Foxl2-GCE Allele Characterization

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Findings: VALIDATED

Our analysis confirms the expression of Foxl2 driven eGFPCreER^{T2} in a small population of cells located in the medulla of the ovary at 14.5-15.5 dpc. The labeled cells are likely granulosa cell precursors. Native GFP expression was not detectable in our samples but could be visualized by immunohistochemistry. Cre dependent R26R-LacZ expression was observed in a subset of GFP positive cells following Tamoxifen treatment.

Crosses

The Foxl2-GCE strain was generated by homologous recombination in ES cells. The expectation is that integration of the Foxl2-GCE DNA into the Forkhead box L2 domain disrupts the allele however we did not intercross heterozygotes to confirm the null phenotype. We crossed two Foxl2$^{GCE/+}$ males with Rosa26R$^{lacZ/+}$ (R26R) female mice and injected with Tamoxifen in order to test correct expression of eGFPCreER^{T2} in Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ embryos at 14.5 and 15.5dpc.

Genotyping

Tail samples of the embryos were collected and incubated in tail digestion buffer overnight at 55ºC. PCR was performed as per the protocol below and the PCR products were run on a 1.5% agarose gel (Fig 1).

Oligonucleotides: for Wt allele Size: 335 bp
DNA sequence (forward): 5'-AGAGAAGAGAGTGAGAGCCG -3'
DNA sequence (reverse 1) 5'-GAGCGCCACGTACGAGTACG -3'

Oligonucleotides: for targeted/transgenic allele Size: 221 bp
DNA sequence (forward): 5'-AGAGAAGAGAGTGAGAGCCG-3'
DNA sequence (reverse 2) 5'-GTCCAGCTCGACCAGGATGG-3'
Amplifies 5' arm into GFP sequence within GFP-Cre region.

Rxn Buffer and Conditions: (25µl reaction)

10X GSB	2.5ul
25mM dNTP	1ul
10uM primer F	1ul
10uM primer R1	1ul
10uM primer R2	1ul
DMSO	2.5ul
2-mercaptoethanol	0.125ul
Amplify Taq	0.3ul (5u/ul)
5x cresol red dye	5ul
Genomic DNA	1ul

94ºC	3min	1 cycle
94ºC	30sec	35cycles
56ºC	30sec	
72ºC	45sec	
72ºC	10min	1 cycle

10X Gitschier Buffer (GSB):
670 mMTris, pH 8.8
166 mM Ammonium Sulfate
65 mM MgCl2
0.1% gelatin

