**SUPPLEMENTARY INFORMATION**

# Luteinizing hormone induces ovulation via tumor necrosis factor α-dependent increases in prostaglandin $F_{2\alpha}$ in a nonmammalian vertebrate

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**Supplementary Table S1. Enrichment of GO classes and KEGG pathways in the list of DEGs obtained in response to sLh (25 ng/mL).**

| Functional group / Pathway | Features[1] | *P*-value[2] | Vocabulary |
|---|---|---|---|
| Acute-phase response | 12 / 32 | 0.000 | GO |
| Basement membrane | 7 / 81 | 0.048 | GO |
| Blood coagulation | 9 / 95 | 0.010 | GO |
| Cell adhesion | 49 / 622 | 0.000 | GO |
| Cell differentiation | 30 / 535 | 0.029 | GO |
| Cell surface receptor linked signal transduction | 9 / 115 | 0.043 | GO |
| Chemotaxis | 9 / 100 | 0.015 | GO |
| Complement and coagulation cascades | 18 / 115 | 0.000 | KEGG |
| ECM-receptor interaction | 34 / 136 | 0.000 | KEGG |
| Endopeptidase inhibitor activity | 7 / 35 | 0.000 | GO |
| Extracellular space | 39 / 438 | 0.000 | GO |
| Focal adhesion | 49 / 352 | 0.000 | KEGG |
| Growth factor activity | 10 / 104 | 0.005 | GO |
| Heparin binding | 16 / 96 | 0.000 | GO |
| Inflammatory response | 22 / 247 | 0.000 | GO |
| Insulin-like growth factor binding | 9 / 25 | 0.000 | GO |
| Integrin complex | 7 / 45 | 0.000 | GO |
| Integrin-mediated signaling pathway | 16 / 89 | 0.000 | GO |
| Intermediate filament | 15 / 68 | 0.000 | GO |
| Lipid binding | 8 / 47 | 0.000 | GO |
| Myosin complex | 7 / 74 | 0.027 | GO |
| Natural killer cell mediated cytotoxicity | 9 / 113 | 0.038 | KEGG |
| Platelet activation | 8 / 42 | 0.000 | GO |
| Regulation of actin cytoskeleton | 22 / 325 | 0.007 | KEGG |
| Regulation of cell growth | 12 / 108 | 0.000 | GO |
| Structural constituent of cytoskeleton | 22 / 166 | 0.000 | GO |
| Transforming growth factor beta receptor signaling pathway | 6 / 24 | 0.000 | GO |
| Transmembrane receptor protein tyrosine kinase signaling pathway | 17 / 105 | 0.000 | GO |

[1] Numbers of genes among DEGs and on the microarray platform. [2] Yate´s corrected chi-square.

