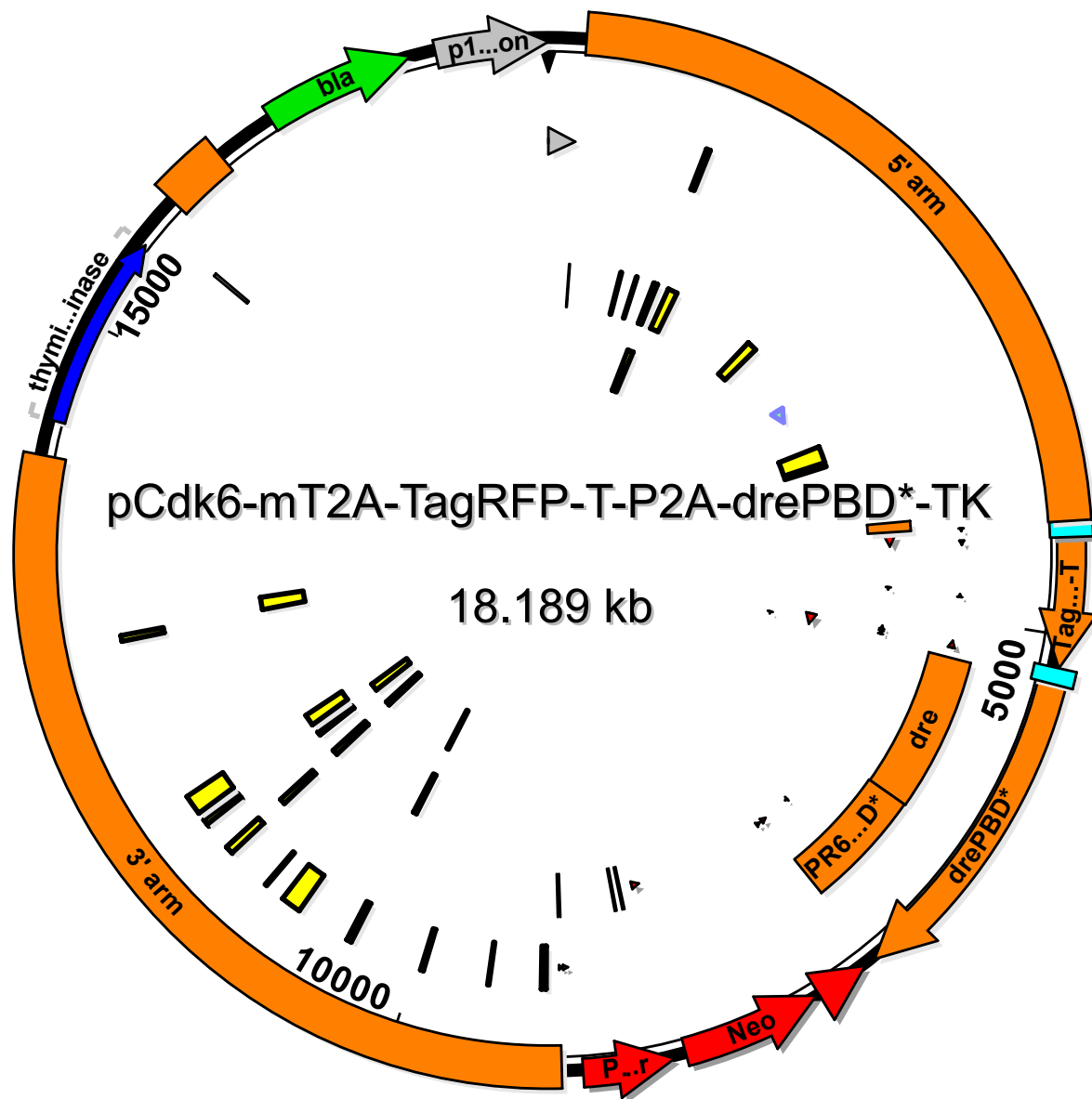
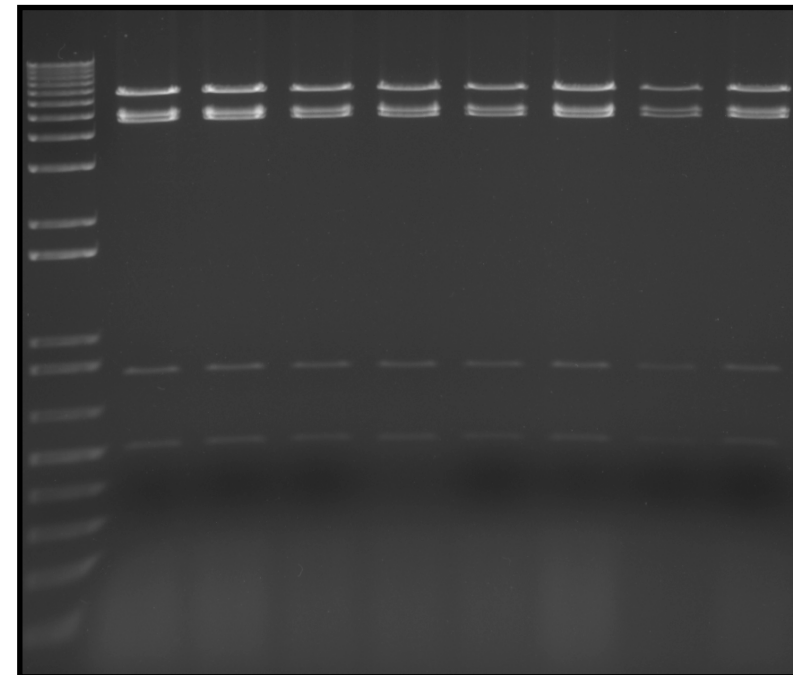