Total volume **25 ul**

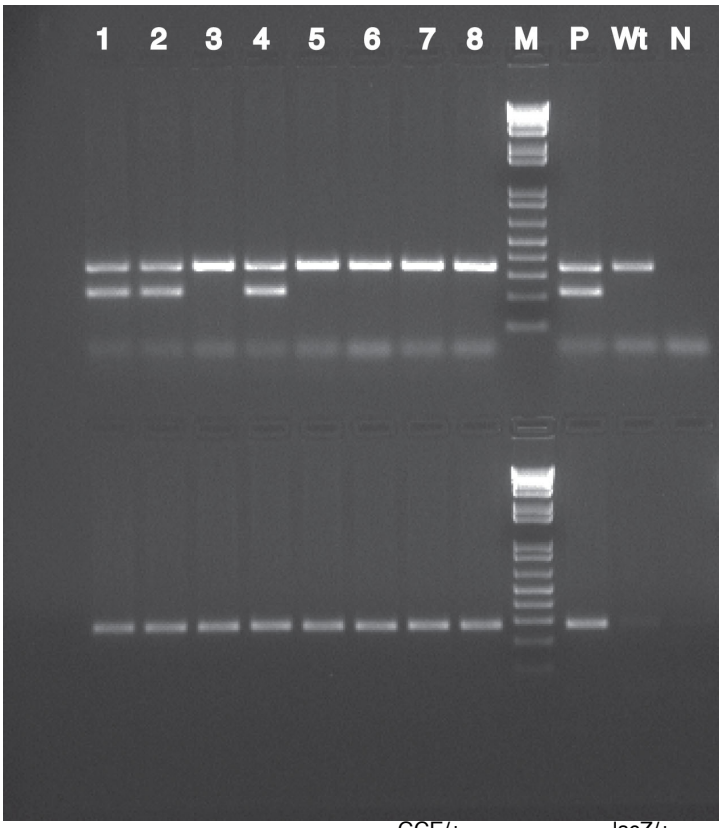

Fig1: Numbers 1, 2, 4 Foxl2$^{GCE/+}$, Rosa26R$^{lacZ/+}$, numbers 3, 5-8 Rosa26R$^{lacZ/+}$, **P:** Foxl2$^{GCE/+}$ positive control; **W**: Wildtype control; **N**: Negative control.

Data:

Native Fluorescence

Whole embryos as well as dissected UGS samples were examined with a fluorescent microscope to view GFP expression. However, GFP was not detectable under these conditions.

Cre-recombinase Activity

Foxl2$^{GCE/+}$ males were mated to Rosa26R$^{lacZ/+}$ (R26R) female mice to generate Foxl2$^{GCE/+}$; Rosa26R$^{lacZ/+}$ embryos. In order to activate ß-galactosidase (β-gal) reporter expression from the R26R$^{lacZ/+}$allele, an intraperitoneal injection of Tamoxifen in corn oil was either: injected into pregnant female mice at 11.5 and 13.5dpc (1mg to 40g body weight) collecting the UGS at 15.5dpc or injected into pregnant female mice at 12.5 (2mg to 40g body) with collections at 14.5 and 15.5dpc. A control group was injected with the same volume of corn oil. Dissected UGS samples were stained with X-gal to assay for β-gal activity. Tamoxifen dependent Cre activity was detected in Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ samples in a cluster of cells in the medulla of the ovary (Fig. 2 and 3).

