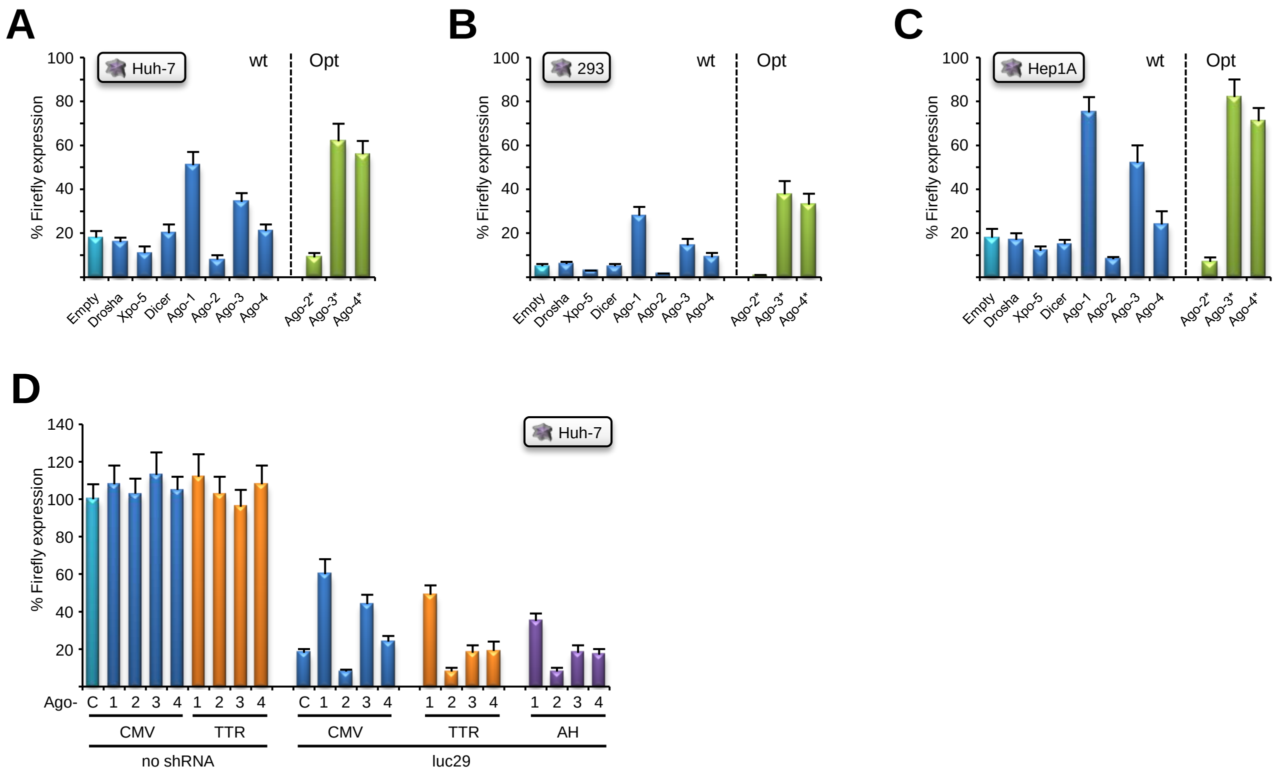
Supplemental Material

Supplemental Figures 1 to 7



Supplemental Figure 1 Confirmation in three further cell lines (**A-C**) that Ago-2 limits shRNA activity (see Figure 1, D and F for details). All values were normalized to a control lacking shRNA and RNAi proteins (not shown, set to 100%). (**D**) Ago-2 expression from various promoters consistently enhanced shRNA activity in Huh-7 cells, as compared to a control (C, not expressing any Ago protein). Wildtype Ago cDNAs were used in this panel. AH, liver/hepatoma-specific ApoE/hAAT enhancer/promoter.

```
wt          ATGTACTCGGGAGCCGGCCCCGCACTTGCACCTCCTGCGCCGCCGCCCCCCATCCAAGGATATGCCTTCAAGCCTCCACCTAGACCCGACTTTGGGACCTCCGGGAGAACAATCAAATTA 120
opt         ATGTACAGCGGAGCCGGCCCTGCCCTGGCCCCTCCTGCCCCCCCTCCCCCCATCCAGGGCTACGCCTTCAAGCCCCCTCCCAGGCCCGACTTCGGCACCAGCGGCCGGACCATCAAGCTG 120
            ******   *********** ** ** ** ******** ** ** *********** ** ** *********** ** ** ** ******** ** ***   ***  * ** *****  *

wt          CAGGCCAATTTCTTCGAAATGGACATCCCCAAAATTGACATCTATCATTATGAATTGGATATCAAGCCAGAGAAGTGCCCGAGGAGAGTTAACAGGGAAATCGTGGAACACATGGTCCAG 240
opt         CAGGCCAACTTCTTCGAGATGGACATCCCCAAGATCGACATCTACCACTACGAGCTGGACATCAAGCCCGAGAAGTGCCCCAGGCGGGTGAACCGGGAGATCGTGGAGCACATGGTCCAG 240
            ******** ******** ************** ** ******** ** ** **  **** ******** *********** *** * ** *** **** ******** ***********

wt          CACTTTAAAACACAGATCTTTGGGGATCGGAAGCCCGTGTTTGACGGCAGGAAGAATCTATACACAGCCATGCCCCTTCCGATTGGGAGGGACAAGGTGGAGCTGGAGGTCACGCTGCCA 360
opt         CACTTCAAGACCCAGATCTTCGGCGACCGGAAGCCCGTGTTCGACGGCCGGAAGAACCTGTACACCGCCATGCCCCTGCCCATCGGCCGGGACAAGGTGGAGCTGGAAGTGACCCTGCCC 360
            ***** ** ** ******** ** ** ************** ****** ******* ** ***** *********** ** ** **  ****************** ** ** *****

wt          GGAGAAGGCAAGGATCGCATCTTCAAGGTGTCCATCAAGTGGGTGTCCTGCGTGAGCTTGCAGGCGTTACACGATGCACTTTCAGGGCGGCTGCCCAGCGTCCCTTTTGAGACGATCCAG 480
opt         GGCGAGGGCAAGGACCGGATCTTCAAGGTGTCCATCAAGTGGGTGTCCTGCGTGAGCCTGCAGGCCCTGCACGACGCCCTGAGCGGCAGGCTGCCCAGCGTGCCCTTCGAGACCATCCAG 480
            ** ** ******** ** *************************************** *******  * ***** ** **    **  ************* ** ** ***** ******

wt          GCCCTGGACGTGGTCATGAGGCACTTGCCATCCATGAGGTACACCCCCGTGGGCCGCTCCTTCTTCACCGCGTCCGAAGGCTGCTCTAACCCTCTTGGCGGGGGCCGAGAAGTGTGGTTT 600
opt         GCCCTGGACGTGGTGATGCGGCACCTGCCCAGCATGCGGTACACCCCCGTGGGCAGAAGCTTCTTCACCGCCAGCGAGGGGTGTTCCAACCCCCTGGGCGGAGGGCGGGAAGTGTGGTTC 600
            ************** *** ***** ****   **** ****************** *   ************   *** ** ** ** ***** ** ***** ** ** ***********

wt          GGCTTCCATCAGTCCGTCCGGCCTTCTCTCTGGAAAATGATGCTGAATATTGATGTGTCAGCAACAGCGTTTTACAAGGCACAGCCAGTAATCGAGTTTGTTGTGAAGTTTTGGATTTT 720
opt         GGCTTCCACCAGAGCGTGCGGCCCAGCCTGTGGAAGATGATGCTGAACATCGACGTGAGCGCCACCGCCTTCTACAAGGCCCAGCCCGTGATCGAGTTCGTGTGCGAGGTGCTGGACTTC 720
            ******** ***  *** *****    ** ***** *********** ** ** ***  ** ** ** ** ******** ***** ** ******** ** ** ** **  **** **

wt          AAAAGTATTGAAGAACAACAAAAACCTCTGACAGATTCCCAAAGGGTAAAGTTTACCAAAGAAATTAAAGGTCTAAAGGTGGAGATAACGCACTGTGGGCAGATGAAGAGGAAGTACCGT 840
opt         AAGAGCATCGAGGAACAGCAGAAGCCCCTGACCGACAGCCAGCGGGTGAAGTTCACCAAAGAGATCAAGGGCCTGAAGGTGGAGATCACCCACTGCGGCCAGATGAAGCGGAAGTACAGA 840
            ** ** ** ** ***** ** ** ** ***** **   ***  **** ***** ******** ** ** ** ** ********** ** ***** ** ********* ******** *

wt          GTCTGCAATGTGACCCGGCGGCCCGCCAGTCACCAAACATTCCCGCTGCAGCAGGAGAGCGGGCAGACGGTGGAGTGCACGGTGGCCCAGTATTTCAAGGACAGGCACAAGTTGGTTCTG 960
opt         GTGTGCAACGTGACCAGAAGGCCCGCCAGCCACCAGACATTTCCACTCCAACAGGAAAGCGGCCAGACCGTCGAGTGCACCGTGGCCCAGTACTTCAAGGACCGGCACAAGCTGGTGCTG 960
            ** ***** ****** *  ********** ***** ***** ** ** ** ***** ***** ***** ** ******** *********** ********* ******** **** ***

wt          CGCTACCCCCACCTCCCATGTTTACAAGTCGGACAGGAGCAGAAACACACCTACCTTCCCCTGGAGGTCTGTAACATTGTGGCAGGACAAAGATGTATTAAAAAATTAACGGACAATCAG 1080
opt         AGATACCCCCACCTCCCCTGCCTGCAAGTCGGGCAGGAACAGAAGCACACCTACCTGCCCCTGGAAGTGTGCAACATCGTGGCCGGCCAGCGGTGCATCAAGAAGCTGACCGACAATCAG 1080
            * ************** **  * ******** ***** ***** *********** ******** ** ** ***** ***** ** **  * ** ** ** **  * ** *********

wt          ACCTCAACCATGATCAGAGCGACTGCTAGGTCGGCGCCCGATCGGCAAGAAGAGATTAGCAAATTGATGCGAAGTGCAAGTTTCAACACAGATCCATACGTCCGTGAATTTGGAATCATG 1200
opt         ACCAGCACCATGATCCGGGCCACCGCCAGAAGCGCCCCTGACAGGCAGGAAGAGATCAGCAAGCTGATGCGGAGCGCCAGCTTCAACACCGACCCCTACGTGCGGGAGTTCGGCATCATG 1200
            ***   ********* * ** ** ** **   ** ** ** **** ******** *****  ******* ** ** ** ******** ** ** ***** ** ** ** ** ******

wt          GTCAAAGATGAGATGACAGACGTGACTGGGCGGGTGCTGCAGCCGCCCTCCATCCTCTACGGGGGCAGGAATAAAGCTATTGCGACCCCTGTCCAGGGCGTCTGGGACATGCGGAACAAG 1320
