Supplemental Information

Supplemental information includes eleven figures, nine tables, supplemental experimental procedures, and supplemental references.
A

Pluripotency genes in A3-1 mESC

- Nanog
- Pou5f1
- Klf4

Relative expression

siRNA: NC1, NC2, Ddx47, Ddx52, Krr1, Pdcd11

B

siNC siDdx47 siDdx52

siSmc1a siKrr1 siPdcd11

C

Krr1/Ssea1/DAPI Esrrb/Ssea1/DAPI Tfcp2l1/Ssea1/DAPI

siNC siKrr1
A

Comparison between DEGs from core TF knockdown and those from Krr1 knockdown

| RNAi set | Overlap with DEGs from siKrr1 | Odd ratio |
|----------|-------------------------------|-----------|
| Esrrb kd | p = 4.094e-62                 | 7.143     |
| Nanog kd | p = 5.521e-47                 | 4.573     |
| Pou5f1 kd| p = 2.678e-55                 | 5.846     |
| Sox2 kd  | p = 1.740e-54                 | 5.187     |
| Tbx3 kd  | p = 5.829e-38                 | 5.492     |
| Tct1 kd  | p = 1.483e-48                 | 8.125     |

One-tailed Fisher’s exact test

B

Changes of the mRNA levels of SSUP genes following core TF knockdown

| Symbol | shEsrrb | shNanog | shPou5f1 | shSox2 | shTbx3 | shTct1 | Average |
|--------|---------|---------|---------|--------|--------|--------|---------|
| Krr1   |         |         |         |        |        |        |         |
| Wdr42  |         |         |         |        |        |        |         |
| Mphoph10|        |         |         |        |        |        |         |
| Ddx10  |         |         |         |        |        |        |         |
| Esf1   |         |         |         |        |        |        |         |
| Rsl1d1 |         |         |         |        |        |        |         |
| Ncl    |         |         |         |        |        |        |         |
| Bms1   |         |         |         |        |        |        |         |
| Dxs15  |         |         |         |        |        |        |         |
| Up11l  |         |         |         |        |        |        |         |
| Up18   |         |         |         |        |        |        |         |
| Ddx52  |         |         |         |        |        |        |         |
| Wdr36  |         |         |         |        |        |        |         |
| Ngn7   |         |         |         |        |        |        |         |
| Ut20   |         |         |         |        |        |        |         |
| Nrp6   |         |         |         |        |        |        |         |
| Ltv1   |         |         |         |        |        |        |         |
| Dxs8   |         |         |         |        |        |        |         |
| Nol6   |         |         |         |        |        |        |         |
| Nhp21l |         |         |         |        |        |        |         |
| Csrrk2a1|        |         |         |        |        |        |         |
| Rrp9   |         |         |         |        |        |        |         |
| Imp6   |         |         |         |        |        |        |         |
| Cdh1a  |         |         |         |        |        |        |         |
| Noc4l  |         |         |         |        |        |        |         |
| Ddx18  |         |         |         |        |        |        |         |
| Ddx47  |         |         |         |        |        |        |         |
| Emgr1  |         |         |         |        |        |        |         |
| A1a1   |         |         |         |        |        |        |         |
| Fbl    |         |         |         |        |        |        |         |
| Pwp2   |         |         |         |        |        |        |         |
| Tbl3   |         |         |         |        |        |        |         |
| Rc1l   |         |         |         |        |        |        |         |
| Ddx49  |         |         |         |        |        |        |         |
| El4a3  |         |         |         |        |        |        |         |
| Heatr1 |         |         |         |        |        |        |         |
| Imp3   |         |         |         |        |        |        |         |
| Fbm19  |         |         |         |        |        |        |         |
| Wdr46  |         |         |         |        |        |        |         |
| Wdr75  |         |         |         |        |        |        |         |

log2 fold change (Kd/Ctr)
A

OSKM transduction with shRNA viruses

AP-staining

MEFs

Day 0

LIF with Dox

Day 5

Day 10

Day 15

LIF + 2i

iPSCs

qRT-PCR: gene expression profiling during reprogramming

Immunostaining and EB formation

2 weeks culture without Dox

B

C

ESC-specific genes

Lineage-specific genes

D

Krr1 (SSUP component)

Nanog (pluripotency gene)

Esrrb (pluripotency gene)

E

p53 (cell cycle repressor)

p21 (cell cycle repressor)
A. Expression of 53 SSUP genes in mouse tissues and cell lines

B. Box plots of log2 gene expression

C. Western blot analysis of Ddx47, Krr1, Mpp10, Wdr46, and Gapdh
Proteome profile during iPSC formation

SSUP genes (n=52)  Others (n=7,500)
Rate of protein synthesis

S\textsuperscript{35}-Met incorporation (% of control)

mESC  NIH3T3  MEF
**A**

|          | ESC | EB 1d | EB 2d | EB 3d | EB 4d | EB 6d |
|----------|-----|-------|-------|-------|-------|-------|
| Nanog    |     |       |       |       |       |       |
| Pou5f1   |     |       |       |       |       |       |
| Actb     |     |       |       |       |       |       |

