Differential Responses to Retinoic Acid and Endocrine Disruptor Compounds of Subpopulations within Human Embryonic Stem Cell Lines

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Supplemental Figures and legends

Supplemental Figure 1. Feeder free hES cells display two distinct cell sub-populations.
Flow cytometry analysis of H1 hES cells grown in feeder free conditions on matrigel sorted by forward/side scatter using flow cytometry. Large and small populations identified by gates in PI negative (viable) cells.

Supplemental Figure 2. Small cell population clones revert to parental cell profiles upon subsequent passaging.
Flow cytometric profiles, forward vs. side scatter of H1 hES small and large clones showing distribution of the two populations after passaging in culture. A) H1 hES small cell clone sort profile at passage 2. B) The same H1 hES small cell clone profile at passage 5. C) H1 hES large cell profile at passage 0. D) H1 hES large cell clone profile at passage 2.

Supplemental Figure 3. Histology of BGN1 large cell population teratoma shows presence of all three primordial germ layers.
A) H&E staining of a BGN1 large hES cell population teratoma. B) H&E staining of a BGN1 whole population teratoma.

Supplemental Figure 43.Retinoic acid receptors mRNA expression in large and small cell populations.
H1 hES small and large cell population mRNA expression after a 6-day treatment of RA or DMSO. Three isoforms of the retinoic acid receptor (RAR), RARA, RARB, RARG, and three isoforms of the retinoid X receptor (RXR) ,RXRA, RXRB, RXRG, were examined in both subpopulations relative to GAPDH.

Supplemental Figure 54. Integrin alpha-6 protein expression in large and small cell population.
A) H1 hES cells that have been hybridized and sorted with FITC labeled antibody to Integrin alpha-6. FITC+ cells are labeled yellow and FITC- cells are green. B) Distribution of Integrin alpha-6-FITC- cells in large and small populations. C) Distribution of Integrin alpha-6-FITC + cells in large and small populations. D) Histograms of unlabeled large cells (control) and hybridized with Integrin-6-FITC or PE+ (phycoerythrin dye, negative control) large cells.

Supplemental Figure 65.mRNA Expression of Primordial Germ Cell Layer Associated Genes.
Relative mRNA expression (of Trophectoderm markers Cdx2 and hCGalpha; Endoderm markers Gata6 and Gata4; Mesoderm markers Enolase and Beta-hemaglobin; and Ectoderm marker Pax6 in H1 hES small and large populations. Values are expressed relative to GAPDH.

Supplemental Figure 76. Effect of RA treatment on mRNA expression of CoupTFs (NR2Fs), Notch signaling components and targets
Quantitative RT-PCR analysis showing mRNA expression of CoupTFs (NR2Fs), notch signaling components and targets in H1 hES small and large cell population after a 6 day treatment with RA or

DMSO. mRNA expression is normalized to DMSO control. A) Small cell population expression. B) Large cell population expression.

