Supplementary information

Supplementary methodology

Dynamic light scattering (DLS)

Dynamic light scattering was performed using a Zetasizer nano ZS (Malvern Panalytical, UK) fitted with a 633 nm laser (detection range-0.3nm-10μm). Vaccines formulated in saline and R10 medium were introduced into polystyrene cuvettes and measured at 25 and 37°C respectively. Data shown in Fig. S2A represents the mean of 5 individual measurements per sample replicate.

Graphite furnace atomic absorption spectroscopy (GFAAS)

Quantification of aluminium recovery was performed using an AAnalyst 600 atomic absorption spectrometer equipped with a transversely heated graphite atomizer (THGA) and AS 800 autosampler (Perkin Elmer, UK). Briefly, vaccines containing Al were subject to size-exclusion filtration as per methodology outlined for those containing L-tyr. Filtrates were acidified to 50%*v/v* using analytical grade HNO_3 (15.8M - Fisher scientific, UK) and digested at 180°C for 40 minutes using a MARS 6 Xpress microwave digester (CEM Corp., US). The instrumentation was calibrated using freshly prepared aluminium standards prior to quantification (max. 60 μg/L Al in 1% HNO_3). Controls included complementary digests of saline and R10 depending upon the particular experiment performed.

Supplementary figures and tables

Table S1: The concentration of constituents used to prepare the three formulations of MIF used in this study.

	Formulation		
Constituents	**Base**	**PO_4**	**Cit**
PIPES	100mM	100mM	100mM
NaCl	120mM	120mM	120mM
KCl	3.2mM	3.2mM	3.2mM
$Mg(OH)_2$	0.7mM	0.7mM	0.7mM
$CaCl_2$	1.6mM	1.6mM	1.6mM
$C_6H_{12}O_6$	3.6mM	3.6mM	3.6mM
Na_2HPO_4	n/a	0.6mM	0.6mM
$Na_3C_6H_5O_7$	n/a	n/a	2.3mM
NaN_3	0.05% *w/v*	0.05% *w/v*	0.05% *w/v*

Table S2: XRD peak intensity and 2θ° values obtained for MCT® and L-tyr.

MCT		L-tyr		MCT		L-tyr		MCT		L-tyr	
2θ°	**Intensity**	**2θ°**	**Intensity**	**2θ°**	**Intensity**	**2θ°**	**Intensity**	**2θ°**	**Intensity**	**2θ°**	**Intensity**
13.47	58.71	13.36	341.82	28.43	276.59	28.35	629.68	44.85	31.57	44.77	67.43
13.81	15.75	13.73	46.36	28.74	97.33	28.66	375.15	45.44	43.13	45.55	83.52
15.31	157.22	15.21	872.34	29.11	49.68	29.00	130.16	45.92	16.05	46.67	36.75
15.76	41.63	15.66	128.79	30.01	23.74	29.93	91.03	46.78	47.08	47.43	55.98
16.21	24.90	16.11	77.36	30.32	33.36	30.21	112.48	47.45	48.33	48.19	59.06
16.79	360.34	16.70	668.35	30.60	20.22	30.52	51.38	48.12	49.57	49.77	59.56
17.22	20.22	17.14	55.91	31.22	34.83	31.11	110.34	48.79	50.82	51.72	63.32
17.34	21.70	17.26	56.99	31.69	106.89	31.71	61.28	49.45	52.06	53.05	63.27
17.98	593.91	17.87	2418.38	32.35	114.06	32.26	267.25	53.68	40.95	53.62	79.90
19.08	15.85	18.98	34.57	32.65	64.53	32.55	201.35	54.65	26.21	54.56	73.10
19.90	83.59	19.80	293.89	33.43	68.94	33.33	176.73	56.12	17.98	55.49	44.78
20.33	221.40	20.23	775.68	33.94	309.17	33.86	399.25	57.00	16.21	56.92	57.95
21.15	135.69	21.07	406.30	34.42	40.05	34.31	107.69	58.30	17.70	58.23	41.12
21.62	67.57	21.52	206.67	35.89	31.48	35.83	82.27				
22.26	23.88	22.18	53.65	36.40	159.07	36.32	323.57				
22.79	15.50	22.73	29.85	37.47	22.81	37.35	58.96				
23.16	13.12	23.14	34.78	38.45	12.71	38.35	30.97				
23.59	19.34	23.47	46.38	39.66	44.55	39.54	125.97				
24.21	17.00	23.74	32.69	40.13	15.86	40.03	45.26				
24.68	635.54	24.60	1552.65	40.65	13.62	40.54	40.89				
25.29	245.18	25.21	359.64	41.47	16.68	41.34	44.67				
25.72	102.63	25.64	457.85	42.43	37.28	42.25	145.99				
26.13	116.09	26.03	318.15	43.54	41.70	43.45	97.21				
27.10	143.19	27.02	592.30	44.07	14.43	43.95	43.62				

