Supplementary information for:

Regulatory Mechanisms of Maternal Imprinting at the Dlk1-Dio3 Domain

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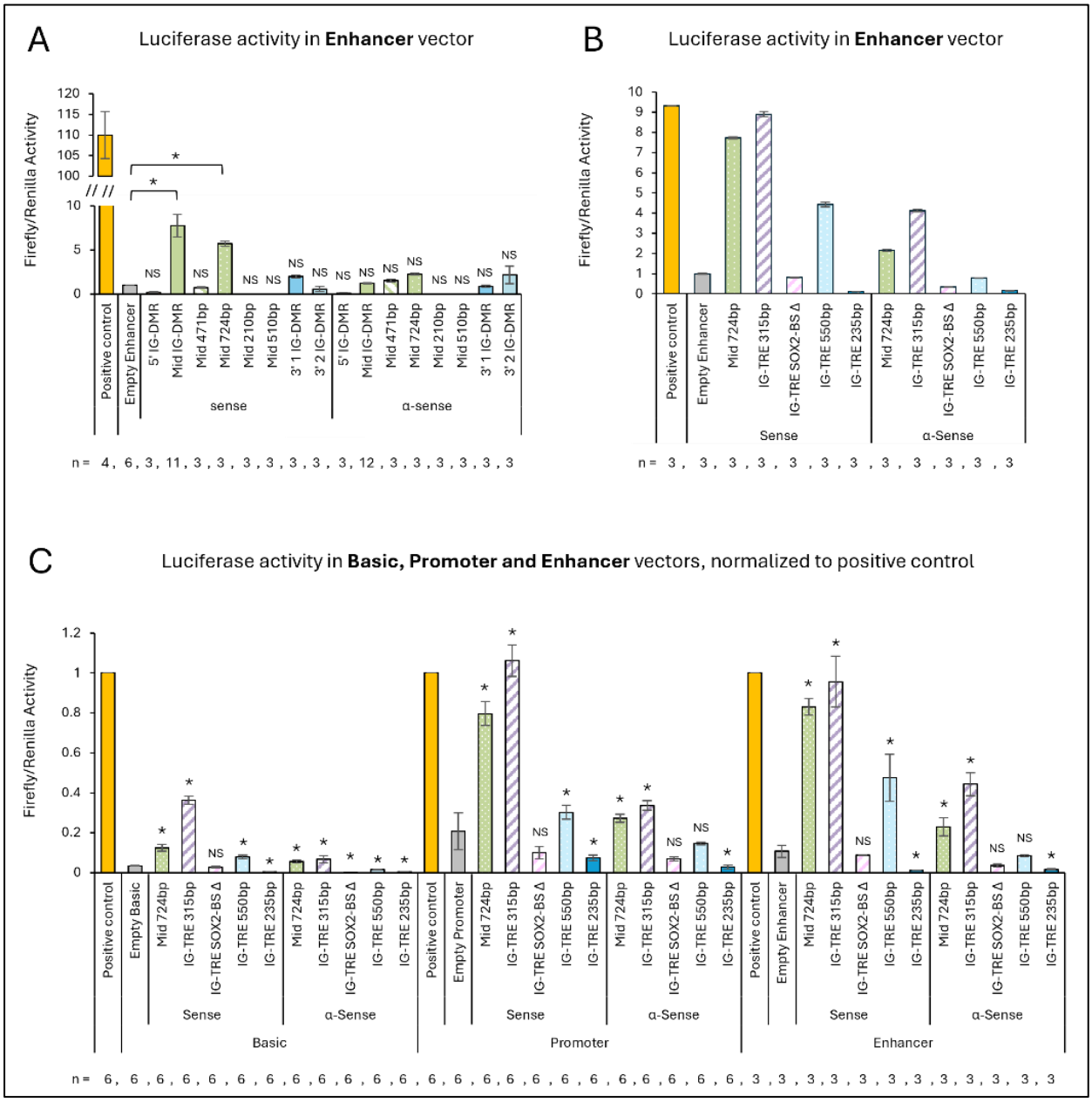

