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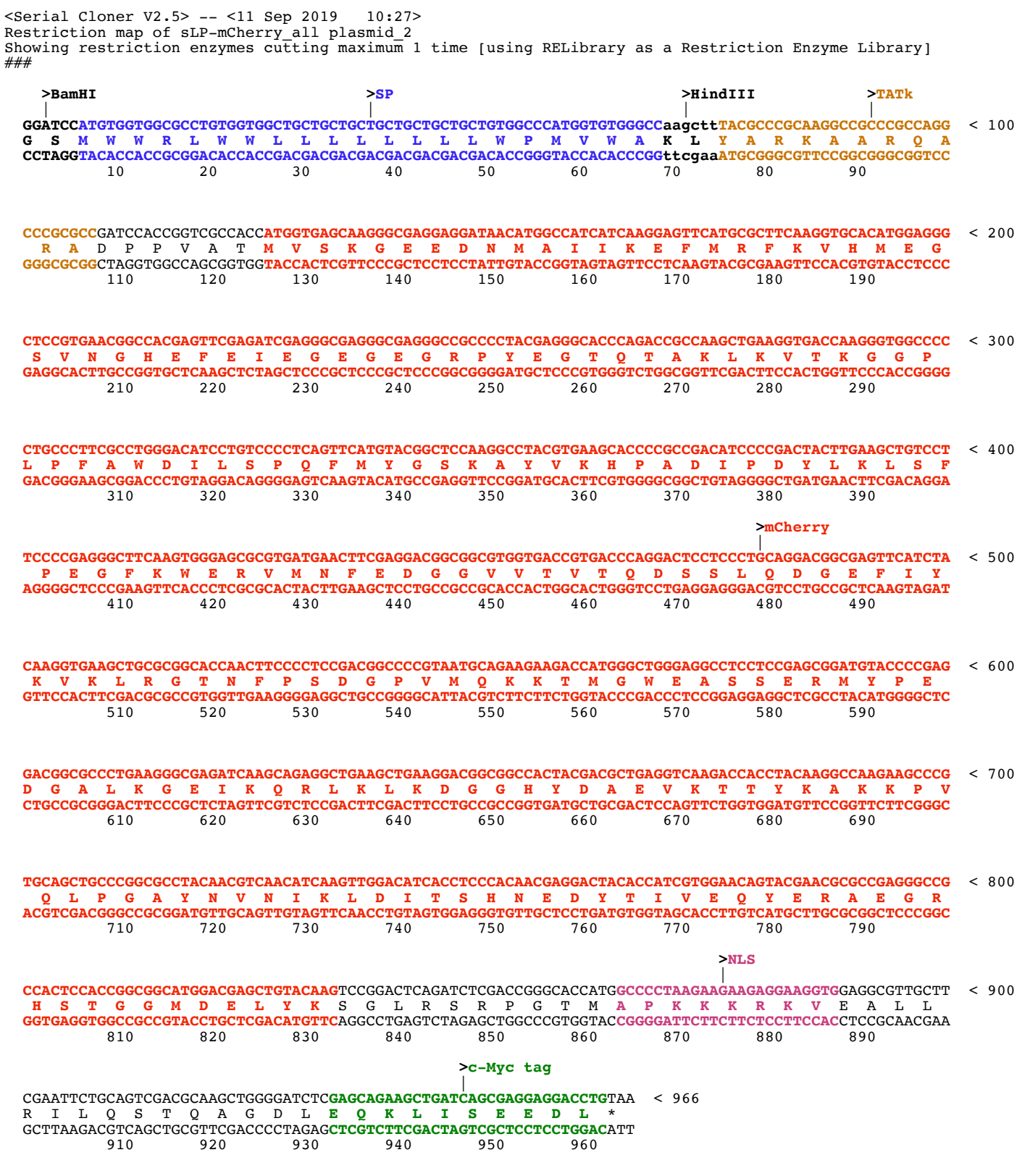
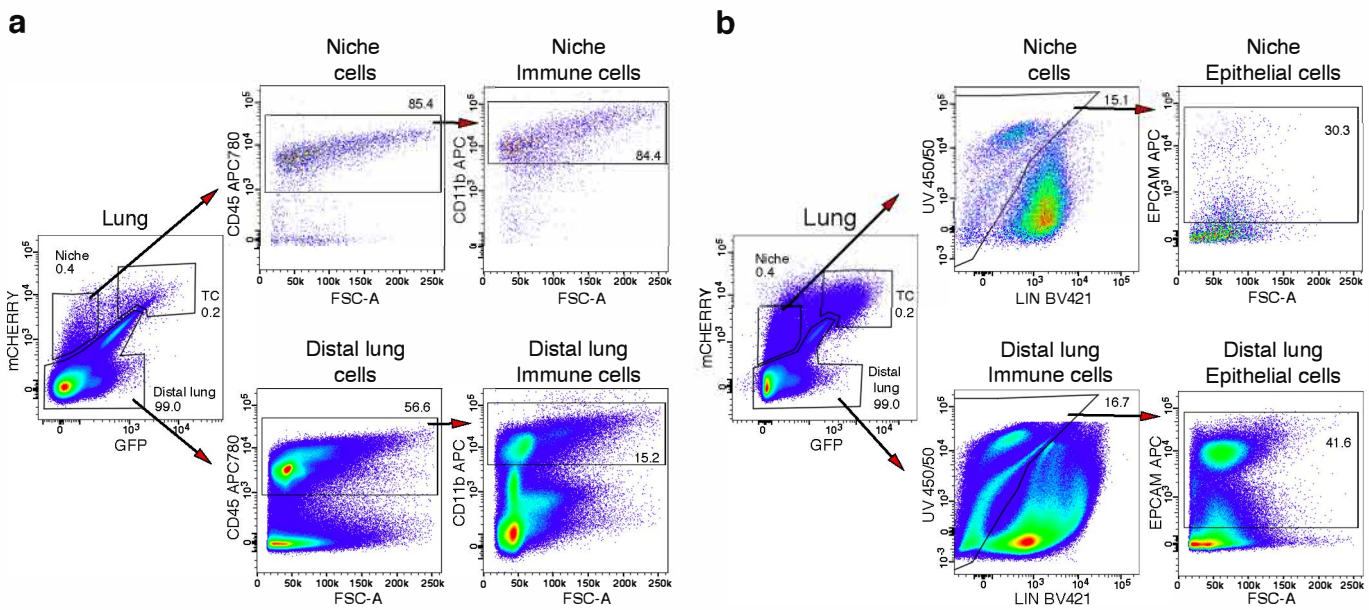
Supplementary information

