Literature Report

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ARTICLES

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Single-cell analysis of regions of interest (SCARI) using a photosensitive tag

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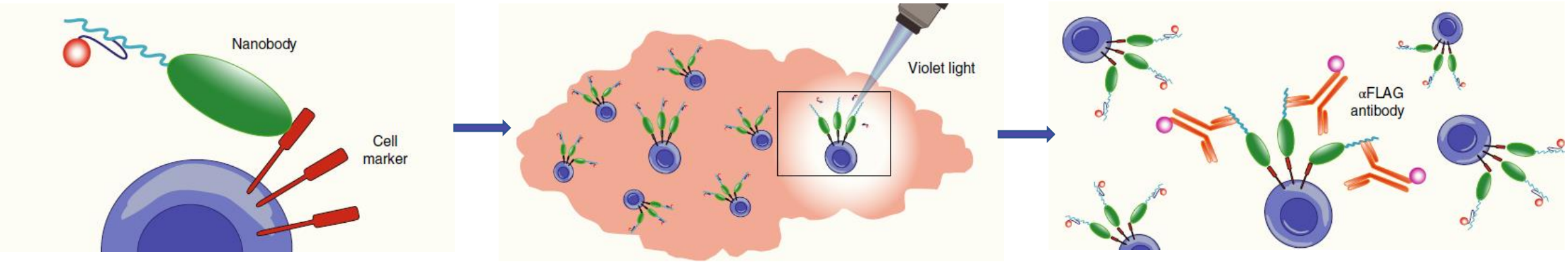


Corresponding author

Ton N. Schumacher

1988-1992 Ph.D. The Netherlands Cancer Institute
1992-1996 Post-doctoral Massachusetts Institute of Technology
Whitehead Institute in Cambridge
1996-now Senior Member, The Netherlands Cancer Institute

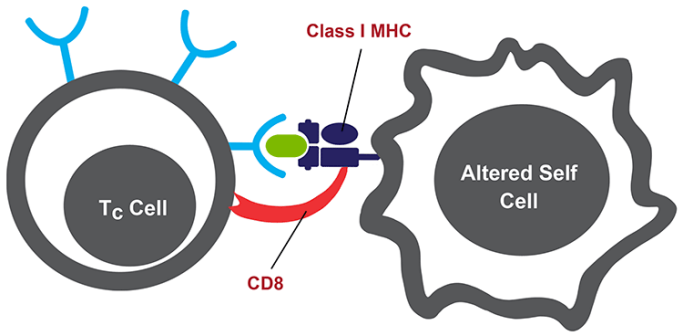
We use a technology-based approach to understand how our T cell-based immune system recognizes cancer cells as foreign, and how T cell recognition of cancer tissue can be enhanced by therapeutic intervention.

Sander I. van Kasteren

2001 MSc Chemistry & Medicinal Chemistry, Faculty of Chemistry, University of Edinburgh
2007 PhD, Faculty of Chemistry, University of Oxford
2007 – 2010 Sir Henry Wellcome Postdoctoral Fellow, University of Dundee
2010 – 2012 Post-doctoral researcher (Veni), Netherlands Cancer Institute
2012 – now Tenure Track Assistant Professor, Leiden Institute of Chemistry, Leiden University

Sander van Kasteren works on the interface between chemistry and immunology. He uses his background in organic synthesis and dendritic cell biology to study and manipulate the uptake and routing of antigen in dendritic cells.

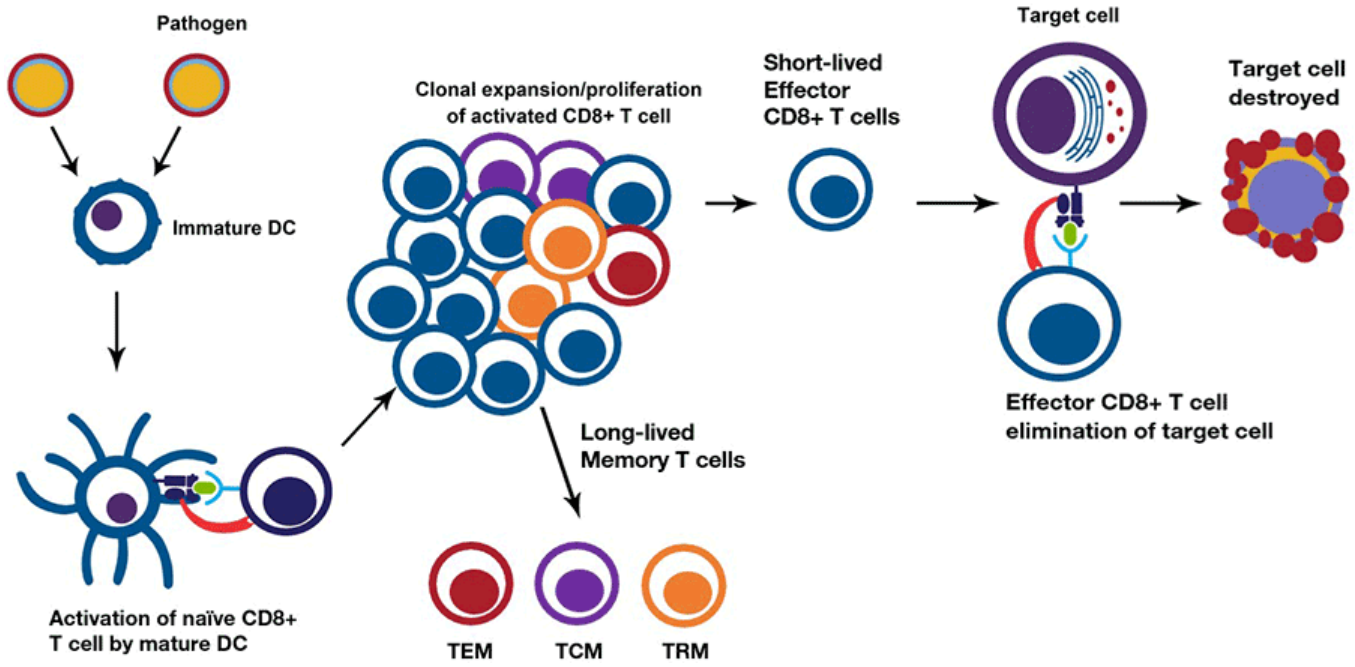
Background

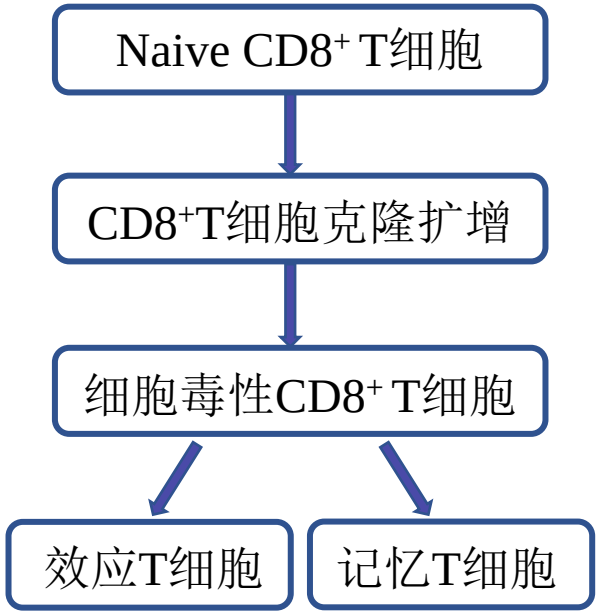


CD8⁺ T细胞（CTL, Tc Cell）

细胞毒性T淋巴细胞，适应性免疫系统的关键组成部分，在免疫防御病毒、细菌、肿瘤等过程中发挥重要作用。

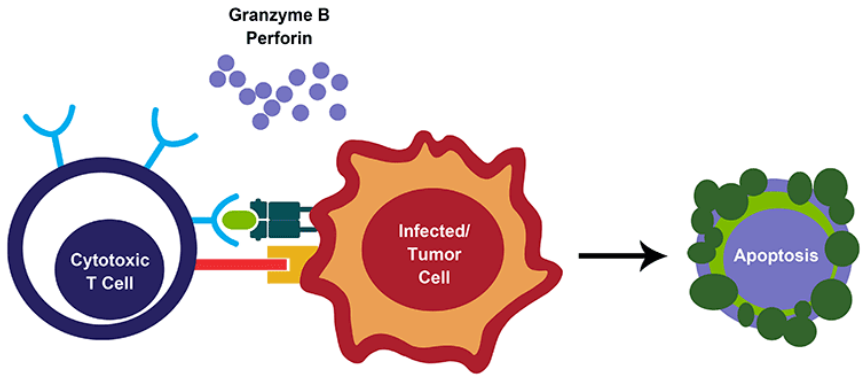
CD8⁺ T细胞缺失会破坏抗肿瘤免疫，功能失调也可能引发过度的免疫反应，导致免疫介导的损伤或疾病。





