**Supplementary Information**

**In vitro formation of Neutrophil Extracellular Traps (NETs) is inhibited by the presence of serum and serum albumin**

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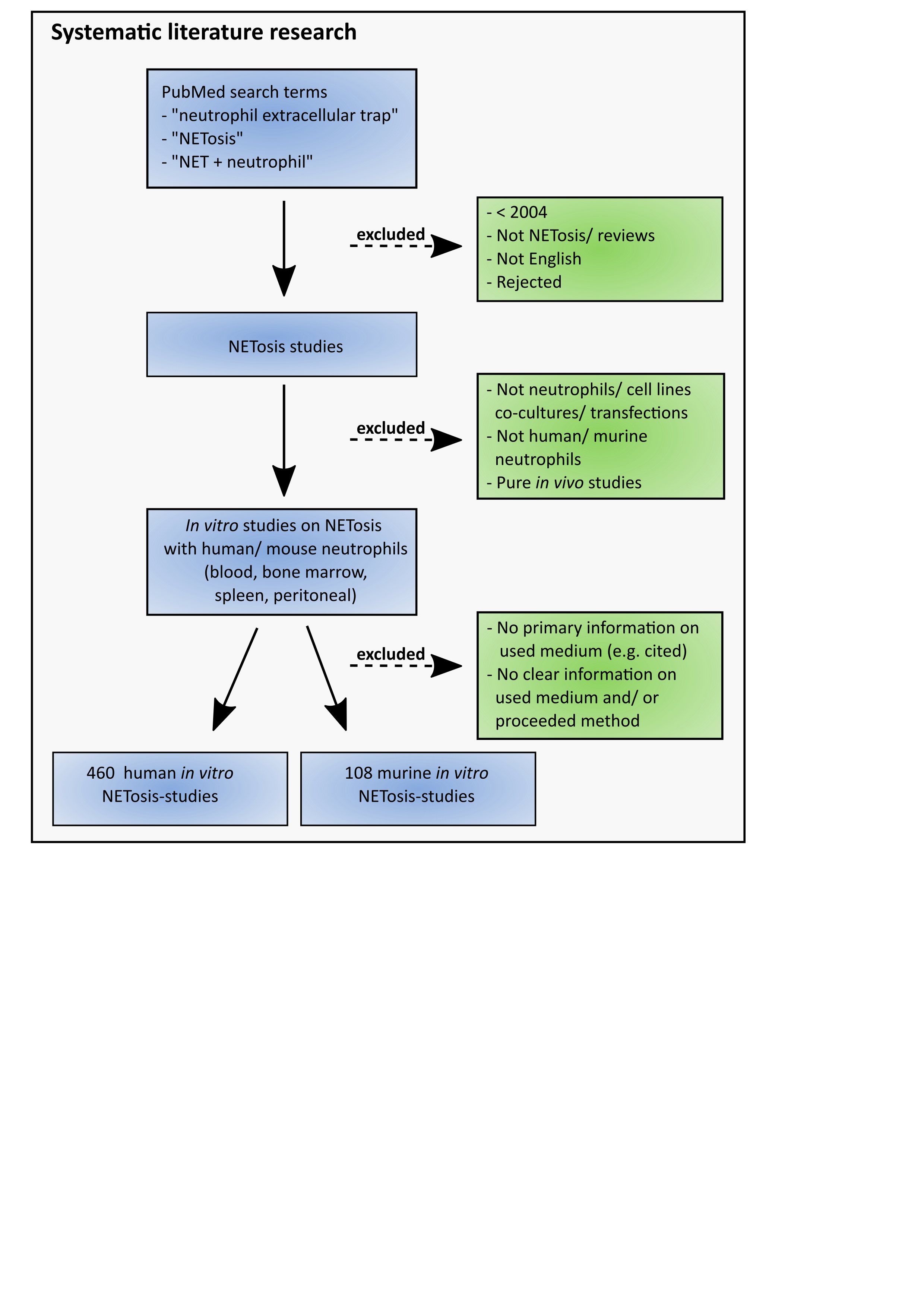
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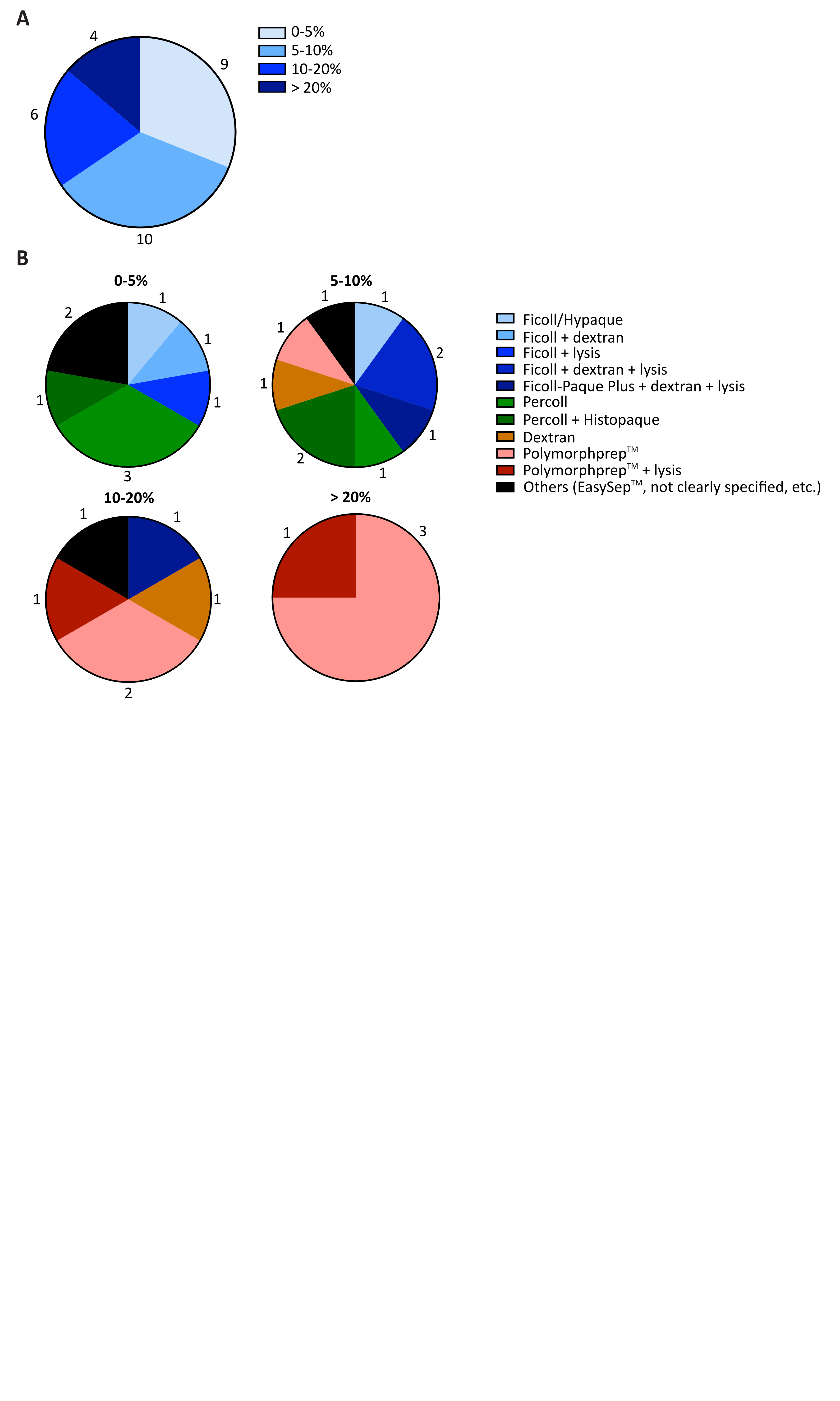
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**Supplementary Figure 1:Literature research**.

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**Supplementary Figure 2: Neutrophil elastase (NE) is released together with extracellular DNA after stimulation.** DNA-bound NE was measured after stimulation of human neutrophils by PMA (100 nM, 3h), CaI (4 µM, 2h) or LPS (100 µg/ml, 3h) in RPMI/HEPES with and without HSA. Neutrophils release DNA-bound NE in response to all stimuli. In media containing 0.5% HSA the release is clearly decreased after LPS and CaI stimulation, but appears stable in response to PMA. Error bars = mean ± SD. N = 2.



**Supplementary Figure 3:Spontaneous NETosis in literature. (A)** Percent of spontaneous NET formation in publications using serum- and albumin-free culture conditions. NETosis without further stimulation of isolated human neutrophils range between 0% and more than 20% NETotic cells. N = 29 publications with clear displayed percentage of NETs (out of all 255 publication without serum-supplements). **(B)** Spontaneous NET formation depending on neutrophil isolation technique. In literature neutrophils isolated by PolymorphprepTM show a higher tendency for spontaneous NET formation than neutrophils isolated by density-gradient techniques.

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**Supplementary Figure 4:HSA does not influence calcium concentrations.** The concentration of calcium in PBS does not significantly change in presence or absence of 0.5% HSA. Statistic: two-tailed t test. ns = not significant. N = 3. Error = SD.

