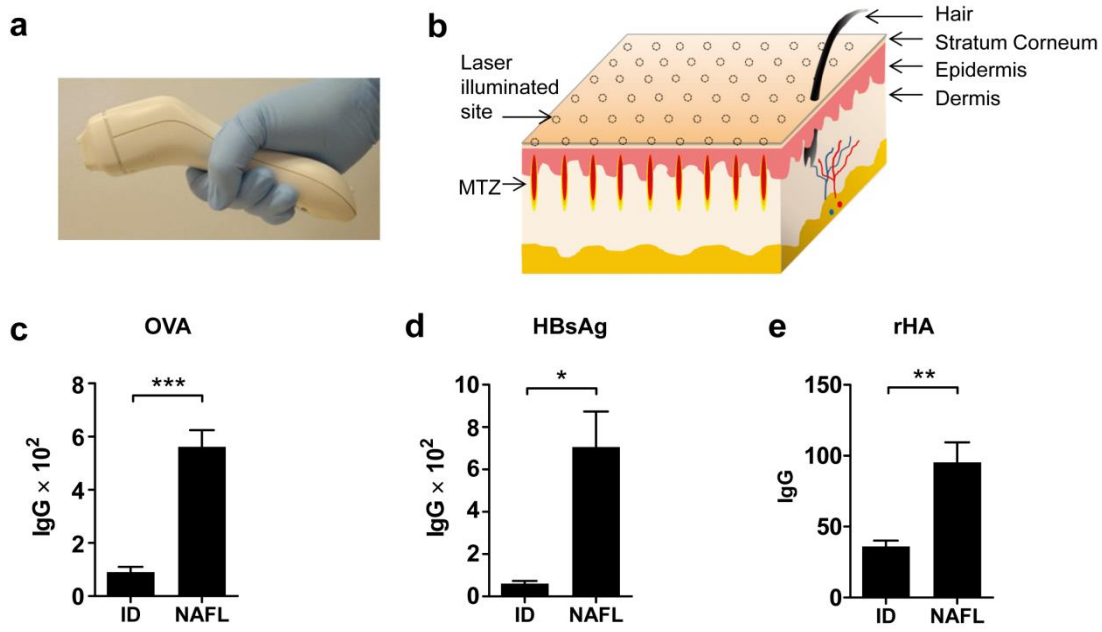
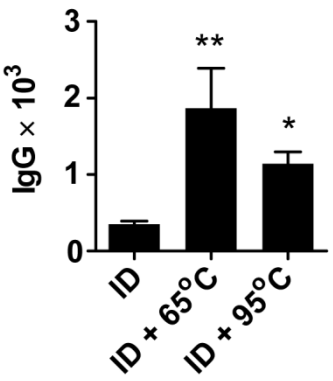
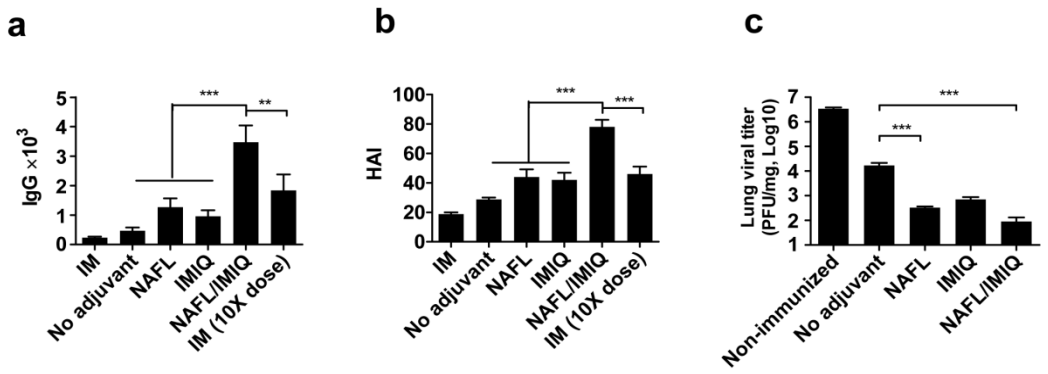


Supplementary Figure 1. NAFL enhanced immunity of other vaccines
(**a**) An over-the-counter, hand-held non-ablative fractional laser (NAFL). (**b**) Depiction of a MTZ array generated by NAFL. (**c-e**) IgG production in BALB/c mice ID immunized with ovalbumin (OVA, 10 μg) (**c**) n=8, HBsAg (0.25 μg) (**d**) n=8, or rHA (A/Brisbane/59/2007 H1N1, 100 ng) n=6 at the site of laser treatment for 4 weeks. Data are presented as mean ± s.e.m. Statistical significance was analyzed by *t*-test.*, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively.

