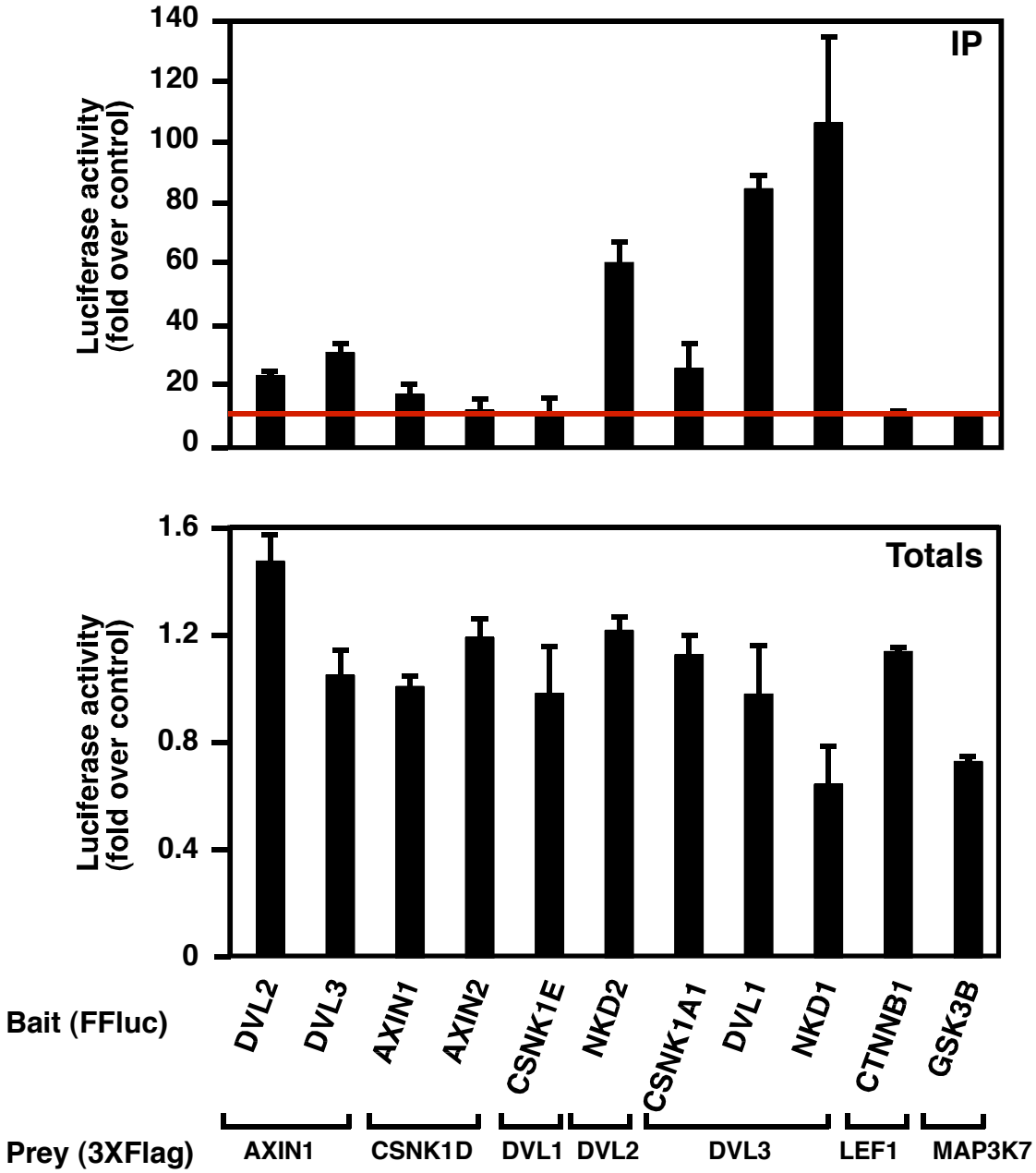
