

# **SUPPLEMENTAL MATERIAL**

**Table S1. Primer information.**

Gene		Sequence (5' -> 3')
COL1A1	Forward	CTCAAGATGTGCCACTCTGACT
	Reverse	GAGGGAGTTTACAGGAAGCAGAC
COL3A1	Forward	TGGCGGCTTTTCACCATATT
	Reverse	ACTCTCTATTTGTCCGTTAACAGACTTG
AURKB	Forward	CAGTGGGACACCCGACATC
	Reverse	GTACACGTTTCCAACTTGCC
CCND1	Forward	GATCGGGCATCACCTGAAAAA
	Reverse	TCGTCTGGTATCTTACCTACTCG
Mki67	Forward	ACGCCTGGTACTATCAAAGG
	Reverse	CAGACCCATTTACTTGTGTTGGA
Ccnd3	Forward	TACCCGCCATCCATGATCG
	Reverse	AGGCAGTCCACTTCAGTGC
Bnp	Forward	CCTCTTCTGGCCTTTTGG
	Reverse	TGTGTTGGACACCGCACTGT
Anp	Forward	GCTGCAGACTCCGGCTTCT
	Reverse	ATCACTTGAGAGGTGGTCCCA
Rrm2	Forward	GCCGAGCTGGAAAGTAAAGC
	Reverse	TCATGGTACTCGATGGGAAAGA
Pclaf	Forward	ATGGTGGGACTAAAGCAGAC
	Reverse	CCTCGATGAAACTGATGTGCAAT
Mcm6	Forward	GAGGAACTGATTCGTCCTGAGA
	Reverse	CAAGGCCCGACACAGGTAAG
LOC100359539	Forward	GGGGCGGTGATCTCTACAC
	Reverse	GGGAGTCGGACATTATTGACCA
TCF19	Forward	GGGGCGGTGATCTCTACAC
	Reverse	GGGAGTCGGACATTATTGACCA
Spc24	Forward	GCCTTCCGCGACATAGAGG
	Reverse	CCTGCTCCTTCGATTGAGA
Ncaph	Forward	GTCCTCGAAGACTTTCCTCAGA
	Reverse	TGAAATGTCAATACTCCTGCTGG
Pbk	Forward	CCAAACATTGTTGGTTATCGTGC
	Reverse	GGCTGGCTTTATATCGTTCTTCT
Prim1	Forward	ATGGAGACGTTTGACCCAC
	Reverse	CGTAGTTGAGCCAGCGATAGT
Smc2	Forward	TCTCAGGTTCCGGCTTCTAAT
	Reverse	CCTGTA CTCTGGTGTGTTGG
Plk4	Forward	AAGCTCGACACTTCATGCACC
	Reverse	GCATTTTCAGTTGAGTTGCCAG
Dynll1	Forward	AGAGATGCAACAGGACTCGGT
	Reverse	CCAGGTGGGATTGTA CTCTTG
Mastl	Forward	GACTGAGGAGGGCGTGAATAG
	Reverse	AGCTTGGACCTGATGAGTCATA
Oxct1	Forward	GTTGGTGGTTTTGGCTATGT
	Reverse	AGACCATGCGTTTTATCTGCTT
Cavin2	Forward	CATCCGGGACA ACTCACAGG
	Reverse	CAGCGTCTAGCATGTTACCA
Tk1	Forward	GGGCAGATCCAGGTGATTCTC
	Reverse	TGTAGCGAGTGTCTTTGGCATA

Khdrbs1	Forward	GGAGCCAGAGAACAAGTACCT
	Reverse	CATGGCGTGAGTGAAGGAC
Dnmt1	Forward	AGGCGGCTCAAAGATTTGGAA
	Reverse	GCAGAAATTCGTGCAAGAGATTC
Top2a	Forward	ACCATTGCAGCCTGTAAATGA
	Reverse	GGGCGGAGCAAAATATGTTCC
Melk	Forward	TCTCCAGTAGCATTCTGCTT
	Reverse	TGATCCAGGGATGGTTCAATAGA
Tuba1b	Forward	ACCTTAACCGCCTTATTAGCCA
	Reverse	CACCACGGTACAACAGGCA
Mms22l	Forward	TGGACACCTATCTGAACCTCCC
	Reverse	ATGCCATGACGGTGGAAAAAG
Uhrf1	Forward	GCCATACCCTCTTCGACTACG
	Reverse	GCCCAATTCCGTCTCATCC

