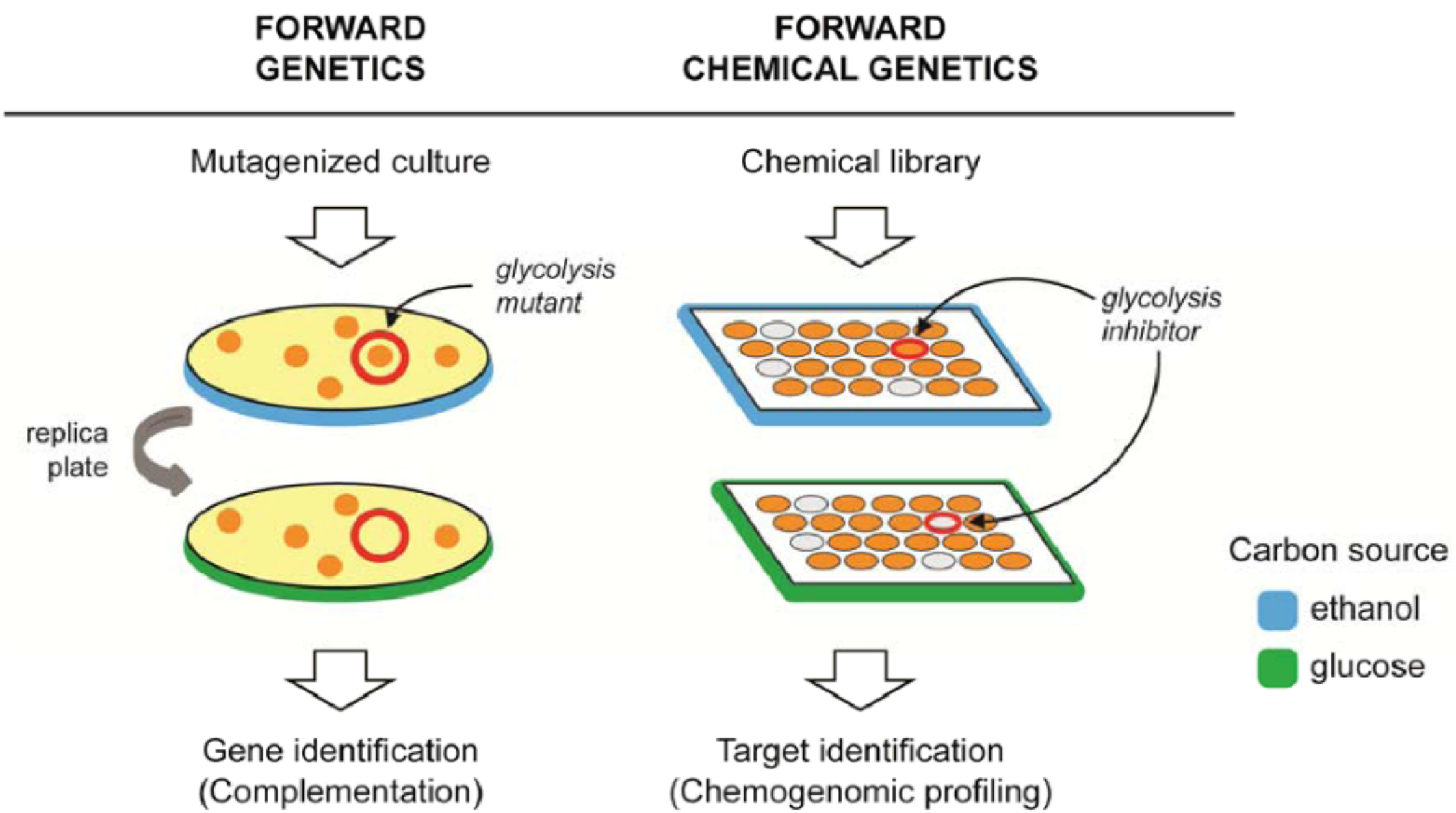
Single-cell biology

Week 6 .

Sc-Based Perturbation Screenings
Spatial Omics techniques

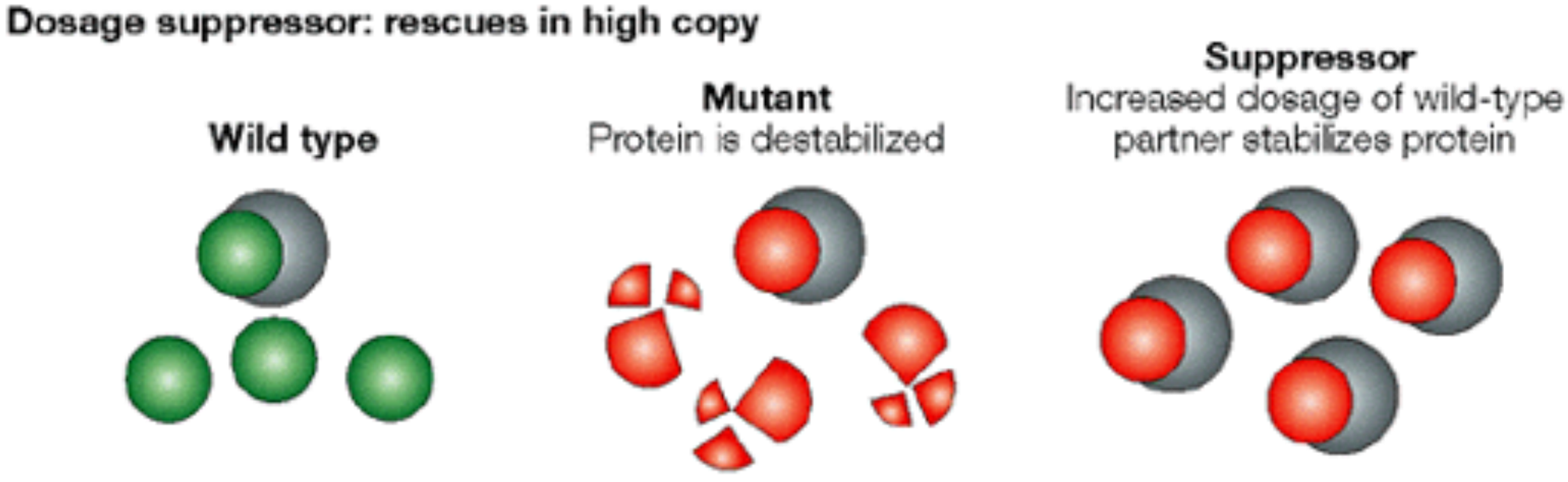
sc-Based Perturb screenings



Forsburg 2001 Nat Rew in Generics

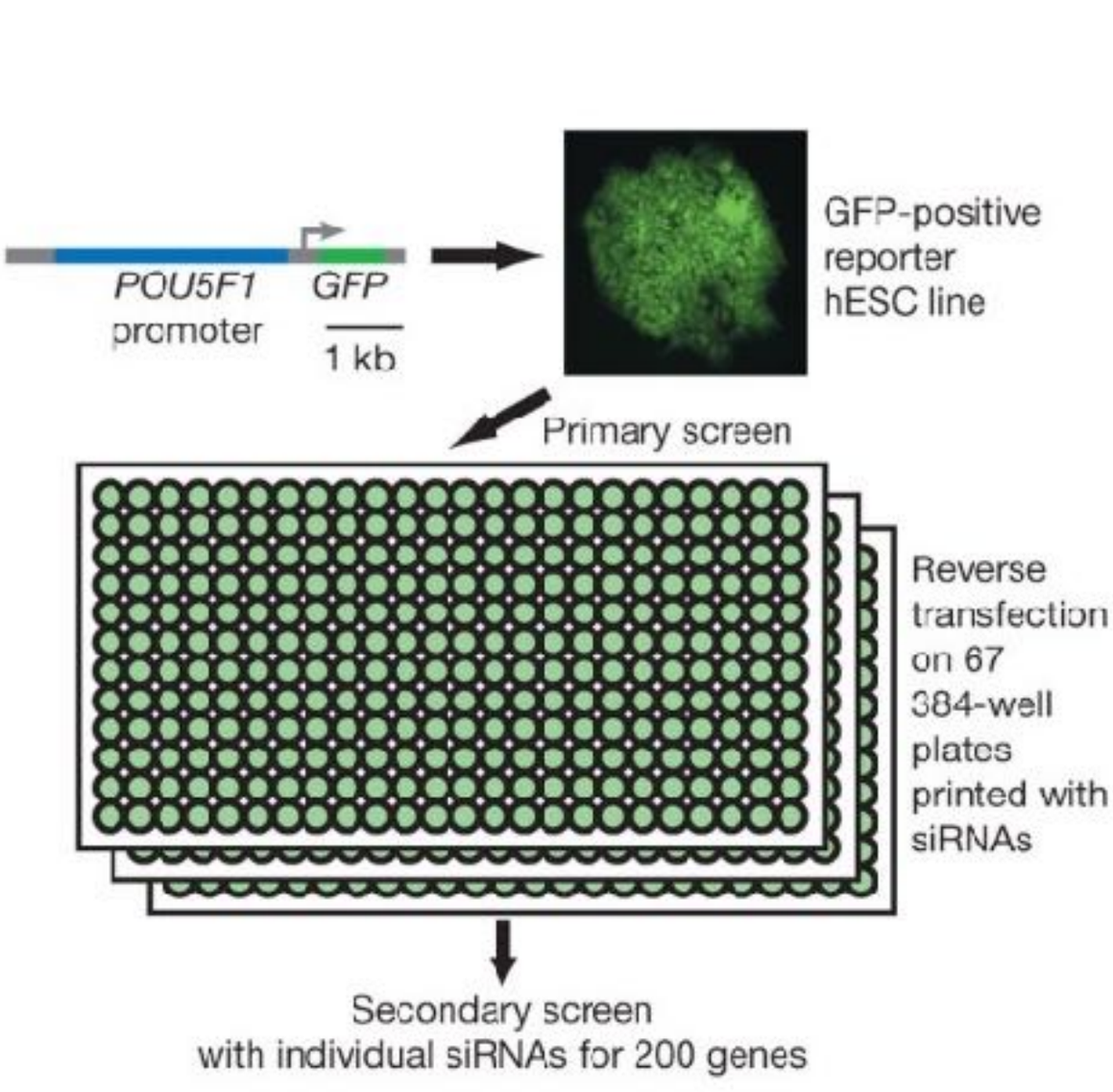
genetics offers unique tools for discovering gene function

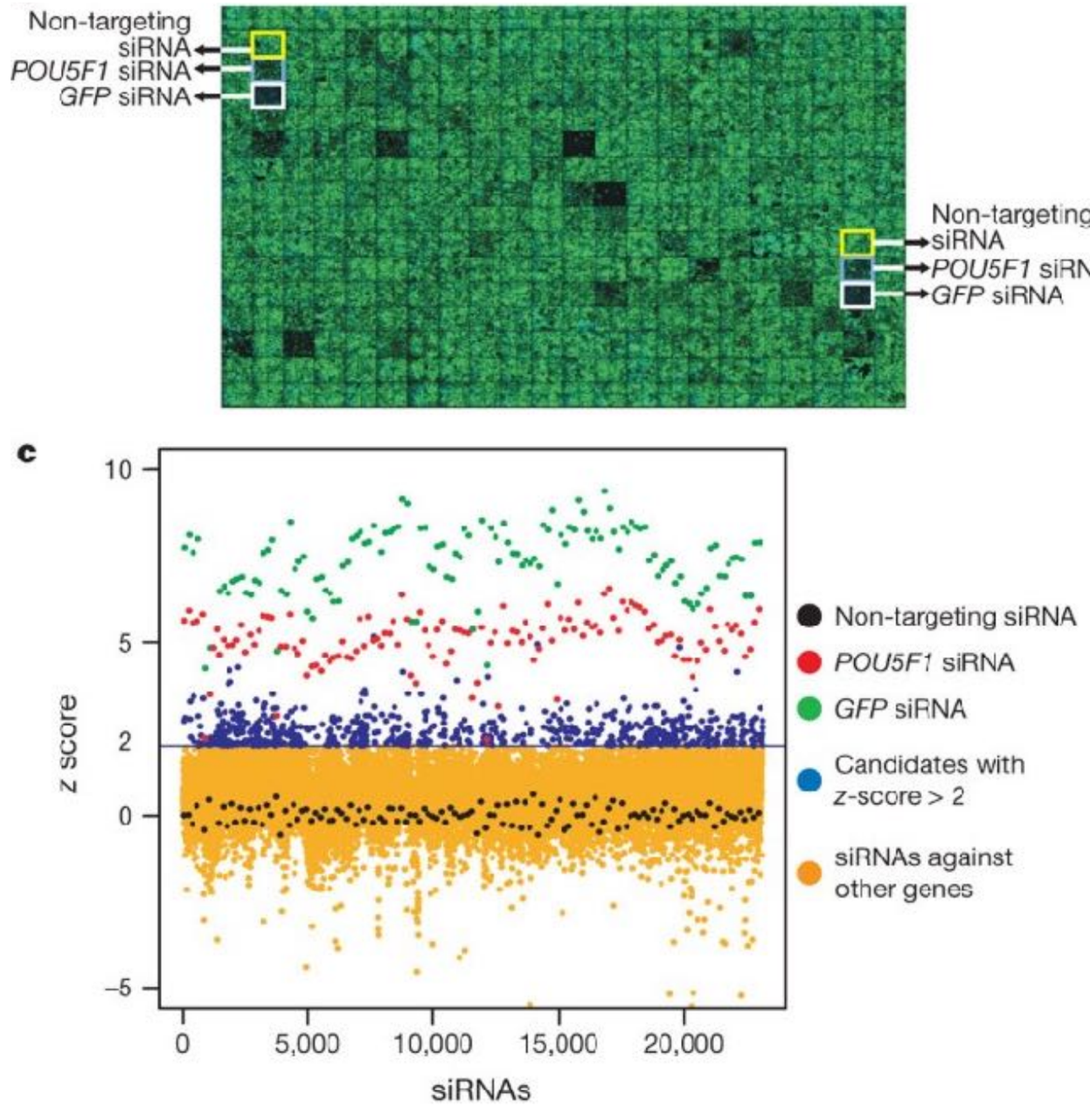
sc-Based Perturb screenings



Forsburg 2001 Nat Rew in Generics

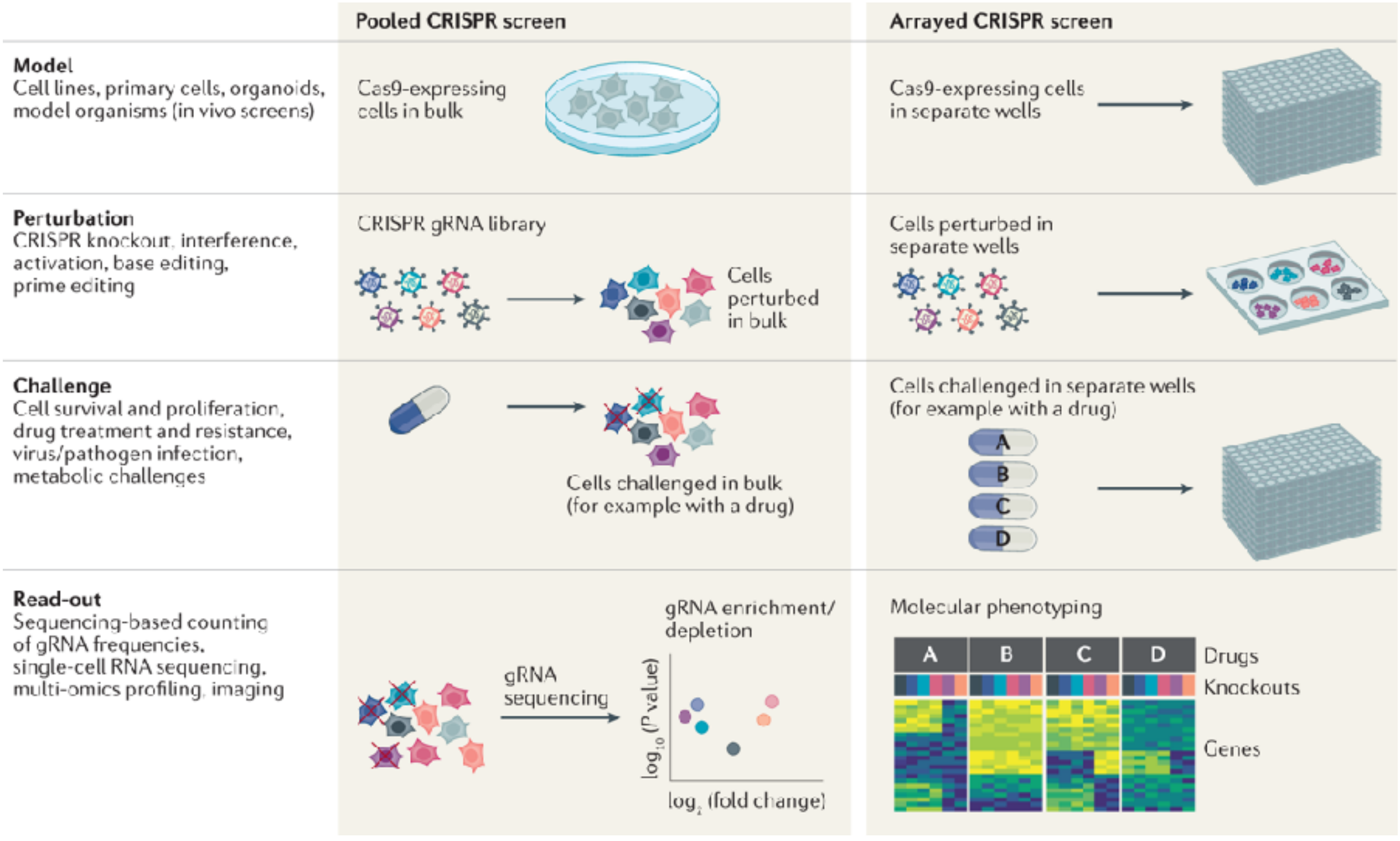
sc-Based Perturb screenings





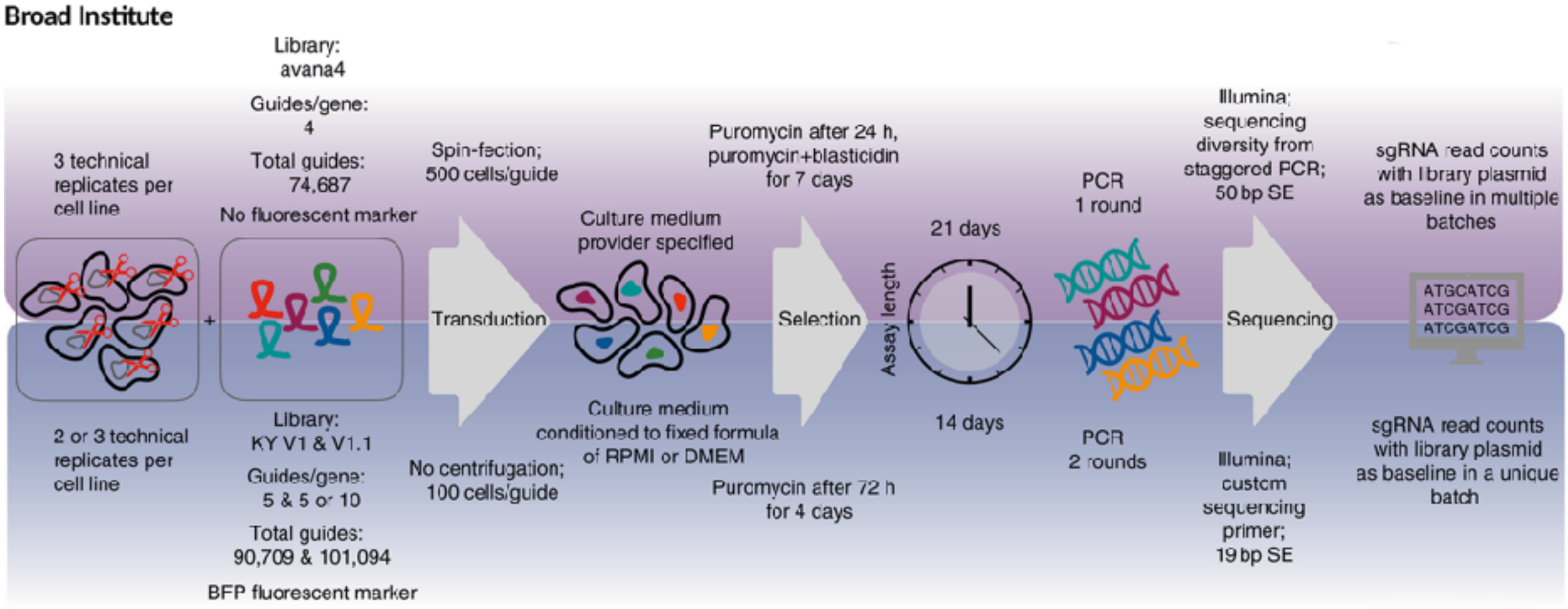
genetic screens are often focused to the assessment of one parameter

