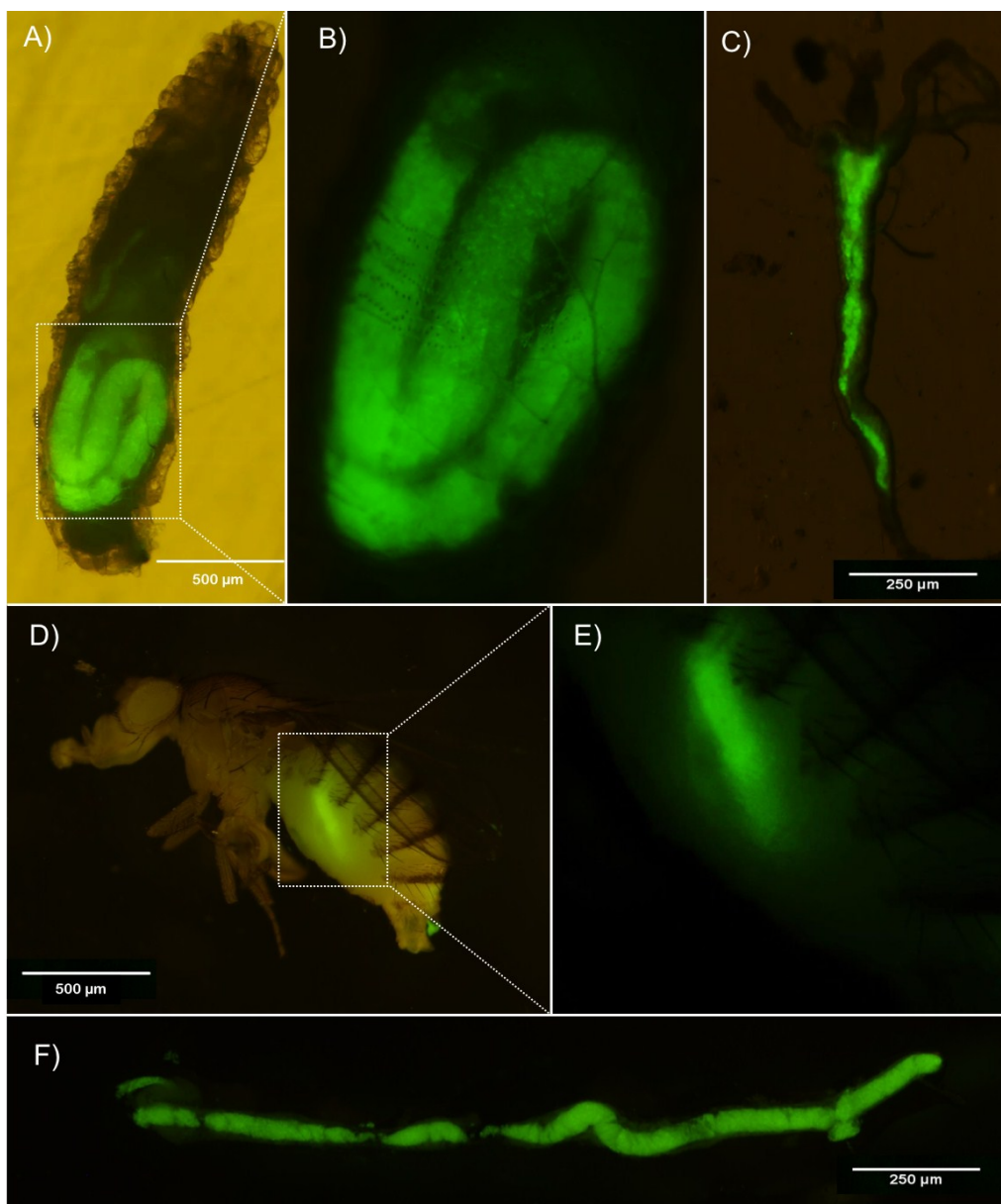


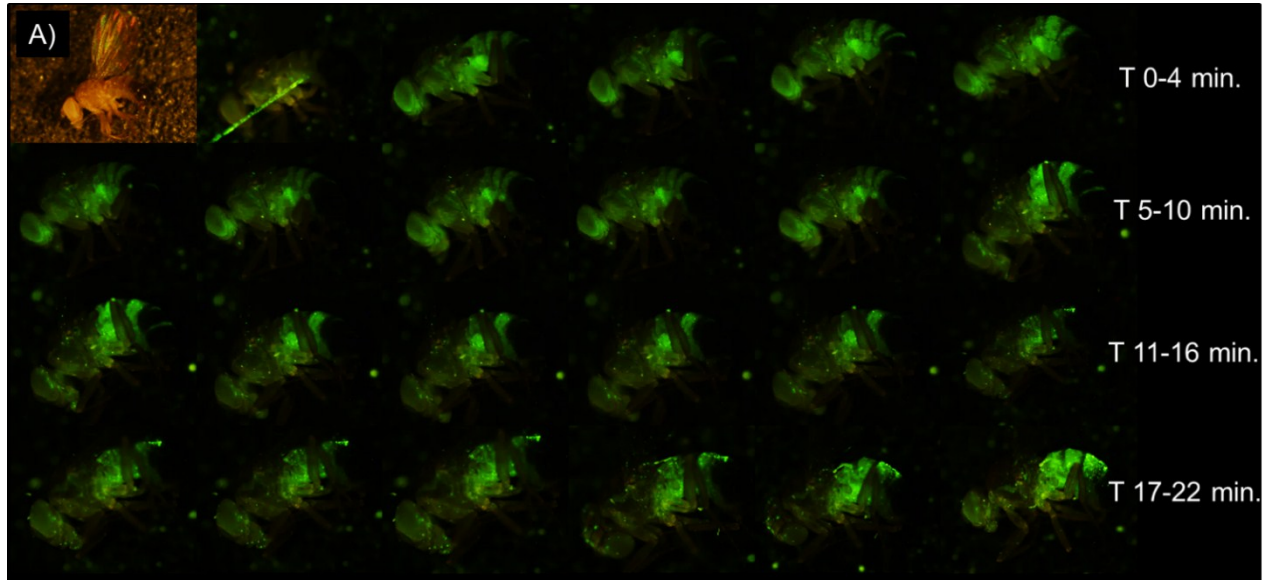
## Supplementary material

### Yeast glucan particles enable intracellular protein delivery in *Drosophila* without compromising the immune system

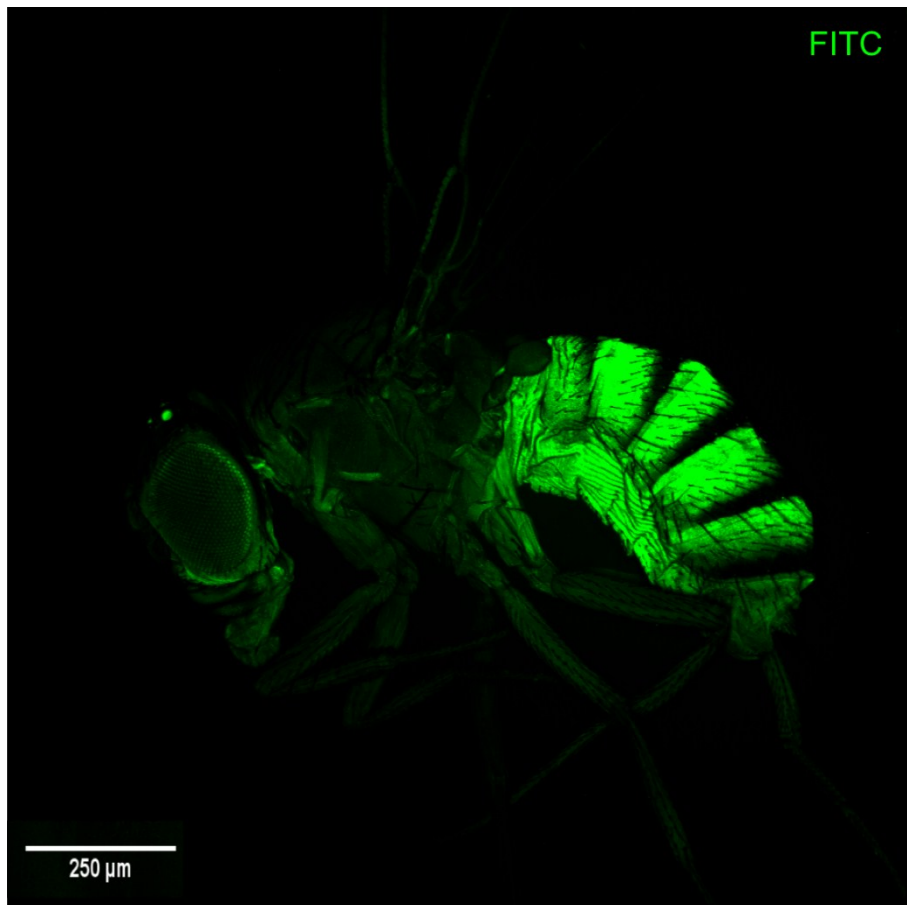
Adam Bajgar, Ivan Saloň, Gabriela Krejčová, Tomáš Doležal, Marek Jindra, František Štěpánek



