**Supplementary Figure 1. Quantification of exocrine pancreas area and relative β-cell mass. A.** *insa*-mCherry expression in β-cells and *ptf1a*-GFP expression in exocrine pancreas in 5dpf Tg(*ptf1a*:GFP/*insa*:mCherry) larvae. **B.** Quantification of the area ($\mu m^2$) of exocrine pancreas from 5 dpf larvae. **C.** Quantification of β-cell mass area relative to area of exocrine pancreas in control and *alms1*-, *bbs1*-, *bbs4*-depleted embryos. **D.** Quantification of β-cell number relative to area of exocrine pancreas in control and *alms1*-, *bbs1*-, *bbs4*-depleted embryos. * indicates p=0.008 and ** represents $p \leq 0.0001$ relative to control after Bonferroni correction for multiple comparisons. $n \geq 20$ per experiment.

**Supplementary Figure 2. Validation of morpholino sensitivity and specificity. (A-C)** Western blot analysis of 5 dpf zebrafish homogenates detecting the expression of Bbs1, Bbs4 or Alms1 proteins. Blot images are representative of two separate experiments per protein. **D.** qRT-PCR quantification of *alms1* transcript relative to *actin* in control and *alms1* morpholino injected embryos at 48 hpf and 5 dpf. **E.** Loss of wildtype *alms1* transcript (2446 bp) and presence of shortened morphant transcript (528 bp) in *alms1* morphants at 2 dpf, 5 dpf and 6 dpf. **F.** Expression of $\Delta 113p^{53}$ transcript relative to *actin* in control, *alms1*, *bbs1* and *bbs4* depleted embryos. Data were represented as percent relative to control. **G.** β-cells in controls, *bbs1* morphants co-injected with *bbs1* mRNA, or *bbs4* morphants co-injected with *bbs4* mRNA. **H.** Quantification of β-cell count in control and rescued embryos. **(I-J)** Area and intensity of mCherry fluorescence in β-cells in 5 dpf F1 progeny of *alms1* or *bbs1* gRNA/Cas9-injected animals. Intensity represents fluorescence intensity per pixel as calculated by ImageJ. (**K-L)** T7-endonuclease

treatment of PCR-amplified target region in *alms1* (K) or *bbs1* (L) gRNA/Cas9-injected embryos (F0) or their progeny (F1). Gel images representative of assays repeated two times. Double bands indicate the presence of mismatch. The arrows indicate the wild type band (top) and the mismatched cleaved band (bottom). * indicates $p<0.001$ and ** represents $p\leq0.0001$ relative to control after Bonferroni correction for multiple comparisons.

**Supplementary Figure 3. Effects of a second splice-blocking *bbs4* morpholino (bbs4 MO2). (A-B)** Area and intensity of β-cell mass in control and *bbs4* CRISPR-targeted animals. **C.** Expression of $\Delta 113p^{53}$ transcript relative to *actin* in control and embryos injected with *bbs4* MO2. Data represented as % relative to control. **D.** mCherry-expressing β-cells in control and *bbs4* MO2-injected animals at 48 hpf or 5 dpf. **E.** Average β-cell counts in control and *bbs4* MO2 animals at 48 hpf and 5 dpf. **F.** *ptf1a*-GFP expression in exocrine pancreas in 5dpf Tg(*ptf1a*:GFP/*insa*:mCherry) embryos. **G.** Quantification of the average area ($\mu m^2$) of exocrine pancreas from 5 dpf embryos. **H.** Quantification of average β-cell number relative to area of exocrine pancreas in control and *bbs4*-depleted embryos. **I.** mCherry-expressing β-cells in 5 dpf controls and morphants grown in the absence and presence of glucose. **J.** Quantification of β-cell number at 48 hpf and 5 dpf under normal and high glucose conditions. Student t-test was performed on the data set. Error bars represent standard deviation. * represents $p=0.0018$ and ** represents $p\leq0.0001$ relative to control after Bonferroni correction for multiple comparisons. $n\geq20$ per experimental group. Scale bars represent 100 μm.

