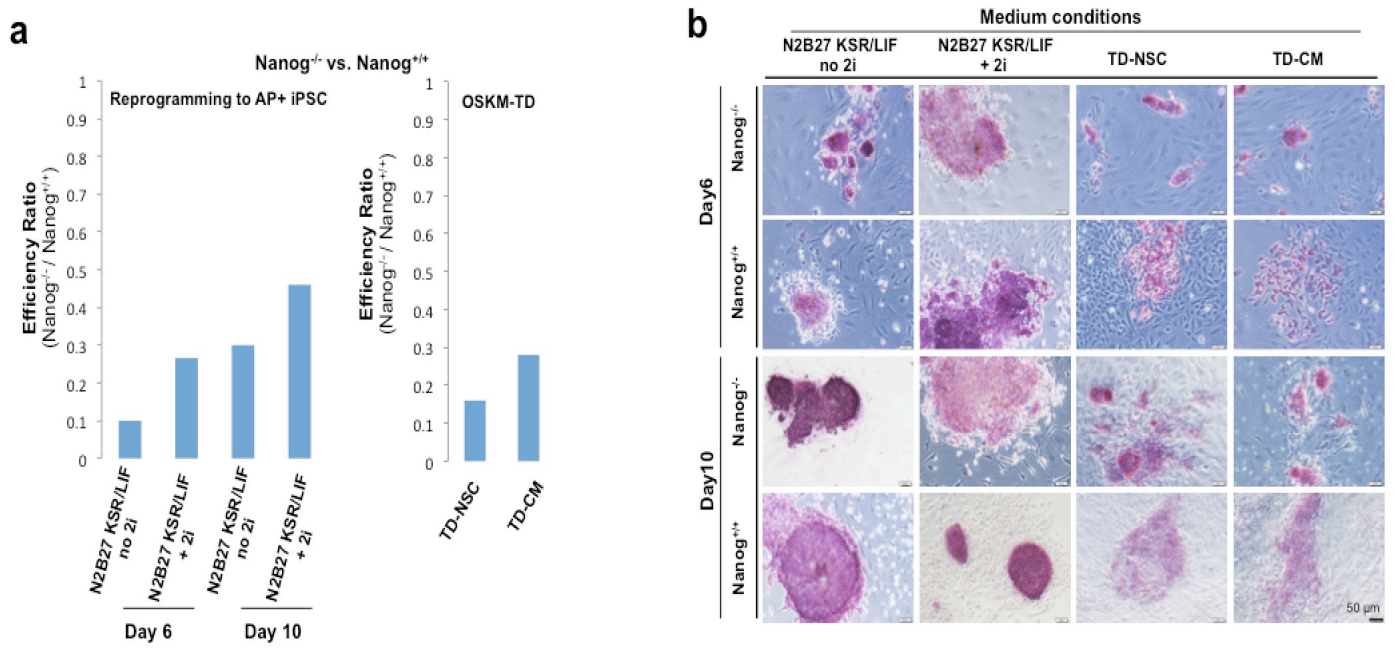
