siRNAs targeted to certain polyadenylation signals promote specific, RISC-independent degradation of messenger RNAs

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Supplementary Figures

Figure S1. Activity is independent of all Ago and P-Body proteins.
Ago2$^{-/-}$ MEF cells were treated with ASOs targeting Agos 1, 3, and 4 or the P-body proteins DCP1, DCP2, GW182, and TNRC6B. The following day cells were seeded in 96 well plates and treated with Il4R-α siRNA 383281 at concentration from 300 pM to 300 nM. RNA was purfied 24 hours later and Il4R-α expression analyzed by qRT/PCR. A) Percent control Ago mRNA expression in ASO treated Ago2$^{-/-}$ cells at 24 hours post transfection. B) Concentration response curves for treatment with Il4R-α siRNA 383281 in control cells (solid line), Ago1-reduced cells (dashed line), Ago3-reduced cells (dotted line), or Ago4-reduced cells (dash-dot). C) Concentration response curves for treatment with siRNA 383281 in control wild-type MEF cells (solid line), DCP1:2-reduced cells (dashed line), GW182-reduced cells (dotted line), or TNRC6B-reduced cells (dash-dot). D) Concentration response curves for treatment with Il4R-α siRNA 383281 in control Ago2$^{-/-}$ MEF cells (solid line), Ago1-reduced cells (dashed line), DCP1:2-reduced cells (dotted line), or TNRC6B-reduced cells (dash-dot).

Figure S2. Il4R-α (383281 site) siRNA does not compete for Eg5 or PTEN siRNA activity in WT or Ago2–/– knockout cells.
A) WT MEF cells were transfected 10 nM Eg5 siRNA alone or in the presence of 30 or 100 nM Il4R-α siRNA for 4 hours. Reduction of Eg5 mRNA was assessed the following by qRT/PCR and plotted as percent control in untreated cells. B) WT MEF cells were transfected 10 nM PTEN siRNA 29592 alone or in the presence of 30 or 100 nM Il4R-α 383281 siRNA for 4 hours. Reduction of PTEN mRNA was assessed the following by qRT/PCR and plotted as percent control in untreated. C) Ago2$^{-/-}$ MEF cells transfected with 10 nM Eg5 siRNA alone or in the presence of 30, or 100 nM Il4R-α siRNA 383281 for 4 hours. Reduction of Eg5 mRNA was assessed the following by qRT/PCR. D) C) Ago2$^{-/-}$ MEF cells transfected with 10 nM PTEN siRNA alone or in the presence of 30, or 100 nM Il4R-α siRNA 383281 for 4 hours. Reduction of PTEN mRNA was assessed the following by qRT/PCR.

