

Cellular responses to low dose radiation

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Outline

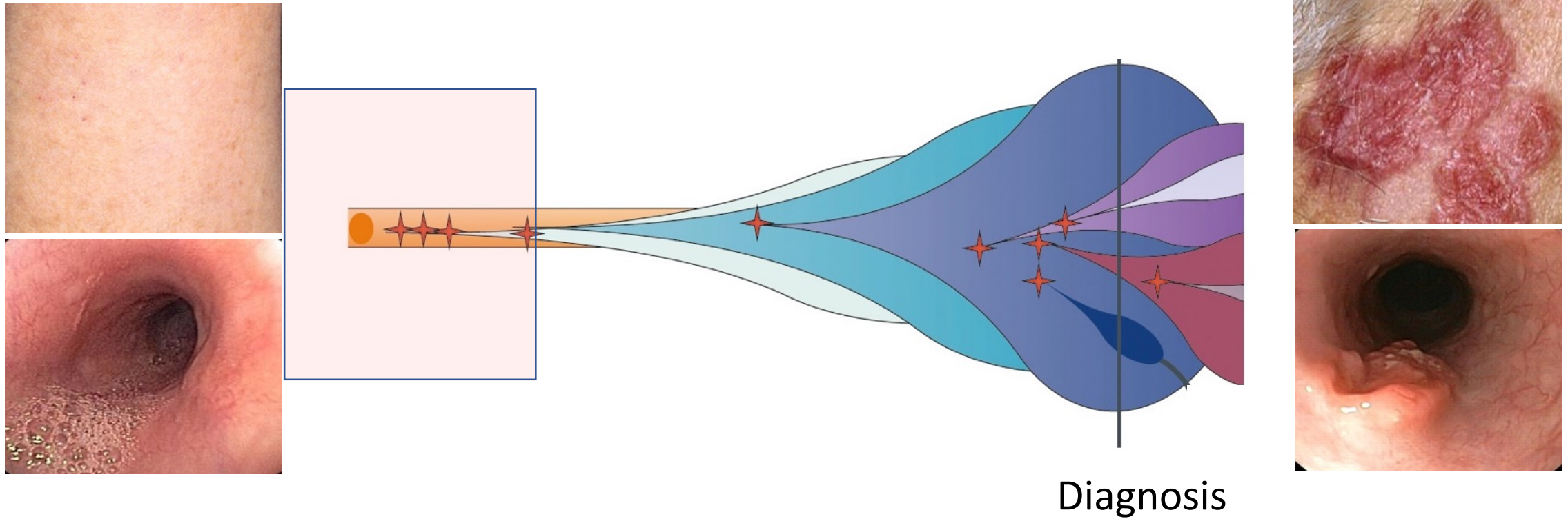
Mapping somatic mutant cells in normal tissues

Application to epidemiology studies?

Somatic mutants in low dose radiation research

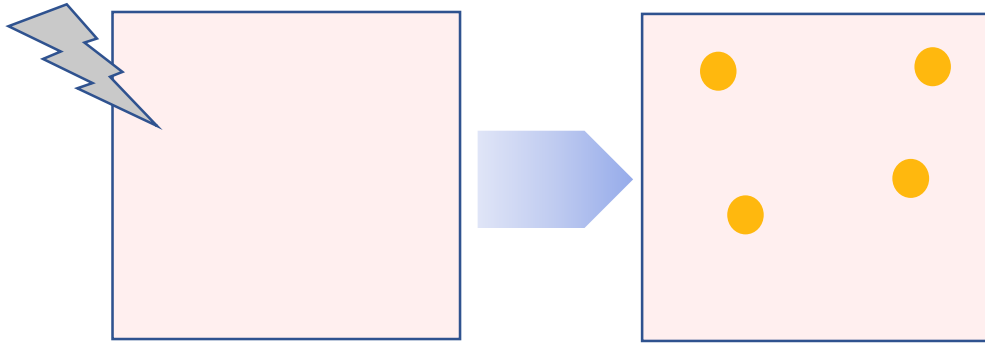
Research priorities

Cancer origins: mutant clones in normal tissue



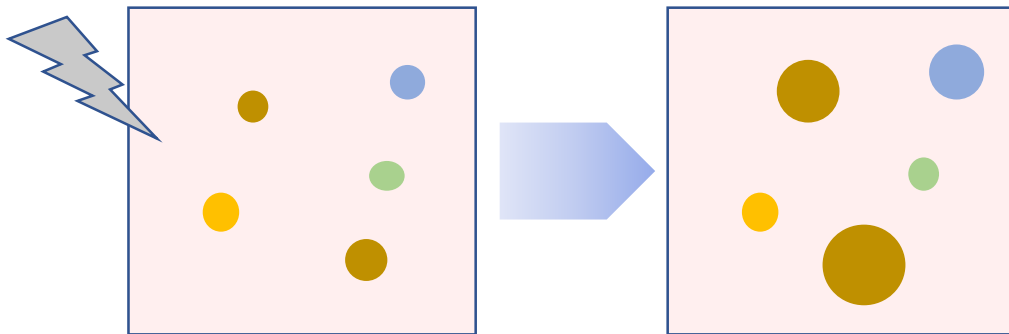
Models of low dose radiation & cancer risk

