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Supplemental Information

Identification of Three Early Phases of Cell-Fate Determination during Osteogenic and Adipogenic Differentiation by Transcription Factor Dynamics

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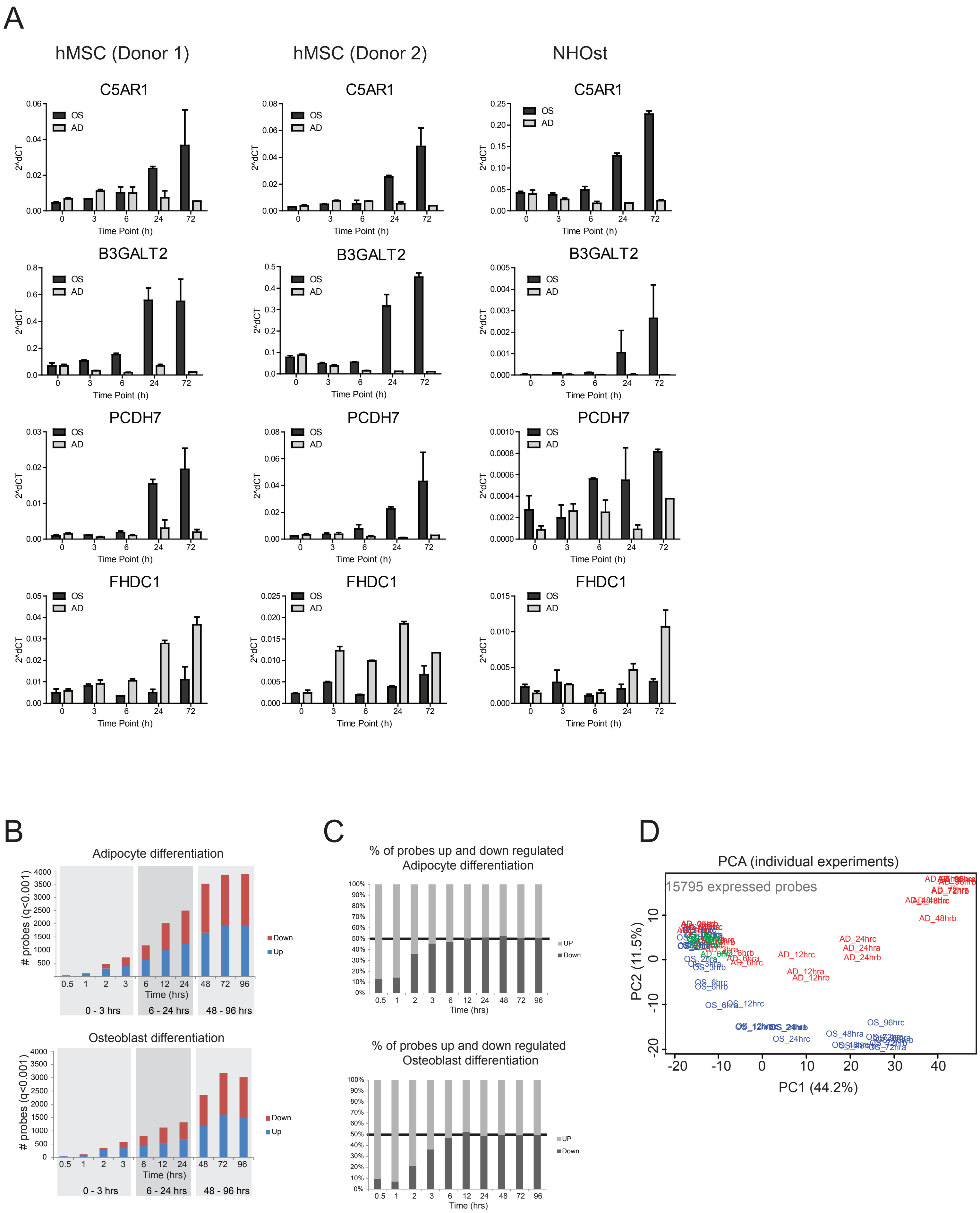
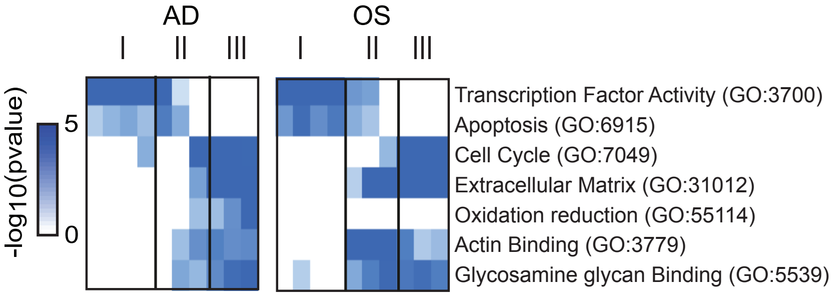
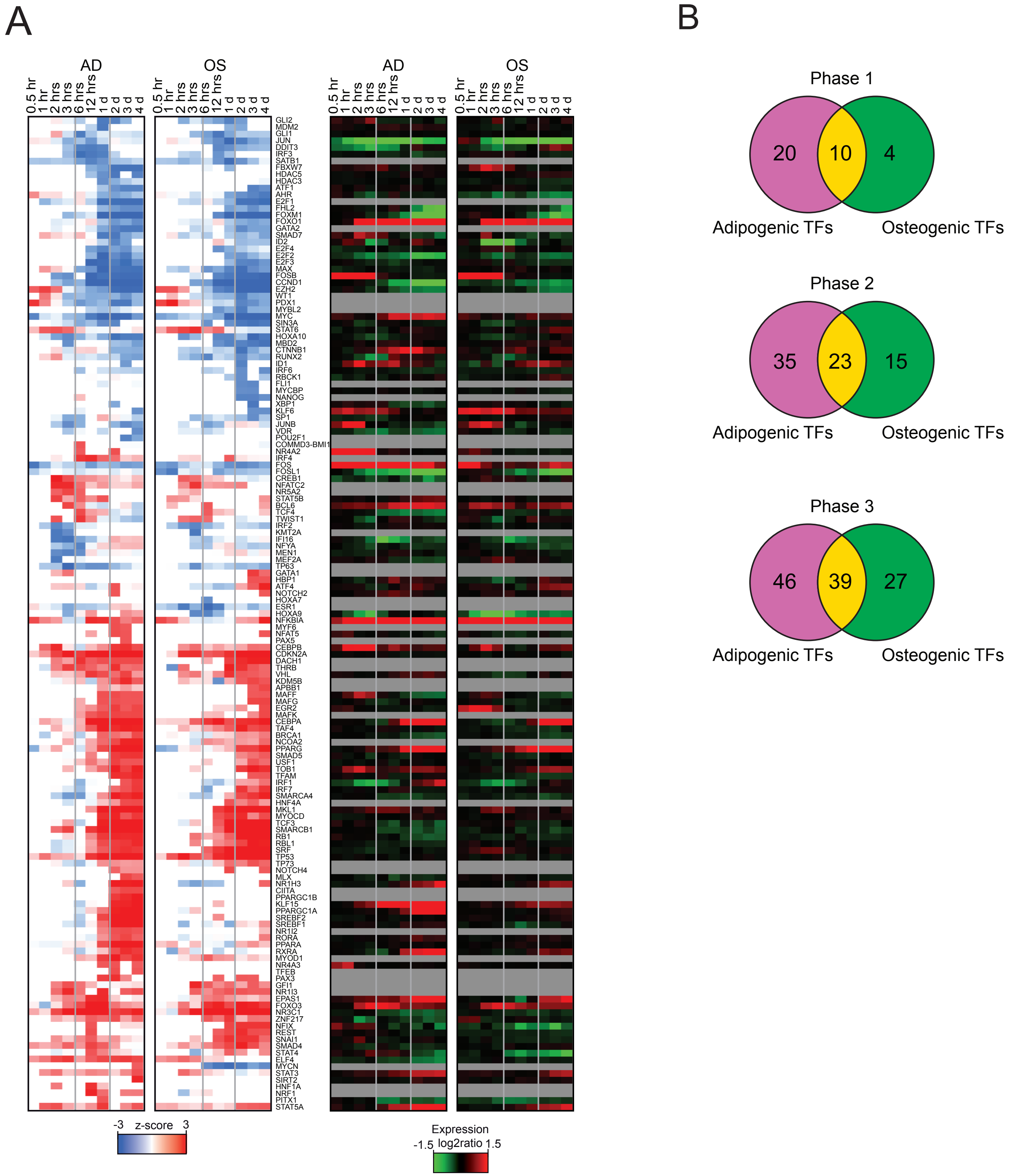
Supplemental Figure 1 van de Peppel et al.



