**Protein profiling identified key chemokines that regulate the maintenance of human pluripotent stem cells**

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# Supplemental figures

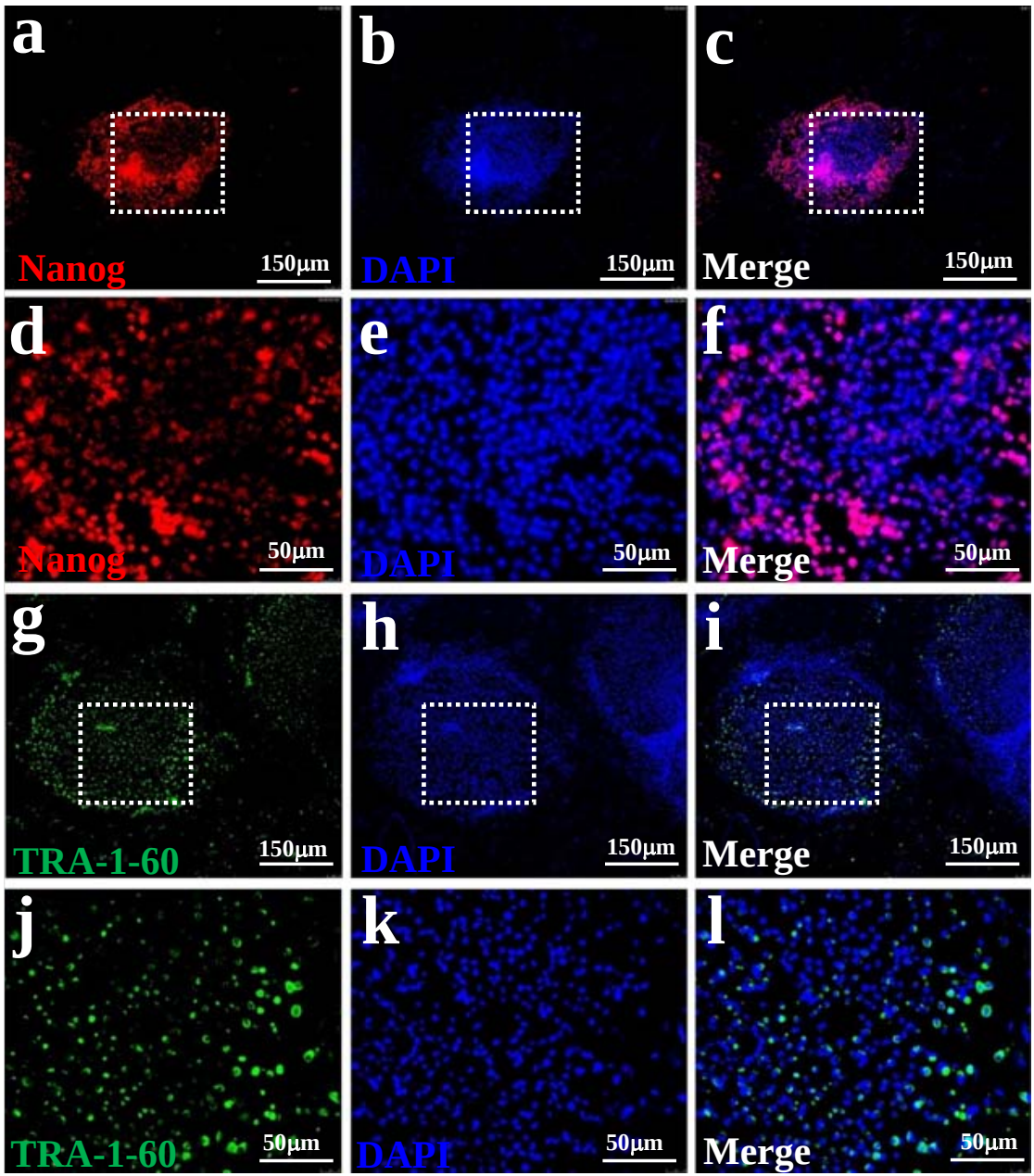


**Figure S1. Protein expressions of Nanog and TRA-1-60 on hESCs cultured on feeder layers in KSR medium. (a-l)** Immunostaining of pluripotency markers Nanog and TRA-1-60 in hESCs-derived clones. Nanog (**a**, **d**); TRA-1-60 (**g**, **j**); DAPI (**b**, **e**, **h**, **k**); and Merge (**c**, **f**, **i**, **l**). Scale bars, 150 μm (**a-c**, **g-i**) and 50 μm (**d-f**, **j-l**).