Figure S1- Enhancer and silencer activity detected at the IG-TRE. A+B. Graphical representation of the luciferase activity in the pGL3 Enhancer vector, normalized to the empty enhancer vector. **C**. Graphical representation of the luciferase activity in the pGL3 Enhancer vector normalized to the positive control vector. The Mid 724bp fragment shows activity in sense orientation only, suggesting promoter activity. The Mid 315bp fragment shows activity similar to the 724bp fragment, suggesting enhancer activity. This activity is lost upon deletion of the SOX2-binding site from within the 315bp fragment. In parallel, the 235bp fragment, downstream of the 315bp region, shows silencing activity, indicating dual activity in a minimal region of 550bp in the IG-TRE with both enhancing and silencing regulation. Each construct was measured twice in triplicates wells. The firefly luciferase values were normalized to the *Renilla* values and then each test construct was normalized to the corresponding empty vector. NS- not significant. Asterisks indicate statistical significance in comparison to the empty vector using a two-tailed unpaired Student's t-test.

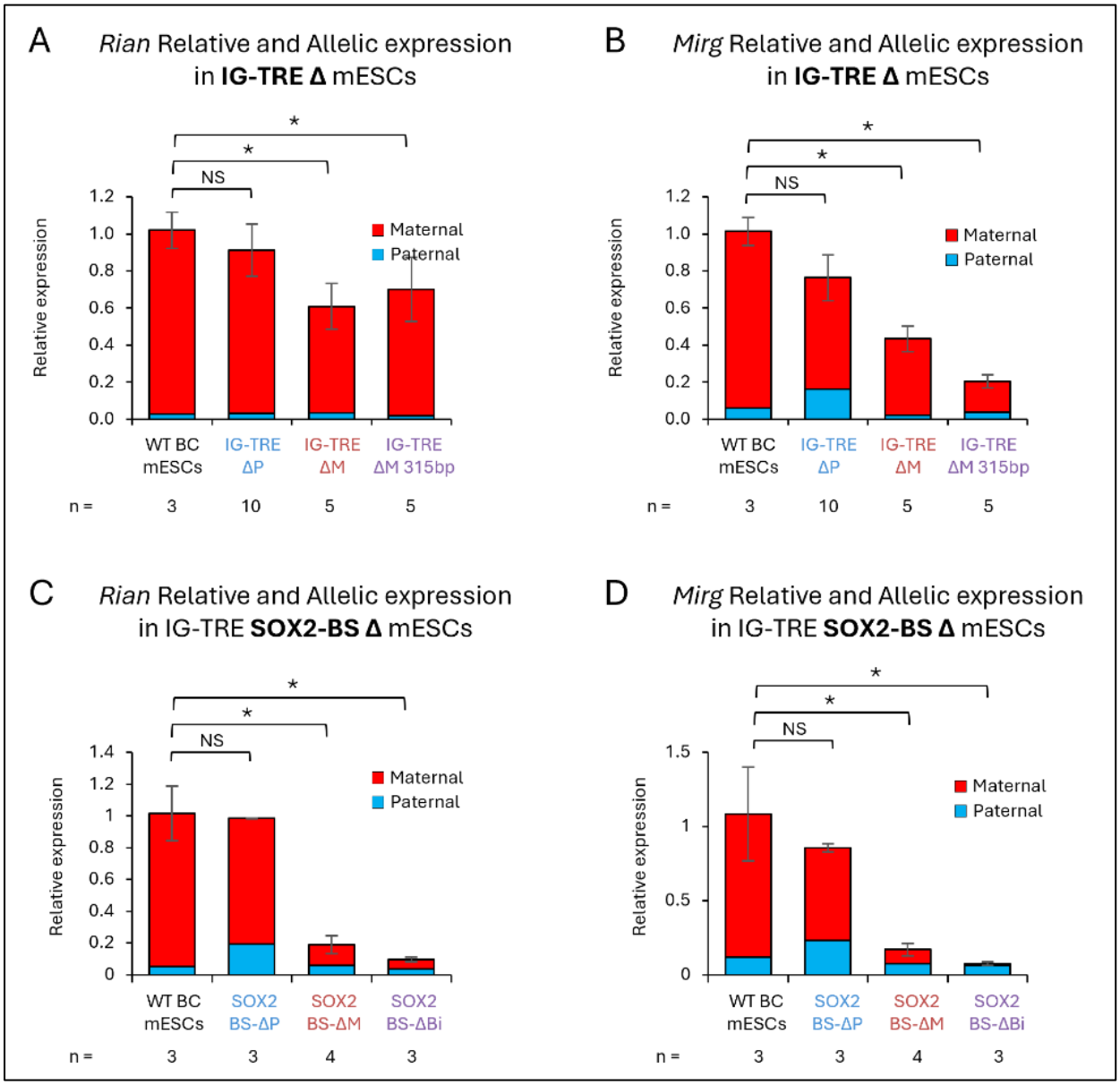

Figure S2- Deleting the entire maternal IG-TRE or even just the SOX2-binding site *in vitro* results in dysregulation of the maternal transcripts *Rian* and *Mirg*. A. Graphical representation of *Rian* expression in mESC clones deleted for the entire IG-TRE (1.3kb) shows loss of *Rian* expression upon maternal or biallelic deletions. **B.