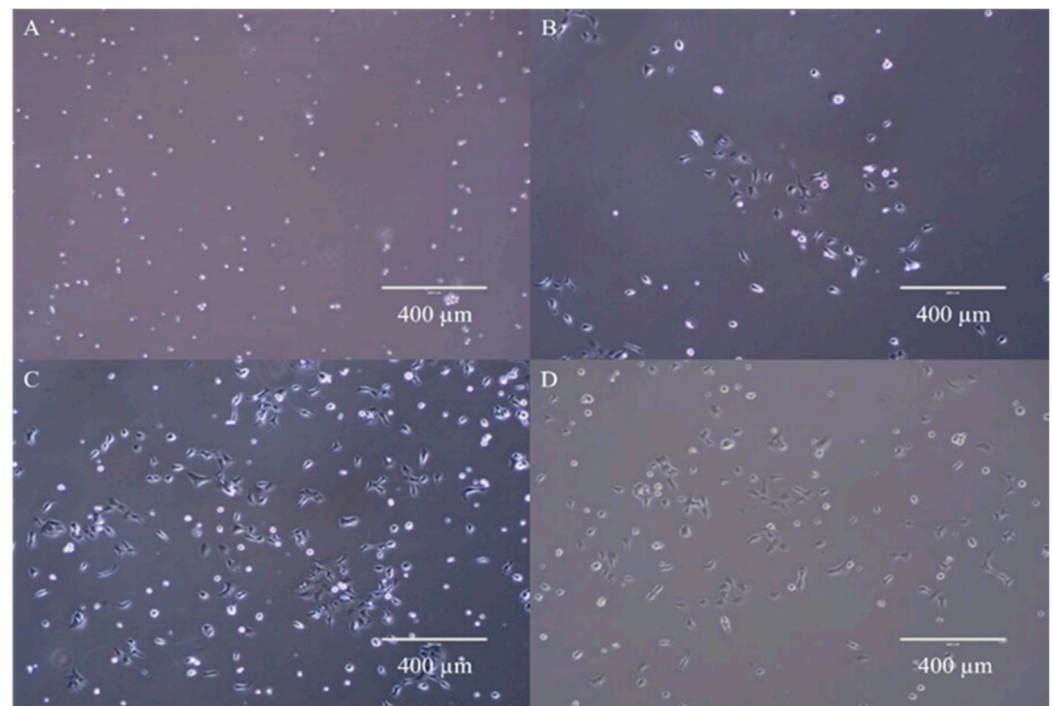
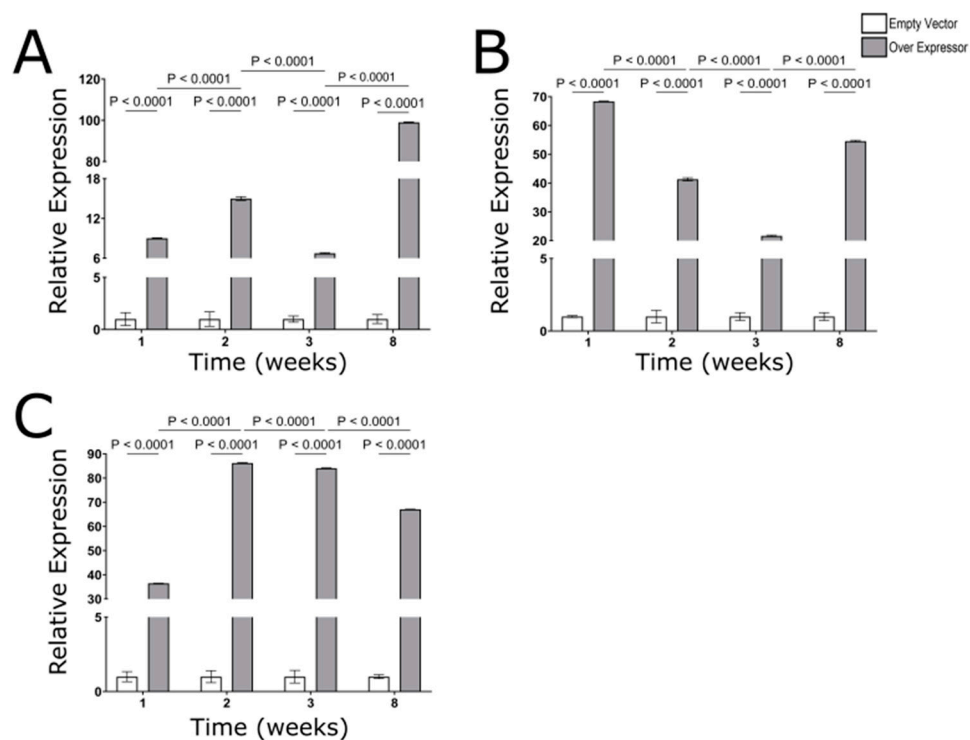


Supplementary Table S1: Oligonucleotides used in this study.