*Cdk6-T2A-TagRFP-T-drePBD** allele targeting vector construction

Constructed by recombineering

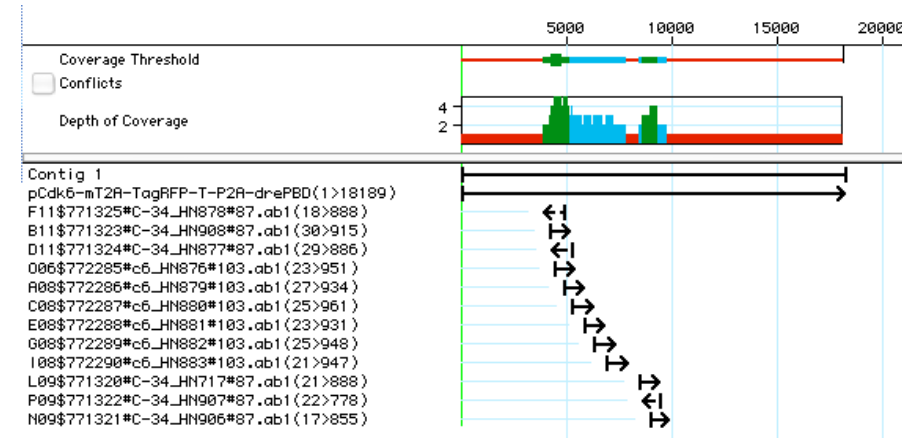


Restriction enzyme digests



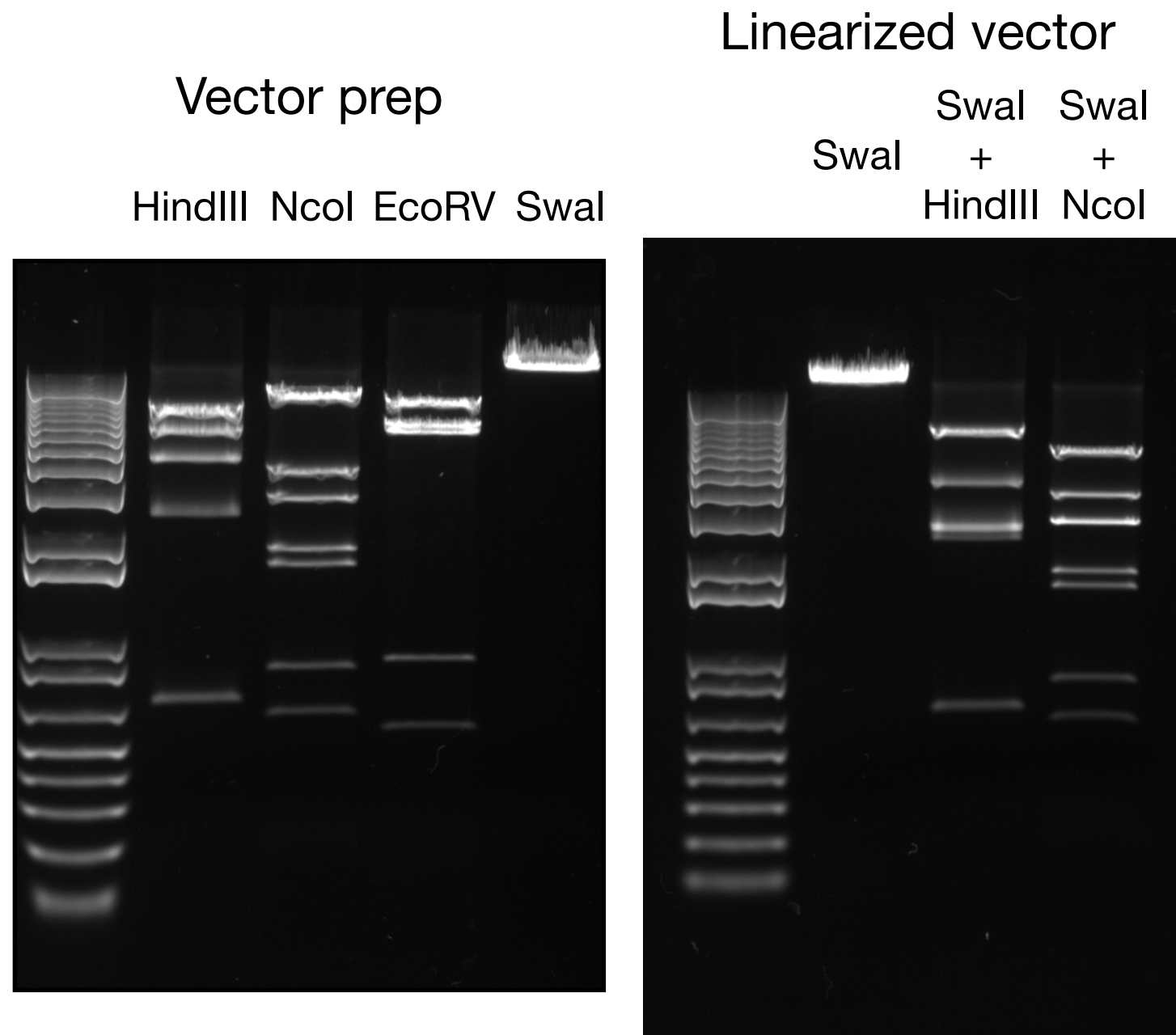
Crude plasmid DNA prep
(no column)

