Electronic Supplementary Information (ESI)

Vaccine adjuvant Platform and Fluorescence Imaging of Amphiphilic γ-PGA-IMQ-LA-FL Conjugates

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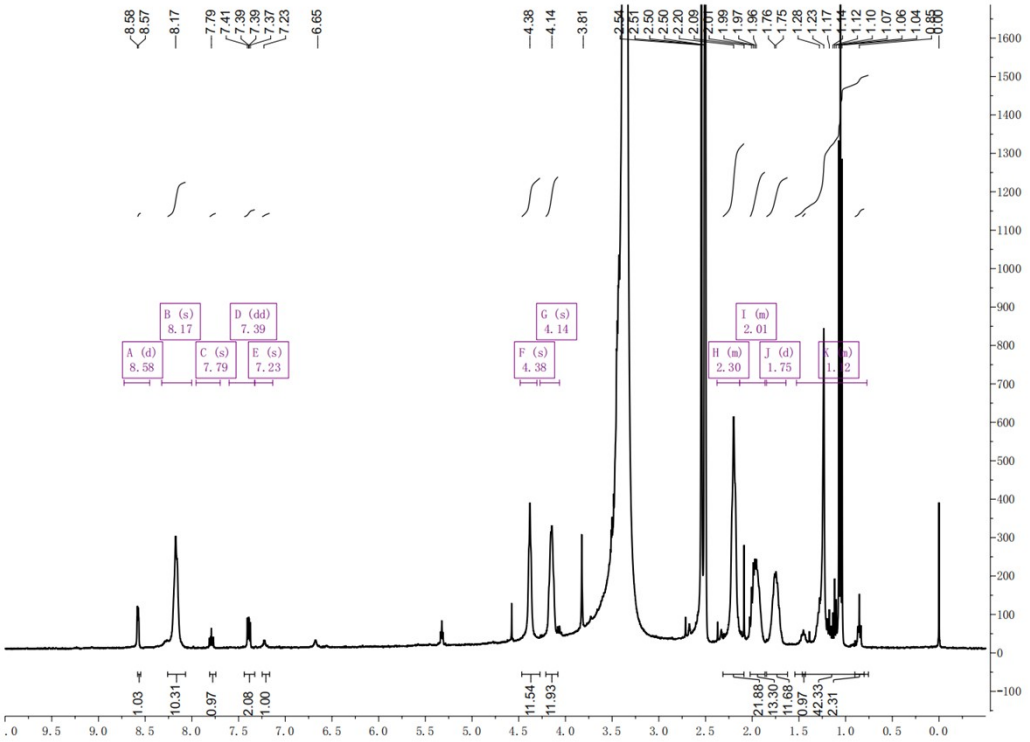
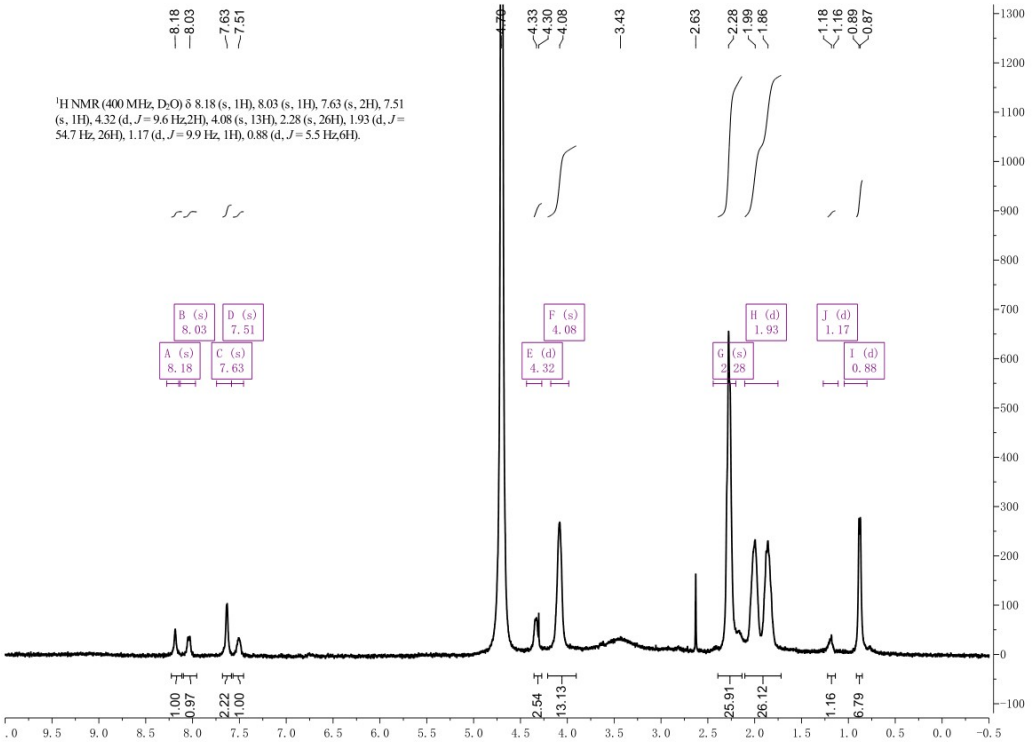
Single ^{1}H NMR full spectra of FIP and other materials

