

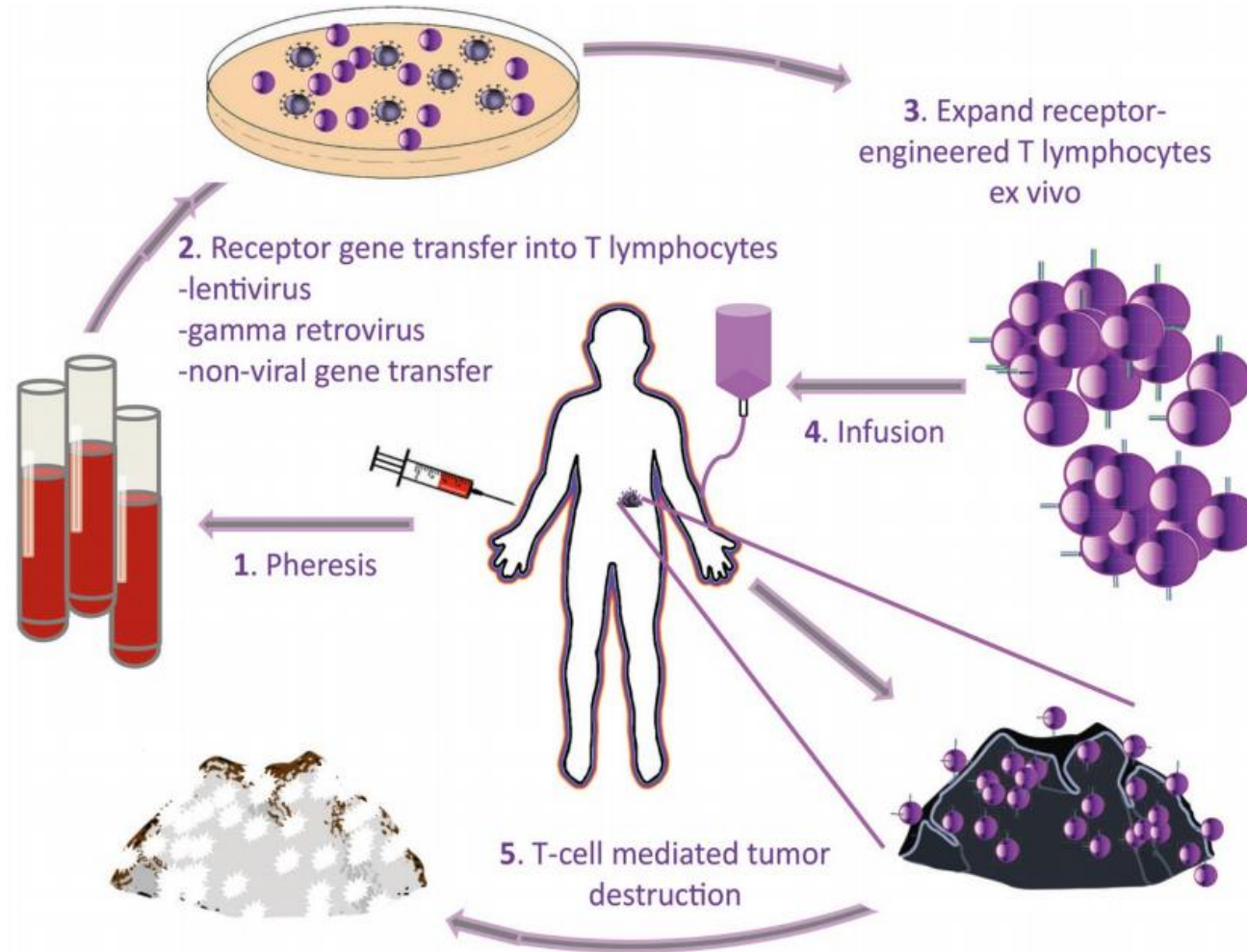
参与肿瘤细胞生长调控的C-Jun蛋白结构与功能分析
Structural and functional analysis of C-Jun
protein involved in tumor cell growth regulation

实用生物分析技术G13

陈佳晓、邓雨菁、蒋柘、张文心

Background

细胞免疫疗法的主要疗程





2012年接受治疗的Emily

- 2005年出生，2010年确诊急性淋巴细胞白血病
- 16个月化疗后，药物失效，疾病复发
- 2012年4月接受CAR-T治疗，5月出院
- 美国费城儿童医院和癌症研究中心CRI



