## Supplementary Data

**Figure S1. A.** Raw counts for bouton area **B.** branch length **C.** active zone number per bouton **D.** Average bouton area and branch length for wild-type ($w^{1118}$) and $foxo^{C431}$. *$P< 0.05$, ** $P<0.01$, ***$P>0.001$, ****$P< 0.0001$, n.s. not significant. $w^{1118}$ n=5; $foxo^{C431}$ n=5. **E.** Averaged bouton area and branch length. **F.** Raw bouton area and branch length for wild-type ($w^{1118}$) and $foxo^{C431}$. *$P< 0.05$, ** $P<0.01$, ***$P>0.001$, ****$P< 0.0001$, n.s. not significant. $w^{1118}$ n=59; $foxo^{C431}$ n=66. **G. F.** Western analysis of FOXO protein abundance in control and $Foxo^{21}$ mutant whole-body tissue.

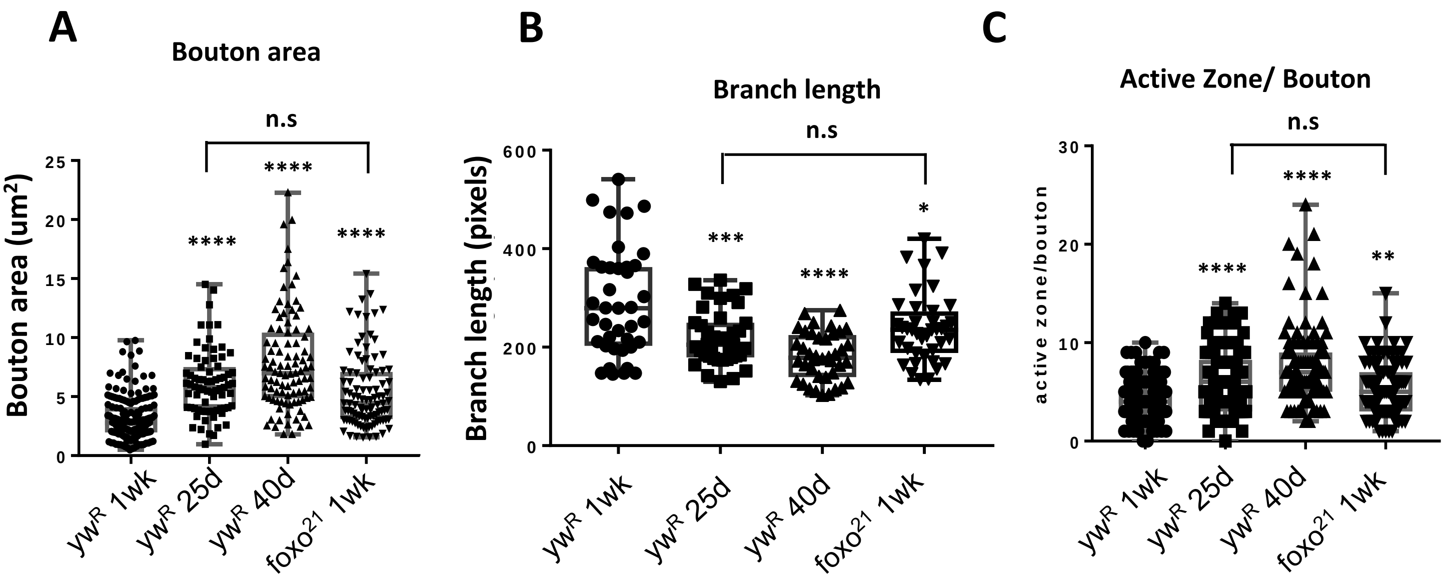
**Figure S2. A.** Representative images of anti-Ac-Tub staining for wildtype ($yw^R$) at 1-week and 25-days of age, and $foxo^{21}$ flies at 1 week of age. **B.** Representative images of anti-Ac-Tub staining for control (Ok6-Gal4> $yw^R$) at 1 week and 25 days of age, and Ok6-Gal4> foxo-RNAi flies at 1 week post eclosion. **C.** Quantification of acetylated alpha-tubulin structure. Significance values are compared to young control for each grouping . *$P< 0.05$, ** $P<0.01$, ***$P>0.001$.