sc-Based Perturb screenings



Bock et al. 2022 Nat Rew Met Prim

sc-Based Perturb screenings

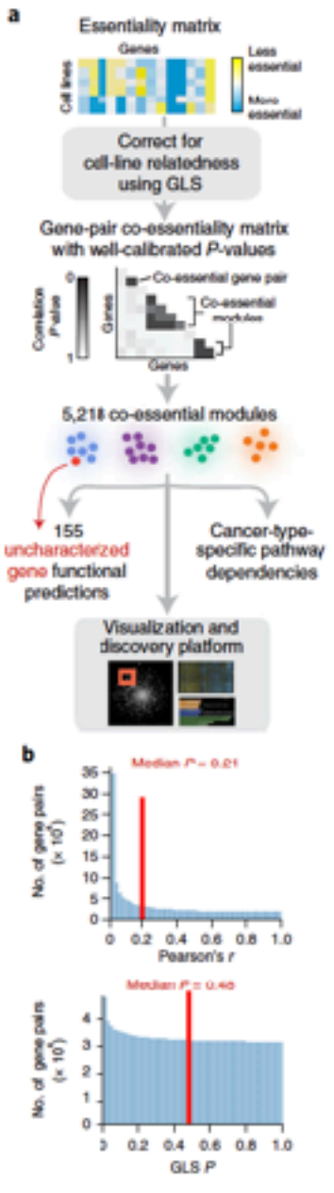


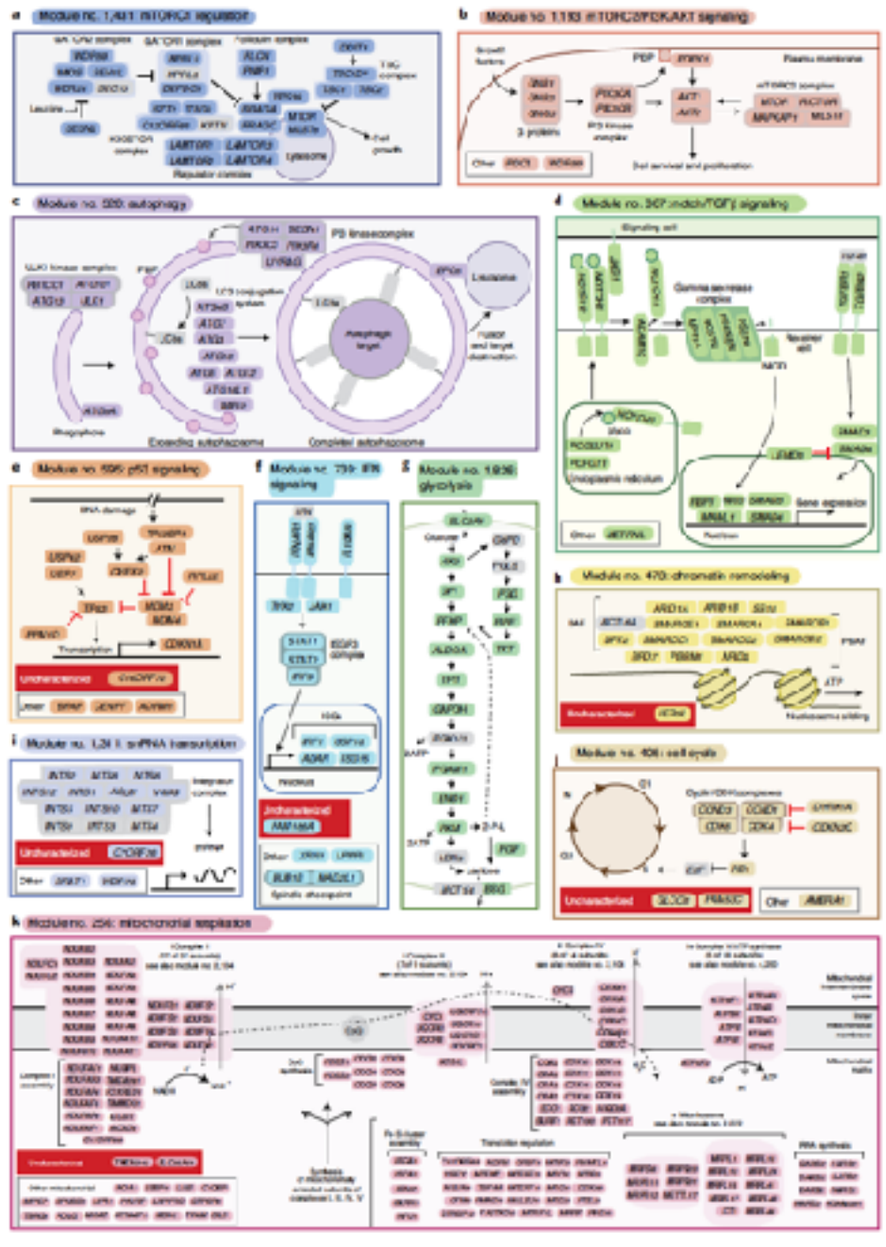
Tsherniak et al. 2017 Cell

Creating a genome-scale catalog of genetic vulnerabilities

A complete map of the vulnerabilities of cancer cell models is a key first step towards identifying therapeutics leads. Therefore, researchers are using genome-wide CRISPR loss-of-function screens to systematically identify essential genes across hundreds of human cancers

sc-Based Perturb screenings

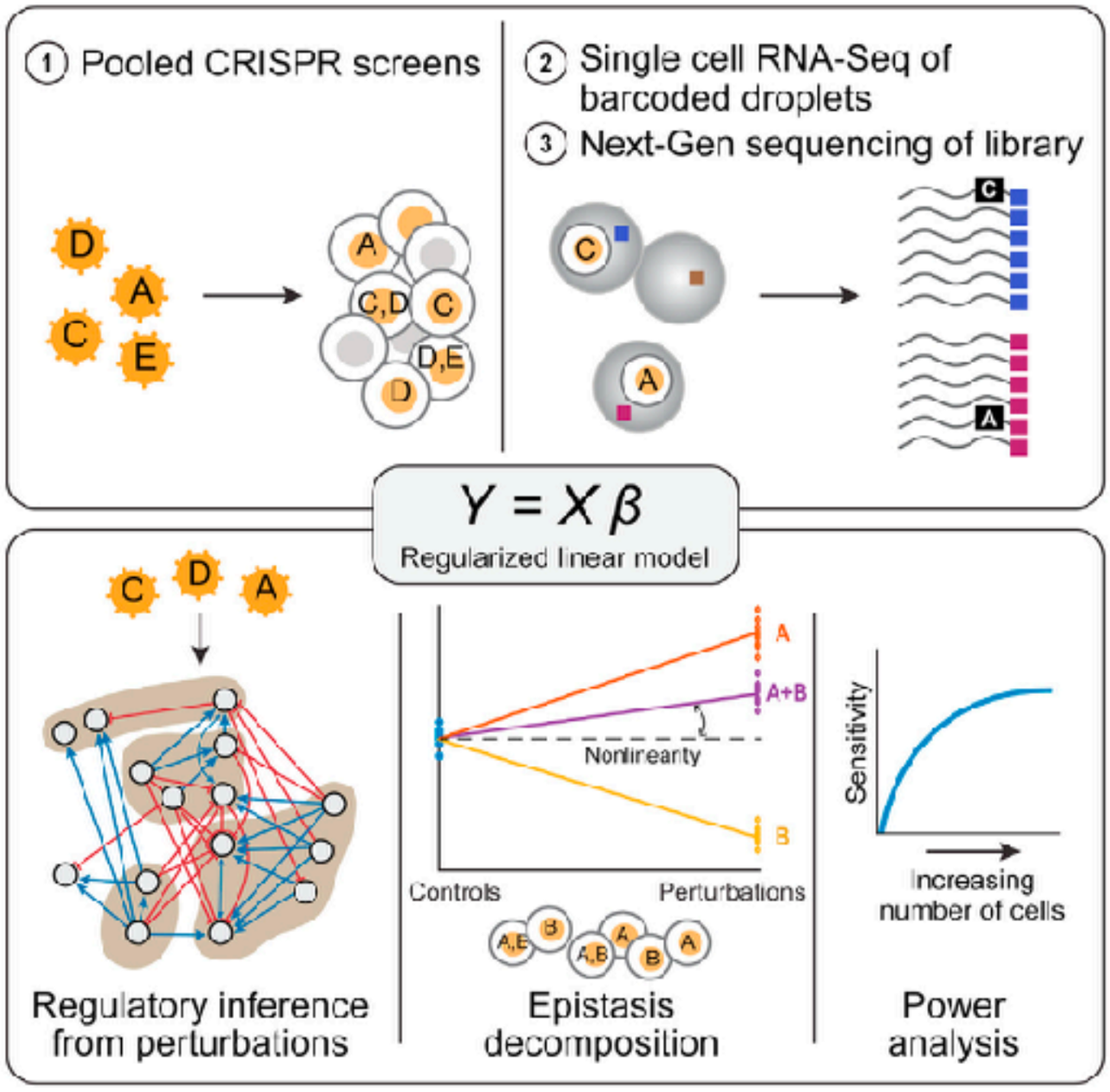




Wainberg et al. 2021 Nat Genetics

Dep_Maps allow the inference of co-functional gene networks (with no priors)

sc-Based Perturb screenings

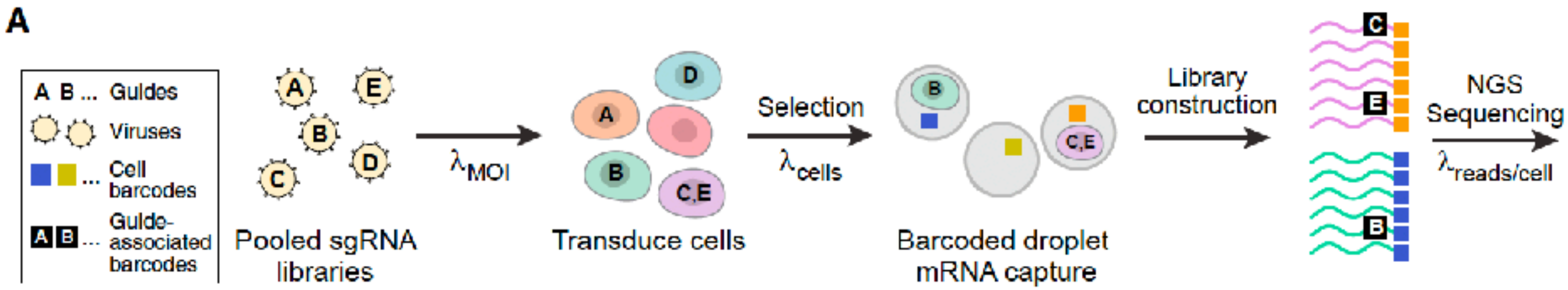


Dixit et al. 2016 Cell

Perturb-seq (also known as **CRISP-seq** and **CROP-seq**) refers to a high-throughput method of performing scRNA-seq on pooled genetic perturbation screens.

Perturb-seq combines multiplexed CRISPR mediated gene inactivations with single cell RNA sequencing to assess comprehensive gene expression phenotypes for each perturbation. Inferring a gene's function by applying genetic perturbations to KD or KD a gene and studying the resulting phenotype is known as reverse genetics. Perturb-seq is a reverse genetics approach that allows for the investigation of phenotypes at the level of the transcriptome, to elucidate gene functions in many cells, in a massively parallel fashion.

sc-Based Perturb screenings



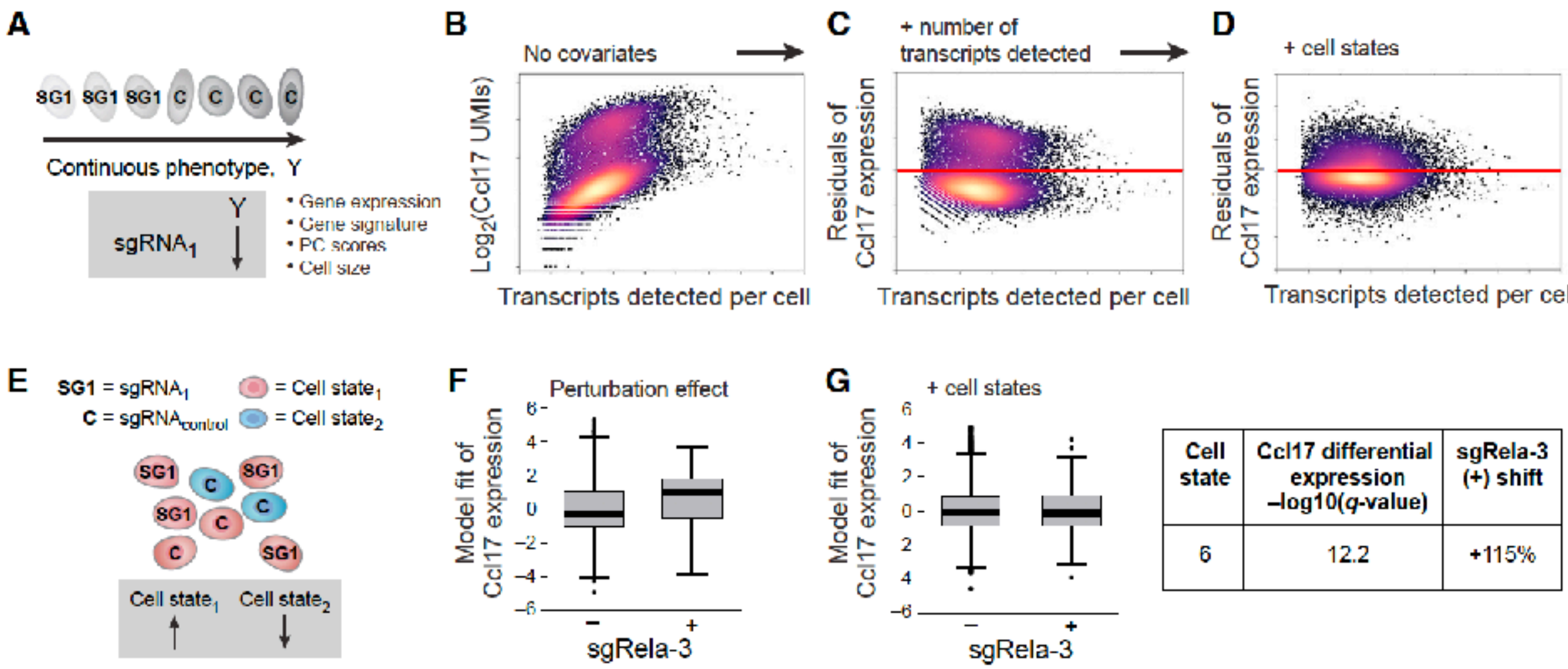
Dixit et al. 2016 Cell

Perturb-seq combines a pooled CRISPR screen with scRNA-seq by encoding the identity of the perturbation on an expressed guide barcode (GBC)

so each cell has 2 barcodes one for cell identity (CBC) and the other for perturbation identity (GBC)

sc-Based Perturb screenings

Bone marrow-derived dendritic cells (BMDCs), they targeted 24 transcription factors (TFs) in ctrl and LPS treated cells

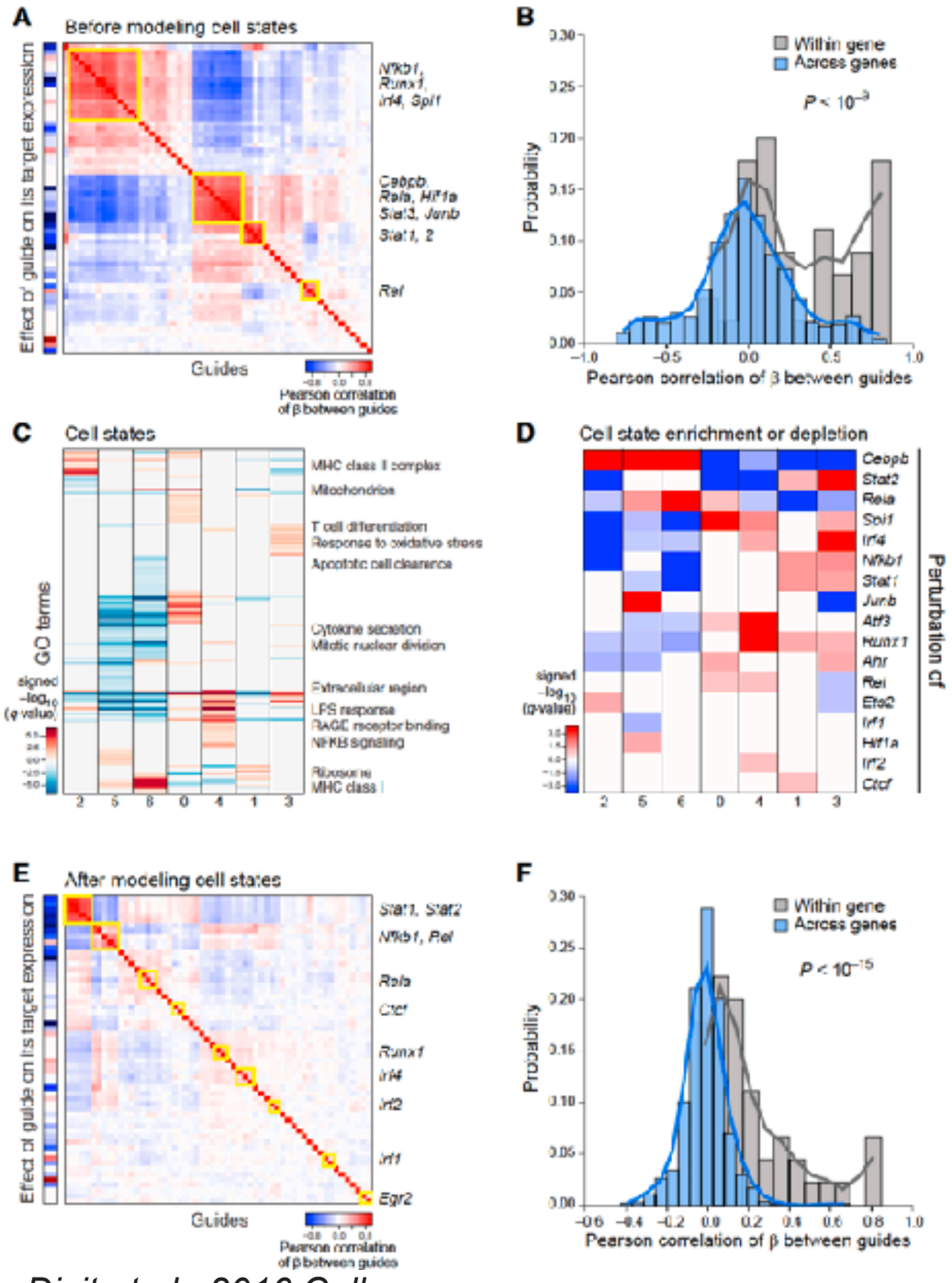


Cell state	Ccl17 differential expression –log10(*q*-value)	sgRela-3 (+) shift
6	12.2	+115%

Dixit et al. 2016 Cell

Allows to discriminate effects on cell state proportions from those on the expression of a given gene

sc-Based Perturb screenings

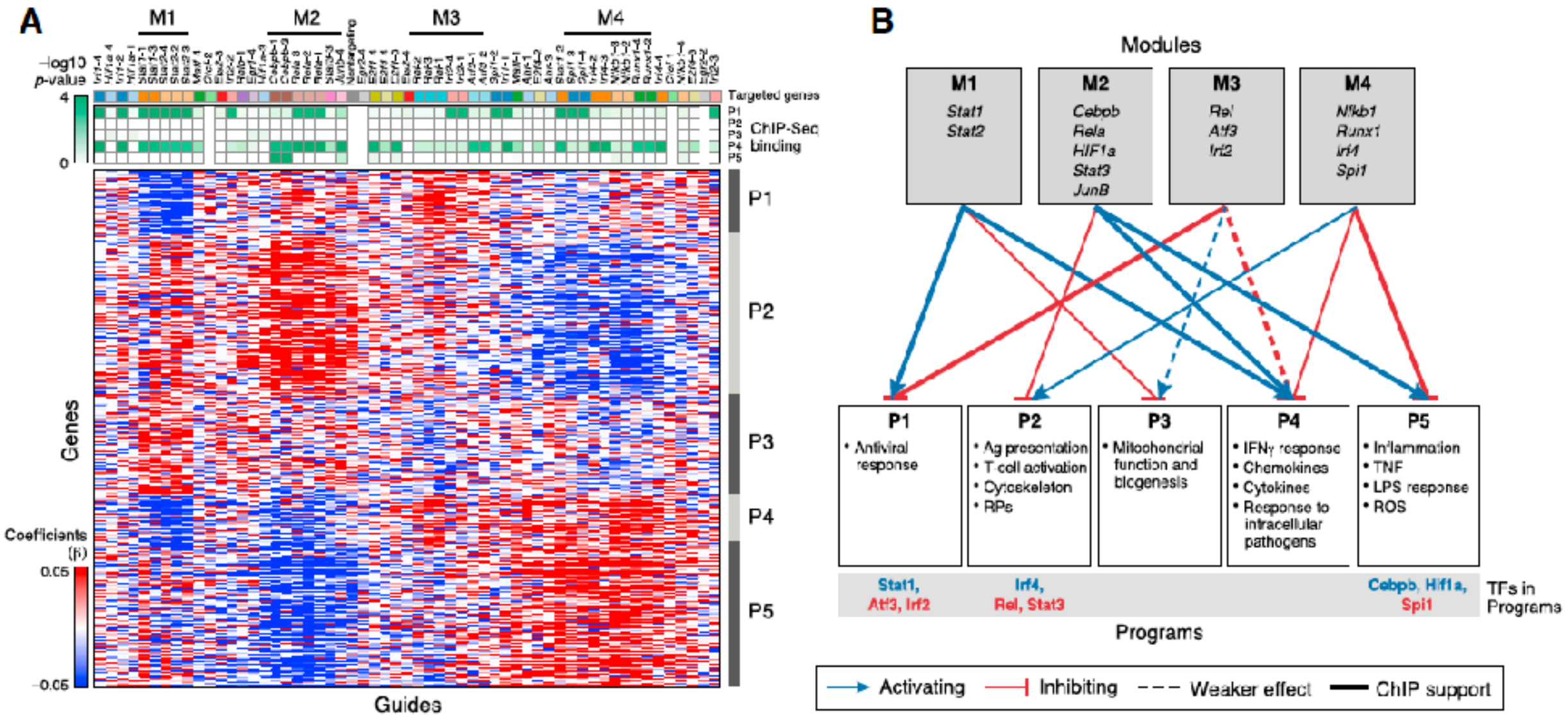


Authors used the regulatory effects of each perturbed TF on each gene, to group TFs into modules by their similar regulatory effects and to group genes into programs by how they are affected by the perturbations.

