

Supplementary Information

Gene	Forward (5'-3')	Reverse (5'-3')
ActB	GCTCTTTTCCAGCCTTCCTT	CGGATGTCAACGTCACACTT
<i>Carm1</i>	CCTGTGGTGGACACATTTGA	TCGCCTTCTTTGGCTTCTAA
<i>Cdx2</i>	TCAAGAAGAAGCAGCAGCAG	GCAAGGAGGTCACAGGACTC
<i>Ecad</i>	AGACTTTGGTGTGGGTCAGG	CATGCTCAGCGTCTTCTCTG
EMK1	TGTGGAACCTCTCCCTGA	CCCATTGACACCATCAACTCT
<i>Par3</i>	AGCCTTCTGGTCTTTCGTCA	GGGTGTGAGAACAACGTCCT
aPKC	TGGGTGGACAGTGAAGGTGAC	GTTGGCTCGGTACAGCTTC
PKCII	as 'aPKC Forward'	ATTGGCTCGGTACGGCTTT

Table 1. Primer pairs used in qRT-PCR (see Materials and Methods)

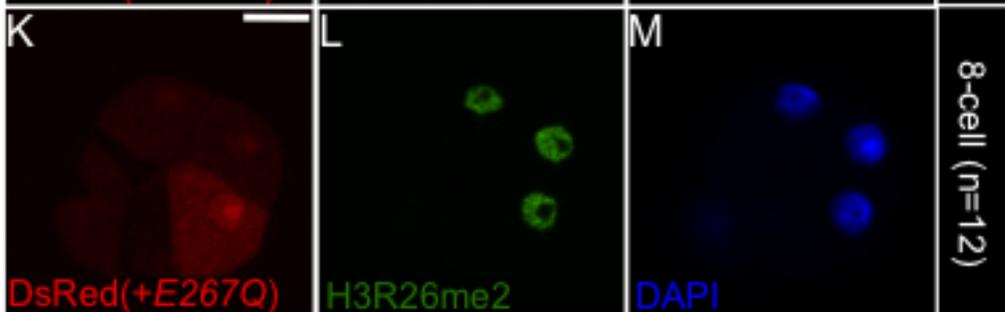
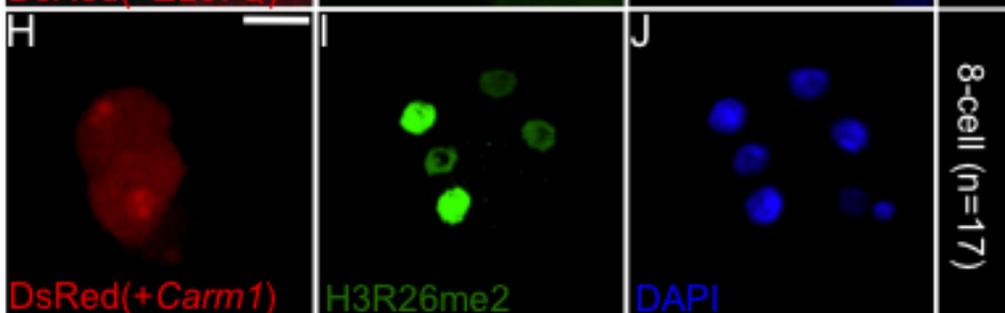
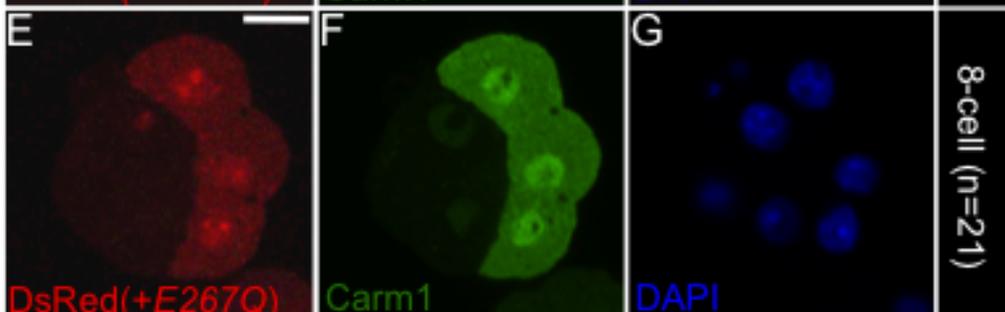
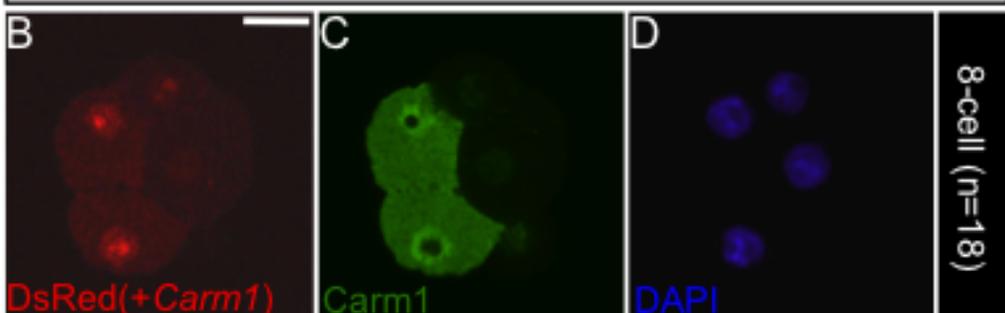
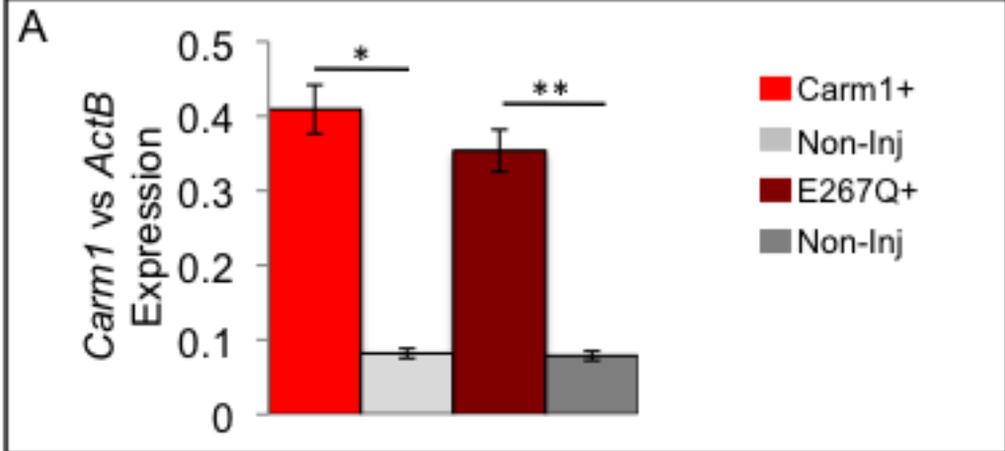
Supplementary Figure 1. Confirmation of functional *Carm1* overexpression. (A) One 2-cell blastomere was injected with *DsRed* and either *Carm1* or *Carm1(E267Q)* mRNAs and cultured until being separated at the mid-8-cell stage into non-labelled/labelled blastomeres. These were pooled separately and used for qRT-PCR. Normalised averages of biological and technical triplicate are shown. Error bars = SEM. Transcripts detected using *Carm1* primers were significantly higher in *Carm1* and *Carm1(E267Q)* injected blastomeres than their non-injected counterparts (Student's T-test; * $p=0.0061$ and ** $p=0.0032$ respectively). (B-M) Embryos were treated as above, but fixed at the 8-cell stage and treated with antibodies for *Carm1* (C and I) or H3R26me2 (F and L). *DsRed* indicates blastomeres injected with *Carm1* (B, E) or *Carm1(E267Q)* mRNA (H, K). Nuclei are visualised with DAPI (D, G, J and M). Scale bar = 20 μ m. *Carm1* protein and H3R26me2 levels were increased after *Carm1* injection (n=18 and 17 embryos respectively). Increased *Carm1* protein was detected after *E267Q* blastomere injection (n=21 embryos), but no change in H3R26me2 was apparent in these blastomeres (n=12 embryos).