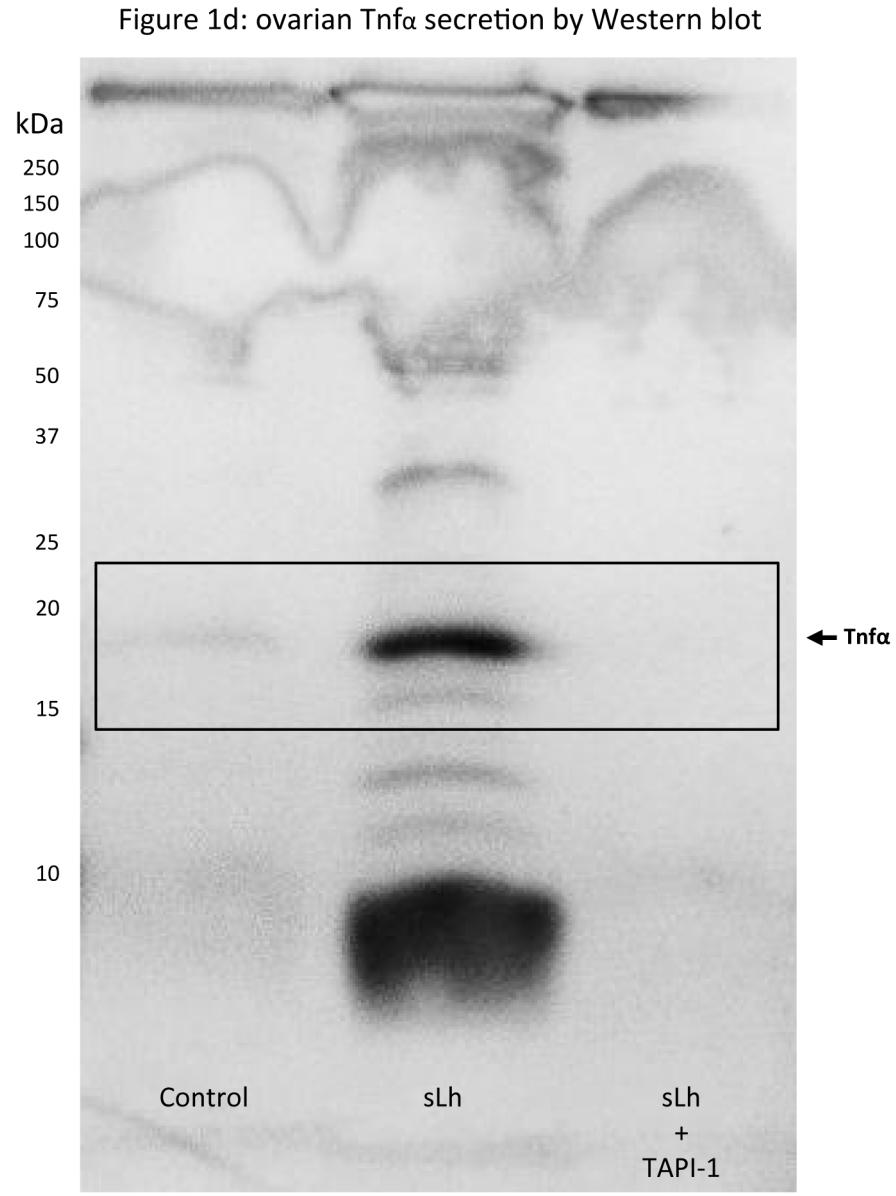
**Supplementary Table S2. Microarray validation by qPCR.** Data are expressed as mean of fold change (FC) ± SEM (n = 4).

| ProbeID | Gene name | Microarray | qPCR |
|---|---|---|---|
| Omy#S15300025 | Immediate early response gene 2 protein | 6.85 ± 2.27 | 2.15 ± 0.15 |
| Omy#S15296827 | Transgelin | 4.93 ± 2.35 | 2.15 ± 0.66 |
| Omy#TC165968 | Alpha 1 type IV collagen preproprotein | 3.50 ± 1.36 | 1.53 ± 0.54 |
| Omy#CX247152 | Forkhead box O3A | 3.08 ± 1.25 | 1.58 ± 0.25 |
| Omy#CX245477 | Testosterone 17-beta-dehydrogenase 3 | 2.86 ± 0.64 | 1.58 ± 0.41 |
| Omy#TC151257 | Matrix metalloproteinase-2 | 1.99 ± 1.01 | 2.35 ± 0.35 |
| Omy#S15244274 | Cyclin-dependent kinase 4 inhibitor B | -2.46 ± 1.34 | -1.03 ± 1.23 |
| Omy#S22164837 | CC chemokine | -2.59 ± 0.91 | -2.28 ± 1.51 |
| Omy#S34311675 | Transferrin | -3.01 ± 1.57 | -1.11 ± 0.69 |
| Omy#S15332001 | Matrix metalloproteinase-9 | -4.03 ± 0.45 | -7.04 ± 1.93 |
| Omy#S34422573 | Type I keratin E7 | -4.23 ± 1.23 | -1.77 ± 0.61 |
| Omy#S22927555 | C-type lectin, superfamily member 14 isoform 1 | -5.54 ± 2.32 | -1.33 ± 1.24 |