opt         GTGAAGGACGAGATGACCGACGTGACCGGCAGAGTCCTGCAGCCTCCCAGCATCCTGTACGGCGGCAGGAACAAGGCCATCGCCACCCCCGTGCAGGGCGTGTGGGACATGCGGAACAAG 1320
            ** ** ** ******** ******** **  * ** ******** ***   ****** ***** ******** ** ** ** ** ***** ** ********* ******************

wt          CAGTTCCACACGGGCATCGAGATCAAGGTGTGGGCCATTGCGTGCTTCGCCCCCCAGCGCCAGTGCACGGAAGTCCATCTGAAGTCCTTCACAGAGCAGCTCAGAAAGATCTCGAGAGAC 1440
opt         CAGTTCCACACCGGCATCGAGATCAAAGTGTGGGCCATCGCCTGCTTCGCCCCCCAGCGGCAGTGCACCGAGGTGCACCTGAAGAGCTTCACCGAGCAGCTGCGGAAGATCAGCCGGGAC 1440
            *********** ************** *********** ** ***************** ******** ** ** ******  ****** ********  * ******    * ***

wt          GCCGGCATGCCCATCCAGGGCCAGCCGTGCTTCTGCAAATACGCGCAGGGGGCGGACAGCGTGGAGCCCATGTTCCGGCACCTGAAGAACACGTATGCGGGCCTGCAGCTGGTGGTGGTC 1560
opt         GCCGGCATGCCTATCCAGGGCCAGCCCTGCTTCTGCAAGTACGCCCAGGGCGCCGACAGCGTGGAGCCCATGTTCAGACACCTGAAGAACACCTACGCCGGCCTCCAGCTGGTCGTGGTG 1560
            *********** ************** *********** ***** ***** ** *********************  * ************** ** ** ***** ******** *****

wt          ATCCTGCCCGGCAAGACGCCCGTGTACGCCGAGGTCAAGCGCGTGGGAGACACGGTGCTGGGGATGGCCACGCAGTGCGTGCAGATGAAGAACGTGCAGAGGACCACGCCACAGACCCTG 1680
opt         ATCCTGCCTGGCAAGACCCCCGTGTACGCCGAGGTGAAGAGAGTGGGCGACACCGTCCTGGGCATGGCCACCCAGTGTGTGCAGATGAAGAACGTGCAGCGGACCACCCCCCAGACCCTG 1680
            ******** ******** ***************** *** * ***** ***** ** ***** ******** ***** ********************* ******* ** *********

wt          TCCAACCTCTGCCTGAAGATCAACGTCAAGCTGGGAGGCGTGAACAACATCCTGCTGCCCCAGGGCAGGCCGCCGGTGTTCCAGCAGCCCGTCATCTTTCTGGGAGCAGACGTCACTCAC 1800
opt         AGCAACCTGTGCCTGAAGATCAACGTGAAGCTGGGCGGCGTGAACAACATCCTGCTGCCCCAGGGCAGGCCTCCCGTGTTCCAGCAGCCTGTGATCTTCCTGGGCGCCGATGTGACCCAC 1800
             ****** ***************** ******** *********************************** ** ************** ** ***** ***** ** ** ** ** ***

wt          CCCCCCGCCGGGGATGGGAAGAAGCCCTCCATTGCCGCCGTGGTGGGCAGCATGGACGCCCACCCCAATCGCTACTGCGCCACCGTGCGCGTGCAGCAGCACCGGCAGGAGATCATACAA 1920
opt         CCCCCTGCCGGCGACGGGAAGAAGCCCAGCATCGCCGCCGTGGTGGGCAGCATGGACGCCCACCCCAACCGGTACTGCGCCACCGTGCGGGTGCAGCAGCACCGGCAGGAAATCATTCAG 1920
            ***** ***** ** ************  *** ************************************ ** *************** ********************* ***** **

wt          GACCTGGCCGCCATGGTCCGCGAGCTCCTCATCCAGTTCTACAAGTCCACGCGCTTCAAGCCCACCCGCATCATCTTCTACCGCGACGGTGTCTCTGAAGGCCAGTTCCAGCAGGTTCTC 2040
opt         GATCTGGCTGCCATGGTCCGGGAGCTGCTGATCCAGTTCTACAAGAGCACCCGGTTCAAGCCCACCCGGATCATCTTCTACCGGGACGGCGTGAGCGAGGGCCAGTTCCAGCAGGTGCTG 2040
            ** ***** *********** ***** ** ****************  *** ** ************** ************** ***** **    ** ***************** **

wt          CACCACGAGTTGCTGGCCATCCGTGAGGCCTGTATCAAGCTAGAAAAAGACTACCAGCCCGGGATCACCTTCATCGTGGTGCAGAAGAGGCACCACACCCGGCTCTTCTGCACTGACAAG 2160
opt         CACCACGAGCTGCTGGCCATCCGGGAAGCTTGCATCAAGCTGGAAAAGGACTACCAGCCCGGCATCACCTTCATCGTCGTGCAGAAGCGGCACCACACCCGGCTGTTCTGCACCGACAAG 2160
            ********* ************* ** ** ** ******** ***** ************** ************** ********* **************** ******** ******

wt          AACGAGCGGGTTGGGAAAAGTGGAAACATTCCAGCAGGCACGACTGTGGACACGAAAATCACCCACCCCACCGAGTTCGACTTCTACCTGTGTAGTCACGCTGGCATCCAGGGGACAAGC 2280
opt         AACGAGCGCGTGGGCAAGAGCGGCAACATCCCTGCCGGCACCACCGTGGACACCAAGATCACACACCCCACCGAGTTCGACTTCTACCTGTGCAGCCACGCCGGCATCCAGGGCACCAGC 2280
            ******** ** ** ** ** ** ***** ** ** ***** ** ******** ** ***** ****************************** ** ***** ********** ** ***

wt          AGGCCTTCGCACTATCACGTCCTCTGGGACGACAATCGTTTCTCCTCTGATGAGCTGCAGATCCTAACCTACCAGCTGTGTCACACCTACGTGCGCTGCACACGCTCCGTGTCCATCCCA 2400
opt         CGGCCCAGCCACTACCACGTGCTGTGGGACGACAACCGGTTCAGCAGCGACGAGCTGCAGATCCTGACCTACCAGCTGTGCCACACCTACGTGAGATGCACCAGAAGCGTGAGCATCCCT 2400
             ****    ***** ***** ** *********** ** ***  *    ** *************** ************** ************ * *****  *   ****  ******

wt          GCGCCAGCATACTACGCTCACCTGGTGGCCTTCCGGGCCAGGTACCACCTGGTGGATAAGGAACATGACAGTGCTGAAGGAAGCCATACCTCTGGGCAGAGTAACGGGCGAGACCACCAA 2520
opt         GCCCCTGCCTACTACGCCCACCTGGTGGCCTTCCGGGCCAGATATCACCTGGTGGACAAAGAGCACGACAGCGCCGAGGGCAGCCACACCTCCGGCCAGAGCAACGGCCGGGATCACCAG 2520
            ** ** ** ******** ********************** ** *********** ** ** ** ** ***** ** ** ** ***** ***** ** ***** ***** ** ** *****

wt          GCACTGGCCAAGGCGGTCCAGGTTCACCAAGACACTCTGCGCACCATGTACTTTGCTTGA 2580
opt         GCCCTGGCCAAGGCTGTGCAGGTGCACCAGGATACCCTGCGGACCATGTACTTCGCCTGA 2580
            ** *********** ** ***** ***** ** ** ***** *********** ** ***
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Supplemental Figure 2 Alignment of wildtype (wt) and codon-optimized (opt) Ago-2.

