Endothelial cell culture
Human microvascular endothelial cells (HMEC-1) were a kind gift of Francisco Candal, Centers for Disease Control, Atlanta, GA. Human coronary artery endothelial cells (HCAEC) were obtained from Cell Applications, Inc. (San Diego, CA), and were grown in endothelial cell growth medium with supplement mix (Promocell, Heidelberg, Germany).

Transcriptional analysis
Confluent HMEC-1 were exposed to indicated amounts of TNF-α for indicated time periods. Total RNA was isolated from by using the NucleoSpin® RNA II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. RNA concentration was measured followed by cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Munich, Germany). Quantification of cDNA was performed by real-time RT-PCR (iCycler; Bio-Rad Laboratories Inc.). Primer sets and PCR conditions are summarized in the following table. Data are expressed as fold change in transcript over unstimulated cells ± SD, and have been calculated relative to internal housekeeping gene (β-actin). Results are derived from three to four experiments in each condition. Human primers of P2 receptors as well as human VCAM-1, ICAM-1, and IL-8 primers were used at 60°C annealing temperature, besides of P2RY2 (64°C), P2RY6 (63°C), and P2RX5 (63°C). The murine P2RY6 primer was used at 63°C annealing temperature. See Tables S1 and S2.

Microarray
Confluent HMEC-1 were exposed to 100ng/ml TNF-α for 4 and 24h. Three separate Petri dishes were pooled and total RNA was isolated. Probes were shipped on dry ice to Miltenyi Biotec. RNA samples were quality checked, and an Agilent Whole Human Genome Oligo Microarray was performed. Results of the Bioanalyzer run were visualized in a gel image and an electropherogram using the Agilent 2100 Bioanalyzer expert software. For the linear T7-based amplification step, 1 μg of each total RNA sample was used as starting material. To produce Cy3- and Cy5-labeled cRNA, the RNA samples were amplified and labeled using the Agilent Low RNA Input Linear Amp Kit (Agilent Technologies) following the manufacturer's protocol. Yields of cRNA and the dye-incorporation rate were measured with the ND-1000 Spectrophotometer (NanoDrop Technologies). The hybridization procedure was performed according to the Agilent 60-mer oligo microarray processing protocol using the Agilent Gene Expression Hybridization Kit (Agilent Technologies). Briefly, 825 ng of the corresponding Cy3- and Cy5-labeled fragmented cRNA were combined and hybridized overnight (17 hours, 65°C) to Agilent Whole Human Genome Oligo Microarrays 4 × 44K using Agilent's recommended hybridization chamber and oven. Finally, the microarrays were washed. Fluorescence signals of the hybridized Agilent Oligo Microarrays were detected using Agilent's DNA microarray scanner (Agilent Technologies). The Agilent Feature Extraction Software (FES) was used to read out and process the microarray image files. The software determines feature intensities and ratios (including background subtraction and normalization), rejects outliers, and calculates statistical confidences (p-values). For determination of differential gene expression FES derived output data files were further analyzed using the Rosetta Resolver gene expression data analysis system (Rosetta Biosoftware).

Microarray data were accepted and published on GEO NCBI, submission number 18102 and can be found at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE18102.

Immunoblotting experiments
HMEC-1 were grown to confluence and were exposed to indicated times of 10ng/ml TNF-α. Cells were lysed 45min at 4°C in RIPA buffer with freshly added Complete (protease inhibitors, Roche). After spinning at 13000g, the pellet was discarded. Protein concentration was measured with a BCA Protein Assay kit (Pierce, Bonn, Germany). The membranes were blocked over night and incubated with polyclonal rabbit anti-human $P2Y_6$ receptor antibody (SantaCruz, CA, USA) over night at 4°C. Subsequently membranes were incubated with the HRP-conjugated goat anti-rabbit IgG secondary antibody (SantaCruz, CA, USA) for 1h at room temperature. As a control for equal protein loading membranes were stripped (Restore Western Blot Stripping Buffer, Pierce, Bonn, Germany) and reprobed for β-actin using rabbit anti-human β-actin monoclonal antibody (Cell Signaling, Danvers, MA, USA) and the above mentioned secondary antibody.

