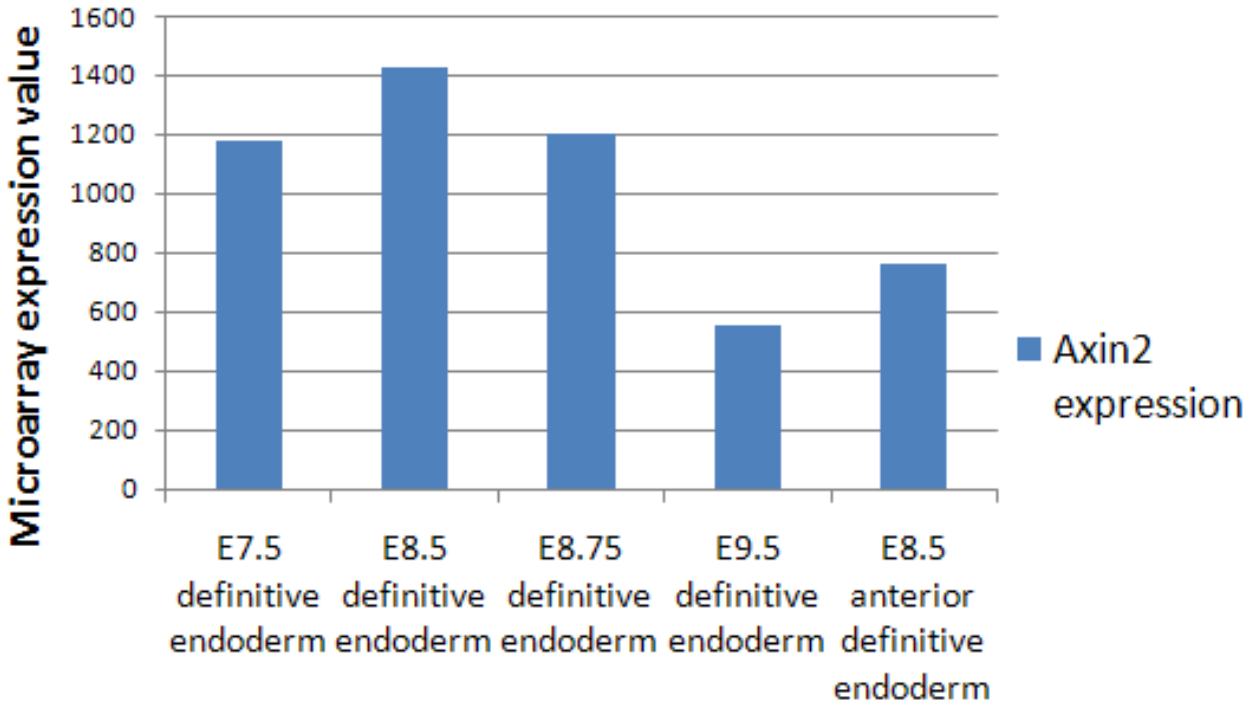
Supplemental Data

Supplemental Figure 1

Axin2 is preferentially expressed in E7.5-E8.75 endoderm and is expressed at lower levels in anterior endoderm.

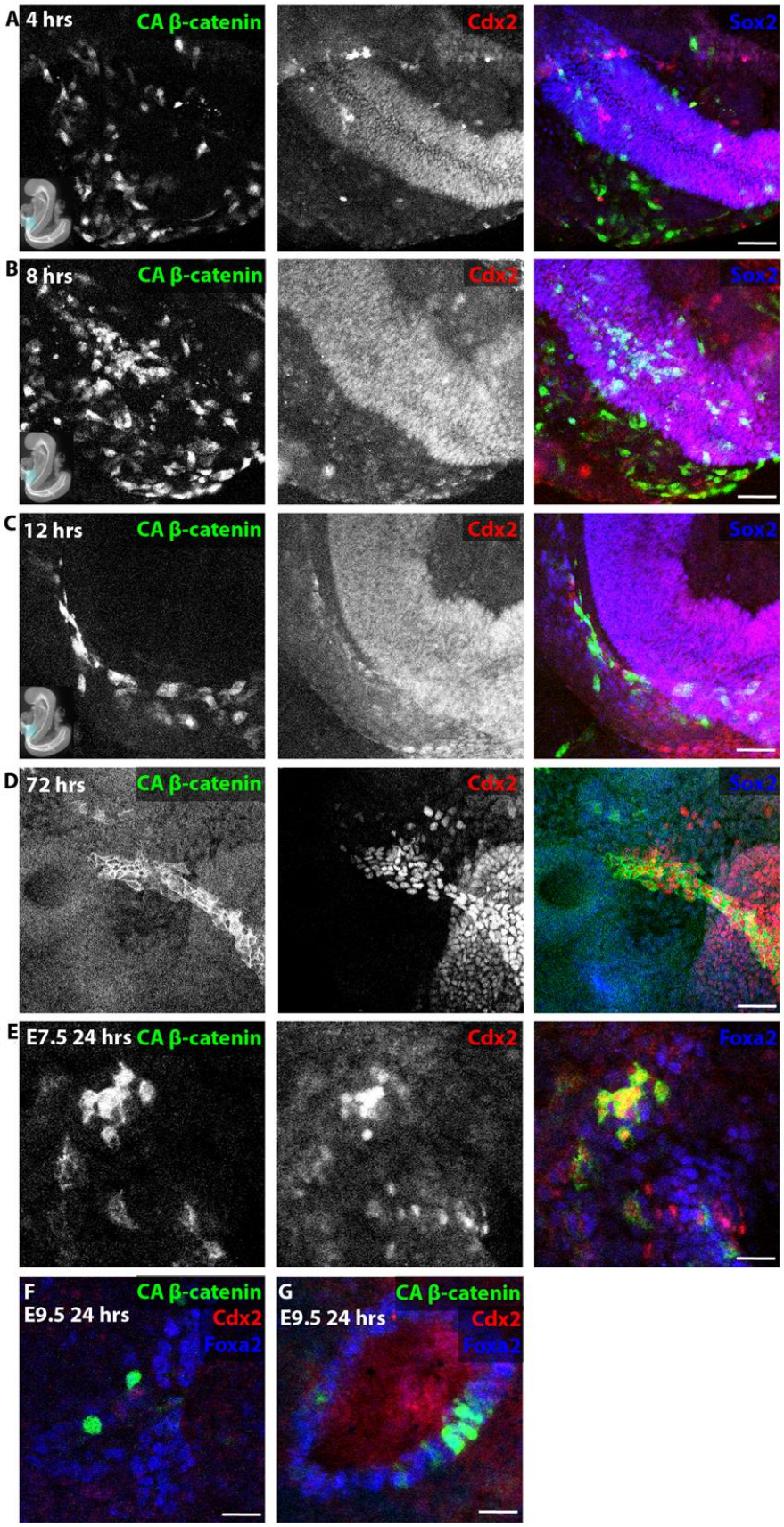
Graph displaying the rank invariant-normalized arbitrary unit microarray expression values of Axin2 in definitive endoderm from multiple embryonic stages and from E8.5 anterior definitive endoderm.