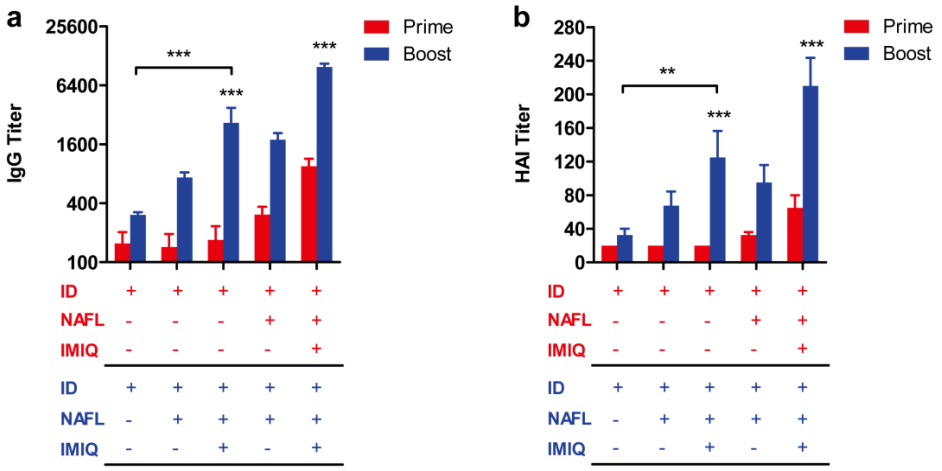


Supplementary Figure 2. Adjuvant effects of heat-killed skin cells

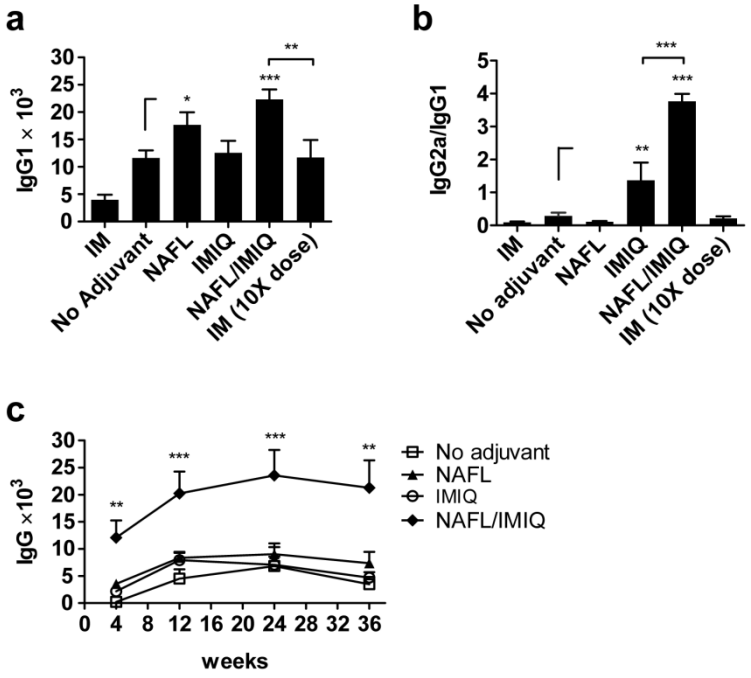
The skin of BALB/c mice was dissected and digested by Dispase II and Collagenase D for 2 hours at 37°C. The single cell suspensions were heated in 65^{o}C or 95^{o}C for 5 minutes. Cognate mice were ID immunized with influenza vaccine (0.06 μg HA) mixed with 1×10^{6} heat-killed skin cells. IgG antibody titers were measured in 2 weeks by ELISA. n=8. Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni.*, $p<0.05$; **, $p< 0.01$, respectively.



