Supplemental Information

Yang et al.: SOX4-mediated repression of specific tRNAs inhibits proliferation of human glioblastoma cells

Supplemental Information: Supplemental Methods

Supplemental Information: Supplemental Figures S1 to S10

Supplemental Information: Supplemental Datasets S1 to S3

www.pnas.org/cgi/doi/10.1073/pnas.1920200117

Supplemental Methods

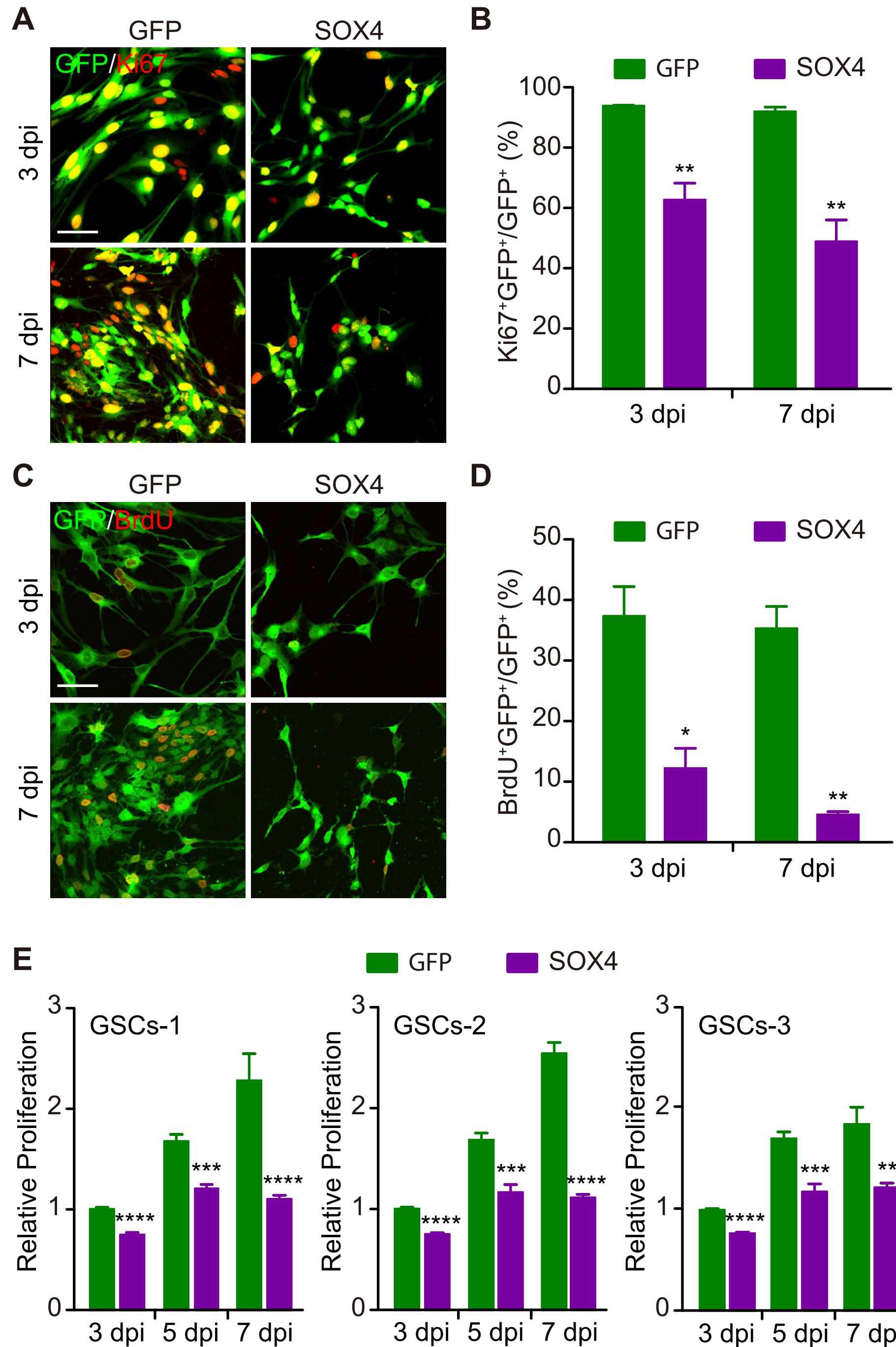
Chromatin immunoprecipitation-sequencing (ChIP-seq) and ChIP-qPCR. ChIP was performed as previously described (1). Briefly, approximately 1E+7 HA-SOX4-expressing U251 cells were crosslinked with methanol-free formaldehyde, followed by quenching with glycine solution. After washing and cell lysis, chromatin was sheared into 100-500 bp length using a Bioruptor (Diagenode). An aliquot of 10 µg sheared chromatin was reserved as input, while 150 µg was used for immunoprecipitation with 5 µg each of the following antibodies: HA (Abcam, ab9110), POLR3A (Abcam, ab96328), TBP (Abcam, ab818), or GTF3C2 (Abcam, ab89113). Chromatin-bound antibodies were subsequently pulled down with 100 µl Magnetic Protein G Dynabeads. After reverse cross-linking and proteinase K-digestion, both input and immunoprecipitated DNA was purified and quantified. For massively parallel DNA sequencing, 10 ng of input chromatin and 10 ng of immunoprecipitated chromatin samples were used to construct libraries. Biological triplicates were prepared and sequenced to generate single-end 50-base reads on an Illumina HiSeq 2500 System. Raw FASTQ files were analyzed using FastQC v0.11.5 and FastQ Screen v0.11.4, and reads were quality-trimmed using fastq-mcf. The trimmed reads were mapped to hg19 with bowtie2 (version 2.3.3.1) with sensitive and end-to-end options and alignments with mapping quality less than 10 were filtered out. Duplicates were removed using picard-tools v2.10. The alignment summary was included in SI Appendix, Table-S1. The ChIP-seq peaks were called using MACS2 (version 2.1.1), with a q-value threshold of 0.05 and using the random background of ChIP samples as controls. Motif discovery (parameter: 150 nucleotide window from peak center), annotation of peaks and motif analysis was performed using HOMER (v4.9.1). ChIP-seq data was visualized via the UCSC Genome Browser. For qPCR, biological triplicates were prepared for each condition. Immunoprecipitated DNA was analyzed by using the SYBR Green chemistry on a 384-well 7900HT System (Applied Biosystem). The PCR program consisted of 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 60 sec at 58°C. Amplification specificity was confirmed by using a heat dissociation protocol during the final cycle consisting of 15 sec each at 95°C, 60°C, and 95°C. Primer sequences are listed in SI Appendix, Table-S3. Results were presented as % enrichment to input or fold enrichment to the GFP control group.

