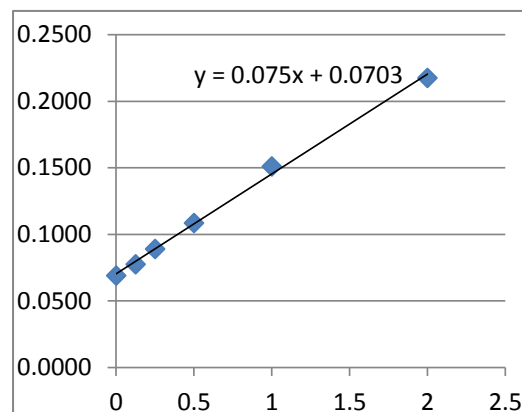


kvantifikace proteinu
mg/ml

0	0.0710	0.0670	prumer	0.0690
0.125	0.0720	0.0830		0.0775
0.25	0.0860	0.0920		0.0890
0.5	0.1120	0.1050		0.1085
1	0.1520	0.1500		0.1510
2	0.2110	0.2240		0.2175

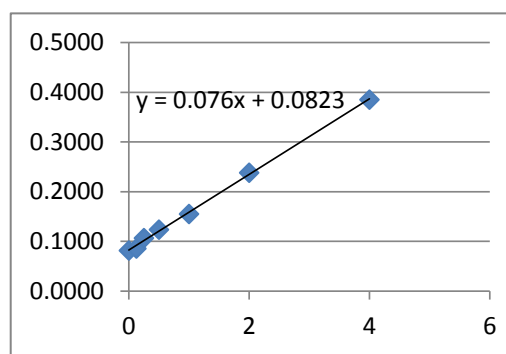


C			prumer mg/ml	prepočet	bfb+merc	
T-	0.1180	0.1320	0.1250	0.729333	65.87	14.59
T-W+	0.1370	0.1250	0.1310	0.809333	81.87	16.19
T-R+	0.1150	0.1140	0.1145	0.589333	37.87	11.79
T-W+R+	0.1150	0.1160	0.1155	0.602667	40.53	12.05

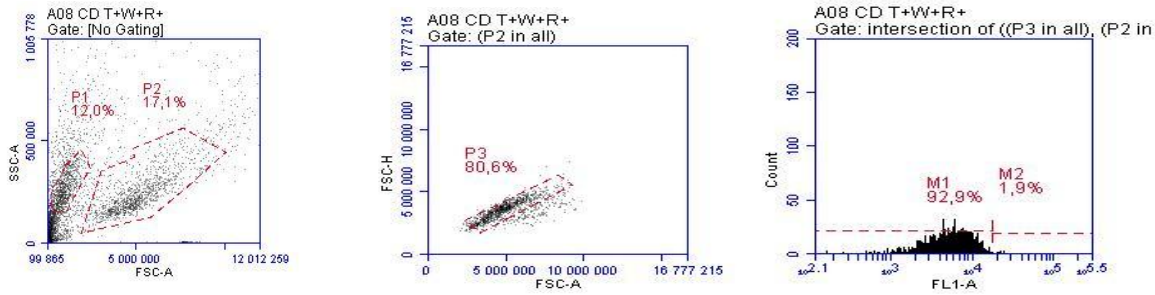
D						
T+	0.1310	0.1380	0.1345	0.686842	57.37	13.74
T+W+	0.1190	0.1190	0.1190	0.482895	16.58	9.66
T+R+	0.1240	0.1110	0.1175	0.463158	12.63	9.26
T+W+R+	0.1070	0.1190	0.1130	0.403947	0.79	8.08

ug/ml

0	0.0760	0.0870	0.0815
0.125	0.0840	0.0870	0.0855
0.25	0.1060	0.1070	0.1065
0.5	0.1200	0.1270	0.1235
1	0.1640	0.1460	0.1550
2	0.2460	0.2300	0.2380
4	0.3700	0.4000	0.3850