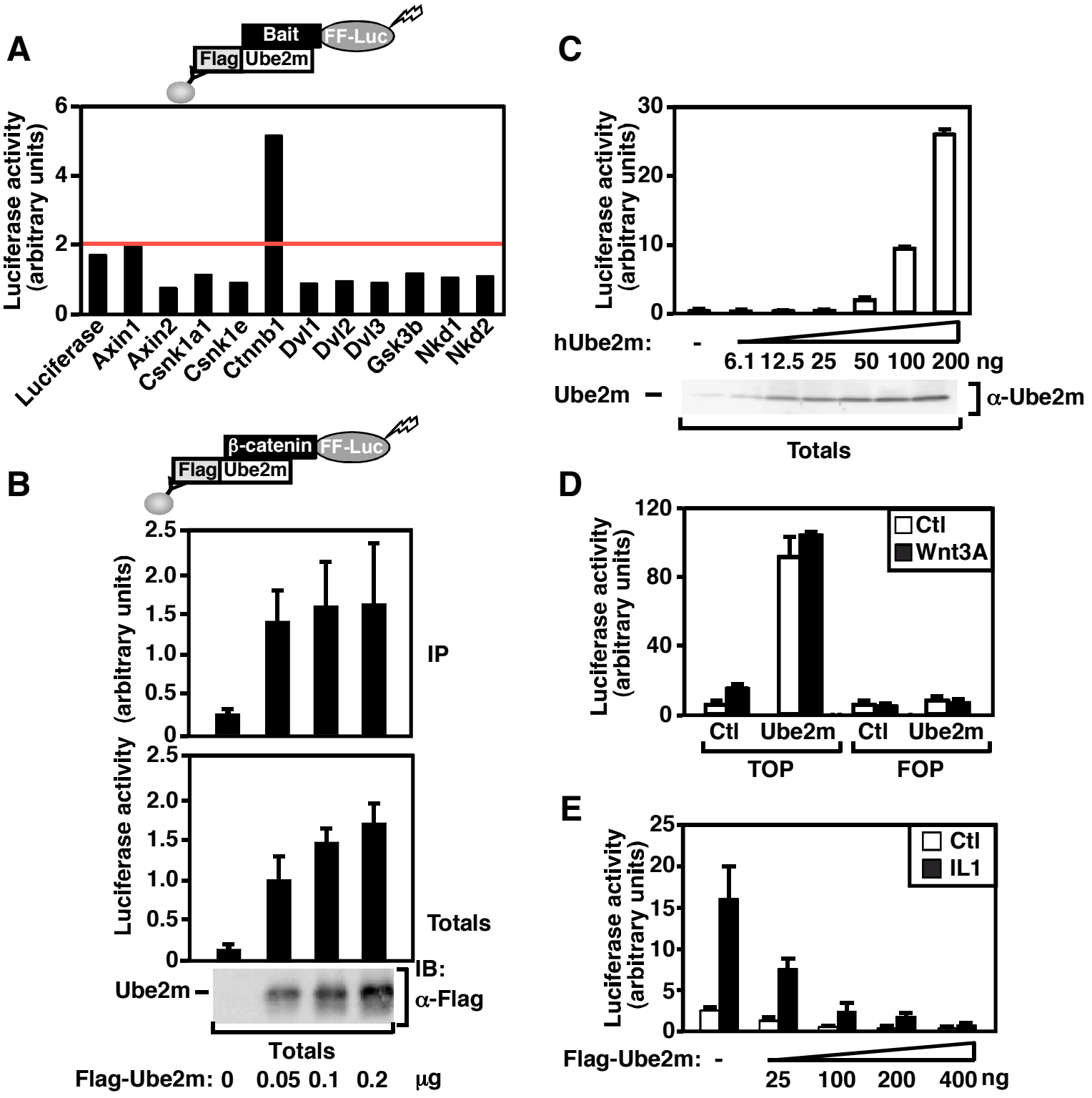
SUPPLEMENTARY MATERIALS

