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Supplemental information

LGL1 binds to Integrin β1 and inhibits downstream signaling to promote epithelial branching in the mammary gland

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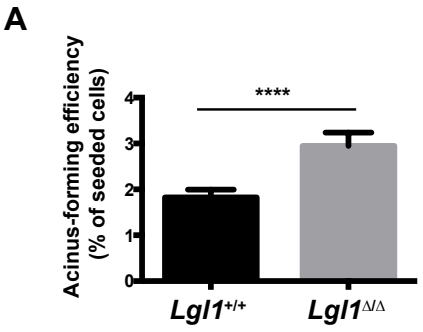

Figure S1. ***Lgl1*** **loss does not affect self-renewal using the acinus-forming assay, Related to Figure 1.**
(A) Plot depicting acini-forming efficiency of *Lgl1* het and *Lgl1* KO basal cells. Data points show paired values, and lines indicate mean (n = 4). Statistical analysis was performed using paired t-test and revealed no significant difference between $Lgl1^{+/+}$ and $Lgl1^{\Delta/\Delta}$ basal cells. ****, P<0.0001.

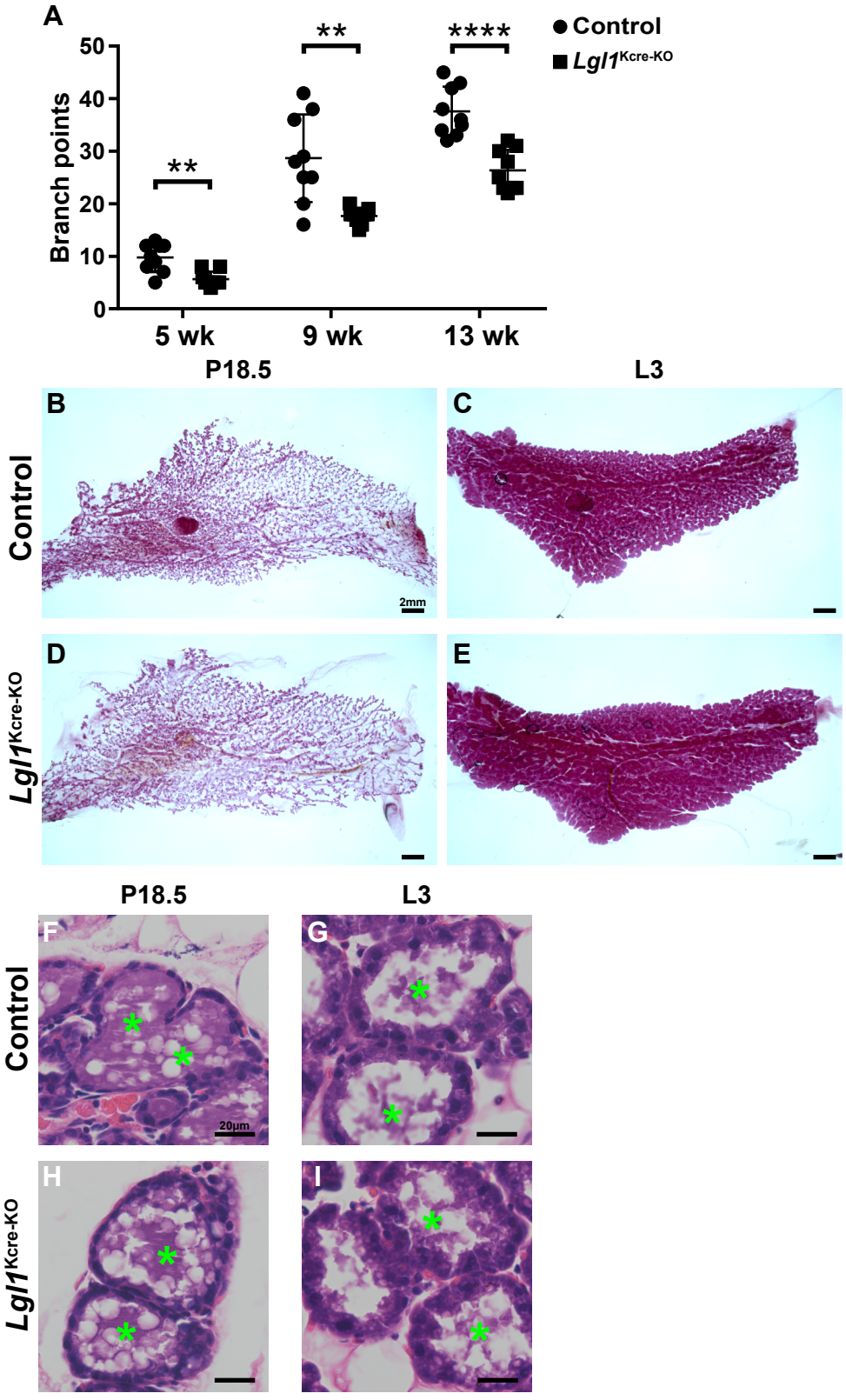

Figure S2. *Lgl1* promotes branching morphogenesis of the mammary gland epithelium, Related to Figure 2.

(A) Quantitative comparisons of branching points between control and mutant glands. Plots show mean ± SD (n ≥ 3/genotype); **, P<0.01; ****, P<0.0001 . Scale bars: 2 mm.

(B-F) Mammary epithelium as revealed by Carmine Red staining on wholemount mammary glands of *Lgl1* null and control mice at the stages indicated. Scale bars: 2 mm.

(G-J) Histological analysis by H&E staining of paraffin sections of the mammary glands of *Lgl1* null and control mice at the stages indicated. Green asterisks indicate the milk inside the lumen. Scale bars: 20 μm.

