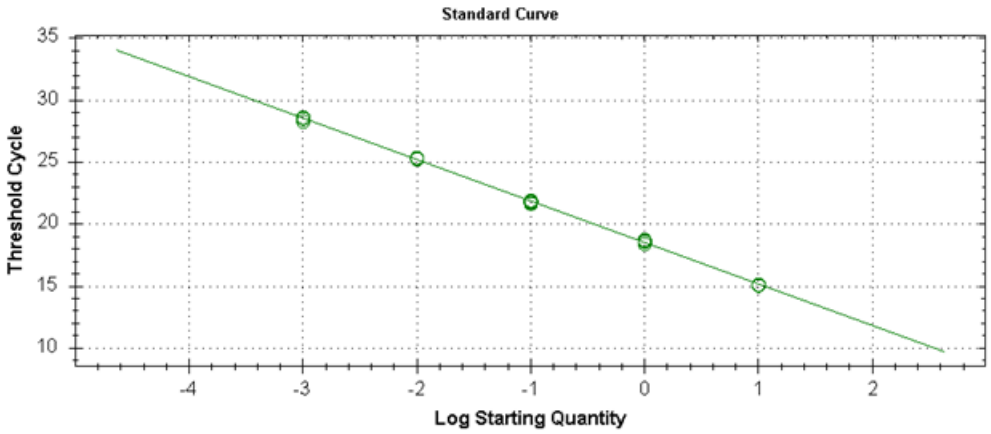
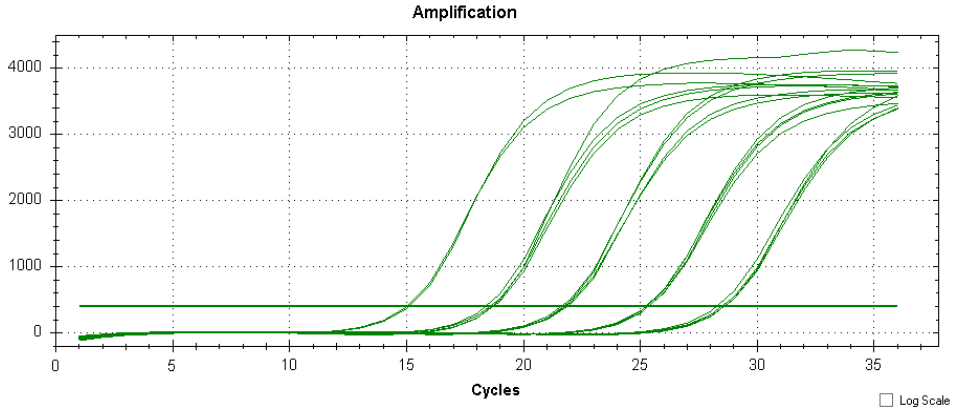
SFig.1. Cell free RQ-TRAP based on partial purified telomerase.

A - Standard calibration curve for the RQ-PCR reaction based on TSR8 oligonucleotide. B - Original amplification curve, obtained by 10-fold serial dilution of 10pM TSR8 oligonucleotide. C - Linearity of signal of RQ-TRAP obtained from serially 2-fold dilution of partial purified telomerase before PCR amplification.