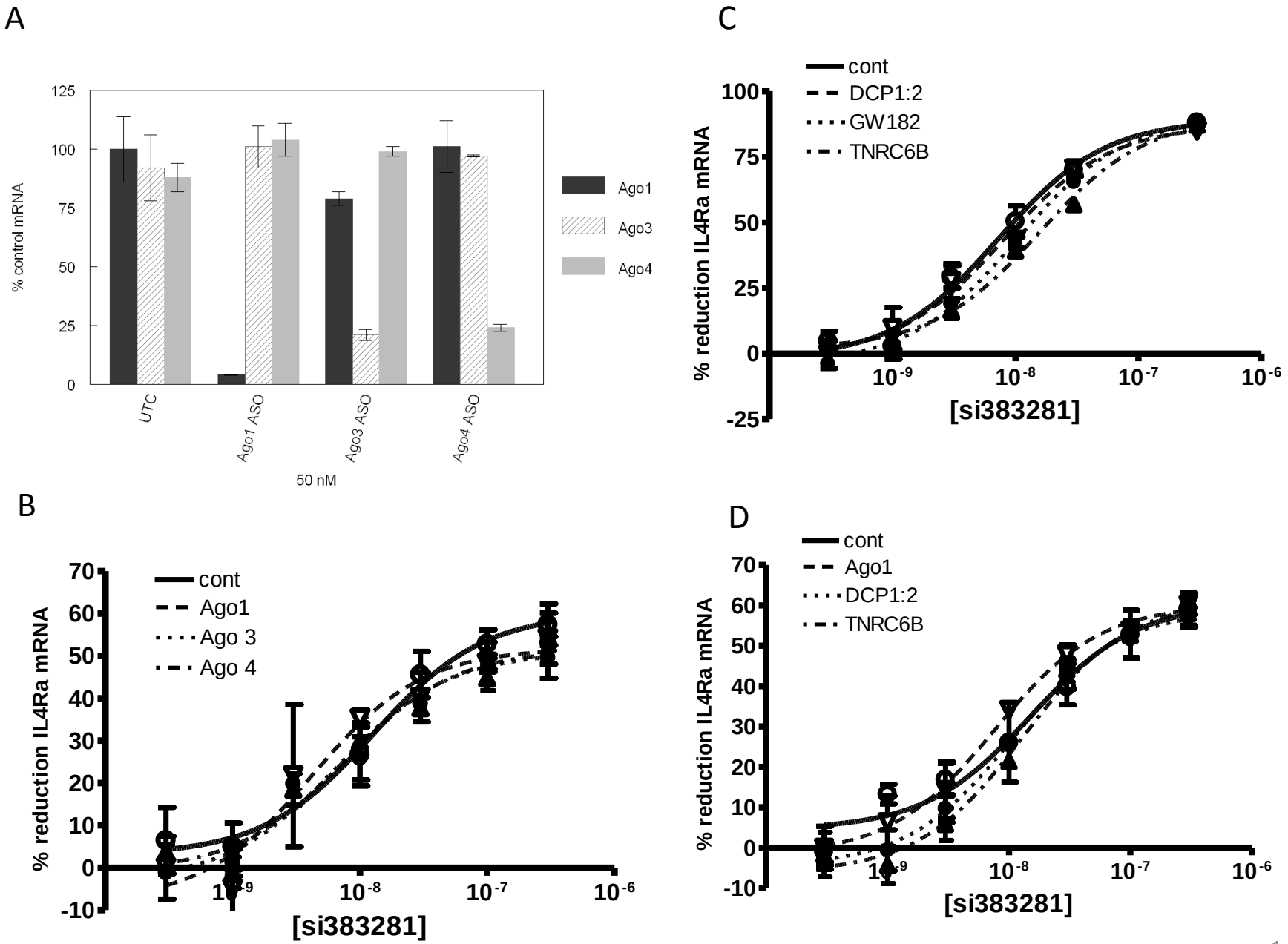
Figure S3. Messenger RNA half-life for alternatively polyadenylated Il4R-α.
Ago2$^{-/-}$ MEF cells in 12-well plates were treated for 18 hours with 100 nM si383281. Cells were harvested from 0-8 hours following the addition of Actinomycin D at 5 ug/ml. Total RNA was isolated and individual mRNA levels quantitated by qRT/PCR. mRNA levels are shown relative to T=0 for each mRNA. Il4R-α primer/probes are as described in Figure 10. Il4R-α polyA #1 (short), solid line; Il4R-α polyA #3 (long), dashed line; PIK3CB, dotted line.

Figure S4. Expression of several genes is increased following reduction of exosomal proteins.
Ago2$^{-/-}$ MEF cells were plated in 10 cm dishes at 50% confluence. The following day cells were transfected with 75 nM each of ISIS 308299 (Exosc9) and ISIS 454373 (SKIV2l) ASOs using Lipofectamine 2000 reagent. Cells were incubated overnight, then RNA purified using RNeasy mini columns (Qiagen). An on-column DNAseI digestion step was included according to the manufacturer's protocol. For the Mouse JAK/STAT PCR Array (PAHS-012), cDNA was generated in 20 ul reactions from 500 ng of total RNA using an RT^2 First Strand kit following the manufacturer's protocol (SABiosciences). The finished first strand cDNA was diluted with 91 ul H_2O, then amplified using RT^2 qPCR Master Mix following the manufacturer's protocol (SABiosciences). Two PCR arrays were run for

each treatment group. Data was analyzed using the $\Delta\Delta C_t$ method comparing ASO/U1 adaptor treated to mock treated control. (A) In exosome reduced cells 7/84 genes, including Il4R-α , were found to be increased by more than 2 fold (panel 1). Actual fold changes are shown below the graph. (B) Confirmation of PCR array results by qRT/PCR. C) Activity of siRNAs targeted to the polyA sites of exosome regulated transcripts in wild-type (dashed lines) or $Ago2^{-/-}$ (solid lines) cells.

Figure S5. Activity of polyA directed siRNAs in wild-type and $Ago2^{-/-}$ MEF cells.
Wild-type or $Ago2^{-/-}$ MEF cells were seeded in 96 well plates and treated with WAF-1 siRNA 549896 at concentrations from 300 pM to 300 nM. RNA was purfied 24 hours later and target RNA expression analyzed by qRT/PCR. Percent control WAF-1 mRNA expression is shown for siRNA treated wild-type (dashed line) or $Ago2^{-/-}$ (solid line) cells.

