

Supplementary Materials

Methods

Fig. S1. Generation of novel Rab27A and Rab27B floxed mouse animals

Fig. S2. Baseline Vav-Rab27A cKO immune cell populations and confirmation of various cKOs

Fig. S3. Baseline CD11c-Rab27A cKO immune cell populations and confirmation of cKOs

Fig. S4. In vivo transfer of miR-146a by BMDC EVs regulates macrophage skewing

Fig. S5. Human CD patients have dysregulated transcription of key exosome loading machinery, cargo and secretion proteins

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Methods

Genotyping:

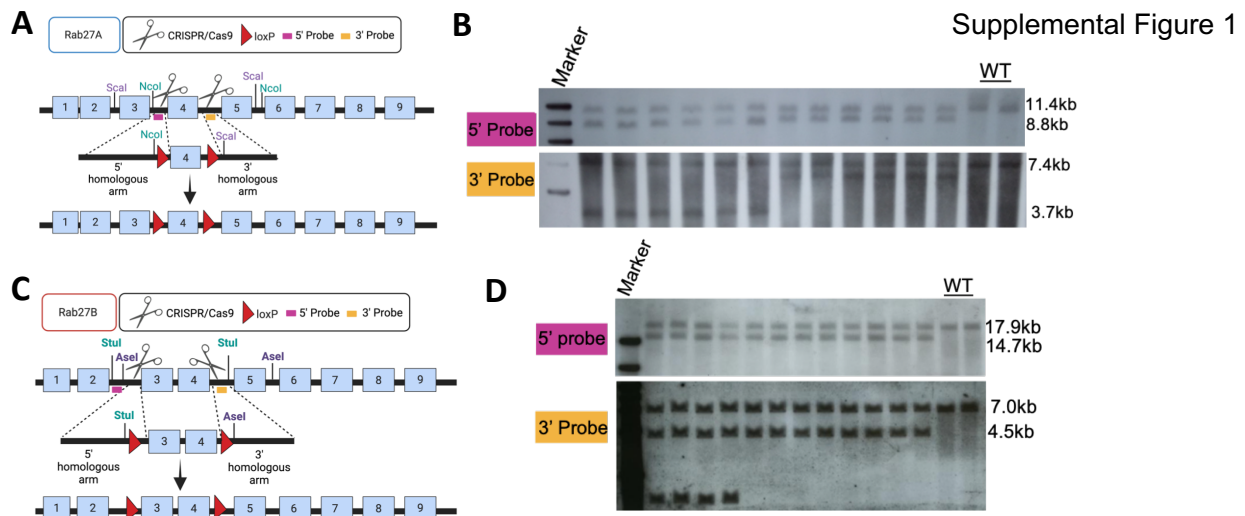
DNA was isolated from mouse ear punches using the ThermoFisher Red Extract System. The following PCR programs were run for indicated genes: *Rab27A fl*: 94°C for 5:00; 30 cycles of 94°C 0:30, 62°C for 0:30, 72°C for 1:00; 72°C 10:00, 4°C hold. *Rab27B fl*: 94°C for 5:00; 35 cycles of 94°C 0:30, 62°C for 0:30, 72°C for 1:00; 72°C 10:00, 4°C hold. *Villin1-Cre*: 95°C for 2:00; 40 cycles of 95°C 0:20, 58°C for 0:20, 72°C for 0:45; 72°C 2:00, 4°C hold. *CD4-Cre*: 95°C for 5:00; 30 cycles of 95°C 1:00, 56°C for 1:30, 72°C for 2:00; 72°C 8:00, 4°C hold. *Vav-iCre*: 94°C for 5:00; 40 cycles of 94°C 0:20, 64°C for 0:20, 72°C for 0:45; 72°C for 5:00, 4°C hold. *CD11c-Cre*: 95°C, 5:00; 10 cycles of 94°C 20 sec, 60°C for 0:15 (-0.5°C / cycle), 68°C for 0:10; 94°C 0:15, 55°C 0:15, 72°C 0:10; 72°C 2:00, 4°C hold. *mTmG fl*: 95°C, 5:00; 10 cycles of 94°C 0:20, 65°C for 0:15 (-0.5°C / cycle), 68°C for 0:10; 94°C 0:15, 60°C 0:15, 72°C 0:10; 72°C 2:00, 4°C hold. PCR products were visualized using 2% gel electrophoresis.