```
wt          ATGGAAATCGGCTCCGCAGGACCCGCTGGGGCCCAGCCCCTACTCATGGTGCCCAGAAGACCTGGCTATGGCACCATGGGCAAACCCATTAAACTGCTGGCTAACTGTTTTCAAGTTGAA 120
opt         ATGGAAATCGGCAGCGCCGGACCCGCCGGAGCCCAGCCCCTGCTGATGGTGCCCAGAAGGCCCGGCTACGGCACCATGGGCAAGCCCATCAAGCTGCTGGCCAACTGCTTCCAGGTGGAG 120
            ************  *** ******** ** *********** ** ************** ** ***** ************** ***** ** ******** ***** ** ** ** **

wt          ATCCCAAAGATTGATGTCTACCTCTATGAGGTAGATATTAAACCAGACAAGTGTCCTAGGAGAGTGAACAGGGAGGTGGTTGACTCAATGGTTCAGCATTTTAAAGTAACTATATTTGGA 240
opt         ATCCCCAAGATCGACGTGTACCTGTACGAGGTGGACATCAAGCCCGACAAGTGCCCCAGGCGGGTGAACCGGGAGGTGGTGGACAGCATGGTGCAGCACTTCAAGGTGACCATCTTCGGC 240
            ***** ***** ** ** ***** ** ***** ** ** ** ** ******** ** *** * ****** ********** ***    ***** ***** ** ** ** ** ** ** **

wt          GACCGTAGACCAGTTTATGATGGAAAAAGAAGTCTTTACACCGCCAATCCACTTCCTGTGGCAACTACAGGGGTAGATTTAGACGTTACTTTACCTGGGGAAGGTGGAAAAGATCGACCT 360
opt         GACAGACGGCCCGTGTACGACGGCAAGCGGAGCCTGTACACCGCCAACCCCCTGCCCGTGGCCACCACCGGCGTGGACCTGGACGTGACCCTGCCTGGCGAGGGCGGCAAGGACCGGCCC 360
            *** *  * ** ** ** ** ** **  * ** ** *********** ** ** ** ***** ** ** ** ** **  * ***** **  * ***** ** ** ** ** ** ** **

wt          TTCAAGGTGTCAATCAAATTTGTCTCTCGGGTGAGTTGGCACCTACTGCATGAAGTACTGACAGGACGGACCTTGCCTGAGCCACTGGAATTAGACAAGCCAATCAGCACTAACCCTGTC 480
opt         TTTAAGGTGTCCATCAAGTTCGTGAGCAGAGTGAGCTGGCACCTGCTGCACGAGGTGCTGACCGGCAGGACACTCCCTGAGCCCCTGGAACTGGACAAGCCCATCTCCACCAACCCCGTG 480
            ** ******** ***** ** **     * ***** ******** ***** ** ** ***** **  ****  * ******** ****** * ******** ***   *** ***** **

