Kourtidis et al., https://doi.org/10.1083/jcb.201612125

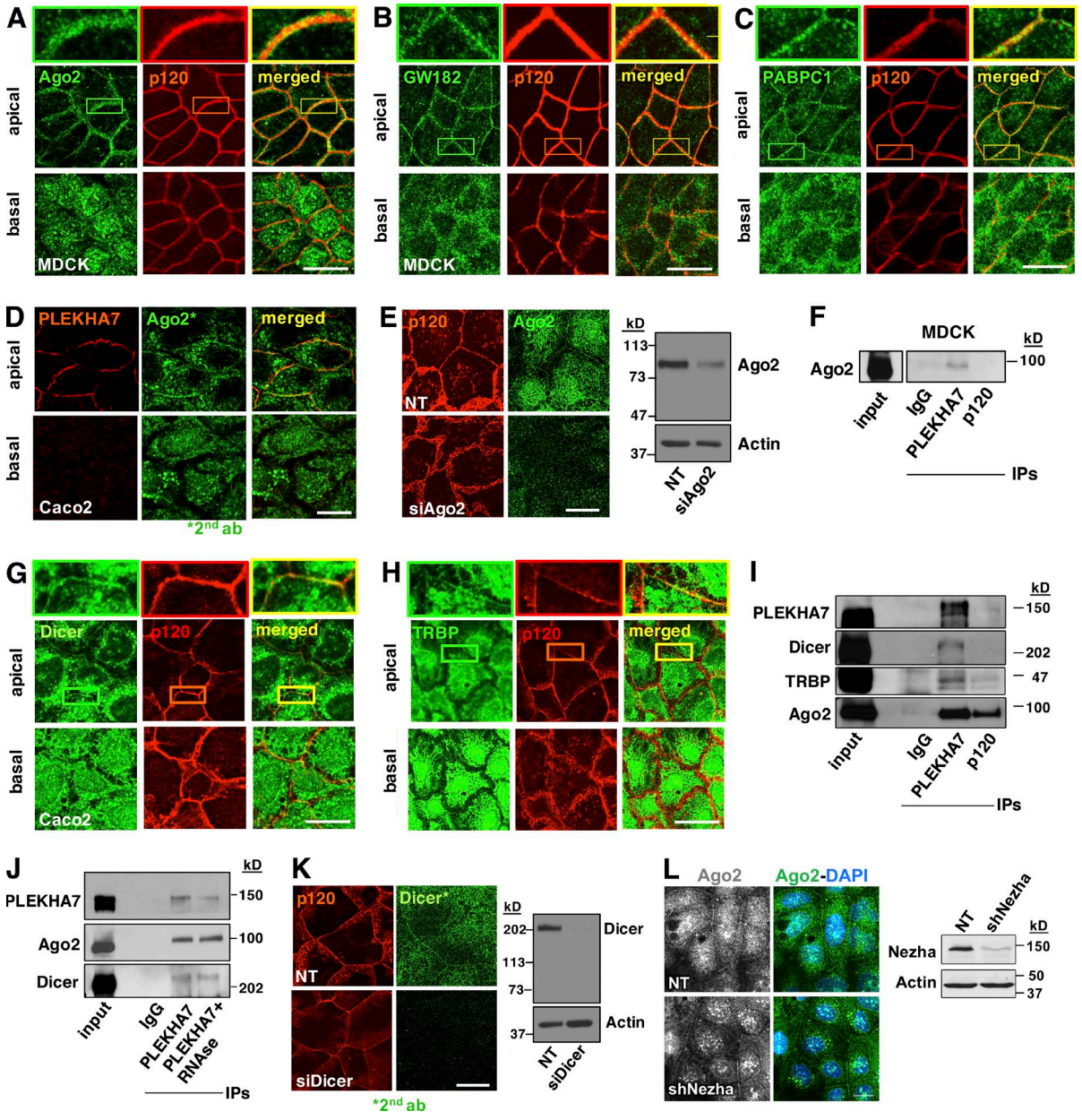

Figure S1. **PLEKHA7 associates with RISC and the miRLC at epithelial apical junctions.** MDCK cells were grown to polarize and subjected to immunofluorescence for p120 and the core proteins of the RISC complex: Ago2 (A); GW182 (B); and PABPC1 (C). Representative apical and basolateral confocal images are shown. Enlarged parts of images on top of each stack indicate areas of cell–cell contact. (D) Caco2 cells were grown to polarize and subjected to immunofluorescence for PLEKHA7 and Ago2 with a different antibody compared with Fig. 1 A and panel A (see also Materials and methods). (E) siRNA-mediated knockdown of Ago2 (siAgo2) in Caco2 cells, shown by both immunofluorescence (left) and Western blot (right). Nontarget (NT) siRNA is the control. p120 was used as a costain for immunofluorescence; Actin is the loading control for the blots; molecular masses (kD) are indicated on the left side of the blot. (F) Western blot of PLEKHA7 and p120 IPs of MDCK cells for Ago2. IgG is the negative control. Molecular masses (kD) are indicated on the right. (G and H) Caco2 cells were grown to polarize and subjected to immunofluorescence