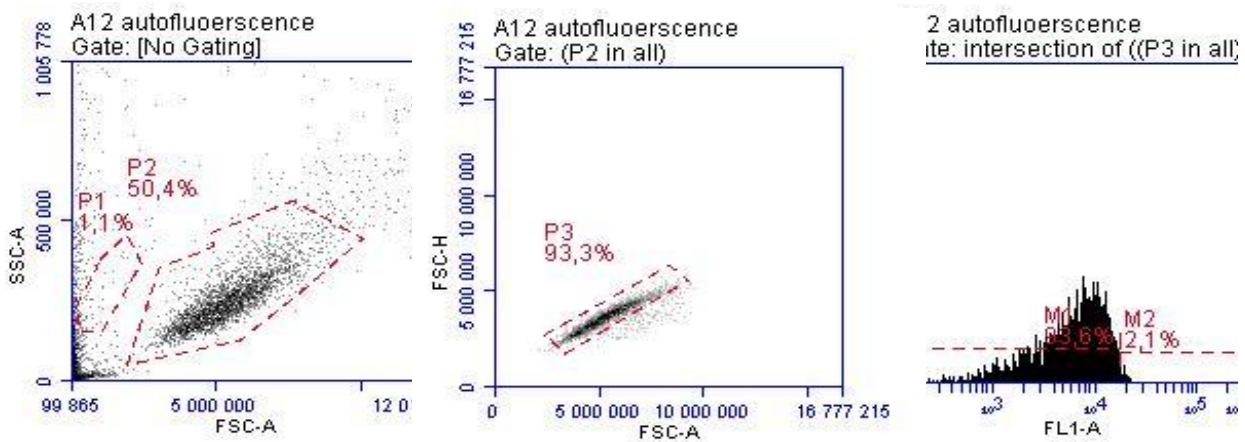
CD hodnoceni ucinnosti transfekce - GFP protein



CD	Plot 1 (FSC-A/SSC-A)		Plot 2 (FL1-A)				
	P1 Count	P2 Count	M1 Count	M2 Count	% of This Plot		
A01 CD T-	361		3,815	3,185	98.67%	4	0.12%
A02 CD T+	957		6,645	5,411	95.67%	31	0.55%
A03 CD T-W+	449		3,237	2,605	96.98%	8	0.30%
A04 CD T+ W+	357		1,216	955	97.35%	6	0.61%
A05 CD T-R+	373		2,590	2,040	96.36%	16	0.76%
A06 CD T+R+	282		922	722	95.25%	10	1.32%
A07 CD T-W+R+	228		1,524	1,247	96.74%	11	0.85%
A08 CD T+W+R+	826		1,178	882	92.94%	18	1.90%

prumerna ucinnost 0.80%

AUTOFLUORESCENCE



koncentrace RNA
C,D

	konc. RNA 800ng/ul		doredit na H2O
D T+	68.85	11.6194626	
D T+R+	98.8	8.097165992	3.403
D T+W+	102.25	7.82396088	3.676
D T+W+R+	94.05	8.506113769	2.994
[RNA] T- C	143.5	5.574912892	5.925
[RNA] T- W+C	118.45	6.753904601	4.746
[RNA] T- R+C	84.9	9.422850412	2.077
[RNA] T- W+R+C	94.8	8.438818565	3.061

Top flash

	FF	R	pomer I x100		
T-	297	37460	0.008	0.792846	
T-W+	211	12504	0.017	1.68746	
T-R+	257	14282	0.018	1.799468	
T-W+R+	3268	22274	0.147	14.67181	
T+	294	44996	0.007	0.653391	
T+W+	587	98452	0.006	0.59623	
T+R+	537	39622	0.014	1.355308	
T+W+R+	4893	72630	0.067	6.736886	

transfekce a následný top flash presne odpovídají očekávání!!!

GAPDH

Pos	Name	Cp	prumer	
A1	T-	24.92	24.51	
A2	T-	24.09		brat nebudeme,
B1	T-W+	24.06	24.02	nebyl tam dost specificky vzorek
B2	T-W+	23.97		
C1	T-R+	23.83	23.86	
C2	T-R+	23.89		
D1	T-W+R+	23.14	23.17	
D2	T-W+R+	23.2		
E1	T+	23.12	23.13	
E2	T+	23.14		
F1	T+R+	37.77	32.01	
F2	T+R+	26.24		
G1	T+W+	23.4	23.65	
G2	T+W+	23.9		
H1	T+R+W+	24.79	25.42	
H2	T+R+W+	26.04		

HPRT

Pos	Name	Cp		
A3	T-		24.98	24.87
A4	T-		24.76	
B3	T-W+		25.3	25.08 do pomeru bereme GAPDH
B4	T-W+		24.85	
C3	T-R+		24.62	24.53
C4	T-R+		24.44	
D3	T-W+R+		24.3	24.3
D4	T-W+R+		24.3	
E3	T+		23.79	23.83
E4	T+		23.86	
F3	T+R+		25.53	25.5
F4	T+R+		25.47	
G3	T+W+		24.09	24.14
G4	T+W+		24.19	
H3	T+R+W+		24.24	24.17
H4	T+R+W+		24.1	

