



Structure and function analysis of MG53



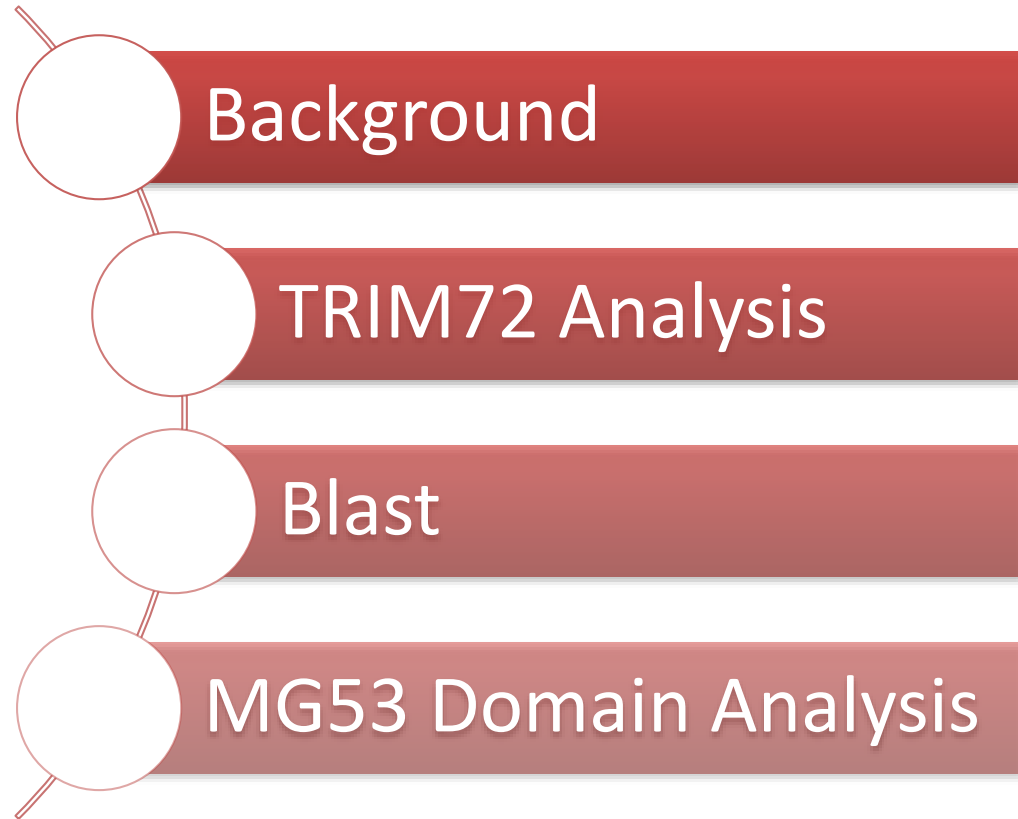
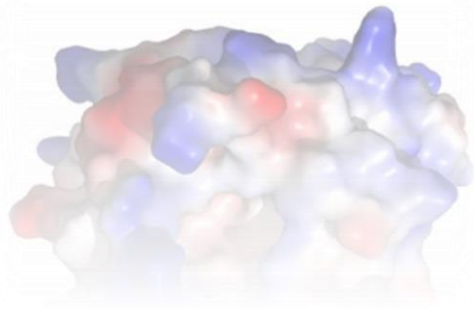
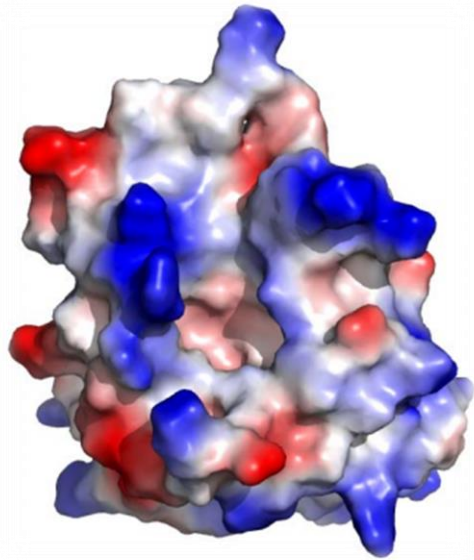
Luotong Wang

Congmin Xu

Xuan Zheng

Xiaojian Song





MG53蛋白的基本信息

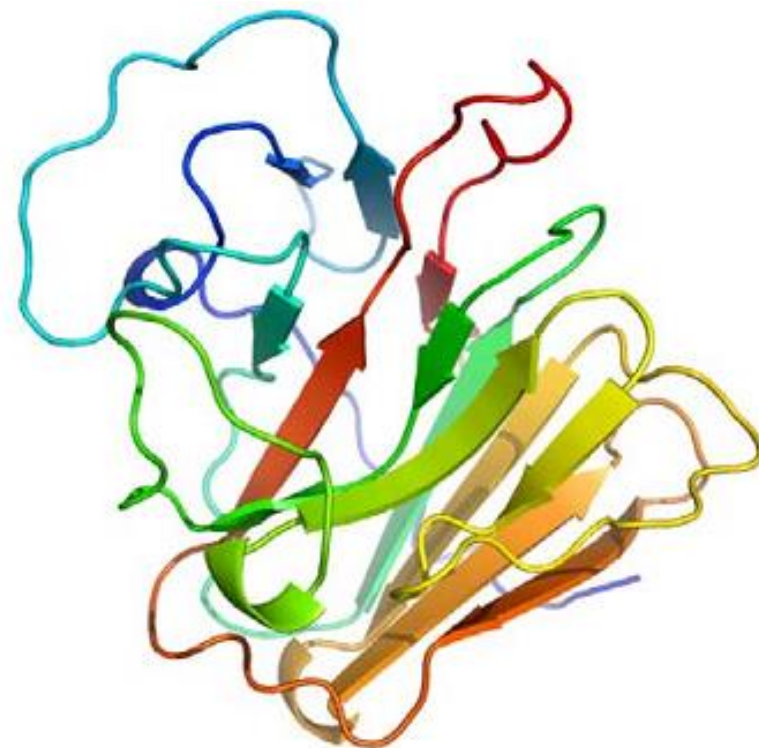
蛋白名: Tripartite motif-containing protein 72