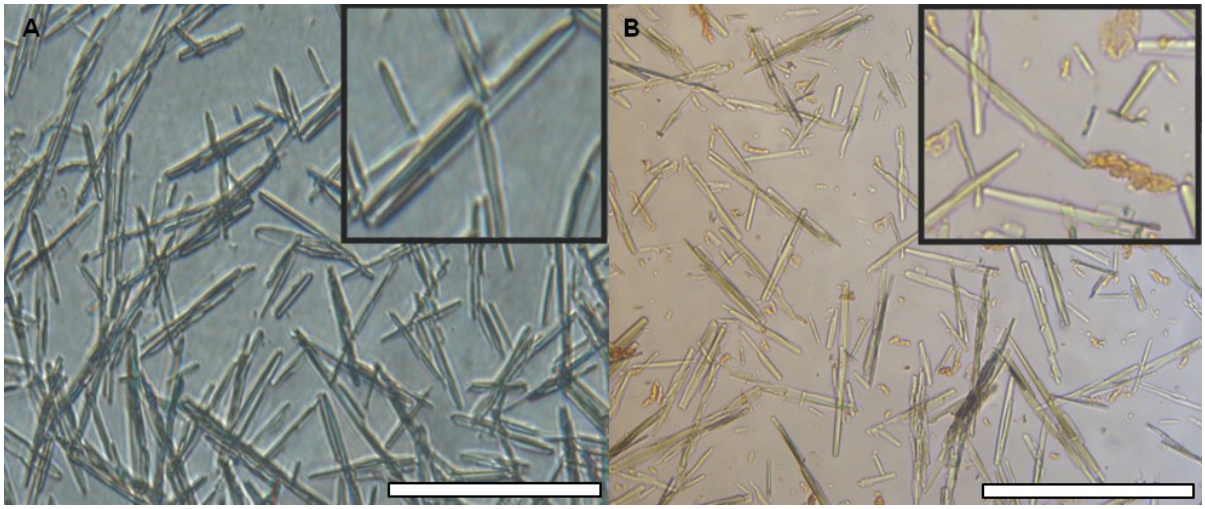

Fig S1: Light images of 0.7mg/mL MCT® in saline (polarised light) (A) and in the presence of 70µg/mL OVA (Congo red staining) (B). Both images were taken at a magnification of 200X (scale bars 100 µm).

