Supplementary Information

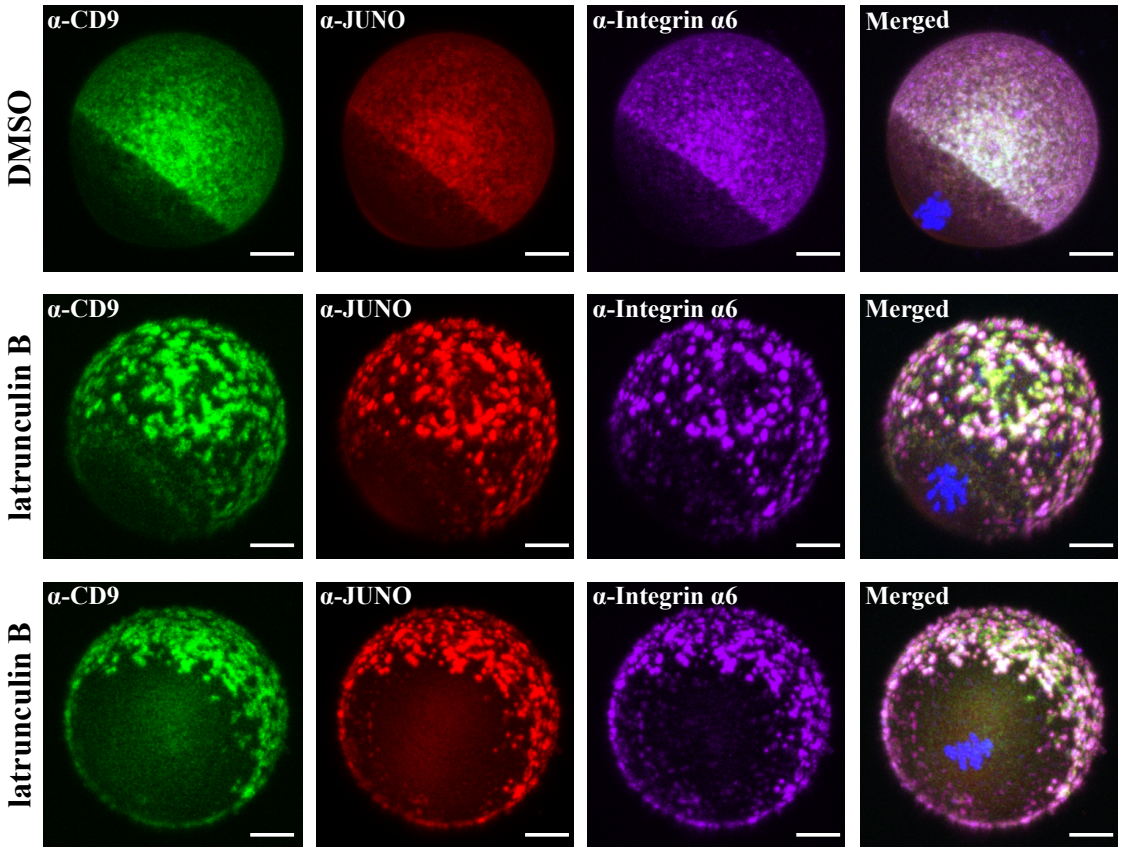


Fig. S1. Latrunculin B treatment in oocytes.

Immunostaining analysis of oocytes after being treated with latrunculin B. The oocytes were treated with 10 μM latrunculin B in TYH medium for 1 hour, then incubated with 0.5 μg ml^{-1} MZ3-FITC (CD9: green), 0.5 μg ml^{-1} TH6-Alexa647 (JUNO: red), 1 μg ml^{-1} GoH3-Alexa546 (Integrin α6: purple) and 1 μg ml^{-1} Hoechst 33342 (nucleus: blue). Two different angles are shown of a latrunculin B-treated oocyte. Scale bars: 10 μm.

