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Supplemental Information

The Hypothalamic Neuropeptide Oxytocin Is Required for Formation of the Neurovascular Interface of the Pituitary

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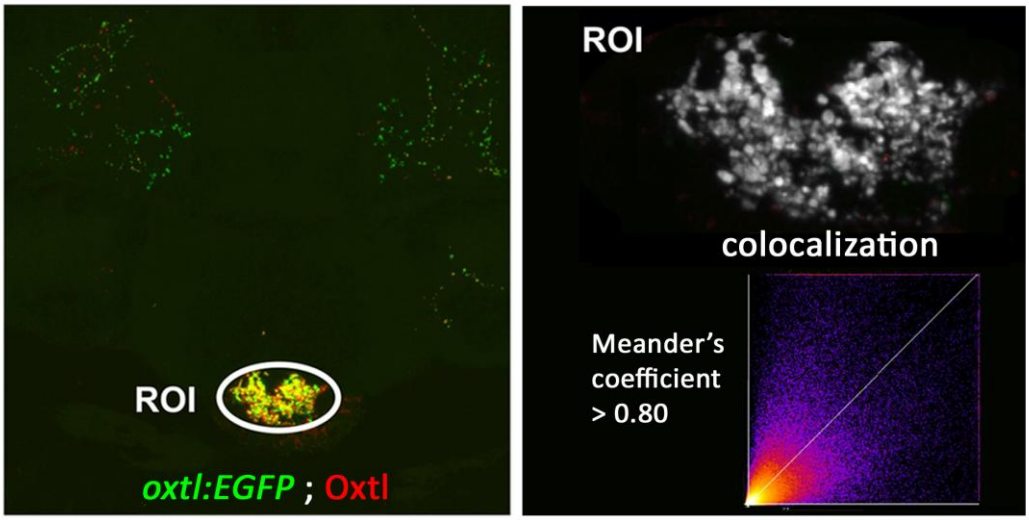

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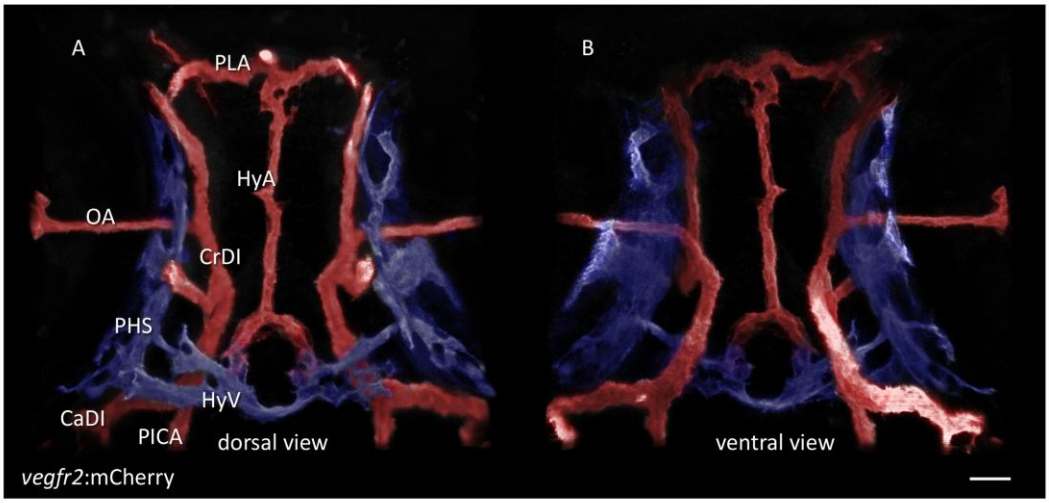

