

## Supplementary material Tables S1-S7

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#### **Comparative studies of urolithins and their phase II metabolites on macrophage and neutrophil functions.**

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**Table S1.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on viability of THP-1 macrophages. PMA-differentiated THP-1 cells were preincubated for 1h with iso-urolithin A, urolithin A and B (iUA, UA, UB), their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M, parthenolide (Parth) at the concentration of 5  $\mu$ M and stimulated with LPS (10 ng/mL) for 24 h. MTT test was performed as described in Materials and methods section.

	viability (%)	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)													
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB						
<b>NST</b>	109,4	3,9	0,999954	0,058926	<b>NST</b>													
<b>LPS</b>	100,0	2,1			<b>LPS</b>	0,380802												
<b>iUA</b>	103,3	11,7	0,986367	0,554204	<b>iUA</b>	0,855963	0,993753											
<b>GiUA</b>	101,8	6,5	0,959475	0,726273	<b>GiUA</b>	0,655934	0,999920	0,999982										
<b>UA</b>	94,8	4,7	0,325675	0,997400	<b>UA</b>	0,026668	0,903563	0,406000	0,655506									
<b>GUA</b>	94,6	5,0	0,300016	0,997917	<b>GUA</b>	0,022926	0,879444	0,368967	0,614636	1,000000								
<b>UB</b>	106,2	11,6	0,998179	0,285092	<b>UB</b>	0,998662	0,912834	0,999304	0,988082	0,282639	0,258121							
<b>GUB</b>	99,9	4,4	0,873140	0,886020	<b>GUB</b>	0,366829	1,000000	0,992357	0,999879	0,912758	0,890002	0,905373						
<b>Parthenolide</b>	99,7	3,3	0,862237	0,895542	<b>Parthenolide</b>	0,343553	1,000000	0,994194	0,999883	0,955880	0,942433	0,891711	1,000000					

**Table S2.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on apoptosis of non-stimulated (A) and LPS-stimulated (100 ng/ml) (B) human primary neutrophils. Neutrophils were incubated with iso-urolithin A, urolithin A and B (iUA, UA, UB), their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M, roscovitine at the concentration of 40  $\mu$ M and/or stimulated with LPS (10 ng/mL) for 20 h. Neutrophils' viability and apoptosis was determined by staining with propidium iodide (PI) and Annexin V-FITC after 6h in non-stimulated cells (A) and after 20h in LPS-stimulated cells.

without LPS (6h)												
	viable (%)	SD	<i>post hoc</i> (Dunnett)				<i>post hoc</i> (Dunnett)				<i>post hoc</i> (Dunnett)	
			<i>p</i> (<NST)	<i>p</i> (>NST)	early apoptosis (%)	SD	<i>p</i> (<NST)	<i>p</i> (>NST)	late apoptosis (%)	SD	<i>p</i> (<NST)	<i>p</i> (>NST)
NST	88,8	4,4			9,1	2,9			1,9	1,8		
LPS	93,2	3,5	0,999945	0,077183	5,2	2,7	0,035004	0,999969	1,4	0,9	0,572742	0,988726
iUA	90,4	2,4	0,986898	0,595769	8,4	2,2	0,730938	0,968313	1,1	0,5	0,397543	0,996664
GiUA	90,5	2,0	0,990130	0,999999	8,7	1,9	0,813820	0,943284	0,7	0,2	0,162098	0,999821
UA	91,2	2,2	0,996629	0,398937	6,9	1,2	0,274710	0,998804	1,7	0,9	0,784393	0,954204
GUA	89,9	2,5	0,972962	0,707114	9,0	1,6	0,869998	0,911451	1,0	0,6	0,308857	0,998373
UB	93,1	1,1	0,999939	0,083205	5,8	0,9	0,074388	0,999947	0,9	0,4	0,291258	0,998608
GUB	90,7	2,6	0,992621	0,508888	8,2	2,1	0,675748	0,977990	0,9	0,4	0,279834	0,998746
Roscovitine	29,3	0,6	0,000019	0,999978	67,4	0,4	0,999978	0,000019	3,1	0,3	0,999086	0,247267

