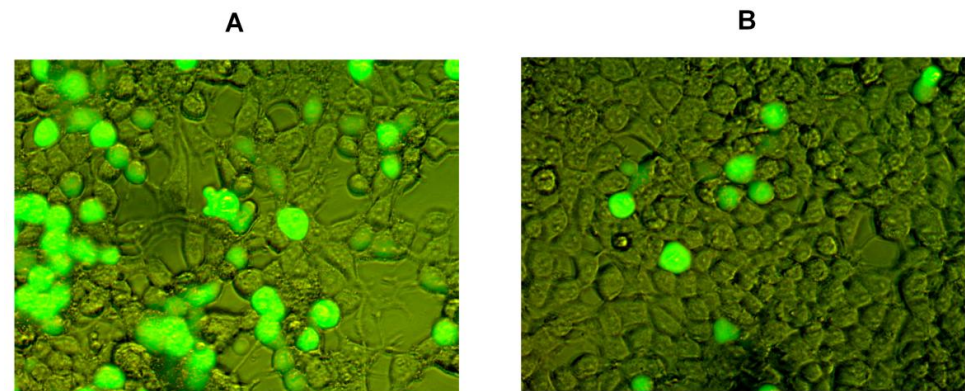


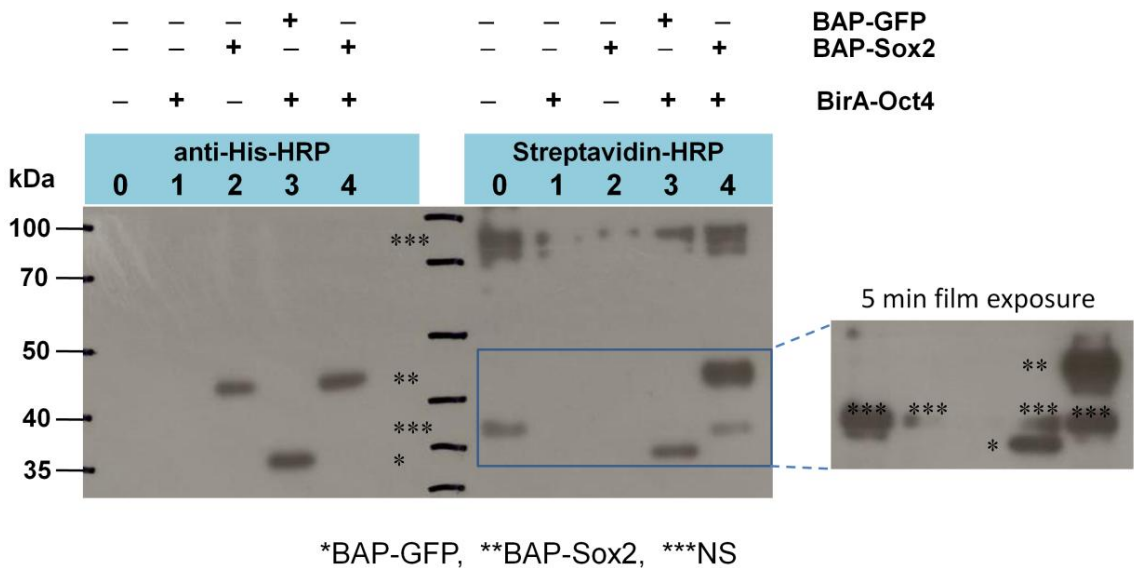
# Detection of Recombinant Proteins SOX2 and OCT4 Interacting in HEK293T Cells Using Real-Time Quantitative PCR

Darkhan Kanayev, Diana Abilmazhenova, Ilyas Akhmetollayev, Alia Sekenova, Vyacheslav Ogay and Arman Kulyyassov\*