Note this structure is largely dictated by effects one cell states

Dixit et al. 2016 Cell

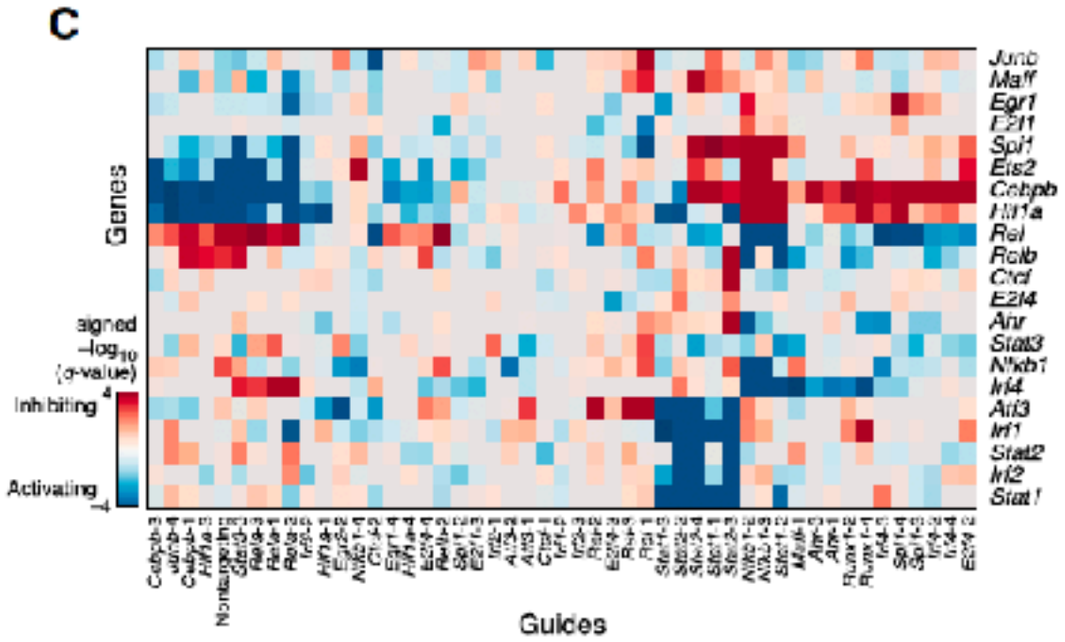
sc-Based Perturb screenings

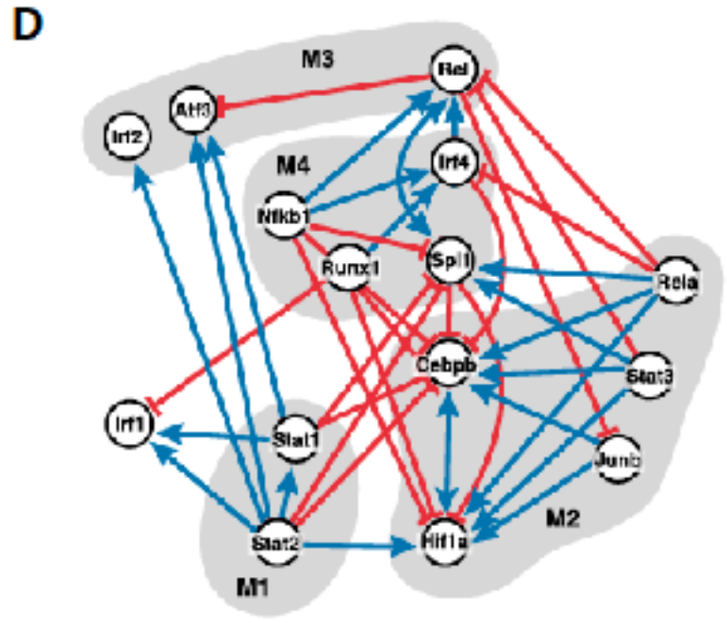


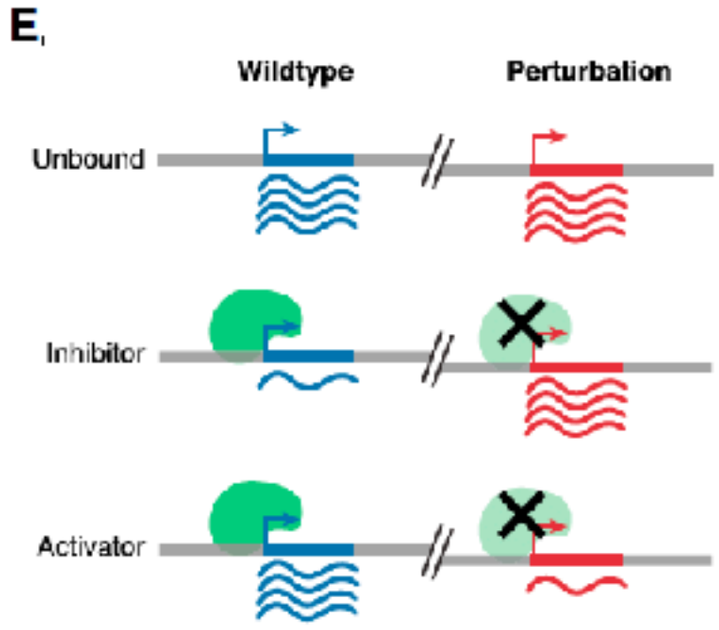
Dixit et al. 2016 Cell

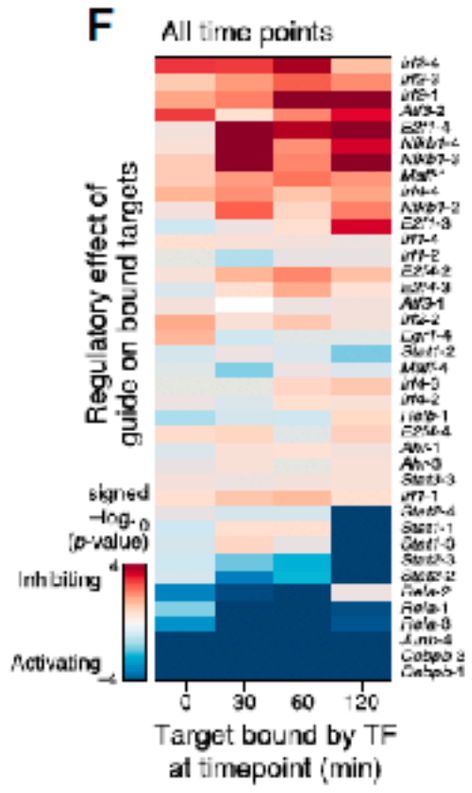
The Genetic Circuit Is Supported by TF Binding Profiles

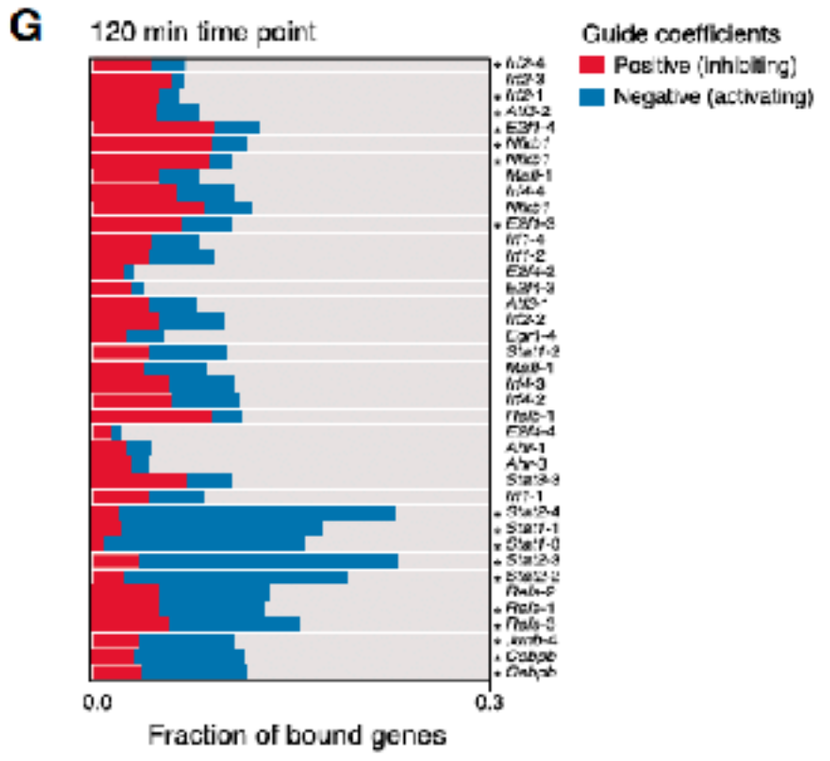
sc-Based Perturb screenings





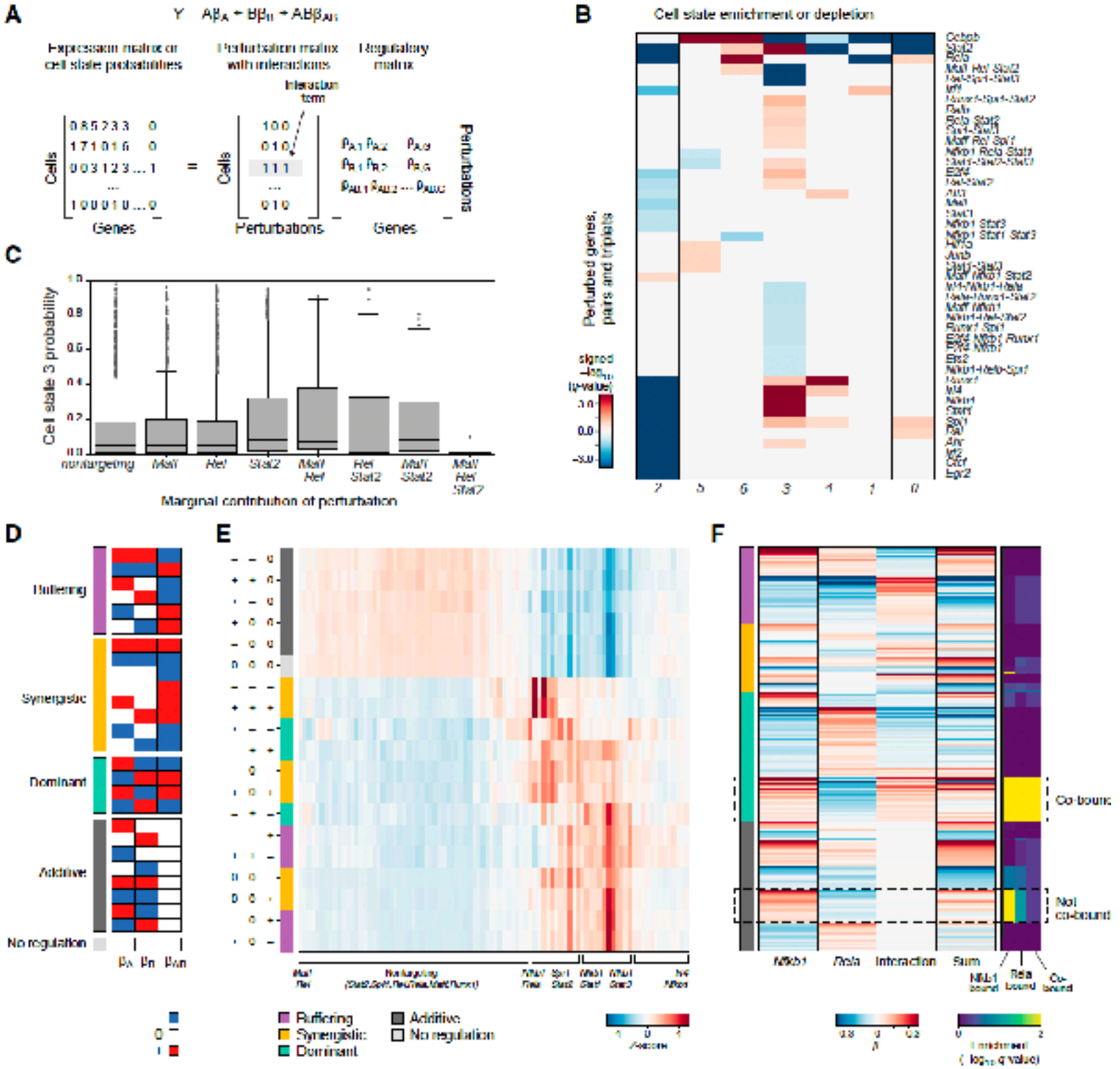






Dixit et al. 2016 Cell

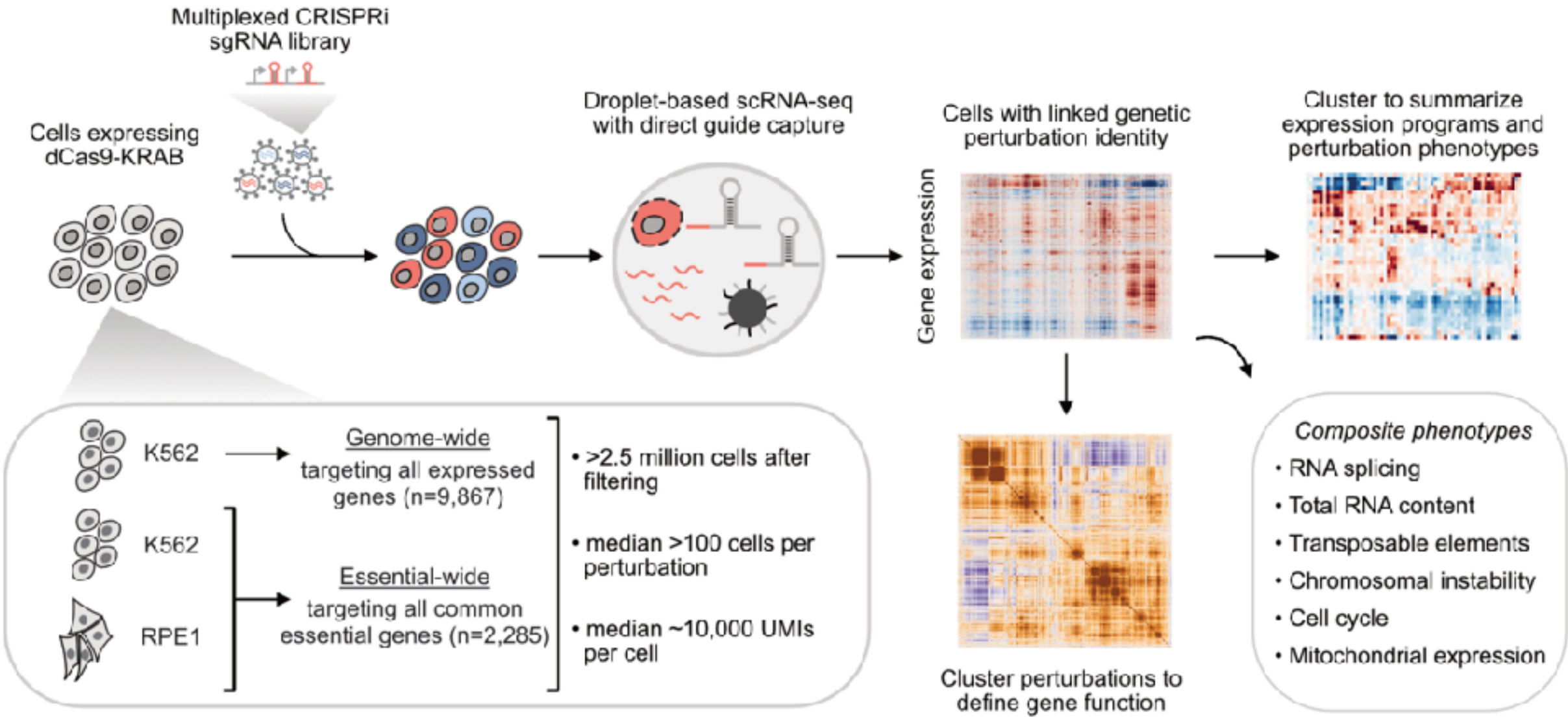
sc-Based Perturb screenings



Genetic Interactions between TFs in BMDCs can be inferred by looking at double or triple infected cells

Dixit et al. 2016 Cell

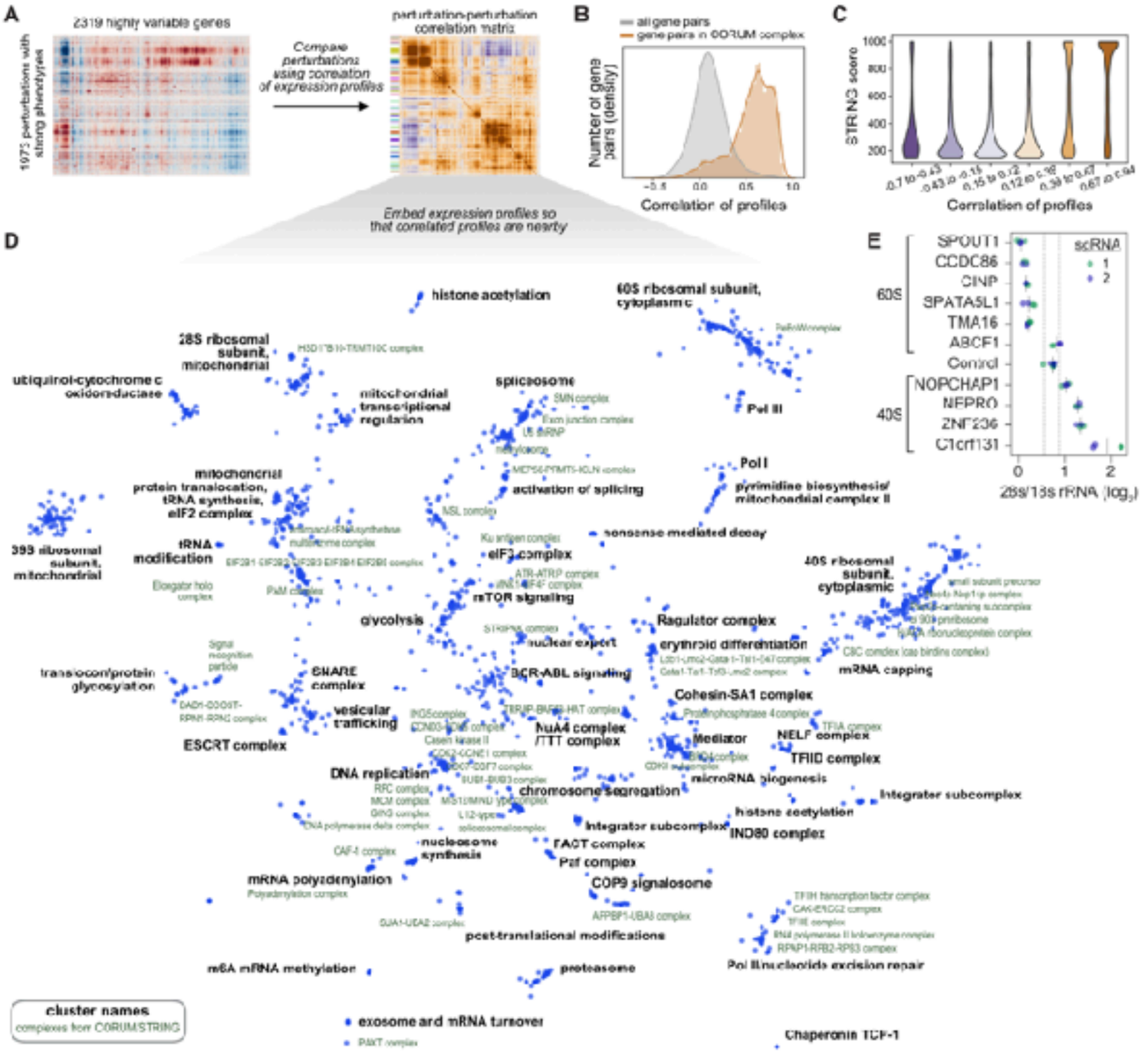
sc-Based Perturb screenings



Replogle et al. 2021 Biorxiv

Whole Genome Perturb Seq

Whole Genome Perturb Seq

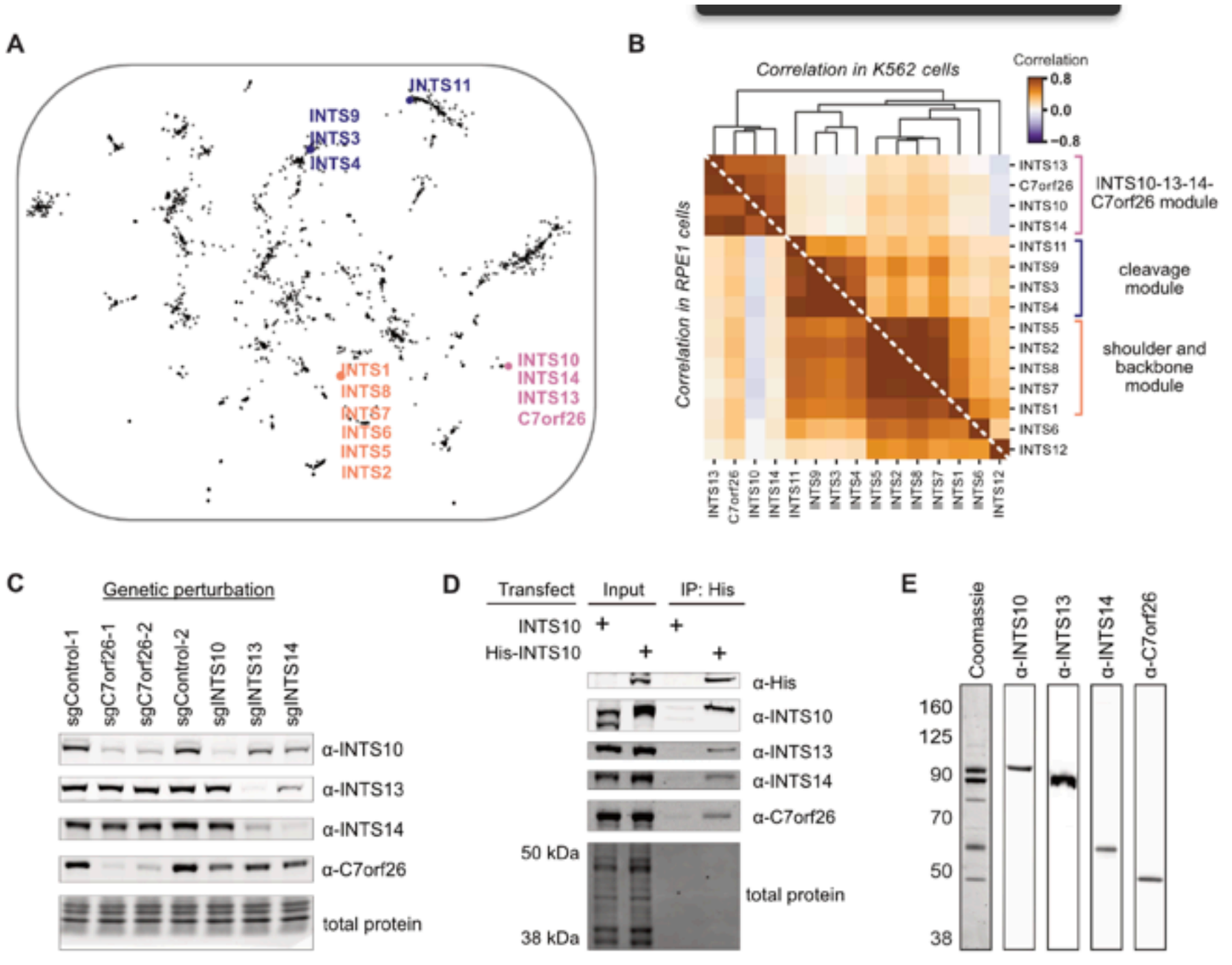


Whole genome Perturb_seq allows the inference of co-functional gene networks active in a give cell type looking at perturbation correlation only

