Biodistribution, pharmacokinetics, and blood compatibility of native and PEGylated tobacco mosaic virus nano-rods and -spheres in mice

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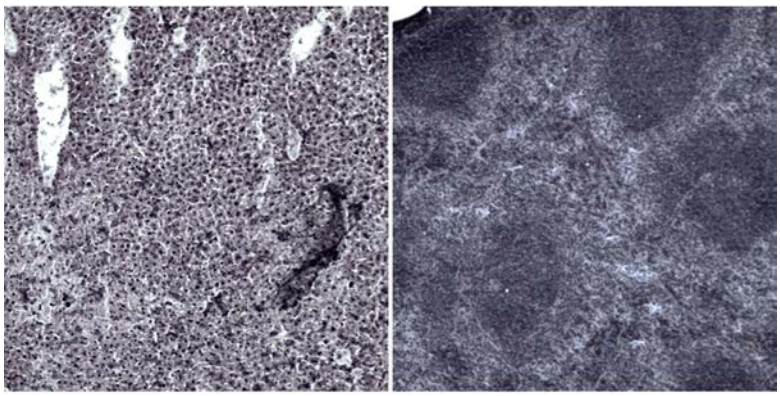
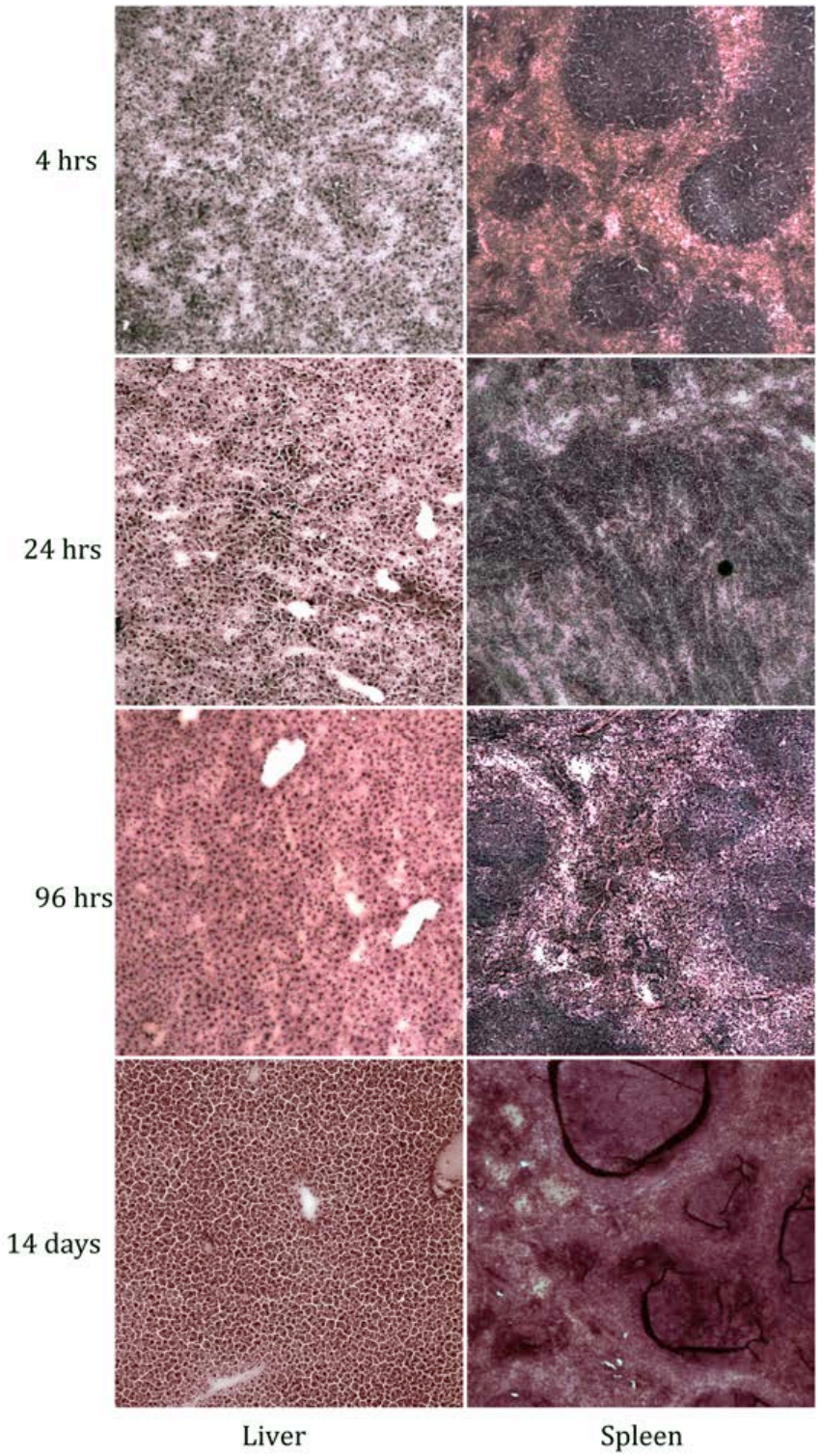
Particle formulation	Time post administration	Tissue analyzed	Cell Stain	M2 co-localization coefficient
Cy5-TMV	4	Liver	F4/80	0.719
Cy5-TMV	24	Liver	F4/80	0.753
Cy5-TMV	96	Liver	F4/80	0.753
Cy5-TMV	4	Spleen	F4/80	0.708
Cy5-TMV	24	Spleen	F4/80	0.172