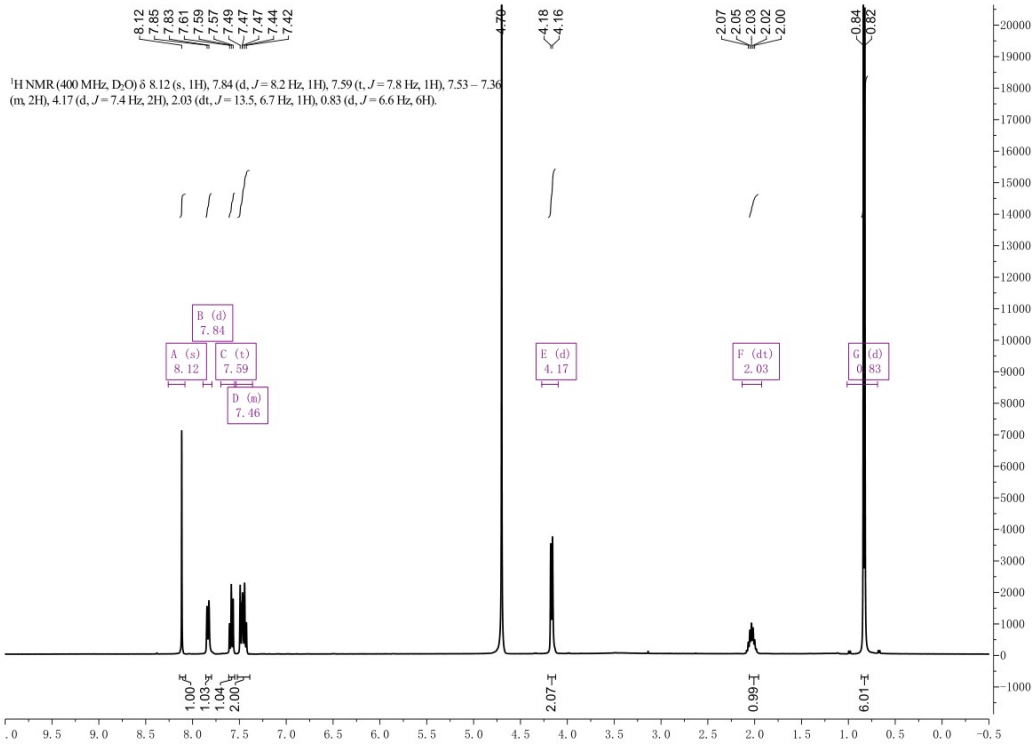
(a)



(b)


8.18
8.03
7.63
7.51
4.79
4.33
4.30
4.08
3.43
2.63
2.28
1.99
1.86
1.18
1.16
0.89
0.87
1H NMR (400 MHz, D2O) δ 8.18 (s, 1H), 8.03 (s, 1H), 7.63 (s, 2H), 7.51 (s, 1H), 4.32 (d, J = 9.6 Hz,2H), 4.08 (s, 13H), 2.28 (s, 26H), 1.93 (d, J = 54.7 Hz, 26H), 1.17 (d, J = 9.9 Hz, 1H), 0.88 (d, J = 5.5 Hz,6H).
B (s) 8.03
D (s) 7.51
A (s) 8.18
C (s) 7.63
F (s) 4.08
E (d) 4.32
H (d) 1.93
G (s) 2.28
J (d) 1.17
I (d) 0.88
1.00
0.97
2.22
1.00
2.54
13.13
25.91
26.12
1.16
6.79

