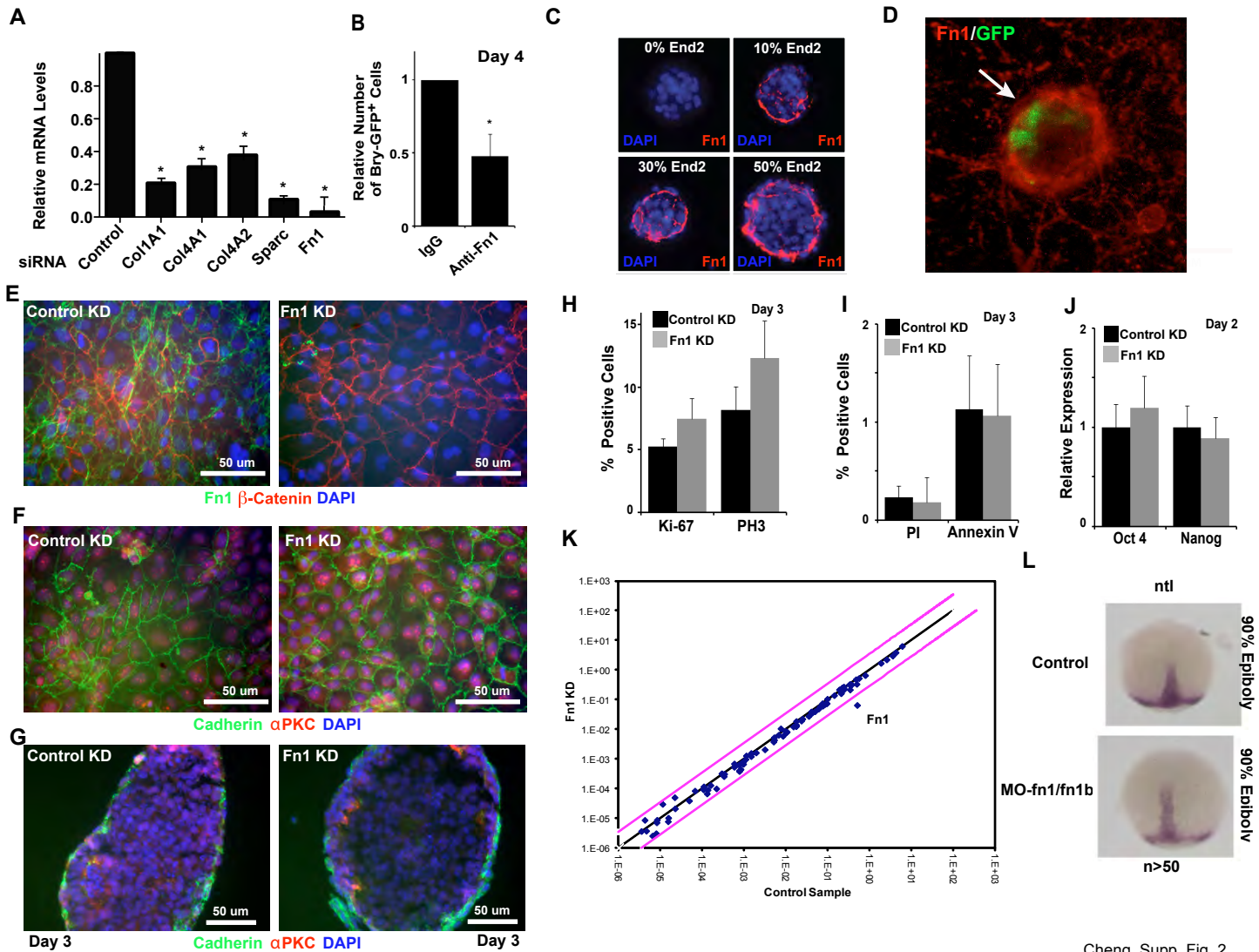
