

## Transcription factor FoxO1 regulates myoepithelial cell diversity and growth

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| Supplemental Table S1. Primer pairs |         | size (bp)                      |     |
|-------------------------------------|---------|--------------------------------|-----|
| GAPDH                               | forward | 5'-AAGCCCATCACCATCTTCCAGGAG-3' | 310 |
|                                     | reverse | 5'-ATGAGCCCTTCCACAATGCCAAAG-3' |     |
| $\alpha$ SMA                        | forward | 5'-GGAGAAGCCCAGCCAGTCGC-3'     | 117 |
|                                     | reverse | 5'-AGCCGGCCTTACAGAGCCCA-3'     |     |
| FoxO1                               | forward | 5'-CTACGAGTGGATGGTGAAGAGC-3'   | 138 |
|                                     | reverse | 5'-CCAGTTCCTTCATTCTGCACTCG-3'  |     |
| p21                                 | forward | 5'-TCGCTGTCTTGCACTCTGGTGT-3'   | 124 |
|                                     | reverse | 5'-CCAATCTGCGCTTGGAGTGATAG-3'  |     |
| p27                                 | forward | 5'-AGCAGTGTCAGGGATGAGGAA-3'    | 133 |
|                                     | reverse | 5'-TTCTTGGGCGTCTGCTCCACAG-3'   |     |
| Eda                                 | forward | 5'-TCAACAGCAGCCTTTGGAACCG-3'   | 132 |
|                                     | reverse | 5'-CGAACACGCCTACTTTCCTCTTC-3'  |     |
| Eda2r                               | forward | 5'-AAGCAGACTCCTTCTTCCGAGG-3'   | 105 |
|                                     | reverse | 5'-CACCAGTGCAACAAGTGTGGCT-3'   |     |

Table S2. Top 30 Up-regulated gene list (FoxO1TPM mean>5, Control TPM mean>1). Related to Fig.3B.

| Gene    | Description  | FoxO1 /Control | FoxO1 mean | Control mean |
|---------|--|----------------|------------|--------------|
| Foxo1   | forkhead box O1  | 21.66          | 595.41     | 27.49        |
| Tmsb15l | thymosin beta 15b like   | 13.07          | 24.04      | 1.84         |
| Ghr     | growth hormone receptor  | 5.79           | 40.23      | 6.95         |
| Ifi209  | interferon activated gene 209                                      | 4.62           | 8.72       | 1.89         |
| Gnai1   | guanine nucleotide binding protein (G protein), alpha inhibiting 1 | 3.96           | 21.79      | 5.50         |
| Ifi44   | interferon-induced protein 44                                      | 3.90           | 8.10       | 2.08         |
| H1f2    | histone cluster 1, H1c   | 3.51           | 31.36      | 8.95         |
| Bdh2    | 3-hydroxybutyrate dehydrogenase, type 2                            | 3.48           | 5.74       | 1.65         |
| Sdcbp2  | syndecan binding protein (syntenin) 2                              | 3.36           | 18.66      | 5.56         |
| Pik3ip1 | phosphoinositide-3-kinase interacting protein 1                    | 3.27           | 10.78      | 3.30         |
| Cpq     | carboxypeptidase Q   | 3.20           | 7.56       | 2.36         |
| Nfe2l3  | nuclear factor, erythroid derived 2, like 3                        | 3.17           | 44.27      | 13.98        |
| Rb1     | RB transcriptional corepressor 1                                   | 3.15           | 11.12      | 3.53         |
| Tmem140 | transmembrane protein 140  | 3.14           | 12.86      | 4.09         |
| Cytip   | cytohesin 1 interacting protein                                    | 3.02           | 7.84       | 2.60         |
| Igfbp2  | insulin-like growth factor binding protein 2                       | 2.98           | 54.15      | 18.18        |
| Irf7    | interferon regulatory factor 7                                     | 2.93           | 5.10       | 1.74         |
| Efhd1   | EF hand domain containing 1  | 2.92           | 41.17      | 14.11        |
| Eda2r   | ectodysplasin A2 receptor  | 2.86           | 6.41       | 2.24         |
| Usp18   | ubiquitin specific peptidase 18                                    | 2.84           | 5.71       | 2.01         |
| Entpd3  | ectonucleoside triphosphate diphosphohydrolase 3                   | 2.80           | 10.43      | 3.73         |
| Nipal1  | NIPA-like domain containing 1                                      | 2.78           | 7.51       | 2.70         |
| Itn2a   | integral membrane protein 2A                                       | 2.76           | 17.16      | 6.22         |
| Plaat3  | phospholipase A and acyltransferase 3                              | 2.73           | 27.94      | 10.25        |
| Cyp4v3  | cytochrome P450, family 4, subfamily v, polypeptide 3              | 2.72           | 8.35       | 3.07         |
| Prss12  | protease, serine 12 neurotrypsin (motopsin)                        | 2.69           | 5.69       | 2.11         |
| Pamr1   | peptidase domain containing associated with muscle regeneration 1  | 2.62           | 6.65       | 2.54         |
| Mmp13   | matrix metalloproteinase 13  | 2.61           | 24.41      | 9.34         |
| Isg15   | ISG15 ubiquitin-like modifier                                      | 2.56           | 21.26      | 8.29         |
| Klf15   | Kruppel-like factor 15   | 2.56           | 19.69      | 7.70         |