(with no priors)

Replogle et al. 2021 Biorxiv

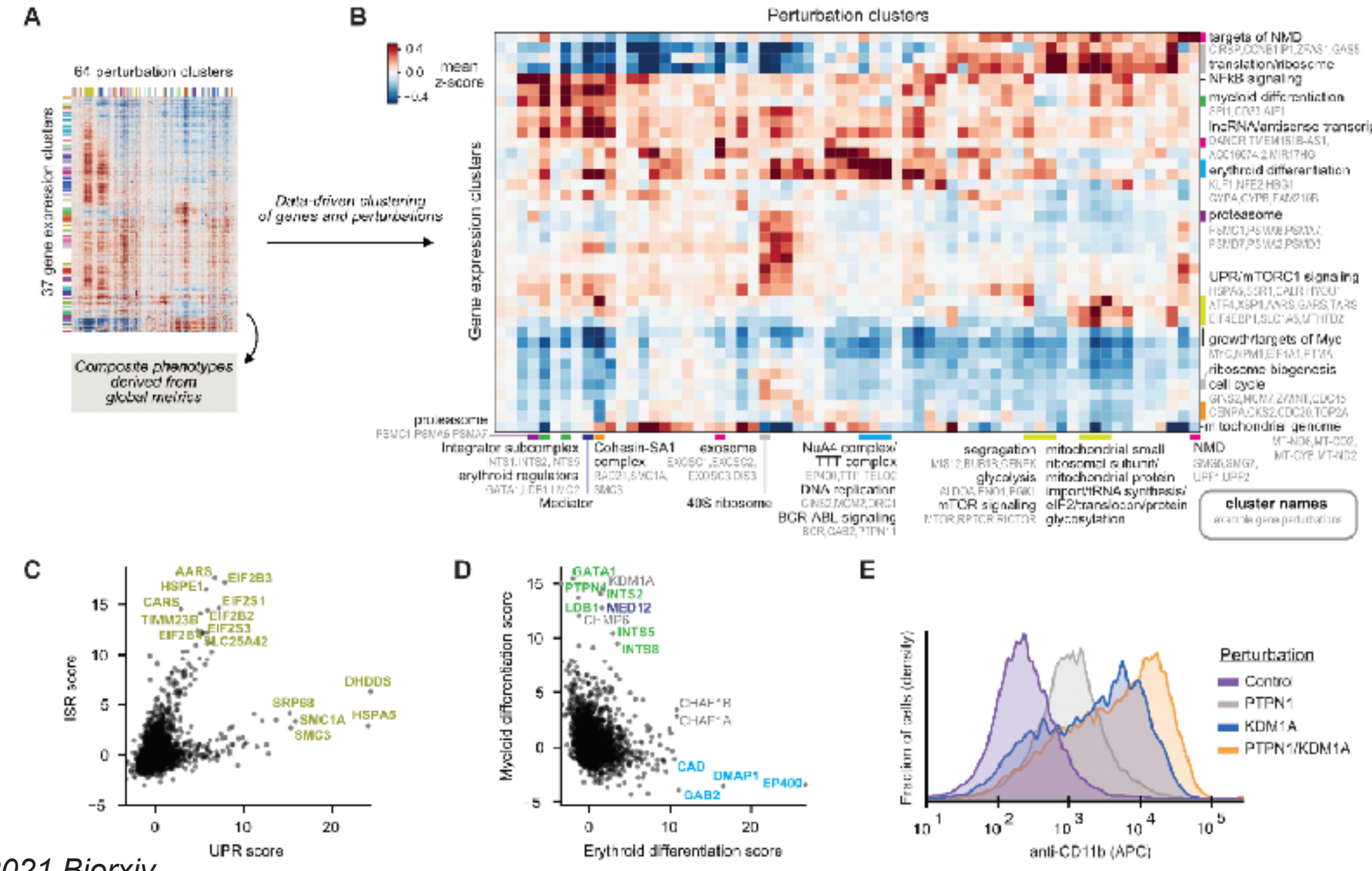
Whole Genome Perturb Seq



Replogle et al. 2021 Biorxiv

Whole Genome Perturb Seq

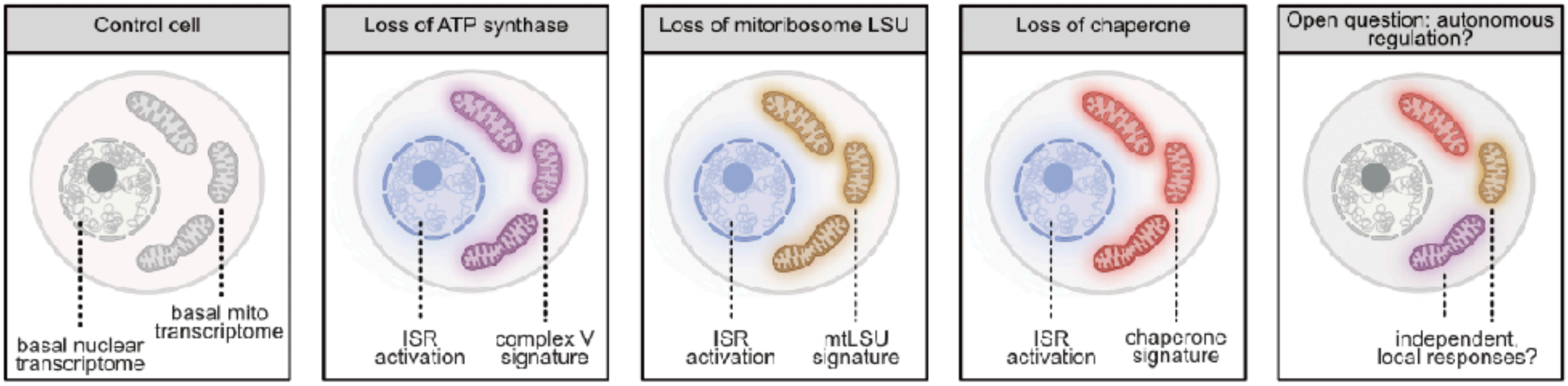
Summarizing genotype-phenotype relationships with Perturb-seq



Replogle et al. 2021 Biorxiv

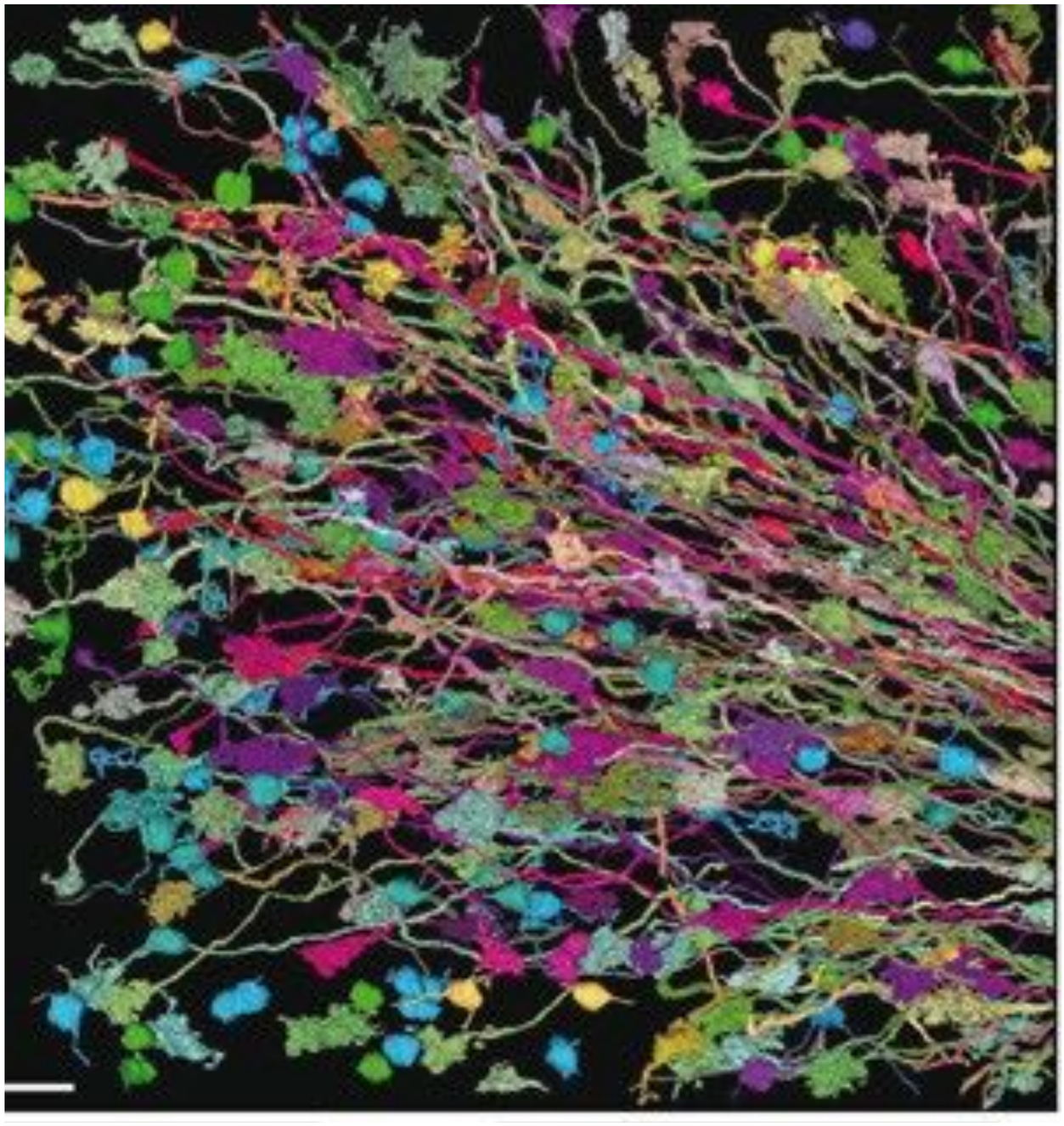
Data-driven definition of transcriptional programs

sc-Based Perturb screenings



Replogle et al. 2021 Biorxiv

Spatial Omics Techniques



Single-cell analyses, especially RNA sequencing and other genomics modalities, have been transformative in revealing new biology.

However, these approaches fail to provide a complete picture of biological processes, as contextual information on cellular location is lost.

New technologies leveraging multiplexed fluorescence, DNA, RNA and isotope labeling enable the detection of tens to thousands molecular biomarkers within their native spatial context.

Spatial Omics Techniques

(1) optical barcoding methods for tracking cell subclones

(2) spatial proteomics methods

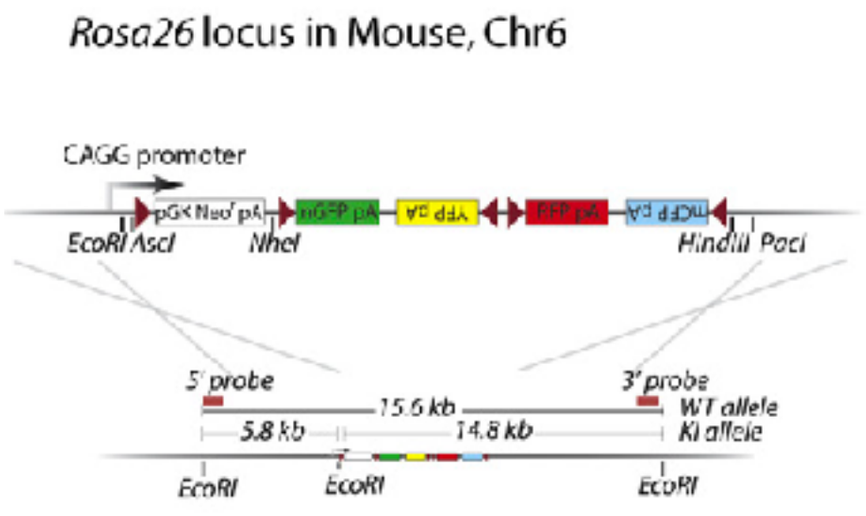
(3) spatial transcriptomic methods

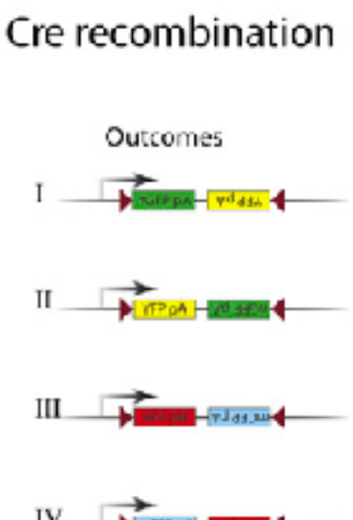
(4) spatial metabolomics methods

(5) computational integration of these modalities

Spatial Omics Techniques

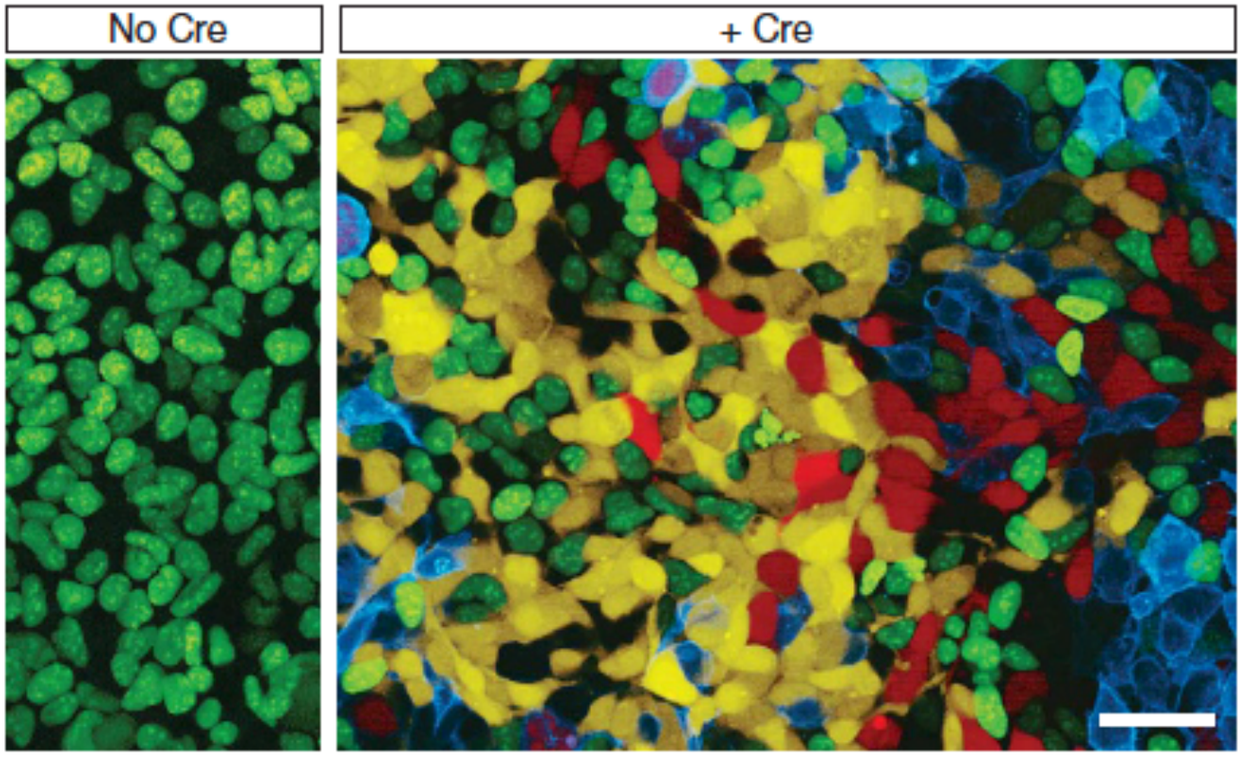
Tracking the spatiotemporal fate of live cells in their tissue context





R26R-Confetti knock-in strategy.

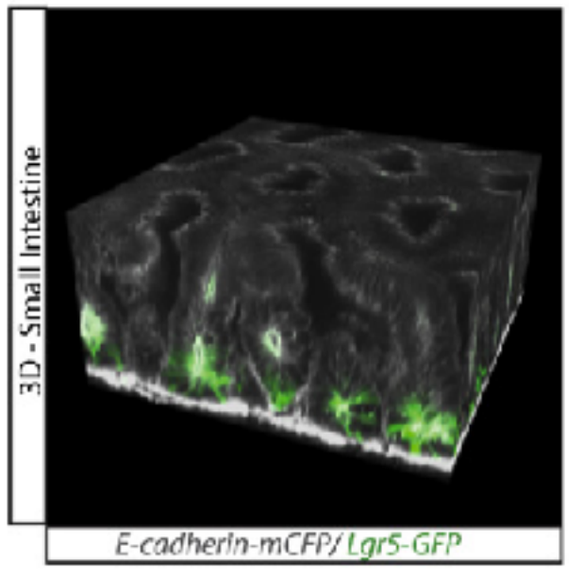
Brainbow2.1 encoding four fluorescent proteins was inserted into the Rosa26 locus. Upstream, the strong CAGG promoter, a LoxP site, and a neomycin resistance roadblock cassette were inserted. Upon cre activation, the neomycin roadblock is excised, while the brainbow2.1 recombines in a random fashion to four possible outcomes.

