

Table S1: Tead4 dependent mtDNA encoded relative nascent transcript levels

Name	Control (%)	TEAD4KD (%)
Complex I		
ND1	100	51.5
ND2	100	30.9
ND3	100	30.6
ND4	100	32.7
ND5	100	44.9
ND6	100	52.5
Complex III		
MT-CYB	100	44.0
Complex IV		
MT-CO1	100	40.5
MT-CO2	100	46.9
Complex V		
ATP6	100	37.4
Transcript Joining two genes		
12SRNA-TrnV	100	39.0
TrnL-ND1	100	42.8
MT-CO1-TrnS	100	83.5
ATP6-MT-CO3	100	33.6
ND6-TrnE	100	65.1
MT-CYB-TrnT	100	44.0

To detect splicing, we used primers that amplify cDNA including the junction between the two genes. For example, 12SRNA-TrnV; primer pair amplifying 12SRNA and the adjacent transfer RNA (Val) (*tRNA(V)*). mtDNA genome organization and primer pairs used for real time PCR (1-7) (E). RT PCR showing POLRMT occupancy along mitochondrial genome, which is affected upon TEAD4 knockdown (F).

Table S2: List of Antibodies

Primary antibody			
	Species raised in	Vendor	Catalog number
anti-TEAD4	Mouse	Abcam	Ab58310
anti-Lamin B	Goat	Santa Cruz Biotechnology	Sc-6216
anti-TFAM	Rabbit	Santa Cruz Biotechnology	Sc-28200
anti-CYTB	Rabbit	Santa Cruz Biotechnology	Sc-11436
anti-TOM20	Rabbit	Cell Signaling	#13929
Anti-POLRMT	Rabbit	Abcam	Ab93102
Anti-POLRMT	Mouse	Abcam	ab167368
Anti-POLRMT	Rabbit	Gift (mouse specific)	(Kuhl et al., 2014)
Anti-Actin	Mouse	Sigma	#AC-74
Purified IgG	Mouse	BD Biosciences	#554121

Secondary antibody			
	Species raised in	Vendor	Catalog number
Alexa Fluor 568 anti-goat IgG	Donkey	Life Technologies	A11057
Alexa Fluor 488 anti-mouse IgG	Donkey	Life Technologies	A21202
anti-Goat IgG-HRP	Donkey	Santa Cruz Biotechnology	Sc-2033
anti-Mouse IgG-HRP	Goat	Santa Cruz Biotechnology	Sc-2005
anti-Rabbit IgG-HRP	Goat	Santa Cruz Biotechnology	Sc-2004

