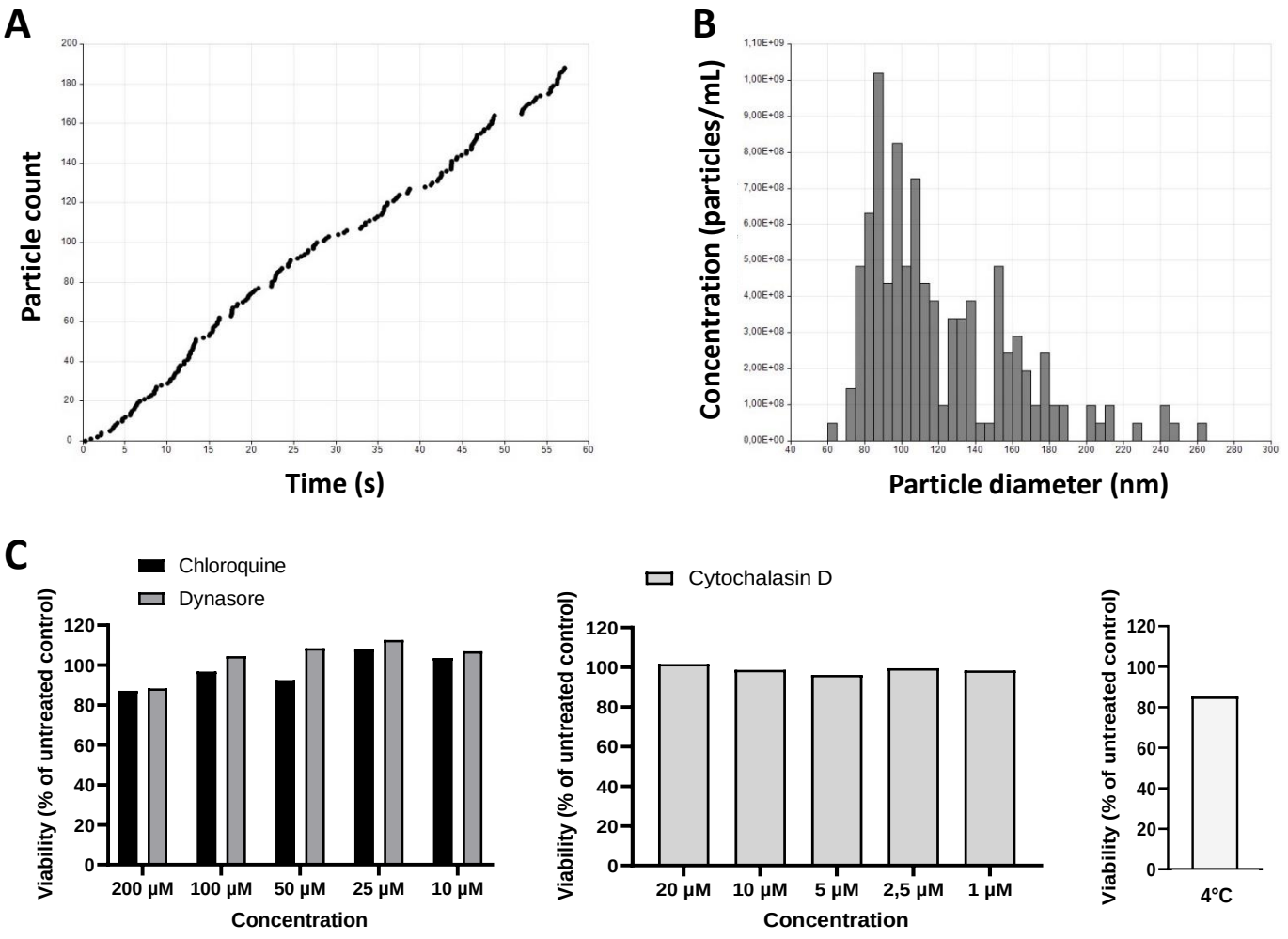
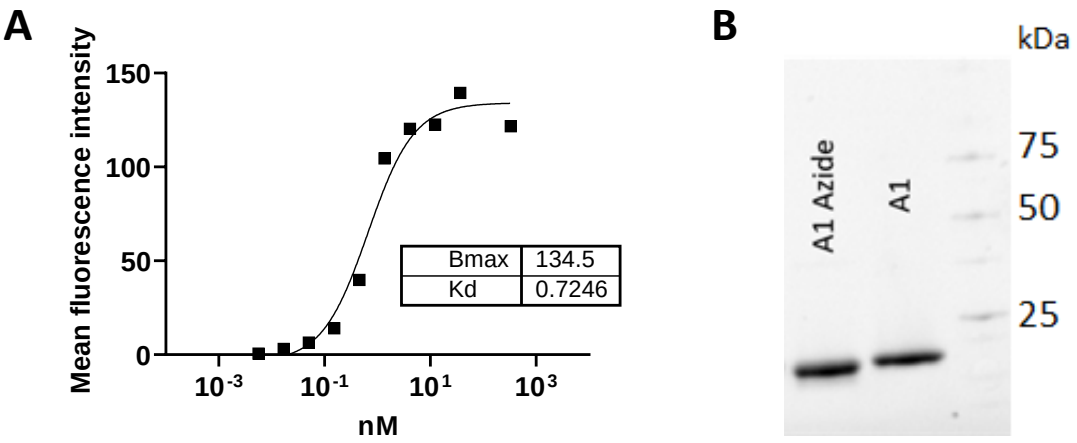
Sup data 1



