Supplamental Figure S1

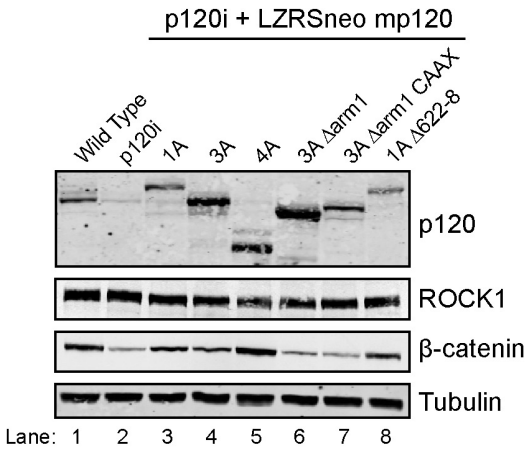

Figure S1: p120 depletion and reconstitution does not affect ROCK1 levels. Western blot analysis of p120, ROCK1, β-catenin, and Tubulin in Wild Type A431 cells, p120-depleted A431 cells (p120i), of p120i cells expressing the indicated murine p120 isoforms. Lane 1: Wild-Type A431 cells, lane 2: p120i A431 cells transduced with LZRS neo vector alone, lanes 3-8: p120i cells expressing mp120 1A (lane 3), mp120 3A (lane 4), mp120 4A (lane 5) mp120 3A Δarm1 (lane 6), mp120 3A Δarm1 CAAX (lane 7), mp120 1A Δ622-628.

