Phosphatase Activity of Small C-terminal domain phosphatase 1 (SCP1) controls the stability of the key neuronal regulator RE1-silencing transcription factor (REST)

Nathaniel Tate Burkholder[1, #], Joshua E. Mayfield[1,2,#], Xiaohua Yu[3, #], Seema Irani[4], Daniel K. Arce[1], Faqin Jiang[5], Wendy Matthews[1], Yuanchao Xue[3], Yan Jessie Zhang[1,6 *]

SUPPORTING INFORMATION

Table S1. Synthetic phosphorylated REST peptide sequences used in malachite green kinetics and X-ray crystallography. Ac represents N-terminal acetyl groups of peptides from synthesis.

Peptide	Sequence (REST 858-869)
REST-pS861/4	Ac-EDLpSPPpSPPLPK-NH_2
REST-pS861	Ac-EDLpSPPSPPLPK-NH_2
REST-pS864	Ac-EDLSPPpSPPLPK-NH_2

Table S2. Sequences of shRNA for knockdown of SCP1 and REST. The underlined sequences in the sense strands correspond with the target gene sequence and are homologous for shRNA hairpin formation.

shRNA	Sequence	TRC Number
shSCP1-1 Sense	CCGGCCTGCCTCCTATGTCTTCCATCTCGAGATGGAAGACATAGGAGGCAGGTTTTTG	TRCN0000343645
shSCP1-1 Antisense	AATTCAAAAACCTGCCTCCTATGTCTTCCATCTCGAGATGGAAGACATAGGAGGCAGG	
shSCP1-2 Sense	CCGGCAGCTCCTTCAAGCCAGTGAACTCGAGTTCACTGGCTTGAAGGAGCTGTTTTTG	TRCN0000343583
shSCP1-2 Antisense	AATTCAAAAACAGCTCCTTCAAGCCAGTGAACTCGAGTTCACTGGCTTGAAGGAGCTG	
shREST-1 Sense	CCGGCTAATCGATATGATCACTATACTCGAGTATAGTGATCATATCGATTAGTTTTTTG	TRCN0000436671
shREST-1 Antisense	CAAAAAACTAATCGATATGATCACTATACTCGAGTATAGTGATCATATCGATTAGCCGG	
shREST-2 Sense	CCGGGCATCCTACTTGTCCTAATAACTCGAGTTATTAGGACAAGTAGGATGCTTTTTG	TRCN0000425937
shREST-2 Antisense	CAAAAAAGCATCCTACTTGTCCTAATAACTCGAGTTATTAGGACAAGTAGGATGCCCGG	
shREST-3 Sense	CCGGTCAACGAATCTACCCATATTTCTCGAGAAATATGGGTAGATTCGTTGATTTTTTG	TRCN0000432087
shREST-3 Antisense	CAAAAAATCAACGAATCTACCCATATTTCTCGAGAAATATGGGTAGATTCGTTGACCGG	

Table S3. Quantitative Real-Time PCR primer sequences.

qRT-PCR Primer	Sequence
SCP1-F	CTGCCTCCTATGTCTTCCATCC
SCP1-R	ACGGCTGAGTTGCTCGAAGAAG
REST-F	CTTTGTCCTTACTCAAGTTCTCAG
REST-R	ACCTGTCTTGCATGGCGGGTTA
TUBB3-F	TCAGCGTCTACTACAACGAGGC
TUBB3-R	GCCTGAAGAGATGTCCAAAGGC
BDNF-F	CATCCGAGGACAAGGTGGCTTG
BDNF-R	GCCGAACTTTCTGGTCCTCATC
BEX-F	GCATAGGCTTGGAGAACCACAG
BEX-R	CCGCAGACTATGACTCAACTGC
USP37-F	TCTCTATTGACCTTCCTCGTAGG
USP37-R	TGCCTGACAAGAGCACACTTCC
DYRK1A-F	CCTTGCCATTGATATGTGGTCCC
DYRK1A-R	GCAGGTGGAATACCCAGAACTTC
UCHL1-F	CAGTTCAGAGGACACCCTGCTG
UCHL1-R	CCACAGAGCATTAGGCTGCCTT
ACTB-F	CACCATTGGCAATGAGCGGTTC
ACTB-R	AGGTCTTTGCGGATGTCCACGT

