Supplemental Materials

Molecular Biology of the Cell

Tuli et al.

Supplementary Figures

Figure S1: Arl8b co-localizes with perforin in YT-Indy cells. Serial *z*-sections were obtained every 0.2 µm, and the crosshairs depict common membrane structures on a representative micrograph containing both Arl8b (green) and Pfn (red). Nucleus was stained with DAPI (blue). Scale bar 10 µm.

Figure S2: Silencing of Arl8b and Rab27a in NK cells. A) Primary NK cells were electroporated with control- or Arl8b- siRNA. After 72 hr, a qRT-PCR analysis was performed using Arl8b and Arl8a specific primers. **B)** Silencing of Rab27a in YT-Indy cells. Stable silencing of Rab27a was achieved in YT-Indy cells by introducing lentiviral vector-driven shRNA against Rab27a and qRT-PCR was performed to check the efficiency of Rab27a silencing.

Figure S3: Arl8a silencing inhibits NK cytotoxicity but to a lesser extent compared to Arl8b. A) qRT-PCR analyses of Arl8b and Arl8a levels in control shRNA-, Arl8a 497 shRNA-, and Arl8a 1685 shRNA-expressing YT-Indy cells. **B)** YT-Indy cells stably expressing control shRNA, Arl8b-specific shRNA (407 and 921) or Arl8a-specific shRNA (497 and 1685) were tested against 721.221 target cells by ^{51}Cr-release assay at various E:T cell ratios. Data show mean ± SEM of triplicates from one representative experiment of three performed.

Figure S4: Arl8b silencing does not prevent F-actin polarization, and Arl8a and Rab27a silencing does not affect lytic granule polarization in NK cells. A) YT-Indy cells stably transduced with control shRNA (left panel) or Arl8b shRNA (right panel) were mixed with anti-LFA1 mAb-coated polystyrene beads for 60 min at 37°C. The cells were then stained with AlexaFluor 488-conjugated phalloidin (green) to visualize F-actin. **B)** The percentage of lytic granule (marked by perforin staining) polarization in control- versus Arl8a-silenced-YT-Indy

cells. Bar graph represents mean ± SD of three independent experiments; at least 50 conjugates were evaluated in each experiment. No significant difference was observed between the groups. **C)** Confocal analyses was performed on YT-Indy cells stably transduced with control shRNA (left panel) or Rab27a-specific shRNA (right panel) and mixed with 721.221 target cells for 20 min at the E:T ratio of 2:1. Conjugates were fixed and stained using anti-perforin antibody (green) and the MTOC was marked by anti-pericentrin antibody staining (red). Scale bar, 10 µm.

Figure S5: Dynein activity is required for perinuclear clustering of lysosomes observed upon Arl8b silencing. A) HeLa cells were treated with control siRNA (left panel), Arl8b siRNA (middle panel) or Arl8b siRNA plus dynein siRNA (right panel) for 72 hr. Following siRNA treatment, cells were fixed and stained with anti-LAMP-1 antibody to visualize lysosomes by confocal microscopy. **B-C)** HeLa treated with control siRNA (B) or Arl8b siRNA (C). Post 48 hr siRNA treatment, cells were transfected with CC1-GFP (green) to inhibit dynein activity. Post 24 hr transfection, cells were fixed and stained with anti-LAMP-1 antibody (red) to visualize lysosomes by confocal microscopy.

Figure S6: List of proteins identified as Arl8b binding partners from NK cell lysates. GST-pull down assay was performed using YT-Indy cell lysates with GST-Arl8b or GST alone. Eluates were run on SDS-PAGE. Bands which appeared specifically in the GST-Arl8b lane were excised, partially digested with trypsin, and analyzed by mass spectrometry. The number of unique peptides identified corresponding to each interaction partner is listed at right. Four unique peptides corresponding to KIF5B motor protein were identified using this approach (highlighted in bold).

Figure S7: Silencing of Kif5B in HeLa and NK cells. A-D) Silencing of Arl8b or Kif5b causes perinuclear clustering of lysosomes. HeLa cells were treated with control siRNA (A), Arl8b siRNA (B) or Kif5B siRNA (C). Following 72 hrs treatment, cells were fixed, stained with anti-LAMP-1 antibody and analyzed by confocal microscopy. The silencing efficiency of Kif5B was assessed by Western blot (D). Actin blot was performed to show equal protein loading. **E)** Stable silencing of Kif5B expression was achieved in YT-Indy cells by introducing lentiviral vector-driven shRNA against Kif5B. Western blot was performed to check the efficiency of Kif5B silencing and β-actin blot was done as a control to show equal protein loading.