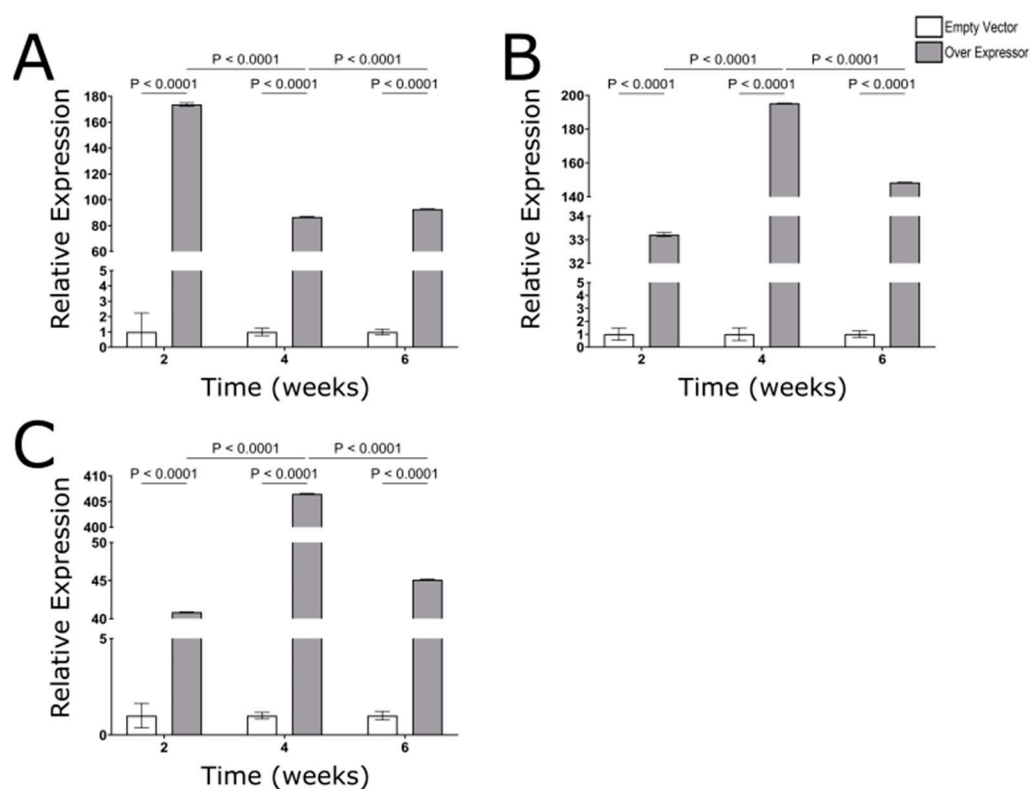
Target	Oligonucleotide sequence (5'-3')
<i>Human circHTT(2-6) qRT-PCR</i>	<i>F (exon 6): GAGACCCGAAGAATCAGTCCA R (exon 2): CACGGTCTTTCTTGGTAGCTG</i>
<i>Human HTT mRNA qRT-PCR</i>	<i>F (exon 64): TTGCTATGGAGCGGGTATC R (exon 65): GTATGGCTGCTGGTTGGAC</i>
<i>Human/ mouse GAPDH qRT-PCR</i>	<i>F: CAGTGAGCTTCCCGTTCAG R: ACCCAGAAGACTGTGGATGG</i>
<i>Human TBP qRT-PCR</i>	<i>F: CACGCCAGCTTCGGAGAGTT R: ATCAGTGCCGTGGTTCGTGG</i>
<i>Mouse mmu_circHTT(2-6) qRT-PCR</i>	<i>F (exon 6): CGGAGGAATCAGTTCAGGAG R (exon 2): TCACACGGTCTTTCTTGGTG</i>