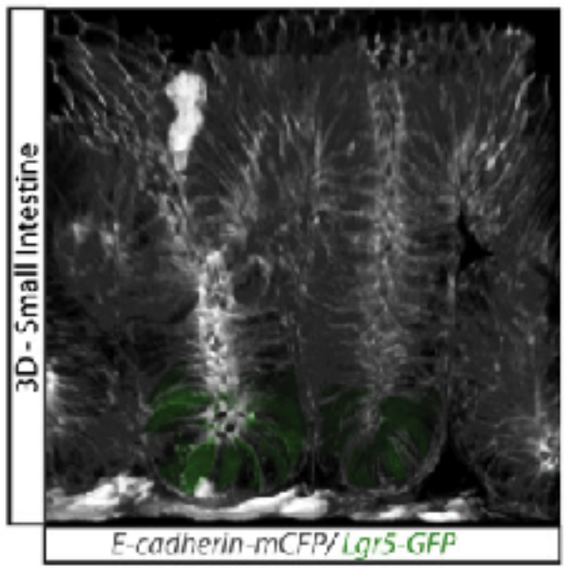


Livet et al Nature 2007

Spatial Omics Techniques

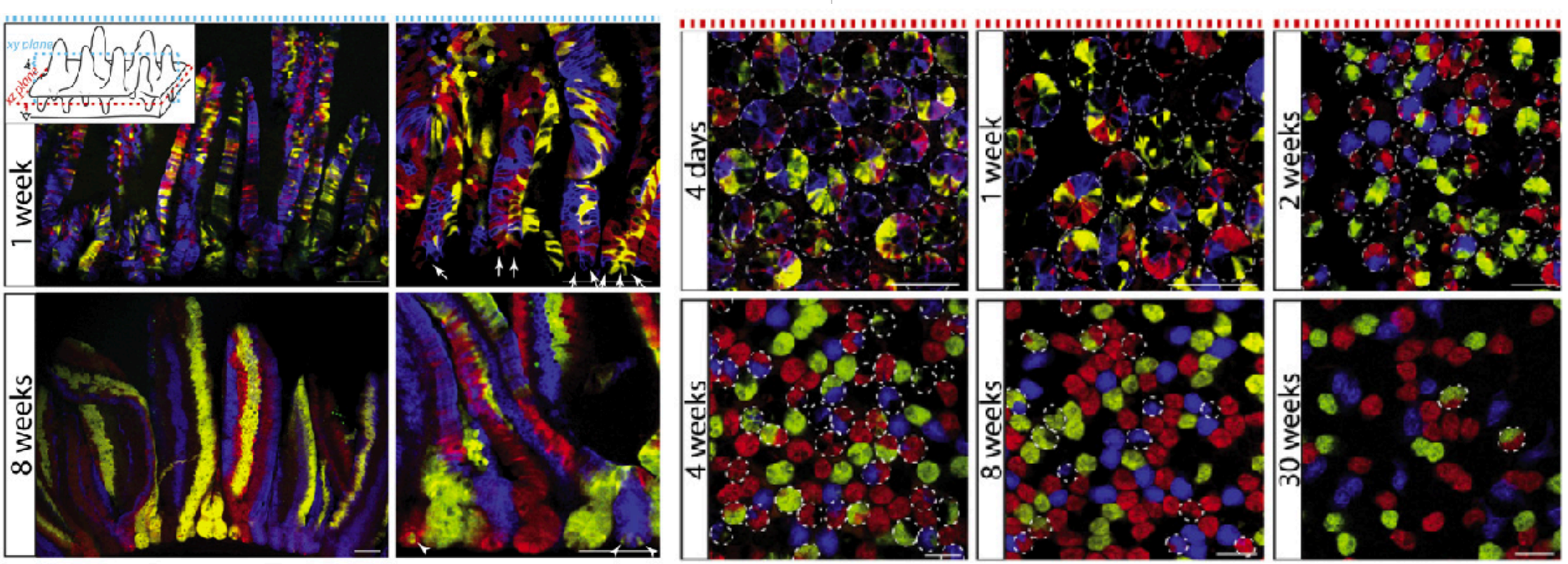
Tracking the spatiotemporal fate of live cells in their tissue context





Intestinal stem cells, characterized by high *Lgr5* expression, reside between Paneth cells at the small intestinal crypt base and divide every day.

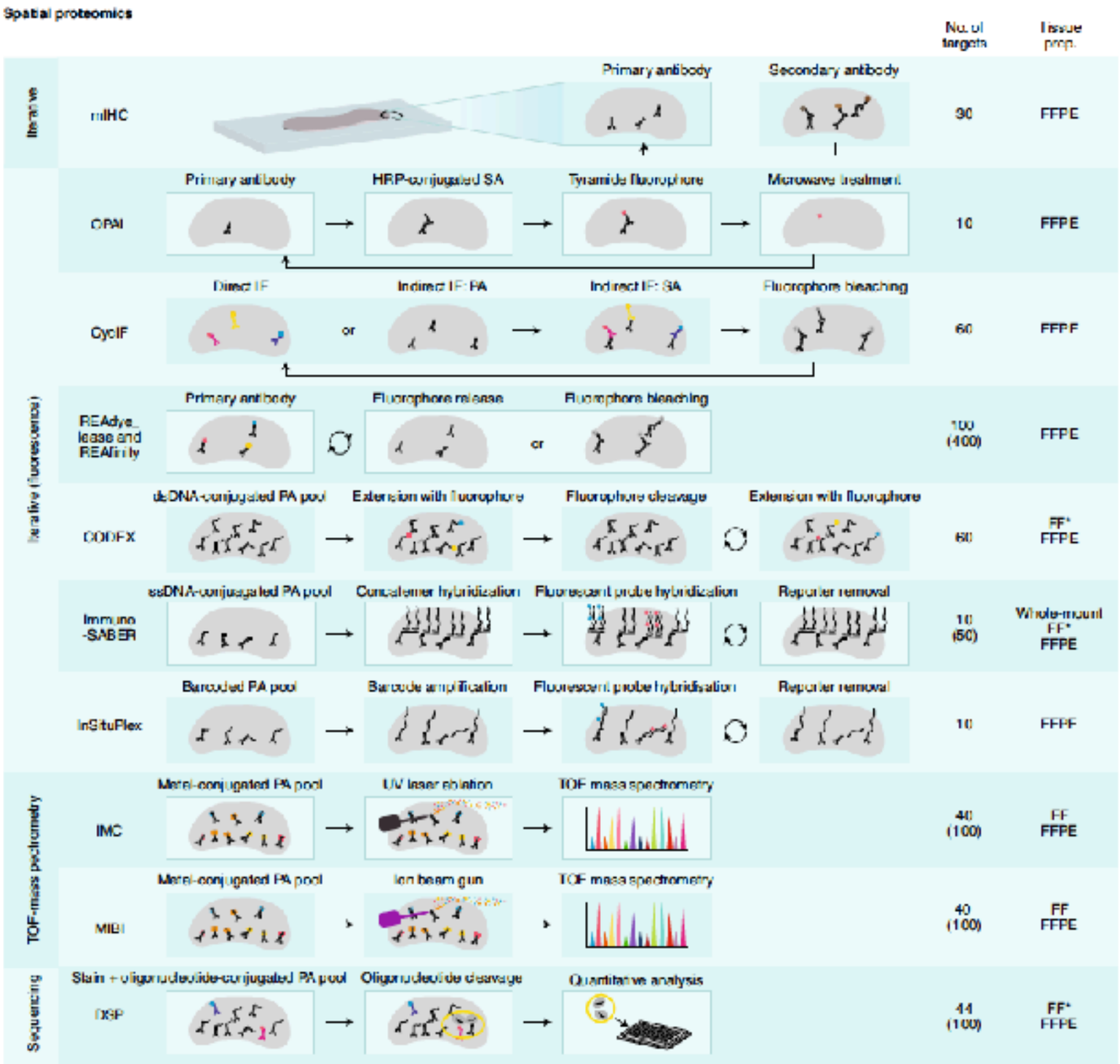
The authors investigated the fate of stem cells in vivo.



Sippert et al Cell 2010

Spatial Omics Techniques

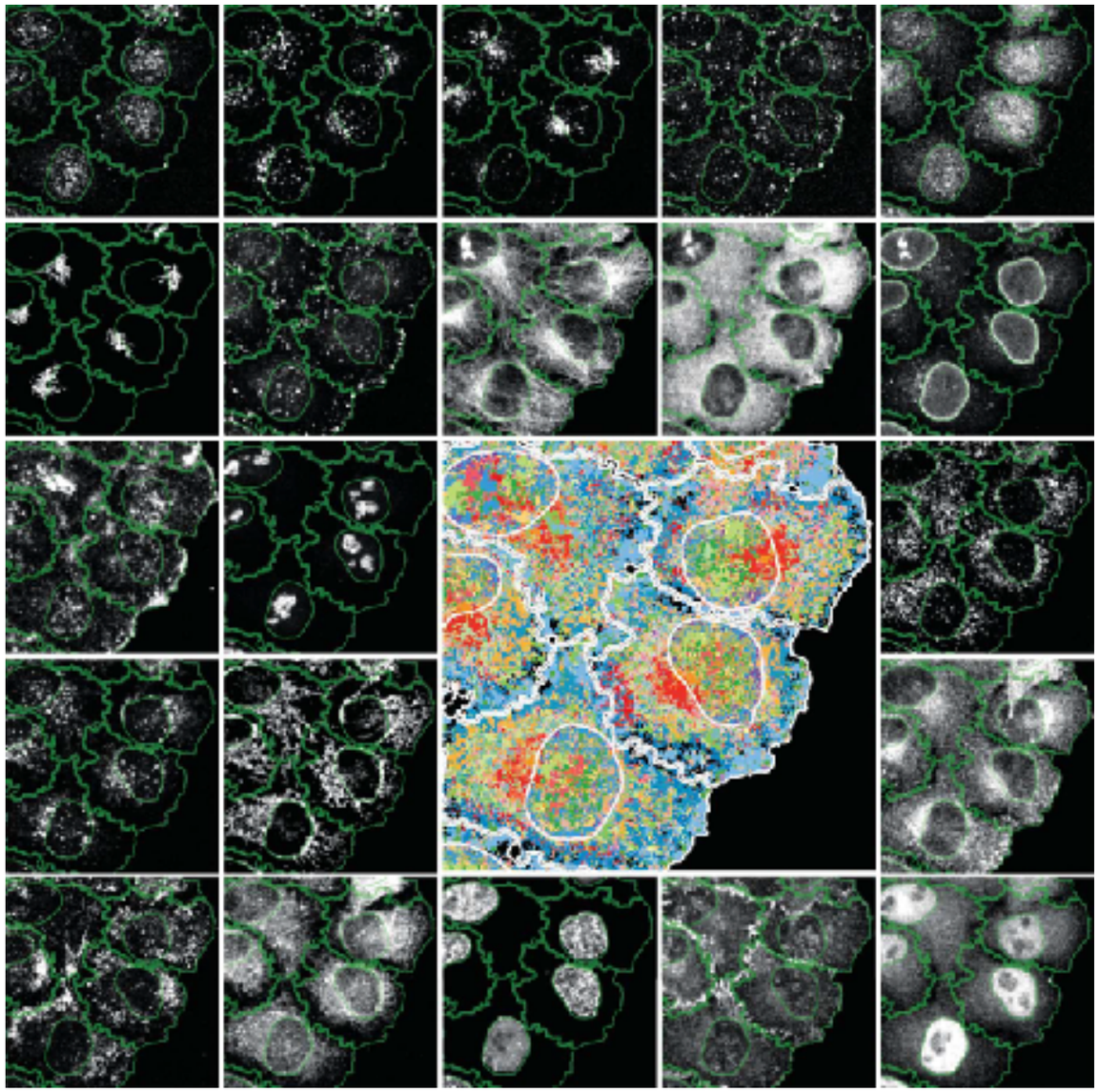
increasing the number of detectable proteins in a spatial context



Spatial Proteomics

Spatial Omics Techniques

iterative staining (4i)

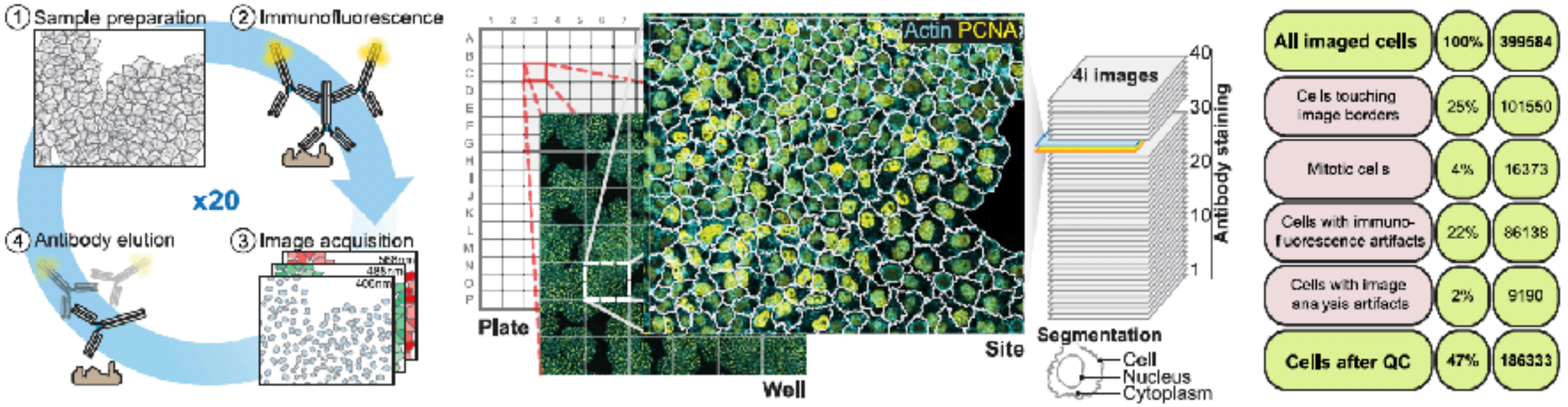


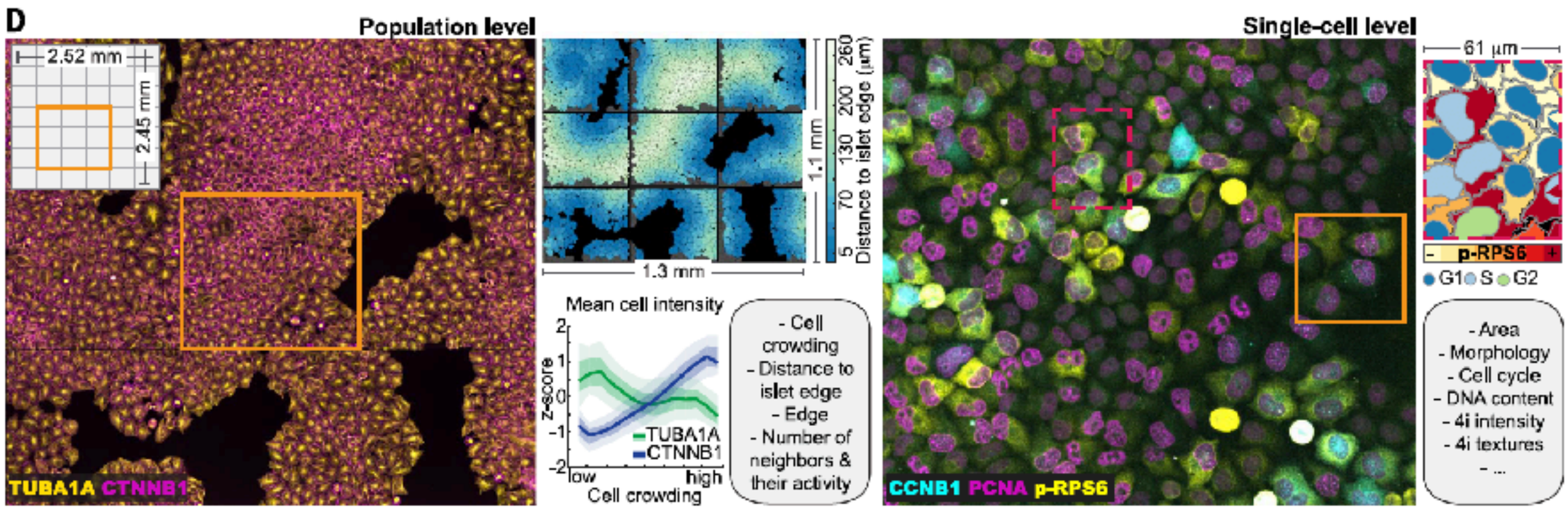
Iterative indirect immunofluorescence imaging (4i), 40-plex protein readouts from biological samples at high-throughput from the millimeter to the nanometer scale.This approach simultaneously captures properties apparent at the population, cellular, and subcellular levels, including microenvironment, cell shape, and cell cycle state. It also captures the detailed morphology of organelles, cytoskeletal structures, nuclear subcompartments, and the fate of signaling receptors in thousands of single cells in situ.

Gut et al Science 2018

Spatial Omics Techniques

iterative staining (4i)

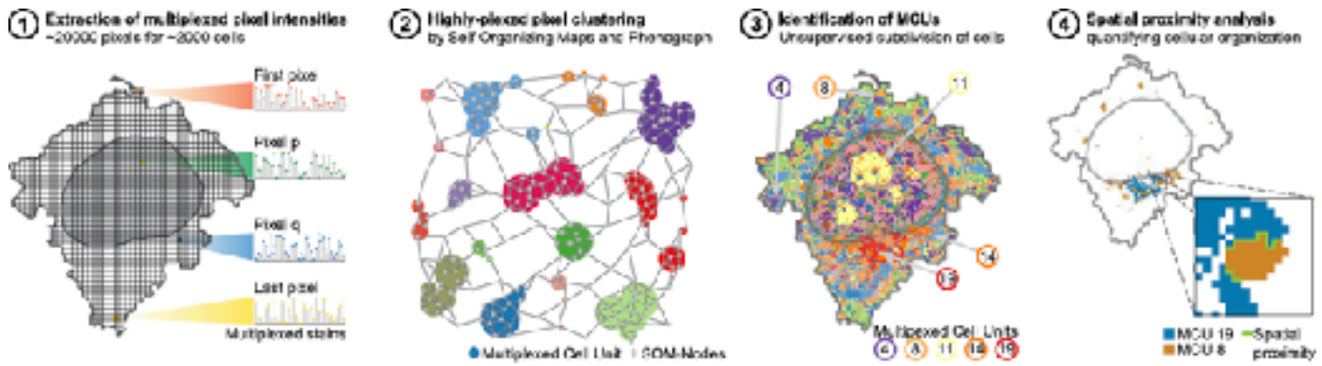


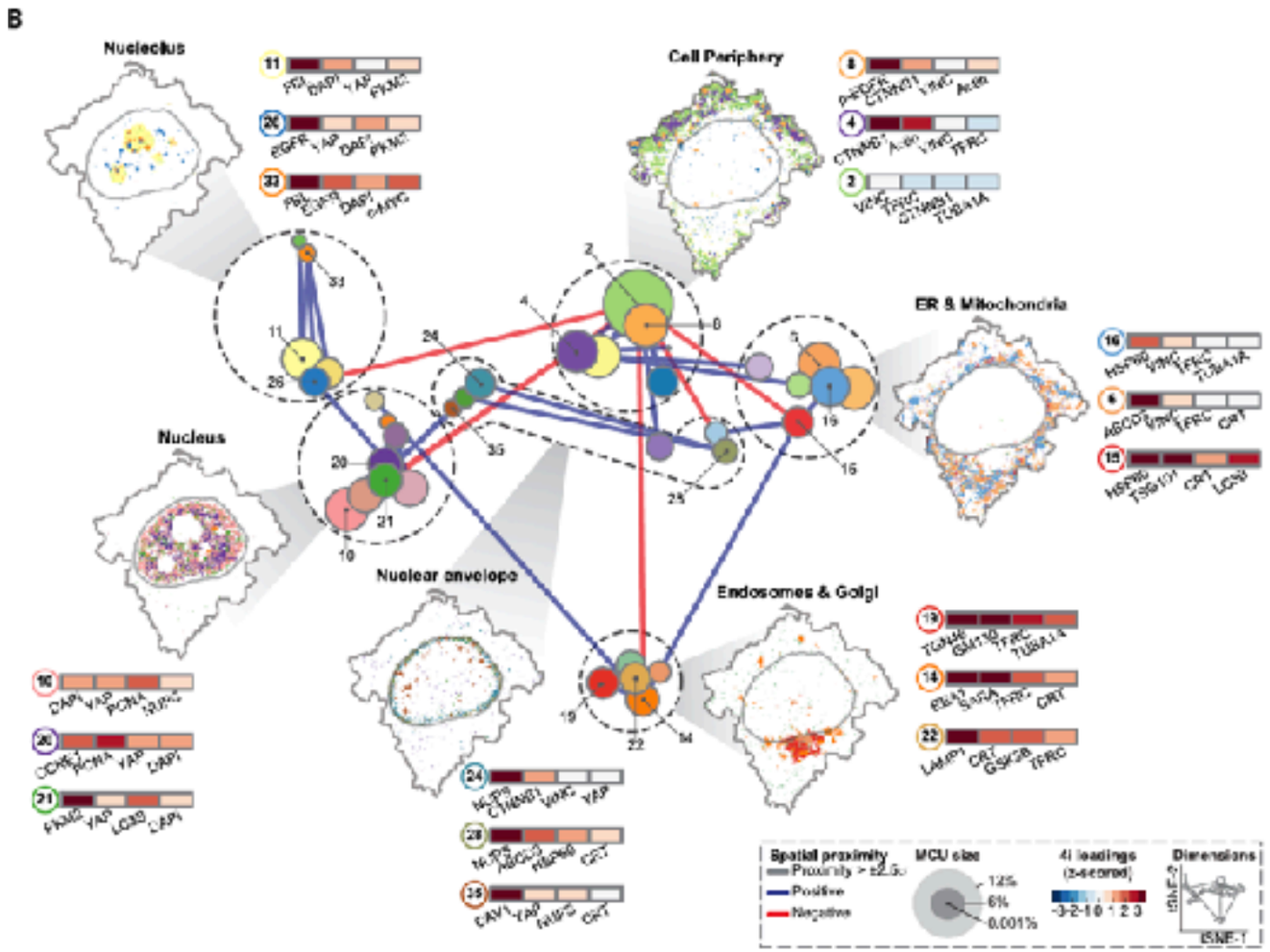


Gut et al Science 2018

Spatial Omics Techniques

iterative staining (4i)



