SUPPLEMENTARY MATERIAL.

SUPPLEMENTARY FIGURE LEGENDS.

SuppFig. 1. Binding of $(CT)_n$ based LNA and PNA ODNs to plasmid. (**A**) Agarose gel, (without EtBr), of 0.023uM plasmid gWiz incubated with 4uM, rhodamine labelled LNA or PNA ODNs. (**B**) as (**A**) with EtBr. (M) 1kb ladder.

SuppFig. 2. Variations in conditions for plasmid: LNA ODN binding. (**A**) Agarose gel, (without EtBr), of 0.023uM plasmid gWiz incubated with 4uM, rhodamine labelled, $(CT)_n$ based LNA ODNs under different conditions at 37^0C. (**B**) as (**A**) with EtBr. (M) 1kb ladder; (1), (5) & (9): at pH5.8; (2), (6) & (10): at pH7.0; (3), (7) & (11): at pH7.0 + 100mM Na Cl; (4), (8) & (12): at pH7.0 + 3M TMAC. (**C**) Agarose gel, (without EtBr), of 0.023uM plasmid gWiz incubated with rhodamine labelled, $(CT)_n$ based LNA ODNs at high (H, 4uM) and low (L, 0.5uM) concentrations at different temperatures. (**B**) as (**A**)) with EtBr. (M) 1kb ladder; (1), (4), (7), (10) & (13): at 4^0C, overnight; (2), (5), (8), (11) & (14): at room temperature for 1hr; (3), (6), (9), (12) & (15): at 37^0C for 1hr.

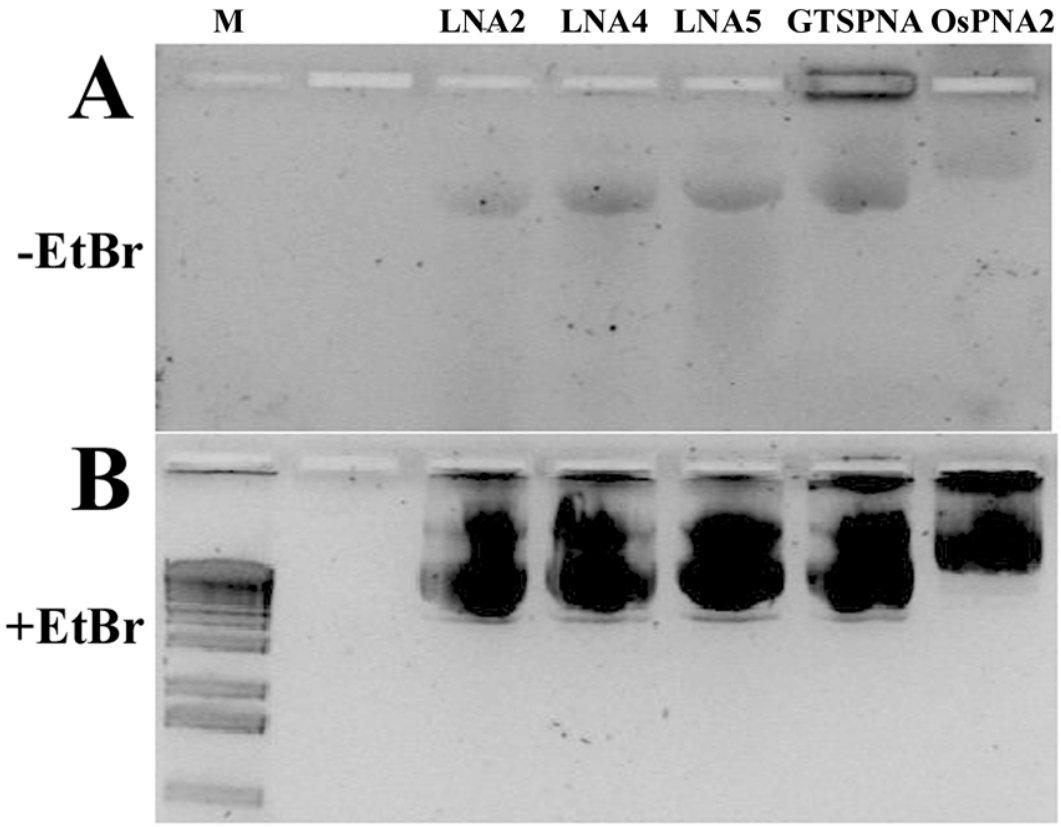
SuppFig. 3. Co-localisation in cells, post-transfection, of LNA ODN bound to plasmid expressing Green Fluorescent Protein, (GFP). Confocal microscopy was performed to demonstrate that when LNA ODNs bind at their cognate binding sites within plasmids, expression of a plasmid-encoded gene is shown. Plasmid pGG2XGFP bound with LNA9R, (Table1), was transfected into CHO cells, after removal of unbound LNA. Cells were analysed for expression of green fluorescent protein, (GFP) and detection of plasmid bound rhodamine-labelled LNA by confocal microscopy, CHO cells expressing GFP derived from transfected pGG2XGFP plasmid that also contained a rhodamine signal from bound LNA9R were readily detectable. This data provides additional validation at the molecular level, to demonstrate that gene expression can occur when LNA ODNs are bound to Gene Grip plasmids. Confocal analysis in CHO cells, 66 hrs post-transfection: (1), pGG2XGFP expressing GFP, FITC channel; (2), DAPI stained nuclei, UV light; (3), LNA9R, TRITC channel; (4), overlay of (1) + (2) + (3).