Table S3. GO term: Developmental process(FoxO1 TPM mean >2.5, Control TPM mean>1). Related to Fig.3C.

| Gene     | Fold Change | Control_1 | Control_2 | Control_3 | FoxO1_1 | FoxO1_2 | FoxO1_3 |
|----------|-------------|-----------|-----------|-----------|---------|---------|---------|
| Foxo1    | 21.66       | 26.77     | 27.46     | 28.25     | 647.11  | 588.69  | 550.42  |
| Ghr      | 5.79        | 7.43      | 6.51      | 6.91      | 40.68   | 39.14   | 40.86   |
| Bdh2     | 3.48        | 1.94      | 1.40      | 1.61      | 5.78    | 5.09    | 6.36    |
| Cpq      | 3.20        | 2.47      | 2.15      | 2.46      | 7.92    | 7.70    | 7.05    |
| Rb1      | 3.15        | 3.20      | 3.61      | 3.79      | 10.89   | 11.92   | 10.56   |
| Arhgdib  | 3.04        | 1.13      | 1.24      | 1.36      | 3.20    | 3.92    | 4.21    |
| Igf2bp2  | 2.98        | 19.75     | 16.20     | 18.60     | 47.83   | 54.63   | 59.99   |
| Efhd1    | 2.92        | 13.05     | 15.67     | 13.61     | 42.04   | 40.01   | 41.46   |
| Grk5     | 2.88        | 1.90      | 1.34      | 1.85      | 4.91    | 5.05    | 4.66    |
| Eda2r    | 2.86        | 2.29      | 2.03      | 2.40      | 5.81    | 7.23    | 6.20    |
| Itih2a   | 2.76        | 6.75      | 6.08      | 5.83      | 15.61   | 17.26   | 18.60   |
| Wdpcp    | 2.74        | 1.15      | 1.72      | 0.87      | 3.23    | 3.56    | 3.42    |
| Plaat3   | 2.73        | 10.14     | 10.26     | 10.35     | 26.83   | 27.99   | 29.00   |
| Mmp13    | 2.61        | 10.00     | 9.19      | 8.84      | 26.54   | 24.74   | 21.96   |
| Isg15    | 2.56        | 8.41      | 7.87      | 8.60      | 17.61   | 26.09   | 20.09   |
| Klf15    | 2.56        | 8.49      | 7.13      | 7.47      | 20.37   | 19.46   | 19.25   |
| Eda      | 2.47        | 0.99      | 1.06      | 1.04      | 3.08    | 2.55    | 2.01    |
| Lratd1   | 2.42        | 1.90      | 1.40      | 1.37      | 3.97    | 3.54    | 3.81    |
| Chn1     | 2.42        | 2.33      | 2.47      | 2.40      | 6.20    | 5.21    | 6.05    |
| Slit2    | 2.41        | 1.30      | 1.33      | 1.52      | 3.77    | 3.06    | 3.16    |
| Ctla2a   | 2.36        | 3.95      | 5.97      | 4.98      | 11.64   | 10.28   | 13.30   |
| Nrp1     | 2.25        | 2.89      | 2.83      | 3.31      | 6.57    | 7.34    | 6.41    |
| Cebpd    | 2.23        | 5.44      | 4.91      | 4.29      | 11.60   | 10.06   | 10.97   |
| Arrdc3   | 2.13        | 2.18      | 2.28      | 2.13      | 4.85    | 4.71    | 4.50    |
| Arhgap18 | 2.12        | 1.39      | 1.09      | 1.39      | 2.84    | 3.30    | 2.07    |
| Btbd2    | 2.09        | 4.33      | 3.95      | 4.87      | 9.23    | 9.34    | 8.93    |
| Eya1     | 2.08        | 1.75      | 1.45      | 1.44      | 3.45    | 3.05    | 3.16    |
| Alcam    | 2.04        | 2.64      | 2.77      | 2.53      | 5.79    | 5.67    | 4.74    |
| Uchl1    | 2.04        | 8.66      | 7.03      | 7.40      | 16.23   | 15.05   | 15.83   |