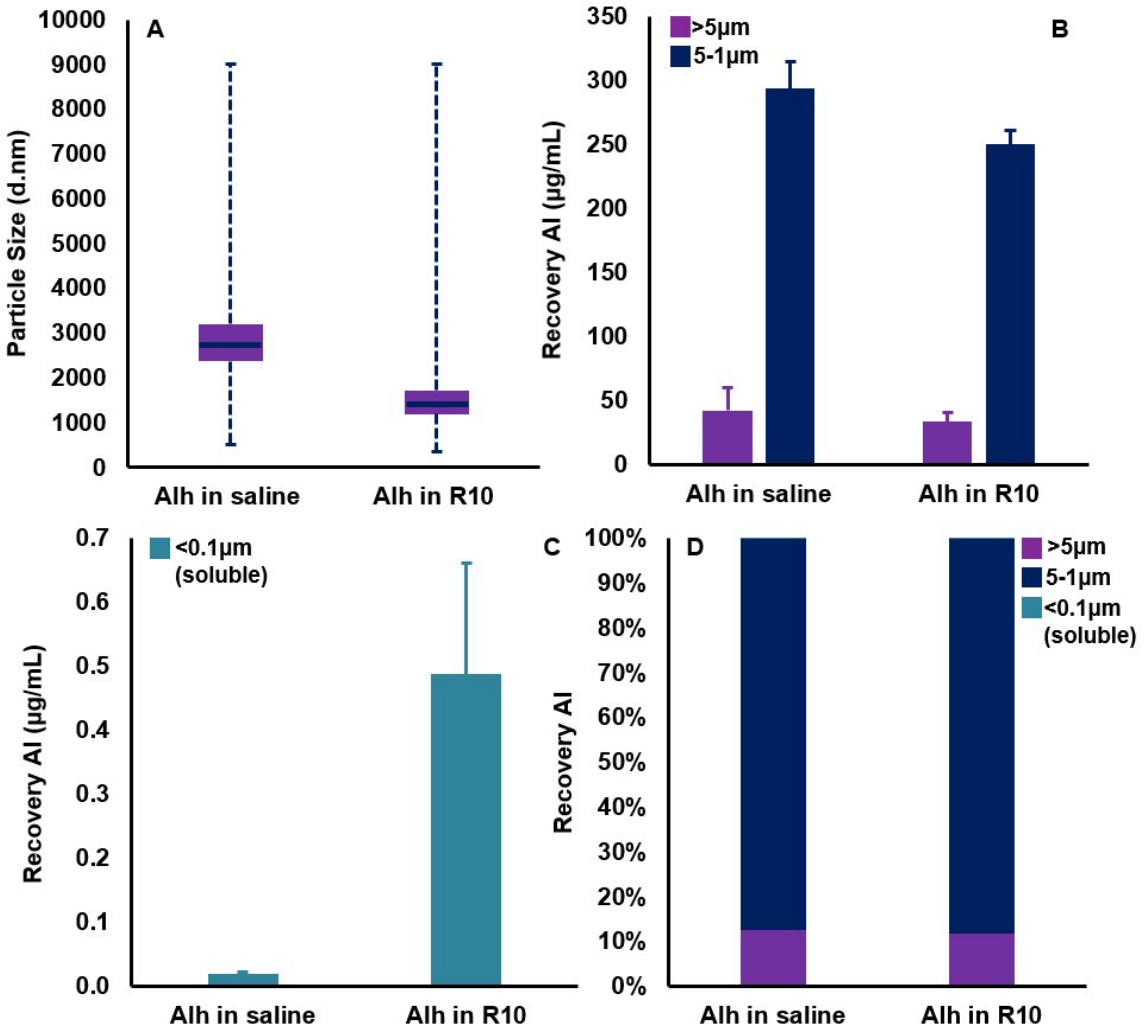

Fig. S2: Determination of the size of particles within vaccines adjuvanted with Alhydrogel® (*ca* 0.3mg/mL Al) formulated in saline, pH 7±0.1. Panel A shows the particle size distribution as determined by dynamic light scattering (DLS). Blue lines represent the d50 of the distribution, purple boxes the interquartile range and blue dotted lines the span. Panel B represents the Al recovery as determined by graphite furnace atomic adsorption spectroscopy >ca 5 µm and between 5-1µm while panel C shows the Al recovery <0.1 µm (soluble). Panel D highlights the relative percentage contribution of each fraction to the whole vaccine. Error bars are representative of ±SD of the measurement where n=5.

