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online data

## SUPPLEMENTARY ONLINE DATA

# Isoform-selective induction of human p110$\delta$ PI3K expression by TNF$\alpha$: identification of a new and inducible *PIK3CD* promoter

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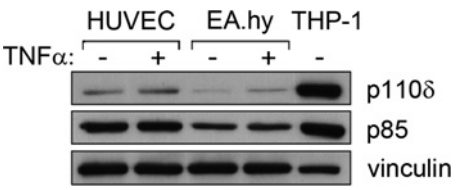


**Figure S1 Comparison of p110$\delta$ expression levels in TNF$\alpha$-stimulated ECs and unstimulated THP-1 cells**

p110$\delta$ protein expression in unstimulated and TNF$\alpha$-stimulated (10 ng/ml for 18 h) HUVECs and EA.hy926 cells compared with basal expression levels in THP-1 cells. Equal amounts (60 $\mu$g) of total cell lysate from each sample were analysed by Western blotting for the indicated proteins.

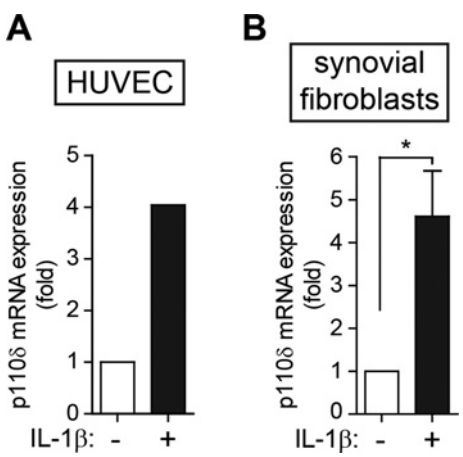


**Figure S2 IL-1$\beta$ stimulates *PIK3CD* mRNA expression in HUVECs and synovial fibroblasts**

*PIK3CD* mRNA expression was analysed by qPCR in HUVECs that were stimulated with 20 ng/ml IL-1$\beta$ for 12 h (**A**) and synovial fibroblasts with 10 ng/ml IL-1$\beta$ for 18 h (**B**). *PIK3CD* transcript expression was normalized to 18S rRNA and shown as fold increase over unstimulated levels. Results are means for two experiments for HUVECs and the means $\pm$ S.E.M. for four independent experiments for synovial fibroblasts. *$P < 0.05$ by two-tailed unpaired Student's *t* test.

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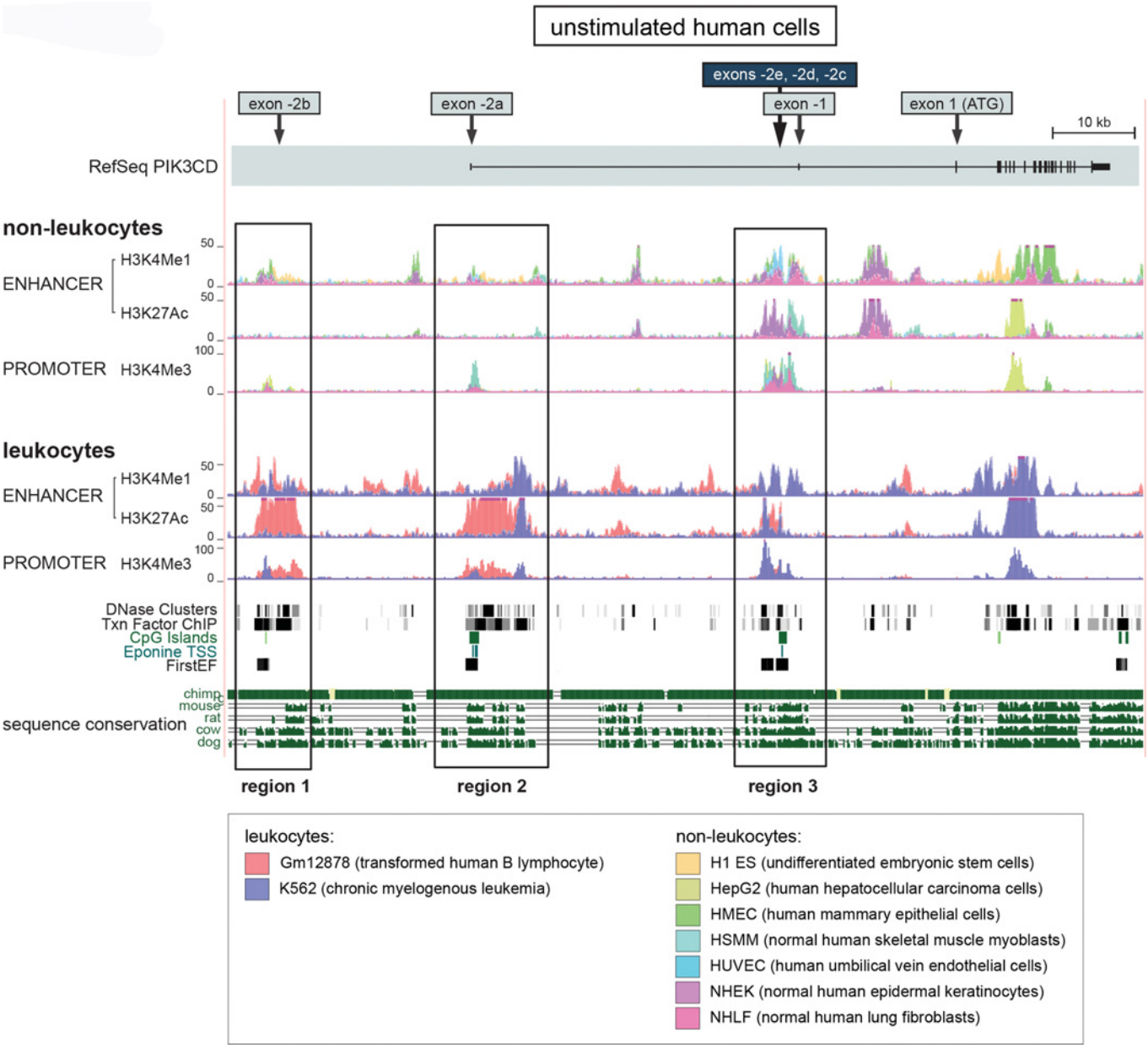


