Electronic supplementary material

Methods

Cell culture and transient transfection INS-1 cells were maintained at 37ºC under 5% CO_2 and at 95% humidity in RPMI supplemented with 10% (vol./vol) heat-inactivated fetal bovine serum, penicillin (100 µg/ml) and streptomycin (0.25 µg/ml). MIN6 cells were maintained as above, in DMEM supplemented with 15% (vol./vol.) fetal bovine serum, penicillin (100 µg/ml) and streptomycin (0.25 µg/ml). Transfections were done with lipofectAMINE2000 (Invitrogen, San Diego, CA, USA).

Cell proliferation assay Proliferation of INS-1 cells was determined by incorporation of BrdU into newly synthesised DNA of proliferating cells. Cells in 96-well plates were treated with SDF-1 (10 nmol/l), exendin-4 (2 nmol/l) or PBS overnight, then pulse-labelled with BrdU for 4 h. BrdU staining was measured with a kit (Delfia Cell Proliferation kit; Perkin Elmer, Wellesley, MA, USA).

MTT assay Growth of INS-1 cells was determined by the MTT system. Serum starved INS-1 cells in 96-well plates were treated with SDF-1 (10 nmol/l), exendin-4 (2 nmol/l) or PBS for 48 h and then subjected to MTT assay (Sigma-Aldrich, St Louis, MO, USA).

Gene expression profiling on focused microarrays INS-1 cells were treated or not with 10 nmol/l SDF-1 for 4 h. Total RNA was isolated and biotin-labelled complementary RNAs were generated using a kit (TrueLabeling-AMP Linear RNA Amplification; SuperArray Bioscience, Frederick, MD, USA). The WNT-signalling pathway-focused microarray filters

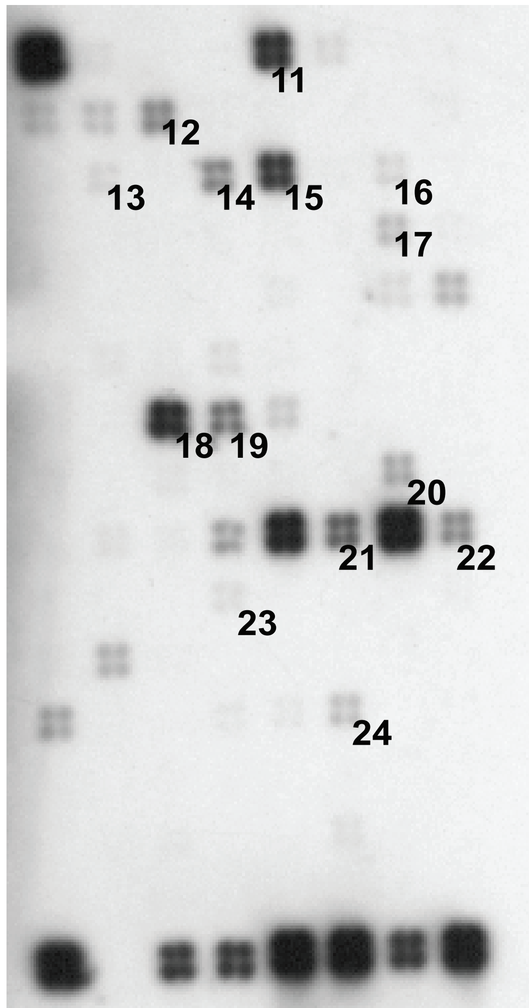
ESM Table 1 Superarray results for mRNAs regulated by SDF-1 in INS-1 cells