** Graphical representation of *Mirg* expression in mESC clones deleted for the entire IG-TRE (1.3kb) shows loss of *Mirg* expression upon maternal or biallelic deletions. **C.** Graphical representation of *Rian* expression in mESC clones deleted for the SOX2-binding site within the 315bp of the IG-TRE shows loss of *Rian* expression upon maternal or biallelic deletions. **D.** Graphical representation of *Mirg* expression in mESC clones deleted for the SOX2-binding site within the 315bp of the IG-TRE shows loss of *Mirg* expression upon maternal or biallelic deletions. NS- not significant. Asterisks indicate statistical significance in comparison to wildtype using a two-tailed unpaired Student's t-test.

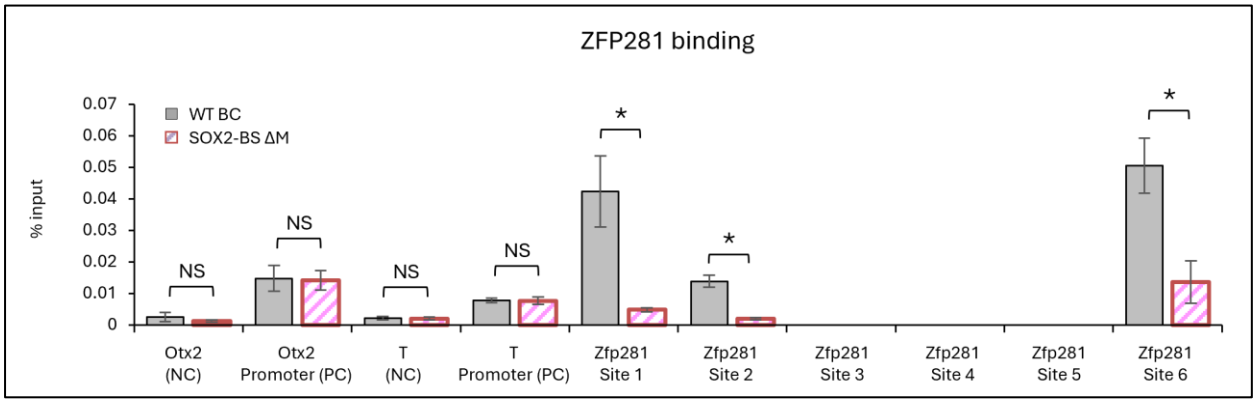

Figure S3- ZFP281 binding is dependent on Sox2 binding on the maternal chromosome. Graphical representation of ZFP281 binding at the IG-TRE, determined by ChIP-qPCR, shows depletion of ZFP281 binding at binding sites 1, 2 and 6, when the SOX2-binding site is deleted from the 315bp region. No binding was observed at predicted binding sites 3, 4 and 5 and no change in binding was observed at control sites and the *Otx2* and *T* genes. NS- not significant. Asterisks indicate statistical significance in comparison to wildtype using a two-tailed unpaired Student's t-test.

