

Table S1. A reinterpretation of mutant mouse phenotypes that include defective AVE formation

Gene	Localization in wild type		Genetic modification	Areas of expression that remain in the mutant		DVE formation (molecular phenotype) at E5.5	DVE migration (molecular phenotype)	AVE formation (molecular phenotype) at E6.5	AVE migration (molecular phenotype)	References
	E5.5	E6.5		E5.5	E6.5					
Nodal signaling										
<i>Nodal</i>	EPI+EmVE	Posterior EPI+EmVE	Null	×	×	× (<i>Cer1; Lefty1; Dkk1</i>)	–	× (<i>Cer1; Lefty1; Dkk1; Hhex</i>)	–	(Brennan et al., 2001; Camus et al., 2006; Mesnard et al., 2006)
			EPI-specific null	EmVE	EmVE	○ (mouse <i>Fz8?</i>)	○ (mouse <i>Fz8?</i>)	× (<i>Hhex; mouse Fz8</i>)	–	(Lu and Robertson, 2004)
			Hypomorphic (severe)	N.D.	Proximal EPI (weak)	N.D.	N.D.	○ (<i>Hesx1</i>)	× (<i>Hesx1</i>)	(Lowe et al., 2001)
			Protease-cleaved mutant	×	Proximal EPI (weak)	N.D.	N.D.	× (<i>Cer1; Hesx1; Lhx1</i>)	–	(Ben-Haim et al., 2006)
			Δ600/-EPI-dependent ASE enhancer upstream of Nodal	N.D.	Proximal EPI (weak)	N.D.	N.D.	○ (<i>Hhex; Lhx1</i>) × (<i>Lefty1</i>)	× (<i>Hhex; Lhx1</i>)	(Norris et al., 2002)
<i>Furin; Pcsk6</i>	Furin: ExE Pcsk6: ExE	ExE ExE	Double null	×	×	N.D.	N.D.	× (<i>Hhex; Hesx1; Lefty1</i>)	–	(Beck et al., 2002; Mesnard et al., 2006)
<i>Smad2</i>	Ubiquitous	Ubiquitous	Null	×	×	× (<i>Hhex; Lhx1; Lefty1</i>)	–	× (<i>Hhex; Lhx1; Lefty1</i>)	–	(Nomura and Li, 1998; Brennan et al., 2001)
<i>Cripto</i>	EPI	Posterior EPI	Null	×	×	× (<i>Cer1; Lefty1</i>)	–	○ (<i>Hhex; Cer1</i>) × (<i>Lefty1</i>)	× (<i>Hhex; Cer1</i>)	(Ding et al., 1998; Kimura et al., 2001; Chu and Shen; 2010)
<i>Foxh1</i>	EPI+EmVE	EPI+EmVE	Null (severe)	×	×	○ (<i>Hhex</i>) × (<i>Lefty1</i>)	× (<i>Hhex</i>)	○ (<i>Cer1; Lhx1; Hhex</i>) × (<i>Lefty1</i>)	× (<i>Cer1; Lhx1; Hhex</i>)	(Yamamoto et al., 2001; Yamamoto et al., 2004)
			Null (mild)	×	×	○ (<i>Hhex</i>) × (<i>Lefty1</i>)	Delay (<i>Hhex</i>)	○ (<i>Cer1; Lhx1; Hhex</i>) × (<i>Lefty1</i>)	Delay (<i>Cer1; Lhx1; Hhex</i>) × (<i>Lefty1</i>)	
<i>Gdf3</i>	EPI	EPI	Null (severe)	×	×	N.D.	N.D.	× (<i>Lefty1; Hhex</i>)	–	(Chen et al., 2006; Andersson et al., 2007)
			Null (mild)			N.D.	N.D.	○ (<i>Lefty1; Hhex</i>)	○ (<i>Lefty1; Hhex</i>)	
<i>Lefty1</i>	DVE	AVE	Null	×	×	Expansion (<i>Hhex; Cer1</i>)	○ (<i>Hhex; Cer1</i>)	Expansion (<i>Hhex; Cer1</i>)	○ (<i>Hhex; Cer1</i>)	(Yamamoto et al., 2004; Trichas et al., 2011)
<i>Lefty1; Cer1</i>	DVE	AVE	Double null	×	×	N.D.	N.D.	○ (<i>Hhex</i>)	Delay (<i>Hhex</i>)	(Perea-Gomez et al., 2002; Yamamoto et al., 2004)
				×	×					

