Supplementary Table 1

Mice plasma (–)-epicatechin metabolites method performance

			Recovery	Repeatability		Intermediate reproducibility	
Analyte	**Number of days * number of replicates**	**Median (nM)**	**(%)**	**SD(r)**	**CV(r) [%]**	**SD(iR)**	**CV(iR) [%]**
(–)-epicatechin-4'-*β*-*D*-glucuronide	6*2	84.3	105.4	7.97	9.5	8.18	9.7
	6*2	815.9	102.4	44.66	5.5	51.18	6.3
	6*2	4150	103.8	104.94	2.5	251.7	6.1
(–)-epicatechin-3'-*β*-*D*-glucuronide	6*2	79.9	99.9	9.02	11.3	6.77	8.5
	6*2	748.9	93.6	32.6	4.4	35.97	4.8
	6*2	3816.7	95.4	179.27	4.7	195.32	5.1
(–)-epicatechin-4'-*O*-sulfate	6*2	52.38	99.9	3.09	5.9	3.19	6.1
	6*2	514.4	97.4	24.11	4.7	40.96	8
	6*2	2721.45	102	136.5	5	235.34	8.6
(–)-epicatechin-3'-*O*-sulfate	6*2	81.33	101.7	8.23	10.1	8.27	10.2
	6*2	804.18	100.5	22.75	2.8	26.07	3.2
	6*2	4074.45	101.9	153.11	3.8	271.64	6.7
3'-*O*-methyl-(–)-epicatechin-4'-*O*-sulfate	6*2	71.95	112.5	3.04	4.2	6	8.3
	6*2	641.7	100.4	21.6	3.4	31.68	4.9
	6*2	3276.8	102.4	167.63	5.1	196.82	6

Supplementary Methods

Isolation of human PBMCs:

Human peripheral blood mononuclear cells (PBMCs) were isolated from buffycoat obtained from the transfusion center of the CHUV (Lausanne). The cells were diluted 1:2 with Hanks balanced salt solution (HBSS) (Sigma, Lachen, Switzerland). After a Histopaque gradient centrifugation (Sigma), mononuclear cells were collected at the interface and washed twice with 40 ml HBSS according to the manufacturer's protocol. Cells were then resuspended in Iscove's Modified Dulbecco's Medium (IMDM, Sigma) supplemented with 10% heat inactivated fetal calf serum (Bioconcept, Paris, France), 1% L-glutamine (Sigma), 1% penicillin/streptomycin (Sigma) and 0.1% gentamycin (Sigma). PBMCs (7 x 10^5 cells/ well) were then incubated with a two different epicatechin metabolites mixtures resembling mice or human profile (2 µM) in 48 well plates. A mix of mice epicatechin metabolites (80% (-)-epicatechin-4'-*O*-glucuronide and 20% (-)-epicatechin-3'-*O*-sulfate) and human epicatechin metabolites (25% (-)-epicatechin-3'-*O*-glucuronide, 25% (-)-epicatechin-3'-*O*-sulfate, 25% 3'-O-methyl-(-)-epicatechin-4'-*O*-sulfate and 25% 3'-O-methyl-(-)-epicatechin-5-*O*-sulfate) were prepared by spiking purified compounds in HBSS. After 36 h incubation, culture plates were frozen and keep at -20 °C until cytokines measurement. The effects of metabolites were tested on PBMCs isolated from 4 individual donors.

Supplementary Figure Legend

Supplementary Figure 1

Murine and human (-) epicatechin metabolites impact immune cytokine production. The concentrations of different cytokines (A) IL-10, (B) TNF-α, (C) IFN-γ from PBMCs following incubation of PBMCs with the mix of epicatechin metabolites. Control un-stimulated (Medium) conditions are depicted in white bars, in light grey bars are PBMCs incubated with a mix of epicatechin metabolites mimicking murine metabolites and in dark grey bars are PBMCs incubated with a mix of epicatechin metabolites mimicking human metabolites. Both mixes induce Th-1 (IFN-g) and immune-regulatory (IL-10) cytokines (Suppl. Fig 1A & 1C). There was no effect on Th-2 cytokines (IL-4, IL-5, IL-13; data not shown)