Supplementary figure 1 (Pendaries et al.)
Supplementary Fig. 1. Localisation of PtdIns(5)P and phosphorylated Akt at the entry sites during early time points of HeLa cells infection by S. flexneri. A, HeLa cells were infected with either the wild-type M90T (WT) (A-C) or the IpgD-deficient (ipgD) (D-F) strains. PtdIns(5)P was localised with the biotinylated GST-PHDx2 recombinant probe and stained with steptavidin-Alexa Fluor594 (A,D). Actin cytoskeleton was stained with phalloidin-Alexa Fluor488 (B,E) and bacteria with anti-LPS antibody and an Alexa Fluor350-conjugated secondary antibody (C,F). B, HeLa cells were incubated for 48 hours with siRNA control (A-C) or against Akt1 isoform (D-F) and infected for 10 min with the wild-type M90T (WT) strain. Akt phosphorylation was detected with the anti-phospho-T308 antibody and an Alexa Fluor594-conjugated secondary antibody (A,D). The knockdown of Akt1 (see Fig.7), which reached 90.5 ± 8% (n=3), allowed to test the specificity of phospho-Akt recognition. Actin cytoskeleton (B,E) and bacteria (C,F) were stained as in A. Calibration bar = 15µm.