Supplemental information

Deficiency of the CD155-CD96 immune checkpoint

controls IL-9 production in giant cell arteritis

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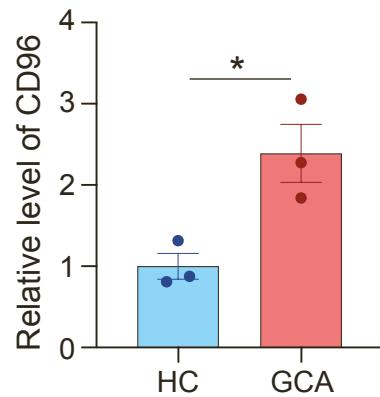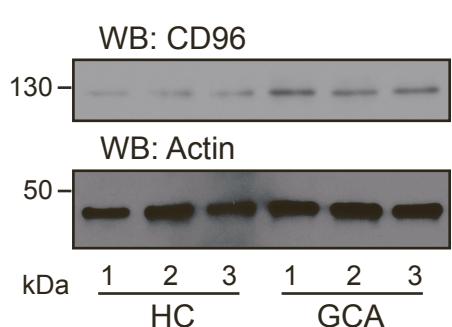
Supplemental Figure S1. Accumulation of CD96^{high} CD4^{+} T cells in GCA patients.

Immunoblotting of CD96 protein in control and patient-derived resting CD4^{+} T cells. b-actin served as a control. Bar graph shows protein quantification. Data from 3 GCA patients and 3 healthy controls. Two-tailed unpaired t test. *<0.05.
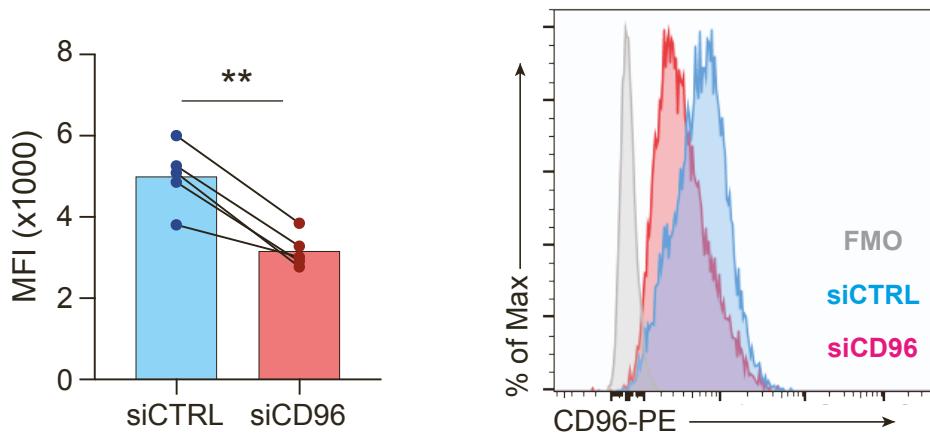
Supplemental Figure S2. Knockdown efficiency for CD96.

CD96 was knocked down by transfecting CD4^{+} T cells with CD96-specific or control siRNA. CD96 protein expression was quantified by FACS (n=5). Two-tailed paired t test. **<0.01.