基因名: TRIM72

审阅状态: Reviewed

Annotation score: 五星级

全长: 477 aas



MG53蛋白的基本信息

□功能:

- 肌肉特异性蛋白可通过核化组装受损位点的修复机制对机体进行膜修复
- 特异性结合磷脂丝氨酸
- 氧化传感器
- 在钙离子封装上游调控过程中起作用
- DYSF转运到细胞损伤位点过程必需
- 调控出芽和胞吐
- 可能参与KCNB1的胞吞过程

□亚细胞定位:

- 细胞膜 > 肌纤维膜
- 胞质膜小泡

□组织特异性:

- 骨骼肌
- 心肌细胞



Song RS, et al. Nature, 2013, 494: 375-381

MG53蛋白的基本信息

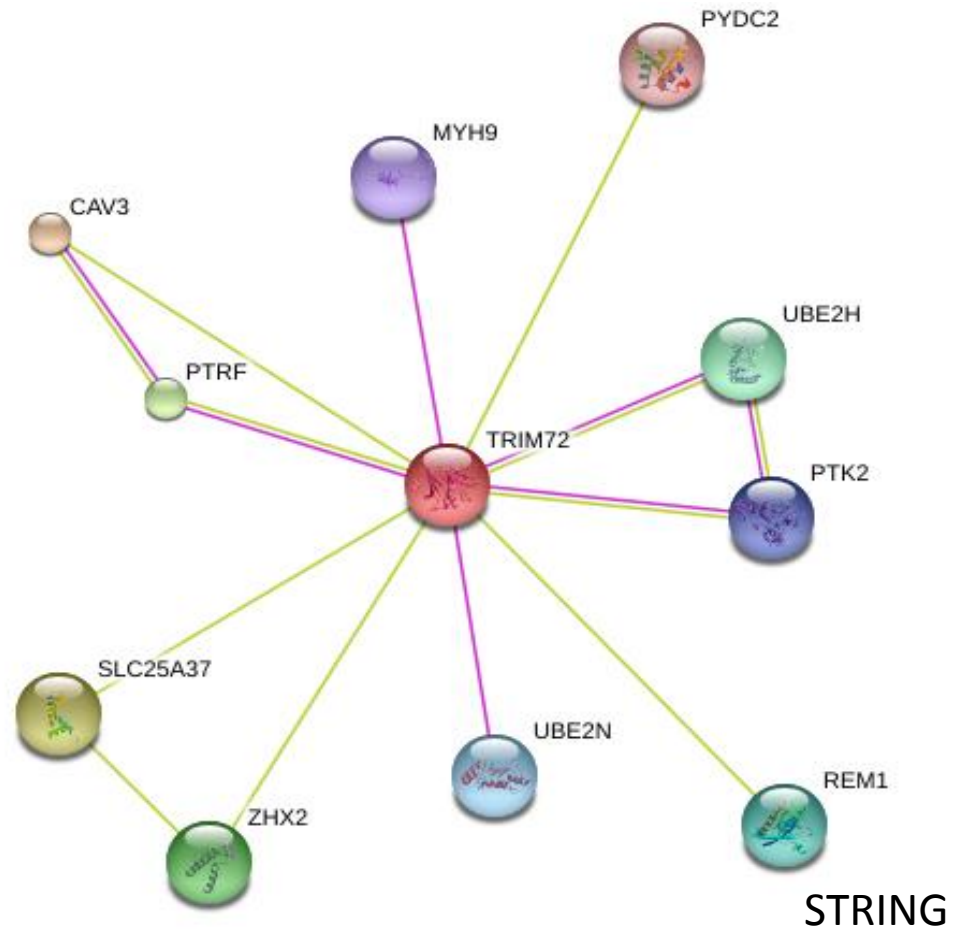
□ 互作蛋白:

- 同源寡聚
- 二硫键结合
- DYSF
- CAV3

□ 剪接变体:

- Q6ZMU5-1
- Q6ZMU5-2

与功能伙伴间的作用关系:



