



Extended Figure S1 (A) Expression of the wild-type *Tdg* gene (top) is abolished by the targeted replacement of exon 6/7 with a neomycin resistance cassette (NeoR). The NeoR-expression is controlled by the MC1 promoter (blue arrow) and a polyA-signal (!) from the herpes simplex thymidine kinase gene (middle). CRISPR/Cas9-mediated removal of the NeoR (bottom) with two gRNAs (purple boxes). **(B)** Proportion of living cells in indicated ESCs after three days of treatment with G418, measured with CCK-8. Shown are means and standard deviations of two biological replicas. **(C)** Top: Immunoblot to estimate the sensitivity of TDG detection. NP-40 whole-cell protein extracts of TDG proficient cells were diluted in extracts of TDG deficient cells. Bottom: Ponceau staining of the membrane as loading control. **(D)** mRNA expression of indicated genes in thymic cells (TCs), and thymic endothelial cells (TECs) obtained from mice with the *miniTdg* and *FoxN1::Cre*. Shown are the mean and standard deviation of three technical replicates. **(E)** Immunoblot of SDS protein extracts from cells as in (C). β -Actin serves as a loading control.

Table S1 Overview of Primers used in this study

Oligo name	Sequence (5'-3')	Application
GT wt/ko TDG F	GGG AGT CCT AAG GGC AAA GTG GTG	Genotyping TDG WT
GT TDG B R	GAT GCA CTC GGG AGA CTT ACA G	Genotyping TDG WT
GT TDG D F	TTG CGT GGG AGT AAC CGA GC	Genotyping TDG KO
GT KO C R	CAT CTT GTT CAA TGG CCG ATC C	Genotyping TDG KO
Comp1 mTDG F	AAA TAC TCT GAG TCC AAA CCG GG	Genotyping miniTdg
TDG C R	TGG TGA ATC CGA TGC CGT ACT TG	Genotyping miniTdg
GT_3'ROSA_FF	CCC GCT CAT GAT CAA ACG CTC T	Screening for Cre inserts
GT_3'ROSA_GR	CTC AGT GGC TCA ACA ACA CTT GG	Screening for Cre inserts
Rosa26-P1_F	CTG CGC AAC GTG GCA GGA A	Southern Blot Probe 1 from pROSA26-ERT2CreERT2
Rosa26-P1_R	CTG GAC TAC TGC GCC CTA CA	Southern Blot Probe 1 from pROSA26-ERT2CreERT2
Rosa26-P2_F	GGA ACT AAA CCA TGG CCA AGC C	Southern Blot Probe 2 from pROSA26-ERT2CreERT2
Rosa26-P2_R	AGG ATC ATC CAG CCG GCG T	Southern Blot Probe 2 from pROSA26-ERT2CreERT2
S_miniTDGex_F	AAC AGA GAC CTA GAC AGC CCC T	miniTdg Excision (Primer 1)
S_miniTDG_F	AGA GAG CCA CGC TTA GTG AAG A	miniTdg Abundance (Primer 2)
pTCO2_CreEx_R	TCC GTC CTG AGG TCT GCA TTA G	miniTdg Excision (Primer 3)
S_TDGnorm_F	TCA GCC TCG ACT GTG CCT TC	Normalizer Excision qPCR (Primer 4)
S_TDGnorm_R	CAC CTA CTC AGA CAA TGC GAT GC	Normalizer Excision qPCR Primer 5)
Chr2neg_F	AGC ACA GCC TGA AGC CTC TA	Normalizer Excision qPCR
Chr2neg_R	ACA CAG CAT GGC ATC TTG AA	Normalizer Excision qPCR
GT TDG D F	TTG CGT GGG AGT AAC CGA GC	NeoR sequencing
3'NptII_rev	AGT ACA AGC CAG TTC CTT TGA CAC	NeoR sequencing
S_RPS13_F	GTG CGG CTT GAT TTC CTG TG	Normalizer Expression Analysis
S_RPS13_R	TTC AGC CAC GTG GGG ACG	Normalizer Expression Analysis
S_EEF1A1_F	CCC AGC CAA GAC ACA TAG TTG C	Normalizer Expression Analysis
S_EEF1A1_R	TCC GTC CTG AGG TCT GCA TTA G	Normalizer Expression Analysis
Nanog_F	CAG GAG TTT GAG GGT AGC TC	Stemness Marker
Nanog_R	CGG TTC ATC ATG GTA CAG TC	Stemness Marker
Bmp4_F	TTC CTG GTA ACC GAA TGC TGA	Mesodermal Marker
Bmp4_R	CCT GAA TCT CGG CGA CTT TTT	Mesodermal Marker
Pax6_F	TCA CAG CGG AGT GAA TCA G	Ectodermal Marker
Pax6_R	CCC AAG CAA AGA TGG AAG	Ectodermal Marker
Nestin_F	TCT CCA GAA GAG GAG GAC CA	Ectodermal Marker
Nestin_R	TTC GAG AGA TTC GAG GGA GA	Ectodermal Marker
GATA6_F	TTG CTC CGG TAA CAG CAG TG	Endodermal Marker
GATA6_R	GTG GTC GCT TGT GTA GAA GGA	Endodermal Marker

Table S2 Overview of ESC lines generated in this study

<u>Clone Name</u>	<u>Tdg locus</u>	<u>ROSA26</u>	<u>Antibiotic resistance</u>	<u>Derived from</u>	<u>Mass spectrometry verification</u>	<u><i>In vitro</i> differentiation capability</u>
Mm01	Tdg ^{-/-}	WT	Tdg::NeoR ROSA26:Blast	Mouse blastocyst	Yes	Yes
Mm02	Tdg ^{-/-}	WT	Tdg::NeoR ROSA26:Blast	Mouse blastocyst	Yes	Not determined
Mm11	Tdg ^{+/-}	WT	Tdg::NeoR ROSA26:Blast	Mouse blastocyst	Yes	Not determined
TDGiKO1	Tdg ^{-/-}	Cre-ERT ^{-/+}	Tdg::NeoR ROSA26:Blast	Mm01	Yes	Yes
TDGiKO4	Tdg ^{-/-}	Cre-ERT ^{-/+}	Tdg::NeoR ROSA26:Blast	Mm02	No	Not determined
TDGiKO5	Tdg ^{-/-}	Cre-ERT ^{-/+}	Tdg::NeoR ROSA26:Blast	Mm02	No	Not determined
TDGiKO6	Tdg ^{+/-}	Cre-ERT ^{-/+}	Tdg::NeoR ROSA26:Blast	Mm11	No	Not determined
TDGiKO1.1	Tdg ^{-/-}	Cre-ERT ^{-/+}	ROSA26:Blast	TDGiKO1	Yes	Yes
TDGiKO1.2	Tdg ^{-/-}	Cre-ERT ^{-/+}	ROSA26:Blast	TDGiKO1	Yes	Yes