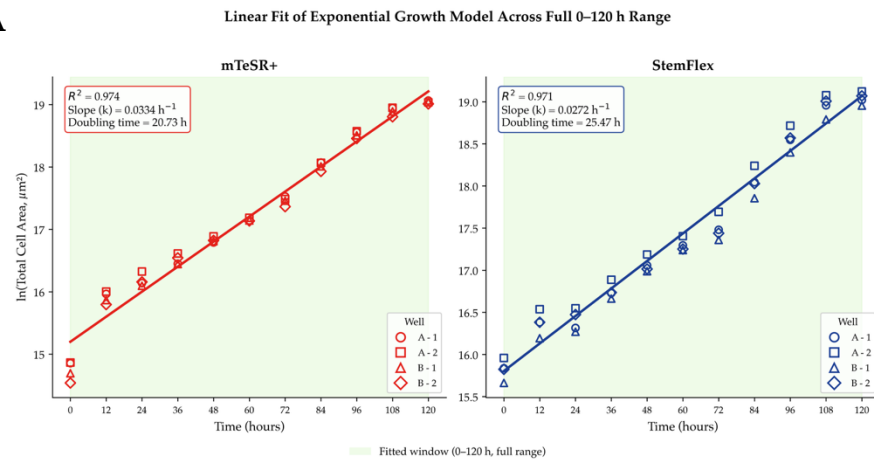
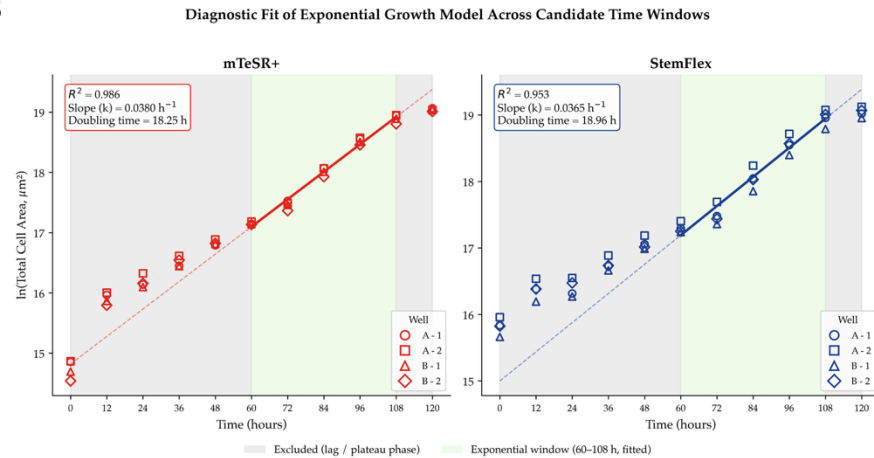