**Figure S3 Three human *PIK3CD* promoter regions revealed by bioinformatic analysis of functional regulatory elements**

The human *PIK3CD* locus in the UCSC genome browser as described in the legend to Figure 4 of the main text including ChIP-seq data for enhancer-associated H3K4Me1 and H3K27Ac shown separately for non-leucocytes and leucocytes.

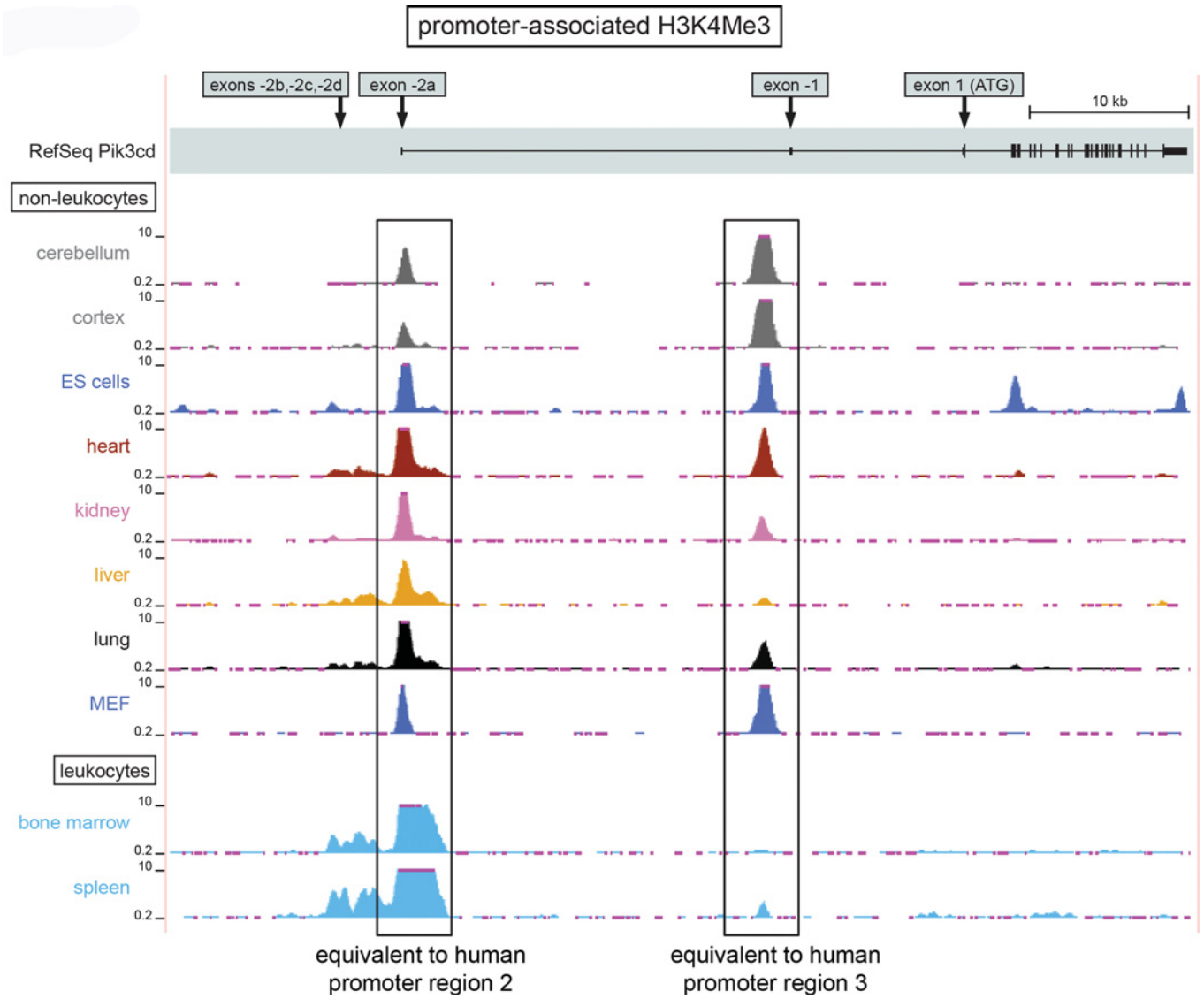


**Figure S4 Bioinformatics analysis of the mouse *Pik3cd* locus**

ChIP-seq data for the enrichment of H3K4Me3 in the mouse *Pik3cd* locus in the UCSC genome browser. The RefSeq *Pik3cd* mRNA transcript (on top) contains the untranslated exons −2a and −1 and all the protein-coding exons. The approximate locations of the untranslated exons −2c, −2d and −2e are indicated with arrows. The boxed regions surrounding exons −2a and −1 correspond to the human *PIK3CD* promoter regions 2 and 3 respectively (Figure 4 of the main text). Data collected from the indicated tissue and cell types are shown as separate tracks.

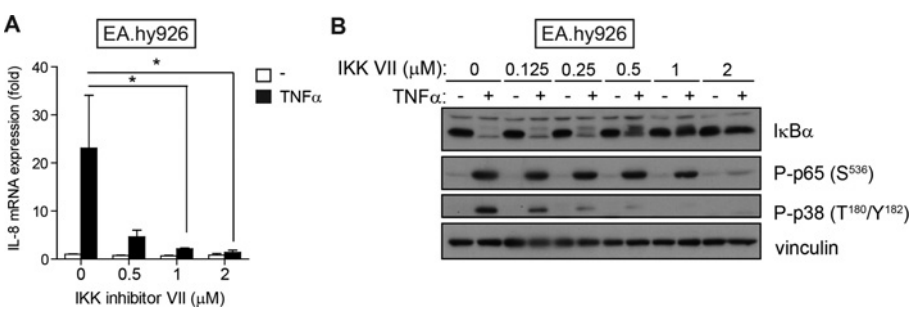


**Figure S5 IKK inhibitor VII prevents TNFα-stimulated NF-κB activation and IL-8 expression in EA.hy926 cells**

(**A**) EA.hy926 cells were pre-treated for 1 h with the indicated concentrations of IKK inhibitor