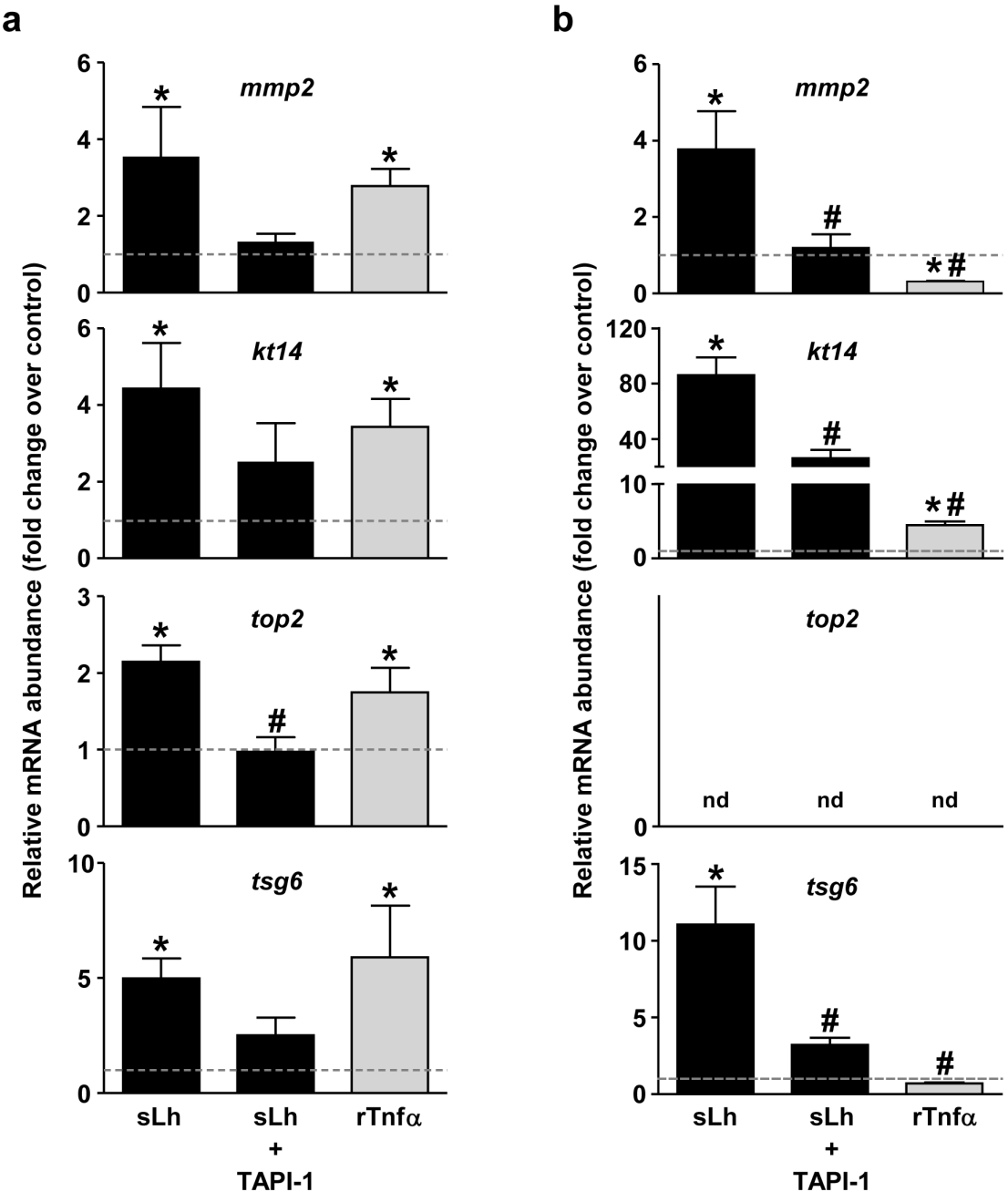
**Supplementary Table S3. Sequences of primers used in gene expression analyses by qPCR.**

