**Supplemental Figure Legends with References**

**Fig. S1.** FLAG-tagged CD151-VR, CD151-Palm, and wild type CD151 constructs were re-expressed in CD151-silenced A431 cells. CD151 expression levels in the three cell lines were measured by FLAG immunoblotting as previously described [1], and quantified using a LiCOR infrared blot scanner. Bars indicate the mean ± S.E.M of values measured in two independent blots.

**Fig. S2.** HaCaT parental and CD151sh3 cells were fixed and stained for E-cadherin (green) and F-actin (magenta).

**Fig. S3.** A431 parental and $\alpha$6 integrin silenced cells expressing E-cadherin-GFP (green) were fixed and stained for $\alpha$6 integrin (red). DAPI-stained nuclei are shown in blue.

**Fig. S4.** A431 parental or laminin-$\alpha$3-silenced cells were plated at 1.5 X $10^5$ cells/well in 96 well plates and allowed to attach overnight. Cells were lysed in Triton X-100 detergent, and the amount of adsorbed laminin-$\beta$3 subunit in each well was measured by ELISA assay. Bars indicate mean ± S.E.M. of values from 6 wells/cell type after subtraction of background staining in cell-free wells. The laminin-silenced cells deposited significantly less laminin-$\beta$3, *$P < 0.001$, ANOVA with Tukey post-tests.

**Fig. S5.** Parental and laminin-$\alpha$3-silenced A431 cells were cultured on their own endogenous extracellular matrices (**A-C**), or replated on the LM-332-rich matrix secreted by the parental A431 cells (**D-F**), on collagen I (**G-I**), on fibronectin (**J-L**) or on Matrigel (**M-O**). Cells were fixed with methanol and stained for E-cadherin. Parental cell matrix (**D-F**), but none of the other matrices, was able to restore junctional organization to the laminin-$\alpha$3-silenced cells. Parental

cells maintained junctional organization on all matrices, suggesting that the matrix they secrete controls junctional organization irrespective of the presence of exogenous matrix proteins. To obtain parental cell matrix for replating experiments, coverslips with freshly confluent parental A431 cells were treated with PBS, 1 mM EDTA for 20 minutes at 37$^{o}$C. Detached parental cells were removed by pipetting and aspirating, and repeated treatments and rinses were performed until all parental cells had been removed.

**Fig. S6**. A431 cells silenced for $\alpha$3 integrin ($\alpha$3sh4) (A), parental A431 cells (B), or $\alpha$3sh4 cells replated on parental cell matrix (C) were cultured overnight and then fixed for visualization of E-cadherin-GFP by fluorescence microscopy. Parental cell matrix was unable to rescue the junctional organization of the $\alpha$3sh4 cells.

**Fig. S7.** LM-332 expression by tumor spheroids in vitro. 10,000 cell spheroids of parental (A-C) or CD151-silenced (D-F) A431 cells were formed and embedded in 3D collagen, as described in Materials and Methods. After 2 days, cultures were fixed and immunostained for LM-332, without permeabilization (anti-laminin-$\beta$3 antibody) magenta. Ecad-GFP is shown in green. (G-I) Tumor spheroid stained with an irrelevant antibody to reveal low background and minimal antibody trapping.

**Fig. S8**. Proliferation of parental and CD151-silenced cells in serum-free medium in (**A**) collagen I-coated wells or (**B**) laminin-332-coated wells was measured by WST assay. On days 2, 4 and 6, CD151-silenced cells proliferated less well on laminin-332, *P<0.001, 2 way ANOVA with Bonferroni t test, n=6 wells/cell type.

**Fig. S9**. Characterization of lung squamous cell carcinoma and ESR1$^{low}$/GATA3$^{low}$ breast cancer cases. (**A**) Graphical representation of lung squamous cell carcinoma cases with alterations in the squamous cell differentiation gene set defined by The Cancer Genome Atlas Research Network [2]. (**B**) Graphical representation of the proportion of breast cancer cases with reduced estrogen receptor $\alpha$ (ESR1) or GATA3 mRNA expression (Z-score < -1 for mRNA levels measured by RNAseq in the Provisional Breast Invasive Carcinoma TCGA database). ESR1$^{low}$ and/or GATA3$^{low}$ cases (blue) represent 288 out of the 1098 cases in the database as of February 2015. Downregulation of ESR1 and GATA3 showed a significant co-occurrence as reported by the cBioportal website ($P < 0.001$, Fisher Exact test). (**C**) The ESR1$^{low}$/GATA3$^{low}$ breast cancer cases were associated with a significant reduction in disease-free survival ($P=0.0014$, logrank test). (**D**) Reverse phase protein array data confirmed significant reductions in GATA3, ESR1, progesterone receptor (PGR) and androgen receptor (AR) proteins in the ESR1$^{low}$/GATA3$^{low}$ cases (p values are from 2 sided student's t tests). P-cadherin (CDH3), cyclin E1 (CCNE1), and cyclin B1 (CCNB1) proteins were significantly upregulated in the ESR1$^{low}$/GATA3$^{low}$ cases. (**E**) Graphical representation of the number of cases with mutations in p53 (TP53) in ESR1$^{low}$/GATA3$^{low}$ cases (61%) versus cases without ESR1 or GATA3 reduction (15% of cases). An increased p53 mutation rate is a feature of aggressive, basal-like breast cancers [3].