Gel-shift assays. Gel-shift assays were conducted by using dsDNA probes as previously described (2). Briefly, dsDNA probes were prepared by annealing partially complementary oligonucleotides and Taq polymerase-dependent end extensions in the presence of α-^{32}P-dCTP (Perkin Elmer). Oligonucleotide sequences are listed in SI Appendix, Table-S3. Probes were purified and measured on a liquid scintillation counter (LS6500, Beckman). For nuclear extract preparation, SOX4-IRES-GFP lentivirus-transduced U251 cells were pelleted by centrifugation for 4 min at 300 x g, resuspended in ice-cold hypotonic buffer (10 mM HEPES-KOH [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.2 mM PMSF), and incubated on ice for 10 min. Nuclei were then pelleted and incubated with shaking in high salt buffer (20 mM HEPES-KOH [pH 7.9], 25% (v/v) glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.3 mM DTT, 0.2 mM PMSF) for 20 min at 4°C. Nuclear extracts were collected by removing cellular debris with high-speed centrifugation (1,200 x g for 10 sec at 4°C). Ten µg of nuclear extracts were then incubated with ~4,000 dpm ^{32}P-labeled probes for 20 min at RT in reaction buffer consisting of 10 mM Tris [pH7.5], 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 5% (v/v) glycerol, and 0.1 µg/µl poly (dI-dC). For antibody-mediated super-shift assays, the reactions were further incubated with a SOX4

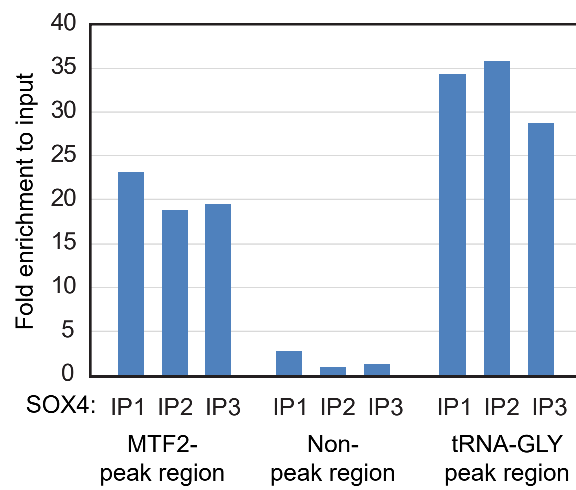
antibody (Abcam, rabbit, ab86809, 1 μg) or a Ctrl-Ab (HA antibody, Abcam, rabbit, ab9110, 1 μg) for another 20 min at RT. The protein-DNA complexes were then loaded onto a 5% non-denaturing acrylamide gel for separation of protein-bound probes from free probes. The gel was then dried and exposed to a film for autoradiography.

