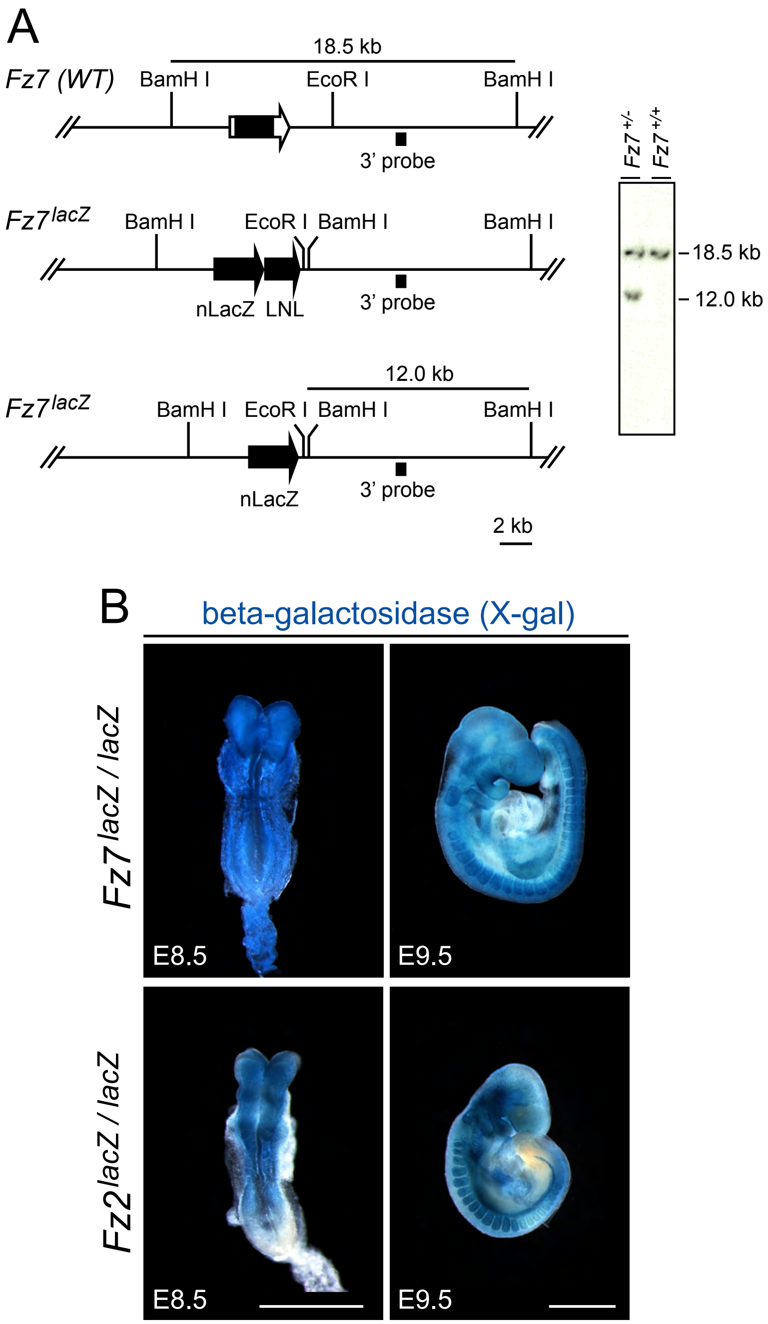

Fig. S1. ***Fz7* knockout strategy and expression patterns of *Fz2*^{lacZ} and *Fz7*^{lacZ} in early embryos.** (**A**) Targeted mutation of *Fz7*, with replacement of the *Fz7* coding region with a nuclear-localized *lacZ* (*nlacZ*) coding region. *LNL*, *loxP-PGK-neo-loxP* cassette, which was excised by germline Cre-mediated recombination. The 3′ Southern blot probe is indicated below the restriction maps, the 18.5 kb (native) and 12.0 kb (targeted) *Bam*HI fragments visualized by Southern blotting with this probe are indicated above the restriction maps, and a representative Southern blot is shown to the right. (**B**) X-Gal staining of *Fz7*$^{lacZ/lacZ}$ and *Fz2*$^{lacZ/lacZ}$ embryos at E8.5 (dorsal view) and E9.5 (side view) shows widespread expression of both genes in ectoderm and mesoderm. Scale bars: 1 mm.