**B**

- UV absorbance (254 nm)
- Sucrose gradient
- Polysome

**C**

- Metabolic labeling
- Coomassie staining

**D**

Protein synthesis rate

ESC | EB 1d | EB 2d | EB 3d | EB 4d | EB 6d
---|-------|-------|-------|-------|-------
0   | 0     | 0     | 0     | 0     | 0

S-Met incorporation
Distribution of TE fold changes

std: 0.016
Supplemental Figure Legends

**Supplemental Fig. S1. RNAi screening and validation of Initial hits, related to Fig. 1 (A)**
The average ranked z-score of each marker from the initial RNAi screen is plotted. The red circles indicate the initial hits. **(B)** Among 247 genes, 28 RBPs are selected as regulators of ESC status from initial screen. **(C)** Representative data from the secondary screen using four individual siRNAs. If more than 2 out of 4 siRNAs induce differentiation, the gene was considered as a positive hit. All the mRNA levels were normalized against Actb mRNA or others (when indicated). **(D)** qRT-PCR shows the relative Nanog expression levels after RNAi of ribosome biogenesis factors. The orange and blue bars denote SSUP and other ribosome biogenesis genes, respectively. All the error bars represent the mean ± SD from triplicates or duplicate experiments (when indicated).

**Supplemental Fig. S2. SSUP genes are involved in regulation of ESC identity, related to Fig. 2**
**(A)** Altered expression of ESC markers upon RNAi of SSUP genes in A3-1 mESCs. **(B)** Morphological changes upon knockdown of SSUP genes. RNAi of Smc1a was used as a control. Bars, 100 μm. **(C)** Immunostaining of Krr1 knockdown R1 mESCs for endogenous Krr1, Esrrb, and Tfcp2l1 (green), Ssea1 (red), and nuclei (blue). Scale bars, 50um.

**Supplemental Fig. S3. Krr1 depletion impairs the expressions of pluripotency gene network, related to Fig. 2**
**(A)** Overlaps between the DEGs from core TF knockdown and the DEGs from Krr1 knockdown. **(B)** Expression of SSUP components such as Krr1, Wdr43, and Mphosph10, upon knockdown of core ESC regulators (Ivanova et al. 2006). Bold symbols denote the SSUP genes that are shown to influence ESC maintenance in this study.
Supplemental Fig. S4. Experimental process of iPSC reprogramming by ectopic expression of defined factors, related to Fig. 2 (A) Schematic diagram of reprogramming procedure. (B) Immunostaining of derived iPSC clones for pluripotency markers. Nanog and Esrrb (green), Ssea1 (red), Sox2 (white), and nuclei (blue). Scale bars, 50um. (C) qRT-PCR shows the relative expression of pluripotency (Pou5f1, Sox2), ectoderm (Fgf5), mesoderm (T/brachyury), endoderm (Gata4), and extra-embryonic (Cdx2) markers during EB differentiation of derived iPSC clones. (D and E) qRT-PCR shows the expression change of several marker genes during reprogramming process of MEFs with shRNAs for negative control (shControl) or Krr1 (shKrr1). All the error bars represent the mean ± SD of triplicates.

Supplemental Fig. S5. SSUP is Preferentially Expressed in Pluripotent Stem Cells, related to Fig. 3 (A) The median expression values of 53 SSUP components in 89 mouse tissues and cell types are shown. Re-analysis of the microarray data from BioGPS, web-based database (Lattin et al. 2008; Wu et al. 2009). The red and blue bars denote stem cells and progenitor cells, respectively. The orange line indicates expression profile of Krr1. (B) Violin plot shows that expression pattern of 53 SSUP genes in representative tissues, which are indicated by an asterisk (*) in (A). Dots indicate the expression level of each SSUP components. (C) Western blotting validates the expression of SSUP genes in R1 mESC, NIH3T3, and MEF.

Supplemental Fig. S6. SSUP is upregulated during iPSC reprogramming, related to Fig. 3 Proteome profiles during iPSC reprogramming indicate that the SSUP components are upregulated in early phase and the enhanced levels are maintained during the reprogramming
process (Hansson et al. 2012). D denotes days after reprogramming, MEF denotes mouse embryonic fibroblast.

**Supplemental Fig. S7.** Krr1 is required for pre-18S rRNA processing in mESCs, related to Fig. 4 (A) Analysis of pre-rRNA processing by Northern blotting. Lentiviruses expressing three different shRNA for Krr1 are transduced in R1 cells, respectively. A schematic representation of pre-rRNA processing intermediates is shown on the right side. The position of ITS-1 probe is indicated as a red bar. (B) Knockdown of Krr1 results in the reduction of the ratio of 28S rRNA versus 18S rRNA as measured by microfluidics-based electrophoresis.