with LPS 20h												
	viable (%)	SD	<i>post hoc</i> (Dunnett)				<i>post hoc</i> (Dunnett)				<i>post hoc</i> (Dunnett)	
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	early apoptosis (%)	SD	<i>p</i> (<LPS)	<i>p</i> (>LPS)	late apoptosis (%)	SD	<i>p</i> (<LPS)	<i>p</i> (>LPS)
NST	34,8	3,8	0,000005	0,999978	43,7	10,0	0,999979	0,000026	20,0	5,1	0,999978	0,000005
LPS	82,6	6,1			13,6	5,0			3,2	0,9		
iUA	79,1	5,9	0,637201	0,970780	17,2	5,2	0,985812	0,530331	2,8	1,1	0,820630	0,908687
GiUA	76,1	10,4	0,385180	0,994085	15,6	4,8	0,959505	0,703248	5,4	3,4	0,990567	0,453445
UA	78,5	5,6	0,585185	0,978580	17,6	4,3	0,988943	0,490735	3,4	1,1	0,889707	0,847534
GUA	76,5	9,7	0,417738	0,992573	16,6	5,1	0,978028	0,601980	5,1	3,2	0,984787	0,528806
UB	86,6	4,7	0,976311	0,602008	9,2	3,3	0,456219	0,991159	3,1	1,7	0,852507	0,885643
GUB	75,5	10,5	0,378365	0,994367	16,2	6,3	0,968035	0,664256	6,8	3,8	0,998470	0,224425
Roscovitine	3,8	2,9	0,000005	0,999978	82,7	0,8	0,999979	0,000024	11,7	1,1	0,999978	0,001460

**Table S3.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on LPS-induced TNF- $\alpha$  and IL-10 production. PMA-differentiated THP-1 cells were preincubated for 1h with iso-urolithin A, urolithin A and B (iUA, UA, UB) and their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M and stimulated with LPS (10 ng/mL).

	TNF- $\alpha$ 3h (%)	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	0,0	0,0	0,000022	0,999963	NST									
LPS	100,0	4,0			LPS	0,000136								
iUA	93,1	11,7	0,108434	0,999962	iUA	0,000136	0,540174							
GiUA	106,3	8,7	0,999921	0,283703	GiUA	0,000136	0,908662	0,115742						
UA	55,7	8,1	0,000022	0,999963	UA	0,000136	0,000136	0,000136	0,000136					
GUA	97,7	8,4	0,751957	0,994840	GUA	0,000136	0,999597	0,959170	0,638124	0,000136				
UB	89,1	8,9	0,004432	0,999963	UB	0,000136	0,054933	0,964476	0,011138	0,000136	0,423909			
GUB	98,1	9,6	0,791211	0,992500	GUB	0,000136	0,999842	0,905742	0,696014	0,000136	1,000000	0,272747		
Parthenolide	33,2	5,5	0,000022	0,999963	Parthenolide	0,000136	0,000136	0,000136	0,000136	0,000166	0,000136	0,000136	0,000136	0,000136

	TNF- $\alpha$ 6h (%)	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	2,9	1,0	0,000021	0,999967	NST									
LPS	100,0	12,5			LPS	0,000144								
iUA	134,6	9,8	0,999967	0,000021	iUA	0,000144	0,000144							
GiUA	109,5	9,9	0,999959	0,139040	GiUA	0,000144	0,717004	0,000777						
UA	75,2	7,7	0,000028	0,999967	UA	0,000144	0,000924	0,000144	0,000145					
GUA	94,1	8,7	0,438330	0,999323	GUA	0,000144	0,973293	0,000144	0,127700	0,024815				
UB	112,1	12,3	0,999967	0,041287	UB	0,000144	0,398475	0,003483	0,999905	0,000144	0,038329			
GUB	101,5	10,1	0,973598	0,862002	GUB	0,000144	0,999999	0,000146	0,863149	0,000440	0,906987	0,574407		
Parthenolide	41,1	8,8	0,000021	0,999967	Parthenolide	0,000144	0,000144	0,000144	0,000144	0,000145	0,000144	0,000144	0,000144	0,000144

	IL-10 24h (%)	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	74,2	21,6	0,000096	0,999962	NST									
LPS	100,0	5,0			LPS	0,001091								
iUA	141,4	21,6	0,999962	0,000023	iUA	0,000134	0,000466							
GiUA	98,3	15,7	0,903330	0,975355	GiUA	0,154255	1,000000	0,000286						
UA	97,5	24,3	0,869079	0,984106	UA	0,081641	0,999997	0,000242	1,000000					
GUA	110,4	7,4	0,999863	0,358578	GUA	0,001233	0,939385	0,019344	0,902516	0,821682				
UB	115,7	15,9	0,999962	0,081222	UB	0,000159	0,534456	0,098237	0,561303	0,332117	0,999316			
GUB	111,2	11,1	0,999939	0,305872	GUB	0,000902	0,909641	0,025232	0,865956	0,768436	1,000000	0,999796		
Parthenolide	69,4	2,8	0,015201	0,999962	Parthenolide	0,999992	0,369049	0,000157	0,450495	0,486247	0,072814	0,024938	0,062485	

**Table S4.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on LPS-induced TNF- $\alpha$  and TGF- $\beta$ 1 mRNA expression in THP-1 macrophages. PMA-differentiated THP-1 cells were preincubated for 1h with iso-urolithin A, urolithin A and B (iUA, UA, UB) and their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M and stimulated with LPS (10 ng/mL) for 24 h. Real-time RT-PCR analysis was performed as described in Materials and methods section. Changes in mRNA expression were normalized to  $\beta$ -actin. Parthenolide at the concentration of 5  $\mu$ M was used as a positive control.