Figure S6. Immunofluorescent imaging of Cy3/Cy5 labeled siRNA 383281.
A). For live cell imaging $Ago2^{-/-}$ MEF cells grown in glass-bottom dishes were transfected with 3'-Cy5 antisense/5'-Cy3 sense siRN383281 using 6 ug/ml RNAiMAX in OptiMEM media. Cells were imaged 4 hours post-transfection with a confocal microscope (Olympus, Fluoview 1000) and images were processed using software FV10-ASW 2.1. Both Cy3 linked antisense and Cy5 linked sense strand of siRNA 383281 were clearly distributed in the nucleus as well as the cytoplasm B) Cy3/Cy5 linked siRNA retains activity. $Ago2^{-/-}$ MEF cells in 96 well plates were treated with the siRNAs at 300 pM to 300 nM using RNAiMax as described in Materials and Methods. The following day total RNA was purified and Il4R-α expression analyzed by qRT/PCR No difference in potency was observed as compared to the unmodified siRNA. C) To rule out that the observed localization was the result of the dye used, cells were also treated with Cy5 or Cy3 linked antisense strand annealed to unmodified sense strand. Live cell imaging of $Ago2^{-/-}$ MEF cells treated for 5 hours Cy3 linked antisense strand annealed to unmodified sense strand of siRNA 383281. D) Live cell imaging of $Ago2^{-/-}$ MEF cells treated for 5 hours Cy5 linked antisense strand annealed to unmodified sense strand of siRNA 383281.


A
125
100
75
50
25
0
% control mRNA
Ago1
Ago3
Ago4
UTC
Ago1 ASO
Ago3 ASO
Ago4 ASO
50 nM
C
cont
DCP1:2
GW182
TNRC6B
100
75
50
25
0
-25
% reduction IL4Ra mRNA
10-9
10-8
10-7
10-6
[si383281]
B
70
60
50
40
30
20
10
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-10
cont
Ago1
Ago 3
Ago 4
% reduction IL4Ra mRNA
10-9
10-8
10-7
10-6
[si383281]
D
70
60
50
40
30
20
10
0
-10
cont
Ago1
DCP1:2
TNRC6B
% reduction IL4Ra mRNA
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10-6
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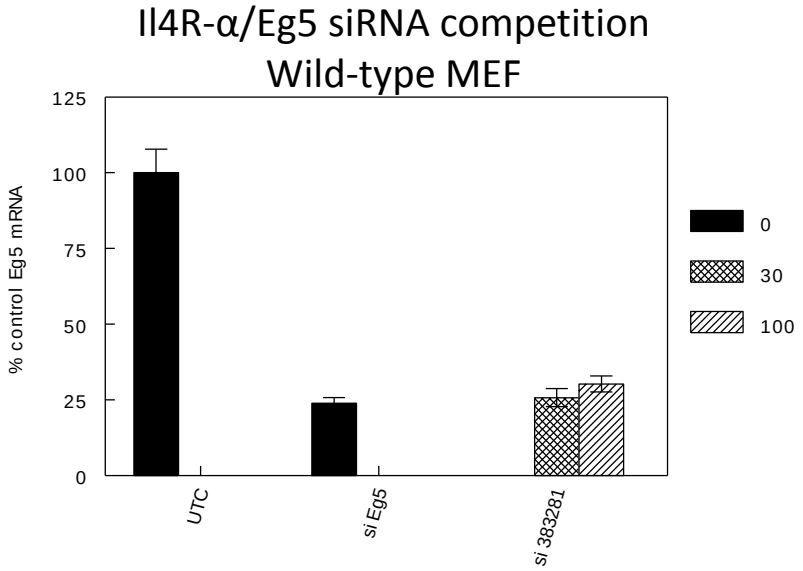
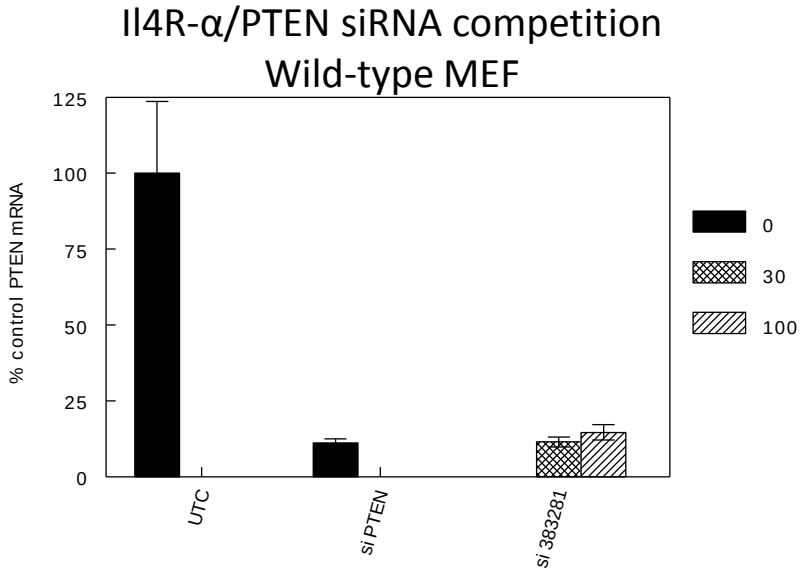
Figure S1

