Gao Delivery Team Summary

Goals

Detect and quantify genome editing in lung airway epithelia following AAV delivery

Examine non-target tissues for evidence of editing

Strategy

Activation of fluorescent tdTomato following CRISPR/Cas9 editing

Experimental Summary

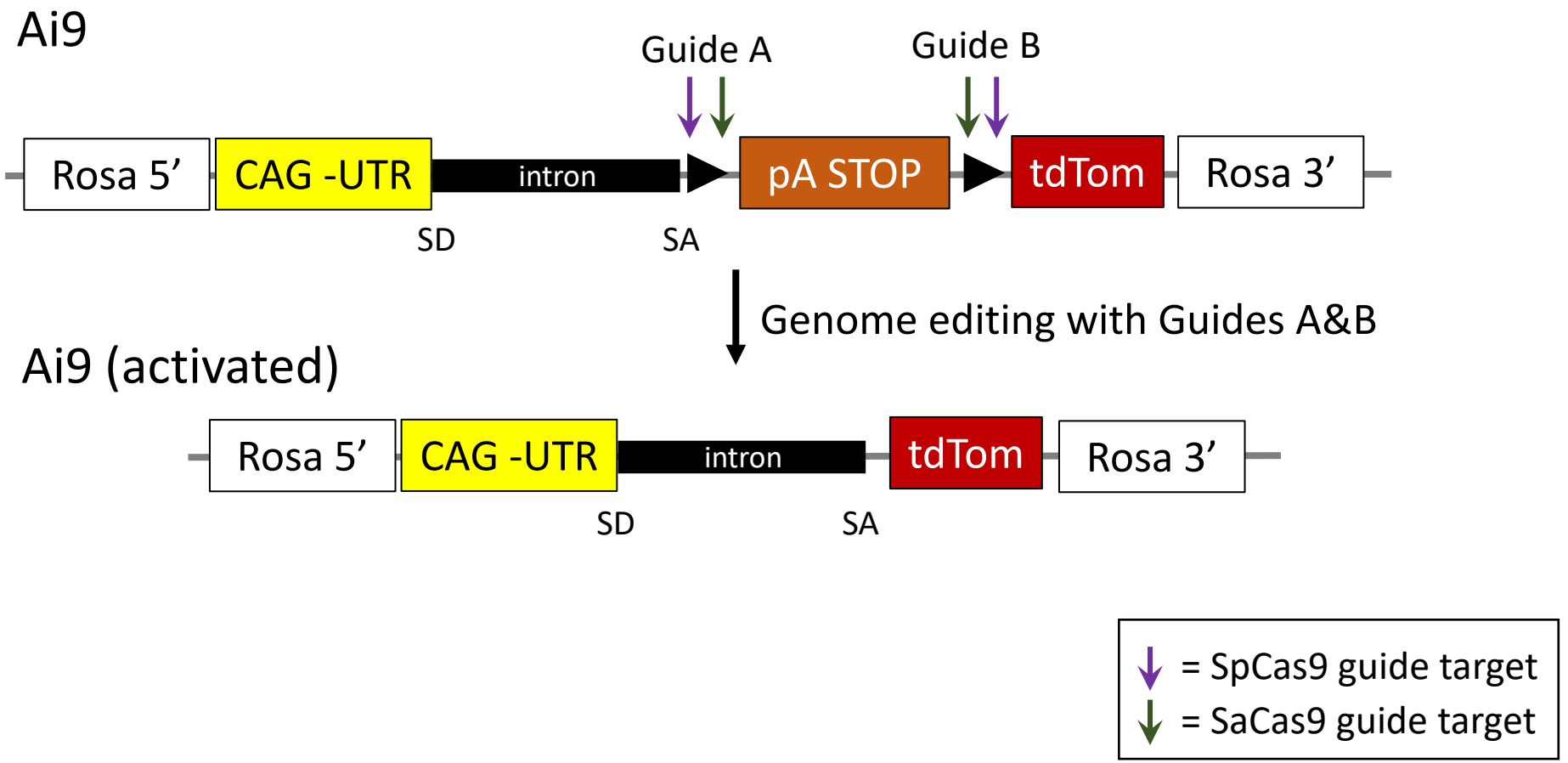
Delivery reagents

Study Arm	Vector	Cargo	Dosage	Solvent	Volume	Delivery Method
1 - Experimental	ssAAV5	B guide + SaCas9	1.7E11 vg	Saline	40 µl	Intratracheal intubation
2 - Experimental	ssAAV5 x 2	1) SpCas9 2) A & B guides + GFP	1) 1.7E11 vg 2) 1.7E11 vg	Saline	40 µl	Intratracheal intubation
3 - Positive control	ssAAV5	Cre recombinase	1.7E11 vg	Saline	40 µl	Intratracheal intubation
4 - Negative control	-	saline	-	Saline	40 µl	Intratracheal intubation

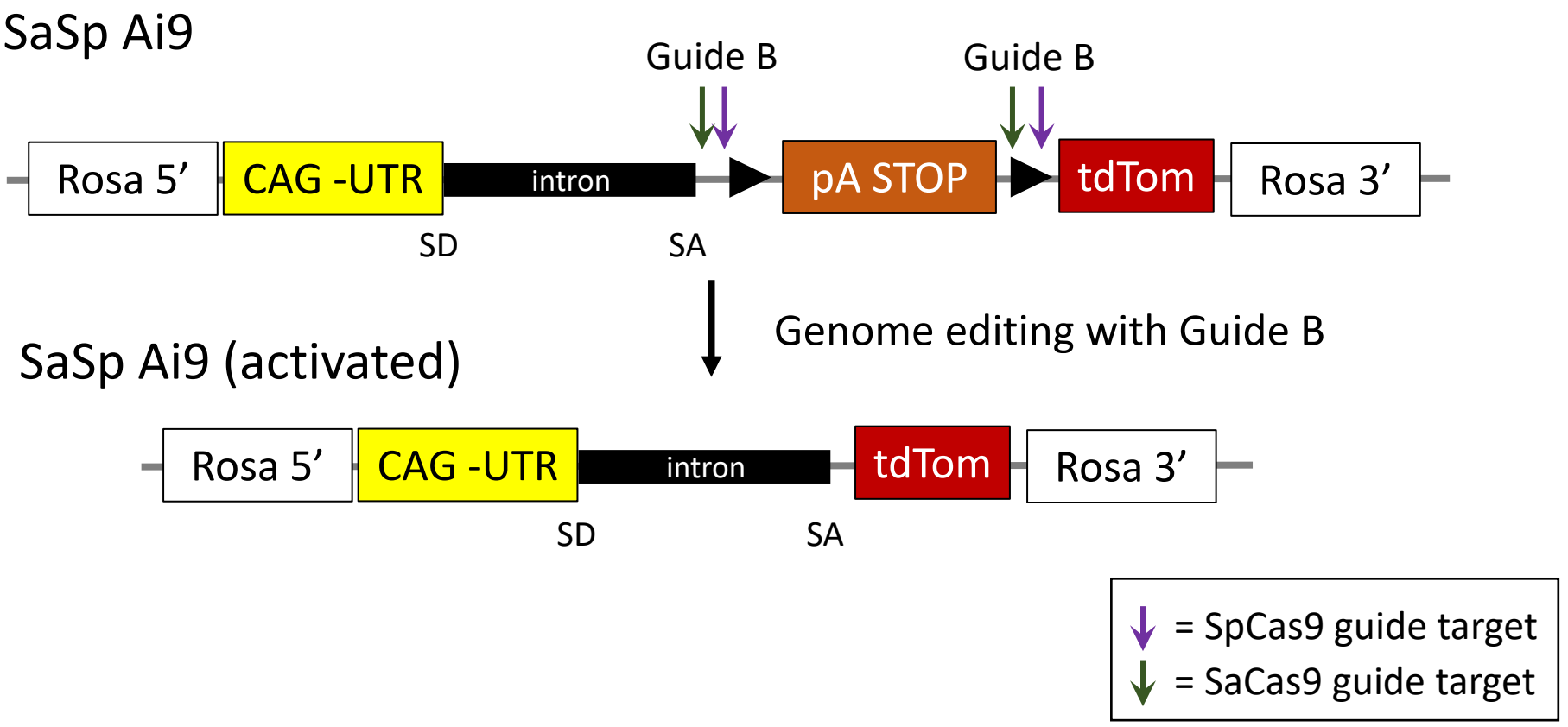
Test mice

Study Arm	Mouse genotype	Age	Incubation time	Number
1 - Experimental	SaSp Ai9/SaSp Ai9	8 weeks	4 weeks	7M 7F
2 - Experimental	SaSp Ai9/SaSp Ai9 and Ai9/Ai9	8 weeks	4 weeks	7M 9F
3 - Positive control	SaSp Ai9/SaSp Ai9	8 weeks	4 weeks	2M 2F
4 - Negative control	SaSp Ai9/SaSp Ai9	8 weeks	4 weeks	3M 3F

