Supplementary Figure Legends

Figure S-1. Serial propagation of RL-CL9 cells and the expression of Cx43, E-cadherin and N-cadherin. **A**. RL-CL9 cells were passaged serially for 6-8 months and imaged at passage 3 (early), 14 (middle) and 28 (late). Note the typical epithelial appearance of early passage cells and appearance of cells that have begun to lose this morphology, marked by red arrows, with serial passage. **B**. RL-CL9 cells at passage 22 were immunostained for N-Cad (red) and Cx43 (green). Note intracellular localization of Cx43 only in cells that express N-Cad (delineated by dotted line) and junctional localization in cells that lack N-Cad. **C**. RL-CL9 cells at passage 14 were immunostained for Cx43 (red) and E-Cad (green). Note the appearance of cells in which Cx43 is intracellular despite the expression of E-Cad (arrows).

Figure S-2. Localization and expression of E-cadherin, N-cadherin, vimentin and actin in RL-CL9 cells at early and late passages. RL-CL9 cells were passaged serially and immunostained at passage 7 (Early) and 25 (Late) for E-Cad and N-Cad (**A**), for vimentin and actin (**B**). Note the emergence of cells that express N-Cad (red) heterogeneously. Note also that some E-Cad shows increased localization at intracellular sites in late passage cells. Note also the increases in the expression level and the altered patterns of localization of vimentin and actin in early versus late passage cells. **C**. Western blot analysis of expression of E-Cad, N-Cad, vimentin and actin in early and late passage cells. Two lanes represent cell lysates independently obtained from early and late passage RL-CL9 cells. Note that the expression level of E-Cad and actin remains unchanged whereas that of N-Cad and vimentin increases.

Figure S-3. Directional migration of RL-NΔE and RL-EΔN cells. RL-EΔN and RL-NΔE cells were seeded in LabTek two well chamber slides and allowed to grow to confluence. Confluent monolayers of cells were wounded and cells imaged as described in “Materials and Methods”. Both cell types were imaged in parallel (see Materials and Methods). Shown are the live cell images of the corresponding time points that were converted to TIFF files. The numbers refer to time in hours and represent the corresponding captured frames.

Note that the scratches in the RL-NΔE cultures were filled earlier compared to RL-EΔN cultures. The wounds were filled within 14 ± 2 h in RL-NΔE cells whereas 22 ± 2 h were required to fill the wounds in RL-EΔN cells (n=3). The mean (± SE) rate of migration was significantly faster in RL-NΔE cells as compared with RL-EΔN cells (P=0.04). A two tailed Student's *t* test was used to calculate P value assuming unequal variance.

Figure S-4. Expression level and localization of myc-tagged E-cadherin and N-cadherin in RL-EΔN and RL-NΔE cells upon retroviral infection. Myc tagged N-Cad and E-Cad were introduced, respectively, into RL-EΔN and RL-NΔE cells as described in Materials and Methods. Pooled polyclonal cultures were immunostained with antibody against Myc for detecting E-Cad in RL-NΔE cells (**A**) and N-Cad in RL-EΔN cells (**B**). Note that myc tagged E-Cad and N-Cad are expressed robustly at the area of cell-cell contact. **C.D**. Western blot analysis of E-Cad and N-Cad expression in RL-NΔE and RL-EΔN cells after retroviral infection. Note robust expression of E-Cad in RL-NΔE cells and N-Cad in RL-EΔN cells upon retroviral transduction of myc-tagged cadherins as detected by anti-myc antibody.

Figure S-5. Cx43 co-localizes with Rab7 and Lamp-1 but not with Rab5 in RL-NΔE cells. RL-NΔE cells were seeded on glass cover slips at a density of 3 x10^5 cells in six well clusters and transfected with Rab5-GFP (Q-L) and Rab7-GFP (Q-L) after 16 h. Cells were fixed after 36 h and immunostained for Cx43 (red, rows 1 and 2) and for Cx43 and Lamp-1 (Bottom row). Note that Cx43 co-localizes partially with Rab7 and extensively with Lamp-1 in as shown in the enlarged images of the marked boxes on the right.