VII or DMSO and stimulated with TNFα (10 ng/ml) for 6 h. Expression of *IL8* mRNA was analysed by qPCR and normalized to 18S rRNA. Results are expressed as fold increase over unstimulated DMSO-treated cells are shown as means ± S.E.M. for three independent experiments. $*P < 0.05$ by two-tailed unpaired Student's *t* test. (**B**) EA.hy926 cells were pre-treated for 1 h with the indicated concentrations of IKK inhibitor VII or DMSO and stimulated for 10 min with 10 ng/ml TNFα. Levels of the indicated proteins were analysed by Western blot.

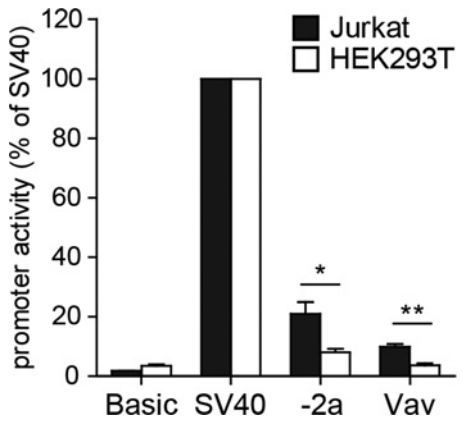


**Figure S6 Human *PIK3CD* exon −2a promoter has higher activity in leucocytes than non-leucocytes**

Promoter activity of the genomic DNA containing human *PIK3CD* exon −2a and its immediate upstream sequence (−139 to +92 relative to the start sites of exon −2a) was analysed in a leucocyte (Jurkat) and a non-leucocyte [HEK (human embryonic kidney)-293T] cell line. The leucocyte-specific *Vav* promoter was used as positive control. Promoter activity is expressed as a percentage of that of the SV40 promoter (set as 100%) in each cell type. Results are means ± S.E.M. for three independent experiments. $*P < 0.05$, $**P < 0.01$ by two-tailed unpaired Student's *t* test.