**Supplementary Table 1: Culture media for stimulation of NETosis in human neutrophils**

|  |  |  |  |
| --- | --- | --- | --- |
| Supplement | **Concentration** | **Medium** | **Activator** |
| None | Not applicable | RPMI +/- sup. | PMA (10 nM [1], 12 nM [2], 16 nM [3], 20 nM [4-24], 24 nM [25], 25 nM [26-58] (TNFα -primed [45, 59]), 0.1 – 25 nM [60], 30 nM [61, 62], 32 nM [63], 40 nM [64-67], 20-40 nM [68], 50 nM [1, 45, 69-81], 100 nM [1, 82-109], 0-100 nM [110, 111], 162 nM [112, 113], 253 nM [114], 500 nM [81, 115, 116], 600 nM [117], 800 nM [118], 16 µM [119], 50 µM [120], 10 mM [121]) [1, 85, 122-125] |
| Calcium ionophore A23187 (2.5 µM [81, 84, 123], 4 µM [42, 43, 53, 100, 126], 5 µM [117], 25 µM [13, 115]); Ionomycin (1.3 µM [3], 5 µm [42, 43, 53, 126], 8 µM [61]) |
| LPS (10 ng/ml [60], 100 ng/ml [47, 54, 55, 70, 127, 128]+/- TNFα [45], 250 ng/ml [61], 0-104 ng/ml [124], 1 μg/ml [5, 10, 13, 60, 129, 130], 2.5 µg/ml [3], 3 µg/ml [39], 5 µg/ml [53, 81], 0-25 µg/ml [52], 25 µg/ml [42, 53], 100 µg/ml [17], 100 nM [131]) [125] |
| Bacteria (*Staphylococcus aureus* [6, 26, 43, 53, 74, 113, 121, 124, 125, 132, 133] (+/- LL-37 [48]), *Neisseria gonorrhoeae* [9], *Pseudomonas aeruginosa* [23, 52, 53, 67, 125, 134, 135], *Streptococcus suis* [29]*, Streptococcus pneumonia* [98], *Streptococcus sanguinis* [136], *Streptococcus mutans* (+combinations of IgG /platelet/P-selectin [101]),  *Escherichia coli* [52], *Yersinia enterocolitica* [31], *Yersinia pseudotuberculosis* [137], *Helicobacter pylori* [68], *Haemophilus influenza* [138], *Aggregatibacter actinomycetemcomitans* [74, 112, 133]*,* *Fusobacterium nucleatum* [74, 133]*, Actinomyces viscosus* [133], *Vibrio cholerae* [96], *Staphylococcus epidermidis* biofilms [139], Periodontal bacteria [77],isolated bacterial cultured from plaques/ mixed bacterial population from plaque [131]) |
| Virus (Respiratory Syncytial Virus (RSV) [70], Dengue virus [113]) |
| Fungi (*Paraoccidioides brasiliensis* [39], *Candida albicans* [39, 56, 83, 104, 140], *Aspergillus* *fumigatus* [50], *Cryptococcus gattii/+ mutants* [141], *Candida glabrata* [141]*, Aspergillus* species [142]) |
| Other pathogens/parasites (*Naegleria fowleri* trophozoites[15], *Brugia malayi* microfilariae [38], *Leishmania amazonensis* promastigotes [82, 102, 109, 127], *Cryptosporidium parvum* sporozoites [143]) |
| Cytokines/ Chemokines (IL-8 [47, 54, 55, 83] +/- TNFα [45], IL17 +/- TNFα [130], IL-18 [10], TNF [61], TNFα [85, 144], platelet activating factor (PAF) [67, 68, 115, 145]) |
| Other stimuli [1-5, 11, 14, 17, 24, 25, 27, 30, 34, 40, 41, 45, 47, 48, 54-56, 59, 61, 63, 66-70, 74, 76-78, 80, 81, 85, 90, 91, 93, 95, 98, 99, 102, 104, 107, 111, 112, 114, 115, 119, 120, 122-124, 128, 130, 143, 144, 146-158] |
| HBSS +/- sup. | PMA (1.5 nM [159, 160], 10 nM [161], 20 nM [134, 162-164], 25 nM [30, 43, 165-172], 40.5 nM [173], 50 nM [174, 175], 100 nM [104, 176-180], 1-100 nM [181], 162 nM [182] [183], 1.6 µM [184], 1-100 nM [185], 10-1000 nM [186], (2 nM - 20 μM) [187], 1.6 mM [139])[135, 188] |
| LPS (100 ng/mL [184], 1 µg/ml [159]) |
| Calcium ionophore (1 µM [189], 4 μM [43]) [188]; Ionomycin (5 μM [43]) |
| Bacteria (*Mycobacterium tuberculosis* [190], *Burkholderia pseudomallei* [162], *Staphylococcus aureus* [162], *Staphylococcus epidermidis* biofilms [139], *Streptococcus pyogenes* [172], *Pseudomonas* *aeruginosa* [191, 192], *Escherichia coli* [137], *Porphyromonas gingivalis* [193], mid-log bacterial cultures [194], *Bacillus anthracis* [171]) |
| Fungi (*Candida albicans* [104]) |
| Other pathogens/Parasites (*Trypanosoma cruzi* and soluble antigen [169]) |