1. Zevian S, Winterwood NE, Stipp CS: **Structure-function analysis of tetraspanin CD151 reveals distinct requirements for tumor cell behaviors mediated by α3β1 versus α6β4 integrin.** *Journal of Biological Chemistry* 2011, **286**:7496–7506.

2. Cancer Genome Atlas Research Network: **Comprehensive genomic characterization of squamous cell lung cancers.** *Nature* 2012, **489**:519–525.

3. Cancer Genome Atlas Network: **Comprehensive molecular portraits of human breast tumours.** *Nature* 2012, **490**:61–70.

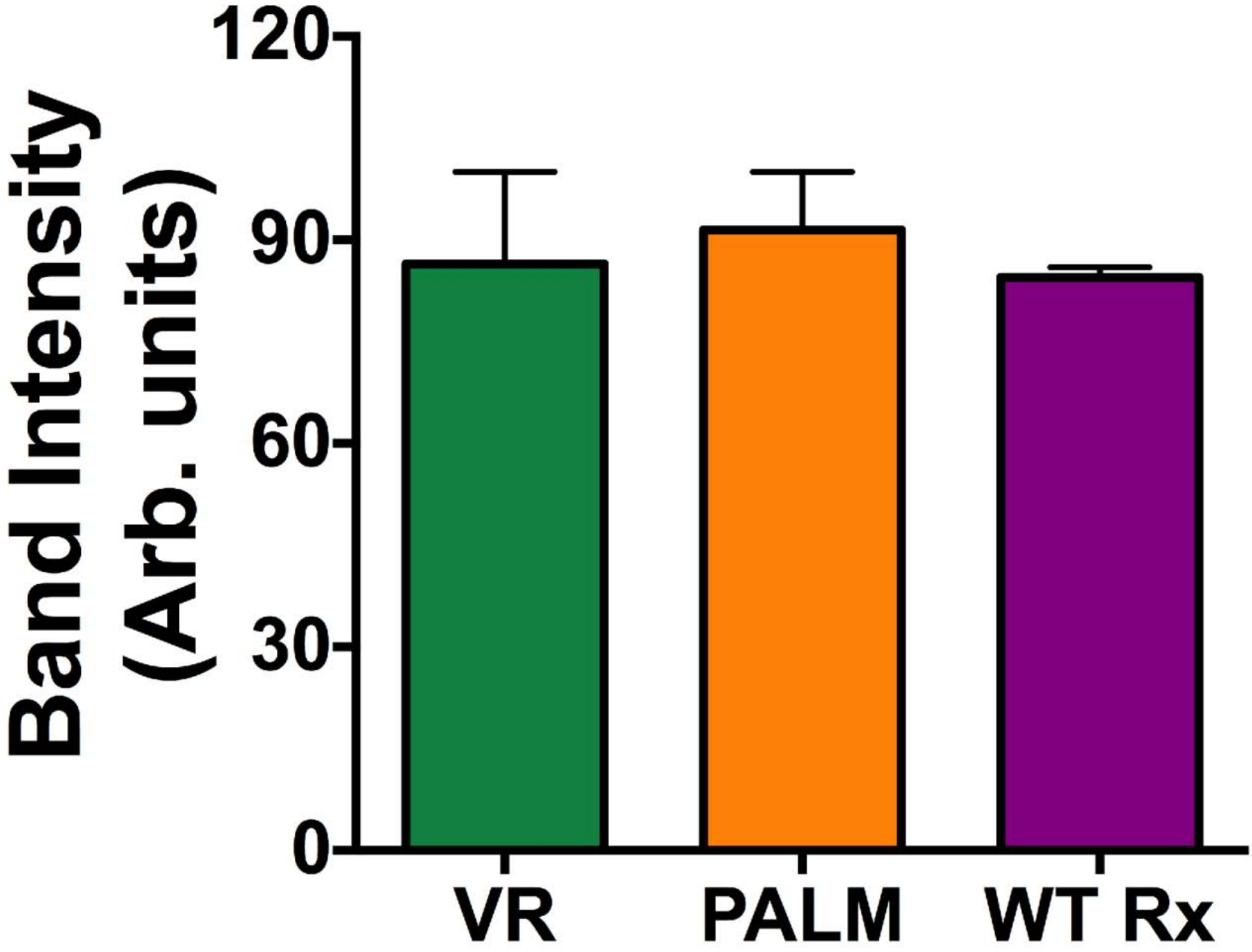
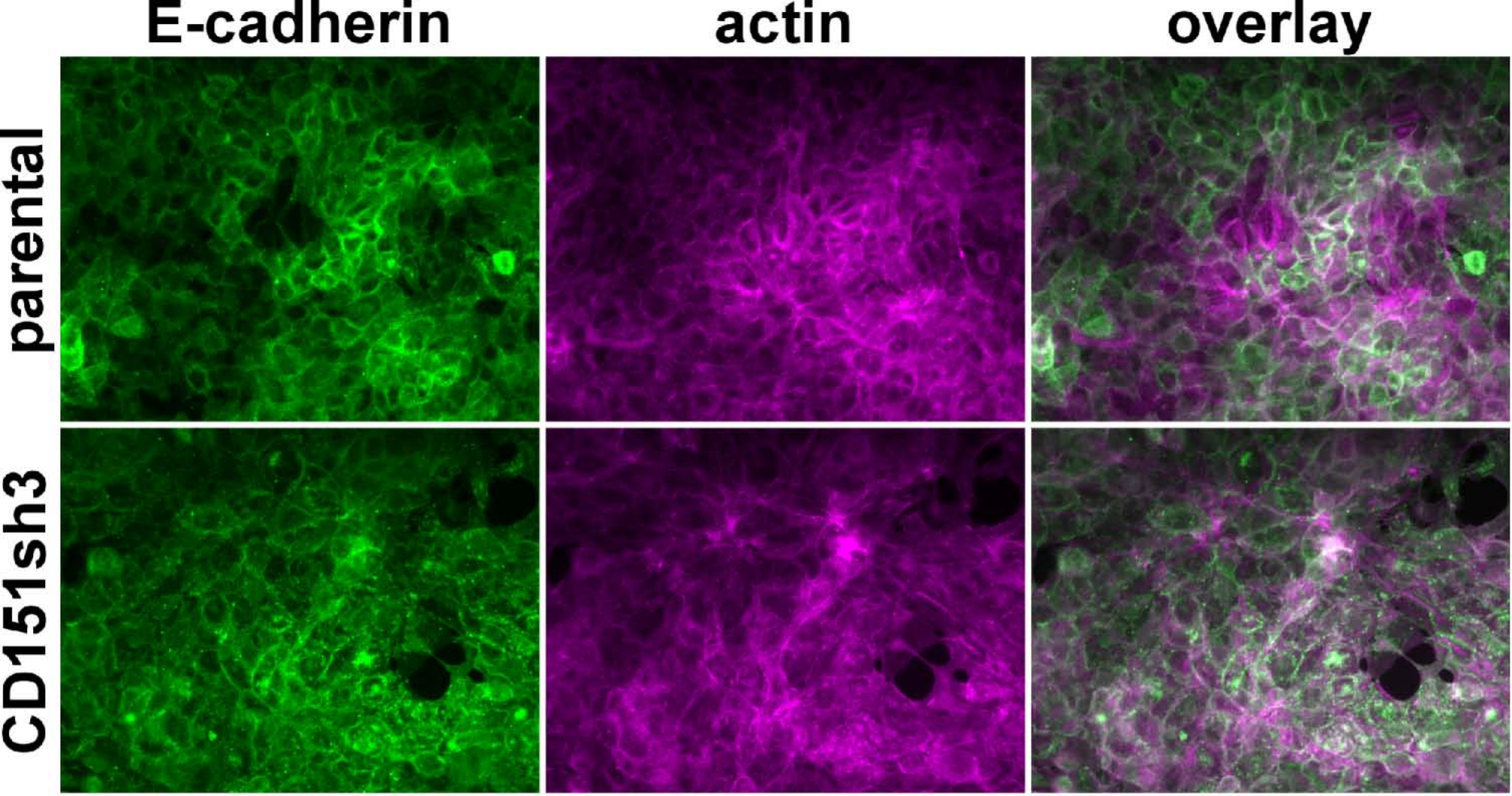