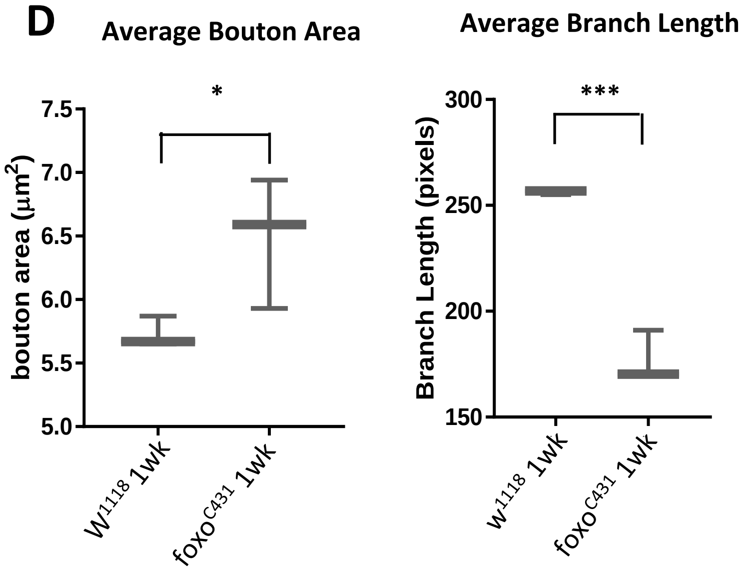
**Figure S3. A.** Rab7 quantification at 1 week for ok6-Gal4>ywR, ok6-Gal4>foxo-RNAi #1, ok6-Gal4> foxo-RNAi #2. **B.** Thor relative expression for whole body tissue. Daughterless-Gal4 activated with 200mM of RU486. **C.** Quantification of FOXO protein intensity in fat body nuclei after RU486 induced knockdown. * $P< 0.05$. **D.** Representative images of fat body tissue for control (S106-GS-GAL4> $w^{1118}$) and FOXO RNAi (S106-GS-Gal4>BL32993). S106-Gal4 activated with 200mM of Mifepristone (RU). Staining with anti-FOXO and DAPI. Scale Bar 10µm**,** n=5**.**

**Figure S4. A.** Rab7 relative intensity quantification at 1 week for ok6-Gal4>ywR, ok6-Gal4>foxo-RNAi. **B.** Rab7 intensity quantification Rab7 quantification at 2-days for ok6-Gal4>ywR, ok6-Gal4>foxo-RNAi **C.** Bouton area quantification at 2-days for ok6-Gal4>ywR, ok6-Gal4>foxo-RNAi **D.** Ac-Alpha tubulin quantification at 2-days for ok6-Gal4>ywR, ok6-Gal4>foxo-RNAi **E. .** Quantification of Rab7 for epistasis flies. Values are set as a percentage with ok6-Gal4>Attp40 as baseline. *$P< 0.05$, n.s. – not significant.

**Figure S5. Representative confocal images from genetic screening. A.** anti-rab7 and anti-HRP immunofluorescence for ok6;$foxo^{RNAi}$> $p38b^{RNAi}$, ok6;$foxo^{RNAi}$> $rl^{RNAi}$, ok6;$foxo^{RNAi}$> $p38a^{RNAi}$. B. anti-rab7 and anti-HRP immunofluorescence for control (ok6-gal4> $yw^R$ ) ; ok6;$foxo^{RNAi}$> $yw^R$ ; ok6;$foxo^{RNAi}$> $babo^{RNAi}$, **C.** anti-Ac-Tub and anti-HRP immunofluorescence for ok6;$foxo^{RNAi}$> $p38b^{RNAi}$, ok6;$foxo^{RNAi}$> $babo^{RNAi}$, ok6;$foxo^{RNAi}$> $yw^R$.