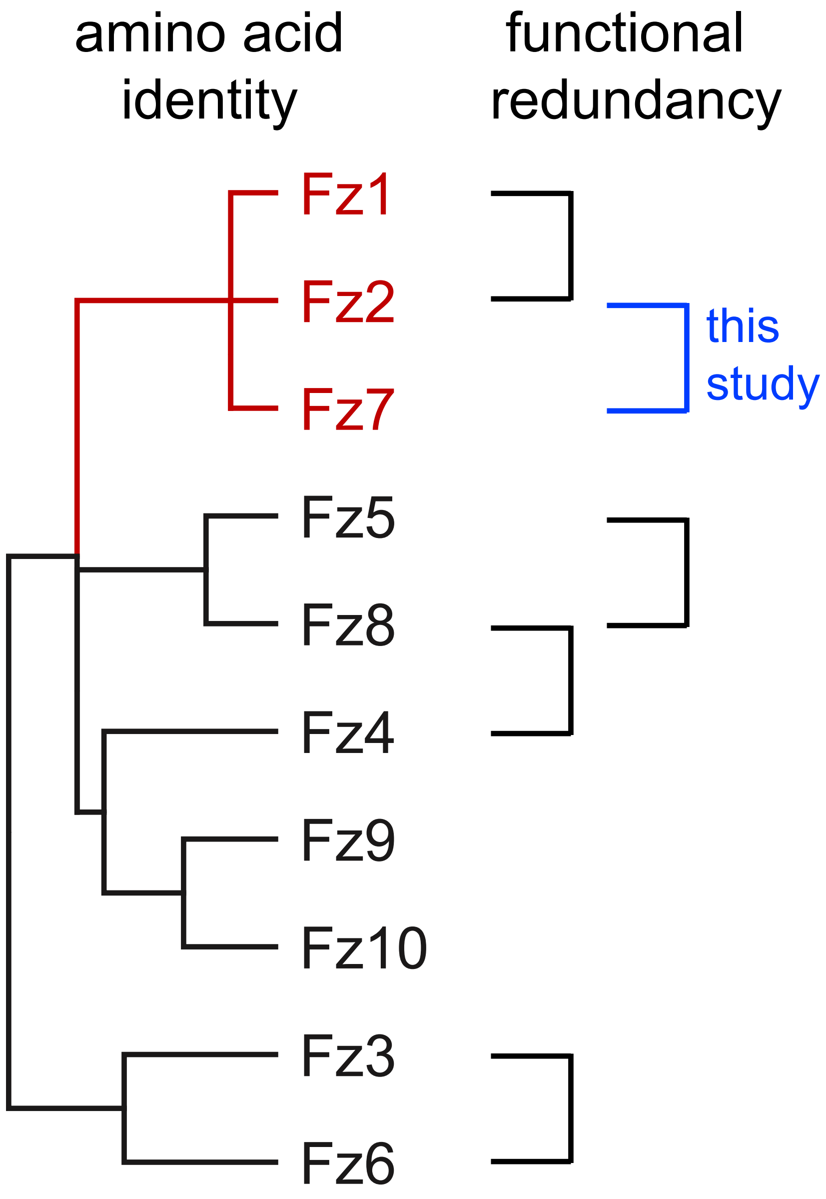

Fig. S2. Dendrogram showing amino acid sequence identity among mouse Frizzled proteins. Fz1, Fz2 and Fz7 (red) constitute a distinct branch within the Frizzled family. The dendrograms were generated from multiple sequence alignments produced by MEGA4 software. Previously described functional redundancies among Frizzled family members are indicated by the brackets and are described in the following references: *Fz1/Fz2* (Yu et al., 2010); *Fz3/Fz6* (Wang et al., 2006b); *Fz4/Fz8* (Ye et al., 2011); *Fz5/Fz8* (Liu et al., 2012). The blue bracket represents the *Fz2/Fz7* redundancy described in the present study.

