Supplementary Figure 1: MCF10A cells are visualized by immunofluorescence of β-tubulin (green) and phalloidin staining of β-actin (red) 72 hours after transfection with miR-222, miR-222CUCU or the 21 and 25nt negative controls (nc21 and nc25). DAPI (blue) was used to stain nuclei.

Supplementary Figure 2: MCF10A cells are shown 72hr after the transfection of the 21 or 25nt controls (nc21 and nc25) or miR-222 isomiRs as indicated.

Supplementary Figure 3: Relative luciferase activity was measured for miR-222 target gene 3'UTR reporters as Figure 3f. miR-222 isomiRs or negative controls were transfected as indicated.

Supplementary Figure 4: MCF10A cells were transfected by single-stranded miRNA mimics of miR-222, miR-222CUCU or the 21nt and 25nt negative controls and visualised 72 hours post-transfection.

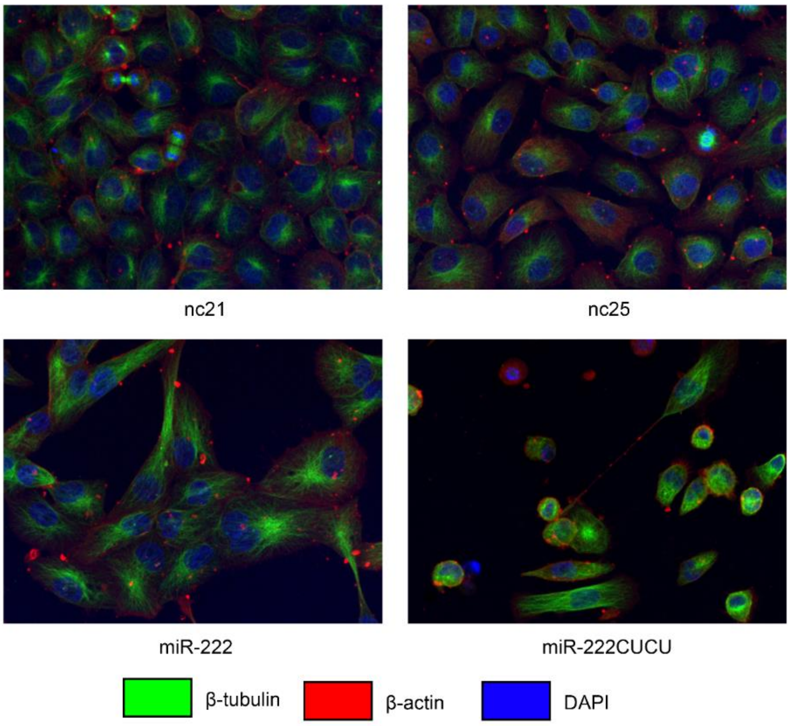
Supplementary Figure 5: The most significantly enriched ontologies of genes that were 2-fold up- and down-regulated 48hr after the transfection of control or miR-222 mimics into MCF10A cells.

Supplementary Figure 6: Differential expression ratios (top) and EISA analysis (bottom) of PIK3/AKT pathway components. MCF10A cells were transfected with miR-222 or control isomiRs for 48hr (as in Supp.Fig 4) at which time poly(A+) RNA was harvested and subjected to RNA sequencing. Relative expression of total (upper) and EISA-analysed (lower) PI3K/AKT pathway members are shown. Due to insufficient intron-mapping depth, determination of the level of AKT2 inhibition by EISA was not possible.

Supplementary Figure 7: Successful fractionation of MCF10A cells as indicated by the exclusive cytoplasmic and nuclear presence of 7SL and 45s rRNA respectively.

Supplementary Table 1: The 15 most significantly altered pathways are listed according to the IMPaLA analysis of deep-sequencing results of cells treated by miR-222CUCU in comparison to miR-222.