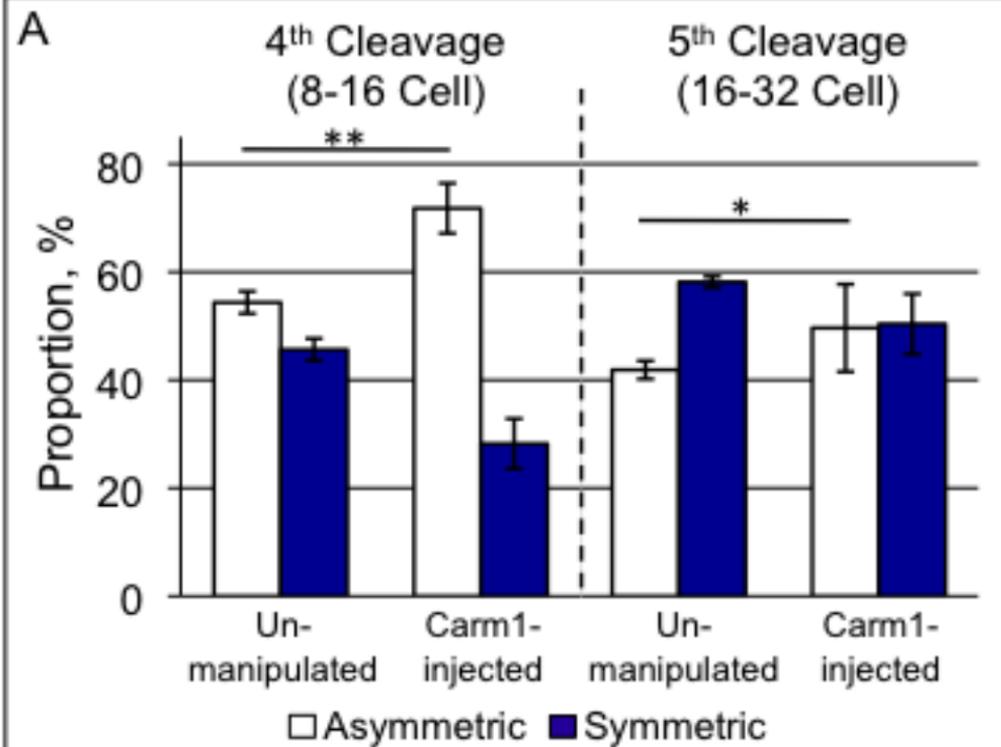
Supplementary Figure 2. *Carm1* overexpression leads to an increased frequency of asymmetric blastomere division and increased inner cells at the blastocyst stage. H2B-EGFP expressing zygotes were injected with mRNA for *dsRed* and *Carm1*, thus artificially elevating the levels of these transcripts in all cells of the embryo. (A) Injected embryos were time-lapse imaged to the blastocyst stage, tracked using Simbiocell© software, and DIC and H2B-EGFP Z-stacks used to determine blastomere positions as in Figure 1C. Average proportions of asymmetric and symmetric divisions during 4th and 5th cleavage for these embryos (n=10) were higher when compared to those of non-manipulated embryos (Bischoff et al., 2008). Error

bars=SEM. Student's T-Tests: ** $p < 0.001$, * $p = 0.04$. **(B)** After time-lapse microscopy, all cells were scored as inner/outer for each embryo and DsRed positive/negative. **(C)** The proportion of inner and outer cells in each blastocyst after *Carm1* overexpression in the zygote or one 2-cell stage blastomere, and *Carm1(E267Q)* in one 2-cell stage blastomere. The embryos injected with *Carm1* at the zygote stage had significantly more inner cells than those injected at the 2-cell stage.

Supplementary Figure 3. *E-Cadherin* expression levels do not change in response to *Carm1* overexpression. Separated 8-cell blastomeres injected with *Carm1* or *Carm1(E267Q)* and *DsRed* mRNAs at the 2-cell stage were pooled into non-injected (Non-Inj) and injected (+) samples used for qRT-PCR. Normalised averages of biological and technical duplicate are shown. Error bars=SEM. *E-Cad* transcripts were statistically equal in *Carm1*-injected, (*Carm1*)*E267Q*-injected and both non-injected samples (Two-way ANOVA, $p = 0.087$).

Supplementary Movie. Time-lapse movie of embryo overexpressing *Carm1* in one 2-cell blastomere, from Figure 1. 3D reconstruction of localisation of blastomeres' nuclei from the 2-cell to blastocyst stage (right panel); DIC movie of the same embryo (left panel). The nuclei from each clone are marked either red (*Carm1* mRNA clone) or white (non-injected clone). From the 16-cell-stage onwards, nuclear centres of inside cells are marked purple (*Carm1* overexpressing clone) or light blue (non-injected clone).