Sanger sequencing



See <https://www.addgene.org/browse/article/28215683/> for reagents

*Cdk6-T2A-TagRFP-T-drePBD** allele targeting vector prep for electroporation



*Cdk6-T2A-TagRFP-T-drePBD** allele targeting vector ES cell electroporation Southern blots

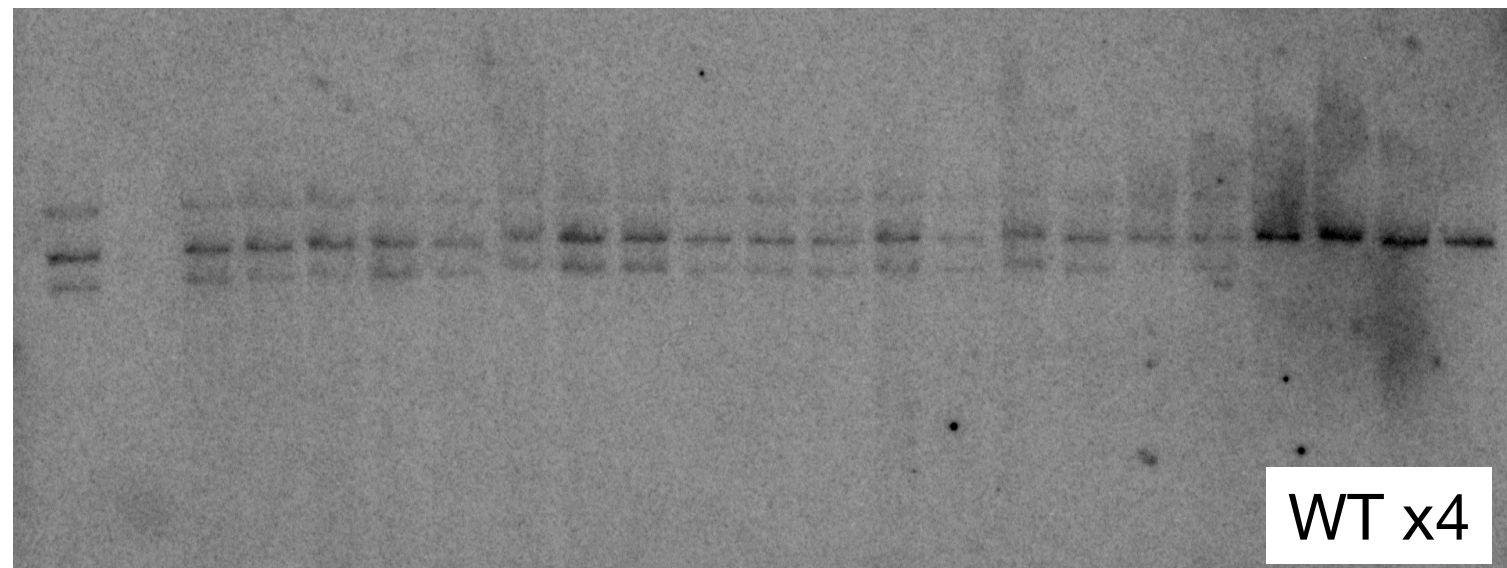
19 positive clones out of 72 clones screened (~26%)

