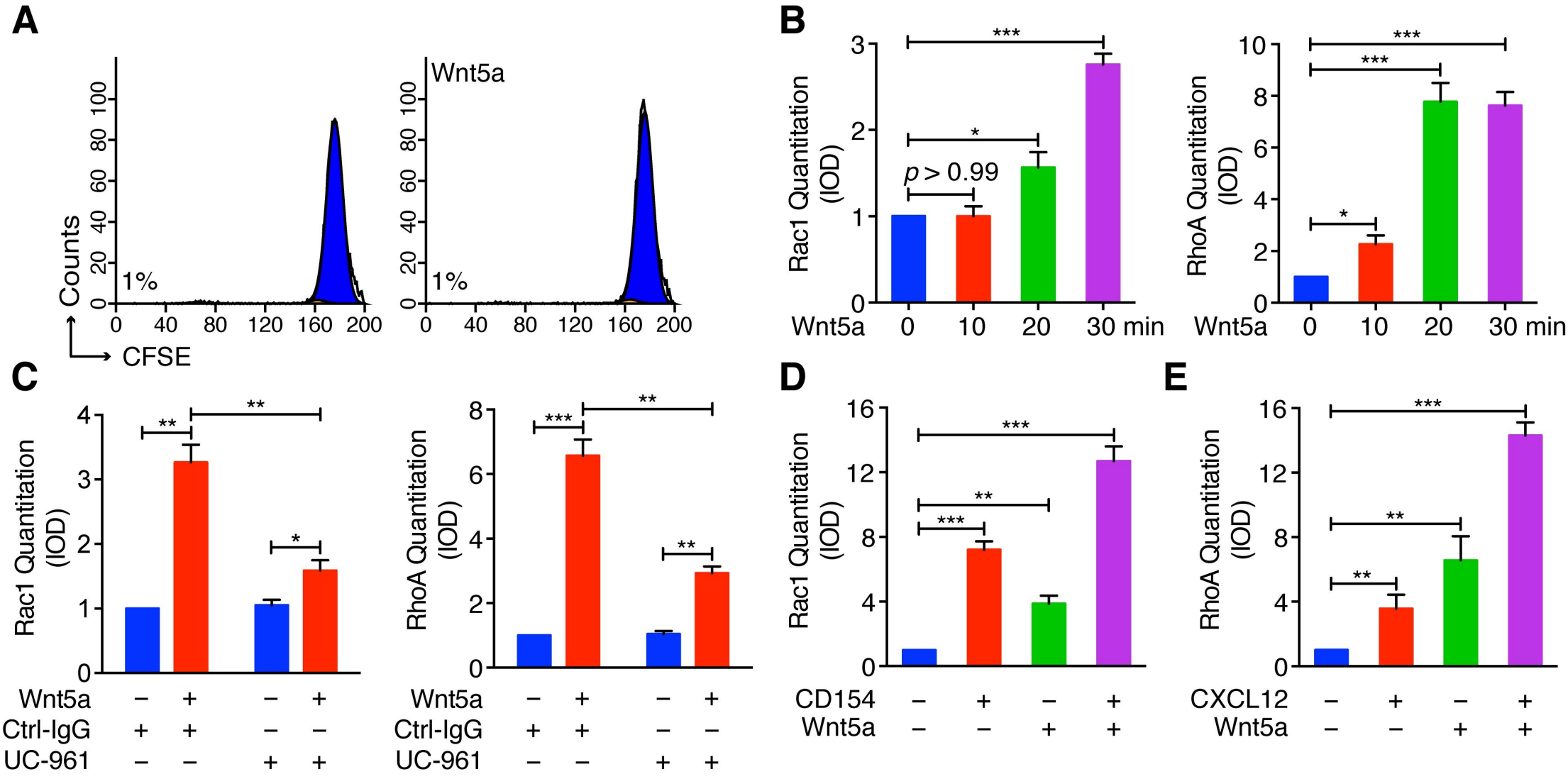
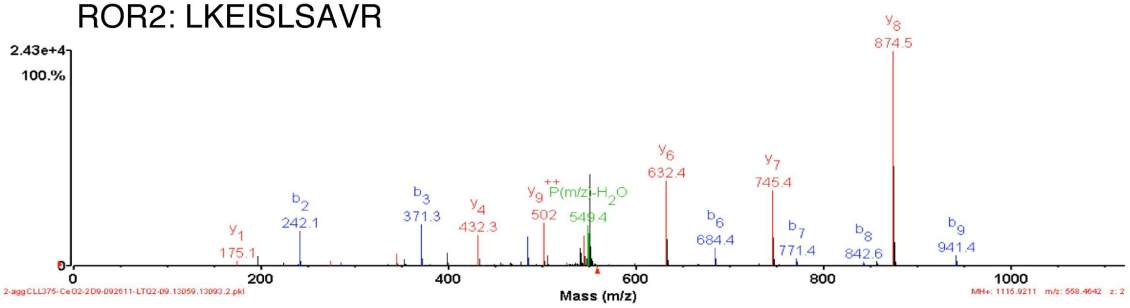

A
Counts
CFSE
1%
Wnt5a
B
Rac1 Quantitation (IOD)
RhoA Quantitation (IOD)
p > 0.99
Wnt5a 0 10 20 30 min
C
Rac1 Quantitation (IOD)
RhoA Quantitation (IOD)
Wnt5a
Ctrl-IgG
UC-961
D
Rac1 Quantitation (IOD)
CD154
Wnt5a
E
RhoA Quantitation (IOD)
CXCL12
Wnt5a

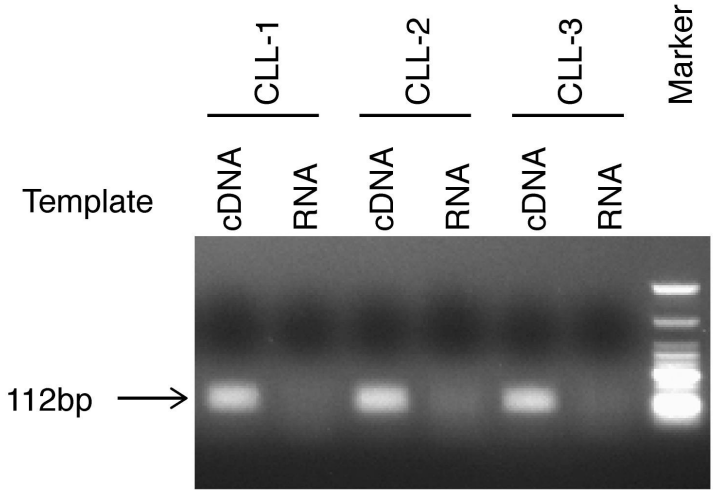
Supplemental Figure 1. **CFSE assay for CLL proliferation**. **(A)** CFSE assay for CLL proliferation induced by Wnt5a without CD154. Fluorescence of CFSE-labeled CLL cells (n=6) co-cultured for 6 days with HeLa cells without (left panel) or with (right panel) exogenous Wnt5a in the presence of IL-4/10. The results of assays on one representative CLL sample are shown with the percent of dividing cells indicated in the lower left of each panel. **(B)** Rac1 or RhoA activation was measured in serum starved CLL cells, which were treated with Wnt5a for the times indicated above each lane. Mean Rac1 or RhoA activation observed in three independent experiments is shown (n=3 per group). **(C)** Wnt5a-induced activation of Rac1 or RhoA in CLL cells treated with Ctrl-IgG or UC-961. Mean Rac1 or RhoA activation observed in three independent experiments is shown (n=3 per group). **(D)** Activated Rac1 in serum-starved CLL cells treated with or without Wnt5a and/or CD154 for 30 min. Mean Rac1 activation observed in three independent experiments is shown (n=3 per group). **(E)** Activated RhoA in serum-starved CLL cells treated with or without Wnt5a and/or CXCL12 for 30 min. Mean RhoA activation observed in three independent experiments is shown (n=3 per group). Data are shown as mean ± SEM; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, as calculated using the Student t test.

