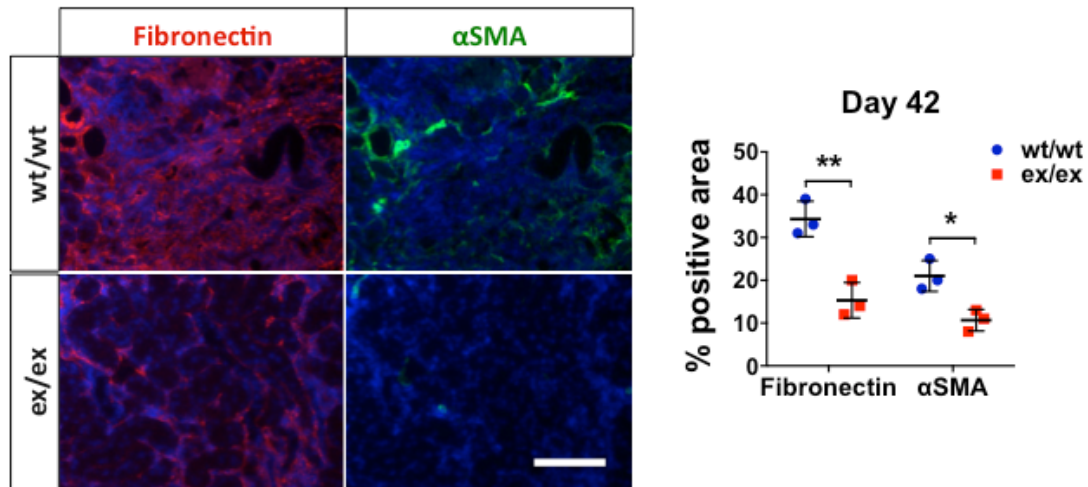
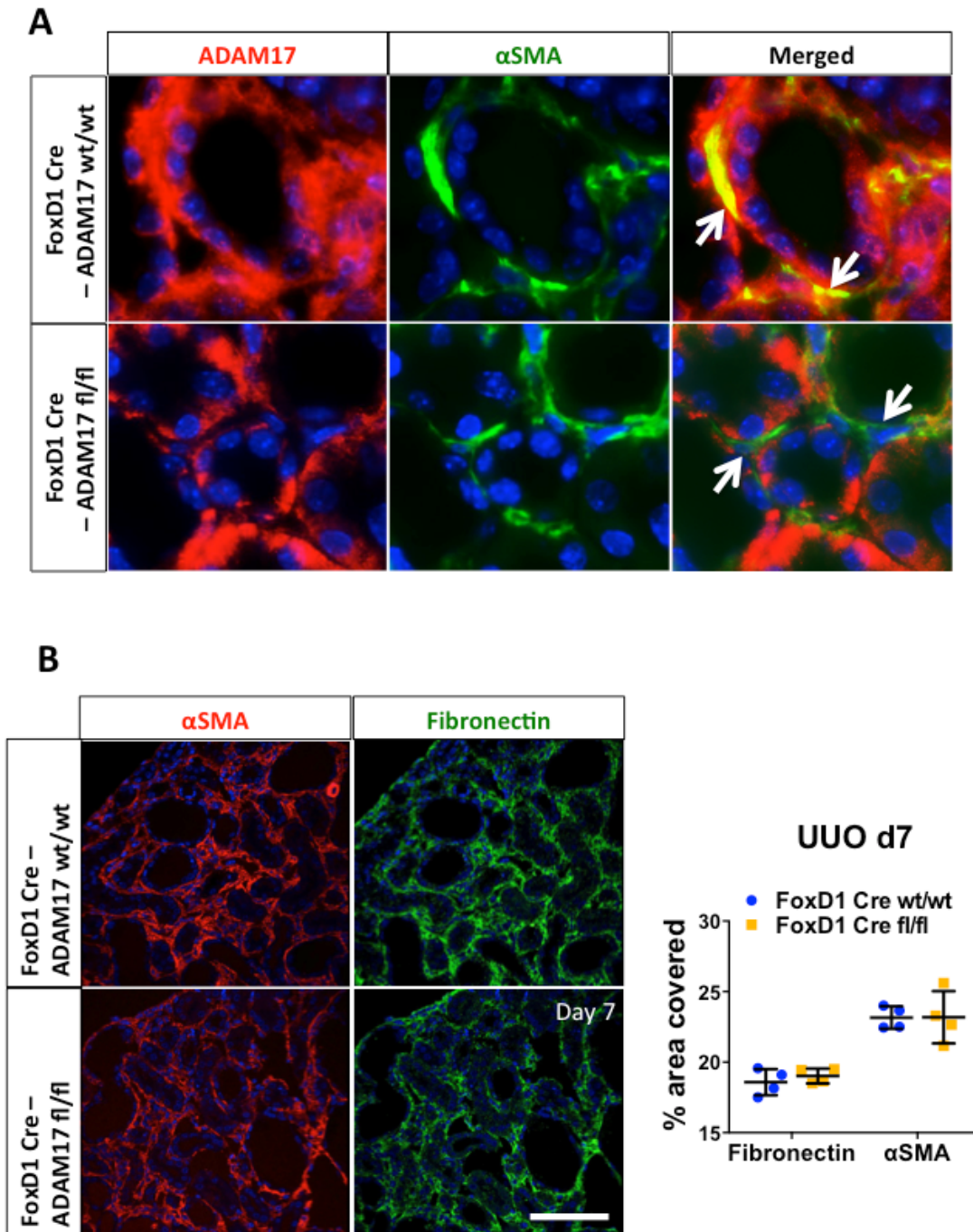