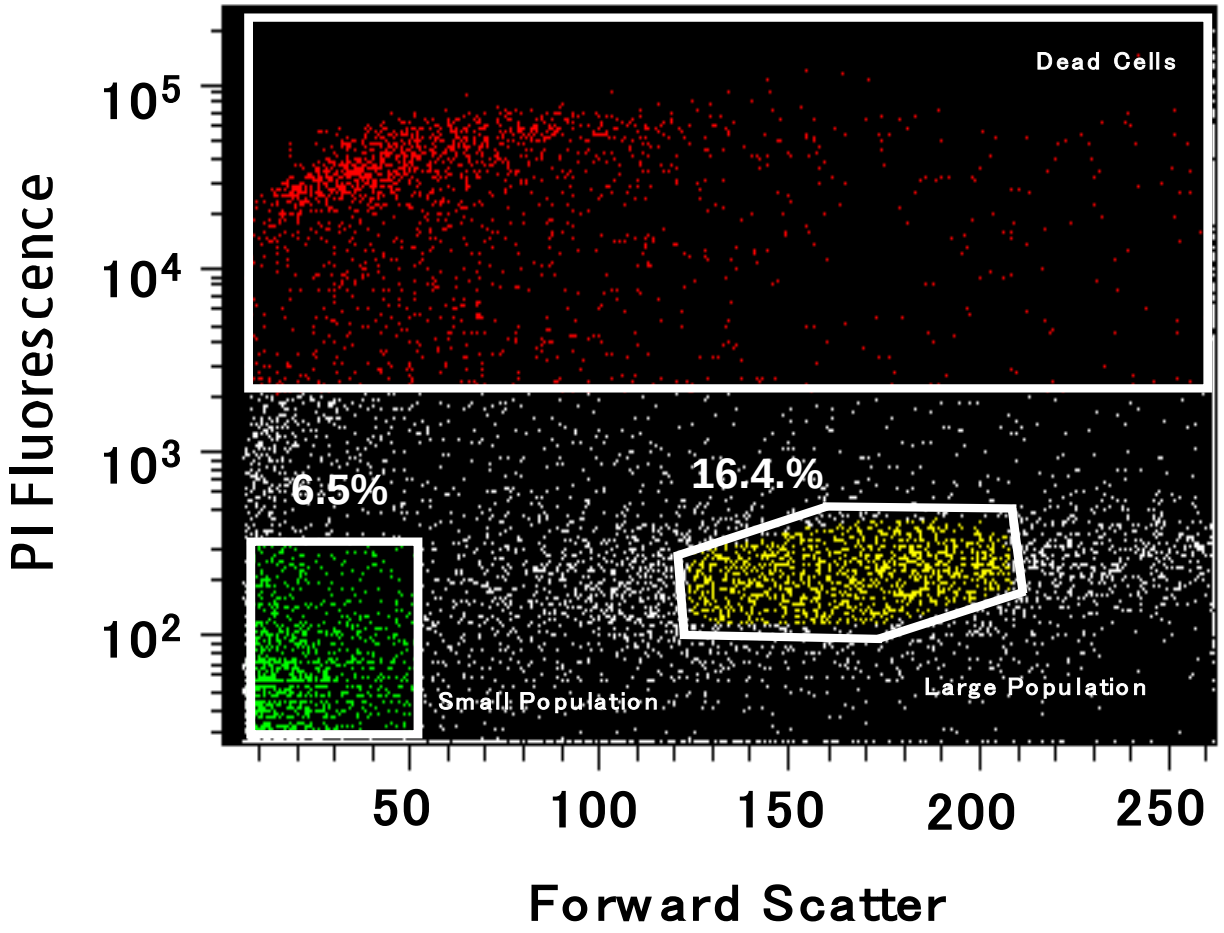
Supplemental Figure 8~~7~~. SWI/SNF subunit mRNA expression upon treatment with RA. Quantitative RT-PCR analysis showing mRNA expression of BRG1 associated factors within the SWI/SNF complex in H1 hES small and large cell populations after a 6 day treatment of RA or DMSO. mRNA expression is normalized to DMSO control. A) Small cell population expression. B) Large cell population expression.

Supplemental Figure 9~~8~~. Changes in Histone Modifying Enzyme mRNA levels after RA Treatment. Quantitative RT-PCR analysis showing mRNA expression of histone modifying enzymes in H1 hES small and large cell populations after a 6 day treatment with RA or DMSO. mRNA expression is normalized to DMSO control. A) Small cell population expression. B) Large cell population expression.

Supplemental Figure 10~~9~~. Alterations of SSEA-3 and Oct-4 protein expression in hESCs upon endocrine disruptor compound (EDC) exposure accompanied by changes in cell morphology. H9 hES cells were treated for 2 days with EDC's, labeled with SSEA-3-FITC and collected for protein. A) Percent SSEA-3 positive viable (PI negative) cells treated with 10 EDC's and controls. B)) Western blot analysis of whole cell lysates probed for Oct-4 expression after 2 day treatment of four EDC's and controls. Tubulin was used for a loading control. C) Microphotographs of H9 hES cells treated for 2 days with four EDC's and controls.

Supplemental Table 1. Teratoma Formation Efficiency of Large and Small Cells.
Teratoma tumor formation in BGN1 hES cells before and after sorting using two methods of disassociation of cells (collengase and trypsin) and injecting 4 x 10^6 cells per site with or without matrigel (M) into Scid-beige Mice.