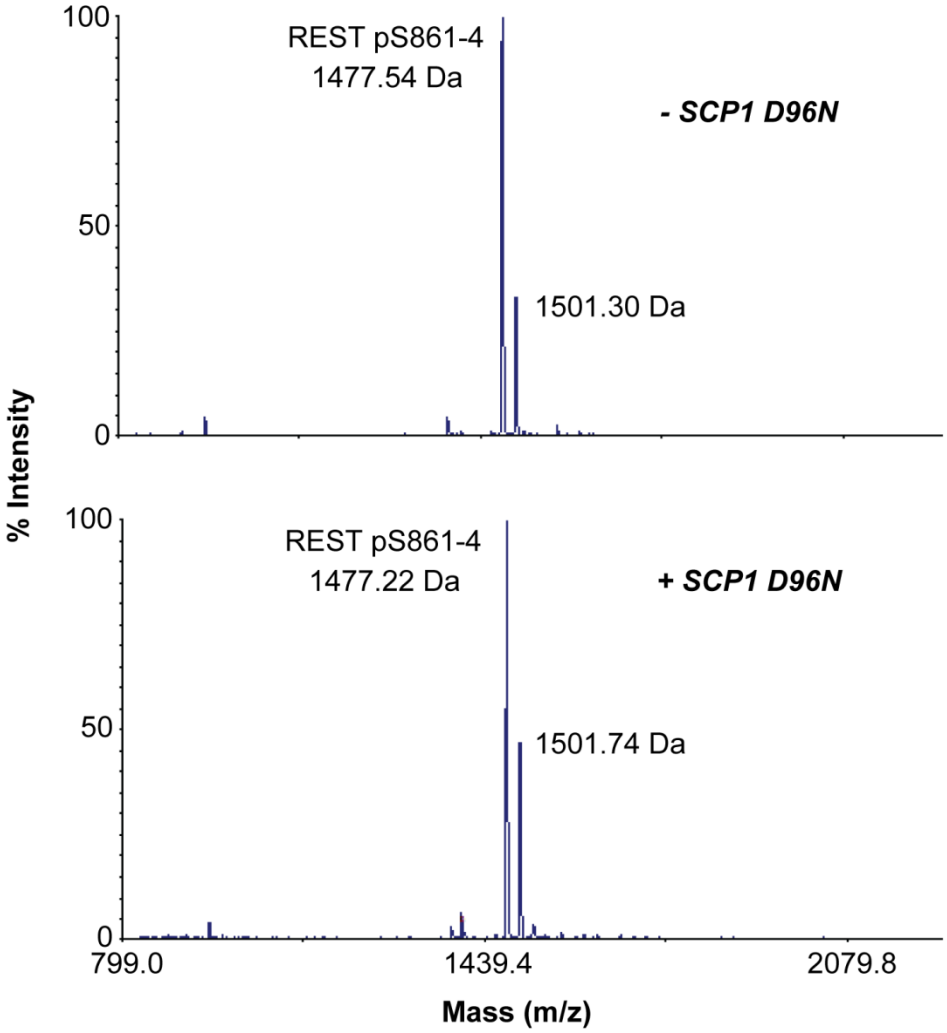

Figure S1. MALDI-TOF of REST pS861-4 peptide treated with (+) or without (-) SCP1 D96N. The predicted molecular weight of the pS861-4 peptide is 1477.5 Da.

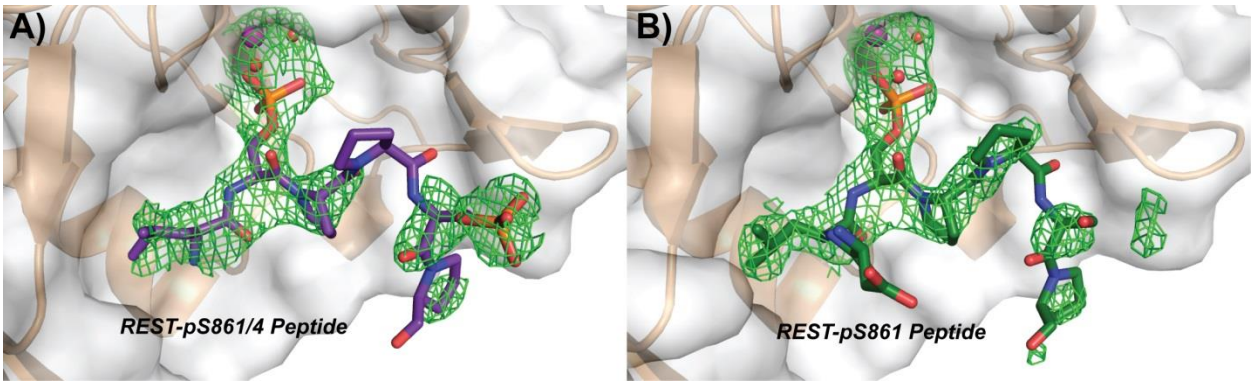

Figure S2. Polder omit maps of REST peptides complexed with SCP1 (phenix.polder). A. Structure of SCP1 (sand) bound to the doubly phosphorylated pS861/4 REST peptide (purple) with polder omit map around the ligand, magnesium (magenta), and coordinated waters (red). The omit map representing positive electron density was contoured to 3σ and illustrated as a light green mesh around the REST peptide. The Van der Waals exterior of SCP1 is represented as a transparent grey surface. B. Structure of SCP1 bound to the pS861 REST peptide (green) with polder omit map around the ligand, magnesium, and coordinated waters.

