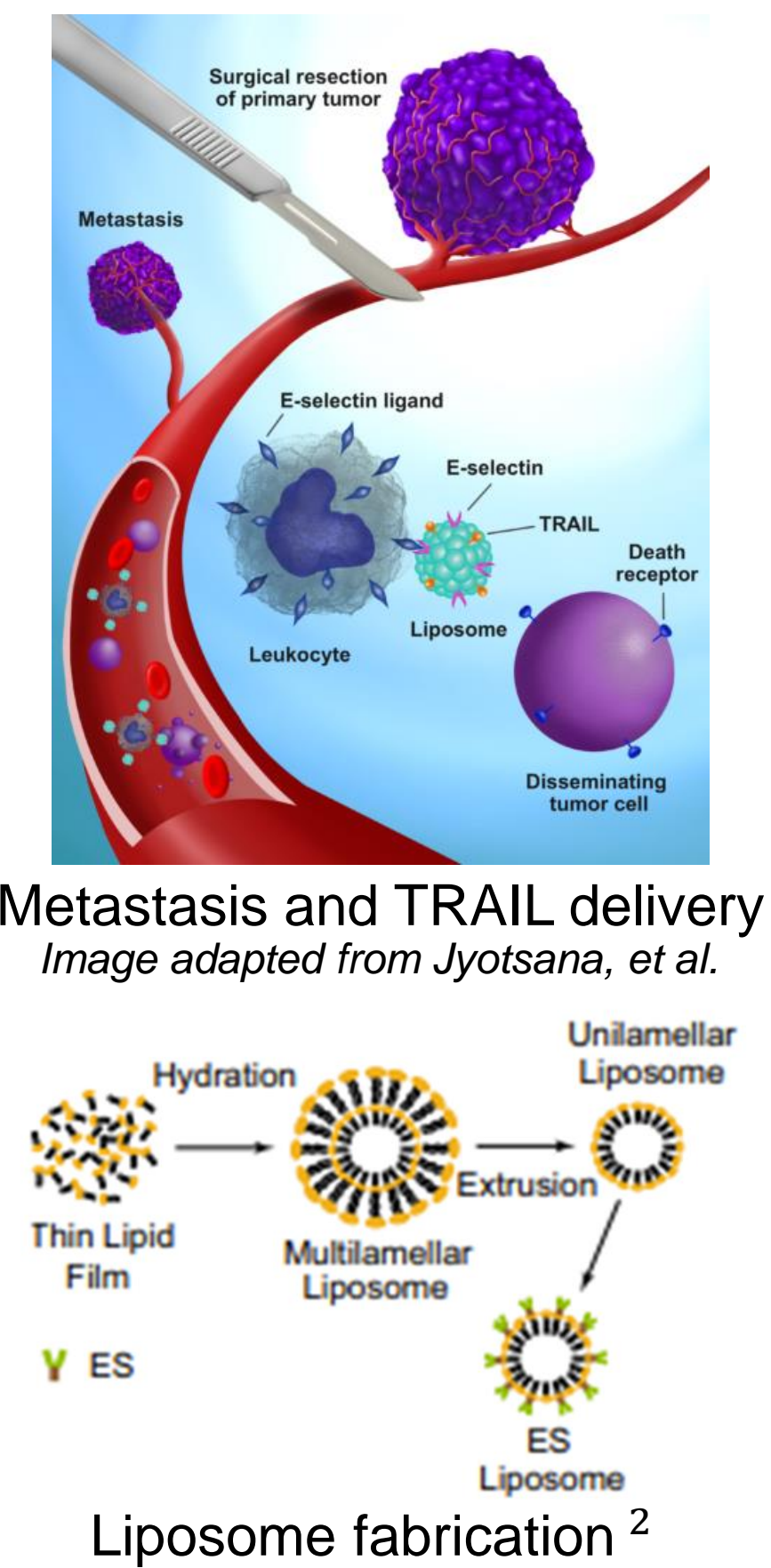


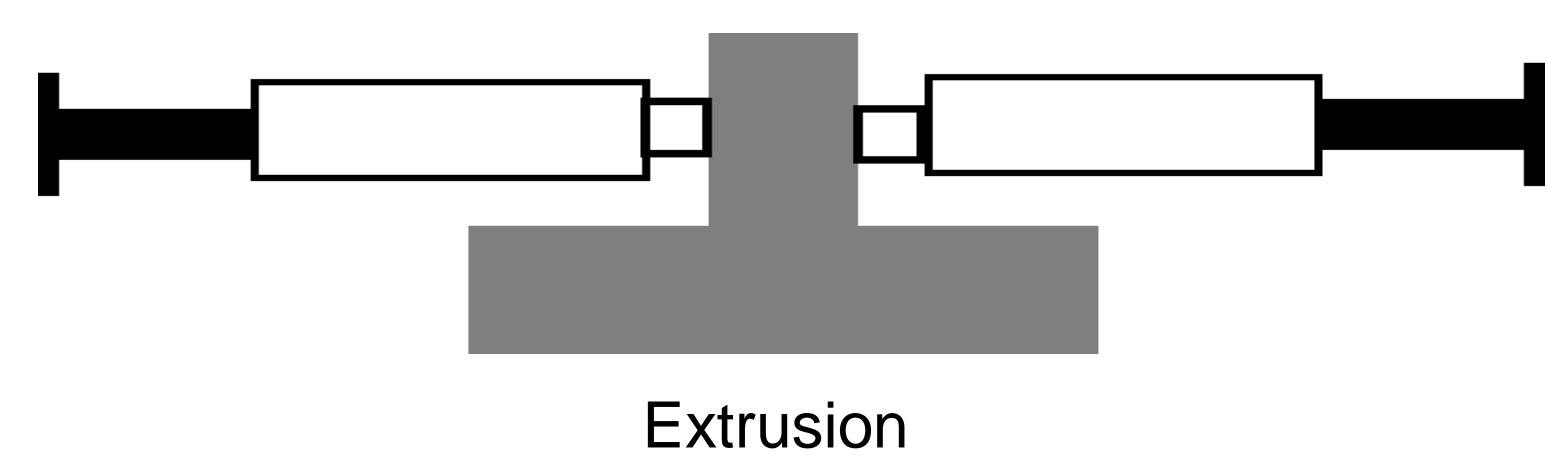
Introduction

- Metastatic tumors release circulating tumor cells (CTCs)¹
- TNF-Related Apoptosis Inducing Ligand (TRAIL) protein kills CTCs¹
- Liposomes bound to leukocytes effectively deliver TRAIL to CTCs²
- E-Selectin (ES) protein allows liposome-leukocyte binding²
- DSE-PEG lipid can increase liposome lifespan in circulation



