Bone & Joint Research

Supplementary Material

10.1302/2046-3758.127.BJR-2022-0279.R2

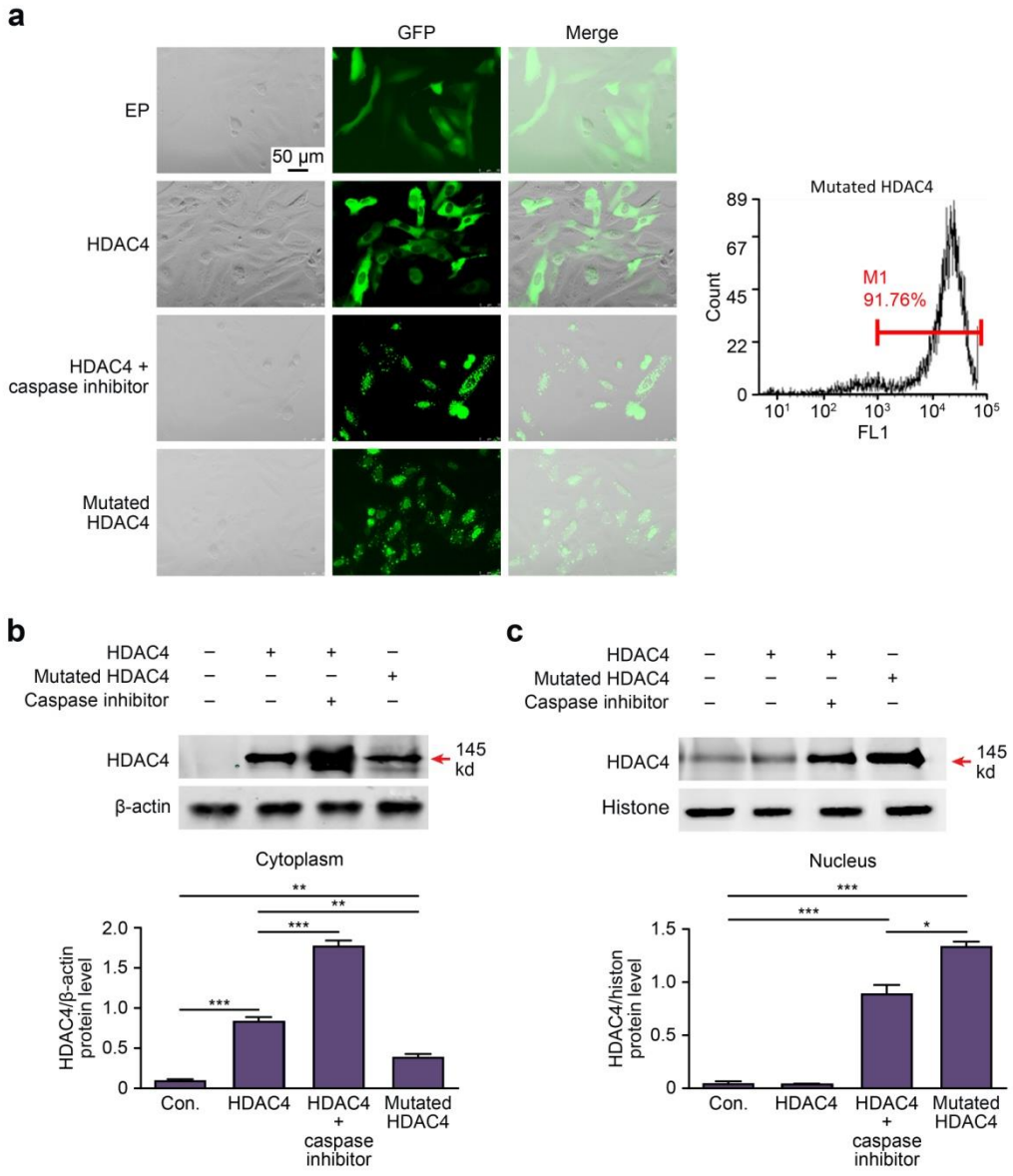


Fig a. a) Fluorescence microscopy illustrated the subcellular location of histone deacetylase 4 (HDAC4), the adenovirus fused to green fluorescent protein (GFP) (green); scale bar: 50 μm, and representative transfection efficiency of mutated-HDAC4 group was detected at 48 hours after transfection by flow cytometry. b) and c) Representative protein expressions of HDAC4 in the

cytoplasm and nucleus; data were quantified by average grey value ratio of HDAC4/β-actin and HDAC4/histone and expressed as the mean (standard deviation (SD)). *p < 0.05, **p < 0.01, ***p < 0.001 (n = 3). Con., control; M1, FITC positive cell rate; FL1, FITC channel.

