SUPPLEMENTAL DATA

SPLICE-SPECIFIC ALTERATIONS OF GEPHYRIN IN GLYCINE RECEPTOR BINDING, FOLDING AND PHOSPHORYLATION

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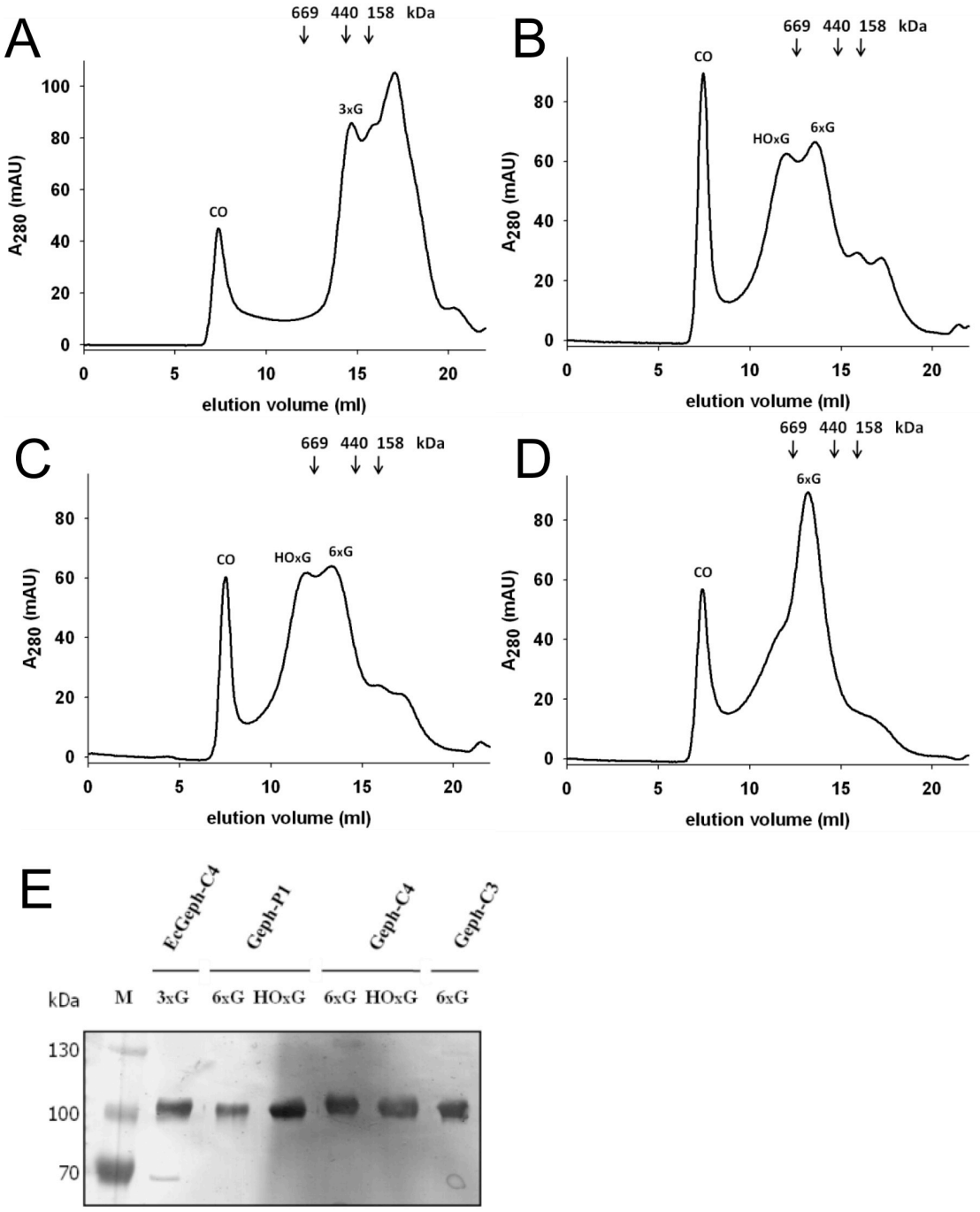
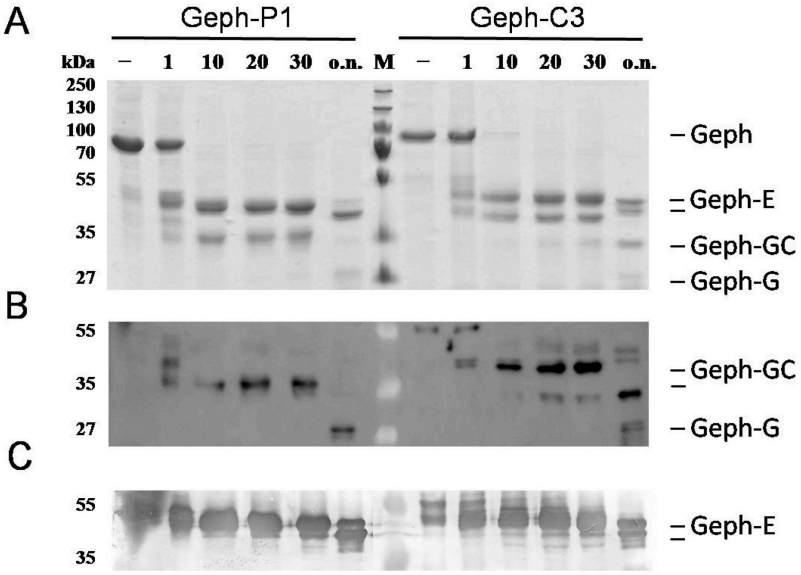
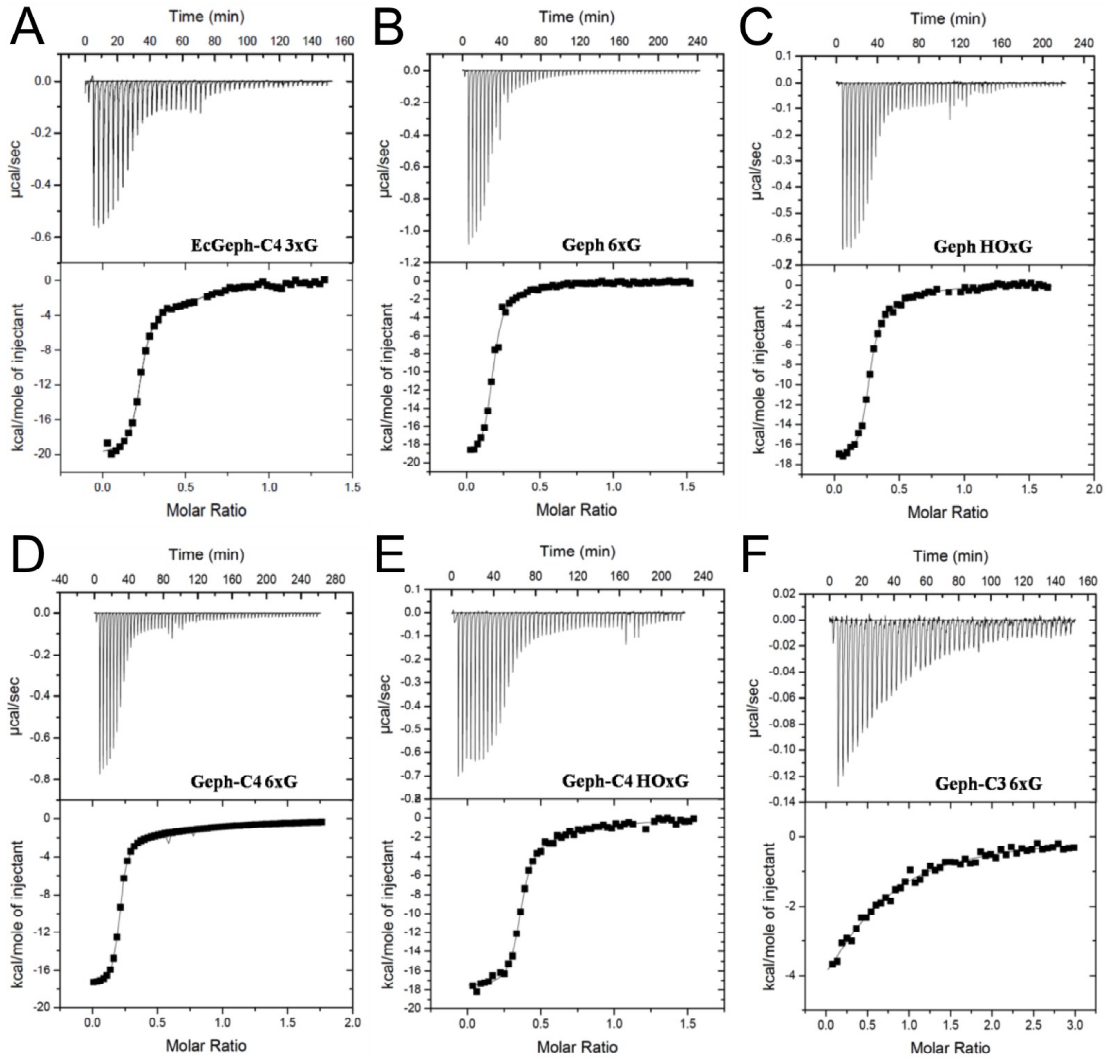
SUPPLEMENTAL FIGURES



