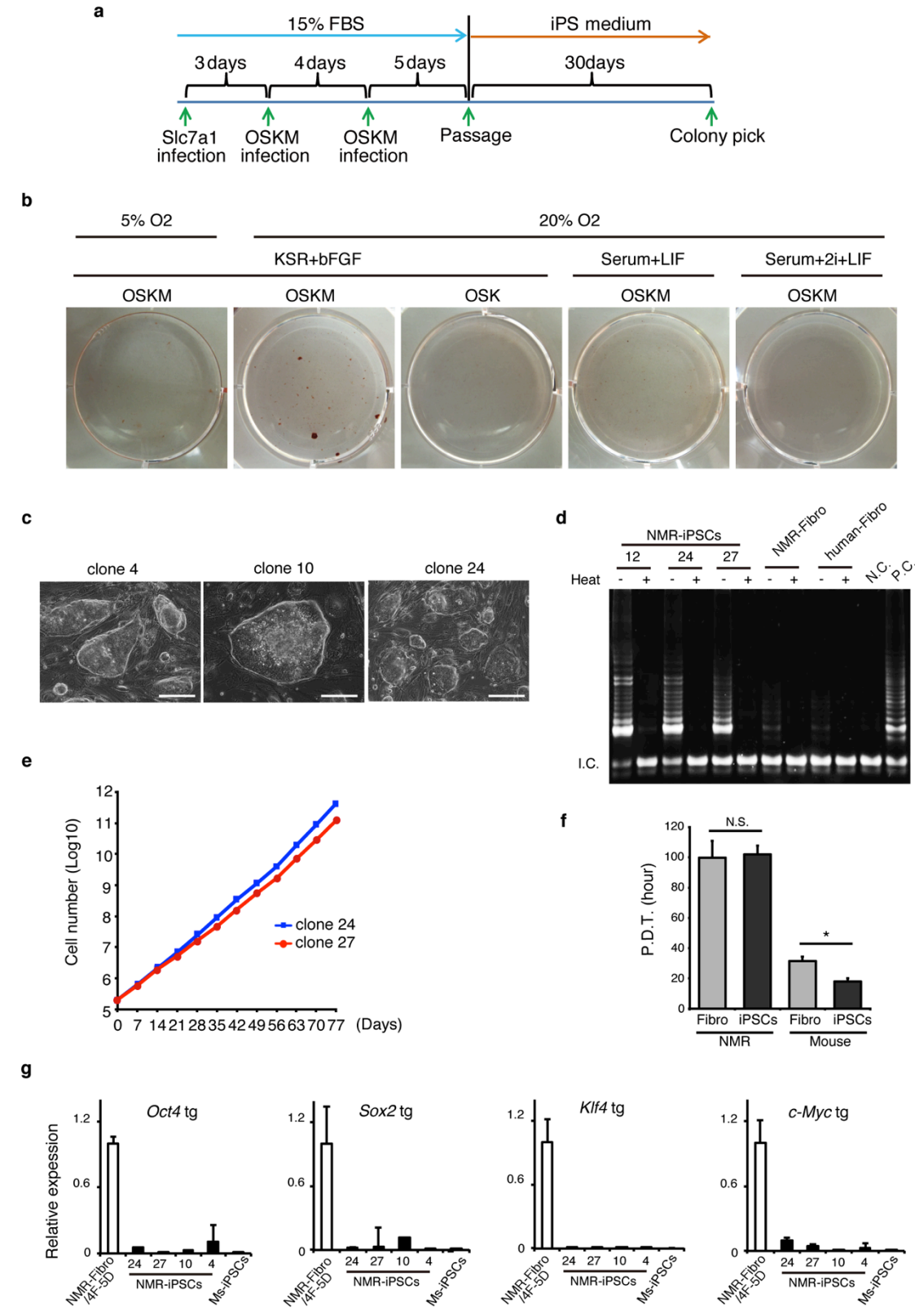
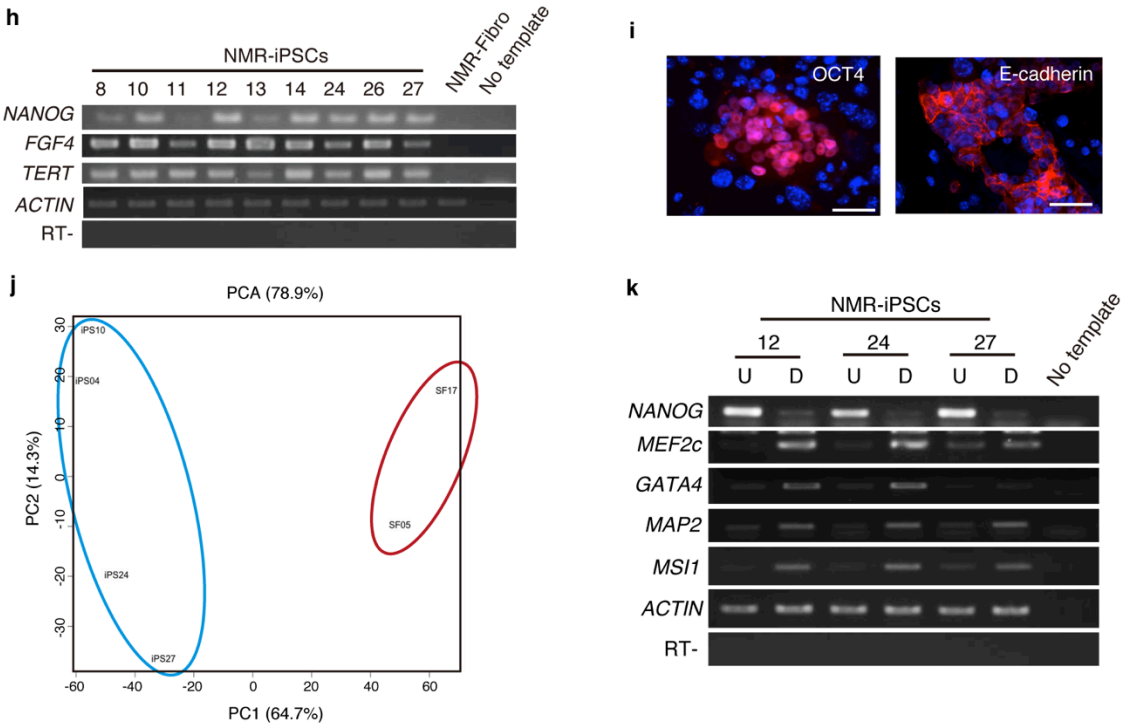
Supplementary Figure 1