A

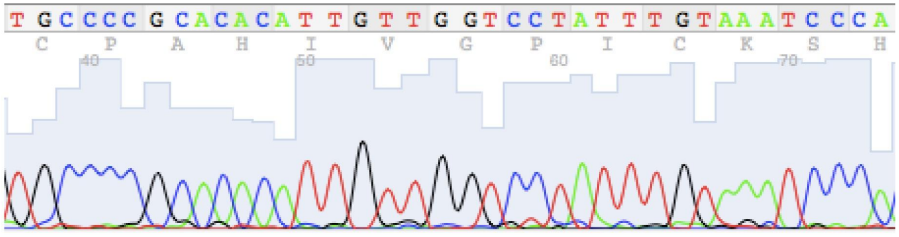


B

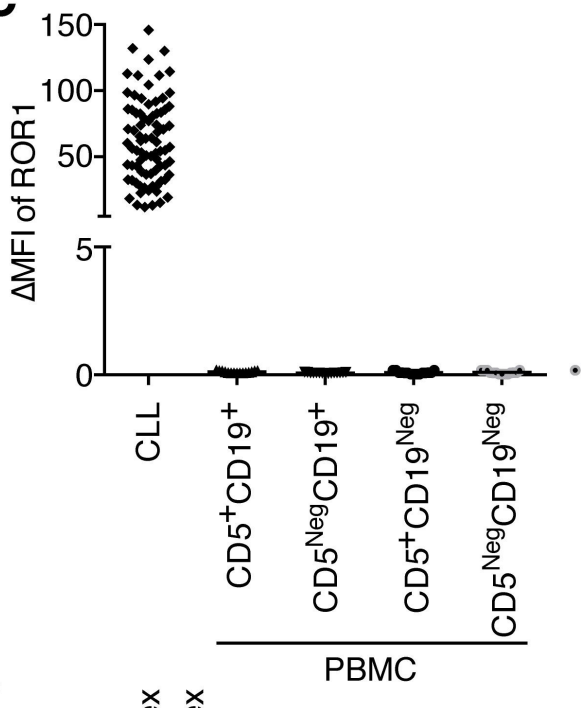
Primer	Sequence
Forward	5'-ACGTGCACATGAGGTCCATT-3'
Reverse	5'-CACCGGGTGTGGGATTTACA-3'



