Supplementary Figures for

Data-modeling identifies conflicting signaling axes governing myoblast proliferation and differentiation responses to diverse ligand stimuli

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Figure S1. Diagram of cues, signals, and responses associated with this study. Ligands, cues, receptors, pathways, and effects on myoblast proliferation and differentiation response phenotypes are summarized from prior literature. Arrows represent pathway influences not direct protein mechanisms. All species labeled with a yellow “p” are measured in the CSR data compendium (see **Fig. 2**).

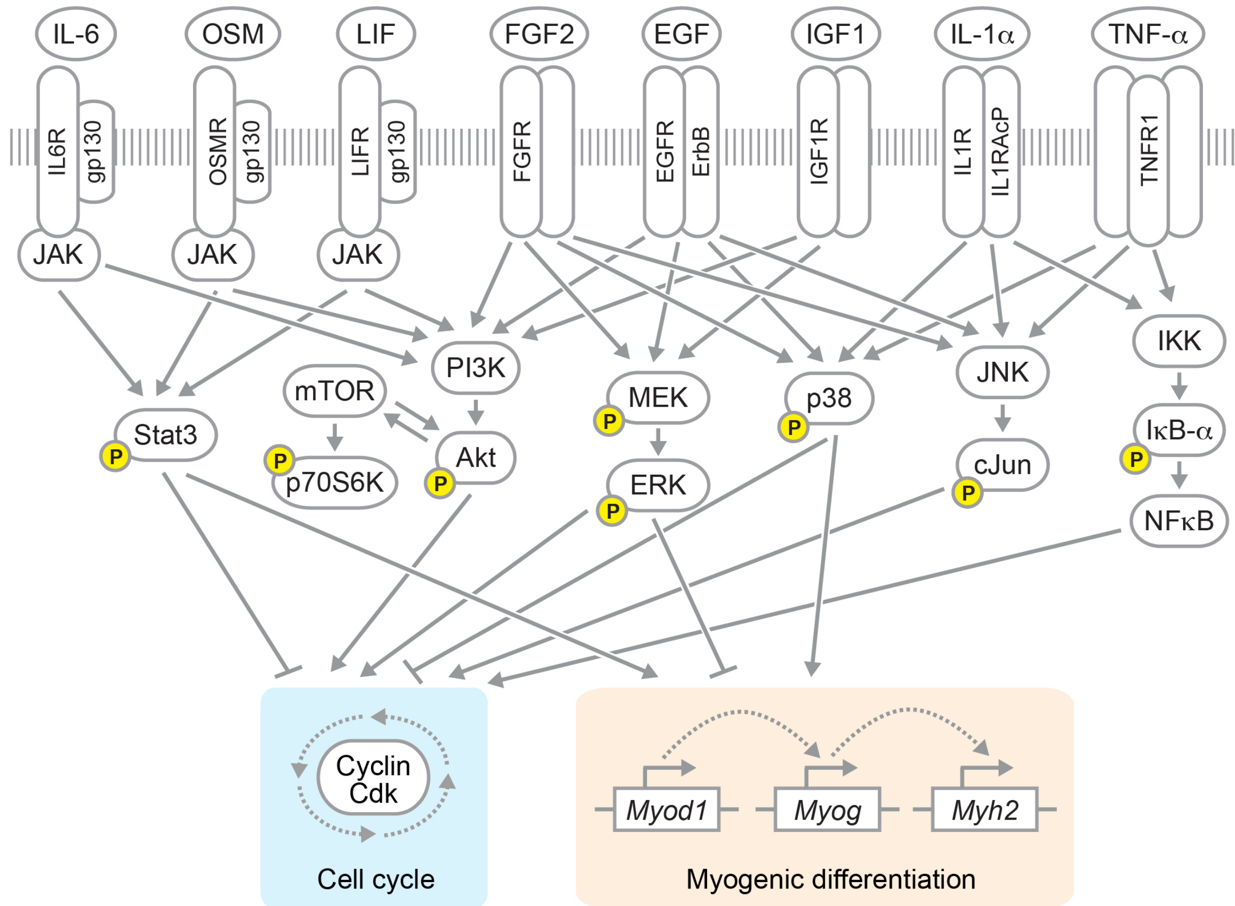

Figure S2. Immunoblot and Luminex optimization for lysate loading mass. (**a**) Representative immunoblot for Gapdh. Primary mouse myoblasts were cultured in growth medium (GM) with 2.5 ng mL^{-1} FGF2 and lysed with NP-40 based lysis buffer. Lanes were loaded with 5, 10, 20, 30, and 40 µg of total protein in 25 µL of loading buffer. (**b**) AUC intensity of Gapdh bands from (**a**), normalized to the AUC intensity of the 5 µg Gapdh band. (**c**) Immunoblot for phospho-STAT3 and Gapdh. Primary myoblasts were cultured in differentiation medium (DM) for 