| GenBank /ProbeID | Gene name | Primer sequence (5´-3´) | Amplicon size (bp) |
|---|---|---|---|
| AF308735 | 18S ribosomal RNA (*18s*) | (F) CGGAGGTTCGAAGACGATCA<br>(R) TCGCTAGTTGGCATCGTTTAT | 62 |
| DY696186 | ADAM metallopeptidase domain 17 (*adam17*) | (F) TGGTGGTCTTCTCGTTGGTCTTC<br>(R) TCCACACAGTGAACCAGGATGC | 58 |
| Omy#TC165968 | Alpha 1 type IV collagen preproprotein (*col4a1*) | (F) CGAGGACGAGGAGATGTTTACGAA<br>(R) TAAGGGTCACAGAGAGGTCAGGG | 134 |
| Omy#S22927555 | C-type lectin, superfamily member 14 isoform 1 (*clec14*) | (F) AAGGGGCGACCAACACTCATTAT<br>(R) CTTCTCGGCTTACCAGGCTTCAT | 129 |
| Omy#S22164837 | CC chemokine (*ccc*) | (F) AGAAGATCACCGTTCCCATCATCG<br>(R) CAGGACTCTTTCCAATGGCTGC | 121 |
| Omy#S48436481 | Cyclin-dependent kinase 4 inhibitor (*cdkn4b*) | (F) TTACACGATGCCGCCAGGAT<br>(R) TTGTCCCGGCTGTTCGGATC | 83 |
| Omy#CX247152 | Forkhead box O3A (*foxo3a*) | (F) CTCACAACCACAACCAGGGCTC<br>(R) CTCCCGTTCAGGAAGAGAGACGA | 160 |
| Omy#S15300025 | Immediate early response gene 2 protein (*ier2*) | (F) AGAGAGCGAGACTTTCACTGGAGC<br>(R) TTTGAGTTAGGGACAGGGGTGGT | 109 |
| AF005026 | Kallikrein trout #14 (*kt14*) | (F) CCAGGAGGTGGAATTAGGATGAGA<br>(R) GCAGAGCAGCAGTCAGGAACA | 167 |
| AB021698 | Matrix metalloproteinase-2 (*mmp2*) | (F) CGTCAAACTGGGCGAGATTACA<br>(R) AGGATAGGCTGGTTGGATAGGGA | 123 |
| Omy#S15332001 | Matrix metalloproteinase-9 (*mmp9*) | (F) CTATGGCACTGTTTCTGGGCTGA<br>(R) GGAGAGACAGAGAATGGCATAGCA | 122 |
| AF158374 | Prostaglandin-endoperoxide synthase 1 (*ptgs1*) | (F) GAGCCGCCACCAAGGAAACA<br>(R) CAGGCATCATAGGTGGACAAGGG | 119 |
| AF158373 | Prostaglandin-endoperoxide synthase 2 (*ptgs2*) | (F) TGTCCGATGGTGTCCTTTCAA<br>(R) TGAGAGCAGCTTCAGAACACTGAG | 202 |
| Ssa#S47624978 | Serine protease 23 (*prss23*) | (F) TGCTGAGGAAGTGAAGTTGACCA<br>(R) GTTGTGTGTTGGCAGGATGTCTC | 134 |
| Omy#CX245477 | Testosterone 17-beta-dehydrogenase 3 (*hsd17b3*) | (F) GGACAAGACCTACGGCAGCATC<br>(R) CCTATGTCCCCACTCTTTTCTTCA | 134 |
| Omy#S34311675 | Transferrin (*tf*) | (F) TGTGTCCATCTTTCTGAGCAGCA<br>(R) TTGTGGAATCCTTGAAGAGCGAG | 112 |
| Omy#S15296827 | Transgelin (*tagln*) | (F) CACACACACTATGACCAACCCAAT<br>(R) GGTTAGTTGCTTCAGTGCCTTCTC | 190 |
| U67854 | Trout ovulatory protein-2 (*top2*) | (F) GGGATGTGTGCGGAGTTATGCT<br>(R) ACTGTGTCGGTGCAATGCAGTCAT | 99 |
| AJ278085 | Tumor necrosis factor α (*tnfα*) | (F) AGCATGGAAGACCGTCAA<br>(R) TTCGTTTACAGCCAGGCT | 271 |
| 209733821 | Tumor necrosis factor α-stimulated gene 6 (*tsg6*) | (F) GGCTGAACAAGAGTGAAAGATGGG<br>(R) CTGCTCATCCTGGTACTCCTCTGG | 131 |
| Omy#S34422573 | Type I keratin E7 (*krt1e7*) | (F) AGGAGTTGGGTCGCATGAAGA<br>(R) TCTCCCGACATCCTCTCCGT | 134 |

F: forward, R: reverse.