	TNF- $\alpha$ mRNA expression	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	0,307	0,100	0,000021	0,999977	NST									
LPS	1,000	0,000			LPS	0,000157								
iUA	1,656	0,357	0,999977	0,000023	iUA	0,000151	0,000167							
GiUA	1,259	0,169	0,999963	0,048627	GiUA	0,000151	0,264518	0,015475						
UA	0,696	0,050	0,019004	0,999974	UA	0,018594	0,118597	0,000151	0,000376					
GUA	1,075	0,099	0,982345	0,628681	GUA	0,000152	0,997784	0,000290	0,687112	0,023592				
UB	0,885	0,037	0,446890	0,994711	UB	0,000303	0,965475	0,000152	0,026219	0,659436	0,647125			
GUB	0,966	0,069	0,793004	0,947672	GUB	0,000166	0,999994	0,000157	0,145385	0,221474	0,974735	0,996228		
Parthenolide	0,573	0,070	0,000996	0,999977	Parthenolide	0,237884	0,007535	0,000151	0,000158	0,948326	0,001252	0,100151	0,016858	

  

	TGF- $\beta$ 1 mRNA expression	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	1,938	0,319	0,999977	0,000020	NST									
LPS	1,000	0,000			LPS	0,000152								
iUA	1,276	0,061	0,999942	0,077760	iUA	0,000366	0,382545							
GiUA	1,024	0,129	0,927998	0,836339	GiUA	0,000152	1,000000	0,498991						
UA	1,603	0,250	0,999977	0,000125	UA	0,167581	0,000972	0,187941	0,001544					
GUA	1,029	0,109	0,935048	0,822715	GUA	0,000152	1,000000	0,526359	1,000000	0,001722				
UB	1,350	0,167	0,999973	0,021201	UB	0,001312	0,130620	0,999332	0,190211	0,494948	0,206003			
GUB	0,980	0,053	0,846782	0,921913	GUB	0,000152	1,000000	0,298611	0,999989	0,000683	0,999973	0,094077		
Parthenolide	1,256	0,184	0,999905	0,105615	Parthenolide	0,000289	0,478509	1,000000	0,602079	0,137741	0,629843	0,996436	0,383650	

**Table S5.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on LPS-induced TNF- $\alpha$ . PBMCs were incubated with iso-urolithin A, urolithin A and B (iUA, UA, UB) and their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M and stimulated with LPS (100 ng/mL).

	TNF- $\alpha$ (%)	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)							
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	
<b>NST</b>	0,528	0,587	<b>0,000024</b>	0,999979	<b>NST</b>							
<b>LPS</b>	100,000	0,000			<b>LPS</b>	<b>0,000150</b>						
<b>iUA</b>	45,587	3,325	<b>0,000029</b>	0,999979	<b>iUA</b>	<b>0,002912</b>	<b>0,000407</b>					
<b>GiUA</b>	95,550	5,463	0,767267	0,982755	<b>GiUA</b>	<b>0,000150</b>	0,999774	<b>0,000963</b>				
<b>UA</b>	8,198	3,478	<b>0,000024</b>	0,999979	<b>UA</b>	0,869576	<b>0,000150</b>	<b>0,017525</b>	<b>0,000150</b>			
<b>GUA</b>	131,429	24,683	0,999979	<b>0,000526</b>	<b>GUA</b>	<b>0,000150</b>	<b>0,003358</b>	<b>0,000150</b>	<b>0,024751</b>	<b>0,000150</b>		
<b>UB</b>	95,059	10,860	0,709656	0,988972	<b>UB</b>	<b>0,000150</b>	0,998317	<b>0,001069</b>	1,000000	<b>0,000150</b>	<b>0,003282</b>	
<b>GUB</b>	101,317	4,807	0,951260	0,883040	<b>GUB</b>	<b>0,000150</b>	1,000000	<b>0,000332</b>	0,998762	<b>0,000150</b>	<b>0,019703</b>	0,992691

**Table S6.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on LPS-induced NF- $\kappa$ Bp65 nuclear translocation and ERK1/2 phosphorylation in THP-1 macrophages. PMA-differentiated THP-1 cells were preincubated for 1h with iso-urolithin A, urolithin A and B (iUA, UA, UB) and their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M and stimulated with LPS (10 ng/mL) for 35 min. Parthenolide at the concentration of 5  $\mu$ M was used as a positive control. Western blotting analysis was performed as described in Materials and methods. Densitometric analysis was performed using ImageJ software. Changes in p65 protein levels in nuclear fractions were normalized to p65 protein levels in cytosolic fractions. The changes in phospho-ERK1/2 were normalized to total ERK1/2.

