Supplemental Information

The Selection of Monoclonal Antibodies Against 6-oxo-M_1dG and Their Use in an LC-MS/MS Assay for the Presence of 6-oxo-M_1dG *in Vivo*

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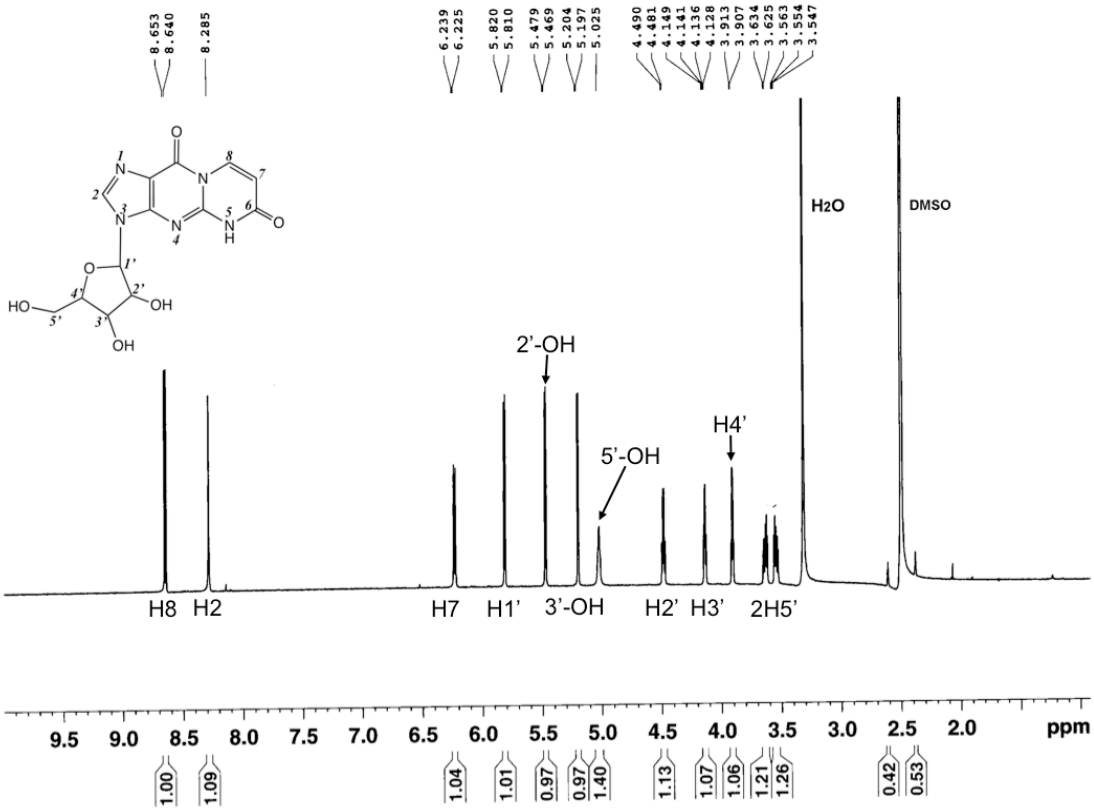
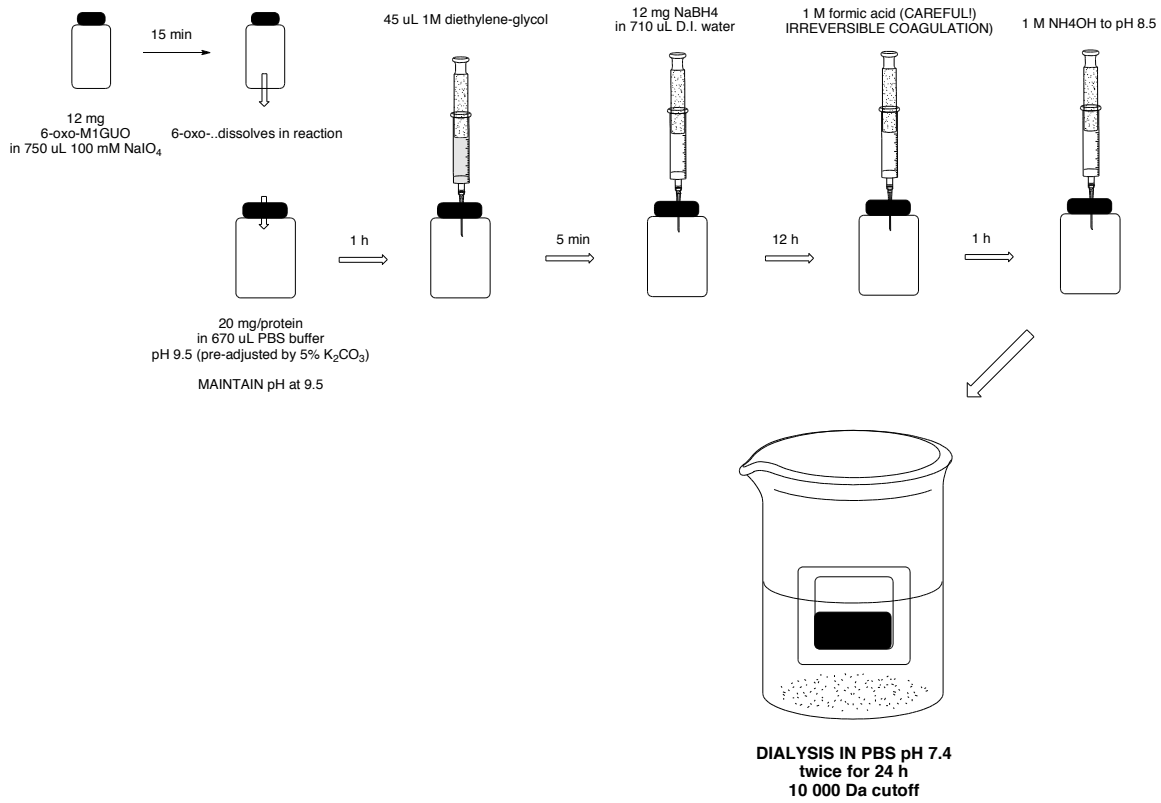
Supplemental Figure A. Proton NMR of 6-oxo-M_1Guo

