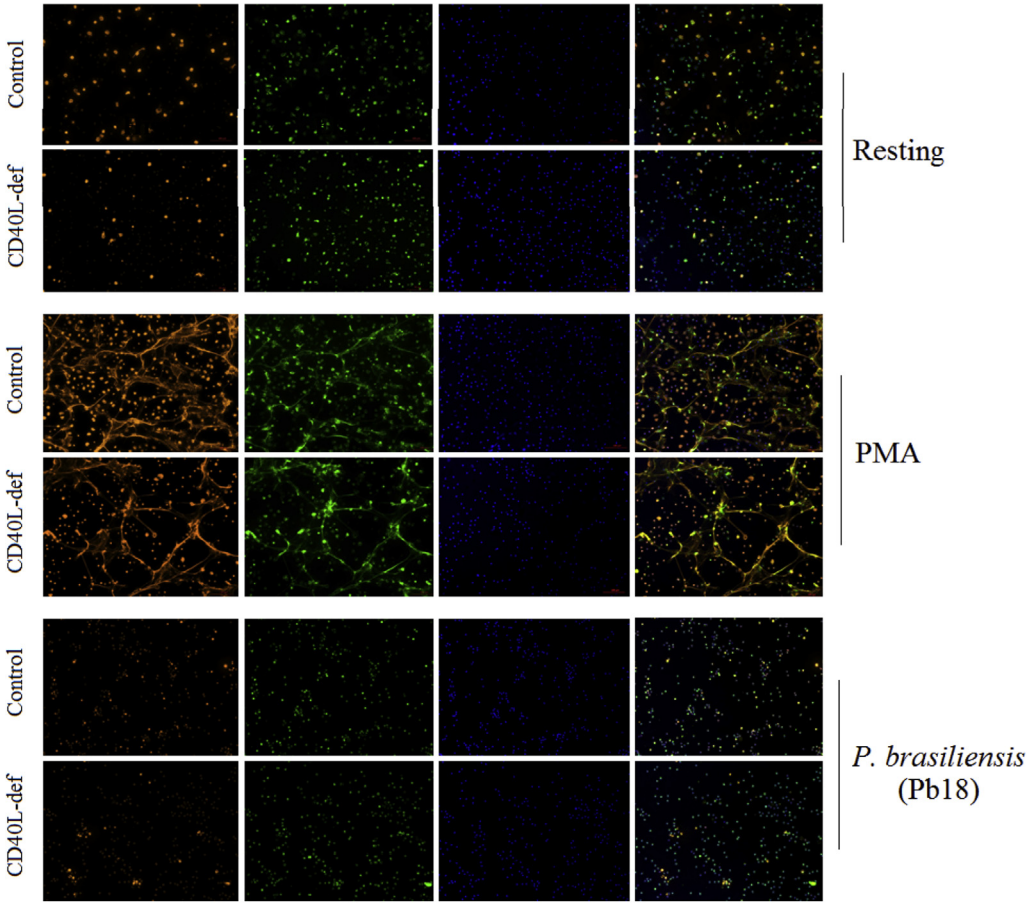

FIG E1. Reduced NET release in patients with CD40L deficiency. NET release by PMA- and *P brasiliensis*–activated neutrophils from patients and healthy control subjects was assessed by using fluorescence microscopy. Histones are shown in orange, neutrophil elastase is shown in *green*, and the nucleus (DNA) is shown in blue. One of 5 independent experiments is shown. Graphic data are shown in Fig 1, *G*.