Figure S1

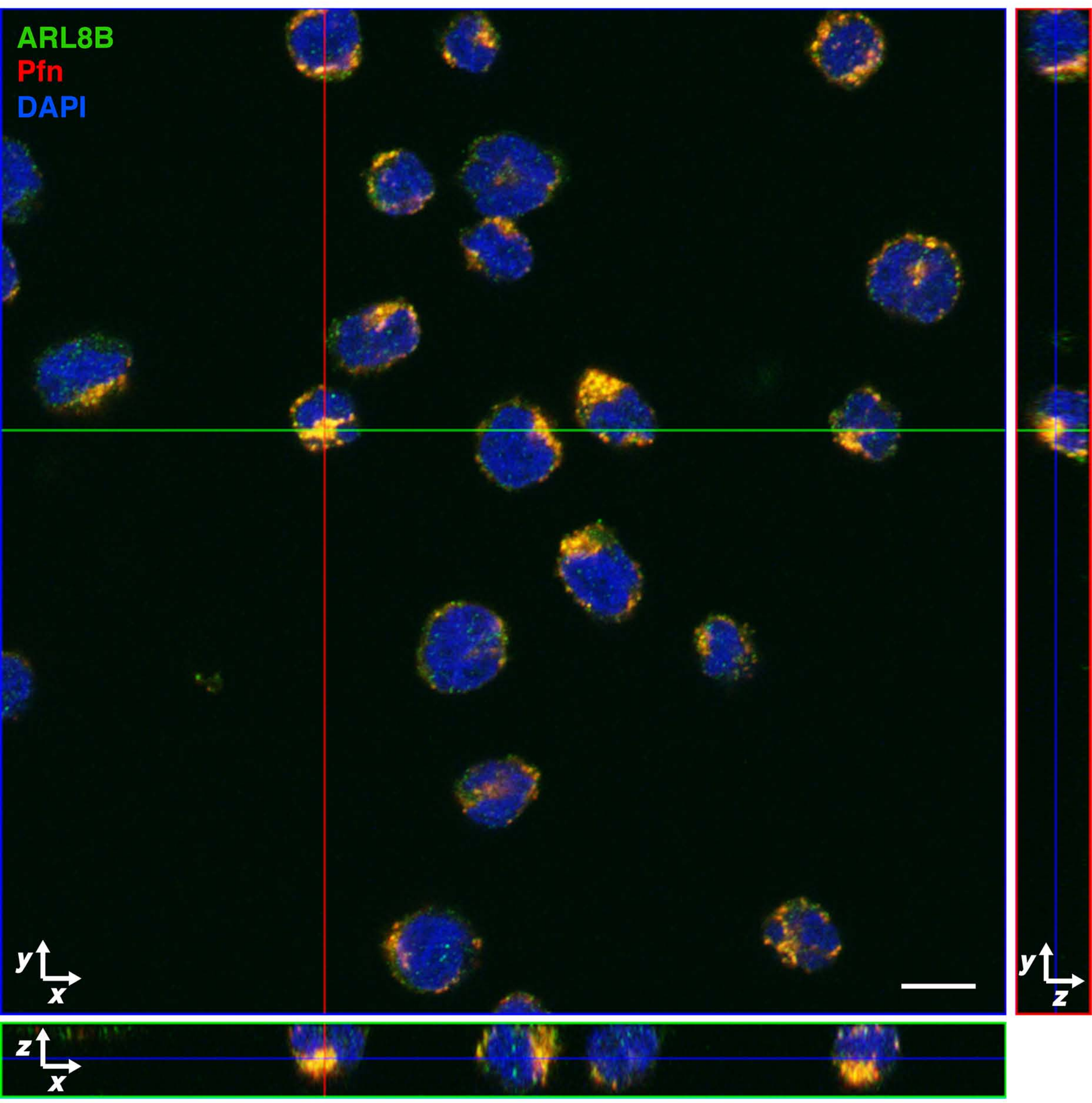
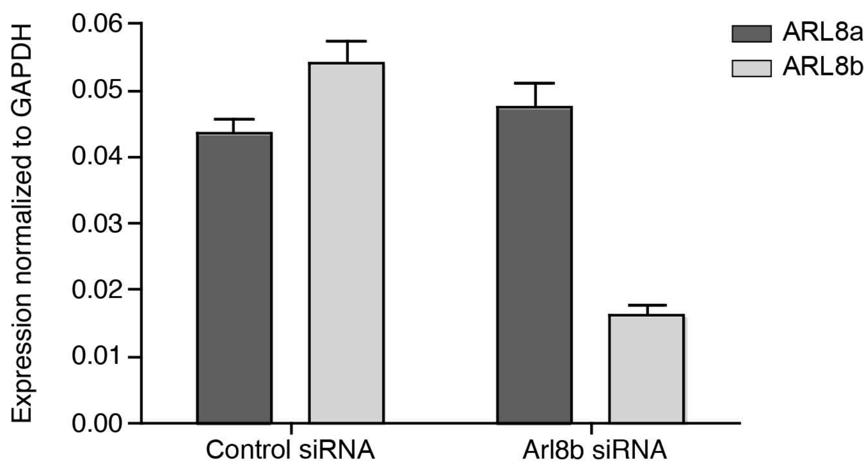


