

1 **Targeting of immune cells by dual TLR2/7 ligands suppresses features of**
2 **allergic T_H2 immune responses in mice**

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12 **Short title: Dual TLR2/7-ligands as allergy adjuvants**

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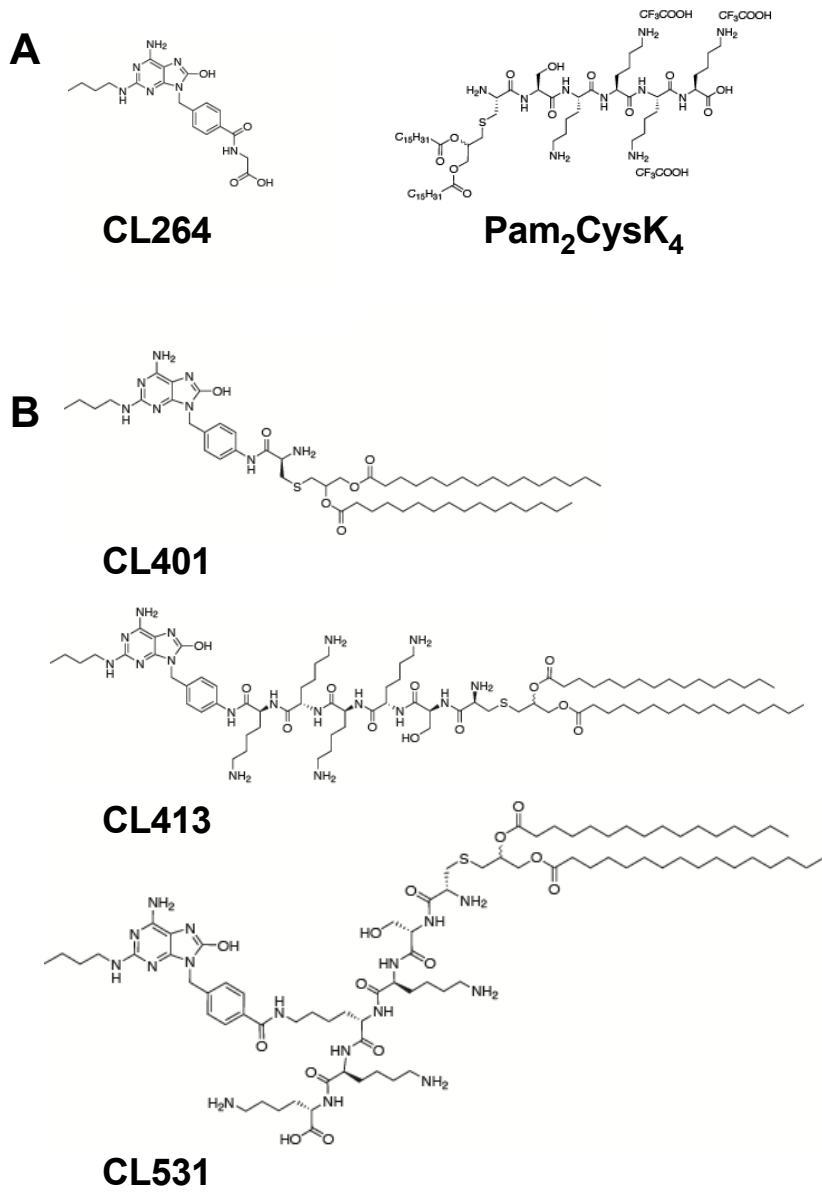
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20 Repository figures:

