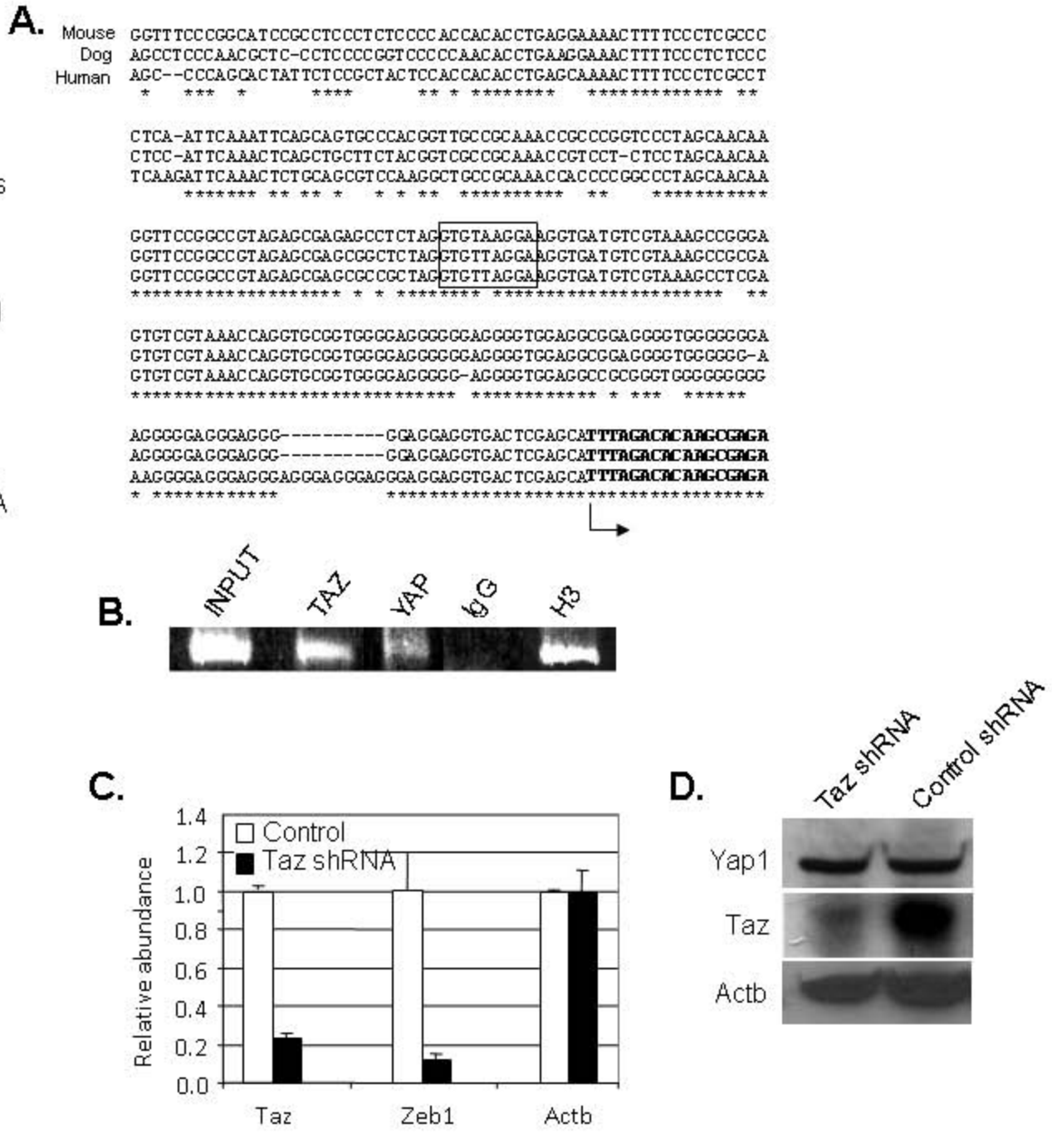
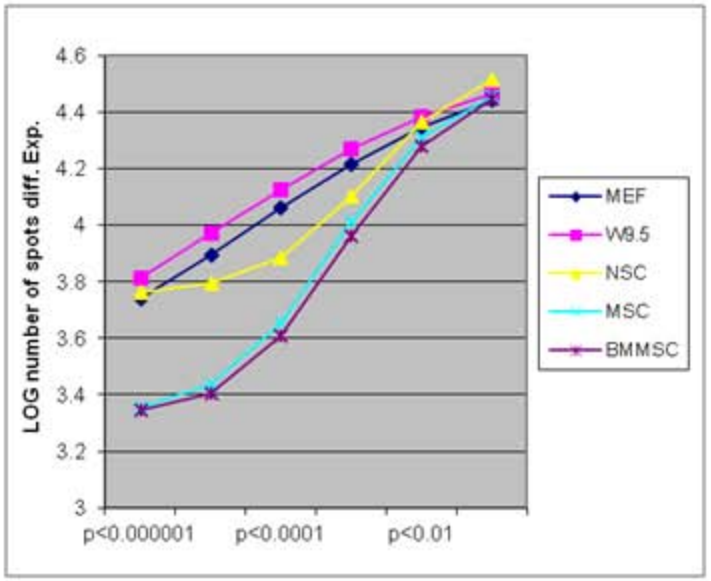
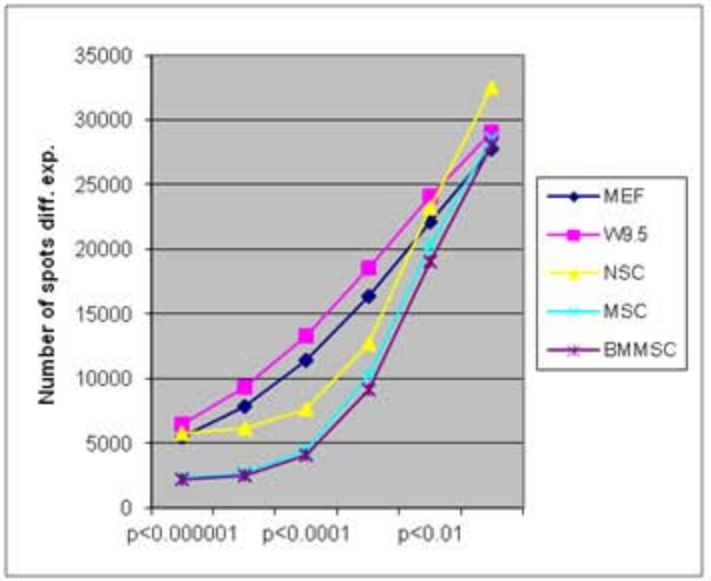
Liu et al Supplemental Fig. 1.