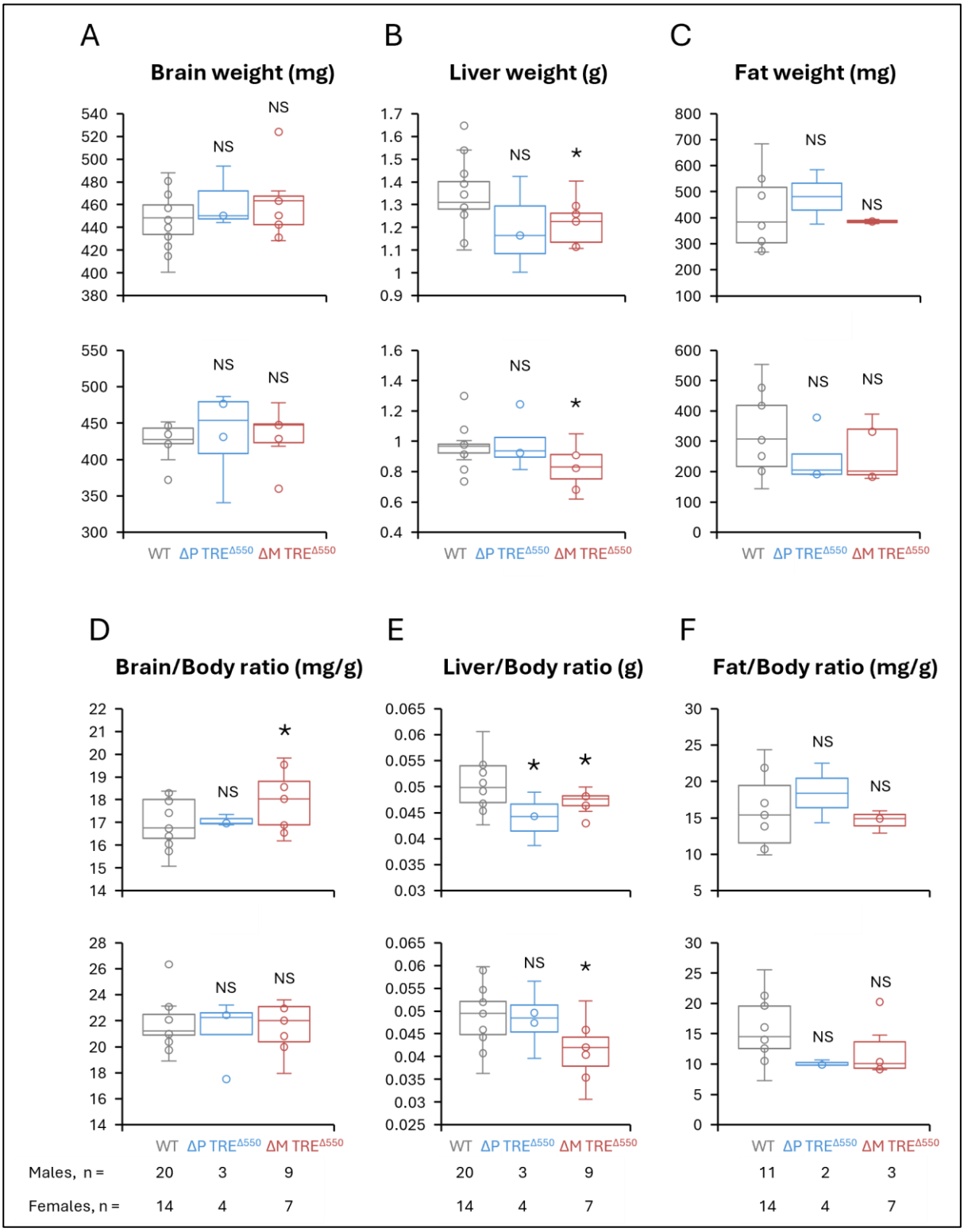

Figure S4- Deletion of the IG-TRE$^{\Delta 550}$ *in vivo* is viable with a slight reduction in adult liver size. A-F. Box plot representation of brain weight (mg), liver weight (g) and fat weight (mg) of 9 week-old adult animals. N_{WT}=20, $N_{\Delta P\text{-}TRE^{\Delta 550}}$=3, $N_{\Delta M\text{-}TRE^{\Delta 550}}$=9 biologically independent males were measured for brain weight, liver weight, brain/body ratio and liver/body ratio; and N_{WT}=11, $N_{\Delta P\text{-}TRE^{\Delta 550}}$=2, $N_{\Delta M\text{-}TRE^{\Delta 550}}$=3 biologically independent males were measured for fat weight and fat/body ratio. N_{WT}=14, $N_{\Delta P\text{-}TRE^{\Delta 550}}$=4, $N_{\Delta M\text{-}TRE^{\Delta 550}}$=7 biologically independent females were measured for all categories. NS- not significant. Asterisks indicate statistical significance in comparison to wildtype using a two-tailed unpaired Student's t-test.