wt          CATGCCGTTGATGTGGTGCTACGACATCTGCCCTCCATGAAATACACACCTGTGGGGCGTTCATTTTTCTCCGCTCCAGAAGGATATGACCACCCTCTGGGAGGGGGCAGGGAAGTGTGG 600
opt         CACGCCGTGGACGTGGTGCTGCGGCACCTGCCCAGCATGAAGTACACCCCCGTGGGCAGAAGCTTCTTCAGCGCCCCTGAGGGCTACGACCACCCCCTGGGCGGAGGGCGGGAAGTGTGG 600
            ** ***** ** ******** ** ** ******  ****** ***** ** *****  *    ** ***  *** ** ** ** ** ******** ***** ** **  **********

wt          TTTGGATTCCATCAGTCTGTTCGGCCTGCCATGTGGAAAATGATGCTTAATATCGATGTTTCTGCCACTGCCTTCTACAAAGCACAACCTGTAATTCAGTTCATGTGTGAAGTTCTTGAT 720
opt         TTCGGCTTCCACCAGAGCGTGAGGCCCGCCATGTGGAAGATGATGCTGAACATCGACGTGAGCGCCACCGCCTTCTACAAGGCCCAGCCCGTGATCCAGTTCATGTGCGAGGTGCTGGAC 720
            ** ** ***** ***   **  **** *********** ******** ** ***** **    ***** *********** ** ** ** ** ** *********** ** ** ** **

wt          ATTCATAATATTGATGAGCAACCAAGACCTCTGACTGATTCTCATCGGGTAAAATTCACCAAAGAGATAAAAGGTTTGAAGGTTGAAGTGACTCATTGTGGAACAATGAGACGGAAATAC 840
opt         ATCCACAACATCGACGAGCAGCCCAGGCCCCTGACCGACAGCCACCGGGTGAAGTTCACCAAAGAGATCAAGGGCCTGAAGGTGGAGGTGACCCACTGCGGCACCATGCGGCGGAAGTAC 840
            ** ** ** ** ** ***** ** ** ***** **    ** ***** ** ************** ** **  ******* ** ***** ** ** ** ** *** * ***** ***

wt          CGTGTTTGTAATGTAACAAGGAGGCCTGCCAGTCATCAAACCTTTCCTTTACAGTTAGAAAACGGCCAAACTGTGGAGAGAACAGTAGCGCAGTATTTCAGAGAAAAGTATACTCTTCAG 960
opt         AGAGTGTGCAACGTGACCCGCAGGCCCGCCAGCCACCAGACATTCCCACTCCAGCTCGAAAACGGCCAGACCGTGGAGCGGACCGTGGCCCAGTACTTCCGGGAGAAGTATACCCTGCAG 960
             * ** ** ** ** **  * ***** ***** ** ** ** ** **  * *** * *********** ** ****** * ** ** ** ***** *** * ** ******** ** ***

wt          CTGAAGTACCCGCACCTTCCCTGTCTGCAAGTCGGGCAGGAACAGAAACACACCTACCTGCCACTAGAAGTCTGTAATATTGTGGCAGGGCAACGATGTATCAAGAAGCTAACAGACAAT 1080
opt         CTGAAGTACCCCCACCTGCCCTGCCTGCAAGTCGGCCAGGAACAGAAGCACACCTACCTGCCCCTGGAAGTGTGCAACATCGTGGCCGGCCAGCGGTGCATCAAGAAGCTGACCGACAAC 1080
            *********** ***** ***** *********** *********** ************** ** ***** ** ** ** ***** ** ** ** ** *********** ** *****

wt          CAGACTTCCACTATGATCAAGGCAACAGCAAGATCTGCACCAGATAGACAAGAGGAAATTAGCAGATTGGTAAGAAGTGCAAATTATGAAACAGATCCATTTGTTCAGGAGTTTCAATTT 1200
opt         CAGACCAGCACCATGATCAAGGCCACCGCCCGGTCCGCCCCTGACAGGCAGGAAGAGATCAGCCGGCTGGTCCGGAGCGCCAACTACGAGACCGACCCCTTCGTGCAGGAATTTCAGTTC 1200
            *****   *** *********** ** **  * ** ** ** ** ** ** ** ** *** *  ****  * ** ** ** ** ** ** ** ** ** ** ***** ***** **

wt          AAAGTTCGGGATGAAATGGCTCATGTAACTGGACGCGTACTTCCAGCACCTATGCTCCAGTATGGGGGACGGAATCGGACAGTAGCAACACCGAGCCATGGAGTATGGGACATGCGAGGG 1320
opt         AAAGTCCGGGACGAGATGGCCCACGTGACCGGCAGAGTGCTGCCTGCCCCCATGCTGCAGTACGGCGGCAGAAACCGGACAGTGGCCACCCCCAGCCACGGCGTGTGGGACATGCGGGGC 1320
            ***** ***** ** ***** ** ** ** **  * ** ** ** ** ***** ***** ** **  * ** ******** ** ** ***** ** ** *********** **

wt          AAACAATTCCACACAGGAGTTGAAATCAAAATGTGGGCTATCGCTTGTTTTGCCACACAGAGGCAGTGCAGAGAAGAAATATTGAAGGGTTTCACAGACCAGCTGCGTAAGATTTCTAAG 1440
opt         AAGCAGTTCCACACAGGCGTGGAGATCAAGATGTGGGCCATCGCCTGCTTCGCCACCCAGCGGCAGTGCCGGGAGGAAATCCTGAAGGGCTTCACCGACCAGCTGCGGAAGATCAGCAAG 1440
            ** ** *********** ** ** ***** ******** ***** ** ** ***** *** ******** * ** *****  ******* ***** *********** *****    ***

wt          GATGCAGGGATGCCCATCCAGGGCCAGCCATGCTTCTGCAAATATGCACAGGGGGCAGACAGCGTAGAGCCCATGTTCCGGCATCTCAAGAACACATATTCTGGCCTACAGCTTATTATC 1560
opt         GACGCCGGCATGCCCATCCAGGGCCAGCCCTGCTTCTGTAAGTACGCTCAGGGCGCCGACAGCGTGGAGCCCATGTTCCGGCACCTGAAGAACACCTACAGCGGGCTGCAGCTGATCATC 1560
            ** ** ** ******************* ******** ** ** ** ***** ** ******** **************** ** ******** **    ** ** ***** ** ***

wt          GTCATCCTGCCGGGGAAGACACCAGTGTATGCGGAAGTGAAACGTGTAGGAGACACACTTTTGGGTATGGCTACACAATGTGTTCAAGTCAAGAATGTAATAAAAACATCTCCTCAAACT 1680
opt         GTGATCCTGCCCGGCAAGACCCCCGTGTACGCCGAGGTGAAGAGAGTGGGCGACACACTGCTGGGCATGGCCACCCAGTGCGTGCAGGTCAAGAACGTGATCAAGACCAGCCCCCAGACC 1680
            ** ******** ** ***** ** ***** ** ** *****  * ** ** ********  **** ***** ** ** ** ** ** ******** ** ** ** **    ** ** **