**Supplemental Fig. S8.** The p53-dependent ribosomal stress pathway is not required for reduction of Nanog in SSUP knockdown cells. Simultaneous knockdown of p53 does not rescue the reduction of Nanog expression in SSUP-depleted cells. Expression level of genes was measured two days after siRNA transfection. Induction of Cdkn1a/p21 expression is a mark of p53 activation. Knockdown of Rpl37 is used as a control that triggers the p53-dependent ribosomal stress response in mESCs (Morgado-Palacín et al. 2012).

**Supplemental Fig. S9.** Mouse ESC has higher protein synthesis rate compared to NIH3T3 and MEF, related to Fig. 4 Protein synthesis rate in different cell lines were measured by S35-methionine incorporation assay.

**Supplemental Fig. S10.** General translational capacity is decreased during exit from pluripotency, related to Fig. 4 (A) Western blotting determines the protein levels of Nanog
and Pou5f1 during EB differentiation. (B) Polysome profiles show the decreased polysome content during early phase of differentiation. (C and D) Metabolic labeling shows the change of protein synthesis rate during EB differentiation.

Supplemental Fig. S11. The distributions of fold change for translation efficiency in response to Krr1-knockdown, related to Fig. 4
Supplemental Tables

Supplemental Table S1. List of 247 RBPs for RNAi screen, related to experimental procedures

Supplemental Table S2. Results of RNAi screen (z-score values for each markers), related to Fig. 1

Supplemental Table S3. Initial 28 hits from the RNAi screen, related to Supplemental Fig. S1

Supplemental Table S4. List of genes excluded due to cell death, related to Supplemental Fig. S1

Supplemental Table S5. List of final 16 RBPs important for pluripotency maintenance, related to Fig. 1

Supplemental Table S6. Putative components of SSUP used in this study

Supplemental Table S7. Differentially expressed genes from RNA-seq, related to Fig. 2

Supplemental Table S8. Gene set enrichment analysis, related to Fig. 2

Supplemental Table S9. Oligonucleotides used in this study, related to experimental procedures

*Supplemental Table S1, S2, S6, and S7 are available as separate Excel files:
Supplemental Table S3. Initial 28 hits from the RNAi screen, related to Supplemental Fig. S1

| Symbol | Accession No.     | Name                                           | Notes (Related to pluripotency or lncRNA)                                                |
|--------|-------------------|------------------------------------------------|------------------------------------------------------------------------------------------|
| Cnot6* | NM_212484         | CCR4-NOT transcription complex, subunit 6      |                                           |
| Cwc15  | NM_023153         | CWC15 homolog (S. cerevisiae)                  |                                           |
| Ddx47  | NM_026360         | DEAD (Asp-Glu-Ala-Asp) box polypeptide 47      | Pou5f1-interaction                                                                         |
| Ddx52  | NM_030096         | DEAD (Asp-Glu-Ala-Asp) box polypeptide 52      |                                           |
| Ddx56  | NM_026538         | DEAD (Asp-Glu-Ala-Asp) box polypeptide 56      |                                           |
| Dkc1   | NM_001030307      | Dyskeratosis congenita 1, dyskerin homolog (human) | Dkc1-mutant iPSCs show telomere shortening and loss of self-renewal                       |
| Exosc8 | NM_027148         | Exosome component 8                            |                                           |
| Exosc9 | NM_019393         | Exosome component 9                            | Necessary for the self-renewal of epidermal progenitor cells                             |
| Hnrnpk*| NM_025279         | Heterogeneous nuclear ribonucleoprotein K      | Regulates lncRNA-p21 or lncRNA TUNA                                                      |
| Hnrnpu*| NM_016805         | Heterogeneous nuclear ribonucleoprotein U      | Interact with lncRNA Firre, Pou5f1-interaction                                           |
| Krr1   | NM_178610         | KRR1, small subunit (SSU) processome component, homolog (yeast) |                                           |
| Nifk*  | NM_026472         | Mki67 (FHA domain) interacting nucleolar phosphoprotein | Suggest the functional importance of nucleolus in pluripotency                          |
| Nol6   | NM_139236         | Nucleolar protein family 6 (RNA-associated)    |                                           |
| Nop2   | NM_138747         | NOP2 nucleolar protein homolog (yeast)         | Interacts with lncRNA-hPVT1                                                              |
| Pabpn1 | NM_019402         | Poly(A) binding protein, nuclear 1             | Promotes lncRNA turnover                                                                |
| Pdcd11 | NM_011053         | Programmed cell death 11                       |                                           |
| Label   | Accession No. | Symbol       | Notes (Related to pluripotency or lncRNA) |
|---------|---------------|--------------|------------------------------------------|
| Poldip3 | NM_178627     | Polymerase (DNA-directed), delta interacting protein 3 |                                          |
| Ptbp1*  | NM_001077363  | Polypyrimidine tract binding protein 1                  | Regulates lncRNA TUNA                    |
| Rbm19   | NM_028762     | RNA binding motif protein 19                            |                                          |
| Rbm28   | NM_133925     | RNA binding motif protein 28                            |                                          |
| Rbm42   | NM_133693     | RNA binding motif protein 42                            |                                          |
| Rrp7a   | NM_029101     | Ribosomal RNA processing 7 homolog A (S. cerevisiae)    |                                          |
| Rrp9    | NM_145620     | RRP9, small subunit (SSU) processome component, homolog (yeast) |                                          |
| Srbd1   | NM_030133     | S1 RNA binding domain 1                                 |                                          |
| Srrm1   | NM_016799     | Serine/arginine repetitive matrix 1                     |                                          |
| Srsf7   | NM_146083     | Serine/arginine-rich splicing factor 7                   |                                          |
| Tardbp* | NM_001003898  | TAR DNA binding protein                                 |                                          |
| Zc3h14  | NM_029334     | Zinc finger CCCH type containing 14                      |                                          |

