

Supporting information “NLRP3 is essential for neutrophil polarization and chemotaxis in response to leukotriene B4 gradient”

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Supporting Video legends

Supporting video 1 Deficiency in NLRP3 results in a marked reduction in neutrophil chemotaxis towards higher concentrations of LTB4. NLRP3^{+/+} (top panel) and NLRP3^{-/-} (bottom panel) were followed through live cell time-lapse imaging in a stable gradient of 4000 pg/mL of LTB4. Cellular movement was quantified by manually tracking neutrophils for a period of one hour. NLRP3^{-/-} neutrophils were stationary in the gradient for the one-hour period while NLRP3^{+/+} neutrophils showed a marked migration towards the highest concentration of LTB4. Scale bar represents 10 μ m.

Supporting video 2 Neutrophil polarization and elongation is critically dependent on the presence of LTB4 and required for neutrophil chemotaxis. In a stable gradient of 4000 pg/mL LTB4, NLRP3^{+/+} neutrophils (left panel) showed a dynamic microtubule cytoskeleton, capable of polarization/elongation followed by chemotaxis. NLRP3^{-/-} neutrophils (right panel) are incapable of polarization/elongation and take on a spread and rounded morphology in the same gradient of LTB4. Scale bar represents 10 μ m.

Supporting video 3 NLRP3 deficiency results in a reduced recruitment of neutrophils towards a site of sterile laser induced liver burn injury. Upon liver burn injury, seen as a bright white mark, neutrophils (stained white) are seen to migrate towards the site of injury in NLRP3^{+/+} mice (left panel). In NLRP3^{-/-} mice (right panel), this recruitment is visibly reduced. Scale bar represents 30 μ m, image acquisition for 150 minutes.

Matlab (R2022b) script for generation of rose and trajectory plots

```
clear all

Data_cellen= xlsread('NLRP3Ctrl.xlsx');

k = length(Data_cellen(:,1));

Off = 10;

o = 1;

for i = 2:k

    if Data_cellen(i,6) > Data_cellen(i-1,6);

        Data_cellen(i,14) = o;

    else

        Data_cellen(i,14) = o+1;

        o = o+1;

    end

end

Data_cellen(1,14) = 1;

Aantal = max(Data_cellen(:,14));

o = 0;

for n = 1:Aantal

    f = find(Data_cellen(:,14)==n);

    l = length(f);

    o=o+1;

    if l < 60

        Min = min(f);

        Max = max(f);

        celx(1:l,o) = Data_cellen(Min:Max,4);

    end

end
```

```

    cely(1:l,o)= Data_cellen(Min:Max,5);

    m = Data_cellen(Max,4);

    n = Data_cellen(Max,5);

    celx(l:60,o) = m;

    cely(l:60,o) = n;

else

Min = min(f);

Max = Min+59;

celx(1:60,o) = Data_cellen(Min:Max,4);

cely(1:60,o) = Data_cellen(Min:Max,5);

end

end

o = 0;

for n = 1:Aantal

    o=o+1;

    orgx = celx(1,n);

    x(:,o) = celx(:,n) - orgx;

    orgy = cely(1,n);

    y(:,o) = cely(:,n) - orgy;

end

figure (1)

plot(x,y,'Linewidth',1)

hold on

xlabel('x (μm)');

ylabel('y (μm)');

xlim([-250 250]);

ylim([-250 250]);

