

Supplementary data to:

Original article:

REMIFENTANIL DOES NOT AFFECT HUMAN MICROGLIAL IMMUNE ACTIVATION IN RESPONSE TO PRO-INFLAMMATORY CYTOKINES

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<https://dx.doi.org/10.17179/excli2022-5667>

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Supplementary Table 1: Raw data concerning Figure 1A and Table 1. C20 microglial cells were stimulated with a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ (TII), each used at the same concentration of 50 pg/ml in the cocktail. Cells were treated for different times, from 2 h up to 24 h. After the incubation period, the concentration of interleukin 6 (IL-6, pg/ml) in the incubation medium was measured by a specific ELISA assay. The amounts of IL-6 measured in the incubation medium after different times of incubation are reported in this table. Data are shown as pg/ml and were obtained from 3 independent experiments, each including 4 biological replicates per treatment. ND = not detectable. The ND values were excluded from the analysis.

Treatments	Experiment 1				Experiment 2				Experiment 3			
	IL-6 concentration (pg/ml)				IL-6 Concentration (pg/ml)				IL-6 Concentration (pg/ml)			
Control (2 h)	22.16	26.85	22.23	18.35	17.67	19.03	33.70	33.86	42.05	32.94	37.45	44.02
TII (2 h)	51.46	79.32	64.09	36.15	68.17	62.44	161.75	183.05	166.49	188.25	218.53	201.96
Control (4 h)	22.38	23.85	19.63	36.18	34.06	32.02	34.96	38.24	39.76	37.96	37.96	42.10
TII (4h)	224.85	234.17	213.50	236.83	269.97	252.75	578.89	500.57	510.41	551.92	624.61	495.40
Control (6 h)	27.88	21.86	20.37	20.45	20.37	22.60	46.32	47.93	43.38	52.65	49.75	61.26
TII (6 h)	318.27	321.59	314.30	340.84	ND	348.16	1230.60	1178.61	1092.12	1290.25	1117.32	1174.12
Control (8 h)	52.86	24.37	25.61	20.75	26.85	30.21	46.49	43.55	52.15	46.97	48.53	48.65
TII (8 h)	352.15	296.43	316.28	347.49	323.58	349.49	2187.18	2224.17	2181.98	2287.14	2839.70	1860.07
Control (16 h)	10.41	8.95	9.90	9.27	10.47	11.77	ND	11.23	11.09	12.85	6.33	10.81
TII (16 h)	2721.11	2703.31	1900.93	2000.71	1150.10	1084.90	1037.36	929.32	1173.02	985.77	825.87	507.40
Control (24 h)	26.05	17.06	16.45	18.50	25.10	24.00	13.77	13.18	15.27	13.45	16.12	19.76
TII (24 h)	599.41	588.37	589.84	552.01	713.13	471.51	3888.00	3653.95	3210.97	4027.18	3818.32	3737.74

Supplementary Table 2: Raw data concerning Figure 1B. C20 microglial cells were stimulated with a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ (TII), each used at the same concentration of 50 pg/ml in the cocktail. Cells were treated for different times, namely 4 h, 16 h and 24 h. After the incubation period, the mRNA expression levels of the interleukin-6 (IL-6) were quantified through real-time qPCR using the comparative quantitation method based upon the $-\Delta\Delta C_t$ method. Relative mRNA concentrations were calculated from the take-off point of reactions (threshold cycle, Ct), using the expression level of the Control as Calibrator (Cal.) and the expression level of β -actin as normalizing gene. Ct values were obtained at fix threshold of 0.15, in order to combine data from different experiments. Each sample was assayed in triplicate. Raw data presented in the table include Ct values, Ct average and Ct standard deviation (SD), relative quantitation to calibrator (Rel. Quant. to Cal.) and melting temperature (Tm) of the amplified products, for both IL-6 and β -actin mRNA. If Ct SD was > 0.5, one outlier value was excluded from the analysis (please refer to numbers in bold). The last column includes the normalized expression values (Normalized Rel. Quant. to Cal.) for the IL-6 mRNA used to generate Figure 1B and reported in the graph as fold variation versus Control at different times.

Experiment (number/biological replicates)	Treatment	Technical Replicates	mRNA IL-6					mRNA β -actin (Normalizing gene)					$-\Delta\Delta C_t$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (4 h)	1	28.73	28.91	0.20	1.13	79.85	15.07	15.01	0.40	0.96	86.6	1.10
		1	28.88	28.91	0.20	1.02	79.85	15.38	15.01	0.40	0.77	86.6	0.99
		1	29.12	28.91	0.20	0.86	79.83	14.58	15.01	0.40	1.35	86.6	0.84
	TII (4 h)	2	24.43	24.89	0.48	22.32	79.83	14.81	15.06	0.22	1.15	86.6	22.86
		2	24.86	24.89	0.48	16.56	79.83	15.11	15.06	0.22	0.93	87.1	16.97
		2	25.39	24.89	0.48	11.47	79.85	15.25	15.06	0.22	0.85	87.1	11.75
2	Control (4 h)	3	29.70	29.30	0.35	0.76	79.83	17.24				86.3	
		3	29.06	29.30	0.35	1.18	79.85	15.50	15.30	0.28	0.87	86.3	1.17
		3	29.15	29.30	0.35	1.11	79.83	15.10	15.30	0.28	1.15	86.8	1.10
	TII (4 h)	4	23.11	23.12	0.06	73.18	79.70	15.36	15.54	0.43	0.96	87.2	84.05
		4	23.18	23.12	0.06	69.71	80.25	16.03	15.54	0.43	0.60	87.15	80.07
		4	23.06	23.12	0.06	75.76	80.20	15.23	15.54	0.43	1.05	87.15	87.02
3	Control (4 h)	5	28.39	28.29	0.11	0.94	79.83	17.18	17.13	0.08	0.97	86.83	0.93
		5	28.31	28.29	0.11	0.99	79.83	17.18	17.13	0.08	0.97	86.8	0.99
		5	28.18	28.29	0.11	1.08	79.83	17.03	17.13	0.08	1.07	86.8	1.08
	TII (4 h)	6	26.33	26.28	0.09	3.90	79.70	17.95	17.83	0.24	0.57	86.8	6.26
		6	26.17	26.28	0.09	4.36	80.25	17.55	17.83	0.24	0.75	86.75	6.99
		6	26.34	26.28	0.09	3.87	80.20	17.98	17.83	0.24	0.55	87.2	6.22
4	Control (4 h)	7	26.84	26.65	0.18	0.88	79.60	15.91	15.63	0.27	0.82	86.60	0.87
		7	26.64	26.65	0.18	1.01	80.10	15.60	15.63	0.27	1.02	86.60	1.00
		7	26.47	26.65	0.18	1.13	80.10	15.38	15.63	0.27	1.19	86.60	1.12
	TII (4 h)	8	22.47	22.39	0.07	18.13	80.10	14.67	14.79	0.28	1.95	87.10	10.00
		8	22.38	22.39	0.07	19.29	80.10	14.59	14.79	0.28	2.06	87.10	10.65
		8	22.32	22.39	0.07	20.11	80.10	15.11	14.79	0.28	1.43	87.10	11.10

Supplementary Table 2: Raw data concerning Figure 1B (cont.)

Experiment (number/biological replicates)	Treatment	Technical Replicates	mRNA IL-6					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (16 h)	1	27.69	27.70	0.05	1.01	79.85	15.51	15.45	0.05	0.96	86.6	1.01
		1	27.66	27.70	0.05	1.03	79.85	15.41	15.45	0.05	1.03	86.6	1.03
		1	27.76	27.70	0.05	0.96	79.85	15.44	15.45	0.05	1.01	86.6	0.96
	TII (16 h)	2	22.71	22.69	0.03	31.85	79.85	17.30	17.16	0.23	0.28	86.6	102.82
		2	22.66	22.69	0.03	32.98	79.83	16.89	17.16	0.23	0.37	86.6	106.45
		2	22.71	22.69	0.03	31.85	79.83	17.28	17.16	0.23	0.28	86.6	102.82
2	Control (16 h)	3	27.95	27.71	0.21	0.84	80.35	14.93	15.05	0.19	1.08	86.83	0.84
		3	27.57	27.71	0.21	1.10	79.83	14.94	15.05	0.19	1.08	87.3	1.09
		3	27.60	27.71	0.21	1.08	79.83	15.27	15.05	0.19	0.86	87.3	1.07
	TII (16 h)	4	22.69	22.66	0.21	32.37	79.85	15.98	16.05	0.14	0.52	87.25	64.70
		4	22.85	22.66	0.21	28.97	79.83	16.21	16.05	0.14	0.45	87.3	57.91
		4	22.43	22.66	0.21	38.76	79.70	15.96	16.05	0.14	0.53	87.2	77.47
3	Control (16 h)	5	28.20	27.95	0.22	0.84	80.33	16.44	16.43	0.13	1.00	86.85	0.84
		5	27.79	27.95	0.22	1.11	80.33	16.56	16.43	0.13	0.92	86.83	1.11
		5	27.85	27.95	0.22	1.07	79.83	16.30	16.43	0.13	1.10	86.83	1.07
	TII (16 h)	6	25.31	25.28	0.10	6.22	79.83	16.69	16.71	0.12	0.84	86.8	7.50
		6	25.36	25.28	0.10	6.01	79.83	16.60	16.71	0.12	0.89	86.8	7.24
		6	25.17	25.28	0.10	6.85	80.25	16.83	16.71	0.12	0.76	86.8	8.26
4	Control (16 h)	7	29.77	29.69	0.24	0.95	80.35	16.44	16.80	0.38	1.28	86.85	0.93
		7	29.89	29.69	0.24	0.87	79.83	16.75	16.80	0.38	1.03	86.8	0.85
		7	29.42	29.69	0.24	1.21	79.83	17.20	16.80	0.38	0.76	86.8	1.18
	TII (16 h)	8	22.93	23.15	0.20	108.63	80.33	16.76	16.70	0.05	1.03	86.83	101.55
		8	23.21	23.15	0.20	89.47	80.33	16.67	16.70	0.05	1.09	86.8	83.64
		8	23.32	23.15	0.20	82.90	80.33	16.67	16.70	0.05	1.09	86.8	77.50

Supplementary Table 2: Raw data concerning Figure 1B (cont.)