for Miller et al., 2009

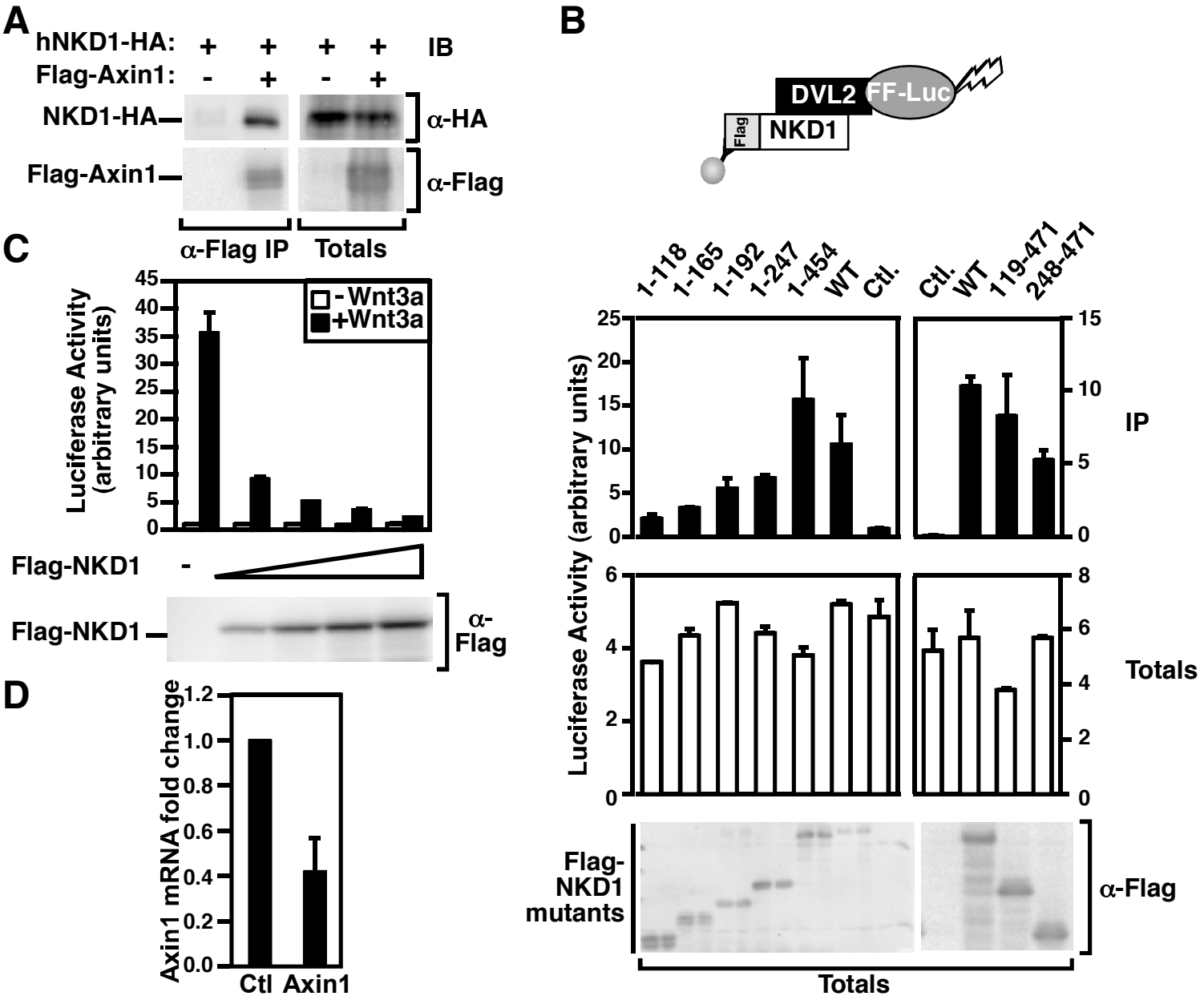
LIST OF SUPPLEMENTARY FIGURES, TABLES AND DATA SETS



Supplementary Figure 1: Manual LUMIER testing of luciferase-tagged baits. HEK293T cells were transfected with the indicated Firefly luciferase tagged baits in the presence and absence of Flag-tagged preys and interactions were detected using the LUMIER assay. Bait expressed in the absence of prey was used as a control and data are expressed as fold over control +/- standard deviation. The 10-fold over control threshold is indicated (red line).


A
Bait
FF-Luc
Flag
Ube2m
Luciferase activity (arbitrary units)
0
2
4
6
Luciferase
Axin1
Axin2
Csnk1a1
Csnk1e
Ctnnb1
Dvl1
Dvl2
Dvl3
Gsk3b
Nkd1
Nkd2
B
β-catenin
FF-Luc
Flag
Ube2m
Luciferase activity (arbitrary units)
0
0.5
1.0
1.5
2.0
2.5
IP
Totals
IB:
α-Flag
Ube2m
Totals
Flag-Ube2m: 0 0.05 0.1 0.2 μg
C
Luciferase activity (arbitrary units)
0
10
20
30
hUbe2m: -
6.1 12.5 25 50 100 200 ng
Ube2m
α-Ube2m
Totals
D
Luciferase activity (arbitrary units)
0
40
80
120
Ctl
Wnt3A
Ctl
Ube2m
Ctl
Ube2m
TOP
FOP
E
Luciferase activity (arbitrary units)
0
5
10
15
20
25
Ctl
IL1
Flag-Ube2m: -
25 100 200 400 ng

