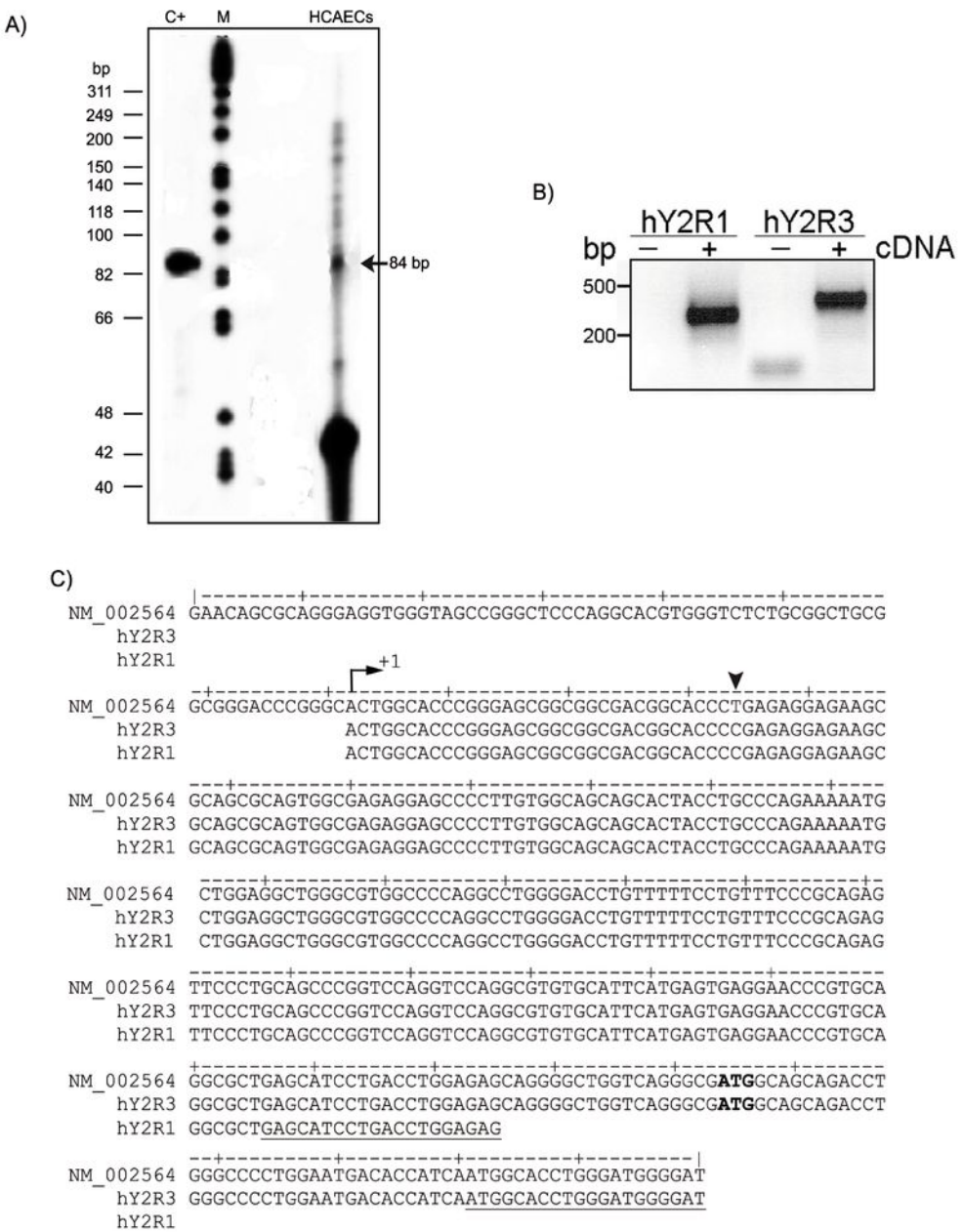
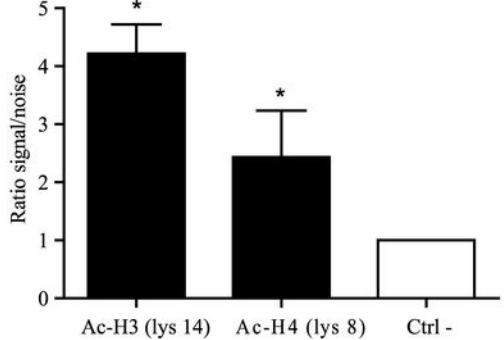


Supplemental-Figure 1. Localization of the transcription start site of the human P2Y$_2$R gene. A) Primer extension analysis revealed one major transcription start site using mRNA from human coronary artery endothelial cells (HCAECs) and some weak intensity products of sizes from 100-230 bp. A positive control (C+) from the Promega kit was included that produced a band at 84 bp. Hinf I digested markers (M) were used as standards to compare the sizes of the products. B) Localization of the TSS was determined by RLM-RACE using mRNA isolated from human Caco-2 intestinal epithelial cells. Electrophoretic analysis of the nested PCR amplification products was performed on a 2% (w/v) agarose gel using total Caco-2 modified cDNAs. The second step of amplification was done with two different inner reverse primers, hY2R1 and hY2R3, respectively. C) Sequence alignment of representative clones obtained after nested PCR with the 5'-UTR sequence of human P2Y$_2$R (NM_002564). Two positive clones obtained with hY2R3 amplification and three out of 12 from hY2R1 amplification were sequenced; all sequences were identical. We localized the TSS (+1 site) at position +72 in exon 1 of the previously described P2Y$_2$R sequence (NM_002564). A mutation at position +32, where a T is replaced with a C, was also observed (arrowhead). The translation start site (ATG) is shown in bold and the primers used for the nested PCR are underlined, namely hY2R1 and hY2R3.