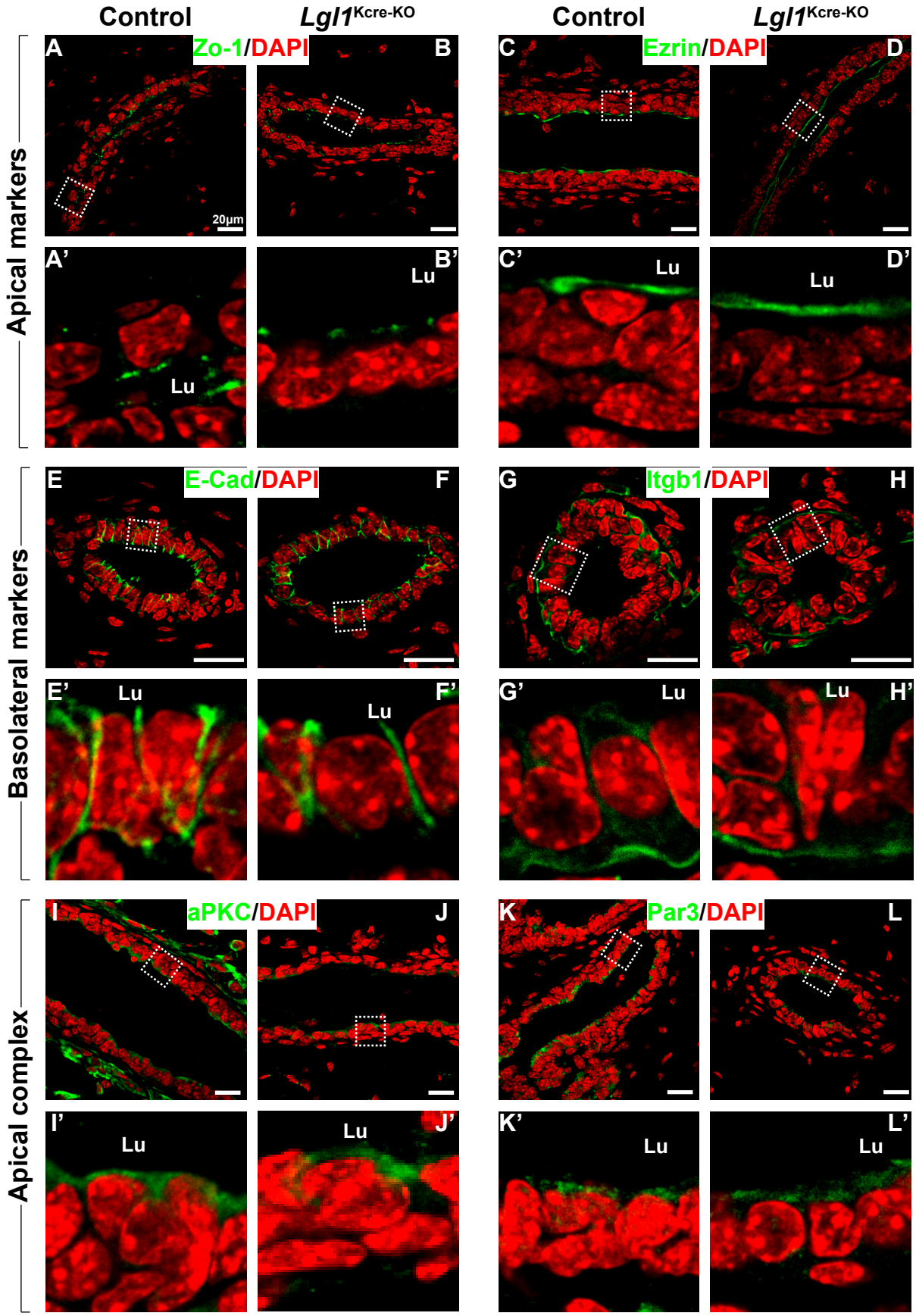

Figure S3. ***Lgl1*** **is not essential for epithelial polarity in the mammary gland, Related to Figure 3.**
(A-L) Immunofluorescence examination of epithelial apical (A-D) and basolateral polarity markers (E-H) and components of the apical polarity complex (I-L) in 9-wk old mammary glands. Insets are close-up views of an area in the white rectangles with dashed lines. Scale bars: 20 μm.

