

Supplementary Data

Supplementary Figure 1. Knock-down of miR-124 target VAMP3 by single-strand mimics, 24h following transfection of HCT-116 at 2nM and 10nM. 2'-OH is denoted in red, 2'-fluoro in green, 2'-o-methyl in black, and 5'-phosphorylation in purple. (A) miR-124 single-strands containing different patterns of 2'-fluoro modifications, with or without 5'-phosphorylation. (B) When single strand length is varied, maximum activity is seen for miR-124 single-strands of length 19 to 22 nucleotides. (C) 2'-o-methyl walkthrough of a 2'-fluoro and 5'-phosphorylated miR-124 single strand shows loss of activity when a 2'-o-methyl is placed at position 2.

Supplementary Figure 2. Activity of the fluorinated and phosphorylated miR-124 single-strand is enhanced by the addition of 5' and 3' passenger fragments, but either fragment alone decreases activity. Dose-dependent response of VAMP3 mRNA in HCT-116 cells, as measured by qRT-PCR. Schematic of annealed strands depicts modified strands and passenger segments, where 2'-OH is denoted in red, 2'-fluoro is green, 2'-o-methyl is black, and 5'-phosphate is purple.

Supplementary Figure 3. Restoration of activity by addition of 3' and 5' passenger strand fragments linked by carbon spacers. HCT-116 cells were transfected at 10 nM, 4 nM, and 2 nM, and VAMP3 mRNA was measured by qRT-PCR. 2'-OH is depicted in red, 2'-fluoro in green, 2'-o-methyl in black, LNA in light blue, 5'-phosphate in purple, and inverted abasic in yellow. The C3-spacer (ChemGenes CLP-9908) is represented by a dash.

Supplementary Figure 4. Activity of miR-124 single-strands with randomized bases at positions 10 through 19. VAMP3 mRNA levels were measured by qRT-PCR following transfection of HCT-116 cells at 10nM.

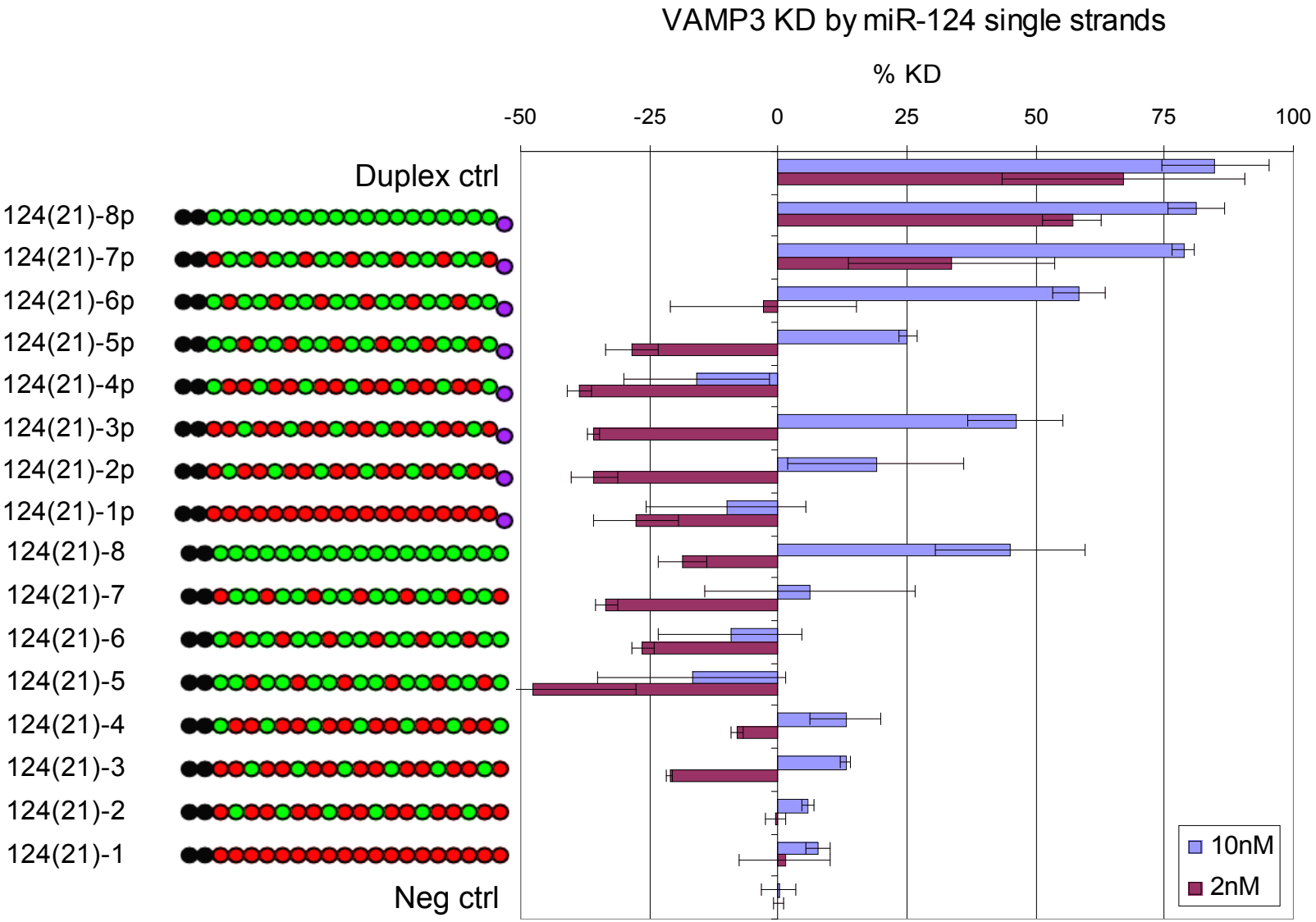
Supplementary Figure 5. Dose response curve of miR-122 target GYS1 to