Supplemental Figure 1



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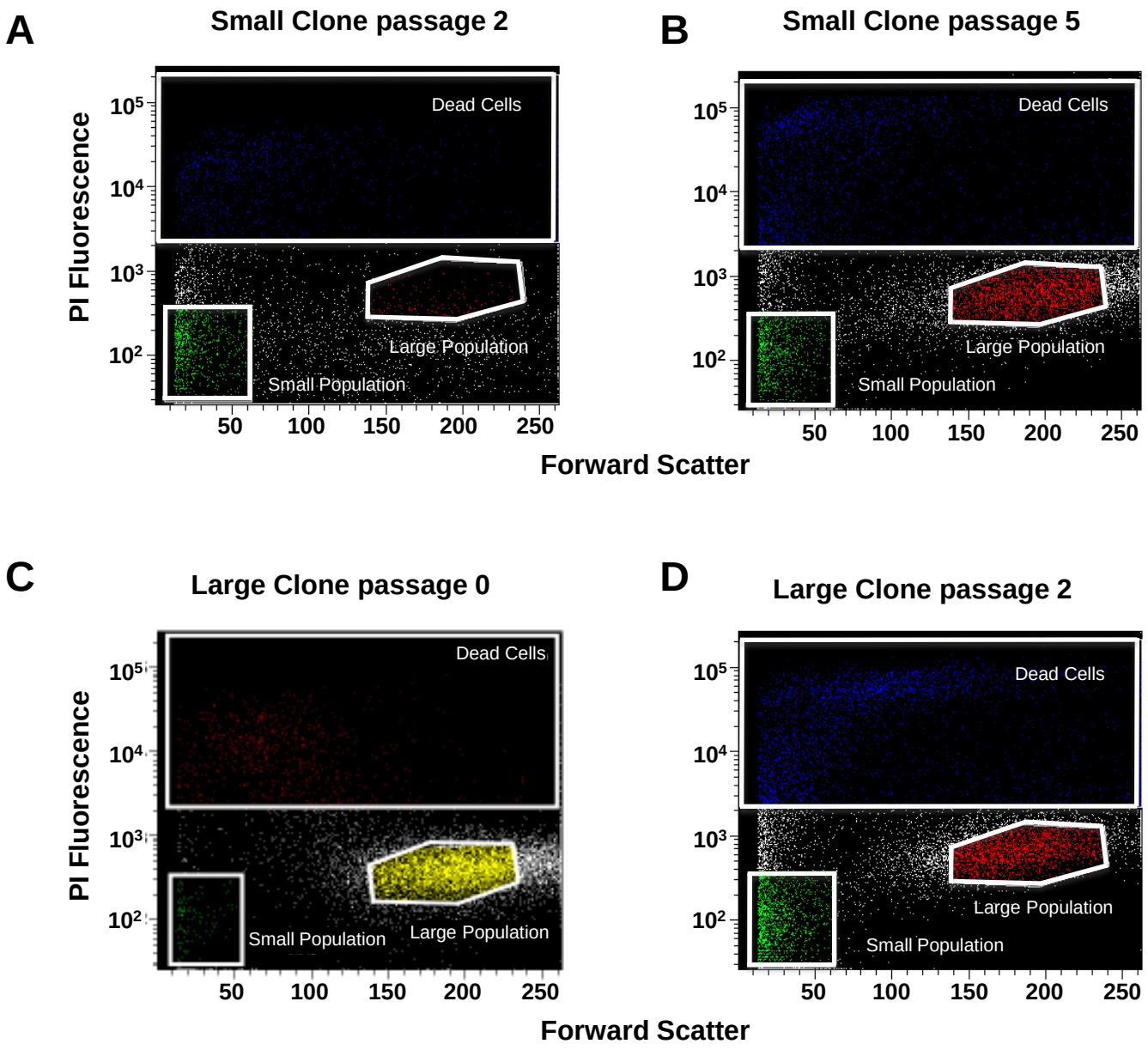
Supplemental Figure 2



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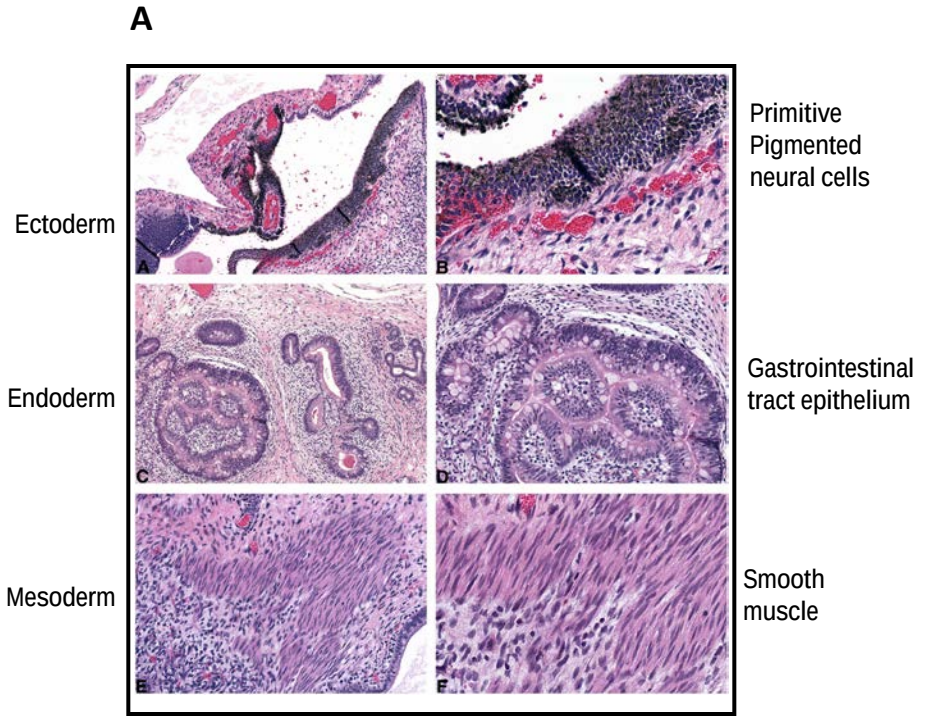
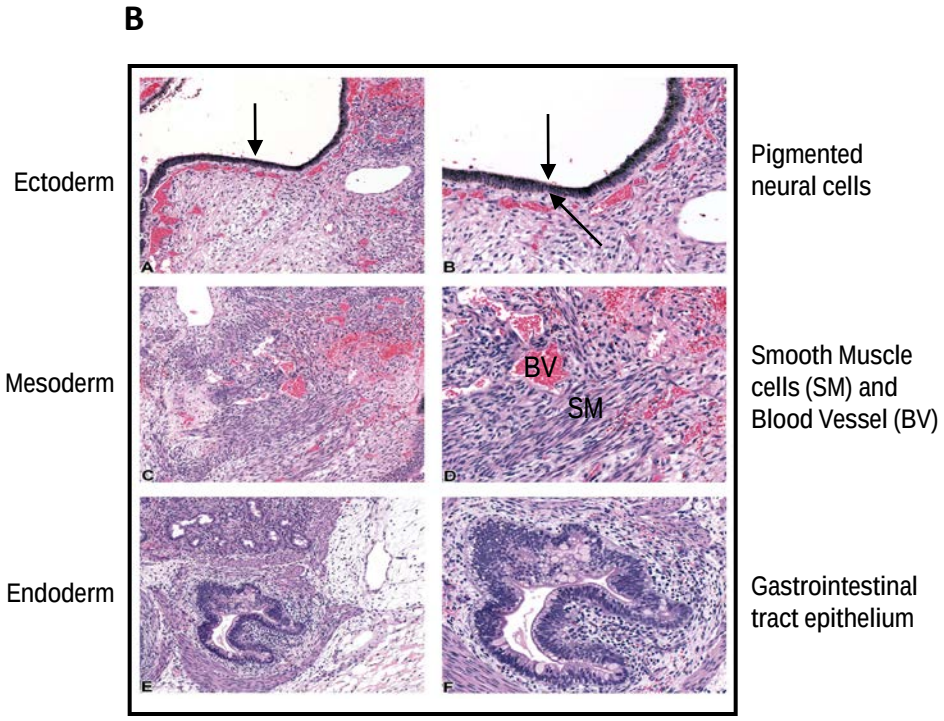
Supplemental Figure 3