Experiment (number/biological replicates)	Treatment	Technical Replicates	mRNA IL-6					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (24 h)	1	28.07	27.93	0.14	0.91	79.85	18.53	18.70	0.30	1.13	86.6	0.90
		1	27.79	27.93	0.14	1.10	80.33	18.52	18.70	0.30	1.13	87.1	1.09
		1	27.94	27.93	0.14	1.00	80.33	19.05	18.70	0.30	0.78	87.1	0.98
	TII (24 h)	2	21.28	21.16	0.12	100.66	79.85	20.16	20.00	0.15	0.36	86.6	247.47
		2	21.14	21.16	0.12	110.92	79.85	19.85	20.00	0.15	0.45	86.6	272.69
		2	21.05	21.16	0.12	118.06	79.85	20.00	20.00	0.15	0.41	86.6	290.24
2	Control (24 h)	3	26.78	26.49	0.26	0.82	80.35	16.68	16.51	0.19	0.89	87.35	0.81
		3	26.40	26.49	0.26	1.06	80.25	16.53	16.51	0.19	0.98	87.7	1.06
		3	26.29	26.49	0.26	1.15	80.20	16.31	16.51	0.19	1.15	87.65	1.14
	TII (24 h)	4	21.31	21.05	0.23	36.25	80.33	16.79	16.83	0.11	0.82	87.3	45.28
		4	20.97	21.05	0.23	45.89	79.83	16.75	16.83	0.11	0.84	87.3	57.31
		4	20.86	21.05	0.23	49.52	79.85	16.95	16.83	0.11	0.74	87.25	61.85
3	Control (24 h)	5	25.89	25.89	0.08	1.00	80.33	17.04	17.04	0.05	1.00	86.85	1.00
		5	25.80	25.89	0.08	1.06	80.25	17.09	17.04	0.05	0.97	86.75	1.06
		5	25.97	25.89	0.08	0.94	80.20	16.99	17.04	0.05	1.04	86.7	0.94
	TII (24 h)	6	23.32	23.10	0.19	5.92	80.33	16.51	16.34	0.15	1.44	86.83	3.64
		6	22.99	23.10	0.19	7.45	79.83	16.24	16.34	0.15	1.74	86.83	4.58
		6	22.98	23.10	0.19	7.50	79.83	16.28	16.34	0.15	1.69	86.8	4.61
4	Control (24 h)	7	28.26	28.17	0.09	0.94	80.15	15.63	15.75	0.35	1.09	86.65	0.92
		7	28.07	28.17	0.09	1.07	80.15	16.15	15.75	0.35	0.76	86.70	1.05
		7	28.18	28.17	0.09	0.99	80.10	15.47	15.75	0.35	1.21	86.60	0.97
	TII (24 h)	8	23.28	23.09	0.32	29.65	79.60	16.39	16.03	0.36	0.64	86.60	35.58
		8	23.26	23.09	0.32	30.06	79.60	16.07	16.03	0.36	0.80	86.60	36.08
		8	22.72	23.09	0.32	43.71	80.10	15.67	16.03	0.35	1.06	87.10	52.46

Supplementary Table 3: Raw data concerning Figure 2A and Table 1. C20 microglial cells were stimulated with a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ (TII), each used at the same concentration of 50 pg/ml in the cocktail. Cells were treated for different times, from 2 h up to 24 h. After the incubation period, the concentration of the monocyte chemoattractant protein-1 (MCP-1, pg/ml) in the incubation medium was measured by a specific ELISA assay. The amounts of MCP-1 measured in the incubation medium after different times of incubation are reported in this table. Data are shown as pg/ml and were obtained from 3 independent experiments, each including 4 biological replicates per treatment. ND = not detectable. The ND values were excluded from the analysis.

Treatments	Experiment n. 1				Experiment n. 2				Experiment n. 3			
	MCP-1 Concentration (pg/ml)				MCP-1 Concentration (pg/ml)				MCP-1 Concentration (pg/ml)			
Control (2 h)	155.37	49.18	53.14	24.69	39.10	43.52	54.88	33.19	ND	22.02	84.02	27.70
TII (2 h)	205.74	313.83	647.02	174.11	305.70	286.63	361.06	344.75	26.45	105.72	69.09	479.08
Control (4 h)	108.44	54.95	212.24	ND	79.83	114.83	119.70	77.83	64.13	43.33	150.49	22.64
TII (4 h)	974.29	1200.50	1064.04	1183.21	1866.78	2119.12	1767.16	1786.42	1715.87	658.92	2175.26	2664.79
Control (6 h)	134.84	42.48	131.48	123.31	215.89	207.13	214.86	236.88	175.91	153.28	126.41	156.54
TII (6 h)	2033.39	1055.43	803.12	1042.91	4789.24	4529.09	4652.77	4049.14	3304.37	3658.35	3606.57	4708.59
Control (8 h)	198.03	194.10	276.82	222.53	226.32	120.59	241.13	183.90	217.53	174.00	266.14	232.66
TII (8 h)	2511.00	2401.00	2570.95	2140.24	4962.82	4383.02	4902.78	5549.02	4934.02	5340.81	5206.32	4725.01
Control (16 h)	149.44	117.27	109.40	110.63	195.93	140.16	151.87	147.62	102.40	111.44	90.61	92.28
TII (16 h)	3779.85	2938.84	2790.47	3531.46	5072.27	7412.37	6045.04	6157.21	5230.03	5438.10	6036.75	6989.28
Control (24 h)	290.51	183.77	183.41	179.90	226.32	264.89	276.98	282.52	161.22	268.23	268.23	279.27
TII (24 h)	8225.66	7667.60	6186.11	4992.82	9768.53	10236.92	10552.39	8453.45	10527.84	12792.65	13155.67	13085.69

Supplementary Table 4: Raw data concerning Figure 2B. C20 microglial cells were stimulated with with a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ (TII), each used at the same concentration of 50 pg/ml in the cocktail. Cells were treated for different times, namely 4 h, 16 h and 24 h. After the incubation period, the mRNA expression levels of the monocyte chemoattractant protein-1 (MCP-1) were quantified through real-time qPCR using the comparative quantitation method based upon the $-\Delta\Delta C_t$ method. Relative mRNA concentrations were calculated from the take-off point of reactions (threshold cycle, Ct), using the expression level of the Control as Calibrator (Cal.) and the expression level of β -actin as normalizing gene. Ct values were obtained at fix threshold of 0.15, in order to combine data from different experiments. Each sample was assayed in triplicate. Raw data presented in the table include Ct values, Ct average and Ct standard deviation (SD), relative quantitation to calibrator (Rel. Quant. to Cal.) and melting temperature (Tm) of the amplified products, for both MCP-1 and β -actin mRNA. If Ct SD was > 0.5 , one outlier value was excluded from the analysis (please refer to numbers in bold). The last column includes the normalized expression values (Normalized Rel. Quant. to Cal.) for the MCP-1 mRNA used to generate Fig. 2B and reported in the graph as fold variation versus Control at different times.

Experiment (number/ biological replicates)	Treatment	Technical Replicates	mRNA MCP-1					mRNA β -actin (Normalizing gene)					$-\Delta\Delta C_t$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (4 h)	1	25.17	24.90	0.23	0.83	83.25	15.07	15.01	0.40	0.96	86.6	0.81
		1	24.76	24.90	0.23	1.10	83.20	15.38	15.01	0.40	0.77	86.6	1.07
		1	24.76	24.90	0.23	1.10	83.20	14.58	15.01	0.40	1.35	86.6	1.07
	TII (4 h)	2	18.71	18.35	0.32	72.84	83.15	14.81	15.06	0.22	1.15	86.6	74.62
		2	18.11	18.35	0.32	110.41	83.65	15.11	15.06	0.22	0.93	87.1	113.10
		2	18.23	18.35	0.32	101.59	83.65	15.25	15.06	0.22	0.85	87.1	104.07
2	Control (4 h)	3	25.83	25.62	0.26	0.86	83.33	17.24				86.3	
		3	25.33	25.62	0.26	1.22	83.33	15.50	15.30	0.28	0.87	86.3	1.21
		3	25.70	25.62	0.26	0.95	83.33	15.10	15.30	0.28	1.15	86.8	0.94
	TII (4 h)	4	19.14	19.16	0.11	89.26	83.30	15.36	15.54	0.43	0.96	87.2	102.53
		4	19.28	19.16	0.11	81.01	83.30	16.03	15.54	0.43	0.60	87.15	93.05
		4	19.06	19.16	0.11	94.35	83.30	15.23	15.54	0.43	1.05	87.15	108.37
3	Control (4 h)	5	27.70	27.76	0.05	1.04	83.30	17.18	17.13	0.08	0.97	86.83	1.04
		5	27.78	27.76	0.05	0.99	83.33	17.18	17.13	0.08	0.97	86.8	0.99
		5	27.80	27.76	0.05	0.97	83.33	17.03	17.13	0.08	1.07	86.8	0.97
	TII (4 h)	6	23.79	23.76	0.03	15.67	83.25	17.95	17.83	0.24	0.57	86.8	25.16
		6	23.76	23.76	0.03	16.00	83.25	17.55	17.83	0.24	0.75	86.75	25.69
		6	23.73	23.76	0.03	16.34	83.25	17.98	17.83	0.24	0.55	87.2	26.23
4	Control (4h)	7	22.42	22.73	0.44	1.24	83.10	15.91	15.63	0.27	0.82	86.6	1.23
		7	23.04	22.73	0.44	0.81	83.10	15.60	15.63	0.27	1.02	86.6	0.80
		7	No Ct				83.10	15.38	15.63	0.27	1.19	86.6	
	TII (4h)	8	17.65	17.40	0.36	33.82	83.60	14.67	14.79	0.28	1.95	87.1	18.67
		8	17.14	17.40	0.36	48.17	83.10	14.59	14.79	0.28	2.06	87.1	26.59
		8	28.30				83.10	15.11	14.79	0.28	1.43	87.1	

Supplementary Table 4: Raw data concerning Figure 2B (cont.)