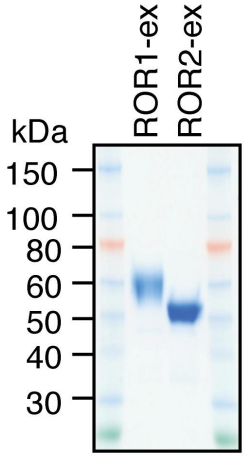
Sequencing



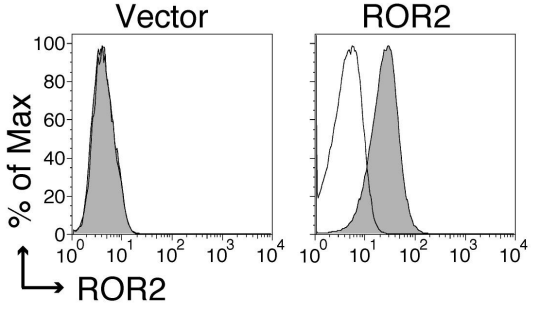
C



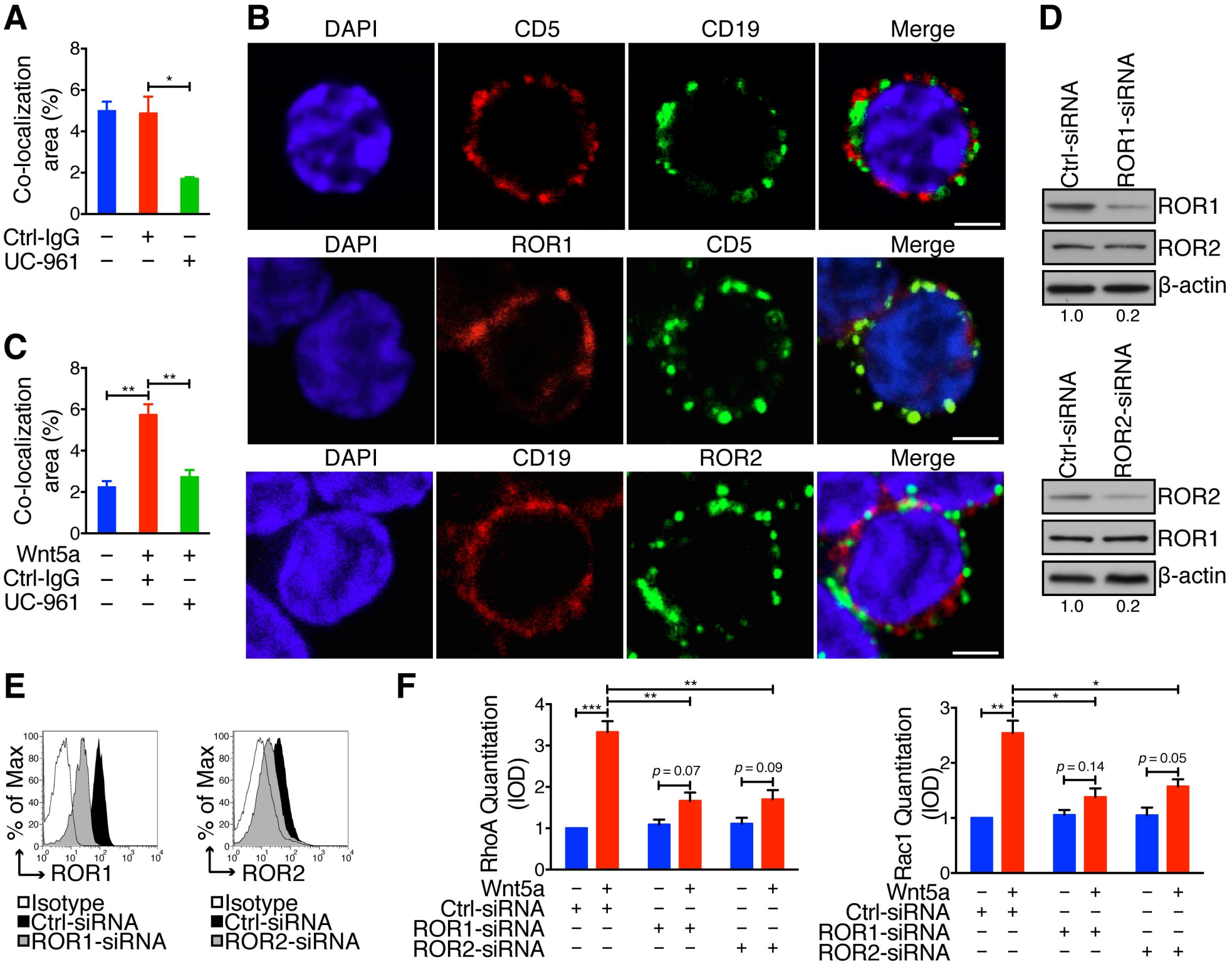
D



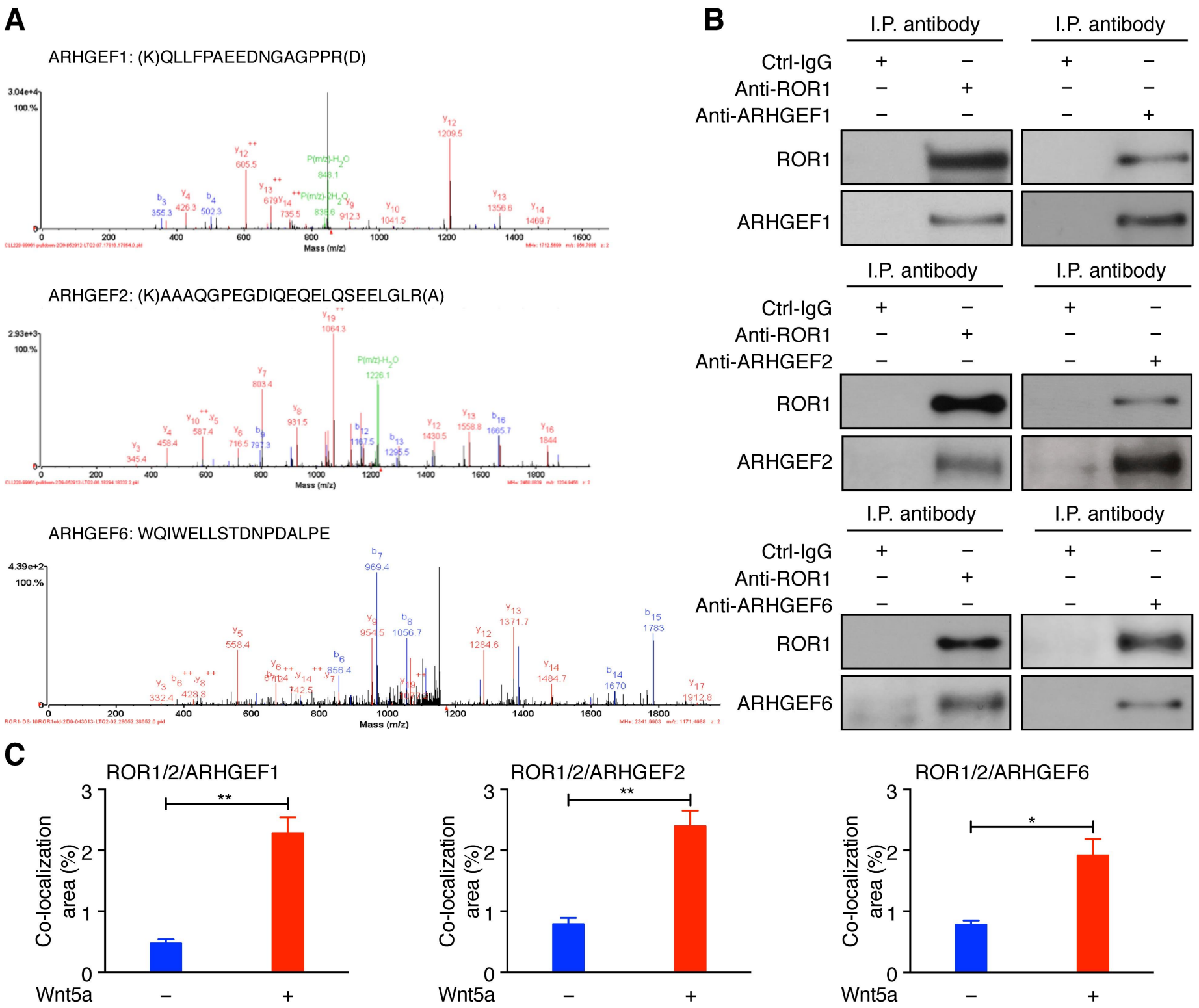
E



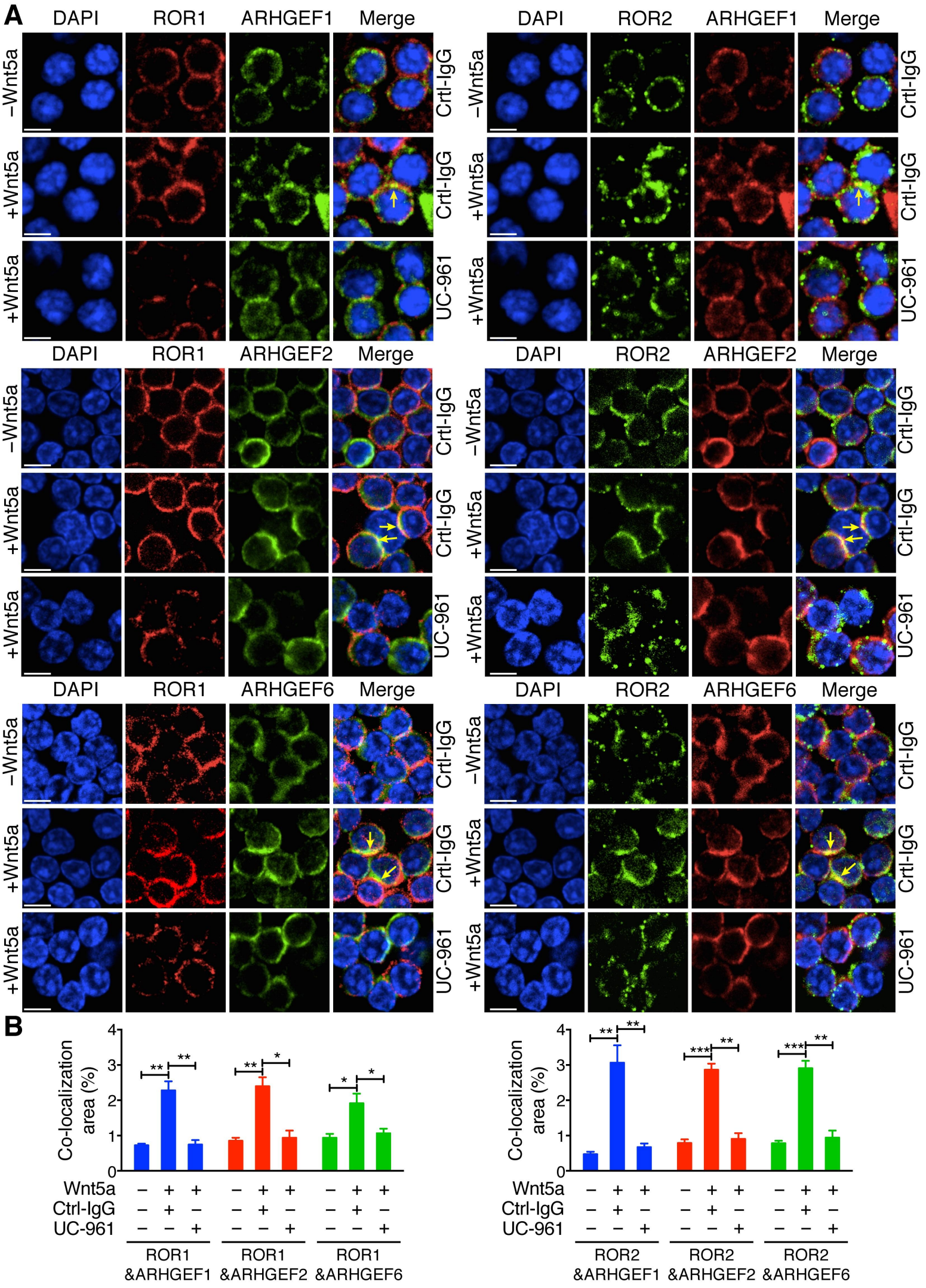
Supplemental Figure 2. **ROR2 expression in CLL cells. (A)** Mass spectrum showing unique peptide of ROR2 identified by 2D-nanoLC-MS/MS from the protein lysates prepared from CLL cells following ip with anti-ROR1 mAb *versus* Ctrl-IgG. **(B)** Total RNA was extracted from each of 3 CLL patient samples. ROR2 cDNA was amplified by RT-PCR and validated by DNA sequence analysis. Total RNA was used as PCR template to control for genomic DNA contamination. **(C)** The ΔMFI for ROR1 of CLL samples (n=80) or each of the gated lymphocyte-subsets in PBMC of healthy adults (n=15), as indicated at the bottom. **(D)** ROR1-ex and ROR2-ex proteins were purified and analyzed by SDS-PAGE and Coomassie staining. **(E)** Chinese hamster ovary (CHO) cells were transfected with pcDNA3.1 (Vector, left panel) or pcDNA3.1-encoding *ROR2* (ROR2, right panel), stained with a Alexa-488-labeled isotype control mAb (open histograms) or an anti-ROR2-Alexa-488 mAb, and then analyzed by flow cytometry.