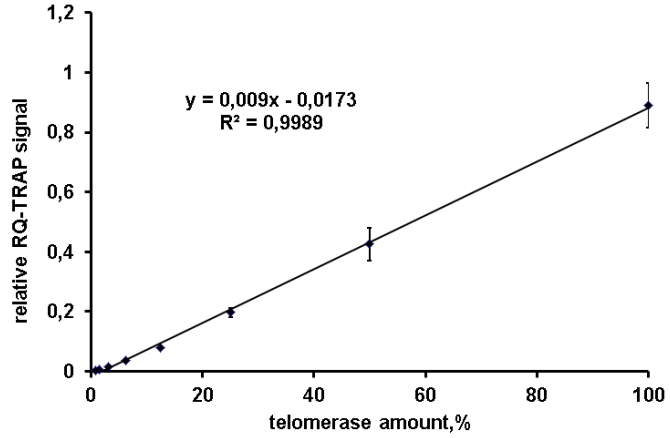
A



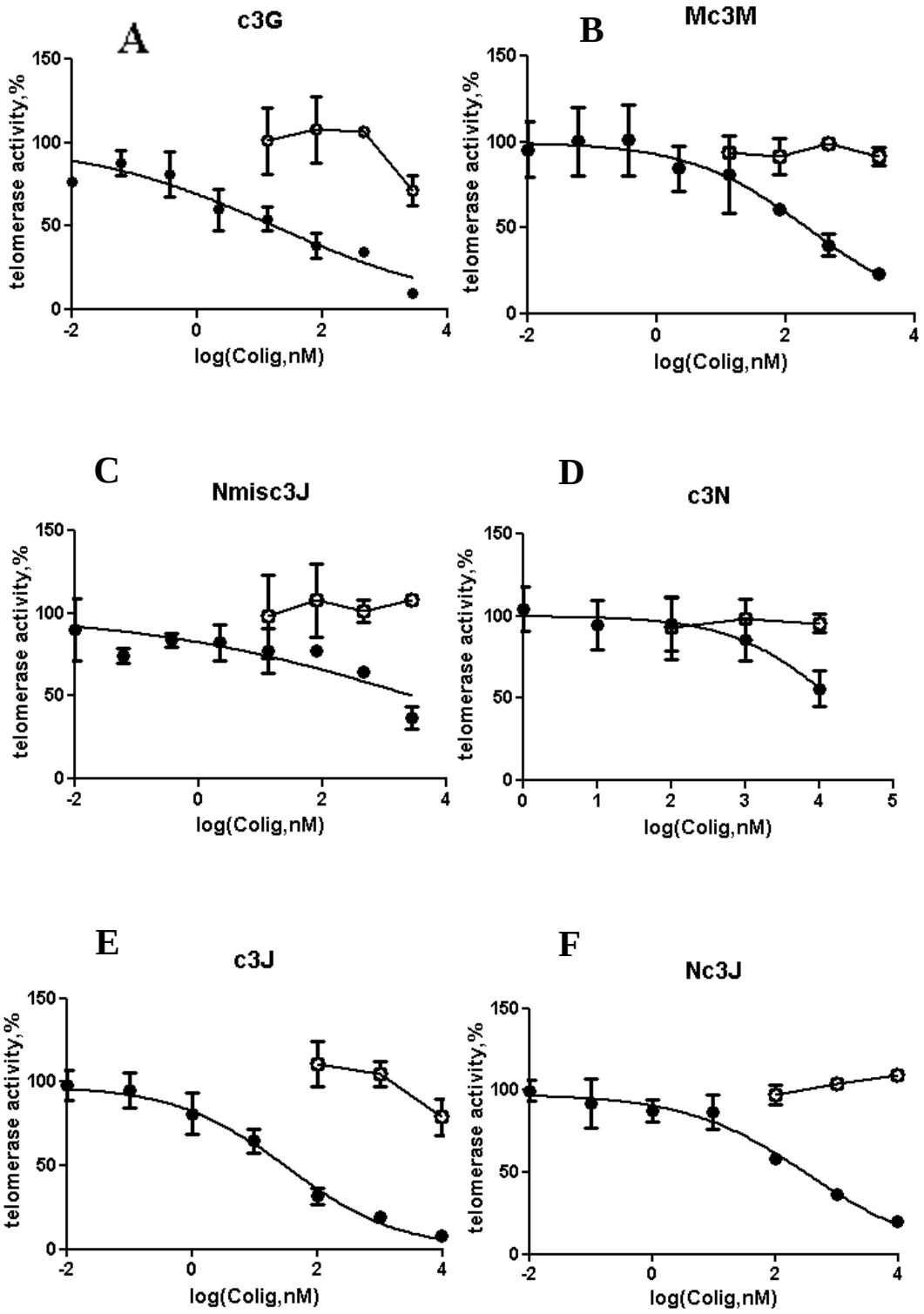
B

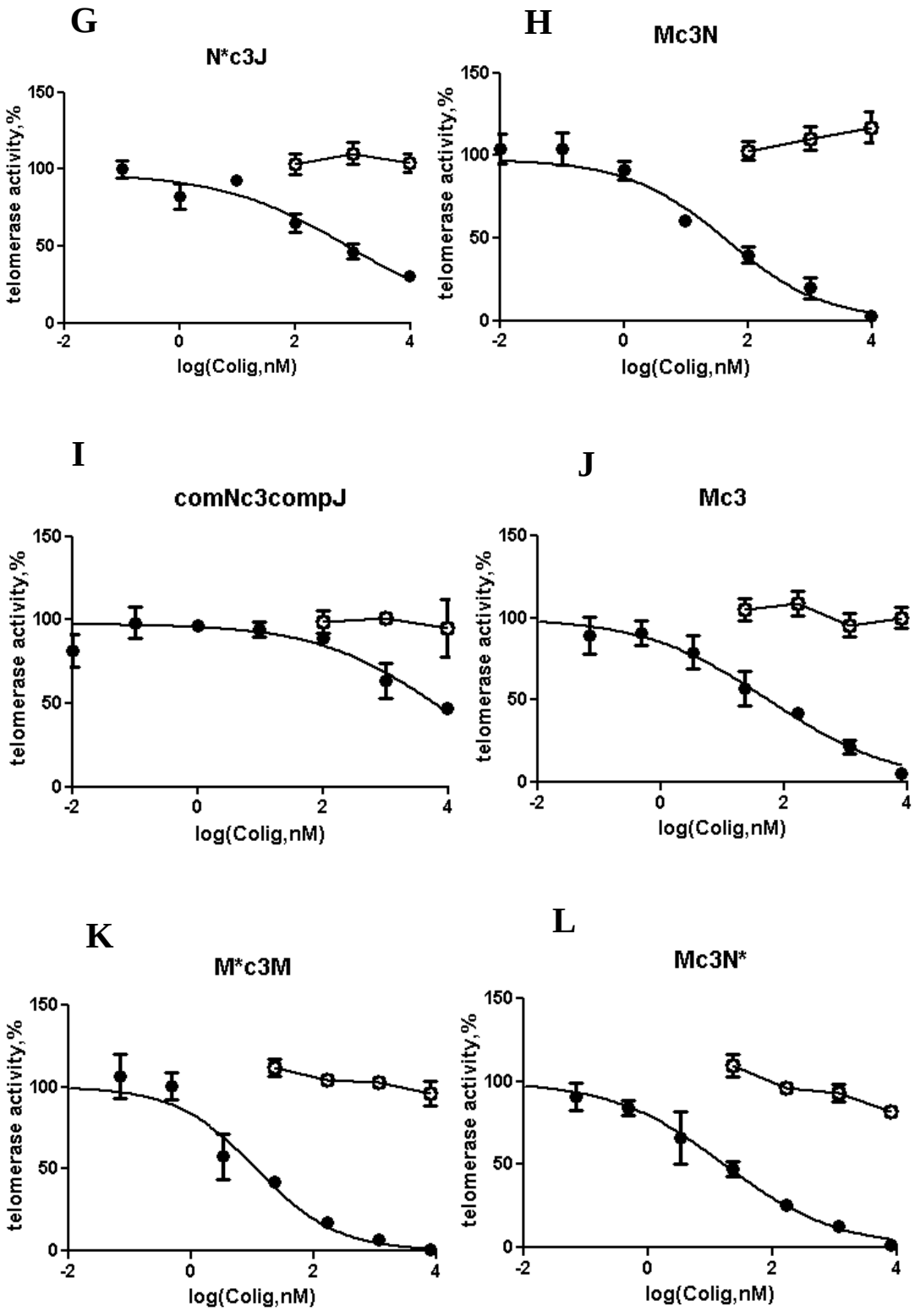


C

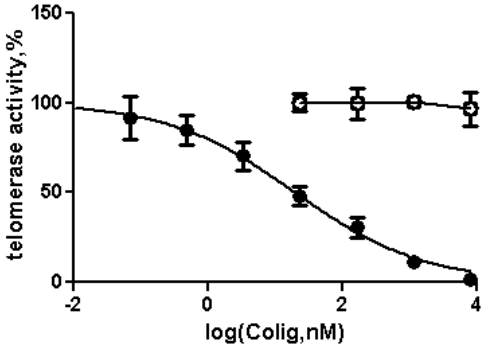


SFig.2. Inhibition of telomerase by modified oligonucleotides *in vitro*. (●) - Inhibition of telomerase, oligonucleotide was added to telomere reaction. (○) - Inhibition of PCR, oligonucleotide was added after telomerase reaction before PCR amplification. A - c3G, B – Mc3M, C – Nmisc3J, D – c3N, E – c3J, F – Nc3J, G – N*c3J, H- Mc3N, I - compNc3compJ, J – Mc3, K - M*c3M, L – Mc3N*, M – M*c3N, N – MMc3, O – Nmisc3Jmis, P – c3MN, Q – Nc3M, R – Nc3Jmis, S – MNc3, T – MmisNmisc3, U – Jc3N, V – c3M**,** W – c3Mc3N, X – c3Mc3M.

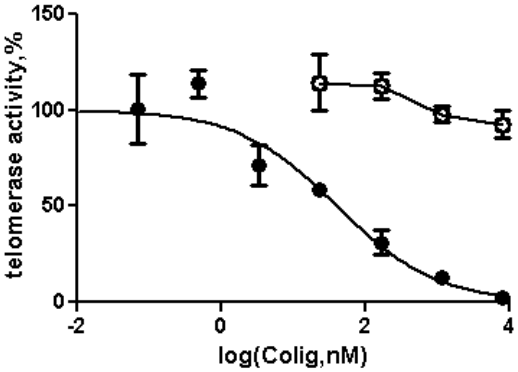



G
N*c3J
telomerase activity,%
log(Colig,nM)
H
Mc3N
telomerase activity,%
log(Colig,nM)
I
comNc3compJ
telomerase activity,%
log(Colig,nM)
J
Mc3
telomerase activity,%
log(Colig,nM)
K
M*c3M
telomerase activity,%
log(Colig,nM)
L
Mc3N*
telomerase activity,%
log(Colig,nM)

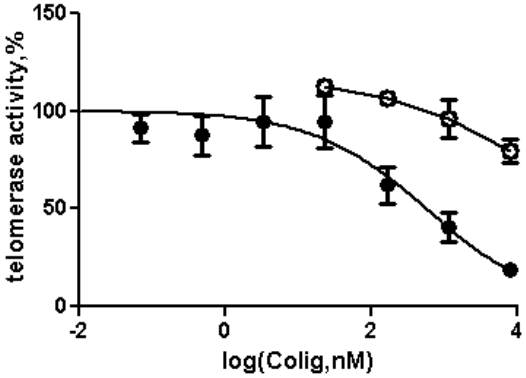
M


M*c3N
telomerase activity,%
150
100
50
0
-2
0
2
4
log(Colig,nM)

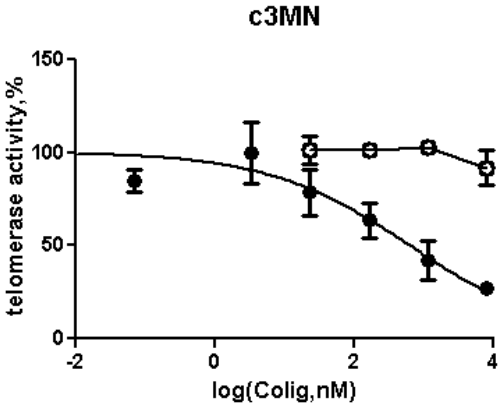
N


MMc3
telomerase activity,%
150
100
50
0
-2
0
2
4
log(Colig,nM)

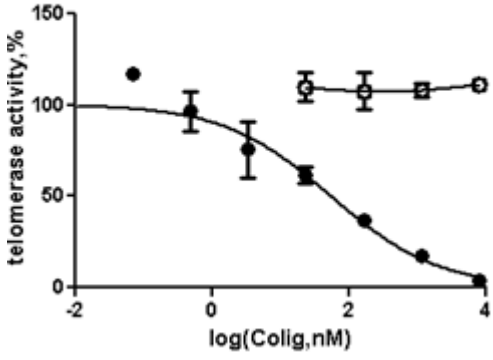
O


Nmisc3Jmis
telomerase activity,%
150
100
50
0
-2
0
2
4
log(Colig,nM)