Figure S1

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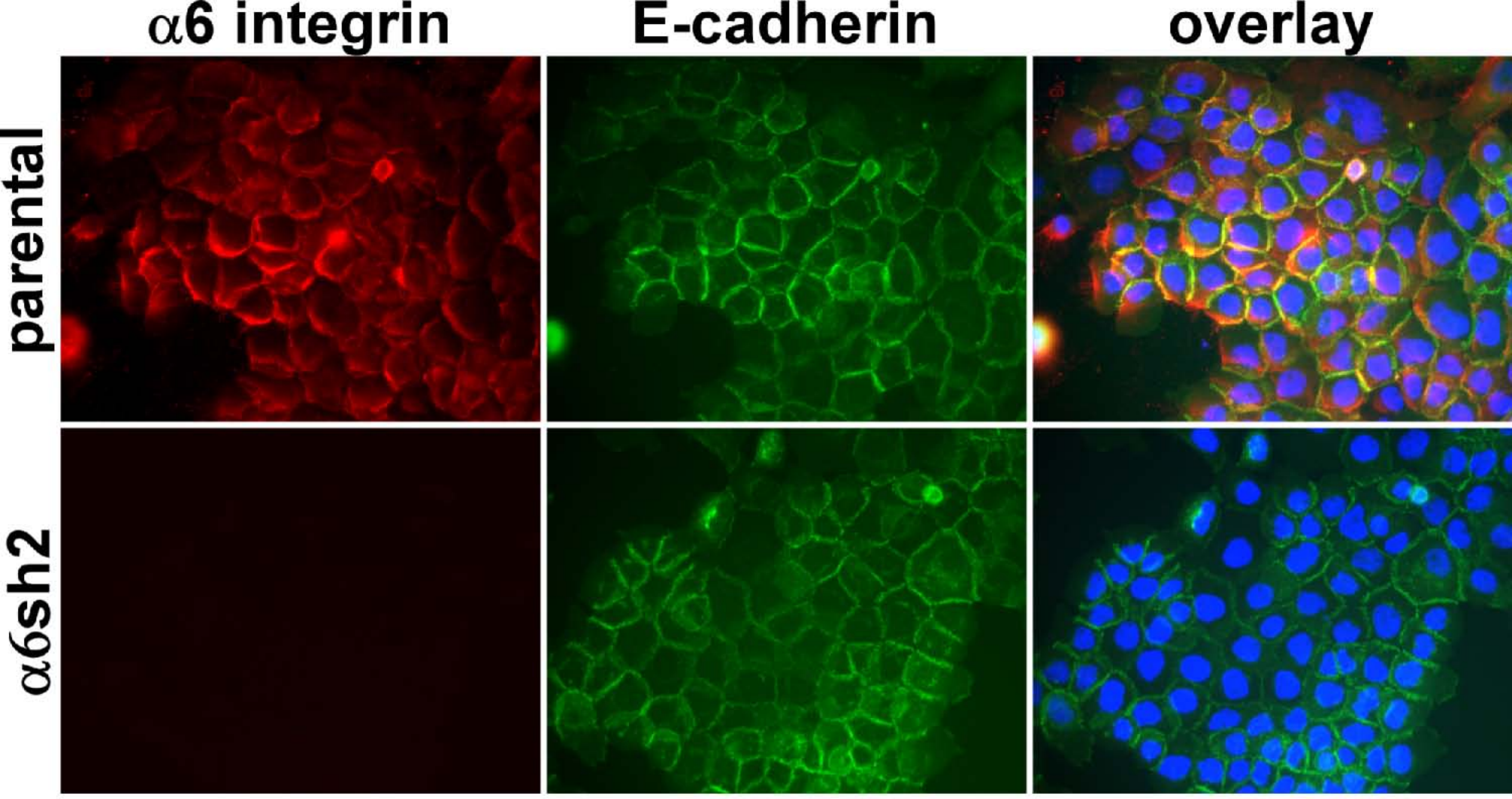


