

## Supplemental Tables

Trim43a, Trim43b, and Trim43c: novel mouse genes expressed specifically in mouse preimplantation embryos

Ilaria Stanghellini, Geppino Falco, Sung-Lim Lee, Manuela Monti and Minoru S. H. Ko

### Supplemental Table S1. Fluorescence expression pattern of pTrim43a(ATG)-mStrawberry-3'UTR injected embryos. Representative images are shown in Fig. 3A.

Developmental stage	Culture period (post-hCG)	No. (% mean±SEM) of positive		No. (% mean±SEM) of negative
		Symmetric <sup>†</sup>	Asymmetric <sup>‡</sup>	
1-cell	~32 h	0 (0.0±0.0) <sup>a</sup>	0 (0.0±0.0) <sup>a</sup>	187 (100.0±0.0)
2-cell ~ 4-cell	48~54h	4 (2.7±0.2) <sup>b</sup>	47 (25.1±10.0) <sup>c</sup>	136 (72.2±10.0)
8-cell ~ morula	62~74h	4 (2.7±0.2) <sup>b</sup>	63 (33.7±16.1) <sup>c</sup>	120 (63.6±16.0)
Blastocyst	86~92h	3 (2.1±0.7) <sup>b</sup>	42 (22.5±4.5) <sup>c</sup>	142 (75.4±3.9)
Hatched blastocysts <sup>¶</sup>	100~108h	0 (0.0±0.0) <sup>a</sup>	0 (0.0±0.0) <sup>a</sup>	187 (100.0±0.0)

Embryo development was monitored in three separate experiments with 42, 50, and 95 injected embryos, respectively. Results of total 187 embryos are shown in this table. We also carried out the injection of total 126 embryos (two separate experiments with 48 and 78 embryos) with pTrim43a(500bp)-Emerald-3'UTR. None of the embryos showed fluorescence signals throughout the preimplantation stages.

<sup>†</sup> or <sup>‡</sup>, symmetric or asymmetric expression of fluorescent signals.

<sup>¶</sup>, this stage included both hatching and failing-to-hatch blastocysts.

<sup>a, b, c</sup> Percentages are statistically different between a and b ( $P < 0.05$ ) as well as between b and c ( $P < 0.01$ ). Statistical analysis was carried out using one-way analysis of variance (ANOVA) by SPSS. Comparisons of mean values (% positive embryos) were performed using Duncan's and Tukey's multiple comparisons test. Data are represented as mean  $\pm$  standard error of the mean (SEM), and differences are considered to be significant when  $P < 0.05$ .

**Supplemental Table S2. Fluorescence expression pattern of pTrim43a(ATG)-Emerald-tkPolyA injected embryos. Representative images are shown in Fig. 4A.**

Developmental stage	Culture period (post-hCG)	No. (% mean±SEM) of positive		No. (% mean±SEM) of negative
		Symmetric <sup>†</sup>	Asymmetric <sup>‡</sup>	
1-cell	~32 h	0 (0.0±0.0)	0 (0.0±0.0)	202 (100.0±0.0)
2-cell ~ 4-cell	48~54h	10 (5.0±0.6)	40 (19.8±9.7)	152 (75.2±10.3)
8-cell ~ morula	62~74h	10 (5.0±0.6)	67 (33.2±4.0)	125 (61.9±4.7)
Blastocyst	86~92h	10 (5.0±0.6)	56 (27.7±4.2)	136 (67.3±4.8)
Hatched blastocysts <sup>d</sup>	100~108h	0 (0.0±0.0)	0 (0.0±0.0)	202 (100.0±0.0)

Embryo development was monitored in three separate experiments with 51, 61, and 90 injected embryos, respectively. Results of total 202 embryos are shown in this table.

<sup>†</sup> or <sup>‡</sup>, symmetric or asymmetric expression of fluorescent signals.

<sup>d</sup>, this stage included both hatching and failing-to-hatch blastocysts.

