

# Identification of small molecule inhibitors of the Lin28-mediated blockage of pre-let-7g processing

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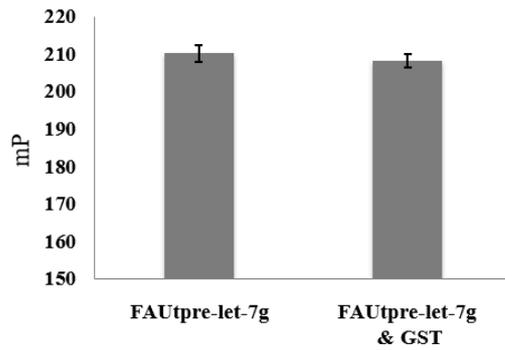
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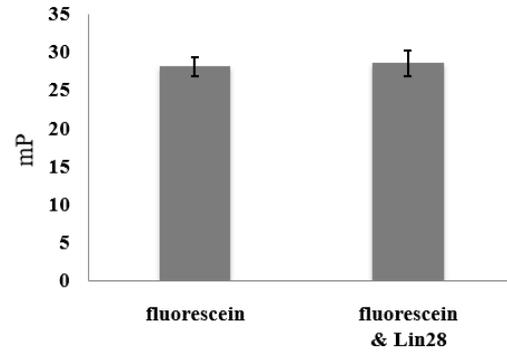
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Key words: Lin28, let-7 miRNA, small molecules, high throughput screening