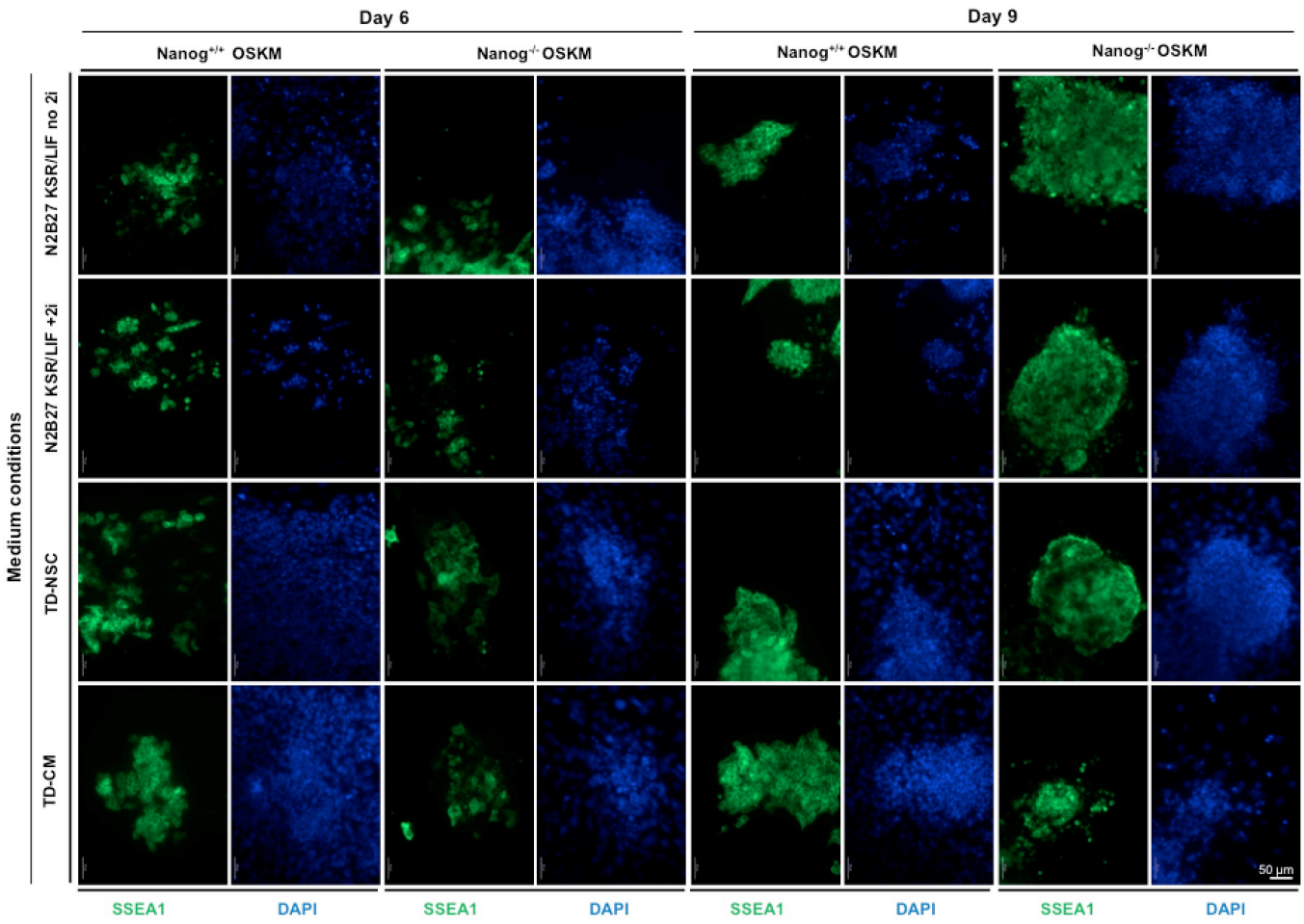