A. Sequence comparison of the *Zeb1* promoter in different species showing conserved Taz/Yap1 binding site (boxed). B. Chromatin immunoprecipitation assay showing binding of Taz and to a lesser degree Yap1 to the *Zeb1* promoter. "IgG" is control anti-serum, and H3 is a positive control of histone H3. C. Knockdown of Taz in MEFs leads to down regulation of ZEB1 mRNA by real time PCR. D. Western blot showing loss of Taz but not Yap1 with Taz knockdown. See ref. 19 for additional details of different Taz shRNA lentiviral constructs.

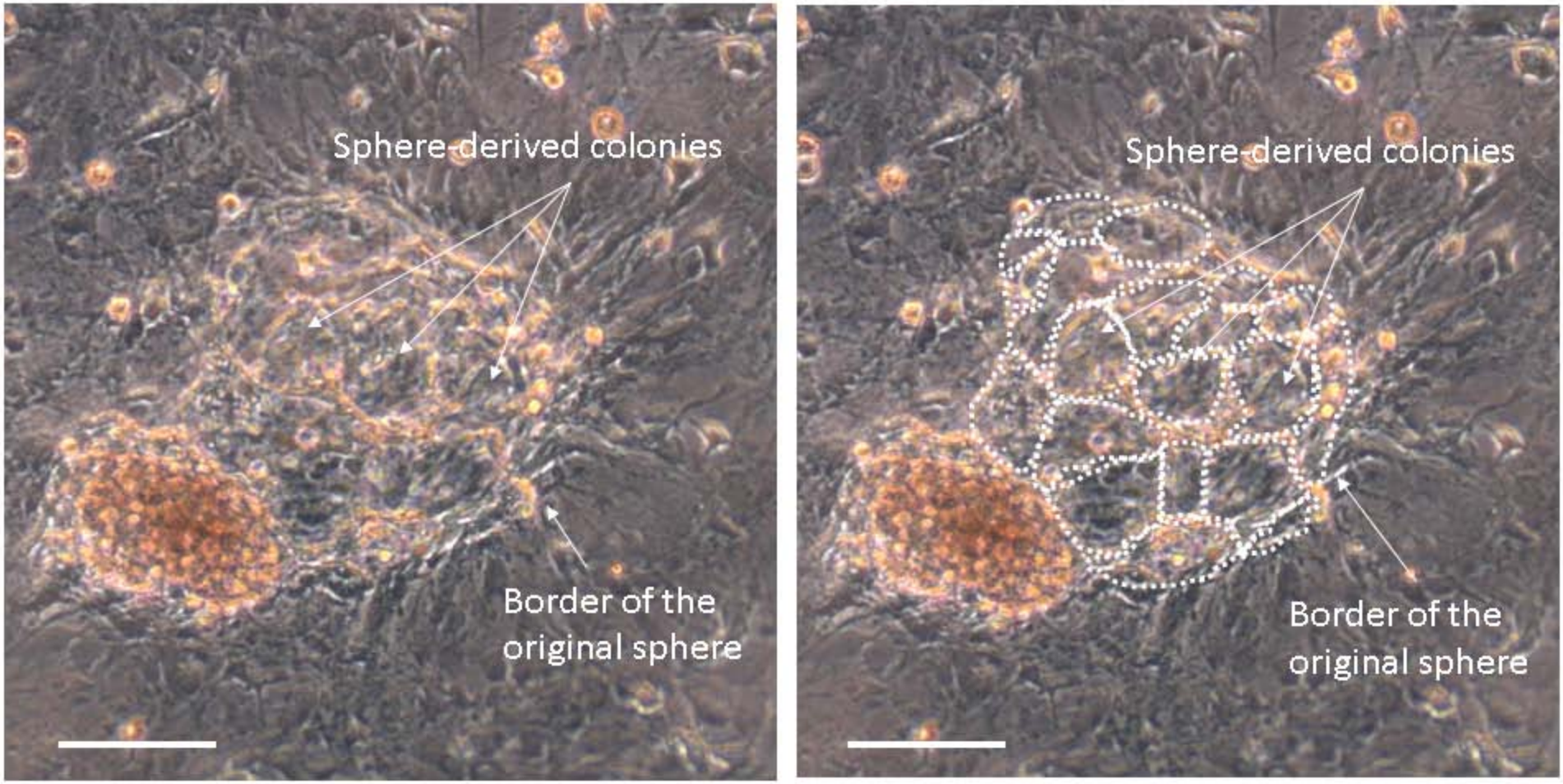




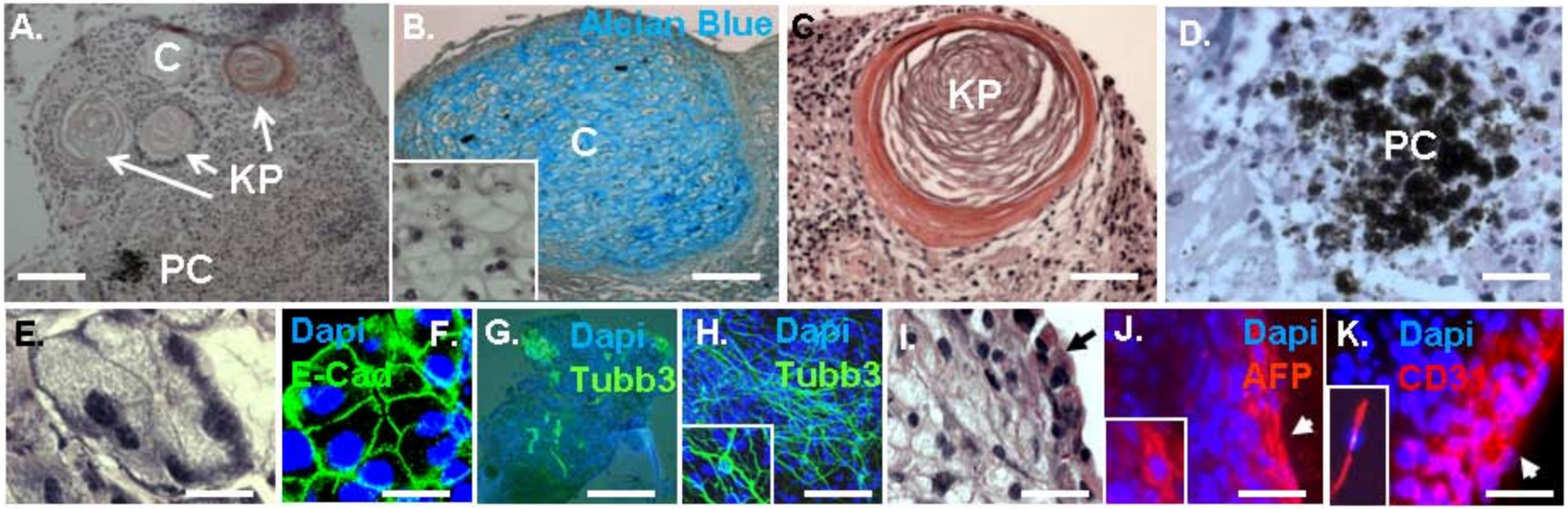


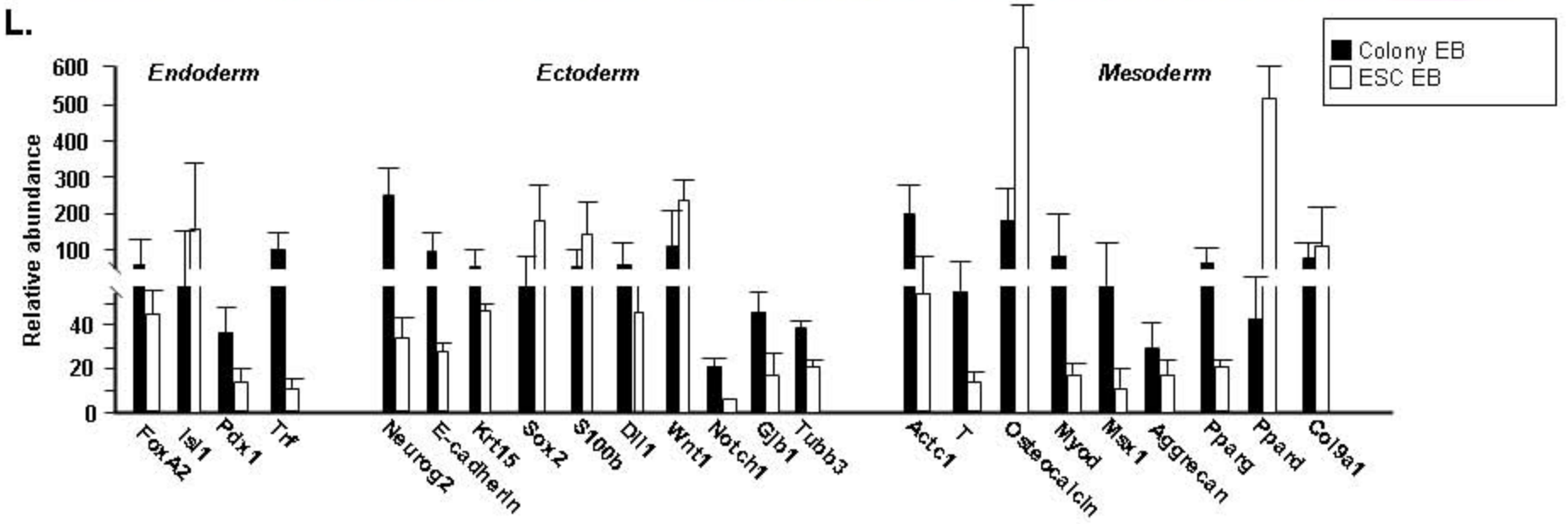
	MEF	W9.5	NSC	MSC	BMMSC
p<0.000001	5466	6482	5786	2260	2208
p<0.00001	7810	9340	6191	2715	2544
p<0.0001	11465	13303	7635	4497	4048
p<0.001	16398	18548	12697	10229	9150
p<0.01	22154	24153	23169	20335	19048
p<0.1	27742	29037	32541	28567	28046

Supplemental Fig. 2. All 45101 spots on the mouse whole genome arrays for MEF, W9.5, NSC, MSC, BMMSC data in Fig. 4 were compared to the sphere derived stem cell like colonies (SDSC). The LOG-transformed data were first normalized to the mean of all the spots, then the value of each spot for SDSC was divided by the value of the same spot for MEF, W9.5, NSC, MSC, BMMSC, respectively. A student T-test of SDSC vs. other samples for each spot is calculated against '1', and the probability (p value) then is assigned for all the tests. Based on the p value the number of differentially expressed spots were counted and plotted on the attached two figures. On right, the y-axis is actual spot number, whereas in the figure on left the y-axis is LOG10 spot number. The fewest significant gene expression changes are seen in the MSC and BMMSC, suggesting that the SDSC are more closely related to MSC.