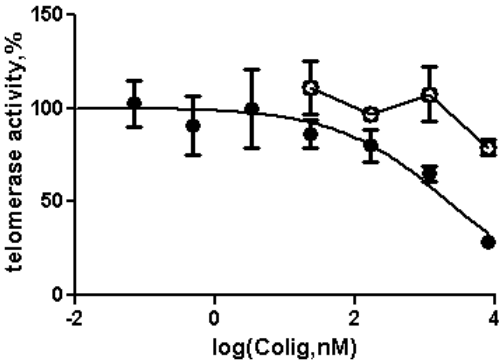
P

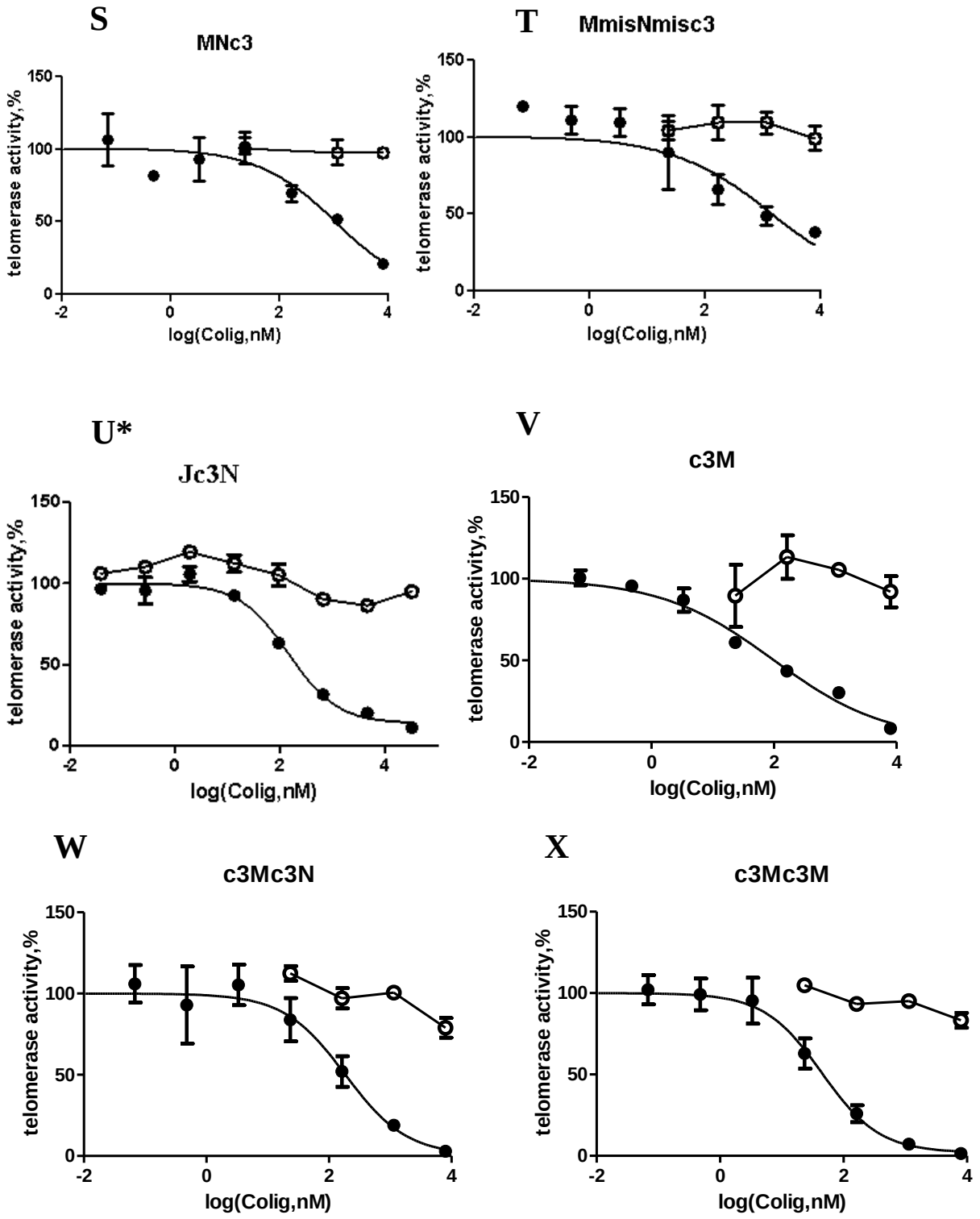

c3MN
telomerase activity,%
150
100
50
0
-2
0
2
4
log(Colig,nM)

Q


Nc3M
telomerase activity,%
150
100
50
0
-2
0
2
4
log(Colig,nM)

R

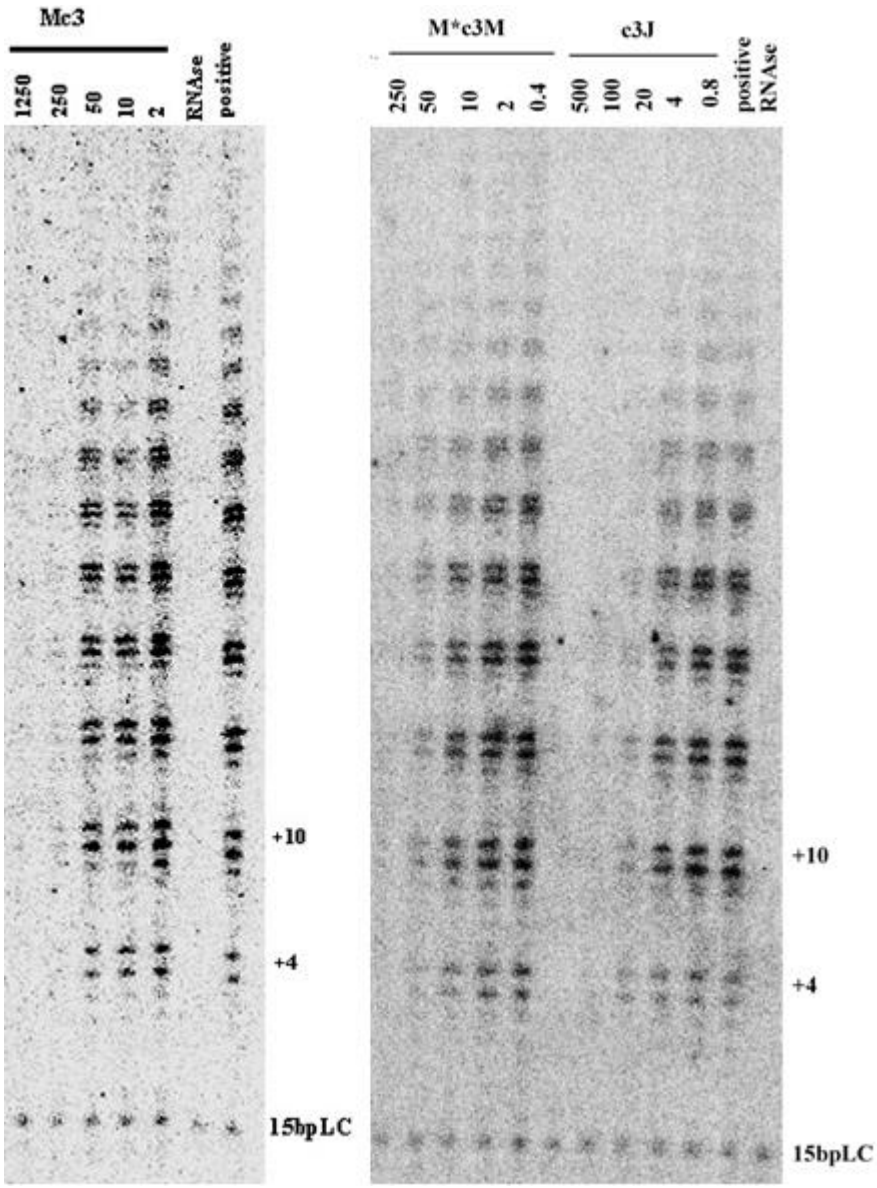

Nc3Jmis
telomerase activity,%
150
100
50
0
-2
0
2
4
log(Colig,nM)

