Supplemental Figure 1: Fluorescence microscopy of fibrinogen and endostatin uptake in mouse megakaryocytes

Mouse fetal liver derived megakaryocytes were incubated with fluorescently tagged fibrinogen and endostatin for two hours, washed, and then fixed 2, 4, and 24 hours after the initial additional of fluorescent proteins. (A) Shows micrographs of megakaryocytes containing Alexa 488-fibrinogen (FG488) and Alexa 568-endostatin (ENDO568) at different timepoints. Scale bar is 10 μm for 2 hours, 15 μm for 4 hours, and 20 μm for 24 hours. Quantification of Pearson's R value (bottom right) 24 hours after the addition of fluorescent proteins. Analyzed 3 round megakaryocytes. (B) Shows micrographs of megakaryocytes containing FG488 and FG568 at different timepoints. Scale bar is 10 μm. Quantification of Pearson's R value (bottom right) 24 hours after addition of fluorescent proteins (n=3). Analyzed 3 round megakaryocytes per timepoint. (C) Shows comparison of an unprocessed widefield image versus a structured illumination image of the same megakaryocyte containing fluorescently tagged fibrinogen and endostatin. Scale bar is 25 μm. (D) Quantification of FG488/FG546 and FG488/ENDO568 from Figure 1A (n=4). Analyzed 4 round megakaryocytes per condition. The Pearson's R values are 0.91 ± 0.06 for FG488/FG546 and 0.29 ± 0.22 for FG488/ENDO568.

Supplemental Figure 2. Fluorescently conjugated protein endocytosis specificity controls

Mouse fetal liver derived megakaryocytes were incubated overnight with (A) Alexa 488-fibrinogen or (B) Alexa 568-endostatin, or (C) both. Megakaryocytes were also incubated with (D) Alexa 488-fibrinogen or (E) Alexa 546-fibrinogen, or (F) both. (A-F) Scale bar is 20 μm.

Supplemental Figure 3. Co-localization of fibrinogen with endosomal markers

Mouse fetal liver derived megakaryocytes were incubated with Alexa-488 fibrinogen overnight and probed with antibodies against Rab5 and Rab7, after 24 hours. Scale bar is 7 μm.

Supplemental Figure 4. Quantification of α-granule contents in mouse proplatelets and human platelets

Mouse proplatelet shafts and resting human platelets were probed with antibodies against bFGF, ENDO, TSP and VEGF. (A) Analysis of proplatelet shaft colocalization was conducted using Pearson's correlation coefficient with R values measuring overlap of pixel intensities between 2 channels (n=3). Analyzed three replicates of one proplatelet shaft – quadruple labeled. (The Pearson's R values are as follows: ENDO/bFGF is 0.53 ± 0.08, ENDO/TSP is 0.47 ± 0.09, ENDO/VEGF is 0.47 ± 0.16, bFGF/TSP is 0.71 ± 0.14, bFGF/VEGF is 0.62 ± 0.19, and TSP/VEGF is 0.79 ± 0.01. (B) Human platelets were stained and analyzed as described above (n=5). Analyzed 5 human platelets per condition. The Pearson's R values for ENDO/bFGF is 0.49 ± 0.14, ENDO/TSP is 0.27 ± 0.08, ENDO/VEGF is 0.65 ± 0.06, bFGF/TSP is 0.28 ± 0.15, bFGF/VEGF is 0.4 ± 0.14, and TSP/VEGF is 0.42 ± 0.06.

Supplemental Figure 5. Immunofluorescence antibody specificity controls

Resting human platelets were probed for VEGF, TSP, ENDO, and bFGF. Antibody specificity was confirmed by repeating immunolabeling procedure without primary antibody or without secondary antibody. Scale bar is 5 μm.

Supplemental Video 1. 3D reconstruction of a megakarycyte co-incubated with fibrinogen-488 and endostatin-568

Movie shows a super resolution 3D reconstruction of a mouse megakaryocyte containing fluorescently tagged endostatin and fibrinogen in separate granules.