MG53蛋白的基本信息

序列结构：一个B box 型锌指结构域；一个B3.0/SPRY结构域；一个RING型锌指结构域。

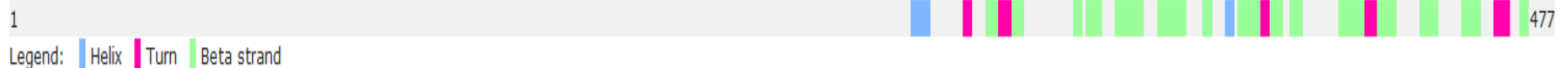


14-57为RING型锌指结构域； 81-122为B box型锌指结构域；

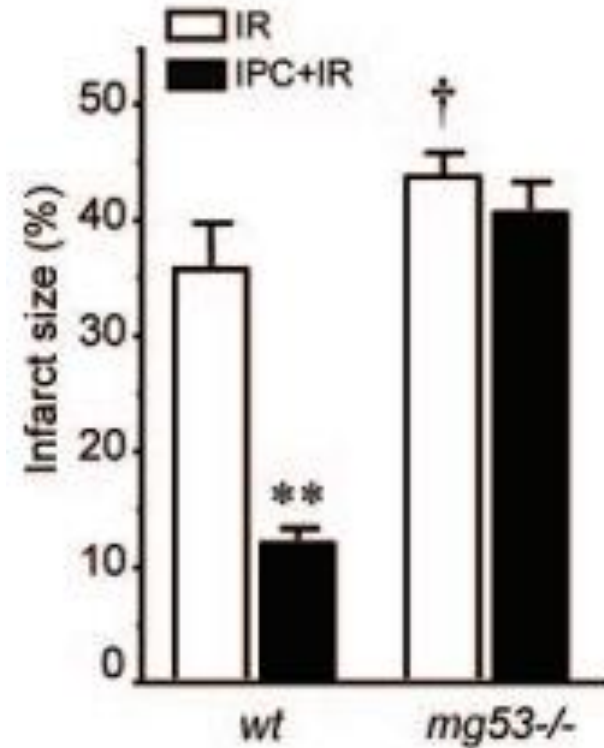
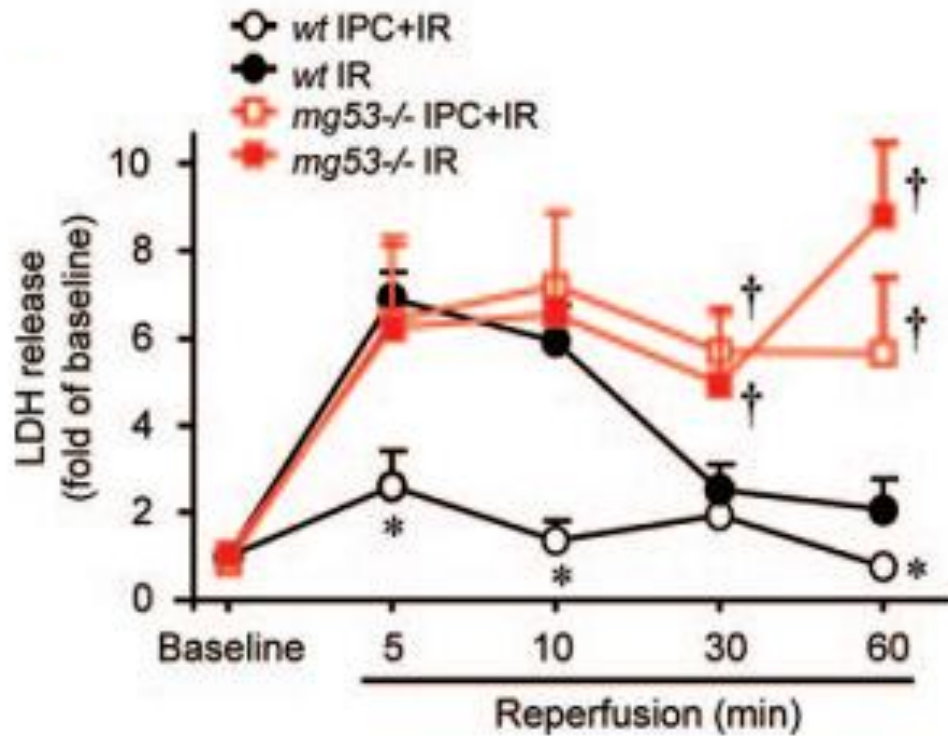
135-169为Coiled coil结构域； 271-475为B30.2/SPRY结构域。