References:

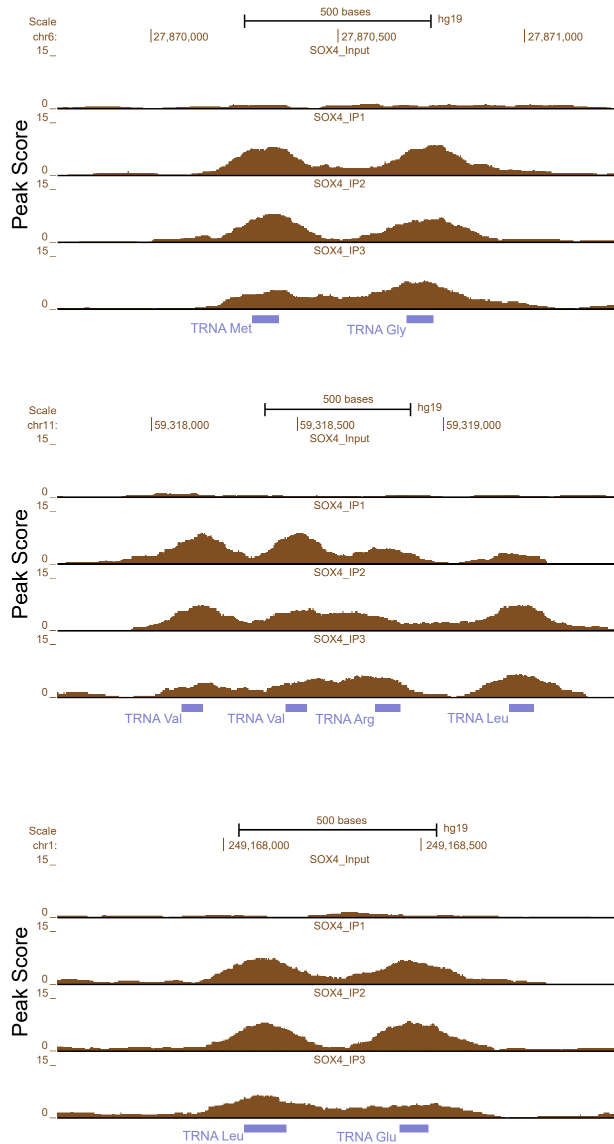
1. D. K. Smith, J. Yang, M. L. Liu, C. L. Zhang, Small Molecules Modulate Chromatin Accessibility to Promote NEUROG2-Mediated Fibroblast-to-Neuron Reprogramming. *Stem cell reports* **7**, 955-969 (2016).
2. C. L. Zhang, Y. Zou, R. T. Yu, F. H. Gage, R. M. Evans, Nuclear receptor TLX prevents retinal dystrophy and recruits the corepressor atrophin1. *Genes & development* **20**, 1308-1320 (2006).