A
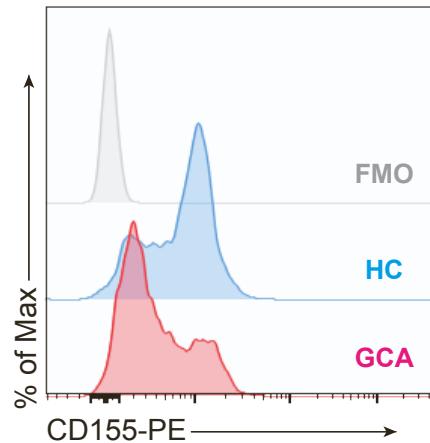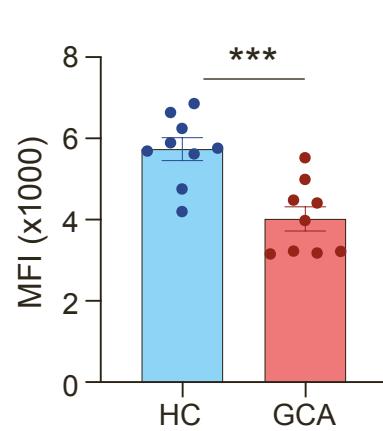
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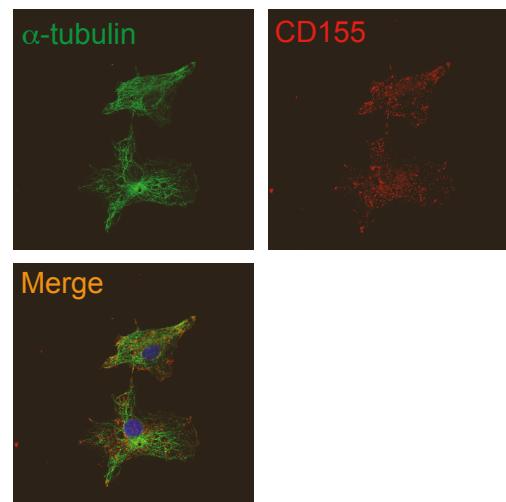
Supplemental Figure S3. CD155 low-expressing monocytes in giant cell arteritis (GCA).