* = identified genes for pluripotency in previous RNAi screens

**Supplemental Table S4.** List of genes excluded due to cell death, related to Supplemental Fig. S1
| Gene ID | Gene Symbol | Description |
|---------|-------------|-------------|
| NM_138669 | Eif4a3 | Regulates lncRNA TUNA, role in p53 activation |
| NM_025513 | Exosc3 | Interacts with KSHV viral lncRNA PAN |
| NM_010760 | Magoh | Interacts with IncRNA and regulates translation of a subset of genes |
| NM_010880 | Ncl* | Regulates proper splicing of ESC factors in hESCs |
| NM_008774 | Pabpc1 | Interact with MALAT1 and organize speckle assembly |
| NM_021568 | Pcbp3 | |
| NM_025443 | Pno1 | |
| NM_026329 | Polr2g | |
| NM_027328 | Prpf31 | |
| NM_138659 | Prpf8 | |
| NM_028364 | Puf60 | |
| NM_025776 | Rbm22 | |
| NM_133242 | Rbm39 | |
| NM_020600 | Rps14 | |
| NM_009091 | Rps15 | |
| NM_026147 | Rps20 | |
| NM_026175 | Sf3a1* | |
| NM_009224 | Snrnp70 | |
| NM_021336 | Snrpa1 | |
| NM_011432 | Snrpc | |
| NM_009227 | Snrpe | |
| NM_027246 | Snrpf | |
| NM_178880 | Son* | |
| NM_173374 | Srsfl | |
| NM_011358 | Srsf2 | |
| NM_013663 | Srsf3* | |
| NM_009297 | Supt6h | |

**Gene IDs:** NM_138669, NM_025513, NM_010760, NM_010880, NM_008774, NM_021568, NM_025443, NM_026329, NM_027328, NM_138659, NM_028364, NM_025776, NM_133242, NM_020600, NM_009091, NM_026147, NM_026175, NM_009224, NM_021336, NM_011432, NM_009227, NM_027246, NM_178880, NM_173374, NM_011358, NM_013663, NM_009297
### Supplemental Table S5.
List of final 16 RBPs important for pluripotency maintenance, related to Fig. 1

| Symbol | Aliases | Name | Known Functions, notes | Subcellular Location |
|--------|---------|------|-------------------------|----------------------|
| Cwc15  | mED1, Cwf15, Ed1 | CWC15 homolog (S. cerevisiae) | splicing | Nu, Mt |
| Ddx47  | E4-DBP, RRP3 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 47 | rRNA processing (SSUP), Pou5f1-interaction | No |
| Ddx52  | HUSSY19, ROK1 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 52 | rRNA processing (SSUP) | Nu, No |
| Ddx56  | DDX21, DDX26, Noh61 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 56 | rRNA processing | Nu, No* |
| Hnrnpu | Hnrpu, SAFA, Sp120 | Heterogeneous nuclear ribonucleoprotein U | splicing, lncRNA regulation, Pou5f1-interaction | Nu |
| Hnrnpk | CSBP, KBBP, NOVA, Hnrpk | Heterogeneous nuclear ribonucleoprotein K | | Nu |
| Krr1   | HRB2, RIP-1 | KRR1, small subunit (SSU) processome component | rRNA processing (SSUP) | Nu, No |
| Nifk   | Mki67ip, Nopp34 | Mki67 (FHA domain) interacting nucleolar phosphoprotein | rRNA processing, role in mitosis | No |
| Nol6   | NRAP, UTP22 | Nucleolar protein family 6 (RNA-associated) | rRNA processing (SSUP) | Nu, No, Mt |
| Pdcd11 | ALG4, NFBP, RRP5, Pdcd7 | Programmed cell death 11 | rRNA processing (SSUP) | No |
| Ptbp1  | HNRPI, PTB-1, PTB2, PTB3, PTB4, Ptb, pPTB | Polypyrimidine tract binding protein 1 | splicing, lncRNA regulation | Nu |
| Rbm28  | ANES, 2810480G15Rik | RNA binding motif protein 28 | rRNA processing | No |
| Rbm42  | 1700003D06Rik | RNA binding motif protein 42 | splicing, 3'UTR binding | Nu, Cy |
| Rrp7a  | Kheg1 | Ribosomal RNA | rRNA processing | Nu, No |