Supplementary Figure 2: Ube2m is a Wnt pathway component. (A) Detection of Ube2m interacting partners by high-throughput LUMIER. Data from a LUMIER screen (Figure 2) are presented as the average fold change over the plate median from single (luciferase alone) or triplicate (all other) runs. The 2-fold threshold is indicated (red line). **(B)** Ube2m interacts with β-catenin. HEK293T cells were transfected with Flag-Ube2m and β-catenin tagged with Firefly luciferase (β-catenin.FF). Following anti-Flag immunoprecipitation, β-catenin.FF was detected by luciferase assay. Data are shown as the mean of triplicates -/+ standard deviation. **(C-E)** HEK293T cells were transfected with TOPflash (C, D), FOPflash (D) or pNF-kB-Luc (E) reporters with human Ube2m (hUbe2m) or Flag-tagged mouse Ube2m (Flag-Ube2m) as indicated. Cells were unstimulated or stimulated with Wnt3A or Interleukin 1 (IL1) overnight. Promoter activity was measured by luciferase assay and the data are shown as the mean of triplicate samples -/+ standard deviation. (C) Increased human Ube2m expression activates TOPflash. (D) Ube2m does not activate FOPflash. (E) Increasing Ube2m expression inhibits an NFkB responsive promoter.



Supplementary Figure 3: Nkd1 cooperates with Axin1 to inhibit Wnt signaling. **(A)** HEK293T cells were transfected with Nkd1-HA in the presence and absence of 3XFlag-Axin1. Cell lysates were subject to anti-Flag immunoprecipitation and associated Nkd1 was detected by anti-HA immunoblotting. **(B)** Internal regions of Nkd1 are required for its association with Dvl2. HEK293T cells were transfected with luciferase tagged Dvl2 and the indicated Flag-tagged Nkd1 constructs. Cell lysates were subject to Flag immunoprecipitation and the presence of Dvl2 was assessed by luciferase assay. Data are expressed as the mean of 2 samples +/- range. **(C)** Nkd1 overexpression inhibits TOPflash activation by Wnt3A. HEK293T cells were transfected with TOPflash and varying amounts of Nkd1 (0 to 0.28 mg in 24 well plates). TOPflash activity was measured by luciferase assay. Data are shown as the mean of 2 samples +/- standard deviation. **(D)** Measurement of siRNA mediated knockdown of Axin1. HEK293T cells were transfected with 20 nM of siRNA targetting Axin1 or non-targetting control siRNA. Axin1 levels were measured by real time PCR and normalised to HPRT. Data are expressed as fold over control and are shown as the average of three independent experiments +/- SEM.

Supplementary Table 1: Verification frequency by manual LUMIER

Average mLIR	Number tested manually	Number positive[1]	Success rate (%)	Cumulative number tested	Cumulative number positive	Cumulative success (%)
>4.00	23	22	96	23	22	96
3.00-3.99	8	6	75	31	28	90
2.00-2.99	17	11	65	48	39	81
1.00-1.99	20	9	45	68	48	71
<1.00	20	2	10	88	50	57

1) Proteins with luciferase activities of at least 3-fold over control were considered positive.

Supplementary Table 2: False negative rate of protein interaction screen.