Supplemental Video 2. 3D reconstruction of a megakaryocyte co-incubated with fibrinogen-488 and endostatin-568

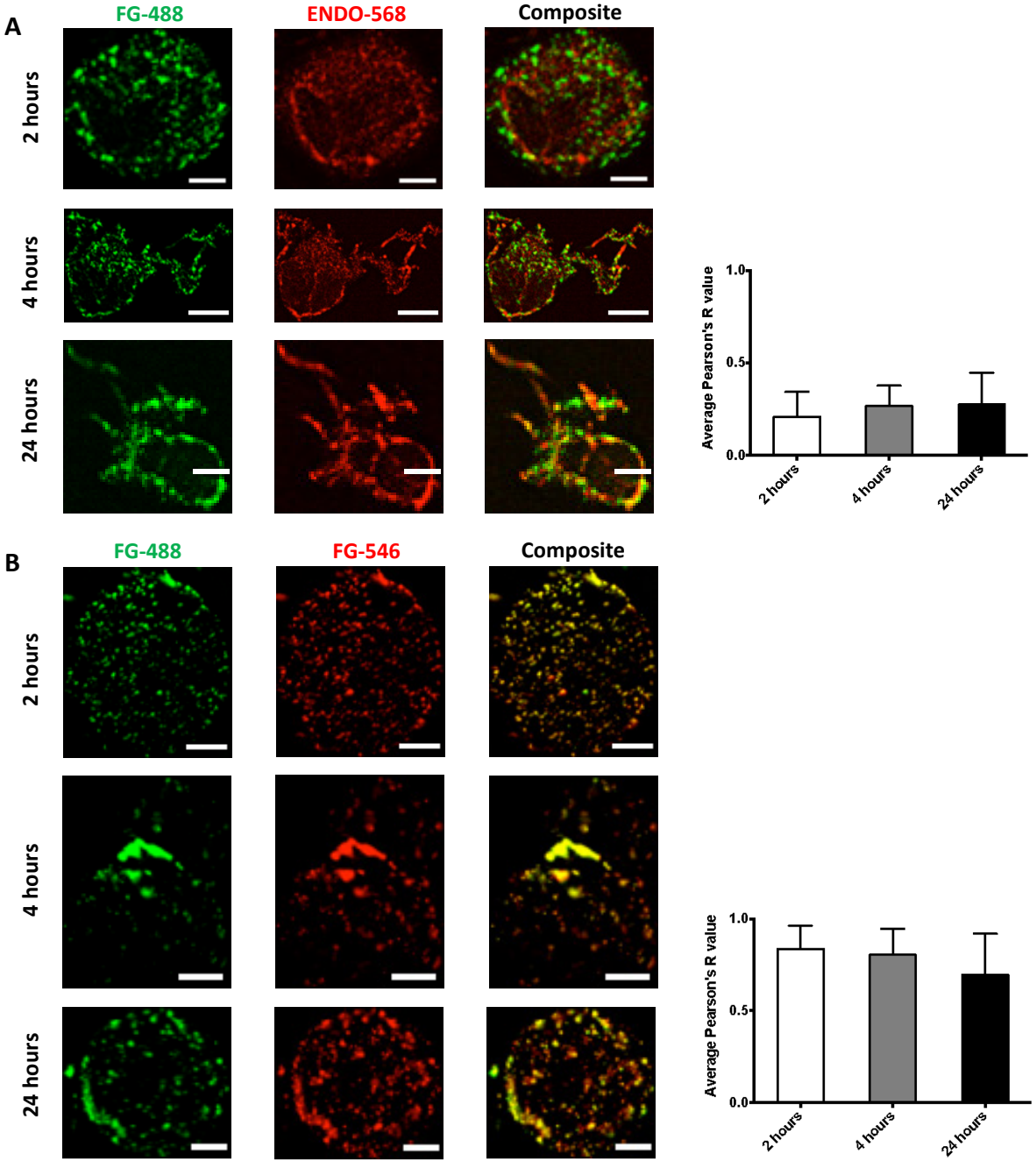
Movie shows a super resolution 3D reconstruction of a mouse megakaryocyte containing fibrinogen-488 and fibrinogen-546 in the same granules.

