Supplemental information

Global characterization of macrophage polarization mechanisms and identification of M2-type polarization inhibitors

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Two-dimensional clustering analysis: Differentially expressed proteins ≥ 2-fold change
Figure S1: Global quantitative proteomics of fully polarized macrophages identify M1-versus M2-type polarization markers.

Related to Figures 1 and 2; Tables S1 and S2.

A. THP-1 cells were treated for 24 hours with PMA and then for 4 days with either IL-4 (M2), IFNγ/LPS (M1) or no further treatment (M0), achieving full polarization of macrophages.

B. Western blotting of THP-1-derived macrophages treated with IL-4 or IFNγ/LPS 24hrs after PMA treatment for indicated times with antibodies against β-tubulin, phospho-ERK1 (T202/Y204), total ERK1/2, phospho-STAT1 (Y701), TGM2 and phospho-STAT6(Y641). In IFNγ/LPS-treated cells phospho-ERK1 (T202/Y204) levels are highest at 4 days. In IL-4-treated cells phosphorylated ERK1 levels are highest at ~2-8 hours (*) and diminished at 24 hours and 4 days. Increasing TGM2 protein levels between 8hrs and 4 days. STAT6 (Y641) phosphorylation occurs rapidly with IL-4 treatment and STAT1 (Y701) phosphorylation with IFNγ/LPS treatment, confirming the phosphoproteomic data (shown for STAT1(Y701) and for STAT6(Y614) in Figure S1G). Values indicate normalization to β-actin loading control and DMSO control sample with no IL-4.

C. Scatter plot shows protein levels of fully polarized THP-1-derived macrophages (treated for 4 days with IL-4 or IFNγ/LPS after PMA treatment), normalized to PMA-only treated macrophages (M0). Proteins in green indicate selected M1-type specific marker proteins. Proteins in red indicate selected M2-type specific marker proteins.

D. Two-dimensional clustering analysis of proteins in IL-4-treated versus IFNγ/LPS-treated macrophages with at least two-fold differences compared to PMA controls.

E. Left: Top-ranking proteins in THP-1-derived macrophages after IL-4-induced full M2-type polarization (1-day PMA followed by 4-day treatment with IL-4) or after IFNγ/LPS-induced full M1-type polarization (1-day PMA followed by 4-day treatment with IFNγ/LPS). Fold changes of protein levels determined by proteomics when comparing IL-4- versus IFNγ/LPS-treated cells (ratio of average of duplicates; n=2/group). Among highest protein levels for M1-markers are FAM26F, IDO1 and CXCL9. Among highest protein levels for M2-markers are MRC1, TGM2 and FABP4. These proteomic data provide a signature motif of proteins that characterize M1- versus M2-type macrophage polarization phases. Right: Proteomic changes that occur in the induction phase of polarization in THP-1 cells after 24 hrs of treatment with either IL-4 or IFNγ/LPS (after 24 hrs of treatment with PMA). Fold changes of protein levels determined by proteomics when comparing groups treated with IL-4 or IFNγ/LPS for 24 hrs versus 30 min.

F. Top: Heatmap shows clear separation of majority of proteins between THP-1-derived macrophages treated with PMA only (controls), IL-4 or IFNγ/LPS in this full polarization proteomics experiment. The data for each protein are shown as log2 ratios relative to its mean level. Bottom left: Principal component analysis shows that treatment with IL-4 or IFNγ/LPS results in very distinct changes in protein levels among these groups.

G. Time-course phosphoproteomics of THP-1-derived macrophages during the first 24 hours of treatment with either IL-4 or IFNγ/LPS shows that phosphorylation of STAT1 (Y701) occurs rapidly after treatment with IFNγ/LPS and declines subsequently. IL-4 treatment does not induce STAT1 (Y701) phosphorylation. In contrast, STAT6 (Y641) occurs selectively and rapidly only with IL-4 treatment. Y-axis shows mass spec (MS)-intensity values (arbitrary units).