Confirmation Southern blots of the positive clones

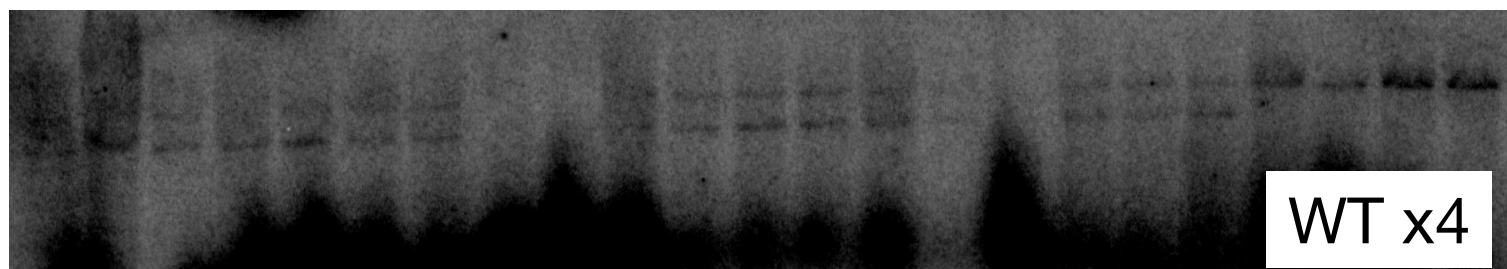
Hybridization buffer dried out in the first attempt