for PLEKHA7 or p120 and Dicer, TRBP. Images were acquired and presented as in Fig. 1. Enlarged parts of images indicate areas of cell–cell contact. (I) Western blot of PLEKHA7 and p120 IPs of Caco2 cells for PLEKHA7, Dicer, TRBP, and Ago2. IgG is the negative IP control. Molecular masses (kD) are indicated on the right. (J) Western blot of PLEKHA7 IPs after treatment with or without RNase, for PLEKHA7, Ago2, and Dicer in Caco2 cells. IgG is the negative IP control. Molecular masses (kD) are indicated on the right. (K) siRNA-mediated knockdown of Dicer (siDicer) in Caco2 cells, shown by both immunofluorescence (left) and Western blot (right). A different antibody compared with G was used here for Dicer (see also Materials and methods). NT siRNA is the control. p120 was used as a costain for immunofluorescence; Actin is the loading control for the blots; molecular masses (kD) are indicated on the left. (L) Caco2 control (NT) and Nezha shRNA-mediated knockdown cells (shNezha) were subjected to immunofluorescence for Ago2. DAPI is the nuclear stain. Western blots confirming the knockdown are shown at right; Actin is the loading control. Molecular masses (kD) are indicated on the right. Bars, 20 µM. Insets are magnified 3×.

