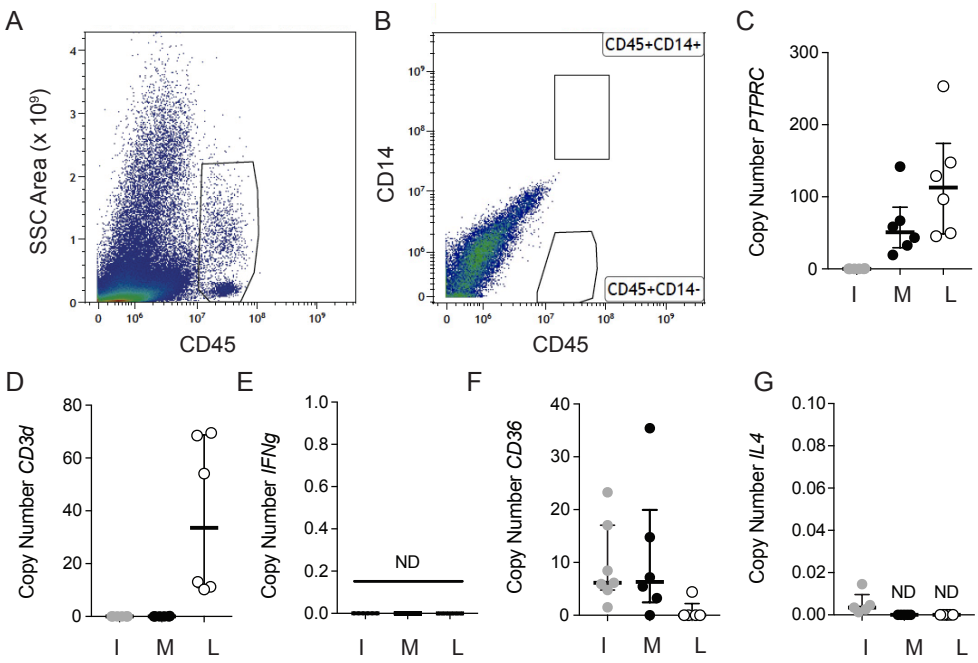
SUPPLEMENTARY MATERIAL

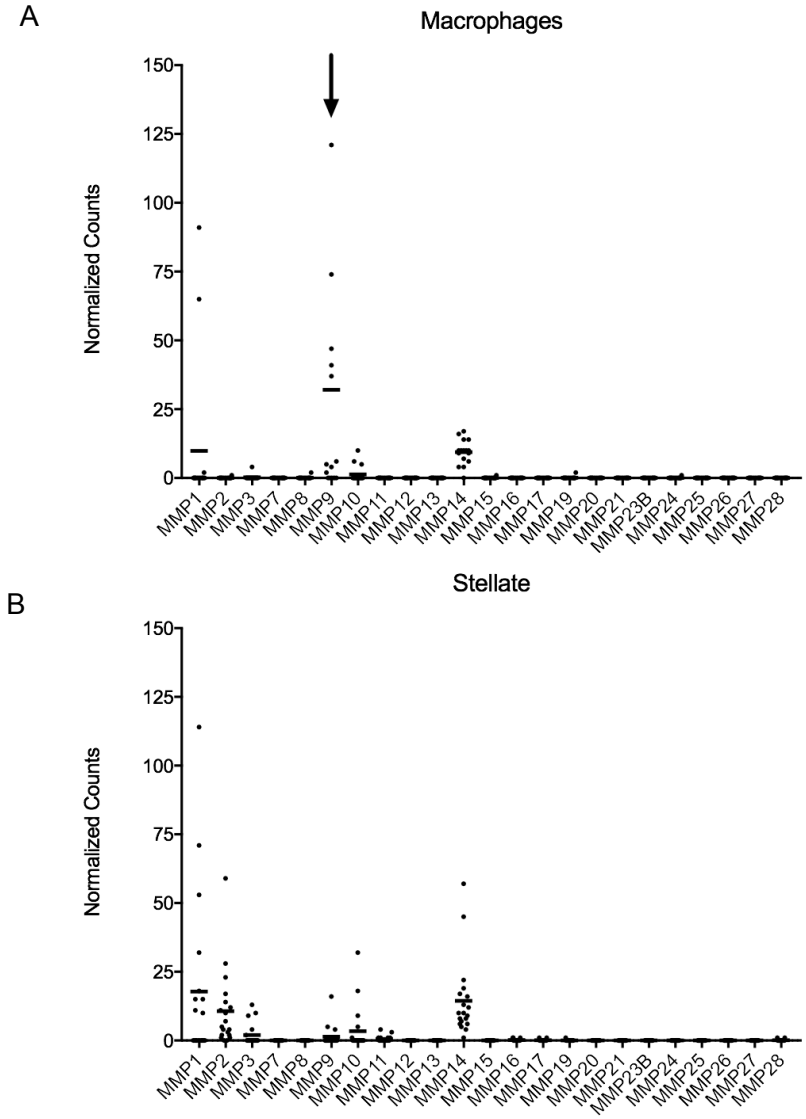
Figure S1



Sorting of unique human islet leukocytes populations

A: Gating on SSC for granularity difference in all leukocytes CD45+ (black box, right panel). *B*: No antibody control. *C-D*: Validation of expression from FACS sorted mixed endocrine cells (CD45-, CD14-), macrophages (CD45+, CD14+), and lymphocytes (CD45+, CD14-). Quantification of mRNA levels used for validation of individual population identity shown in; leukocytes (macrophages and lymphocytes) *PTPRC* (ie., CD45) (*C*) and lymphocytes *CD3d* (*D*). Values are from n = 6 independent donor isolations (median ± interquartile range; *P < 0.05 ANOVA followed by Tukey's test for multiple comparisons). *E-G*: Differences in gene expression between endocrine cells, macrophages, and lymphocytes for the following genes; *IFNγ* (*E*)*, CD36* (*F*) *and IL4* (*G*). Values are from n = 6 independent donor isolations (median ± interquartile range; *P < 0.05 ANOVA followed by Tukey's test for multiple comparisons).

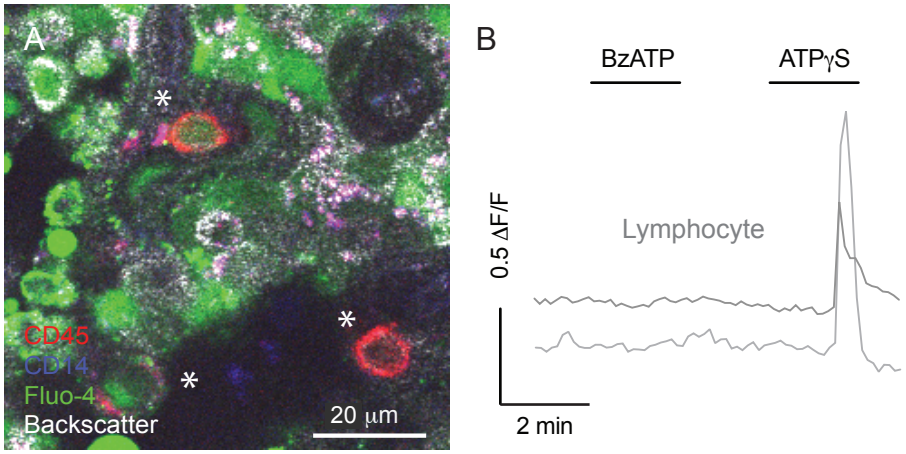
Figure S2



Matrix metalloproteinase expression in human pancreas cell subsets

A and *B*: Differences in macrophage (*A*) and activated stellate cell (*B*) gene counts in a subset of randomized single cells sorted from 4 human donors.