## Supplementary Materials:



**Supplemental Figure 1: Profibrotic markers in wt/wt or ex/ex mouse kidneys 42 days post IRI.** The levels of fibronectin and  $\alpha$ SMA were examined by immunostaining in ADAM17 wt/wt or ADAM17 ex/ex mouse kidneys 42 days post ischemia (left: representative images, right: quantification, n=4, scale bar: 100  $\mu$ m). \*: P<0.05; \*\*: P<0.01 as determined by an unpaired 2-tailed Student's *t* test.

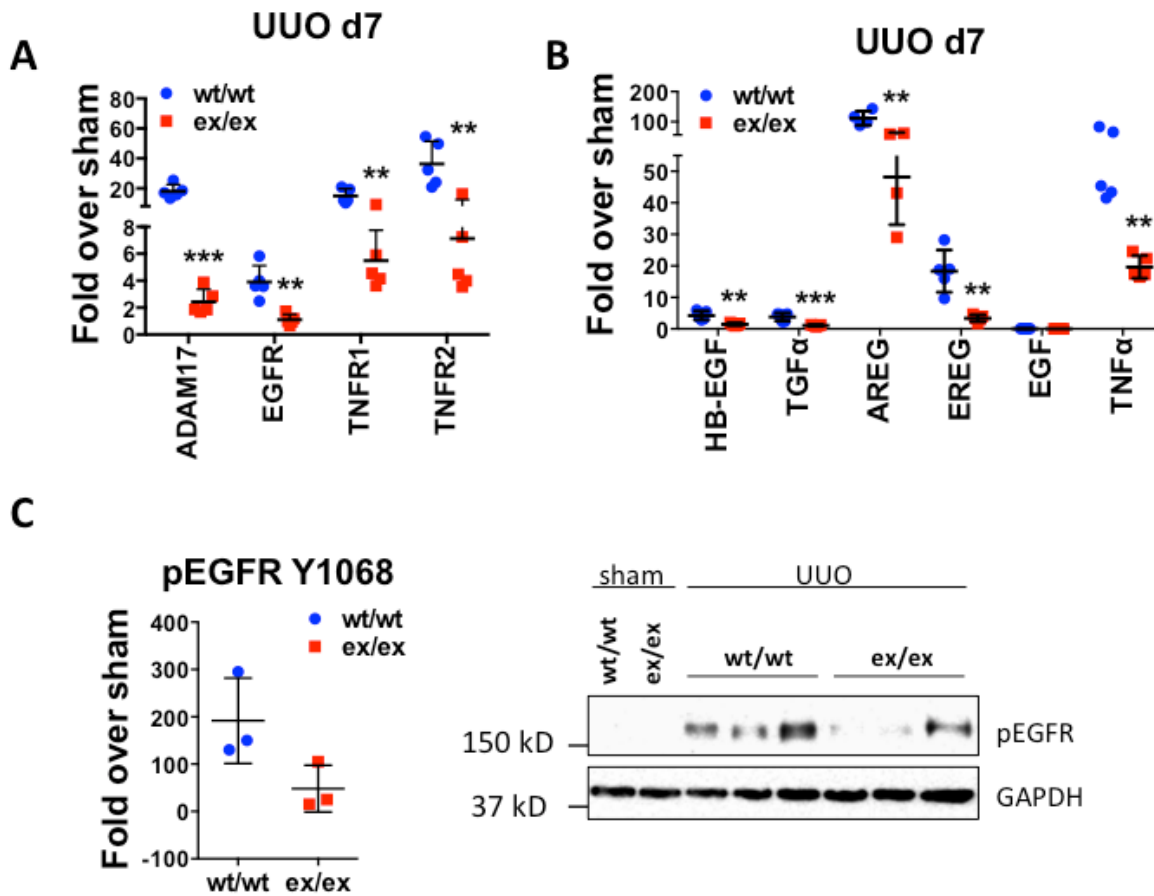
ADAM17: a-disintegrin-and-metalloprotease-17, IRI: ischemia-reperfusion-injury,  $\alpha$ SMA: alpha smooth muscle actin