Figure S-6. Expression of E-cadherin and N-cadherin and the junctional and non-junctional fate of Cx43 in NMuMG rat mammary cells. Localization of E-Cad, N-Cad and Cx43 was examined in NMuMG cells that express both E-Cad and N-Cad (top row, NMuMG), in NMuMG derived subclone, Clone E9, that expresses nearly equal levels of both E-Cad and N-Cad (middle row), and in NMuMG derived subclone, Clone E11, that expresses high level of N-Cad and no detectable level of E-Cad (bottom row). Note that in parental NMuMG cells, cells that express high levels of E-Cad assemble GJs. Note also that in Clone E9 cells, both junctional and non-junctional localization of Cx43 is observed whereas in Clone E11, Cx43 remains intracellular and is not assembled into GJs.

Table S-1. Expression of E-cadherin and N-cadherin in isogenic clones derived from early and late passage RL-CL9 cells and the junctional and non-junctional localization of Cx43.

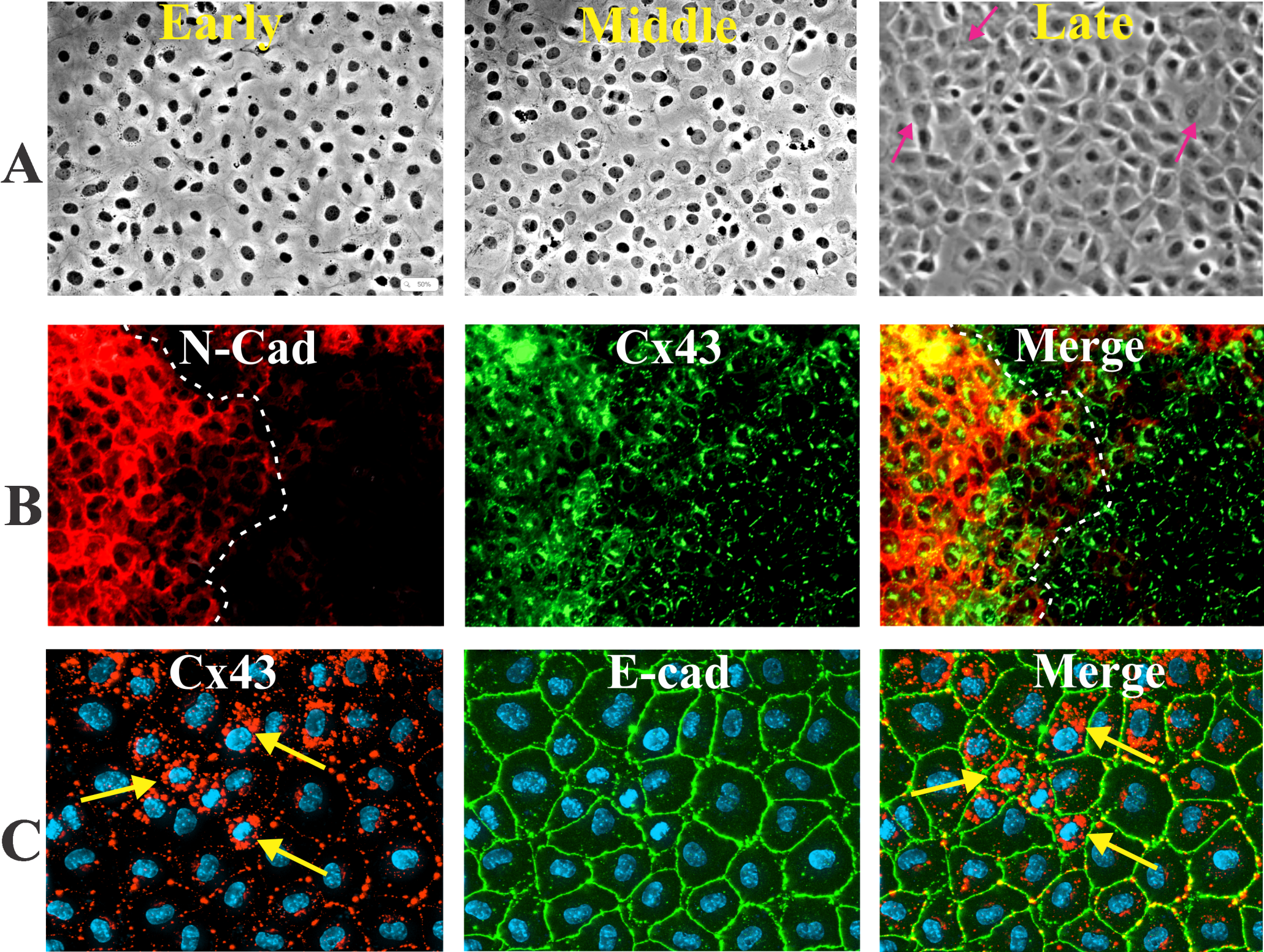
	RL-EARLY	**RL-LATE**
Number of clones	92	63
E-Cadherin +ve **(% +ve)**[a]	30/43 **(70)**	5/30 **(17)**
N-Cadherin +ve **(% +ve)**[a]	14/49 **(30)**	20/33 **(79)**
Junctional(Intercellular) Cx43 **(% +ve)**[a]	56/92 **(61)**	16/63 **(25)**
Non-junctional (Intracellular) Cx43 **(% +ve)**[a]	0/92 **(0)**	32/63 **(51)**
Junctional and Non-junctional Cx43 **(% +ve)**[a]	36/92 **(32)**	15/63 **(24)**