Figure S3

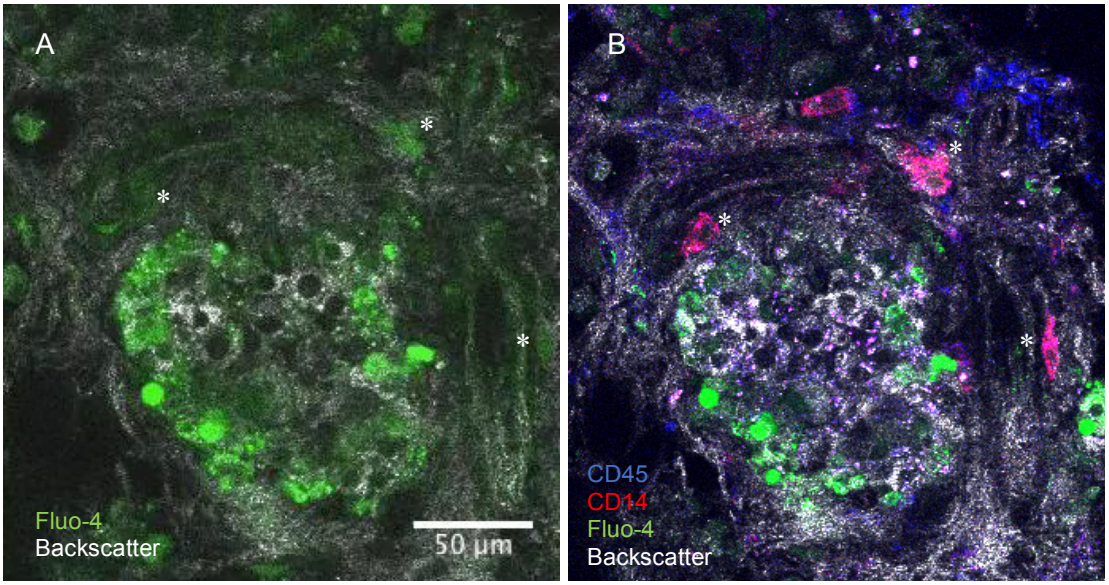


Imaging of human tissue lymphocytes (CD45+, CD14-) in the pancreatic tissue microenvironment

A: Confocal image taken during a live video recording of a living pancreatic tissue slice. The pancreatic tissue slice was incubated with the $[Ca^{2+}]_i$ indicator Fluo-4 (green), as well as CD14 (blue), and CD45 (red). The reflective cellular structures (islet backscatter) represent endocrine tissue (grey). Stars (*) denoting lymphocytes (CD45+, CD14-) were loaded with Fluo-4 (green).

B: Representative traces of individual pancreatic lymphocytes showing $[Ca^{2+}]_i$ increases when exposed to ATPγS (100 M) but not to BzATP (10 M).

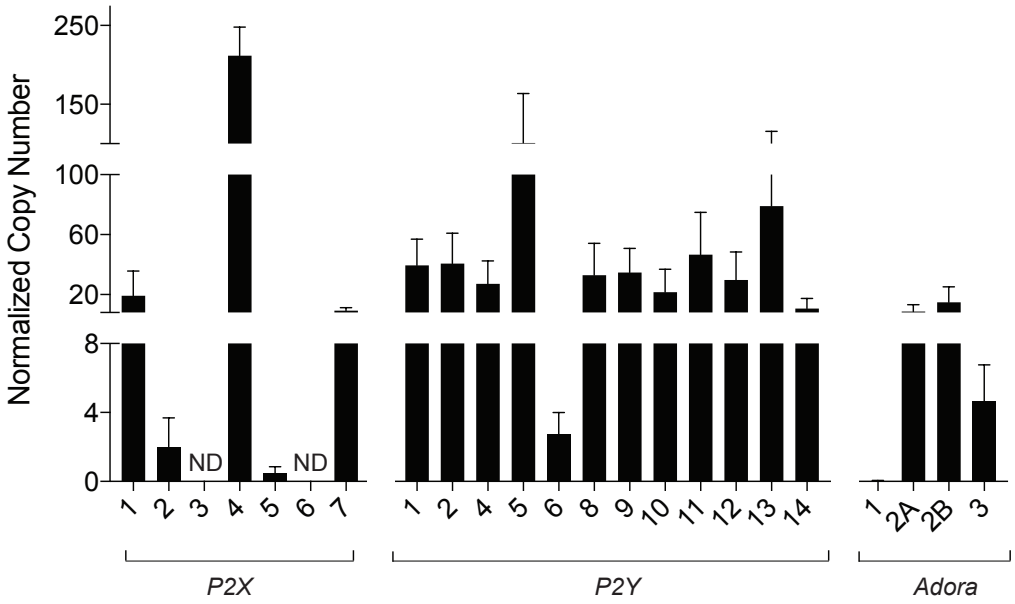
Movie S4



Live $[Ca^{2+}]_i$ imaging of macrophage responses in a living human pancreatic tissue slice.

A: Still frame image of a video recording with the $[Ca^{2+}]_i$ indicator Fluo-4 (green). The reflective cellular structures (islet backscatter) represent endocrine tissue (grey). Stars (*) denoting macrophages (CD45+, CD14+) were loaded with Fluo-4 (green). Video time starts at 00:00 displayed in minutes where 01:00 = 1 minute. *B*: Confocal image taken during a live video recording of a living pancreatic tissue slice. The pancreatic tissue slice was incubated with the $[Ca^{2+}]_i$ indicator Fluo-4 (green), as well as CD14 (red), and CD45 (blue). The reflective cellular structures (islet backscatter) represent endocrine tissue (grey). Stars (*) denoting macrophages (CD45+, CD14+) were loaded with Fluo-4 (green).

