Supplementary Material

Immunosuppressive activity of an aqueous *Viola tricolor* herbal extract

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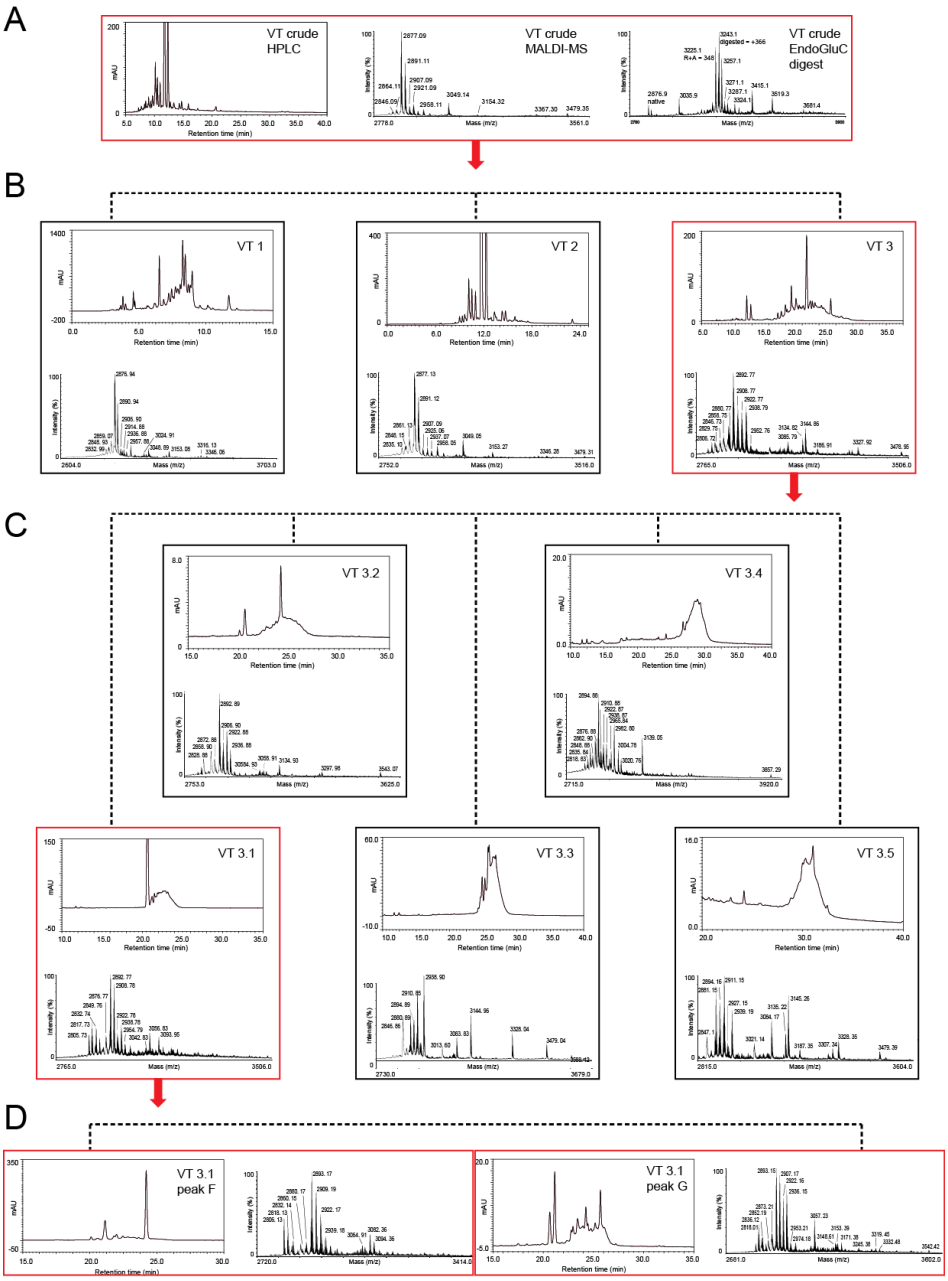
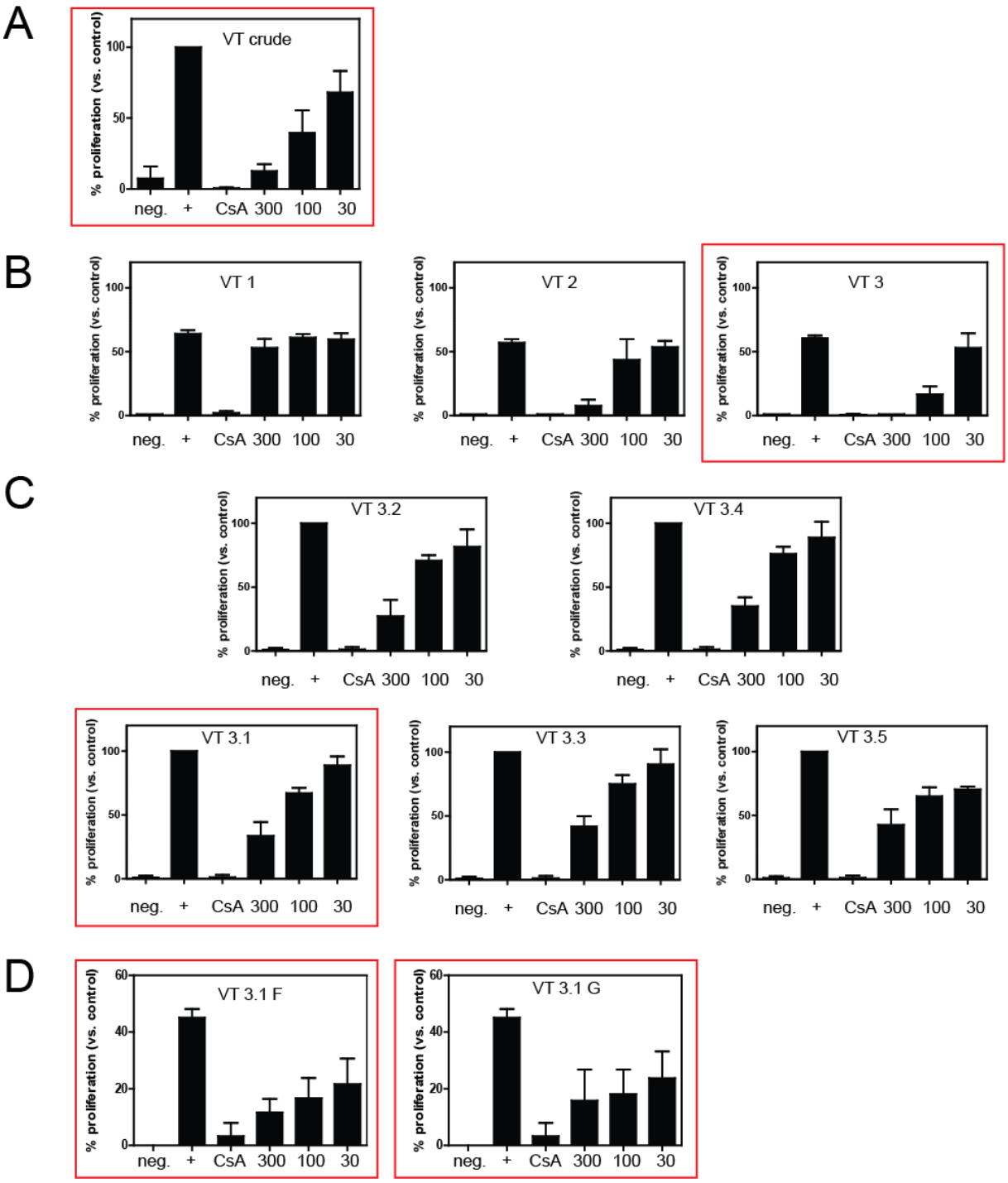


Supplementary Figure 1: Purification scheme for the bioactivity guided fractionation of aqueous *Viola tricolor* extracts. (A) The aqueous extracts were subjected to UV-HPLC analysis and additionally analyzed by MALDI-TOF to give evidence of the presence of cyclotides. An aliquote of extract in 0.1 M ammonium hydrogencarbonate buffer was reduced by dithiothreitol, the thioles were alkylated using iodoacetamide and the sample was digested by endoproteinase GluC. A mass shift of 366 Da of the corresponding peaks in the native crude extracts compared to the alkylated and digested sample provides indication for the presence of six cysteines and one glutamic acid – both are specific identification criteria for cyclotides. (B-D) Bioactivity guided multi-step sub-fractionation revealed to cyclotides containing samples VT3.1.F and VT3.1.G with were analyzed as described above.



Supplementary Figure 2: Influence of all tested *Viola tricolor* fractions on the proliferation of activated human T-lymphocytes. (A) According to the bioactivity guided fractionation approach, all extracts and chromatographed fractions were applied to a proliferation assay as described in the material and method section. (B-C) Subsequently the most active fraction of each stage was subjected to further chromatographic sub-fractionation to yield highly enriched cyclotides in fraction VT3.1.F and VT3.1.G (D).