二级结构：

Secondary structure

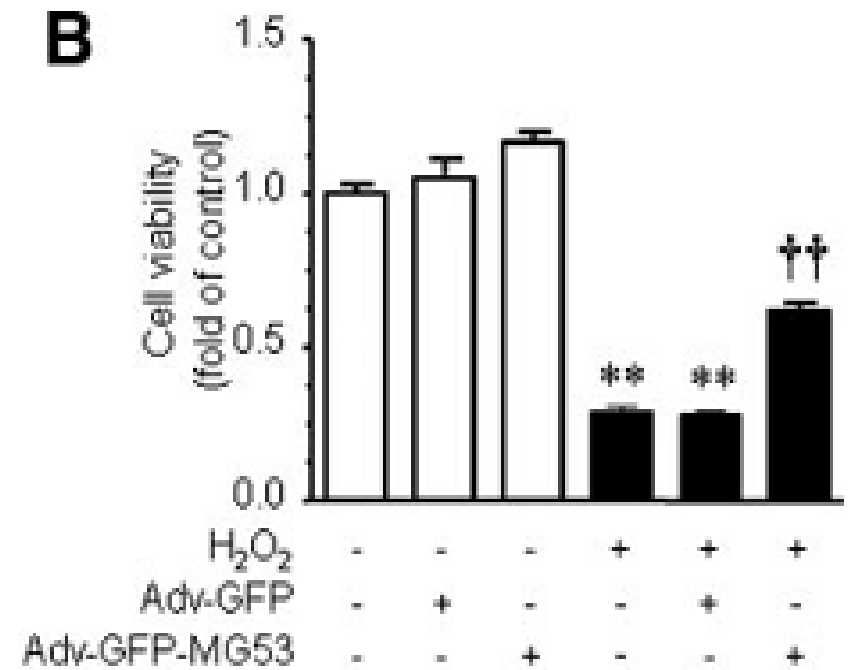
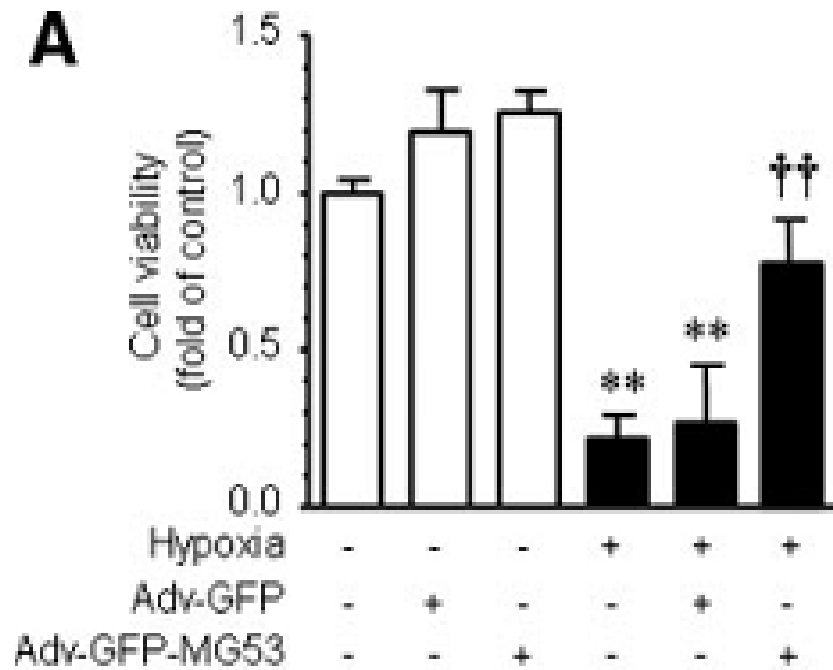