increasing amounts of the indicated oligos. Red: miR-122 control duplex. Black: non-targeting control siRNA. Green: miR-122 sequence in the “21-8p” modification pattern. Blue: miR-122 seed sequence fused with the “9Y_2” pyrimidine-rich 3' cassette sequence, also in the 21-8p modification pattern.

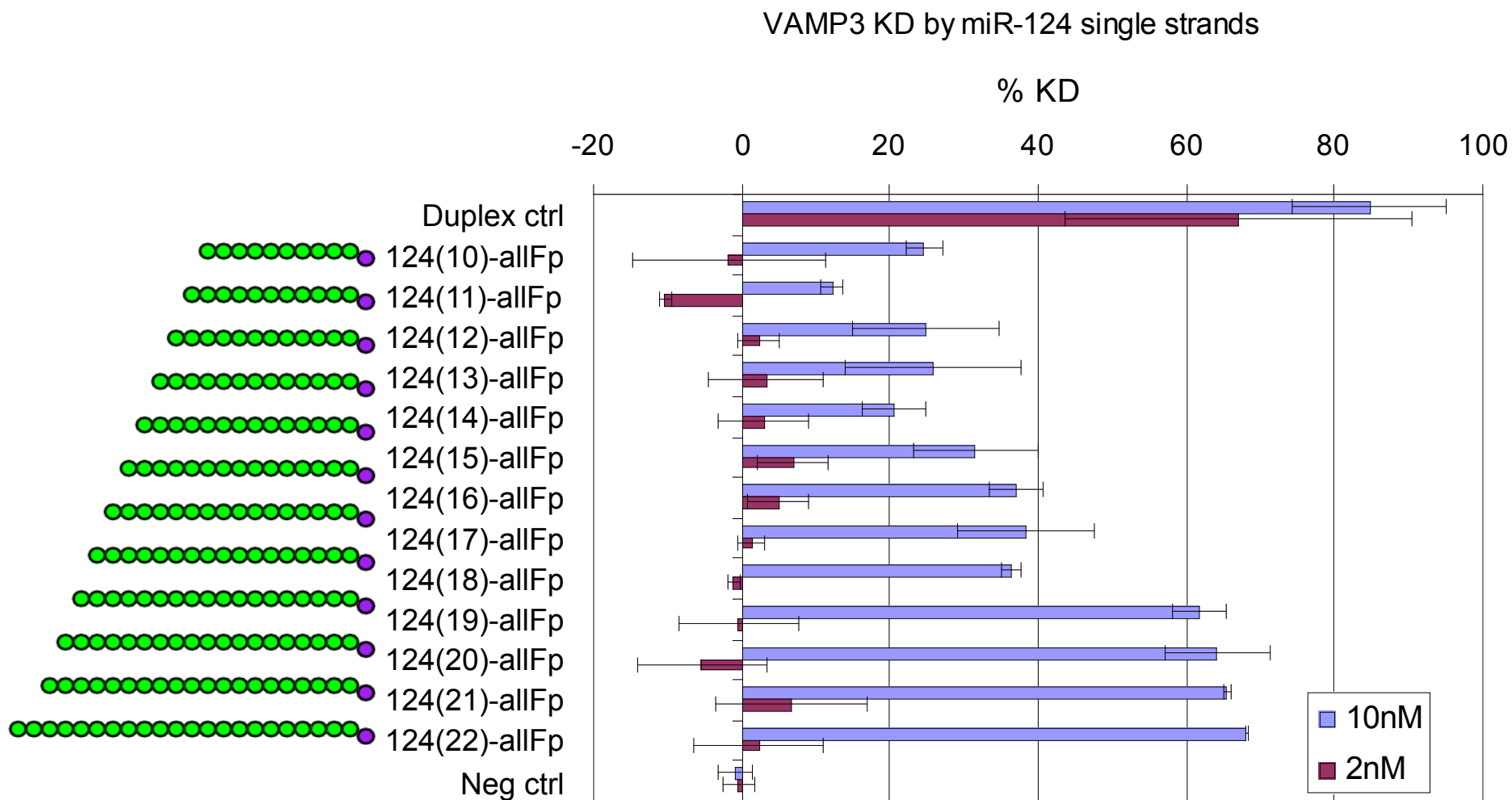
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Supplementary Figure 6. 9Y_2 cassette does not show extensive matching to target sites as compared to wildtype sequence. Binding sites and 3' pairing were predicted by running the Targetscan algorithm (Grimson et al, 2007) using the indicated UTR and miRNA sequences. miRNA sequences (shown 3'->5') are at the bottom of each pairing. A. miR-122 9Y_2 cassette compared with mir122-8p. B. miR-124 9Y_2 cassette compared with miR-124 21-8p.

