

Supporting Information

Supplemental Table

Lrp (#1)	P'type in 1° Scrtn	ΔNLrp6	fold change	no induc	fold change	predicted/determined localization
CG1135	<i>increased</i>	334	3.5	320	3.2	nucleus
CG18041	<i>increased</i>	302	3.2	339	3.4	unknown
Bx42	<i>increased</i>	290	3.0	267	2.7	nucleus
bel	<i>increased</i>	281	3.0	323	3.2	nucleus/cytoplasm
CG31992	<i>increased</i>	257	2.7	136	1.4	cytoplasm
Trn	<i>increased</i>	243	2.6	248	2.5	nuclear
CG6686	<i>increased</i>	242	2.5	213	2.1	membrane/cytoplasm
CG1017	<i>increased</i>	229	2.4	160	1.6	nucleus/cytoplasm
CG15432	<i>increased</i>	219	2.3	757	7.6	cytoplasm
CG11848	<i>increased</i>	218	2.3	144	1.4	extracellular
CG16903	<i>increased</i>	218	2.3	139	1.4	unknown
Pp1-13C	<i>increased</i>	218	2.3	651	6.5	plasma
CG8435	<i>increased</i>	202	2.1	213	2.1	membrane/cytoskeleton
ash1	<i>increased</i>	199	2.1	93	0.9	nucleus/cytoplasm
Fs(2)Ket	<i>increased</i>	199	2.1	157	1.6	nucleus
Hsc70-3	<i>increased</i>	198	2.1	136	1.4	nucleus/cytoplasm
enok	<i>increased</i>	198	2.1	171	1.7	ER, plasma membrane, nucleolus, cytoplasm
						nucleus

Table S1.

Table showing dsRNA targeting eighteen genes with known human orthologs that increased reporter activation two-fold or greater.

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(0.01 MB DOC)

A

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mouse      1  -----EAGG-----
human      1  -----DDEG-----
zebrafish  1  -----EADD-----
fly        1  MAASGNASCLGGLPDMGANMMSNASALCPLSDLRSPSGVLTNSSTYMPQHKKCNNDASQ
worm       1  -----MDAPLNDNSTIGLVEGRKRLSETRNPGLPTLPN-----YGLKDKSGGCI

mouse      7  -----NAGQGPAERCHRSVSVGARAAD-----VLVTLADDEVVPAVCHTSSISAH
human      7  -----SAGQGPAERCHRSVSVGARAAD-----VLVTLADDEVVPAVCHTSSISAH
zebrafish  8  -----FPPESEHSLCQGGVAVSVTRAAD-----LVVTLANDSAVHTLGGGCMNQ
fly        61 NPAIQKRLMATSSSTSNFATAGKAAANLTRLQNVIPTCVYMSRVAVHMEIGTAGCPSQ
worm       45 MPWTPIGQLRATHNQSTETLGSPPVKKFSIYFSRGERLSNEENGIEENISGGKTDATK

mouse      56 ELHRAVE-----VYQIDFVLEAFALMLVSPLELVQKKPKOPYLCHOMPELLAFPSA
human      56 ELHRAVE-----VYQIDFVLEAFALMLVSPLELVQKKPKOPYLCHOMPELLAFPSA
zebrafish  57 ELGSEV-----SALNIDNSAADVFAPFCSELLEQKKPKOPYLCHOMPELLAFPSA
fly        121 VMLAALGCEELGINKLLAQSVGLMTALLESQLAHNCPIIVRVANENLAKQNSNS
worm       105 KPLFHLAK-----CGIDYSAFSEMFALMISPLLEVQLSYHNPCEKQGSTATEFAKE

