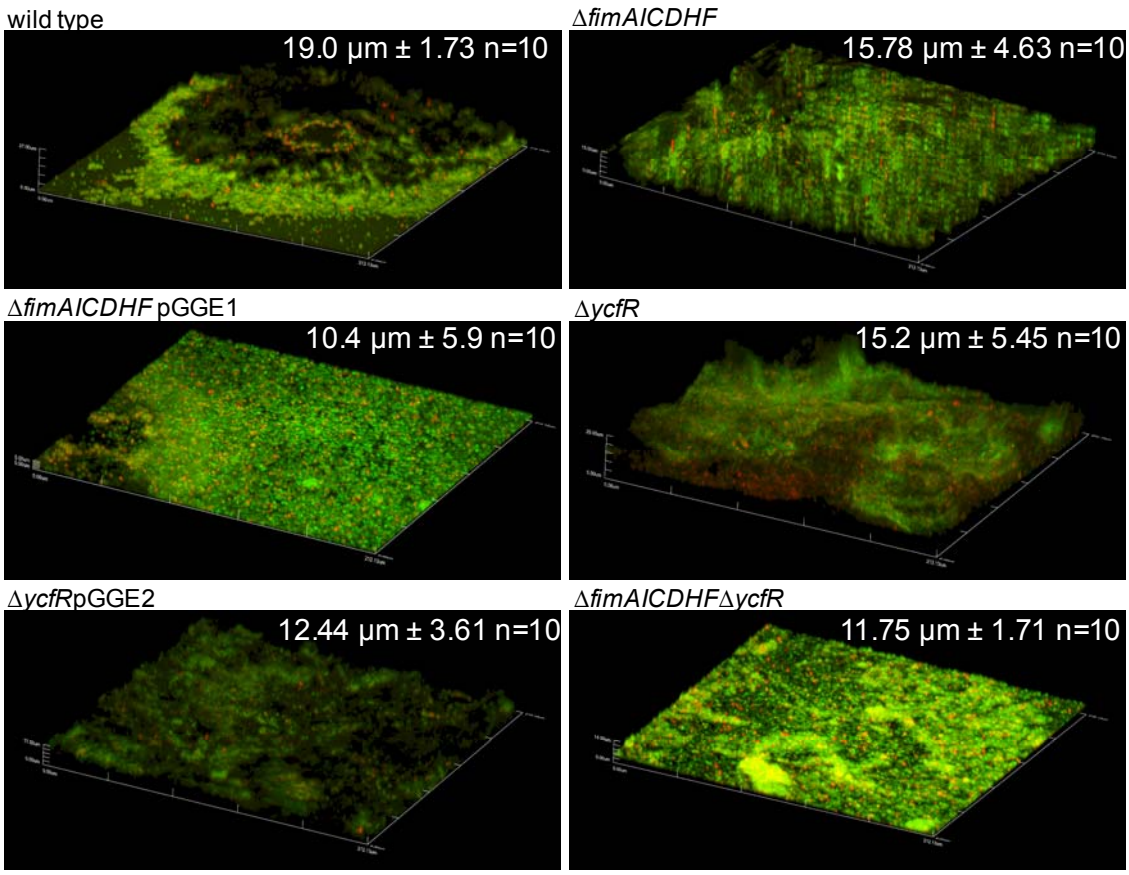

Figure S1. Biofilms from Type 1 fimbriae and *ycfR* mutants at 72h post-inoculation. Representative confocal images of biofilms on cholesterol coated-surfaces produced by the wild type, mutants and complemented strains after 72h of flow in the presence of bile. Biofilms were stained with live/dead stain (Invitrogen), fixed with 4% paraformaldehyde and observed by confocal microscopy. All strains harbor the empty vector (pWSK29 or pWSK129) or the respective complementation vector (pGGE1 and pGGE2 for *fimAICDHF* and *ycfR*, respectively). All images have a magnification of 40x. All mutants showed more biomass but similar thickness as the wild-type. Δ*ycfR* also showed increased cell damage/death (increased red staining). The complemented strains demonstrated a reduction of biofilm thickness and biomass.

