# SUPPLEMENTAL MATERIAL

Williams et al., http://www.jem.org/cgi/content/full/jem.20131768/DC1

## Table S1. Chemical details of the LDHs used in this study

| Layered double hydroxide formula | Abbreviation\(^a\) | Particle size |
|----------------------------------|---------------------|---------------|
| \([\text{LiAl}(\text{OH})_2]\)\(\text{Cl}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | LiAl\(_2\)-Cl | TEM: Particles from <100 nm to several micrometers. DLS: Polydisperse, particles from 250 to 300 nm to several micrometers. |
| \([\text{LiAl}(\text{OH})_2]\)(\text{CO}_3)^{0.5}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | LiAl\(_2\)-CO\(_3\) | TEM: 50–100 nm, plus larger aggregates 100 nm to 1μm. DLS: Polydisperse aggregates 300 nm to 2 μm. |
| \([\text{LiAl}(\text{OH})_2]\)\(\text{NO}_3\)^{y}\(\text{H}_2\)\(\text{O}\) | LiAl\(_2\)-NO\(_3\)^{b} | Estimated from XRD to be approximately the same as LiAl\(_2\)-Cl. |
| \([\text{Ca}_2\text{Al}(\text{OH})_3]\)\(\text{Cl}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | Ca\(_2\)Al-Cl | Estimated from XRD to be approximately the same as Ca\(_2\)Al-NO\(_3\). |
| # \([\text{Ca}_2\text{Al}(\text{OH})_3]\)\(\text{NO}_3\)^{y}\(\text{H}_2\)\(\text{O}\) | Ca\(_2\)Al-NO\(_3\) | TEM: Aggregates below 100 nm, also larger aggregates. DLS: Polydisperse aggregates. |
| \([\text{Mg}_2\text{Al}(\text{OH})_3]\)\(\text{Cl}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | Mg\(_2\)Al-Cl\(^b\) | XRD: ~185 nm. |
| \([\text{Mg}_2\text{Al}(\text{OH})_3]\)(\text{CO}_3)^{0.5}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | Mg\(_2\)Al-CO\(_3\) | TEM: 100–200 nm. DLS: 120 nm plus larger aggregates. |
| \([\text{Mg}_2\text{Al}(\text{OH})_3]\)\(\text{NO}_3\)^{y}\(\text{H}_2\)\(\text{O}\) | Mg\(_2\)Al-NO\(_3\) | TEM: 30–200 nm. DLS: ~100 nm. |
| \([\text{Mg}_2\text{Fe}(\text{OH})_3]\)\(\text{Cl}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | Mg\(_2\)Fe-Cl | TEM: Particles 50–100 nm, aggregates of 200–500 nm. DLS: ~145 nm plus larger aggregates. |
| \([\text{Mg}_2\text{Fe}(\text{OH})_3]\)(\text{CO}_3)^{0.5}\)\(_y\)\(\text{H}_2\)\(\text{O}\) | Mg\(_2\)Fe-CO\(_3\) | XRD: ~180 nm. |
| \([\text{Mg}_2\text{Fe}(\text{OH})_3]\)\(\text{NO}_3\)^{y}\(\text{H}_2\)\(\text{O}\) | Mg\(_2\)Fe-NO\(_3\) | XRD: ~100 nm. |

\(^a\) Indicates that the \([\text{Ca}_2\text{Al}(\text{OH})_3]\)(\text{CO}_3)^{0.5}\)\(_y\)\(\text{H}_2\)\(\text{O}\) LDH could not be synthesized in phase-pure form and therefore could not be included in this study. DLS, dynamic light scattering; TEM, transmission electron micrographs; XRD, X-ray diffraction.

\(^b\) For clarity, stoichiometric indices are not subscripted in figures.

\(^c\) LDHs synthesized for de novo predictions of immunological activities.
## Table S2. Responses of human monocyte-derived DCs to LDHs and alums (tabulation of values presented in Fig. 2)

| Compound       | IL-1β Response | IL-1β In response | IL-1β SE | IL-1β In SE | IL-6 Response | IL-6 In response | IL-6 SE | IL-6 In SE | TNF-α Response | TNF-α In response | TNF-α SE | TNF-α In SE |
|----------------|----------------|-------------------|---------|------------|--------------|----------------|---------|------------|----------------|-----------------|----------|------------|
| Cells alone    | 4.92           | 1.91              | 1.51    | 0.42       | 38.84        | 10.11          | 3.62    | 0.27       | 24.69          | 7.55            | 3.16     | 0.32       |
| LiAl₂-CO₃      | 174.17         | 67.72             | 5.07    | 0.42       | 2716.17      | 706.94         | 7.87    | 0.27       | 3802.80        | 1162.79         | 8.19     | 0.32       |
| LiAl₂-Cl       | 26.88          | 10.45             | 3.21    | 0.42       | 199.00       | 51.79          | 5.26    | 0.27       | 491.75         | 150.36          | 6.15     | 0.32       |
| Ca₂Al-Cl       | 5.96           | 2.61              | 1.67    | 0.49       | 99.36        | 25.86          | 4.56    | 0.27       | 248.96         | 79.76           | 5.46     | 0.34       |
| Ca₂Al-NO₃      | 7.85           | 3.05              | 1.97    | 0.42       | 52.23        | 13.59          | 3.92    | 0.27       | 123.34         | 37.72           | 4.76     | 0.32       |
| Mg₂Al-NO₃      | 17.71          | 6.91              | 2.79    | 0.43       | 126.99       | 33.05          | 4.81    | 0.27       | 298.59         | 91.30           | 5.65     | 0.32       |
| Mg₂Al-CO₃      | 12.97          | 5.06              | 2.48    | 0.43       | 86.04        | 22.39          | 4.42    | 0.27       | 173.18         | 52.95           | 5.10     | 0.32       |
| Mg₂Fe-CO₃      | 14.55          | 6.38              | 2.56    | 0.49       | 305.44       | 79.50          | 5.69    | 0.27       | 412.45         | 132.14          | 5.97     | 0.34       |
| Mg₂Fe-Cl       | 9.67           | 3.76              | 2.18    | 0.42       | 94.10        | 24.49          | 4.51    | 0.27       | 140.16         | 42.86           | 4.89     | 0.32       |
| Mg₂Fe-NO₃      | 3.83           | 1.68              | 1.23    | 0.49       | 103.63       | 26.97          | 4.61    | 0.27       | 166.62         | 53.38           | 5.06     | 0.34       |
| Imject         | 18.82          | 7.32              | 2.85    | 0.42       | 85.42        | 22.23          | 4.41    | 0.27       | 333.82         | 102.07          | 5.76     | 0.32       |
| Alhydrogel     | 25.60          | 9.95              | 3.16    | 0.42       | 150.54       | 39.18          | 4.98    | 0.27       | 246.47         | 75.36           | 5.46     | 0.32       |

