Stem Cell Reports, Volume *5*

Supplemental Information

α-5 Laminin Synthesized by Human Pluripotent Stem Cells Promotes Self-Renewal

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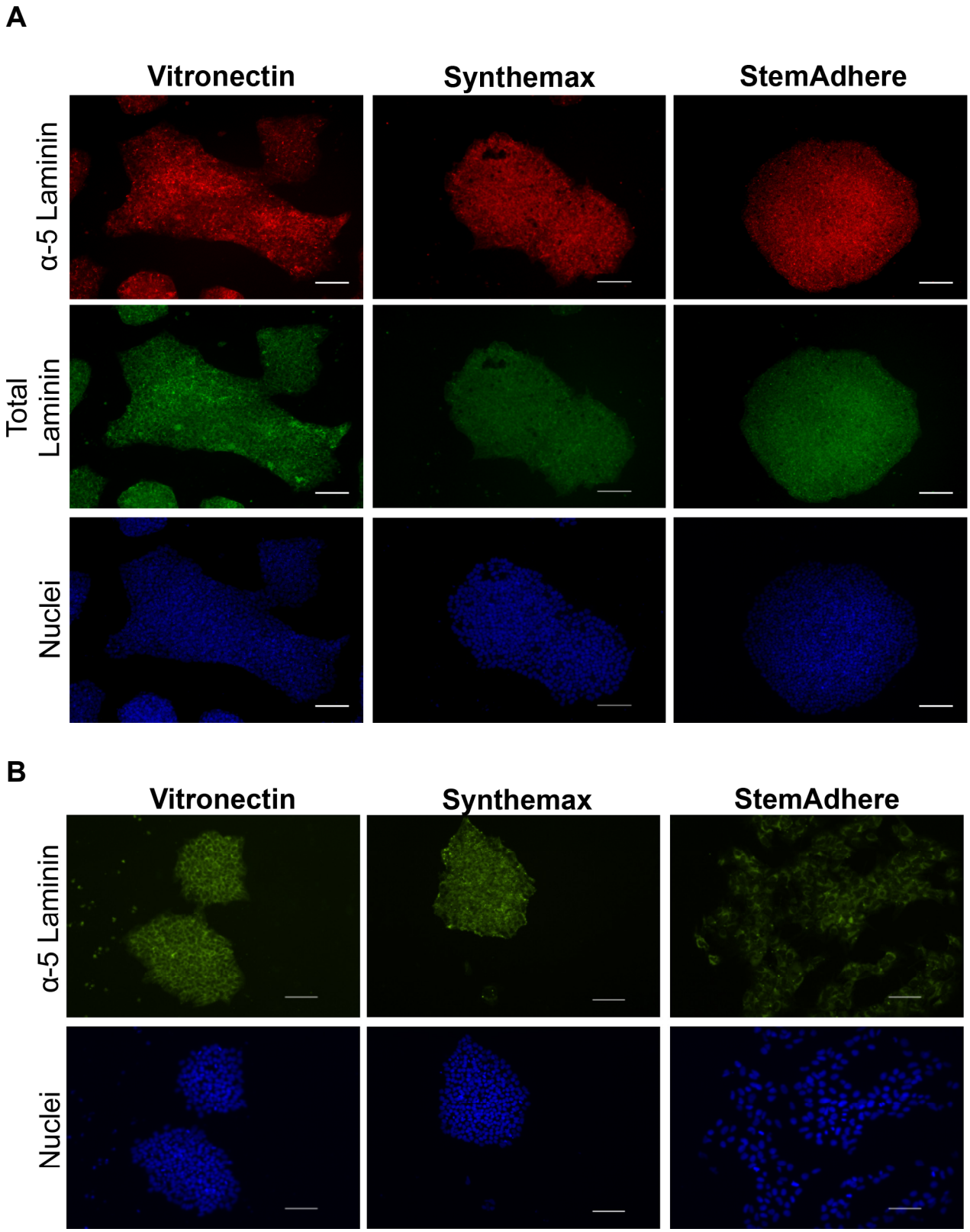