SUPPLEMENTAL-FIGURE 2. Schematic representation of the P2Y$_2$R core promoter organization. Alignment of the core promoter sequences cloned from Caco-2 and HCAEC cells shows a homology of 99.2%. Sp1 and NFκB potential binding sites are indicated in red and purple, respectively. The green region represents 130bp of exon 1. Transcription start site found using primer extension assay is indicated in pink while transcription start site found using 5'RLM-RACE is indicated in orange. Mismatched base pairs are indicated by blue stars.



SUPPLEMENTAL-FIGURE 3. Transcriptional activation of the $P2Y_2R$ gene by NF-κB p65 is located in a chromosomic region of active transcription. Chromatin immunoprecipitation assays were performed on Caco-2 cell extracts. Chromatin was immunoprecipitated with a mouse IgG antibody (Ctrl -) or antibody against acetylated histone H3 (lys 14) and H4 (lys 8). Samples were verified by quantitative RT-PCR analysis with oligonucleotides amplifying the -221 bp to -155 bp region of the $P2Y_2R$ promoter and expressed as fold increase over negative control normalized to input. Representative results from 3 independent experiments are shown. Statistical analysis were performed an unpaired *t* test where *; $p < 0.05$ when compared to samples immunoprecipitated with the normal mouse IgG antibody.

Table 1. Oligonucleotides used for Real-Time PCR of $P2Y_2R$

Oligonucleotide	Sequence (5' → 3')
$P2Y_2R$ Upstream [a]	CGGTGGACTTAGCTCTGAGG
$P2Y_2R$ Downstream [a]	GCCTCCAGATGGGTCTATGA
$P2Y_2R$ Upstream [b]	ATGTCGCTTCAACGAGGACT
$P2Y_2R$ Downstream [b]	TGCCAGGTGAAACATGTAGG
COX-2 Upstream [a]	TGAAACCCACTCCAAACAC
COX-2 Downstream [a]	AACTGATGCGTGAAGTGCTG
TBP Upstream [a]	TGAGGATAAGAGAGCCACGAA
TBP Downstream [a]	GAGCACAAGGCCTTCTAACCT
TBP Upstream [b]	TATAATCCCAAGCGGTTTGC
TBP Downstream [b]	CCCACCATGTTCTGGATCTT

Oligonucleotide primers targeting: [a] *Homo sapiens*; [b] *Rattus norvegicus*

Table 2. $P2Y_2R$ primers for RLM-RACE assays

Oligonucleotide	Sequence (5' → 3')
hY2R1 (296 bp to 315 bp)[c]	GAGCATCCTGACCTGGAGAG
hY2R3 (371 bp to 390 bp)[c]	ATGGCACCTGGGATGGGGAT
hY2R4 (675 bp to 694 bp)[c]	CACCAACCTTTACTGCAGCA

[c] given position relative to GenBank NM_002564.2

Table 3. P2Y$_2$R promoter cloning

Oligonucleotide	Sequence (5' → 3')
P2Y$_2$/NheI (upstream)	GCTAGCCAGGAATGCAGTTTCCTCAGCTG
P2Y$_2$/BglII (downstream)	AGATCTCGCCTTGTTCCCTCCAGA

Underlined are the annealing sequences

Table 4. Oligonucleotides used for the construction of P2Y$_2$R promoter deletion mutants

Oligonucleotide	Sequence (5' → 3')
P2Y$_2$Δ-400bp (-359bp to -339bp)	AAGCTGGCTAGCGGCTGACCTGGCAAAACTTT
P2Y$_2$Δ-800bp (-794bp to -774bp)	AAGCTGGCTAGCAGCAGGAAGAAGGCAGACCT
P2Y$_2$Δ-1200bp (-1172bp tp -1152bp)	AAGCTGGCTAGCCACTATGGGCTGTGTGTCCA

Underlined are the annealing sequences

Table 5. Oligonucleotides used for the deletions of the putative NFκB-p65 binding motif

Oligonucleotide	Sequence (5' → 3')
P2Y$_2$ΔNBM1 (-290 to -260) [d]	GTGAGGGAGGAATTCCCTAGGCTTCTGGCC
P2Y$_2$ΔNBM2 (-192 to -162) [d]	CTTGTCGCTGTTAATTAAGAATTCGCGGGT
P2Y$_2$ΔNBM3 (-145 to -115) [d]	GGGCGGGTTGAATTCACGCGTAGGGTCAGT

[d] *Sense strands only are shown*

Table 6. ChIP assays

Oligonucleotide	Sequence (5' → 3')
$P2Y_2C$ Upstream (-221bp to -201bp)	*ACCGGTACAGACACGCTGAC*
$P2Y_2C$ Downstream (-123bp to -104bp)	*GGCGGACTTTTGACTGACC*

Table 7. List of oligonucleotides used for EMSA[a]

Oligonucleotide	Sequence (5'→ 3') [e]
NBM1 (-282 bp to -268 bp)	*CCGGGAGGCCTCCTT*
NBM2 (-184 bp to -169 bp)	*GGGCGGCGTCCCGGA*
NBM3 (-138 bp to -124 bp)	*GAGGGCGGTGCCAGG*

[e] *Sense strands only are shown. Mutated nucleotides are underlined.*