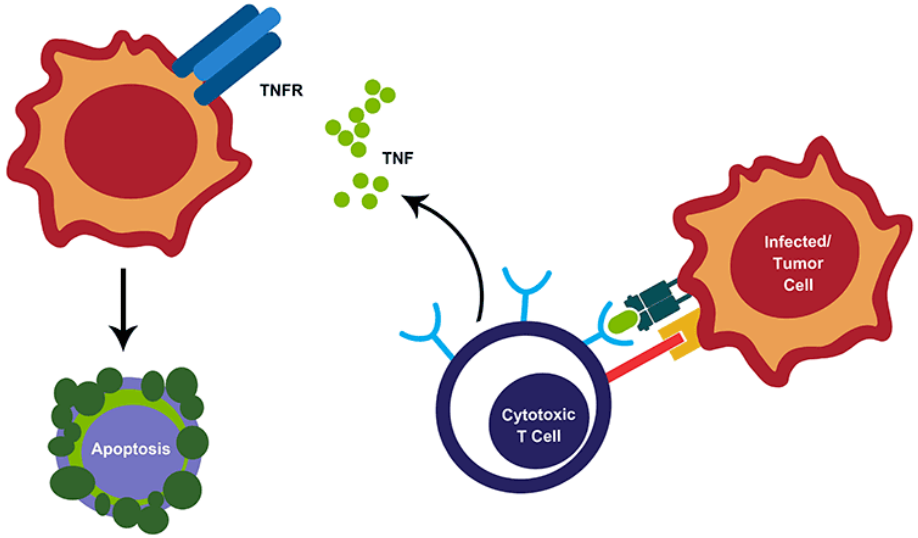
Background

- CD8⁺ T细胞介导的直接杀伤
  细胞直接接触，释放细胞溶解酶引起靶细胞凋亡



- CD8⁺ T细胞介导的间接杀伤
  CD8⁺ T细胞的Fas配体与靶细胞受体Fas相互作用，通过酶依赖性途径导致靶细胞凋亡

- CD8⁺ T细胞的远距离作用
  分泌细胞因子间接诱导邻近的肿瘤细胞死亡。



单细胞的空间位置、环境

↓

环境信号区域性差异

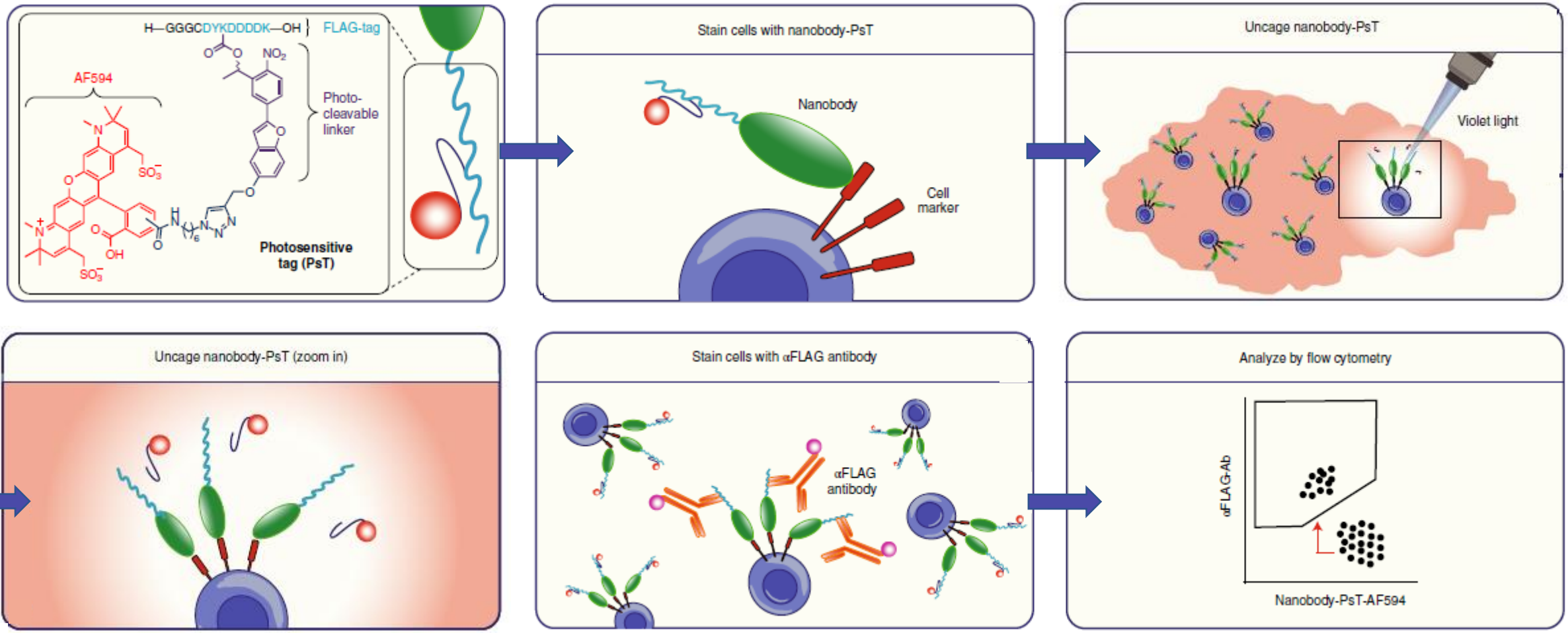
↓

细胞状态和细胞分化

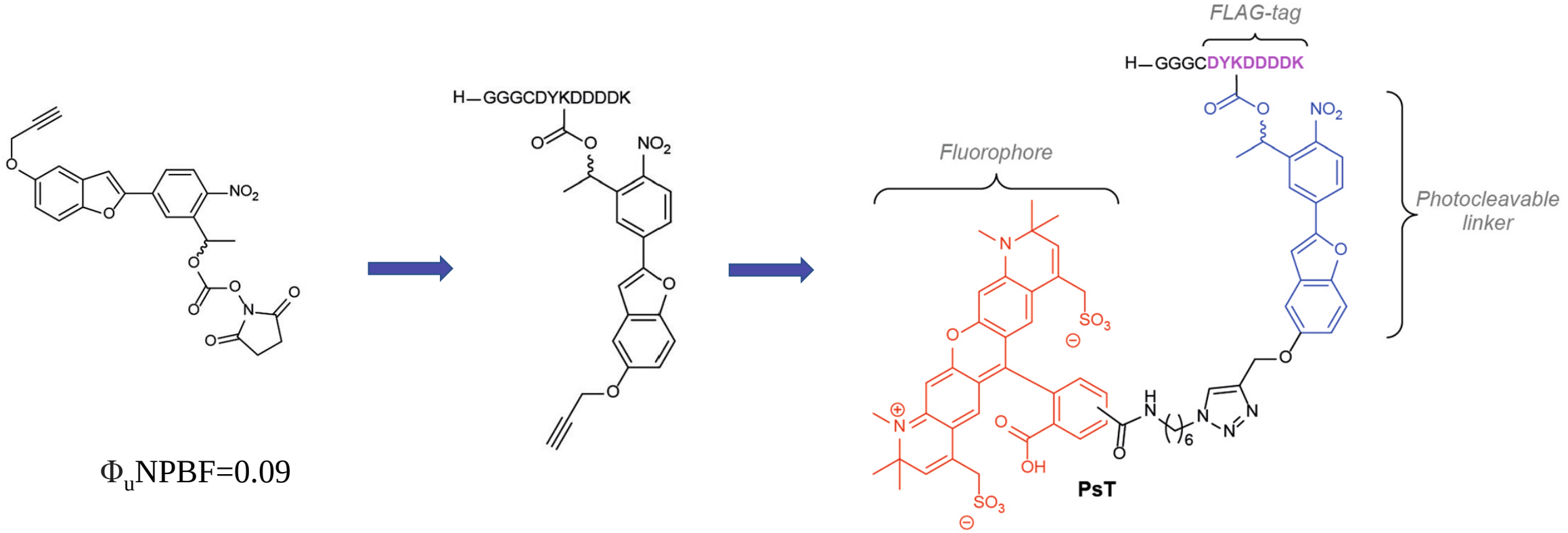
Single-cell analysis of regions of interest

