



Supplemental Fig. S1. Photolabelling of CERT, StarD7 and StarD7^{R189Q} with pacCer in the presence of excess ceramide.

(A) The START domains of wild-type CERT or ceramide-binding deficient mutant CERT^{N504A} were mixed with maltose-binding protein (MBP) and incubated with liposomes containing 0.5 or 0.25 mol% of pacCer for 30 min at 37°C. Samples were UV-irradiated for the indicated time, click-reacted with Alexa Fluor647-N₃ and then analysed by in-gel fluorescence (IGF) and Coomassie staining (CB). (B) Quantitative analysis of photolabelling of CERT or CERT^{N504A} START domains by pacCer as in (A). Data shown are the means ± error range of two independent experiments. (C) CERT, StarD7 or PC-binding mutant StarD7^{R189Q} START domains were mixed with MBP and incubated with liposomes containing 0.5 mol% of pacCer and 0, 2.25, 5 or 20 mol% of natural C16:0-

ceramide (Cer) for 30 min at 37°C (pacCer: Cer = 1:0, 1:2.5, 1:10 or 1:40). Samples were UV-irradiated for 90 sec and then processed and analysed as in (A).

(D) Quantitative analysis of photolabelling of CERT, StarD7 and StarD7^{R189Q} START domains by pacCer as in (C). Data shown are the means \pm SD (CERT, StarD7) or error range (StarD7^{R189Q}) of the indicated number of independent experiments.