Inventory of Supplemental Data

Figure S1, related to Figure 2. Quantification of side branching in outgrowths generated from decreasing dilutions of transplanted cells.

Table S1, related to Figure 2. Overview of mammary fat pad filling at decreasing dilutions of transplanted cells.

Figure S2, related to Figure 3. FACS analysis and colony formation assays. (A) Analysis of CD29 and CD24 surface markers in MECs. (B) Cyclin D1 is expressed in luminal but not in myoepithelial cells.

Figure S3, related to Figure 4. CD24/CD49f sorted populations. (A) Cytokeratin-14 (K14)/E-cadherin co-staining of $CD24^{Med}CD49f^{H}$ - and $CD24^{Hi}CD49f^{Low}$- derived colonies. (B) CD61 expression in $CD24^{Med}CD49f^{H}$ and $CD24^{Hi}CD49f^{Low}$ populations. (C) The $CD24^{Hi}CD49f^{Low}$ population contains bi-potential progenitor activity.

Figure S4, related to Figure 5. Defects in luminal epithelial differentiation. (A) Luminal differentiation defects in Cyclin $D1^{KE/KE}$ outgrowths. (B) Characterization of $CD24^{High}$ and $CD24^{Low}$ populations.

Supplemental Data

Legends for Supplemental Figures and Table.

Figure S1, related to Figure 2. Quantification of side branching in outgrowths generated from decreasing dilutions of transplanted cells. The graph shows the number of side sprouts per mm of duct (the number of sprouts observed between a major ductal bifurcation and a terminal end bud (TEB), or between two major ductal bifurcations, were counted and the number divided by the distance in mm). Although the differences in side branching were significant between WT and KE outgrowths for all dilutions analyzed (p values < 0.0001), there was not intra-group variation between different dilutions. Error bars indicate mean ± SD.

Table S1, related to Figure 2. Overview of mammary fat pad filling at decreasing dilutions of transplanted cells. The table shows the percentage of transplants that grew but did not fill the fat pad when 50K, 10K, or 2K cells of either genotype were transplanted (K=1,000).

Figure S2, related to Figure 3. FACS analysis and colony formation assays. (A) Analysis of CD29 and CD24 surface markers in MECs. Flow cytometry analysis for the expression of the surface markers CD29 and CD24 does not reveal a significant difference in the percentage of the $CD24^{Med}CD29^{Hi}$ cells when cyclin $D1^{KE/KE}$ MECs are compared to cyclin $D1^{+/+}$ MECs. These profiles have been gated for CD45(-) cells. Error bars indicate mean ± SD. **(B) Cyclin D1 is expressed in luminal but not in myoepithelial cells.** Immunofluorescence staining of a myoepithelial and a luminal colony for SMA (green) and cyclin D1 (red) shows that

myoepithelial cells do not stain positive for cyclin D1. However, cyclin D1 was readily detected in luminal cells from the luminal colony. Scale bar, 50μm.

Figure S3, related to Figure 4. CD24/CD49f sorted populations. (A) Cytokeratin-14 (K14)/E-cadherin co-staining of $CD24^{Med}CD49f^{H}$ - and $CD24^{Hi}CD49f^{Low}$- derived colonies. Freshly sorted $CD24^{Hi}CD49f^{Low}$ and $CD24^{Med}CD49f^{Hi}$ cells from either genotype were directly embedded in matrigel. After 5 days, acinar and solid colonies were subjected to immunofluorescence for the detection of the myoepithelial marker keratin-14 (K14, green) and the luminal epithelial marker E-cadherin (red). Scale bar, 50μm. **(B) CD61 expression in $CD24^{Med}CD49f^{H}$ and $CD24^{Hi}CD49f^{Low}$ populations.** Expression of CD61 in $CD24^{Hi}CD49f^{Low}$ and $CD24^{Med}CD49f^{Hi}$ populations was analyzed by qRT-PCR. The $CD24^{Hi}CD49f^{Low}$ population derived from cyclin $D1^{KE/KE}$ mammary glands shows reduced CD61 mRNA levels compared to the same population derived from cyclin $D1^{WT/WT}$ mammary glands (p value < 0.05). Error bars indicate mean ± SD. **(C) The $CD24^{Hi}CD49f^{Low}$ population contains bi-potential progenitor activity.** $CD24^{Hi}CD49f^{Low}$ and $CD24^{Med}CD49f^{Hi}$ populations derived from wild-type mammary glands were transplanted in either cleared fat pads of nulliparous (virgin) mice, or cleared fat pads of pregnant hosts. The number of successful outgrowths upon transplantation of $5x10^4$ sorted cells and the percent of fat pad filling is indicated.

