

Supplementary Information

Inhibition of lysophosphatidic acid receptors 1 and 3 attenuates atherosclerosis development in LDL-receptor deficient mice

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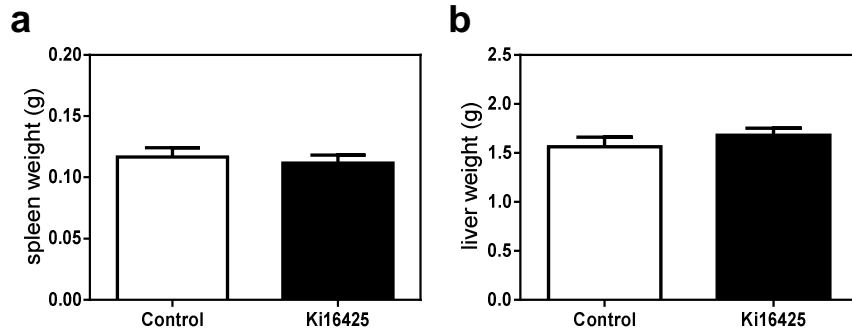


Figure 1: Treatment with Ki16425 does not affect the spleen or liver weight of mice. (a) The spleen weight between the control and Ki16425 treated group showed no significant difference ($P=0.63$) at the end of the study. **(b)** Similarly the liver weight between the control and Ki16425 groups was not substantially different ($P=0.38$). All values ($n=13$ /grp; spleen and $n=6$ /grp; liver) are depicted as mean \pm SEM.

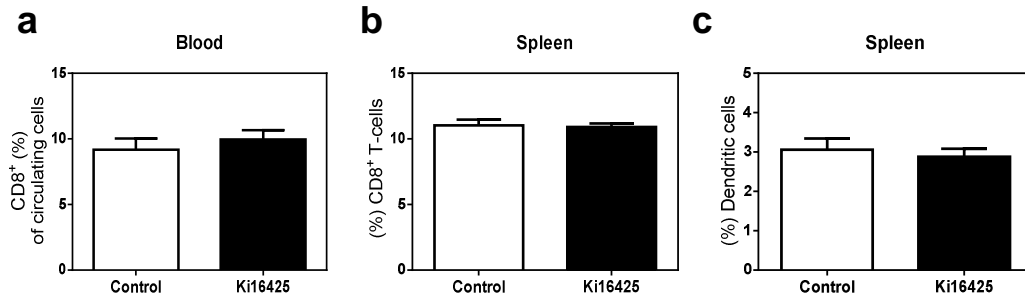


Figure 2. LPA_{1/3} inhibition did not affect the amount of CD8⁺ T-lymphocytes or dendritic cells. The amount of CD8⁺ T-cells in the (a) circulation and (b) spleen was not affected by treatment with Ki16425. (P=0.50) and (P=0.78). (c) The overall percentage of dendritic cells present in the spleen showed no differences among the control and treated mice (P=0.60). (All values (n=12/grp) are depicted as mean±SEM).

<i>Gene</i>	<i>Forward primer (5'-3')</i>	<i>Reverse primer (3'-5')</i>
CCL2	GCATCT GCCCTAAGGTCT TCA	TTCACTGTCACACTGGTCACTCCTA
CD68	TGCCTGACAAGGGACACTTCGGG	GCGGGTGATGCAGAAGGCGATG
CXCL1	TTGACCCTGAAGCTCCCTTG	AGGTGCCATCAGAGCAGTC
I-CAM 1	GTCCGCTTCCGCTACCATCA	GGTCCTGCCTACTTGCTGCC
Rpl27	CGCCAAGCGATCCAAGATCAAGTCC	AGCTGGGTCCCTGAACACATCCTTG
β -actin	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA

Supplementary Table 1. qPCR gene primer sequences: All gene expression analysis was performed using two housekeeping genes (β -actin and Rpl27). Abbreviations: Chemokine C-C motif ligand 2 (CCL2); cluster of differentiation 68 (CD68); chemokine C-X-C motif ligand 1 (CXCL1); Intercellular adhesion molecule-1 (ICAM-1); 60s ribosomal protein ligand 27 (Rpl27).

Antibody	Fluorochrome	Clone	Company
CD11b	eFluor 450	M1/70	Ebioscience
Ly6C	PercP Cy5.5	HK1.4	Ebioscience
Ly6G	PE	1A8	BD Biosciences
CCR2	APC	#475301	R&D Systems
CD4	PercP	Rm4-5	BD Biosciences
CD25	FITC	PC61.5	Ebioscience
NK1.1	FITC	PK136	Ebioscience
CD8a	PercP	53-6.7	BD Biosciences
MHC-II	eFluor 450	AF6-120.1	Ebioscience
CD11c	APC	N418	Ebioscience
<i>FoxP3</i>	<i>eFluor 450</i>	<i>FJK-16s</i>	Ebioscience
<i>T-bet</i>	<i>Alexa -Fluor 660</i>	<i>eBio4B10</i>	Ebioscience
<i>Helios</i>	<i>Alexa-Fluor 647</i>	<i>22F6</i>	Ebioscience

Supplementary Table 2. List of extracellular and *intracellular* antibodies used.