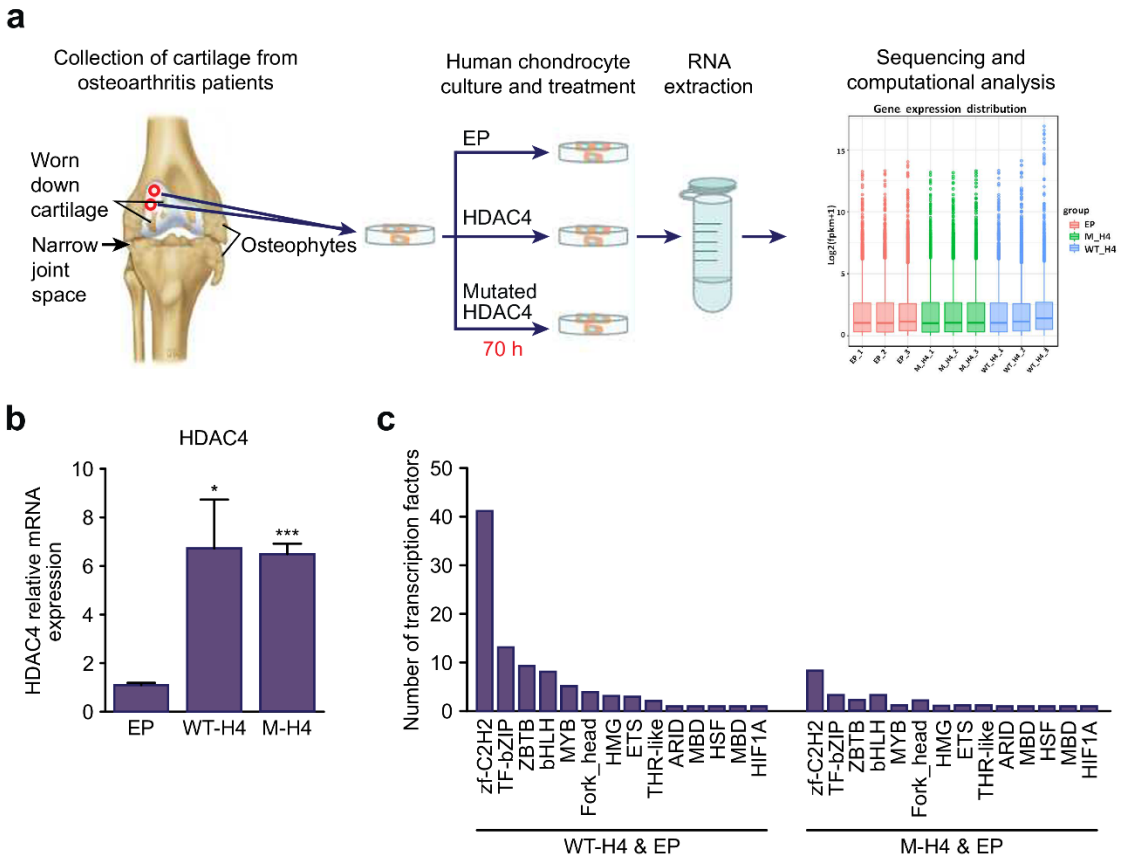


Fig b. a) Schematic of RNA sequencing (RNA-seq). b) the RNA expression of histone deacetylase 4 (HDAC4) in empty adenovirus (EP), HDAC4, and mutated-HDAC4 groups were detected by RT-PCR, the data was quantified and expressed as the mean (standard deviation (SD)). *p < 0.05, ***p < 0.001 (n = 3). c) The results show changes in the transcription factor families; the Y axis shows the number of members with significant changes, and the X axis shows the different transcription factor families. mRNA, messenger RNA; M-H4, mutated-HDAC4; WT-H4, wild-type HDAC4.