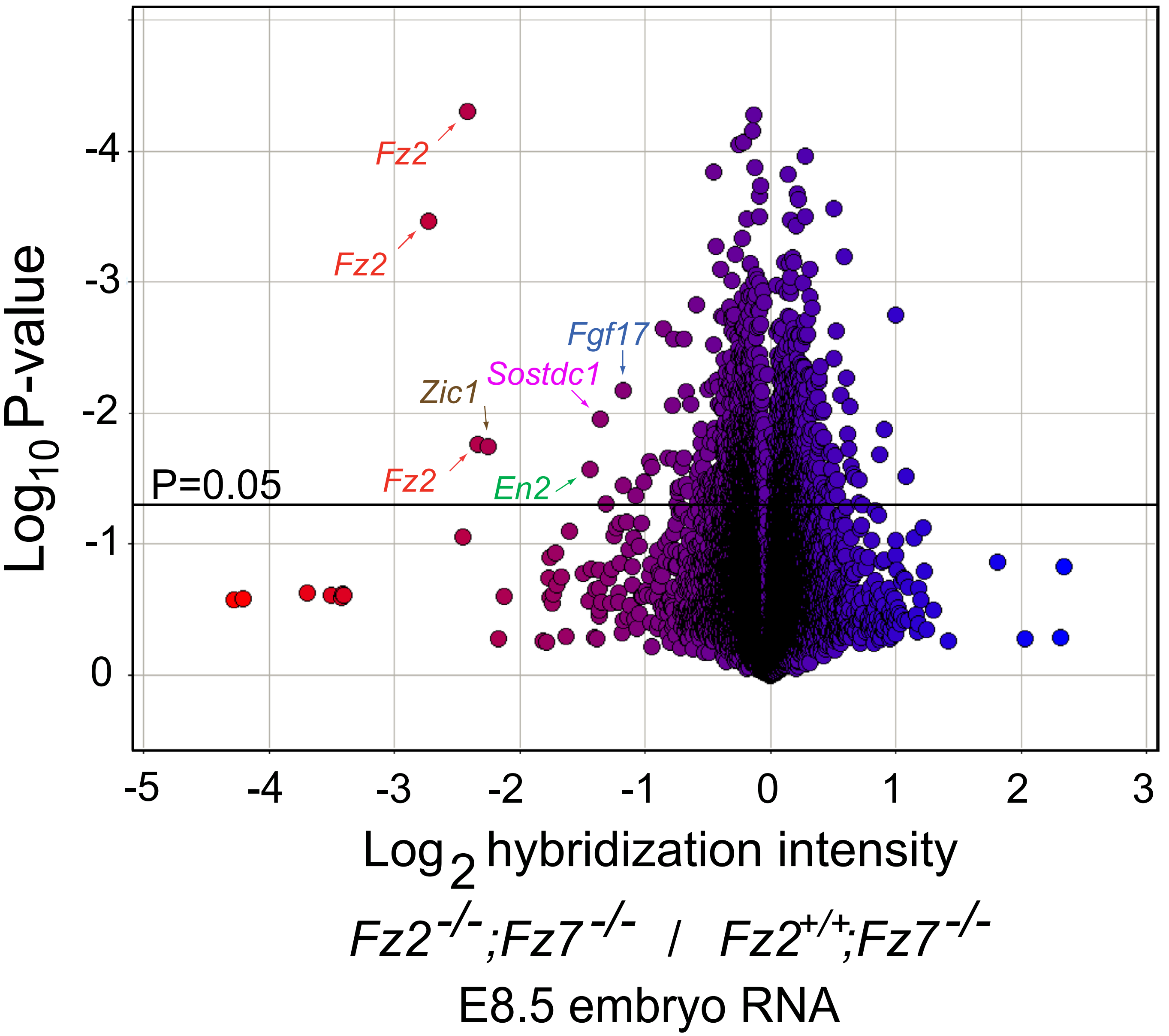

Fig. S3. Microarray hybridization comparison of E8.5 littermate *$Fz2^{-/-};Fz7^{-/-}$* and *$Fz2^{+/+};Fz7^{-/-}$* transcriptomes. Volcano plot showing the result of three biologically independent hybridizations to Affymetrix 430 2.0 microarrays, with hybridization intensity ratios plotted on a $\log_2$ scale, and *P*-values plotted on a $\log_{10}$ scale. The horizontal black line represents P=0.05. Each transcript is generally represented by multiple sets of oligonucleotide targets, multiple entries in the Affymetrix spreadsheet, and, therefore, multiple points on the volcano plot. The volcano plot shows the expected decrease in the abundance of *Fz2* transcripts, which are absent in $Fz2^{-/-};Fz7^{-/-}$ embryos. The primary data are available at the Gene Expression Omnibus under accession number GSE37221.