Damage



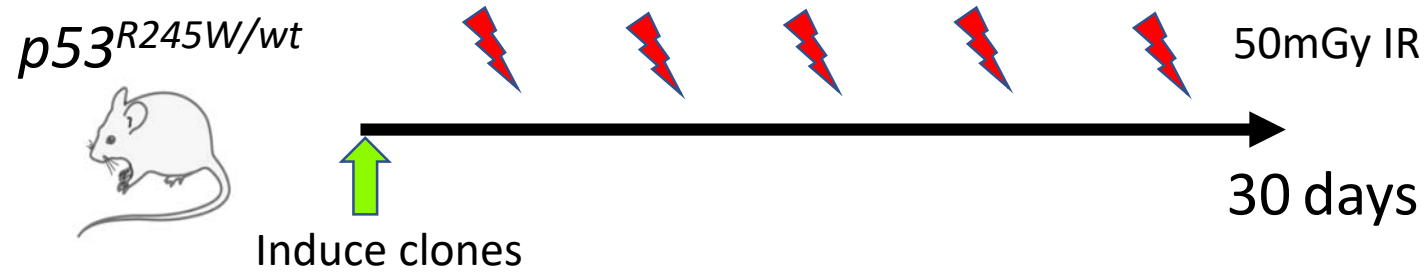
Genome damage induces new clones

Selection



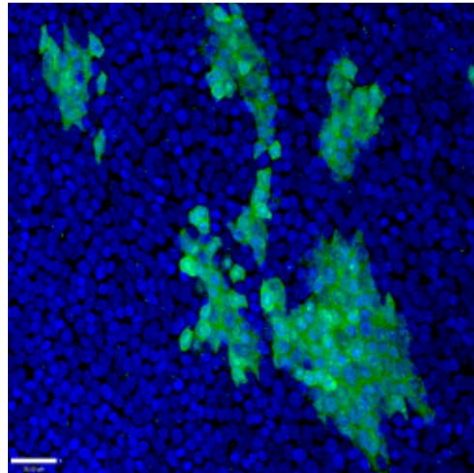
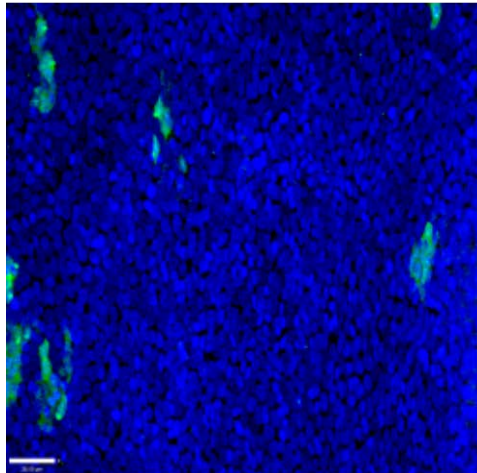
Environmental stress expands pre-existing clones

