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Supplemental Information

Mutations in an Innate Immunity Pathway Are Associated with Poor Overall Survival Outcomes and Hypoxic Signaling in Cancer

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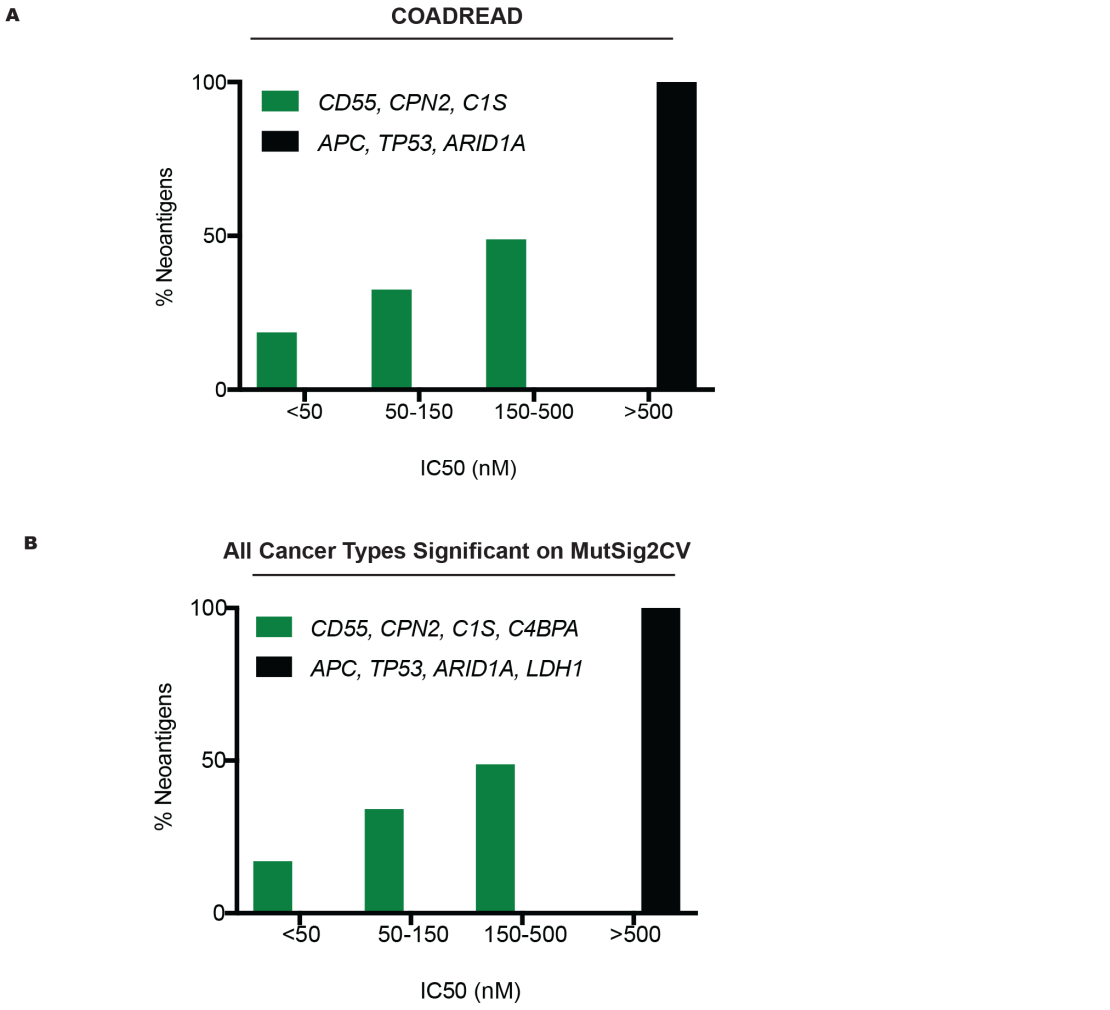
A
COADREAD
100
50
0
% Neoantigens
CD55, CPN2, C1S
APC, TP53, ARID1A
<50
50-150
150-500
>500
IC50 (nM)
B
All Cancer Types Significant on MutSig2CV
100
50
0
% Neoantigens
CD55, CPN2, C1S, C4BPA
APC, TP53, ARID1A, LDH1
<50
50-150
150-500
>500
IC50 (nM)