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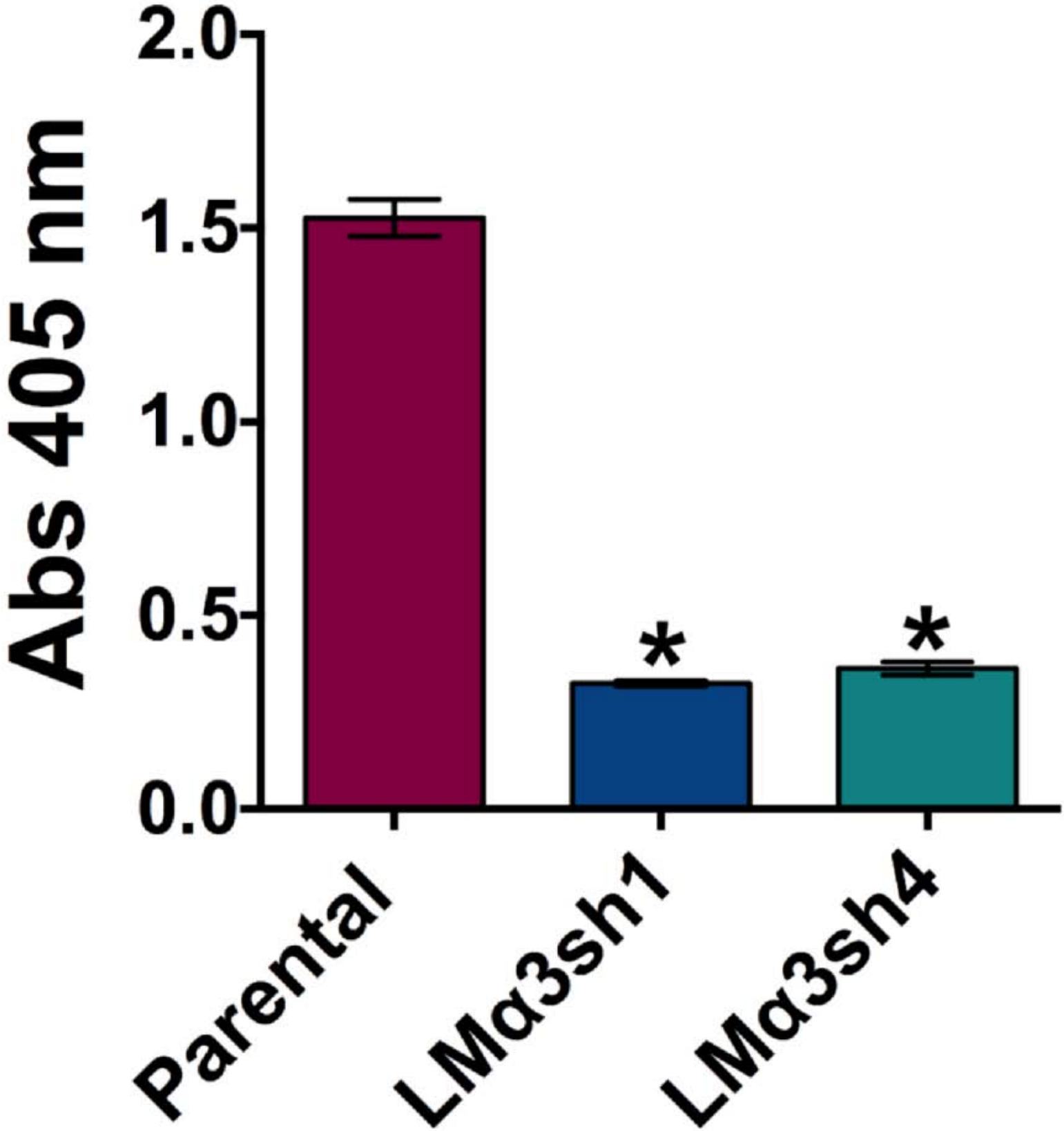