Supplemental Figure 2

Timecourse and age-dependence of Cdx2 induction by CA β-catenin

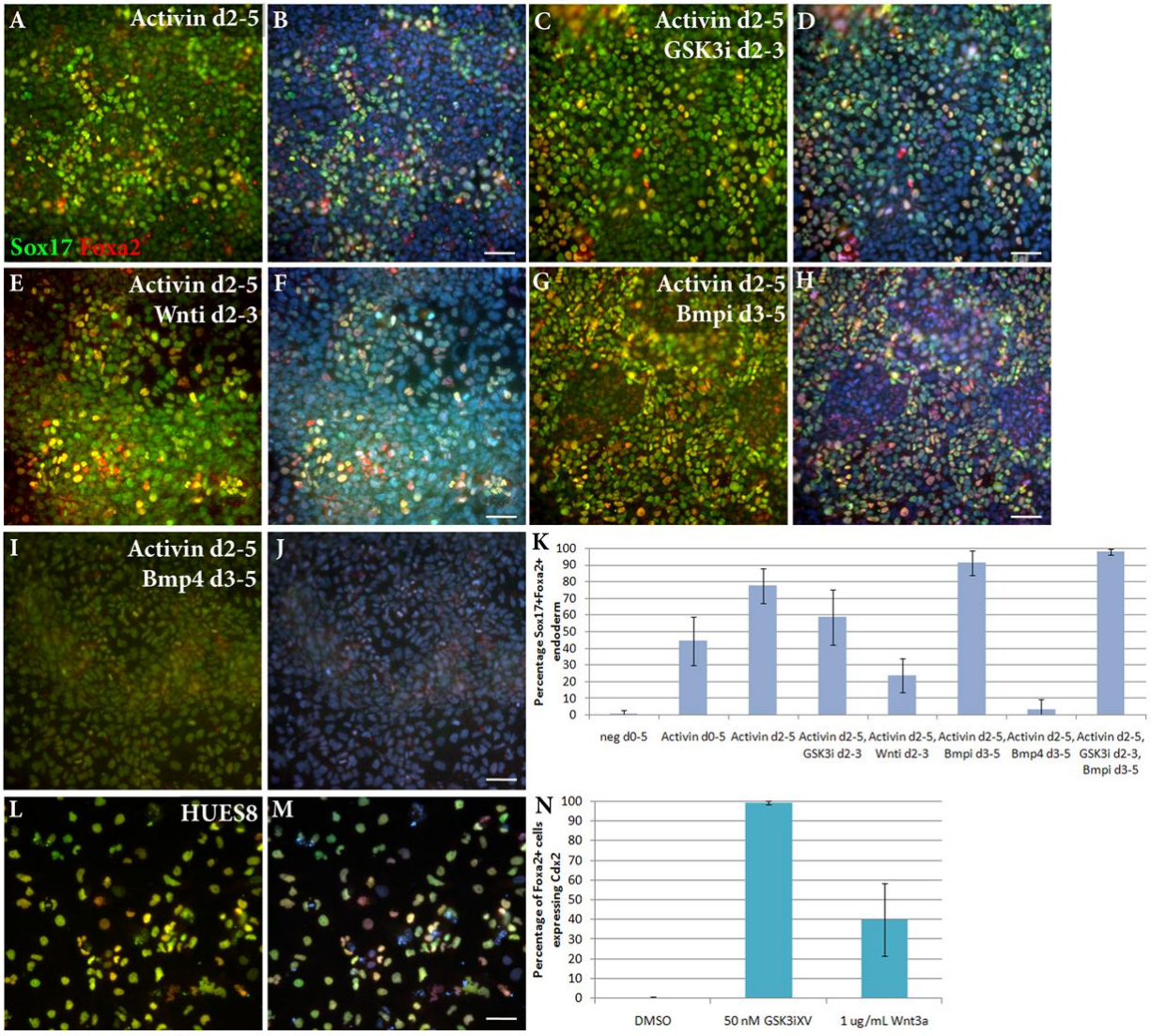
(A-C) Wholemount confocal immunofluoresence images of E8.25 foregut and midgut 4 hours (A), 8 hours (B), 12 hours (C), and 72 hours (D) after electroporation with CA β-catenin and placement in whole embryo culture (A-C) or whole embryo culture for 24 hours followed by explants culture for 48 hours (D). Embryos are stained for GFP (green), Cdx2 (red) and Sox2 (blue). (E-G) Wholemount confocal immunofluorescence images of E7.75 anterior endoderm (E) and E9.5 stomach (F-G) electroporated with CA β-catenin and placed in explant culture for 24 hours. Embryos are stained for GFP (green), Cdx2 (red), and Foxa2 (blue). Scale bar equals 200 μm in A-D, 50 μm in E-G.


