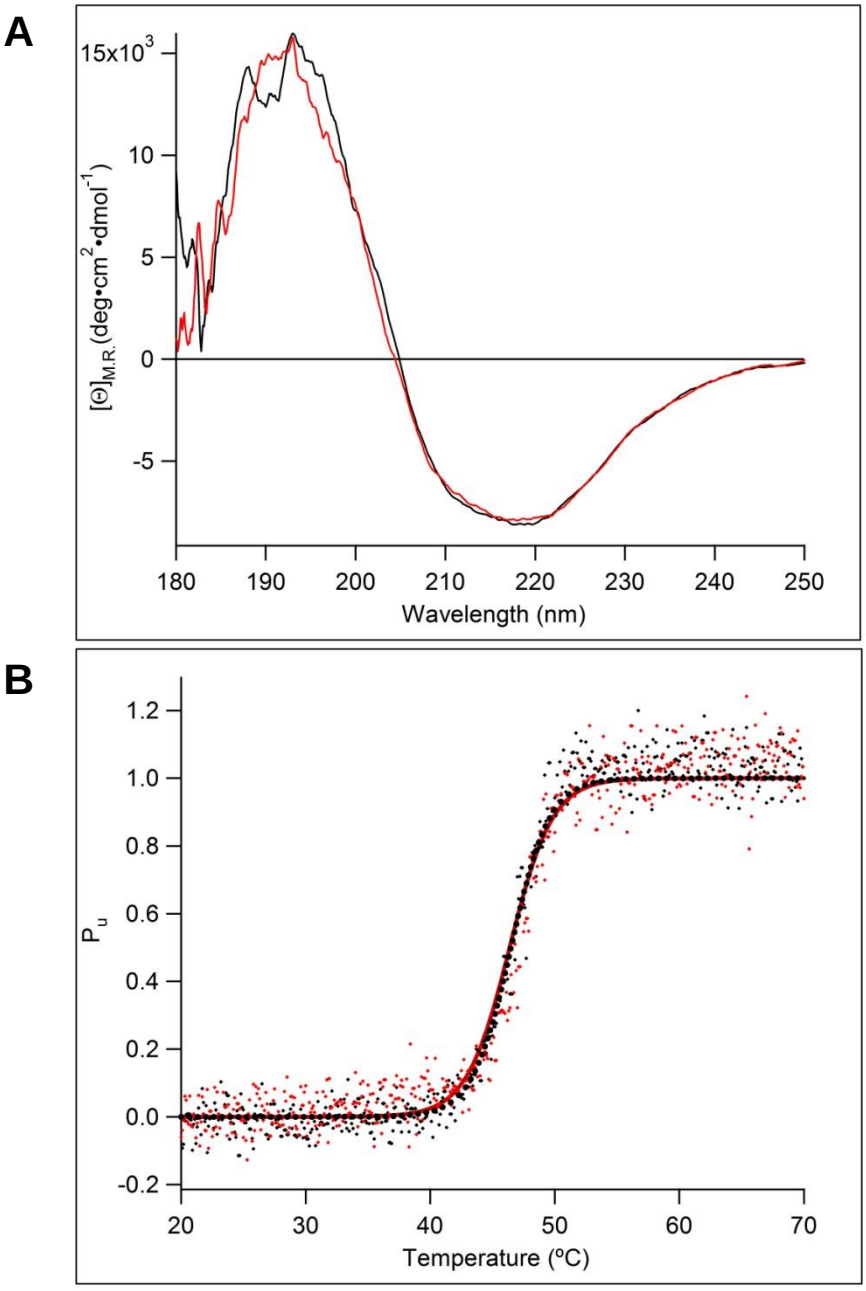

Figure S1: Far-UV CD spectrum and temperature denaturation monitored by CD of STARD5 in presence of 1:1 cholesterol. (a) Far-UV CD spectra of apo-STARD5 (black) and STARD5 with cholesterol at 1:1 molar ratio (red) **(b)** Temperature-dependence of the population of unfolded state (P_u); black: apo-STARD5 and red: STARD5 with cholesterol at 1:1 molar ratio. The concentration of STARD5 and cholesterol were 10 µM in sodium phosphate buffer 10 mM at pH 7.4. Cholesterol was added as described in Roostaee et al. (1)

