SUPPORTING INFORMATION

**Immune profiling and tracking of two-dimensional transition metal dichalcogenides in cells and tissues**

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**Table of contents:**

1. Supplementary Tables
2. Supplementary Figures
3. **Supplementary Tables**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Analyte** |  | **ConA** | | |  | **LPS** | | |  | **G** | | |  | **MoS2** | | |  | **WS2** | | |  | **Unt** | | |
| MIF |  | 15,9 | 16,5 | 16,5 |  | 15,8 | 15,9 | 16,1 |  | 16,3 | 15,6 | 15,0 |  | 17,2 | 16,2 | 16,3 |  | 15,8 | 15,5 | 15,7 |  | 15,4 | 16,0 | 15,7 |
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| IL4 |  | 7,6 | 7,4 | 7,7 |  | 5,3 | 4,7 | 5,4 |  | 8,8 | 8,6 | 8,6 |  | 3,6 | 3,6 | 3,6 |  | 3,6 | 3,6 | 3,6 |  | 3,6 | 4,2 | 3,6 |
| CCL15 |  | 6,5 | 6,5 | 6,4 |  | 5,9 | 5,8 | 5,6 |  | 7,1 | 6,9 | 6,6 |  | 4,1 | 4,0 | 3,5 |  | 3,9 | 3,5 | 3,2 |  | 3,5 | 4,2 | 2,9 |
| CXCL1 |  | 12,5 | 12,9 | 13,8 |  | 15,6 | 12,7 | 12,8 |  | 13,0 | 13,4 | 13,1 |  | 9,7 | 10,1 | 9,4 |  | 6,3 | 6,6 | 6,0 |  | 5,3 | 7,3 | 6,7 |
| CXCL8 |  | 12,8 | 13,3 | 14,3 |  | 14,3 | 13,8 | 13,9 |  | 13,9 | 14,5 | 14,9 |  | 12,3 | 12,9 | 11,8 |  | 9,4 | 9,8 | 9,2 |  | 9,1 | 9,8 | 9,7 |
| CCL3 |  | 11,8 | 12,4 | 12,9 |  | 12,9 | 12,8 | 13,0 |  | 9,8 | 9,2 | 8,6 |  | 4,0 | 4,1 | 3,6 |  | 3,8 | 3,6 | 3,0 |  | 3,6 | 7,3 | 4,3 |
| IL6 |  | 12,3 | 14,7 | 14,8 |  | 13,8 | 14,8 | 13,7 |  | 9,6 | 9,0 | 8,7 |  | 4,0 | 4,2 | 3,7 |  | 3,7 | 3,5 | 3,3 |  | 3,0 | 6,6 | 4,7 |
| TNFa |  | 12,3 | 12,4 | 12,4 |  | 11,3 | 10,9 | 10,8 |  | 8,9 | 8,3 | 8,3 |  | 5,4 | 5,1 | 5,0 |  | 5,4 | 5,1 | 4,7 |  | 4,9 | 5,5 | 4,8 |
| CX3CL1 |  | 9,4 | 9,4 | 9,4 |  | 8,5 | 8,4 | 8,3 |  | 7,7 | 7,5 | 7,3 |  | 6,0 | 5,8 | 5,5 |  | 5,6 | 5,6 | 5,3 |  | 5,5 | 6,2 | 5,2 |
| CXCL13 |  | 8,4 | 8,2 | 8,0 |  | 8,7 | 8,5 | 8,0 |  | 5,6 | 5,6 | 5,3 |  | 3,3 | 3,3 | 2,7 |  | 3,3 | 3,0 | 2,6 |  | 3,5 | 4,1 | 2,9 |
| IL-1b |  | 12,2 | 11,9 | 11,9 |  | 12,8 | 12,0 | 11,8 |  | 7,9 | 7,4 | 7,1 |  | 4,0 | 4,2 | 3,9 |  | 3,3 | 3,4 | 3,0 |  | 3,1 | 4,8 | 3,8 |
| CCL20 |  | 10,8 | 10,7 | 10,5 |  | 11,2 | 10,8 | 9,8 |  | 7,2 | 6,8 | 6,3 |  | 3,9 | 4,3 | 3,8 |  | 3,8 | 3,1 | 3,1 |  | 3,4 | 3,6 | 1,8 |
| CXCL6 |  | 9,0 | 8,8 | 8,6 |  | 9,7 | 9,5 | 8,7 |  | 7,1 | 6,9 | 6,2 |  | 4,6 | 4,8 | 4,4 |  | 4,8 | 4,5 | 3,9 |  | 4,5 | 4,7 | 2,9 |
| IL10 |  | 10,9 | 10,9 | 10,7 |  | 12,2 | 11,9 | 11,6 |  | 8,0 | 7,8 | 7,5 |  | 7,5 | 7,5 | 7,2 |  | 6,1 | 6,3 | 5,8 |  | 5,5 | 6,1 | 5,5 |
| CCL19 |  | 11,9 | 12,0 | 12,0 |  | 12,5 | 12,5 | 12,3 |  | 11,0 | 11,0 | 10,7 |  | 10,0 | 10,3 | 9,8 |  | 9,5 | 9,5 | 8,9 |  | 9,1 | 9,3 | 8,6 |
| CXCL2 |  | 13,7 | 14,1 | 15,1 |  | 14,6 | 14,8 | 14,6 |  | 12,0 | 11,7 | 11,9 |  | 9,7 | 9,7 | 9,4 |  | 8,8 | 8,6 | 8,6 |  | 8,2 | 8,4 | 8,1 |
| CCL27 |  | 9,5 | 9,6 | 9,6 |  | 9,3 | 9,3 | 9,2 |  | 7,1 | 6,4 | 6,4 |  | 3,3 | 2,5 | 3,2 |  | 3,5 | -0,6 | 3,0 |  | -0,6 | 4,3 | -0,6 |
| CCL1 |  | 10,6 | 10,6 | 10,5 |  | 10,0 | 10,1 | 9,9 |  | 10,4 | 9,7 | 9,6 |  | 8,4 | 8,5 | 8,3 |  | 8,2 | 8,1 | 7,7 |  | 7,9 | 8,2 | 7,7 |