mutant *p53* & low dose radiation in mouse oesophagus



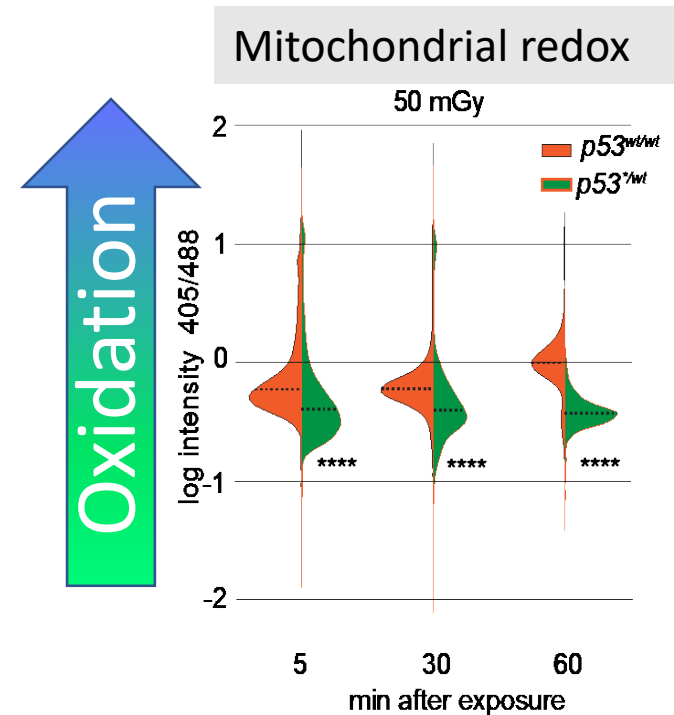
0 mGy

50 mGy x5

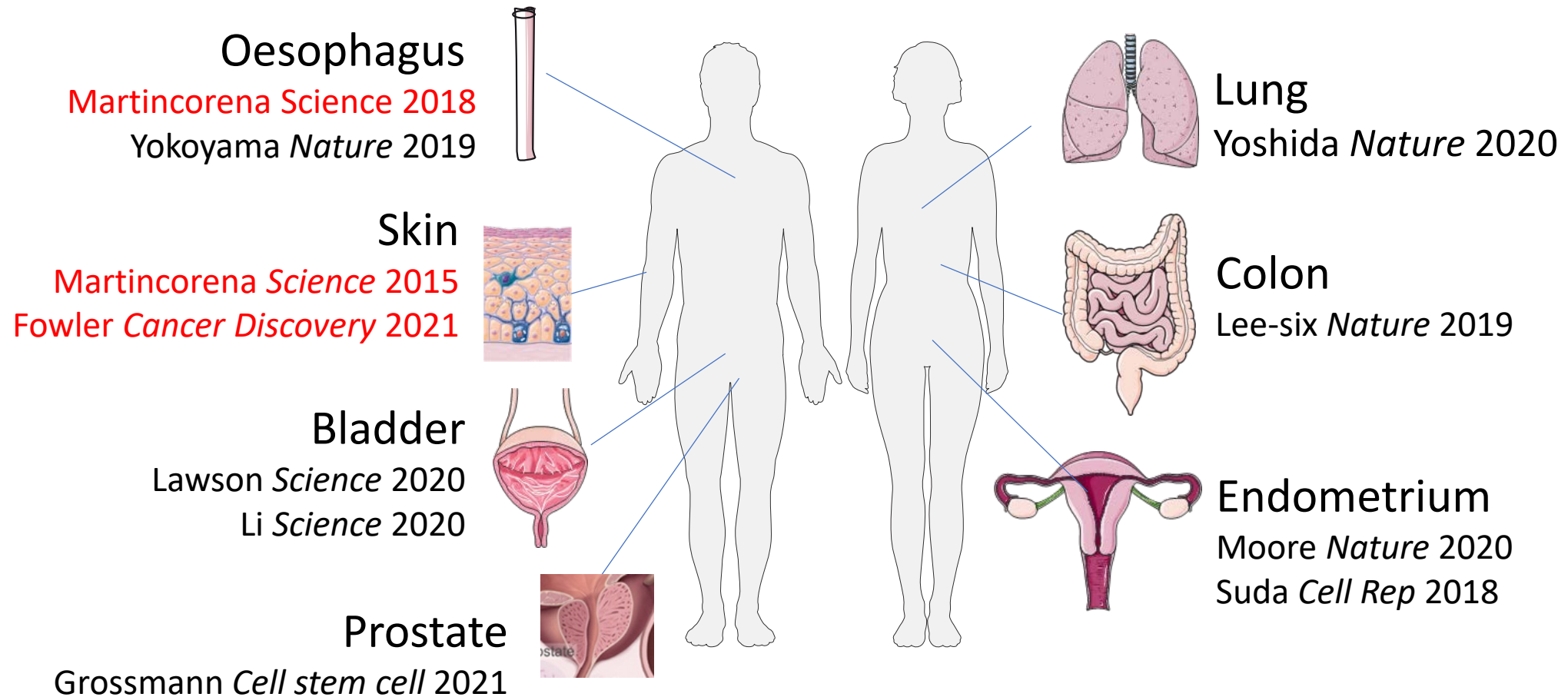


Top down views
esophagus
p53 mutant clones

p53 mutation is a gatekeeper to esophageal cancer



Normal aging epithelia carry somatic mutants....



.....many are linked to cancer

Mapping mutants in normal tissues



Micro dissect
clonal units

Whole
Genome
Sequence

Mutational burden
Mutational signatures
Copy number alterations
Mutant prevalence & selection

Lee-six *Nature* 2019
Moore *Nature* 2020
Lawson *Science* 2020



Small area of
epithelium

Deep
targeted
sequence

Spatial map
Mutant prevalence & selection
Mutational signatures
(Mutational burden)
Limited copy no. information

Martincorena *Science* 2015
Martincorena *Science* 2018



Micro-biopsy

Nanoseq

Mutational burden
Mutational signatures

Abscal *Nature* 2021

Mapping mutants in normal tissues



Micro dissect
clonal units

Whole
Genome
Sequence

Laborious
Expensive
High quality data, but few clones

Lee-six *Nature* 2019
Moore *Nature* 2020
Lawson *Science* 2020



Sheet of
epithelium

Deep
targeted
sequence

Epithelial sheets only
Cheap
Many 1000's of mutants
Limited clonal & copy no. info
Very sensitive at detecting selection
& changes in mutant prevalence