2021年5月 最新状态

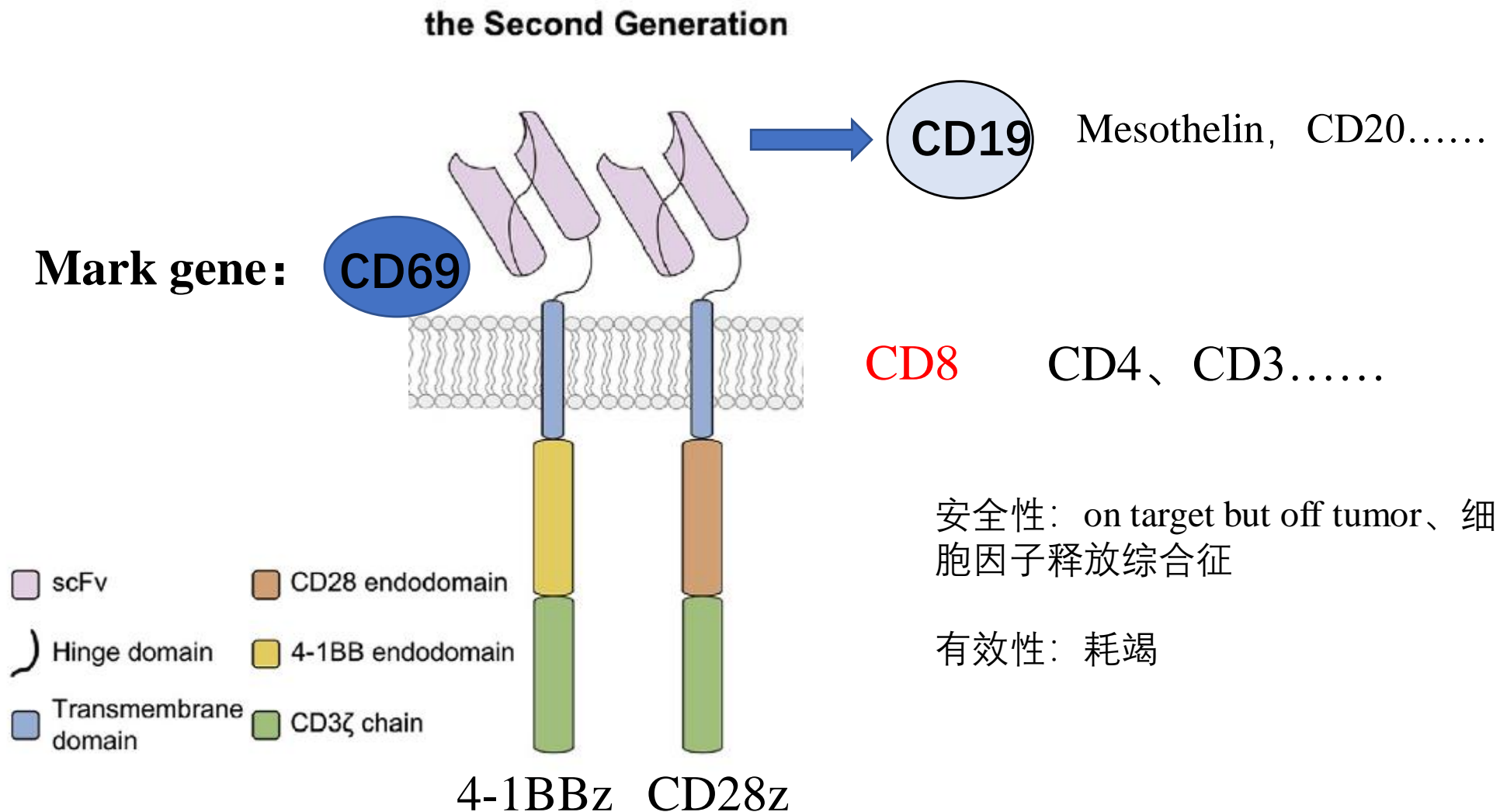


Emily每一年的“打卡照”

- **CAR-T适应症：血液肿瘤（骨髓瘤、淋巴瘤）**
- **美国FDA已批准上市：诺华Kymriah，吉利德Yescarta，百时美施贵宝Breyanzi等（约40万\$）**
- **中国批准上市，只有2款：（约200万RMB）**
2021年6月·复星凯特·奕凯达，9月·药明巨诺·倍诺达

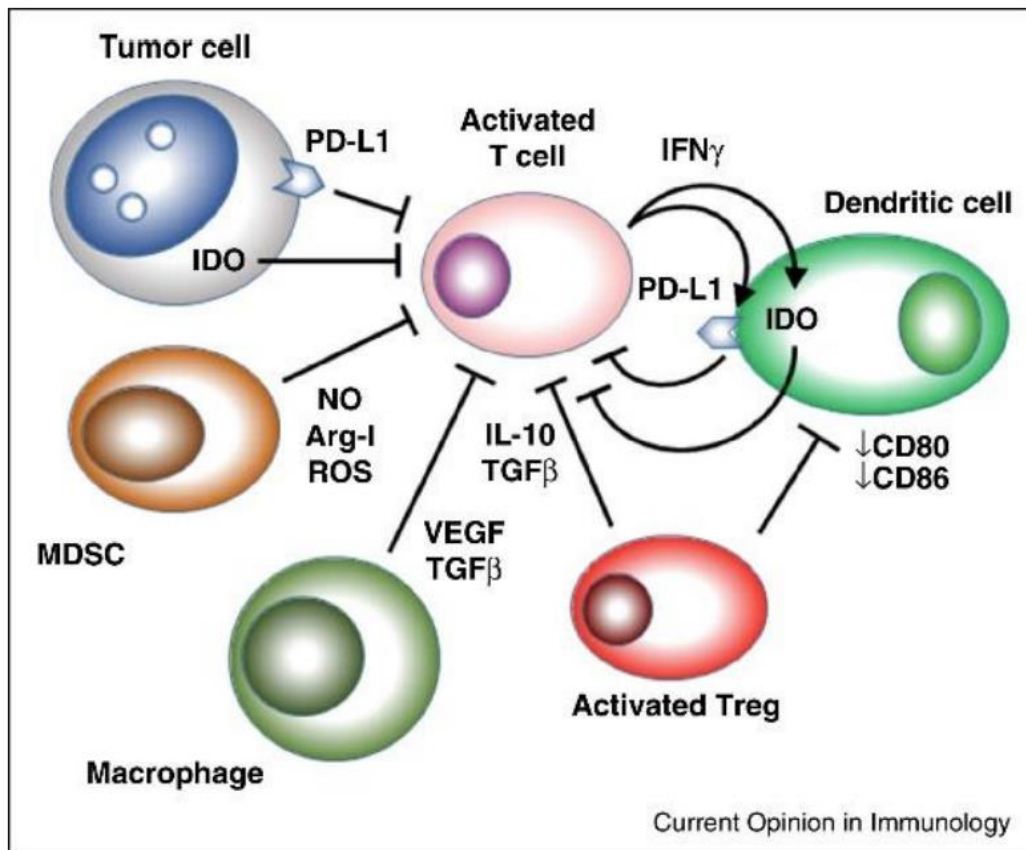
嵌合抗原受体结构

CART: Chimeric antigen receptor T cells



CART 疗法的有效性——免疫肿瘤微环境(TME)对T细胞的抑制

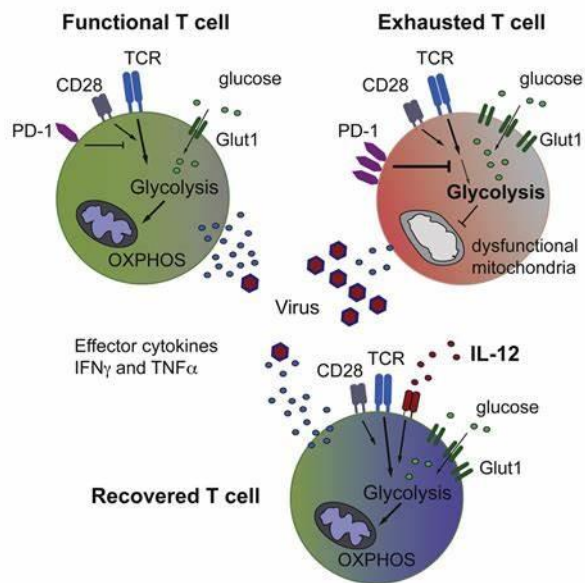
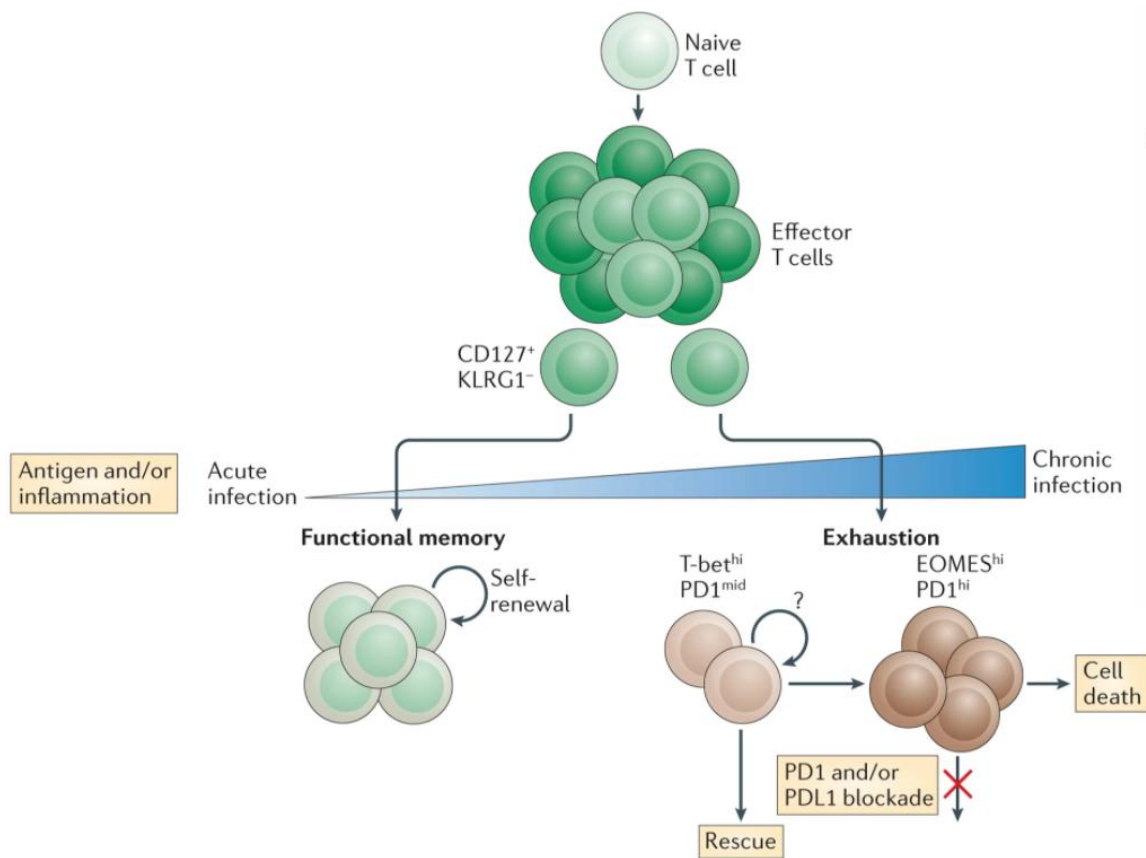
目前, CAR-T 在治疗癌症, 上已经取得了 重大的进步。但 CAR-T 仍然存在一定疗效上和安全上的问题。恶性病变和局部淋巴组织的炎症、免疫和代谢过程, 构成免疫肿瘤微环境(TME)。