BGN1 Large ES Cell Teratoma



BGN1 ES Cell Teratoma

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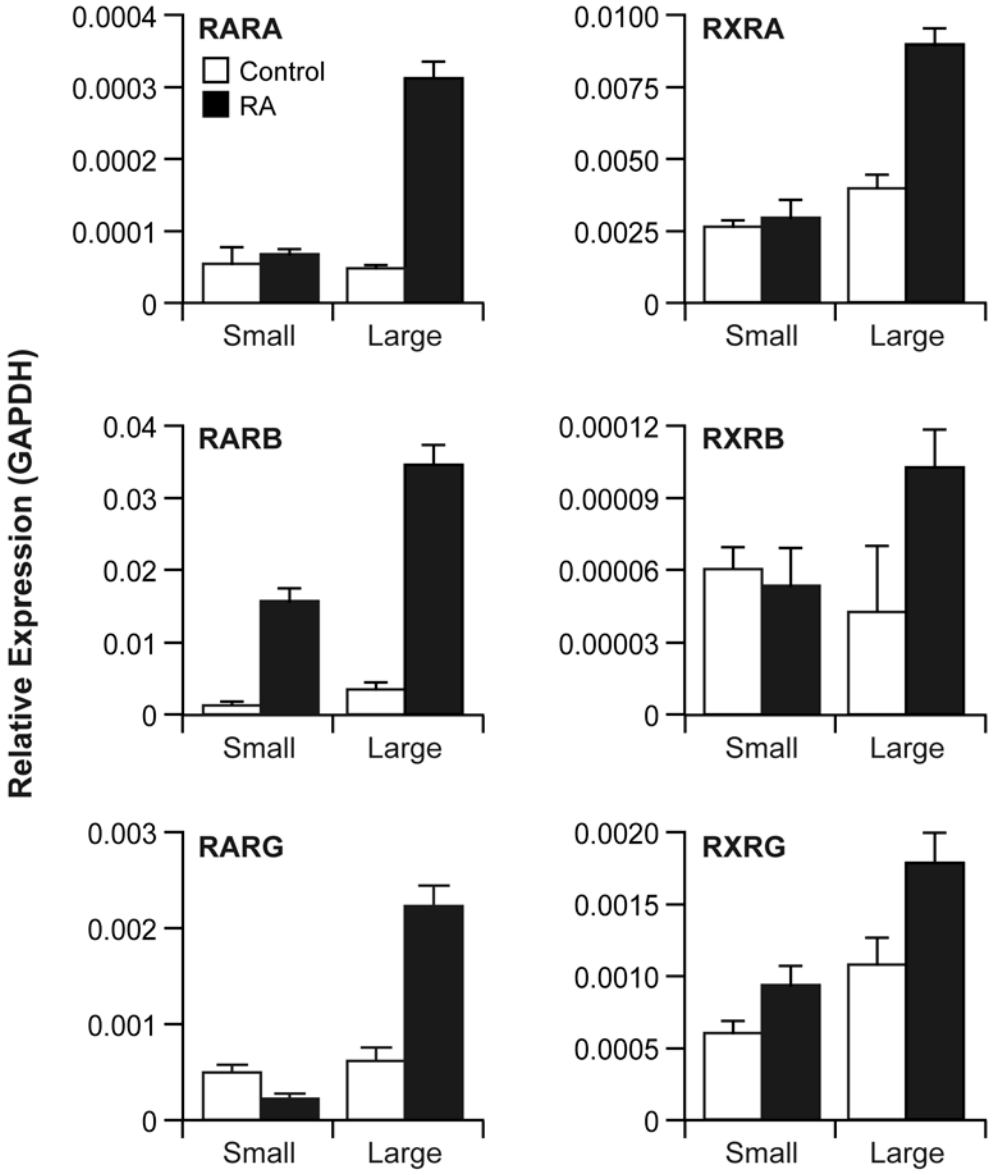
Supplemental Figure 4