Repression of inflammatory cytokine mRNA by MRS2578, a specific P2Y6 receptor antagonist
HMEC-1 were preincubated with 10μM MRS2578 for 30min. 10ng/ml TNF-α were added, and cells were lysed after indicated time points. mRNA levels of NF-κB–induced genes were determined using the primer sets summarized in the table above.

Intravenous LPS injection
C57BL/6 mice (Charles River Laboratories) and $P2Y_6^{-/-}$ mice on C57Bl/6 background weighing 20–25g were used. Experimental groups were matched in weight, gender, and age. Mice received an intraperitoneal injection of 3mg of ketamine (Ketamine ratiopharm®), 20μg of atropine and 1mg of xylazine. After disinfection, the jugular vein was dissected, and the mice received either 300μg LPS (E.coli O26:B6) or saline in the jugular vein. The vein was closed by bipolar coagulation, and the skin was sutured. For $P2Y_6$ receptor expression studies, mice were killed 24h after administration with an overdose of anesthetic and were exsanguiated with 10ml of 0.9%NaCl. Organs were immediately frozen in liquid nitrogen or added in 4% formaline for immunfluorescence. Animal experiments were approved by the local animal ethics committee (Regierungspraesidium Tübingen and Freiburg) and performed according to the respective guidelines.

Data analysis
Data were compared by Student's *t*-test. Values are expressed as the mean ± SD from at least three separate experiments.

Table S1. Transcriptional analysis

	Sense	Antisense
ß-actin, 190bp	GGAGAAAATCT GGCACCACA	AGAGGCGTACAG GGATAGCA
$P2RY_1$, 117bp	ACCTCTATGGC AGCATCTTG	CGCTGATACAGA TCGCATTC
$P2RY_2$, 100bp	CCGTGGCGCTC TACATCTTC	GAGGCCGCATAC AGTGCATC
$P2RY_4$, 134bp	ATCCTGCTGC CTGTGAGCTA	CTGACAATGC CAGGTGGAAC
$P2RY_6$, 110bp	CCACAGGCATC CAGCGTAAC	CAGCAGGAAGCC GATGACAG
$P2RY_{11}$, 133bp	GÀGCATGGCAGC CAACGTCT	CACGGCCACCAGG AACTCAA
$P2RY_{12}$, 146bp	CAGATGCCACT CTGCAGGTT	AACACCAGTCTGTG CACCAG
$P2RY_{13}$, 106bp	CCAGCTCCTCCAC CTTCATC	GGTGCCAGGTGTGA GTCAGA
$P2RY_{14}$, 156bp	GGCCTCTGCCT TCAGAAGTT	CCTGACACTCCA TTGAGTAG
$P2RX_1$, 102bp	GGCTACGT GGTGCAAGAGTC	GTGCCAGTC CAGGTCACAGT
$P2RX_2$, 116bp	GCTTCACAGA GCTCGCACAC	GTCAAGCCT CCGGAAGT
$P2RX_3$, 140bp	CCAAGTCGGTG GTTGTGAAG	CGAGGACTCAA TGGCTGTGT
$P2RX_4$, 120bp	GGATGTGGCGGA TTATGTGA	GTGGTCGCATCT GGAATCTC
$P2RX_5$, 120bp	TGGCGTACCTGGT CGTATGG	GATCCGAGGTGT TGGTGAAG
$P2RX_6$, 197bp	ACTTCAGGACAGCCACTCAC	CAGCAGTAGCAGGTCACAGA
$P2RX_7$, 166bp	CAAGAGCAGCG GTTGTGTCC	GGCACCAGGC AGAGACTTCA
VCAM, 229bp	GACCACATCTACGCTGACAA	GTCTATCTCCAG CCTGTCAA
ICAM, 117bp	AACCAGAGCCA GGAGACACT	GGCCTCACACTT CACTGTCA
IL-8, 171bp	CTCTTGGCAGC CTTCCTGAT	TTCTGTGTTGGC GCAGTGTG