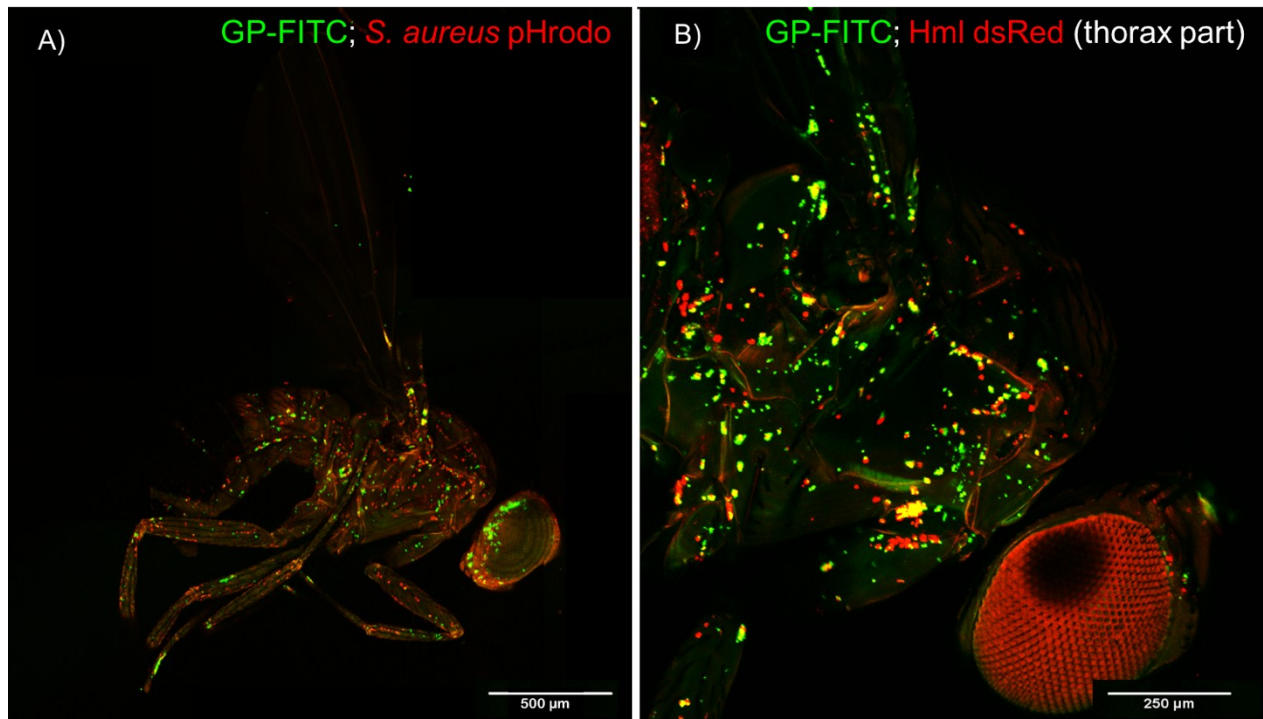
**Fig. S1.** Since flies possess a functionally developed gastro-intestinal tract, it was tried to deliver glucan particles into the *Drosophila* circulation by feeding (100 uL of 1% GP-FITC particle suspension mixed with a cornmeal diet in ratio 1:9). However, this simple way of delivery system was not effective. Even in individuals held on a high fat diet, resulting in higher permeabilisation of the gut wall, the glucan particles remained in the gut and were excreted after a few hours. **(A, B)** Binocular microscope image of GP-FITC fed larvae. **(C)** The same is documented also on dissected gut. **(D, E)** The same pattern has been observed also in adult flies where GP-FITC particles cannot get through the gut wall barrier similarly as in larvae. **(F)** The same is documented also on the dissected gut. The confocal images are representative of more than 15 analysed individuals.