* = identified genes for pluripotency in previous studies
### Supplemental Table S8. Gene set enrichment analysis, related to Fig. 2

| Category                                      | GO Term                                           | P-value   |
|-----------------------------------------------|---------------------------------------------------|-----------|
| **GO_Functional Annotation Clustering with Downregulated Genes in RNAseq** | GO:0043228~non-membrane-bounded organelle          | 1.99E-06  |
|                                               | GO:0048863~stem cell differentiation              | 5.06E-04  |
|                                               | GO:0065003~macromolecular complex assembly        | 1.64E-05  |
|                                               | GO:0006412~translation                            | 6.39E-04  |
|                                               | GO:0001568~blood vessel development               | 9.39E-04  |
|                                               | GO:0003700~transcription factor activity          | 0.00127   |
|                                               | GO:0001829~trophectodermal cell differentiation    | 0.00464   |
|                                               | GO:0009792~embryonic development ending in birth or egg hatching | 0.00557    |
| **GO_Functional Annotation Clustering with Upregulated Genes in RNAseq**  | GO:0007155~cell adhesion                          | 1.29E-13  |
|                                               | GO:0070161~anchoring junction                     | 4.71E-10  |
|                                               | GO:0001944~vasculature development               | 7.70E-05  |
|                                               | GO:0043067~regulation of programmed cell death    | 1.72E-05  |
|                                               | GO:0035239~tube morphogenesis                     | 6.44E-05  |

Nu = Nucleus, No = Nucleolus, Cy = Cytoplasm, Mt = Mitochondria, * = by similarity
### GO:0004672~protein kinase activity 3.48E-05

### GO:0007010~cytoskeleton organization 3.99E-04

### GO:0016477~cell migration 3.83E-04

**Supplemental Table S9.** Oligonucleotides used in this study, related to experimental procedures

| Name     | Note   | Sequence               |
|----------|--------|------------------------|
| Nanog    | forward| TTGCTTACAAGGGTCTGCTACT |
|          | reverse| ACTGGTAGAAGAATCAGGGCT  |
| Fgf5     | forward| TGTGTTCAGGGGATTGTAGG   |
|          | reverse| AGCTGTTTTTCTTGAATCTCTCC|
| Cdx2     | forward| CAAGGACGTGAGCATGTATCC  |
|          | reverse| GTAACCACCGTAGGTCCGGTA  |
| Klf4     | forward| AGGAACTCTCTCACAATGAAAGC|
|          | reverse| GGTCGTTGAACCTCCTCGGTC  |
| Tbx3     | forward| ACTCGGGGTTCTCGAACTGAA  |
|          | reverse| GGAGGGGGCGATTTTGTTTT   |
| Pou5f1   | forward| CACCATCTGCGCTTCGAG    |
|          | reverse| AGGGCTCCGTAGATTTGCTATCT|
| Brachyury| forward| CTCGGATTACATCGTGAGAG  |
|          | reverse| AAGGCTTAAAGCAGTGTTGTA  |
| Gata6    | forward| TTGCTCCTGTAACAGCAGTG   |
|          | reverse| GTGGTCGCTTGTCAGAAGG    |
| Gapdh    | forward| AGGTCGGTGAACGGATTGG    |
|          | reverse| TGTAGACCATGAGGTTGAGGTA |
| Name          | Note | Sequence                      |
|---------------|------|-------------------------------|
| Actb          |      | forward GGCTGTATTCCCTCCATCG    |
|               |      | reverse CCAGTTGGTAAACATGCCATGT |
| 45S pre-rRNA  |      | forward GGTTGAGGGCCACCTATTTT  |
| 5'ETS         |      | reverse GGAGGCATGTGGAAGAAAGA  |
| 45S pre-rRNA  |      | forward ACGACCGTGTTGGTGTTTG   |
| 3'ETS         |      | reverse CTCCCAACCCCAAGGAGAG   |

