frontiers

Supplementary Material

Disruption of endosomal trafficking with EGA alters TLR9 cytokine response in human plasmacytoid dendritic cells

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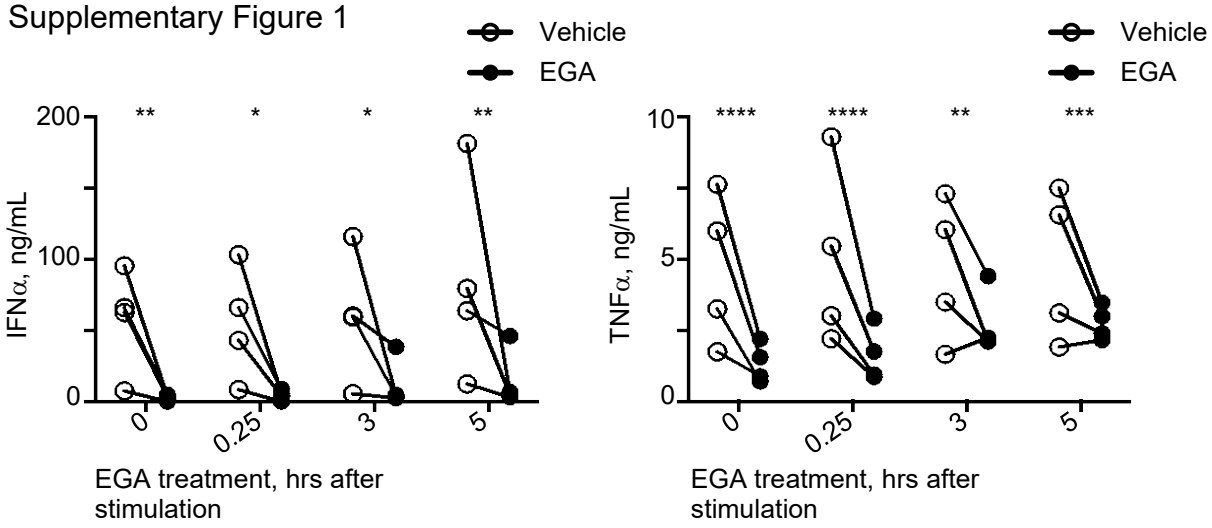
Supplementary Table 3. Antibodies utilized in confocal microscopy in this study.

Supplementary Table 4. Information of SLE Patients recruited in this study.