Cy5-TMV	96	Spleen	F4/80	0.358
Cy5-TMV	4	Spleen	B220	0.351
Cy5-TMV	24	Spleen	B220	0.972
Cy5-TMV	96	Spleen	B220	0.981
PEG-Cy5-TMV	4	Liver	F4/80	0.567
PEG-Cy5-TMV	24	Liver	F4/80	0.963
PEG-Cy5-TMV	96	Liver	F4/80	0.013
PEG-Cy5-TMV	4	Spleen	F4/80	0.542
PEG-Cy5-TMV	24	Spleen	F4/80	0.98
PEG-Cy5-TMV	96	Spleen	F4/80	0.483
PEG-Cy5-TMV	4	Spleen	B220	0.828
PEG-Cy5-TMV	24	Spleen	B220	1
PEG-Cy5-TMV	96	Spleen	B220	1
Cy5-SNP	4	Liver	F4/80	1
Cy5-SNP	24	Liver	F4/80	0.959
Cy5-SNP	96	Liver	F4/80	0
Cy5-SNP	4	Spleen	F4/80	0.762
Cy5-SNP	24	Spleen	F4/80	0.254
Cy5-SNP	96	Spleen	F4/80	0.763
Cy5-SNP	4	Spleen	B220	0.602
Cy5-SNP	24	Spleen	B220	0.872
Cy5-SNP	96	Spleen	B220	1

Supporting Table 1 Manders' M2 colocalization of Cy5-TMV, PEG-Cy5-TMV, and Cy5-SNP with selected cell markers in cryosectioned spleens and livers.

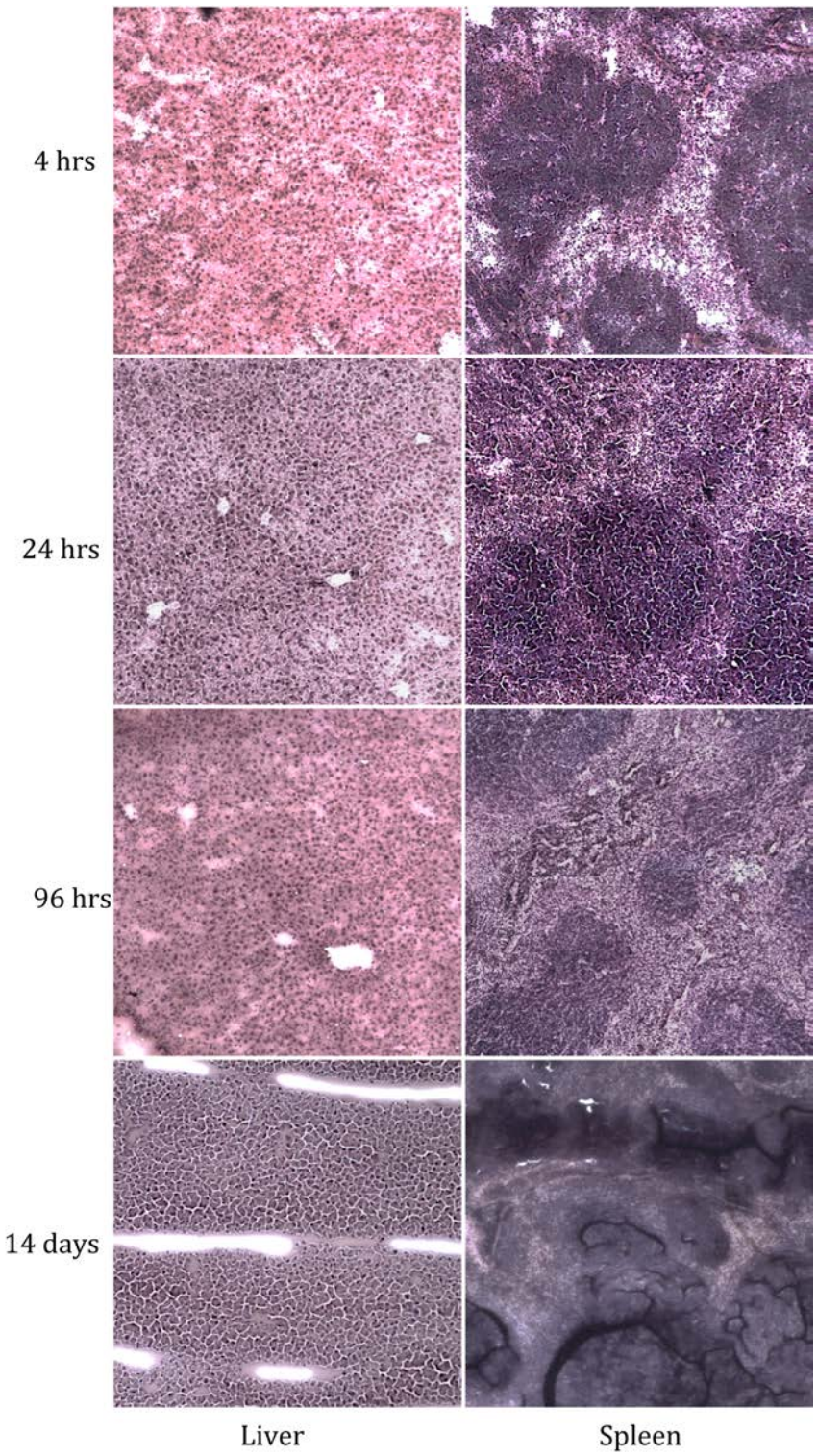


Liver Spleen

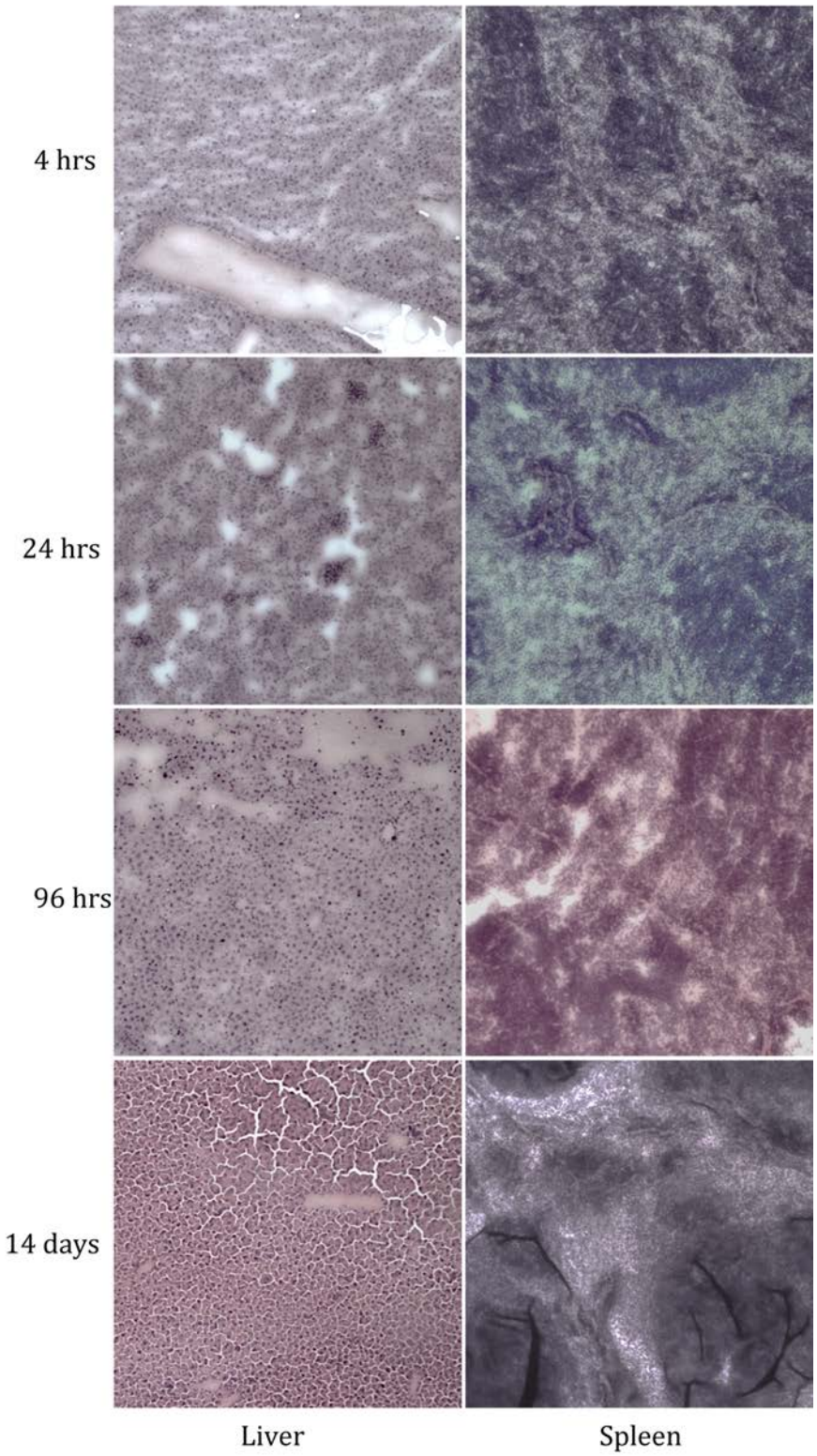
Supporting Figure 1 Excised liver and spleens from mice injected with PBS were fixed and stained using H&E. Images are 650 x 650 μm.