mouse      112 SDDOVA-MDEPSDFARNVFFFRRELOIDE-----VLRLITEAKGVLTARTFCDLDD
human      112 PDDOVA-MDEPSDFARNVFFFRRELOIDE-----VLRLITEAKGVLTARTFCDVDD
zebrafish  113 PTEDIS-LDEPSDFARNVFFFRRELOIDE-----VLRLITEAKGVLTARTFCDPH
fly        181 SPSDRK-FDEPMIVLRNVVFSKDDSEKMDHR-----IDLLITEARNVLTGATIMEPH
worm       162 IDIQLFNQDEPMIVLRNVVFSKDDSEKMDHR-----IDLLITEARNVLTGATIMEPH

mouse      168 CEVLGGVYCEVVOGPTQPGQPAACDIL-EKIDSLFPAHCHRGHFAAFKRGAKTSPG
human      168 CEALGALVCEVVOGPTQPGQPAACDIL-EKIDSLFPAHCHRGHFAAFKRGAKTSPG
zebrafish  169 WETLGAALSCAIEGTELDQALTAALERKLSLFPAAALCGGFLSTKRGKGNNAEM
fly        237 SLMGGGQALIEGPTNSHTHTIGFPP-ENQARLPPVAAGSTWMLPISQKNS---A
worm       222 TIRVALPLFCLQFQNFDETRHDVAFIL-NHIDEILERTCDSIVATTIFGKAINKKT-VQ

mouse      227 EGGALNAYRQVKEVTGNNSEREATLGSHYATLLKCHLFFPGCAFFGEVDKP-AQGFL
human      227 EGGALNAYRQVKEVSSDGG-CEAALGTHYATLLKCHLFFPGCAFFGEVDKP-AQGFL
zebrafish  229 EONLVKECSVCSSAASGS--SQEPIALLOVYRSCHNLFFPGCAFFGEVDKP-AQGFL
fly        292 EVKLEQFKRVPQTATRK-----LMRK--TFEFCWALFFPGCAFFGEVDKP-VRGIM
worm       280 ERLVSEKKNISKIGDKSK-----KENDLLMELTONSSCTGAAHLSAHDEVKRRAST

mouse      286 HRCGRK--PVVAISLEG-VHVIDNREKVVLLGLRFQELSWDETSFEE-----EPVLM
human      285 HRCGRK--PVVAISLEG-VHVIDNREKVVLLGLRFQELSWDETSFEE-----EPVLM
zebrafish  286 HRCGRK--PVVAISLEG-VHVIDNREKVVLLGLRFQELSWDETSFEE-----EPVLM
fly        343 ALVHQDMEVLLVNERQ-VFVIDPYCTELGLRLQELSWDYAKSASDDPECLTCEFL
worm       333 RNFCHGFAIEVVGINDRFLTVESNSGTLVHPLESITVLEGLQPDQMP--YLVN

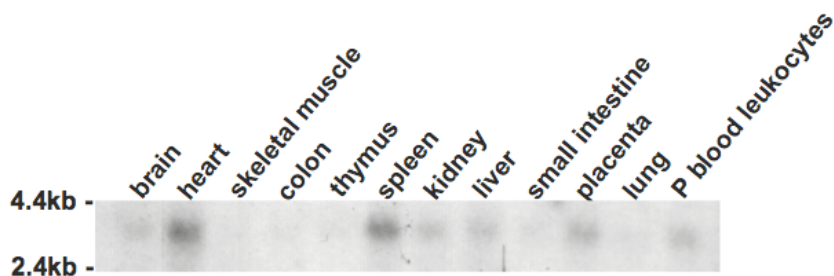
mouse      338 EFDQDS-EQTFVKKLLRITSKQAEMLSGLIECIEFSQAAPTLSESASGPHEAPSPSP
human      337 EFDQDS-EQTFVKKLLRITSKQAEMLSGLIECIEFSQAAPAGPDSATGSPSPSSSL
zebrafish  340 EFDQEE-AQTFVKKLLRITSKQAEMLSGLIECIEFSRVSLSAATGTDGEVTFSHFPTSP
fly        402 QFDAVE-NGIQSKLMOHFSKQAMIDALLSHFTDQIRNKK---QEGNTQEFHDEPNP
worm       391 SFEIEIKNGNSTIDISNETTQFLMITQNTFLIDALLKMTGRRLDRSSSDSSSSSDSS