C-MYC

cely divny,dvojband

Pos	Name	Cp	prumer HPRT	ΔCp	$2^{-\Delta Cp}$	x1000		
A5	T-		35.16	34.99	24.87	10.115	0.000902	0.090174
A6	T-		34.81					
B5	T-W+		34.78	35.99	25.075	10.915	0.000518	0.051791
B6	T-W+		37.2					
C5	T-R+		32.81	32.99	24.53	8.455	0.00285	0.284965
C6	T-R+		33.16					
D5	T-W+R+		34.63	34.47	24.3	10.165	0.000871	0.087102
D6	T-W+R+		34.3					
E5	T+		31.58	31.25	23.825	7.425	0.005819	0.581905
E6	T+		30.92					
F5	T+R+		31.29	31.12	25.5	5.615	0.020404	2.040406
F6	T+R+		30.94					
G5	T+W+		31.05	31.02	24.14	6.88	0.00849	0.849012
G6	T+W+		30.99					
H5	T+R+W+		31.05	31.05	24.17	6.88	0.00849	0.849012

AXIN2

Pos	Name	Cp						
A7	T-		25.61	25.57	24.87	0.695	0.617709	61.77093
A8	T-		25.52					
B7	T-W+		25.46	25.58	25.075	0.505	0.70466	70.46604
B8	T-W+		25.7					
C7	T-R+		25.1	25.11	24.53	0.58	0.668964	66.89638
C8	T-R+		25.12					
D7	T-W+R+		24.79	24.84	24.3	0.535	0.690159	69.01587
D8	T-W+R+		24.88					
E7	T+		24.58	24.54	23.825	0.71	0.61132	61.13201
E8	T+		24.49					
F7	T+R+		25.86	25.84	25.5	0.335	0.792784	79.27841
F8	T+R+		25.81					
G7	T+W+		24.66	24.68	24.14	0.535	0.690159	69.01587
G8	T+W+		24.69					

H7	T+R+W+	24.7	24.74	24.17	0.565	0.675955	67.59554
H8	T+R+W+	24.77					

CYCLIN D1

Pos	Name	Cp					
A9	T-		26.43	26.36	24.87	1.485	0.357249 35.72485
A10	T-		26.28				
B9	T-W+		26.59	26.57	25.075	1.49	0.356013 35.60125
B10	T-W+		26.54				
C9	T-R+		26.5	26.46	24.53	1.93	0.262429 26.24292
C10	T-R+		26.42				
D9	T-W+R+		26.45	26.45	24.3	2.145	0.226095 22.60948
D10	T-W+R+		26.44				
E9	T+		26.31	26.24	23.825	2.415	0.187505 18.75049
E10	T+		26.17				
F9	T+R+		26.47	26.23	25.5	0.73	0.602904 60.29039
F10	T+R+		25.99				
G9	T+W+		26.5	26.59	24.14	2.45	0.183011 18.30107
G10	T+W+		26.68				
H9	T+R+W+		26.66	26.62	24.17	2.445	0.183646 18.36461
H10	T+R+W+		26.57				

