

# Profiling Adipokines Secreted During Differentiation of Human Preadipocytes and Mouse 3T3-L1 Adipocytes

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## Abstract

Adipose tissue secretes a variety of bioactive peptides, called adipokines, that act in an autocrine, paracrine, or endocrine manner to regulate energy metabolism. *In vitro* cultured primary adipocytes and cell lines, such as 3T3-L1 mouse embryonic fibroblast adipose-like cells, are commonly used models for studying adipocyte functions. In order to better understand adipogenesis in these model systems, we analyzed adipokine secretion during *in vitro* differentiation of human preadipocytes and mouse 3T3-L1 cells. 3T3-L1 cells were untreated or differentiated with methylisobutylxanthine (115 mg/mL), insulin (10 mg/mL), and dexamethasone (390 ng/mL) for 48 hours, and then with additional insulin (10 mg/mL) for the next 48 hours. The cells were then grown in standard media that was changed every 48 hours for 96 hours. Human subcutaneous preadipocytes (BMI=30, female) were differentiated into primary human adipocytes using differentiation media from ZenBio. Phase-contrast microscopy revealed the formation of visible lipid droplets during the differentiation of 3T3-L1 cells and human preadipocytes. Proteome Profiler™ Mouse Adipokine Antibody Arrays were used to screen 3T3-L1 cell culture supernates collected on days 2, 4, and 6. Proteome Profiler™ Human Adipokine Antibody Arrays were used to screen adipocyte cell culture supernates collected on days 0, 7, 14, 21, and 28. Array data for select adipokines were confirmed using Quantikine® ELISAs. In conclusion, our data suggest that the combined use of antibody arrays and ELISAs is an effective method by which to study adipokine secretion during mouse and human *in vitro* adipocyte differentiation.

## Materials & Methods

### Cell Culture

3T3-L1 mouse embryonic fibroblast adipocyte-like cells were untreated or differentiated with methylisobutylxanthine (115 mg/mL), insulin (10 mg/mL), and dexamethasone (390 ng/mL) for 48 hours, followed by additional insulin (10 mg/mL) for the next 48 hours. The cells were then grown in standard media that was changed every 48 hours for 96 hours. Human subcutaneous preadipocytes (BMI=30, female) were differentiated into primary human adipocytes using differentiation media from ZenBio.

### Proteome Profiler™ Antibody Array Analysis

Human Adipokine Antibody Arrays (R&D Systems, Catalog # ARY024) were used to screen adipocyte cell culture supernates collected on days 0, 7, 14, 21, and 28. Mouse Adipokine Antibody Arrays (R&D Systems, Catalog # ARY013) were used to screen 3T3-L1 cell culture supernates collected on days 2, 4, and 6. 500 µL of cell culture supernate was run on each array.

### Quantikine® ELISA Analysis

Adipocyte cell culture supernates collected on days 0, 14, and 28 were analyzed using the Human Leptin ELISA Kit (R&D Systems, Catalog # DLP00), the Human IGFBP-2 ELISA Kit (R&D Systems, Catalog # DGB200), and the Human Adiponectin/Acrp-30 ELISA Kit (R&D Systems, Catalog # DRP300).

