## SUPPLEMENTAL MATERIAL

## Reduction in E-cadherin expression fosters migration of

## Xenopus laevis primordial germ cells

Thilo Baronsky<sup>*a*,†</sup>, Aliaksandr Dzementsei<sup>*b*,†</sup>, Marieelen Oelkers<sup>*a*</sup>, Juliane Melchert<sup>*c*</sup>,

Tomas Pieler<sup>c\*</sup> and Andreas Janshoff<sup>a\*</sup>

<sup>a</sup> Institute of Physical Chemistry, Tammannstr. 6, 37077 Göttingen, Germany, Tel: 049 0551 39 10633; E-mail: ajansho@gwdg.de

<sup>b</sup> The Danish Stem Cell Center, Laboratory Blegdamsvej 3b, 2200 København N, Denmark

<sup>c</sup> Department of Developmental Biochemistry, Göttingen Center for Molecular Biosciences, Georg-August-University, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

<sup>†</sup> These authors contributed equally to the manuscript.

\* Corresponding authors (ajansho@gwdg.de; tpieler@gwdg.de)



**Suppl. Fig. 1. Number of PGCs per embryo in individual E-cadherin overexpression and knock-down experiments.** Embryos were injected at 2-cell stage with *GFP\_E-cadherin\_DELE* mRNA (E-cadh-GFP-DE) or E-cadherin morpholino oligonucleotide (E-cad MO). For each experiment control injections with *GFP\_DE* mRNA or control morpholino oligonucleotide (CoMO) were performed. Embryos were fixed at stage 28-30 and used for whole mount *in situ* hybridisation with antisense *Xpat* RNA as a PGC marker. Average numbers of PGCs per embryo were calculated in each individual experiment. *N* corresponds to the number of embryos analysed. Error bars represent standard deviation.



Suppl. Fig. 2. Knock-down and overexpression of E-cadherin in *X. laevis* embryos. Embryos were injected at 2-cell stage with control morpholino oligonucleotides (Contr MO), E-cadherin morpholino oligonucleotides (E-cad MO) or *GFP\_E-cadherin\_DELE* mRNA (E-cad GFP). (A) Western Blot analysis of endodermal explants obtained from the injected and uninjected (uninj) embryos. Expression of GAPDH was used as a positive control, M corresponds to the marker lane. Staining with  $\alpha$ GFP antibody was used to detect GFP-E-cadherin expression, while staining with  $\alpha$ E-cadherin antibody revealed endogenous E-cadherin expression in addition to GFP-E-cadherin. (B-D) Expression of GFP-E-cadherin (green) in dissociated endodermal cells. B – merged image, C – GFP channel, D – transmitted white light. Scale bar – 20 µm.



**Suppl. Fig. 3.** Expression of GFP-E-cadherin in PGCs. Embryos were injected at 2cell stage with *GFP\_DELE* mRNA or *GFP\_E-cadherin\_DELE* mRNA (green), both coinjected with *membrane RFP* mRNA (red). Cells were isolated from endodermal explants of stage 28-30 embryos by brief incubation in CMFM (3-5 min). White arrows indicate remaining cell-cell contacts via filopodia-like protrusions. **(A)** GFP is localized to the cytoplasm excluding yolk granules and is not present in the filopodia-like protrusions. **(B,C)** Overexpressed GFP-E-cadherin is localized on the plasma membrane, filopodia-like protrusions and is enriched at the sites of cell-cell contacts.



## Number of PGCs per embryo

Suppl. Fig. 4. Number of PGCs is not decreased at stage 17-19 upon E-cadherin overexpression and knock-down. Embryos were injected at 2-cell stage with *GFP\_E-cadherin\_DELE* mRNA (E-cadh-GFP-DE) or E-cadherin morpholino oligonucleotide (E-cad MO) as indicated. Control injections with *GFP\_DE* mRNA or control morpholino oligonucleotide (CoMO) were performed. Embryos from the same injection series were fixed at stages 17-19 and 28-30 and used for whole mount *in situ* hybridisation with antisense *Xpat* RNA as a PGC marker. Average numbers of PGCs per embryo were calculated in each individual experiment. Exp6 and Exp7 correspond to *Suppl. Fig. 1.* N - number of analysed embryos. Error bars represent standard deviation. \*\* - p<0.05; \*\*\* - p<0.01 (2-tailed homoscedastic t-test).