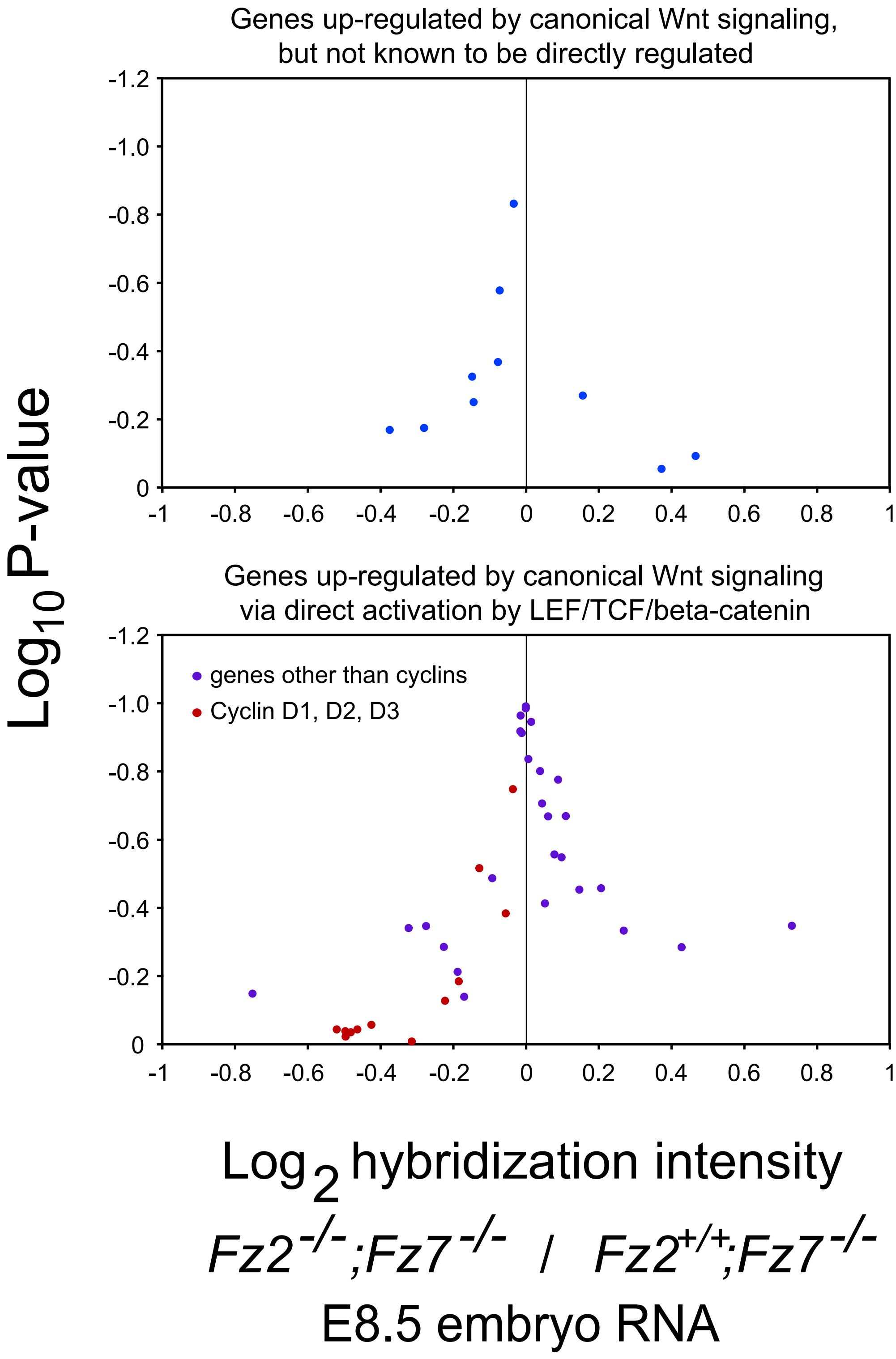

Fig. S4. Microarray hybridization comparison of E8.5 littermate *Fz2*$^{-/-}$;*Fz7*$^{-/-}$ and *Fz2*$^{+/+}$;*Fz7*$^{-/-}$ for a subset of 25 genes induced by canonical Wnt signaling. Volcano plots showing the result of three biologically independent hybridizations to Affymetrix 430 2.0 microarrays, with hybridization intensity ratios plotted on a $\log_2$ scale, and *P*-values plotted on a $\log_{10}$ scale. The 25 Wnt-responsive genes are divided into two plots for those that are (lower plot) or are not (upper plot) known to be direct targets of LEF/TCF/β-catenin signaling (see summary at http://www.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes). Each transcript is generally represented by multiple sets of oligonucleotide targets, multiple entries in the Affymetrix spreadsheet, and, therefore, multiple points on the volcano plot (see Table S1). Data points corresponding to Cyclin D family members, which have the largest number of probe sets, are color-coded red.