MG53 knockout hearts are vulnerable to IR injury and resistant to IPC protection

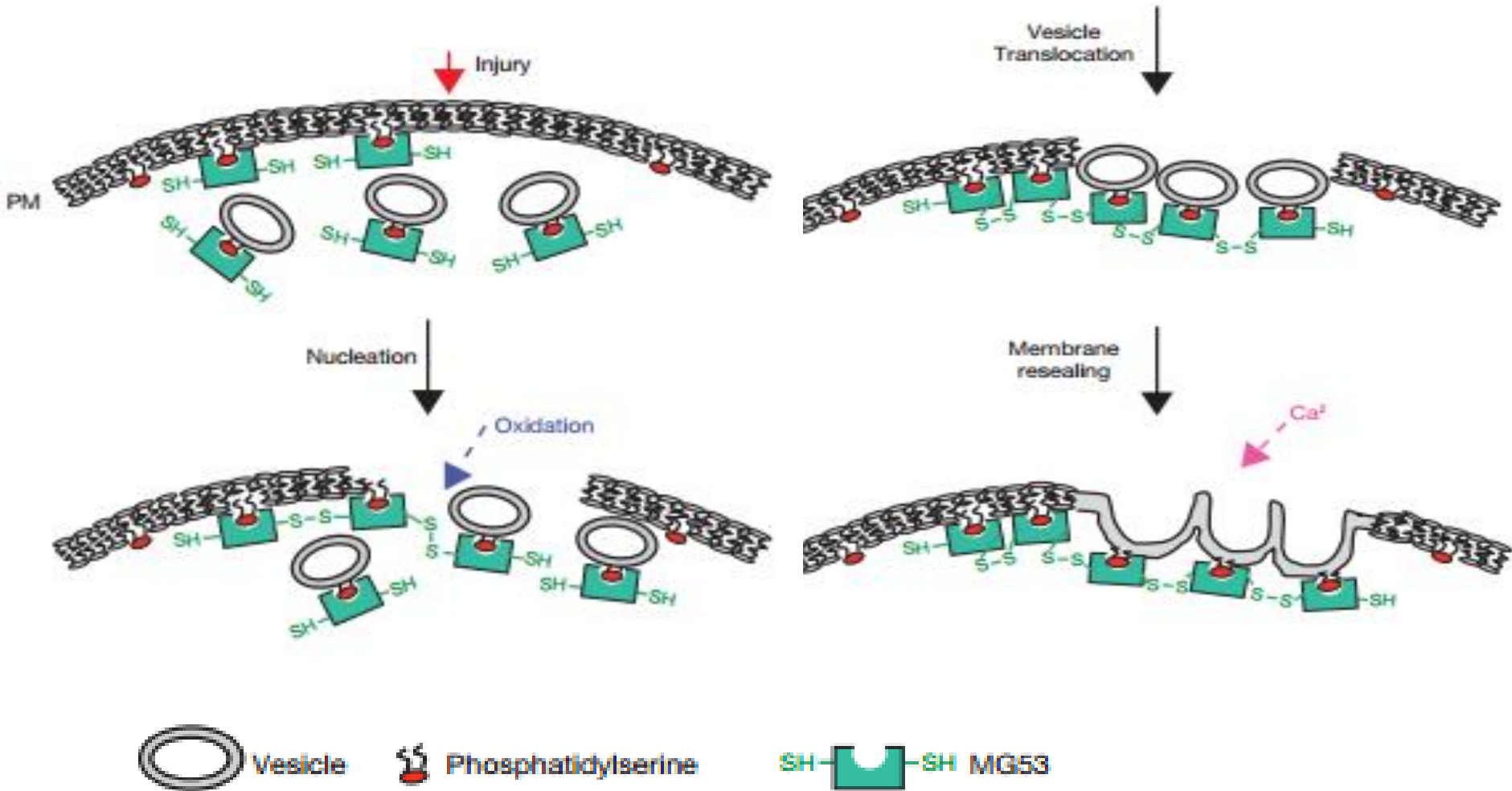


Cao CM, et al. Circulation, 2010, 121: 2565-2574

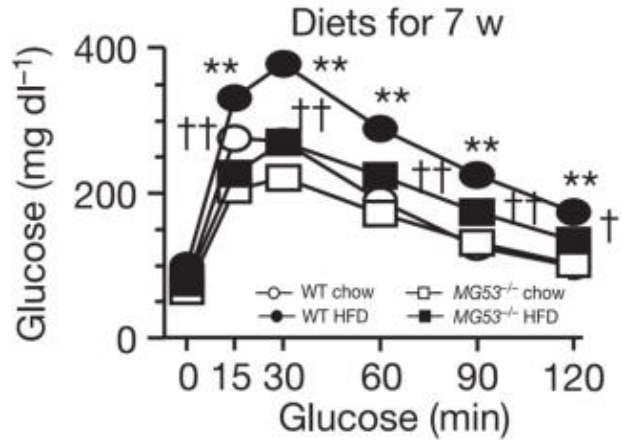
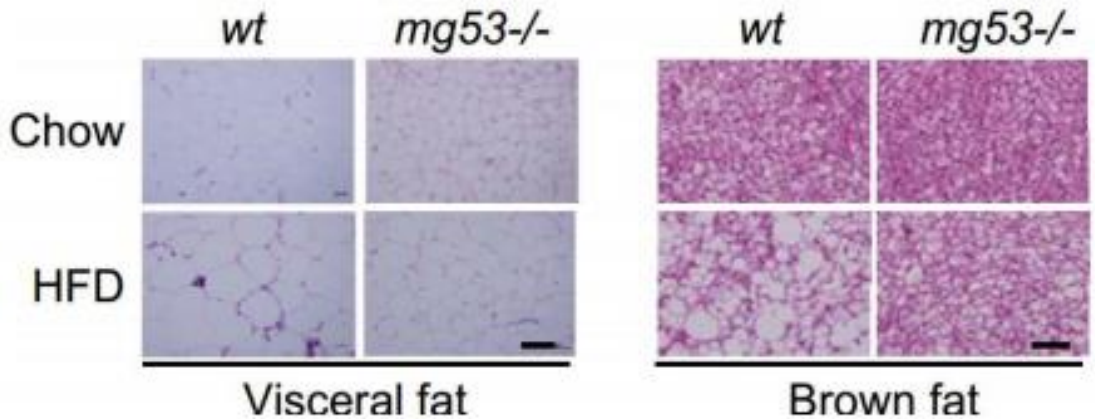
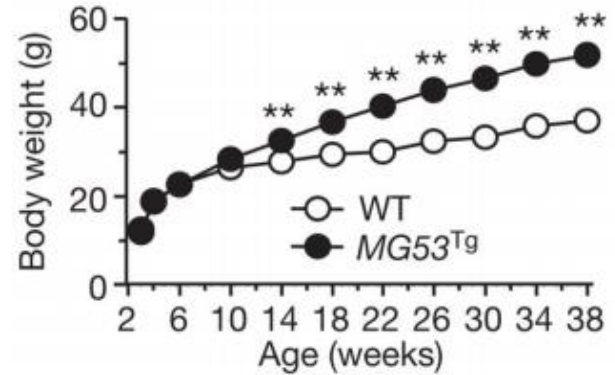
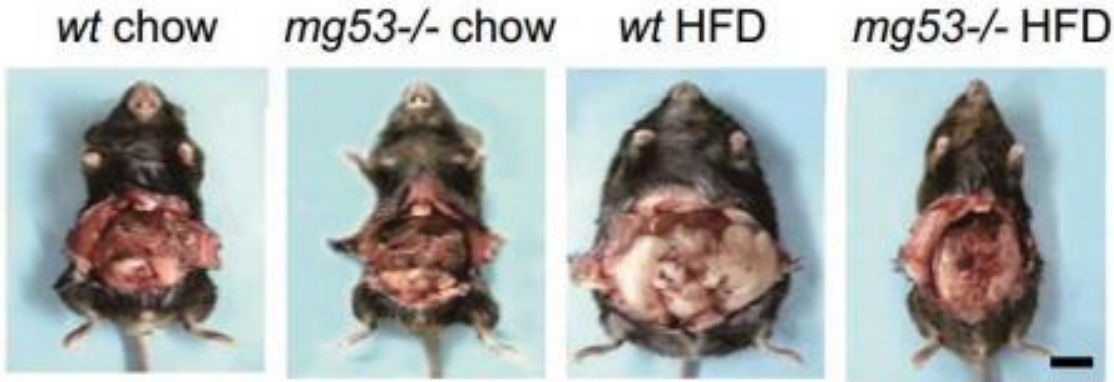
Overexpression of MG53 protects cardiomyocytes against hypoxia and oxidative stress