Reporter mouse model: conventional Ai9



Reporter mouse model: single-guide SaSp Ai9



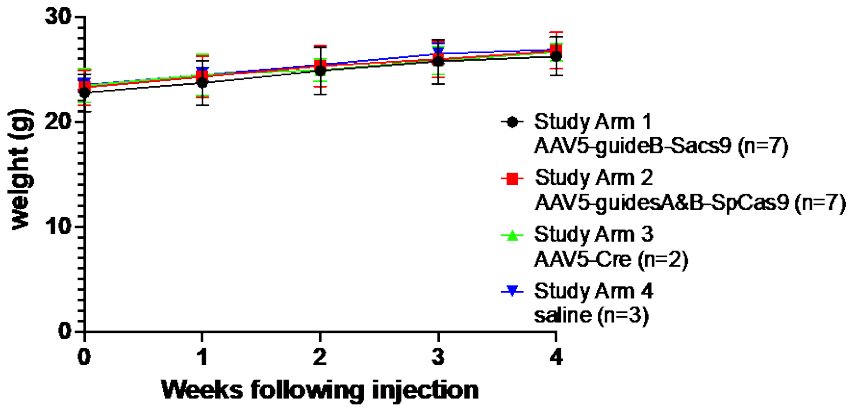
Animal Health data

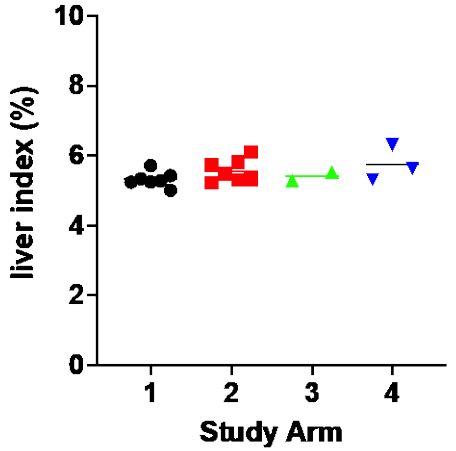
Body weight

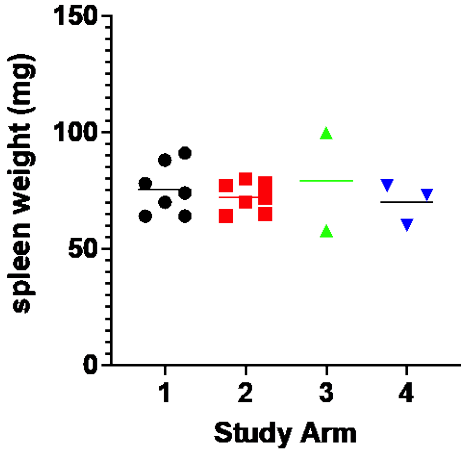
Liver

Spleen

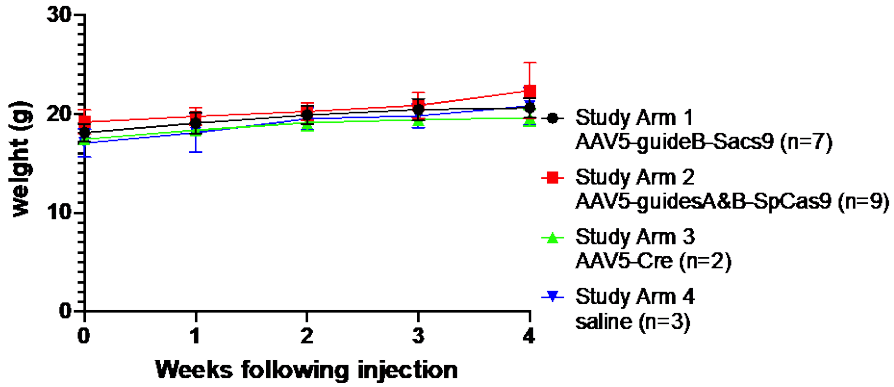
Males

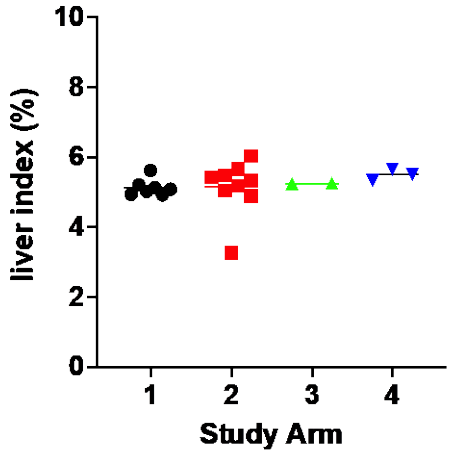


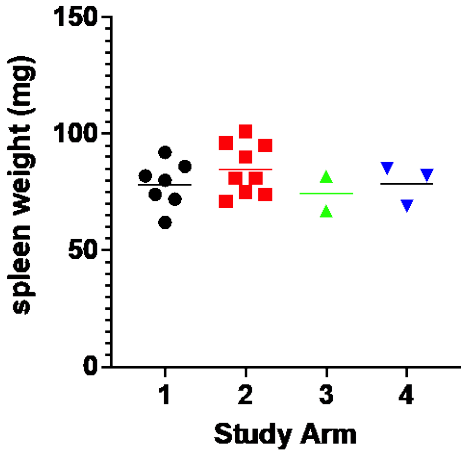




Females







H&E stains

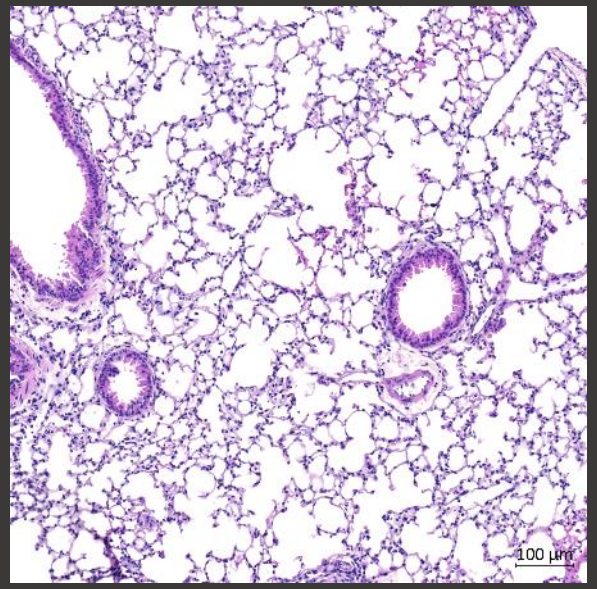
Lung

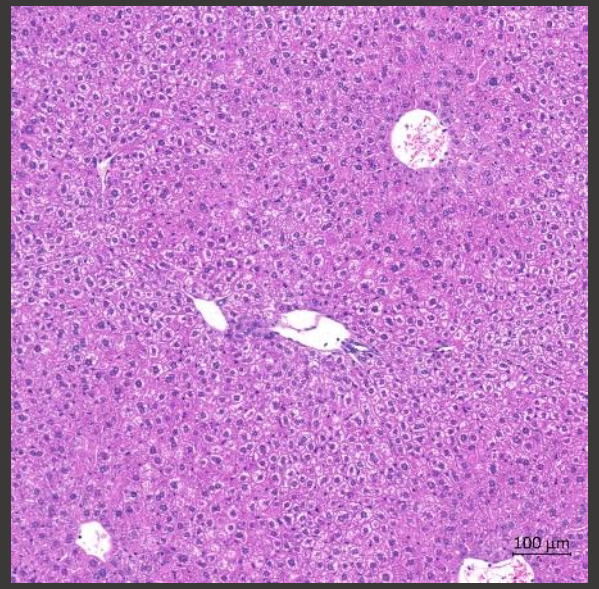
Liver

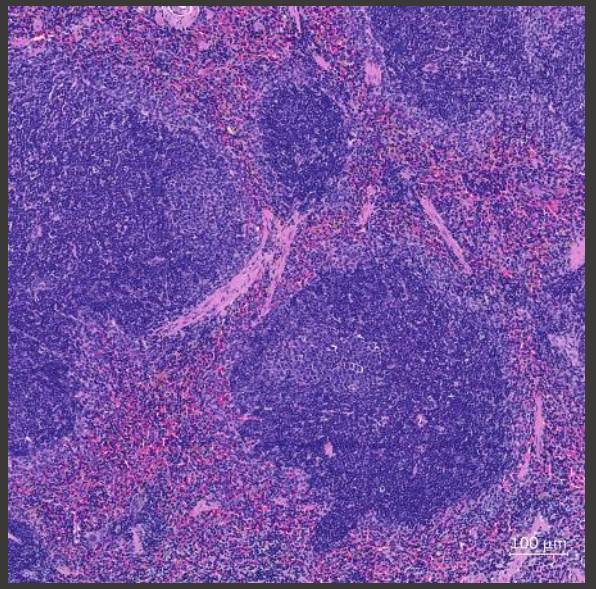
Spleen

Male

Study Arm 2
Experimental
AAV5-SpCas9 &
AAV5-Guides A&B-GFP
2857

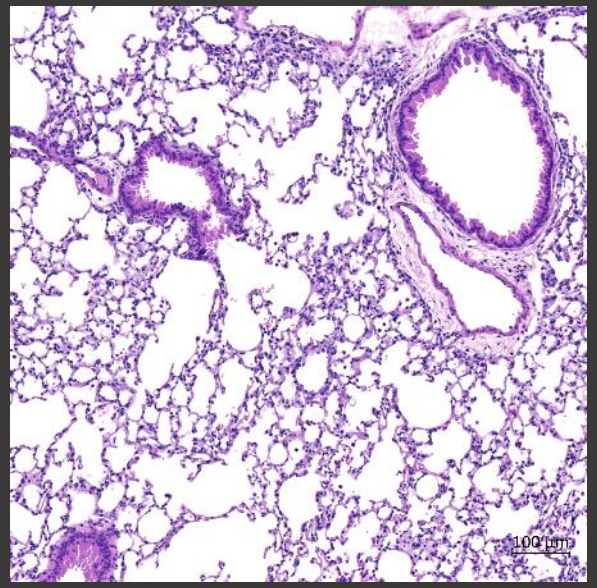


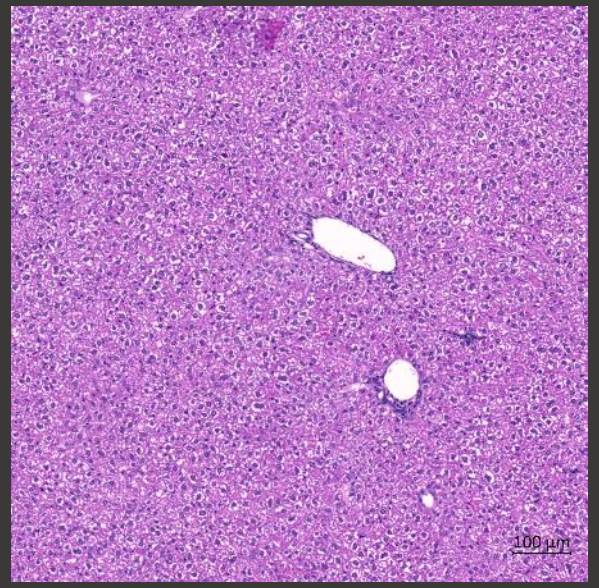


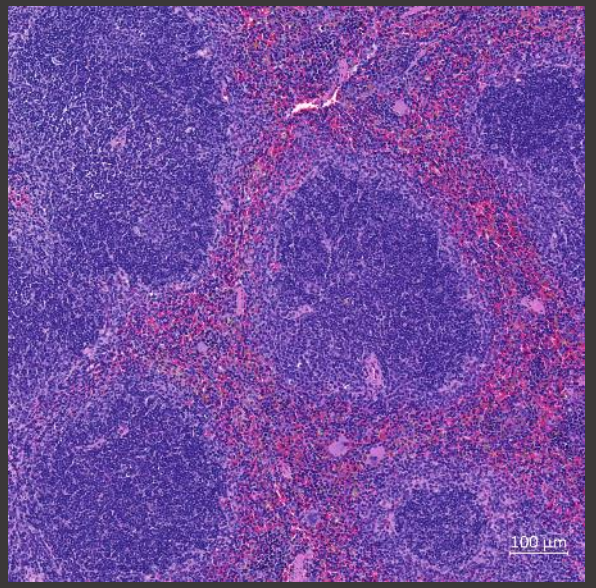


Female

Study Arm 2
Experimental
AAV5-SpCas9 &
AAV5-Guides A&B-GFP
2875







Animal health summary

No anomalies were observed with respect to body weight, liver weight or spleen weight in any mouse, regardless of the treatment

Microscopic examination of the lung (target tissue), liver and spleen revealed normal histologies, with no evidence of inflammation or toxicity

Imaging Protocol

Following dissection, organs and removal of small samples for molecular analysis, organs were fixed overnight in 4% paraformaldehyde, then saturated overnight in 30% sucrose. Samples were frozen in OCT and stored at -80 C

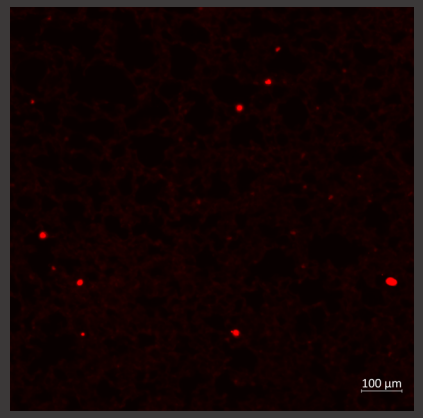
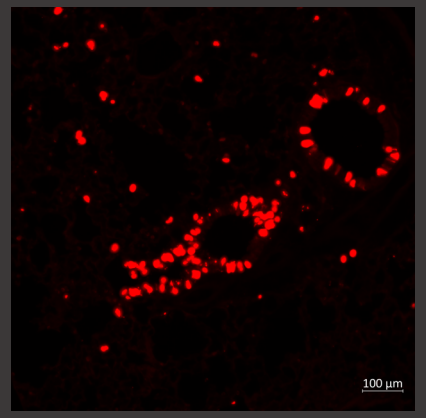
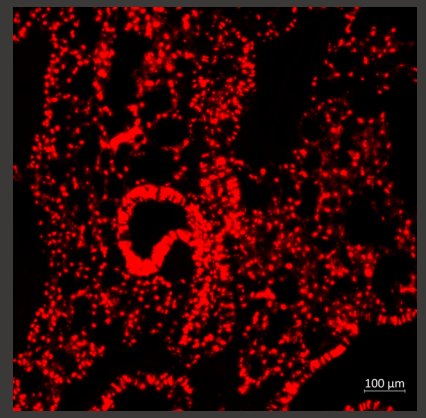
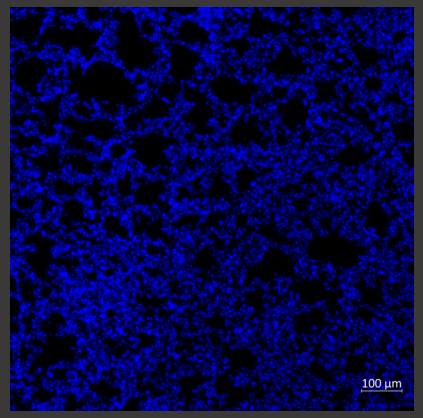
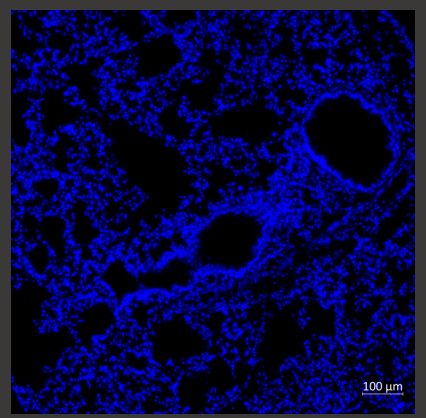
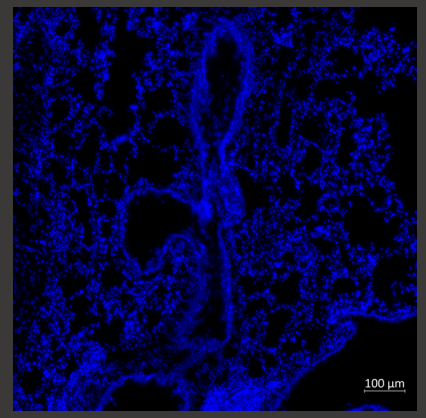
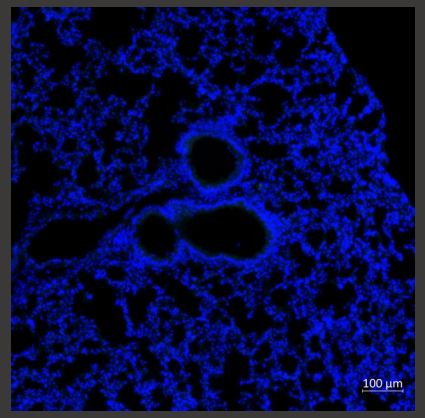
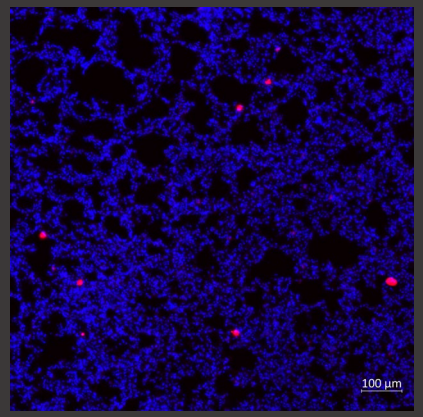
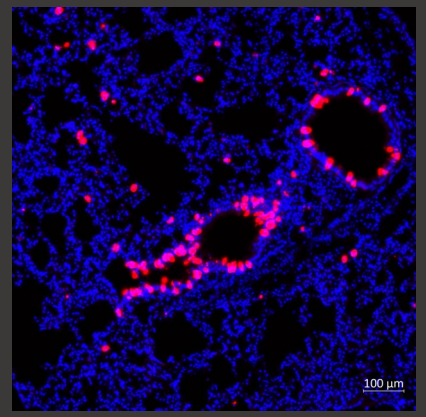
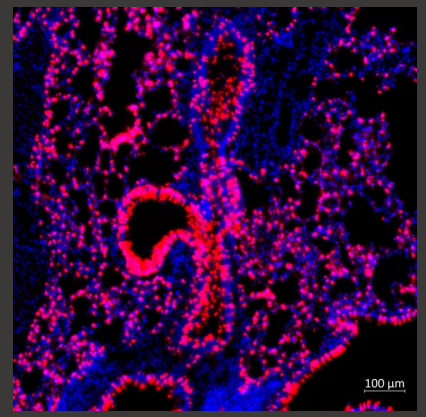
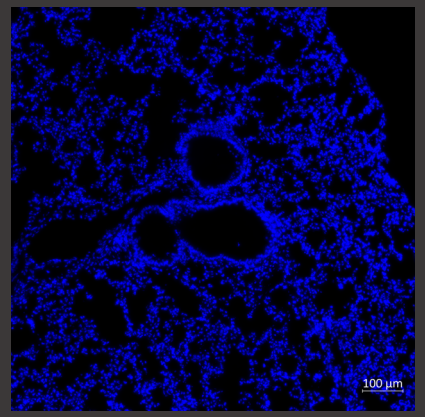
Lung and other organ samples were sectioned at 14 μm and imaged on an AxioScan.X1 slide scanner with a 20X objective

Each section was imaged for tdTomato, indicating a successful editing event, and DAPI to highlight nuclei and identify airways

Some sections were also imaged for GFP, to mark successful AAV delivery (not shown)

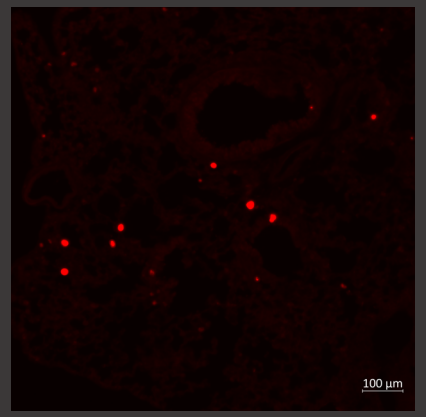
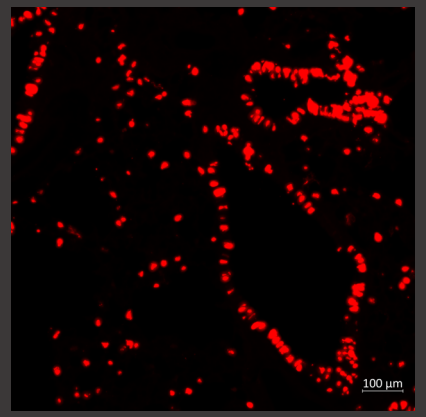
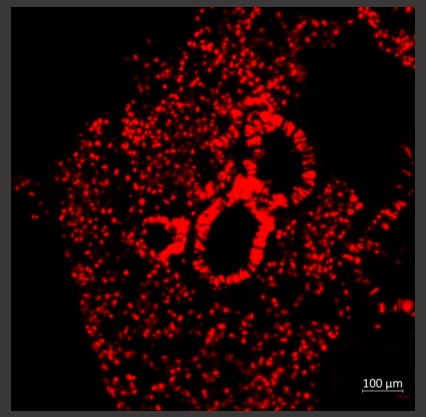
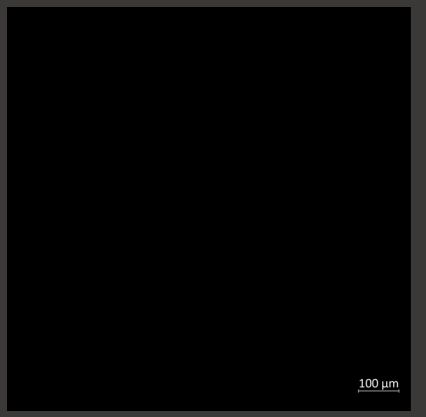
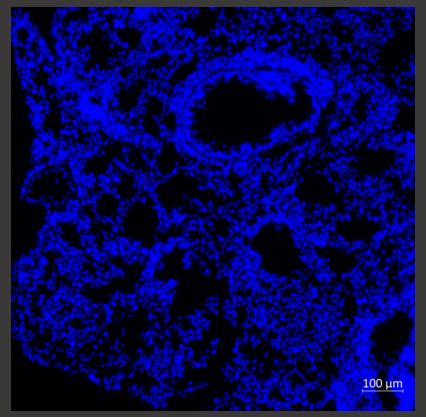
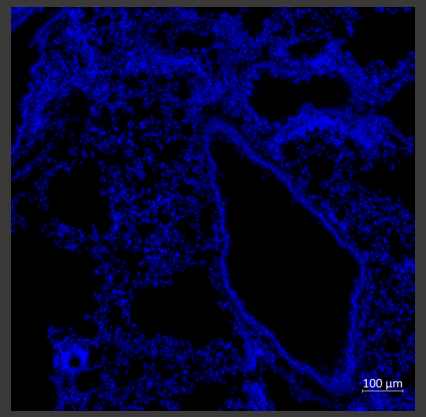
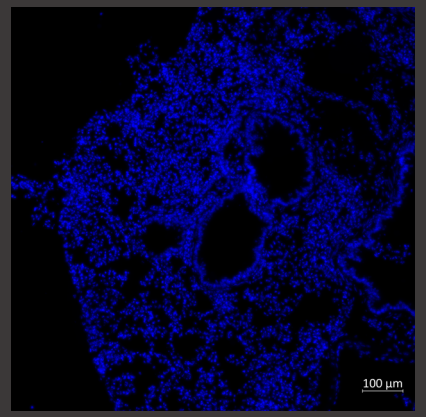
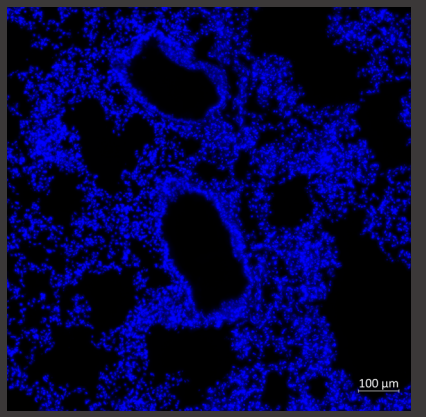
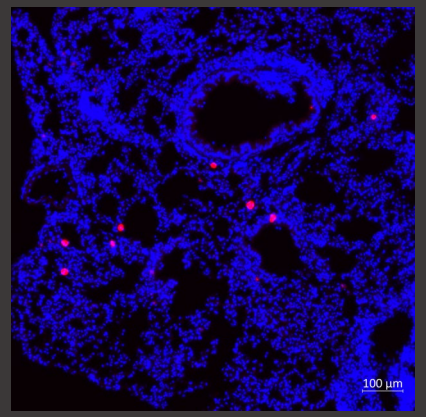
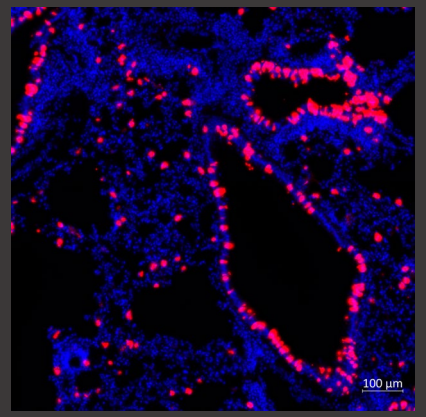
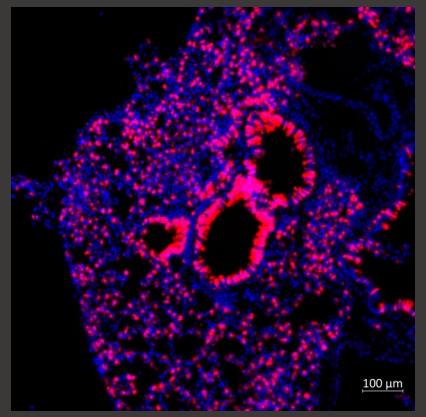
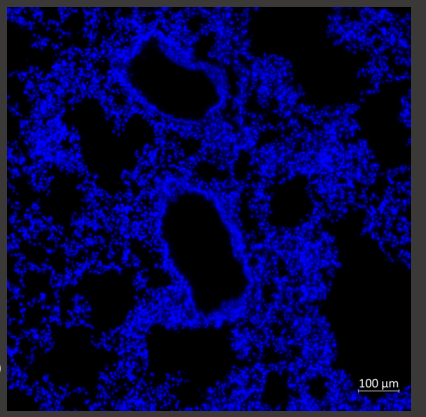
Males

Target tissue
Lung airway epithelia

	Study Arm 1 Experimental AAV5-guideB-SaCas9	Study Arm 2 Experimental AAV5-SpCas9 + AAV5-guidesA&B-GFP	Study Arm 3 Positive Cont. AAV5-Cre	Study Arm 4 Negative Cont. Saline
tdTomato				
DAPI				
Merged				
	2868	2857	2845	2844

Females

Target tissue
Lung airway epithelia

	Study Arm 1 Experimental AAV5-guideB-SaCas9	Study Arm 2 Experimental AAV5-SpCas9 + AAV5-guidesA&B-GFP	Study Arm 3 Positive Cont. AAV5-Cre	Study Arm 4 Negative Cont. Saline
tdTomato				
DAPI				
Merged				
	3304	2875	2847	2846

Lung imaging results

Study Arm 1 - Experimental: AAV5 – guideB-SaCas9

Few to no red cells observed localized to airway epithelia, indicating no significant editing of target.
Five out of 14 (1 M, 4 F) showed sporadic red cells (>5 per section) not localized to airways.

