

Supplemental Table S1. Cx43 phosphorylation in NP-UAEC with VEGF-165, VEGF-E or PIGF and without or with VEGFR kinase inhibitor (VEGFRi).¹

| | Control | | VEGF-165 | | VEGF E | | PIGF | |
|------------------------|---------|------------|------------|--------------|------------|--------------|-------------|-------------|
| | -VEGFRi | +VEGFRi | -VEGFRi | +VEGFRi | -VEGFRi | +VEGFRi | -VEGFRi | +VEGFRi |
| Ser-279 | | | | | | | | |
| 46 kDa | 1.00 | 1.15±0.06+ | 1.26±0.08* | 1.00±0.06+/# | 1.26±0.10* | 0.97±0.06+/# | 0.95±0.11§ | 1.00±0.09 |
| Tyr-265 | | | | | | | | |
| 82-78 kDa | 1.00 | 0.98±0.09 | 1.13±0.02* | 0.91±0.08+ | 0.97±0.10 | 0.90±0.08 | 0.91±0.09 | 0.76±0.06* |
| 82 kDa | 1.00 | 0.96±0.07 | 1.07±0.08 | 0.91±0.10 | 0.96±0.11 | 0.96±0.14 | 0.92±0.12 | 0.74±0.07* |
| 80 kDa | 1.00 | 0.93±0.08 | 1.11±0.07 | 0.88±0.09+ | 0.98±0.13 | 0.90±0.12 | 0.92±0.11 | 0.74±0.06* |
| 78 kDa | 1.00 | 1.02±0.07 | 1.12±0.10 | 0.96±0.07+ | 0.95±0.10 | 0.95±0.11 | 0.94±0.09 | 0.79±0.05* |
| 46 kDa | 1.00 | 0.84±0.08 | 0.91±0.06 | 0.88±0.07 | 0.95±1.10 | 0.86±0.10 | 0.85±0.13 | 0.79±0.05 |
| 42 kDa | 1.00 | 0.90±0.08 | 0.96±0.09 | 0.93±0.09 | 0.98±0.14 | 0.90±0.15 | 0.82±0.17 | 0.81±0.11 |
| Ser-368 | | | | | | | | |
| 46 kDa | 1.00 | 1.00±0.06 | 1.04±0.08 | 0.82±0.07*+ | 0.95±0.05 | 0.90±0.04* | 0.96±0.03 | 0.84±0.03*+ |
| 42 kDa ² | 1.00 | 0.88±0.04+ | 1.00±0.07 | 0.77±0.09* | 0.91±0.06 | 0.83±0.10 | 0.90±0.06 | 0.67±0.06*+ |
| Ser-262 | | | | | | | | |
| All bands | 1.00 | 1.03±0.07 | 1.03±0.08 | 0.98±0.06 | 1.01±0.05 | 0.92±0.05 | 1.01±0.04 | 0.99±0.04 |
| 42-46 kDa | 1.00 | 1.03±0.12 | 1.02±0.09 | 0.95±0.09 | 0.97±0.08 | 0.92±0.08 | 1.02±0.06 | 0.97±0.12 |
| 46 kDa | 1.00 | 1.06±0.11 | 1.05±0.06 | 1.01±0.08 | 0.99±0.07 | 1.00±0.09 | 1.05±0.05 | 1.05±0.12 |
| 44 kDa | 1.00 | 0.99±0.15 | 0.89±0.11 | 0.89±0.08 | 0.95±0.09 | 0.86±0.08 | 0.89±0.07 | 0.92±0.16 |
| 42 kDa | 1.00 | 1.09±0.09 | 0.99±0.13 | 0.95±0.08 | 0.97±0.09 | 0.86±0.06* | 0.97±0.13 | 0.88±0.12 |
| 36 kDa | 1.00 | 1.05±0.06 | 1.02±0.08 | 0.98±0.04 | 1.00±0.06 | 0.84±0.06* | 0.99±0.08 | 0.87±0.10 |
| 32 kDa | 1.00 | 0.99±0.06 | 1.44±0.10* | 1.12±0.13+ | 1.33±0.12* | 1.20±0.10* | 1.05±0.13§ | 1.05±0.12 |
| 28 kDa | 1.00 | 1.08±0.06 | 1.03±0.08 | 1.12±0.05 | 1.07±0.09 | 0.98±0.06 | 0.97±0.19 | 1.02±0.05 |
| Total Cx43 | | | | | | | | |
| All bands | 1.00 | 1.13±0.03+ | 1.14±0.04* | 1.03±0.03+ | 1.11±0.03* | 1.11±0.04* | 1.17±0.05*# | 1.02±0.10 |
| 46-47 kDa | 1.00 | 1.13±0.05+ | 1.17±0.03* | 1.11±0.05* | 1.18±0.05* | 1.16±0.07* | 1.22±0.06*# | 1.09±0.12 |
| 42-43 kDa | 1.00 | 1.13±0.03+ | 1.11±0.06 | 0.98±0.03 | 1.08±0.03* | 1.06±0.04 | 1.16±0.08 | 1.01±0.11 |
| CT1³ | | | | | | | | |
| 42-43 kDa | 1.00 | 1.06±0.05 | 1.01±0.02 | 1.04±0.05 | 1.05±0.03 | 1.00±0.04 | 1.06±0.03 | 0.99±0.04 |