Figure S1: ECM deposition by hPSCs on three defined substrates. (A) H9 hESCs and (B) 19-9-11 iPSCs were cultured on the indicated substrates for 5 days in E8 medium. Aberrant morphology on StemAdhere is due to the low seeding density (25,000 cells per well) employed in this assay and is no longer present when cells are plated at higher (>50,000 cells per well) densities. Scale bars = 100 μm. n=3 independent samples/condition

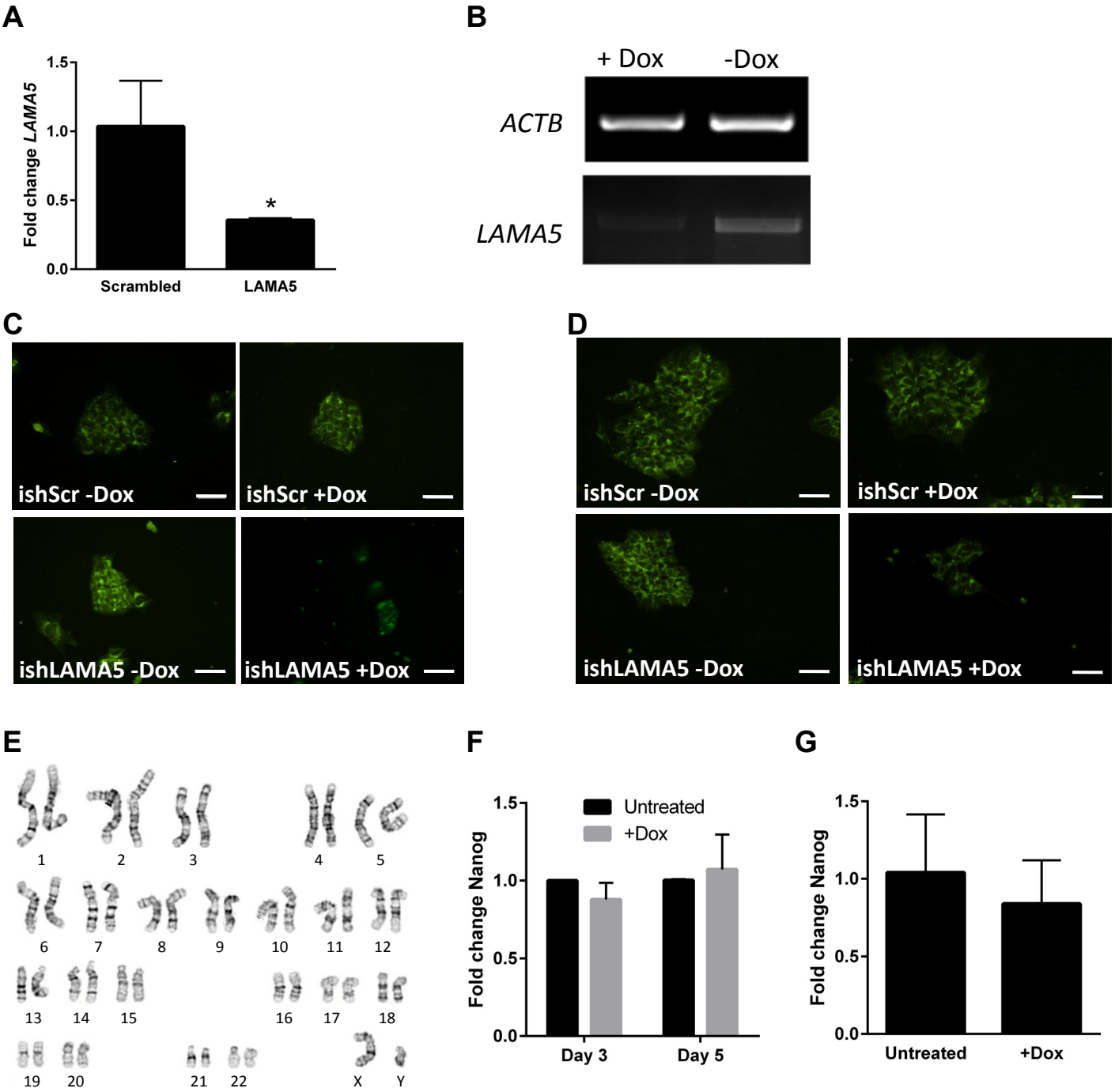

Figure S2: Generation of H9 and 19-9-11 doxycycline (dox) inducible lines. (A) *LAMA5* expression by H9s transfected with *LAMA5* siRNA at 48 hrs post-transfection. (B) RT-PCR showing reduction in *LAMA5* expression upon addition of 5μM dox. (C) α-5 laminin (green) deposition by H9 *LAMA5* knockdown (ishLAMA5) and scrambled (ishScr) cells. (D) α-5 laminin (green) deposition by 19-9-11 *LAMA5* knockdown (ishLAMA5) and scrambled (ishScr) cells. (E) Karyotype analysis of 19-9-11 ishLAMA5 cells. (F) qRT-PCR demonstrating *NANOG* expression by H9 ishLAMA5 cells cultured on Synthemax. (G) qRT-PCR demonstrating *NANOG* expression by 19-9-11 ishLAMA5 cells cultured on Synthemax. *p<0.05 vs. Scrambled. Scale bars = 50 μm. n=3 independent samples/condition