**Fig. S2.** Time progress of a typical injection event. FITC labeled glucan particles spread through the body and within twenty minutes they are distributed in all the body compartments including distal parts. Binocular microscope images were collated into a time series. The image sequence is representative images of more than 15 analysed individuals.



**Fig. S3.** Fly injected with a 6M FITC solution in PBS. Colocalisation is not seen in this case and the body of the fruit fly is uniformly fluorescent.



**Fig. S4.** Colocalisation of labeled glukan particles with macrophages and phagocytic spots was verified by two approaches. **(A)** Fly coinjected with GP-FITC particles and pHrodo labeled *S. aureus* cell wall lysates. High level of colocalisation can be seen in this case. **(B)** GP-FITC particles were injected into the fly with a genomic construct Hml dsRed marking the nuclei of macrophages by a red fluorescent protein. Both confocal images are representative of more than 15 analysed individuals.

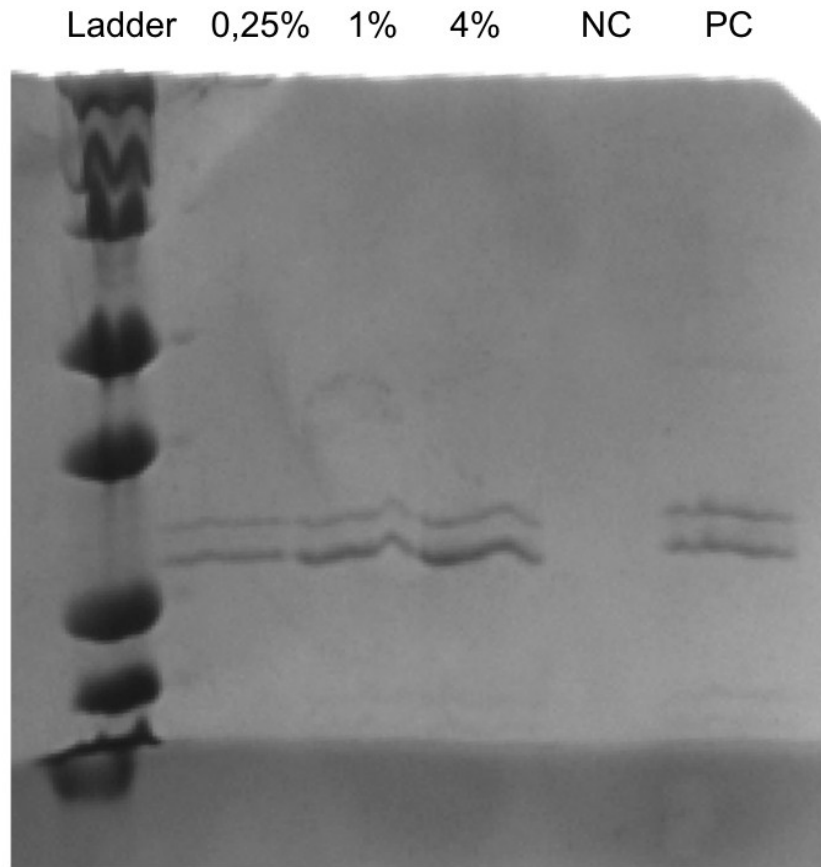


Fig. S5. The amount of mCherry protein produced in response to GP-FITC Gal4 loaded particles was determined by pull down assay and analysis of extracted proteins on SDS PAGE using RFP Trap (Chromotec) as described in Section 5.12. The observed size of the trapped protein corresponds to the expected size (34 kDa) of the protein and the data show that the amount of the protein is increasing with the number of injected particles into the living flies. The GP-FITC Gal4 loaded particles were injected to the flies in concentrations of 0.25 %, 1 % and 4 % (w/w) as indicated. The negative control (NC) were flies without mCherry expression, the positive control (PC) were flies naturally expressing mCherry.

