

Supporting Information

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SI Materials and Methods

Cell Culture. Cell culturing, Western blots, reverse transcription, and ChIP assays were performed as previously described (1). Antibodies used were 8WG16 α -CTD (Covance), N20 α -RNAP II largest subunit (Santa Cruz Biotechnology), N-12 α -TBP (Santa Cruz Biotechnology), BL1355 α -TLS (Bethyl Laboratories), and α -actin (Sigma). For knockdown experiments, siRNA targeting either TLS (Dharmacon D-009497-06) or luciferase (Dharmacon D-001219-02) as a negative control was used. For overexpression experiments, the TLS gene was cloned into the p3X-FLAG-CMV-14 vector (Sigma), or empty vector expressing Flag alone was used.

PCR. Radioactive PCR amplifications contained 0.5 units of Taq polymerase (Invitrogen), 1.5 mM MgCl₂, 0.2 mM each dNTP, 10% (vol/vol) DMSO, α -³²P dCTP, and primers corresponding to the indicated genes for 25 or 30 cycles. Reactions were separated on 8.3 M urea 10% (weight/vol) acrylamide gels and analyzed using a Storm 860 PhosphorImager (Molecular Dynamics).

EMSA. Single-stranded DNA probes were end labeled with γ -[³²P] ATP and polynucleotide kinase, then gel purified and eluted overnight using 0.5 M NaCl and 0.1% SDS at 4 °C. After ethanol precipitation, radiolabeled ssDNA (5 ng per reaction) was incubated with 0–0.1 μ g protein in 20- μ L reactions containing 10 mM

Hepes (pH 7.9), 100 mM KCl, 10% (vol/vol) glycerol, 1 mM DTT, and 50 ng sonicated salmon sperm DNA for 10 min at 4 °C. Samples were loaded on a prerun native 5% (weight/vol) acrylamide gel containing 2.5% (vol/vol) glycerol and run at 200 V for 120 min at 4 °C in 0.5 \times Tris-glycine buffer (25 mM Tris, 190 mM glycine, 1 μ M EDTA). Gels were dried then analyzed using a Storm 860 PhosphorImager and ImageQuant software (Molecular Dynamics).

Protein Purification. GST-TLS was expressed in Rosetta BL21 cells. Cells were grown in 400 mL LB media containing 100 μ g/mL ampicillin at 37 °C, with shaking until OD₆₀₀ = 0.4, then induced with isopropyl β -D-1-thiogalactopyranoside (0.5 mM final concentration) and grown for another 16 h at 16 °C with shaking. Cells were harvested, then resuspended and sonicated in 20 mM Tris (pH 8.0), 1 mM EDTA, and 100 mM NaCl (NET buffer) before addition of Nonidet P-40 0.5% (final concentration). After centrifugation at 6,800 \times g for 20 min at 4 °C, the supernatant was passed through a 0.8- μ m filter and bound to prepared glutathione Sepharose beads (Amersham) for 4 h at 4 °C. After extensive washing with NET + 0.5% Nonidet P-40 (NETN), protein was eluted in NETN + 50 mM reduced glutathione, and subsequently dialyzed twice against 40 vol of buffer D [20 mM Hepes (pH 7.9), 20% (vol/vol) glycerol, 0.2 mM EDTA, 0.5 mM DTT, 100 mM KCl] at 4 °C, for at least 3 h each time.