肿瘤微环境 (TME) 对 T 细胞杀伤效果的抑制

Munn DH, Bronte V. Immune suppressive mechanisms in the tumor microenvironment. *Current opinion in immunology*. 2015;2016;39:1-6.

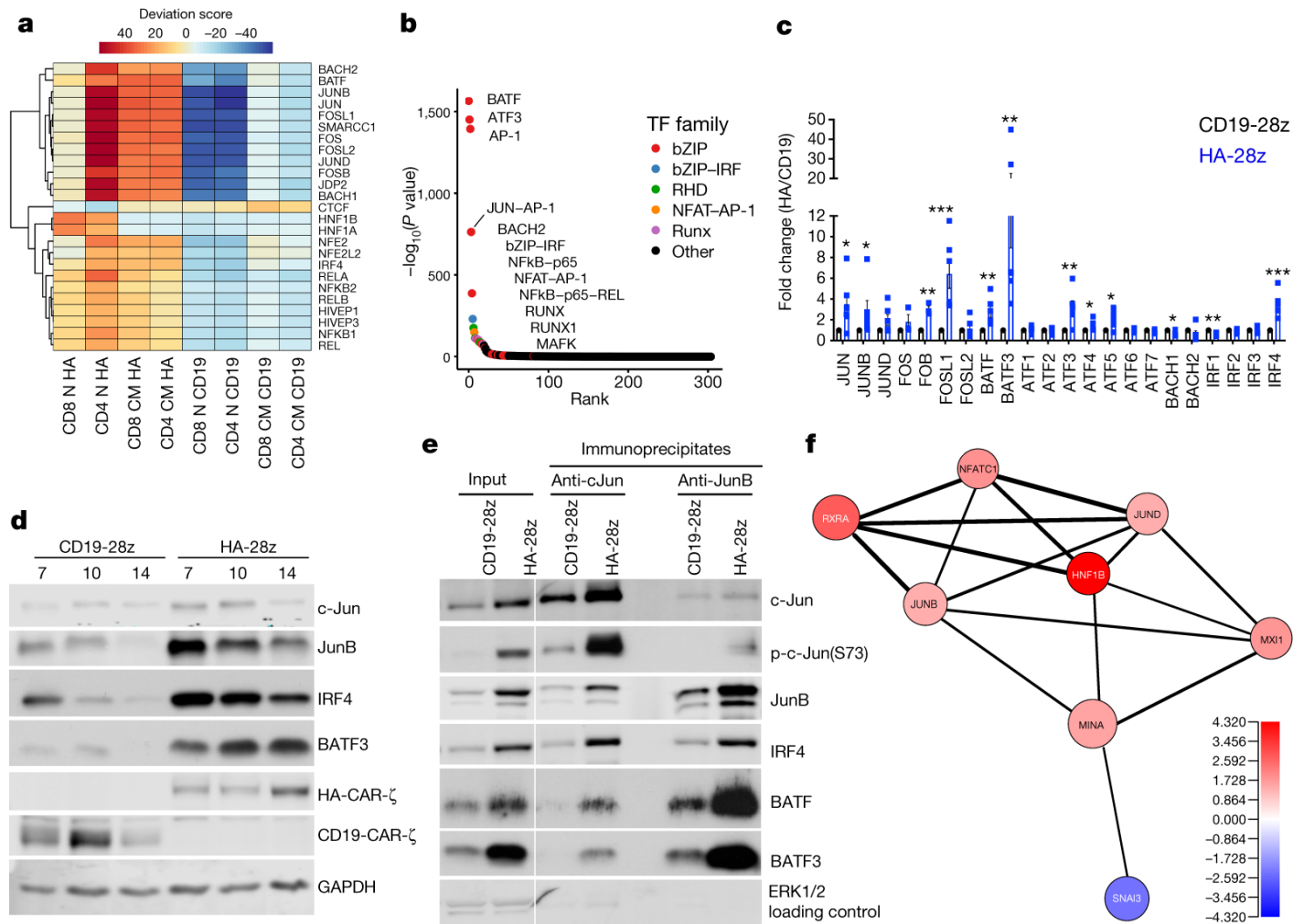
CART 疗法的安全性——T细胞功能的衰退 (exhaustion)



- progressive and hierarchical loss of effector functions
- sustained upregulation and co-expression of multiple inhibitory receptors
- altered expression and use of key transcription factors
- metabolic derangements
- a failure to transition to quiescence and acquire antigen-independent memory T cell homeostatic responsiveness

Feature	Functional memory T cell	Exhausted T cell
Proliferative potential	+++	+/-
Cytokine production	+++	+/-
Memory markers (e.g. CD44, CD62L, CD127 or CXCR3)	+++	+/-
Inhibitory receptors (e.g. PD1, LAG3, CD160 or 2B4)	-	+++
IL-7- and/or IL-15-driven self-renewal	++	-
Antigen dependency	-	+