### Table S2 (continued)

| Compound       | IL-12p70 Response | IL-12p70 In response | IL-12p70 SE | IL-12p70 In SE | IL-15 Response | IL-15 In response | IL-15 SE | IL-15 In SE | IFN-α2 Response | IFN-α2 In response | IFN-α2 SE | IFN-α2 In SE |
|----------------|-------------------|----------------------|-------------|----------------|----------------|-------------------|---------|-------------|----------------|-----------------|----------|-------------|
| Cells alone    | 2.34              | 0.52                 | 0.82        | 0.23           | 1.06           | 0.24              | 0.03    | 0.23        | 2.51           | 0.59            | 0.89     | 0.24        |
| LiAl₂-CO₃      | 20.98             | 4.28                 | 3.02        | 0.21           | 2.35           | 0.52              | 0.83    | 0.23        | 6.36           | 1.41            | 1.83     | 0.23        |
| LiAl₂-Cl       | 4.24              | 0.93                 | 1.42        | 0.22           | 1.62           | 0.36              | 0.46    | 0.23        | 4.61           | 1.03            | 1.50     | 0.23        |
| Ca₂Al-Cl       | 5.04              | 1.89                 | 1.54        | 0.41           | 1.92           | 0.57              | 0.61    | 0.31        | 3.40           | 0.90            | 1.19     | 0.27        |
| Ca₂Al-NO₃      | 4.48              | 1.00                 | 1.47        | 0.23           | 1.20           | 0.27              | 0.15    | 0.23        | 3.18           | 0.72            | 1.13     | 0.23        |
| Mg₂Al-NO₃      | 3.78              | 0.86                 | 1.30        | 0.23           | 1.60           | 0.36              | 0.44    | 0.23        | 3.52           | 0.78            | 1.23     | 0.23        |
| Mg₂Al-CO₃      | 4.80              | 1.07                 | 1.54        | 0.23           | 1.58           | 0.35              | 0.43    | 0.23        | 3.46           | 0.76            | 1.22     | 0.22        |
| Mg₂Fe-CO₃      | 4.99              | 1.87                 | 1.53        | 0.41           | 1.70           | 0.44              | 0.50    | 0.27        | 4.44           | 1.10            | 1.46     | 0.26        |
| Mg₂Fe-Cl       | 4.97              | 1.71                 | 1.36        | 0.37           | 1.36           | 0.30              | 0.28    | 0.23        | 3.60           | 0.81            | 1.25     | 0.23        |
| Compound     | IL-8 Response | IL-8 SE | MCP-1 Response | MCP-1 SE | MIP-1β Response | MIP-1β SE |
|--------------|---------------|---------|----------------|----------|----------------|----------|
| Mg₂Fe-NO₃   | 6.68          | 2.80    | 1.80           | 0.46     | 0.34           | 0.31     |
| Imject      | 5.51          | 1.23    | 1.68           | 0.23     | 0.32           | 0.23     |
| Alhydrogel  | 1.61          | 0.36    | 0.45           | 0.23     | 0.23           | 0.23     |

Table S2 (continued)

| Compound     | CD40 Response | CD40 SE | CD86 Response | CD86 SE | CD274 Response | CD274 SE |
|--------------|---------------|---------|---------------|---------|----------------|----------|
| Cells alone  | 1197.40       | 479.20  | 505.51        | 122.72  | 1048.34        | 297.41   |
| LiAl₂-CO₃    | 1963.50       | 785.90  | 1575.45       | 382.48  | 3114.77        | 884.52   |
| LiAl₂-Cl     | 1658.04       | 663.64  | 794.68        | 192.88  | 1154.54        | 327.54   |

Table S2 (continued)
Responses with standard errors (SE) for cytokine and chemokine secretion are in pg/ml, and values for CD40, CD86, and CD274 are shown as mean channel fluorescence intensities. Natural logarithms of response values (ln response) are shown in the columns highlighted in gray, along with the respective SE.