Fecal LCN2

For fecal LCN2 isolation, feces were weighed in a pre-weighed eppie tube. For every 10 mg of feces, 10 ul of 1X HBSS with Ca, Mg was added. The fecal pellet was then mashed with a 1000 ul pipette tip. This was vortexed and spun down at 60 x g for 5 minutes. The supernatant was transferred to a new tube and spun at 8000 x g for 5 minutes and supernatant containing free LCN2 was saved.

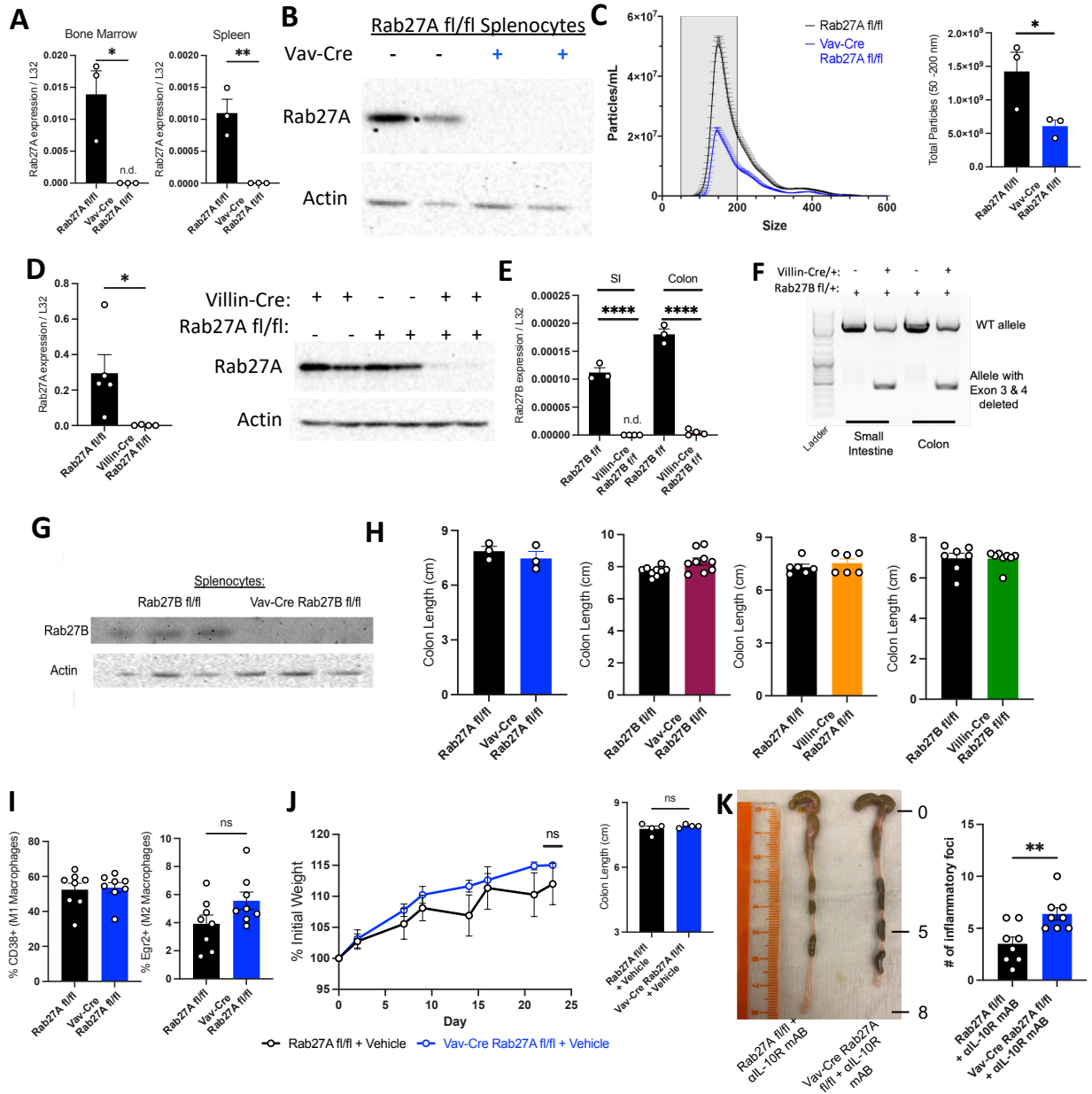
Immunoblotting

Cellular pellets were resuspended in 100 μ l of fresh protein lysis buffer (1X RIPA and 1% protease inhibitor) and sonicated with the following program: 20 seconds on, 30 seconds off, for 5 cycles. Tubes were spun at 21,000 \times g for 3 minutes at 4C and supernatant was retained. Protein concentration was measured (Pierce BCA Protein Assay Kit). Sonicated proteins were separated via SDS-PAGE and transferred