Expanded View Figures

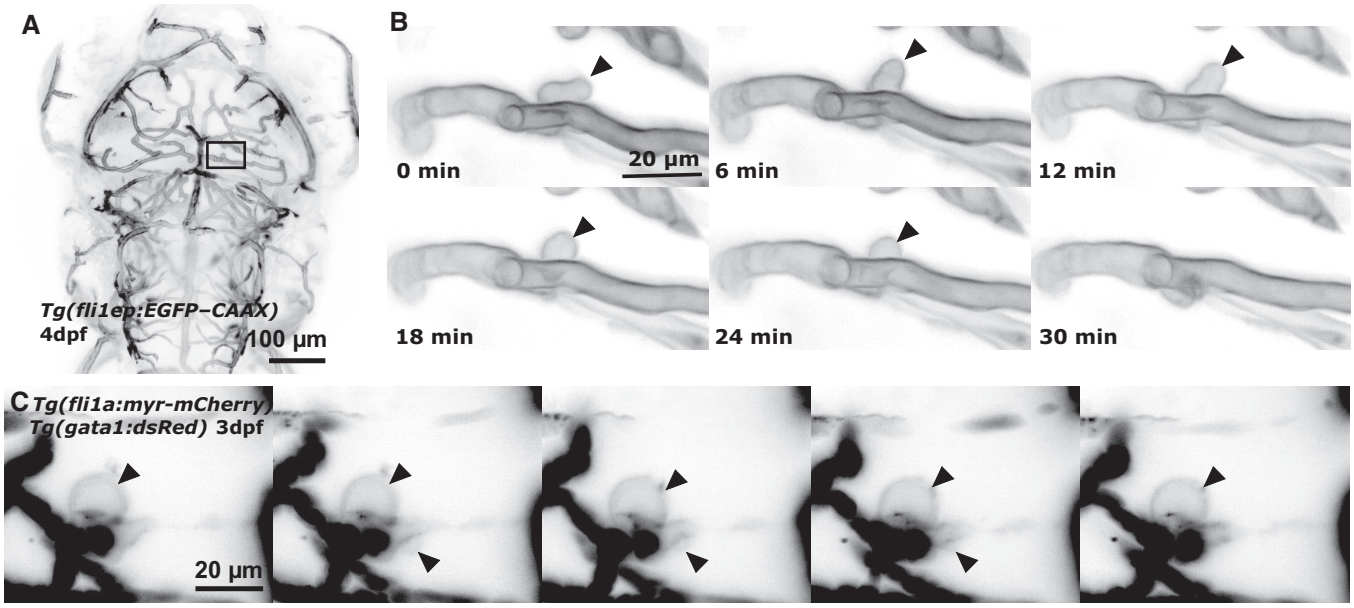

Figure EV1. *Kugeln* were observed in the transgenic *Tg(fli1aep:eGFP-CAAX)*.

A MIP of cerebral vessels of 4 dpf *Tg(fli1aep:eGFP-CAAX)* embryo.

B Time-lapse acquisitions showed that *kugeln* were dynamic (black arrowhead) as in *Tg(kdrl:HRAS-mCherry)*^{s916}, showing protrusion, shape changes, oscillation and retraction.

C Additional time-lapse acquisitions in the *Tg(fli1a:myr-mCherry), Tg(gata1:dsRed)* showed equally dynamics of *kugeln* (black arrowhead; images grey LUT; inverted).