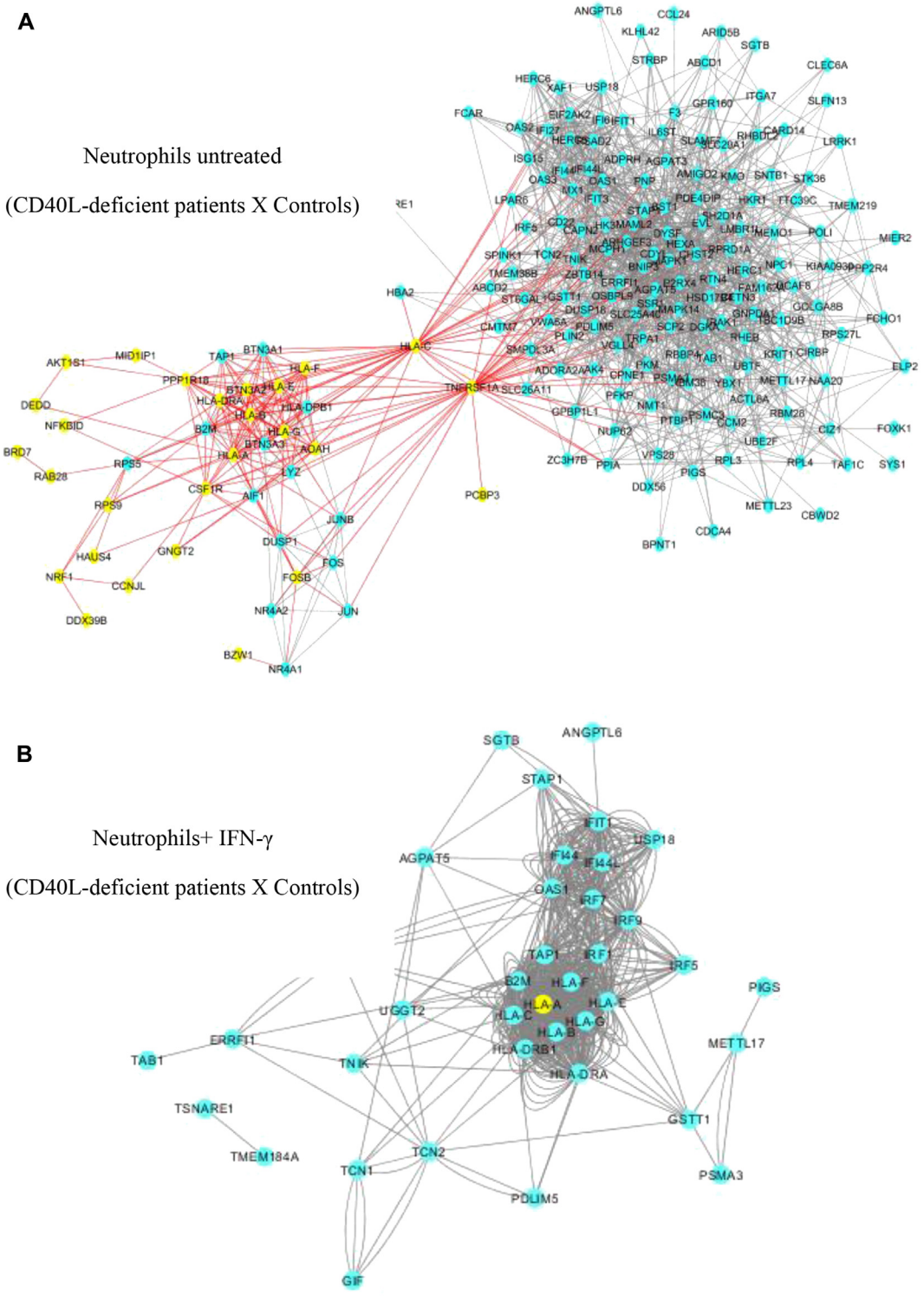

FIG E2. Interaction network of genes upregulated and downregulated in neutrophils from CD40L-deficient patients compared with those from control subjects. The network is shown as predicted by GeneMania and visualized by Cytoscape.[72] The images show the network of dysredulated genes when comparing neutrophils from CD40L-deficient patients and healthy controls in the absence **(A)** or after **(B)** *in vitro* treatment with IFN-γ. Blue represents upregulated genes, and yellow represents downregulated genes. The interaction network is based on features shared by genes, such as coexpression, colocalization, genetic interactions, signaling pathways, and physical interactions. *Red lines* show connections among downregulated genes or between downregulated and upregulated genes, whereas *gray lines* display connections only between upregulated genes. Gene Ontology categories are described in Table I.