Table S3. Significance data for human DC responses to LDHs (presented in Fig. 2)

| Compound       | IL-1β (n = 10) | IL-6 (n = 18) | TNF-α (n = 22) |
|----------------|----------------|---------------|----------------|
|                | DC alone       | Imject AH     | DC alone       | Imject AH     | DC alone       | Imject AH     |
| LiAl₂-CO₃      | 0.000          | 0.000         | 0.000          | 0.000         | 0.000          | 0.000         |
| LiAl₂-Cl       | 0.000          | 0.192 0.858   | 0.027 0.302 0.167 | 0.000 0.000 0.000 |
| Ca₂Al-Cl       | 0.658 0.002 0.000 | 0.385 0.809 0.666 | 0.000 0.319 0.053 |
| Ca₂Al-NO₃      | 0.088 0.002 0.000 | 0.399 0.044 0.099 | 0.000 0.116 0.733 |
| Mg₂Al-NO₃      | 0.000 0.824 0.181 | 0.005 0.100 0.047 | 0.000 0.443 0.010 |
| Mg₂Al-CO₃      | 0.001 0.177 0.014 | 0.002 0.063 0.028 | 0.000 0.004 0.000 |
| Mg₂Fe-CO₃      | 0.004 0.440 0.109 | 0.000 0.000 0.000 | 0.000 0.008 0.000 |
| Mg₂Fe-Cl       | 0.014 0.015 0.000 | 0.314 0.864 0.854 | 0.002 0.010 0.555 |
| Mg₂Fe-NO₃      | 0.449 0.000 0.000 | 0.309 0.697 0.563 | 0.012 0.742 0.532 |
| Imject         | 0.000 NA 0.260 | 0.238 NA 0.723 | 0.000 NA 0.062 |
| Compound       | IL-12p70 (n = 8) | IL-15 (n = 8) | IFN-α2 (n = 7) |
|---------------|------------------|---------------|----------------|
|               | DC alone | Imject | AH | DC alone | Imject | AH | DC alone | Imject | AH |
| LiAl₂-CO₃     | 0.000    | 0.000  | 0.000   | 0.000    | 0.000  | 0.000   | 0.000    | 0.262  | 0.000 |
| LiAl₂-Cl      | 0.015    | 0.287  | 0.000   | 0.001    | 0.283  | 0.081   | 0.004    | 0.583  | 0.007 |
| Ca₂Al-Cl      | 0.086    | 0.760  | 0.010   | 0.020    | 0.246  | 0.131   | 0.262    | 0.088  | 0.332 |
| Ca₂Al-NO₃     | 0.009    | 0.405  | 0.000   | 0.341    | 0.192  | 0.530   | 0.268    | 0.016  | 0.358 |
| Mg₂Al-NO₃     | 0.057    | 0.135  | 0.001   | 0.002    | 0.349  | 0.107   | 0.104    | 0.052  | 0.149 |
| Mg₂Al-CO₃     | 0.004    | 0.568  | 0.000   | 0.002    | 0.378  | 0.120   | 0.118    | 0.041  | 0.167 |
| Mg₂Fe-CO₃     | 0.084    | 0.755  | 0.009   | 0.013    | 0.341  | 0.156   | 0.019    | 0.507  | 0.028 |
| Mg₂Fe-Cl      | 0.031    | 0.198  | 0.000   | 0.051    | 0.762  | 0.707   | 0.093    | 0.073  | 0.132 |
| Mg₂Fe-NO₃     | 0.034    | 0.744  | 0.004   | 0.220    | 0.956  | 0.683   | 0.144    | 0.149  | 0.189 |
| Imject        | 0.001    | NA     | 0.000   | 0.025    | NA     | 0.497   | 0.001    | NA     | 0.001 |
| Alhydrogel    | 0.133    | 0.000  | NA      | 0.115    | 0.497  | NA      | 0.841    | 0.001  | NA   |

Table S3 (continued)

| Compound       | IL-8 (n = 7) | MCP-1 (n = 8) | MIP-1β (n = 8) |
|---------------|--------------|---------------|----------------|
|               | DC alone    | Imject | AH | DC alone | Imject | AH | DC alone | Imject | AH |
| LiAl₂-CO₃     | 0.000    | 0.018  | 0.000   | 0.000    | 0.034  | 0.000   | 0.000    | 0.001  | 0.000 |
| LiAl₂-Cl      | 0.000    | 0.700  | 0.000   | 0.000    | 0.121  | 0.003   | 0.000    | 0.025  | 0.000 |
| Ca₂Al-Cl      | 0.000    | 0.562  | 0.000   | 0.000    | 0.174  | 0.011   | 0.000    | 0.032  | 0.006 |
| Compound          | CD40 (n = 8) | CD86 (n = 16) | CD274 (n = 6) |
|-------------------|--------------|---------------|---------------|
|                   | DC alone     | Imject        | AH            | DC alone     | Imject        | AH            | DC alone     | Imject        | AH            |
| Ca$_2$Al-NO$_3$   | 0.000 0.019  0.000 | 0.000 0.130  0.996 | 0.000 0.005  0.001 |
| Mg$_2$Al-NO$_3$   | 0.000 0.072  0.000 | 0.000 0.338  0.577 | 0.000 0.000  0.049 |
| Mg$_2$Al-CO$_3$   | 0.000 0.024  0.000 | 0.017 0.000  0.003 | 0.000 0.000  0.865 |
| Mg$_2$Fe-CO$_3$   | 0.000 0.109  0.000 | 0.000 0.255  0.935 | 0.000 0.230  0.000 |
| Mg$_2$Fe-Cl       | 0.000 0.000  0.006 | 0.002 0.000  0.021 | 0.000 0.000  0.999 |
| Mg$_2$Fe-NO$_3$   | 0.000 0.558  0.000 | 0.003 0.010  0.176 | 0.001 0.000  0.812 |
| Imject            | 0.000 NA    0.000 | 0.000 NA    0.133 | 0.000 NA    0.000 |
| Alhydrogel        | 0.008 0.000  NA | 0.000 0.133  NA | 0.000 0.000  NA |