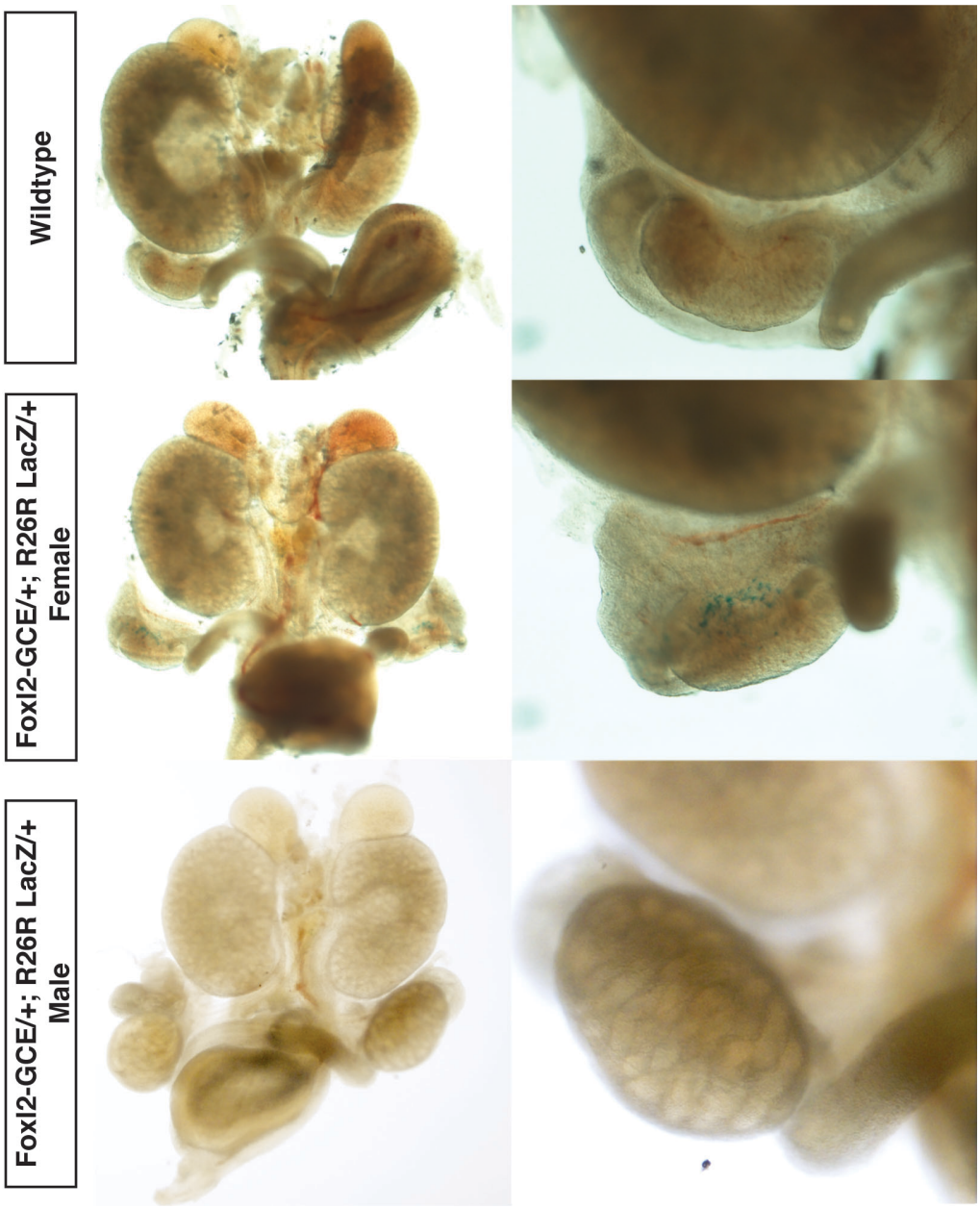

Fig 2. Cre-dependent β-gal activity in Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ UGS samples. A single dose of Tamoxifen (1mg/40g) results in a small number of X-gal positive cells in granulosa cell precursors located in the ovarian medulla of Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ embryos at 15.5dpc.