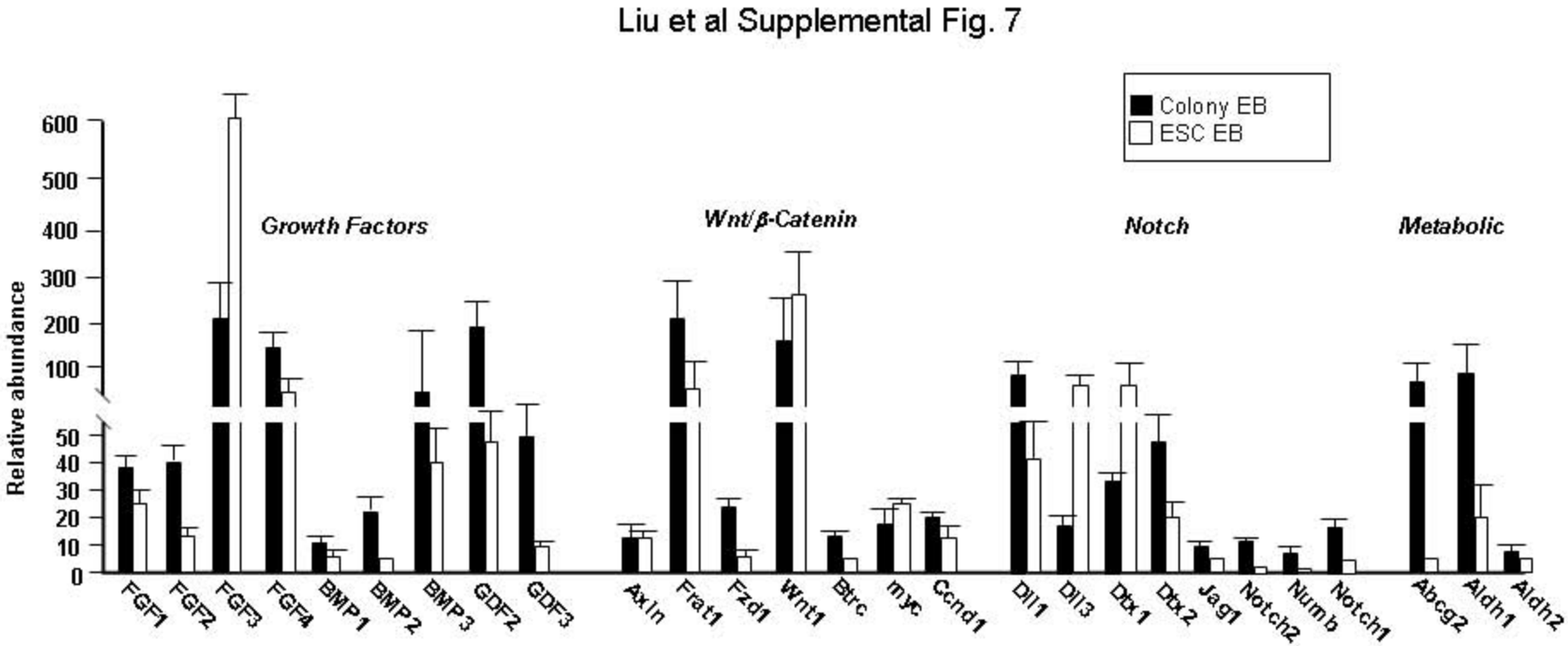


Supplemental Fig. 3. After 4 days in suspension culture, MEF spheres were allowed to adhere to Matrigel coated plates. Cells in the spheres migrated out onto the plate, and a colony was evident at the site of sphere adhesion after 10 days. Note that this colony is actually composed of a number of tightly packed colonies, which are outlined in the panel on the right. The bar is 30 μm.