A



B



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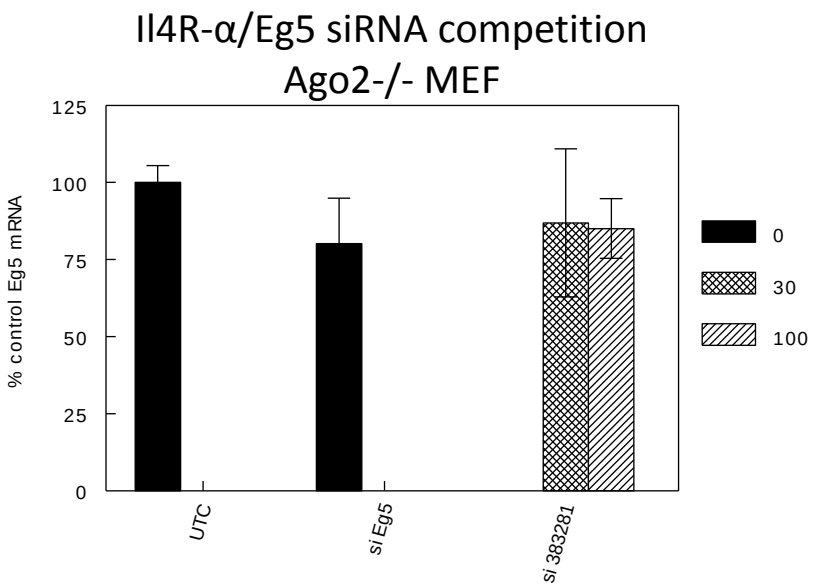

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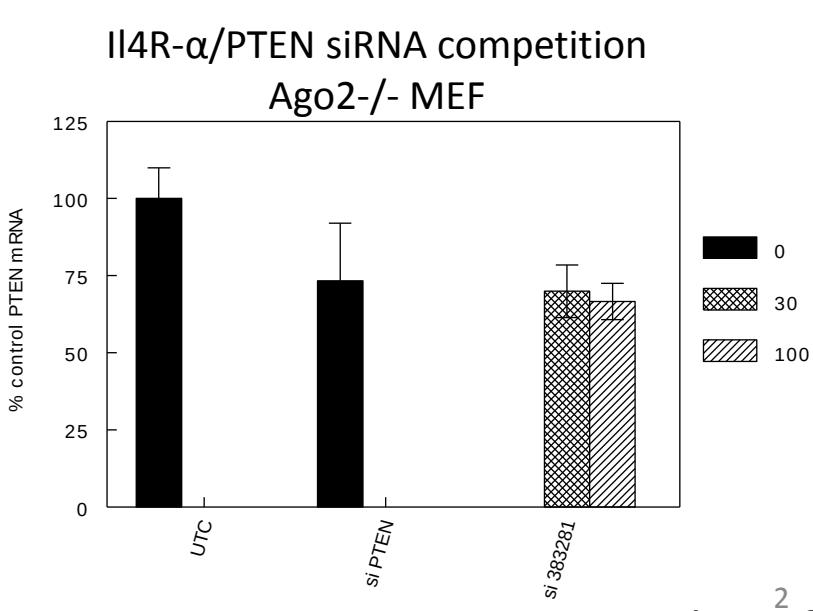