The p-values for the respective DC responses generated by LDHs with respect to cells alone, Imject, and Alhydrogel (denoted AH) are shown. The number (n) of biological replicates (donor DCs) evaluated for each response is indicated. Numbers of experiments are as follows. Cytokines and chemokines: IFN-α2, 7; IL-1β, 10; 5

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Table S3 (continued)
IL-6, 18; IL-8, 7; IL-12p70, 8; IL-15, 8; MCP-1, 8; MIP-1β, 8; and TNF-α, 22. Membrane molecules: CD40, 8; CD86, 18; and CD274, 6. Numbers in bold are where P < 0.05, and those highlighted in gray are where P < 0.01.

Table S4. Selected physicochemical properties of the LDHs used in this study

| Property | LiAl₂CO₃ | LiAl₂Cl | Ca₂Al-Cl | Ca₂Al-NO₃ | Mg₂Al-NO₃ | Mg₂Al-CO₃ | Mg₂Fe-CO₃ | Mg₂Fe-Cl | Mg₂Fe-NO₃ | LiAl₂-NO₃ | Mg₂Al-Cl |
|----------|---------|---------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| Ionic radius (M⁺ or M²⁺) (Å) | 0.760 | 0.760 | 1.000 | 1.000 | 0.720 | 0.720 | 0.720 | 0.720 | 0.720 | 0.760 | 0.720 |
| Ionic radius (M³⁺) (Å) | 0.540 | 0.540 | 0.540 | 0.540 | 0.540 | 0.540 | 0.550 | 0.550 | 0.550 | 0.540 | 0.540 |
| Electronegativity (M⁺ or M²⁺) | 0.980 | 0.980 | 1.000 | 1.000 | 1.310 | 1.310 | 1.310 | 1.310 | 1.310 | 0.980 | 1.310 |
| Electronegativity (M³⁺) | 1.610 | 1.610 | 1.610 | 1.610 | 1.610 | 1.610 | 1.830 | 1.830 | 1.830 | 1.610 | 1.610 |
| Standard electrode potential (M⁺ or M²⁺) (V) | -3.040 | -3.040 | -2.870 | -2.870 | -2.370 | -2.370 | -2.370 | -2.370 | -2.370 | -3.040 | -2.370 |
| Standard electrode potential (M³⁺) (V) | -1.660 | -1.660 | -1.660 | -1.660 | -1.840 | -0.040 | -0.040 | -0.040 | -1.660 | -1.660 |
| a-parameter (Å) | 5.100 | 5.100 | 5.740 | 5.740 | 3.050 | 3.050 | 3.100 | 3.100 | 3.100 | 5.100 | 3.050 |
| Interlayer spacing (Å) | 7.65 | 7.65 | 7.50 | 8.60 | 8.00 | 7.60 | 7.75 | 8.00 | 7.85 | 8.85 | 7.75 |
| pH of 10 μg/ml suspension in PBS | 6.820 | 6.020 | 11.360 | 11.410 | 7.920 | 8.890 | 9.350 | 8.960 | 7.840 | 5.630 | 7.840 |
| Zeta potential (mV) | -19.093 | -12.037 | 14.850 | 17.628 | -14.274 | -14.556 | -13.483 | -15.008 | -11.400 | -15.255 | -13.348 |

Shannon-Prewitt definitions are used for the ionic radii of the metal cations (M⁺ or M²⁺ and M³⁺). Electronegativity is given in the Pauling convention. Standard electrode potentials refer to the process Mⁿ⁺ + ne⁻ → M(0). These parameters were taken from the literature (Lide, 2000). The a-parameter defines the unit cell of the LDH perpendicular to the layer stacking, and the interlayer spacing is the distance between the LDH layers (see Fig. 1). These parameters were calculated from the positions of the non-basal (a-parameter) and 00l (interlayer spacing) reflections in x-ray diffraction patterns. The zeta potential is a measure of the electrical potential difference between the surface of the LDH particles and the PBS medium in which they were suspended; LDH suspensions at 10 mg/ml in PBS (initial pH 7.2) were prepared at least in triplicate for determination of pH and zeta potentials, and the data are given as mean values (standard deviations for zeta potential measurements are typically 0.500–1.00 mV).
b. Standardized properties