Supplementary Figure 16

Reprogramming efficiencies of Nanog$^{-/-}$ MEFs by either iPSCs or OSKM-TD growth conditions.

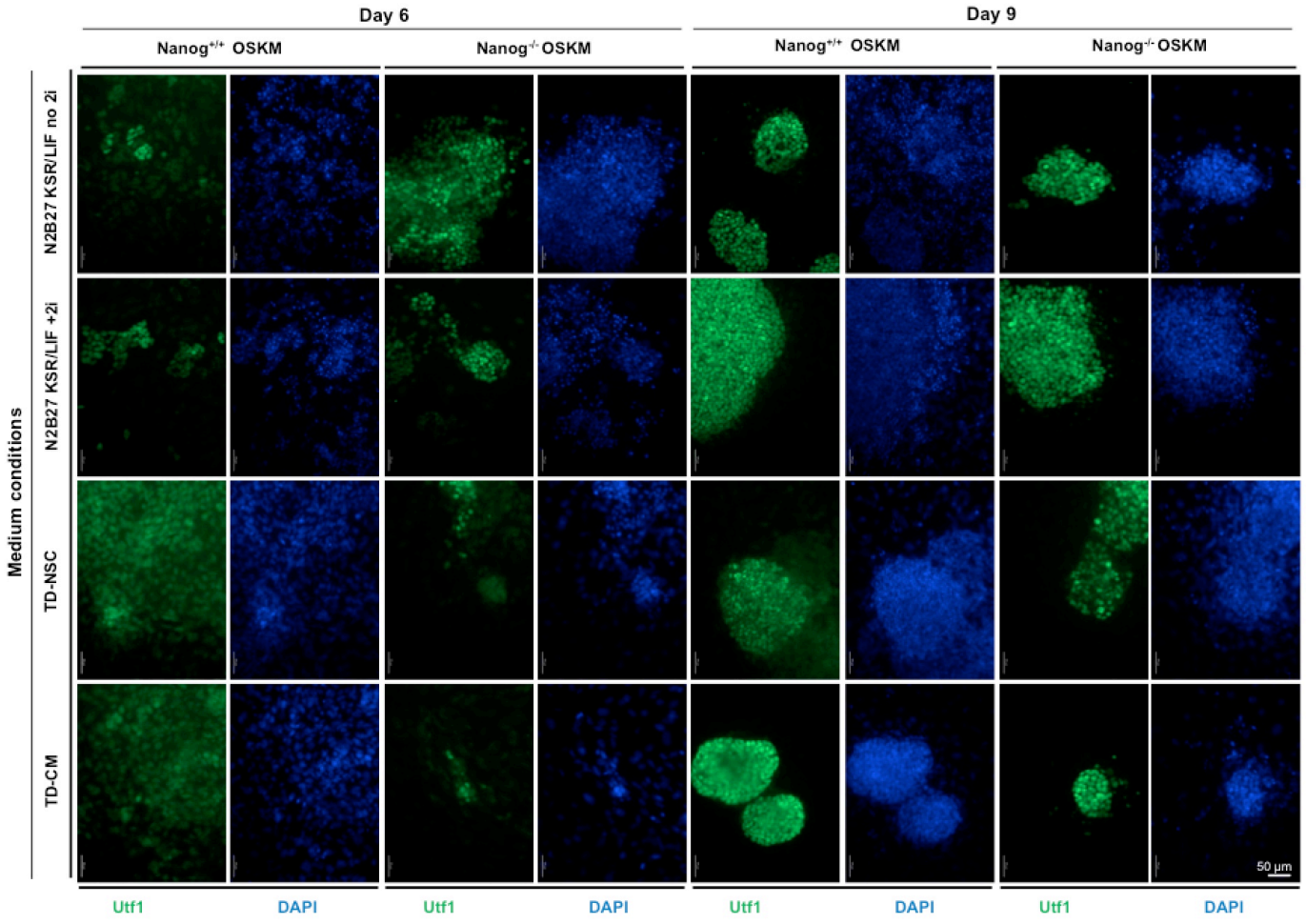
a. Reduction of reprogramming efficiencies of Nanog$^{-/-}$ MEFs to iPSCs, TD-NSCs and TD-CMs, as compared to control Nanog$^{+/+}$ MEFs, evaluated by alkaline phosphatase stain and Sox1 or Myosin immunostaining, respectively. Bar plot indicates ratio reduction of reprogramming efficiency, evaluated by number of iPSCs colonies per 1,000 plated cells. Noticeably, reprogramming efficiency was similarly reduced in Nanog$^{-/-}$ MEFs when reprogrammed to iPSCs without 2i at day 6 and 10, compared to Nanog$^{-/-}$ MEFs reprogrammed to NSCs (quantified after 6 days Dox activation) and TD-CMs (quantified after 10 days Dox activation). **b.** Representative images of alkaline phosphatase stain of Nanog$^{-/-}$ and Nanog$^{+/+}$ MEFs at day 6 and 10 of reprogramming to either iPSCs, TD-NSCs or TD-CMs.