mouse      397 PPAQRP-----LLRRQGSVVCRRQHLSTIDIVEDGCGIRVKKPKRTSFFSRQLSSSQ
human      396 APVQRP-----LLRRQGSVVCRRQHLSTIDIVEDGCGIRVKKPKRTSFFSRQLSLGQ
zebrafish  399 ETNNKTRERRQG-LLRRQGSVVCRRVHSLSTINIVDGGCHRLNDPRAASFFRQAQPP-
fly        457 IQNNGN-----C-VLCNKLRLATLFDGGRICIGQMSLSIGT
worm       451 TSSTKH-----SRIEKLDFKNCTAGCNKRLCFAKFQGGKCEVAGSFKAFTS

mouse      451 GSTVVCPTDSDLEQS
human      450 GSTVVCPTDSDLEQS
zebrafish  458 -TISAVCVTESLEQS
fly        -----
worm       500 HGOGL-----

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B



C



Figure S1.

(A) Sequence alignment of Bili protein shows it is well conserved phylogenically. (B) Human Multi Tissue Northern blot show mRNA expression. (C) Illustration of Bili protein structure and domains.

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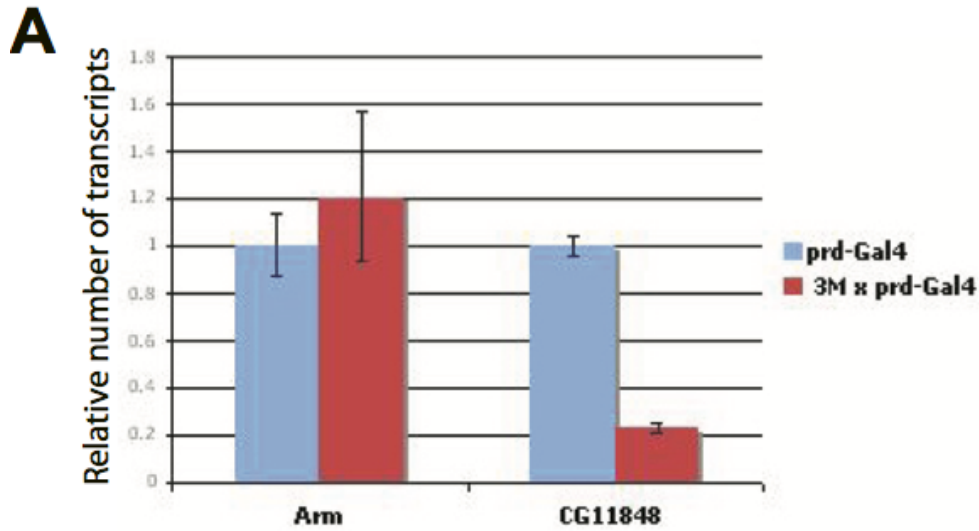


Figure S2.

(A) qRT-PCR analysis of dBili knockdown. Expression of short-hairpin (shRNA) dbili (#3M) under the control of prd-GAL4 in the *Drosophila* embryo results in >75% knockdown in message level (Red bar) as compared to prd-GAL4 control embryos (Blue bar). As a control, Armadillo mRNA was not affected in embryos expressing dBili shRNA. (B) Embryos expressing dBili shRNA under the control of even skipped stripe 3/7-GAL4: These embryos display a partial lack/disruption of the 2nd or 3rd denticle belt which coincides with the eve-stripe 3 expression. This phenotype is consistent with a localized increase in Wingless signaling activity in the region around eve-stripe 3. The same however was not observed for stripe 7 (posterior end of the embryo), perhaps due to differential expression of GAL4 in stripe 3 versus stripe 7.