Study Arm 2 - Experimental: AAV5-SpCas9 + AAV5-guidesA&B-GFP

In 7 animals (1 M, 6 F), significant number of red cells in airway epithelia, indicating successful editing.
In remaining animals, few to no red cells observed.

Study Arm 3 - Positive control: AAV5-Cre

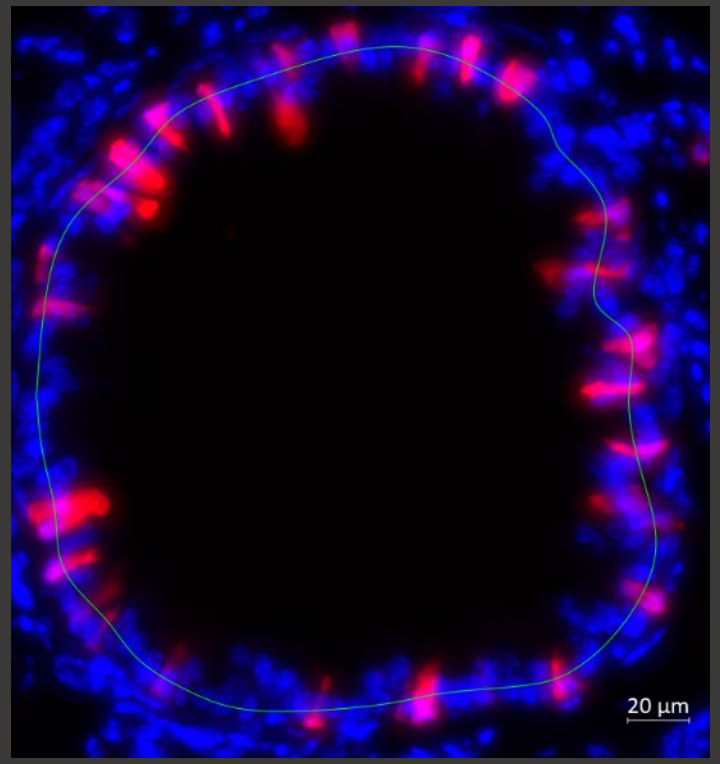
Significant red cell accumulation observed, as expected.

Study Arm 4 - Negative control: saline

No red cells detected, as expected.

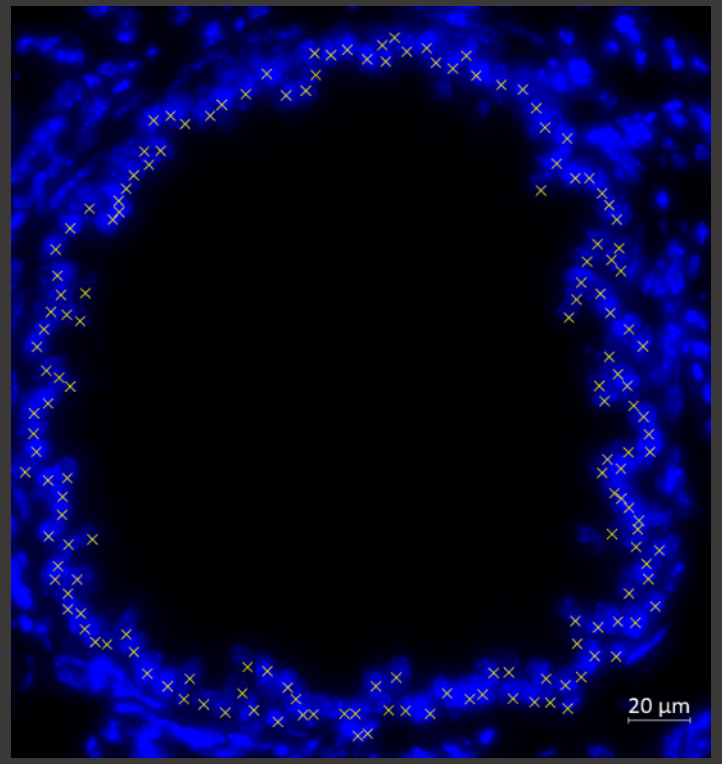
Quantification of editing in target tissue - lung airway epithelia

Merged image



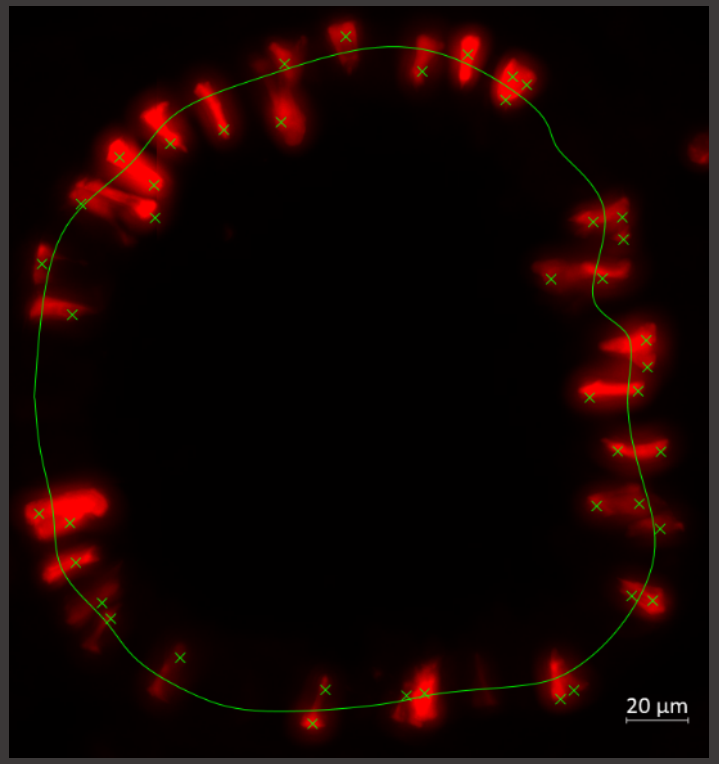
2857 M

Nuclei



163 nuclei

Positive cells



43 tdTomato+ cells

Therefore, 26.3% editing efficiency, in a 34960 μm^2 airway

Quantification results

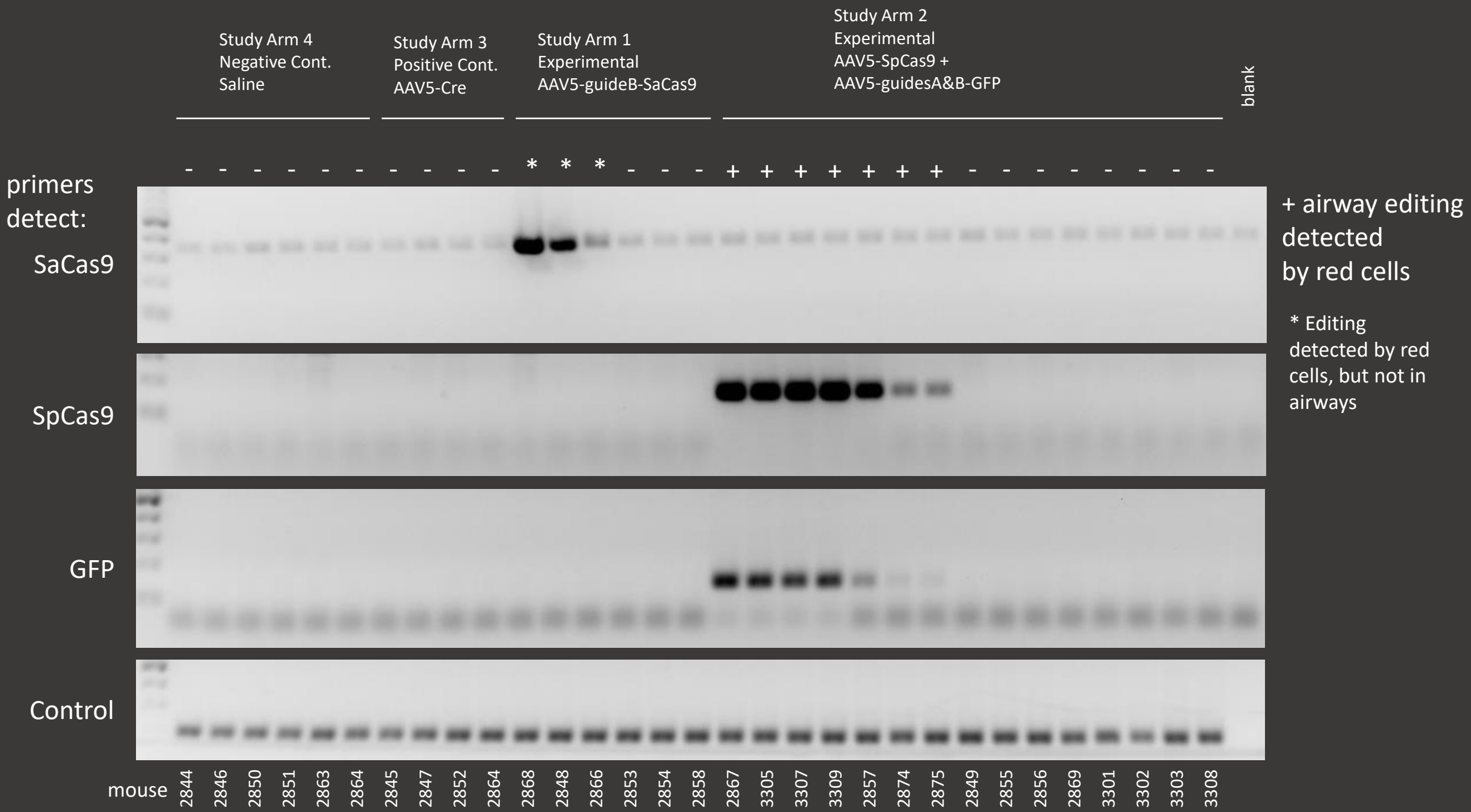
Mouse	Small airways (n=3) %	Large airways (n=3) %
2857 M	21.5 ± 4.6	17.6 ± 6.8
2867 F	17.6 ± 2.5	14.5 ± 1.0
2874 F*	22.1 ± 3.7	19.3 ± 5.7
2875 F*	25.3 ± 3.7	20.6 ± 5.8
3305 F	21.2 ± 3.1	11.6 ± 3.2
3307 F	17.3 ± 3.7	13.9 ± 1.3
3309 F	18.2 ± 1.1	14.6 ± 2.5

* Conventional Ai9, not SaSp Ai9

Molecular analysis: PCR to detect AAV genomes

- PCR was performed to detect the presence of vector genomes in genomic DNA isolated from lung samples
- Primers were specific for SaCas9, SpCas9 or GFP transgenes
- Presence of AAV genomes serves as confirmation of successful intratracheal infusion

Result: presence of AAV genomes correlated very well with detection of tdTomato+ cells


Study Arm 4
Negative Cont.
Saline
Study Arm 3
Positive Cont.
AAV5-Cre
Study Arm 1
Experimental
AAV5-guideB-SaCas9
Study Arm 2
Experimental
AAV5-SpCas9 +
AAV5-guidesA&B-GFP
blank
- - - - - - - - - - * * * - - - + + + + + + + - - - - - - - -
primers
detect:
SaCas9
SpCas9
GFP
Control
mouse
2844 2846 2850 2851 2863 2864 2845 2847 2852 2864 2868 2848 2866 2853 2854 2858 2867 3305 3307 3309 2857 2874 2875 2849 2855 2856 2869 3301 3302 3303 3308
+ airway editing
detected
by red cells
* Editing
detected by red
cells, but not in
airways

Analysis of genome editing in non-target tissues

- From every animal in all four study arms, a full panel of non-target organs was dissected and prepared for imaging
  - Brain, Eye, Heart, Trachea, Liver, Kidney, Pancreas, Spleen, Stomach, Small Intestine (Duodenum, Jejunum, Ileum), Large Intestine, Muscle (Gastrocnemius, Soleus, TA, EDL and Diaphragm), White Adipose (Subcutaneous and Perigonadal), Brown Adipose, Testes or Ovary, Epididymis or Uterus

- For those animals showing significant editing in the target tissue, the non-target organs were sectioned and imaged as above
  - Three non-consecutive sections from each organ were imaged

- Images below are from one male and one female mouse, each of which showed significant editing in the target tissue

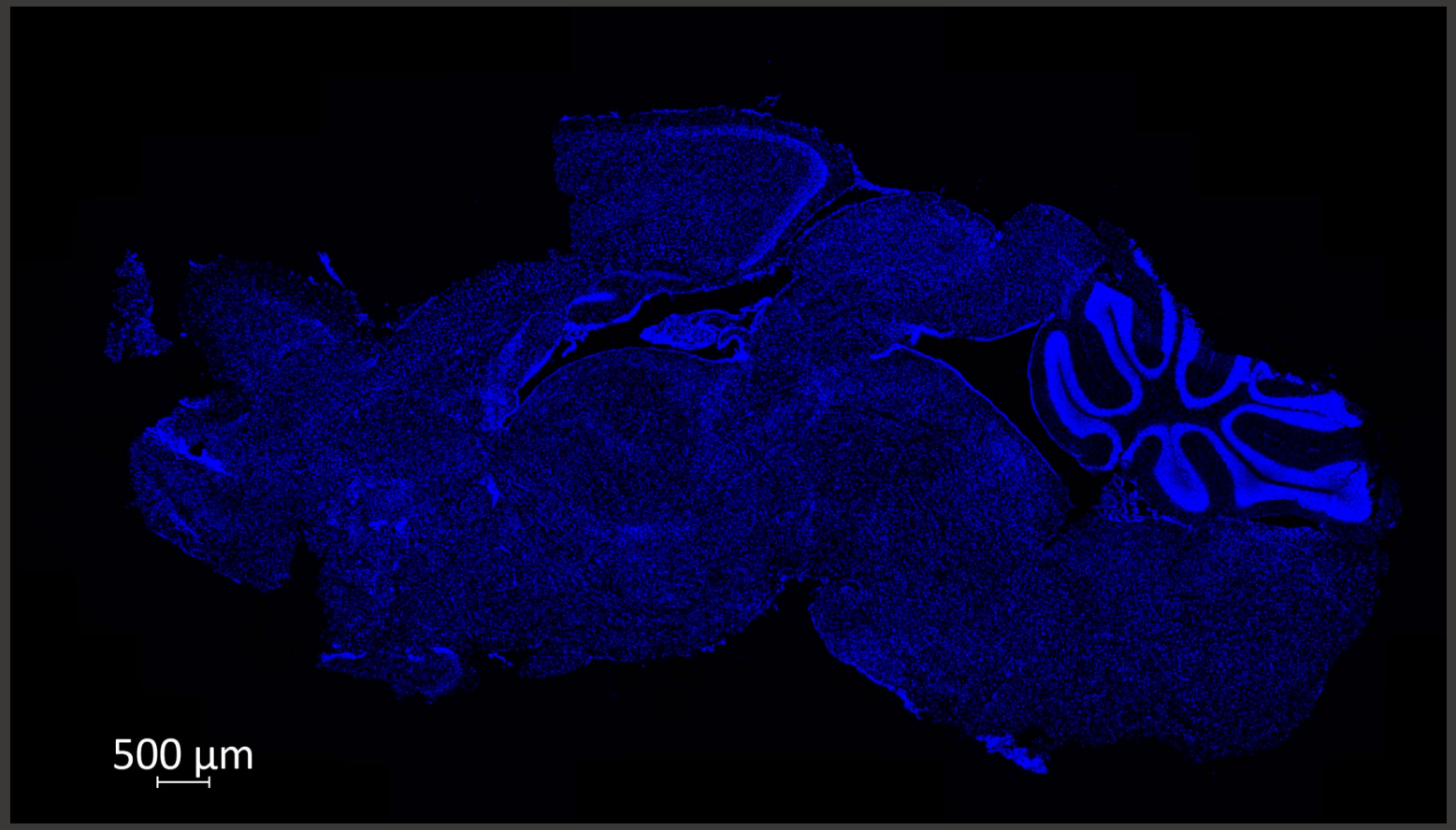
Results: Very rare red cells were observed in some non-target tissues.

Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Brain

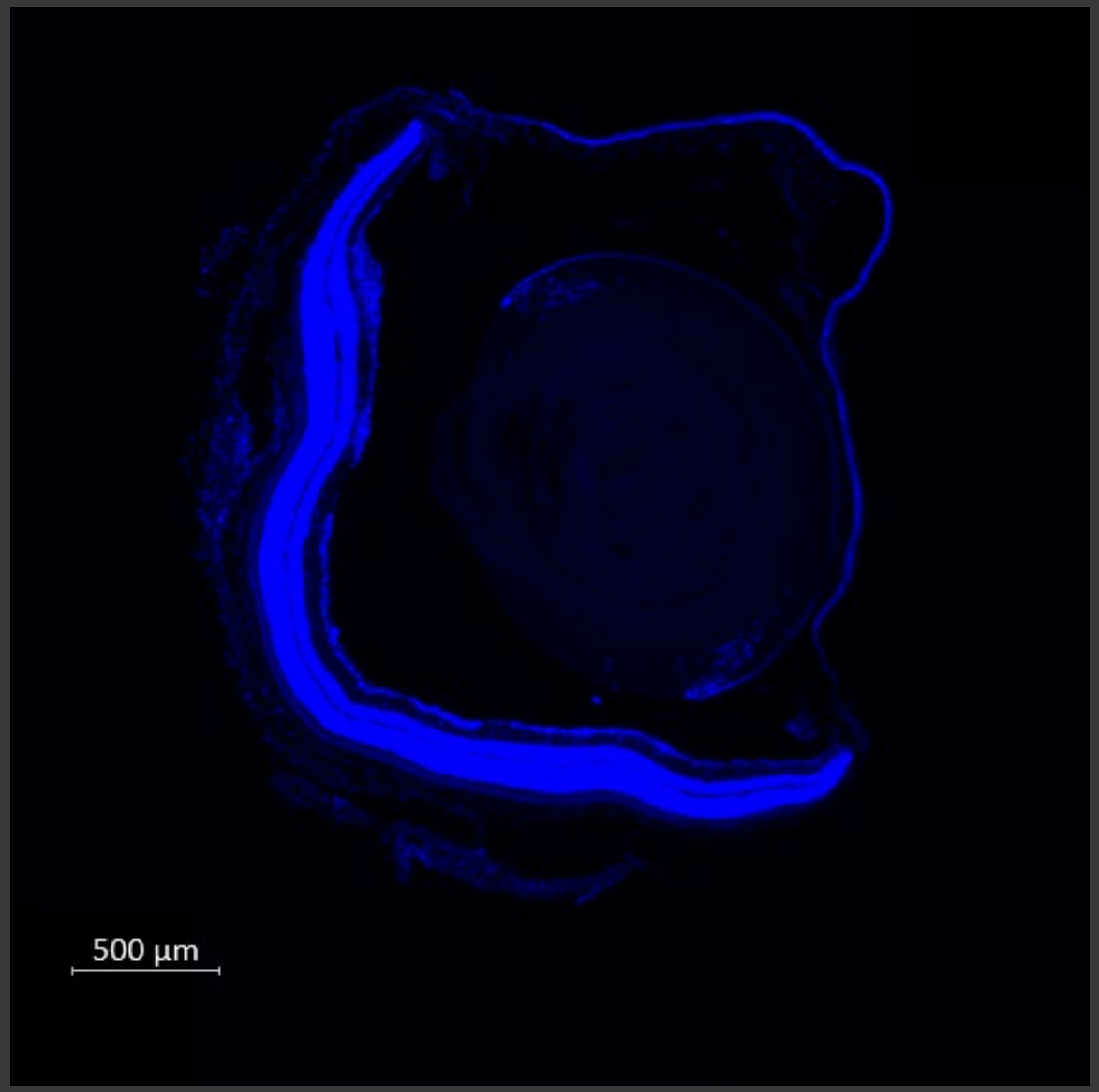


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Eye

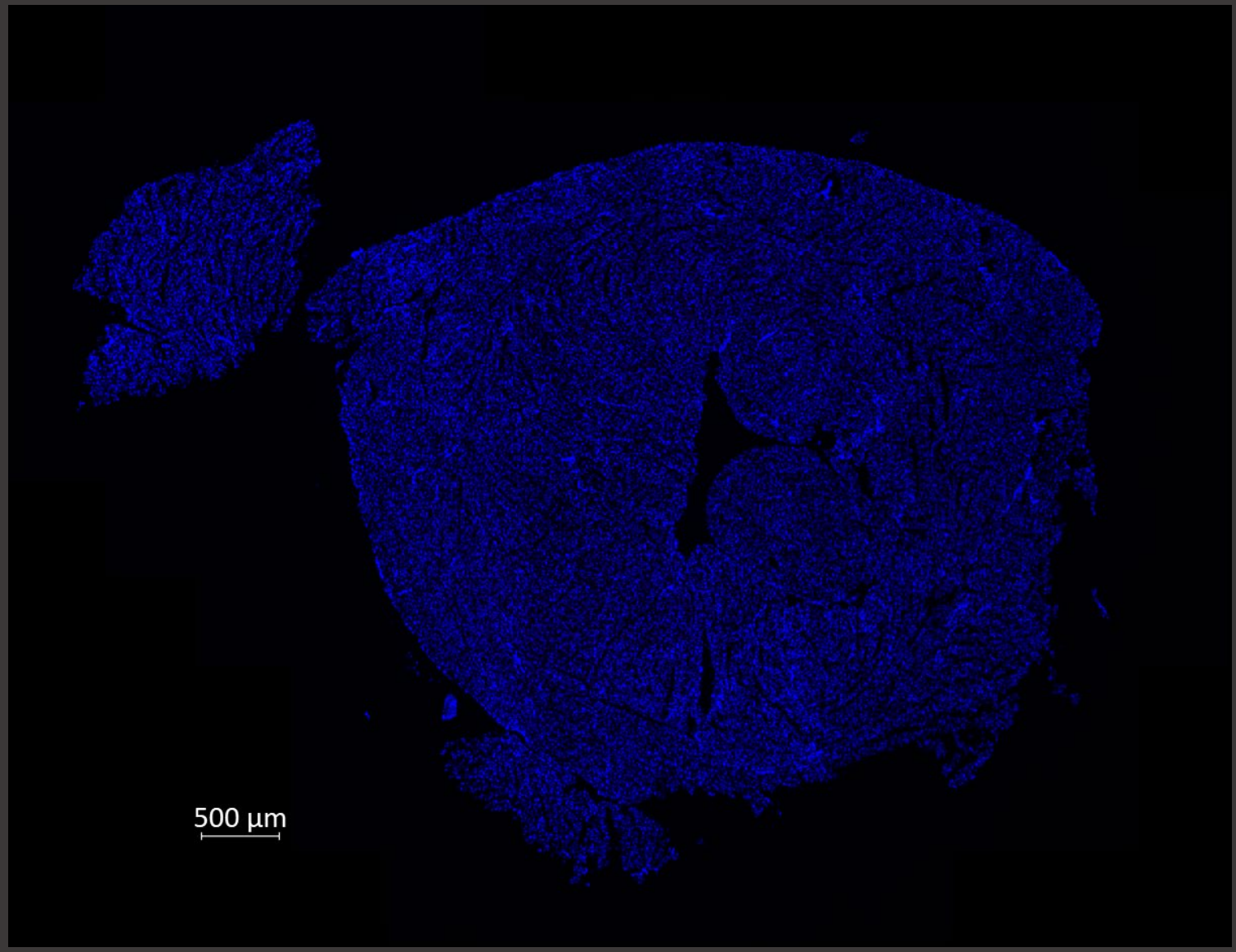


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Heart

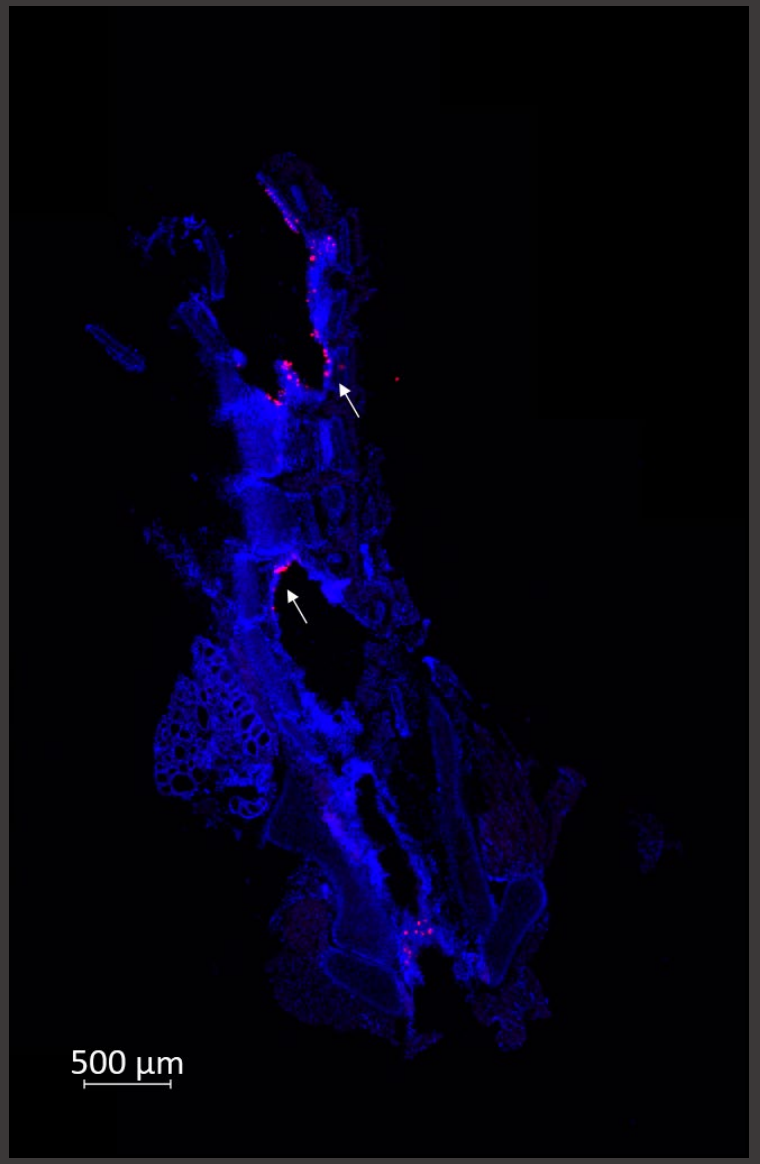


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Trachea

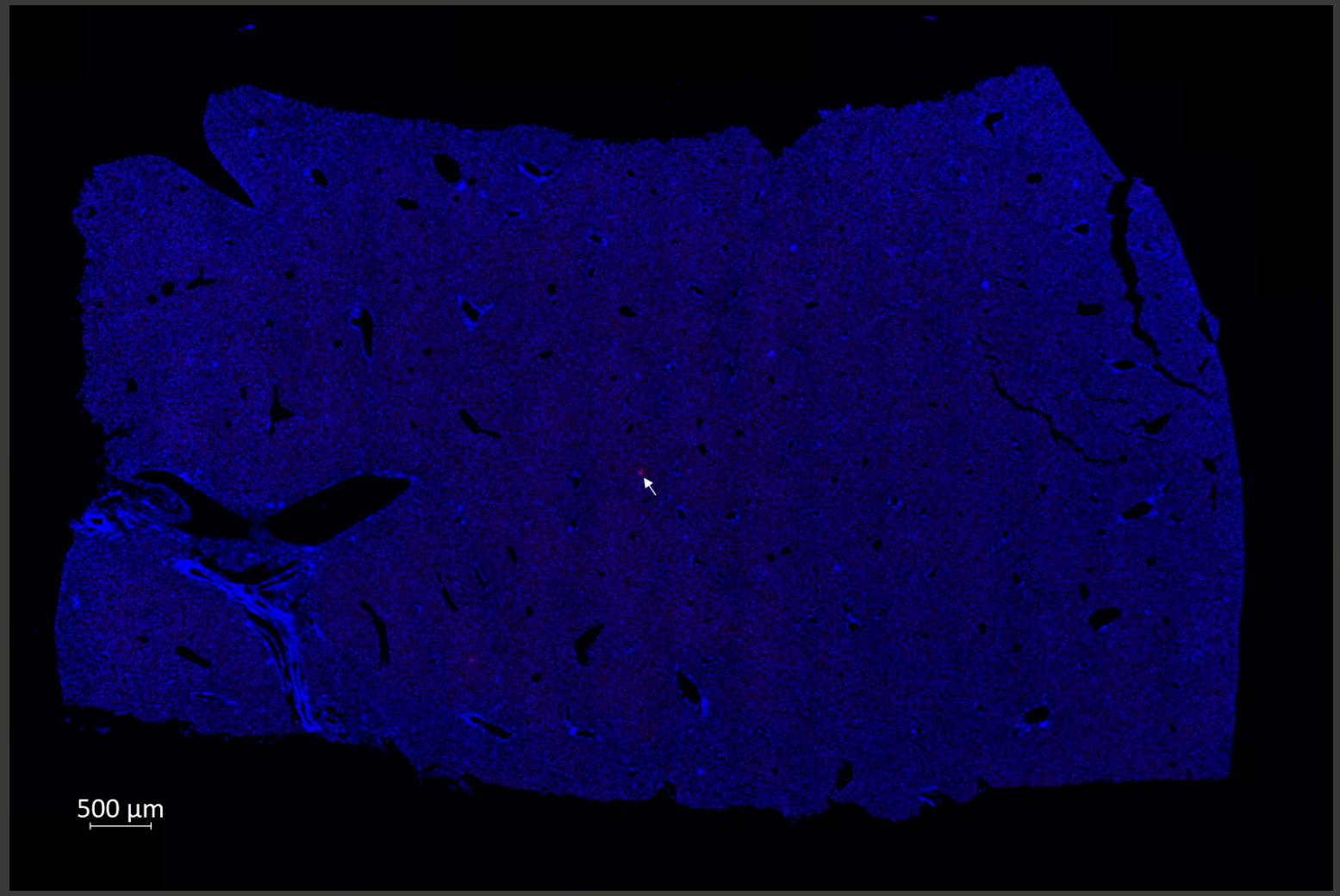


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Liver

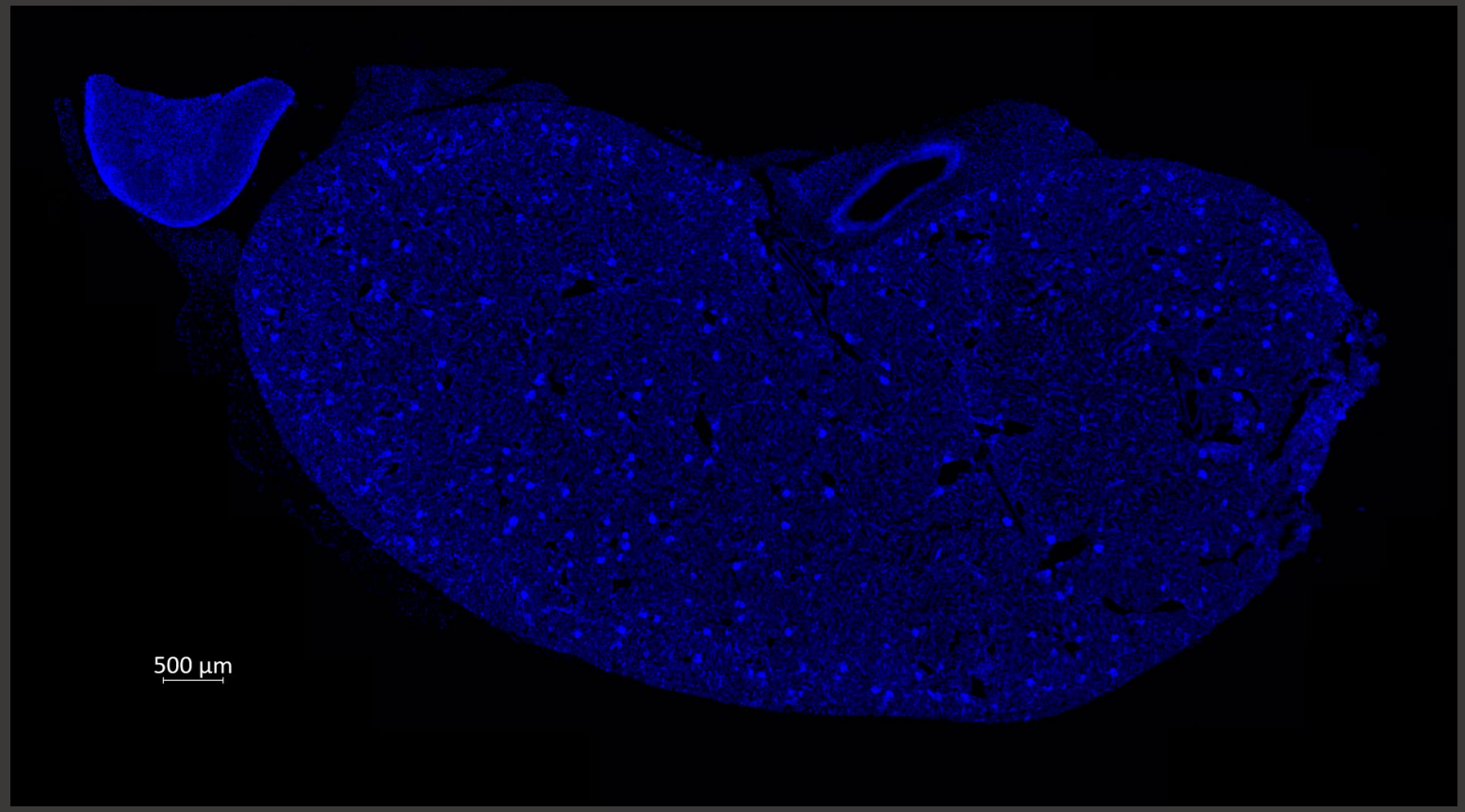


