

SUPPORTING INFORMATION

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Supplemental for Figure 1

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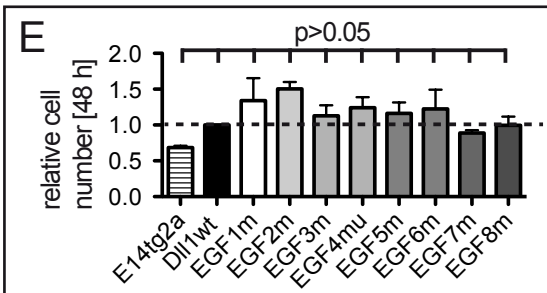
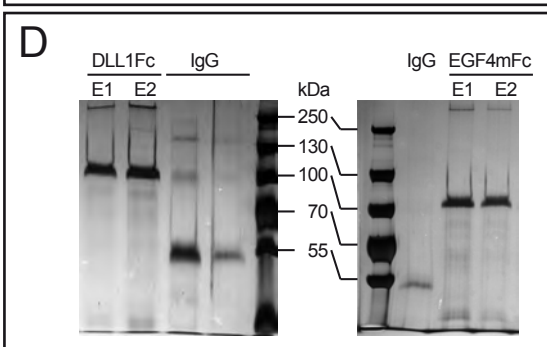
