Supplemental Information

Methods

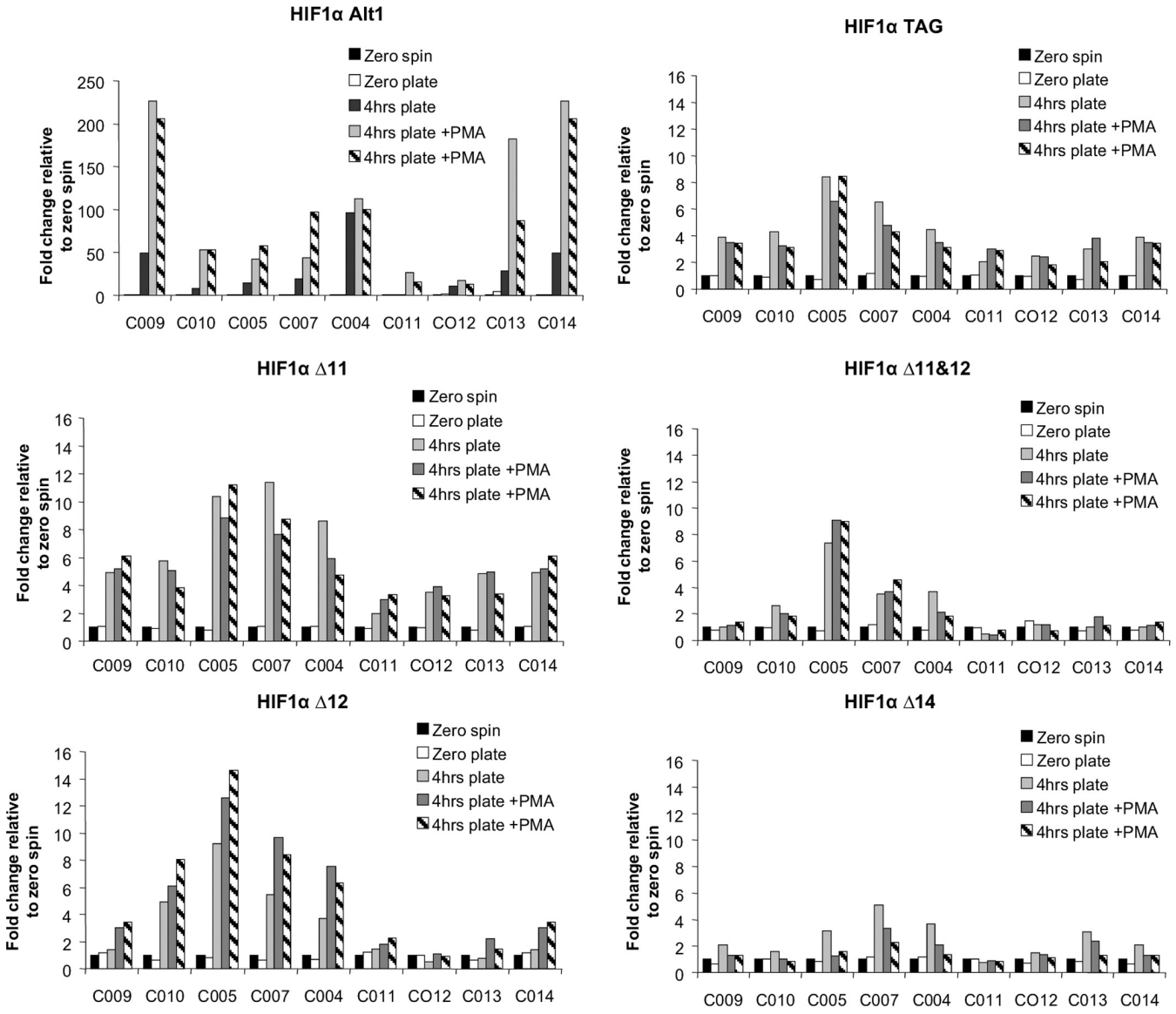
SuperArray screening

PBMCs ($0.8x10^4$) from a healthy donor were cultured in a 60mm cell culture dish for 4hrs with 100nM PMA or harvested at time zero following initial plating. RNA was isolated using Qiagen RNeasy kit, followed by treatment with DNase I. The cDNA was prepared from 1ug of RNA using the RT2 first strand kit (SABiosciences) as per manufacturer's instructions. Real-time PCR was then performed using the Human Hypoxia Signalling Pathway PCR RT^2 