Supplementary Figure 3. Adjuvant effects of NAFL/IMIQ in outbred mice
Swiss Webster mice at 6 weeks of age were ID immunized with influenza vaccine with or without indicated adjuvants as **Fig. 1**. IgG (**a**) and HAI (**b**) titers were measured in 4 weeks. The mice were challenged with 10× LD_{50} of mouse-adapted A/California/7/2009 H1N1 viruses 5 weeks post-immunization. Mice were euthanized 4 days after the infection, and lung viral titers were determined by $TCID_{50}$ using MDCK cells. n=8, except for the IM or IM (10× dose) group (n=6). Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively.

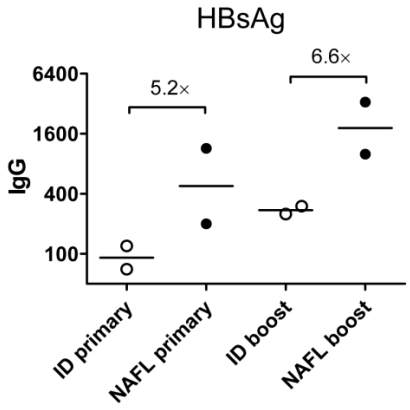


Supplementary Figure 4. NAFL/IMIQ is effective in a booster immunization.
Swiss Webster mice at 6 weeks of age were primed with influenza vaccine with or without indicated adjuvants shown in red, and boosted two weeks after primary immunization with same vaccine with or without indicated adjuvants shown in blue. IgG (**a**) and HAI (**b**) titers were measured on day 14 (prime) and 21 (boost). n=8. Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively.



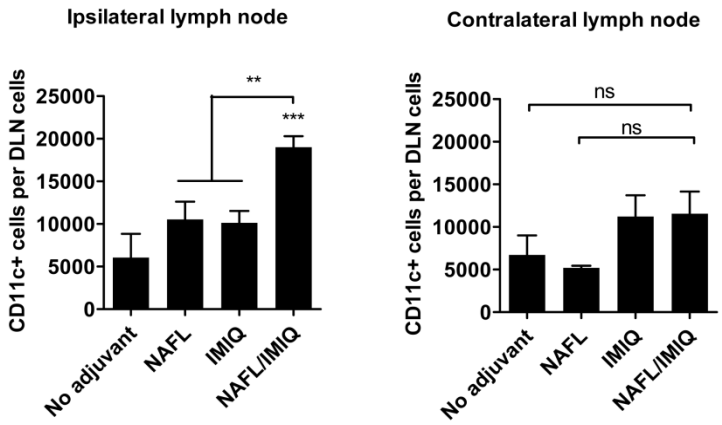
Supplementary Figure 5. NAFL/IMIQ enhanced vaccine-induced humoral immune responses

BALB/c mice were ID immunized as **Fig. 1**. IgG1 (**a**) and ratios of IgG2a to IgG1(**b**) were determined 4 weeks after immunizations. (**c**) IgG titers were further followed up for a period of 9 months. n=8, except for NAFL and NAFL/IMIQ groups (n=10). Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively. All experiments were repeated twice with similar results.

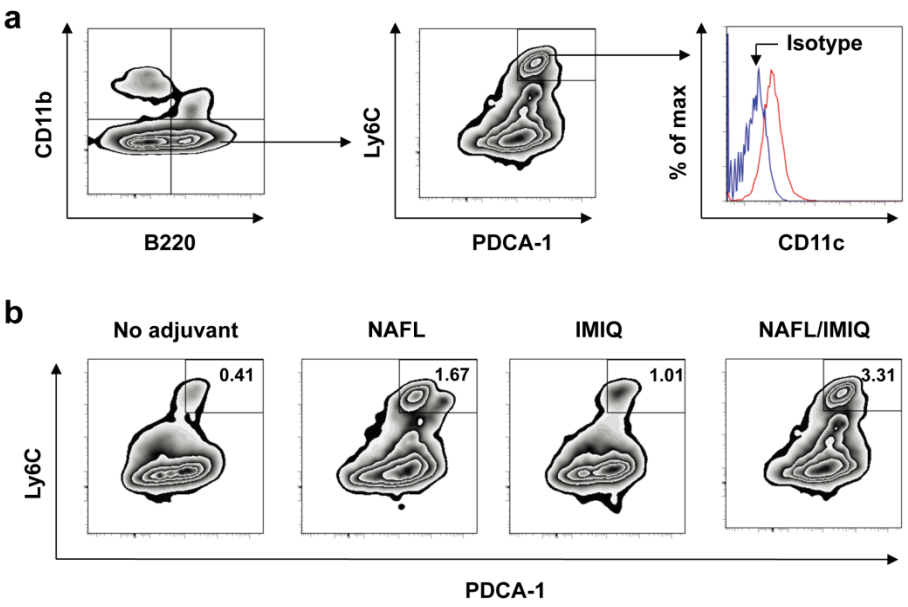


Supplementary Figure 6. NAFL augments HBsAg-induced immune response in pigs.