Supplementary Figure 17

SSEA1 expression during iPSC or TD reprogramming of Nanog$^{-/-}$ cells.

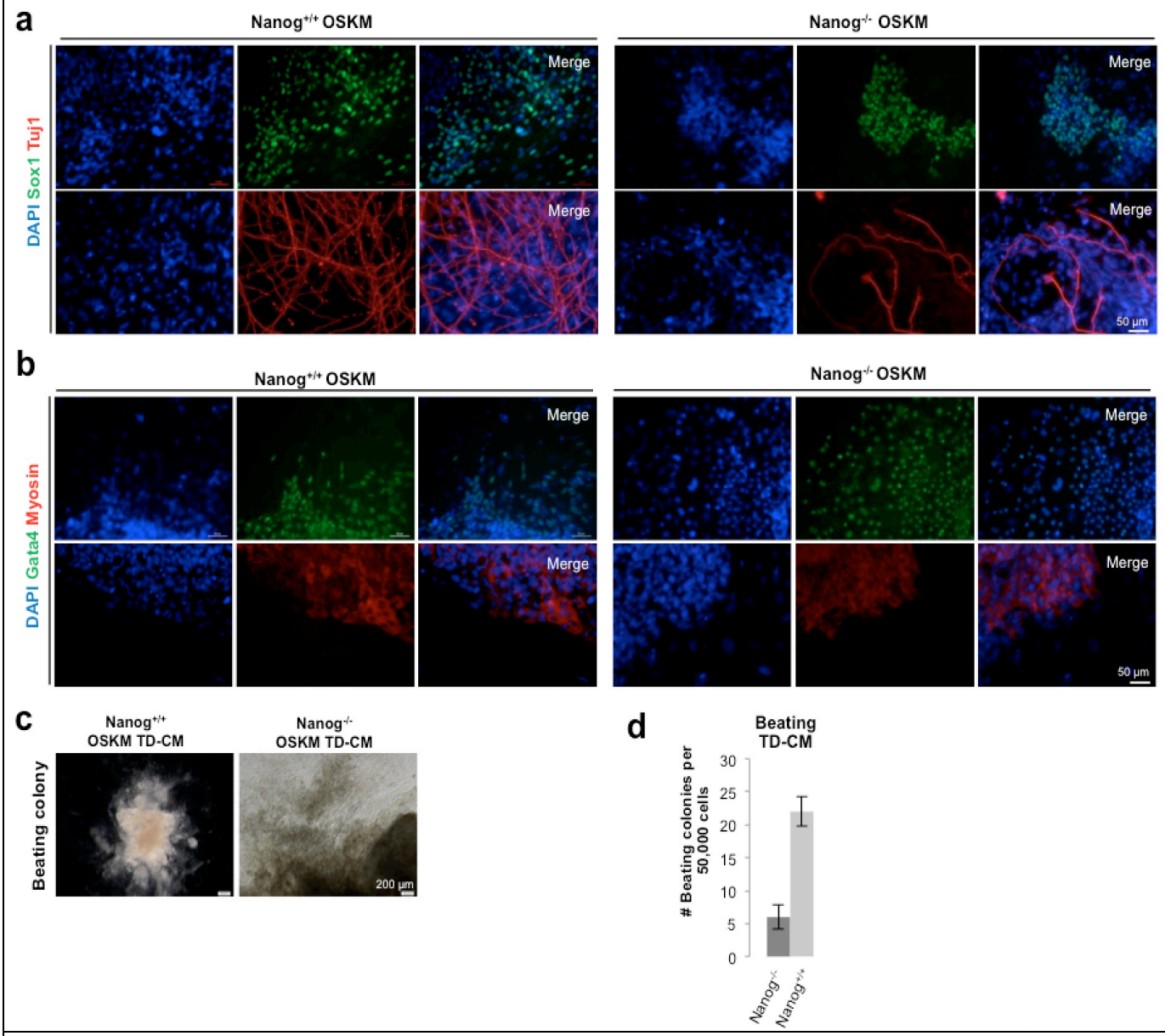
Reprogramming cultures of Nanog$^{-/-}$ and Nanog$^{+/+}$ MEFs to either iPSCs, TD-NSCs or TD-CMs, were subjected to SSEA1 immunostaining at day 6 and 9 during the protocol, respectively.