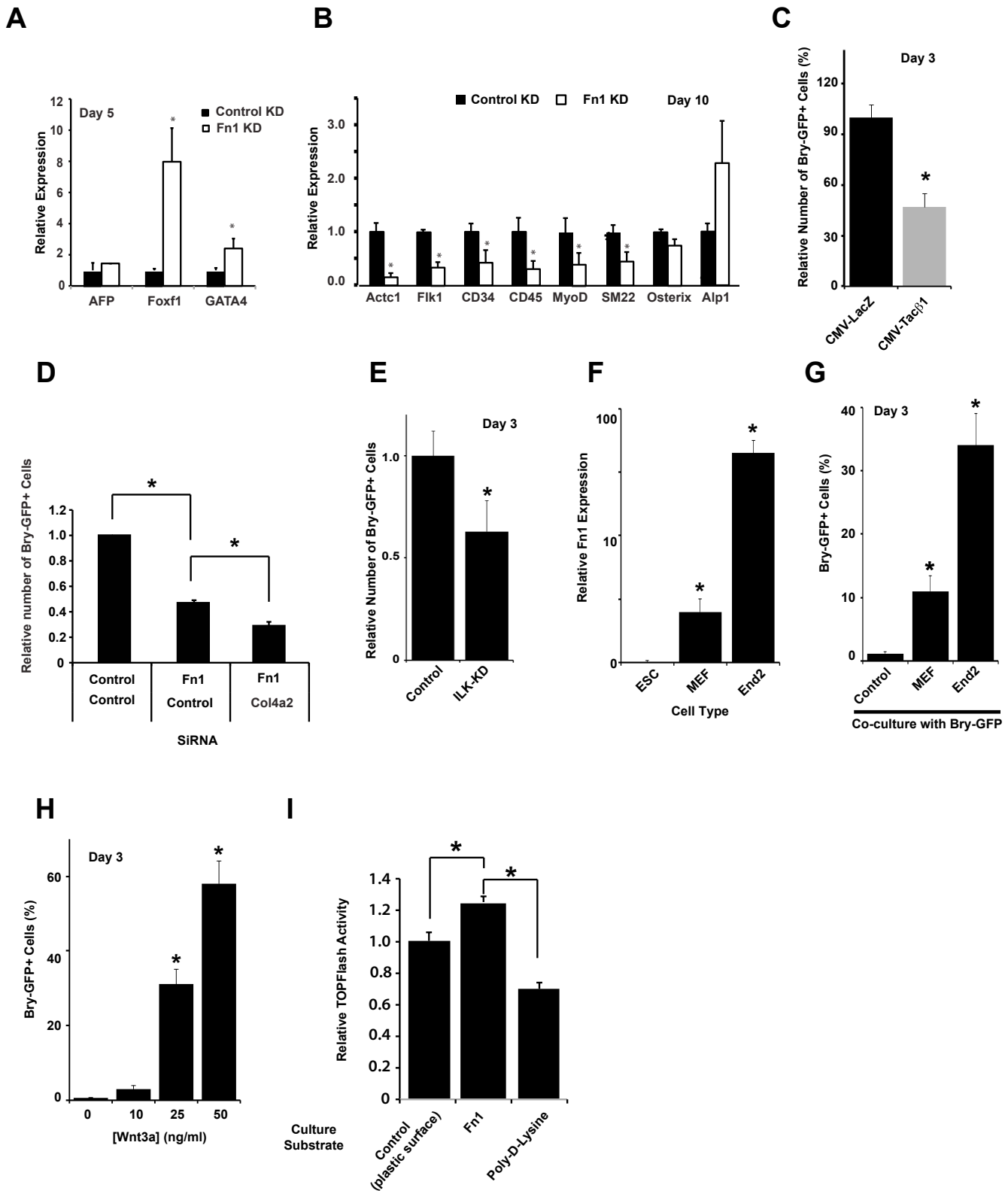