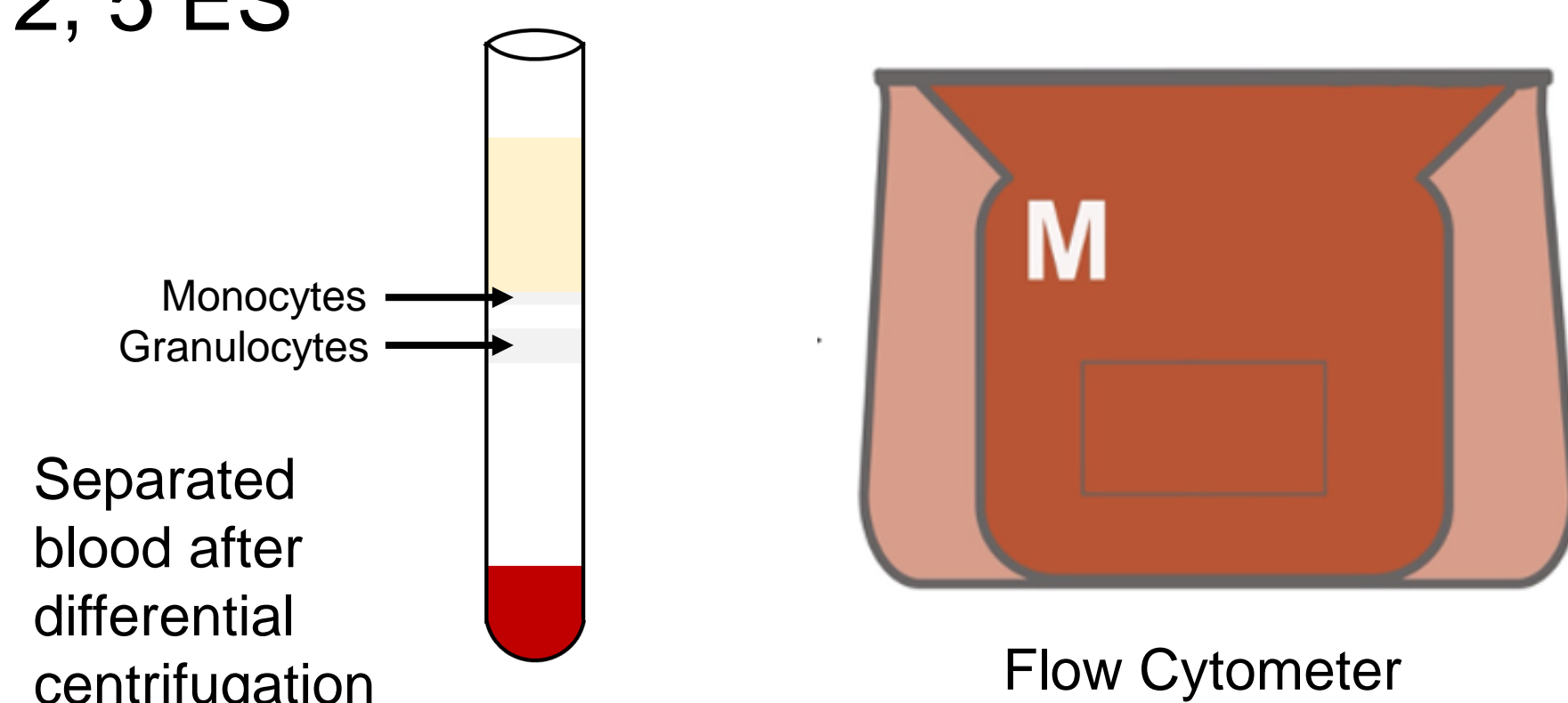
Objective: Investigate how E-Selectin liposomes can functionalize two subpopulations of leukocytes—monocytes and granulocytes—to yield a better understanding of how E-Selectin can assist in the delivery of TRAIL in antimetastatic therapies.

Materials and Methods



Liposome Fabrication

- Extrude a Dil-dyed lipid solution through 100 nm Whatman Nuclepore Track-Etch Membrane
 - Yields uniform-sized fluorescent liposomes with and without DSPE-PEG
- Varying numbers of E-Selectin/liposome
 - 0, 1, 2, 5 ES



Leukocyte Incubation and Analysis

- Using healthy patient blood, isolate monocytes and granulocytes via differential centrifugation
- Incubate leukocytes with fluorescent ES-liposomes
- Measure the fluorescence present on leukocytes using Guava easyCyte HT flow cytometer
- Gating and analysis performed via FlowJo and Prism