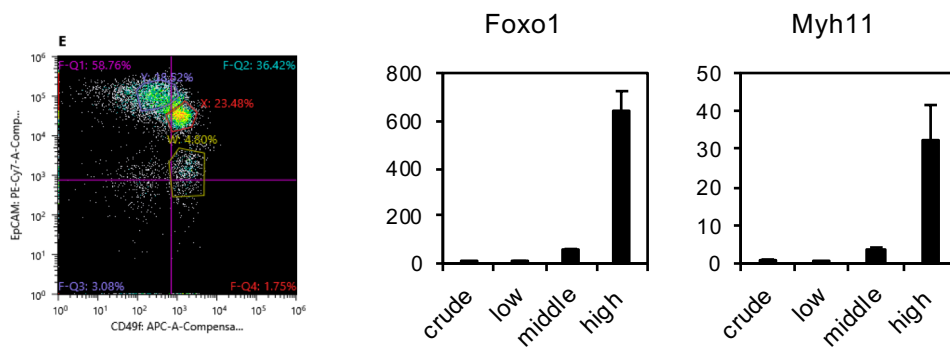
Table S4. Down-regulated gene list (FoxO1 TPM mean<10, Control TPM mean>5). Related to Fig.3B.

| Gene         | Description  | FoxO1 /Control | FoxO1 mean | Control mean |
|--------------|--|----------------|------------|--------------|
| Txnip        | thioredoxin interacting protein  | -3.86          | 2.84       | 10.97        |
| Cpa4         | carboxypeptidase A4  | -3.57          | 1.48       | 5.27         |
| Mmp12        | matrix metalloproteinase 12  | -3.15          | 6.74       | 21.23        |
| Cyba         | cytochrome b-245, alpha polypeptide  | -3.00          | 2.29       | 6.88         |
| Ulbp1        | UL16 binding protein 1   | -2.67          | 4.54       | 12.13        |
| Sprp1b       | small proline-rich protein 1B  | -2.50          | 2.79       | 6.97         |
| Snora73b     | small nucleolar RNA, H/ACA box 73b   | -2.49          | 2.06       | 5.13         |
| Snord68      | small nucleolar RNA, C/D box 68  | -2.48          | 2.69       | 6.67         |
| LOC115488444 | U6 spliceosomal RNA  | -2.46          | 2.23       | 5.48         |
| Shcbp1       | Shc SH2-domain binding protein 1   | -2.40          | 2.20       | 5.27         |
| Adamts8      | a disintegrin-like and metalloproteinase (reprolysin type) with thrombospondin type 1 motif, 8 | -2.33          | 7.18       | 16.76        |
| LOC115489087 | small nucleolar RNA SNORD31  | -2.28          | 4.40       | 10.03        |
| Dlgap5       | DLG associated protein 5   | -2.24          | 5.51       | 12.34        |
| Kifc1        | kinesin family member C1   | -2.23          | 7.78       | 17.37        |
| LOC115489726 | uncharacterized LOC115489726   | -2.22          | 3.09       | 6.87         |
| LOC115490091 | U6 spliceosomal RNA  | -2.21          | 2.87       | 6.34         |
| Nusap1       | nucleolar and spindle associated protein 1   | -2.20          | 5.17       | 11.36        |
| Sprp2j-ps    | small proline-rich protein 2J, pseudogene  | -2.17          | 2.88       | 6.26         |
| Cp           | ceruloplasmin  | -2.14          | 9.68       | 20.71        |