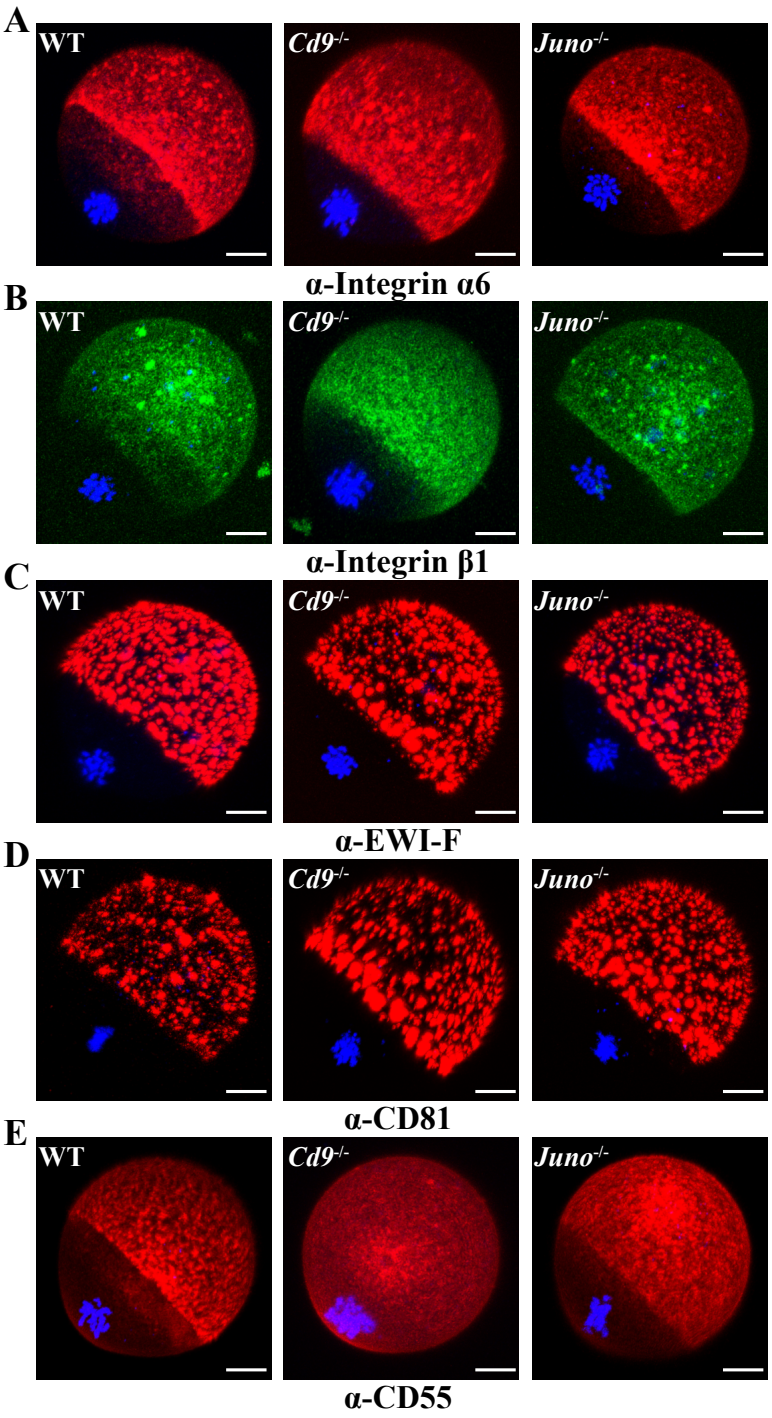


Fig. S2. Immunofluorescence of oocytes with antibodies against membrane-anchored proteins. WT, *Cd9*$^{-/-}$ and *Juno*$^{-/-}$ oocytes were incubated in TYH medium with 1 μg ml^{-1} GoH3-Alexa546 (Integrin α6: **A**), 5 μg ml^{-1} HMb1-1-FITC (Integrin β1: **B**), 2 μg ml^{-1} CD315 antibody (EWI-F: **C**, visualization: Alexa Fluor 647–labeled α-Sheep IgG), 5 μg ml^{-1} Eat2 (CD81: **D**, visualization: Alexa Fluor 647–labeled α-Armenian hamster IgG) and 1 μg ml^{-1} RIKO-3-Alexa647 (CD55: **E**). Scale bars: 10 μm.

