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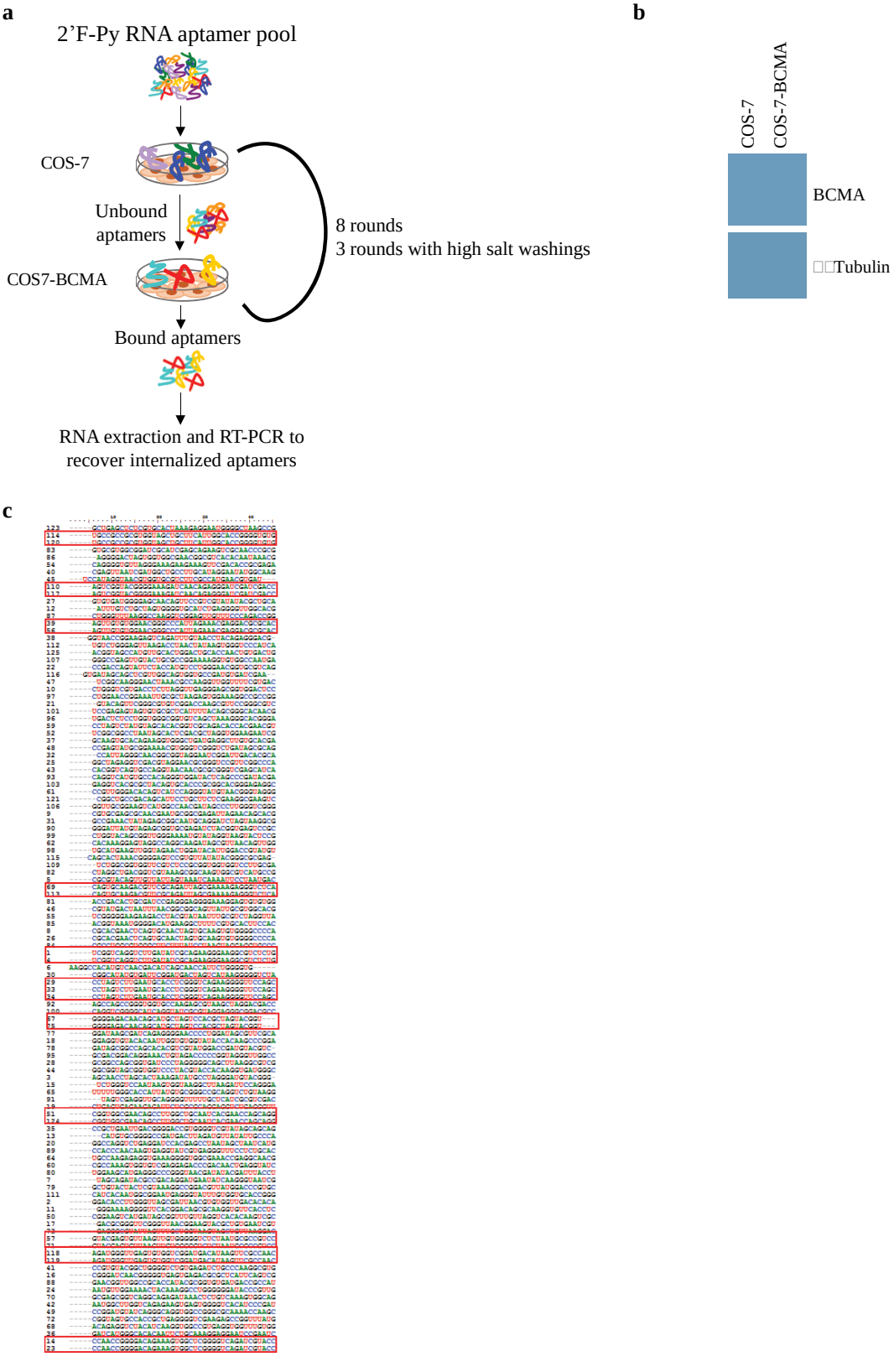
Supplemental Information