| CCL26 |  | 10,9 | 11,0 | 11,1 |  | 10,3 | 10,5 | 10,2 |  | 9,5 | 9,2 | 9,2 |  | 7,1 | 7,4 | 7,1 |  | 6,8 | 6,5 | 6,4 |  | 6,0 | 7,0 | 5,4 |
| CCL13 |  | 11,1 | 11,8 | 12,0 |  | 10,4 | 10,9 | 11,0 |  | 8,5 | 8,6 | 8,2 |  | 5,3 | 5,6 | 5,2 |  | 1,7 | 1,7 | 1,7 |  | 1,7 | 1,7 | 1,7 |
| CCL25 |  | 12,9 | 13,2 | 13,0 |  | 12,4 | 12,6 | 12,3 |  | 12,0 | 11,9 | 11,8 |  | 11,4 | 11,2 | 11,1 |  | 10,5 | 10,3 | 10,3 |  | 9,9 | 10,5 | 10,0 |
| CCL21 |  | 13,1 | 13,2 | 13,1 |  | 12,7 | 12,7 | 12,6 |  | 12,3 | 12,2 | 12,2 |  | 11,5 | 11,6 | 11,4 |  | 10,5 | 10,4 | 10,3 |  | 9,7 | 10,7 | 10,1 |
| CCL23 |  | 8,0 | 8,0 | 7,2 |  | 9,0 | 9,0 | 8,5 |  | 6,9 | 6,3 | 6,1 |  | 0,9 | -0,1 | -0,1 |  | 2,7 | 1,9 | -0,1 |  | -0,1 | 0,9 | -0,1 |
| CXCL5 |  | 15,8 | 16,2 | 16,3 |  | 16,2 | 16,2 | 15,8 |  | 15,5 | 15,2 | 15,2 |  | 9,5 | 9,7 | 11,1 |  | 9,5 | 9,5 | 9,5 |  | 9,5 | 9,5 | 9,5 |
| CXCL10 |  | 13,6 | 13,8 | 13,7 |  | 8,9 | 8,9 | 8,0 |  | 9,3 | 9,2 | 8,5 |  | 5,4 | 5,1 | 5,0 |  | 7,6 | 6,9 | 5,5 |  | 8,0 | 7,4 | 7,2 |
| CCL22 |  | 13,5 | 13,3 | 13,7 |  | 11,4 | 11,1 | 10,7 |  | 11,9 | 12,2 | 11,5 |  | 8,3 | 8,4 | 8,1 |  | 10,7 | 9,7 | 9,6 |  | 11,0 | 10,5 | 10,1 |
| CCL11 |  | 8,3 | 8,4 | 8,5 |  | 7,7 | 7,7 | 7,5 |  | 7,9 | 7,8 | 7,6 |  | 6,3 | 6,2 | 5,9 |  | 7,1 | 6,4 | 6,3 |  | 7,0 | 7,0 | 6,4 |
| CCL8 |  | 12,4 | 12,4 | 14,2 |  | 8,1 | 8,2 | 7,7 |  | 9,8 | 9,3 | 9,2 |  | 6,2 | 6,1 | 5,0 |  | 4,1 | 3,8 | 3,4 |  | 3,2 | 3,5 | 3,1 |
| CCL7 |  | 13,8 | 13,9 | 13,8 |  | 10,8 | 11,0 | 10,5 |  | 12,7 | 11,8 | 11,8 |  | 9,2 | 9,4 | 8,6 |  | 7,8 | 7,8 | 7,0 |  | 7,8 | 7,4 | 6,4 |
| CXCL11 |  | 8,3 | 8,0 | 8,4 |  | 6,4 | 6,3 | 6,4 |  | 6,0 | 5,7 | 5,5 |  | 3,3 | 3,5 | 3,0 |  | 3,5 | 2,8 | 2,1 |  | 3,1 | 3,4 | 1,7 |
| CXCL12 |  | 13,1 | 13,3 | 13,2 |  | 10,9 | 10,8 | 10,5 |  | 10,3 | 10,1 | 9,7 |  | 8,7 | 8,8 | 8,0 |  | 8,6 | 7,7 | 6,7 |  | 8,3 | 8,7 | 6,3 |
| IL-2 |  | 11,7 | 11,7 | 12,1 |  | 5,4 | 5,6 | 5,4 |  | 6,6 | 7,0 | 6,8 |  | 4,6 | 4,7 | 3,8 |  | 4,0 | 5,4 | 3,3 |  | 4,0 | 3,0 | 4,8 |
| CXCL9 |  | 14,5 | 14,5 | 14,5 |  | 8,8 | 8,9 | 8,7 |  | 8,9 | 8,9 | 8,9 |  | 6,9 | 6,9 | 6,6 |  | 7,1 | 7,1 | 6,0 |  | 7,2 | 7,4 | 6,6 |
| CCL17 |  | 11,8 | 11,8 | 11,5 |  | 8,0 | 8,0 | 7,6 |  | 7,9 | 7,6 | 7,0 |  | 6,4 | 6,2 | 6,1 |  | 6,9 | 6,6 | 6,2 |  | 7,0 | 7,1 | 5,2 |
| IFN-g |  | 16,0 | 16,0 | 15,4 |  | 10,2 | 10,1 | 10,0 |  | 10,0 | 9,9 | 9,6 |  | 8,9 | 9,1 | 8,8 |  | 7,6 | 7,5 | 6,9 |  | 7,3 | 7,5 | 5,8 |
| GM-CSF |  | 11,7 | 11,6 | 11,5 |  | 9,1 | 9,0 | 8,7 |  | 8,5 | 8,4 | 8,1 |  | 7,9 | 7,8 | 7,0 |  | 7,9 | 8,1 | 7,5 |  | 7,4 | 7,7 | 5,1 |
| IL-16 |  | 8,6 | 8,6 | 8,5 |  | 7,7 | 7,6 | 7,4 |  | 7,9 | 7,8 | 7,4 |  | 7,7 | 7,7 | 7,3 |  | 8,1 | 7,8 | 7,6 |  | 7,6 | 7,7 | 7,0 |
| CCL24 |  | 10,2 | 10,0 | 9,7 |  | 8,3 | 8,2 | 7,3 |  | 14,6 | 14,5 | 14,2 |  | 4,9 | 5,3 | 5,3 |  | 8,7 | 8,6 | 7,0 |  | 11,0 | 10,1 | 9,7 |
| CXCL16 |  | 9,5 | 9,3 | 8,8 |  | 8,0 | 8,0 | 7,3 |  | 10,3 | 10,2 | 9,6 |  | 9,5 | 9,3 | 8,7 |  | 9,9 | 9,8 | 9,2 |  | 9,7 | 10,0 | 9,4 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