Experiment (number/ biological replicates)	Treatment	Technical Replicate s	mRNA MCP-1					mRNA β -actin (Normalizing gene)					- $\Delta\Delta C_t$
			Ct value s	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (16 h)	1	23.12	23.14	0.08	1.01	83.83	15.51	15.45	0.05	0.96	86.6	1.01
		1	23.23	23.14	0.08	0.94	83.80	15.41	15.45	0.05	1.03	86.6	0.94
		1	23.07	23.14	0.08	1.05	83.25	15.44	15.45	0.05	1.01	86.6	1.05
	TII (16 h)	2	17.50	17.49	0.02	49.87	83.75	17.30	17.16	0.23	0.28	86.6	160.97
		2	17.47	17.49	0.02	50.91	83.70	16.89	17.16	0.23	0.37	86.6	164.35
		2	17.51	17.49	0.02	49.52	83.65	17.28	17.16	0.23	0.28	86.6	159.86
2	Control (16 h)	3	24.36	24.38	0.13	1.01	83.35	14.93	15.05	0.19	1.08	86.83	1.01
		3	24.52	24.38	0.13	0.91	83.33	14.94	15.05	0.19	1.08	87.3	0.90
		3	24.26	24.38	0.13	1.09	83.33	15.27	15.05	0.19	0.86	87.3	1.08
	TII (16 h)	4	18.41	18.29	0.12	62.68	83.33	15.98	16.05	0.14	0.52	87.25	125.28
		4	18.16	18.29	0.12	74.54	83.33	16.21	16.05	0.14	0.45	87.3	148.98
		4	18.29	18.29	0.12	68.12	83.30	15.96	16.05	0.14	0.53	87.2	136.14
3	Control (16 h)	5	25.99	25.96	0.11	0.98	83.35	16.44	16.43	0.13	1.00	86.85	0.98
		5	25.84	25.96	0.11	1.09	83.33	16.56	16.43	0.13	0.92	86.83	1.08
		5	26.05	25.96	0.11	0.94	83.30	16.30	16.43	0.13	1.10	86.83	0.94
	TII (16 h)	6	21.52	21.51	0.11	21.71	83.33	16.69	16.71	0.12	0.84	86.8	26.18
		6	21.40	21.51	0.11	23.59	83.30	16.60	16.71	0.12	0.89	86.8	28.45
		6	21.62	21.51	0.11	20.25	83.25	16.83	16.71	0.12	0.76	86.8	24.42
4	Control (16 h)	7	25.19	25.23	0.08	1.03	83.25	16.44	16.80	0.38	1.28	86.85	1.00
		7	25.18	25.23	0.08	1.04	83.25	16.75	16.80	0.38	1.03	86.8	1.01
		7	25.32	25.23	0.08	0.94	83.25	17.20	16.80	0.38	0.76	86.8	0.92
	TII (16 h)	8	18.10	18.14	0.22	140.07	83.85	16.76	16.70	0.05	1.03	86.83	130.94
		8	17.94	18.14	0.22	156.50	83.85	16.67	16.70	0.05	1.09	86.8	146.29
		8	18.37	18.14	0.22	116.16	83.25	16.67	16.70	0.05	1.09	86.8	108.59

Supplementary Table 4: Raw data concerning Figure 2B (cont.)

Experiment (number/ biological replicates)	Treatment	Technical Replicates	mRNA MCP-1					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (24 h)	1	23.76	23.80	0.07	1.03	83.83	18.53	18.70	0.30	1.13	86.6	1.01
		1	23.88	23.80	0.07	0.94	83.65	18.52	18.70	0.30	1.13	87.1	0.93
		1	23.75	23.80	0.07	1.03	83.65	19.05	18.70	0.30	0.78	87.1	1.02
	TII (24 h)	2	19.54	19.58	0.15	19.12	83.80	20.16	20.00	0.15	0.36	86.6	47.00
		2	19.75	19.58	0.15	16.53	83.75	19.85	20.00	0.15	0.45	86.6	40.63
		2	19.46	19.58	0.15	20.21	83.20	20.00	20.00	0.15	0.41	86.6	49.68
2	Control (24 h)	3	24.19	24.20	0.08	1.01	83.85	16.68	16.51	0.19	0.89	87.35	1.00
		3	24.29	24.20	0.08	0.94	83.30	16.53	16.51	0.19	0.98	87.7	0.94
		3	24.13	24.20	0.08	1.05	83.80	16.31	16.51	0.19	1.15	87.65	1.05
	TII (24 h)	4	19.27	19.15	0.10	30.55	83.33	16.79	16.83	0.11	0.82	87.3	38.16
		4	19.13	19.15	0.10	33.67	83.33	16.75	16.83	0.11	0.84	87.3	42.05
		4	19.06	19.15	0.10	35.34	83.33	16.95	16.83	0.11	0.74	87.25	44.14
3	Control (24 h)	5	26.41	26.43	0.13	1.01	83.85	17.04	17.04	0.05	1.00	86.85	1.01
		5	26.56	26.43	0.13	0.91	83.25	17.09	17.04	0.05	0.97	86.75	0.91
		5	26.31	26.43	0.13	1.08	83.25	16.99	17.04	0.05	1.04	86.7	1.08
	TII (24 h)	6	20.73	20.66	0.10	51.86	83.80	16.51	16.34	0.15	1.44	86.83	31.89
		6	20.70	20.66	0.10	52.95	83.30	16.24	16.34	0.15	1.74	86.83	32.56
		6	20.54	20.66	0.10	59.16	83.33	16.28	16.34	0.15	1.69	86.8	36.38
4	Control (24 h)	7	24.71	24.35	0.38	0.78	83.65	15.63	15.75	0.36	1.09	86.65	0.76
		7	23.96	24.35	0.38	1.31	83.65	16.15	15.75	0.36	0.76	86.70	1.28
		7	24.37	24.35	0.38	0.98	83.15	15.47	15.75	0.36	1.21	86.60	0.97
	TII (24 h)	8	19.18	19.14	0.06	35.92	83.10	16.39	16.04	0.36	0.64	86.60	43.11
		8	19.16	19.14	0.06	36.42	83.10	16.07	16.04	0.36	0.80	86.60	43.71
		8	19.07	19.14	0.06	38.76	83.10	15.67	16.04	0.36	1.06	87.10	46.52

Supplementary Table 5: Raw data concerning Figure 2C. C20 microglial cells were stimulated with a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ (TII), each used at the same concentration of 50 pg/ml in the cocktail. Cells were treated for different times, from 2 h up to 24 h. After the incubation period, the concentration of interleukin 8 (IL-8, pg/ml) in the incubation medium was measured by a specific ELISA assay. The amounts of IL-8 measured in the incubation medium after different times of incubation are reported in this table. Data are shown as pg/ml and were obtained from 4 independent experiments, each including 4 biological replicates per treatment. ND = not detectable. The ND values were excluded from the analysis.

Treatments	Experiment n. 1				Experiment n. 2				Experiment n. 3				Experiment n. 4			
	IL-8 Concentration (pg/ml)				IL-8 Concentration (pg/ml)				IL-8 Concentration (pg/ml)				IL-8 Concentration (pg/ml)			
Control (2 h)	7.39	5.85	5.96	5.05	16.54	15.92	16.14	16.69	11.82	11.82	12.81	10.35	11.36	12.33	11.45	10.61
TII (2 h)	58.68	76.36	74.23	87.66	82.86	99.68	87.71	91.70	64.37	72.92	84.52	83.17	77.15	73.17	84.25	80.50
Control (4 h)	11.11	10.09	10.06	9.66	17.56	16.71	17.75	17.22	13.17	15.46	11.82	15.05	16.97	17.20	12.50	15.67
TII (4 h)	2371.80	1852.86	1730.25	1984.92	357.05	359.76	408.47	391.13	564.62	377.39	645.86	678.27	1242.22	920.95	1877.53	1733.37
Control (6 h)	21.15	13.65	12.52	12.85	18.01	19.10	19.02	18.06	13.64	13.08	17.20	12.42	23.27	18.00	15.05	16.04
TII (6 h)	1801.25	1294.81	1759.89	2541.08	1008.43	777.30	860.00	876.81	1658.97	735.18	771.12	3820.19	1151.77	1812.76	1019.58	ND
Control (8 h)	23.60	15.49	13.15	20.19	18.36	17.48	19.10	18.58	14.31	15.21	18.60	15.88	24.52	17.14	34.82	23.41
TII (8 h)	2712.21	2096.94	1942.62	2290.50	1098.31	984.75	1046.62	1140.65	4280.23	5289.99	2932.39	1342.23	1503.25	4367.09	5940.35	10244.15
Control (16 h)	12.67	9.66	8.71	7.53	17.88	17.38	18.04	18.22	16.69	21.26	26.53	18.42	29.44	15.83	17.88	19.51
TII (16 h)	3665.34	3332.44	4388.97	6756.02	1064.73	1039.81	1251.35	1232.87	1807.31	1730.38	1807.31	1837.96	5781.34	4063.77	6928.46	6095.61
Control (24 h)	16.88	14.40	9.89	11.11	18.63	18.69	17.72	17.25	26.13	17.94	24.52	101.28	25.51	19.51	21.53	20.53
TII (24 h)	9051.22	8463.53	6186.25	5944.69	1776.25	1812.46	1758.45	1716.94	9866.09	4130.58	2804.56	3086.85	9005.83	3868.52	10328.83	13246.23