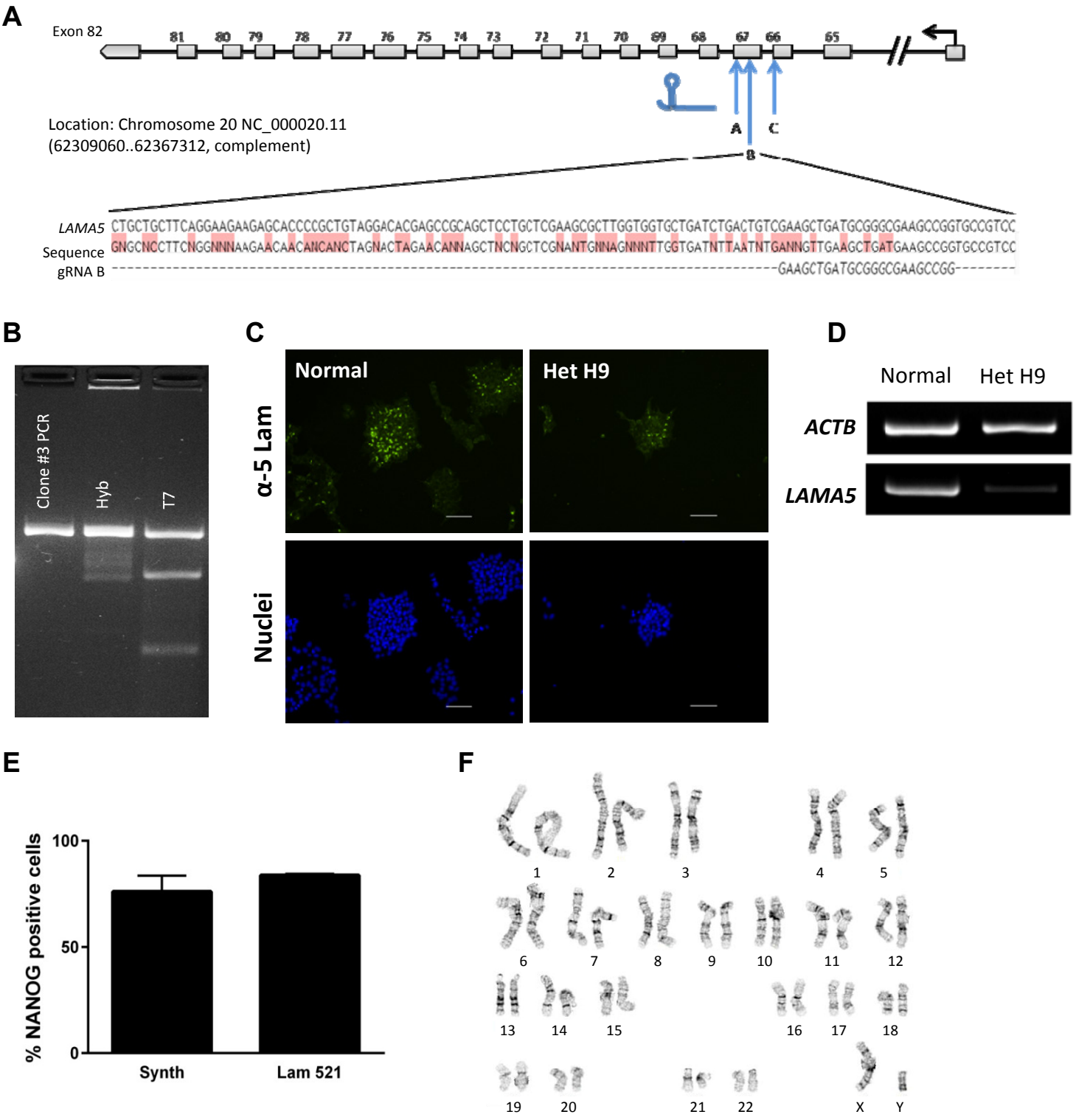

Figure S3: Generation of H9 Cas9 heterozygous *LAMA5* knockout line. (A) Schematic showing gRNA locations and Sanger sequence for Het H9 line. (B) T7 Assay demonstrating heterozygosity of the Het H9 line. (C) Immunocytochemical staining for α-5 laminin deposition after 3 days of H9 and Het H9 culture on Synthemax. (D) *LAMA5* expression by Het H9 cells on Synthemax, as measured by RT-PCR. (E) NANOG expression by Het H9 cells, as measured by flow cytometry after 5 days of culture on the indicated substrates. (F) Karyotype analysis of homozygous *LAMA5* 19-9-11 knockout line. Scale bars = 100 μm. n=3 independent samples/condition

