OMTN, Volume *35*

Supplemental information

mRNA vaccine trafficking and resulting protein expression after intramuscular administration

Kimberly J. Hassett, Ivana Liric Rajlic, Kapil Bahl, Rebecca White, Kristen Cowens, Eric Jacquinet, and Kristine E. Burke

Supplemental Information

Table S1. Surface antibodies used in flow cytometry to identify specific cell types expressing protein in NHP

Marker	Clone	Fluorophore
Live/Dead (L/D)		FVD 780
CD3	SP34-2	AF700
CD20	L27	PerCPCy5.5
HLA-DR	L243	BV711
CD123	7G3	BV421
CD11c	3.9	PE-Cy7
CD86	2331 (FUN-1)	APC
CD14	M5E2	BV510
CD16	3G8	PECF594
CD1c	AD5-8E7	PE
CD163	GHI/61	BV605
eGFP		eeGFP

Table S2. Primary markers for all NHP cell populations characterized.

NHP Subsets	Primary Markers
Classical Monocytes	L/D- HLA-DR+ CD3- CD20- CD14+ CD16-
Intermediate Monocytes	L/D- HLA-DR+ CD3- CD20- CD14+ CD16+
Non-classical Monocytes	L/D- HLA-DR+ CD3- CD20- CD14- CD16+
CD1c+ Myeloid DCs	L/D- HLA-DR+ CD3- CD20- CD14- CD16-CD163- CD11c+ CD123- CD1c+
CD1c- Myeloid DCs	L/D- HLA-DR+ CD3- CD20- CD14- CD16-CD163- CD11c+ CD123- CD1c-
Plasmacytoid DCs	L/D- HLA-DR+ CD3- CD20- CD14- CD16-CD163- CD11c- CD123+
Activation	CD86
B cells	L/D- CD20+
T cells	L/D- CD3+
Macrophages	L/D- CD3- CD20- HLA-DR+ CD163+

