

Supplementary Figures

Continual proteomic divergence of HepG2 cells as a consequence of long-term spheroid culture

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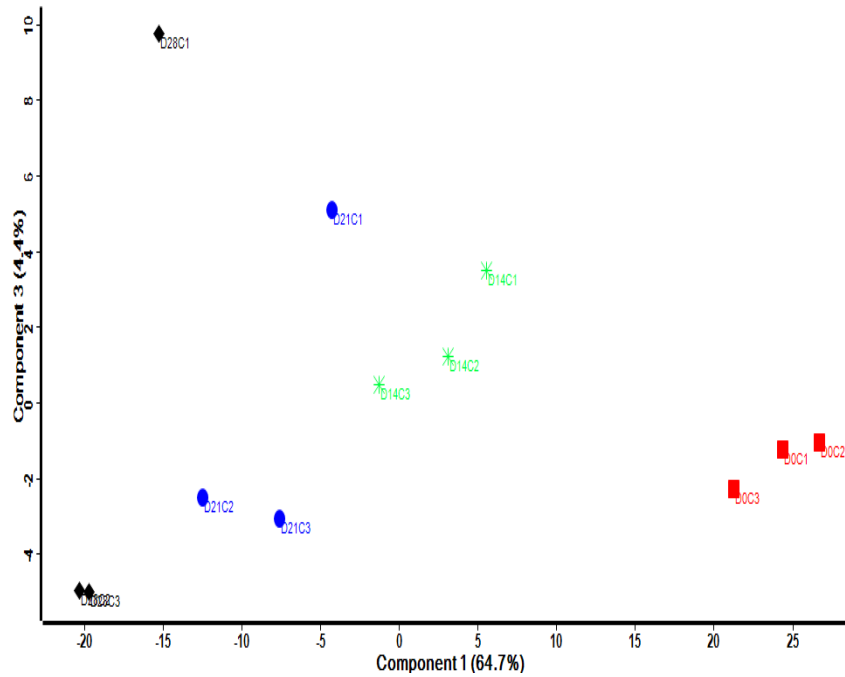
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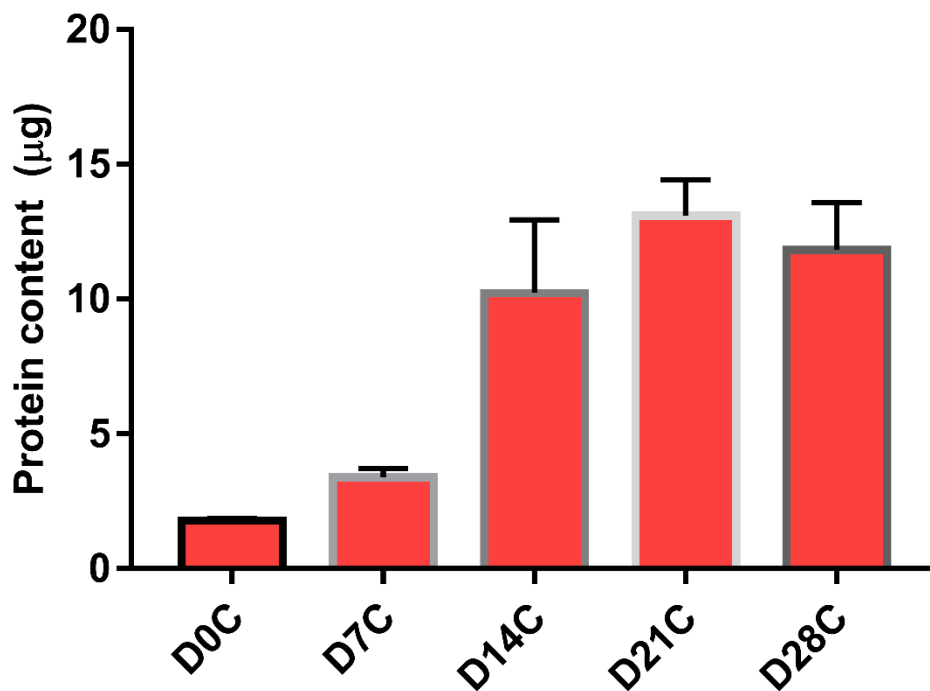
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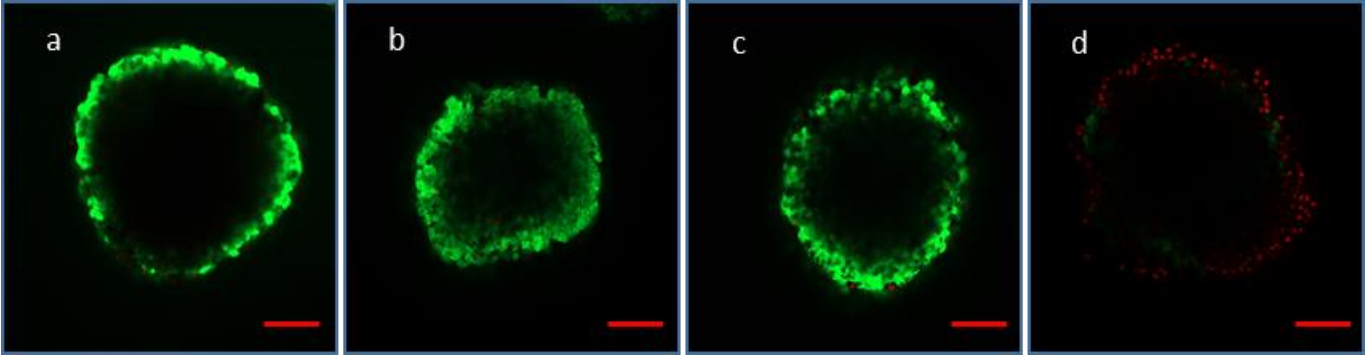
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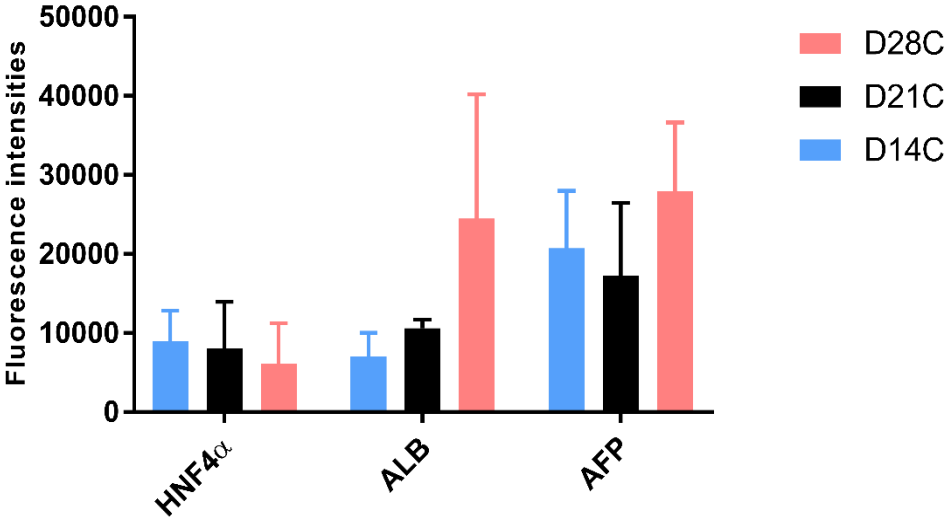
Supplementary Fig 1: PCA of HepG2 cell monolayers (red) and spheroids at Days 14 (green), 21 (blue), and 28 (black) comparing Component 1 versus Component 3. Figure generated using Perseus v. 1.6.7.0 software²⁸.