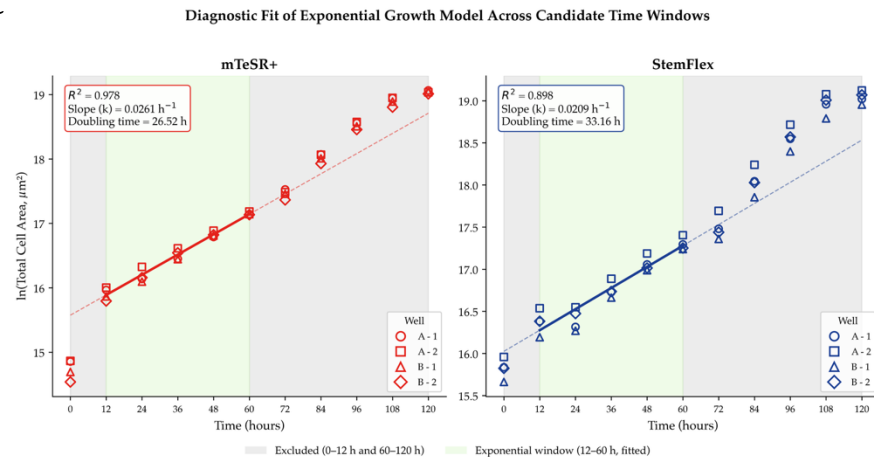
A



B

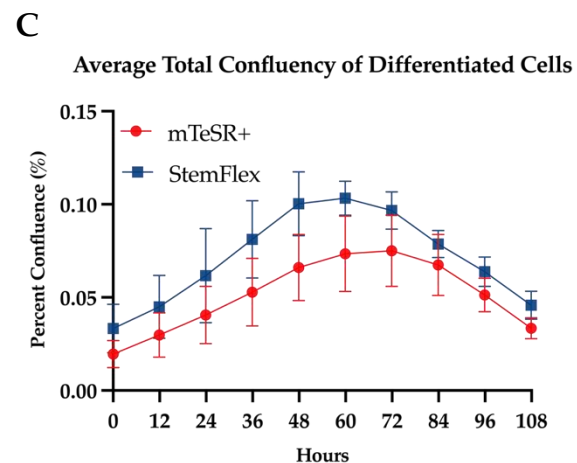
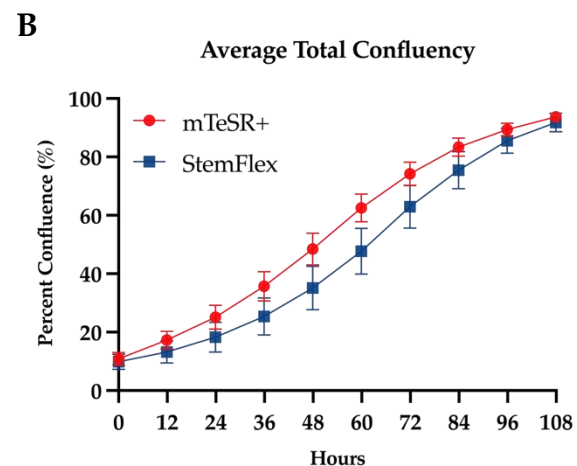
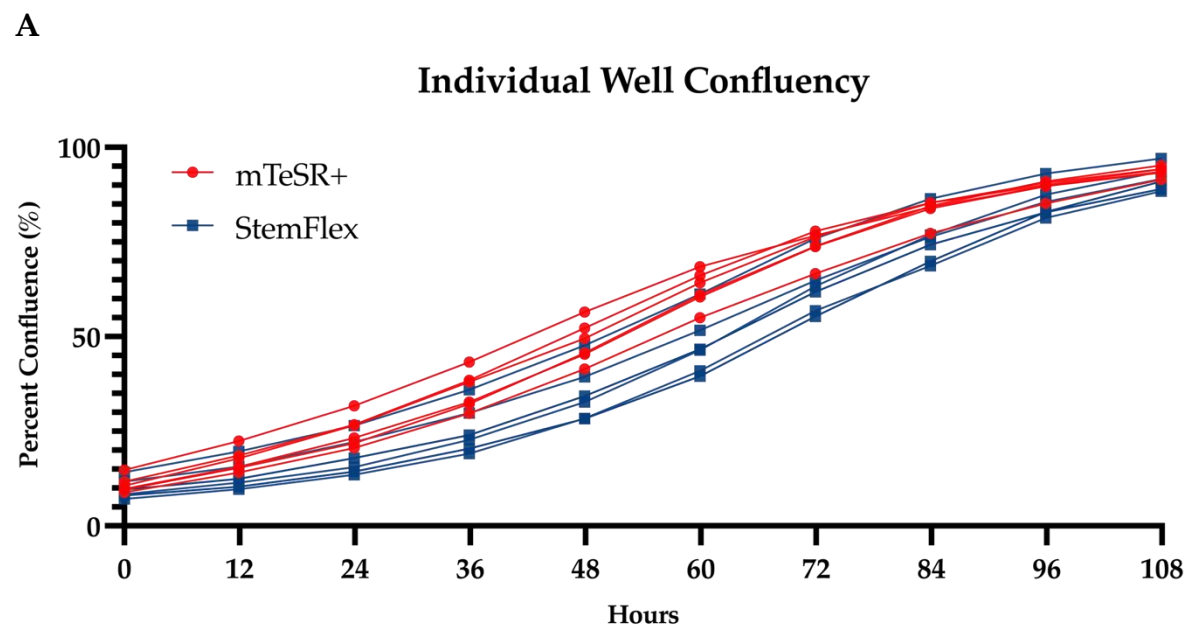


