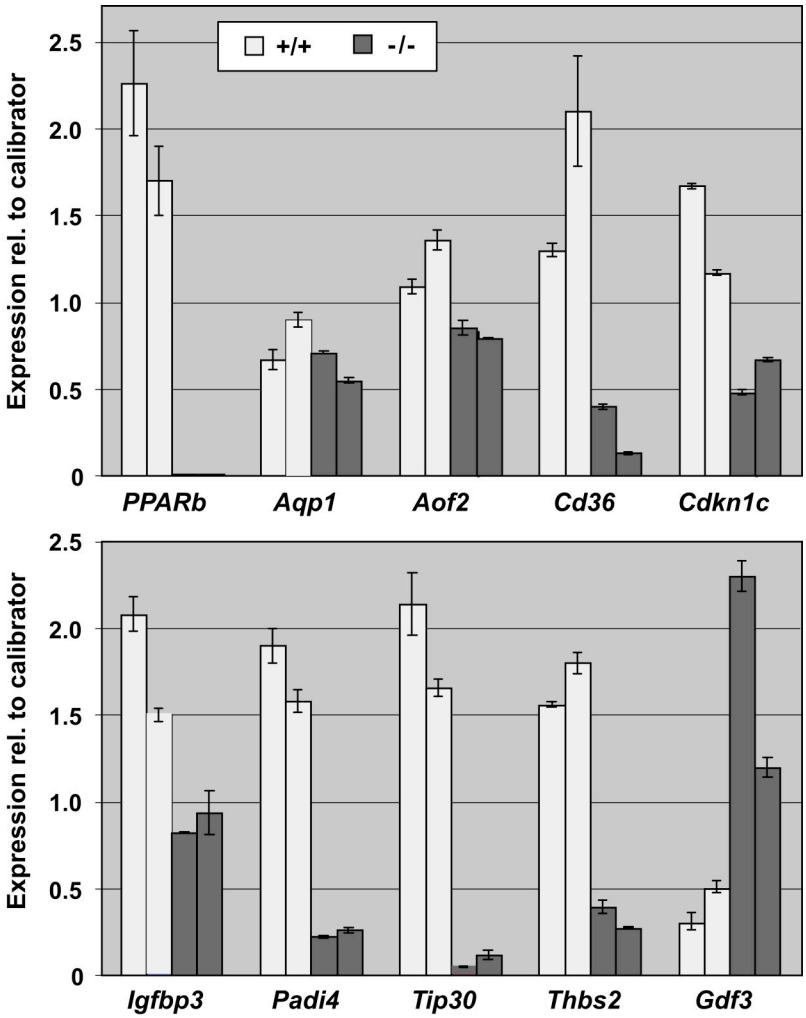

Figure S5: Verification of microarray results. Gene expression patterns of matrigel-invading cells from *Pparb*$^{+/+}$ and *Pparb*$^{-/-}$ mice 3 days after implantation were analyzed by qPCR. Data are shown for 2 individual plugs from each genotype. Data represent relative expression values calibrated with RNA from pooled cultured aortic ECs and normalized to *Arp0* (triplicates ± S.D). The qPCR data confirm the microarray results in Supplemental Table I.

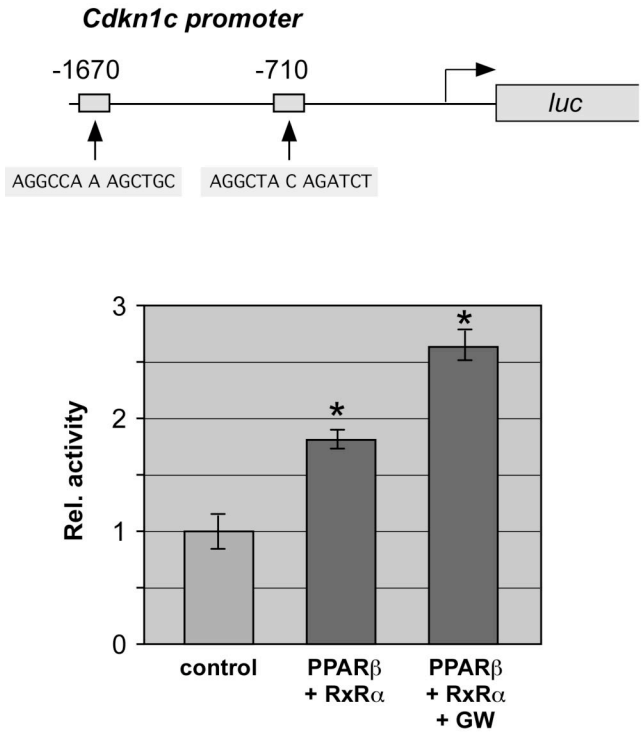

Figure S6: Regulation of the murine *Cdkn1c* promoter by PPARβ. Two motifs fitting the PPRE consensus sequence were identified at positions –1670 and –710 by Genomatix MatInpector. A 1.7 kb *Cdkn1c* promoter fragment harboring these elements was cloned in front of a luciferase reporter gene and analyzed in the mouse EC line 2H11 (Walter-Yohrling et al., 2004) by transient transfection as described (Fauti et al., 2006). Transfections were performed with 5 μg of reporter plasmid and 50 ng of each expression plasmid (PPARβ, RxRα) or empty vector (control). Right-most bar: cells were treated with 1 μM GW501516 after transfection. Values indicate RLUs normalized to 1 for control cells (transfected with empty vector only) and represent the mean of triplicates. Error bars show the standard deviation, asterisks denote statistically significant differences relative to the control (t-test; $P<0.05$).

References

Fauti, T., Müller-Brusselbach, S., Kreutzer, M., Rieck, M., Meissner, W., Rapp, U., Schweer, H., Komhoff, M. and Müller, R. (2006) Induction of PPARβ and prostacyclin (PGI_2) synthesis by Raf signaling: failure of PGI_2 to activate PPARβ. *Febs J*, 273, 170-179.

Walter-Yohrling, J., Morgenbesser, S., Rouleau, C., Bagley, R., Callahan, M., Weber, W. and Teicher, B.A. (2004) Murine endothelial cell lines as models of tumor endothelial cells. *Clin Cancer Res*, **10**, 2179-2189.