Supplementary Figure 1A: increased activity from single strands that are phosphorylated and fluorinated

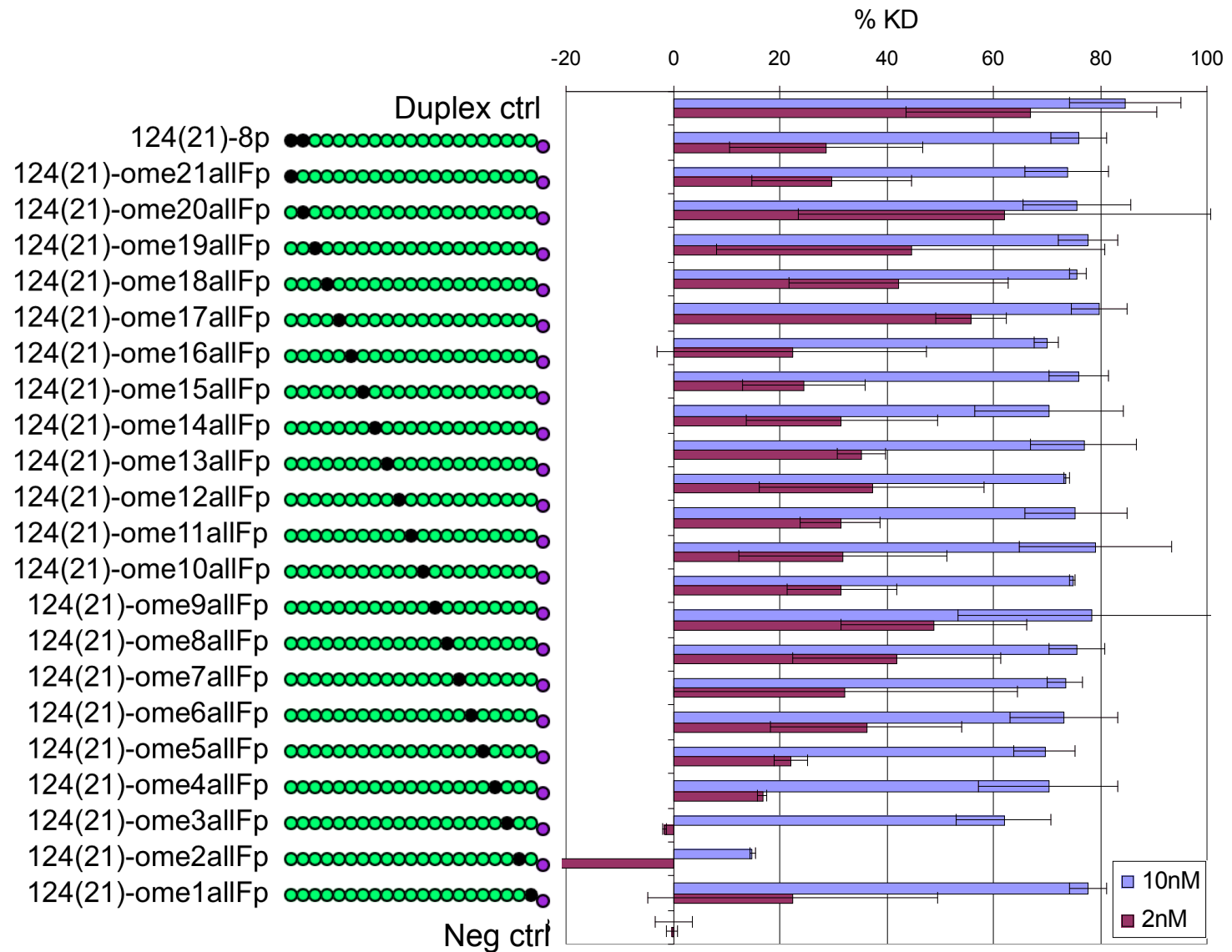


Supplementary Figure 1B: Maximum activity is observed for strands that are 19-22 nucleotides long

