Supporting Information

Supplemental Table

Lrp (#1)	P'type in 1˚ Scrn	ΔNLrp6	fold change	no induc	fold change	predicted/determined localization
CG1135	*increased*	334	3.5	320	3.2	nucleus
CG18041	*increased*	302	3.2	339	3.4	unknown
Bx42	*increased*	290	3.0	267	2.7	nucleus
bel	*increased*	281	3.0	323	3.2	nucleus/cytoplasm
CG31992	*increased*	257	2.7	136	1.4	cytoplasm
Trn	*increased*	243	2.6	248	2.5	nuclear membrane/cytoplasm
CG6686	*increased*	242	2.5	213	2.1	nucleus/cytoplasm
ran	***increased***	**229**	**2.4**	**309**	**3.1**	**cytoplasm**
CG1017	*increased*	229	2.4	160	1.6	extracellular
CG15432	*increased*	219	2.3	757	7.6	unknown
CG11848	***increased***	**218**	**2.3**	**144**	**1.4**	**plasma membrane/cytoskeleton**
CG16903	*increased*	218	2.3	139	1.4	nucleus/cytoplasm
Pp1-13C	*increased*	218	2.3	651	6.5	nucleus/cytoplasm
CG8435	*increased*	202	2.1	213	2.1	unknown
ash1	*increased*	199	2.1	93	0.9	nucleus
Fs(2)Ket	*increased*	199	2.1	157	1.6	nucleus/cytoplasm
Hsc70-3	*increased*	198	2.1	136	1.4	ER, plasma membrane, nucleolus, cytoplasm
enok	*increased*	198	2.1	171	1.7	nucleus

Table S1.
Table showing dsRNA targeting eighteen genes with known human orthologs that increased reporter activation two-fold or greater.
doi:10.1371/journal.pone.0006129.s001
(0.01 MB DOC)