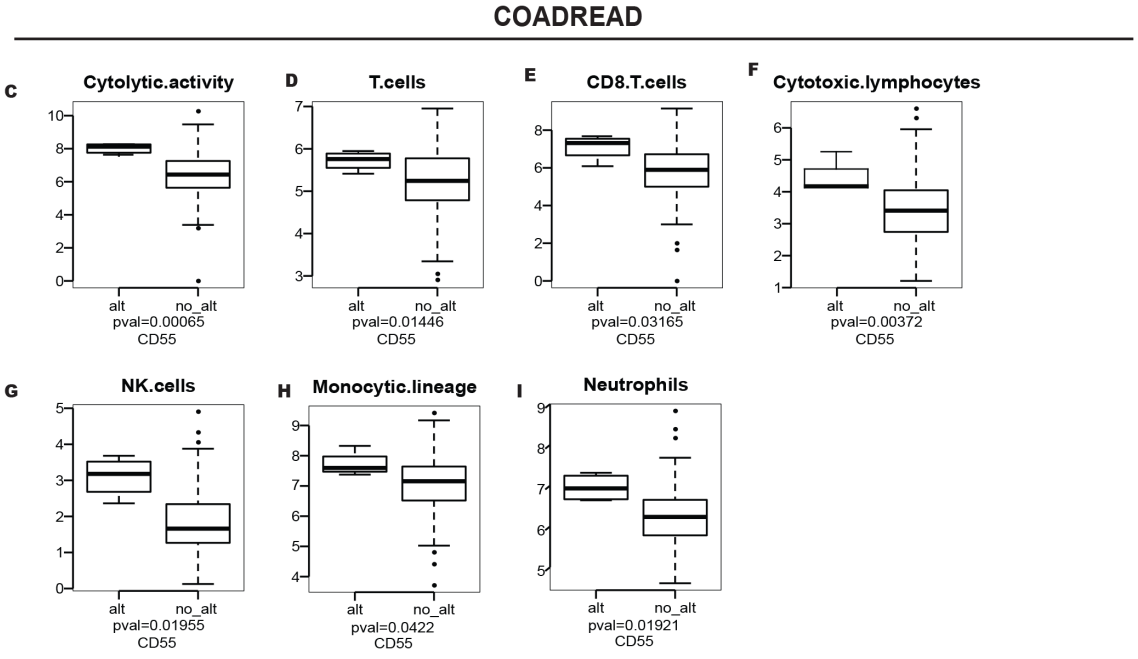
COADREAD
C
Cytolytic.activity
alt
no_alt
pval=0.00065
CD55
D
T.cells
alt
no_alt
pval=0.01446
CD55
E
CD8.T.cells
alt
no_alt
pval=0.03165
CD55
F
Cytotoxic.lymphocytes
alt
no_alt
pval=0.00372
CD55
G
NK.cells
alt
no_alt
pval=0.01955
CD55
H
Monocytic.lineage
alt
no_alt
pval=0.0422
CD55
I
Neutrophils
alt
no_alt
pval=0.01921
CD55

Figure S1: Complement mutations predicted to be "drivers" can give rise to predicted neoantigens. Related to Figure 1.

(A) Graph shows the % neoantigens binding with either strong (<50 nM), moderate (50-150 nM), weak (150-500 nM) and very weak (>500 nM) affinity for predicted neoantigens derived from three complement mutations (*CD55*, *CPN2* and *C1S*) and neoantigens derived from *APC*, *TP53* and *ARID1A* mutations in COADREAD. 41 predicted true complement mutation-derived neoantigens were compared to 41 predicted true neoantigens derived from *APC*, *TP53* and *ARID1A* mutations in COADREAD.

(B) Graph shows the % neoantigens binding with either strong (<50 nM), moderate (50-150 nM), weak (150-500 nM) and very weak (>500 nM) affinity for predicted neoantigens derived from complement mutations (*CD55*, *CPN2*, *C1S and C4BPA*), and neoantigens derived from *APC*, *TP53*, *ARID1A* and *LDH1* mutations. 43 predicted true complement mutation-derived neoantigens (from *CD55*, *CPN2*, *C1S* mutations from COADREAD and *C4BPA* from LGG) were compared to 43 predicted true neoantigens derived from *APC*, *TP53* and *ARID1A* mutations in COADREAD and *LDH1* in LGG.

(C) -(I) Differential predicted immune infiltration profiles for patients with or without *CD55* mutations in COADREAD are shown.