Martincorena *Science* 2015
Martincorena *Science* 2018



Micro-biopsy

Nanoseq

Single molecule resolution
Burden/signatures only
Works on small samples

Abscal *Nature* 2021

Examples: middle aged skin & esophagus

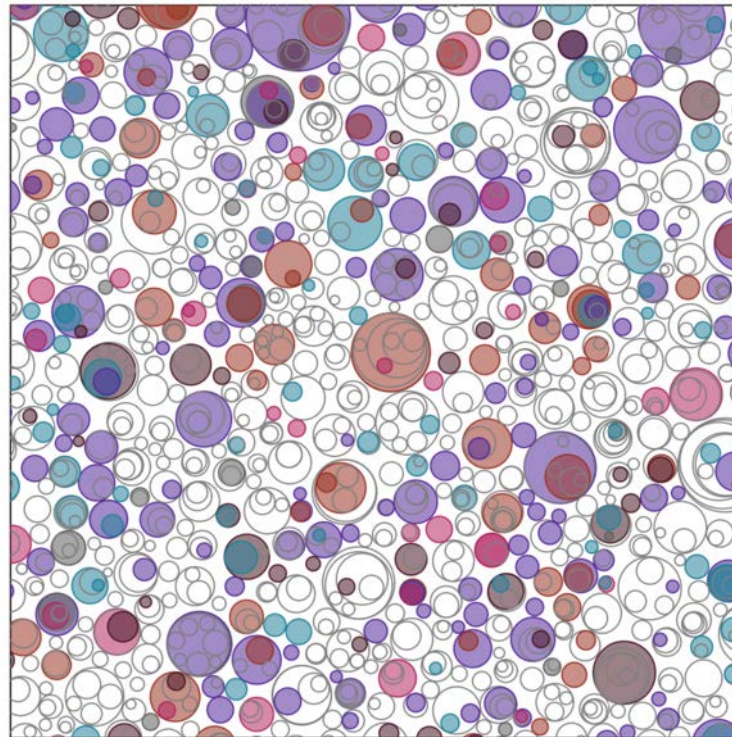
Eyelid

esophagus

Maps from deep
targeted sequencing

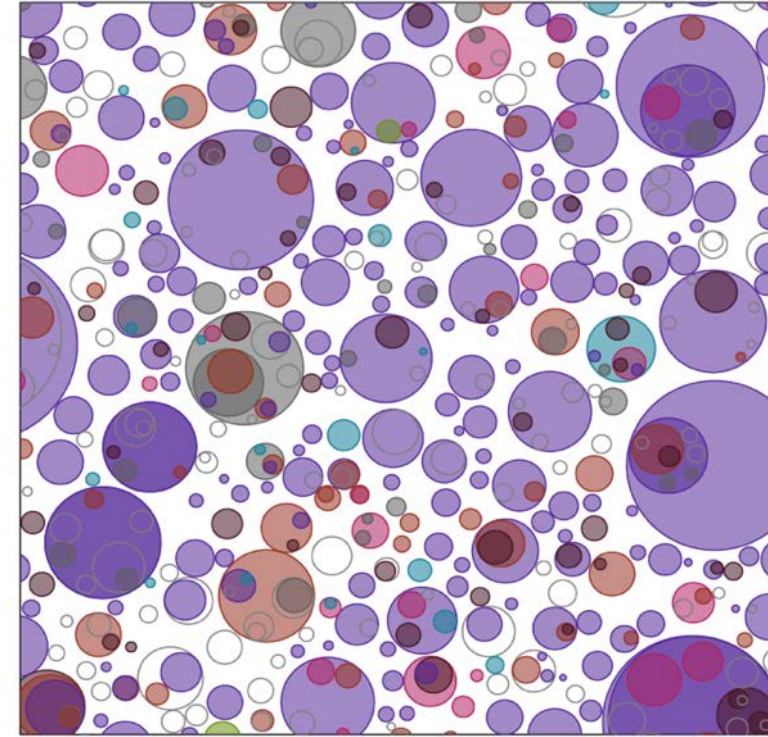
Top down views:
mutant clones in
1cm² tissue

Coloured circles:
positively selected
mutants



TP53 NOTCH1 NOTCH2 NOTCH3 FAT1
AJUBA / ARID2 Other genes

Martincorena Science 2015
Martincorena Science 2018



TP53 NOTCH1 NOTCH2 NOTCH3 FAT1 ARID1A
KMT2D / CUL3 / AJUBA / PIK3CA / ARID2 / TP63 / NFE2L2 / CCND1 Other genes

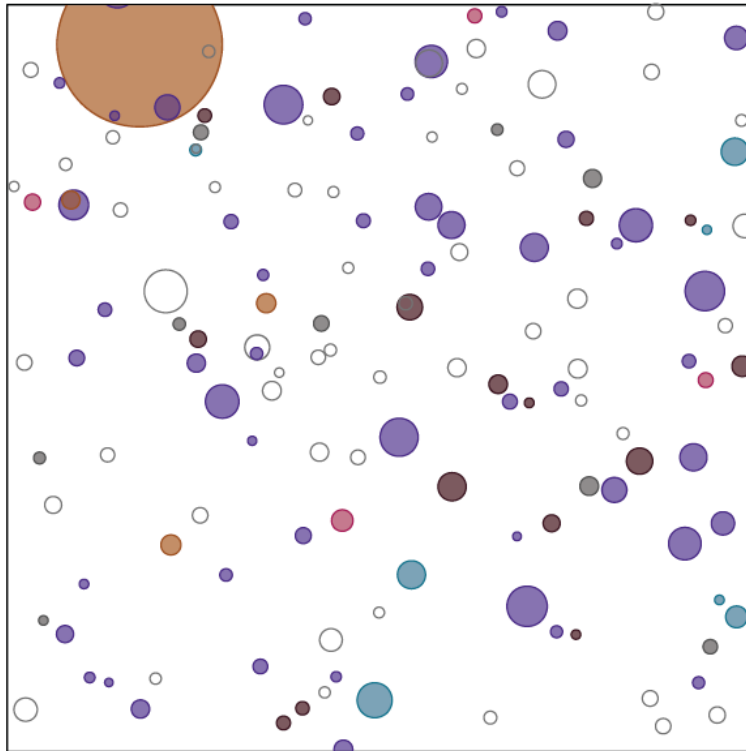
Fowler Cancer Discovery 2021

Esophagus: Mutants accumulate with age

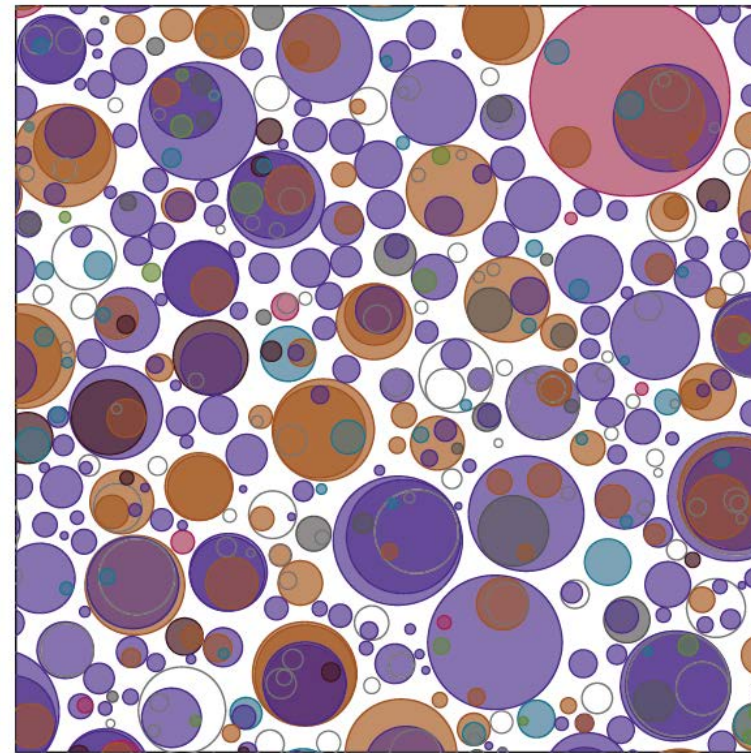
Maps from deep
targeted sequencing

Top down views of
esophagus.
Coloured circles:
positively selected
mutants

23yr male (non-smoker)