(A) Flow cytometric analysis of CD155 expression on $CD14^+$ monocytes from GCA patients and age-matched controls. Representative histograms and MFI are shown. Each dot represents one patient or one healthy individual.
(B) Monocyte-derived macrophages were induced from GCA patients and stained for CD155 (red) and α-tubulin (green) as structure marker.
Two-tailed unpaired t test. ***<0.001
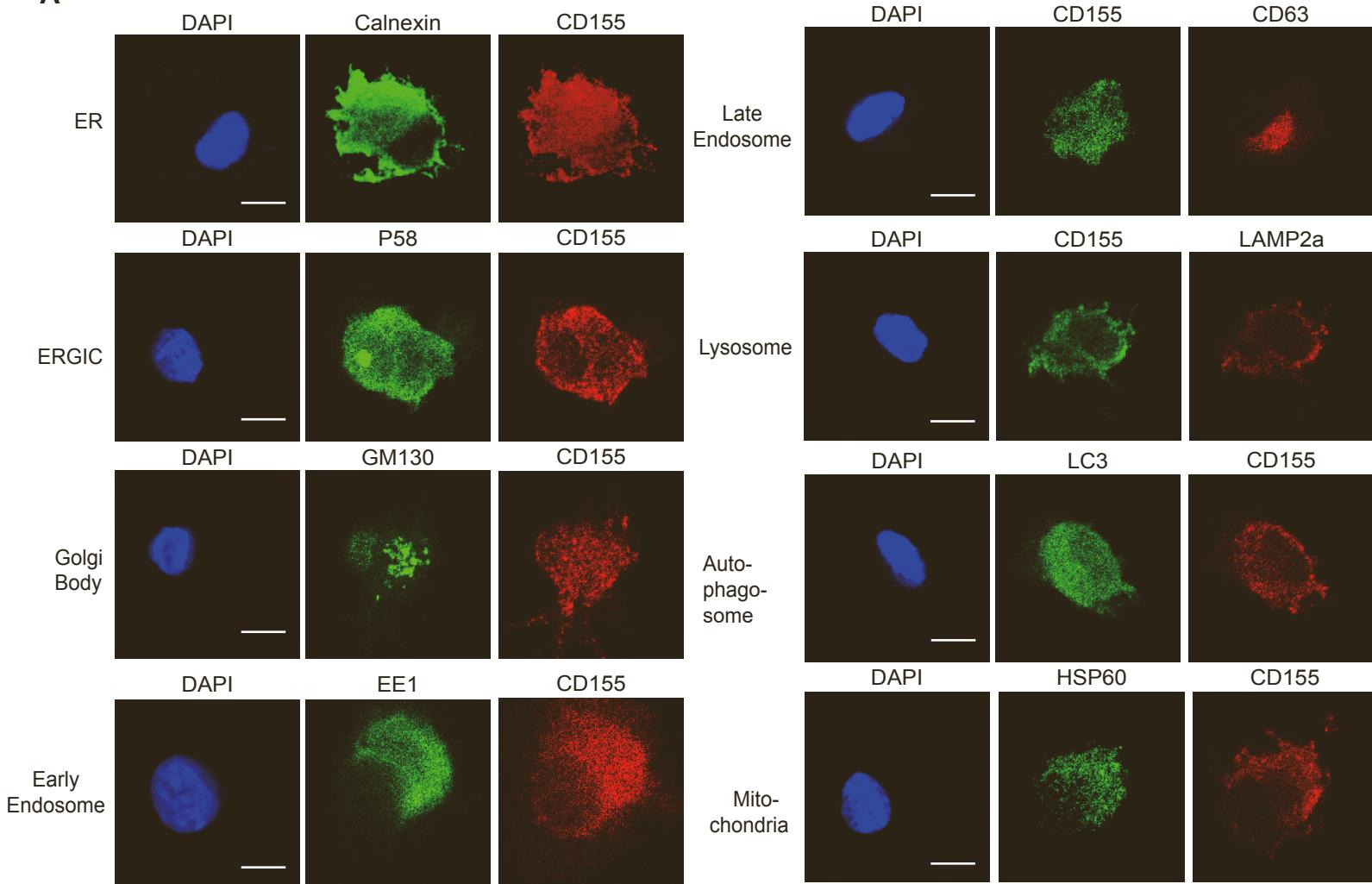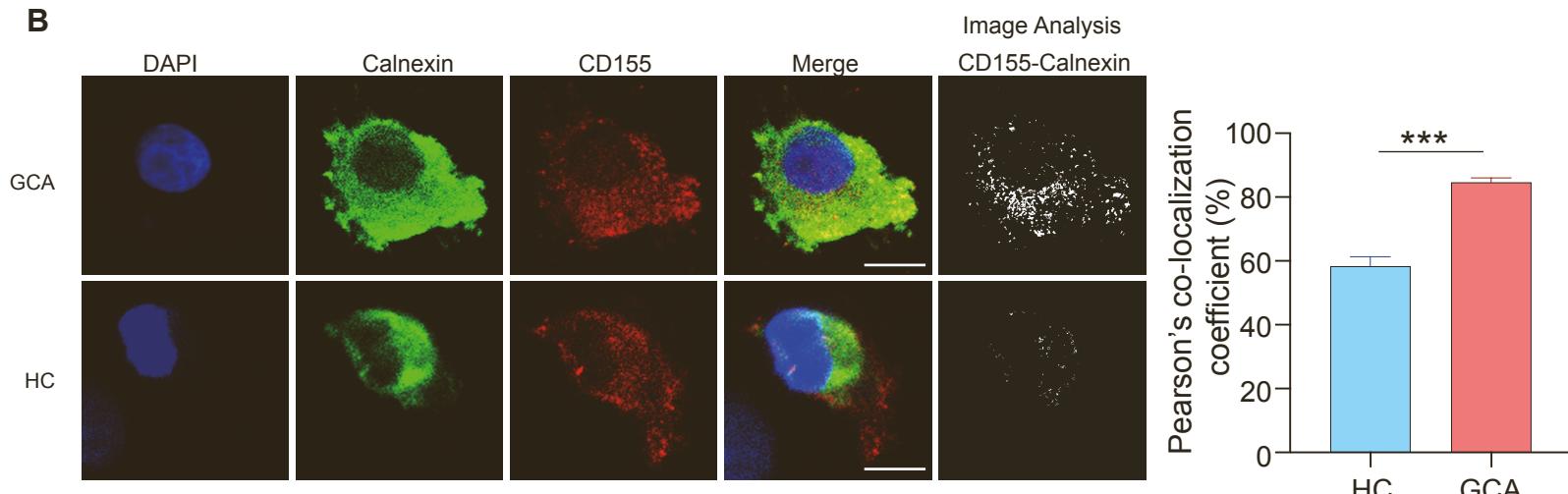
Supplemental Figure S4. GCA macrophages under ER stress trap CD155 in the cytoplasm.