Blot membranes stripped and re-probed

5' blot, HpaI + AscI digested



3' blot, SpeI digested



*Cdk6-T2A-TagRFP-T-drePBD** allele ESC chimeras

Injected 2 positive ESC clones into blastocysts and bred the chimeras

Clone #92

Males

6 – 100%

2 – 90%

2 – 70%

1 – 60%

1 – 40%

2 – 30%

Females

1 – 35%

1 – 20%

Agouti pups

Clone #95

Males

1 – 30%

1 – 25%

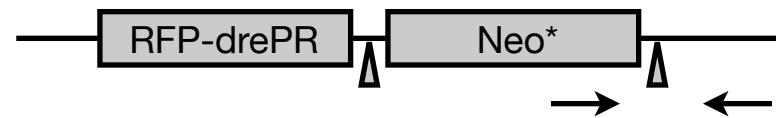
Females

1 – 20%

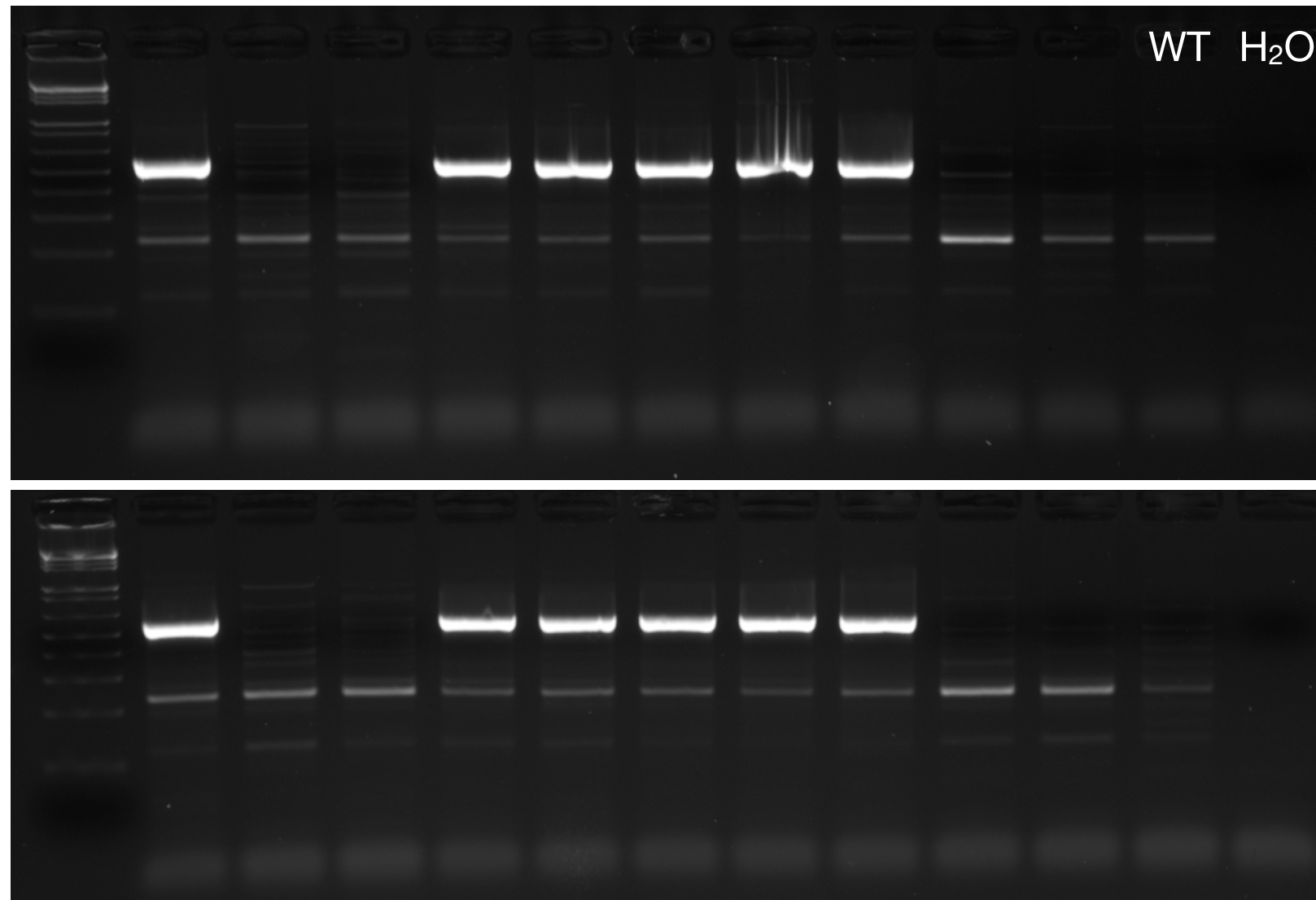
1 – 15%

Black pups only

PCR genotyping to check for germline transmission



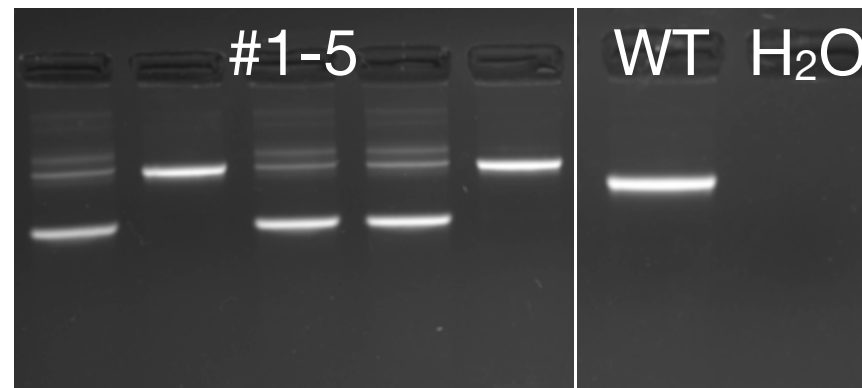
Pups from cross of clone #92 chimera x B6J female
Duplicate reactions



Germline transmission

FRT Neo^{*} FRT loop out

Multiplex PCR

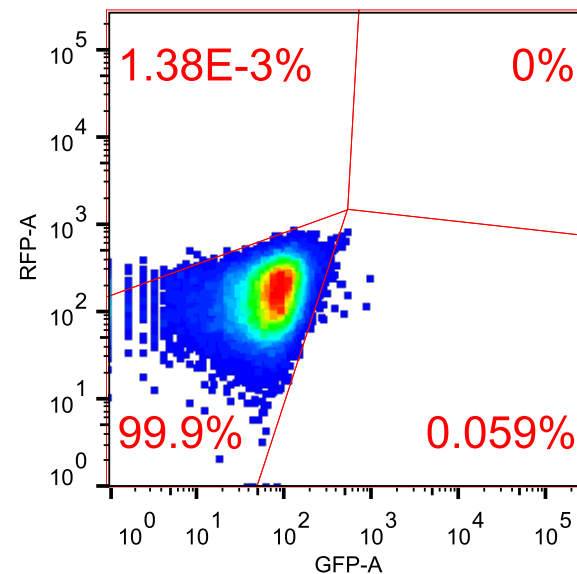


Test of drePBD* activity in mouse ES cells

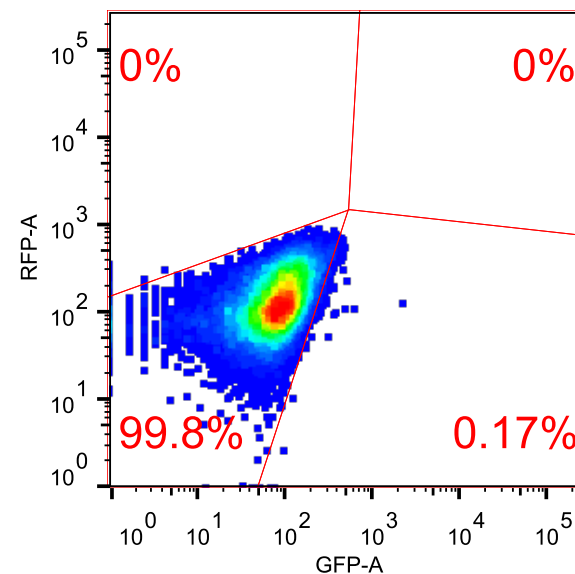
Cdk6-RFP-drePR/+ ESC clone #95 electroporated
20 μ g of GFP dre reporter plasmid and ± 0.5 μ M of
RU-486 for 1 day