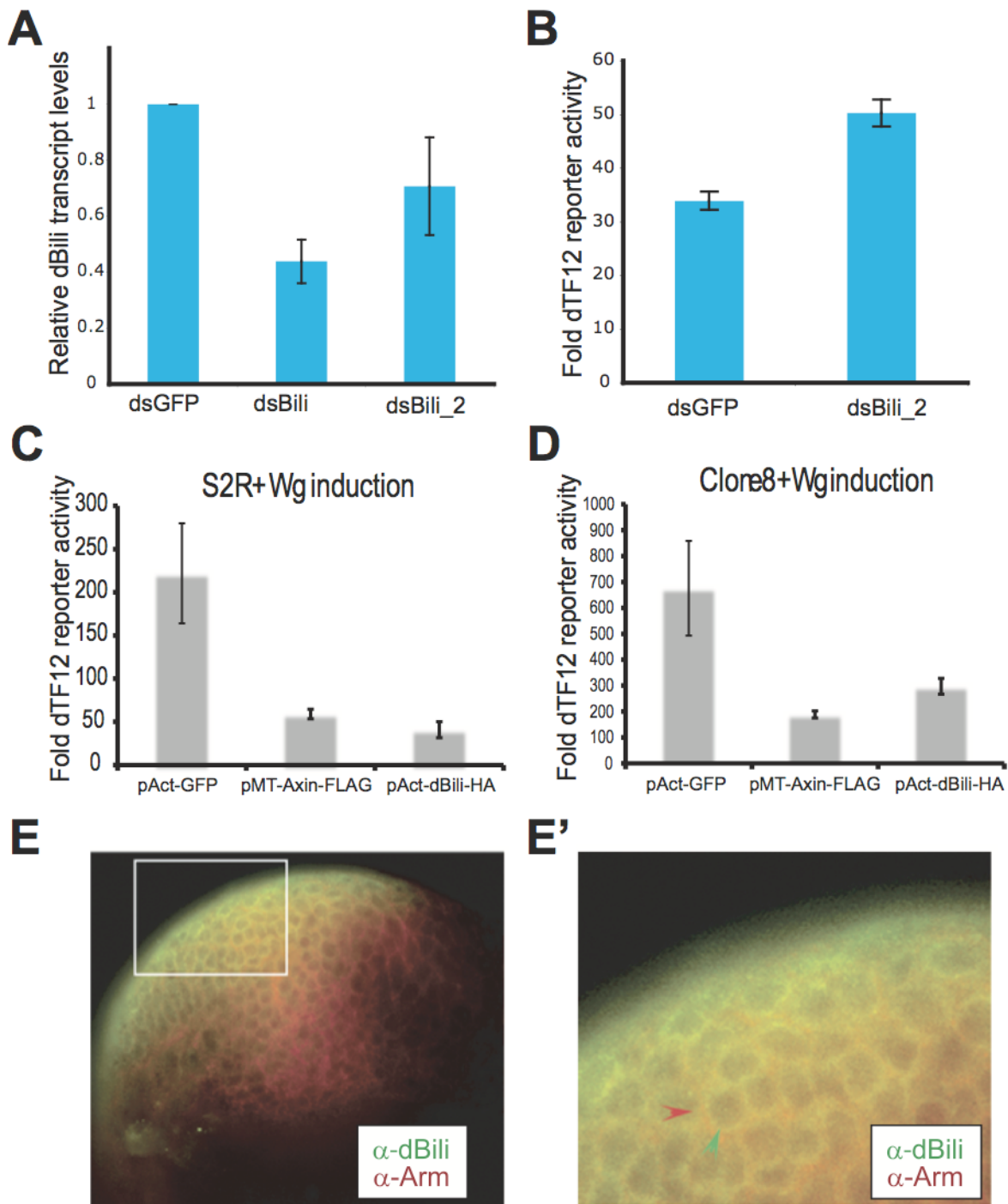


Figure S3.