### Proteome Profiler™ Array Assay Principle

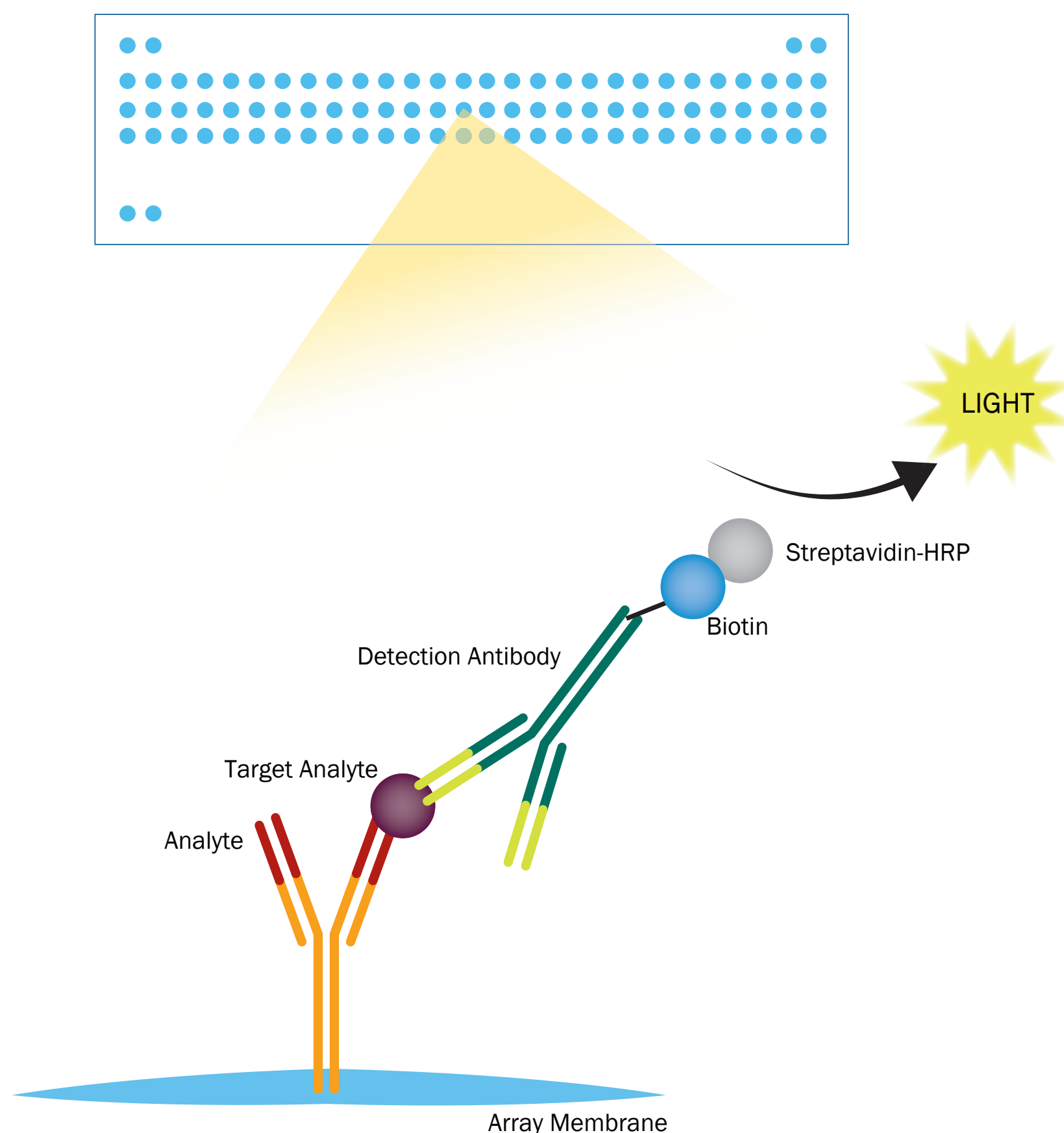