to a 0.45 μ M nitrocellulose membrane O/N at 4 C. After blocking in 1% BSA, antibody staining was performed with CD9 (Abcam EPR2949), CD63 (Santa Cruz Biotechnology, sc-15363), Rab27A (Cell Signaling Technology, D7Z9Q), Rab27B (Proteintech 13412-1-AP) and mouse β -Actin (Cell Signaling Technology, 3700S) antibodies. An HRP conjugated secondary antibody was detected with Amersham ECL reagent (GE) with a BioRad GelDoc and ImageLab 6.1 Software.



Supplemental Figure 1: Generation of novel Rab27A and Rab27B floxed mouse animals

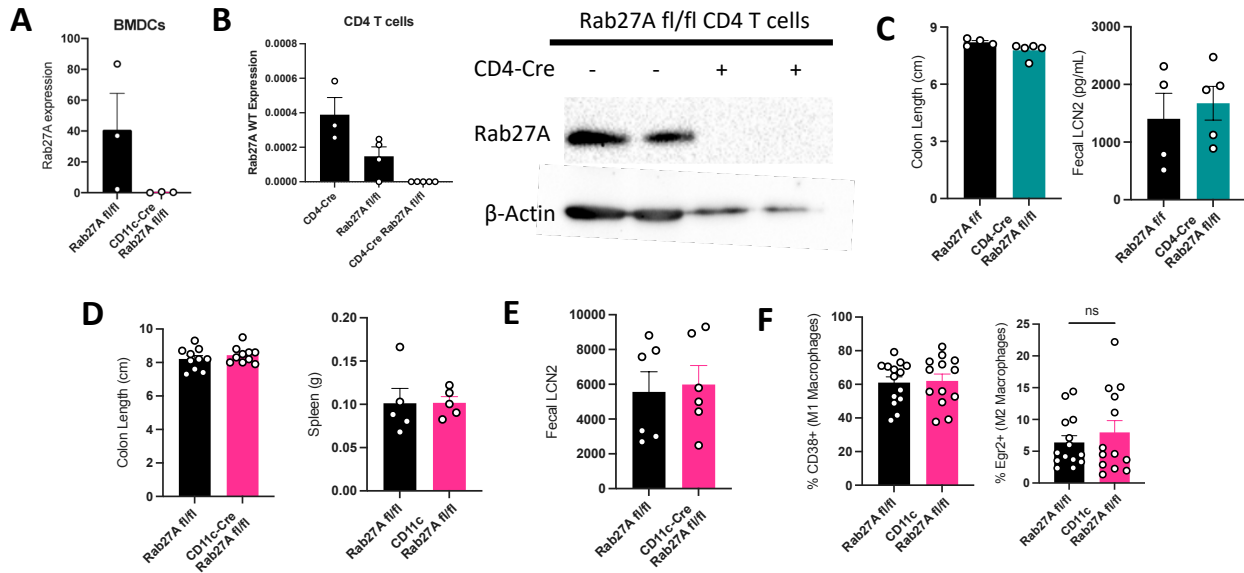
A) Schematic of loxP site insertion for *Rab27A* by CRISPR/Cas9 technology. **B)** Southern blot for 5' and 3' probe to select founder mice of floxed lines. **C)** Schematic of loxP site insertion for *Rab27B*. **D)** Southern blot for 5' and 3' probe to select founder mice of floxed lines.