(c)


8.12
7.85
7.83
7.61
7.59
7.57
7.49
7.47
7.47
7.44
7.42
4.79
4.18
4.16
2.07
2.05
2.03
2.02
2.00
0.84
0.82
1H NMR (400 MHz, D2O) δ 8.12 (s, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.53 – 7.36 (m, 2H), 4.17 (d, J = 7.4 Hz, 2H), 2.03 (dt, J = 13.5, 6.7 Hz, 1H), 0.83 (d, J = 6.6 Hz, 6H).
B (d) 7.84
A (s) 8.12
C (t) 7.59
D (m) 7.46
E (d) 4.17
F (dt) 2.03
G (d) 0.83
1.00
1.03
1.04
2.00
2.07
0.99
6.01

(d)

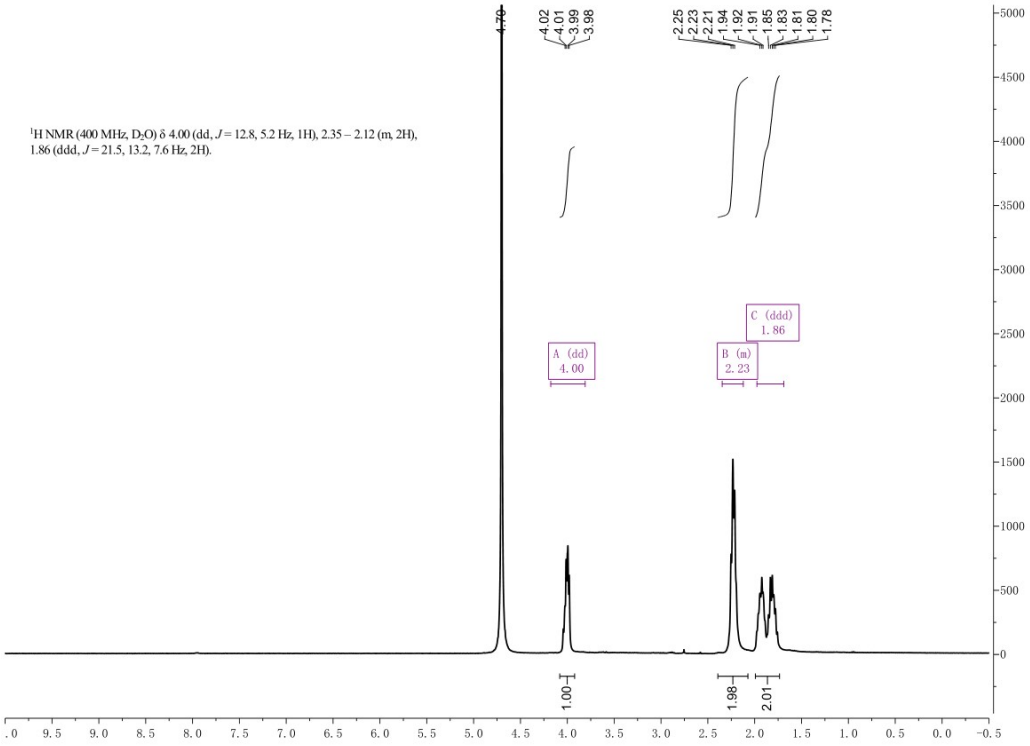


Figure S1. The 1H NMR full spectra of (a) FIP,(b) γ-PGA-IMQ,(c) IMQ and (d) γ-PGA

DLS and PDI information of FIP

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 284.7	Peak 1:	354.3	97.4	203.5
PdI: 0.265	Peak 2:	4831	2.6	712.1
Intercept: 0.941	Peak 3:	0.000	0.0	0.000
Result quality : Good				

