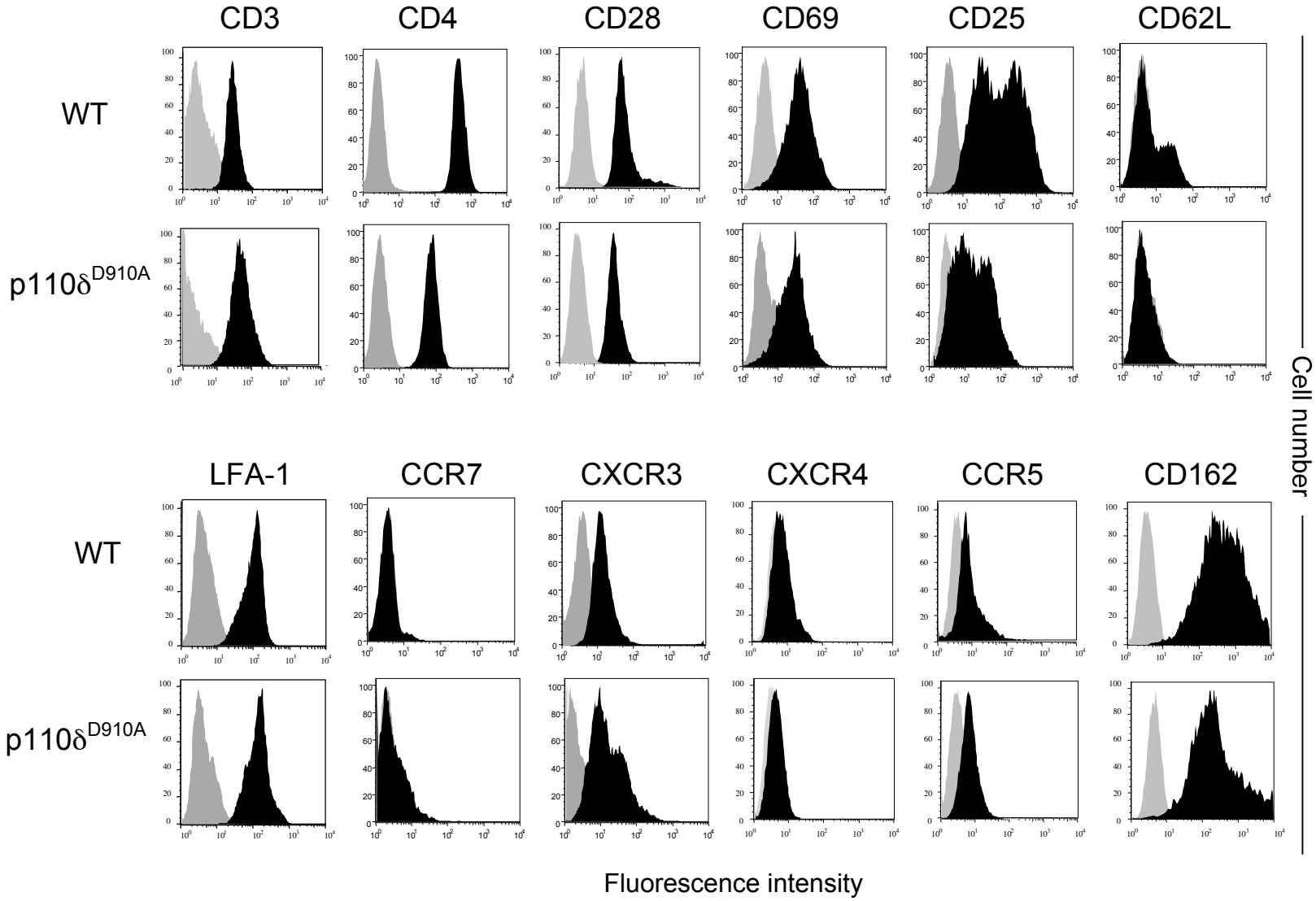
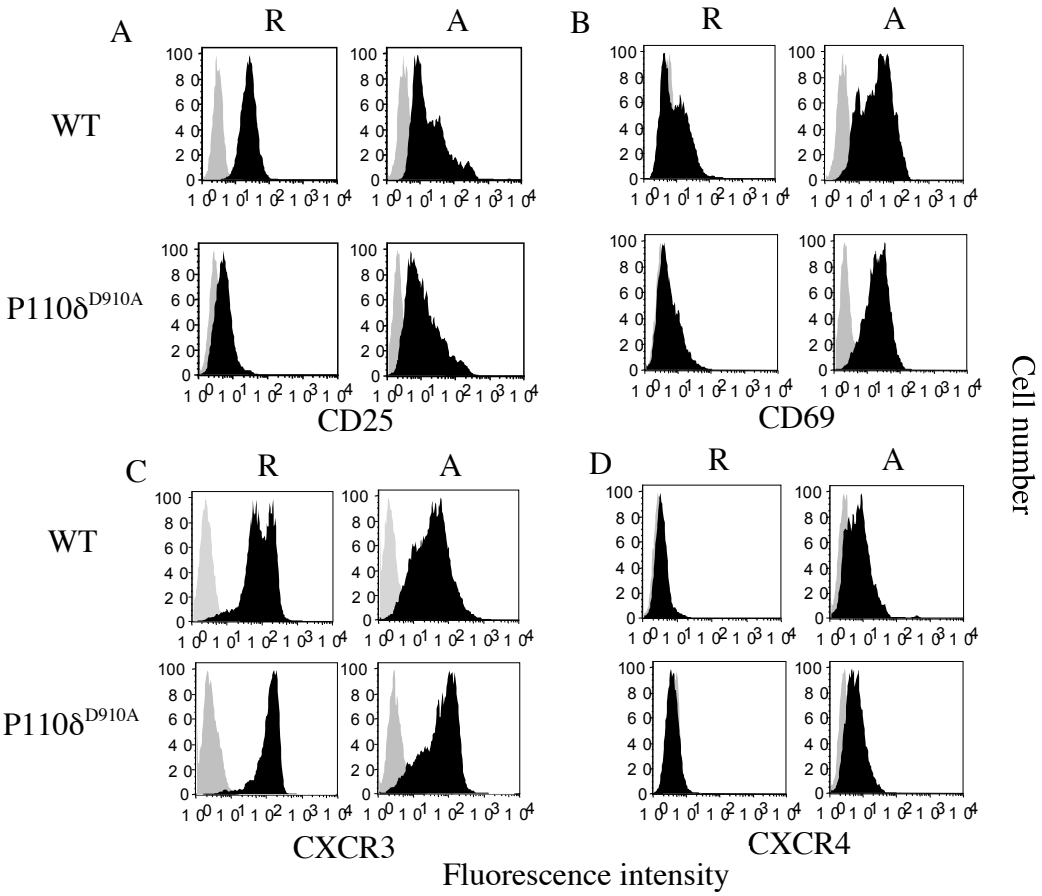
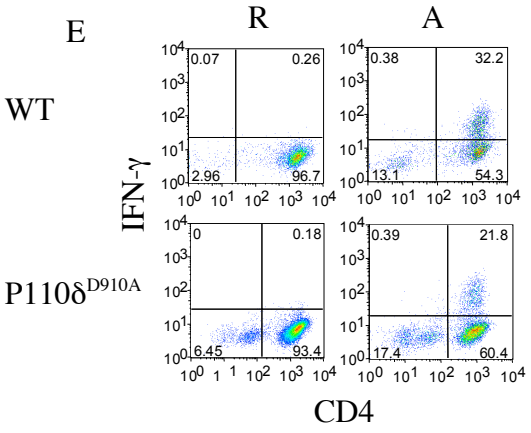
Supplemental Figure 1. *Phenotype of HY-specific T cells at the time of injection.*



