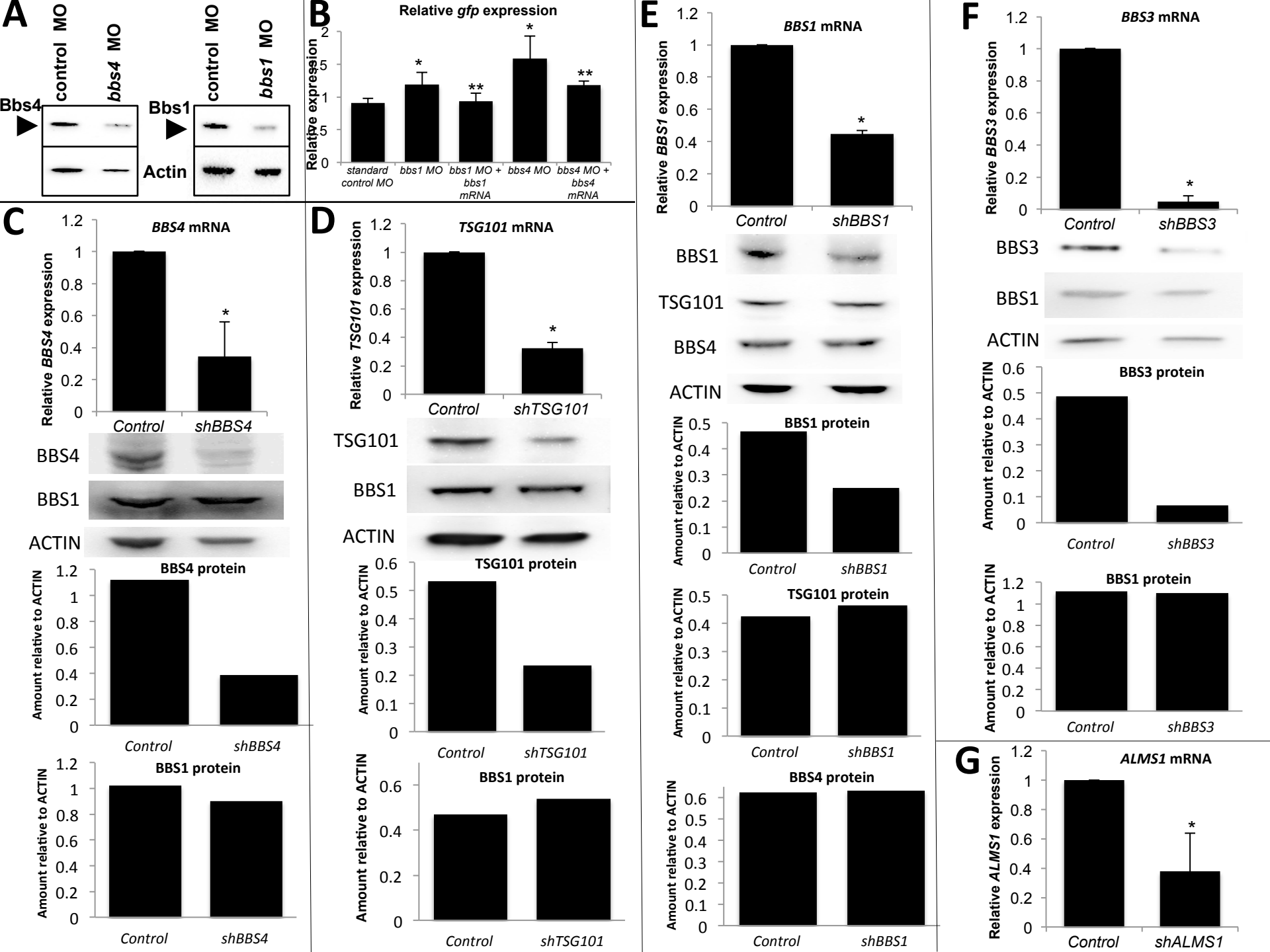