Supplementary Figure 1



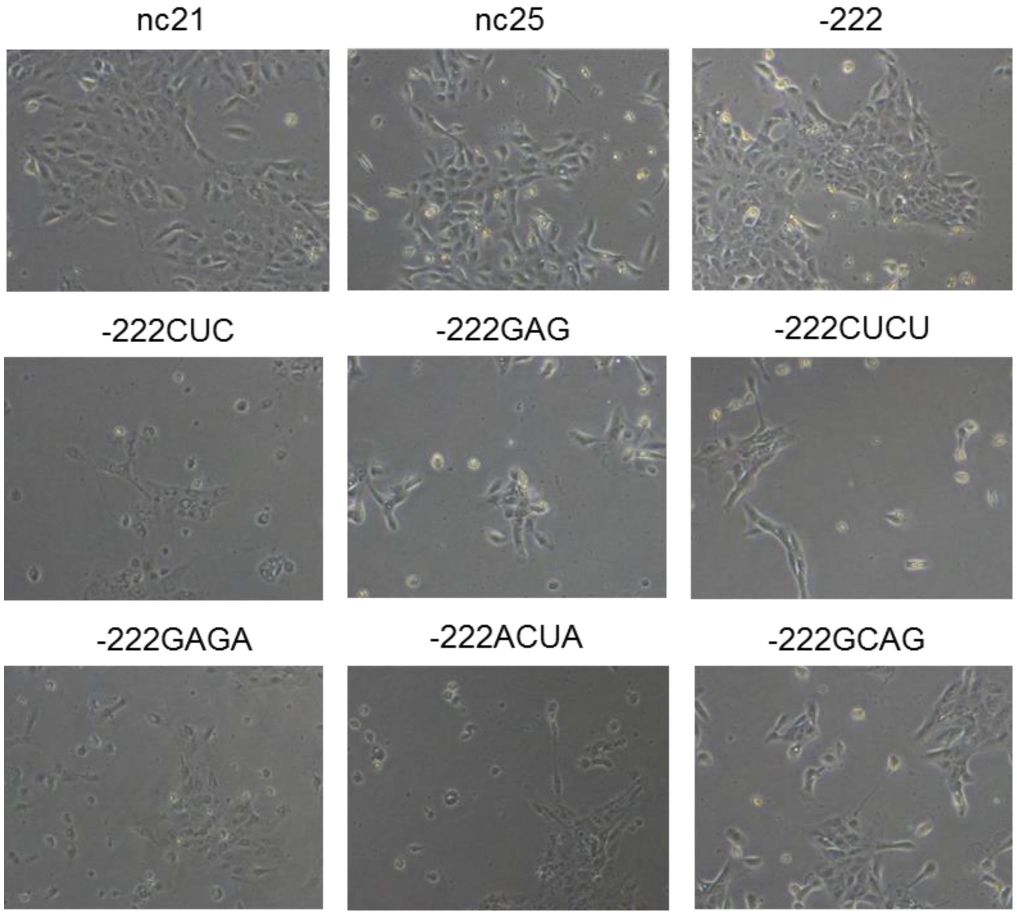
nc21

nc25

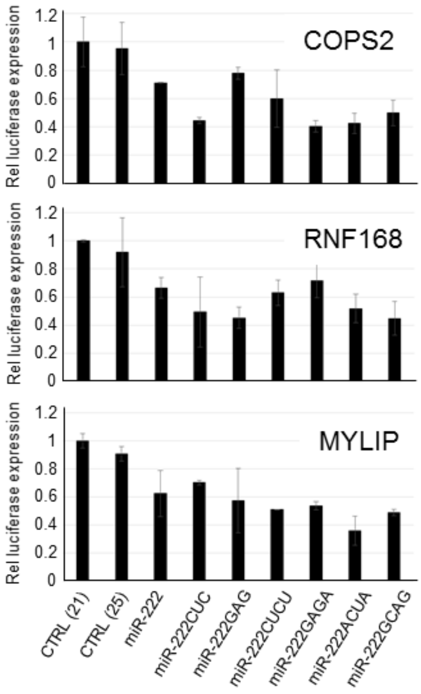
miR-222

miR-222CUCU

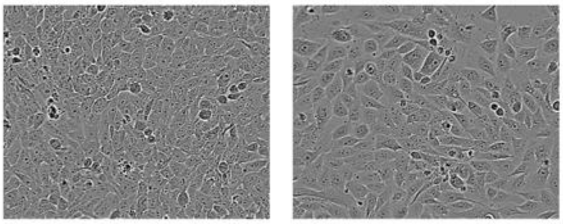
Supplementary Figure 2



Supplementary Figure 3

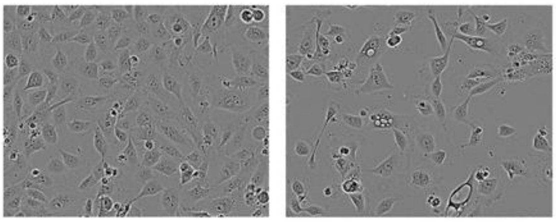


Supplementary Figure 4



nc21, single stranded

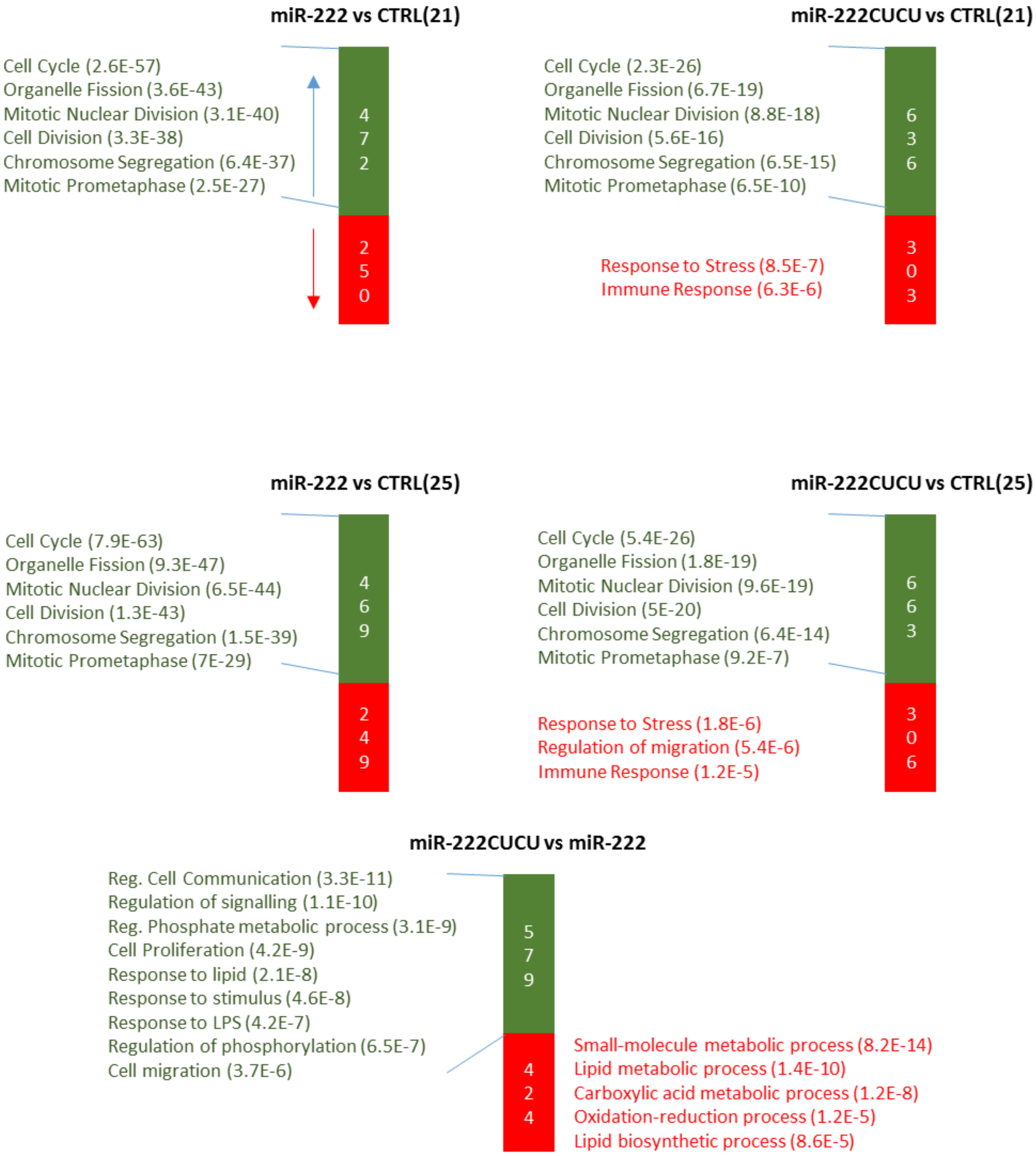
nc25, single stranded



miR-222, single stranded