3 hr and stimulated for 15 min with IL-6+OSM+LIF (IOL). Lanes were loaded with 10 and 20 µg of total protein in 25 µL of loading buffer. (**d**) AUC intensity of phospho-STAT3 and Gapdh bands from (**c**), normalized to the AUC intensity of the 10 µg bands. (**e-i**) Luminex background-subtracted fluorescent intensity plots. Primary mouse myoblasts were cultured in DM for 3 hr and stimulated for 15 min with or without TNF-α/FGF2/IL-6 (TFI). Wells were loaded with 2, 4, 8, 12, and 16 µg of total protein. (**e**) β-tubulin fluorescent intensity. (**f**) phospho-cJun fluorescent intensity. (**g**) Data from (**f**) normalized to β-tubulin data from (**e**). (**h**) phospho-cJun fluorescent intensity. (**i**) Data from (**h**) normalized to β-tubulin data from (**e**). In (**a-d**), cells were lysed with NP-40 based lysis buffer, and protein concentration was determined via BCA assay. In (**e-i**), cells were lysed with Millipore lysis buffer. and protein concentration was determined via BCA assay. (**j**) Percentage of cells with apoptotic bodies at 72 hr post-treatment. In (**b**, **d**), n = 2 replicates are plotted as mean ± s.e.m. In (**e-j**), n = 3 replicates are plotted as mean ± s.e.m.

