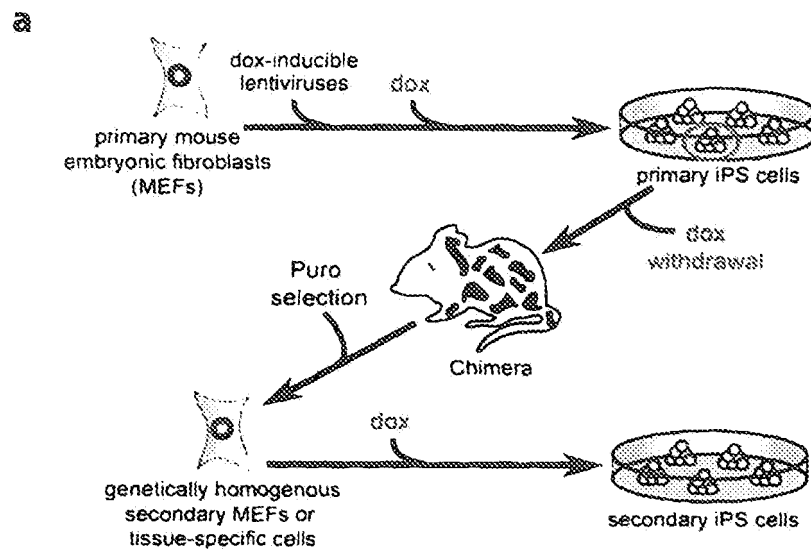
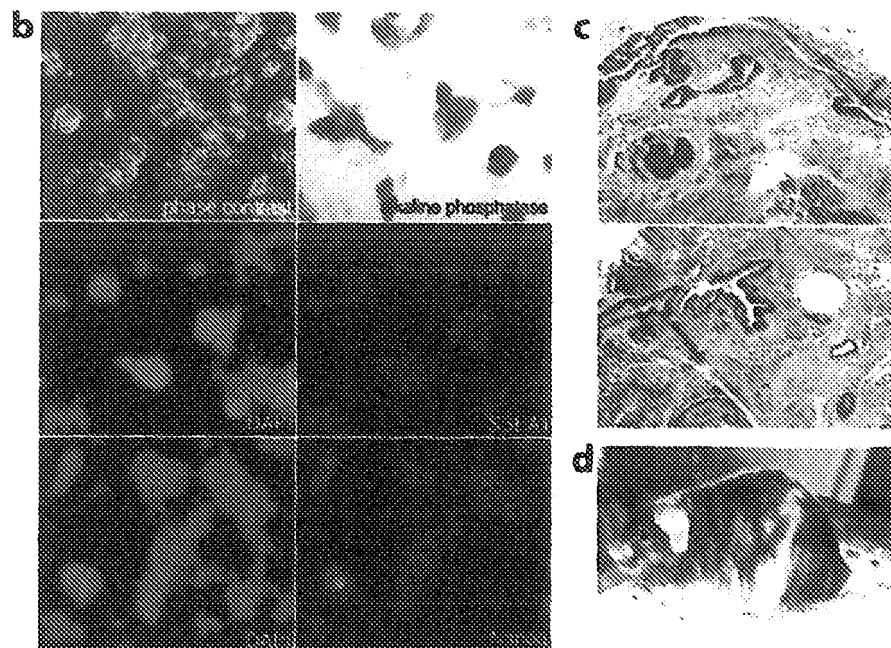




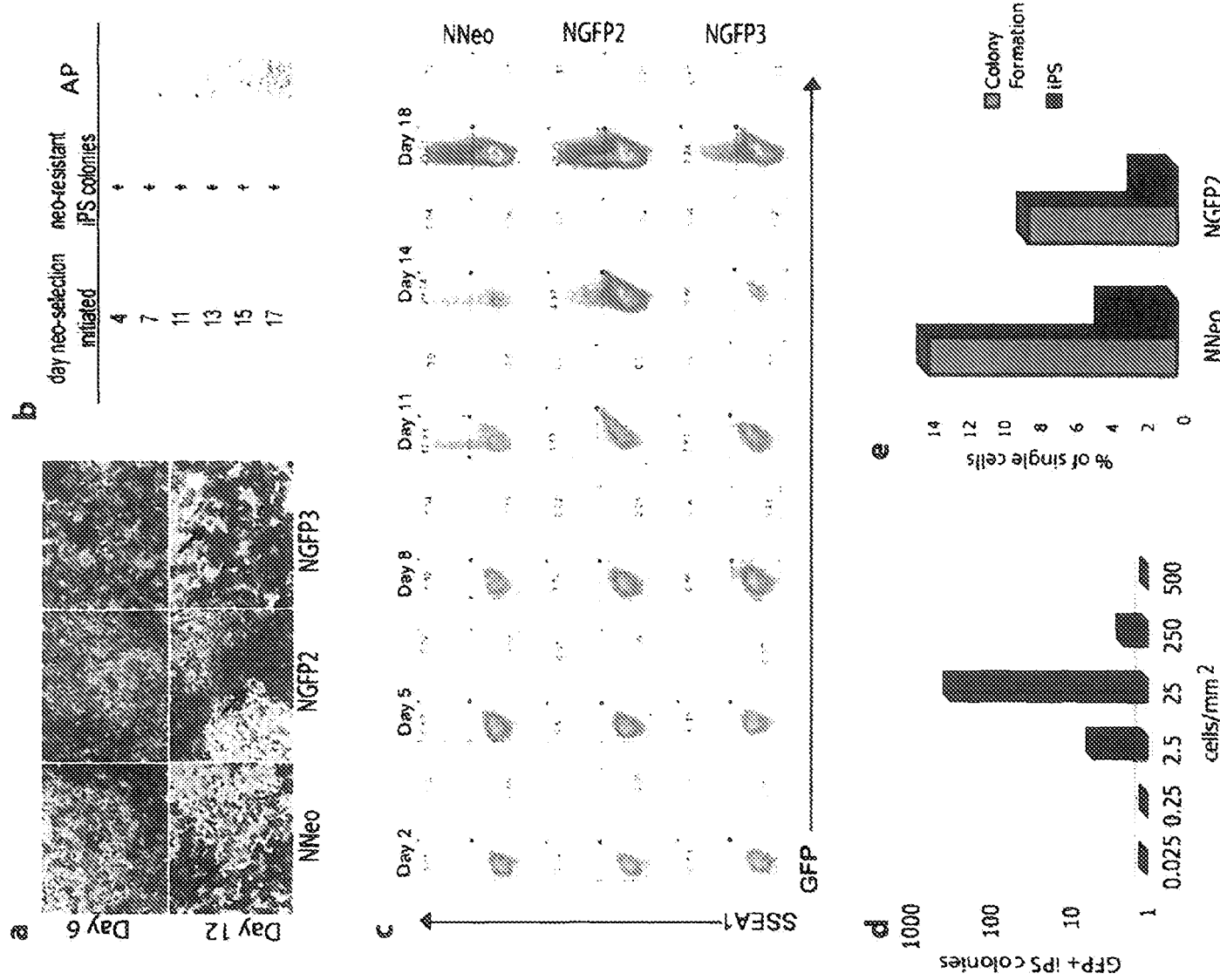
Background of MEFs used for primary infections:

Nanog-neo MEFs: Rosa26-rTA/ pgk-puro
Collagen1A1-tetO-Oct4
Nanog-neo

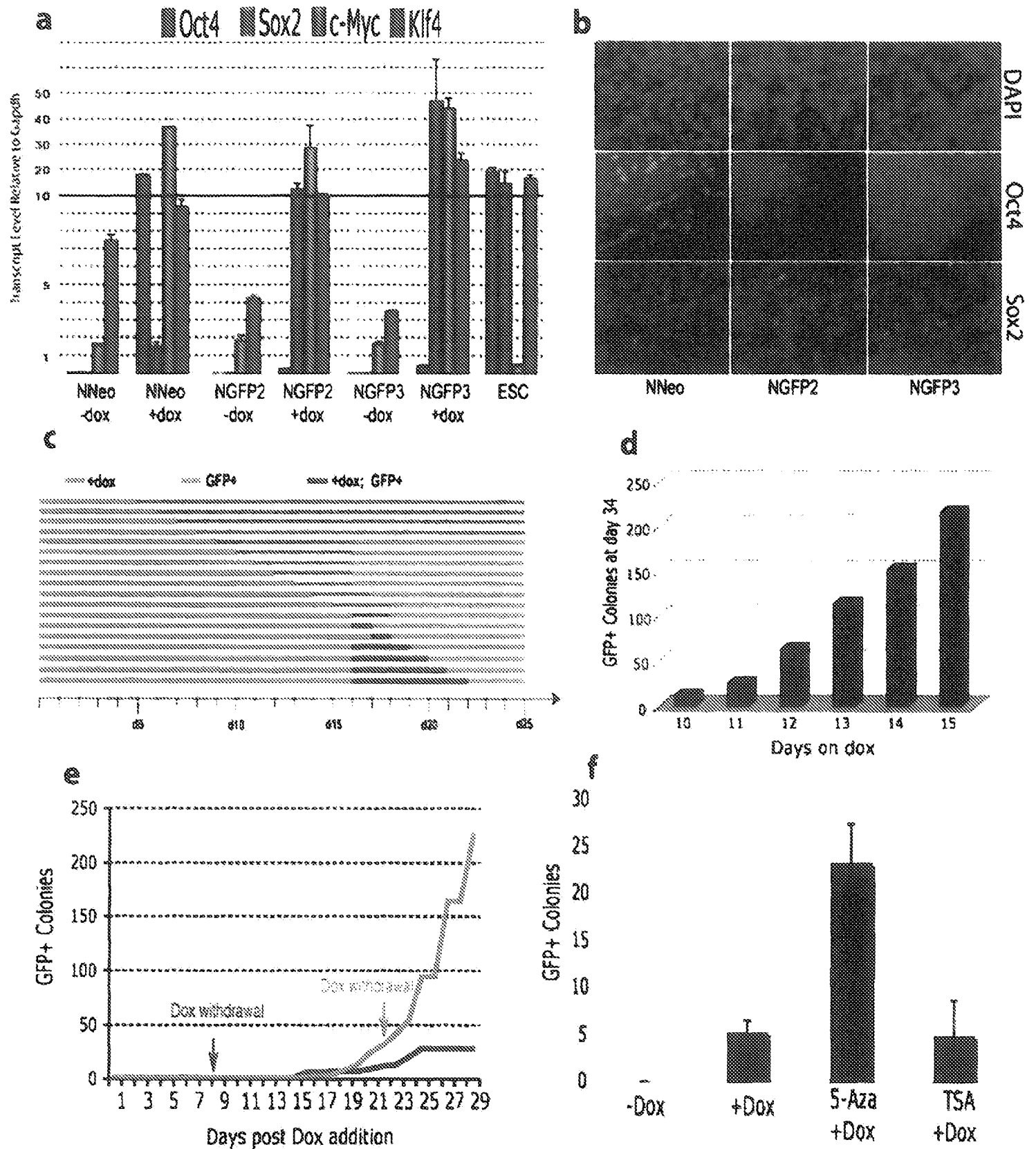
Nanog-GFP MEFs: Rosa26-rTA/ pgk-puro
Nanog-GFPiresPuro