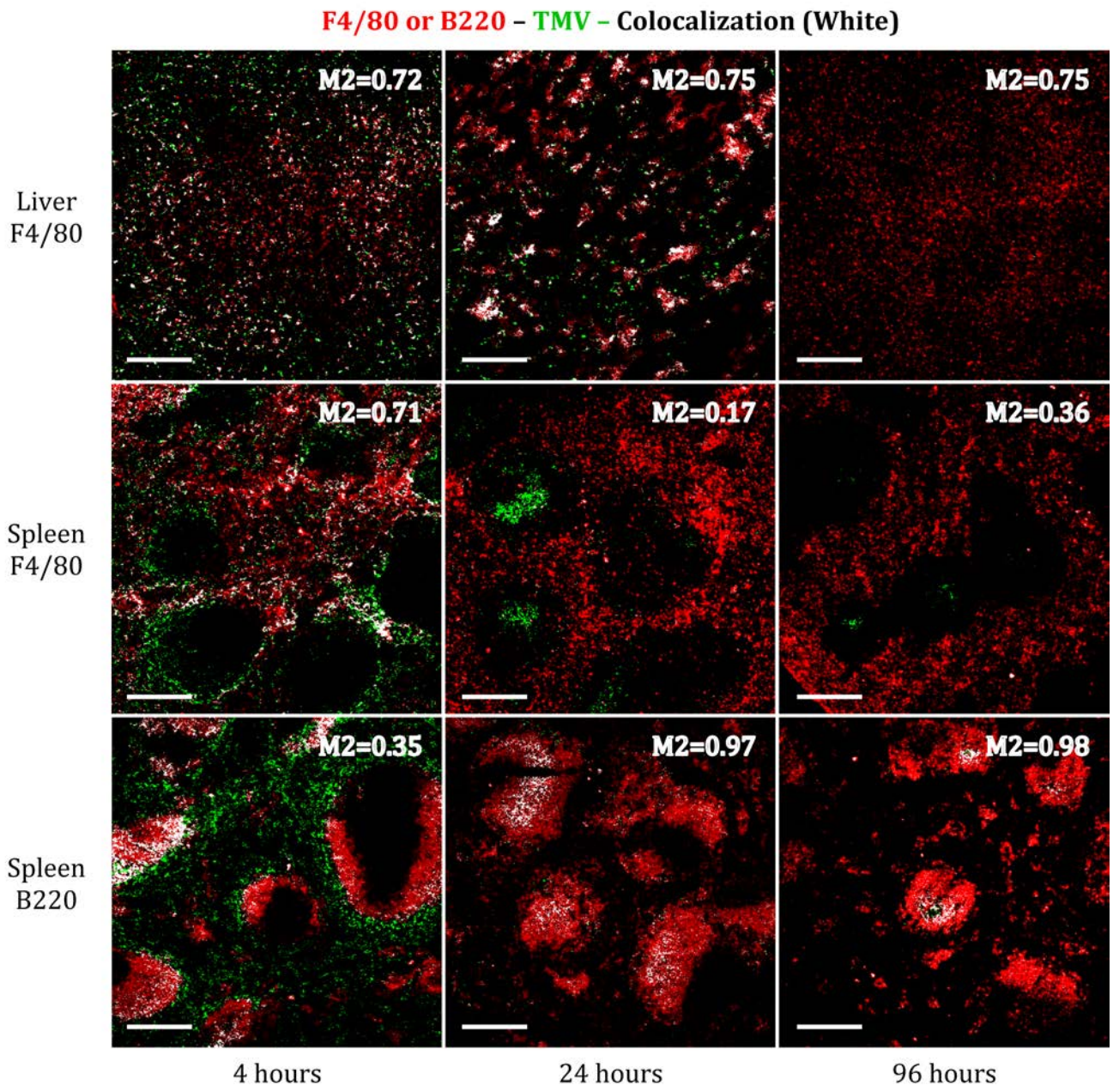
Supporting Figure 2 Excised liver and spleens from mice 4, 24, 96 hours, and 14 days after being injected with Cy5-TMV were fixed and stained using H&E. No visible signs of toxicity were noticed. Images are 650 x 650 μm.