wt          CTGTCAAACTTGTGCCTAAAGATAAATGTTAAACTCGGAGGGATCAATAATATTCTTGTACCTCATCAAAGACCTTCTGTGTTCCAGCAACCAGTGATCTTTTTGGGAGCCGATGTCACT 1800
opt         CTGAGCAACCTGTGCCTGAAGATCAACGTGAAGCTGGGCGGCATCAACAACATCCTGGTGCCCCACCAGAGGCCCAGCGTGTTCCAGCAGCCTGTGATCTTCCTGGGCGCCGACGTGACC 1800
            ***   *** ******* ***** ** ** ** ** ** ** ***** ** ** ** ** ** ** ** ** ** **    *********** ** ********  **** ***** ** **

wt          CATCCACCTGCTGGTGATGGAAAGAAGCCTTCTATTGCTGCTGTTGTAGGTAGTATGGATGCACACCCAAGCAGATACTGTGCCACAGTAAGAGTTCAGAGACCCCGACAGGAGATCATC 1920
opt         CACCCCCCTGCCGGCGACGGCAAGAAGCCCAGCATCGCCGCCGTGGTCGGCTCTATGGATGCCCATCCCTCCCGGTATTGCGCCACCGTGCGGGTGCAGCGGCCCAGGCAGGAAATCATC 1920
            ** ** ***** ** ** ** ********    ** ** ** ** ** **   ********* ** **   * * ** ** ***** **  * ** *** * *** * ***** ******

wt          CAGGACTTGGCCTCCATGGTCCGGGAACTTCTTATTCAATTTTATAAGTCAACTCGGTTCAAGCCTACTCGTATCATCTTTTATCGGGATGGTGTTTCAGAGGGGCAGTTTAGGCAGGTA 2040
opt         CAGGACCTGGCCTCCATGGTCCGGGAGCTGCTGATCCAGTTTTACAAGAGCACCCGGTTCAAGCCCACCCGGATCATCTTCTACCGGGACGGCGTGAGCGAGGGCCAGTTCCGGCAGGTG 2040
            ****** ****************** ** ** ** ** ***** ***   ** *********** ** ** ******** ** ***** ** **    ***** *****  *******

wt          TTATATTATGAACTACTAGCAATTCGAGAAGCCTGCATCAGTTTGGAGAAAGACTATCAACCTGGAATAACCTACATTGTAGTTCAGAAGAGACATCACACTCGATTATTTTGTGCTGAT 2160
opt         CTGTACTACGAACTGCTGGCCATCCGGGAGGCCTGCATCAGCCTGGAAAAGGACTACCAGCCCGGCATCACCTATATCGTGGTGCAGAAGCGGCACCACACCCGGCTGTTCTGCGCCGAC 2160
             * ** ** ***** ** ** ** ** ** ***********  **** ** ***** ** ** ** ** ***** ** ** ** ****** * ** ***** **  * ** ** ** **

wt          AGGACAGAAAGGGTTGGAAGAAGTGGCAATATCCCAGCTGGAACAACAGTTGATACAGACATTACACACCCATATGAGTTCGATTTTTACCTCTGTAGCCATGCTGGAATACAGGGTACC 2280
opt         CGGACCGAGCGCGTGGGCCGGTCCGGCAACATCCCTGCCGGCACCACCGTGGACACCGACATCACCCACCCCTACGAGTTCGACTTCTACCTGTGCAGCCACGCCGGCATCCAGGGCACC 2280
             **** **  * ** **  *    ***** ***** ** ** ** ** ** ** ** ***** ** ***** ** ******** ** ***** ** ***** ** ** ** ***** ***

wt          AGTCGTCCTTCACACTATCATGTTTTATGGGATGATAACTGCTTTACTGCAGATGAACTTCAGCTGCTAACTTACCAGCTCTGCCACACTTACGTACGCTGTACACGATCTGTTTCTATA 2400
opt         AGCCGGCCCAGCCACTACCACGTGCTGTGGGACGACAACTGCTTCACCGCCGACGAGCTGCAGCTGCTGACCTACCAGCTGTGCCACACCTACGTGCGGTGCACCCGGTCCGTGAGCATC 2400
            ** ** **    ***** ** **  * ***** ** ******** ** ** ** ** ** ******** ** ******** ******** ***** ** ** ** ** ** **    **

wt          CCTGCACCAGCGTATTATGCTCACCTGGTAGCATTTAGAGCCAGATATCATCTTGTGGACAAAGAACATGACAGTGCTGAAGGAAGTCACGTTTCAGGACAAAGCAATGGGCGAGATCCA 2520
opt         CCAGCCCCAGCCTACTACGCCCACCTGGTGGCCTTCCGGGCCAGATATCACCTGGTGGACAAAGAGCACGACAGCGCCGAGGGCAGCCACGTGAGCGGCCAGAGCAACGGCAGGGACCCC 2520
            ** ** ***** ** ** ** ******** ** **  * *********** ** *********** ** ***** ** ** ** ** *****    ** ** ***** **  * ** **