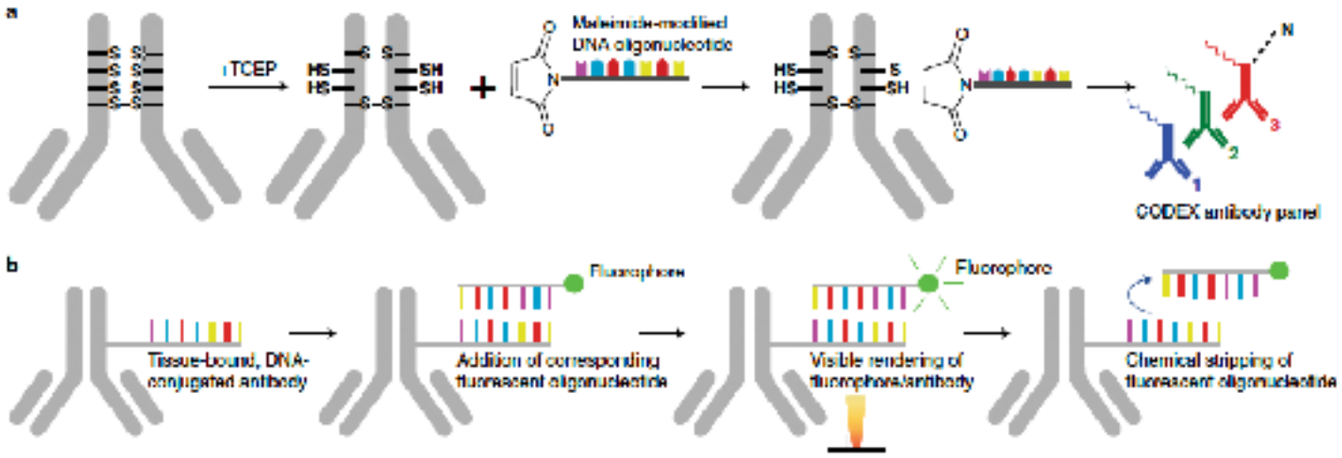


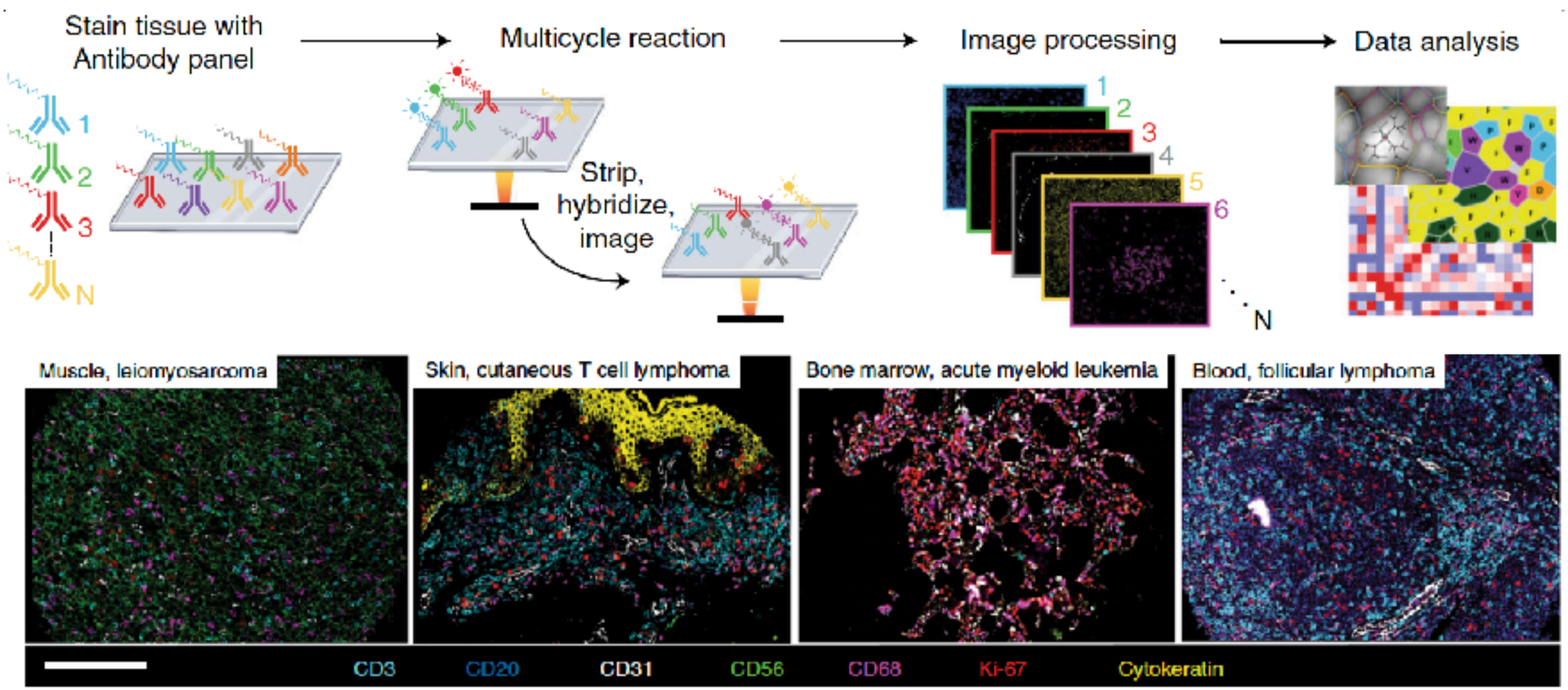
4i quantifies subcellular organization at high spatial detail in thousands of single cells based on multiplexed single-pixel profiles. 4i builds on a well-established highthroughput multivariate imaging platform

Gut et al Science 2018

Spatial Omics Techniques

iterative staining CODEX

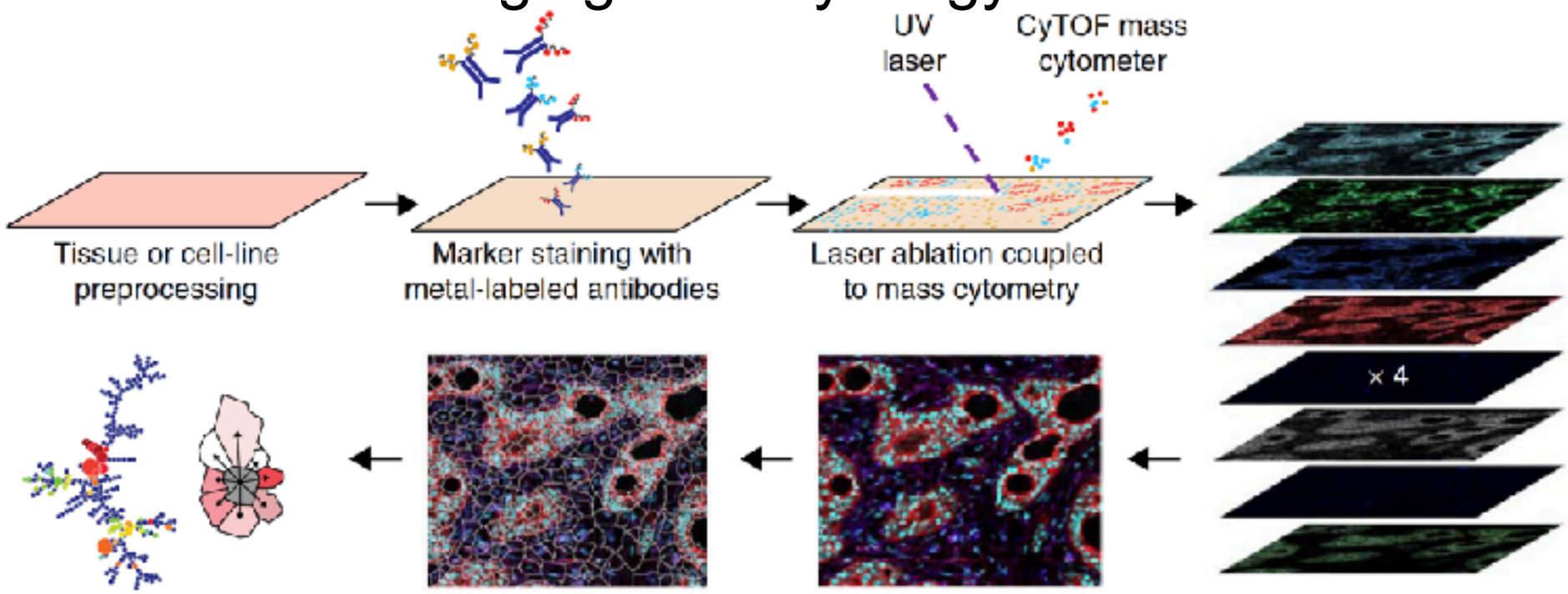


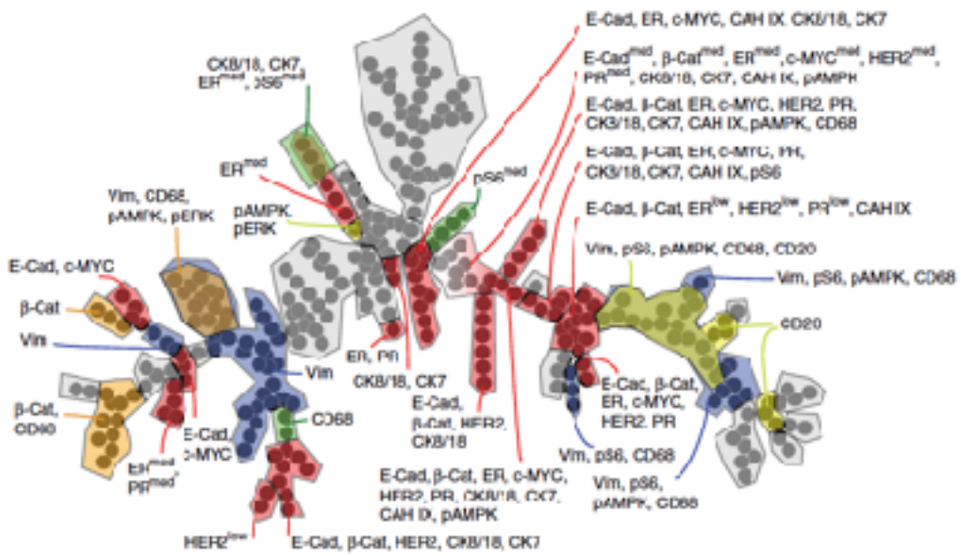


Black et al Nat Prot 2021

Spatial Omics Techniques

MS based methods - ImagingMassCytology



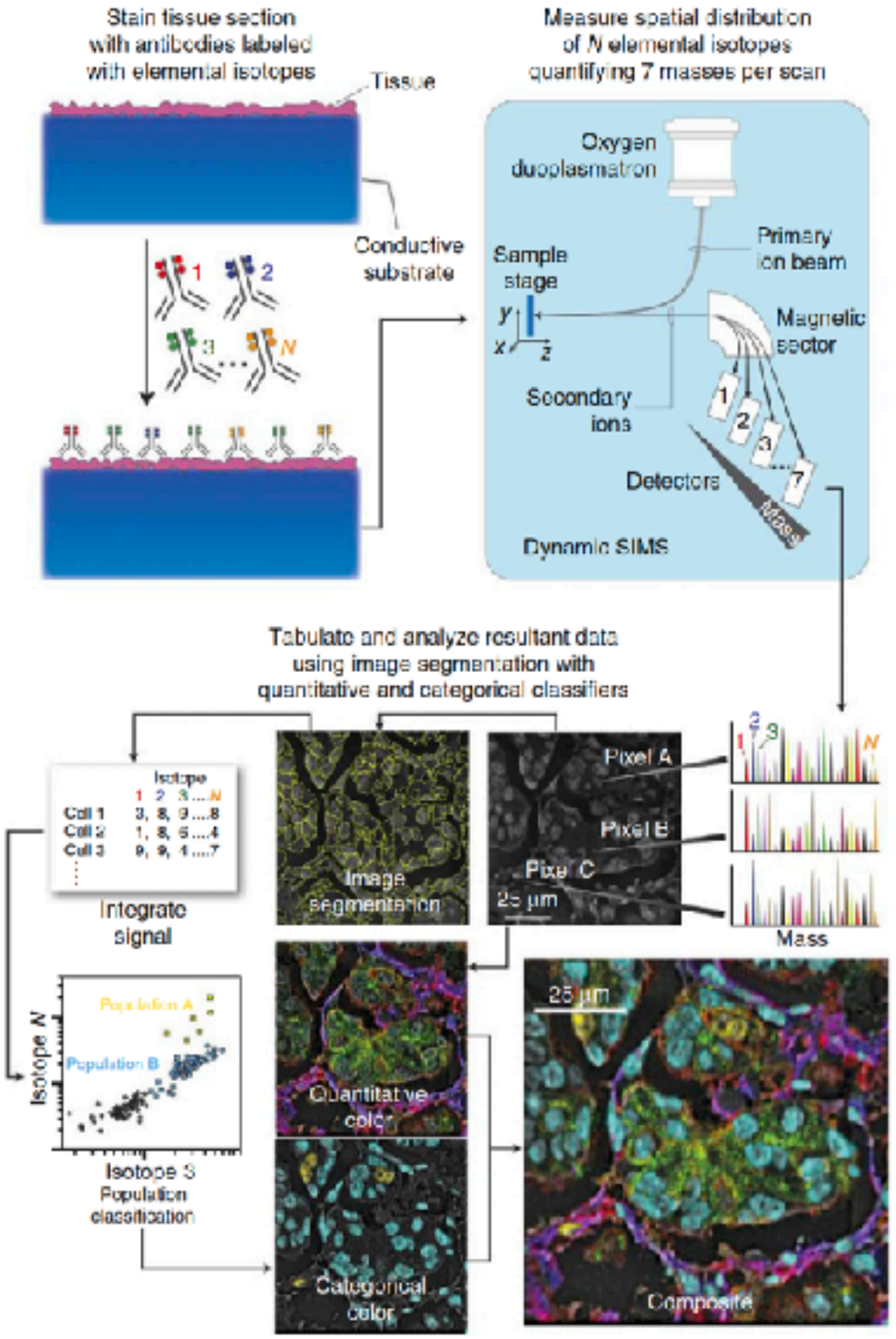


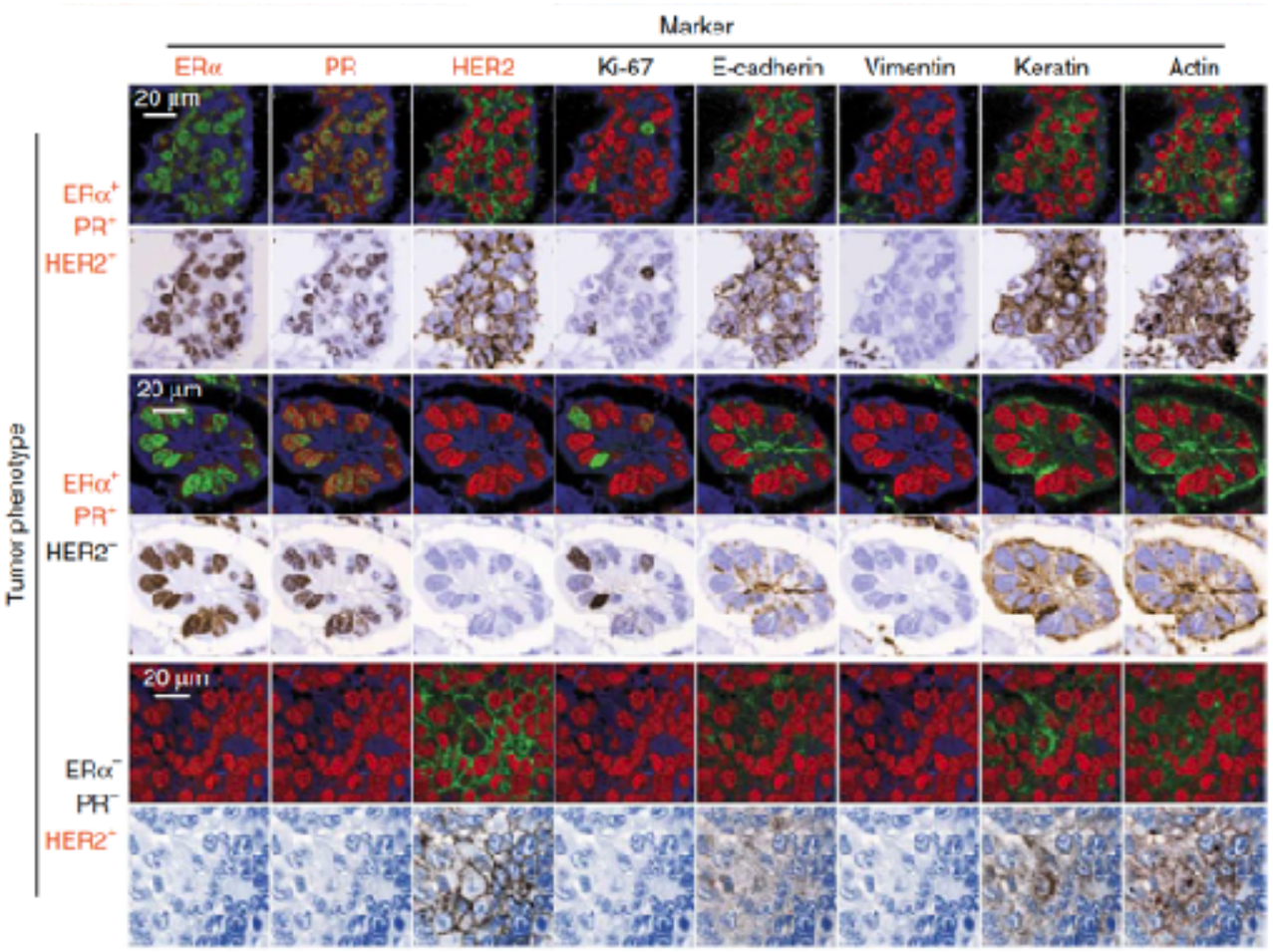
Imaging mass cytometry (IMC) uses TOF - MS as a readout combined with a high-energy, short-wavelength laser beam for tissue ablation that is focused to a spot size of 1 µm in diameter. This beam is rastered across the tissue, point by point, and at each spot the vaporized tissue is carried by an inert gas into the mass spectrometer for analysis.

Giesen et al Nat Met 2018

Spatial Omics Techniques

MS based methods - MIBI



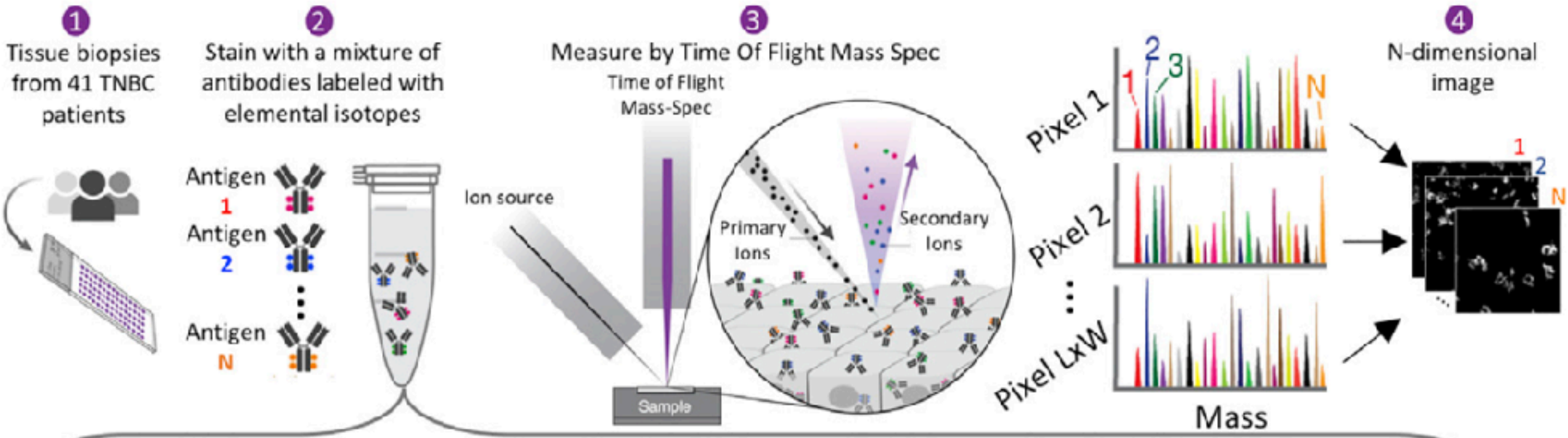


multiplexed ion beam imaging (MIBI) can image upwards of 40 proteins in tissue. The primary ion beam is rastered across the tissue to generate secondary ions for detection by TOF mass spectrometry at defined spatial coordinates. In theory, the ion beam in MIBI can be reduced to a spot size of well below 500 nm in diameter