<i>Mouse mmu_HTT mRNA qRT-PCR</i>	<i>F (exon 66): CTGTCCCTGTCCAACTTCAC R (exon 67): TTCCTCCTCTATCTGGTGTCTG</i>
<i>HD (CAG)_n direct test reporting primers</i>	<i>HD1 (F): 6'FAM-ATGAAGGCCTTCGAGTCCCTCAAGTCCTTC 447X (R): GGCGGCGGTGGCGGCTGTTG</i>



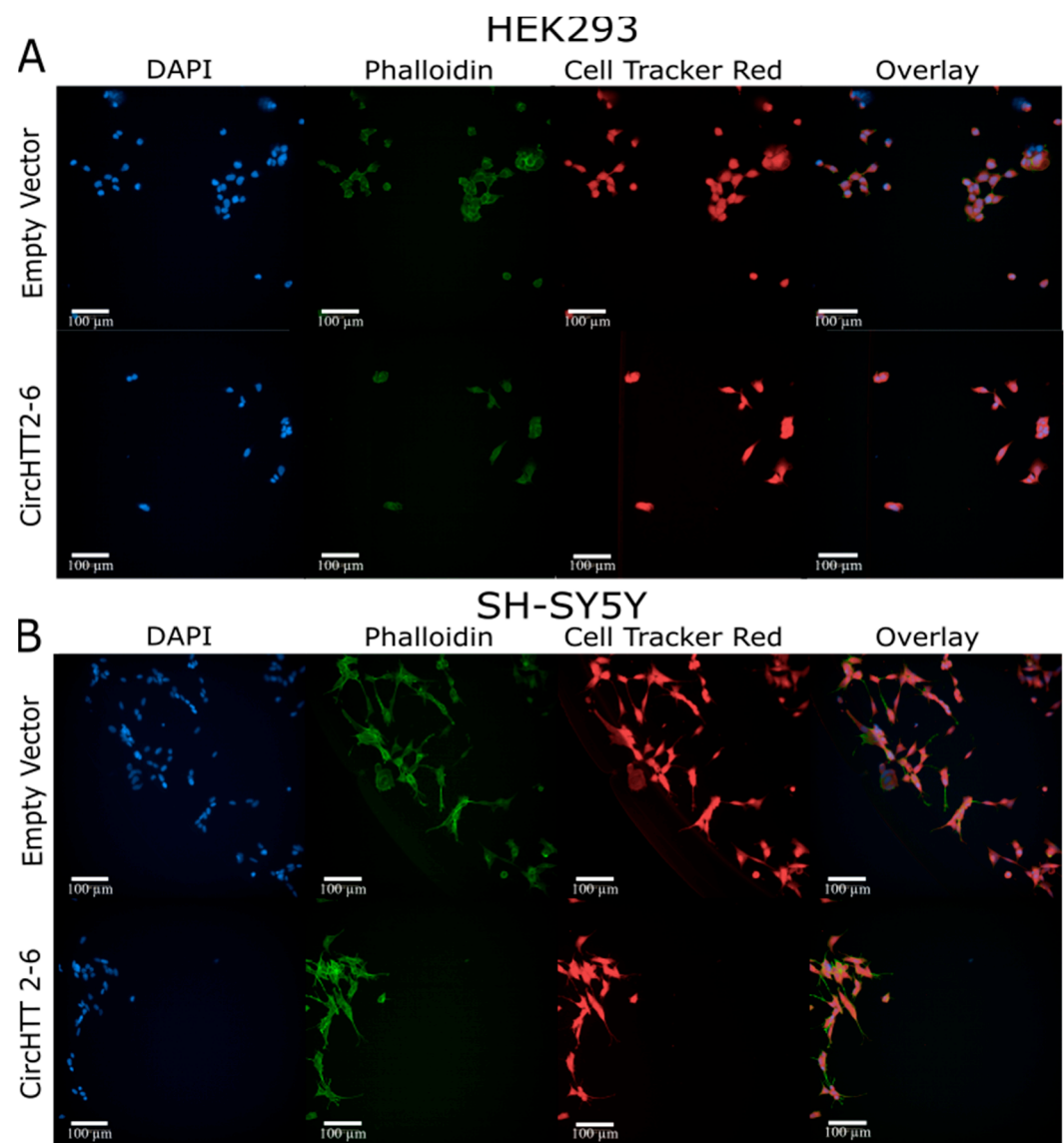
Supplementary Figure S1: Figure shows transfected HEK293 cells 1 week after G418 selection. (A) Non-transfected HEK293 cells killed after seven days of selection, with HEK293 cells surviving following transfection with (B) circHTT(2-6) (C) circNFASC(26-27) and (D) empty vector (pcDNA3.1). Magnification 10 X, scale bar = 400 μ M.