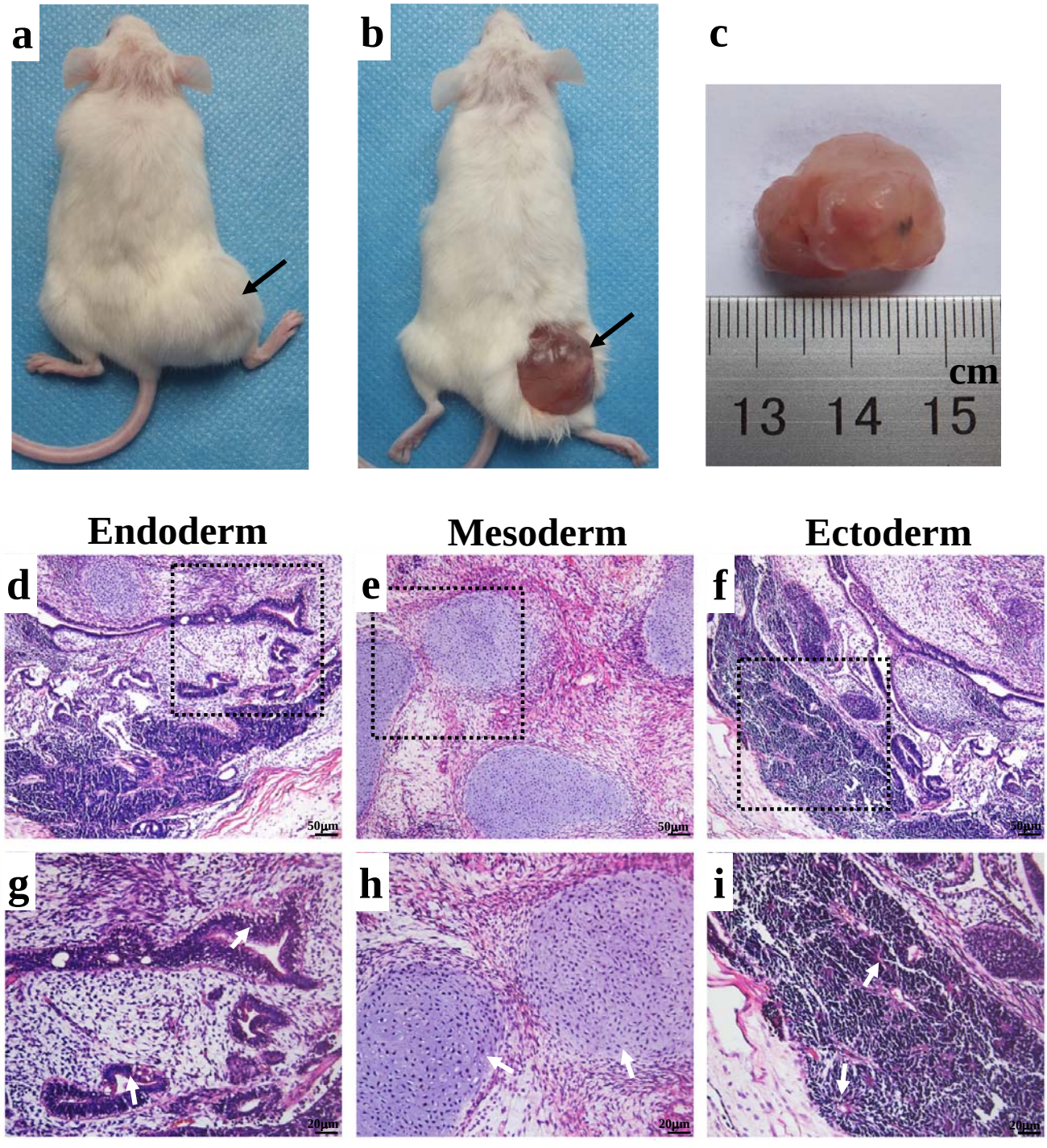


**Figure S2. Differentiation of three germ layers of hESCs *in vivo*.** (**a-c**) Teratomas (arrows) formed after hESCs were subcutaneously transplanted into NOD SCID mice for seven weeks. (**d-h**) Histopathological analysis of three germ layers of teratoma derived from hESCs by hematoxylin and eosin staining. Endoderm (**d**, **g**); mesoderm (**e**, **h**); ectoderm (**f**, **i**). Scale bars, 50 μm (**d**, **e**, **f**) and 20 μm (**g**, **h**, **i**).

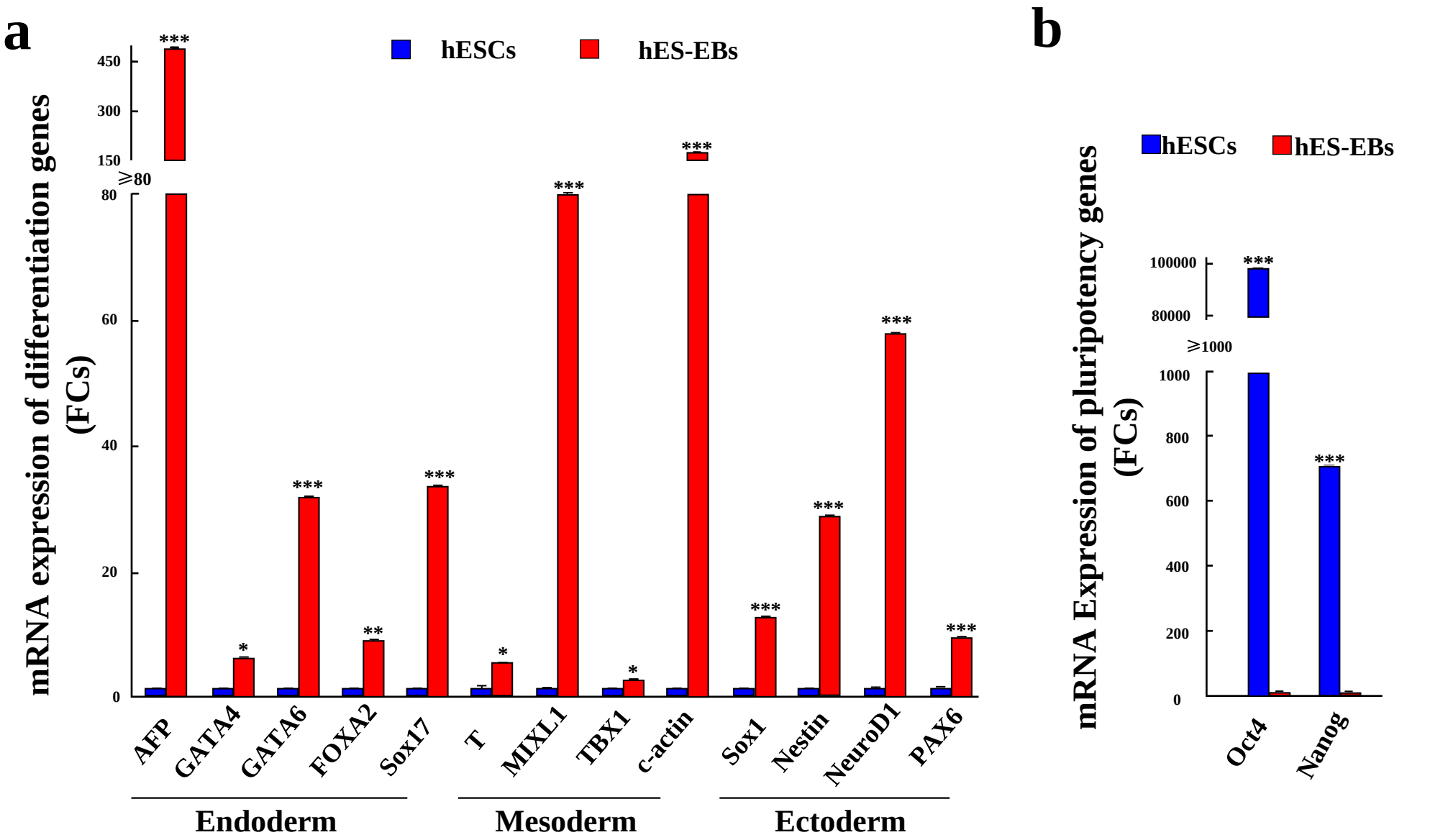


**Figure S3. Quantitative PCR analysis of three germ layers and pluripotency gene markers in hESC-derived embryonic bodies (EBs).** (**a**) *AFP*, *GATA4*, *GATA6*, *FOXA2*, and *Sox17* (endoderm); *T*, *MIXL1*, *TBX1*, and *c-actin* (mesoderm); *Sox1*, *Nestin*, *NeuroD1*, and *PAX6* (ectoderm). (**b**) *Oct4* and *Nanog*. Values on graphs represent means ± sem, n ≧ 3 individual experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

