(A)

5-LOX (Amino Acid 1-671) Sequence coverage 81.8%
MPSYTVTVAT GSQEHAGTDD YIYLSLVGSA GCSEKHLLDK GSFERGAVDS YDVTVDEELG
EIQLVRIEKR KYGSNDDWYL KYITLKTPHG DYIEFPCYRW ITGDVEVVLR DGRAKLARDD
QIHILKQHRR KELETRQKQY RWMEWNPGFP LSIDAKCHKD LPRDIQFDSE KGVDFVLNYS
KAMENLFINR FMHMFQSSWN DFADFEKIFV KISNTISERV MNHWQEDLMF GYQFLNGANP
VLIRRCTELP EKLPVTTEMV ECSLERQLSL EQEVQQGNIF IVDFELLDGI DANKTDPCTL
QFLAAPICLL YKNLANKIVP IAIQLNQIPG DENPIFLPSD AKYDWLLAKI WVRSSDFHVH
QTITHLLRTH LVSEVFGIAM YRQLPAVHPI FKLLVAHVRF TIAINTKARE QLICECGLFD
KANATGGGGH VQMVQRAMKD LTYASLCFPE AIKARGMESK EDIPYYFYRD DGLLVWEAIR
TFTAEVVDIY YEGDQVVEED PELQDFVNDV YVYGMRGRKS SGFPKSVKSR EQLSEYLTVV
IFTASAQHAA VNFGQYDWAS WIPNAPPTMR APPPTAKGVV TIEQIVDTLP DRGRSCWHLG
AVWALSQFQE NELFLGMYPE EHFIEKPVKE AMARFRKNLE AIVSVIAERN ENLQLPYYYL
DPDRIPNSVA I

(B)

TLR4 (Amino Acid 23-629) Sequence coverage 84.8%
PESWEPCVEVVPNITYQCMELNFYKIPDNLPFSTKNLDLSFNPLRHLGSYSFFSFPELQV
LDLSRCEIQTIEDGAYQSLSHLSTLILTGNPIQSLALGAFSGLSSLQKLVAVETNLASLE
NFPIGHLKTLKELNVAHNLIQSFKLPEYFSNLTNLEHLDLSSNKIQSIYCTDLRVLHQMP
LLNLSLDLSLNPMNFIQPGAFKEIRLHKLTLRNNFDSLNVMKTCIQGLAGLEVHRLVLGE
FRNEGNLEKFDKSALEGLCNLTIEEFRLAYLDYYLDDIIDLFNCLTNVSSFSLVSVTIER
VKDFSYNFGWQHLELVNCKFGQFPTLKLKSLKRLTFTSNKGGNAFSEVDLPSLEFLDLSR
NGLSFKGCCSQSDFGTTSLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSEFSV
FLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMAGNSFQENFLPDIFTELRNLTFLD
LSQCQLEQLSPTAFNSLSSLQVLNMSHNNFFSLDTFPYKCLNSLQVLDYSLNHIMTSKKQ
ELQHFPSSLAFLNLTQNDFACTCEHQSFLQWIKDQRQLLVEVERMECATPSDKQGMPVL
S
LNITCQM