Monocyte-derived macrophages were generated from age-matched controls and GCA patients and stimulated with LPS/IFN-γ.

(A) Macrophages were stained for CD155 and cellular organelles and imaged by fluorescence microscopy. Markers represent the following organelles: (Calnexin; ER), (P58; ERGIC), (GM130; Golgi body), (EE1; early endosome), (CD63; late endosome), (LAMP2a; lysosome), (LC3; autophagosome), (HSP60; mitochondria), (DAPI; nucleus).

(B) HC macrophages and GCA macrophages were stained for CD155 and Calnexin. Cellular stains were analyzed by imaging software to reveal co-localization as white dots (right panel). Scale bar; 5 μm. Representative images for each subcellular organelle are shown.

Supplemental Table 1. Clinical characteristics of patients with giant cell arteritis

Parameters	Patients (n=144)
Age (year, mean ± SD)	73.60 ± 8.70
Female	101 (70.1%)
Ethnicity	
Caucasian	121 (84.0%)
African American	6 (4.2%)
Hispanic	8 (5.6%)
Asian	6 (4.2%)
Other	3 (2.1%)
Disease Duration (months, mean ± SD)	6.31 ± 7.86
Erythrocyte sedimentation rate (mean ± SD, mm/h)	36.76 ± 27.81
C-reactive protein (mean ± SD, mg/dL)	7.94 ± 13.95
Headaches	70 (48.6%)
Eye involvement	50 (34.7%)
Aortic/large vessel involvement	84 (58.3%)
Polymyalgia rheumatica	92 (63.9%)
Treatment of GCA	
Untreated	44 (30.6%)
Prednisone (mg/day, mean ± SD)	5.17 ± 7.47
Second immunosuppressant	37 (25.7%)

Supplemental Table 2. PCR primers used in this study

Genes	Forward	Reverse
β-actin	GATCATTGCTCCTCCTGAGC	CGTCATACTCCTGCTTGCTG
IL-1β	AAGTACTGAGCTCGCCAGTGAAA	TTGCTGTAGTGGTGGTCGGAGATT
IL-2	AACTCCTGTCTTGCATTGCAC	GCTCCAGTTGTAGCTGTGTTT
IL-4	TACAGCCACCATGAGAAGGACACT	TTCCTGTCGAGCCGTTTCAGGAAT
IL-5	TGGAGCTGCCTACGTGTATG	TTCGATGAGTAGAAAGCAGTGC
IL-6	AGCCACTCACCTCTTCAGAACGAA	AGTGCCTCTTTGCTGCTTTCACAC
IL-9	GGGATCCTGGACATCAACTTC	GAAGCATGGTCTGGTGCAGTT
IL-9	CTCTGTTTGGGCATTCCCTCT	GGGTATCTTGTTTGCATGGTGG
IL-10	TCCTTGCTGGAGGACTTTAAGGGT	TGTCTGGGTCTTGGTTCTCAGCTT
IL-13	CAACGCTCATTGCTCTCACTTGCC	CCTTGTGCGGGCAGAATCCGCTCA
IL-17A	AACCGATCCACCTCACCTTGGAAT	TTCATGTGGTAGTCCACGTTCCCA
IL-18	TCTTCATTGACCAAGGAAATCGG	TCCGGGGGTGCATTATCTCTAC
IL-21	TCCTGGCAACATGGAGAGGATTGT	AGCTGGCAGAAATTCAGGGACCAA
IL-22	CCTATATCACCAACCGCACCTTC	AGATTGAGGGAACAGCACTTCTTC
IL-9 receptor	GCAACATCAGTTCTGGCCCAC	TGCTTCCAGGCTCCCCGA
IFN-γ	ACTAGGCAGCCAACCTAAGCAAGA	CATCAGGGTCACCTGACACATTCA
TNF-α	GGTGGTGCCATCAGAGGGCC	GAGCACATGGGTGGAGGGGC
TNF-α	GGGACCTCTCTCTAATCAGCC	GTTATCTCTCAGCTCCACGCC
GM-CSF	GGGAGCATGTGAATGCCATC	GGCTCCTGGAGGTCAAACAT
TGF-β	ACTTGCACCACCTTGGACTTC	GGTCATCACCGTTGGCTCA
T cell receptor	CCTTCAACAACAGCATTATTATTCCAG	CGAGGGAGCACAGGCTGTCTTA
CD80	ATGGTGGGCACAGAAGTAGC	AGGAAATCTGGGTTCTGGCG
CD86	TGGTCAGGGAGGGGTTTTGG	GCCCCGGGTGATCTGTGTCT
CD96	CAAACACAGACAGTAGGCTTCTT	GGGGATGATAGACAGCAATCAG
CD155	TGGAGGTGACGCATGTGTC	GTTTGGACTCCGAATAGCTGG
ATF4	ATGACCGAAATGAGCTTCCTG	GCTGGAGAACCCATGAGGT
ATF6	TCCTCGGTCAGTGGACTCTTA	CTTGGGCTGAATTGAAGGTTTTG
BiP	GAAAGAAGGTTACCCATGCAGT	CAGGCCATAAGCAATAGCAGC
CHOP	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC
TRIB3	AAGCGGTTGGAGTTGGATGAC	CACGATCTGGAGCAGTAGGTG
XBP1	CCCTCCAGAACATCTCCCCAT	ACATGACTGGGTCCAAGTTGT