Profiler™ Array (PAHS-032, SA Biosciences) according to manufacturer's instructions, with the ABI Prism 7300 Real-time PCR system. Data was analysed using the RT^2 Profiler PCR Array Data Analysis web portal provided by the manufacturer. Relative expression of each gene was normalised to the housekeeping genes; B2M (beta-2-microglobulin), HPRT1 (hypoxanthine phosphoribosyltransferase 1), RPL13A (ribosomal protein L13a) and ACTB (beta actin).

Results

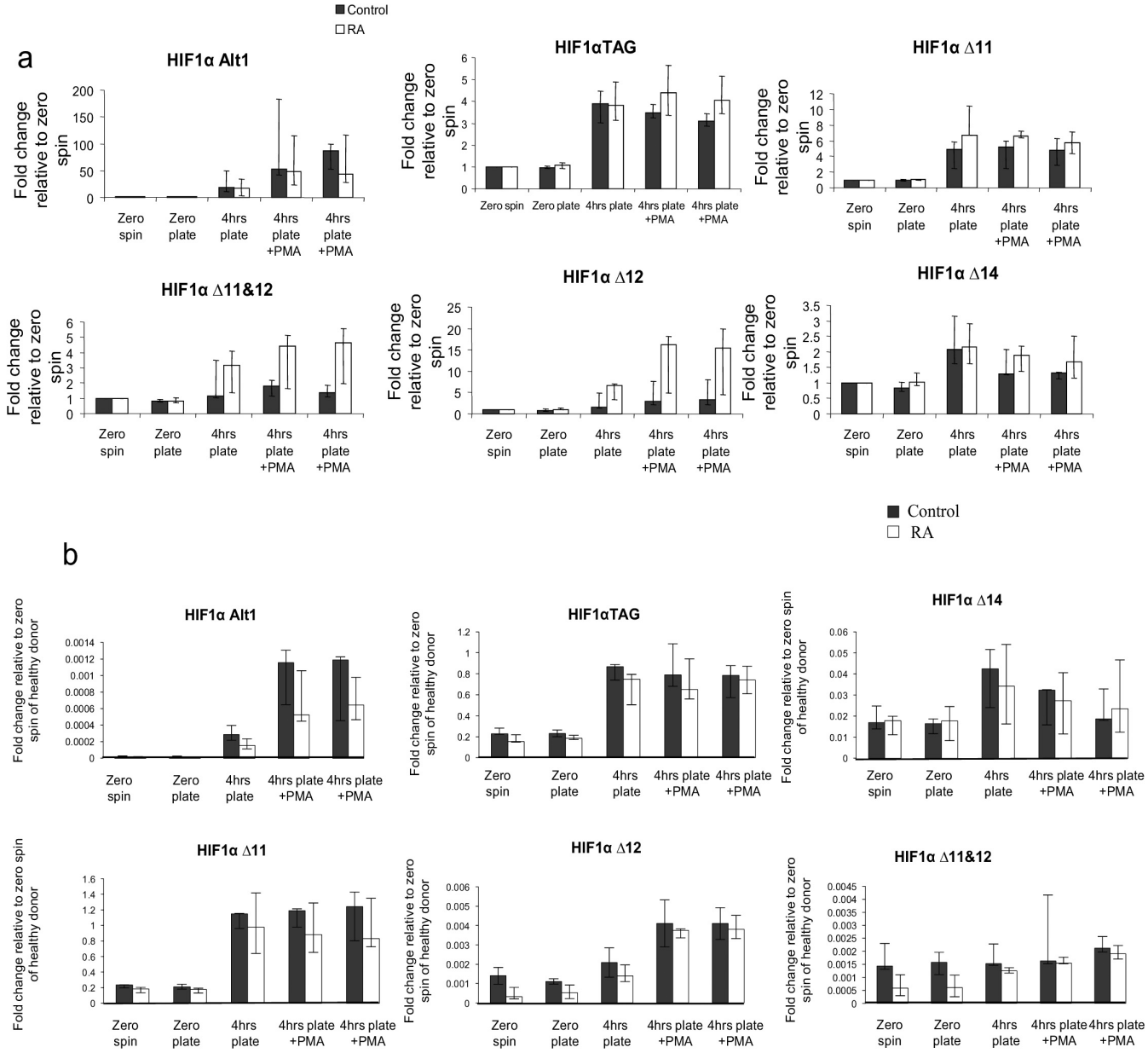
HIF1α target genes in PBMCs are upregulated by PMA