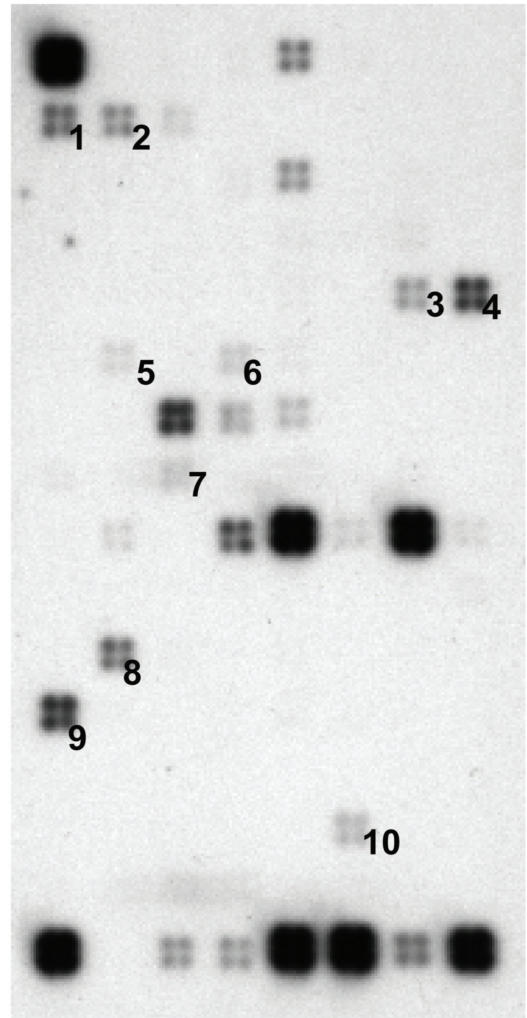
Number	Genebank number	Symbol	Description	mRNA change by SDF-1[a]
1	NM_053357	*Ctnnb1*	Catenin (cadherin associated protein), beta 1	1.99
2	NM_171992	*Ccnd1*	Cyclin D1	1.48
3	NM_012953	*Fosl1*	Fos-like antigen 1	1.72
4	XM_220632	*Foxn1*	Forkhead box N1	1.66
5	NM_001007597	*Fshb*	Follicle stimulating hormone beta	1.69
6	NM_172035	*Fzd2*	Frizzled homologue 2	1.43
7	XM_215187	*Lrp5*, predicted	Low density lipoprotein receptor-related protein 5 (predicted)	1.72
8	NM_021594	*Slc9a3r1*	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1	1.43
9	NM_133524	*Tcfe2a*	Transcription factor E2a	1.61
10	NM_001009695	*Wnt7b*	Wingless-related MMTV integration site 7B	1.59
11	NM_024405	*Axin1*	Axin1	−2.58
12	NM_022267	*Ccnd2*	Cyclin D2	−2.29
13	NM_053824	*Csnk2a1*	Casein kinase II, alpha 1	−1.42
14	NM_031021	*Csnk2b*	Casein kinase 2, beta subunit	−3.03
15	NM-019201	*Ctbp1*	C-terminal binding protein 1	−2.32
16	NM-053342	*Cxxc4*	CXXC finger 4	−1.43
17	NM_031820	*Dvl1*	Dishevelled, dsh homologue 1	−1.92
18	NM_017344	*Gsk3a*	Glycogen synthase kinase 3 alpha	−1.67
19	NM-032080	*Gsk3b*	Glycogen synthase kinase 3 beta	−1.51
20	NM-012603	*Myc*	Myc	−2.55
21	NM-017040	*Ppp2cb*	Protein phosphatase 2, catalytic subunit, beta isoform	−2.12
22	NM_001025418	*Ppp2r1b*	Protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	−1.71
23	NM_057132	*Rhoa*	Ras homologue gene family, member A	−1.32
24	NM_053738	*Wif1*	Wnt inhibitory factor 1	−1.92

Quantification of expression of WNT target genes regulated by SDF-1 was achieved by measuring intensity of signal of each spot:subtracted average intensity of *Gapdh*