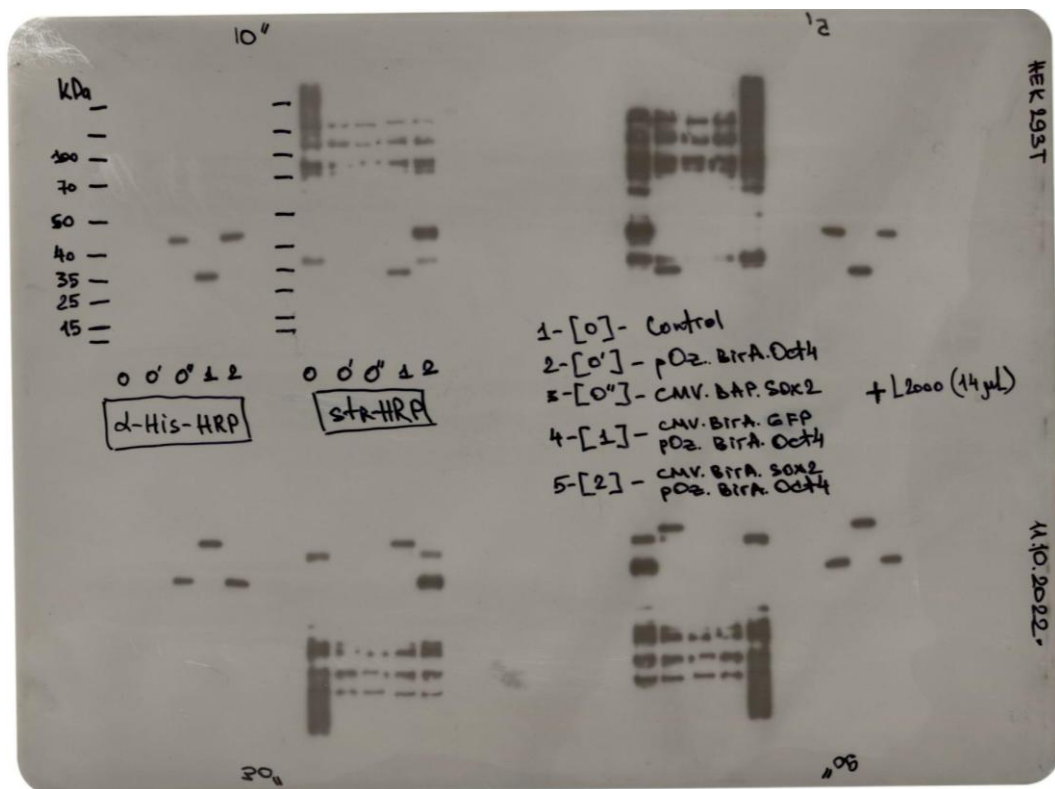
<sup>1</sup> Limited liability partnership “National Center for Biotechnology” Ministry of Healthcare of the Republic of Kazakhstan, 3/5, Kurgalzhynskoye Road , Astana, 010000, Kazakhstan  
\* Correspondence: kulyyassov@biocenter.kz; Tel.: +7-7172-707534



