**Supplementary methods.**

**Immunofluorescence (IF)**

Cells were fixed for 15 min with 100% ice-cold methanol, washed for 15 min with PBS, 0.1% Triton-X-100 and blocked for 15 min in PBS with 2% BSA and 2% horse serum (blocking solution). The cells were incubated with primary antibodies in blocking solution washed with PBS, 0.1% Triton-X-100 and incubated for 40 min with respective secondary antibodies. DAPI (4',6-Diamidin-2'-phenylindol) nuclear counter stain was used as described by the manufacturer (Sigma, D-9542).

**Flow cytometry and FACS**

Flow cytometric analysis and fluorescence-activated cell sorting (FACS) of cultured cells was performed using antibodies DECMA-1 (N-term. E-cadherin, abcam) and SSEA-1. For flow cytometry and FACS, cells were washed once with ice cold PBS, harvested and centrifuged. For labeling 2.5 x $10^5$ cells were diluted in a total volume of 200 µl and primary antibodies (15 min, on ice) were added, followed by secondary antibodies (5 min, 4°C).

**Plasmids**

Annealed small hairpin-forming oligos with the E-cadherin targeting sequence: 5'-AGCTTAAAAAGAACCTGGTTCAGATCAAATTCTCTTGAAATTTGATCTG AACAGGTTCTGGG-3' (top) and 5'-GATCCCCAGAACCTGGTTCAGATCAAATTTCAAGAGAATTTGATCTGAAC CAGGTTCTTTTTA-3' (bottom) were cloned into pMSCV (Clontech).

**Southern-blotting**

10 µg of DNA were digested overnight at 37 °C with BamHI and separated on a 0.8% agarose gel at 60 V for 6 hours and blotted overnight on a hybond$^+$-nylon membrane (Amersham). Generation of an Oct4-probe was performed using specific primers for Oct4-s 5'-ATGGCTGGACACCTGGCTTCAG-3' and Oct4-as 5'-CTCCTCGGGAGTTGGTTCCACC-3' amplifying a 422 bp fragment from exon 1 of the Oct4 gene. The probe was radioactively labeled using Klenow for incorporation of $^{32}$P-dCTP. Membrane was incubated at 65 °C for overnight.

**Global gene expression analysis**

For analysis of global gene expression, RNA extraction was done with Trizol (Invitrogen) followed by a purification step using the RNeasy MinElute Cleanup Kit (Qiagen), cRNA synthesis and labeling was done with the Illumina TotalPrep RNA Amplification Kit (Ambion). Scatter plots were generated with ArrayStar 4 (Lasergene).

**Quantitative real-time PCR and Western analysis**

Antibodies used were against E-cadherin (BD), N-cadherin (BD), Oct4 (Santa Cruz), Nanog (abcam), α-Tubulin (Sigma) and SSEA-1 (R&D). Secondary antibodies were used (Zymed and Jackson Laboratories).

