Supplementary material

Title: Development of Caco-2 cells expressing four CYPs via a mammalian artificial chromosome

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Supplementary Figure

Figure S1. Comparison of gene expression between human small intestine and day 22 culture of Caco-2 and Caco-2 4CYPs-MAC #2.
### Supplementary Tables

#### Table S1. Primer sequences for genomic PCR and RT-qPCR

| Aim   | Target gene | Primer name       | Sequence (5’-3’)                      |
|-------|-------------|-------------------|---------------------------------------|
| gPCR  | CYP2C9      | gPCR hCYP2C9_F    | ATGGATTTCTCTTGTGTTGCCTT               |
|       |             | gPCR hCYP2C9_R    | TCAGACAGGAATGAAGCAACA                 |
|       | CYP2C19     | qPCR hCYP2C19_F   | ACTTGAGAGCTGGGACAGAGA                 |
|       |             | CAGpA_rev1764     | CAGCCACCCCTTCTGATAGG                  |
|       | CYP2D6      | qPCR hCYP2D6_F    | TGTAGAGAAACCTGCGCAGTAG                |
|       |             | CAGpA_rev1801     | CTCAGTGTTATTTGTGAGCC                 |
|       | CYP3A4      | qPCR hCYP3A4_F    | TGTAGAGAAACCTGCGCAGTAG                |
|       |             | CAGpA_rev1801     | CTCAGTGTTATTTGTGAGCC                 |
|       | POR         | qPCR hPOR_F       | TGGAGAGGACTTCATCACCC                  |
|       |             | CAGpA_rev1801     | CTCAGTGTTATTTGTGAGCC                 |
|       | GAPDH       | GAPDH real F      | AGCCACATCGCTACAGACAC                 |
|       |             | GAPDH real R      | GCCCAATACGACCAAAATCC                 |
| RT-qPCR | CYP2C9    | qPCR hCYP2C9_F    | CCACATGCCCTACACAGATG                  |
|       |             | qPCR hCYP2C9_R    | TGCCCTTGGGAATGAAGATAG                 |
|       | CYP2C19     | qPCR hCYP2C19_F   | ACTTGAGAGCTGGGACAGAGA                 |
|       |             | qPCR hCYP2C19_R   | CATCTGTGAGGGACATGTG                   |
|       | CYP2D6      | qPCR hCYP2D6_F    | TGTAGAGAAACCTGCGCAGTAG                |
|       |             | qPCR hCYP2D6_R    | CCTCATGCATCGTCGATCT                   |
|       | CYP3A4      | qPCR hCYP3A4_F    | TGTGAGGCTTTTATGATG                  |
|       |             | qPCR hCYP3A4_R    | CCTCCGGTTTGTAAGACAG                  |
|       | POR         | qPCR hPOR_F       | TGGAGAGGACTTCATCACCC                  |
|       |             | qPCR hPOR_R       | ACAAGCCTGACGCGCAAT                   |
|       | GAPDH       | GAPDH real F      | AGCCACATCGCTACAGACAC                 |
|       |             | GAPDH real R      | GCCCAATACGACCAAAATCC                 |
Table S2. LC-MS/MS analysis conditions (MDZ, 1'-OH MDZ)

|                      | HPLC condition                                                                 | MS/MS condition            |
|----------------------|--------------------------------------------------------------------------------|-----------------------------|
| column               | TSK gel ODS-100V column (50 mm x 0.2 mm, 3μm, TOSOH)                           | Ionization method          |
| mobile phase         | A: 0.1% formic acid prepared with MQ                                           | curtain gas1               |
|                      | B: 0.1% formic acid prepared with acetonitrile                                 | curtain gas2               |
| gradient condition   | A:B=80:20 (v/v)                                                                | collision gas              |
| flow velocity        | 0.2ml/minute                                                                   | Ionspray voltage           |
| column temperature   | 40°C                                                                            | Declustering Potential (DP) |
| injection amount     | 10μl                                                                            | Entrance potential         |
|                      |                                                                                 | Collision energy           |
|                      |                                                                                 | Collision cell exit        |
|                      |                                                                                 | potential                  |
|                      |                                                                                 | Ion source temp            |

|                      |                                                                                 | 30psi                       |
|                      |                                                                                 | 40psi                       |
|                      |                                                                                 | 9                           |
|                      |                                                                                 | 5500V                       |
|                      |                                                                                 | 100V                        |
|                      |                                                                                 | 10V                         |
|                      |                                                                                 | 40V                         |
|                      |                                                                                 | 10V                         |
|                      |                                                                                 | 10V                         |
|                      |                                                                                 | 650°C                       |