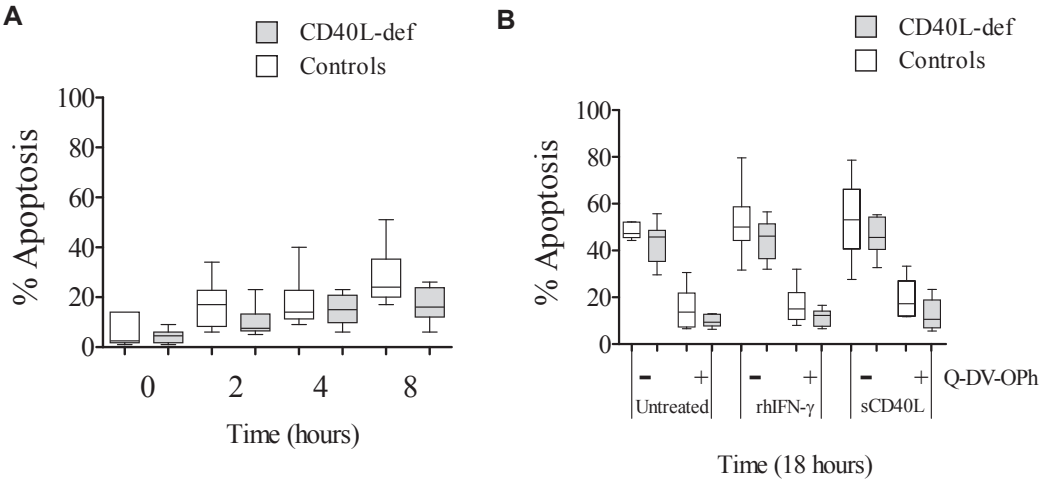

FIG E3. Normal phosphatidylserine levels on the outer leaflet of the cell membrane of neutrophils from CD40L-deficient patients. Kinetic measurement of phosphatidylserine on the outer leaflet of the cell membrane using the Annexin V–FITC single staining assay, as previously described.[44,45] The data suggest no alterations in patients' neutrophil apoptosis over a short (0-8 hours; **A**) or long (18 hours; **B**) period in the presence of IFN-γ or sCD40L when compared with healthy control subjects. Neutrophils were analyzed by using flow cytometry at different time points in the presence or absence of Q-VD-OPh (10 μmol/L; MP Biomedicals), which is a broad-spectrum caspase inhibitor with potent antiapoptotic properties.[46]

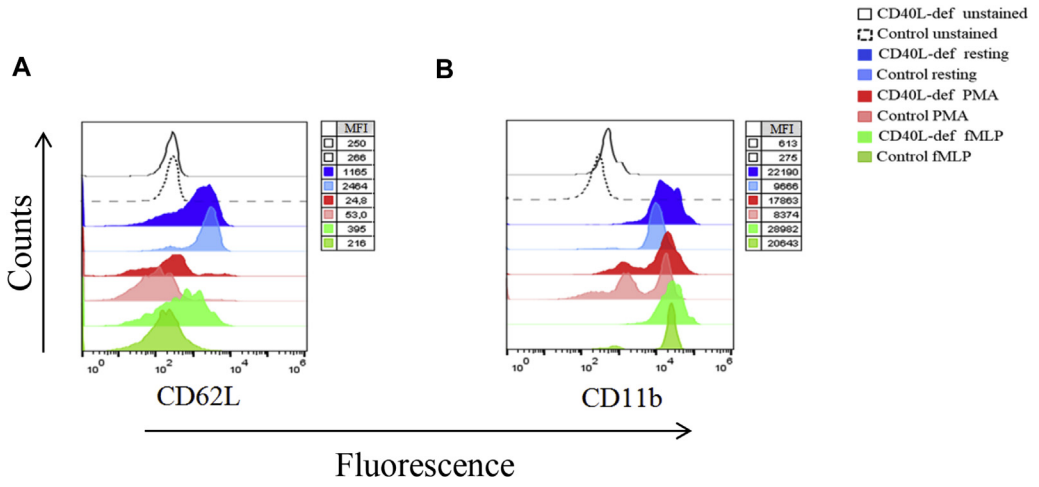

FIG E4. Expression of CD62L **(A)** and CD11b **(B)** by neutrophils from patient P4. Resting and PMA (300 ng/mL)–activated or fMLP (50 nmol/L)–activated neutrophils were analyzed by using flow cytometry.