**Supplemental Figure 2: Kidney stroma-specific ADAM17 knockout does not protect against fibrosis after UOU.** **A.** Stroma-specific knock out (FoxD1-Cre) of ADAM17 was examined by immunostaining in the UOU model 7 days post obstruction.  $\alpha$ SMA (a myofibroblast marker) is strongly co-localized with ADAM17 in kidney stroma in *wt* but not in

ADAM17<sup>fl/fl</sup> - FoxD1 Cre<sup>+/-</sup> mice. **B.** Induction of fibronectin and  $\alpha$ SMA was examined by Immunostaining (left: representative images, right: quantification, n=6-7, scale bar: 100  $\mu$ m).

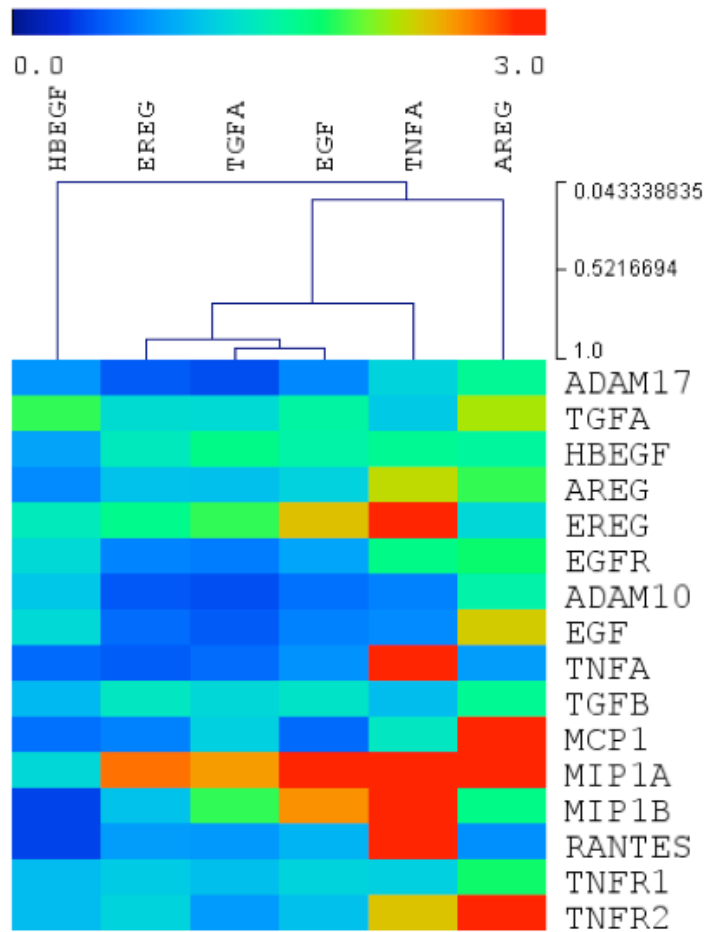
ADAM17: a-disintegrin-and-metalloprotease-17,  $\alpha$ SMA: alpha-smooth-muscle-actin, UUO: unilateral-ureteral-obstruction