Table S2. Transcriptional analysis

	Sense	Antisense
ß-actin, 107bp	GGCTCCTAGCA CCATGAAGA	ACATCTGCTGGA AGGTGGAC
$P2Y_6$, 265bp	AGGCGGTTGCT GTGTCAGAG	CCTATGCTCGG AGAGTCTGT

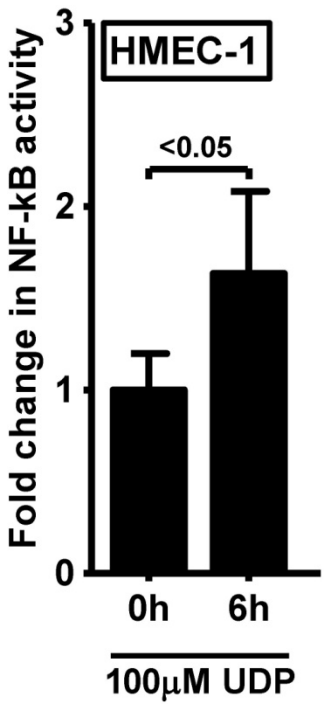
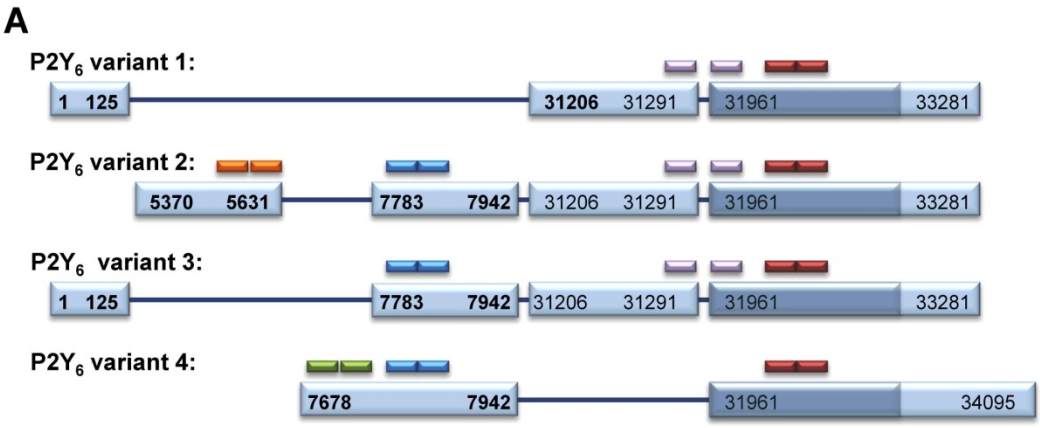


Figure S1. Effect of $P2Y_6$ receptor agonist UDP on endothelial NF-κB activity.
Human microvascular endothelia (HMEC-1) were transfected with 0,25μg of either NF-κB promoter reporter (Clontech, Mountain View, CA, USA) or control pGL3 vector. Cells were exposed to the $P2Y_6$-receptor agonist UDP and NF-κB activity was determined relative to the total protein concentration after 6h of incubation (n=3).



B

	Sense	Antisense
V1-4, 110bp	CCACAGGCATCCAGCTAAC	CAGCAGGAAGCCCGATGACAG
V1-3, 185bp	ACCTCTGCCAGAAGAACCAT	TTGAAGTTCTCGCGGTAGAC
V2, 132bp	TCGGAAGAACTGGTTCTGTG	CAGATGACAGGCAAGCAGAA
V2-4, 100bp	CAGAACATTGCACGCGACAG	TCGCTTGCAGTCCGTGAAGT
V4, 64bp	CTTCAGGGTTCTCGGGATTC	AACTTGCCAAGCCGATGACAG