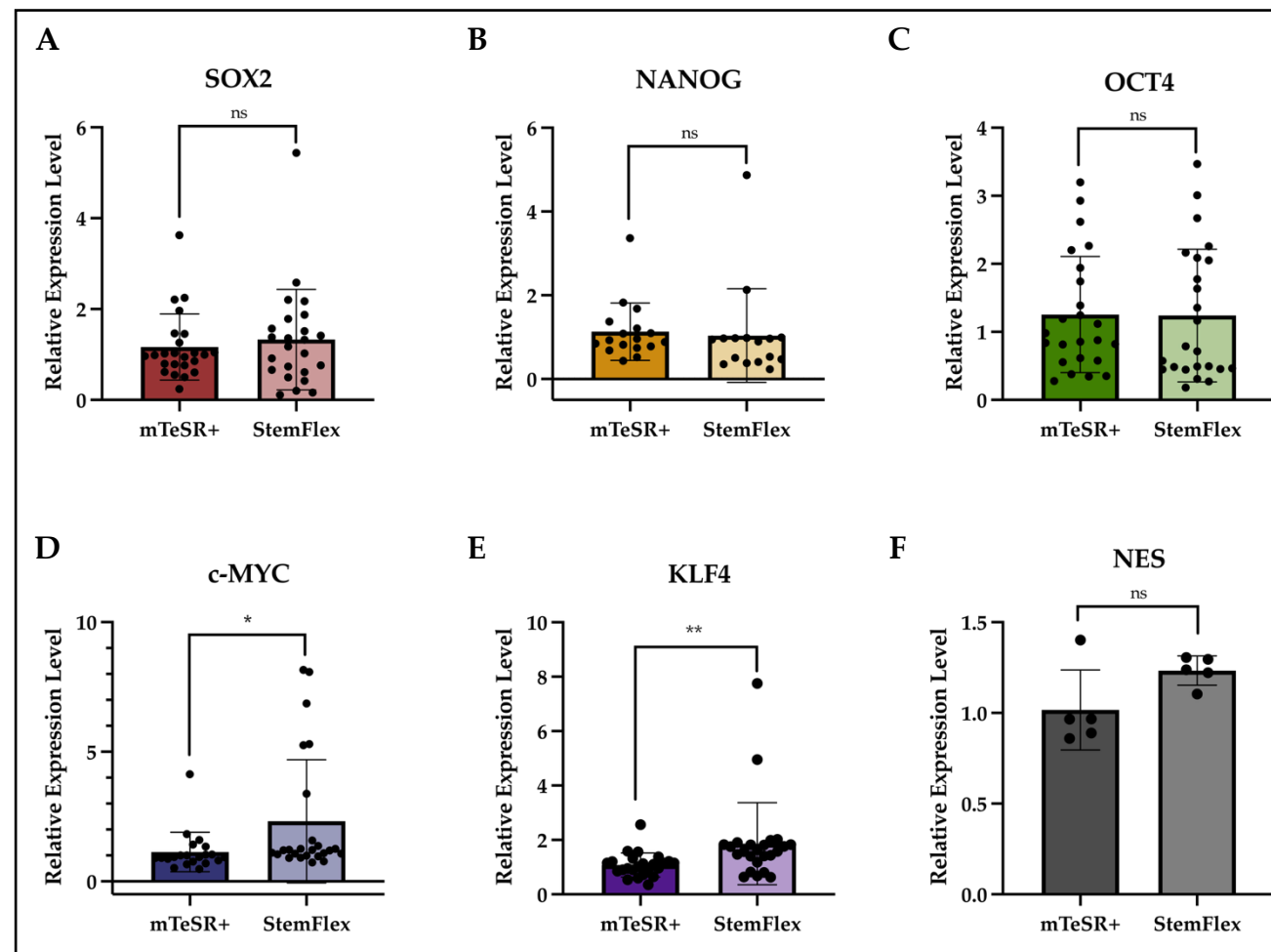
C



Supplementary Figure S1. Diagnostic assessment of linear regression fit supporting selection of the exponential growth window. Total cell area (μm^2) was log-transformed and plotted against time for each biological replicate well cultured in mTeSR+ (red) or StemFlex (blue) medium. Ordinary least squares linear regression was fit to three candidate time windows to evaluate goodness of fit. For each panel, the fitted regression line is displayed as a solid line within the fitted window (shaded region) and extrapolated outside this window for visual reference (dashed line). Individual markers indicate distinct biological replicate wells (A-1, A-2, B-1, B-2). (A) Full 0–120 h range: mTeSR+ ($R^2 = 0.974$, slope = 0.0334 h^{-1} , doubling time = 20.73 h); StemFlex ($R^2 = 0.971$, slope = 0.0272 h^{-1} , doubling time = 25.47 h). (B) 60–108 h window: mTeSR+ ($R^2 = 0.986$, slope = 0.0380 h^{-1} , doubling time = 18.25 h); StemFlex ($R^2 = 0.953$, slope = 0.0365 h^{-1} , doubling time = 18.96 h). (C) 12–60 h window: mTeSR+ ($R^2 = 0.978$, slope = 0.0261 h^{-1} , doubling time = 26.52 h); StemFlex ($R^2 = 0.898$, slope = 0.0209 h^{-1} , doubling time = 33.16 h).