\pm RU-486
Day 0 1 2
EP Flow

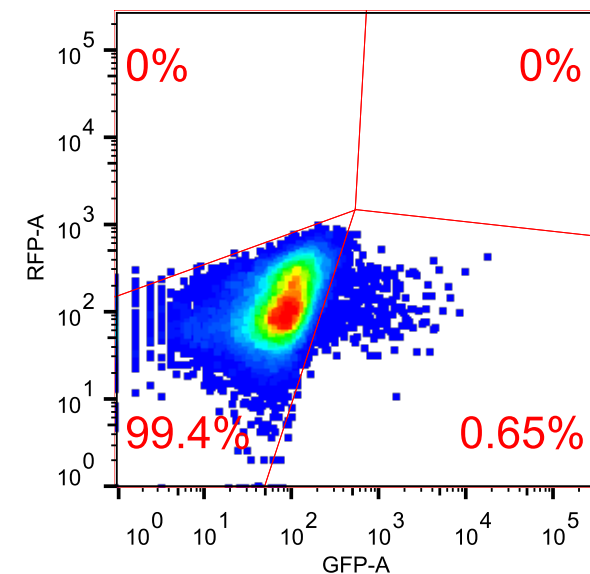
Wildtype ESC



EP #1 -RU-486

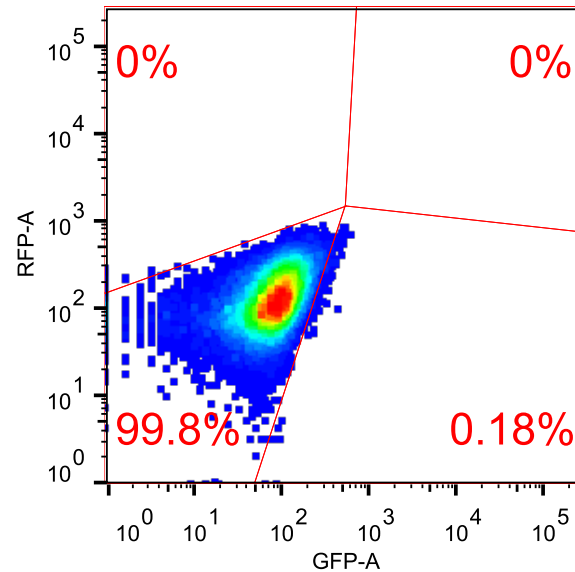


+RU-486

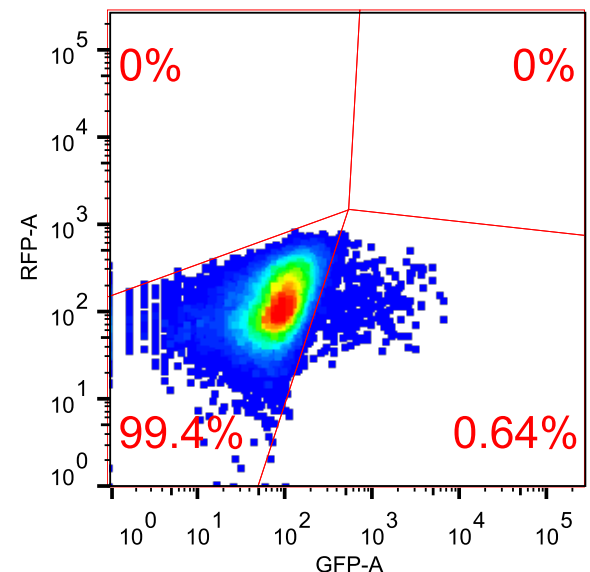


Cdk6-RFP-drePR/+

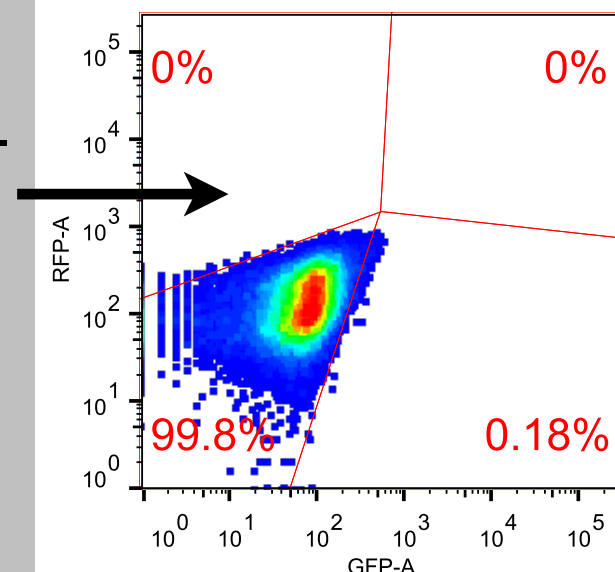
EP #2 -RU-486



+RU-486



No
CDK6-
RFP+
cells



drePBD* seemed to work, not very leaky

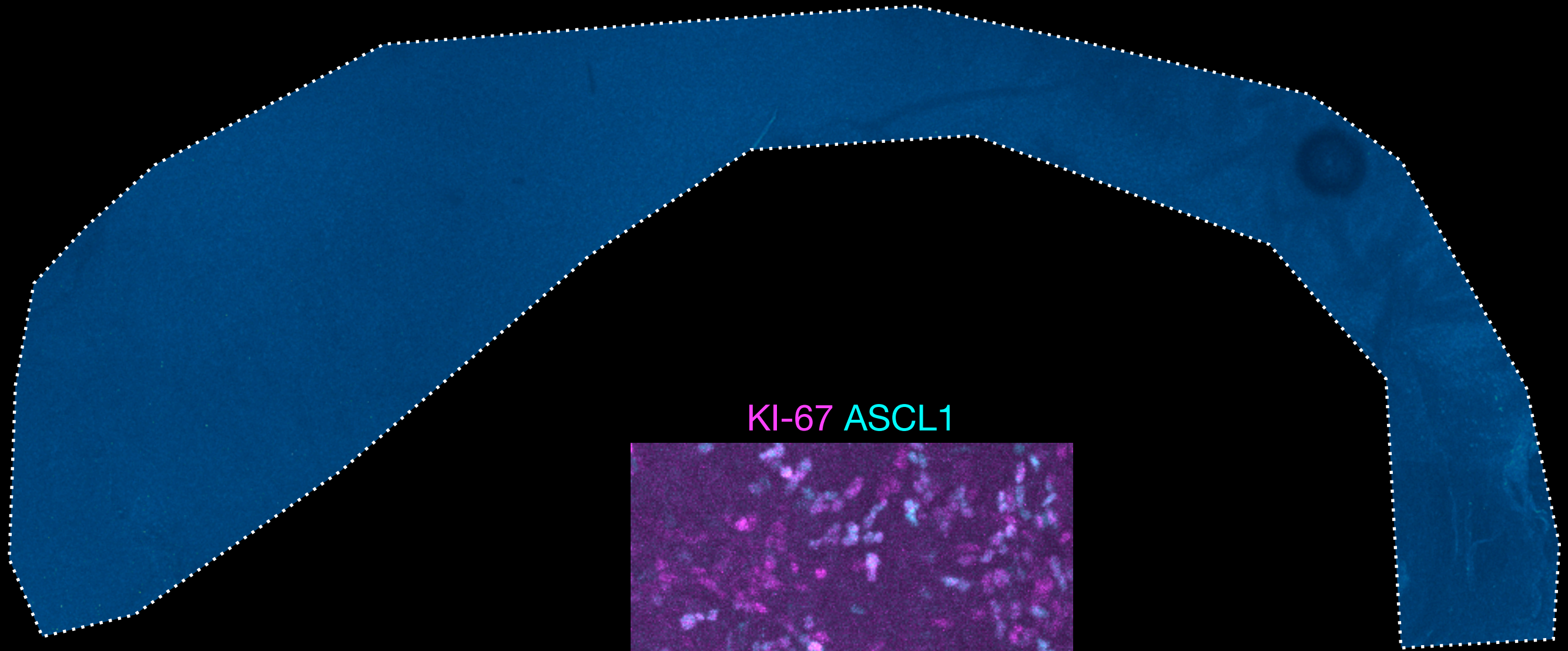
- Bred *Cdk6-T2A-TagRFP-T-drePBD^{*/+}; Hipp11-dre reporter td-sfGFP/+*
- Both lines backcrossed separately to the B6J background
- RU-486 - didn't solubilize at 40 mg/ml in corn oil/EtOH, solubilized at 20 mg/ml, less soluble than tamoxifen in the same vehicle
- Injected intraperitoneally 70 μ l of the vehicle or the 20 mg/ml formulation (~24 g male mouse \rightarrow ~58.3 mg/kg - a low dose)
- Perfused at day 3 after the injection