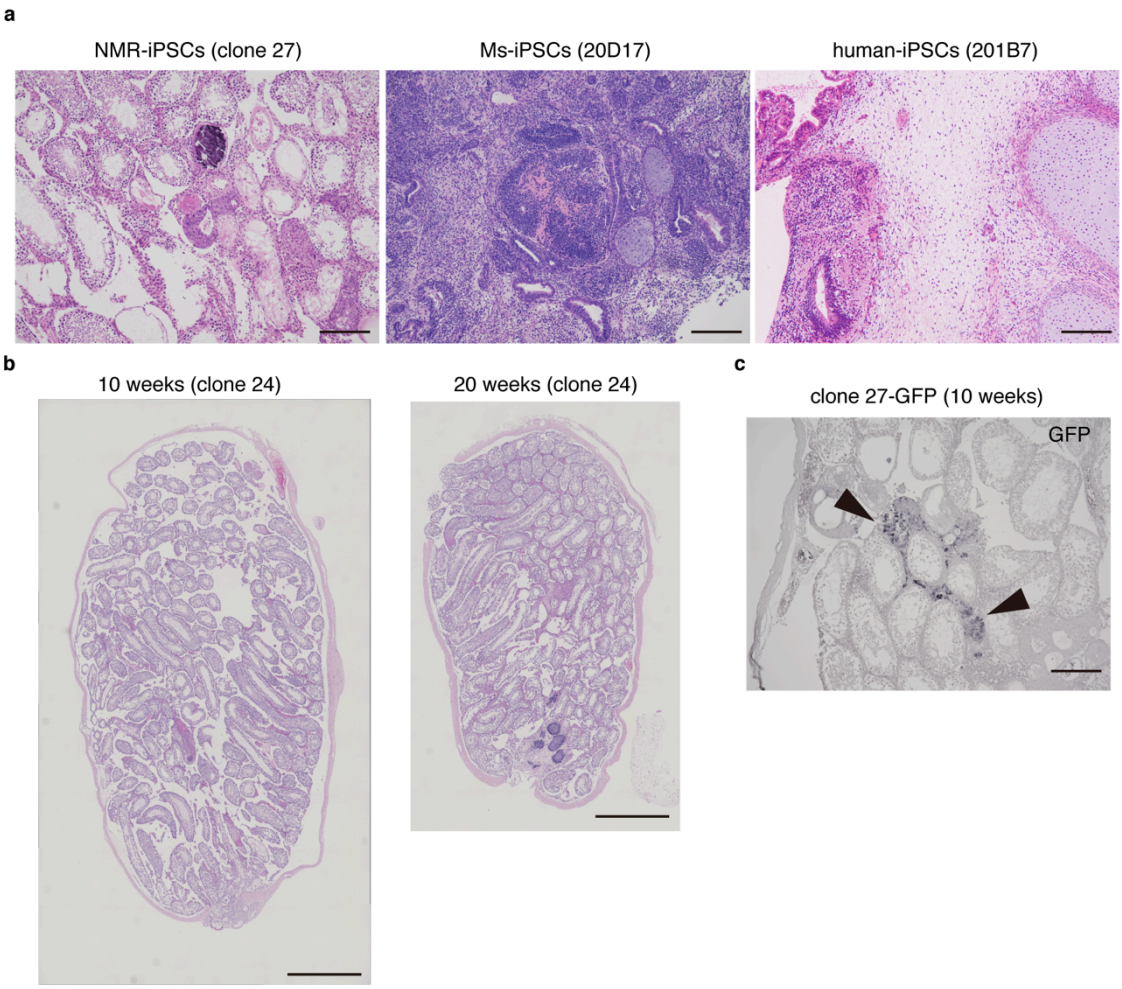
Supplementary Figure 1




**Supplementary Figure 1 | Generation and characterization of NMR-iPSCs.**

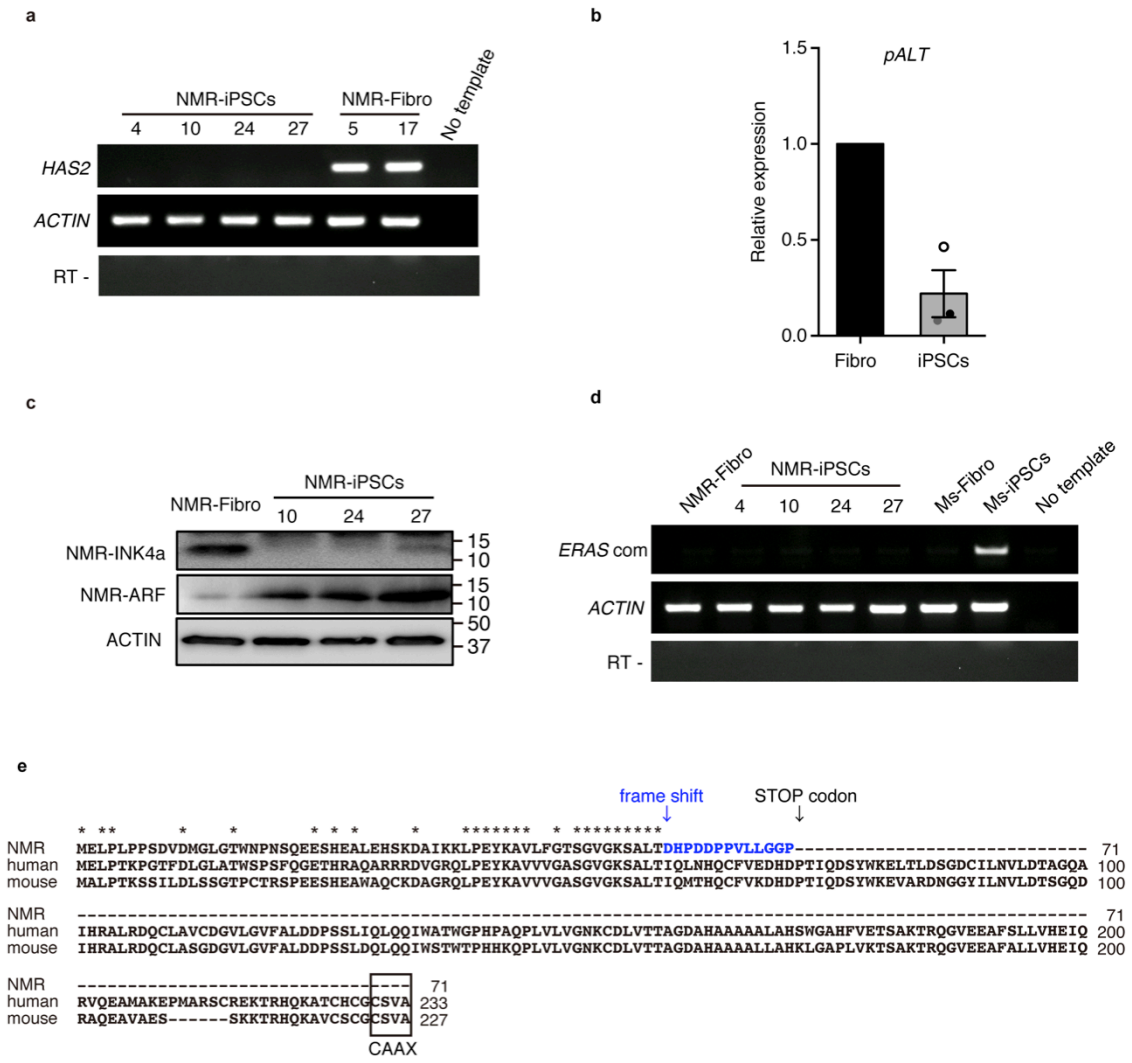
**a**, iPSC generation from NMR-fibroblasts. **b**, Culture conditions. AP activity 37 days after introduction of OSKM. **c**, Morphology of NMR-iPSCs (clones 4, 10 and 24). Scale bar, 200 μm. **d**, Telomerase activities of NMR-iPSCs. NMR-Fibro, NMR-fibroblasts; human-Fibro, human skin fibroblasts (TIG113); N.C., Heat-inactivated (+) samples; I.C., internal control; P.C., positive control. $n$ = 3 clones. **e**, Proliferation of NMR-iPSCs (clones 24 and 27). **f**, Population doubling times (PDT). $n$ = 3 clones. *$P$ < 0.05; N.S., not significant ($t$-test). **g**, qRT-PCR analysis of transgene expression in NMR-iPSCs. NMR-Fibro/4F-5d, NMR-fibroblasts 5 days after the transduction with OSKM. Ms-iPSCs, 20D17. $n$ = 4 clones. Results are represented as mean ± SD. **h**, RT-PCR analysis of pluripotency markers in NMR-iPSCs and NMR-fibroblasts. **i**, Immunofluorescence analysis of the expression of pluripotency markers OCT4 and E-cadherin. Hoechst dye (blue), nuclei. Scale bar, 100 μm. **j**, Principal component analysis (PCA) of global gene expression patterns of four NMR-iPSC clones and two NMR-fibroblast lines. **k**, RT-PCR analysis of the expression of pluripotency and differentiation markers in EBs. *MEF2c,* mesoderm; *GATA4*, endoderm; *MAP2* and *MSI1*, ectoderm. U, undifferentiated; D, differentiated. $n$ = 3 clones.