Supplementary Table 6: Raw data concerning Figure 2D. C20 microglial cells were stimulated with with a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ (TII), each used at the same concentration of 50 pg/ml in the cocktail. Cells were treated for different times, namely 4 h, 16 h and 24 h. After the incubation period, the mRNA expression levels of the monocyte chemoattractant protein-1 (MCP-1) were quantified through real-time qPCR using the comparative quantitation method based upon the $-\Delta\Delta C_t$ method. Relative mRNA concentrations were calculated from the take-off point of reactions (threshold cycle, Ct), using the expression level of the Control as Calibrator (Cal.) and the expression level of β -actin as normalizing gene. Ct values were obtained at fix threshold of 0.15, in order to combine data from different experiments. Each sample was assayed in triplicate. Raw data presented in the table include Ct values, Ct average and Ct standard deviation (SD), relative quantitation to calibrator (Rel. Quant. to Cal.) and melting temperature (Tm) of the amplified products, for both MCP-1 and β -actin mRNA. If Ct SD was > 0.5 , one outlier value was excluded from the analysis (please refer to numbers in bold). Values for Control and TII at 4 h derived from Experiment 4 were excluded from the analysis since SD remained > 0.5 despite the exclusion of 1 outlier (last column, values in bold). The last column includes the normalized expression values (Normalized Rel. Quant. to Cal.) for the IL-8 mRNA used to generate Figure 2D and reported in the graph as fold variation versus Control at different times.

Experiment (number/biological replicates)	Treatment	Technical Replicates	mRNA IL-8					mRNA β -actin (Normalizing gene)					$-\Delta\Delta C_t$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (4 h)	1	No Ct				81.3	15.07	15.01	0.40	0.96	86.6	
		1	29.57	29.13	0.62	0.74	81.75	15.38	15.01	0.40	0.77	86.6	0.72
		1	28.69	29.13	0.62	1.36	81.75	14.58	15.01	0.40	1.35	86.6	1.32
	TII (4 h)	2	19.10	19.17	0.09	1045.52	81.6	14.81	15.06	0.22	1.15	86.6	1071.05
		2	20.61				81.6	15.11	15.06	0.22	0.93	87.1	
		2	19.23	19.17	0.09	955.43	82.1	15.25	15.06	0.22	0.85	87.1	978.76
2	Control (4 h)	3	29.34	29.24	0.22	0.94	81.33	17.24				86.3	
		3	29.40	29.24	0.22	0.90	81.8	15.5	15.30	0.28	0.87	86.3	0.89
		3	28.99	29.24	0.22	1.19	81.8	15.1	15.30	0.28	1.15	86.8	1.18
	TII (4 h)	4	18.81	18.93	0.12	1382.76	81.85	15.36	15.54	0.43	0.96	87.2	1588.23
		4	18.92	18.93	0.12	1281.25	81.85	16.03	15.54	0.43	0.60	87.15	1471.64
		4	19.05	18.93	0.12	1170.84	81.83	15.23	15.54	0.43	1.05	87.15	1344.83
3	Control (4 h)	5	29.41	29.61	0.17	1.14	81.85	17.18	17.13	0.08	0.97	86.83	1.14
		5	29.68	29.61	0.17	0.95	81.85	17.18	17.13	0.08	0.97	86.8	0.95
		5	29.72	29.61	0.17	0.92	81.8	17.03	17.13	0.08	1.07	86.8	0.92
	TII (4 h)	6	25.38	25.31	0.14	18.68	81.8	17.95	17.82	0.24	0.57	86.8	29.99
		6	25.14	25.31	0.14	22.06	81.8	17.55	17.82	0.24	0.75	86.75	35.42
		6	25.39	25.31	0.14	18.55	81.83	17.98	17.82	0.24	0.55	87.2	29.78
4	Control (4 h)	7	28.47	27.79	0.97	0.62	81.10	15.91	15.63	0.27	0.82	86.60	0.62
		7	No Ct				79.60	15.60	15.63	0.27	1.02	86.60	
		7	27.10	27.79	0.97	1.61	81.60	15.38	15.63	0.27	1.19	86.60	1.59
	TII (4 h)	8	28.30				81.10	14.67	14.79	0.28	1.95	87.10	
		8	22.57	20.43	3.03	37.14	81.60	14.59	14.79	0.28	2.06	87.10	20.50
		8	18.28	20.43	3.03	726.59	81.60	15011	14.79	0.28	1.43	87.10	401.03

Supplementary Table 6: Raw data concerning Figure 2D (cont.)

Experiment (number/biological replicates)	Treatment	Technical Replicates	mRNA IL-8					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (16 h)	1	28.54	28.64	0.09	1.07	81.83	15.51	15.45	0.05	1.03	86.6	1.05
		1	28.72	28.64	0.09	0.95	81.85	15.41	15.45	0.05	1.01	86.6	0.93
		1	28.66	28.64	0.09	0.99	81.83	15.44	15.45	0.05	0.28	86.6	0.97
	TII (16 h)	2	19.47	19.12	0.33	576.03	81.75	17.3	17.16	0.23	0.37	86.6	1859.42
		2	19.04	19.12	0.33	776.05	81.75	16.89	17.16	0.23	0.28	86.6	2505.08
		2	18.83	19.12	0.33	897.64	81.6	17.28	17.16	0.23	1.03	86.6	2897.59
2	Control (16 h)	3	28.19	28.39	0.43	1.15	81.85	14.93	15.05	0.19	1.08	86.83	1.14
		3	28.88	28.39	0.43	0.71	81.3	14.94	15.05	0.19	1.08	87.3	0.71
		3	28.09	28.39	0.43	1.23	81.3	15.27	15.05	0.19	0.86	87.3	1.22
	TII (16 h)	4	19.94	19.84	0.08	348.90	81.85	15.98	16.05	0.14	0.52	87.25	697.29
		4	19.80	19.84	0.08	384.45	81.8	16.21	16.05	0.14	0.45	87.3	768.35
		4	19.79	19.84	0.08	387.13	81.78	15.96	16.05	0.14	0.53	87.2	773.70
3	Control (16 h)	5	29.79	29.30	0.45	0.71	81.85	16.44	16.43	0.13	1.00	86.85	0.71
		5	28.91	29.30	0.45	1.31	81.85	16.56	16.43	0.13	0.92	86.83	1.31
		5	29.20	29.30	0.45	1.07	81.35	16.3	16.43	0.13	1.10	86.83	1.07
	TII (16 h)	6	23.71	23.54	0.17	48.17	81.85	16.69	16.7	0.12	0.84	86.8	58.09
		6	23.37	23.54	0.17	60.97	81.8	16.6	16.7	0.12	0.89	86.8	73.53
		6	23.55	23.54	0.17	53.82	81.8	16.83	16.7	0.12	0.76	86.8	64.90
4	Control (16 h)	7	32.66					16.44	16.8	0.38	1.28	86.85	
		7	31.39	31.29	0.15	0.93	81.25	16.75	16.8	0.38	1.03	86.8	0.91
		7	31.18	31.29	0.15	1.08	81.25	17.2	16.8	0.38	0.76	86.8	1.05
	TII (16 h)	8	19.46	19.28	0.28	3628.10	81.83	16.76	16.7	0.05	1.03	86.83	3391.51
		8	18.96	19.28	0.28	5130.91	81.8	16.67	16.7	0.05	1.09	86.8	4796.33
		8	19.43	19.28	0.28	3704.34	81.8	16.67	16.7	0.05	1.09	86.8	3462.78

Supplementary Table 6: Raw data concerning Figure 2D (cont.)