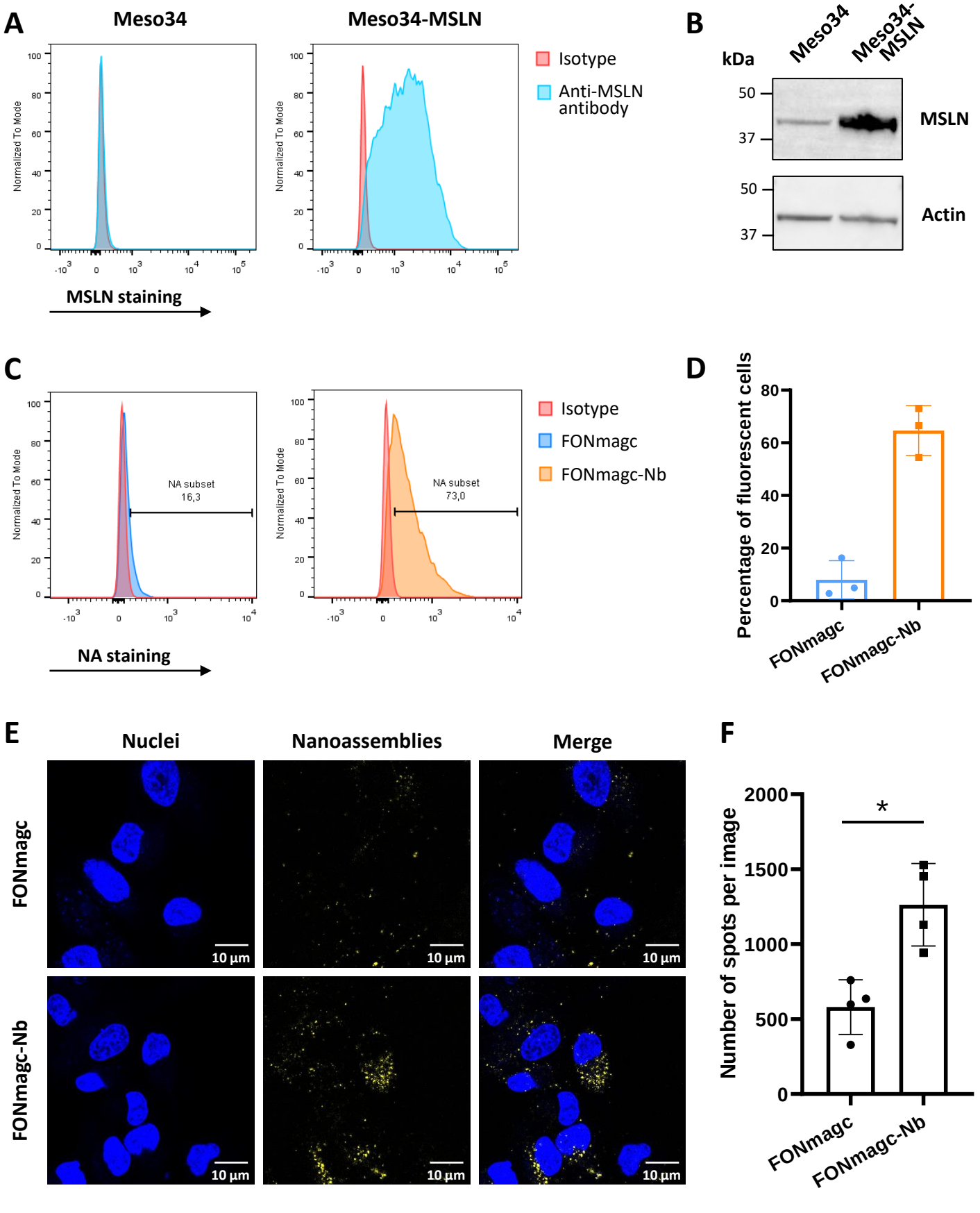
Supplementary figure 1: Quantification of the nanoassemblies and cytotoxicity of endocytosis inhibitors. A-B) The concentration and the average size of the FONmagc suspension was measured by Tunable Resistive Pulse Sensing on a qNano device. C) Meso34-MSLN cells were treated with the endocytosis inhibitors or incubated at 4°C for 4 h and cell viability was assessed by bioluminescence with Celltiter Glo (Promega). n=1.