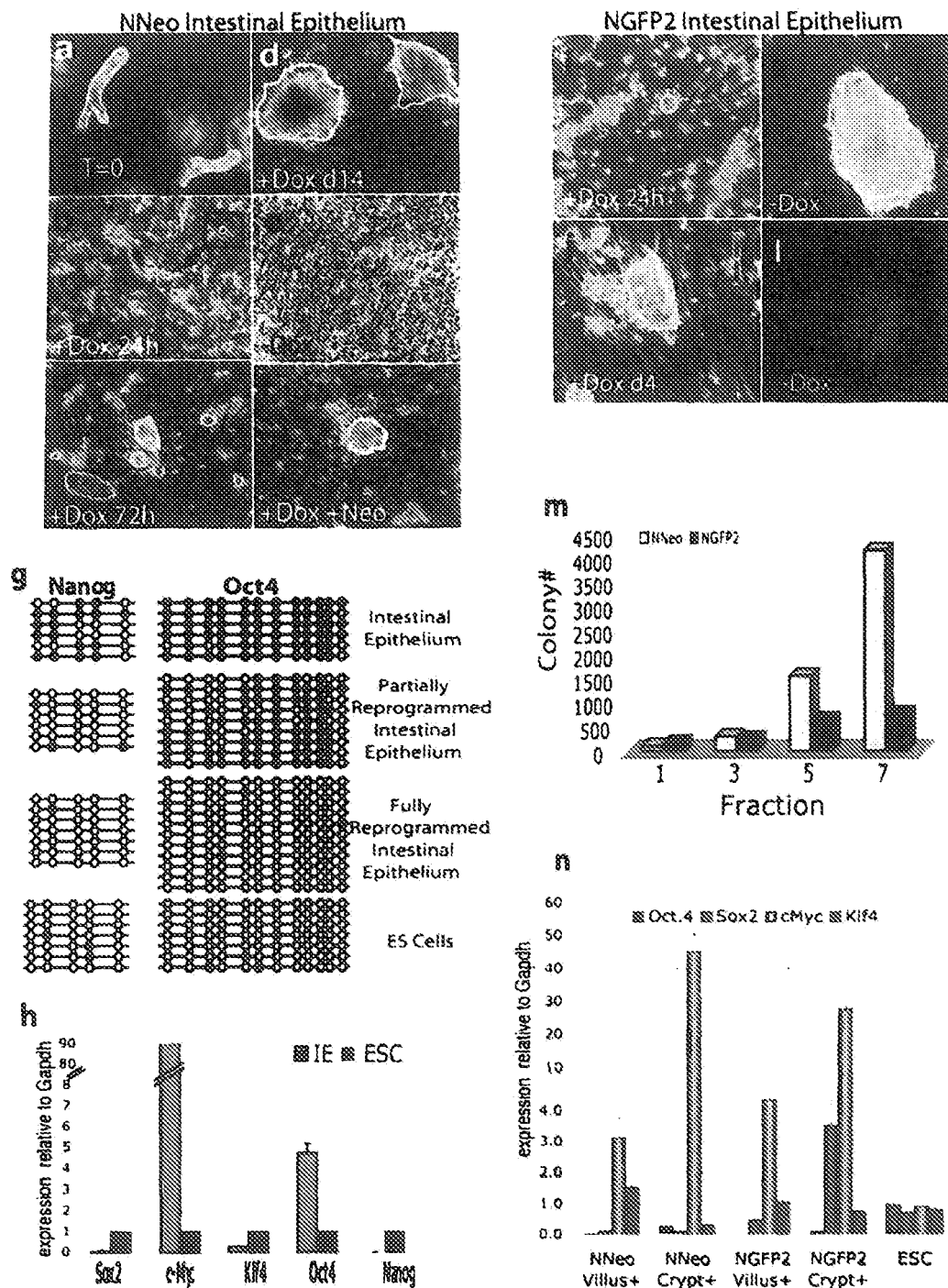
Figs. 1A-AD



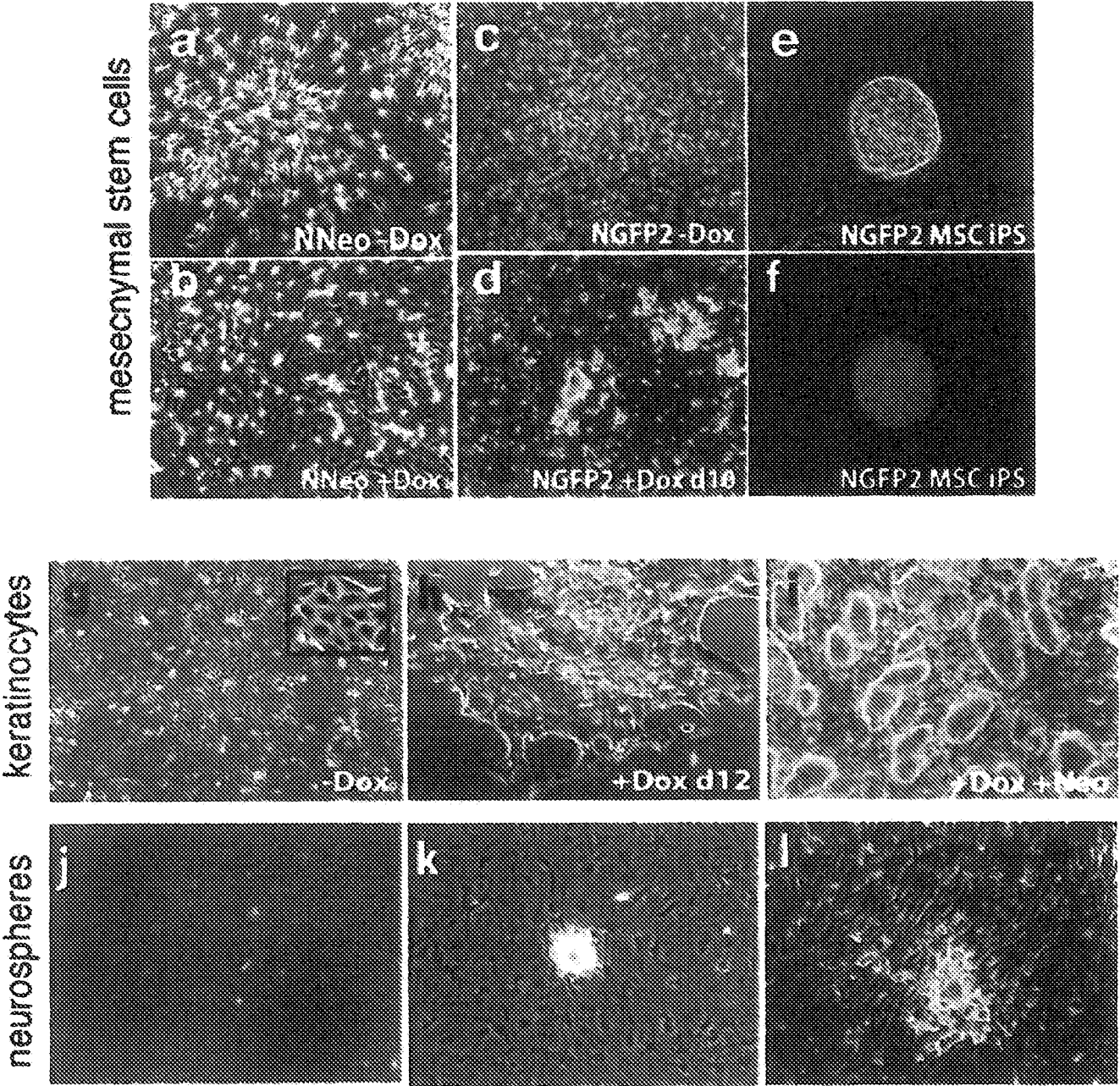
Figs. 2A-2E



Figs. 3A-3F

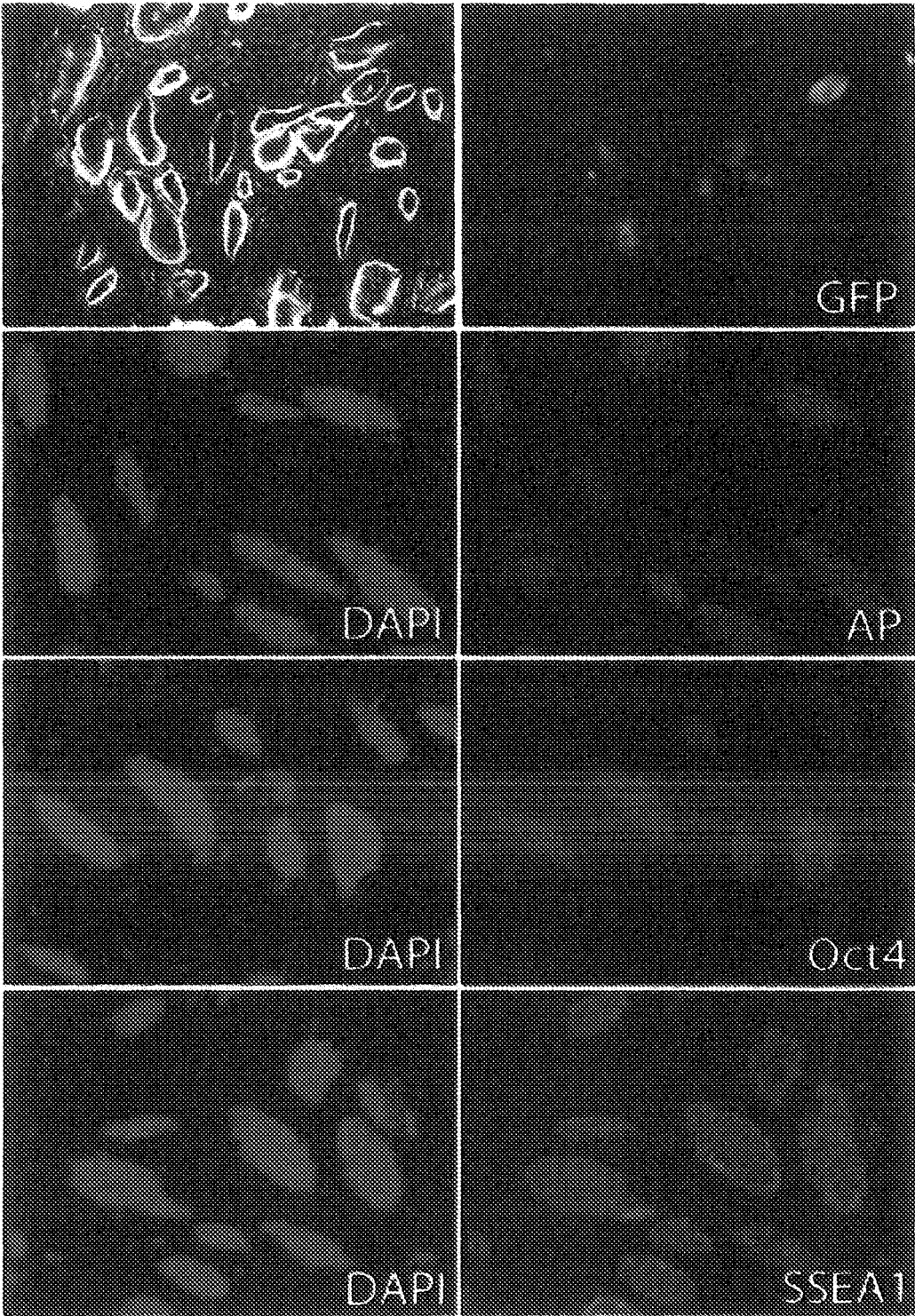


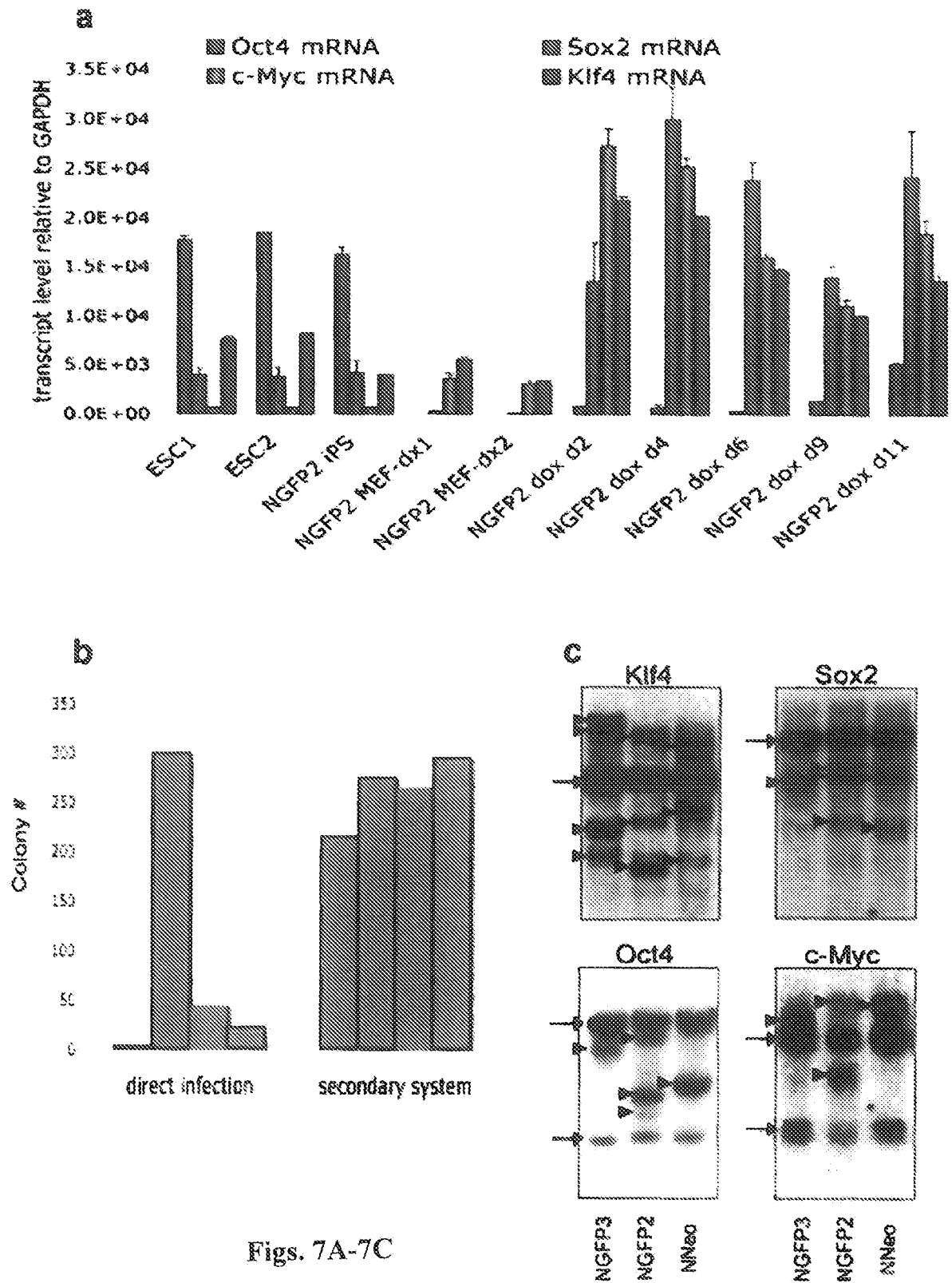
Figs. 4A-4N



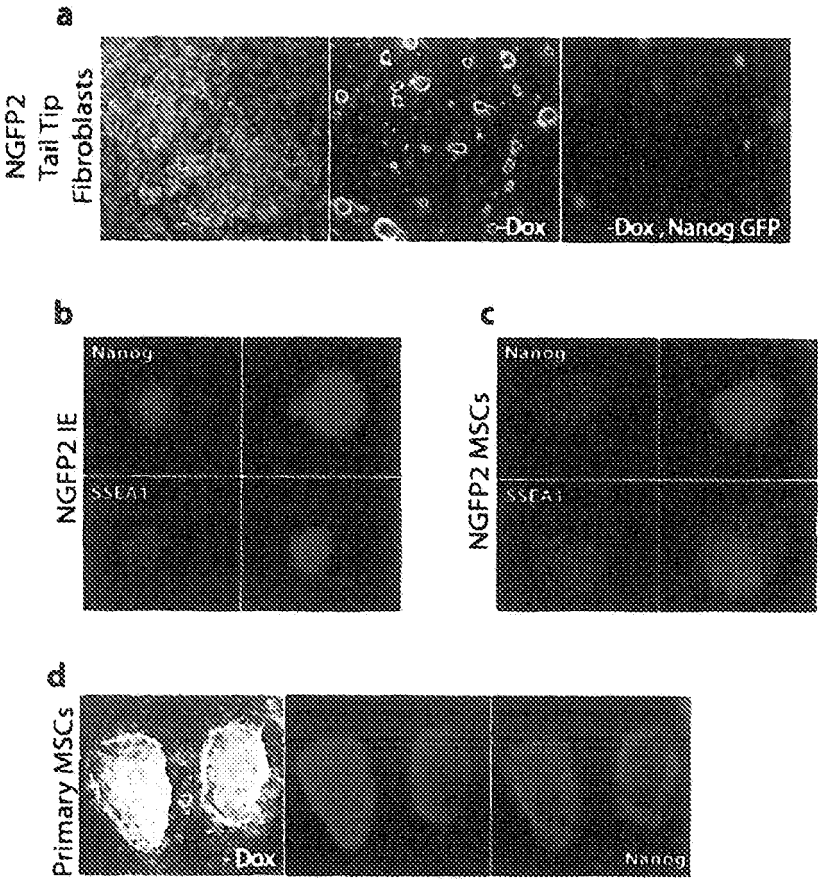