**Supplementary table 1: Primer sequences used in quantitative real-time PCR.**

| *Gene* | *Accession No.* | *Sequence* |
|---|---|---|
| **Ecad-total-s** | NM_009864 | TTGAGGCCAAGCAGCAATACATCC |
| **Ecad-total-as** | | AGATGTGATTTCCTGACCCACACC |
| **Ecad-endo-s** | NM_009864 | CTCAGTGTTTGCTCGGCGTCTGC |
| **Ecad-endo-as** | | CAGGACATGGCCTCTCTCCAGGT |
| **Ecad-viral-s** | | ACGGCGGTGGTGAGGACGACTAG |
| **Ecad-viral-as** | | ATCCTGTTTGGCCCATATTCAGCT |
| **Ncad-s** | NM_007664 | TTGCTTCTGACAATGGAATCCCGC |
| **Ncad-as** | | AAGGAAAGATCAAACGCGAACGGC |
| **Klf4, total-s** | NM_010637 | CACCATGGACCCGGGCGTGGCTGCCAGAAA |
| **Klf4, total-as** | | TTAGGCTGTTCTTTTCCGGGGCCACGA |
| **Klf4, endo-s** | NM_010637 | GCGAACTCACACAGGCGAGAAACC |
| **Klf4, endo-as** | | TCGCTTCCTCTTCCTCCGACACA |
| **c-Myc, total-s** | NM_010849 | CAGAGGAGGAACGAGCTGAAGCGC |
| **c-Myc, total-as** | | TTATGCACCAGAGTTTCGAAGCTGTTCG |
| **c-Myc, endo-s** | NM_010849 | GACCTAACTCGAGGAGGAGCTGGAATC |
| **c-Myc, endo-as** | | AAGTTTGAGGCAGTTAAAATTATGGCTGAAGC |
| **Oct4, total-s** | NM_002701 | CTGAGGGCCAGGCAGGAGCACGAG |
| **Oct4, total-as** | | CTGTAGGGAGGGCTTCGGGCACTT |
| **Oct4, endo-s** | NM_002701 | TCTTTCCACCAGGCCCCCGGCTC |
| **Oct4, endo-as** | | TGCGGGCGGACATGGGGAGATCC |
| **Oct4, viral-s** | | AAGAACCTAGAACCTCGCTGGAAAGG |
| **Oct4, viral-as** | | CTGTAGGGAGGGCTTCGGGCACTT |
| **Sox2, total-s** | NM_011443 | GGTTACCTCTTCCTCCCACTCCAG |
| **Sox2, total-as** | | TCACATGTGCGACAGGGGCAG |
| **Sox2, endo-s** | NM_011443 | TAGAGCTAGACTCCGGGCGATGA |
| **Sox2, endo-as** | | TTGCCTTAAACAAGACCACGAAA |
| **Afp-s** | NM_007423 | AATGACTAGCGATGTGTTGGCTGC |
| **Afp-as** | | TTCATGTGCTTTGCAACTCTCGGC |
| **Brachyury (T)-s** | NM_009309 | ACCACCGCTGGAAATATGTGAACG |
| **Brachyury (T)-as** | | AACTCTCACGATGTGAATCCGAGG |
| **β-Actin-s** | NM_007393 | TCGTGCGTGACATCAAAGAGAAGC |
| **β-Actin-as** | | ATGGATGCCACAGGATTCCATACC |
| **Dppa5-s** | NM_025274 | GAAATATCTGTTTGGCCCACAGGG |
| **Dppa5-as** | | GCCATGGACTGAAGCATCCATTTAGC |
| **GATA4-s** | NM_008092 | TCACAAGATGAACGGCATCAACCG |
| **GATA4-as** | | GCAGGCATTACATACAGGCTCACC |
| **Isl-1-s** | NM_021459 | TGATTTCCCTGTGTGTTGGTTGCG |
| **Isl-1-as** | | ATCACGAAGTCGTTCTTGCTGAAGCC |
| **Krt8-s** | NM_031170 | AGCATTCATACGAAGACCACCAGC |
| **Krt8-as** | | TTGGACACGACATCAGAAGACTCG |
| **Mef2c-s** | NM_025282 | AGCAGCAGCACCTACATAACATGC |
| **Mef2c-as** | | ATGCGCTTGACTGAAGGACTTTCC |
| **MMP2-s** | NM_008610 | AGATGCAGAAGTTCTTTGGGCTGC |
| **MMP2-as** | | AAAGCATCATCCACGGTTTCAGGG |
| **Msx2-s** | NM_013601 | AAATCTGGTTCCAGAACCGAAGGG |
| **Msx2-as** | | CATGGTAGATGCCATATCCAACCG |
| **Myl7-s** | NM_022879 | TTCAGCTGCATTGACCAGAACAGG |

| | | |
|---|---|---|
| **Myl7-as** | | TGAGGAAGACGGTGAAGTTGATGG |
| **Nanog-s** | NM_028016 | AACCAAAGGATGAAGTGCAAGCGG |
| **Nanog-as** | | TCTGGTTGTTCCAAGTTGGGTTGG |
| **Nr5a2-s** | NM_030676 | ACACAGAAGTCGCGTTCAACAACC |
| **Nr5a2-as** | | TAGTTGCAAACCGTGTAGTCCAGC |
| **Sox1-s** | NM_009233 | ATGCACAACTCGGAGATCAGCAAGC |
| **Sox1-as** | | AGTACTTGTCCTTCTTGAGCAGCG |
| **Tbx5-s** | NM_011537 | CAAATGGTCCGTAACTGGCAAAGC |
| **Tbx5-as** | | ACGCAGTGTTCTTTGAACCGAACC |
| **TropT2-s** | NM_001130174 | TCCAACATGATGCACTTTGGAGGG |
| **TropT2-as** | | AGGTTGTGAATACTCTGCCACAGC |
| **Oct4-s (bis. seq)** | | GATTTTGTAGGTGGGATTAATTGTGAATTT |
| **Oct4-as (bis. seq)** | | ACCAAAAAAACCCACACTCATATCAATATA |
| **Nanog-s (bis. seq)** | | TGGGTTGAAATATTGGGTTTATTT |
| **Nanog-as (bis. seq)** | | CTAAAACCAAATATCCA ACCATA |
| **Ecad-s (bis. seq)** | | TGTTATTAATTATAGATAGGGGTGGAG |
| **Ecad-as (bis. seq)** | | ATCTAACAAAAATTCTTAAAAACTCAATAA |

PCR primers were designed with http://eu.idtdna.com/Scitools/Applications/Primerquest/ using the whole coding sequence of the target genes (NCBI). Primers were designed as follows: Primer size 20 – 27 bp; $T_M$ 58 – 62 °C; GC% 35 – 65; Product size 150 – 250 bp; GC Clamp 2. For detection of viral expression of Klf4, Sox2 and c-Myc, the Oct4 viral-s primer was used in combination with the corresponding antisense primers of these genes.