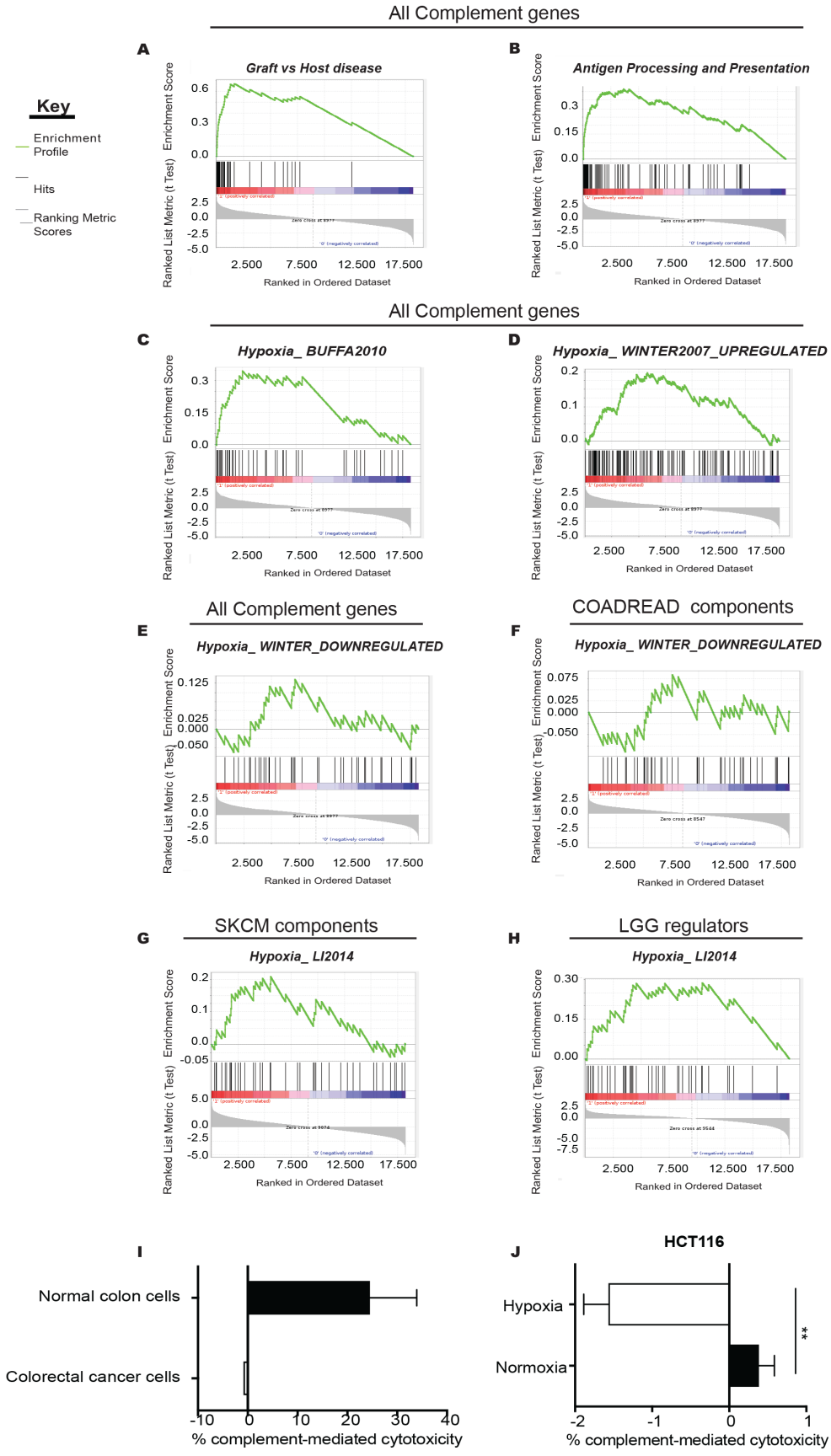
All Complement genes
A
Graft vs Host disease
B
Antigen Processing and Presentation
Key
Enrichment Profile
Hits
Ranking Metric Scores
Enrichment Score
Ranked List Metric (t Test)
Ranked in Ordered Dataset
All Complement genes
C
Hypoxia_ BUFFA2010
D
Hypoxia_ WINTER2007_UPREGULATED
All Complement genes
E
Hypoxia_ WINTER_DOWNREGULATED
COADREAD components
F
Hypoxia_ WINTER_DOWNREGULATED
SKCM components
G
Hypoxia_ LI2014
LGG regulators
H
Hypoxia_ LI2014
I
Normal colon cells
Colorectal cancer cells
% complement-mediated cytotoxicity
HCT116
J
Hypoxia
Normoxia
**
% complement-mediated cytotoxicity

Figure S2: Hypoxia inhibits complement-mediated cytotoxicity (CMC) in colorectal cancer. Related to Figure 3.

(A) GSEA plot for "Graft vs host disease "in COADREAD patients with any complement mutation.