共聚焦显微镜成像
空间分辨

单细胞转录组学
细胞的激活和分化状态



Photosensitive tag



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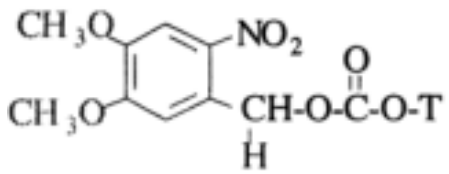

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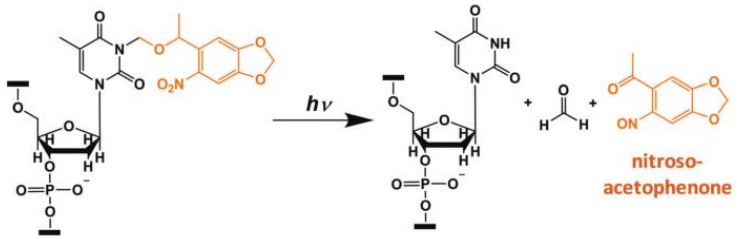

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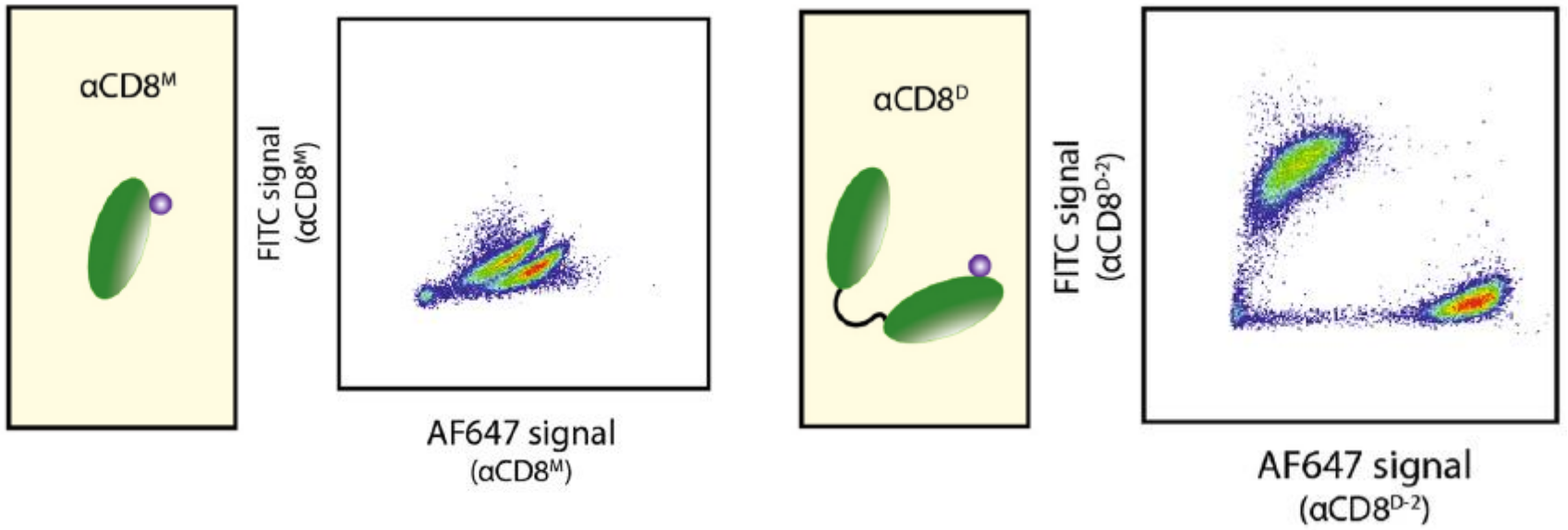
- 光解效率
- 有毒副产物