A
4 hrs
CA β-catenin
Cdx2
Sox2
B
8 hrs
CA β-catenin
Cdx2
Sox2
C
12 hrs
CA β-catenin
Cdx2
Sox2
D
72 hrs
CA β-catenin
Cdx2
Sox2
E
E7.5 24 hrs
CA β-catenin
Cdx2
Foxa2
F
CA β-catenin
E9.5 24 hrs
Cdx2
Foxa2
G
CA β-catenin
E9.5 24 hrs
Cdx2
Foxa2

Supplemental Figure 3

Optimization of ES cell differentiation toward definitive endoderm

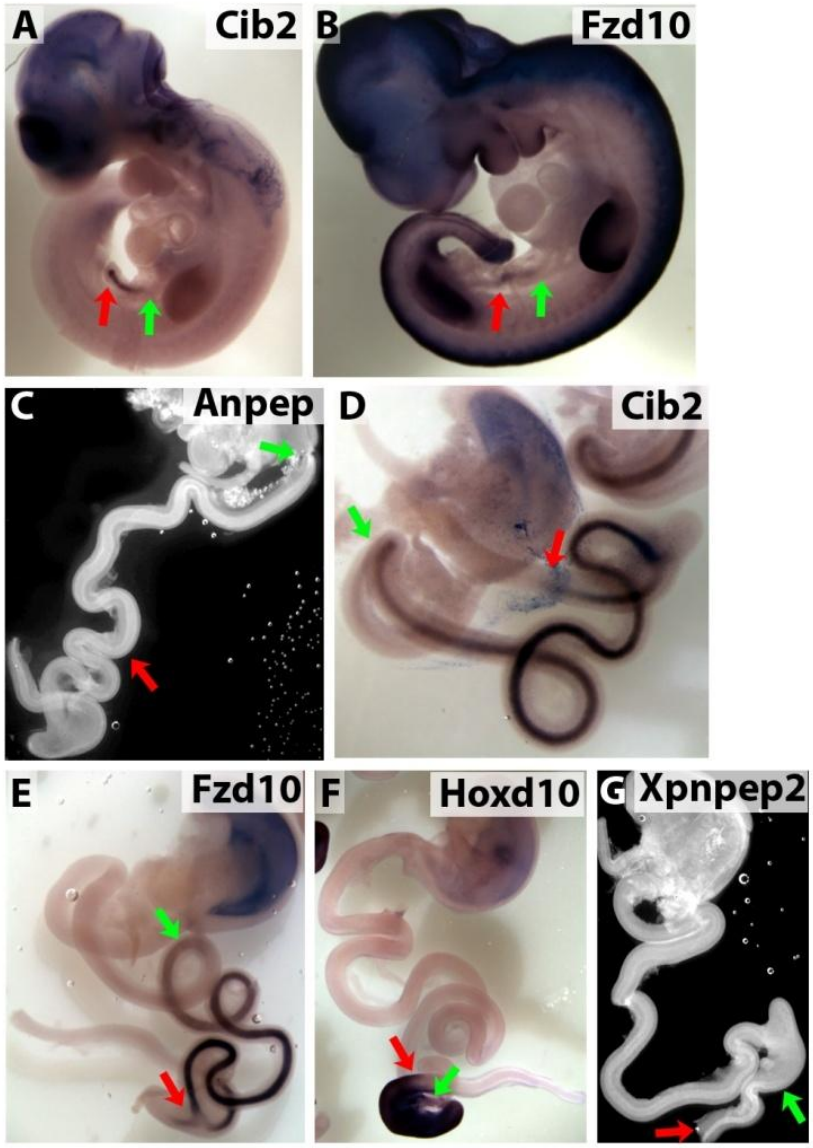
(A-J) Immunofluorescence analysis of ES cells treated with Activin A from days 2-5 (A-B), Activin A from days 2-5 and GSK3iXV from day 2-3 (C-D), Activin A from days 2-5 and the Wnt inhibitor PNU74654 from day 2-3 (E-F), Activin A from days 2-5 and the Bmp inhibitor Dorsomorphin from days 3-5 (G-H), or Activin A from days 2-5 and Bmp4 from days 3-5 (I-J) before fixation and stained for Sox17 (green) and Foxa2 (red). Nuclei are stained with Hoechst 33342 (B, D, F, H, J). (K) Graph displaying percentage of $Sox17^{+}Foxa2^{+}$ endoderm at day 5 following indicated treatment. (L-M) Immunofluorescence analysis of HUES8 human ES cell-derived endoderm stained for Sox17 (green) and Foxa2 (red). Nuclei are stained for Hoechst 33342 (M). (N) Graph displaying percentage of $Foxa2^{+}$ endoderm cells that are $Cdx2^{+}$ after 24 hours of the indicated treatment. Scale bar equals 50 μm in B, D, F, H, J, and M.