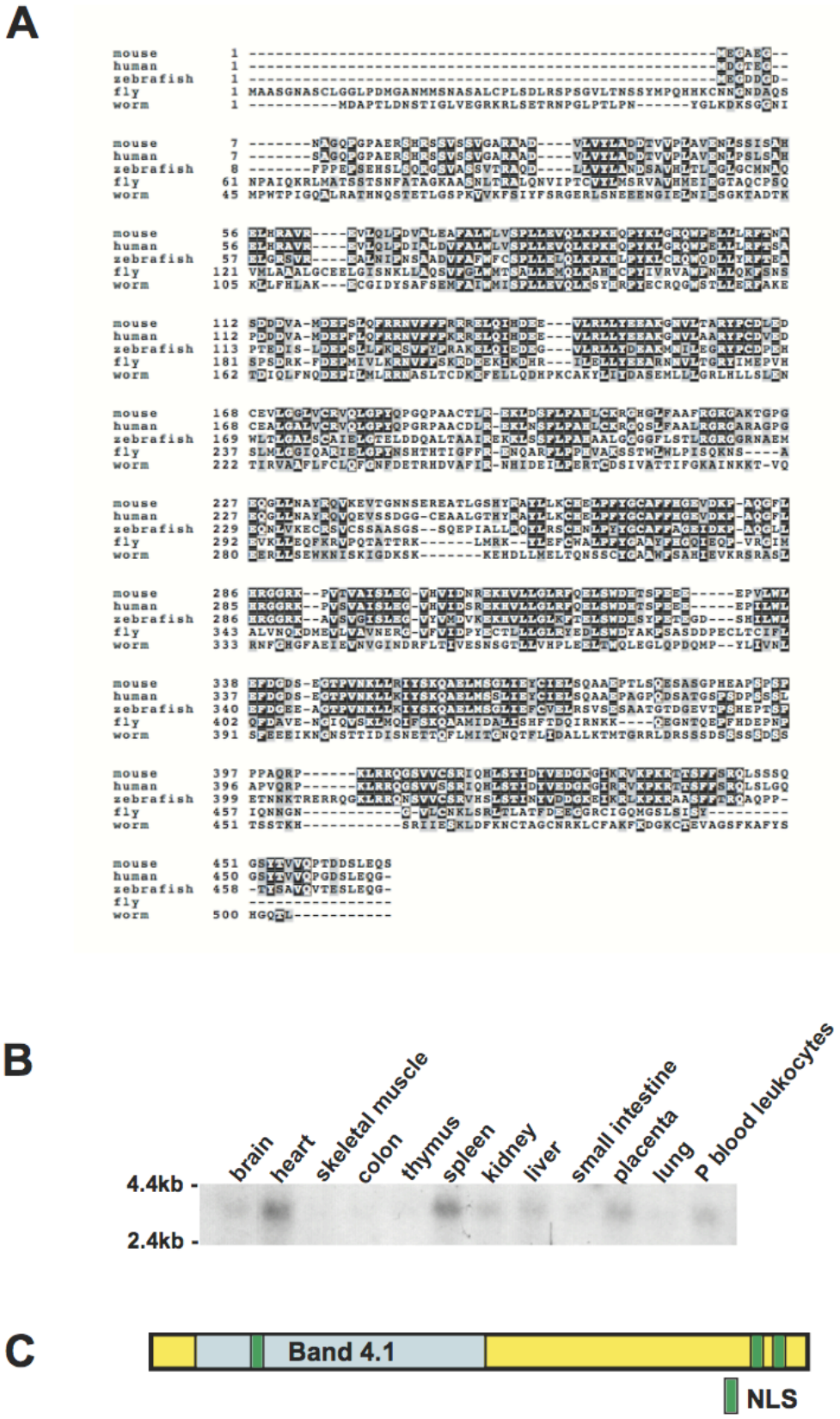

Figure S1.

(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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(2.14 MB EPS)