H. Left: Graph of time-course proteomics data shows highest upregulated proteins at 24 hours after induction of M2-type macrophage polarization with IL-4. ALDH1A2 is specifically and highly upregulated at 24 hrs after treatment with IL-4 but not after IFNγ/LPS treatment. PD-L1 (CD274) and VCAM1 upregulation was observed in both IFNγ/LPS-treated and IL-4-treated macrophages. Right: Graph shows key proteins that are associated selectively with the induction of M1-type macrophage polarization during the first 24 hours of polarization. Y-axes show mass spec (MS)-intensity values (arbitrary units).
### Proteomics

| datasets                        | experimental groups       | treatment duration |
|--------------------------------|---------------------------|--------------------|
| quantitative proteomics         | vehicle (M0)              | 4 days             |
| fully polarized THP-1-derived macrophages | IL-4 (M2) | 4 days             |
| treatment after 24 hours of PMA | IL-4+JQ1                  | 4 days             |
|                                | IFNγ+LPS (M1)             | 4 days             |
| quantitative time-course proteomics | vehicle (M0)          | 0 min              |
| THP-1-derived macrophages       | IL-4 (M2)                 | 10 min             |
| treatment after 24 hours of PMA | IFNγ+LPS (M1)             | 30 min             |
|                                |                           | 1 hour             |
|                                |                           | 2 hours             |
|                                |                           | 4 hours             |
|                                |                           | 8 hours             |
|                                |                           | 24 hours            |
| quantitative time-course phosphoproteomics | vehicle (M0)     | 0 min              |
| THP-1-derived macrophages       | IL-4 (M2)                 | 10 min             |
| treatment after 24 hours of PMA | IFNγ+LPS (M1)             | 30 min             |
|                                |                           | 1 hour             |
|                                |                           | 2 hours             |
|                                |                           | 4 hours             |
|                                |                           | 8 hours             |
|                                |                           | 24 hours            |
| quantitative proteomics         | IL-4+DMSO (M2)            | 24 hours            |
| effects of trametinib and GDC-0879 | IL-4+Trametinib (M2)     | 24 hours            |
| THP-1-derived macrophages       | IL-4+DMSO-0879 (M2)       | 24 hours            |
| treatment after 24 hours of PMA | IFNγ+LPS+DMSO (M1)        | 24 hours            |
|                                | IFNγ+LPS+Trametinib (M1)  | 24 hours            |
|                                | IFNγ+LPS+GDC-0879 (M1)    | 24 hours            |
| quantitative phosphoproteomics  | IL-4+DMSO (M2)            | 24 hours            |
| effects of GDC-0879             | IL-4+GDC-0879 (M2)        | 24 hours            |
| THP-1-derived macrophages       | IFNγ+LPS+DMSO (M1)        | 24 hours            |
| treatment after 24 hours of PMA | IFNγ+LPS+GDC-0879 (M1)    | 24 hours            |
| MassIVE repository              | MSV000084672              |                    |

### RNA-Seq/ChIP-Seq

| datasets                  | experimental groups   | treatment duration |
|---------------------------|-----------------------|--------------------|
| H3K27ac ChIP-Seq          | DMSO (M0)             | 24 hours            |
| human PBMC-derived macrophages | Panobinostat (M0)  | 24 hours            |
|                           | IL-4+DMSO (M2)        | 24 hours            |
|                           | IFNγ+LPS+DMSO (M1)    | 24 hours            |
|                           | IFNγ+LPS+Panobinostat (M1) | 24 hours    |
| H3K27ac ChIP-Seq          | DMSO (M0)             | 24 hours            |
| human THP-1-derived macrophages | Panobinostat (M0)  | 24 hours            |
| treatment after 24 hours of PMA | Trametinib (M0)     | 24 hours            |
|                           | IL-4+DMSO (M2)        | 24 hours            |
|                           | IL-4+Panobinostat (M2) | 24 hours    |
|                           | IL-4+Trametinib (M2)  | 24 hours            |
|                           | IFNγ+LPS+DMSO (M1)    | 24 hours            |
|                           | IFNγ+LPS+Trametinib (M1) | 24 hours |
| RNA-Seq                   | DMSO (M0)             | 24 hours            |
| human PBMC-derived macrophages | Panobinostat (M0)  | 24 hours            |
|                           | Trametinib (M0)       | 24 hours            |
|                           | IL-4+DMSO (M2)        | 24 hours            |
|                           | IL-4+Panobinostat (M2) | 24 hours    |
|                           | IL-4+Trametinib (M2)  | 24 hours            |
|                           | IFNγ+LPS+DMSO (M1)    | 24 hours            |
|                           | IFNγ+LPS+Trametinib (M1) | 24 hours |
| RNA-Seq                   | DMSO (M0)             | 24 hours            |
| human THP-1-derived macrophages | Panobinostat (M0)  | 24 hours            |
| treatment after 24 hours of PMA | Trametinib (M0)     | 24 hours            |
|                           | IL-4+DMSO (M2)        | 24 hours            |
|                           | IL-4+Panobinostat (M2) | 24 hours    |
|                           | IL-4+Trametinib (M2)  | 24 hours            |
|                           | IFNγ+LPS+DMSO (M1)    | 24 hours            |
|                           | IFNγ+LPS+Trametinib (M1) | 24 hours |
| GEO repository             | GSE154347             |                    |

