Neutrophil extracellular traps mediate articular cartilage damage and enhance cartilage components immunogenicity in rheumatoid arthritis

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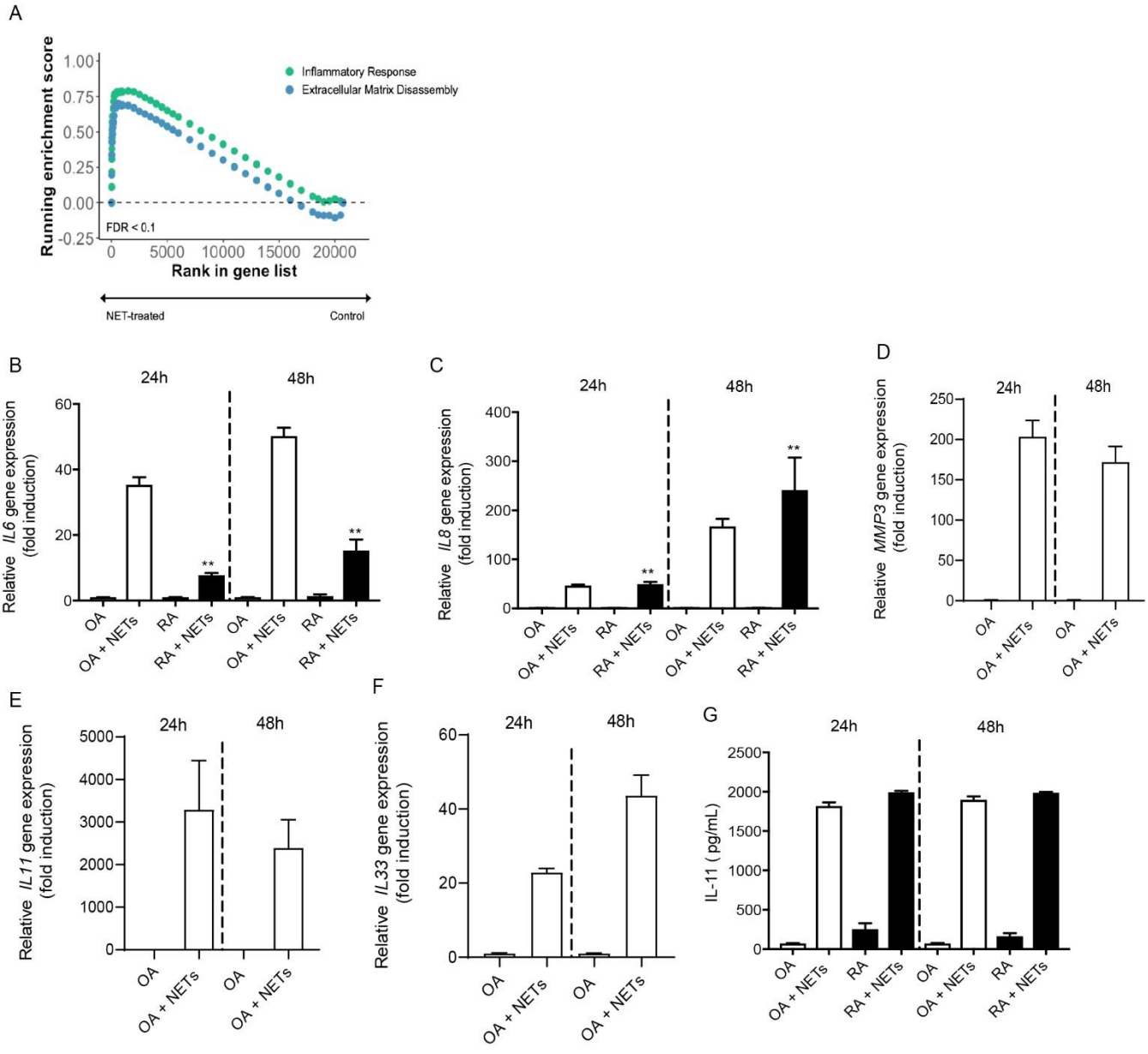
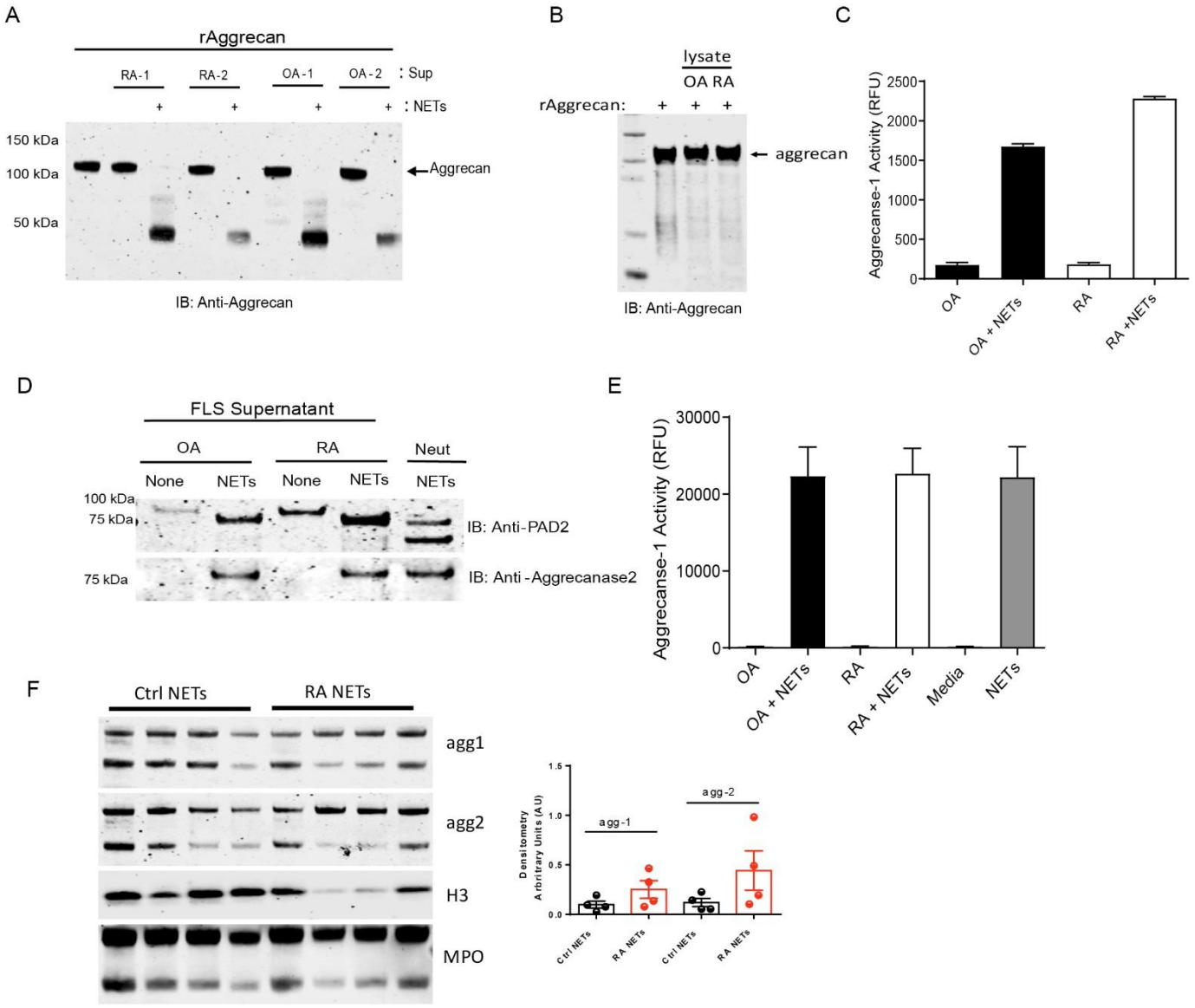
Supplementary Figures