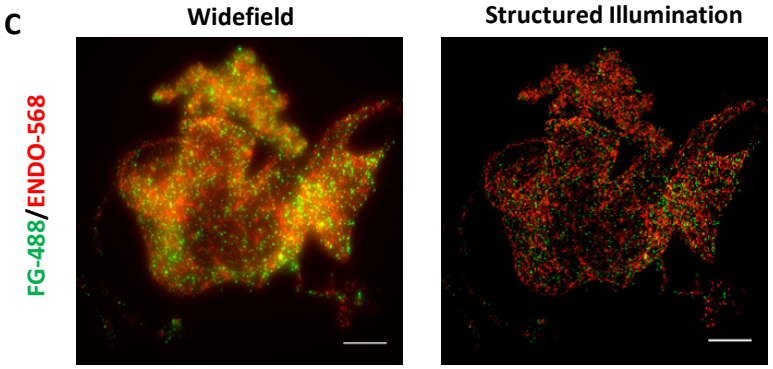
Supplemental Video 3. Live cell imaging of a proplatelet containing fibrinogen-488 and endostatin-568

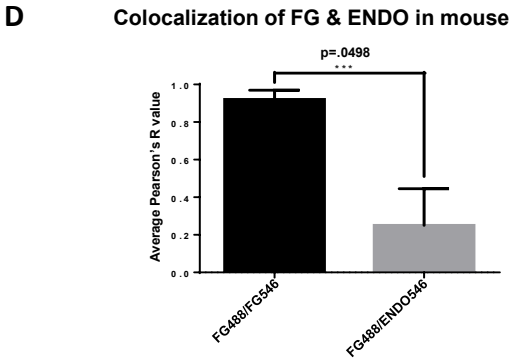
(A) Live cell imaging of a proplatelet derived from megakaryocytes that were co-incubated with Alexa 488-fibrinogen (green) and Alexa 568-endostatin (red). Images were taken every 5 minutes for 1 hour. Timestamp is displayed as minutes:seconds. Scale bar is 25 μm. (B) and (C) show separate fibrinogen and endostatin channels, respectively.

Supplemental Video 4. Zoomed in live cell imaging of a proplatelet containing fibrinogen-488 and endostatin-568