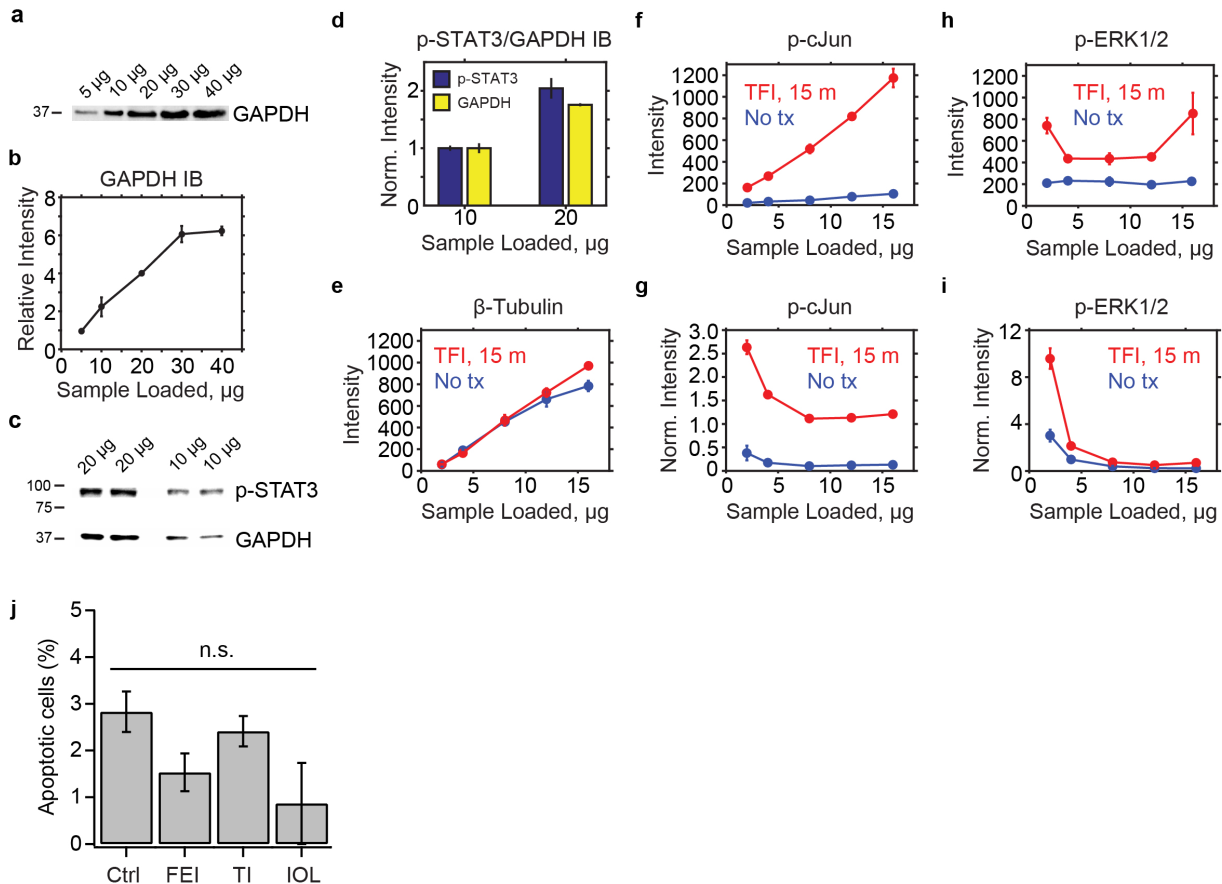

Figure S3. A dense time-course of myoblast phosphoprotein Luminex signals for PLS model training. Primary mouse myoblasts were cultured in differentiation medium (DM only) or pre-treated for 3 hours with 0.1% DMSO (Control). Cells were stimulated with either FGF2+EGF+IGF1 (FEI), TNF-α+IL-1α (TI), IL-6+OSM+LIF (IOL), or their two-way combinations. At the following time-points: 0 min, 5 min, 15 min, 60 min, 4 hr, 24 hr, cells were lysed with Millipore lysis buffer and protein concentration was quantified via BCA assay. Luminex wells were loaded with 3 µg of total protein. Background-subtracted fluorescent intensities for p-Akt, p-p70 S6K, p-MEK1, p-ERK1/2, p-p38, p-IκBα and p-cJun were normalized to β-tubulin fluorescent intensity. Each normalized intensity was normalized to the 0 min time-point for the given phosphoprotein, generating a relative fold change value plotted here. n = 2-3 biological replicates with n = 1 technical replicate are plotted with mean in red.