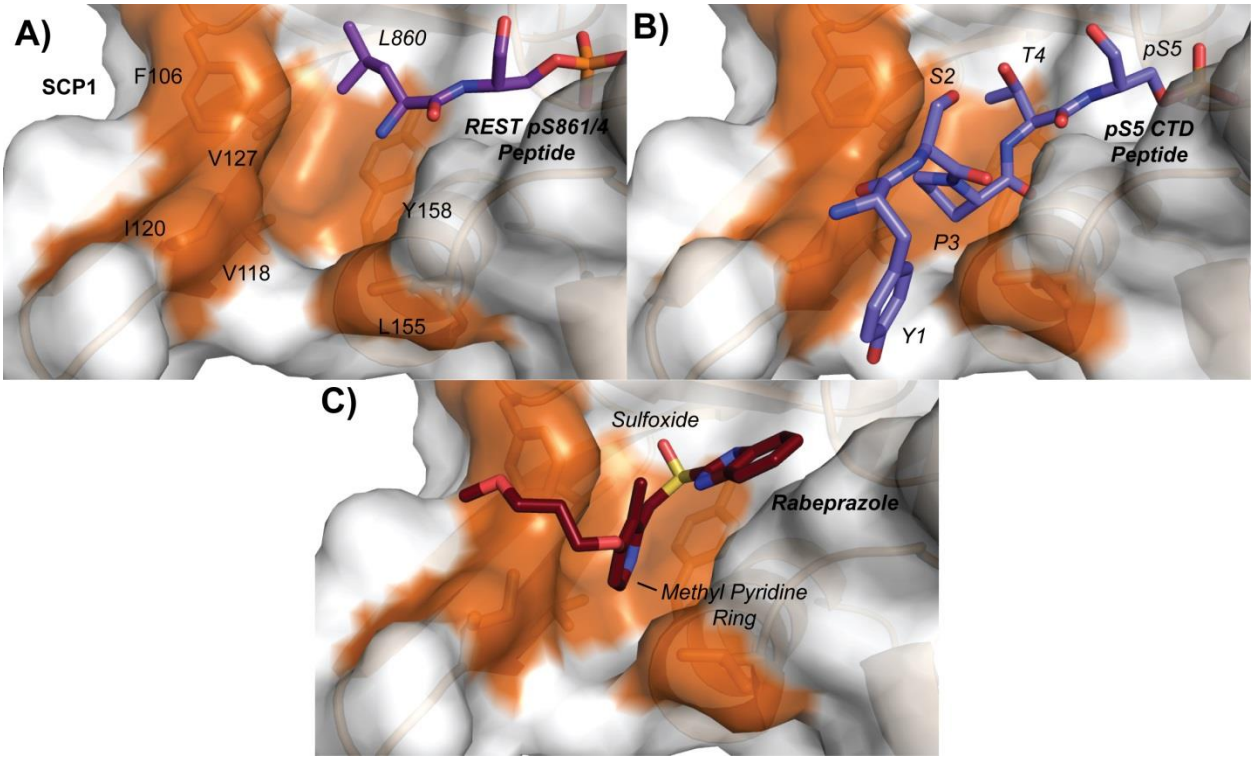

Figure S3. Comparison of SCP1 hydrophobic pocket binding to different molecules. A. Binding of the doubly phosphorylated pS861/4 REST peptide (purple) to the SCP1 hydrophobic pocket. Residues to the C-terminus of the phosphorylated S861 site were hidden for clarity. Ligand residues are listed in italics. The Van der Waals exterior of SCP1 is represented as a transparent grey surface with orange surfaces indicating hydrophobic patches. B. Binding of the pS5 RNA polymerase II CTD peptide (slate) to the SCP1 hydrophobic pocket (PDB:2GHT). Residues to the C-terminus of the phosphorylated S5 site and to the N-terminus of Y1 were hidden for clarity. C. Binding of the small molecule inhibitor rabeprazole (crimson) to the SCP1 hydrophobic pocket (PDB:3PGL).