Expression of the molecules indicated above each set of panels by wild type (WT) and p110δ^{D910A} HY-specific T cells was assessed at the time of injection (i.e., 7 days following stimulation in vitro) by flow cytometry. Histograms are representative of a number of routinely performed analyses carried out prior to injection. The comparison of individual molecules was performed on cells injected in the same experiment. Staining with an isotype-matched control antibody is indicated by the grey profiles. **Although variations in the expression of some of the surface molecules indicated was occasionally observed, these were not found to be statistically significant when data from all the analyses performed were averaged (as differential MFI between the experimental sample and the isotype-control).**

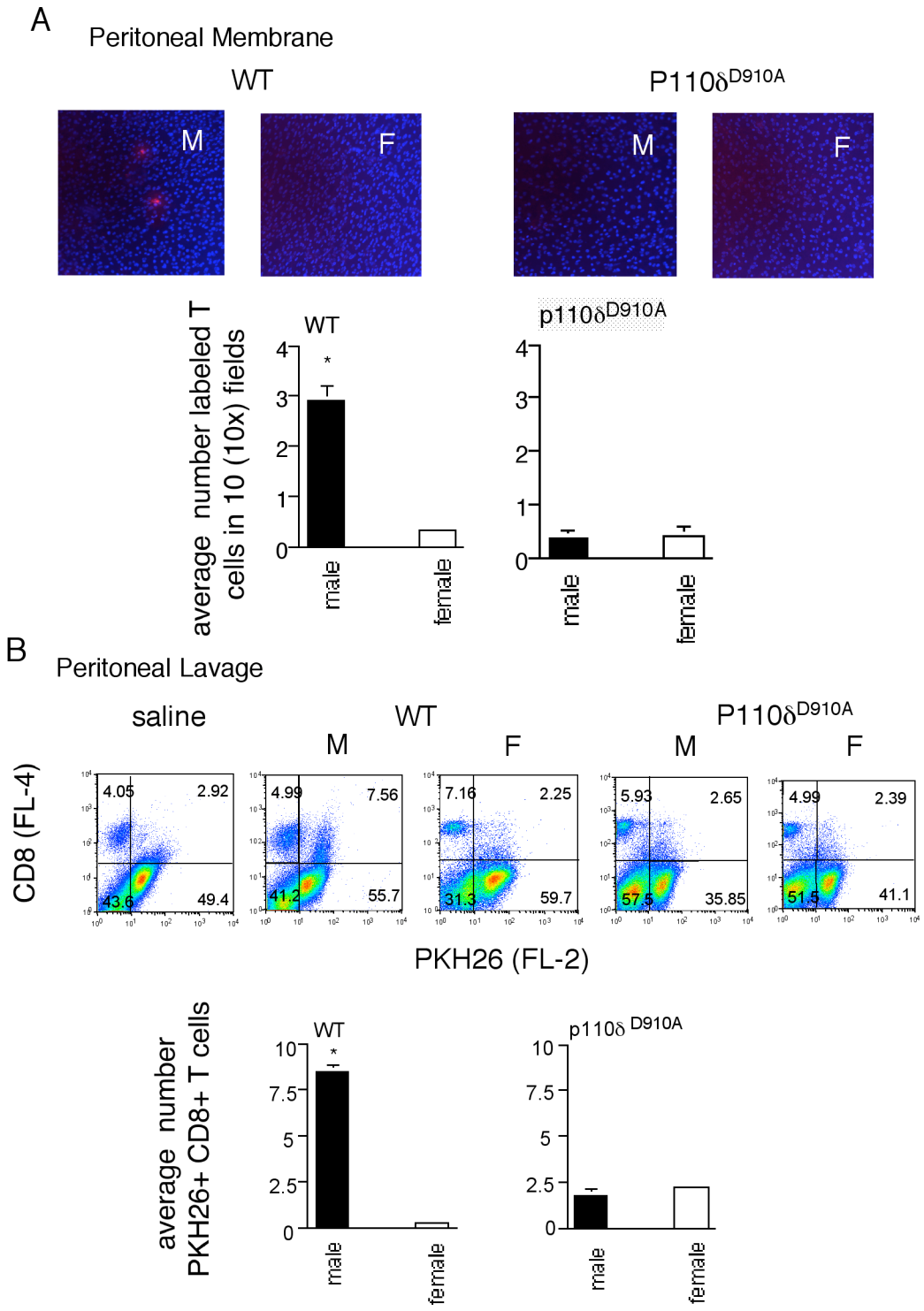
Supplemental figure 2. Phenotypic and functional changes induced by antibody-activation in WT and p110δ^{D910A} T cells.