(B) GSEA plot for "Antigen processing and presentation" in COADREAD patients with any complement mutation.

(C) GSEA plot for "Hypoxia_ Buffa2010" in COADREAD patients with any complement mutation.

(D) GSEA plot for "Hypoxia_Winter2007 upregulated" in COADREAD patients with any complement mutation.

(E) GSEA plot for "Hypoxia_Winter downregulated" in COADREAD patients with any complement mutation.

(F) GSEA plot for "Hypoxia_Winter downregulated" in COADREAD patients with *component* mutations.

(G) GSEA plot for "Hypoxia_Li2014" in SKCM patients with *component* mutations.

(H) GSEA plot for "Hypoxia_Li2014" in LGG patients with *regulator* mutations.

(I) Graph represents the % CMC/total lysis in fetal human normal colon (FHC) cells and HCT116 colorectal cancer cells. CMC/total lysis was assessed by calcein release/total lysis following treatment with either normal human serum or heat inactivated normal human serum. Error bars represent the SEM for a representative experiment. n=2

(J) Graph represents the % CMC/total lysis in HCT116 colorectal cancer cells exposed to normoxia (21% O_2) or hypoxia (1% O_2) for 24 hr. CMC/total lysis was assessed by calcein release/total lysis following treatment with either normal human serum or heat inactivated normal human serum. ** = p-value <0.01, unpaired t-test, two-tailed. Error bars represent the SEM for a representative experiment. n=3

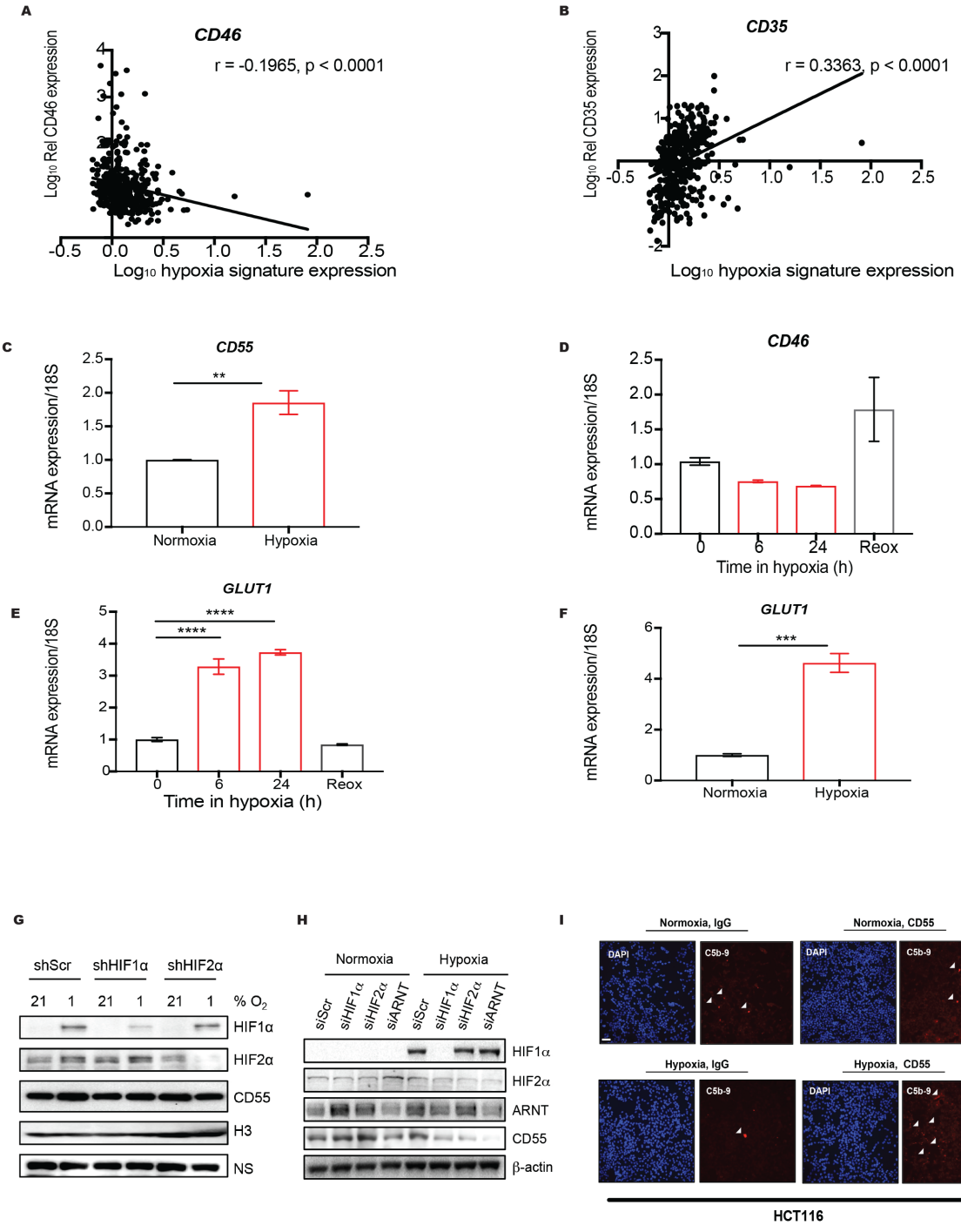
A
CD46
r = -0.1965, p < 0.0001
Log10 Rel CD46 expression
Log10 hypoxia signature expression
B
CD35
r = 0.3363, p < 0.0001
Log10 Rel CD35 expression
Log10 hypoxia signature expression
C
CD55
mRNA expression/18S
Normoxia
Hypoxia
D
CD46
mRNA expression/18S
Time in hypoxia (h)
E
GLUT1
mRNA expression/18S
Time in hypoxia (h)
F
GLUT1
mRNA expression/18S
Normoxia
Hypoxia
G
shScr
shHIF1α
shHIF2α
% O2
HIF1α
HIF2α
CD55
H3
NS
H
Normoxia
Hypoxia
siScr
siHIF1α
siHIF2α
siARNT
HIF1α
HIF2α
ARNT
CD55
β-actin
I
Normoxia, IgG
Normoxia, CD55
Hypoxia, IgG
Hypoxia, CD55
DAPI
C5b-9
HCT116