An Anti-BCMA RNA Aptamer for miRNA Intracellular Delivery

Silvia Catuogno, Maria Teresa Di Martino, Silvia Nuzzo, Carla Lucia Esposito, Pierfrancesco Tassone, and Vittorio de Franciscis

Round Number	RNA pool (pmoles)	Number of cells at the day before incubation	Washes after selection	Counter-selection steps	Incubation time (min)	High salt washings
I	600	$2{,}67 \times 10^6$	1	1	30' selection 30' counter-selection	no
II	600	$2{,}67 \times 10^6$	2	1	30' selection 30' counter-selection	no
III	600	$2{,}67 \times 10^6$	2	1	30' selection 30' counter-selection	no
IV	600	$2{,}67 \times 10^6$	3	1	30' selection 30' counter-selection	no
V	600	$0{,}9 \times 10^6$	3	1	30' selection 30' counter-selection	no
VI	600	$0{,}7 \times 10^6$	4	1	30' selection 30' counter-selection	no
VII	600	$0{,}7 \times 10^6$	5	2	30' selection 30' counter-selection 30' counter-selection	no
VIII	600	$0{,}32 \times 10^6$	5	2	15' selection 15' counter-selection 15' counter-selection	no
IX	600 + polyinosinic acid 0.1 ug/ul (during the selection step)	$0{,}32 \times 10^6$	5	2	15' selection 15' counter-selection 15' counter-selection	yes
X	600 + polyinosinic acid 0.1 ug/ul (15' pretreatment before selection and during the selection step)	$0{,}32 \times 10^6$	6	2	15' selection 15' counter-selection 15' counter-selection	yes
XI	**600 + polyinosinic acid 0.1 ug/ul (30' pretreatment before selection and during the selection step)**	**$0{,}32 \times 10^6$**	**6**	**2**	**15' selection 15' counter-selection 15' counter-selection**	**yes**

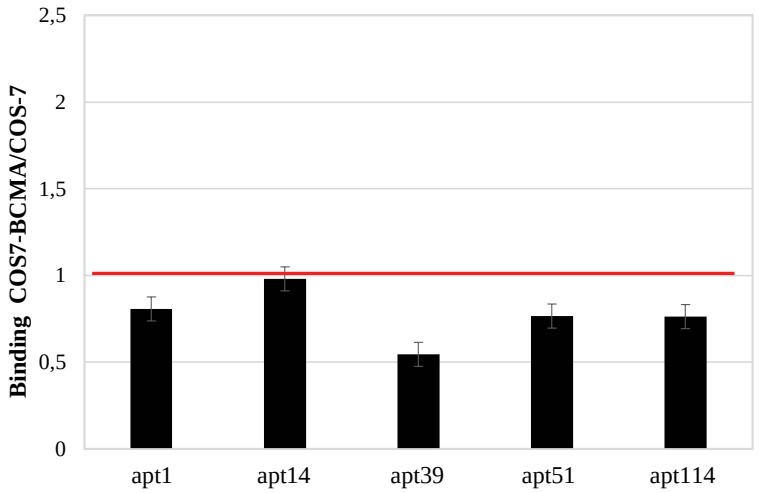
Supplementary Table 1 Cell-internalizing SELEX conditions



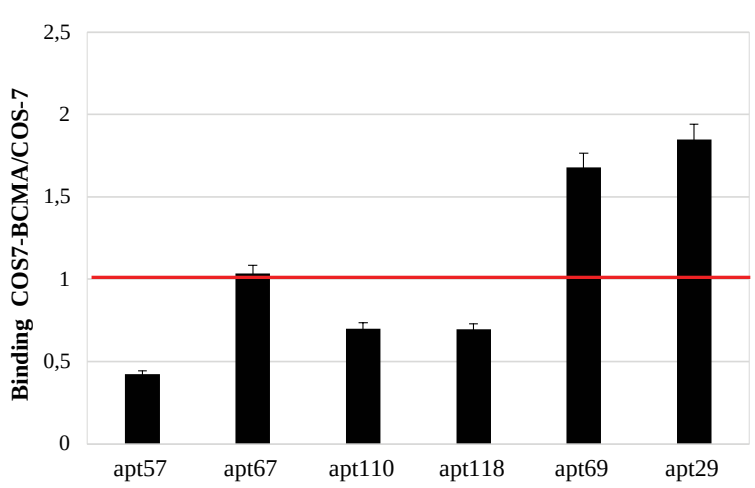
Supplementary Figure 1 Cell-internalizing SELEX. (a) Scheme of cell-internalizing SELEX protocol. We performed eight rounds in which the 2'F-Py RNA aptamer pool was: incubated with parental COS-7 cells (counter-selection); unbound aptamers were recovered and incubated with COS7-BCMA cells (positive selection); bound aptamers were recovered by RNA extraction and RT-PCR. In three additional rounds, downstream the positive selection step three high slat washings were performed to remove cell surface-bound