**Table S1. Cytokine analysis on human peripheral blood mononuclear cells (PBMCs).** Analysis of inflammatory mediators released by PBMCs treated with 50 μg/ml of G, MoS2, or WS2 for 24 h or left untreated (Unt) performed by Luminex assay. As positive controls, cells were exposed to ConA (10 μg/mL) and LPS (2 μg/mL). Values represent the Log2 concentration for each cytokine. Three independent samples were analyzed for each sample group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target** | **Metal** | **Clone** | **Catalog #** | **Company** |
| CD45 | 089Y | HI30 | 201325 | Fluidigm |
| CD196/CCR6 | 141Pr | G034E3 | 201325 | Fluidigm |
| CD235a/b | 141Pr | HIR2 | 201304 | Fluidigm |
| IL-4 | 142Nd | MP4-25D2 | 3142002B | Fluidigm |
| CD19 | 142Nd | HIB19 | 201304 | Fluidigm |
| CD123 (IL-3R) | 143Nd | 6H6 | 201325 | Fluidigm |
| IL-5 | 143Nd | TRFK5 | 201308 | Fluidigm |
| CD19 | 144Nd | HIB19 | 201325 | Fluidigm |
| IL-4 | 144Nd | MP4-25D2 | 201308 | Fluidigm |
| CD4 | 145Nd | RPA-T4 | 201325 | Fluidigm |
| CD8a | 146Nd | RPA-T8 | 201325 | Fluidigm |
| CD11c | 147Sm | Bu15 | 201325 | Fluidigm |
| CD20 | 147Sm | 2H7 | 201304 | Fluidigm |
| CD16 | 148Nd | 3G8 | 201325 | Fluidigm |
| CD45RO | 149Sm | UCHL1 | 201325 | Fluidigm |
| CD66 | 149Sm | CD66a-B1.1 | 201304 | Fluidigm |
| CD45RA | 150Nd | HI100 | 201325 | Fluidigm |
| MIP1β | 150Nd | D21-1351 | 201308 | Fluidigm |
| CD161 | 151Eu | HP-3G10 | 201325 | Fluidigm |
| CD123 | 151Eu | 6H6 | 201304 | Fluidigm |
| CD194/CCR4 | 152Sm | L291H4 | 201325 | Fluidigm |
| TNFα | 152Sm | Mab11 | 201308 | Fluidigm |
| CD25 | 153Eu | BC96 | 201325 | Fluidigm |
| CD27 | 154Sm | O323 | 201325 | Fluidigm |
| CD45 | 154Sm | HI30 | 201304 | Fluidigm |
| CD57 | 155Gd | HCD57 | 201325 | Fluidigm |
| CD183/CXCR3 | 156Gd | G025H7 | 201325 | Fluidigm |
| IL-6 | 156Gd | MQ2-13A5 | 201308 | Fluidigm |
| CD185/CXCR5 | 158Gd | J252D4 | 201325 | Fluidigm |
| IL-2 | 158Gd | MQ1-17H12 | 201308 | Fluidigm |
| GM-CSF | 159Tb | BVD2-21C11 | 3159008B | Fluidigm |
| CD11c | 159Tb | Bu15 | 201304 | Fluidigm |
| CD28 | 160Gd | CD28.2 | 201325 | Fluidigm |
| CD14 | 160Gd | M5E2 | 201304 | Fluidigm |
| CD38 | 161Dy | HB-7 | 201325 | Fluidigm |
| CD56 (NCAM) | 163Dy | NCAM16.2 | 201325 | Fluidigm |
| TCRgd | 164Dy | B1 | 201325 | Fluidigm |
| IL-17A | 164Dy | N49-653 | 201308 | Fluidigm |
| IFNg | 165Ho | B27 | 3165002B | Fluidigm |
| CD61 | 165Ho | VI-PL2 | 201304 | Fluidigm |
| IL-17F | 166Er | SHLR17 | 201308 | Fluidigm |
| CD27 | 167Er | O323 | 201304 | Fluidigm |
| CD294 (CRTH2) | 166Er | BM16 | 201325 | Fluidigm |
| CD197/CCR7 | 167Er | G043H7 | 201325 | Fluidigm |
| CD14 | 168Er | 63D3 | 201325 | Fluidigm |
| FNγ | 168Er | B27 | 201308 | Fluidigm |
| CD45RA | 169Tm | HI100 | 201304 | Fluidigm |
| CD3 | 170Er | UCHT1 | 201325 | Fluidigm |
| CD20 | 171Yb | 2H7 | 201325 | Fluidigm |
| Granzyme B | 171Yb | GB11 | 201308 | Fluidigm |
| CD66b | 172Yb | G10F5 | 201325 | Fluidigm |
| CD38 | 172Yb | HIT2 | 201304 | Fluidigm |
| HLA-DR | 173Yb | LN3 | 201325 | Fluidigm |
| IgD | 174Yb | IA6-2 | 201325 | Fluidigm |
| HLA-DR | 174Yb | L243 | 201304 | Fluidigm |
| TNFa | 175Lu | Mab11 | 3175023B | Fluidigm |
| Perforin B | 175Lu | D48 | 201308 | Fluidigm |
| CD127 (IL-Ra) | 176Yb | A019D5 | 201325 | Fluidigm |
| DNA | 191Ir | n/a | 201192B | Fluidigm |
| DNA | 193Ir | n/a | 201192B | Fluidigm |
| Cisplatin  Viability | 195Pt | n/a | 201064 | Fluidigm |

**Table S2.** Antibody conjugation for CyTOF analysis. A summary of antibodies, staining, and conjugated metals used for CyTOF analysis.