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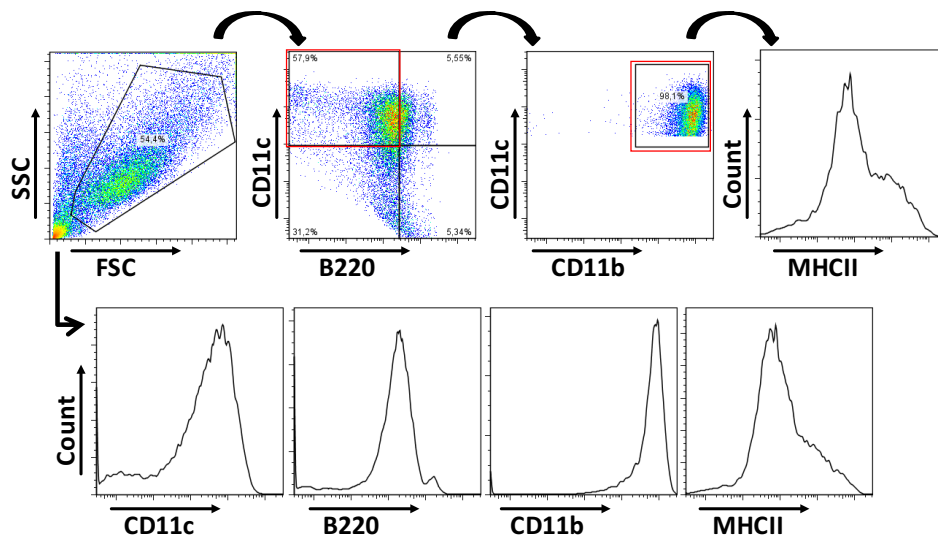


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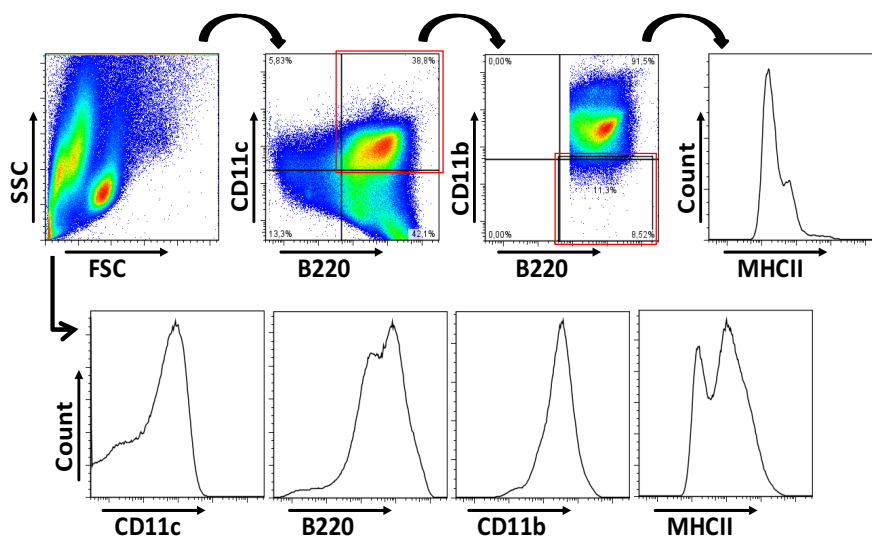
23 Repository figure 1: Chemical structures of single (Pam₂CysK₄ and CL264, A)

24 and dual (CL401, CL413, and CL431, B) TLR-ligands used in this study.

A Gating strategy and phenotype of myeloid dendritic cells ($CD11b^+CD11c^+B220^-$)



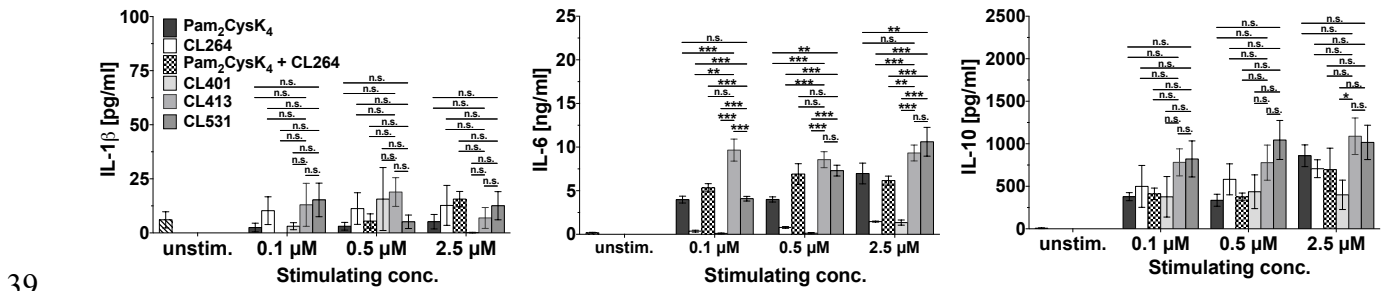
B Gating strategy and phenotype of plasmacytoid dendritic cells ($CD11b^-CD11c^+B220^+$)



