Supplemental data

Table 1. Number of Endothelial Cells after Incubation with SMC-Conditioned Medium after 24h and 48h Exposure in Normal Gravity and Simulated Microgravity.

| Medium          | 0h  | 24h                  | 48h                  |
|-----------------|-----|----------------------|----------------------|
| EC (Cow 1)      |     |                      |                      |
| DMEM            | 20000 | 39938 ± 1452       | 67500 ± 1369        |
| SMC CM+1g       | 20000 | 37125 ± 2054       | 63188 ± 1663        |
| SMC CM+MG       | 20000 | 33750 ± 1591       | 56813 ± 1281        |
| EC (Cow 2)      |     |                      |                      |
| DMEM            | 20000 | 40469 ± 1041       | 87500 ± 3536        |
| SMC CM+1g       | 20000 | 39047 ± 2585       | 82500 ± 2041        |
| SMC CM+MG       | 20000 | 32266 ± 1094       | 63125 ± 4270        |
| EC (Cow 3)      |     |                      |                      |
| DMEM            | 20000 | 37708 ± 1301       | 70208 ± 2818        |
| SMC CM+1g       | 20000 | 36875 ± 3903       | 63542 ± 955         |
| SMC CM+MG       | 20000 | 23958 ± 955        | 41458 ± 2009        |

Table 2. The Number of Migrated Endothelial Cells after Incubation with SMC-Conditioned Medium after 24h Exposure in Normal Gravity and Simulated Microgravity.

| Medium          | 24h  |
|-----------------|------|
| EC (Cow 1)      |      |
| DMEM            | 94±5 |
| SMC CM+1g       | 113±8|
| SMC CM+MG       | 163±13|
| EC (Cow 2)      |      |
| DMEM            | 98±9 |
| SMC CM+1g       | 112±9|
| SMC CM+MG       | 151±20|
| EC (Cow 3)      |      |
| DMEM            | 86±5 |
| SMC CM+1g       | 109±4|
| SMC CM+MG       | 138±17|
Table 3. Number of Smooth Muscle Cells after Incubation with EC-Conditioned Medium after 24h Exposure in Normal Gravity and Simulated Microgravity.

| Medium      | 0h    | 24h       | 48h       |
|-------------|-------|-----------|-----------|
| **SMC (Cow 1)** |       |           |           |
| DMEM        | 20000 | 40500 ± 1472 | 68625 ± 1652 |
| EC CM+1g    | 20000 | 30625 ± 854  | 54625 ± 2689  |
| EC CM+MG    | 20000 | 35125 ± 1548 | 65000 ± 2160  |
| **SMC (Cow 2)** |       |           |           |
| DMEM        | 20000 | 49594 ± 986   | 142625 ± 5441 |
| EC CM+1g    | 20000 | 40594 ± 1161  | 94500 ± 4041  |
| EC CM+MG    | 20000 | 47344 ± 1120  | 122500 ± 5152 |
| **SMC (Cow 3)** |       |           |           |
| DMEM        | 20000 | 41500 ± 3000  | 102083 ± 5052 |
| EC CM+1g    | 20000 | 28167 ± 2566  | 54583 ± 1909  |
| EC CM+MG    | 20000 | 38167 ± 2021  | 87917 ± 2602  |

Table 4. The Number of Migrated Smooth Muscle Cells after Incubation with EC-Conditioned Medium after 24h Exposure in Normal Gravity and Simulated Microgravity.

| Medium      | 24h  |
|-------------|------|
| **SMC (Cow 1)** |      |
| DMEM        | 173±10 |
| EC CM+1g    | 122±9  |
| EC CM+MG    | 221±6  |
| **SMC (Cow 2)** |      |
| DMEM        | 169±7  |
| EC CM+1g    | 129±5  |
| EC CM+MG    | 208±15 |
| **SMC (Cow 3)** |      |
| DMEM        | 147±11 |
| EC CM+1g    | 113±6  |
| EC CM+MG    | 188±15 |
Table 5 Primer List and RT-PCR Amplification Condition