光敏性αCD8纳米抗体

αCD8： $CD8^+$ T细胞标记物

纳米抗体：尺寸小(15 k Da ~ 150 k Da)，具有优越的组织穿透能力
利于具有致密细胞和细胞外结构的完整组织染色

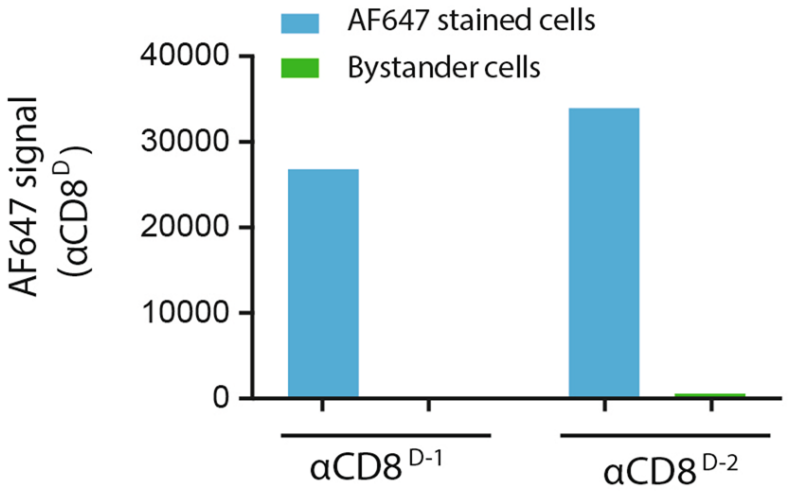
光敏性纳米抗体标记稳定性



单体αCD8纳米抗体 $\alpha CD8^M$

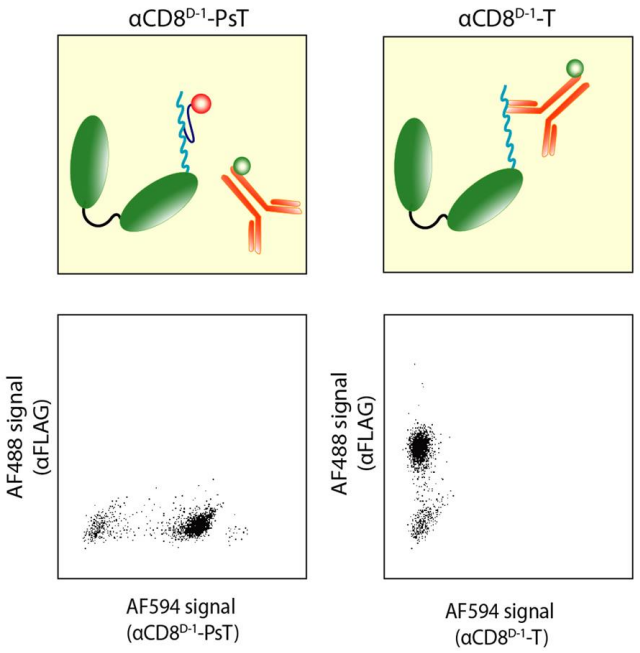
二聚体αCD8纳米抗体 $\alpha CD8^D$

高度稳定的细胞结合



光敏性αCD8纳米抗体试剂

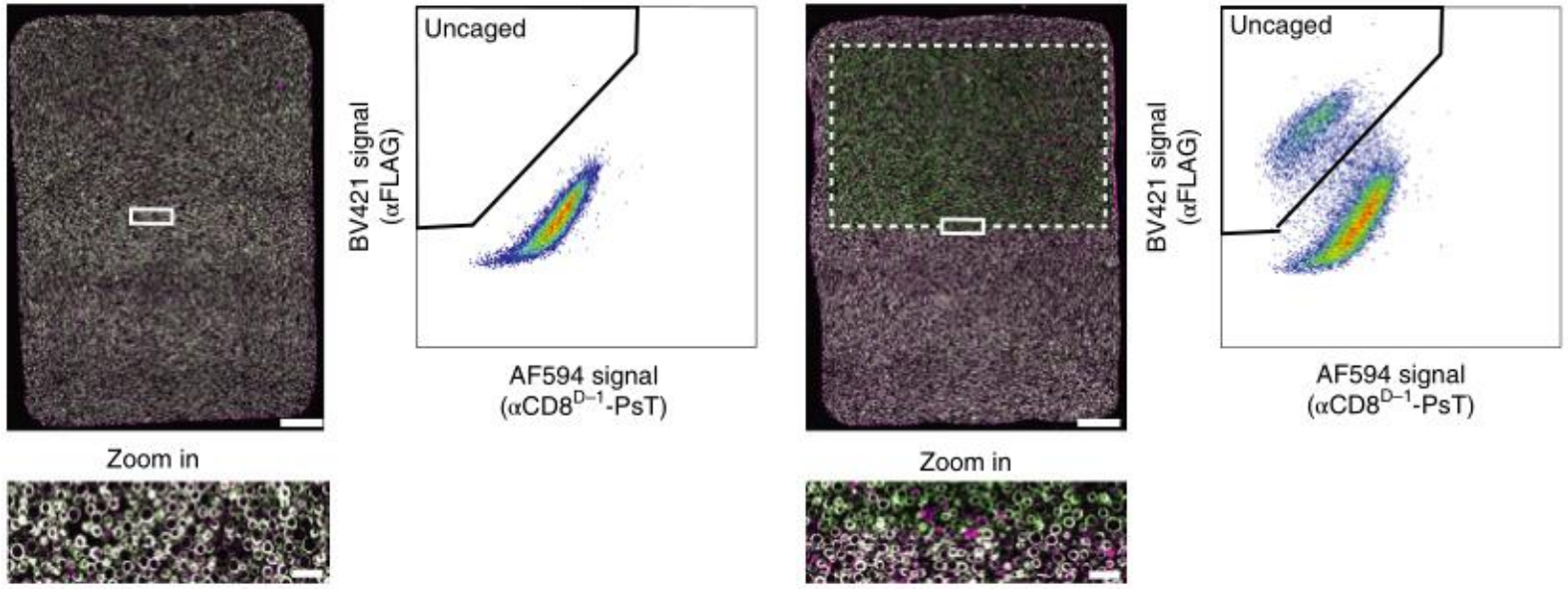
光敏标签有效阻止αFLAG的结合

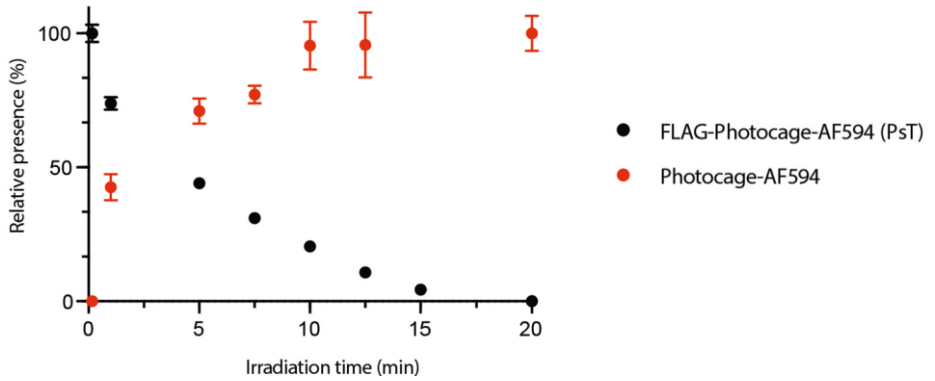


光敏标签的有效光解

Caged: $AF594^{high}\alpha FLAG^{low}$

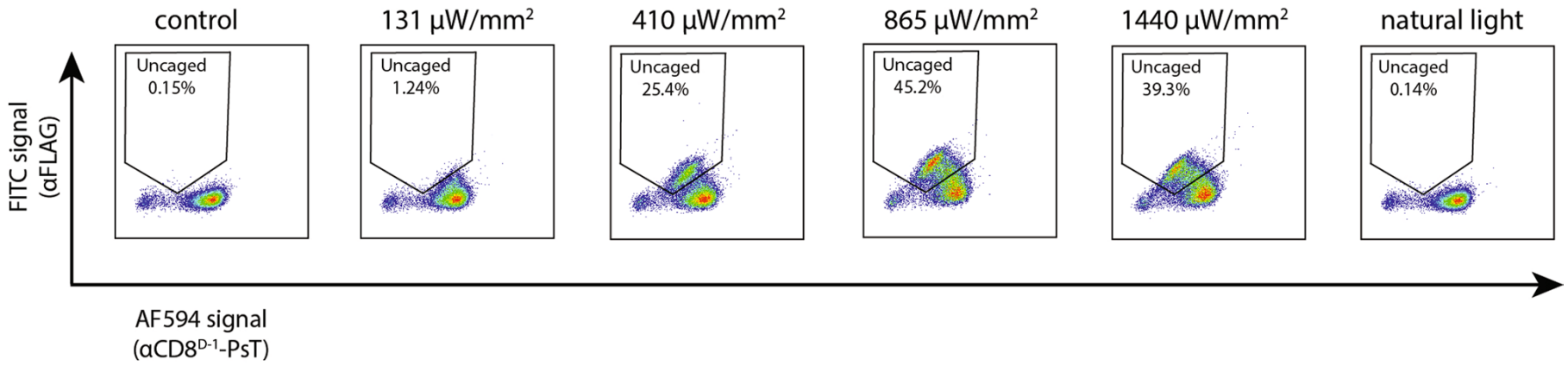
Uncaged: $AF594^{low}\alpha FLAG^{high}$