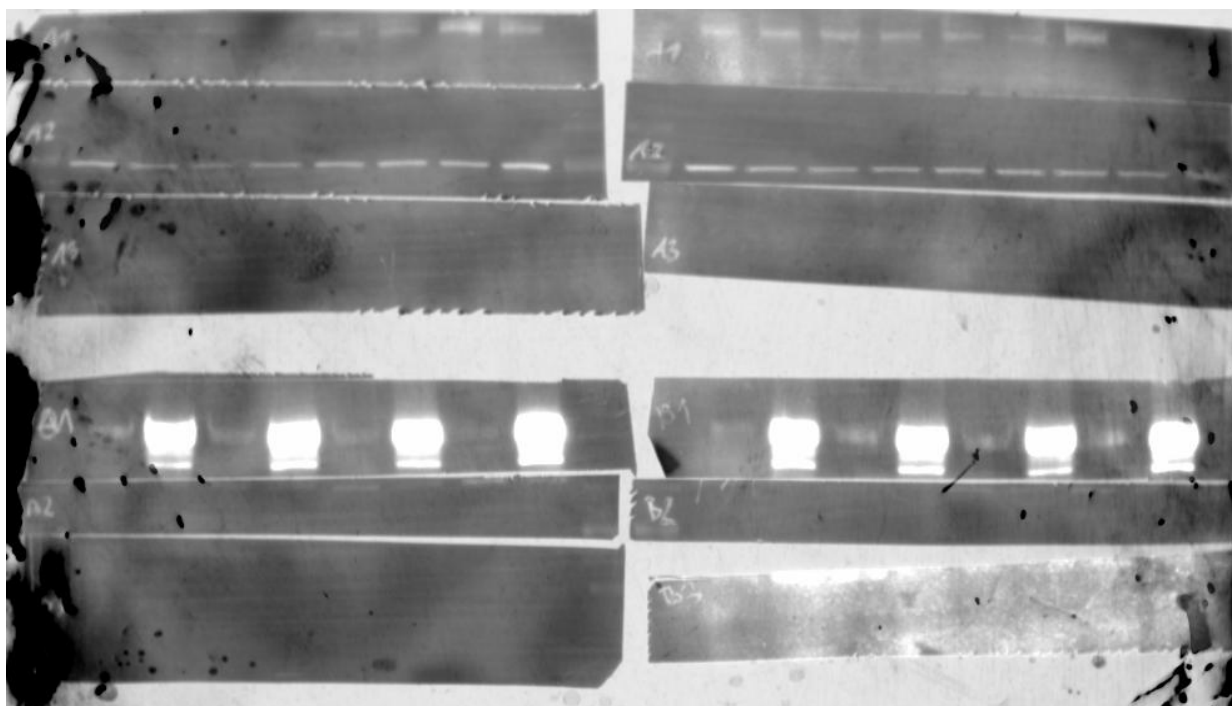
RNF43

Pos	Name	Cp					
A11	T-		25.37	25.34	24.87	0.465	0.724471 72.44711
A12	T-		25.3				
B11	T-W+		24.97	25.06	25.075	-0.015	1.010451 101.0451
B12	T-W+		25.15				
C11	T-R+		24.91	24.92	24.53	0.39	0.76313 76.31296
C12	T-R+		24.93				
D11	T-W+R+		24.26	24.27	24.3	-0.03	1.021012 102.1012
D12	T-W+R+		24.28				
E11	T+		24.02	24.02	23.825	0.195	0.873573 87.35729
E12	T+		24.02				
F11	T+R+				25.5	-25.5	47453133 4.75E+09
F12	T+R+						
G11	T+W+		24.31	24.29	24.14	0.15	0.90125 90.12505
G12	T+W+		24.27				
H11	T+R+W+		24.41	24.38	24.17	0.21	0.864537 86.45372
H12	T+R+W+		24.35				

	cMyc	Axin2	cyclinD	RNF43
T-	0.09	61.77	35.72	72.45
T-W+	0.05	70.47	35.60	101.05
T-R+	0.28	66.90	26.24	76.31
T-W+R+	0.09	69.02	22.61	102.10
T+	0.58	61.13	18.75	87.36
T+R+	2.04	79.28	60.29	
T+W+	0.85	69.02	18.30	90.13
T+R+W+	0.84	67.60	18.36	86.45
divnz	nevychazi	nevychazi	nevyslo, hodnoty T+	jsou mal0

Western blotting

pS1490-LRP6 150-250 kDa (cs-2568, 1:1 000) R	kDa	RNF43- HAtag 130 kDa(Biolegend 901514)
-	250	-
DVL2 70-100 kDa (sc-8026, 1:1 000), M	130	Aktivní β catenin (ABC), 92 kDa (Millipore M)
-	100	-
-	72	Tubulin α 55 kDa (CS5335, 1:1000) R
Aktin β 42 kDa (sc-1615, 1:1 000) G	55	
	35	



hezky vysel RNF43, pLRP6, trosku tušime aktivní beta catenin. Disheveled by mel mit dva bandy, aktin a tubulin jsou asi spatna protilatka...

λ_1 (P2 in



5.5

1, 1:2000) M

35665, 1:1000)

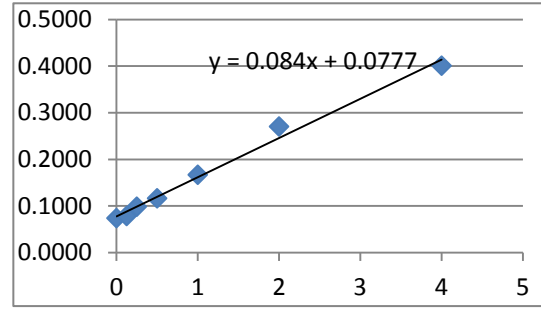


kvantifikace proteinu
mg/ml

0	0.0710	0.0770
0.125	0.0780	0.0790
0.25	0.0980	0.0990
0.5	0.1150	0.1180
1	0.1450	0.1890
2	0.2640	0.2770
4	0.4010	

prumer

0.0740
0.0785
0.0985
0.1165
0.1670
0.2705
0.4010



A

T-	0.1240	0.1150
T-W+	0.1510	0.1150
T-R+	0.1440	0.1300
T-W+R+	0.1090	0.1240

A

mg/ml	prepocet bfb na 0,5 mg/ml
0.1195	0.497619 0 8
0.1330	0.6583333 25.33333 10.53333
0.1370	0.7059524 32.95238 11.29524
0.1165	0.4619048 0 8
	32.3