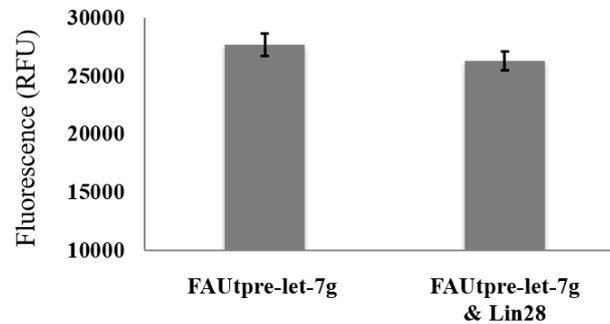
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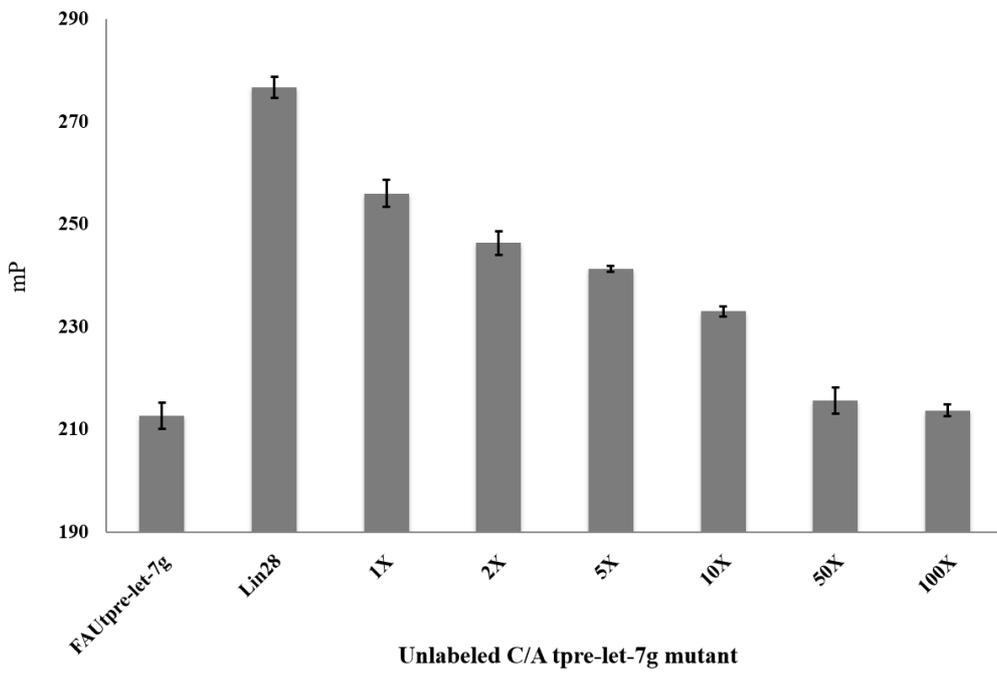
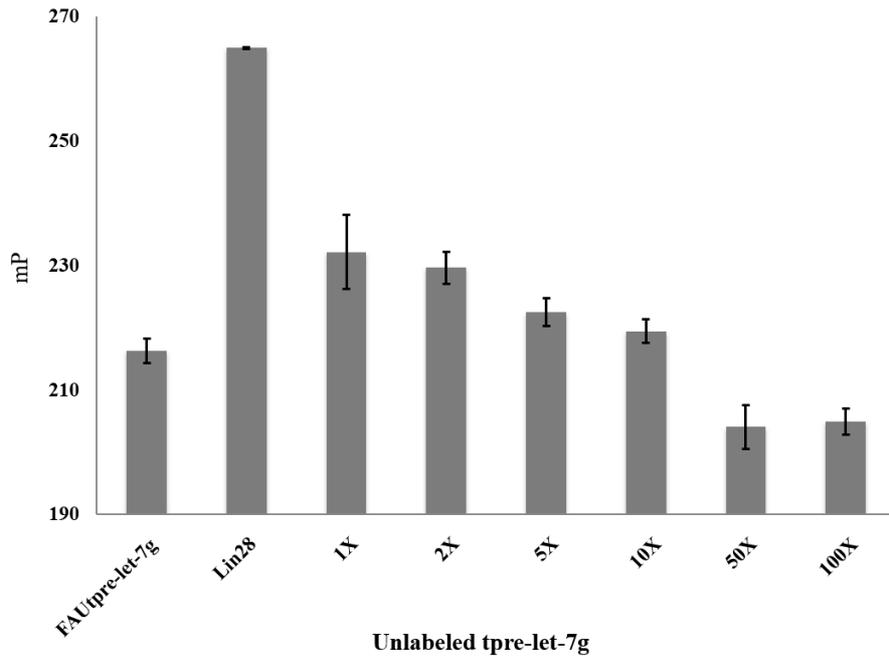
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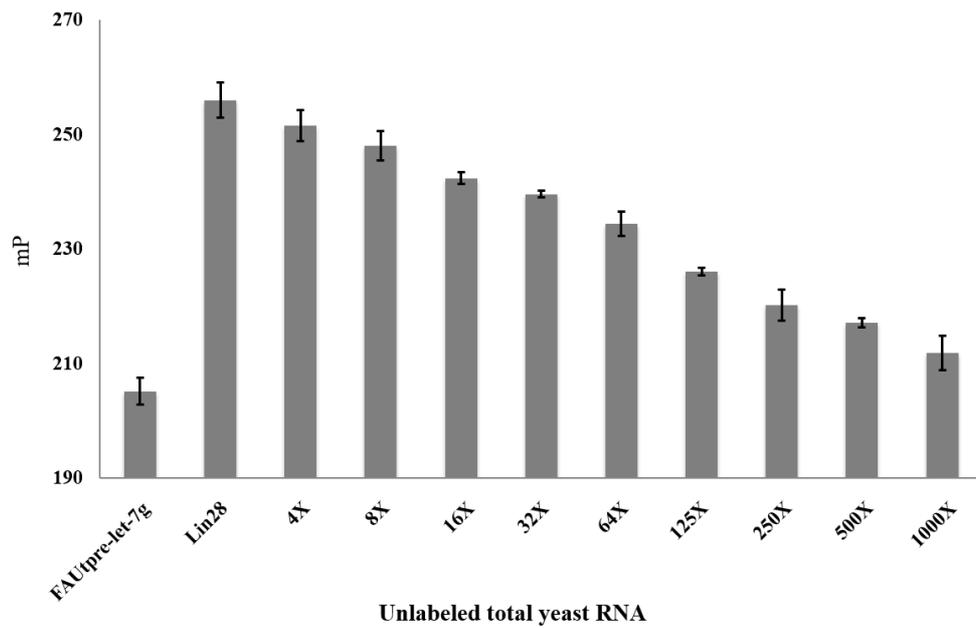


