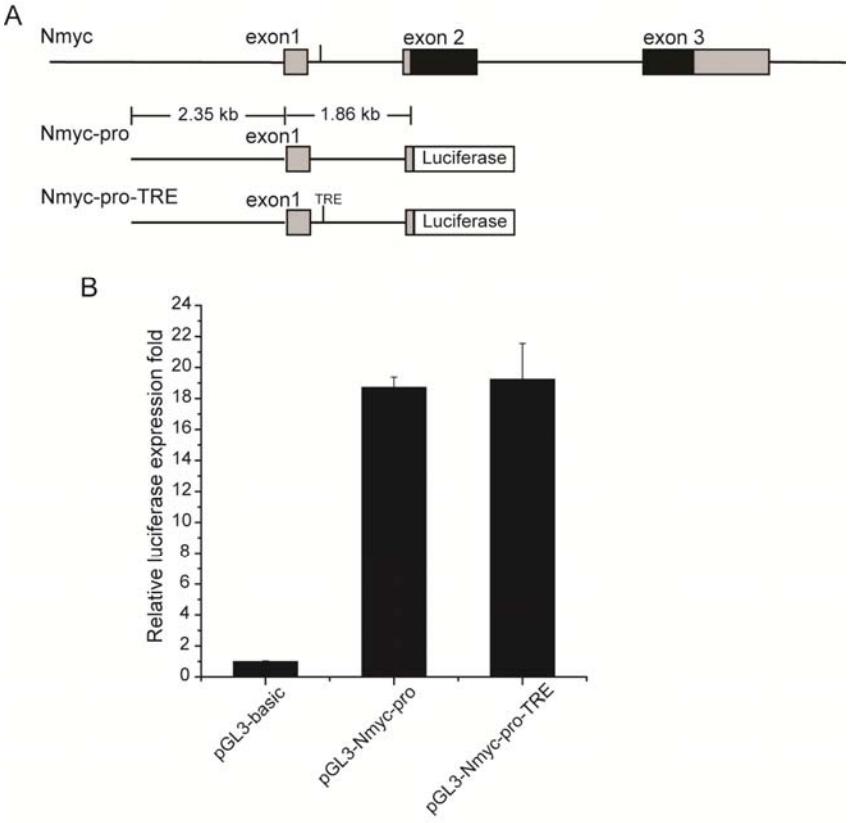
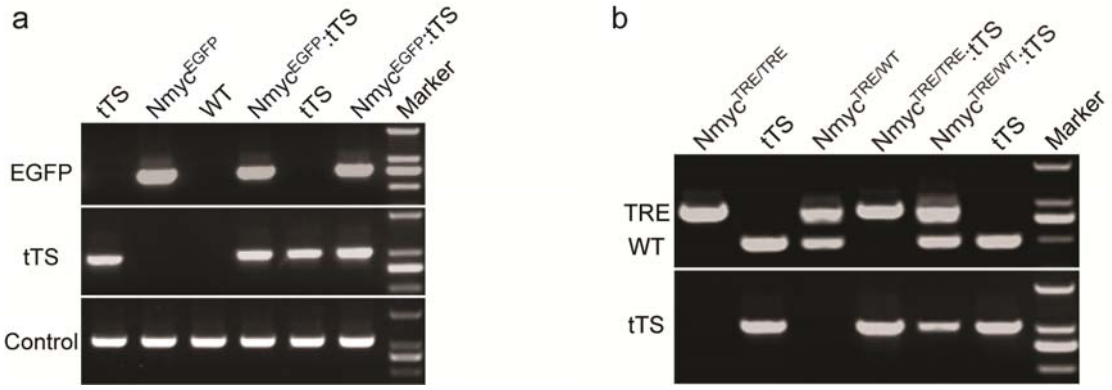
Supplementary Figure 1



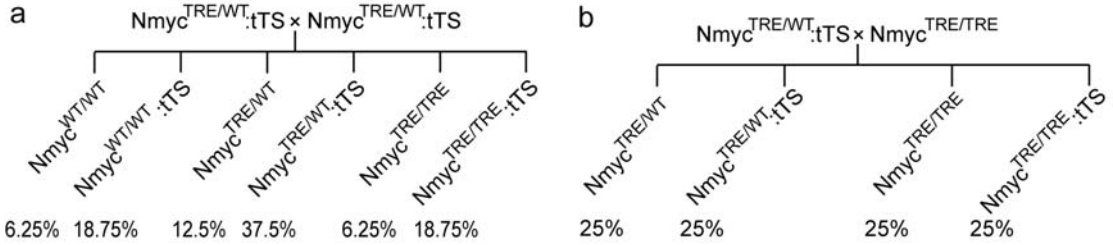
Transient transfection assay define the effect of TRE insertion to normal expression of Nmyc promoter. A: The structure of Nmyc gene, pGL3-Nmyc-pro plasmid and pGL3-Nmyc-pro-TRE plasmid. B: Plasmid of pGL3-basic, pGL3-Nmyc-pro and pGL3-Nmyc-pro-TRE were transfected into Neuro-2a cell. Luciferase activity was normalized to Renilla luciferase activity encoded by cotransfected control plasmid pRL-SV40 and then normalized to the pGL3-basic plasmid.