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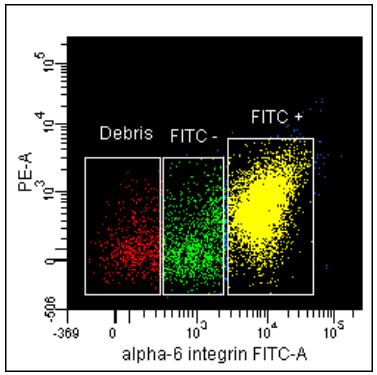
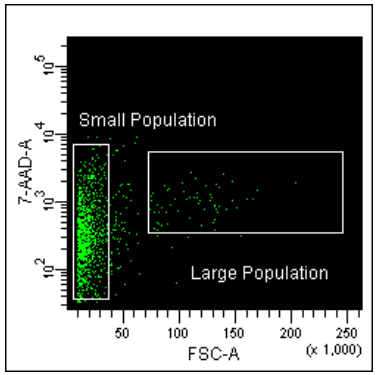
Supplemental Figure 5

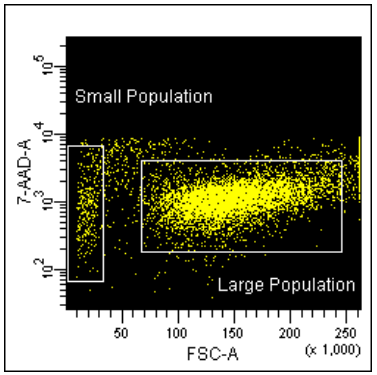
A Integrin alpha-6 FITC labeled Cells



B FITC neg Population Analyzed for Cell Size



C FITC pos Population Analyzed for Cell Size



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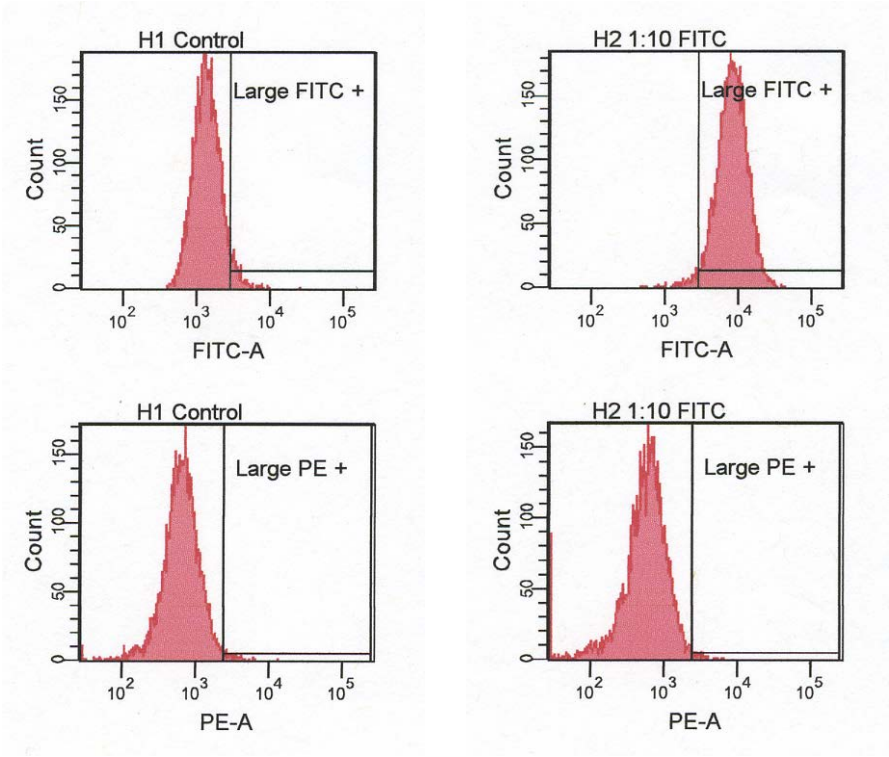