SuppFig. 4. TNFalpha ELISA data for dose response curve of PTO ODN: CpG1826, compared to GpC1745, and to basal levels of TNFalpha induced by gWiz plasmid transfected into RAW264.7. Plasmid dose was converted into equivalent ODN dose (μM) as described.

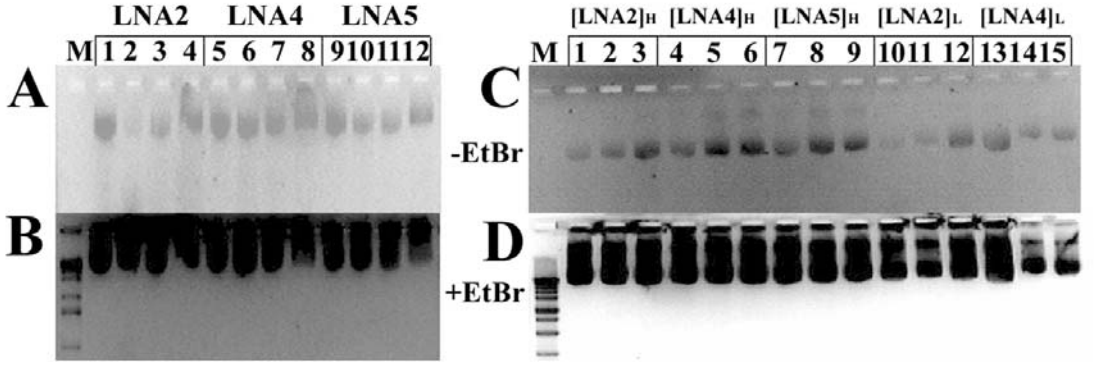
SuppFig. 5. Luciferase activity in RAW264.7 cells transfected with gWiz plasmid ± bound PTOCpG-LNA or PTOGpC-LNA ODNs or plasmid mixed with ODNs. **(A)** Luciferase activity, 24 hrs post-transfection, of cells transfected by FuGENE6, with gWiz ± bound PTOCpG-LNA or PTOGpC-LNA ODNs. Values are a mean of three transfections, with standard errors shown. **(B)** Luciferase activity, 24 hrs post-transfection of cells transfected by FuGENE6, with gWiz, mixed with increasing amounts of PTO ODN CpG1745.