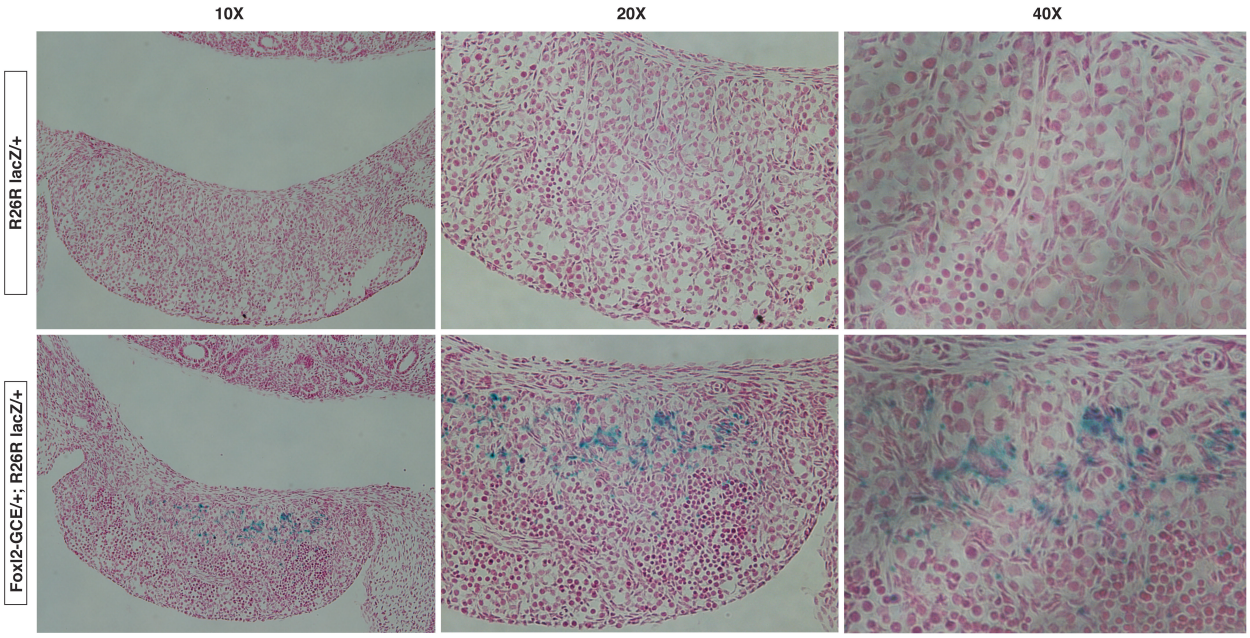

Fig 3. Cre-dependent β-gal activity in Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ UGS samples. A single dose of Tamoxifen (1mg/40g) results in a small number of X-gal positive cells in the medulla of the ovaries of Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ embryos at 15.5dpc

Immunohistochemistry

Immunohistochemistry was performed to examine if the eGFPCreER^{T2} allele was expressed in the expected Foxl2 domain. To test for Cre function, 14.5-15.5dpc Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ embryos from Tamoxifen and corn oil injected mice were assayed.

Whole UGS samples were fixed in 4% paraformaldehyde at 4ºC 2 hours, washed 3 times in PBS, equilibrated in 30% sucrose overnight, embedded in OCT and flash frozen on dry ice. The samples were sectioned at 16μm and probed with chicken-anti-GFP/ rat-anti-E-Cadherin/ rabbit-anti-Laminin and rabbit-anti-ß-gal/chicken-anti-GFP/ Rat-anti-E-Cadherin (Table 1).

Construct	Primary Antibody	Company	Catalog	Dilution	Secondary	Company	Dilution
Foxl2-GCE	Chicken-anti-GFP	Aves Labs, Inc	GFP-1020	1/500	Goat-anti-chicken-A488	Invitrogen	1/500
	Rabbit-anti-ß-gal	MP Biomedicals, LLC	55976	1/20,000	Donkey-anti-rabbit-A555	Invitrogen	1/500
	Rat-anti-e-Cadherin (DECMA-1)	Sigma	U3254	1/1000	Goat-anti-rat-A633	Invitrogen	1/250
	Rabbit anti-Laminin	Sigma	L9393	1/500	Donkey-anti-rabbit-A555	Invitrogen	1/500

Table 1. Summary of antibodies used to screen Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ 14.5 dpc embryo sections.

GFP was detected in $Foxl2^{GCE/+}$; $R26R^{lacZ/+}$ embryos in a cluster of somatic cells in the medulla of the ovary, distinct from E-Cadherin positive germ cells at 14.5dpc (Fig 4 and 5). Co-localization of a small number of GFP and ß-gal positive cells were detected upon Tamoxifen induction in granulosa precursor cells in the developing ovary (Fig 6).