**Oligonucleotides for construction of shRNA vectors**

| Name          | Note | Sequence                      |
|---------------|------|-------------------------------|
| Krr1 shRNA-1  |      | forward CCGGGGCTCTCTAGTGAGAAATAAGCTCGAGCTTTATTTTC TCACTAGAGAGCTTTTTG |
|               |      | reverse AATTCCAAAAAGCTCTCTATGGAAGAAATAAGCTCGAGCTTTATTTTC ATTTCTCACTAGAGAGC |
| Krr1 shRNA-2  |      | forward CCGGGGCAGAGAGTCAGATTGATTAGTAAACTCGAGTTATTAATCAATC TGACTCTCTTGGCTTTTTG |
|               |      | reverse AATTCCAAAAAGCCAGAGAGTCAGATTGACTCGAGTTAATCTG ACTCGACTCTCTTGG |
| Ddx47 shRNA-1 |      | forward CCGGGGGATTATATCCATCGAGTTGGCTCGAGCACAATCGG ATGGATATAATCCTTTTTG |
|               |      | reverse AATTCCAAAAAGCTCTCTATCAGTGACTGACTCGAGCACAATCGG TCACTATGGAAGATATC |
| Ddx47 shRNA-2 |      | forward CCGGGGTCACGGAACGTGTAATGACTCGAGTCATTCAATCAGTGGGCTCGAGCCA ACTCGATGGAATATC |
|               |      | reverse AATTCCAAAAAGGCTACTCAGAAACTGTAATGACTCGAGTCATTCAATCAGTGGGCTCGAGCCA AACCACTG |
| Ddx52 shRNA-1 |      | forward CCGGGGGACTAGACTTCTTCGGAACACTCGAGGTTTCCGA AGAAGTCTAGTCTTTTTG |
|               |      | reverse AATTCCAAAAAGGACTAGACTTCTTCGGAACACTCGAGGTTTCCGA AGAAGTCTAGTCTTTTTG |
| Ddx52 shRNA-2 |      | forward CCGGGGAGAGAAGAAGTTAACTTCACTCGAGTGAAGTTAACCTCTTCTCTTCTTTTTG |
|               |      | reverse AATTCCAAAAAGGACTAGACTTCTTCGGAACACTCGAGGTTTCCGA AGAAGTCTAGTCTTTTTG |
| Pdcd11 shRNA-1|      | forward CCGGACACCAAGTCCCGAGGAAATACACTCGAGGTATTTTCT CCGACTTTGGGTCTTTTTT |
|               |      | reverse AATTCCAAAAACACCAAGTCCCGAGGAAATACACTCGAGGTATTTTCT CCGACTTTGGGTCTTTTTT |
Supplemental Experimental Procedures

**Cell Culture and Differentiation**

R1 mouse embryonic stem cells were maintained on 0.1% gelatin-coated dishes with ESC media (DMEM containing 15% FBS (Gibco), 1X nonessential amino acids (Gibco), 100 uM 2-mercaptoethanol (Sigma), and 1000 U/ml LIF (Millipore)). For retinoic acid (RA) differentiation, ESC cultured with ESC media without LIF, and containing 1 uM RA. For embryoid body (EB) differentiation culture, ESCs were suspension cultured on petri dishes with ESC media without LIF and split every 2 days. For inhibition of general translation, ESCs were cultured 3 days in ESC culture media containing 50 uM 4EGI-1.

**RNAi Library Construction and RNAi Screen**

Among 443 annotated RBPs in the uniprot database, we selected 247 RBPs according to its expression level in mESCs (See also Supplemental Table S1). We designed 4 different 21-mer RNA duplex for each of the 247 RBPs using block-it RNAi designer (Invitrogen). R1 cells were reverse transfected as follows: final 20 nM siRNA and one ul Lipofectamine 2000 were mixed with 100 ul Opti-MEM in each well of a 24-well plate. After 15 min incubation, 1.0
x $10^4$ cells with 300 ul medium were seeded in each well. Luciferase siRNA, negative control siRNA (Bioneer), or without siRNA (Lipofectamine only) were used as negative controls. Smc1a siRNA was used as a positive and normalization control for plate batch variation. Cells were incubated in ESC medium for 4 days and harvested for RNA extraction. When we observed the apoptotic cell debris by light microscopy and the total amount of RNA is lower than 10 % of control set, we designated the siRNA as causing “severe” cell death and hence excluded them from the subsequent experiments even when the marker expression indicated differentiation (Table S4).

**Quantitative Real-Time PCR**

RNA was isolated the illustra RNAspin Kit (GE Healthcare), according to the manufacturer’s instructions, and cDNA was produced with the Primescript RT Kit (Takara). Quantitative Real-time PCR reactions were set up in duplicate or triplicate with the Brilliant II SYBR Premix EX Taq Kit (Takara) and run on a StepOnePlus System (Applied Biosystems). Relative expression level of each gene was calculated using the comparative $Ct$ method with $Actb$ or $Gapdh$ as a normalization control. Primer sequences for qPCR were taken from PrimerBank (Wang and Seed 2003) and are listed in Supplemental Table S9.