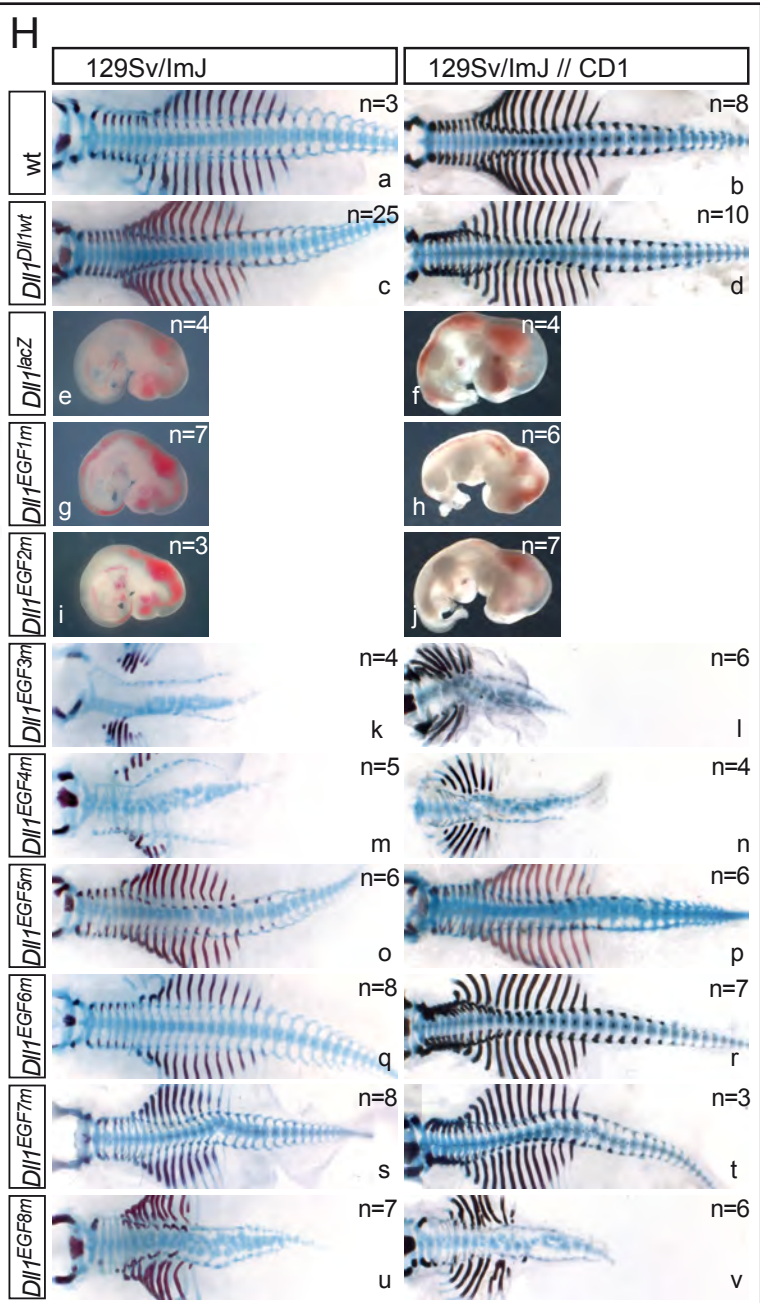
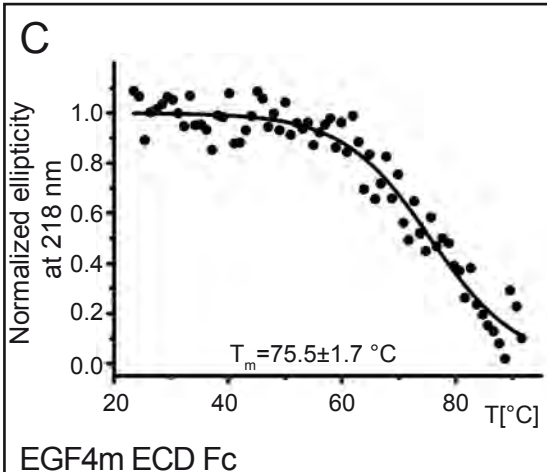
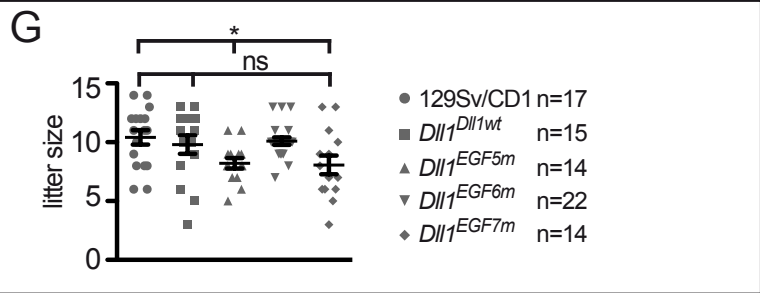
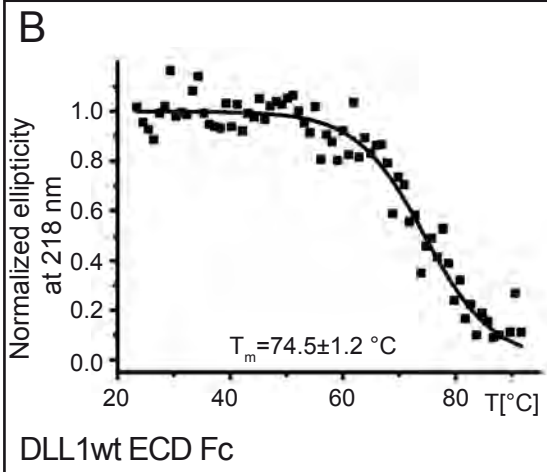
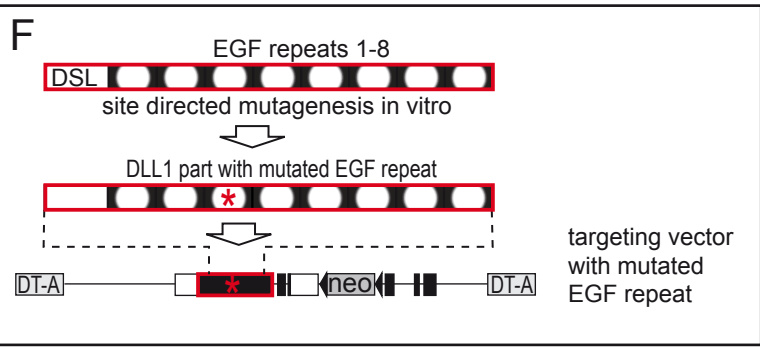
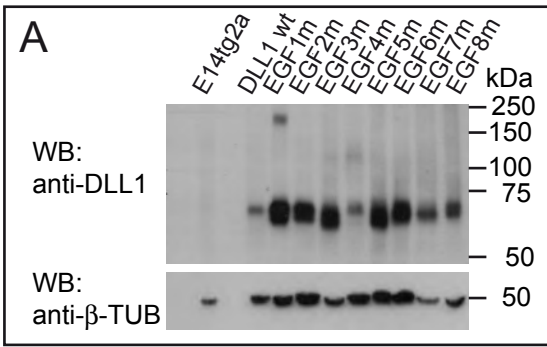


