

Supplementary information

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

In the format provided by the authors and unedited

<Serial Cloner V2.5> -- <11 Sep 2019 10:27>
 Restriction map of sLP-mCherry_all plasmid 2
 Showing restriction enzymes cutting maximum 1 time [using RELibrary as a Restriction Enzyme Library]
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    >BamHI                >SP                >HindIII        >TATk
GGATCCATGTGGTGGCGCTGTGGTGGCTGTGTGTGTGTGTGTGTGTGGCCATGGTGTGGCCaagcttTACGCCCGCAAGGCCGCCCGCCAGG < 100
G S M W W R L W W L L L L L L L L L W P M V W A K L Y A R K A A R Q A
CCTAGGTACACCACCGCGACACCACCGACGACGACGACGACGACGACGACGACCGGTTcgaatAGCGGGCGTTCGGCGGGCGGCTCC < 110
10 20 30 40 50 60 70 80 90

CCCAGCCGATCCACCGGTTCGCCACCATGGTGTAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGG < 200
R A D P P V A T M V S K G E E D N M A I I K E F M R F K V H M E G
GGGCGGGCTAGGTGGCCAGCGGTGGTACCACCTCGTTCGCCCTCCTCTATTGTACCAGGTAGTAGTTCCTCAAGTACGCGAAGTTCACAGTGTACCTCC < 120
110 120 130 140 150 160 170 180 190

CTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGACCGCAAGCTGAAGGTGACCAAGGTTGGCCCC < 300
S V N G H E F E I E G E G E G R P Y E G T Q T A K L K V T K G G P
GAGGCATTGCGCGTGTCAAGCTCTAGCTCCCGCTCCCGCTCCCGCGGGGATGCTCCCGTGGGTCTGGCGGTTTCGACTTCCACTGGTTCACCGGGG < 210
210 220 230 240 250 260 270 280 290

CTGCCCCGCGCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCGACATCCCCGACTACTTGAAGCTGTCTT < 400
L P F A W D I L S P Q F M Y G S K A Y V K H P A D I P D Y L K L S F
GACGGGAAGCGGCCCTGTAGGACAGGGGAGTCAAGTACATGCCGAGTTCGGATGCATTCGTGGGGCGGCTGTAGGGGCTGATGAACCTCGACAGGA < 310
310 320 330 340 350 360 370 380 390

TCCCCGAGGGCTTCAAGTGGGAGCGCGTGTGAAGTTCGAGGACGGCGCGTGGTGCACCGAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTA < 500
P E G F K W E R V M N F E D G G V V T V T Q D S S L Q D G E F I Y
AGGGCTCCCGAAGTTCACCCCTCGCGACTACTTGAAGCTCCTGCCCGCCACCACTGGCACTGGGTCTGAGGAGGGACGCTCGCCGCTCAAGTAGAT < 410
410 420 430 440 450 460 470 480 490

CAAGGTGAAGCTGCGCGCACCAACTTCCCCTCCGACGGCCCGTAAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAG < 600
K V K L R G T N F P S D G P V M Q K K T M G W E A S S E R M Y P E
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510 520 530 540 550 560 570 580 590

GACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCG < 700
D G A L K G E I K Q R L K L K D G G H Y D A E V K T T Y K A K K P V
CTGCGCGGGACTTCCCGCTTAGTTCGTTCTCCGACTTCGACTTCCCTGCGGCGGTGATGCTGCGACTCCAGTTCGGTGGATGTTCCGGTTCCTCGGGC < 610
610 620 630 640 650 660 670 680 690

TGCAGCTGCCCGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCACACGAGGACTACACCATCGTGGAACAGTACGAACGCGCGCGAGGGCCG < 800
Q L P G A Y N V N I K L D I T S H N E D Y T I V E Q Y E R A E G R
ACGTCGACGGGCGCGGATGTTGAGTGTAGTTCAACCTGTAGTGGAGGGTGTGCTCCTGTATGGTAGCACCTTGTATGCTTGGCGCGCTCCCGGC < 710
710 720 730 740 750 760 770 780 790

CCAATCCACCGGGCGCATGGACGAGCTGTACAAGTCCGGACTCAGATCTCGACCGGGCACCATGCCCTAAGAAGAAGGAAGGTGGAGGCGTTGCTT < 900
H S T G G M D E L Y K S G L R S R P G T M A P K K K R K V E A L L
GGTGAAGTGGCGGCTACCTGCTCGACATGTTTAGGCCCTGAGTCTAGAGCTGGCCCGTGGTACCGGGGATTCCTTCTCTCCCTCCACCTCCGCAACGAA < 810
810 820 830 840 850 860 870 880 890