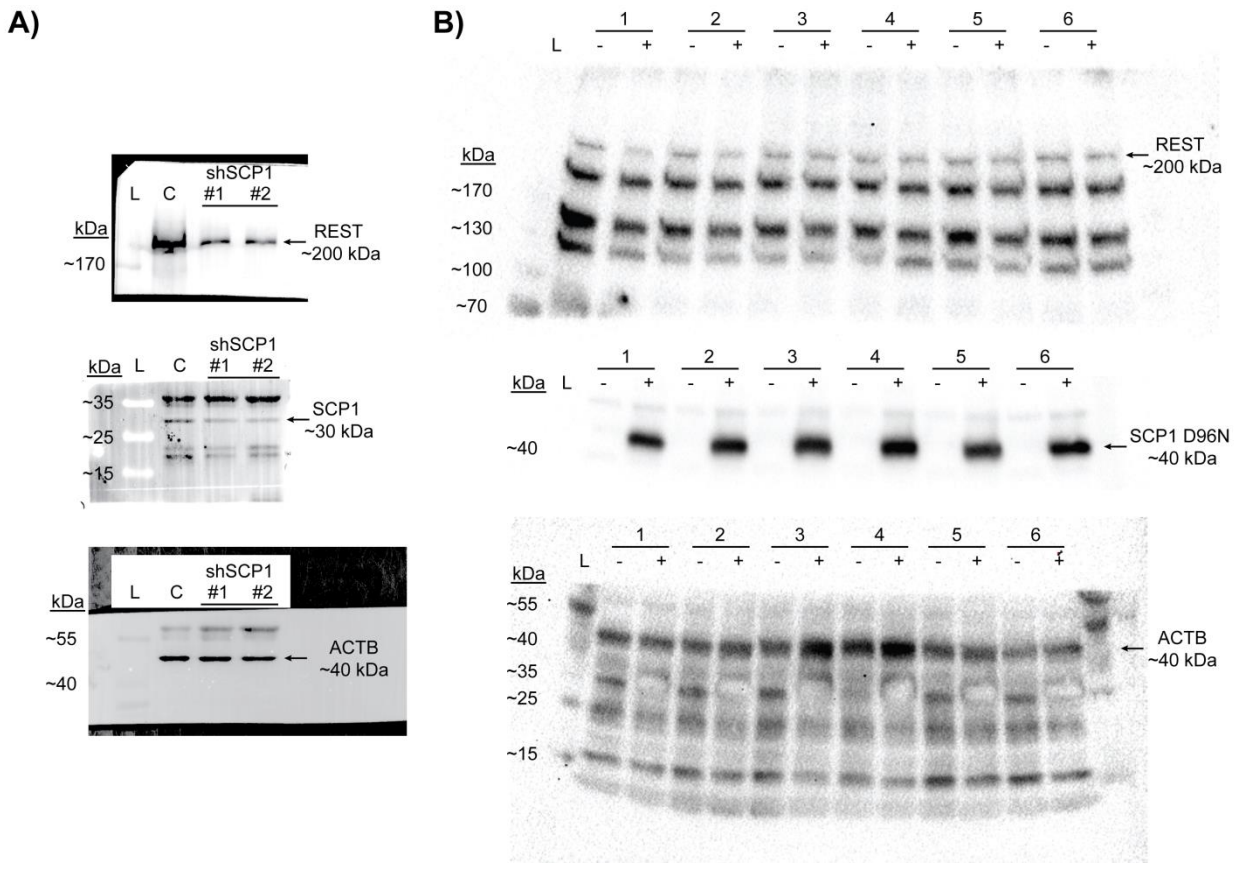

Figure S4. Raw western blots for shSCP1 knockdown and SCP1 D96N overexpression. A) Representative blots for shSCP1 transfected cells. Blot lanes were labeled L for PageRuler Prestained Protein Ladder (Thermo), C for control samples transfected with hygromycin pLKO.1 vector, and 1-2 for each shSCP1 construct tested. Ladder molecular weights and expected REST, SCP1, and ACTB sizes were labeled to the sides of the blots. The blots were cut before probing for REST, SCP1, and ACTB. B) Blots for SCP1 D96N transfected cells. Blot lanes were labeled L for PageRuler Prestained Protein Ladder (Thermo), "-" for empty pcDNA3 plasmid controls, "+" for SCP1 D96N transfected samples, and 1-6 for each set of biological replicates. Ladder molecular weights and expected REST, SCP1 D96N, and ACTB sizes were labeled to the sides of the blots. The blots were first cut in half before probing for REST and ACTB, whereas the bottom part of the blot was later destained in a low pH buffer (0.1 M glycine, 50 mM KCl, 20 mM $MgCl_2$, pH 2.2) and re-probed for SCP1 D96N (the ladder bands faded after destaining).