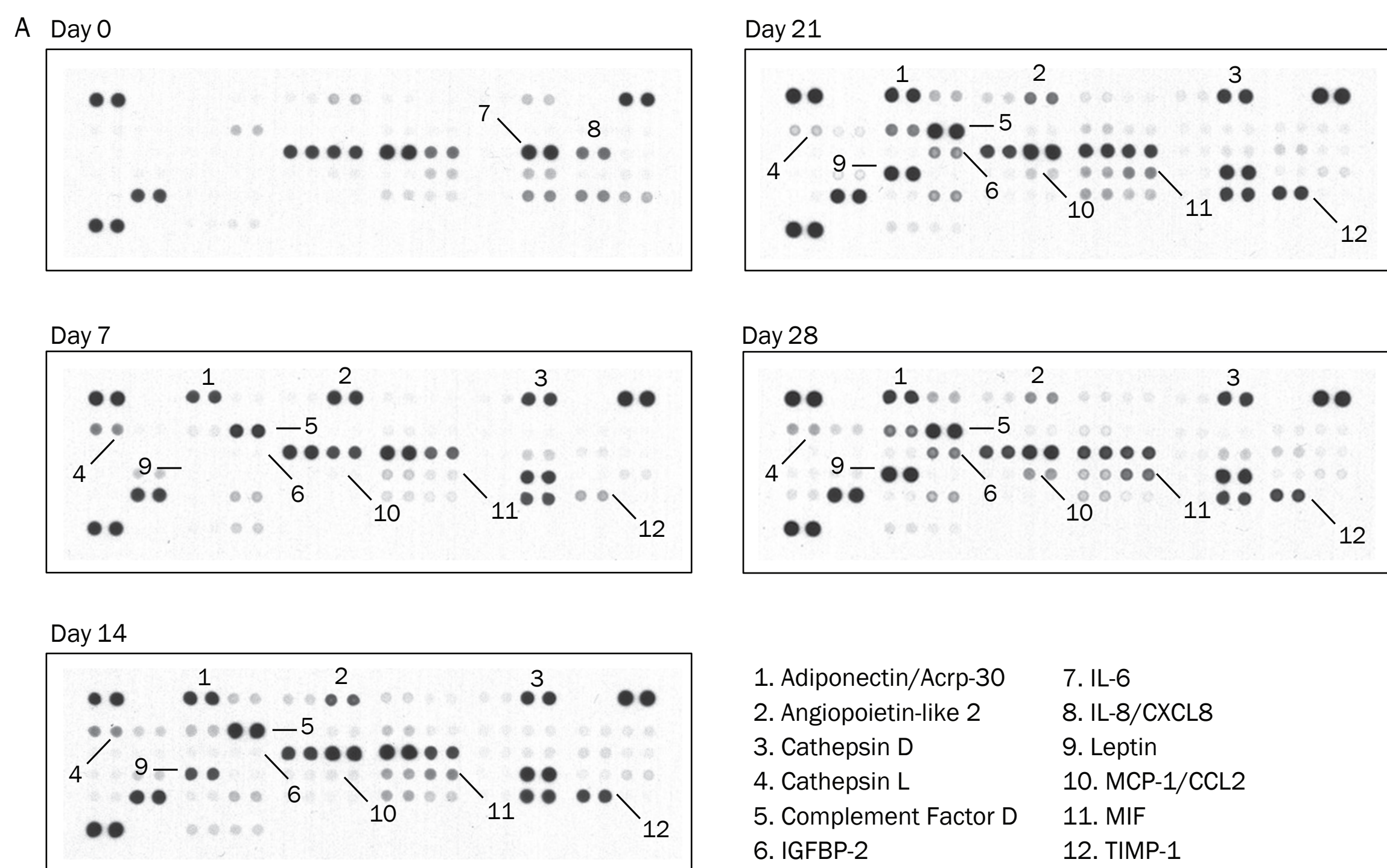
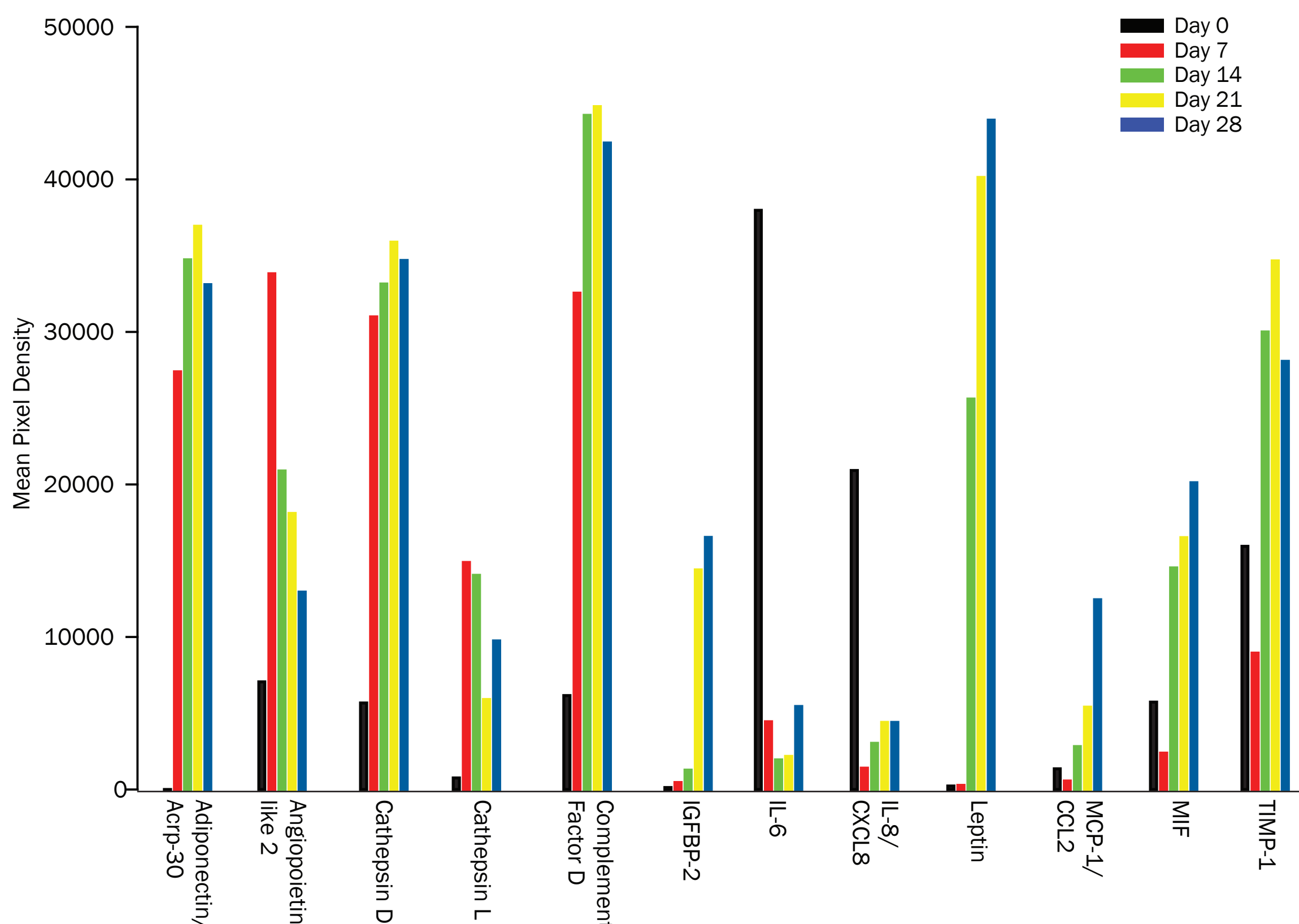