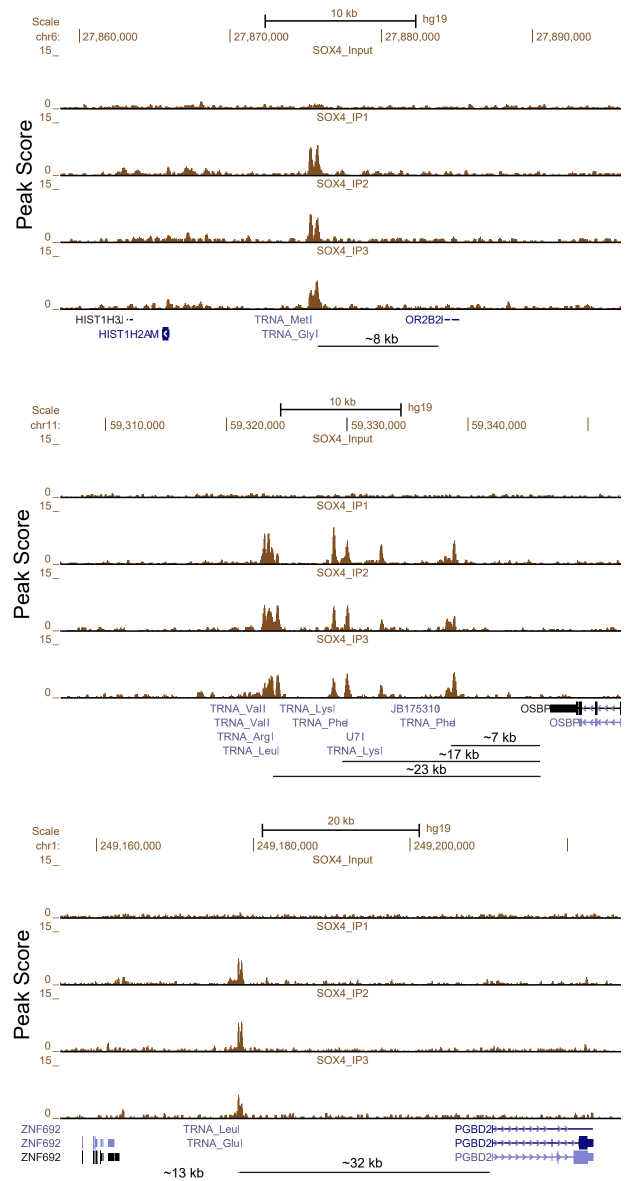


Supplemental Figure S1. SOX4 inhibits proliferation of primary human glioblastoma stem cells.
(A) Confocal images of proliferating glioblastoma stem cells (GSCs) indicated by Ki67-staining. Scale bar: 50 µm.
(B) Quantification of Ki67-positive proliferating GSCs (Mean ± SEM; n=3; **p < 0.01).
(C) Confocal images of proliferating GSCs indicated by BrdU-incorporation. Scale bar: 50 µm.
(D) Quantification of BrdU-positive proliferating GSCs (Mean ± SEM; n=3; *p < 0.05 and **p < 0.01).
(E) Proliferation of GSCs measured by ATP-dependent luminescence. GSCs-1, GSCs-2, and GSCs-3 represent three independent isolates of human glioblastoma stem cells (Mean ± SEM; n=6; **p < 0.01, ***p < 0.001, and ****p < 0.0001).

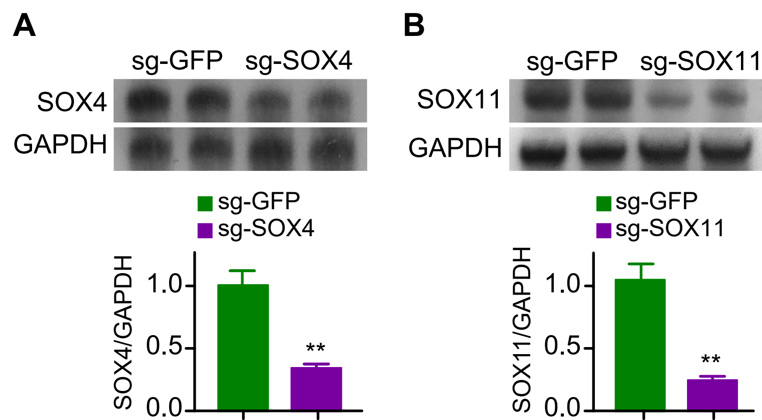


Supplemental Figure S2. ChIP-qPCR validation of SOX4-targeted genes.