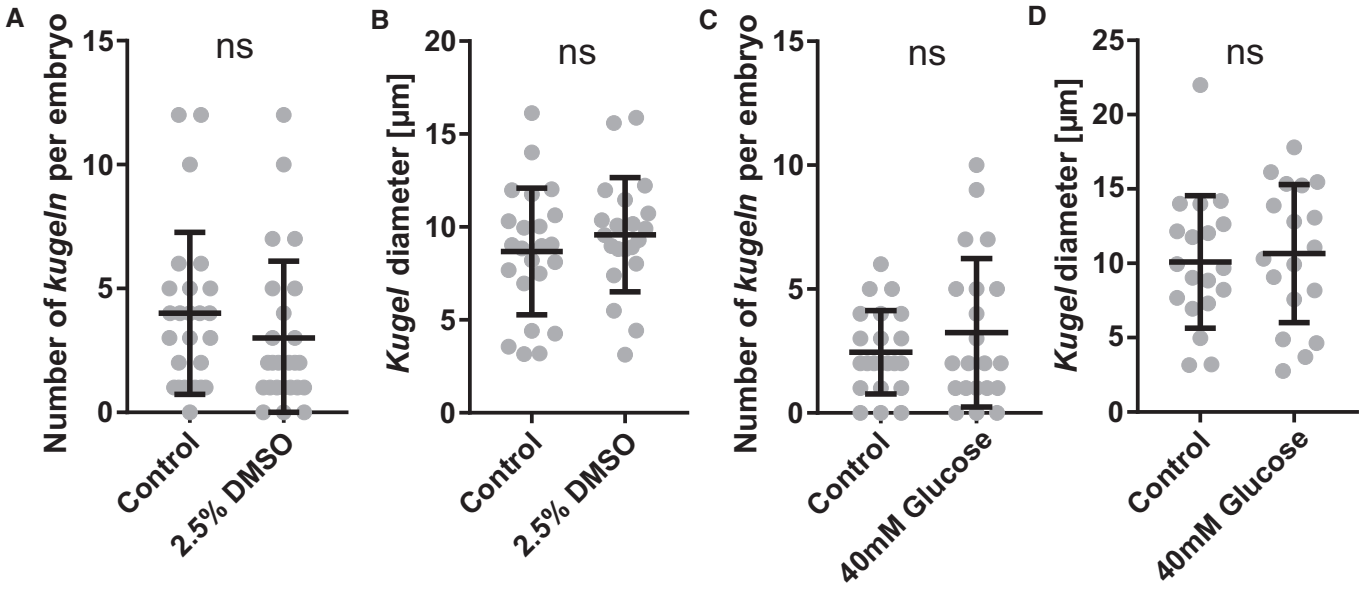

Figure EV2. *Kugeln* number or diameter is not altered by changes of membrane permeability or osmotic pressure.

A The influence of membrane permeability increase was studied by application of DMSO; the number of *kugeln* was not statistically significantly changed (2.5% DMSO 24 h; $P = 0.1596$; control $n = 25$ embryos 4.00 ± 0.65 (mean ± s.e.m.), DMSO $n = 25$ embryos 3.00 ± 0.62 (mean ± s.e.m.); 4 dpf; three experimental repeats; Mann–Whitney *U*-test).

B Diameter of *kugeln* was not statistically significantly different after incubation with DMSO ($P = 0.3665$; control $n = 97$ *kugeln* from 25 embryos 8.68 ± 0.71 (mean ± s.e.m.), DMSO $n = 75$ *kugeln* from 25 9.76 ± 0.67 (mean ± s.e.m.); 4 dpf; three experimental repeats; Student's *t*-test).

C The impact of osmotic pressure on *kugeln* was studied by application of glucose; no statistically significant difference was observed (40 mM glucose 24 h; $P = 0.7371$; control $n = 22$ embryos 2.46 ± 0.36 (mean ± s.e.m.), glucose $n = 21$ embryos 3.24 ± 0.65 (mean ± s.e.m.); 4 dpf; two experimental repeats; Mann–Whitney *U*-test).

D *Kugel* diameter was not statistically significantly different after incubation with glucose ($P = 0.7060$; control $n = 54$ *kugeln* from 22 embryos 10.09 ± 1.02 (mean ± s.e.m.), glucose $n = 67$ *kugeln* from 21 embryos 10.66 ± 4.65 (mean ± s.e.m.); 4 dpf; two experimental repeats; Student's *t*-test).