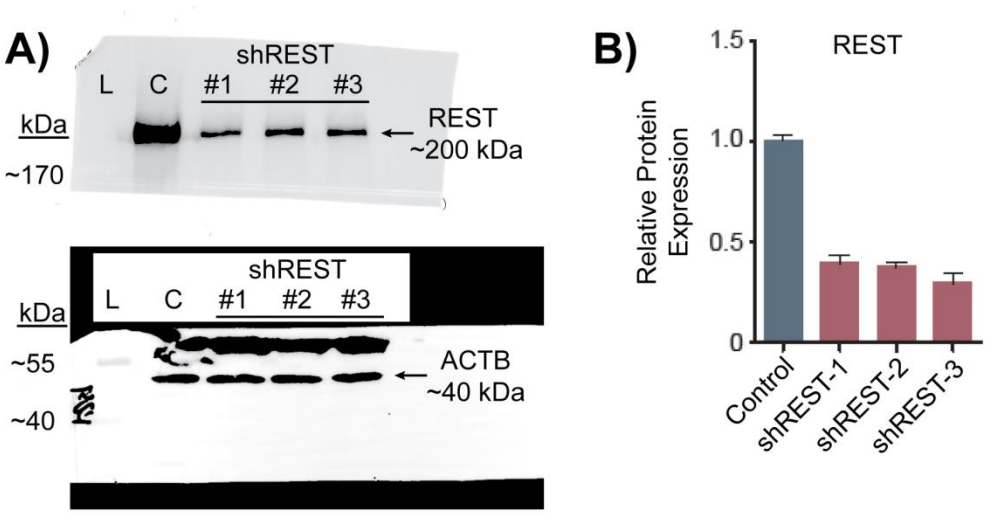

Figure S5. Western blot analysis of REST protein levels in cells transfected with shREST vectors. A) Representative raw western blots for REST knockdown in HEK293 cells. Blot lanes were labeled L for PageRuler Prestained Protein Ladder (Thermo), C for control samples transfected with empty pLKO.1 vector, and 1-3 for each shREST construct tested. Ladder molecular weights and expected REST and ACTB sizes were labeled to the sides of the blots. The blots were cut before probing for REST and ACTB. B) Analysis of REST protein levels in REST knockdown samples using ACTB for normalization. Protein samples from cell extracts were run in biological triplicate and compared to pLKO.1 hygromycin vector transfected controls.

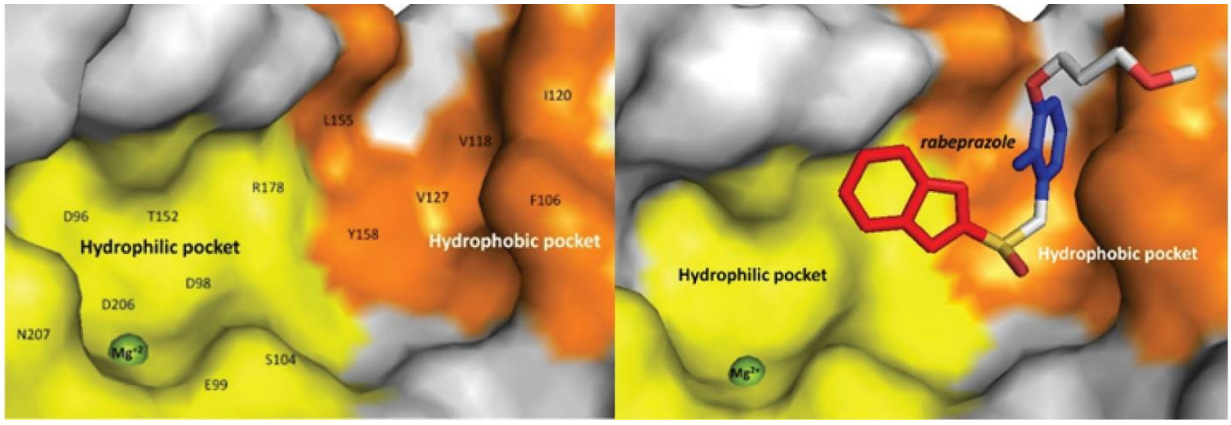

Figure S6. SCP1 binding pocket. A. SCP1 binding pocket in complex with Mg^{2+} (green). The surface and residue composition of the hydrophilic pocket (yellow) and hydrophobic pocket (orange) have been illustrated. B. SCP1 hydrophobic pocket binding to the small molecule inhibitor rabeprazole.