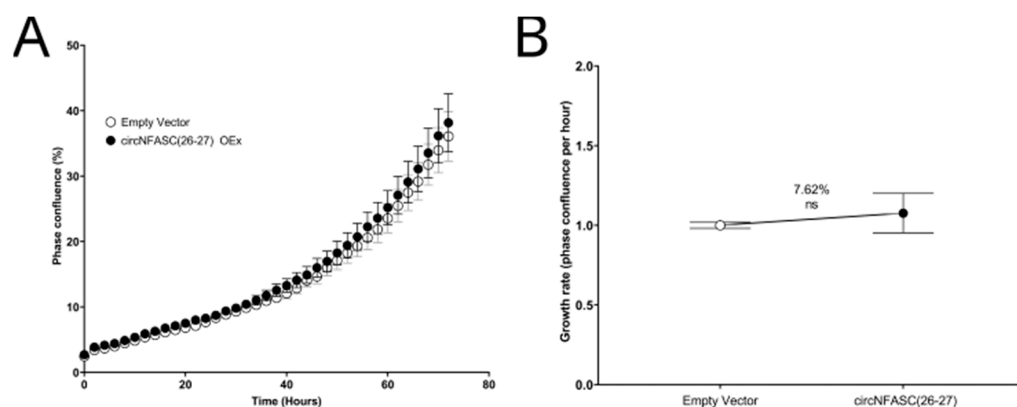
Supplementary Figure S2: circHTT(2-6) is significantly over expressed in HEK293 cells over eight weeks in 3 biological replicates (one-way ANOVA). Paired empty vector (pcDNA3.1) and circHTT(2-6) overexpression at 1, 2, 3 and 8 weeks after selection of HEK293 cells for **A**) biological replicate 1, **B**) biological replicate 2 and **C**) biological replicate 3. Data shows mean circHTT(2-6) expression and standard deviation normalised to EV, week 2.