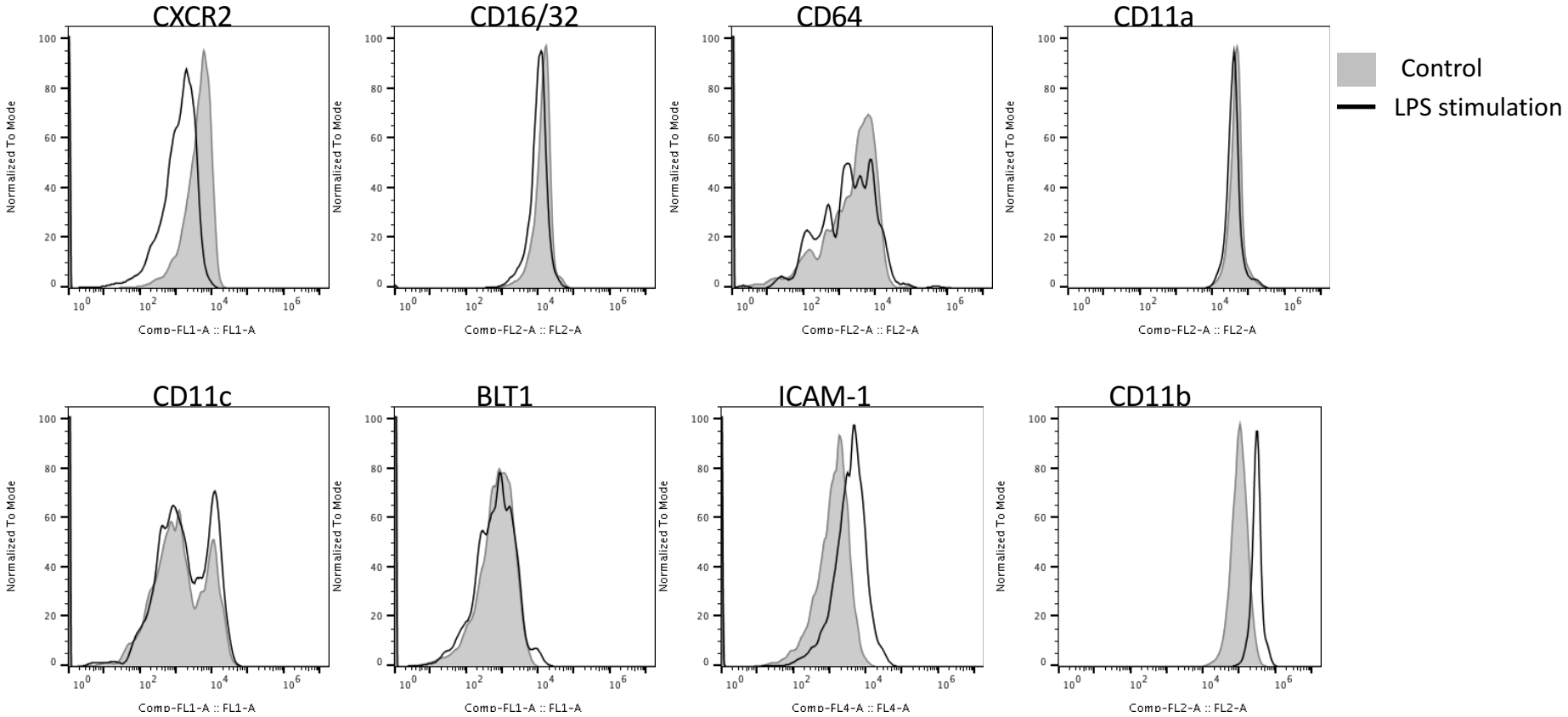
MD-2 (Amino acid 19-160) Sequence coverage 94.4%
EAQKQYWVCNSSDASISYTYCDKMQYPISINVNPCIELKGSKGLLHIFYIPRRDLKQLYF
NLYITVNTMNLPKRKEVICRGSDDDYSFCRALKGETVNTTISFSFKGIKFSKGKYKCVVE
AISGSPEEMLFCLEFVILHQPNSN

(C)

TLR4 (Amino acid 23-629) Sequence coverage 84.8%
PESWEPCVEVVPNITYQCMELNFYKIPDNLPFSTKNLDLSFNPLRHLGSYSFFSFPELQV
LDLSRCEIQTIEDGAYQSLSHLSTLILTGNPIQSLALGAFSGLSSLQKLVAVETNLASLE
NFPIGHLKTLKELNVAHNLIQSFKLPEYFSNLTNLEHLDLSSNKIQSIYCTDLRVLHQMP
LLNLSLDLSLNPMNFIQPGAFKEIRLHKLTLRNNFDSLNVMKTCIQGLAGLEVHRLVLGE
FRNEGNLEKFDKSALEGLCNLTIEEFRLAYLDYYLDDIIDLFNCLTNVSSFSLVSVTIER
VKDFSYNFGWQHLELVNCKFGQFPTLKLKSLKRLTFTSNKGGNAFSEVDLPSLEFLDLSR
NGLSFKGCCSQSDFGTTSLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSEFSV
FLSLRNLIYLDISHTHTRVAFNGIFNGLSSLEVLKMAGNSFQENFLPDIFTELRNLTFLD
LSQCQLEQLSPTAFNSLSSLQVLNMSHNNFFSLDTFPYKCLNSLQVLDYSLNHIMTSKKQ
ELQHFPSSLAFLNLTQNDFACTCEHQSFLQWIKDQRQLLVEVERMECATPSDKQGMPVLS
LNITCQM