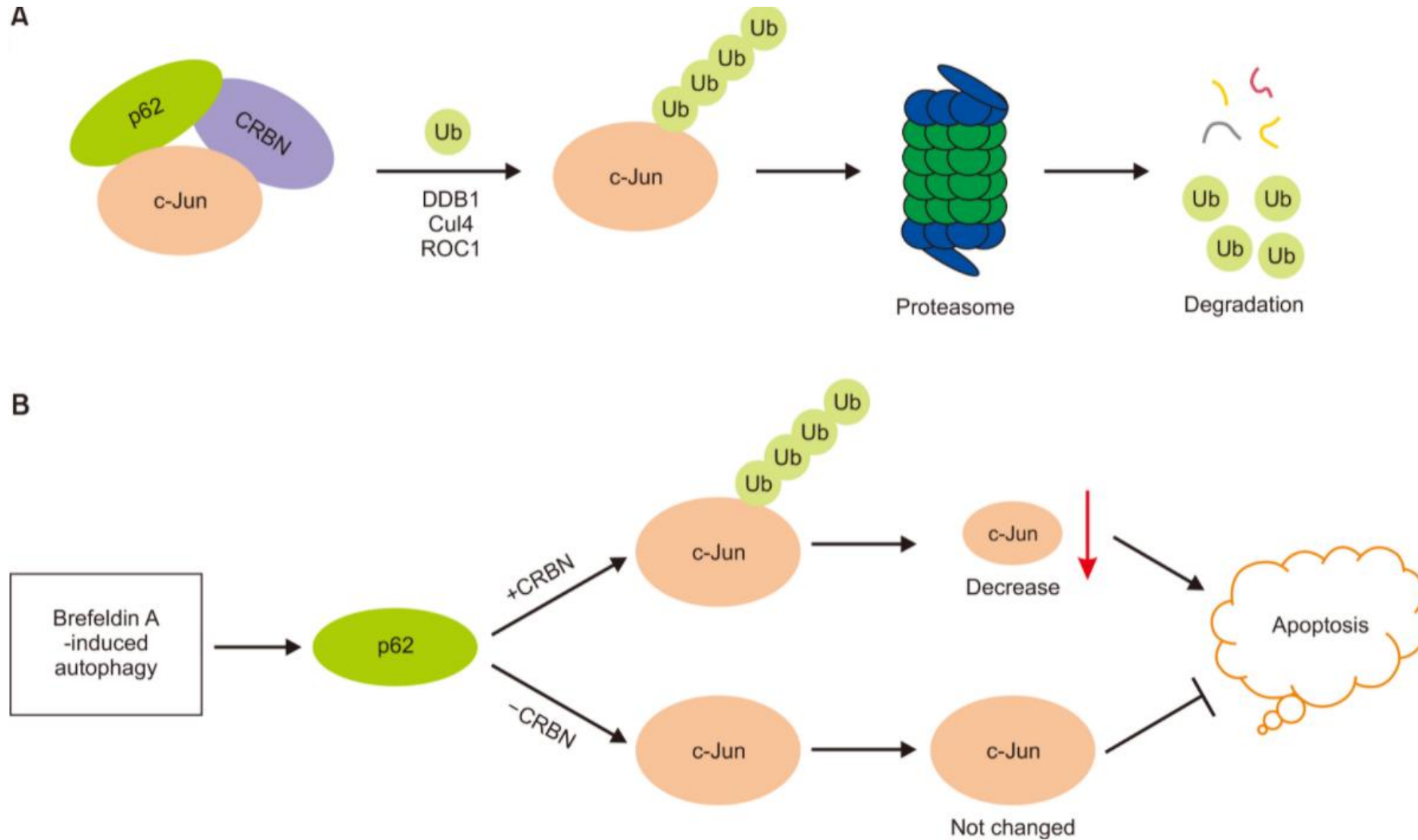
CART 细胞中过表达 C-Jun 可以有效提升其抗耗竭能力



陈佳晓

Lynn RC, Weber EW, Sotillo E, Gennert D, Xu P, Good Z, et al. c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature (London)*. 2019;576(7786):293-300.

内质网应力介导的p62下调通过上调c-Jun抑制细胞凋亡



Workflow

- 介绍了细胞免疫疗法的主要疗程，以及首个使用CART疗法的临床病例。
- 介绍了CAR嵌合抗原受体的结构，以及但 CAR-T 仍然存在一定疗效上和安全上的问题。
- 熟悉C-Jun蛋白的性质，掌握结构、通路、进化关系、同源性等内容。
- 找出同样影响CART细胞激活的基因。

C-Jun的结构与功能介绍——蛋白翻译后修饰（化学修饰）

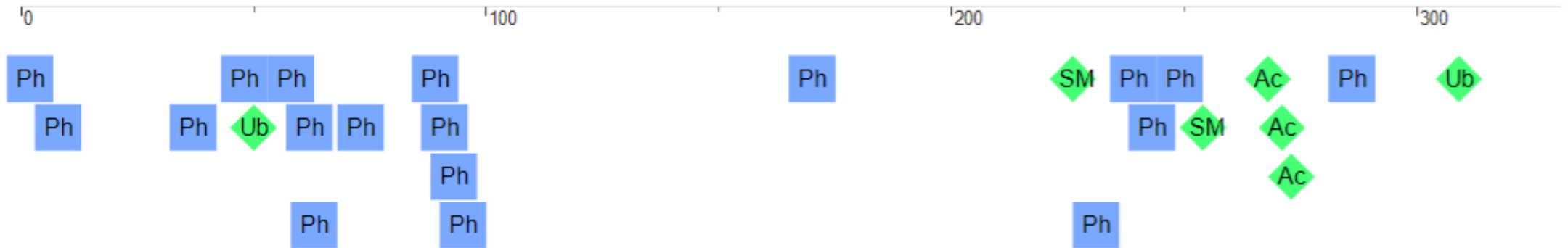
There are 33 PTMs annotated in this protein:

	PTM	Count
磷酸化	Ph Phosphorylation	20
泛素化	Ub Ubiquitination	6
乙酰化	Ac Acetylation	4
类泛素化	Sm SUMOylation	3

- Phosphorylation

- by CaMK4/PRKDC/HIPK3...
- enhances the transcriptional activity
- reduces or increases its ability to bind DNA

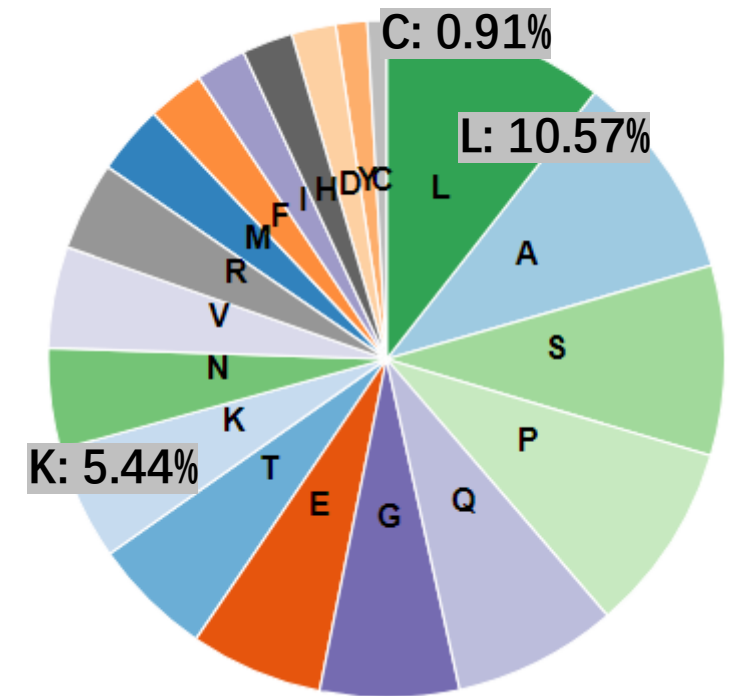
全长331 aa



C-Jun的结构与功能介绍——重要位点 (突变敏感性)