wt          CAAGCTCTTGCCAAGGCTGTACAGATTCACCAAGATACCTTACGCACAATGTACTTCGCTTAA 2583
opt         CAGGCTCTGGCCAAAGCTGTCCAGATCCACCAGGACACCCTGCGGACCATGTACTTCGCCTGA 2583
            ** ***** ***** ***** ***** ***** ** *** * ** ** ***********
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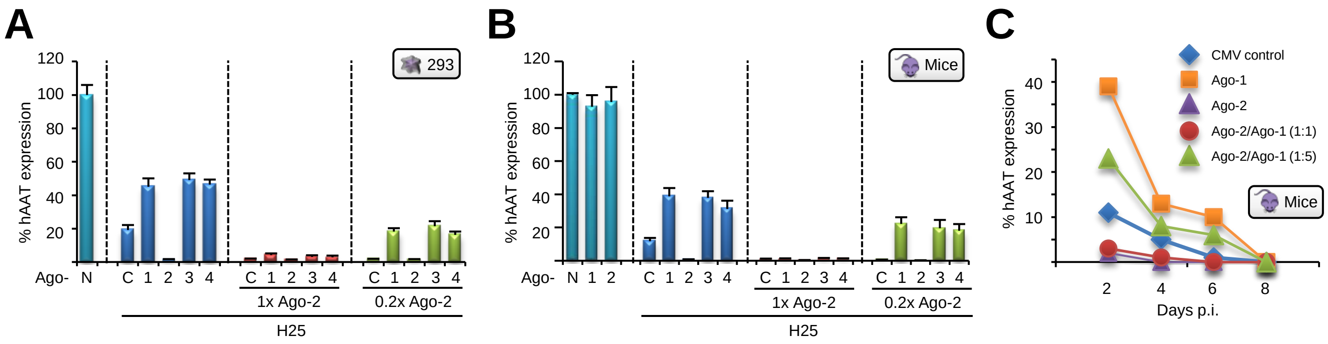
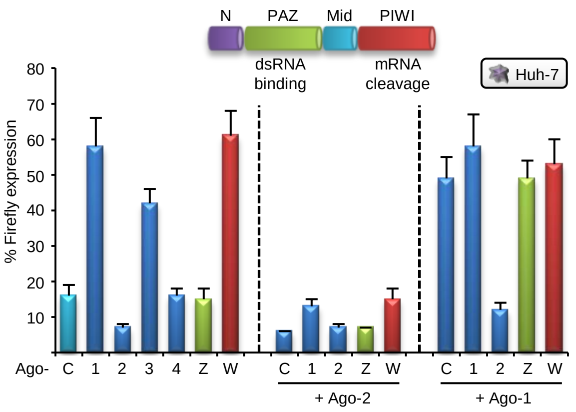
Supplemental Figure 3 Alignment of wildtype (wt) and codon-optimized (opt) Ago-3.

```
wt     ATGGAGGCGCTGGGACCCGGACCTCCGGCTAGCCTGTTTCAGCCACCTCGTCGTCCTGGCCTTGGAACTGTTGGAAAACCAATTCGACTGTTAGCCAATCATTTTCAGGTTCAGATTCCT 120
opt    ATGGAAGCCCTGGGCCCTGGCCCTCCCGCCAGCCTGTTCCAGCCCCCCAGGCGGCCTGGCCTGGGCACCGTGGGCAAGCCCATCCGGCTGCTGGCCAACCACTTTCAGGTCCAGATCCCC 120
       ***** ** ***** ** ** ***** ** ******** ***** **  * ** ******** ** ** ** ** ** ** ** ** *** * ***** ** ******** ***** **

wt     AAAATAGATGTGTATCACTATGATGTGGATATTAAGCCTGAAAAACGGCCTCGTAGAGTCAACAGGGAGGTAGTAGATACAATGGTGCGGCACTTCAAGATGCAAATATTTGGTGATCGG 240
opt    AAGATCGACGTGTACCACTACGACGTGGACATCAAGCCCGAGAAGCGGCCCAGGCGGGTGAACCGGGAGGTGGTGGACACCATGGTCCGGCACTTCAAGATGCAGATCTTCGGCGACCGG 240
       ** ** ** ***** ***** ** ***** ** ***** ** ** *****  *  * ** *** ******* ** ** ** ***** **************** ** ** ** ** ***

wt     CAGCCTGGGTATGATGGCAAAAGAAACATGTACACAGCACATCCACTACCAATTGGACGGGATAGGGTTGATATGGAGGTGACTCTTCCAGGCGAGGGTAAAGACCAAACATTTAAAGTG 360
opt    CAGCCCGGCTACGACGGCAAGCGGAACATGTACACCGCCCACCCCCTGCCCATCGGCCGGGACCGGGTGGACATGGAAGTGACCCTGCCCGGCGAGGGCAAGGACCAGACCTTCAAGGTG 360
       ***** ** ** ** *****  * *********** ** ** ** ** ** ** *****  **** ** ***** ***** ** ** ******** ** ***** ** ** ** ***

wt     TCTGTTCAGTGGGTGTCAGTTGTGAGCCTTCAGTTGCTTTTAGAAGCTTTGGCTGGGCACTTGAATGAAGTCCCAGATGACTCAGTACAAGCACTTGATGTTATCACAAGACACCTTCCC 480
opt    TCCGTGCAGTGGGTGTCCGTGGTGTCCCTGCAGCTGCTGCTGGAAGCTCTCGCTGGACATCTGAACGAGGTGCCCGACGACAGCGTGCAGGCCCTGGACGTGATCACCCGGCACCTGCCC 480
       ** ** *********** ** ***  *** *** ****  * ****** * ***** **  **** ** ** ** ** ***   ** ** ** ** ** ** *****  * ***** ***

wt     TCCATGAGGTACACCCCAGTGGGCCGTTCCTTTTTCTCACCCCCGGAAGGTTACTACCACCCTCTGGGAGGGGGCAGGGAGGTCTGGTTTGGTTTTCATCAGTCTGTGAGACCTGCCATG 600
opt    AGCATGCGGTACACCCCCGTGGGCAGAAGCTTCTTCAGCCCCCCTGAGGGCTACTACCACCCCCTGGGCGGAGGGCGGGAAGTGTGGTTCGGCTTCCACCAGAGCGTGAGGCCCGCCATG 600
         **** ********** ****** *   *** ***   ***** ** ** *********** ***** ** **  **** ** ***** ** ** ** ***   ***** ** ******

wt     TGGAATATGATGCTCAACATTGATGTATCTGCAACTGCTTTCTACCGGGCTCAGCCTATCATTGAGTTCATGTGTGAGGTTTTAGACATTCAGAACATCAATGAACAGACCAAACCTCTA 720
opt    TGGAACATGATGCTGAACATCGACGTGAGCGCCACCGCCTTCTACAGAGCCCAGCCCATCATCGAGTTCATGTGCGAGGTGCTGGACATCCAGAACATCAACGAGCAGACCAAGCCCCTG 720
       ***** ******** ***** ** **    ** ** ** ****** * ** ***** ***** *********** *****  * ***** *********** ** ******** ** **

wt     ACAGACTCCCAGCGTGTCAAATTTACCAAAGAAATCAGAGGTCTCAAAGTTGAGGTGACCCACTGTGGACAGATGAAACGAAAATACCGAGTTTGTAATGTGACTAGACGGCCAGCCAGT 840
opt    ACCGACAGCCAGCGGGTGAAGTTCACCAAAGAGATCCGGGGCCTGAAGGTGGAGGTGACCCACTGCGGCCAGATGAAGCGGAAGTACAGAGTGTGCAACGTGACCAGAAGGCCCGCCAGC 840
       ** ***  ****** ** ** ** ******** *** * ** ** ** ** ************** ** ******** ** ** *** **** ** ** ***** *** **** *****

wt     CATCAAACTTTTCCTTTGCAGCTAGAAAACGGTCAAGCTATGGAATGTACAGTAGCTCAATATTTTAAGCAAAAGTATAGTCTGCAACTGAAATACCCCCATCTTCCCTGTCTCCAAGTG 960
opt    CACCAGACATTCCCACTGCAGCTGGAAAACGGCCAGGCCATGGAATGCACCGTGGCCCAGTACTTCAAGCAGAAGTACAGCCTGCAGCTGAAGTACCCCCACCTGCCCTGCCTGCAAGTC 960
       ** ** ** ** **  ******* ******** ** ** ******** ** ** ** ** ** ** ***** ***** ** ***** ***** ******** ** ***** ** *****

wt     GGACAAGAACAAAAGCATACATACTTGCCACTCGAGGTCTGTAATATAGTGGCAGGACAGCGATGTATCAAGAAGCTCACAGACAATCAGACTTCCACAATGATCAAAGCTACAGCAAGA 1080
opt    GGCCAGGAACAGAAGCACACCTACCTGCCTCTCGAAGTCTGCAACATCGTCGCAGGGCAGCGGTGCATCAAGAAGCTGACCGACAATCAGACCAGCACCATGATCAAGGCCACCGCCAGA 1080
       ** ** ***** ***** ** *** **** ***** ***** ** ** ** ***** ***** ** *********** ** ***********   *** ******** ** ** ** ***

wt     TCTGCTCCTGACAGACAGGAAGAGATCAGTAGACTGGTGAAGAGCAACAGTATGGTGGGTGGACCTGATCCATACCTTAAAGAATTTGGTATTGTTGTCCACAATGAAATGACAGAGCTC 1200
opt    AGCGCCCCTGACAGGCAGGAAGAGATCAGCCGGCTGGTGAAGAGCAACAGCATGGTCGGCGGACCCGACCCCTACCTGAAAGAATTTGGCATCGTGGTGCACAACGAGATGACCGAGCTG 1200
          ** ******** *************  * ***************** ***** ** ***** ** ** ***** *********** ** ** ** ***** ** ***** *****

wt     ACAGGCAGGGTACTTCCAGCACCAATGCTGCAATATGGAGGCCGGAATAAAACAGTAGCCACACCCAACCAGGGTGTCTGGGACATGCGAGGAAAGCAGTTTTATGCTGGCATTGAAATT 1320
opt    ACCGGCAGAGTGCTGCCTGCCCCCATGCTGCAGTACGGCGGCAGAAACAAGACCGTGGCCACCCCCAACCAGGGCGTGTGGGACATGCGGGGCAAGCAGTTCTACGCCGGCATCGAGATC 1320
       ** ***** ** ** ** ** ** ** ******** ** ** *** * ** ** ** ** ***** *********** ** *********** ** ******** ** ** ***** ** **

wt     AAAGTTTGGGCAGTTGCTTGTTTTGCACCTCAGAAACAATGTAGGGAAGATTTACTAAAGAGTTTCACTGACCAGCTGCGTAAAATCTCTAAGGATGCAGGAATGCCCATCCAGGGTCAG 1440
opt    AAAGTGTGGGCCGTGGCCTGCTTCGCCCCCCAGAAGCAGTGCCGGGAGGACCTGCTGAAGAGCTTCACCGACCAGCTGCGGAAGATCAGCAAGGACGCCGGCATGCCCATCCAGGGCCAG 1440
       ***** ***** ** ** ** ** ** ** ***** ** **  **** **  * ** ***** ***** *********** ** ***   ***** ** ** ************** ***

wt     CCATGTTTCTGCAAGTATGCACAAGGTGCAGACAGTGTGGAGCCTATGTTTAAACATCTGAAAATGACTTATGTGGGCCTACAGCTAATAGTGGTTATCCTGCCTGGAAAGACACCAGTA 1560
opt    CCCTGCTTCTGTAAGTACGCTCAGGGCGCCGACAGCGTGGAGCCCATGTTCAAGCACCTGAAGATGACCTACGTGGGGCTGCAGCTGATCGTGGTGATCCTGCCTGGCAAGACCCCCGTG 1560
       ** ** ***** ***** ** ** ** ** ***** ******** ***** ** ** ***** ***** ** ***** ** ***** ** ***** *********** ***** ** **

wt     TATGCGGAGGTGAAACGTGTTGGAGATACCCTTCTAGGTATGGCCACACAGTGTGTCCAGGTAAAAAATGTAGTGAAGACCTCACCTCAAACCCTTTCCAATCTTTGCCTGAAGATAAAT 1680
opt    TACGCCGAGGTGAAGAGAGTGGGCGACACACTGCTGGGCATGGCCACCCAGTGCGTGCAGGTCAAGAACGTGGTGAAAACCAGCCCCCAGACCCTGAGCAACCTGTGCCTGAAGATCAAC 1680
       ** ** ********  * ** ** ** ** ** ** ** ******** ***** ** ***** ** ** ** ***** ***    ** ** *****    *** ** *********** ** **

wt     GCAAAACTTGGAGGAATTAACAATGTGCTTGTGCCTCATCAAAGGCCCTCGGTGTTCCAGCAGCCTGTCATCTTCCTGGGAGCGGATGTCACACACCCCCCAGCAGGGGATGGGAAGAAA 1800
opt    GCCAAGCTGGGCGGCATCAACAACGTGCTGGTGCCCCACCAGAGGCCCTCCGTGTTCCAGCAGCCCGTGATCTTCCTGGGCGCCGACGTGACCCACCCCCCTGCCGGCGACGGGAAGAAG 1800
       ** ** ** ** ** ** ***** ***** ***** ** ** ********* ************** ** *********** ** ** ** ** ******** ** ** ** ********

wt     CCTTCCATTGCTGCTGTGGTTGGCAGTATGGATGGCCACCCCAGCCGGTACTGTGCCACCGTTCGGGTGCAGACTTCCCGGCAGGAGATCTCCCAAGAGCTCCTCTACAGTCAAGAGGTC 1920
opt    CCCAGCATCGCCGCCGTGGTGGGCAGCATGGACGGCCACCCCAGCCGGTACTGCGCCACCGTGCGGGTGCAGACCAGCCGGCAGGAAATCAGCCAGGAACTGCTGTACAGCCAGGAAGTG 1920
       **   *** ** ** ***** ***** ***** ******************** ******** ***********   ********* *****  *** ** ** ** ***** ** ** **

wt     ATCCAGGACCTGACTAACATGGTTCGAGAGCTGCTGATTCAGTTCTACAAATCCACACGCTTCAAACCCACTCGGATCATCTATTACCGTGGAGGGGTATCTGAGGGACAAATGAAACAG 2040
opt    ATCCAGGACCTGACCAACATGGTCCGGGAGCTGCTGATCCAGTTCTACAAGAGCACCCGGTTCAAGCCCACCCGGATCATCTACTACAGAGGCGGCGTGAGCGAGGGGCAGATGAAGCAG 2040
       ************** ******** ** *********** ***********   *** ** ***** ***** *********** *** * ** ** **    ***** ** ***** ***

wt     GTAGCTTGGCCAGAACTAATAGCAATTCGAAAGGCATGTATTAGCTTGGAAGAAGATTACCGGCCAGGAATAACTTATATTGTGGTGCAAAAAAGACATCACACACGACTCTTCTGTGCA 2160
opt    GTGGCCTGGCCCGAGCTGATCGCCATCCGGAAGGCCTGCATCAGCCTGGAAGAGGACTACAGGCCCGGCATCACCTACATTGTGGTGCAGAAGCGGCACCACACCCGGCTGTTCTGCGCC 2160
       ** ** ***** ** ** ** ** ** ** ***** ** *** ******* ** *** **** ** ** ** ** *********** **  * ** ***** ** ** ***** ** **

wt     GATAAAACAGAAAGGGTAGGGAAAAGTGGCAATGTACCAGCAGGCACTACAGTGGATAGTACCATCACACATCCATCTGAGTTTGACTTTTACCTCTGTAGTCATGCAGGAATACAGGGA 2280
opt    GACAAGACCGAGCGCGTGGGCAAGAGCGGCAACGTGCCTGCCGGCACCACCGTGGACAGCACCATCACCCACCCCAGCGAGTTCGACTTCTACCTGTGCAGCCACGCCGGCATTCAGGGC 2280
       ** ** ** **  * ** ** ** ** ***** ** ** ** ***** ** ***** ** ******** ** **   ***** ***** ***** ** ** ** ** ** ** *****

wt     ACCAGCCGTCCCTCACATTACCAGGTCTTGTGGGATGACAACTGCTTCACTGCAGATGAACTCCAGCTACTGACTTACCAGCTGTGTCACACCTATGTGAGGTGCACTCGCTCAGTCTCT 2400
opt    ACAAGCAGGCCCAGCCACTACCAGGTGCTGTGGGACGACAACTGCTTCACCGCCGACGAACTCCAGCTGCTCACCTACCAGCTGTGCCACACCTACGTGCGGTGCACCCGGTCCGTGAGC 2400
       ** *** * ***   ** ********  ******* ************** ** ** *********** ** ** ************ ******** *** ******* ** ** **

wt     ATTCCAGCCCCTGCATATTATGCCCGGCTTGTAGCATTTAGGGCAAGGTATCATCTGGTGGATAAAGATCATGACAGTGCGGAAGGCAGTCATGTGTCAGGACAGAGCAACGGCCGAGAT 2520
opt    ATCCCTGCCCCTGCCTACTACGCCAGACTGGTGGCCTTCCGGGCCAGATACCACCTGGTGGACAAGGACCACGACAGCGCCGAGGGCAGCCACGTGAGCGGCCAGAGCAACGGCAGGGAC 2520
       ** ** ******** ** ** *** * ** ** ** **  **** ** ** ******** ** ** ** ***** ** ** ***** ** ***    ** ************ * **

wt     CCTCAGGCCTTGGCTAAGGCTGTGCAAATCCACCATGATACCCAGCACACGATGTATTTTGCCTGA 2586
opt    CCCCAGGCTCTGGCCAAGGCCGTGCAGATCCATCACGACACACAGCACACCATGTACTTCGCCTGA 2586
       ** *****  **** ***** ***** ***** ** ** ** ******** ***** ** ******
```