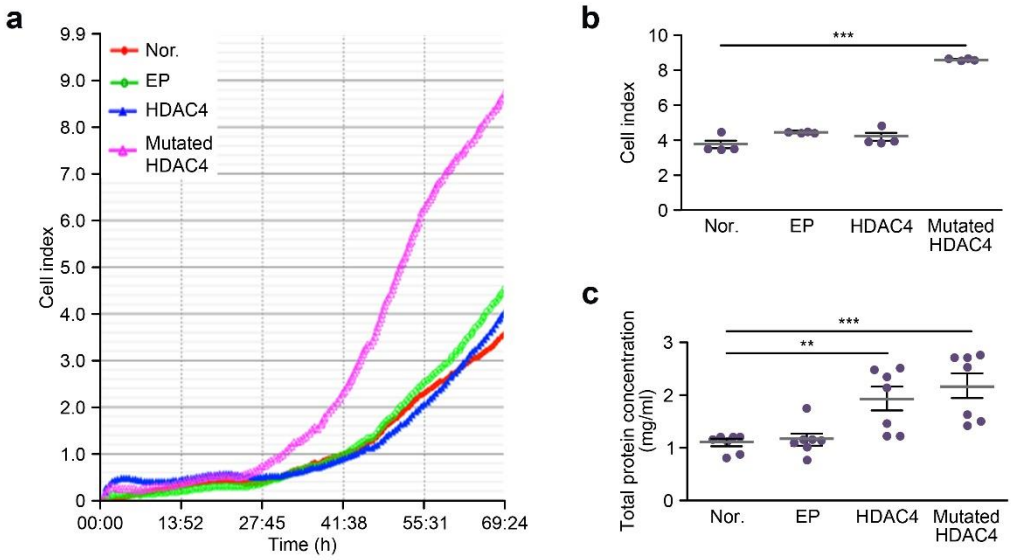


Fig c. a) Representative cell survival rates of groups were detected by real-time cell analysis (RTCA) assay. b) The data were quantified by mean cell index (n = 4). c) Total protein concentration of groups was quantified (n = 7). The data were quantified and expressed as the mean (standard deviation (SD)). **p < 0.01, ***p < 0.001. EP, empty adenovirus; HDAC4, histone deacetylase 4; Nor., normal.

Table i. Differentially expressed genes for empty adenovirus versus histone deacetylase 4 (top 20).

ID	WT_H4	EP	log2FoldChange	p-value	p-adj	gene_name	gene_strand	gene_length	gene_biotype
ENSG00000184321	370.3555	12.25719	4.916292	9.69E-20	1.98E-15	OR51J1	+	951	unprocessed_pseudogene
ENSG00000185164	134.7703	1491.163	-3.4717	1.64E-17	1.68E-13	NOMO2	-	6801	protein_coding
ENSG00000240184	101.2777	1019.076	-3.32232	5.74E-16	3.90E-12	PCDHGC3	+	5432	protein_coding
ENSG00000226928	426.4683	3.060448	7.141764	3.22E-15	1.64E-11	RPS14P4	-	418	processed_pseudogene
ENSG00000229807	1004.965	6826.696	-2.76337	9.54E-15	3.89E-11	XIST	-	19961	lincRNA
ENSG00000278301	758.2212	21.15179	5.154084	3.03E-13	1.03E-09	GRAMD4P3	+	1669	processed_pseudogene
ENSG00000225877	307.887	9.798637	4.943762	7.62E-13	2.22E-09	PSG8-AS1	+	1873	lincRNA
ENSG00000125107	834.4249	4882.945	-2.54949	1.50E-12	3.82E-09	CNOT1	-	10852	protein_coding
ENSG00000001630	8.169469	247.472	-4.69851	1.97E-12	4.47E-09	CYP51A1	-	4074	protein_coding
ENSG00000236824	1092.923	105.4616	3.370199	3.61E-12	6.94E-09	BCYRN1	+	200	scRNA
ENSG00000206448	172.841	2.235797	6.268546	3.75E-12	6.94E-09	PPIAP30	-	519	processed_pseudogene
ENSG00000264895	390.9229	5.391029	6.162667	6.23E-12	1.05E-08	AC006141.1	-	2170	sense_intronic
ENSG00000037749	65.592	533.0344	-3.02663	6.71E-12	1.05E-08	MFAP3	+	4792	protein_coding
ENSG00000143631	38.95201	423.7209	-3.50415	9.05E-12	1.18E-08	FLG	-	12747	protein_coding

ENSG00000257604	429.8749	9.4349	5.510962	9.38E-12	1.18E-08	AC027288.2	-	243	processed_pseudogene
ENSG00000198467	5570.571	296.6418	4.230287	9.65E-12	1.18E-08	TPM2	-	3248	protein_coding
ENSG00000110344	192.8697	1241.98	-2.6947	9.83E-12	1.18E-08	UBE4A	+	6576	protein_coding
ENSG00000259207	64.55574	511.6728	-2.9974	1.58E-11	1.72E-08	ITGB3	+	4394	protein_coding
ENSG00000176986	223.0172	1334.8	-2.58107	1.60E-11	1.72E-08	SEC24C	+	5341	protein_coding
ENSG00000140526	380.3612	2163.817	-2.51017	2.57E-11	2.62E-08	ABHD2	+	10345	protein_coding

EP, empty adenovirus; WT_H4, wild-type HDAC4; p-adj, p-adjusted.