One hundred RL-CL9 cells at passage 4 and 30 were seeded in replicate 10 cm dishes in 10 ml complete medium and allowed to grow into colonies for 3-4 weeks. Individual clones were picked using glass cylinders, expanded and frozen. Each clone was examined for the expression of E-Cad and N-Cad and for the junctional versus non-junctional localization of Cx43 by immunocytochemical analysis as described in Materials and Methods. **a** = Number of positive clones versus total number of clones analyzed. In parenthesis = % of positive clones.

Table S-2 Cell-cell adhesion remains intact in RL-EΔN and RL-NΔE cells.

Cell Line	Exp #	Mean Area (SE)	# Aggregates
RL-EΔN	**1**	**1775 ∀ 322**	**40**
	2	**1910 ∀ 378**	**32**
RL-NΔE	**1**	**1940 ∀ 415**	**39**
	2	**2165 ∀ 445**	**33**

Five thousand cells, suspended in a 20 Φl of complete medium and placed on the lids of petri dishes, were allowed to aggregate as described in Materials and Methods. The size of aggregates from four random snapshots obtained from two independent experiments was measured and the average aggregate size was calculated and plotted as described (see Methods). As assessed visually, aggregates formed of RL-EΔN cells appeared to be more compact and regular compared to RL-NΔE