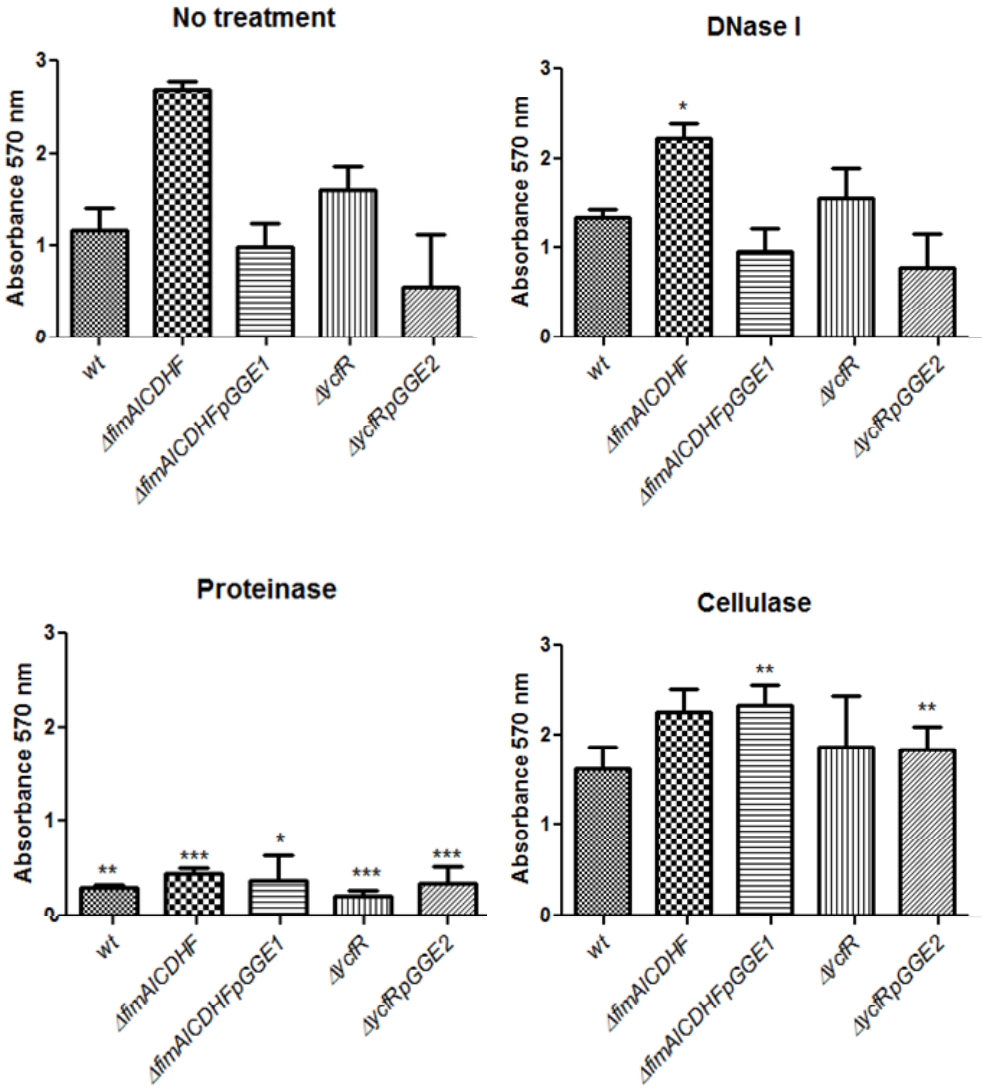

Figure S2. Proteinase treatment of established biofilms drastically affected biofilm formation of the wild-type, type 1 fimbriae and *ycfR* mutants. Inhibitory properties of DNase I, proteinase and cellulase on 24 h biofilms formed by the wild-type, mutants and complemented strains on cholesterol surfaces (plus bile). Enzymatic treatment of established biofilms was performed for 16 h at 37°C. All strains harbor the empty vector (pWSK29 or pWSK129) or the respective complementation vector (pGGE1 and pGGE2 for *fimAICDHF* and *ycfR*, respectively). Biofilm formation was determined by the crystal violet staining method. Experiments were performed by triplicate and repeated twice. Means between conditions with no treatment and with the respective enzyme treatment were compared by a Student's *t* test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