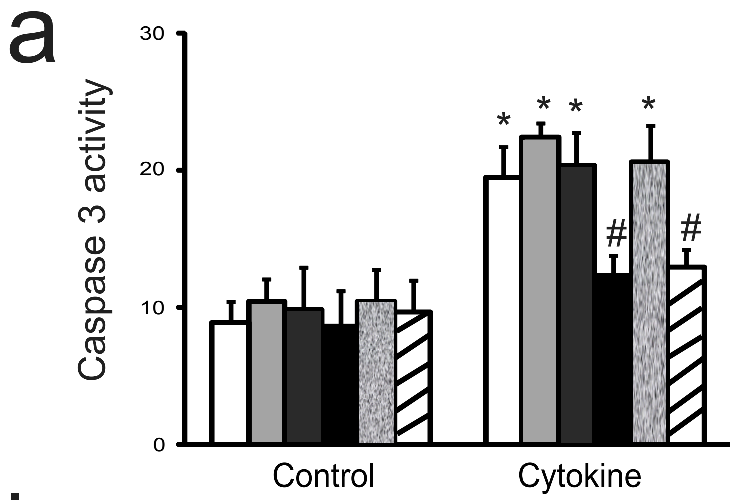
Superarray results, see also ESM Fig. 3

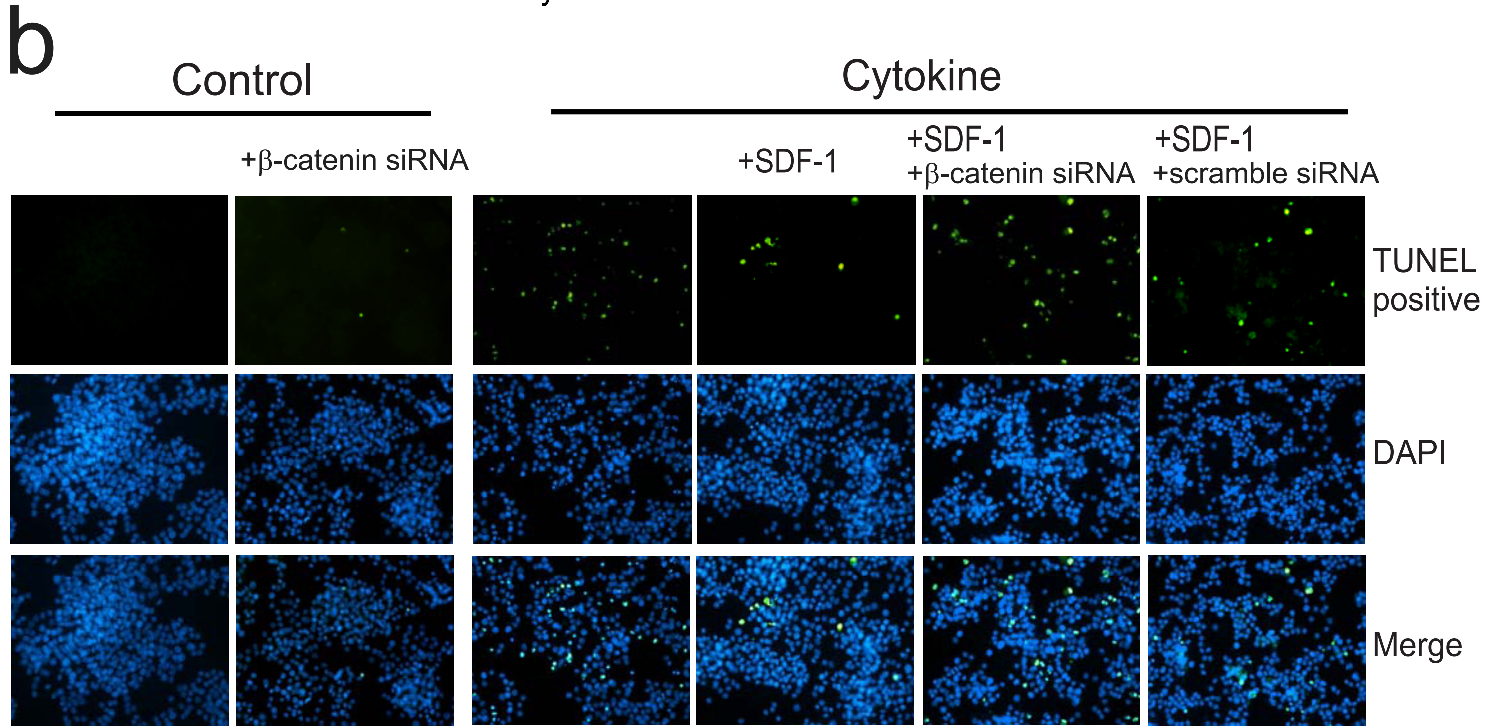
[a]Fold change

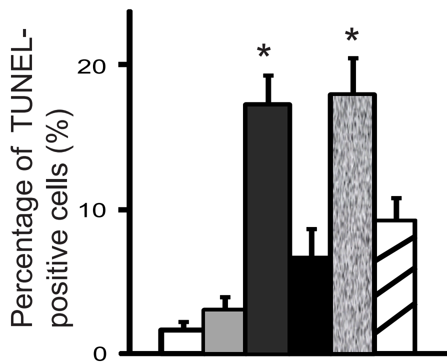




ESM Figure 5.