**Supplemental Figure 3: ADAM17 pathway induction by UUO in wt/wt or ex/ex mouse kidneys.** **A, B.** qPCR analysis of mRNA expression levels of ADAM17 pathway components in ex/ex or wt/wt mice at 7 days of UUO, expressed as fold over respective sham-injured mice (n=5). **C.** UUO (7 days) -induced EGFR phosphorylation (Y1068) was examined by western blot in wt/wt or ex/ex mice (left: quantification, n=3, right: representative blot). GAPDH was used as loading control. \*: P<0.05; \*\*: P<0.01;\*\*\*:P<0.001 as determined by an unpaired 2-tailed Student's *t* test.

ADAM17: a-disintegrin-and-metalloprotease-17, AREG: amphiregulin, EGF: epidermal-growth-factor, EGFR: epidermal-growth-factor-receptor, EREG: epiregulin, HB-EGF: heparin-binding-

epidermal-growth-factor-like growth factor, TGF $\alpha$ : transforming-growth-factor-alpha, TNF $\alpha$ : tumor-necrosis-factor-alpha, TNFR: tumor-necrosis-factor-receptor, UUO: unilateral-ureteral-obstruction



**Supplemental Figure 4: ADAM17 pathway and proinflammatory markers induction by different EGFR ligands in HPTC.** qPCR analysis of ADAM17 pathway components and proinflammatory marker mRNA expression after addition of equimolar amounts of different EGFR ligands (HB-EGF, EREG, TGF $\alpha$ , EGF, AREG) or of TNF $\alpha$  for 24h. Average results of 3 independent experiments are shown as fold over control after hierarchical clustering of the added factors.

ADAM10: a-disintegrin-and-metalloprotease-10, ADAM17: a-disintegrin-and-metalloprotease-17, AREG: amphiregulin, EGF: epidermal-growth-factor, EGFR: epidermal-growth-factor-receptor, EREG: epiregulin, HB-EGF: heparin-binding-epidermal-growth-factor-like growth

factor, MCP1: monocyte-chemoattractant-protein-1, MIP1A: macrophage-inflammatory-protein-1-alpha, MIP1B: macrophage-inflammatory-protein-1-beta, RANTES: regulated-on-activation-normal-T-cell-expressed-and-secreted,  $TGF\alpha$ : transforming-growth-factor-alpha,  $TGF\beta$ : transforming-growth-factor-beta,  $TNF\alpha$ : tumor-necrosis-factor-alpha, TNFR: tumor-necrosis-factor-receptor.