Experiment (number/biological replicates)	Treatment	Technical Replicates	mRNA IL-8					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$
			Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct values	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (24 h)	1	28.86	28.85	0.31	0.99	81.83	18.53	18.7	0.30	1.13	86.6	0.98
		1	29.15	28.85	0.31	0.81	81.6	18.52	18.7	0.30	1.13	87.1	0.80
		1	28.53	28.85	0.31	1.25	81.65	19.05	18.7	0.30	0.78	87.1	1.23
	TII (24 h)	2	19.61	19.41	0.2	603.27	81.85	20.16	20	0.15	0.36	86.6	1483.16
		2	19.40	19.41	0.2	697.80	81.83	19.85	20	0.15	0.45	86.6	1715.55
		2	19.21	19.41	0.2	796.02	81.75	20	20	0.15	0.41	86.6	1957.04
2	Control (24 h)	3	27.79	28.17	0.42	1.30	81.85	16.68	16.51	0.19	0.89	87.35	1.30
		3	28.11	28.17	0.42	1.04	81.83	16.53	16.51	0.19	0.98	87.7	1.04
		3	28.62	28.17	0.42	0.73	81.75	16.31	16.51	0.19	1.15	87.65	0.73
	TII (24 h)	4	19.25	19.06	0.17	485.50	81.83	16.79	16.83	0.11	0.82	87.3	606.39
		4	18.97	19.06	0.17	589.49	81.8	16.75	16.83	0.11	0.84	87.3	736.28
		4	18.96	19.06	0.17	593.59	81.85	16.95	16.83	0.11	0.74	87.25	741.40
3	Control (24 h)	5	28.06	28.04	0.03	0.99	81.35	17.04	17.04	0.05	1.00	86.85	0.99
		5	28.05	28.04	0.03	0.99	81.8	17.09	17.04	0.05	0.97	86.75	0.99
		5	28.01	28.04	0.03	1.02	81.83	16.99	17.04	0.05	1.04	86.7	1.02
	TII (24 h)	6	21.24	21.07	0.19	111.43	81.85	16.51	16.34	0.15	1.44	86.83	68.52
		6	20.86	21.07	0.19	145.01	81.85	16.24	16.34	0.15	1.74	86.83	89.17
		6	21.13	21.07	0.19	120.26	81.85	16.28	16.34	0.15	1.69	86.8	73.95
4	Control (24 h)	7	30.80				81.70	15.63	15.75	0.36	1.09	86.65	
		7	28.34	28.26	0.11	0.95	81.60	16.15	15.75	0.36	0.76	86.70	0.93
		7	28.18	28.26	0.11	1.06	81.60	15.47	15.75	0.36	1.21	86.60	1.04
	TII (24 h)	8	20.50	20.60	0.11	216.77	81.60	16.39	16.04	0.36	0.64	86.60	260.14
		8	20.60	20.60	0.11	202.25	81.60	16.07	16.04	0.36	0.80	86.60	242.72
		8	20.71	20.60	0.11	187.40	81.60	15.67	16.04	0.36	1.06	87.10	224.90

Supplementary Table 7: Raw data concerning Figure 3A. C20 cells were treated with different concentrations of remifentanyl (RF) alone or in combination with TII for 6 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at 50 pg/ml. After the incubation period, cell viability was assed using the MTS assay. Raw data are expressed as Net Optical Density (OD), calculated by subtracting the absorbance values measured at 655 nm to the absorbance values measured at 490 nm (Net OD = OD₄₉₀ – OD₆₅₅). Two readings were performed, at time 0 (soon after the addition of the MTS solutions, t=0) and after 3 h of incubation at 37 °C in a humidified atmosphere containing 5 % CO₂. Each reading is corrected by the average Net OD measured for the Blanks, i.e. plain medium, not exposed to the cells and incubated for 3 h in the same conditions of the experimental samples. These data are then normalized as percentage of controls. In the table, we reported the Net OD values: [(Net OD at 3 h - Average Net OD for the Blanks at 3h) - (Net OD at t=0 - Average Net OD for the Blanks at t=0)]. These values were used to calculate the normalized data, shown in the table as Normalized Values. The latter were used to generate Figure 3A. RF=remifentanyl; TII= mixture of TNF α , IL-1 β and IFN γ ; Net OD= Net Optical Density

Treatments	Experiment n. 1					Experiment n. 2					Experiment n. 3			
	Net OD					Net OD					Net OD			
Control	0.476	0.507	0.544	0.311	0.444	0.355	0.362	0.371	0.427	0.364	1.120	1.167	1.194	1.111
1.25 ng/ml RF	0.383	0.516	0.437	0.570	0.800	0.376	0.428	0.378	0.442	0.360	1.088	0.990	1.003	0.934
2.5 ng/ml RF	0.393	0.544	0.561	0.778	0.574	0.389	0.42	0.432	0.394	0.383	0.946	1.096	0.985	1.114
5 ng/ml RF	0.503	0.526	0.507	0.631	0.625	0.356	0.406	0.449	0.403	0.423	0.884	0.837	0.823	1.117
10 ng/ml RF	0.328	0.410	0.521	0.685	0.705	0.351	0.372	0.36	0.358	0.331	0.862	0.830	0.877	0.950
20 ng/ml RF	0.270	0.382	0.434	0.355	0.421	0.356	0.33	0.362	0.34	0.352	0.865	0.840	0.871	0.873
TII	0.634	0.583	0.407	0.583	0.608	0.366	0.406	0.383	0.375	0.394	0.815	0.787	0.764	0.767
TII + 1.25 ng/ml RF	0.773	0.610	0.633	0.618	0.605	0.456	0.399	0.422	0.388	0.434	0.844	0.806	0.790	0.765
TII + 2.5 ng/ml RF	0.764	0.591	0.632	0.656	0.524	0.403	0.414	0.387	0.427	0.402	0.932	0.827	0.931	0.782
TII + 5 ng/ml RF	0.657	0.607	0.655	0.612	0.573	0.431	0.438	0.44	0.45	0.427	0.862	0.992	1.010	0.834
TII + 10 ng/ml RF	0.333	0.599	0.616	0.597	0.393	0.393	0.4	0.436	0.422	0.416	1.151	1.167	1.175	1.214
TII + 20 ng/ml RF	0.39	0.439	0.594	0.559	0.451	0.336	0.393	0.422	0.355	0.384	1.157	1.082	1.012	0.960

Supplementary Table 7: Raw data concerning Figure 3A (cont.)

Treatments	Experiment n. 1					Experiment n. 2					Experiment n. 3			
	Normalized values					Normalized values					Normalized values			
Control	104.29	111.09	119.19	68.14	97.28	94.47	96.33	98.72	113.62	96.86	97.56	101.65	104.01	96.78
1.25 ng/ml RF	83.92	113.06	95.75	124.89	175.28	100.05	113.89	100.59	117.62	95.80	94.78	86.24	87.37	81.36
2.5 ng/ml RF	86.11	119.19	122.92	170.46	125.76	103.51	111.76	114.95	104.84	101.92	82.41	95.47	85.81	97.04
5 ng/ml RF	110.21	115.25	111.09	138.25	136.94	94.73	108.04	119.48	107.24	112.56	77.01	72.92	71.70	97.30
10 ng/ml RF	71.87	89.83	114.15	150.08	154.47	93.40	98.99	95.80	95.26	88.08	75.09	72.30	76.40	82.76
20 ng/ml RF	59.16	83.70	95.09	77.78	92.24	94.73	87.81	96.33	90.47	93.67	75.36	73.18	75.88	76.05
TII	138.91	127.74	89.18	127.74	133.21	97.39	108.04	101.92	99.79	104.84	71.00	68.56	66.56	66.82
TII + 1.25 ng/ml RF	169.37	133.65	138.69	135.40	132.56	121.34	106.17	112.29	103.25	115.49	73.53	70.22	68.82	66.65
TII + 2.5 ng/ml RF	167.39	129.49	138.47	143.73	114.81	107.24	110.16	102.98	113.62	106.97	81.19	72.05	81.10	68.13
TII + 5 ng/ml RF	143.95	132.99	143.51	134.09	125.55	114.69	116.55	117.08	119.74	113.62	75.09	86.42	87.98	72.66
TII + 10 ng/ml RF	72.96	131.24	134.97	130.80	86.11	104.58	106.44	116.02	112.29	110.70	100.26	101.65	102.35	105.75
TII + 20 ng/ml RF	85.45	96.19	130.15	122.48	98.82	89.41	104.58	112.29	94.47	102.18	100.78	94.25	88.16	83.63

Supplementary Table 8: Raw data concerning Figure 3B. C20 cells were treated with RF alone at different concentration and in combination with TII for 16 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration (50 pg/ml). After the incubation period, cell viability was assessed using the MTS assay. Raw data are expressed as Net Optical Density (OD), calculated by subtracting the absorbance values measured at 655 nm to the absorbance values measured at 490 nm (Net OD = OD₄₉₀ – OD₆₅₅). Two readings were performed, at time 0 (soon after the addition of the MTS solutions, t=0) and after 3 h of incubation at 37 °C in a humidified atmosphere containing 5 % CO₂. Each reading is corrected by the average Net OD measured for the Blanks, i.e. plain medium, not exposed to the cells and incubated for 3 h in the same conditions of the experimental samples. These data are then normalized as percentage of controls. In the table, we reported the Net OD values: [(Net OD at 3 h – Average Net OD for the Blanks at 3 h) - (Net OD at t=0 – Average Net OD for the Blanks at t=0)]. These values were used to calculate the normalized data, shown in the table as Normalized Values. The latter were used to generate Figure 3B. RF=remifentanyl; TII= mixture of TNF α , IL-1 β and IFN γ ; Net OD= Net Optical Density

Treatments	Experiment n. 1					Experiment n. 2					Experiment n. 3			
	Net OD 490 nm					Net OD 490 nm					Net OD 490 nm			
Control	1.022	1.126	1.095	1.115	0.949	0.764	0.634	0.644	0.565	0.603	1.244	1.154	1.367	1.336
1.25 ng/ml RF	1.022	1.063	1.225	1.254	1.210	0.642	0.625	0.661	0.565	0.600	1.404	1.318	1.783	1.684
2.5 ng/ml RF	1.114	1.031	1.083	1.212	1.229	0.581	0.579	0.545	0.540	0.574	1.175	1.234	1.192	1.274
5 ng/ml RF	1.033	1.080	1.082	1.210	1.220	0.586	0.580	0.558	0.785	0.801	1.188	1.121	1.089	1.239
10 ng/ml RF	1.042	1.020	1.018	1.123	1.176	0.628	0.595	0.600	0.587	0.579	1.186	1.475	1.464	1.338
20 ng/ml RF	1.013	1.071	1.026	1.026	1.128	0.574	0.546	0.569	0.581	0.591	0.998	1.023	1.351	1.388
TII	0.989	1.034	1.005	0.996	1.078	0.592	0.663	0.582	0.561	0.569	1.634	1.563	1.696	1.638
TII + 1.25 ng/ml RF	1.252	1.287	1.063	0.968	1.010	0.617	0.585	0.609	0.578	0.548	1.446	1.340	1.419	1.376
TII + 2.5 ng/ml RF	1.296	1.290	1.283	1.019	1.090	0.604	0.572	0.533	0.504	0.461	1.405	1.141	1.204	1.156
TII + 5 ng/ml RF	1.244	1.340	1.298	1.301	1.049	0.807	0.716	0.667	0.618	0.662	1.299	1.290	1.178	1.196
TII + 10 ng/ml RF	1.318	1.345	1.261	1.149	1.052	0.610	0.587	0.600	0.608	0.581	1.209	1.367	1.045	0.986
TII + 20 ng/ml RF	1.419	1.213	1.232	1.081	1.142	0.580	0.575	0.560	0.529	0.583	1.334	1.354	1.327	1.260

Supplementary Table 8: Raw data concerning Figure 3B (cont.)