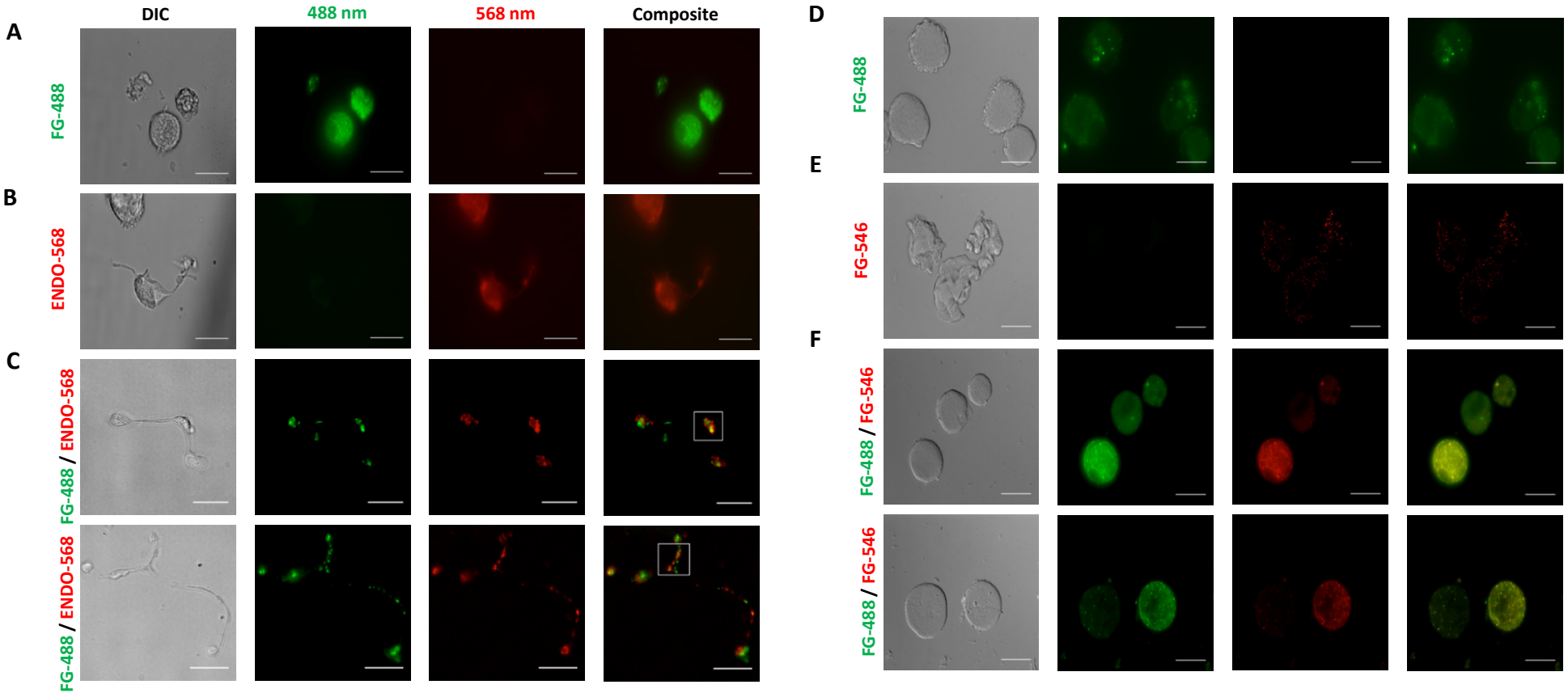
The movie shows a zoomed in view of boxed region shown in Supp. Video 3A. Scale bar is 5 μm.