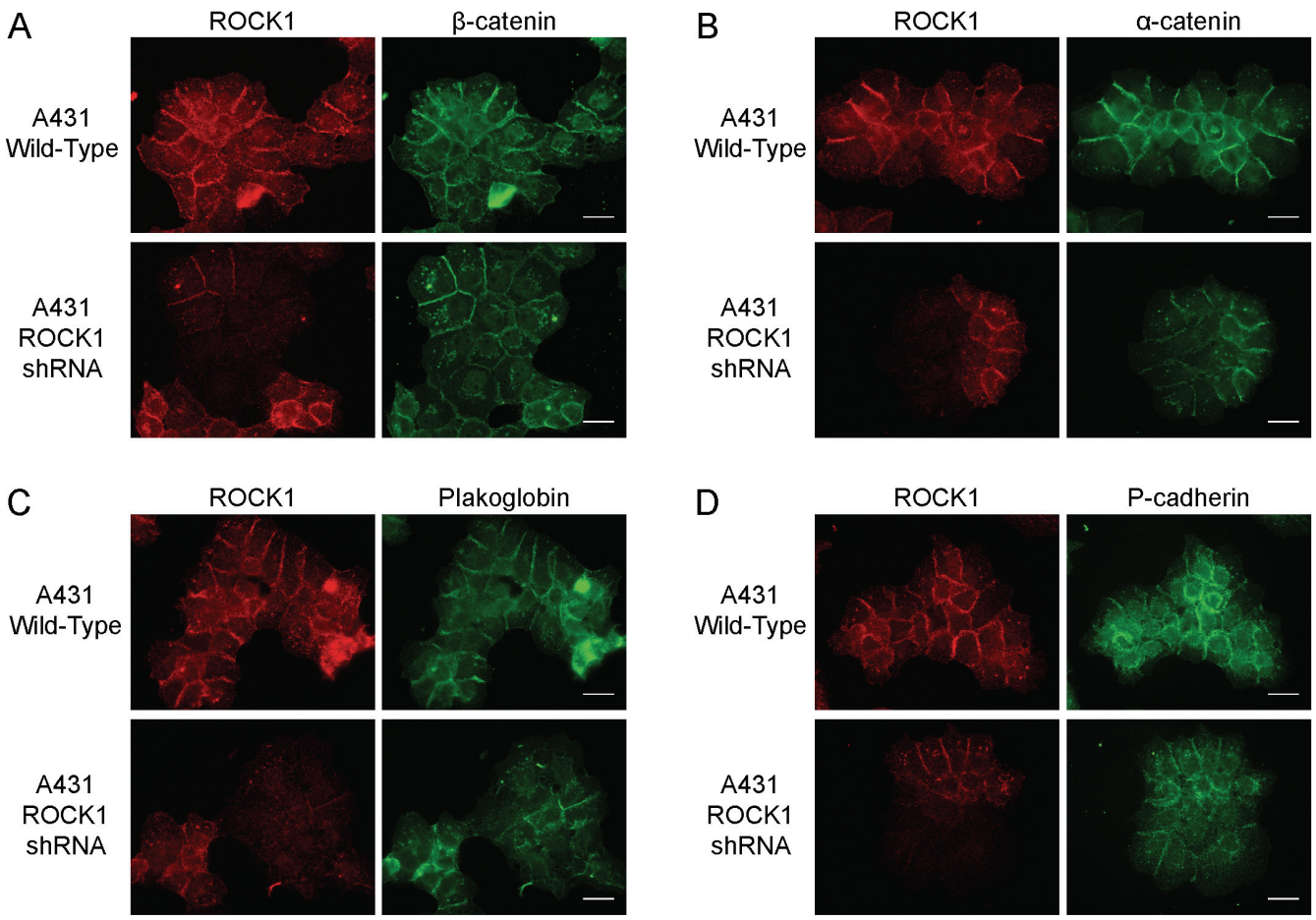

Figure S2: ROCK1 depletion affects the entire cadherin complex.
Immunofluorescence analysis of ROCK1 (red) and β-catenin **(A)**, α-catenin **(B)**, Plakoglobin **(C)**, and P-cadherin **(D)** in wild-type A431 cells and A431 cells expressing ROCK1 shRNA. Scale bar represents 10 μm.

Supplamental Figure S3

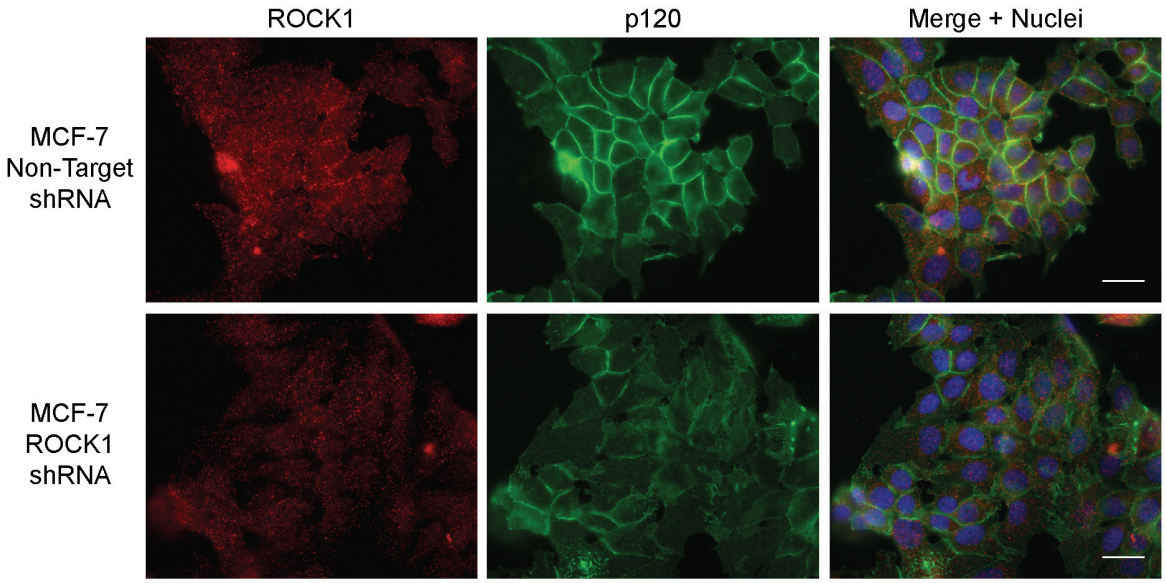

Figure S3: ROCK1 depletion in MCF-7 cells affects p120 distribution. Immunofluorescence analysis of ROCK1 (red) and p120 (green) in MCF-7 cells expressing Non-Target shRNA or ROCK1 shRNA. Scale bar represents 10 μm.