Supplementary Figure 2



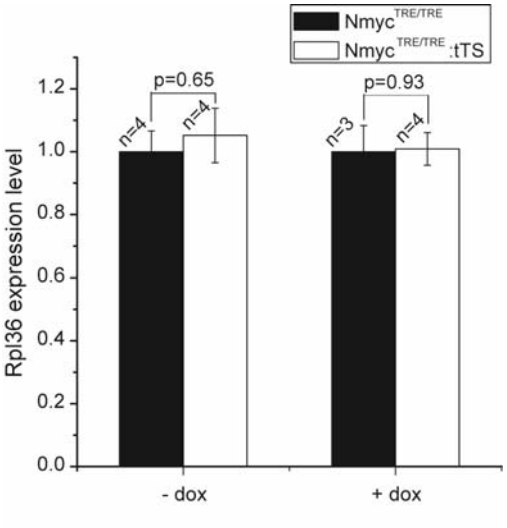
PCR confirmed mouse genotypes. (a) The expected size of PCR products were 782 bp for $Nmyc^{EGFP}$ and 1027 bp for tTS. A 1051 bp product was the positive control. (b) The expected size of PCR products were 880 bp for the $Nmyc^{TRE}$, 495 bp for the wild type Nmyc, and 1027 bp for tTS.

Supplementary Figure 3



Expected genotypic frequencies in the progeny of (a) a cross between Nmyc$^{TRE/WT}$:tTS individuals and (b) a cross between Nmyc$^{TRE/WT}$:tTS and Nmyc$^{TRE/TRE}$ mice.

Supplementary Figure 4



Quantitative real-time PCR of Rpl36 expression level in Nmyc$^{TRE/TRE}$ and Nmyc$^{TRE/TRE}$:tTS E10.5d embryos. Pregnant mice were reared on drinking water containing 0 mg/ml dox (-dox) or 2mg/ml dox (+dox) since E0.5d.

Supplementary Material and Methods

Primer information described in our experiments.

Primer	**Sequence (5'-3')**	**Fragment size (bp)**
Primer I	ATTGGGTCTTAAGATGTGAACAAAGTC	Nmyc^{EGFP} allele: 3528; wt allele: 3144
Primer II	TTTCTTTTCGCTAAAGCTATTTCTCTG	
Primer III	CCTCCCCCGTGCCTTCCTTGAC	Nmyc^{EGFP} allele: 5408
Primer IV	GATGTTCGCGATTGTCTCGGAAGC	
tTS-F	TCGACGCCTTAGCCATTGAGAT	tTS(+) allele: 1027
tTS-R	GCGATGCAATTTCCTCATTT	
Primer V	GTTGGCGCGACTCTGCTGCTCTCC	Nmyc^{EGFP} allele: 782
Primer VI	CGGCGGCGGTCACGAACTCC	
Genome-control-F	AATTCGGTGCAACACGTTGCTCTCG	1051
Genome-control-R	TGGCAGTACTCGCATGTAATCCTA	
Primer VII	CCGCCGCCGCTCCCTCAG	Nmyc^{TRE} allele: 880;

Primer VIII	GCTTCGCCTAACCCCTTTTCCTTCTT	WT allele: 495
Cre-F	GCGCGGTCTGGCAGTAAAAAC	Cre(+) allele: 779
Cre-R	TCAGCAGGCGCACCATTG	

Cell culture, transfection, and reporter gene assays.

Neuro 2a (mouse neuroblastoma) cell lines were grown in DMEM (Hyclone) with 10% fetal calf serum supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin (Invitrogen). Cells were transfected in Opti-MEM (Invitrogen) with Lipofectamine 2000 (Invitrogen). Cells were harvested 48 hours post-transfection and assayed for reporter gene activity with Dual-luciferase Reporter Assay System (Promega).