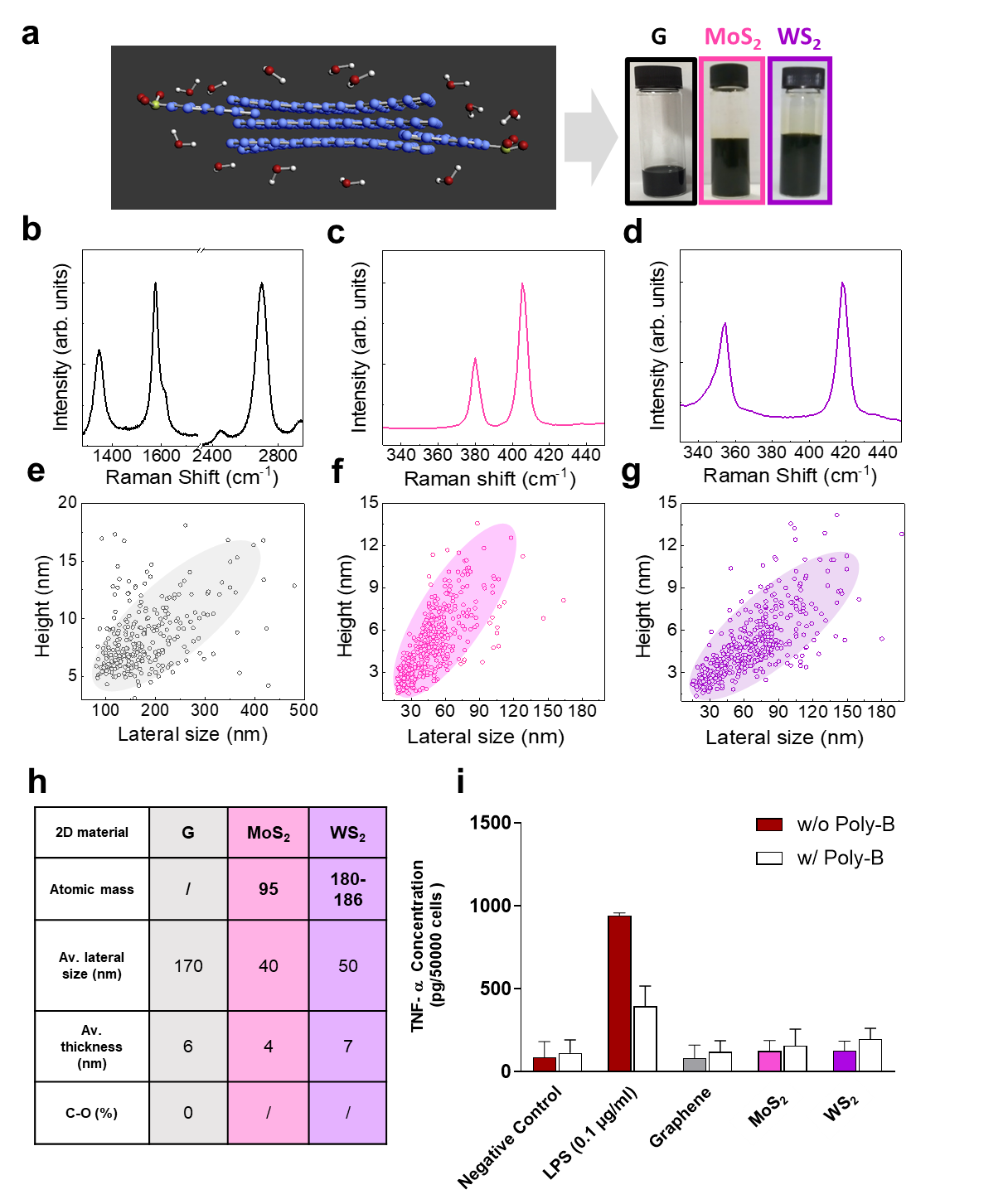
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target** | **Metal** | **Clone** | **Catalog #** | **Company** |
| CD45 | 89Y | 30-F11 | 3089005B | Fluidigm |
| Ly-6C | 150Nd | HK1.4 | 128002 | Biolegend |
| CD11c | 142Nd | N418 | 3142003B | Fluidigm |
| TCR-b | 143Nd | H57-597 | 3143010B | Fluidigm |
| CD11b | 148Nd | M1/70 | 101202 | Biolegend |
| CD19 | 145Nd | 6D5 | 115502 | Biolegend |
| CD8a | 146Nd | 53-6.7 | 3153012B | Fluidigm |
| NK1.1 | 165Ho | PK136 | 108702 | Biolegend |
| CD4 | 172Yb | RM4-5 | 3172003B | Fluidigm |
| CD117 | 173Yb | 2B8 | 3173004B | Fluidigm |
| Ly-6G | 141Pr | 1A8 | 127602 | Biolegend |
| DNA | 191Ir | n/a | 201192B | Fluidigm |
| DNA | 193Ir | n/a | 201192B | Fluidigm |
| Cisplatin  Viability | 195Pt | n/a | 201064 | Fluidigm |

**Table S3**. **Antibody conjugation for *in vivo* biodistribution analysis.** A summary of antibodies, staining, and conjugated metals used for *in vivo* biodistribution analysis.