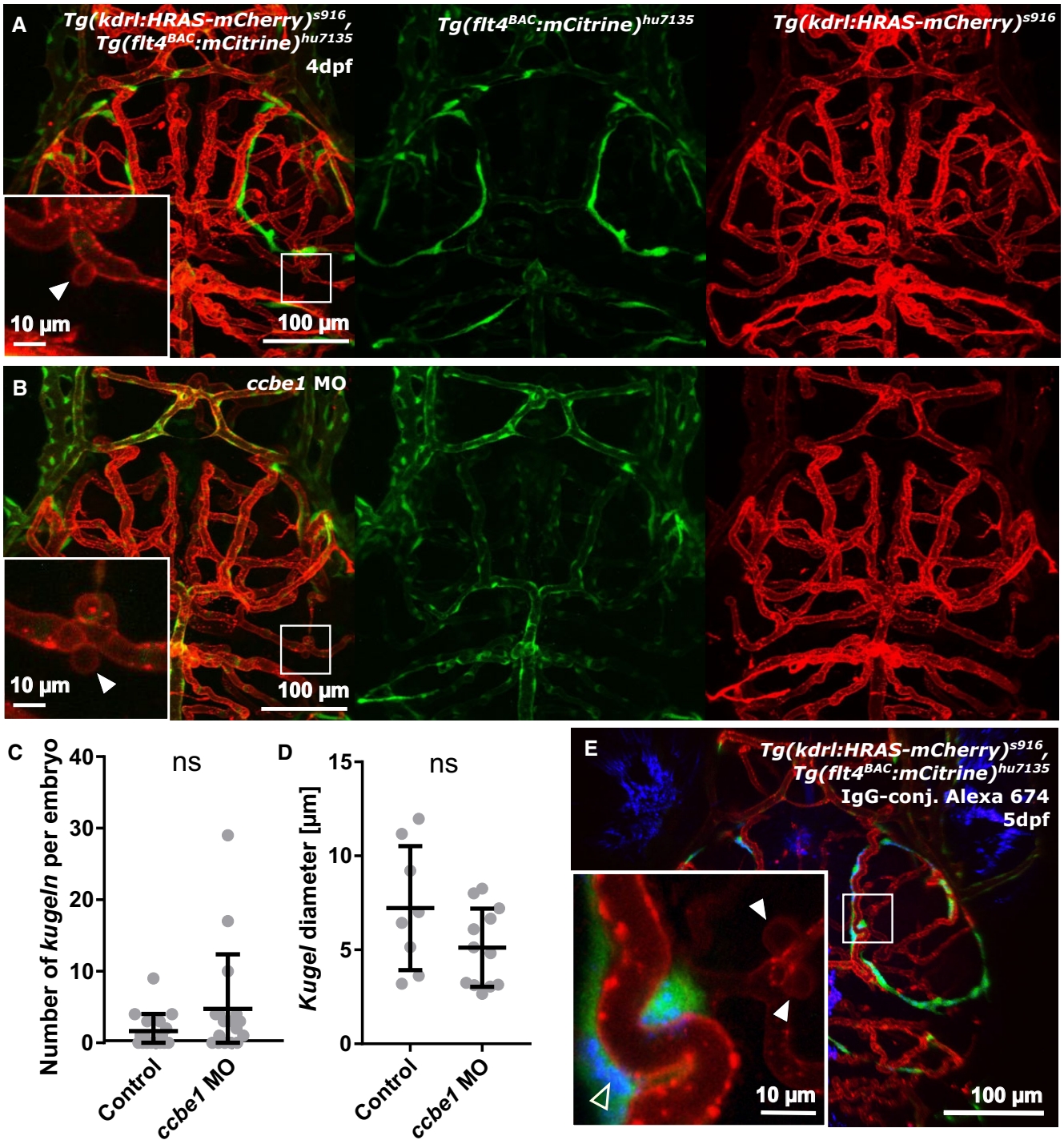

Figure EV3. *Kugeln* do not interact with BLECs in *Tg(flt4^{BAC}:mCitrine)^{hu7135}*, *Tg(kdrl:HRAS-mCherry)^{s916}*.

A *Kugeln* (white arrowhead) were additionally studied in the transgenic *Tg(flt4^{BAC}:mCitrine)^{hu7135}*, *Tg(kdrl:HRAS-mCherry)^{s916}*, which was more specific for BLECs, using confocal microscopy to allow higher resolution imaging.

B *Ccbe1* morpholino (MO) injection leads to loss of BLECs, while *kugeln* were still observed (white arrowhead).