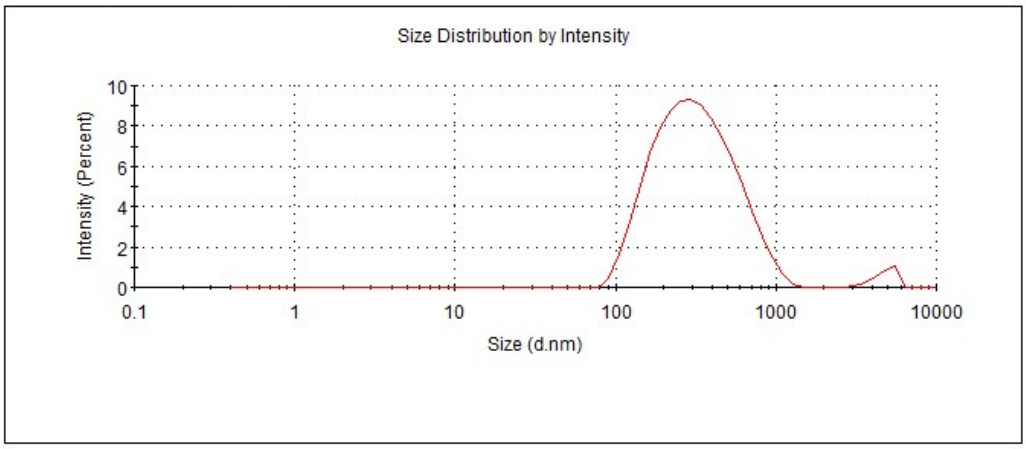
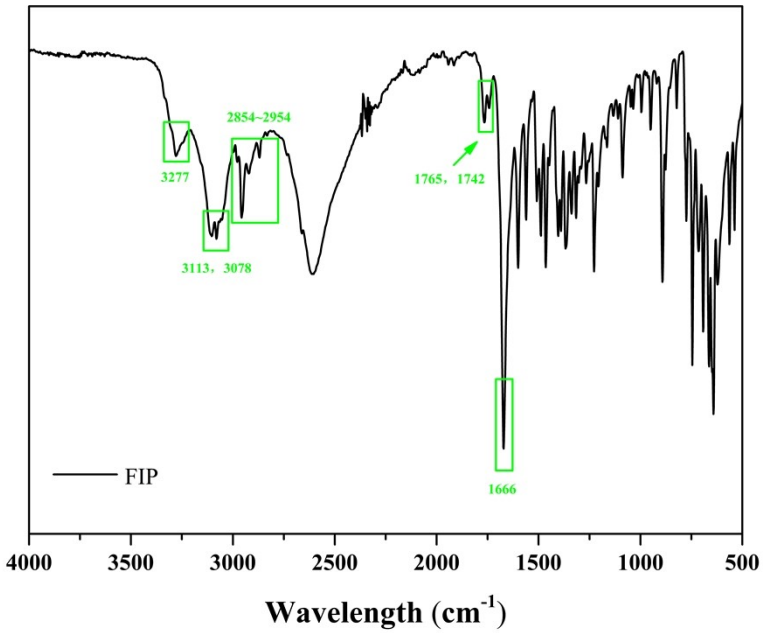


Figure S2. the average diameter of FIP was detected by DLS

FI-IR spectra of FIP and γ-PGA-LA

(a)



(b)