**Supplemental Table 1: Patient characteristics of AKI, CKD patients and healthy volunteers included in urinary ELISA measurements.**

| Healthy controls |          |     |      |            |         |          |    |       |     |     |
|------------------|----------|-----|------|------------|---------|----------|----|-------|-----|-----|
| Gender           | Race     | Age |      |            |         |          |    |       |     |     |
| F                | White    | 49  |      |            |         |          |    |       |     |     |
| M                | White    | 50  |      |            |         |          |    |       |     |     |
| M                | White    | 50  |      |            |         |          |    |       |     |     |
| F                | Asian    | 41  |      |            |         |          |    |       |     |     |
| F                | Black    | 28  |      |            |         |          |    |       |     |     |
| M                | White    | 27  |      |            |         |          |    |       |     |     |
| F                | Asian    | 28  |      |            |         |          |    |       |     |     |
| F                | White    | 37  |      |            |         |          |    |       |     |     |
| M                | White    | 28  |      |            |         |          |    |       |     |     |
| F                | Asian    | 28  |      |            |         |          |    |       |     |     |
| CKD patients     |          |     |      |            |         |          |    |       |     |     |
| Gender           | Race     | Age | eGFR | Protein/Cr | ACE/ARB | Diuretic | DM | Cause | SBP | DBP |
| Male             | Black    | 40  | 12   | 1.68       | 1       | 1        | 0  | FSGS  | 134 | 85  |
| Female           | Hispanic | 75  | 13   | 2.44       | 1       | 1        | 1  | DM    | 154 | 66  |

|        |          |    |    |        |   |   |   |                   |     |    |
|--------|----------|----|----|--------|---|---|---|-------------------|-----|----|
| Female | Asian    | 50 | 19 | 0.16   | 1 | 1 | 0 | Chronic nephritis | 131 | 82 |
| Female | Black    | 70 | 22 | 0.23   | 1 | 1 | 1 | DM/HTN            | 139 | 55 |
| Female | White    | 35 | 20 | 0.44   | 1 | 1 | 0 | CAKUT             | 124 | 88 |
| Male   | Hispanic | 39 | 6  |        | 0 | 0 | 0 | Chronic nephritis | 136 | 98 |
| Female | White    | 65 | 15 | 0.544  | 0 | 0 | 0 | LITHIUM           | 127 | 76 |
| Female | Black    | 70 | 22 | 0.23   | 1 | 1 | 1 | DM/HTN            | 139 | 55 |
| Male   | Black    | 56 | 12 | 0.4536 | 0 | 1 | 1 | DM                | 126 | 72 |
| Female | White    | 55 | 23 | 5.48   | 0 | 1 | 1 | DM                | 185 | 76 |

#### AKI patients

| Admission Creatinine | Baseline sCr | Peak sCr | Reason for AKI                        |
|----------------------|--------------|----------|---------------------------------------|
| 0.85                 | 0.85         | 2.3      | Hypotension/hypoperfusion, likely ATN |
| 1                    | 1            | 3.59     | ATN                                   |
| 2.83                 | 2-2.1        | 4.44     | ATN, hemodynamic changes intra-op     |
| 1.3                  | 1.2-1.3      | 5.23     | ATN                                   |
| 1.2                  | 1.2-1.3      | 3.56     | ATN (post-op)                         |

|      |         |      |                                             |
|------|---------|------|---------------------------------------------|
| 0.7  | 0.7     | 5.02 | ATN (sepsis, hypotension)                   |
| 1.04 | 0.9     | 9.9  | ATN (post-operative, hemodynamic, vascular) |
| 0.59 | 0.6     | 4.87 | ATN, i.v. contrast                          |
| 1    | 0.8     | 6.41 | Cardiogenic shock, ATN                      |
| 1.4  | 1.2-1.4 | 4.31 | Post-operative AKI                          |

