

Supplementary Figure S3. MH-S cells were seeded and grown in media (changed daily) for seven days, with varying durations of exposure to IFN- γ (25ng/ml/day) lasting between 0 and 6 days prior to inoculation with a GFP expressing D39 pneumococcus (MOI of 15). Fifty minutes following bacterial inoculation, cells were gently washed 3 times with 1 ml ice-cold PBS to remove unbound bacteria immediately before fixing with 4% paraformaldehyde. The phagocytic index was calculated as the fraction of GFP positive MH-S cells per field (e.g. % MH-S cells associated with at least one bacterium) multiplied by the mean fluorescence intensity (MFI; representing both bound and internalized bacteria) per cell. Two representative images taken of controls (A) and IFN- γ exposed for 4 days (B) are shown. Note the apparent lack of internalization in (B), demonstrated by the absence of GFP 'lighting up' the cytoplasmic portions of the cells – an effect seen when the GFP is internalized and the light is refracted within the cell membrane.