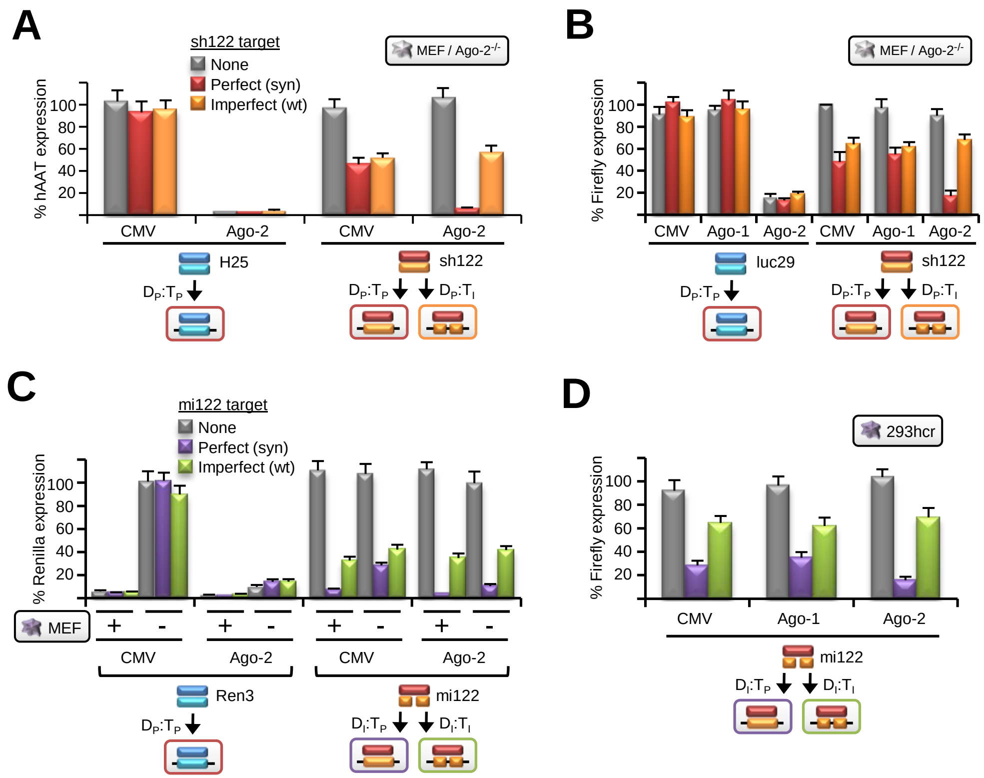
Supplemental Figure 4 Alignment of wildtype (wt) and codon-optimized (opt) Ago-4.