**Supplemental Table 2: Primers used for qPCR.**

| Target gene | Synonym | Species | Direction | Sequence                    |
|-------------|---------|---------|-----------|-----------------------------|
| ADAM10      |         | human   | Forward   | GCTGTGATTGCCCAGATATC<br>CA  |
| ADAM10      |         | human   | Reverse   | CACCATGAAACTGATGTTAC<br>GGA |
| ADAM17      | TACE    | human   | Forward   | AGTGCAGTGACAGGAACAGT<br>C   |
| ADAM17      | TACE    | human   | Reverse   | GGACACGCCTTTGCAAGTAG        |
| AREG        |         | human   | Forward   | TCGGCTCAGGCCATTATGC         |
| AREG        |         | human   | Reverse   | AATCCATCAGCACTGTGGTC<br>C   |
| CCL2        | MCP1    | human   | Forward   | AGCATGAAAGTCTCTGCCGC        |
| CCL2        | MCP1    | human   | Reverse   | GGCATTGATTGCATCTGGCT<br>G   |
| CCL3        | MIP1A   | human   | Forward   | TTCCGTCACCTGCTCAGAAT        |
| CCL3        | MIP1A   | human   | Reverse   | GCAGCAAGTGATGCAGAGAA<br>C   |
| CCL4        | MIP1B   | human   | Forward   | CCCAGCCAGCTGTGGTATTC        |
| CCL4        | MIP1B   | human   | Reverse   | AGCAGCTCAGTTCAGTTCCA        |

|       |        |             |         |                            |
|-------|--------|-------------|---------|----------------------------|
| CCL5  | RANTES | human       | Forward | CTGCTTTGCCTACATTGCCC       |
| CCL5  | RANTES | human       | Reverse | TCGGGTGACAAAGACGACTG       |
| EGF   |        | human       | Forward | CTTGGGAGCCTGAGCAGAAA       |
| EGF   |        | human       | Reverse | TGCACAAGTGTGACTGGAGG       |
| EGFR  |        | human       | Forward | CGAATGGGCCTAAGATCCCG       |
| EGFR  |        | human       | Reverse | CTTCGCATGAAGAGGCCGAT       |
| EREG  |        | human       | Forward | ACGTGTGGCTCAAGTGTCAA       |
| EREG  |        | human       | Reverse | CACTTCACACCTGCAGTAGT<br>TT |
| GAPDH |        | human/mouse | Forward | ACCACAGTCCATGCCATCAC       |
| GAPDH |        | human/mouse | Reverse | TCCACCACCCTGTTGCTGTA       |
| HBEGF |        | human       | Forward | TTGTGCTCAAGGAATCGGCT       |
| HBEGF |        | human       | Reverse | CAACTGGGGACGAAGGAGTC       |
| PRLP0 |        | human       | Forward | CGTCCTCGTGGAAGTGACAT       |
| PRLP0 |        | human       | Reverse | TAGTTGGACTTCCAGGTCGC       |
| TGFA  |        | human       | Forward | GTAAAATGGTCCCCTCGGCT       |
| TGFA  |        | human       | Reverse | GGGTCTGCACTCAGCGG          |
| TGFB1 |        | human       | Forward | GGAAATTGAGGGCTTTCGCC       |
| TGFB2 |        | human       | Reverse | CCGGTAGTGAACCCGTTGAT       |
| TNF   | TNFA   | human       | Forward | GCCCATGTTGTAGCAAACCC       |

|          |              |       |         |                             |
|----------|--------------|-------|---------|-----------------------------|
| TNF      | TNFA         | human | Reverse | TATCTCTCAGCTCCACGCCA        |
| TNFRSF1A | TNFR1        | human | Forward | ACGAGTGTGTCTCCTGTAGT        |
| TNFRSF1A | TNFR1        | human | Reverse | CACTGTGGTGCCTGAGTCC         |
| TNFRSF1B | TNFR2        | human | Forward | CCCAGGTGAAAAAGAAGCCC        |
| TNFRSF1B | TNFR2        | human | Reverse | CAGGTGCTGCTGCTCGG           |
| Acta2    | $\alpha$ SMA | mouse | Forward | AGCCATCTTTCATTGGGATG<br>GA  |
| Acta2    | $\alpha$ SMA | mouse | Reverse | TACCCCCTGACAGGACGTTG        |
| Adam10   |              | mouse | Forward | TGCCTGGCCTATGTCTTCAC        |
| Adam10   |              | mouse | Reverse | TATTCCCCCAGAGCTTCCTGA       |
| Adam17   | Tace         | mouse | Forward | TCTGAAGAGTTTGTTCGTCG<br>AGT |
| Adam17   | Tace         | mouse | Reverse | CTTCTCCACGGCCCATGTAT        |
| Areg     |              | mouse | Forward | GCTGAGGACAATGCAGGGTA<br>A   |
| Areg     |              | mouse | Reverse | GTGACAACTGGGCATCTGGA        |
| Ccl2     | Mcp1         | mouse | Forward | CACTCACCTGCTGCTACTCA        |
| Ccl2     | Mcp1         | mouse | Reverse | GCTTGGTGACAAAACTACA<br>GC   |
| Ccl3     | Mip1A        | mouse | Forward | CAGCCAGGTGTCATTTTCCTG<br>A  |