B

T+	0.1110	0.1070
T+W+	0.0840	0.0840
T+R+	0.0790	0.0840
T+W+R+	0.0810	0.0770

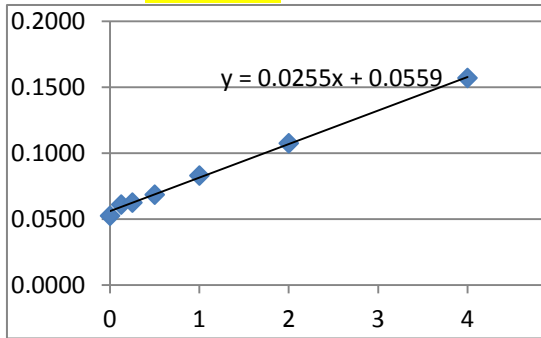
B

0.1090	2.0823529 253.1765 33.31765
0.0840	1.1019608 96.31373 17.63137
0.0815	1.0039216 80.62745 16.06275
0.0790	0.9058824 64.94118 14.49412

mg/ml

0	0.0520	0.0530
0.125	0.0600	0.0620
0.25	0.0630	0.0620
0.5	0.0670	0.0700
1	0.0830	0.0830
2	0.1070	0.1080
4	0.1520	0.1620

0.0525
0.0610
0.0625
0.0685
0.0830
0.1075
0.1570



kvantifikace RNA

Sample ID	ng/ul	A260	A280	260/280
T-	28.69	0.717	0.417	Jan-72
T-	24.99	0.625	0.315	Jan-98
T-	21.91	0.548	0.293	Jan-87
T+	78.81	1.970	0.966	2-Apr
T+	78.17	1.954	0.969	2-Feb
T- W+	62.21	1.555	0.761	2-Apr
T- W+	62.67	1.567	0.758	2-Jul
T+W+	22.55	0.564	0.314	Jan-80
T+W+	21.41	0.535	0.265	2-Feb
T-R+	176.09	4.402	2.207	2.00
T-R+	199.04	4.976	2.492	2.00
T+R+	Aug-13	0.203	0.082	Feb-47
T+R+	Sep-28	0.232	0.123	Jan-88
T+R+	Sep-45	0.236	0.114	2-Aug
T-R+W+	207.60	5.190	2.605	Jan-99
T-R+W+	203.08	5.077	2.592	Jan-96
T+R+W+	95.21	2.380	1.182	2-Jan
T+R+W+	100.98	2.524	1.284	Jan-97
T-R+ 30x	49.83	1.246	0.652	Jan-91
T-R+ 30x	49.09	1.227	0.649	Jan-89
T-R+W+ 30x	52.21	1.305	0.697	Jan-87
T-R+W+ 30x	51.36	1.284	0.681	Jan-89

kvantifikace RNA

	prumer		ng/ul do realu		1000 ng	doredit na 11,5
T-	28.69	24.99	26.84	268.4000	3.73	7.77
T+	78.81	78.17	78.49	784.9000	1.27	10.23
T-W+	62.21	62.67	62.44	624.4000	1.60	9.90
T+W+	22.55	21.41	21.98	219.8000	4.55	6.95
T-R+	49.83	49.09	49.46	1483.8000	0.67	10.83
T+R+	9.28	9.45	9.37	93.6500	10.68	0.82
T-W+R+	52.21	51.36	51.79	1553.5500	0.64	10.86
T+W+R+	95.21	100.98	98.10	980.9500	1.02	10.48

Topflash

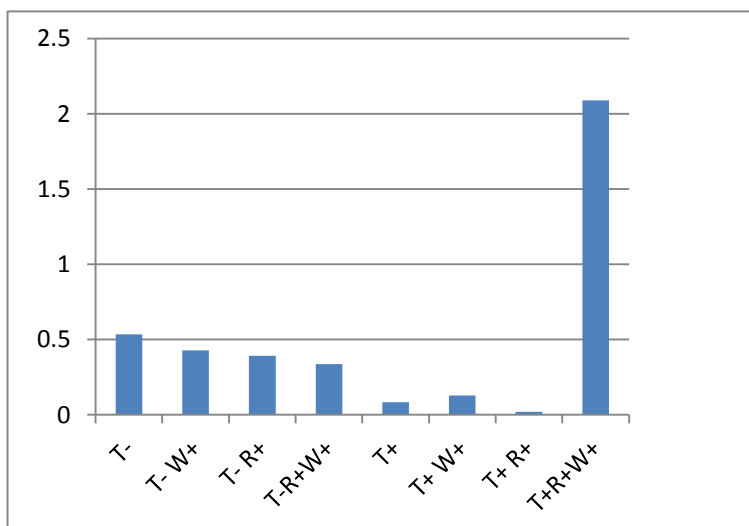
B

	T+	T+ W+	T+ R+	T+R+W+
TF	143	141	151	5403
Ren	1722	1093	7356	2587
result	0.083043	0.1290027	0.020527461	2.0885195