(A) Knockdown of dBili with a second dsRNA, ds11848_2, revealed only ~30% knockdown of endogenous message compared to the original dsRNA (DRSC1.0-CG11848) which robustly knocked down message levels by >50%. Primer sequence used for the generation of ds11848_2 PCR is as follows: Forward primer: 5'-GTAATACGACTCACTATAGGGAGA GAAGATAACAAGTGAGGCATTC-3' and Reverse primer: 5'-GTAATACGACTCACTATAGGGAGAGGCAAATAAAATATCTGATGGGTGCGTGG-3' (B) Knockdown of dBili with ds11848_2 displayed a modest increase (~35–40%) in dTF12 (Wg-reporter) activity. (C & D) Expression of dBili-HA strongly inhibits dTF12-reporter activity when the pathway is activated by Wg overexpression. The inhibitory effect of dBili is comparable to that of Axin expression in both S2R+ (C) and clone8 (D) cells. (E) dBili protein (in GREEN) is localized in a concentric ring,

just inside and abutting Arm protein (in RED) at the membrane of cells in the drosophila embryo. (E')
Magnitified view of boxed region in panel E.
[doi:10.1371/journal.pone.0006129.s004](https://doi.org/10.1371/journal.pone.0006129.s004)
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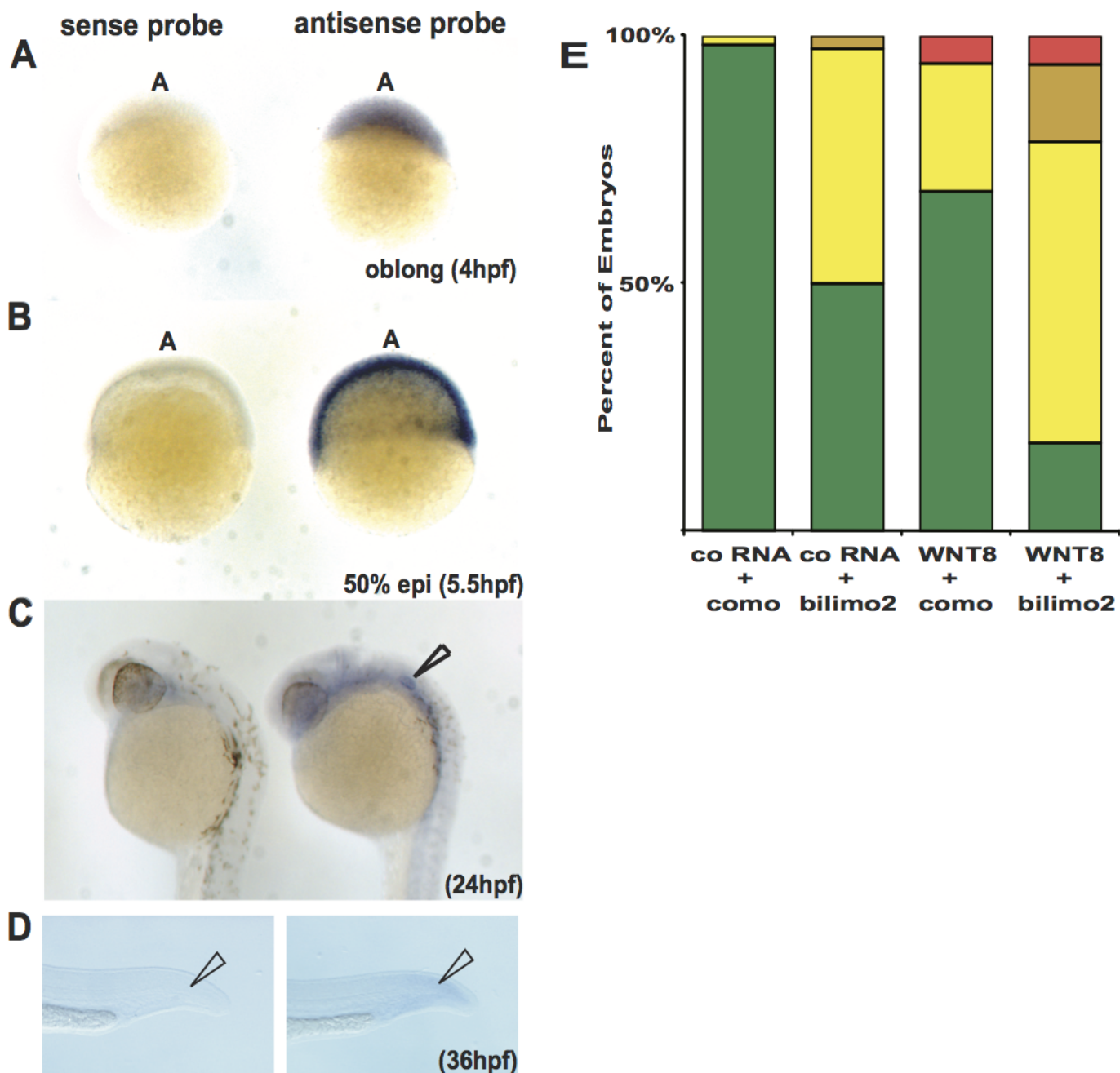