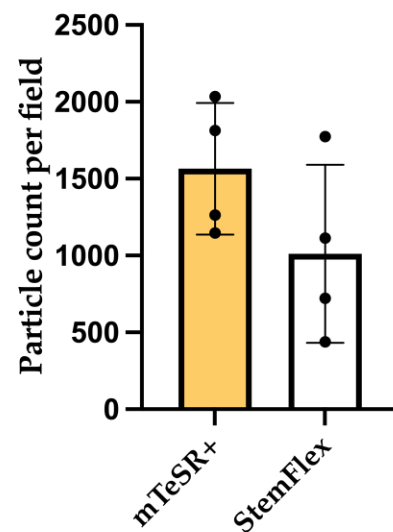
Supplementary Figure S2. Secondary quantitative analysis of hiPSC growth kinetics and differentiated cell burden in mTeSR+ and StemFlex media measured by automated live-cell imaging. iP11N cells were maintained in mTeSR+ (red, circles) or StemFlex (blue, squares) medium and imaged every 12 hours over 108 hours using the CellXpress.ai automated imaging platform at 4× magnification. Machine learning-based segmentation models were applied in IN Carta to quantify cellular growth and differentiation-associated metrics. **(A)** Individual well confluence trajectories for biological replicates cultured in mTeSR+ and StemFlex conditions. **(B)** Total confluence (%) over time. **(C)** Mean total confluence (%) of differentiated cells over time. Data are presented as mean ± SD.



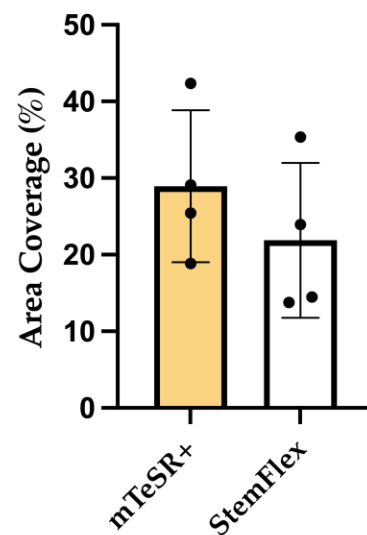


Supplementary Figure S3. Quantitative RT-qPCR analysis of pluripotency and neural marker gene expression in iP11N hiPSCs cultured in StemFlex or mTeSR+ medium prior to automated culture. Relative gene expression levels were normalized to a standardized amount of cDNA input. Data are presented as mean $\pm$ SD with individual data points shown. As several genes did not meet the assumption of a Gaussian distribution, statistical comparisons were performed using the Mann-Whitney U test, with resulting p-values corrected for multiple comparisons across the six genes assessed using the Holm-Bonferroni method; ns = not significant. **(A)** SOX2 expression (mTeSR+ n=24, StemFlex n=24; adjusted p=1.000, ns). **(B)** NANOG expression (mTeSR+ n=17, StemFlex n=16; adjusted p=0.781, ns). **(C)** OCT4 expression (mTeSR+ n=24, StemFlex n=24; adjusted p=1.000, ns). **(D)** c-MYC expression (mTeSR+ n=21, StemFlex n=25; adjusted p=0.032, *). **(E)** KLF4 expression (mTeSR+ n=24, StemFlex n=24; adjusted p=0.007, **). **(F)** NES expression (mTeSR+ n=5, StemFlex n=5; adjusted p=0.603, ns). Statistically significant differences in expression were detected for c-MYC and KLF4, with all other genes remaining non-significant between culture conditions after correction for multiple comparisons.

A. SOX2/GFP Particle Count



B. SOX2/GFP Area Coverage



Supplementary Figure S4. Quantitative ImageJ analysis of SOX2-positive particle count and area coverage in iP11N hiPSCs cultured in mTeSR+ and StemFlex media. Representative 20× ICC images from four wells per culture condition were analyzed using ImageJ. The SOX2/GFP channel was segmented using Otsu automatic thresholding followed by particle analysis. **(A)** SOX2-positive particle count per field and **(B)** percentage area (%Area) were quantified for mTeSR+ and StemFlex cultures. Data are presented as individual well values with mean $\pm$ SD ($n = 4$ wells per condition). StemFlex cultures showed a mean particle count of $1,012 \pm 579$ per field and mean area coverage of $21.88 \pm 10.11\%$, compared with $1,565 \pm 428$ particles per field and $28.94 \pm 9.90\%$ area coverage for mTeSR+ cultures.