cells. The differences in the mean sizes of the aggregates among RL-EΔN and RL-NΔE cells were not statistically significant (P ∃ 0.8**).**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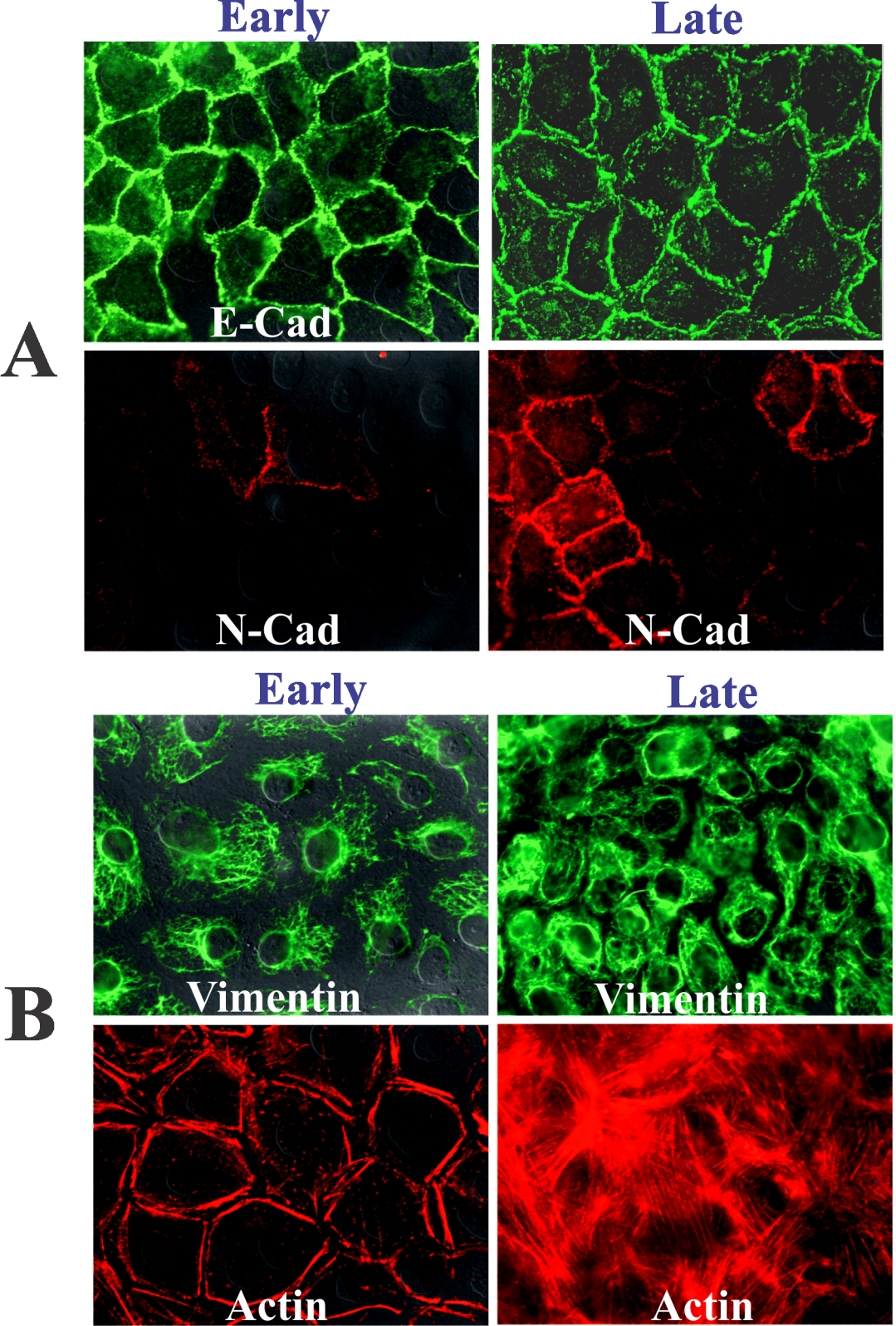
Early
Middle
Late
B
N-Cad
Cx43
Merge
C
Cx43
E-cad
Merge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Late
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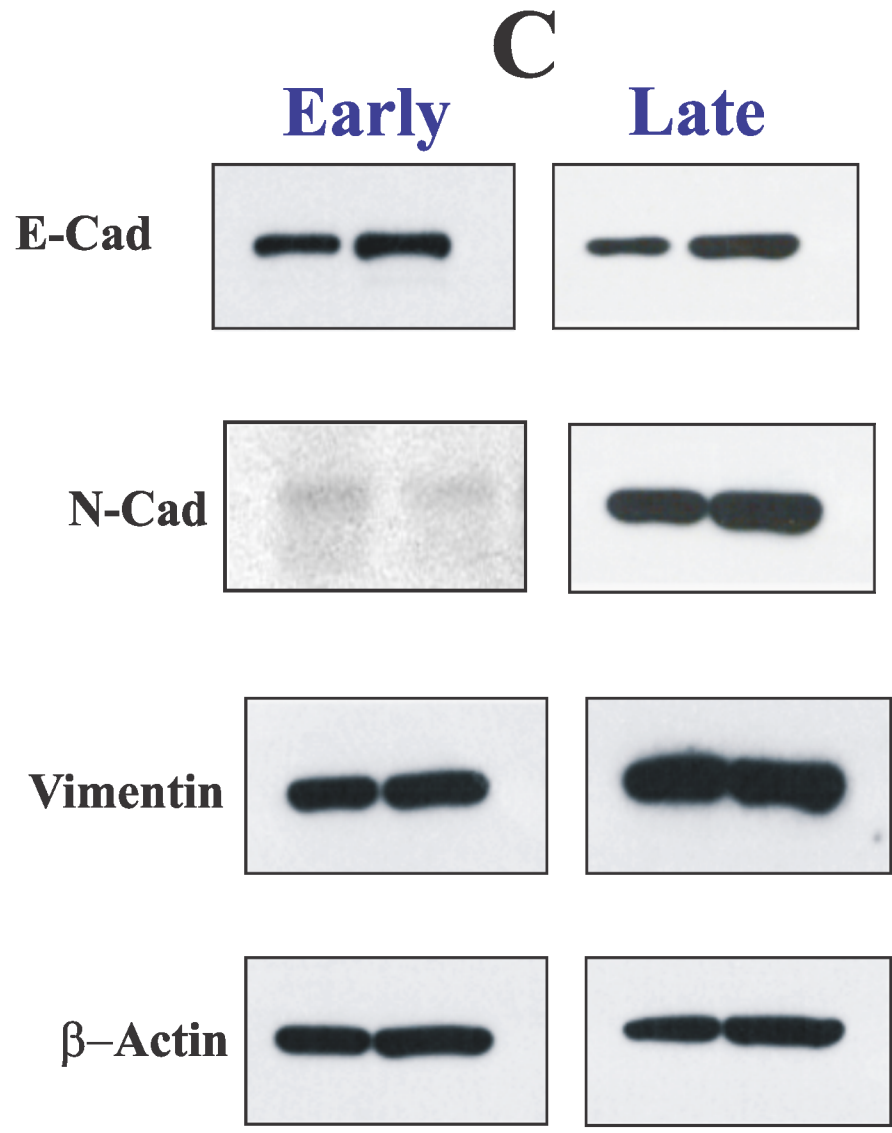
E-Cad
N-Cad
N-Cad
B
Early
Late
Vimentin
Vimentin
Actin
Actin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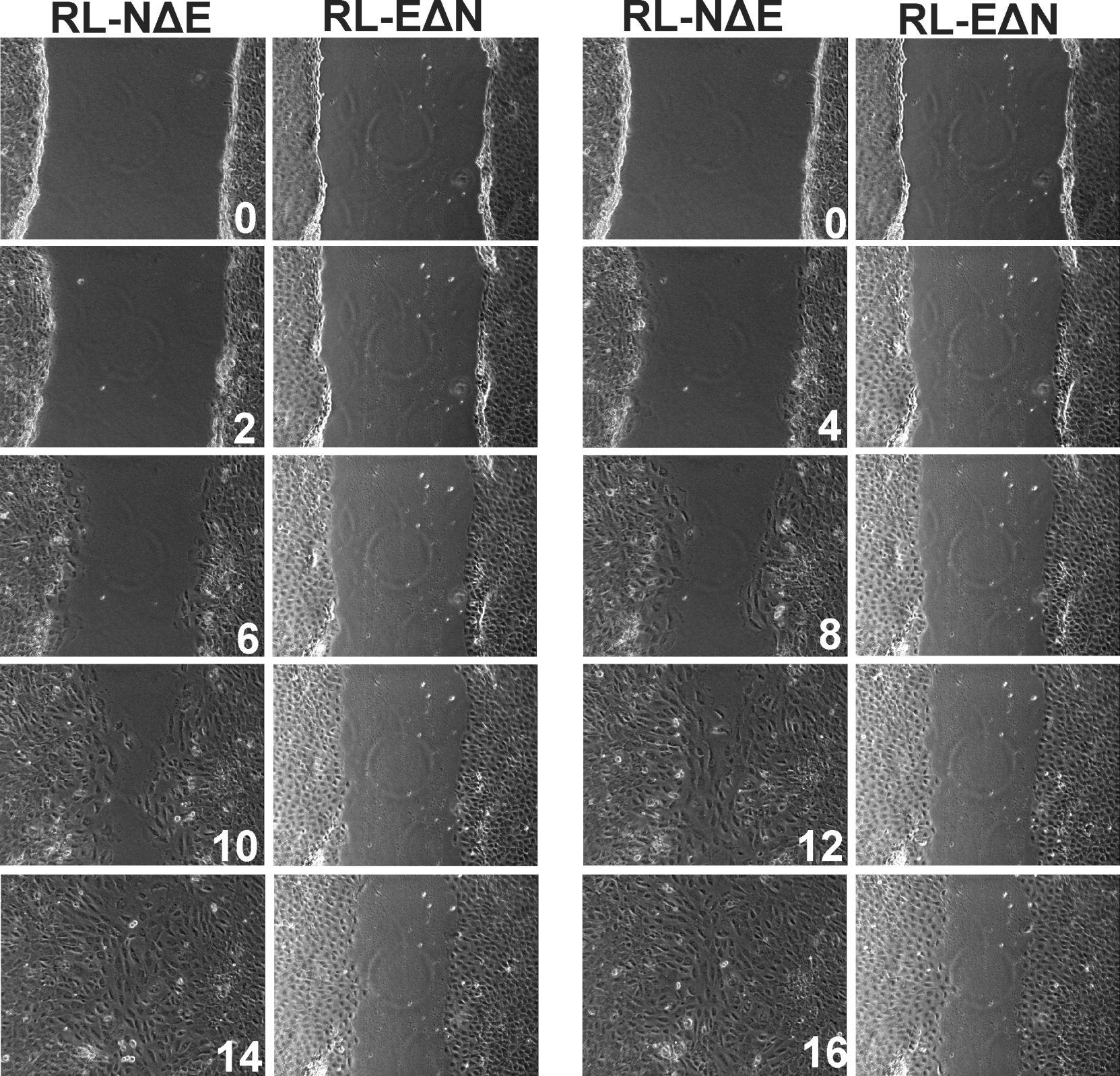
Late
E-Cad
N-Cad
Vimentin
β–Actin