Treatments	Experiment n. 1					Experiment n. 2					Experiment n. 3			
	Normalized values					Normalized values					Normalized values			
Control	96.29	106.09	103.17	105.05	89.41	118.99	98.75	100.31	88.01	93.93	97.55	90.49	107.19	104.76
1.25 ng/ml RF	96.29	100.15	115.41	118.15	114.00	100.00	97.35	102.96	88.01	93.46	110.09	103.35	139.81	132.04
2.5 ng/ml RF	104.96	97.14	102.04	114.19	115.79	90.50	90.19	84.80	84.12	89.41	92.14	96.77	93.47	99.90
5 ng/ml RF	97.32	101.75	101.94	114.00	114.94	91.28	90.35	86.82	122.26	124.75	93.16	87.91	85.40	97.16
10 ng/ml RF	98.17	96.10	95.91	105.80	110.80	97.82	92.68	93.46	91.44	90.19	93.00	115.66	114.80	104.92
20 ng/ml RF	95.44	100.90	96.66	96.66	106.27	89.41	85.05	88.64	90.50	92.06	78.26	80.22	105.94	108.84
TII	93.18	97.42	94.69	93.84	101.56	92.22	103.27	90.66	87.39	88.64	128.12	122.56	132.98	128.44
TII + 1.25 ng/ml RF	117.96	121.25	100.15	91.20	95.16	96.11	91.13	94.86	89.93	85.37	113.39	105.08	111.27	107.90
TII + 2.5 ng/ml RF	122.10	121.54	120.88	96.01	102.69	94.08	89.00	82.93	78.41	71.72	110.17	89.48	94.41	90.65
TII + 5 ng/ml RF	117.20	126.25	122.29	122.57	98.83	125.69	111.52	103.89	96.26	103.11	101.86	101.16	92.38	93.79
TII + 10 ng/ml RF	124.18	126.72	118.81	108.25	99.11	95.02	91.44	93.46	94.60	90.40	94.81	107.19	81.95	77.32
TII + 20 ng/ml RF	133.69	114.28	116.07	101.85	107.59	90.35	89.57	87.13	82.30	90.71	104.61	106.17	104.06	98.80

Supplementary Table 9: Raw data concerning Figure 3C. C20 cells were treated with RF alone at different concentration and in combination with TII for 16 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration (50 pg/ml). After the incubation period, cytotoxicity was assessed by measuring the activity of lactate dehydrogenase (LDH) released in the incubation media. Raw data were collected as Optical Density (OD) at 490 nm (OD₄₉₀). In the table the net OD₄₉₀ is reported per each sample. It was calculated by subtracting the average OD₄₉₀ values measured for the blanks, *i.e.* incubation medium not exposed to the C20 cells, to the absorbance values measured for each sample. Average OD₄₉₀ for the blanks was 0.138 (\pm 0.005 SD, n=3) in experiment n. 1; 0.127 (\pm 0.002 SD, n=2) in Experiment n. 2; and 0.108 (\pm 0.003 SD, n=3) in experiment n. 3. Data were then normalized as percentage of controls and to generate Figure 3C. Data in bold were considered outliers and excluded from the analysis. RF=remifentamil; TII= mixture of TNF α , IL-1 β and IFN γ ; OD= Optical Density.

Treatments	Experiment n. 1					Experiment n. 2					Experiment n. 3			
	net OD ₄₉₀					net OD ₄₉₀					net OD ₄₉₀			
Control	0.022	0.022	0.017	0.027	0.024	ND	ND	0.004	0.023	0.005	0.033	0.020	0.018	0.032
1.25 ng/ml RF	0.018	0.021	0.015	0.020	0.020	0.008	0.010	0.015	0.019	0.015	0.046	0.022	0.026	0.011
2.5 ng/ml RF	0.028	0.021	0.022	0.026	0.022	0.011	0.020	0.012	0.011	0.015	0.029	0.025	0.016	0.016
5 ng/ml RF	0.027	0.018	0.018	0.017	0.052	0.010	0.007	0.004	0.008	0.006	0.028	0.028	0.039	0.021
10 ng/ml RF	0.019	0.021	0.020	0.035	0.044	0.008	0.011	0.007	0.010	0.004	0.046	0.030	0.036	0.048
20 ng/ml RF	0.047	0.017	0.024	0.025	0.031	0.012	0.006	0.007	0.006	0.007	0.029	0.026	0.025	0.019
TII	0.023	0.020	0.020	0.024	0.019	0.010	0.001	0.001	0.004	ND	0.029	0.030	0.022	0.031
TII + 1.25 ng/ml RF	0.019	0.021	0.023	0.019	0.018	0.009	0.003	0.001	0.007	0.041	0.025	0.022	0.026	0.037
TII + 2.5 ng/ml RF	0.026	0.023	0.026	0.026	0.023	0.012	0.007	0.003	0.011	0.006	0.021	0.030	0.025	0.028
TII + 5 ng/ml RF	0.031	0.024	0.019	0.030	0.024	0.013	0.004	0.010	0.009	0.004	0.034	0.033	0.025	0.042
TII + 10 ng/ml RF	0.022	0.021	0.021	0.037	0.029	0.015	0.003	0.007	0.008	0.018	0.048	0.033	0.032	0.026
TII + 20 ng/ml RF	0.022	0.020	0.019	0.029	0.046	0.068	0.005	0.006	0.004	0.007	0.029	0.032	0.034	0.036

Supplementary Table 9: Raw data concerning Figure 3C (cont.)

Treatments	Experiment n. 1					Experiment n. 2					Experiment n. 3			
	Normalized values					Normalized values					Normalized values			
Control	98.21	98.21	75.89	120.54	107.14	ND	ND	37.50	215.63	46.88	128.48	76.54	70.74	124.23
1.25 ng/ml RF	80.36	93.75	66.96	89.29	89.29	75.00	93.75	140.63	178.13	140.63	178.60	85.17	102.62	41.65
2.5 ng/ml RF	125.00	93.75	98.21	116.07	98.21	103.13	187.50	112.50	103.13	140.63	111.00	96.46	62.92	62.92
5 ng/ml RF	120.54	80.36	80.36	75.89	232.14	93.75	65.63	37.50	75.00	56.25	109.77	107.82	149.86	80.02
10 ng/ml RF	84.82	93.75	89.29	156.25	196.43	75.00	103.13	65.63	93.75	37.50	177.66	117.32	141.23	186.60
20 ng/ml RF	209.82	75.89	107.14	111.61	138.39	112.50	56.25	65.63	56.25	65.63	111.38	101.59	98.90	74.94
TII	102.68	89.29	89.29	107.14	84.82	93.75	9.38	9.38	37.50	ND	114.54	115.24	84.91	119.58
TII + 1.25 ng/ml RF	84.82	93.75	102.68	84.82	80.36	84.38	28.13	9.38	65.63	384.38	96.87	86.12	100.77	143.04
TII + 2.5 ng/ml RF	116.07	102.68	116.07	116.07	102.68	112.50	65.63	28.13	103.13	56.25	80.80	114.90	96.11	108.94
TII + 5 ng/ml RF	138.39	107.14	84.82	133.93	107.14	121.88	37.50	93.75	84.38	37.50	132.10	126.48	97.10	162.88
TII + 10 ng/ml RF	98.21	93.75	93.75	165.18	129.46	140.63	28.13	65.63	75.00	168.75	184.99	126.93	122.43	101.41
TII + 20 ng/ml RF	98.21	89.29	84.82	129.46	205.36	637.50	46.88	56.25	37.50	65.63	112.70	124.34	133.55	140.62

Supplementary Table 10: Raw data concerning Figure 4A. C20 cells were treated with remifentanil (RF) at different concentrations alone or in combination with 50 pg/ml TII for 6 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration (50 pg/ml) in the cocktail. After the incubation period, the concentrations of IL-6 (pg/ml) in the incubation media were measured by a specific ELISA assay. The amount of IL-6 was measured in the incubation media after 6 h. Data are shown as pg/ml and derive from 3 independent experiments, each including 4 biological replicates per treatment.