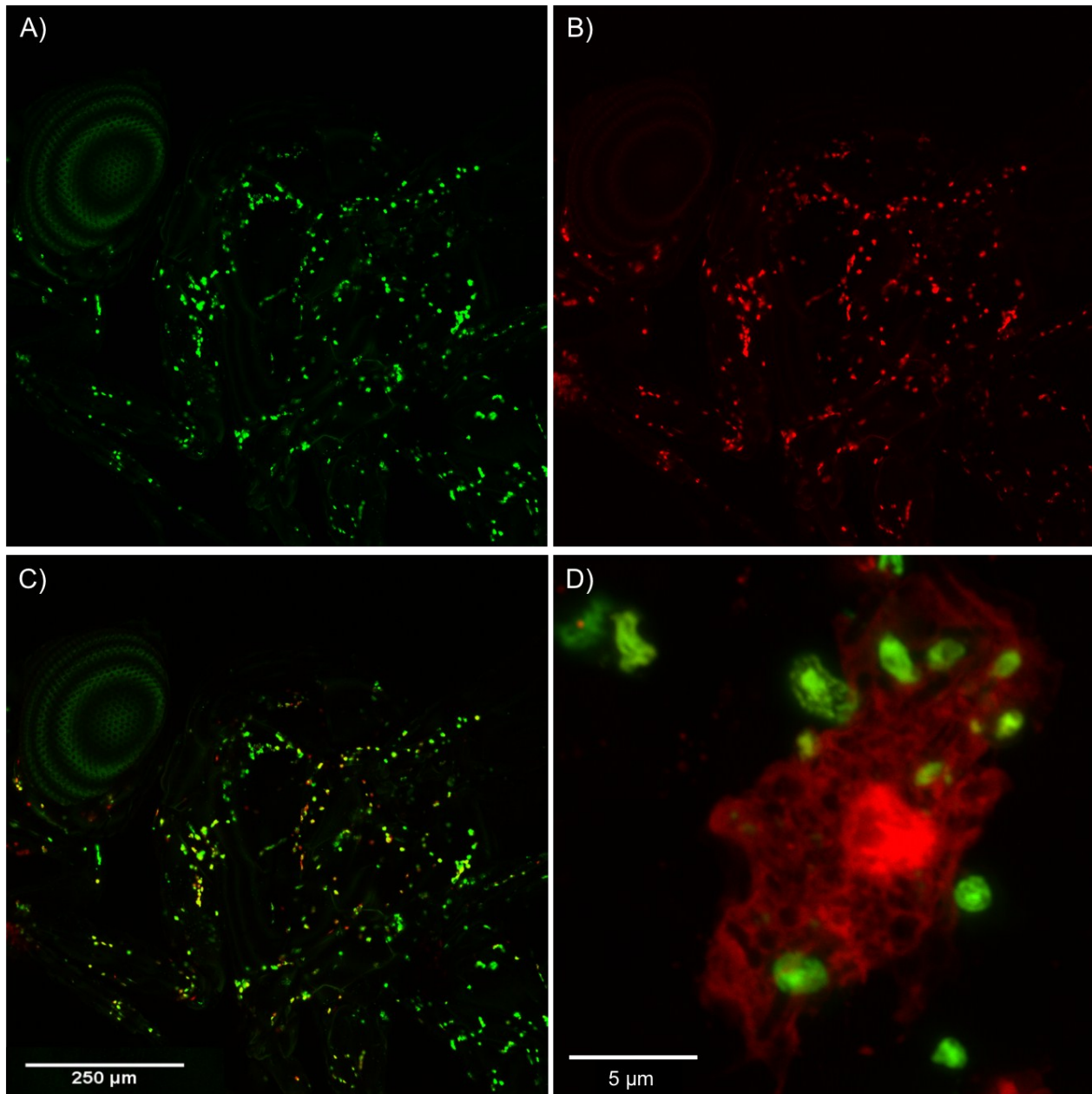


Fig. S6. Delivery of Gal4 by GP-FITC into macrophages with mCherry expression. A) Green signal of GP-FITC. B) Red signal of mCherry expressing cells. C) Merge of green and red signal. D) Detail of one macrophage targeted with GP-FITC and expressing mCherry.