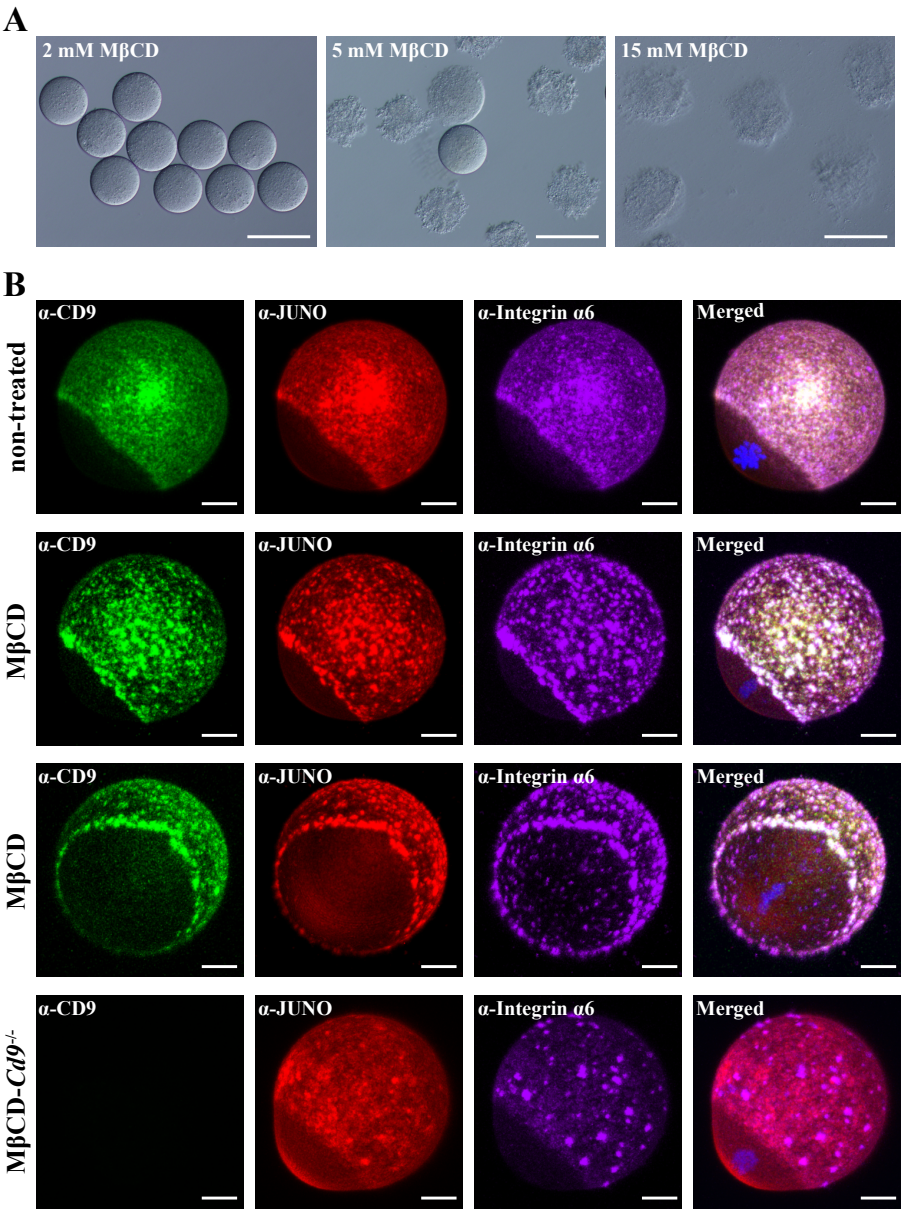


Fig. S3. Methyl-β-cyclodextrin (MβCD) treatment in oocytes.

(**A**) Cholesterol depletion of zona-free oocytes. The zona-free oocytes were incubated with different concentrations of MβCD for 30 min at 37°C. In this assay, all oocytes survived in the 2 mM MβCD treatment only. Scale bars, 100 µm. (**B**) Immunostaining analysis of oocytes after treatment with MβCD. The oocytes were incubated with 0.5 µg ml^{-1} MZ3-FITC (CD9: green), 0.5 µg ml^{-1} TH6-Alexa647 (JUNO: red), 1 µg ml^{-1} GoH3-Alexa546 (Integrin α6: purple) and 1 µg ml^{-1} Hoechst 33342 (nucleus: blue) in TYH medium for 90 min, then treated with 2 µM MβCD in FHM medium for 30 min. Two different angles are shown of a MβCD-treated WT and *Cd9*$^{-/-}$ oocytes. Scale bars: 10 µm.

