# Validation of Common Housekeeping Genes as Reference for qPCR Gene Expression Analysis During iPS Reprogramming Process

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## Supplementary Tables

| Cell type | Gene | Pair # | Primer sequence | Slope | E (%) | $R^2$ |
|---|---|---|---|---|---|---|
| Fully reprogrammed iPS cells | Gusb | 1 | F: AACAACACACTGACCCCTCA<br>R: ACCACAGATCGATGCAGTCC | 1.054 | 93 | 0.953 |
| | Gusb | 2 | F: TGGCTGGGTGTGGTATGAAC<br>R: GGTGACCTCCCTCATGTTCC | 0.722 | 161 | 0.931 |
| | Gusb | 3 | F: GGTGGAACATGAGGGAGGTC<br>R: AGGGTATGAGGGGTCAGTGT | 0.926 | 111 | 0.983 |
| | Gusb | 4 | F: GGCCTCTAGATAGCCTTGAGC<br>R: ACACGCACTCCATTTTAGGGA | 0.342 | 660 | 0.736 |
| | Hprt | 1 | F: GTTGGGCTTACCTCACTGCT<br>R: TAATCACGACGCTGGGACTG | 0.526 | 274 | 0.923 |
| | Hprt | 2 | F: GATCAGTCAACGGGGGACAT<br>R: GGTCCTTTTCACCAGCAAGC | 0.529 | 271 | 0.886 |
| | Hprt | 3 | F: ACAGGCCAGACTTTGTTGGA<br>R: ACTTGCGCTCATCTTAGGCT | 0.699 | 170 | 0.962 |
| | Hprt | 4 | F: CAGTCCCAGCGTCGTGATTA<br>R: TGGCCTCCCATCTCCTTCAT | 1.01 | 99 | 0.995 |
| | Tfrc | 1 | F: AAACTGGCTGAAACGGAGGA<br>R: AGATCCAGCCTCACGAGGAG | 1.157 | 82 | 0.997 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Tfrc | 2 | F: AAGAGCTGCTGCAGAAAAGC<br>R: ACGGTCTGGTTCCTCATAACC | 1.049 | 94 | 0.997 |
| | Tfrc | 3 | F: GTTCGTACAGCAGCGGAAGT<br>R: GGAAGTAGTCTCCACGAGCG | 1.073 | 91 | 0.985 |
| | Tfrc | 4 | F: AGCAAAGTCTGGCGAGATGAA<br>R: CCACATAACCCTCGGGAGAC | 1.189 | 79 | 0.989 |
| Parental cells (N31) | Gusb | 1 | F: AACAACACACTGACCCCTCA<br>R: ACCACAGATCGATGCAGTCC | -2.39 | 163 | 0.991 |
| | Gusb | 2 | F: TGGCTGGGTGTGGTATGAAC<br>R: GGTGACCTCCCTCATGTTCC | -1.69 | 289 | 0.988 |
| | Gusb | 3 | F: GGTGGAACATGAGGGAGGTC<br>R: AGGGTATGAGGGGTCAGTGT | -2.32 | 170 | 0.980 |
| | Gusb | 4 | F: GGCCTCTAGATAGCCTTGAGC<br>R: ACACGCACTCCATTTTAGGGA | -1.30 | 486 | 0.955 |
| | Hprt | 1 | F: GTTGGGCTTACCTCACTGCT<br>R: TAATCACGACGCTGGGACTG | -2.23 | 181 | 0.979 |
| | Hprt | 2 | F: GATCAGTCAACGGGGGACAT<br>R: GGTCCTTTTCACCAGCAAGC | -1.49 | 367 | 0.976 |
| | Hprt | 3 | F: ACAGGCCAGACTTTGTTGGA<br>R: ACTTGCGCTCATCTTAGGCT | -1.40 | 423 | 0.960 |
| | Hprt | 4 | F: CAGTCCCAGCGTCGTGATTA<br>R: TGGCCTCCCATCTCCTTCAT | -2.60 | 143 | 0.995 |
| | Tfrc | 1 | F: AAACTGGCTGAAACGGAGGA<br>R: AGATCCAGCCTCACGAGGAG | -3.16 | 107 | 0.987 |
| | Tfrc | 2 | F: AAGAGCTGCTGCAGAAAAGC<br>R: ACGGTCTGGTTCCTCATAACC | -3.02 | 114 | 0.999 |
| | Tfrc | 3 | F: GTTCGTACAGCAGCGGAAGT<br>R: GGAAGTAGTCTCCACGAGCG | -3.08 | 111 | 0.972 |
| | Tfrc | 4 | F: AGCAAAGTCTGGCGAGATGAA<br>R: CCACATAACCCTCGGGAGAC | -3.28 | 102 | 0.991 |