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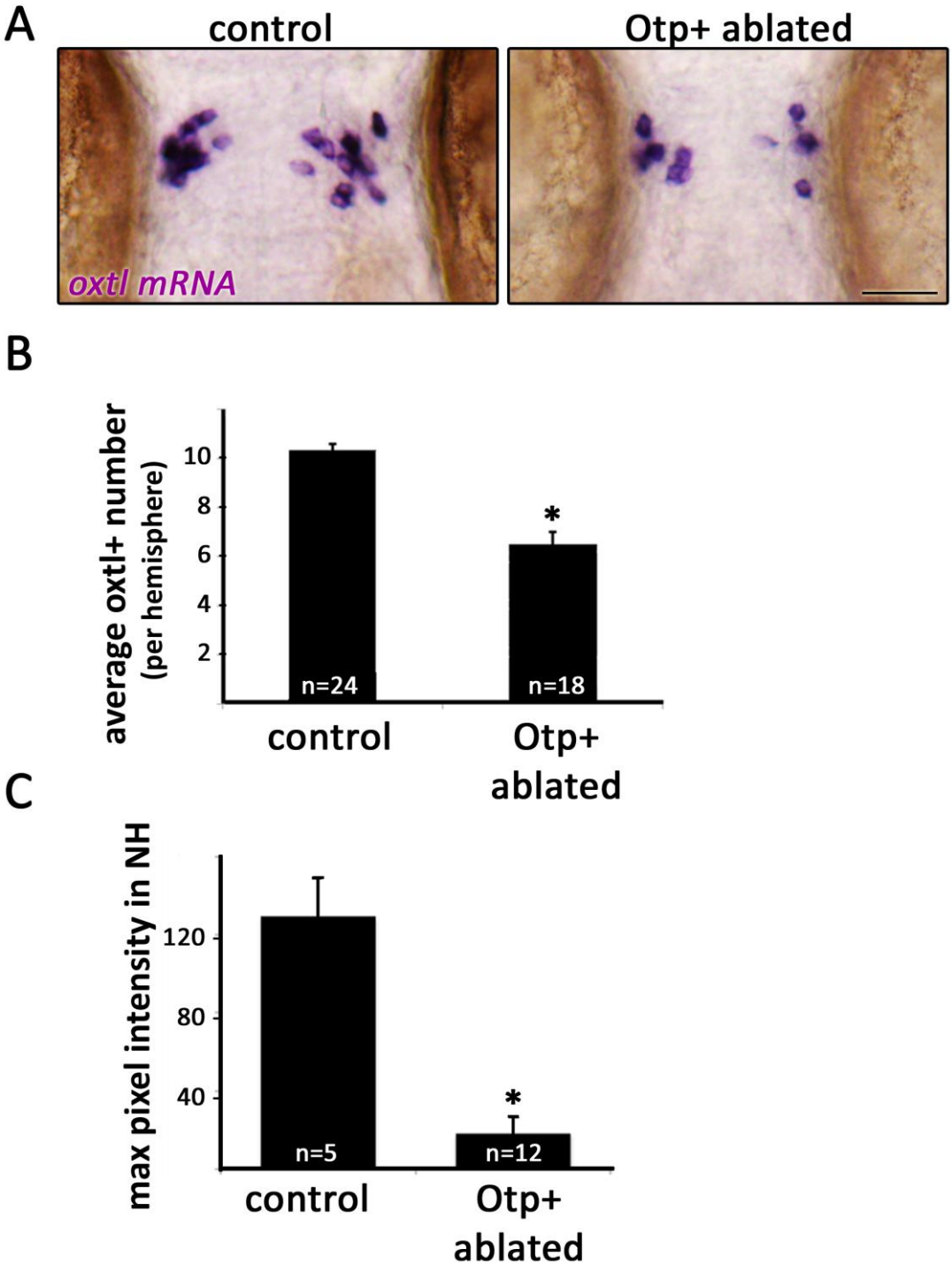