Supplemental Figure 5 Validation of the key role of human Ago proteins and of their competition for shRNA activity. Co-transfection of individual Ago proteins, or of equimolar (1x) or sub-molar (0.2x) amounts of Ago-2 with the other three human Ago proteins, into cells (**A**) or mice (n = 3 to 5, day 2) (**B**). Also added were hAAT expression and H25 shRNA plasmids. Note the dominant positive effect of Ago-2 on shRNA activity that could only be out-competed with the other Agos when they were delivered in excess. N; non-shRNA-transfected control. (**C**) Kinetics of competition and hAAT knockdown in murine livers (n = 2) hydrodynamically transfected with hAAT expression plasmid, H25 shRNA and the indicated Ago combinations. In all assays (**A-C**), the presence of exogenous Ago-2 markedly enhanced shRNA activity, validating and extending the data in Figure 1.



Supplemental Figure 6 Competition of wildtype and cleavage-deficient Ago-2 for shRNA activation (confirmation with an alternative reporter, see Figure 1, G and H for details). Huh-7 cells were co-transfected with a Firefly reporter, luc29 shRNA and the indicated Ago constructs ("+" indicates a four-fold excess of competitor).



Supplemental Figure 7 Further evidence for varying Ago-2 dependency of RNAi triggers. (**A**) Figure 2, B-D suggested that shRNAs against imperfect 3'UTR targets depend less on Ago-2 than those against perfect targets (ORF or 3'UTR), at least under transient conditions. This was substantiated here in Ago-2 knockout cells (MEF/Ago-$2^{-/-}$), where anti-ORF D_P:T_P-H25 was inactive, while anti-3'UTR D_P:T_P- and D_P:T_I-sh122 were similarly potent (compare red and orange bars in the absence of Ago-2 ("CMV")). Ago-2 co-delivery enhanced D_P:T_P-H25 and D_P:T_P-sh122, but not D_P:T_I-sh122 activities. This was further confirmed with another reporter (Firefly, **B**). (**C**) For evaluation with miRNAs, we transfected Ago-2-positive (+) or -negative (-) MEFs with D_I-miR-122 expression plasmid or anti-ORF D_P-shRNA (Ren3, same results were obtained with two other shRNAs, Ren1/2), together with luciferase reporters tagged with perfect or imperfect miR-122 sites. The fact that D_I:T_P and D_I:T_I miRNA activity were conserved in Ago-$2^{-/-}$ cells in the absence of exogenous Ago-2 ("-/CMV"), while the control shRNA was inactive (as noted before, **A,B** above), validated that miRNAs principally act independently of Ago-2. Notably, D_I:T_P miRNA activity was consistently enhanced upon Ago-2 co-delivery both in wildtype and Ago-$2^{-/-}$ cells. This implied that with artificial perfect targets, high miRNA levels (estimated $>10^5$ copies / transfected cell) can also saturate Ago-2. This is in line with our in vivo data in Figure 2G and was further validated in 293 cells inducibly expressing ~25.000 miR-122 copies (293hcr), where D_I:T_P activity also increased with Ago-2 expression (**D**, purple). Mutant cells with an inactive miR-122 served as negative control (not shown). Again validating the data in Figure 2G, D_I:T_I activity was always unaffected by ectopic Ago-2, suggesting no Ago-2 saturation with imperfect triggers against imperfect targets (**C,D**, green bars). Alas, as discussed in the main text, this combination is not therapeutically useful.