CGAATTCTGCAGTCGACGCAAGCTGGGATCTCGAGCAGAAGCTGATCAGCGAGGAGGACCTGTAA < 966
R I L Q S T Q A G D L E Q K L I S E E D L *
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910 920 930 940 950 960

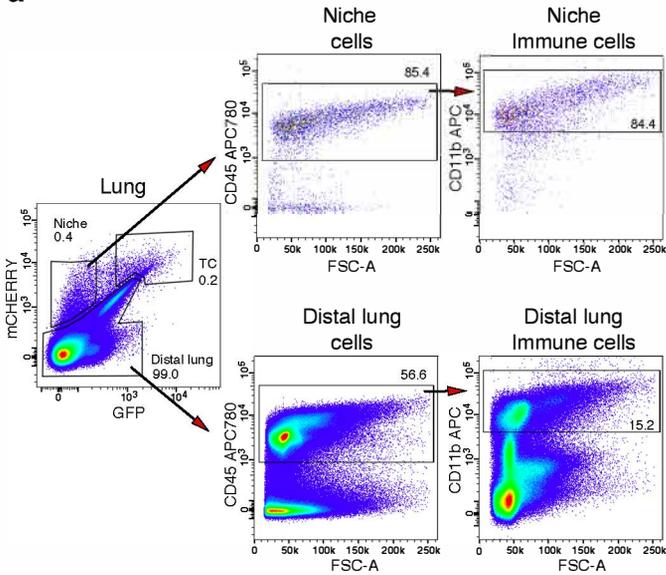
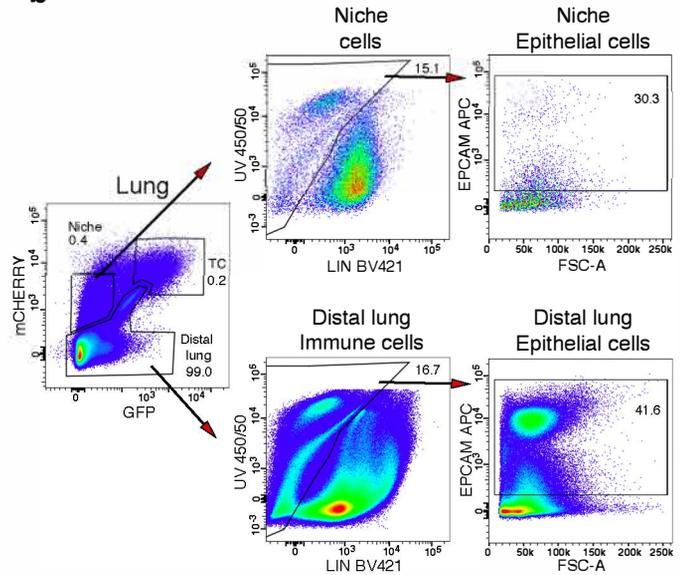
    >c-Myc tag

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Features :

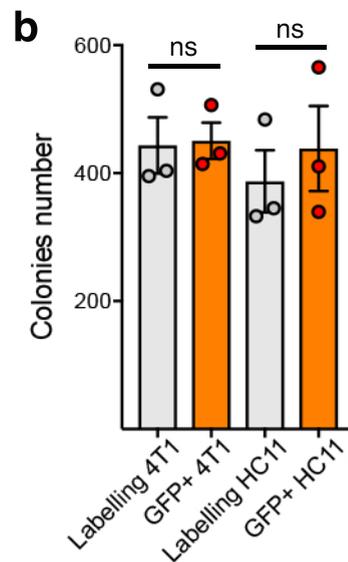
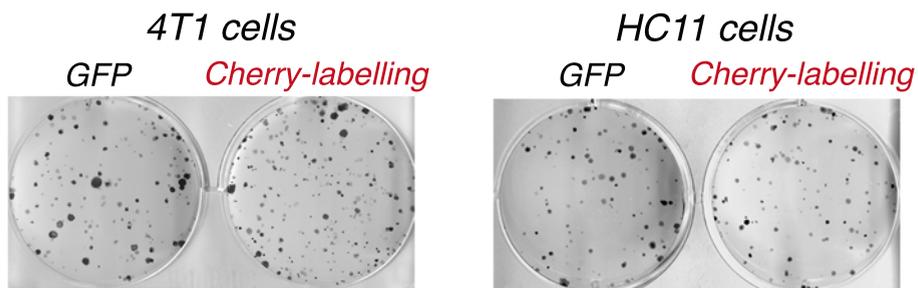
- BamHI : [1 : 6 - CW]
- SP : [7 : 69 - CW]
- HindIII : [70 : 75 - CW]
- TATk : [76 : 108 - CW]
- mCherry : [127 : 834 - CW]
- NLS : [865 : 888 - CW]
- c-Myc tag : [934 : 963 - CW]

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 publication¹.