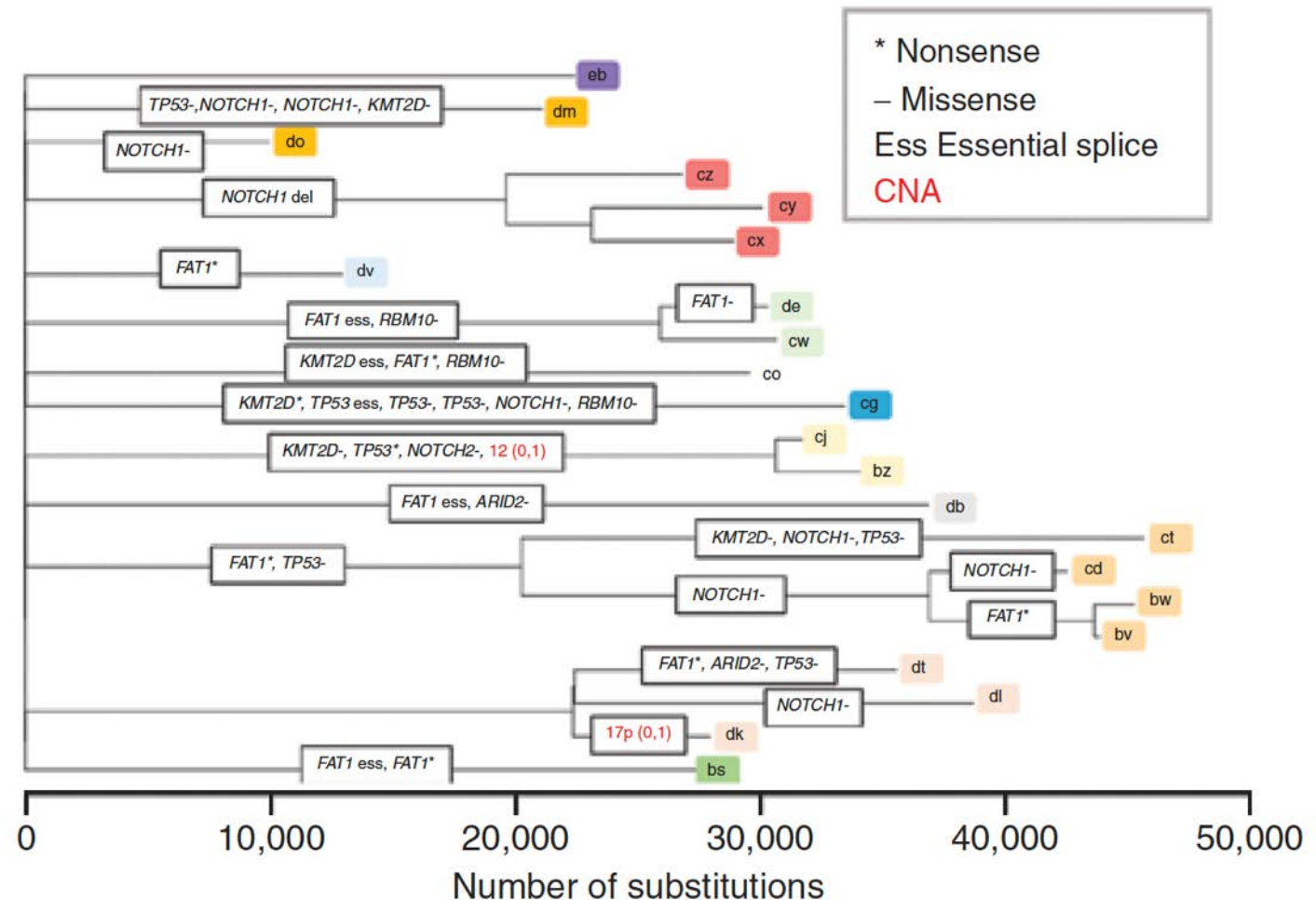
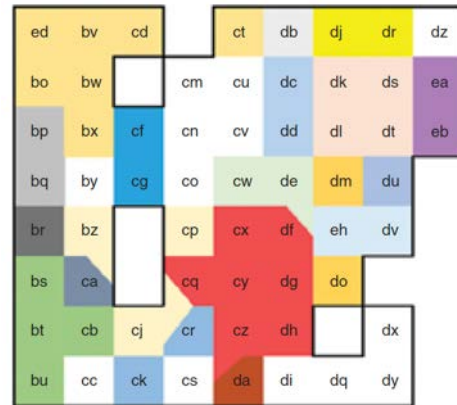
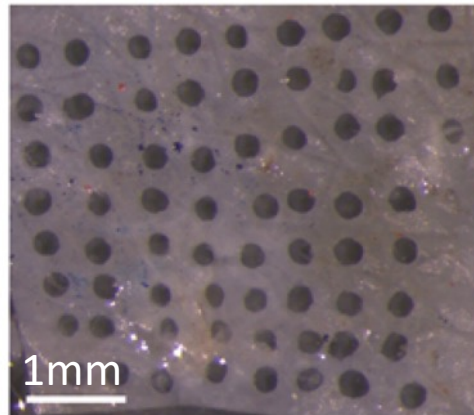
75yr male (non-smoker)



Martincorena,
Fowler et al,
Science 2018



Normal skin 75 year old: clonal genomes



Mapping mutants in normal tissues

No single simple assay for all tissues

Variation: with age
 within tissues
 across tissues
 between individuals

What shapes the landscape?