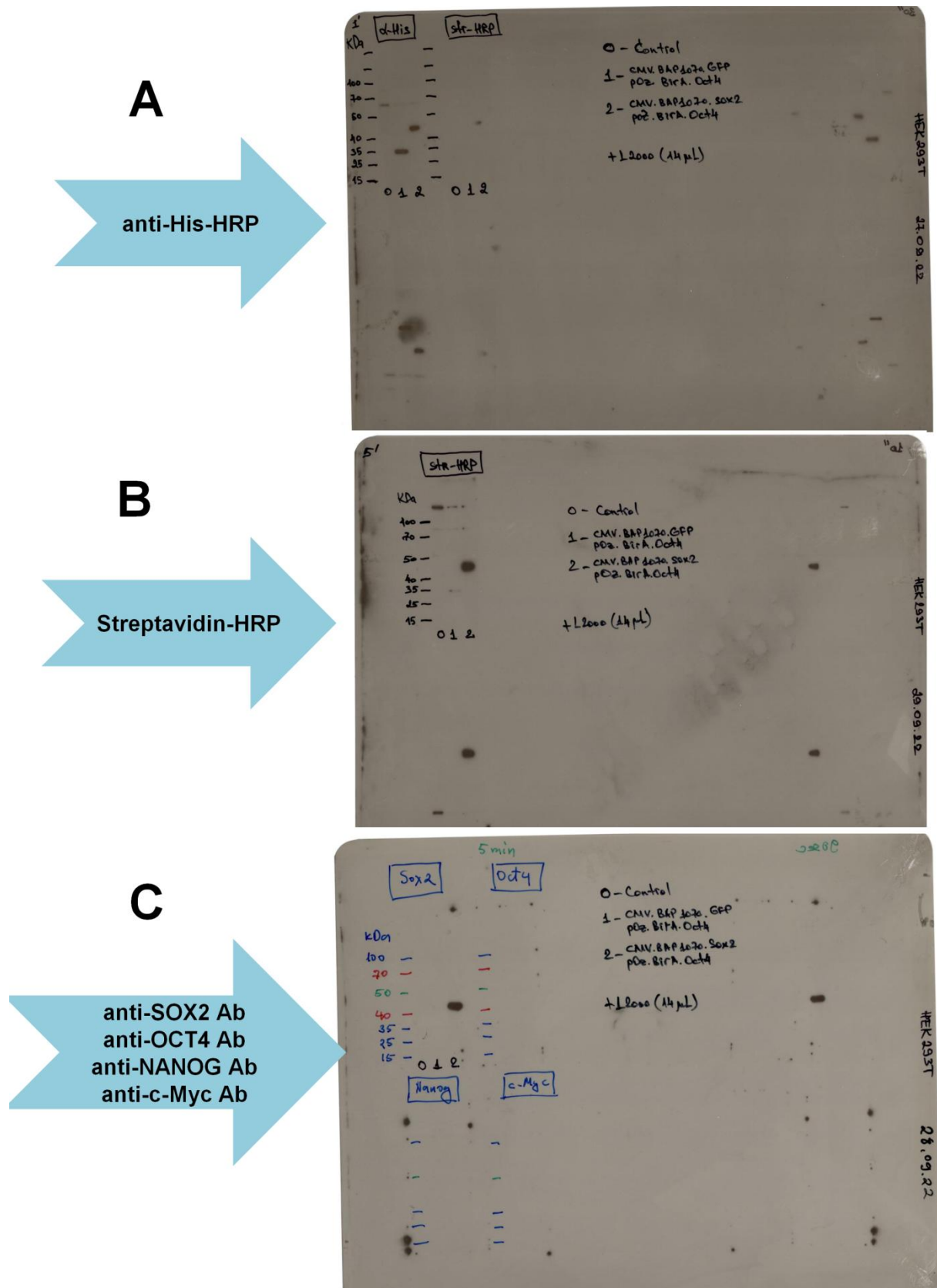
**Figure S1.** Comparison of expression of eGFP under strong CMV (A) and weak MoMuLV (B) promoters. HEK293T cells were transfected by corresponding plasmids (CMV-BirA-GFP or MoMuLV-BirA-GFP) using Lipofectamine.