Figure S2: Overview of the different experimental groups and OMICs approaches used in this study (proteomics, RNA-Seq and ChIP-Seq).
Related to Figures 1, 2, 4 and 5; Tables S1, S2, S4 and S5.
Figure S3: GO pathway analyses show an increase in PPAR/RA signaling, insulin receptor signaling, glycosaminoglycan metabolism, oxidative phosphorylation and HDAC activities during M2-type polarization.

Related to Figure 1; Table S2.

Heatmaps list statistically significant GO terms from the GSEA of the time-course proteomics data. Data for each GO term are shown as log2 ratios relative to its mean level.

A. Top 50 up- or downregulated GO terms with the highest differential between any timepoint in the M1 versus the M2 groups for GO terms with a p-value <0.001. Among the strongest upregulated GO pathways with M2-type polarization but downregulation with M1-type polarization included PPAR signaling and RA metabolism, glucose metabolism, glycosaminoglycan metabolism, fatty acid metabolism, insulin receptor signaling and histone deacetylation. Pathways upregulated with M1- but downregulated with M2-type polarization included NFκB signaling, IFNγ signaling, TLR signaling, tryptophan catabolic process and cAMP metabolic process.

B. An increase in GO terms associated with PPAR signaling and retinoid metabolism during M2-type polarization and a downregulation with M1-type polarization is observed.

C. An increase in GO terms associated with glycosaminoglycan metabolism during M2-type polarization and a downregulation with M1-type polarization is observed.

D. Insulin receptor signaling and GO terms associated with oxidative phosphorylation are increased during the process of M2-type polarization.

E. Changes in triglyceride and fatty acid metabolism occur with M2-type polarization, including an increase in fatty acid oxidation.

F. A moderate increase in HDAC activities between 1-4 hours after IL-4 treatment is observed in macrophages undergoing M2-type polarization, whereas a lesser increase is observed with M1-type polarization.

C. to F. All GO terms with p-value <0.01. Select pathways highlighted with underlining.
GO pathway analyses of time-course proteomics data: metabolic pathways (M1 or M2 N min vs M0 N min) * p value < 0.05

**Insulin receptor signaling**

**Retinoic acid signaling**

**Glycan, glycerolipid, glycolysis**

**Amino acid metabolism**

**Lipid metabolism**

**Fatty acid metabolism**

Figure S4
Figure S4: GO enrichment analysis shows differences in activation of metabolic pathways during M1- versus M2-type polarization.

Related to Figure 1; Table S2.

Heatmaps show selected GO pathways involved in cell metabolism based on analysis of time-course proteomic data. M2-type polarization is associated with increases in insulin receptor signaling, retinoic acid signaling, proteoglycan metabolism or lipid oxidation. M1-type polarization shows increased glycolysis and significant differences in lipid metabolism when compared to M2-type polarization. Color coding indicates -log10 p-values when comparing the M1 or M2 group at the indicated timepoint with the M0 group (after 24-hour PMA treatment only) of the corresponding same timepoint. * indicates p-values <0.05. Timepoints are: 10 min, 30 min, 1 hr, 2hr, 4hr, 8hr, 24hr and 4 days after initiation of INFγ/LPS or IL-4 treatment. Select GO terms are underlined.
Figure S5: Dose-dependent inhibition of MRC1-driven luciferase activity by MEK, pan-Raf or HDAC inhibitors.
Related to Figure 3; Table S2 and S3.

A. A small molecule screen for regulators of IL-4-induced MRC1-driven luciferase expression in THP-1-derived macrophages identifies several classes of pharmacologic inhibitors as being able to potently reduce MRC1 expression. This table shows a selection of identified inhibitors in which a dose-dependent inhibitory effect on MRC1-driven luciferase expression was confirmed in a separate set of experiments. Column "Avg" indicates the average reduction in luciferase activity observed in the initial small molecule screen (performed in duplicate). The molecular target of the small molecule inhibitor is indicated, as well as the clinical trial phase of the compound. Among the most potent inhibitors of MRC1-driven luciferase activity were MEK/pan-Raf inhibitors, HDAC inhibitors, JAK inhibitors, BET bromodomain inhibitors and HSP90 inhibitors. Inhibition was also observed for the PPARγ inhibitor GW9662.

