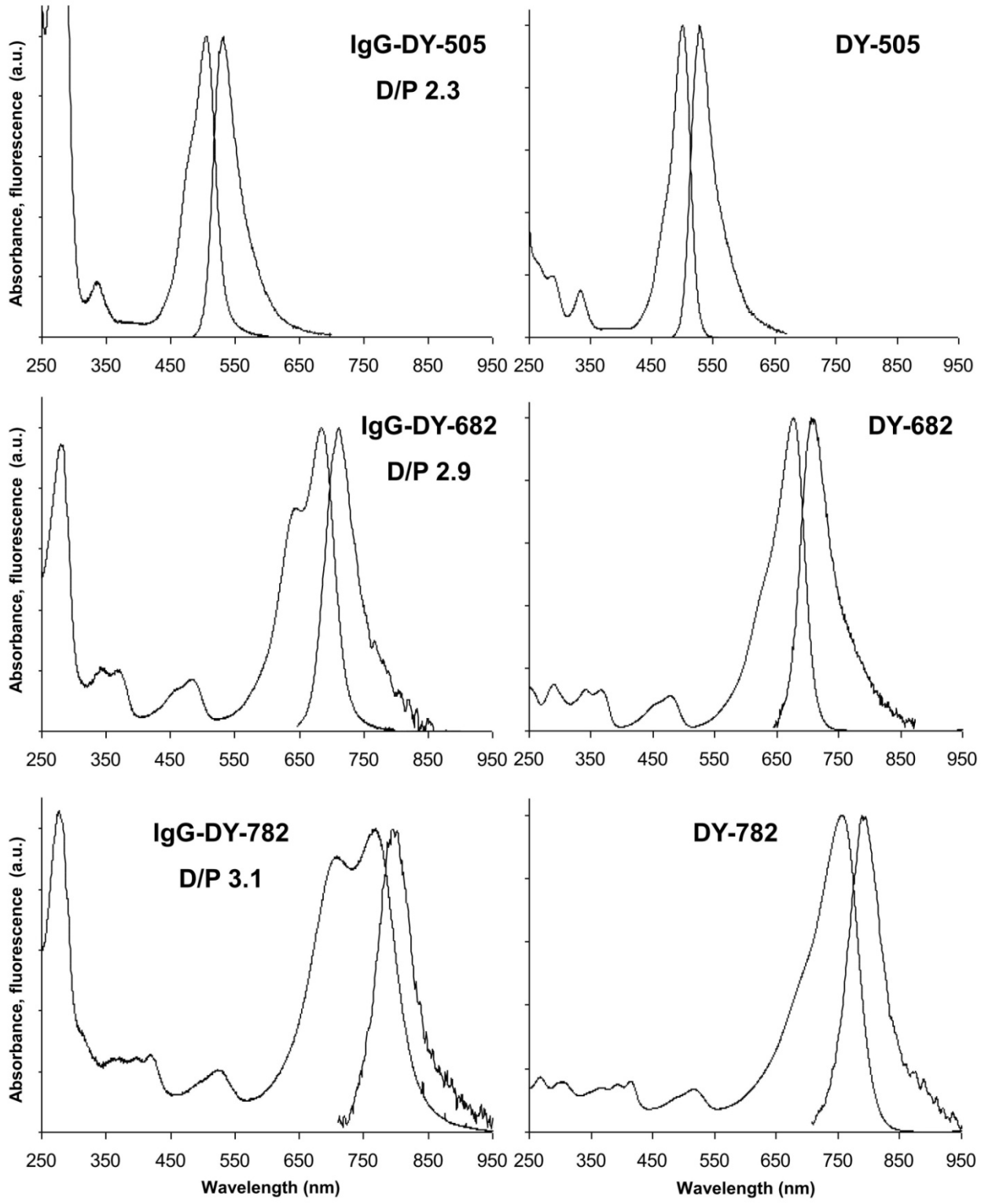
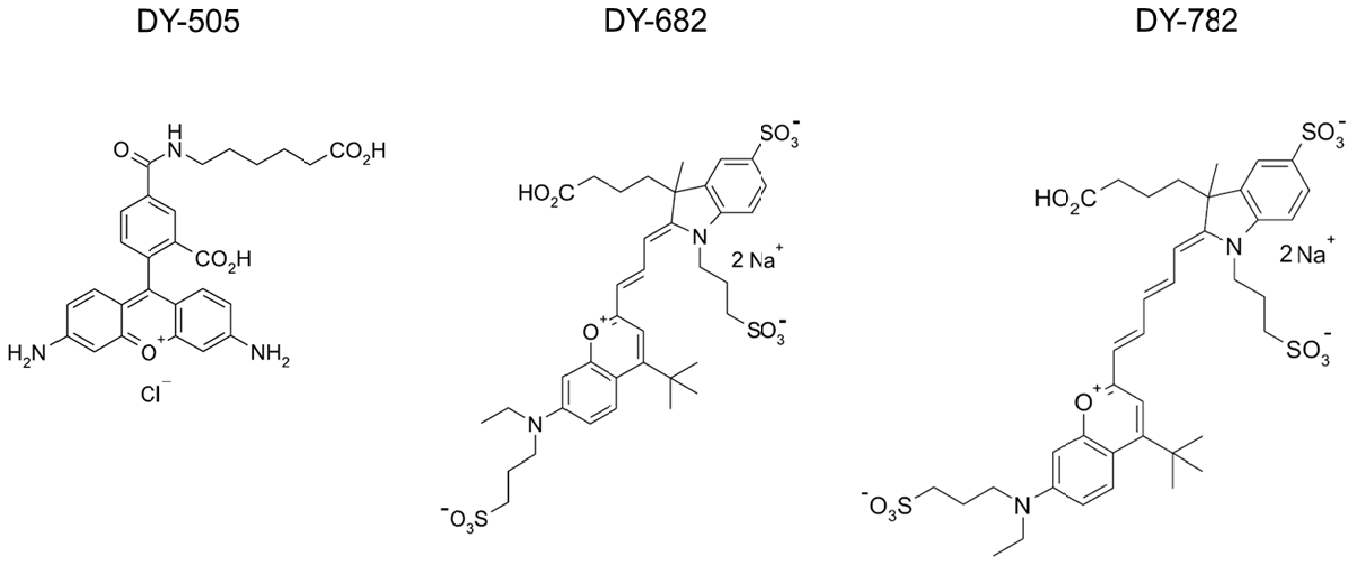
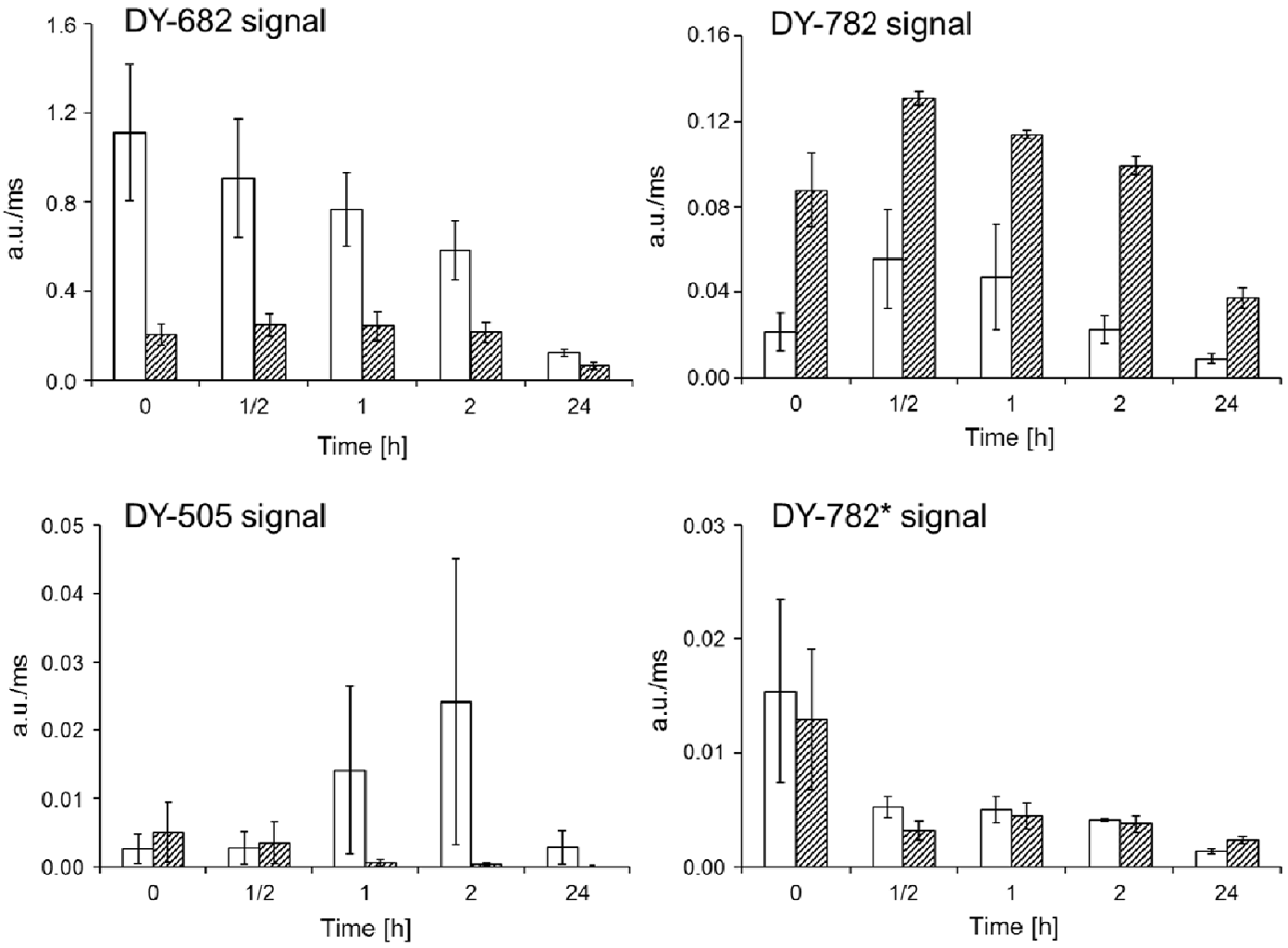


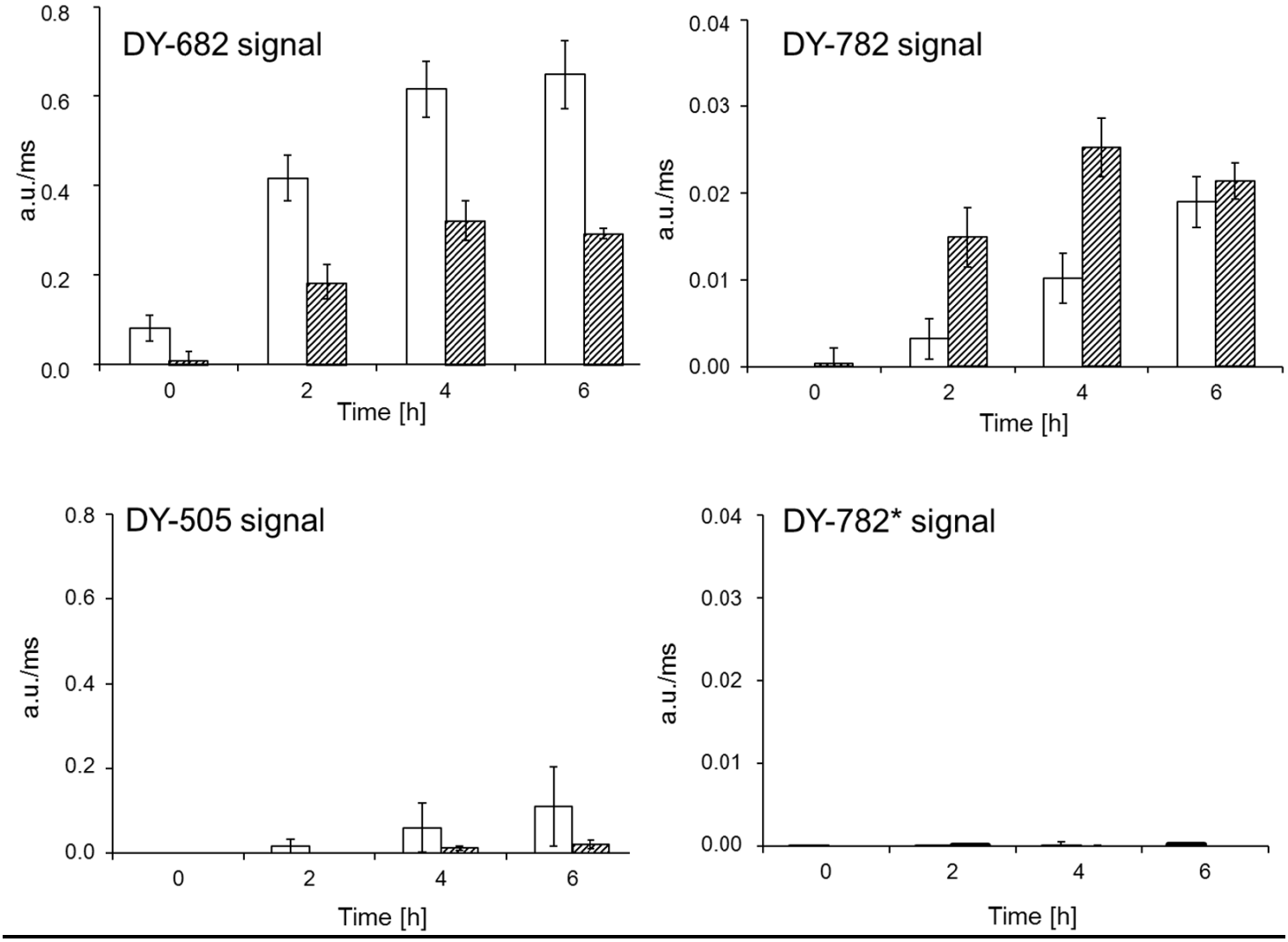
Supplemental Figure 1. Normalized absorption and fluorescence spectra of the IgG-dye conjugates (left panels) and the non-conjugated dyes (right panels) in PBS. Excitation was always at the vibronic shoulder of the longest wavelength absorption maximum. The dye-to-protein (D/P) ratios of the conjugates were determined photometrically.



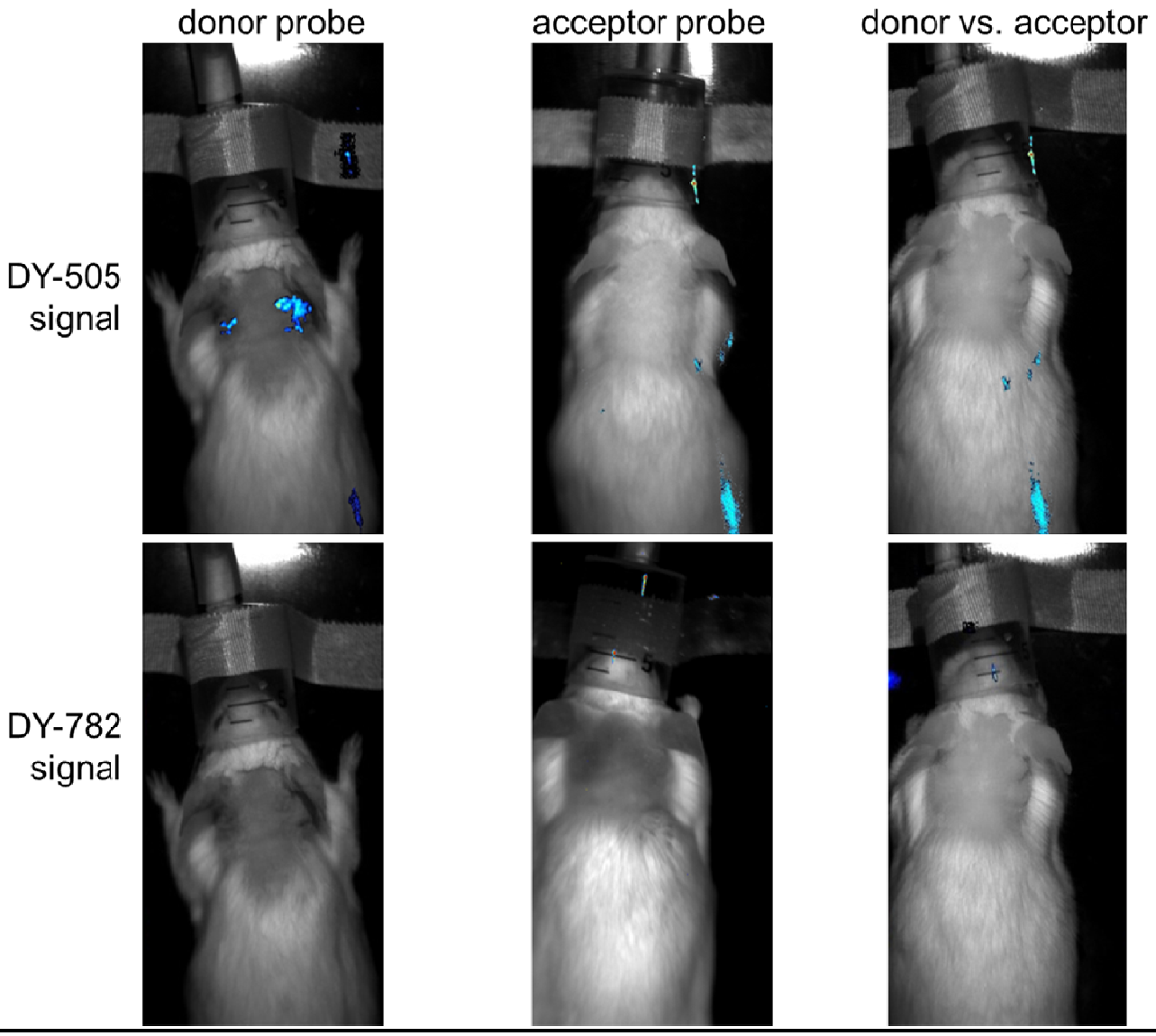