Figs. 5A-5L

Fig. 6

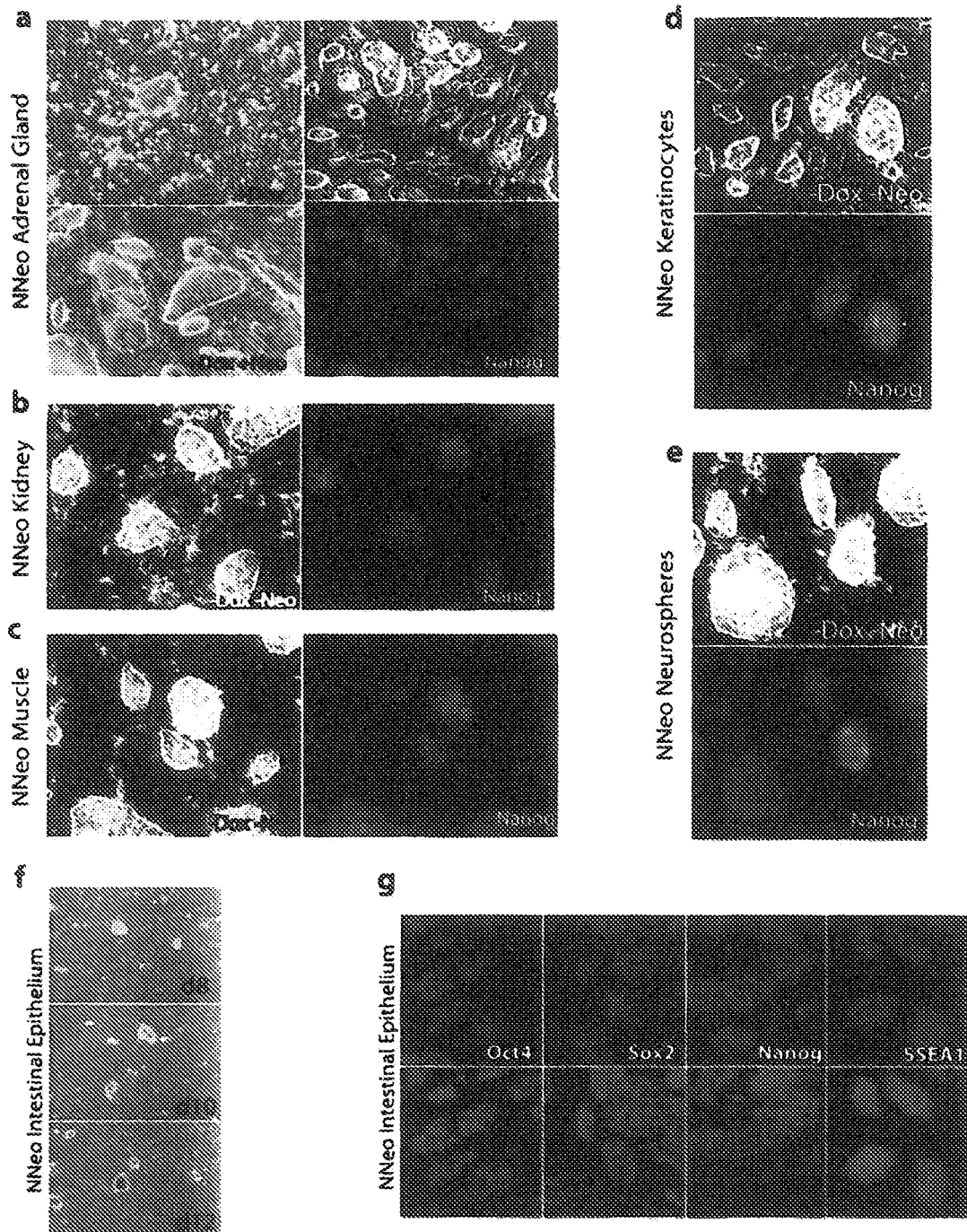




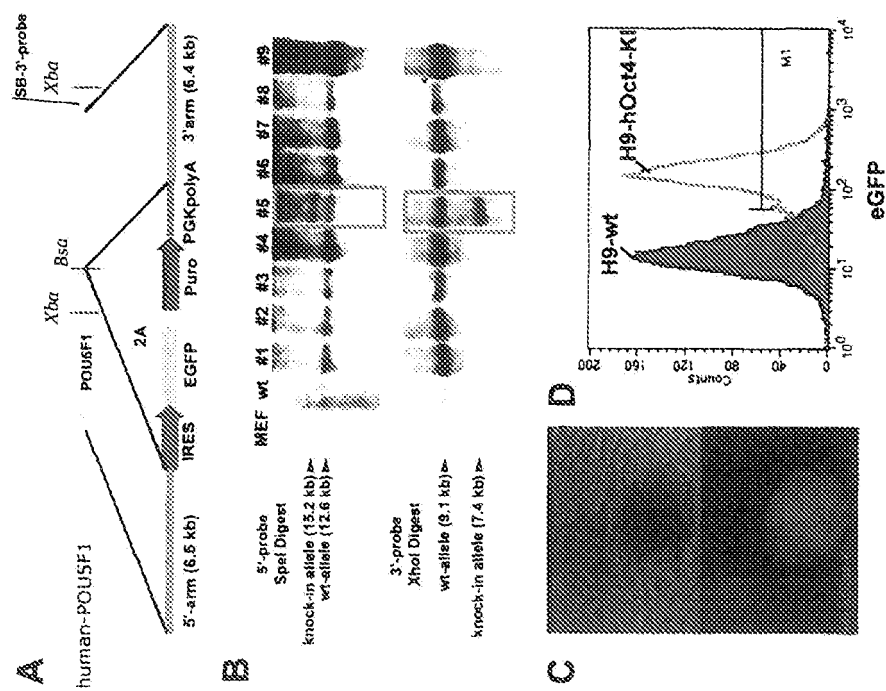
Figs. 7A-7C



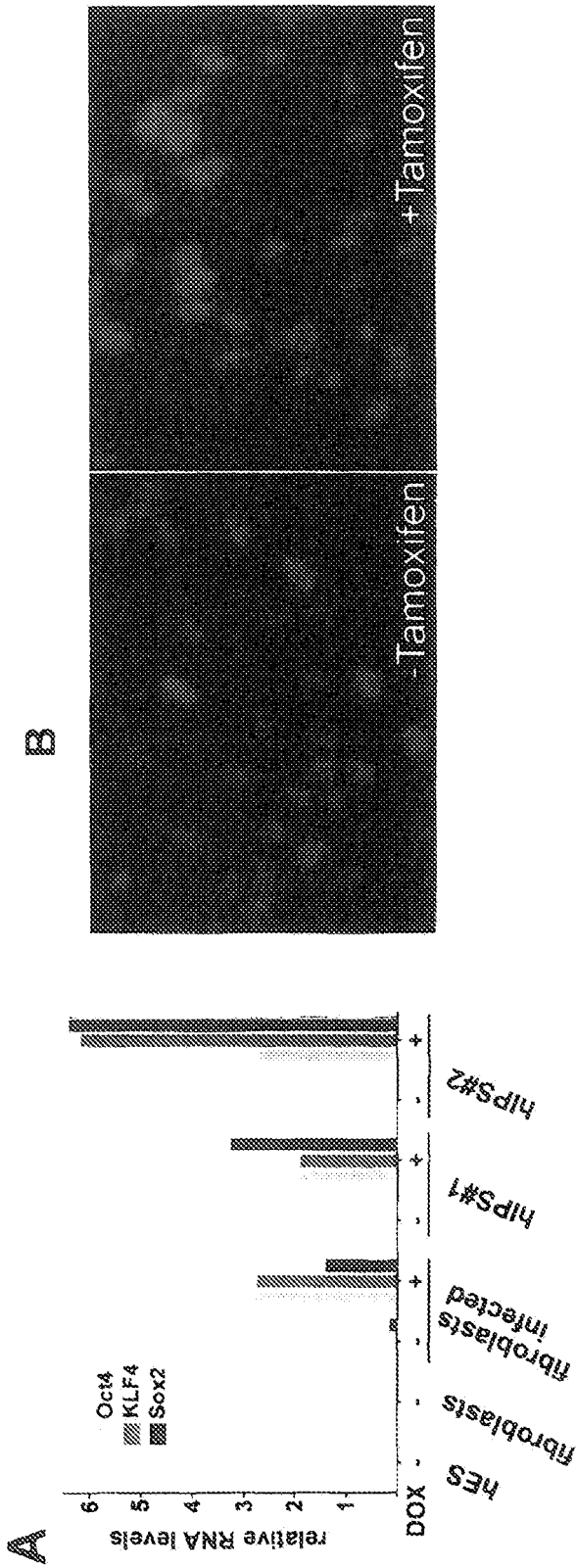
Figs. 8A-8D



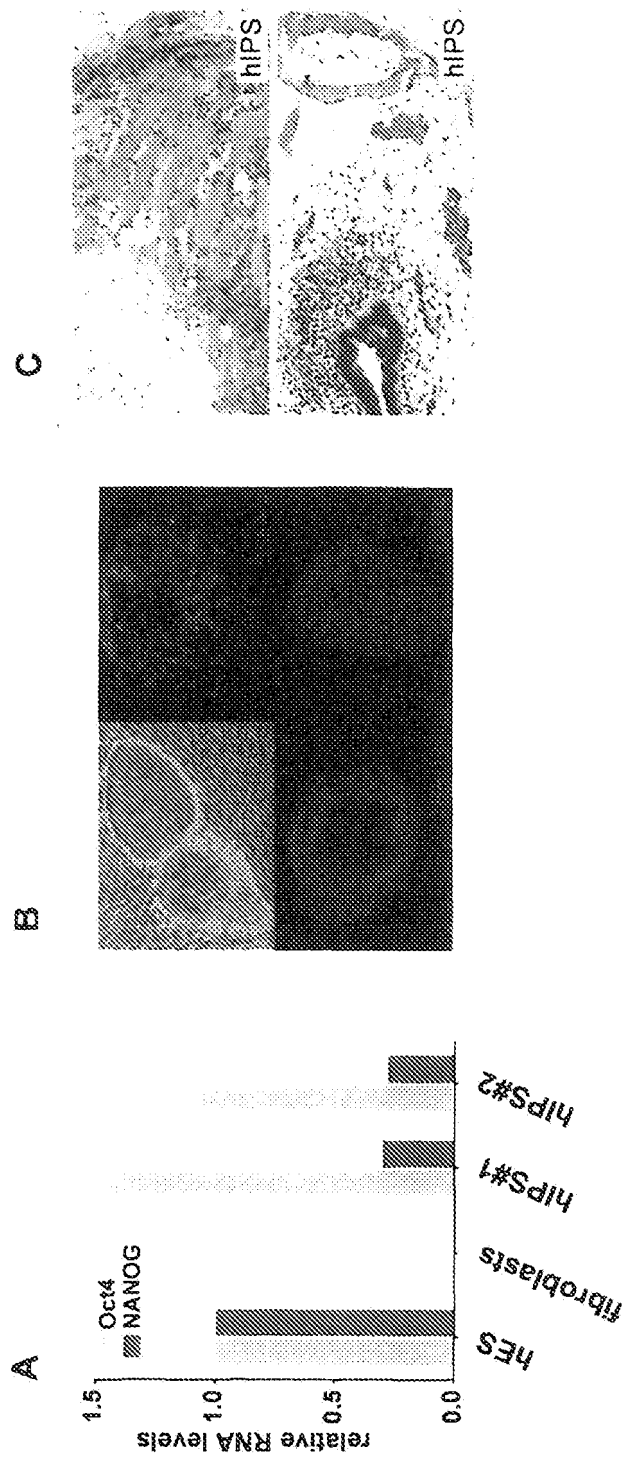
Figs. 9A-9G