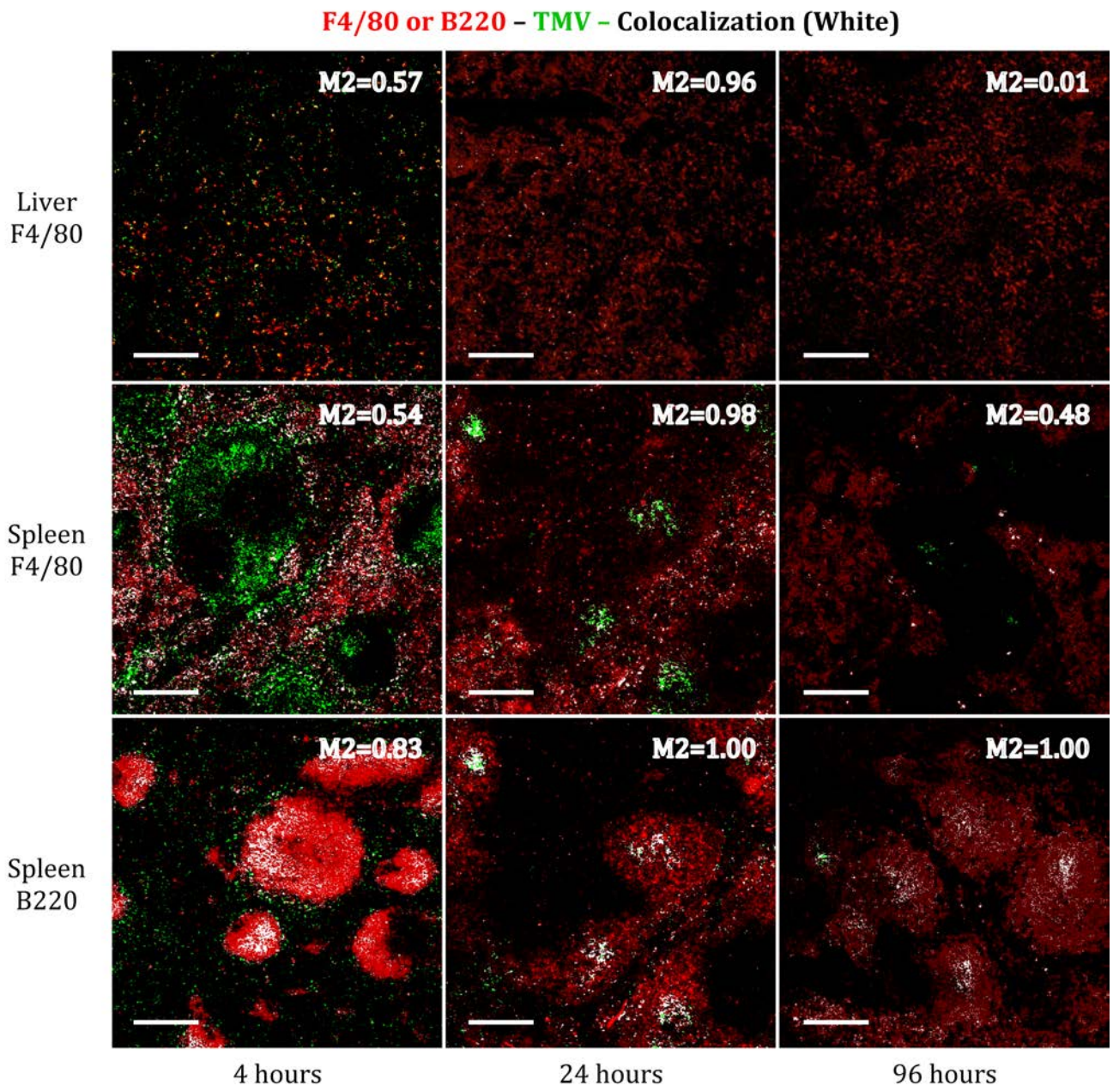
Supporting Figure 3 Excised liver and spleens from mice 4, 24, 96 hours, and 14 days after being injected with PEG-Cy5-TMV were fixed and stained using H&E. No visible signs of toxicity were noticed. Images are 650 x 650 μm.