Cell membrane repair



E3 ubiquitin ligase



Song RS, et al. *Nature*, 2013, 494: 375-381

序列比对

□ 人、小鼠、大鼠MG53蛋白质序列比对结果

物种 [↵] Species [↵]	登录号 [↵] Accession [↵]	得分 [↵] Score [↵]	相同氨基酸 [↵] Identity [↵]	相同和相似氨基酸 [↵] Similarity [↵]	空位 [↵] Gaps [↵]
人/小鼠 [↵]	Q6ZMU5- TRI72/	2293.0 [↵]	435/477	456/477	0/477
	Q1XH17- TRI72 [↵]		(91.2%) [↵]	(95.6%) [↵]	(0.0%) [↵]
人/大鼠 [↵]	Q6ZMU5- TRI72/	2274.0 [↵]	433/477	451/477	0/477
	A0JPQ4- TRI72 [↵]		(90.8%) [↵]	(94.5%) [↵]	(0.0%) [↵]
小鼠/大鼠 [↵]	Q1XH17- TRI72/	2380.0 [↵]	455/477	465/477	0/477
	A0JPQ4- TRI72 [↵]		(95.4%) [↵]	(97.5%) [↵]	(0.0%) [↵]

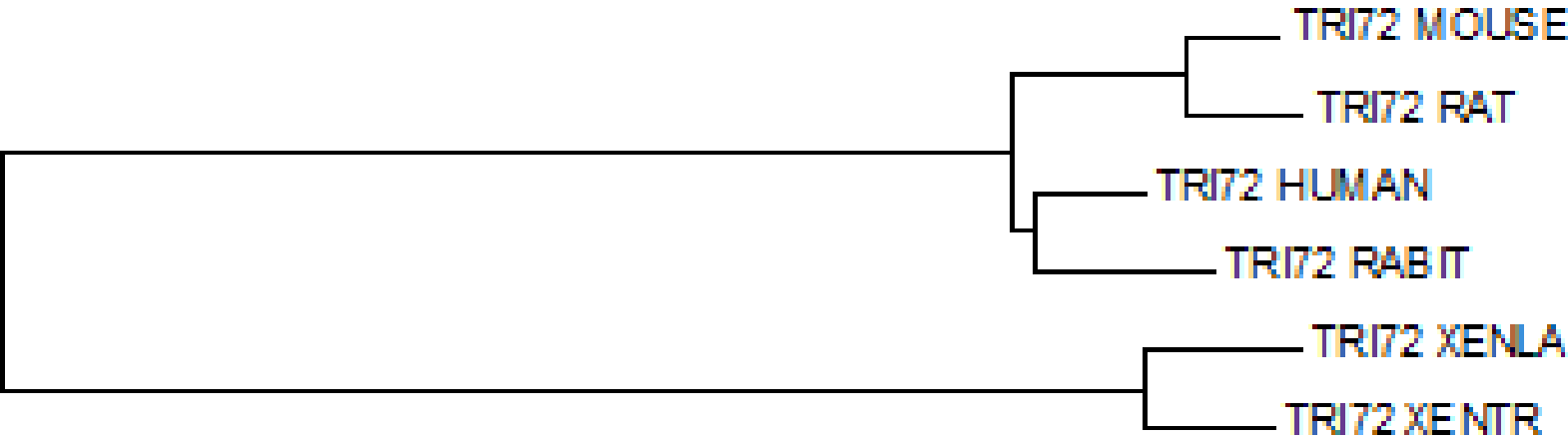
序列比对



□ 人、小鼠、大鼠MG53蛋白基因编码区序列比对结果

物种	编码区序列 登录号	编码区序列相同位 点	氨基酸序列相同位点
人/小鼠	NM_001008274/NM_001079932	1234/1436 (85.9%)	435/477 (91.2%)
人/大鼠	NM_001008274/NM_001077675	1216/1434 (84.8%)	433/477 (90.8%)
小鼠/大鼠	NM_001079932/NM_001077675	1341/1435 (93.4%)	455/477 (95.4%)

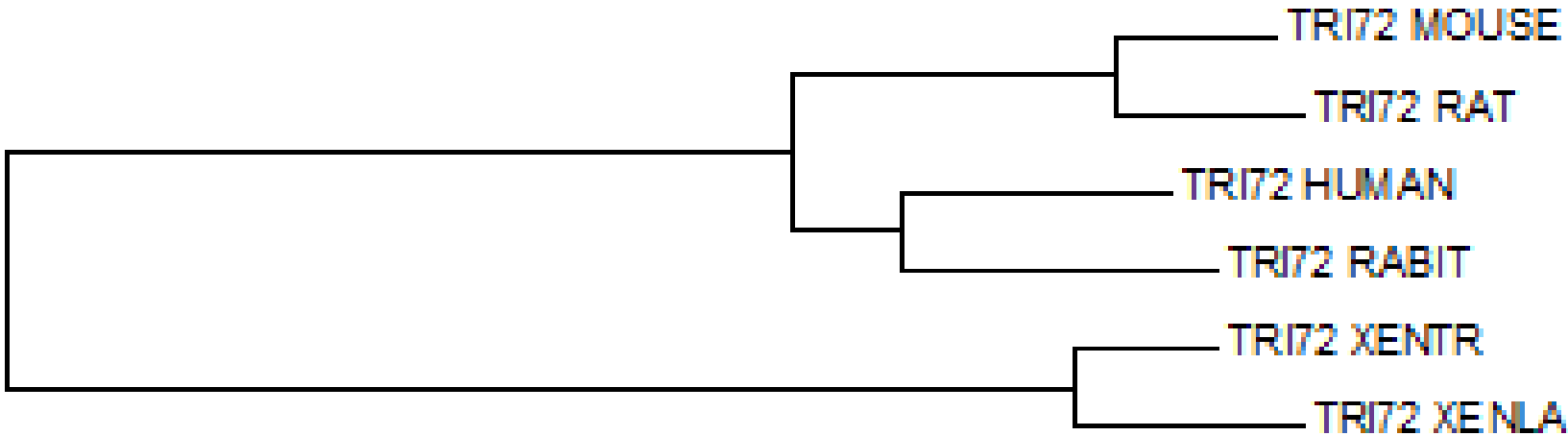
phylogenetic tree (Protein)