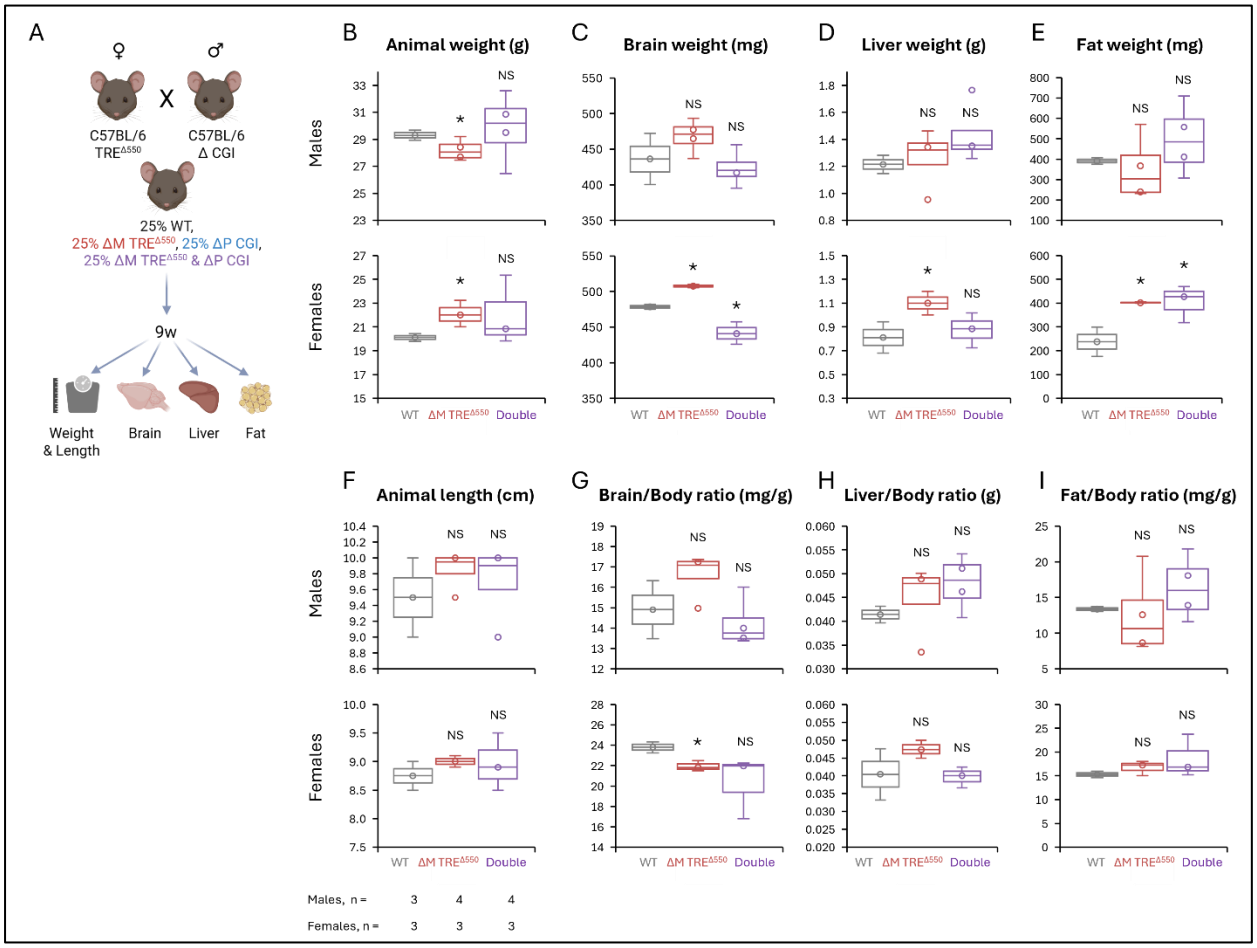

Figure S5- IG-CGI$^{\Delta P}$ and IG-TRE$^{\Delta 550}$ double deletion embryos are normal in size and rescue the lethality of the paternal IG-CGI deletion *in vivo*. A. Schematic representation of the predicted outcome of crossing IG-CGI$^{\Delta P}$ and IG-TRE$^{\Delta 550}$ mouse strains. In each litter, 25% of the pups are wildtype littermates (pure C57BL/6) and kept as controls. Tissues were collected for expression at 9 weeks. **B-I.** Box plot representation of total body weight (g), brain weight (mg), liver weight (g), fat weight (mg) and body length (cm) of 9 week-old adult animals. N_{WT}=3, $N_{\Delta M\text{-}TRE^{\Delta 550}}$=4 and $N_{\Delta M\text{-}TRE^{\Delta 550}\&\Delta P\text{-}CGI}$=4 biologically independent males and N_{WT}=3, $N_{\Delta M\text{-}TRE^{\Delta 550}}$=3 and $N_{\Delta M\text{-}TRE^{\Delta 550}\&\Delta P\text{-}CGI}$=3 biologically independent females. NS- not significant. Asterisks indicate statistical significance in comparison to wildtype using a two-tailed unpaired Student's t-test.