Germline Variation

Mutagen exposure

Age

Cell lineage (selection varies markedly across tissues)

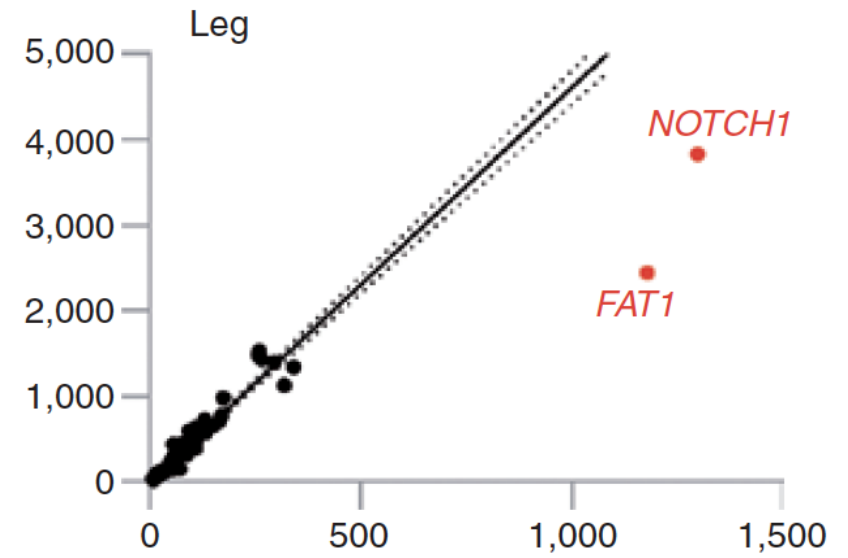
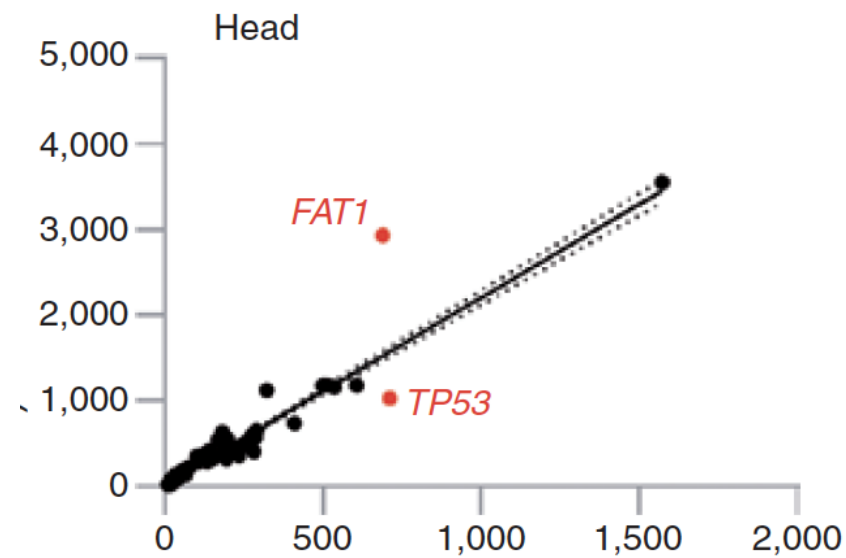
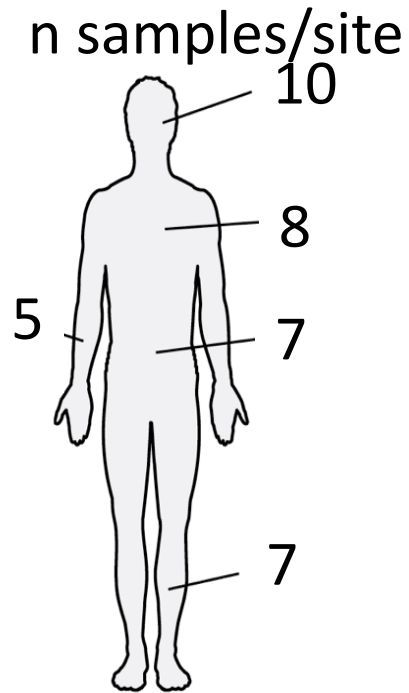
Tissue architecture (crypt, gland or epithelial sheet)

Disease (inflammatory bowel disease, cirrhosis)

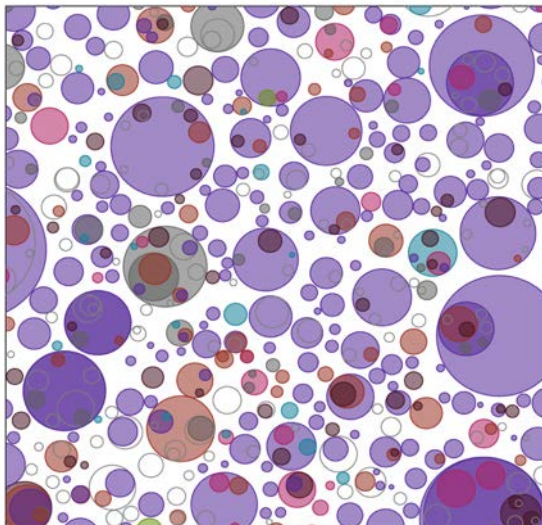
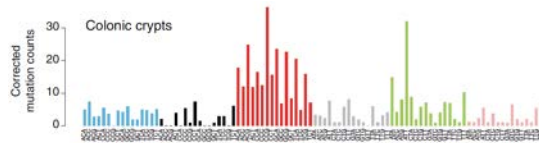
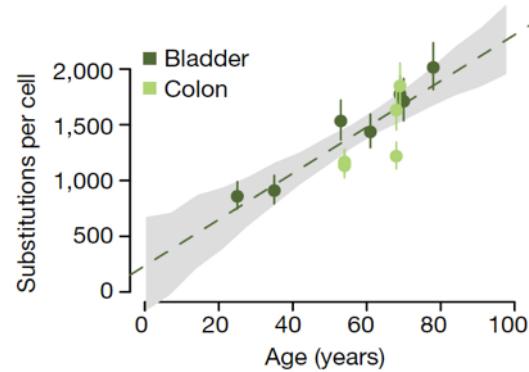
Density of competing clones (less competition=larger clones)

Environmental factors altering fitness UV, IR?