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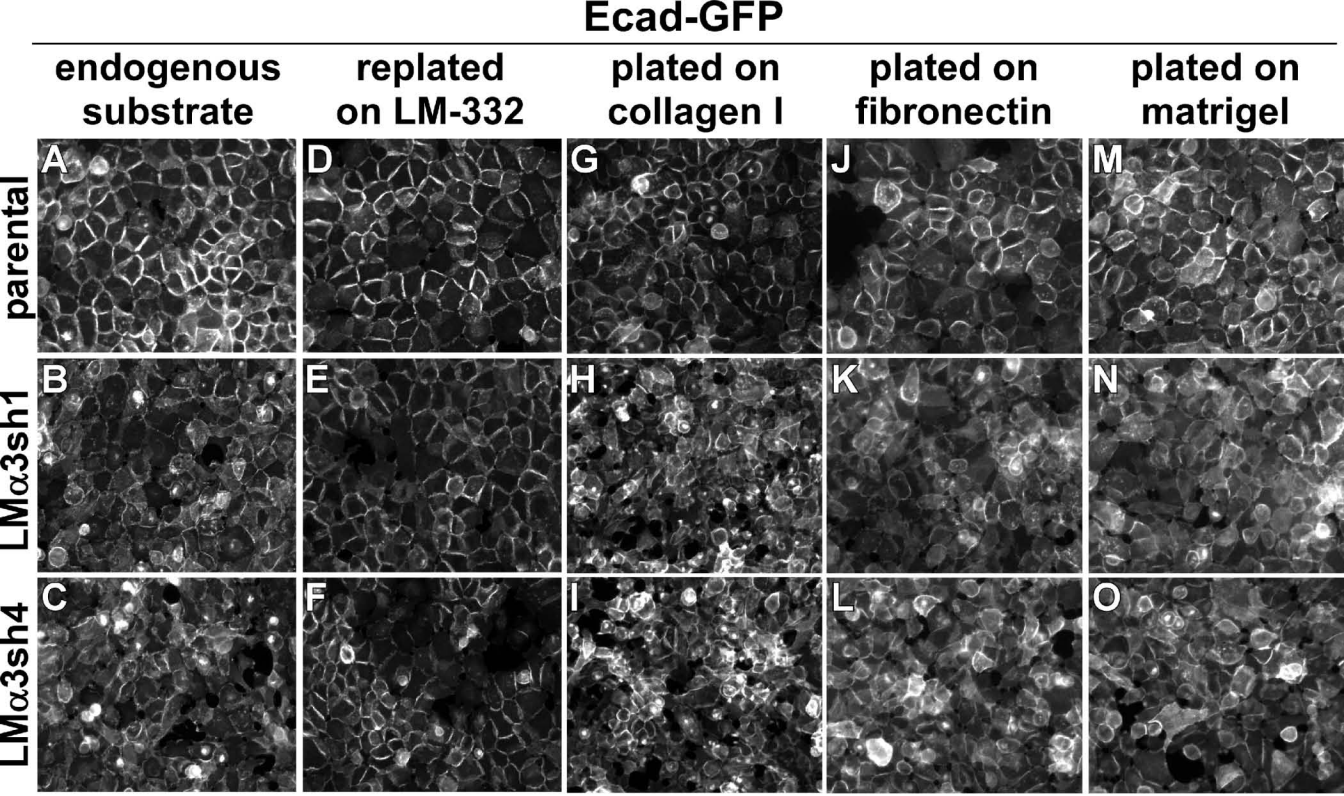

Ecad-GFP
endogenous substrate
replated on LM-332
plated on collagen I
plated on fibronectin
plated on matrigel
parental
LMα3sh1
LMα3sh4
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O