Figure S1 Characterization of mutant proteins and alleles on different genetic backgrounds

(A) Western blot analysis of DLL1 wt and EGF mutant proteins separated under non-reducing conditions. Melting curves of Fc-fusion proteins of the ECD of DLL1 wild type (B) and EGF4 mutant (C) as determined by circular dichroism (CD) spectroscopy at 218 nm. Circular Dichroism for wildtype and mutant protein was followed along a temperature gradient from 23.4°C to 91.6°C. Data curves are normalized and fitted according to the model of Boltzmann. [1.0] displays the folded state whereas [0.0] is attributed to the unfolded state. The melting temperatures are $74.5 \pm 1.2^\circ\text{C}$ for the wild type protein and $75.5 \pm 1.7^\circ\text{C}$ for the EGF4 mutant, respectively. (D) Silver stained SDS PAGE gel of human IgG and purified DLL1ECD-Fc and EGF4mECD-Fc used for CD spectroscopy. (E) Cell numbers of ES cells expressing DLL1 proteins from the Hprt locus after 48 h of culture normalized to DLL1 wt expressing cells. (F) Schematic representation of the strategy to introduce mutations in individual EGF repeats into the same targeting vector. The region encoding the DSL domain and the EGF repeats in the cDNA portion of the mini gene is flanked by unique restriction sites, can be readily excised by restriction enzymes and replaced by the same region of DLL1 after site directed mutagenesis in vitro. (G) Breeding performance of homozygous viable *Dll1* alleles on a hybrid 129Sv/ImJ/CD1 background. *Dll1*^{*Dll1*wt} (square), *Dll1*^{*EGF6m*} (triangle downward), breed essentially like wt (circle), *Dll1*^{*EGF5m*} (triangle upward) and *Dll1*^{*EGF7m*} (diamond) show reduced breeding performance ($p \leq 0.05$). Each symbol represents one litter. (H) Comparison of either external (E10.5) or skeletal phenotypes (E15.5) of homozygous mutants on an isogenic 129Sv/ImJ and mixed 129Sv/ImJ/CD1 genetic background. Severity and range of phenotypes are virtually identical on both genetic backgrounds. Alleles are indicated at the left, genetic backgrounds at the top.

Table S1. Quantification of steady state levels of DLL1 variant proteins.

WB #	DLL1 wt	EGF1m	EGF2m	EGF3m	EGF4m	EGF5m	EGF6m	EGF7m	EGF8m
1	1	4,598	1,468	2,551	1,344	2,077	1,844	1,554	2,141
2	1	3,110	0,942	1,689	0,847	1,971	1,576	1,337	2,413
3	1	4,955	1,596	1,907	0,716	1,540	0,801	0,862	1,783
4	1	5,805	2,081	4,789	1,749	2,913	2,085	1,920	3,780
Mean	1,000	4,617	1,522	2,734	1,164	2,125	1,577	1,418	2,529

Protein levels of EGF mutants relative to wt DLL1 determined from 4 independent Western Blots of ES cell lysates using β -tubulin as loading control. These values were used to generate the bar graph in Fig. 1E and for statistical analyses. The correction factors used for calculations in Fig. 1 I and K are indicated in bold.

Table S2. Quantification of surface levels of DLL1 EGF mutant proteins.