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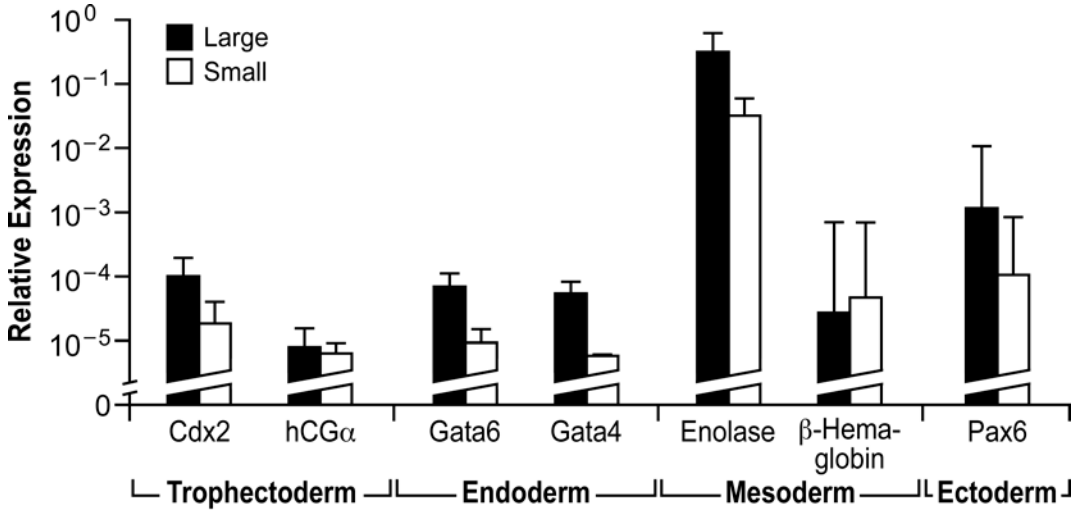
Supplemental Figure 6



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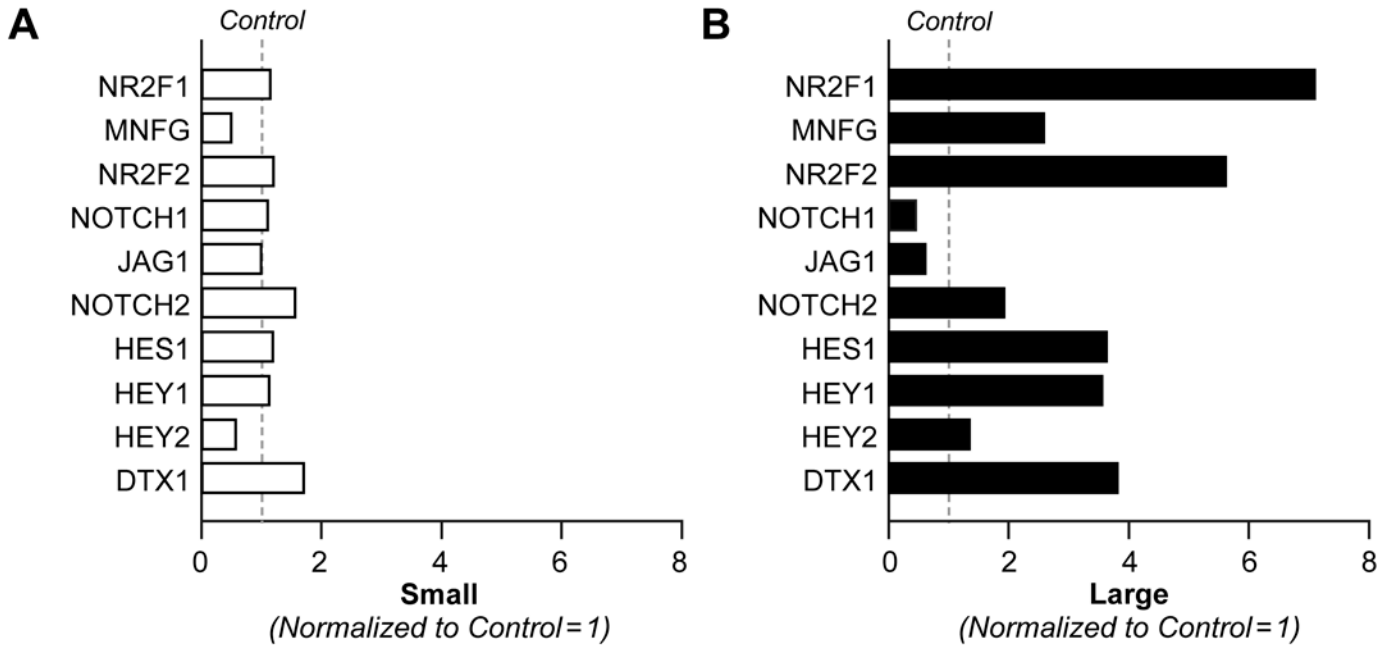
Supplemental Figure 7