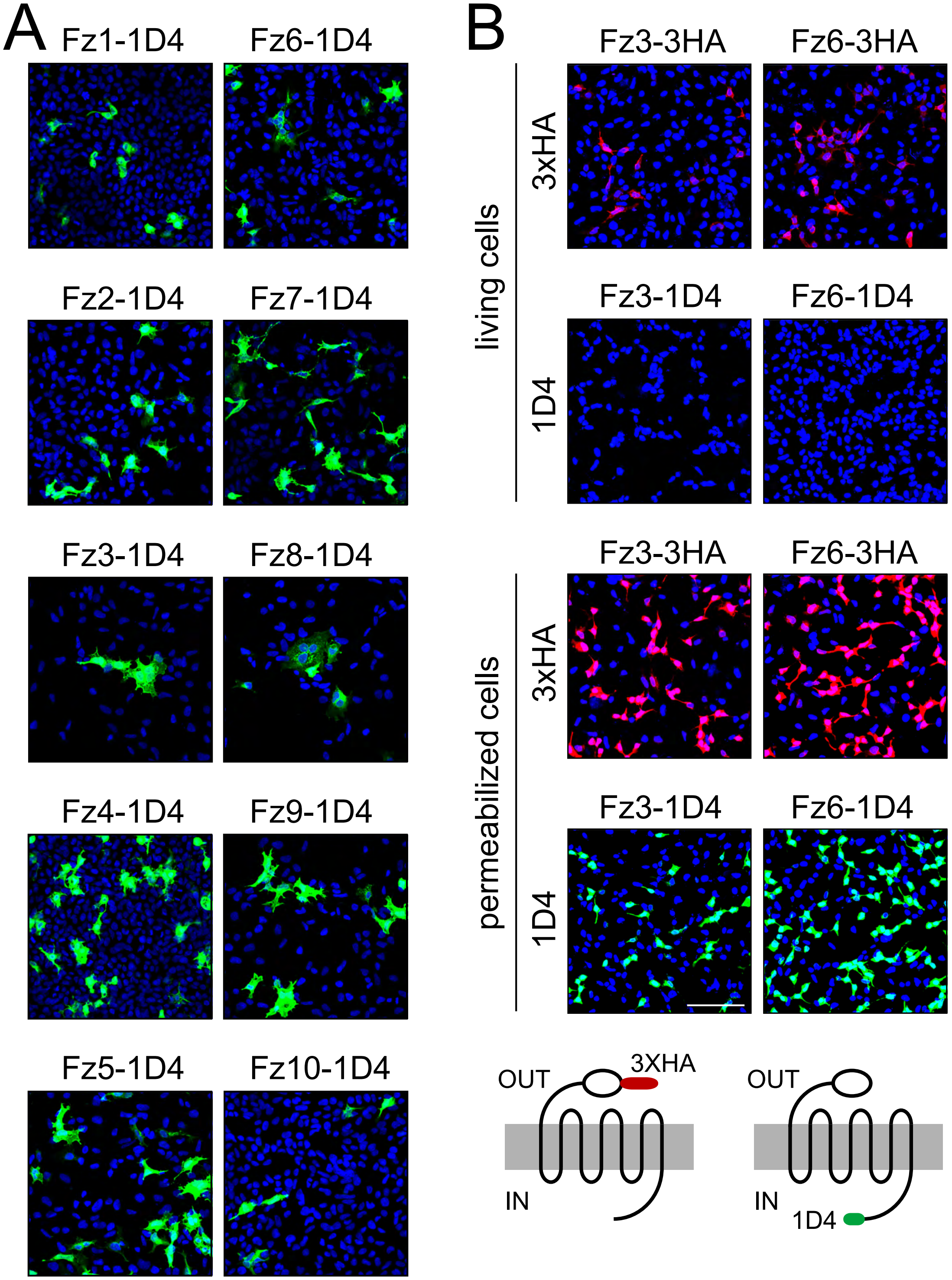

Fig. S5. Analysis of expression of Fz1-Fz10 and cell surface localization of Fz3 and Fz6 in HEK293 cells. (**A**) HEK293 cells transiently transfected with plasmids coding for 1D4 C-terminal epitope-tagged Fz1 through Fz10 were fixed, permeabilized with Triton X-100, and immunostained with mAb 1D4. Transfection and immunostaining were performed in parallel with common reagent mixes, confocal imaging was performed with identical settings, and image processing was performed with identical parameters. (**B**) HEK293 cells transiently transfected with plasmids coding for epitope-tagged Fz3 or Fz6 proteins (diagramed beneath) were incubated with primary antibodies under one of two conditions: (1) as living cells, followed by paraformaldehyde fixation or (2) following paraformaldehyde fixation and permeabilization. For all samples, secondary antibody binding was conducted under permeabilizing conditions. For each antibody, permeabilized and non-permeabilized confocal imaging was performed with identical settings. For all images, image processing was performed with identical parameters. The failure of mAb 1D4 to stain living cells is consistent with the intracellular localization of this C-terminal epitope. The lower level of 3×HA binding to living cells compared with fixed and permeabilized cells is likely to reflect a combination of the accumulation of the majority of Fz3 and Fz6 within the secretory pathway and the ~15× shorter primary antibody incubation time used for living cells compared with permeabilized cells. Scale bar: 100 μm.

Table S1. Transcript abundance changes in *Fz2*$^{-/-}$ versus *Fz2*$^{-/-}$;*Fz7*$^{-/-}$ E8.5 embryos among 25 genes known to be induced by canonical Wnt signaling
Shown are Affymetrix probe set ID and gene symbol, the average hybridization intensity changes based on three independent sets of *Fz2*$^{-/-}$ versus *Fz2*$^{-/-}$;*Fz7*$^{-/-}$ E8.5 embryos, and the *P*-value of the resulting fold change versus the null hypothesis of no change. The numbers to the left refer to the row number in the full microarray hybridization data set (GEO accession number GSE37221). The 25 genes are divided into those known to be directly regulated by canonical Wnt signaling (i.e. by LEF/TCF/beta-catenin binding to DNA target sequences) (19/25 genes, upper table) and those not known to be directly regulated (6/25 genes, lower table); Wnt induction data are summarized at http://www.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes.