Generation of neighbor-labeling cells to study intercellular interactions in vivo

In the format provided by the authors and unedited



Suppl. Fig. 1 | Sequence of the mCherry protein (sLP-Cherry) containing a soluble peptide (s) and a TATk (LP). This can be also found in our previous publication[1].



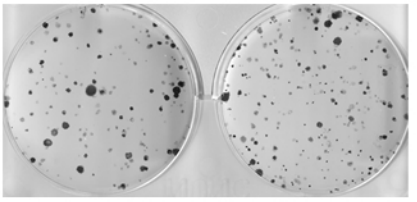
Suppl. Fig. 2 | Examples of gating strategy to define the identity of labelled cells in vivo. The gating strategy here follows doublets and cell death exclusion as described in Figure 6a. Gate sequence is indicated by red arrows. **a**, CD45$^+$ immune cells are gated distinctively from niche and distal lung cells, then CD11b$^+$ myeloid cells are gated from CD45$^+$ cells. **b**, Lineage$^-$ (CD45$^-$CD31$^-$Ter119$^-$) cells are gated distinctively from niche and distal lung cells. The channel used for the Lin BV421 is plotted against the UV 450/50, which allows you to capture LIN$^-$ cells that are auto-fluorescent (this strategy is only possible if DAPI$^+$ cells have been previously excluded as shown in Figure 6). Next, epithelial Epcam$^+$ cells are gated from Lin$^-$ cells. Myeloid and epithelial cells from lungs harbouring labelling 4T1 cells have been identified using the same strategy and results have been previously published[1], using data generated from independent experimental replicates.

a

Cherry-labelling expression does not affect proliferation of cancer or normal cells