Angelo et al Nat Med 2013

Spatial Omics Techniques

MS based methods - MIBI

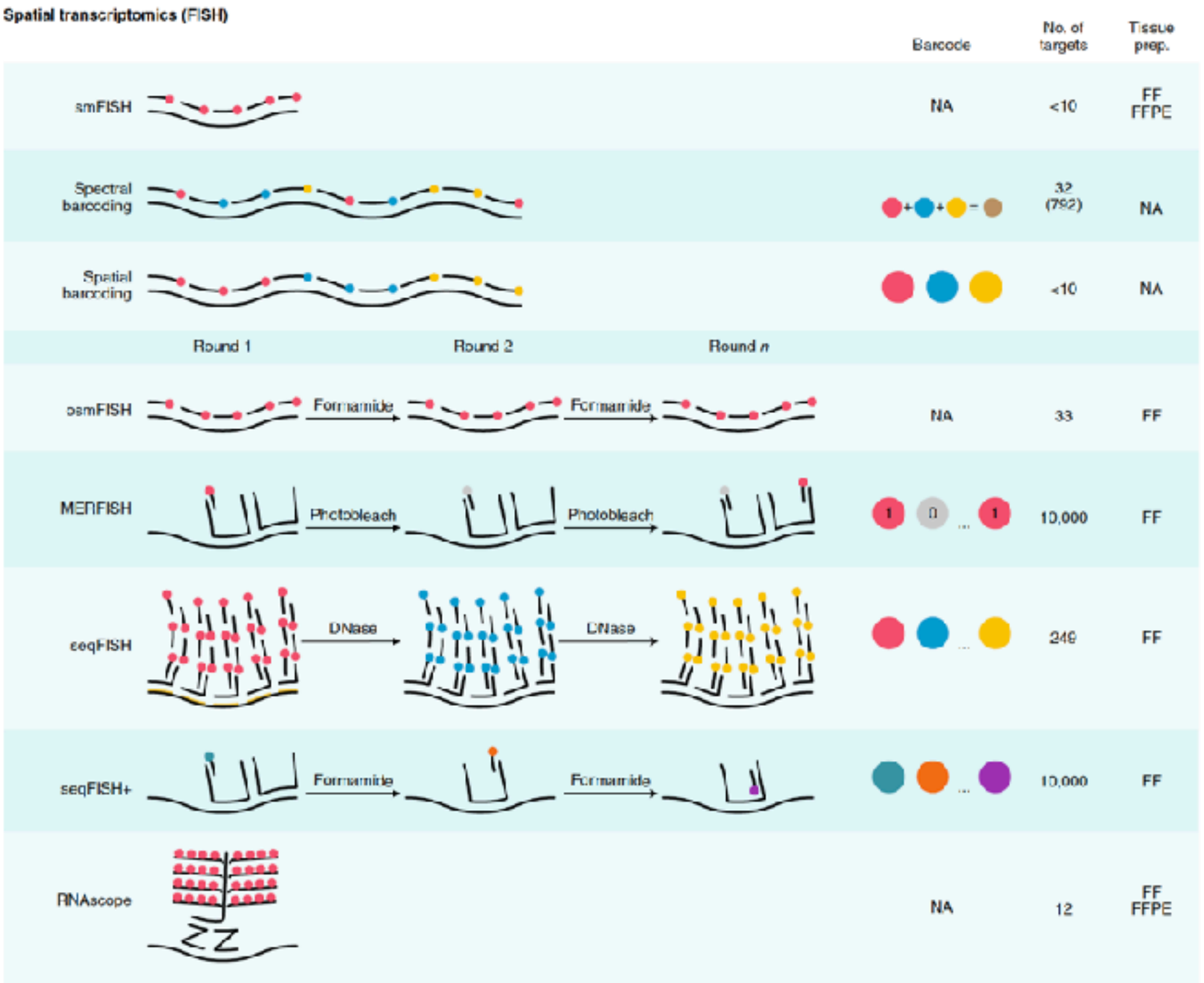


Tumor	Cell status	Immune cell types				Immune regulation	Stroma
		Lymphocytes		Monocytes	Antigen presentation		
Pan-keratin	dsDNA	CD45	CD20	CD11b	HLA-DR	Lag3	Vimentin
Keratin 6	H3K9ac	CD3	CD45RO	CD11c	HLA1	PD1	SMA
Keratin 17	H3K27me3	CD8	CD56	CD68	CD209	PDL1	CD31
p53	pS6	CD4	CD16	CD63		IDO	
EGFR	Ki-67	FoxP3	CD138	Neutrophils			
Beta catenin				MPO			

Keren et al Cell 2018

Spatial Omics Techniques

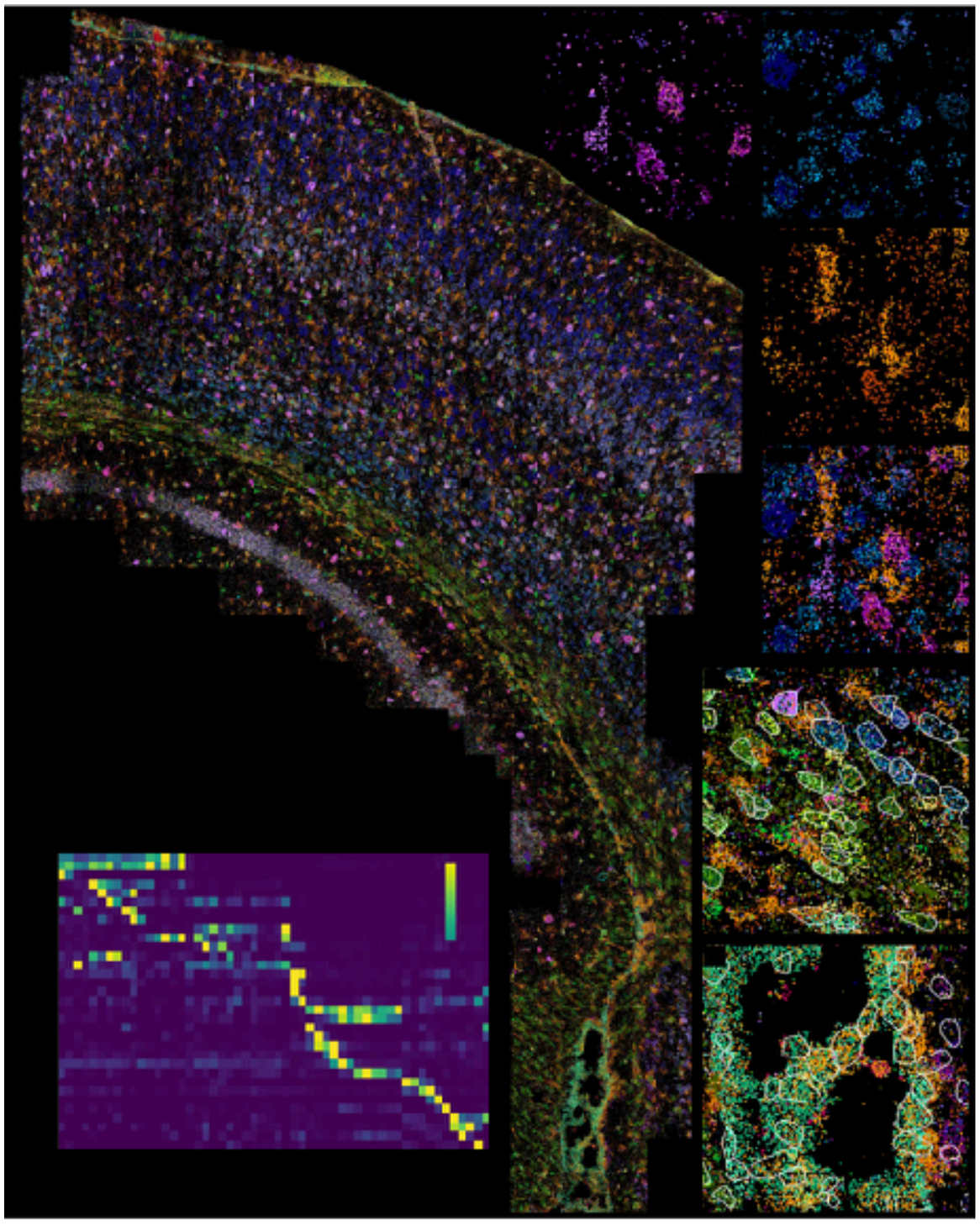
FISH based methods



Spatial Transcriptomics

Spatial Omics Techniques

osmFISH

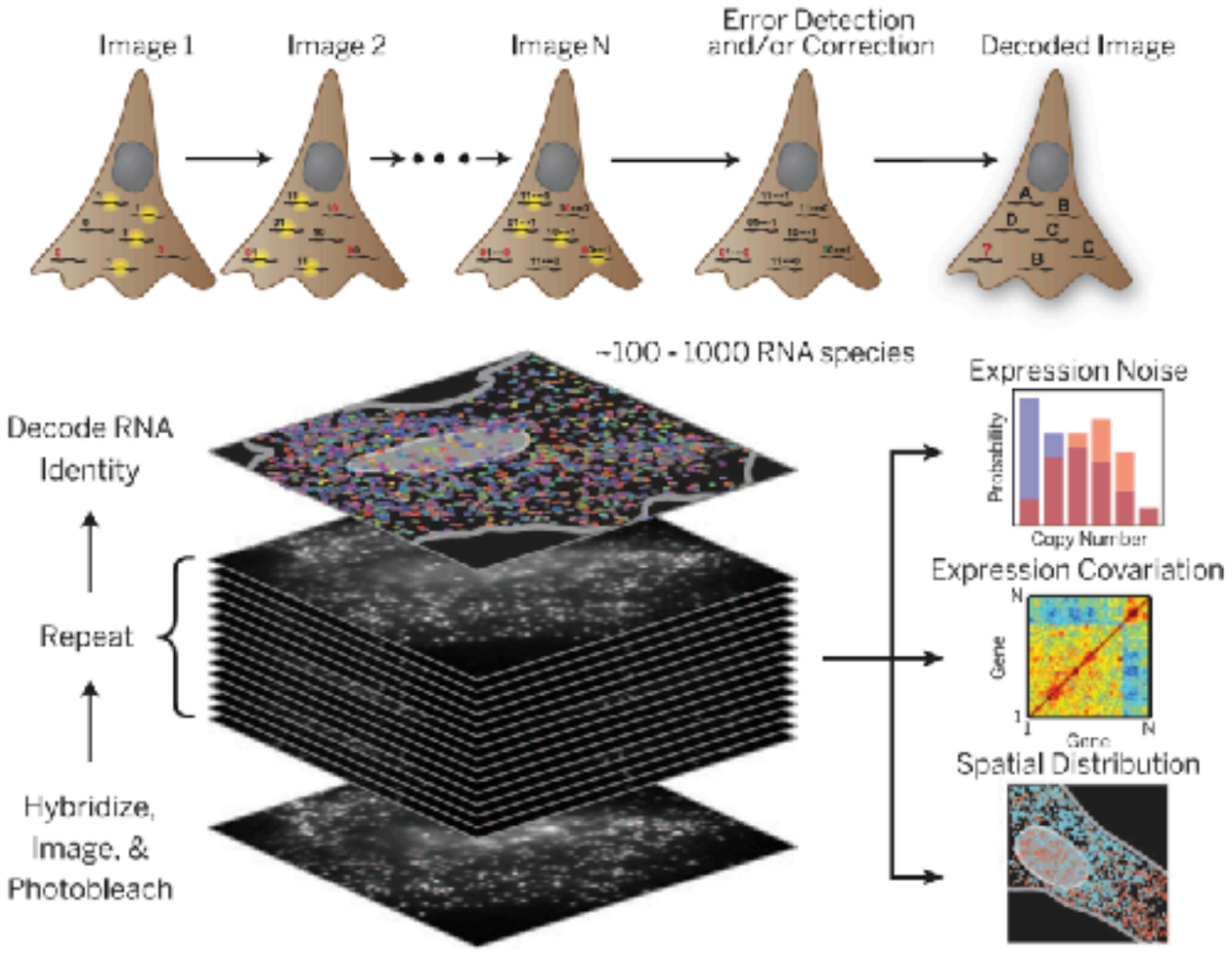


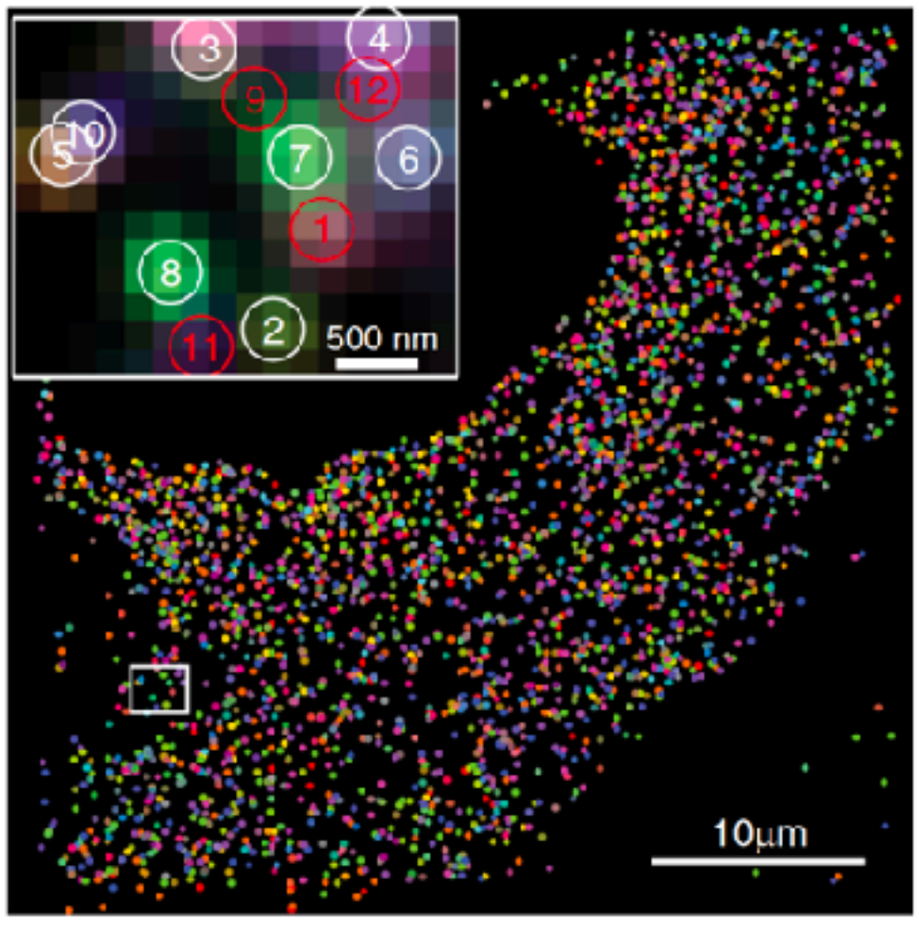
Sequential imaging can increase the multiplexing capacity of smFISH methods. Ouroboros single-molecule FISH (osmFISH) is a non-barcoded form of cyclic smFISH that targets transcripts in successive rounds of hybridization. Both osmFISH and other iterative fluorescence methods are efficient in the process of fluorophore stripping and quenching. While fluorophore brightness and tissue integrity may be marginally reduced with increasing numbers of hybridization rounds, samples generally maintain stability for an extended period of time.

Codeluppi et al Nat Met 2018

Spatial Omics Techniques

MERFISH (Barcoding)



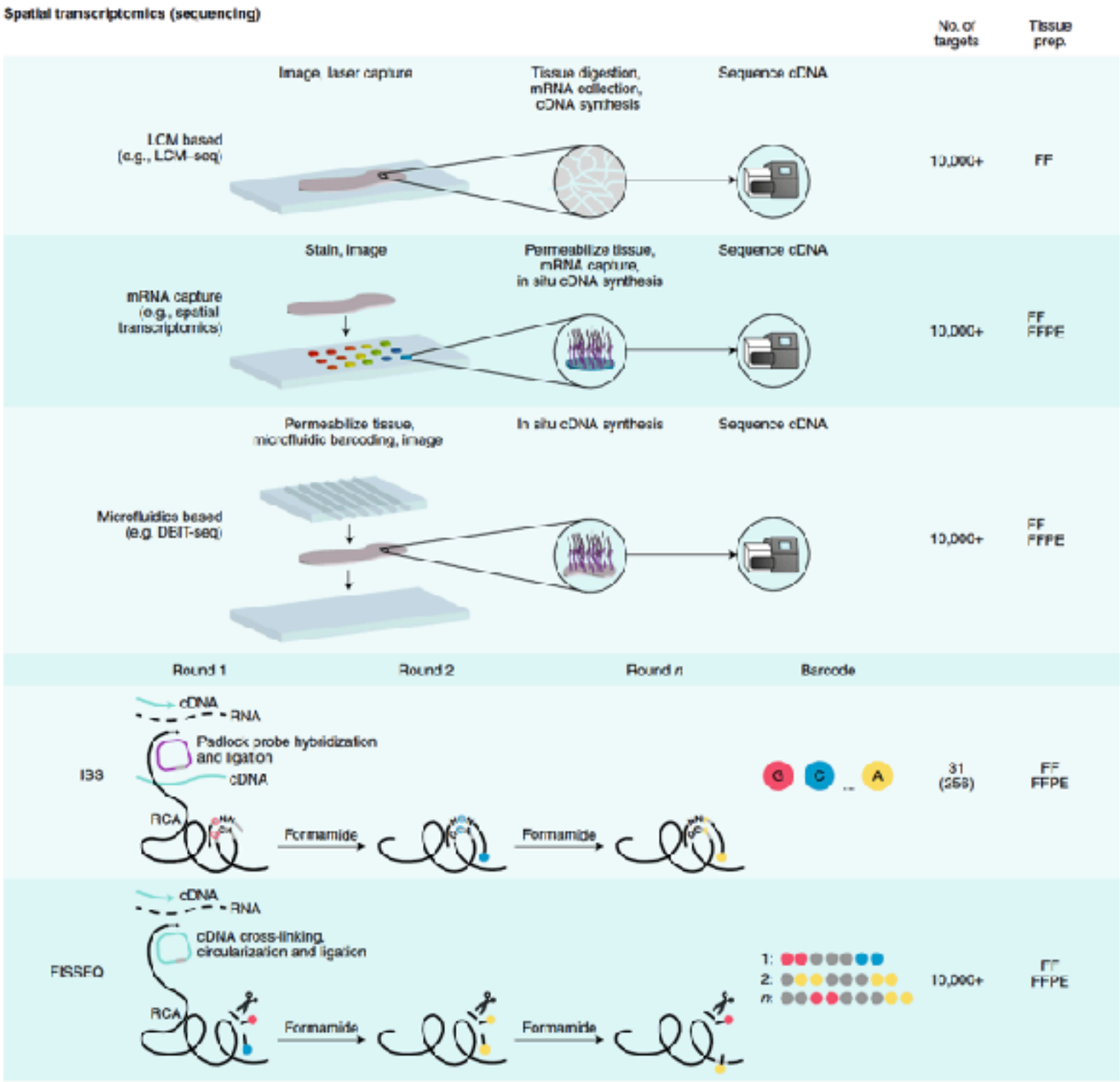


MERFISH is a temporal barcoded smFISH method that measures **100–1,001** genes with high spatial resolution and detection efficiency. This is a single-molecule imaging approach that uses combinatorial labeling and sequential imaging with encoding schemes capable of detection and/or correction of errors. This highly multiplexed measurement of individual RNAs can be used to compute the gene expression profile and noise, covariation in expression among different genes, and spatial distribution of RNAs within single cells.