¹Values shown are Means ± S.E.M. for 5–6 independent experiments (for the 28 kDa band of s262, n=3). Following frequently used nomenclature, 42 kDa=P0, 43 kDa=P1, 46 kDa=P2; 47 kDa=P3. Because some bands were difficult to measure separately due to their proximity, they were measured together.

²This band increases with alkaline phosphatase treatment.

³CT1 antibody recognizes Cx43 when Ser-365 is not phosphorylated.

* P<0.05 compared with control, + P<0.05 compared with same stimulated without inhibitor. # P<0.05 compared with P-UAEc, § P<0.05 compared with VEGF165.

Supplemental Table S2. Cx43 phosphorylation in P-UAEC with VEGF-165, VEGF-E or PIGF and without or with VEGFR kinase inhibitor (VEGFRi)¹

| | Control | | VEGF-165 | | VEGF E | | PIGF | |
|------------------------|---------|------------|------------|-------------|------------|-------------|------------|------------|
| | -VEGFRi | +VEGFRi | -VEGFRi | +VEGFRi | -VEGFRi | +VEGFRi | -VEGFRi | +VEGFRi |
| Ser-279 | | | | | | | | |
| 46 kDa | 1.00 | 0.96±0.10 | 1.41±0.07* | 1.35±0.11*# | 1.51±0.14* | 1.50±0.14*# | 1.06±0.08§ | 0.97±0.17§ |
| Tyr-265 | | | | | | | | |
| 82-78 kDa | 1.00 | 1.05±0.07 | 1.26±0.12* | 1.13±0.12+ | 1.26±0.11* | 1.10±0.11+ | 1.18±0.12 | 1.11±0.11 |
| 82 kDa | 1.00 | 1.05±0.15 | 1.26±0.19 | 1.24±0.21 | 1.26±0.18 | 1.13±0.20 | 1.13±0.18 | 1.18±0.18 |
| 80 kDa | 1.00 | 0.94±0.09 | 1.19±0.14 | 1.08±0.15 | 1.18±0.13 | 1.00±0.12+ | 1.02±0.10 | 0.99±0.09 |
| 78 kDa | 1.00 | 0.95±0.09 | 1.05±0.10 | 1.06±0.16 | 1.17±0.14 | 1.00±0.11+ | 1.04±0.10 | 0.99±0.10 |
| 46 kDa | 1.00 | 0.80±0.07+ | 0.93±0.07 | 0.85±0.07 | 0.95±0.10 | 0.79±0.08 | 0.89±0.08 | 0.90±0.08 |
| 42 kDa | 1.00 | 0.78±0.10+ | 0.92±0.09 | 0.76±0.10 | 0.88±0.11 | 0.71±0.08 | 0.77±0.13 | 0.72±0.09 |
| Ser-368 | | | | | | | | |
| 46 kDa | 1.00 | 0.94±0.06 | 0.95±0.07 | 0.92±0.06 | 0.94±0.06 | 0.96±0.11 | 0.98±0.08 | 0.92±0.06 |
| 42 kDa ² | 1.00± | 1.08±0.10 | 1.23±0.15 | 1.00±0.17 | 1.22±0.16 | 1.03±0.11 | 1.04±0.15 | 0.88±0.15 |
| Ser-262 | | | | | | | | |
| All bands | 1.00 | 1.04±0.07 | 1.06±0.07 | 1.06±0.06 | 1.14±0.11 | 1.13±0.11 | 1.04±0.06 | 1.09±0.10 |
| 42-46 kDa | 1.00 | 1.04±0.09 | 1.07±0.07 | 1.06±0.06 | 1.06±0.10 | 1.06±0.11 | 1.06±0.05 | 1.10±0.10 |
| 46 kDa | 1.00 | 1.00±0.07 | 1.06±0.05 | 1.09±0.03 | 1.05±0.07 | 1.07±0.06 | 1.11±0.03* | 1.13±0.05* |
| 44 kDa | 1.00 | 1.01±0.10 | 1.09±0.11 | 1.07±0.09 | 1.02±0.12 | 0.98±0.18 | 1.05±0.11 | 1.08±0.16 |
| 42 kDa | 1.00 | 1.07±0.14 | 1.05±0.12 | 1.09±0.10 | 1.10±0.15 | 1.07±0.20 | 1.04±0.11 | 1.16±0.18 |
| 36 kDa | 1.00 | 1.00±0.07 | 1.00±0.06 | 0.99±0.05 | 1.04±0.10 | 1.07±0.10 | 0.96±0.07 | 0.98±0.07 |
| 32 kDa | 1.00 | 0.99±0.12 | 1.40±0.08* | 1.39±0.18* | 1.66±0.23* | 1.57±0.24* | 1.27±0.12* | 1.18±0.11 |
| 28 kDa | 1.00 | 1.15±0.13 | 1.10±0.14 | 1.05±0.16 | 1.09±0.14 | 1.05±0.13 | 0.87±0.05 | 0.96±0.09 |
| Total Cx43 | | | | | | | | |
| All bands | 1.00 | 1.07±0.06 | 1.12±0.08 | 0.93±0.07 | 1.05±0.07 | 1.03±0.07 | 0.99±0.06# | 1.00±0.09 |
| 46-47 kDa | 1.00 | 1.04±0.03 | 1.14±0.06* | 1.04±0.08 | 1.09±0.06 | 1.04±0.04 | 1.01±0.05# | 0.99±0.09 |
| 42-43 kDa | 1.00 | 1.12±0.11 | 1.11±0.12 | 0.88±0.08 | 1.03±0.09 | 1.01±0.09 | 1.00±0.10 | 1.01±0.09 |
| CT1³ | | | | | | | | |
| 42-43 kDa | 1.00 | 1.01±0.06 | 1.08±0.06 | 1.08±0.08 | 1.02±0.04 | 0.97±0.07 | 1.08±0.07 | 1.00±0.08 |