Table S3: List of primers used for ChIP, transcript and genotyping

RT PCR primers for mouse transcript		
	Forward Primer (5'-3')	Reverse Primer (5'-3')
TEAD4	ATCCTGACGGAGGAAGGCA	GCTTGATATGGCGTGCAT
18SrRNA	AGTTCCAGCACATTTGCGAG	TCATCCTCCGTGAGTTCTCCA
ND1	CATTGCAGACGCCATAAAA	TGATAGGGTGGGTGCAATAA
ND2	AACCCACGATCAACTGAAGC	GTACGATGCCAGGAGGATA
MT-CO1	GCAACCCTACACGGAGGTAA	CCGGTTAGACCACCAACTGT
MT-CO2	TCTCCCCTCTACGCATTC	TCATTGGTGCCTATGGTTT
MT-CYB	TGAGGGGGCTCTCAGTAGA	TAGGGCCGCGATAATAATG
ATP6	CCTTCCACAAGGAACTCCAA	TGCTAATGCCATTGGTGAA
ND3	GCATTCTGACTTCCCCAAAT	TGCAGAGCTGTAGGGTCAA
ND4	GGAACCAAACGTAAACGCCCTA	ATGAGGGCAATTAGCAGTGG
ND5	TAGAAGGCCCTACCCAGTT	AGTCGTGAGTGGGTGGAATC
ND6	TTGGCATTAAAGCCTTCACC	TCCACCAAACCTAAAACCA
ATP6-MT-CO3	GCCTACGTATTCACCCCTCCT	CAGTTAATGGTCATGGACTTGG
TrnL-ND1	AGCCAGGAAATTGCGTAAGA	TAGAATGGGGACGAGGAGTG
MT-CYB-TrnT	TCTTATACCAATCTCAGGAATTATC G	TTCATTTAGGTTACAAGACCA
12SrRNA-TrnV	CCGTTATGAGAGGAGATAAGTCG	GGGTGTAGGCCAGATGCTT
MT-CO1-TrnS	CCCTCCACCATATCACACATT	GGCTTGAACCAATTAGGG
ND6-TrnE	AATGCTAACCAAGACAACCA	TCATGTCATTGGTCGCAGTT
POLRMT	TGGGCGAAAAGCTAGAGG	GTGAAGGGTCCAGAACTCCTG
Nrf1	TATGGCGGAAGTAATGAAAGACG	CAACGTAAGCTCTGCCTTGTT
Sirt3	ATCCCGACTTCAGATCCCC	CAACATGAAAAAGGGCTTGGG
Yap1	TGGCCAAGACATCTCTGGT	GCCATGTTGTTGTCTGATCG
Pgc1a	TATGGAGTGACATAGAGTGTGCT	CCACTTCATCCACCCAGAAAG
Cdx2	GCAGTCCCTAGGAAGCCAAGTGA	CTCTCGAGAGGCCAGTGTG
Gata3	CGGGTTCGGATGTAAGTCGA	GTAGAGGTTGCCCGCAGT
Elf5	ATGTTGGACTCCGTAACCCAT	GCAGGGTAGTAGTCTTCATTGCT
Gcm1	CTGACTGGTCCAGGAGTGG	TGTCGTCCGAGCTGTAGATG
Ascl2	AAGTGGACGTTGCACCTCA	AAGCACACCTTGACTGGTACG
Prl3b1	GGGGCACT CCTGTTGCTGGCA	GGACTTGCTCGCTGTTCTGGA
TFAM	ATTCCGAAGTGTGTTCCAGCA	TCTGAAAGTTTGATCTGGGT
ChIP primers for rat Genome		
1	CCTGTCCCCAATTGGTCTCT	TATAGTCACCCCCAGGACGA
2	TCCCGACACAAAATCTTCC	TGCTTGCTTGTATTAAAGCTACA
3	CGGCGTAAACGTGCCACT	ATTACTTCGTTATTGGCTTAGG
4	ATACCGCCATCTCAGCAAA	CCATTCTTCCGCTTCATT
5	TATGACCAACTAATGCACCTCCT	GGTCAATTCTATTGTCCTAGAAA
6	GAAGCCACTCTAATCCCAACA	GGGATGGAGCCAATTAGTGT

ChIP primers for mouse Genome		
1(ND6)	AAACCTCTATAATCACCCCAAT	GGGATGTTGGTTGTGTTGG
2(MT-CYB)	GCAATCGTTCACCTCCTCTT	TTGTATAGTAGGGGTGAAATGGAA
3(D-Loop)	ATCAAACCTATGTCCTGATCAAT	TTTGGTTCACGGAACATGA
4(12S rRNA)	CGGCGTAAAACGTGTCAACT	AGAATTACTTCGTTATTGAGTTAG
5(ND1)	CATTTGCAGACGCCATAAAA	TGATAGGGTGGGTGCAATAA
6(MT-CO1)	GCAACCCTACACGGAGGTAA	CCGGTTAGACCACCAACTGT
7(MT-CO3)	TAACCCTGGCCTACTCACC	ATAGGAGTGTGGTGGCCTTG
Genotyping primers		
TEAD4FF	CTAGCATTAAAGGAATGTCCCGA	CGTATAGCATACATTATACGAAG
TEAD4KO	CTCAACATACAGTTGAAGCAC	GTGTTCTTAGAGGTACAGTCA
Cre	CATTTGGGCCAGCTAACAT	CCCGGCAAAACAGGTAGTTA
Internal Control		
Interleukin 2	CTAGGCCACAGAATTGAAAGATCT	GTAGGTGGAAATTCTAGCATCATCC
For mtDNA quantitation		
mtND2	CGCCCCATTCCACTTCTGATTACC	TTAAGTCCTCCTCATGCCCTATG
β 2microglobulin	CCTTCAGCAAGGACTGGTCT	CAGTCTCAGTGGGGTGAAT