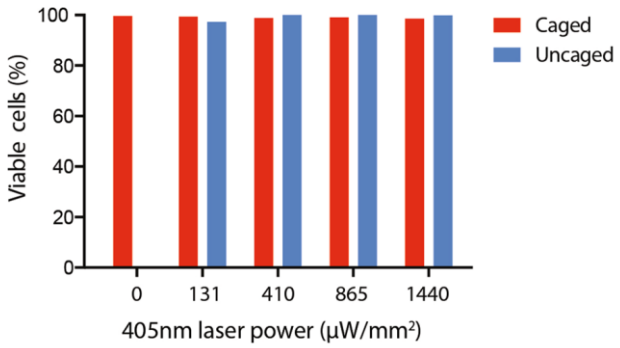


光敏性αCD8纳米抗体试剂

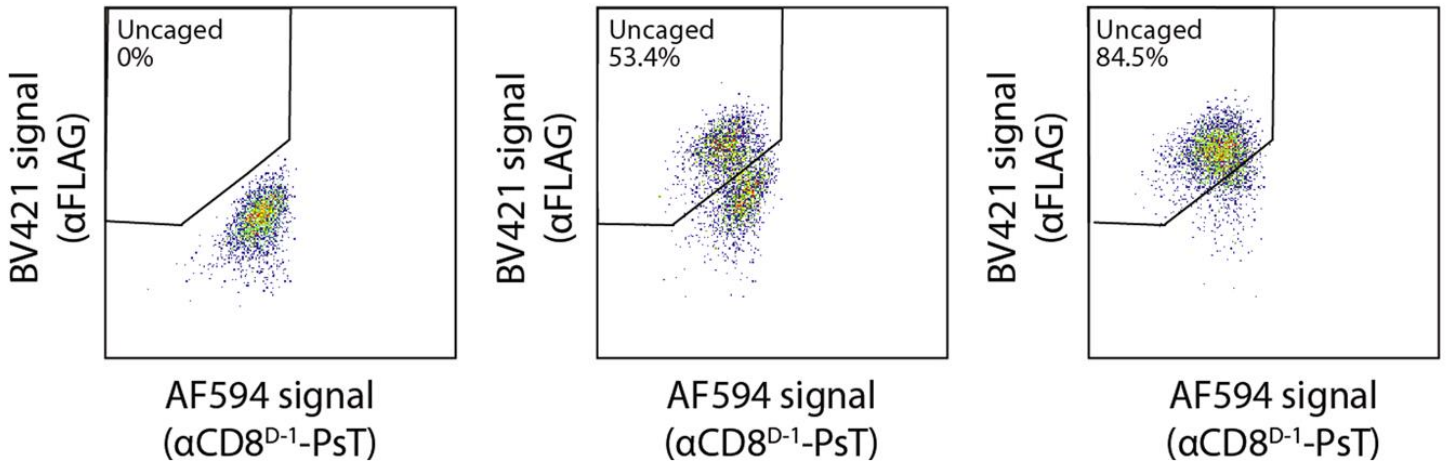
光敏标签的有效光解

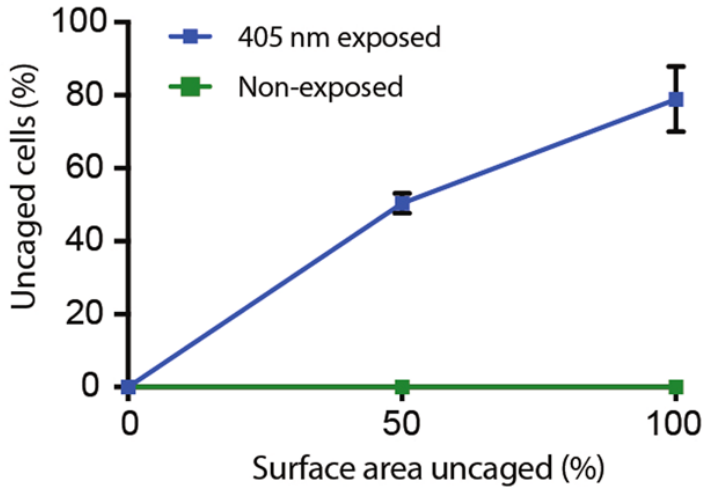


光解后细胞存活率高



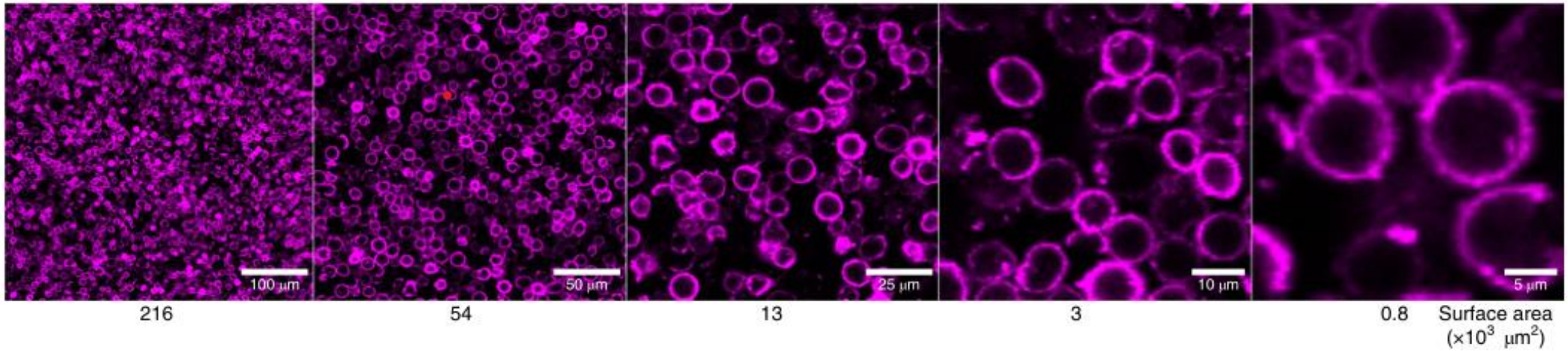
光敏标签光解的特异性

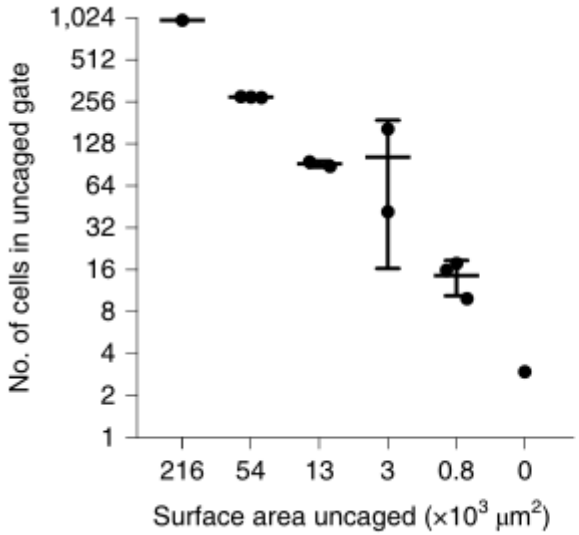


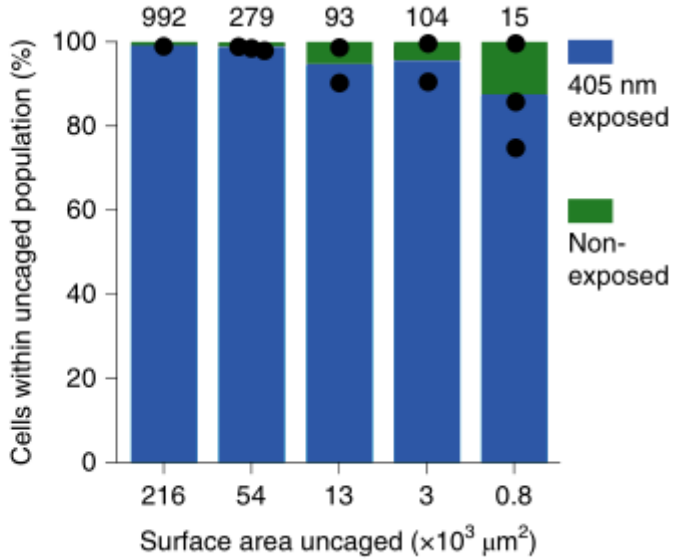


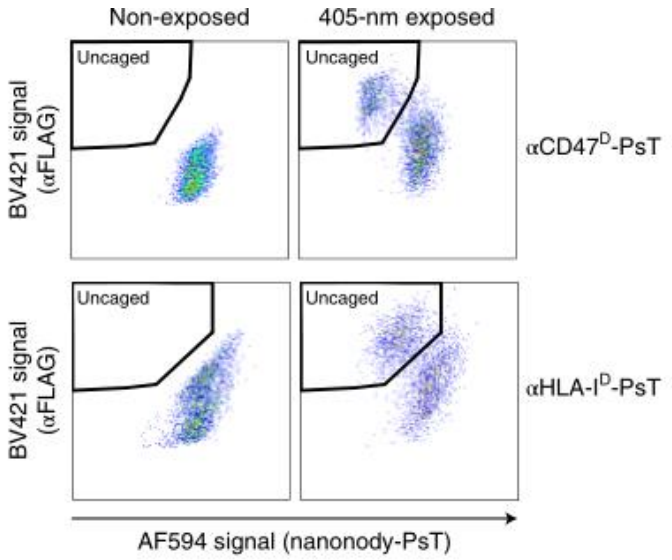
光敏性αCD8纳米抗体试剂

光敏性纳米抗体标记分辨率：3*10^3 μm^2（ 58*58 μm），对应30 - 60个细胞