Table ii. Gene Ontology enrichment analysis for empty adenovirus versus histone deacetylase 4 (top 10).

Category	ID	Description	GeneRatio	BgRatio	p-value	p-adj	Count
BP	GO:0009205	purine ribonucleoside triphosphate metabolic process	35/807	132/7400	3.51E-07	0.000637	35
BP	GO:0009144	purine nucleoside triphosphate metabolic process	36/807	138/7400	3.70E-07	0.000637	36
BP	GO:0009199	ribonucleoside triphosphate metabolic process	35/807	134/7400	5.20E-07	0.000637	35
BP	GO:0009150	purine ribonucleotide metabolic process	54/807	253/7400	6.88E-07	0.000637	54
BP	GO:0019693	ribose phosphate metabolic process	56/807	268/7400	8.79E-07	0.000637	56
BP	GO:0006163	purine nucleotide metabolic process	55/807	263/7400	1.07E-06	0.000637	55
BP	GO:0009141	nucleoside triphosphate metabolic process	37/807	150/7400	1.14E-06	0.000637	37
BP	GO:0006119	oxidative phosphorylation	20/807	58/7400	1.46E-06	0.000669	20
BP	GO:0009259	ribonucleotide metabolic process	54/807	260/7400	1.69E-06	0.000669	54
CC	GO:0005924	cell-substrate adherens junction	45/810	202/7702	6.48E-07	0.00016	45
CC	GO:0005925	focal adhesion	45/810	202/7702	6.48E-07	0.00016	45
CC	GO:0030055	cell-substrate junction	45/810	205/7702	1.00E-06	0.000165	45
CC	GO:0098800	inner mitochondrial membrane protein complex	21/810	65/7702	1.50E-06	0.000184	21
CC	GO:0005912	adherens junction	51/810	252/7702	2.55E-06	0.000251	51
CC	GO:0070161	anchoring junction	52/810	262/7702	3.71E-06	0.000305	52
CC	GO:0005789	endoplasmic reticulum membrane	74/810	430/7702	9.45E-06	0.000665	74
CC	GO:0042175	nuclear outer membrane-endoplasmic reticulum membrane network	75/810	442/7702	1.38E-05	0.000848	75
CC	GO:0070469	respiratory chain	16/810	48/7702	1.70E-05	0.000919	16
CC	GO:0098798	mitochondrial protein complex	21/810	75/7702	1.86E-05	0.000919	21
MF	GO:0008137	nicotinamide adenine dinucleotide reduced form (NADH) dehydrogenase (ubiquinone) activity	10/811	21/7455	2.52E-05	0.006571	10
MF	GO:0050136	NADH dehydrogenase (quinone) activity	10/811	21/7455	2.52E-05	0.006571	10
MF	GO:0022890	inorganic cation transmembrane transporter activity	47/811	238/7455	3.01E-05	0.006571	47
MF	GO:0003954	NADH dehydrogenase activity	10/811	23/7455	6.68E-05	0.009256	10
MF	GO:0008324	cation transmembrane transporter activity	52/811	284/7455	9.56E-05	0.009256	52
MF	GO:0015078	hydrogen ion transmembrane transporter activity	16/811	53/7455	0.000101	0.009256	16

MF	GO:0016655	oxidoreductase activity, acting on nicotinamide adenine dinucleotide phosphate reduced form (NAD(P)H), quinone, or similar compound as acceptor	10/811	24/7455	0.000103	0.009256	10
MF	GO:0050840	extracellular matrix binding	9/811	20/7455	0.000113	0.009256	9
MF	GO:0016887	ATPase activity	38/811	193/7455	0.000183	0.013344	38
MF	GO:0015075	ion transmembrane transporter activity	61/811	370/7455	0.000507	0.032937	61

ATP, adenosine triphosphate; BP, biological process; CC, cell component; GO, Gene Ontology; MF, molecular function; p-adj, p-adjusted.

Table iii. Kyoto Encyclopedia of Genes and Genomes pathway analysis for empty adenovirus versus histone deacetylase 4 (top 20).