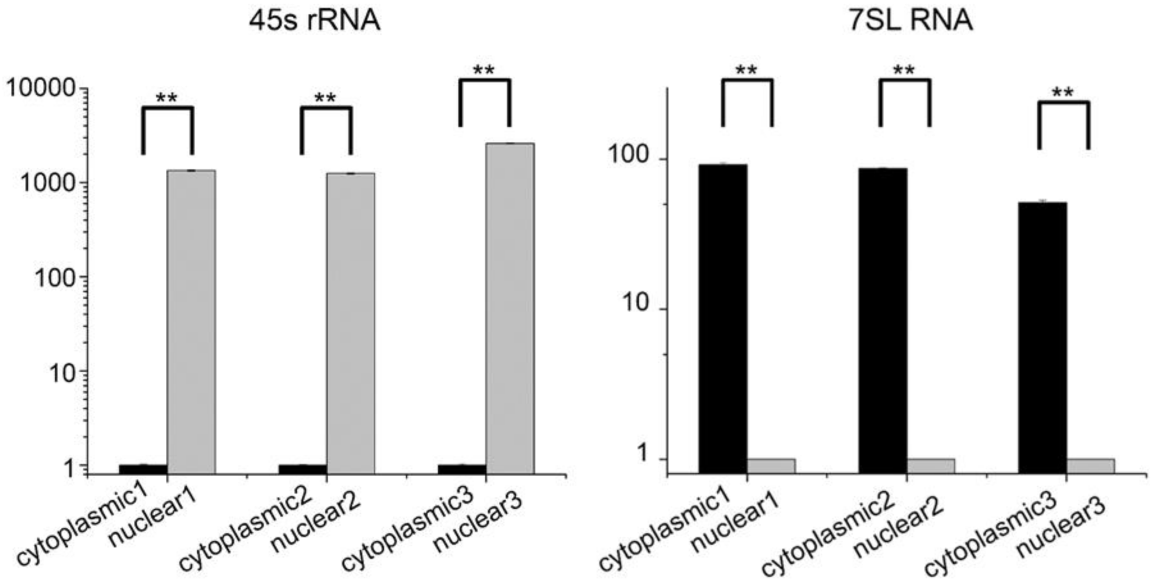
miR-222CUCU, single stranded

Supplementary Figure 5



Supplementary Figure 6

Supplementary Figure 7



Supplementary Table 1

Pathway name	Overlapping genes	All genes	p value	q value
Metabolism	383	1427	1.32E-12	5.15E-09
Nuclear Receptors Meta-Pathway	99	307	9.37E-08	0.00018
Non-integrin membrane-ECM interactions	22	42	2.3E-06	0.00304
EGFR1	126	447	6.7E-06	0.0065
Metabolism of lipids and lipoproteins	136	497	1.5E-05	0.0118
Peroxisome - Homo sapiens	32	81	3E-05	0.0144
Diseases of glycosylation	70	226	3.2E-05	0.0144
Metabolism of carbohydrates	77	256	4.1E-05	0.0144
TGF Beta Signaling Pathway	24	55	4.43E-05	0.0144
Laminin interactions	13	22	5.42E-05	0.0152
Pre-NOTCH Processing in Golgi	9	12	5.46E-05	0.0152
Glucocorticoid Receptor Pathway	27	67	8.24E-05	0.0215