MD-2 (Amino acid 19-160) 80.6%
EAQKQYWVCNSSDASISYTYCDKMQYPISINVNPCIELKGSKGLLHIFYIPRRDLKQLYF
NLYITVNTMNLPKRKEVICRGSDDDYSFCRALKGETVNTTISFSFKGIKFSKGKYKCVVE
AISGSPEEMLFCLEFVILHQPNSN

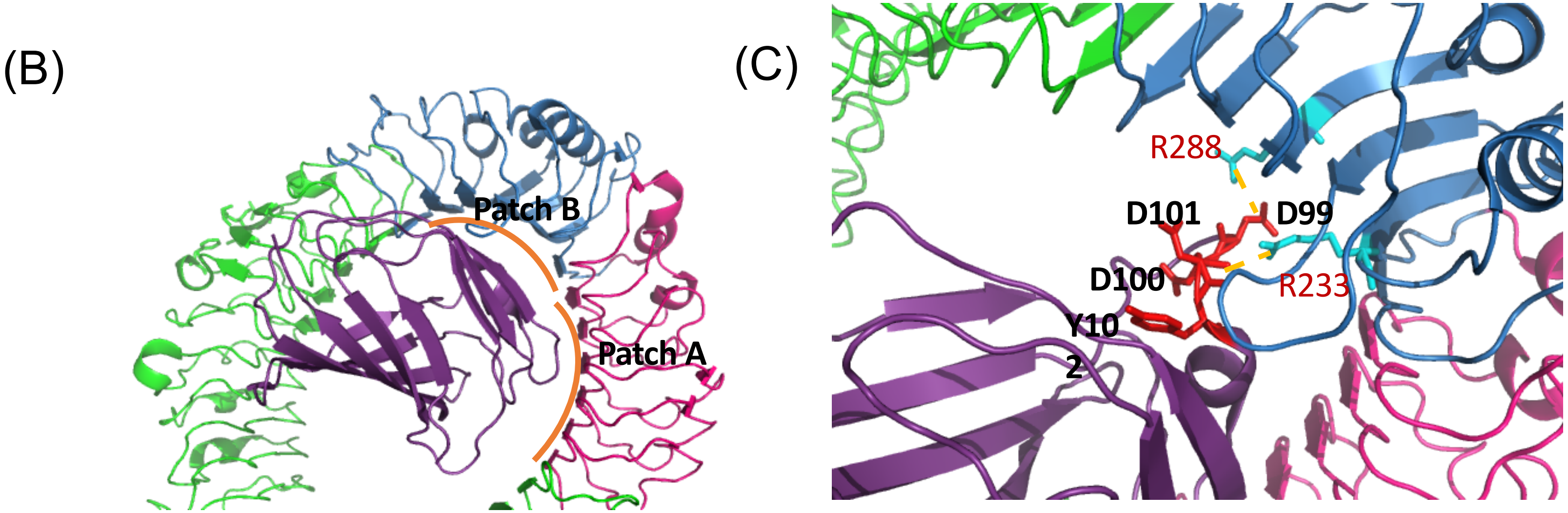
Suppl Fig. 1. Photolabeling experiment of 5-lipoxygenase and TLR4-MD-2 complex
(A). Stable 5-lipoxygenase protein was subjected to photolabeling experiment using azi-isoflurane. Amino acids shown in orange were sequence-covered in mass spectrometry analysis. No adducted residues were found. (B,C). Sequence coverage and adducted amino acids of TLR4-MD-2 complex photolabeled with photoactivatable isoflurane (B) and sevoflurane (C) are shown (azi-isoflurane, azi-sevoflurane, respectively). Organ indicates covered sequences and red indicates adducted amino acids.



Suppl Fig. 2. The effect of LPS stimulation on the expression of adhesion receptors, chemotaxis receptors, and phagocytosis receptors on neutrophils
Whole blood was stimulated with LPS. The expression of adhesion receptors CD11a, CD11b, CD11c, chemotaxis receptors BLT1 and CXCR2, complement receptors CD11b, CD11c and Fc receptors FcR II/III (CD16/32) and FcR I (CD64) on neutrophils was examined. Representative figures are shown.