**Table S2. Reagents.**

Name	Company	Catalog No.
Triton X-100	Sigma-Aldrich	T9284
HBSS	ThermoFisher Scientific, Pittsburgh, PA, USA	14175103
DNase	Worthington, Lakewood, NJ, USA	9003-98-9
RNase	Worthington, Lakewood, NJ, USA	9001-99-4
paraformaldehyde(PFA)	Sigma-Aldrich	158127
4',6-diamidino-2-phenylindole(DAPI)	ThermoFisher Scientific)	D3571
EdU Kit	Invitrogen	C10337
PureLink RNA Micro Scale Kit	Life Technologies, Carlsbad, California, USA	12183016
PrimeScript™ reagent kit	Takara Bio, Kusatsu, Japan	RR037A
SYBR Green Power Premix Kits	Applied Biosystems, Foster City, California	4368577
RIPA lysis buffer	Beyotime, Shanghai, China	P0013B
NEB Next® Ultra™ RNA Library Prep Kit	NEB, USA	E7760
TruSeq PE Cluster Kit	Illumina	v3-cBot-HS

**Table S3. Antibodies.**

Name	Company	Catalog No.
cardiac troponin T	Abcam	ab8295
sarcomeric $\alpha$ -actinin	Abcam	ab9465
Ki67	Abcam	ab15580
phosphorylated histone H3	Milipore	06-570
aurora B	Abcam	ab2254
collagen IV	Abcam	ab6585

collagen II	Abcam	ab34712
GAPDH	Abcam	ab8245

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**Table S4. Top 24 upregulated genes.**

gene_id	OWOP1	OWOP2	OWOP3	OWOP4	OWOP5	OWOP6	OWCON1
ENSRNOG00000016561	273.4829	231.5473	357.1895	353.1868	319.7178	278.3281	47.80062
ENSRNOG00000054286	683.7071	622.605	933.4951	561.2283	626.8788	538.7381	95.60125
ENSRNOG00000003703	919.6343	877.307	1083.575	828.5375	880.9144	850.5133	296.1931
ENSRNOG00000008450	520.9656	379.0515	623.3306	482.608	439.4913	351.1951	66.57944
ENSRNOG00000058288	211.8529	198.9592	209.1109	263.6805	230.8537	191.1266	69.14019
ENSRNOG00000029862	404.4465	316.448	450.2388	400.359	379.6046	322.5261	132.3053
ENSRNOG00000012051	691.4109	617.4595	837.4442	764.4317	733.1294	597.2706	262.0498
ENSRNOG00000015308	376.5204	300.1539	384.2038	338.6723	332.2747	272.3554	63.16511
ENSRNOG00000031993	816.5967	794.9791	995.528	897.4815	898.3009	870.8205	371.3084
ENSRNOG00000022325	1196.969	1234.061	1464.777	1285.745	1206.428	950.8548	444.7165
ENSRNOG00000011654	364.9648	346.4634	453.2404	373.749	437.5595	359.5569	163.0343
ENSRNOG00000011222	3485.943	2979.242	3413.811	2742.036	2752.858	2569.458	1438.287
ENSRNOG00000054474	285.0385	238.408	297.1576	310.8527	251.1379	222.1847	84.50467
ENSRNOG00000043094	7277.148	8242.226	8049.269	6368.248	6687.029	4183.283	1916.293
ENSRNOG00000025895	4321.8	4195.294	4551.414	5081.293	4689.517	4191.645	2407.956
ENSRNOG00000047314	1208.525	899.6042	1207.641	918.0437	1083.757	973.5511	392.648
ENSRNOG00000046794	2315.938	2319.761	2327.234	2371.915	2237.059	2236.181	1678.997
ENSRNOG00000039859	1518.6	1354.981	1645.873	1331.708	1453.702	1380.89	704.2056
ENSRNOG00000053047	3165.275	2788.859	2936.558	2590.843	2705.528	2094.031	1224.037
ENSRNOG00000013598	268.668	212.6805	299.1587	258.8424	225.0582	236.5192	72.55452
ENSRNOG00000053468	11191.61	9963.395	12668.72	11016.52	10956.37	8328.341	4733.115
ENSRNOG00000006996	376.5204	367.9029	479.2542	445.1121	372.8432	352.3897	114.3801
ENSRNOG00000005659	854.1524	689.4964	891.4728	783.7844	747.6182	677.3049	365.3333
ENSRNOG00000048411	723.1888	610.5988	793.4208	576.9524	738.9249	659.3867	194.6168