A
Co-localization area (%)
Ctrl-IgG
UC-961
B
DAPI
CD5
CD19
Merge
ROR1
ROR2
C
Wnt5a
D
Ctrl-siRNA
ROR1-siRNA
ROR2-siRNA
β-actin
1.0
0.2
E
% of Max
Isotype
F
RhoA Quantitation (IOD)
Rac1 Quantitation (IOD)
p = 0.07
p = 0.09
p = 0.14
p = 0.05

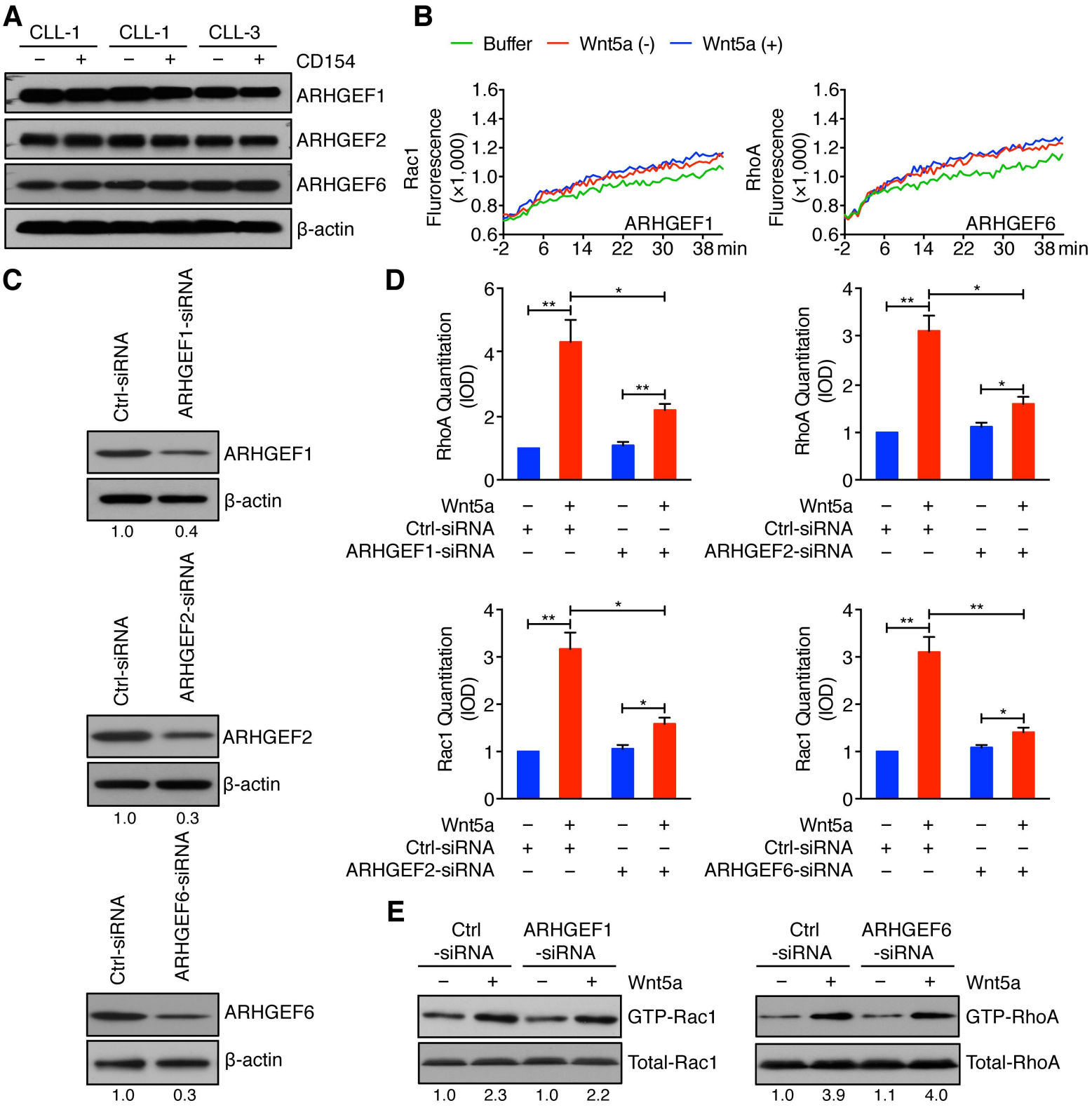
Supplemental Figure 3. **ROR1 and ROR2 conjoin in response to Wnt5a. (A)** Percentage of co-localization area of ROR1 and ROR2 in freshly isolated CLL cells +/− Ctrl-IgG or UC-961 (n=3). **(B)** Negative control for co-localization analysis by confocal microscopy. CLL cells were stained with anti-CD5-Alexa-647 and anti-CD19-Alexa-488; anti-ROR1-Alexa-647 and anti-CD19-Alexa-488; or anti-CD5-Alexa-647 and anti-ROR2-Alexa-488 and then evaluated by confocal microscopy (Objective: ×100; scale bar: 2 μm). There was no co-localization of CD5 (Red) with CD19 (Green), ROR1 (Red) with CD19 (Green), or CD5 (Red) with ROR2 (Green). **(C)** Percentage of co-localization area of ROR1 and ROR2 in serum-starved CLL cells after treatment with (+) or without (-) Wnt5a and Ctrl-IgG or UC-961 (n=3). **(D)** CLL cells were transfected with non-specific control siRNA (Ctrl-siRNA), or siRNA specific for *ROR1* (ROR1-siRNA) or *ROR2* (ROR2-siRNA), as indicated at the top of each lane. The cells were examined 48 h later for ROR1 or ROR2 via immunoblot analysis. The numbers beneath each lane is the ratio of band densities for ROR1 or ROR2 versus β-actin normalized with respect to that of Ctrl-siRNA treated samples. **(E)** Flow cytometry of CLL cells transfected with Ctrl-siRNA (black histogram) or either ROR1-siRNA or ROR2-siRNA (grey histograms) and then stained with anti-CD5-Alexa-647 (left panel) or anti-ROR2-Alexa-488 (right panel). The open histograms are representative of the fluorescence of control or transfected CLL cells stained with an Alexa-488-labeled isotype Ctrl-IgG (Isotype). **(F)** Activated RhoA or Rac1 was measured by Rho-family protein activity pull-down assays on lysates of CLL cells transfected with Ctrl-siRNA or siRNA specific for *ROR1* or *ROR2* and cultured with or without Wnt5a. Mean activated RhoA or Rac1 observed in three independent experiments is shown (n=3 per group). Data are presented as mean ± SEM; **P* < 0.05; ***P* < 0.01; ****P* < 0.001, as calculated using the Student t test.