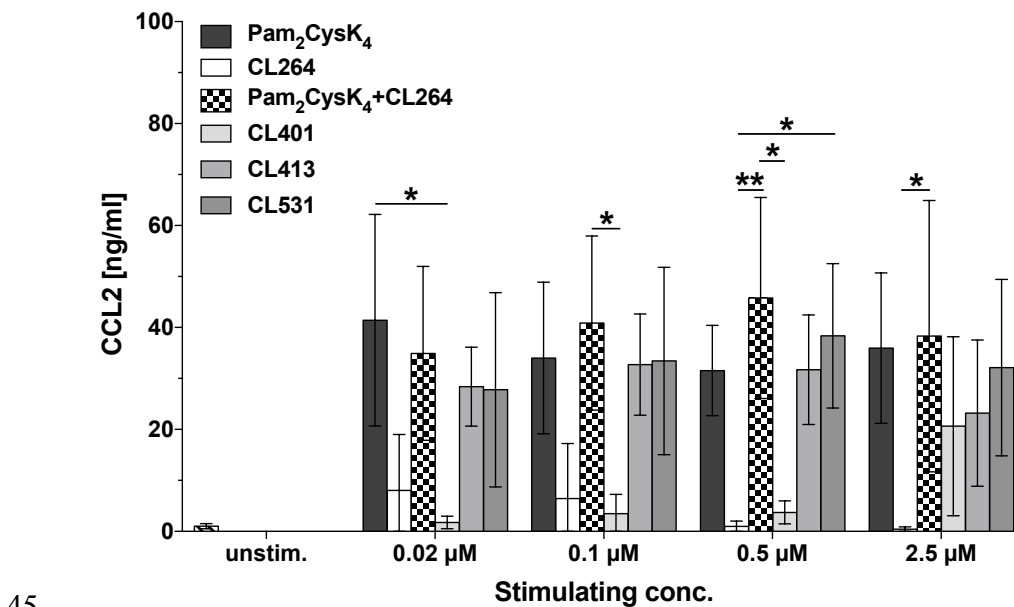
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26 **Repository Fig. 2: Gating strategies and phenotypes of bone marrow-derived**
27 **myeloid and plasmacytoid dendritic cells used for this study.** Gating strategies
28 for bone marrow-derived myeloid (**A**, BMmDC, $CD11b^+CD11c^+B220^-$) and
29 plasmacytoid (**B**, **BMpDC**, $CD11b^-CD11c^+B220^+$) dendritic cells. The upper row
30 demonstrates the gating strategy, whereas the lower row shows the expression levels
31 of the indicated surface markers in ungated mDC and pDC cultures on day 8 of
32 differentiation, respectively. For the identification of $CD11b^+CD11c^+B220^-$ BMmDCs
33 GM-CSF-cultures were first gated on $B220^-CD11c^+$ cells (indicated by the red box),

34 BMmDCs were then identified within this gate as CD11c⁺CD11b⁺ double positive cells
 35 (A). For the identification of CD11b⁻CD11c⁺B220⁺ BMpDCs Flt-3L-cultures were first
 36 gated on B220⁺CD11c⁺ cells (indicated by the red box), BMpDCs were then identified
 37 within this gate as CD11c⁺CD11b⁻ cells (B).
 38



40 **Repository Fig. 3: The dual TLR2/7-ligands CL413 and CL531 also activate**
 41 **dendritic cells in Flt-3L-cultures.** Cytokine secretion from BALB/c-derived Flt-3L-
 42 cultures stimulated as indicated after 8 days of differentiation. Data are mean results
 43 of three independent experiments ± SD.
 44

