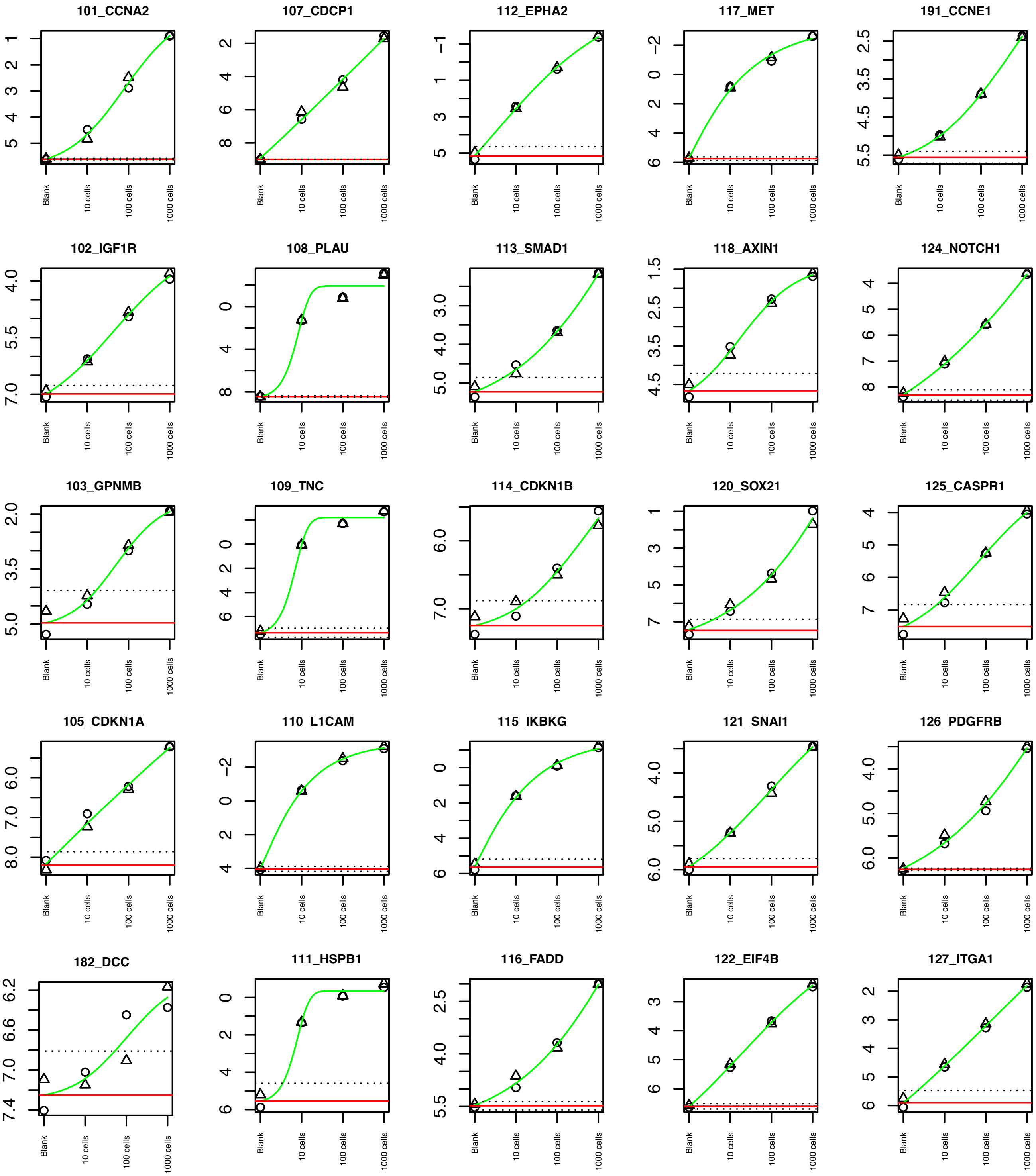
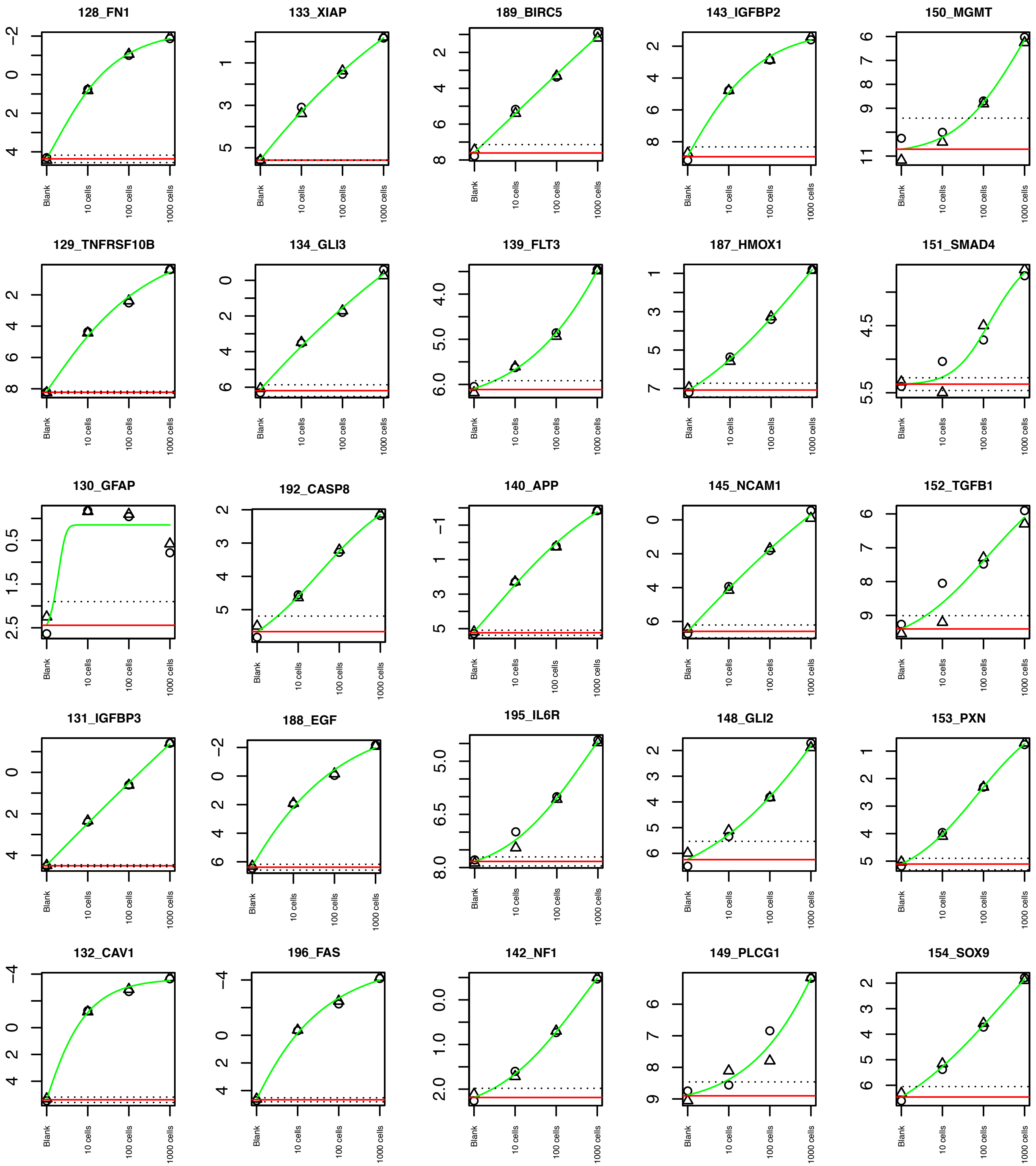
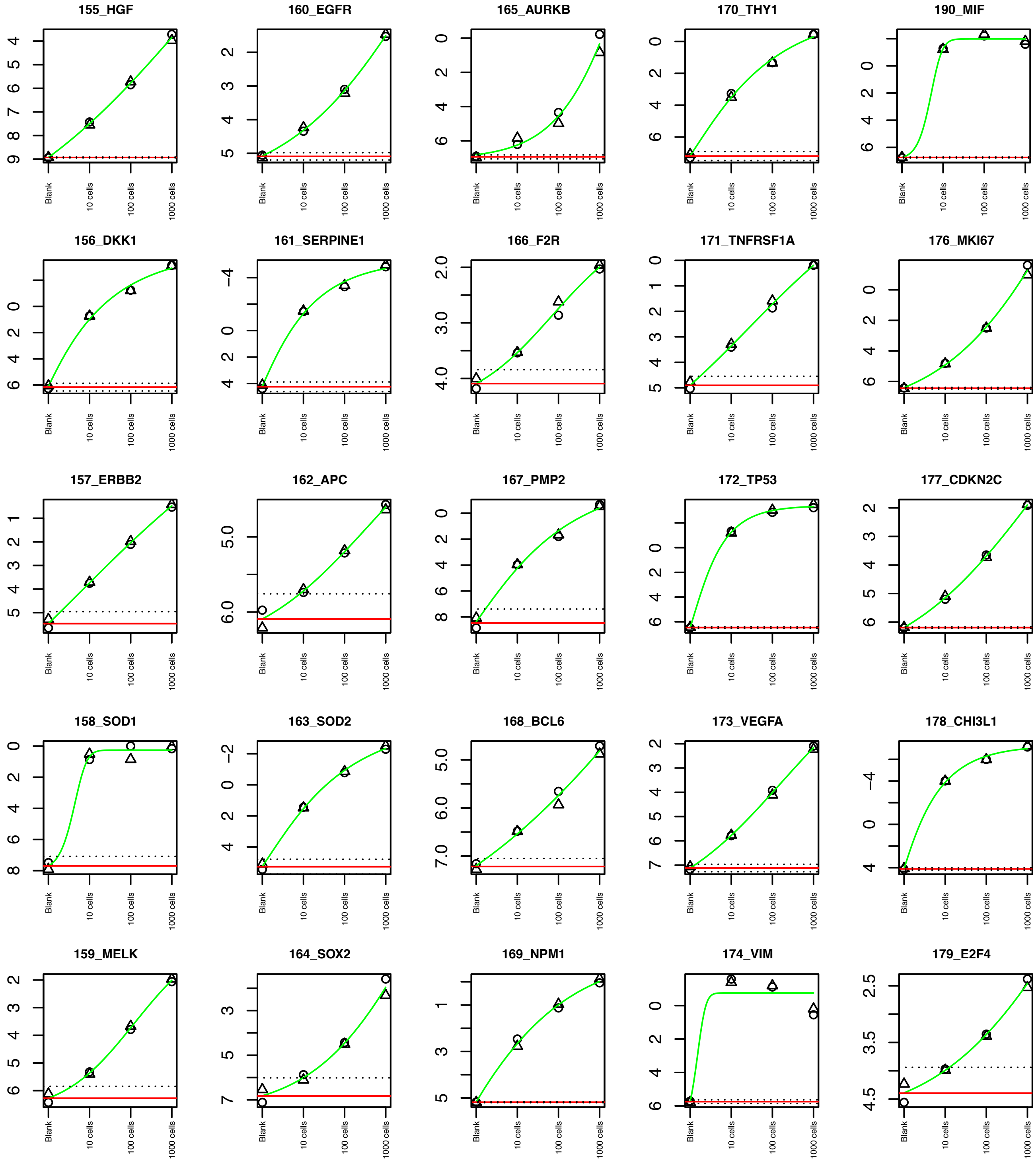
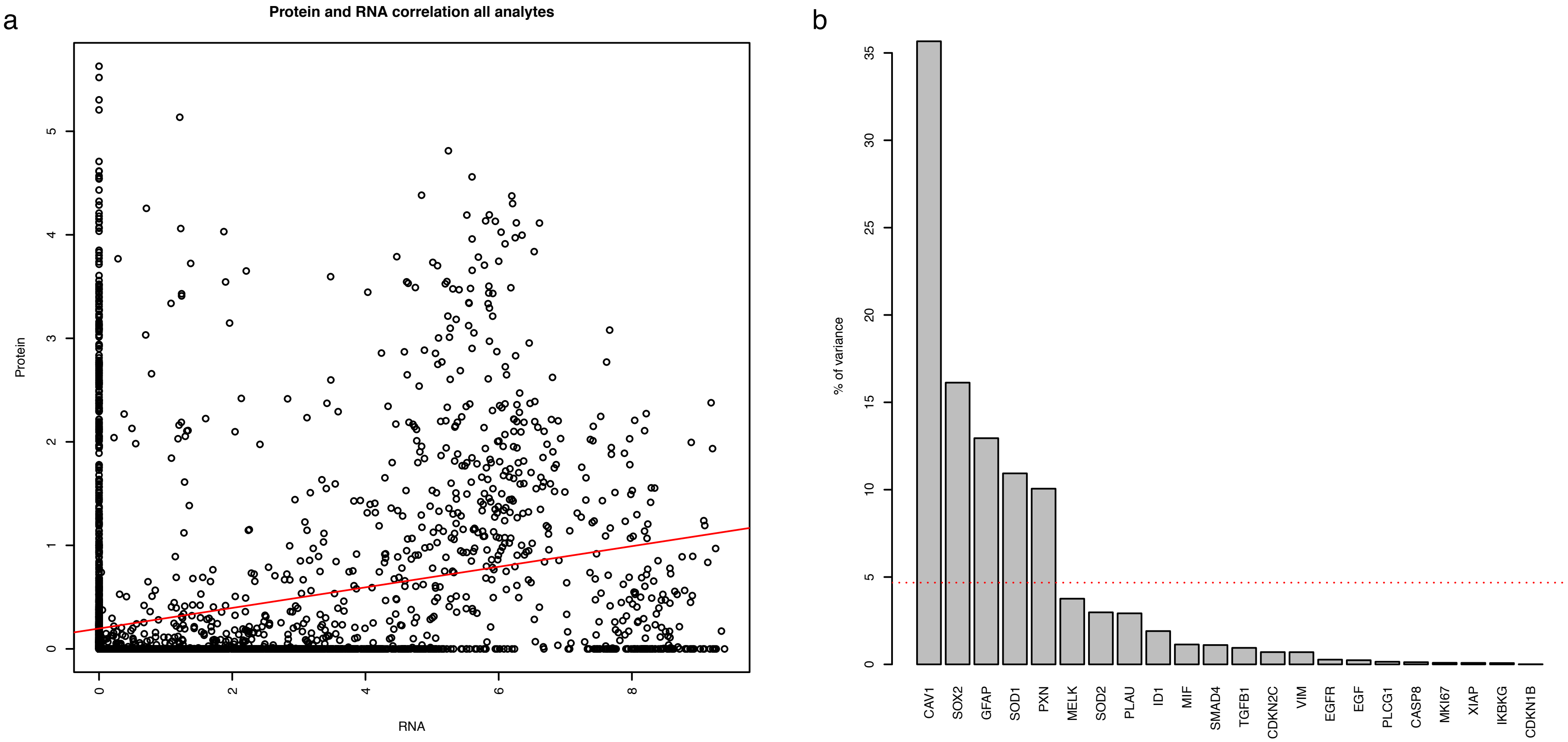
Supplemental Figure 1, Related to Figure 1



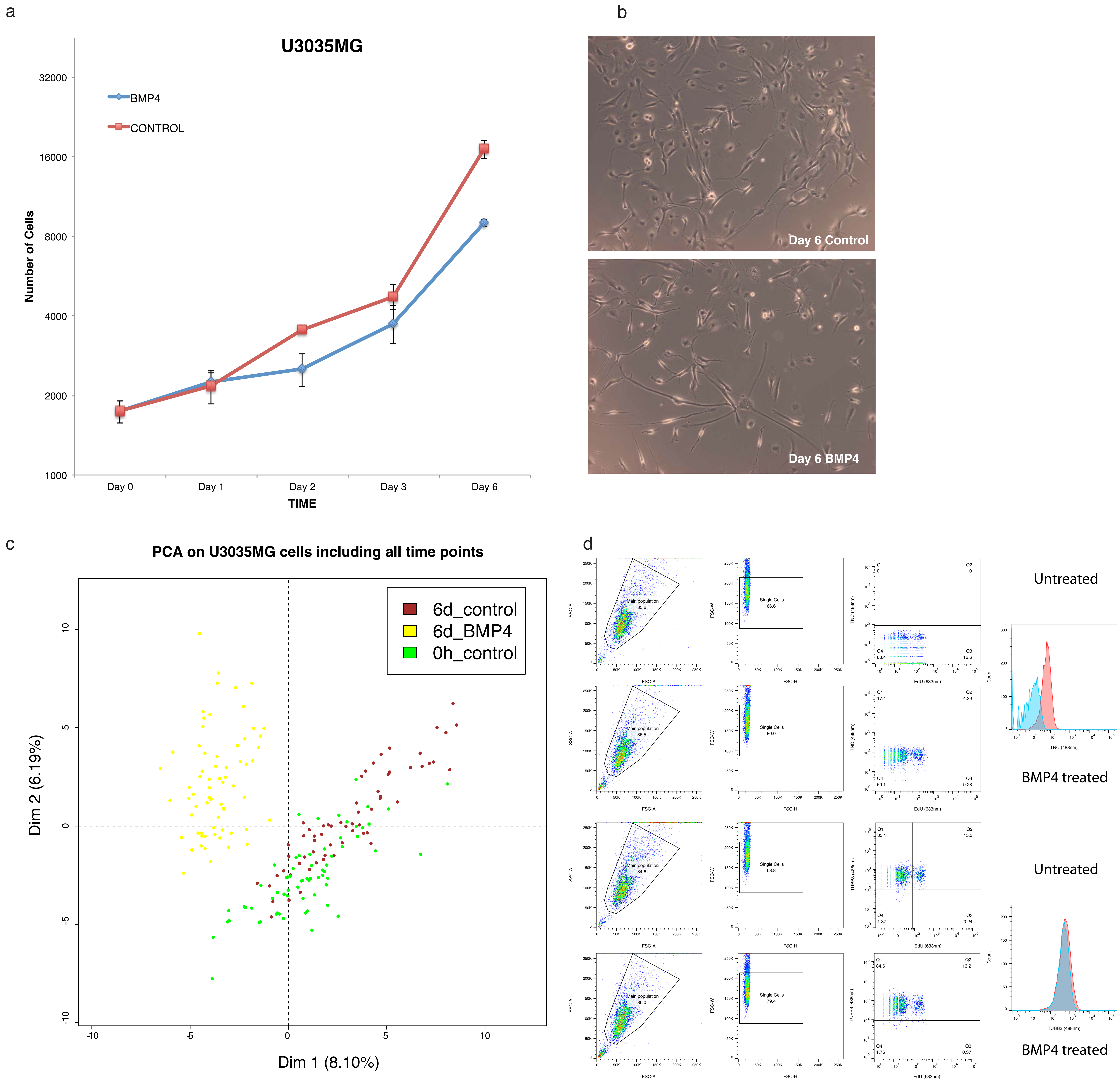

128_FN1
−2
0
2
4
133_XIAP
1
3
5
189_BIRC5
2
4
6
8
143_IGFBP2
2
4
6
8
150_MGMT
6
7
8
9
11
129_TNFRSF10B
2
4
6
8
134_GLI3
0
2
4
6
139_FLT3
4.0
5.0
6.0
187_HMOX1
1
3
5
7
151_SMAD4
4.5
5.5
130_GFAP
0.5
1.5
2.5
192_CASP8
2
3
4
5
140_APP
−1
1
3
5
145_NCAM1
0
2
4
6
152_TGFB1
6
7
8
9
131_IGFBP3
0
2
4