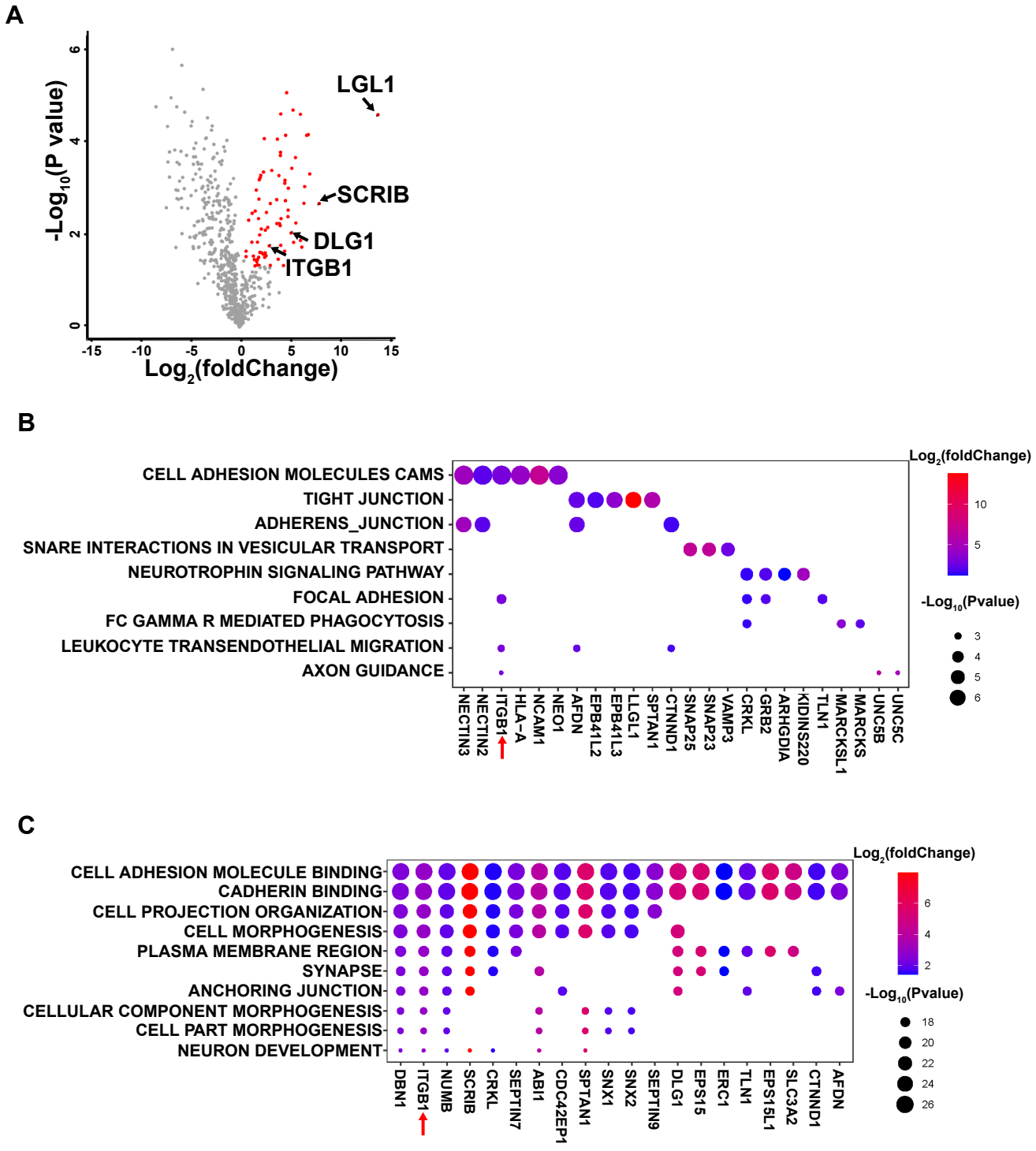

Figure S4. LGL1 was bound to and co-localized with Integrin, Related to Figure 4.
(A) Volcano plot showing potential targets from BioID screen. Red arrows indicate known protein partners of LGL1 and ITGB1. (B) Gene Ontology analysis of the differentially ($P < 0.05$) expressed genes with to determine the biological processes (with full description) with which these genes might be involved. (C) KEGG analysis of the differentially ($P < 0.05$) expressed genes to determine the biological processes(with full description) with which these genes might be involved.