Sup data 2



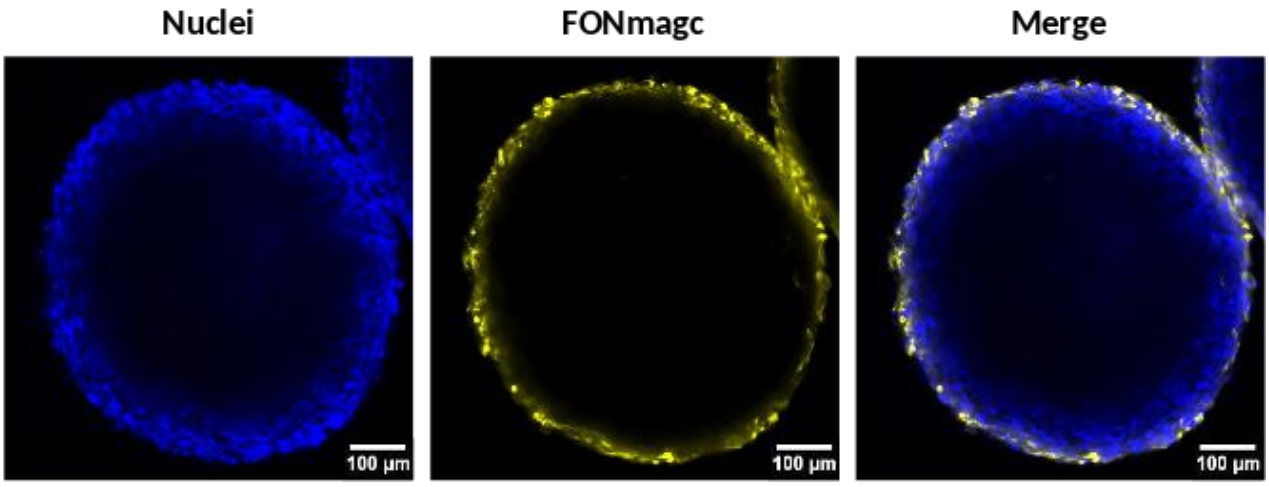
Supplementary figure 2: Coupling of an azide function to the anti-mesothelin A1 nanobody. A) Binding of azide-A1 nanobody on MSLN-transfected HEK 293 T cells. Cells were incubated with a concentration range of the nanobody and analyzed by flow cytometry. B) SDS-PAGE (4-12%) of A1 and azide-A1 nanobodies.