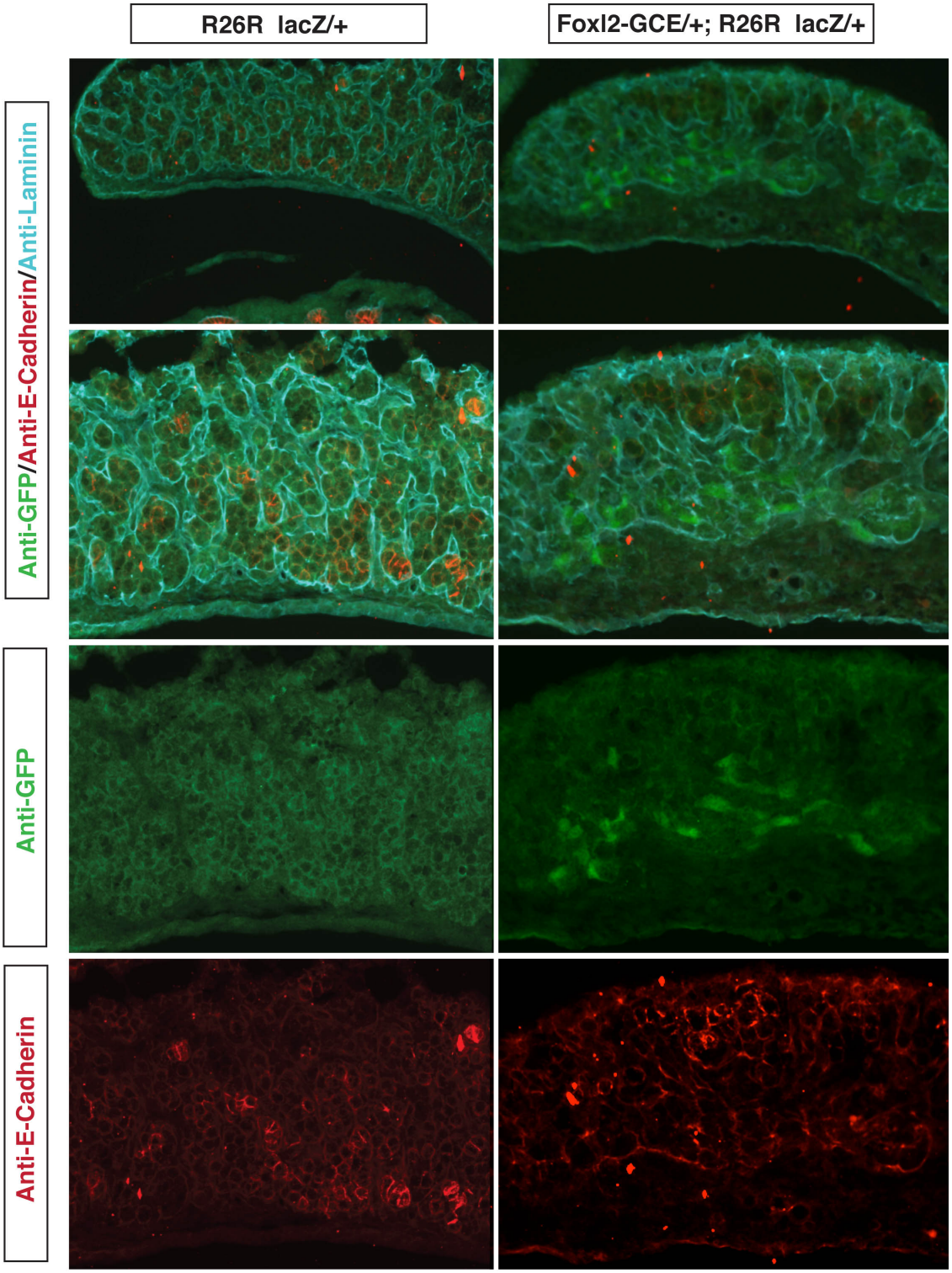

Fig 4. GFP is detected in cells adjacent to E-Cadherin positive germ cells in the medulla of the ovary in Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ embryos at 14.5dpc.
Antibodies: chicken-anti-GFP/ rat-anti-E-Cadherin / rabbit-anti-Laminin.