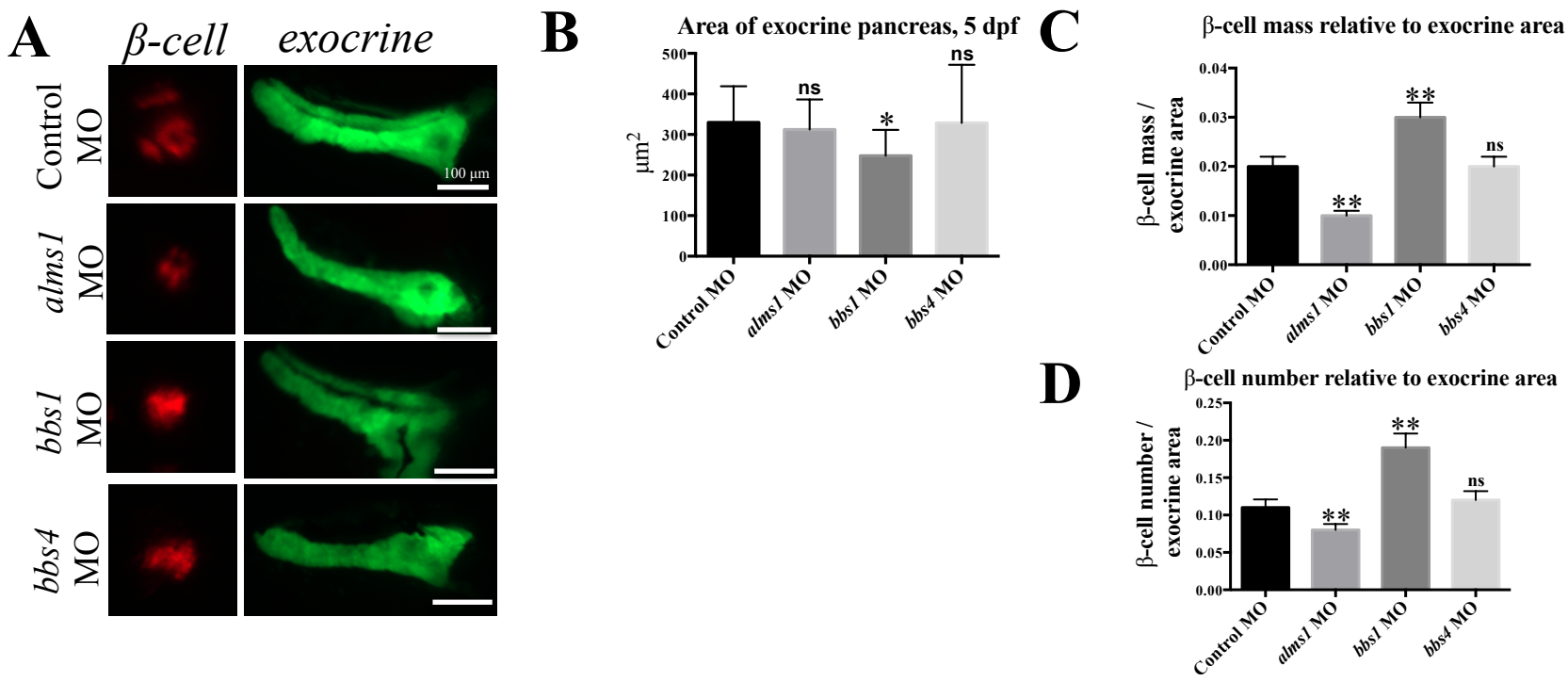
A
β-cell
exocrine
Control MO
almsl MO
bbs1 MO
bbs4 MO
100 µm
B
Area of exocrine pancreas, 5 dpf
µm2
500
400
300
200
100
0
ns
ns
*
Control MO
almsl MO
bbs1 MO
bbs4 MO
C
β-cell mass relative to exocrine area
β-cell mass / exocrine area
0.04
0.03
0.02
0.01
0.00
**
**
ns
Control MO
almsl MO
bbs1 MO
bbs4 MO
D
β-cell number relative to exocrine area
β-cell number / exocrine area
0.25
0.20
0.15
0.10
0.05
0.00
**
**
ns
Control MO
almsl MO
bbs1 MO
bbs4 MO