**Figure S2.** Experiment with additional controls for testing specificity of biotinylation. Sample 0 – no plasmid were added to the cells. BirA-OCT4 and BAP-SOX2 were expressed separately in samples 1 and 2 correspondingly. Biotin labeling time was 9h. The positions of the BAP-fusions and nonspecific signals (NS) are indicated as asterisks. No biotinylation bands except nonspecific bindings were observed in lines 0-2. Biotinylation level of BAP-SOX2 in sample 4 is much higher than BAP-GFP in sample 3. No biotinylation was observed in case of separate BAP-SOX2 or BirA-OCT4 expressions (lanes 1 and 2 on streptavidin blot).

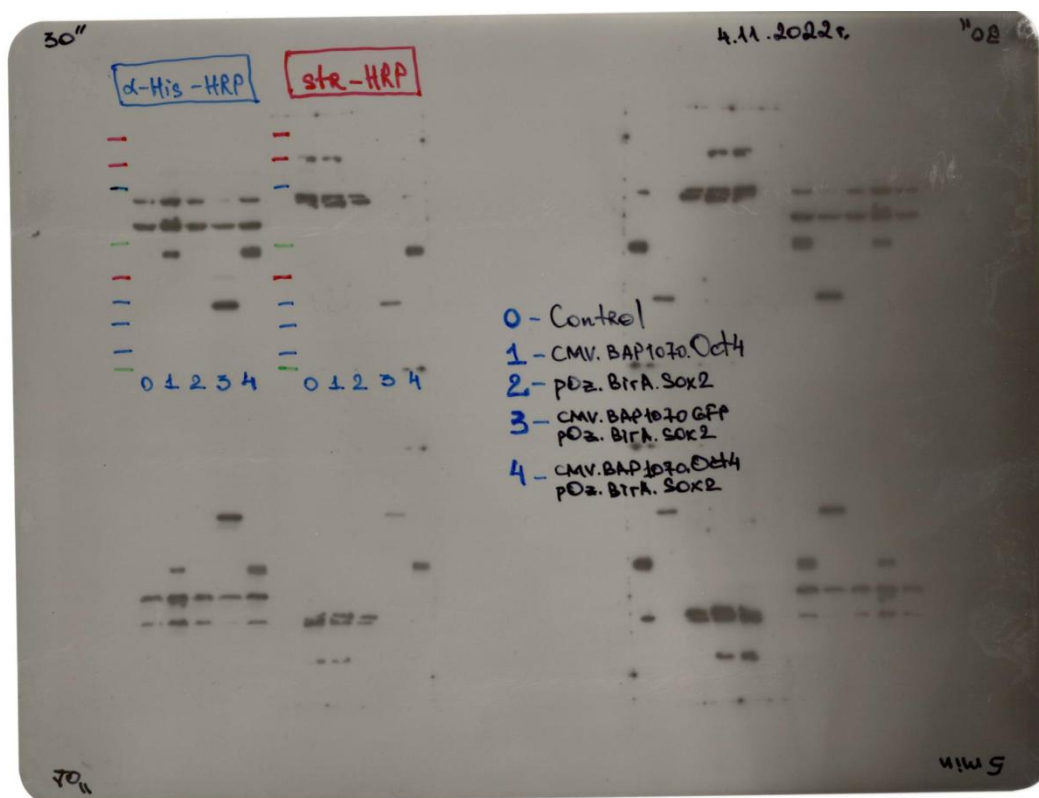


**Figure S3.** Uncropped blot from [Figure S2](#). Testing the specificity of biotinylation for BAP/BirA pair in HEK293T cells. Time of film exposure: 10 sec, 30, sec, 90 sec and 5 min.

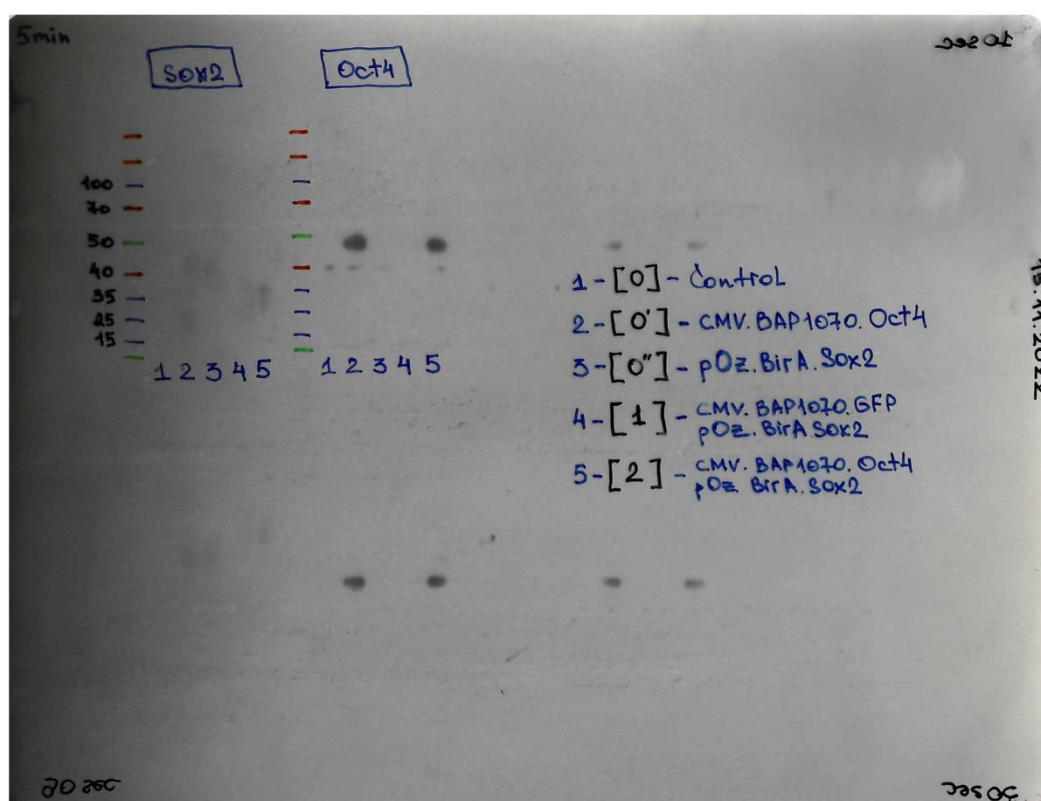


**Figure S4.** Uncropped blot from [Figure 1B](#) of manuscript. Sample 0 – nontransfected cells, sample 1 - BAP-GFP+BirA-OCT4, sample 2 - BAP-SOX2+BirA-OCT4. (A) anti-His antibody. (B) Streptavidin-HRP conjugate. (C) Checking the level of expression of endogenous SOX2, OCT4, NANOG and c-Myc proteins in cells.