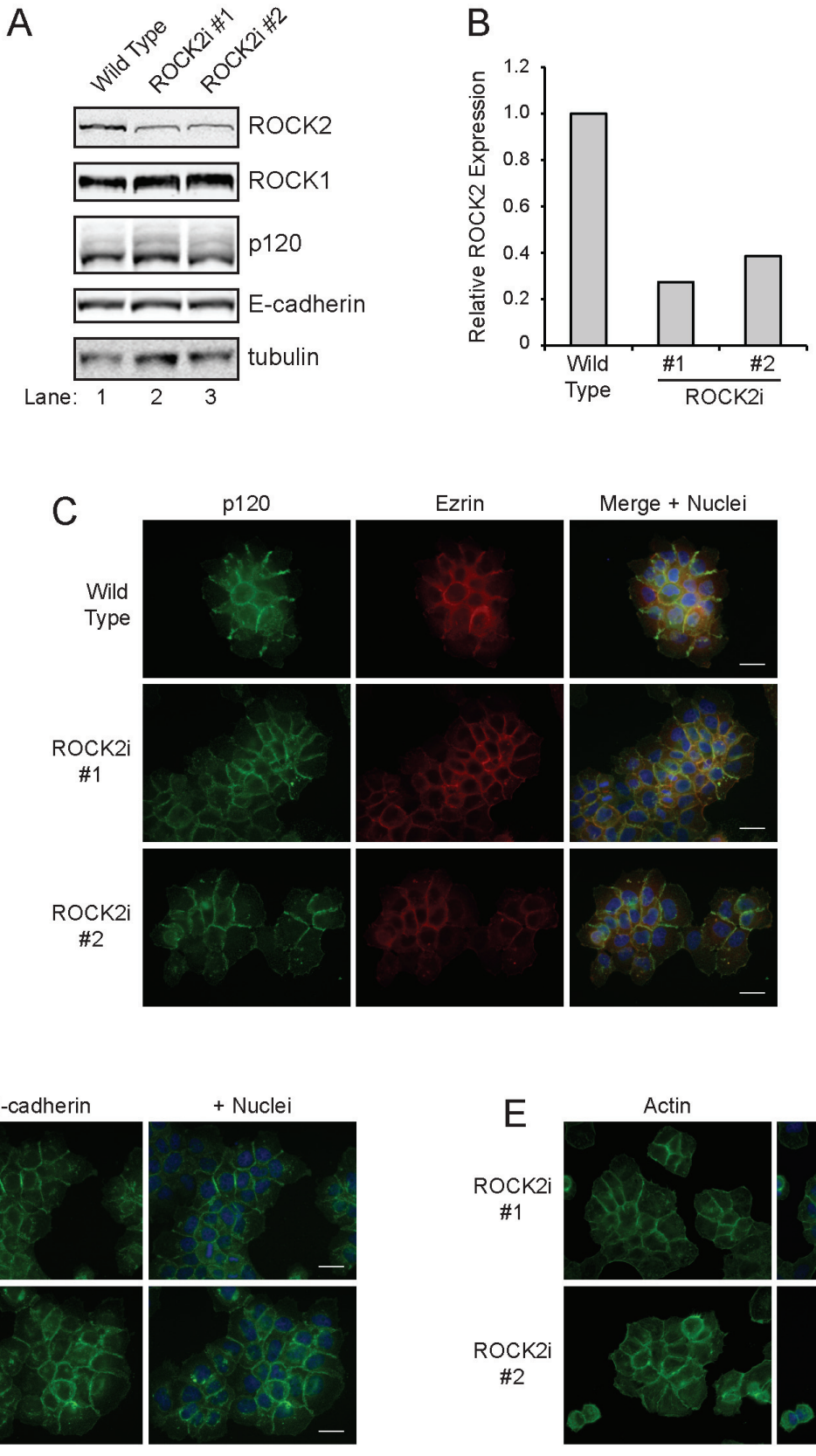

Figure S4: Knockdown of ROCK2 does not affect cell-cell adhesion. **(A)** Western blot analysis of wild-type A431 cells (lane 1) and those expressing two different ROCK2 shRNA constructs (ROCK2i #1 & #2, lanes 2 & 3). **(B)** Relative levels of ROCK2 from cells in **(A)**, normalized to tubulin. **(C-E)** Effects of ROCK2 depletion. Immunofluorescence analysis of p120 and Ezrin **(C)**, E-cadherin **(D)**, and actin **(E)** in wild-type and ROCK2 knockdown cells. Scale bars represent 10 μM.

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Table S1: ROCK1 peptides detected in MCF-7, MCF-10A, and Caco-2 cells by ReCLIP.

MCF-7

Peptide	*Position*	*ROCK Domain*
AESEQLAR	899-906	Coiled-coil
VSQNSQLANEK	529-539	Coiled-coil
ITSLQEEVK	631-639	Coiled-coil
LEEANSMLTK	941-950	Coiled-coil (RBD)
LLLQNELK	784-791	Coiled-coil
SISQLESLNR	574-583	Coiled-coil
GAFGEVQLVR	85-94	Kinase
VSYDVTSAR	1285-1293	PH
YLSSANPNDNR	405-415	Coiled-coil
LADFGTCMK	214-222	Kinase
DRGHDSEMIGDLQAR	616-630	Coiled-coil
SLQESLQK	423-430	Coiled-coil

MCF10A

Peptide	*Position*	*ROCK1 Domain*