Supplementary Figure 18

Utf1 expression during iPSC or TD reprogramming of Nanog$^{-/-}$ cells.

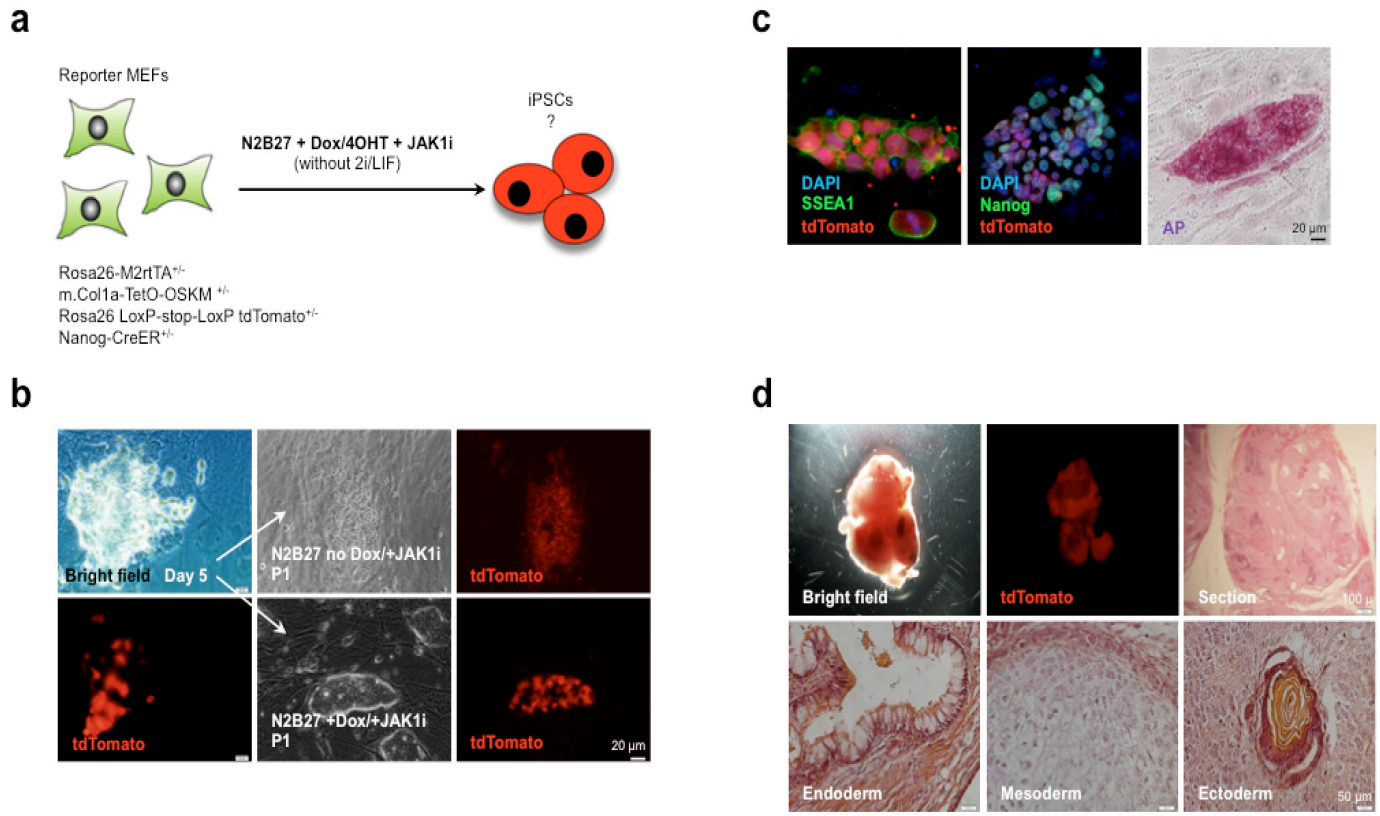
Reprogramming cultures of Nanog$^{-/-}$ and Nanog$^{+/+}$ MEFs to either iPSCs, TD-NSCs or TD-CMs, were subjected to Utf1 immunostaining at day 6 and 9 during the protocol, respectively.