A

	T-	T- W+	T- R+	T-R+W+
TF	182	150	130	113
Ren	341	351	331	335
result	0.533724	0.4273504	0.392749245	0.3373134

T-	T- W+	T- R+	T-R+W+	T+	T+ W+	T+ R+	T+R+W+
0.53372434	0.42735	0.3927492	0.337313433	0.083043	0.129003	0.020527	2.08852

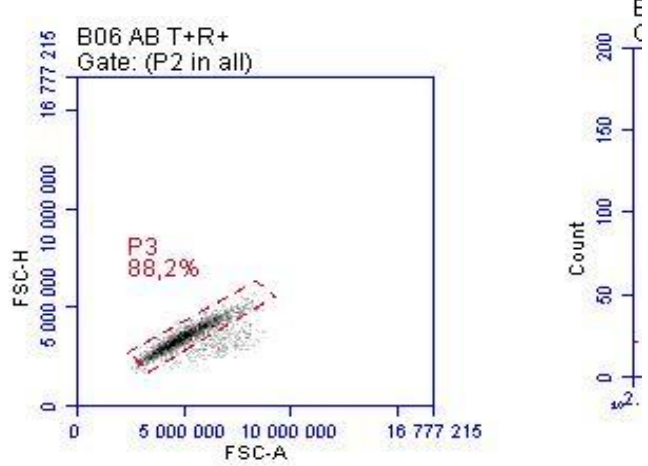
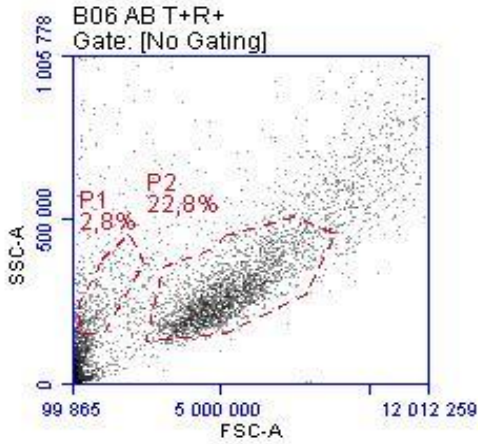


ucinnost transfekce

Plot 1 (FSC-A/SSC-A) Plot 2 (FL1-A)
P1 P2 M1

M2

	Count	Count	Count	% of This PI Count	% of This Plot
B01 AB T-	238	3,508	3,209	98.16%	45 1.38%
B02 AB T+	241	4,121	3,655	94.35%	170 4.39%
B03 AB T-W+	189	3,838	3,525	97.38%	75 2.07%
B04 AB T+ W+	407	4,503	3,981	92.80%	190 4.43%
B05 AB T-R+	147	2,365	2,108	97.05%	33 1.52%
B06 AB T+R+	260	2,126	1,689	90.08%	114 6.08%
B07 AB T-W+R	118	2,491	2,098	95.41%	50 2.27%
B08 AB T+W+R	127	3,804	3,135	91.67%	144 4.21%



vyhodnoceni qRT PCR
GAPDH

Pos	Name	Cp	
A1	T+	13.86	14.52
A2	T+	15.18	
B1	T+ W+	24.6	24.61
B2	T+ W+	24.62	
C1	T+ R+	25.31	25.255
C2	T+ R+	25.2	
D1	T+ W+ R+	15.58	15.455
D2	T+ W+ R+	15.33	
E1	T-	19.12	19.065
E2	T-	19.01	
F1	T- w+	17.11	17.205
F2	T- w+	17.3	
G1	T- R+	15.94	15.595
G2	T- R+	15.25	
H1	T- W+ R+	14.81	14.765
H2	T- W+ R+	14.72	

HPRT

Pos	Name	Cp	
A3	T+	19.45	19.46
A4	T+	19.47	
B3	T+ W+	25.77	25.735
B4	T+ W+	25.7	
C3	T+ R+	26.75	26.83
C4	T+ R+	26.91	
D3	T+ W+ R+	19.63	19.53
D4	T+ W+ R+	19.43	

E3	T-	23.17	26.245
E4	T-	29.32	
F3	T- w+	20.52	20.575
F4	T- w+	20.63	
G3	T- R+	19.66	19.6
G4	T- R+	19.54	
H3	T- W+ R+	18.57	18.66
H4	T- W+ R+	18.75	