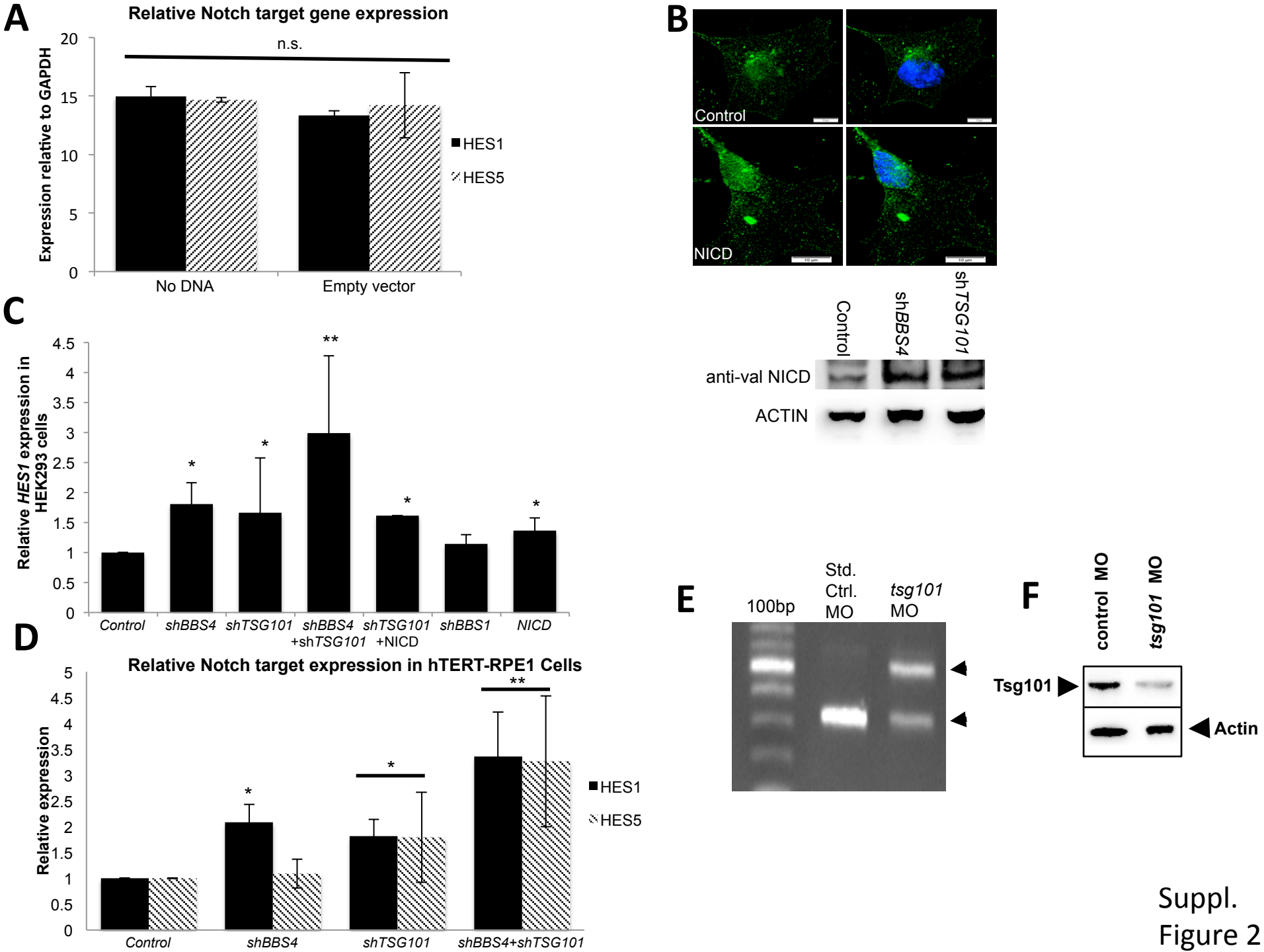
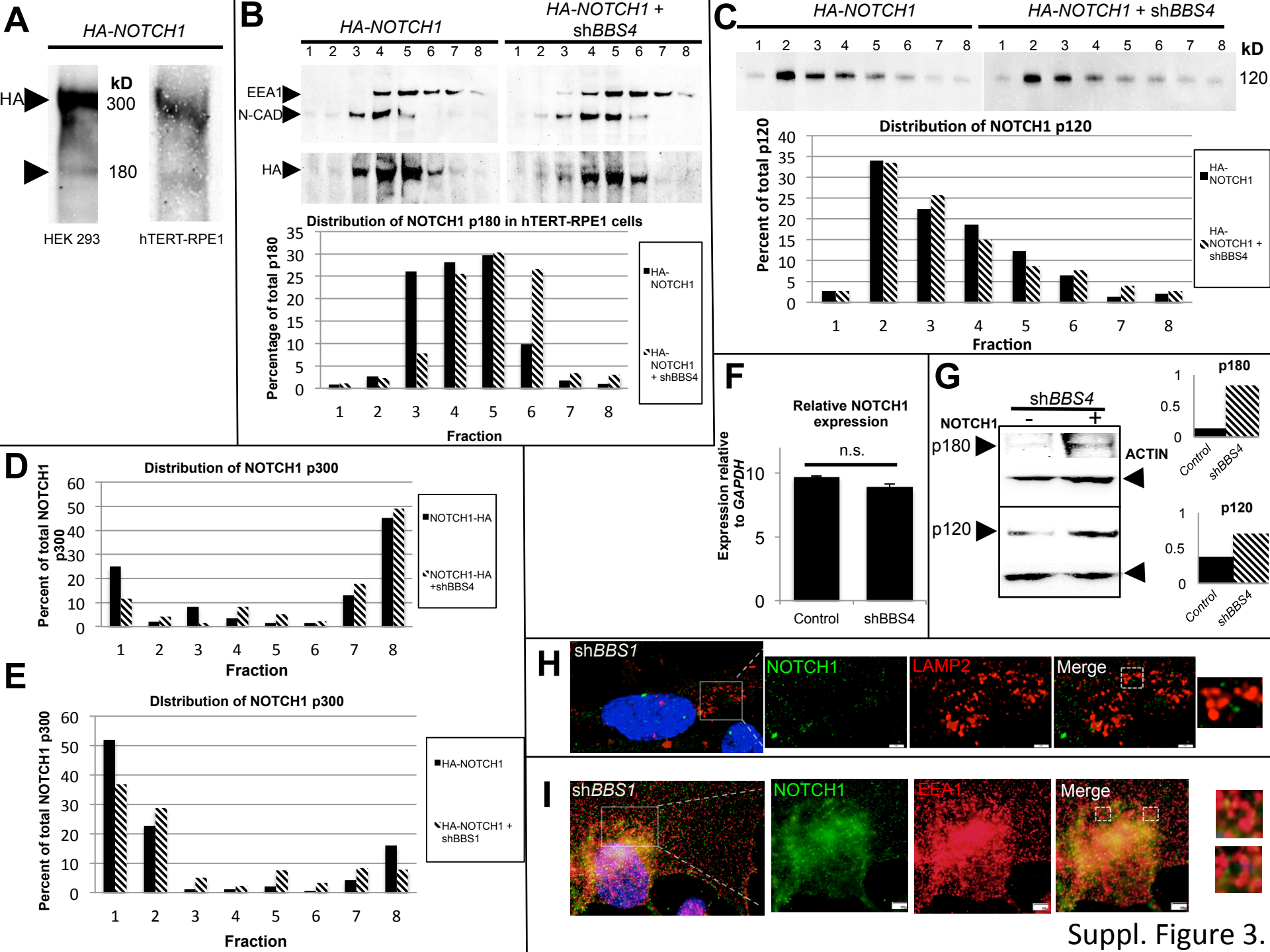