0.05

MAGA 6.06

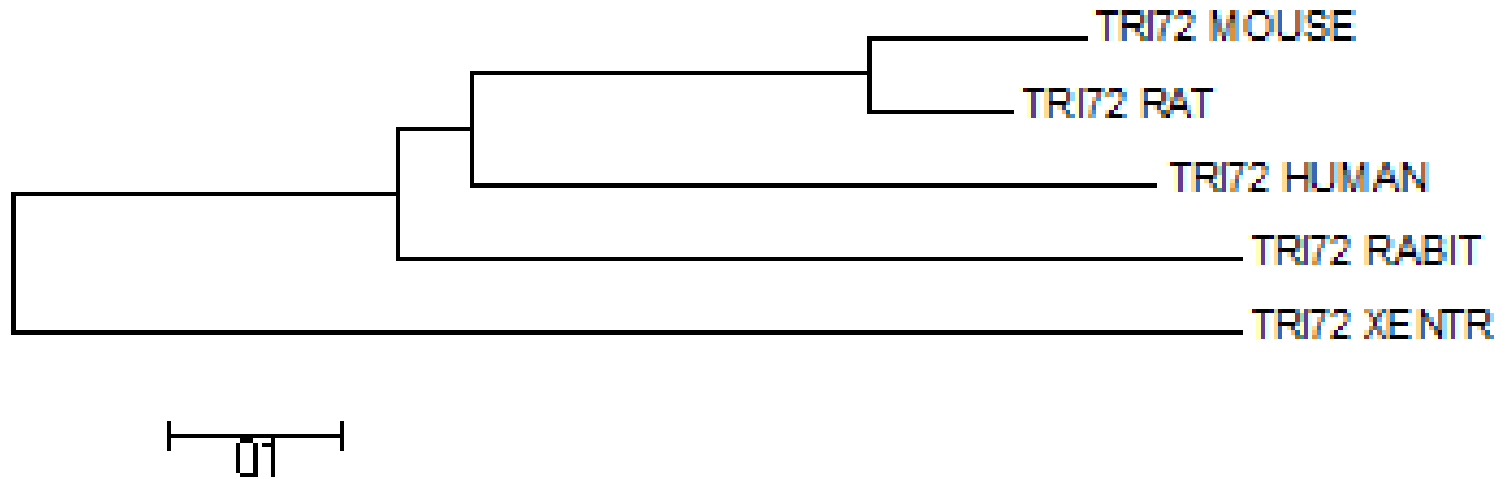
phylogenetic tree (mRNA)