| Property                                      | LiAl₂-CO₃ | LiAl₂-Cl | Ca₂Al-Cl | Ca₂Al-NO₃ | Mg₂Al-NO₃ | Mg₂Al-CO₃ | Mg₂Fe-CO₃ | Mg₂Fe-Cl | Mg₂Fe-NO₃ | LiAl₂-NO₃ | Mg₂Al-Cl |
|----------------------------------------------|-----------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| Ionic radius (M⁺ or M²⁺) (Å)                | -0.200    | -0.200   | 1.995    | 1.995     | -0.565    | -0.565    | -0.565    | -0.565    | -0.565    | -0.200    | -0.565   |
| Ionic radius (M³⁺) (Å)                      | -0.584    | -0.584   | -0.584   | -0.584    | -0.584    | -0.584    | 1.557     | 1.557     | 1.557     | -0.584    | -0.584   |
| Electronegativity (M⁺ or M²⁺)               | -1.091    | -1.091   | -0.972   | -0.972    | 0.870     | 0.870     | 0.870     | 0.870     | 0.870     | -1.091    | 0.870    |
| Electronegativity (M³⁺)                      | -0.584    | -0.584   | -0.584   | -0.584    | -0.584    | -0.584    | 1.557     | 1.557     | 1.557     | -0.584    | -0.584   |
| Standard electrode potential (M⁺ or M²⁺) (V) | -1.239    | -1.239   | -0.708   | -0.708    | 0.856     | 0.856     | 0.856     | 0.856     | 0.856     | -1.239    | 0.856    |
| Standard electrode potential (M³⁺) (V)      | -0.584    | -0.584   | -0.584   | -0.584    | -0.584    | -0.584    | 1.557     | 1.557     | 1.557     | -0.584    | -0.584   |
| a-parameter (Å)                              | 0.816     | 0.816    | 1.344    | 1.344     | -0.876    | -0.876    | -0.835    | -0.835    | -0.835    | 0.816     | -0.876   |
| Interlayer spacing (Å)                       | -0.649    | -0.649   | -1.000   | 1.574     | 0.170     | -0.766    | -0.415    | 0.170     | -0.181    | 2.159     | -0.415   |
| pH of 10 µg/mL suspension in PBS            | -0.816    | -1.238   | 1.578    | 1.605     | -0.236    | 0.276     | 0.518     | 0.313     | -0.278    | -1.444    | -0.278   |
| Zeta potential (mV)                          | -0.828    | -0.265   | 1.883    | 2.105     | -0.443    | -0.466    | -0.380    | -0.502    | -0.214    | -0.522    | -0.369   |

The properties in Table S4 a were standardized to have mean 0 and unit variance in order to facilitate development of models linking physicochemical properties and immunological responses; from these, Eq. 1 (see Results and discussion) was derived, and predictions were made.

Table S5. Coefficients for DC responses

| Response | A   | B   | C for standardized properties |
|----------|-----|-----|-------------------------------|
|          | Ionic radius (M⁺ or M²⁺) | Interlayer spacing | Zeta potential |
| IL-1β    | 2.790 | 2.941 | 0.911 | 0.087 | -1.054 |
| IL-6     | -3.244 | 1.899 | 0.883 | 0.053 | -1.050 |
| TNF-α    | -1.602 | 2.262 | 1.008 | 0.036 | -1.122 |
| IL-12p70 | 1.747 | 1.223 | 1.033 | 0.060 | -1.122 |
| IL-15    | 0.442 | 0.442 | 1.064 | -0.116 | -1.107 |
| IFN-α2   | 1.375 | 0.390 | 0.626 | -0.150 | -0.812 |
Responses of human DC to LDHs in vitro can be expressed by Eq. 1. Values for the constants $A$ and $B$ and the coefficients $C_i$ are shown for each respective DC response measured. Use of the values above in Eq. 1 with the appropriate standardized properties in Table S4b generates calculated values for the ln responses. Note that for TNF-α and IL-6 the formula calculates ln (response/ng ml$^{-1}$), for other cytokines and chemokines, it gives ln (response/pg ml$^{-1}$), and for the membrane molecules ln (response/MFI). For any given response, the relative magnitudes of $C_i$ for the three standardized properties are proportional to the influence that the respective properties have in controlling that response.

Table S6.  Actual versus predicted responses to two newly-synthesised LDHs

| Response | LiAl$_2$-NO$_3$ | Mg$_2$Al-Cl |
|----------|-----------------|-------------|
|          | Actual | Predicted | Lower PI | Upper PI | Residual | %CV | Actual | Predicted | Lower PI | Upper PI | Residual | %CV |
| IL-1β    | 4.86  | 4.80   | 3.70   | 5.90  | 0.06  | 1.23 | 2.66  | 2.10   | 1.19   | 3.01  | 0.56   | 21.05 |
| IL-6     | -2.02 | -2.74  | -3.98  | -1.50 | 0.73  | 35.98 | -3.48 | -3.50  | -4.67  | -2.32 | 0.02   | 0.50  |
| TNF-α    | -0.10 | -1.26  | -2.32  | -0.20 | 1.16  | 1159.84 | -1.72 | -2.04  | -3.08  | -1.00 | 0.32   | 18.60 |
| IL-12p70 | 2.53  | 2.35   | 1.36   | 3.33  | 0.18  | 7.11 | 1.54  | 1.46   | 0.58   | 2.34  | 0.08   | 5.19  |
| IL-15    | 0.58  | 0.29   | 0.10   | 0.48  | 0.29  | 50.00 | 0.25  | 0.40   | 0.22   | 0.59  | -0.15  | 60.00 |
| IFN-α2   | 1.3   | 1.59   | 1.28   | 1.89  | -0.29 | 22.31 | 1.76  | 1.29   | 1.01   | 1.57  | 0.47   | 26.70 |
| IL-8     | 7.94  | 7.22   | 6.16   | 8.28  | 0.72  | 9.07 | 7.77  | 7.64   | 6.71   | 8.57  | 0.13   | 1.67  |
| MCP-1    | 8.33  | 7.29   | 6.56   | 8.02  | 1.04  | 12.48 | 7.46  | 7.08   | 6.33   | 7.83  | 0.38   | 5.09  |
| MIP-1β   | 7.77  | 7.43   | 6.25   | 8.61  | 0.34  | 4.38 | 6.15  | 6.04   | 4.97   | 7.10  | 0.11   | 1.79  |
| CD40     | 7.43  | 7.34   | 7.12   | 7.56  | 0.09  | 1.21 | 7.29  | 7.29   | 7.07   | 7.51  | 0.00   | 0.00  |
| CD86     | 7.17  | 7.24   | 6.81   | 7.66  | -0.07 | 0.98 | 6.61  | 6.50   | 6.15   | 6.86  | 0.11   | 1.66  |
| CD274    | 7.23  | 7.51   | 7.00   | 8.01  | -0.28 | 3.87 | 7.23  | 7.13   | 6.65   | 7.60  | 0.10   | 1.38  |
Actual (measured) DC ln responses to LiAl₂-NO₃ and Mg₂Al-Cl and predicted values from Eq. 1 using the respective property values in Table S4 b (for standardized properties) and coefficients in Table S5; residual = actual value − predicted value, and % coefficient of variation (CV) = 100 × (|residual|/actual). Upper and lower 95% CIs have been calculated for the predictions and are included above as lower and upper predictive intervals (PI). Note that for TNF-α and IL-6 the formula calculates ln (response/ng ml⁻¹), for other cytokines and chemokines it gives ln (response/pg ml⁻¹), and for the membrane molecules ln (response/MFI).