Figure S4, related to Figure 5. Defects in luminal epithelial differentiation. (A) Luminal differentiation defects in Cyclin $D1^{KE/KE}$ outgrowths. Immunofluorescence staining of ducts from transplant outgrowths shows that cytokeratin-6 (CK6) positive cells reside in the inner layer of ducts and co-express GATA-3 but not SMA. Notice that cyclin $D1^{KE/KE}$ ductal outgrowths do

not exhibit any detectable CK6 positive cells and also display reduced staining for GATA-3. Arrow indicates a CK6 positive cell also expressing GATA-3 as shown at higher magnification in the inset. Scale bar, 40μm. **(B) Characterization of CD24^{High} and CD24^{Low} populations.** Immunofluorescence staining for SMA and E-cadherin (staining done separately and both in green) of cyclin D1$^{+/+}$ and cyclin D1$^{KE/KE}$ MECs that were sorted for CD24 expression and cytospun directly onto slides. CD24L=CD24^{Low}; CD24H= CD24^{High}. Scale bar, 50μm.

Supplemental Text and Figures

Click here to download Supplemental Text and Figures: Supplemental.pdf

Table S1

	Number of Cells Transplanted					
	50K		10K		2K	
Genotype	WT	KE	WT	KE	WT	KE
% Non-filling	25 (1/4)	100 (4/4)	20 (1/5)	75 (3/4)	25 (1/4)	75 (3/4)

Table S1

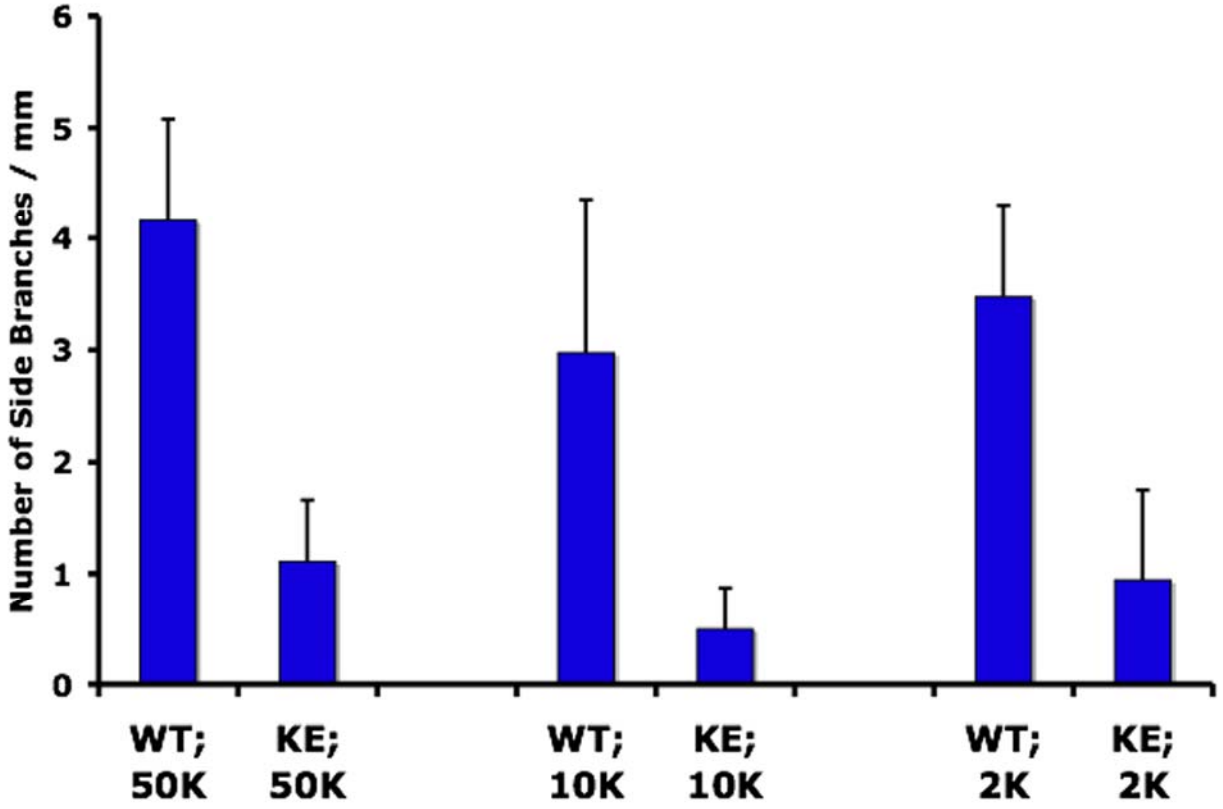

Number of Side Branches / mm
0
1
2
3
4
5
6
WT; 50K
KE; 50K
WT; 10K
KE; 10K
WT; 2K
KE; 2K