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Kidney

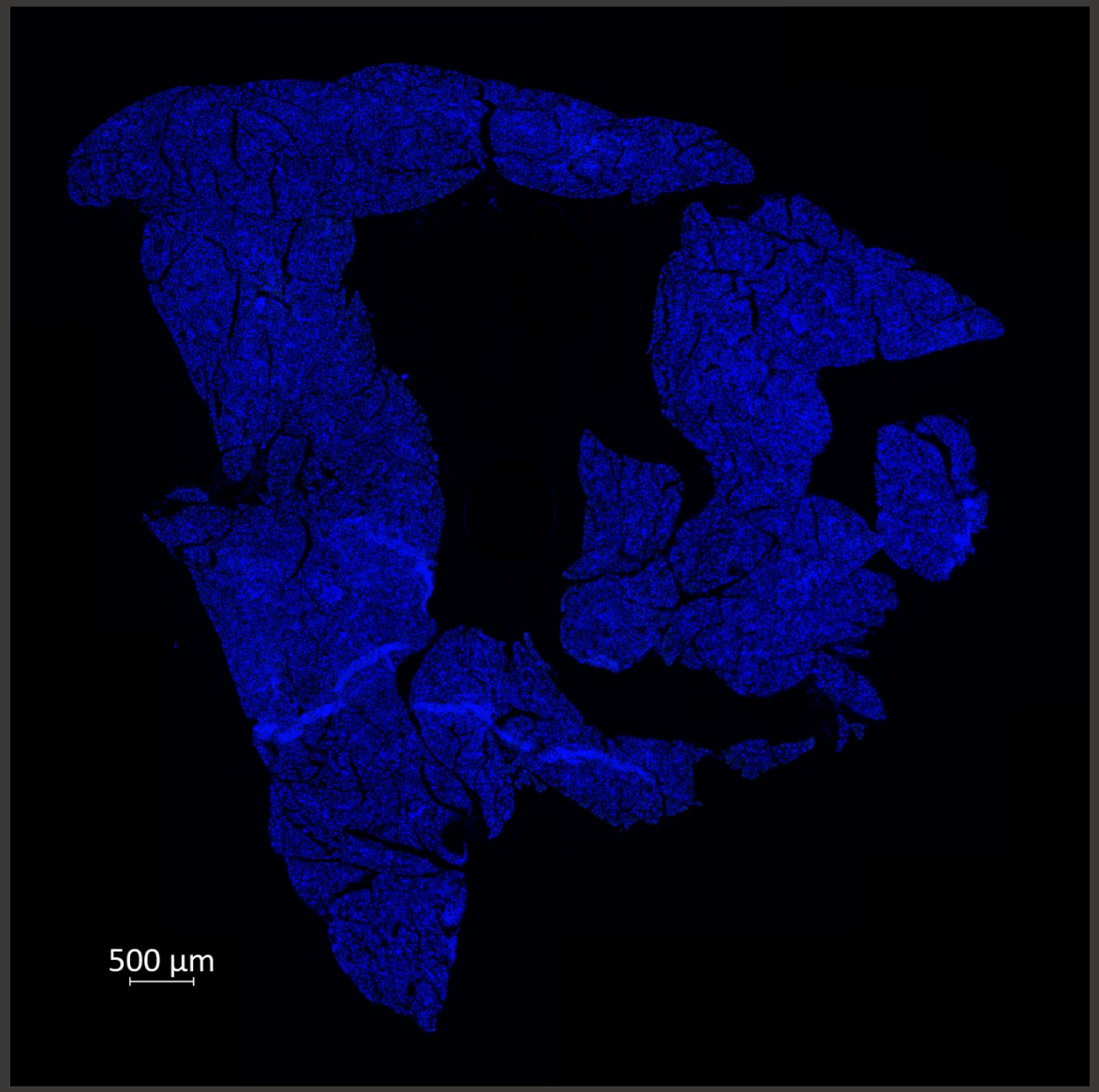


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Pancreas

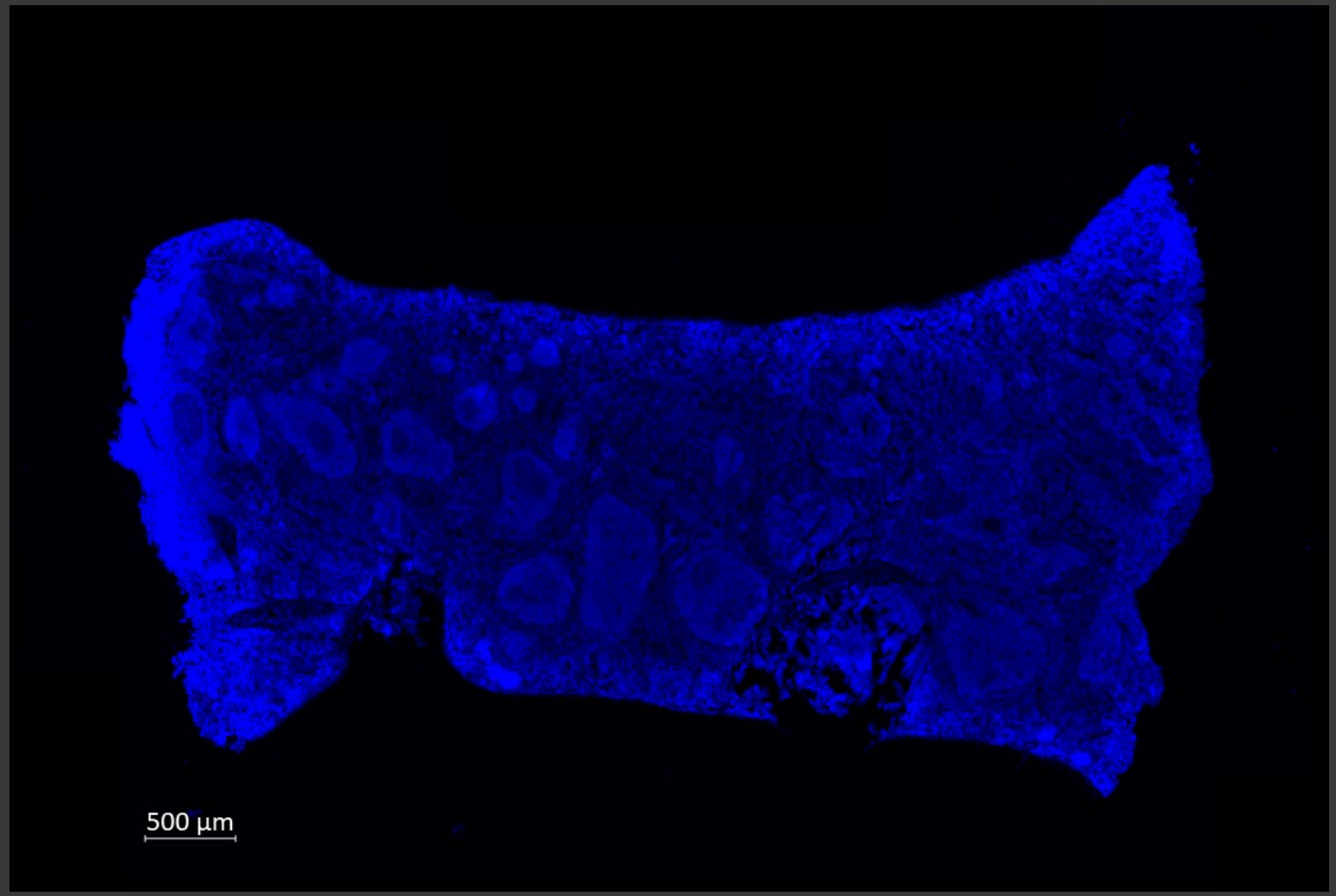


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Spleen

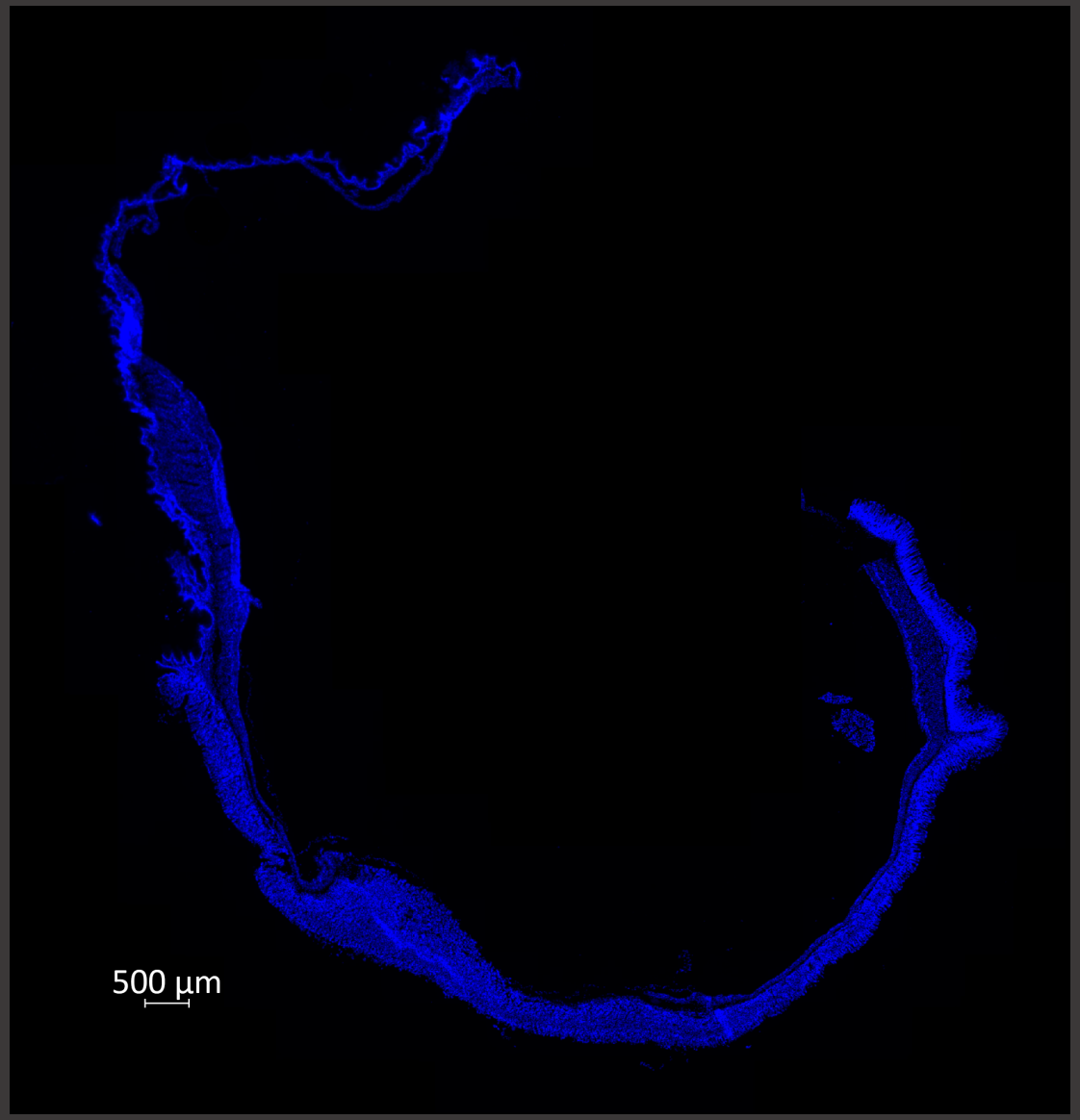


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Stomach

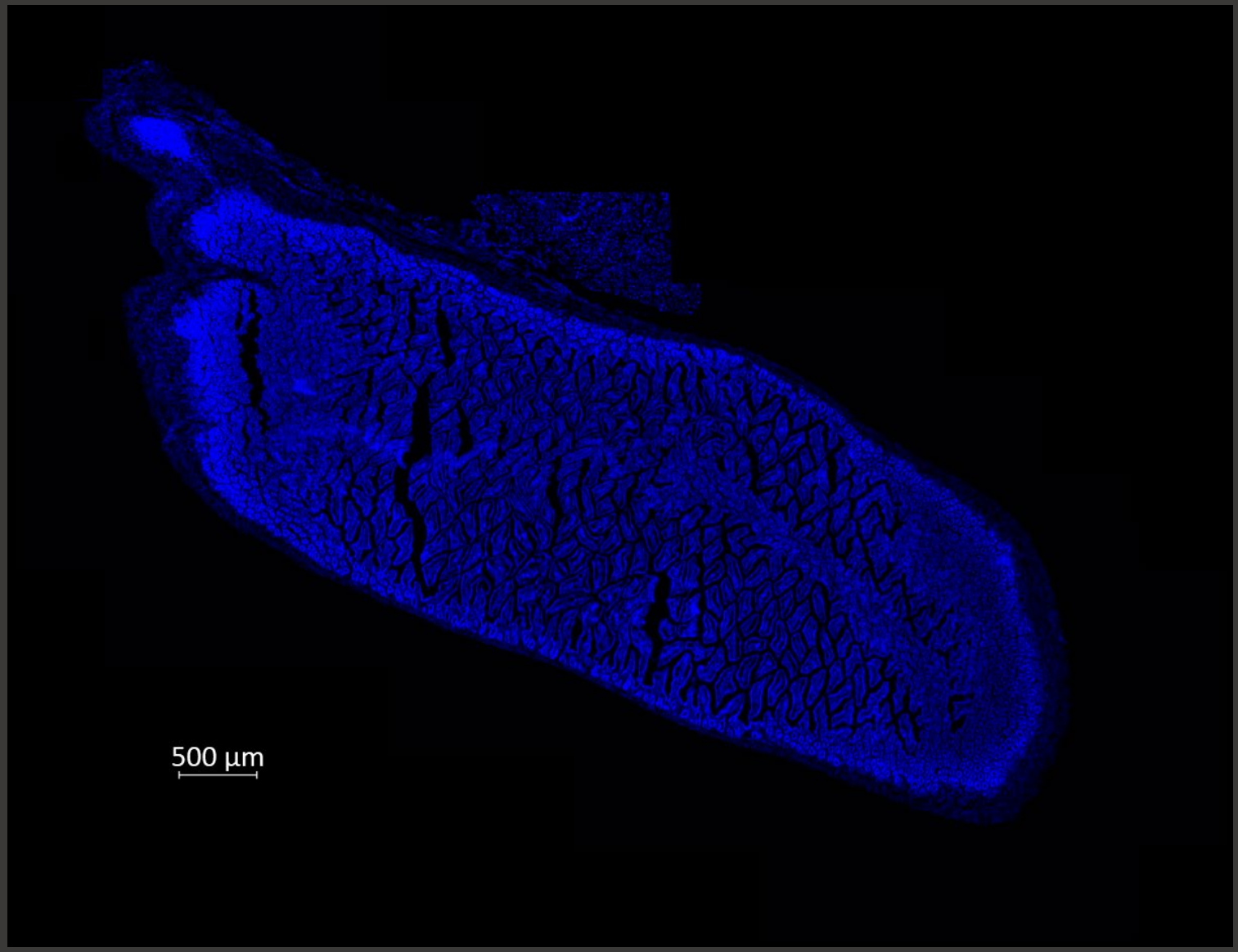


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Small Intestine (Duodenum)