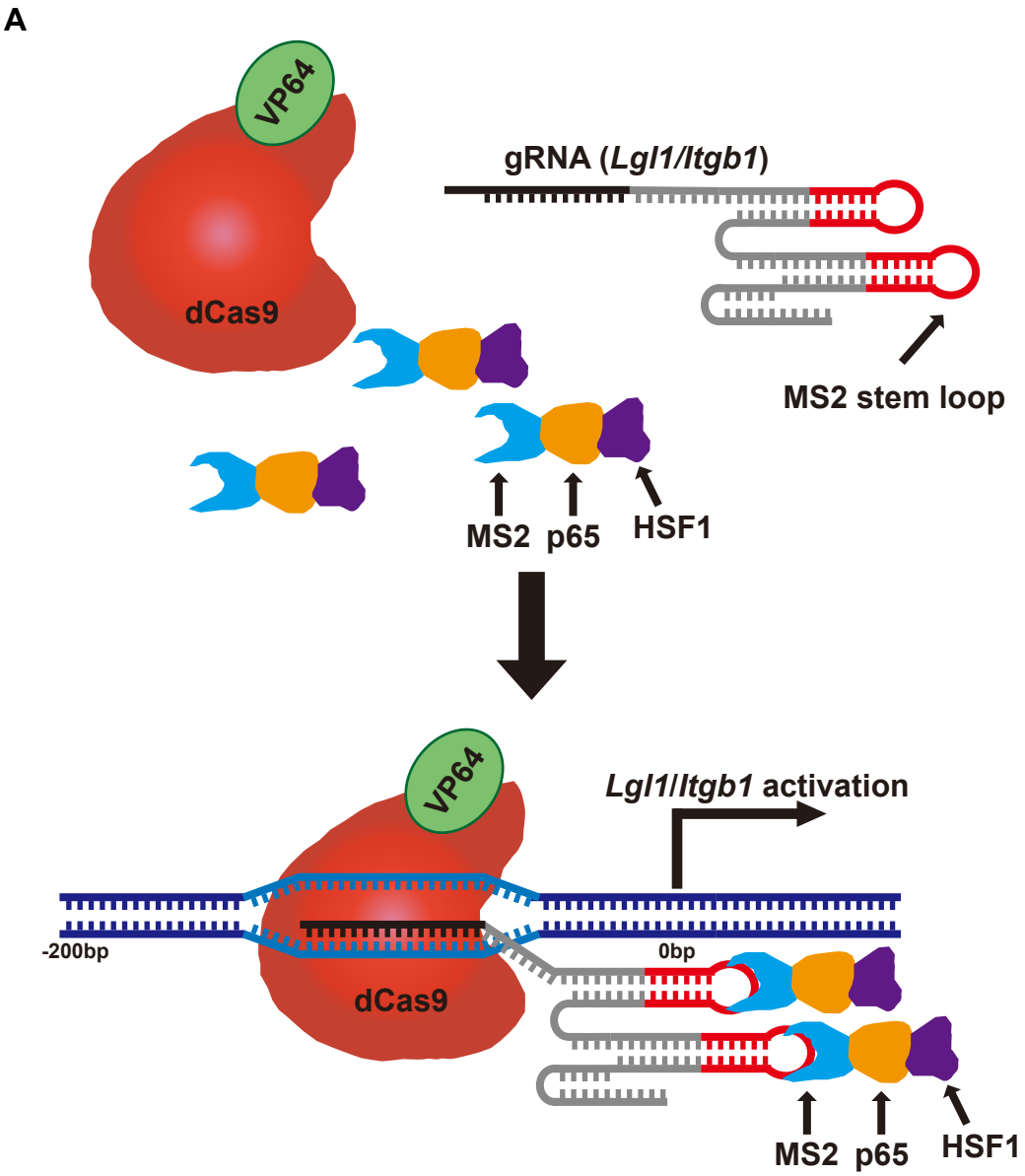

Figure S5. Overexpression strategy for *Lgl1* and *Itgb1*, Related to Figure 5.
(A) Gain-of-function of *Lgl1* and *Itgb1* was based on a modified CRISPR technique in which a deactivated Cas9 protein was fused to the transcriptional activator VP64. Target specificity was achieved by using the sgRNA guide sequence from the promoter region of *Lgl1* or *Itgb1*.

Gene name	Forward sequence (5' → 3')	Reverse sequence (5' → 3')
Lgl1	gggtgatgtccacgtcttct	gtgagaagcgctcaaattcc
Itgb1	atgccaaatcttgcggagaat	tttgctgcgattggtgacatt
Etv4	ccaccaggatcaagaaggaa	ttgtctgggggagtcatagg
Etv5	aggaccccaggctgtacttt	tggccgattcttctggatac
Mkp3	tcgggctgctgctcaagaaac	cggtcaaggtcagactcaatgtcc
Actb	ggctgtattcccctccatcg	ccagttggtaacaatgccatgt
Gapdh	ttcaccaccatggagaaggc	ccctttggctccaccct

Table S1. Primers used in qPCR, Related to STAR Methods.

Total RNA was harvested from mammary glands at the stages indicated. cRNA was prepared as described in Materials and Methods and used as templates for quantitative RT-PCR.

	Pearson's Coefficient	Overlap Coefficient	Manders' Coefficients M1	M2
LGL1 and ITGB1 colocalization	0.55±0.11	0.65±0.09	0.74±0.12	0.29±0.19

Table S2. Colocalization coefficients of LGL1 and ITGB1, Related to Figure 4.

Note based on these coefficients, if n=1, it means two proteins have perfect colocalization; whereas if n=0, it means they do not colocalize.