		Average mLIR[2]				
Protein 1[1]	**Protein 2**	**>4**	**>3**	**>2**	**>1.5**	**>1**
AXIN1	ANKRD6	Y	Y	Y	Y	Y
AXIN1	AXIN1	Y	Y	Y	Y	Y
AXIN1	CDK2	N	N	N	N	N
AXIN1	DAB2	N	N	N	N	N
AXIN1	DVL2	Y	Y	Y	Y	Y
AXIN1	PIAS1	N	N	N	N	Y
AXIN1	SENP2	N	N	N	N	Y
AXIN1	SMAD3	N	N	N	Y	Y
AXIN1	SMAD6	N	N	N	N	N
AXIN1	SMAD7	N	N	N	N	N
AXIN2	ANKRD6	Y	Y	Y	Y	Y
CSNK1A1	AXIN1	Y	Y	Y	Y	Y
CSNK1E	ANKRD6	Y	Y	Y	Y	Y
CSNK1E	AXIN1	Y	Y	Y	Y	Y
CSNK1E	DVL1	Y	Y	Y	Y	Y
CSNK1E	DVL3	Y	Y	Y	Y	Y
CTNNB1	AXIN1	Y	Y	Y	Y	Y
CTNNB1	AXIN2	Y	Y	Y	Y	Y
CTNNB1	BTRC	Y	Y	Y	Y	Y
CTNNB1	CDK2	N	N	N	N	N
CTNNB1	CSNK1A1	Y	Y	Y	Y	Y
CTNNB1	CTNNB1	Y	Y	Y	Y	Y
CTNNB1	CTNNBIP1	Y	Y	Y	Y	Y
CTNNB1	DVL2	N	N	Y	Y	Y
CTNNB1	DVL3	Y	Y	Y	Y	Y
CTNNB1	FHL2	N	N	N	N	Y
CTNNB1	GSK3B	N	N	N	N	Y
CTNNB1	LEF1	Y	Y	Y	Y	Y
CTNNB1	MAGI2	N	N	Y	Y	Y
CTNNB1	NCOA2	N	N	N	N	Y
CTNNB1	PCP2	N	N	N	N	N
CTNNB1	PIN1	N	N	N	N	Y
CTNNB1	PKP2	N	N	N	N	Y
CTNNB1	PTEN	N	N	Y	Y	Y
CTNNB1	SMAD7	N	N	N	N	Y
CTNNB1	TAX1BP3	N	Y	Y	Y	Y
CTNNB1	TCF7	Y	Y	Y	Y	Y
DVL1	AXIN1	Y	Y	Y	Y	Y
DVL1	CSNK2A2	N	N	Y	Y	Y
DVL1	DVL1	Y	Y	Y	Y	Y
DVL1	DVL3	Y	Y	Y	Y	Y
DVL1	FRAT1	N	N	N	N	N
DVL1	PAK1	N	Y	Y	Y	Y
DVL1	RAC1	N	N	N	N	Y
DVL1	SMAD1	N	N	N	N	N
DVL2	ARRB2	N	N	N	N	N
DVL2	CSNK2A2	N	Y	Y	Y	Y
DVL2	RAC1	N	N	N	N	N
DVL2	RHOA	N	N	Y	Y	Y
DVL3	DAB2	N	N	N	N	N
GSK3B	AXIN1	Y	Y	Y	Y	Y
GSK3B	AXIN2	Y	Y	Y	Y	Y
GSK3B	DVL2	N	Y	Y	Y	Y
GSK3B	DVL3	N	N	Y	Y	Y
GSK3B	FRAT1	Y	Y	Y	Y	Y
GSK3B	FRAT2	N	N	N	N	N
GSK3B	TP53	N	N	N	N	N
NKD1	DVL1	Y	Y	Y	Y	Y
False negative rate		**33/58=57%**	**29/58=50%**	**23/58=40%**	**22/58=38%**	**13/58=22%**
Total interactions		**230**	**395**	**945**	**1717**	**3723**
Total interactions		**211**	**358**	**829**	**1509**	**3407**

1) Listed interactions were identified from literature searches using immunoprecipitation from mammalian cell lysates in multiple reports, or in a single report published since 2002.

2) Detection (Y) or not (N) of interactions at the indicated thresholds in the protein interaction screen are shown.

Supplementary Table 3: Verification of LUMIER screen interactions

Protein1	Protein2	LUMIER[1]	IP-Western[1]	Mass Spectrometry[2, 3]
AXIN1	CSNK1A1			+
AXIN1	CSNK1D	+		
AXIN1	CTNNB1			+
AXIN1	ENC1	+		
AXIN1	GSK3A			+
AXIN1	GSK3B			+
AXIN1	KIAA103	+		
AXIN1	SOCS6	+		
AXIN1	TAX1BP3	+		
AXIN1	WWTR1		+	
AXIN2	CSNK1D	+		
CSNK1A1	DVL1	+		
CSNK1A1	DVL3	+		
CTNNB1	AXIN2			+
CTNNB1	CTNNBIP1			+
CTNNB1	FRAT2	+		
CTNNB1	LEF1			+
CTNNB1	NKD2	+		
CTNNB1	PIAS1	+		
CTNNB1	PPP2CB	+		
CTNNB1	UBE2M	+		
CTNNB1	WWTR1		+	
DVL1	ING2	+		
DVL1	LOC51035	+		
DVL1	PLEKHB1	+		
DVL1	SMAD6	+		
DVL1	SQSTM1	+		
DVL1	STAT1	+		
DVL1	STUB1	+		
DVL1	WWTR1		+	
DVL2	CSNK1E			+
DVL2	DVL3			+
DVL2	NKD1	+		+
DVL2	NKD2	+		
DVL2	NKD2-v1	+		
DVL2	PARD6A		+	
DVL2	SMURF2		+	
DVL2	WWTR1		+	
DVL3	CSNK1D	+		
DVL3	CSNK2A2			+
DVL3	NKD1	+		
DVL3	NKD2	+		
DVL3	NKD2-v1	+		
DVL3	WWTR1		+	
GSK3B	MAP3K7	+		
GSK3B	SMURF1		+	
NKD1	AXIN1	+	+	
NKD1	AXIN2	+		
NKD1	NKD1	+		
NKD1	PAK1	+		
NKD1	PARD6	+		
NKD1	PLEKHA2	+		
NKD1	PLEKHB1	+		
NKD1	SQSTM1	+		
NKD2	AXIN1	+		
NKD2	MAP3K7	+		
NKD2	NKD1	+		
NKD2	NKD2	+		