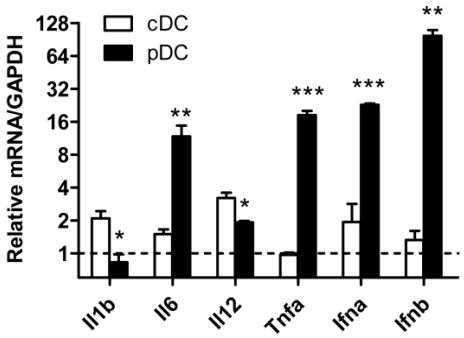
Yorkshire pigs were ID immunized with 5 μg HBsAg alone or in the presence of NAFL as **Fig. 3**. The immunization was repeated on day 10. IgG titers were measured 10 days after each immunization. Each symbol represents data from individual animals and horizontal lines indicate the mean. The numbers indicate fold increases in the presence vs. the absence of NAFL treatment.



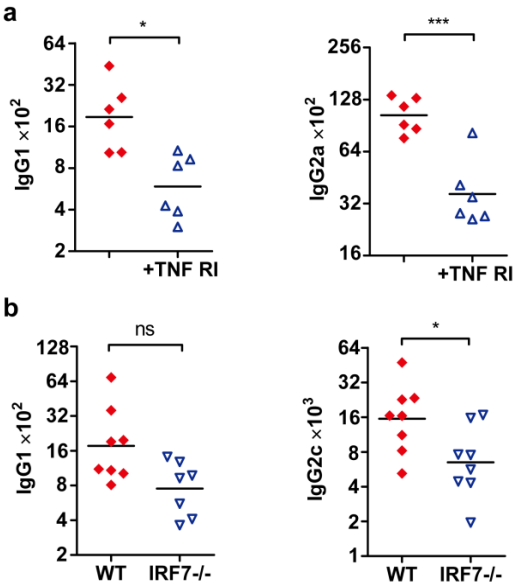
Supplementary Figure 7. Flow cytometric analysis of APCs in lymph nodes
BALB/c mice were immunized as **Fig.1**. $CD11c^{+}$ cells were analyzed 6 hours post-immunization in ipsilateral and contralateral lymph nodes. n=4. Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively.



Supplementary Figure 8. Identification of pDCs by flow cytometry
The skin at the inoculation site was collected and digested by collagenase and dispase at 37^{o}C for 2 hours, and the resultant single cell suspensions were incubated with anti-CD16/32 antibody to mask Fc receptors on the cells followed by staining with indicated antibodies to mark pDCs. Representative profiles of flow cytometry are shown in (**a**), in which cells were first gated by B220^{+}CD11b^{-}, then by Ly6C^{+}PDCA-1^{+}. pDCs were also confirmed by expression of CD11c. (**b**) Flow cytometric profiles of pDCs in skin receiving different treatments.