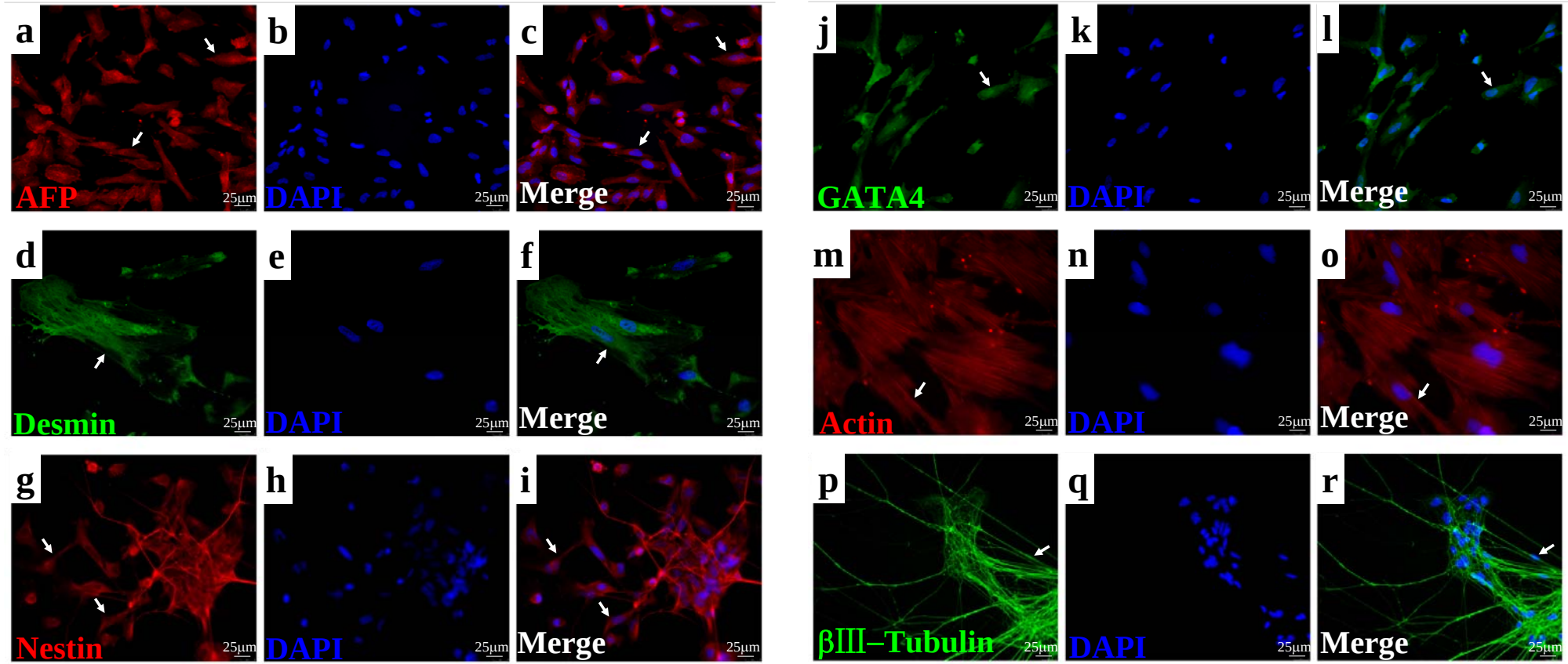


**Figure S4. Immunostaining of three germ layer protein markers in EBs.** (**a-r**) Endoderm (AFP, **a-c** and GATA4, **j-l**); Ectoderm (desmin, **d-f** and actin, **m-o**); Ectoderm (nestin, **g-i** and β-III tubulin, **p-r**). Cells were counterstained by DAPI. Scale bar, 25 μm.

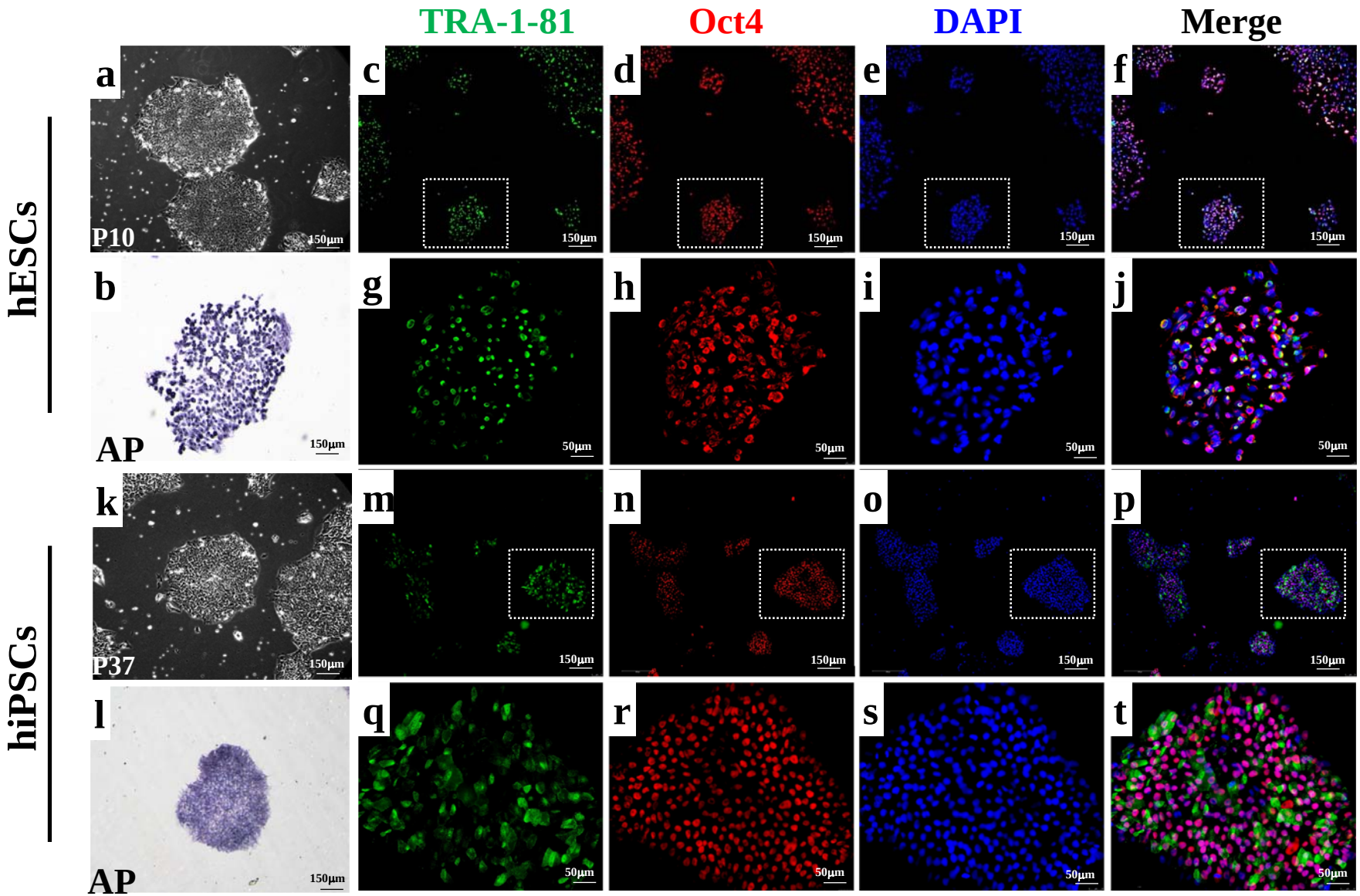


**Figure S5. Maintenance of hESCs and hiPSCs in mTeSR1 medium. (a-j)** hESC-derived clones. Scale bar, 150 mm. **(k-t)** hiPSC-derived clones. Both hESCs and hiPSCs were cultured in mTeSR1 medium. TRA-1-81 (**c**, **g**, **m**, **q**); Oct4 (**d**, **h**, **n**, **r**); DAPI (**e**, **i**, **o**, **s**); and Merge (**f**, **j**, **p**, **t**). Scale bars, 150 μm (**c-f**, **m-p**) and 50 μm (**g-j**, **q-t**).