**Supplementary table 2: Volumes of Ecad$^{high}$-iPSC, Ecad$^{low}$-cell and 129/Sv3 mESC derived tumors.**

Tumor volume [cm$^3$]/ at days of growth

| | 12 | 18 | 21 |
|---|---|---|---|
| iPS, Ecad$^{high}$ | 0.1490 | 0.2750 | 0.5000 |
| | 0.1300 | 0.9060 | 1.4820 |
| | 0.2150 | 1.2530 | 2.0330 |
| **Average** | 0.1647 | 0.8113 | 1.3383 |
| **SD** | 0.0446 | 0.4958 | 0.7765 |
| | | | |
| | **12** | **18** | **21** |
| iPS, Ecad$^{low}$ | 0.0850 | 0.0640 | 0.2280 |
| | 0.0220 | 0.0190 | 0.0270 |
| | 0.0140 | 0.0630 | 0.0900 |
| **Average** | 0.0403 | 0.0487 | 0.1150 |
| **SD** | 0.0389 | 0.0257 | 0.1028 |
| | 0.0850 | 0.0640 | 0.2280 |
| | | | |
| | **12** | **18** | **21** |
| 129/Sv3 | 0.2430 | 1.9370 | 2.4110 |
| | 0.0980 | 0.6730 | 1.3700 |
| | 0.1880 | 0.6180 | 1.5810 |
| **Average** | 0.1763 | 1.0760 | 1.7873 |
| **SD** | 0.0732 | 0.7462 | 0.5503 |
| | 0.2430 | 1.9370 | 2.4110 |

**Supplementary table 3: Volumes of ESKM-iPSC and SKM-cell derived tumors.**

**Tumor volume [$cm^3$]/ at days of growth**

| | **7** | **12** | **18** | **21** | **25** |
|---|---|---|---|---|---|
| ESKM#2, 1 | 0.0020 | 0.0270 | 0.3230 | 0.9000 | 2.4820 |
| ESKM#2, 2 | 0.0020 | 0.0180 | 0.1110 | 0.2690 | 0.9150 |
| ESKM#2, 3 | 0.0060 | 0.0130 | 0.0450 | 0.4250 | 0.9400 |
| **Average** | 0.0033 | 0.0193 | 0.1597 | 0.5313 | 1.4457 |
| **SD** | 0.0023 | 0.0071 | 0.1452 | 0.3287 | 0.8976 |
| | | | | | |
| | **7** | **12** | **18** | **21** | **25** |
| ESKM#3, 1 | 0.0010 | 0.0080 | 1.1530 | * | - |
| ESKM#3, 2 | 0.0010 | 0.0020 | 0.0350 | 0.2290 | 1.1290 |
| ESKM#3, 3 | 0.0010 | 0.0050 | 1.0770 | * | - |
| | | | | | |
| | **7** | **12** | **18** | **21** | **25** |
| SKM#1, 1 | 0.0010 | 0.0010 | 0.0010 | 0.0010 | 0.0010 |
| SKM#1, 2 | 0.0010 | 0.0010 | 0.0010 | 0.0010 | 0.0010 |
| SKM#1, 3 | 0.0010 | 0.0020 | 0.0020 | 0.0020 | 0.0020 |
| **Average** | 0.0010 | 0.0013 | 0.0013 | 0.0013 | 0.0013 |
| | | | | | |

*Tumors were dissected at day 18.