**Fig. S1. Endoderm-like (End2) cells promote the emergence of mesoderm in ES cells through a short-range signal.** (A) Relative expression of cardiac genes *Actc1* and *Hand2* in embryoid bodies (EBs) formed with increasing ratios of End2 cells relative to mES cells (ESCs). (B) Percentage of beating foci observed in a mixed culture of EBs formed with (green/red) or without (yellow) End2 cells. (C) Number of mES cells and End2 cells at day 0 and after 5 days of co-culture. (D) Percentage of Bry-GFP<sup>+</sup> mesodermal cells in EBs differentiated with End2 cells, End2-conditioned medium, or conditioned medium concentrated via a 10 or 30  $\mu$ m filter. \* $P < 0.05$ , error bars indicate s.e.m.



Cheng, Supp. Fig. 2

**Fig. S2. Fibronectin promotes End2-mediated induction of mesoderm and precardiac mesoderm *in vitro* and *in vivo*.** (A) Knockdown efficiency of the indicated siRNAs assayed by qPCR. (B) Percentage of Bry-GFP<sup>+</sup> cells in day-4 EBs cultured with End2 cells with control (IgG) or anti-Fn1 antibody. (C) EBs with increasing ratio of End2 cells stained with DAPI and Fn1 antibody. (D) Adherent culture of mES<sup>Bry-GFP</sup> EBs plated with End2 cells and stained for Fn1 (red) and GFP (green). (E) End2 cells 48 hours after transfection with scrambled siRNA or siRNA against Fn1, stained for Fn1 (green) and  $\beta$ -catenin (red). (F) End2 cells 48 hours after transfection with scrambled siRNA or siRNA against Fn1, stained for pan-cadherin (green) and  $\alpha$ PKC (red). (G) EBs containing End2 cells transfected with scrambled siRNA or siRNA against Fn1, stained for pan-cadherin (green) and  $\alpha$ PKC (red). (H) FACS analysis of cell proliferation as assayed by Ki-67 or phosphohistone H3 (PH3) staining among Bry-GFP<sup>+</sup> cells in EBs aggregated with control or fibronectin-deficient End2 cells. (I) FACS analysis of cell death as assayed by propidium iodide (PI)<sup>+</sup> and annexin V staining among Bry-GFP<sup>+</sup> populations in EBs aggregated with control or fibronectin-deficient End2 cells. (J) Expression of the pluripotent genes *Oct4* and *Nanog* in day-2 EBs with control or Fn1-deficient End2 cells. (K) Scatter plot of ECM mRNA array from EBs co-cultured with control or Fn1 knockdown End2 cells. (L) Whole-mount *in situ* hybridization of *ntl* in control or Fn1/Fn3 knockdown (MO-Fn1/3) zebrafish embryos at 90% epiboly (9 hpf). \**P*<0.05, error bars indicate s.e.m.



**Fig. S3. Fibronectin augments mesoderm induction through integrin-dependent activation of Wnt/ $\beta$ -catenin signaling.**

(A) Expression of various endodermal markers in cells differentiated from Bry-GFP<sup>+</sup> cells isolated from EBs with control or Fn1 knockdown (KD). (B) Expression of various mesodermal markers in cells differentiated from Bry-GFP<sup>+</sup> cells isolated from EBs with control or Fn1-deficient End2 cells. (C) Relative number of Bry-GFP<sup>+</sup> cells induced by End2 cells in differentiating mES<sup>Bry-GFP</sup> cells with overexpression of the integrin signaling mutant Tac $\beta$ 1 or control (*lacZ*). (D) Relative number of Bry-GFP<sup>+</sup> cells induced by End2 cells transfected with control and/or Fn1/Col4a2 siRNA. (E) Relative number of Bry-GFP<sup>+</sup> cells in End2 co-cultures with mES<sup>Bry-GFP</sup> cells transfected with control or Ilk siRNA. (F) Relative *Fnl* expression in ESCs, MEFs or End2 cells. (G) Percentage Bry-GFP<sup>+</sup> cells observed in differentiation with ESCs alone, or co-culture with MEFs or End2 cells. (H) Percentage Bry-GFP<sup>+</sup> cells induced by increasing concentrations of Wnt3a. (I) Relative TOP-flash activity in differentiated ESCs cultured on Fn1 or poly-D-lysine. \* $P < 0.05$ ,  $n = 3$  or greater. Error bars indicate s.e.m.