Sup data 3


A
Meso34
Meso34-MSLN
Normalized To Mode
Isotype
Anti-MSLN antibody
MSLN staining
B
kDa
Meso34
Meso34-MSLN
50
37
MSLN
Actin
C
NA subset 16,3
NA subset 73,0
Isotype
FONmagc
FONmagc-Nb
NA staining
D
Percentage of fluorescent cells
FONmagc
FONmagc-Nb
E
Nuclei
Nanoassemblies
Merge
FONmagc
FONmagc-Nb
10 µm
F
Number of spots per image
*
FONmagc
FONmagc-Nb

Supplementary figure 3: Characterization of MSLN expression in Meso34 and Meso34-MSLN cell lines and confirmation of FONmagc-Nb enrichment in the Meso34-MSLN. A) Meso34 and Meso34-MSLN were stained for 30 min at 4°C with an anti-human MSLN-PE conjugated antibody. Cells were washed and cellular fluorescence was analyzed by flow cytometry. Histograms represent the results of a representative experiment. B) One million Meso34 and Meso34-MSLN cells were lyzed in RIPA buffer (Thermo Fischer Scientific 89901) and supplemented with a protease inhibitors cocktail (Sigma Aldrich S8820). Samples protein concentration was determined by BCA (Interchim UP40840A). Twenty micrograms of proteins of each cellular lysate were denatured for 5 min at 95°C in Laemmli buffer (Biorad 1610747) and deposited on a 4-12% acrylamid gel (Genscript M00652). Samples were then electrophorized by SDS-PAGE and transferred on a PVDF membrane. The membrane was blocked for 1 hour at room temperature with 5% non-fat milk (Régilait). The membrane was incubated overnight at 4°C with 0.1 µg/mL of mouse anti-human actin (Invitrogen MA5-11869) and 1 µg/mL of rat anti-human MSLN (R et D Systems MAB32652) primary antibodies, followed by a 1 hour incubation at room temperature with 1 µg/mL of goat anti-mouse-A647 (Invitrogen A21236) and 1 µg/mL of goat anti-rat-A488 (Cell Signaling Technology 4416S) secondary antibodies. Protein expression was then assessed using a ChemiDocMP Imaging System (Biorad). C-D) Meso34-MSLN MPM cells were stained with FONmagc or FONmagc-Nb for 30 min at 4°C and NA fixation was quantified by flow cytometry. The histogram in D) displays the percentage of cells stained with the two types of NA (n=3) and representative flow cytometry results are presented in C). E-F) Meso34-MSLN cells were incubated for 15 h at 37°C with FONmagc or FONmagc-Nb NA. Cells were then fixed, stained with Hoechst and analyzed by confocal microscopy. Representative images are presented in E) and the quantification, in several confocal images using the FiJi software, of the number of fluorescent spots corresponding to NA in cells is presented in F).

Sup data 4



Supplementary figure 4: Evaluation of the diffusion of FONmagc in MCTS. FONmagc were added on pre-formed multicellular tumor spheroids of MPM cells expressing MSLN for 24 h at 37°C. The penetration of the NA in the 3D structures was assessed by confocal microscopy after transparization and nuclei staining (x25 obj). Blue: nuclei; yellow: FONmagc.