	p65 nuclear translocation	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	0,000	0,000	0,000020	0,999971	NST									
LPS	1,000	0,000			LPS	0,000143								
iUA	0,683	0,020	0,006501	0,999971	iUA	0,000143	0,046530							
GiUA	0,514	0,112	0,000049	0,999971	GiUA	0,000232	0,000387	0,715946						
UA	0,782	0,032	0,045704	0,999923	UA	0,000143	0,186041	0,981363	0,153234					
GUA	0,405	0,117	0,000020	0,999971	GUA	0,003801	0,000146	0,120306	0,964913	0,008669				
UB	0,831	0,042	0,192656	0,999520	UB	0,000143	0,712987	0,832413	0,047095	0,999862	0,001964			
GUB	0,483	0,112	0,000029	0,999971	GUB	0,000418	0,000223	0,501859	0,999995	0,073272	0,996111	0,019834		
Parthenolide	0,000	0,000	0,000020	0,999971	Parthenolide	1,000000	0,000143	0,000143	0,000232	0,000143	0,003801	0,000143	0,000418	

  

	ERK1/2 phosphorylation	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
NST	0,170	0,094	0,000082	0,999553	NST									
LPS	1,000	0,000			LPS	0,661334								
iUA	1,921	0,423	0,999869	0,049058	iUA	0,015567	0,043506							
GiUA	1,379	0,260	0,987035	0,580605	GiUA	0,205182	0,994464	0,950585						
UA	3,153	0,777	0,999977	0,000226	UA	0,000160	0,001774	0,187516	0,013816					
GUA	1,140	0,214	0,944748	0,800609	GUA	0,468306	0,999997	0,726788	0,999793	0,003760				
UB	1,042	0,077	0,908841	0,865942	UB	0,603145	1,000000	0,593587	0,997531	0,002220	1,000000			
GUB	0,791	0,064	0,744861	0,962440	GUB	0,898517	0,999927	0,278225	0,923218	0,000623	0,996912	0,999712		
Parthenolide	0,793	0,092	0,746568	0,962007	Parthenolide	0,896919	0,999932	0,280234	0,924549	0,000629	0,997031	0,999729	1,000000	

**Table S7.** Effects of tested urolithins and respective glucuronides at the concentration of 40  $\mu$ M on f-MLP-induced  $\beta$ -glucuronidase release from human primary neutrophils. After isolation, neutrophils were resuspended in HBSS with iso-urolithin A, urolithin A and B (iUA, UA, UB) and their respective glucuronides (GiUA, GUA, GUB) at the concentration of 40  $\mu$ M primed with cytochalasin B (10  $\mu$ M) for 5 min and then stimulated with f-MLP (1  $\mu$ M) for 10 min. Genistein at the concentration of 40  $\mu$ M was used as a positive control.

	$\beta$ -glucuronidase (%)	SD	<i>post hoc</i> (Dunnett)		<i>post hoc</i> (Tukey)									
			<i>p</i> (<LPS)	<i>p</i> (>LPS)	NST	LPS	iUA	GiUA	UA	GUA	UB	GUB		
<b>NST</b>	60,9	7,6	<b>0,013047</b>	0,999972	<b>NST</b>									
<b>LPS</b>	100,0	0,0			<b>LPS</b>	0,087996								
<b>iUA</b>	96,2	3,5	0,788193	0,926403	<b>iUA</b>	0,145624	0,999999							
<b>GiUA</b>	79,2	6,8	0,308796	0,996707	<b>GiUA</b>	0,721582	0,888322	0,961493						
<b>UA</b>	40,8	14,6	<b>0,000917</b>	0,999978	<b>UA</b>	0,994139	<b>0,013853</b>	<b>0,006582</b>	0,248087					
<b>GUA</b>	85,4	8,4	0,457707	0,990301	<b>GUA</b>	0,466331	0,984274	0,994364	0,999965	<b>0,044542</b>				
<b>UB</b>	61,8	8,3	<b>0,030522</b>	0,999958	<b>UB</b>	0,999640	0,253034	0,213364	0,956335	0,781710	0,665537			
<b>GUB</b>	101,6	7,6	0,896030	0,839290	<b>GUB</b>	0,070802	1,000000	0,999963	0,843751	<b>0,002479</b>	0,934993	0,097136		
<b>Genistein</b>	92,2	14,6	0,679385	0,962420	<b>Genistein</b>	0,233997	0,999809	0,999997	0,992350	<b>0,013444</b>	0,999779	0,349463	0,997926	