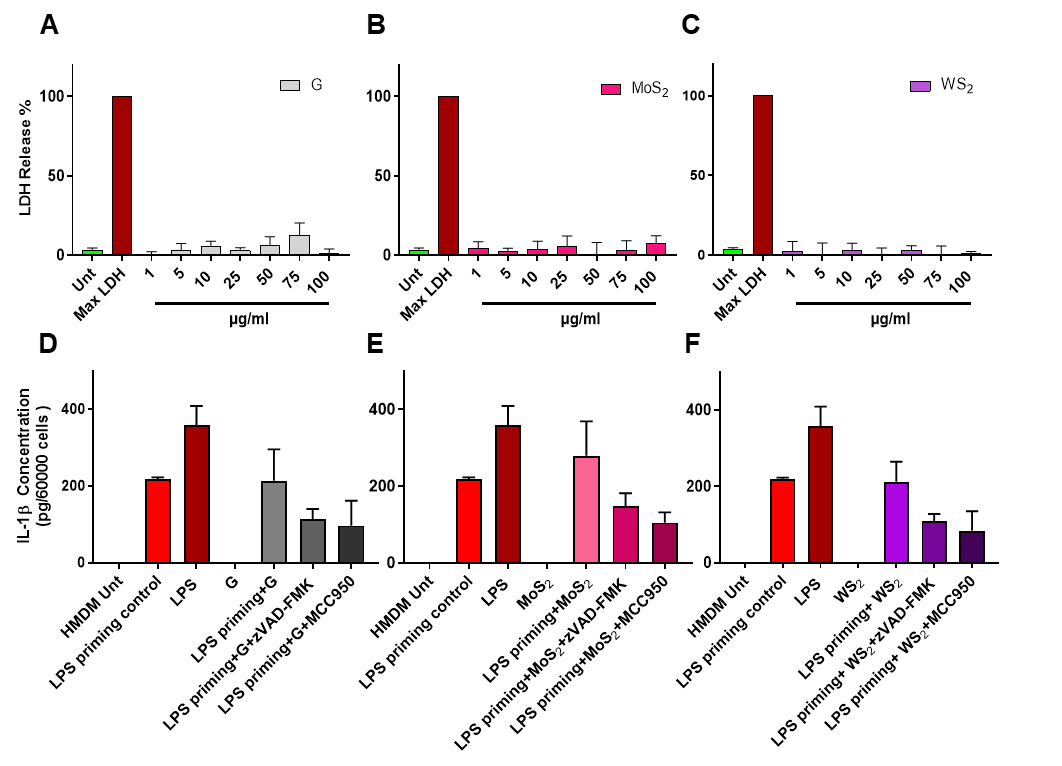
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antibody target | Clone | Source | Cat# | Conjugated metal | Concentration (µg/mL) | Incubation conditions |
| CD3 | SP162 | abcam | ab245731 | 159Td | 1.67 | 4˚C overnight |
| CD31 | EPR17259 | abcam | ab225883 | 174yb | 2.5 | 4˚C overnight |
| CD74 | In1/CD74 | Biolegend | 151002 | 172Yb | 2.5 | 4˚C overnight |
| COL1A1 | E8F4L | CST | 72026 | 113In | 1.25 | 4˚C overnight |
| dsDNA | 35I9 DNA | Ionpath | 708901 | 89Y | 0.375 | RT, 1 hour |
| F4/80 | D2S9R | CST | 70076 | 161Dy | 10 | 4˚C overnight |
| Na-K-ATPase | EP1845Y | Ionpath | 717603 | 176Yb | 1 | 4˚C overnight |
| SMA | SP171 | abcam | ab242395 | 115Ln | 0.5 | RT, 1 hour |
| Vimentin | D21H3 | CST | 5741 | 168Er | 1.25 | 4˚C overnight |

**Table S4. Antibody conjugation for MIBI-TOF analysis.** A summary of antibodies, staining concentrations, and conjugated metals used for MIBI-TOF analysis.