| Cytokines/ Chemokines (IL8 [184]) |
| Other stimuli [3, 30, 139, 159, 160, 163, 166, 170, 172, 173, 178, 179, 182, 193, 195-201] |
|  |  | Other media and buffer | PMA (0.32 nM [202], 5 nM [203, 204], 20 nM [16, 205-208] (+/- TGF-β-pretreated [14]), 25 nM [37, 209], 40 nM [210], 50 nM [211], 81 nM [212, 213], 100 nM [162, 208, 214-220], 50-100 nM [221], 150 nM [222], 162 nM [202, 223], 600 nM [224], 649 nM [225, 226], 1 µM [227], 1.6µM [210]) [228] |
|  |  | Calcium ionophore (5 µM [229], 1 µM [230], 25 µM [231]) |
|  |  | LPS (0.1 ng/ml [232], 100 ng/ml [207, 232-235], 0.1-100 ng/mL [228, 236], 6 or 8 pg/neutrophil (TNFα/ IL-6/ IFNα-primed) [218]) |
|  |  | Virus (Influenza A virus (Phil82) (+/-LL-37-preincubation [225, 226]), dengue virus [207] |
| Bacteria (*Staphylococcus aureus* [207, 208, 228, 230, 237-239], *Escherichia coli* [228, 237], *Pseudomonas aeruginosa* [227, 230], *Mycoplasma pneumoniae* [222],  *Mycobacterium tuberculosis* [209]) |
| Fungi(*Candida albicans* (+/- fibronectin-precoating) [240, 241], *Aspergillus fumigatus* or *nidulans* [242]) |
| Cytokines/ Chemokines (PAF [228], IL-8 [223, 243, 244]) |
| Other stimuli [14, 204, 206, 212, 215, 220, 223, 228, 235, 240, 241, 243-255] |
| Fetal calf serum (FCS) | 0.05% | HBSS +/- sup. | PMA (2-250 nM [256]) |
| Calcium ionophore (0.2-25 µM [256]) |
| 0.5% | RPMI +/- sup. | PMA (10 nM [257, 258], 20 nM [259, 260], 25 nM [261, 262], 30 nM [263], 10–50 nM [264], 50 nM [265], 100 nM [266]) |
| Cytokines/ Chemokines (PAF [265], IL-8 [257, 265], TNFα [257, 258, 265], IL-1β [257, 267]) |
| Other stimuli [257, 259-261, 263, 264, 266, 268] |
| HBSS +/- sup. | PMA (2-250 nM [269]) |
| Calcium ionophore (5 µM [270]) |
| 1% | RPMI +/- sup. | PMA (20 nM [271], 25 nM [272, 273], 100 nM [274], 600 nM [275]) |
| LPS (0.7–100 μg/ ml [271], 2 -200 µg/mL [276]) [277] |
| Fungi (*Candida albicans* [274] |
| Cytokines/ Chemokines (TNFα [277]) |
| Other stimuli [272, 277] |
| 2% | RPMI +/- sup. | PMA (4 nM [278], 8 nM [279], 10 nM [280, 281], 20 nM [15, 280, 282-288], 25 nM [289], 32 nM [290], 40 nM [291], 50 nM [78], 100 nM [292-294], 120 nM [295], 600 nM [296]) |
| Ionomycin (0.626–5 µg/ml [285], 5 µM [288]) |
| LPS (100 ng/ml [278, 279], 300 ng/ml [287]) |
| Bacteria (*Staphylococcus aureus* [285, 289, 291], *Escherichia coli* [285]*, Pseudomonas aeruginosa* [285], *Wolbachia* (soluble, from supernatants of infected mosquito cells, conjugated fluorescent beads [296]) |
| Fungi (*Aspergillus fumigatus* [278], *C. albicans* [297] (biofilm and planktonic cells [294])) |
| Virus (RSV (+/- PMA) [295]) |
| Other pathogens/parasites (*Naegleria fowleri* [15]) |
| Cytokines/ Chemokines (IL-8 [278, 287, 298, 299], IL-17 [287], PAF [287, 288]) |
| Other stimuli [78, 281, 286, 291, 300] |
| Other media and buffer | PMA (20 nM [301], 25 nM [302], 100 nM [303]) |
| Bacteria (*Pseudomonas aeruginosa* [302], *Porphyromonas gingivalis* [303]) |
| Other stimuli [301] |
| 3% | RPMI +/- sup. | PMA (500 nM [304, 305]) |
| 4% | RPMI +/- sup. | PMA (50 nM [149], 100 nM [306]) |
| Other stimuli [306] |
| 5% | RPMI +/- sup. | PMA (10 nM [180, 307], 20 nM [308], 50 nM [309], 0-100 nM [310], 16.2 – 1620 nM [311], 1.62 µM (+/- retinoic acid) [312]) |
| LPS (1 µg/ml [311]) |
| Fungi (*Arthroderma benhamiae* [313]) |
| Other stimuli [4, 307, 309, 312] |
| 7.5% | Other media and buffer | PMA (100 nM [314]) |
| Other stimuli [314] |
| 10 % | RPMI +/- sup. | PMA (16 nM [315, 316], 10 nM [317], 20 nM [318], 32 nM [315], 50 nM [318, 319], 100 nM [104, 320, 321], 200 nM [136, 322], 600 nM [323]) |
| Ionomycin (5 μM [324]) [325] |
| LPS (200 ng/ml [319], LPS + IL-8 [325]) |