A



B



**Figure S5.** Uncropped blot from [Figure 1C](#) of manuscript (Reciprocal experiment). Sample 0 – nontransfected cells, sample 1 - BAP-OCT4 alone, sample 2 - BirA-SOX2 alone, sample 3 - BAP-GFP+BirA-SOX2, sample 4 – BAP-OCT4 +BirA-SOX2. (A) anti-His antibody and Streptavidin-HRP conjugate. (B) Checking the level of expression of endogenous SOX2 and OCT4 proteins in cells.

## Real time quantitative RT PCR

**Table S1.** List of Gene Primers (the Forward and Reverse Sequences and TaqMan probes), Annealing Conditions, and Region of Amplification for the RT PCR.

No	Gene	Sequence 5'-3'	Annealing temperature °C	Primer concentration $\mu$ M	NCBI accession ID
1	ACTB	TCACCATTGGCAATGAG CCACGTCACACTTCATG ROX-ACTCTTCCAGCCTTCCTTCC-BHQ2	58	0.5	NM_001101
2	SOX2	CTCCGGGACATGATCAGC CTGGGACATGTGAAGTCTGC FAM-TTCCGGCACCTCGGCG-BHQ1	64	0.5	NM_003106
3	OCT4	GGGTTCTATTTGGAAGG TCCTGAAGATTTTCATGTTG FAM-ACGACCATCTGCCGCT-BHQ1	58	0.5	NM_001173531

- a. The  $\Delta C_T$  value is determined by subtracting the average OCT4  $C_T$  value from the average Housekeeping (Actin)  $C_T$  value.

$$\Delta C_T = C_{T,target} - C_{T,housekeeping}$$

The standard deviation of the difference is calculated from the standard deviations of the target and housekeeping values.  $s = \sqrt{s_{target}^2 + s_{housekeeping}^2}$

- b. The calculation of  $\Delta\Delta C_T$  involves subtraction by the  $\Delta C_T$  calibrator value (control).

$\Delta\Delta C_T = \Delta C_{T,sample} - \Delta C_{T,control}$  This is subtraction of an arbitrary constant, so the standard deviation of  $\Delta\Delta C_T$  is the same as the standard deviation of the  $\Delta C_T$  value.