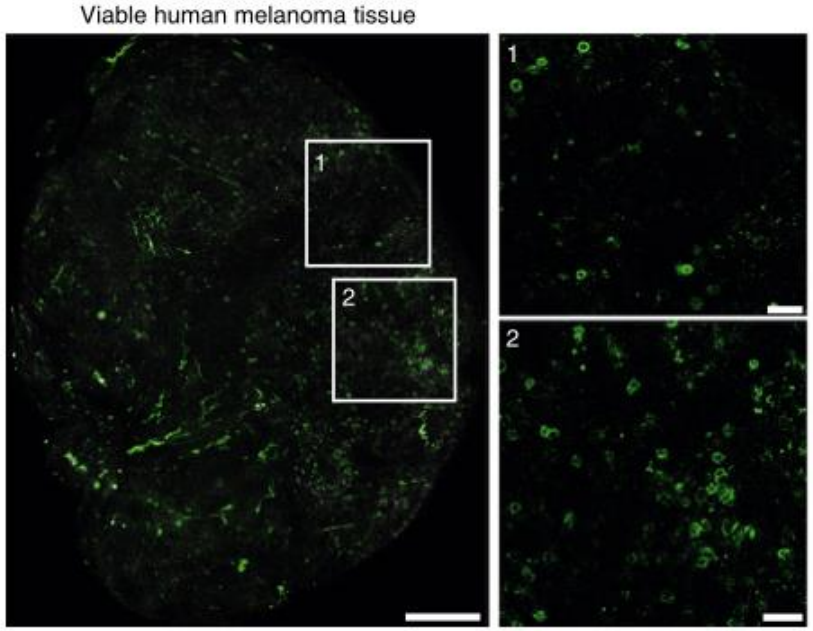


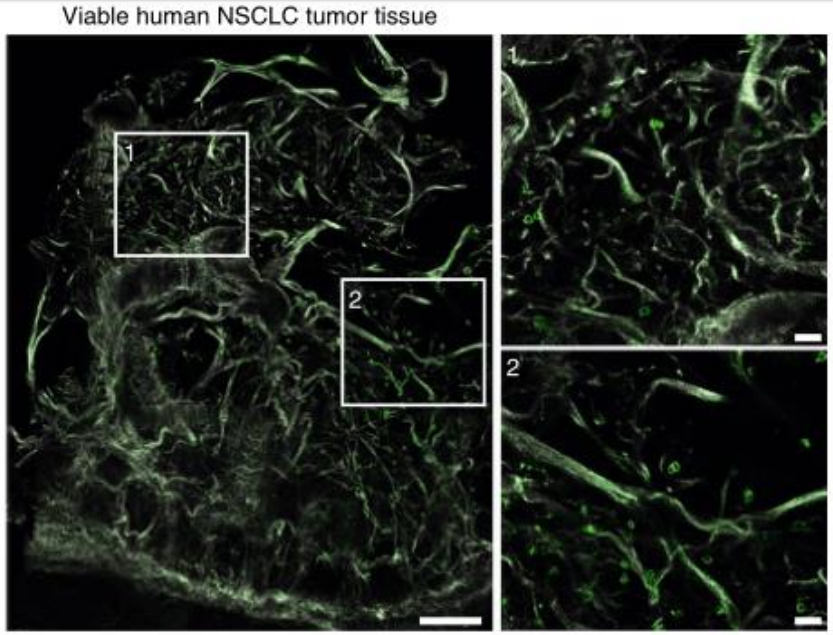


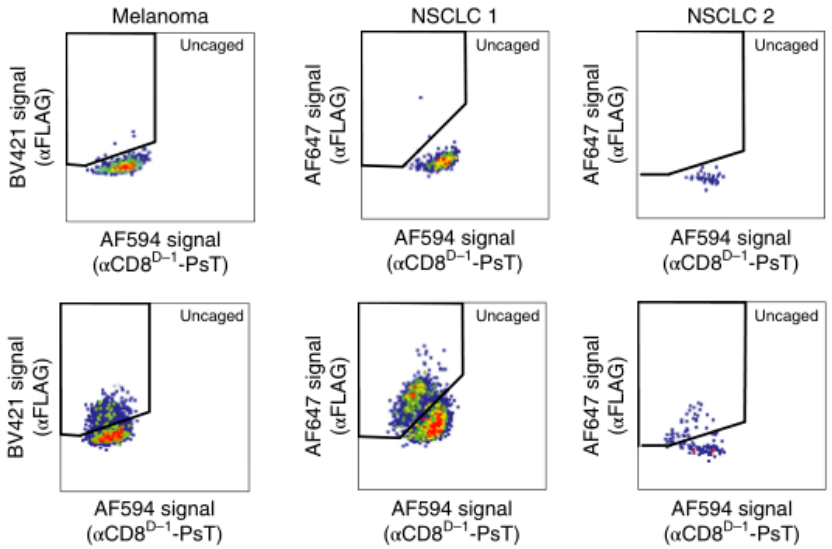


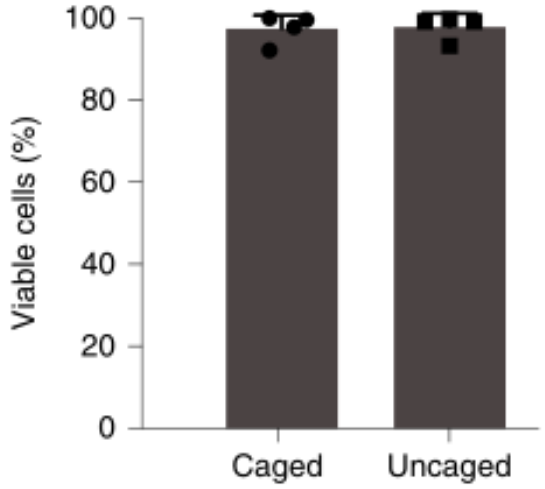


人肿瘤组织标记、局部光解

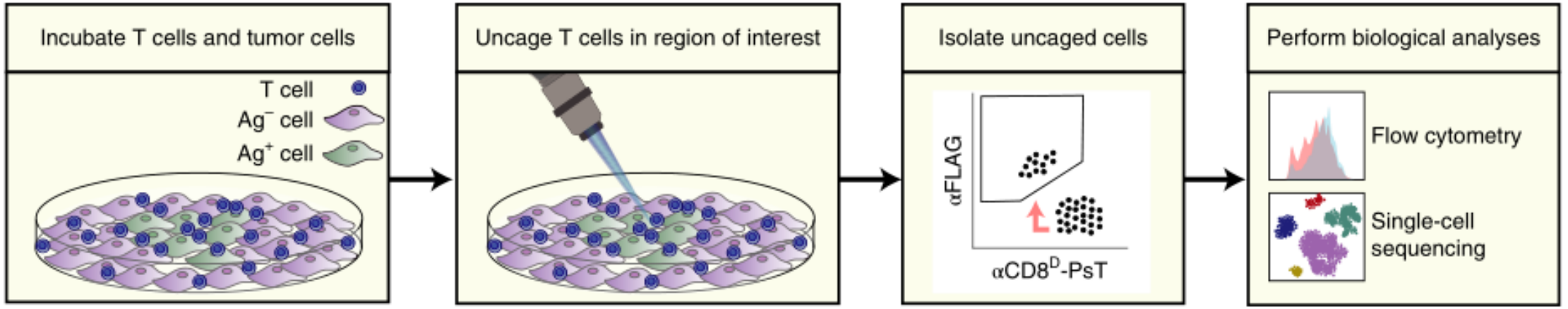




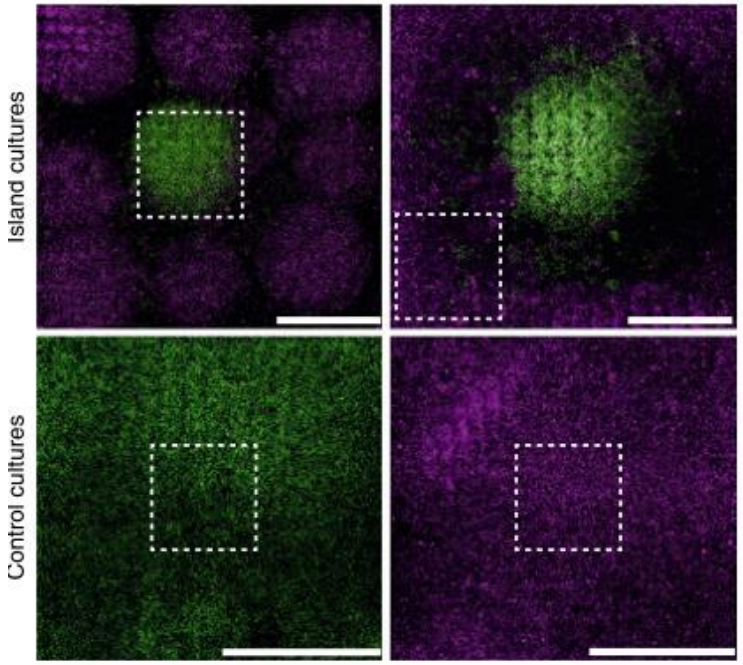


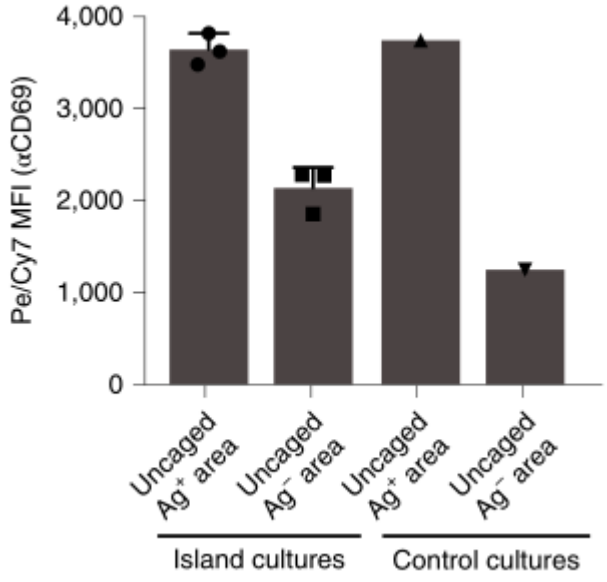


细胞状态的空间差异



- Ag^+区：表达HLA I类限制性 $CDK4_{R>L}$新抗原（GFP阳性）
- Ag^-区：缺乏表达HLA I类限制性 $CDK4_{R>L}$新抗原（ Katushka阳性）
- CD69：T细胞活化标志物