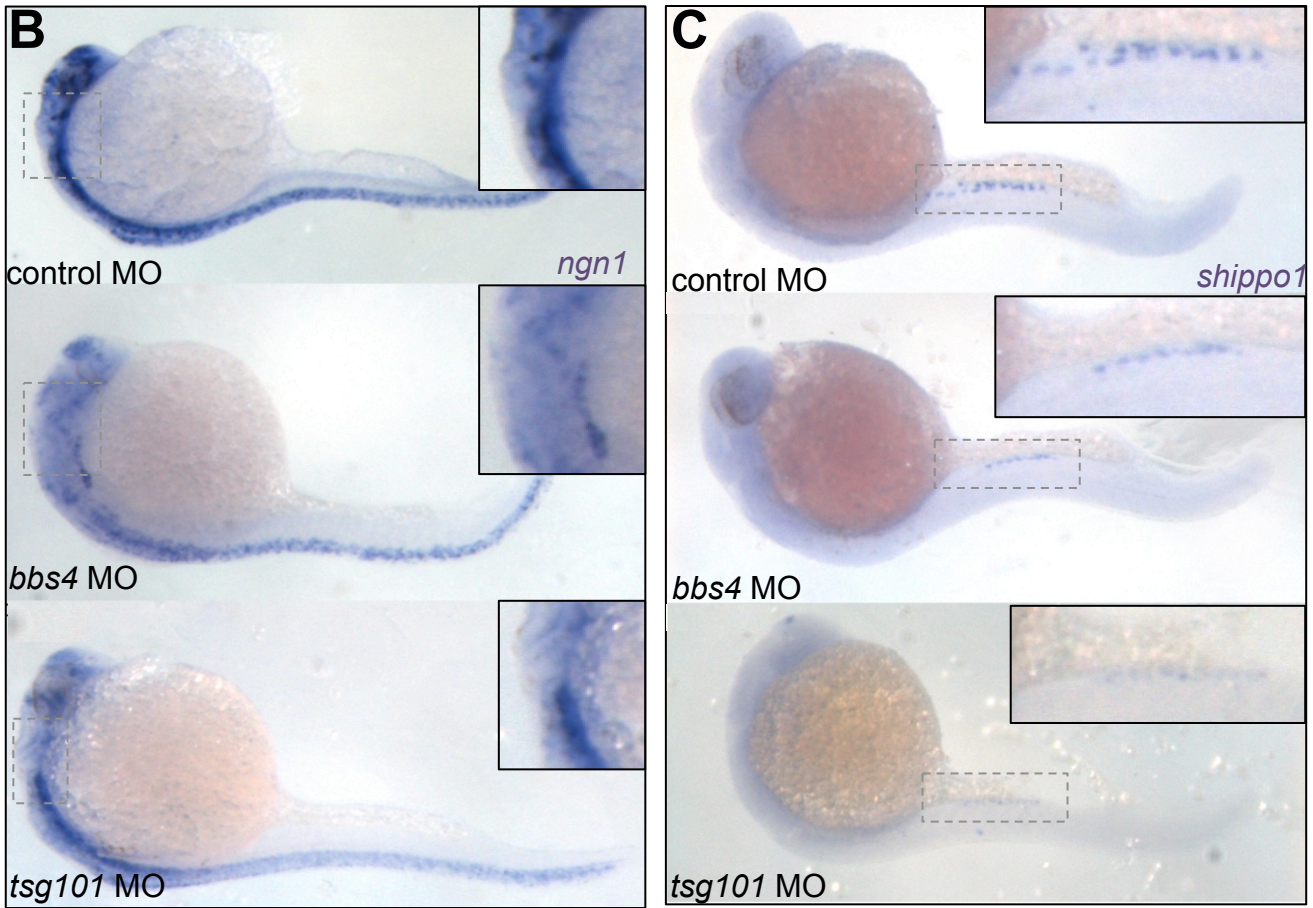