Supplemental Figure 2: Baseline Vav-Rab27A cKO immune cell populations and confirmation of various cKOs

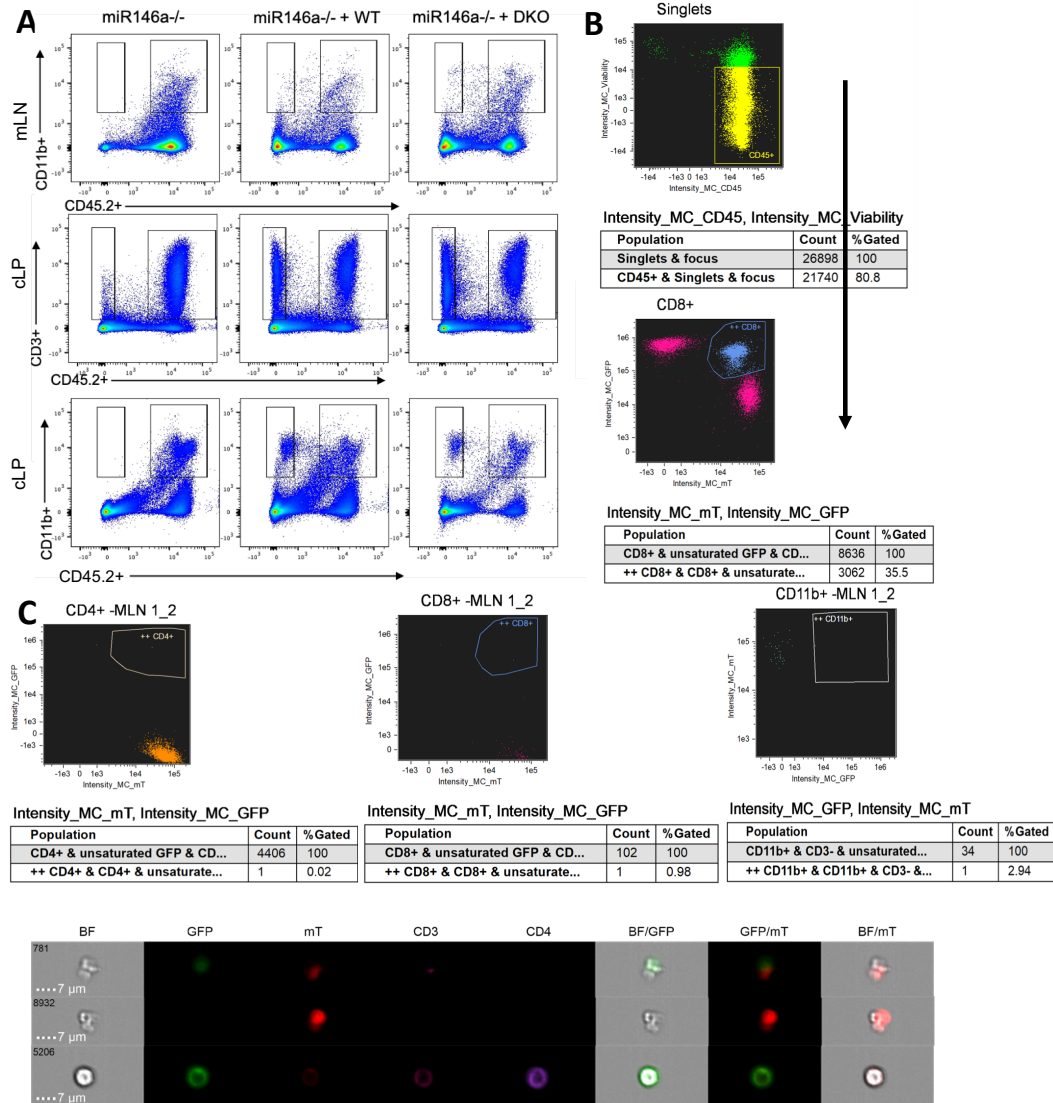
A) *Rab27A* expression in BM cells and splenocytes of WT and KO animals (n=3). **B)** IB of *Rab27A* and Actin within baseline splenocytes of WT and Vav-iCre *Rab27A* fl/fl mice. **C)** NTA of vesicles isolated from WT and Vav-Rab27A cKO BMDCs. Exosome sized (50-200 nm) particle quantification from NTA (n=3). **D)** *Rab27A* expression in IECs from floxed and Villin-*Rab27A* cKO mice and IB for *Rab27A* and Actin in Villin-Cre only, floxed and Villin-*Rab27A* cKO IECs (n=4). **E)** *Rab27B* expression in IECs of the SI and colon of WT and Villin-*Rab27B*