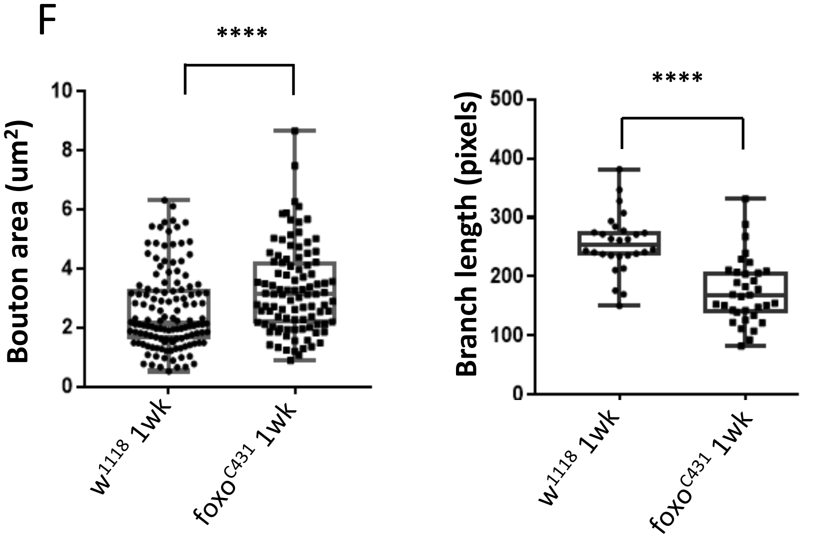
# Figure S1

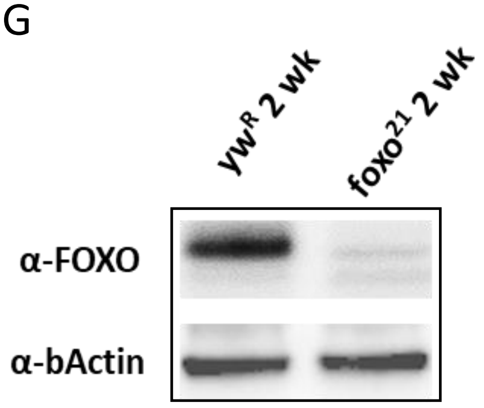




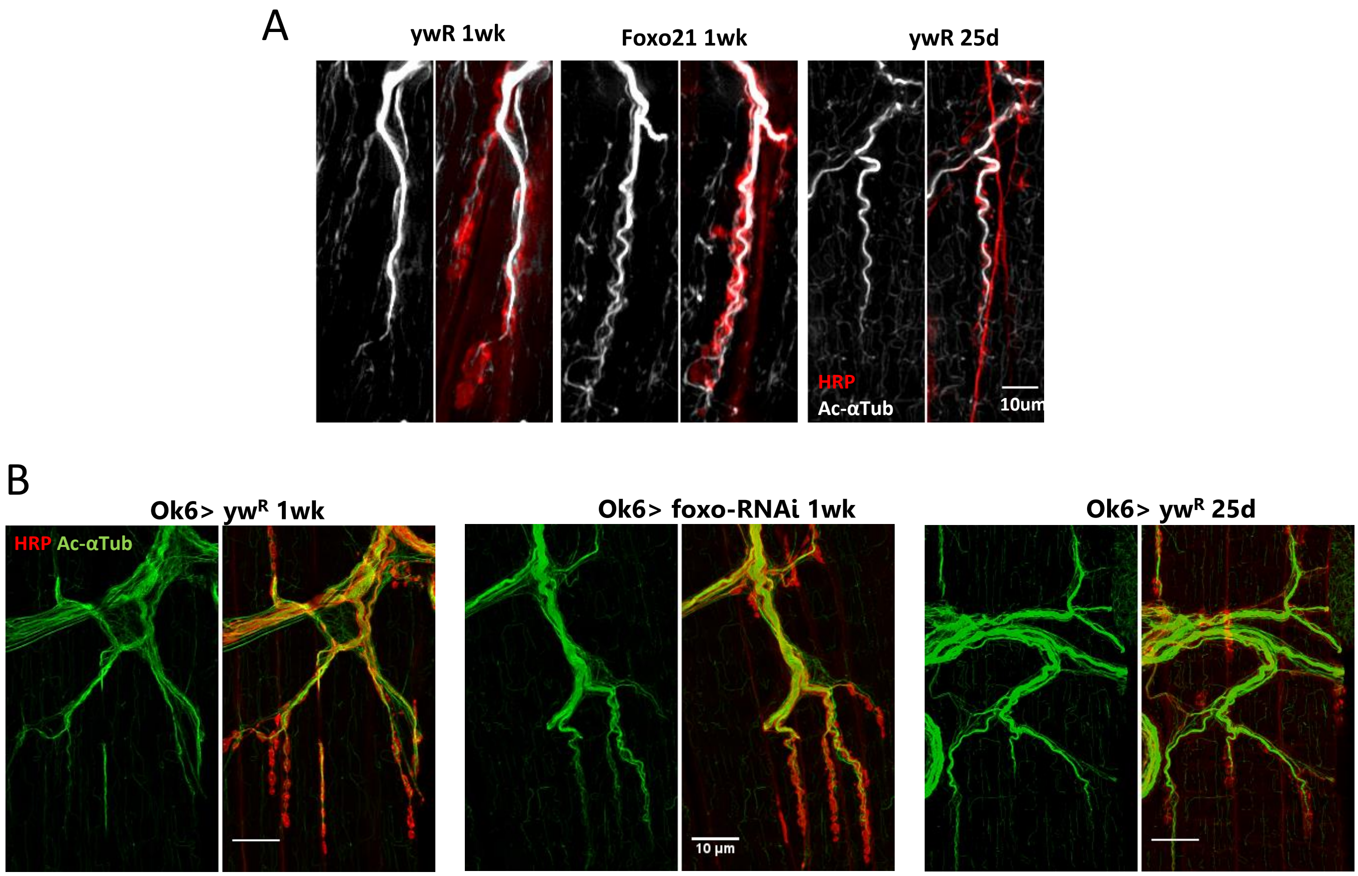
E

| | Size of boutons (um$^2$) | Branch length (pixels) |
|---|---|---|
| w$^{1118}$ 1wk | 5.53 ± 2.47 | 256.4 ± 49.85 |
| foxo$^{C431}$ 1wk | 6.59 ± 2.94 | 175.2 ± 55.32 |





# Figure S2



C

| | Straight | Undulating |
|---|---|---|
| ywR 1wk | 69.24% | 30.76% |
| yw$^R$ 25d | 40.38%* | 59.62%* |
| yw$^R$ 40d | 30.00%** | 70.00%** |
| foxo$^{21}$ 1wk | 46.83%* | 53.17%* |
| ok6 > control 1wk | 67% | 33% |
| ok6 > foxo-RNAi 1wk | 34.27%*** | 65.73%*** |
| ok6 > control 25d | 38.64%** | 61.36%** |