Supplementary Figure 2

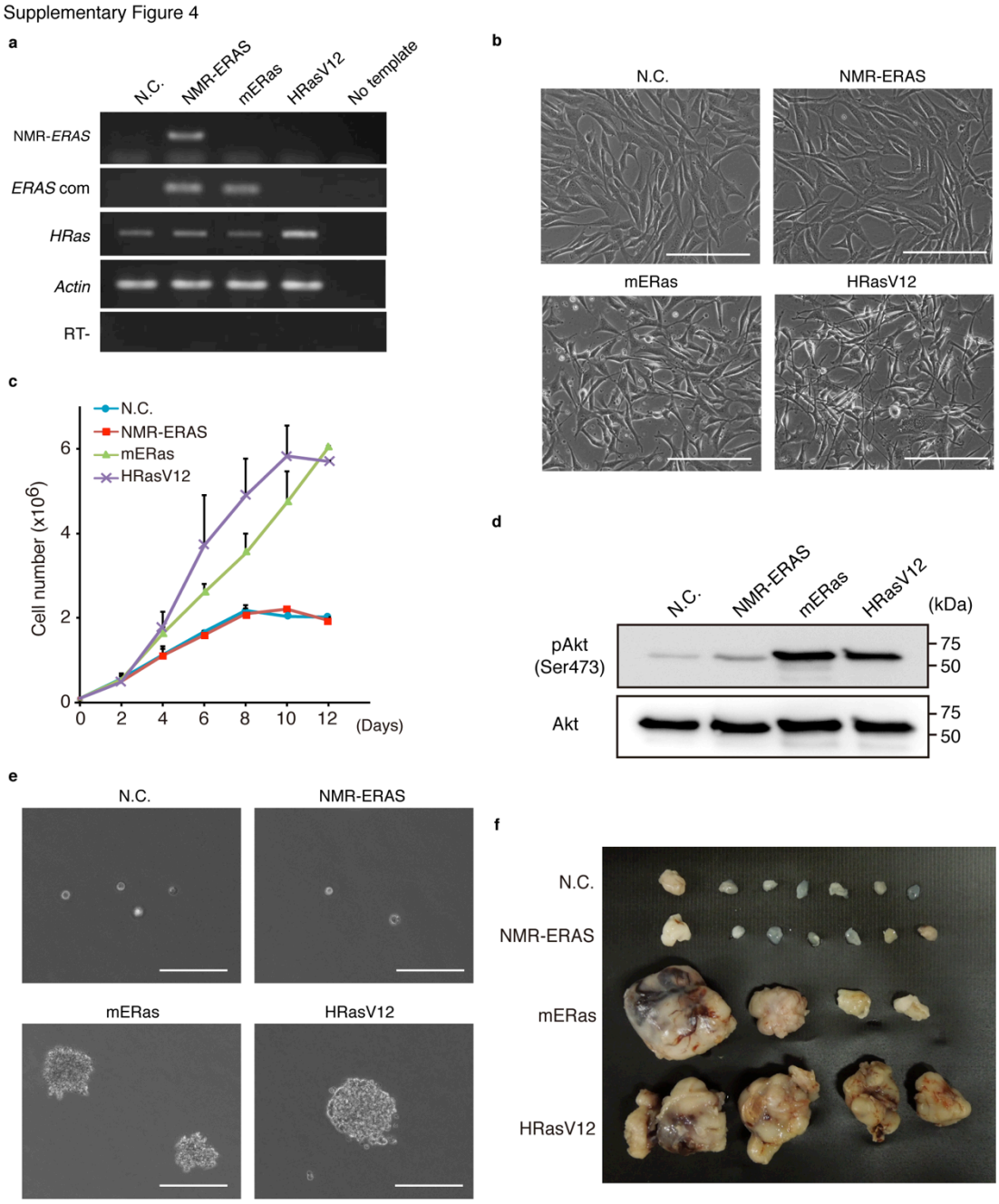



**Supplementary Figure 2 | Histopathological analysis of tumours and testes after transplantation of iPSCs. a** and **b**, Haematoxylin and eosin staining. Sections of tumours and testes of mice transplanted with NMR-iPSCs (clone 27), Ms-iPSCs (20D17) or human-iPSCs (201B7) (**a**). Scale bar, 200 μm. Testes injected with NMR-iPSCs (clone 24) 10 or 20 weeks after transplantation (**b**). Scale bar, 1 cm. **c**, Immunohistochemical analysis of GFP. Transplanted NMR-iPSCs were lentivirally labelled with GFP. Arrowhead, area of the engrafted site. Scale bar, 200 μm.

Supplementary Figure 3

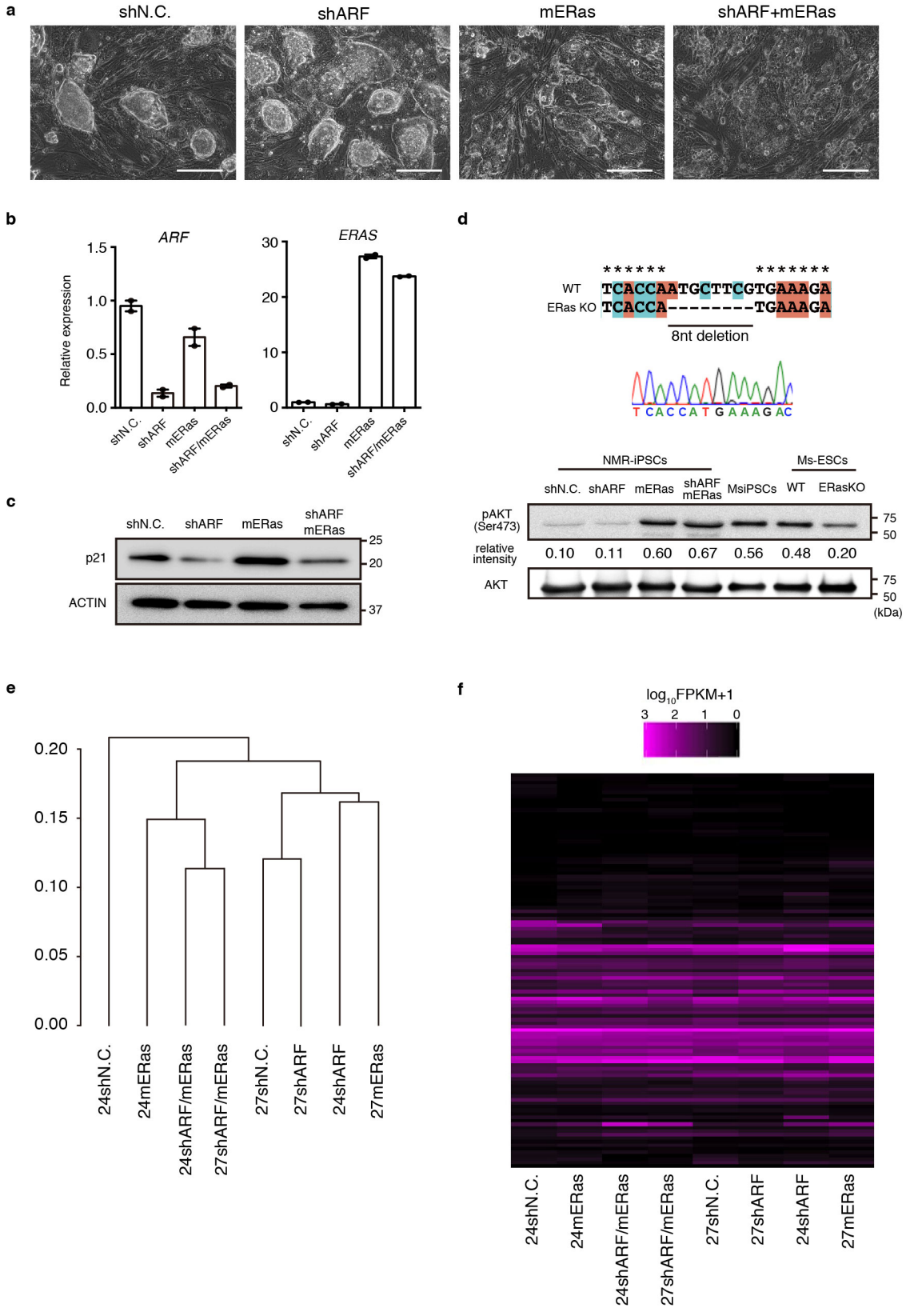



**Supplementary Figure 3 | Activation of *ARF* and frameshift mutation of *ERAS* in NMR-iPSCs. a**, RT-PCR analysis of *HAS2* expression in NMR-iPSCs. **b**, Expression of *pALT*. Results are presented as mean ± SEM. *n* = 3 clones. **c**, Western blotting of INK4a and ARF expression in NMR-iPSCs and NMR-fibroblasts. *n* = 3 clones. **d**, RT-PCR analysis of *ERAS* expression in NMR-iPSCs. Ms-iPSCs (20D17), positive control. *ERAS* com, primer-set designed to amplify the sequence shared by NMR-*ERAS* and *mERas*. **e**, Amino acid sequence comparison among Ms-, human- and NMR-*ERAS* genes. Blue arrow: frameshift mutation. Box: CAAX motif.




**Supplementary Figure 4 | Transforming potential of NMR-ERAS in NIH-3T3 cells.** NIH-3T3 cells were infected with lentiviral vectors expressing NMR-ERAS or mERas. HRasV12, positive oncogenic control; N.C., EGFP as negative control. **a**, RT-PCR analysis of transgene expression. **b**, Cell morphology. Scale bar, 200 μm. **c**, Cell proliferation. Cells ($1 \times 10^5$) were plated on 10 cm dish and counted every other day. Data are represented as mean ± SD. **d**, Western blotting for AKT or phosphorylated-AKT expression. **e**, Soft agar growth assay. Scale bar, 200 μm. **f**, Tumour formation in nude mice. Cells ($1 \times 10^6$) were subcutaneously injected and tumours were dissected 25 days later.