Figure S2

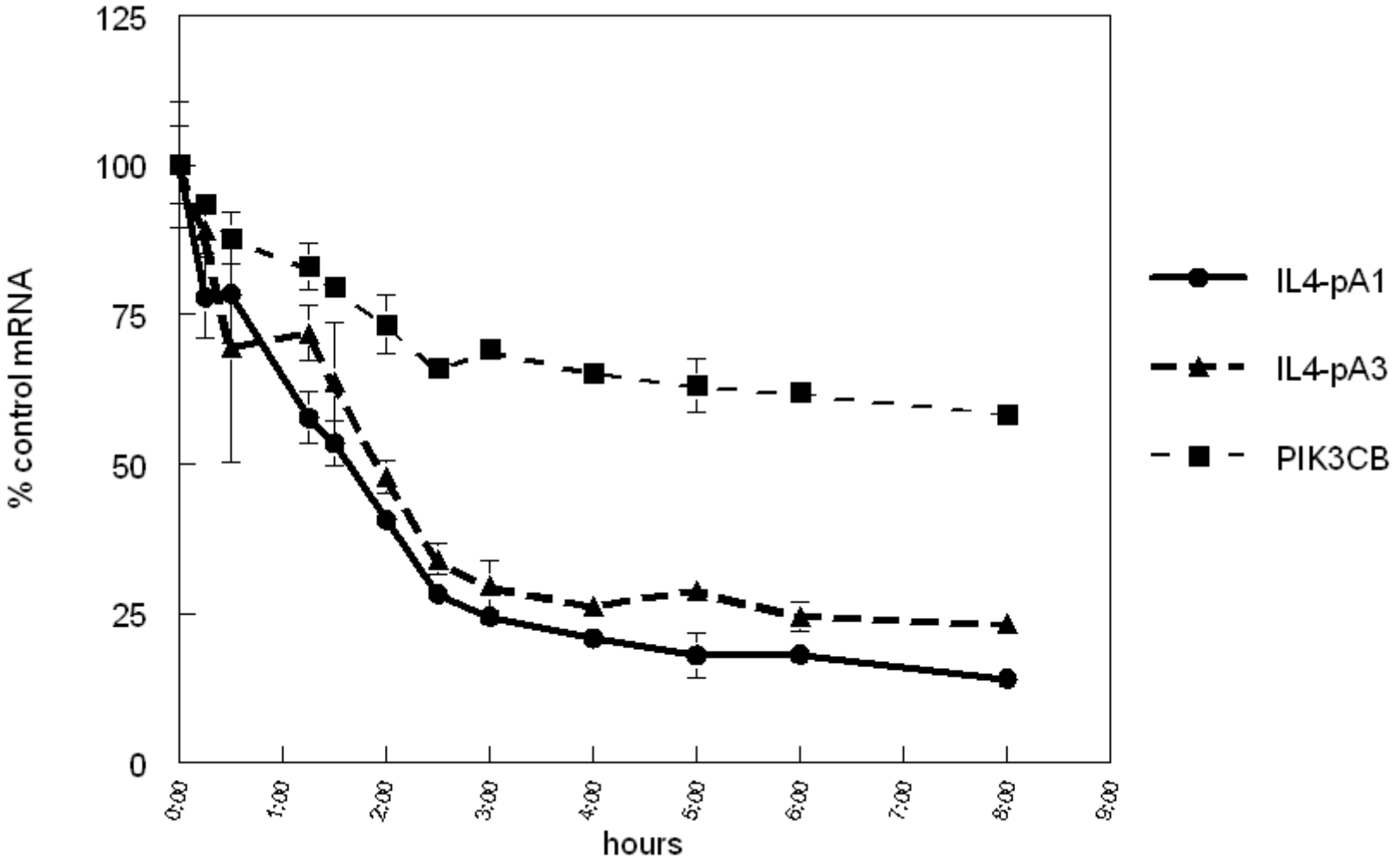
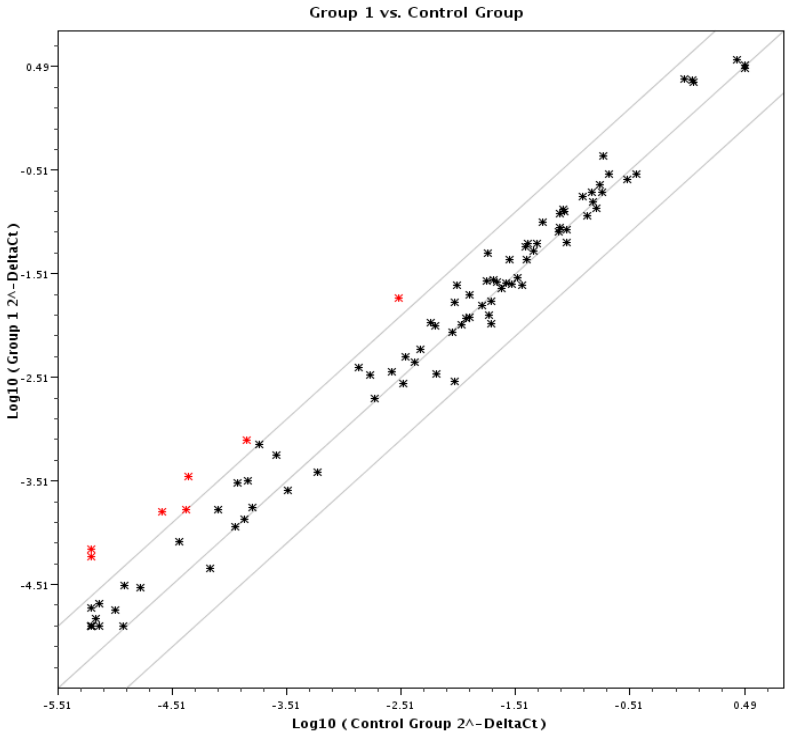

125
100
75
50
25
0
% control mRNA
0:00
1:00
2:00
3:00
4:00
5:00
6:00
7:00
8:00
9:00
hours
IL4-pA1
IL4-pA3
PIK3CB

Figure S3

A



Symbol	Fold Change
Isg15	6.1
Gata3	3.0
Irf9	2.8
Stat2	2.5
Cdkn1a	2.4
Csf1r	2.2
Il4ra	1.9