**c.**

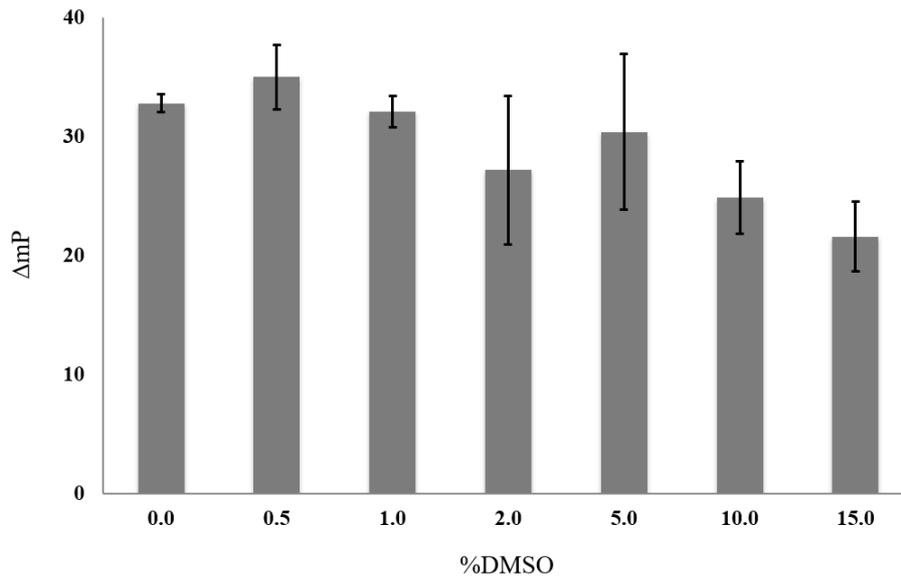


Supplementary Figure 1: Assay development. Fluorescence polarisation of **a.** FAUtpre-let-7g in the presence and absence of GST ( $0.300 \mu\text{M}$ ) and **b.** Fluorescein in the presence and absence of Lin28 ( $0.300 \mu\text{M}$ ). **c.** Intensity of FAUtpre-let-7g in the presence and absence of Lin28 ( $0.300 \mu\text{M}$ ). Data shown was taken at  $t = 40$  min and is representative of measurements taken every 20 min for 6 h. Error bars represent the standard deviation of three independent experiments.

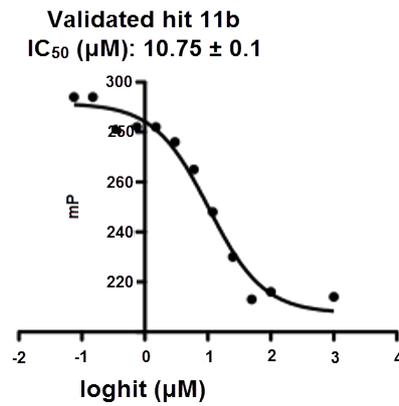
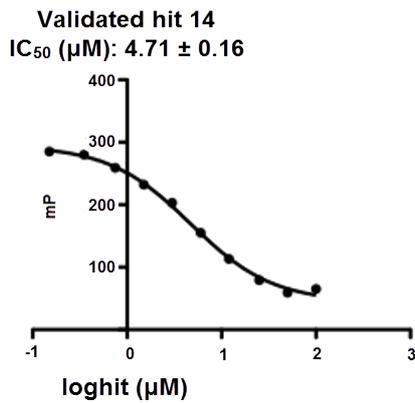
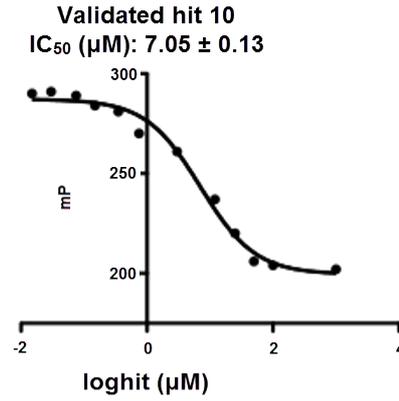
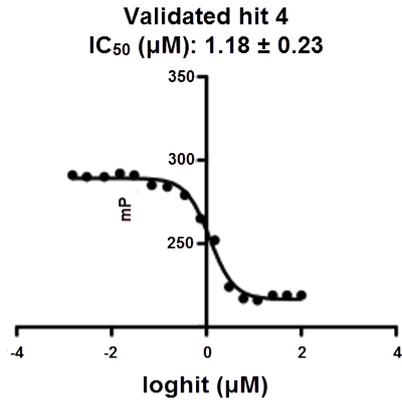




Supplementary Figure 2: Assay development. The effect of unlabeled competitors, tpre-let-7g (specific), C/A tpre-let-7g mutant and total yeast RNA (non-specific), on fluorescence polarisation of FAUtpre-let-7g in the presence of Lin28. Competitor concentrations represented as the amount of competitor relative to FAUtpre-let-7g. Error bars represent the standard deviation of at least two independent experiments.