6-oxo-M_1Guo was synthesized as described in Materials and Methods. The purified product was analyzed using proton NMR. The proton assignment is displayed in the structure of 6-oxo-M_1Guo.



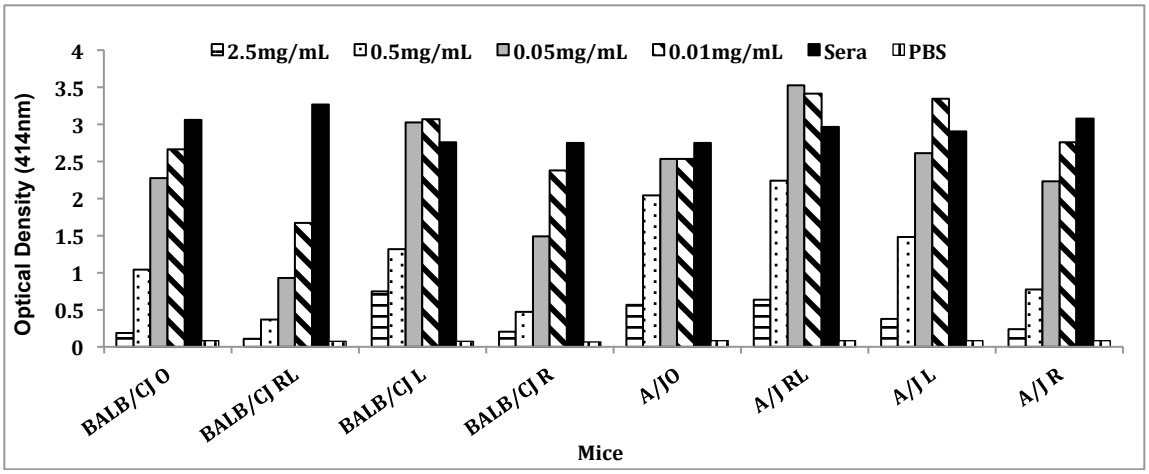
Supplemental Figure B. Scheme of the generation of the 6-oxo-M_1Guo/protein conjugate.

6-oxo-M_1Guo is conjugated to either BSA or KLH as described in the Materials and Methods section, resulting in a pure protein conjugate suitable for murine innoculation or use in ELISA analysis.