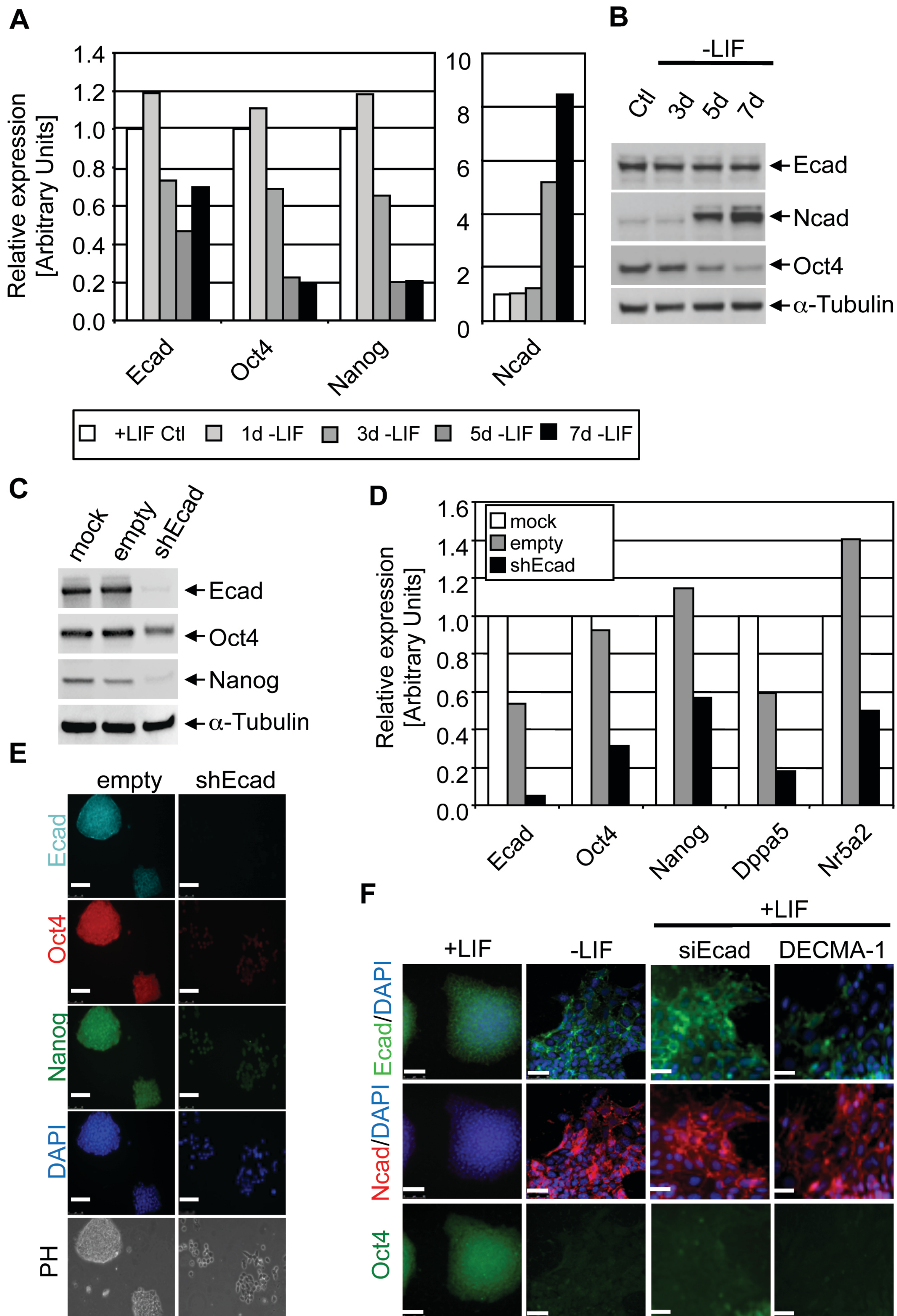
**Supplementary table 4 a: Summary of blastocyst injections of $Ecad^{high}$- and $Ecad^{low}$ cells.**

| Stage inj. | Cell clone | Cells (inj.) | Newborn pubs | $GFP^{pos}$ |
|---|---|---|---|---|
| 8-Zell | $SSEA1^{+}$, Clone11 | 11 (1) | 4 | 1/4 (25%) |
| 8-Zell | $SSEA1^{-}$, Clone1 | 10 (1) | 13 | - |
| Blastocyst | $SSEA1^{+}$, Clone11 | 11 (2) | 13 | 1/13 (7.7%) |
| Blastocyst | $SSEA1^{-}$, Clone1 | 12 (2) | 8 | - |
| Blastocyst | $SSEA1^{+}$, Clone11 | 12 (1) | 7 | - |
| Blastocyst | $SSEA1^{-}$, Clone1 | 11 (2) | 4 | - |

**Supplementary table 4 b: Summary of blastocyst injections of ESKM-derived iPSCs.**

| Factor comb. | clone | Cells/blastocyst | blastocysts | Pubs | $GFP^{pos}$ |
|---|---|---|---|---|---|
| ESKM | #2 | 30 | 36 | 15 | 7 (47%) |
| ESKM | #3 | 30 | 36 | 1 | 1 (100%) |
| ESKM | #3 | 15 | 24 | 4 | 3 (75%) |
| OSKM | #1 | 30 | 36 | 8 | - |
| OSKM | #1 | 15 | 24 | 3 | - |