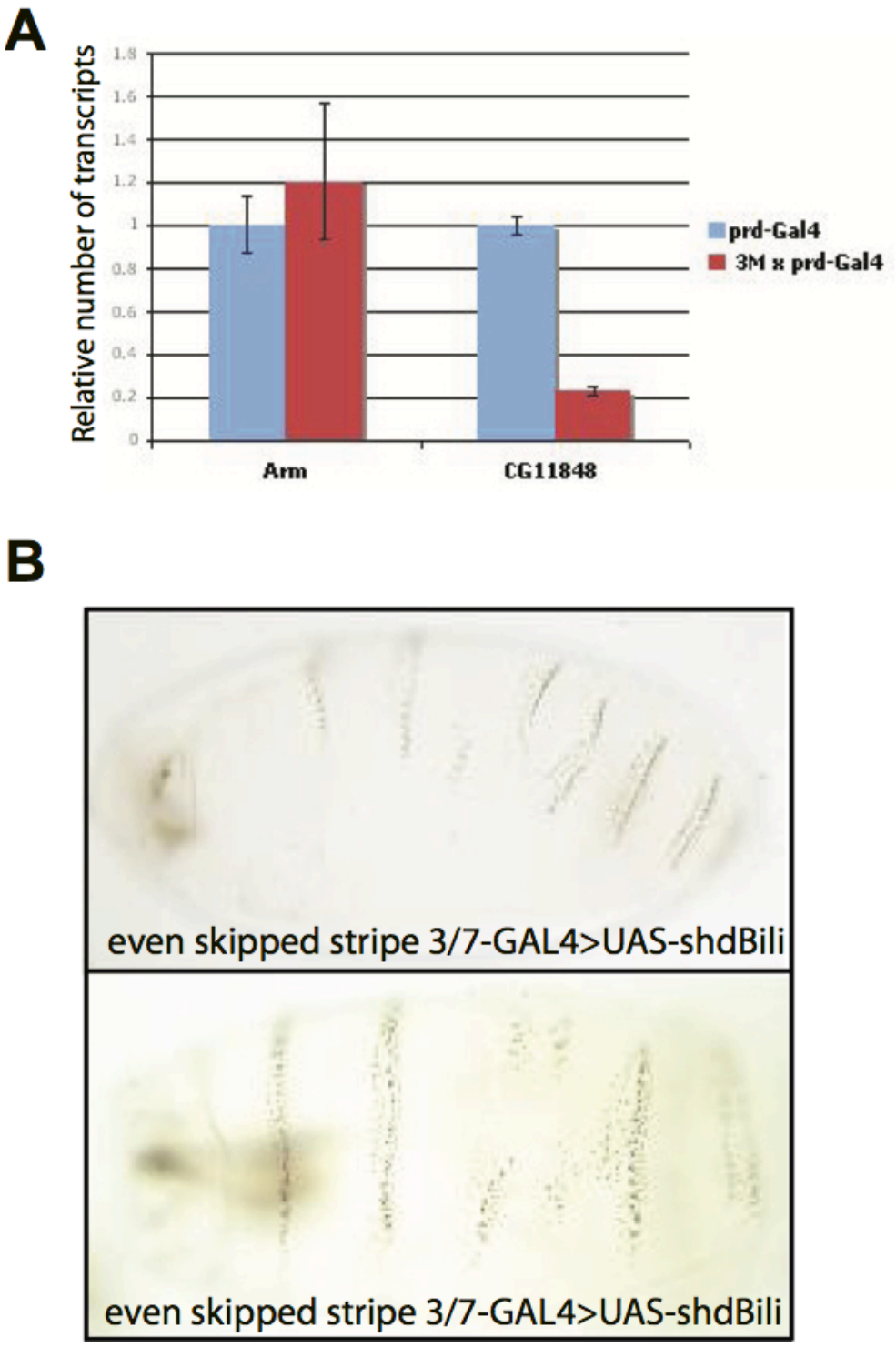

Figure S2.
(A) qRT-PCR analysis of dBili knockdown. Expression of short-hairpin (shRNA) dbili (#3M) under the control of prd-GAL4 in the Drosophila embryo results in >75% knockdown in message level (Red bar) as compared to prd-GAL4 control embryos (Blue bar). As a control, Armadillo mRNA was not affected 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.

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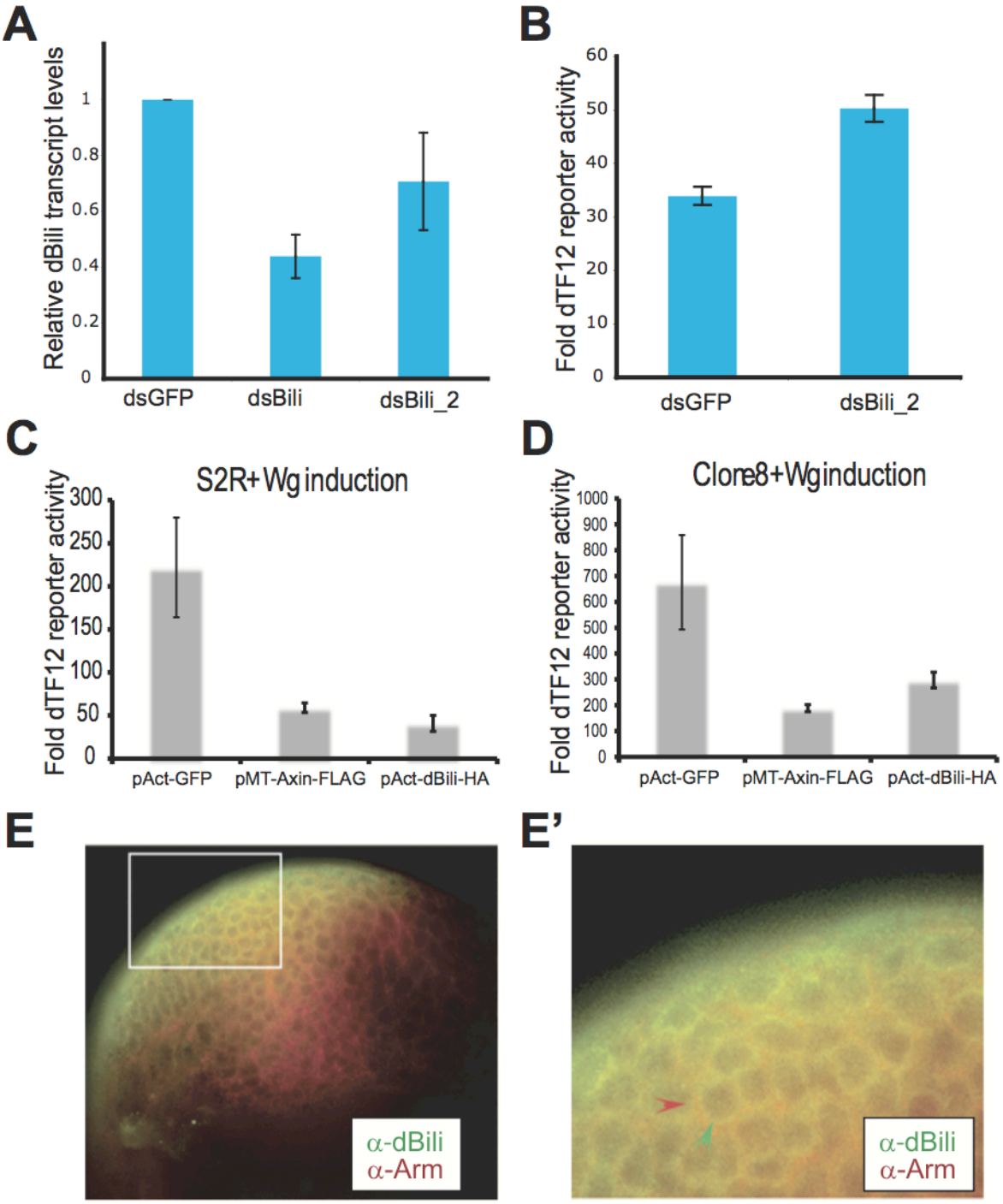