| Mki67        | antigen identified by monoclonal antibody Ki 67  | -2.13          | 5.64       | 12.02        |
| Snord14a     | small nucleolar RNA, C/D box 14A   | -2.11          | 3.26       | 6.89         |
| Hmmr         | hyaluronan mediated motility receptor (RHAMM)  | -2.10          | 2.67       | 5.62         |
| Snord35a     | small nucleolar RNA, C/D box 35A   | -2.07          | 2.63       | 5.43         |
| Mirt1        | myocardial infarction associated transcript 1  | -2.03          | 2.97       | 6.03         |
| Kif18b       | kinesin family member 18B  | -1.99          | 2.87       | 5.72         |
| Cenpk        | centromere protein K   | -1.99          | 3.86       | 7.67         |
| Tubb3        | tubulin, beta 3 class III  | -1.99          | 9.25       | 18.36        |

Table S5. GO term: Mitotic cell cycle. Related to Fig.3D.

| Gene   | Fold change | Control_1 | Control_2 | Control_3 | FoxO1_1 | FoxO1_2 | FoxO1_3 |
|--------|-------------|-----------|-----------|-----------|---------|---------|---------|
| Dact1  | -2.65       | 0.7       | 1.2       | 1.2       | 0.3     | 0.3     | 0.6     |
| Kifc1  | -2.23       | 14.2      | 19.4      | 18.5      | 7.4     | 8.8     | 7.2     |
| Nusap1 | -2.20       | 11.1      | 11.5      | 11.6      | 4.0     | 5.3     | 6.2     |
| Mki67  | -2.13       | 10.7      | 12.3      | 13.0      | 5.1     | 6.3     | 5.6     |
| Ticrr  | -2.12       | 1.4       | 1.8       | 1.8       | 0.7     | 0.8     | 0.9     |
| Ska1   | -2.12       | 0.5       | 0.7       | 0.5       | 0.2     | 0.3     | 0.3     |
| Cdc6   | -2.06       | 2.9       | 2.3       | 2.7       | 1.3     | 1.1     | 1.5     |
| Cenpf  | -2.03       | 1.8       | 2.7       | 2.5       | 1.1     | 1.2     | 1.2     |
| Sapcd2 | -2.07       | 0.9       | 1.6       | 1.6       | 0.6     | 0.9     | 0.4     |
| Eme1   | -1.94       | 1.0       | 1.4       | 1.0       | 0.6     | 0.8     | 0.3     |
| Cenpk  | -1.99       | 7.7       | 7.6       | 7.7       | 4.0     | 3.6     | 4.0     |
| Kif18b | -1.99       | 5.2       | 6.4       | 5.5       | 2.8     | 2.8     | 3.0     |
| Tubb3  | -1.99       | 16.1      | 19.2      | 19.8      | 8.5     | 10.5    | 8.7     |