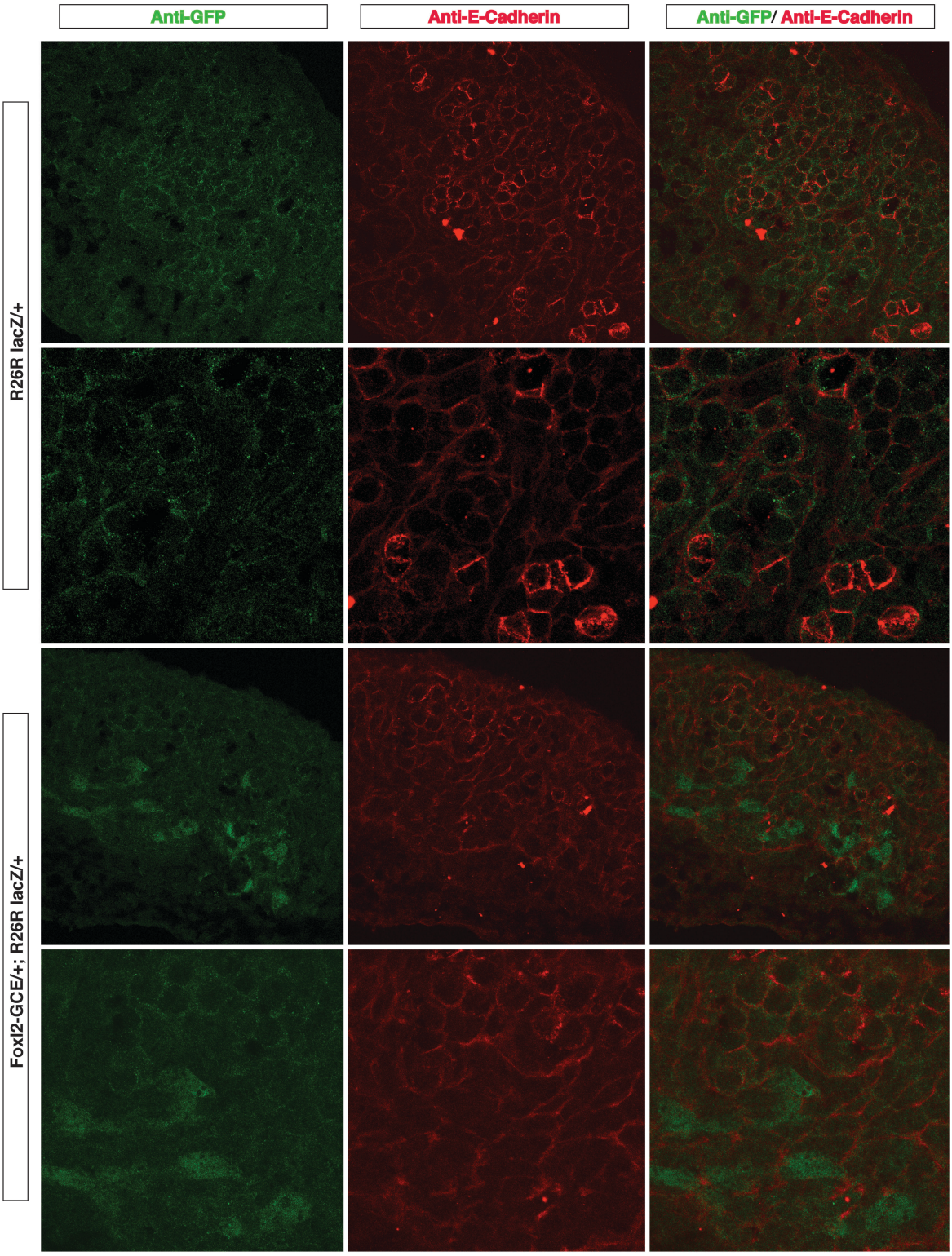

Fig 5. GFP is detected in granulosa precursor cells distinct from E-Cadherin positive germ cells, in the ovary of Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ embryos at 14.5dpc. Antibodies: chicken-anti-GFP/ rat-anti-E-Cadherin.