RL-NΔE
RL-EΔN
RL-NΔE
RL-EΔN
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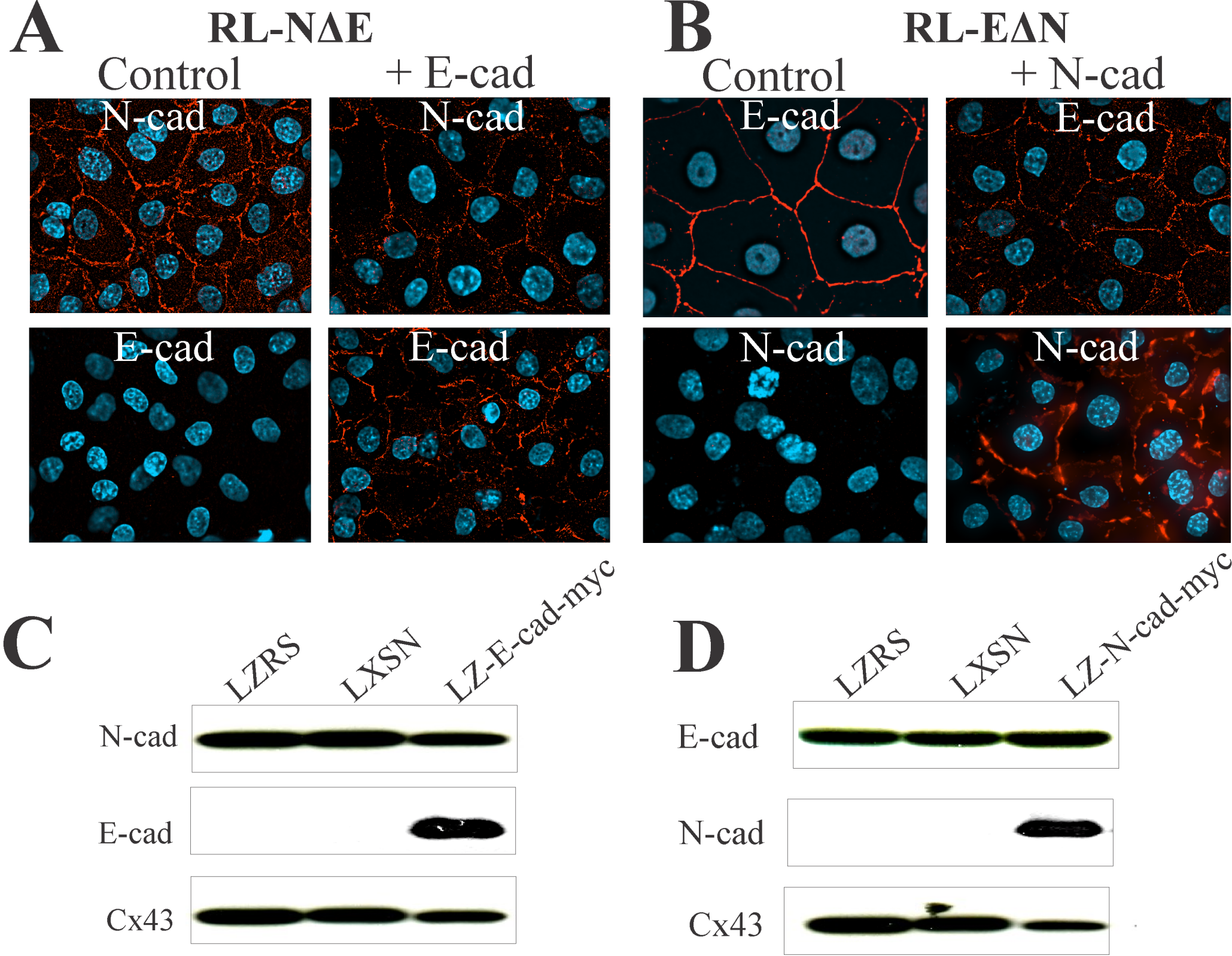
6
8
10
12
14
16