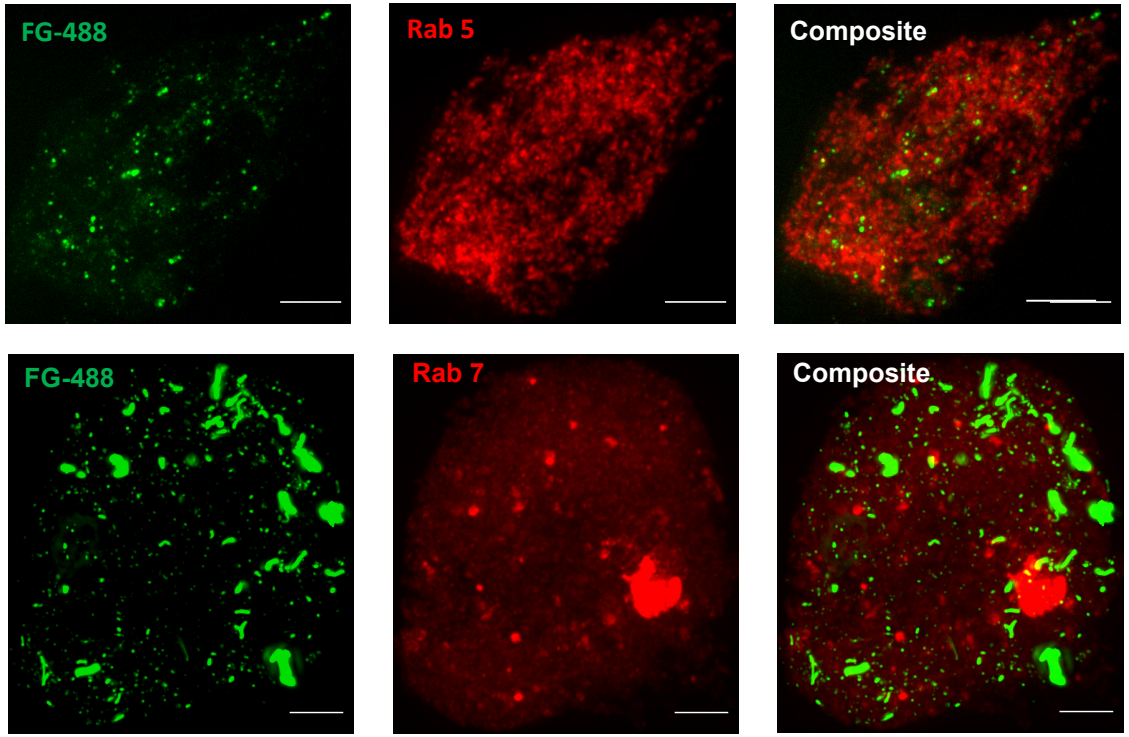




Supplemental Figure 1

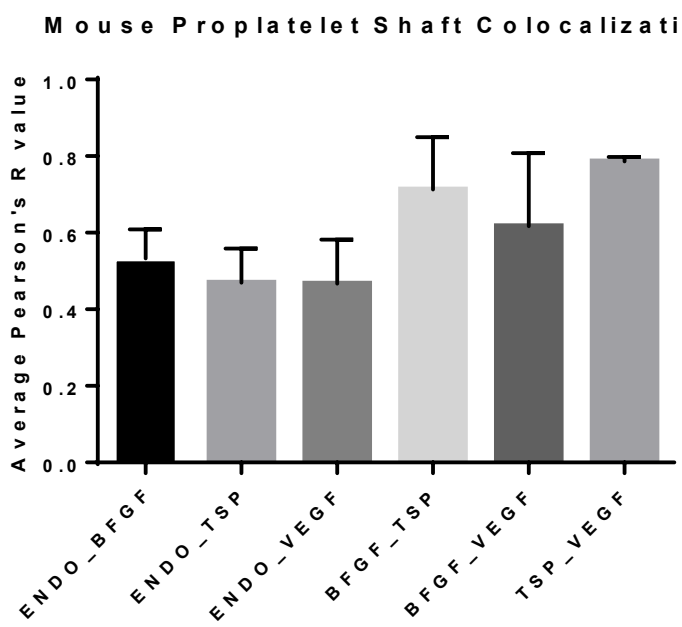


Supplemental Figure 2



Supplemental Figure 3

A