**Supplemental Table S3. Fluorescence expression pattern of pTrim43a(ATG)-mStrawberry-3'UTR and Zscan4 promoter-Emerald co-injected embryos. Representative images are shown in Fig. 4C.**

Developmental stage	Culture period (post-hCG)	No. (%) of Emerald positive			No. (%) of Strawberry positive			No. (%) of co-expression <sup>F</sup>
		Sym. <sup>†</sup>	Asym. <sup>‡</sup>	total	Sym. <sup>†</sup>	Asym. <sup>‡</sup>	total	
1-cell	~32 h	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
2-cell ~ 4-cell	48~54h	8 (17.4)	0 (0.0)	8 (17.4)	2 (4.3)	4 (8.7)	6 (13.0)	6 (13.0)
8-cell ~ morula	62~74h	7 (15.2)	0 (0.0)	7 (15.2)	1 (2.2)	7 (15.2)	8 (17.4)	7 (15.2)
Blastocyst	86~92h	5 (10.9)	0 (0.0)	5 (10.9)	1 (2.2)	6 (13.0)	7 (15.2)	5 (10.9)
Hatched blastocysts <sup>¥</sup>	100~108h	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Development of 46 injected embryos was monitored throughout the preimplantation period.

<sup>†</sup> or <sup>‡</sup>, symmetric or asymmetric expression of fluorescent signals.

<sup>F</sup>, both fluorescent signals (Emerald + Strawberry) were observed in each embryo.

<sup>¥</sup>, this stage included both hatching and failing-to-hatch blastocysts.

**Supplemental Table S4. Fluorescence expression pattern of embryos injected with Trim43-strawberry without insulator (a representative image shown in Fig. 4D) or with insulator (a representative image shown in Fig. 4E).**

Developmental stage	Trim43-strawberry <b>without Insulator</b>			Trim43-strawberry <b>with Insulator</b>		
	No. (%) of positive			No. (%) of positive		
	Sym. <sup>†</sup>	Asym. <sup>‡</sup>	total <sup>£</sup>	Sym. <sup>†</sup>	Asym. <sup>‡</sup>	total <sup>£</sup>
1-cell	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
2-cell	2 (4.9)	3 (7.3)	5 (12.2)	4 (14.8)	5 (18.5)	9 (33.3)
8-cell ~ morula	2 (4.9)	14 (34.1)	16 (39.0)	4 (14.8)	5 (18.5)	9 (33.3)
Blastocyst	2 (4.9)	10 (24.4)	12 (29.3)	4 (14.8)	4 (14.8)	8 (29.6)

Development of 41 embryos injected with Trim43-Strawberry without insulator and 27 embryos injected with Trim42-Strawberry with insulator was monitored throughout the preimplantation period.

<sup>†</sup> or <sup>‡</sup>, symmetric or asymmetric expression of fluorescent signal.

<sup>£</sup>, total number of fluorescent positive embryos.

**Supplemental Table S5. Fluorescence expression pattern of embryos injected with Trim43-strawberry with Insulator (experiments carried out in addition to those presented in Supplemental Table S4). Representative images are shown in Fig. 4E.**

Developmental stage	No. (%) of positive		No. (%) of negative
	Symmetric <sup>†</sup>	Asymmetric <sup>‡</sup>	
1-cell	0 (0.0)	0 (0.0)	0 (0.0)
2-cell ~ 4-cell	12 (10.0)	14 (11.7)	26 (21.7)
8-cell ~ morula	17 (14.2)	22 (18.3)	39 (32.5)
Blastocyst	10 (8.3)	14 (11.7)	24 (20.0)
Hatched blastocysts <sup>¶</sup>	0 (0.0)	0 (0.0)	0 (0.0)

Embryo development was monitored in two separate experiments with 50 and 70 injected embryos, respectively. Results of total 120 embryos are shown in this table.

<sup>†</sup> or <sup>‡</sup>, symmetric or asymmetric expression of fluorescent signal.

<sup>¶</sup>, this stage embryos are included hatching or failed hatch blastocysts.