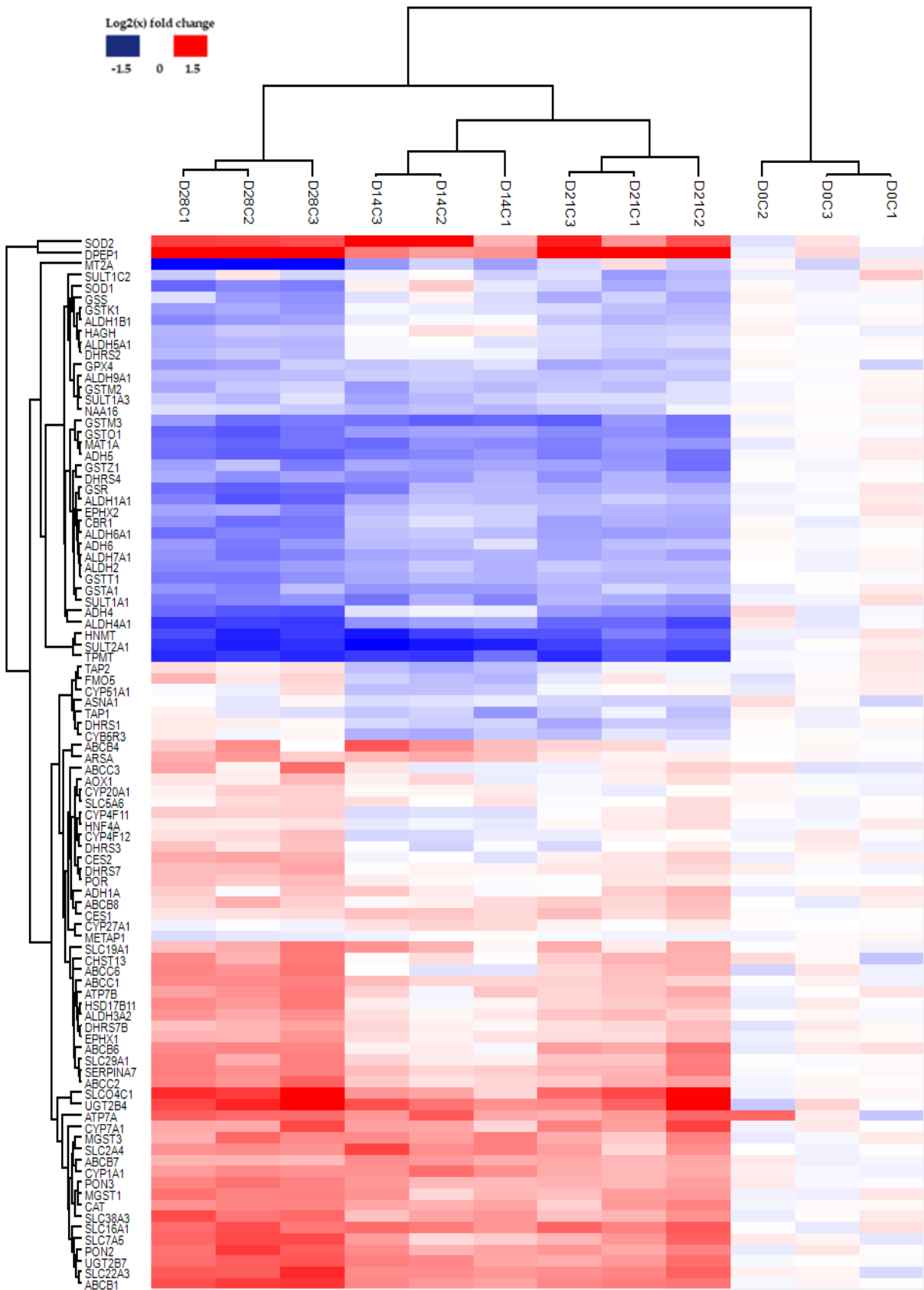
Supplementary Fig 2: Protein content per spheroid (mean ± SEM), used as an inference for cellular biomass, measured over the 28-day time course. Generated using GraphPad Prism windows software V7.0.0 (www.graphpad.com).