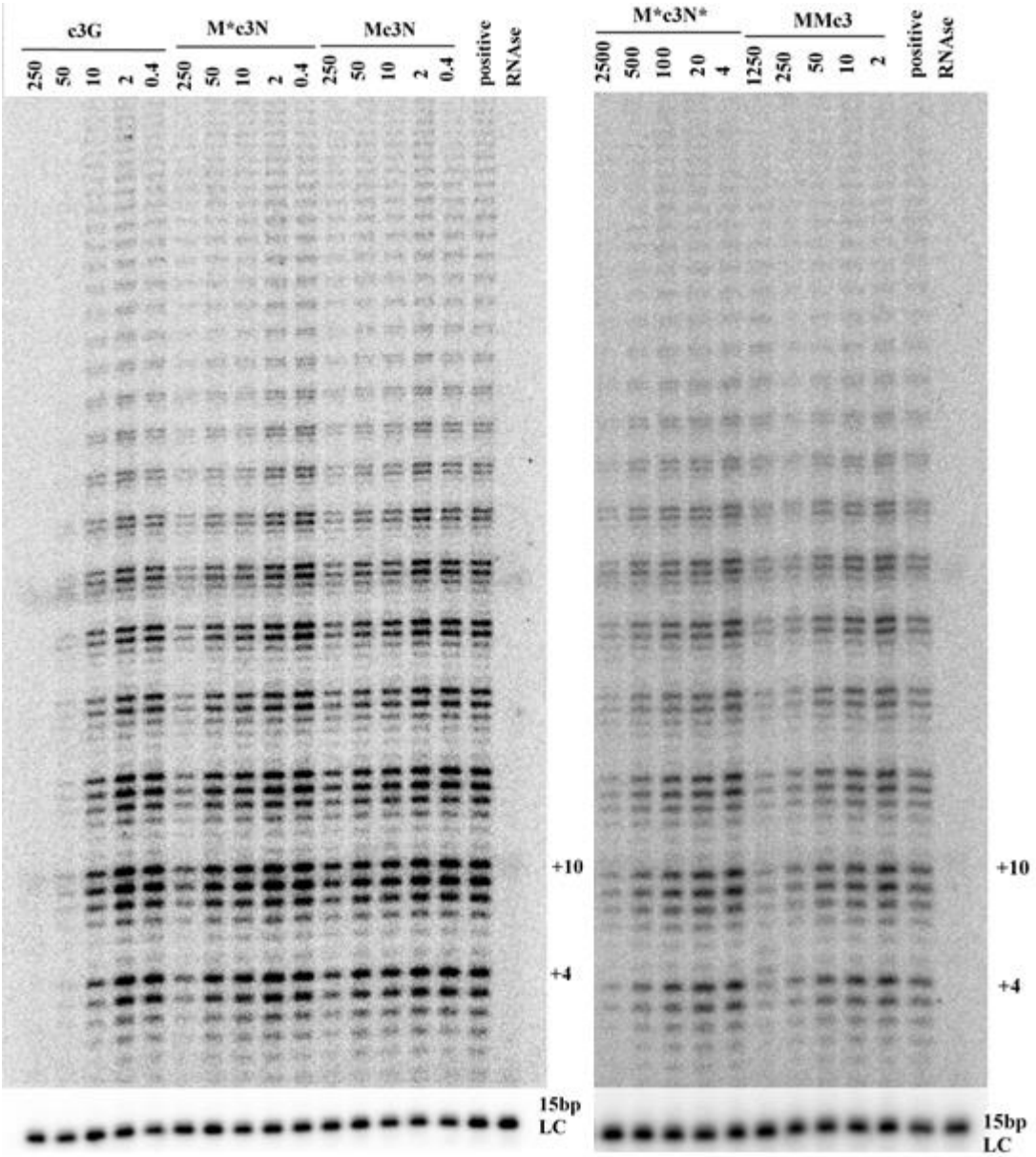


Graphics made by GraphPad Prism program. Errors are the standard deviation of three measurements. Telomerase activity was calculated by RQ-TRAP signal of inhibited telomerase normalized to RQ-TRAP signal of telomerase without inhibition. IC_{50} calculated by GraphPad Prism5 software by choosing step by step: nonlinear regression, dose-response – inhibition, log (inhibitor) vs. response, variable slope.

U*- for Jc3N 100-fold dilution of telomerase product by 1 x TRAP buffer before PCR was used.

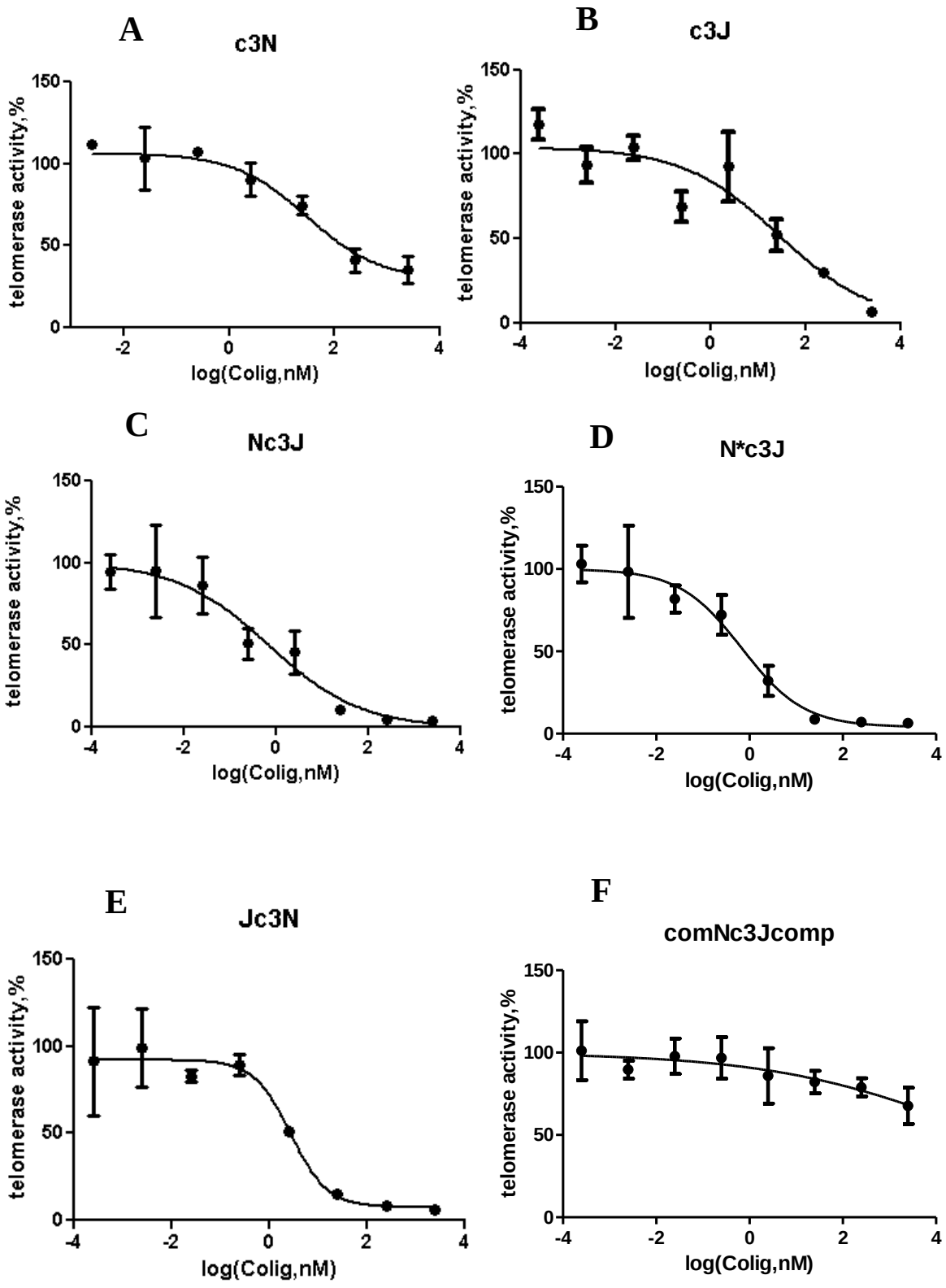
SFig.3. Analysis of inhibition effect to telomerase activity by direct telomerase assay. The numbers on the right of the gel indicate the number of nucleotides added to the 3'-end of the primer. The numbers on the top of the gel indicate the concentrations (in nM) of oligonucleotides in assay. **positive** – uninhibited telomerase activity. **RNAse** – is telomerase which treated with RNAse A (2mg/ml in sample) before analysis.



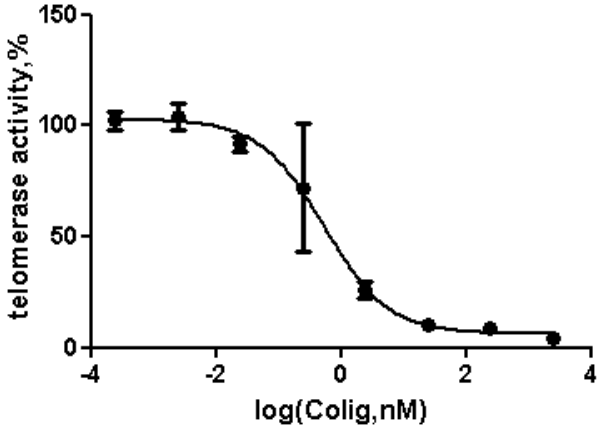


Intensity of each lane quantified by TotalLab Quant software, then normalized to intensity of loading control (15bp LC) which was used as recovery control and was added before DNA purification (a 5'-radiolabeled and 3'-biotinylated 15-mer oligonucleotide). IC_{50} calculated by GraphPad Prism5 software by choosing next buttons: Nonlinear regression (curve fit) → Dose-response – inhibition → log (inhibitor) vs. response - Variable slope. The intensity obtained from RNAse control lane was used as background subtraction.