A
Activin d2-5
Sox17 Foxa2
B
C
Activin d2-5
GSK3i d2-3
D
E
Activin d2-5
Wnti d2-3
F
G
Activin d2-5
Bmpi d3-5
H
I
Activin d2-5
Bmp4 d3-5
J
K
Percentage Sox17+Foxa2+ endoderm
100
90
80
70
60
50
40
30
20
10
0
neg d0-5
Activin d0-5
Activin d2-5
Activin d2-5, GSK3i d2-3
Activin d2-5, Wnti d2-3
Activin d2-5, Bmpi d3-5
Activin d2-5, Bmp4 d3-5
Activin d2-5, GSK3i d2-3, Bmpi d3-5
L
HUES8
M
N
Percentage of Foxa2+ cells expressing Cdx2
100
90
80
70
60
50
40
30
20
10
0
DMSO
50 nM GSK3iXV
1 ug/mL Wnt3a

Supplemental Figure 4

Additional examples of genes expressed in distinct anterior-posterior regions of the intestine.

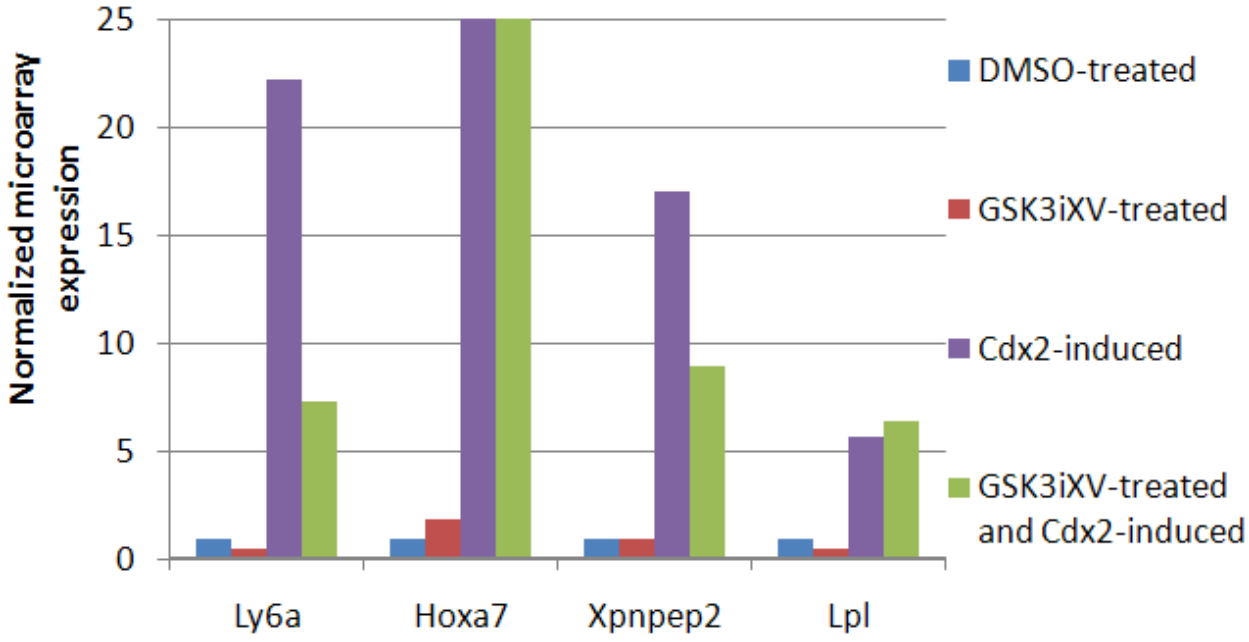
(A-B, D-F) Wholemount in situ hybridization images of E10.5 embryos (A-B) and E14.5 gut (D-F) showing expression of posterior small intestine-specific Cib2 (A, D) and Fzd10 (B, E) and cecum-specific Hoxd10 (F). (C, G) Wholemount immunofluorescence images of E14.5 gut showing expression of anterior small intestine-specific Anpep (C) and large intestine-specific Xpnpep2 (G). Green arrows refer to the anterior border of the intestinal staining and red arrows refer to the posterior border of the intestinal staining.