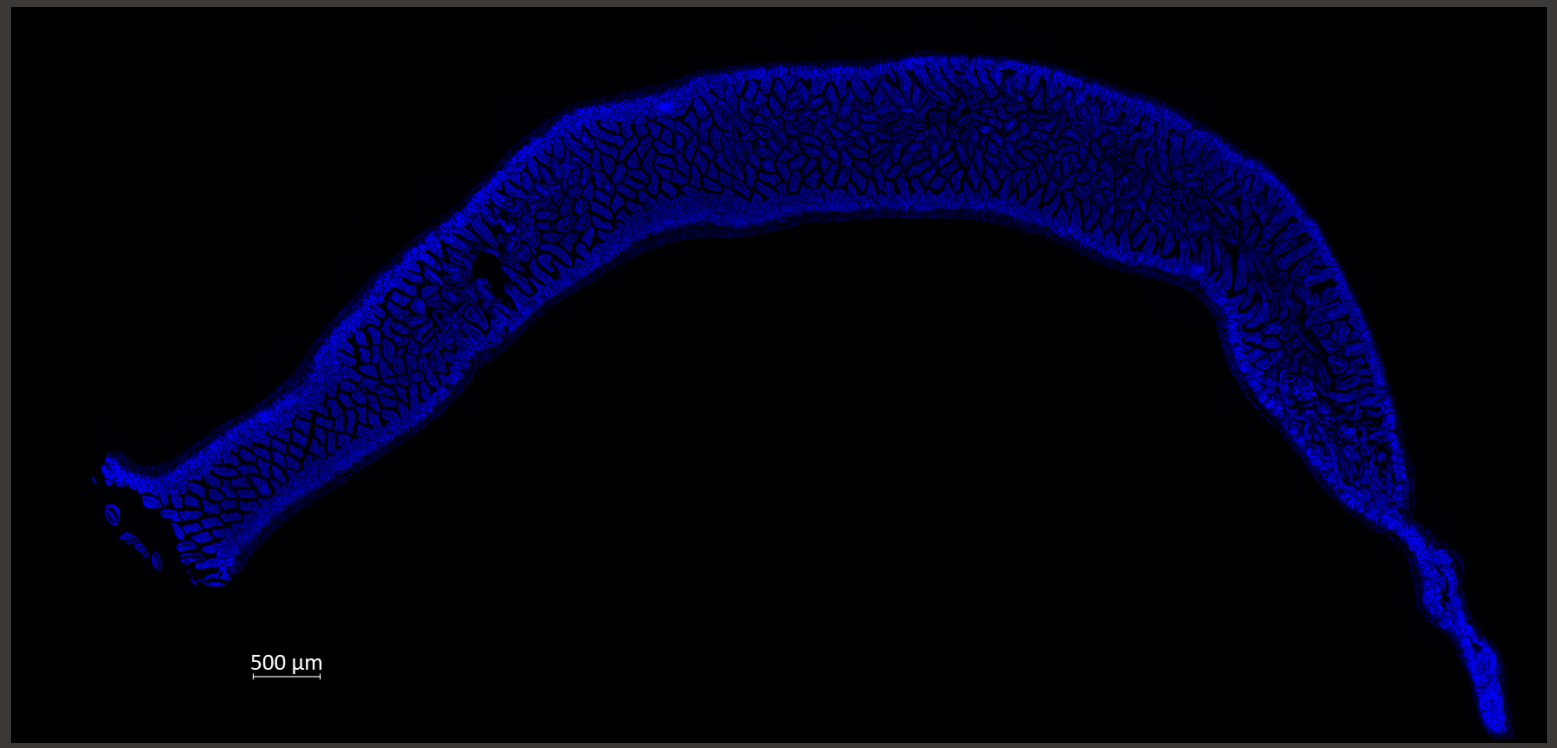


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Small Intestine (Jejunum)



Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

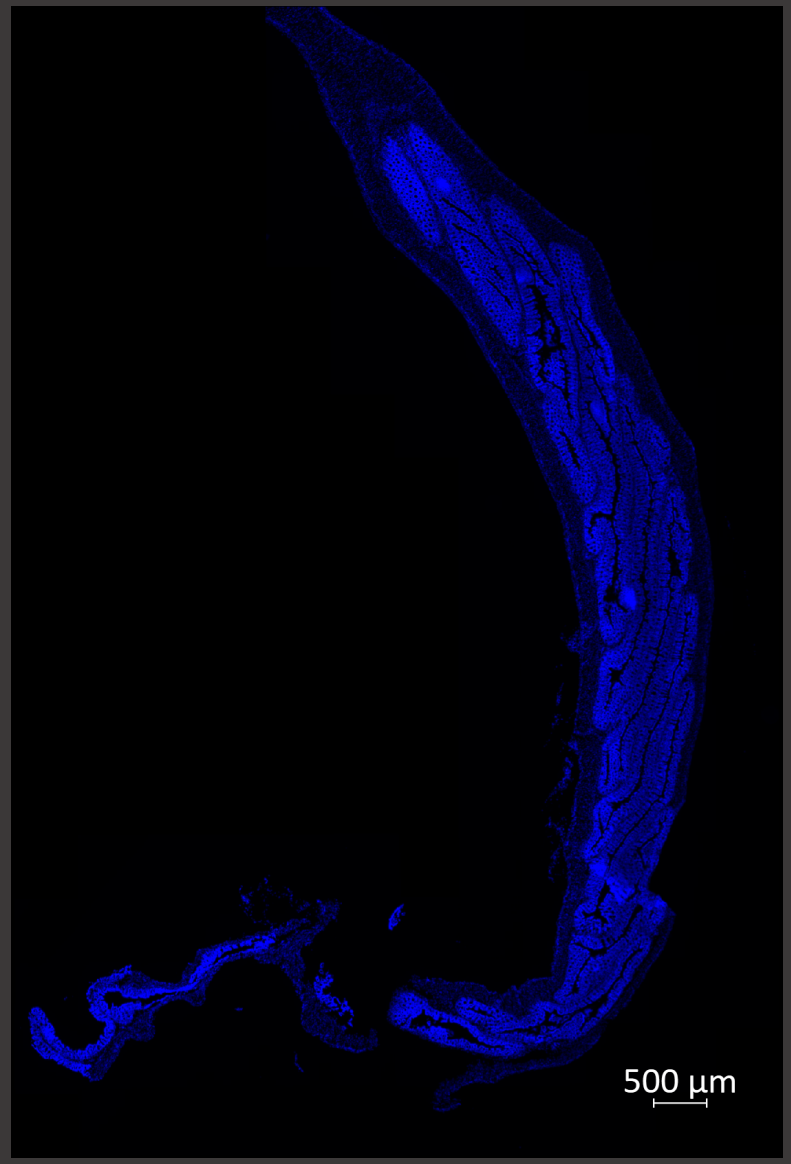
Small Intestine (Ileum)

Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Large Intestine

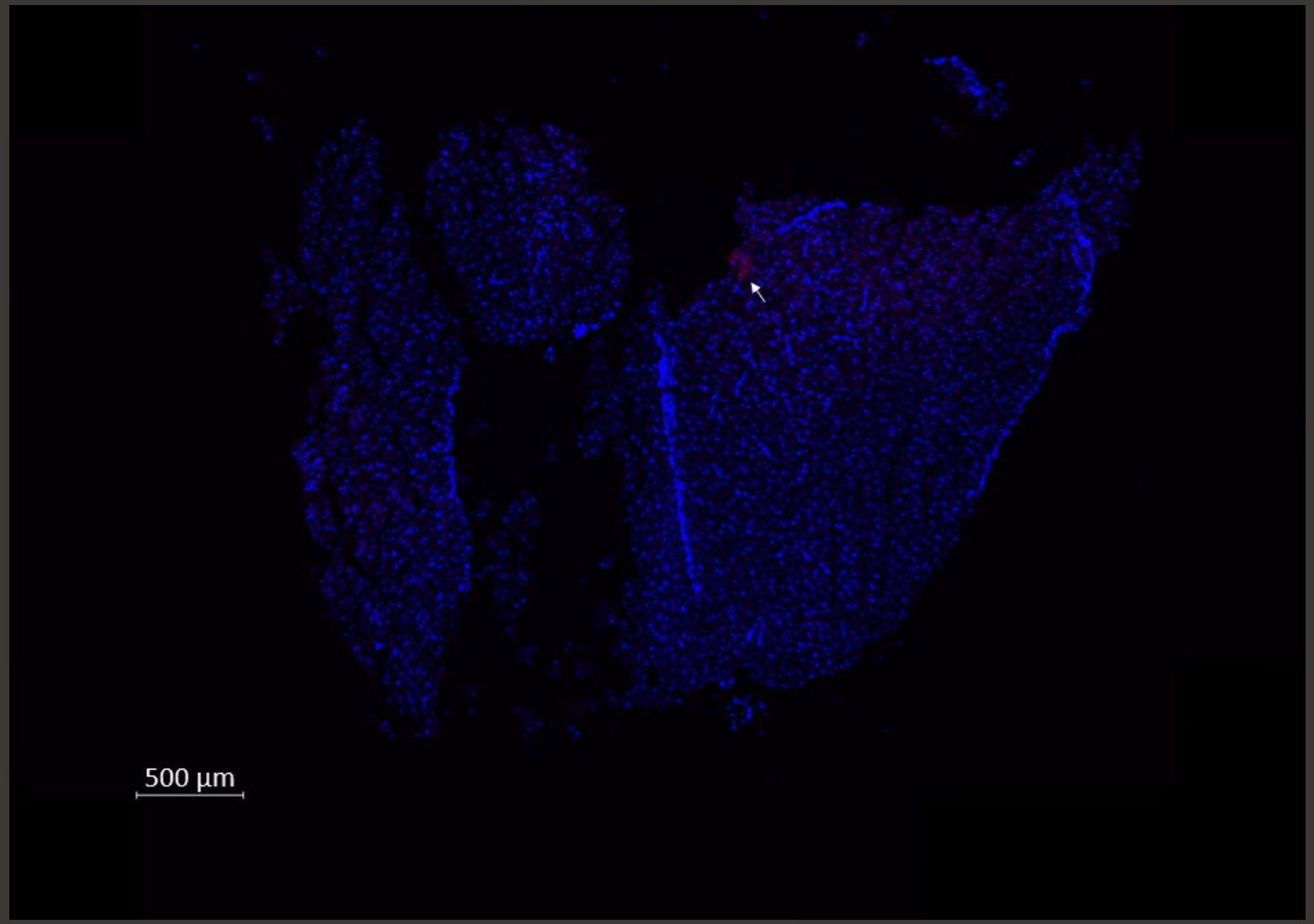


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Muscle (Gastrocnemius)

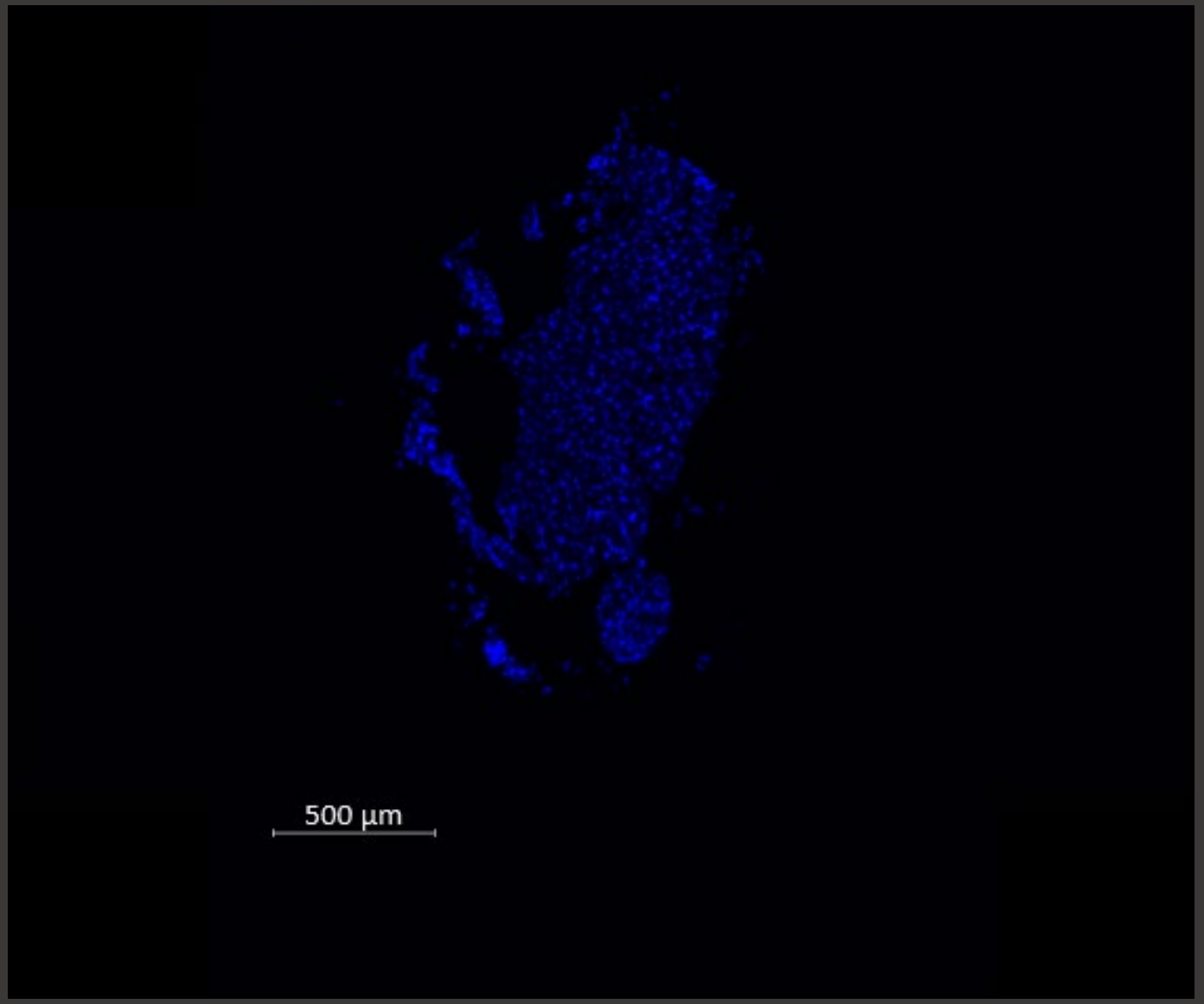


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Muscle (Soleus)

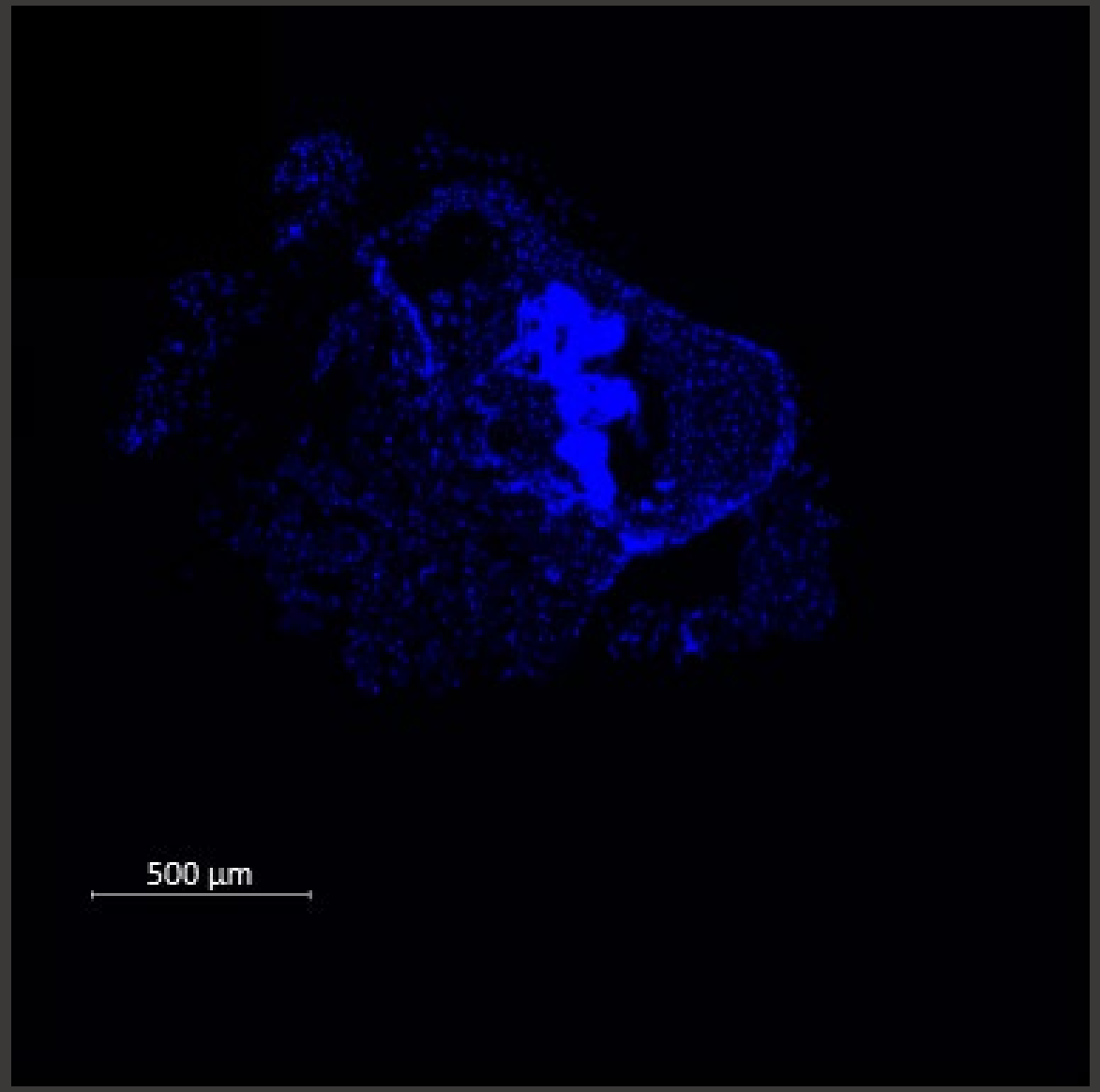


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Muscle (TA)