Position	Amino acid	Function
2	T → A	Complete loss of PAK2-mediated phosphorylation
8	T → A	Complete loss of PAK2-mediated phosphorylation
63	S → A	Greatly reduced ATF7-mediated transcriptional activity
73	S → A	Greatly reduced ATF7-mediated transcriptional activity
89	T → A	Complete loss of PAK2-mediated phosphorylation
91	T → A	Abolishes interaction with FBXW7
93	T → A	Complete loss of PAK2-mediated phosphorylation
243	S → A	Abolishes phosphorylation by DYRK2
272	R → V	Abolishes the synergistic activity with SMAD3 to activate TGF-β-mediated transcription
286	T → A	Complete loss of PAK2-mediated phosphorylation

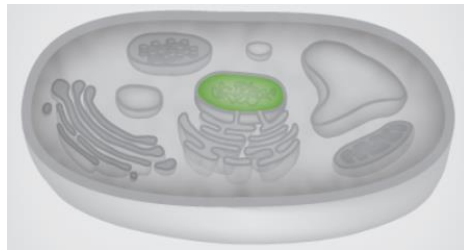
Amino Acid composition



C-Jun的结构与功能介绍——主要结构域



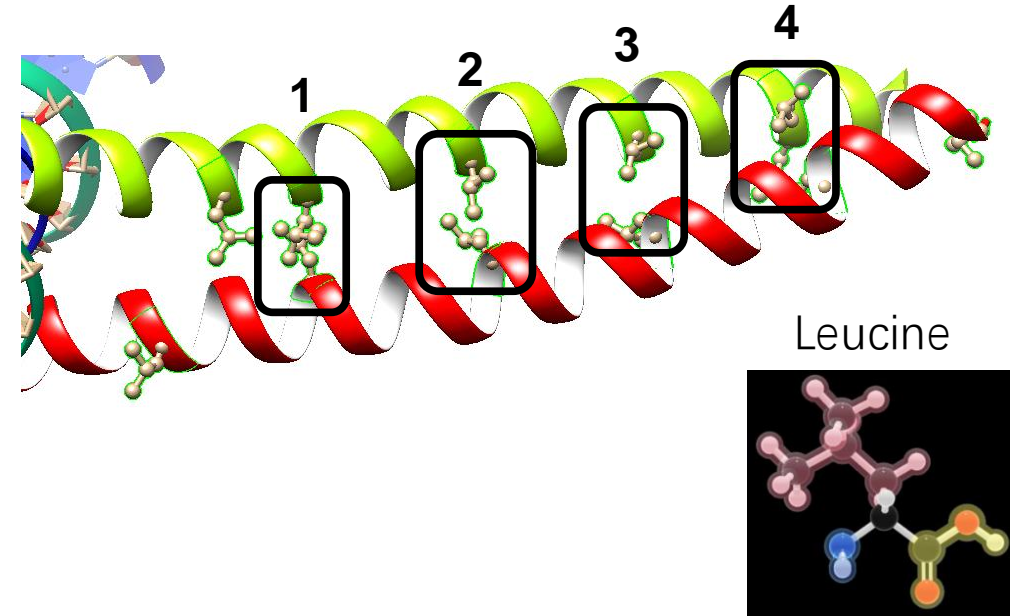
Feature key	Position	Function	Length	
domain	around 252-315	bZIP (basic leucine zipper)	about 64 aa	
region	139-162	low complexity region	24 aa	<u>GAGMVAPAVASVAGGSGSGGFSAS</u>
region	178-196	low complexity region	19 aa	<u>PGALSSGGGAPSYGAAGLA</u>
region	198-220	low complexity region	23 aa	<u>PAQPQQQQPPHLLPQQMPVQHP</u>



Subcellular Localization: Nucleus

C-Jun的结构与功能介绍——重要结构域BRLZ

- basic region leucin zipper, or **bZIP** (basic-leucine zipper domain) 亮氨酸拉链
- α -helix has 3.6 amino acid residues per turn
- one Leucine in every 7 amino acid residues
- ~ 65 aa