Treatments	Experiment n. 1				Experiment n. 2				Experiment n. 3			
	IL-6 Concentration (pg/ml)				IL-6 Concentration (pg/ml)				IL-6 Concentration (pg/ml)			
Control	9.43	9.61	10.63	8.94	8.25	4.67	3.65	4.17	4.67	3.65	ND	ND
1.25 ng/ml RF	4.92	3.82	6.30	5.09	3.91	ND	ND	ND	1.51	ND	ND	1.51
2.5 ng/ml RF	4.34	3.91	4.92	5.66	5.98	ND	3.27	ND	ND	ND	ND	3.30
5 ng/ml RF	6.14	5.42	4.42	5.01	5.98	ND	ND	ND	ND	ND	5.56	ND
10 ng/ml RF	6.14	6.22	5.25	4.76	4.25	2.06	3.81	1.87	1.42	4.03	5.44	ND
20 ng/ml RF	5.50	5.90	5.82	5.58	6.62	1.51	ND	ND	1.31	ND	ND	22.64
TII	453.73	426.72	427.28	378.09	379.70	236.70	244.88	ND	219	243.25	233.96	ND
TII + 1.25 ng/ml RF	391.03	365.79	352.53	388.87	514.39	234.25	238.34	ND	226.79	220.68	244.88	ND
TII + 2.5 ng/ml RF	394.83	363.66	368.99	349.37	409.03	ND	217.33	206.04	227.90	240.52	213.40	ND
TII + 5 ng/ml RF	473.22	404.10	378.62	379.70	356.23	249.22	ND	222.91	226.79	260.53	255.15	ND
TII + 10 ng/ml RF	362.60	363.13	346.74	365.79	398.10	250.84	ND	ND	250.84	174.55	386.82	235.60
TII + 20 ng/ml RF	366.85	451.46	365.26	338.87	421.72	245.42	210.58	ND	233.41	239.98	236.15	ND

Supplementary Table 11: Raw data concerning Figure 4B. C20 cells were treated with remifentanyl (RF) at different concentrations alone or in combination with 50 pg/ml TII for 4 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration (50 pg/ml). After the incubation period, the concentration of MCP-1 (pg/ml) in the incubation media were measured by a specific ELISA assay. The amounts of MCP-1 measured in the incubation media after 6 h are reported. Data are shown as pg/ml and derive from 3 independent experiments, each including 4 biological replicates per treatment.

Treatments	Experiment n. 1				Experiment n. 2				Experiment n. 3			
	MCP-1 concentration (pg/ml)				MCP-1 Concentration (pg/ml)				MCP-1 Concentration (pg/ml)			
Control	18.50	32.49	32.69	22.77	57.93	58.84	68.39	63.07	44.57	66.63	33.26	31.57
1.25 ng/ml RF	21.01	24.92	39.11	43.85	20.33	44.23	50.93	36.61	25.06	19.63	13.88	11.86
2.5 ng/ml RF	10.12	18.04	26.60	21.89	42.15	35.83	45.35	32.29	36.10	36.56	62.46	51.03
5 ng/ml RF	12.59	29.48	35.64	37.96	40.07	46.10	41.02	29.89	51.69	35.16	49.48	65.97
10 ng/ml RF	ND	21.01	29.48	18.50	35.05	39.49	49.82	34.07	17.31	25.32	22.67	38.19
20 ng/ml RF	18.74	37.77	74.49	50.74	42.15	39.49	42.15	33.29	28.37	33.02	48.15	23.48
TII	667.51	955.17	908.21	959.42	563.36	603.65	540.41	558.54	470.24	735.65	636.05	768.60
TII + 1.25 ng/ml RF	1158.86	909.56	936.95	1205.18	480.30	523.48	449.91	571.11	669.33	614.12	740.07	705.37
TII + 2.5 ng/ml RF	791.38	542.31	760.71	815.63	565.29	671.76	687.86	635.11	531.33	722.54	550.16	756.47
TII + 5 ng/ml RF	949.53	733.20	867.28	975.18	578.92	483.02	477.59	502.17	645.24	672.05	746.00	647.88
TII + 10 ng/ml RF	571.11	1384.26	1096.47	997.08	256.54	465.02	429.69	465.02	483.51	615.39	524.36	546.60
TII + 20 ng/ml RF	1080.26	827.98	844.27	934.18	454.34	420.98	405.42	406.28	411.28	609.03	657.20	530.16

Supplementary Table 12: Raw data concerning Figure 4C. C20 cells were treated with remifentanyl (RF) at different concentrations alone or in combination with 50 pg/ml TII for 6 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration (50 pg/ml). After the incubation period, the concentration of IL-8 (pg/ml) in the incubation media were measured by a specific ELISA assay. The released amount of IL-8 measured in the incubation media after 6 h are reported in the table. Data are shown as pg/ml and derive from 4 independent experiments, each including 4 biological replicates per treatment.

Treatments	Experiment n. 1				Experiment n. 2				Experiment n. 3				Experiment n. 4			
	IL-8 Concentration (pg/ml)				IL-8 Concentration (pg/ml)				IL-8 Concentration (pg/ml)				IL-8 Concentration (pg/ml)			
Control	ND	1.50	2.76	ND	4.56	8.88	7.21	4.72	ND	ND	ND	ND	ND	1.62	5.85	ND
1.25 ng/ml RF	2.41	4.55	9.35	7.67	2.59	3.00	3.83	3.09	ND	ND	ND	ND	ND	ND	9.11	2.77
2.5 ng/ml RF	52.21	7.67	8.37	6.66	5.29	4.40	10.07	2.42	ND	ND	ND	ND	ND	ND	1.36	2.56
5 ng/ml RF	10.85	4.23	7.24	7.38	4.32	6.01	4.80	6.01	ND	ND	ND	ND	ND	7.26	10.51	13.53
10 ng/ml RF	11.12	8.79	6.51	2.93	6.49	4.64	4.80	88.24	ND	ND	ND	ND	1.40	36.86	3.48	46.19
20 ng/ml RF	7.81	6.07	6.51	9.35	75.53	31.03	10.94	82.92	25.99	ND	ND	33.41	60.45	63.57	9.51	183.44
TII	1432.55	753.48	769.82	953.64	983.51	1263.95	1161.64	1359.97	1795.62	2479.78	1782.83	4244.294	6428.01	4125.50	6958.49	7113.28
TII + 1.25 ng/ml RF	2773.53	829.88	835.06	865.11	1067.60	1136.05	1118.25	1250.97	1823.91	2252.78	2114.88	1981.55	10506.28	4246.80	5035.19	7197.23
TII + 2.5 ng/ml RF	2647.09	816.97	865.11	900.90	1241.30	1215.83	1420.32	1327.27	1607.42	1772.62	2084.56	2221.40	3982.67	3139.11	3350.56	3946.11
TII + 5 ng/ml RF	2920.45	795.19	1124.55	763.52	3383.39	919.51	972.85	1204.83	2092.81	1997.64	1336.93	2109.35	4195.35	3355.86	5156.83	6948.29
TII + 10 ng/ml RF	3080.37	907.59	981.19	945.44	4021.37	1124.16	1045.04	1285.31	2112.112	1749.73	1737.07	1847.18	6428.01	3994.92	8812.63	7186.68
TII + 20 ng/ml RF	2717.19	833.77	978.42	933.21	3053.43	1436.71	1334.09	1300.28	4437.917	1989.59	1519.69	2212.89	5989.41	3556.78	6523.19	6024.84

Supplementary Table 13: Raw data concerning Figure 5A. C20 cells were treated with 5 ng/ml remifentanyl (RF) alone or in combination with 50 pg/ml TII for 16 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration of 50 pg/ml in the cocktail. After the incubation period, the mRNA expression levels of the monocyte chemoattractant protein-1 (MCP-1) were quantified through real-time qPCR using the comparative quantitation method based upon the $-\Delta\Delta C_t$ method. Relative mRNA concentrations were calculated from the take-off point of reactions (threshold cycle, Ct) taking the expression level of the Control as Calibrator (Cal.) and the expression level of β -actin as normalizing gene. Ct values were obtained at fix threshold of 0.15, in order to combine data from different experiments. Each sample was assessed in triplicate. Raw data presented in the table include Ct values, Ct average and Ct standard deviation (SD), relative expression to calibrator (Rel. Quant. to Cal.) and melting temperature (Tm) of the amplified product, for both MCP-1 and β -actin mRNA. The last column includes the normalized expression values (Normalized Rel. Quant. to Cal.) for the MCP-1 mRNA used to generate Figure 5A and reported in the graph as fold variation versus Control.

Experiment (number/biological replicate)	Treatment	Technical Replicates	mRNA MCP1					mRNA β -actin (Normalizing gene)					$-\Delta\Delta C_t$
			Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (16 h)	1	25.19	25.23	0.08	1.03	83.25	16.44	16.80	0.38	1.28	86.85	1.00
		1	25.18	25.23	0.08	1.04	83.25	16.75	16.80	0.38	1.03	86.80	1.01
		1	25.32	25.23	0.08	0.94	83.25	17.20	16.80	0.38	0.76	86.80	0.92
	5 ng/ml RF (16 h)	2	25.24	25.14	0.16	0.99	83.25	16.39	16.38	0.03	1.33	86.85	0.74
		2	25.22	25.14	0.16	1.01	83.60	16.35	16.38	0.03	1.36	86.85	0.75
		2	24.95	25.14	0.16	1.21	83.60	16.40	16.38	0.03	1.32	86.85	0.91
	TII (16 h)	3	18.10	18.14	0.22	140.07	83.85	16.76	16.70	0.05	1.03	86.83	130.94
		3	17.94	18.14	0.22	156.50	83.85	16.67	16.70	0.05	1.09	86.80	146.29
		3	18.37	18.14	0.22	116.16	83.25	16.67	16.70	0.05	1.09	86.80	108.59
	TII + 5 ng/ml RF (16 h)	4	18.58	18.63	0.07	100.43	83.25	17.20	17.24	0.04	0.76	86.85	136.84
		4	18.71	18.63	0.07	91.77	83.25	17.26	17.24	0.04	0.73	86.85	125.05
		4	18.60	18.63	0.07	99.04	83.80	17.27	17.24	0.04	0.72	86.80	134.95

Supplementary Table 13: Raw data concerning Figure 5A (cont.)