|        |        |       |         |                            |
|--------|--------|-------|---------|----------------------------|
| Ccl3   | Mip1A  | mouse | Reverse | TCTCAGGCATTCA GTTCCAG<br>G |
| Ccl4   | Mip1B  | mouse | Forward | GCCAGCTGTGGTATTCCTGA       |
| Ccl4   | Mip1B  | mouse | Reverse | AGCTGCTCAGTTCAACTCCA       |
| Ccl5   | Rantes | mouse | Forward | CTCACCATATGGCTCGGACA       |
| Ccl5   | Rantes | mouse | Reverse | CGACTGCAAGATTGGAGCAC       |
| Egf    |        | mouse | Forward | AGCATACTCAGCGTCACAGC       |
| Egf    |        | mouse | Reverse | GCAGGACCGGCACAAGTC         |
| Egfr   |        | mouse | Forward | ACCTCTCCCGGTCAGAGATG       |
| Egfr   |        | mouse | Reverse | CTTGTGCCTTGGCAGACTTTC      |
| Ereg   |        | mouse | Forward | TGCTTTGTCTAGGTTCCCACC      |
| Ereg   |        | mouse | Reverse | GGCGGTACAGTTATCCTCGG       |
| Hbegf  |        | mouse | Forward | TCTGGCCGCAGTGTTGTCC        |
| Hbegf  |        | mouse | Reverse | GGTTTGTGGATCCAGTGGGA<br>G  |
| Pdgfrb | Pdgfr1 | mouse | Forward | GTCAATGTCCCCGTCCGTG        |
| Pdgfrb | Pdgfr2 | mouse | Reverse | CTGCAGGTAGACCAGGTGAC       |
| Prpl0  |        | mouse | Forward | CTCTCGCTTTCTGGAGGGTG       |
| Prpl0  |        | mouse | Reverse | ACGCGCTTGTACCCATTGAT       |
| Tgfa   |        | mouse | Forward | CTCTGCTAGCGCTGGGTATC       |
| Tgfa   |        | mouse | Reverse | TGGGCACTTGTTGAAGTGAG       |

| A        |       |       |         |                      |
|----------|-------|-------|---------|----------------------|
| Tgfb1    | Tgfβ  | mouse | Forward | CTGCTGACCCCCACTGATAC |
| Tgfb1    | Tgfβ  | mouse | Reverse | AGCCCTGTATTCCGTCTCCT |
| Tnf      | Tnfa  | mouse | Forward | ATGGCCTCCCTCTCATCAGT |
| Tnf      | Tnfa  | mouse | Reverse | CTTGGTGGTTTGCTACGACG |
| Tnfrsf1a | Tnfr1 | mouse | Forward | ACGAATCACTCTGCTCCGTG |
| Tnfrsf1a | Tnfr1 | mouse | Reverse | TCGCAAGGTCTGCATTGTCA |
| Tnfrsf1b | Tnfr2 | mouse | Forward | GAGATGCCAAGGTGCCTCAT |
| Tnfrsf1b | Tnfr2 | mouse | Reverse | AACTGGGTGCTGTGGTCAAC |