B-C. Luciferase assays show a dose-dependent inhibition of MRC1 promoter-driven luciferase (red) activity at concentrations that do not affect cell viability (green: CTG) for the following chemicals: MEK inhibitors PD0325901, MEK-162, selumetinib, pimasertib, PD184352, and the pan-Raf inhibitors AZ-628, TAK-632 and sorafenib, as well as HDAC inhibitors pracinostat, LAQ824, nexturastat A and givinostat. Concentrations of chemicals used indicated in nM on the x-axis; values indicated in the y-axis normalized to the control carrier (DMSO). Red: luciferase activity; Green: cell viability assay (CTG). Graphs represent data as mean ± STD. N=4/group.

D. Effects of targeting MEKs, BRAF or HDACs on M2-type macrophage polarization in murine BMDMs. IL-4 treatment for 4 days. Expression levels of Arg1 normalized to the expression of CD68 are shown in murine BMDMs after treatment with small molecule inhibitors, assessed by semiquantitative RT-PCR. HDAC inhibitors (panobinostat, pracinostat, dacinostat), MEK inhibitors (trametinib) and pan-Raf inhibitors (TAK-632, AZ 628, Sorafenib) potently suppress Arg1 expression. In contrast, the B-Raf inhibitor GDC-0879 induces Arg1 expression. Graphs represent data as mean ± SEM. N=3/group (each in triplicate).
### GSEA-KEGG M1 vs M0

| Macrophage state | proteome | transcriptome |
|------------------|----------|---------------|
|                  | upregulated | significantly enriched at FDR < 25% | significantly enriched at nominal p-value < 1% | significantly enriched at nominal p-value < 5% | upregulated | significantly enriched at FDR < 25% | significantly enriched at nominal p-value < 1% | significantly enriched at nominal p-value < 5% |
| THP-1_M0         | 53 / 129 gene sets | 2 gene sets | 4 gene sets | 132 / 178 gene sets | 12 gene sets | 13 gene sets | 21 gene sets |
| THP-1_M1         | 76 / 129 gene sets | 25 gene sets | 12 gene sets | 46 / 178 gene sets | 23 gene sets | 19 gene sets | 21 gene sets |

### GSEA-KEGG M2 vs M0

| Macrophage state | proteome | transcriptome |
|------------------|----------|---------------|
|                  | upregulated | significantly enriched at FDR < 25% | significantly enriched at nominal p-value < 1% | significantly enriched at nominal p-value < 5% | upregulated | significantly enriched at FDR < 25% | significantly enriched at nominal p-value < 1% | significantly enriched at nominal p-value < 5% |
| THP-1_M0         | 83 / 128 gene sets | 5 gene sets | 4 gene sets | 11 gene sets | 145 / 178 gene sets | 60 gene sets | 28 gene sets | 49 gene sets |
| THP-1_M2         | 45 / 128 gene sets | 9 gene sets | 4 gene sets | 10 gene sets | 33 / 178 gene sets | 7 gene sets | 7 gene sets | 8 gene sets |

### GSEA-KEGG M1 vs M2

| Macrophage state | proteome | transcriptome |
|------------------|----------|---------------|
|                  | upregulated | significantly enriched at FDR < 25% | significantly enriched at nominal p-value < 1% | significantly enriched at nominal p-value < 5% | upregulated | significantly enriched at FDR < 25% | significantly enriched at nominal p-value < 1% | significantly enriched at nominal p-value < 5% |
| THP-1_M1         | 88 / 131 gene sets | 22 gene sets | 13 gene sets | 17 gene sets | 104 / 178 gene sets | 54 gene sets | 27 gene sets | 44 gene sets |
| THP-1_M2         | 43 / 131 gene sets | 1 gene sets | 1 gene sets | 4 gene sets | 74 / 178 gene sets | 18 gene sets | 7 gene sets | 16 gene sets |

### Pearson correlation analysis between proteome and transcriptome based on fold change

**M1-type compared to M0**

\[ r = 0.89, p < 2.2e-16 \]

**M2-type compared to M0**

\[ r = 0.23, p < 2.2e-16 \]
Figure S6: GSEA shows overlap between proteomic and transcriptomic datasets in THP-1-derived macrophages undergoing M1- or M2-type polarization.

Related to Figure 4; Table S2, S4 and S5.