Supplementary Figure 5



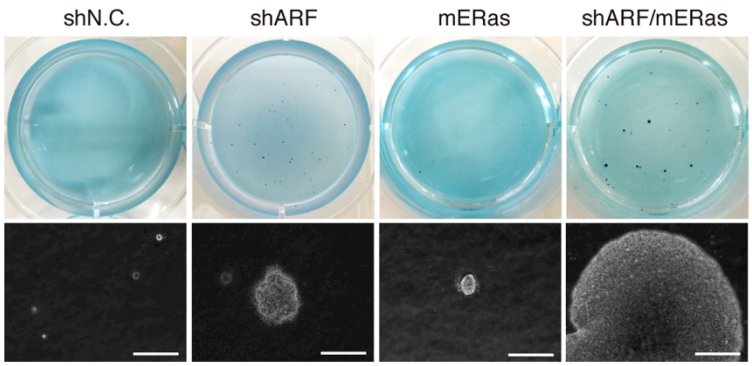
Supplementary Figure 5

**g**

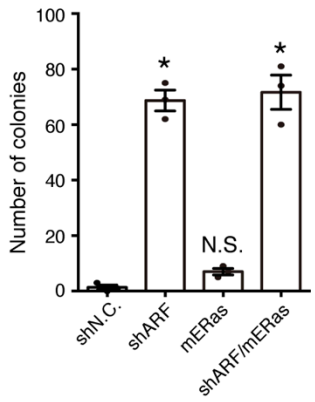
Gene List

| Undifferentiated | | Mesoderm | Endoderm | Ectoderm |
|---|---|---|---|---|
| hDNMT3B | DNMT3L | BMP2 | AFP | OTX2 |
| DPPA4 | KRT17 | BMP4 | FOXA1 | SLC6A3 |
| ERAS | DPPA2 | CDH1 | FOXA2 | CHAT |
| FBXO15 | TERT | EOMES | KRT19 | LMX1B |
| FGF4 | IFITM1 | HAND1 | SOX17 | GFAP |
| KLF4 | NODAL | FABP4 | TTR | MAP2 |
| LIN28A | GRB7 | VEGFA | GATA4 | PAX6 |
| LIN28B | PODXL | MEF2C | PDX1 | NES |
| NANOG | CD9 | NKX2-5 | KRT8 | MSI1 |
| NR0B1 | BRIX1 | ACTA2 | ALB | GFAP |
| POU5F1 | ESRRB | FLT1 | PDGFRA | REST |
| SALL4 | RNF17 | CDH11 | HNF4A | TH |
| SOX2 | NR6A1 | RUNX1 | HNF1B | SYP |
| UTF1 | NUMB | MESDC2 | SERPINA1 | MNX1 |
| ZFP42 | REST | MYOD1 | CPS1 | |
| ZNF296 | LIFR | WT1 | TAT | |
| ZSCAN4 | T | NPPA | LAMC1 | |
| TDGF1 | MYC | HBB | INS | |
| FGF5 | MYCN | RUNX2 | FN1 | |
| | | COL2A1 | PAX4 | |
| | | | SST | |