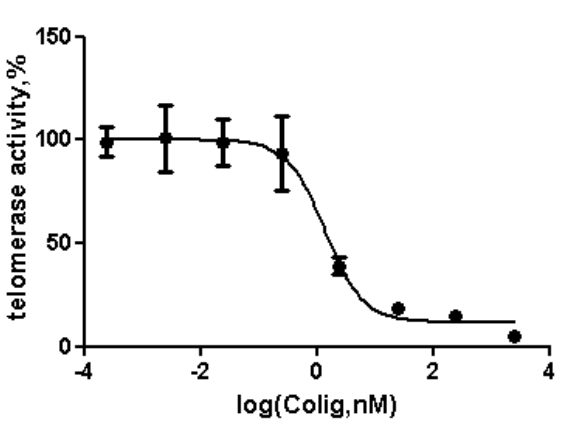
SFig.4. Inhibition of telomerase by modified oligonucleotides *in vivo*. A - c3N, B – c3J, C – N3J, D – N*3J, E – Jc3N, F – compNc3compJ, G – M*c3N, H- Mc3N, I - Nmisc3Jmis, J – Mc3N*, K - Nc3M, L – MmisNmisc3, M – c3G, N – c3MN, O – Mc3M, P – MNc3, Q – M*c3M, R – MMc3, S – Nmisc3J, T – Nc3Jmis, U –c3M, V – Mc3, W- c3Mc3M, X – c3Mc3N



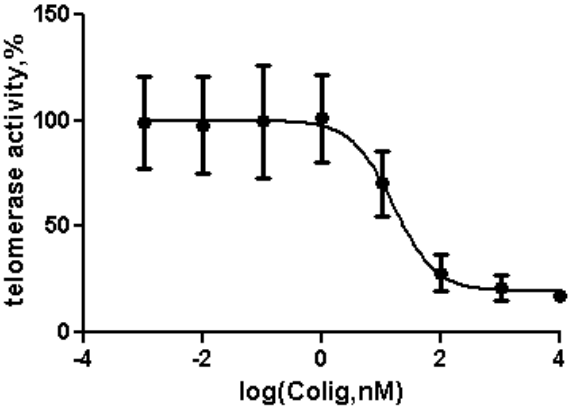
G


M*c3N
telomerase activity,%
150
100
50
0
-4
-2
0
2
4
log(Colig,nM)

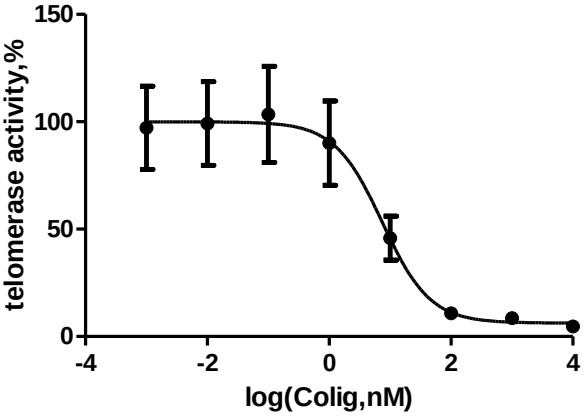
H


Mc3N
telomerase activity,%
150
100
50
0
-4
-2
0
2
4
log(Colig,nM)

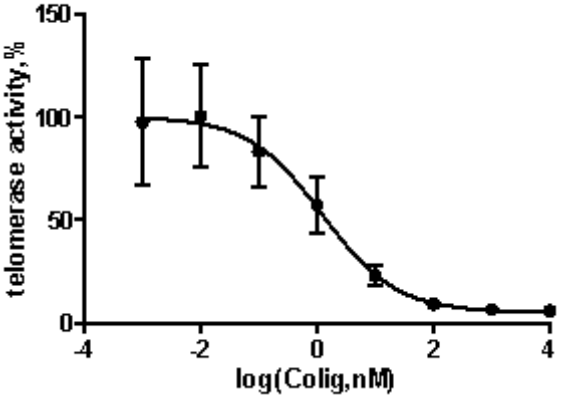
I


Nmisc3Jmis
telomerase activity,%
150
100
50
0
-4
-2
0
2
4
log(Colig,nM)

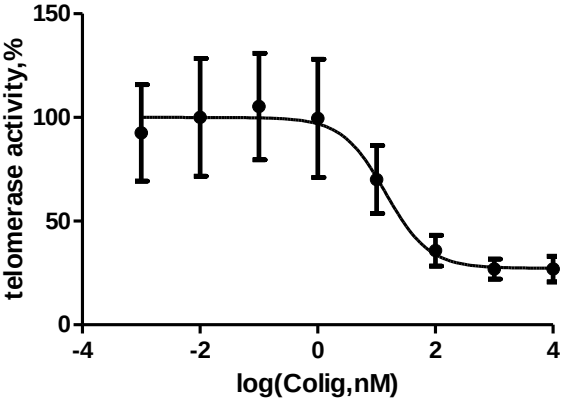
J

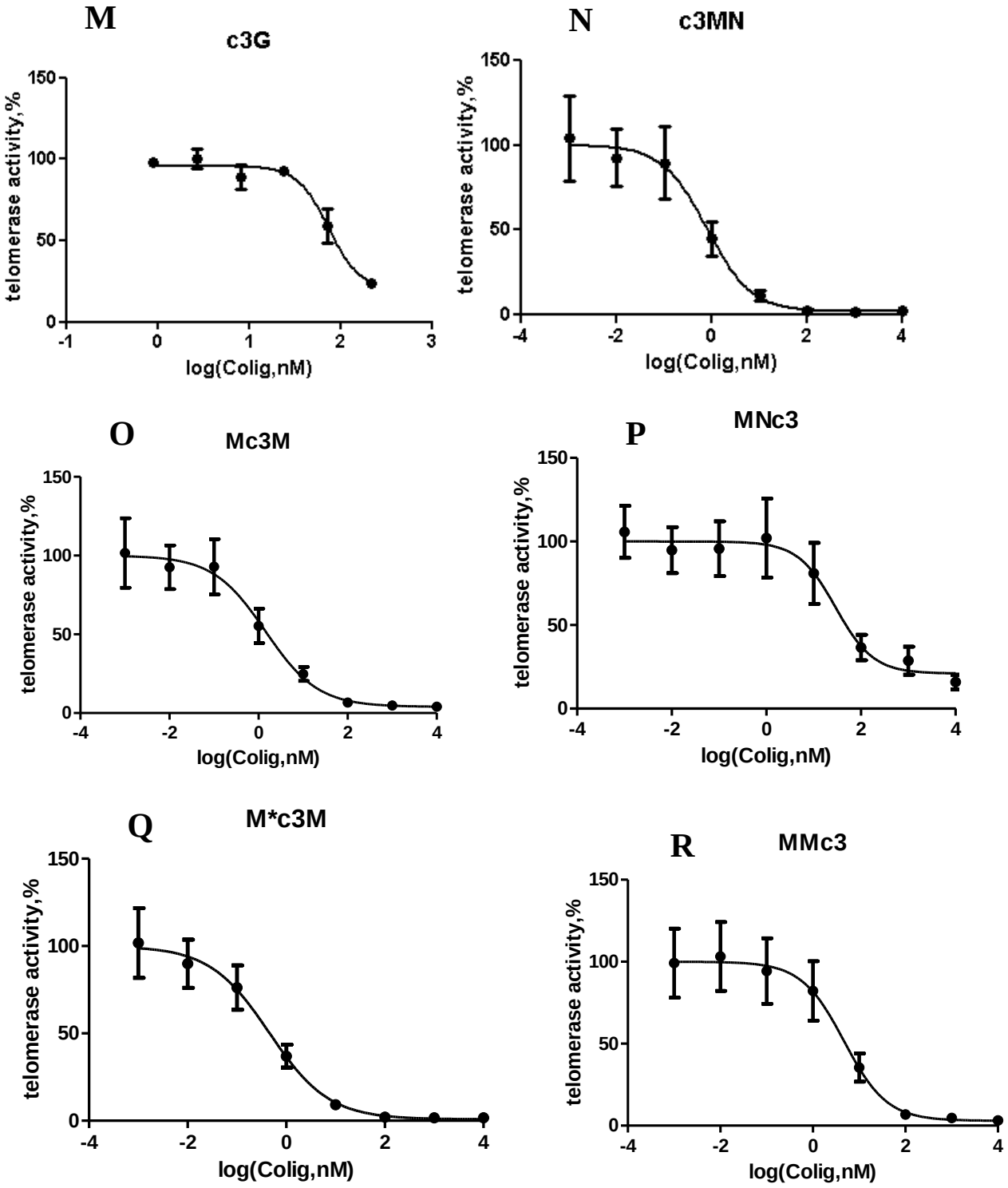

Mc3N*
telomerase activity,%
150
100
50
0
-4
-2
0
2
4
log(Colig,nM)