Figure S1

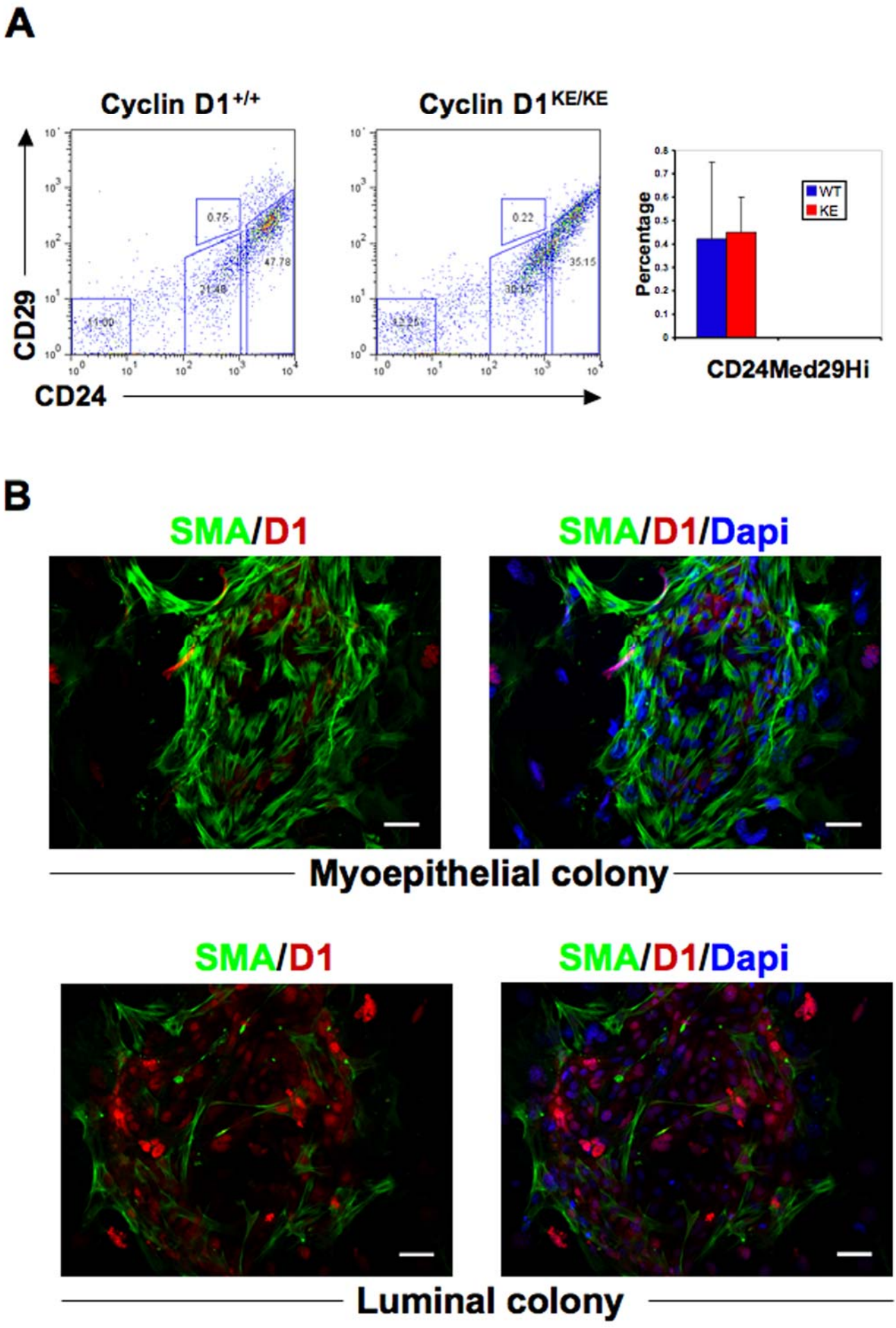