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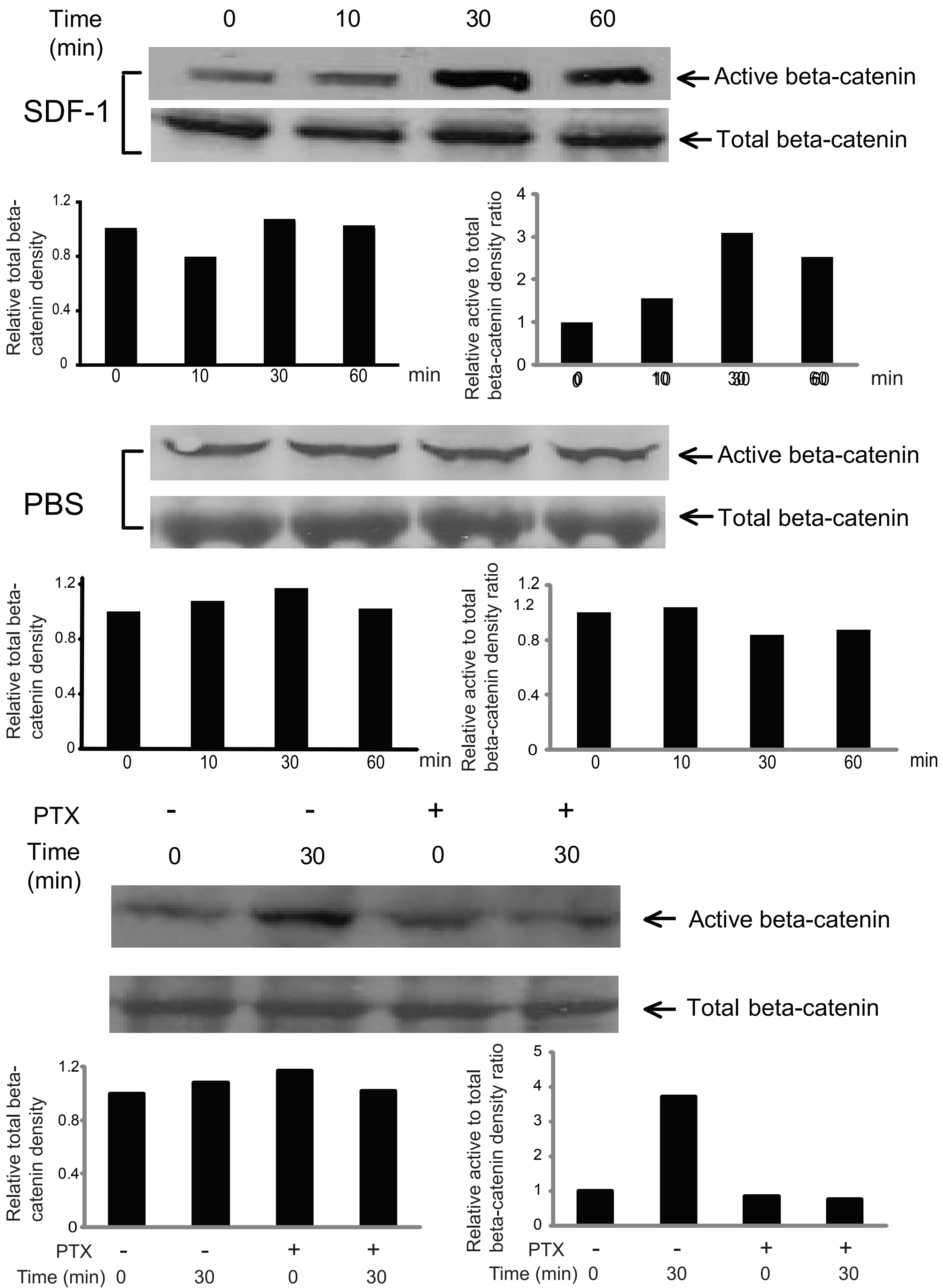
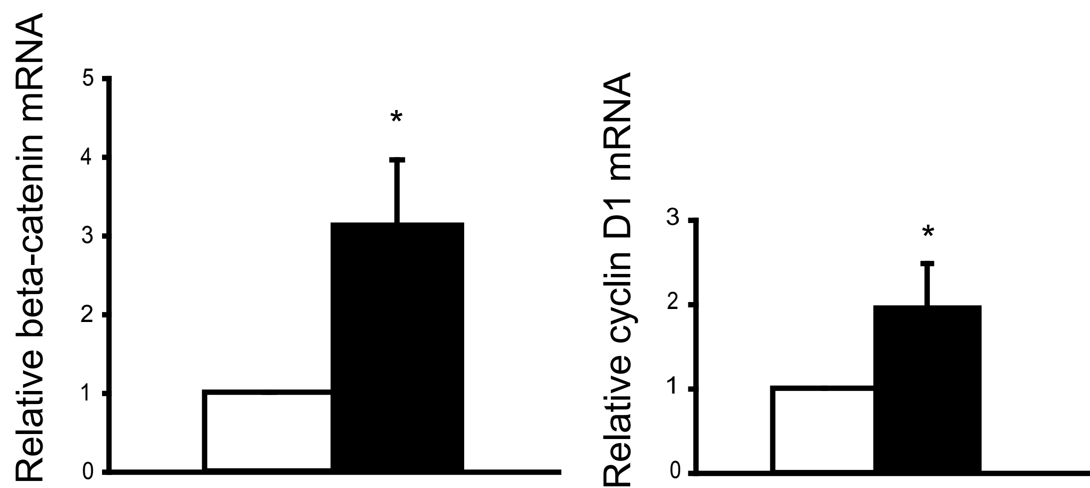