Figure S2



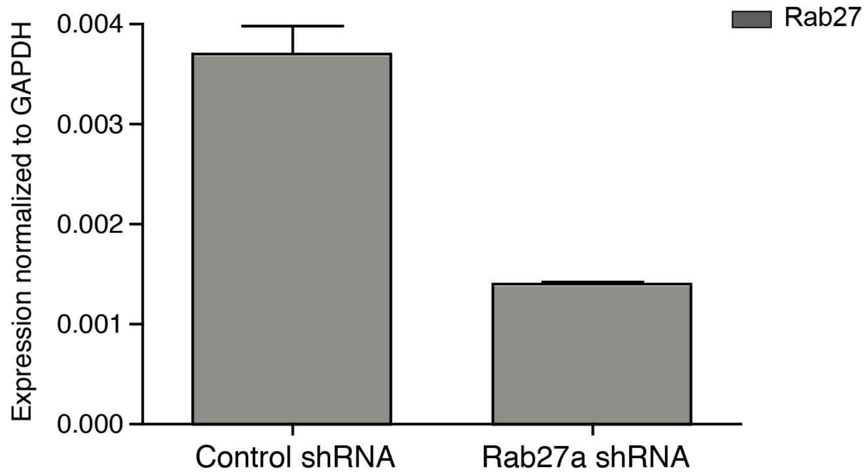
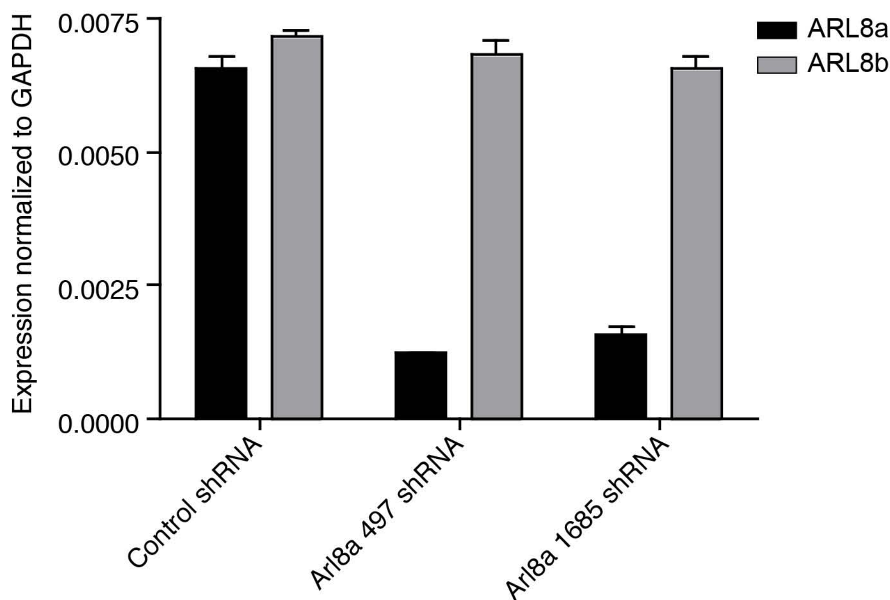