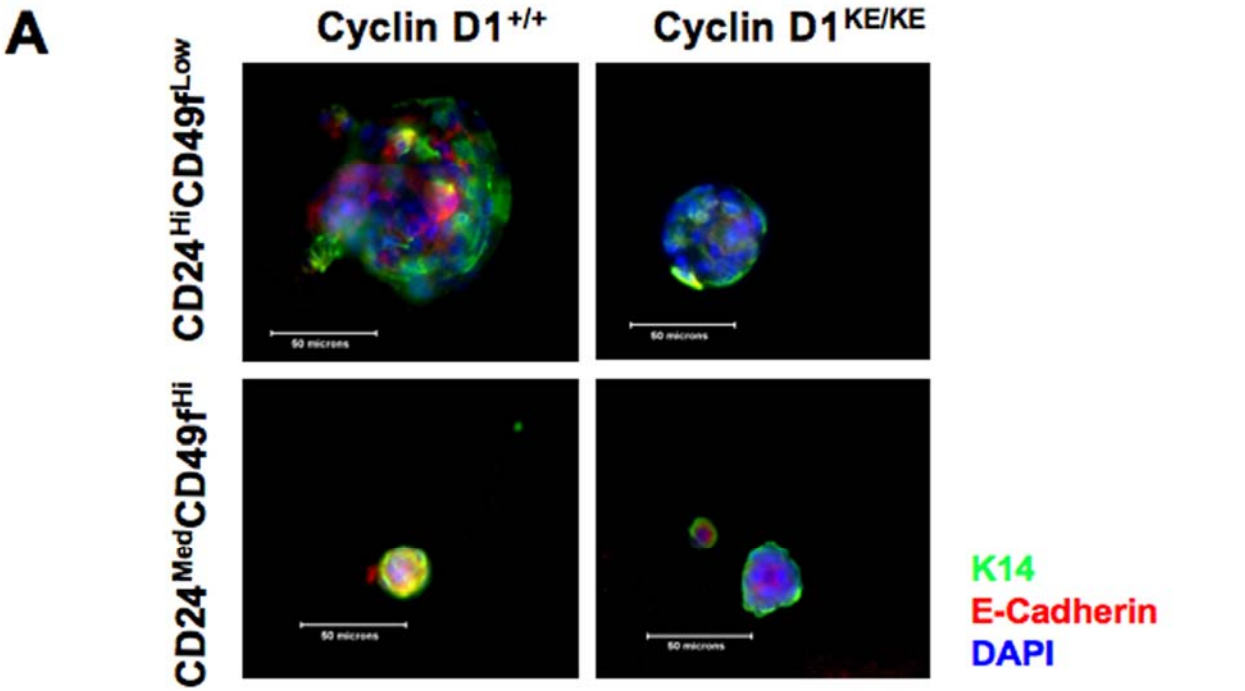
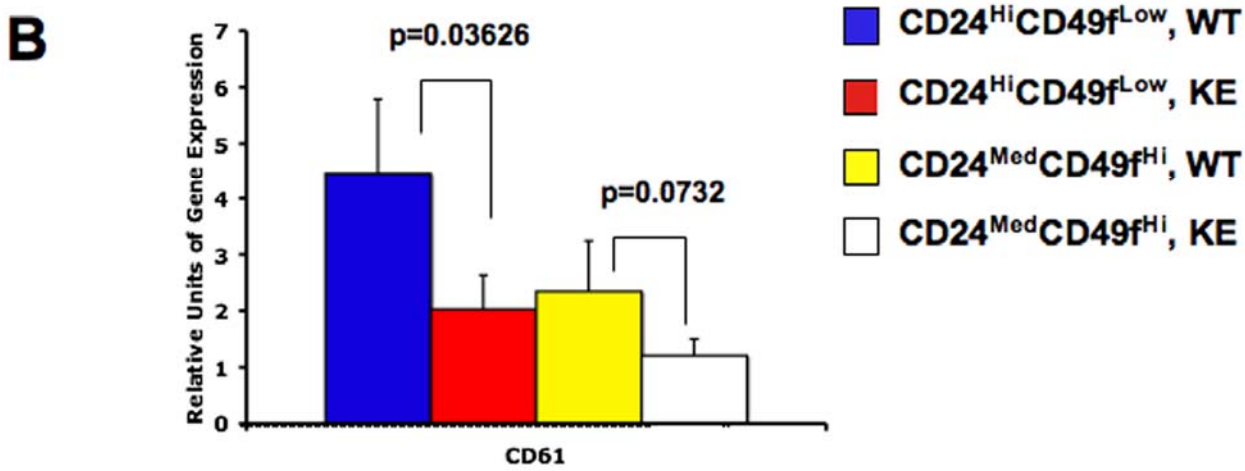