ESM Figure 3.

a



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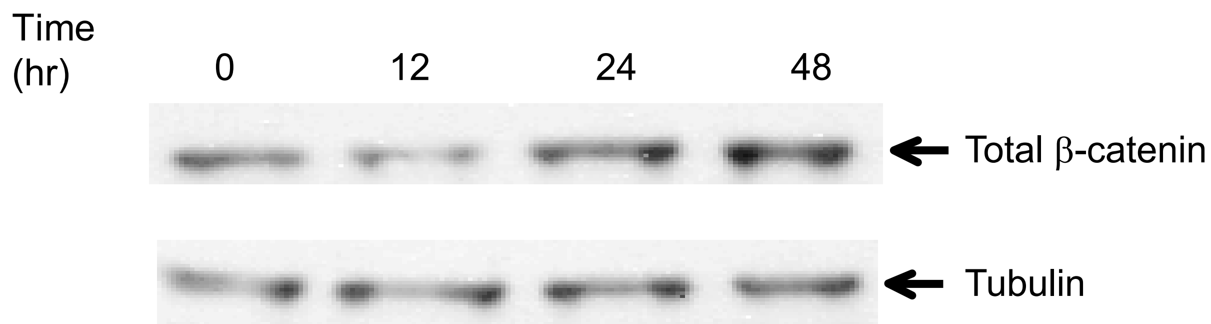


ESM Figure 2.