OWCON2	OWCON3	OWCON4	OWCON5	OWCON6	Opt1w	Con1w	log2FoldCh	pvalue
53.82467	49.06721	49.1797	58.37299	39.2052	302.2421	49.57507	2.611407	1.05E-59
106.4532	99.22481	91.69096	127.8167	75.73733	661.1087	99.42071	2.737093	1.65E-59
267.9272	278.0475	265.0702	206.3183	211.1735	906.7469	254.1217	1.835709	2.31E-55
102.8649	71.96525	92.52451	89.57234	98.90404	466.107	87.06842	2.42227	2.78E-44
55.02077	55.60951	63.35012	54.34726	52.57062	217.5973	58.33974	1.891693	3.72E-43
123.1987	119.9421	105.8614	107.6881	98.90404	378.9372	114.6499	1.726325	9.30E-41
235.6324	240.9745	270.9051	266.7042	209.3914	706.8577	247.6096	1.512346	2.19E-40
77.74674	77.41716	41.67771	86.55305	51.67959	334.0301	66.37323	2.341963	9.86E-40
316.9675	432.8819	385.9356	387.4759	359.084	878.9511	375.6089	1.225466	2.25E-39
434.1857	444.8761	539.3095	459.9389	432.1483	1223.139	459.1958	1.412882	3.42E-37
147.1208	152.6536	160.876	140.9003	151.4747	389.2557	152.6766	1.348381	1.79E-36
1304.949	1286.651	1293.676	1200.672	1182.393	2990.558	1284.438	1.219458	3.38E-35
95.6883	85.04984	91.69096	94.6045	71.28219	267.4632	87.13674	1.62034	1.07E-34
1716.409	2039.015	1589.588	2066.202	1660.875	6801.201	1831.397	1.893055	1.07E-33
2285.754	2107.709	2629.03	2483.871	2344.293	4505.161	2376.436	0.922225	1.40E-32
391.1259	358.7358	290.0768	330.1093	240.5774	1048.52	333.8789	1.652762	2.28E-31
1560.915	1612.676	1508.733	1581.103	1579.792	2301.348	1587.036	0.536062	1.13E-30
810.9583	704.3871	721.8579	630.0257	609.4627	1447.625	696.8162	1.056133	1.58E-30
1246.34	1068.575	1017.77	1164.44	982.8032	2713.515	1117.328	1.280817	1.76E-30
64.5896	55.60951	89.1903	57.36656	42.76931	250.1545	63.67997	1.968585	6.51E-30
5069.088	5077.911	5079.679	4490.694	4429.297	10687.49	4813.297	1.150927	8.85E-30
133.9636	137.3882	116.6976	73.46945	82.86555	399.0037	109.7941	1.864984	1.70E-29
367.2038	288.9514	326.7532	377.4116	326.116	773.9715	341.9616	1.178754	4.78E-29
300.222	246.4264	225.8932	282.8071	201.3722	683.7454	241.8896	1.503354	1.52E-28