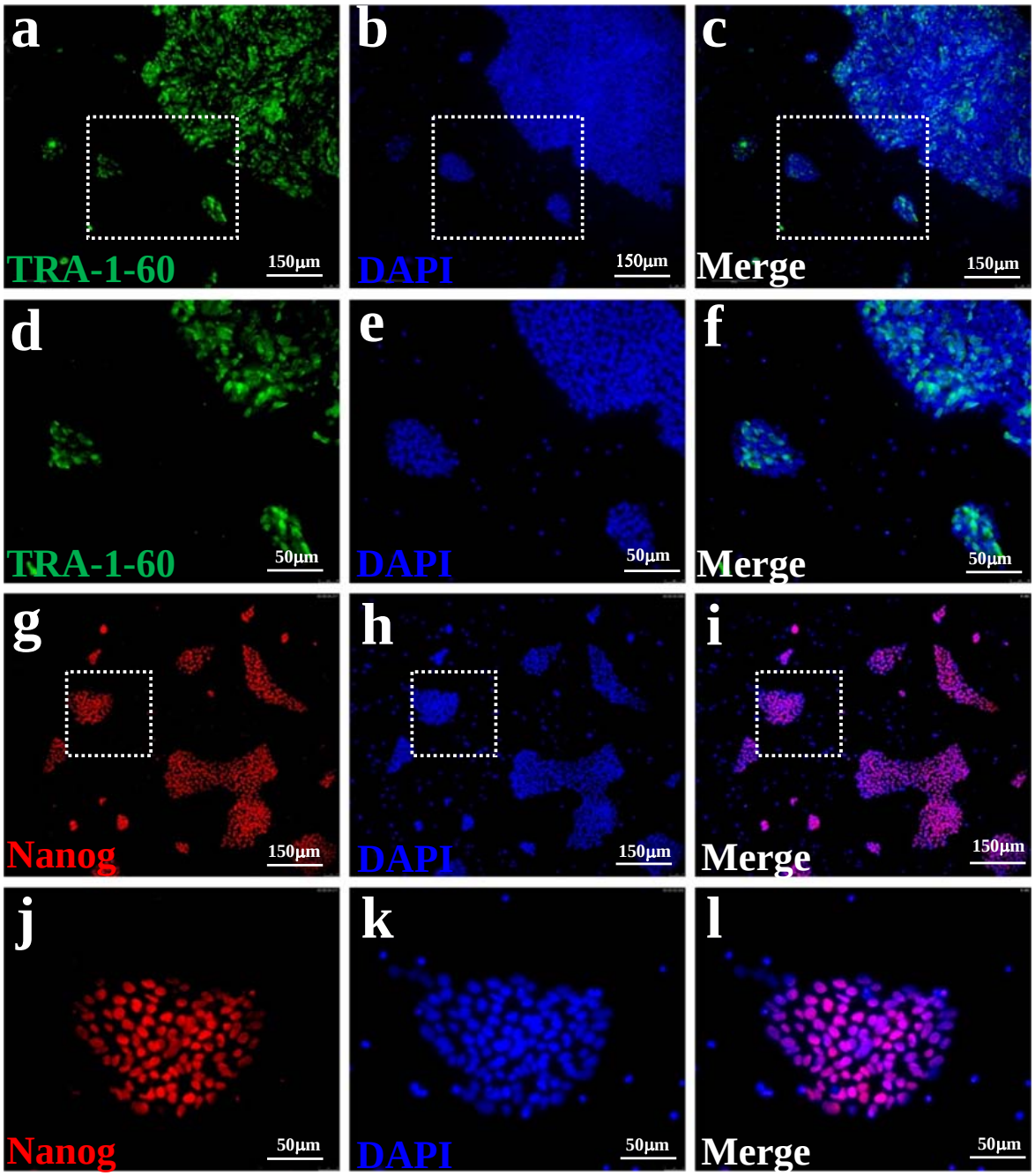


**Figure S6. Protein expressions of Nanog and TRA-1-60 in hESCs cultured in mTeSR1 medium. (a-l)** Immunostaining of pluripotency markers Nanog and TRA-1-60 in hESCs-derived clones. Nanog (**a**, **d**); TRA-1-60 (**g**, **j**); DAPI (**b**, **e**, **h**, **k**); and Merge (**c**, **f**, **i**, **l**). Scale bars, 150 μm (**a-c**, **g-i**) and 50 μm (**d-f**, **j-l**).

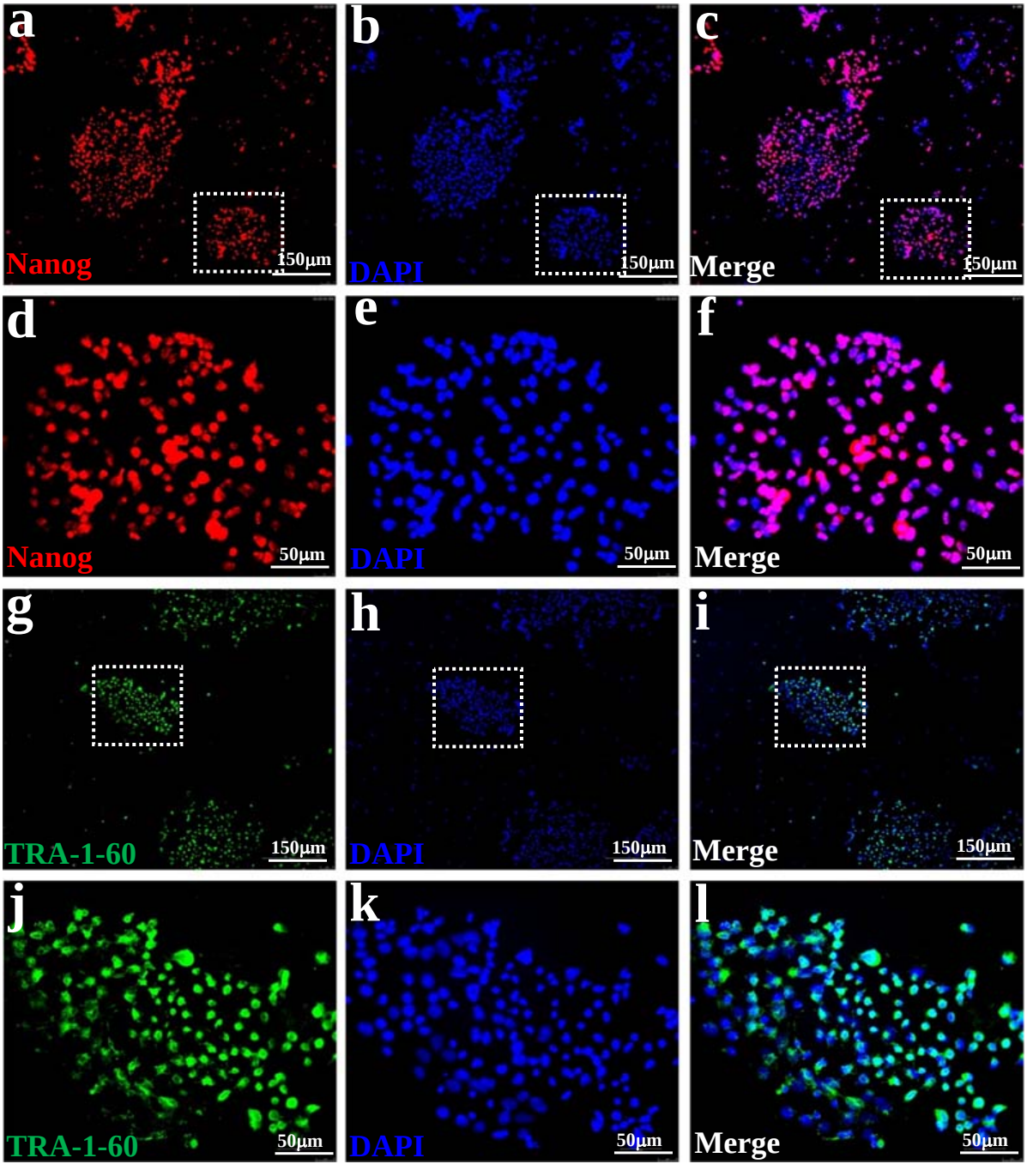


**Figure S7. Protein expressions of Nanog and TRA-1-60 in hiPSCs cultured in mTeSR1 medium. (a-l)** Immunostaining of pluripotency markers Nanog and TRA-1-60 in hESCs-derived clones. Nanog (**a**, **d**); TRA-1-60 (**g**, **j**); DAPI (**b**, **e**, **h**, **k**); and Merge (**c**, **f**, **i**, **l**). Scale bars, 150 μm (**a-c**, **g-i**) and 50 μm (**d-f**, **j-l**).