**Supplementary Figure S1. Full-length blot image for Figure 1.** Box indicates the cropped image shown in Figure 1d.




**Supplementary Figure S2. Regulation of proteolytic gene expression by Lh and Tnfα in isolated theca (a) and granulosa layers (b) from brown trout preovulatory follicles.** Follicles were incubated with sLh (25 ng/mL) in the absence or presence of TAPI-1 (50 μM), or with rTnfα (50 ng/mL). The relative expression of *mmp2*, *kt14*, *top2* and *tsg6* was determined by qPCR and normalized to the abundance of *18s*. The results from ovarian tissue from three separate brown trout females assayed in triplicate are expressed as mean of fold change ± SEM with respect to the control that was set at 1. * $P < 0.05$, with respect to control; # $P < 0.05$, with respect to sLh; nd, no detectable expression.

Figure 2d: Mmp2 activity by gelatin zymography

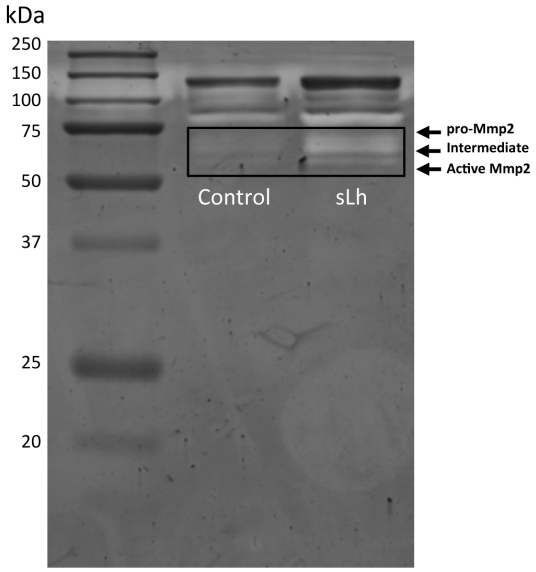
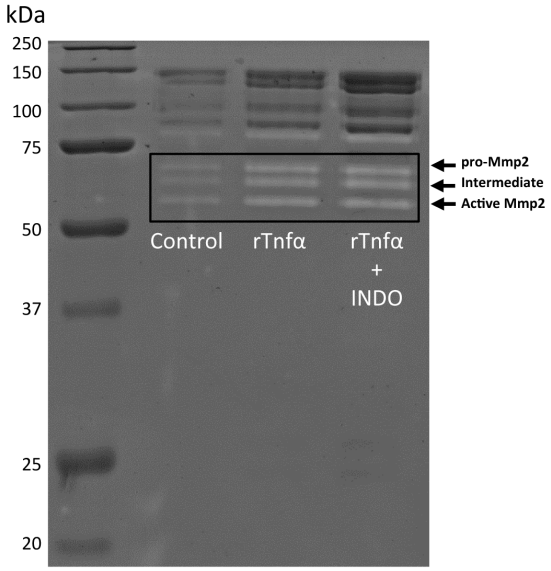
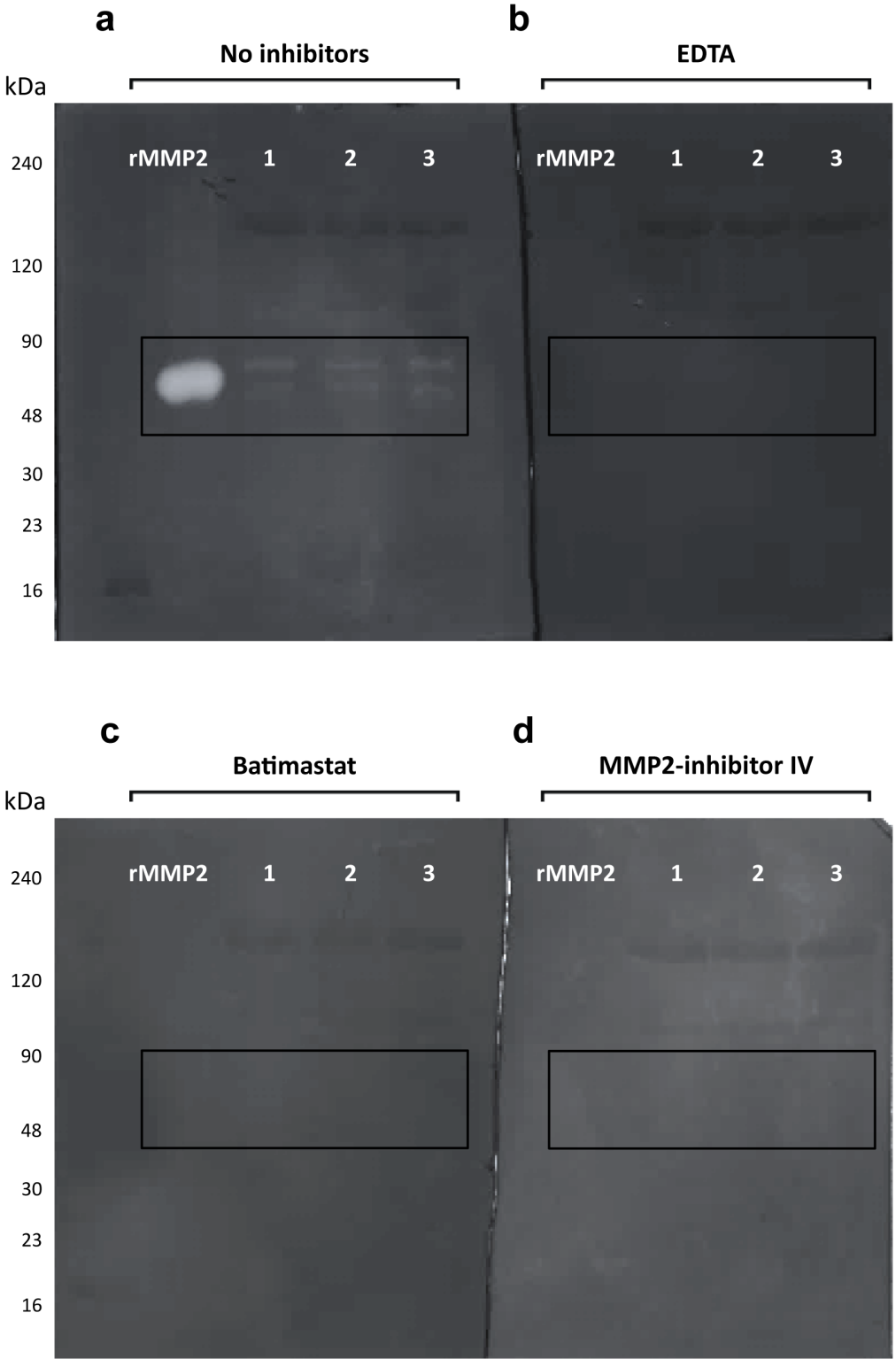