Figure S4.

The zebrafish homolog of Bili (zfBili) is expressed during embryogenesis. In situ hybridization using a sense (left column) and antisense probes (right column) were used to detect mRNA. (A) zfBili is expressed maternally as it is detected in the animal pole 4 h post-fertilization. (B) zfBili continues to be expressed ubiquitously at 50% epiboly. (C) 24 hpf zfBili remains ubiquitous but shows specific staining in the otic vesicle (arrow head). (D) Weak Bili expression in the tail at 36 hpf. (E) A second morpholino targeting zfBili (bilimo2) but not a control morpholino (como) enhances the Wnt8 overexpression phenotype (n=50 for each condition). Embryos were scored as wt (green), small eyes (yellow), no eyes (orange) or severe (red). Data shown is representative from four independent experiments.

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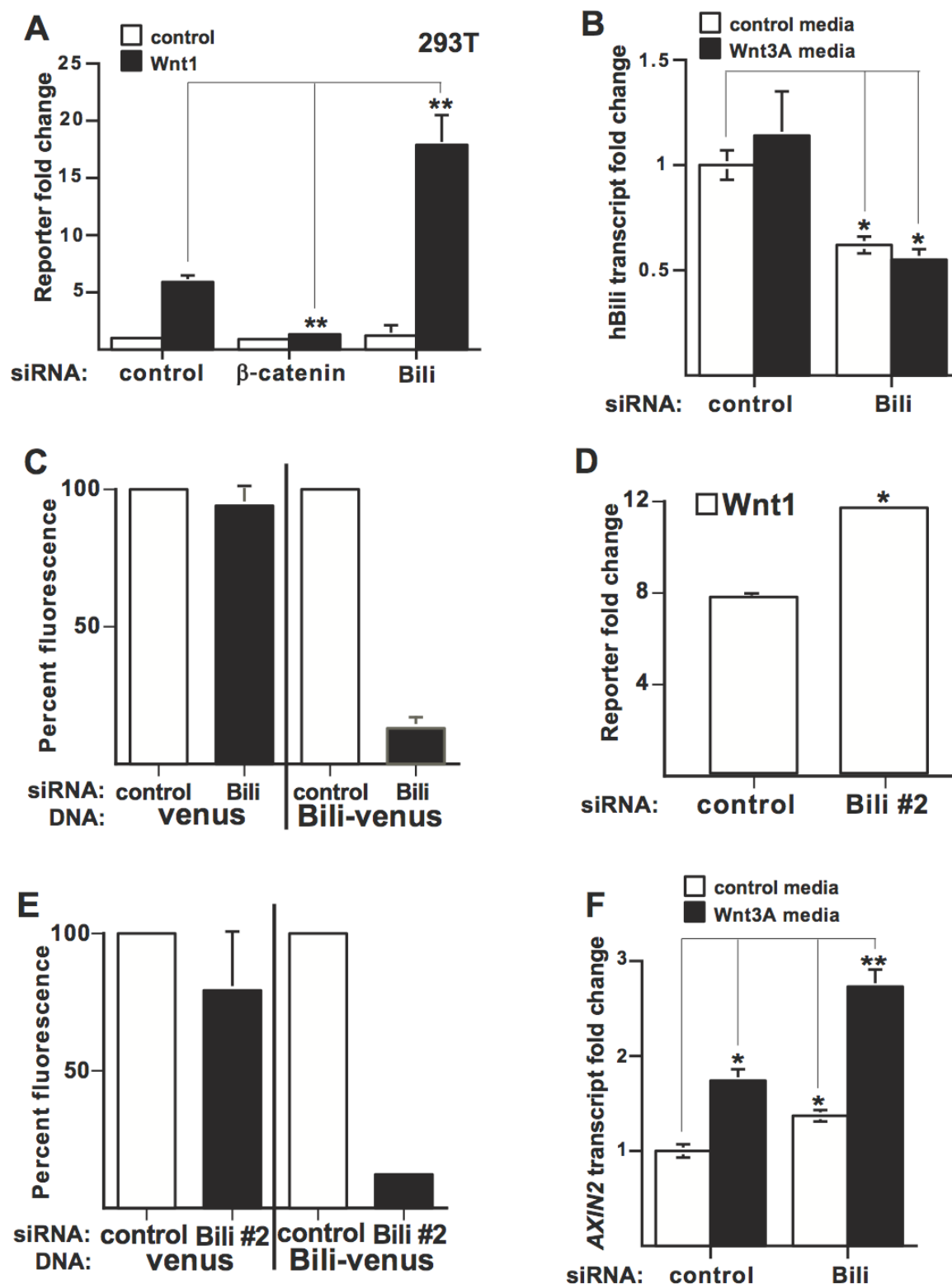