68 **Table S1**- primers used to generate and sequence luciferase constructs

Fragment	Primer	Sequence 5' → 3'
IG-DMR 5'	SF8 (SacI) forward	CACGGCACAGAGCAGTAGCG
	SR9 (SacI) reverse	ACTGCCATTTAGAATTTGAG
IG-DMR Mid	IG-DMR Mid 1F NheI forward	AACTACCGCTACGGTTCATA
	IG-DMR Mid 1R NheI reverse	CATCCCACGAGGAACTGGT
	Mid forward	AACTACCGCTACGGTTCATA
	Mid NheI reverse	GGCCGCTAGCCATCCCACGAGGAACTGGT
	Mid KpnI reverse	GGCCGGTACCCATCCCACGAGGAACTGGT
IG-DMR 3'1	IGDMR 3'1F NheI forward	CCTTAGGGCTTCCAAGC
	IGDMR 3'1R NheI reverse	CTGGCGAGCAACAGTGAA
IG-DMR 3'2	IGDMR 3'2F NheI forward	GCTTCACTGTTGCTCGCC
	IGDMR 3'2R NheI reverse	CATGCTCAGAGCCAGCAG
315bp	NheI forward (also used for 550bp)	GGCCGCTAGCCGGTCTCGGAGTTCTTGTC
	NheI reverse	GGCCGCTAGCAGACGAGTTGGCAGGACG
	KpnI forward (also used for 550bp)	GGCCGGTACCCGGTCTCGGAGTTCTTGTC
	KpnI reverse	GGCCGGTACCAGACGAGTTGGCAGGACG
550bp	NheI reverse (also used for 235bp)	GGCCGCTAGCTGGTCAACAAAAGCATCTCTGT
	KpnI reverse (also used for 235bp)	GGCCGGTACCTGGTCAACAAAAGCATCTCTGT
235bp	NheI forward	GGCCGCTAGCGAGCCCTGTGGTTAACTAAGC
	KpnI forward	GGCCGGTACCGAGCCCTGTGGTTAACTAAGC
pGL3 empty vector	RV primer forward	CTAGCAAAATAGGCTGTCCC
	GL primer reverse	TATGTTTTTGGCGTCTTCCA
pGL3 promoter vector	Forward	AGGTACCGAGCTCTTACGC
	Reverse	GCCGGGCCTTTCTTTATGTT
pGL3 enhancer vector	Forward	GGGAGGTGTGGGAGGTTTT
	Reverse	AAGGAGCTGACTGGGTTGAA

69 **Table S2**- gRNAs and genotyping primers for CRISPR in mESCs

Target	Primer	Sequence 5' → 3'
5' IG-TRE	gRNA 1 forward	CACCGCTGTAGCCTTGCTAGACTG
	gRNA 1 reverse	AAACCAGTCTAGCAAGGCTACAGC
3' IG-TRE	gRNA 2 forward	CACCgCCATGGCTTACAAGCTACTG
	gRNA 2 reverse	AAACCAGTAGCTTGTAAGCCATGGC
SOX2-BS	gRNA forward	CACCgAGAAGGGATGAGACTCCTCT
	gRNA reverse	AAACAGAGGAGTCTCATCCCTTCTC
	single-stranded oligo donor (ssODN)	ACGGCTCTTTCCCAGAATCCACTGGGCCTGTTTTGGGGGCAGCTTA GTTAACCACAGGGCTCTGGTTAGAAGGGATGAtACgCCTCTAGGATT CCCAGAGTTTCTCGGTCTTCT
IG-TRE Δ	Genotyping forward	TGACTTCCTTCAGCCACAGT
	Genotyping reverse	GGCAAACCCCAACTTAGCAA
	Genotyping internal reverse	CAGGATGCCAAGGGTTGTG
SOX2-BS Δ	Genotyping forward	CGGTCTCGGAGTTCTTGTC
	Genotyping reverse	AGACGAGTTGGCAGGACG