Figure S3.

(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 GAAGATACAAGTGAGGCATTC-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') Magnitifed view of boxed region in panel E.
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(5.47 MB EPS)

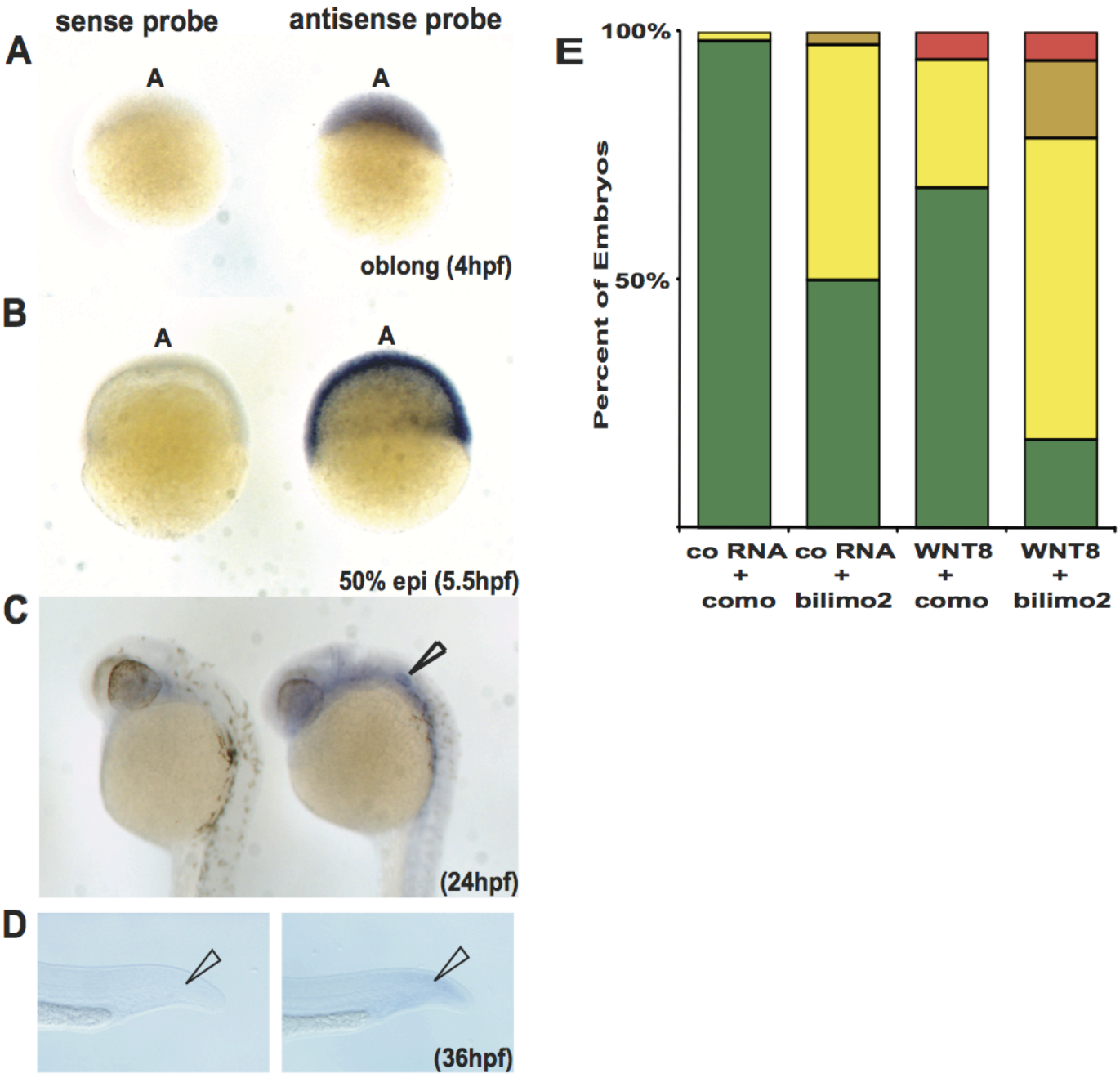

Figure S4.
The zebrafish homolog of Bili (zfBili) is expressed during embryogenesis. In