| Name       | Forward Primer (5'→3') | Size [bp] | Ta [°C] | Mg²⁺ [mM] | Cycle |
|------------|------------------------|-----------|---------|-----------|-------|
| P2X1       | TCT AYG AGA AGG GCT ACC AGA CC TCC ACC TCC ACG GGG CAC CAG | 361       | 60      | 2.5       | 45    |
| P2X2       | CCA TCA TCA CCA ARG TCA AGG TGG GGA TAG TGG ATG CTG TT | 391       | 53      | 2.5       | 45    |
| P2X3       | GCC GCT GGG TSA ACT ACA GGT GGG RAT GAT GTT GAA CT | 560       | 52      | 2.5       | 45    |
| P2X4       | TTC CTG TTC GAG TAC GAC AGG GAA TAT GGG GCA GAA GGG ATC | 659       | 56      | 2.5       | 40    |
| P2X5       | GTC ATC GCC AAG AAC AAG AAG GTG CAG TCG GAA GAT GGG GCA GTA G | 639       | 60      | 2       | 40    |
| P2X6       | GTT CTG CTG GTT GAC CAA CTT CC CGG ATY CCA TAG AGC A | 678       | 58      | 2       | 42    |
| P2X7       | GGT GTG AAA AAG GGA TGG W TG G ACA GCA CT T GCA MCA MCA GGA ATA A | 723       | 55      | 3       | 45    |
| P2Y1       | CTT CCT GGG CAA CAG CGT GGC CAG CAC CAA GGG GAC ACA CAA CAT | 502       | 61      | 3       | 40    |
| P2Y2       | TGT GCC GCC TCA AGA CCT GGA A GAA GGA GTA GTA GAG GGT GCG G | 638       | 60      | 1.5     | 40    |
| P2Y4       | GAK TTC AAG TTC ATC CTG CTG TGA GYC CAT AGC ARA CAA GAG T | 574       | 52      | 2       | 45    |
| P2Y6       | CGG GGY GCC TGG CTA GTG TG TTC GTG TGG TGR AAA GGA AGG AGA C | 354       | 61      | 2       | 45    |
| P2Y11      | AGC GTC ATC TTC ATC ACC TG CAT GTA GAG TAG MGG GTG GA | 621       | 52      | 2       | 45    |
| P2Y12      | ATC GCT ACC AGA ARA CCA CCA GGC CGG GCA AAA TGG AAW GGA ACA AAA CA | 406       | 60      | 2.5     | 45    |
| P2Y13      | TGA GCA ACA AGG AAG CAA CAC CAT C ACA CAA AGA CAG CCA CGA CAA C | 258       | 60      | 3       | 45    |
| P2Y14      | ATG TAC GTG ATG GAT GTG TGC TT TAG GGG ATT CTG CTA GAC ARA TGG T | 455       | 56      | 2       | 45    |
| VEGFR2     | GAG AGG TGC TGC TTYM GAT TT GGA AGG AAC TCT CAT TAG GA | 649       | 52      | 1.5     | 40    |
| PECAM-1    | GGT RAT AGC CCC RGT GGA TGA TGG GCC TGG GTT TTC CTC AG | 411       | 59      | 2.5     | 42    |
| VE-cadherin| CTG CAT CTC CAC CAT CAC AGT CTC GTA GCC GTA GAT GTG CAG | 384       | 61      | 1       | 40    |
| Calponin   | RCA GAT GGG CAG CAA CAA GG ATT TAT TG TCT CCA GGT AAR TAG AA | 443       | 51      | 3.5     | 35    |
| SMA-α      | CAG GGC TGT TTT CCC ATC CCA TCT GCC GAA TGG AAG GAC A | 393       | 51      | 1.5     | 30    |
| MYH-11     | GGG CAG AGC AAA ATC TTC TT GTT TCT GTA TGG CTT CAG | 658       | 51      | 2       | 42    |
| GAPDH      | CGT ATT GGG CGC CTG GTG ACC GCC AGT GAG CCT CCC GTT CAG C | 653       | 61      | 2.5     | 22    |

The amplification conditions for each specific product were conducted as followed: the initial denaturation was performed at 94°C for 3 min and cyclic denaturation was run for 30 seconds. The cyclic annealing step was performed for 30 seconds with the respective annealing temperatures (see table 5). The target gene was elongated at 72°C for 45 seconds. The cyclic amplification was repeated.
with different numbers (see table 5). A final extension of 3 min at 72°C was set with subsequent cooling down to 4°C. GAPDH served as the housekeeping gene controls, which were adjusted to equal levels prior to the comparison of genes of interest. The RT-PCR product was evaluated with 1% agarose gel electrophoresis. The images of gels were taken with a Bio-Rad Chemidoc machine.

The cell line HMEC-1 and C2 were used as positive controls to confirm the EC and SMC specific markers, respectively. Since no single cell type can expresses all P2 receptor subtypes, different positive controls were used here: HMEC-1 for P2X3, P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y11, P2Y12; MG-63 for P2X6, P2Y6 and U-87 MG for P2X1, P2X2, P2Y13, P2Y14. The GAPDH of the positive control is only given for HMEC-1 as representative for all three positive controls.