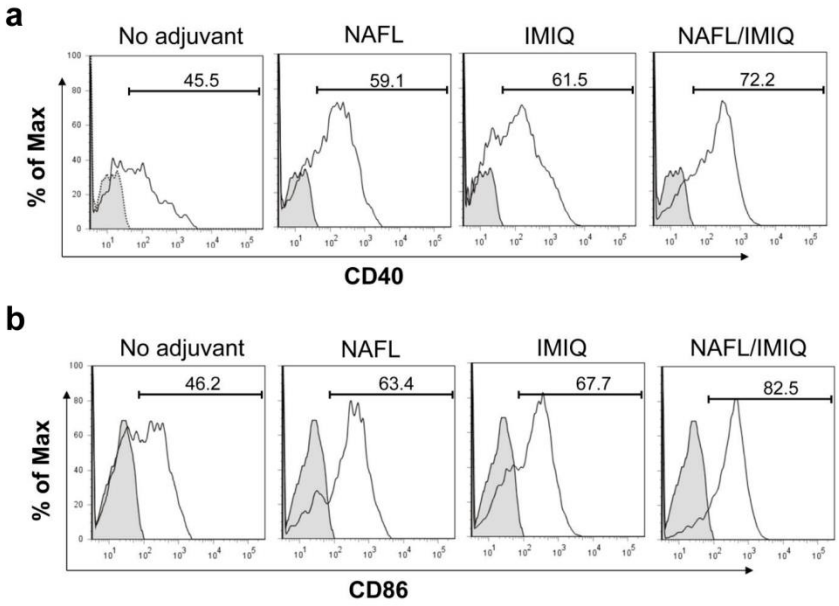


Supplementary Figure 9. Cytokine production of pDCs upon IMIQ stimulation
Bone marrow cells from C57BL/6 mice were cultured in the presence of GM-CSF and IL-4 generating primarily conventional cDCs, but not pDCs. In contrast, when the cells were differentiated in the presence of Flt3-ligand, pDCs were produced mainly. The resultant cDCs and pDCs were stimulated by IMIQ (10 μg ml^{-1}) overnight and harvested for qPCR analysis. mRNA expression levels in stimulated cells were first normalized to GAPDH, then to un-stimulated samples. n=4. Data are presented as mean ± s.e.m. Statistical significance was analyzed by *t*-test. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively.

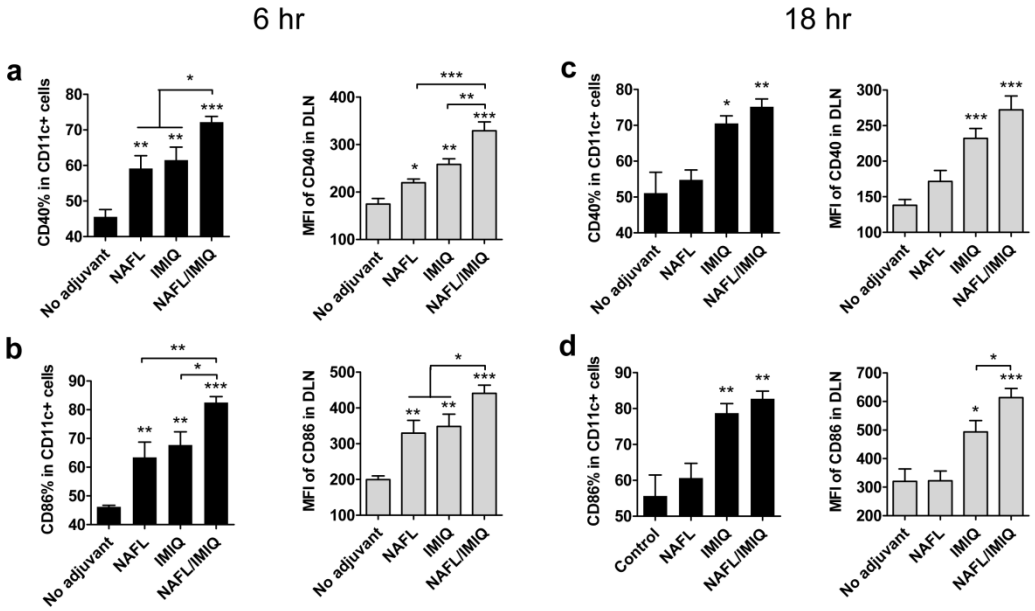


Supplementary Figure 10. Adjuvant effects of NAFL/IMIQ depend on TNF-α and type I interferon

(**a**) Balb/c mice were immunized with H1N1 influenza vaccine and NAFL/IMIQ, followed by ID injection of TNF-α inhibitor (TNF RI) into the vaccination site at 0, 6, and 24 hours after immunization. IgG1 and IgG2a were measured by ELISA in two weeks. (**b**) IRF7-/- and control C57BL/6 mice were immunized with H1N1 influenza vaccine adjuvantated by NAFL/IMIQ. IgG1 and IgG2c (C57BL/6 mice express IgG2c in place of IgG2a) titers were measured 2 weeks later by ELISA. Each symbol represents data from individual animals and horizontal lines indicate the mean. Statistical significance was analyzed by *t*-test. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively. ns, not significant.