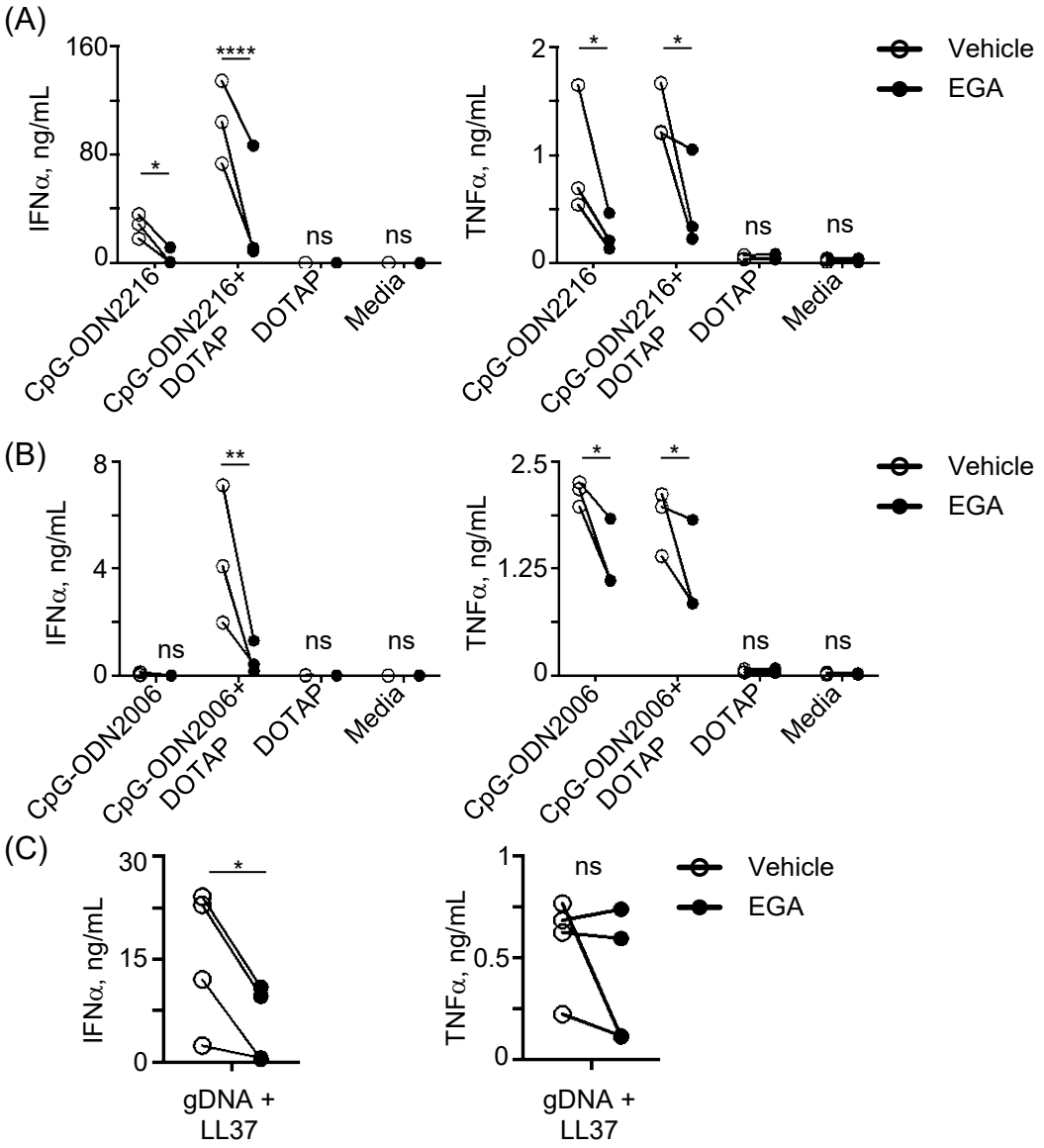
Supplementary Figure 1



Supplementary Figure 1. Kinetics of EGA-mediated suppression of cytokine expression in CpG-ODN2216-stimulated pDCs

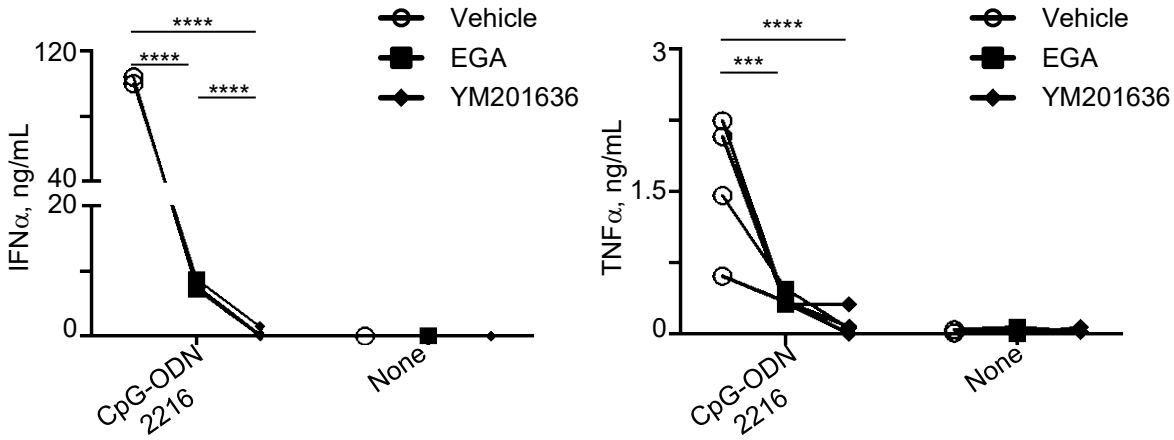
pDCs stimulated with CpG-ODN2216 overnight with 20 μM EGA or vehicle added at time points post-CpG-ODN2216 stimulation. IFNα and TNFα concentrations in supernatants after overnight stimulation. Data from 4 different subjects analyzed by two-way ANOVA with Sidak multiple comparison test. **P*< 0.05, ***P*< 0.01, ****P*< 0.001, *****P*<0.0001, for comparison between groups.