Figs. 10A-10D



Figs. 11A-11B



Figs. 12A-12C

A

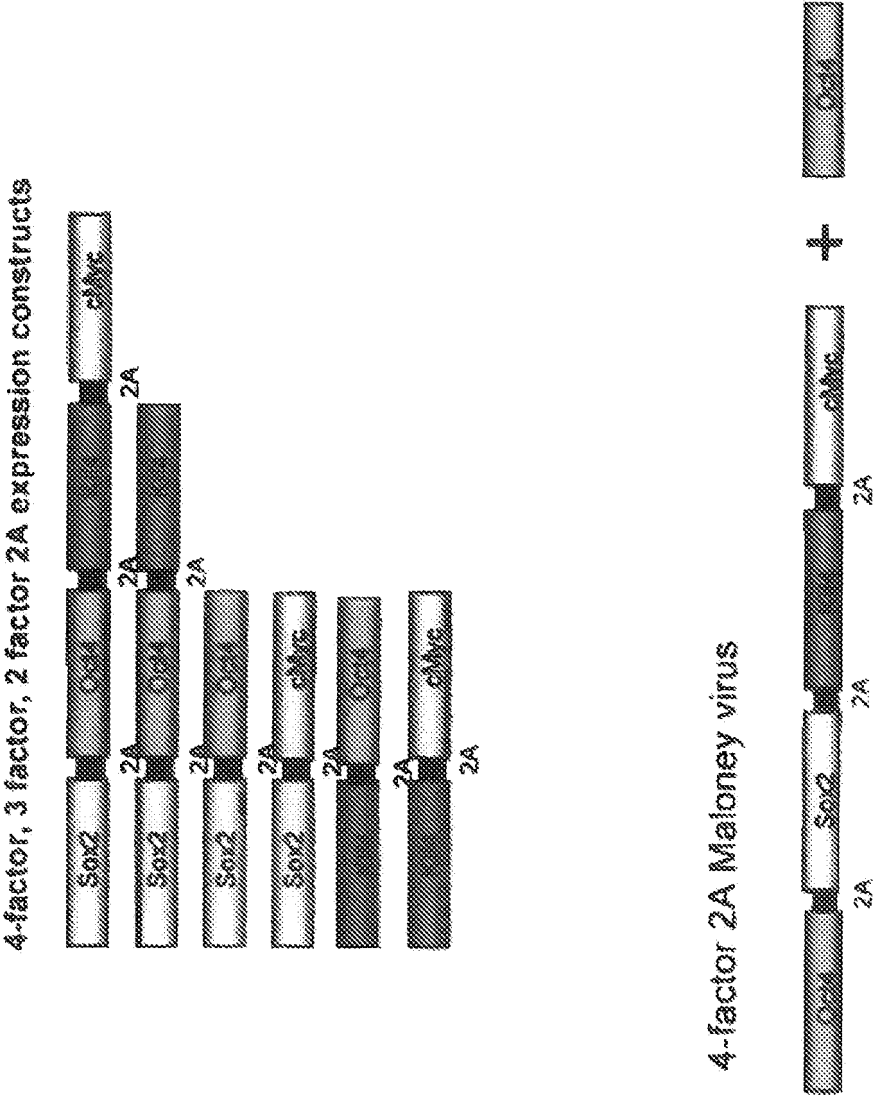


Fig. 13A

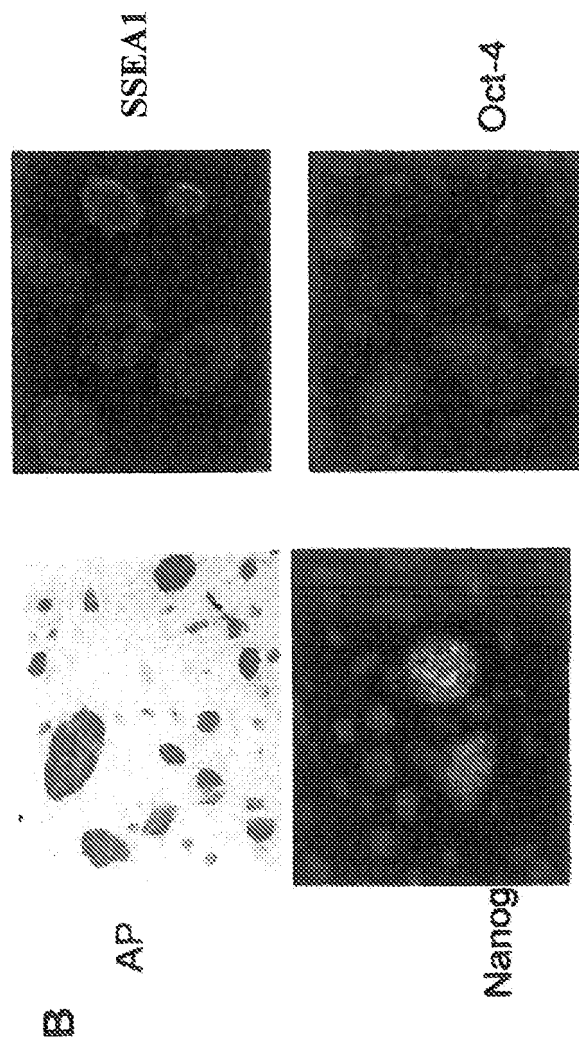


Fig. 13B

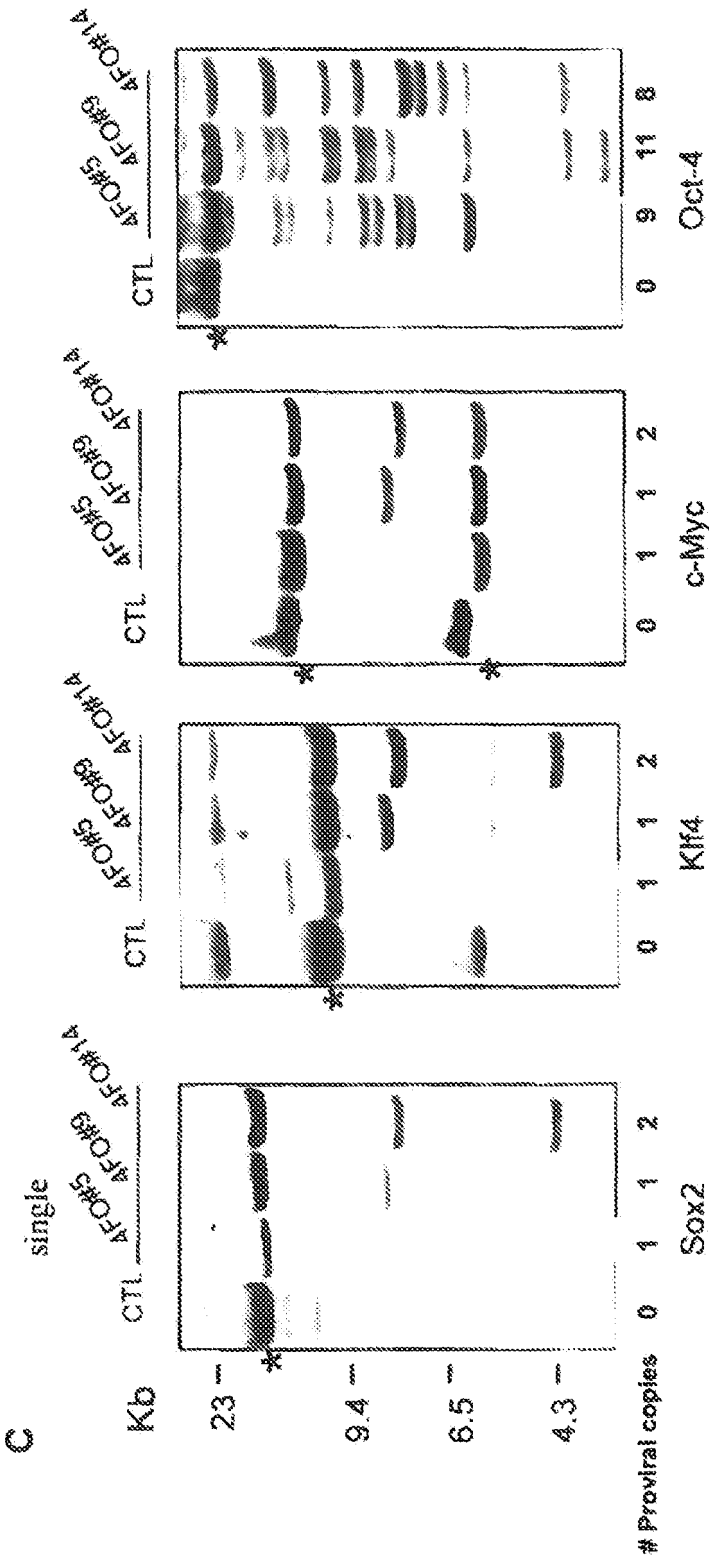
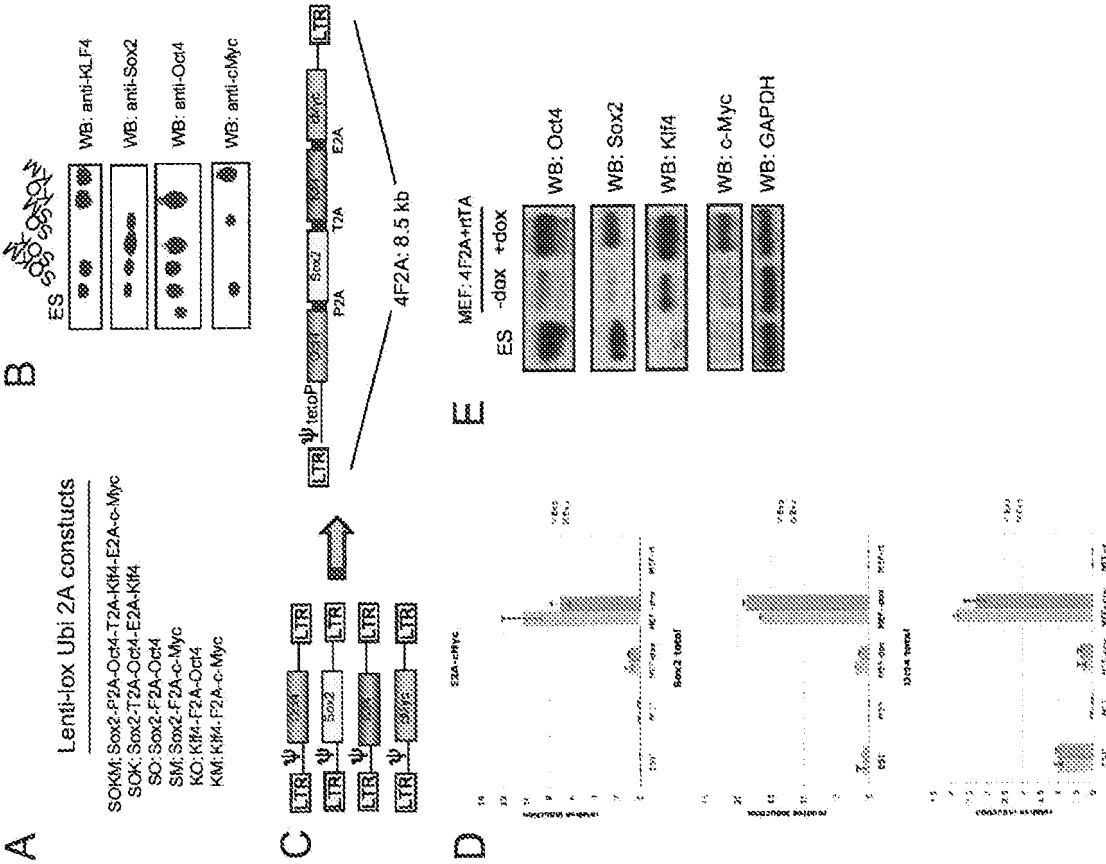