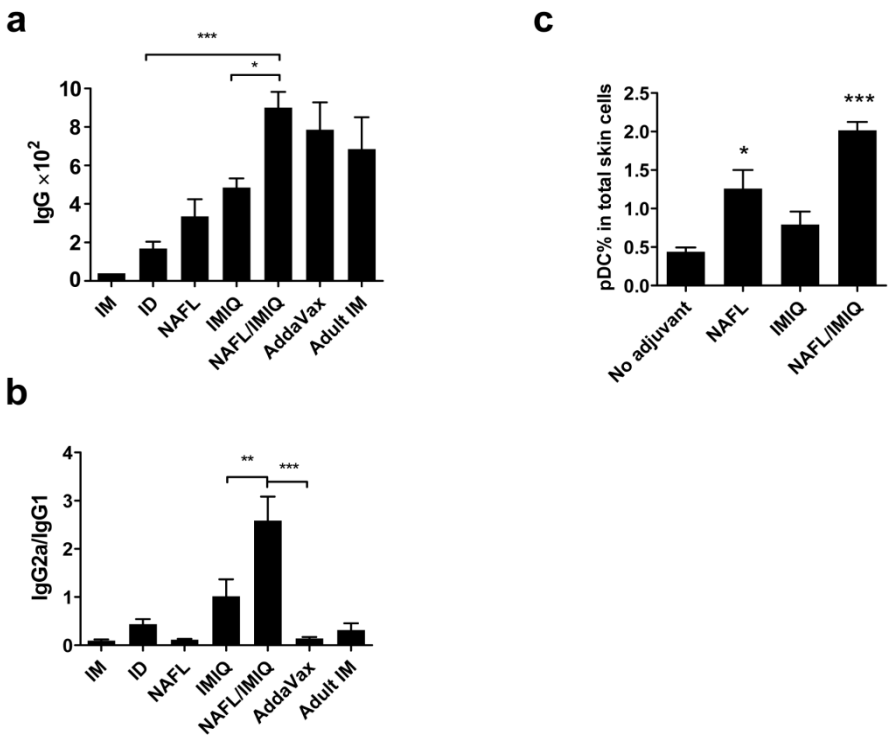


Supplementary Figure 11. Flow cytometric analysis of mature DCs in dLNs
BALB/c mice were immunized as **Fig. 5 e, f**. The cells prepared from dLNs were analyzed 6 hours post-immunization for mature DCs on a FACSAria, with a first gate of $CD11c^+$, then $CD40^+$ (**a**) or $CD86^+$ (**b**) cells, respectively.



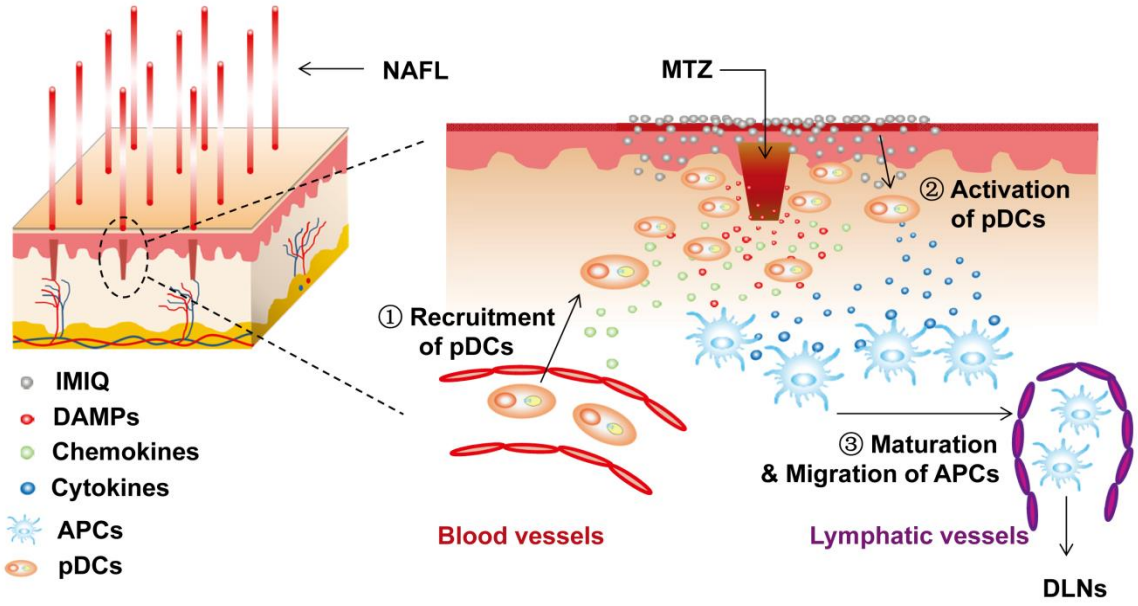
Supplementary Figure 12. NAFL/IMIQ increases maturation of APCs