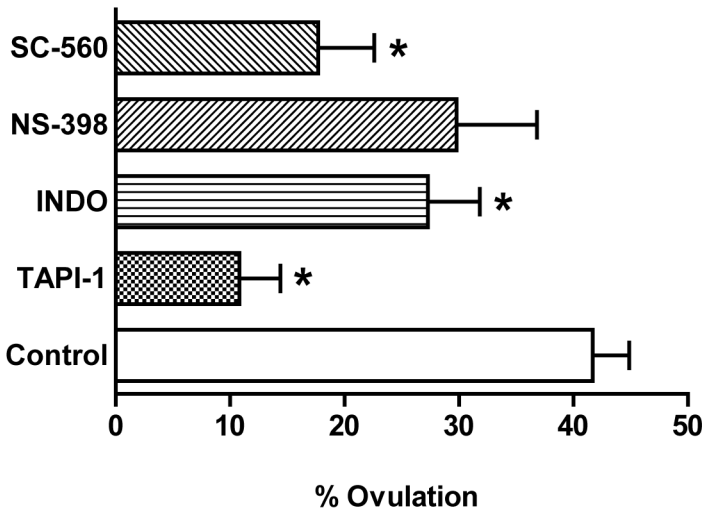

Figure 2e: Mmp2 activity by gelatin zymography



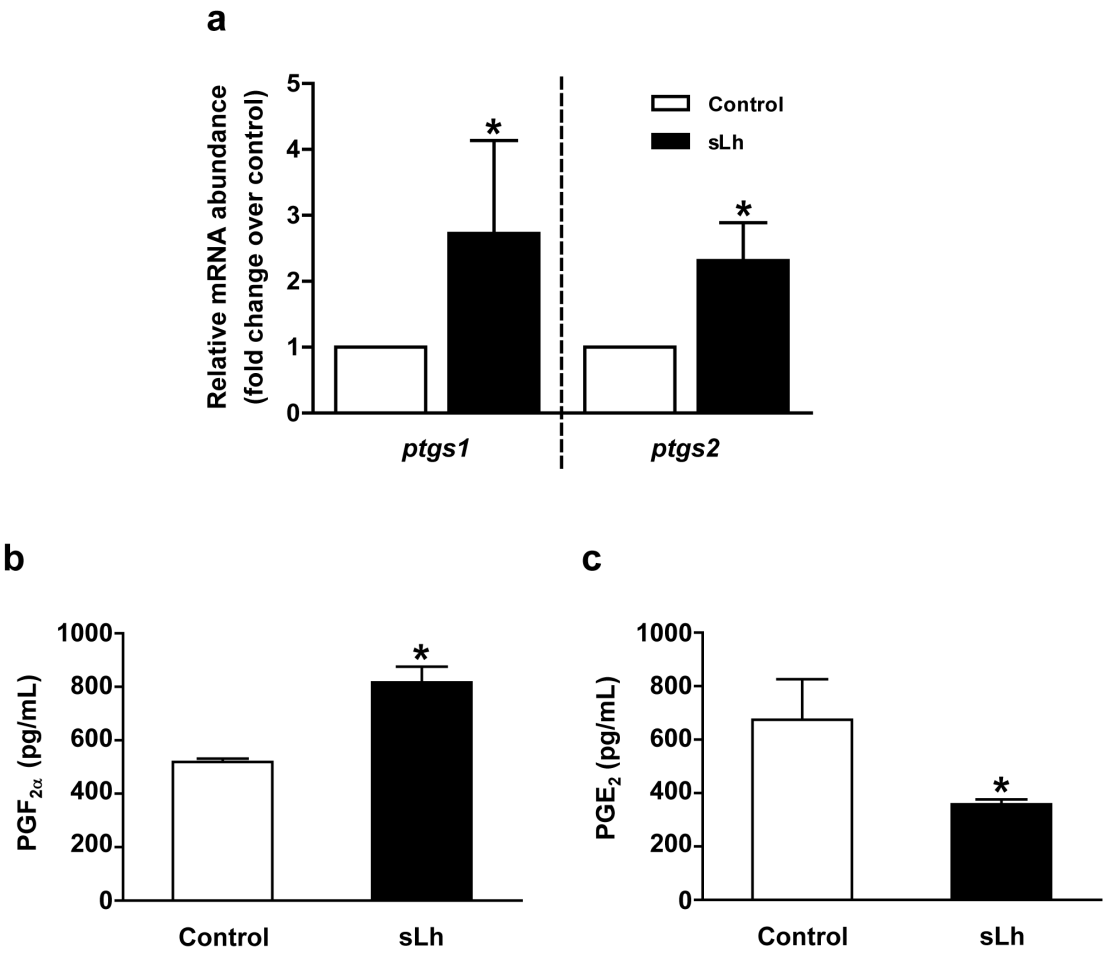

**Supplementary Figure S3. Full-length gel images for Figure 2.** Boxes indicate the cropped images shown in Figures 2d and 2e.