188_EGF
−2
0
2
4
6
195_IL6R
5.0
6.5
8.0
148_GLI2
2
3
4
5
6
153_PXN
1
2
3
4
5
132_CAV1
−4
0
2
4
196_FAS
−4
0
2
4
142_NF1
0.0
1.0
2.0
149_PLCG1
6
7
8
9
154_SOX9
2
3
4
5
6
Blank
10 cells
100 cells
1000 cells



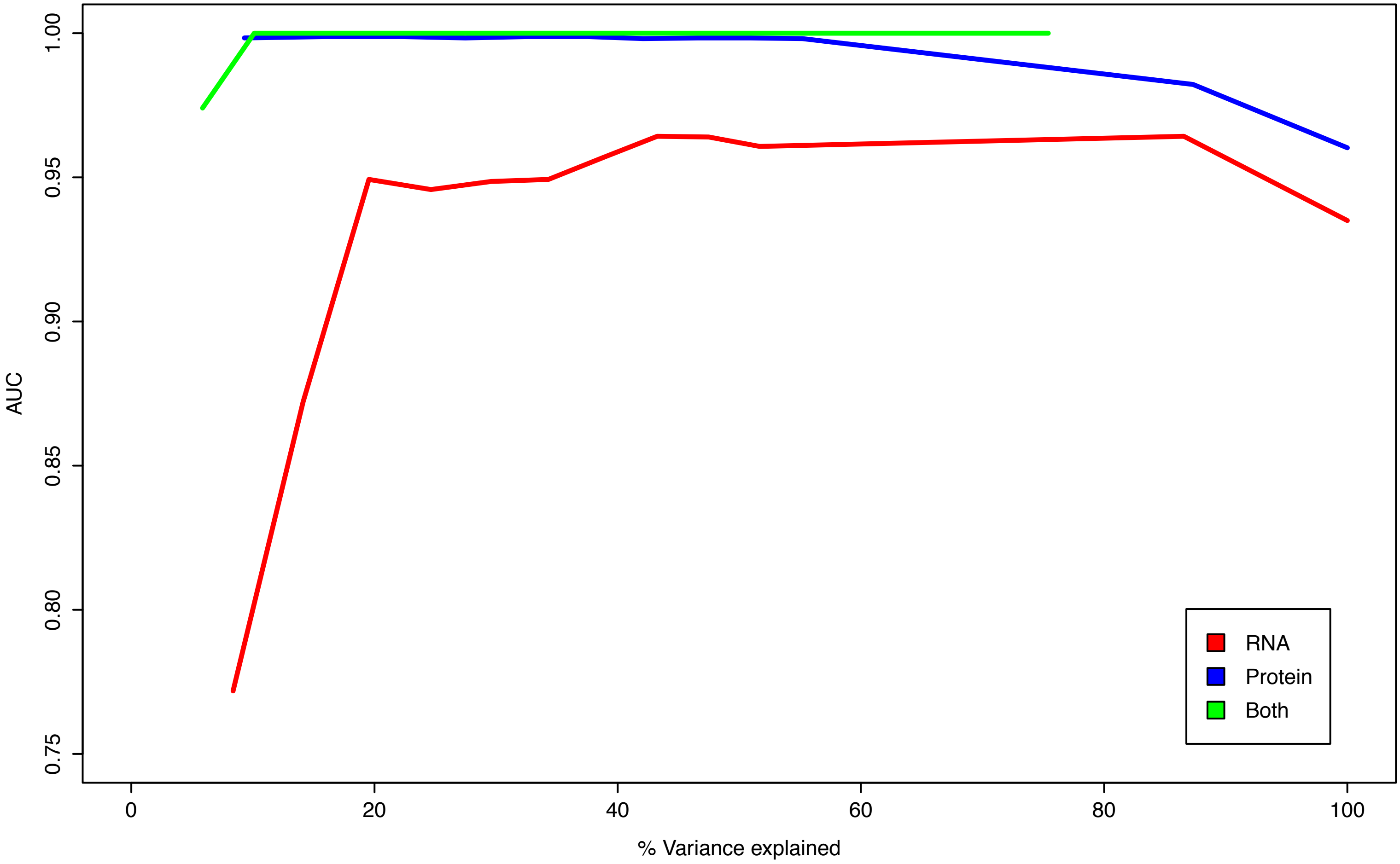
Standard curves for the glioblastoma single cell protein panel using HGCC U3035MG cell lysates. Four data points are shown for each curve corresponding to 0 (blank),10,100 and 1000 cells. The circle and triangle data points represent biological replicates. The red horizontal bar denotes the mean