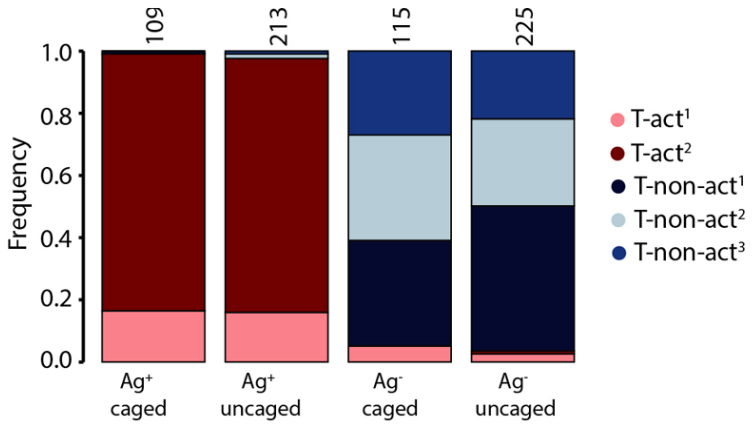
不同空间的CD8⁺T细胞单细胞分析

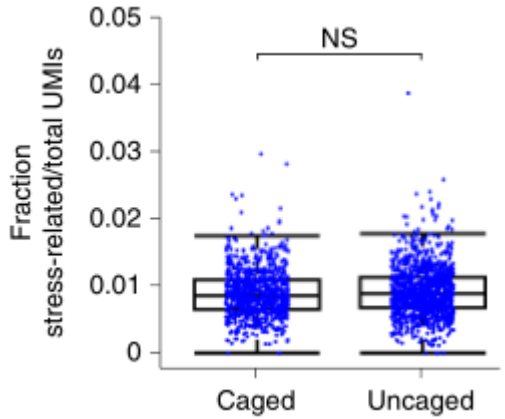
Ag⁺区和Ag⁻区分离的CD8⁺T细胞进行大规模平行单细胞mRNA测序区域（MARS-seq）

MetaCell算法将所有条件下的T细胞分成具有相似基因表达模式的细胞组：

①表达T细胞活化标记物的T细胞（T-act）　②缺乏T细胞活化标记物基因表达的T细胞（T-non-act）

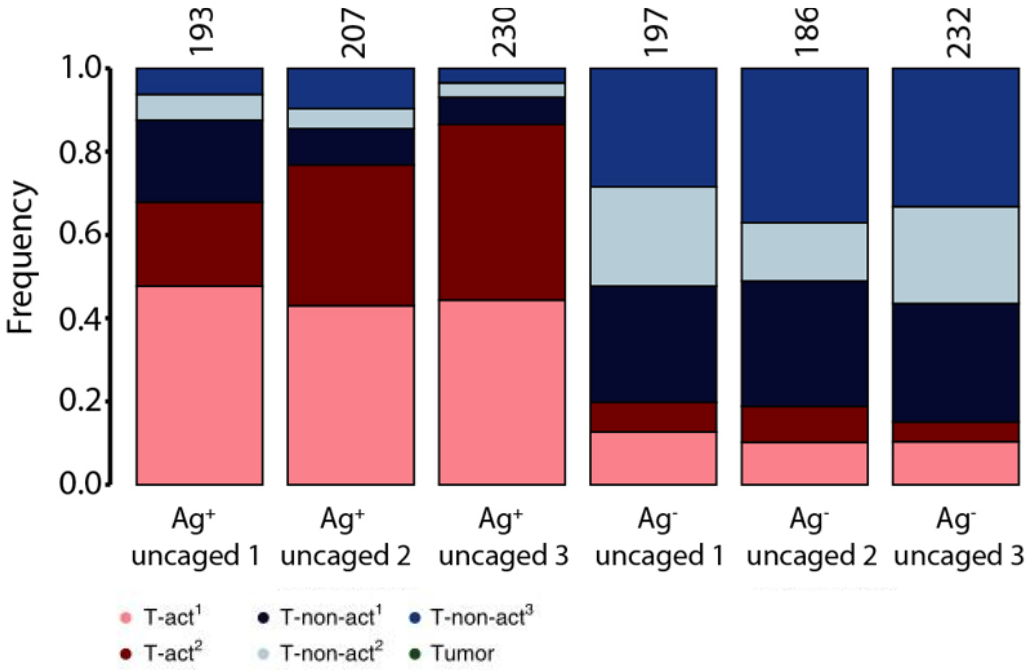
光解未诱导应激基因的表达

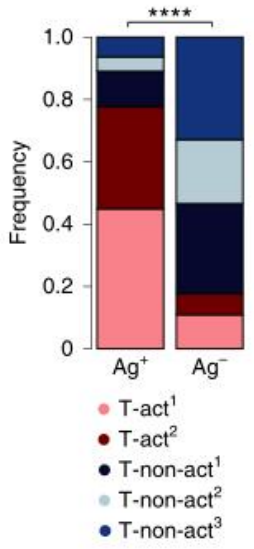




T-act细胞在Ag⁺区高度富集(77 %)