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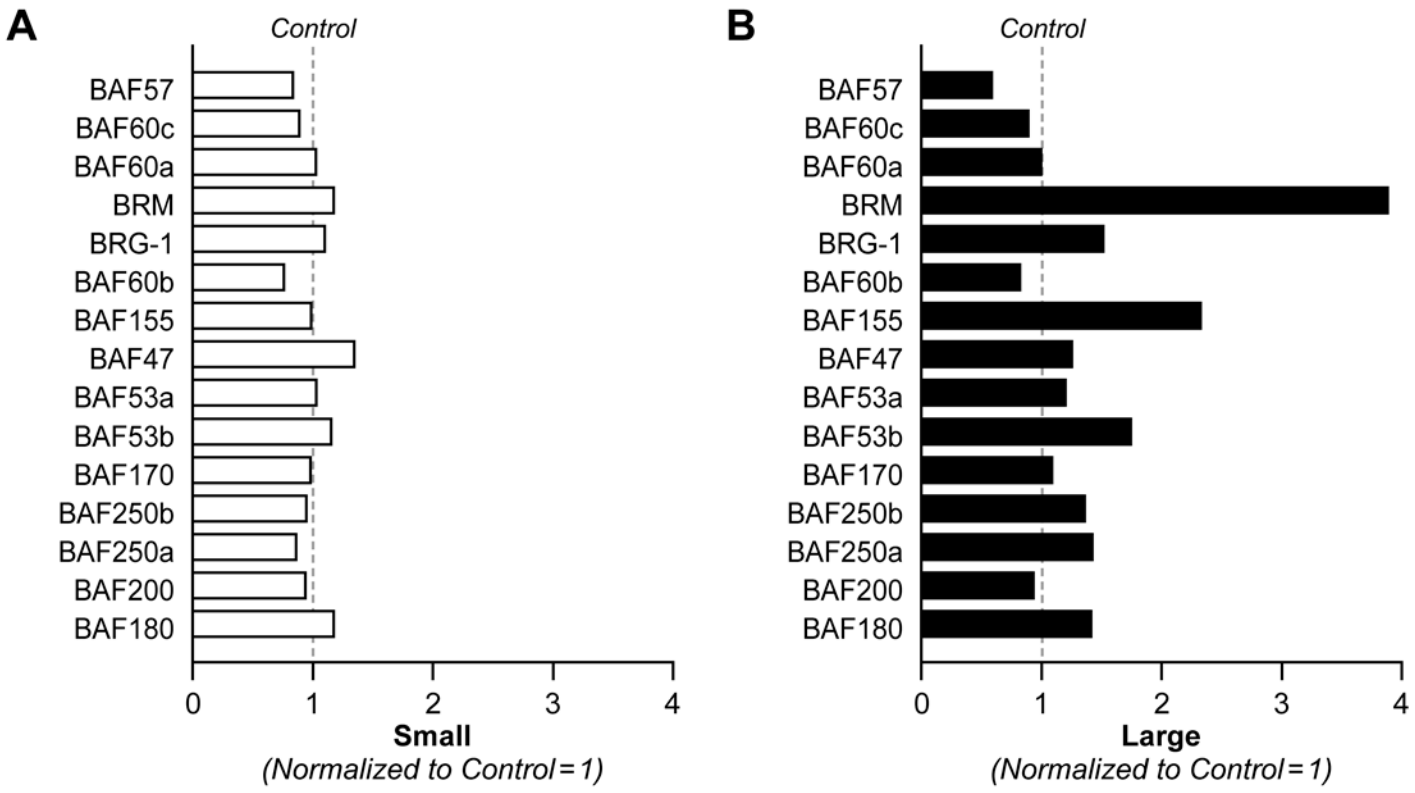
Supplemental Figure 8