B

Embryo	No. of Cells	No. of Inner Cells (% of total)	No. of Outer Cells (% of total)
Zygote 1	32	13 (41)	19 (59)
Zygote 2	32	15 (47)	17 (53)
Zygote 3	32	15 (47)	17 (53)
Zygote 4	32	12 (38)	20 (62)
Zygote 5	33	14 (42)	19 (58)
Zygote 6	31	14 (45)	17 (55)
Zygote 7	32	15 (47)	17 (53)
Zygote 8	32	14 (44)	18 (56)
Zygote 9	32	14 (44)	18 (56)
Zygote 10	34	14 (42)	20 (58)
MEAN	32.2	14.0 (43)	18.2 (57)
SD	0.8	0.9	2.9

C

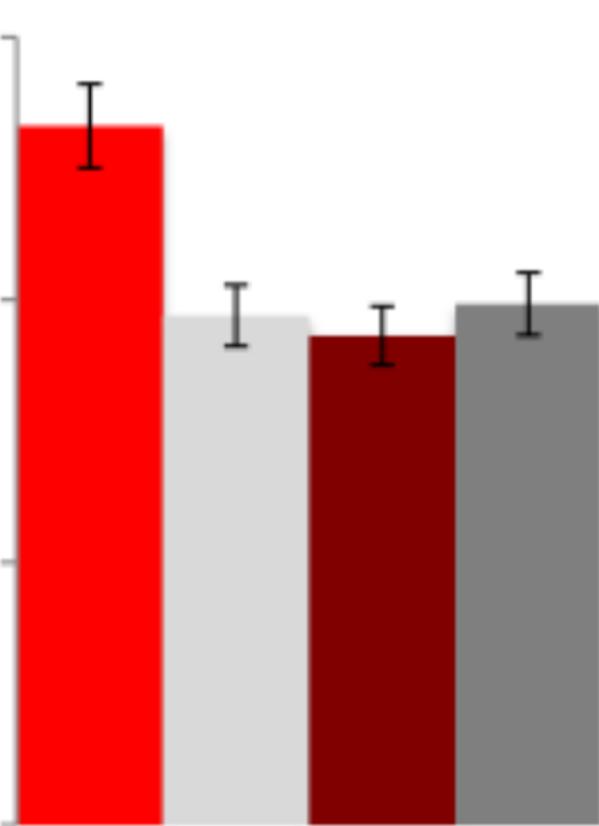
Stage of embryos injected (mRNA)	Inner cells (% ±SD)	Outer cells (% ±SD)
Zygote (<i>Carm1</i>)	43.5 ±2.9 ^{**}	56.5 ±2.9
2-Cell (<i>Carm1</i>)	39.0 ±4.9 [*]	60.3 ±4.9
2-Cell (<i>E267Q</i>)	38.2 ±2.0 [^]	61.8 ±2.2

Student's T-Test: *p=0.0097, ^p=0.0002

Ecad expression (vs ActB)

0.012
0.008
0.004
0

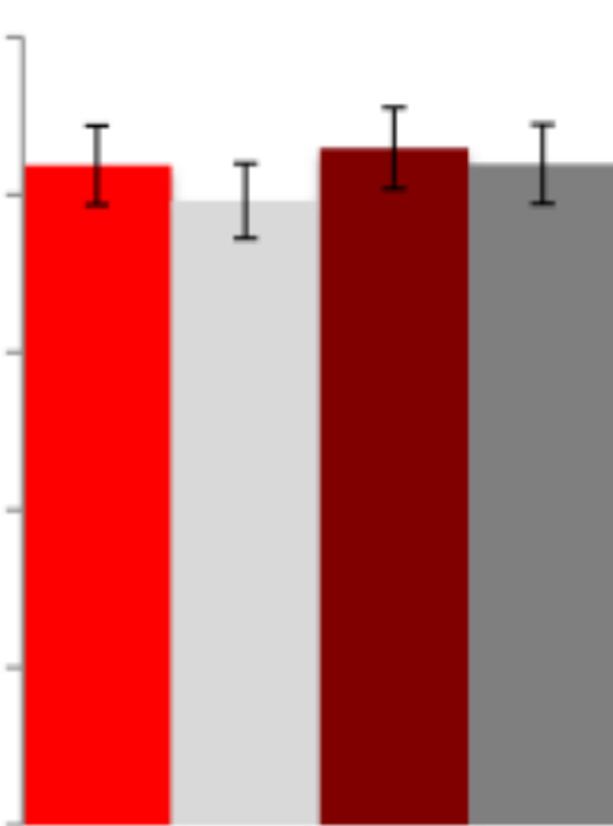
8-cell



Ecad expression (vs ActB)

0.005
0.004
0.003
0.002
0.001
0

16-cell



■ Carm1+
■ Non-Inj
■ E267Q+
■ Non-Inj