



Article

Human Colonoid–Myofibroblast Coculture for Study of Apical Na^+/H^+ Exchangers of the Lower Cryptal Neck Region

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Supplementary Materials

Table S1. Sequence of primers used for RT-qPCR experiments.

Gene	Forward Primer	Reverse Primer
<i>ACTB</i>	CGAGGACTTTGATTGCACATTGTT	TGGGGTGGCTTTTAGGATGG
<i>AE2</i>	TGCCAAAGGTTCACACA	CAACTCATTAGCTCCACAAAC
<i>ALPI</i>	CGCTTTAACCAGTGCAACAC	GTCACCACTCCTACTGACTTTC
<i>CFTR</i>	CTACCACTGGTGCATACTCTAATC	ACGTGTTGAGGGTTGACATAG
<i>CLDN1</i>	CTGGGAGGTGCCCTACTTTG	ACACGTAGTCTTCCCGCTG
<i>CLDN2</i>	GATCCTACGGGACTTCTACTCA	CAGGGAGAACAGGGAAGAAATAA
<i>CLDN3</i>	ACGCGAGAAGAAGTACACGG	TAGACGTAGTCCTTGCGGTC
<i>CLDN4</i>	TTCTACAATCCGCTGGTGGC	GCGGAGTAAGGCTTGTCTGT
<i>CLDN7</i>	GGGGGAGACGACAAAGTGAA	CATACCAGGAGCAAGCTACCA
<i>CLDN8</i>	TGCCCAAAAACGTGAGCTTG	TGTGCGATGGGAAGGTATCG
<i>GAPDH</i>	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
<i>GREM1</i>	GCAAGCCCAAGAAATTCCTACTAC	TGCAACGACACTGCTTCA
<i>KI67</i>	GACCTCAAACCTGGCTCCTAATC	GCTGCCAGATAGAGTCAGAAAG
<i>LGR5</i>	CCTGCTTGACTTTGAGGAAGACC	CCAGCCATCAAGCAGGTGTTCA
<i>LYZ</i>	GGCTTGTCTCTCTTCTGTTA	GTAGCCATCCATTCCCAATCT
<i>MUC2</i>	AGTTTGGGGAGCACTTCGAG	TCTTCCACGCAGTGGGTAAC
<i>NBCe1</i>	CCGGCTTTGTTGGTCACTAT	CAAGTGATACCCTGCTCCTTTC
<i>NBCn1</i>	CTGCTATTCTGCTTTGCTTTG	GTGATAGCCAGCTCCTTTCTT
<i>NHE1</i>	GCTGGTGGCAGACCCCTACGA	ATAGGCCAGTGGGTCTGAGCCGA
<i>NHE2</i>	TGTCTACCGTGGGCAAGAAC	AACGCAAAACAGATGGCACC
<i>NHE3</i>	ACCGTGCGCTACACCATGAAGATG	ATGCGGTAGCGGTTGAGAAGCC
<i>NHE8</i>	CATGTGTGTTTGCAATTTCTTGGC	AGCACTATGCACCAGATGACA
<i>NKCC1</i>	AAAGGAACATTCAAGCACAGC	CTAGACACAGCACCTTTTCGTG
<i>OCN</i>	TCGACCAATGCTCTCTCAGC	CTCCTGGAGGAGAGGTCCAT
<i>PDGFRA</i>	CTTGTGTCAGTGTGCCTCTTG	GTTACCTTCTGTGGCCTATTA
<i>RSPO3</i>	GAAAGAGGAGAAAGGAAGGGAAG	GGCTGCCGATGTATTCCATAA
<i>SI</i>	CGCTACACCTTATTACCCTTCC	CCAGCTGTTTCGTATCCTCATAAA
<i>SLC26A3</i>	CCAGCGTCTATTCCCTCAAAT	TCCCAGCAAATCCTCTGAATAC
<i>SLC26A6</i>	AGAAACTGCTCAAGAAGCAGGA	CCATCTTATCTCCTGAGCTCACC
<i>THY1 (CD90)</i>	TGACCCGTGAGACAAAGAAG	GCTAGTGAAGGCGGATAAGTAG
<i>WNT2B</i>	CCTTGGAGTGGTAGCCATAAG	AACGCTGACTGTGTAGGTATG
<i>WNT5a</i>	GTGATGCAGATAGGCAGCCG	GCCATAGTCGATGTTGTCGC
<i>ZO-1</i>	CCTGAGTTTGACAGTGGAGTT	GCTGAAGGACTCACAGGAATAG

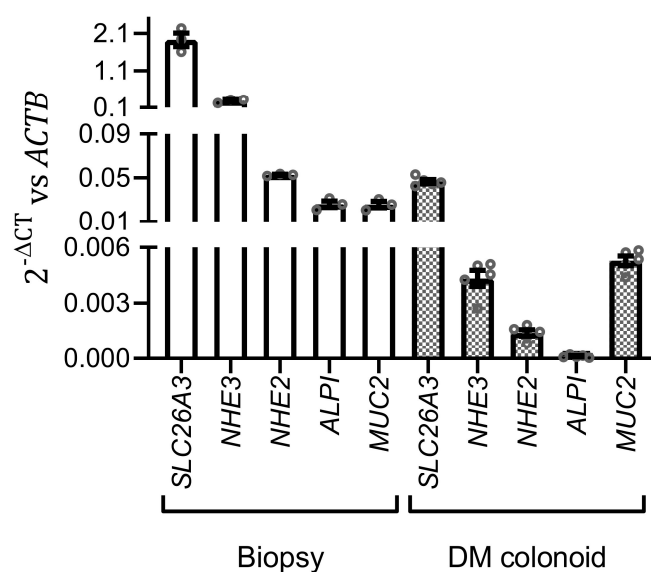


Figure S1. *SLC26A3* mRNA is highly expressed in human colon. RT-qPCR analysis of human transverse colon biopsy material from which the colonoids were derived shows that among the selected panel of genes, *SLC26A3* has substantially higher expression levels. Similarly in differentiated colonoid monolayers, *SLC26A3* expression is drastically higher than other differentiation marker genes including *NHE3*, *MUC2* or *ALPI*.