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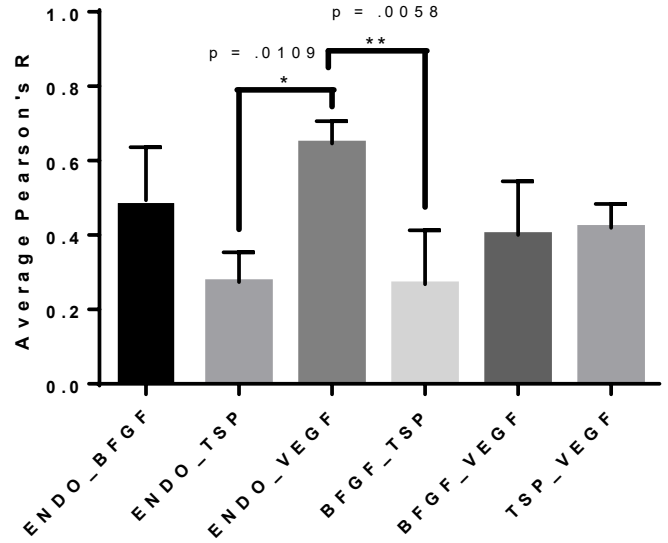
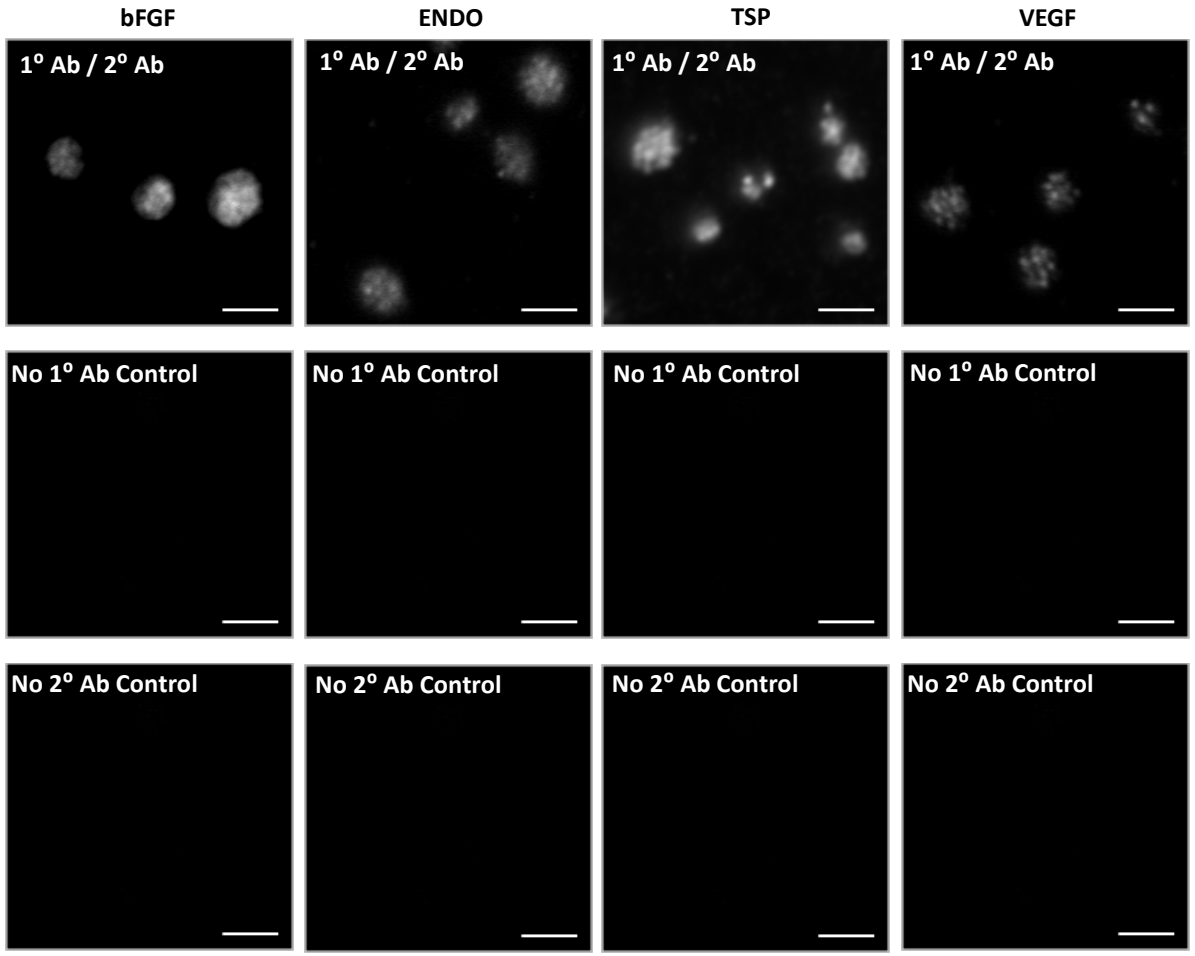


Supplemental Figure 4



Supplemental Figure 5