padj	gene_name	gene_chr	gene_start	gene_end	gene_stran	gene_length
1.55E-55	Pclaf	8	71514281	71526182	+	333
1.55E-55	Rrm2	6	43884678	43889515	+	1173
1.45E-51	Mcm6	13	45042882	45068077	-	2917
1.31E-40	LOC100359539	19	25391700	25394229	+	2530
1.41E-39	Tcf19	20	3744395	3746964	+	838
2.93E-37	Spc24	8	22780725	22785671	-	865
5.90E-37	Ncaph	3	1.2E+08	1.2E+08	-	2848
2.33E-36	Pbk	15	42489377	42500395	+	1830
4.71E-36	Prim1	7	2435553	2451165	+	1596
6.45E-34	Smc2	5	68717519	68763850	+	5373
3.07E-33	Plk4	2	1.28E+08	1.28E+08	+	3466
5.31E-32	Dynll1	12	47074200	47076572	+	652
1.56E-31	Mastl	17	89742140	89876825	+	5433
1.45E-30	Oxct1	2	53859737	54007756	+	3331
1.77E-29	Cavin2	9	55243255	55256340	-	3189
2.69E-28	Tk1	10	1.07E+08	1.07E+08	-	2218
1.25E-27	Khdrbs1	5	1.48E+08	1.48E+08	-	2622
1.65E-27	Dnmt1	8	21922515	21968495	-	5917
1.66E-27	Top2a	10	86901007	86932154	-	5589
5.85E-27	Melk	5	59783890	59844356	+	2842
7.60E-27	Tuba1b	7	1.41E+08	1.41E+08	-	1608
1.39E-26	Mms22l	5	38844488	38961035	+	4483
3.76E-26	Aurkb	10	55626741	55634991	+	4404
1.12E-25	Uhrf1	9	10738222	10757954	-	3856

gene_biotype	gene_description
protein_coding	PCNA clamp associated factor [Source:RGD Symbol;Acc:1303041]
protein_coding	ribonucleotide reductase regulatory subunit M2 [Source:RGD Symbol;Acc:15983]
protein_coding	minichromosome maintenance complex component 6 [Source:RGD Symbol;Acc:1302974]
protein_coding	ribonucleotide reductase M2 polypeptide [Source:RGD Symbol;Acc:2323655]
protein_coding	transcription factor 19 [Source:RGD Symbol;Acc:1302974]
protein_coding	SPC24, NDC80 kinetochore complex component [Source:RGD Symbol;Acc:13076]
protein_coding	non-SMC condensin I complex, subunit H [Source:RGD Symbol;Acc:1596799]
protein_coding	PDZ binding kinase [Source:RGD Symbol;Acc:1309565]
protein_coding	DNA primase subunit 1 [Source:RGD Symbol;Acc:621380]
protein_coding	structural maintenance of chromosomes 2 [Source:RGD Symbol;Acc:1305227]
protein_coding	polo-like kinase 4 [Source:RGD Symbol;Acc:1305390]
protein_coding	dynein light chain LC8-type 1 [Source:RGD Symbol;Acc:619866]
protein_coding	microtubule associated serine/threonine kinase-like [Source:MGI Symbol;Acc:MI000000000]
protein_coding	3-oxoacid CoA transferase 1 [Source:RGD Symbol;Acc:1584008]
protein_coding	caveolae associated protein 2 [Source:RGD Symbol;Acc:1359345]
protein_coding	thymidine kinase 1 [Source:RGD Symbol;Acc:621014]
protein_coding	KH RNA binding domain containing, signal transduction associated 1 [Source:RGD Symbol;Acc:1305227]
protein_coding	DNA methyltransferase 1 [Source:RGD Symbol;Acc:620979]
protein_coding	DNA topoisomerase II alpha [Source:RGD Symbol;Acc:62048]
protein_coding	maternal embryonic leucine zipper kinase [Source:RGD Symbol;Acc:1308974]
protein_coding	tubulin, alpha 1B [Source:RGD Symbol;Acc:1565476]
protein_coding	MMS22-like, DNA repair protein [Source:RGD Symbol;Acc:1304693]
protein_coding	aurora kinase B [Source:RGD Symbol;Acc:621625]
protein_coding	ubiquitin-like with PHD and ring finger domains 1 [Source:RGD Symbol;Acc:1595227]