1. Tan AY, Manley JL (2010) TLS inhibits RNA polymerase III transcription. *Mol Cell Biol* 30: 186–196.

Chromosome	Accession	Description
chr1	BC031662.2 GI:34783269	CHIA-like pseudogene, mRNA
chr1	NM_138796.2 GI:37059739	spermatogenesis associated 17 (SPATA17)
chr1	NM_023015.3 GI:39995083	integrator complex subunit 3 (INTS3)
chr2	NM_017991.4 GI:169234785	KIAA1310
chr2	NM_001077269.1 GI:116284397	WAS/WASL interacting protein family, member 1 (WIPF1)
chr2	AK055967.1 GI:16550828	mRNA AK055967
chr2	NM_001083615.1 GI:134288914	myosin IIIB (MYO3B), transcript variant 1
chr3	NM_018098.4	epithelial cell transforming sequence 2 oncogene (ECT2)
chr3	NM_138345.1	von Willebrand factor A domain containing 5B2 (VWA5B2)
chr3	NM_002343.2 GI:54607119	lactotransferrin (LTF)
chr4	NM_001203.2 GI:169790809	bone morphogenetic protein receptor, type IB (BMPRI1B)
chr4	NM_001042690.1 GI:111548659	chromosome 4 open reading frame 44 (C4orf44)
chr5	AK096220.1 GI:21755655	mRNA from NT2 neuron after the differentiation of NT2 neuronal precursor cells
chr5	NM_014899.3 GI:115430107	Rho-related BTB domain containing 3 (RHOBTB3)
chr5	NM_018912.2 GI:14196453	protocadherin gamma subfamily A, 1 (PCDHGA1)
chr5	NM_018047.1 GI:8922327	RNA binding motif protein 22 (RBM22)
chr6	NM_030752.2 GI:57863256	t-complex 1 (TCP1), molecular chaperone for folding actin, tubulin, and others
chr6	NM_001003690.1 GI:51243062	MAD2 mitotic arrest deficient-like 1 binding protein (MAD2L1BP)
chr6	NM_001135650.1	eukaryotic translation elongation factor 1 epsilon 1 (EEF1E1)
chr6	NM_005074.3 GI:154800442	solute carrier family 17 (sodium phosphate) member 1 (SLC17A1)
chr6	NM_000595.2 GI:6806892	lymphotoxin alpha (TNF superfamily, member 1) (LTA)
chr6	NM_032744.2 GI:141802076	chromosome 6 open reading frame 105 (C6orf105)
chr7	NM_018059.4 GI:148612824	Rap GTPase interactor (RADIL), mediates migration of neural crest precursors
chr8	NM_003909.3 GI:197245388	copine III (CPNE3), Calcium-dependent membrane-binding protein
chr9	BC140739.1 GI:223462791	olfactory receptor, family 13, subfamily F (OR13F1)
chr10	NM_145202.3 GI:48976058	proline-rich acidic protein 1 (PRAP1)
chr10	NM_001098175.1 GI:147905699	ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1)
chr10	BC039000.1 GI:25058520	cyclin Y-like 2 (CCNYL2)
chr11	NM_013299.3 GI:118918404	SAC3 domain containing 1 (SAC3D1)
chr11	NM_018095.3 GI:32189315	kelch repeat and BTB domain containing 4 (KBTBD4)
chr11	NM_004788.2 GI:38327028	ubiquitination factor E4A homolog (UBE4A)
chr12	NM_001093.3	acetyl-Coenzyme A carboxylase beta (ACACB)
chr12	NM_024078.1 GI:13129067	nucleolar complex associated 4 homolog (NOC4L)
chr12	AK122911.1 GI:34528356	highly similar to calcium channel beta-3 subunit (CACNB3)
chr13	NM_001101320.1	nexin-related serine protease inhibitor (SERPINE3)
chr17	NM_001164405.1	basic helix-loop-helix family member a9 (BHLHA9)
chr17	NM_178170.2 GI:31342341	Never In Mitosis gene A- related kinase 8 (NEK8)
chr17	NM_004583.2 GI:41393544	RAB5C, member RAS oncogene family (RAB5C)
chr18	NM_015295.2 GI:148839304	structural maintenance of chromosomes flexible hinge domain containing 1 (SMCHD1)
chr18	NM_032347.1 GI:14150142	zinc finger protein 397 (ZNF397)
chr19	NM_134444.4 GI:194018481	NLR family, pyrin domain containing 4 (NLRP4)
chr19	BC068066.1 GI:45751650	zinc finger protein 85 (ZNF85)
chr20	NM_001033087.1 GI:74325775	MACRO domain containing 2 (MACROD2)
chr21	NM_015565.1 GI:31657110	zinc finger protein 294 (ZNF294)
chr21	NM_006272.2 GI:114520588	S100 calcium binding protein B (S100B)
chrX	NM_145305.1 GI:21687150	solute carrier family 25, member 43 (SLC25A43)
chrX	NM_181532.2 GI:31581555	ES cell expressed Ras (ERAS)
chrX	NM_001110792.1 GI:160707949	methyl CpG binding protein 2 (MECP2)

Fig. S1. Table of chromosome location, accession number, and description for the 48 promoters enriched for TLS occupancy with a cutoff of $P < 0.01$.

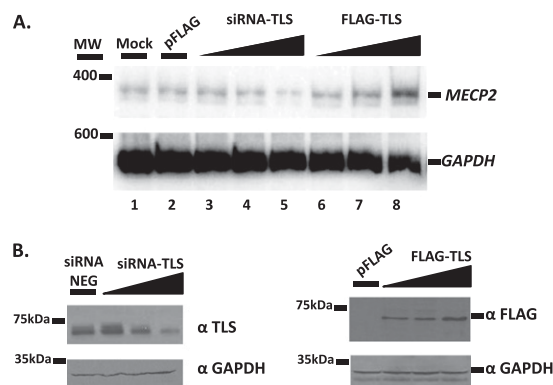


Fig. S2. TLS regulates the expression of MECP2. (A) U87 glioblastoma cells were transfected with mock (transfection medium alone, lane 1), empty vector (pFLAG, lane 2), increasing amounts of siRNA targeting TLS (lanes 3–5), or increasing pFLAG-TLS (lanes 6–8) for 48 h. RNA was extracted and subjected to oligo (dT)-mediated radioactive PCR. (B) TLS and GAPDH protein levels were analyzed by Western blot.