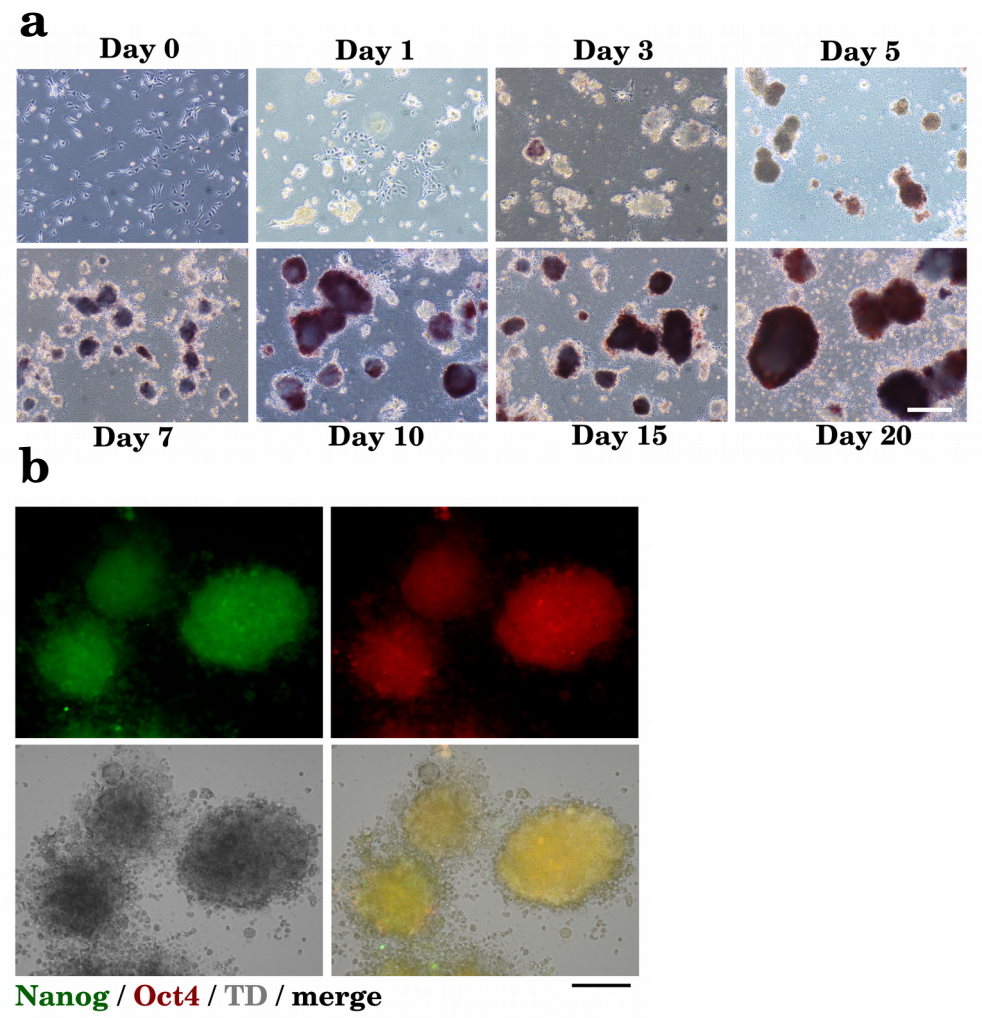
**Supplementary Table S1. Assay performance characteristics on 4 different primer pairs for Gusb, Hprt and Tfrc evaluated in parental cell line and in fully reprogrammed iPS cells.**
PCR efficiency *E*, slope, and associated correlation coefficient $R^2$ are shown. The serial dilutions