Genes directly upregulated by LEF/TCF/beta-catenin

Number	$\log_2$ fold change	Fold change	*P*-value	Probe set ID	Gene symbol
9249	-0.223984329	-1.167954708	0.28289708	1424942_a_at	*Myc*
17778	-0.090327432	-1.064611778	0.48436968	1433471_at	*Tcf7*
34757	0.001656169	1.001148628	0.988533869	1450461_at	*Tcf7*
5606	0.090918542	1.065048066	0.773563439	1421299_a_at	*Lef1*
39030	-0.185546083	-1.137247358	0.210292884	1454734_at	*Lef1*
7221	0.208727406	1.155668325	0.45537026	1422914_at	*Sp5*
34550	0.054642591	1.038601772	0.410765538	1450254_at	*Tert*
5648	0.046504224	1.032759424	0.703458327	1421341_at	*Axin2*
21152	-0.013186043	-1.009181764	0.915578236	1436845_at	*Axin2*
1741	-0.272225812	-1.207669602	0.344053198	1417409_at	*Jun*
32995	-0.320745097	-1.248975431	0.338574059	1448694_at	*Jun*
28826	0.001448836	1.001004761	0.98269741	1444519_at	*Lgr5*
35284	-0.01272021	-1.008855962	0.96171315	1450988_at	*Lgr5*
3636	0.270671496	1.206369196	0.331107416	1419304_at	*T*
4644	0.429942093	1.347179502	0.28178972	1420337_at	*Gbx2*
33883	0.733043422	1.662141749	0.344896614	1449582_at	*Cdx1*
253	0.100157776	1.07189068	0.545620612	1415921_a_at	*Tnfrsf19*
9519	0.148432793	1.108364796	0.451139341	1425212_a_at	*Tnfrsf19*
32448	0.016499704	1.011502373	0.94265723	1448147_at	*Tnfrsf19*
33779	0.008333558	1.005793097	0.833733354	1449478_at	*Mmp7*
10010	-0.00946854	-1.006584676	0.910097025	1425703_at	*Ppard*
24104	-0.167938773	-1.123452224	0.136555917	1439797_at	*Ppard*
10719	0.062984217	1.044624334	0.66564989	1426412_at	*Neurod1*
10720	0.080008756	1.057024456	0.554530867	1426413_at	*Neurod1*
5419	0.112143264	1.080832726	0.666845523	1421112_at	*Nkx2-2*
22858	-0.749572674	-1.681294758	0.14571802	1438551_at	*Neurog1*
35132	0.040716663	1.028624672	0.798325162	1450836_at	*Neurog1*
1751	-0.125847545	-1.091148565	0.514050518	1417419_at	*Ccnd1*
1752	-0.22043166	-1.165082131	0.125036228	1417420_at	*Ccnd1*
32999	-0.31180544	-1.241260085	0.006021336	1448698_at	*Ccnd1*
454	-0.479296827	-1.39406403	0.032819728	1416122_at	*Ccnd2*
455	-0.461771304	-1.37723171	0.041111246	1416123_at	*Ccnd2*