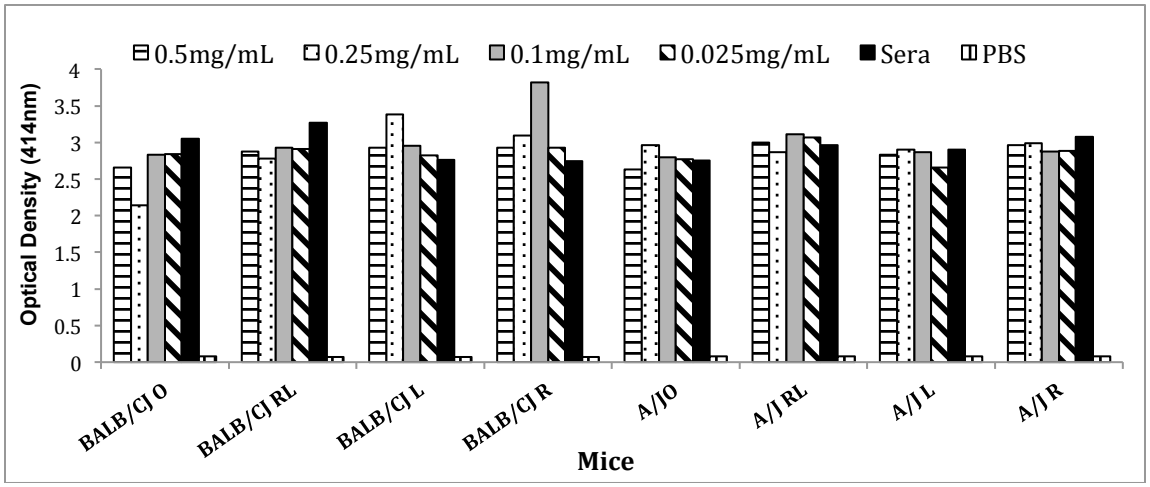
Supplemental Figure C. Sera Reactivity with 6-oxo-M_1dG

The reactivity of diluted sera (1:5000) in the presence of non-BSA bound 6-oxo-M_1dG was analyzed using competitive ELISA analysis. Optical density readings were taken 15 and 30 minutes after adding ABTS substrate and averaged. Sera and antigen were co-incubated with multiple concentrations of non-BSA bound 6-oxo-M_1dG as indicated for 60 minutes.



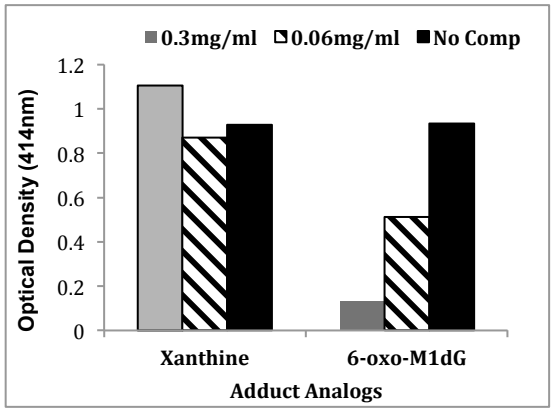
Supplemental Figure D. Lack of Reactivity of Sera with M_1dG

The reactivity of diluted sera (1:5000) with M_1dG was analyzed using competitive ELISA analysis. Optical density readings were taken 15 and 30 minutes after adding ABTS substrate and averaged. Sera and antigen were co-incubated with multiple concentrations of non-BSA bound M_1dG or sera as indicated for 60 minutes.



Supplemental Figure E. Specificity of Sera Antigens for 6-oxo-M_1dG

The reactivity of 6C9 hybridoma subclone (6C9BA4) supernatants were analyzed using competitive ELISA analysis. Xanthine and non-BSA bound 6-oxo-M1dG were examined and bars represent the level of antigen recognition as indicated by optical density readings.



Supplemental Figure F. Limit of Detection and Linearity of the LC-MS/MS system. The LC-MS/MS chromatogram (top) shows a 20 µL injection of a 0.50 nM solution of 6-oxo-M_1dG on the described LC-MS/MS system. This results in 10 fmol of 6-oxo-M_1dG on-column. The graph (bottom) is a plot of response (6-oxo-M_1dG peak area ÷ [$^{15}N_5$]-6-oxo-M_1dG peak area) versus 6-oxo-M_1dG concentration. The 6-oxo-M_1dG concentration ranged from 1.0 to 5,000 nM and [$^{15}N_5$]-6-oxo-M_1dG was present in all solutions at 10 nM.