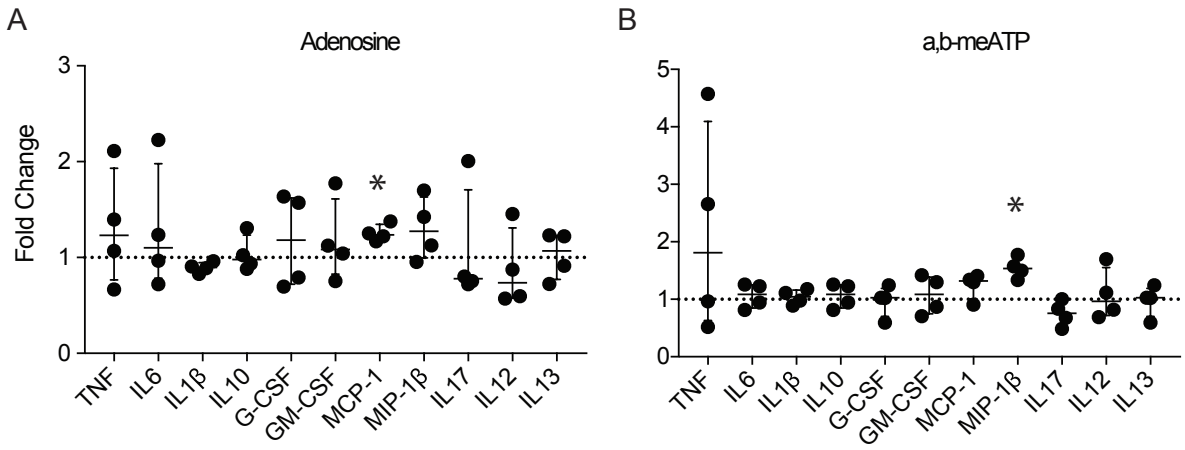
Figure S5



Purinergic gene expression in human islet macrophages

Gene expression of purinergic (*P2RX, P2RY and Adora*) receptors found in human islet macrophages from n = 7 independent donors. Data are presented as mean +/- SEM.