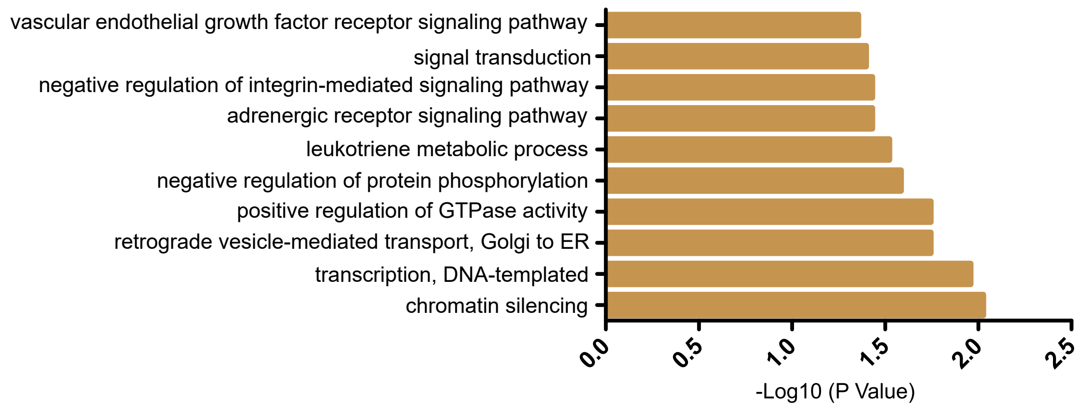


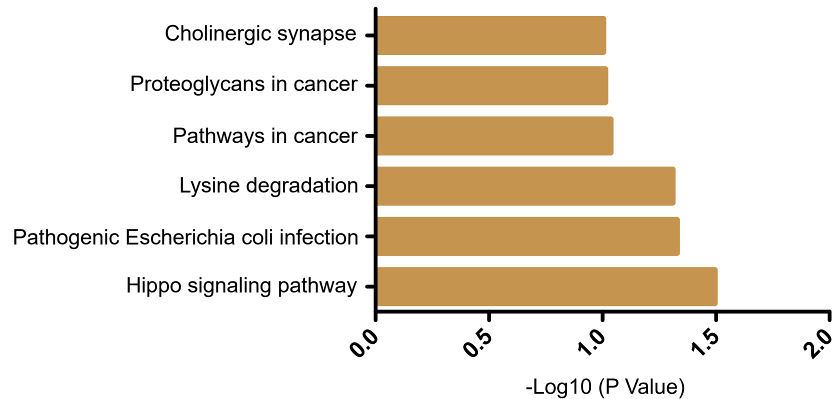


Supplemental Figure S3. ChIP-seq signal tracks for SOX4-targeted genes.
(A) Zoomed-in views of density plots of mapped fragments surrounding SOX4-targeted tRNA genes.
(B) Zoomed-out views of signal tracks surrounding SOX4-targeted tRNA genes. Relative distances between tRNA genes and the nearest Pol II-dependent genes are indicated.

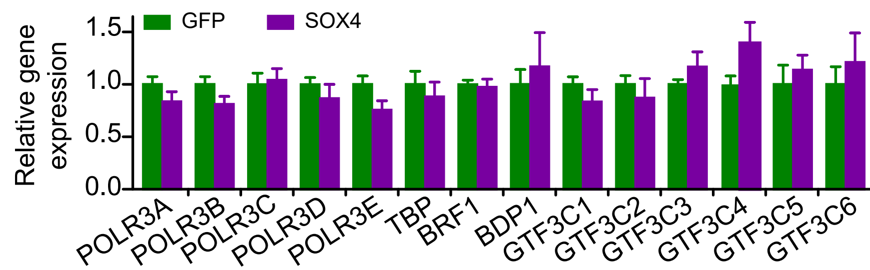


Supplemental Figure S4. Downregulation of SOX4 and SOX11 through CRISPR/Cas9.
(A) Western-blotting analysis of SOX4 expression in U251 cells at 3 dpi (Mean ± SEM; n=3; **p < 0.01).
(B) Western-blotting analysis of SOX11 expression in U251 cells at 3 dpi (Mean ± SEM; n=3; **p < 0.01).