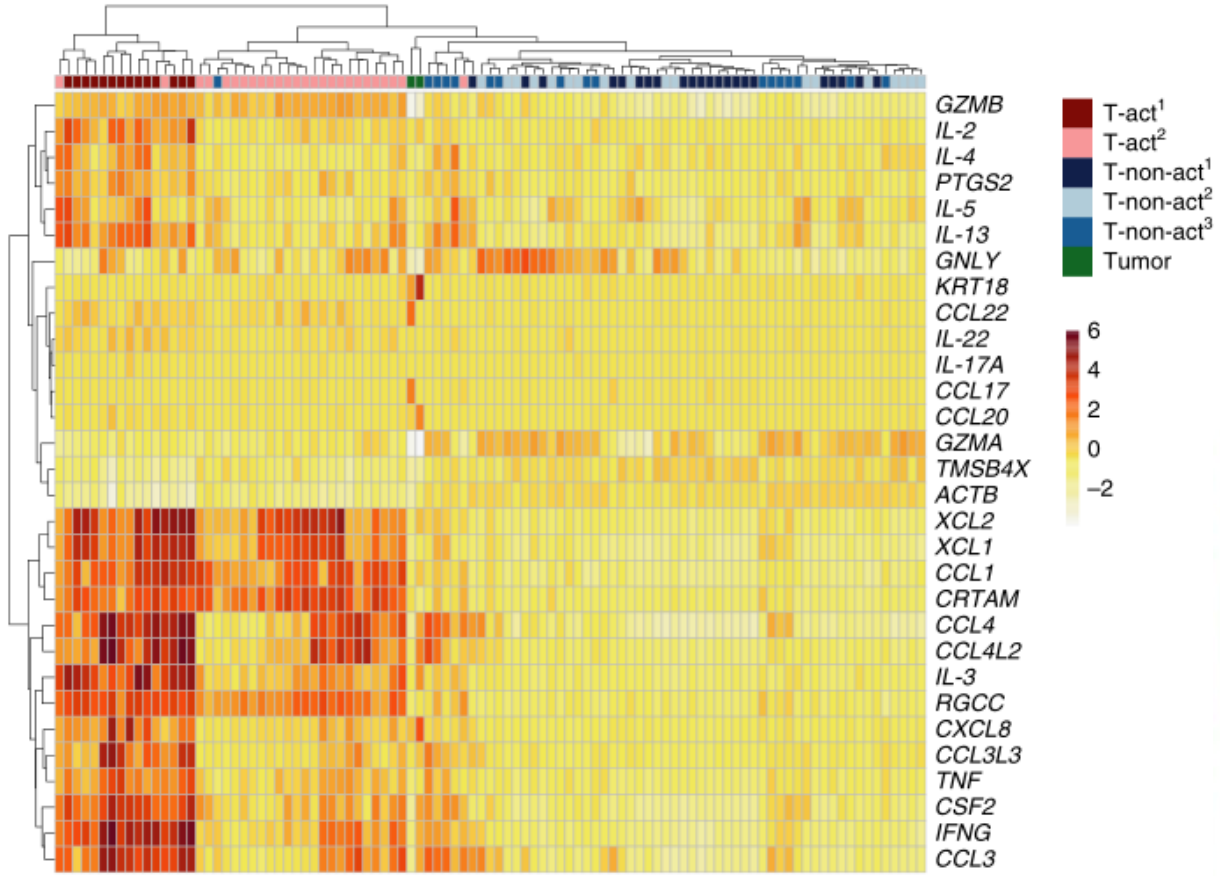
T-non-act细胞在Ag⁻区高度富集（82%）





不同空间的CD8⁺T细胞单细胞分析

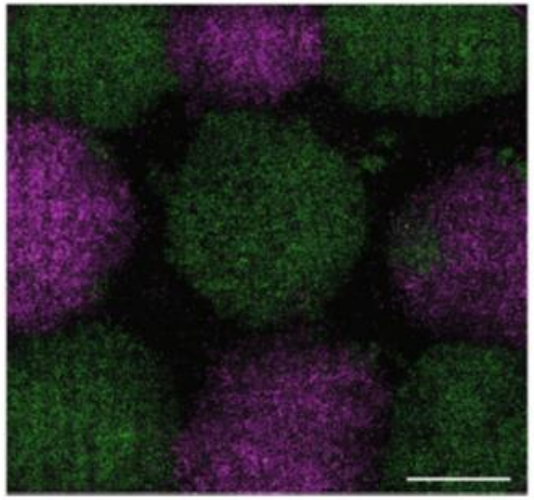
基因数据的变异性映射细胞的位置差异

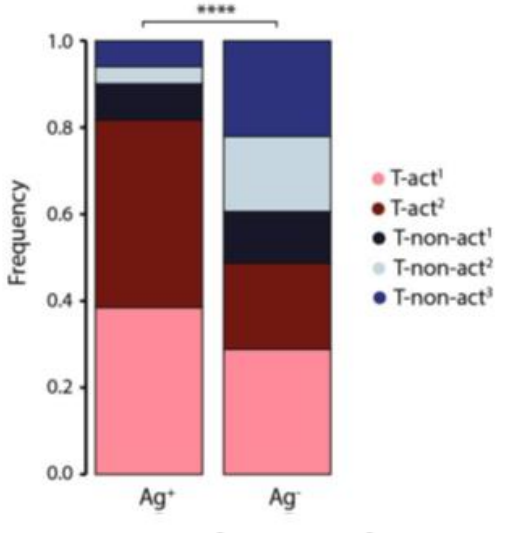


数据集中方差最大的前30个基因

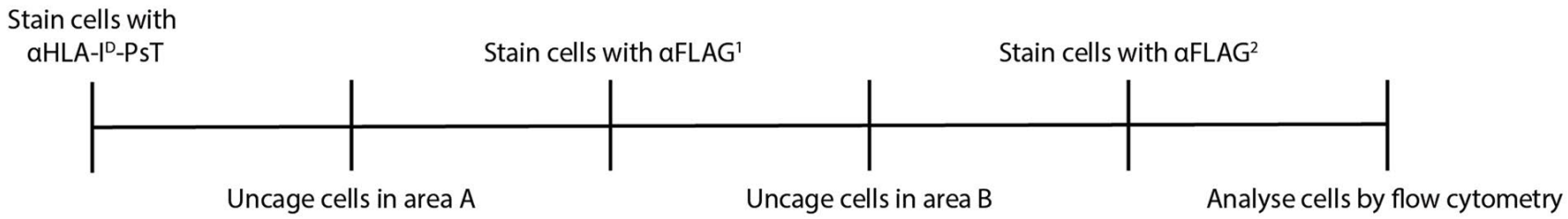
- 包含大量可溶性介质的基因，如干扰素-γ（*IFNG*）、*CCL4*、*CXCL8*
- 67%的基因表达增加

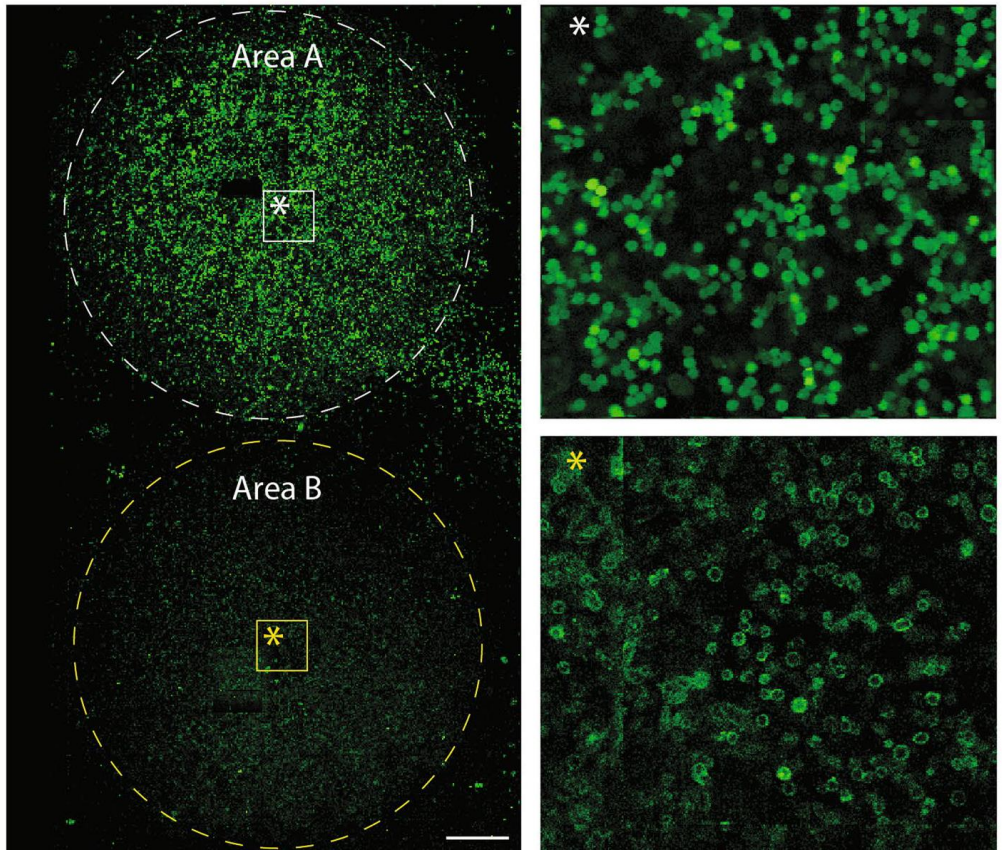
复杂细胞系统中细胞状态的差异

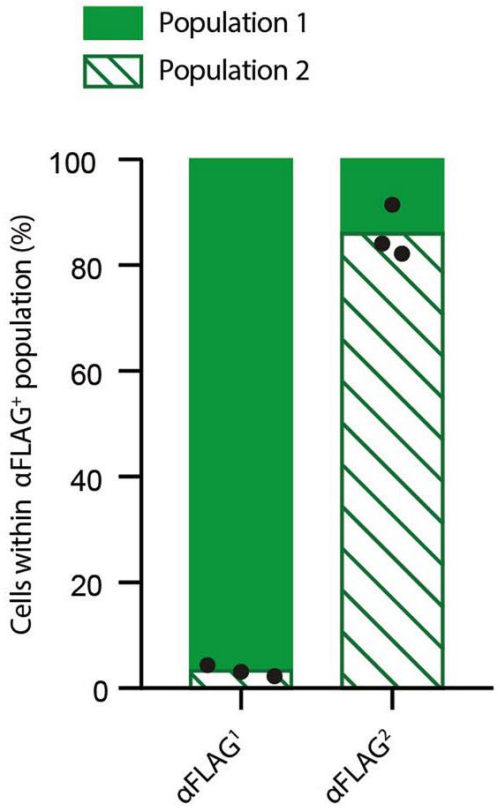




同一样品不同空间细胞多重分析







Summary

- 开发了一种利用光敏标签的纳米抗体标记技术，能够从人类原代组织的目标区域选择性、低毒性地稳定标记细胞，并分离细胞，进行单细胞转录组学分析，剖析局部微环境对细胞状态的影响。
- 有效实现从最低至30–60个细胞的组织结构中分离出光解标签标记的细胞，NPBF生色团的光解可以使用低强度紫外光，细胞毒性低，利于敏感的细胞类型和组织的分析。
- 除了针对CD8作为标记物，开发稳定结合其他细胞标记物的纳米体也可行。
- 利用此技术探究免疫细胞和其他细胞的激活和分化状态与环境信号的相互作用，识别驻留在特定肿瘤或肿瘤周围环境的细胞类型，有助于进一步了解人体组织中细胞位置和细胞状态之间的关系。