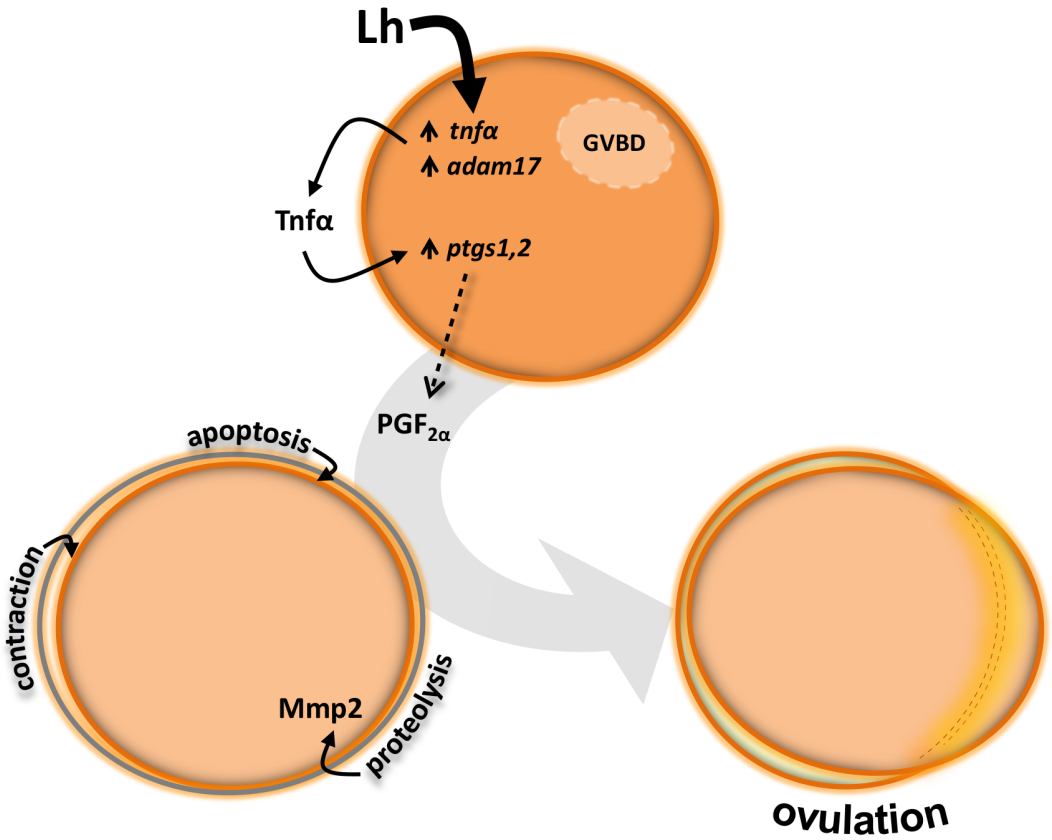

**Supplementary Figure S4. Abrogation of gelatinolytic activity by MMP inhibitors.** A set of purified follicle homogenates (1, 2 and 3) was run in parallel under the same conditions and gels were subsequently incubated in the absence (**a**) or presence of different MMP inhibitors (broad, **b** and **c**; and MMP2-specific, **d**). Boxes indicate the expected size of MMP2 gelatinolytic activity. Clear bands represent enzymatic digestion of gelatin. rMMP2, recombinant human MMP2.




**Supplementary Figure S5. Basal effects of various inhibitors on *in vitro* ovulation in brook trout preovulatory follicles.** Follicles were incubated for 36 h at 12°C in the presence or absence of TAPI-1 (50 μM), INDO (10 μg/mL), NS-398 (10 μg/mL) and SC-560 (10 μg/mL). Each bar represents the mean ± SEM from four independent experiments, each performed with preovulatory follicles from a different female and assayed in triplicate. * $P < 0.05$, with respect to control.




**Supplementary Figure S6. Regulation of prostaglandin synthesis by Lh in brook trout preovulatory follicles.** (**a**) *In vitro* effects of sLh (25 ng/mL) on the expression of *ptgs1* and *ptgs2* as determined by qPCR and normalized to the abundance of *18s*. The results from ovarian tissue from four separate brown trout females for each group are expressed as mean of fold change ± SEM with respect to the control that was set at 1. * $P < 0.05$, with respect to control. Effects of sLh (25 ng/mL) *in vitro* $PGF_{2\alpha}$ (**b**) and $PGE_2$ (**c**) production by intact brook trout ovarian follicles. Each bar represents the mean ± SEM of four independent experiments, each with ovarian tissue from a separate female and assayed in triplicate. * $P < 0.05$, with respect to control.




**Supplementary Figure S7. Proposed model of the pro-ovulatory actions of Lh in the trout ovary.** Evidence from this study strongly supports the notion that Lh orchestrates the complex events leading to ovulation involving the stimulation of proteolysis, apoptosis and contraction of the follicle wall through the stimulation of the local production of $PGF_{2\alpha}$ in a process that is dependent on the ability of Lh to stimulate intraovarian Tnfα production.