**h**

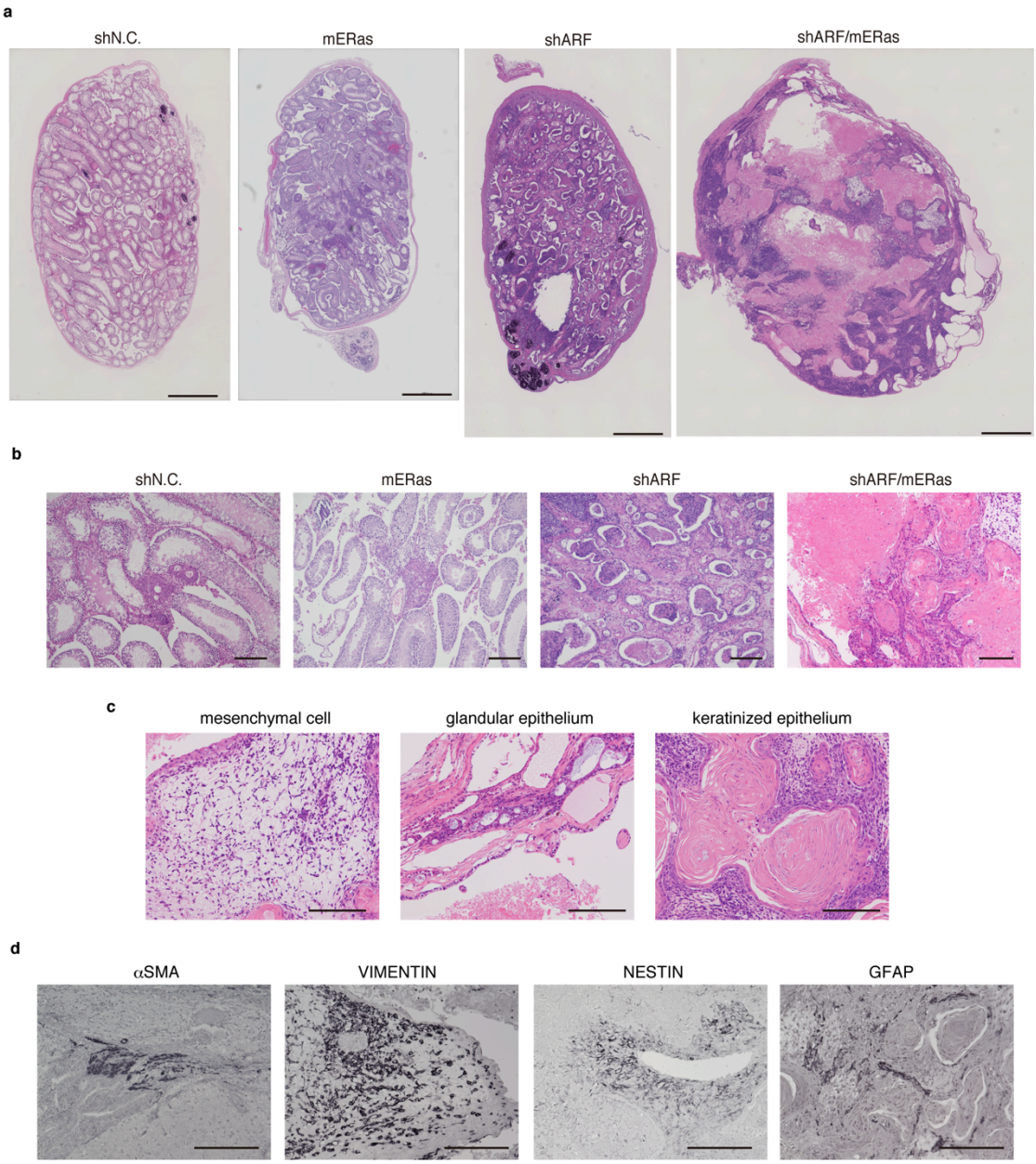



**i**



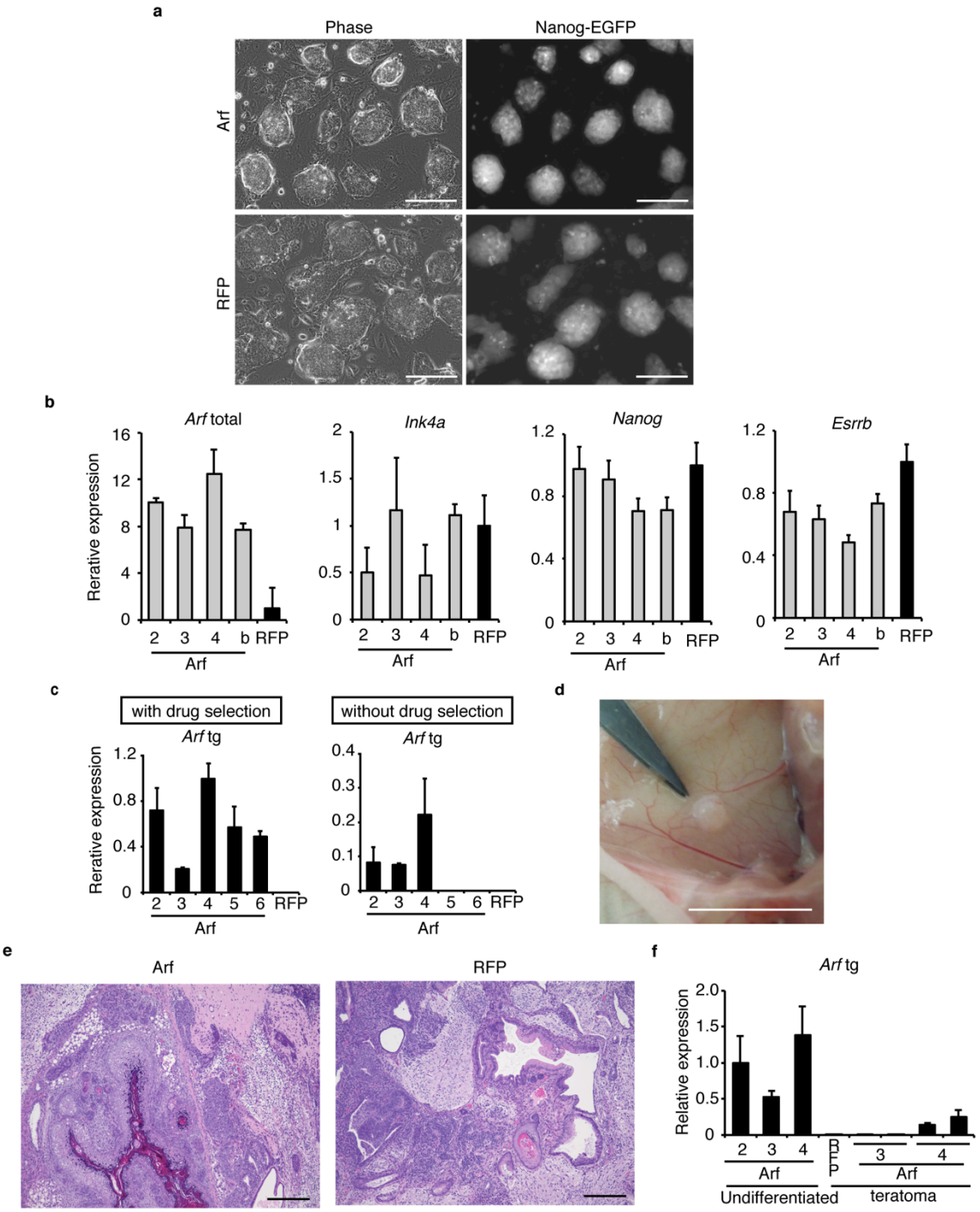

**Supplementary Figure 5 | Characterization of NMR-iPSCs expressing mERas and/or shARF. a**, Morphology of NMR-iPSCs (clone 24) transduced with mERas and/or shARF. Scale bar, 200 μm. **b**, qRT-PCR analysis of *ARF* and *mERas* expression in NMR-iPSCs. $n = 2$ clones. Data are represented as mean ± SEM. **c**, Western blotting for p21. **d**, The genomic sequence of *ERas* in *ERas* knock-out Ms-ES cell line (EGR-G101). Western blotting for AKT or phosphorylated-AKT expression in the indicated cell lines. Values indicate the relative intensity of AKT expression and phosphorylation. **e**, Hierarchical clustering analysis. #24, NMR-iPSC clone 24; #27, NMR-iPSC clone 27. **f**, Heat map of the selected markers of undifferentiated and differentiated cells. **g**, Selected genes shown in Supplementary Fig. 5e and f**. h**, Soft agar growth assay of NMR-iPSCs expressing mERas and/or shARF. **i**, Number of colonies. $n = 2$. Results are presented as mean ± SD for three experimental replicates. $*P < 0.05$ (one way ANOVA). N.S., not significant. N.C., negative control. Scale bar, 200 μm (**a** and **g**).

Supplementary Figure 6




**Supplementary Figure 6 | Histopathological analysis of tumours derived from NMR-iPSCs expressing mERas and/or shARF. a, b** and **c,** Haematoxylin and eosin staining. Sections of tumours and testes 10 weeks after transplantation of NMR-iPSCs expressing mERas and/or shARF. Testes and tumours (**a**). Scale bar, 1 cm. High magnification (**b**). Scale bar, 200 μm. Representative images of teratomas formed by mERas/shARF-NMR-iPSCs (**c**). Scale bar, 200 μm. **d**, Immunohistochemical analysis of teratomas formed by mERas/shARF-NMR-iPSCs. αSMA, mesoderm; VIMENTIN, mesoderm and parietal endoderm; NESTIN and GFAP, ectoderm. Scale bar, 200 μm.

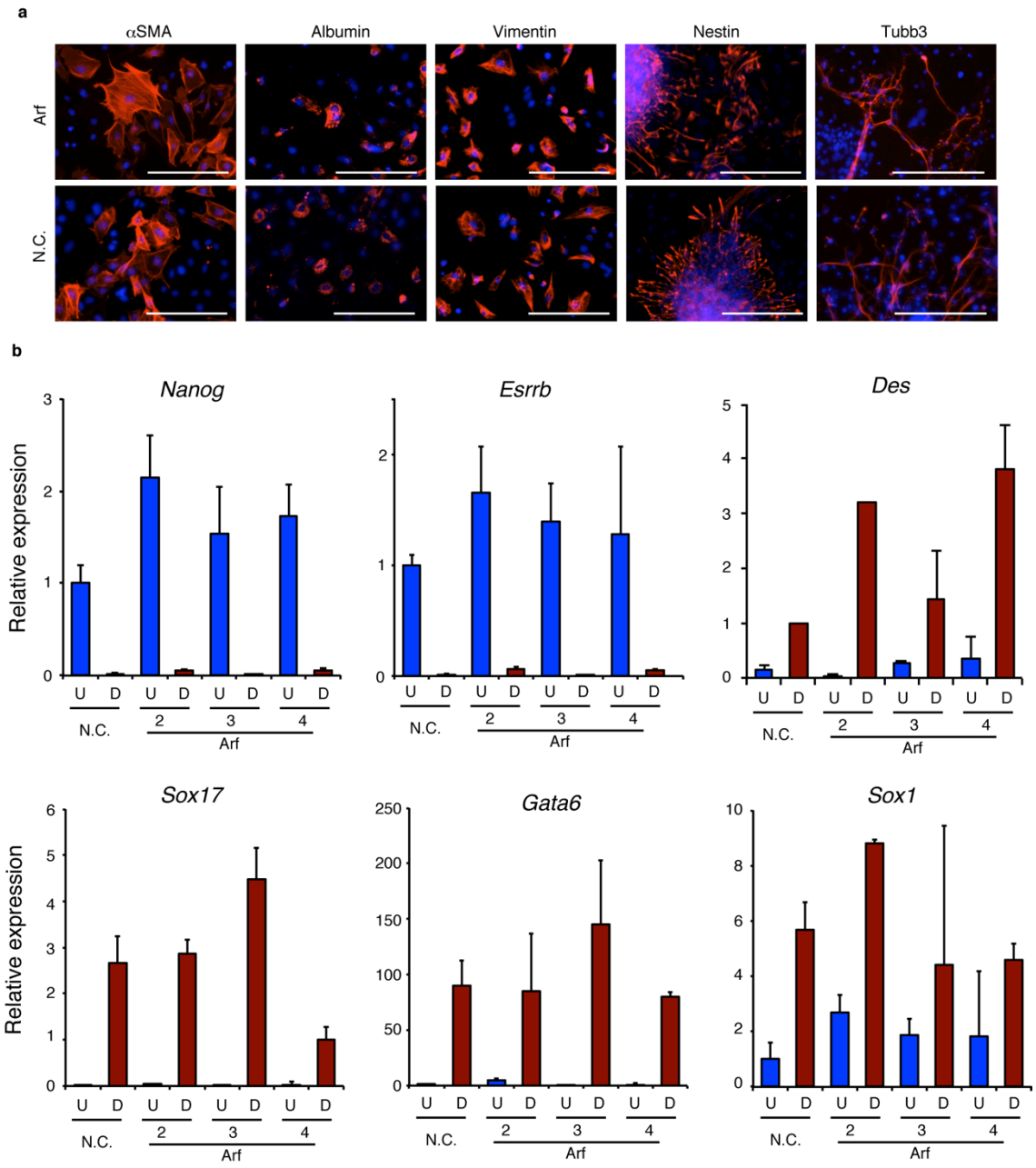
Supplementary Figure 7




**Supplementary Figure 7 | Characterization of Ms-iPSCs expressing Arf. a**, Morphology of Ms-iPSCs (20D17) expressing Arf. GFP: Nanog reporter (Nanog-GFP). Scale bar, 200 μm. **b**, qRT-PCR analysis of total *Arf* (endogenous and transgenic), *Ink4a*, *Nanog* and *Esrrb* expression in Arf-Ms-iPSCs. *n* = 3 clones (2, 3 and 4). b, bulk culture. **c**, Expression of the *Arf* transgene (*ARF* tg) in Arf-Ms-iPSC clones with (left panel) or without (right panel) hygromycin selection.

