Development of an Immunologically Tolerated Combination of Fluorescent Proteins for *In vivo* Two-photon Imaging

Selamawit Gossa[†], Debasis Nayak[†], Bernd H. Zinselmeyer and Dorian B. McGavern

MOVIE LEGENDS:

Movie 1. Two-photon imaging of a suboptimal color combination. Time lapses were captured by two-photon microscopy in the lymph nodes of day 7 LCMV-infected CD11c-YFP mice seeded with CFP^+ and GFP^+ P14 cells. The four individual channels (Ch1 to 4) show the fluorescence emission light received by the NDD4 detector array following separation by dichroic mirrors (see Fig. 3A). The upper and lower panels depict a representative time lapse before (upper) and after (lower) spectral unmixing. Note the failure of the unmixing algorithm to spectrally reassign the colors to their original channels.

Movie 2. Two-photon time lapses depicting optimal spectral reassignment. Time lapses were captured by two-photon microscopy in the lymph nodes of day 7 LCMV-infected CD11c-YFP mice seeded with $mTFP1^+$ and $mOrange^+$ P14 cells. The upper and lower panels depict a representative time lapse before (upper) and after (lower) spectral unmixing. Note the unmixing algorithms ability to reassign all of colors to four spectrally distinct channels (Ch1 – grayscale, Ch2 – blue, Ch3 – red, Ch4 – blue).