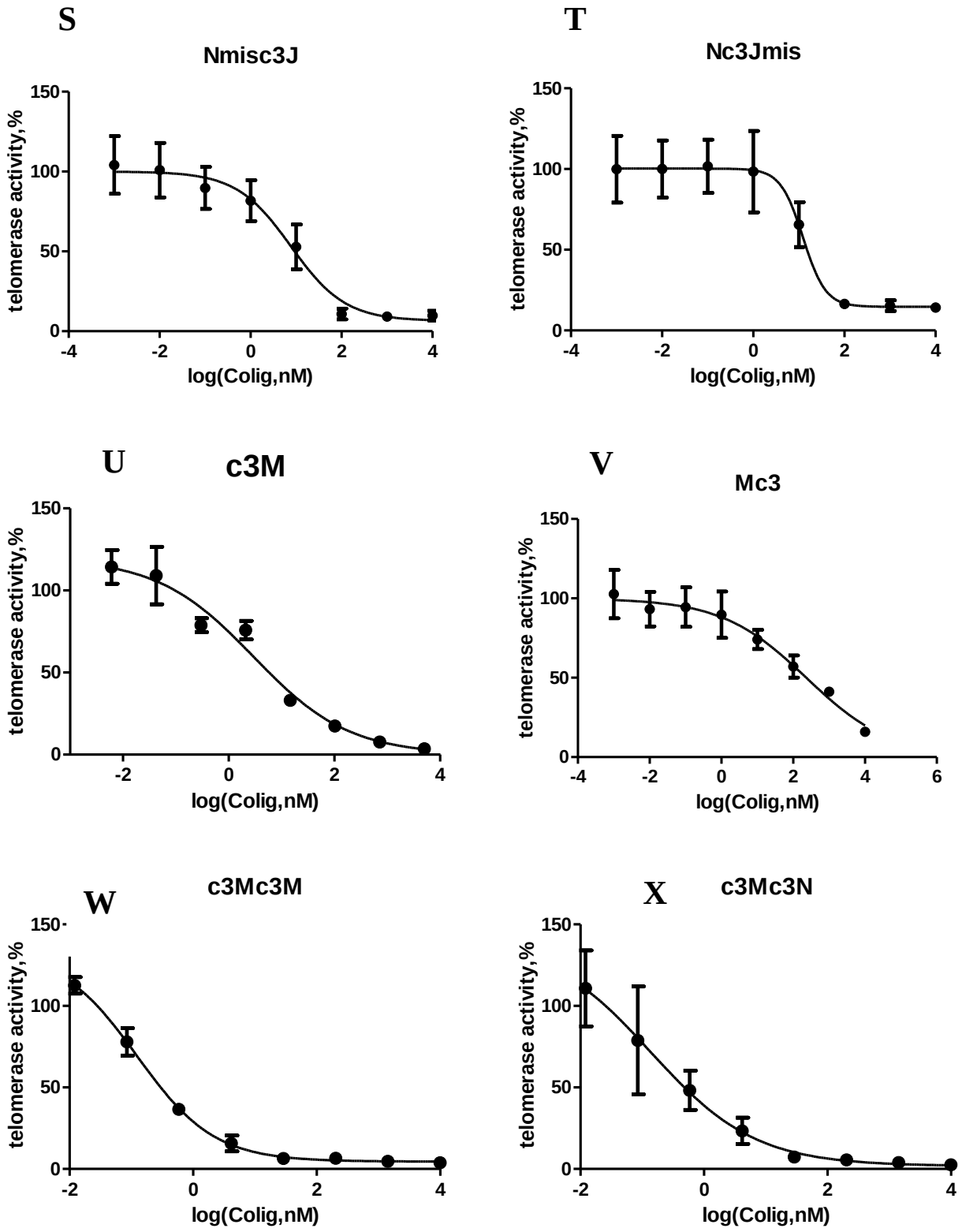
K


Nc3M
telomerase activity,%
150
100
50
0
-4
-2
0
2
4
log(Colig,nM)

L


MmisNmisc3
telomerase activity,%
150
100
50
0
-4
-2
0
2
4
log(Colig,nM)


M
c3G
telomerase activity,%
log(Colig,nM)
N
c3MN
telomerase activity,%
log(Colig,nM)
O
Mc3M
telomerase activity,%
log(Colig,nM)
P
MNc3
telomerase activity,%
log(Colig,nM)
Q
M*c3M
telomerase activity,%
log(Colig,nM)
R
MMc3
telomerase activity,%
log(Colig,nM)



Graphics made by GraphPad Prism program. Errors are the standard deviation of three measurements. Telomerase activity was calculated by RQ-TRAP signal of inhibited telomerase normalized to RQ-TRAP from transfected cell extract normalized to protein concentration, then secondly normalized to RQ-TRAP signal of untransfected cell extract. IC_{50} calculated by GraphPad Prism5 software by choosing step by step: nonlinear regression, dose-response – inhibition, log (inhibitor) vs. response, variable slope.

SFig.5. Influence of linker length and position in chimeric oligonucleotide for telomerase inhibition *in vivo*.

