## Cholesterol increases the E3 ligase MARCH6, controlling protein demolition

Laura J. Sharpe<sup>1,†</sup>, Vicky Howe<sup>1,†</sup>, Nicola A. Scott<sup>1</sup>, Winnie Luu<sup>1</sup>, Lisa Phan<sup>1</sup>, Jason M. Berk<sup>2</sup>, Mark Hochstrasser<sup>2</sup>, and Andrew J. Brown<sup>1,\*</sup>

## **Contents:**

Figure S1: Endogenous MARCH6 is stabilized by cholesterol

Figure S2: Genomic characterisation of CRISPR cell-lines

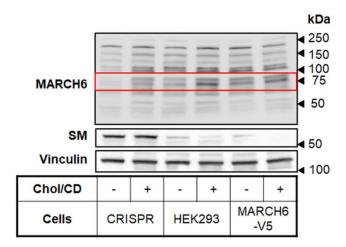
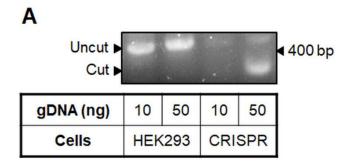


Figure S1: Endogenous MARCH6 is stabilized by cholesterol

HEK293-CRISPR, HEK293, or HEK293-MARCH6-V5 cells were treated for 4 h with or without 20  $\mu$ g/ml Chol/CD before harvesting. Protein levels of endogenous MARCH6, endogenous SM and vinculin were analyzed by Western blotting. The red box indicated the MARCH6 band.



В

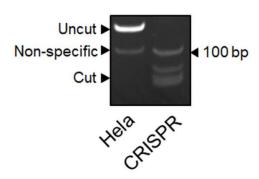


Figure S2: Genomic characterisation of CRISPR cell-lines

Genomic DNA from (**A**) HEK293 or HEK293-CRISPR cell-lines and (**B**) Hela or Hela-CRISPR cell-lines was subjected to PCR to confirm deletion of the targeted region. PCR products were separated by (**A**) agarose gel or (**B**) 6% acrylamide TBE gel electrophoresis and visualized with EtBr staining.