situ hybridization using a sense (left column) and antisense probes (right column) were used to detect mRNA. (A) zfBili is expressed maternally as it is detected in the animal pole 4 h post-fertilization. (B) zfBili 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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(10.82 MB EPS)

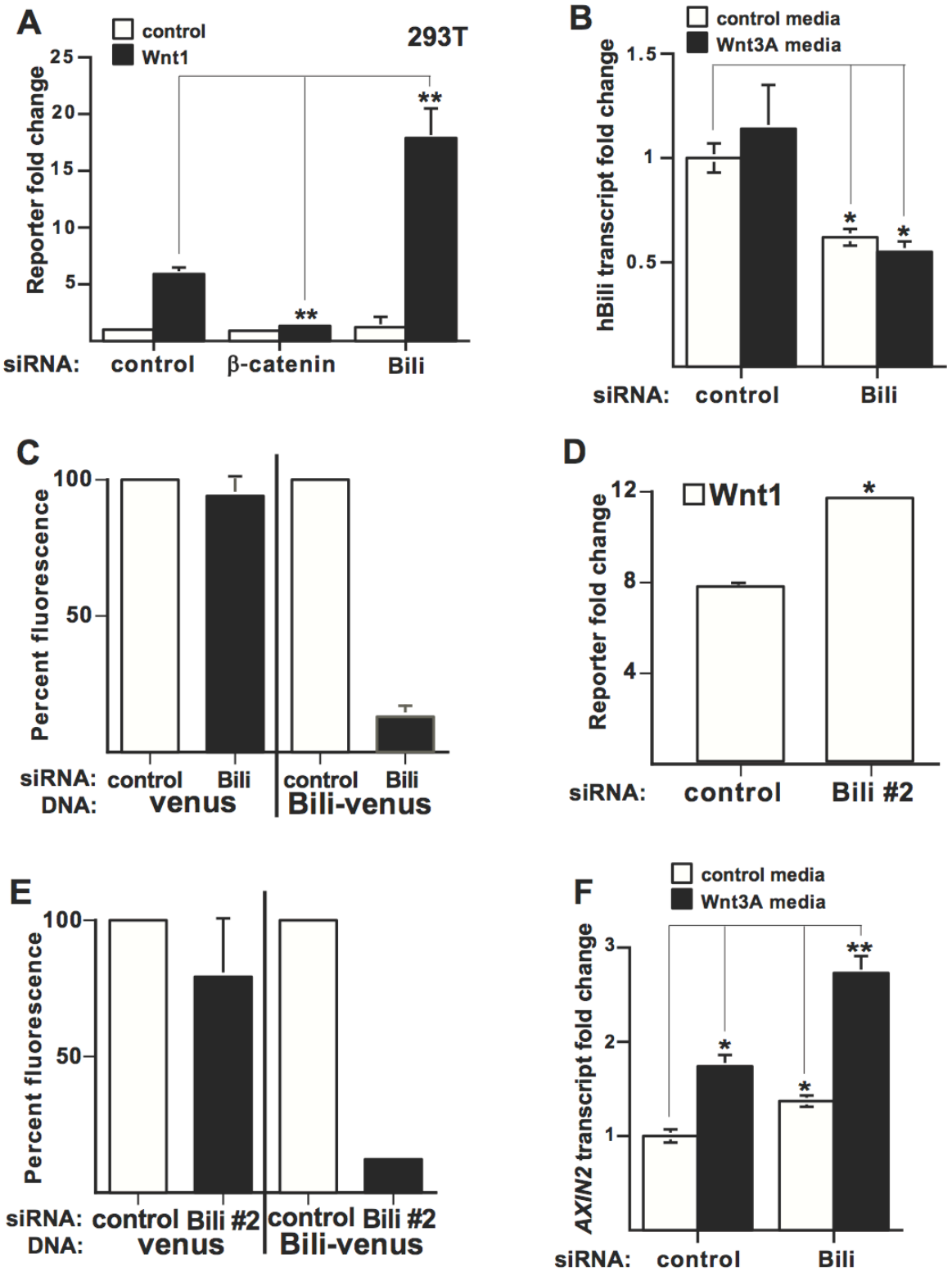

Figure S5.