SUPPLEMENTARY FIGURES.

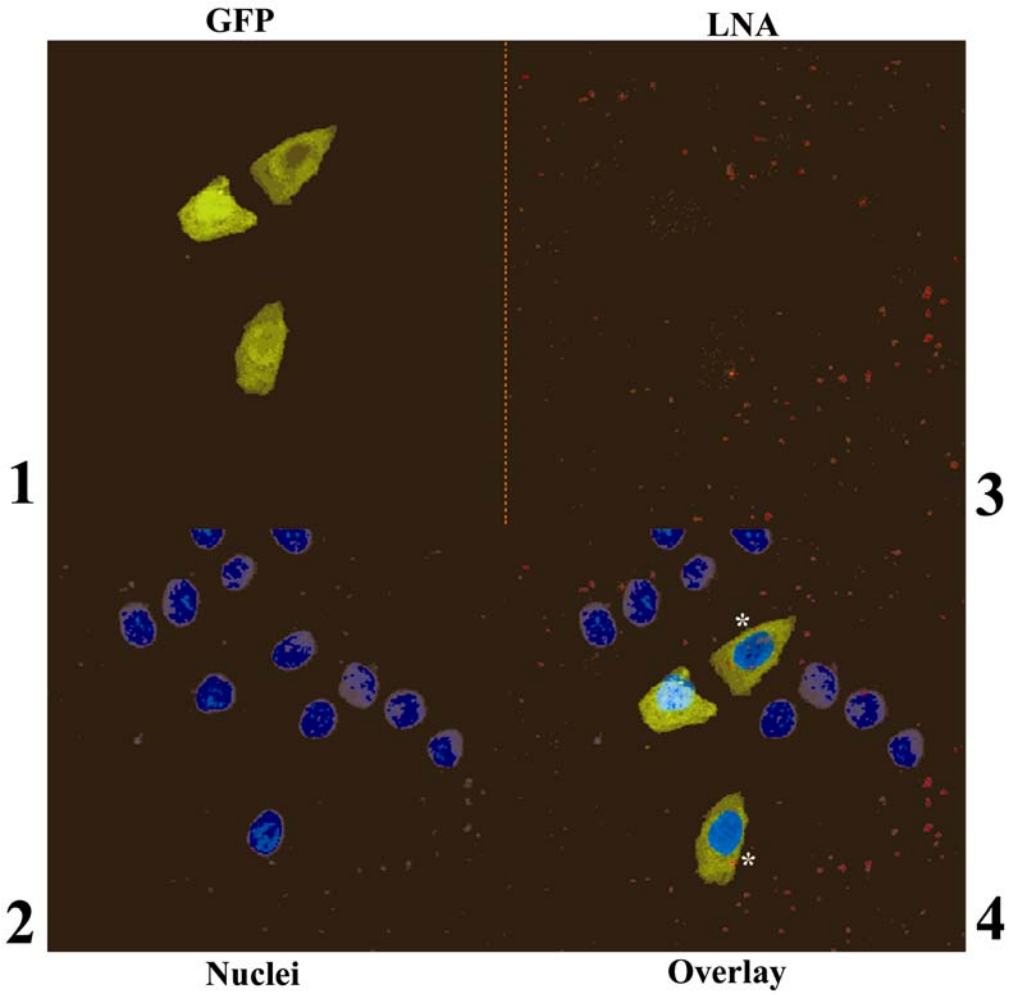
SuppFig. 1



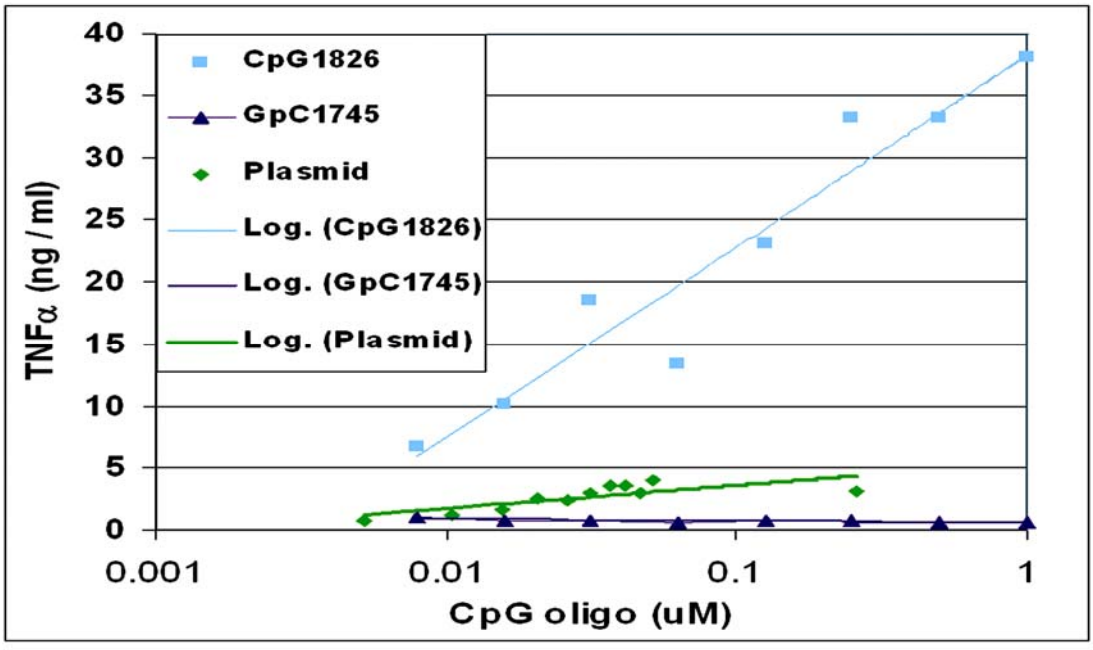
SuppFig. 2



SuppFig. 3

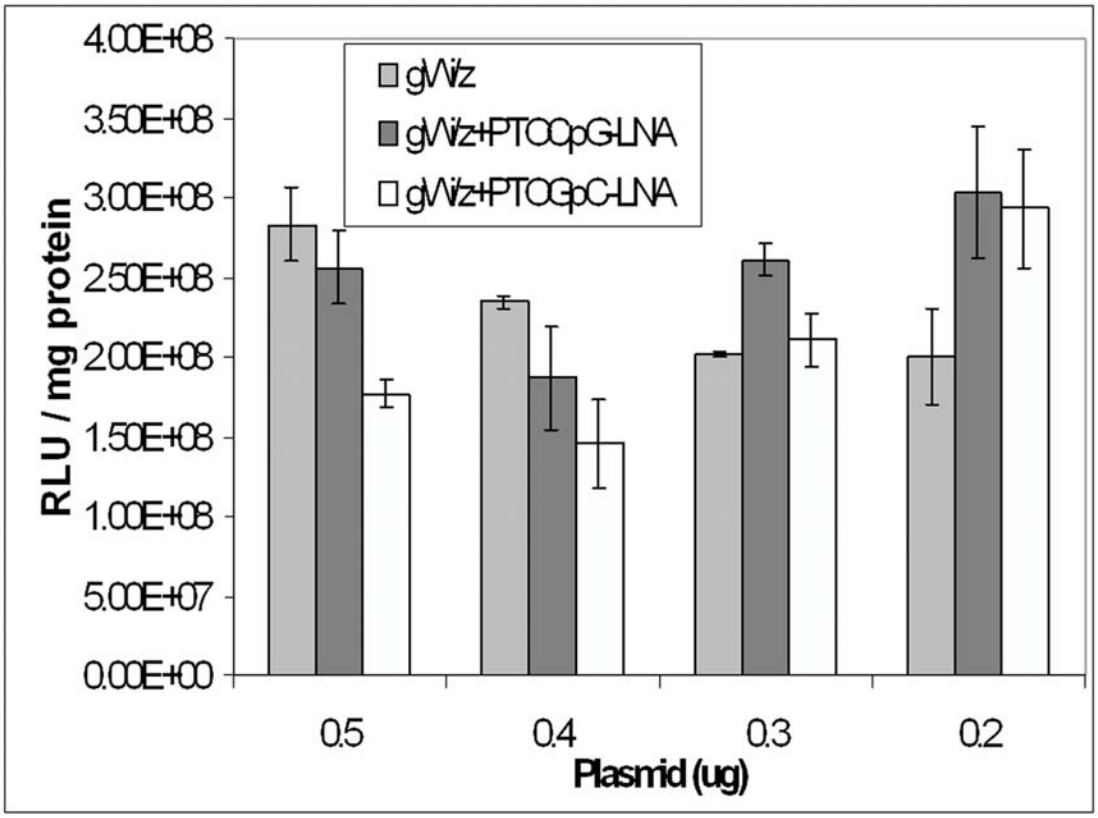


SuppFig. 4



SuppFig. 5

