Supplemental Material

Multiple sclerosis: Molecular mimicry of an anti-myelin HLA class-I restricted T cell receptor

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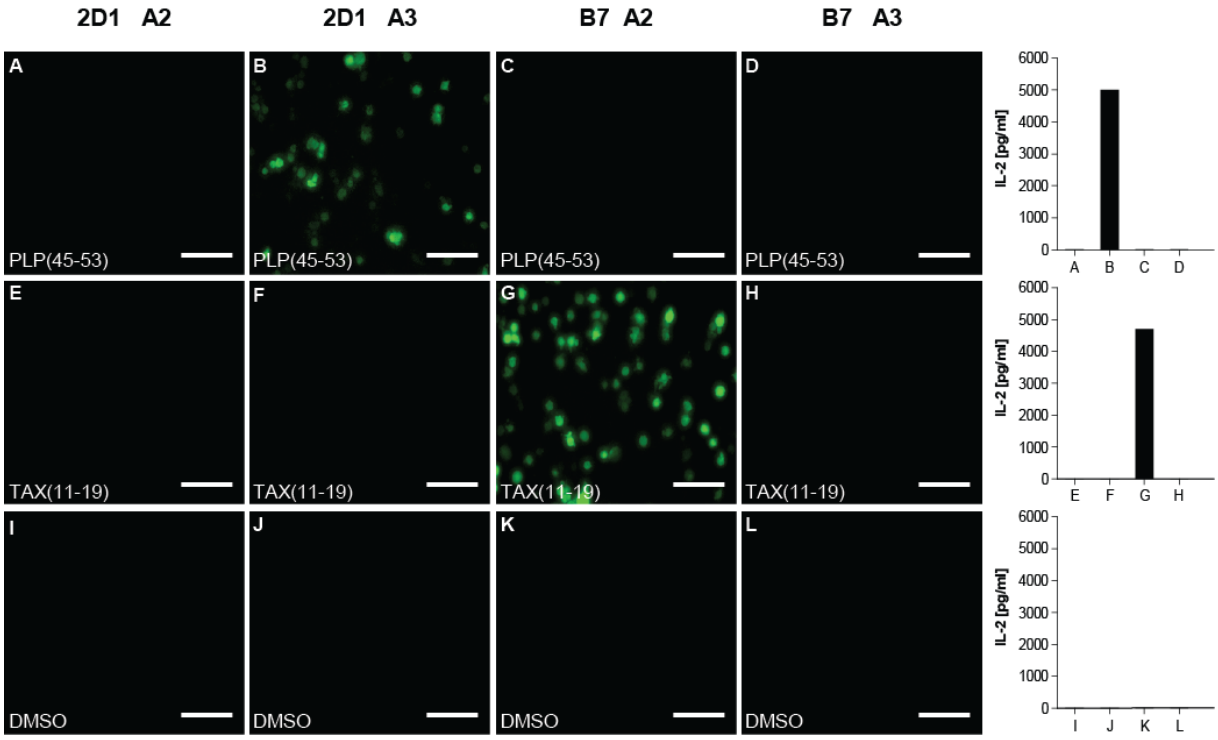

Figure e-1: **Specific activation of TCR-transfected T hybridoma cells by HLA-transfected COS-7 cells and synthetic peptides.**

Adherent COS-7-A2 (A2) or COS-7-A3 (A3) cells were incubated with 0.1 µg/µl PLP(45-53) peptide, TAX(11-19) peptide or pure DMSO which served as solvent for the peptides. COS-7 cells were superposed with 58-2D1-CD8-sGFP (2D1) and 58-B7-CD8-sGFP (B7) cells and T cell activation was analyzed by fluorescence microscopy (left panel) and by IL-2 ELISA (right panel). TCR 2D1 was tested with COS-7-A2 (**A, E, I**) and COS-7-A3 (**B, F, J**). TCR B7 was tested with COS-7-A2 (**C, G, K**) and COS-7-A3 (**D, H, L**). The peptides are given as inserts. Scale bar represents 50 µm. Error bars represent standard deviation of the mean. Green 58-2D1-CD8-sGFP and 58-B7-CD8-sGFP cells may only be observed when the specific synthetic peptides PLP(45-53) and TAX(11-19) were presented by COS-7-A3 or COS-7-A2 cells, respectively (left panel). An identical pattern of TCR activation was observed by measuring secreted IL-2 by ELISA (right panel).