Exp.		Dll1 wt Flag		EGF1m flag		EGF2m flag		EGF3m flag		EGF4m flag		EGF5m flag		EGF6m flag		EGF7m flag		EGF8m flag	
		Input	IP	Input	IP	Input	IP	Input	IP	Input	IP	Input	IP	Input	IP	Input	IP	Input	IP
1	signal	185	784	1863	106	834	5387	1034	4240	977	3661	1142	7628	1101	5513	372	1434	915	665
	total Input (x 100)	18500	1568	186300	212	83400	10774	103400	15802	97700	7322	114200	15256	110100	11026	37200	2868	91500	1330
	total IP (x2)																		
	IP/input		0,085		0,001		0,129		0,153		0,075		0,134		0,100		0,077		0,015
	normalized surface level		1		0,013		1,524		1,803		0,884		1,576		1,182		0,910		0,171
2	signal	1562	5338	3838	504	957	4042	1677	1022	933	2270	3727	6525	2112	7020	1696	5064	3898	2566
	total Input (x 100)	156200	10676	383800	1008	95700	8084	167700	2044	93300	4540	372700	13050	211200	14040	169600	10128	389800	5132
	total IP (x2)																		
	IP/input		0,068		0,003		0,084		0,012		0,049		0,035		0,066		0,060		0,013
	normalized surface level		1		0,038		1,236		0,178		0,712		0,512		0,973		0,874		0,193
3	signal	1127	3539	5533	962	2017	6538	3492	5958	69	502	488	2441	346	1767	1583	4845	2909	3298
	total Input (x 100)	112700	7078	553300	1924	201700	13076	349200	11916	6900	1004	48800	4882	34600	3534	158300	9690	290900	6596
	total IP (x2)																		
	IP/input		0,063		0,003		0,065		0,034		0,146		0,100		0,102		0,061		0,023
	normalized surface level		1		0,055		1,032		0,543		2,317		1,593		1,626		0,975		0,361
4	signal	165	2358	3196	1463	1071	6244	1930	6539	596	3826	1046	4944	750	6994	235	3302	720	1959
	total Input (x 100)	16500	4716	319600	2926	107100	12488	193000	13078	59600	7652	104600	9888	75000	13988	23500	6604	72000	3918
	total IP (x2)																		
	IP/input		0,286		0,009		0,117		0,068		0,128		0,095		0,187		0,281		0,054
	normalized surface level		1		0,032		0,408		0,237		0,449		0,331		0,653		0,983		0,190
	Mean		1		0,035		1,050		0,690		1,091		1,003		1,108		0,935		0,229

Protein surface levels of EGF mutants relative to wt DLL1 determined from 4 independent surface biotinylation experiments. 1% of lysate (input), and 50% of immuno precipitated material were loaded and quantitated. Surface levels of DLL1 wt were set to one and values normalized to DLL1 wt. These values were used to generate the bar graph in Fig. 1F and for statistical analyses.

Table S3. Quantification of Notch1 and Notch2 steady state levels.

WB		Notch1 Δ C-Flag (S1 product)	Flag background band	Notch1 Δ C-Flag/ background-Flag	Notch2-Flag (S1 product)	Flag background band	Notch2-Flag/ background-Flag
1	signal	3106	1326	2,342	3798	928	4,093
2	signal	3482	1660	2,098	4100	1191	3,442
3	signal	3278	1467	2,234	3683	1340	2,749
4	signal	4337	1673	2,592	4577	888	5,154
5	signal	3426	2866	1,195	2675	848	3,154
6	signal	3071	2203	1,394	2123	1252	1,696
7	signal	2929	949	3,086	2944	459	6,414
	mean			2,135			3,815
	N1/2						0,560

Protein levels of Notch1 and Notch2 normalized to a Flag background band determined from 7 independent Western Blots of ES cell lysates using β -tubulin as loading control. These values were used to generate the bar graph in Fig. 1H and for statistical analyses.

Table S4. Notch1 and Notch2 activation in cocultures of Notch1 Δ CFlag-RBPluc or H-Notch2Flag-RBPluc ES cells with ES cells expressing DLL1 variants (normalized to E14tg2a).

assay	E14tg2a	E14tg2a Dll1 wt	E14tg2a EGF1m	E14tg2a EGF2m	E14tg2a EGF3m	E14tg2a EGF4m	E14tg2a EGF5m	E14tg2a EGF6m	E14tg2a EGF7m	E14tg2a EGF8m
NOTCH1 1	1	5,512	1,097	1,294	1,250	1,480	2,552	5,089	3,617	4,435
NOTCH1 2	1	10,296	1,356	1,218	1,593	2,782	4,690	6,852	6,153	7,894
NOTCH1 3	1	6,445	1,019	1,100	1,412	1,640	4,024	5,123	4,716	4,261
NOTCH1 4	1	11,818	1,279	1,353	1,870	3,260	5,019	9,584	5,338	3,888
NOTCH1 5	1	8,913	1,593	0,915	0,764	1,314	3,539	6,225	3,523	2,926
NOTCH1 6	1	5,839	1,351	1,167	1,036	1,798	2,976	5,018	3,976	3,214
NOTCH1 7	1	6,779	1,083	1,207	1,517	1,634	3,214	6,421	4,524	4,779
NOTCH1 8	1	14,021	0,958	1,367	1,829	4,696	7,638	13,483	9,654	8,750
NOTCH1 9	1	14,740	1,104	1,012	1,444	2,632	3,844	9,644	6,892	7,052
NOTCH1 10	1	22,103	1,390	1,507	1,315	1,623	6,144	15,082	9,877	8,925
Mean		10,647	1,223	1,214	1,403	2,286	4,364	8,252	5,827	5,612
NOTCH2 1	1	9,621	0,553	0,943	1,634	3,167	5,548	7,023	6,639	6,172
NOTCH2 2	1	8,198	0,555	1,367	1,449	3,603	5,449	5,706	5,814	5,647
NOTCH2 3	1	16,376	0,955	1,158	1,835	4,459	9,466	12,045	9,444	13,737
NOTCH2 4	1	8,528	0,638	0,557	1,301	2,542	6,707	6,345	6,043	6,438
NOTCH2 5	1	2,913	0,188	0,256	0,548	1,211	1,969	2,283	2,757	2,297
NOTCH2 6	1	9,193	0,861	1,213	1,520	2,993	5,436	6,720	4,554	5,939
NOTCH2 7	1	8,908	1,039	0,546	1,033	2,095	4,102	4,938	4,493	3,526
NOTCH2 8	1	5,033	0,770	0,863	1,383	2,721	4,240	5,836	5,377	5,475
NOTCH2 9	1	11,494	0,690	0,981	1,468	3,646	6,886	11,633	8,076	8,076
Mean		8,918	0,694	0,876	1,352	2,937	5,534	6,948	5,911	6,367