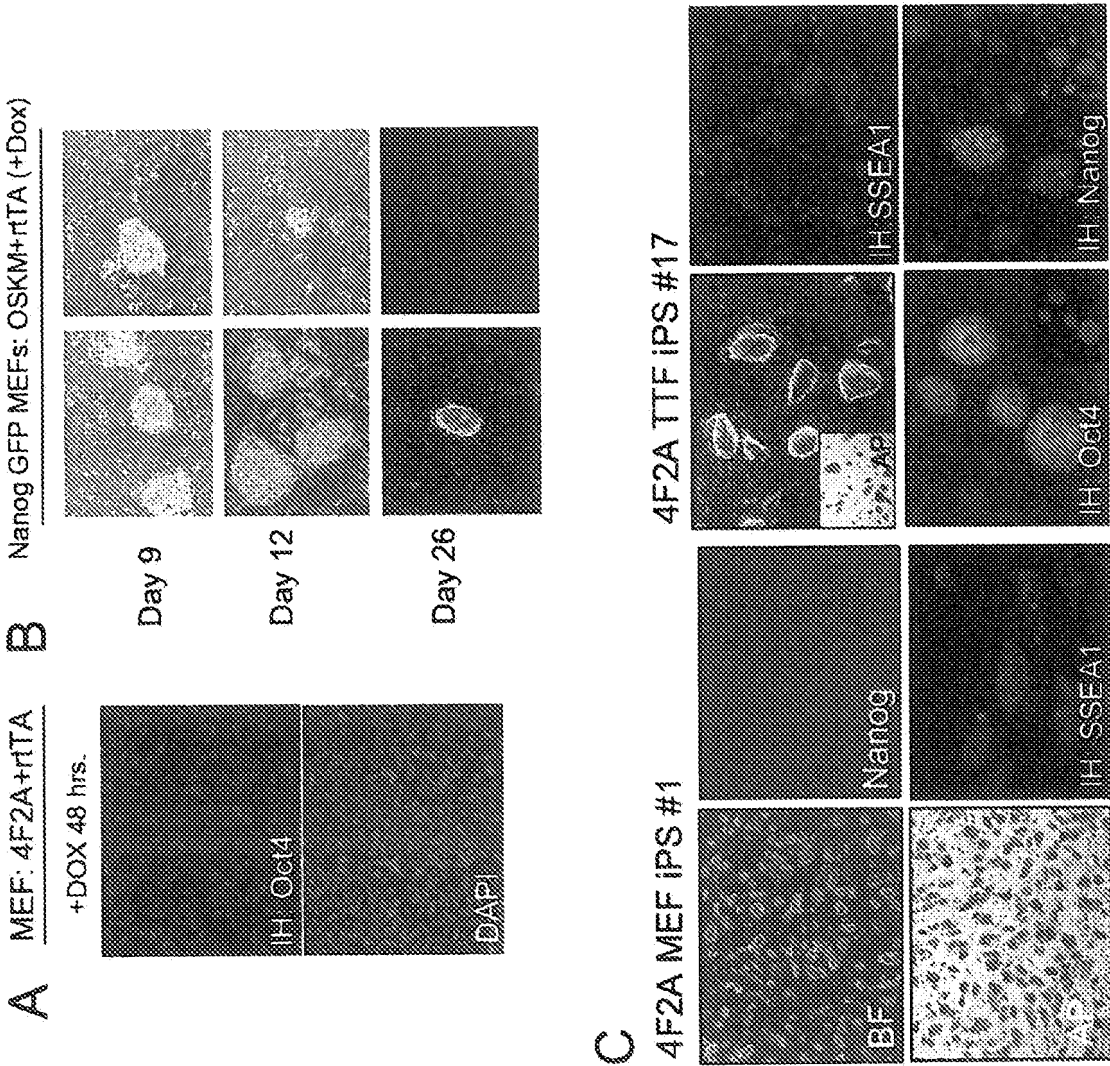
Fig. 13C

Generation of murine iPS cells using a single 4F2A polycistronic virus

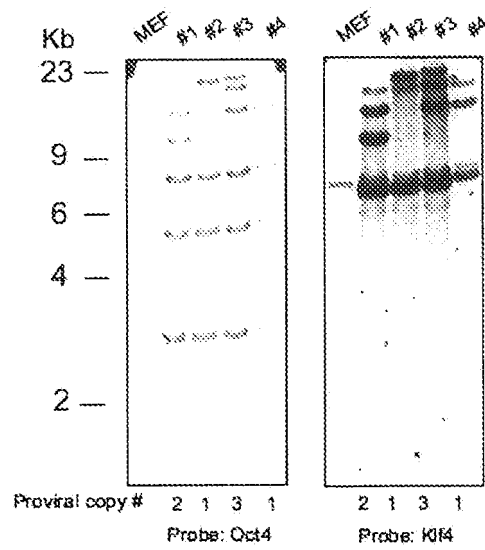


Figs. 14A-14E

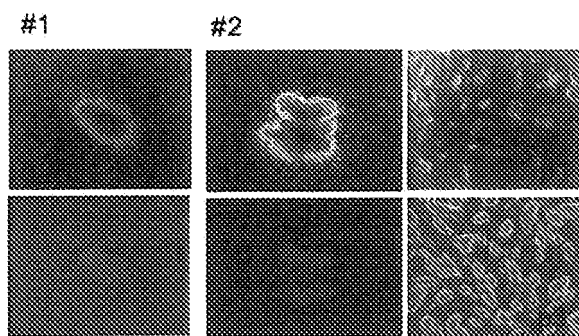
4F2A iPS cells express pluripotency markers