```

```
plot([0 0], ylim, 'k');  
plot(xlim, [0 0], 'k');  
grid on  
str = {Aantal 'cells tracked'};  
text(130,200, str, 'FontSize', 12)  
title(['WT+Noc'])  
set(gca, 'FontSize', 25);  
hold off
```

```
%% Circular bar plot
```

```
cx = x(60,:);  
cy = y(60,:);  
co = [cx;cy];  
c = 0;  
k1 = 0;  
k2 = 0;  
k3 = 0;  
k4 = 0;  
k5 = 0;  
k6 = 0;  
k7 = 0;  
k8 = 0;  
k9 = 0;  
k10 = 0;  
k11 = 0;  
k12 = 0;  
k13 = 0;  
k14 = 0;  
k15 = 0;
```

```
k16 = 0;
```

```
for n = 1:Aantal
```

```
    if abs(x(60,n)) < Off && abs(y(60,n)) < Off;
```

```
        c = c+1;
```

```
    else
```

```
        if x(60,n) > 0 && y(60,n) > 0;
```

```
            if abs(y(60,n)) < abs(x(60,n))*0.4
```

```
                k1 = k1+1;
```

```
            elseif abs(y(60,n)) < abs(x(60,n))
```

```
                k2 = k2+1;
```

```
            elseif abs(y(60,n)) < abs(x(60,n))*2.4
```

```
                k3 = k3+1;
```

```
            else
```

```
                k4 = k4+1;
```

```
        end
```

```
    elseif x(60,n) > 0 && y(60,n) < 0;
```

```
        if abs(y(60,n)) < abs(x(60,n))*0.4
```

```
            k16 = k16+1;
```

```
        elseif abs(y(60,n)) < abs(x(60,n))
```

```
            k15 = k15+1;
```

```
        elseif abs(y(60,n)) < abs(x(60,n))*2.4
```

```
            k14 = k14+1;
```

```
        else
```

```
            k13 = k13+1;
```

```
    end
```

```
    elseif x(60,n) < 0 && y(60,n) > 0;
```

```
        if abs(y(60,n)) < abs(x(60,n))*0.4
```

```
            k8 = k8+1;
```

```
        elseif abs(y(60,n)) < abs(x(60,n))
```

```

    k7 = k7+1;

elseif abs(y(60,n)) < abs(x(60,n))*2.4

    k6 = k6+1;

else

    k5 = k5+1;

end

else

if abs(y(60,n)) < abs(x(60,n))*0.4

    k9 = k9+1;

elseif abs(y(60,n)) < abs(x(60,n))

    k10 = k10+1;

elseif abs(y(60,n)) < abs(x(60,n))*2.4

    k11 = k11+1;

else

    k12 = k12+1;

end

end

end

end

p = [c k1 k2 k3 k4 k5 k6 k7 k8 k9 k10 k11 k12 k13 k14 k15 k16]./Aantal;

pl = [k1 k2 k3 k4 k5 k6 k7 k8 k9 k10 k11 k12 k13 k14 k15 k16]./Aantal;

Percentage = pl.*100;

Relatief = p.*100

xt = -250:1:250;

figure (2)

scatter (cx,cy)

hold on

```

```

plot(xt,xt,'--k');
plot(xt,-xt,'--k');
plot(xt,xt*0.4,'--k');
plot(xt,-xt*0.4,'--k');
plot(xt,2.4*xt,'--k');
plot(xt,-2.4*xt,'--k');
xlabel('x (μm)');
ylabel('y (μm)');
xlim([-250 250]);
ylim([-250 250]);
plot([0 0], ylim, 'k');
plot(xlim, [0 0], 'k');
grid on
hold off

%% Rose
dtheta = 360/16;
r = c*100/(Aantal*16);
Pc = Percentage + r;

figure (3)
xt = -250:1:250;
plot(xt,xt,'--k');
hold on
plot(xt,-xt,'--k');
plot(xt,xt*0.4,'--k');
plot(xt,-xt*0.4,'--k');
plot(xt,2.4*xt,'--k');
plot(xt,-2.4*xt,'--k');

```

```
for i=1:16
    theta = linspace((i-1)*dtheta, (i)* dtheta);
    patch([0 Pc(i)*cosd(theta)], [0 Pc(i)*sind(theta)], 'g');
end
axis equal
xlim([-25 25]);
ylim([-25 25]);
plot([0 0], ylim, 'k');
plot(xlim, [0 0], 'k');

ang=0:0.01:2*pi;
xp=r*cos(ang);
yp=r*sin(ang);
plot(xp,yp, 'c', 'Linewidth',1);
title(['NLRP3'])
xlabel ('% of cells')
ylabel ('% of cells')
set(gca, 'FontSize', 15);
hold off
```