background value whereas the dashed lines are the mean +/-3SD. Y-axis values represent extension control normalized Ct values. Certain assays (e.g. VIM) displayed a "hook" effect. The effect is evident when concentration of the target protein exceeds a threshold at which most PEA probes occupy separate target molecules as opposed to the same one. This results in reduction of signal due to a reduction in the number of proximal events.



Supplemental Figure 2, Related to Figure 2. a) Correlation plot between protein and RNA of all cells (n=210) for all common protein and RNA (n=22). b) Coefficient of determination, expressed as the fraction of variance in protein levels explained by RNA levels for all common RNA and protein assays measured across all cells.



Supplemental Figure 3, Related to Figure 3: a) Growth kinetics of the low passage GBM U3035MG cells grown in neural stem cell conditions +/- BMP4; b) U3035MG cells grown for 6 days with or without BMP4 (10X magnification). Minimal differences in morphology were detected in the different treatment groups. c) PCA analysis of three populations of U3035MG cells. Included populations are i) treated cells after 6 days of culture (yellow), ii) untreated cells after 6 days of culture (red) and iii) untreated cells at baseline (green). d) FACS analysis of TNC and TUBB3 on U3035MG cells. Cells were either treated or untreated with BMP4 for 6 days. Acquired events (n=10000) were gated on FCS-A and SSC-A, followed by doublet discrimination using FCS-H and FCS-W. Gating positive events on the 488nm and 633nm channel was performed using a negative control that contained only the fluorescently labelled secondary antibody.



Supplemental Figure 4, Related to Figure 4. AUC values derived after performing LDA on different number of principal components explaining increasing amount of variance using single cells at day 6 treated or untreated with BMP4. PCA was performed using information from RNA (red), protein (blue) or both analytes (green).

Cell Reports

Supplemental Information

Simultaneous Multiplexed Measurement of RNA and Proteins in Single Cells

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