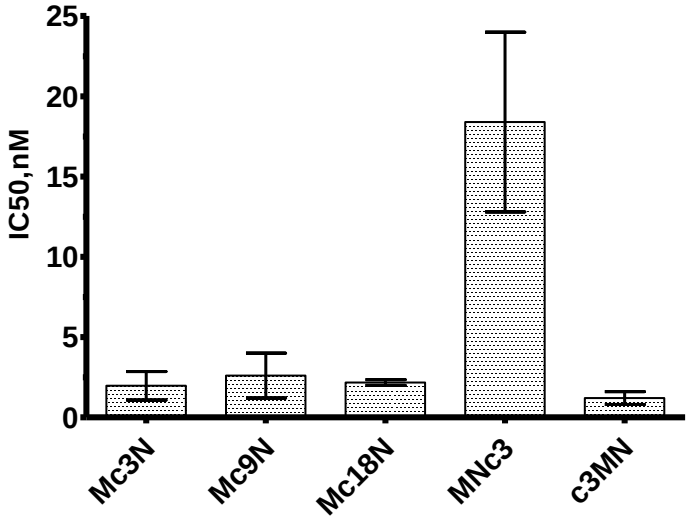
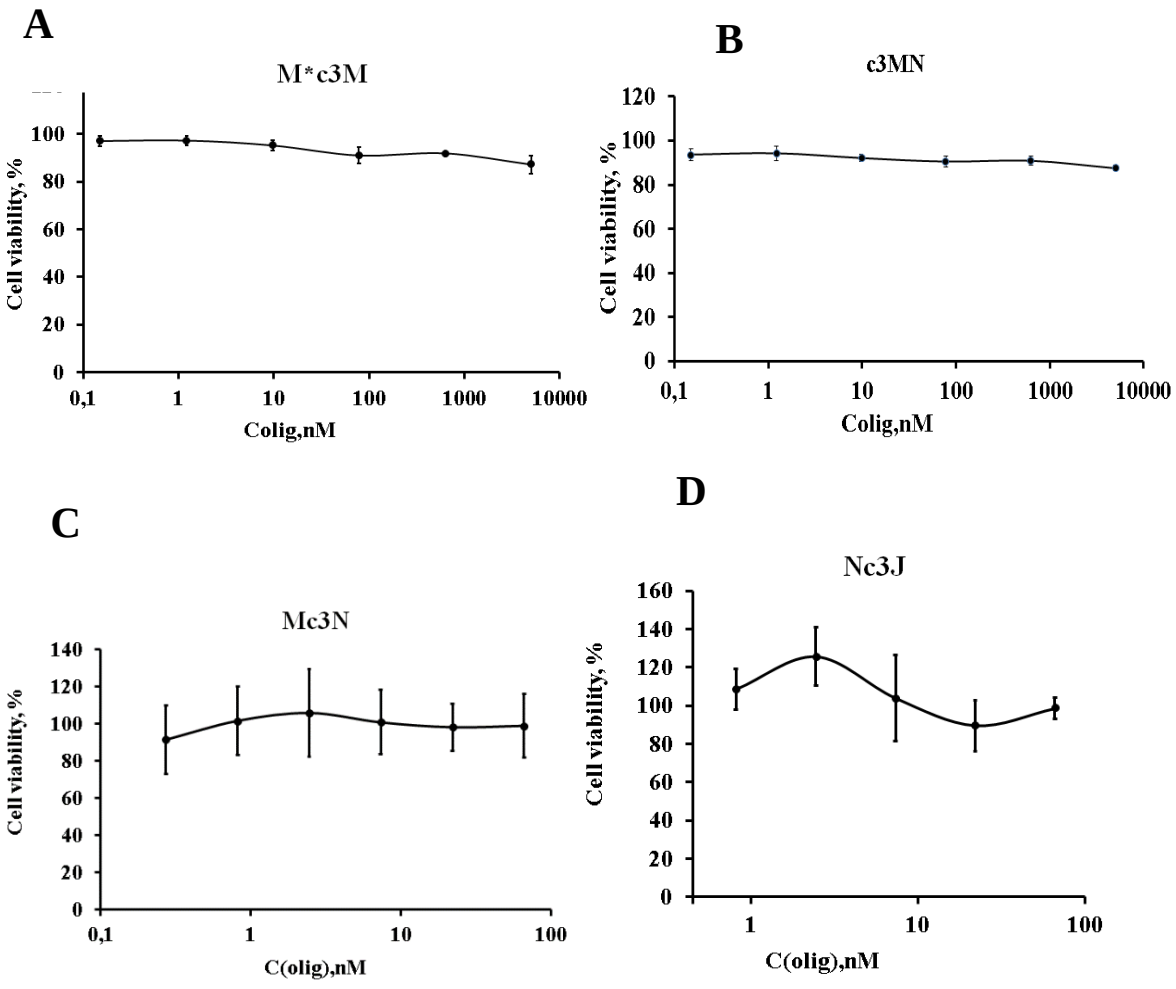
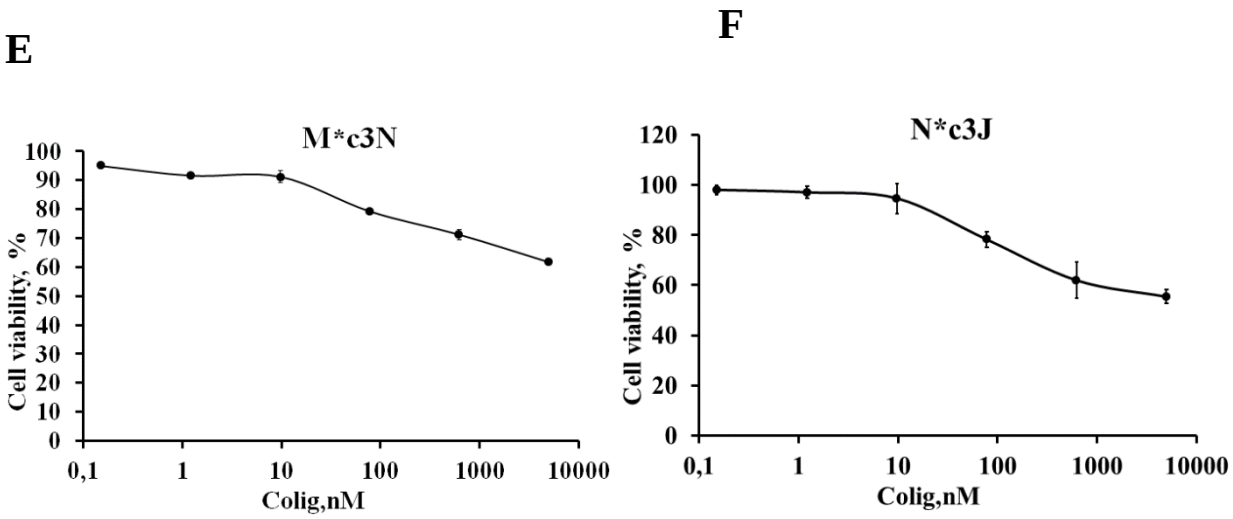
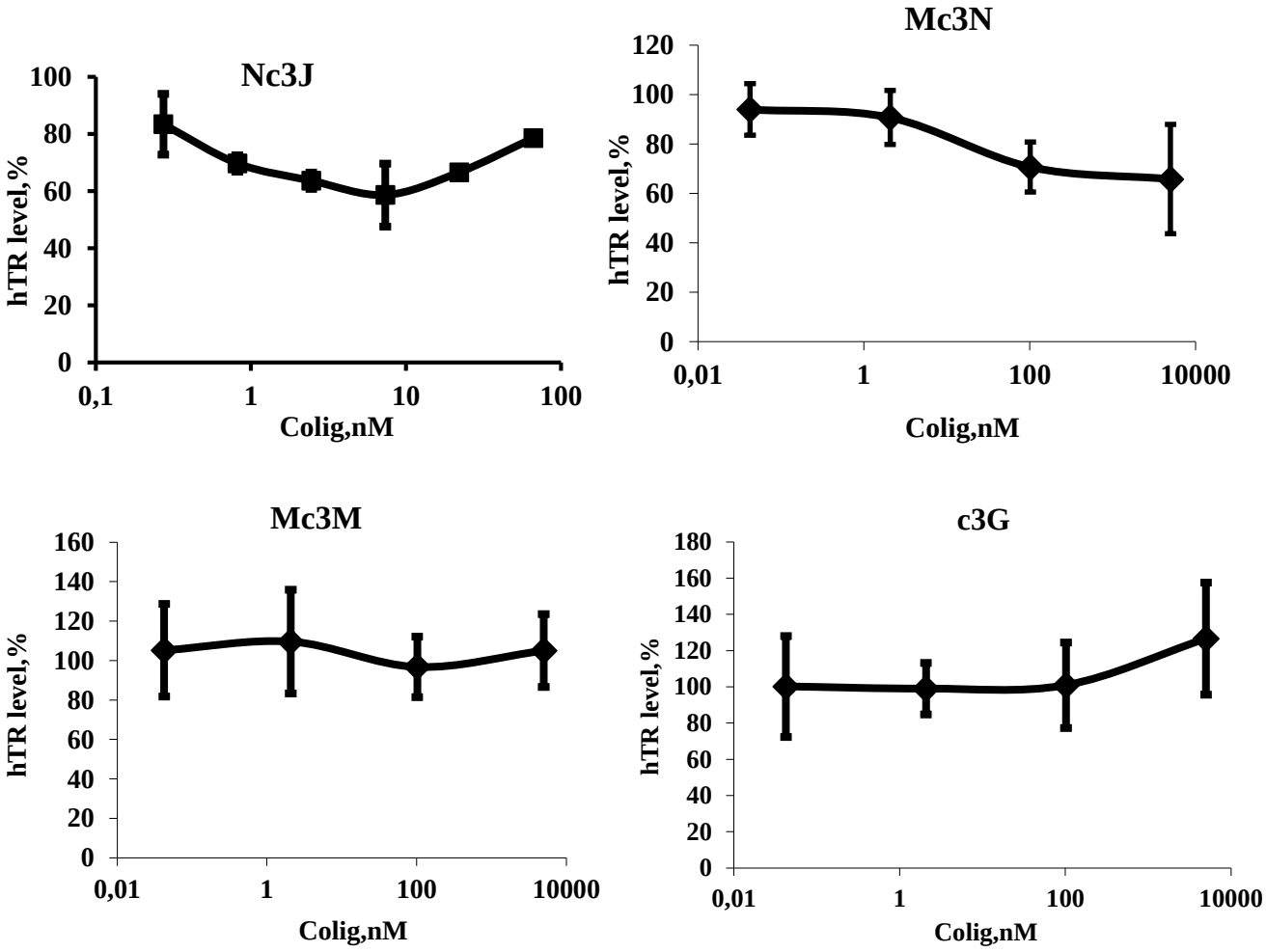


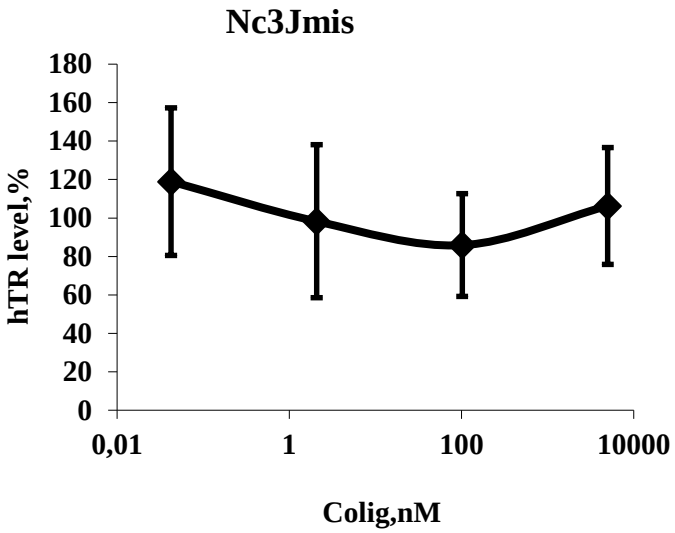
Fig S6. MTT assay for cells transfected and incubated with inhibitory oligonucleotides at various concentrations. A - M*c3M, B – c3MN, C- Mc3N, D – Nc3J, E – M*c3N, F – N*c3J.