Figure S1

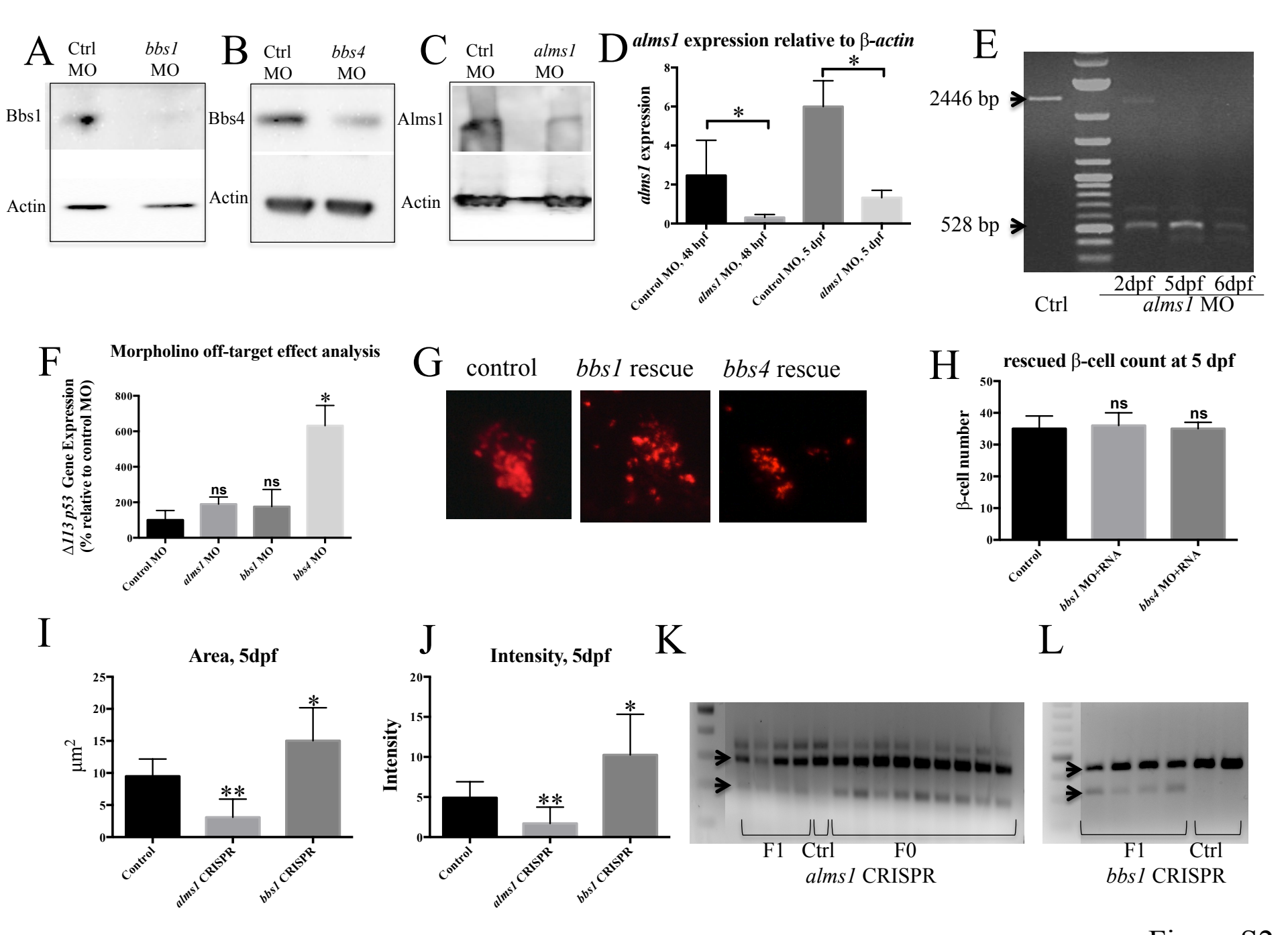
A
Ctrl MO
bbs1 MO
Bbs1
Actin
B
Ctrl MO
bbs4 MO
Bbs4
Actin
C
Ctrl MO
alms1 MO
Alms1
Actin
D
alms1 expression relative to β-actin
alms1 expression
Control MO, 48 hpf
alms1 MO, 48 hpf
Control MO, 5 dpf
alms1 MO, 5 dpf
E
2446 bp
528 bp
2dpf 5dpf 6dpf
Ctrl
alms1 MO
F
Morpholino off-target effect analysis
Δ113 p53 Gene Expression (% relative to control MO)
Control MO
alms1 MO
bbs1 MO
bbs4 MO
ns
G
control
bbs1 rescue
bbs4 rescue
H
rescued β-cell count at 5 dpf
β-cell number
Control
bbs1 MO+RNA
bbs4 MO+RNA
I
Area, 5dpf
μm2
Control
alms1 CRISPR
bbs1 CRISPR
J
Intensity, 5dpf
Intensity
K
F1 Ctrl F0
alms1 CRISPR
L
F1 Ctrl
bbs1 CRISPR