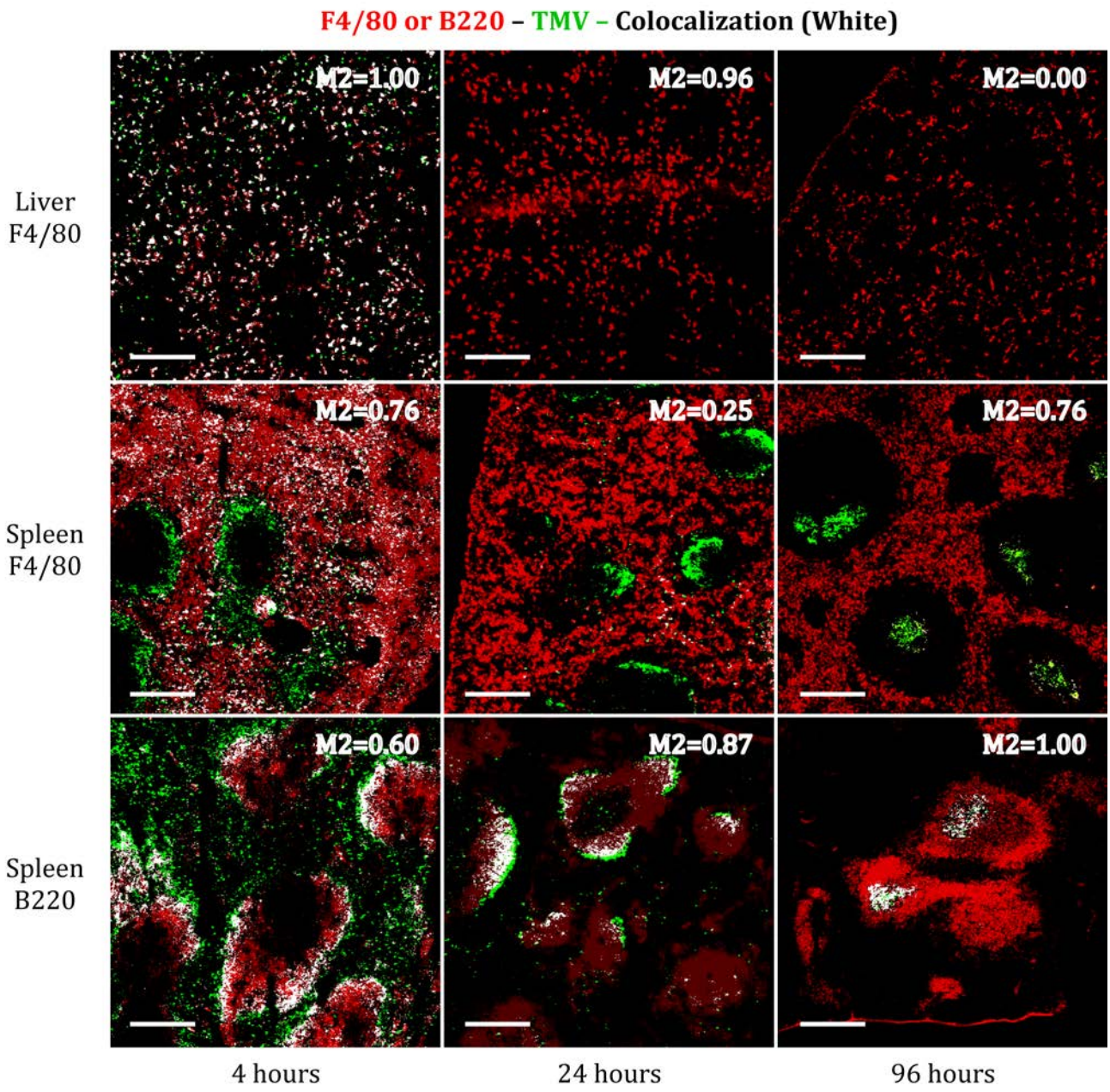
Supporting Figure 4 Excised liver and spleens from mice 4, 24, 96 hours, and 14 days after being injected with Cy5-SNP were fixed and stained using H&E. No visible signs of toxicity were noticed. Images are 650 x 650 μm.



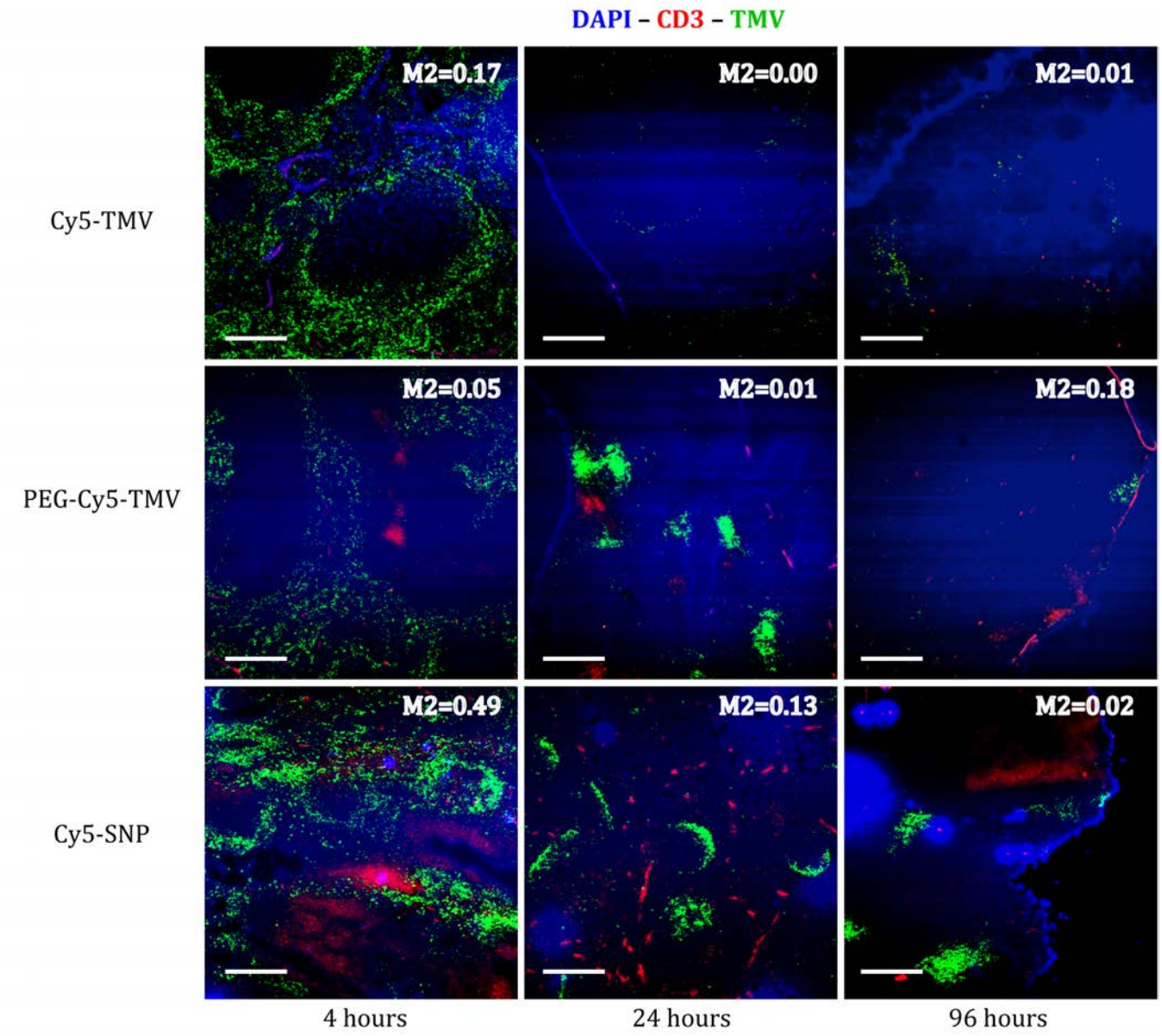
Supporting Figure 5 Liver and spleen sections 4, 24, and 96 hours after i.v. administration of Cy5-TMV. Liver sections were stained for macrophages marker F4/80 (red). Spleen sections were stained for B-cell marker B220 (red) and