456	-0.518002713	-1.431971434	0.041384851	1416124_at	*Ccnd2*
14343	-0.49468802	-1.409016025	0.036310447	1430127_a_at	*Ccnd2*
19052	-0.4226797	-1.340414966	0.054573644	1434745_at	*Ccnd2*
32530	-0.493894403	-1.408241148	0.020480653	1448229_s_at	*Ccnd2*
40252	-0.493286247	-1.40764764	0.033099878	1455956_x_at	*Ccnd2*
239	-0.18298923	-1.135233627	0.18261158	1415907_at	*Ccnd3*
23112	-0.034170701	-1.02396805	0.745531307	1438805_at	*Ccnd3*
28630	-0.053213277	-1.037573312	0.381066095	1444323_at	*Ccnd3*

Genes upregulated by canonical Wnt signaling, but not known to be directly controlled by LEF/TCF/beta-catenin

Number	$\log_2$ fold change	Fold change	*P*-value	Probe set ID	Gene symbol
10229	-0.031643168	-1.022175677	0.834837614	1425922_a_at	*Mycn*
10230	-0.074839608	-1.053243935	0.370151246	1425923_at	*Mycn*
2119	0.157889939	1.115654208	0.272391433	1417787_at	*Dkkl1*
9257	-0.372285003	-1.294401337	0.171519097	1424950_at	*Sox9*
18196	-0.069787667	-1.0495622	0.580000591	1433889_at	*Sox9*
35834	-0.277903713	-1.212431897	0.177249344	1451538_at	*Sox9*
5964	0.374839047	1.296694882	0.05674946	1421657_a_at	*Sox17*
13484	0.468411795	1.383585496	0.095193624	1429177_x_at	*Sox17*
3536	-0.141376469	-1.102956941	0.252686238	1419204_at	*Dll1*
3065	-0.14528704	-1.105950673	0.327187884	1418733_at	*Twist1*

Table S2. STF reporter assay of canonical Wnt signaling
Data are the averages of three transfections and have been normalized for the cotransfected *Renilla* luciferase.

Fz+Lrp5

Fz1	0.48
Fz2	0.37
Fz3	0.76
Fz4	2.54
Fz5	0.46
Fz6	0.7
Fz7	1.02
Fz8	0.85
Fz9	16.3
Fz10	0.26

Wnt+Lrp5

Wnt1	140.8
Wnt2	4.08
Wnt2b	0.19
Wnt3	11.18
Wnt3a	13.57
Wnt4	0.35
Wnt5a	0.18
Wnt5b	0.14
Wnt6	0.204
Wnt7a	0.715
Wnt7b	2.082
Wnt8a	0.342
Wnt8b	0.351
Wnt9a	0.193
Wnt9b	1.329
Wnt10a	0.356
Wnt10b	14.706
Wnt11	0.18
Wnt16	0.583

Wnt+Fz+Lrp5 (not normalized for Wnt+Lrp5 and Fz+Lrp5)

	Fz1	Fz2	Fz3	Fz4	Fz5
Wnt1	92.91	82.82	140.46	87.6	107.9
Wnt2	11.38	25.87	1.74	27.06	42.54
Wnt2b	1.43	1.26	1.18	6.69	12.39
Wnt3	102.8	75.06	5.22	44.43	76.9
Wnt3a	102	63.5	14.11	32.8	63.4
Wnt4	0.72	0.44	0.37	2.56	0.51
Wnt5a	0.59	0.41	0.27	4.1	1.2
Wnt5b	0.68	0.68	0.7	7.14	1.21
Wnt6	5.39	2.87	0.45	13.09	18.59
Wnt7a	9.97	8.44	1.15	9.07	75.82
Wnt7b	37	27.9	2.79	40.35	152.3