70 **Table S3**- primers for bisulfite sequencing

Target	Primer	Sequence 5' → 3'
IG-CGI With biotin for qPCR and pyro methylation analysis	Forward	GTGGTTTGTTATGGGTAAGTTT
	Reverse	CCCTTCCCTCACTCCAAAAATTAA
	Sequencing	TGGTTTATTGTATATAATGT
IG-TRE With biotin for qPCR and pyro methylation analysis	Forward	GTTGGGGTTTGTTAGTTATTTATATGTTAT
	Reverse	AAAACATACTCTCCACTATAACTAATT
	Sequencing	CTATAACTAATTACAACACCAC
Gtl2-DMR With biotin for qPCR and pyro methylation analysis	Forward	AGTTATTTTTTGTTTGAAAGGATGTGTA
	Reverse	CTAACTTTAAAAAAAAATCCCCAACACT
	Sequencing	GAAAGGATGTGTAAAAATGA

71 **Table S4**- primers for qPCR for gene expression

Target	Primer	Sequence 5' → 3'
GAPDH- housekeeping gene (ex2F+3R)	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	TGTAGACCATGTAGTTGAGGTCA
B2M- housekeeping gene (ex1F+2R)	Forward	GGCTGTATTCCCCTCCATCG
	Reverse	CCAGTTGGTAACAATGCCATGT
Dlk1- ex5 With biotin for qPCR and pyro SNP analysis	Forward	CGCAAGAAGAAGAACCTCCTGT
	Reverse	ACGCTGCTTAGATCTCCTCATCA
	Sequence	CAGCCTCCTTGTTGAA
Gtl2- ex10 With biotin for qPCR and pyro SNP analysis	Forward	AGCCACCTATTTACAAATGGACTC
	Reverse	CATCCCCATGAGAAACCTGTT
	Sequence	CATAGAGACACAAACATAGT
Mirg- ex16 With biotin for qPCR and pyro SNP analysis	Forward	CTCAGGAGCGGATGTTCAAG
	Reverse	GATGTCCCCAGTGGAATGTC
	Sequence	GGAACCCTGCCTATG
Rian- ex4 With biotin for qPCR and pyro SNP analysis	Forward	GAGACCTTGGCAGTGACCG
	Reverse	CCTGGCCCAAAAAGCCTC
	Sequence	CTTGGCAGTGACCGC
Dio3- ex4 With biotin for qPCR and pyro SNP analysis	Forward	GAGGGATGCGAGAACTTTTTG
	Reverse	GCGCTTTCTGCCTAGGACT
	Sequence	TTTTGGAGAAGGGATT

72 **Table S5**- primers for ChIP-qPCR

Target	Primer	Sequence 5' → 3'
SOX2 Binding site at IG-TRE	Forward	TCCAATCTTCAAACACCTCCTG
	Reverse	TCCACTGGGCCTGTTTTGG
SOX2 Binding site at IG-TRE With biotin for qPCR and pyro SNP analysis	Forward	CCAATCTTCAAACACCTCCTG
	Reverse	CCACTGGGCCTGTTTTGG
	Sequence	CAAAACAATAAGAAGGGA
SOX2 Binding at Scmh1- positive control (from: Boumahdi *et al*. PMID: 24909994)	Forward	AGCCAACAACGGCACTAAGA
	Reverse	CATGGAAGGATGGAGTGGGT
SOX2 Binding at Ceacam1- negative control (from: Boumahdi *et al*. PMID: 24909994)	Forward	CTGAAGAGTGGATGGTAAGG
	Reverse	CACACCGCAAGGTCAGAATG
ZFP281 Binding site #1a at TRE With biotin for qPCR and pyro SNP analysis	Forward	CCAATAGCATGGAGCTCAGGTTAG
	Reverse	CGAGACCGGTGTTGTAAGTGC
	Sequence	GTGCATGCAGGATCGC