Figure S3

A



B

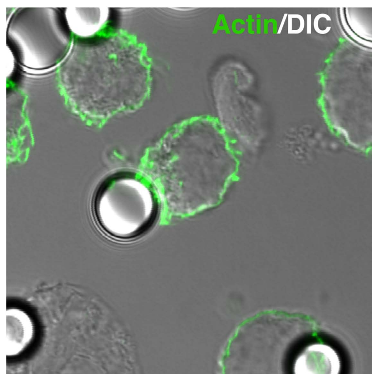
% specific lysis
100
90
80
70
60
50
40
30
20
10
0
30:1
10:1
3:1
1:1
Control shRNA
Arl8b 407 shRNA
Arl8b 921 shRNA
Arl8a 497 shRNA
Arl8a 1685 shRNA

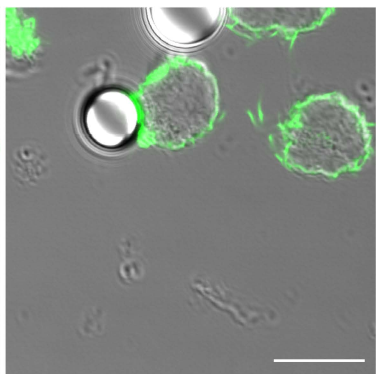
Figure S4

A

YT-Indy-Control shRNA
+ anti-CD18 coated beads

YT-Indy-Arl8b shRNA
+ anti-CD18 coated beads





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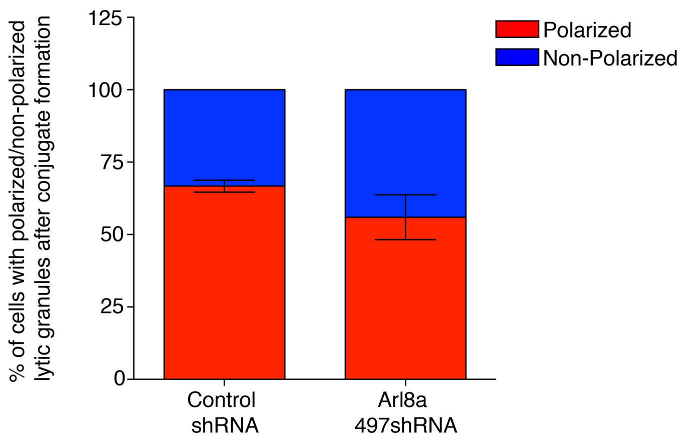

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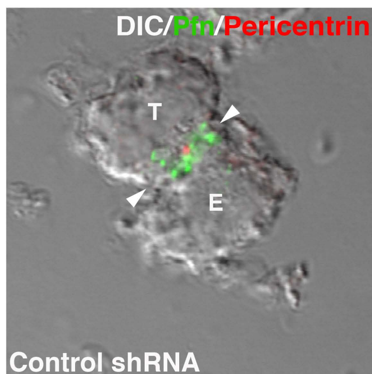