ID	Description	GeneRatio	BgRatio	p-value	p-adj	Count
hsa03010	Ribosome	37/725	128/6149	1.01E-07	3.00E-05	37
hsa00190	Oxidative phosphorylation	34/725	125/6149	1.59E-06	0.000236	34
hsa05010	Alzheimer's disease	38/725	162/6149	1.84E-05	0.001822	38
hsa05414	Dilated cardiomyopathy (DCM)	23/725	80/6149	2.98E-05	0.00221	23
hsa05165	Human papillomavirus infection	53/725	286/6149	0.000439	0.026055	53
hsa04213	Longevity regulating pathway - multiple species	15/725	54/6149	0.00106	0.041171	15
hsa04510	Focal adhesion	38/725	195/6149	0.001072	0.041171	38
hsa04919	Thyroid hormone signalling pathway	24/725	107/6149	0.00124	0.041171	24
hsa04915	Oestrogen signalling pathway	21/725	89/6149	0.001248	0.041171	21
hsa05012	Parkinson's disease	28/725	134/6149	0.00162	0.045802	28
hsa04211	Longevity regulating pathway	19/725	80/6149	0.001931	0.045802	19
hsa05205	Proteoglycans in cancer	36/725	188/6149	0.001981	0.045802	36
hsa04611	Platelet activation	24/725	111/6149	0.002109	0.045802	24
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	17/725	69/6149	0.002159	0.045802	17
hsa05110	*Vibrio cholerae* infection	13/725	47/6149	0.002346	0.046449	13
hsa04142	Lysosome	25/725	120/6149	0.00294	0.052095	25
hsa05410	Hypertrophic cardiomyopathy (HCM)	18/725	77/6149	0.003027	0.052095	18
hsa04141	Protein processing in endoplasmic reticulum	30/725	153/6149	0.003157	0.052095	30
hsa04512	ECM-receptor interaction	18/725	78/6149	0.003514	0.054931	18
hsa04750	Inflammatory mediator regulation of transient receptor potential (TRP) channels	20/725	91/6149	0.00394	0.057601	20

hsa, Homo sapiens (human); p-adj, p-adjusted.

Table iv. Differentially expressed genes for empty adenovirus versus mutated-histone deacetylase 4 (top 20).

ID	M_H4	EP	log2FoldChange	p-value	p-adj	gene_name	gene_strand	gene_biotype
ENSG00000068024	1808.46	308.5345	2.556429	1.39E-81	1.34E-77	HDAC4	-	protein_coding
ENSG00000138735	11171.01	17813.64	-0.6732	5.10E-25	2.45E-21	PDE5A	-	protein_coding
ENSG00000128283	1696.157	987.9488	0.779261	5.29E-14	1.69E-10	CDC42EP1	+	protein_coding
ENSG00000159335	4095.245	2586.953	0.662837	1.26E-13	3.03E-10	PTMS	+	protein_coding
ENSG00000159840	5232.112	3515.5	0.573803	1.66E-13	3.19E-10	ZYX	+	protein_coding
ENSG00000116285	5344.08	8486.067	-0.66669	3.39E-13	5.43E-10	ERRFI1	-	protein_coding
ENSG00000115268	4538.513	2862.287	0.665387	8.66E-13	1.19E-09	RPS15	+	protein_coding
ENSG00000169908	5056.08	7520.538	-0.57249	1.50E-12	1.80E-09	TM4SF1	-	protein_coding
ENSG00000033867	3809.555	5440.249	-0.51367	8.17E-11	8.72E-08	SLC4A7	-	protein_coding
ENSG00000105640	5249.612	3353.519	0.646418	1.33E-10	1.28E-07	RPL18A	+	protein_coding
ENSG00000169047	3532.289	2409.808	0.551943	2.11E-10	1.84E-07	IRS1	-	protein_coding
ENSG00000050820	2832.386	1938.653	0.54741	3.04E-10	2.44E-07	BCAR1	-	protein_coding
ENSG00000233927	1413.24	775.4355	0.865068	8.39E-10	5.93E-07	RPS28	+	protein_coding
ENSG00000062716	7942.76	10902.55	-0.45675	8.64E-10	5.93E-07	VMP1	+	protein_coding
ENSG00000099814	2154.693	1456.617	0.564845	1.20E-09	7.70E-07	CEP170B	+	protein_coding
ENSG00000170454	800.7615	421.9235	0.928273	2.44E-09	1.42E-06	KRT75	-	protein_coding
ENSG00000164889	2786.329	1819.163	0.614909	2.51E-09	1.42E-06	SLC4A2	+	protein_coding
ENSG00000071655	1513.325	978.7796	0.628277	3.72E-09	1.99E-06	MBD3	-	protein_coding
ENSG00000178209	24023.63	18195.35	0.40097	4.42E-09	2.23E-06	PLEC	-	protein_coding
ENSG00000132361	2061.911	1387.529	0.572636	5.79E-09	2.78E-06	CLUH	-	protein_coding

EP, empty adenovirus; M_H4, mutated HDAC4; p-adj, p-adjusted.