|  | Bacteria (*Streptococcus mutans* [320], *Streptococcus sanguinis* [136]) |
|  | Other pathogens/parasites (*Entamoeba histolytica* trophozoites [326]) |
|  | Cytokines/Chemokines (C5a (IFNα-, IFNγ- or GM-CSF-primed) [327], IL-6 [323], IL-9 [328]) |
| Other stimuli [136, 324, 329-331] |
| HBSS +/- sup. | PMA (100 nM [104, 332]) |
| Other media and buffer | PMA (81 nM [237], 40 nM (TNFα -primed [195]), 405 nM [237]) |
| LPS (10, 100, 1000, 5000 ng/ml [237], 0.2 μg/ml [333]) |
| Ionomycin (3 and 5 µg/ml [237]) |
| Other stimuli [195, 237, 333] |
| Heat inactivated FCS (hiFCS) | 0.1% | RPMI +/- sup. | PMA (100 nM[334]) |
| LPS (1 µg/ml [334]) |
| Virus (Hantaan virus (HTNV) and HTNV-infected supernatant (Vero E6 cells) [334], Vaccinia virus (VV) [334], Adenovirus [334]) |
| Other stimuli [334] |
| 0.5% | RPMI +/- sup. | PMA (50 nM (TNFα-primed) [335]) |
| LPS (100 ng/ml (TNFα-primed) [335]) |
| Other stimuli [335] |
| 1% | RPMI +/- sup. | PMA (20 nM [336]) |
| Bacteria (*Escherichia coli* [337]) |
| Other stimuli [337-339] |
| 2% | RPMI +/- sup. | PMA (40 nM [124, 150], 25 nM [35, 340-342] (normal/high glucose) [343], 50 nM [344], 100 nM [345-347] (normal/high glucose or mannitol) [343]) |
| LPS (1 µg/ml [124], 2 µg/ml (normal/high glucose or mannitol) [343]) |
| Ionomycin (normal/high glucose or mannitol [348]) |
| Bacteria (*Staphylococcus aureus* [124] (+ PMA [341])) |
| Fungi (*Candida albicans* biofilms [345], *candida glabrata* (biofilms and planktonic) [347]) |
| Cytokins/Chemokins (TNFα (normal/high glucose) [343], IL-6 (normal/high glucose) [343], IL-8 [346]) |
| Other stimuli [124, 150, 196, 343, 346] |
| 4% | RPMI +/- sup. | PMA (25 nM [349], 100 nM [350]) |
| LPS (100 ng/ml [351]) |
| Cytokines/chemokines (IL-8 [351]) |
| 5% | RPMI +/- sup. | PMA (25 nM [35], 81 nM [352], 100 nM [353], 3.2 µM [352]) |
| Bacteria (*Neisseria gonorrhoeae* [352]) |
| Fungi (*Candida albicans* [353], *Aspergillus fumigatus* [354]) |
| 10% | RPMI +/- sup. | PMA (5 nM [355], 20 nM [356], 25 nM [35], 32 nM[357], 40 nM [325] 65 nM [357], 162 nM [358, 359], 50 nM [355]) |
| LPS (100 ng/ml TNFα-or IL-8-primed [325]) |
| Ionomycin (1 µM [325]) |
| Fungi (*Paracoccidioides brasiliensis* [358, 359]) |
| Cytokins/Chemokins (IL-8 [325]) |
| Other stimuli [325, 360] |
| Other media and buffer | PMA (20 nM [361], 100 nM [361]) |
| Bacteria (*Pseudomona aeruginosa* [361]*, Bordetella parapertussis* [361]) |
| Human plasma (HP) | 3% | RPMI +/- sup. | Fungi (*Candida albicans* [274]) |
| HBSS +/- sup. | Bacteria (*Mycobacterium bovis* [362]) |
| Fungi (C*andida albicans* [362]) |
| 10% | PPMI +/- sup. | Cytokines/Chemokines (IL-8 [363]) |
| RPMI or HBSS +/- sup. | Fungi (*Candida. albicans* [104]) |
| Other media and buffer | LPS (5 µg/ml [247]) |
| 20% | RPMI +/- sup. | PMA (100 nM [98]) |
| Bacteria (*Streptococcus pneumonia* [98]) |
| Other stimuli [98] |
| 100% | - | PMA (100 nM [98]) |
| Bacteria (*Streptococcus pneumonia* [98]) |
| Other stimuli [98, 254, 364] |
| hiHP | 2% | RPMI +/- sup. | PMA (25 nM [365-367], 600 nM [368]) |
| Bacteria (group A *Streptococcus* (GAS) [365, 367, 369], *Lactococcus lactis* [367, 369]*, Pseudomonas aeruginosa* [366]) |
| Other pathogens/parasites (*Schistosoma japonicum* eggs, *soluble egg antigen or excretory/secretory products of* S. japonicum *eggs* [368]) |
| Other Stimuli [367] |
| 5% | RPMI +/- sup. | PMA (25 nM [37]) |
| Human Serum (HS) | 0.2% | HBSS +/- sup. | Other stimuli [370] |
| 1% | HBSS +/- sup. | PMA (100 nM [192, 371-374]) |
| Bacteria (*Pseudomonas aeruginosa* [192, 371, 372], *Escherichia coli* [375]) |
| Other stimuli [373, 374] |