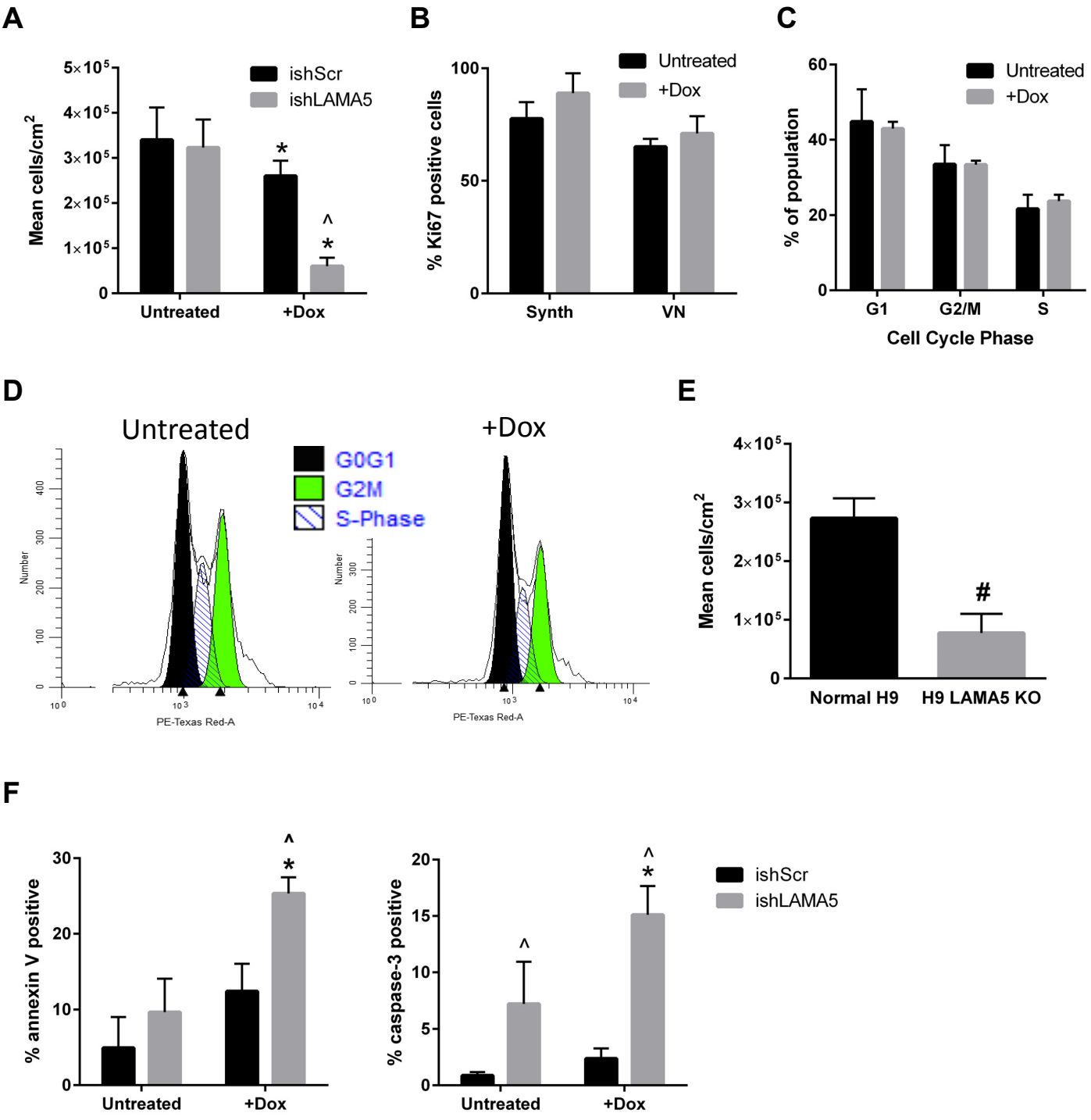

Figure S4: Characterization of cell number and apoptosis in H9 and 19-9-11knockout lines. (A) Mean cell density of ishLAMA5 and ishScr H9 cell lines after 5 days of culture on Synthemax. Cells were seeded at 2,630 cells/cm^2. (B) Ki67 expression by H9 ishLAMA5 cells, as measured by flow cytometry after 5 days of culture on the indicated substrates. (C) Cell cycle distribution of 19-9-11 ishLAMA5 cells cultured on Synthemax for 3 days. (D) Example distribution fits for cell cycle analysis. DNA content was measured using propidium iodide and distribution was determined using ModFit LT software. (E) Normal and Het H9 mean cell density after 5 days of culture on Synthemax. Cells were seeded at 2,630 cells/cm^2. (F) Annexin V and cleaved caspase 3 expression by ishLAMA5 and ishScr H9 cell lines, as measured by flow cytometry after 5 days of culture on Synthemax. *p< 0.001 vs. untreated; ^p<0.001 vs. ishScr; #p<0.001 vs. Normal H9. n=3 independent samples/condition