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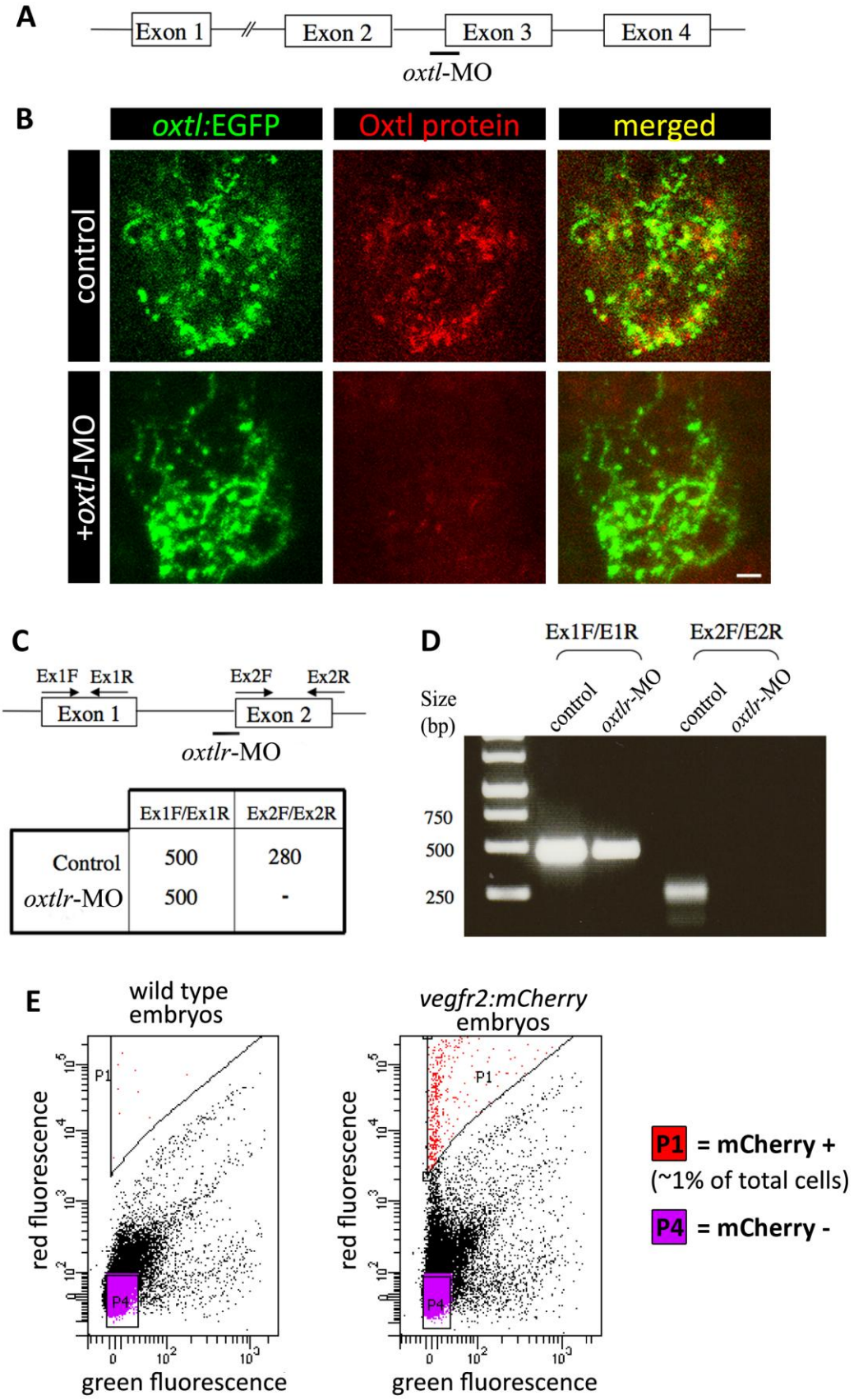

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Legends for Supplemental Figures:

Figure S1 (related to Figure 1). Co-localization of Oxytocin and EGFP in the *oxtl:*EGFP transgene

A representative confocal Z-stack image used for co-localization analysis following double staining of 14 day-old *oxtl:EGFP* transgenes with anti-EGFP and anti-Oxytocin antibodies. Confocal image stacks were taken with a 63x objective (HCX PL APO 63x/1,2 NA Water) on a Leica TCS-SP5 confocal microscope. Images were taken at 300 nm XZ intervals and with a 61.5 nm XY resolution. To minimize bleed-through the separate channels were obtained with sequential scanning. Unbiased co-localization analysis of EGFP+ and Oxtl+ fibers in the hypophysis was done by using the methods of Manders and Costes for spatial intensity, automatic thresholding and statistical significance testing (see: http://pacific.mpi-cbg.de/wiki/index.php/ColocalizationAnalysis). The Manders correlation coefficient indicates the proportion of each channel colocalized with the other channel. It can range from +1 for complete correlation to −1 for complete exclusion of two fluorophores. We obtained an average Meanders coefficient >0.80 for both channels (n=4, 7 scans each). This shows that the EGFP and Oxtl are significantly co-localized in neurohypophyseal fibers. ROI, region of interest.