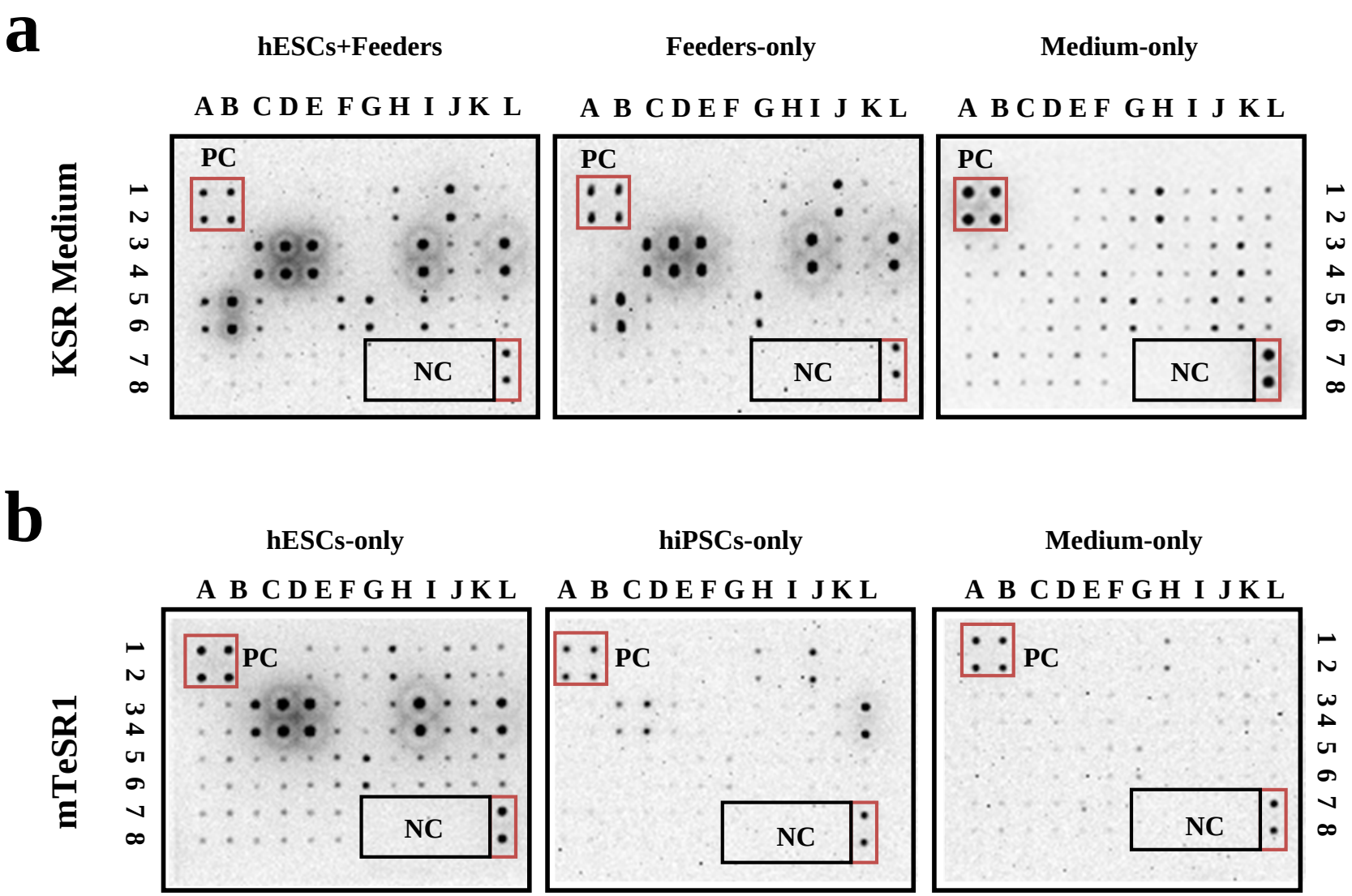


**Figure S8. Signal intensities of chemokines in protein arrays. (a-b)** Chemokines from six different supernatants were evaluated. KSR medium (**a**); mTeSR1 medium (**b**); Positive controls (PCs); Negative controls (NCs). Relative signal intensity (RSI) was defined as per the normalized grey intensities listed in Table 1.

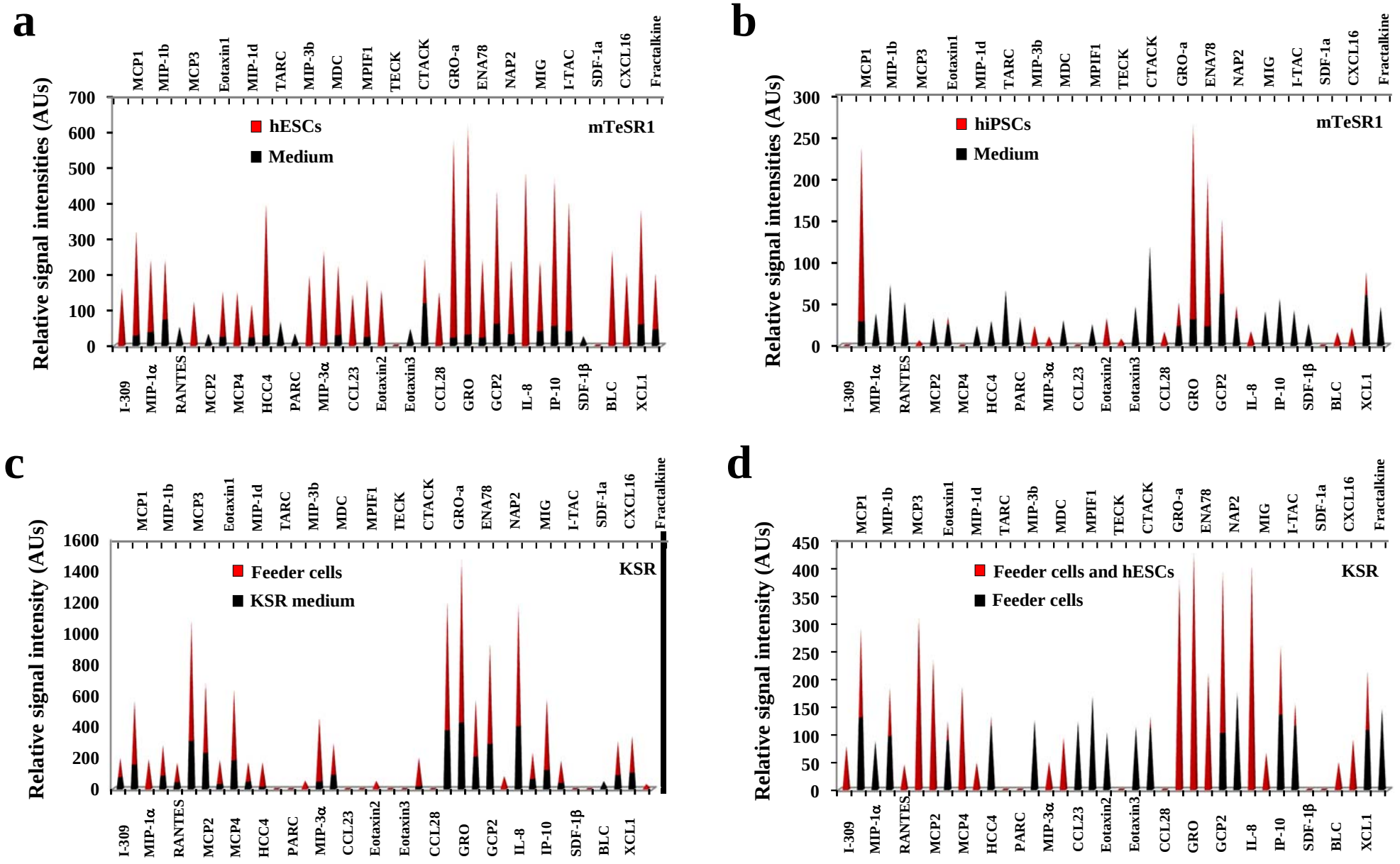


**Figure S9. Comparison of chemokine secretion of hPSCs under different conditions.** hESCs vs mTeSR1 medium (**a**); hiPSCs vs mTeSR1 medium (**b**); Feeder cells vs KSR medium (**c**); and Feeder cells/hESCs vs KSR medium (**d**). AUs, arbitrary units.

