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30 **Supplemental Figure 4.** In the absence of IpaC, membrane-associated IpaB C-
 31 terminal cysteine substitution does not react to PEG5000-maleimide. Gel migration of
 32 PEG5000-maleimide labeled (IpaB-PEG) or unlabeled (IpaB) IpaB in plasma membrane
 33 enriched fractions. *S. flexneri* $\Delta ipaB$ or $\Delta ipaBC$ expressing wildtype (pWT) IpaB or the
 34 IpaB cysteine substitution derivative S454C (pIpaB S454C). Positive control, *S. flexneri*
 35 $\Delta ipaB$ expressing IpaB S454. IpaB S454C lies in the C-terminal extracellular domain
 36 and is accessible to PEG5000-maleimide labeling in membrane inserted translocons
 37 (Fig. 3). Caveolin-1, plasma membrane marker; GroEL, bacterial cytosolic protein.