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**SUPPLEMENTARY MATERIAL**

**corresponding to:**

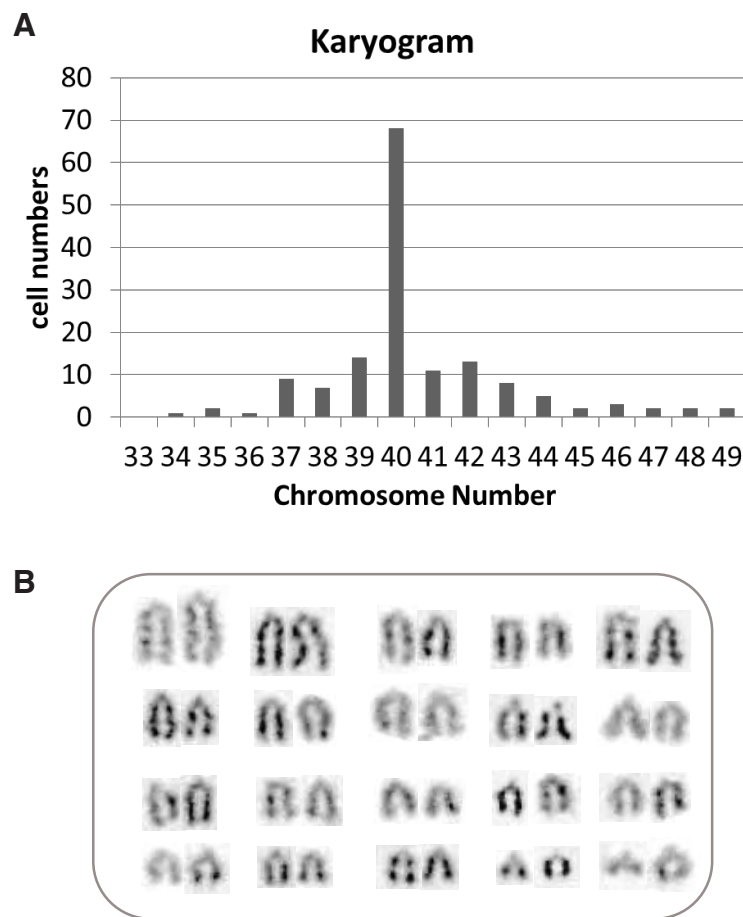
**Long-term serial cultivation of mouse induced pluripotent stem cells in serum-free and feeder-free defined medium**

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**Supplementary Fig. S1. Chromosome analysis of mouse iPS cells maintained in ESF7 at passage 195. (A)** The distribution of chromosome numbers per metaphase is plotted. **(B)** Karyotype analysis was performed with giemsa staining. Almost all of the mouse iPS cells maintained in ESF7 had a normal karyotype.

**Supplementary Movie 1. Beating cardiomyocytes observed following spontaneous differentiation of mouse iPS cells maintained in ESF7.** Photographed with a 400 x objective. (Passage 54). In this paper passage numbers indicate the number of passages after purchase from Riken Bio Resource Center. miPS cells were obtained from Riken Bio Resource Center, at passaged 11. Available at: <http://dx.doi.org/10.1387/ijdb.130173to>

SUPPLEMENTARY TABLE S1

THE PRIMERS USED IN THIS STUDY

| Gene Name         | Primer sequence  | Accession    | Reference          |
|-------------------|--|--------------|--------------------|
| endogenous Sox2   | 5'-TAGAGCTAGACTCCGGGCGATGA-3'<br>5'-TTGCCTTAACAAGACCACGAAA-3'              | NM_011443    | Yamanaka S et al.  |
| endogenous Nanog  | 5'-CAGGTGTTTGAGGGTAGCTC-3'<br>5'-CGGTTTCATCATGGTACAGTC-3'                  | AB093574     | Yamanaka S et al.  |
| endogenous Oct3/4 | 5'-TCTTTCCACCAGGCCCCCGGCTC-3'<br>5'-TGCGGGCGGACATGGGGAGATCC-3'             | NM_013633    | Yamanaka S. et al. |
| Esg-1             | 5'-GAAGTCTGGTTCCTTGGCAGGATG-3'<br>5'-ACTCGATACACTGGCCTAGC-3'               | NM_025274    | Yamanaka S. et al. |
| Nestin            | 5'-AGTCAGGACTCCGGGAAGTT-3'<br>5'-TCTGGATCCACCTTTTCTGGA-3'                  | NM_016701    | Aihara Y. et al.   |
| Otx2              | 5'-CGACGTTCTGGAAGCTCTGTTTGCCAAGAC-3'<br>5'-TGGCAGGCTCACTTTGTCTGACCTCCAT-3' | NM_144841    |                    |
| AP-2              | 5'-CAGAGGGGCAAATCCGATCA-3'<br>5'-GGCATTAGGGGTGTGGGACA-3'                   | NM_001122948 | Aihara Y. et al.   |
| FGF-5             | 5'-CAGGGGATTGTAGGAATACGAGGAG-3'<br>5'-ACTCTCGGCCTGTCTTTTCAGTTCT-3'         | NM_010203    | Aihara Y. et al.   |
| Musashi           | 5'-GTTTCGGGGAGGTGAAAGAGT-3'<br>5'-CTTGGGGTCAATTGTTTTGG-3'                  | NM_008629    | Aihara Y. et al.   |
| Snail             | 5'-CTTGTGTCTGCAGCACCTGT-3'<br>5'-CTTCACATCCGAGTGGGTTT-3'                   | NM_011427    | Aihara Y. et al.   |
| Slug              | 5'-AACATTTCAACGCCTCCAAG-3'<br>5'-GCCGACGATGCCATACAGT-3'                    | NM_011415    | Aihara Y. et al.   |
| Nkx2.5            | 5'-CAGTGGAGCTGGACAAAGCC-3'<br>5'-TAGCGACGGTTCTGGAACCA-3'                   | NM_008700    | Makino S. et al.   |
| GATA-4            | 5'-CTGTCATCTCACTATGGGCA-3'<br>5'-CCAAGTCCGAGCAGGAATTT-3'                   | NM_008092    | Makino S. et al.   |
| MEF-2c            | 5'-AGCAAGAATACGATGCCATC-3'<br>5'-GAAGGGGTGGTGGTACGGTC-3'                   | NM_001170537 | Makino S. et al.   |
| αMHC              | 5'-GGAAGAGTGAGCGGCATCAAGG-3'<br>5'-CTGCTGGAGGTTATTCCTCG-3'                 | M38127       | Makino S. et al.   |
| GAPDH             | 5'-ACCCAGAAGACTGTGGATGG-3'<br>5'-CACATTGGGGTAGGAACAC-3'                    | NM_008084    | Aihara Y. et al.   |

MEF, muscle enhancement factor; MHC, myosin heavy chain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.