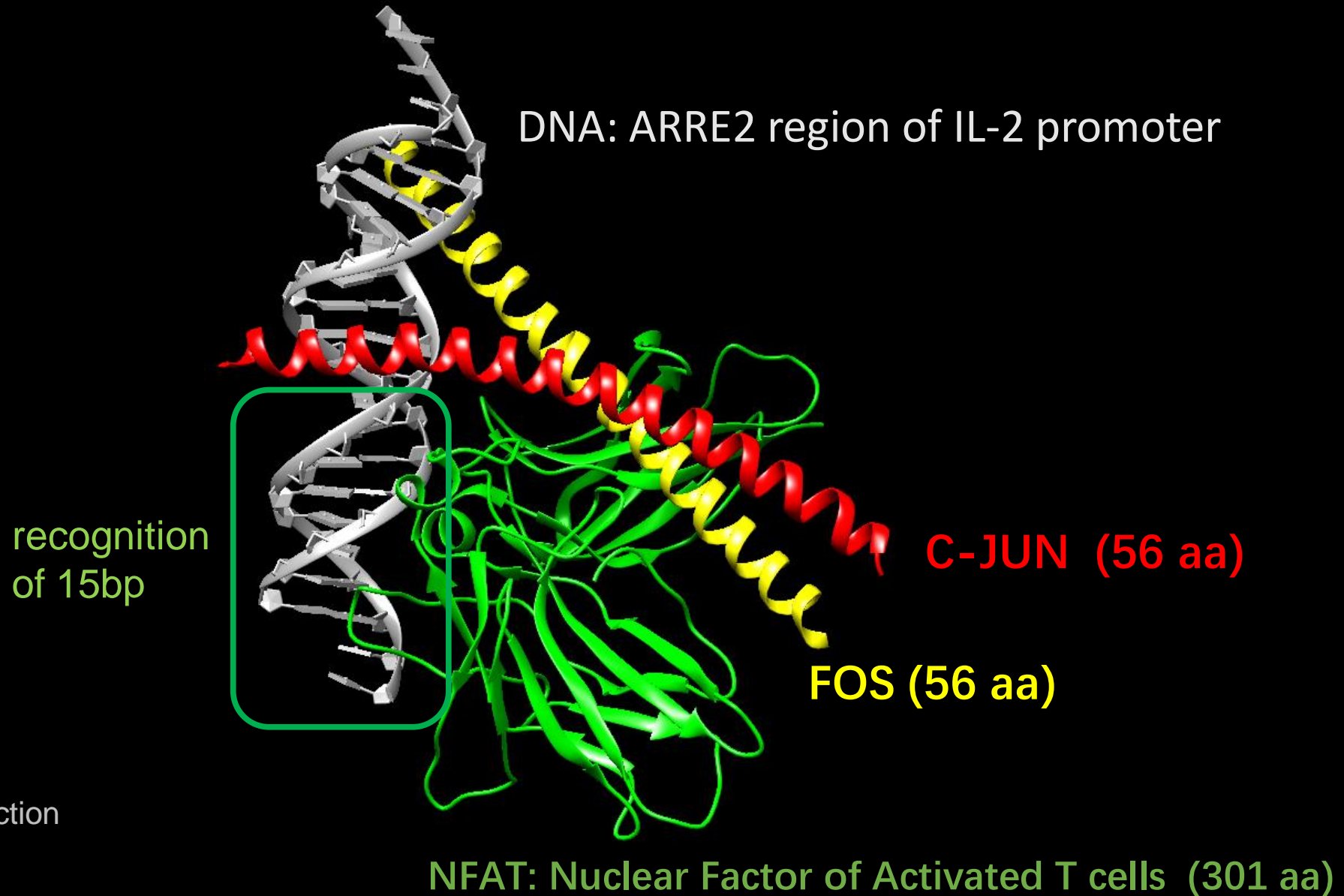


	1	2	3	4	
33 Q39333/18-9	QAE CE -QL QQ	R V ES L TSEN Q	S R DE L QR	-L SG CE KLK	TQ NS S
08 Q43508/18-9	QAE CE -E LQR	R V EAL S HEH	S K DE L QR	-L SE CE KLK	SEN L
EMPI_WHEAT/18-	Q Q CE -E LAQ	K V SE L TAA G	T R SE L DQ	-L KK CK TME	TEN KK
API_DROME/18-9	LER IS -K IED	R V KV L KGE NV	D L AS I VKN	-L KD H V A HVK	Q Q V ME
87 Q65887/18-9	Q A E T E-E LSV	K V D A VA E EM	T L RS KL G Q	-L ND E SE KLR	LE NE A
ATGBF3_1/18-9	Q A E T E-E LAR	K V EAL T AEM	A L RS E L N Q	-L NE K S DK L	GA NA T
CPR1_PETCR/18-	Q A E A E-E LAI	K V D S L T AEM	A L KA E INR	-L TL T A E KL T	ND NS R
86 Q40586/18-9	Q A E A E-E LAI	R V Q S L T GEN	T L K S E L NK	-L M EN S E K L	LE NA A
57 Q41557/18-9	Q Q E CE -E LSR	K V A E L T TEN	A L R T E L DQ	-L KK A C E D M	A Q N A R
HBPA_WHEAT/18-	Q A E CE -E LQ	R A E A L K SEN S	S L R I E L DR	-L IK K E Y E E L	S K N T S
09 Q41109/18-9	Q A E T E-E LAR	K V E M L T AEM	S L K S E I TQ	-L T E G S E Q M	ME NS A
25 Q40625/18-9	Q A E CE -E LAQ	R A E V L K Q E NT	S L R D E V NR	-L IR K E Y D E L	S K N S S
49 Q43449/18-9	Q A E CE -E LQK	R V ES L G S EN Q	T R E E L Q R	-V S E E C K K L	SE ND S
88 Q82288/18-9	Q A E CD -E LAQ	R A E V L N E E NT	H L R A E L NK	-L KS Q C E E L	TE NT S
35 Q41735/18-9	Q A E T E-E LAT	Q V ES L AA E NT	S L R S E I TGR	-L T E S S E K L	LE NS A
36 Q49936/18-9	Q A E T E-E LAR	R V ES L S A EM	A L K S E V NL	-L V EN S Q K L	LE NA A
CPR3_PETCR/18-	Q A K S D-E LQE	R L D M L S K E NR	I L R K N L QR	-L I S E A C A E V	SE N H S
JUNE_HUMAN/18-	LER I A-R IED	K V K T L K A E NA	G L S S T A GL	-L R E Q V A Q L K	Q K V M T
33 Q04233/18-9	Q A E CD -E LAQ	R A D V L S E E NA	S L R A E L SR	-L IK S E H A K A L	A E N A A
REACSJUN_1/18	LER I A-R IEE	K V K T L K A Q NS	E L A S T A N M	-L R E Q V A Q L K	Q K V M N

- 在真核生物中保守
- 牛、褐家鼠、原鸡、果蝇、玉米、烟草...

SMART alignment for the BRLZ domain (consensus: 60%)