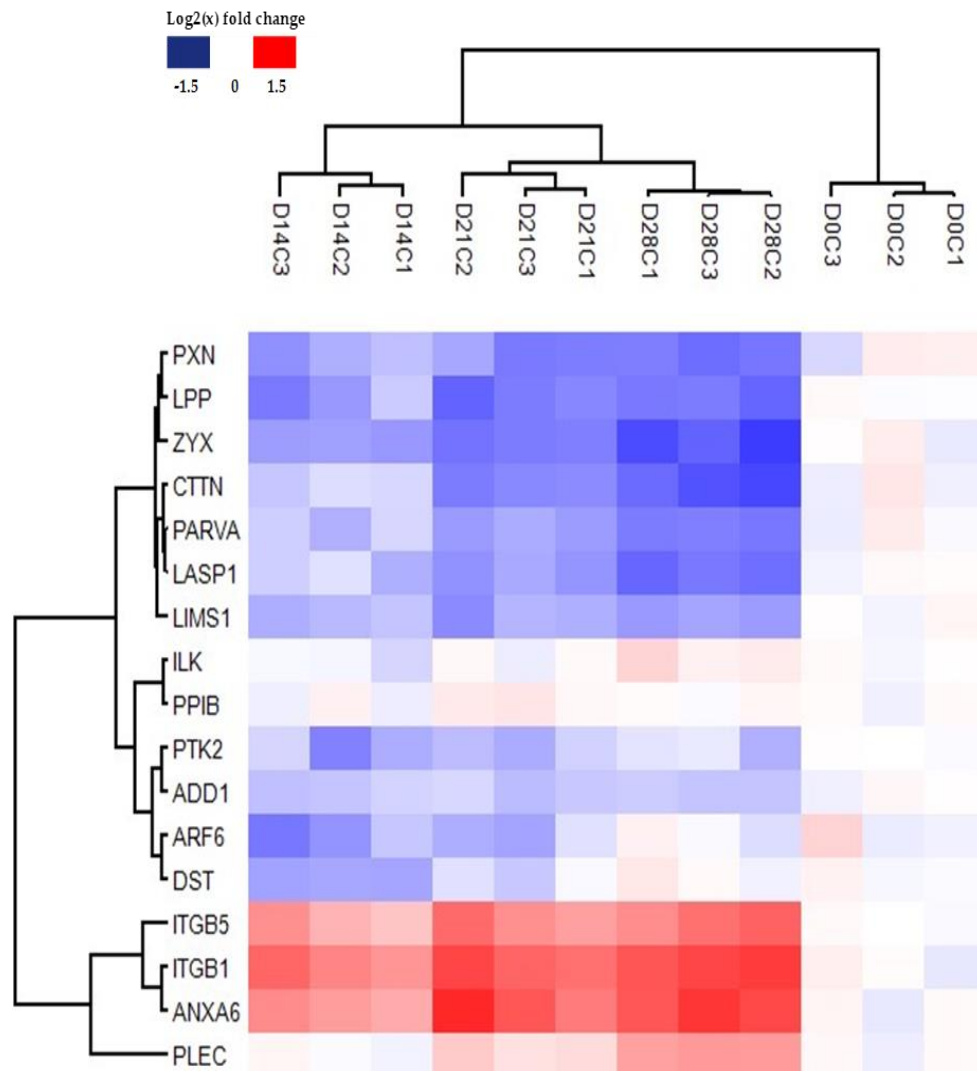
Supplementary Fig 3: Confocal micrographs of spheroids stained with FDA (green) and PI (red) at Days 14 (a), 21 (b), 28 (c), and Day 28 positive control exposed to 10 µg/mL Puromycin (d), Scale bars 100 µm. Generated using Zeiss Zen Blue software 3.0 (<https://www.zeiss.com/microscopy/int/products/microscope-software/zen.html>).