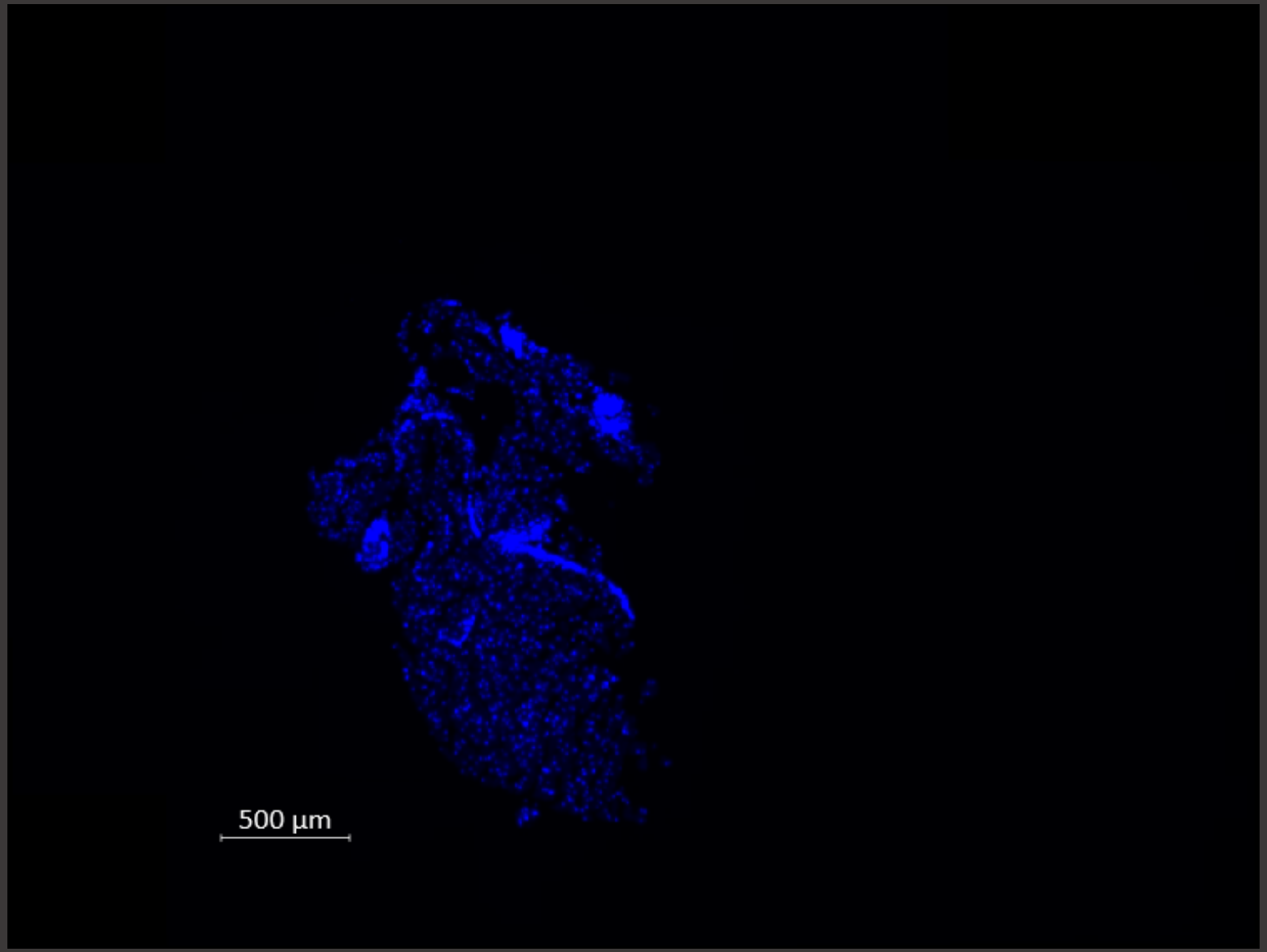


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Muscle (EDL)

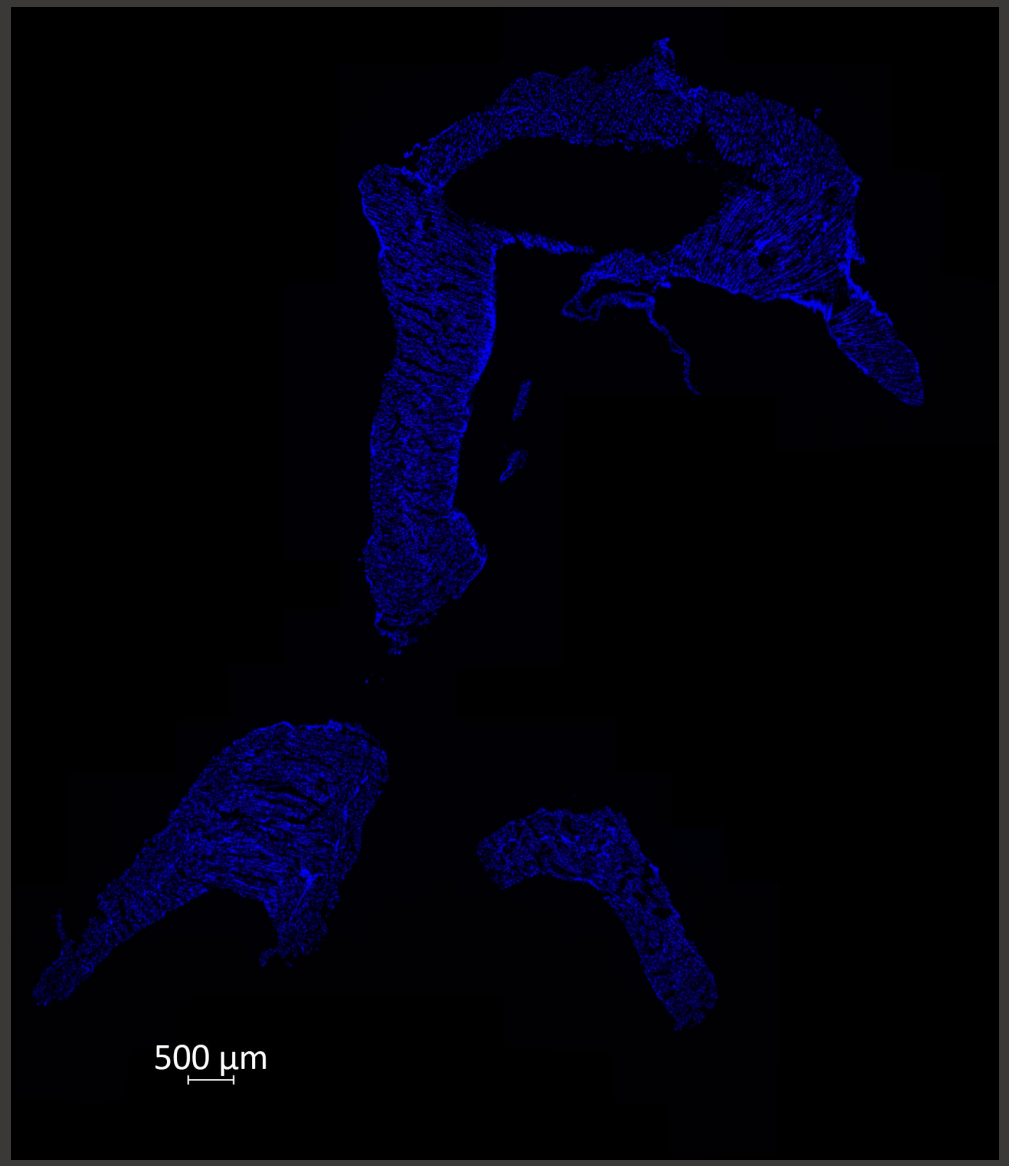


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Muscle (Diaphragm)

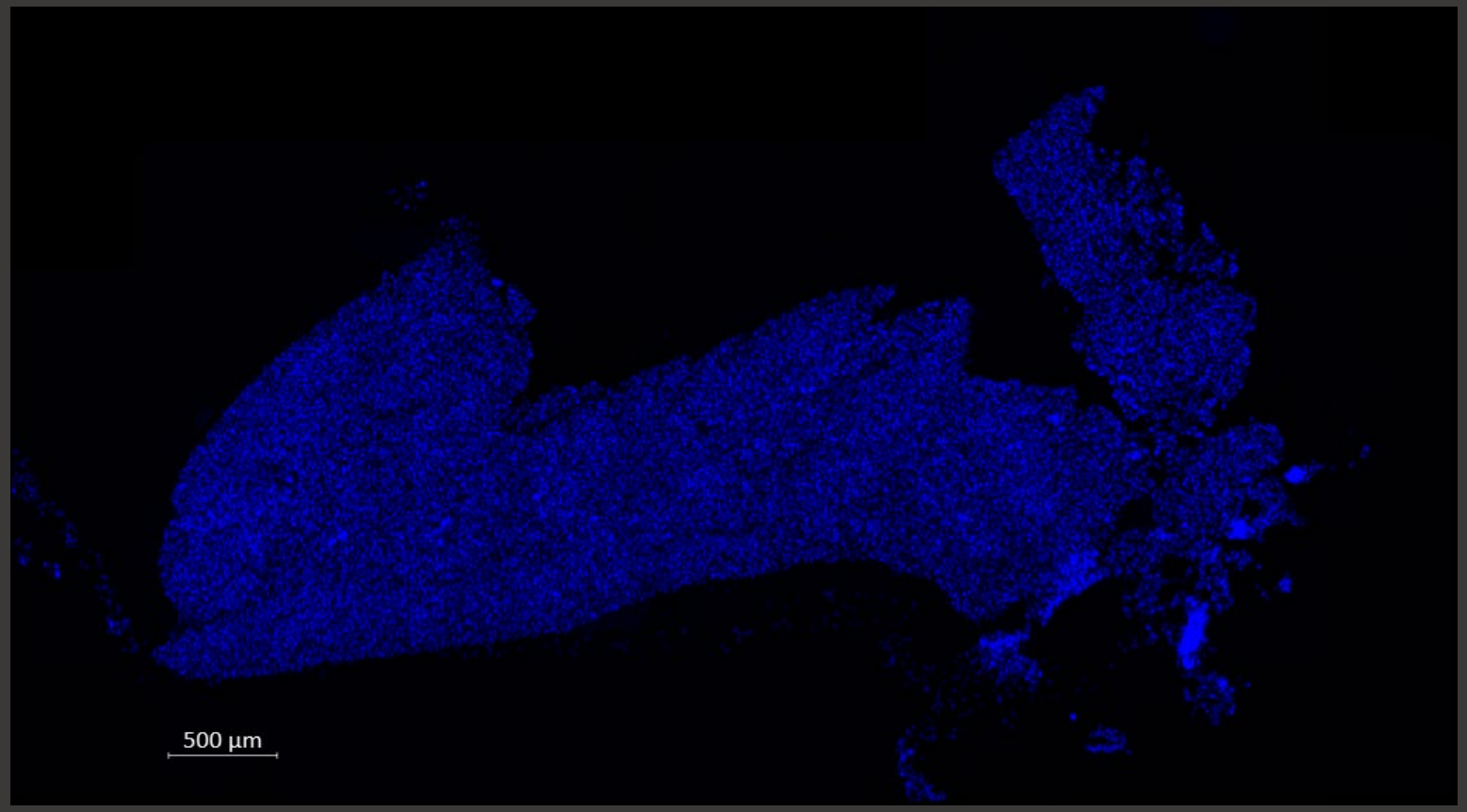


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Brown Adipose Tissue

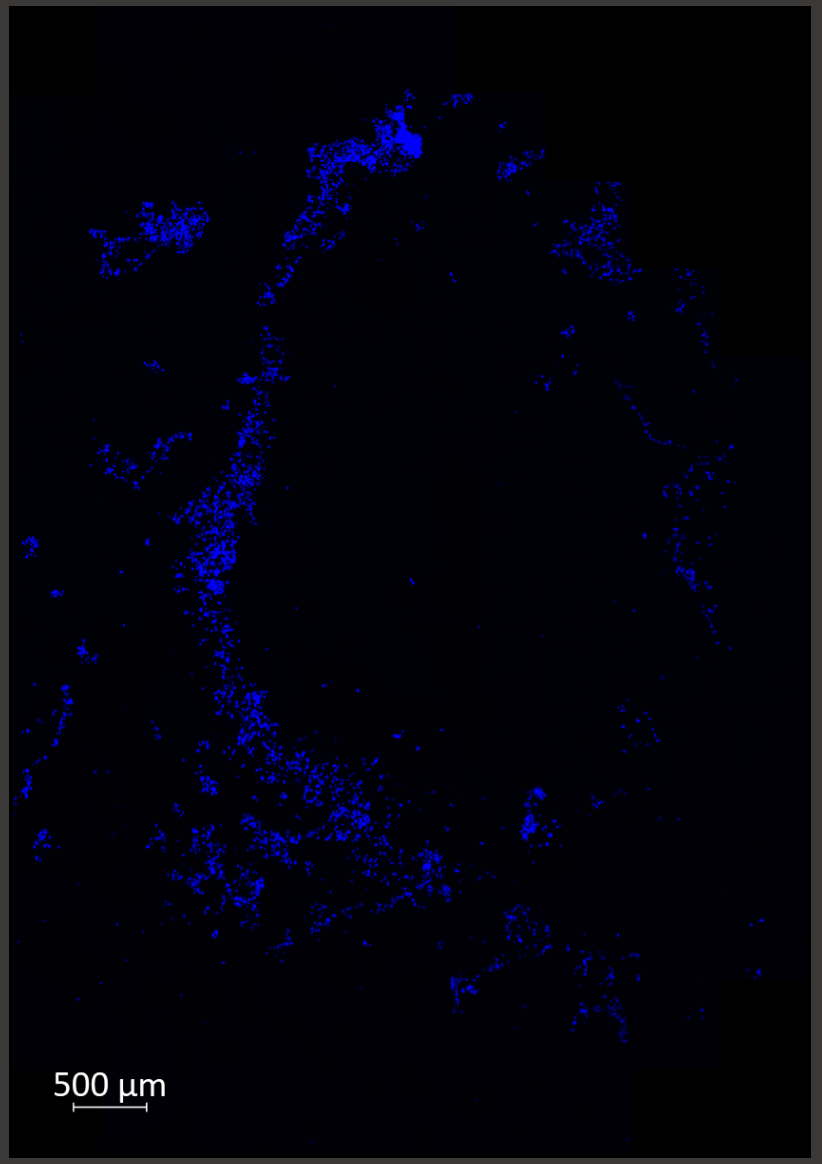


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

White Adipose (Perigonadal)

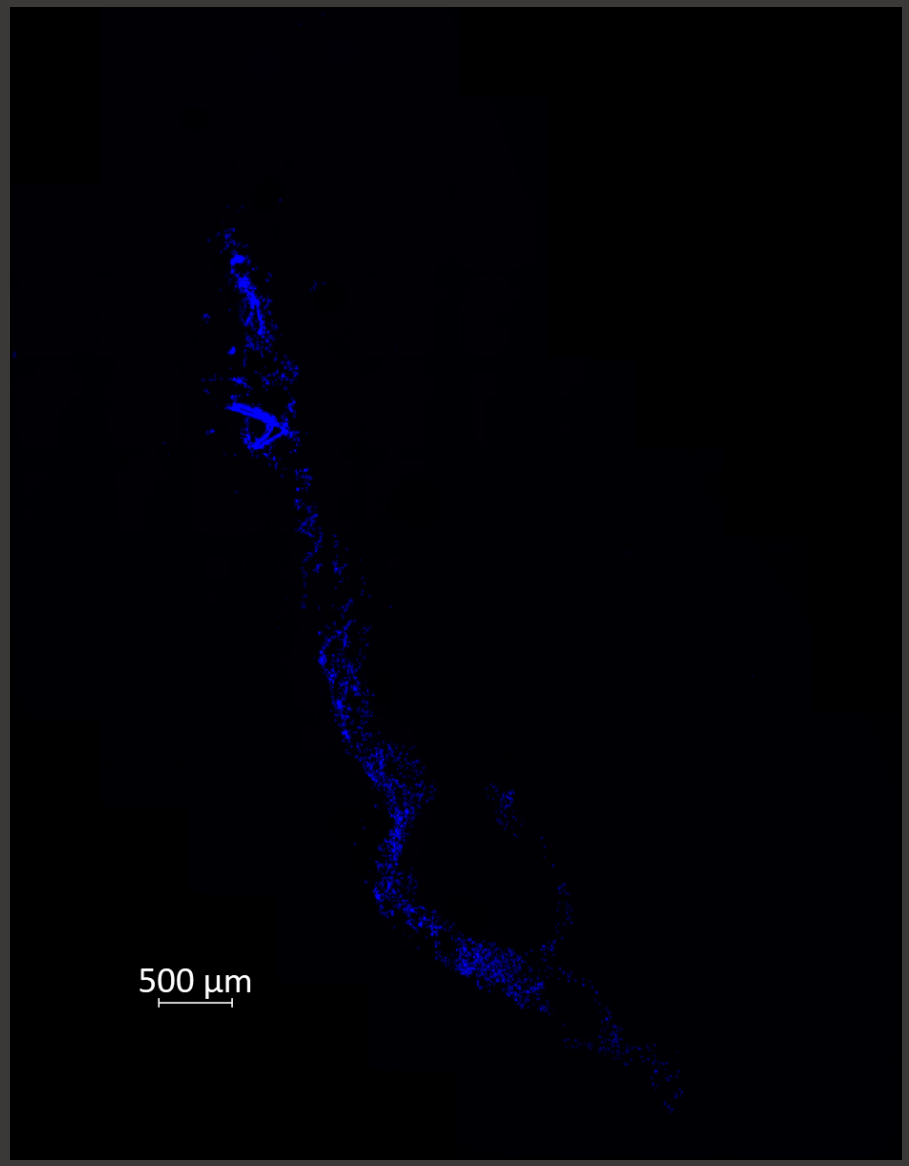


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

White Adipose (Subcutaneous)