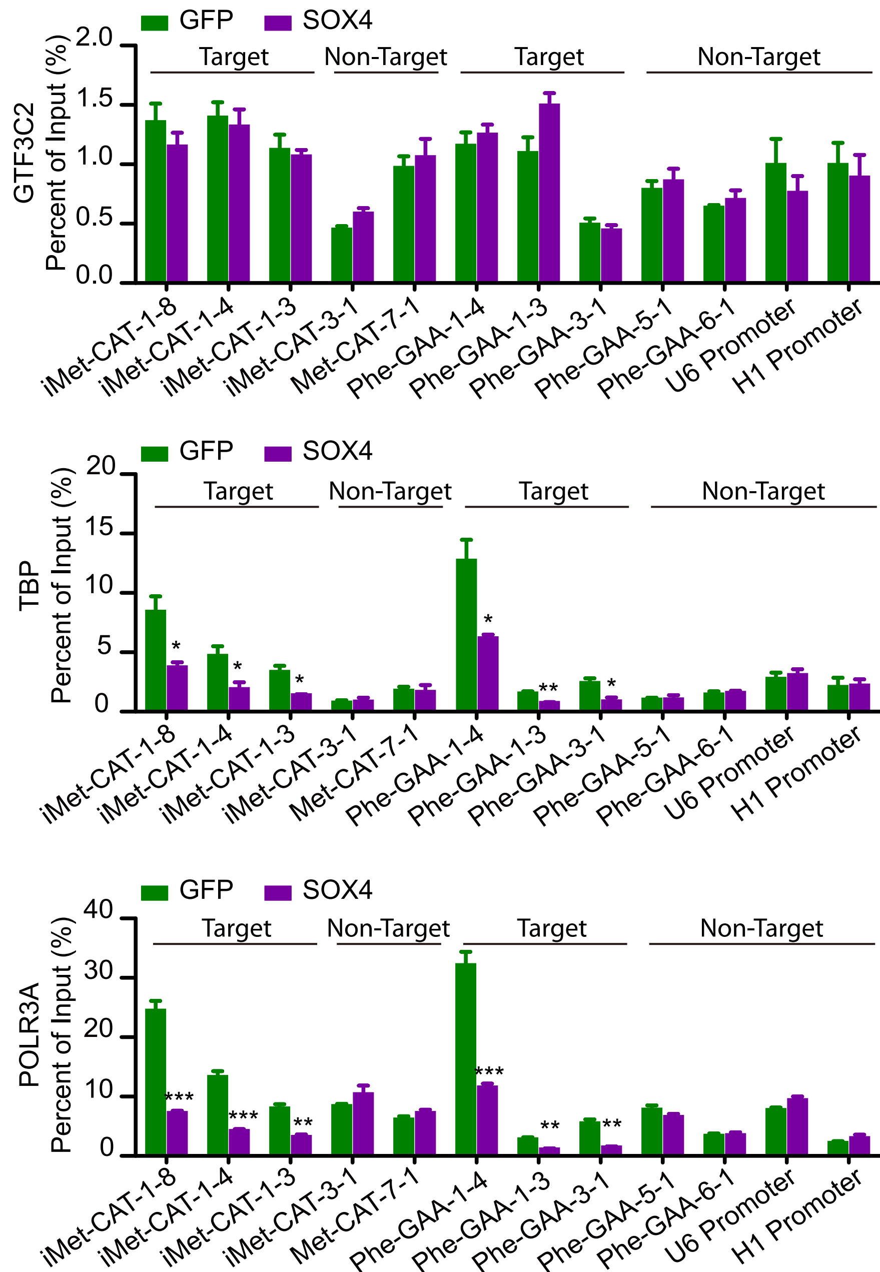




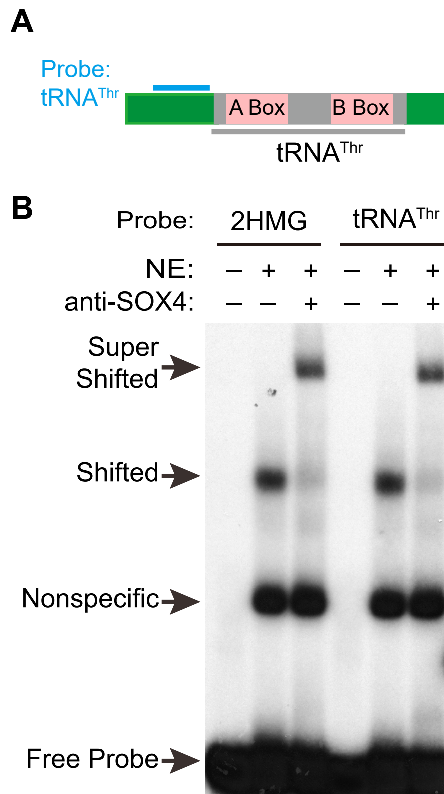
Supplemental Figure S5. SOX4-regulated biological processes and pathways.
Gene ontology analysis was performed on SOX4-targetted non-tRNA genes from ChIP-seq data.