A
ARHGEF1: (K)QLLFPAEEDNGAGPPR(D)
ARHGEF2: (K)AAAQGPEGDIQEQELQSEELGLR(A)
ARHGEF6: WQIWELLSTDNPDALPE
Mass (m/z)
B
I.P. antibody
Ctrl-IgG
Anti-ROR1
Anti-ARHGEF1
Anti-ARHGEF2
Anti-ARHGEF6
ROR1
ARHGEF1
ARHGEF2
ARHGEF6
C
ROR1/2/ARHGEF1
ROR1/2/ARHGEF2
ROR1/2/ARHGEF6
Co-localization area (%)
Wnt5a
**
*

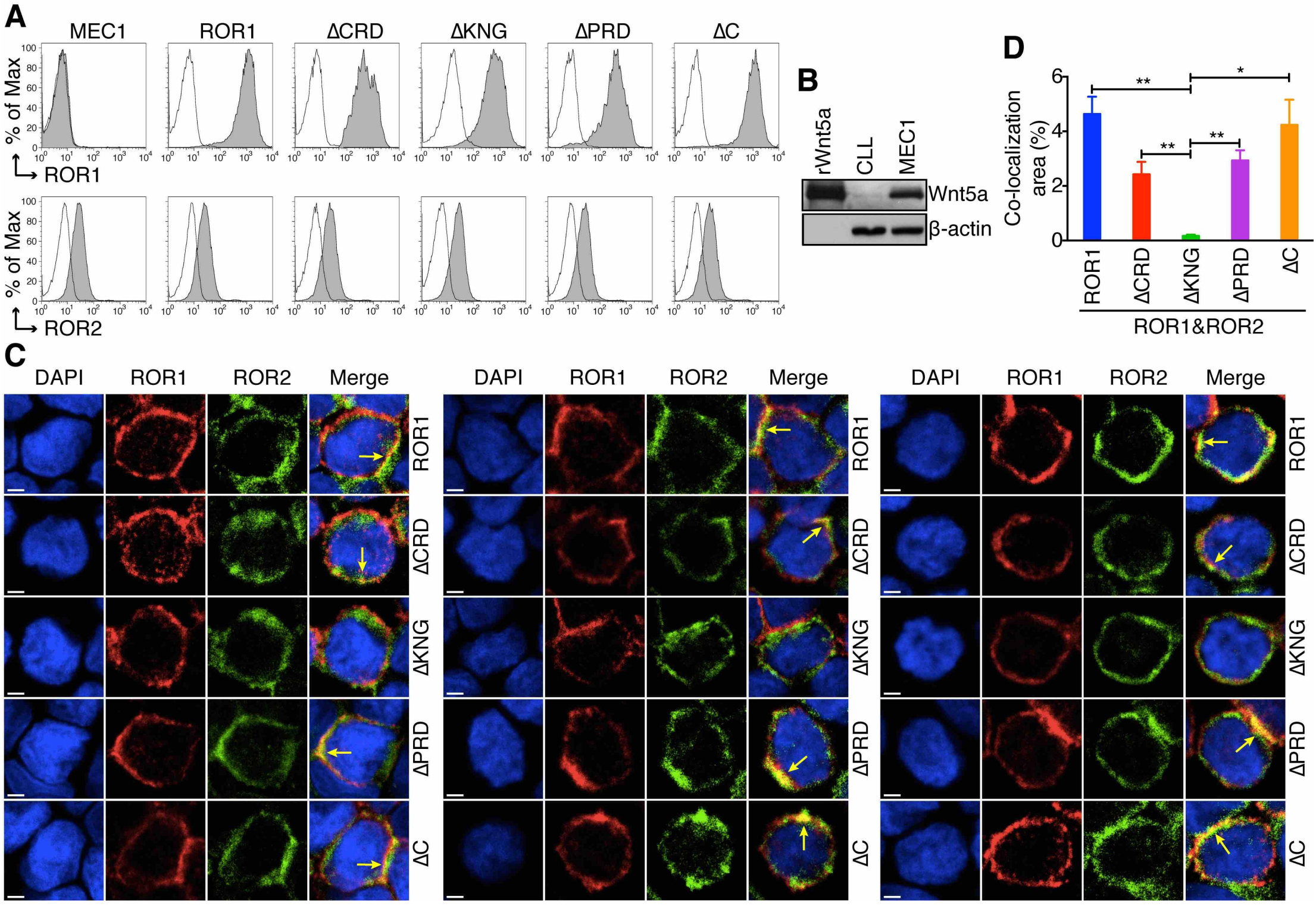
Supplemental Figure 4. **ROR1/ROR2 complex Recruits ARHGEF1, ARHGEF2, or ARHGEF6. (A)** Mass spectra showing unique peptides of ARHGEF1, ARHGEF2, or ARHGEF6 identified by 2D-nanoLC-MS/MS from the protein lysates prepared from CLL cells following ip with anti-ROR1 mAb *versus* Ctrl-IgG. **(B)** Immunoblot analysis of ip generated using lysates of freshly isolated CLL cells with a mAb specific for ROR1, ARHGEF1, ARHGEF2, or ARHGEF6, as indicated above each lane. The immunoblots were probed with antibodies specific for ROR1, ARHGEF1, ARHGEF2, or ARHGEF6, as indicated on the left margin of each subpanel. **(C)** Percentage of co-localization area of ARHGEF1, ARHGEF2, or ARHGEF6 with ROR1 and ROR2 in CLL cells cultured without (−) or with (+) Wnt5a (n=3). Data are shown as mean ± SEM; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, as calculated using the Student t test.