macrophage marker F4/80 (red). Areas of colocalization between Cy5-TMV and cell markers appears white. Scale bars = 250 μm.



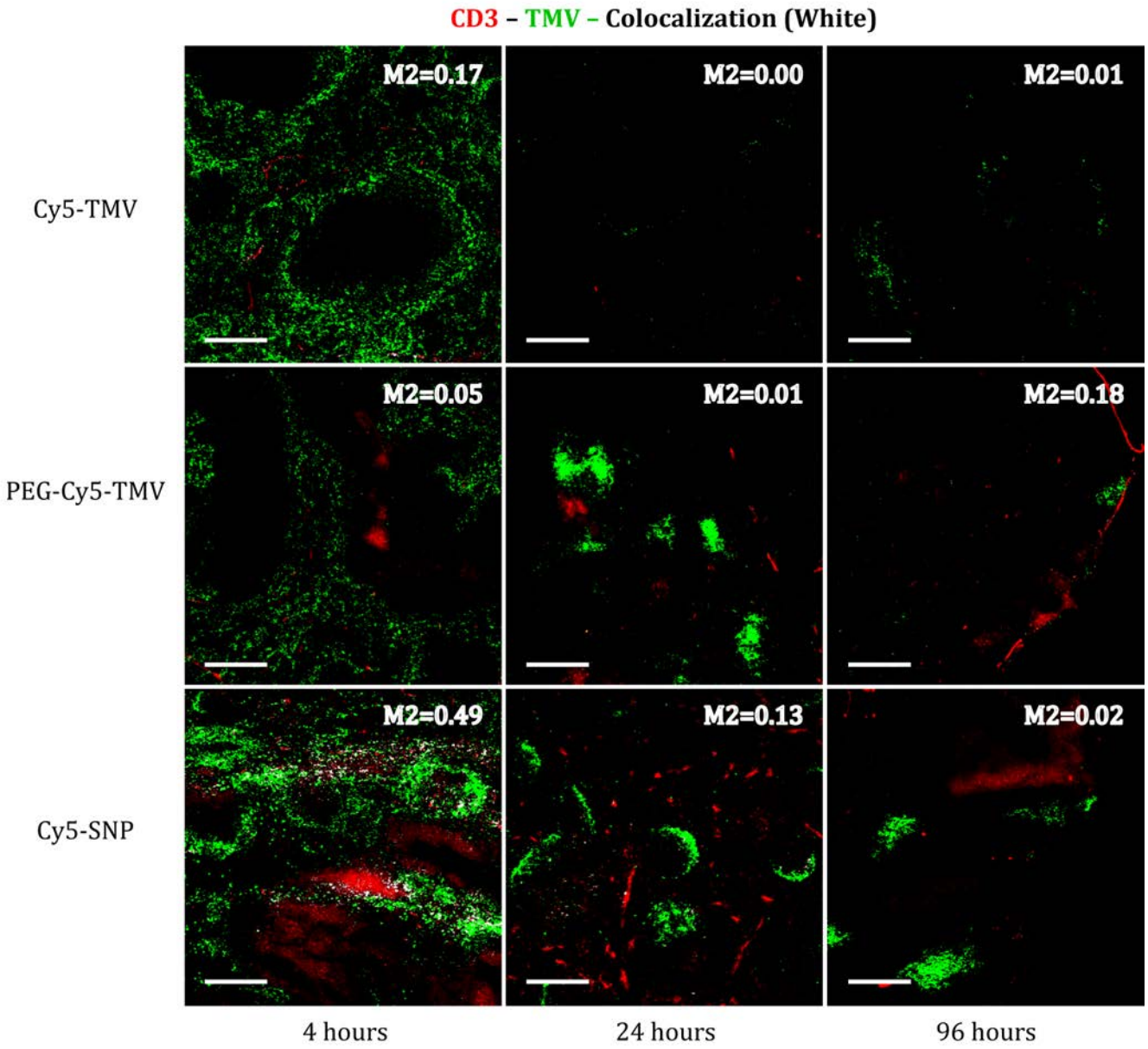
Supporting Figure 6 Liver and spleen sections 4, 24, and 96 hours after i.v. administration of PEG-Cy5-TMV. Liver sections were stained for macrophages marker F4/80 (red). Spleen sections were stained for B-cell marker B220 (red) and macrophage marker F4/80 (red). Areas of colocalization between PEG-Cy5-TMV and cell markers appears white. Scale bars = 250 μm.