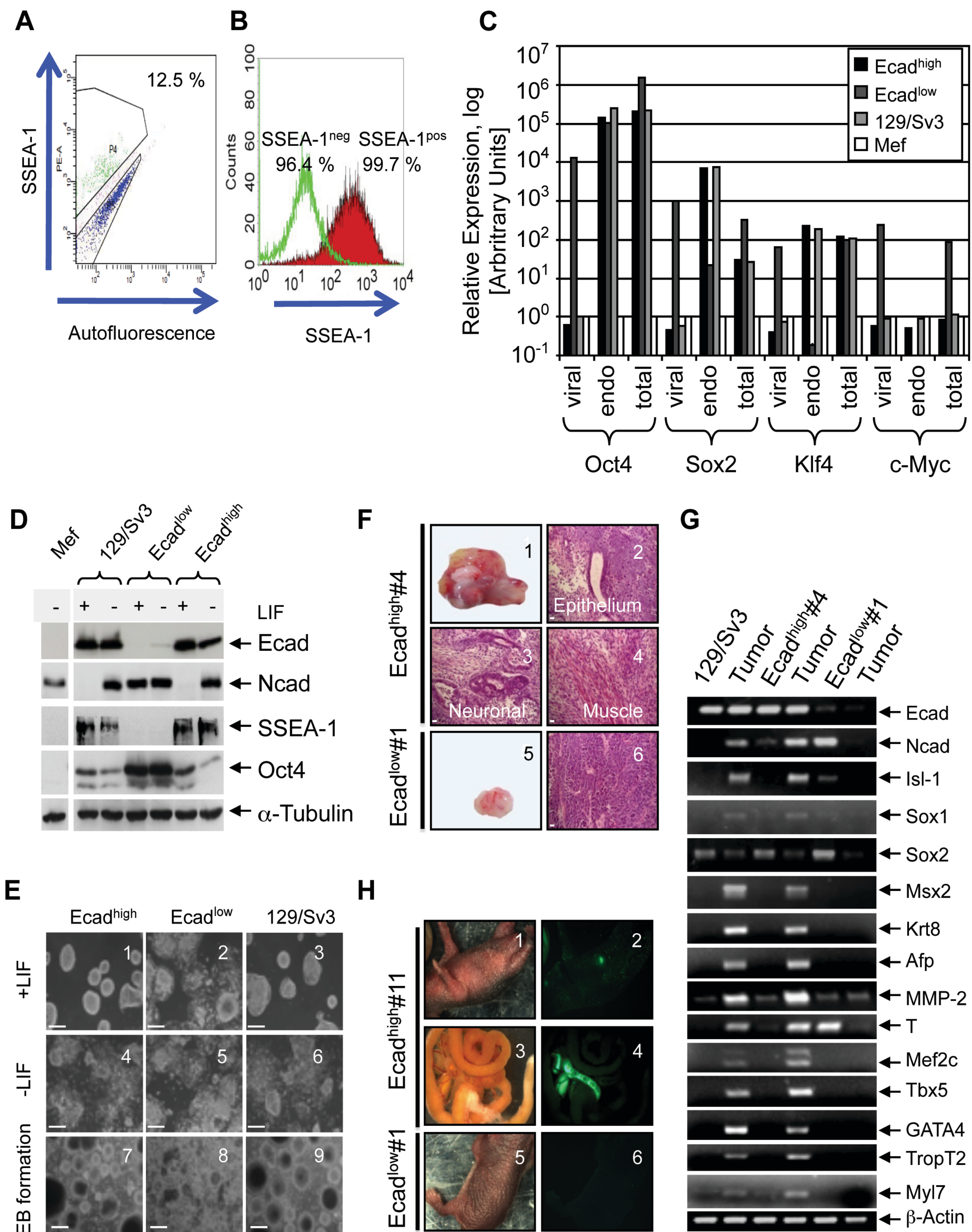
## Supplementary Figures




Redmer et.al., Supplementary Figure 1

**Fig S1 | E-cadherin expression is required for maintenance of pluripotency**.

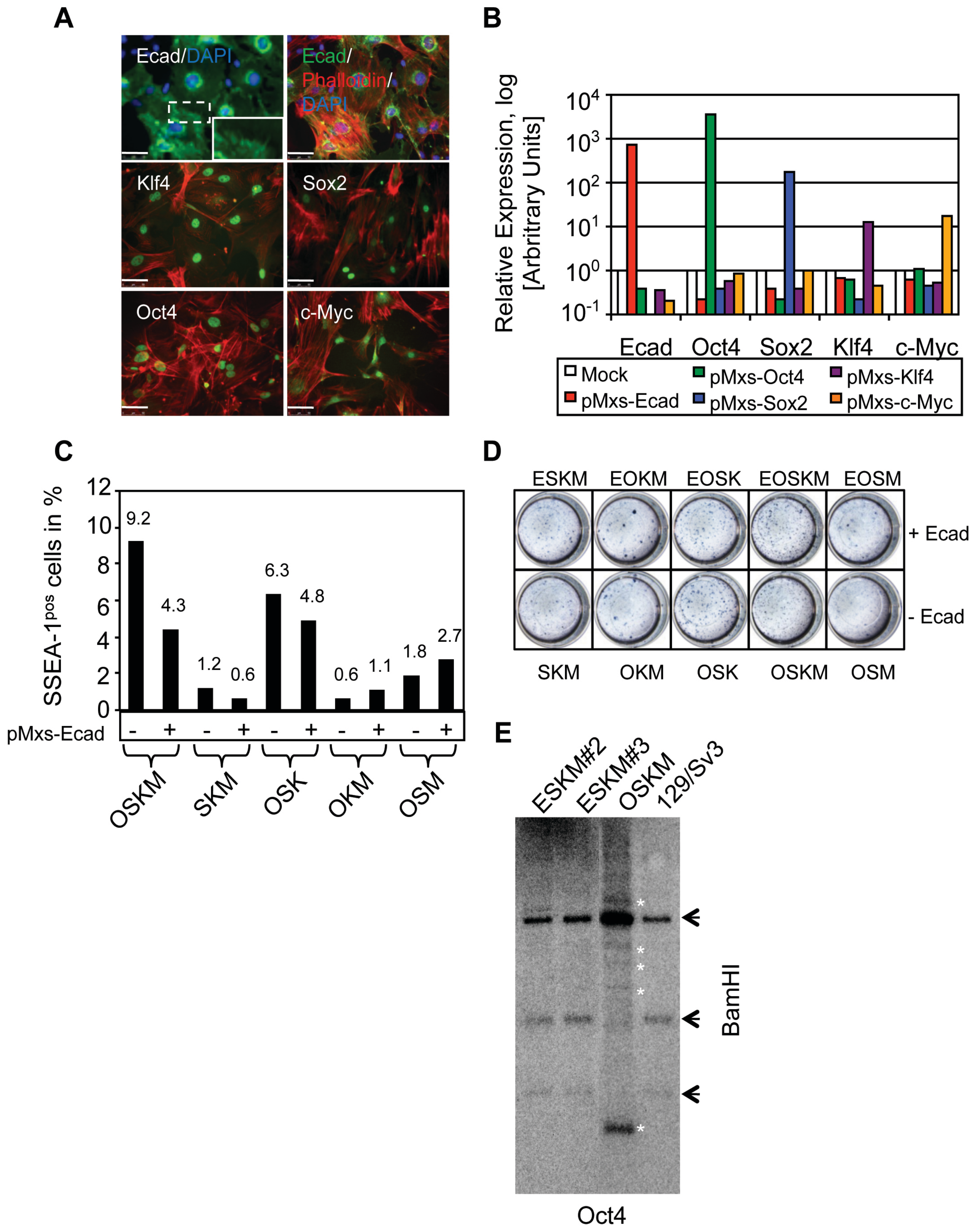
(A) Quantitative real-time RT-PCR for E-cadherin (Ecad), Oct4 and Nanog and N-cadherin (Ncad) in 129/Sv3 mESCs, undifferentiated control (+LIF Ctl) or differentiated in the absence of LIF (as indicated in days (d)). The mRNA expression levels were normalized to β-actin, and values for undifferentiated mESCs were set to 1. Median values from biological duplicates are presented as bars, shown is a representative experiment out four independent experiments. (B) Analysis of protein extracts from 129/Sv3 cells undifferentiated or differentiated for 3, 5 or 7 days with antibodies against Ecad, Ncad, Oct4 and as loading control α-Tubulin. (C) Analysis of Ecad, Oct4 and Nanog protein levels by Western blotting of 129/Sv3 mESCs in LIF not transduced (mock) or 4 days after viral transduction with a pMSCV vector (empty) or a vector constitutively expressing shRNA against E-cadherin (shEcad). Ecad knock down by shRNA was carried out in three independent experiments and a representative result is shown. (D) Real-time RT-PCR of 129/Sv3 mESCs in LIF not transduced (mock) or transduced as in (C) for Ecad, Oct4, Nanog, Dppa5 and Nr5a2. Median values from biological duplicates are presented as bars, shown is a representative experiment out three independent experiments. (E) Immunofluorescence of 129/Sv3 mESCs in LIF not transduced (mock) or transduced as in (C) for Ecad, Oct4 and Nanog. Nuclei were stained with DAPI. Phase contrast (PH) images are shown. Magnification 400x, bars 50 μm. (F) Immunofluorescence of 129/Sv3 mESCs undifferentiated (+LIF) and differentiated in absence of LIF for 7 days (-LIF) or transfected with siRNA against Ecad (Dharmacon, siEcad) for 3 days or a neutralizing antibody DECMA-1 for 3 days both in presence of LIF with antibodies against Ecad (light green fluorescence) and Ncad (red fluorescence) and Oct4 (dark green fluorescence) as indicated. DAPI nuclear staining (blue) was performed to visualize cell nuclei. Pictures were taken with a magnification of 400x, bars, 50 μm.