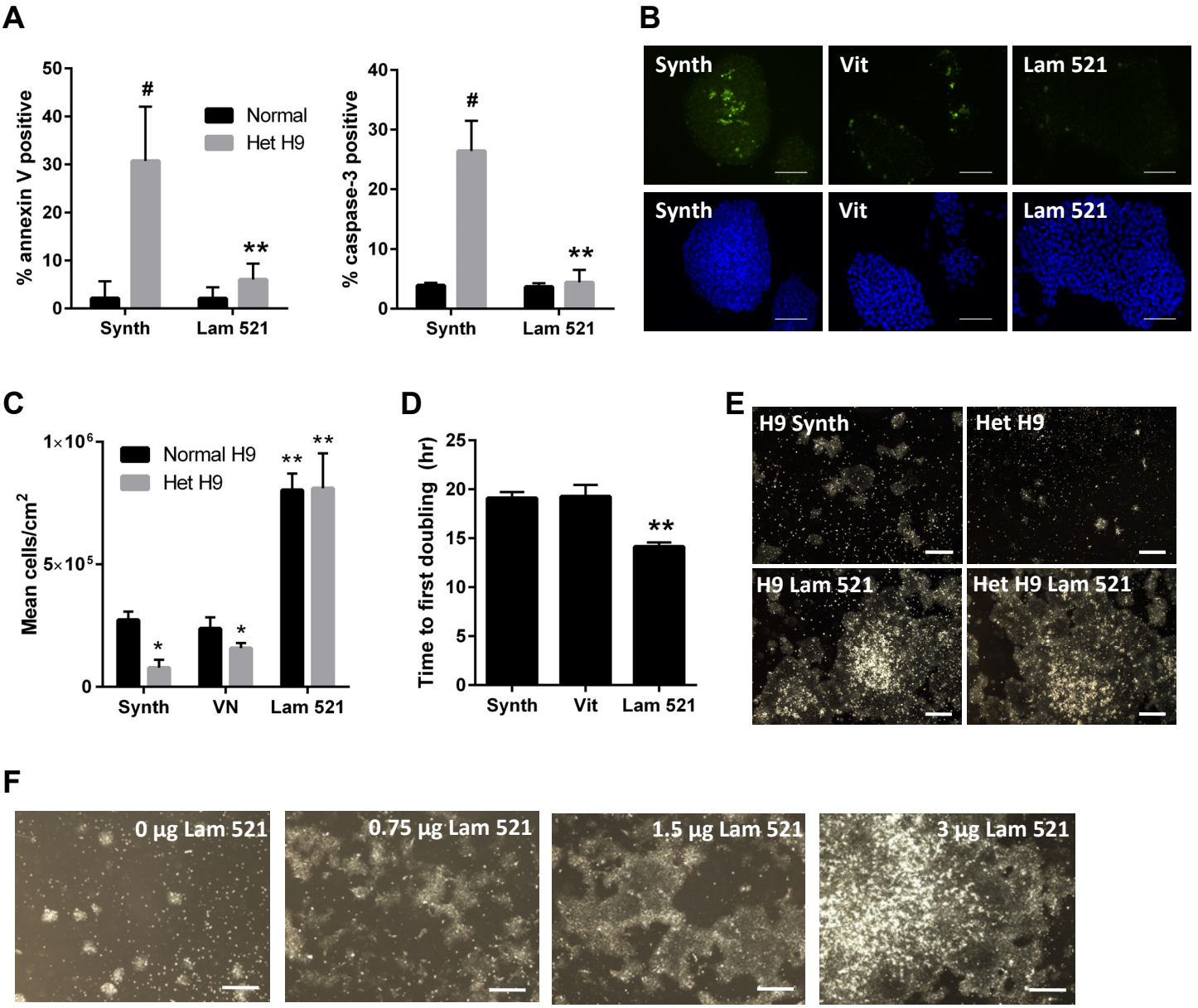

Figure S5: Partial restoration of self-renewal in Het H9s via culture on exogenous laminin 521. (A) Annexin V and cleaved caspase 3 expression by normal and Het H9 cells, as measured by flow cytometry after 5 days of culture on the indicated substrates. (B) Immunocytochemical staining showing cleaved caspase-3 expression (green) in Het H9 cells on the indicated substrates after 3 days of culture; scale bar = 100 µm. (C) Normal and Het H9 mean cell density after 5 days of culture on indicated substrates. Cells were seeded at 2,630 cells/cm^2. (D) Average time from seeding to first observed doubling in Het H9 cells cultured on the indicated substrates. Cells were synced with nocodazole 24 hrs prior to seeding. (E) Brightfield images of normal and Het H9 cells cultured on Synthemax or laminin 521 for 3 days; scale bar = 500 µm. (F) Brightfield images of Het H9 cells cultured on increasing densities of laminin-521 for 5 days, demonstrating dose-response of cell rescue by exogenous laminin-521; scale bar = 100 µm. *p<0.001 vs. Normal H9; #p<0.001 vs. Normal H9; **p<0.001 vs. same cells on other surface(s). n=3 independent samples/condition