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10]  
61967]

31]

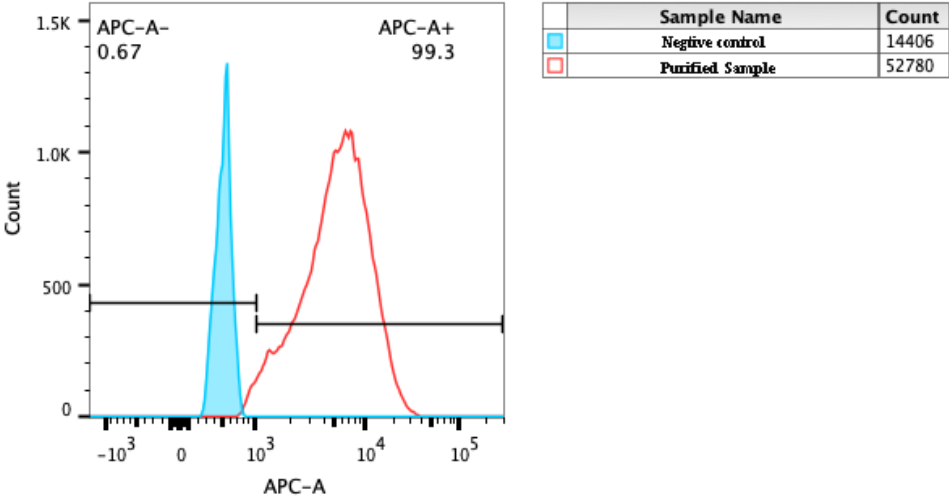
GI:1914371]

Symbol;Acc:621459]

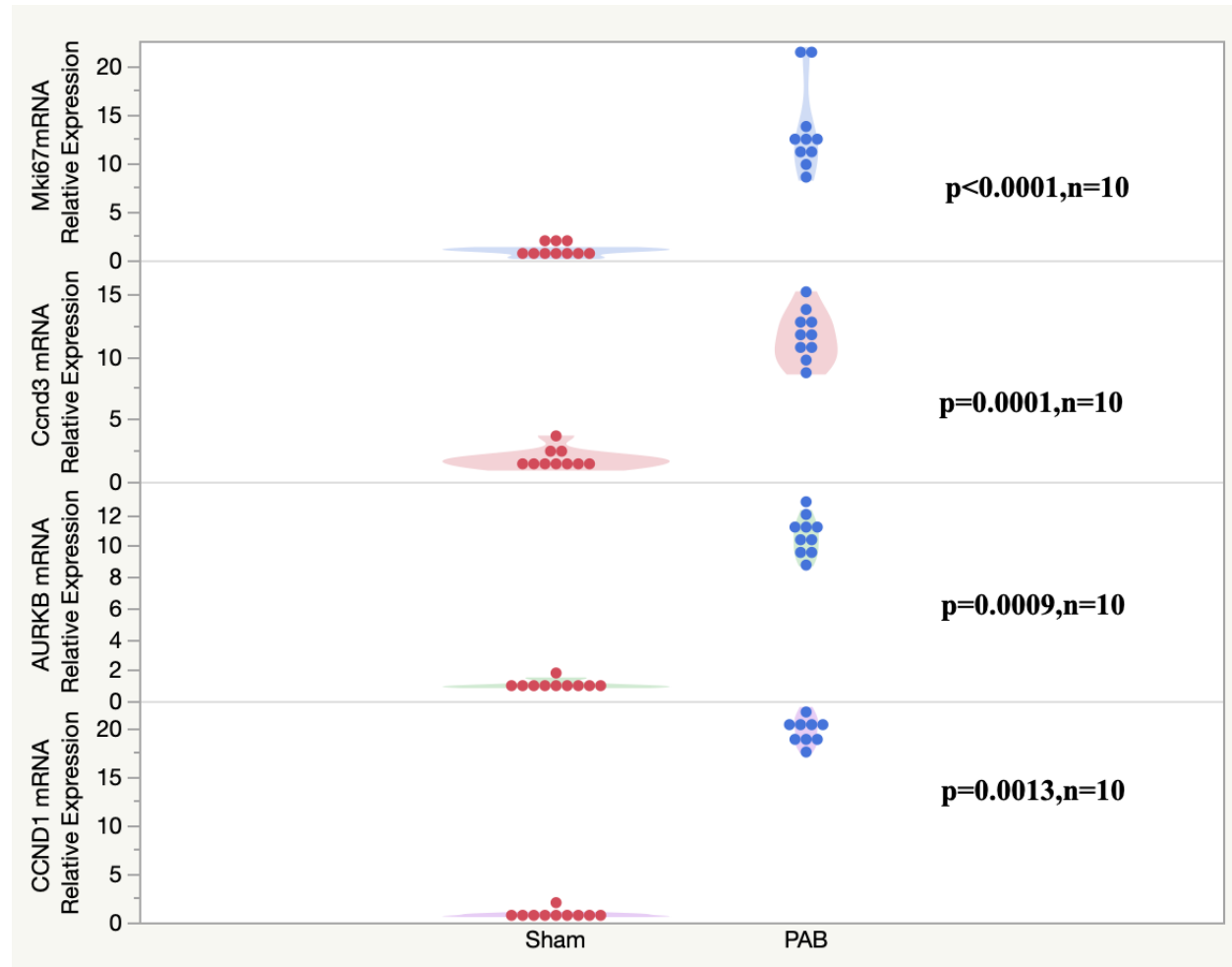
855]