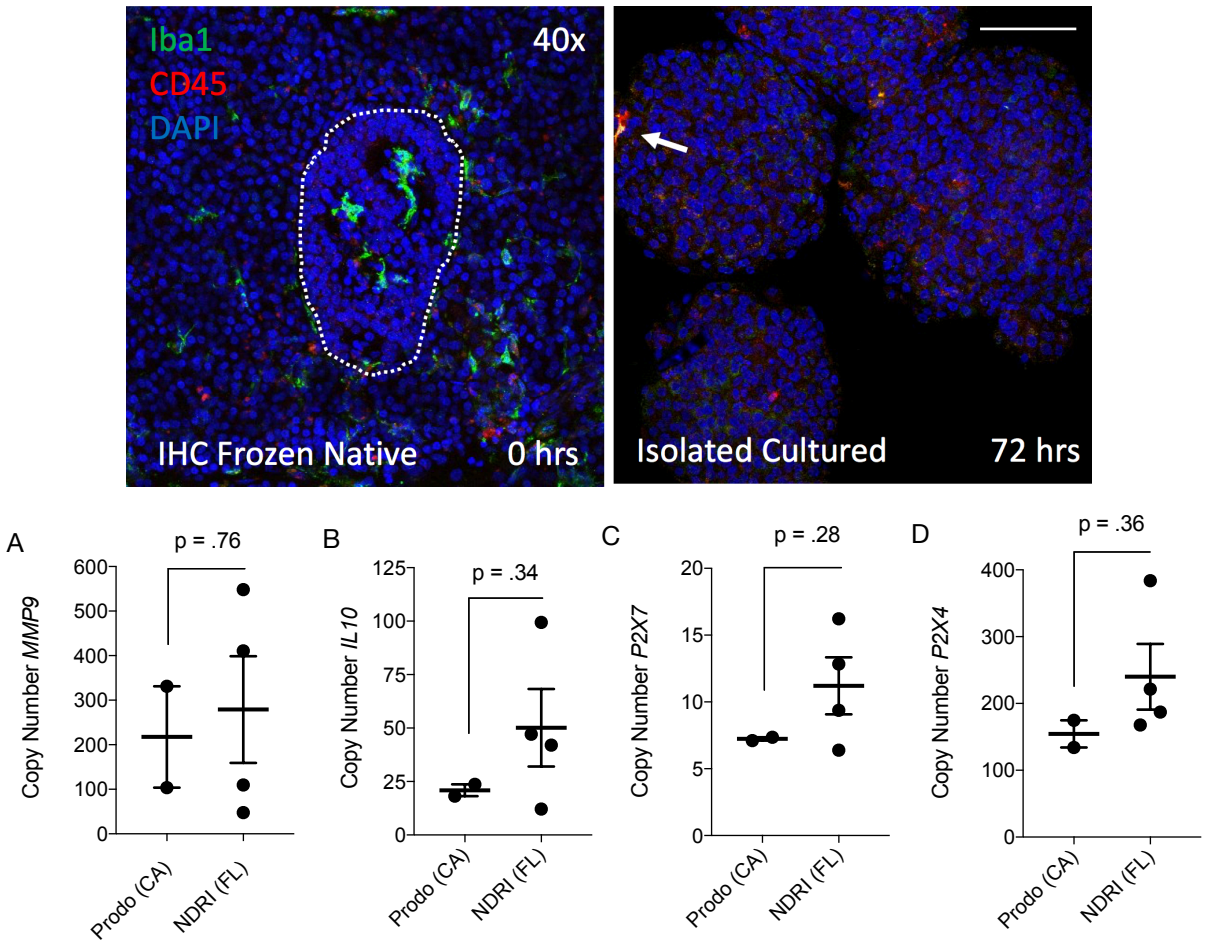
Figure S6



Changes in macrophages secretory products during stimulation of purinergic signals

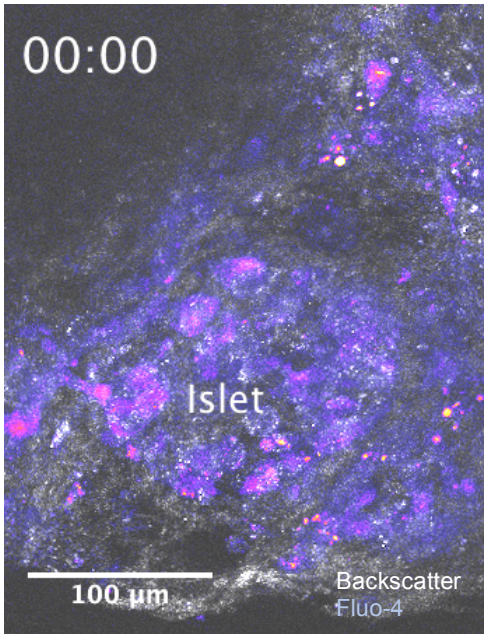
A and *B*: Cytokine secretion from isolated human islets. Concentrations of drugs (Adenosine 100 M), (α,β-meATP 1 M). *P < 0.05, One sample t-test normalized to the control with a hypothetical value of 1, followed by Wilcoxon Signed Rank Test. Data are presented as mean +/- interquartile range.

Figure S7



A and *B*: Isolating pancreatic islets induces loss of islet leukocytes. Macrophage (iba1, green) and leukocyte (CD45, red) markers are present in islets in slices but not in isolated islets cultured for 72 hours (B). Dotted line in A denotes pancreatic islet. Scale bar = 40 m, applies to A and B. *C*: No differences could be detected in macrophage gene expression between islets from Prodo in California and the local facility at the Diabetes Research Institute. There were no differences in gene expression (CT $2^{-[\text{CT gene(x)} - \text{CT (housekeeping)}]}$) for *MMP9*, *IL10*, *P2X7* and *P2X4*. For islets shipped from California, a "rest" period was added after shipping for a total 16-24 hours. Islets obtained in Miami were incubated at 22°C for 16-24 hours. Notice that sample sizes were small.

Movie S8



Live imaging of human islet lymphocyte translational movement via a vasculature scaffold. Still frame image of a video recording of a maximal projection image taken during a live video recording of a living pancreatic tissue slice. The $[Ca^{2+}]_i$ indicator Fluo-4 is displayed in a pseudocolor scale where the amount of $[Ca^{2+}]_i$ is related to the fluorescent intensity. The reflective cellular structures (islet backscatter) represent endocrine tissue (grey). Stars (*) denoting lymphocytes (CD45+, CD14-) were identified *a priori*, based on antibody labeling. Video time starts at 00:00 displayed in minutes where 01:00 = 1 minute.