A
control MO
bbs4 MO
control MO
bbs1 MO
Bbs4
Bbs1
Actin
B
Relative gfp expression
Relative expression
standard control MO
bbs1 MO
bbs1 MO + bbs1 mRNA
bbs4 MO
bbs4 MO + bbs4 mRNA
C
BBS4 mRNA
Relative BBS4 expression
Control
shBBS4
BBS4
BBS1
ACTIN
BBS4 protein
Amount relative to ACTIN
BBS1 protein
D
TSG101 mRNA
Relative TSG101 expression
shTSG101
TSG101
TSG101 protein
E
BBS1 mRNA
Relative BBS1 expression
shBBS1
BBS1 protein
F
BBS3 mRNA
Relative BBS3 expression
shBBS3
BBS3
BBS3 protein
G
ALMS1 mRNA
Relative ALMS1 expression
shALMS1



Suppl. Figure 2.



Suppl. Figure 3.



Suppl. Figure 4.

Supplementary Figure 1. Knockdown verification in zebrafish embryos and short hairpin transfected cells. (A) Western blot analysis of homogenates from zebrafish embryos injected with control MO compared to *bbs4* or *bbs1* MO probed with antibodies for either protein. (B) qRT-PCR analysis of relative *gfp* mRNA expression in *bbs* morphants and MO+mRNA injected embryos. *significant difference (p≤0.01; student's t-test) compared to standard control MO. **significant difference from MO (p≤0.01; student's t-test). (C-G) Cells transfected with short hairpins against (C) *BBS4,* (D) *TSG101,* (E) *BBS1,* (F) *BBS3* or (G) *ALMS1* exhibit decreased mRNA levels, detected by qRT-PCR relative to GAPDH *significant (p≤0.005; student's t-test) from control. Proteins targeted by each short hairpin were also decreased, as detected by western blot. Protein levels of other tested proteins were not changed. Protein quantification reflects quantification of bands by ImageJ gel analysis relative to Actin.