Supplemental Figure 5

High-levels of Cdx2 upregulate large intestinal genes in ES-derived endoderm.

Graph displaying the normalized microarray expression values of large-intestine specific genes in ES-derived endoderm treated with DMSO (blue), GSK3iXV (red), Doxycycline to induce ectopic Cdx2 (purple) or GSK3iXV and Doxycycline (green).



Supplemental Table 1

Complete list of genes significantly affected by GSK3iXV treatment in E8.25 foregut endoderm.

Expression of these genes is changed >3-fold in $EpCAM^{+}$ endoderm by 24-hour treatment of E8.25 foregut endoderm with 10 mM GSK3iXV beads.

Genes upregulated in GSK3iXV-treated E8.25 endoderm					Genes downregulated in GSK3iXV-treated E8.25 endoderm	
0610039N19Rik	AW061290	Epha3	Mod1	S100a3	Sep5	Itga11
0610041B22Rik	AW125753	Epha7	Mogat2	S100a6	4930519N16Rik	Itih2
1110012M11Rik	Axin2	Evx1	Mrpl2	Sca1	5330439J01Rik	Ldb2
1110020C13Rik	AY761185	Fabp3	Mst1r	Scamp2	Acas2l	Ldoc1
1110032A13Rik	BC018465	Fbxo6b	Msx1	Scamp5	Accn1	Lpl
1190002C06Rik	BC030476	Fgf17	Msx2	Scap	Acsm3	Magee1
1200009O22Rik	BC049816	Fgfbp3	Mt1	Scnm1	Adrb2	Mcm6
1600002K03Rik	Bdh	Flt1	Mt2	Sct	Agpt2	Meox1
1700011I11Rik	Bhmt2	Foxq1	Mta3	Sdcbp2	Aldh1a3	MGC106740
1700029G01Rik	Bop1	Fst	Myo9b	Sepx1	Ambp	Myl2
2300002D11Rik	C130034I18Rik	Fut2	Nbl1	Shf	Ankrd1	Myl7
2300002D11Rik	C2	Fzd1	Neu1	Siglech	Anxa11	Nkx2-3
2310042N02Rik	C86987	Fzd10	Neurl	Sirt7	Apoa2	Nkx2-5
2310046K01Rik	Car11	Gababrbp	Nfe2l3	Slc27a3	Arg1	Nkx2-6
2400003C14Rik	Car13	Gad1	Ngfr	Slc29a1	B3galnt1	Nkx3-1
2410001C21Rik	Cbln1	Gadd45g	Nin	Slc3a2	Bmper	Nnat
2410080H04Rik	Ccdc3	Gba	Nkd1	Slc7a3	C630028C02Rik	Npr3
2510009E07Rik	Cd27	Gbx2	Nme5	Smpd3	Cldn11	Nptx2
2610027C15Rik	Cdkn1a	Gch1	Nos3as	Snca	Col2a1	Nrn1
2610033H07Rik	Cdx1	Gjb2	Notch1	Snx17	Corin	Nsbp1
2700017M01Rik	Cdx2	Gjb6	Nspc1	Soat1	Crabp2	Nsg2
2700050C12Rik	Cerk	Gna14	Nt5c	Sox17	Cxcl12	Olfm1
2810417J12Rik	Ch25h	Gnefr	Nt5e	Sp5	Cyp24a1	Onecut2
2900024C23Rik	Chrd	Gng2	Odz4	Sp6	Dab1	Paqr9
2900046G09Rik	Cited1	Golga2	Optn	Sp7	Dab2	Pax9
2900093B09Rik	Cklfsf4	Gorasp2	Ovol1	Sp8	Dcn	Pdcd4
3010003L21Rik	Clic6	Gpc2	Pde10a	Spo11	Dmrt3	Pitx2
4732456N10Rik	Cog8	Gpnmb	Pdgfc	Stat3	Edn1	Pknox2
4732481H14Rik	Crabp1	Gpr156	Pdlim4	Stat5a	Efcab1	Pmp22
4930511J11Rik	Crym	Gpr49	Pet112l	Surf4	Ets1	Prkwnk1
5031410I06Rik	Cyfip2	Gpr83	Pex2	Svopl	Fgf10	Ptpre
5730593N15Rik	D11Moh35	Gpx2	Pgls	T	Fgf13	Pyy
8430438D04Rik	D15Ertd682e	Gulo	Phlda2	Tacstd2	Fgf15	Raet1b
9330161F08Rik	D15Mgi27	Hdac6	Plk3	Tbrg1	Fgf16	Rarb
9630019K15Rik	Decr1	Hoxa7	Polr3b	Tbx3	Fhl2	Reln
9930017A07Rik	Defcr	Hoxc9	Pon2	Tcf7	Foxa1	Ret
A630042L21Rik	Defcr26	Hoxd8	Por	Tcfap2c	Foxc1	Rgs5
A730017C20Rik	Defcr-rs10	Hoxd9	Pou4f1	Tex264	Foxc2	Rsn
Abcg5	Defcr-rs2	Hspb1	Ppap2b	Tiam1	Foxd4	Serpina1b
Abhd2	Defcr-rs7	Ifitm1	Ppp1r1c	Tiam2	Foxe1	Serpinf2
Abhd2	Dkk1	Ifngr2	Pqlc2	Timm50	Foxg1	Sertad4
Acta1	Dkk3	Ilvbl	Prf1	Tirap	Foxi2	Sfrp1
Actb	Dkk4	Itm2b	Prkcabp	Tlx2	Frat2	Six1
Ada	Dlx2	Jmjd3	Psd2	Tnfrsf19	Fzd4	Slc35f3
Adssl1	Dnajc6	Klf5	Psmf1	Tnrc11	Glrx	Sox2
Agpat3	Dncic1	Klhl8	Ptn	Trib3	Gpx3	Ssbp2
AI481500	Dok4	Kremen2	Ptpla	Trim29	H1fx	Tbx1
Anxa2	Dpp6	Lgals3	Ptpn1	Trp63	Hey1	Tgm2
Apcdd1	Dpp7	Lmo2	Pygl	Twist2	Hhex	Thbd
Aplp2	Dpp8	LOC622404	Rabggta	Uap1l1	Hoxb1	Titf1
Aqp1	Dtx2	Lrp4	Rbpjl	Uck1	Hs3st6	Tmem46
Aqp11	E430025E21Rik	Lrrc15	Rec8L1	Wbscr16	Hsd11b2	Vgll2
Arfip2	Edar	Lrrn1	Renbp	Wif1	Igfbp3	Vtn
Aspscr1	Efnb1	Lrrn3	Rhbdl2	Wnt10a	Igfbp5	
Atf5	EG665378	Mal	Rims3	Wnt3a		
Atox1	Egfr	Mapkbp1	Rin2	Wnt6		
Atp10a	Elovl1	Matn4	Rod1	Zdhhc4		
Atp13a1	Emid2	Mccc1	Rpl14	Zfp259		
Atp6v0b	En2	MGC99845	Rps6kl1	Zic1		
Atp6v1f	Epb4.9	Mglap	Runx1	Zxda		