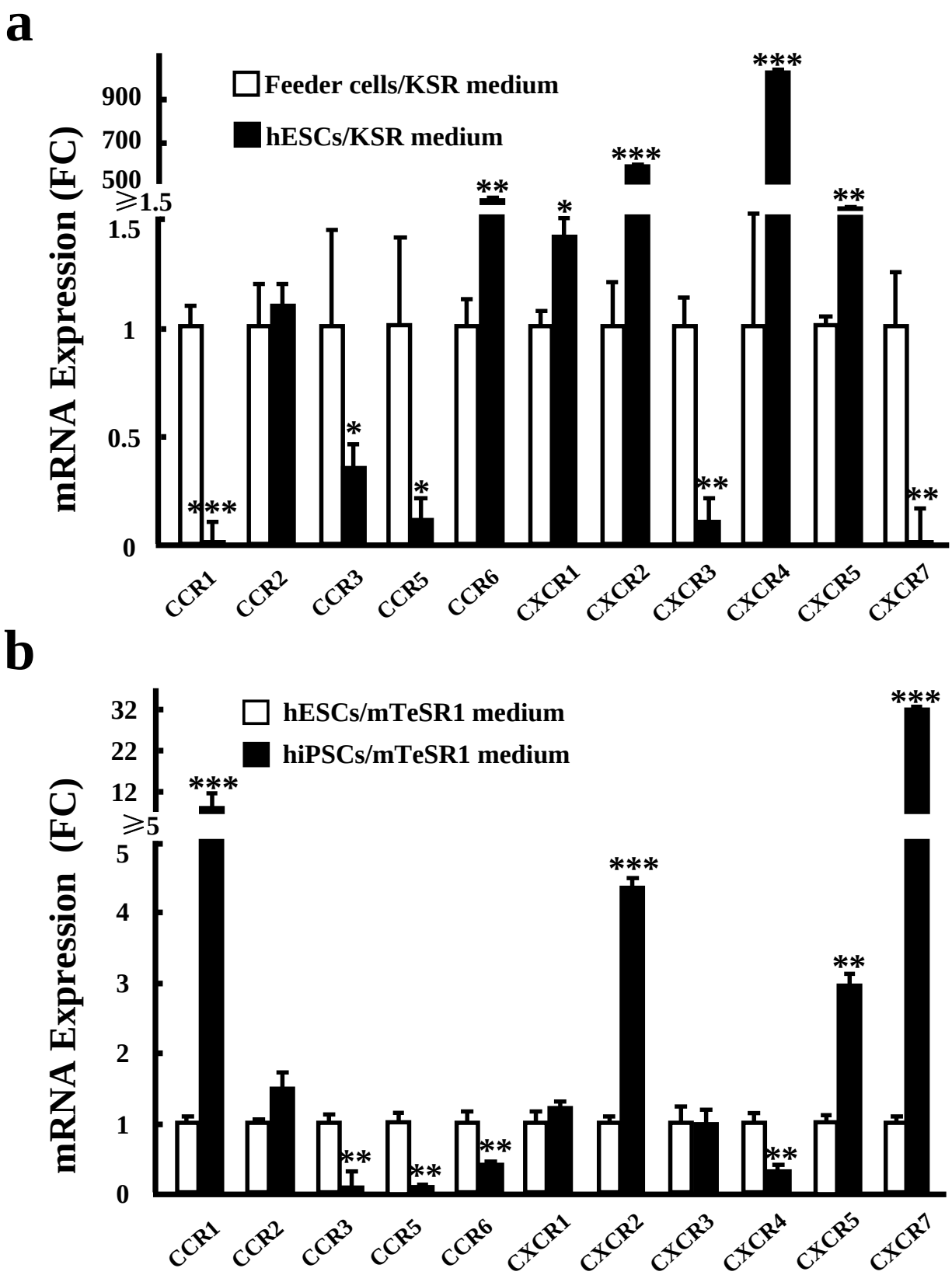


**Figure S10. mRNA expression of CC/CXC chemokine receptors in hPSCs.**

**(a)** Feeder cells vs hESCs in KSR medium; **(b)** hESCs vs hiPSCs in mTeSR1 medium. Values on graphs represent means ± sem; FC, fold change; n = 3 individual experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

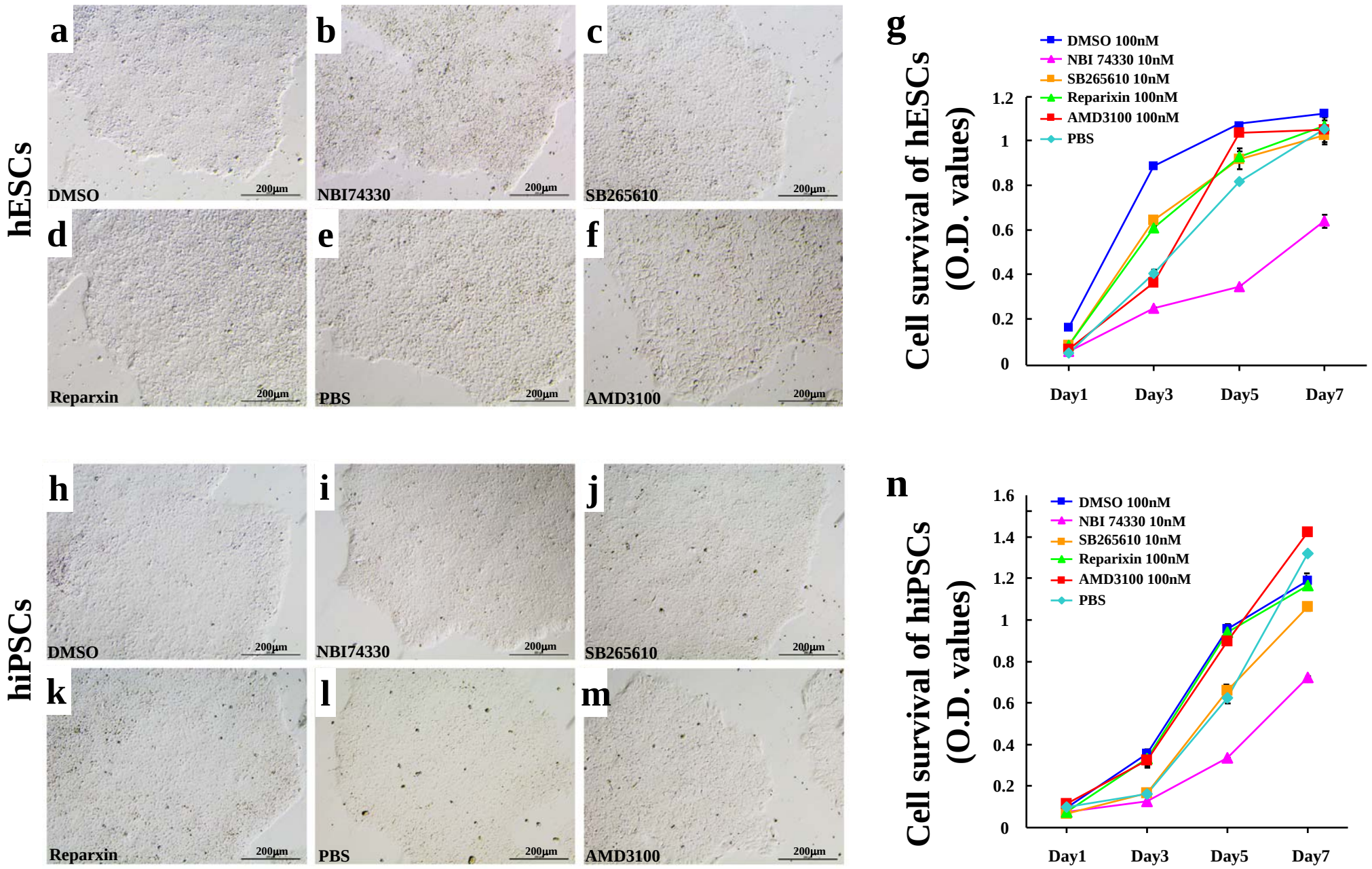


**Figure S11. Effects of antagonists on both hESCs and hiPSCs.** (**a-f**, **h-m**) Phase microscope images of hPSCs treated with several receptor-specific antagonists for 7 d. hESCs (**a-f**); hiPSCs (**h-m**). Scale bar, 200 μm. (**g**, **n**) MTT assay of hPSC survival and toxicity responses to receptor-specific antagonists. hESCs (**g**); hiPSCs (**n**).