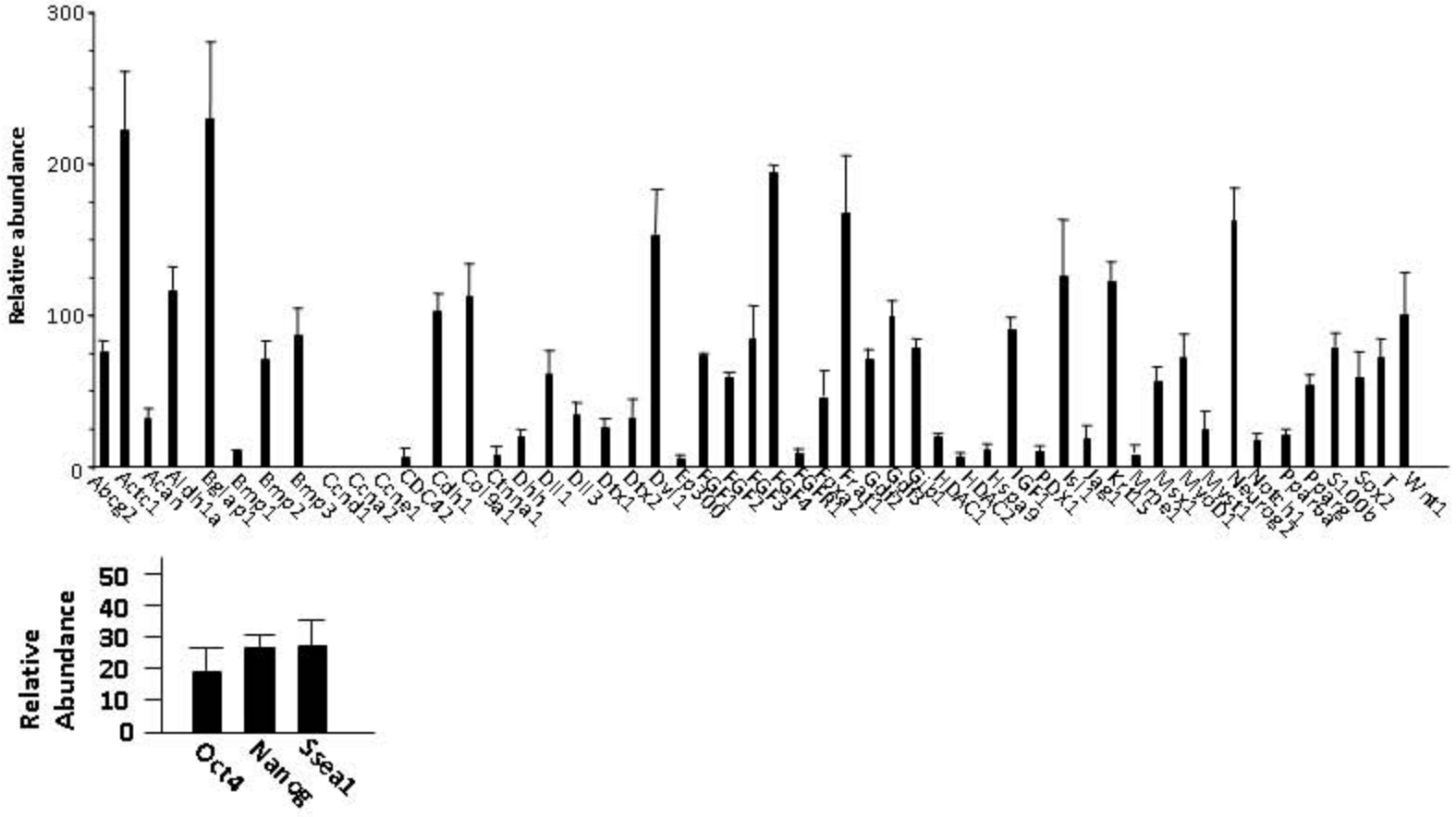

A.
C
KP
PC
B.
Alcian Blue
C
C.
KP
D.
PC
E.
F.
Dapi
E-Cad
G.
Dapi
Tubb3
H.
Dapi
Tubb3
I.
J.
Dapi
AFP
K.
Dapi
CD31


L.
Colony EB
ESC EB
Endoderm
Ectoderm
Mesoderm
Relative abundance
600
500
400
300
200
100
40
20
0
Fox A2
Isl1
Pdx1
Trf
Neurog2
E-cadherin
Krt15
Sox2
S100b
Dll1
Wnt1
Notch1
Gjb1
Tubb3
Actc1
T
Osteocalcin
Myod
Msx1
Aggrecan
Pparg
Ppard
Col9a1

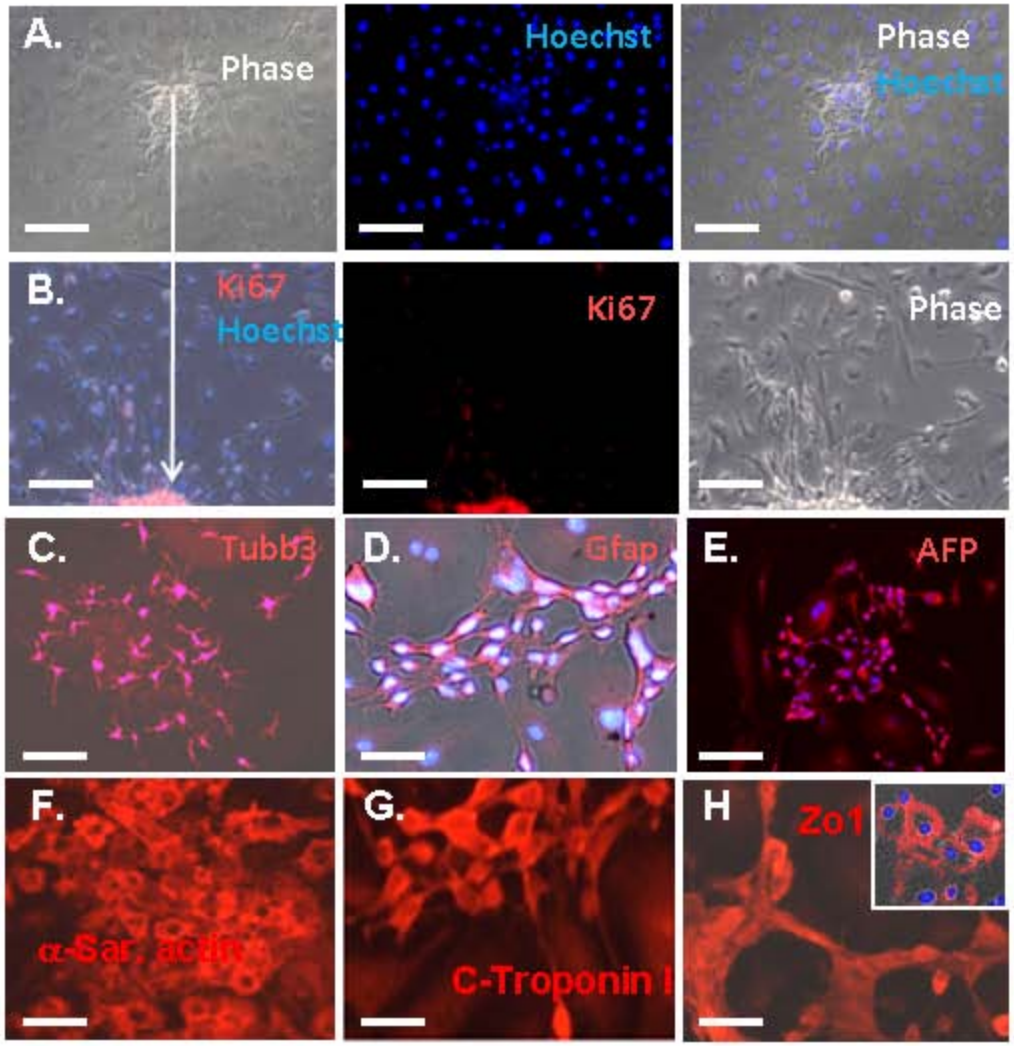
Supplemental Fig. 4. Embryoid body formation with sphere-derived colonies. Three week old embryoid bodies were sectioned for H&E and immunostaining. A. Keratin pearls (KP), cartilage (C) and pigmented cells (PC). B. Region of cartilage stained with alcian blue. The insert shows a higher power view prior to alcian blue staining. C. Higher power view of a keratin pearl. D. Higher power view of pigmented cells. E. A region resembling secretory epithelium. F. Immunostaining of a section adjacent to that in panel E for E-cadherin (E-Cad). G. Low power view of an aggregate of spheres immunostained for the neuronal marker Tubb3. H. Higher power view of Tubb3 immunostaining. I. Edge of an embryoid body containing a region resembling primitive endoderm. J-K. Immunostaining of sections adjacent to that in panel I for AFP and CD31. Arrows indicate the edge of the embryoid body. The bar represents 200 μm in panel A; 75 μm in panel B-C; 400 μm in panel G; 50 μm in panels D and H-K; 30 μm in panels E-F. L. Real time PCR array comparing express in three week old embryoid bodies formed from sphere-derived colonies and ESC (Liu cell stem cell). Results are normalized to b-actin (Actb) mRNA and both samples are compared to fibroblasts maintained in monolayer culture whose mRNA levels are set to 1.0. An average of the values from three independent experiments is shown along with standard deviations. The bar is 150 μm in Panel A and B.