Supplemental Table 2

Complete list of genes significantly affected by GSK3iXV treatment in ES cell-derived endoderm.

Expression of these genes is changed >3-fold by treatment of ES cell-derived endoderm with 50 nM GSK3iXV.

Genes upregulated in GSK3iXV-treated ES-derived endoderm		Genes downregulated in GSK3iXV-treated ES-derived endoderm		
1200015N20Rik	Fst	0610041G09Rik	Gata3	Peg3
1700011H14Rik	Fzd10	1190007F08Rik	Gckr	Pem
2010016F14Rik	Gad1	1200009O22Rik	Gfod1	Pga5
2510009E07Rik	Gas6	1200013B22Rik	Ghrl	Pgam2
2610001E17Rik	Gbx2	1700007G11Rik	Gkn1	Pik3r3
2610019F03Rik	Gjb2	1700027A23Rik	Glrx	Plxna2
2610318G18Rik	Gpr114	1810036H07Rik	Gpc4	Ppnr
2810003C17Rik	Gpr49	2810417M05Rik	Gpr120	Ppp1r1a
4930517K23Rik	Gpx2	4631426J05Rik	Gpr23	Prg
5730593N15Rik	Hoxa1	6330403K07Rik	Gsc	Pyy
9930023K05Rik	Idb4	6530413N01Rik	H19	Rab27a
AA175286	Ifitm1	9130213B05Rik	Hesx1	Rfxdc1
Acot1	Igfbp4	Acta2	Hhex	Rin3
Actb	Kel	Aff1	Hs3st1	Robo1
Ada	Lmo2	Agpt2	Id2	Sbk
Adcy8	LOC212390	AI427138	Igf2	Sec14l2
Add3	Mal	Apoa1	Igfbp5	Sema3f
Ahsg	Matn4	Atp10d	Irx5	Sema6d
Alox12e	Mcc	AW548124	Isl1	Sfrp1
Alox15	Mixl1	B230104P22Rik	Kcnc4	Sh3tc1
Ambp	Mllt3	BC019731	Kirrel3	Shroom3
Apcdd1	Mogat2	Bcl2l11	Lama1	Slc12a2
Aqp11	Msx1	Bmper	Lamc1	Slc7a8
Ass1	Nefm	Cadps2	Leftb	Slco2a1
Atoh8	Ngfr	Cbln1	Lhfp	Sparc
Atp1a2	Pcsk9	Cd59a	Lhx1	Spin2
Axin2	Pdk1	Cdkn2b	Lmyc1	Srd5a2
AY761185	Pglyrp1	Cer1	LOC434197	Tal2
B830021E24Rik	Phlda1	Cfc1	Lrp12	Tbx1
BC018465	Phlda2	Chga	Lrpap1	Tcf7l2
BC040774	Ppl	Chi3l1	Ltbp1	Tgfb2
Bhlhb2	Psd2	Chst1	Matn1	Tgfbr2
Card10	Ptk7	Cish	Mbp	Tgfbr3
Ccnd1	Punc	Cldn3	Mertk	Thbd
Cdx1	Rec8L1	Cntnap2	Mest	Tm6sf1
Cdx2	Rfx4	Cxxc4	MGC106740	Tmem46
Cpn1	Rps6kl1	Cyp26a1	Mt2	Tmem63a
Cpz	Scara5	Cyp2j9	Mxi1	Tnnc1
Csn3	Sepp1	Cyr61	Myl7	Tnnt1
Cyp1b1	Serpina5	D030013I16Rik	Myocd	Tph1
Defcr	Slc16a10	Dgkk	Nkx2-3	Tpm1
Defcr26	Slc1a4	Dmbx1	Nptx2	Tspan7
Defcr4	Smox	Dpysl3	Otx2	Ttr
Defcr6	Sp5	Ephb1	Pard6g	Tuba8
Defcr-rs1	Sp8	Fgf5	Pcdh21	Tulp1
Defcr-rs10	Spo11	Fgfbp1	Pcdh7	Vav3
Defcr-rs12	Spon1	Foxd4	Pde1b	Zfpm1
Defcr-rs2	Tcf2	Fzd7		
Defcr-rs7	Tcf7			
Dkk3	Tm4sf12			
Dkk4	Tmem77			
Dpp4	Tmprss2			
EG545370	Tnfrsf19			
Emid1	Unc5c			
Emid2	Wnt5b			
Evx1	Wnt6			
Fgfbp3	Wnt7a			

