Supplemental Material to:

Phadwal K, Alegre-Abarrategui J, Watson AS, Pike L, Anbalagan S, Hammond EM, Wade-Martins R, McMichael A, Klenerman P, Simon AK

A novel method for autophagy detection in primary cells: Impaired levels of macroautophagy in immunosenescent T cells

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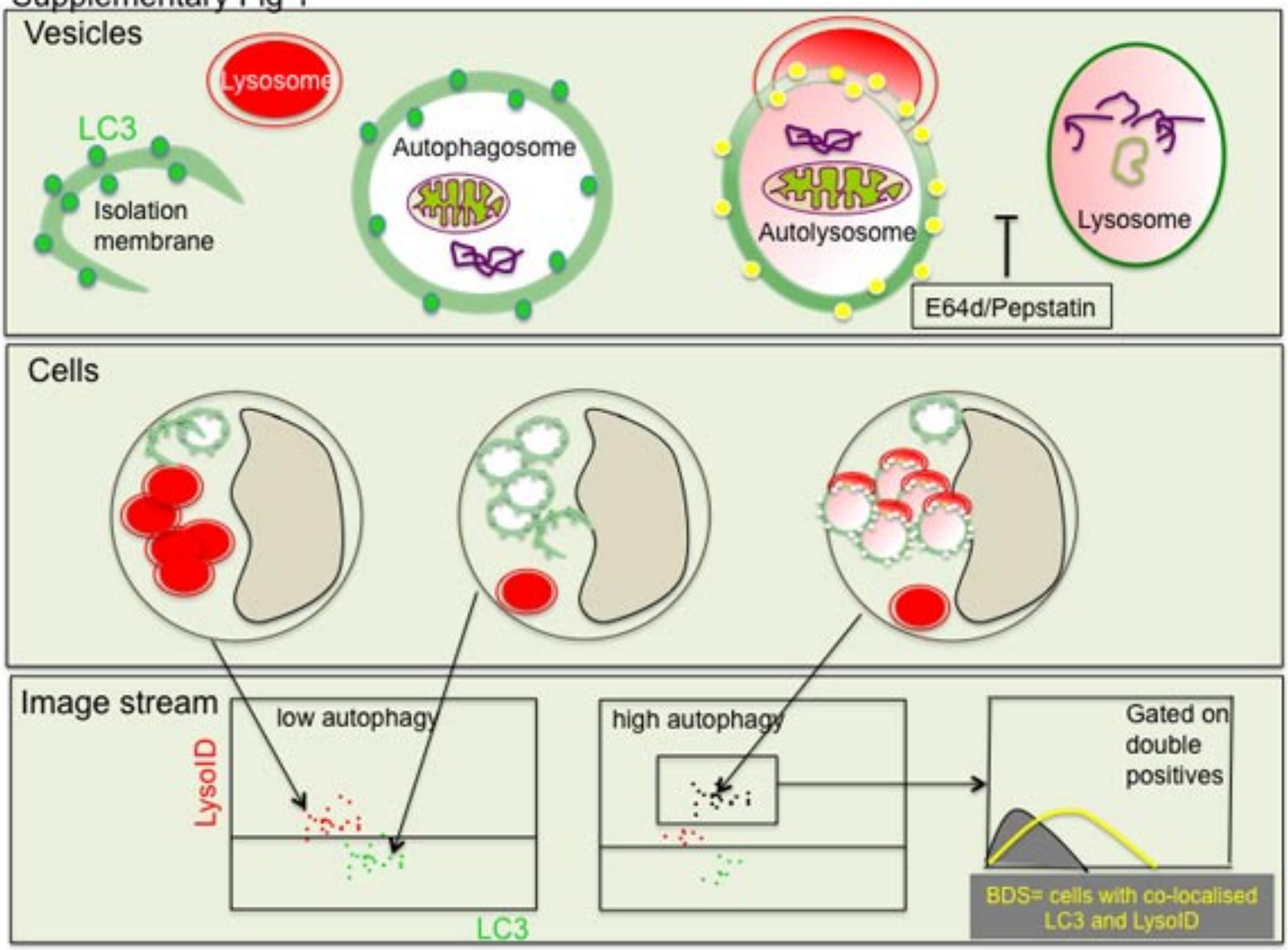
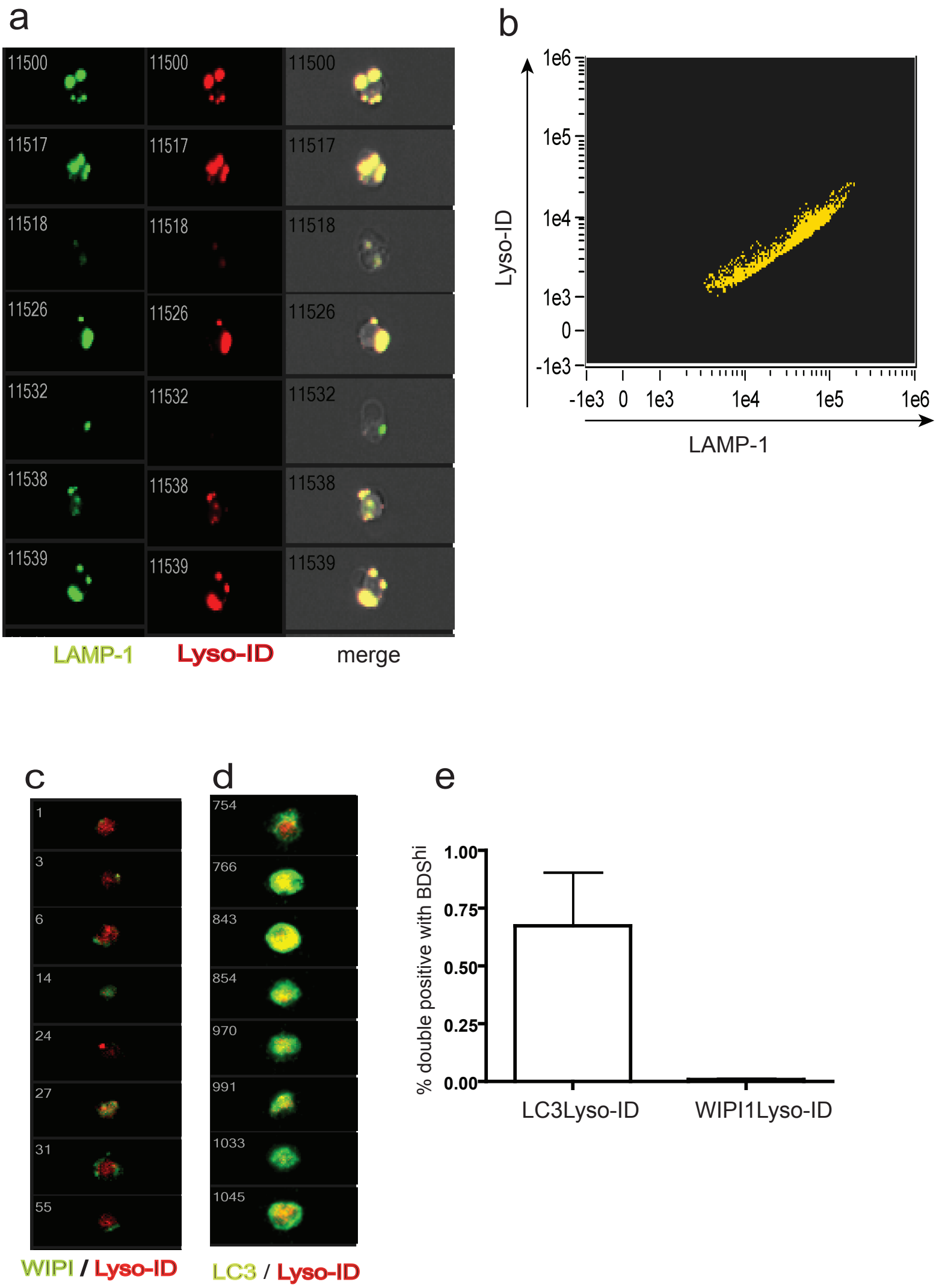
Supplementary Fig 1



Supplementary Figure 1. Image stream-based autophagy detection technique. Upper panel: Vesicles involved in the autophagy process, lysosomes are depicted in red, autophagosomes in green decorated with LC3 (green dots). Addition of lysosomal inhibitors E64d/PepA allows arrest of autophagic flux. Middle panel: Single cells from left to right containing many lysosomes/ few autophagosomes, few lysosomes/many autophagosomes or upon induction of autophagy many autolysosomes. Lower Panel: Representative Image stream dot plots showing location of cells in dot plots, and gating strategy for BDS in histograms.

Supplementary Fig 2



Supplementary Figure 2. Lysosomal markers correlate on Image stream. **(a)** Merge (yellow) of images of PBMCs stained with two lysosomal markers Lyso-ID (red) and LAMP-1 (green) **(b)** Correlation of Lyso-ID and LAMP-1 intensity dot plot. Representative co-localized images of PBMCs stained for **(c)** WIPI-1 (green) and Lyso-ID (red) **(d)** LC3 (green) and Lyso-ID (red). **(e)** BDS (represented as % total double positives, BDS^{hi}) between WIPI-1 Lyso-ID and LC3 Lyso-ID stained PBMCs (mean ± SEM., *n*=4., *p=0.0286).