YLSSANPNDNR	405-415	Coiled-coil
STANQSFR	1336-1343	C-terminus
VSYDVTSAR	1285-1293	PH
NELQMQLASK	1074-1083	Coiled-coil
DRGHDSEMIGDLQAR	616-630	Coiled-coil
VSQNSQLANEK	529-539	Coiled-coil
ITSLQEEVK	631-639	Coiled-coil
TQAFEADNLK	792-801	Coiled-coil
DVKPDNMLLDK	198-208	Kinase
NSLTFPDDNDISK	295-307	Kinase
SISQLESLNR	574-583	Coiled-coil
GAFGEVQLVR	85-94	Kinase

Caco-2

Peptide	*Position*	*ROCK1 Domain*
TQAFEADNLK	792-801	Coiled-coil
SISQLESLNR	574-583	Coiled-coil
VSYDVTSAR	1285-1293	PH
GAFGEVQLVR	85-94	Kinase

Unique peptides detected by LC-MS/MS in each cell line are shown with the corresponding amino-acid position and ROCK1 domain.

Table S2: p190A RhoGAP peptides identified in MCF-7 by ReCLIP.

p190A RhoGAP Peptide	*Position*
GDLSYLDQGHR	1054-1064
NLNLVSSTASIK	764-775
MGVIQDVLGEEQR	517-529
NLQVVETSAR	222-231
STALQPYIK	110-118
FVSNLYNQLAK	180-190
ESLSYVVESIEK	669-680
NEEENIYSVPHDSTQGK	1099-1115
NSLSDPNIDR	588-597
DLADVDLR	776-783
INLVILGK	598-605

Unique p190A RhoGAP peptides detected by LC-MS/MS across four separate p120 ReCLIP experiments in MCF-7 cells are shown with the corresponding amino-acid position.