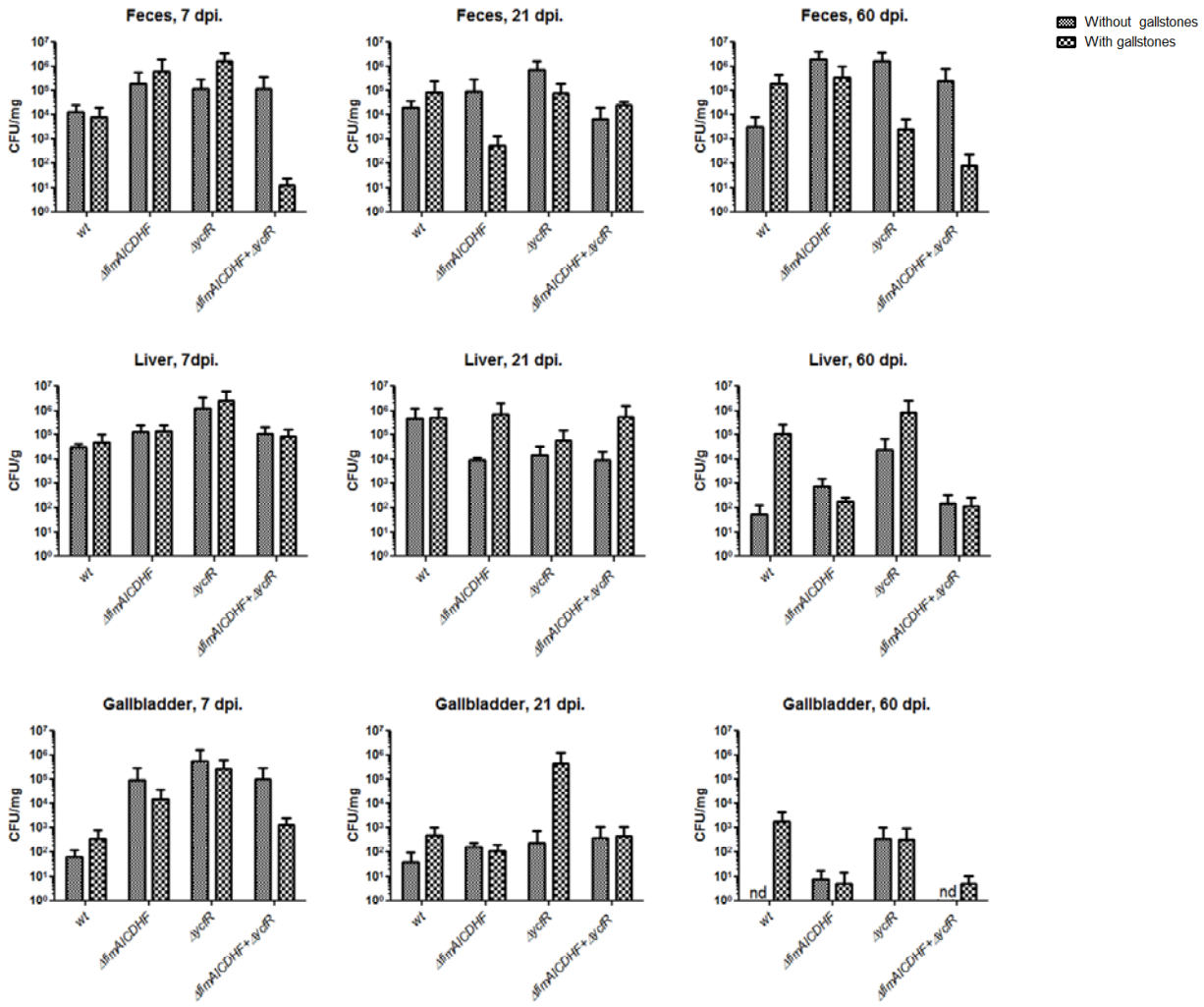

Figure S3. Type 1 fimbriae and *ycfR* mutants colonized *Nramp1*$^{+/+}$ mice during early and chronic time points regardless of the presence of gallstones. CFU enumeration of wild-type *S*. Typhimurium and mutants in the feces, liver and gallbladder at 7, 21 and 60 dpi of *Nramp1*$^{+/+}$ mice. Although the *ycfR* and type 1 fimbriae mutants were recovered in higher numbers than the wild-type in the gallbladder at 7 dpi, this difference was not statistically significant by using Student's *t* test (p <0.5; nd, not detected.