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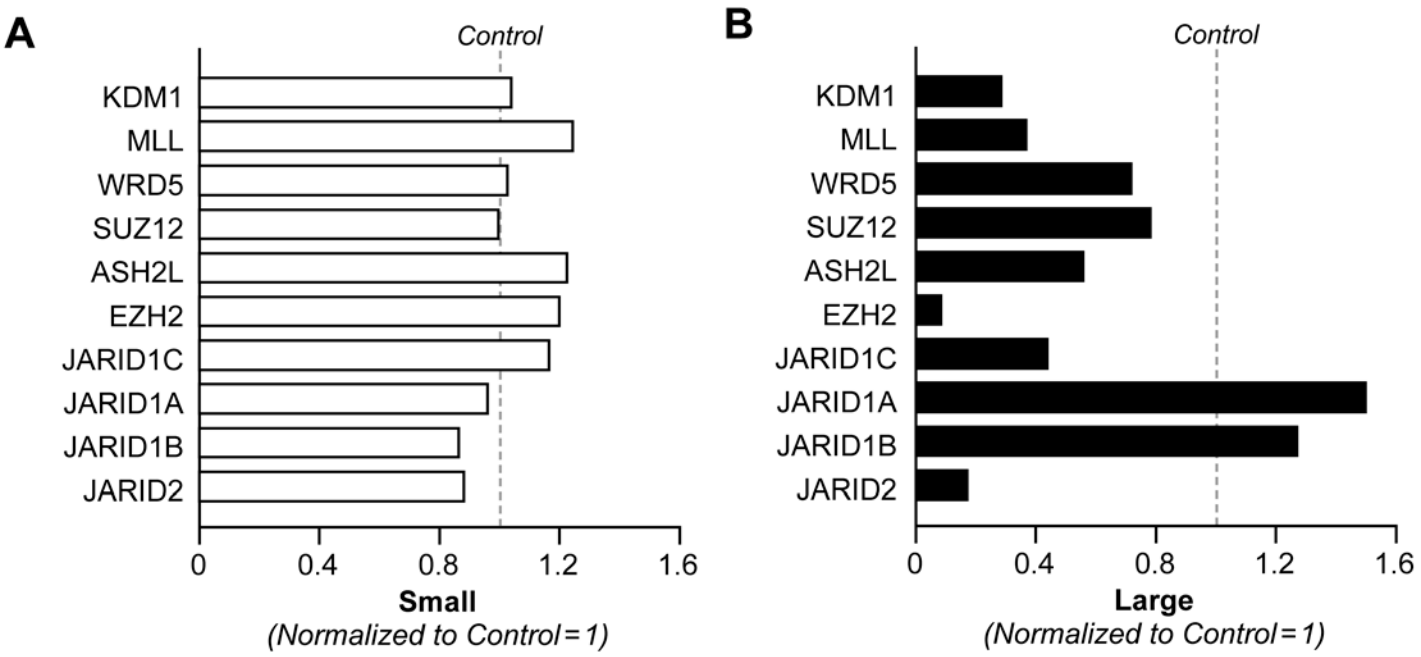
Supplemental Figure 9



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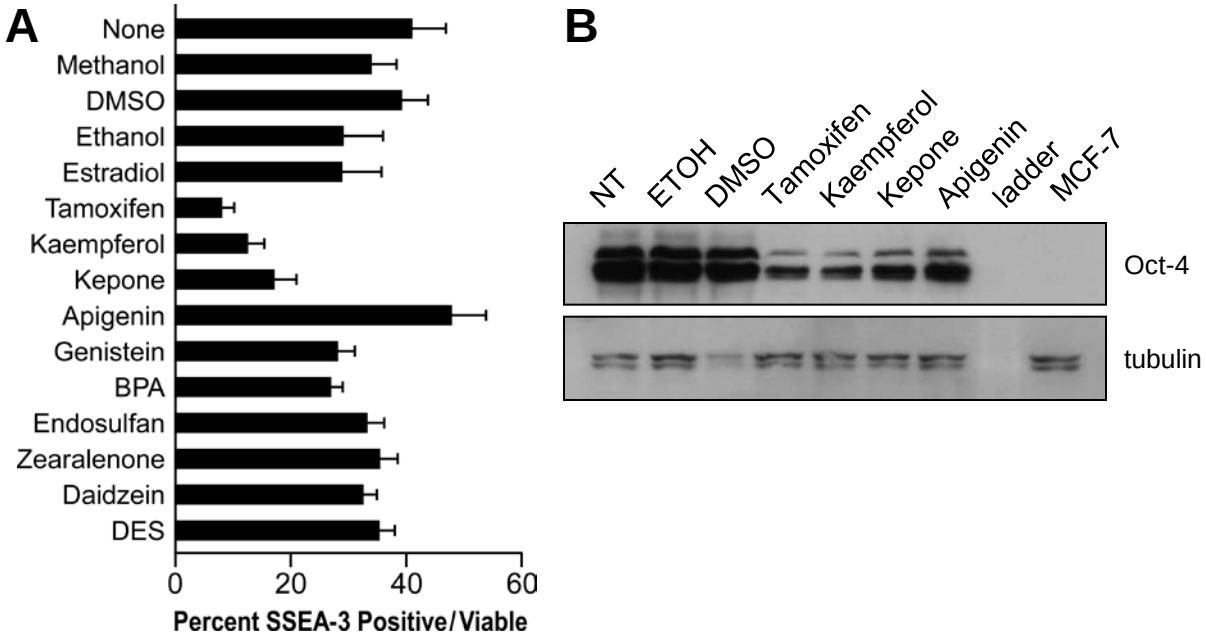
Supplemental Figure 10



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Teratoma Formation

hES line/Treatment	No. tumors/Injection sites	Latency
Unstorted		
BGN1 collagenase	7/10 (70%)	38-70 days
BGN1 trypsin	2/6 (33%)	45-49 days
Sorted		
BGN1 Large	0/6	> 9 mos.
BGN1 Large+M	1/6 (17%)	68 days
BGN1 Small	0/3	> 9 mos.
BGN1 Small+M	0/8	> 9 mos.
BGN1 Large+Small	1/2 (50%)	126 days