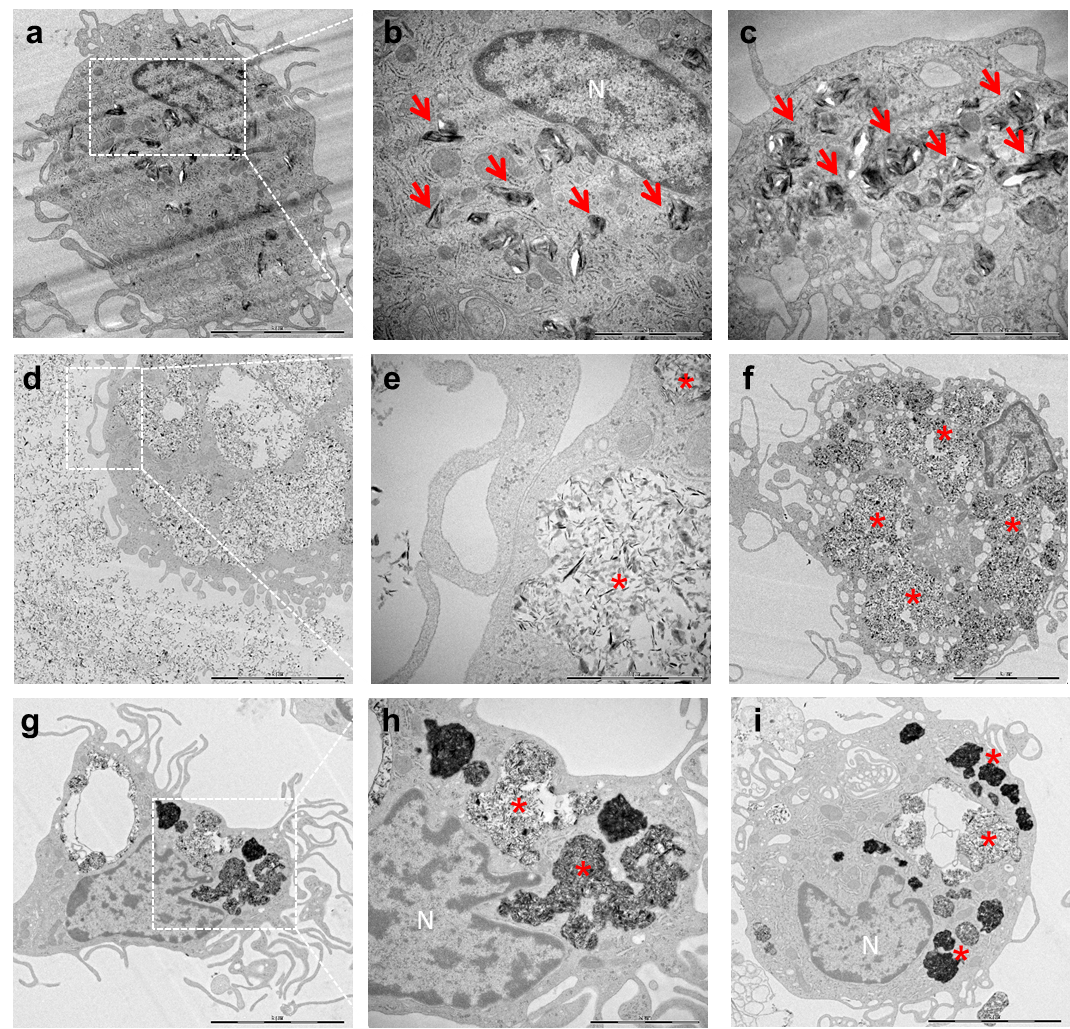
**2. Supplementary Figures**

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**Supplementary Figure S1. Characterization of graphene and TMDs.** Schematic representation of the exfoliation assisted by PS1 for production of aqueous dispersions of G, MoS2, and WS2, shown in the optical pictures **a)**. Representative Raman spectra of G (**b**), MoS2 (**c**), and WS2 (**d**). Statistical distribution plots of lateral size and height of exfoliated nanosheets measured by AFM for the dispersions of **e)** G, **f)** MoS2, and **g)** WS2. **h)** Table illustrating graphene and TMDs in terms of molecular structure, detectable masses, lateral size, thickness, and C-O (%) of G, MoS2, and WS2. **i)** Endotoxin-free 2D materials. HMDMs were exposed to 25 µg/mL of graphene, MoS2, and WS2 in the presence or absence of the endotoxin inhibitor, polymyxin-B (10 µM) as described in Mukherjee *et al*.[39] and TNF-α production was determined by using an ELISA. LPS (0.1 µg/mL) ± polymyxin-B was included as a control.

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**Supplementary Figure S2. Cell viability and cytokine secretion. (A-C)** Primary human macrophages were exposed for 24 h to (**A**) G, (**B**) MoS2, or (**C**) WS2 at the indicated concentrations or left untreated (Unt) and cell viability was measured using the LDH release assay. The data in panel **B** and **C** are reproduced from: Peng G. et al. Small (2022). (**D-F**) IL‐1β secretion in LPS‐primed macrophages. Human monocyte‐derived macrophages, primed or not with LPS (0.1 µg/mL) for 2 h prior to exposure were incubated with (**D**) G, (**E**) MoS2, or (**F**) WS2 (25 µg/mL) for 24 h. IL‐1β secretion was determined by using a specific ELISA. Cells were preincubated with a pan‐caspase inhibitor, zVAD‐FMK (20 µM), or NLRP3 inhibitor, MCC950 (10 µM).

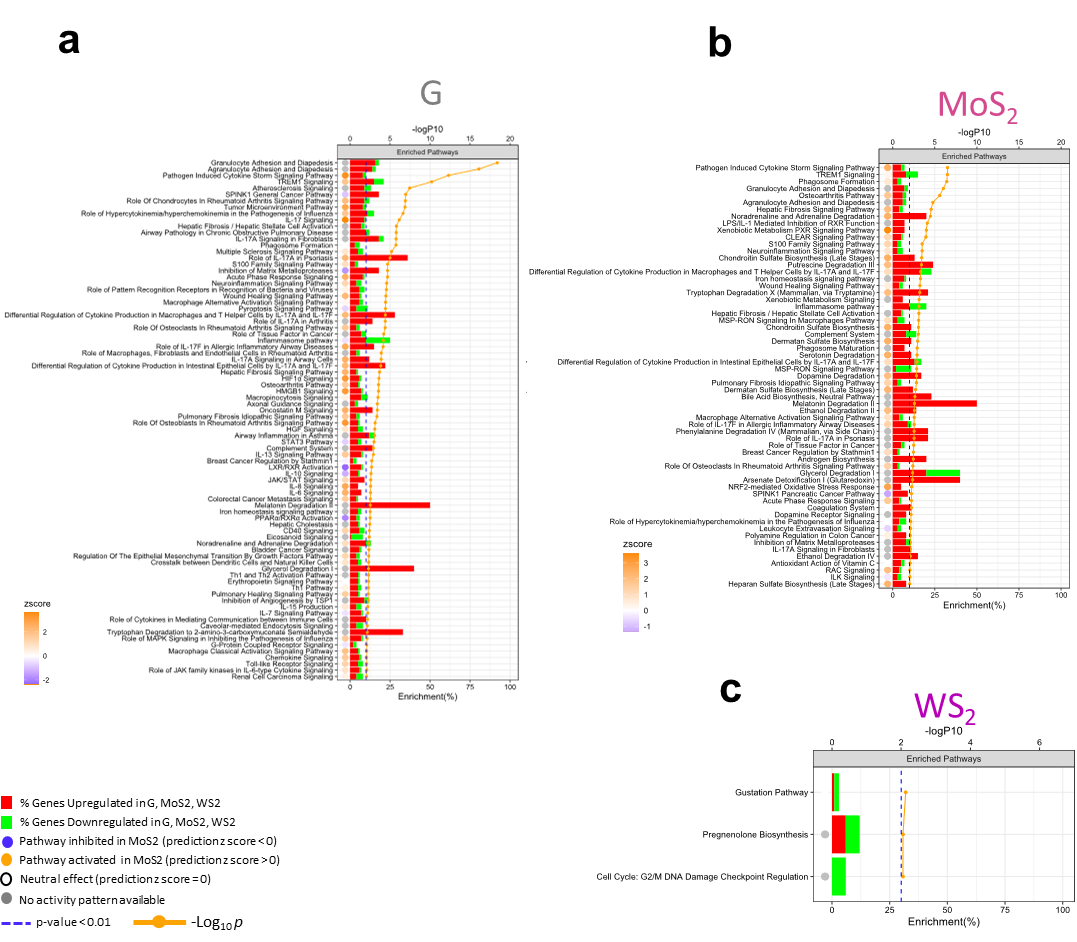


**Supplementary Figure S3. Macrophage uptake of 2D materials.** Human monocyte-derived macrophages were incubated for 24 h with a non-cytotoxic concentration (25 µg/mL) of G (**a–c**), MoS2 (**d–f**), or WS2 (**g–i**). Red arrows in the TEM micrographs point towards cytoplasmic ‘bundles’ of G (b–c), whereas the MoS2 (**e-f**) and WS2 (**h-i**) nanosheets are present in large, membrane-enclosed vacuoles, as indicated with asterisks in the respective TEM micrographs. Scale bars: 5 (**a, d, f, g,** and **i**), 2 (**b, c,** and **h**), and 1 µm (**e**). N, nucleus.