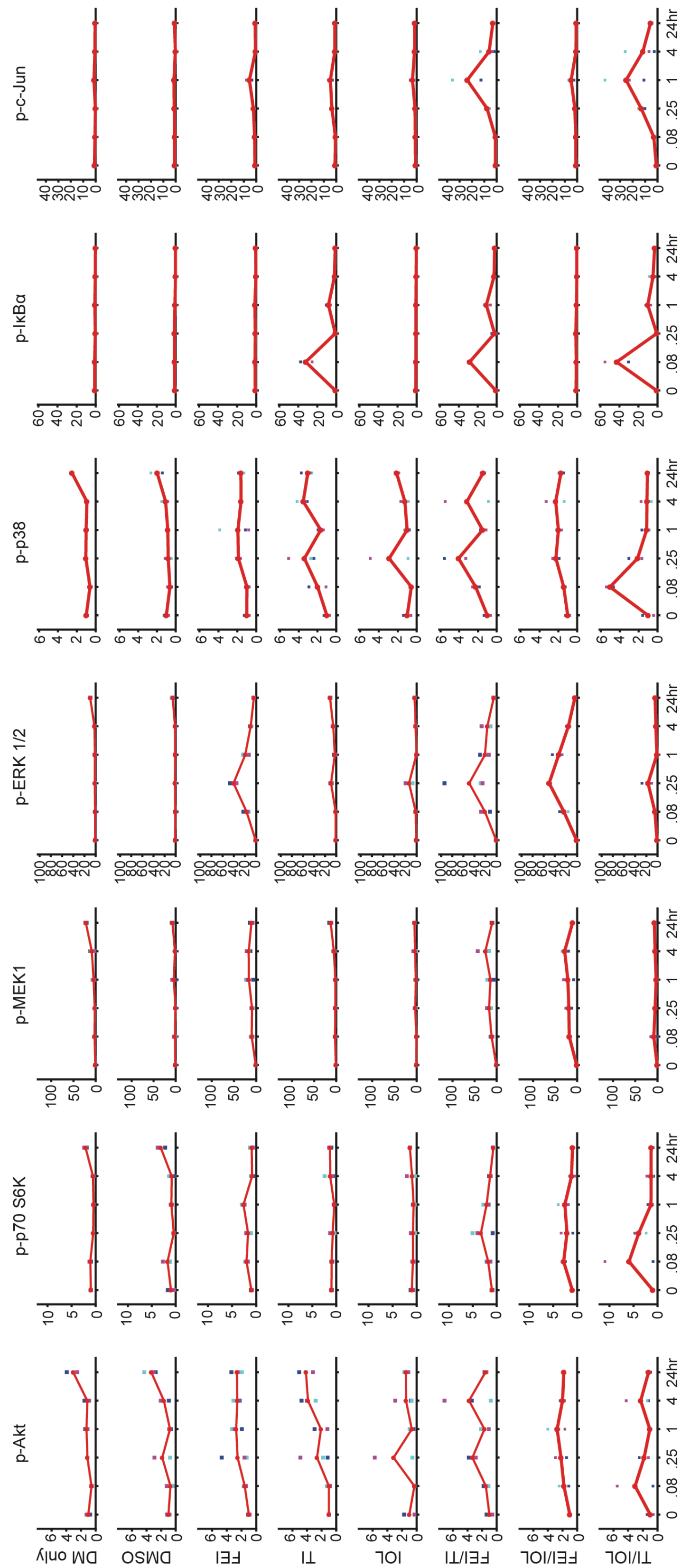

Figure S4. Partial least-squares regression model calibration and interpretation. (**a-c**) The fitness of a four principal component PLS regression model (see **Fig. 3**) to predict response outcomes based on signaling data. Model fitness (R^2; see insets) in predicting Myogenin expression by qPCR (**a**), *Myh2* expression by RT-qPCR (**b**), and Myosin Heavy Chain (MHC) by immunoblot (**c**). Mean ± s.e.m. of n = 3 replicates is reported for observations. (**d-e**) PLS model loadings (w*c) for all signaling metrics (gray) and responses (blue) in PC1 versus PC2 (**d**) and PC3 versus PC4 (**e**). Blue vector overlays are to denote interpretations of axes of cellular response outcomes in the PLS model. (**f-g**) Heatmaps of PLS model signaling loadings in PC2 (**f**) and PC4 (**g**). See **Fig. 4b-c** for PC1 and PC3 heatmaps.