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**Figure S1. Flow cytometry indicates that ~99% of purified cells were cTnT- positive.**

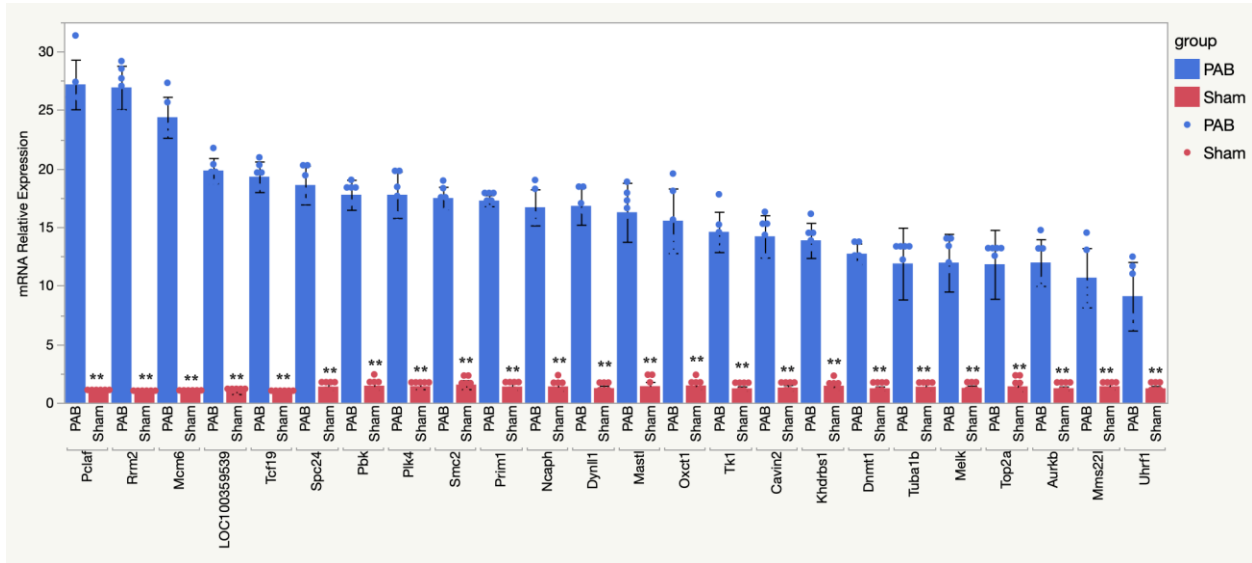


**Figure S2. qRT-PCR confirms the differentially expressed genes of RNA-seq data.**



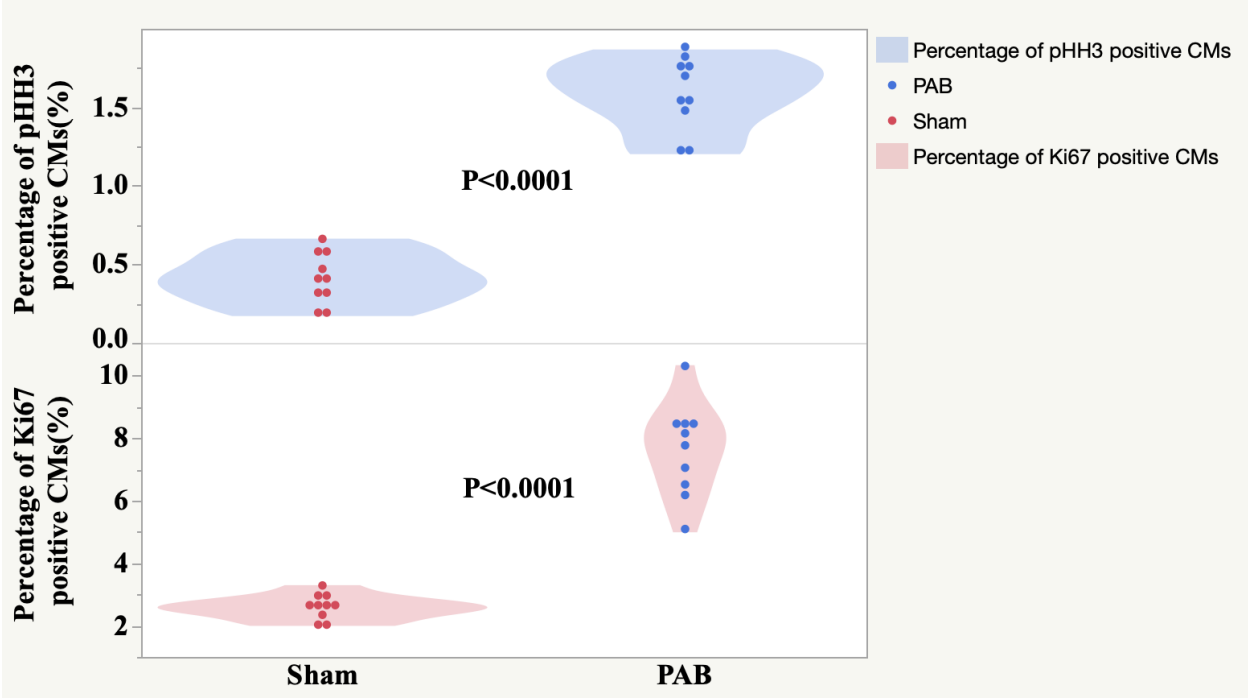
*AURKB* is one of the most important markers of cell division. RNA-seq data (GSE139561) showed that the average FPKM of *AURKB* in the PAB group was  $9.54 \pm 1.07$  and in the sham group was  $4.21 \pm 0.42$ . Qrt-PCR results showed that the relative mRNA level of *AURKB* increased ~10 fold. RNA-seq data (GSE139561) showed that the average FPKM of *CCND1* in the PAB group was  $22.8 \pm 6.2$  and in the sham group was  $7.78 \pm 1.12$ . qRT-PCR results showed that the relative mRNA level of *CCND1* increased ~20 fold. The other two cell cycle related genes *Mki67* and *Ccnd3* were also significantly increased.