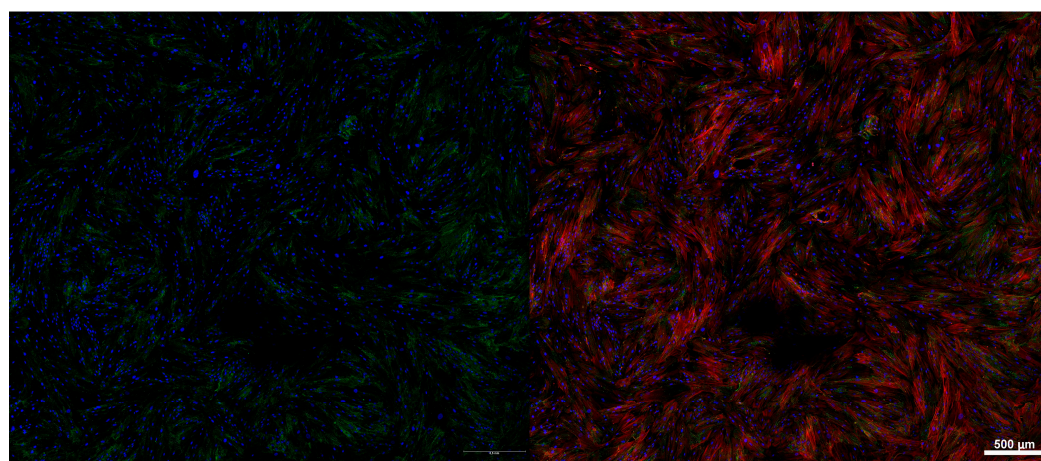


Figure S2. Majority of the cells in the myofibroblast culture used in CM-CE cocultures are CD90-positive. This immunofluorescent image shows intestinal myofibroblasts from the lamina propria of human transverse colon stained for nuclei (blue), CD90 (green) and F-actin (red). The image is generated by stitching multiple tile scan acquisitions to cover a broad area of the culture. The left and right panels are identical, except that in the left panel the F-actin signal is excluded for a better visualization of the CD90 signal. The majority of the myofibroblasts are positively stained for CD90.

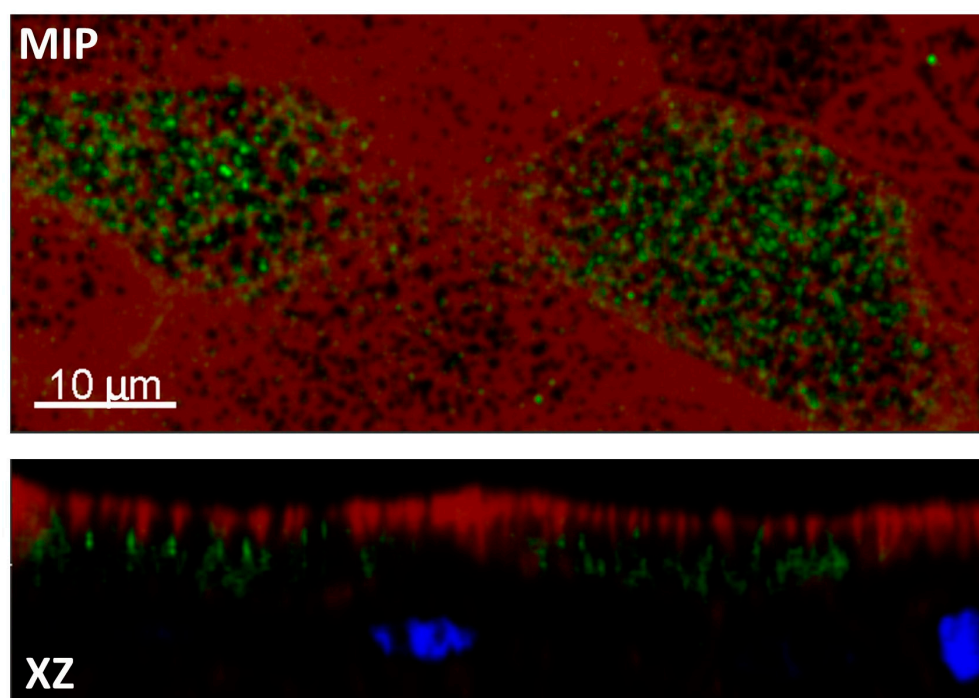


Figure S3. immunohistochemical staining of NHE8 in human CM-CE monolayers. Maximum intensity projection (MIP) and XZ cross section of CM-CE monolayer. NHE8 is detected as organellar structure mainly distributed between nucleus and subapical region. Green: NHE8, Red: F-actin, Blue: nuclei (excluded in MIP).