additional model whereby natural restriction factors may act by preventing capsid disassembly. In turn, compounds that cross-link capsid components, such as multi-functional reagents designed to target the central channel of the hexamer, might be expected to inhibit retroviral replication. □

Methods

Protein production

The DNA sequence coding for the NTD of N-MLV (capsid residues Pro 1 to Ser 132) was amplified by polymerase chain reaction (PCR) using a plasmid containing the proviral DNA[26] as a template. The PCR product was inserted into a pET22b expression vector (Novagen) between the *Nde*I and *Xho*I restriction sites in order to produce a C-terminal hexa-histidine fusion and the sequence Met-Pro at the N terminus. The protein was expressed in the *Escherichia coli* strain BL21 (DE3) and purified using ion exchange, immobilized metal ion affinity and gel filtration chromatography. Verification of the processing of the N-terminal methionine to produce the mature form of the protein was analysed by electrospray ionization mass spectrometry (ESI-MS). The double substitution mutant L4M L126M was prepared using the Quickchange site-directed mutagenesis kit (Stratagene). Seleno-methionine-substituted protein was prepared by expressing the protein in the *E. coli* methionine auxotroph B834 (DE3) grown on seleno-methionine-substituted media.

Crystallization and structure solution

Proteins were crystallized using the vapour batch method. A 16–22 mg ml^{-1} solution of N-MLV(NTD) or N-MLV(NTD/L4M L126M) in 150 mM NaCl, 20 mM Tris-HCl pH 8.0 was mixed with an equal volume of crystallization solution containing 13–16% PEG 3350, 100 mM sodium citrate pH 5.6. Two-microlitre droplets were dispensed into 96-well vapour batch plates (Douglas instruments) covered with 6 ml of 'Al's oil' (Hampton Research) using an IMPAX 1-5 robot (Douglas instruments). A solution of 10% (v/v) aqueous isopropanol was placed in the side wells of the tray and the drops equilibrated overnight. Next day, the 10% isopropanol solution was replaced with a 20% (v/v) isopropanol solution and crystals typically grew to $0.2 \times 0.2 \times 0.1$ mm^3 in one to two weeks. Crystals were collected by transfer into fresh crystallization solution supplemented with 10% (v/v) isopropanol and 15% (v/v) 1,2-propanediol as a cryoprotectant then flash-frozen in liquid nitrogen. The crystals belong to the space group $P2_1$ ($a = 86.0$ Å, $b = 78.3$ Å, $c = 85.76$ Å, $\beta = 118.9°$) with six capsid NTDs in the asymmetric unit. The structure was solved by a three-wavelength MAD experiment using seleno-methionine-containing crystals of the double-methionine-substituted N-MLV(NTD/L4M L126M) on Station 14.2 at the Synchrotron Radiation Source (SRS), Daresbury, UK. All data sets were reduced using the HKL suite of processing software. Twelve selenium atoms were located and the phases refined using the program SOLVE[28], resulting in an overall figure of merit (FoM) of 0.61. The local six-fold operators were identified using information from a self-rotation function and the six pairs of Se sites, and were used for averaging and density modification in DM[29]. The quality of this initial map was good enough to build a polyalanine model into a single monomer using the program O. The hexamer was generated by application of the non-crystallographic symmetry operators to the monomer and the fit improved using real-space rigid-body refinement in the program O. Further refinement against a high-resolution data set collected on the SeMet crystal using REFMAC and ARPP[30] resulted in a map into which an essentially complete model was built. The data collection, phasing and refinement statistics are reported in Table 1.

Received 7 July; accepted 5 August 2004; doi:10.1038/nature02915.

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Acknowledgements We thank S. Gamblin for assistance in crystal handling.

Competing interests statement The authors declare that they have no competing financial interests.

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corrigendum

Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity

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Nature **431,** 200–205 (2004).

In Fig. 4e of this Letter, the arrowhead from TSC to Rheb should be a horizontal bar (as from PKB to TSC). In addition, the phosphorylation sites of IRS1 for human should be S312 and S636/639 and for mouse the corresponding phosphorylation sites should be IRS1 S307 and S632/S635. This does not affect any of the results or conclusions of the paper. □