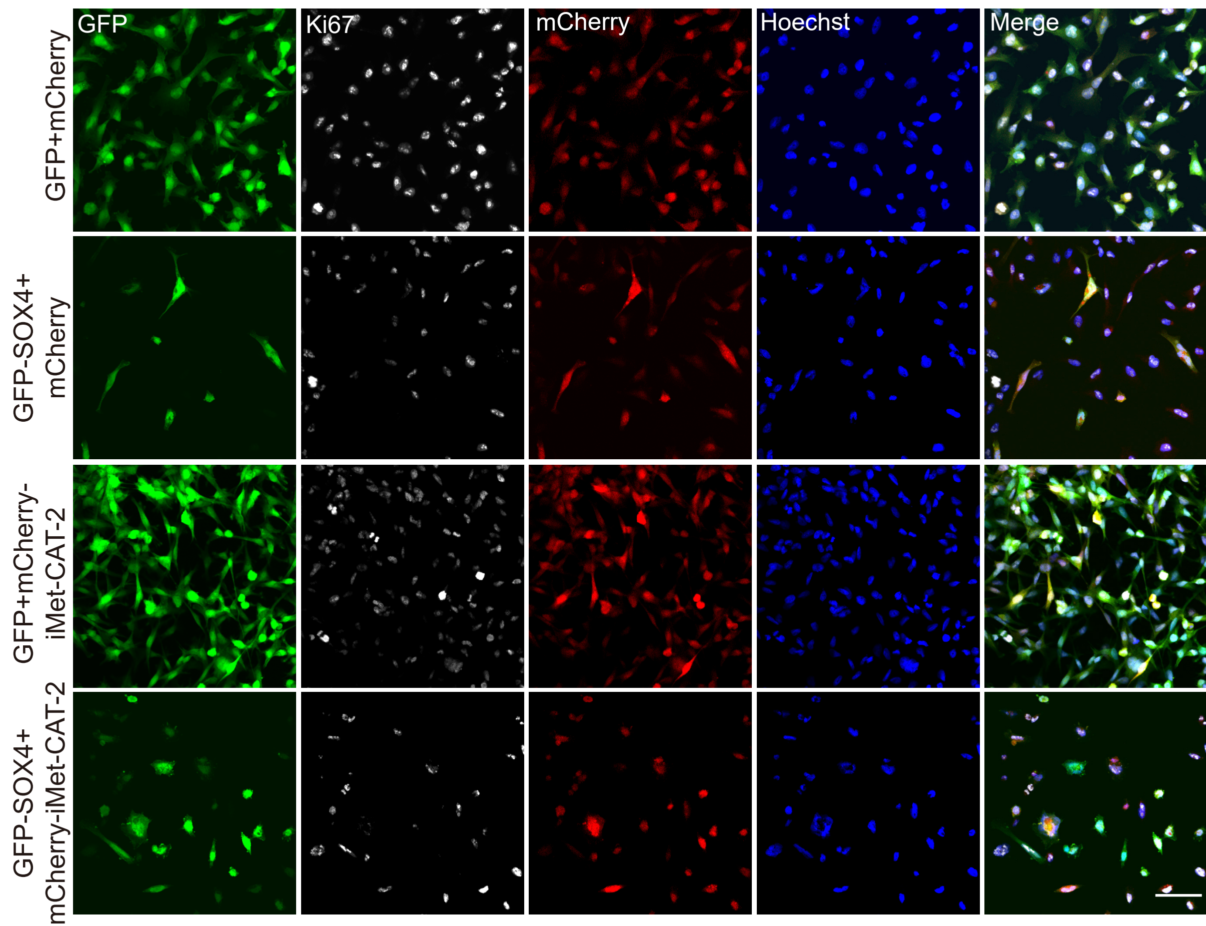
Supplemental Figure S6. The effect of SOX4 on expression of genes in Pol III complex.
Gene expression was analyzed by qRT-PCR on virus-transduced U251 cells at 3 dpi. The expression was normalized to that of HPRT and then to the GFP control.