Bili negatively regulates Wnt/{capital 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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(1.48 MB EPS)

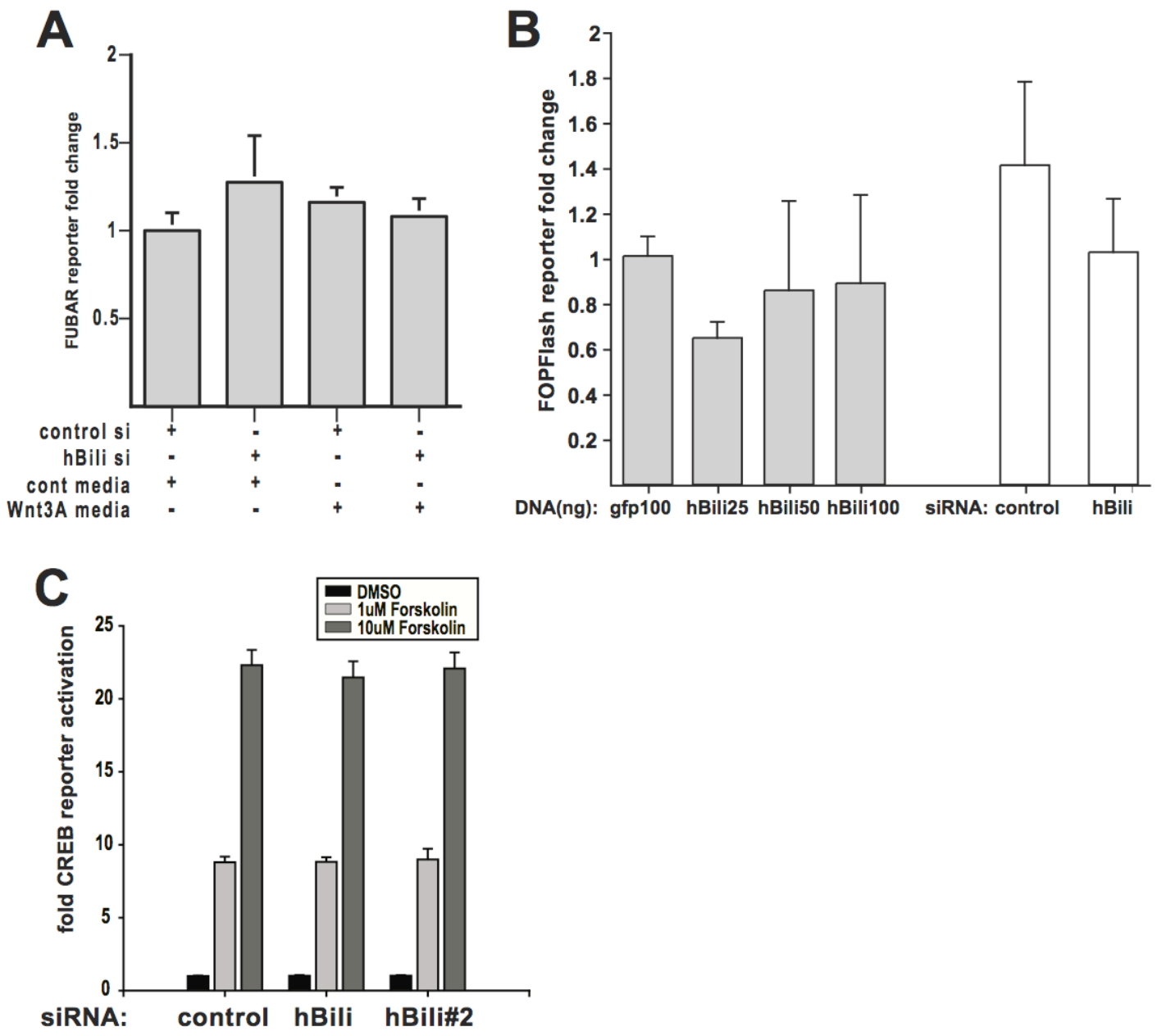

Figure S6.

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 uM forskolin, or 10 uM forskolin. Error bars represent STDEV.

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(1.14 MB EPS)