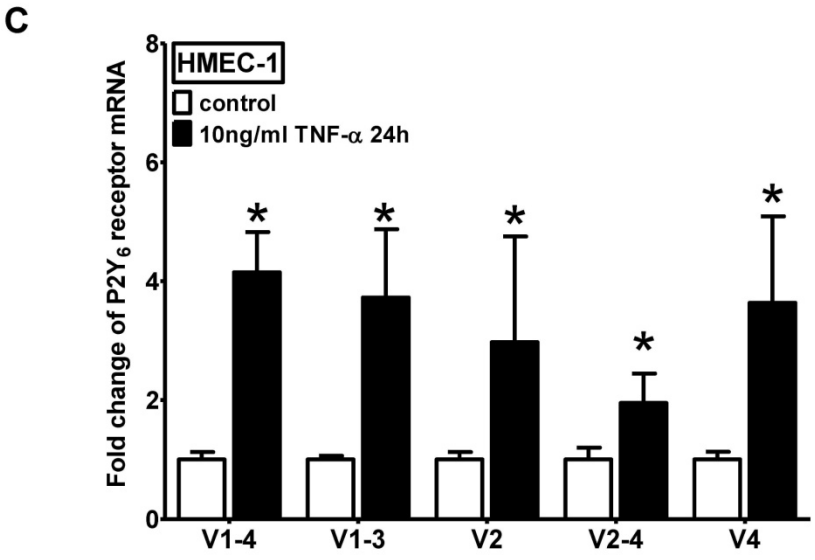


Figure S2. $P2Y_6$ receptor transcript variants and expression.
(A) Four different variants for the $P2Y_6$ receptor have been previously described in human. (B) To assess selective transcriptional responses of the different variants, primers for the four different variants of $P2Y_6$ receptor were designed. (C) Transcriptional responses of different variants to inflammatory stimulation. Data were calculated relative to the internal housekeeping gene β-actin and are expressed as mean fold change compared to the control ± SD (n= 3–4; * $p<0.05$).