A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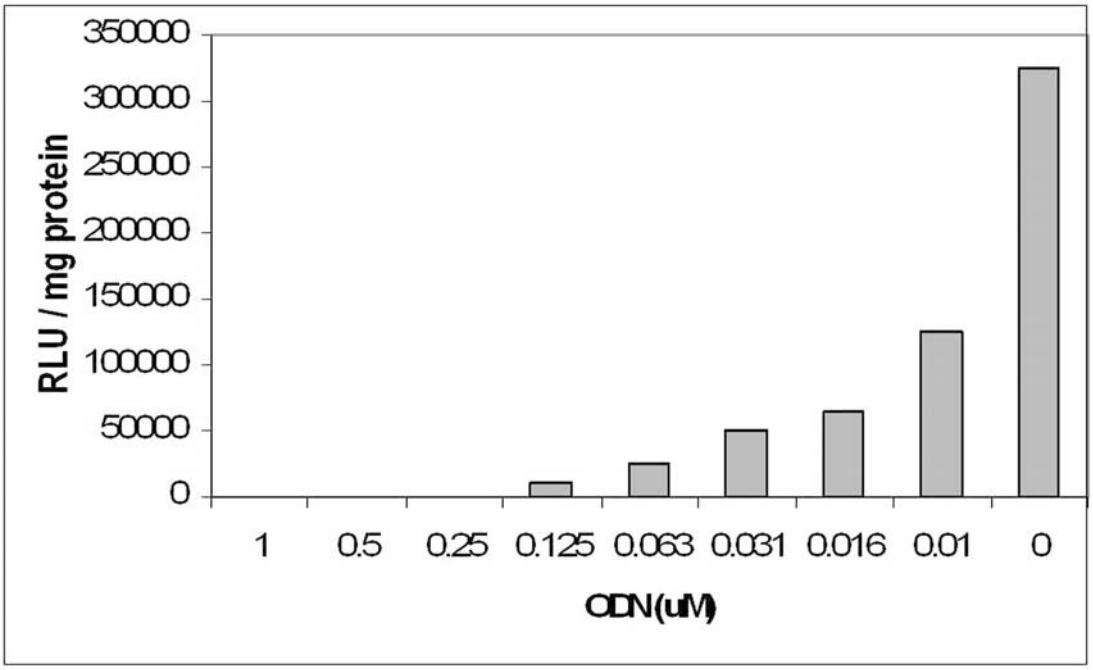
3.00E+08
2.50E+08
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
RLU / mg protein
gWiz
gWiz+PTOGpG-LNA
gWiz+PTOGpC-LNA
0.5
0.4
0.3
0.2
Plasmid (ug)

B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200000
150000
100000
50000
0
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0.125
0.063
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0.016
0.01
0
ODN (uM)