Supplemental Fig. S1: Molecular mass determination of Ni-NTA purified gephyrin splice variants A-D, size exclusion chromatography of EcGeph-C4 derived from *E. coli* (A), Geph (B), Geph-C4 (C) and Geph-C3 (D) derived from Sf9 cells using a 20 ml Superose 6 column. Molecular masses of gephyrin oligomers were determined by comparison to standard proteins as described under Experimental Procedures. Elution volumes of standard proteins with their corresponding molecular masses are highlighted by arrows. Different oligomerization states are indicated by symbols: 3xG, trimer; 6xG, hexamer; HOxG, high-oligomer. Identification of gephyrin oligomers was confirmed by Western blot using the m3B11 antibody (E).
CO = cut-off peak



Supplemental Fig. S2: Partial proteolysis of Geph and Geph-C3. Sf9 cell-derived Geph (left) and Geph-C3 (right) were digested (6 µg each) with trypsin at the indicated gephyrin/protease molar ratio and separated by a 12% SDS-PAGE (A) together with equal amounts of untreated protein (–). Identification of gephyrin fragments was confirmed by Western blot using domain specific antibodies detecting the G domain (B; "puszta serum"), and E-domain (C; m3B11).



Supplemental Fig. S3: Determination of binding affinity between gephyrin splice variants and GlyR ß-loop using isothermal titration calorimetry (ITC). A-D, titration of purified trimeric EcGeph-C4 (A), hexameric Geph (B,) high-oligomeric Geph (C) hexameric Geph-C4 (D), high-oligomeric Geph-C4 (E) and hexameric Geph-C3 (F) with the GlyR ß-loop showing the heats of injection (top) and the binding isotherm (bottom) with the experimental data points (*black squares*) and determined curve fitting (*thin line*) as described in the Results section. All experiments were carried out under the same conditions. For data analysis, the heat release of the first injection in each experiment was omitted.