Results

Fluorescence increases in granulocytes with greater concentrations of E-Selectin

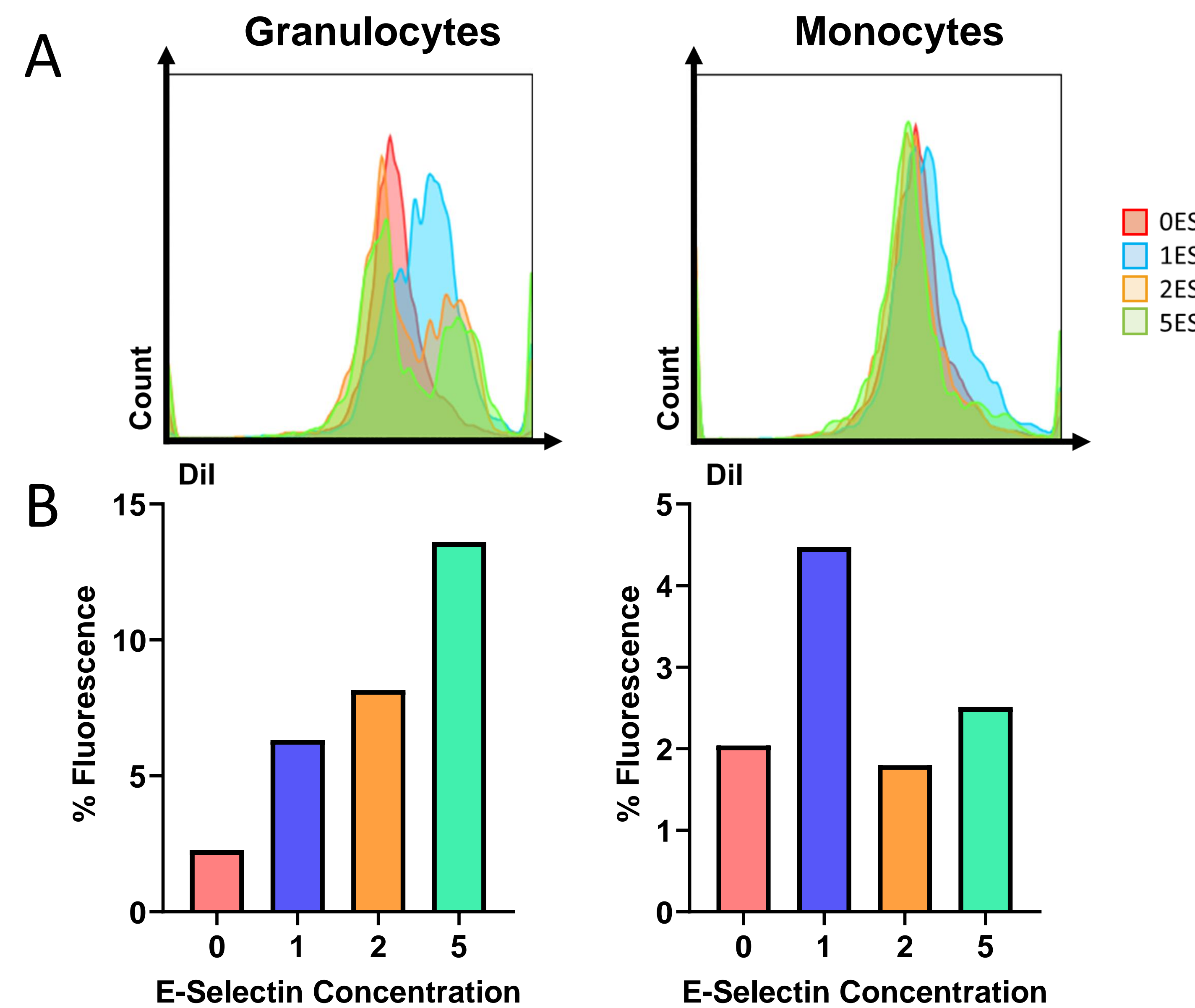


Fig. 1: Analyzing presence of fluorescent liposome binding with varying amounts of ES using flow cytometry. (A) Histogram demonstrates shift of fluorescence with increased ES in granulocytes, indicating higher liposome binding. (B) Percent fluorescence of varying ES concentrations.