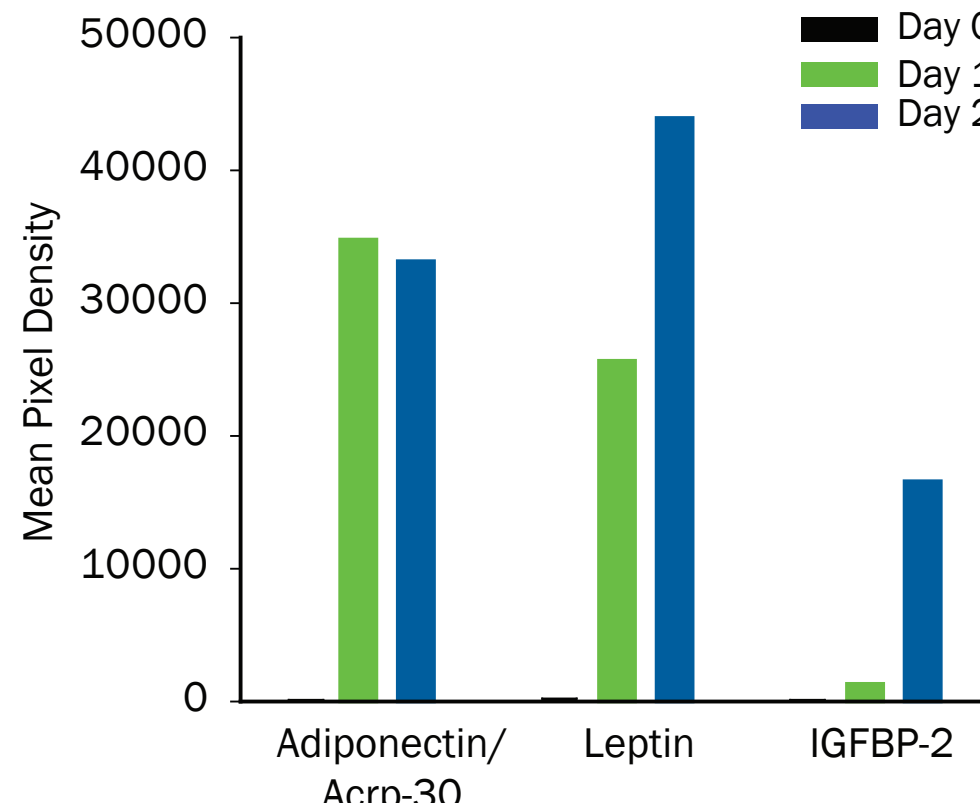
Figure 1. Profiling Human Adipokines Secreted During Adipocyte Differentiation