HY-specific WT and p110δ^{D910} CD4^{+} T cells were isolated by density centrifugation 7 days after the last stimulation in vitro, and subsequently rested for 48 hours in medium alone (indicated as R). This led to a further down-regulation of both CD25 (panel A) and CD69 (panel B), To induce activation (indicated as A), T cells (10^6/well) were then incubated for 24 hours at 37ºC with plastic bound anti-CD3 (1μg/ml, clone 2C11, BD Biosciences, Oxford, UK) and anti-CD28 (5μg/ml, clone 37.51, BD Biosciences) in 24 well plates. Expression of the surface molecules indicated in each panels was then assessed by flow cytometry. Both WT and p110δ^{D910} up-regulated CD25 (A), CD69 (B) and CXCR4 expression following antibody activation. CXCR3 expression was not modified by activation. In panel D, IFN-γ production by resting (R) and antibody-activated (A) T cells was measured by intracellular staining (R&D Systems, Abingdon, UK).

Supplemental Figure 3. **PI3K P110δ activity is required for efficient antigen-dependent localization of specific CD8⁺ T cells to antigenic non-lymphoid tissue.**



HY-specific T cells were generated by intraperitoneal immunization of female WT or $p110^{dD910A}$ C57Bl/6 recipients with C57Bl/6 male-derived splenocytes, as previously described. $CD8^+$ T cells were isolated by negative immuno-magnetic selection and, following a further two rounds of stimulation with male splenocytes in vitro, about 80% of the T cell population was CD8-positive. 70-80% of this population was specific to the D^b Uty HY epitope, while 7-10% recognized the Smcy D^b–restricted epitope (data not shown), in line with what we and others have previously described (4, 36). To establish whether PI3K P110δ activity was also instrumental to the antigen dependent recruitment of HY-specific $CD8^+$ T cells, we compared the recruitment of HY-specific WT and $p110\delta^{D910A}$ $CD8^+$ T cells from the circulation into the peritoneum of male and female WT mice. H2-A^b-restricted HY-specific WT (filled bars), $p110\delta^{D910A}$ (empty bars) $CD8^+$ T cells were injected i.v. into male mice (10^7/mouse) that had received an i.p. injection of IFNγ (600U) 48 hours earlier. The following day mice were sacrificed, and the presence of fluorescently labelled cells in the peritoneal membrane (A) and cavity (B) was assessed by wide field fluorescence microscopy and flow cytometry, respectively. Due to the presence of an autofluorescent non-T cell population detected in FL-2 also in saline solution-injected control mice (panel B, first dot-plot on the left-hand site), cells were double-stained with an APC-conjugated anti-CD8 antibody following harvesting. Significant amounts of WT T cells were detected in the peritoneal membrane (panel A) and cavity (panel B) of male mice. By contrast, HY-specific $p110\delta^{D910A}$ T cells failed to localize to the peritoneal membrane (panel A) and were not found in the peritoneal lavage of IFNγ-treated male mice. As expected, neither WT nor $p110\delta^{D910A}$T cells

migrated the peritoneal membrane or cavity of IFNγ-treated female mice. These data suggest that PI3K p110δ activity is essential for TCR-driven CD8+ T cell trafficking.

Samples from a representative experiments are shown in the top part of each panel. The mean T cell number ± SEM observed in samples from at least three animals are shown in a summary.

*p, : Panel A: significative versus WT female recipient mice ($p < 0.02$). Panel B: significative versus female WT mice ($p < 0.05$).