Figs. 15A-15C

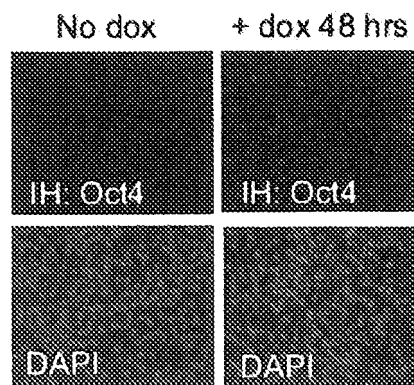
a. XbaI digest**b.**

8 days of DOX exposure



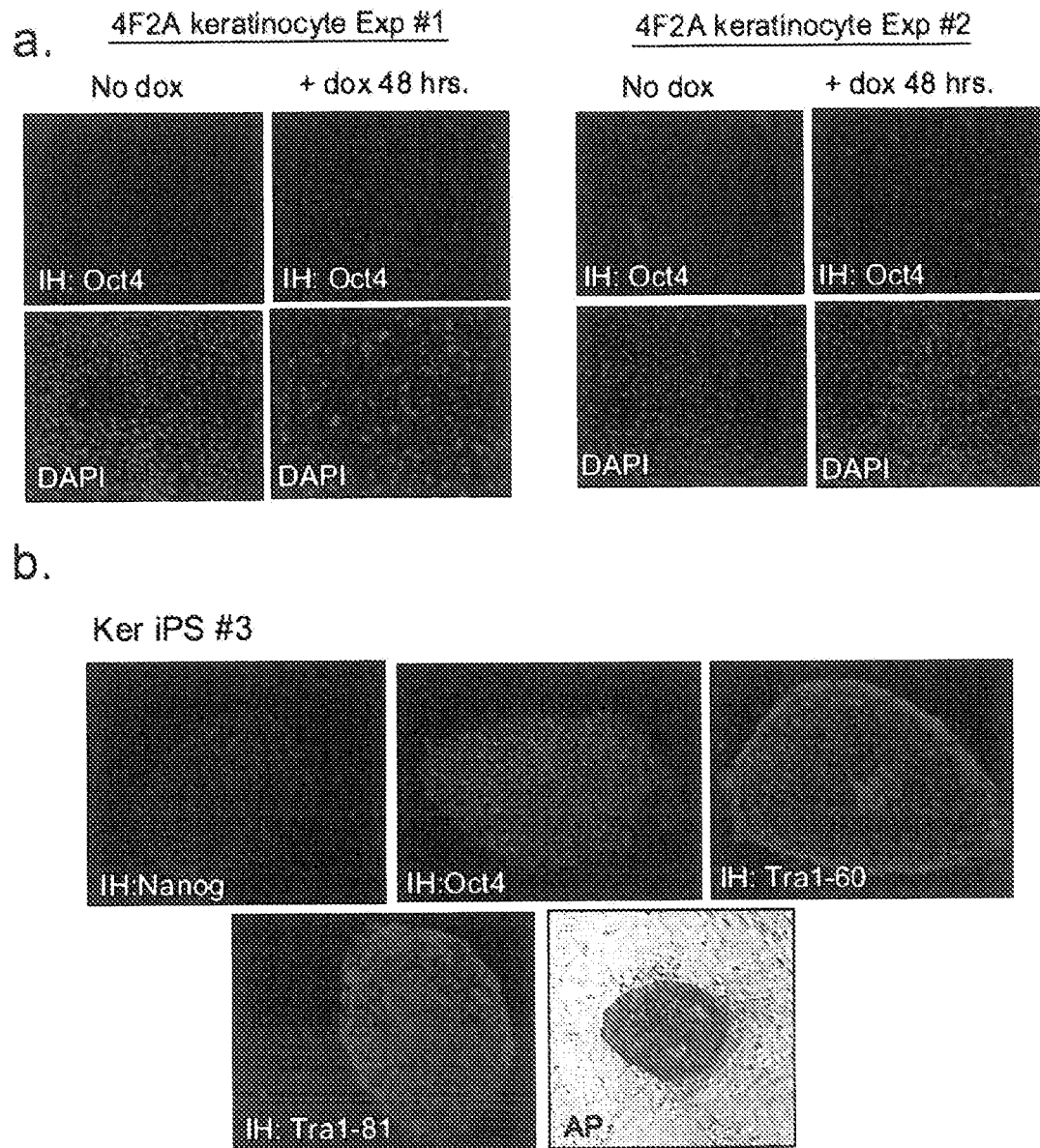
Figs. 18A-18B

MEF exp #2: 4F2A relative

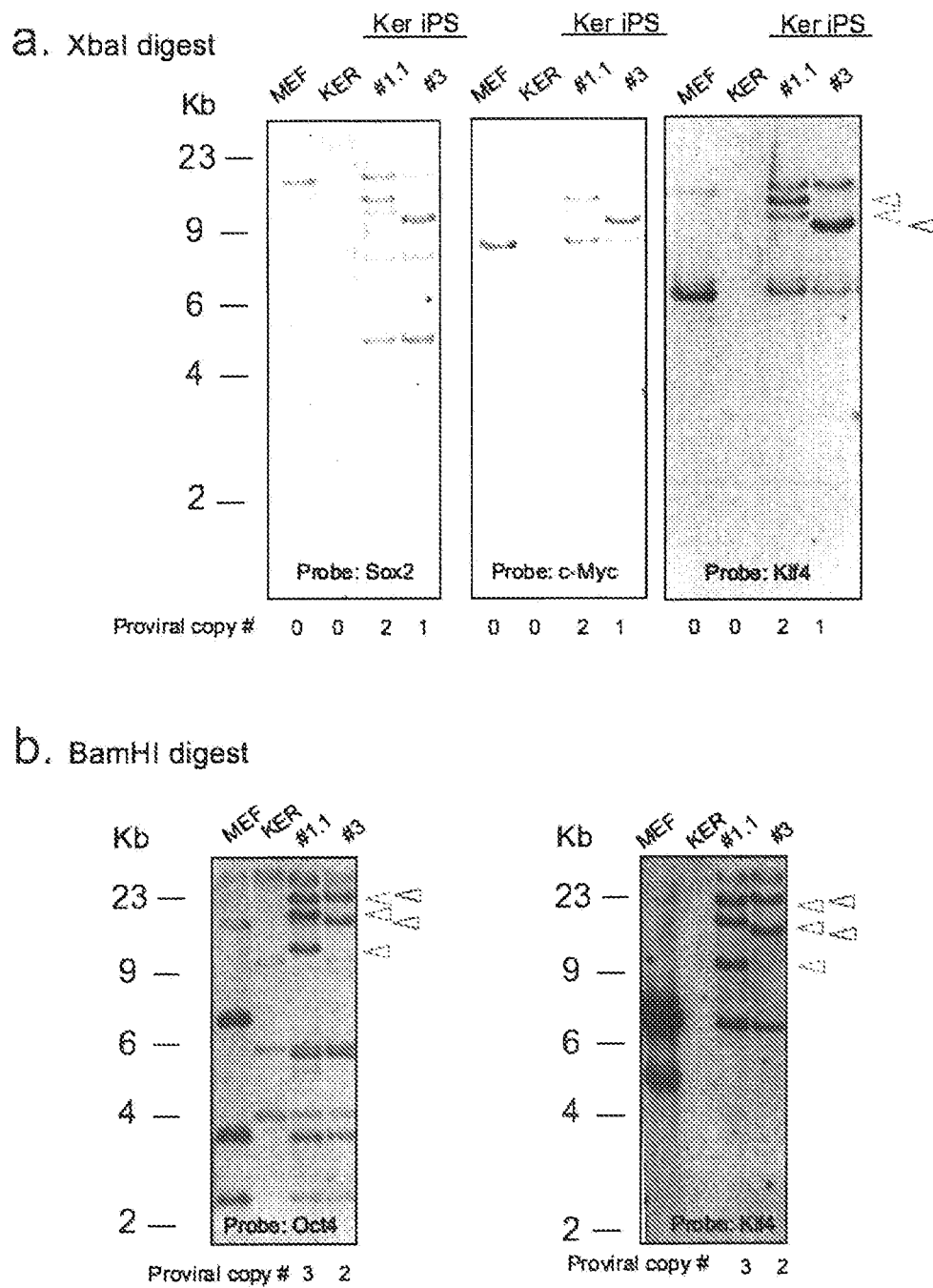


250,000 x 70% infection = 175,000
12.5 GFP + colonies/ 175,000 = ~ 0.0001%

Fig. 19



Figs. 20A-20B



Figs. 21A-21B

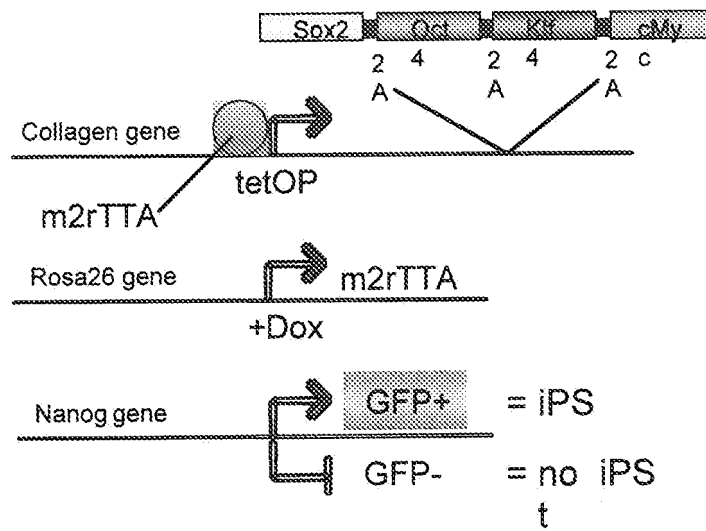
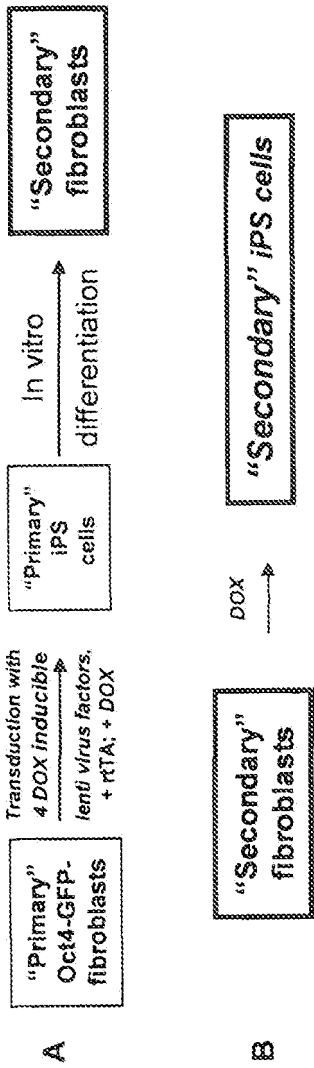


Fig. 22



Figs. 23A-23B

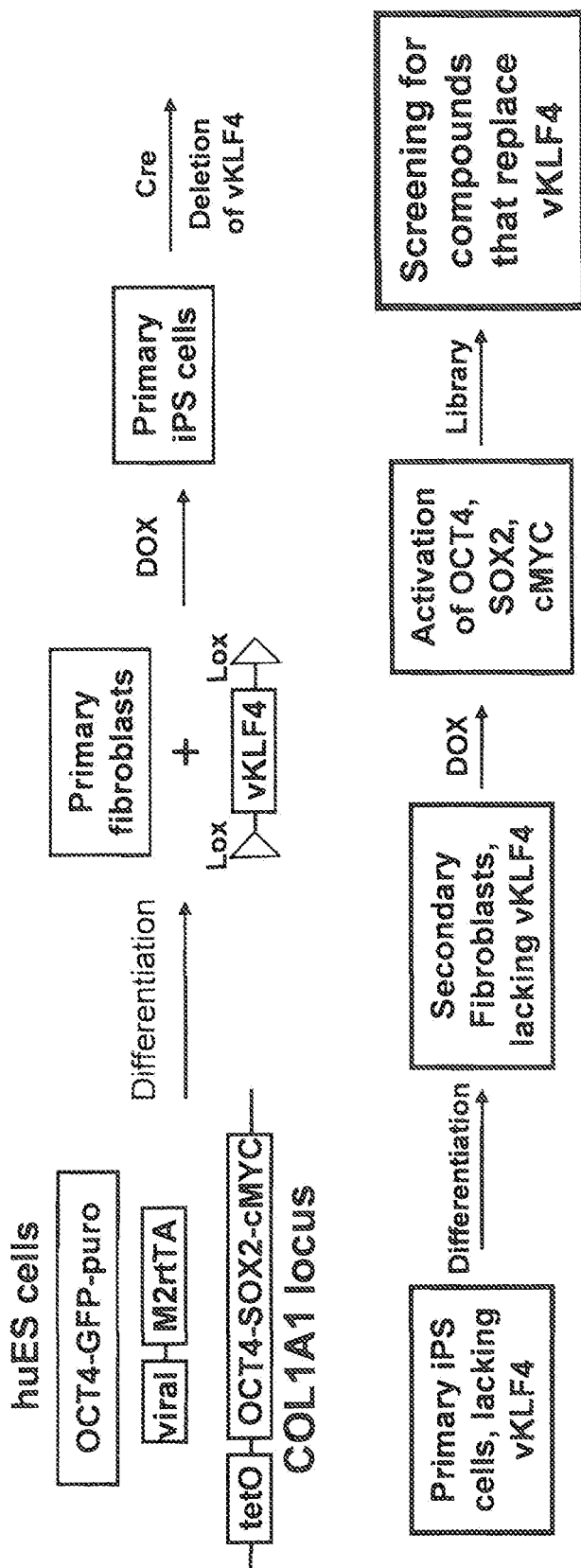


Fig. 24

Activation of GFP serves as screen for (i) small molecules, (ii) insertional activation of endogenous gene or (iii) transient transfection to replace KLF4