Figure S5

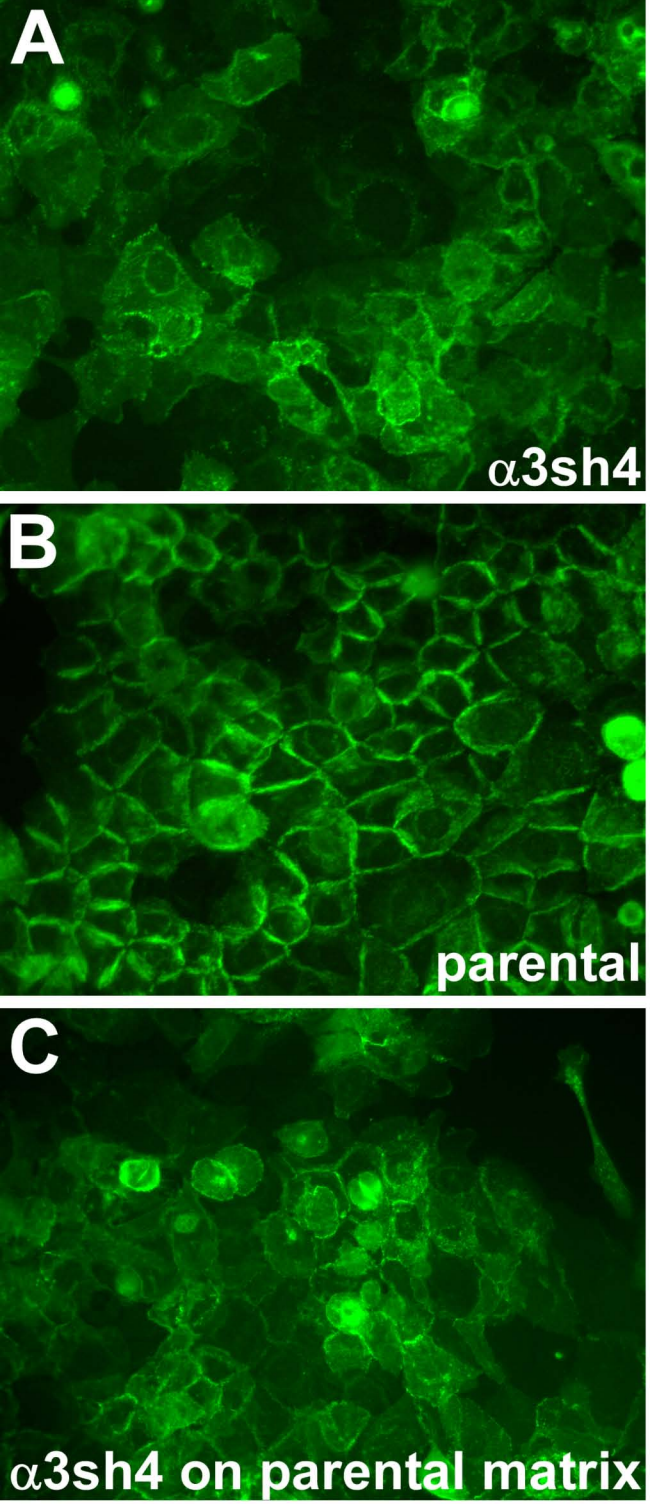



Figure S6

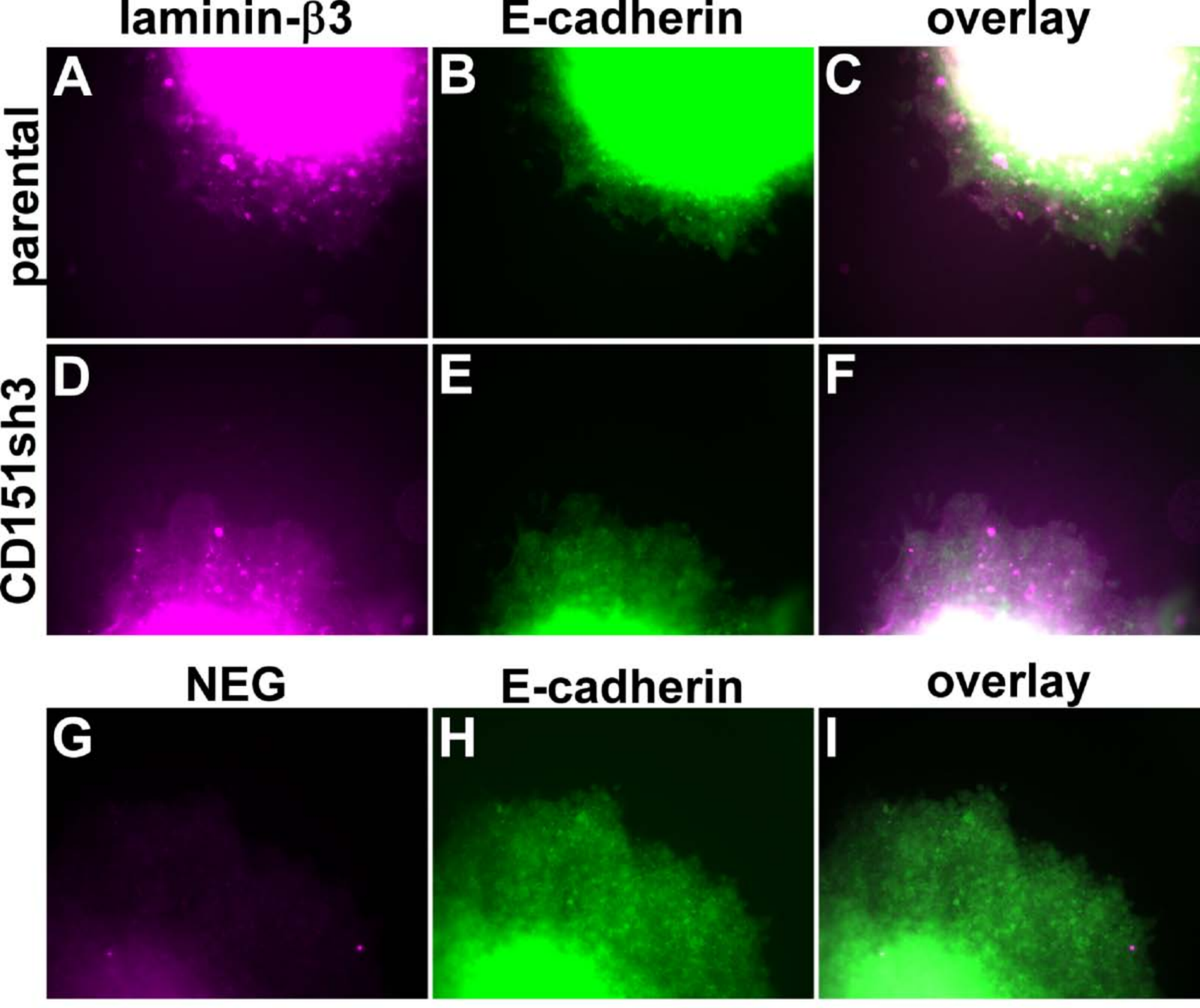

laminin-β3
E-cadherin
overlay
parental
A
B
C
CD151sh3
D
E
F
NEG
E-cadherin
overlay
G
H
I