**Figure S3. The top 24 differentially expressed genes from RNA-seq data.**

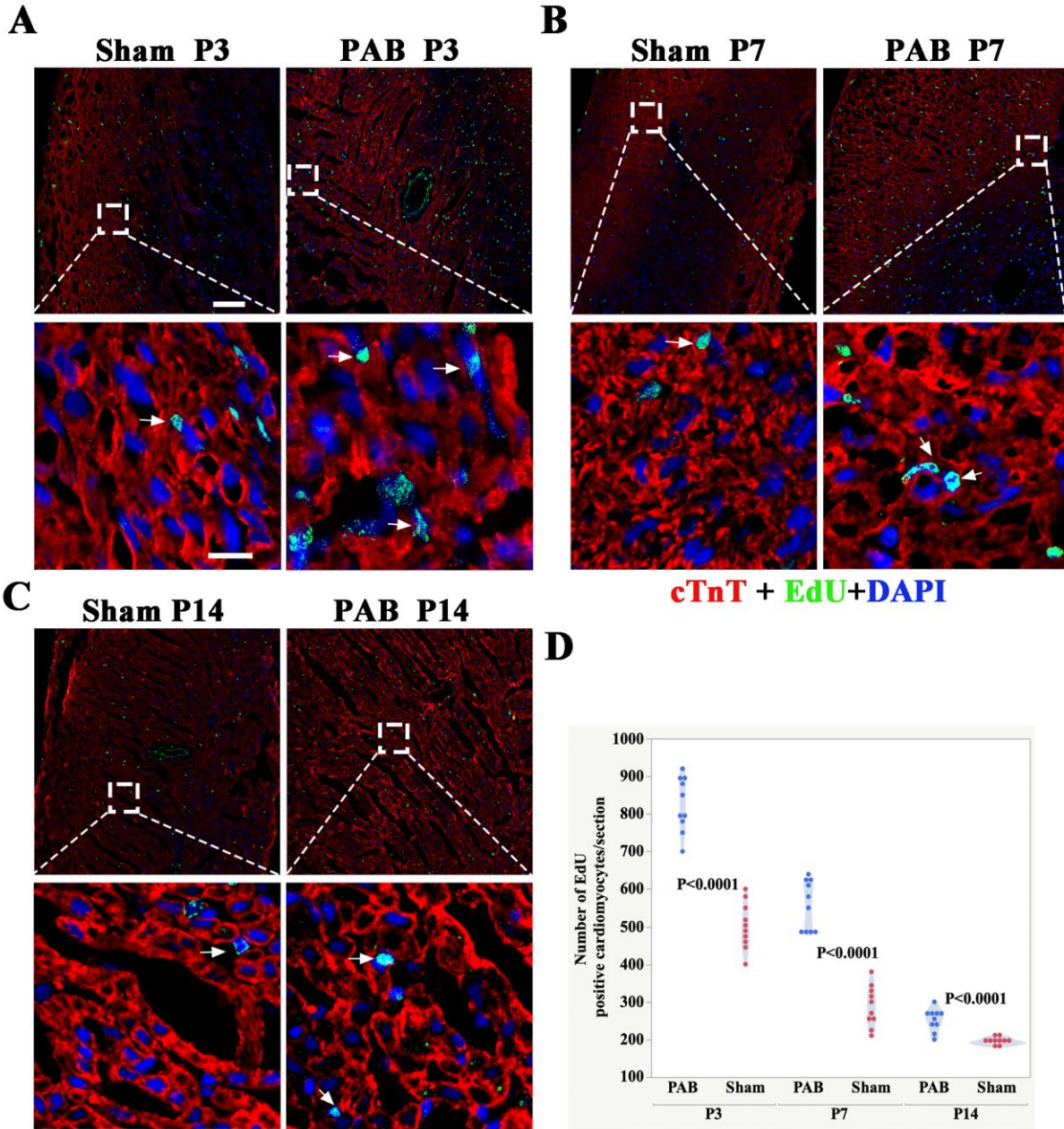


\*\*P<0.01,n=10.

**Figure S4 Quantification of the percentage of Ki67 and pHH3 positive cardiomyocytes in sham and PAB rats at P7.**



**Figure S5. Nucleoside uptake confirms that pressure overload greatly promotes RVCM proliferation.**



Representative graph of EdU-positive cardiomyocytes at P3(A), at P7(B), and at P14(C). (D) Quantification of EdU -positive cardiomyocytes at P7. Arrows indicate proliferating cardiomyocytes. EdU (green), cardiac troponin T (red) and DAPI (blue).