**Table S7.** Mouse OVA-specific antibody isotype responses to LDHs and alums (presented in Fig. 4)

### a. After prime-boost

| Treatment       | IgE OD450 | IgE ln OD450 | IgE ln SE | IgG1 OD450 | IgG1 ln OD450 | IgG1 ln SE | IgG2c OD450 | IgG2c ln OD450 | IgG2c ln SE |
|-----------------|-----------|--------------|-----------|------------|---------------|------------|-----------|---------------|------------|
| Saline          | 0.04      | -3.46        | 0.70      | 0.07       | -2.83         | 0.57       | 0.05      | -3.14         | 0.54       |
| OVA alone       | 0.07      | -2.85        | 0.67      | 0.05       | -3.06         | 0.53       | 0.07      | -2.85         | 0.52       |
| LiAl₂-CO₃       | 0.46      | -0.98        | 0.69      | 1.62       | 0.34          | 0.55       | 0.18      | -1.87         | 0.53       |
| LiAl₂-Cl        | 0.49      | -0.93        | 0.69      | 1.34       | 0.15          | 0.55       | 0.32      | -1.28         | 0.53       |
| Ca₂Al-NO₃      | 0.31      | -1.38        | 0.69      | 3.21       | 1.02          | 0.55       | 0.49      | -0.86         | 0.53       |
| Mg₂Al-NO₃      | 0.13      | -2.22        | 0.69      | 0.19       | -1.81         | 0.55       | 0.10      | -2.43         | 0.53       |
| Mg₂Al-CO₃      | 0.48      | -0.95        | 0.69      | 1.97       | 0.53          | 0.55       | 0.26      | -1.47         | 0.53       |
| Mg₂Fe-CO₃      | 0.34      | -1.34        | 0.76      | 2.77       | 0.83          | 0.65       | 0.44      | -0.99         | 0.58       |
| Mg₂Fe-Cl       | 0.05      | -3.24        | 0.69      | 0.07       | -2.85         | 0.56       | 0.14      | -2.07         | 0.53       |
| Imject         | 0.24      | -1.63        | 0.67      | 2.21       | 0.66          | 0.53       | 0.32      | -1.27         | 0.52       |
| Alhydrogel     | 0.26      | -1.58        | 0.69      | 1.87       | 0.48          | 0.55       | 0.17      | -1.90         | 0.53       |

### b. After challenge

| Treatment       | IgE OD450 | IgE ln OD450 | IgE ln SE | IgG1 OD450 | IgG1 ln OD450 | IgG1 ln SE | IgG2c OD450 | IgG2c ln OD450 | IgG2c ln SE |
|-----------------|-----------|--------------|-----------|------------|---------------|------------|-----------|---------------|------------|
| Saline          | 0.06      | -2.85        | 0.50      | 0.05       | -3.24         | 0.65       | 0.07      | -2.86         | 0.68       |
| OVA alone       | 0.04      | -3.23        | 0.48      | 0.05       | -3.10         | 0.63       | 0.07      | -2.90         | 0.66       |
| LiAl₂-CO₃       | 0.41      | -1.01        | 0.49      | 1.86       | 0.43          | 0.65       | 0.17      | -1.98         | 0.67       |
| LiAl₂-Cl        | 0.32      | -1.25        | 0.49      | 1.42       | 0.16          | 0.65       | 0.20      | -1.81         | 0.67       |
Responses with standard errors (SE) are shown, with ln response (and ln SE) values in columns highlighted in gray, after prime-boost alone (a) and after a final challenge (b). Data are from two replicate experiments, each with at least five mice per experimental group. All responses are shown as OD450 units from ELISA assays.