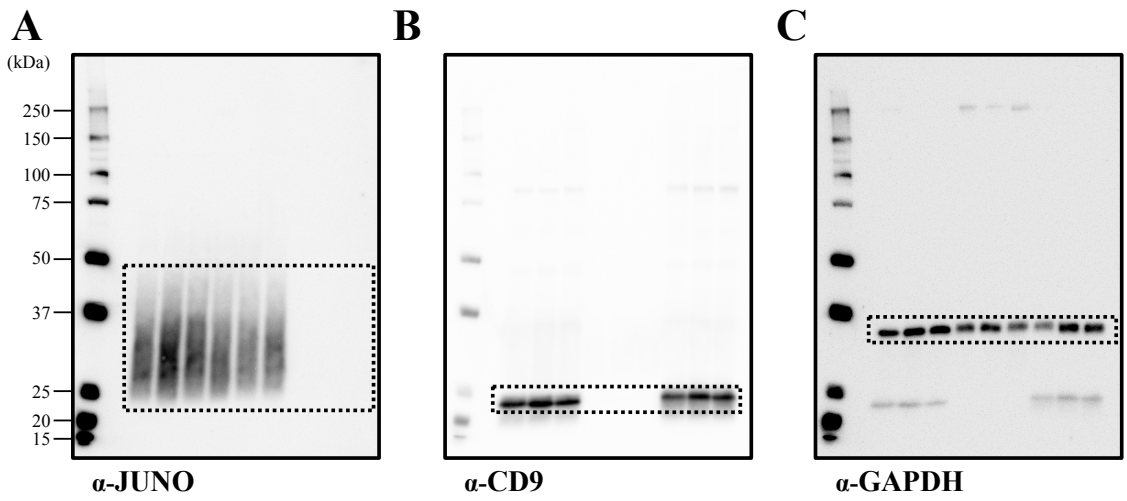
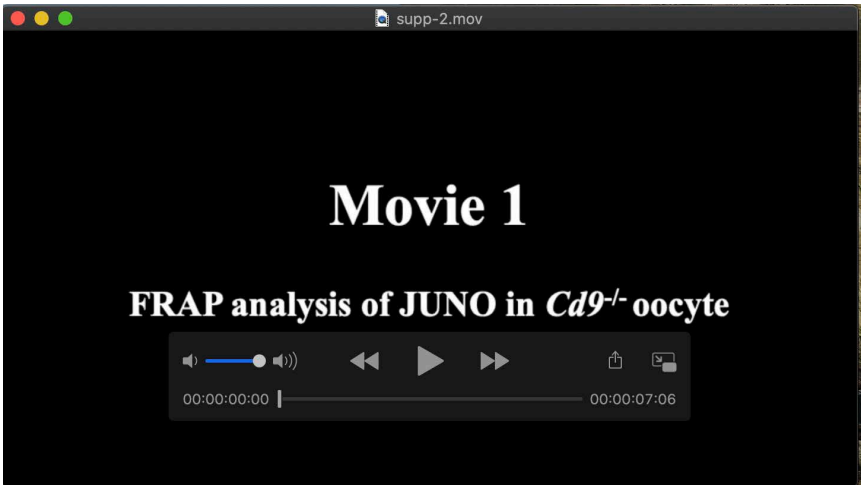


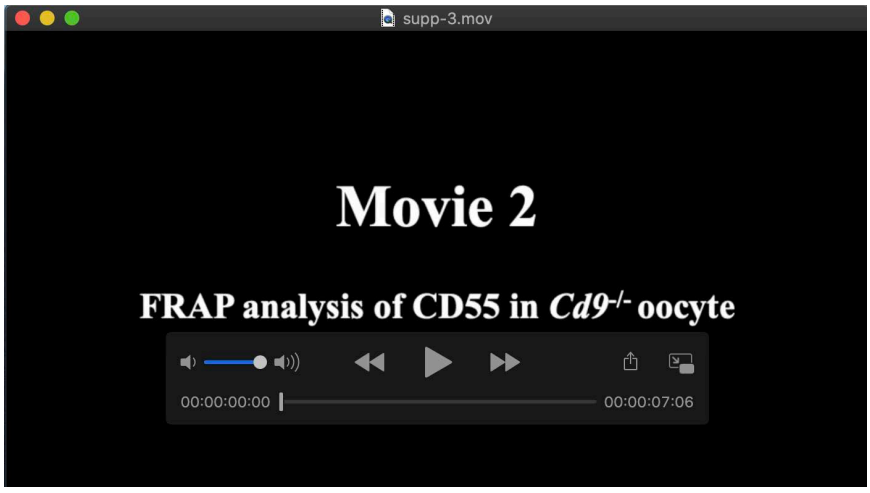
Fig. S4. Original western blot images.

Entire images of blots of Fig. 2B. Blots were incubated with 1 μg ml^{-1} 12A5 (JUNO: **A**), 1 μg ml^{-1} EM-04 (CD9: **B**) and 1 μg ml^{-1} 5A12 (GAPDH: **C**).



Movie 1. FRAP analysis of JUNO in *Cd9*$^{-/-}$ oocyte, related to Fig. 5B.

Oocytes are shown in Gray Scale (upper panels) and Fire LUT (bottom panels) of Fiji. The JUNO fluorescence images were taken at 2 min intervals over 58 min after photobleaching the cortical actin cap region.



Movie 2. FRAP analysis of CD55 in *Cd9*$^{-/-}$ oocyte, related to Fig. 5B.

Oocytes are shown in Gray Scale (upper panels) and Fire LUT (bottom panels) of Fiji. The CD55 fluorescence images were taken at 2 min intervals over 58 min after photobleaching the cortical actin cap region.