B

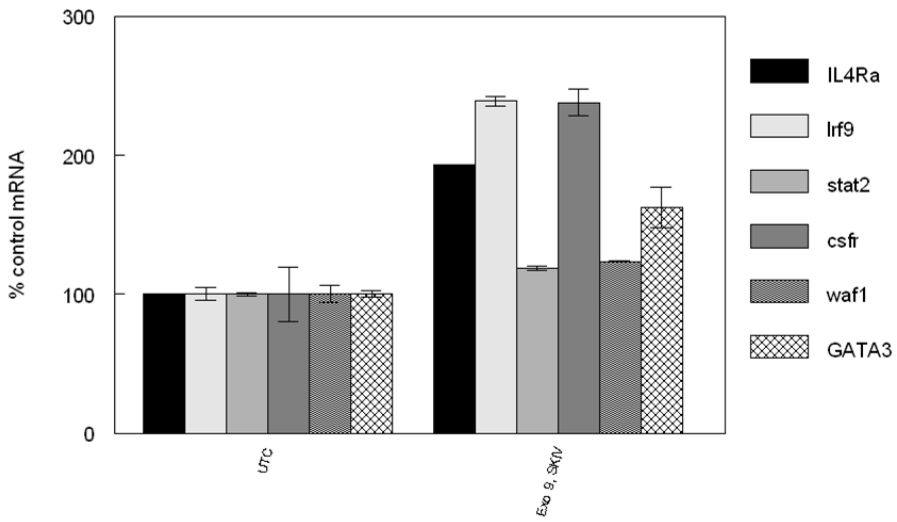
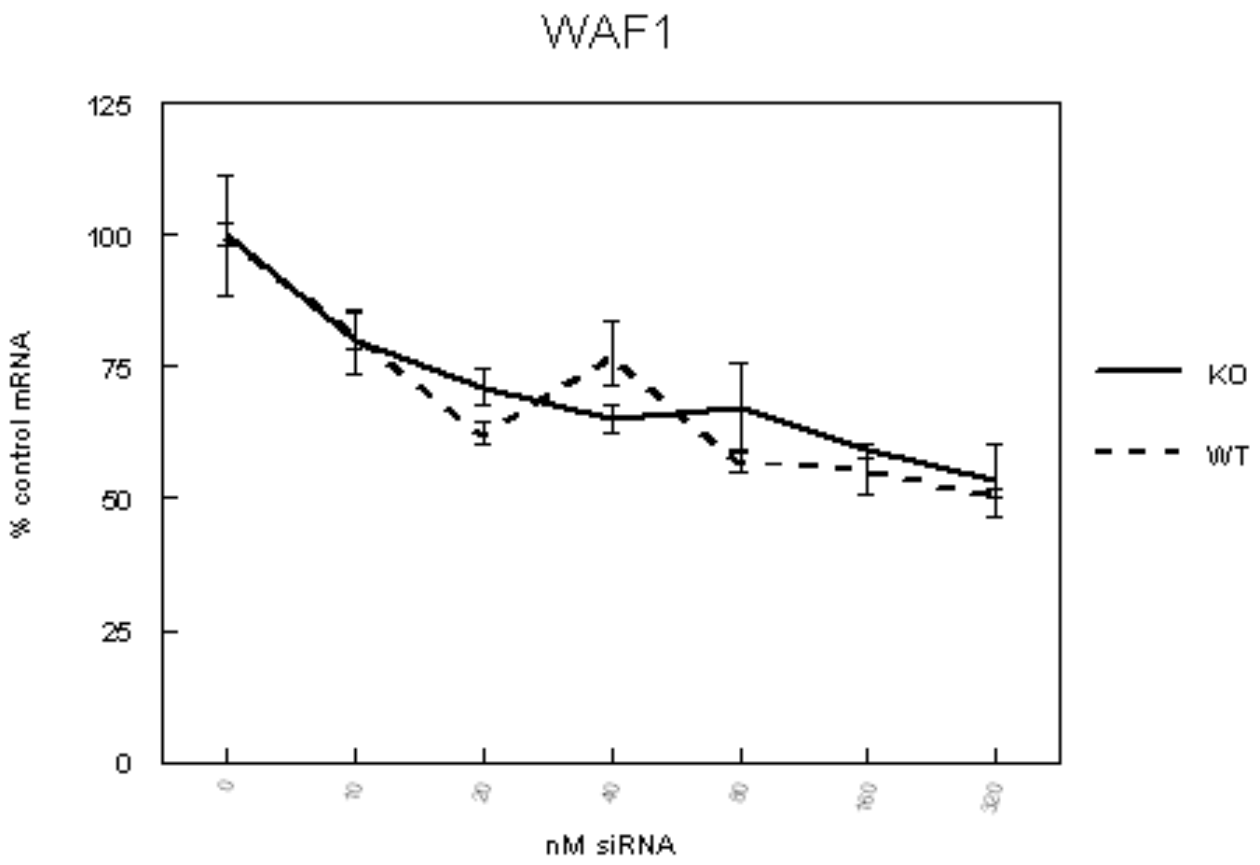