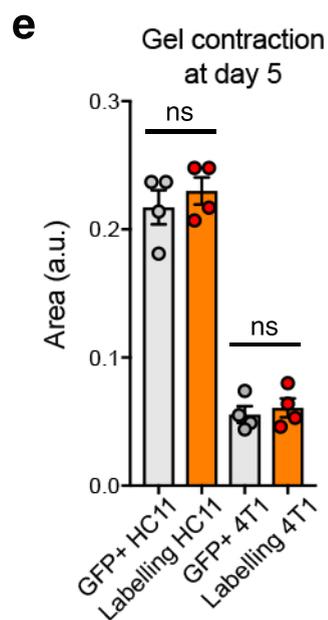
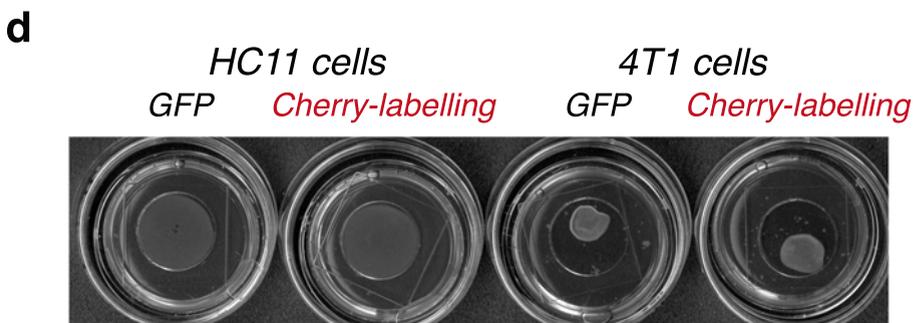
a**b**

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 published¹, using data generated from independent experimental replicates.

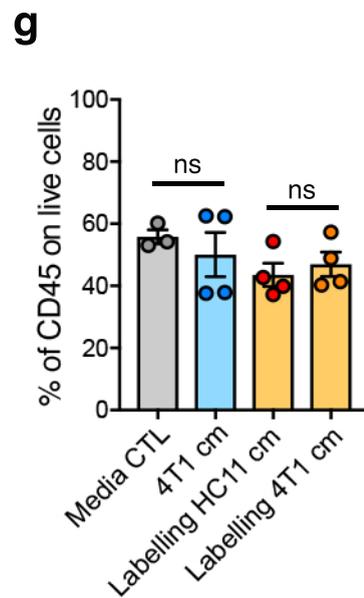
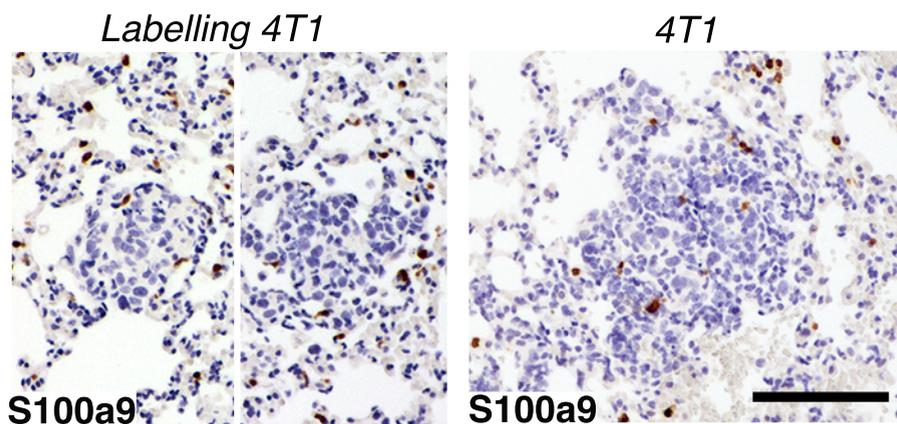
a *Cherry-labelling expression does not affect proliferation of cancer or normal cells*



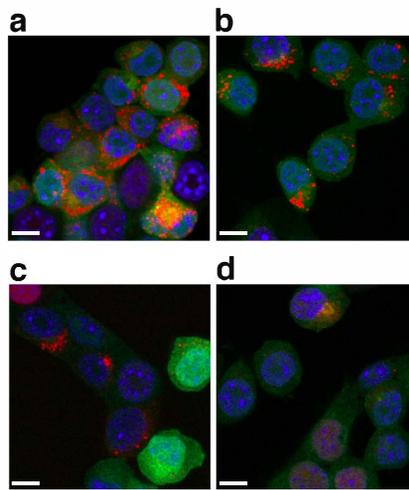
c *Conditioned media from labelling cells does not influence fibroblast activation*



f *Conditioned media from labelling cells does not elicit an inflammatory response*



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 100 μ m. **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 \pm 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.