C MYC

Pos	Name	Cp	prumer Cp	HPRT	ΔCp	$2^{-\Delta Cp}$	x1000
A5	T+	27.77	28.175	19.46	8.715	0.00238	2.379708
A6	T+	28.58					
B5	T+ W+			25.735			
B6	T+ W+	35					
C5	T+ R+	35		26.83			
C6	T+ R+	35					
D5	T+ W+ R+	27.57	27.525	19.53	7.995	0.00392	3.919812
D6	T+ W+ R+	27.48					
E5	T-	35		26.245			
E6	T-	35					
F5	T- w+	29.21	29.4	20.575	8.825	0.002205	2.205009
F6	T- w+	29.59					
G5	T- R+	27.33	27.38	19.6	7.78	0.00455	4.549741
G6	T- R+	27.43					
H5	T- W+ R+	26.9	26.965	18.66	8.305	0.003162	3.161884
H6	T- W+ R+	27.03					

AXIN 2

Pos	Name	Cp					
A7	T+	27.62	27.455	19.46	7.995	0.00392	3.919812
A8	T+	27.29					
B7	T+ W+	26.88	26.935	25.735	1.2	0.435275	435.2753
B8	T+ W+	26.99					
C7	T+ R+	27.64	27.365	26.83	0.535	0.690159	690.1587
C8	T+ R+	27.09					
D7	T+ W+ R+	27.24	27.285	19.53	7.755	0.004629	4.629269
D8	T+ W+ R+	27.33					
E7	T-	26.85	26.69	26.245	0.445	0.734584	734.5843
E8	T-	26.53					
F7	T- w+	27.92	27.99	20.575	7.415	0.00586	5.859527
F8	T- w+	28.06					
G7	T- R+	27.73	27.795	19.6	8.195	0.003412	3.412394
G8	T- R+	27.86					
H7	T- W+ R+	28.55	28.38	18.66	9.72	0.001186	1.185737
H8	T- W+ R+	28.21					

CYCLIN D1

Pos	Name	Cp					
A9	T+	24.64	24.64	19.46	5.18	0.027584	27.58447
A10	T+	24.64					
B9	T+ W+	27.24					
B10	T+ W+	26.98					
C9	T+ R+	27.3					
C10	T+ R+	27.76					
D9	T+ W+ R+	24.15	24.125	19.53	4.595	0.041378	41.37778

D10	T+ W+ R+	24.1						
E9	T-	27.07	27.04	26.245	0.795	0.576343	576.3432	
E10	T-	27.01						
F9	T- w+	25.23	25.24	20.575	4.665	0.039418	39.41804	
F10	T- w+	25.25						
G9	T- R+	23.71	23.845	19.6	4.245	0.052738	52.73849	
G10	T- R+	23.98						
H9	T- W+ R+	23.94	24.915	18.66	6.255	0.013094	13.09355	
H10	T- W+ R+	25.89						

RNF43

Pos	Name	Cp						
A11	T+	27.55	28.7	19.46	9.24	0.001654	1.653799	
A12	T+	29.85						
B11	T+ W+	26.52	26.61	25.735	0.875	0.545254	545.2539	
B12	T+ W+	26.7						
C11	T+ R+	26.63	26.865	26.83	0.035	0.976032	976.0318	
C12	T+ R+	27.1						
D11	T+ W+ R+	26.88	26.88	19.53	7.35	0.00613	6.129563	
D12	T+ W+ R+							
E11	T-	26.22	26.37	26.245	0.125	0.917004	917.004	
E12	T-	26.52						
F11	T- w+	27.53	27.545	20.575	6.97	0.007977	7.976657	
F12	T- w+	27.56						
G11	T- R+	27.55	27.49	19.6	7.89	0.004216	4.215735	
G12	T- R+	27.43						
H11	T- W+ R+	26.91	26.83	18.66	8.17	0.003472	3.472042	
H12	T- W+ R+	26.75						

	c Myc	Axin2	cyclin D1	RNF43
T+	2.38	3.92	27.58	1.65
T+W+		435.28		545.25
T+R+		690.16		976.03
T+W+R+	3.92	4.63	41.38	6.13
T-		734.58	576.34	917.00
T-W+	2.21	5.86	39.42	7.98
T-R+	4.55	3.41	52.74	4.22
T-W+R+	3.16	1.19	13.09	3.47