Figure S4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% control mRNA
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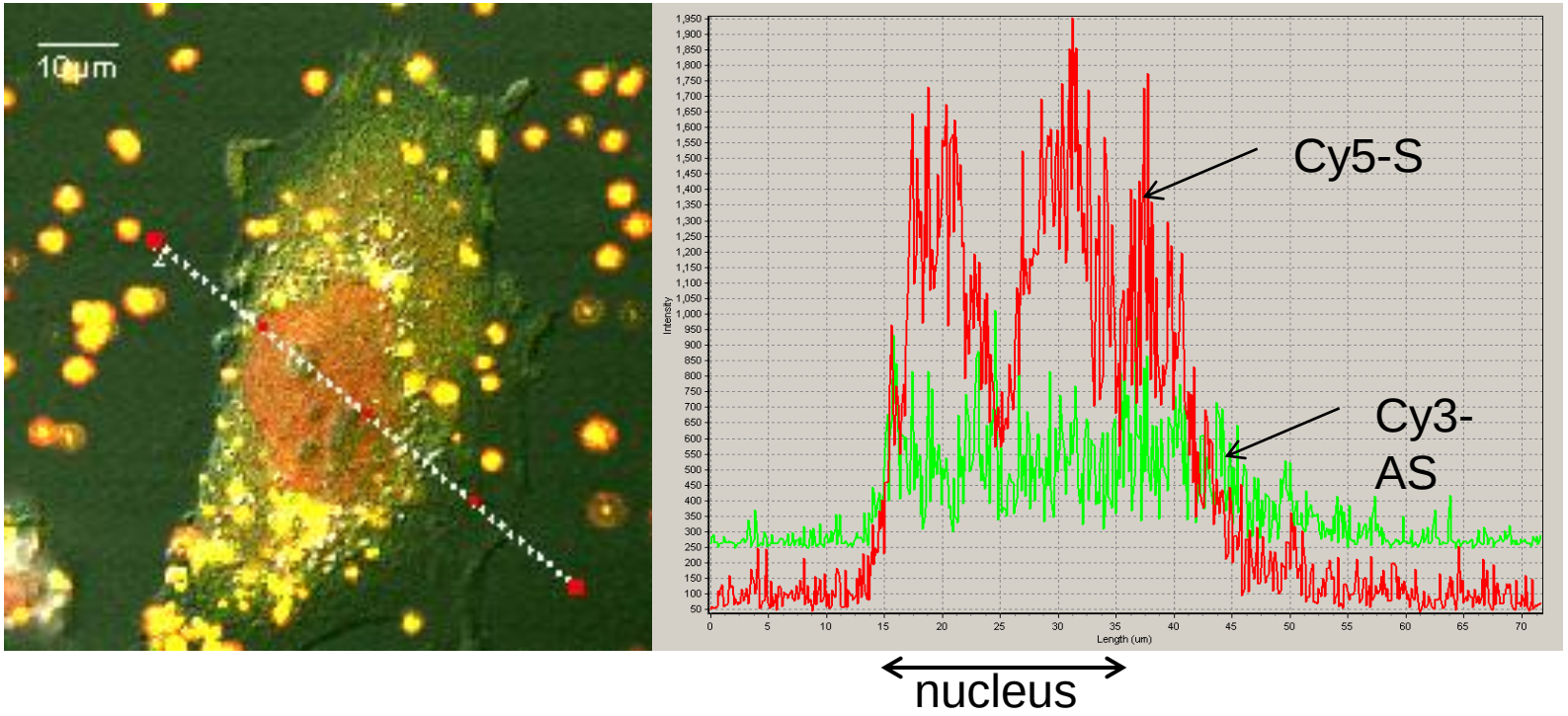
10
20
40
80
160
320
nM siRNA
KO
WT