Supplementary Figure 2



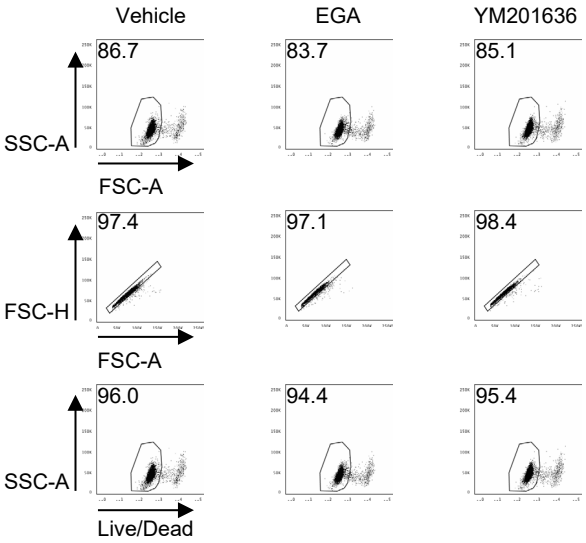
Supplementary Figure 2. EGA diminishes IFNα and TNFα secretion by CpG-DOTAP- and gDNA/LL37-stimulated pDCs.
IFNα and TNFα concentrations in supernatants of pDCs pre-treated with 20 μM EGA or vehicle and stimulated with (A) CpG-ODN2216 with/out DOTAP, (B) CpG-ODN2006 with/out DOTAP, or (C) gDNA/LL37 overnight. Data from 3-4 different subjects analyzed by two-way ANOVA with Sidak multiple comparison test (A and B) or two-tailed paired t-test (C). *P< 0.05, **P< 0.01, ****P< 0.0001, ns – not significant for comparison between groups.

Supplementary Figure 3



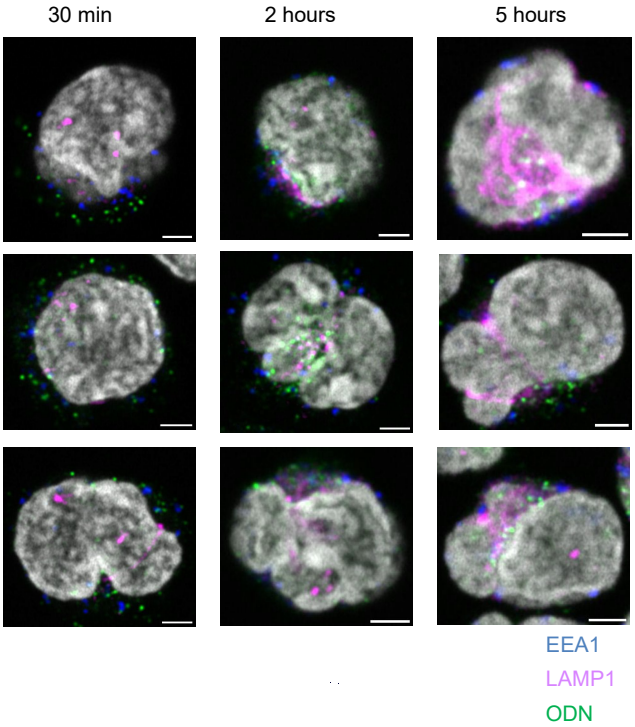
Supplementary Figure 3. EGA and PIKfyve inhibitors reduce IFNα and TNFα secretion by CpG-ODN2216-stimulated pDCs.
IFNα and TNFα levels in supernatants of pDCs pre-incubated with 20 μM EGA, 1 μM YM201636, or vehicle, and stimulated with CpG-ODN2216 overnight. Data were generated with pDCs isolated from 4 different subjects Two-way ANOVA with Tukey multiple comparison test. ****P*< 0.001, *****P*<0.0001, for comparison between groups.