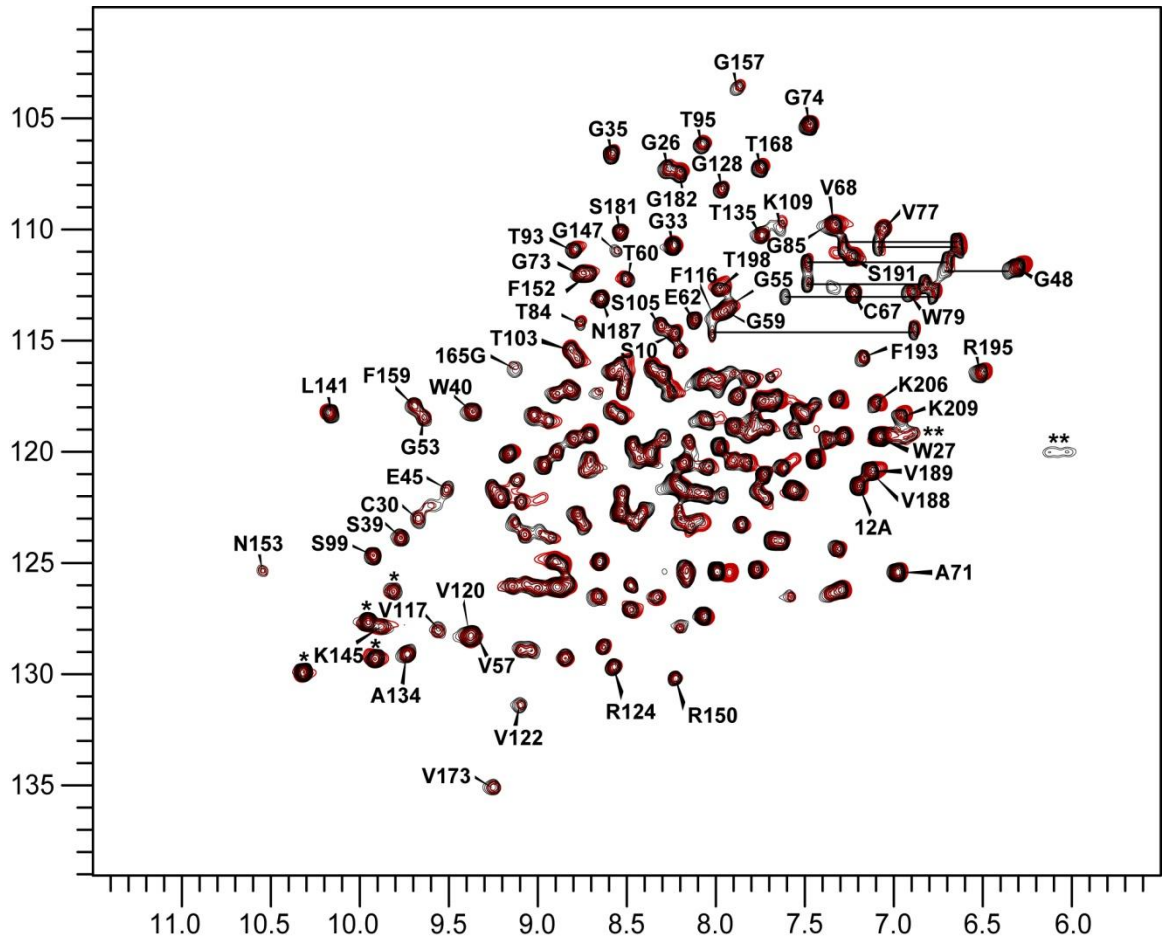

Figure S2: Binding assay of cholesterol to STARD5 by NMR. Overlain of ^{1}H-^{15}N HSQC spectra of STARD5 alone (black) and in the presence (red) of soluble cholesterol (in complex with methyl-β-cyclodextrin). Assignments of the cross-peaks are given with the residue number and one-letter code for amino acids. Horizontal lines connect the side-chain resonances of asparagines and glutamines. Tryptophan side-chain indole NH cross-peaks are marked with an asterisk (*). The double asterisks (**) denote protected guanidino (Hη) protons from arginine side-chains. STARD5 was preincubated with methyl-β-cyclodextrin (black), then soluble cholesterol (cholesterol complexed with methyl-β-cyclodextrin) (red) was used in a 2:1 ratio of cholesterol:StAR. The complex was left at room temperature for 1 hour to reach equilibrium before data acquisition. STARD5 alone (black) shows a good peak dispersion indicating a well-

folded protein. After the addition of cholesterol (red), there are no chemical shift displacements as observed for STARD1 (2).

REFERENCES

1. Roostaee, A., E. Barbar, J. G. Lehoux, and P. Lavigne. 2008. Cholesterol binding is a prerequisite for the activity of the steroidogenic acute regulatory protein (StAR). *Biochem J* **412**: 553-562.
2. Barbar, E., J. G. Lehoux, and P. Lavigne. 2009. Toward the NMR structure of StAR. *Mol Cell Endocrinol* **300**: 89-93.