Figure S7

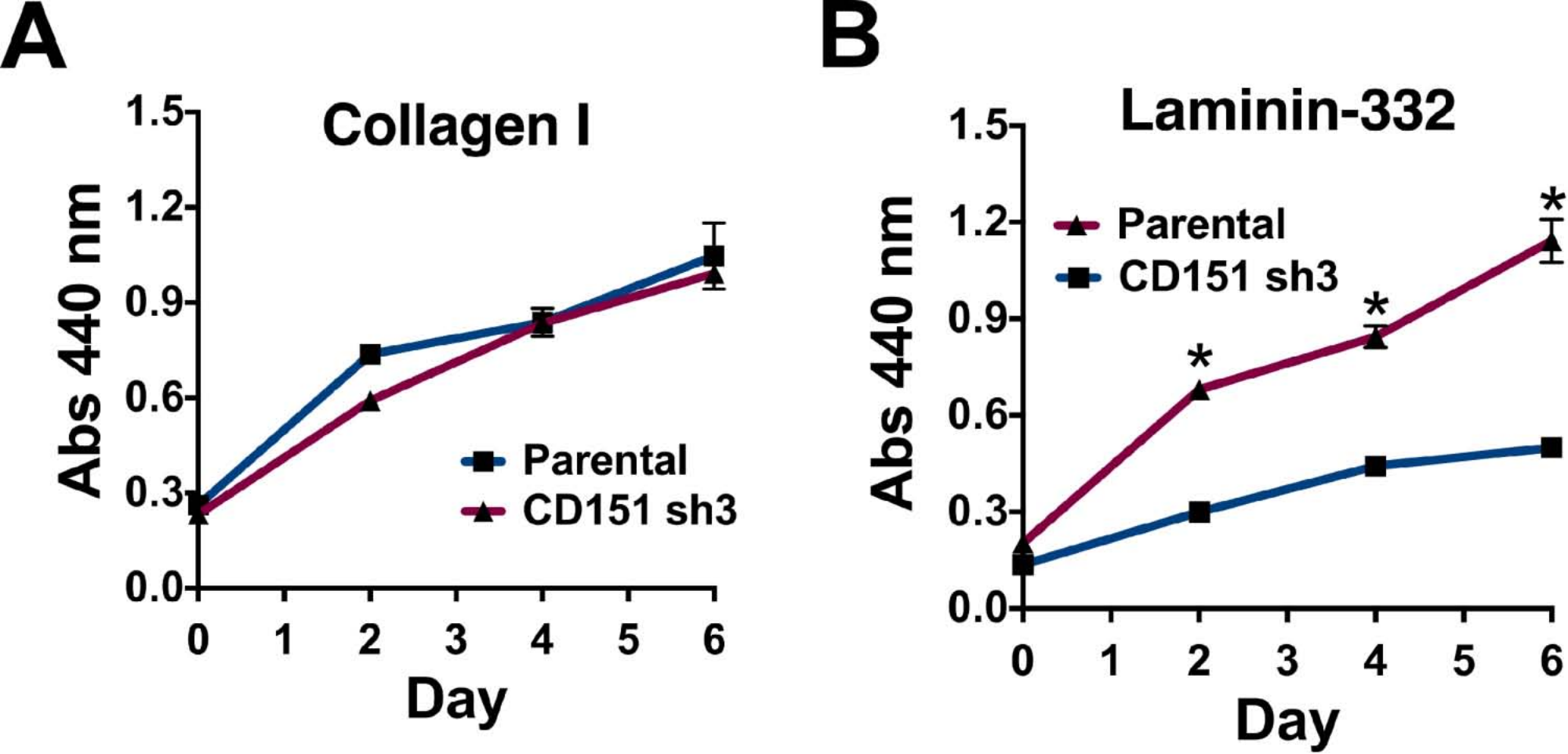
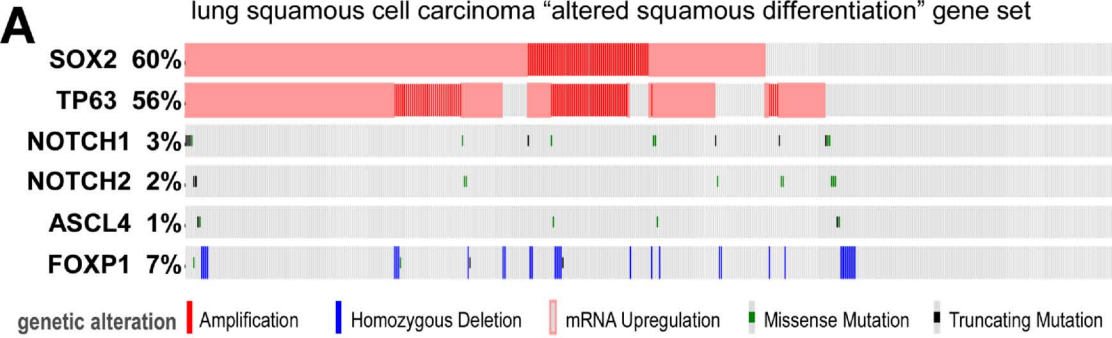
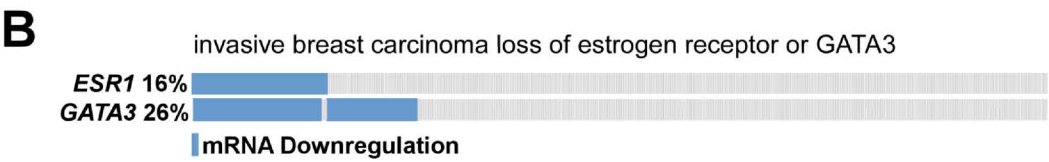
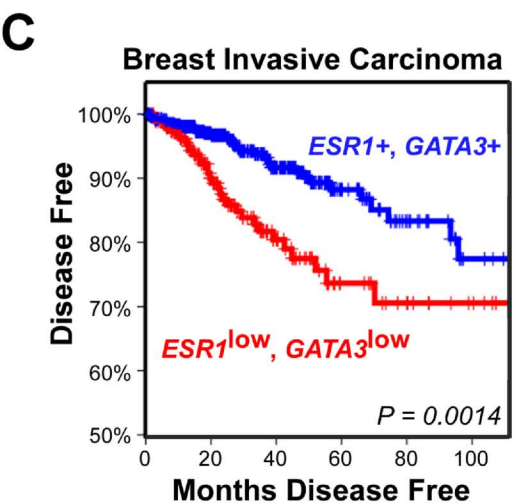