Supplementary Figure 4



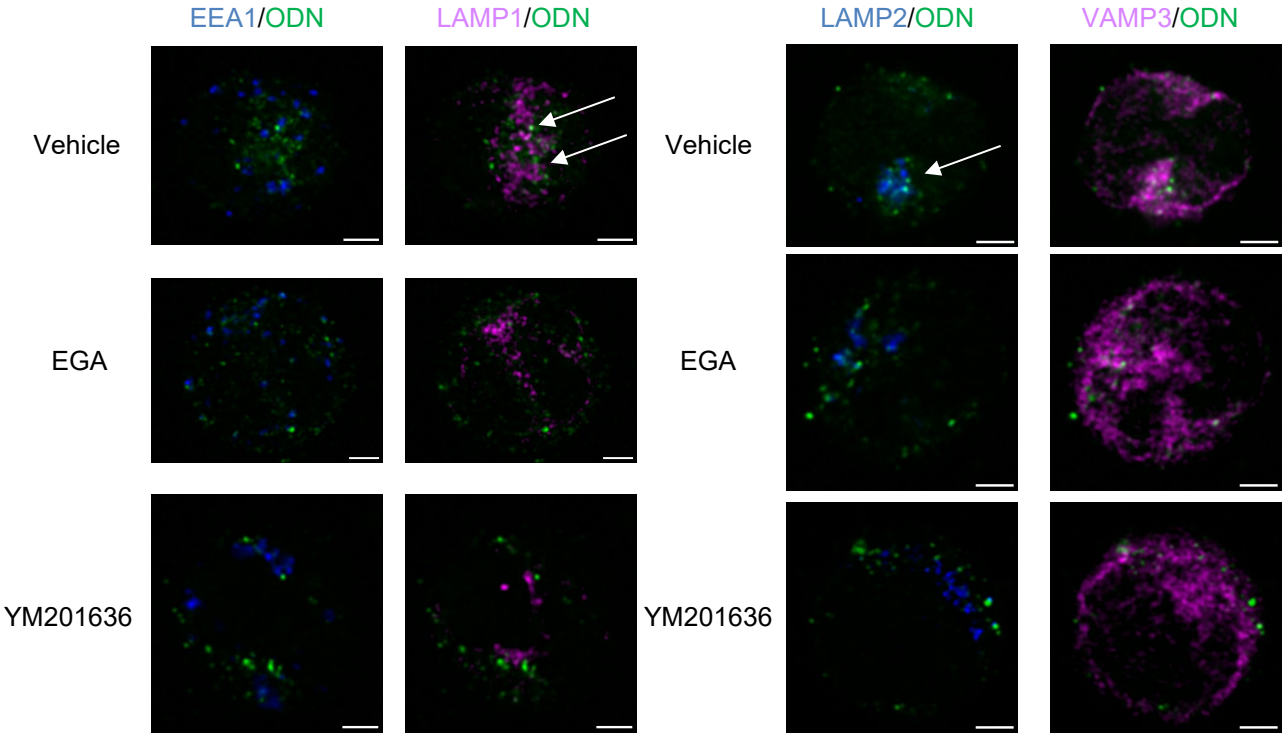
Supplementary Figure 4. EGA and PIKfyve inhibitors do not affect blood pDC viability.
Representative viability assessment of pDCs pre-incubated with 20μM EGA, 1μM YM201636, or vehicle, and stimulated with CpG-ODN2216 overnight.

Supplementary Figure 5



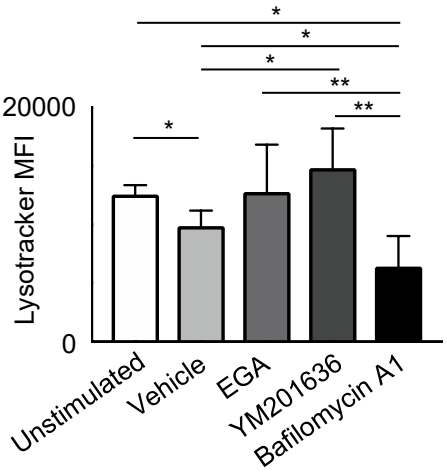
Supplementary Figure 5. Trace of CpG-ODN2216 in pDCs.
pDCs were incubated with 1μM CpG-ODN2216 – FITC. Three representative images of CpG-ODN overlap with endo-lysosomal markers (EEA1, LAMP1) at each time point. Scale bar – 2μm

Supplementary Figure 6



Supplementary Figure 6. EGA- and YM201636-treatment decrease colocalization of CpG-ODN2216-FITC with LAMP1/2^{+} compartments in pDCs.
pDCs were pre-incubated with 20μM EGA, 1μM YM201636, or vehicle and stimulated with 1μM CpG-ODN2216-FITC. Representative images of colocalization of CpG-ODN2216-FITC with endo-lysosomal marker proteins (EEA1, VAMP3, LAMP2, LAMP1) at 2 hours post-stimulation. Scale bar – 2μm

Supplementary Figure 7



Supplementary Figure 7. EGA and YM201636 do not affect endo-lysosomal acidification.

pDCs were pre-incubated with 20 μM EGA, 1 μM YM201636, 1 μM Bafilomycin A1, or vehicle and stimulated with CpG-ODN2216 for 12 hours. CpG-ODN was washed out and cells were labeled with LysoTracker Deep Red and analyzed by flow cytometry. Data representative from 5 donors analyzed by one-way ANOVA with Tukey multiple comparison test. *P< 0.05, **P< 0.01, for comparison between groups.