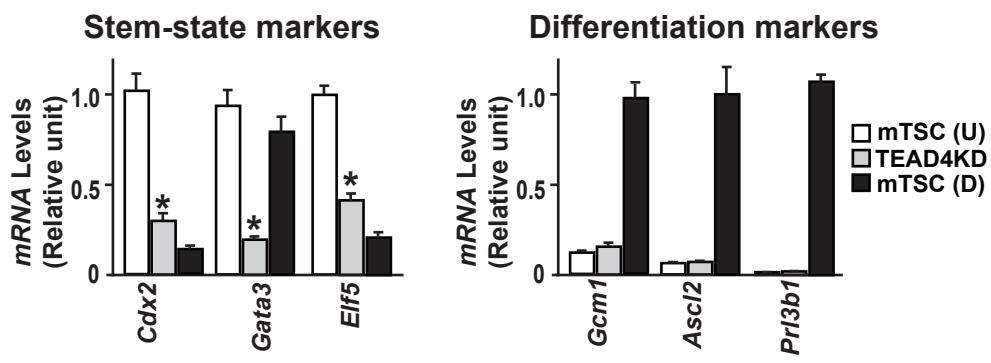


Figure S1: RT-PCR analyses showing mRNA expressions of stem-state and differentiation markers in control and TEAD4KD mouse TSCs. For stem-state markers, mRNA expression in undifferentiated mouse TSCs [mTSC(U)] were used as standard. For differentiation markeres, mRNA expression in differentiated mouse TSCs [mTSC (D)] were used as standard (mean + SEM, three independent experiments, $p \leq 0.01$).