Supplemental Figures

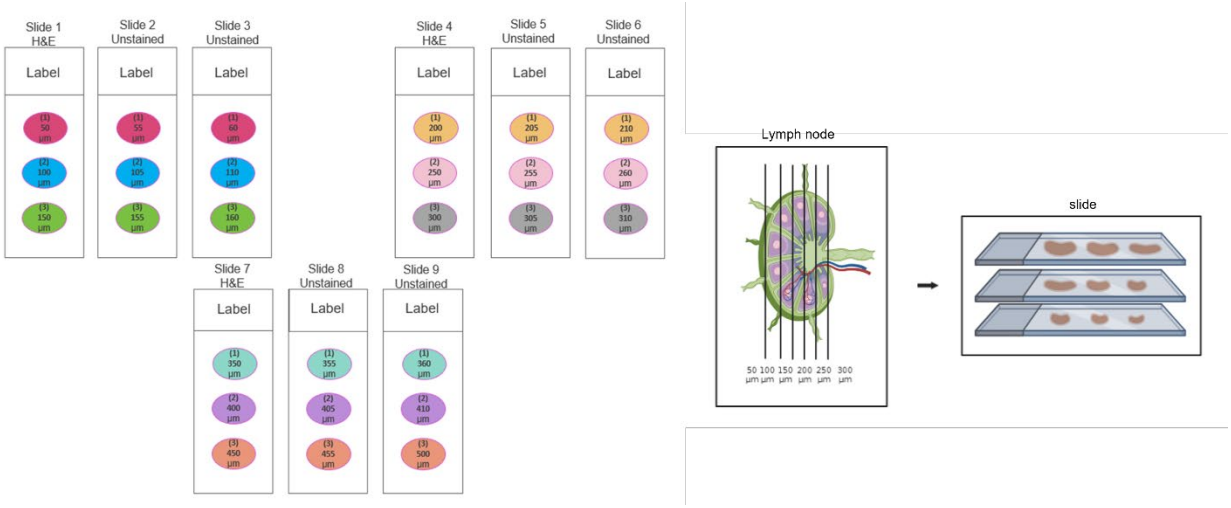
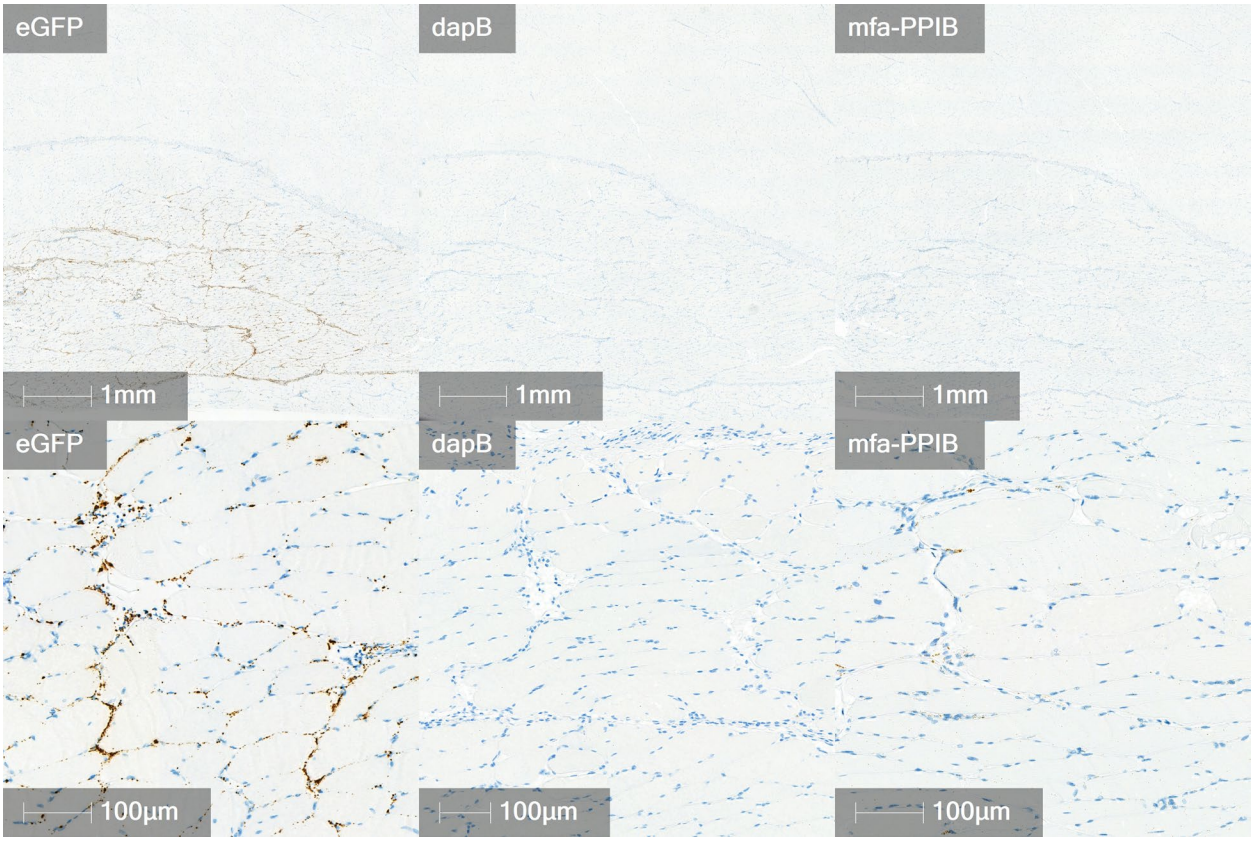


Figure S1. Diagram of step-sectioning technique. The figure illustrates step sectioning through the entire lymph node (LN) to determine the percentage of eGFP protein expression. Sections were taken at regular 50µm intervals to capture changes in protein expression as we cut deeper into the tissue. This information will help understand the distribution and variation of the eGFP protein within the LN. Some elements were created using BioRender.com. Scalebars range from 50 µm to 500 µm.