Table v. Gene Ontology enrichment analysis for empty adenovirus versus mutated-histone deacetylase 4 (top 10).

Category	ID	Description	GeneRatio	BgRatio	p-value	padj	Count
BP	GO:0016071	mRNA metabolic process	43/339	393/7382	6.66E-08	9.42E-05	43
BP	GO:0045047	protein targeting to ER	14/339	53/7382	6.69E-08	9.42E-05	14
BP	GO:0072599	establishment of protein localization to endoplasmic reticulum	14/339	54/7382	8.66E-08	9.42E-05	14
BP	GO:0006613	cotranslational protein targeting to membrane	13/339	51/7382	3.14E-07	0.000256	13
BP	GO:0070972	protein localization to endoplasmic reticulum	14/339	61/7382	4.48E-07	0.000292	14
BP	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	12/339	48/7382	1.13E-06	0.000615	12
BP	GO:0006612	protein targeting to membrane	15/339	82/7382	3.77E-06	0.001654	15
BP	GO:0016072	rRNA metabolic process	21/339	150/7382	4.26E-06	0.001654	21
BP	GO:0006364	rRNA processing	19/339	127/7382	4.56E-06	0.001654	19
BP	GO:0006401	RNA catabolic process	20/339	141/7382	5.90E-06	0.001926	20
CC	GO:0030055	cell-substrate junction	27/347	204/7677	4.14E-07	0.000145	27
CC	GO:0005924	cell-substrate adherens junction	26/347	201/7677	1.07E-06	0.000145	26
CC	GO:0005925	focal adhesion	26/347	201/7677	1.07E-06	0.000145	26
CC	GO:0005912	adherens junction	29/347	251/7677	2.67E-06	0.000273	29
CC	GO:0070161	anchoring junction	29/347	262/7677	6.32E-06	0.000514	29
CC	GO:0030529	intracellular ribonucleoprotein complex	37/347	382/7677	7.82E-06	0.000514	37
CC	GO:1990904	ribonucleoprotein complex	37/347	384/7677	8.82E-06	0.000514	37
CC	GO:0022626	cytosolic ribosome	10/347	47/7677	3.62E-05	0.001833	10
CC	GO:0005730	nucleolus	38/347	427/7677	4.04E-05	0.001833	38
CC	GO:0038201	TOR complex	5/347	11/7677	6.76E-05	0.002758	5
MF	GO:0045296	cadherin binding	21/340	144/7437	2.04E-06	0.00112	21

BP, biological process; CC, cell component; ER, endoplasmic reticulum; GO, Gene Ontology; MF, molecular function; mRNA, messenger RNA; rRNA, ribosomal RNA; SRP, signal recognition particle.

Table vi. Kyoto Encyclopedia of Genes and Genomes pathway analysis for empty adenovirus versus mutated-histone deacetylase 4 (top 20).

ID	Description	GeneRatio	BgRatio	p-value	-log10(padj)	padj	Count
hsa03010	Ribosome	23/352	128/6155	6.98E-07	3.72314	0.000189	23
hsa03020	RNA polymerase	6/352	29/6155	0.005186	0.15326	0.702657	6
hsa00240	Pyrimidine metabolism	12/352	98/6155	0.009605	0.06167	0.867626	12
hsa04152	AMP-activated protein kinase (AMPK) signalling pathway	12/352	111/6155	0.024059	0.00002	0.999961	12
hsa03008	Ribosome biogenesis in eukaryotes	9/352	76/6155	0.028401	0.00002	0.999961	9
hsa05130	Pathogenic *Escherichia coli* infection	7/352	53/6155	0.030015	0.00002	0.999961	7
hsa00250	Alanine, aspartate, and glutamate metabolism	5/352	35/6155	0.04699	0.00002	0.999961	5
hsa03030	DNA replication	5/352	35/6155	0.04699	0.00002	0.999961	5
hsa04140	Autophagy – animal	12/352	123/6155	0.047844	0.00002	0.999961	12
hsa04510	Focal adhesion	17/352	196/6155	0.055362	0.00002	0.999961	17
hsa04216	Ferroptosis	5/352	39/6155	0.069386	0.00002	0.999961	5
hsa00760	Nicotinate and nicotinamide metabolism	4/352	28/6155	0.07279	0.00002	0.999961	4
hsa05219	Bladder cancer	5/352	40/6155	0.075765	0.00002	0.999961	5
hsa00100	Steroid biosynthesis	3/352	18/6155	0.079966	0.00002	0.999961	3
hsa04110	Cell cycle	11/352	122/6155	0.088521	0.00002	0.999961	11
hsa05166	Human T-cell leukaemia virus type I (HTLV-I) infection	18/352	225/6155	0.091971	0.00002	0.999961	18
hsa05211	Renal cell carcinoma	7/352	68/6155	0.092104	0.00002	0.999961	7
hsa04979	Cholesterol metabolism	5/352	43/6155	0.096722	0.00002	0.999961	5
hsa00220	Arginine biosynthesis	3/352	20/6155	0.102844	0.00002	0.999961	3
hsa04141	Protein processing in endoplasmic reticulum	13/352	155/6155	0.105355		0.999961	13

hsa, Homo sapiens (human).