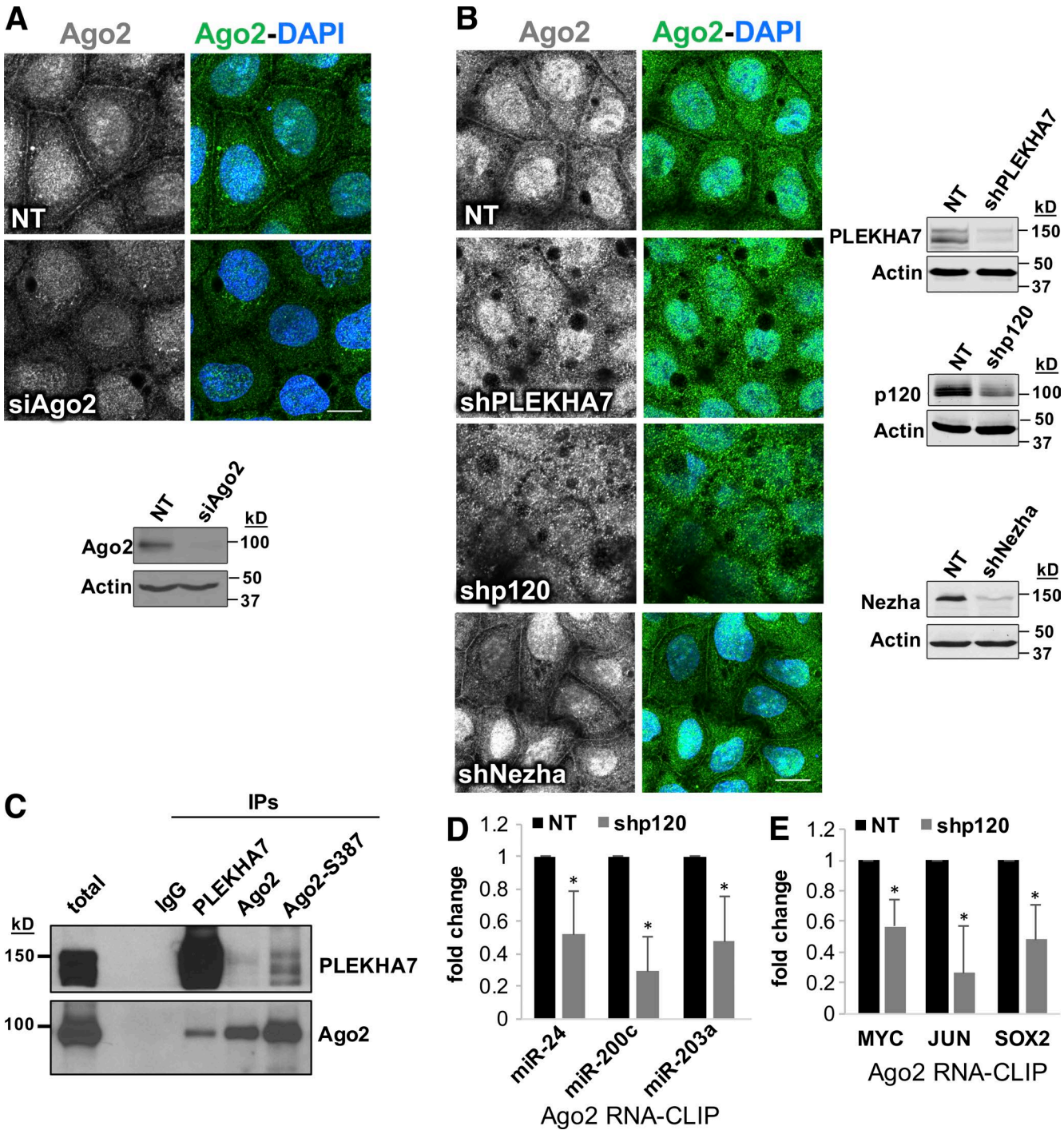

Figure S2. **Cadherin complexes regulate RISC at the junctions.** (A) Caco2 control (nontarget; NT) and Ago2 siRNA-mediated Ago2 knockdown cells (siAgo2) were subjected to immunofluorescence for the phosphorylated Ago2-S387 site. DAPI is the nuclear stain. Western blot confirming the Ago2 knockdown is shown at right; Actin is the loading control. Molecular masses (kD) are indicated on the right. (B) Caco2 control (NT) and shRNA-mediated knockdown cells for PLEKHA7 (shPLEKHA7), p120 (shp120), and Nezha (shNezha) were subjected to immunofluorescence for the phosphorylated Ago2-S387 site. DAPI is the nuclear stain. Western blots confirming all the related knockdowns are shown at right; Actin is the loading control. Molecular masses (kD) are indicated on the right. (C) Western blot of PLEKHA7, Ago2, and Ago2-S387 IPs of Caco2 cells for PLEKHA7 and Ago2. IgG is the negative IP control. Molecular masses (kD) are indicated on the left. (D and E) qRT-PCR for the indicated miRNAs and mRNAs after Ago2 RNA-CLIP of p120 knockdown (shp120) or control (NT) Caco2 cells (mean ± SD from *n* = three independent experiments; *, $P < 0.05$, Student's two-tailed *t* test). Bars, 20 µM.

Table S1. **Pathway analysis of PLEKHA7 immunoprecipitated hits, identified by mass spectrometry (top networks)**

ID	Associated network functions	Score
1	RNA posttranscriptional modification, cancer, organismal injury and abnormalities	53
2	Cellular assembly and organization, cell-to-cell signaling and interaction, reproductive system development and function	53
3	Developmental disorder, skeletal and muscular disorders, cell-to-cell signaling and interaction	53
4	Cellular assembly and organization, cellular function and maintenance, cellular movement	45
5	Protein synthesis, gene expression, RNA posttranscriptional modification	43

Provided online are Tables S2–S4 in Excel. Table S2 includes the full list of the mRNAs that were identified by the PLEKHA7 RNA-CLIP. Table S3 includes the full TLDA miRNA qPCR array data after PLEKHA7 RNA-CLIP. Table S4 shows all the validated interactions between the PLEKHA7-associated miRNAs and mRNAs, mined from MirTarBase.