A.


eGFP
dapB
mfa-PPIB
1mm
1mm
1mm
eGFP
dapB
mfa-PPIB
100µm
100µm
100µm

B.

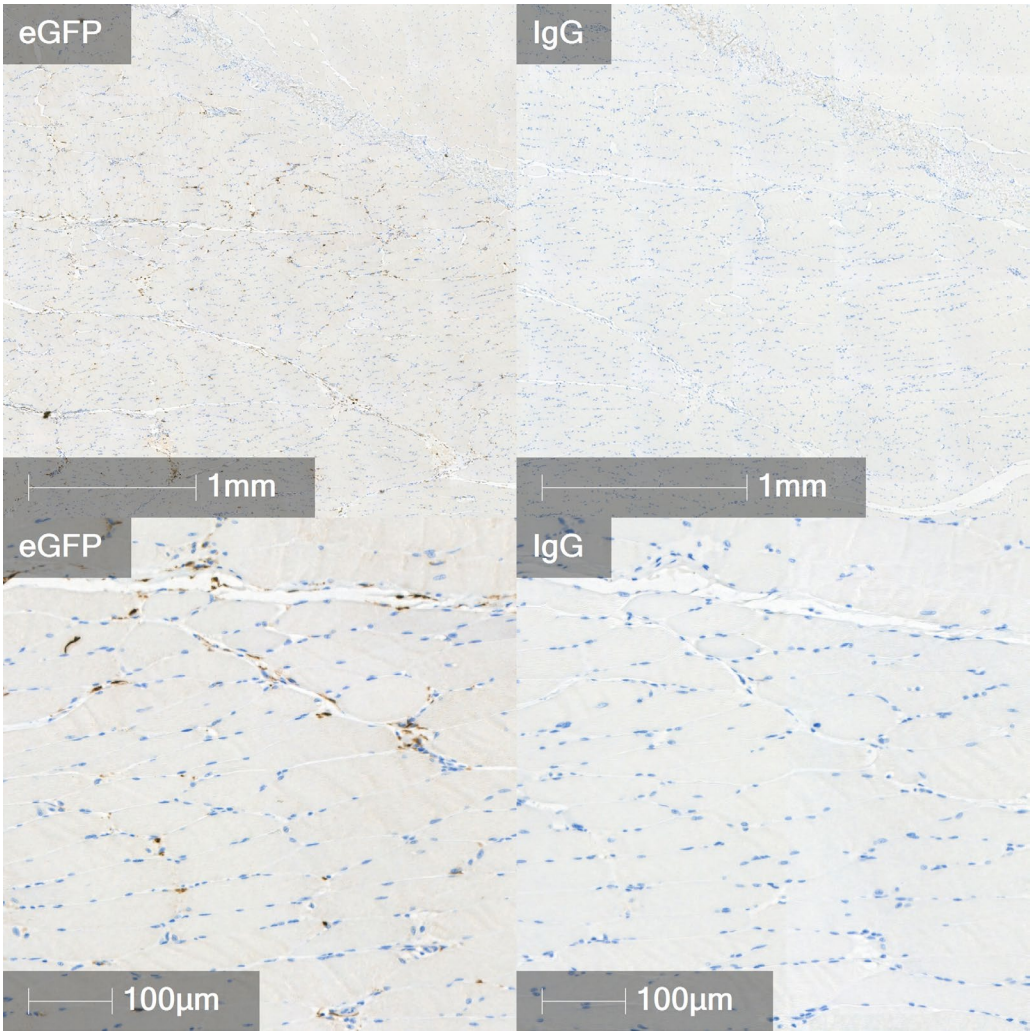
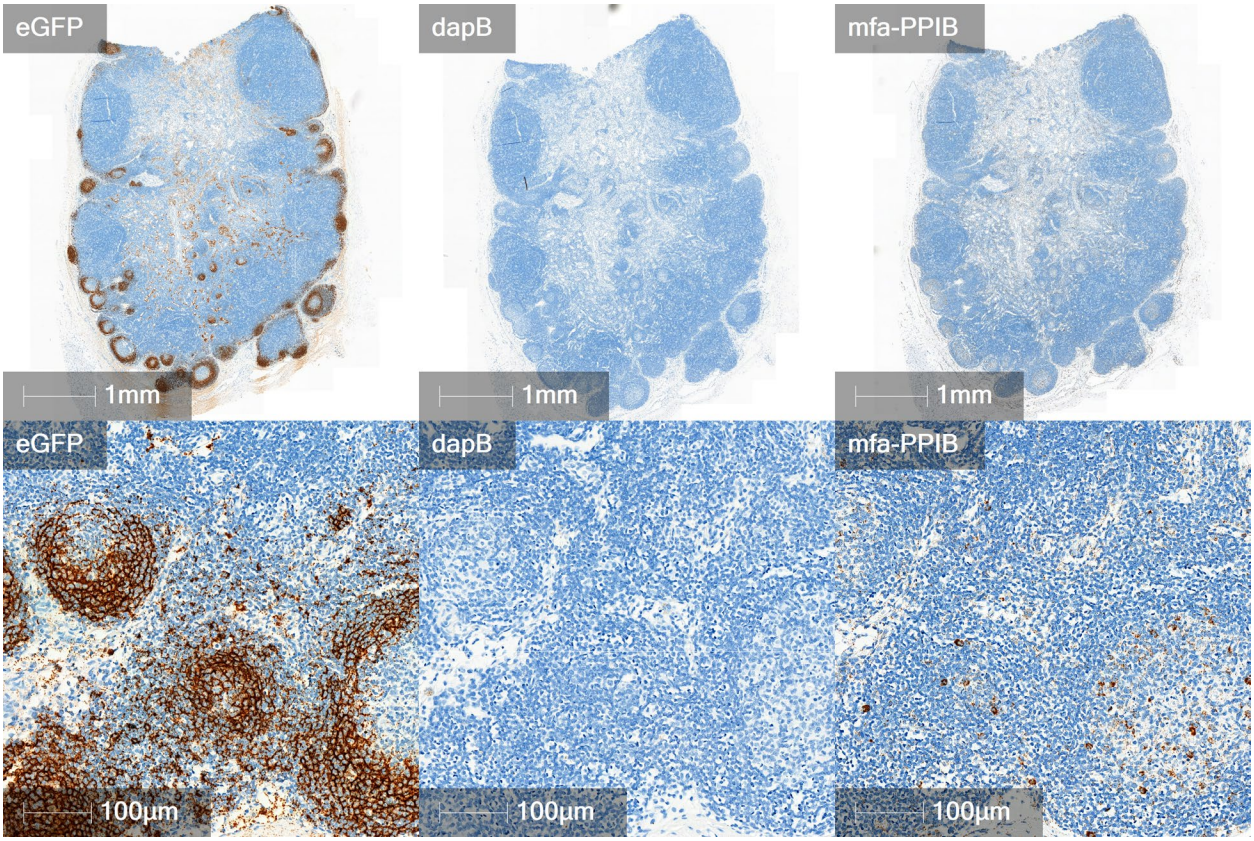