Redmer et.al., Supplementary Figure 2

**Fig S2 | Ecad$^{high}$ cell clones formed teratomas and participated in embryonic development.**

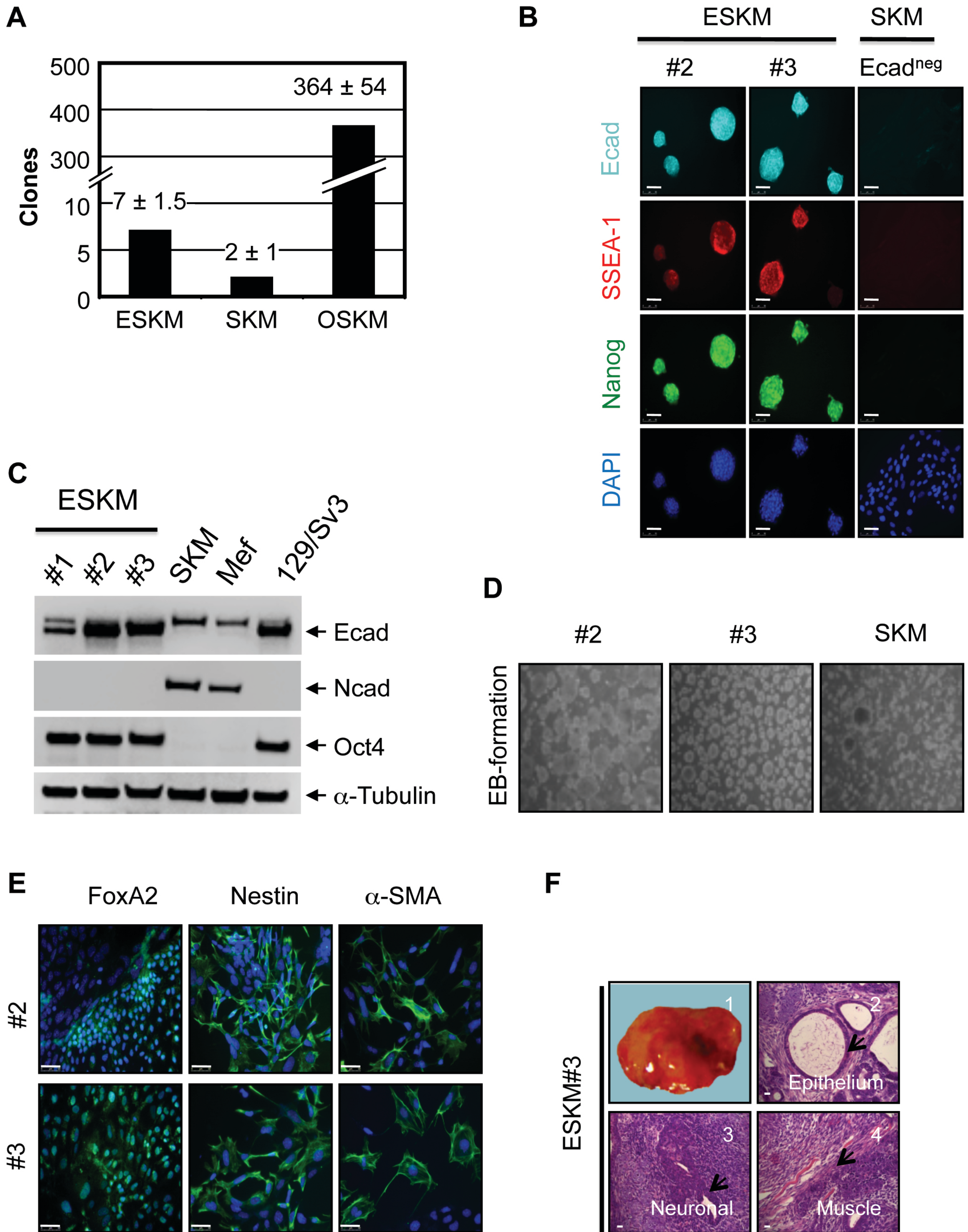
(A) Sorting of MEFs (10.000 cells) eight days after viral transduction (OSKM) using SSEA-1 (CD15) as cell surface marker into SSEA-1 positive (12.5% of total cells) and negative cells (87.5%). (B) Flow cytometry of SSEA-1 positive and negative cells cultured further on feeder cells for 8 days to established stable cell clones confirmed the presence and absence of SSEA-1 on SSEA-1$^{pos}$ cells and on SSEA-1$^{neg}$ cells, respectively. (C) Analysis of mRNA expression levels of Ecad$^{high}$ and Ecad$^{low}$ expressing cells, 129/Sv3 mESCs and MEFs for viral, endogenous and total levels of Oct4, Sox2, Klf4 and c-Myc. mRNA expression levels were normalized to the respective β-actin expression and values for MEFs were set to 1. Median values from biological duplicates are presented as bars, shown is a representative experiment out three independent experiments. (D) Protein levels of Ecad, Ncad, SSEA-1 and Oct4 in MEFs, 129/Sv3 mESCs, Ecad$^{low}$ cells and Ecad$^{high}$ iPS cells cultured in presence or absence of LIF. Equal amounts of total protein were verified by α-Tubulin. (E) Morphological changes of Ecad$^{high}$ iPS cells (1 and 4) and 129/Sv3 mESCs (3 and 6) of cells grown in presence of LIF (1 and 3) and following spontaneous differentiation in absence of LIF for 4 days (4 and 6). Morphology of Ecad$^{low}$ cells (2 and 5) did not change under differentiating conditions when compared to growth in presence of LIF. EB formation of Ecad$^{high}$ cells (7) and 129/Sv3 cells (9) was observable, whereas Ecad$^{low}$ cells did not form EBs (8). The magnification is 100x, bars, 100 μm. (F) Tumor formation of Ecad$^{high}$ and Ecad$^{low}$ cells following subcutaneous injection in NOD/SCID mice (n = 3/cell clone). Ecad$^{high}$ cells formed a large tumor by day 12 (0.17 +/- 0.04 $cm^3$; 1), whereas Ecad$^{low}$ cells produced very small tumors (0.04 +/- 0.04 $cm^3$ ; 5). Compare supplementary table S2 online for further details. Histological analysis of tissue sections by H&E staining of differentiated tumors (Ecad$^{high}$#4) comprising epithelial (2), neuronal (3) and muscle derived (4) structures, or of an undifferentiated tumor (Ecad$^{low}$#1; 6). Magnification 25x (H&E staining), bars 500 μm. (G) Expression of differentiation marker genes of teratoma analyzed by agarose gel electrophoresis of RT-PCR products comprising representative markers of all three germ layers. Expression levels of E-cadherin (Ecad, epithelial), N-cadherin (Ncad, mesoderm/neuroectoderm), Isl-1 (endoderm, neuroectoderm), Sox1 (neuroectoderm), Sox2 (neuroectoderm), Msx2 (mesoderm/neuroectoderm), Cytokeratin 8 (Krt8, non-neural ectoderm), α-Fetoprotein (Afp, visceral and definitive endoderm), MMP-2 (mesoderm), Brachyury (T, pan-mesoderm), Mef2c (myogenic), Tbx5 (cardiomyogenic), GATA4 (definitive endoderm), Troponin T2 (cardiomyogenic) and Myl7 (Myosin-light-chain 7, myogenic). (H) Chimera formation following injection of GFP-labeled Ecad$^{high}$#11 cells in mouse blastocysts (n = 3/cell clone) and analysis of newborn pups (1) for GFP positive areas by fluorescence (2). GFP-labeled organs like the gut (3) were detected by fluorescence (4). No GFP-labeled Ecad$^{low}$ cells were visible (5 and 6). Compare supplementary table S4 a online for further details.