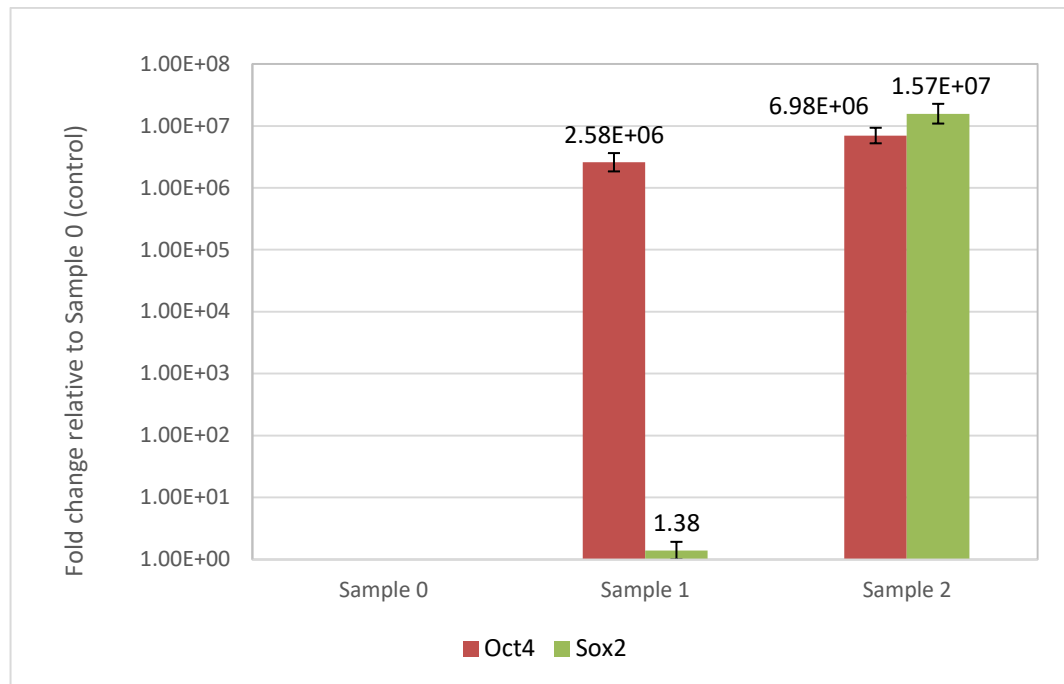
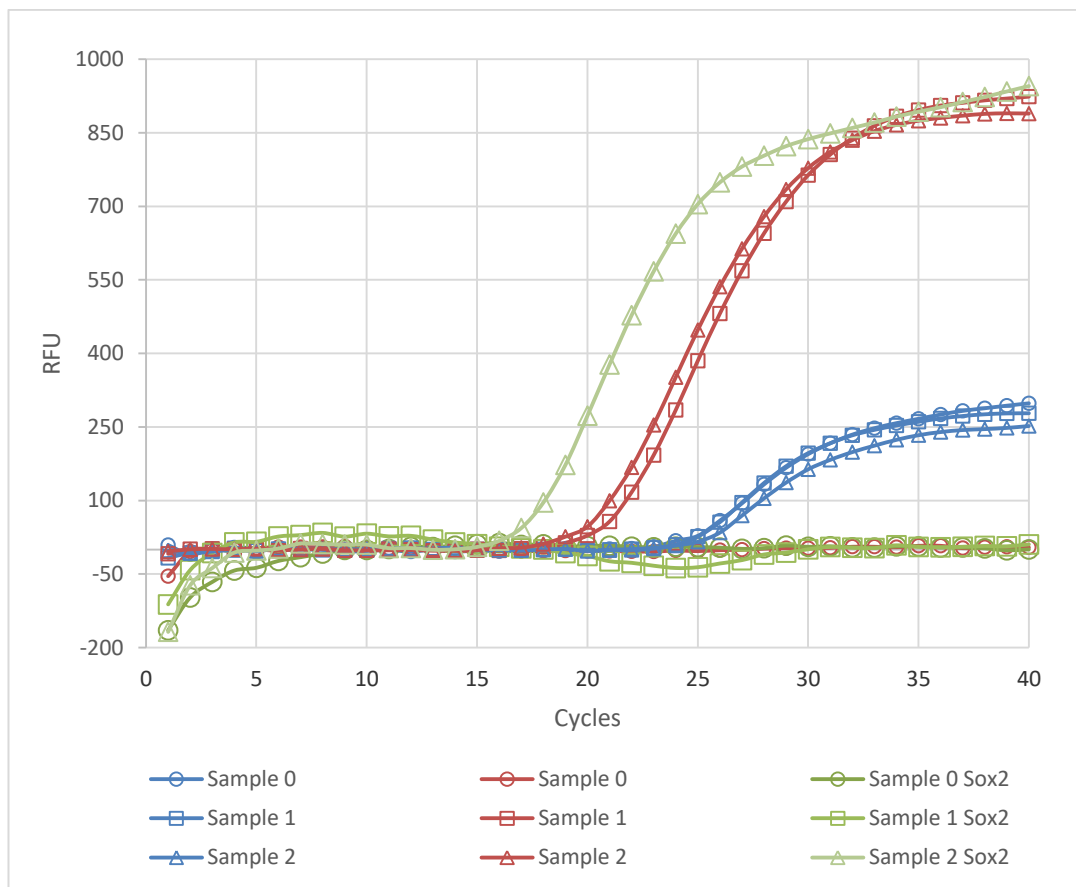
- c. The range given for the expression of target gene relative to Sample 0 is determined by evaluating the expression:  $2^{\Delta\Delta C_T}$  with  $\Delta\Delta C_T + s$  and  $\Delta\Delta C_T - s$ , where  $s$  = the standard deviation of the  $\Delta\Delta C_T$  value.

**Table S2.** Calculation of OCT4 expression in samples 0-2.

Sample	Housekeeping (Actin) Average C <sub>t</sub>	OCT4 Average C <sub>T</sub>	ΔC <sub>T</sub>	ΔΔC <sub>T</sub>	OCT4 Rel. to Sample 0
Sample 0	23,75 ± 0,04	40,00 ± 0,00	16,25 ± 0,04	0,00 ± 0,04	1,00 (0,97 – 1,03)
Sample 1	24,22 ± 0,47	19,17 ± 0,14	-5,05 ± 0,49	-21,30 ± 0,49	2,58×10 <sup>6</sup> (1,84×10 <sup>6</sup> – 3,63×10 <sup>6</sup> )
Sample 2	24,79 ± 0,36	18,31 ± 0,22	-6,48 ± 0,42	-22,73 ± 0,42	6,98×10 <sup>6</sup> (5,23×10 <sup>6</sup> – 9,31×10 <sup>6</sup> )