1) This study

2) Angers S, Thorpe CJ, Biechele TL, Goldenberg SJ, Zheng N, MacCoss, MJ, Moon RT (2006) The KLHL12-Cullin-3 ubiquitin ligase negatively regulates the Wnt-beta-catenin pathway by targeting Dishevelled for degradation. *Nat Cell Biol* **8:** 348-357

3) Major MB, Camp ND, Berndt JD, Yi X, Goldenberg SJ, Hubbert C, Biechele TL, Gingras AC, Zheng N, Maccoss MJ, Angers S, Moon RT (2007) Wilms tumor suppressor WTX negatively regulates WNT/beta-catenin signaling. *Science* **316:** 1043-1046

Supplementary Table 4: Detection of indirect interactions by LUMIER.

		Average mLIR				
Protein 1	**Protein 2**	**>4**	**>3**	**>2**	**>1.5**	**Reference**
CTNNB1	ACP1	No	No	No	Yes	1
GSK3B	ANKRD6	No	No	No	No	2
CTNNB1	PPM1A	No	No	No	No	3
DVL3	PPM1A	No	No	No	No	3
AXIN1	AKT1	No	No	No	No	4
DVL1	CTNNB1	No	No	No	No	5
DVL1	GSK3B	No	No	No	No	6

1) Brady-Kalnay SM, Rimm DL, Tonks NK (1995) Receptor protein tyrosine phosphatase PTPmu associates with cadherins and catenins in vivo. *J Cell Biol* **130:** 977-986

2) Schwarz-Romond T, Asbrand C, Bakkers J, Kuhl M, Schaeffer HJ, Huelsken J, Behrens J, Hammerschmidt M, Birchmeier W (2002) The ankyrin repeat protein Diversin recruits Casein kinase Iepsilon to the beta-catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling. *Genes Dev* **16:** 2073-2084

3) Strovel ET, Wu D, Sussman DJ (2000) Protein phosphatase 2Calpha dephosphorylates axin and activates LEF-1-dependent transcription. *J Biol Chem* **275:** 2399-2403

4) Fukumoto S, Hsieh CM, Maemura K, Layne MD, Yet SF, Lee KH, Matsui T, Rosenzweig A, Taylor WG, Rubin JS, Perrella MA, Lee, M.E. (2001) Akt participation in the Wnt signaling pathway through Dishevelled. *J Biol Chem* **276:** 17479-17483

5) Song DH, Sussman DJ, Seldin DC (2000) Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J Biol Chem* **275:** 23790-23797

6) Liu X, Rubin JS, Kimmel AR (2005) Rapid, Wnt-induced changes in GSK3beta associations that regulate beta-catenin stabilization are mediated by Galpha proteins. *Curr Biol* **15:** 1989-1997

Supplementary Table 5: True positive and corresponding maximum false positive rates for CPS, LUMIER, cDNA and RNAi

True Positive Rate	False Positive Rate for:				Representative CP Score
	CPS	LUMIER	cDNA	RNAi	
0.421	0.000	0.000	0.000	0.210	25.48
0.526	0.000	0.015	0.000	0.350	24.02
0.632	0.000	0.055	0.035	0.445	21.27
0.737	0.000	0.090	0.060	0.465	20.36
0.789	0.005	0.130	0.065	0.490	18.92
0.842	0.015	0.575	0.135	0.520	18.03
0.895	0.025	0.760	0.365	0.520	15.30