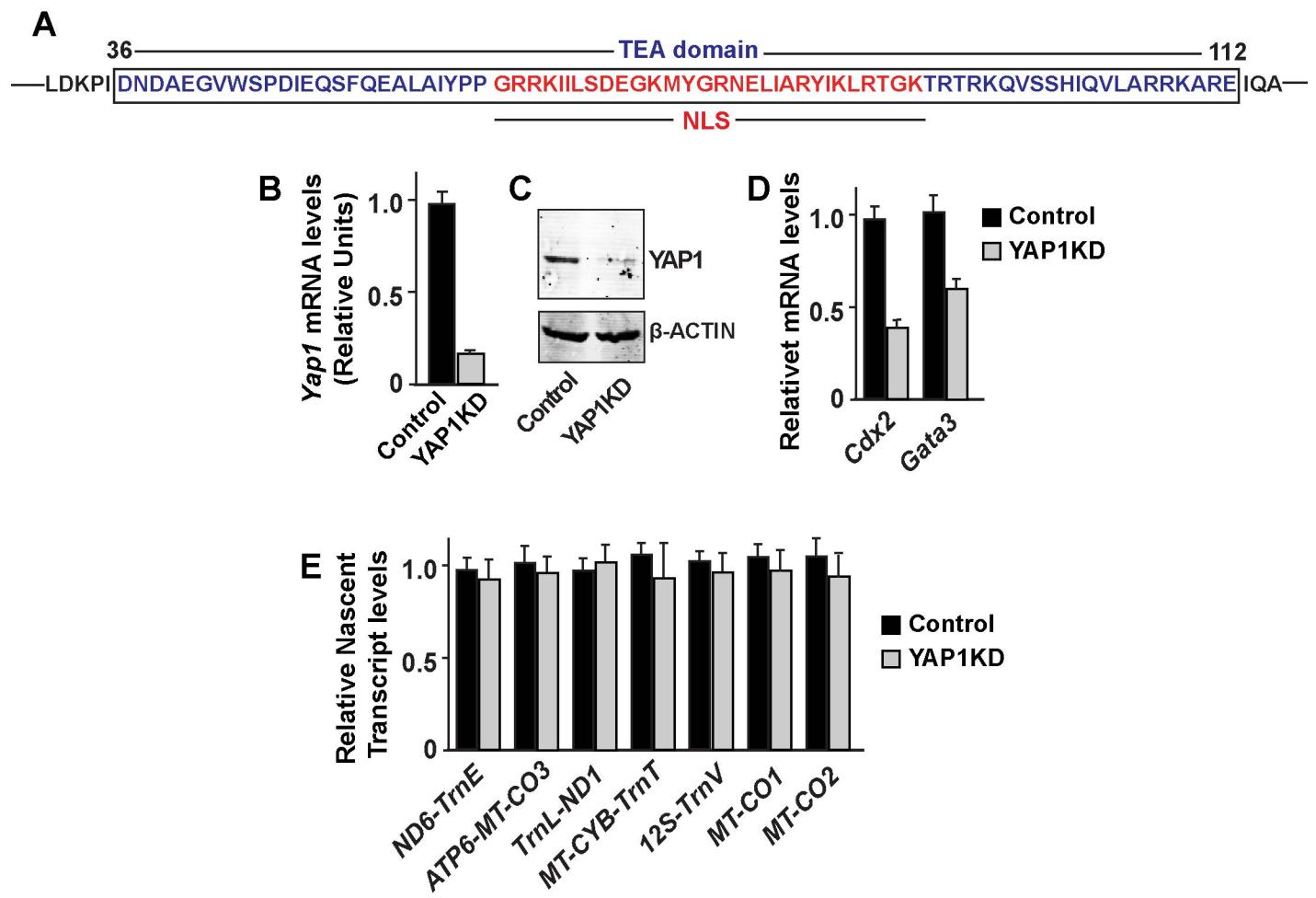


Figure S2: (A) A region of mouse TEAD4 protein showing amino acid sequences with overlapping TEA domain (in blue) and NLS (in red). (B and C), mRNA and western blot analyses showing efficient knockdown of YAP1 expression in mouse TSCs. (D) RT-PCR analyses showing loss of mRNA expression of TSC marker genes in YAP1-depleted mouse TSCs. (E) RT-PCR analyses showing relative transcript levels of mtDNA encoded ETC components in control and YAP1-depleted mouse TSCs. (mean + SEM, three independent experiments).