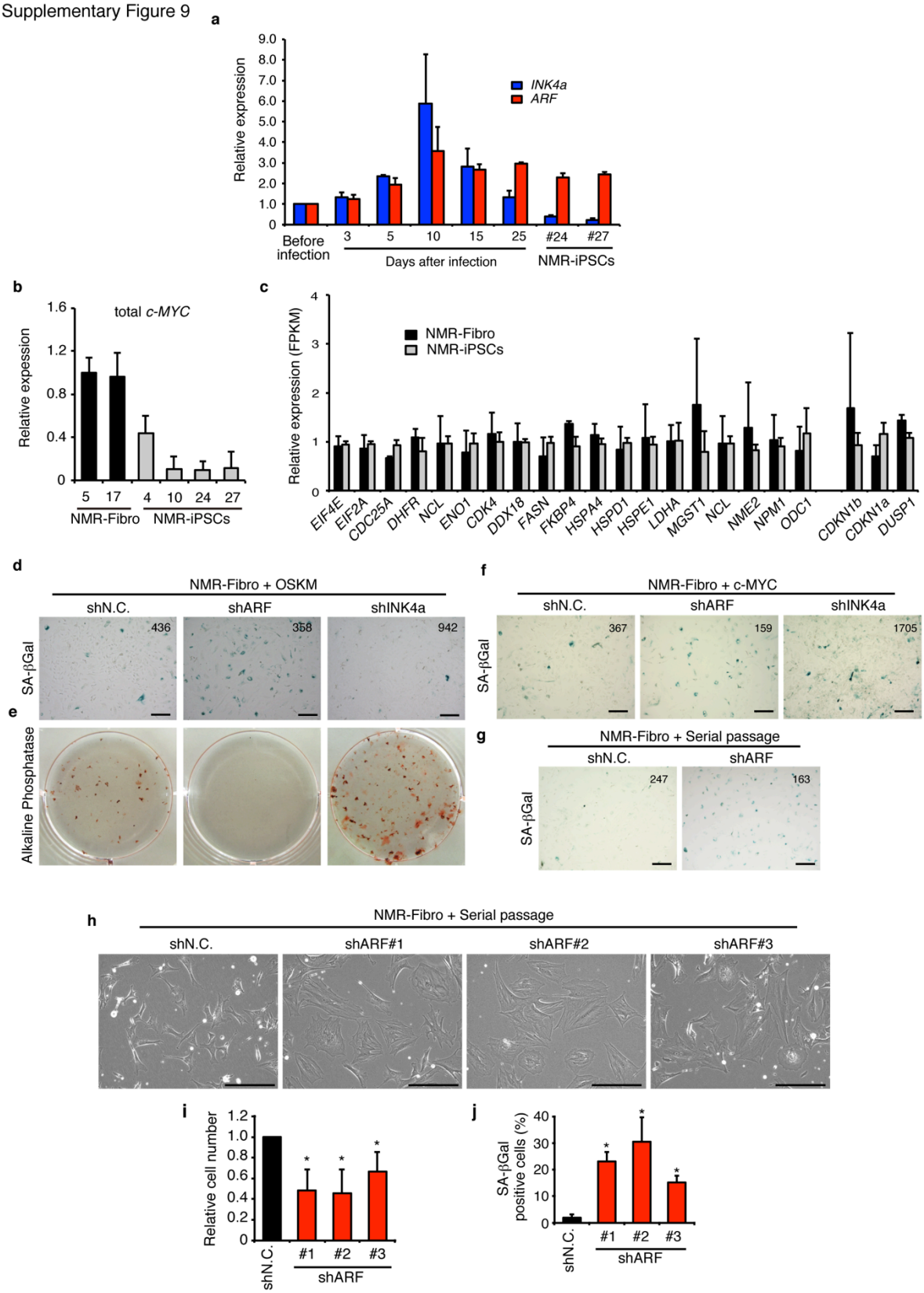
Experimental duplicate. **d**, Small teratomas observed 10 weeks after transplantation of the High-ARF-group clone 3. Scale bar, 1 cm. **e**, Haematoxylin and eosin staining of a teratoma derived from High-ARF-group clone 3 (12 weeks) or control Ms-iPSCs (3 weeks) after transplantation. **f,** qRT-PCR analysis of *Arf* transgene expression in teratomas or undifferentiated iPSCs. Scale bar, 200 μm. Data represent the mean ± SD (**b**, **c** and **f**).

Supplementary Figure 8




**Supplementary Figure 8 | Differentiation potential of Arf-Ms-iPSCs. a**, Immunocytochemical analysis of differentiated cells from the High-Arf-group clone 4. mesoderm (αSMA), endoderm (Albumin and Vimentin), ectoderm (Nestin and Tubb3). Scale bar, 200 μm. **b**, qRT-PCR analysis of differentiated cells from Arf-Ms-iPSCs (clone 2, 3, 4). pluripotent marker genes (*Nanog* and *Esrrb*) and differentiation marker genes (*Des*, *Sox17*, *Gata6* and *Sox1*). U, undifferentiated; D, differentiated. Data represent the mean ± SD.

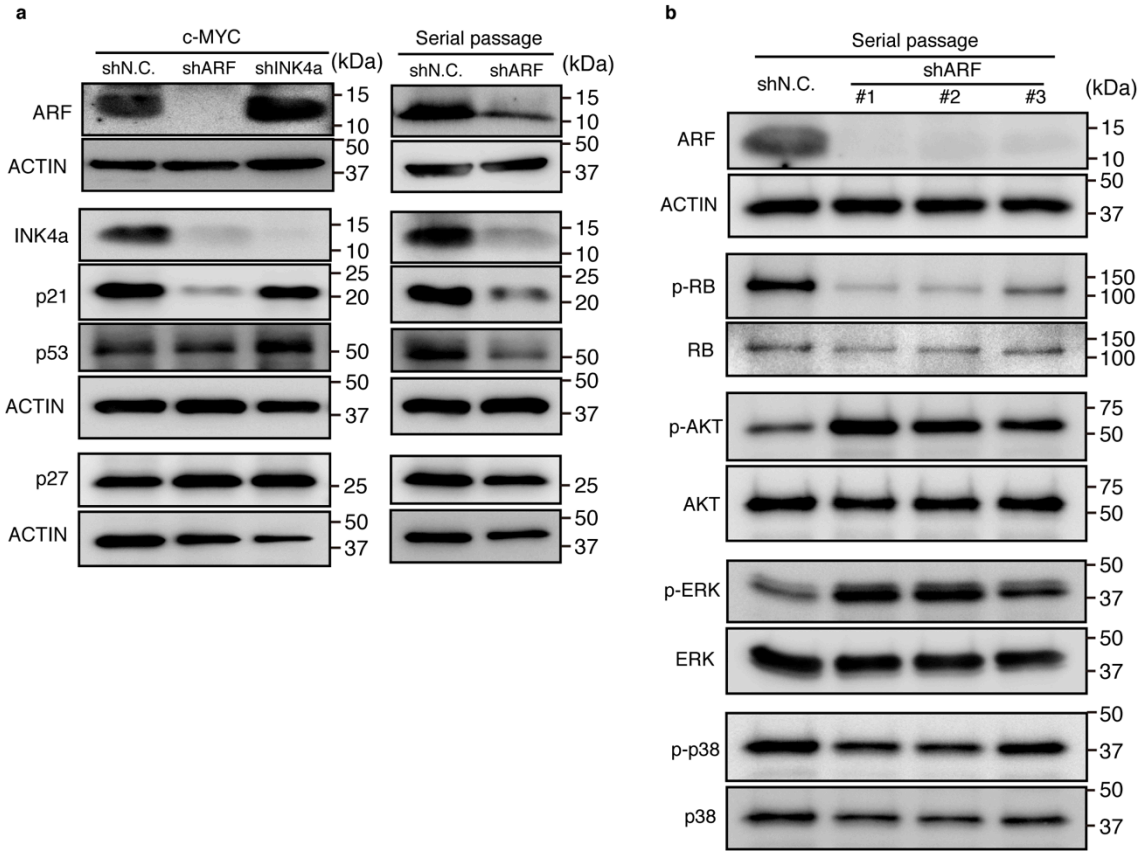
Supplementary Figure 9




**Supplementary Figure 9 | ASIS as a safeguard against reprogramming and oncogenic transformation. a**, qRT-PCR analysis of the kinetics of *INK4a* and *ARF* expression during

reprogramming of NMR-fibroblasts. Experimental triplicate. **b**, qRT-PCR analysis of the expression of total *c-MYC* in NMR-iPSCs and parental NMR-fibroblasts. The primers amplified endogenous and transgenic *c-Myc*. $n = 4$ clones. **c**, RNA-seq of the levels of c-MYC target genes. Nineteen genes on the left, upregulated genes by c-Myc; three on the right, downregulated genes by c-Myc. Y-axis: expression level (FPKM) of NMR-fibroblasts relative to NMR-iPSCs clone 4. **d**, SA-βGal activity of NMR-fibroblasts expressing shARF and/or shINK4a and OSKM 14 days after infection. **e**, AP activity of NMR-fibroblasts expressing shARF or shINK4a and OSKM 37 days after infection. **f**, SA-βGal activity of NMR-fibroblasts expressing shARF or shINK4a and c-MYC. **g**, Transduction of shARF of fibroblasts with derepressed ARF expression induced by serial passage. SA-βGal activity 14 days after transduction. **h, i** and **j**, Reproducibility of the experiment of ASIS in serial passaged NMR fibroblasts using three independent short hairpins RNA against ARF. Cell morphology (**h**). Cell growth (**i**). SA-βGal-positive cells (%) (**j**). Data are represented as mean ± SD. $*P < 0.05$ (*t*-test). Scale bar, 200 μm. The number in the right upper corner indicates Hoechst-positive cells.