A
RL-NΔE
Control
+ E-cad
N-cad
N-cad
E-cad
E-cad
B
RL-EΔN
Control
+ N-cad
E-cad
E-cad
N-cad
N-cad
C
LZRS
LXSN
LZ-E-cad-myc
N-cad
E-cad
Cx43
D
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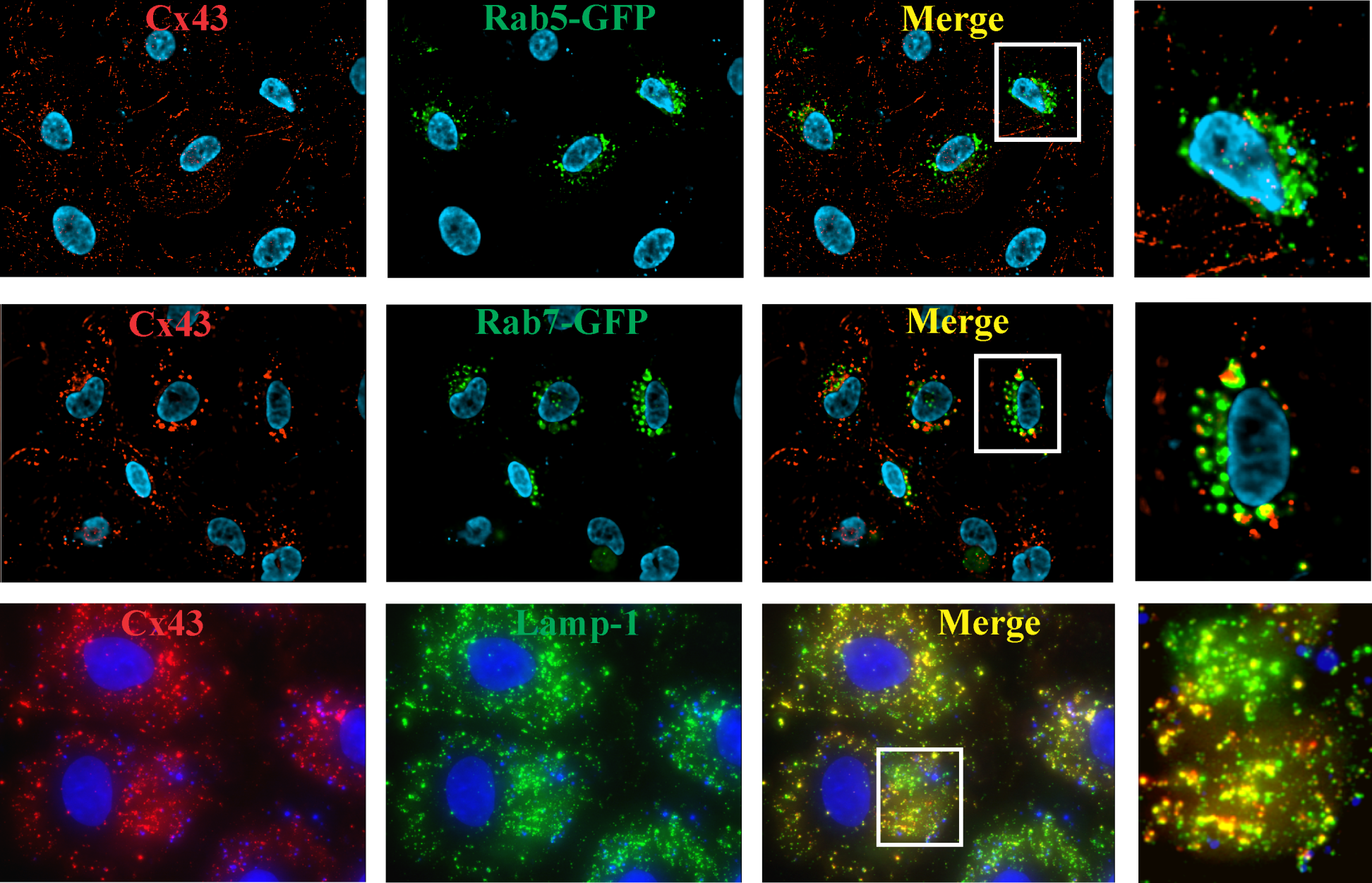
E-cad
N-cad