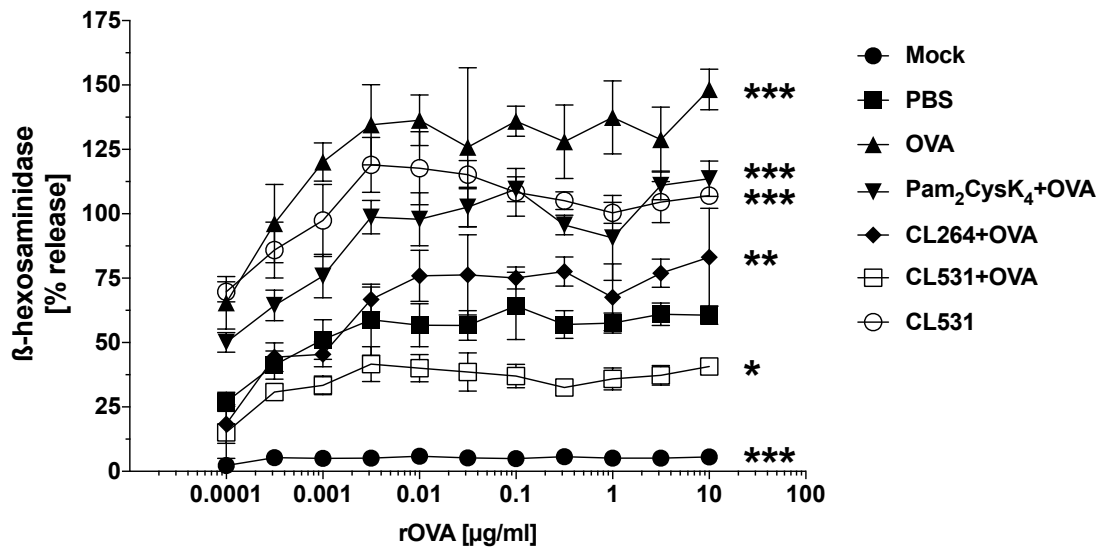


46 **Repository figure 4: CL413 and CL531, but not CL401 or CL264, induce levels of**
 47 **CCL2 secretion from mouse epithelial cells similar to that induced by**

48 **Pam₂CysK₄**. CCL2 secretion from LA-4 epithelial cells stimulated for 24 h with
 49 equimolar amounts of the different TLR2/7-ligands. Data are mean results of two
 50 independent experiments \pm SD.
 51

Group name	Vaccination (i.n.)	Sensitization (i.p.)	Challenge (via food pellets)
Mock	PBS	PBS	Conventional diet
PBS	PBS	2 x OVA (10 μ g) + Alum (2 mg)	Egg white diet
OVA	rOVA (10 μ g)	2 x OVA (10 μ g) + Alum (2 mg)	Egg white diet
Pam ₂ CysK ₄ +OVA	Pam ₂ CysK ₄ (0.5 mM) + rOVA (10 μ g)	2 x OVA (10 μ g) + Alum (2 mg)	Egg white diet
CL264+OVA	CL264 (0.5 mM) + rOVA (10 μ g)	2 x OVA (10 μ g) + Alum (2 mg)	Egg white diet
CL531	CL531 (0.5 mM)	2 x OVA (10 μ g) + Alum (2 mg)	Egg white diet
CL531+OVA	CL531 (0.5 mM) + rOVA (10 μ g)	2 x OVA (10 μ g) + Alum (2 mg)	Egg white diet

52
 53 **Repository figure 5: Experimental groups in prophylactic vaccination**
 54 **experiment.**



56
 57 **Repository figure 6: Sera obtained after prophylactic vaccination with CL531**
 58 **induce reduced mediator release from RBL 2H3 cells upon crosslinking with**

59 **OVA.** β -hexosaminidase release from RBL 2H3 cells upon crosslinking with OVA was
60 performed with sera from the final bleed. Results are means of three technical
61 replicates measured using the same serum pool. Indicated are the statistical
62 differences from the PBS group for the highest stimulation concentration (10 $\mu\text{g}/\text{mL}$
63 OVA).