| 2% | RPMI +/- sup. | PMA (20 nM [376], 25 nM [377], 65 nM [158], 200 nM [378], 600 nM [379]) [147, 380] |
| Ionophore (25 µM [376]) |
| Bacteria (*Escherichia coli* (+/- PMA) [378], *Acinetobacter baumannii* [381] (+/- PMA) [378], *Pseudomonas aeruginosa* [381]) |
| Cytokines/Chemokines (IL-1β [152], TNFα [379]) |
| Other stimuli [147, 148, 151, 152, 158, 376, 377, 380] |
| Other media and buffer | PMA (25 nM [382]) |
| Bacteria (*Yersinia enterocolitica/ Y.pestis* [382]) |
| 5% | RPMI +/- sup. | Other stimuli [383, 384] |
| 6% | RPMI +/- sup. | Bacteria (*E. coli* +/- TNFα/ G-CS/ IL-1β [385]) |
| Other stimuli [385] |
| 10% | RPMI +/- sup. | PMA (25 nM [229]) |
| LPS (50 ng/mL (+/- gallic acid) [386]) |
| Calcium ionophore (5 µM [229]) |
| Bacteria (*Mycobacterium bovis* bacillus Calmette–Guérin Pasteur [229], *Listeria monocytogenes* [387], *Klebsiella pneumoniae* [388]) |
| Other stimuli [386, 387] |
|  | HBSS +/- sup. | Fungi (*Candida albicans* [332]) |
| 25% | Other media and buffer | PMA [389] |
| Bacteria (*Klebsiella pneumoniae* [389]) |
| 100% | - | PMA (100 nM) [390] |
| Other pathogens/parasites (*Strongyloides stercoralis* infective larvae [390]) |
| hiHS | 0.1% | HBSS +/- sup. | Other stimuli [391] |
| 0.5% | HBSS +/- sup. | Other stimuli [392] |
| Other media and buffer | LPS (100 ng/ml [393-395]) |
| Ionophore [394] |
| Bacteria (*Staphylococcus aureus* [396]) |
|  | Other stimuli [393, 394] |
| 2% | RPMI +/- sup. | PMA (25 nM [397], 25 nM [398-400], 32 nM [401, 402], 10-50 nM [403]) [404] |
| LPS (2 µg/ml [401]) |
| Ionomycin (1–5 µM [403]) |
| Bacteria (GAS [397],Streptococcus pneumoniae [404] (PMA-pretreated) [399, 400]) |
|  | Fungi (*C. albicans* [33]) |
| Other pathogens/parasites (*Leishmania donovani/ major* [405]) |
| Cytokines/Chemokines (IL-8 [403], TNFα [398], IL-6 [401]) |
| Other stimuli [398, 401, 405] |
| HBSS +/- sup. | Other stimuli [60] |
| Other media and buffer | Cytokines/Chemokines [406] |
| 100% | - | PMA (100 nM [390]) |
| Other pathogens/parasites (*Strongyloides stercoralis* infective larvae [390]) |
| Human serum albumin (HSA) | 0.05% | RPMI +/- sup. | PMA (50 nM [407], 100 nM [191]) |
| Calcium ionophore (5 µM [407]) |
| Bacteria (GBS [407], *Staphylococcus aureus* [407]) |
| Fungi (*C. albicans* [407]) |
| Other stimuli [191, 407] |
| 0.1% | RPMI +/- sup. | Other stimuli [191] |
| HBSS +/- sup. | PMA (100 nM [197]) |
| Other stimuli [197] |
| Other media and buffer | PMA (50 µM [407]) |
| Calcium ionophore (5 µM [407]) |
| Bacteria (GBS [407]) |
| Fungi (*C. albicans* [407]) |
| Other stimuli [407] |
| 0.2% | RPMI +/- sup. | PMA (20 nM [408, 409], 100 nM [410]) |
| Bacteria (*Staphylococcus aureus* [408, 409], *Escherichia coli* [409]) |
|  | Fungi (*Candida albicans* [410]) |
| Other stimuli [408, 409] |
| HBSS +/- sup. | PMA (50 nM [411]) |
| 0.5% | RPMI +/- sup. | PMA (20 nM [412-414], 25 nM [415], 50 nM [416]) |
| Bacteria (*Pseudomonas aeruginosa* [416], [417]) |
| Cytokines/ Chemokines (IL-1β/TNFα/G-CSF [416]) |
| Other stimuli [414-416] |
| HBSS +/- sup. | PMA (60 nM [418]) |
| Other pathogens/parasites (*toxoplasma gondii* [418]) |
| Other media and buffer | PMA (162 nM [419]) |
| 1% | RPMI +/- sup. | PMA (10 nM [420], 5 nM - 50 nM [421]) |
| Bacteria (*Neisseria meningitidis* [421]) |
| Other stimuli [421] |
| 2% | RPMI +/- sup. | PMA (10-20 nM [396], 25 nM [194, 209, 422, 423], 100 nM [194, 424-426], 200 nM [322]) |
| LPS (1 μg/ml + LPS- binding protein [396]) |
| Bacteria (mid-log bacterial cultures [194], GBS [423], *Mycobacterium tuberculosis* [209]) |
| Fungi (*C. albicans* [33]) |
| Other pathogens/parasites (*Entamoeba histolytica* trophozoites [422]) |