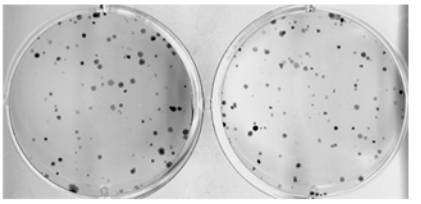
4T1 cells

GFP Cherry-labelling

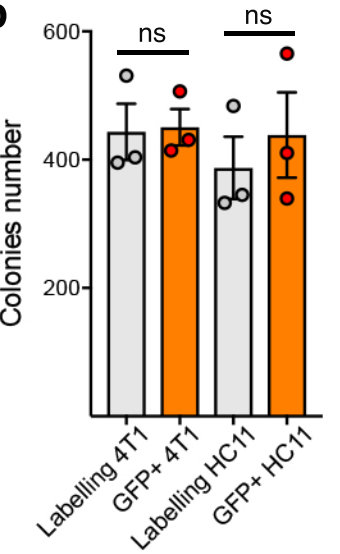


HC11 cells

GFP Cherry-labelling



b



c

Conditioned media from labelling cells does not influence fibroblast activation

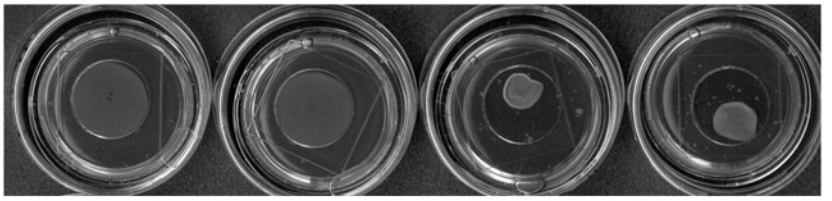
d

HC11 cells

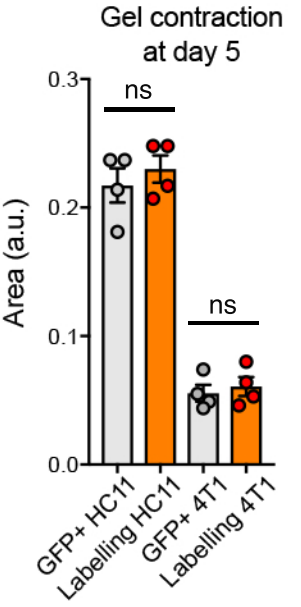
GFP Cherry-labelling

4T1 cells

GFP Cherry-labelling



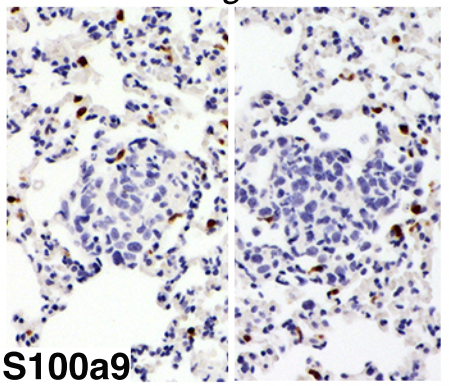
e



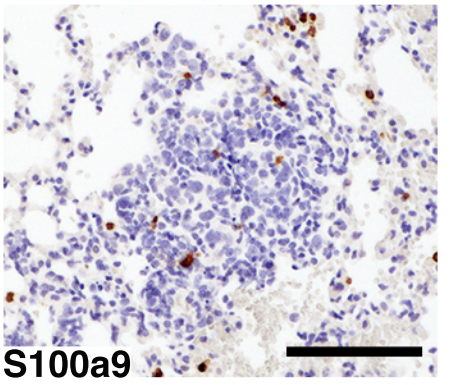
f

Conditioned media from labelling cells does not elicit an inflammatory response

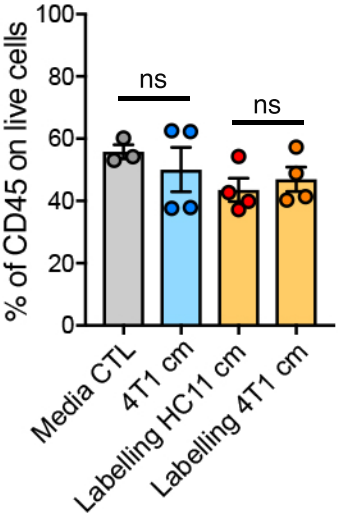
Labelling 4T1



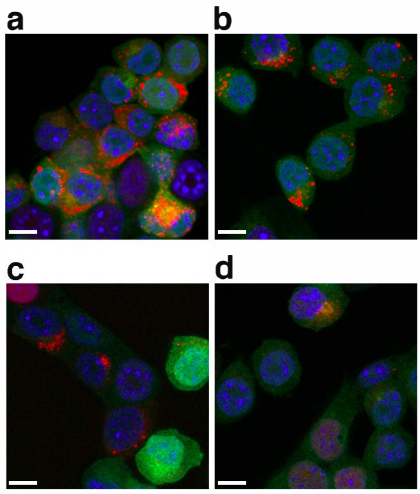
4T1



g



Suppl. Fig. 3 | Functional analysis to exclude specific mCherry-dependent effects on labelling and recipient cells. a, **b**, In vitro proliferation of 4T1 and HC11 cells (GFP or labelling). Representative images showing Crystal violet stained cells (**a**) and quantification (**b**). **c**-**e**, Gel contraction assay of fibroblasts co-cultured with HC11 and 4T1 cells (GFP or labelling): schematic images of co-culture (**c**); representative images (**d**); and quantification of gel area (**e**). **f**, Representative IHC on lung tissue sections stained with S100a9 to detect neutrophils from Balb/c mice injected with either labelling 4T1 or 4T1 cells. Scale bar 100μm. **g**, $CD45^+$ cell frequency in lungs from Balb/c mice injected with control media or conditioned media from 4T1, labelling HC11 and labelling 4T1 cells by FACS. **b**,**e**,**g**, Data are represented as mean ±SEM and statistical analysis are performed by unpaired two-tailed t-test.



Suppl. Fig. 4 | Effect of permeabilization on mCherry detection. a-d, Representative images of labelling 4T1 cells by confocal microscopy. Labelling 4T1 cells (**a**) fixed in 4% PFA for 10 min or permeabilised for 7 min with (**b**) Saponin 0.1%, (**c**) Tween20 0.1% or (**d**) Triton X100 0.1% (**a-d** scalebar 10 μm). The use of strong detergents, such as Tween20 and Triton X-100, results in a large loss of the mCherry signal.