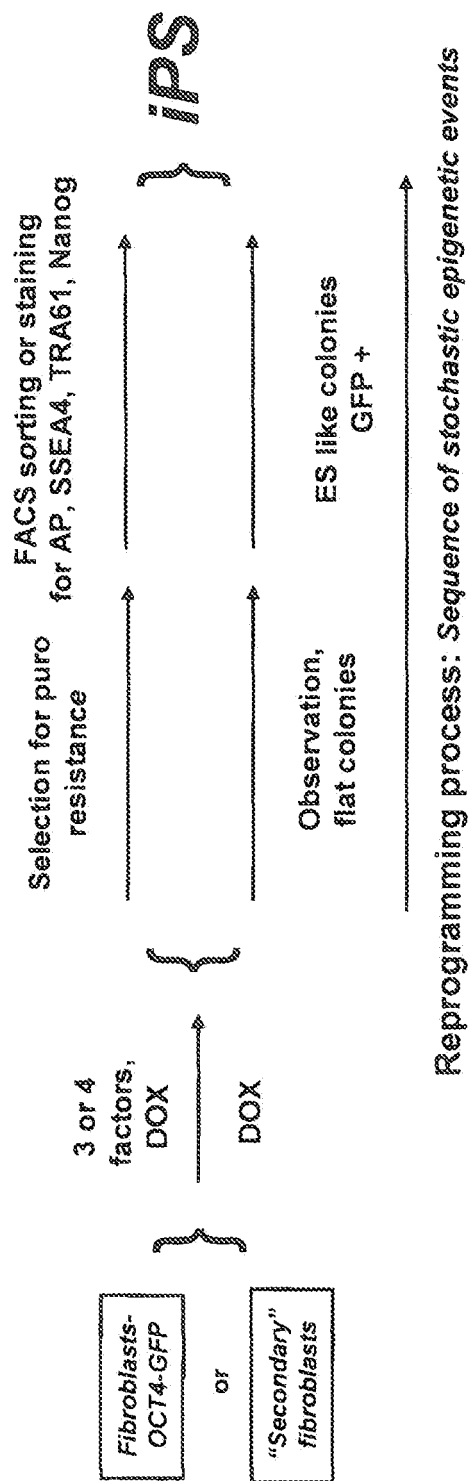


Fig. 25

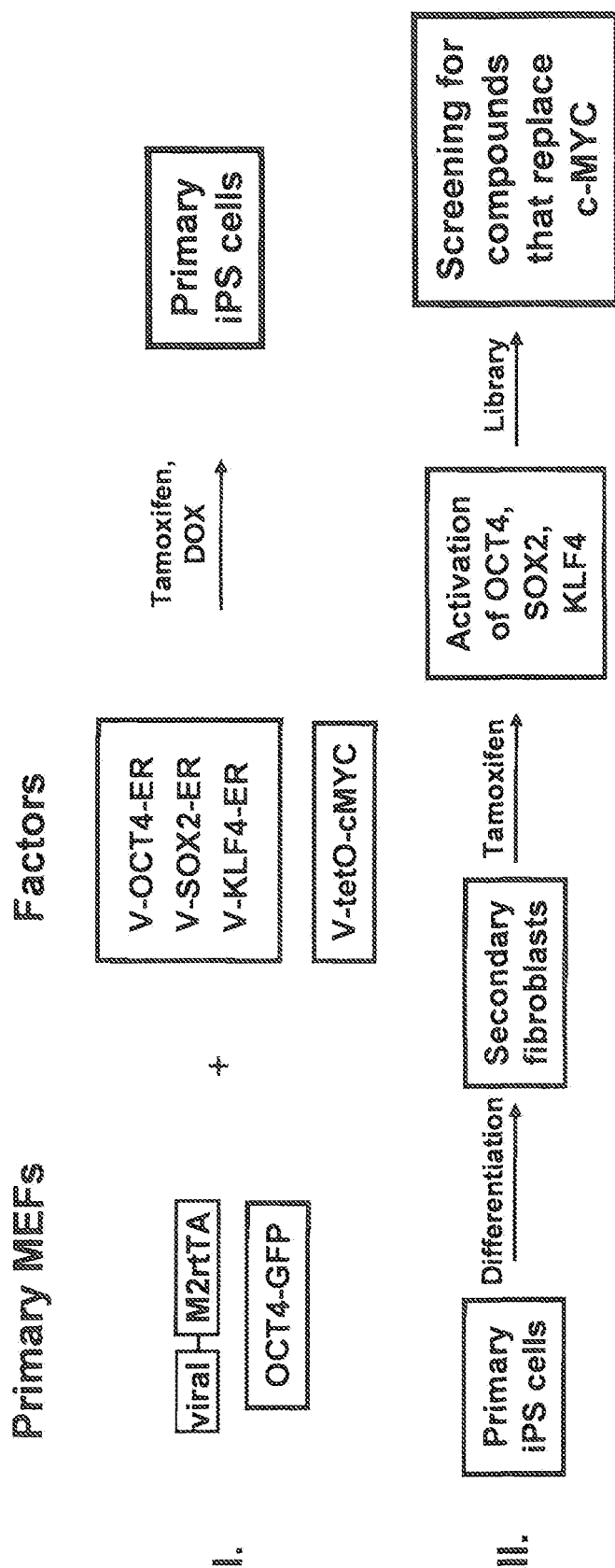
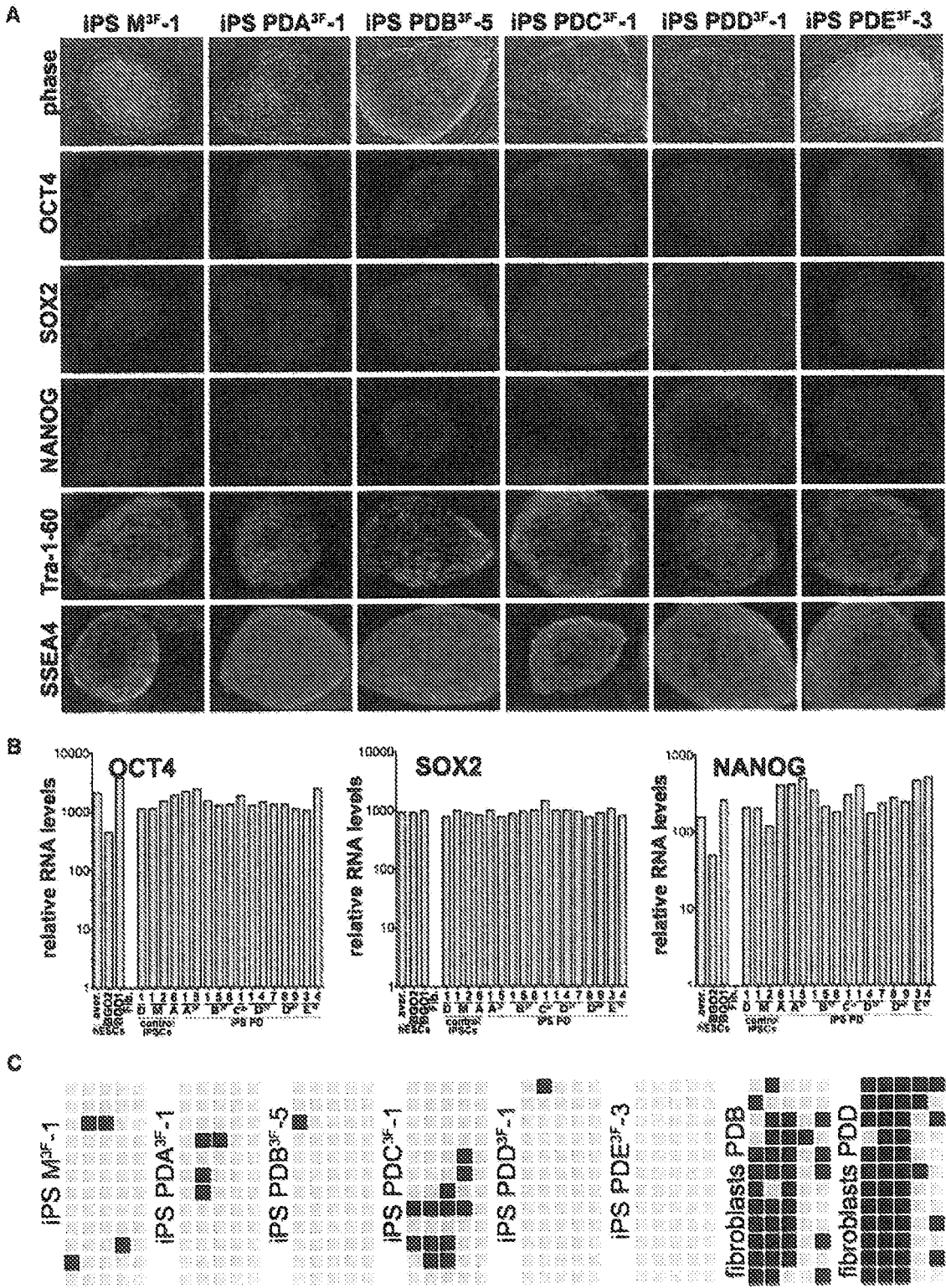
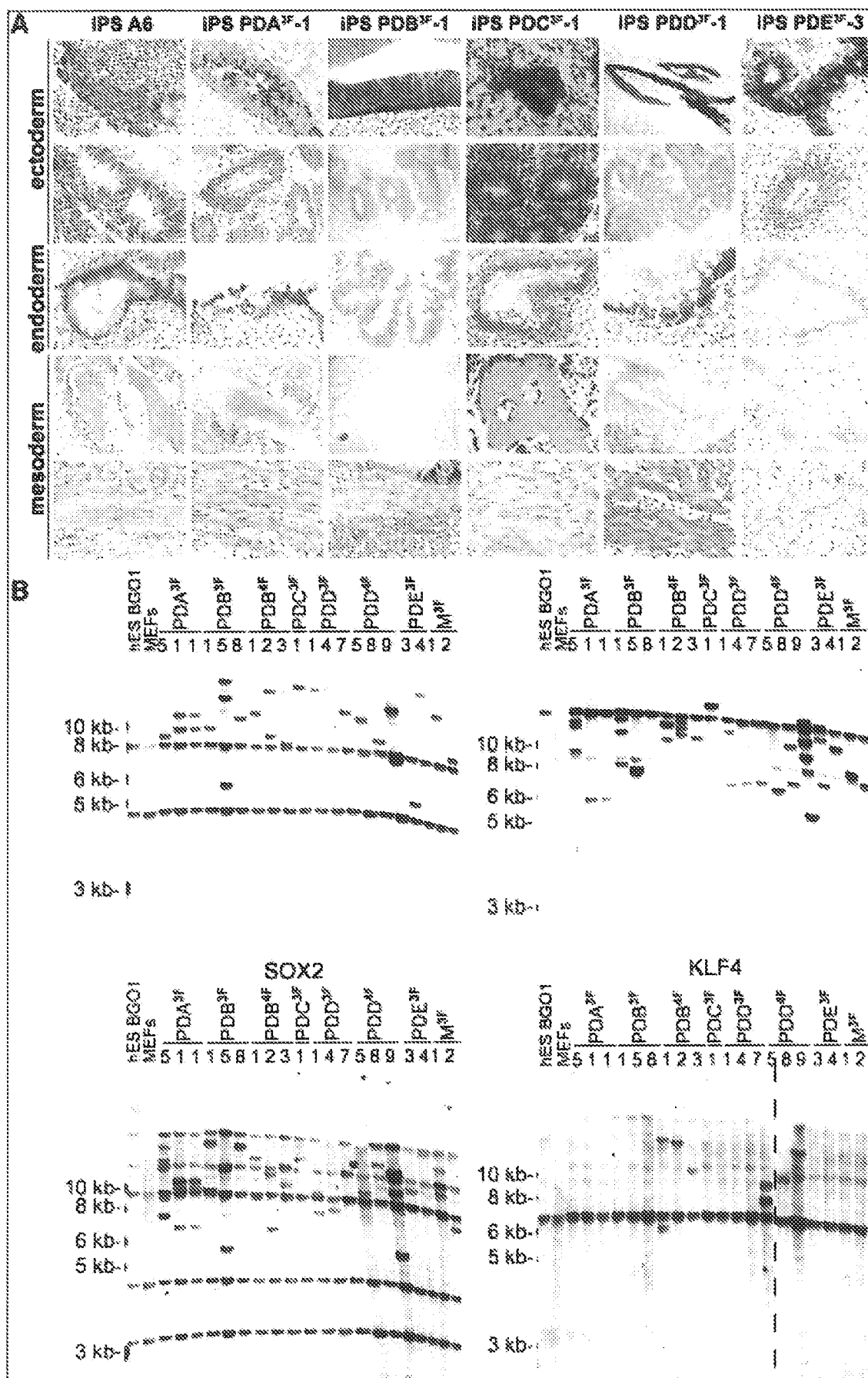


Fig. 26

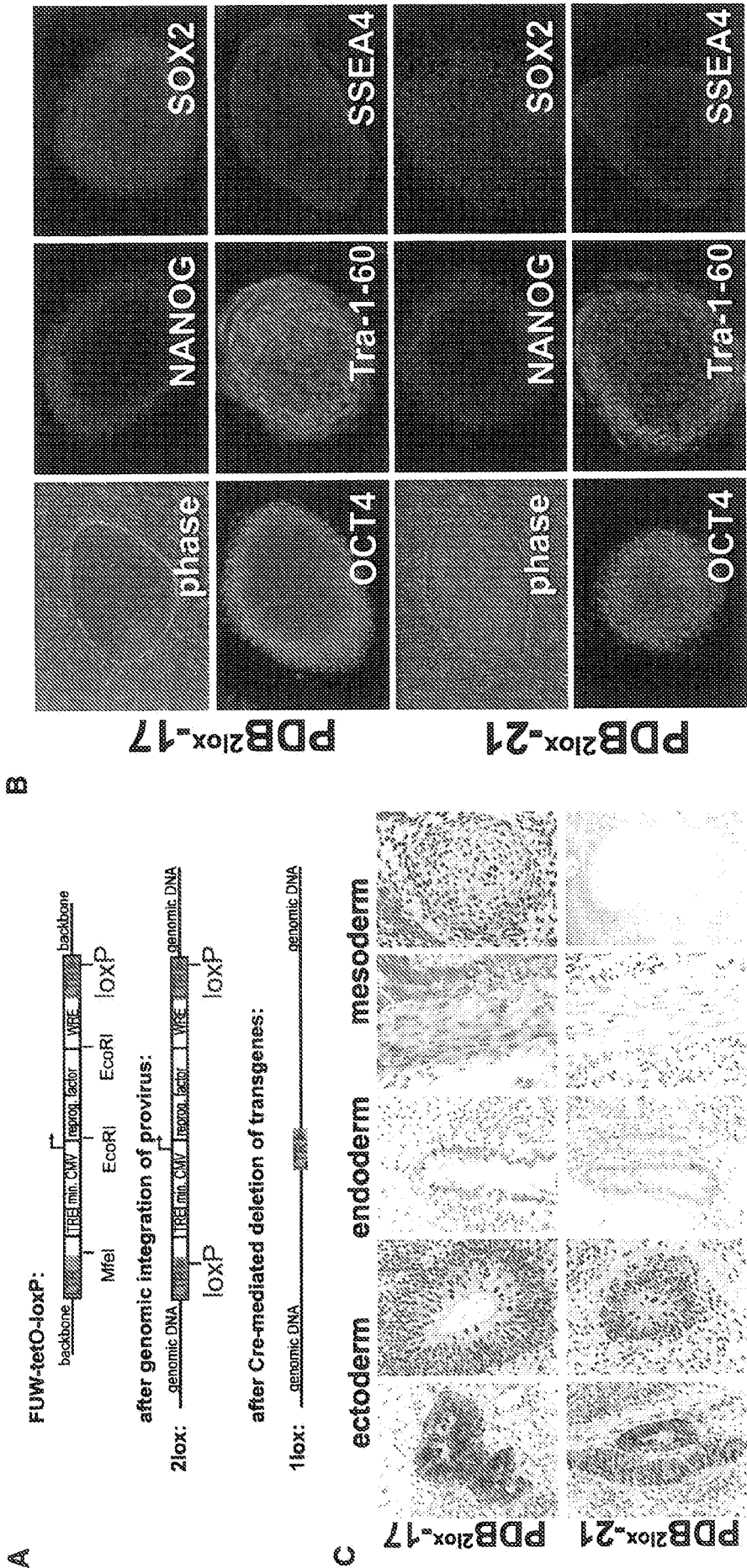


Figs. 27A-27C



Figs. 28A-28B





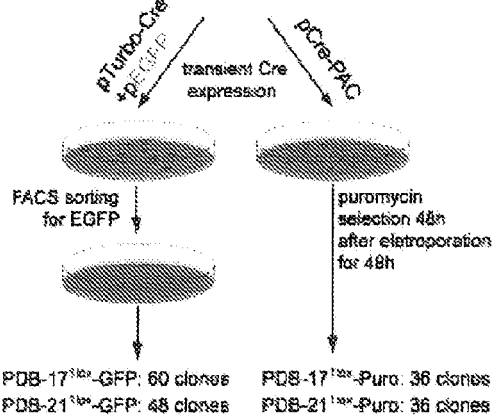
Figs. 29A-C

A

1. Generation of PDB^{2lox} iPSCs (n=24; efficiency ~ 0.005% of infected PDB fibroblasts):

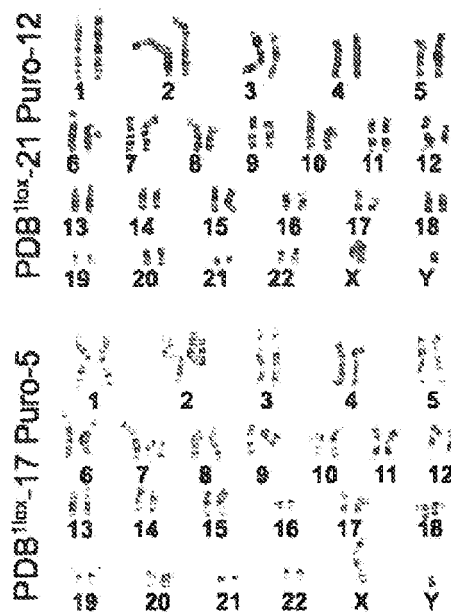


2. Electroporation of 1×10^7 IPS cells of two IPS clones ($PDB-17^{2lox}$ and $PDB-21^{2lox}$):



3. Southern blot analysis for transgene excision:
 -48 out of 180 clones excised KLF4 (EcoRI digest)
 -16 out of those 48 clones excised all copies of OCT4, SOX2 and KLF4 (XbaI and MfeI digest)

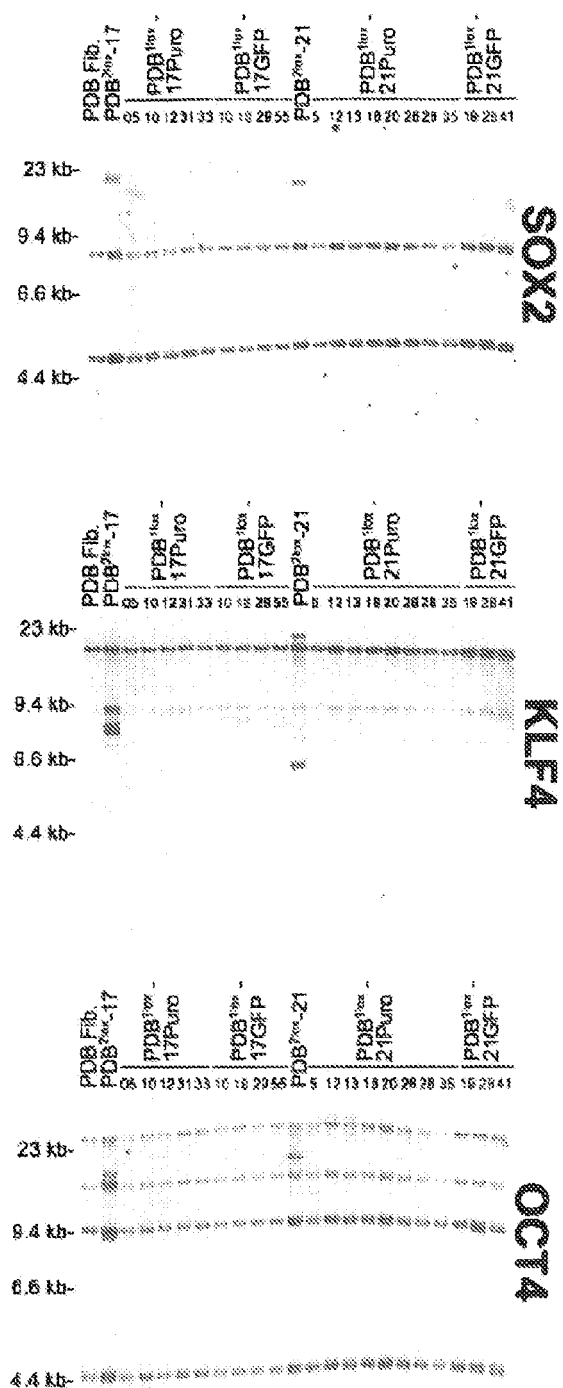
C



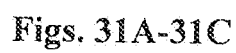
D

parental cell line	reprogramming factors	# of iPSC clones characterized	# of factor free iPSC clones	iPSC clone ID
AG28442 (PDB)	FUW-tetO-loxP; 3 factors (OCT4, SOX2, KLF4)	4/24	-	PDB^{2lox} -5,17,21,22
PDB^{2lox} -17	removed by Cre-recombinase	96	2 5	PDB^{1lox} -17GFP-29,55 PDB^{1lox} -17Puro-5,10,12,31,33
PDB^{2lox} -21	removed by Cre-recombinase	84	2 7	PDB^{1lox} -21GFP-19,41 PDB^{1lox} -21Puro-5,12,13,18,20,26,28