Cdk6^{T2A-drePBD⁺/+}; *Hipp1*^{dre reporter/+}, 10 week old mouse

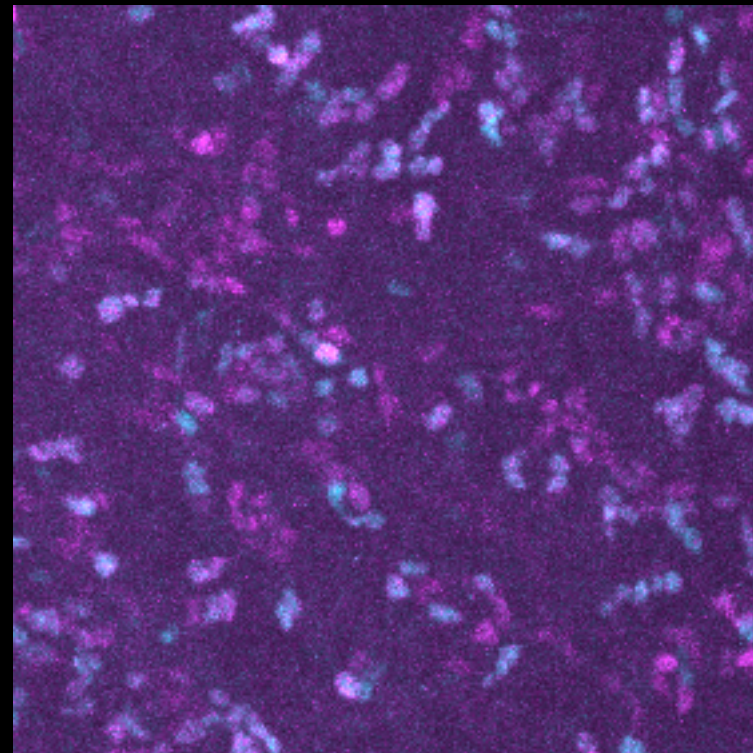
Vehicle 1x i.p. injection

Whole mount immunofluorescence 3 days after

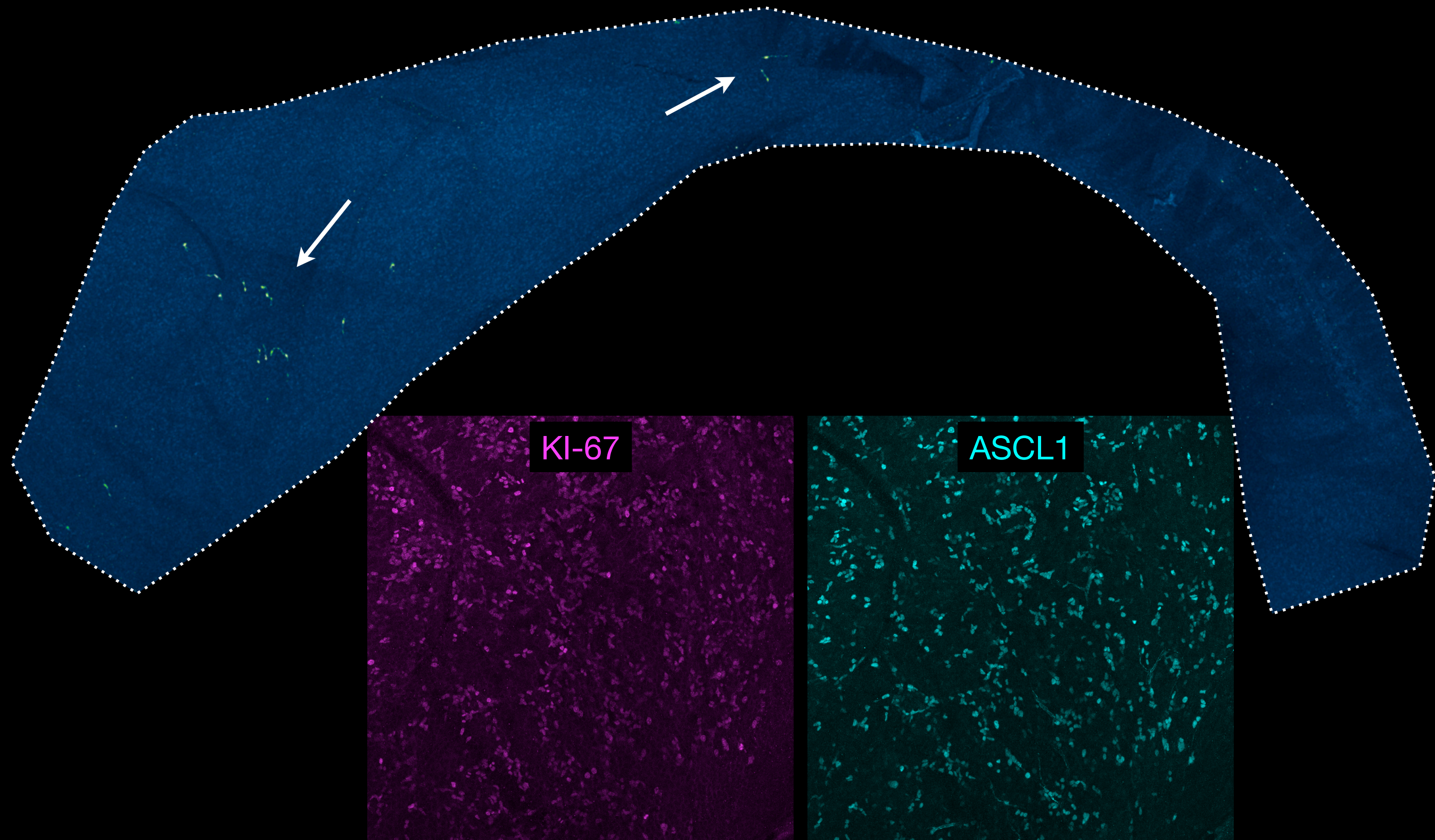
td-sfGFP



KI-67 ASCL1



Cdk6^{T2A-drePBD⁺/+}; *Hipp1*^{dre reporter/+}, 10 week old mouse
RU-486 1x i.p. injection ~58.3 mg/kg in ~70 ul volume (low dose)
Whole mount immunofluorescence 3 days after
td-sfGFP



td-sfGFP

GFP+ cells shown here are KI-67- and ASCL1- → probably neuroblasts not C cells
The anti-GFP antibody is compatible with the sfGFP variant used in this mouse line

td-sfGFP

GFP+ cells shown here are KI-67- and ASCL1- → probably neuroblasts not C cells
The anti-GFP antibody is compatible with the sfGFP variant used in this mouse line