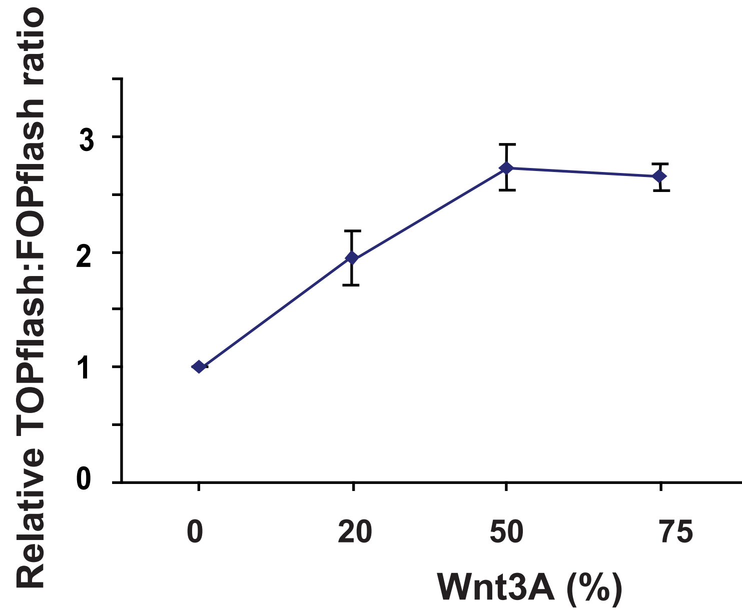
(118 probes) (SuperArray) were hybridised with these biotin-labelled targets (5 μg/array at 60 C overnight). The filters were washed and subsequently incubated with alkaline phosphatase-conjugated streptavidin and CDPStar substrate. The chemiluminescent images were captured using a digital station (Kodak 440). For quantification, the spot intensity was measured and normalised to the value of the housekeeping gene *Gapdh*. Fold-changes in gene expression levels were determined by comparing hybridisation densities of SDF-1-treated versus untreated INS-1 cells. Real-time RT-PCR was carried out with a kit (SYBR Green QPCR; Stratagene). Briefly, INS-1 cells were treated with 10 nmol/l SDF-1 or PBS vehicle control for 4 h. Total RNA was reverse-transcribed to cDNA using Super-Script II reverse transcriptase (Invitrogen). Real-time PCR was performed to amplify cyclin D1 and beta-catenin with the primers as described [1].

Statistical analysis Statistical analysis was done using an independent samples *t* test. Differences were considered statistically significant at $p<0.05$.

Reference

1. Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of TCF7L2-dependent WNT signaling enhances pancreatic beta cell proliferation. J Biol Chem 283:8723–8735

a



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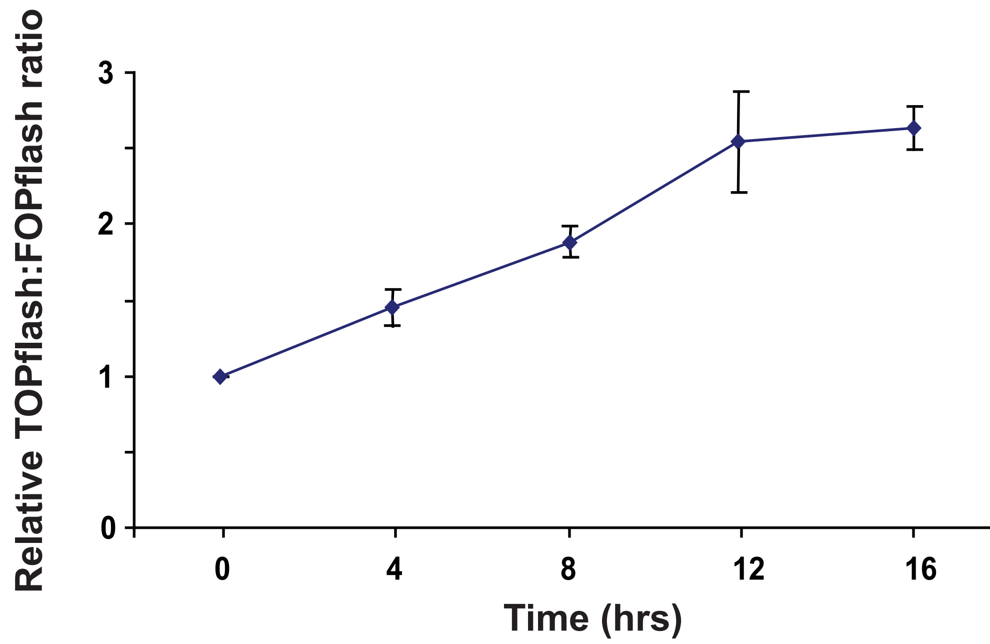

ESM Figure 1.