Liposomes without DSPE-PEG conjugate to both subpopulations of leukocytes

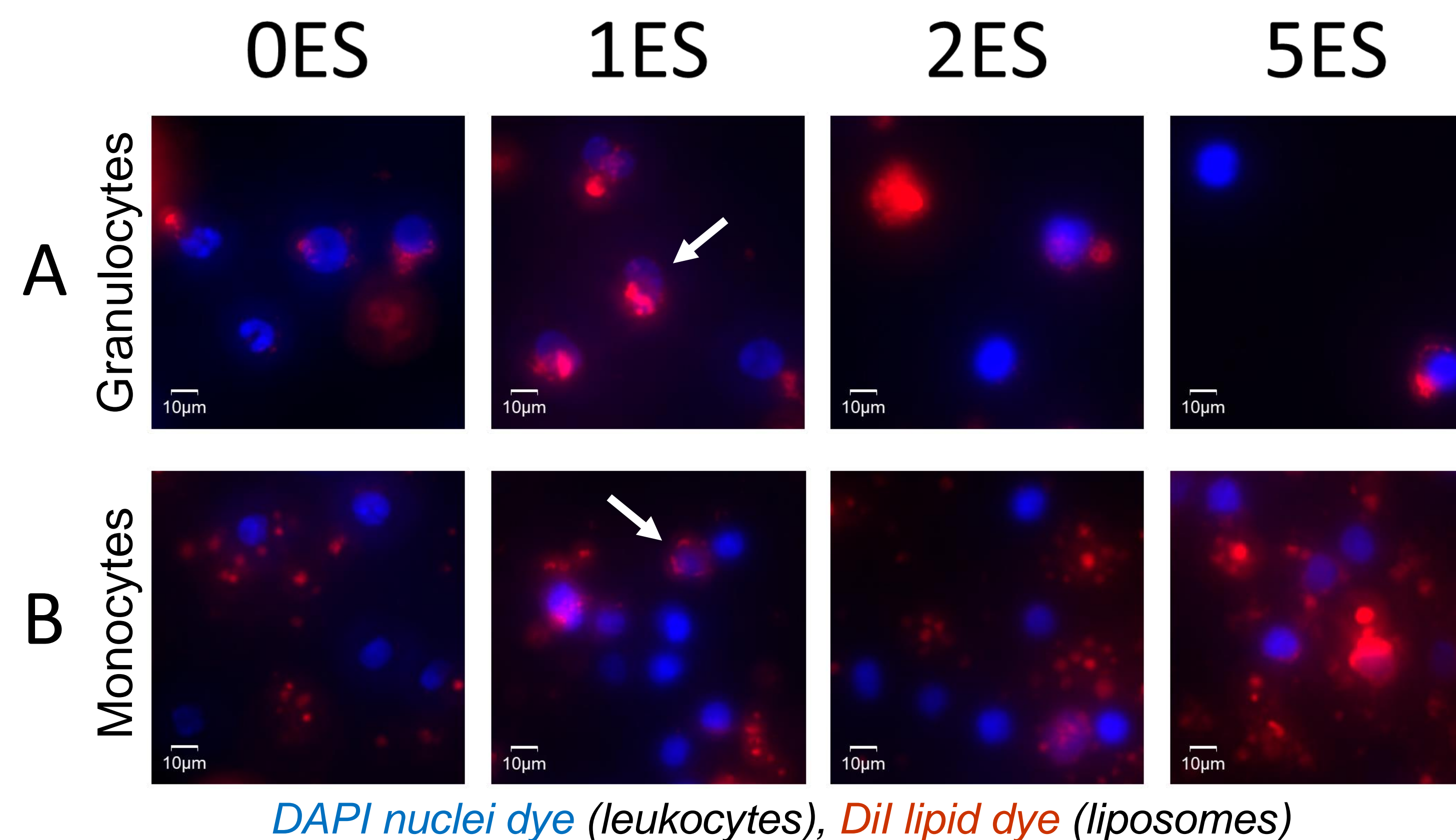


Fig 2: Fluorescence microscopy of leukocytes with liposomes without DSPE-PEG and varying concentrations of E-Selectin. White arrows indicate liposome-bound leukocytes. (A) Select granulocytes conjugate to liposomes despite presence of ES. (B) With ES present, monocytes conjugate to liposomes.

Results

Absence of DSE-PEG in liposome fabrication yields a subpopulation of increased conjugation to granulocytes