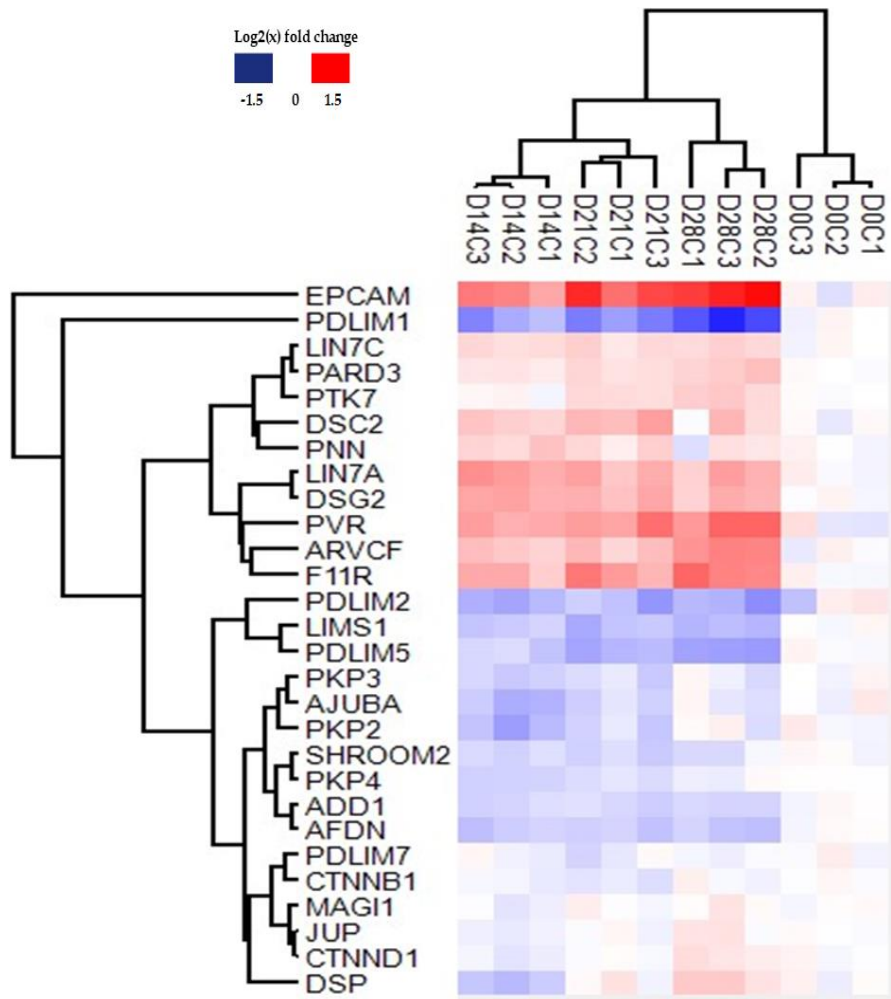
Supplementary Fig 4: Quantified relative fluorescent intensities (mean \pm SD) of spheroids labelled with hepatic nuclear factor 4 alpha (HNF4 α), albumin (ALB) and alpha-fetoprotein (AFP) normalised to DAPI. Generated using GraphPad Prism windows software V7.0.0 (www.graphpad.com).