Supplemental Fig. 5. Real time PCR array as in Supplemental Fig. 2 comparing expression of genes in signaling pathway important for reprogramming in embryoid bodies (EB) formed from sphere-derived colonies and ESC. Expression in MEFs in monolayer culture is normalized to 1.0.

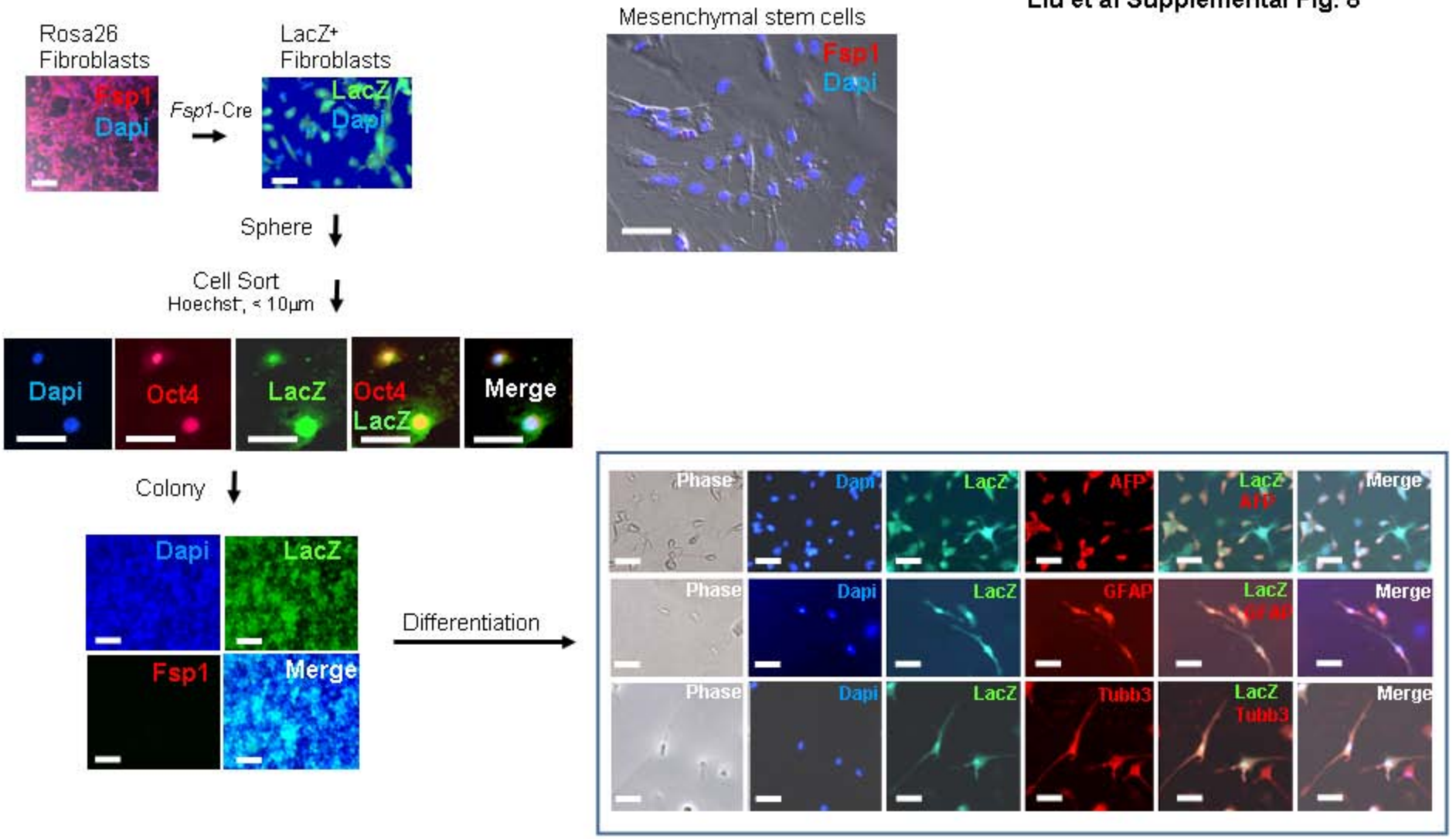


Supplemental Fig. 6. Real time PCR array showing induction of mRNA in 8 day old MEFs spheres compared to monolayer MEFs as in ref 20. Results are normalized to Actb.

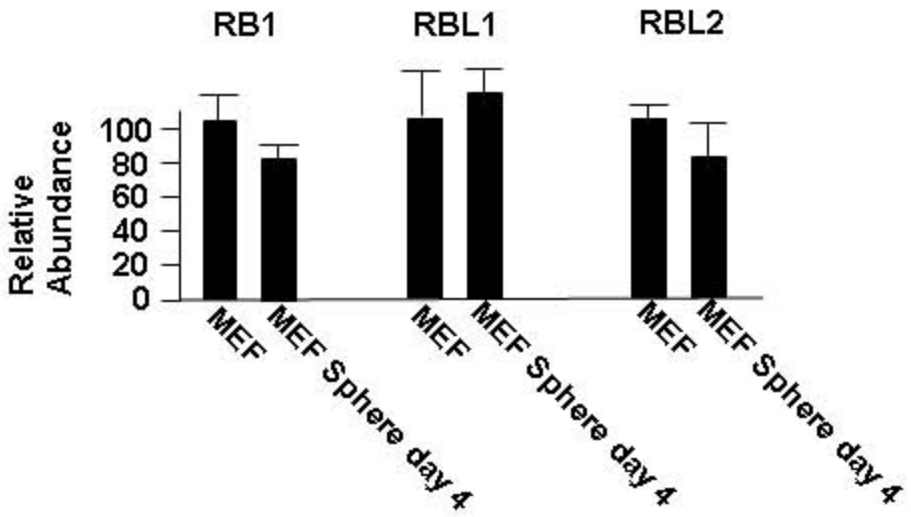


Supplemental Fig. 7. After 8 days in suspension culture, MEF spheres were allowed to adhere to Matrigel coated plates. Cells migrated from the spheres, and a colony arose at the site of the sphere attachment. The colony expressed the proliferation marker Ki67, but most of the cells migrating away from the sphere did not express Ki67, and did not exclude Hoechst dye (these are MP cells). These MP cells were then immunostained for markers of differentiation three days after plating of the spheres. The bar is 20 μm in Panel A, B, C, and D, 25 μm in Panel E, 15 μm in Panel F, 10 μm in Panel G, 20 μm in Panel H.

Liu et al Supplemental Fig. 8