ZFP281 Binding site #1b at TRE With biotin for qPCR and pyro SNP analysis	Forward	AGTGAGGGAAGGGCTGCATTA
	Reverse	GCTAACCTGAGCTCCATGCTATTG
	Sequence	ATGCCTTGAGCACAG
ZFP281 Binding site #2 at TRE With biotin for qPCR and pyro SNP analysis	Forward	TGACTTCCTTCAGCCACAGTTC
	Reverse	GATGCTCAGAAAGGCAGTGG
	Sequence	GTGCATGCAGGATCGC
ZFP281 Binding site #3 downstream of TRE With biotin for qPCR and pyro SNP analysis	Forward	TGATTCACCACAATTCGTTCTG
	Reverse	CGGTCATCAATTTGCCATTTCT
	Sequence	CGTTCTGGTTTGTGATTA
ZFP281 Binding site #4 upstream of Gtl2 With biotin for qPCR and pyro SNP analysis	Forward	TTCGTGTTAGCGAGACAACATT
	Reverse	CCACCCCCAGGAGAAAGTATATC
	Sequence	TGTTAGCGAGACAACATT
ZFP281 Binding site #5 at Gtl2-DMR With biotin for qPCR and pyro SNP analysis	Forward	ATGGGGGTGCATAGCGAC
	Reverse	AGACTCCAATAGCCCAACCACC
	Sequence	GCCCAACCACCTGAG
ZFP281 Binding site #6 at Gtl2-DMR	Forward	TCATCTGTACCCCTCCCATTG
	Reverse	AGGGAAATGGGTAGGGGC
ZFP281 Binding at Otx2 promoter- positive control (from: Huang *et al*. PMID: 29168693)	Forward	CCCGAGGAAAGCAATTACAA
	Reverse	AAATGGCCCCAATCAAGTTT
ZFP281 Binding at Otx2- negative control (from: Huang *et al*. PMID: 29168693)	Forward	AATGCCTGGCTAAAACTGGA
	Reverse	CTTCAATGCTGACTGCTTGG
ZFP281 Binding at T promoter- positive control (from: Huang *et al*. PMID: 29168693)	Forward	GCTTCAAGGAGCTAACTAACGAG
	Reverse	CCAGCAAGAAAGAGTACATGGC
ZFP281 Binding at T- negative control (from: Huang *et al*. PMID: 29168693)	Forward	CCTAGCTGTTTTGGCTTTGG
	Reverse	GCTGAGGCAGGAGAATCAAG

73 **Table S6**- gRNAs and genotyping primers for CRISPR in mice

Target	Primer	Sequence 5' → 3'
5' IG-TRE	sgRNA 1	CAGTCTAGCAAGGCTACAGC, PAM: AGG
	sgRNA 2	ATATAGTCCACAGTCTAGCA, PAM: AGG
3' IG-TRE	sgRNA 3	ATATAGTCCACAGTCTAGCA, PAM: TGG
	sgRNA 4	TTGGTTTACCAGTTCCTCGT, PAM: GGG
single-stranded oligo donor (ssODN)	CCTTCAGCCACAGTTCCCAGGCAGCCCTTGGCTGGAGGCATTGCGATCCT GCATGCACTTACAACACCGGTCTCGGAGTTCTTGTCTCCCTGCTGTAGCCG TGTCTGCTGCAGTGTGCCAGTTTTTTCGTGGTACACAGAGATGCTTTTGTTG ACCACAACCCTTGGCATCCTGGCTCACCGTTGTCTAGAAACACTTTT	
IG-TRE Δ550	Genotyping forward	GCCGCTATGCTATGCTGTTTC
	Genotyping reverse	GTGCCTCAGTGCCGTGTTAG
	Sequencing forward	CCCAGGACCTCCAATAGCAT
Off target 1: chr12:54356165-54356187 (Intergenic) ATA**C**AG**G**C**T**ACAGTCTAGCA TGG	Off target 1 forward	TACAGAGGGACTCTGGTATCTACA
	Off target 1 reverse	GGGATTTGTGGGTCTCGCA
	Off target 1 sequence forward	TCTACATGCAAAAGATGAACTGC
Off target 2: chr12:109960424-109960446 (Intergenic) A**A**A**G**AGT**T**C**C**CAGTCTAGCA GGG	Off target 2 forward	ATGACCTCAGTCTGCAAGGC
	Off target 2 reverse	TCCAGGGCAGGCATTACAAG
	Off target 2 sequence forward	ACCTGGAGTGGTGAGCCTAT
IG-TRE copy count taqman assay	Forward	CCCAGGTTGCCCTTAGTAAAT
	Reverse	CCAGAGTTTCTCGGTCTTCTTT
	Probe (FAM)	TGCGGGAGACACCCTGTTTCTTTA
Dot1l copy count reference taqman assay	Forward	GCCCCAGCACGACCATT
	Reverse	TAGTTGGCATCCTTATGCTTCATC
	Probe (VIC)	CCCAACAGGCCTGGATTCTCAATGC

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