BALB/c mice were immunized and mature DCs in dLNs were analyzed in 6 (**a**, **b**) or 18 (**c**, **d**) hours post-immunization by flow cytometry as above. The percentages (left) of $CD40^+$ (**a, c**) or $CD86^+$ (**b, d**) and MFI (right) of these cells were summarized. n=6, except for the NAFL/IMIQ group (n=8). Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively.



Supplementary Figure 13. NAFL/IMIQ greatly augments a protective immunity in old mice

Old BALB/c mice were immunized as **Fig. 6**. IgG (**a**) titer and ratio of IgG2a to IgG1 (**b**) were measured by ELISA 4 weeks post-immunization. n= 6, except for the NAFL/IMIQ group (n=13). (**c**) Percentages of pDCs at the vaccination site were determined 24 hours post-immunization as **Fig. 5b**. n=4; Data are presented as mean ± s.e.m. Statistical significance was analyzed by ANOVA/Bonferroni. *, $p<0.05$; **, $p< 0.01$ or ***, $p<0.001$, respectively. All experiments were repeated twice with similar results.



Supplementary Figure 14. Postulated mechanisms of NAFL/IMIQ adjuvant
NAFL generates a MTZ array in skin (left) and the laser-damaged cells in MTZs release DAMPs (damage associated molecular patterns) that stimulate chemokine production presumably by keratinocytes or other surrounding cells (right). The chemokines recruit pDCs from blood into the inoculation site ① where topical IMIQ penetrates across the skin and activate these pDCs via TLR7 receptor-mediating signaling ②. The activated pDCs release high levels of pro-inflammatory cytokines, including IFN-α/β and TNF-α and so on, resulting in enhanced maturation of dermal APCs *in situ* and emigration of the mature APCs into dLNs via lymphatic vessels ③.

Supplementary Table 1 Primers for qPCR Analysis

Gene	Forward	Reverse
Gapdh	atcaagaaggtggtgaagca	agacaacctggtcctcagtgt
Ccl2	ggctcagccagatgcagttaa	cctactcattgggatcatcttgct
Ccl3	tgtaccatgacactctgcaac	caacgatgaattggcgtggaa
Ccl5	gcccacgtcaaggagtatttcta	acacacttggcggttccttc
Ccl7	gatctctgccacgcttctgt	atagcctcctcgacccactt
Ccl12	gtcctcaggtattggctgga	cactggctgcttgtgattct
Ccl20	actgttgcctctcgtacataca	gaggaggttcacagccctttt
Cxcl9	ggagttcgaggaaccctagtg	gggatttgtagtggatcgtgc
Cxcl10	ccaagtgctgccgtcattttc	tccctatggccctcattctca
Cxcl12	tgcatcagtgacggtaaacca	cacagtttggagtgttgaggat
Chemerin	gtgcacaatcaaaccaaacg	ggcaaactgtccaggtagga
Ifna2	ggatgcgatctgcctcacac	cttcaggcaggagagaaaggg
Ifnb1	agctccaagaaaggacgaaca	gccctgtaggtgaggttgat
Tnfa	cctgtagcccacgtcgtag	gggagtagacaaggtacaaccc
Il12	ctgtgccttggtagcatctatg	gcagagtctcgccattatgattc
Il6	tagtccttcctaccccaatttcc	ttggtccttagccactccttc
Ifng	atgaacgctacacactgcatc	ccatccttttgccagttcctc
Il1a	tgctgaaggagttgccagaaa	tgcacccgactttgttctttg
Il1b	acatcagcacctcacaagca	ttagaaacagtccagcccata
Il18	tcaaagtgccagtgaacccc	ggtcacagccagtcctcttac
Il33	tccaactccaagatttccccg	catgcagtagacatggcagaa
Tslp	gctaagttcgagcaaatcgagg	gccagggataggattgagagta