Cx43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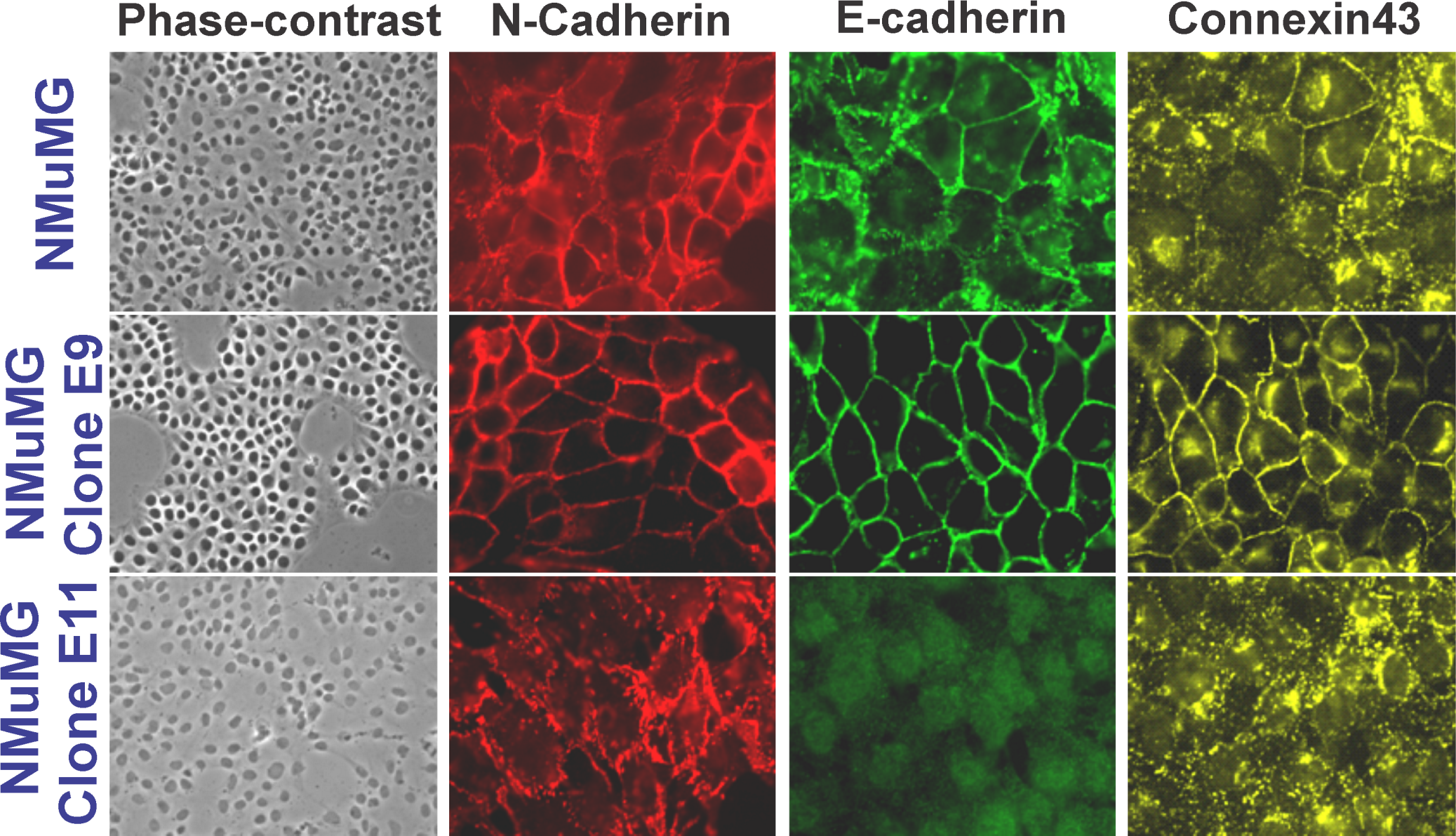
Rab5-GFP
Merge
Cx43
Rab7-GFP
Merge
Cx43
Lamp-1
Merge


Phase-contrast
N-Cadherin
E-cadherin
Connexin43
NMuMG
NMuMG Clone E9
NMuMG Clone E11

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