in fully reprogrammed iPS cells were twofold.The efficiency for twofold dilutions was calculated using the formula E=2^(1/slope)-1*100. The serial dilutions in parental cells were tenfold, and the formula for calculating efficiency was E=10^(1/slope)-1*100. In fully reprogrammed iPS cells, the primer pair closest to 100% efficiency was #1 for Gusb (E=93%), #4 for Hprt (E=99%), and #2 for Tfrc (E=94%). The efficiency for the same sets of primers differed in parental cells, giving E=163% for Gusb, E=143% for Hprt, and E=114% for Tfrc. The best pair of primers for each gene (Gusb #1, Hprt #4 and Tfrc #2) was chosen for the main experiment.

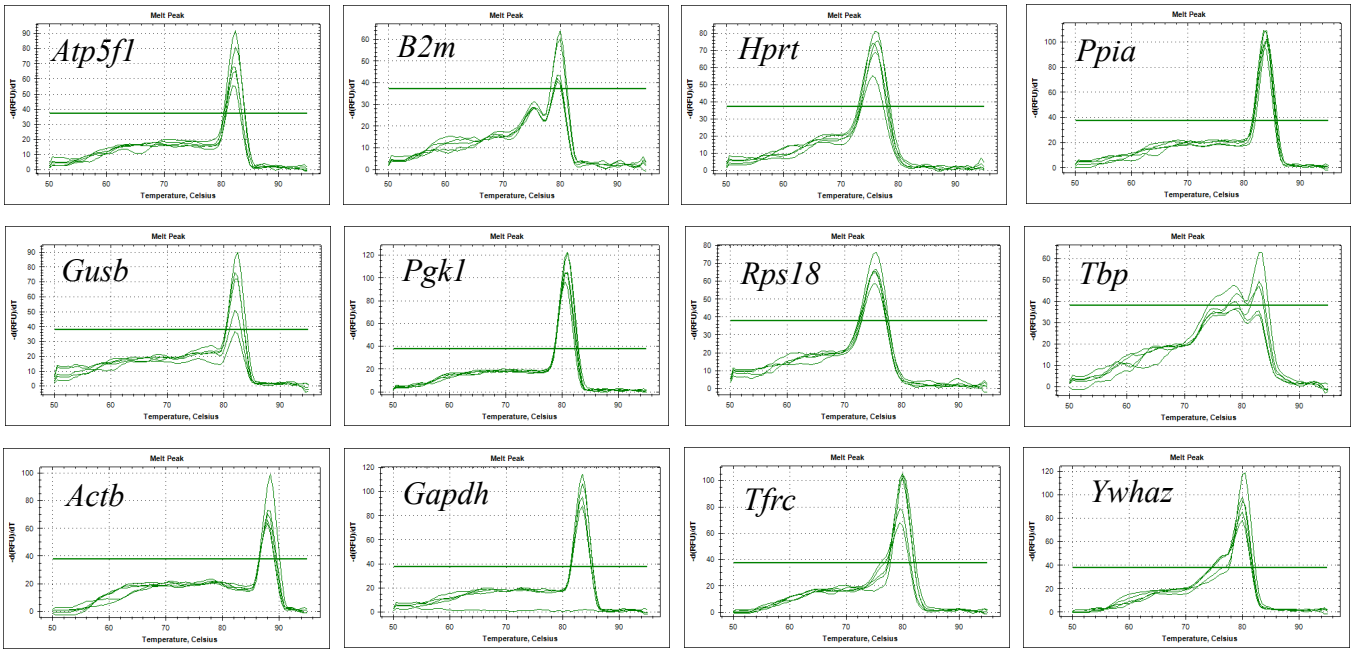
| Gene | Comprehensive Ranking | | Delta Ct | | geNorm | | NormFinder | | BestKeeper | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Value | Rank | SD aver. | Rank | M value | Rank | Stability | Rank | SD | Rank |