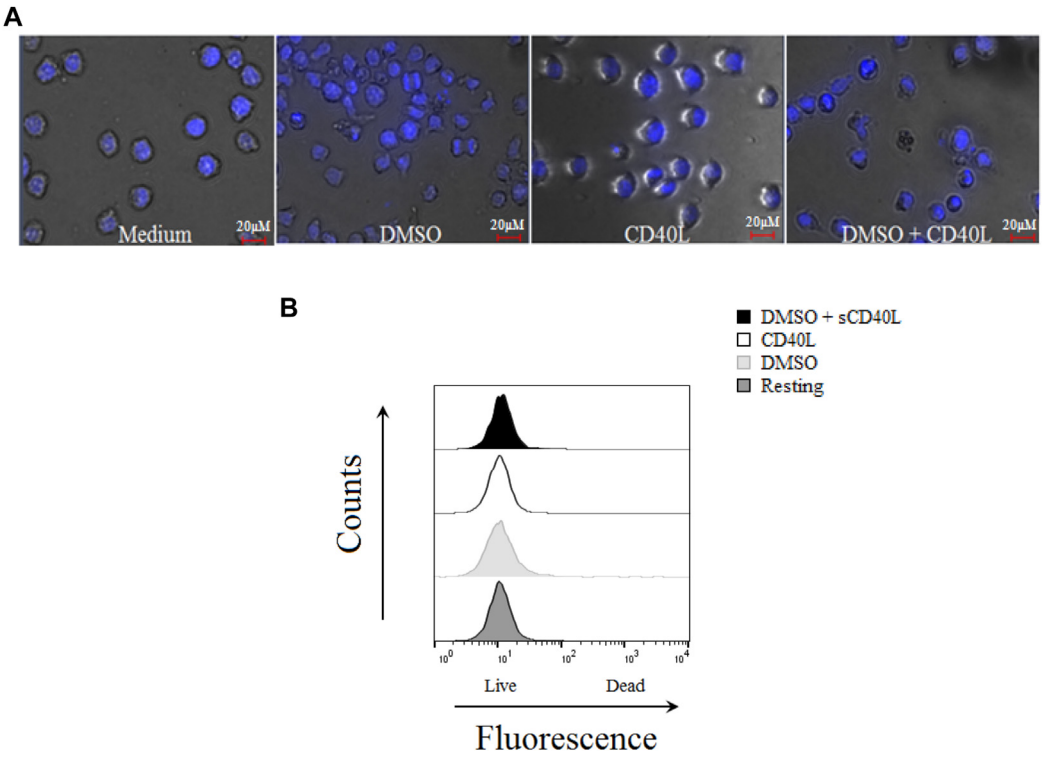

FIG E5. sCD40L does not affect HL-60 cell viability. **A,** Fluorescence microscopy images (using Axio Vert.A1) of HL-60 cells were obtained in the presence or absence of DMSO, sCD40L, or both. After 6 days of culture, HL-60 cells were harvested and counted, and cell viability was analyzed after nuclear staining by using Hoechst dye, which is shown in blue. One of 3 individual experiments is shown. **B,** Results were confirmed by using Trypan blue exclusion and the LIVE/DEAD Viability/Cytotoxicity Kit, according to the manufacturer's instructions (Thermo Fisher Scientific). Results are representative of 3 independent experiments performed in triplicates.