Figure S2

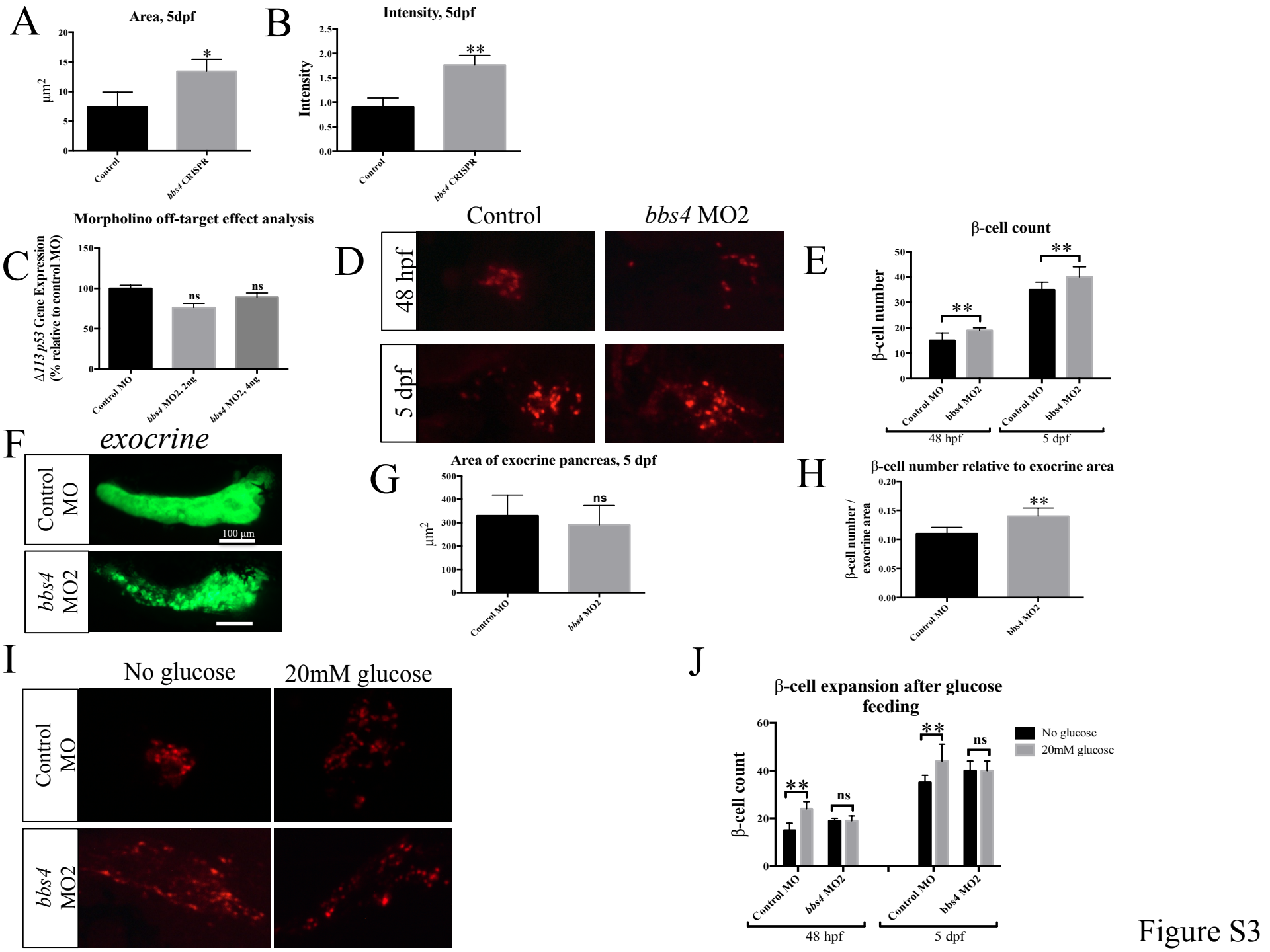
A
Area, 5dpf
μm2
Control
bbs4 CRISPR
B
Intensity, 5dpf
Intensity
Control
bbs4 CRISPR
C
Morpholino off-target effect analysis
Δ113 p53 Gene Expression (% relative to control MO)
ns
ns
Control MO
bbs4 MO2, 2ng
bbs4 MO2, 4ng
D
Control
bbs4 MO2
48 hpf
5 dpf
E
β-cell count
β-cell number
Control MO
bbs4 MO2
48 hpf
5 dpf
F
exocrine
Control MO
bbs4 MO2
100 μm
G
Area of exocrine pancreas, 5 dpf
μm2
ns
Control MO
bbs4 MO2
H
β-cell number relative to exocrine area
β-cell number / exocrine area
Control MO
bbs4 MO2
I
No glucose
20mM glucose
Control MO
bbs4 MO2
J
β-cell expansion after glucose feeding
β-cell count
No glucose
20mM glucose
Control MO
bbs4 MO2
48 hpf
5 dpf

Figure S3