**Table S3.** Calculation of SOX2 expression in samples 0-2.

Sample	Housekeeping (Actin) Average C <sub>t</sub>	SOX2 Average C <sub>T</sub>	ΔC <sub>T</sub>	ΔΔC <sub>T</sub>	SOX2 Rel. to Sample 0
Sample 0	23,75 ± 0,04	40,00 ± 0,00	16,25 ± 0,04	0,00 ± 0,04	1,00 (0,97 – 1,03)
Sample 1	24,22 ± 0,47	40,00 ± 0,00	15,78 ± 0,47	-0,47 ± 0,47	1,38 (1,00 – 1,92)
Sample 2	24,79 ± 0,36	17,14 ± 0,39	-7,65 ± 0,53	-23,91 ± 0,53	1,57×10 <sup>7</sup> (1,09×10 <sup>7</sup> – 2,27×10 <sup>7</sup> )

**A****B**

**Figure S6.** Bar chart diagram and overlay of amplification plots for samples 0-2. Sample 0 (control without plasmids), Sample 1 (BAP-GFP + BirA-OCT4), Sample 2 (BAP-SOX2 + BirA-OCT4): (A) Relative normalized expression of target genes; (B) real-time PCR amplification curves



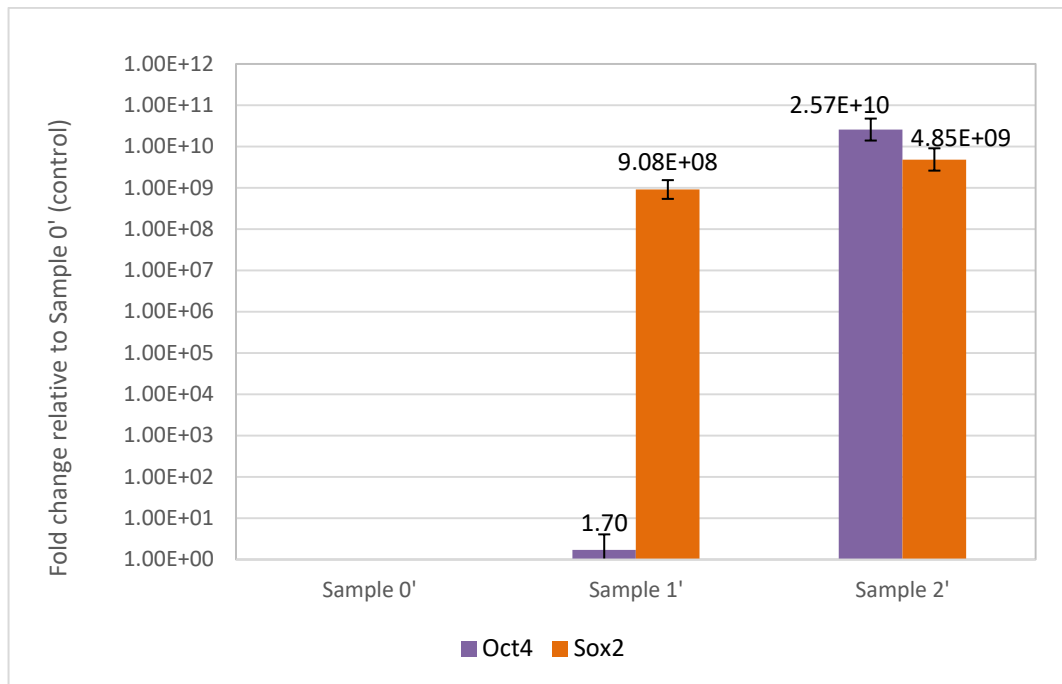
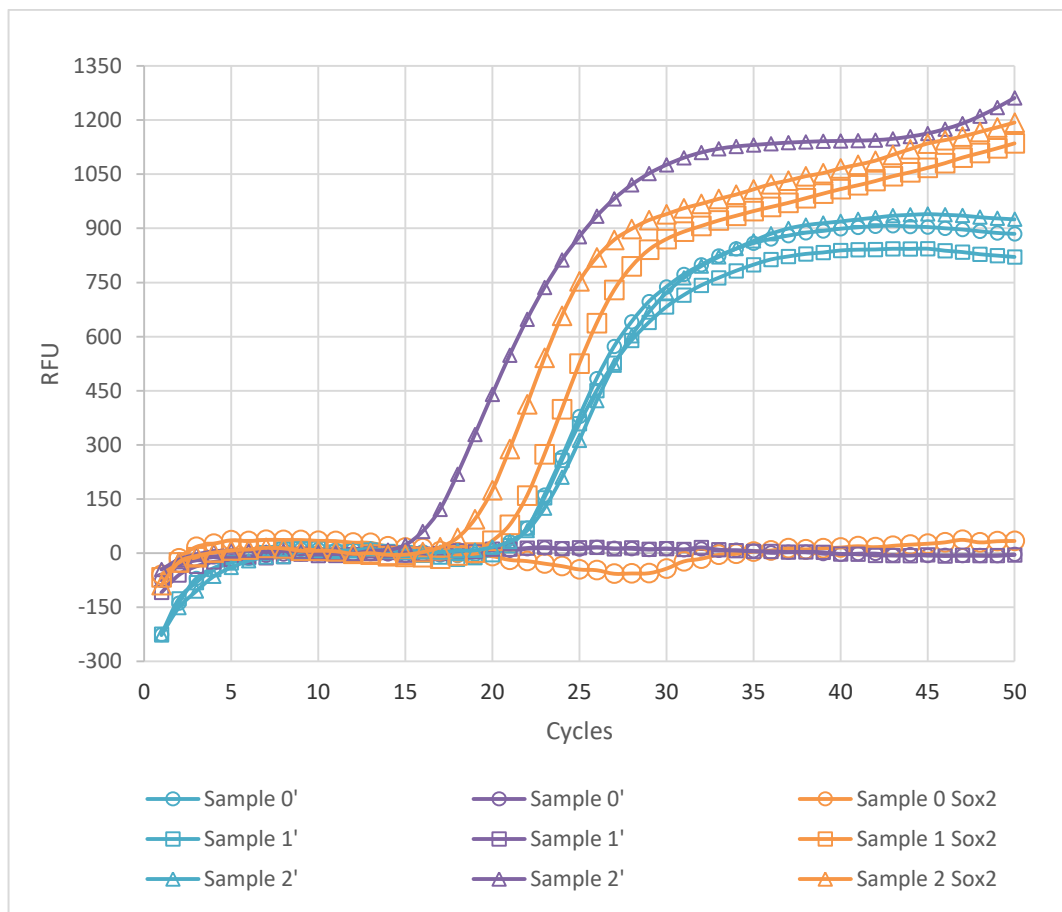
**Table S4.** Calculation of OCT4 expression in samples 0'-2'.