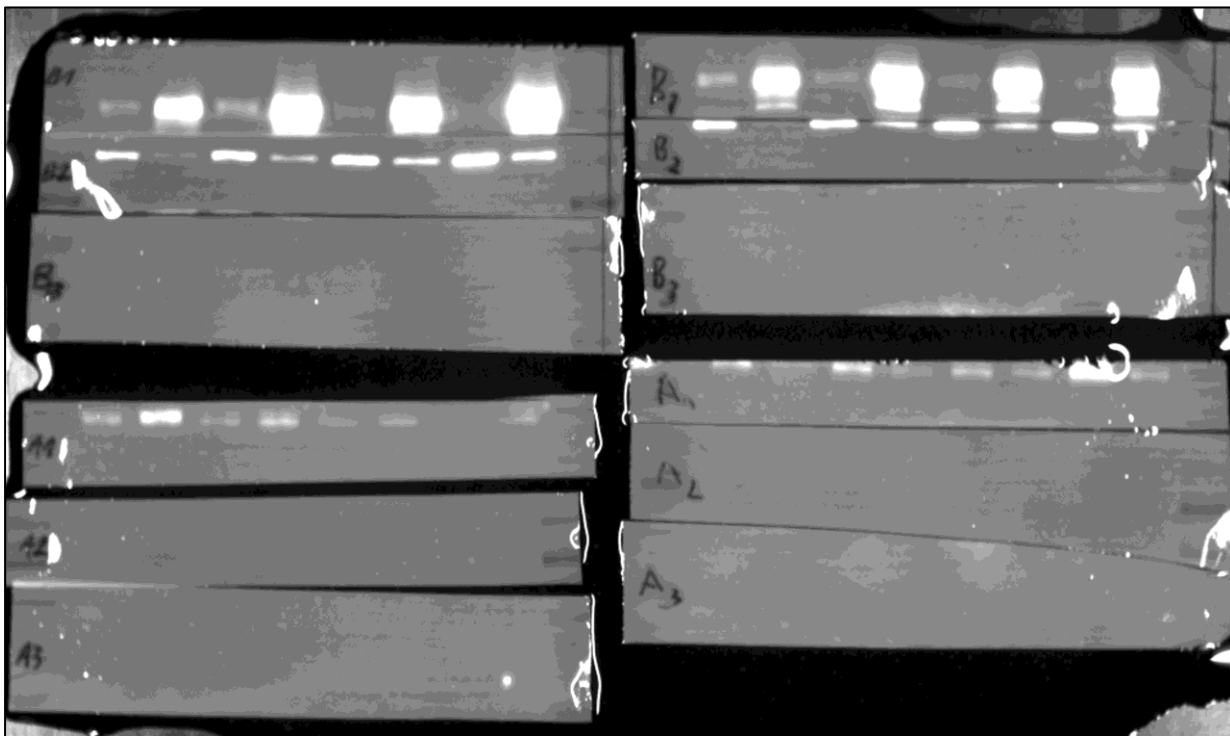
upravy dle RNF 43

	c Myc	Axin2	cyclin D1	RNF43
T+	2.379708	3.9198115	27.58446863	1.6537994
T+W+		435.27528		545.25387
T+R+		690.15868		976.03176
T+W+R+		734.58432	576.3431734	917.00404
T-	3.919812	4.6292686	41.37777845	6.1295633
T-W+	2.205009	5.8595273	39.41804403	7.9766572
T-R+	4.549741	3.4123941	52.73848726	4.2157353
T-W+R+	3.161884	1.1857372	13.09354898	3.4720417

bohuzel doslo asi k prehozeni vzorku (podle RNF43), ale celkove to vyslo podivne, i kdyz technicky je to oK

Western blotting

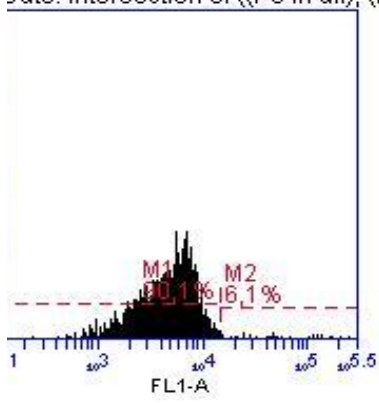
pS1490-LRP6 150-250 kDa (cs-2568, 1:1 000) R	kDa	RNF43- HAtag 130 kDa(Biolegend 90151)
-	250	-
DVL2 70-100 kDa (sc-8026, 1:1 000), M	130	Aktivní β catenin (ABC), 92 kDa (Millipore M)
-	100	-
-	72	Tubulin α 55 kDa (CS5335, 1:1000) R
Aktin β 42 kDa (sc-1615, 1:1 000) G	55	
	35	



hezky vysel pLRP6, RNF43 a aktivní bcatenin, to vše dle očekávání, ostatní byla asi nevhodná protilátka

306 AB T+R+

Gate: intersection of ((P3 in all), (P2 in



4, 1:2000) M

re5665, 1:1000)