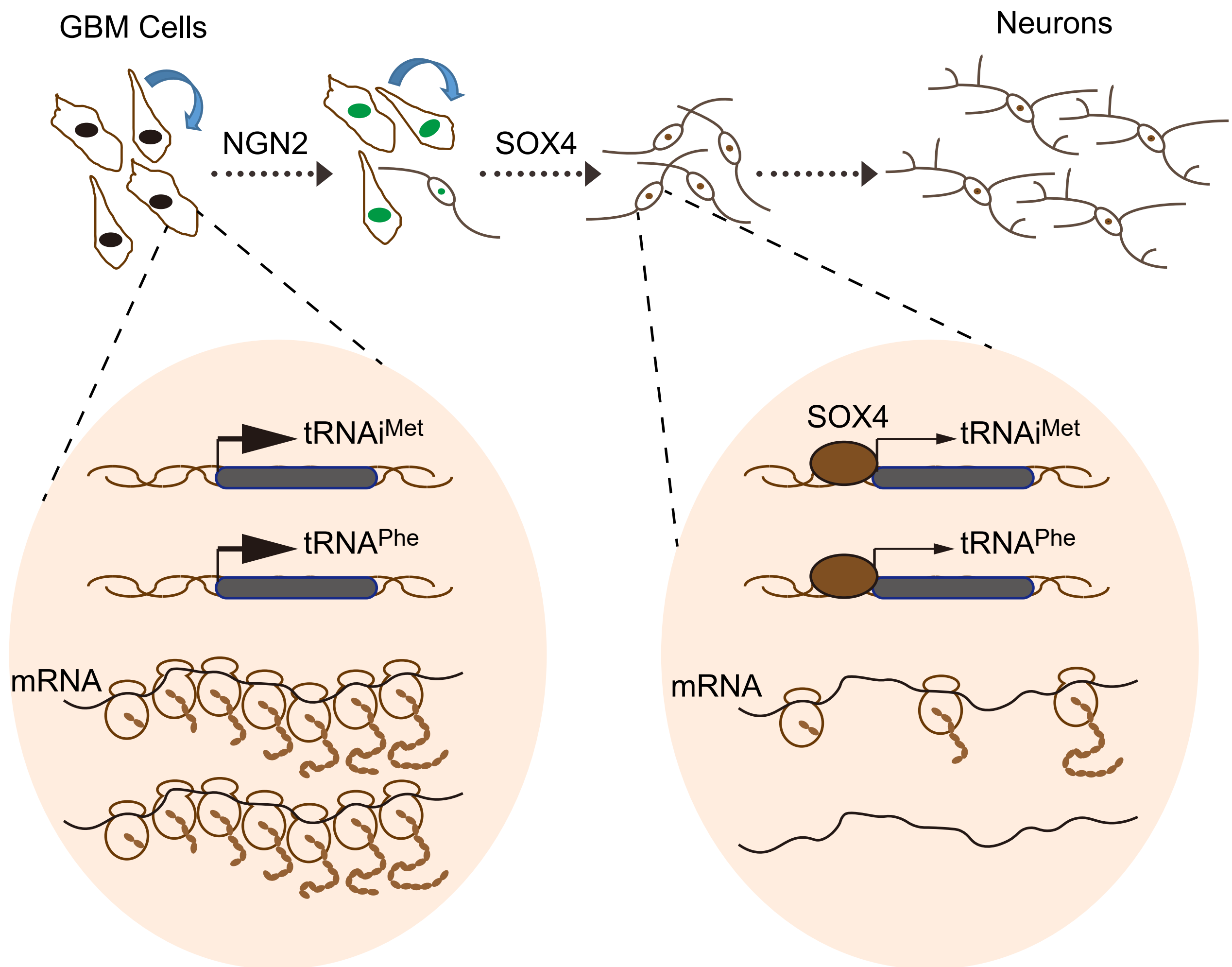
Supplemental Figure S7. SOX4 blocks recruitment of Pol III to specific tRNA genes.
ChIP-qPCR assays for the indicated factors and genes. U6, H1, and some of the tRNA genes that are not targeted by SOX4 were used as controls (mean ± SEM; n = 3; *p < 0.05, **p < 0.01 and ***p < 0.001).



Supplemental Figure S8. Gel-shift assays showing specific binding of SOX4 to tRNA genes.
(A) A schematic diagram showing key elements of the tRNA gene, Thr-AGT-2-1, and probe location. A box and B box, intragenic control sequence blocks for tRNA expression.
(B) Gel-shift using the indicated probes. A DNA probe containing a tandem repeats of HMG-box protein-binding sequences from the CD3E promoter was used as a positive control. NE, nuclear extracts from U251 glioblastoma cells.



Supplemental Figure S9. Ectopic tRNAi^{Met} rescues SOX4-mediated inhibition of U215 proliferation. Representative images of U251 cells transduced with the indicated lentivirus at 7 dpi. Scale bar: 50 µm.



Supplemental Figure S10. A schematic depicting the role of SOX4 in reprogramming of human glioblastoma cells.

Robust tRNA expression and protein translation are required for rapid proliferation of human glioblastoma (GBM) cells. GBM cells can be efficiently reprogrammed into neurons by ectopic expression of NGN2 and SOX4. A key role of SOX4 is to promote cell-cycle exit through direct suppression of a subset of tRNAs and thereby reducing protein translation.