Experiment (number/biological replicate)	Treatment	Technical Replicates	mRNA MCP1					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$	
			Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.	
2	Control (16 h)	5	27.19					16.65					86.8	
		5	24.73	24.70	0.05	0.98	83.25	14.71	15.04	0.45	1.25	86.85	0.96	
		5	24.67	24.70	0.05	1.02	83.25	15.36	15.04	0.45	0.80	87.3	1.00	
	5 ng/ml RF (16 h)	6	24.59	24.90	0.30	1.08	83.2	17.79	17.59	0.28	0.15	87.2	6.28	
		6	25.18	24.90	0.30	0.72	83.65	17.39	17.59	0.28	0.20	87.15	4.17	
		6	24.93	24.90	0.30	0.85	83.7	14.51				87.33	4.96	
	TII (16 h)	7	16.89	16.92	0.05	224.41	83.85	15.13	15.25	0.16	0.94	86.85	258.75	
		7	16.9	16.92	0.05	222.86	83.83	15.36	15.25	0.16	0.80	86.8	256.96	
		7	16.98	16.92	0.05	210.84	83.8	14.11				86.8	243.10	
	TII + 5 ng/ml RF (16 h)	8	16.78	16.79	0.11	242.19	83.25	15.06	14.61	0.40	0.98	87.2	175.95	
		8	16.68	16.79	0.11	259.57	83.7	14.28	14.61	0.40	1.69	86.8	188.58	
		8	16.9	16.79	0.11	222.86	83.65	14.49	14.61	0.40	1.46	86.85	161.91	
3	Control (16 h)	9	27.48					15.89	16.00	0.11	1.08	86.85		
		9	25.99	25.82	0.24	0.89	83.60	16.12	16.00	0.11	0.92	86.85	0.88	
		9	25.64	25.82	0.24	1.13	83.60	15.99	16.00	0.11	1.01	86.83	1.13	
	5 ng/ml RF (16 h)	10	26.62	26.25	0.32	0.57	83.60	15.68	15.90	0.46	1.25	86.80	0.52	
		10	26.04	26.25	0.32	0.86	83.60	15.59	15.90	0.46	1.33	87.28	0.77	
		10	26.08	26.25	0.32	0.83	83.60	16.43	15.90	0.46	0.74	87.25	0.75	
	TII (16 h)	11	20.73	20.66	0.13	33.94	83.65	16.04	15.97	0.08	0.97	86.85	33.13	
		11	20.51	20.66	0.13	39.53	83.60	15.87	15.97	0.08	1.09	86.85	38.58	
		11	20.75	20.66	0.13	33.47	83.60	15.99	15.97	0.08	1.01	86.85	32.67	
	TII + 5 ng/ml RF (16 h)	12	21.05	20.98	0.23	27.19	83.60	16.86	16.63	0.20	0.55	86.85	41.92	
		12	20.73	20.98	0.23	33.94	83.60	16.49	16.63	0.20	0.71	86.83	52.33	
		12	21.17	20.98	0.23	25.02	83.60	16.55	16.63	0.20	0.68	86.80	38.57	

Supplementary Table 14: Raw data concerning Figure 5B. C20 cells were treated with 5 ng/ml remifentanyl (RF) alone or in combination with 50 pg/ml TII for 16 h. TII consists in a mixture of proinflammatory cytokines, including TNF α , IL-1 β and IFN γ , each used at the same concentration of 50 pg/ml. After the incubation period, interleukin 8 (IL-8) gene expression was quantified through real-time qPCR using the comparative quantitation method based upon the $-\Delta\Delta C_t$ method. Relative mRNA concentrations were calculated from the take-off point of reactions (threshold cycle, Ct) taking the expression level of the Control as Calibrator (Cal.) and the expression level of β -actin as normalizing gene. Ct values were obtained at fix threshold of 0.15, in order to combine data from different experiments. Each sample was assessed in triplicate. Raw data presented in the table include Ct values, Ct average and Ct standard deviation (SD), relative expression to calibrator (Rel. Quant. to Cal.) and melting temperature (Tm) of the amplified product, for both IL-8 and β -actin mRNA. The last column includes the normalized expression values (Normalized Rel. Quant. to Cal.) for the IL-8 mRNA used to generate Figure 5B and reported in the graph as fold variation versus Control.

Experiment (number/biological replicate)	Treatment	Technical Replicates	mRNA IL-8					mRNA β -actin (Normalizing gene)					$-\Delta\Delta C_t$
			Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
1	Control (16 h)	1	32.66				81.25	16.44	16.80	0.38	1.28	86.85	
		1	31.39	31.29	0.15	0.93	81.25	16.75	16.80	0.38	1.03	86.80	0.91
		1	31.18	31.29	0.15	1.08	81.25	17.20	16.80	0.38	0.76	86.80	1.05
	5 ng/ml RF (16 h)	2	30.71	30.43	0.24	1.49	81.70	16.39	16.38	0.03	1.33	86.85	1.12
		2	30.26	30.43	0.24	2.03	81.65	16.35	16.38	0.03	1.36	86.85	1.52
		2	30.32	30.43	0.24	1.95	81.65	16.40	16.38	0.03	1.32	86.85	1.46
	TII (16 h)	3	19.46	19.28	0.28	3628.10	81.83	16.76	16.70	0.05	1.03	86.83	3391.51
		3	18.96	19.28	0.28	5130.91	81.80	16.67	16.70	0.05	1.09	86.80	4796.33
		3	19.43	19.28	0.28	3704.34	81.80	16.67	16.70	0.05	1.09	86.80	3462.78
	TII + 5 ng/ml RF (16 h)	4	19.90	19.90	0.02	2674.40	81.75	17.20	17.24	0.04	0.76	86.85	3644.07
		4	19.88	19.90	0.02	2711.73	81.70	17.26	17.24	0.04	0.73	86.85	3694.94
		4	19.93	19.90	0.02	2619.36	81.70	17.27	17.24	0.04	0.72	86.80	3569.07

Supplementary Table 14: Raw data concerning Figure 5B (cont.)

Experiment (number/biological replicate)	Treatment	Technical Replicates	mRNA IL-8					mRNA β -actin (Normalizing gene)					$-\Delta\Delta Ct$
			Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Ct	Ct Average	Ct SD	Rel. Quant. to Cal.	Tm	Normalized Rel. Quant. to Cal.
2	Control (16 h)	5	28.12	28.66	0.47	1.45	81.8	16.65				86.8	1.42
		5	28.91	28.66	0.47	0.84	81.75	14.71	15.04	0.45	1.25	86.85	0.82
		5	28.95	28.66	0.47	0.82	81.75	15.36	15.04	0.45	0.80	87.3	0.80
	5 ng/ml RF (16 h)	6	28.65	28.77	0.12	1.01	81.65	17.79	17.59	0.28	0.15	87.2	5.86
		6	28.77	28.77	0.12	0.93	81.65	17.39	17.59	0.28	0.20	87.15	5.39
		6	28.89	28.77	0.12	0.85	81.6	14.51				87.33	4.96
	TII (16 h)	7	17.54	17.36	0.24	2225.63	81.83	15.13	15.25	0.16	0.94	86.85	2566.21
		7	17.45	17.36	0.24	2368.90	81.83	15.36	15.25	0.16	0.80	86.8	2731.39
		7	17.09	17.36	0.24	3040.30	81.8	14.11				86.8	3505.54
	TII + 5 ng/ml RF (16 h)	8	16.62	16.77	0.13	4211.15	81.75	15.06	14.61	0.4	0.98	87.2	3059.34
		8	16.84	16.77	0.13	3615.55	81.75	14.28	14.61	0.4	1.69	86.8	2626.64
		8	16.85	16.77	0.13	3590.58	81.65	14.49	14.61	0.4	1.46	86.85	2608.50
3	Control (16 h)	9	30.99					15.89	16.00	0.11	1.08	86.85	
		9	29.40	29.26	0.21	0.90	81.30	16.12	16.00	0.11	0.92	86.85	0.90
		9	29.11	29.26	0.21	1.11	81.33	15.99	16.00	0.11	1.01	86.83	1.10
	5 ng/ml RF (16 h)	10	30.04	29.68	0.35	0.58	81.30	15.68	15.90	0.46	1.25	86.80	0.52
		10	29.35	29.68	0.35	0.94	81.80	15.59	15.90	0.46	1.33	87.28	0.85
		10	29.65	29.68	0.35	0.76	81.80	16.43	15.90	0.46	0.74	87.25	0.69
	TII (16 h)	11	19.78	19.52	0.31	711.64	81.85	16.04	15.97	0.08	0.97	86.85	694.53
		11	19.59	19.52	0.31	811.81	81.83	15.87	15.97	0.08	1.09	86.85	792.29
		11	19.18	19.52	0.31	1078.64	81.83	15.99	15.97	0.08	1.01	86.85	1052.71
	TII + 5 ng/ml RF (16 h)	12	19.86	19.78	0.09	673.25	81.85	16.86	16.63	0.20	0.55	86.85	1037.90
		12	19.80	19.78	0.09	701.84	81.85	16.49	16.63	0.20	0.71	86.83	1081.98
		12	19.67	19.78	0.09	768.02	81.85	16.55	16.63	0.20	0.68	86.80	1184.00