A
Cyclin D1+/+
Cyclin D1KE/KE
CD29
CD24
0.75
47.78
21.48
11.00
0.22
35.15
Percentage
WT
KE
CD24Med29Hi
B
SMA/D1
SMA/D1/Dapi
Myoepithelial colony
SMA/D1
SMA/D1/Dapi
Luminal colony

Figure S2


A
Cyclin D1+/+
Cyclin D1KE/KE
CD24HiCD49fLow
CD24MedCD49fHi
50 microns
K14
E-Cadherin
DAPI


B
p=0.03626
p=0.0732
Relative Units of Gene Expression
0
1
2
3
4
5
6
7
CD61
CD24HiCD49fLow, WT
CD24HiCD49fLow, KE
CD24MedCD49fHi, WT
CD24MedCD49fHi, KE

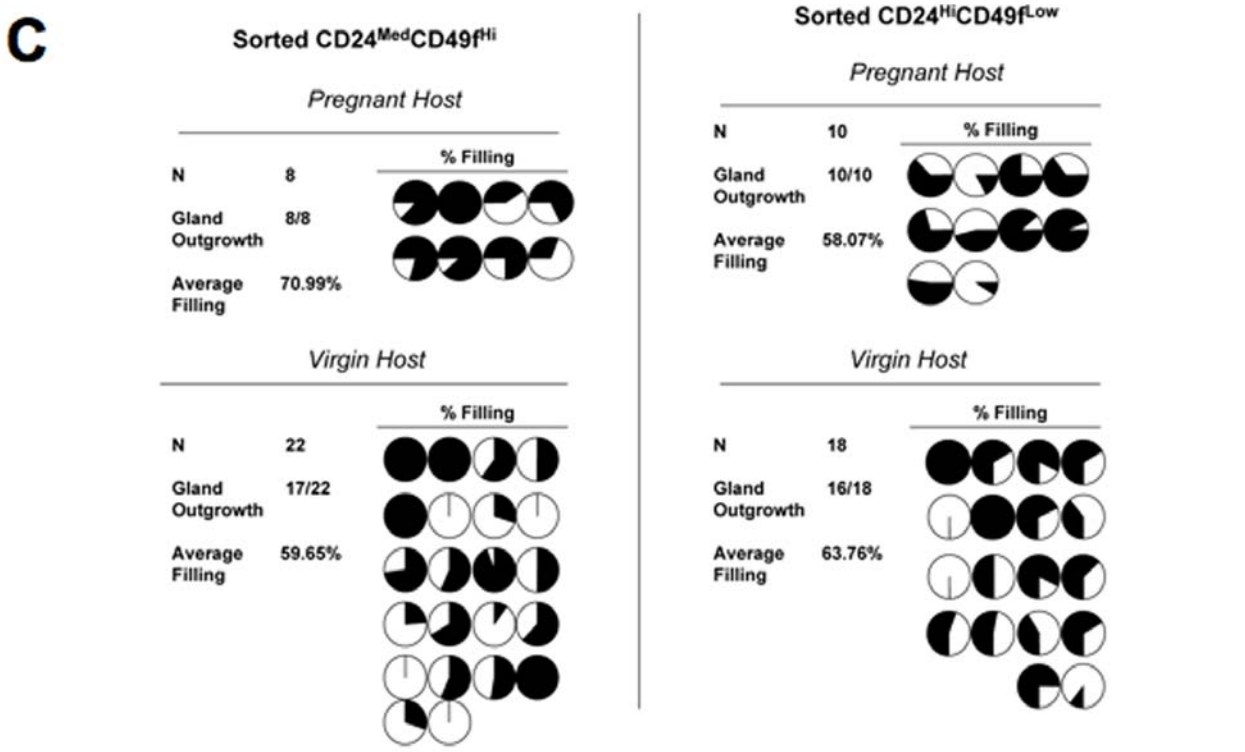

C
Sorted CD24MedCD49fHi
Pregnant Host
N 8
Gland Outgrowth 8/8
Average Filling 70.99%
% Filling
Virgin Host
N 22
Gland Outgrowth 17/22
Average Filling 59.65%
% Filling
Sorted CD24HiCD49fLow
Pregnant Host
N 10
Gland Outgrowth 10/10
Average Filling 58.07%
% Filling
Virgin Host
N 18
Gland Outgrowth 16/18
Average Filling 63.76%
% Filling