**Table S1 Primer details**

Sequence details of all the PCR primers and TaqMan qPCR assays used in the present study. The left-hand column indicates the experiment in which a given primer was used. FAM, 6-carboxyfluorescein.

| Experiment | Primer name | Primer sequence (5′→3′) |
|---|---|---|
| PIK3CD-specific primers for 5′RACE | OP2 (outer primer) | TCGCTGCCCTCAAACTTAACGTT |
| | IP2 (inner primer) | AATGGTACCAGGAGTCAAACTCGTGGAGGCCTT |
| Cloning pGL3-exon-2a reporter | pGL3-exon-2a for | GACTCTCGAGGCCCTGGAGGACCTGTTGTT |
| | pGL3-exon-2a rev | GACTAGATCTGAGTGAGCCTCGAGGGAGGG |
| Cloning pGL3-exon-2e reporter | R1-F6 for | ATTAACGCGTGGGGAATGGGGGTGGAGGAAC |
| | R1-R2 rev | ATTAAGATCTGAAGTCACACACAGACATTCACTCAA |
| κB site mutation in pGL3-exon-2e reporter | R1_M1_for | TGGCTGATTTCTCATCTTGGTTCAAGGAGCGCCCTGGGGGGGTCCGGT |
| | R1_M1_rev | ACCGGACCCCCCCAGGGCGCTCCTTGAACCAAGATGAGAAATCAGCCA |
| PIK3CD exon −1/1 TaqMan assay | Forward primer | ACTCATTGATTCTAAAGCATCTT |
| | Reverse primer | GCATCCTGCGTTGTTACTTC |
| | Probe (FAM) | ACTATTCCAGAGAGGACAACTGTCATCT |
| PIK3CD exon −2a/1 TaqMan assay | Forward primer | CGAGCAGAGCCGCCCA |
| | Reverse primer | AAGATGCTTTAGAATCAATGAGT |
| | Probe (FAM) | AGCTGCGCCGGGACATAAGGAGT |
| PIK3CD exon −2c/1 TaqMan assay | Forward primer | CCCCTGGGCAACTGTCT |
| | Reverse primer | CCGCCCTGGCCTGA |
| | Probe (FAM) | CTCCTTATCGGGTGTCGCT |
| PIK3CD exon −2d/1 TaqMan assay | Forward primer | CGCACCCCGCTTCCT |
| | Reverse primer | TGCTTTAGAATCAATGAGTGTCATCCC |
| | Probe (FAM) | CCTGACTCCTTATCTTTGC |
| PIK3CD exon −2e/1 TaqMan assay | Forward primer | CCCGGGATCTGTGAAAGCA |
| | Reverse primer | CGCCCTGGCCTGACT |
| | Probe (FAM) | CCTTATCCGCCCGCACCC |

**Table S2 Different cell types analysed for p110δ expression upon TNFα stimulation**

Listed are all the different human and mouse cell types that were analysed for the expression of p110δ upon stimulation with TNFα.

| Cell name | Cell type | Effect of TNFα on p110δ expression |
|---|---|---|
| THP-1 | Human acute monocytic leukaemia cell line | 2-Fold increase (mRNA), no effect (protein) |
| Jurkat | Human immortalized T-cell line | No effect (protein) |
| RAW264.7 | Mouse macrophage-like cell line | No effect (protein) |
| 4T1 | Mouse mammary tumour cell line | No effect (protein) |
| MDA-MB-468 | Human breast cancer cell line | No effect (mRNA) |
| MDA-MB-231 | Human breast cancer cell line | No effect (protein) |
| MCF-7 | Human breast cancer cell line | 2-Fold increase (mRNA, protein) |
| HeLa | Human cervical cancer cell line | No effect (protein) |
| HEK-293T | Human embryonic kidney cell line | No effect (mRNA, protein) |
| BMM | Mouse primary bone marrow macrophage | No effect (mRNA, protein) |
| pCEC | Mouse primary cardiac endothelial cells | No effect (protein) |
| iCEC | Mouse immortalized cardiac endothelial cell line | No effect (mRNA, protein) |
| Lung EC | Mouse primary lung endothelial cells | No effect (mRNA, protein) |
| bEND5 | Mouse brain endothelial cell line | No effect (mRNA, protein) |
| MEF | Mouse primary embryonic fibroblasts | 2-Fold increase (mRNA), no effect (protein) |
| NIH-3T3 | Mouse fibroblast cell line | No effect (mRNA, protein) |

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