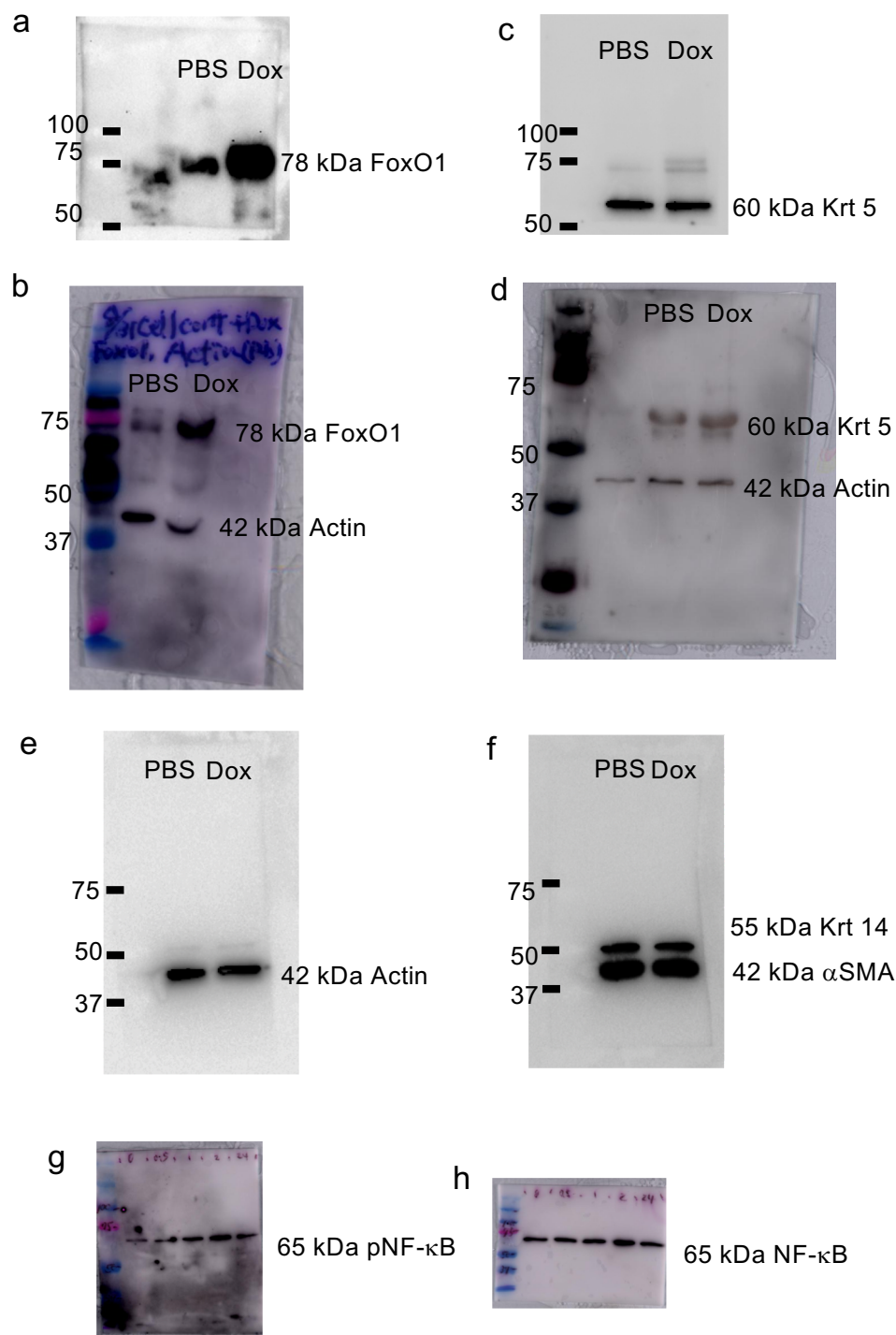
↑ Tokumasu et al. Supplementary Fig. S1, related to Fig. 1



**Figure S1. FoxO1 expressed in Myh11-positive cells, related to Fig. 1.**

Scatter plot of CD49f (x-axis) and EpCAM (y-axis). The expression of Myh11 was high in EpCAM<sup>low</sup>CD49f<sup>high</sup>-cells (W), and that of FoxO1 also was high.