Figure S3

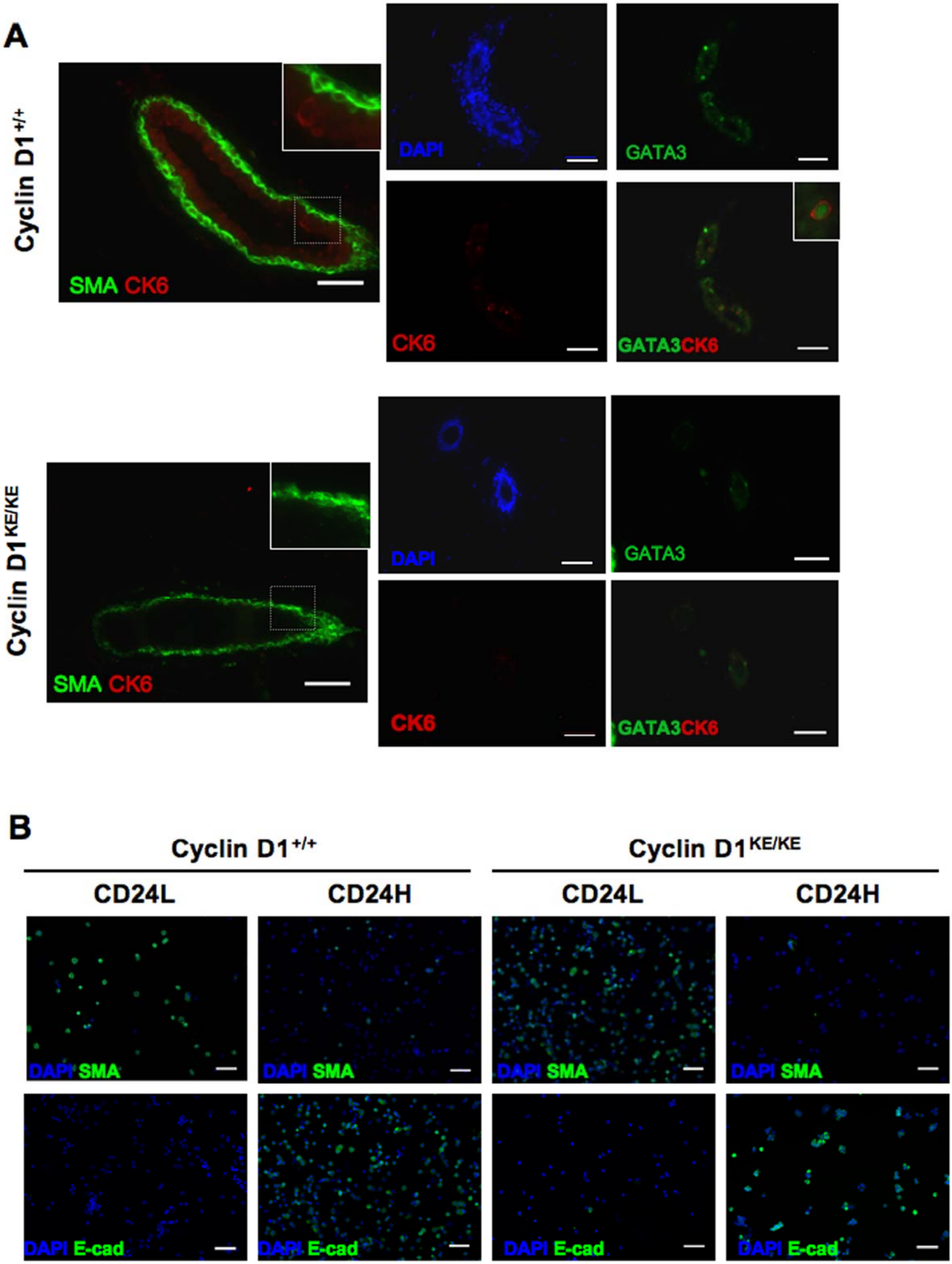

A
Cyclin D1+/+
SMA CK6
DAPI
GATA3
CK6
GATA3CK6
Cyclin D1KE/KE
SMA CK6
DAPI
GATA3
CK6
GATA3CK6
B
Cyclin D1+/+
Cyclin D1KE/KE
CD24L
CD24H
CD24L
CD24H
DAPI SMA
DAPI SMA
DAPI SMA
DAPI SMA
DAPI E-cad
DAPI E-cad
DAPI E-cad
DAPI E-cad

Figure S4