Supplementary Materials for

**In vivo** fermentation production of humanized noncoding RNAs carrying payload miRNAs for targeted anticancer therapy

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Table S1. Sequences of target recombinant ncRNAs.

Table S2. Primers used for cloning ncRNA expression plasmids.

Figure S1. Body weights of animals during the therapy study.
Table S1. Sequences of humanized recombinant ncRNAs bearing RNAi warheads. Underlined, tRNA; Red and green: payload miRNA or siRNA sequence and complementary sequence, respectively; orange, aptamer.

| Target ncRNA          | # nts | Sequence (5’ to 3’)                                                                                                                                                                                                 | MW (Da) |
|-----------------------|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| htRNA/pre-miR-34a     |       | GCCUGAGAUAGCUAGUGAGAGAGCAUCAGACUGCCAGCAGCU UGGAGUGUUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 58,889  |
| htRNA/pre-miR-34a     |       | GGUCCCAUGUGUGUAUGGUAUGCAGACUGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 58,465  |
| htRNA/pre-miR-34a     |       | GGUGGCAUGAGCUAGUGAGAGCAUCAGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 58,543  |
| htRNA/pre-miR-34a     |       | GGUUGAUGUUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 58,905  |
| htRNA/pre-miR-34a     |       | GCGCCAGCGUGAGUUGGUGAUAGCAGACUGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 61,787  |
| htRNA/pre-miR-34a     |       | ACCAGGAUGUCGCGAGUGUUAAGCGUUGAGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 62,110  |
| htRNA/pre-miR-34a     |       | ACCAGGAUGUCGCGAGUGUUAAGCGUUGAGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 62,049  |
| htRNA/pre-miR-34a     |       | ACCAGGAUGUCGCGAGUGUUAAGCGUUGAGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 62,133  |
| htRNA/pre-miR-34a     |       | ACCAGGAUGUCGCGAGUGUUAAGCGUUGAGACUGCCAGCAGCU UGGAGUGUUCUUGGCGAUGUGUCUGUGUGUGUGAG CAAUAGAUAGGAAGCAUCCAGAQAUAUGCACUAGAAGU GCUGACGGUUGGCGCCAUCAUCAGGGCCAGAGUCAG UCUCUUAGGACGCCCAA                                                                 | 62,167  |
htRNA\textsuperscript{Leu}/pre-miR-34a/miR-141-3p

192

\begin{verbatim}
ACCAGGAUGGCCAGUGGUUAAGGCGUUGGACU
GUGAGGUUUCUUUAACACUGUGGUAAGGAUGUGUAG
CAAUAUGAGGAACUCUUCAAAGAUAGGUGCAUAGUAG
GUGGAGCUGUUGGCCCAGAUCAUAUGUGCCCGC
UGGUUUUGAACCCACUCCUGUGUACCA
\end{verbatim}

62,063

htRNA\textsuperscript{Leu}/pre-miR-34a/miR-142-3p

194

\begin{verbatim}
ACCAGGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UAACACUGUCUGGUAAAGAUGG
UGUGAG
CAAUAUGAGGAAACUCUUCAAAGAUAGGUGCAUAGUAG
GUGGAGCUGUUGGCCCAGAUCAUAUGUGCCCGC
UGGUUUUGAACCCACUCCUGUGUACCA
\end{verbatim}

62,674

htRNA\textsuperscript{Leu}/pre-miR-34a/miR-194-3p

192

\begin{verbatim}
ACCAGGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UGUAACAGCAAUCUCCAUGUGGA
UGUGAG
CAAUAUGAGGAAACUCUUCAAAGAUAGGUGCAUAGUAG
GUGGAGCUGUUGGCCCAGAUCAUAUGUGCCCGC
UGGUUUUGAACCCACUCCUGUGUACCA
\end{verbatim}

62,094

htRNA\textsuperscript{Leu}/pre-miR-34a/miR-205-5p

192

\begin{verbatim}
ACCAGGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UCCUUCAUUCCACCGGAGUCUG
UGUGAG
CAAUAUGAGGAAACUCUUCAAAGAUAGGUGCAUAGUAG
GUGGAGCUGUUGGCCCAGAUCAUAUGUGCCCGC
UGGUUUUGAACCCACUCCUGUGUACCA
\end{verbatim}