Figure e-1

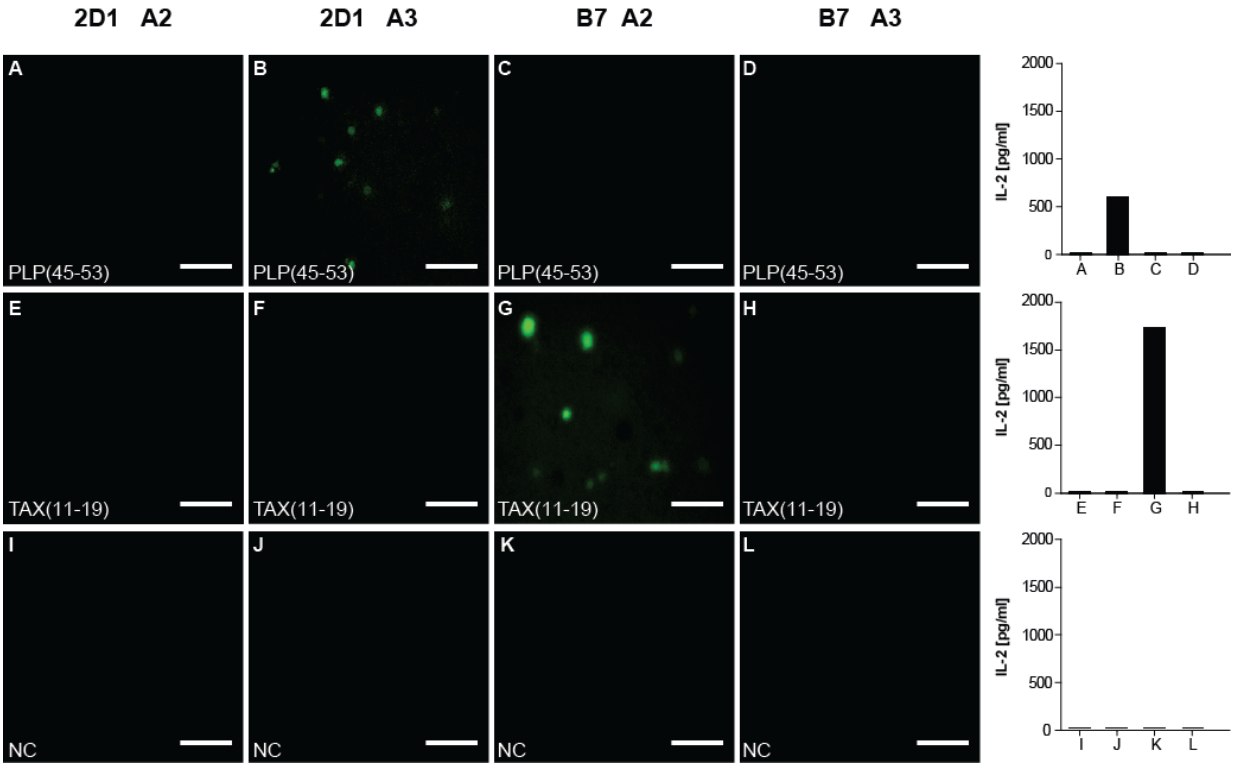

Figure e-2: **Activation of TCR-transfected T hybridoma cells by HLA-transfected COS-7 cells and plasmid-encoded peptides.**

Adherent COS-7-A2 (A2) or COS-7-A3 (A3) cells were transiently transfected with 500 ng PLP(45-53) peptide-encoding, TAX(11-19) peptide-encoding or empty plasmid (NC). COS-7 cells were superposed with 58-2D1-CD8-sGFP (2D1) and 58-B7-CD8-sGFP (B7) cells. T cell activation was analyzed by fluorescence microscopy (left panel) and by IL-2 ELISA (right panel). See legend to **Fig 1** for details. Scale bar represents 50 µm. Error bars represent standard deviation of the mean. As observed for synthetic peptides, 58-2D1-CD8-sGFP and 58-B7-CD8-sGFP were only activated by their specific HLA allele and plasmid-encoded peptide combination.

Figure e-2

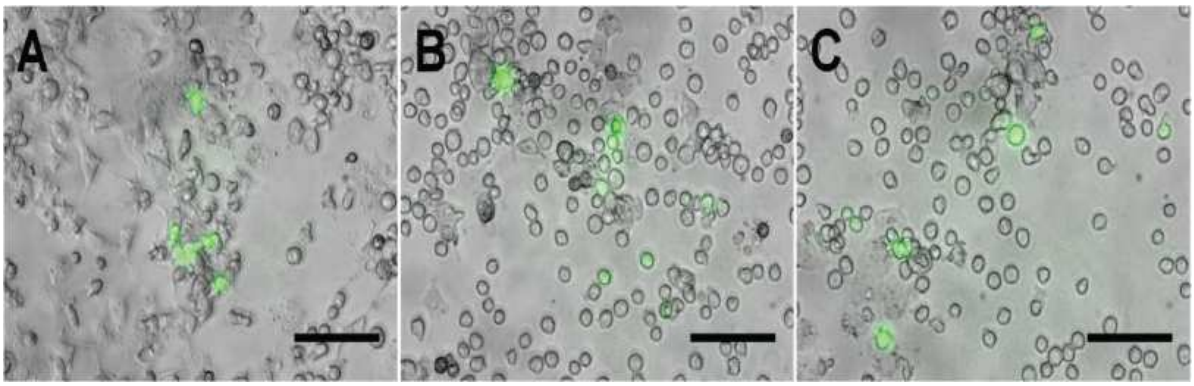

Figure e-3: Clusters of activated 58-2D1-CD8-sGFP cells revealing mimotope presenting COS-7-A2 cells.

Merged fluorescence and transmitted light microscopy images of co-cultured 58-2D1-CD8-sGFP and $8X^{8L}$ PECP library-transfected COS-7-A2 cells. Upon TCR activation 58-2D1-CD8-sGFP cells express sGFP. Thus, underlying COS-7-A2 cells contain PECP library plasmids that code for antigenic peptides for the TCR 2D1. Often clusters of several cells were observed. The clusters arise upon division of activated 58-2D1-CD8-sGFP cells during the experiment (see movie #1 in ref[22]), by division of COS-7-A2 cells before addition of 58-2D1-CD8-sGFP cells, or by concomitant activation of several 58-2D1-CD8-sGFP cells by one or more COS-7-A2 cells. (**A**) Cluster revealing mimotope LIGEVFVL, (**B**) cluster revealing mimotope LLGEVFEL, (**C**) cluster revealing mimotope LVGEVWGL. Scale bar indicates 50 µm.

Figure e-3