Figure S5.

Bili negatively regulates Wnt/ β -catenin signaling in mammalian cells. (A) siRNA mediated knockdown of hBili enhances Wnt1 mediated induction of a β -catenin responsive reporter. HEK293T cells were transfected with siRNA targeting control, β -catenin, or hBili. Cells were then transfected with WNT1 cDNA and a β -catenin responsive reporter and assayed the following day. (B) hBili siRNA effectively knocked down hBili transcripts in HEK293T as measured by qRT-PCR. (C) hBili siRNA effectively decreased the expression of a hBili-Venus fusion protein as assayed by fluorescence

measurement. hBili siRNA had no effect on Venus expression. (D) A second siRNA (Bili #2) targeting hBili enhanced WNT1 mediated activation of a β -catenin responsive reporter. (E) Bili #2 siRNA also effectively decrease expression of a hBili-Venus fusion protein with no effect on Venus alone. (F) hBili knockdown in HEK293T cells with hBili siRNA enhanced the WNT3A mediated transcriptional induction of endogenous AXIN2 transcripts as assayed by qRT-PCR. Error bars represent STDEV (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$).

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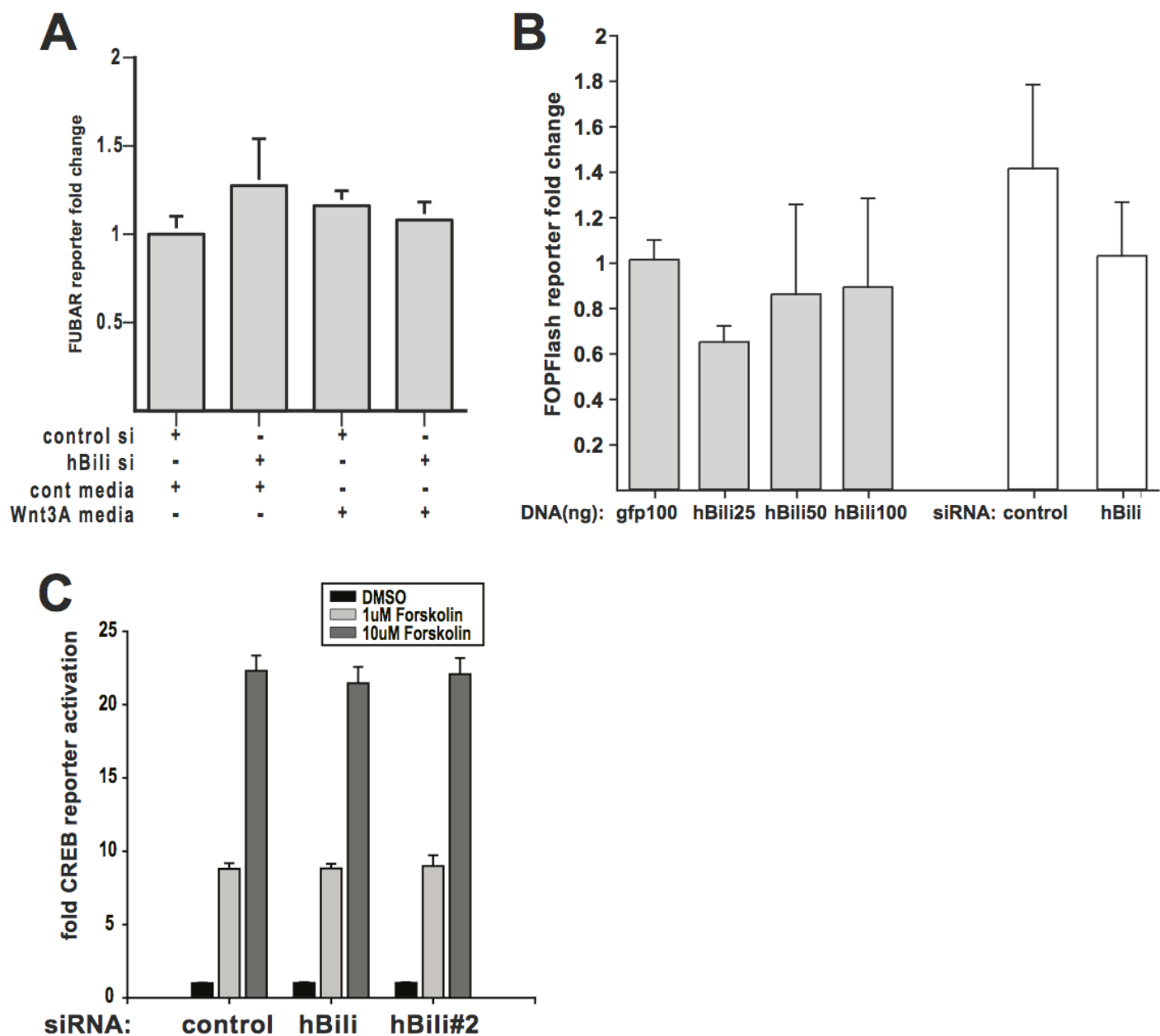


Figure S6.

Bili does not affect control reporters or CREB responsive reporters. (A) HEK293T cells were transfected with FUBAR, a control reporter not responsive to Wnt/ β -catenin signaling, renilla luciferase normalization, and control or hBili siRNA. FUBAR was not responsive to WNT3A conditioned media (right two bars) and hBili siRNA had no effect. (B) HEK293T cells were transfected with FOPFlash, another control reporter not responsive to Wnt/ β -catenin signaling, renilla luciferase normalization, and GFP cDNA or increasing doses of hBili cDNA or control siRNA or hBili siRNA. Neither hBili cDNA control nor hBili siRNA had an effect on the reporter. (C) hBili siRNA HEK293T cells stably expressing a CREB responsive reporter were transfected with renilla luciferase normalization, and control, hBili, or hBili#2 siRNA and treated with DMSO, 1 μ M forskolin, or 10 μ M forskolin. Error bars represent STDEV.

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