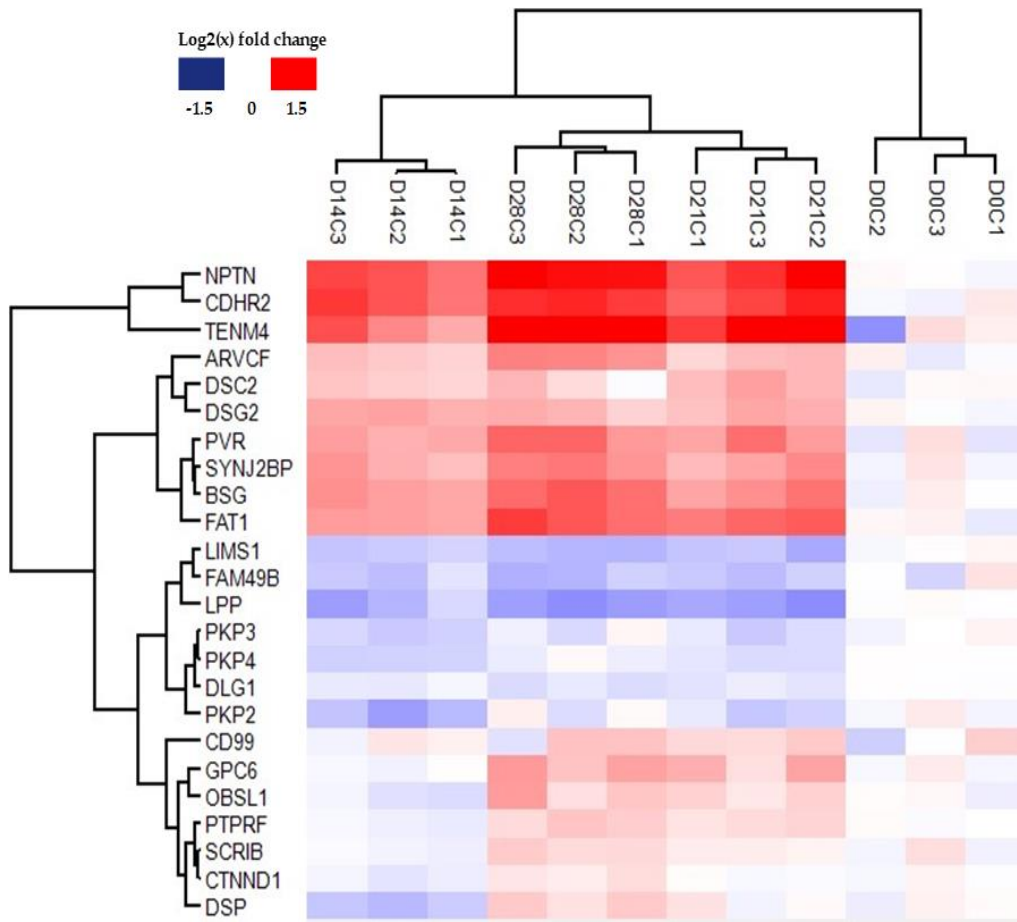
Supplementary Fig 5: Hierarchical clustering of proteins involved in ADME processes. Generated using Perseus v. 1.6.7.0 software.



Supplementary Fig 6: Hierarchical clustering of proteins involved in Focal Adhesions (GO:0005925). Generated using Perseus v. 1.6.7.0 software.



Supplementary Fig 7: Hierarchical clustering of proteins involved in cell-cell junctions (GO 0005911). Generated using Perseus v. 1.6.7.0 software.



Supplementary Fig 8: Hierarchical clustering of proteins involved in cell-cell adhesion (GO 0098609). Generated using Perseus v. 1.6.7.0 software.