Supplemental Table 3

Complete list of genes preferentially expressed in specific E14.5 gut segments.

These genes have expression >3-fold higher in one E14.5 gut segment as compared to all other segments.

Stomach	Anterior small intestine	Posterior small intestine	Large intestine
2210413P10Rik	Amica1	Cdo1	1700011H14Rik
Bace2	Anpep	Cib2	4732472I07Rik
Corin	Areg	Fzd10	Abi3
Creb3l4	Btc	Gpr49	Abp1
D930038M13Rik	Crp	Guca2a	Adh1
Dmn	Dlk1	Ly64	Afp
Ern2	Dmbt1	Mamdc4	Aim2
Evi1	Dusp4	Olfml1	Anxa3
Gsta4	Egln3	Osr2	Atp12a
Idb4	Enpp1	Prodh2	Atp2a3
Ly6h	Eps8l3	Sct	BC025446
Mds1	Gpr128	Sdh1	Bcas1
Rasgrp3	Gpx3	Snca	Crim2
Sox21	Igsf1	Vim	D030013I16Rik
	Lgals3bp		Emp2
	Onecut2		Evx1
	Ppargc1b		Fabp3
	Prap1		Fxyd4
	Siat8c		Gcnt1
	Smoc1		Gdnf
	Spp1		Gna14
	Tm4sf4		Gne
	Tulp2		Hoxa7
	Upk3b		Hoxb7
			Liph
			LOC233038
			Lor
			Lpl
			Ly6a
			Nrn1
			Pdzk1
			Ppp1r1b
			Prkcdbp
			Schip1
			Serpinb1a
			Slc5a8
			Sri
			Stxbp1
			Upk1a
			Xpnpep2

Supplemental Table 4

Complete list of genes significantly affected by GSK3iXV treatment in ES cell-derived endoderm, grouped based on whether or not they are coordinately changed by Cdx2 induction.

Expression of these genes is changed >2.5-fold by treatment of ES cell-derived endoderm with 50 nM GSK3iXV.