↑ Tokumasu et al. Supplementary Fig. S2, Fig. 2E and 2I

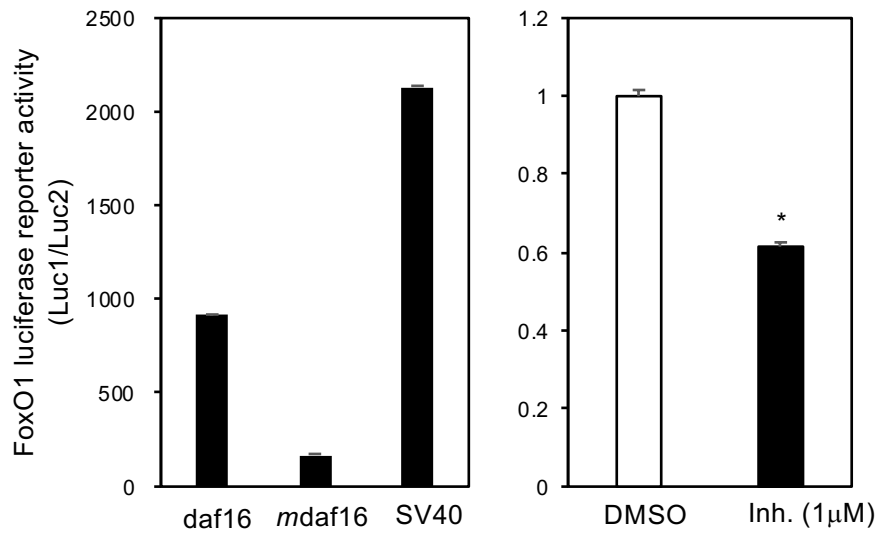


**Figure S2. Raw data of Western Blot results, related to Fig. 2E, 2I.**

(a-f) Immunoblotting for FoxO1 (a, b), Krt5 (c, d), β-actin (e), αSMA (f), and Krt14 (f) in ME<sup>PB-FoxO1</sup> cells treated with and without Dox for 72 h, related to Fig. 2E. (b, d) The replicates of entire membranes of a. and c. showed in b. and d. because the membranes of a. and c. were separated by 50 kDa. (g-h) Immunoblotting for NF-κB/p65 and phospho-NF-κB/p65 in ME<sup>PB-FoxO1</sup> cells treated with and without Dox at the indicated time-points. The full uncropped blot from Western Blot results, related to Fig. 2I.



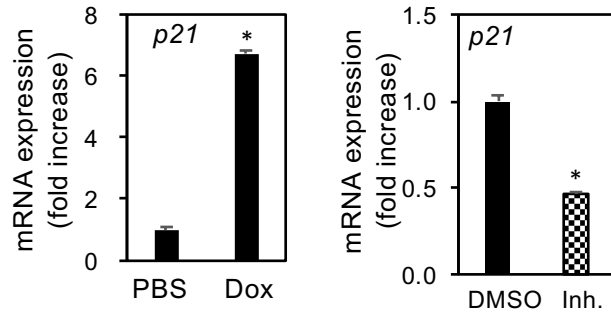
↑ Tokumasu et al. Supplementary Fig. S3, related to Fig. 2F



**Figure S3. FoxO1 reporter activity, related to Fig. 2F.**

Endogenous FoxO1 expression decreased in the presence of FoxO1 inhibitor (Inh; AS1842856, 1µM) (right). Base level of FoxO1 luciferase reporter activity was detected. SV40 (pGL4.13 luc2/SV40, #E6681, Promega), reporter vector was used for positive control (left). \* $P < 0.05$ .