A
DAPI
ROR1
ARHGEF1
Merge
ROR2
ARHGEF2
ARHGEF6
–Wnt5a
+Wnt5a
Crtl-IgG
UC-961
B
Co-localization area (%)
Wnt5a
Ctrl-IgG
UC-961
ROR1 &ARHGEF1
ROR1 &ARHGEF2
ROR1 &ARHGEF6
ROR2 &ARHGEF1
ROR2 &ARHGEF2
ROR2 &ARHGEF6

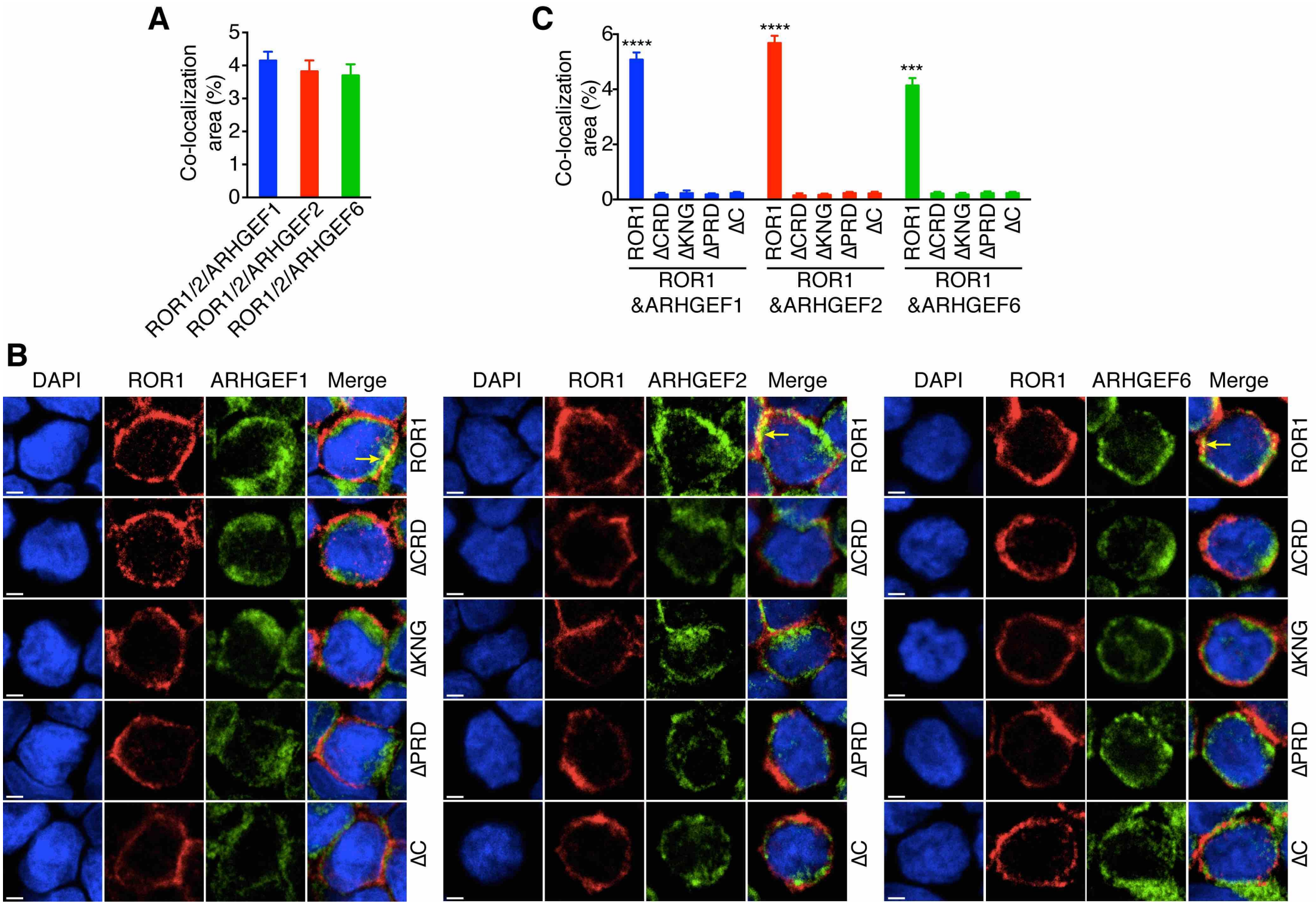
Supplemental Figure 5. **UC-961 inhibits co-localization of ARHGEF1, ARHGEF2, or ARHGEF6 with ROR1 or ROR2. (A)** Co-localization (Yellow, with arrow) of ROR1 or ROR2 with ARHGEF1, ARHGEF2, or ARHGEF6 detected by confocal microscopy in serum-starved CLL cells +/− Wnt5a (as indicated on the left margin) and Ctrl-IgG or UC-961 (Objective: ×100; scale bar: 5 μm). **(B)** Percentage of co-localization area of ROR1 or ROR2 with ARHGEF1, ARHGEF2, or ARHGEF6 detected by confocal microscopy in serum-starved CLL cells +/− Wnt5a and Ctrl-IgG or UC-961 (n=3). Data are shown as mean ± SEM; **P* < 0.05; ***P* < 0.01; ****P* < 0.001, as calculated using the Student t test.


A
CLL-1
CLL-1
CLL-3
CD154
ARHGEF1
ARHGEF2
ARHGEF6
β-actin
B
Buffer
Wnt5a (-)
Wnt5a (+)
Rac1 Flurorescence (×1,000)
RhoA Flurorescence (×1,000)
ARHGEF1
ARHGEF6
min
C
Ctrl-siRNA
ARHGEF1-siRNA
ARHGEF2-siRNA
ARHGEF6-siRNA
1.0
0.4
0.3
D
RhoA Quantitation (IOD)
Rac1 Quantitation (IOD)
Wnt5a
Ctrl-siRNA
ARHGEF1-siRNA
ARHGEF2-siRNA
ARHGEF6-siRNA
E
Ctrl -siRNA
ARHGEF1 -siRNA
ARHGEF6 -siRNA
GTP-Rac1
Total-Rac1
GTP-RhoA
Total-RhoA
1.0
2.3
1.0
2.2
1.0
3.9
1.1
4.0