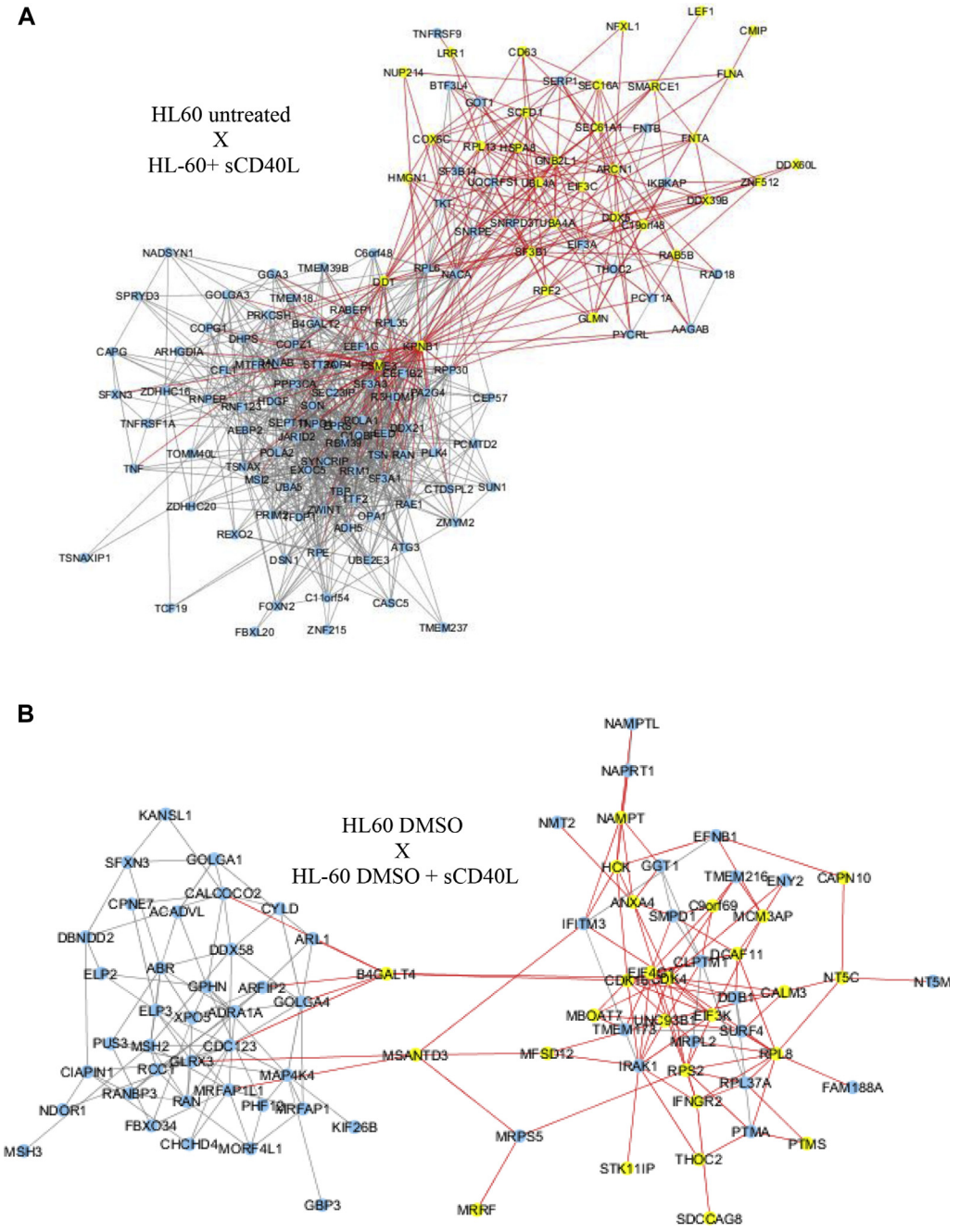

FIG E6. Genes upregulated or downregulated by sCD40L in HL-60 cells. Interaction network of genes upregulated and downregulated by sCD40L. The network is shown as predicted by GeneMania and visualized by Cytoscape.[72] Blue represents upregulated genes, and yellow represents downregulated genes. The interaction network is based on features shared by the genes, such as coexpression, colocalization, genetic interactions, signaling pathways, and physical interactions. *Red lines* show connections among downregulated genes or between downregulated and upregulated genes, whereas *gray lines* display connections only between upregulated genes. The Gene Ontology categories of sCD40L-inducible genes are described in Table I. We evaluated the gene expression of untreated HL-60 cells compared with those cultured in the presence of sCD40L **(A)**, whereas DMSO-differentiated cells were evaluated in comparison with cells cultivated in the presence of DMSO plus sCD40L **(B)**. The gene expression profile was obtained by using RNAseq from a pool of 3 independent cultures of HL-60 cells in which every experimental condition (resting, DMSO, sCD40L, and DMSO plus sCD40L) was explored in triplicates.

TABLE E1. Isolated pathogens and CD40L mutations identified in CD40L-deficient patients

Patient	Birth year	Isolated pathogens			Infections with unidentified pathogens	cDNA mutation†	Predicted effect on protein
		Fungi	Intracellular bacteria and protozoa	Virus			
P1	2007	—	*Mycobacterium tuberculosis*	—	Pneumonia, otitis,	c.419G>A	p. W140*
P2	2005	*Pneumocystis jirovecii*	—	HPV, herpes simplex	Otitis, sinusitis, pneumonia	c.157_160del	p.I53Kfs*13
P3	2007	*P jirovecii, Candida albicans*	*Cryptosporidium parvum*	—	Otitis, sinusitis, pneumonia	c.157_160del	p.I53Kfs*13
P4‡	1993	*Paracoccidioides brasiliensis, P jirovecii*	*M tuberculosis*		Pneumonia, otitis, sinusitis	c.289_346del	p.D97Vfs*12
P5	2004	*Aspergillus* species, *P jirovecii*	*C parvum*	—	Diarrhea	c.del of 170 nucleotides in 5′ UTR, affecting promoter region	No mRNA, no protein expression
P6	2006	—	*M tuberculosis*	—	Pneumonia, otitis urinary tract infection	c.574_577dup	p.L193Qfs*9

HPV, Human papillomavirus; *UTR*, untranslated region.

*Stop codon.

†Nomenclature for the description of mutations is according to guidelines of the Human Genome Variation Society[41,42] and Mutalyzer 2.0.26–Name Checker (https://mutalyzer.nl/).

‡Patient P4 died recently in a cachectic state after recurrent diarrhea caused by refractory *C parvum* and concomitant severe *M tuberculosis* infection. Patient P6 had severe refractory leishmaniosis and is currently hospitalized. Patients P1 to P3 underwent hematopoietic stem cell transplantation during the last trimester of 2017.