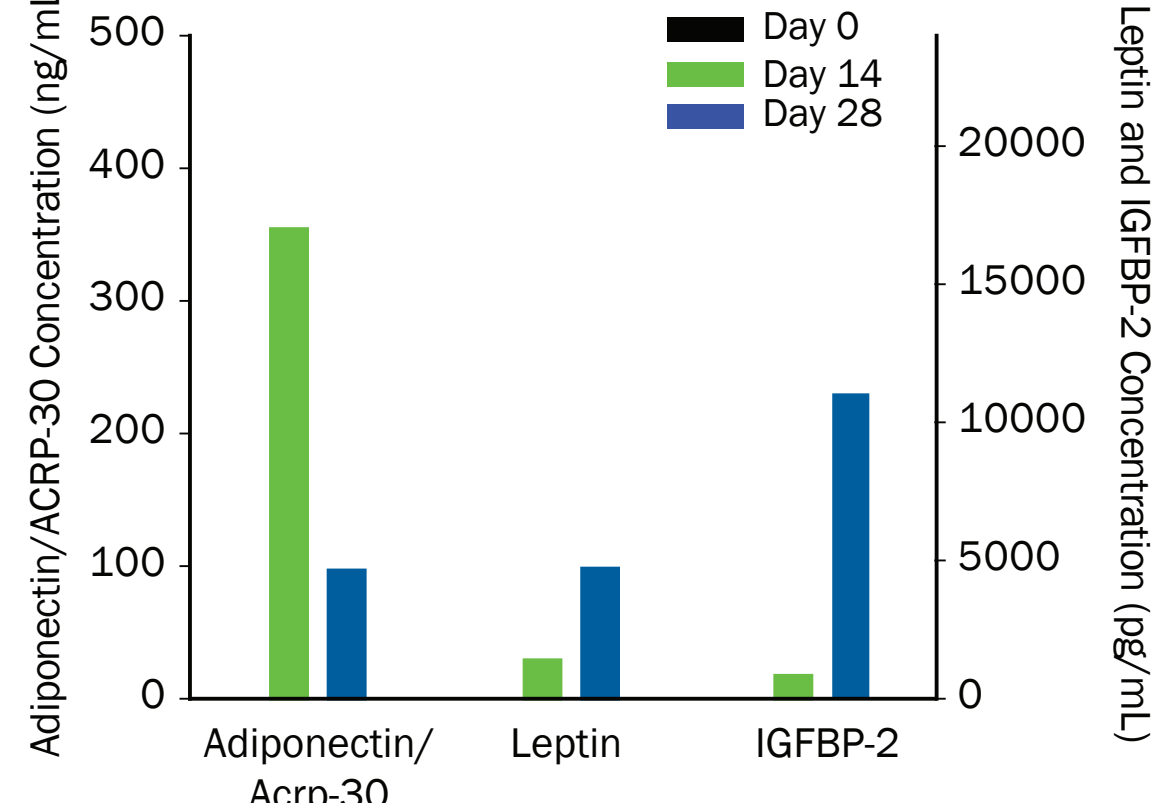
B Human Adipokine Secretion During Adipocyte Differentiation