C The number of *kugeln* was not statistically significantly altered by *ccbe1* morpholino (MO) knockdown (P = 0.1472; control n = 17 embryos 1.65 ± 0.58 (mean ± s.e.m.), *ccbe1* MO n = 17 embryos 4.77 ± 1.85 (mean ± s.e.m.); 4 dpf; two experimental repeats; Mann–Whitney U-test).

D Diameter of *kugeln* was not statistically significantly altered upon *ccbe1* MO injection (P = 0.0962; control n = 27 *kugeln* from 17 embryos 7.22 ± 1.17 (mean ± s.e.m.), *ccbe1* MO n = 81 *kugeln* from 17 embryos 5.12 ± 0.60 (mean ± s.e.m.); two experimental repeats; Student's t-test).

E Injection of IgG-conjugated Alexa 647 into the tectum (unfilled arrowhead) showed no uptake of IgG-conjugated Alexa 647 by *kugeln* (n = 52 *kugeln* from 5 5 dpf embryos, *kugeln* indicated by white arrowheads).

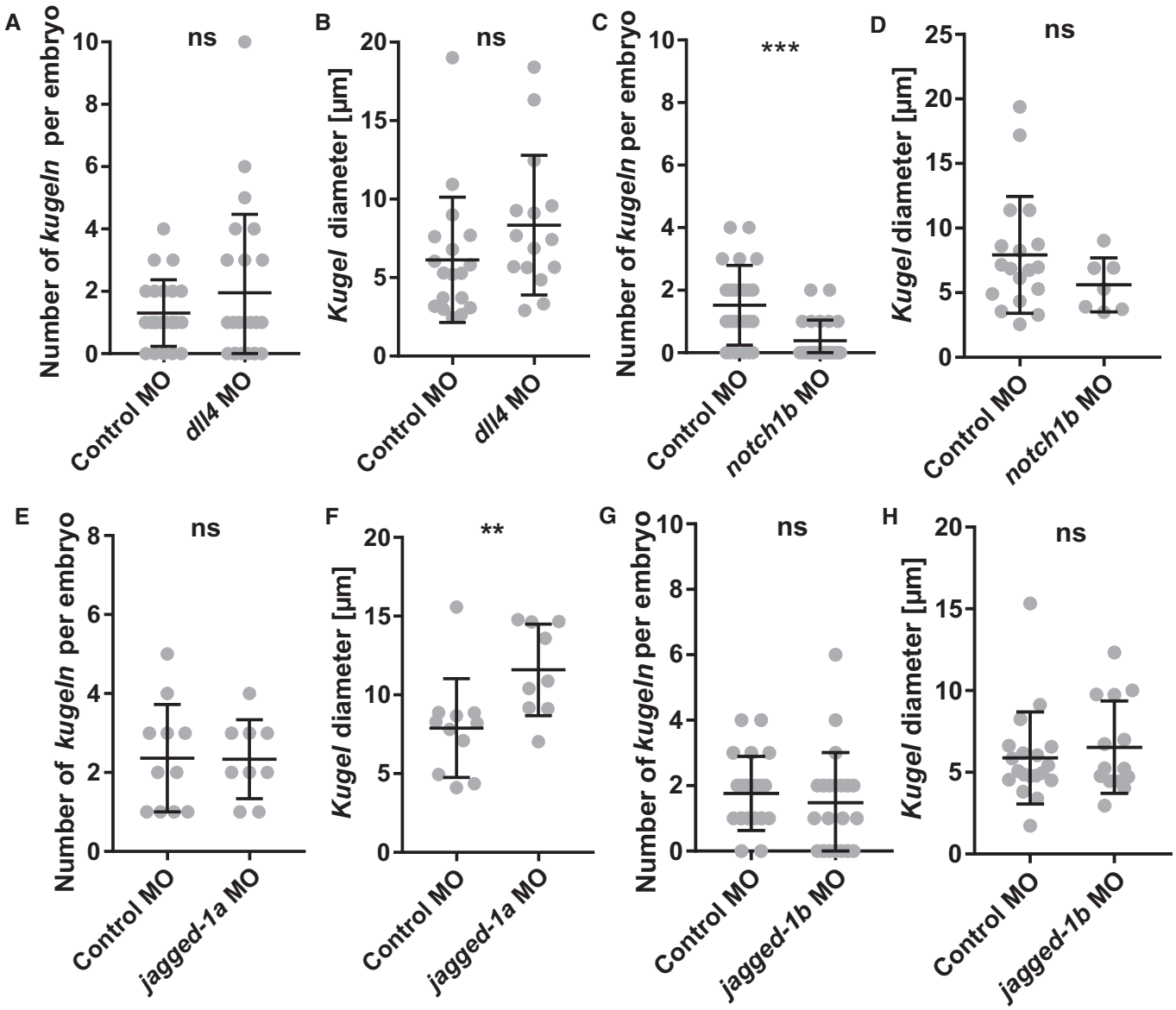

Figure EV4. The effect of morpholino knockdown of *dll4*, *notch1b*, *jagged-1a* and *jagged-1b* on *kugeln* number and diameter.

A Number of *kugeln* per embryo was not statistically significantly changed upon *dll4* MO injection ($P = 0.9639$; control $n = 23$ embryos 1.30 ± 0.22 (mean ± s.e.m.), *dll4* MO $n = 23$ embryos 1.96 ± 0.52 (mean ± s.e.m.); 3 dpf; three experimental repeats; Mann–Whitney *U*-test).

B *Kugel* diameter was not statistically significantly changed upon *dll4* MO injection ($P = 0.0843$; control $n = 30$ *kugeln* from 23 embryos 6.13 ± 0.94 (mean ± s.e.m.), *dll4* MO $n = 45$ *kugeln* from 23 embryos 8.35 ± 1.15 (mean ± s.e.m.); 3 dpf; three experimental repeats; Mann–Whitney *U*-test).