Wnt8a	4.49	4.49	0.92	11.75	28.72
Wnt8b	0.98	2	1.34	9.49	21.01
Wnt9a	0.89	0.47	0.58	2.01	0.64
Wnt9b	6.77	2.79	1.26	27.35	68.8
Wnt10a	3.43	1.65	1.22	9.08	8.03
Wnt10b	29.61	34.37	18.38	47.73	53.23
Wnt11	0.44	0.56	1.27	17.5	0.82
Wnt16	0.77	0.87	0.96	8.58	1.27

	Fz6	Fz7	Fz8	Fz9	Fz10
Wnt1	98.8	57.89	170.79	129.5	170.1
Wnt2	1.05	25.43	72.58	55.23	7.46
Wnt2b	0.86	2.69	22.4	18.23	0.94
Wnt3	4.19	76.14	49.12	37.36	18.94
Wnt3a	21.4	79.36	55.09	37.73	39.17
Wnt4	0.9	0.98	0.5	11.18	0.37
Wnt5a	0.82	1.02	0.93	15.34	0.19
Wnt5b	1.75	1.49	1.15	17.3	0.27
Wnt6	0.85	3.85	17.8	9.83	1.91
Wnt7a	0.97	13.39	55.36	23.5	1.15
Wnt7b	1.04	35.95	118.9	49.3	4.38
Wnt8a	0.89	6.94	39.55	22.13	2.29
Wnt8b	0.94	2.74	47.96	17.9	0.49
Wnt9a	0.88	0.92	1.08	13.04	0.63
Wnt9b	1.96	4.85	37.51	126.61	15.7
Wnt10a	0.84	5.16	2.73	20.21	0.78
Wnt10b	5.2	36.19	36	58.5	32.03
Wnt11	0.83	1.64	1.9	18.04	0.64
Wnt16	0.8	2.26	0.74	32.25	0.77

Wnt+Fz+Lrp5 (normalized for Wnt+Lrp5 and Fz+Lrp5)

	Fz1	Fz2	Fz3	Fz4	Fz5
Wnt1	0.85	0.76	1.03	0.83	1.03
Wnt2	3.22	6.49	0.49	4.45	11.41
Wnt2b	2.03	2.07	1.37	2.83	18.46
Wnt3	11.98	7.68	0.56	4.05	8.55
Wnt3a	8.13	5.71	0.83	2.15	5.13
Wnt4	0.82	0.51	0.37	0.99	0.53
Wnt5a	0.89	0.64	0.44	1.3	1.12
Wnt5b	0.95	1.12	0.79	2.95	1.7
Wnt6	9.45	6.07	0.64	11.28	34.93
Wnt7a	4.39	5.6	0.73	1.51	46.97
Wnt7b	12.45	10.86	0.87	5.63	51.27
Wnt8a	6.27	6.41	0.84	4.81	40.56
Wnt8b	1.34	2.98	1.23	2.95	27.1
Wnt9a	1.58	0.95	0.64	0.75	0.99
Wnt9b	4.03	1.95	0.75	8.99	38.78
Wnt10a	4.73	2.59	1.08	2.83	10.06
Wnt10b	4.52	2.75	1.28	2.92	4.37
Wnt11	0.91	1.28	1.51	7.03	1.28

Wnt16	0.85	0.98	0.81	3.35	1.13

	Fz6	Fz7	Fz8	Fz9	Fz10
Wnt1	0.7	0.53	1.34	1.05	1.34
Wnt2	0.28	5.21	17.63	2.81	1.93
Wnt2b	0.66	2.11	21.16	1.49	1.96
Wnt3	0.95	8.11	6.09	1.3	2.08
Wnt3a	0.91	5.67	4.28	1.5	3.06
Wnt4	0.7	0.78	0.39	0.81	0.53
Wnt5a	0.76	0.81	0.77	1.12	0.42
Wnt5b	22.25	1.15	0.98	1.15	0.72
Wnt6	0.38	5.38	20.4	1.11	4.5
Wnt7a	1.11	4.74	22.66	0.87	1.25
Wnt7b	1.51	8.27	30.13	1.57	1.81
Wnt8a	0.89	5.72	32.45	1.54	3.72
Wnt8b	1.23	1.83	35.82	1.09	1.03
Wnt9a	1.02	0.82	0.92	0.87	1.43
Wnt9b	0.89	2.15	18.12	8.39	13.26
Wnt10a	0.63	3.77	2.23	1.37	1.63
Wnt10b	0.8	2.42	3.13	1.89	2.54
Wnt11	1.06	1.29	1.41	1.2	1.68
Wnt16	0.48	1.67	0.42	2.21	0.86