Biochem. J. (2010) **430**, 39–48 (Printed in Great Britain) doi:10.1042/BJ20091758

online data

SUPPLEMENTARY ONLINE DATA

Calmodulin-dependent nuclear import of HMG-box family nuclear factors: importance of the role of SRY in sex reversal

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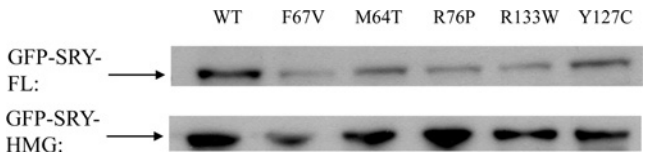

Figure S1 Immunoblot analysis of whole-cell lysates

COS-7 cells were transiently transfected to express WT and mutant GFP–SRY-FL and GFP–SRY-HMG, and whole-cell lysates were produced as described previously [1] 24 h p.t. Briefly, intact cells were harvested using 0.02 % EDTA, washed twice with PBS, and resuspended in cell lysis buffer [10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 0.5 mM EDTA, 0.5% Nonidet P40 and Complete™ EDTA-free protease inhibitor cocktail tablets] for 30 min and insoluble materials were removed by centrifugation at 20000 ***g*** at 4 °C for 15 min. Protein levels were assessed by Western blot analysis using anti-GFP antibodies; in all cases, proteins were expressed in intact form, and to comparable levels within the limits of transfection efficiency variability.

REFERENCE

1 Glover, D. J., Leyton, D. L., Moseley, G. W. and Jans, D. A. (2010) The efficiency of nuclear plasmid DNA delivery is a critical determinant of transgene expression at the single cell level. J. Gene Med. **12**, 77–85

Received 16 November 2010/24 May 2010; accepted 9 June 2010
Published as BJ Immediate Publication 9 June 2010, doi:10.1042/BJ20091758

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