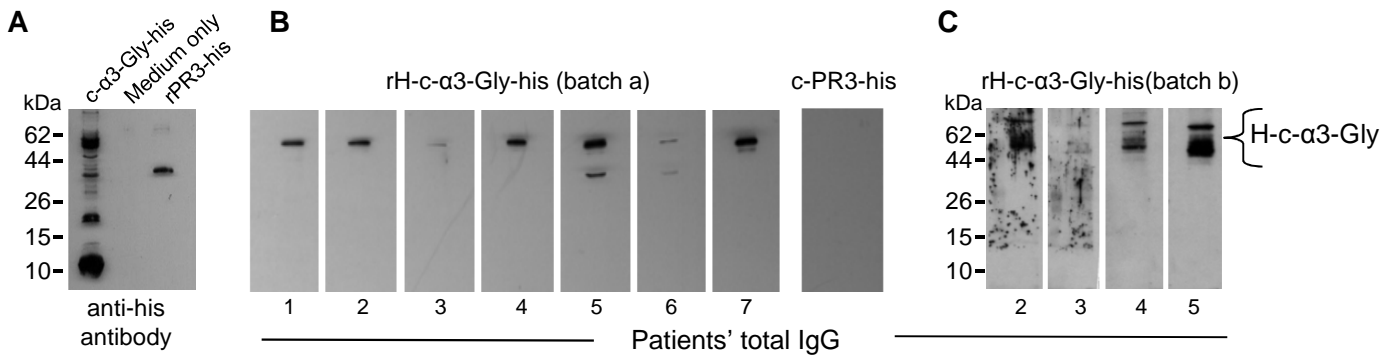


Appendix 1 Supplementary data



**Appendix 1.** Recombinant human-c- $\alpha$ 3-Gly-his protein (rH-c- $\alpha$ 3-Gly-his) is recognized by antibodies from patients with anti-GBM disease. **(A)** Representative immunoblot showing rH-c- $\alpha$ 3-Gly-his produced in HEK293-C18 cells. Precipitation of histidine-tagged proteins from cell culture medium, using nickel resin, followed by SDS-PAGE and immunoblotting with a rabbit-anti-hexahistidine antibody revealed multimeric forms of rH-c- $\alpha$ 3-Gly-his protein. Conditioned medium alone was negative. Recombinant proteinase-3-his (rPR3), which was also produced in HEK293 cells, revealed a single form indicating the multiple forms of rH-c- $\alpha$ 3-Gly-his were unlikely due to the his tag. **(B)** Anti-GBM patients' total IgG was reactive with rH-c- $\alpha$ 3-Gly-his of approx. 40 and 60 kD by western immunoblotting. Anti-GBM patient IgG did not react with recombinant PR3-his. **(C)** Using an alternate purification procedure (batch b), rH-c- $\alpha$ 3-Gly-his protein was present in multimeric forms. Reproducibly, the same patients' IgG recognized proteins of 40 and 60 kD molecular weight.