Supplemental Figure 2. Chemical structures of DY-505, DY-682, and DY-782.



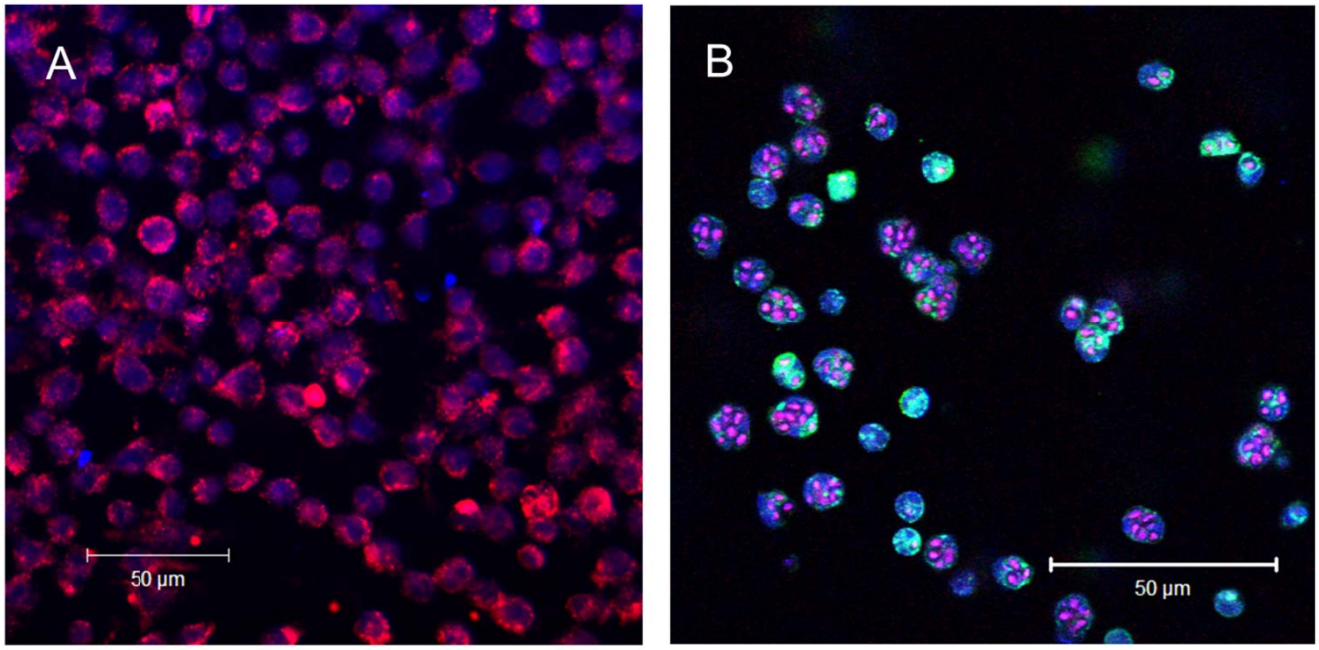
<u>Supplemental Figure 3.</u> FRET effects are detectable for a period of 24 h in a local *in vivo* mouse model. Fluorescence intensity was monitored from over a period of 24 h after injection of labeled macrophages. Semi quantitative analysis of donor fluorescence (682 signal) and acceptor fluorescence intensity (782 signal and DY-782* signal, for details see Methods) alone (white bars) or in combination (donor vs. acceptor, striated bars). Values are mean average values of three different mice and standard error of the mean. Top: analysis of IgG-DY-682 & IgG-DY-782; bottom: analysis of control couple IgG-DY-505 & IgG-DY-782.



Supplemental Figure 4. **Semiquantitative analysis of fluorescence intensities after systemic probe application in an inflammatory ear edema *in vivo* mouse model.** After injection of both zymosan in the left ear lobe and fluorescent probes in the tail vein (55 nmol dye per kg body weight), mice were imaged using a whole body NIRF imaging system. Semiquantitative analysis of the fluorescence intensities of donor (682 signal) and acceptor (782 signal and DY-782* signal) was carried out for each probe alone (white bars) and in combination (donor vs. acceptor, striated bars). The fluorescence intensity is presented as the average signal per exposure time (ms). The values present mean average values of 3 different mice and standard error of the mean. Top: IgG-DY-682 & IgG-DY-782; bottom: control couple IgG-DY-505 & IgG-DY-782.



Supplemental Figure 5. **After systemic application of IgG-DY-505 & IgG-DY-782, no FRET effects occur in an inflammatory ear edema *in vivo* mouse model.** Representative images of DY-505 and DY-782 signals of representative mice injected with IgG-DY-505 and IgG-DY-782 (55 nmol dye per kg body weight) obtained 6 h post application using a whole body NIRF imaging system. Overlay of fluorescence (false colors: blue: low and red: high fluorescence intensity) and white light images. From left to right: localization of the donor (left), the acceptor (middle), and the donor in the presence of the acceptor (right).



Supplemental Figure 6. Phagocytes internalize IgG-DY-probes. A) J774 macrophages after 24 h of incubation with 1.2 nmol/ml IgG-DY-682 and B) monocytes/macrophages isolated from edematous areas after local application of zymosan A and intravenous application of IgG-DY-682 into mice (55 nmol/kg body weight) and isolation of mononuclear cells. Blue: DNA staining with Hoechst33258 dye, green: marker for monocytes/macrophages (MOMA-2), red: IgG-DY-682.