Supplementary Figure 2. Amplification of Notch signaling in cultured cells. (A) Expression of *HES1* (solid bars) or *HES5* (striped bars) relative to GAPDH in cells transfected with No DNA or transfected with empty vector. n.s.=no significant difference. (B) Control or NICD-transfected hTERT-RPE1 cells immunostained with antibody directed against the C-terminal protion of NOTCH1. Western blot shows protein levels of activated cleaved-NICD detected using an antibody that recognizes valine 1744 of NOTCH1-NICD. Scale bar = 10 μm. (C) Relative *HES1* expression in HEK293 cells expressed as fold-change expression relative to control. *significant difference (p≤0.01; student's t-test) relative to control cells. **significant difference (p≤0.01; student's t-test) compared to either sh*BBS4* or sh*TSG101* alone. (D) Relative *HES1* or *HES5* expression in hTERT-RPE1 cells expressed as fold-change expression relative to control. *significant difference (p≤0.005; student's t-test) in relative to control cells. **significant difference compared to either sh*BBS4* or sh*TSG101* alone. Error bars indicate standard deviation. (E) RT-PCR amplification from standard control MO and *tsg101* MO injected embryos. Primers target sequences in exon 3 and exon 5. Arrowheads indicate bands at 300 and 500 bp. Intron 4 (included intron) is

212bp. (F) Western blot analysis of homogenates from zebrafish embryos injected with control MO compared to *tsg101* MO probed with antibodies for Tsg101 protein.

Supplementary Figure 3. Notch receptor fragment localization and accumulation in *BBS*-depleted cells. (A) Western blot detection of HA in whole cell lysate from HA-NOTCH1 transfected HEK293 or hTERT-RPE1 cells showing detection of full-length p300 protein and post-S1 cleavage p180 fragment. 30µg protein loaded, quantified by BCA assay. (B) Sucrose gradient fractionation of hTERT-RPE1 cells transfected with *HA-NOTCH1* alone or *HA-NOTCH1*+sh*BBS4* and quantification of the percentage of p180 fragment found in each lane relative to total across all lanes. (C) Sucrose gradient fractionation of HEK293 cells transfected with *HA-NOTCH1* alone or *HA-NOTCH1*+sh*BBS4* probed with antibody against the C-terminal domain of NOTCH1. Chart shows quantification of band in each fraction represented as a proportion of the total across all fractions. (D-E) Quantification of the full length p300 NOTCH1 protein in each sucrose gradient fraction represented as a proportion of the total across all fractions. **(**F) qRT-PCR detection of *NOTCH1* mRNA in HEK293 cells relative to GAPDH mRNA. n.s., no significant difference from control. Error bars indicate standard deviation. (G) Western blot detection of N-terminal (p180) NOTCH1 receptor fragment by HA detection in HA-NOTCH1-transfected cells and detection of C-terminal domains (p120) of endogenous NOTCH1 in whole cell lysate of hTERT-RPE1 cells transfected with or without sh*BBS4*. Graphs show the quantification of band intensity for each, measured using ImageJ software, relative to Actin. (H) Double immunostaining of hTERT-RPE1 cells for NOTCH1 (green) and the lysosomal marker, LAMP2 (red), at 100X magnification with enlarged regions denoted by white box and enlarged individual vesicles denoted by dashed box. (I) Double immunostaining of hTERT-RPE1 cells with NOTCH1 (green) and the early endosomal marker, EEA1 (red). Scale bars = 2 µm.

Supplementary Figure 4. Notch1 localization in ciliated and unciliated cells and suppression of Notch-inhibited cell type markers in *bbs4* morphants. (A) hTERT-RPE1 cells doubled immunostained for detection of NOTCH1 (green) and ARL13B (red) and transfected with or without sh*BBS4.* 40X magnification. Cilia in ciliated cells (+) magnified in inset boxes. Scale bars = 20 μm. (B) Expression of *neurogenin1* in control MO-, *bbs4* MO- and *tsg101* MO-injected embryos at 24 hpf detected by whole mount *in situ* hybridization. (C) Expression of *shippo1* in control MO-, *bbs4* MO- and *tsg101* MO-injected embryos at 24 hpf. Insets indicate magnification of dashed boxes.

Supplementary Table 1. Proteins targeted and assayed

Protein	Structure
BBS1	Basal body protein; BBSome component
BBS4	Basal body protein; BBSome component
BBS3	Basal body protein; not part of BBSome
ALMS1	Basal body protein; not part of BBSome
TSG101	ESCRT protein; MVB marker
NOTCH1	Notch receptor
DeltaA	Ligand to Notch receptor
NICD	Activated/signaing portion of Notch receptor
N-CADHERIN	Plasma membrane marker
EEA1	Early/sorting endosome marker
RAB11	Recycling endosome marker
RAB7	Late endosome marker
ARL13B	Ciliary axoneme marker
Acteylated tubulin	Ciliary axoneme marker
γ-tubulin	Basal body marker