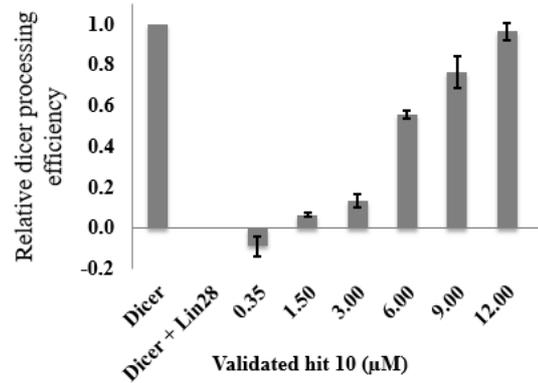
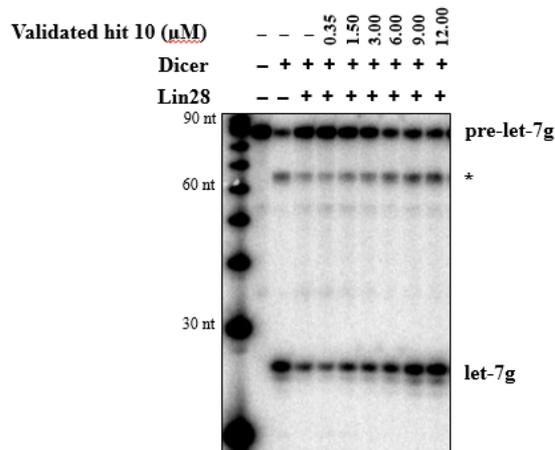


Supplementary Figure 3: Assay development. Effect of DMSO on the change in fluorescence polarisation of FAUpre-let-7g in the presence of Lin28. Error bars represent the standard deviation of three independent experiments.

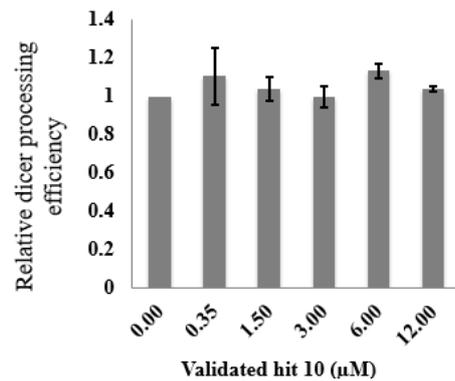
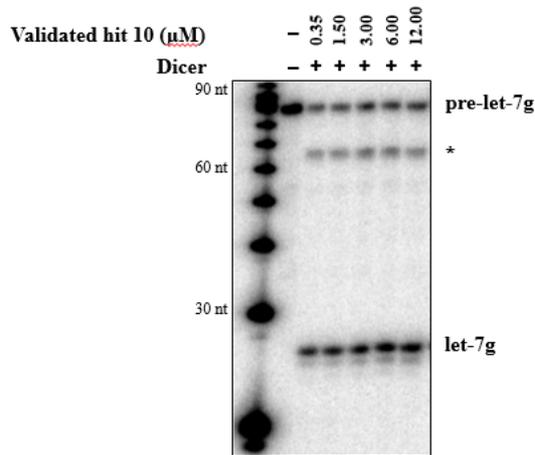


Supplementary Figure 4: Dose response curves for the effect of validated hits on the interaction between FAUp<sub>pre</sub>-let-7g and Lin28 in the fluorescence polarization assay. The polarisation was measured in three independent experiments. A non-linear curve-fitting program (Prism) was used to fit a dose response curve to a plot of polarisation versus the log of the hit concentration ( $\mu M$ ). The  $IC_{50}$  values were calculated.