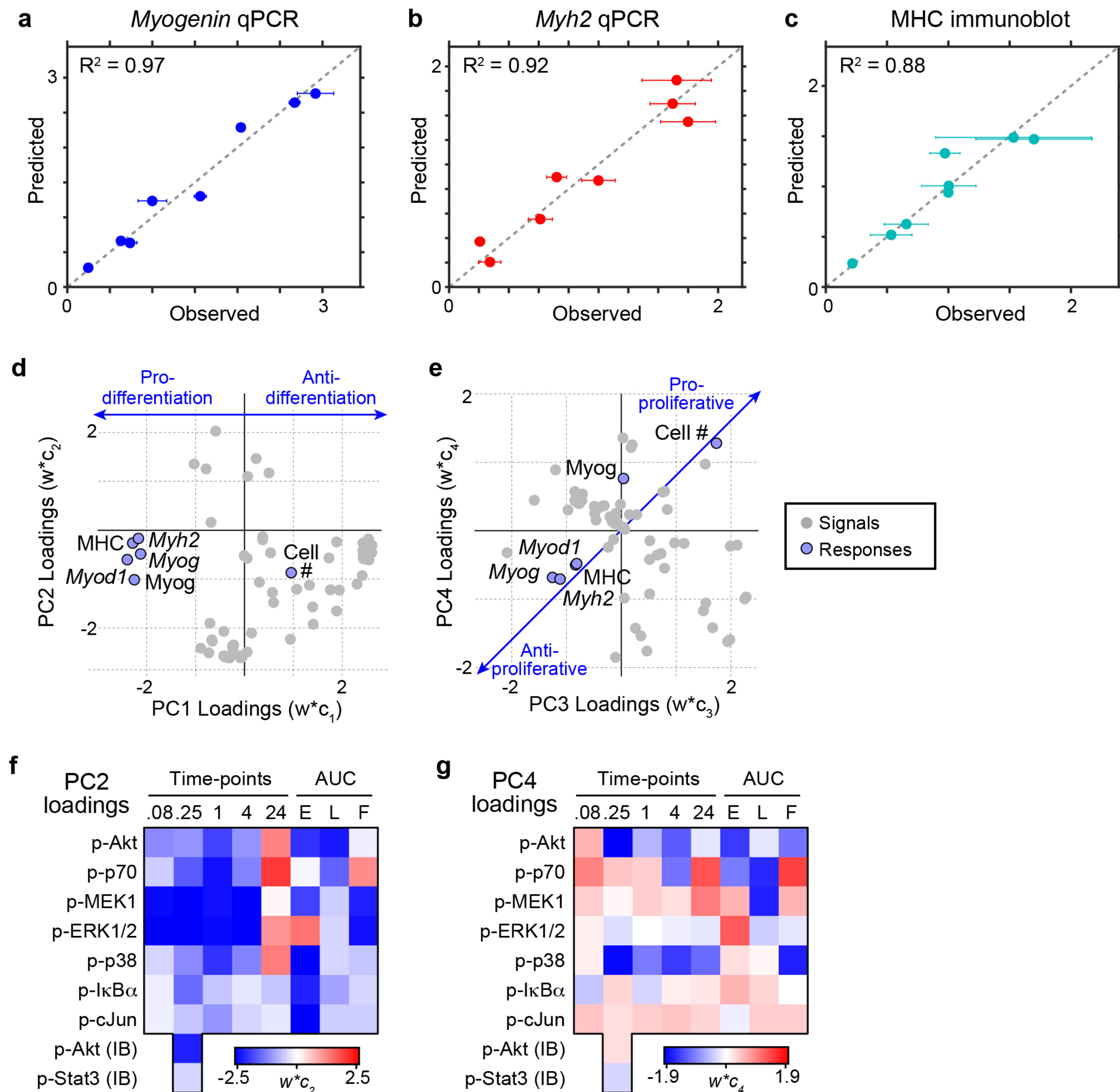

Figure S5. Predictive accuracy of the trained PLS model on a new CSR compendium. The trained PLS model tested in predicative accuracy in a second "test" myoblast CSR compendium, containing both repeated (Control, FEI, TI) and new (FEI with the MEK inhibitor PD0325901 or p38 inhibitor SB203580 and TI with PD0325901, SB203580 or the JNK inhibitor SP600125). Inhibitors were added 1 hr before stimulation. Left, measured signaling data heatmap. Right, measured (top) and model-predicted (bottom) proliferation and differentiation response data heatmaps. All data are normalized, scaled and presented as in **Fig. 2c**. Cumulative model fitness (R^2) for all response variables is shown in the inset.