| Cytokines/Chemokines (IL-8 [396]) |
| Other stimuli [396, 422] |
| HBSS +/- sup. | PMA (50 nM [427, 428], 100 nM [427]) |
| Other stimuli [428] |
| Other media and buffer | PMA (50 nM [429]) |
| Ionophore (5 µM [429]) |
| Other stimuli [429] |
| unknown | X-VivoTM 15 | PMA (20 nM – 81 nM (+/- human Ig coated well plates [287]), 20 nM [288], 25 nM [430-433], 600 nM [431]) |
| LPS (0.3 µg/mL (GM-CSF-primed) [430], 100 ng/ml (GM-CSF-primed) [432]) |
| Bacteria (*Escherichia coli* [430]) |
| Cytokines/Chemokines (C5a (GM-CSF-primed) [430, 432, 433]) |
| Other stimuli [431] |
| hiHSA | 2% | Other media and buffer | PMA (12.5–25 nM [434]) |
| Other stimuli [434] |
| Bovine serum albumin (BSA) | 0.1% | Other media and buffer | PMA (10 nM [435]) |
| 0.2% | RPMI +/- sup. | PMA (81 nM [436]) |
| Other stimuli [436] |
| 0.25% | HBSS +/- sup. | PMA (100 nM [437]) |
| 0.3% | Other media and buffer | PMA (50 nM [438]) |
| 0.5% | RPMI +/- sup. | PMA (10 nM [439], 25 nM [440], 27 nM [441], 50 nM [442], 100 nM [443], 250 nM [443]) |
| Bacteria (*Streptococcus gordonii* [442]*, Peptoanaerobacter stomatis* [442]*, Filifactor alocis* (+/- TNFα-primed)[442]) |
| Fungi (*Aspergillus fumigatus* [439]) |
| Other stimuli [440, 441] |
| 1% | RPMI +/- sup. | PMA (20 nM [444], 600 nM [117]) |
| Calcium ionophore (5 µM [117]) |
| Bacteria (GBS strains or hemolytic GBS pigment [444], *Escherichia coli* [445]) |
| Other stimuli [445] |
| HBSS +/- sup. | PMA (10 nM [446, 447] (PAF-pretreated) [448]) |
| Other stimuli [3, 446, 448] |
| Other media and buffer | Bacteria (*Escherichia coli* [445]) |
| Other stimuli [445] |
| 2% | RPMI +/- sup. | PMA (16 nM [449], 20 nM [450, 451], 50 nM [452-454], 81 nM [455]) |
| LPS (1 µg/ml [449]) |
| Bacteria (*Leptospira interrogans and biflexa* [455], *Staphylococcus aureus* [454]) |
| Cytokines/ Chemokines [452] |
| Other media and buffer | PMA (100 nM [219, 456]) |
| Albumin | 0.2% | RPMI +/- sup. | PMA (20 nM [457]) |
| Other stimuli [457] |
| 0.5% | Other media and buffer | PMA [458] |
| Fungi (*Aspergillus fumigatus* [458]) |
| 2% | RPMI +/- sup. | PMA (5-20 nM [459]) |
| Other stimuli [459] |
| 5% | RPMI +/- sup. | PMA (5-20 nM [459]) |
| Other stimuli [459] |
| Low serum | -- | RPMI +/- sup. | PMA (64 nM [460]) |
| Other stimuli [460] |

**Supplementary Table 2: Culture media for stimulation of NETosis in murine neutrophils**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serum/**  **serum albumin** | **Concentration** | **Medium** | **Activator** |
| **None** | Not applicable | RPMI +/- supp. | PMA (20 nM [1, 2], 25 nM [3, 4], 50 nM [5-10], 100 nM [4, 11-17], 200 nM [18], 2.5 µM [13], 16.2 µM [19], 100 mM [20]) [21-23] |
| LPS (0.2 µg/ml [24], 0.5 µg/ml [25], 1 µg/ml [9], 10 µg/ml [10, 26] (TNFα-primed [27]), 2.5-25 µg/ml [28], 25 µg/ml [29]) [22] |
| Ionomycin (4 µM [4, 12, 15, 16, 20, 26, 30, 31], 1 µg/ml [19]); Ionophore (5 µM [9]) |
| Bacteria(*Streptococcus pneumoniae* +/- BALF after infection with PR8 virus [32], *Escherichia coli* +/- PMA [9], *Haemophilus influenzae* [33]) |
| Fungi(*Aspergillus fumigatus* [34]) |
| Parasites(*Plasmodium berghei* [35]) |
| Cytokines/ chemokines (MIP-2 [10], PAF [10, 20]) |
| Other stimuli [1, 3, 9-11, 13, 17, 19-22, 32, 36, 37] |
| HBSS +/- supp. | PMA (20 nM [38], 32 nM [39], 50 nM [40], 100 nM [41-43] (TNF-α primed [44]), 160 nM [45]) |
| LPS (0.1 µg/ml [42], 1 µg/mL [46], 10 µg/ml [28]) |
| Ionomycin (1 µg/mL [45]) |
| Bacteria (*Klebsiella pneumoniae* [47], *Streptococcus aureus* [41], commensal/ probiotic/ enterohemorrhagic *Escherichia coli* [41], TSB/ LB/ MRS broth fecal bacteria [48, 49]*, Pseudomonas aeruginosa* [50]) |