C-Jun的结构与功能介绍——复合体结构

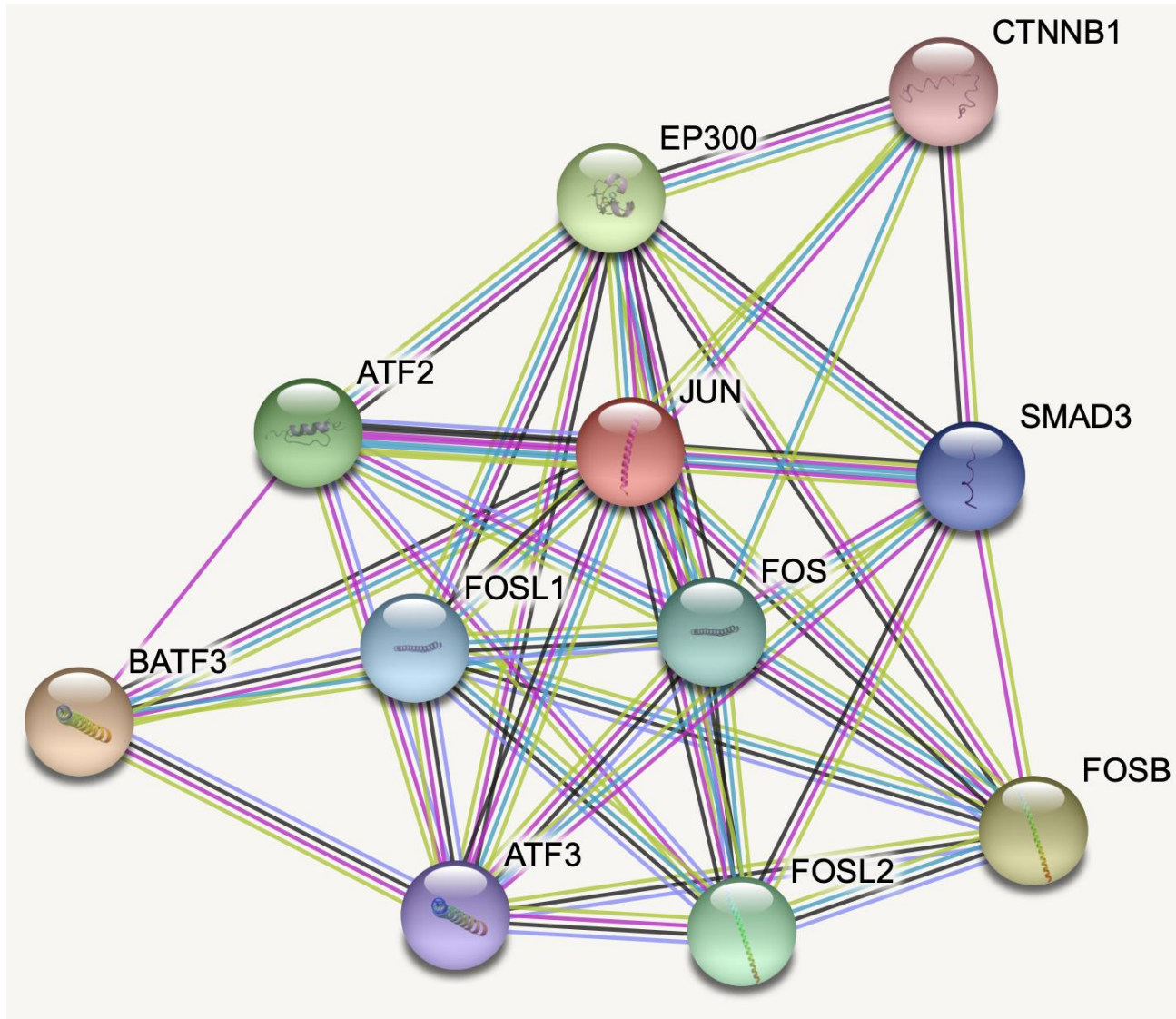


•PDB entry: 1A02

•Method: X-Ray Diffraction

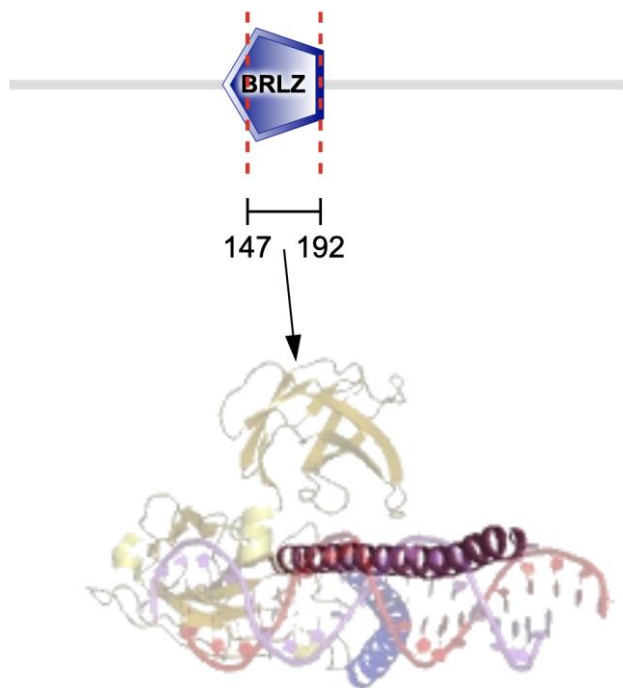
•Resolution: 2.70 Å

蛋白质互作网络



- 在JUN的蛋白互作网络中，其他蛋白通过与JUN结合而获得与DNA结合的能力，进而起到调控下游通路的作用

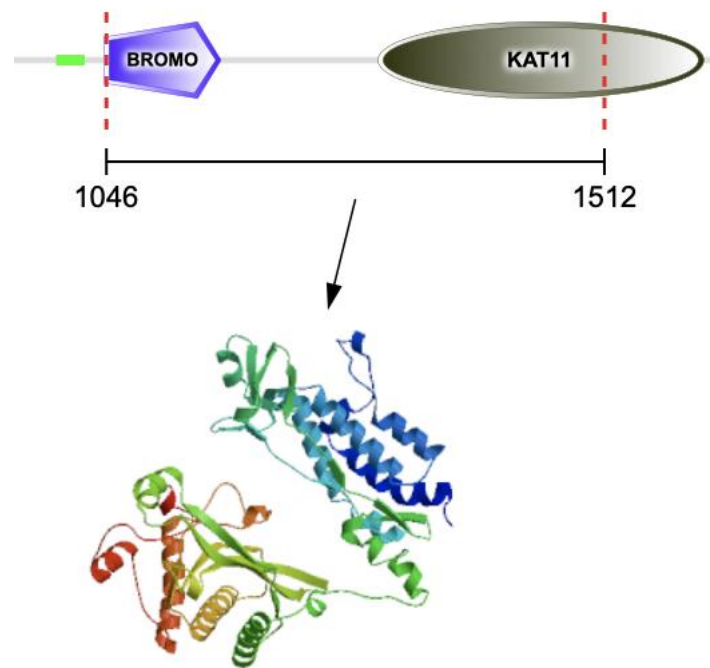
FOS



- FOS为细胞核内的一个磷酸化蛋白
- 与JUN通过非共价作用紧密连接
- 在TGF-beta激活过程中，通过在AP1/SMAD结合位点形成SMAD3/SMAD4/JUN/FOS四元复合物，来调节TGF-beta介导的信号通路

蛋白质互作网络

EP300



- EP300为一组蛋白乙酰转移酶，通过增加组蛋白上的乙酰化程度来激活转录过程

通过blast根据同源性筛选具有相似结构和功能的蛋白

使用linux系统操作PSI-BLAST进行序列比对:

下载PSI-BLAST后, 将软件添加到路径:

```
export PATH=/home/test_zd/software/ncbi-blast-2.12.0+/bin/:$PATH
```

对下载的数据库建库: `makeblastdb -in swissprot.fasta -dbtype prot -parse_seqids -out swissprot -logfile logfile.txt`

运行命令进行序列比对:

```
blastp -query C-Jun.fasta -out orgin_out/result.blast -db /sprot-homo -outfmt 6 -evaluate 1e-5 -num_threads 8
```

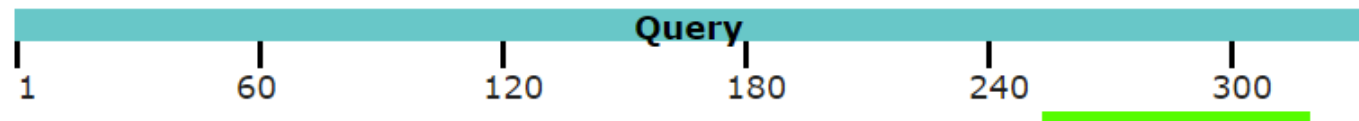
P05412	100.000	331	0	0	1	331	1	331	0.0	675	
P17535	58.029	274	100	4	60	331	87	347	3.02e-81	250	
P17275	44.595	370	143	12	1	331	1	347	8.24e-70	221	
P17544	28.455	246	138	9	90	315	168	395	1.44e-10	62.0	
P15336	38.028	71	41	1	256	323	353	423	1.76e-07	52.4	
Q02930	39.683	63	35	1	256	315	376	438	1.04e-06	50.1	
Q9BYV9	40.984	61	36	0	248	308	642	702	1.42e-06	50.1	
Q16621	30.986	71	42	1	253	316	267	337	4.15e-06	48.1	