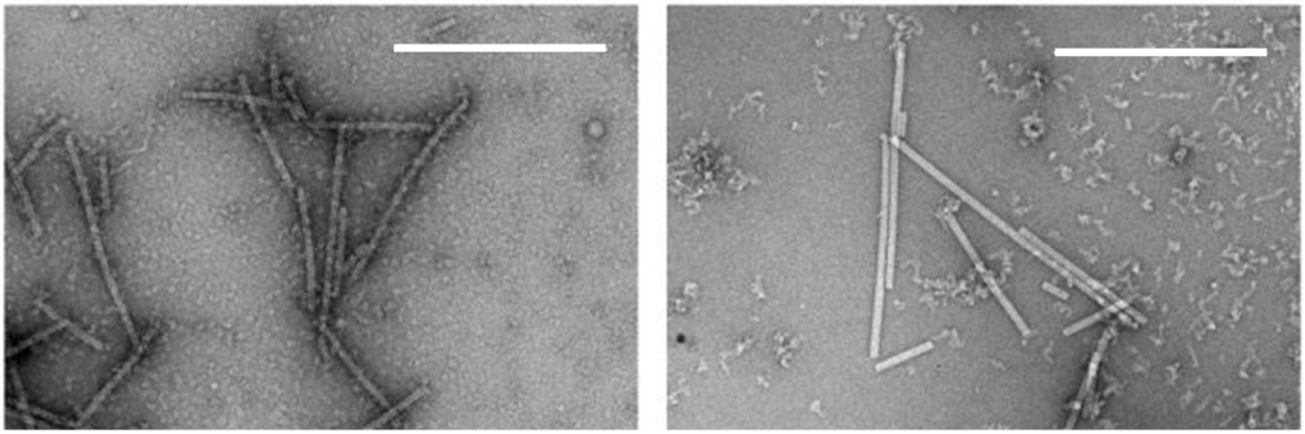
Supporting Figure 7 Liver and spleen sections 4, 24, and 96 hours after i.v. administration of Cy5-SNP. Liver sections were stained for macrophages marker F4/80 (red). Spleen sections were stained for B-cell marker B220 (red) and macrophage marker F4/80 (red). Areas of colocalization between Cy5-SNP and cell markers appears white. Scale bars = 250 μm.



Supporting Figure 8 Spleen sections 4, 24, and 96 hours after i.v. administration of Cy5-TMV, PEG-Cy5-TMV, and Cy5-SNP (colored green). Spleen sections were stained for T-cell marker CD3 (red). Cell nuclei are stained with DAPI (blue). Inset into each image is the Manders' M2 colocalization coefficient (M2=1.0 is attributed to 100% colocalization). Scale bars = 250 μm.



Supporting Figure 9 Spleen sections 4, 24, and 96 hours after i.v. administration of Cy5-TMV, PEG-Cy5-TMV, and Cy5-SNP (colored green). Spleen sections were stained for T-cell marker CD3 (red). . Areas of colocalization between Cy5-SNP and cell markers appears white. Scale bars = 250 μm.



Supporting Figure 10 TMV rods were incubated with plasma from mice. Native plasma (left panel) and heat inactivated plasma (right panel) was considered. The TMV samples were exposed to plasma for three hours, then collected through ultracentrifugation, followed by subsequent analysis by TEM imaging. Negatively-stained (UAc) samples were collected on a Tecnai G-2 Spirit (FEI Co.) equipped with a 2Kx2K CCD camera (TVIPS F224) at 29,000x magnification. The scale bar is 500 nm.