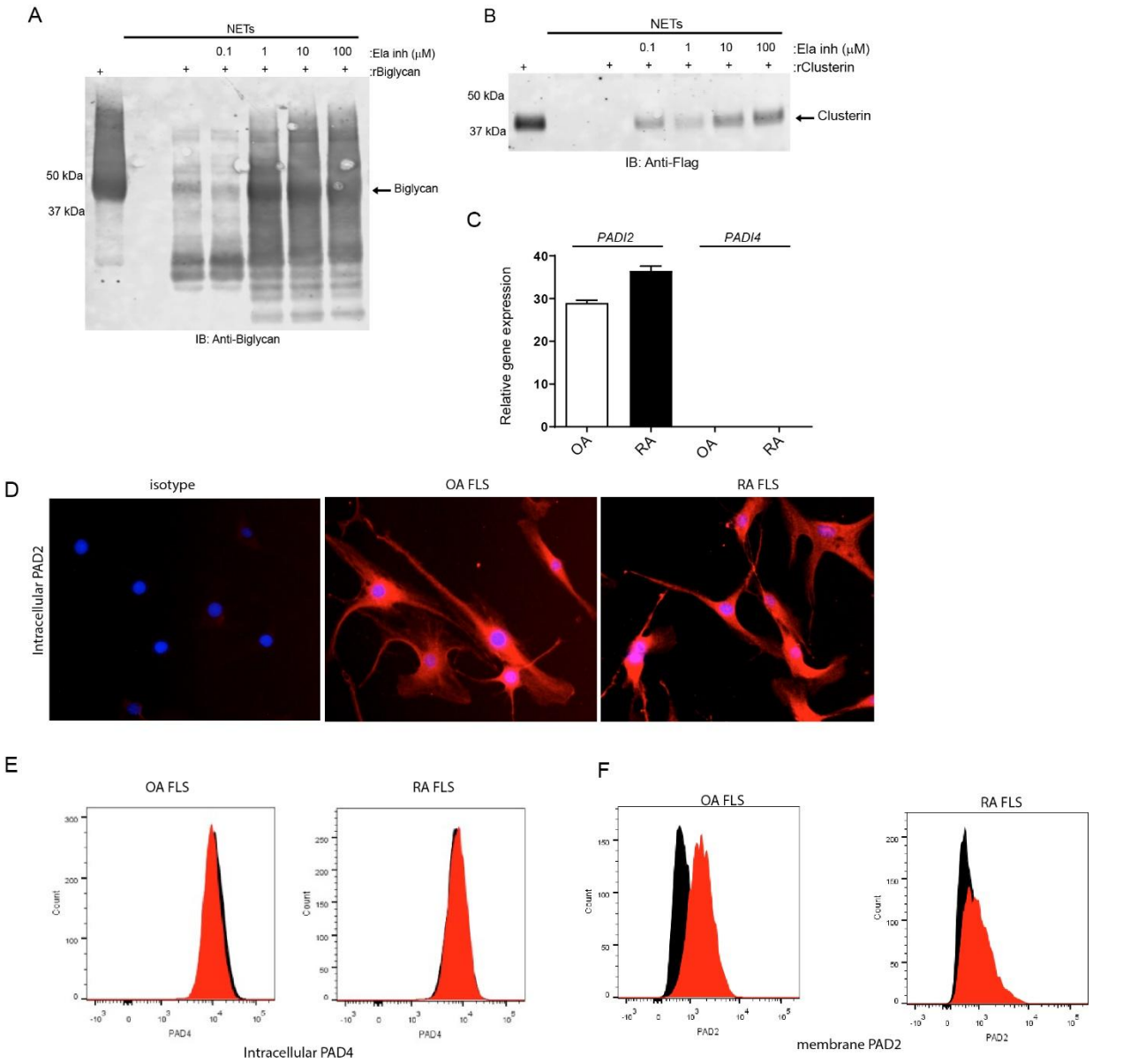


Supplementary Fig s1. FLS exposed to NETs upregulate proinflammatory cytokines and enzymes involved in extracellular remodeling. A. Gene enrichment analysis of the inflammatory

response and extracellular matrix disassembly pathways from RNA sequencing in RA FLS treated with NETs vs. RA FLS for 48 hours. **B-F.** Quantitative PCR to confirm differential expression of *IL6, IL8, MMP3, IL11, IL33* in RA or OA FLS in the presence or absence of NETs at 24 and 48 hours. Results are the mean +/- SEM of 3-6 independent experiments. Mann-Whitney U test was used. **p<0.01. **G.** IL-11 was measured in supernatants by ELISA from RA or OA FLS in the presence or absence of NETs at 24 or 48 hours. Results are the mean +/- SEM of 3 independent experiments. Mann-Whitney U test was used.



Supplementary Fig s2. NETs contain aggrecanases. A. Western blot analysis of aggrecan degradation in the presence of OA and RA FLS supernatants incubated with NETs. **B.** Western blot analysis of aggrecan degradation in the presence of OA and RA lysate. Representative of 2 independent experiments. **C.** Aggrecanase-1 activity in OA and RA supernatants incubated in the presence or absence of NETs. Results represent 2 independent experiments. **D-E.** Detection of PAD2 and Aggrecanase-1 and of Aggrecanase-1 activity in NETs and OA and RA FLS supernatants in the presence or absence of NETs. Results represent 2 independent experiments **F.** Western blot analysis to detect aggrecanase-1 (agg1) and aggrecanase-2 (agg2) in NETs isolated from control (Ctrl) or RA neutrophils. Histone H3 (H3) and Myeloperoxidase (MPO) were used as NET loading controls. Aggrecanase-1 (agg1) and aggrecanases-2 (agg2) were normalized to MPO and expressed as arbitrary units (AU). Ctrl n=4 and RA n=4.



Supplementary Fig s3. NET-bound neutrophil elastase mediates biglycan and clusterin degradation and PAD2, but not PAD4, is expressed by FLS. A-B Western blot analysis of NET-mediated degradation of recombinant biglycan or clusterin in the presence or absence of increasing concentrations of elastase inhibitor. Representative of 2 independent experiments. **C.** Quantitative PCR analysis of PADI2 and PAD14 in OA and RA FLS. Results are the mean +/- SEM of 3 independent experiments. Mann-Whitney U test was used **D.** Immunofluorescence analysis of PAD2 protein expression in permeabilized OA and RA FLS. Isotype antibody was used as control. Nuclei are stained in blue. **E.** Flow cytometry analysis for intracellular PAD4 protein expression in OA and RA FLS. **F.** Detection of PAD2 protein in the membrane of OA and RA FLS by flow cytometry. Results are representative of 2 independent experiments.