| Atp5f1 | 1.57 | 1 | 0.49 | 1 | 0.333 | 5 | 0.099 | 1 | 0.077 | 1 |
| Pgk1 | 1.68 | 2 | 0.50 | 2 | 0.168 | 1 | 0.251 | 2 | 0.247 | 2 |
| Gapdh | 3.44 | 3 | 0.55 | 5 | 0.168 | 1 | 0.417 | 7 | 0.322 | 4 |
| Ppia | 3.66 | 4 | 0.56 | 3 | 0.197 | 2 | 0.353 | 4 | 0.323 | 5 |
| Gusb | 4.53 | 5 | 0.59 | 4 | 0.300 | 4 | 0.311 | 3 | 0.345 | 7 |
| Tbp | 5.18 | 6 | 0.60 | 6 | 0.467 | 7 | 0.400 | 5 | 0.285 | 3 |
| Tfrc | 6.88 | 7 | 0.62 | 7 | 0.253 | 3 | 0.458 | 8 | 0.422 | 10 |
| Ywhaz | 7.20 | 8 | 0.64 | 8 | 0.415 | 6 | 0.414 | 6 | 0.367 | 8 |
| Rps18 | 8.80 | 9 | 0.65 | 10 | 0.532 | 9 | 0.564 | 10 | 0.337 | 6 |
| Hprt | 9.00 | 10 | 0.70 | 9 | 0.501 | 8 | 0.464 | 9 | 0.395 | 9 |
| Actb | 11.00 | 11 | 0.74 | 11 | 0.560 | 10 | 0.631 | 11 | 0.429 | 11 |
| B2m | 12.00 | 12 | 1.03 | 12 | 0.638 | 11 | 0.961 | 12 | 0.879 | 12 |