Supplementary Table 1. Antibodies utilized in flow cytometry in this study.

Antibody	Clone	Source	Catalog
Anti-Human HLA-DR	L243	Biolegend	307618
Anti-Human HLA-DR	G46-6	BD	560651, 561359
Anti-Human Lineage-1	Various	BD, Biolegend	340546, 363601
Anti-Human CD3	UCHT1	BD, Biolegend	555335, 300412
Anti-Human CD3	Sk7	BD	340440
Anti-Human CD14	MϕP9	BD	557831
Anti-Human CD19	HIB19	BD	555415
Anti-Human CD20	2H7	eBioscience	17-0209-42
Anti-Human CD56	B159	BD	555518
Anti-Human CD123	9F5	BD	551065, 563161
Anti-Human CD123	7G3	BD	560826
Anti-Human CD11c	B-ly6	BD	560369, 561355, 562393
Anti-Human IFNα	LT27:295	Miltenyi Biotec	130-092-601, 130-123-708
Anti-Human TNFα	Mab11	Biolegend	502932, 502936
Mouse IgG1 Isotype	MOPC-21	Biolegend	400114, 400158, 400162

Supplementary Table 2. Antibodies utilized in immunoblotting in this study.

Antibody	Clone	Catalog	Host	Source
Anti - ATF3	E9J4N	18665	Rabbit	CST
Anti - p-p65 (S536)	93H1	3033	Rabbit	CST
Anti - p65	L8F6	6956	Mouse	CST
Anti - p-IKKα/β (S176/S177)	C84E11	2078	Rabbit	CST
Anti - IKβa	44D4	4812	Rabbit	CST
Anti - p-Akt (S473)	N/A	9271	Rabbit	CST
Anti - Akt	C67E7	4691	Rabbit	CST
Anti - p-p38 (T180/Y182)	3D7	9215	Rabbit	CST
Anti - p38	N/A	9212	Rabbit	CST
Anti - pSTAT1 (Y701)	58D6	9167	Rabbit	CST
Anti - STAT1	D1K9Y	14994	Rabbit	CST
Anti - IRF7	N/A	4920	Rabbit	CST
HRP - Anti - Mouse IgG	N/A	7076	Horse	CST
HRP - Anti - Rabbit IgG	N/A	7074	Goat	CST

Supplementary Table 3. Antibodies utilized in confocal microscopy in this study.

Antibody	Clone	Catalog	Host	Source	Dilution
Anti-EEA1	14	610456	Mouse	BD	1:250
Anti-VAMP3	EPR16866	ab200657	Rabbit	Abcam	1:500
Anti-LAMP1	N/A	ab24170	Rabbit	Abcam	1:1000
Anti-LAMP2	H4B4	354301	Mouse	Biolegend	1:500
Rabbit Polyclonal IgG	N/A	LS-C149375	Rabbit	LSBio	1:5000
Mouse IgG1	MOPC-21	400102	Mouse	Biolegend	1:500
Alexa Flour 488 Goat- Anti FITC	N/A	A11096	Goat	ThermoFisher	1:1000
Alexa Flour 568-Goat-Anti-Rabbit IgG	N/A	A11036	Goat	ThermoFisher	1:1000
Alexa Flour 647-Goat-Anti-Mouse IgG	N/A	A21235	Goat	ThermoFisher	1:1000

Supplementary Table 4. Information of SLE Patients recruited in this study.

Patient	Age	Gender	Ethnicity	SLEDAI - 2k	Clinical Status
1	59	Female	White	8	2
2	39	Female	White	8	2
3	44	Female	White	12	2
4	57	Male	White	12	2
5	63	Female	White	8	2
6	37	Female	White	0	2
7	50	Male	Pacific Islander	7	2

Clinical Status

1: No disease activity, off medications

2: Disease in remission, on medication

3: Disease, relapsing

4: Treatment resistant