Table S8. Significance data for mouse antibody isotype responses (tabulation of P-values presented in Fig. 4)

| Treatment       | OVA-specific IgE OVA alone | Imject | AH | OVA-specific IgG1 OVA alone | Imject | AH | OVA-specific IgG2c OVA alone | Imject | AH |
|-----------------|---------------------------|--------|----|----------------------------|--------|----|-----------------------------|--------|----|
| Saline alone    | 0.126                     | **0.000** | 0.000 | 0.533                     | **0.000** | 0.000 | 0.337                      | **0.000** | 0.000 |
| LiAl2-CO3       | **0.000** | 0.093 | 0.156 | **0.000** | 0.356 | 0.714 | **0.001** | **0.044** | 0.925 |
| LiAl2-Cl        | **0.000** | 0.069 | 0.122 | **0.000** | 0.138 | 0.376 | **0.000** | 0.973 | 0.057 |
| Ca2Al-NO3       | **0.000** | 0.516 | 0.637 | **0.000** | 0.296 | 0.152 | **0.000** | 0.158 | **0.002** |
| Mg2Al-NO3       | 0.103 | 0.122 | 0.124 | **0.000** | **0.000** | **0.000** | 0.157 | **0.000** | 0.102 |
| Mg2Al-CO3       | **0.000** | 0.078 | 0.135 | **0.000** | 0.710 | 0.889 | **0.000** | 0.504 | 0.182 |
| Mg2Fe-CO3       | **0.003** | 0.558 | 0.644 | **0.000** | 0.731 | 0.492 | **0.000** | 0.457 | **0.024** |
| Mg2Fe-Cl        | 0.313 | **0.000** | **0.000** | 0.553 | **0.000** | **0.000** | **0.010** | **0.008** | 0.591 |
| Imject          | **0.001** | NA | 0.893 | **0.000** | NA | 0.601 | **0.000** | NA | 0.035 |
| Alhydrogel      | **0.001** | 0.893 | NA | **0.000** | 0.601 | NA | **0.002** | **0.035** | NA |
b. After challenge

| Treatment          | OVA-specific IgE  | OVA-specific IgG1 | OVA-specific IgG2c |
|--------------------|-------------------|-------------------|-------------------|
|                    | OVA alone | Imject   | AH | OVA alone | Imject   | AH | OVA alone | Imject   | AH |
| Saline alone       | 0.182     | 0.000    | 0.000 | 0.724     | 0.000    | 0.000 | 0.892     | 0.000    | 0.000 |
| LiAl$_2$-CO$_3$    | 0.000     | 0.248    | 0.107 | 0.000     | 0.889    | 0.597 | 0.003     | 0.003    | 0.053 |
| LiAl$_2$-Cl        | 0.000     | 0.427    | 0.000 | 0.000     | 0.359    | 0.238 | 0.000     | 0.015    | 0.155 |
| Ca$_3$Al-NO$_3$    | 0.000     | 0.019    | 0.000 | 0.000     | 0.819    | 0.542 | 0.000     | 0.099    | 0.021 |
| Mg$_3$Al-NO$_3$    | 0.009     | 0.000    | 0.001 | 0.005     | 0.000    | 0.000 | 0.148     | 0.000    | 0.001 |
| Mg$_2$Al-CO$_3$    | 0.000     | 0.051    | 0.000 | 0.000     | 0.017    | 0.819 | 0.000     | 0.755    | 0.592 |
| Mg$_2$Fe-CO$_3$    | 0.000     | 0.083    | 0.000 | 0.000     | 0.737    | 0.980 | 0.000     | 0.000    | 0.000 |
| Mg$_2$Fe-Cl        | 0.075     | 0.000    | 0.000 | 0.004     | 0.000    | 0.000 | 0.109     | 0.000    | 0.000 |
| Imject             | 0.000     | NA       | 0.539 | 0.000     | NA       | 0.662 | 0.000     | NA       | 0.370 |
| Alhydrogel         | 0.000     | 0.539    | NA   | 0.000     | 0.662    | NA   | 0.000     | 0.370    | NA   |

The p-values for the respective OVA-specific, antibody isotype responses induced by LDHs with respect to OVA alone, Imject, and Alhydrogel (denoted AH) are shown. Values in bold are where $P < 0.05$ and those highlighted in gray where $P < 0.01$. Data are from two replicate experiments, each with at least five mice per experimental group. Note that data for total IgG2c induced after prime-boost and for total IgG1 and IgG2c induced after challenge also reached significance, with responses conforming to Eq. 1; these data are omitted for simplicity.

**Table S9. Coefficients for antibody responses**

| OVA-specific isotype | $A$  | $B$  | $C_i$ for standardized properties |
|----------------------|------|------|-----------------------------------|
|                      |      |      | Ionic radius (M$^+$ or M$^{2+}$) | Interlayer spacing | Zeta potential |
| Prime-boost          |      |      |                                  |                    |                |
| IgE                  | -1.570 | -1.965 | -0.428 | 0.945 | -0.344 |
| IgG1                 | -0.196 | -3.245 | -0.286 | 0.889 | -0.562 |
| IgG2c                | -1.526 | -1.271 | 0.235  | 0.779 | -1.053 |
| Challenge            |      |      |                                  |                    |                |
Mouse antibody responses to LDHs in vivo can be expressed by Eq. 1. Values for the constants \( A \) and \( B \) and the coefficient \( C_i \) are shown for each respective antigen-specific antibody response measured. Use of the values above, with the appropriate standardized properties in Table S4.b, in Eq. 1 generates calculated values for the \( \ln \) responses given in Table S6.