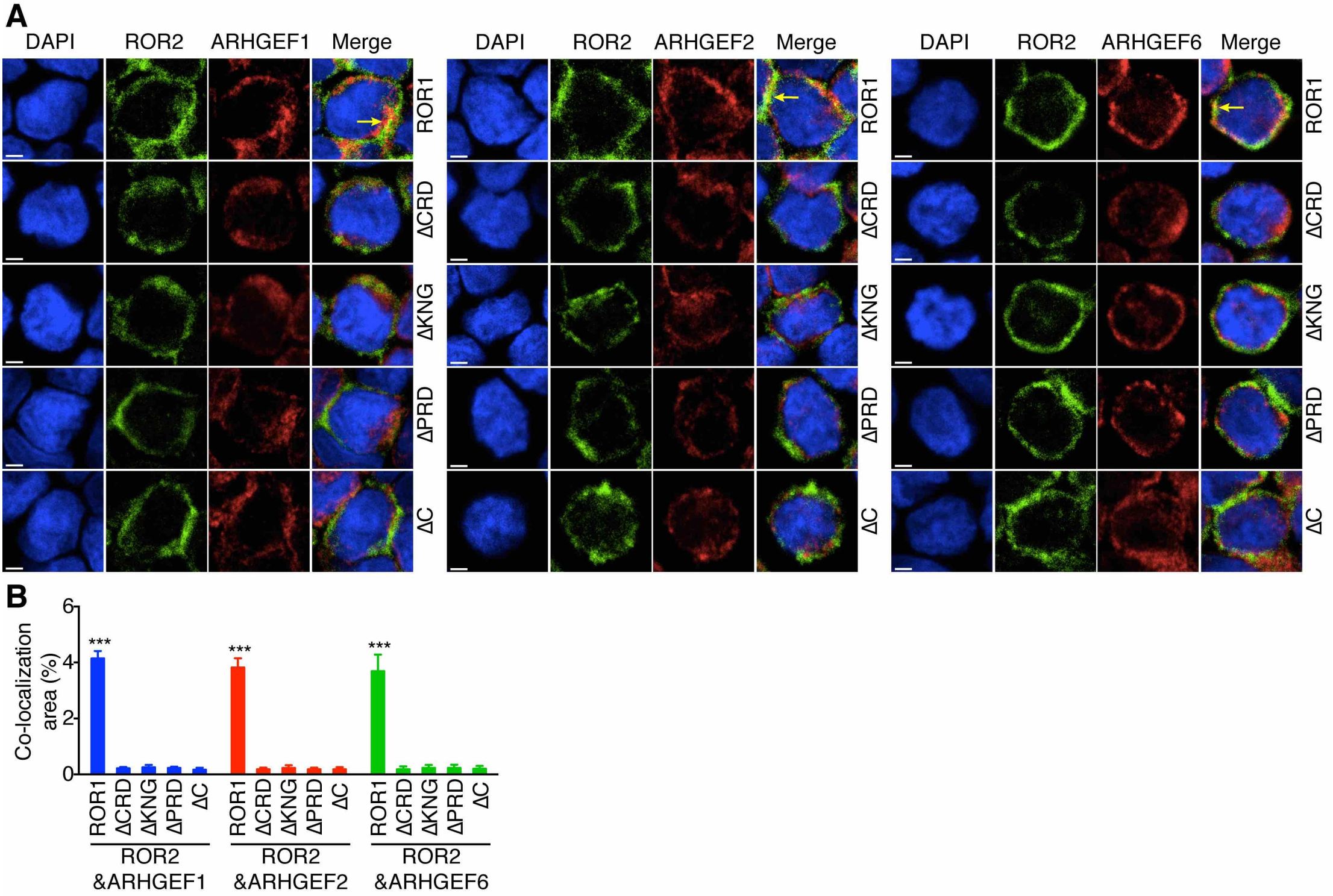
Supplemental Figure 6. Activation of **Rac1 or RhoA by ARHGEF1, ARHGEF2, or ARHGEF6. (A)** Expression of ARHGEF1, ARHGEF2, or ARHGEF6 in CLL cells treated with or without CD154 for 2 days (n=3). **(B)** *In vitro* exchange over time (in minutes) of Rac1 (left panel) or RhoA (right panel) in ip of mAbs specific for ARHGEF1 (left subpanel) or ARHGEF6 (right subpanel) using lysates of CLL cells cultured with (blue line) or without (red line) Wnt5a, as indicated in the bottom right of each graph. The green line depicts GTPase-activation using buffer alone. **(C)** CLL cells were transiently transfected with non-specific Ctrl-siRNA or siRNA specific for *ARHGEF1* (ARHGEF1-siRNA), *ARHGEF2* (ARHGEF2-siRNA), or *ARHGEF6* (ARHGEF6-siRNA), as indicated at the top of each lane. The cells were examined 48 h later for expression of ARHGEF1, ARHGEF2, or ARHGEF6 by immunoblot analysis. The numbers beneath each lane is the ratio of band densities for each GEF versus β-actin normalized with respect to Ctrl-siRNA samples. **(D)** Activation of RhoA or Rac1 following treatment without (−) or with (+) Wnt5a of CLL cells transfected with Ctrl-siRNA or siRNA specific for *ARHGEF1*, *ARHGEF2*, or *ARHGEF6*. Mean activated RhoA or Rac1 observed in three independent experiments is shown (n=3 per group). Data are shown as mean ± SEM; $*P < 0.05$; $**P < 0.01$. **(E)** Activation of Rac1 or RhoA in CLL cells transfected with Ctrl-siRNA, ARHGEF1-siRNA, or ARHGEF6-siRNA following treatment without (−) or with (+) Wnt5a, as indicated at the top of the lanes. Whole-cell lysates also were examined via immunoblot analysis for total Rac1 or RhoA. The number beneath each lane is the ratios of band densities for activated versus total GTPase normalized to that of untreated, Ctrl-siRNA-transfected samples.


A
MEC1
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC
% of Max
ROR1
ROR2
B
rWnt5a
CLL
MEC1
Wnt5a
β-actin
D
Co-localization area (%)
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC
ROR1&ROR2
C
DAPI
ROR1
ROR2
Merge