Figure S2. RNAscope and IHC eGFP labeling of NHP muscle at the injection site with technical controls. (A) Comparison of eGFP and control RNAScope probes which include the bacterial gene DapB (negative control) and the housekeeping gene *Macaca fascicularis* peptidylprolyl isomerase B (cyclophilin B) (mfa-PPIB) (positive control). (B) Comparison of anti-eGFP antibody and IgG isotype control staining at injection site. Area shown in insets is indicated in the low magnification images. Scalebars, 1 mm and 100 µm.

A.


eGFP
dapB
mfa-PPIB
1mm
1mm
1mm
eGFP
dapB
mfa-PPIB
100µm
100µm
100µm

B.

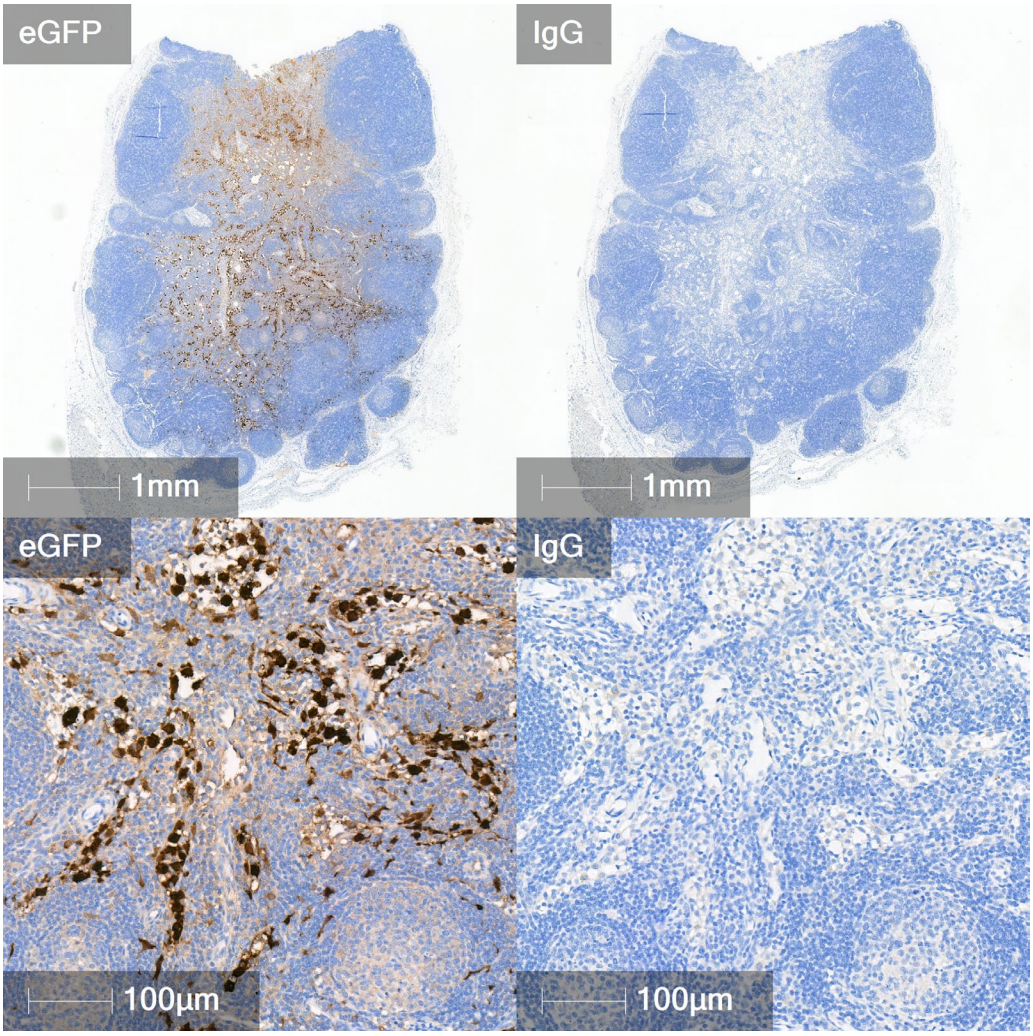


Figure S3. RNAscope and IHC eGFP labeling of NHP lymph node tissue with technical controls. (A) Comparison of eGFP and control RNAScope probes which include the bacterial gene DapB (negative control) and the housekeeping gene *Macaca fascicularis* peptidylprolyl isomerase B (cyclophilin B) (mfa-PPIB) (positive control). (B) Comparison of anti-eGFP antibody and IgG control staining in lymph node. Area shown in insets is indicated in the low magnification images. Scalebars, 1 mm and 100 µm.