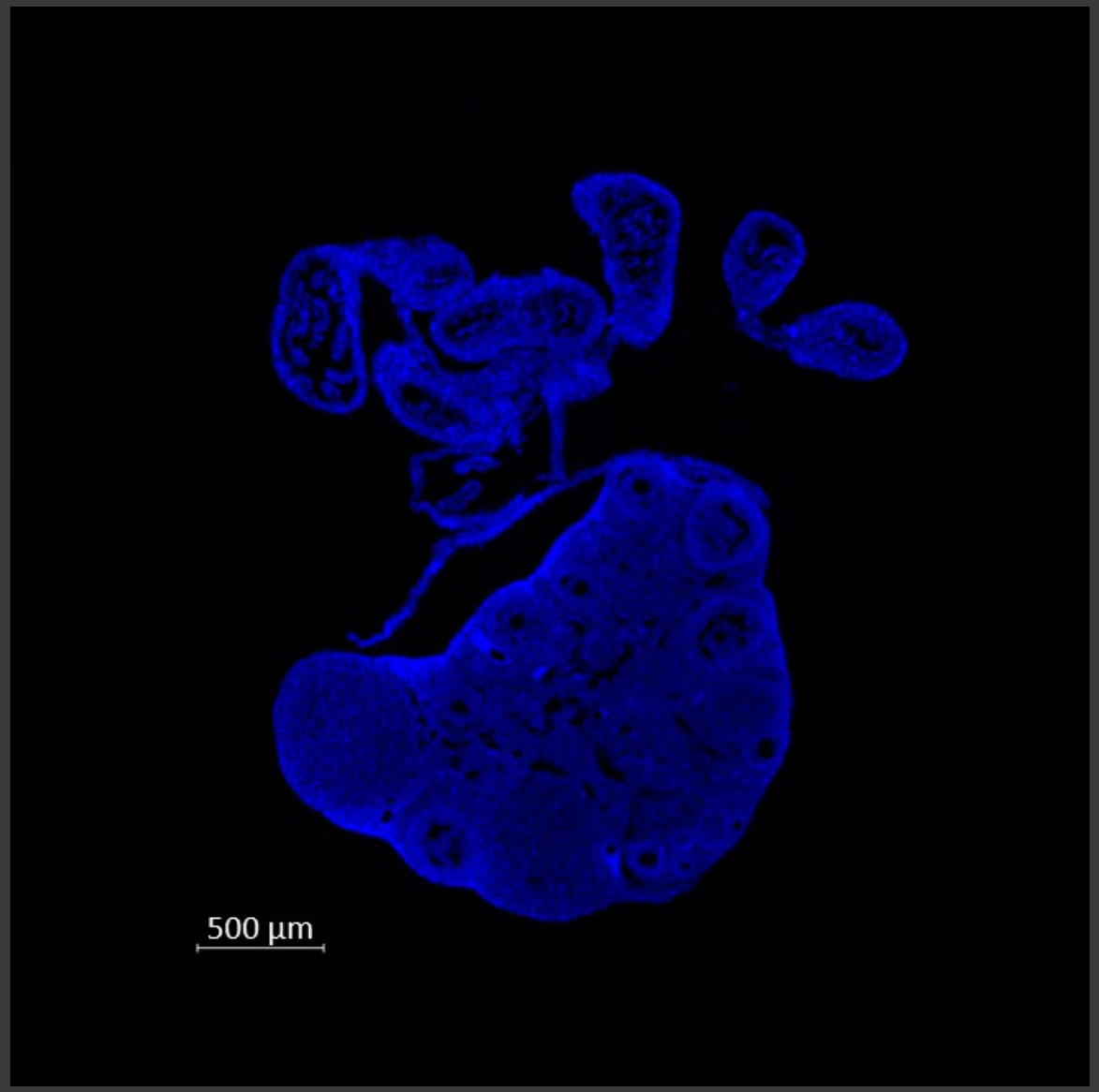


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Ovary

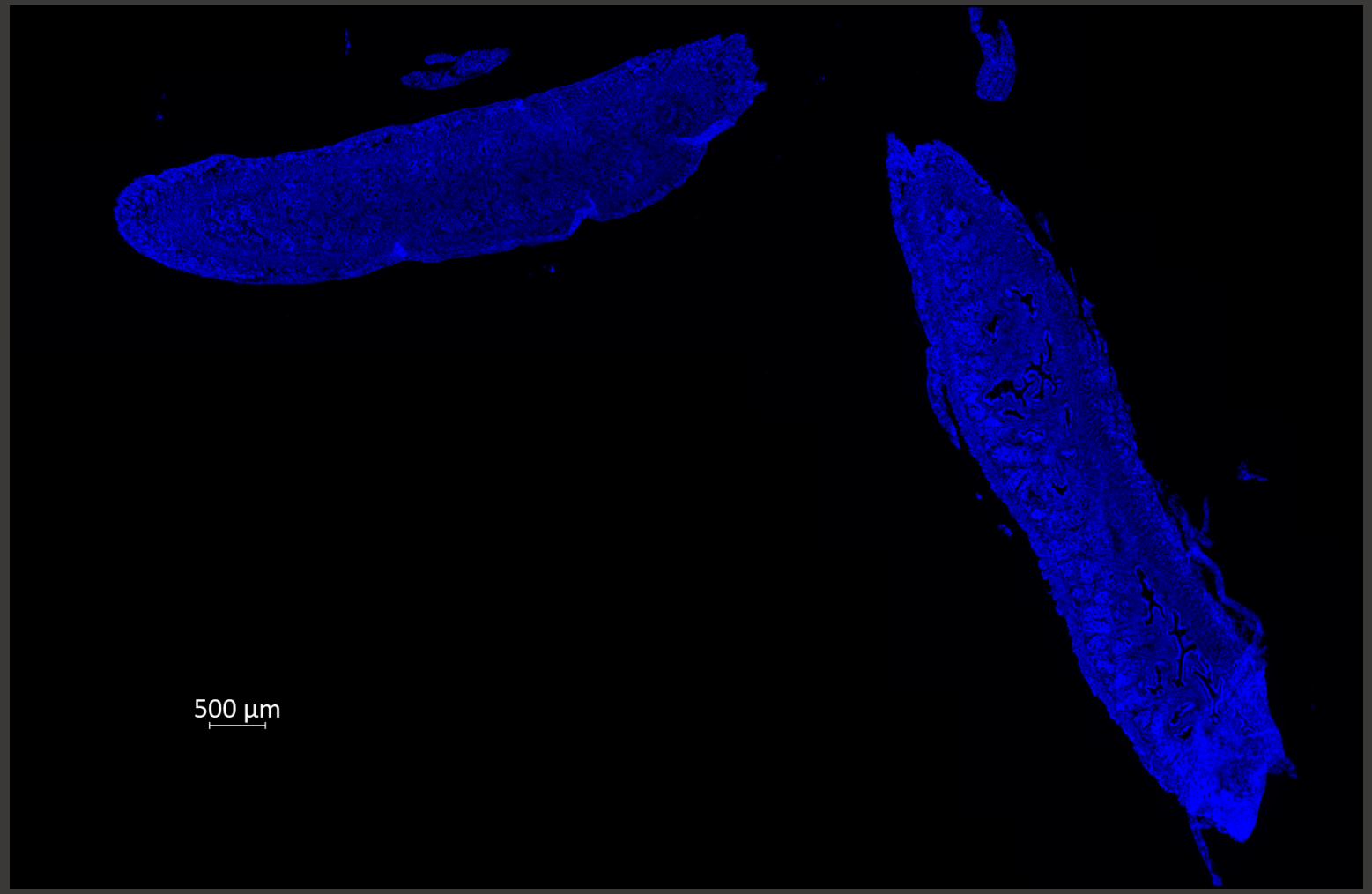


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

3305 F

Uterus

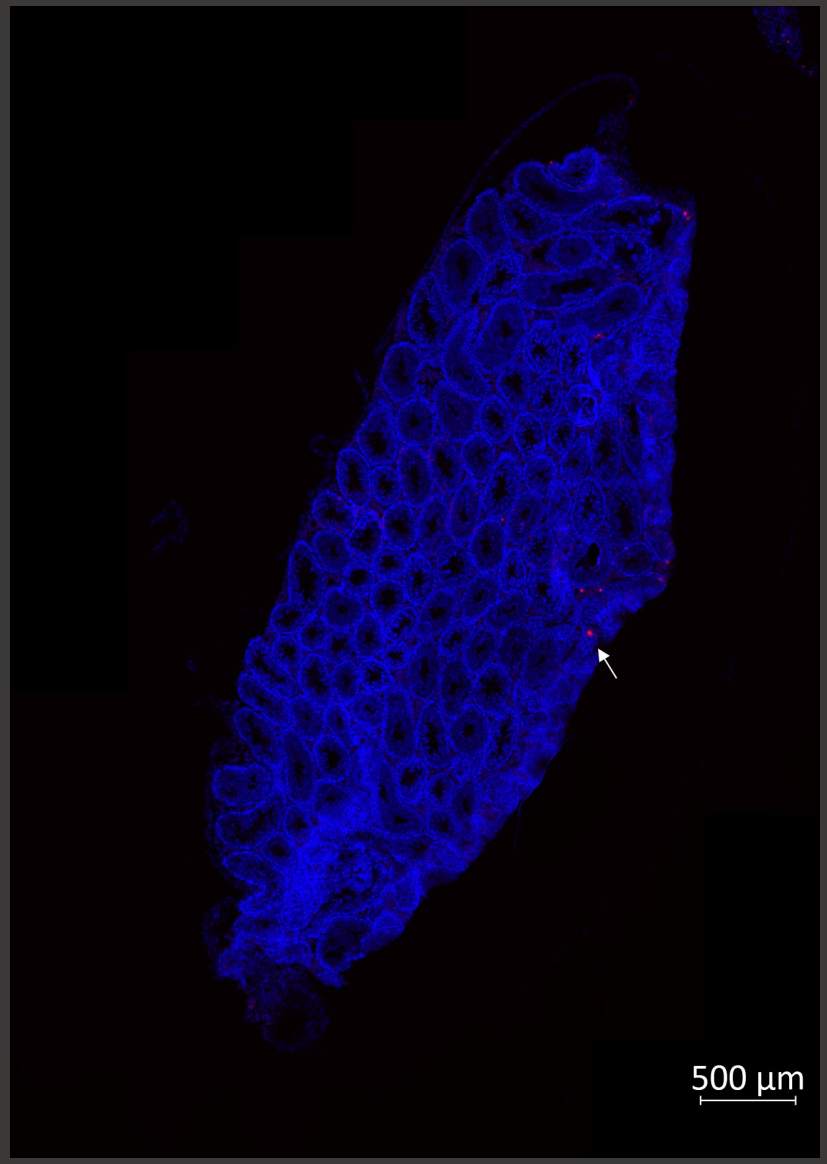


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

2857 M

Testes

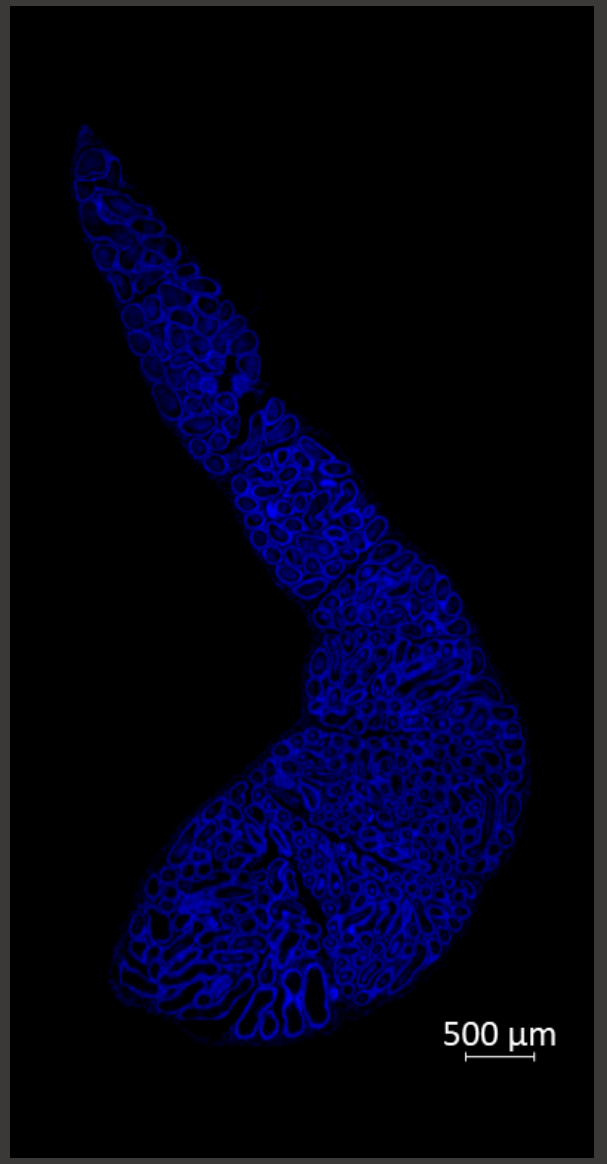


Non-target tissues

Study Arm 2
Experimental
AAV5-SpCas9

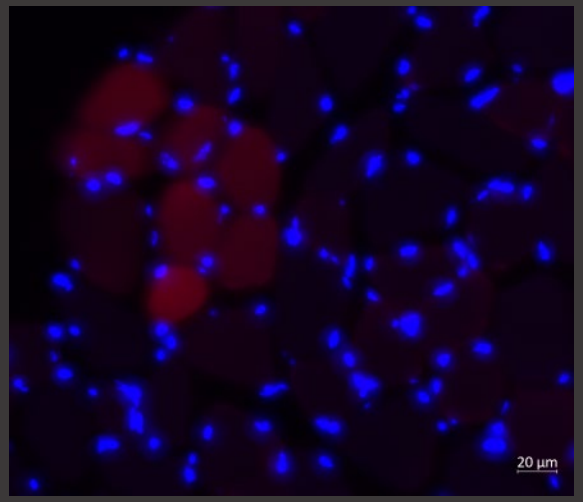
2857 M

Epididymis



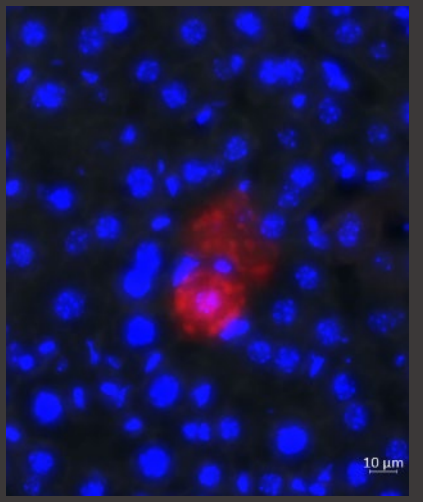
Zoomed images of red cells in non-target tissues

Gastroc



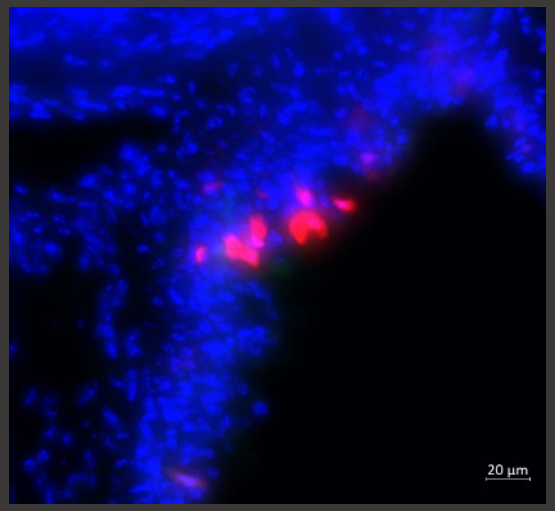
3305 F

Liver



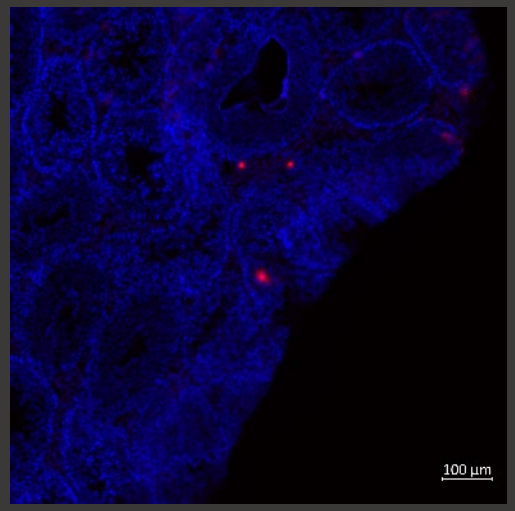
3305 F

Trachea



3305 F

Testes



2857 M

Conclusion: Extremely rare red cells are detectable in non-target tissues