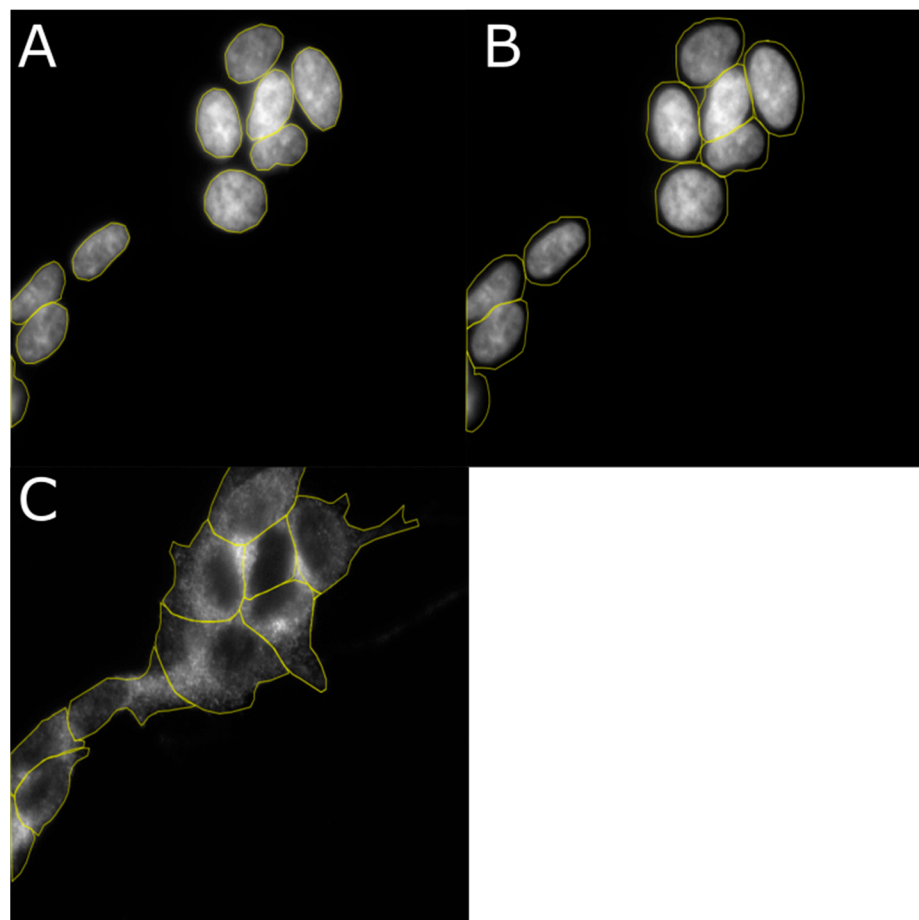
Supplementary Figure S3: *circHTT(2-6)* is significantly over expressed in SH-SY5Y cells over six weeks in 3 biological replicates (two-way ANOVA). Paired empty vector (pcDNA3.1) and *circHTT(2-6)* overexpression at 2, 4 and 6 weeks after selection of SH-SY5Y cells for **A)** Biological replicate 1, **B)** Biological replicate 2 and **C)** Biological replicate 3. Data shows mean *circHTT(2-6)* expression and standard deviation normalised to EV, week 2.



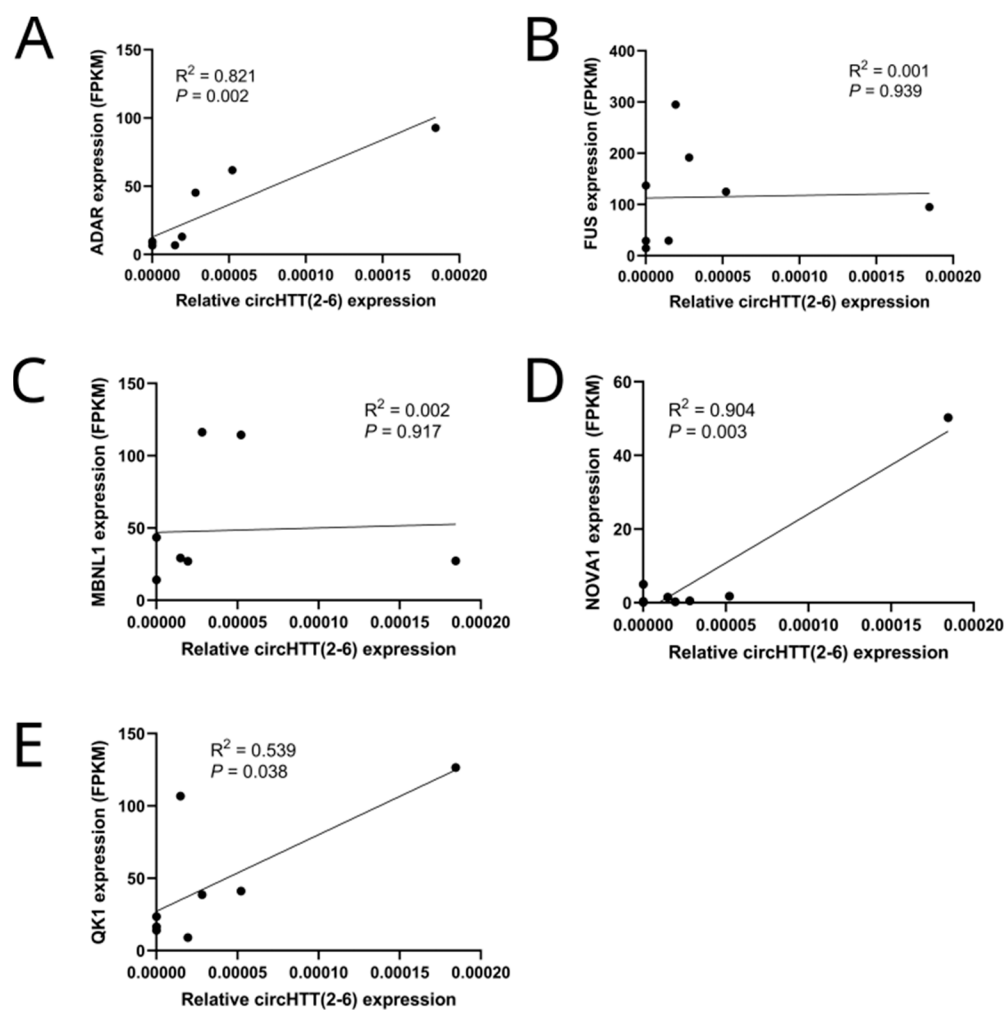
Supplementary Figure S4: Operetta images of representative biological replicate of (A) HEK293 and (B) SH-SY5Y cells empty vector (pcDNA3.1) and circHTT(2-6) overexpression lines four weeks after selection, stained with DAPI (blue), CellTracker Deep Red (red), Phalloidin (green). 40X magnification, scale bar = 100 μm.



Supplementary Figure S5: Overexpression of circNFASC(26-27) in HEK293 cells does not affect cell proliferation. **(A)** One biological replicate of empty vector (pcDNA3.1, unfilled circles) and circNFASC(26-27) overexpression lines (filled circles) two weeks after selection of HEK293 cells. Data shows line graph over 72 hours for $n = 2000$ individual cells from each sample. **(B)** Overall phase object confluence (%) at exponential growth phase (exponential growth phase 10-hour window of linear growth preceding plateauing growth rate between 50–60hrs) for EV and circNFASC(26-27) OEx, showing unaffected cell proliferation rate. ns = not significant. Statistical analysis performed by one-way ANOVA.



Supplementary Figure S6: Subcellular boundaries used to measure HTT immunofluorescence. Figure shows one biological replicate of empty vector SH-SY5Y cells six weeks after selection. (A) Nuclear boundary, (B) perinuclear boundary and (C) whole cell boundary (cytoplasm was calculated from this by subtracting the nuclear signal).



Supplementary Figure S7: Correlation between expression of *mmu_circHTT(2-6)* across mouse tissues with known circRNA biogenesis factors. QRT-PCR of *mmu_circHTT(2-6)* from this manuscript was correlated with RNAseq expression (FPKM) for known circRNA biogenesis factors in mice, or their orthologues (A) ADAR (B) FUS (C) MBNL1 (D) NOVA1 (E) QK1 with linear regression line fitted and Pearson's correlation coefficient shown as R^2 (and P-value).