Sample	Housekeeping (Actin) Average C <sub>t</sub>	OCT4 Average C <sub>T</sub>	ΔC <sub>T</sub>	ΔΔC <sub>T</sub>	OCT4 Rel. to Sample 0'
Sample 0'	20,28 ± 0,33	40,00 ± 0,00	29,72 ± 0,33	0,00 ± 0,33	1,00 (0,79 – 1,26)
Sample 1'	21,05 ± 1,25	40,00 ± 0,00	28,95 ± 1,25	-0,77 ± 1,25	1,70 (0,72 – 4,03)
Sample 2'	20,75 ± 0,87	15,88 ± 0,17	-4,87 ± 0,88	-34,58 ± 0,88	2,57×10 <sup>10</sup> (1,39×10 <sup>10</sup> – 4,74×10 <sup>10</sup> )

**Table S5.** Calculation of SOX2 expression in samples 0'-2'.

Sample	Housekeeping (Actin) Average C <sub>t</sub>	SOX2 Average C <sub>T</sub>	ΔC <sub>T</sub>	ΔΔC <sub>T</sub>	SOX2 Rel. to Sample 0
Sample 0'	20,28 ± 0,33	40,00 ± 0,00	29,72 ± 0,33	0,00 ± 0,33	1,00 (0,79 – 1,26)
Sample 1'	21,05 ± 1,25	20,51 ± 0,53	-0,04 ± 0,75	-29,76 ± 0,75	9,08×10 <sup>8</sup> (5,38×10 <sup>8</sup> – 1,53×10 <sup>9</sup> )
Sample 2'	20,75 ± 0,87	18,29 ± 0,24	-2,46 ± 0,90	-32,18 ± 0,90	4,85×10 <sup>9</sup> (2,60×10 <sup>9</sup> – 9,04×10 <sup>9</sup> )



**A****B**

**Figure S7.** Bar chart diagram and overlay of amplification plots for samples 0'-2'. Sample 0' (control without plasmids), Sample 1' (BAP-GFP + BirA-SOX2), Sample 2 (BAP-OCT4 + BirA-SOX2): (A) Relative normalized expression of target genes; (B) real-time PCR amplification curves.