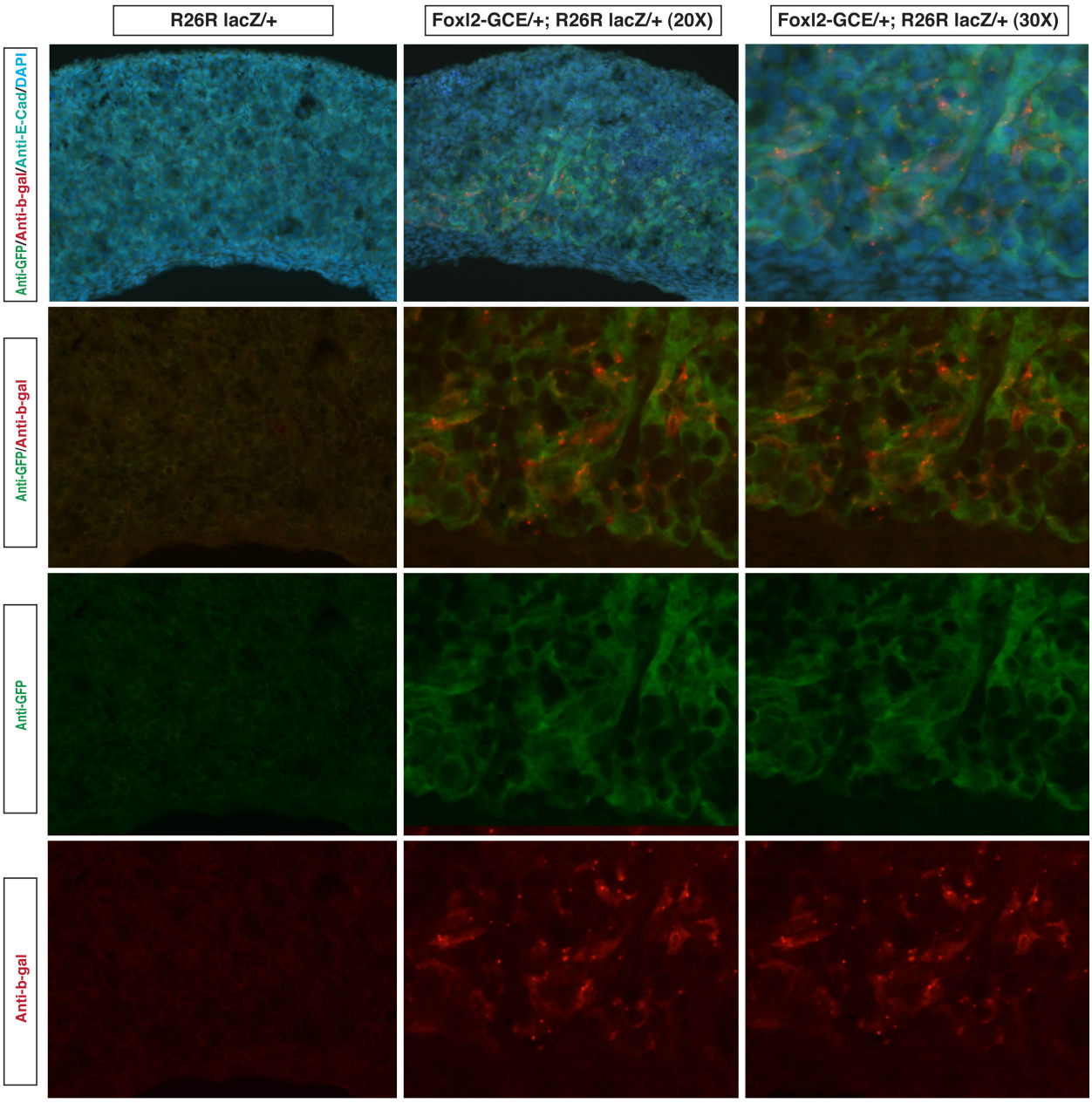

Fig 6. Co-localization of β-gal positive cells and GFP positive cells following Tamoxifen induction of Foxl2 driven GCE. Co-localization is observed in putative granulosa precursor cells in the center of the medulla in Foxl2$^{GCE/+}$; R26R$^{lacZ/+}$ ovaries at 14.5dpc.
Antibodies: chicken-anti-GFP / rabbit-anti-β-gal / rat-anti-E-Cadherin.