¹Values shown are Means ± S.E.M for 5–6 independent experiments (for the 28 kDa band of Ser-262, n=3). Following frequently used nomenclature, 42 kDa=P0, 43 kDa=P1, 46 kDa=P2; 47 kDa=P3. Because some bands were difficult to measure separately due to their proximity, they were measured together.

²This band increases with alkaline phosphatase treatment.

³CT1 antibody recognizes Cx43 when Ser-365 is not phosphorylated.

* P<0.05 compared with control, + P<0.05 compared with same stimulated without inhibitor. # P<0.05 compared with NP-UAEC, § P<0.05 compared with VEGF 165.

Supplemental Table S3. Cx43 phosphorylation in NP-UAEC with PMA, ATP or VEGF-165 and without or with U0126¹

| | Control | | PMA | | ATP | | VEGF-165 | |
|---------------------|---------|------------|-------------|-------------|------------|------------|------------|-------------|
| | -U0126 | +U0126 | -U0126 | +U0126 | -U0126 | +U0126 | -U0126 | +U0126 |
| Ser-279 | | | | | | | | |
| 46 kDa | 1.00 | 1.08±0.08 | 2.14±0.10*# | 0.94±0.10+ | 0.89±0.08 | 0.93±0.06 | 1.19±0.04* | 0.95±0.05+ |
| Tyr-265 | | | | | | | | |
| 82–78 kDa | 1.00 | 1.14±0.06+ | 1.40±0.04* | 1.44±0.07* | 1.14±0.10 | 1.19±0.09 | 1.14±0.08* | 1.09±0.07 |
| 82 kDa | 1.00 | 1.10±0.09 | 1.77±0.20* | 1.47±0.19* | 1.14±0.11 | 1.14±0.14 | 1.20±0.12 | 1.04±0.11 |
| 80 kDa | 1.00 | 1.14±0.05+ | 1.51±0.08* | 1.56±0.08* | 1.13±0.09 | 1.17±0.10 | 1.15±0.10 | 1.11±0.06 |
| 78 kDa | 1.00 | 1.12±0.06 | 1.01±0.09 | 1.28±0.09* | 1.15±0.12 | 1.21±0.11 | 1.15±0.10 | 1.12±0.07 |
| 46 kDa | 1.00 | 1.02±0.06 | 1.06±0.08 | 0.98±0.07 | 0.96±0.12 | 1.00±0.10 | 1.04±0.10 | 1.05±0.07 |
| Ser-368 | | | | | | | | |
| 46 kDa | 1.00 | 1.11±0.06 | 1.56±0.11* | 1.37±0.09* | 1.03±0.08 | 1.00±0.11 | 1.24±0.15 | 1.05±0.10 |
| 42 kDa ² | 1.00 | 1.00±0.05 | 3.04±0.19* | 2.80±0.18* | 1.45±0.09* | 1.21±0.11 | 1.29±0.15* | 1.09±0.18 |