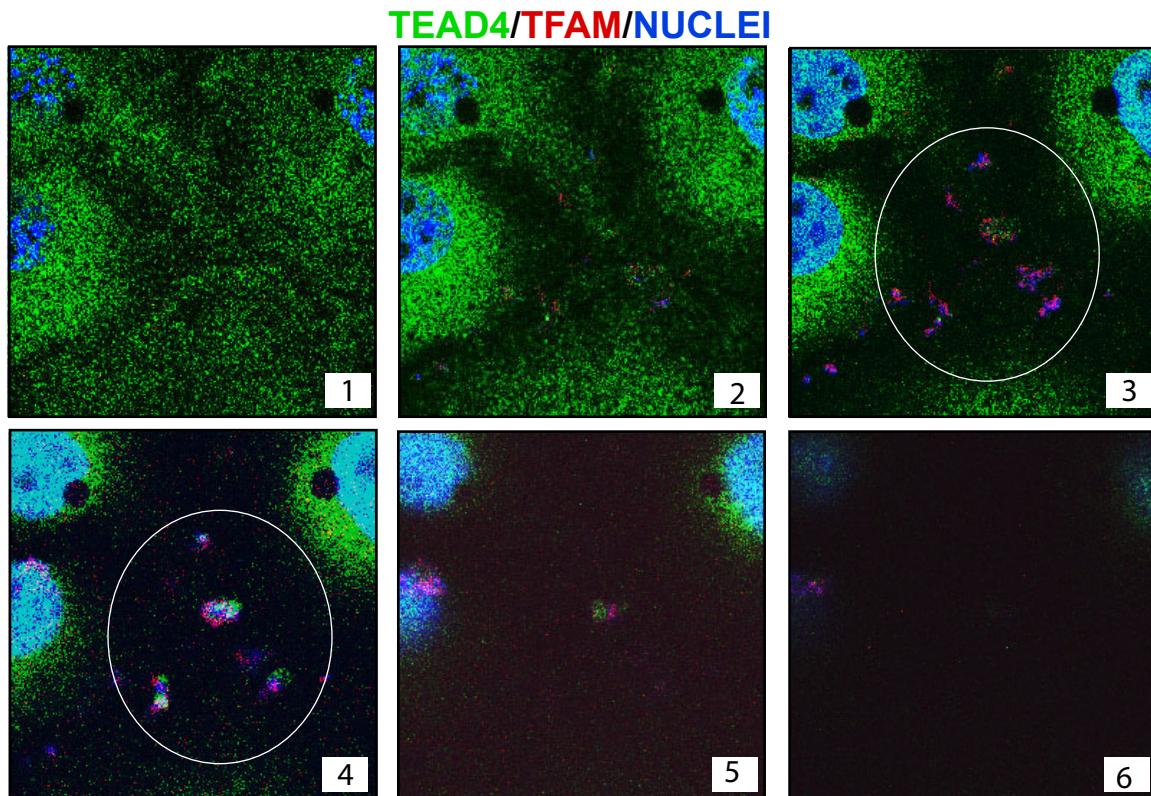


Figure S3: Z-Stack confocal images showing localizations of TEAD4 and TFAM in mouse TSCs. Six merged stacks are shown. TEAD4 localization in nuclei are evident from stacks 1-4. Cytoplasmic localization of TEAD4 are evident in stacks 1-2, whereas mitochondrial localization is evident from stacks 3 and 4 (white ring). Unlike TEAD4, TFAM is predominantly localized within mitochondria (Stacks 3 and 4). Z-stack 4 is used for panels figure 5A of the main manuscript.