We next sought to investigate the downstream effects of the HIF1α by measuring expression of a panel of HIF1α target genes. PBMCs were isolated from a healthy donor and treated with PMA for 4 hours or harvested at time zero. Total RNA was isolated and used as a template for a PCR array consisting of primers designed to detect a panel of hypoxia responsive genes, including a profile of HIF1α targets (Fig. 4).

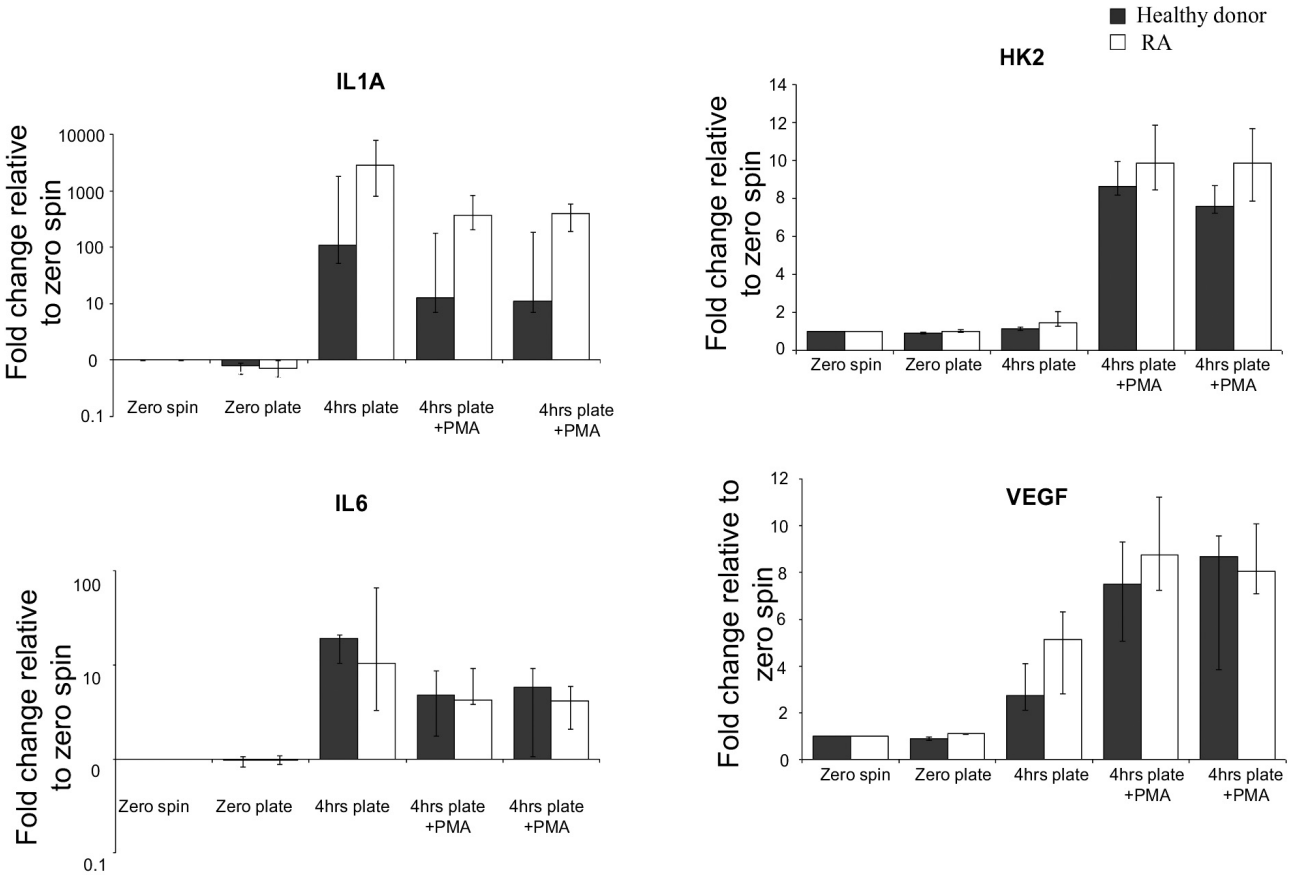


Supplementary Fig. 1. HIF1α splice variants are variably upregulated in activated PBMCs. PBMCs were isolated from healthy donors and activated by either plating on plastic for 4hrs (4hrs plated) or plating in combination with PMA treatment for 4hrs (4hrs plated + PMA). As controls, PBMCs were harvested at time zero with and without the effects of plating (zero plate and zero spun respectively). Real time RT-PCR was performed with Taqman probes designed to detect HIF1α splice variants. Results are presented relative to mRNA levels in cells harvested at zero without plating (zero spun). Each healthy donor is represented by C009-C014.

HIF1α targets ADM (Adrenomedullin), CYGB (cytoglobin), ENO1 (Enolase 1), HK2 (hexokinase), IL6 (Interleukin 6), LEP (Leptin) and VEGFA were all upregulated in response to PMA treatment. The HIF1α target SLC2A1 was downregulated in response to PMA. Importantly, HIF1α itself was upregulated 6-fold and HIF2α (EPAS1) 15-fold, confirming the results of our earlier qRT-PCR assays. In addition, IL1-A, a proinflammatory cytokine, was also upregulated 17-fold