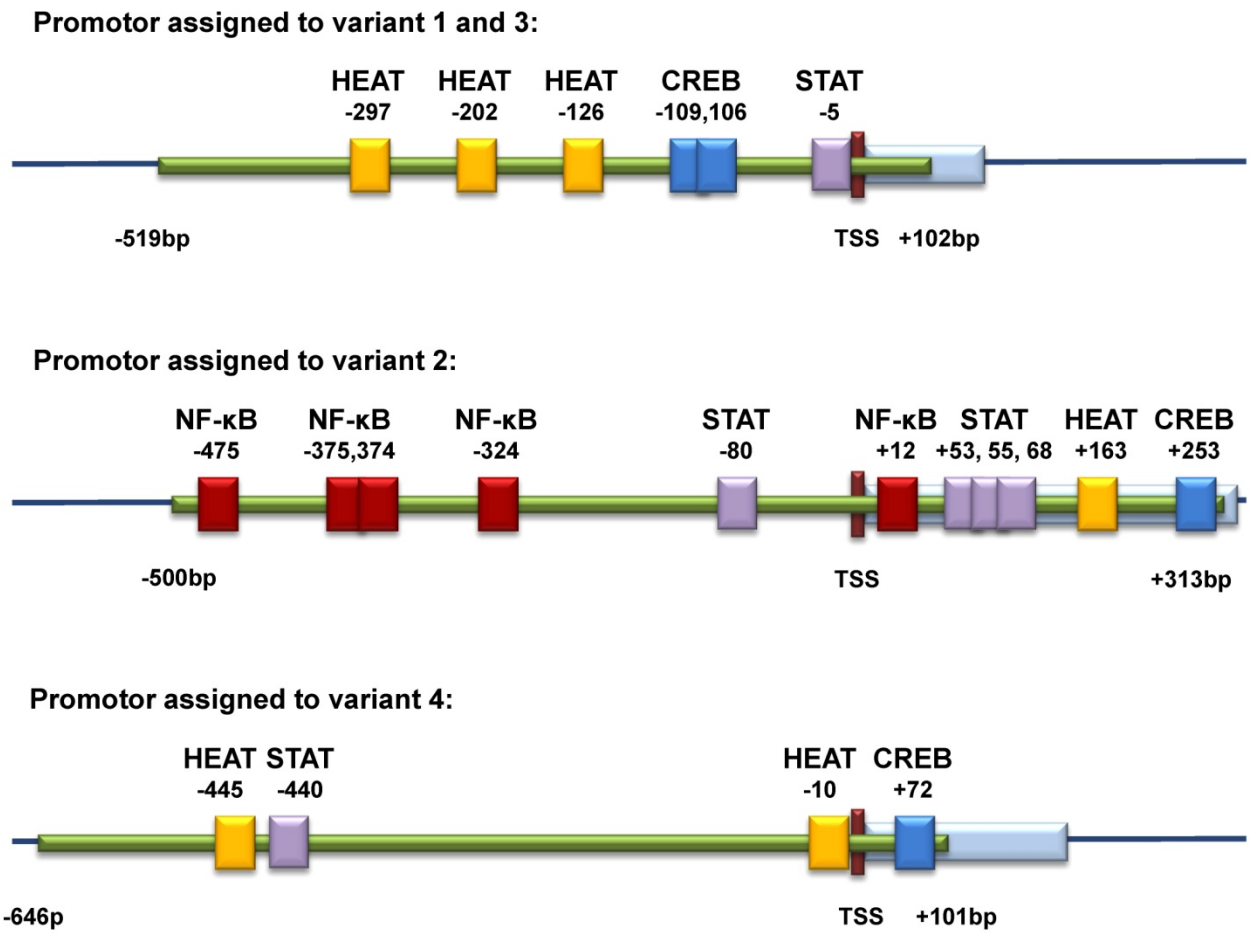


Figure S3. $P2Y_6$ receptor promotor regions.
$P2Y_6$ receptor promoter regions were analyzed for potential transcription factor binding sites for TNF-α, LPS, and IL-1α induced transcription factors. Only the promoter of transcription variant 2 contained NF-κB binding sites. Study of the 3 genomatix proposed variants of $P2Y_6$ promotors revealed the following shared transcripton factor binding regions: HAND (Twist subfamily of class B bHLH transcription factors), MYOD (Myoblast determining factors), HOXH (HOX-MEIS1 heterodimers. Meis1a/b and Hoxa9 form heterodimeric binding complexes on target DNA), HOXF (Factors with moderate activity to homeo domain consensus sequence), E4FF (Ubiquitous GLI-Krueppel like zinc finger involved in cell cycle regulation), BRN5 (Brn-5 POU domain factors), BRNF (Brn POU domain factors), FKHD (Fork head domain factors), SORY (SOX/SRY-sex/testis determinig and related HMG box factors), OCT1 (Octamer binding protein), GREF (Glucocorticoid responsive and related elements), NF1F (Nuclear factor 1), ABDB (Abdominal-B type homeodomain transcription factors), MAZF (Myc associated zinc fingers), TALE (TALE homeodomain class recognizing TG motifs), HEAT (Heat shock factors), PAX6 (PAX-4/PAX-6 paired domain binding sites), EVI1 (EVI1-myleoid transforming protein), NEUR (NeuroD, Beta2, HLH domain), KLFS (Krueppel like transcription factors), ZBPF(Zinc binding protein factors), EGRF (EGR/nerve growth factor induced protein C & related factors), GLIF (GLI zinc finger family), MAZF (Myc associated zinc fingers), RXRF (RXR heterodimer

binding sites), ETSF (RXR heterodimer binding sites), CREB (cAMP-responsive element binding proteins), RBPF (RBPJ-κ), STAT (Signal transducer and activator of transcription), GCMF (Chorion-specific transcription factors with a GCM DNA binding domain), GRHL (Grainyhead-like transcription factors), and NRSF (Neuron-restrictive silencer factor). Of these shared transcription factor binding sites, CREB, STAT, and HSPs are known to be activated through TNF-α, LPS, and IL-1α. Hoxa9 has shown to be inactivated through LPS and TNF-α. Data about the other transcription factors and their activation through TNF-α, LPS, and IL-1α were barely found.