62,068

htRNA\textsuperscript{Ser}/pre-miR-34a

118

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
AGUAAUUU
ACGUCGACGGUGACGUCGAUGGUUGCGG
AAUCCAAUGGGGU
CUCCCCCGCGAAAUCUAAUGGGU
\end{verbatim}

38,302

htRNA\textsuperscript{Ser}/pre-miR-34a/BCL2-siRNA

191

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UACAGUUCCACAAAGGCAUCCU
UGUGAGC
AAUAGUAAGGAAG
GGAUGCCUUGUGGGAACUGUCU
\end{verbatim}

61,809

htRNA\textsuperscript{Ser}/pre-miR-34a/AGR2-siRNA

191

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UUAAAGCUUGACUGUGUGGGCU
UGUGAG
CAAUAUGAGGAAGGCCACACGUCUAAGCUUUACU
\end{verbatim}

61,747

htRNA\textsuperscript{Ser}/pre-miR-34a/let-7c-5p

192

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UAAUACUGCCUGGUAAUGAUGA
UGUGAG
CAAUAUGAGGAAGGCCACACGUCUAAGCUUUACU
\end{verbatim}

61,726

htRNA\textsuperscript{Ser}/pre-miR-34a/miR-124-3p

191

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UAAGGCACGCGGUGAAUGCCGU
UGUGAG
CAAUAUGAGGAAGGCCACACGUCUAAGCUUUACU
\end{verbatim}

61,809

htRNA\textsuperscript{Ser}/pre-miR-34a/miR-328-3p

191

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
CUGGCCCUCUCUGCCCUUCCGU
UGUGAG
CAAUAUGAGGAAGGCCACACGUCUAAGCUUUACU
\end{verbatim}

61,879

htRNA\textsuperscript{Ser}/pre-miR-34a/miR-200b-3p

191

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
UAAUACUGCCUGUAAGUAUGAG
CAAUAUGAGGAAGGCCACACGUCUAAGCUUUACU
\end{verbatim}

61,726

htRNA\textsuperscript{Ser}/pre-miR-34a/miR-22-3p

191

\begin{verbatim}
GCAGCGAUGGCCAGUGGUUAAGGCGUUGGACU
GGCCAGCU
GUGAGUGUUUCCU
AAAGCUGCCAGUGUAAGUAUGAG
\end{verbatim}

61,755
|       | Sequence                                                                 |
|-------|--------------------------------------------------------------------------|
| htRNA<sup>Ser</sup>/pre-miR-34a/miR-6775-3p | CAAUAGUAAGGAAAGCAAAGUCUAGUGCUGCUAGACAGUAGGCUGCACGUGCUUGGCCCAAUCCAAUGGGUCUCUCCCGGCAAGGUGUUGAACCACUGCUGCUGCGCA |
|       | GCAGCGAUGGCGAGGUGUUAAGGCUGUUGGACUGCCACGCAAGGGUGAAGGAGCUGCGUGGCGUUCCGCUCCGCG |
|       | CAAUAGUAAGGAAGCAAAGUCUAGUGCUGCUAGACAGUAGGCUGCACGUGCUUGGCCCAAUCCAAUGGGUCUCUCCCGGCAAGGUGUUGAACCACUGCUGCUGCGCA |
| htRNA<sup>Ser</sup>/pre-miR-34a/NRF2-siRNA | CAAUAGUAAGGAAAGCAAAGUCUAGUGCUGCUAGACAGUAGGCUGCACGUGCUUGGCCCAAUCCAAUGGGUCUCUCCCGGCAAGGUGUUGAACCACUGCUGCUGCGCA |
|       | GCAGCGAUGGCGAGGUGUUAAGGCUGUUGGACUGCCACGCAAGGGUGAAGGAGCUGCGUGGCGUUCCGCUCCGCG |
|       | CAAUAGUAAGGAAGCAAAGUCUAGUGCUGCUAGACAGUAGGCUGCACGUGCUUGGCCCAAUCCAAUGGGUCUCUCCCGGCAAGGUGUUGAACCACUGCUGCUGCGCA |