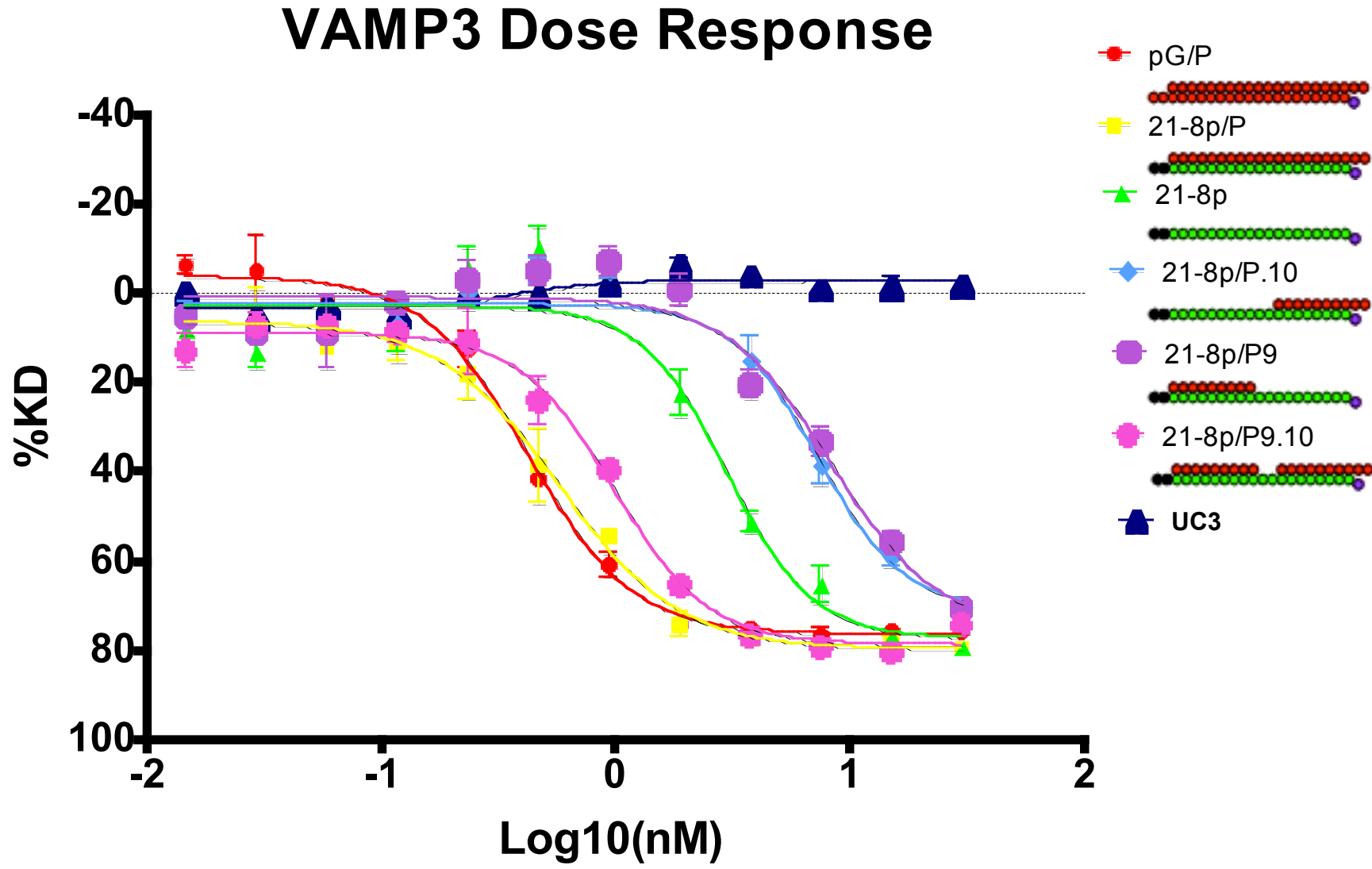


Supplementary Figure 1C: 2-o-methyl modification walkthrough shows loss