Table vii. Gene set enrichment analysis of Gene Ontology enrichment analysis for empty adenovirus versus mutated-histone deacetylase 4 (top 20).

Term	ES	NES	p-value	FDR	geneset_size	matched_size
RNA polymerase II core binding	0.719292	3.482342	0.006289	0.96817	26	17
Cellular pigmentation	-0.13251	-0.66321	1	0.999996	23	23
Non-coding RNA (ncRNA) processing	0.448925	2.819373	0	1	237	203
rRNA metabolic process	0.478695	2.988312	0	1	176	150
rRNA processing	0.486937	2.928654	0	1	149	127
ribosome biogenesis	0.458395	2.92344	0	1	192	160
ribonucleoprotein complex biogenesis	0.407961	2.540099	0.003604	1	279	231
Negative regulation of tumour necrosis factor production	-0.67695	-3.47837	0.006316	1	40	23
ncRNA metabolic process	0.374116	2.448103	0.007326	1	341	290
RNA polymerase core enzyme binding	0.688741	3.137772	0.007905	1	28	18
Positive regulation of protein kinase B signalling	-0.53276	-3.33258	0.010661	1	73	59
Natural killer cell mediated cytotoxicity	-0.63362	-3.30375	0.012605	1	66	25
Negative regulation of tumour necrosis factor superfamily cytokine production	-0.66459	-3.61502	0.013129	1	41	24
Intraciliary transport particle	0.700577	3.004099	0.013462	1	16	16
Activation of cysteine-type endopeptidase activity involved in apoptotic process	0.553096	2.893405	0.013566	1	45	37
Negative regulation of interleukin-6 production	-0.70539	-3.50644	0.015217	1	25	16
Basal transcription machinery binding	0.623407	2.835554	0.016698	1	34	25
Basal RNA polymerase II transcription machinery binding	0.623407	2.891453	0.018692	1	34	25
Chondroitin sulphate proteoglycan biosynthetic process	0.706432	2.9218	0.020484	1	16	15
Ensheathment of neurons	-0.5172	-3.02946	0.020619	1	50	46

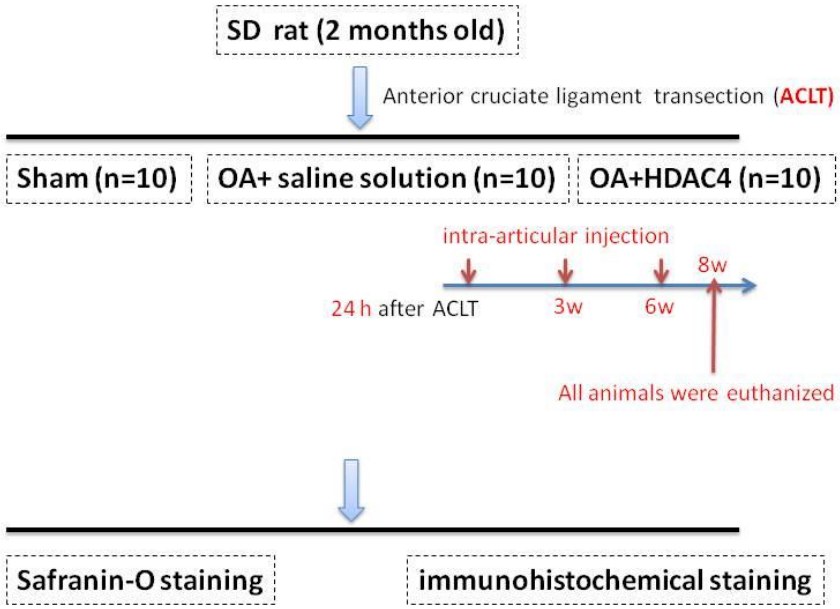
ES, Enrichment Score; FDR, false discovery rate; NES, Normalized Enrichment Score; rRNA, ribosomal RNA.

Table viii. Gene set enrichment analysis of Kyoto Encyclopedia of Genes and Genomes pathway analysis for empty adenovirus versus mutated-histone deacetylase 4 (top 20).