**Movie 1. Spontaneous contraction of cardiomyocytes differentiated from End2-induced precardiac mesoderm.**

**Table S1. Expression of ECM genes in End2 cells versus ES and ectodermal cells**

Symbol	Log <sub>2</sub> (FC)	P-value	Expressed around egg-cylinder and gastrulation stage
<b><i>Fn1</i></b>	<b>2.36832369</b>	<b>0.000482225</b>	+
<i>Lama3</i>	-3.69437791	0.004787302	
<b><i>Col4a1</i></b>	<b>5.39362669</b>	<b>0.006466226</b>	+
<b><i>Col4a2</i></b>	<b>2.25451884</b>	<b>0.009827216</b>	+
<b><i>Timp3</i></b>	<b>4.78813179</b>	<b>0.010284841</b>	-
<i>Itgav</i>	1.81114939	0.011167394	
<b><i>Sparc</i></b>	<b>2.88647209</b>	<b>0.013530666</b>	+
<b><i>Cdh2</i></b>	<b>2.00801929</b>	<b>0.013980868</b>	-
<b><i>Sgce</i></b>	<b>2.51501439</b>	<b>0.017630714</b>	-
<i>Ctnnb1</i>	-1.62105666	0.01829935	
<b><i>Timp2</i></b>	<b>4.80258464</b>	<b>0.020218724</b>	-
<b><i>Col1a1</i></b>	<b>6.11512519</b>	<b>0.020504982</b>	+
<i>Cntn1</i>	-1.52143081	0.021217884	
<i>Thbs3</i>	-1.55693326	0.024294494	
<i>Vcam1</i>	6.87848319	0.028849818	
<i>Adamts5</i>	2.66148604	0.029912537	
<i>Mmp2</i>	2.41154834	0.03266755	
<i>Ctnna2</i>	2.24932944	0.036734739	
<i>Thbs2</i>	-3.59531056	0.041485655	
<i>Col3a1</i>	6.66594809	0.04231551	
<i>Col5a1</i>	1.55671699	0.047171288	
<i>Itgam</i>	-2.18766771	0.052602835	
<i>Tnc</i>	3.28507064	0.05851667	
<i>Itgb1</i>	0.90042484	0.058915566	
<i>Hapln1</i>	-2.41886931	0.063244531	
<i>Cdh3</i>	1.52066139	0.064073183	
<i>Itgb3</i>	4.04664894	0.071629966	
<i>Mmp13</i>	3.58515104	0.072185608	
<i>Cd44</i>	4.93159714	0.081185984	
<i>Pecam1</i>	-5.15819076	0.082377403	
<i>Thbs1</i>	4.53810729	0.084706461	
<i>Postn</i>	2.96244189	0.090221262	
<i>Adamts1</i>	2.73405889	0.099148625	
<i>Spp1</i>	-5.03487621	0.113469299	
<i>Ncam1</i>	0.81139574	0.121124338	
<i>Lamb2</i>	0.73336334	0.135373586	
<i>Itgb4</i>	-1.00554206	0.150664561	
<i>Mmp14</i>	1.39571854	0.155233801	
<i>Col6a1</i>	7.11230019	0.165616311	
<i>Itgal</i>	-2.57583136	0.167756088	
<i>Selp</i>	1.47631989	0.173485089	
<i>Mmp8</i>	2.99186449	0.182355545	
<i>Mmp9</i>	-3.36491961	0.187441341	
<i>Lamb3</i>	-2.97790276	0.191517903	
<i>Sele</i>	1.88041984	0.193782813	

<i>Adamts2</i>	1.15955854	0.203361496
<i>Col4a3</i>	1.39159194	0.209170667
<i>Col2a1</i>	-4.03470811	0.209645434
<i>Cdh1</i>	-4.95819166	0.223356991
<i>Spock1</i>	-3.01631216	0.240265978
<i>Mmp15</i>	-1.47104581	0.240576219
<i>Icam1</i>	-0.11250206	0.243198795
<i>Adamts8</i>	-1.85968281	0.25723376
<i>Mmp11</i>	-0.56176436	0.259450606
<i>Ctgf</i>	0.28752319	0.269014355
<i>Syt1</i>	1.51337214	0.270920915
<i>Emilin1</i>	-2.35108266	0.292417197
<i>Mmp1a</i>	1.29737074	0.300237402
<i>Vcan</i>	1.09386404	0.319549642
<i>Itga3</i>	1.11160969	0.321351939
<i>Ncam2</i>	-1.48765866	0.343283912
<i>Ecm1</i>	0.62412304	0.360233824
<i>Lama2</i>	0.28008739	0.367725047
<i>Mmp7</i>	1.40926059	0.371188088
<i>Itga2</i>	0.90122809	0.376339274
<i>Sell</i>	0.80391174	0.384569169
<i>Tgfb1</i>	-2.19130066	0.394459931
<i>Timp1</i>	-1.43928676	0.40153966
<i>Lama1</i>	-4.47066411	0.408702404
<i>Hc</i>	0.09429884	0.426772266
<i>Mmp12</i>	0.27556214	0.435886078
<i>Ctnna1</i>	-0.05077756	0.53954357
<i>Mmp3</i>	0.61166869	0.543160669
<i>Itgax</i>	0.04080364	0.549823798
<i>Itga5</i>	0.24840284	0.561258646
<i>Lamc1</i>	0.01268754	0.605250367
<i>Itga4</i>	-0.62558746	0.638477331
<i>Itgb2</i>	-0.84664986	0.652115056
<i>Cdh4</i>	-4.15708061	0.744720352
<i>Fbln1</i>	-0.77668296	0.748958051
<i>Itgae</i>	-0.10477356	0.773917062
<i>Mmp10</i>	0.91885374	0.794369608
<i>Entpd1</i>	-0.15355406	0.831367709
<i>Vtn</i>	0.00488484	0.89528792

Cut-offs were set at >4-fold increase,  $P > 0.025$ .