Figure S5

A


10μm
Cy5-S
Cy3-
AS
Intensity
Length (um)
nucleus

B

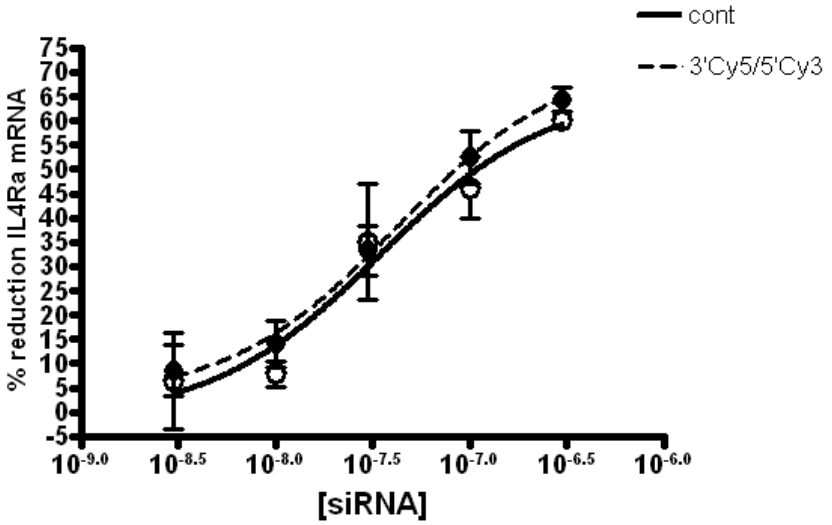
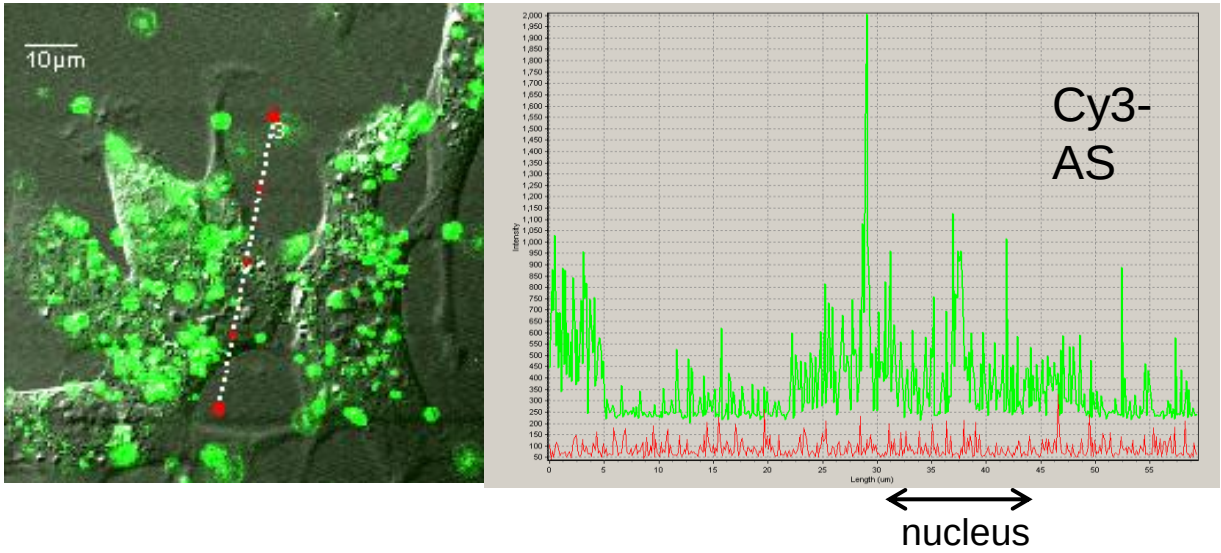

cont
3'Cy5/5'Cy3
% reduction IL4Ra mRNA
[siRNA]

Figure S6

C



D

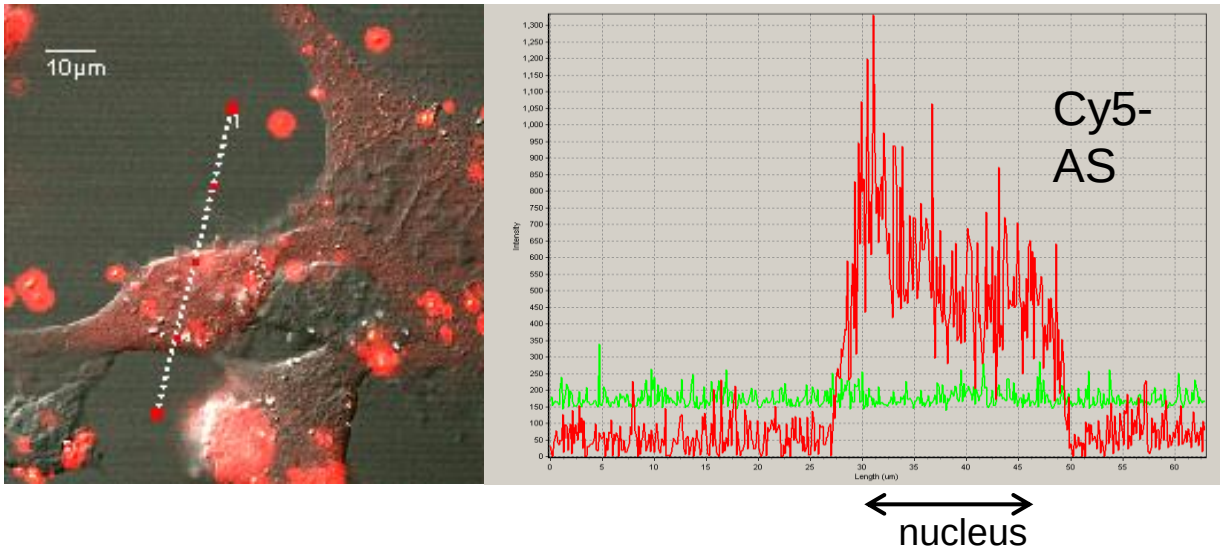


Figure S6