Figure S9

Donor – Islet Isolation	Age	Gender	Height	Weight	BMI	COD	Source	Diabetic
HP2297	56	F	67	170	26.6	Stroke	DRI	No
HP2298	49	M	70	181	26	ICH/Stroke	DRI	No
HP18232-CTRL	33	F	68	200	30.3	Head Trauma	Prodo	No
HP2299	49	M	65	169.6	28.3	Anoxia	DRI	No
HP2303	48	M	69	165	24.4	Head Trauma	DRI	No
HP-18310-01	38	M	69	190	28	Head Trauma	Prodo	No
HP1695	55	F	x	x	32	CNS Tumor	DRI	No
HP1697	50	M	x	x	27	Brain Tumor	DRI	No
HP18212-01T2D	40	M	69	185	27.7	Stroke	Prodo	Yes
HP18243-01T2D	51	M	65	228	37.1	Anoxia	Prodo	Yes
HP-18275-01T2D	30	F	64	233	40.1	Asthma	Prodo	Yes
HP-19051-01T2D	53	M	65	190	30.1	Head Trauma	Prodo	Yes
Donor - Tissue Slices								
HP6462	13	F	x	x	15.2	Anoxia	nPOD	No
HP2298	49	M	70	181	26	ICH/Stroke	DRI	No
HP2299	49	M	65	169.6	28.3	Anoxia	DRI	No
HP2303	48	M	69	165	24.4	Head Trauma	DRI	No
HP6468	16	M	x	x	15.8	Anoxia	nPOD	No
Donor - IHC								
HP2111A	31	M	75	254	31.7	Head Trauma	NE Organ Bank	No
HP2096A	51	F	63	145	25.7	Head Trauma	NE Organ Bank	No
HP2102A	43	F	62	129.3	23.6	Stoke	Live Share of Carolina	No
HP2116A	53	M	69	196	28.9	Head Trauma	NDRI	No
HP2117A	28	M	74	165.3	21.1	Head Trauma	IIAM	No
HP2119A	69	F	64	204	35	Stroke	Live Link of Florida	Yes

Figure S10

Surface Marker	Citation
CD11b	Butcher et al., 2014
CD11c	Butcher et al., 2014
MHC-II	Ehses et al., 2007
CD68	Ehses et al., 2007; Richardson et al., 2009; Kamata et al., 2014, Eguchi et al., 2016
CD14	**
CD45	Butcher et al., 2014, **
CD86	Butcher et al., 2014
CD163	Ehses et al., 2007; Kamata et al., 2014
CD204	Kamata et al., 2014
CD206	**
** identified in current manuscript	

Figure S11

Gene ID	Probe	Gene ID	Probe
p2rx1	Hs01039860_g1	ADORA2B	Hs00386497_m1
p2rx2	Hs04176268_g1	ADORA3	Hs00181232_m1
p2rx3	Hs01125554_m1	IL1B	Hs01555410_m1
p2rx4	Hs00902156_g1	TNF	Hs00174128_m1
p2rx5	Hs01112471_m1	IFNy	Hs00989291_m1
p2rx6	Hs01003997_m1	IL6	Hs00174131_m1
p2rx7	Hs00175721_m1	iNOS	Hs01075529_m1
p2ry1	Hs00704965_s1	IL-10	Hs00961622_m1
p2ry2	Hs01856611_s1	IL-4	Hs00174122_m1
p2ry4	Hs00267404_s1	ARG	Hs00163660_m1
p2ry5	Hs00271758_s1	PTPRC	Hs04189704_m1
p2ry6	Hs00366312_m1	CSF1R	Hs00911250_m1
p2ry8	Hs01938524_s1	CD3D	Hs00174158_m1
p2ry9	Hs00271072_s1	CD19	Hs01047413_g1
p2ry10	Hs00274326_s1	GAPDH	Hs02786624_g1
p2ry11	Hs00267414_s1	ITGAM	Hs00167304_m1
p2ry12	Hs01881698_s1	MMP9	Hs01548727_m1
p2ry13	Hs00256749_s1	MMP2	Hs00957562_m1
p2ry14	Hs01848195_s1	AMYLIN	Hs00169095_m1
ADORA1A	Hs00181231_m1	CD36	Hs00354519_m1
ADORA2A	Hs00169123_m1	INS	Hs00355773_m1