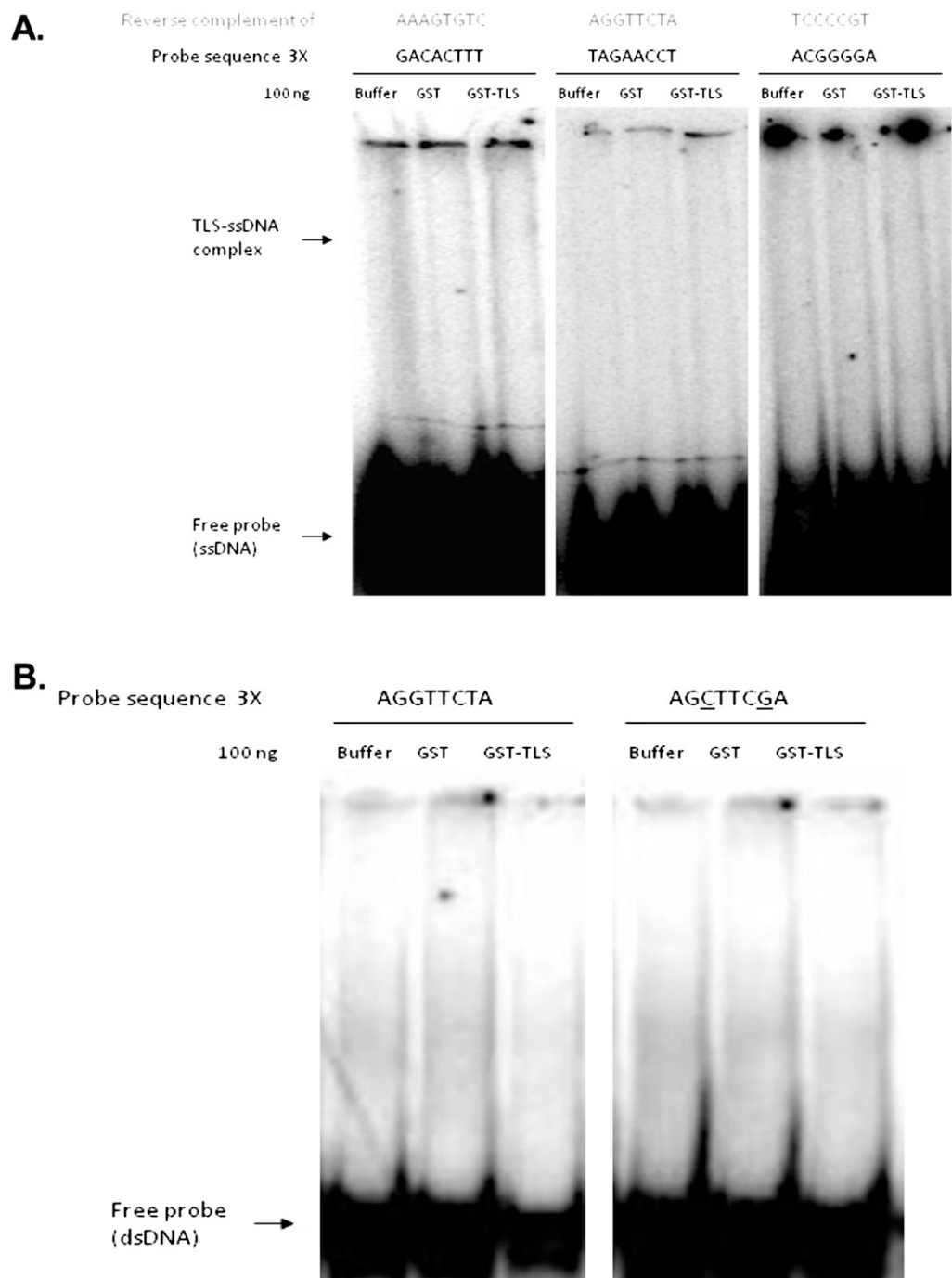


Fig. S3. (A) GST or GST-TLS was added to ssDNA containing three copies of the reverse complement of the consensus motifs. (B) GST or GST-TLS was added to dsDNA containing three copies of AGGTTCTA or AGCTTCGA.