Wnt signaling										
β -Catenin	Ubiquitous?	Ubiquitous?	Null	×	×	N.D.	N.D.	\times (<i>Hhex; Lefty1</i>) ○ (<i>Cer1; Hesx1</i>)	\times (<i>Cer1; Hesx1</i>)	(Huelsken et al., 2000; Morkel et al., 2003; Kimura-Yoshida et al., 2005)
<i>Otx2</i>	EPI+VE	EPI+VE	Null	×	×	\times (<i>Hhex; Cer1; Lefty1</i>) \times (<i>Dkk1</i>)	\times (<i>Hhex; Cer1; Lefty1</i>)	\times (<i>Hhex; Cer1; Lefty1</i>) \times (<i>Dkk1</i>)	\times (<i>Hhex; Cer1; Lefty1</i>)	(Kimura et al., 2000; Kimura et al., 2001)
<i>Apc</i>	Ubiquitous?	Ubiquitous?	Multiple intestinal neoplasia	×	×	\times (<i>Hhex; Lhx1</i>)	–	\times (<i>Cer1</i>)	–	(Kielman et al., 2002; Chazaud et al., 2006)
BMP signaling										
<i>Bmpr1a</i>	VE+EPI	VE+EPI	Null	×	×	N.D.	N.D.	○ (<i>Hhex; Cer1</i>) \times (<i>Dkk1</i>)	\times (<i>Hhex; Cer1</i>)	(Mishina et al., 1995; Miura et al., 2010)
			EPI-specific null	VE	VE	○ (<i>Hhex; Cer1; Dkk1</i>)	Abnormal (<i>Hhex; Cer1; Dkk1</i>)	○ (<i>Hhex; Hesx1; Cer1; Dkk1</i>)	Abnormal (<i>Hhex; Hesx1; Cer1; Dkk1</i>)	(Miura et al., 2010)
<i>Bmpr2</i>	VE+EPI	VE+EPI		×	×	\times (<i>Cer1; Hhex; Lefty1; Lhx1</i>)	–	–	–	(Beppu et al., 2000; Yamamoto et al., 2009)
<i>Bmp4</i>	ExE	ExE	Knock down by dsRNA at E5.2	\times (after E5.2)	\times ?	Expansion (<i>Cer1</i>)	\times (<i>Cer1</i>)	Expansion (<i>Cer1</i>)	\times (<i>Cer1</i>)	(Soares et al., 2008)
Others										
<i>Nckap1</i>	N.D.	N.D.	Null	×	×	N.D.	N.D.	○ (<i>Hhex; Cer1; Lhx1</i>)	\times (<i>Hhex; Cer1; Lhx1</i>)	(Rakeman and Anderson, 2006)
			Null	×	×	N.D.	N.D.	\times (<i>Hhex; Cer1</i>)	\times (<i>Hhex; Cer1</i>)	(Sugihara et al., 1998; Migeotte et al., 2010)
<i>Rac1</i>	N.D.	N.D.	VE-specific null	×	×	○ (<i>Hhex</i>)	\times (<i>Hhex</i>)	○ (<i>Hhex; Cer1; Lhx1</i>)	\times (<i>Hhex; Cer1; Lhx1</i>)	(Migeotte et al., 2010)
DVE-ablated embryo	–	–	–	–	–	\times (<i>Cer1; Hhex; Lefty1</i>)	–	○ (<i>Cer1; Hhex; Lefty1</i>)	\times (<i>Cer1; Hhex; Lefty1</i>)	(Miura and Mishina, 2007; Takaoka et al., 2011)

The phenotypes of various mouse mutants are summarized on the basis of four criteria: (1) whether the DVE forms; (2) whether DVE migrates; (3) whether the AVE forms; and (4) whether the AVE migrates. Our aim is to distinguish the DVE and AVE where possible; although they can be clearly distinguished in some studies, in others it is not clear which of these two lineages is defective. The DVE and AVE markers used to examine each criterion are indicated. ○, normal/present; ×, absent/impaired; –, not applicable, N.D. not determined.

AVE, anterior visceral endoderm; DVE, distal visceral endoderm; EmVE, embryonic visceral endoderm; EPI, epiblast; ExE, extra-embryonic ectoderm; VE, visceral endoderm.

Apc, adenomatosis polyposis coli; *Bmp*, bone morphogenetic protein; *Bmpr*, bone morphogenetic protein receptor; *Cer1*, cerberus 1 homolog; *Cripto*, cryptic family 1; *Dkk1*, dickkopf homolog 1; *Foxh1*, forkhead box H1; *Fz8*, frizzled 8; *Gdf3*, growth differentiation factor 3; *Hesx1*, homeobox gene expressed in ES cells; *Hhex* (Hex), hematopoietically expressed homeobox; *Lefty1*, left right determination factor 1; *Lhx1* (Lim1), LIM homeobox protein 1; *Nckap1* (Nap1), NCK-associated protein 1; *Otx2*, orthodenticle homolog 2; *Pcsk6* (Pace4), proprotein convertase subtilisin/kexin type 6; *Rac1*, RAS-related C3 botulinum substrate 1; *Smad2*, MAD homolog 2.