A. GSEA (KEGG pathways) of proteomic data at 24 hours of treatment with either vehicle (M0), IL-4 (M2) or IFNγ/LPS (M1) (from time-course proteomics dataset), as well as transcriptomic data at the same time-point (RNA-Seq dataset). Left: Tables show number of upregulated KEGG pathways in each group (M0 vs M1, M0 vs M2, M1 vs M2) and statistical significance. Right: Venn diagrams show how many upregulated KEGG pathways overlap (and % of overlap relative to total upregulated pathways in each group is indicated above Venn diagrams).

B. Pearson correlation plots show degree of correlation between proteomic and transcriptomic data from these groups (based on fold change) (R and p-values are shown).
Figure S7: Several key pathways associated with M1- versus M2-type polarization that were identified in the GSEA of the proteomics data were also identified in the GSEA of the RNA-Seq data.

Related to Figure 4; Table S2, S4 and S5.

GSEA enrichment plots show similar results for specific functional KEGG pathways when comparing proteomics and transcriptomics 24 hours after initiation of polarization to the M1- or M2-type (groups shown in Figure S6). For example, M1-type polarization is linked to the RIG-I-like receptor signaling pathway, apoptosis, NOD-like receptor signaling pathway, TLR-signaling pathway, or chemokine signaling. Pathways associated with M2-type polarization include PPAR signaling, oxidative phosphorylation, ribosome, RNA polymerase or proteasome. Venn diagrams show overlap between number of proteins and genes associated with these specific pathways. GSEA plots for oxidative phosphorylation and PPAR signaling also shown in Figure 4B.
Figure S8: KEGG pathway enrichment analysis of highly upregulated genes (lg FC>2) found in RNA-Seq data after 24 hours of treatment with IFNγ/LPS in THP-1-derived macrophages or primary human macrophages.

Related to Figure 4; Tables S4 and S5.

Similar pathways are activated in both groups of macrophages (underlined), including cytokine-cytokine receptor interaction, NOD-like receptor signaling, TNF signaling, NFκB signaling, systemic lupus erythematosus, and TLR signaling. GeneRatio shows percentage of DEGs in the given term. "Count" shows number of DEGs in the given term.
Figure S9: GSEA of RNA-Seq data shows effects of trametinib or panobinostat on M2-type gene programs.

Related to Figure 4; Table S4 and S5.

A. Selection of highly significantly enriched GSEA-identified terms for IL-4-treated THP-1-derived macrophages and human PBMC-derived macrophages for M2-type genes that were inhibited by trametinib or panobinostat. These included MAPK signaling, retinol metabolism, oxidative phosphorylation, PPAR signaling or histone deacetylase activity. Data based on RNA-Seq after 24 hours of IL-4 treatment.

B. Pathway analysis of M2-type genes inhibited by panobinostat (left) or trametinib (right) greater 2-fold (lg FC >1). Panobinostat inhibits genes associated with MAP kinase signaling and PPAR signaling. Among top ranking pathways trametinib inhibits genes associated with MAP kinase signaling and histone modification. Data based on RNA-Seq after 24 hours of IL-4 treatment of THP-1-derived macrophages. GeneRatio shows percentage of DEGs in the given term. “Count” shows number of DEGs in the given term.

C. Left: Heatmaps of IL-4-induced genes involved in retinol metabolism and inhibited by panobinostat and trametinib. Log2 fold change (Lg FC) of gene expression values is shown. Expression of ALDH1A2 is strongly reduced by trametinib as well as by panobinostat. Right: Enrichment plots of KEGG term retinol metabolism show that panobinostat and trametinib inhibit expression of genes linked to retinol metabolism.

D. Left: Heatmap of IL-4-induced genes involved in PPAR signaling and inhibited by panobinostat. Log2 fold change (Lg FC) of gene expression values is shown. Right: Enrichment plot of KEGG term PPAR signaling shows that panobinostat inhibits gene expression of genes linked to PPAR signaling.

E. Trametinib and panobinostat both inhibit enhancer occupancy for MRC1, ALDH1A2, PPARγ and TGM2 in IL-4-treated THP-1-derived macrophages (H3K27Ac ChIP-Seq data).
Figure S10: Effects of trametinib or panobinostat on cellular pathways in M2-type macrophages.

Related to Figure 4; Table S4 and S5.

