**Supplemental Figure legends**

**Supplemental Figure S1:** **A)** Analysis of the amino acid sequence of the proteins encoded by circZNF609 and ZNF609. Both proteins share the first 249 aa. The last amino acid Q250 of circZNF609 emerges from the codon spanning circZNF609 junction, thus leading to the only difference between the two proteins besides the length of the polypeptide. The varying amino acid is displayed by red letter. **B)** Northern blot analysis of the expression of overexpressed circZNF609. Membrane used in Figure 1C was stripped and the signal was detected with linear circZNF609 probe, which does not span the splice junction. **C)** Northern blot of the corresponding membrane of RNase R-digested RNA using linear circZNF609 probe. The membrane used in Figure 1D was stripped and used for this northern blot. **D)** Northern blot of HEK293T cells transfected with different overexpressed circZNF609 AUG mutants. The blot was detected with a probe spanning circZNF609 junction. **E)** Scheme of two different constructs for expressing linearized circZNF609 ORF with Flag/HA tag at either N-terminal (FH-circZNF609 ORF-linear) or C-terminal (circZNF609 ORF-FH linear). **F)** Western blot of HEK293T cells transfected with different overexpressed circZNF609 ORF linearized constructs. Tagging Flag/HA at the C-terminal of circZNF609 (circZNF609 ORF-FH linear) gives two protein bands (lane 3).

**Supplemental Figure S2**: **A)** Schematic overview of CRISPR/Cas9 strategy for targeting METTL3 using 2 different gRNA. Both gRNAs target exon 3 of METTL3.

**Supplemental Figure S3: A)** Northern blot analysis of the expression of overexpressed circZNF609 and the fragments after digestion with restriction enzymes. Membrane used in Figure 3D was stripped and the signal was detected with a probe targeting Flag sequence, which does not span the splice junction. B) Northern blot of the membrane used Figure 3G, after stripping using a probe spanning circZNF609 junction.

**Supplemental Figure S4:** Northern blot analysis of the expression of overexpressed circZNF609 5’ UTR mutants. **A) and B)** Northern blot of RNA isolated from HEK293T cells transfected with different overexpressed 5’ UTR deletion mutants. The blot was detected with a probe spanning circZNF609 junction.

**Supplemental Figure S5:** The self-splicing permutated intron–exon (PIE) method for production of circular RNA. **A)** Scheme of the pGEM-3E5-T7t vector containing all necessary sequence for PIE method. The PIE is located between the T7 promoter sequence and the T7 terminator sequence. The PIE sequence consists of the 3’ half intron, sequence of the desired exon for circularization and the 5’ half intron sequence. **B)** After purifying the in vitro transcribed RNA reaction, the RNA was loaded 1% MOPS Agarose gel (lane 1, 2, 3) for visualization of different products. To detect the correct products corresponding to circZNF609, a northern blot was performed. 10 ng of RNA after purified from IVT reaction was loaded on 1% MOPS Agarose gel. The RNA was then transferred to a membrane. First, the RNA was detected by a probe spanning circZNF609 junction (lane 4, 5, 6). Then, the membrane was stripped and detected with a probe targeting the 5’ intron downstream of circZNF609 (lane 7, 8, 9). **C)** To confirm the circular nature of the IVT circZNF609, 10 ng of RNA was digested with RNase R. The samples were then loaded directed to 1% MOPS Agarose without purification. The membrane was detected with a probe spanning circZNF609 junction.

**Supplemental Figure S6: A)** Schematic overview of different circZNF609-Flag constructs and the 5’ UTR deletion constructs (Del 121, Del 122, Del 123) with deletion of splice sites (delSS) and AluSz located in the ZKSCAN1 left intron downstream of circZNF609. **B)** Northern blot of HEK293T cells transfected with different deletion mutants. The signal was detected with a probe spanning circZNF609 junction. **C)** Western blot of HEK293T cells transfected with different deletion mutants detected with anti-Flag antibody. GAPDH served as loading control.