**Construction of RNA-seq and Ribosome Profiling Library**

RNA-seq and Ribosome profiling libraries were prepared as described previously (Cho et al. 2012), according to the protocol of ARTseq Ribosome Profiling Kit (Epicentre) with minor modifications. Briefly, R1 cells were transfected with three different siRNAs targeting controls and 3 different siRNAs targeting Krr1 for 48 hrs. Cell lysates were divided equally for RNA-seq library and ribosome profiling library. Lysates were digested by RNase I (Ambion) and
gel purified in the range of 30 nt for ribosome profiling or 40-60 nt for RNA-seq library. The purified RNAs were subjected to reverse-transcription, after ligated with 3’ and 5’ adapter. The cDNA was used as a template for PCR amplification using Phusion polymerase (NEB). Reactions were separated by acrylamide gel and purified again. Quantify and characterize the purified cDNA libraries using bioanalyzer (Agilent) and Kapa library quantification kit, respectively. The libraries were sequenced on Illumina HiSeq 2000 (multiplexing 50 base single-end). All adapters and primers were synthesized by IDT.

Classification of SSUP components

About 72 proteins were previously reported to be required for 18S rRNA processing and associated with U3 snoRNA and Mpp10 (Phipps et al. 2011). Among 72 putative SSUP components, 54 genes showed the defects of pre-rRNA processing upon RNAi transfection in human cells (Tafforeau et al. 2013). We used these 54 genes as components of SSUP in this study (Supplemental Table S6).

Bioinformatics Analysis

To analyze the mRNA profile during EB differentiation presented in Fig. 3B, we used the published data from previous RNA-seq study (Liu et al. 2011). For the proteome profile during reprogramming into iPSCs, we used the published proteome data (Hansson et al. 2012). These data were imported into the R program for further analysis shown in Fig. 3B and Supplemental Fig. S65.

Expression levels of SSUP genes in various tissues are re-analyzed based on the preprocessed version of microarray data from GSE10246, provided by BioGPS (Lattin et al. 2008; Wu et al. 2009). Probe annotations are obtained from the platform GPL1261, and
probe sets suffixed by "_s_at" and "_x_at" are excluded to guarantee that the one gene per one probe. The median of probe intensities are assigned to each gene if a certain gene has multiple probes. Among 54 SSUP genes, Nom1 is excluded from this analysis because of no probe annotation is available. Summarized expression levels are then subjected to show SSUP gene expression profiles in various tissues (Fig. 3C and Supplemental Fig. S54).

Sequencing reads from RNA-seq firstly preprocessed to keep only high-quality reads. Preprocess carried out by applying trimming low-quality bases from 3′ end, adapter clipping, filtering out low-quality or artifacts, sequentially, using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Remained high-quality reads are then aligned to mouse genome, mm10, with STAR aligner (Dobin et al. 2013). We used Illumina iGenome as a reference annotation, and counted reads for each gene using HTSeq (Anders et al. 2015). Read counts are further normalized by upper quartile. RPF sequences are processed in the same manner, and translational efficiency is calculated as simply normalized RNA-seq read counts over normalized RPF read counts (Fig. 4E, 4F, and Supplemental Fig. S119).

For DEG analysis, lipofectamine-only, siGFP, negative control siRNA samples are considered as replicates for the control, and the rest as replicates for Krr1-depleted. The categorized read counts are then fed up to DESeq R package to obtain the list of differentially expressed genes (Anders and Huber 2010) (Fig. 2D). To examine how significant the overlap between our DEGs and the affected genes from depletion of pluripotency factors is (Ivanova et al. 2006), we compared the gene lists to generate 2x2 contingency table and then performed one-tailed Fisher’s exact test (Supplemental Fig. S43A).

Gene Ontology analysis was performed with the web-based bioinformatics resources DAVID 6.7 (Huang da et al. 2009) using the functional annotation clustering for biological process, molecular function, and cellular component. We selected top 8 annotation cluster based on the enrichment score. Most significant GO term of each cluster are listed in
Supplemental Table S8.

**S\textsuperscript{35}-Methionine Metabolic Labeling**

R1 mESCs transfected with siRNA targeting control or RBPs for 48 hr, and incubated with media lacking methionine for 40 min. S\textsuperscript{35}-labeled methionine was added to the media and incubated for additional 30 min. Cells were harvested and the protein concentration were determined by BCA assay in triplicates. Equal amount of protein were separated by 10% SDS-PAGE and exposed to an imaging plate (Fujifilm). Radioisotope signals were read with the BAS-2500 system and the signal intensities were quantified using Multi Gauge (Fujifilm).