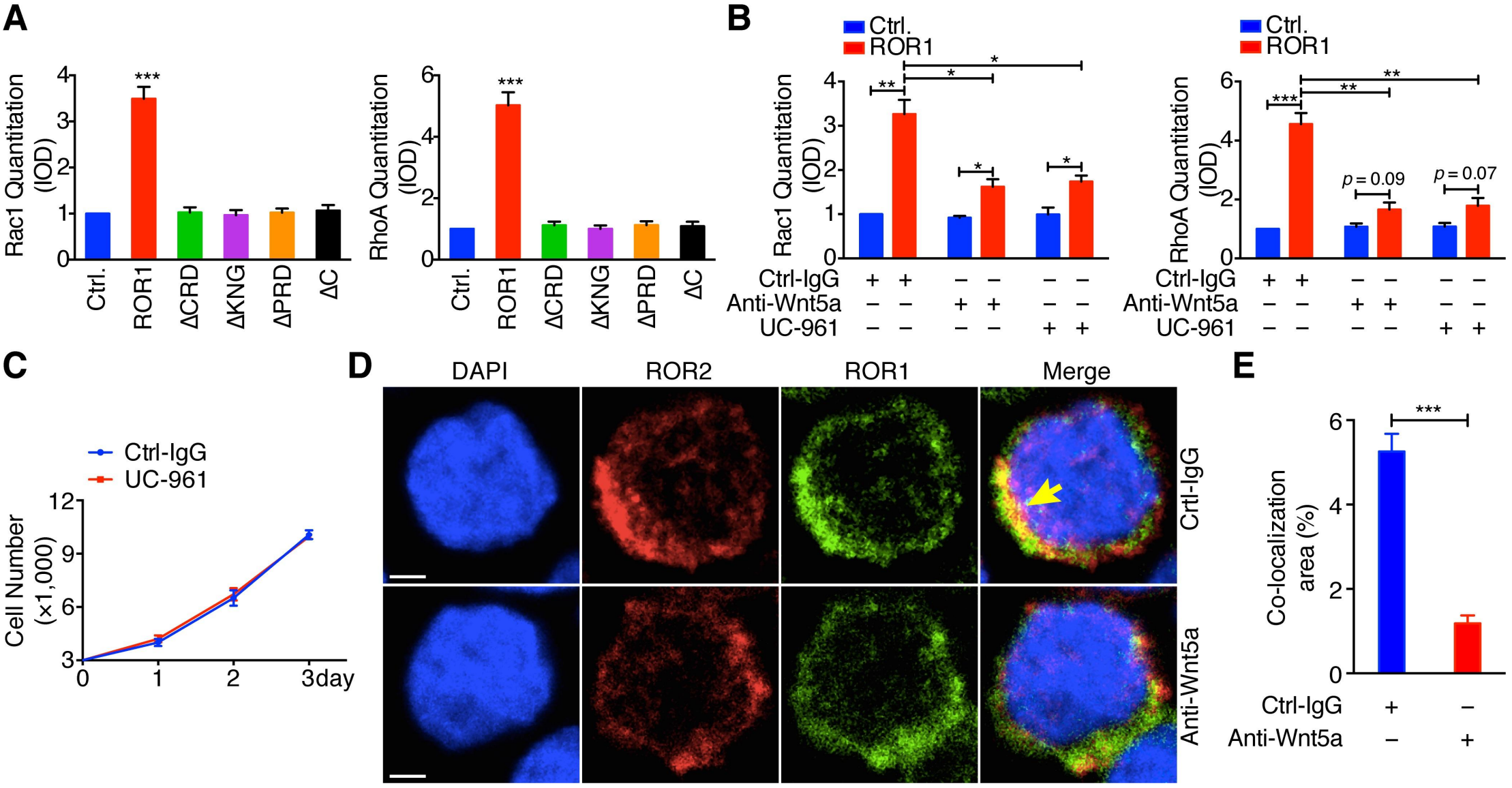
Supplemental Figure 7. ROR1 and ROR2 conjoin in MEC1-ROR1 cells. **(A)** Fluorescence of MEC1 cells, or MEC1 cells transfected with vectors encoding *ROR1* or truncated forms of *ROR1*, stained with a fluorochrome-labeled isotype control mAb (open histogram) or 4A5-Alexa-647 (top row) or anti-ROR2-Alexa-488 (bottom row) (shaded histograms). **(B)** Expression of Wnt5a was determined by immunoblot analysis of lysates from primary CLL cells or MEC1 cells. Recombinant Wnt5a (100 ng) was used as a positive control. Duplicate samples were run on parallel gels to detect β-actin. **(C)** Co-localization (Yellow, with arrow) of ROR1 (Red) and ROR2 (Green) in MEC1 cells transfected with each of the various ROR1 constructs (Objective: ×100; scale bar: 2 μm). **(D)** Percentage of co-localization area of ROR1 and ROR2 in MEC1 cells transfected with each of the various ROR1 constructs (n=3). Data are shown as the mean ± SEM; **P* < 0.05; ***P* < 0.01, as calculated using the Student t test.


A
Co-localization area (%)
0
1
2
3
4
5
ROR1/2/ARHGEF1
ROR1/2/ARHGEF2
ROR1/2/ARHGEF6
C
Co-localization area (%)
0
2
4
6
****
****
***
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC
ROR1 &ARHGEF1
ROR1 &ARHGEF2
ROR1 &ARHGEF6
B
DAPI
ROR1
ARHGEF1
Merge
DAPI
ROR1
ARHGEF2
Merge
DAPI
ROR1
ARHGEF6
Merge
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC

Supplemental Figure 8. Co-localization of ROR1 and GEFs in MEC1-ROR1 cells. (A) Percentage of co-localization area of ARHGEF1, ARHGEF2 or ARHGEF6 with ROR1 and ROR2 in MEC1-ROR1 cells (n=3). **(B)** Co-localization (Yellow, with arrow) of ROR1 (Red) and ARHGEF1, ARHGEF2, or ARHGEF6 (Green) in MEC1 cells transfected with each of the various ROR1 constructs (Objective: ×100; scale bar: 2 μm). (**C**) Percentage of co-localization area of ROR1 and ARHGEF1, ARHGEF2, or ARHGEF6 in MEC1 cells transfected with each of the various ROR1 constructs (n=3). Data are shown as mean ± SEM; ***$P < 0.001$; ****$P < 0.0001$, as calculated using the Student t test.


A
DAPI
ROR2
ARHGEF1
Merge
DAPI
ROR2
ARHGEF2
Merge
DAPI
ROR2
ARHGEF6
Merge
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC
B
Co-localization area (%)
0
2
4
6
***
***
***
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC
ROR2 &ARHGEF1
ROR2 &ARHGEF2
ROR2 &ARHGEF6

Supplemental Figure 9. Co-localization of ROR2 and GEFs in MEC1-ROR1 cells. **(A)** Percentage of co-localization area of ROR2 and ARHGEF1, ARHGEF2, or ARHGEF6 in MEC1 cells transfected with each of the various ROR1 constructs (n=3). (**B**) Percentage of co-localization area of ROR1 and ARHGEF1, ARHGEF2, or ARHGEF6 in MEC1 cells transfected with each of the various ROR1 constructs (n=3). Data are shown as the mean ± SEM; ***$P < 0.001$, as calculated using the Student t test.


A
Rac1 Quantitation (IOD)
RhoA Quantitation (IOD)
Ctrl.
ROR1
ΔCRD
ΔKNG
ΔPRD
ΔC
B
Ctrl.
ROR1
Rac1 Quantitation (IOD)
RhoA Quantitation (IOD)
Ctrl-IgG
Anti-Wnt5a
UC-961
p = 0.09
p = 0.07
C
Ctrl-IgG
UC-961
Cell Number (×1,000)
3day
D
DAPI
ROR2
ROR1
Merge
Ctrl-IgG
Anti-Wnt5a
E
Co-localization area (%)
Ctrl-IgG
Anti-Wnt5a

Supplemental Figure 10. Inhibition of RhoA and Rac1 activation by UC-961 or anti-Wnt5a in MEC1-ROR1 cells. **(A)** Activated Rac1 or RhoA were measured in MEC1 (Ctrl.), MEC1-ROR1, or MEC1 cells expressing each of the truncated forms of ROR1 (n=3). **(B)** Inhibition of Rac1 or RhoA activation by UC-961 or anti-Wnt5a in MEC1 or MEC1-ROR1 cells (n=3). **(C)** The mean numbers of MEC1 cells cultured with Ctrl-IgG (blue circles) or UC-961 (red squares) in triplicate wells at the times indicated (n=3). **(D)** Co-localization (Yellow, with arrow) of ROR1 and ROR2 detected by confocal microscopy in MEC1-ROR1 cells treated with Ctrl-IgG or anti-Wnt5a (Objective: ×100; scale bar: 2 μm). **(E)** Percentage of co-localization area of ROR1 and ROR2 in MEC1-ROR1 cells treated with Ctrl-IgG or anti-Wnt5a (n=3). Data are shown as the mean ± SEM; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, as calculated using the Student t test.