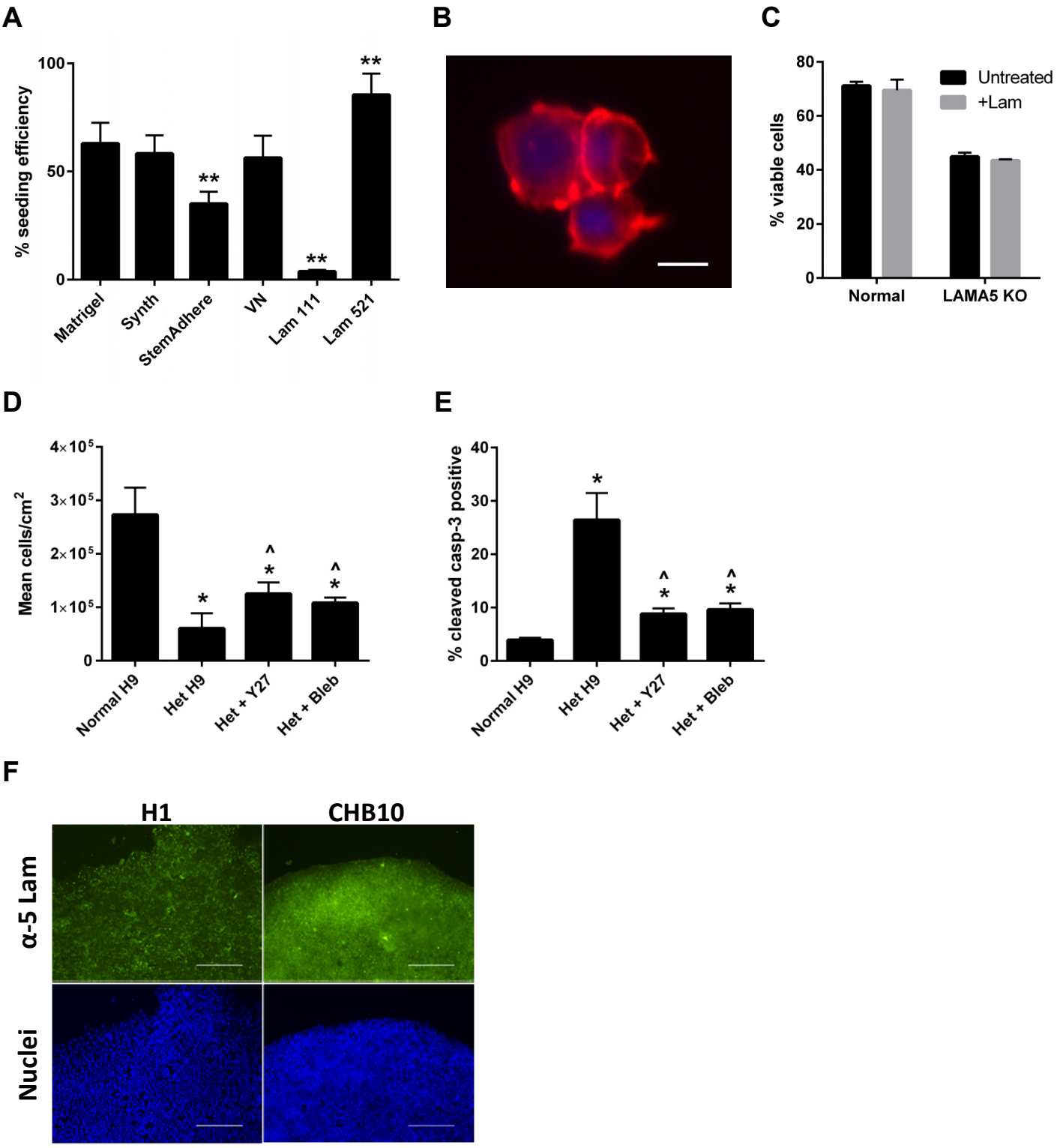

Figure S6: Partial restoration of self-renewal in Het H9s via inhibition of contractility. (A) Attachment efficiency (expressed as % of initially seeded cells) of normal 19-9-11 cells seeded on indicated substrates in the presence of 5 μM Y27639. (B) F-actin immunocytochemistry illustrating blebbing in Het H9 cells cultured on Synthemax for 48 hrs; scale bar = 10 μm. (C) Viability of normal and KO 19-9-11 cells cultured in suspension for 3 hours with or without soluble laminin 521. (D) Mean cell density and (E) cleaved caspase-3 expression for Het H9 cells cultured on Synthemax with the indicated inhibitors. (F) α-5 laminin deposited by H1 and CHB10 hESCs after 5 days of culture on TCPS coated with the defined synthetic polymer PMEDSAH. Scale bars = 100 μm. *p<0.001 vs. Normal H9; ^p<0.001 vs. untreated Het H9 condition; **p<0.001 vs. culture on Synthemax. n=3 independent samples/condition

Supplemental Table S1: Sequences of siRNA against LAMA5 and shRNA LAMA5 construct cloned into Addgene plasmids 12260 and 12259

RNAi	Sequence
siRNA	
Sense	5’ ACAGGGCAGTGCTACTGTAtt 3’
Antisense	5’ TACAGTAGCACTGCCCTGTga 3’
shRNA	
Sense	5’CCGGACAGGGCAGTGCTACTGTATTCTCGAGAATACAGTAGCACTGCCCTGTTTTTG 3’
Antisense	5’AATTCAAAAAACAGGGCAGTGCTACTGTATTCTCGAGAATACAGTAGCACTGCCCTG 3’

Supplemental Table S2: Sequences and chromosomal locations of all Cas9 *LAMA5* guide RNAs used

Guide RNAs	
gRNA A	
Chromosome location	chr20:60888167-60888189
Gene name	LAMA5