These values were used to generate the bar graphs in Fig.1 I and K, and for statistical analyses.

Table S5. Notch1 and Notch2 activation of EGFm proteins normalized to DLL1wt and corrected for protein levels.

assay	wt	EGF1 norm	EGF2 norm	EGF3 norm	E3 norm corr [= E3/2.7]	EGF4 norm	EGF5 norm	E5 norm corr [= E5/2.1]	EGF6 norm	EGF7 norm	EGF8 norm	E8 norm corr [= E8/2.6]
NOTCH1 1	1,0	0,021	0,065	0,055	0,020	0,106	0,344	0,164	0,906	0,580	0,761	0,293
NOTCH1 2	1,0	0,038	0,023	0,064	0,024	0,192	0,397	0,189	0,630	0,554	0,742	0,285
NOTCH1 3	1,0	0,003	0,018	0,076	0,028	0,118	0,555	0,264	0,757	0,682	0,599	0,230
NOTCH1 4	1,0	0,026	0,033	0,080	0,030	0,209	0,372	0,177	0,793	0,401	0,267	0,103
NOTCH1 5	1,0	0,075	-0,011	-0,030	-0,011	0,040	0,321	0,153	0,660	0,319	0,243	0,093
NOTCH1 6	1,0	0,073	0,035	0,007	0,003	0,165	0,408	0,194	0,830	0,615	0,458	0,176
NOTCH1 7	1,0	0,014	0,036	0,089	0,033	0,110	0,383	0,182	0,938	0,610	0,654	0,252
NOTCH1 8	1,0	-0,003	0,028	0,064	0,024	0,284	0,510	0,243	0,959	0,665	0,595	0,229
NOTCH1 9	1,0	0,008	0,001	0,032	0,012	0,119	0,207	0,099	0,629	0,429	0,440	0,169
NOTCH1 10	1,0	0,018	0,024	0,015	0,006	0,030	0,244	0,116	0,667	0,421	0,376	0,145
Mean		0,027	0,025	0,045	0,017	0,137	0,374	0,178	0,777	0,528	0,514	0,198
NOTCH2 1	1,000	-0,052	-0,007	0,074	0,027	0,251	0,528	0,251	0,699	0,654	0,600	0,231
NOTCH2 2	1,000	-0,062	0,051	0,062	0,023	0,362	0,618	0,294	0,654	0,669	0,646	0,248
NOTCH2 3	1,000	-0,003	0,010	0,054	0,020	0,225	0,551	0,262	0,718	0,549	0,828	0,318
NOTCH2 4	1,000	-0,048	-0,059	0,040	0,015	0,205	0,758	0,361	0,710	0,670	0,722	0,278
NOTCH2 5	1,000	-0,424	-0,389	-0,236	-0,087	0,110	0,507	0,241	0,671	0,918	0,678	0,261
NOTCH2 6	1,000	-0,017	0,026	0,063	0,023	0,243	0,541	0,258	0,698	0,434	0,603	0,232
NOTCH2 7	1,000	0,005	-0,057	0,004	0,001	0,138	0,392	0,187	0,498	0,442	0,319	0,123
NOTCH2 8	1,000	-0,057	-0,034	0,095	0,035	0,427	0,803	0,382	1,199	1,085	1,110	0,427
NOTCH2 9	1,000	-0,030	-0,002	0,045	0,017	0,252	0,561	0,267	1,013	0,674	0,674	0,259
Mean		-0,039	-0,016	0,044	0,016	0,245	0,573	0,273	0,751	0,620	0,678	0,261