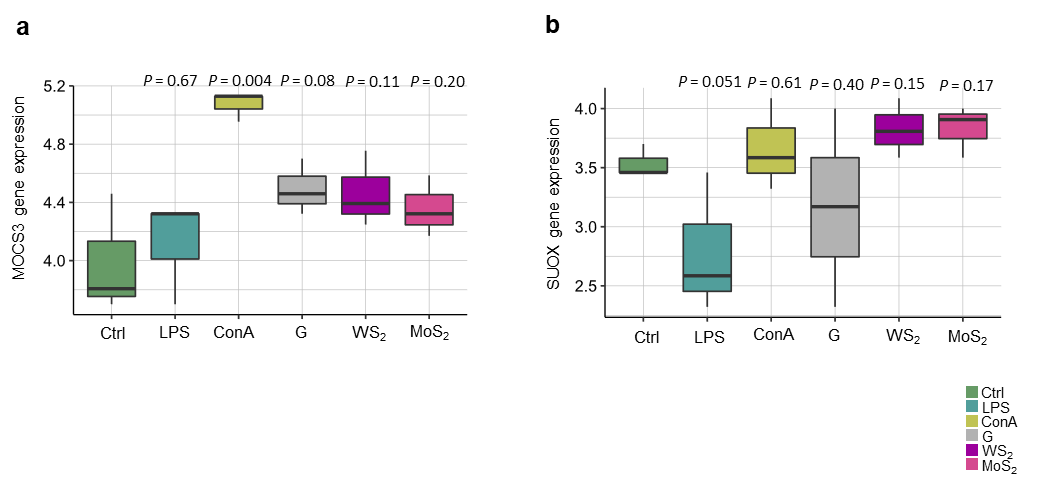
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**Supplementary Figure S4. Viability on PBMCs by calcein assay and dye 780 staining. a)** PBMCs were treated with different concentrations (25, 50, and 100 µg/mL)of G, MoS2, or WS2 for 24 h, and cell viability was analysed using the LIVE/DEAD® Viability/Cytotoxicity Kit discriminating live from dead cells by simultaneously staining with green-fluorescent calcein-AM to indicate intracellular esterase activity and red-fluorescent ethidium homodimer-1 to indicate loss of plasma membrane integrity. Plasma membrane integrity and esterase activity were measured by using TECAN fluorescence microplate reader. **b)** PBMCs were treated with different concentrations (25, 50, and 100 µg/mL)of G, MoS2, or WS2for 24 h, and cell viability was analysed by flow cytometry using Fixable Viability Dye 780 staining. Data are presented as mean ± SD of three independent samples. Comparison between groups was performed by one-way ANOVA, followed by a Tukey’s post hoc multiple comparison.

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**Supplementary Figure S5. Gene expression of PBMCs treated with TMDs.** Gene expression analysis by mRNA-Seq of PBMCs after treatment with 50 µg/mLof of G, MoS2, or WS2 for 24 h. **a-c**) Pathway analysis associated with the list of DEG (FDR < 0.05, Pathway *p-*value < 0.01) for **a**) G (n=483) **b**) MoS2 (n=451) and **c**) WS2 (n=184). The orange line represents the enrichment p value, the dotted blue line represents the *p* value of 0.01 (-Log10 P value = 2). Histograms represent the proportion (%) of DEGs upregulated (red) or downregulated (green) in G, MoS2 and WS2 versus untreated. The circles represent the pathway activation status. The blue circle indicates the pathway is inhibited with a negative score, the orange circle represents a pathway is activated with a positive score, the white circle represents the pathway is neutral with zero score, while a gray circle indicates that the pathway activity is unknown.



**Supplementary Figure S6. MoS2 modulates Mo-dependent enzymes.** PBMCs were left untreated or exposed to 50 µg/mLof G, MoS2, or WS2 for 24 h. Gene expression was determined by mRNA-Seq. a) Boxplot reporting the gene expression analysis of *MOCS3* gene and b) *SUOX* gene. (limma test *p* value).

**A screen shot of a game

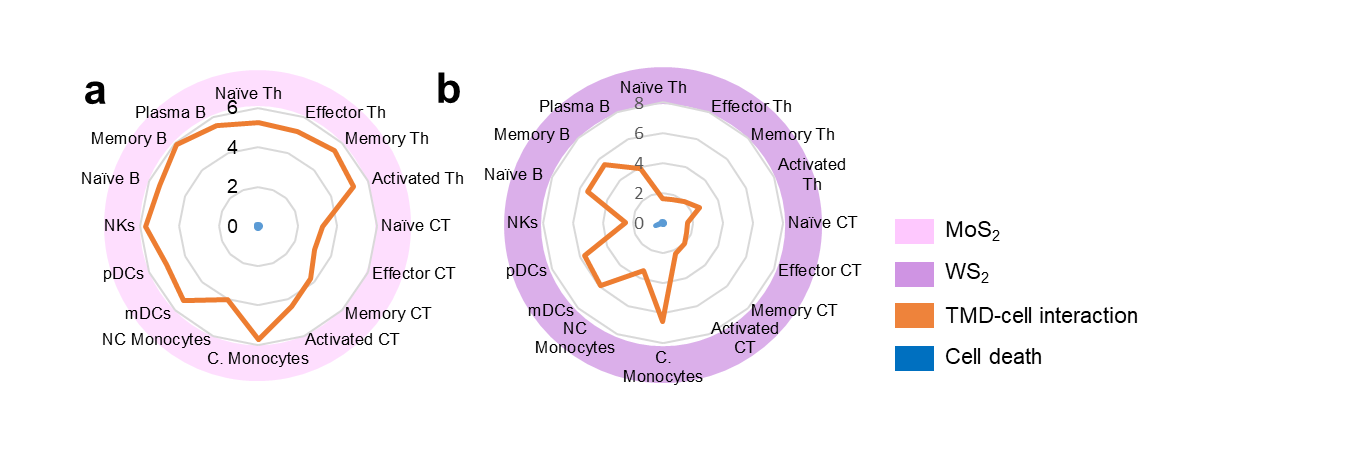
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**Supplementary Figure S7**. **Immune cell subpopulation gating strategy for CyTOF analysis**. Dot plots showing the gating strategy used for the identification of the different immune cell subpopulations by CyTOF.