Figure S2 (related to Figure 3). Structure of the hypophyseal vasculature at 3 days.

Ray-traced 3D rendering of the hypophyseal vascular structure of an optically sectioned 3-day old zebrafish embryo carrying the *vegfr2*:mCherry transgene (anterior up; **A,** dorsal view; **B,** ventral view). Arteries and veins are pseudo-colored in red and blue, respectively.

The hypophyseal artery is dense and structurally well defined, while the veins are thinner, less taut and with wider lumens. CaDI, caudal division of the internal carotid artery; CrDI, cranial division of the internal carotid artery; HyA, hypophyseal artery, HyV, hypophyseal veins, OA, optic artery; PHS, primary head sinus; PICA, primitive internal carotid artery; PLA, palatocerebral artery. Scale bar, 20μm.

Figure S3 (related to Figure 5). Genetic cell ablation of Otp+ cells of the neurosecretory preoptic nucleus (NPO).

A, Micrographs of 3-day old embryos that underwent Otp+ cell ablation and their unablated siblings (see '*RESULTS*' section). Embryos were subjected to *in-situ* hybridization with *oxtl* mRNA probe. Scale bar, 50µm.

B, Bar histogram depicting *oxtl*+ cell counts in control and Otpb+ cell ablated embryos. *p<0.001.

C, Oxtl protein was stained by immunofluorescence in 3-day old embryos that underwent Otp+ cell ablation and in their unablated siblings. Maximal pixel intensity (between 0-255) was recorded from confocal images of the NH region of each embryo and normalized for background pixel intensity. Oxtl staining was found to be reduced by ~85% in embryos that had undergone Otpb+ ablation. *p<0.001.

Figure S4 (related to Figure 6). Effects of *oxtl* and *oxtlr* gene knockdowns

A, Scheme depicting *oxtl* gene structure indicating the binding site of a splice blocking antisense oligonucleotide (*oxtl*-MO).

B, Reduction of Oxytocin (Oxtl) protein expression by *oxtl*-directed antisense oligonucleotide (*oxtl*-MO). Anti-oxytocin immunostaining of the neurohypophysis of 3-day old transgenic *oxtl:EGFP* embryos. Injection of *oxtl*-MO causes a marked reduction in Oxtl but not EGFP protein levels.

C, Scheme depicting *oxtlr* gene structure indicating the binding site of a splice blocking antisense oligonucleotide (*oxtlr*-MO) as well as the PCR primers used to amplify the respective exons (Ex) of the various mRNA products. A table summarizing the expected mRNA sizes in the control and followed *oxtlr* knockdown is shown at the bottom.

D, Gel electrophoresis analysis of PCR-amplified mRNA species in control and in embryos injected with *oxtlr*-MO. Primer pairs used to amplify the respective exons (Ex) are indicated at the top. Amplification of exon 1 (Ex1F/Ex1R) was used as control to demonstrate untargeted constitutive exon, which was not affected by the knockdown reagent.

E, Scatterplot representation of the fluorescence profiles of cell suspensions from dissociated 3-day old *vegfr2:mCherry* embryos (right) and their non-transgenic

siblings (left), prior to FACS sorting. P1 is the proportion of cells that exhibit high red fluorescence (defined as 'mCherry+' cells, ~1% of the total population). P4 is the proportion of cells that exhibit no fluorescence (defined as 'mCherry-' cells, ~80% of the total population).

Table S1. Oligonucleotide primers for RT-PCR analysis

Gene	Primers	Fragment size (bp)
oxtlr	tctgaacagctgctgcaacccct tgtgatggaggtttgggtgatgct	214
vegfr2	actcctcctctgaaatatatcagacc acaggaaatgtgaatctctgaatgacc	713
otpb	tcgctgggtcgacagcaggcgat cggcgaagggaagcgatacta	260
β-actin	gaggctctcttccagccttc cggatgtccacgtcgcacttc	95