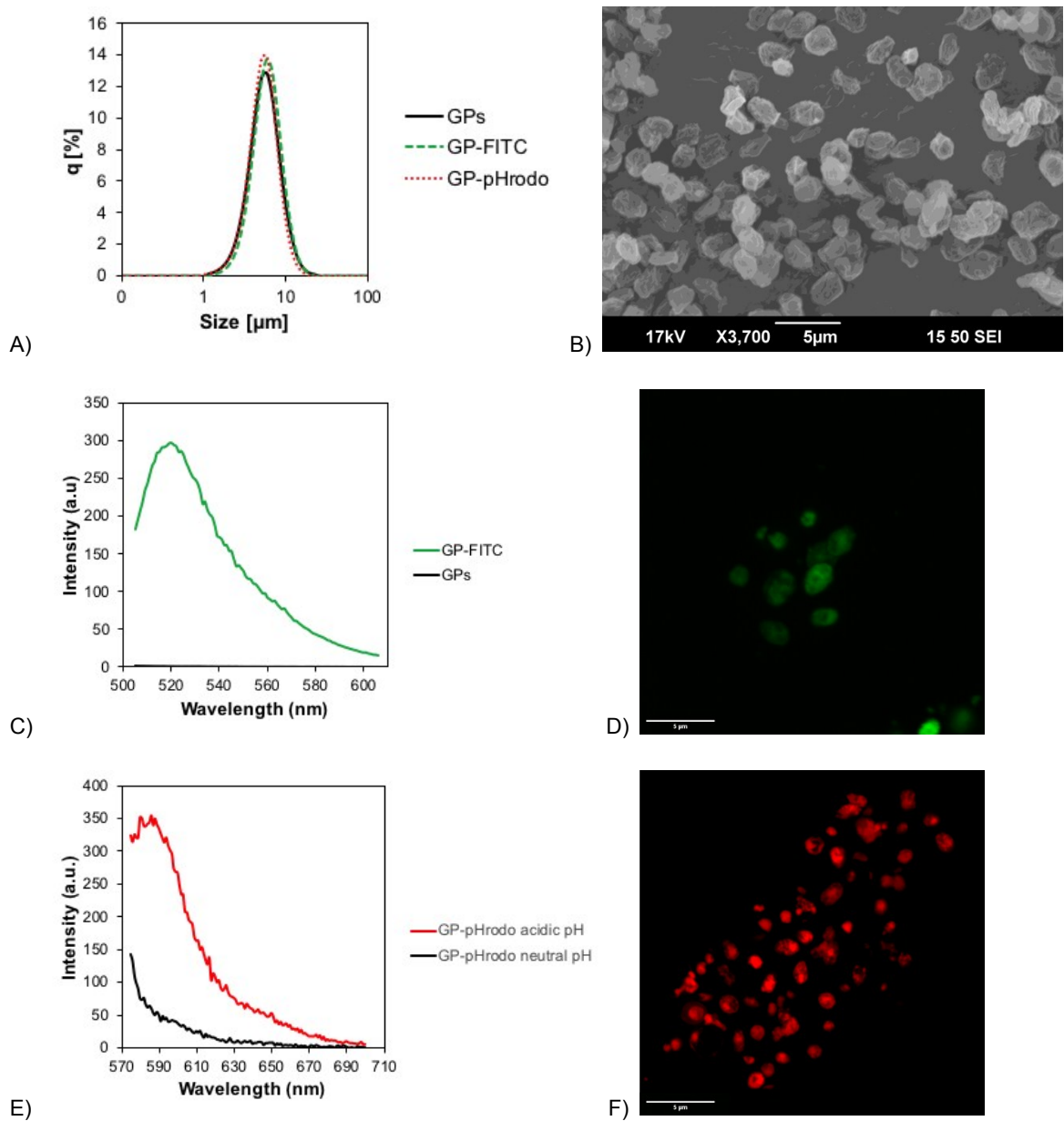


Fig. S7. A) Comparison of particle size distribution of plain and fluorescently labeled GPs, demonstrating that the attachment of fluorescent probes did not influence the colloidal stability of glucan particles. The size distributions were obtained by static light scattering (Horiba Partica LA 950/S2) in the wet state after dispersion in PBS by sonication. B) SEM micrograph of plain GPs for reference. C), E) Emission spectra of plain and fluorescently labeled GPs, demonstrating the successful attachment of fluorescent probes. GPs were suspended in PBS and fluorescence spectra were acquired by a Carry Eclipse (Agilent Technologies) fluorescence spectrophotometer with excitation wavelengths 488 nm for GP-FITC and 560 nm for GP-pHrodo. D), F) LSCM micrographs of fluorescently labeled GPs, demonstrating the successful attachment of fluorescent probes without affecting the particle size or morphology.