| Cytokines/ chemokines (PAF [51]) |
| Other stimuli [39, 41, 42, 44, 48, 49, 52] |
| Other media and buffer | PMA (20 nM [53, 54], 25 nM [55, 56], 50 nM [57], 100 nM [14, 58], 1 µM [59]) |
| LPS (100 ng/ml [55], 1 µg/ml [56, 60], 500 µg/ml [61]) |
| Calcium ionophore (4 µM [59]) |
| Bacteria (*Escherichia coli*, *Shigella flexneri* or GAS (IL-8-primed) [56]) |
| Cytokines/ chemokines (C5a (GM-CSF-primed) [55], IL8 [58]) |
| Other stimuli [53, 56-59, 61-63] |
| **FCS** | 0.5% | RPMI +/- supp. | PMA (10 nM [64]) |
| LPS (100 ng/ml [64]) |
| Other stimuli [64] |
| HBSS +/- supp. | PMA (500 nM [65]) |
| 1% | RPMI +/- supp. | PMA (100 nM [66]) |
| LPS (10 µg/ml [67]) |
| Other stimuli [66, 68, 69] |
| 2% | RPMI +/- supp. | PMA (100 nM (TNFα-primed [27], GM-CSF-primed [70]), 600 nM [71], 160 µM [72]) |
| LPS (10 µg/ml (GM-CSF-primed) [70]) |
| Bacteria (*Streptococcus aureus* (GM-CSF-primed) [70]*, Escherichia coli* (GM-CSF-primed)[70]*,* GBS [72], Wolbachia containing supernatant [71]) |
| Other stimuli [71] |
| Other media and buffer | PMA (20 nM [73], 50 nM [74], 20-80 nM [75], 100 nM [76]) [77] |
| Parasites (*Plasmodium berghei* lysate [74], *Pseudomonas aeruginosa* (+/- PMA) [76]) |
| Cytokines/ chemokines (IFNγ + C5a [73]) |
| Other stimuli [74] |
| 5% | RPMI +/- supp. | PMA (100 nM [78]) [79] |
| LPS (100 µg/mL [78]) |
| Fungi (*Candida albicans* [80]) |
| Other stimuli [78, 79] |
| 10% | RPMI +/- supp. | PMA (25 nM [81], 0-100 nM [82]) |
| Bacteria (*Streptococcus agalactiae* [83], *Escherichia coli* [84], *Staphylococcus aureus* [84]) |
| HBSS +/- supp. | PMA (100 nM [85]) |
| Other media | PMA (40 nM (TNFα –primed) [86]) |
| Other stimuli [86] |
| unknown | Other media and buffer | Other stimuli [87] |
| **hiFCS** | 0.1% | RPMI +/- supp. | PMA (3-2000 nM [88]) |
| Virus (Hantaan virus [88]) |
| Other stimuli [88] |
| 0.5% | RPMI +/- supp. | PMA [89] |
| Other stimuli [89] |
| 2% | RPMI +/- supp. | PMA (200 nM [90]) |
| Bacteria (*Streptococcus suis* (planktonic/ bacteria from biofilm matrix/ biofilms) +/- PMA [90]) |
| 10% | Other media and buffer | PMA (324 nM [91]) |
| **BSA** | 0.1% | RPMI +/- supp. | LPS (100 ng/ml [92]) |
| 0.5% | RPMI +/- supp. | PMA (324 nM [93]) |
| LPS (100 ng/ml (TNFα-priming) [93]) |
| Other Stimuli [93] |
| 1% | RPMI +/- supp. | PMA (162 nM (GM-CSF-primed) [70]) |
| LPS (10 µg/ml (GM-CSF-primed) [70]) |
| HBSS +/- supp. | LPS (100 ng/ml [94]) |
| 2% | RPMI +/- supp. | PMA (16 nM [60], 100 nM [95-98]) [99] |
| Other stimuli [95, 96, 98, 99] |
| Other media and buffer | Bacteria (*Staphylococcus aureus* (methicillin-resistant) [5]) |
| **HSA** | unknown | X-VIVO 15 | PMA (25 nM [100]) |
| LPS (100 ng/ml (GM-CSF-primed) [100]) |
| Cytokines/ chemokines (C5a (GM-CSF-primed [100]) |
| Other stimuli [100] |
| **Mouse serum (MS)** | 1% | RPMI +/- supp. | PMA (100 nM [101-103]) |
| Ionomycin (4 µM [103]) |
| Bacteria (*Staphylococcus aureus* (methicillin-resistant) [103] |
| 10% | RPMI +/- supp. | Bacteria (*Listeria monocytogenes* [104]) |
| 100% | - | PMA (100 nM [105]) |
| Parasites (*Strongyloides stercoralis* infective larvae [105]) |
| **DNase (-/-) MS** | 2% | RPMI +/- supp. | PMA (100 nM [106]) |
| Fungi (*Candida albicans* (heat killed) [106]) |
| HBSS +/- supp. | PMA (100 nM [107]) |
| Fungi (*Candida albicans* [107]) |
| Bacteria (*Listeria monocytogenes* [107]) |
| **hiMS** | 100% | - | PMA (100 nM [105]) |
| Parasites (*Strongyloides stercoralis* infective larvae [105]) |
| **Bovine growth serum (BGS)** | 2% | Other media and buffer | PMA (600 µM [108]) |
| Parasites (*Toxoplasma gondii* [108]) |

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