cKO mice (n=3). **F**) PCR analysis of new floxed allele with exons 3 & 4 deleted in the presence of Villin-Cre in SI and colon IECs. **G**) IB of Rab27B and Actin in floxed and Vav-Rab27B cKO splenocytes (n=3). **H**) Baseline colon lengths of all cKO lines in Figure 2. **I**) % of M1 and M2-like macrophages in the cLP of baseline WT and Vav-Rab27A cKO animals. **J**) Weight and colon length of control mice from Figure 2Q given only vehicle. **K**) Representative colon images and length from Figure 2Q and quantification of inflammatory aggregates. Unpaired two-tailed t test for all bar graphs.



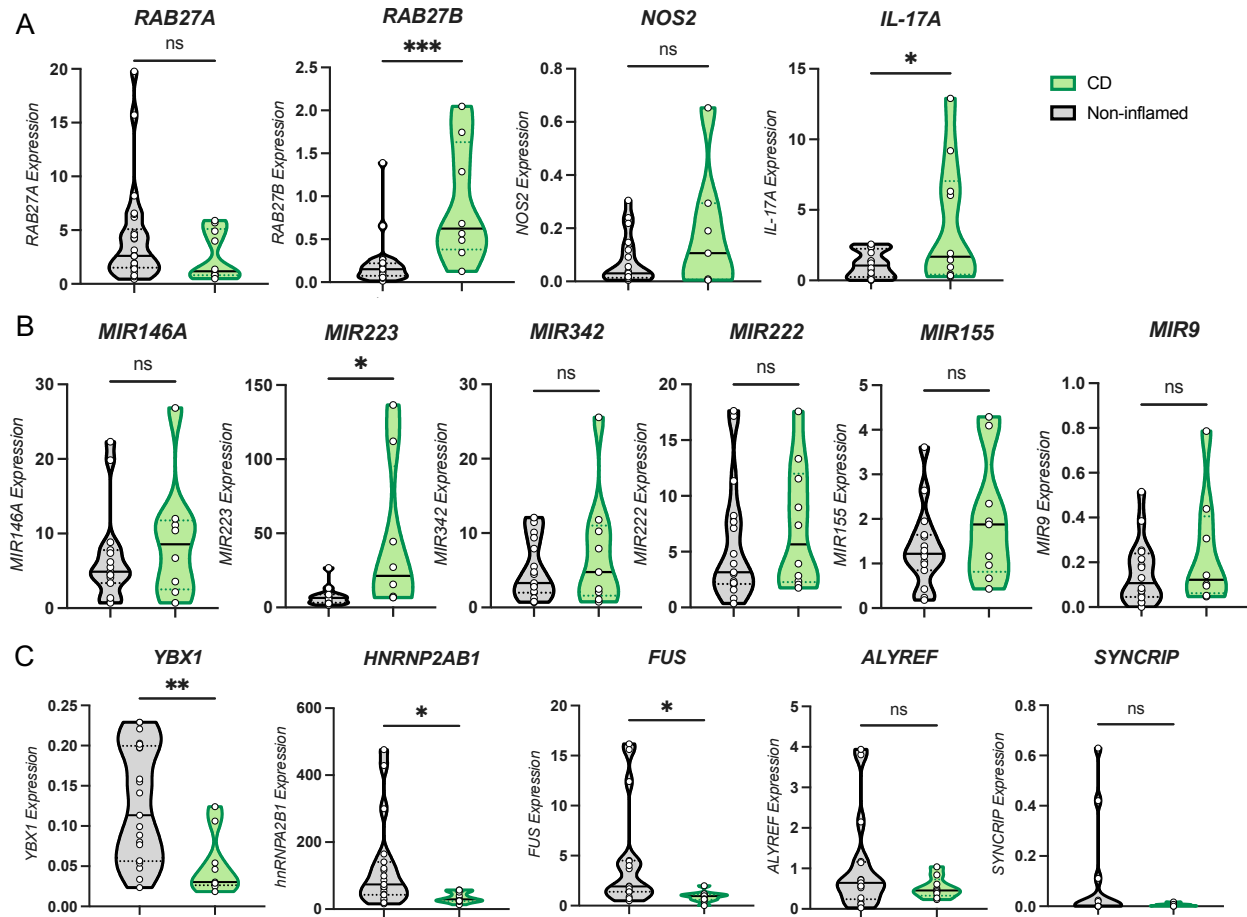
Supplemental Figure 3: Baseline CD11c-Rab27A cKO immune cell populations and confirmation of cKOs

A) *Rab27A* expression in BMDCs from WT and CD11c-Rab27A cKO mice. **B**) *Rab27A* expression in column sorted CD4 T cells from CD4-Cre, floxed and CD4-Cre Rab27A fl/fl animals (n= 3-5). IB of Rab27A and Actin in sorted CD4+ T cells from floxed or CD4-Rab27A cKO animals. **C**) Baseline colon length and fecal LCN2 levels from WT and CD4-Rab27A cKO. **D**) Baseline colon length