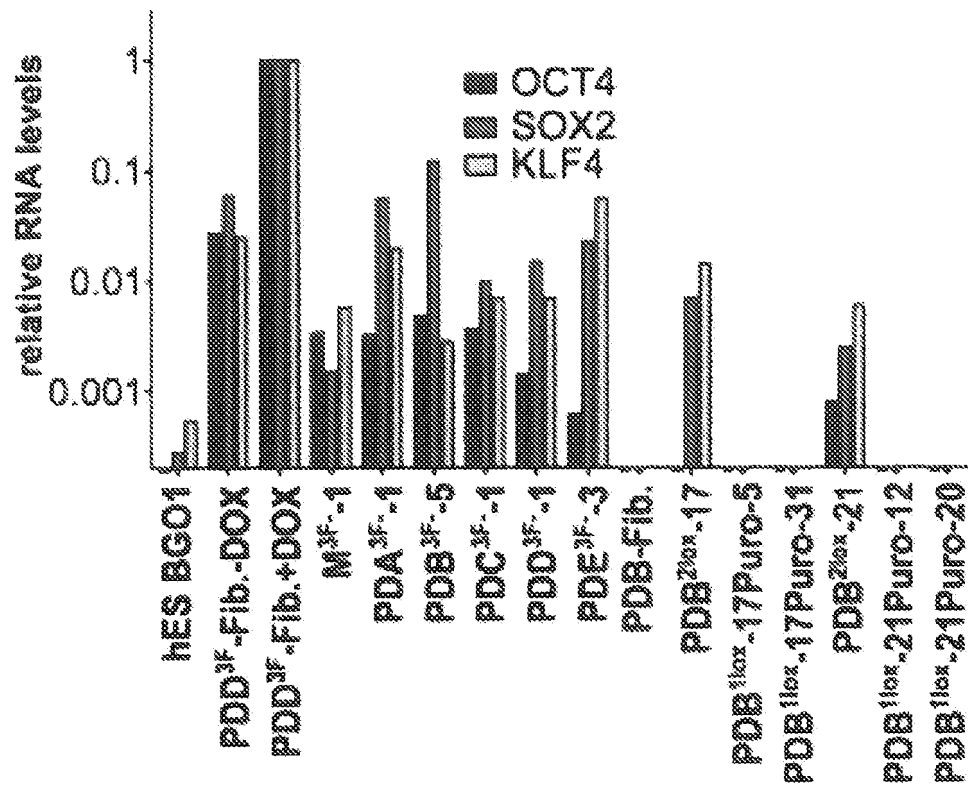
B



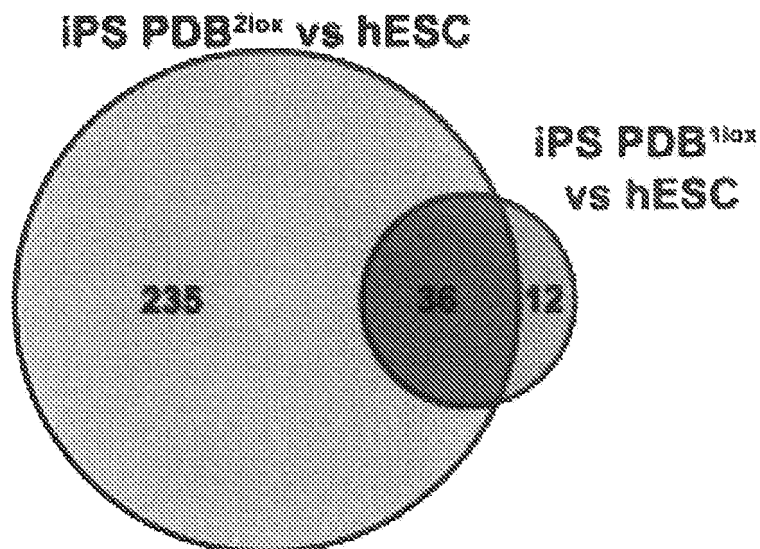
Figs. 30A-30D



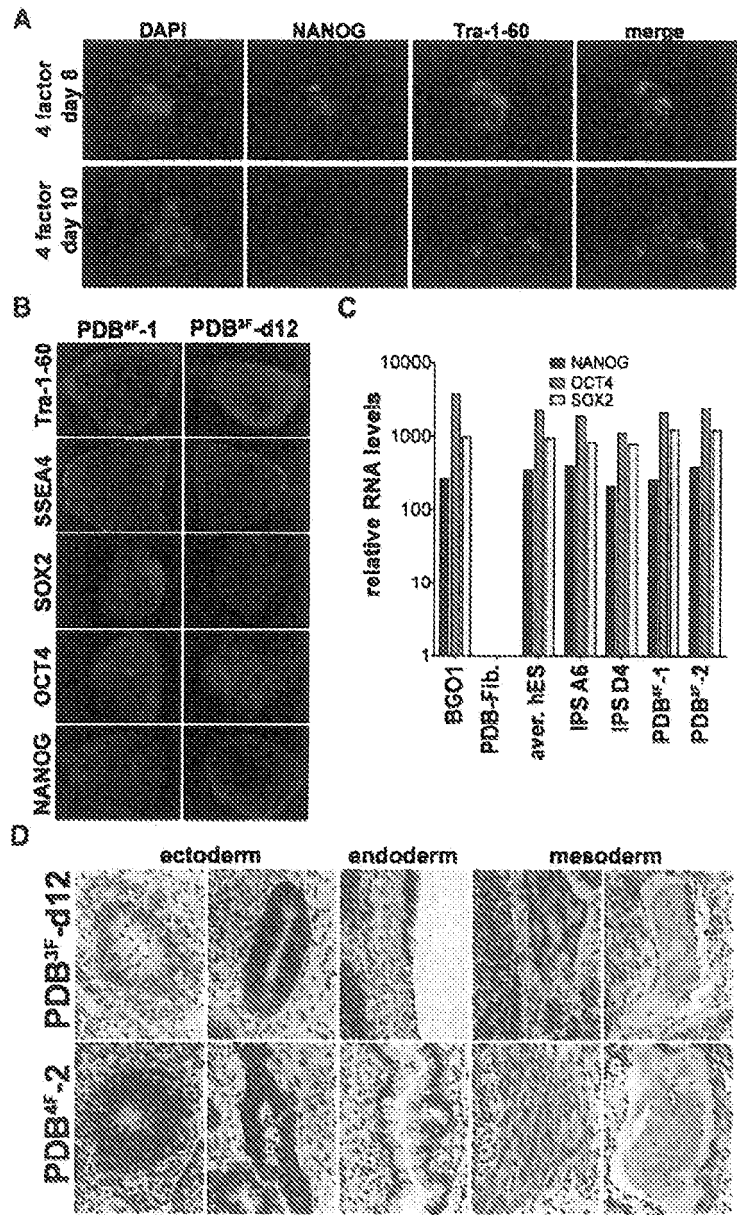
D



E



Figs. 31D-31E



Figs. 32A-32D

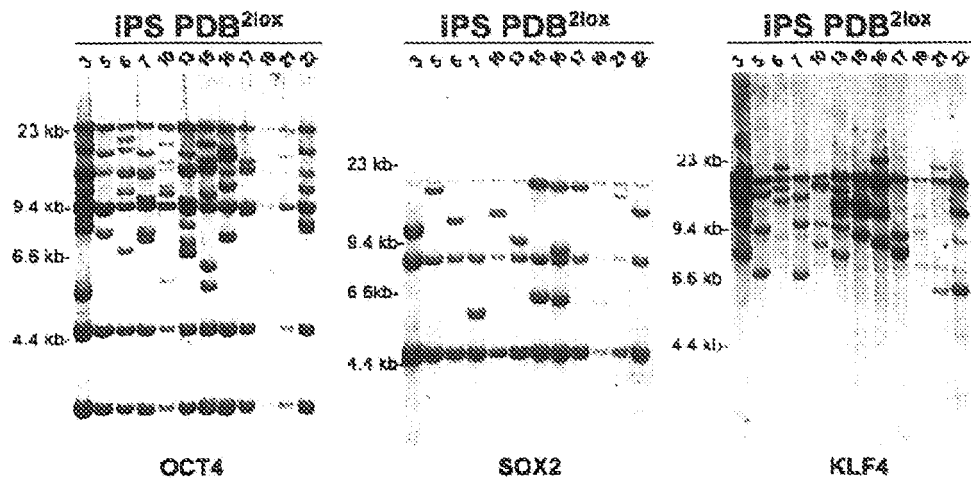


Fig. 33

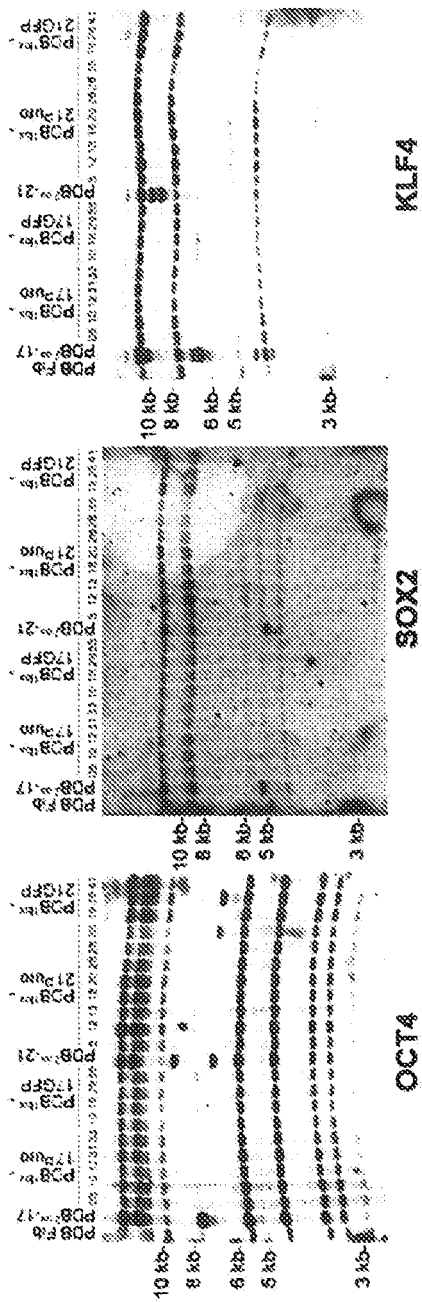


Fig. 34

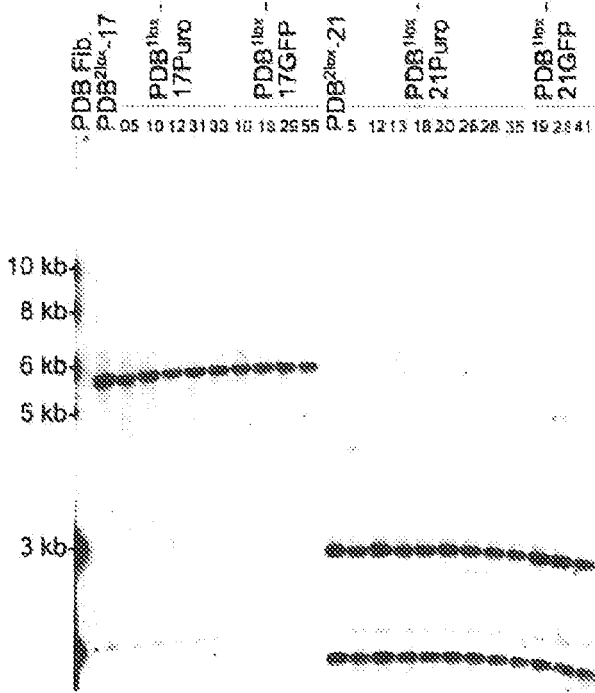


Fig. 35