Chen et al Science 2015

Spatial Omics Techniques

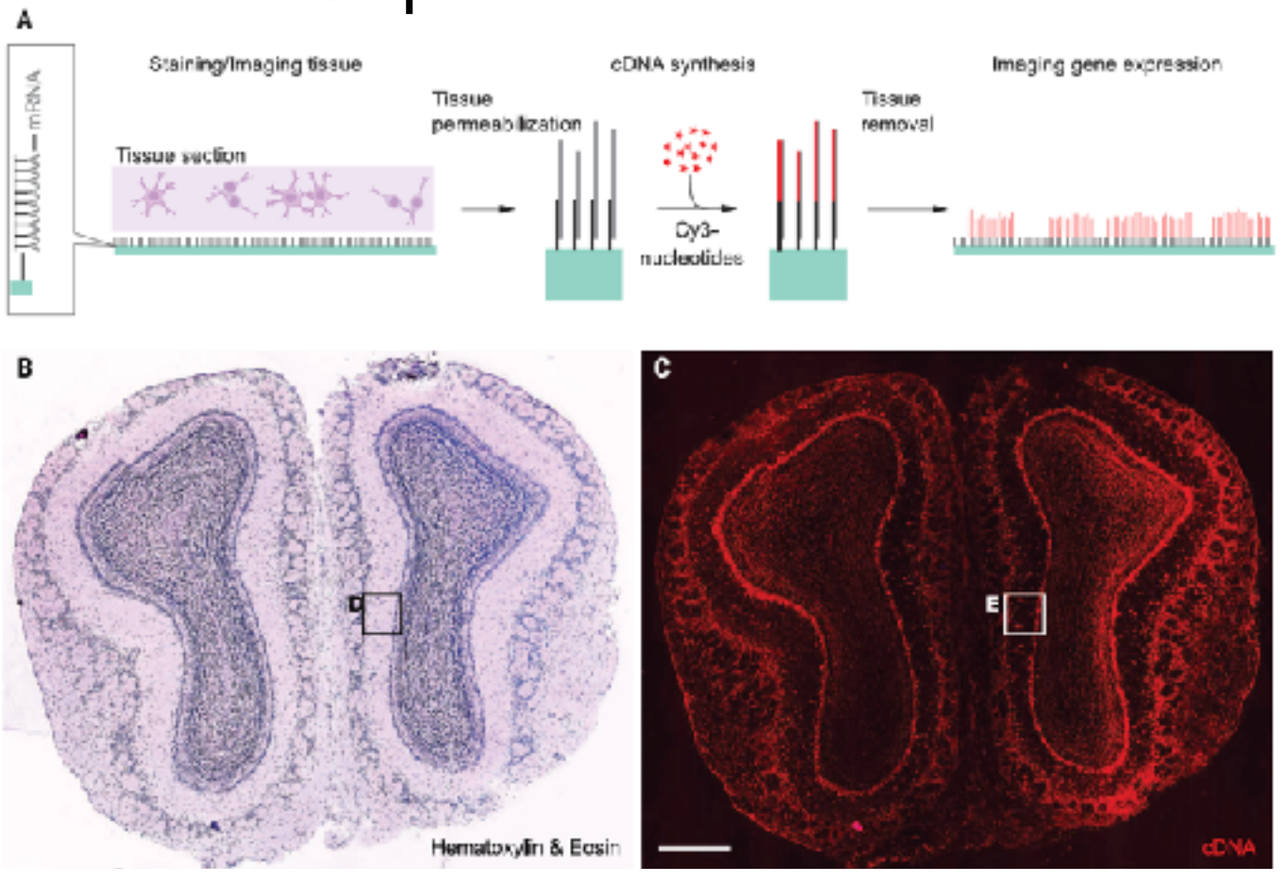
Sequencing methods

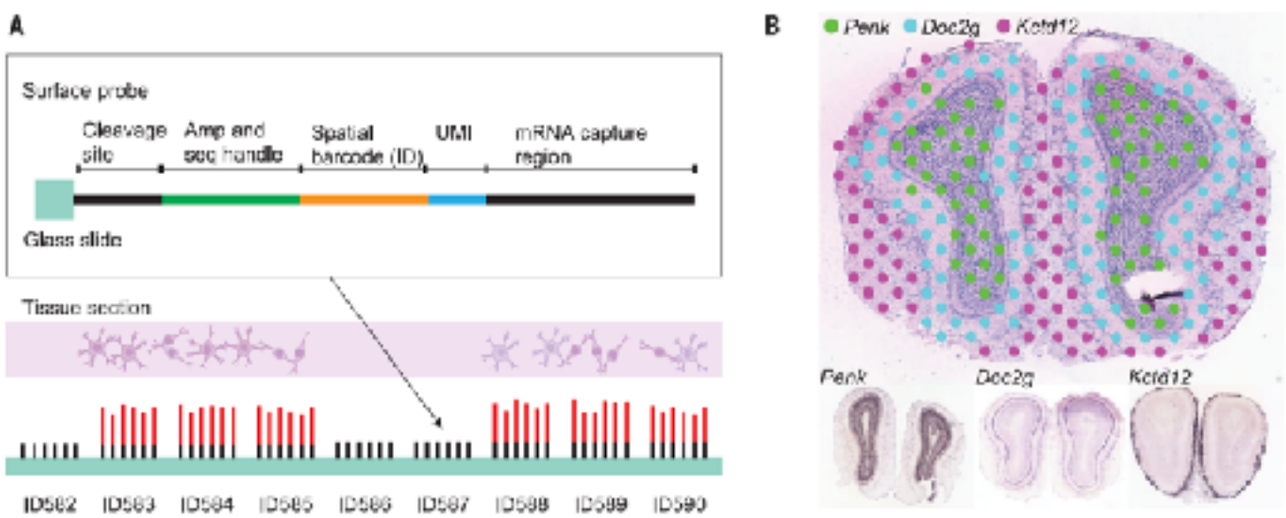


Spatial Transcriptomics

Spatial Omics Techniques

mRNA-Capture





The recent emergence of sequencing-based spatially resolved transcriptomics, as pioneered by ‘spatial transcriptomics’ has heralded the ability to determine the unbiased transcriptome of multiple regions in an entire tissue section. Here gridded areas of a slide contain or receive poly(dT) capture probes with identifier barcode sequences enabling later spatial assignment. After probes bind to the total mRNA present within the tissue, they are converted to cDNA, incorporating the spatial barcode.Gene expression is then traced back to a specific tissue location by way of the associated spatial barcode and through the use of fiducial markers on a corresponding histological image.

Ståhl et al Science 2016

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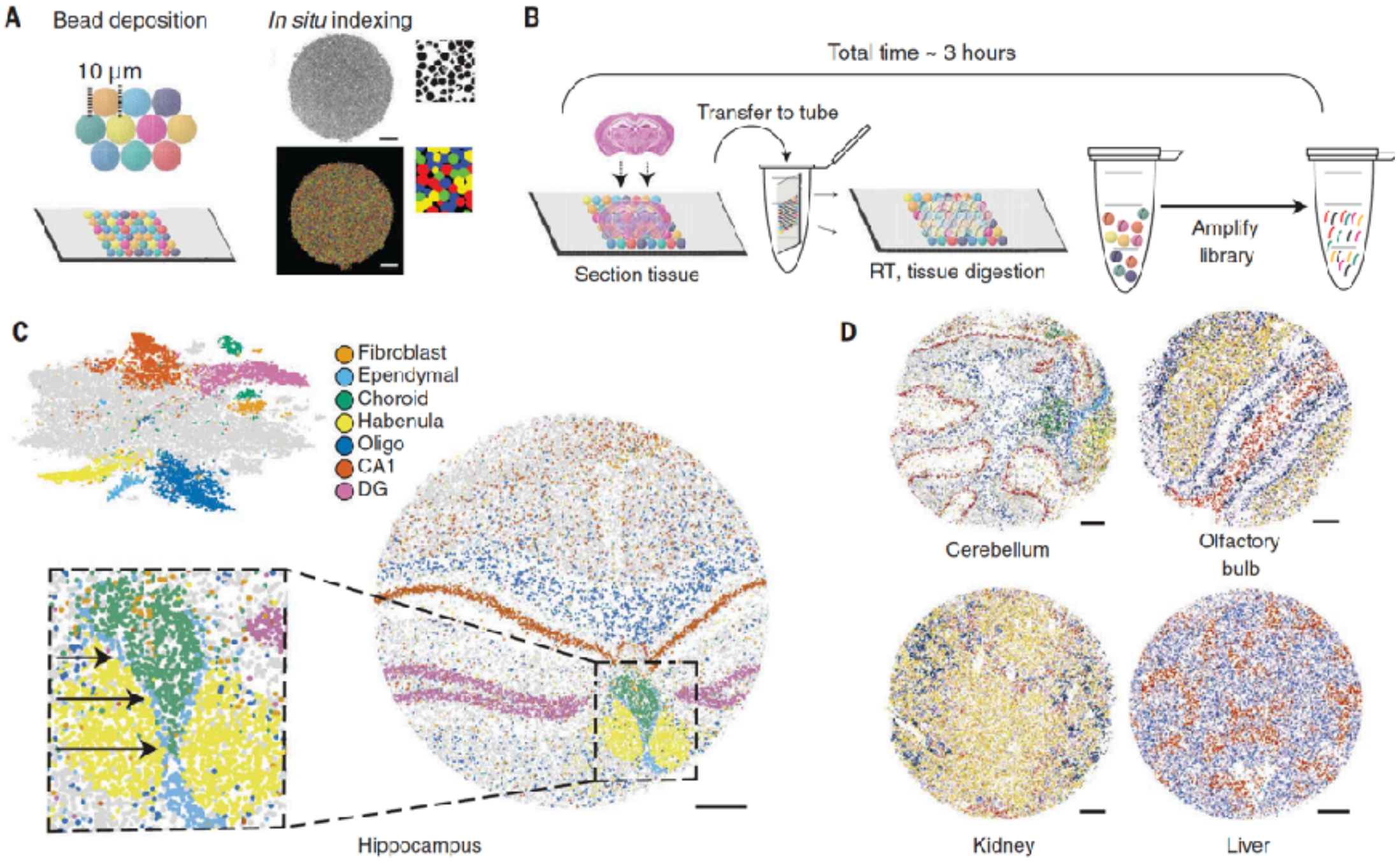
Slide-seq



Rodriques et al Science 2019

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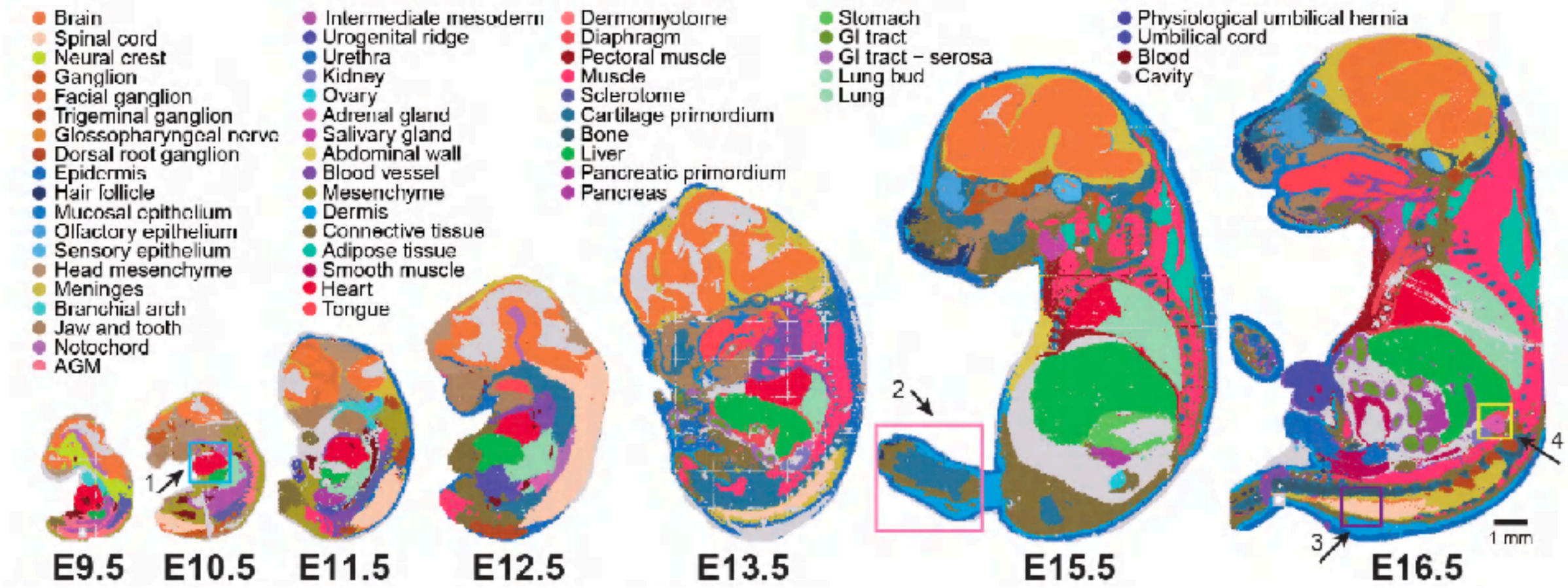
Stereo-seq

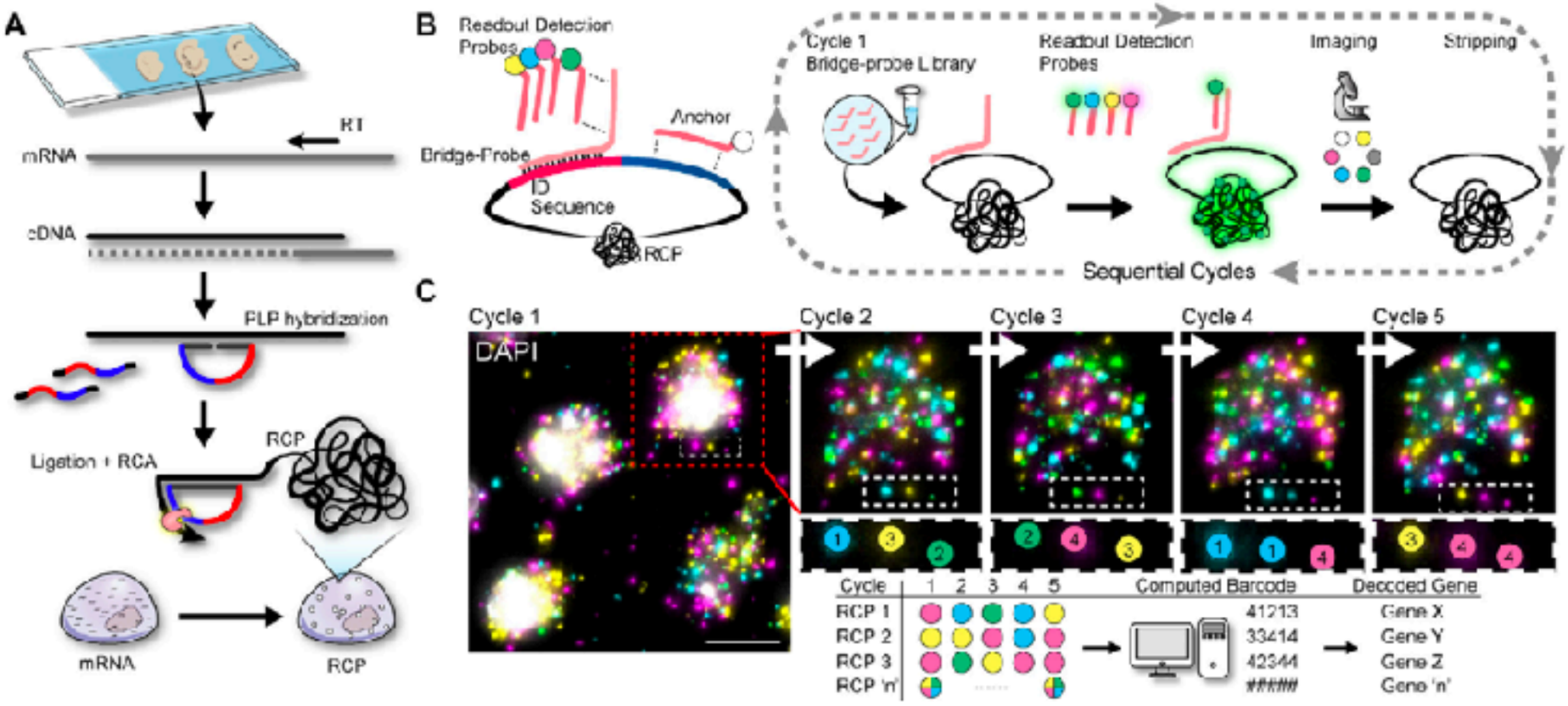


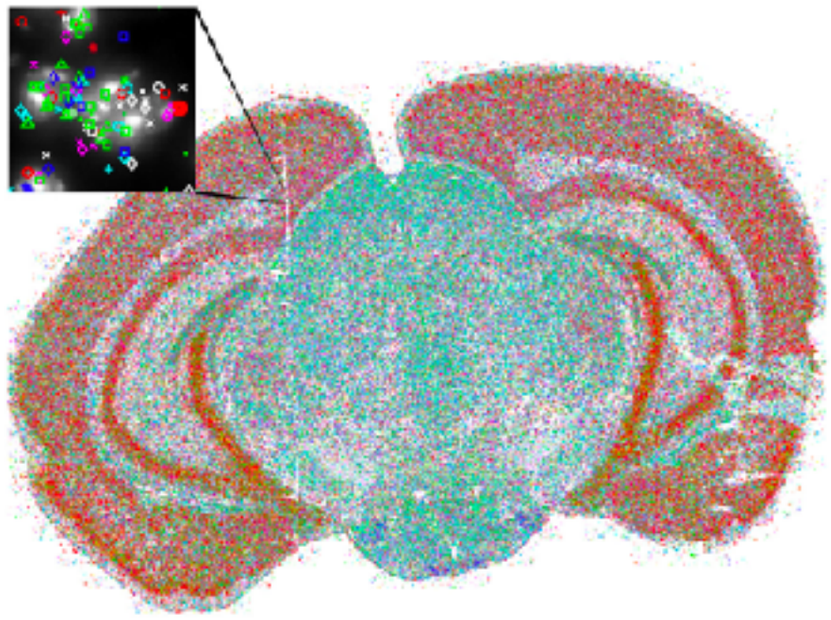
https://doi.org/10.1101/2021.01.17.427004

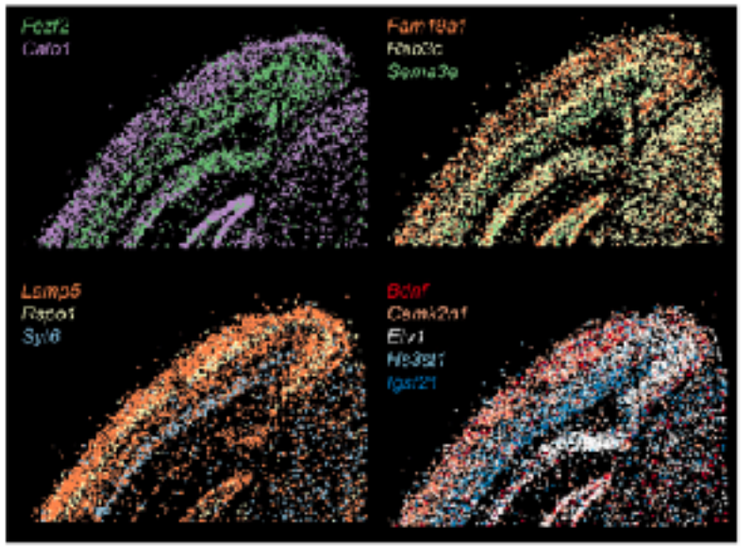
Chen et al Biorxiv 2021

Spatial Omics Techniques

HybISS (Hybridization-based *in situ* sequencing)







Gyllborg et al NAR 2020

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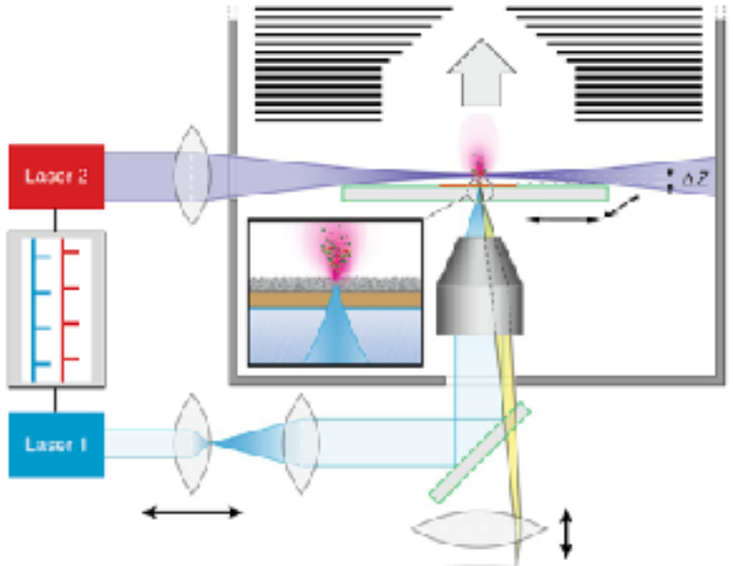
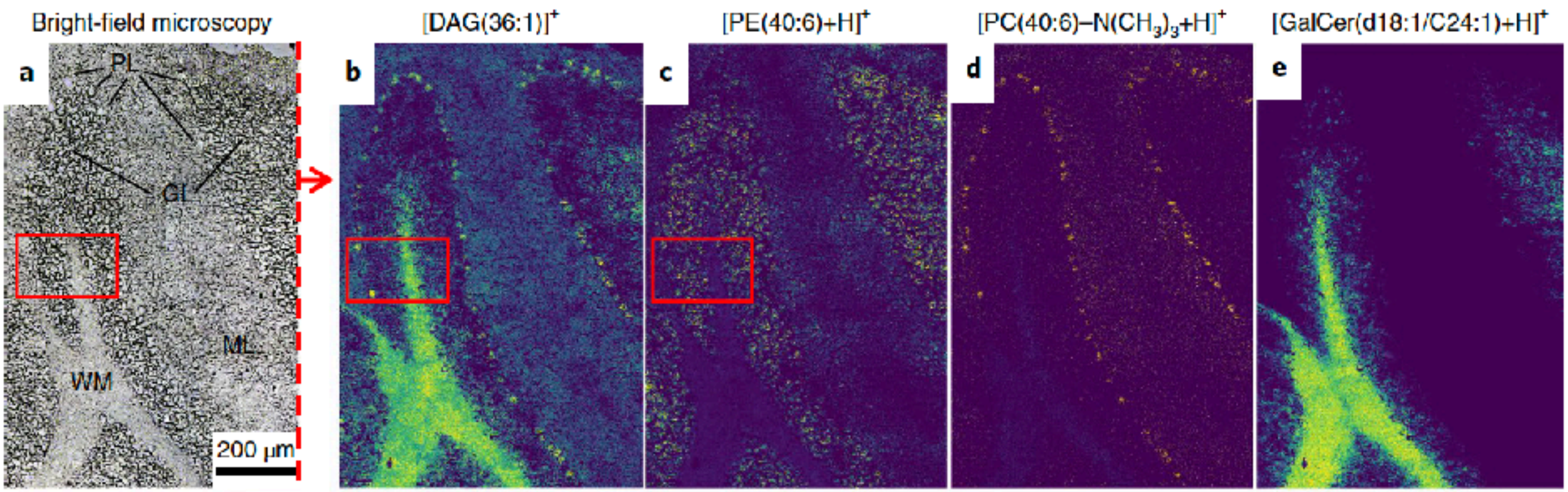
MALDI-2-IMS



Matrix-assisted laser desorption–ionization mass spectrometry imaging in transmission-mode geometry (t-MALDI–MSI) can provide molecular information with a pixel size of 1 µm and smaller, which makes this label-free method highly interesting for characterizing the chemical composition of tissues and cells on a (sub)cellular level. laser-induced postionization (MALDI-2) increase the sensitivity of this technique to allow in situ single cell metabolomics



Niehaus et al Nat Met 2019