A. Left: GO pathway analysis of IL-4-induced genes that are inhibited by panobinostat greater 2-fold ($\log[\text{M2/M2 + Pano}] > 1$) in THP-1-derived macrophages. Among the pathways strongly associated with these genes are carboxylic acid biosynthetic process and ERK1/ERK2 cascade. Right: IL-4-induced genes inhibited by panobinostat in THP-1-derived macrophages >2-fold that are linked to the GO term ERK1/ERK2 cascade or the KEGG term MAP kinase signaling. These include the transcription factor MYC and the proangiogenic factors IL-1$\beta$ and PDGFB.

B. GO pathway analysis of IL-4-induced genes that are inhibited by panobinostat greater 2-fold ($\log[\text{M2/M2 + Pano}] > 1$) in PBMC-derived macrophages. Among the pathways strongly associated with these genes are regulation of inflammatory response.

C. GO pathway analysis of IL-4-induced genes that are inhibited by trametinib greater 2-fold ($\log[\text{M2/M2 + Tram}] > 1$) in PBMC-derived macrophages. Among the pathways strongly associated with these genes are leukocyte migration, extracellular matrix organization, regulation of focal adhesion assembly, regulation of adherens junction organization and phospholipid biosynthetic process.

D. KEGG pathway analysis of IL-4-induced genes that are inhibited by trametinib greater 2-fold ($\log[\text{M2/M2 + Tram}] > 1$) in PBMC-derived macrophages. Among the pathways strongly associated with these genes are PI3K/AKT signaling, Ras signaling, focal adhesion and ECM-receptor interactions.

E. KEGG pathway analysis of IL-4-induced genes that are inhibited by trametinib greater 2-fold ($\log[\text{M2/M2 + Tram}] > 1$) in THP-1-derived macrophages. Among the pathways strongly associated with these genes are MAPK signaling and focal adhesion.

GeneRatio shows percentage of DEGs in the given term. “Count” shows number of DEGs in the given term. Data based on RNA-Seq after 24 hours of IL-4 treatment.
Figure S11: GSEA on effects of trametinib or panobinostat on IL-4-induced M2-type gene expression.

Related to Figure 4; Table S4 and S5.

Left: Select heatmaps of genes and enrichment plots showing downregulation of IL-4-induced gene expression with panobinostat or trametinib in THP-1-derived macrophages for GO/KEGG terms MAPK signaling pathway, histone binding, histone deacetylase complex, oxidative phosphorylation, and respiratory chain complex. Log2 fold change (Lg FC) of gene expression values is shown. Data based on RNA-Seq after 24 hours of IL-4 treatment.

Right: Select heatmaps of genes and enrichment plots showing downregulation of IL-4-induced gene expression with panobinostat or trametinib in PBMC-derived primary human macrophages for GO/KEGG terms histone binding, MAPK signaling pathway, citrate cycle/TCA cycle, oxidative phosphorylation, and mitochondrial respiratory chain complex I. Log2 fold change (Lg FC) of gene expression values is shown. Data based on RNA-Seq after 24 hours of IL-4 treatment.
Figure S12: Time-course proteomics and phosphoproteomics show temporal activation pattern during macrophage polarization.

Related to Figure 5; Table S2.

A. IRS2 activation in response to IL-4 with an increase in tyrosine phosphorylation (red arrows) and a decrease in serine phosphorylation (blue arrows). A decrease in IRS2 (S365; S388; S391) is observed already at 10-30 minutes and an increase of IRS2 (Y823) at 30 minutes. IRS2 protein levels are reduced at 24 hours (blue arrow).

B. An increase in phosphorylation of the p85α subunit of PI3K at Y467 and Y580 is observed at 10-30 minutes of IL-4 treatment (red arrow).

C. Activating phosphorylation events of BRAF (e.g. at S446) occur at ~1 hour of IL-4 treatment (red arrow) whereas inhibiting phosphorylation events (e.g. T440) are reduced at that time (blue arrow).

D. Activating phosphorylation event at Y182 of p38α increase at 4-8 hours after IL-4 treatment (red arrows).

E. Motif analysis of H3K27Ac ChIP-Seq data of IL-4-induced M2-type polarization shows enrichment of AP-1 transcription factor binding sites in both primary human macrophages and THP-1-derived macrophages.

F. PPARγ total protein levels increase at ~8 hours of IL-4 treatment (red arrow), followed by a strong decrease at 24 hours (blue arrow). In contrast, PPARγ (S112) phosphorylation that leads to its inactivation and degradation is increased at 24 hours of IL-4 treatment (red arrow).

G. Early activation of STAT6/AKT1/ERK signaling occurs in a first phase of the M2-type polarization process and leads to increased PPARγ protein levels, which further promotes M2-type polarization in a second phase by stimulating RA signaling.