aptamers. Internalized aptamers were recovered by RNA extraction and RT-PCR. **(b)** Western blot analysis of COS-7 parental cells compared to COS-7 transiently transfected with BCMA. **(c)** Multiple Alignment of aptamer identified following cloning of the last SELEX rounds and sequencing. Multiple Sequence Alignment (ClustalW2) tool of EMBL-EBI was used.

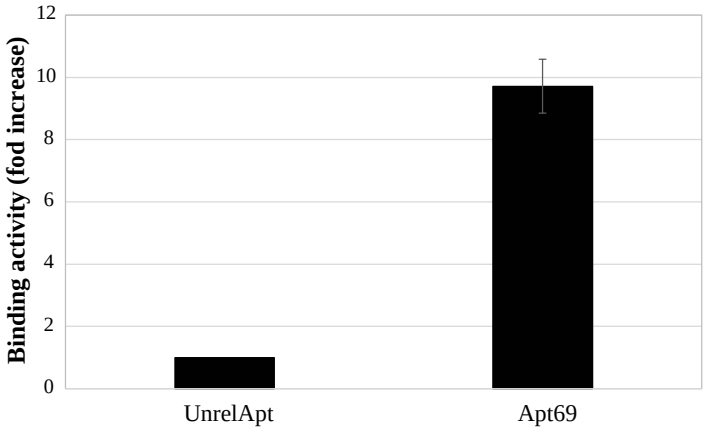
a



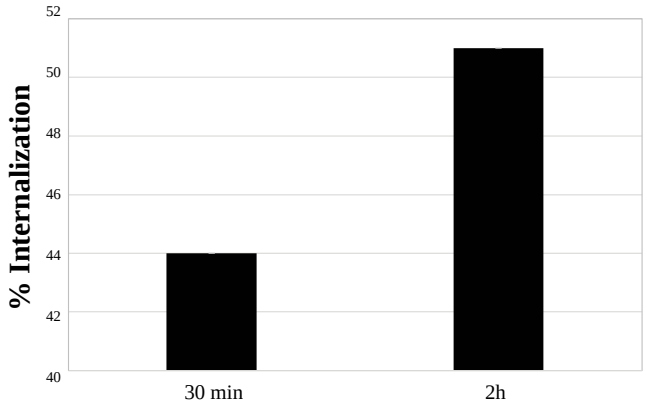
b



Supplementary Figure 2 Binding activity analysis. (a,b) Binding of 11 RNA aptamers obtained by SELEX procedure. Aptamers (100 nM) were incubated with COS-7 parental cells or with COS7-BCMA cells for 30 min at 37°C. Binding values were measured by RT-qPCR and here reported as fold increase (COS7-BCMA/ COS-7).



Supplementary Figure 3 Binding activity of apt69 compared to a control aptamer. Apt69 or control aptamer (UnrelApt) were incubated with COS7-BCMA cells for 30 min at 37°C. Binding values were measured by RT-qPCR and here reported as fold increase.



Supplementary Figure 4 Internalization of apt69.T. Apt69.T was incubated with U266 cells for 30 min or 2 hours at 37°C. Cells were recovered following three washings with DPBS (total) or with DPBS 0,5 M NaCl (internalized) in order to remove aptamer on the cell surface. The quantities of total and internalized aptamer was evaluated by RT-qPCR and reported as percentage of internalized aptamer. Bars show the mean ± SEM values.