**Table S10.** Inflammatory cytokine secretion by human macrophages stimulated by LDHs in vitro

a. Responses with standard errors (SE), with \( \ln \) responses (with \( \ln \) SE) in columns highlighted in gray, for cytokine secretion in pg/ml

| Compound        | Macrophages, IL-6 Response | Macrophages, IL-6 \( \ln \) response | Dermal macrophages, IL-6 Response | Dermal macrophages, IL-6 \( \ln \) response |
|-----------------|-----------------------------|--------------------------------------|-----------------------------------|---------------------------------------------|
|                 | SE                          | \( \ln \) SE                          | SE                                | \( \ln \) SE                                  |
| Cells alone     | 26.34                       | 10.22                                | 3.19                              | 0.42                                        |
| LiAl\(_2\)-CO\(_3\) | 247.70                      | 99.61                                | 5.42                              | 0.44                                        |
| LiAl\(_2\)-Cl   | 38.47                       | 14.92                                | 3.56                              | 0.42                                        |
| Ca\(_2\)Al-NO\(_3\) | 28.08                       | 10.89                                | 3.25                              | 0.42                                        |
| Mg\(_2\)Al-NO\(_3\) | 27.02                       | 10.65                                | 3.21                              | 0.43                                        |
| Mg\(_2\)Al-CO\(_3\) | 112.38                      | 43.60                                | 4.64                              | 0.42                                        |
| Mg\(_2\)Fe-Cl   | 34.79                       | 13.50                                | 3.46                              | 0.42                                        |
| Imject          | 18.73                       | 7.26                                 | 2.84                              | 0.42                                        |
| Alhydrogel      | 33.50                       | 13.00                                | 3.43                              | 0.42                                        |

**Table S10 a (Continued)**

| Compound        | Macrophages, TNF-\( \alpha \) Response | Macrophages, TNF-\( \alpha \) \( \ln \) response | Dermal macrophages, TNF-\( \alpha \) Response | Dermal macrophages, TNF-\( \alpha \) \( \ln \) response |
|-----------------|----------------------------------------|--------------------------------|---------------------------------------------|---------------------------------------------|
|                 | SE                                    | \( \ln \) SE                          | SE                                          | \( \ln \) SE                                  |
| Cells alone     | 19.27                                  | 10.07                                | 2.78                                          | 0.62                                        |
| LiAl\(_2\)-CO\(_3\) | 1240.42                                  | 648.22                              | 6.95                                          | 0.62                                        |
Human macrophages or dermal macrophages derived from monocytes were cultured without or with the indicated LDH or commercial adjuvant preparation for a period of 24 hours, and cytokine secretion determined by ELISA.

**b. Significance data for human macrophage responses to LDHs**

| Compound    | Macrophages, IL-6 (n=4) | Dermal macrophages, IL-6 (n=3) | Macrophages, IL-6 (n=4) | Dermal macrophages, IL-6 (n=3) |
|-------------|-------------------------|-------------------------------|-------------------------|-------------------------------|
| LiAl₂-Cl    | 0.000 0.000 0.000       | 0.000 0.000 0.000             | 0.000 0.000 0.000       | 0.000 0.000 0.000             |
| LiAl₂-NO₃   | 0.261 0.034 0.680       | 0.237 0.089 0.010             | 0.000 0.000 0.000       | 0.005 0.204 0.000             |
| Ca₂Al-NO₃   | 0.849 0.229 0.599       | 0.132 0.045 0.005             | 0.001 0.157 0.037       | 0.012 0.389 0.001             |
| Mg₂Al-NO₃   | 0.949 0.295 0.528       | 0.348 0.144 0.020             | 0.003 0.347 0.109       | 0.469 0.333 0.097             |
| Mg₂Al-CO₃   | 0.000 0.000 0.001       | 0.001 0.000 0.000             | 0.000 0.000 0.000       | 0.004 0.180 0.000             |
| Mg₂Fe-Cl    | 0.408 0.068 0.910       | 0.007 0.001 0.000             | 0.002 0.270 0.078       | 0.707 0.186 0.190             |
| Imject      | 0.311 NA 0.086         | 0.584 NA 0.353               | 0.034 NA 0.488          | 0.089 NA 0.008               |
| Alhydrogel  | 0.474 0.086 NA         | 0.142 0.353 NA               | 0.147 0.488 NA          | 0.353 0.008 NA               |

The number (n) of biological replicates (donor macrophages or dermal macrophages) evaluated for each response is indicated. Values given in the table are p-values; values in bold are where P < 0.05 and those highlighted in gray are where P < 0.01.

**c. Coefficients for macrophage responses**

| Macrophage | A | B | Cᵢ for standardized properties |
|------------|---|---|-------------------------------|
|             | Ionic radius, (M⁺ or M²⁺) | Interlayer spacing | Zeta potential |

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### IL-6

|                | Macrophages |     |     |     |     |
|----------------|-------------|-----|-----|-----|-----|
|                |             |     |     |     |     |
| Dermal macrophages | -2.019      | 1.032 | 1.429 | -0.005 | -1.486 |

### TNF-α

|                | Macrophages |     |     |     |     |
|----------------|-------------|-----|-----|-----|-----|
|                |             |     |     |     |     |
| Dermal macrophages | -1.654      | -1.473 | -1.436 | 0.334 | 1.120 |

Responses of human macrophages to LDHs in vitro can be expressed by Eq. 1. Values for the constants $A$ and $B$ and the coefficient $C_i$ are shown for each macrophage response measured. Use of the values above in Eq. 1, with the appropriate standardized properties in Table S4 b, generates calculated values for the In responses. Note that the formula calculates ln (response/ng ml$^{-1}$).

**Reference**

Lide, D.R., editor. 2000. CRC Handbook of Chemistry and Physics. 81st edition. CRC Press, Boca Raton, FL. 2556 pp.