Detecting selection: UV exposed human skin



Epidemiology: Cancer risk prediction



Mutational burden?

Mutational signatures?

Proportion of oncogenic mutants?

Genetic background?

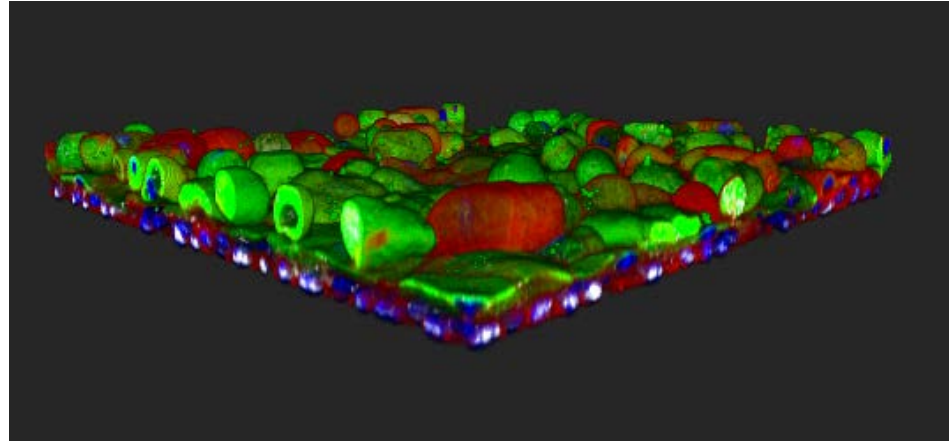
Tissue type?

Somatic mutations in low dose research

Challenging the selection hypothesis:

- Pre and post exposure sequencing of aged tissues
- Organotypic primary cultures with gene edited cells
Live imaging, reporters, spatial omics.....
- Genetics of response: Crispr/Cas9 fitness screens +/- LDIR
- Mice: Lineage tracing, complex mutational landscapes

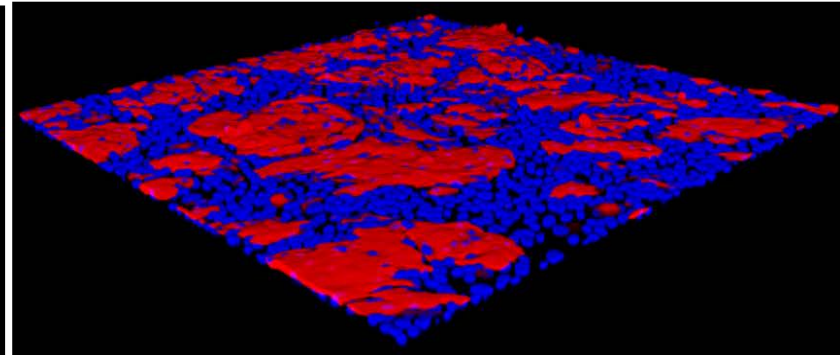
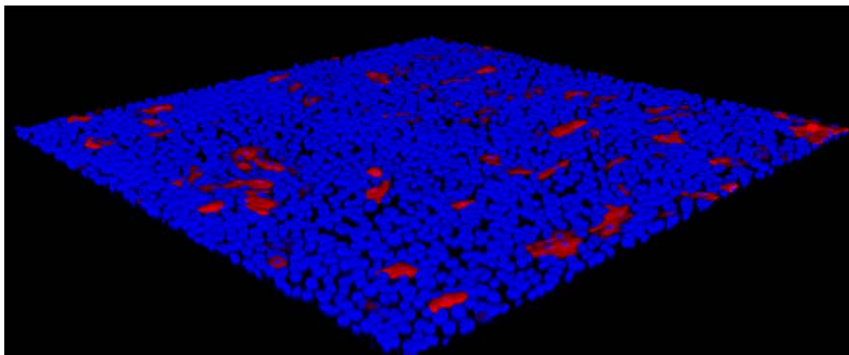
Organotypic Culture: esophagus