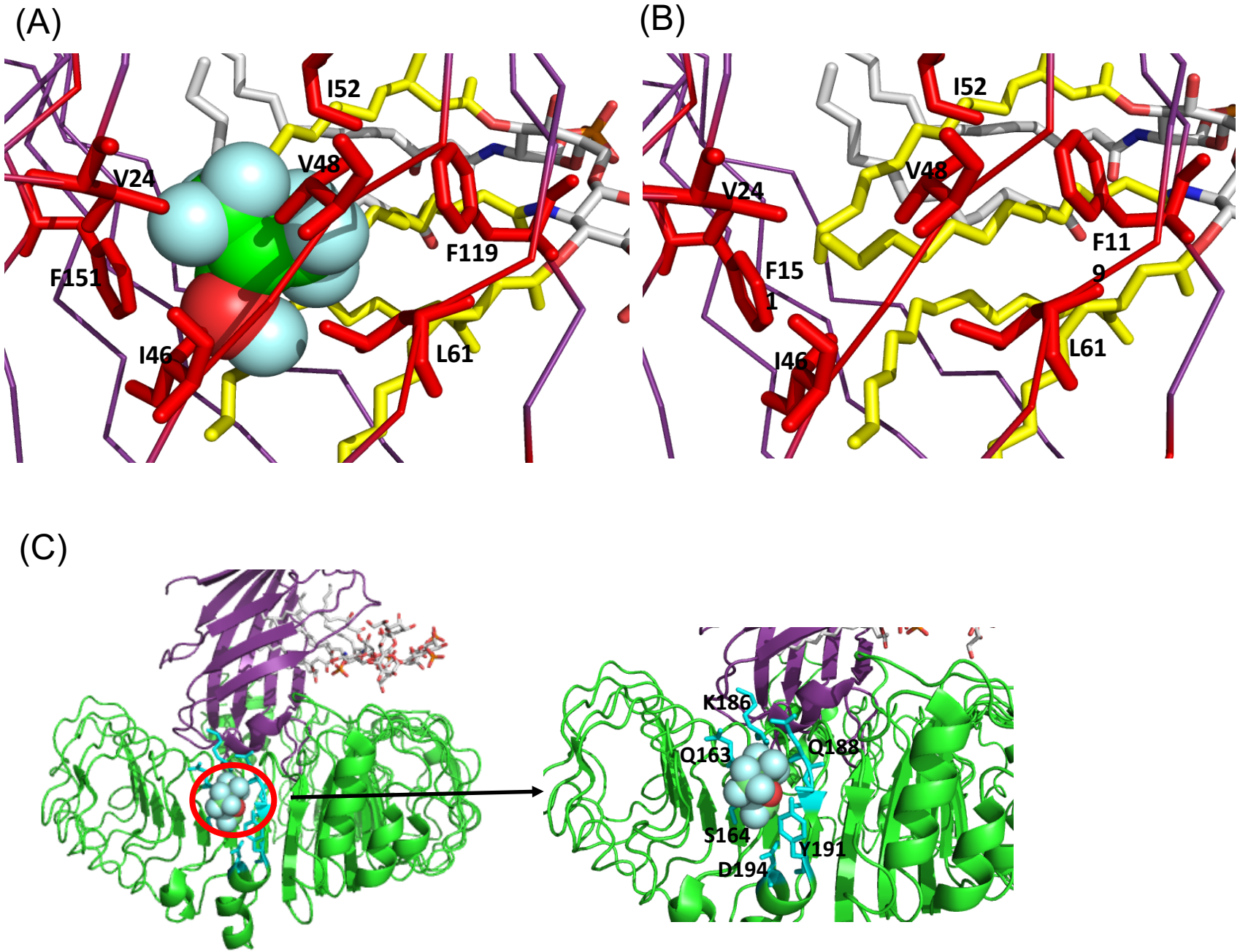
(A)

	80	90	100	110	120
Human MD-2	LKQLYFNLYI	TVNTMNLPKR	KEV ICRGS**DD**	**D**YSFC**R**AL**K**G	ETVNTT ISFS
Mouse MD-2	LKYLYFNLFI	SVNS I ELPKR	KEVLCHGH**DD**	**D**YSFC**R**AL**K**G	ETVNTS IPFS



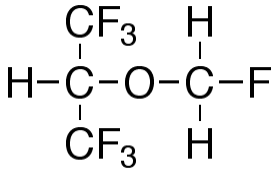
Suppl Fig. 3 The interaction of MD-2 with TLR4

(A) Sequence alignment of human and mouse MD-2. Residues known to be involved in TLR4 binding are shown in bold letter. Underlined amino acids showed the ones near docked isoflurane and sevoflurane. Cysteines that form disulfide bond are highlighted in yellow. (B) Hydrophobic patch was shown in hotpink (Patch A) and marine blue (patch B). The negative charged residues in patch A interact with the positive charged residues in MD-2 and the positive charged residues in patch B interact with the negative charged residues in MD-2. (C) Polar interactions were shown in orange.