Supplementary Figure 19

OSKM-TD reprogramming of Nanog$^{-/-}$ MEFs show full differentiation potential.

a. OSKM-TD of Nanog$^{-/-}$ and Nanog$^{+/+}$ MEFs produced Sox1 positive NSCs and Tuj1 positive neurons. Representative images of immunostained Nanog$^{-/-}$ and Nanog$^{+/+}$ TD-NSCs and differentiated neurons. **b.** OSKM-TD of Nanog$^{-/-}$ and Nanog$^{+/+}$ MEFs produce Gata4 positive early-stage TD-CMs and Myosin positive fully developed TD-CMs. Representative images of immunostained Nanog$^{-/-}$ and Nanog$^{+/+}$ TD-CM. **c.** Bright-field images of beating colonies of Nanog$^{-/-}$ and Nanog$^{+/+}$ TD-CM colonies. **d**. OSKM-TD of Nanog$^{-/-}$ MEFs to cardiomyocytes produced beating colonies at a lower efficiency than Nanog$^{+/+}$ MEFs. Bar plot indicates number of beating colonies developed per 50,000 plated MEFs. Error bars indicate s.e.m of biological triplicates (1 out of 2 representative experiments is shown). 5-10% of Nanog$^{-/-}$ OSKM Myosin positive TD-CM colonies were beating colonies, while 15-20% of Nanog$^{+/+}$ OSKM Myosin positive TD-CM colonies were beating colonies.



Supplementary Figure 20

OSKM exogenous transgenes maintain iPSCs pluripotency independent of exogenous LIF/Stat3 signaling.

a. Schematic representation of secondary OSKM transgenic Nanog-CreER reporter MEFs reprogramming to iPSCs with JAK1 inhibitor (J1i) and LIF-free conditions. **b**. Representative images of reprogrammed reporter MEFs at day 5 reprogramming and established clones with or without Dox maintained in the growth medium, with activation of tdTomato reporter. At the first passage the colonies differentiated after Dox withdrawal (upper panel), however ES-like colonies can be maintained on Dox (lower panel). **c**. Alkaline phosphatase staining and Immunostaining for pluripotency markers SSEA1 and Nanog in iPSCs reprogrammed in J1i and LIF free conditions from reporter MEFs, showing overlay with tdTomato. **d**. Evaluation of pluripotency potential of iPSCs reprogrammed with J1i and in LIF free conditions from reporter MEFs, as evident by teratoma formation assay. Images of Hematoxylin & Eosin stain of teratoma sections with representation of all three germ layers: endoderm (glandular epithelium), mesoderm (cartilage) and ectoderm (squamous epithelium). The results validate induction and maintenance of functional pluripotency in mouse iPSCs by exogenous OSKM transgene expression in the absence of LIF/Stat3 activation.