| Ser-262 | | | | | | | | |
| All bands | 1.00 | 0.83±0.11 | 1.40±0.10* | 1.04±0.05+ | 0.98±0.06 | 0.92±0.07 | 1.06±0.05 | 0.89±0.07 |
| 44–46 kDa | 1.00 | 0.80±0.08+ | 1.08±0.04 | 0.90±0.07 | 0.92±0.07 | 0.95±0.06 | 1.00±0.04 | 0.96±0.06 |
| 46 kDa | 1.00 | 0.85±0.08 | 1.06±0.04 | 0.89±0.07 | 0.91±0.05 | 0.94±0.05 | 0.99±0.05 | 1.01±0.07 |
| 45 kDa | 1.00 | 0.78±0.08+ | 1.09±0.04 | 0.92±0.08 | 0.95±0.07 | 0.97±0.06 | 1.04±0.04 | 0.94±0.06 |
| 44 kDa | 1.00 | 0.83±0.13 | 1.19±0.09* | 0.94±0.12 | 0.94±0.13 | 0.95±0.12 | 0.97±0.04 | 0.96±0.11 |
| 36 kDa | 1.00 | 0.78±0.05+ | 1.01±0.08 | 0.95±0.13 | 0.80±0.06* | 0.85±0.04* | 0.91±0.12 | 0.97±0.08 |
| 32 kDa | 1.00 | 0.60±0.12+ | 1.38±0.17* | 0.66±0.09*+ | 1.16±0.12 | 0.86±0.10 | 1.02±0.08 | 0.70±0.11*+ |
| 28 kDa | 1.00 | 0.89±0.09 | 2.18±0.32* | 2.06±0.32* | 0.89±0.09 | 0.85±0.12 | 1.07±0.09 | 0.97±0.16 |
| Total Cx43 | | | | | | | | |
| All bands | 1.00 | 0.98±0.06 | 1.10±0.07 | 1.03±0.07+ | 1.16±0.07* | 1.11±0.07 | 1.08±0.06 | 0.98±0.06 |
| 46–47 kDa | 1.00 | 0.95±0.06 | 1.78±0.19* | 1.20±0.08*+ | 1.18±0.11 | 1.10±0.08 | 1.26±0.12* | 1.08±0.08 |
| 42–43 kDa | 1.00 | 1.00±0.06 | 0.88±0.06* | 0.99±0.07+ | 1.18±0.07 | 1.15±0.07 | 1.04±0.06 | 0.98±0.05 |

¹Values shown are Means ± S.E.M for 5–6 independent experiments. Following frequently used nomenclature, 42 kDa=P0, 43 kDa=P1, 46 kDa=P2; 47 kDa=P3. Because some bands were difficult to measure separately due to their proximity, they were measured together.

²This band increases with alkaline phosphatase treatment.

*P<0.05 compared with control, [†]P<0.05 compared with same stimulated without inhibitor. #P<0.05 compared with P-UAEC.

Supplemental Table S4. Cx43 phosphorylation in P-UAEc with PMA, ATP or VEGF-165 and without or with U0126¹