# Figure S3

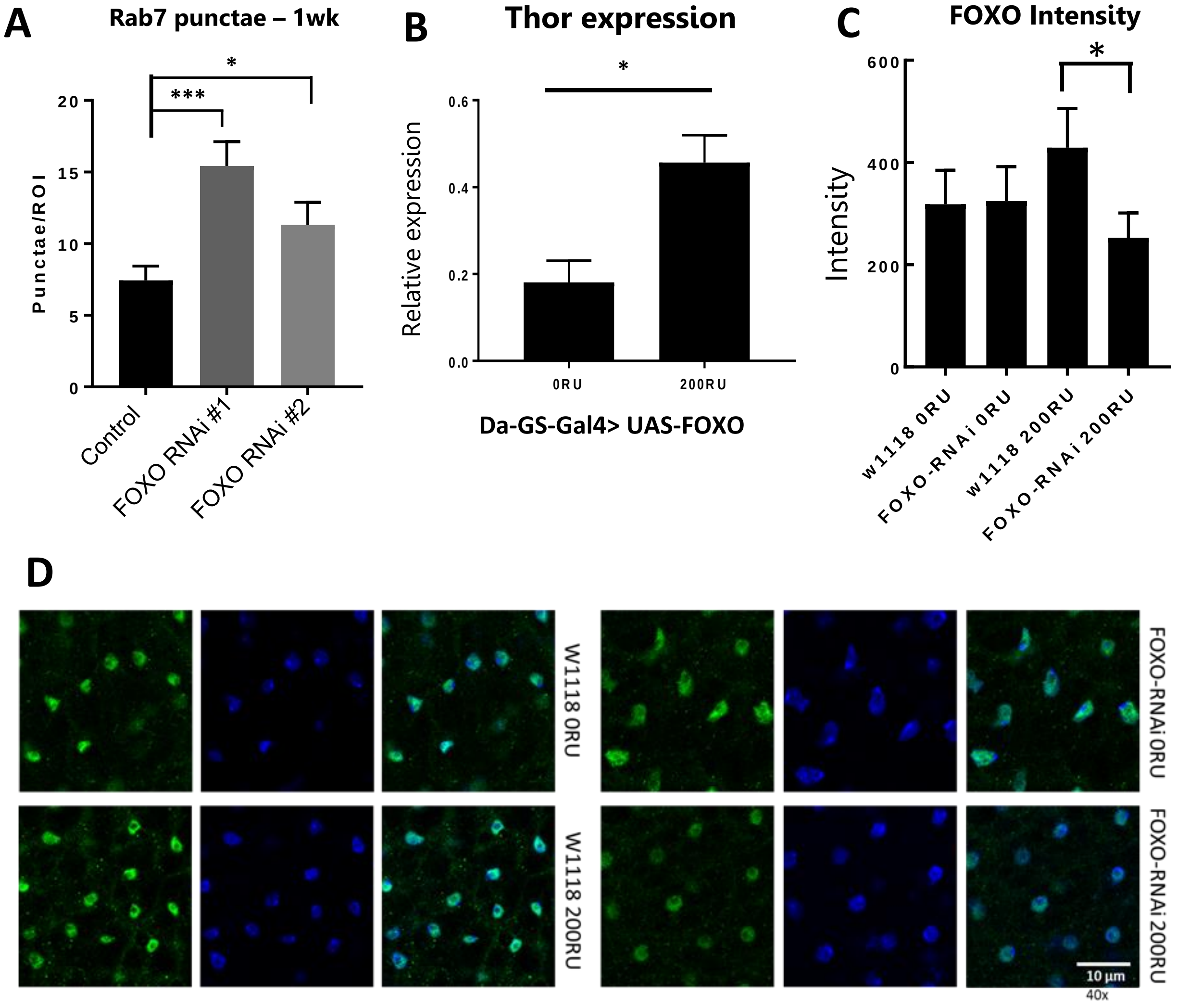

A
Rab7 punctae – 1wk
Punctae/ROI
0
5
10
15
20
***
*
Control
FOXO RNAi #1
FOXO RNAi #2
B
Thor expression
Relative expression
0.0
0.2
0.4
0.6
*
0RU
200RU
Da-GS-Gal4> UAS-FOXO
C
FOXO Intensity
Intensity
0
200
400
600
*
w1118 0RU
FOXO-RNAi 0RU
w1118 200RU
FOXO-RNAi 200RU
D
W1118 0RU
W1118 200RU
FOXO-RNAi 0RU
FOXO-RNAi 200RU
10 µm
40x