Supplemental Figure 2 van de Peppel et al.



Supplemental Figure 3 van de Peppel et al.



Supplemental Figure 4 van de Peppel et al.

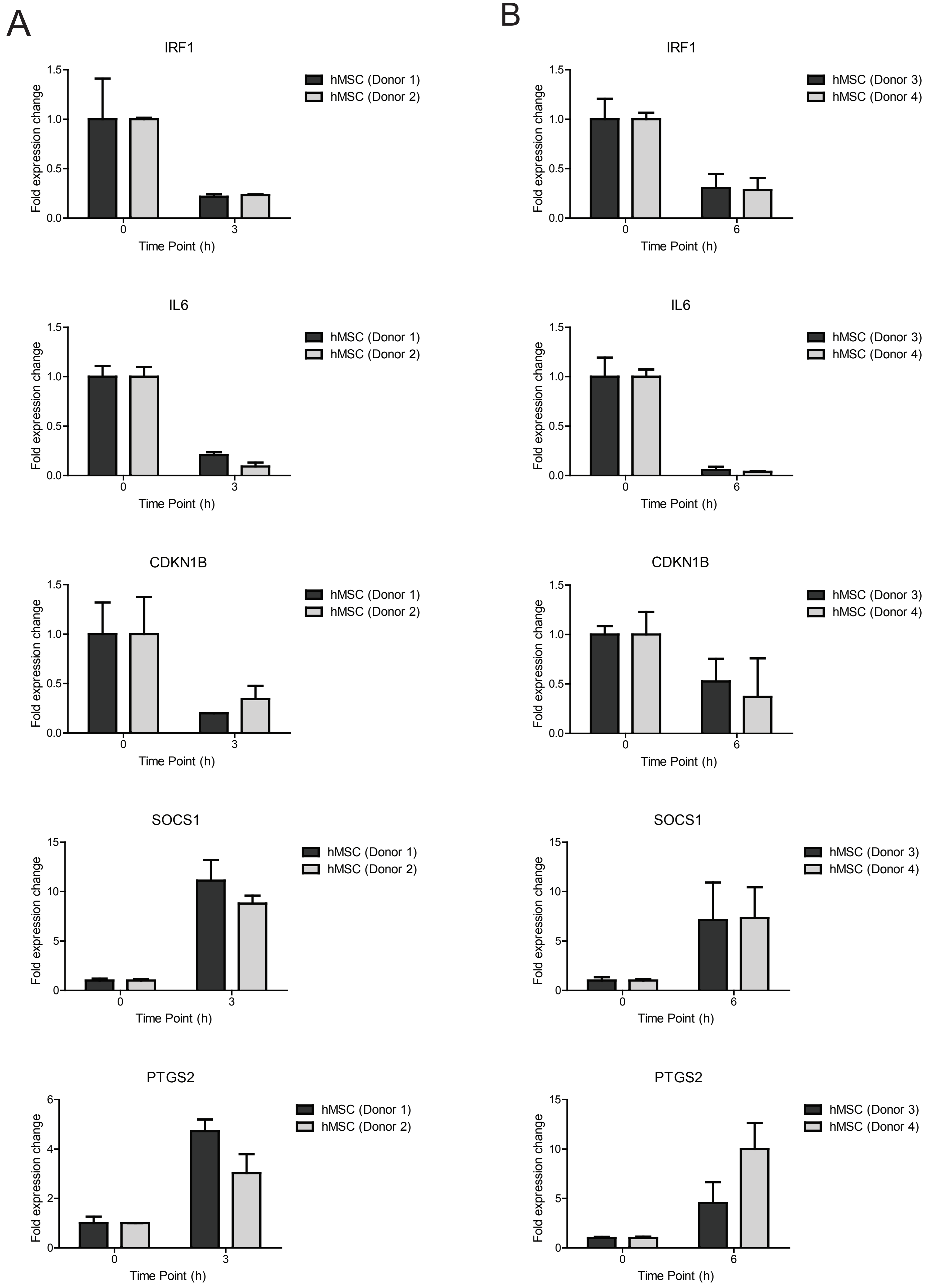


Figure S1 Validation of gene expression profiling in adipogenic and osteogenic differentiating cell.

A. qPCR validation of 3 genes (ie. C5AR1, B3GALT2, PCDH7) that were specifically upregulated during osteogenic differentiation (OS) and 1 gene (ie. FHDC1) that was specifically upregulated during adipogenic differentiation (AD). Validation was carried out in the same donor that was used in the gene expression profiling experiment (Donor 1), a different hMSCs donor (Donor 2) and a primary osteoblast cell line (NHOst) with 2 independent experiments per donor, treatment and time point. **B.** The number of significantly regulated probes per time-point compared to t=0 ($q<0.001$). In red are the number of upregulated probes and in blue the number of downregulated probes. On the left the data from the adipocyte differentiating cells and on the right the osteogenic differentiating cell. Based on the same 3 independent replicated samples as in Figure 1C **C**. Data from **Figure S1B** presented as percentage of total significantly regulated probes at each time-point ($q<0.001$). Based on the same 3 independent replicated samples as in Figure 1C. **D.** To couple gene expression with the dynamics of adipogenic and osteogenic differentiation of hMSC we performed unsupervised cluster analyses of all samples within the first 4 days. All biological replicates cluster close together indicating the reproducibility of our experiments. (blue = osteogenic, red = adipogenic).

Figure S2 Functional annotation of significant regulated probes.

Selection of functional Gene Ontology categories that were similarly or lineage specifically enriched from Figure 2. The cluster diagram depicts the -log10(pvalue) of the enrichment (see M&M for detailed procedure).

Figure S3 Clustering of all TFs that change activity within 4 days of differentiation.

A. Cluster diagram of all transcription factors that had at least once z-score >2 (red) or <-2 (blue) in either lineages within the first 4 days of osteogenic and adipogenic differentiation. Next to the cluster of activity patterns is the relative expression (red is upregulated, green is down regulated relative to undifferentiated cells at t=0) during differentiation for these TFs. Expression patterns of genes that are grey, were not expressed or not present on the array and therefore no expression data was available. **B.** Venn diagram illustrating the number and overlap of the transcription factors that were identified during osteoblast differentiation in the 3 differentiation phases.

Figure S4 Validation gene expression changes of IRF2 target genes in MSCs from different donors.

qPCR validation of 5 IRF2 target genes in MSCs obtained from different donors after 3 hours (**A**) or 6 hours (**B**) of osteogenic differentiation. Donor 1 was used for the gene expression profiling experiment. Average and standard deviation of 2 independent experiments for each donor and time point.