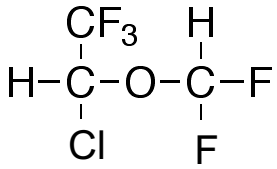


Suppl Fig 4. Sevoflurane binding to TLR4-MD-2 complex
(A) Sevoflurane binds to the interface between MD-2 and Lipid A. Nearby residues in MD-2 and Lipid A from sevoflurane were shown in red and yellow, respectively. (B) Sevoflurane was removed from the image in A to show interaction between MD-2 and Lipid A. (C). The landscape view of sevoflurane binding site near TLR4-Gln-163 is shown on the left. Blow-out image is shown on the right. Amino acids near sevoflurane are shown in cyan.

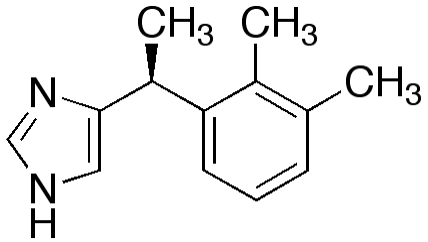
(A)



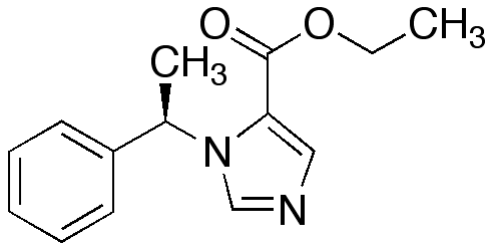
Sevoflurane



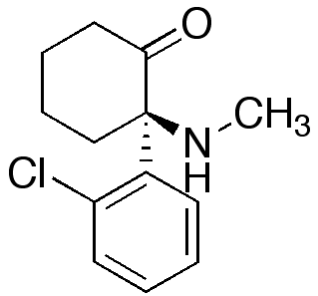
Isoflurane



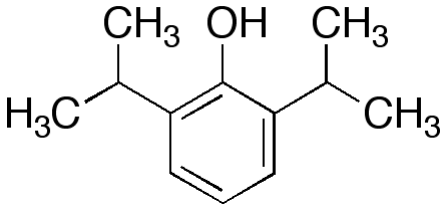
Dexmedetomidine



Etomidate



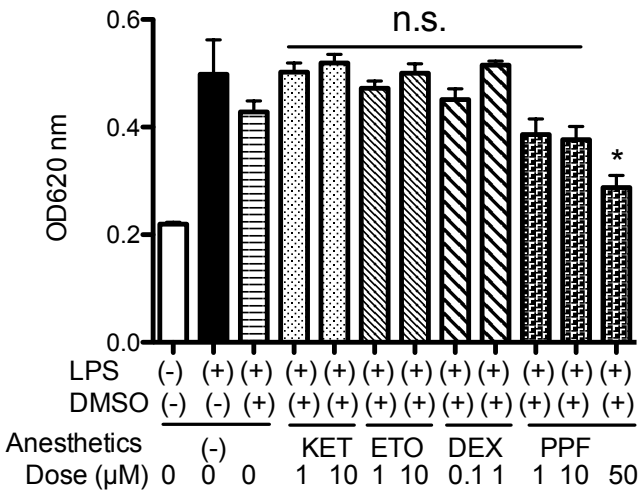
Ketamine



Propofol

Volatile anesthetics | **Intravenous anesthetics**

(B)



Suppl Fig 5. Structure of volatile and intravenous anesthetics

Suppl Fig. 5. The effect of intravenous anesthetics on TLR4 activation
(A) Structures of volatile anesthetics (isoflurane, sevoflurane) and intravenous anesthetics (dexmedetomidine, etomidate, ketamine, propofol) are shown. (B) TLR4 NFκB activation was tested under various intravenous anesthetics at different concentrations. Highest concentrations for each drug are above clinically relevant concentrations. Data were shown as mean +/- S.D. of quadruplicates. Statistical analysis was performed using one-way ANOVA with Bonferroni *post hoc* analysis. n.s. = not significant versus LPS, dimethylsulfoxide (DMSO) positive condition. KET, ketamine; ETO, etomidate; DEX, dexmedetomidine; PPF, propofol.