Supplementary Figure 10

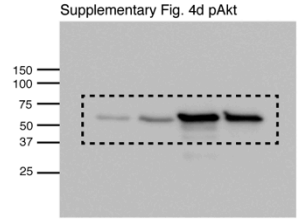



**Supplementary Figure 10 | Expression status of senescence-associated genes in NMR fibroblast undergoing ASIS.**

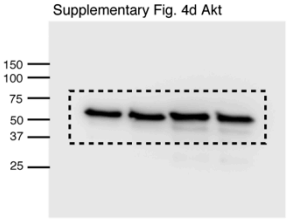
**a**, Western blotting of ARF, INK4a, p21 and p53 expression in stressed NMR-fibroblasts expressing shARF or shINK4a. **b**, Western blotting of RB, AKT and MAPK (ERK and p38) expression in NMR fibroblast undergoing ASIS.

## Supplementary Figure 11

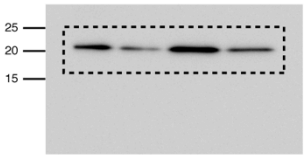
Supplementary Fig. 4d pAkt



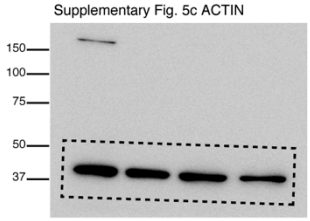

Supplementary Fig. 4d Akt



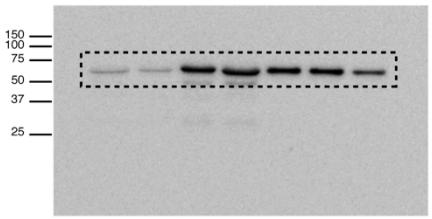

Supplementary Fig. 5c p21



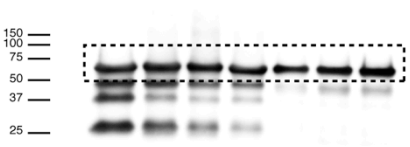

Supplementary Fig. 5c ACTIN



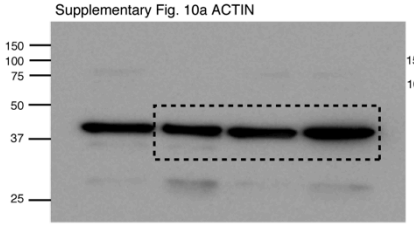

Supplementary Fig. 5d pAKT



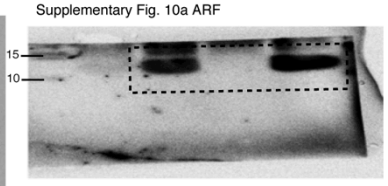

Supplementary Fig. 5d AKT



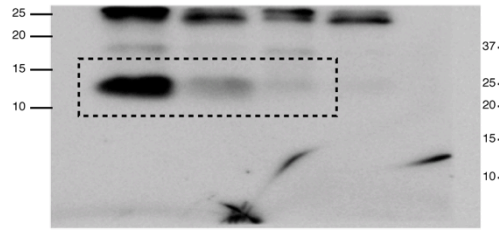

Supplementary Fig. 10a ACTIN



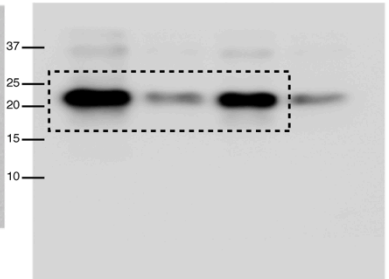

Supplementary Fig. 10a ARF



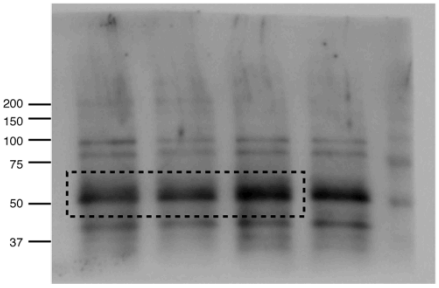

Supplementary Fig. 10a INK4a



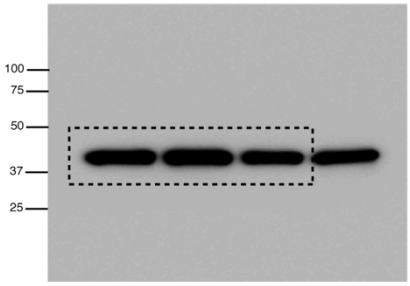

Supplementary Fig. 10a p21

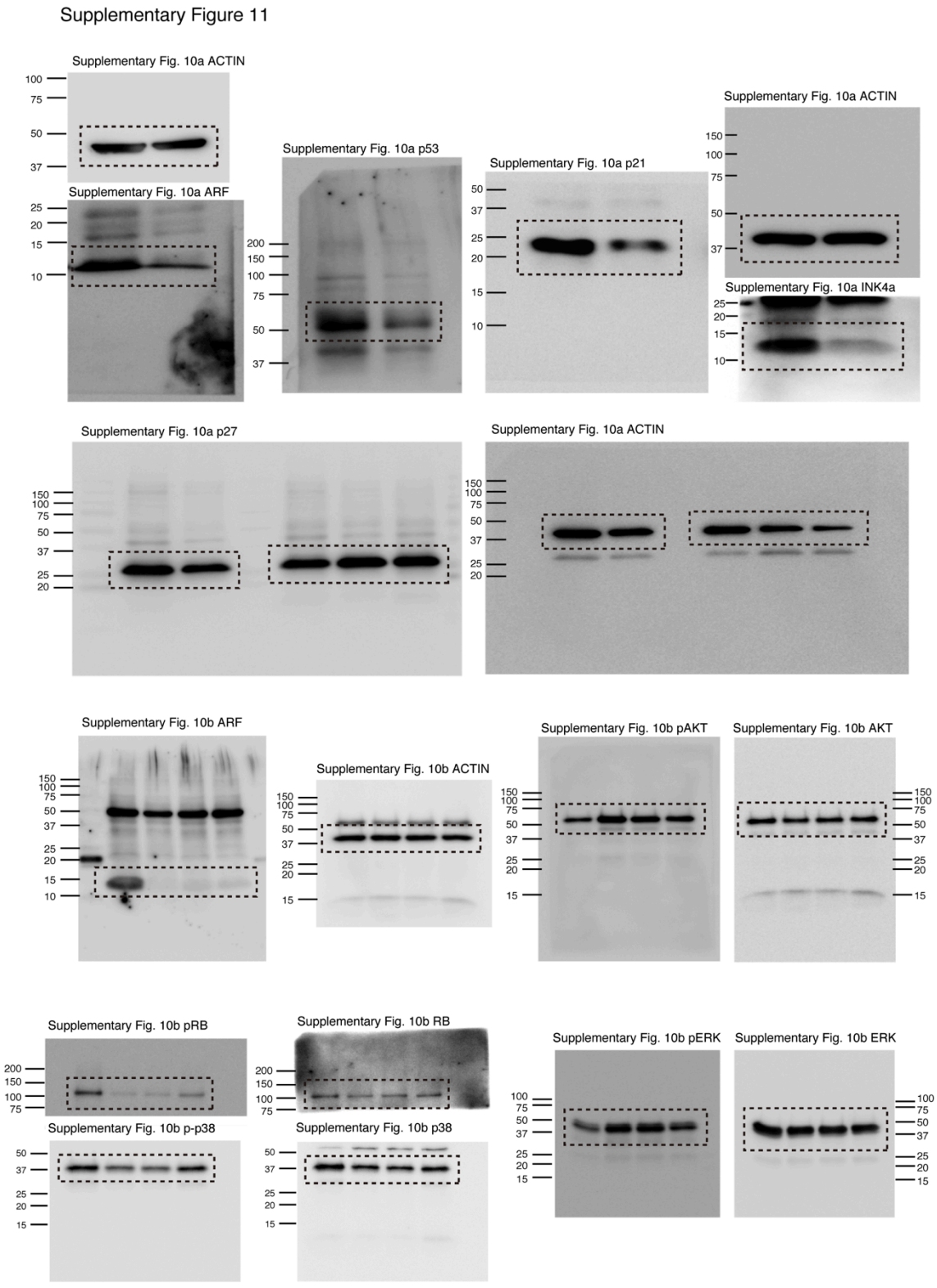



Supplementary Fig. 10a p53




Supplementary Fig. 10a ACTIN






**Supplementary Figure 11 | Uncropped scans of western blot results displayed in this study**

## Supplementary Table 1 | Primers sequences

| | | |
|---|---|---|
| NMR-INK4a-F | GACCCGAACTGCGCTGACCCT | qRT-PCR for NMR-INK4a |
| NMR-INK4a-R | CCGCGTCATGCACCGGTAGTGTGA | |
| NMR-ARF-F | CCATCGTGTGTGCGGGCTTTCGT | qRT-PCR for NMR-ARF |
| NMR-ARF-R | CTGCTGCCCTCTCCGGTGTCT | |
| NMR-INK4b-F | AACGCCGTCAATCGCTTCGGGAG | qRT-PCR for NMR-INK4b |
| NMR-INK4b-R | TCCTCAGCTAGGTCCACGGGCAA | |
| NMR-CDKN1a-F | ACCTGTCGCTGTCCTGCACCCTTG | qRT-PCR for NMR-p21 |
| NMR-CDKN1a-R | CGTCATGCTGGTCTGCCGCCGTT | |
| pMXs-Oct4-F | GACTGCCGGATCTAGCTAGTT | qRT-PCR for Oct4 tg |
| pMXs-Oct4-R | GGGGTGAGAAGGCGAAGTCTGA | |
| pMXs-Sox2-F | CATGGCCCAGCACTACCAGA | qRT-PCR for Sox2 tg |
| pMXs-Sox2-R | CCTTACGCGAAATACGGGCAGA | |
| pMXs-Klf4-F | GCCGACACCAGACTAAGAACC | qRT-PCR for Klf4 tg |
| pMXs-Klf4-R | GGAGAAGGACGGGAGCAGAG | |
| pMXs-cMyc-F | GGAATTCGCCCTTCACCATGC | qRT-PCR for c-Myc tg |
| pMXs-cMyc-R | TGGGAAGCAGCTCGAATTTCT | |
| NMR-NANOG-F | AAGTACCTCAGCCTGCAGCAGATGC | RT-PCR for NMR-NANOG |
| NMR-NANOG-R | TTTTCTGCCACCGCTTACATTTCAT | |
| NMR-FGF4-F | CGTGAGCATCTTTGGAGTGGCCAGC | RT-PCR for NMR-FGF4 |
| NMR-FGF4-R | CAGCCTGGGGAGAAAGTGGGTGAC | |
| NMR-TERT-F | CCTGCTCAAGCTGGGTCATCACCGTG | RT-PCR for NMR-TERT |
| NMR-TERT-R | TCAGTCCAGGATGGTCTTGAAGT | |