**htRNA<sup>Ser</sup>/pre-miR-34a/miR-6775-3p**

**htRNA<sup>Ser</sup>/pre-miR-34a/NRF2-siRNA**
Table S2. Primers used for the construction of RNA expression plasmids.

| Target ncRNA      | Cloning Primers (5’ to 3’)                      |
|-------------------|-------------------------------------------------|
| **htRNA/pre-miR-34a** |                                                 |
| htRNA<sup>1/2</sup>/pre-miR-34a | FTTGTAACGCTGATATTCAAGGTCGCCAGTGTGTTGTTAGCGACTCGAGTAGCAGCTGATGCCAAGCGTGTGAATG |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
| htRNA<sup>2/3</sup>/pre-miR-34a | FTTGTAACGCTGATATTCTGAGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
| **htRNA/pre-miR-34a** |                                                 |
| htRNA<sup>3/4</sup>/pre-miR-34a | FTTGTAACGCTGATATTCAAGGTCGCCAGTGTGTTGTTAGCGACTCGAGTAGCAGCTGATGCCAAGCGTGTGAATG |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
| htRNA<sup>4/5</sup>/pre-miR-34a | FTTGTAACGCTGATATTCAAGGTCGCCAGTGTGTTGTTAGCGACTCGAGTAGCAGCTGATGCCAAGCGTGTGAATG |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
| **htRNA/pre-miR-34a** |                                                 |
| htRNA<sup>5/6</sup>/pre-miR-34a | FTTGTAACGCTGATATTCAAGGTCGCCAGTGTGTTGTTAGCGACTCGAGTAGCAGCTGATGCCAAGCGTGTGAATG |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
| **htRNA/pre-miR-34a** |                                                 |
| htRNA<sup>6/7</sup>/pre-miR-34a | FTTGTAACGCTGATATTCAAGGTCGCCAGTGTGTTGTTAGCGACTCGAGTAGCAGCTGATGCCAAGCGTGTGAATG |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
| **htRNA/pre-miR-34a** |                                                 |
| htRNA<sup>7/8</sup>/pre-miR-34a | FTTGTAACGCTGATATTCAAGGTCGCCAGTGTGTTGTTAGCGACTCGAGTAGCAGCTGATGCCAAGCGTGTGAATG |
|                    | R CTTCTGCTAAGGATCGTGGGAGCTTGAACAGGGGACTTCCTAGCTGAGCTCAGATTGGGCCAAACAAGCGTGC |
htRNA<sup>sr</sup>/pre-miR-34a

| Primer Forward | Primer Reverse |
|---------------|---------------|
| htfRNA<sup>sr</sup>/pre-miR-34a/miR-124-3p | uses the same primer as in htfRNA<sup>sr</sup>/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| htfRNA<sup>sr</sup>/pre-miR-34a/miR-7-7c-5p | uses the same primer as in htfRNA<sup>sr</sup>/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| htfRNA<sup>sr</sup>/pre-miR-34a/miR-328-3p | uses the same primer as in htfRNA<sup>sr</sup>/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| htfRNA<sup>sr</sup>/pre-miR-34a/miR-200b-3p | uses the same primer as in htfRNA<sup>sr</sup>/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| htfRNA<sup>sr</sup>/pre-miR-34a/miR-22-3p | uses the same primer as in htfRNA<sup>sr</sup>/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| htfRNA<sup>sr</sup>/pre-miR-34a/miR-148-3p | uses the same primer as in htfRNA<sup>sr</sup>/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| Genotype | Function | Primer | Template |
|----------|----------|--------|----------|
| htRNA\textsuperscript{Ser}/pre-miR-34a/miR-6775-3p | F | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| R | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| htRNA\textsuperscript{Ser}/pre-miR-34a/NRF2-siRNA | F | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
| R | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template | uses the same primer as in htRNA\textsuperscript{Ser}/pre-miR-34a plus corresponding btRNA/hsa-miR-34a plasmid as template |
Figure S1. Animal body weights did not differ between different treatment groups (not significant between any groups; 2-way ANOVA with Bonferroni post-tests).