Supplemental Fig. 8. Marking fibroblasts to assess the cell origin of sphere-derived colonies. Microarray analysis showed expression of Fsp1 in MEFs but not in MCS or bone marrow derived MSC (Fig. 4D), and accordingly immunostaining for Fsp1 showed uniform expression in MAFs and MEFs, but no expression on adipose MSC. Fibroblasts were isolated from tail tips of Rosa26 mice at birth and the cells were infected with a lentivirus expressing *Cre* driven by the *Fsp1* promoter. This construct has been shown to drive fibroblast-specific *Cre* expression in vivo and in culture. We followed LacZ recombination efficiency in the infected Rosa26 fibroblasts by immunostaining for LacZ. Many of the cells expressed LacZ demonstrating that they had efficiently recombined the LacZ locus, which then permanently marked the fibroblasts. These LacZ$^+$ fibroblast where then used to generate spheres, and Oct4$^+$ SP cells were sorted. These Oct4$^+$ SP cells co-expressed LacZ, demonstrating that they were derived from fibroblasts. Then colonies of these cells were subjected to differentiation protocols as in Fig. 7, and the differentiated cells were co-immunostained for LacZ and lineage markers. LacZ-positive cells were evident among those expressing each marker. We conclude that these various lineages also arise from fibroblasts. Bars are 20 μm.



Supplemental Fig. 9. Real time PCR comparing the levels of Rb1 family mRNAs in MEFs in monolayer culture to cells in four-day old spheres. Results are normalized to Actb.