C Array Human Analyte Profiles

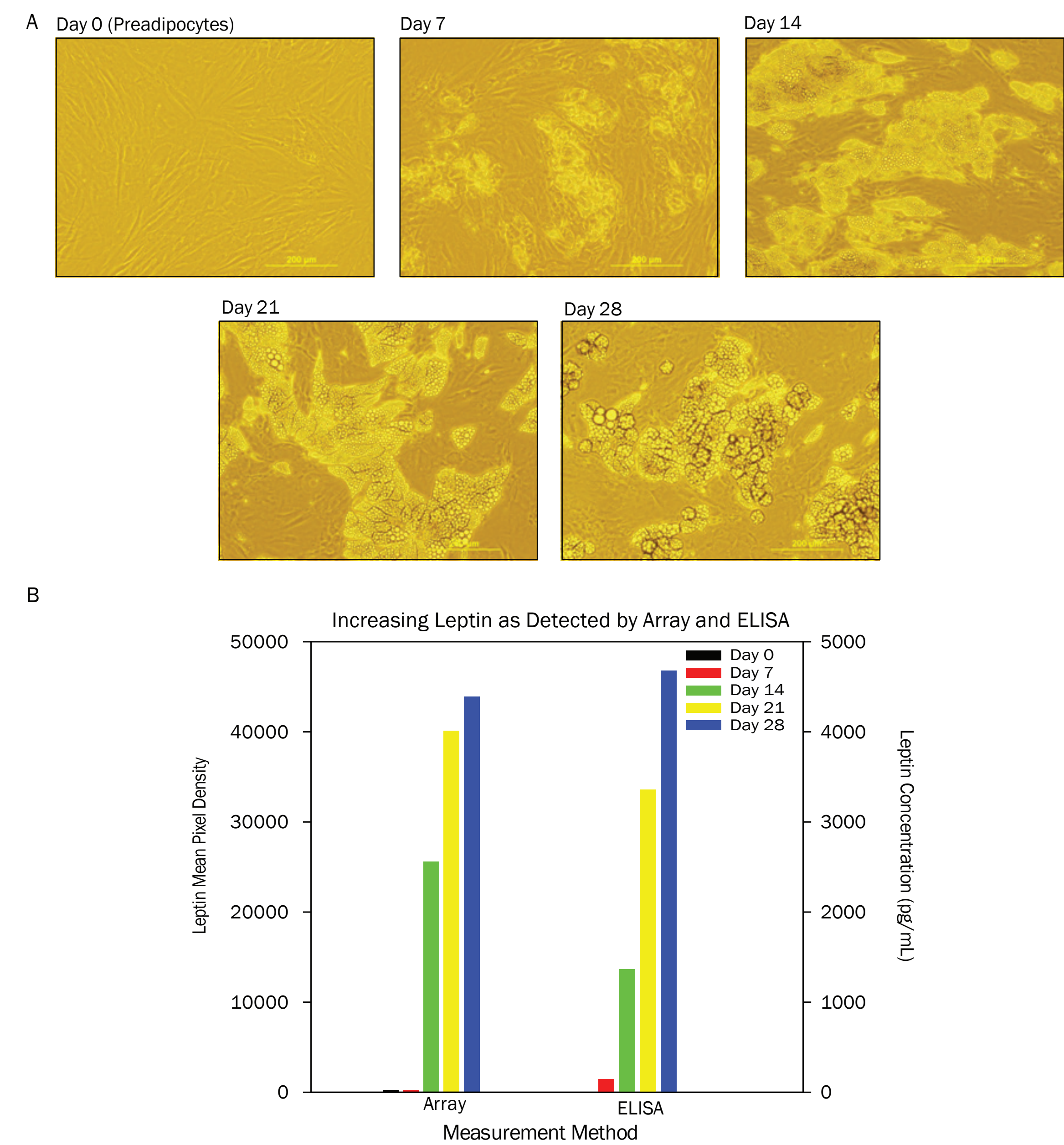


ELISA Human Analyte Concentrations



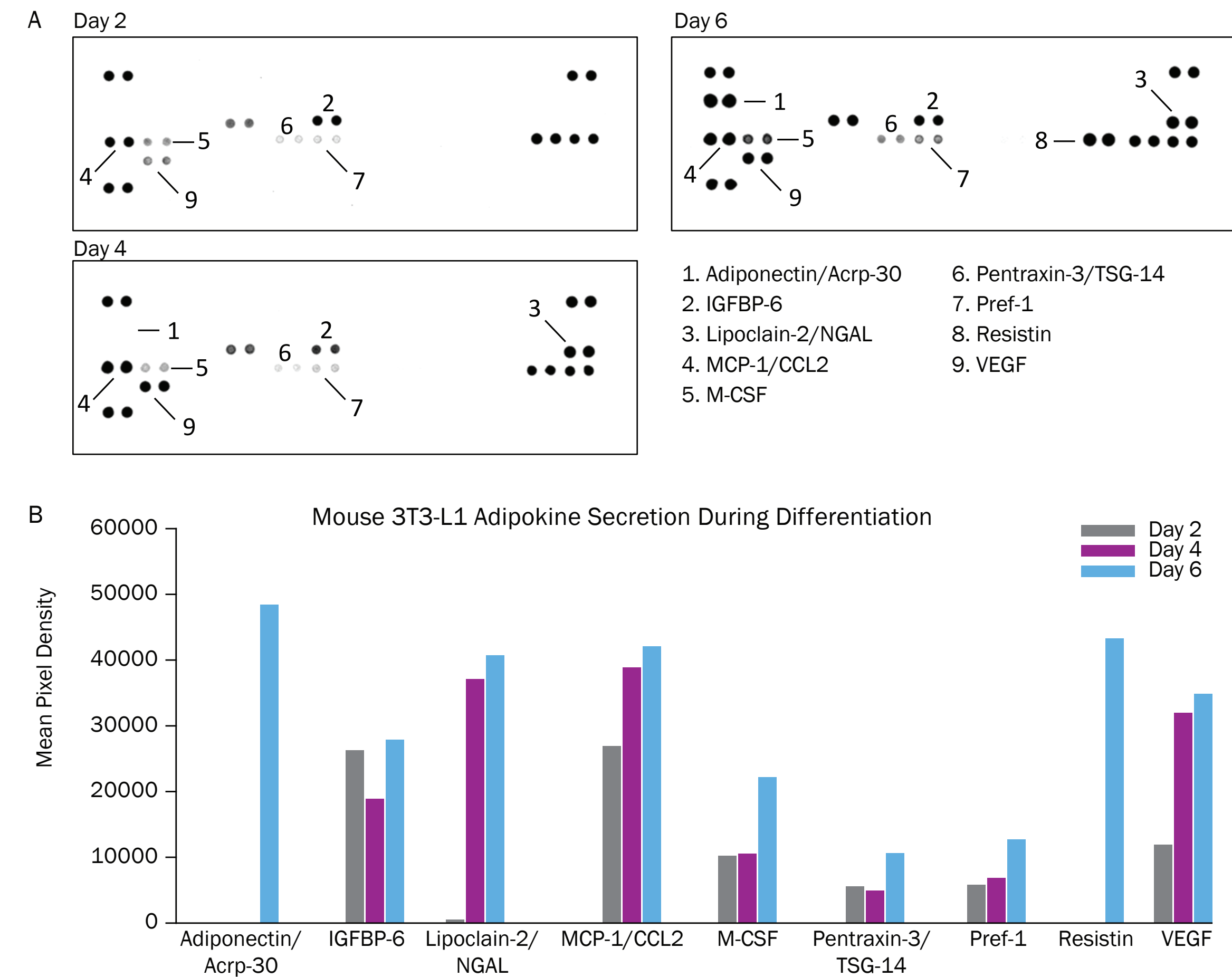
Cell culture supernates from human undifferentiated preadipocytes and adipocytes were analyzed semi-quantitatively using the Human Adipokine Array Kit and quantitatively using ELISA kits. (A) Arrays incubated with supernates collected on days 0, 7, 14, 21, and 28 of differentiation are shown (2 minute X-ray film exposure). (B) Histogram profiles of mean spot pixel density for select analytes are shown. (C) The mean pixel densities (left) for Adiponectin/Acrp-30, Leptin, and IGFBP-2 on days 0, 14, and 28 are compared to the concentrations (right) measured by ELISA kits.