A
Collagen I
Abs 440 nm
1.5
1.2
0.9
0.6
0.3
0.0
0 1 2 3 4 5 6
Day
Parental
CD151 sh3
B
Laminin-332
Abs 440 nm
1.5
1.2
0.9
0.6
0.3
0.0
0 1 2 3 4 5 6
Day
Parental
CD151 sh3
*
*
*

Figure S8




D

| Protein | Average Abundance (Z-score) | | p-value |
|---|---|---|---|
| | *ESR1+/ GATA3+ cases* | *ESR1$^{low}$/ GATA3$^{low}$ cases* | |
| GATA3 | 0.49 | -1.17 | 1.07e-73 |
| ESR1 | 0.49 | -1.18 | 1.52e-67 |
| PGR | 0.32 | -0.78 | 3.89e-44 |
| AR | 0.37 | -0.90 | 2.24e-27 |
| CDH3 | -0.34 | 0.83 | 7.11e-20 |
| CCNE1 | -0.35 | 0.84 | 5.46e-19 |
| CCNB1 | -0.28 | 0.67 | 5.79e-18 |

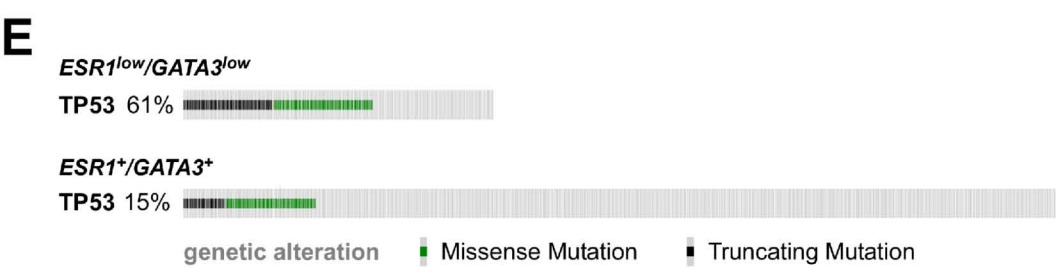



Figure S9

## Table S1. Data from The Cancer Genome Atlas (TCGA) and KM-plotter used in Figure 10

***TCGA Databases***

| Cancer Type | CD151[a] expression | Number of Cases | Cases Deceased | Median Months Survival | P-Value | Panel in Figure 10 |
|---|---|---|---|---|---|---|
| Lung squamous cell carcinoma with altered squamous differentiation | low (Z < -1) | 53 | 14 | 98 | 0.0079 | A |
| | not low (Z > -1) | 219 | 77 | 47 | | |
| Breast (ESR1+/GATA3+) | hi (Z > 1) | 56 | 5 | 146 | 0.026 | B |
| | not hi (Z < 1) | 605 | 75 | 112 | | |
| Breast (ESR1+/GATA3+) | low (Z < -1) | 110 | 22 | 89 | 0.016 | C |
| | not low (Z > -1) | 551 | 58 | 115 | | |
| Breast ($ESR1^{low}$/$GATA3^{low}$) | low (Z < -1) | 69 | 9 | 114 | 0.162 | D |
| | not low (Z > -1) | 171 | 28 | 97 | | |
| Breast ($ESR1^{low}$/$GATA3^{low}$) | hi (Z > 1) | 26 | 5 | 78 | 0.013 | E |
| | not hi (Z < 1) | 213 | 31 | 114 | | |

***KM-plotter Datasets***

| Breast Cancer Type | CD151 expression[b] | Number of Cases | Expression Value Cutoff[c] | Expression Range of the probe | P-Value | Panel in Figure 10 |
|---|---|---|---|---|---|---|
| Luminal A | high | 1277 | 1010 | 35-4946 | 0.029 | F |
| | low | 487 | | | | |
| Luminal B | high | 562 | 1184 | 59-7533 | 0.44 | G |
| | low | 439 | | | | |
| Her2+ | high | 116 | 1020 | 122-3612 | 0.002 | H |
| | low | 92 | | | | |
| Basal | high | 435 | 647 | 63-3851 | 0.033 | I |
| | low | 146 | | | | |

[a]CD151 mRNA expression by RNAseq V2 from TCGA database via cBioportal. Comparisons are either (i) between CD151-low cases (CD151 mRNA expression reduced as defined by Z-score of < -1) and cases in which CD151 was not low (Z-score > -1), *or* (ii) between CD151-hi cases (CD151 expression increased as defined by Z-score > 1) and cases in which CD151 was not high (Z-score < 1).

[b]CD151 expression was dichotomized as high versus low based on the Affymetrix probe expression values for CD151.

[c]The probe expression value determined by KM-plotter to be the best cutoff for the survival curve analysis.