Redmer et.al., Supplementary Figure 3

**Fig S3 | E-cadherin overexpression during iPS cell induction did not increase efficiency**

(A) Immunofluorescence of MEFs transduced with pMXs-Ecad (upper panels), pMXs-Klf4 and pMXs-Sox2 (middle panels) and with pMXs-Oct4 and pMXs-c-Myc (lower panels) with antibodies specifically recognizing the proteins (green; Klf4: R&D, AF3158; Oct4; Sox2, R&D, AF2018 or c-Myc, Sigma, C3956), confirmed proper expression and localization of factors. TRITC-labeled Phalloidin (red, Sigma) was used for visualization of the cytoskeleton and DAPI for visualization of the nucleus. (B) Transduced MEFs were analyzed for expression of indicated mRNAs, 2 days after viral transduction, shown is a representative experiment out of three. (C) Flow cytometric analysis for SSEA-1 expression of MEFs following 8 days after viral transduction with different combinations of OSKM in presence or absence of pMXs-Ecad. SSEA-1 positive cells are shown in % of 10,000 analyzed cells. The experiment was performed twice and a representative experiment is shown. (D) Alkaline phosphatase assay of cells treated like in (C). For determination of alkaline phosphatase activity, cells were washed once with PBS and fixed with PBS/4% PFA. Cells were washed and alkaline phosphatase activity was visualized using both substrates NBT and BCIP. (E) Southern-blot analysis of digested DNA of ESKM cells (clone#2 and #3), OSKM cells and 129/Sv3 mESCs with an Oct4 specific probe. Arrows indicate fragments of the endogenous gene and stars indicate viral integration sites.




Redmer et.al., Supplementary Figure 4

**Supplementary Figure 4. Characterization of ESKM-iPS cell clones**

(A) Number of colonies of two independent transduction series and re-seeding in duplicates to 2 x $10^5$ cells per 10 cm dish. Median values from biological duplicates are presented as bars. (B) Immunofluorescence analysis of established ESKM (#2, #3) and SKM cells for expression of Ecad, SSEA-1 and Nanog, as indicated. (C) Characterization of three established ESKM-clones in comparison to ESKM iPS cells, SKM-induced cells, 129/Sv3 cells and MEFs, respectively. Expression of Ecad, Ncad and Oct4 was determined by Western blotting as indicated, α-Tubulin was used as loading control. (D) Pictures of EB formed by ESKM clones (#2 and #3). No EBs were detected for the SKM clones. (E) Outgrowth of EBs derived from ESKM clones (#2 and #3, Fig. S5D) was analyzed for marker genes of all three germ layers. Using immunofluorescence, expression of FoxA2 (endoderm; antibody from Abcam, AB40874), Nestin (ectoderm; antibody was a gift from C. Birchmeier) and α-smooth-muscle actin (α-SMA; antibody from abcam, ab5694) was detectable in outgrown EBs of both iPS cell clones. DAPI was used for nuclear staining, the magnification is 400x. Bars, 50 µm. (F) Teratomas grown after injection of ESKM-iPS cells (clone#3, 1) in NOD/SCID mice. Sections were stained with H&E and typical structures were indicative for derivatives of endoderm (2), ectoderm (3) and mesoderm (4).