23 bp Target Sequence	GCGGCTCGTGTCCTACAGCGGGG
gRNA B	
Chromosome location	chr20:60888231-60888253
Gene name	LAMA5
23 bp Target Sequence (no spaces)	GAAGCTGATGCGGGCGAAGCCGG
gRNA C	
Chromosome location	chr20:60888373-60888395
Gene name	LAMA5
23 bp Target Sequence (no spaces)	GGCCTTGTGCCCGGTGCGTGTGG

Supplemental Table S3: Inhibitors tested against *LAMA5* KO cells

Name	Target	Concentration used
Bax inhibiting peptide	Bax	200 µM
PD 98059	MEK	1 µM
SU 5402	VEGFR and FGFR	2 µM
Y27632	ROCK	5 µM
Blebbistatin	Myosin contractility	10 µM
Z-IETD-FMK	Caspase 8	40 µM
NS 3694	Caspase 9	50 µM

Supplemental Table S4: Sequences of all *LAMA5* mRNA primers used for *LAMA5* detection

LAMA5 mRNA Primers	Sequence (5'->3')
Primer pair 1	
Forward primer	CAGGCTAAGGAGGAGCTGGA
Reverse primer	TGCTTGTCTCGTCTGTGTCC
Product length	535
Primer pair 2	
Forward primer	AGGCTAAGGAGGAGCTGGA
Reverse primer	AGCATGGCCTCTTCTAGTGC
Product length	385
Deletion Detection Primer	
Forward primer	GCCAGCCCCAAAGTCATACA
Reverse primer	CGCATCAGCTTCGACAGTCA
Product length	242

Supplemental Table S5: Sequences of all genomic *LAMA5* primers used to detect and sequence Cas9-induced mutations

Genomic DNA Primers	Sequence (5'->3')
Primer pair 1	
Forward primer	CAGAAACAGGGCAGGGTTAGT
Reverse primer	ATCGAGATGGACACGCTGAA
Product length	408
Primer pair 2	
Forward primer	AGAAACAGGGCAGGGTTAGTG
Reverse primer	TCAGTCCGTCCTGCTGTAGT
Product length	540
Primer pair 3	
Forward primer	CCCATCGTTCCATCTCCTCT
Reverse primer	TGGTCAGCCTCTACAACTTCG
Product length	558