| | Control | | PMA | | ATP | | VEGF-165 | |
|---------------------|---------|------------|-------------|-------------|------------|-------------|------------|-------------|
| | -U0126 | +U0126 | -U0126 | +U0126 | -U0126 | +U0126 | -U0126 | +U0126 |
| Ser-279 | | | | | | | | |
| 46 kDa | 1.00 | 0.84±0.07 | 2.85±0.14*# | 0.93±0.09+ | 1.03±.010 | 1.00±0.09 | 1.24±0.10* | 0.84±0.05*+ |
| Tyr-265 | | | | | | | | |
| 82–78 kDa | 1.00 | 1.08±0.04 | 1.33±0.11* | 1.41±0.13* | 1.10±0.08* | 1.14±0.11* | 1.19±0.06 | 1.20±0.06* |
| 82 kDa | 1.00 | 1.01±0.06 | 1.31±0.07* | 1.28±0.08* | 1.12±0.07 | 1.15±0.11 | 1.15±0.12 | 1.11±0.15 |
| 80 kDa | 1.00 | 1.06±0.02+ | 1.48±0.11* | 1.49±0.07* | 1.07±0.04 | 1.15±0.11 | 1.07±0.10 | 1.06±0.16 |
| 78 kDa | 1.00 | 1.07±0.02+ | 1.09±0.08 | 1.21±0.05* | 1.04±0.05 | 1.15±0.13 | 1.04±0.11 | 1.09±0.19 |
| 46 kDa | 1.00 | 1.12±0.06 | 1.09±0.07 | 1.10±0.06 | 1.12±0.06 | 1.12±0.10 | 1.23±0.14 | 1.10±0.14 |
| Ser-368 | | | | | | | | |
| 46 kDa | 1.00 | 1.12±0.05* | 1.26±0.09* | 1.12±0.05*+ | 0.95±0.07 | 0.92±0.09 | 1.01±0.14 | 0.86±0.15 |
| 42 kDa ² | 1.00 | 1.21±0.06+ | 2.69±0.21* | 2.60±0.25* | 1.43±0.18* | 1.29±0.13* | 1.26±0.23* | 1.15±0.18 |
| Ser-262 | | | | | | | | |
| All bands | 1.00 | 1.00±0.04 | 1.32±0.12* | 1.07±0.09+ | 1.14±0.10 | 1.17±0.09 | 1.19±0.11 | 0.91±0.08 |
| 44–46 kDa | 1.00 | 1.17±0.09 | 1.37±0.16* | 0.87±0.13+ | 1.01±0.12 | 1.26±0.11 | 0.99±0.05 | 0.82±0.09 |
| 46 kDa | 1.00 | 1.19±0.10 | 1.28±0.15 | 0.87±0.12 | 1.03±0.12 | 1.22±0.13 | 1.06±0.12 | 0.83±0.12 |
| 45 kDa | 1.00 | 1.17±0.12 | 1.42±0.18* | 0.86±0.12+ | 0.94±0.06 | 1.32±0.13*+ | 1.11±0.15 | 0.83±0.07* |
| 44 kDa | 1.00 | 1.18±0.09 | 1.36±0.10* | 1.07±0.20 | 1.01±0.10 | 1.25±0.13 | 0.93±0.08 | 0.77±0.07* |
| 36 kDa | 1.00 | 0.96±0.05 | 1.17±0.05* | 0.96±0.15 | 0.93±0.06 | 1.05±0.04 | 1.11±0.10 | 0.85±0.07 |
| 32 kDa | 1.00 | 0.76±0.15 | 1.75±0.12* | 0.89±0.12+ | 1.15±0.04 | 1.09±0.17 | 1.48±0.18* | 1.04±0.14+ |
| 28 kDa | 1.00 | 1.07±0.17 | 2.21±0.34* | 2.23±0.70* | 1.13±0.24 | 0.86±0.16 | 1.09±0.17 | 0.95±0.04 |
| Total Cx43 | | | | | | | | |
| All bands | 1.00 | 0.98±0.05 | 1.10±0.04* | 1.06±0.03 | 1.21±0.06* | 1.15±0.05* | 1.21±0.11 | 0.98±0.07 |
| 46–47 kDa | 1.00 | 0.98±0.05 | 1.44±0.08* | 1.11±0.05*+ | 1.09±0.06 | 1.00±0.08 | 1.13±0.14 | 0.92±0.12 |
| 42–43 kDa | 1.00 | 0.98±0.04 | 0.92±0.05 | 1.03±0.02 | 1.21±0.06* | 1.16±0.04 | 1.17±0.11 | 0.96±0.07 |

¹Values shown are Means ± S.E.M for 5–6 independent experiments (for the 28 kDa band of Ser-262, n=3). Following frequently used nomenclature, 42 kDa=P0, 43 kDa=P1, 46 kDa=P2; 47 kDa=P3. Because some bands were difficult to measure separately due to their proximity, they were measured together.

²This band increases with alkaline phosphatase treatment.

* P<0.05 compared with control. + P<0.05 compared with same stimulated without inhibitor. # P<0.05 compared with NP-UAEc.