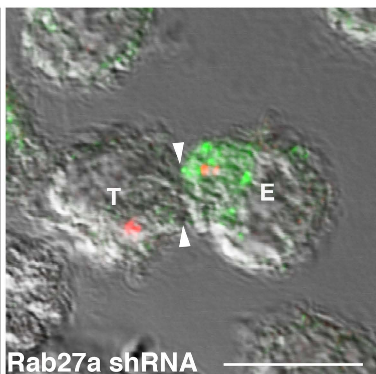

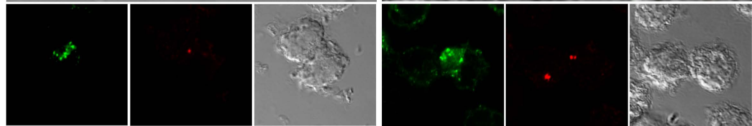

Figure S5

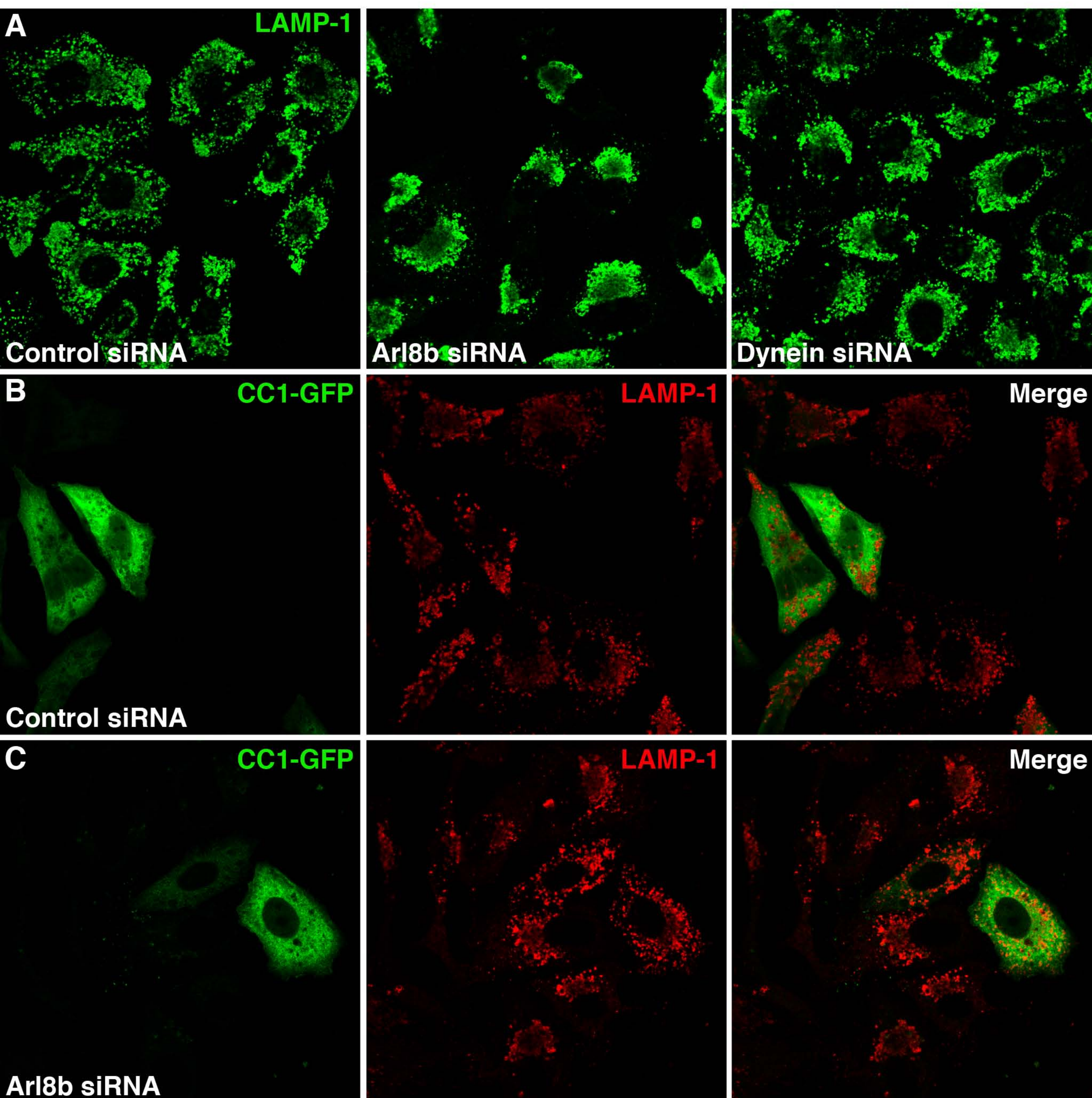

Figure S6

Arl8b-interacting proteins	# of unique peptides
TUBB2C Tubulin beta-2C chain	30
TUBA4A Tubulin alpha-4A chain	28
TUBB3 Tubulin beta-3 chain	11
TBCD Isoform 4 of Tubulin-specific chaperone D	9
CCT2 T-complex protein 1 subunit beta	9
ATP6V1A V-type proton ATPase catalytic subunit A	7
KANK2 KN motif and ankyrin repeat domains 2	4
KIF5B Kinesin-1 heavy chain	**4**
FAF2 FAS-associated factor 2	3
LMNA Isoform A of Lamin-A/C	3
IFI16 Isoform 1 of Gamma-interferon-inducible protein Ifi-16	3
VIM Vimentin	3
Vps16 Isoform 1 of Vacuolar protein sorting-associated protein 16 homolog	2
Vps18 of Vacuolar protein sorting-associated protein 18 homolog	2
ANXA2P2 Putative annexin A2-like protein	2

Figure S7