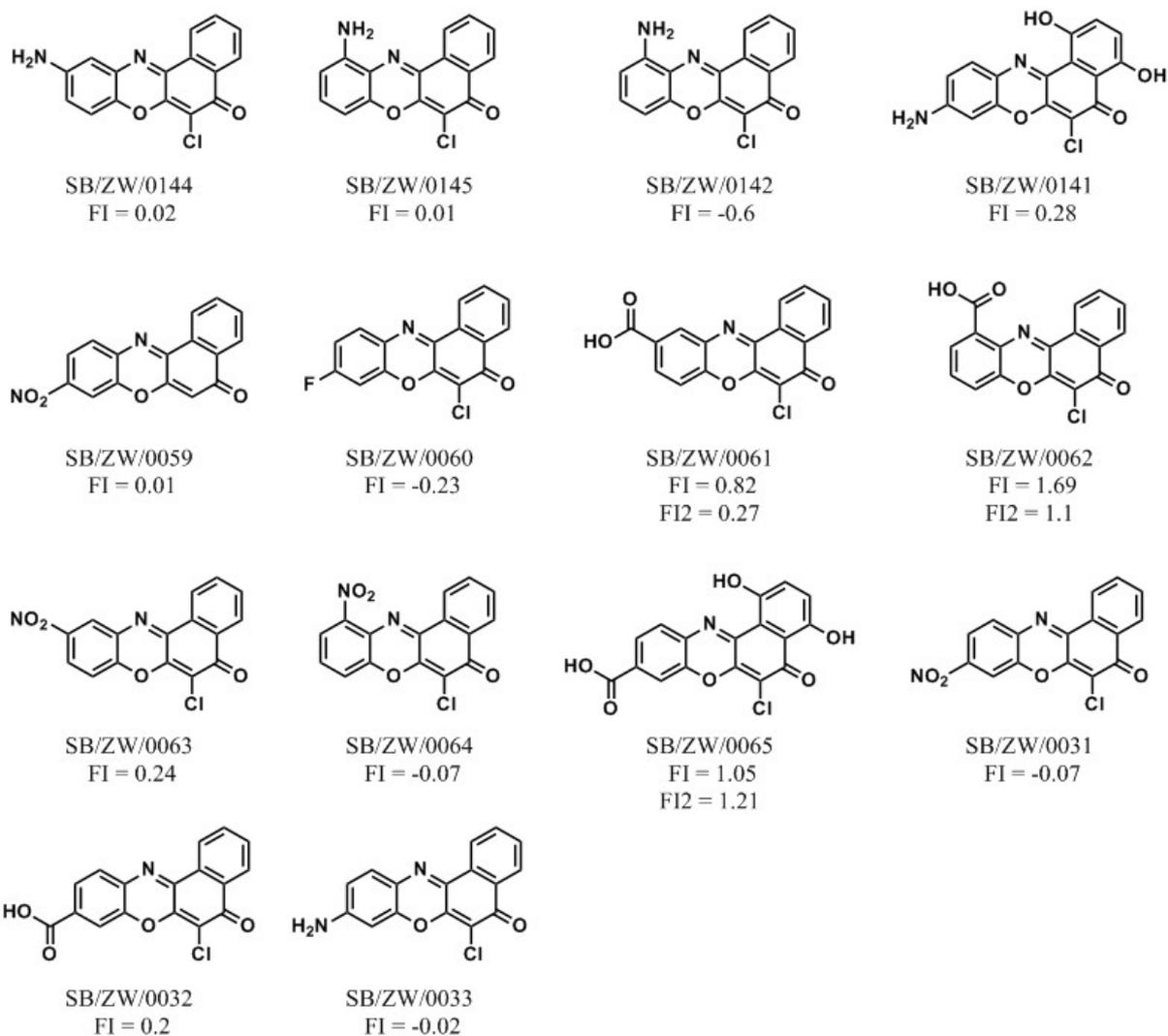
**a.**



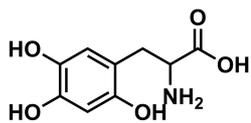
**b.**



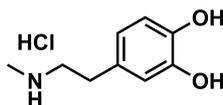
Supplementary Figure 5. Dose dependent effects of 6-hydroxy *DL*-DOPA (validated hit 10) on **a.** Lin28-mediated inhibition of pre-let-7g processing by Dicer and **b.** Dicer processing in the absence of Lin28. The extent of Dicer processing was quantified by calculating the relative Dicer processing efficiency. Results were normalized relative to the Dicer processing efficiency obtained for [ $^{32}\text{P}$ ]-pre-let-7g alone and in the presence of Lin28 (**a.**). \*: initial Dicer cleavage product (single cleavage). Error bars represent the standard deviation of two independent experiments.



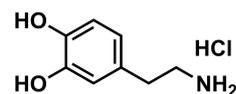
Supplementary Figure 6: Analogues of validated hit 14 (SB/ZW/0065) from the primary FP screen. FI= Fraction inhibition (FP) from primary screen. FI2= Fraction inhibition (FP) from secondary screen.



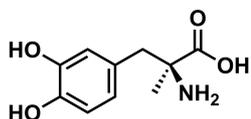
6-Hydroxy-DL-DOPA  
FI = 0.98  
FI2 = 0.99



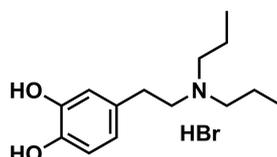
N-Methyldopamine hydrochloride  
FI=-0.06



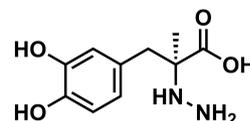
Dopamine hydrochloride  
FI=-0.04



L-alpha-Methyl DOPA  
FI=-0.03



Dipropyldopamine hydrobromide  
FI=-0.07



S-(-)-Carbidopa  
FI= -0.12

Supplementary Figure 7: Analogues of validated hit 10 (6-Hydroxy-DL-DOPA) from the primary FP screen. FI= Fraction inhibition (FP). FI2= Fraction inhibition (FP) from secondary screen.