in response to PMA treatment, confirming PBMC activation. ARNT2 (HIF2beta), an alternative binding partner of HIF1α, was also dramatically upregulated in response to PMA treatment, and we found that ARNT2 protein was also induced (Fig. 4).



Supplementary Fig. 2. HIF 1α splice variant expression in RA cases and controls. (**a**) PBMCs were isolated from nine healthy donors and nine RA patients and activated as described in fig 2. Cells were harvested post treatment or at zero with and without the effects of plating and total RNA isolated. Real time RT-PCR was performed with Taqman probes designed to detect mRNA of HIF1α splice variants. Results are presented as the median of at least 8 patients relative to mRNA levels in cells harvested at time zero without plating (zero spin). Error bars represent plus and minus the interquartile range. (**b**) The same as (**a**) but analysis of the results are presented relative to mRNA levels in cells of healthy donors harvested at time zero without plating (zero spin).



Supplementary Fig. 3. HIF1α target gene expression in RA cases and controls. PBMCs were isolated from 15 healthy donors and 14 RA patients (DAS>4) and activated with PMA treatment and / or plating, cells harvested as indicated and total RNA isolated. Real time RT PCR was performed with Taqman probes designed to detect HK2, IL6, VEGF and IL1A mRNA. Results are presented as the median relative to mRNA levels in cells harvested at zero without plating (zero spin). Error bars represent plus and minus the interquartile range.