Figure 2. Leptin Concentrations Correlate with Increasing Lipid Droplets



The Leptin concentration in cell culture supernates from human undifferentiated preadipocytes (Day 0) and adipocytes on days 7, 14, 21, and 28 of differentiation was measured semi-quantitatively using the Human Adipokine Array Kit and quantitatively using the ELISA kit. (A) Phase-contrast microscopy images of the human preadipocytes (Day 0) and human adipocytes (Days 7, 14, 21, and 28) that were used to obtain the cell culture supernates from Figure 1. Lipid droplets increase in number and size over the 28 day culture period. (B) Leptin concentrations measured by array and ELISA increase over the 28 day culture period.

Figure 3. Profiling Mouse Adipokines Secreted During 3T3-L1 Differentiation



Cell culture supernates from 3T3-L1 mouse embryonic fibroblast adipocyte-like cells were analyzed using the Mouse Adipokine Array kit. (A) Arrays incubated with supernates collected on days 2, 4, and 6 of differentiation are shown (5 minute X-ray film exposure). (B) Histogram profiles of mean spot pixel density for select analytes are shown.

## Summary

The Human and Mouse Proteome Profiler™ Adipokine Array Kit detected changes in adipokine secretion during the differentiation of adipocytes and adipocyte-like cells.

- Leptin, Adiponectin/Acrp30, Lipocalin-2/NGAL, and Resistin protein levels increased during differentiation.
- IL-6 and IL-8 protein levels decreased during differentiation.

Changes in analyte concentration for Adiponectin/Acrp-30, Leptin, and IGFBP-2 correlated well between array and quantitative ELISA data.

The combined use of Proteome Profiler™ Antibody Arrays and Quantikine® ELISAs is an effective method to study adipokine secretion during mouse and human *in vitro* adipocyte differentiation.