- Transcription factor NF-E2 45 kDa subunit
- Transcription facjun-D
- Cyclic AMP-dependent transcription factor ATF-7
- Cyclic AMP-dependent transcription factor ATF-2
- Cyclic AMP-responsive element-binding protein 5
- Transcription regulator protein BACH2
- Transcription factor NF-tor E2 45 kDa subunit

Two sequence alignment

Alignment Scores < 40 40 - 50 50 - 80 80 - 200 >= 200

Distribution of the top 1 Blast Hits on 1 subject sequences



Range 1: 267 to 339 [Graphics](#)

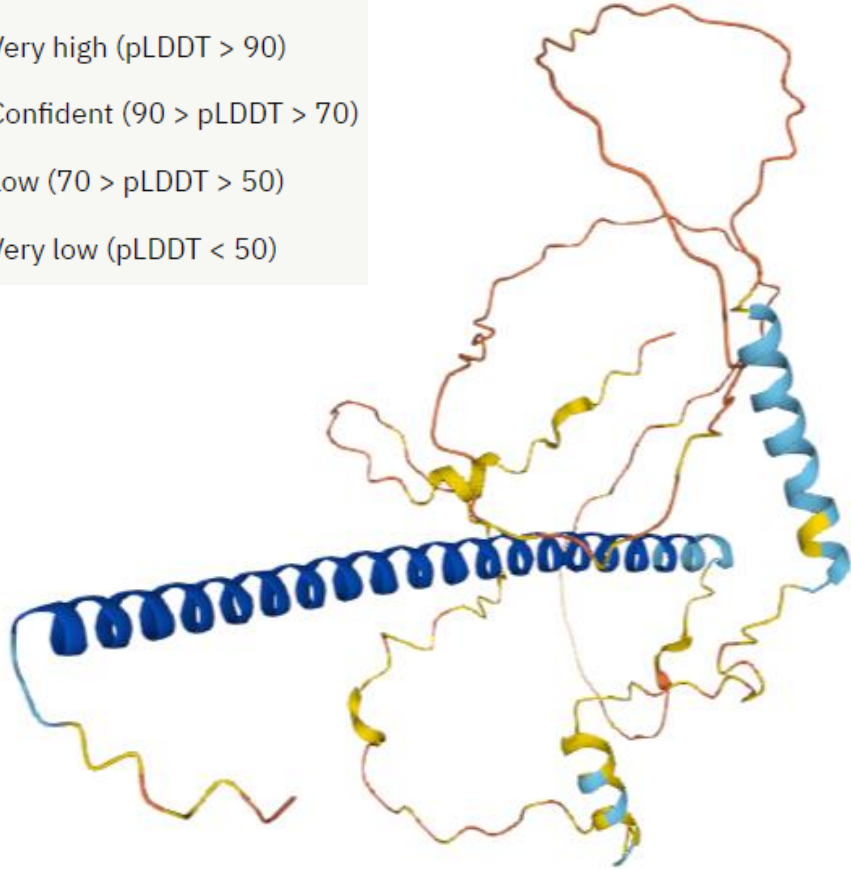
▼ [Next Match](#) ▲ [P](#)

Score	Expect	Method	Identities	Positives	Gaps
50.8 bits(120)	1e-11	Compositional matrix adjust.	23/73(32%)	42/73(57%)	7/73(9%)
Query 253	IKAERKRMNRNRIAASKCRKRKLERIARLEEKVKTLKAQNSEL-----ASTANMLREQV				305
	++ R+R +N++AA CRKRKLE I +LE +++ L ++ L T ++R+Q+				
Sbjct 267	VRDIRRRGKNKVAQAQNCRCRKRKLETIVQLERELERLSSSERERLLRARGEADRTLEVMRQQL				326
Query 306	AQLKQKVMNHVNS	318			
	A+L + H+				
Sbjct 327	AELYHDIFQHLRD	339			

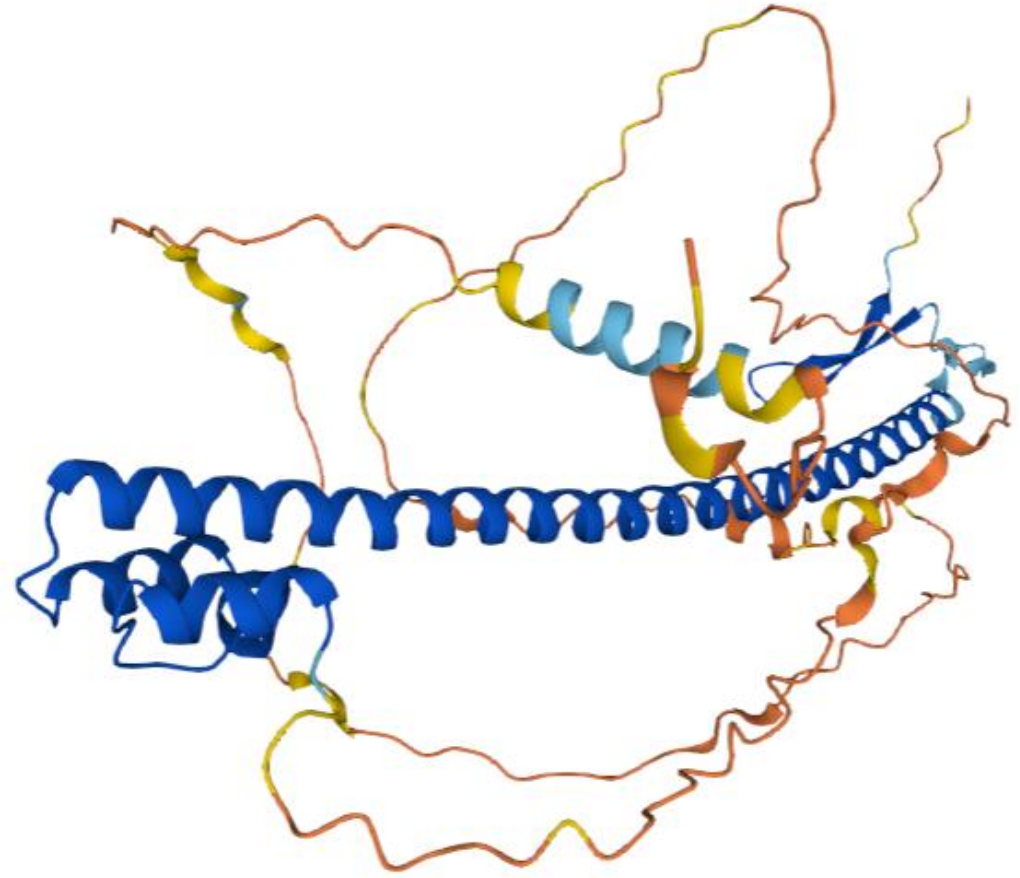
AlphaFold2 prediction

Model Confidence:

- Very high (pLDDT > 90)
- Confident (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)



Individual C-Jun



Basic leucine zipper (bZIP) transcription factor atfB

AlphaFold2网络架构概览

MSA (多序列比对) 信息的预处理:

节省运算内存并同时保持序列的多样性，MSA序列会被随机删除的操作。

AlphaFold2利用mask策略，每个位置有15%的概率被mask,然后按照一个具体的规则替换，最后看这个被mask的residue的恢复度。