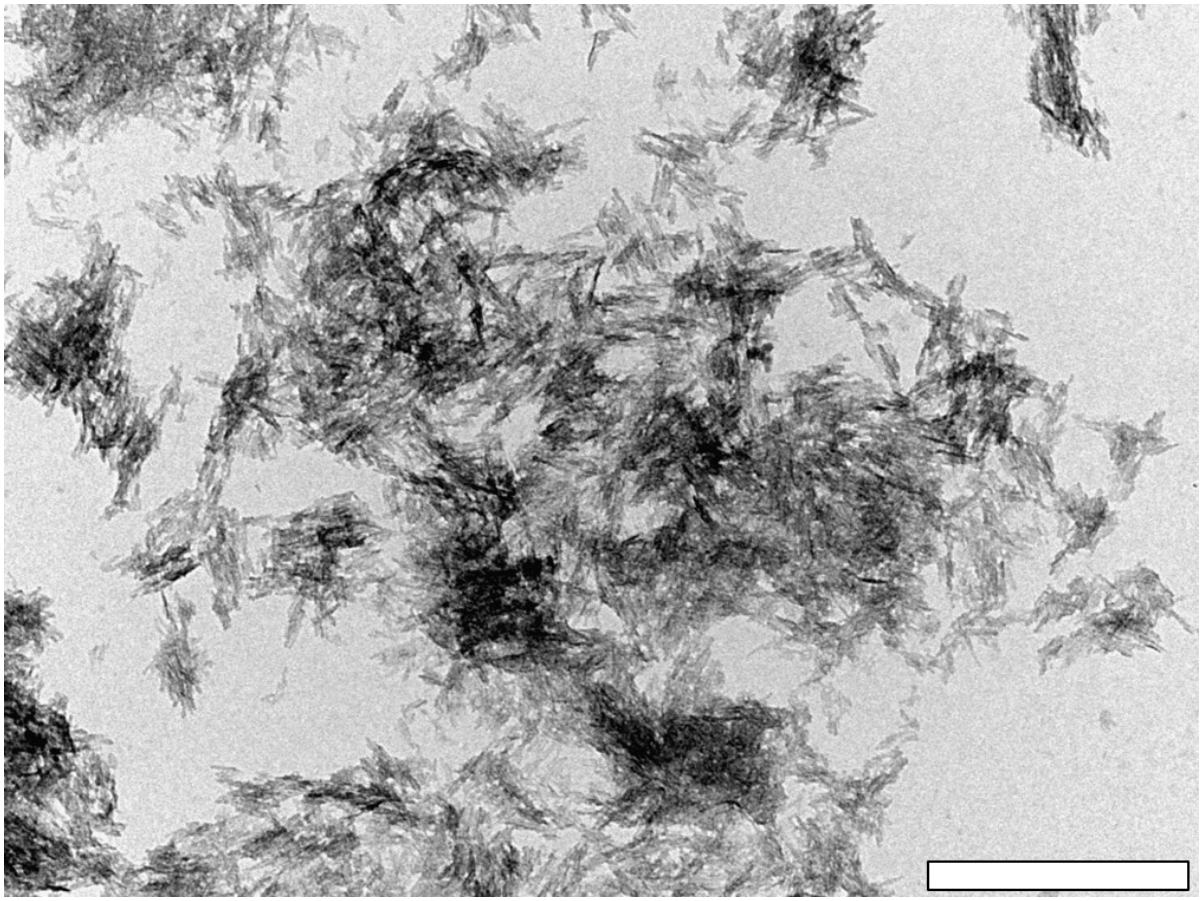

Fig. S3: TEM image of Alhydrogel® prepared at a concentration of 0.1mg/mL in saline plus 2% uranyl acetate. The image was taken at a magnification of 60k and the scale bar represents 0.5µm.