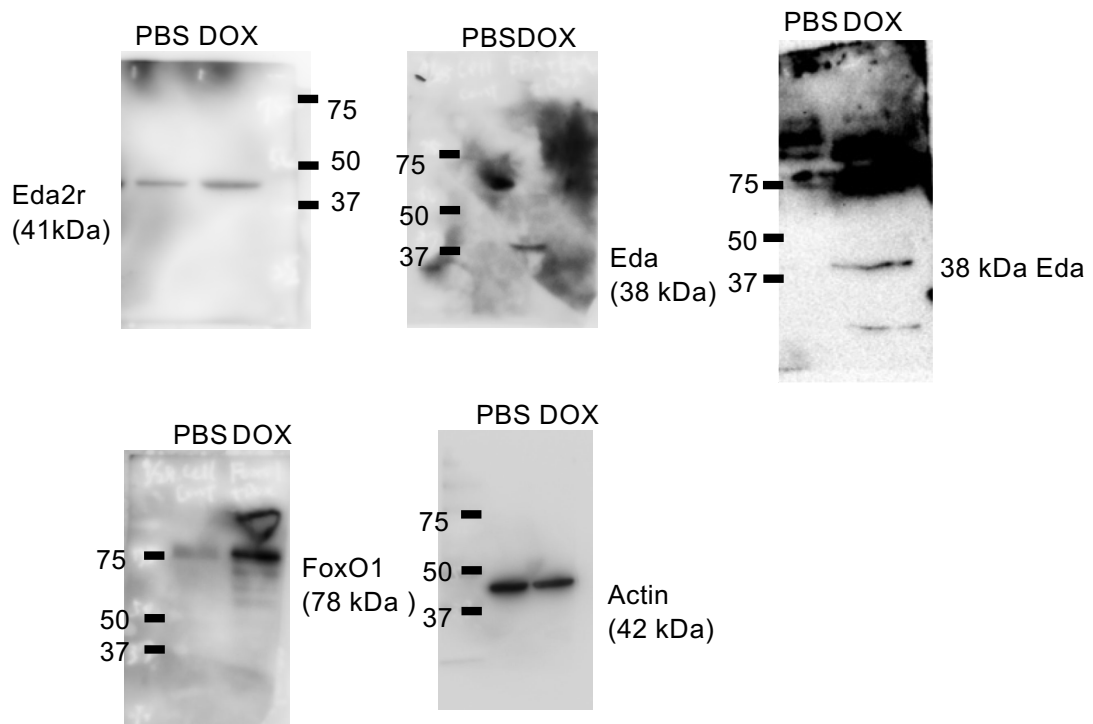
↑ Tokumasu et al. Supplementary Fig. S4, related to Fig. 4C-4E



**Figure S4. Gene expression of p21, related to Fig.4C-4E.**

Expression of *p21*(*CIP/WAF1*) in ME<sup>PB-FoxO1</sup> cells treated with and without Dox (2  $\mu$ g/mL) for 72 h (left), and with and without FoxO1 inhibitor (Inh.; AS1842856, 1  $\mu$ M) pretreatment for 24 h (right). \* $P$ <0.05.

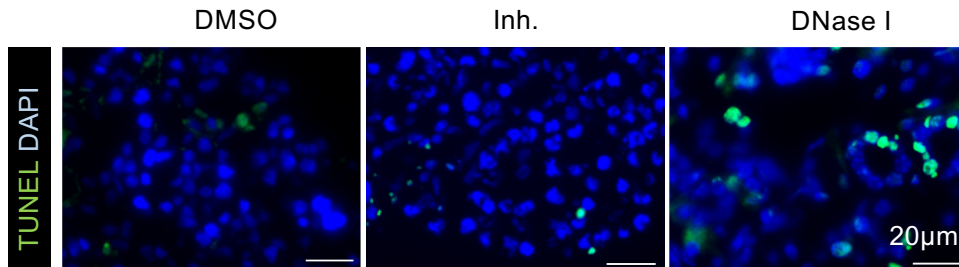
↑ Tokumasu et al. Supplementary Fig. S5, related to Fig. 5B



**Figure S5. Raw data of Western Blot results, related to Fig. 5B.**

(Fig5. B) Immunoblotting of Eda and Eda2r in ME<sup>PB-FoxO1</sup> cells treated with and without Dox (2  $\mu$ g/mL) for 24 h. The full uncropped blot from Western Blot results.