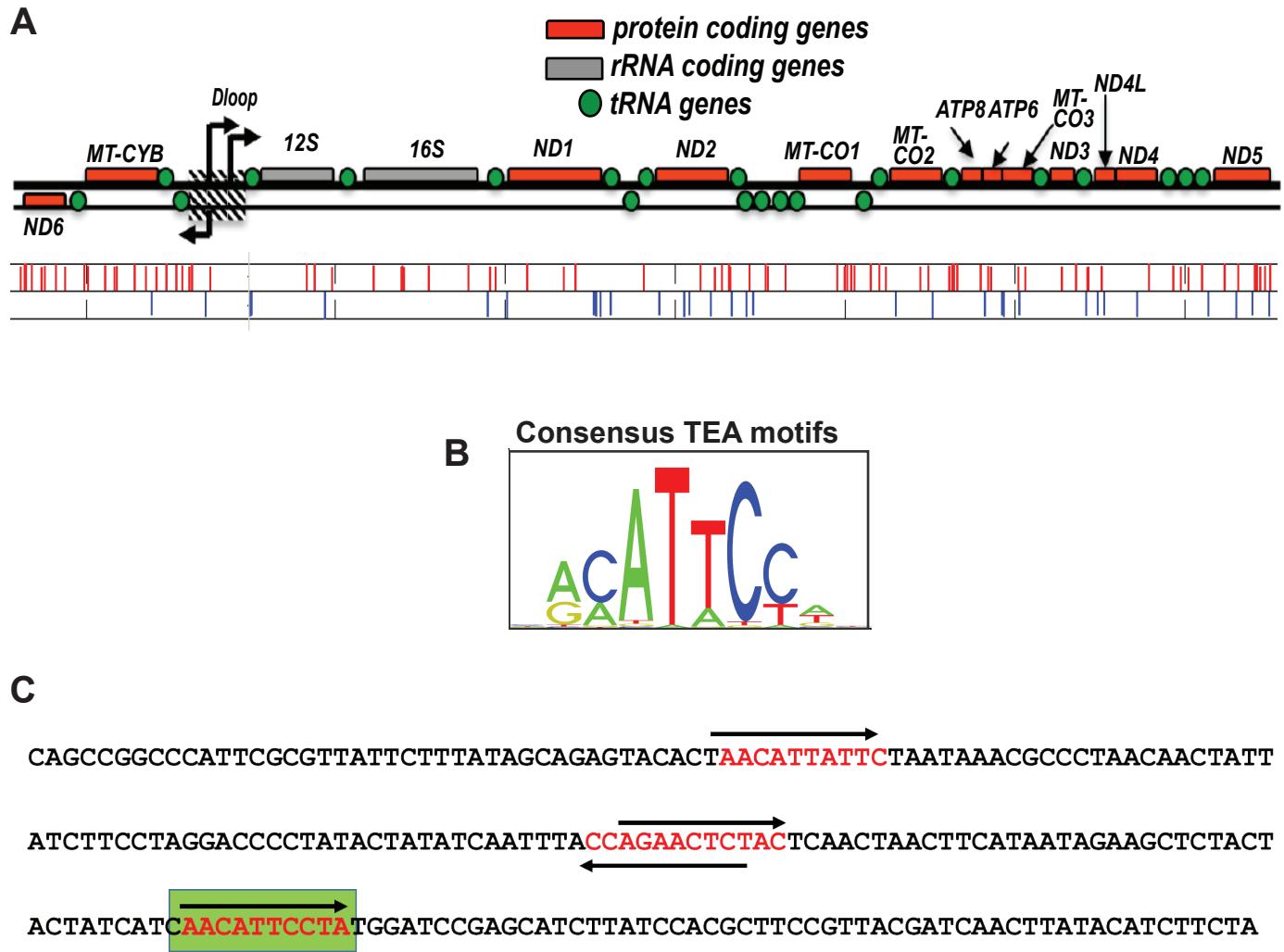


Figure S4: Endogenous TEAD4 physically interacts with POLRMT in mouse TSC.
 (A-B) mouse mtDNA genome showing putative TEA motifs, identified using the JASPAR database. (C) 200 bp *mtND1* fragment from mouse mtDNA genome, which was used for EMSA. PutativeTEA motifs are highlighted.