of activity at position 2

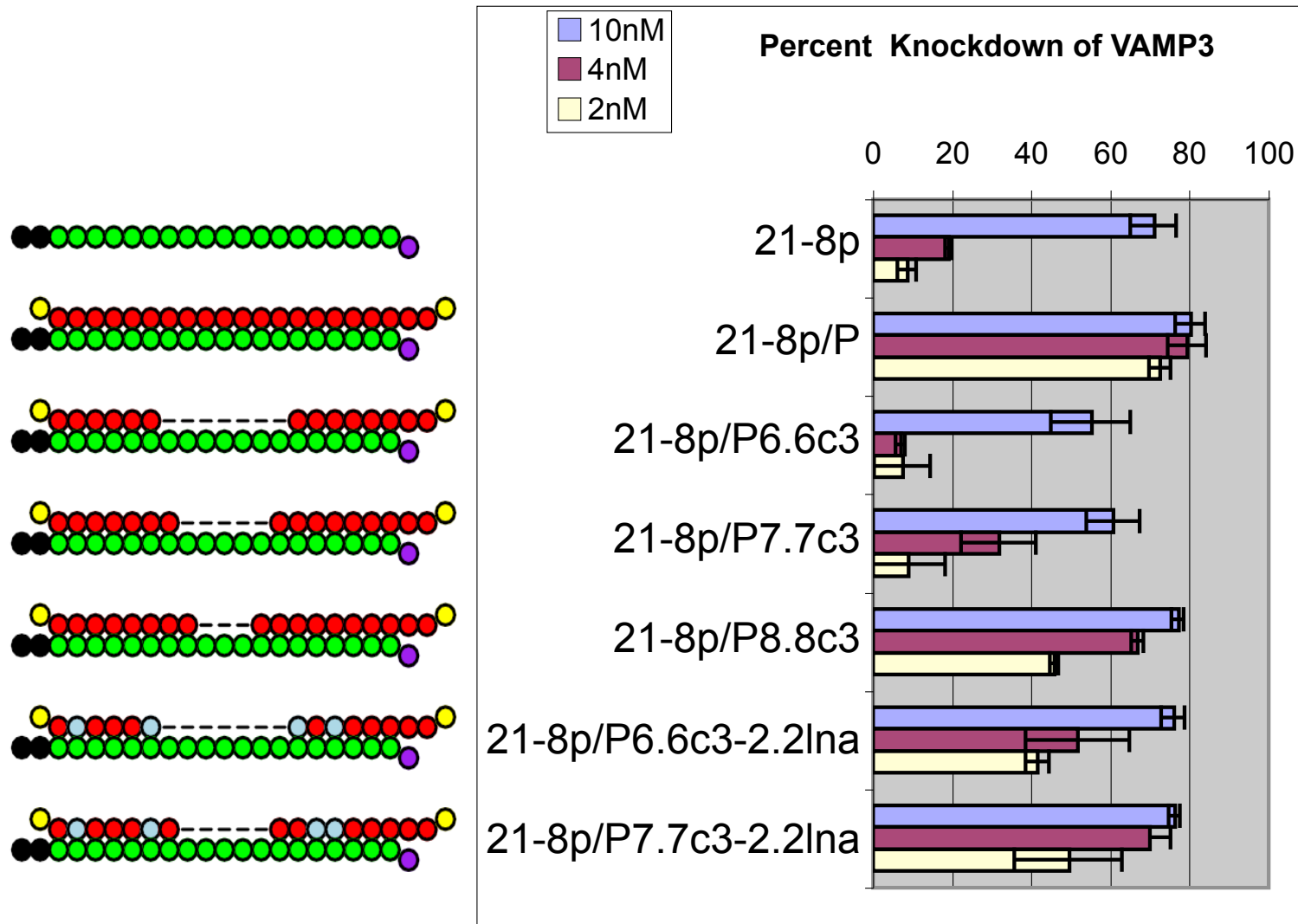
VAMP3 KD by miR-124 2' O-methyl modifications