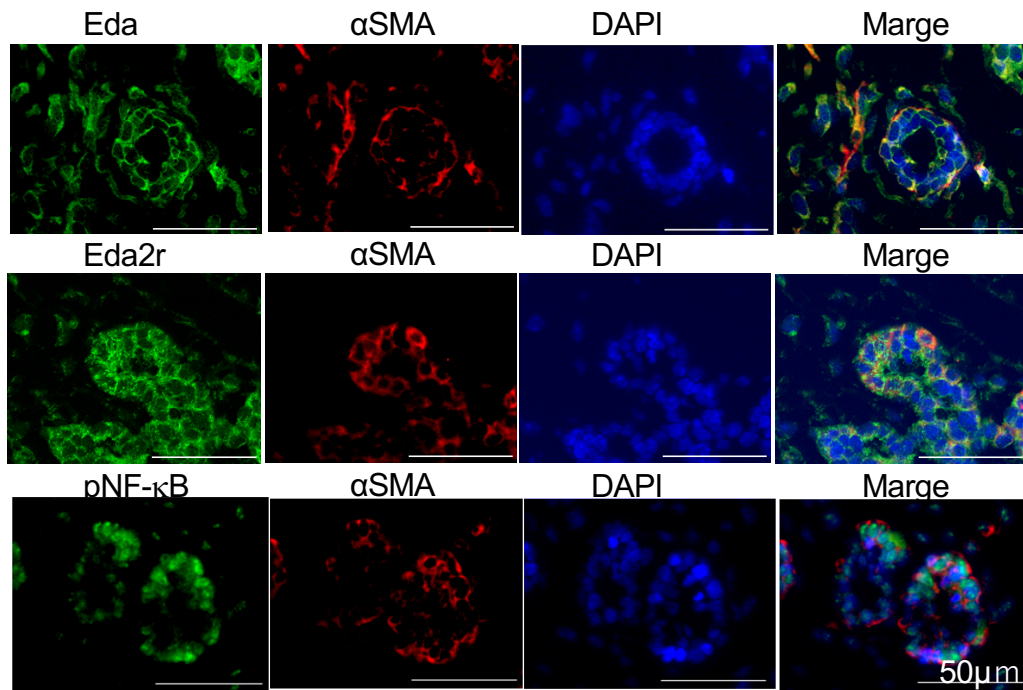
↑ Tokumasu et al. Supplementary Fig. S6, related to Fig. 6 and 7



**Figure. S6. TUNEL Assay to examine apoptosis after FoxO1 inhibitor treatment in organ culture of primordial SMGs, related to Fig. 6 and 7.**

The SMG epithelium from E14.5 SMGs eliminated mesenchyme was cultured for 1 day with and without AS1842856 (10  $\mu$ M). The SMG epithelium was fixed in 4 % paraformaldehyde at 4 °C for O/N, followed by cryopreservation with sucrose. The cryosections were 4  $\mu$ m. We used In Situ Cell Death Detection Kit, Fluorescein (#11684795910, Roche). Fix tissue section with 4% paraformaldehyde in PBS, pH 7.4, for 20 minutes at room temperature. After wash in PBS, the tissues were permeabilized in 0.1% Triton X-100 for 2 minutes on ice. Then add 50  $\mu$ l TUNEL reaction mixture on sample and incubate for 60 minutes at 37 °C in a humidified atmosphere in the dark. Rinse slides 3 times with PBS. Nuclei were stained with DAPI . Images were captured using a BZ- 9000 fluorescence microscope. No TUNEL signal were observed in the control (left) and AS1842856 (10 $\mu$ M) additions (middle). Tissue sections treated with DNase I (10 $\mu$ g/ml) showed positive TUNEL images (right). bar=20 $\mu$ m.

↑ Tokumasu et al. Supplementary Fig. S7, related to Fig. 6 and 7



**Figure S7. Localization of phospho-NF-κB, Eda, and Eda2r in ME cells of SMGs, related to Fig. 6 and 7.** IF of αSMA, Eda, Eda2r, and phospho-NF-κB (pNF-κB) in SMGs from E16 WT mice. Nuclei were stained with DAPI. bar =50 μm.