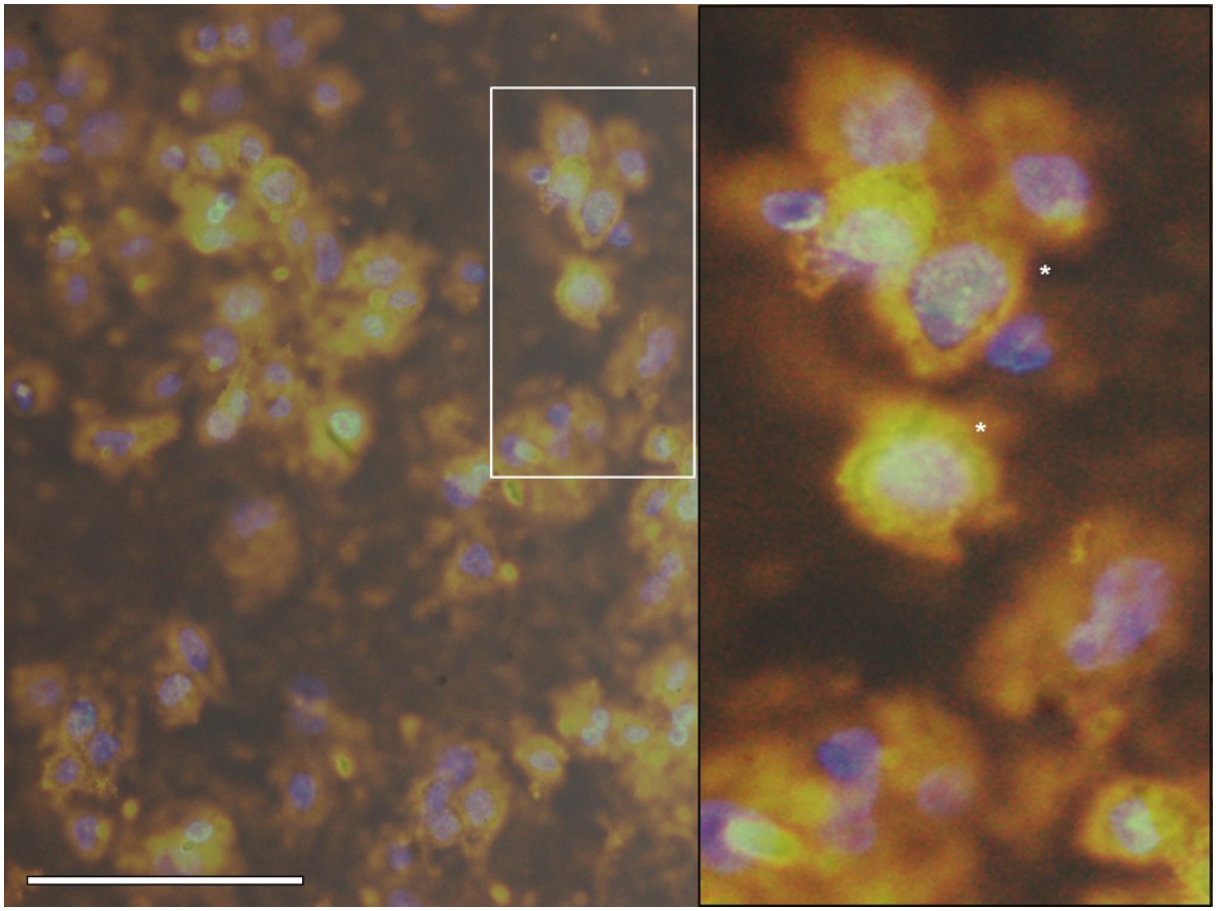

Fig. S4: Light, NIB and WU overlay images of THP-1 differentiated macrophages in R10 medium treated with Alhydrogel (0.3mg/mL Al) and stained using lumogallion (100µM) for 1hr. Positive lumogallion staining (orange fluorescence) indicates the presence of Al. Positive DAPI staining (blue fluorescence) indicates cell nuclei. The image was taken at a magnification of 200X (scale bar 100µm). The area used as an insert is identified by a white box and cells marked with an asterisk highlight the marked cytoplasmic loading of Al observed throughout the sample.