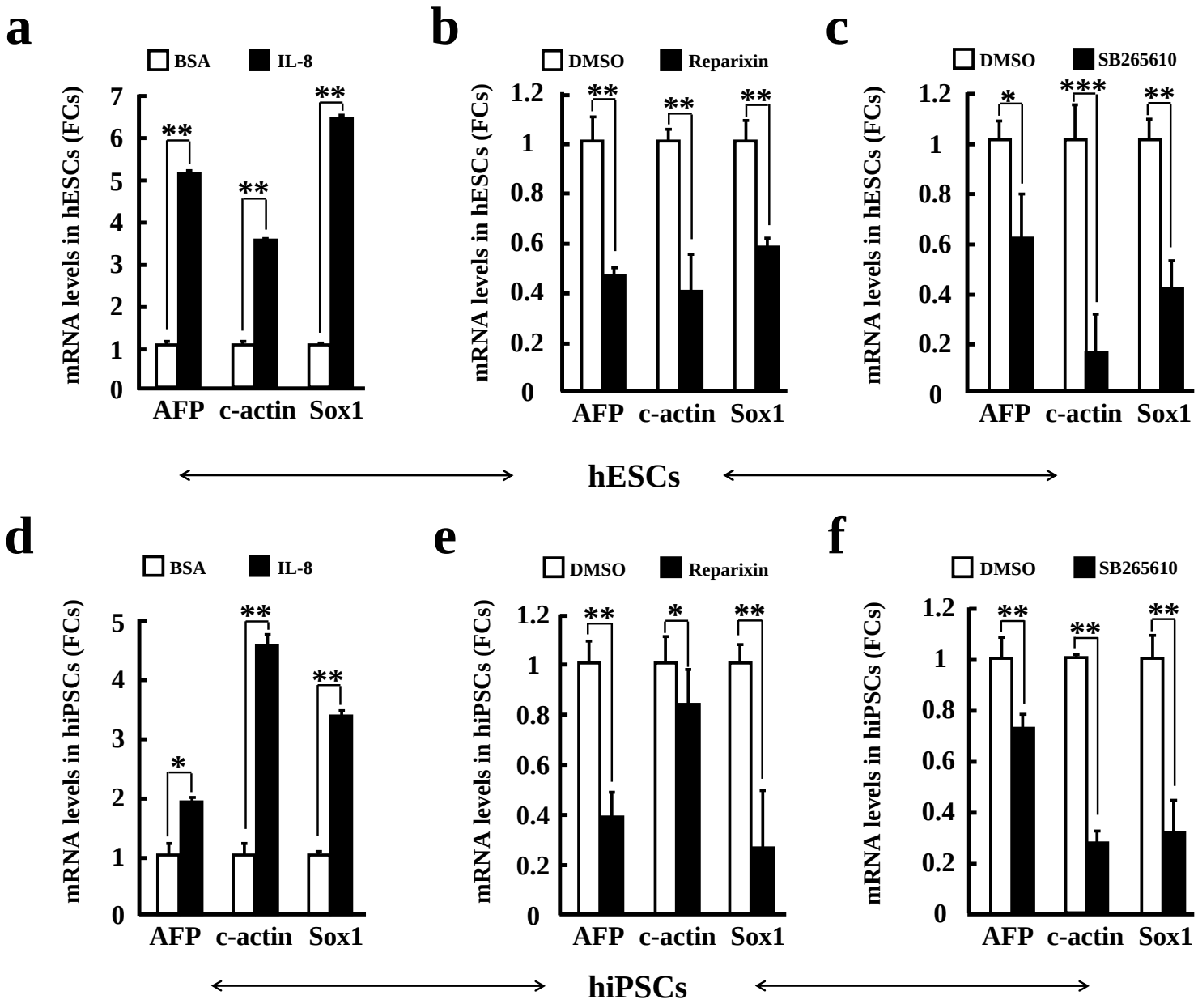


**Figure S12. Chemokine IL-8 signaling in the *in vitro* differentiation of hESCs and hPSCs.** Differentiation of hESCs (**a-c**) and hiPSCs (**d-f**) was evaluated by the expressions of three germ genes (*AFP*, *c-actin*, and *Sox1*) after treatment with IL-8 and CXCR2 antagonists reparixin/SB265610. hESCs (**a-c**); hiPSCs (**d-f**); IL-8 (**a**, **d**); Reparixin (**b**, **e**); and SB265610 (**c**, **f**). Values on graphs represent means ± sem, n ≧ 3 individual experiments. *P < 0.05, **P < 0.01, ***P < 0. 001.