Term	ES	NES	p-value	FDR	geneset_size	matched_size
Primary immunodeficiency	-0.54341	-3.07014	0.044872	0.843095	35	29
Oestrogen signalling pathway	0.112726	0.644121	1	0.999994	98	88
Drug metabolism - cytochrome P450	-0.49054	-2.95479	0.026915	1	70	52
Ascorbate and aldarate metabolism	-0.64066	-3.14222	0.029046	1	27	18
Ribosome	0.409813	2.364414	0.030411	1	152	128
Antifolate resistance	-0.56051	-3.07443	0.035865	1	31	29
Chemical carcinogenesis	-0.46732	-3.1209	0.045249	1	82	62
Circadian rhythm	-0.52048	-2.76824	0.060729	1	31	30
Metabolism of xenobiotics by cytochrome P450	-0.46504	-2.81515	0.076605	1	73	53
Mineral absorption	-0.47019	-2.82044	0.084052	1	51	43
Porphyrin and chlorophyll metabolism	-0.50153	-2.76405	0.096234	1	42	32
Systemic lupus erythematosus	0.399369	2.333946	0.131931	1	132	80
Inositol phosphate metabolism	-0.40051	-2.49133	0.135135	1	73	70
Glycosaminoglycan degradation	-0.54362	-2.73183	0.137634	1	19	19
Base excision repair	0.471815	2.38927	0.158915	1	33	33
Cell adhesion molecules (CAMs)	0.353706	2.18174	0.174905	1	145	114
Spliceosome	0.349005	2.172974	0.176583	1	133	120
Alcoholism	0.344717	2.046578	0.185455	1	180	145
Retinol metabolism	-0.41523	-2.59558	0.207127	1	64	48
Nicotinate and nicotinamide metabolism	-0.45797	-2.4771	0.207983	1	29	28

ES, Enrichment Score; NES, Normalized Enrichment Score.

Study design and sample size



HDAC4, histone deacetylase 4; OA, osteoarthritis; SD, Sprague Dawley.

Inclusion and exclusion criteria

The animals were included in the study if they underwent successful anterior cruciate ligament transection (ACLT) operation, defined by Safranin O staining (evaluating whether the articular cartilage structure was destroyed and whether the cartilage matrix was degraded). The animals were excluded if the animal died prematurely, preventing the collection of behavioural and histological data. In this study, no animal died.

Blinding

For each animal, three different investigators were involved as follows: a first investigator (LG) administered the treatment based on the randomization table. This investigator was the only person aware of the treatment group allocation. A second investigator (HG) was responsible for the anaesthetic and surgical procedures. Finally, a third investigator (GW) (also unaware of treatment) assessed Safranin O staining, immunohistochemical staining, histological score (Mankin's score), and positive cell rate statistics.

Outcome measures

The following changes were evaluated:

1) Safranin-O staining: cartilage structure, cellularity, cartilage matrix, and Tidemark integrity.
2) Immunohistochemical staining: CCR4-NOT Complex 1 (CNOT1) positive cell rate.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods:The verification of RNA-seq in vivo: a pilot study Methods:The verification of RNA-seq in vivo: a pilot study
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Methods: The verification of RNA-seq in vivo: a pilot study Methods: Histology
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Supplementary material: Inclusion and exclusion criteria Supplementary material: Inclusion and exclusion criteria Figure legends
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods:The verification of RNA-seq in vivo: a pilot study Supplementary material: 1. Study design and 2. sample size
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Supplementary material: 4. Blinding
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Supplementary material: 5. Outcome measures Supplementary material: 5. Outcome measures
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods:Statistical analysis Methods;Statistical analysis
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Mehtods:The verification of RNA-seq in vivo: a pilot study Method;The verification of RNA-seq in vivo: a pilot study
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Methods:The verification of RNA-seq in vivo: a pilot study Method;The verification of RNA-seq in vivo: a pilot study Methods：The verification of RNA-seq in vivo: a pilot study Methods：The verification of RNA-seq in vivo: a pilot study
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Figure legends of Fig 2,3,5,6,7,a,b,c Figure legends

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	The relevant contents were recorded in the Me
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction Results: In vitro and in vivo validation
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Abstract
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Ethical review statement
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	The rats were housed underspecific pathogen-f
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Animals were anesthetized with inhaled isoflurane, the skin was prepped with a topical antiseptic no adverse events Methods:The verification of RNA-seq in vivo: a pilot study
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Results: In vitro and in vivo validation Discussion
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	The findings of this study are likely to generalize to other species.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	N/A
Data access	20	Provide a statement describing if and where study data are available.	Data sharing
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	ICMJE COI statement Funding information