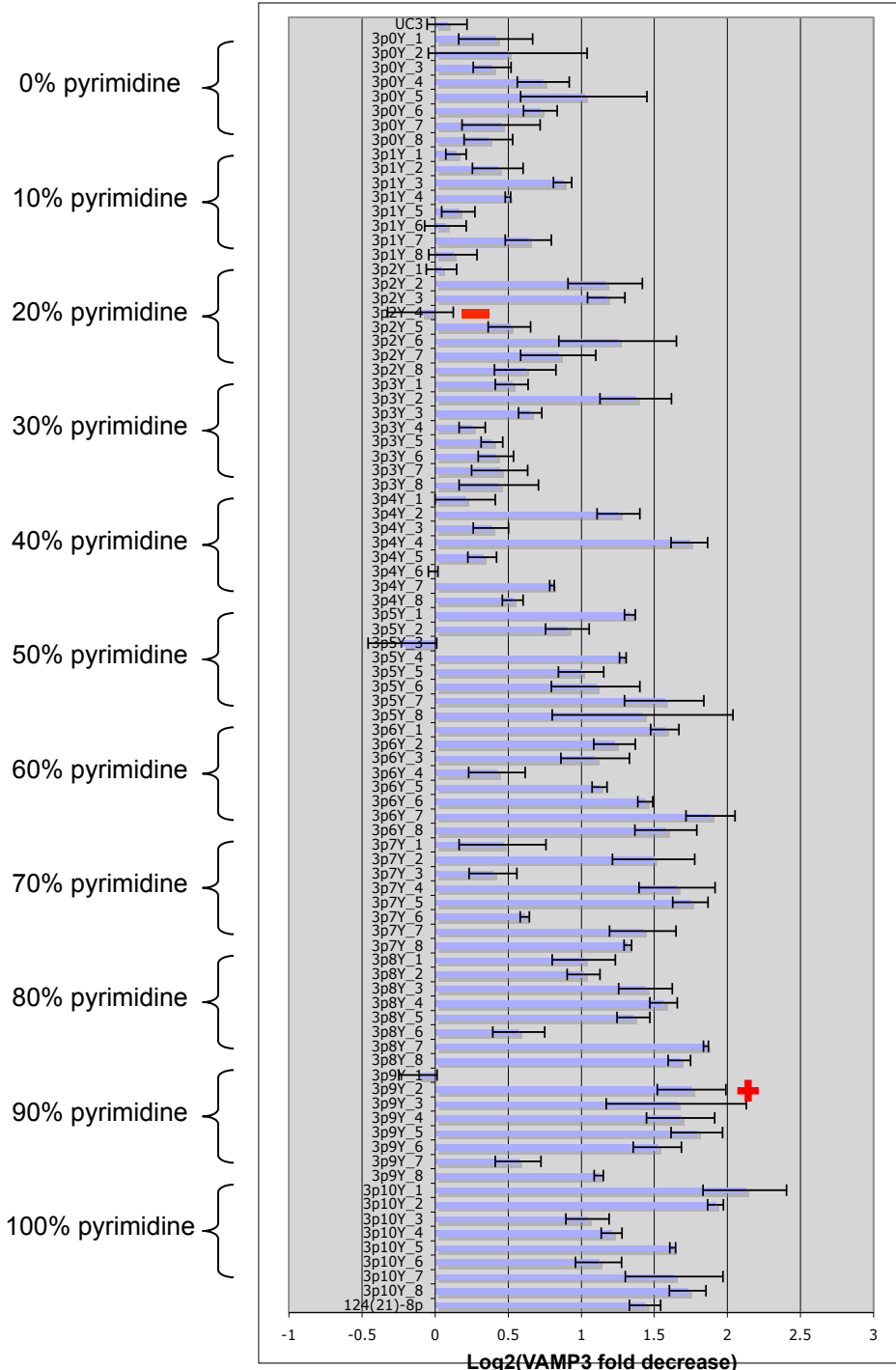
Supplementary Figure 2: 5' and 3' passenger fragments hurt 21-8p activity by themselves, but help activity when combined