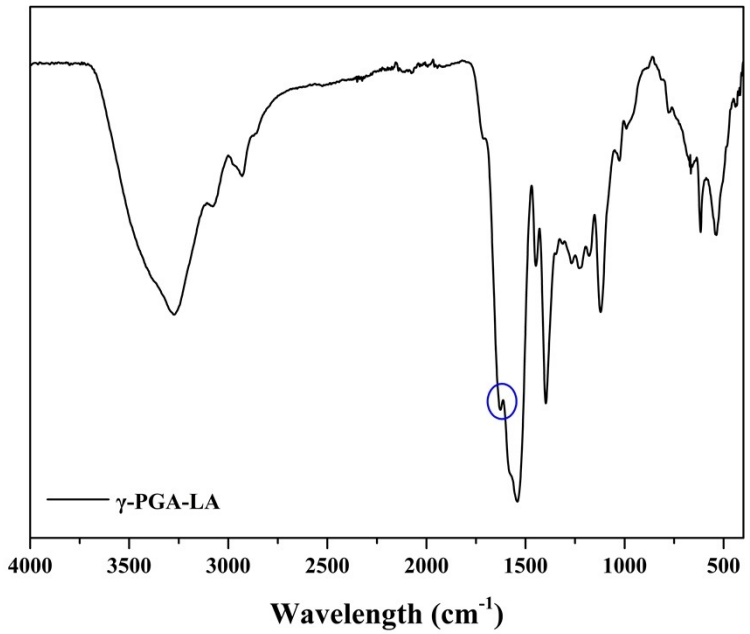


Figure S3. the FI-IR images of (a) FIP and (b) γ-PGA-LA

Cytokines Detection

To further evaluate the effect of FIP on RAW 264.7 activation, the concentration of cytokines in supernatants of RAW 264.7 stimulated with OVA, OVA+FIP was measured by ELISA kits.

RAW 264.7 cells were first cultured in DMEM supplemented with 10% fetal bovine serum, then diluted to the final cell concentration of 2×10^5 cells/mL, transferred to 24-well plants at 200 μL per well, and cultured at 37 °C for 24 h. After discarding the supernatant in the wells, 200 μL of OVA+FIP, OVA (diluted in DMEM culture) were added into the wells, followed by incubation at 37 °C for 24 h. Finally, The amounts of the inflammatory cytokines (IL-2, 4, 6) and macrophage activating factor (IFN-γ) in the cell culture supernatants were determined by ELISA (Figure S1). All data demonstrated that the production of IL-2, 4, 6 and IFN-γ were significantly improved after OVA+FIP treatment. Activation of RAW 264.7 cells by FIP can induce RAW 264.7 cells maturation and stimulate the expression of pro-inflammatory cytokines. The performance of FIP are favorable for vaccine adjuvant and subsequent adaptive immune responses. [1].

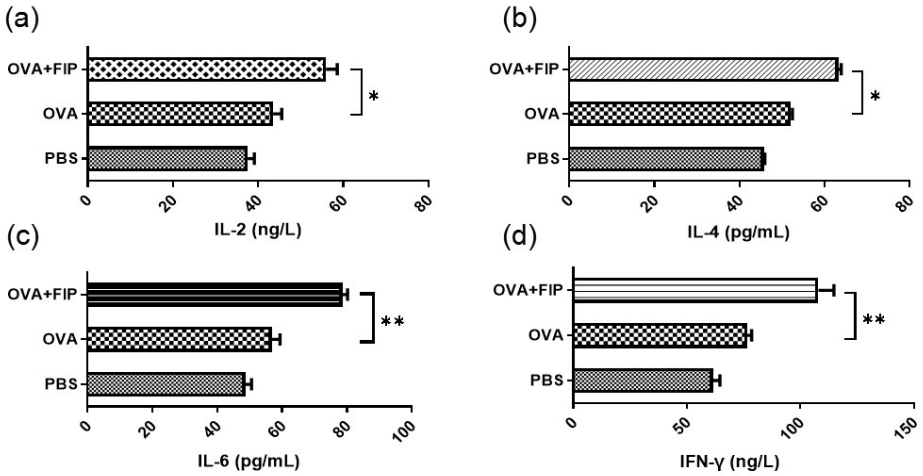


Figure S4. The secretion of cytocines by RAW264.7 cells stimulated with OVA, OVA+FIP or PBS. (a) IL-2, (b) IL-4, (c) IL-6 and (d) IFN-γ

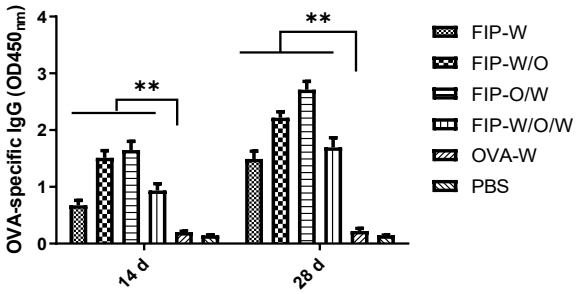


Figure S5. the OVA-special IgG of mice immunized OVA+FIP with different formulations at 14 and 28 dpv

References

[1] J. P. Vasilakos, M. ATomai, *Vaccines*, 2013, **12 (7)**, 809-819.