and spleen weight of WT and CD11c-Rab27A cKO mice. **E**) Baseline fecal LCN2 of WT and CD11c-Rab27A cKO animals. **F**) Baseline percentage of CD38+ M1-like macrophages and Egr2+ M2-like macrophages in floxed and CD11c-Rab27A cKO mLNs.



Supplemental Figure 4: In vivo administration of miR-146a by BMDC EVs regulates macrophage skewing

A) Representative flow plots of sorted CD45.2+ cells from intestinal tissues. **B)** Gating strategy for Imagestream analysis, progressing from Singlets, selecting CD45+, Ghost Dye- and then gating on CD3+ CD4 or CD8+ and then mTomato+ and mGFP+. **C)** Representative plots of mTmG fl/fl animals as negative controls, any doublets are shown.



Supplemental Figure 5: CD patients have dysregulated expression of key exosome loading machinery, cargo and secretion proteins

A) qPCR of *RAB27A*, *RAB27B*, *IL-17A* and *NOS2* in non-IBD (n = 27) and CD patient (n = 10) colonic samples. **B)** miRNA qPCR of miR-146a, miR-223, miR-342, miR-222, miR-155 and miR-9 in A. **C)** qPCR of *YBX1*, *HNRNP2AB1*, *FUS*, *ALYREF* and *SYNCRIP* in A. Unpaired two-tailed t test of all violin plots.