**Calendar

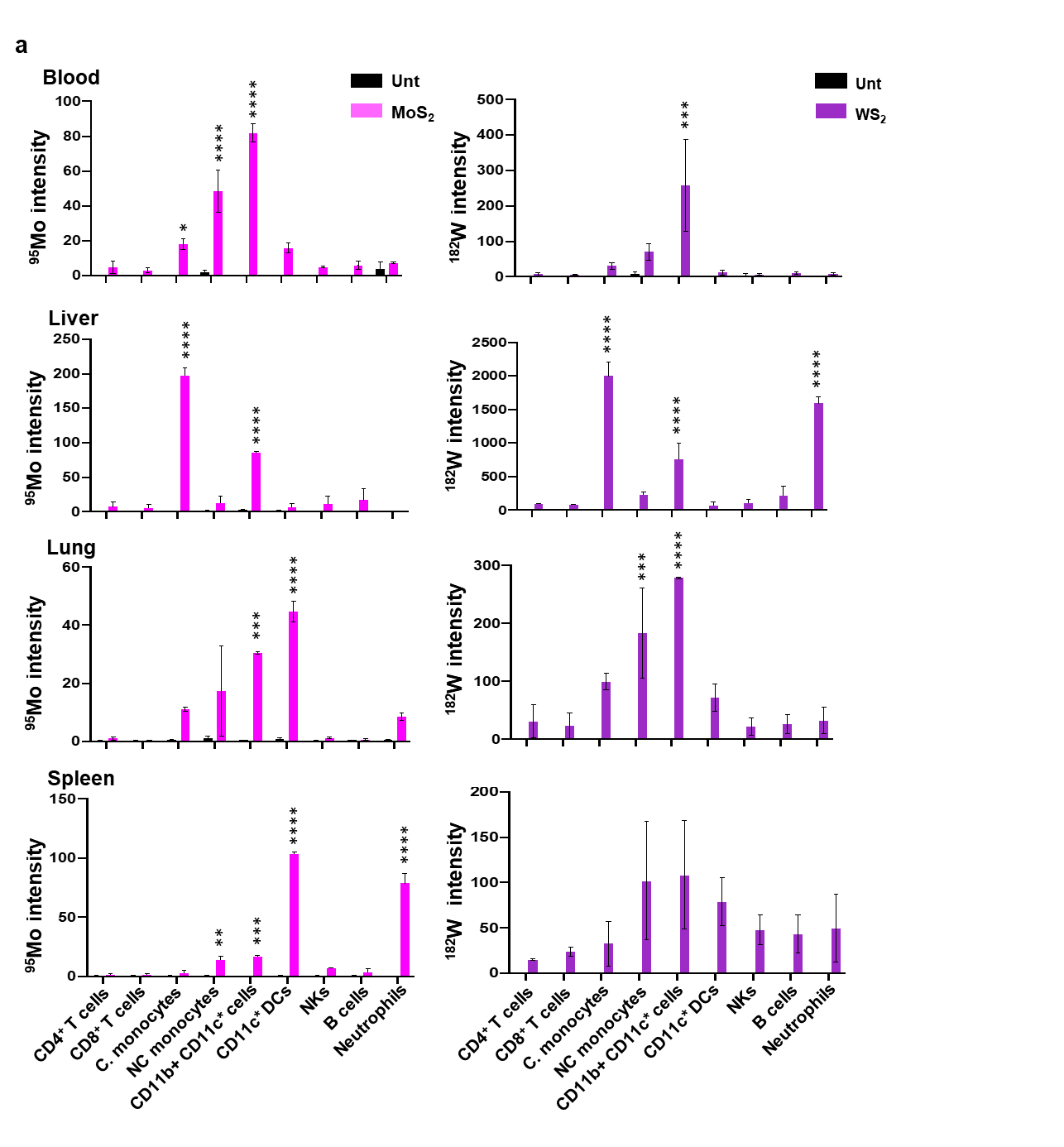
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**Supplementary Figure S8. Single-cell tracking analysis of MoS2 and WS2 on human PBMC subpopulations. a-c)** t-SNE distribution of CD4+ cells, CD8+ cells, **d-f)** B cells and **g-i)** CD3- CD19- cells. Plots representing the use of t-SNE to obtain a comprehensive single-cell view of the PBMC subpopulations treated with MoS2 or WS2 showing the median intensity of Molybdenum (95Mo) Tungsten (182W) signal in CD4+ cells, CD8+ cells, B cells, monocytes, mDCs and NKs. A representative t-SNE graph is shown out of three biological replicates.

**Supplementary Figure S9. Viability and TDMs cell binding correlation.** a, b) Spider charts represent the impact on viability (blue), expressed as Cisplatin (Cis) median intensity, vs the 2D material cell binding (orange) expressed for sixteen immune cell types after exposure of PBMCs to MoS2 (a) or WS2 (b) (50 µg/mL) for 24 h. Data are expressed as log2 fold change. Data are presented as mean ± ST.D. of three independent samples. \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001 by two-way ANOVA Tukey’s multiple comparison test.

**A picture containing graphical user interface

Description automatically generatedSupplementary Figure S10. CyTOF detection of TMDs in human immune cells.** a-e) Signal intensity comparison of MoS2 and WS2 in T cells, (b) monocytes, (c) DCs, (d) NKs and (e) B cells as detected by CyTOF.



**Supplementary Figure S11. Single-cell biodistribution analysis of TMDs *in vivo.*** Total TMD intensity detected per immune cell population in each analysed organ.