C Number of *kugeln* was statistically significantly reduced after *notch1b* MO injection (***$P = 0.0008$; control $n = 23$ embryos 1.52 ± 0.27 (mean ± s.e.m.), *notch1b* MO $n = 23$ embryos 0.39 ± 0.14 (mean ± s.e.m.); 3 dpf; 3 experimental repeats; Mann–Whitney *U*-test).

D *Kugel* diameter was not statistically significantly changed upon *notch1b* MO injection ($P = 0.3198$; control $n = 37$ *kugeln* from 23 embryos 7.93 ± 1.07 (mean ± s.e.m.), *notch1b* MO $n = 9$ *kugeln* from 23 embryos 5.61 ± 0.79 (mean ± s.e.m.); 3 experimental repeats; Mann–Whitney *U*-test).

E Number of *kugeln* was not statistically significantly changed after *jagged-1a* MO injection ($P = 0.9563$; control $n = 11$ embryos 2.36 ± 0.41 (mean ± s.e.m.), *jagged-1a* MO $n = 9$ embryos 2.33 ± 0.33 (mean ± s.e.m.); 3 dpf; 2 experimental repeats; Student's *t*-test).

F *Kugel* diameter was found to be statistically significantly changed upon *jagged-1a* MO injection (**$P = 0.0097$; control $n = 23$ *kugeln* from 11 embryos 7.89 ± 0.94 (mean ± s.e.m.), *jagged-1a* MO $n = 20$ *kugeln* from 9 embryos 11.58 ± 0.97 (mean ± s.e.m.); 3 dpf; 2 experimental repeats; Mann–Whitney *U*-test).

G Number of *kugeln* per embryo was not statistically significantly changed upon *jagged-1b* MO injection ($P = 0.3042$; control $n = 21$ embryos 1.76 ± 0.25 (mean ± s.e.m.), *jagged-1b* MO $n = 21$ embryos 1.48 ± 0.34 (mean ± s.e.m.); 3 dpf; 3 experimental repeats; Mann–Whitney *U*-test).

H *Kugel* diameter was not statistically significantly changed upon *jagged-1b* MO injection ($P = 0.7060$; control $n = 19$ *kugeln* from 21 embryos 5.88 ± 0.65 (mean ± s.e.m.), *jagged-1b* MO $n = 14$ *kugeln* from 21 embryos 6.53 ± 0.76 (mean ± s.e.m.); 3 dpf; 3 experimental repeats; Mann–Whitney *U*-test).