Supplemental Table 3. Antibodies used in this study

Antigen	Conjugated	Source	Catalog No
Control mouse IgG	None	BioLegend	400124
Control mouse IgG	None	Thermo Fischer Scientific	02-6502
Mouse IgG	Alexa Fluor 594	Thermo Fisher Scientific	A-11032
Mouse IgG	PE	Thermo Fisher Scientific	P-852
Rabbit IgG	FITC	abcam	ab97050
Rabbit IgG	Alexa Fluor 488	Thermo Fisher Scientific	A-11008
CD3	None	Dako	A0452
CD3	FITC	Biolegend	344804
CD4	FITC	BioLegend	300506
CD4	FITC	BioLegend	357406
CD8	BV480	BD Biosciences	566121
CD14	FITC	BioLegend	325604
CCR7	BV650	Biolegend	353234
CD45	PE-Cy7	BioLegend	368532
CD45RA	BV570	Biolegend	304132
CD68	None	Thermo Fisher Scientific	PA5-32331
CD68	None	Cell Signaling Technology	76437
CD163	None	Thermo Fisher Scientific	MA5-11458
CD96	None	Thermo Fisher Scientific	PA5-97568
CD96	None	BioLegend	338402
CD96	PE	BioLegend	338406
CD96	BV421	Biolegend	338418
PD-1	PE	Biolegend	329906
TIGIT	PE/Dazzle594	Biolegend	372716
CD226	BV711	Biolegend	338334
TIM3	PE/Cy5	Biolegend	345052
LAG3	PE/Cy7	Thermo Fischer Scientific	25-2239-42
CD155	None	Thermo Fischer Scientific	MA5-13493
CD155	None	BioLegend	337502
CD155	None	Thermo Fisher Scientific	MA5-29762
CD155	PE	BioLegend	337507
CD63	None	Santa Cruz Biotechnology	sc-5275
GM130	None	abcam	ab52649
ERGIC-53/p58	None	Sigma	E1031
LAMP2A	None	abcam	ab18528
Calnexin	None	Abclonal	A15631
ee1	None	Abclonal	A0592
LC3	None	MBL	PM036
HSP60	None	Santa Cruz Biotechnology	sc-13115
GRP78	None	Thermo Fischer Scientific	PA1-014A
IFN-γ	PerCP/Cy5.5	BioLegend	502526
IL-9	None	BioLegend	507704
IL-9	None	proteintech	66144-Ig
IL-9	BV421	BD Bioscience	564254