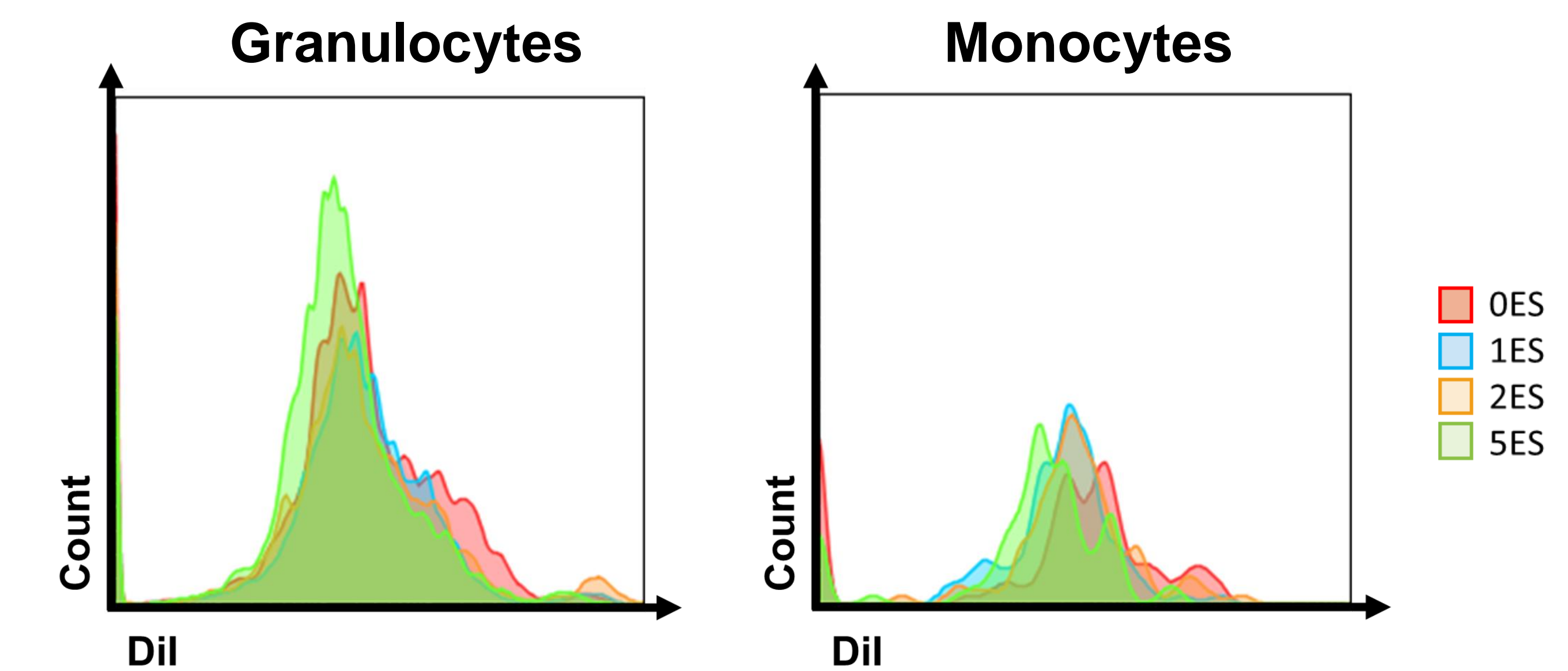


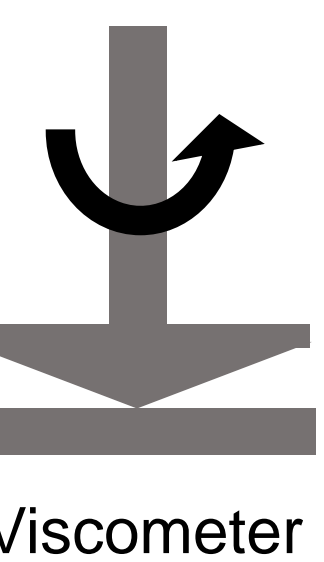
Fig. 3: Flow cytometry results of fluorescent E-Selectin liposome-bound leukocytes without DSE-PEG. Histograms indicate presence of fluorescence and binding to leukocytes. In granulocytes, there is a subpopulation in which there is higher fluorescence, indicating increased binding.

Conclusions

- Liposome attachment is dependent on the average number of E-Selectin per liposome in granulocytes, but not in monocytes
- Absence of DSPE-PEG can:
 - Cause attachment with high concentrations of E-Selectin in monocytes
 - Increase liposome attachment to a granulocyte subpopulation
 - Cause attachment to platelets

Future Directions

- Analyze effectiveness of TRAIL with varying E-Selectin concentrations
- Introduce fluid shear stress to mimic blood flow
- Investigate how liposomes without DSPE-PEG:
 - Affect monocyte conjugation with increased E-Selectin
 - Attach to different types of granulocytes



References and Acknowledgements

- [1] Jyotsana, et al., *Science Advances*, 2019, 5, 1-11
 [2] Mitchell, et al., *PNAS*, 2013, 3, 1-6



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