Supplementary Figure 3: Reconstitution of activity by adding back linked passenger strand fragments



Supplementary Figure 4: Testing of 3' ends with differing levels of pyrimidine composition demonstrates importance of sequence composition on single strand activity



— “2Y_4”, low performer

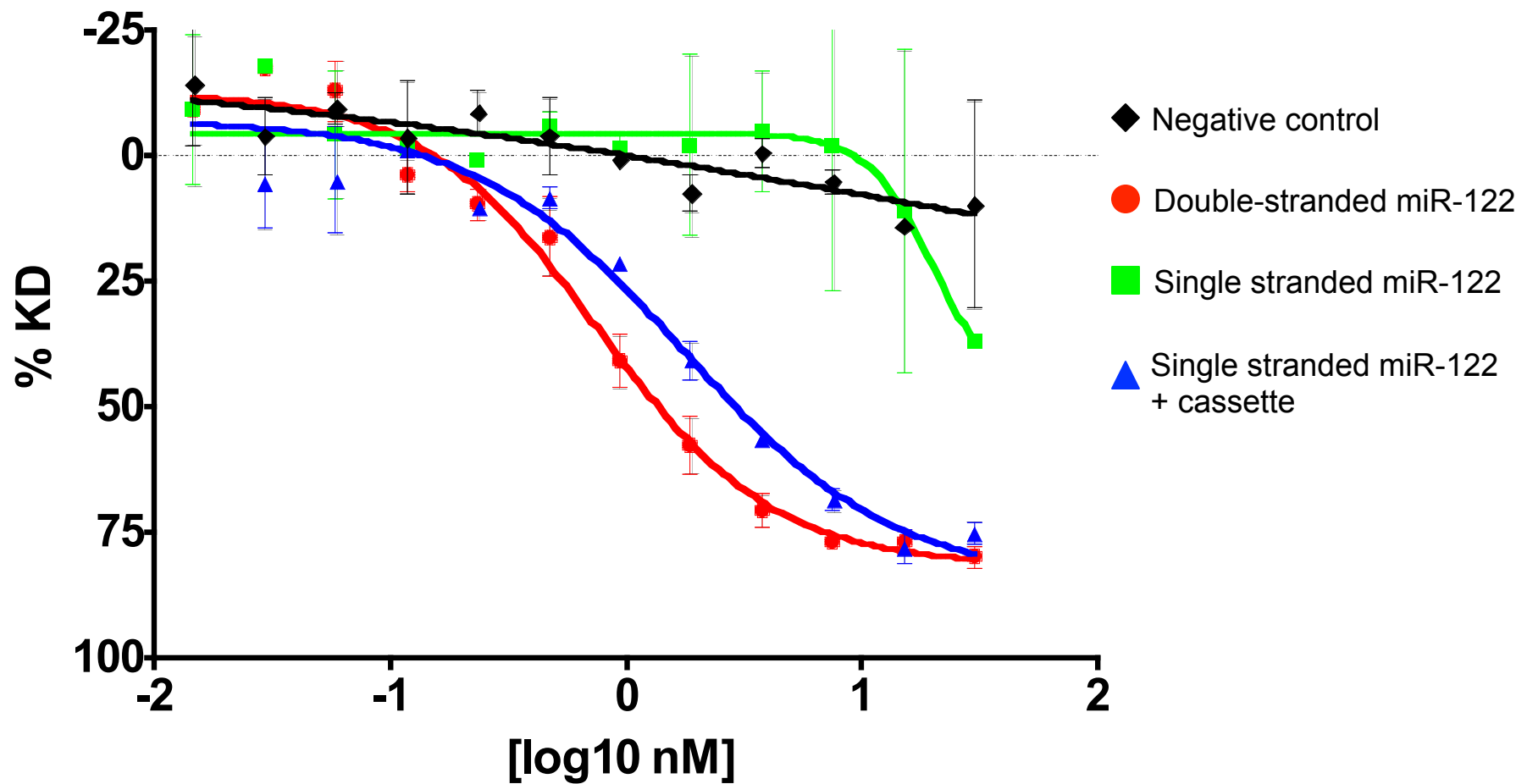
pUAAGGCACGGAGGGCAAUGUU
 miR-124 seed 20% pyrimidine cassette