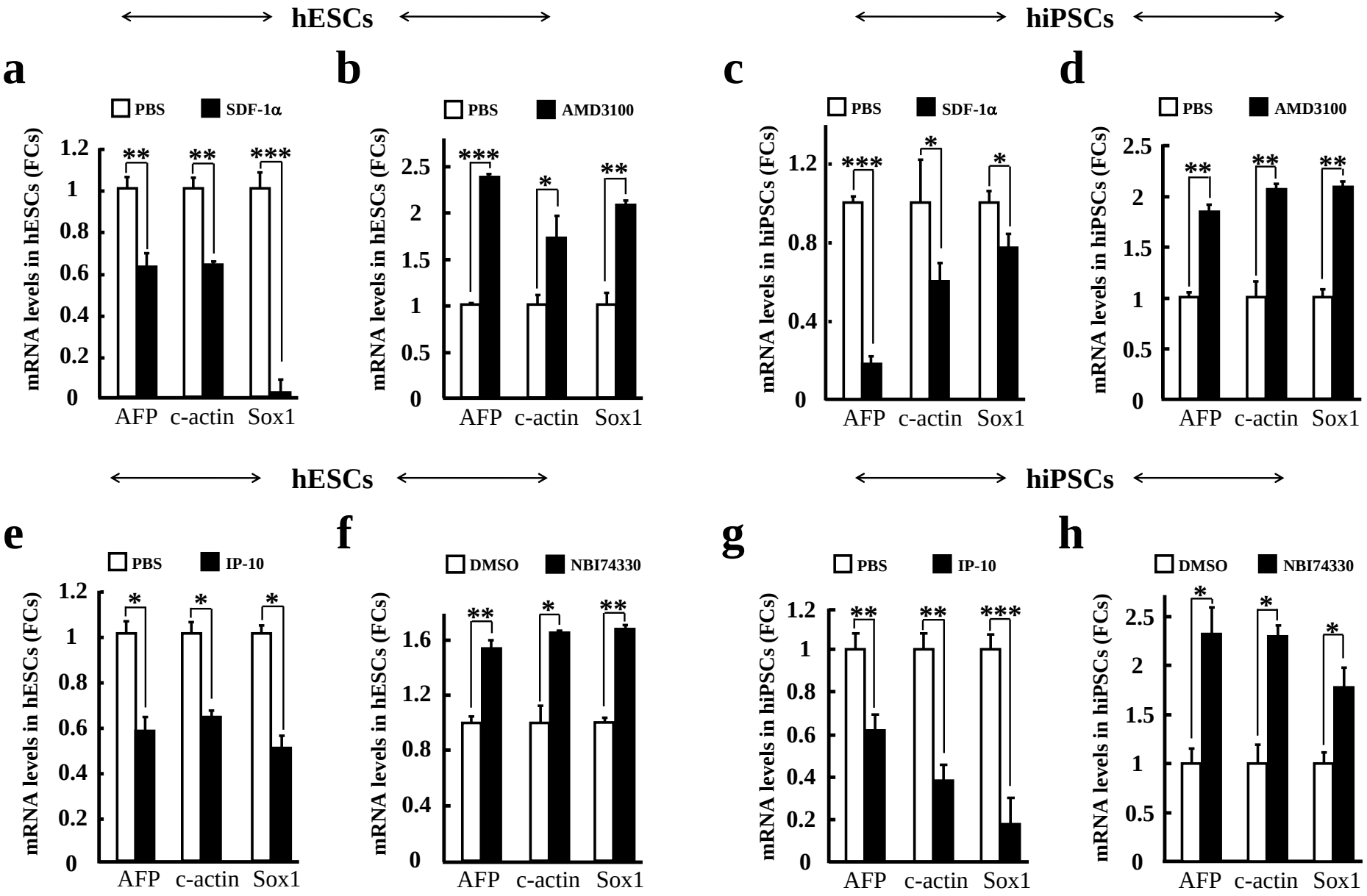


**Figure S13. Chemokines SDF-1a and IP-10 in the *in vitro* differentiation of hESCs and hPSCs.** Differentiation of hPSCs was evaluated by the expressions of three germ genes (*AFP*, *c-actin*, and *Sox1*) after treatment with AMD3100/NBI 74330. hESCs (**a**, **b**, **e**, **f**); hiPSCs (**c**, **d**, **g**, **h**); SDF-1a (**a**, **c**); CXCR4 antagonist AMD3100 (**b**, **d**); IP-10 (**e**, **g**); and CXCR3 antagonist NBI74330 (**f**, **h**). Values on graphs represent means ± sem, n ≧ 3 individual experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

**Supplementary table 1.**

**Oligo primers of chemokine receptors and transcription factors.**

| Name | Sequence |
|---|---|
| **Chemokine receptors** | |
| CCR1 forward | 5'-GGCTGGTATTGCCTTTGTTG-3' |
| CCR1 reverse | 5'-GGTCCAAATGTCTGCTCTGC-3' |
| CCR2 forward | 5'-ACGGTGCTCCCTGTCATAAA-3' |
| CCR2 reverse | 5'-CATTCCCAAAGACCCACTCAT-3' |
| CCR3 forward | 5'-CTGTCTCGTTCTCCCTCTGCT-3' |
| CCR3 reverse | 5'-GCTCCGCTCACAGTCATTTC-3' |
| CCR5 forward | 5'-GGGGTGGTGACAAGTGTGAT-3' |
| CCR5 reverse | 5'-GATGATTCCTGGGAGAGACG-3' |
| CCR6 forward | 5'-AGGCAGTTCTCCAGGCTATTT-3' |
| CCR6 reverse | 5'-GGCATTGCTGAAAACCCAC-3' |
| CXCR1 forward | 5'-CCAGTCCAGTTTGCTATGAGG-3' |
| CXCR1 reverse | 5'-AGCAGGACCAGGTTGTAGGG-3' |
| CXCR2 forward | 5'-ACCTACTCTTTGCCCTGACCT-3' |
| CXCR2 reverse | 5'-ACAGACCCCAGATGCTGAGAC-3' |
| CXCR3 forward | 5'-ATCAACTTCTACGCAGGAGCC-3' |
| CXCR3 reverse | 5'-AGGAAGATGAAGTCTGGGAGG-3' |
| CXCR4 forward | 5'-TCTTTGTCATCACGCTTCCC-3' |
| CXCR4 reverse | 5'-CGCCAACATAGACCACCTTT-3' |
| CXCR5 forward | 5'-TGGGAACTGGACAGATTGGA-3' |
| CXCR5 reverse | 5'-GTCTCCGTGGAACTGCGTGT-3' |
| CXCR7 forward | 5'-CTTCCTTGTCTGCTGGTTGC-3' |
| CXCR7 reverse | 5'-GCTTGGTGAGCCCTGTTTT-3' |
| **Transcription Factors** | |
| Oct4 forward | 5'- AGCGACTATGCACAACGA-3' |
| Oct4 reverse | 5'- AGTGGTGACGGAGACAGG-3' |
| Nanog forward | 5'-ACCTATGCCTGTGATTTGTGG-3' |
| Nanog reverse | 5'-CGGGACCTTGTCTTCCTTTT-3' |
| Rex-1 forward | 5'-ATGGCGTCCAAGACTACCAC-3' |
| Rex-1 reverse | 5'-ACTTTGCCCCCAAACTCTTT-3' |

| | |
|---|---|
| **Three germ layer genes** | |
| AFP forward | 5'-AGCGGCTGACATTATTATCG-3' |
| AFP reverse | 5'-GCAGGAGGGACATATGTTTC-3' |
| GATA4 forward | 5'-GCCAGTCTACGTGCCCACA-3' |
| GATA4 reverse | 5'-GGGTGTAAGCGGCTCCGTC-3' |
| GATA6 forward | 5'-TCCACTCGTGTCTGCTTTTG-3' |
| GATA6 reverse | 5'-CCCTTCCCTTCCATCTTCTC-3' |
| Sox17 forward | 5'-CCTGGGTTTTTGTTGTTGCT-3' |
| Sox17 reverse | 5'-GAGGAAGCTGTTTTGGGACA-3' |
| FOXA2 forward | 5'-CTACGCCAACATGAACTCCA-3' |
| FOXA2 reverse | 5'-CGGTAGAAGGGGAAGAGGTC-3' |
| Brachyury T (T) forward | 5'-ACCCAGTTCATAGCGGTGAC-3' |
| Brachyury T (T) reverse | 5'-ATGAGGATTTGCAGGTGGAC-3' |
| MIXL1 forward | 5'-GGTACCCCGACATCCACTT-3' |
| MIXL1 reverse | 5'-GCCTGTTCTGGAACCATACCT-3' |
| TBX1 forward | 5'-CGCCGGTGAAGAAGAACG-3' |
| TBX1 reverse | 5'-CACTTGGAAGGTGGGAAACA-3' |
| c-actin forward | 5'-TGTGCTAGACAGGAACTCAGAT-3' |
| c-actin reverse | 5'-GATGAGTCCAGAGTACTCCAAA-3' |
| Nestin forward | 5'-AACAGCGACGGAGGTCTCTA-3' |
| Nestin reverse | 5'-TTCTCTTGTCCCGCAGACTT-3' |
| Sox1 forward | 5'-GGACTCTCTCTGAGGTTCTTTG-3' |
| Sox1 reverse | 5'-GGCCCACATCCTAATCTTGA-3' |
| NeuroD1 forward | 5'-ATCTTGCACAGGGAGTCACC-3' |
| NeuroD1 reverse | 5'-TACTGCCGTCCAGTCCCATA-3' |
| Pax6 forward | 5'-GCTCGGTGGTGTCTTTGTCA-3' |
| Pax6 reverse | 5'-CAGAATTCGGGAAATGTCGC-3' |
| **Control** | |
| 18S RNA forward | 5'-CAGCCACCCGAGATTGAGCA-3' |
| 18S RNA reverse | 5'-TAGTAGCGACGGGCGGTGTG-3' |