Figure S3: Hypoxia-induced expression of complement regulator CD55 contributes to inhibition of CMC. Related to Figure 4.

(A) Relative expression of *CD46* (Log_{10} conversion) in COADREAD patients is shown against hypoxia signature expression (Log_{10} conversion)(Li et al., 2014). Two-tailed p-value is shown for the Pearson r (correlation coefficient).

(B) Relative expression of *CD35* (Log_{10} conversion) in COADREAD patients is shown against hypoxia signature expression (Log_{10} conversion)(Li et al., 2014). Two-tailed p-value is shown for the Pearson r (correlation coefficient).

(C) mRNA expression of *CD55/18S* is shown. qPCR was carried out following treatment of RKO colorectal cancer cells with 0 or 24 hr of hypoxia (1% O_2). ** = p-value <0.01, unpaired t-test, two-tailed. Error bars represent the SEM for a representative experiment. n=3.

(D) mRNA expression of *CD46/18S* is shown. qPCR was carried out following treatment of HCT116 cells with 0, 6 or 24 hr of hypoxia (1% O_2) or 24 hr of hypoxia followed by 1 hr reoxygenation (21% O_2). Error bars represent the SEM for a representative experiment. n=3.

(E) mRNA expression of GLUT1/18S is shown. qPCR was carried out following treatment of HCT116 colorectal cancer cells with 0, 6 or 24 hr of hypoxia (1% O_2) or 24 hr of hypoxia followed by 1 hr reoxygenation (21% O_2). **** = p-value <0.0001, 1-way ANOVA with Tukey's multiple comparisons test. Error bars represent the SEM for a representative experiment. n=3.

(F) mRNA expression of GLUT1/18S is shown. qPCR was carried out following treatment of RKO colorectal cancer cells with 0 or 24 hr of hypoxia (1% O_2). *** = p-value <0.001, unpaired t-test, two-tailed. Error bars represent the SEM for a representative experiment. n=3.

(G) HCT116 cells were transduced with either scramble (Scr), HIF1α or HIF2α shRNA and exposed to normoxia (21% O_2) or hypoxia (1% O_2) for 24 hr. WB was carried with the antibodies indicated. H3 = loading control. N.S = non-specific band that can indirectly provide an indication of loading. n=3.

(H) HCT116 cells were transfected with either scramble (Scr), HIF1α, HIF2α or ARNT/HIF1β siRNA and exposed to normoxia (21% O_2) or hypoxia (1% O_2) for 24 hr. WB was carried with the antibodies indicated. β-actin = loading control. n=3.

(I) HCT116 cells were treated as in (Figure 4G/H). Immunofluorescence staining for membrane attack complex (C5b-9) was performed as a marker for CMC. C5b-9 = red, DAPI = blue. White arrows indicate areas of C5b-9 staining. Scale bar in white = 59.7 μM.