Genes upregulated in GSK3iXV-treated ES-derived endoderm			Genes downregulated in GSK3iXV-treated ES-derived endoderm			
Induced by Ectopic Cdx2	Not Induced by Ectopic Cdx2		Downregulated by Ectopic Cdx2		Not Downregulated by Ectopic Cdx2	
2510009E07Rik	1200015N20Rik	Hebp1	1190002A17Rik	Lhx1	1110003E01Rik	Irx5
2610318G18Rik	1700019D03Rik	Hoxa1	1700027A23Rik	LOC620807	1110035L05Rik	Itpr3
4930511J11Rik	2610019F03Rik	Hoxb1	2310045A20Rik	Loxl1	1190007F08Rik	Krt1-18
Adcy8	2900093B09Rik	Hoxb2	2410012C07Rik	Ly6h	1200009O22Rik	Lasp1
Aldh4a1	3632451O06Rik	Hoxb4	3110001A13Rik	Matn1	1200013B22Rik	Leftb
Ambp	4632425D07Rik	Hoxb5	9130213B05Rik	Mertk	1600023A02Rik	Lgals3bp
Arid5b	5730593N15Rik	Ifi30	Adamts4	Mfap4	1700007G11Rik	Lhfp
Atp1b1	6330505N24Rik	Ifitm1	AI427138	Nkx2-3	2310010M24Rik	Lmyc1
B430218L07Rik	A730017C20Rik	Ihh	Alcam	Nkx2-5	2810046M22Rik	LOC434197
B4galnt2	A930010I20Rik	Kcnj4	Amn	Nkx3-1	4631426J05Rik	Mbp
B830021E24Rik	Abhd2	Kdelr3	Apoa1	Nptx2	6330403K07Rik	Mest
Chst7	Acot1	LOC212390	BC011468	Nsg2	8430427H17Rik	Mfap2
Cyp1b1	Adcy2	Lrig1	BC037527	Nxf7	Acaa2	Mical1
Evx1	AI413582	Matn4	BC055811	Otx2	Acad10	Mmp17
Gprc5a	Aldh1a2	Mcc	Bmper	Pacsin1	Acta2	Mreg
Hoxa5	Aldh5a1	Mixl1	Car14	Pde1b	Actc1	Mtap1b
Lmo2	Aldoc	Mllt3	Car2	Pdyn	Aff1	Mxi1
Msx1	Apcdd1	Nkd1	Cer1	Peg3	Agpt2	Myl4
Msx2	Aqp11	Nkd2	Chi3l1	Pou3f1	Akr1c19	Myl7
Ngfr	Ass1	Nkx1-2	Chst1	Prkch	Ankrd1	Nuak1
Ptk7	Atp1a2	Nme5	Clip1	Pyy	Anxa6	Pcdh7
Punc	Axin2	Nos3as	Coch	Rab27a	Art5	Pcsk1n
Sh3gl2	AY761185	Nrarp	Col2a1	Rarb	Atp10d	Pdlim3
Smarca2	B3gnt7	Nup210	Cplx2	Rasgrp3	AW548124	Pga5
Tcf2	BC018465	Pcsk9	Crx	Rax	Axud1	Pgam2
Tmem77	BC040774	Pdgfra	Cyp26a1	Rfx2	B230104P22Rik	Pik3r3
Tmprss2	Bhlhb2	Pglyrp1	Dnmt3b	Rfxdc1	BC019731	Plac1
	Cdx1	Phlda2	Efcbp2	Rsn	Bok	Plekha6
	Cdx2	Plcg2	Eomes	Sfrp1	C630028C02Rik	Pls3
	Chst2	Ppp1r3c	Fgf5	Sgk	Cacna2d3	Ppnr
	Cited1	Ptplb	Foxd4	Shh	Cadps2	Ppp1r1a
	Cpn1	Rab11fip5	Foxh1	Six1	Capn6	Prnd
	Cpz	Rec8L1	Fzd2	Slc12a2	Cbln1	Prss19
	Crtac1	Sepp1	Ghrl	Slc1a3	Ccdc68	Qprt
	Cxx1a	Serpina5	Gjb3	Slc7a8	Cd24a	Rasl11a
	Cxx1c	Serpinb6c	Gpr23	Soat2	Cd59a	Rbp4
	Cyp27a1	Sesn3	Gsc	Sox9	Chga	Rem2
	Defcr	Slc16a10	H19	Sst	Cish	Rgs5
	Defcr26	Slc1a4	Has2	Tal2	Ckb	Rin3
	Defcr-rs1	Slc24a3	Hesx1	Tbx1	Cldn3	Robo1
	Defcr-rs10	Slc38a4	Hhex	Tmem46	Clu	Rtn2
	Defcr-rs2	Smox	Hsd11b2	Tnrc9	Cntnap2	Sall1
	Defcr-rs7	Sostdc1	Igf2	Tph1	Ctgf	Satb1
	Dll1	Sp5	Isl1	Ttr	Ctsc	Sbk
	Dpp4	Sp8	Kctd12b	Utf1	Cxxc4	Sec14l2
	Dpysl4	Spo11	Laptm5	Wisp1	D030013I16Rik	Sema3f
	Efhd1	Srrp		Zfpm1	Dab1	Sertad4
	Emid1	Stard8			Ddah1	Sh3tc1
	Epha1	Stmn4			Ddc	Shrm
	Etv4	Stra6			Dgkk	Shroom3
	Fgf17	Syngr2			Dpysl3	Slc38a5
	Fgfbp3	Sytl1			Ebaf	Slit2
	Foxb1	Tbx6			Efna5	Spdef
	Fst	Tcf7			Eppk1	Spin2
	Fzd10	Tm4sf12			Eras	Spon2
	Gad1	Tnfrsf19			F2r	Syt11
	Gas6	Unc5c			Fgd2	Syt5
	Gbx2	Wnt10a			Fgfbp1	Tcf21
	Gjb2	Wnt3a			Flrt2	Tcf3
	Gna14	Wnt5b			Fos	Tcf7l2
	Gpc6	Wnt6			Frat2	Tdrd7
	Gpr49	Wnt7a			Fzd7	Tgfb2
	Gpx2	Wnt8a			Galnt10	Tgfbr2
		Zic1			Garnl4	Tgfbr3
					Gata3	Tle4
					Gckr	Tmem108
					Glrx	Tmem2
					Gng8	Tnnc1
					Gpc4	Tnnt1
					Gpr120	Tspan7
					Gulp1	Unc5b
					Hemp1	Vav3
					Idb1	Vwf
					Igfbp5	Ypel1