0 mGy

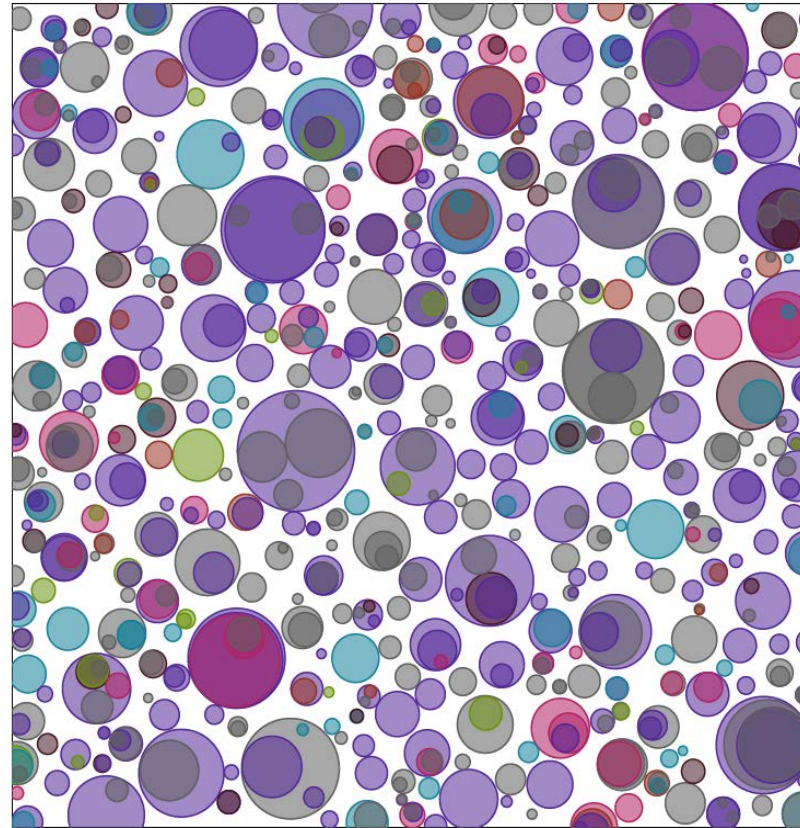
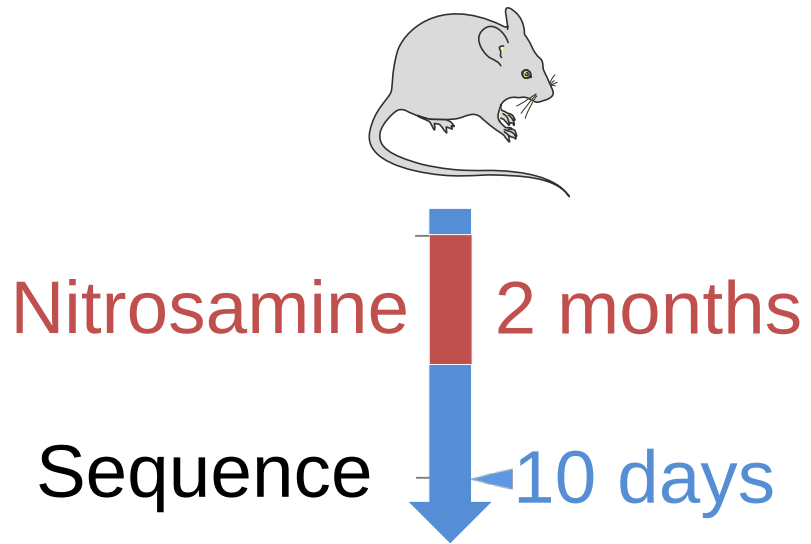
50 mGy

3D view



DAPI KRT4 (differentiation marker)

Replicating aged human esophagus in mice



- Notch1
- Notch2
- Ripk4
- Adam10
- Trp53
- Atp2a2
- Cul3
- Arid1a
- Kdm6a
- Chuk
- Fat1

Research Priorities

Basic biology of normal tissue responses is an urgent need

Engage a wider community in low dose research to diversify perspectives and technologies

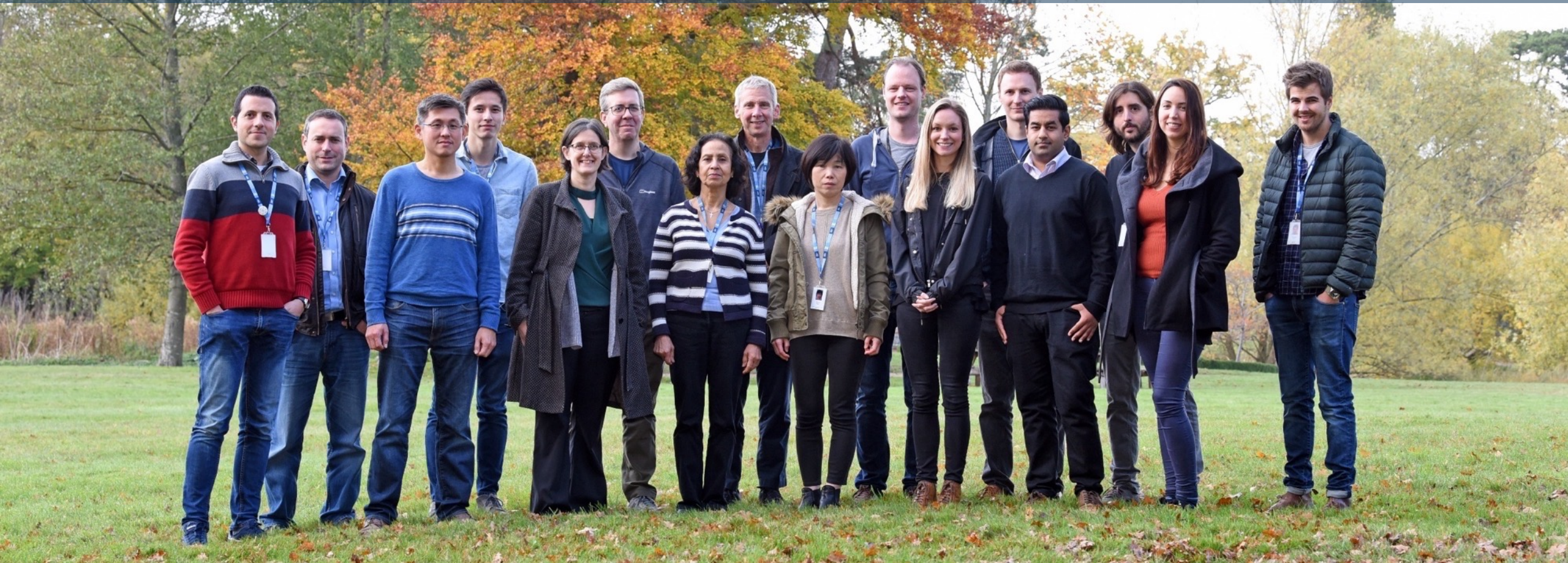
Build collaborative groups linking radiation researchers with those new to the field

Diversity of models and tissues is an advantage

Ensure access to low dose irradiation facilities

Include cutting edge genomic and gene editing experts and data scientists

Thanks to...



Emilie Abby
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