Figure S4

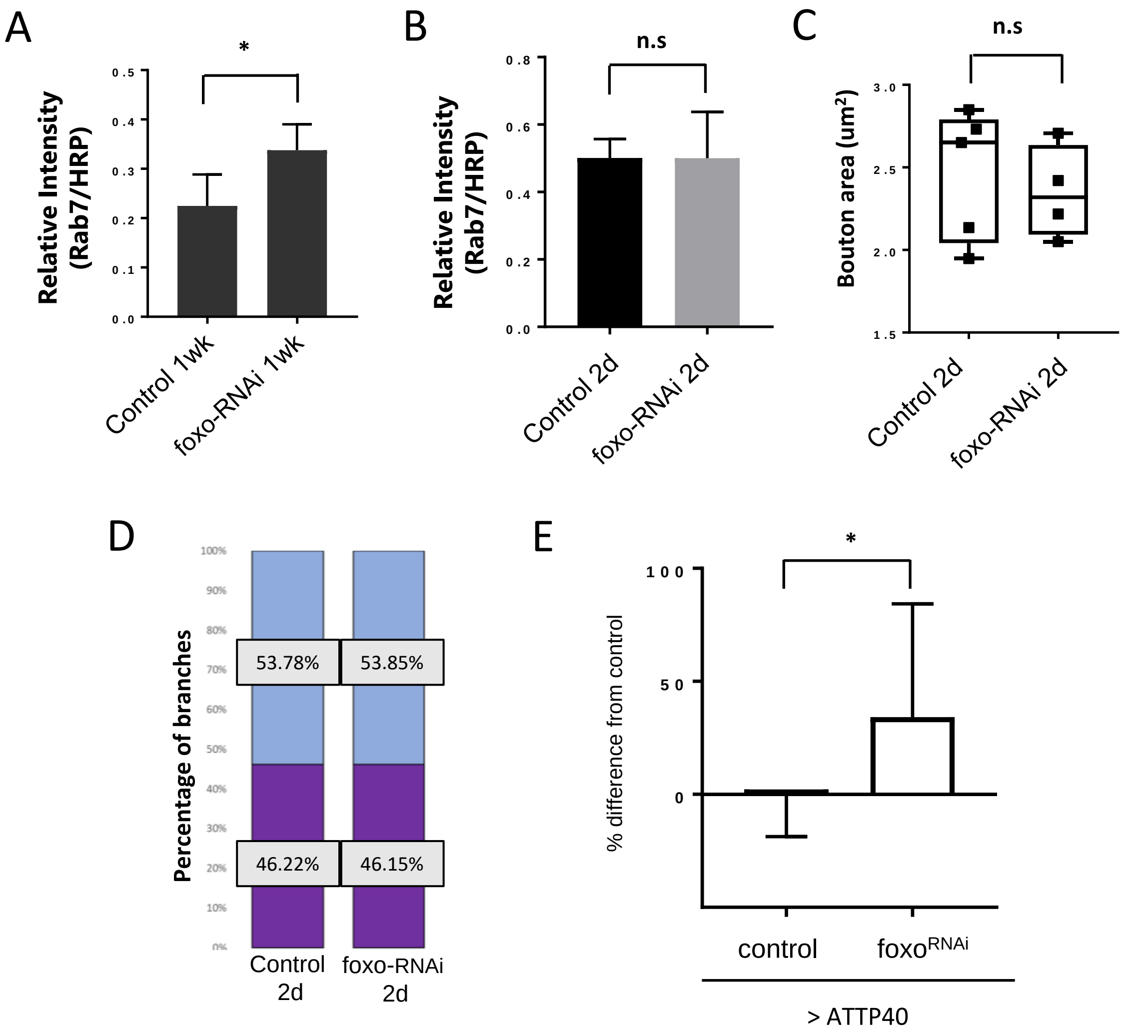
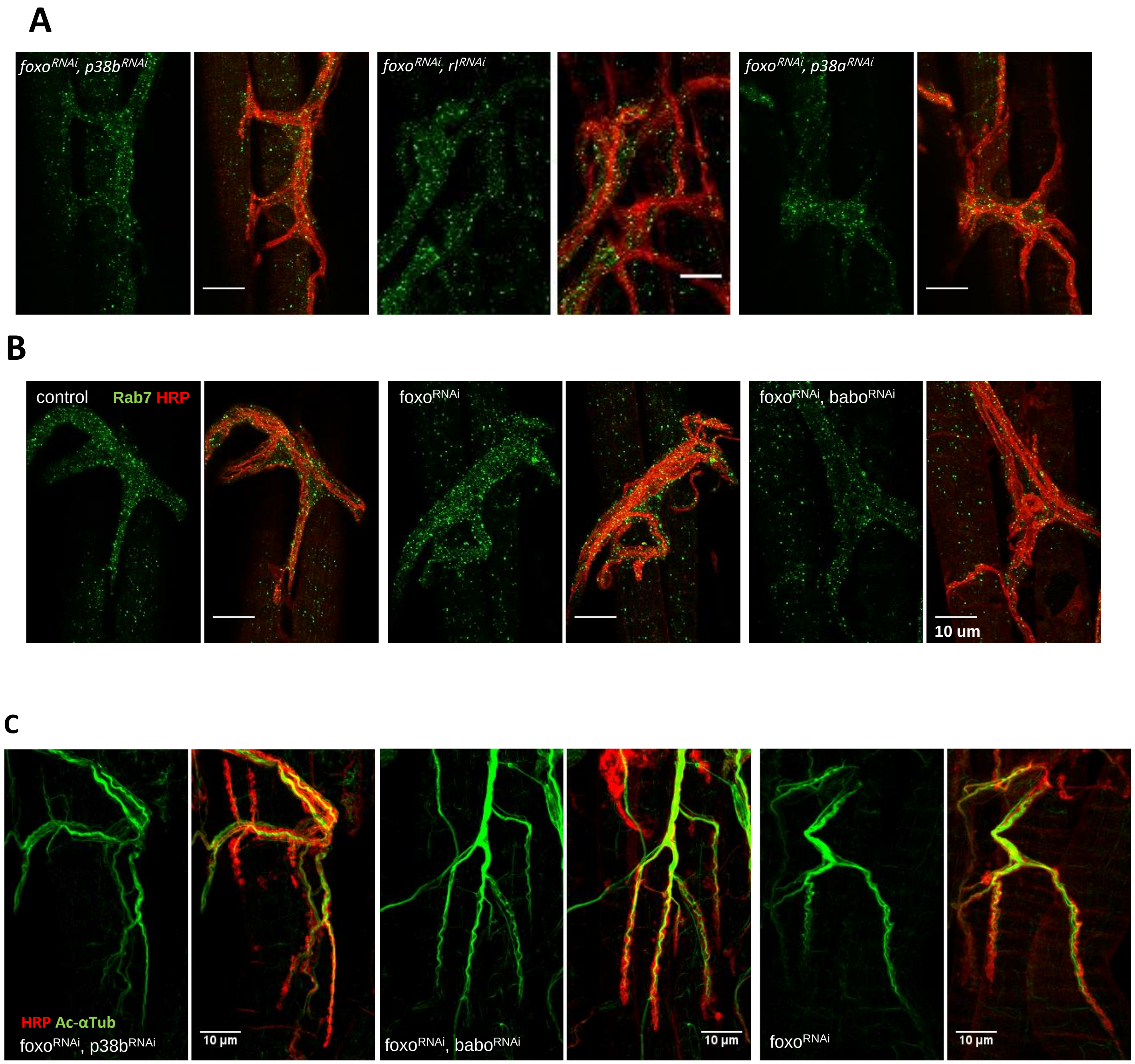

A
*
0.5
0.4
0.3
0.2
0.1
0.0
Relative Intensity (Rab7/HRP)
Control 1wk
foxo-RNAi 1wk
B
n.s
0.8
0.6
0.4
0.2
0.0
Relative Intensity (Rab7/HRP)
Control 2d
foxo-RNAi 2d
C
n.s
3.0
2.5
2.0
1.5
Bouton area (um²)
Control 2d
foxo-RNAi 2d
D
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of branches
53.78%
53.85%
46.22%
46.15%
Control 2d
foxo-RNAi 2d
E
*
100
50
0
% difference from control
control
foxoRNAi
> ATTP40

Figure S5


A
foxo$^{RNAi}$, p38b$^{RNAi}$
foxo$^{RNAi}$, rl$^{RNAi}$
foxo$^{RNAi}$, p38a$^{RNAi}$
B
control
Rab7 HRP
foxo$^{RNAi}$
foxo$^{RNAi}$, babo$^{RNAi}$
10 um
C
HRP Ac-αTub
foxo$^{RNAi}$, p38b$^{RNAi}$
10 µm
foxo$^{RNAi}$, babo$^{RNAi}$
10 µm
foxo$^{RNAi}$
10 µm