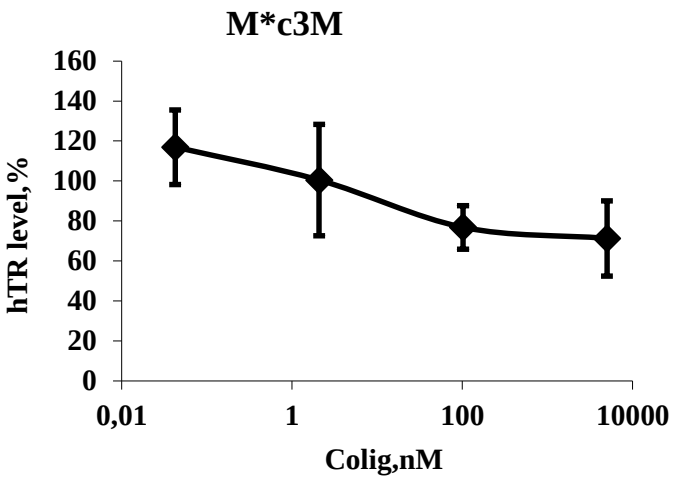


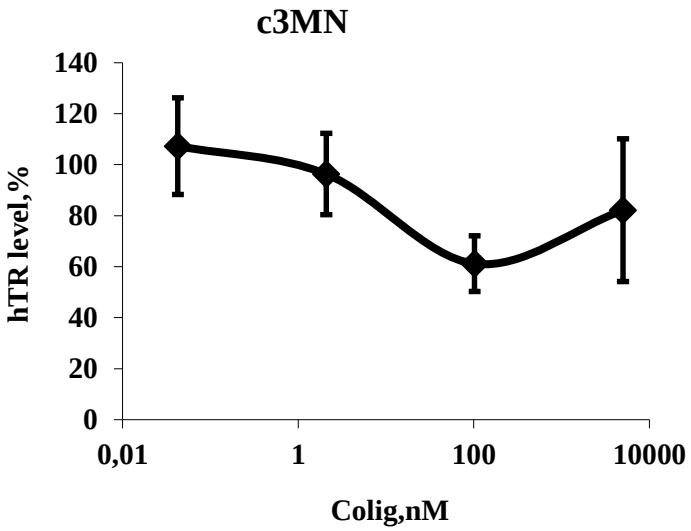


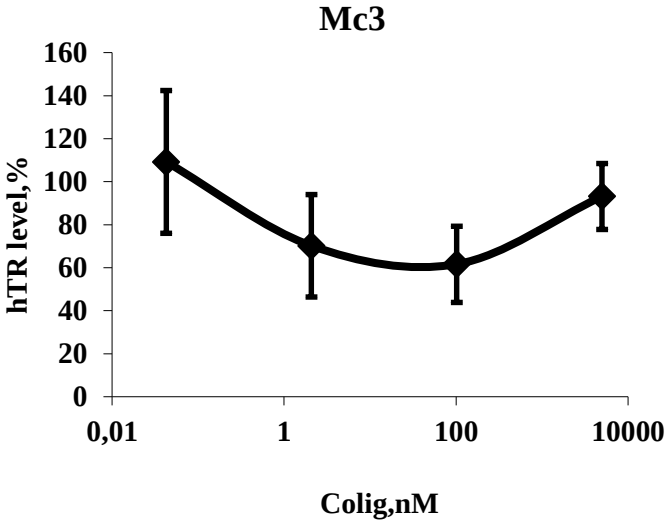
SFig 7. Telomerase RNA is not degraded *in vivo* after transfection of chimeric oligonucleotides.

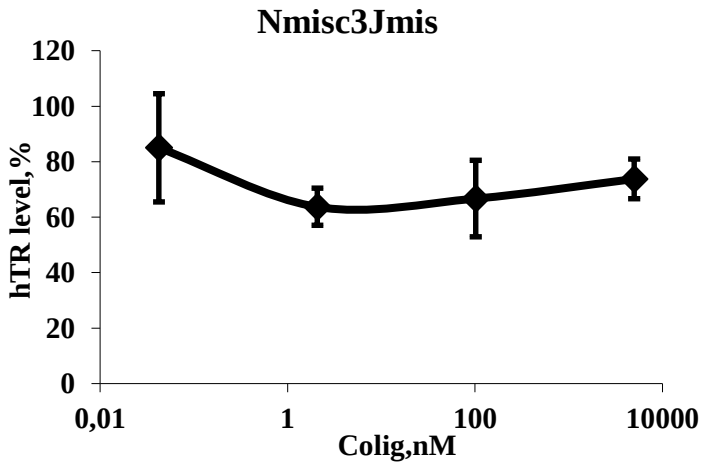






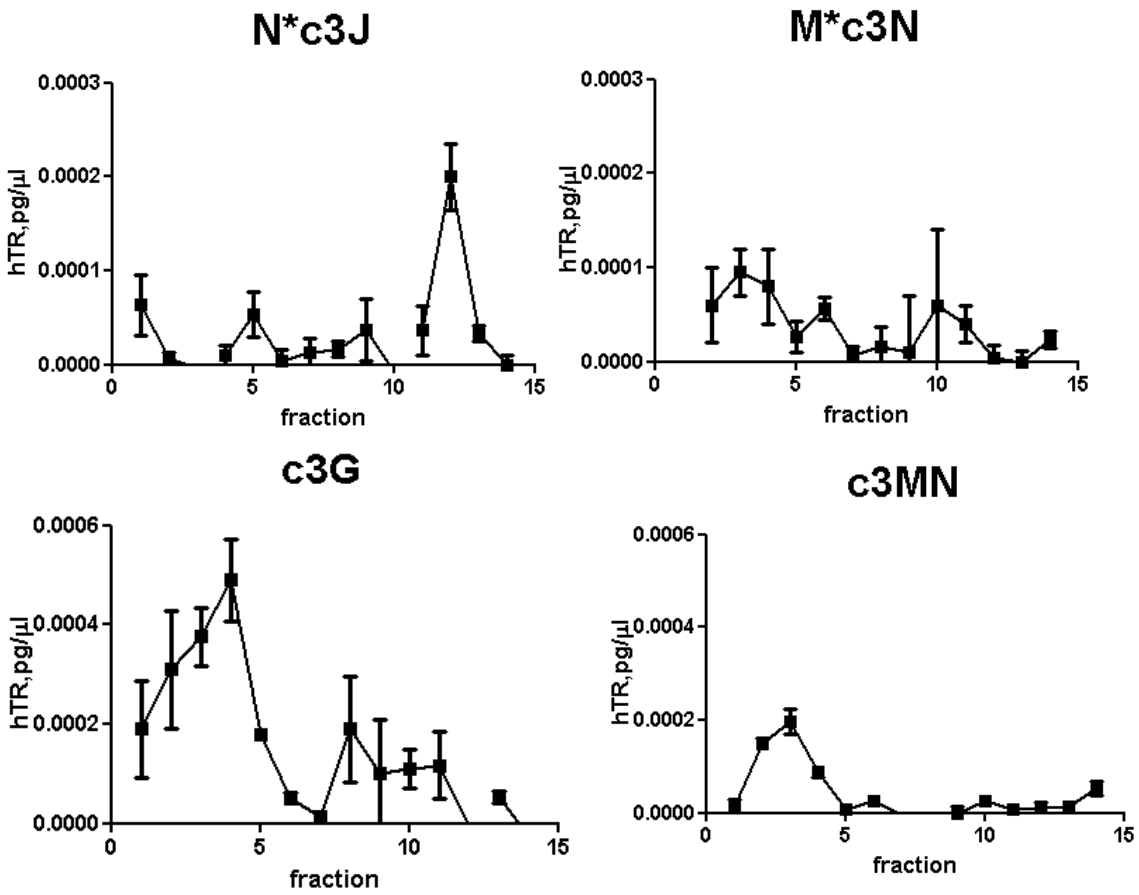






hTR level % is hTR amount in treated cell extract determined by RT-qPCR normalized to GAPDH amount, then secondly normalized to hTR amount of untransfected cell extract. The propagation of errors by GAPDH normalization was calculated by equation: $\sqrt{((\Delta hTR/hTR)^2+(\Delta GAPDH/GAPDH)^2)}*(hTR/GAPDH)$ that was suggested by authors Gerhard Bohm and Günter Zech in "Introduction to Statistics and DataAnalysis for Physicists", DESY, 2010.

SFig.8. Analysis of telomerase assembly.



Cellular extract was centrifuged through sucrose gradient and hTR amount in each fraction was determined by RT-qPCR. Percentage of assembled telomerase calculated as described next: area under the curve was calculated by GraphPad Prism5 software. Then area under the peak, which coincides with telomerase activity, was normalized to assembled telomerase area obtained from untransfected cell lysate.

STab. 1. Evaluation of the degree of purification of telomerase of HEK 293E through precipitation.

	protein, µg	Total telomerase activity, AU	activity/ µg protein	Purification factor based on activity	hTR, relative units	hTR/ µg protein	Concentration factor based on hTR
S16	5.99	12	2	1	12	2	1
Partially purified telomerase	0.46	6.64	14.4	7.2	3.73	8.19	4.1

STab. 2. Test of inhibition of PCR for cell based RQ-TRAP.

Oligonucleotide	activity,%#
c3N	95±3
c3M	89±10
c3J	87±10
Nc3M	103±13
Jc3N	85±9
Nmisc3J	94±3
Nmisc3Jmis	109±3
compNc3J	100±20
M*c3M	95±15

#- relative activity of partial purified telomerase mixed with cell extracts obtained from HEK293 cells transfected with oligonucleotides (% compared to telomerase mixed with 1xTRAP buffer).

Analysis of influence of cell extracts obtained from HEK293 cells transfected with oligonucleotides on PCR was done for highest concentration of oligonucleotides used for transfection. Telomerase activity of 1 µl purified telomerase sample without dilution was 1352 u.a., Telomerase activity of 1 µl of total extract HEK293 cells was 4 u.a. Telomerase activity of 2.5 µl 200 fold diluted purified telomerase was 17 u.a., the telomerase activity of 2.5 µl 10 fold diluted total extract of HEK was 1 u.a. Dilution in 200 times of purified telomerase was chosen to arrange the activity of purified telomerase higher than total activity of the extract in the reaction mixture so that the latter was in the error range compared with the first. For the experiment 2.5 µl of 10 fold diluted by 1x TRAP buffer total cell extract obtained from HEK293 cells transfected with 220 µM was added to 2.5 µl 200 fold diluted by 1xTRAP buffer partial purified telomerase. The mix was incubated for 10 min at room temperature. 5 µl mix 2 was added to reaction mix on ice and amplification was performed as described in Material and Methods.