Values from Table S4 were normalized to DLLwt $[(Act\ EGFm - 1 [Act\ E14tg2a]) / (Act\ DLL1wt - 1 [Act\ E14tg2a])]$ and in case of EGF3m, EGF5m and EGF8m divided by the correction factors from Table S1. These values were used to generate the bar graph in Fig. 1 J and L, and for statistical analyses. Act = Activation.

Table S6. Number of *Nodal* expressing six somite-stage embryos.

	Mating	# embryos analyzed at ss 0-6	expected # of mutant embryos	# embryos w/o <i>Nodal</i> expression
wild type	x wild type	28	0	0
<i>Dll1</i> ^{lacZ/+}	x <i>Dll1</i> ^{lacZ/+}	43	10.75	6
<i>Dll1</i> ^{Dll1ki/Dll1ki}	x <i>Dll1</i> ^{Dll1ki/Dll1ki}	85	85	0
<i>Dll1</i> ^{EGF1m/+}	x <i>Dll1</i> ^{EGF1m/+}	25	6.25	6
<i>Dll1</i> ^{EGF2m/+}	x <i>Dll1</i> ^{EGF2m/+}	49	12.25	8
<i>Dll1</i> ^{EGF3m/+}	x <i>Dll1</i> ^{EGF3m/+}	76	19	7
<i>Dll1</i> ^{EGF4m/+}	x <i>Dll1</i> ^{EGF4m/+}	117	29.25	0
<i>Dll1</i> ^{EGF5m/EGF5m}	x <i>Dll1</i> ^{EGF5m/EGF5m}	21	21	0
<i>Dll1</i> ^{EGF6m/EGF6m}	x <i>Dll1</i> ^{EGF6m/EGF6m}	41	41	0
<i>Dll1</i> ^{EGF7m/EGF7m}	x <i>Dll1</i> ^{EGF7m/EGF7m}	24	24	0
<i>Dll1</i> ^{EGF8m/+}	x <i>Dll1</i> ^{EGF8m/+}	54	13.50	0

Table S7. *Pitx2* expression and heart looping in embryos homozygous for *Dll1* alleles.

Genotype*	<u><i>Pitx2</i> expression</u>			<u>Heart looping</u>		
	analyzed	w left lpm expression	w abnormal expression [%]	analyzed	w normal looping	w abnormal looping [%]
wild type	24	24	0 [0]	12	12	0 [0]
<i>Dll1^{lacZ/lacZ}</i>	11	1	10 [81]	20	1	19 [95]
<i>Dll1^{Dll1ki/Dll1ki}</i>	6	6	0 [0]	22	22	0 [0]
<i>Dll1^{EGF1m/EGF1m}</i>	14	2	12 [86]	35	12	23 [66]
<i>Dll1^{EGF2m/EGF2m}</i>	6	0	6 [100]	22	3	19 [87]
<i>Dll1^{EGF3m/EGF3m}</i>	6	1	5 [83]	48	29	19 [40]
<i>Dll1^{EGF4m/EGF4m}</i>	10	10	0 [0]	44	44	0 [0]
<i>Dll1^{EGF5m/EGF5m}</i>	6	6	0 [0]	23	23	0 [0]
<i>Dll1^{EGF6m/EGF6m}</i>	6	6	0 [0]	33	33	0 [0]
<i>Dll1^{EGF7m/EGF7m}</i>	7	7	0 [0]	21	21	0 [0]
<i>Dll1^{EGF8m/EGF8m}</i>	20	20	0 [0]	49	49	0 [0]

* Homozygous *Dll1^{lacZ}*, *Dll1^{EGF1m}*, *Dll1^{EGF2m}*, and *Dll1^{EGF3m}* embryos were identified based on their irregular somites, *Dll1^{Dll1wt}*, *Dll1^{EGF5m}*, *Dll1^{EGF6m}*, and *Dll1^{EGF7m}* embryos were obtained from homozygous matings.