| ACTB-F | ACAACGGCTCCGGCATGTGCAA | RT-PCR and qPCR for ACTIN common Ms and NMR |
| ACTB-R | CATTGTAGAAGGTGTGGTGCCAGA | |
| NMR-MEF2c-F | GTTTAACACAGCCAGTGCGCTTC | RT-PCR for NMR-MEF2c |
| NMR-MEF2c-R | CTCTCTGTCGCTGCCGTCGTA | |
| NMR-GATA4-F | CCCTCCATCCACCCGGTCCTC | RT-PCR for NMR-GATA4 |
| NMR-GATA4-R | ACGCAGTGATTATGTCCCCGTGACT | |
| NMR-MAP2-F | GATTCTCCTATGCCAAGTCCCT | RT-PCR for NMR-MAP2 |
| NMR-MAP2-R | CTGTTTCTGCCACTTTATCAGGT | |
| NMR-MSI1-F | AGCTCGACTCCAAAACAATTGACCC | RT-PCR for NMR-MSI1 |
| NMR-MSI1-R | CATCCACCTTCCCGAACTGCT | |
| NMR-HAS2-F | GAAAAGGGTCCTGGTGAGACGGATGAG | RT-PCR for NMR-HAS2[14] |
| NMR-HAS2-R | TTCACCATCTCCACAGATGAGGCAGG | |
| pALT-p15S-F | CAGGAAAAGCCCGGAACTAACTAC | qRT-PCR for NMR-pALT[16] |
| pALT-p16AS-R | GGTGACAGGGTCAGCGCAGTTCG | |
| NMR-ERAS-Cl-F | CACCATGGAGCTGCCACTGCCACCTAGT | NMR-ERAS cloning |
| NMR-ERAS-Cl-R | TCATGGTCCTCCAAGAAGCACT | |
| ERAS-com-F | CACAGAGCAGCCACAGCTACAC | RT-PCR for Ms and NMR ERAS |
| ERAS-com-R | GGCAAGGTGTGGAGGAAGCCTT | |
| NMR-ERAS-F | TTCCCACCTGCTTCTGCCAT | RT-PCR for NMR-ERAS |
| NMR-ERAS-R | GCTCCCTTCGTGGAAACCTCA | |
| HRasV12-F | AAGAGTGCGCTGACCATCCAG | RT-PCR for HRasV12 |
| HRasV12-R | TTTGATCTGCTCCCTGTACTGGTG | |
| Ms-Ink4a-F | GTGTGCATGACGTGCGGG | qRT-PCR for Ms-Ink4a |
| Ms-Ink4a-R | GCAGTTCGAATCTGCACCGTAG | |
| Ms-Arf-F | GCTCTGGCTTTCGTGAACATG | qRT-PCR for Ms-Arf |
| Ms-Arf-R | TCGAATCTGCACCGTAGTTGAG | |
| Ms-Ink4b-F | AGATCCCAACGCCCTGAAC | qRT-PCR for Ms-Ink4b |
| Ms-Ink4b-R | CCCATCATCATGACCTGGATT | |
| Ms-Cdkn1a-F | TCCCGTGGACAGTGAGCAGTTG | qRT-PCR for Ms-p21 |
| Ms-Cdkn1a-R | CGTCTCCGTGACGAAGTCAAAG | |
| Ms-Nanog-F | TTCTTGCTTACAAGGGTCTGC | qRT-PCR for Ms-Nanog |
| Ms-Nanog-R | AGAGGAAGGGCGAGGAGA | |
| Ms-Esrrb-F | CTGCCGATTTCCCCACCTG | qRT-PCR for Ms-Esrrb |
| Ms-Esrrb-R | TGAGGAACACAAGCTCCCGAT | |
| Arf-tg-F | CCAAGAGCGGGGACATCAAGACA | qRT-PCR for Arf tg |
| Arf-tg-R | CCACACCAGCCACCACCTT | |
| Ms-Des-F | AGCTCTCCCGTGTTCCCTC | qRT-PCR for Ms-Des |
| Ms-Des-R | CAGCGACCCCAAGCCTCC | |
| Ms-Sox17-F | GTGGACCGCACGGAATTCGAA | qRT-PCR for Ms-Sox17[45] |
| Ms-Sox17-R | GCAATAGTAGACCGCTGAGCTA | |
| Ms-Gata6-F | ACCTTATGGCGTAGAAATGCTGAGGGTG | qRT-PCR for Ms-Gata6 |
| Ms-Gata6-R | CTGAATACTTGAGGTCACTGTTCTCGGG | |
| Ms-Sox1-F | TGAACGCCTTCATGGTGTGGTC | qRT-PCR for Ms-Sox1[45] |
| Ms-Sox1-R | GCGCGGCCGGTACTTGTAAT | |
| cMYC-com-F | TGCTCCACCTCCAGCCTGTACCT | pRT-PCR for Ms and NMR c-MYC |
| cMYC-com-R | CCTCATCCTCTTGCTCTCCTTCAG | |

**Supplementary Table 2 | shRNA sequences**

| Target gene | Sequence |
|---|---|
| NMR-ARF #1 | 5'-GGGCTTTCGTGGTGCAGATCC -3' |
| NMR-ARF #2 | 5'-GCGGGCTTTCGTGGTGCAGAT-3' |
| NMR-ARF #3 | 5'-GGCCCTCTTGCTGATGCTAGT-3' |
| NMR-INK4a | 5'-GGTCCAGGAGGTACGCGAGCT-3' |