+ “9Y_2”, high performer

pUAAGGCACGCGCUUCUCUUUU
 miR-124 seed 90% pyrimidine cassette

Supplementary Figure 5: Pyrimidine rich 3' cassette can enhance single-strand miR-122 activity

SLC7A1



Supplementary Figure 6: 9Y_2 cassette does not show extensive matching to target sites when compared to wildtype sequence

A

		mir122-8p	mir122-9Y_2
GYS1 3'UTR (NM_002103)	Pos 134	CACCCAGUCCGCCAAACACUCCA	CACCCAGUCCGCCAAACACUCCA
		UUUGUGGUAACAGUGUGAGGU	UUUUCUCUUCGCGUGUGAGGU
	Pos 172	GUUUCCAAGUUCCUGCACUCCAG	GUUUCCAAGUUCCUGCACUCCAG
		UUUGUGGUAACAGU--GUGAGGU	UUUUCUCUUCGCGUGUGAGGU
SLC7A1 3'UTR (NM_003045)	Pos 1090	GUCCAUGAAAUUGUGACACUCCA	GUCCAUGAAAUUGUGACACUCCA
		UUUGUGGUAACAGUGUGAGGU	UUUUCUCUUCGCG--UGUGAGGU
	Pos 1363	AGCACCAUUUCACACACACUCCU	AGCACCAUUUCACACACACUCCU
		UUUGUGGUAACAG---UGUGAGGU	UUUUCUCUUCGCGUGUGAGGU
	Pos 4109	GUGGUGCAAGAAGCUACACUCCA	GUGGUGCAAGAAGCU-ACACUCCA
		UUUGUGGUAACAGUGUGAGGU	UUUUCUCUUCGCGUGUGAGGU

B

miR-124 21-8p

miR-124 3p9Y_2

CD164 3'UTR
(NM_006016)

Pos 650

AGAAUAUAAAGAACAGUGCCUUA

||||||

ACCGUAAGUGGCGCACGGAU

Pos 1651

ACUUA AUGUUUCAA---GUGCCUUA

|||| ||||||

ACCGUAAGUGGCGCACGGAU

AGAAUAUAAAGACA--GUGCCUUA

|||| ||||||

UUUUCUCUUCGCGCACGGAU

ACUUA AUGUUUCAAGUGCCUUA

||||||

UUUUCUCUUCGCGCACGGAU

VAMP3 3'UTR
(NM_004781)

Pos 140

GAAUUUCUAGGAAACGUGCCUUU

||||||

ACCGUAAGUGGCGCACGGAU

Pos 202

UCCACAUUUUGCACAGUGCCUUU

|||| ||||||

ACCGUAAGUGGCG-CACGGAU

Pos 437

UGAUAACAACAGGCUGUGCCUUA

||||||

ACCGUAAGUGGCGCACGGAU

GAAUUUCUAGGAAAC--GUGCCUUU

||| ||||||

UUUUCUCUUCGCGCACGGAU

UCCACAUUUUGCACAGUGCCUUU

||||||

UUUUCUCUUCGCGCACGGAU

UGAUAACAACAGGCUGUGCCUUA

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UUUUCUCUUCGCGCACGGAU