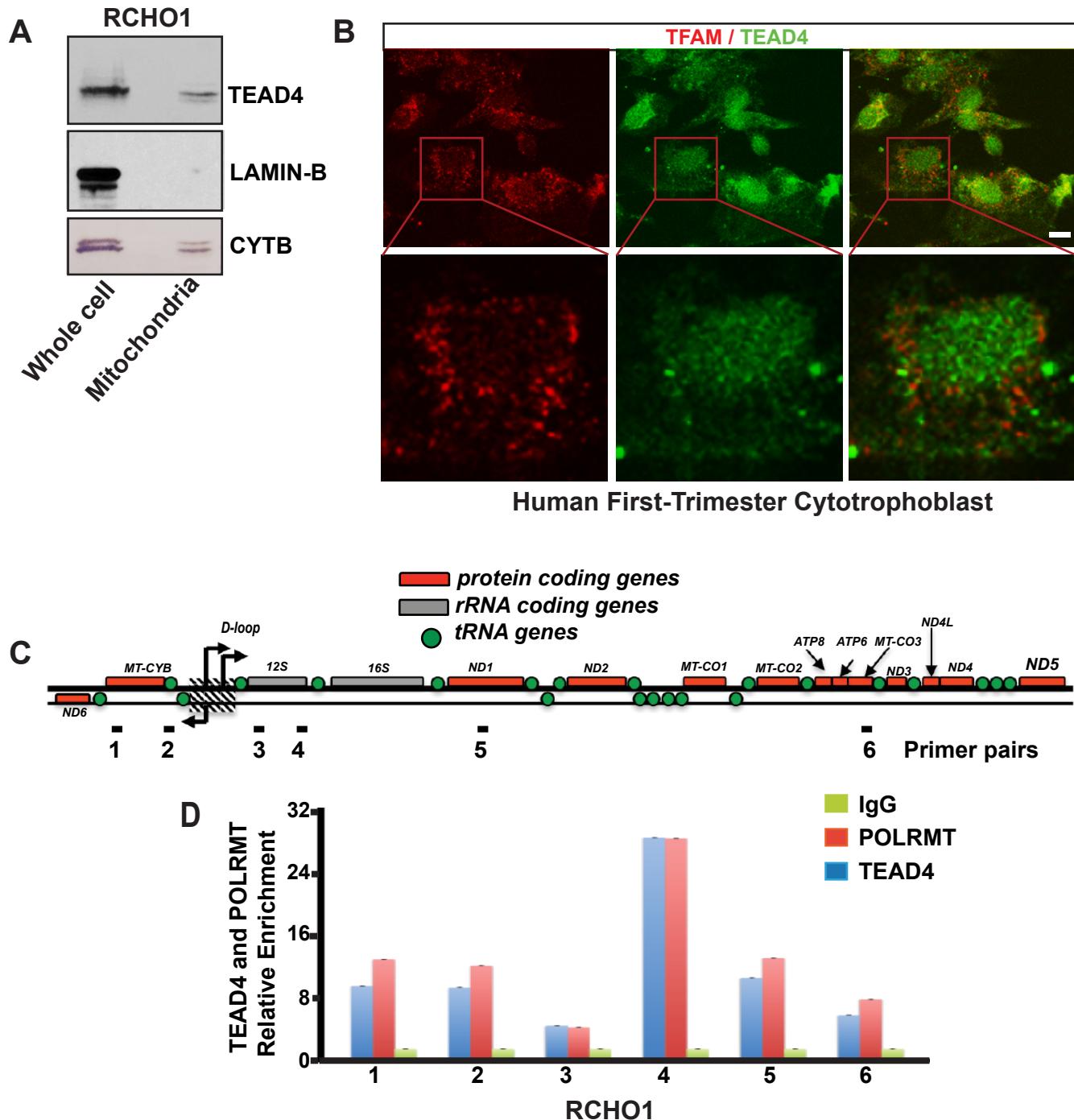


Figure S5. Endogenous TEAD4 localizes to mitochondria in rat trophoblast stem cell line (RCHO-1) and human primary cytotrophoblast cells. (A) Western blot showing TEAD4 in mitochondrial fraction in rat RCHO-1 cells. (B) Human first trimester cytotrophoblasts were stained with TEAD4 (green) and mitochondria specific transcription factor TFAM (red) showing TEAD4 localization in mitochondria (scale bar: 10μm). (C) Schematic diagram of mtDNA and localization of primer pairs (1-6) that were used for quantitative ChIP analyses in RCHO-1 cells. (D) Plot shows quantitative assessment of TEAD4 and POLRMT occupancy at different regions of mtDNA in RCHO-1 cells.

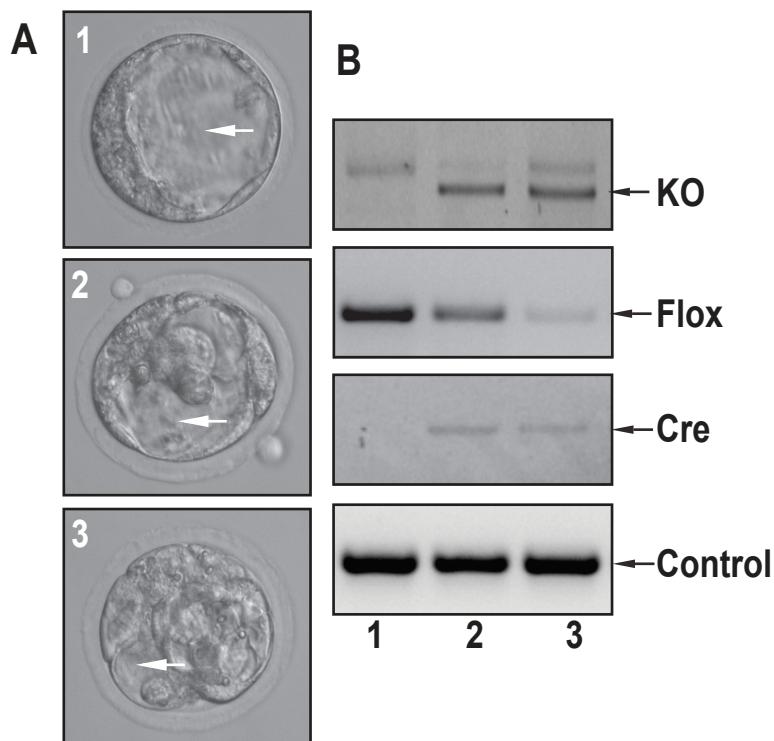


Figure S6: Differential recombination efficiency of *Tead4*-floxed allele is associated with differential blastocyst maturation. (A and B) In-vitro culture and genotyping of mouse *Tead4^{FF}:UbcERT2-Cre* or *Tead4^{FF}* preimplantation embryos in the presence of tamoxifen. Representative images of blastocysts with mixed phenotype are shown. *Tead4^{FF}* embryo formed a matured blastocyst (Embryo 1, white arrow in A) in the presence of tamoxifen. In contrast, *Tead4^{FF}:UbcERT2-Cre* embryos showed mixed phenotype with tamoxifen due to differential recombination efficiency of the floxed *Tead4* alleles. Recombination efficacy was low in embryo 2, resulting in maintenance of the floxed *Tead4* allele (panels 2 in B) and matured blastocyst with a defined but less expanded blastocoel cavity. High recombination efficiency in embryo 3 resulted in an immature blastocyst with very small blastocoel cavity (White arrow in embryo 3 in A).