**Supplementary Table S2. Ranking of the candidate reference genes' stability during reprogramming according to five different evaluation methods in mouse embryonic fibroblasts (MEFs).** Atp5f1, Pgk1 and Gapdh were ranked as the most stable candidate reference genes overall, while Hprt, Actb and B2m were designated as the least stable ones.

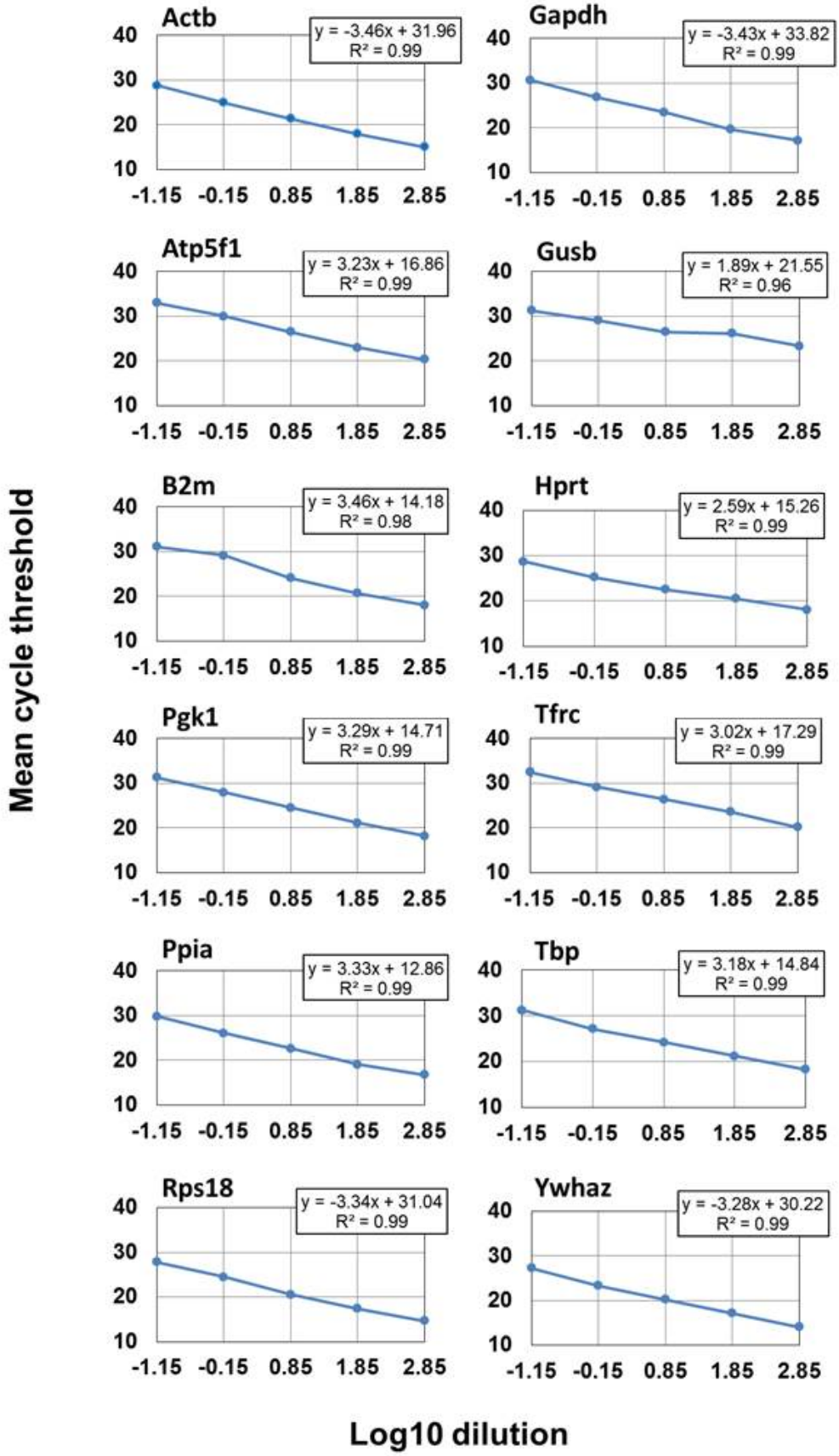
## Supplementary figures



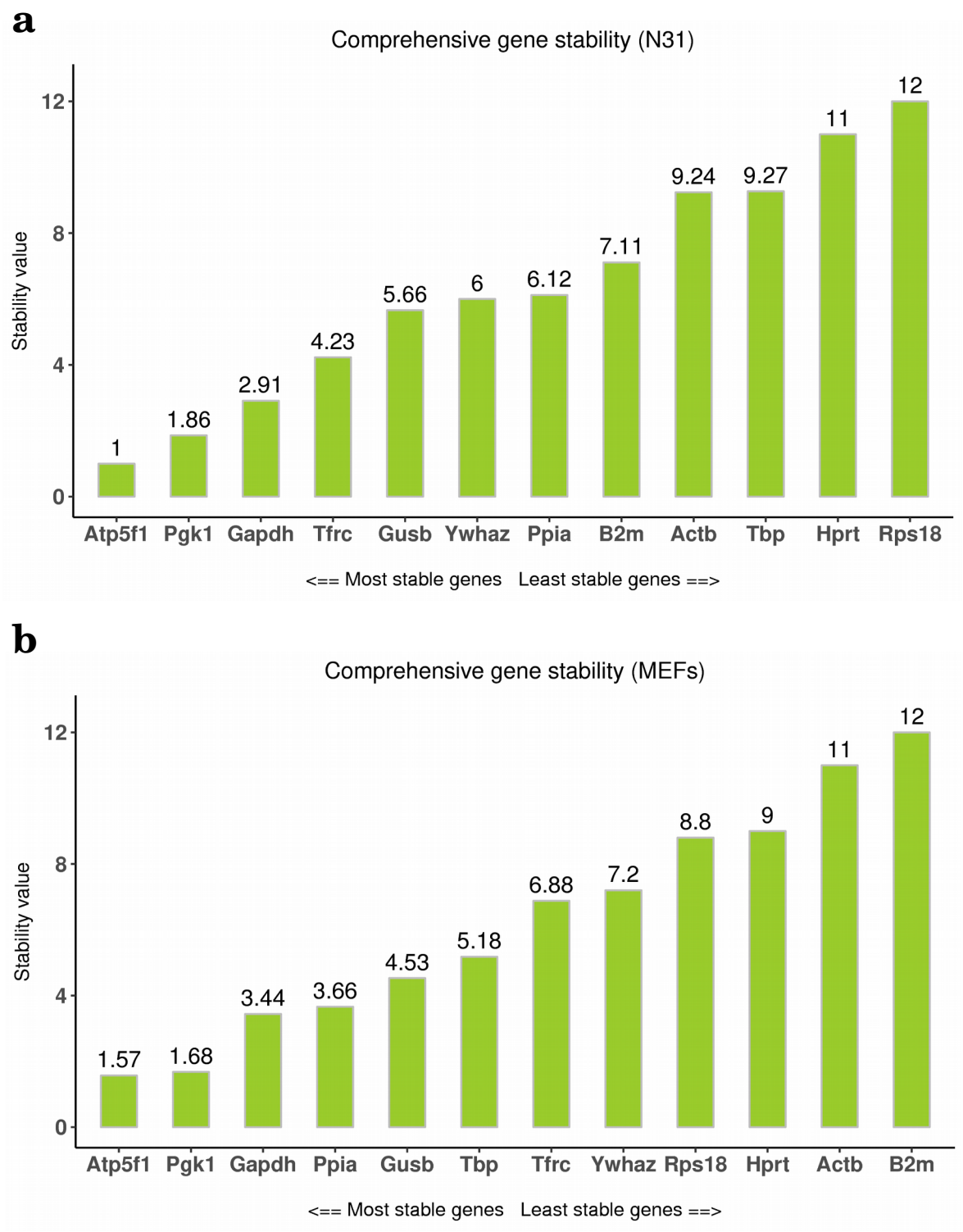

**Supplementary Figure S1. Pluripotency markers check in the reprogramming process of N31 cell line.** (a) Alkaline phosphatase staining of nascent iPS colonies over the course of iPS reprogramming. (b) Immunostaining of fully reprogrammed iPS colonies at day 20 with pluripotency markers anti-Nanog and anti-Oct4.




**Supplementary Figure S2. Melting curves of candidate reference genes.** Curves from log 10 dilution series are included for demonstration.




**Supplementary Figure S3. Log 10 dilution curves of candidate reference genes.** The log 10 dilution values were plotted against cycle threshold values for evaluation of linearity. Correlation coefficients are included for each curve.




**Supplementary Figure S4. Comprehensive stability ranking of candidate reference genes during iPS reprogramming of neural stem cells (N31) or mouse embryonic fibroblasts (MEFs).** The stability is expressed as a comprehensive RefFinder value which is a geometric mean of the overall weight value for four algorithms: Genorm, Normfinder, Bestkeeper and Delta Ct (see Materials and Methods). Atp5, Pgk1 and Gapdh are showing the best stability in both cell lines. The lowest stability values differed in two cell lines, the lowest score belonging to Rps18, Hprt and Tbp in N31 cells (a) and to B2m, Actb and Hprt in MEFs (b).