**Reprogramming Assay and Alkaline Phosphatase Staining**

To produce infectious viral particles, 293T cells cultured on 10 cm dishes were transfected with the TetO-FUW-OSKM, FUW-M2rtTA (Addgene plasmid 20321 and 20342) (Takahashi and Yamanaka 2006; Carey et al. 2009) or pLKO-shRNA vectors together with the packaging plasmids psPAX2 and pMD2.G (Addgene plasmid 10878, 12260 and 12259) using Fugene HD (Roche). Viral supernatants were harvested on three consecutive days starting 24 hrs after transfection, concentrated by centrifugation followed mixing with Lenti-X concentrator (Clontech). Carefully remove supernatant, viral particles were resuspended in Opti-MEM, and stored at -80°C in single-use aliquots.

For reprogramming, MEFs were infected with lentiviruses containing TetO-FUW-OSKM (containing *Pou5f1*, *Sox2*, *Klf4*, and *c-Myc*) or FUW-M2rtTA in ESC medium containing LIF and doxycycline (Dox). For SSUP knockdown experiment, MEFs were re-infected with lentiviruses encoding shRNAs for Ddx47, Ddx52, Krr1, Pdcd11, and controls, 2 days later of OSKM transduction. Sequences of the shRNAs used are listed in Supplemental
Table S9. Fifteen days after Dox induction, mESCs were fixed 4% paraformaldehyde (PFA) and colonies were stained for alkaline phosphatase (AP) Activity. The number of AP positive colonies were counted by Image J. For immunostaining of iPSCs, reprogrammed cells were further cultured for 2 weeks without Dox treatment.

**Western Blotting**

Samples were resolved on 10% SDS-polyacrylamide gels or 4%–12% NuPAGE gels (Invitrogen) and transferred to Immobilon-P transfer membrane (Millipore) or Hybond C extra-western membrane (Amersham). Primary antibodies used in this study were goat anti-Sox2 (Santa Cruz), mouse anti-Gapdh (Santa Cruz), mouse anti-Mpp10 (Santa Cruz), mouse anti-Ncl (Santa Cruz), mouse anti-Ubf (Santa Cruz), rabbit anti-Actb (Santa Cruz), rabbit anti-Esrrb (Proteintech), rabbit anti-Klf4 (Cell Signaling), rabbit anti-Krr1 (Santa Cruz), rabbit anti-Nanog (Bethyl), rabbit anti-Pou5f1 (Abcam), rabbit anti-Tfcp2l1 (Abcam), and rabbit anti-Wdr46 (Proteintech).

**Polysome profiling**

For polysome fractionation, confluent R1 mESCs in 150mm dish were incubated with 100 mg/ml of cycloheximide for 10 min to arrest ribosome on mRNAs before harvesting the cells. Cells were resuspended in 1 ml of 50 mM MOPS-NaOH at pH 7.4, 150 mM NaCl, 15 mM MgCl2, 0.25% Triton X-100, 1 mg/ml Heparin, 100 mg/ml cycloheximide with 5 ul protease inhibitor cocktail (Calbiochem) and 5 ul RNasin (Promega). Cells were incubated in the buffer for 10 min at 4°C, and subjected to centrifugation for 10 min at 12,000 rpm to obtain clarified lysate. The supernatant was loaded on the 10%-50% sucrose gradient and centrifuged at 36,000 rpm for 2 hr 30 min at 4°C with SW41T rotor (Beckman). Then, the UV
absorbance of gradient were measured at 260 nm by BioLogic LP system (BioRad).

Northern Blotting

Five ug of total RNA were resolved on agarose denaturing gels (6% formaldehyde/1.2% agarose in HEPES-EDTA buffer). Agarose gels were transferred by capillarity overnight in 10 x SSC buffer. Membrane and RNAs were irradiated by CL-1000 UV cross-linker and pre-hybridized for 30 min at 65°C in Hybrid solution containing sperm DNA. Sequence of ITS-1 probe, which is complementary to the ITS1 region of 45S pre-rRNA, is 5′-ACACACAAGACGGGGAGA-3′. The P32-labeled DNA probe was added and incubated for 1 hr at 65°C and washing with first (high salt) and second (low salt) washing solution. Radioisotope signals were read with the BAS-2500 system and the signal intensities were quantified using Multi Gauge (Fujifilm).

Immunostaining

R1 cells or iPSCs were fixed in 4% formaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 2% BSA in PBS. They were incubated with the primary antibodies Nanog (Bethyl), and Krr1 (Santa Cruz), Sox2 (Santa Cruz), Esrrb (Proteintech), Tfcp2l1 (Abcam), and Ssea1 (Cell signaling) for 2 hr at RT. The fixed R1 cells on the slide were visualized with standard immunofluorescence technique and a fluorescence microscope (LSM700, Zeiss). The Alexa Fluor-conjugated secondary antibodies (Invitrogen) were used for visualization (1:1000). DAPI (Vector Laboratories) was used to visualize nuclei, Phalloidin (Invitrogen) was used for actin staining.
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