0.05

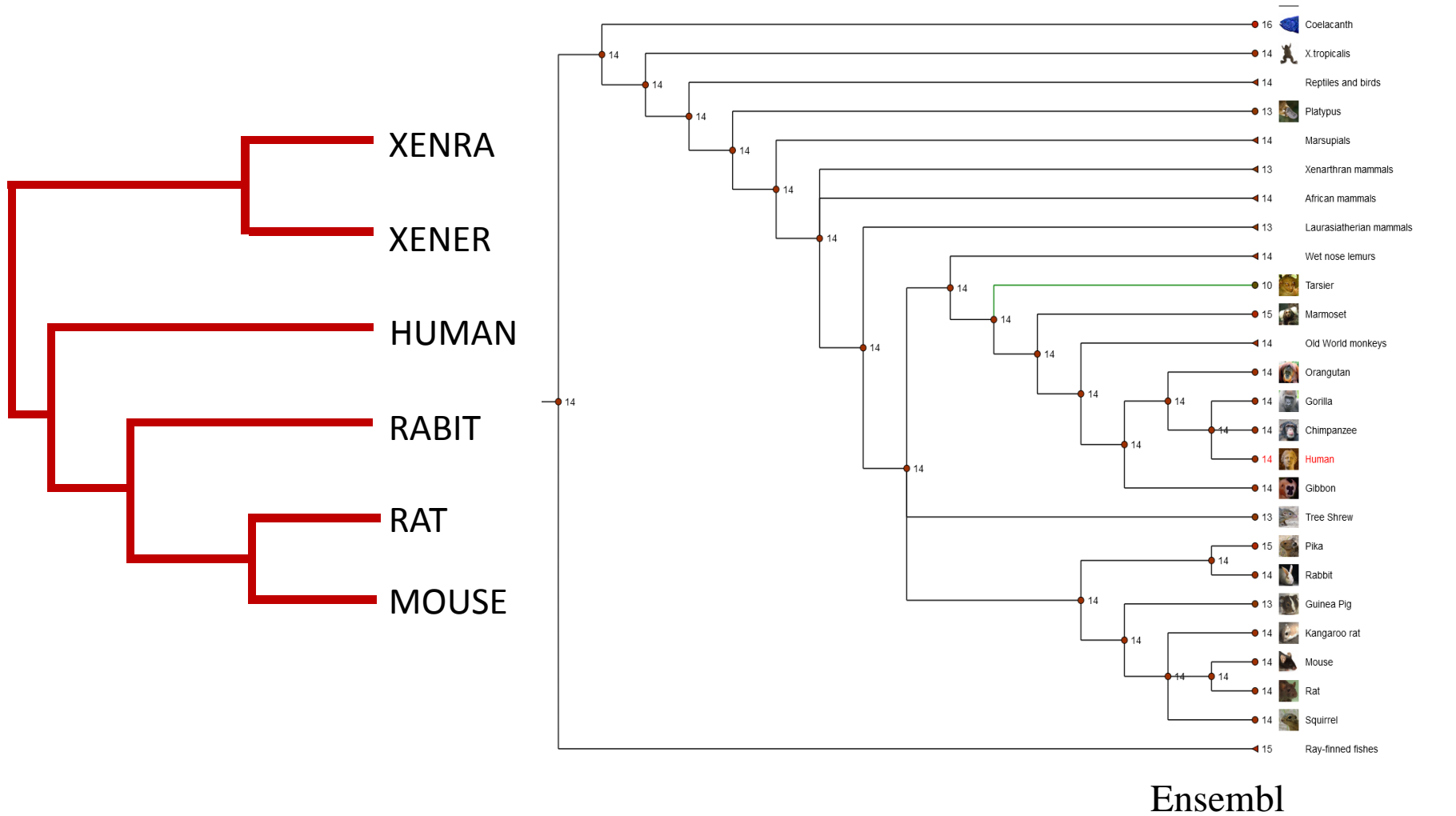
MAGA 6.06

phylogenetic tree (gene)



MAGA 6.06

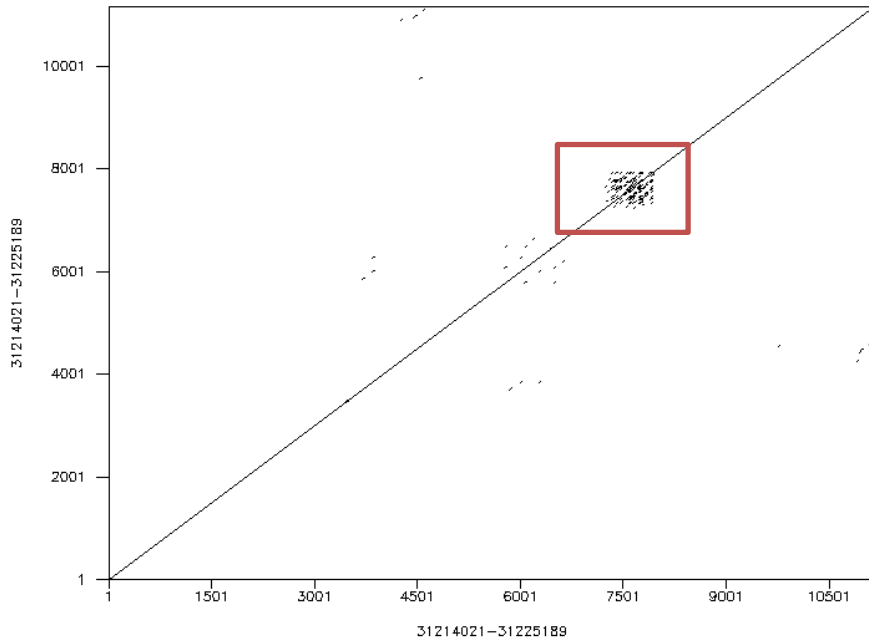
phylogenetic tree (species)



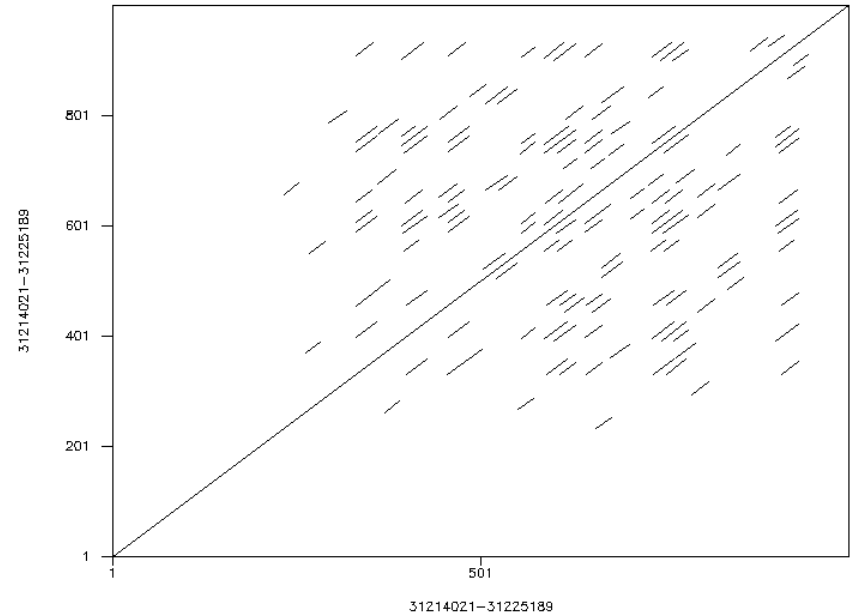
TRIM72 GENE ANALYSIS



Dottup: fasta::855337:31214021-31225189 vs fasta::8553
Thu 25 Jun 2015 01:21:40

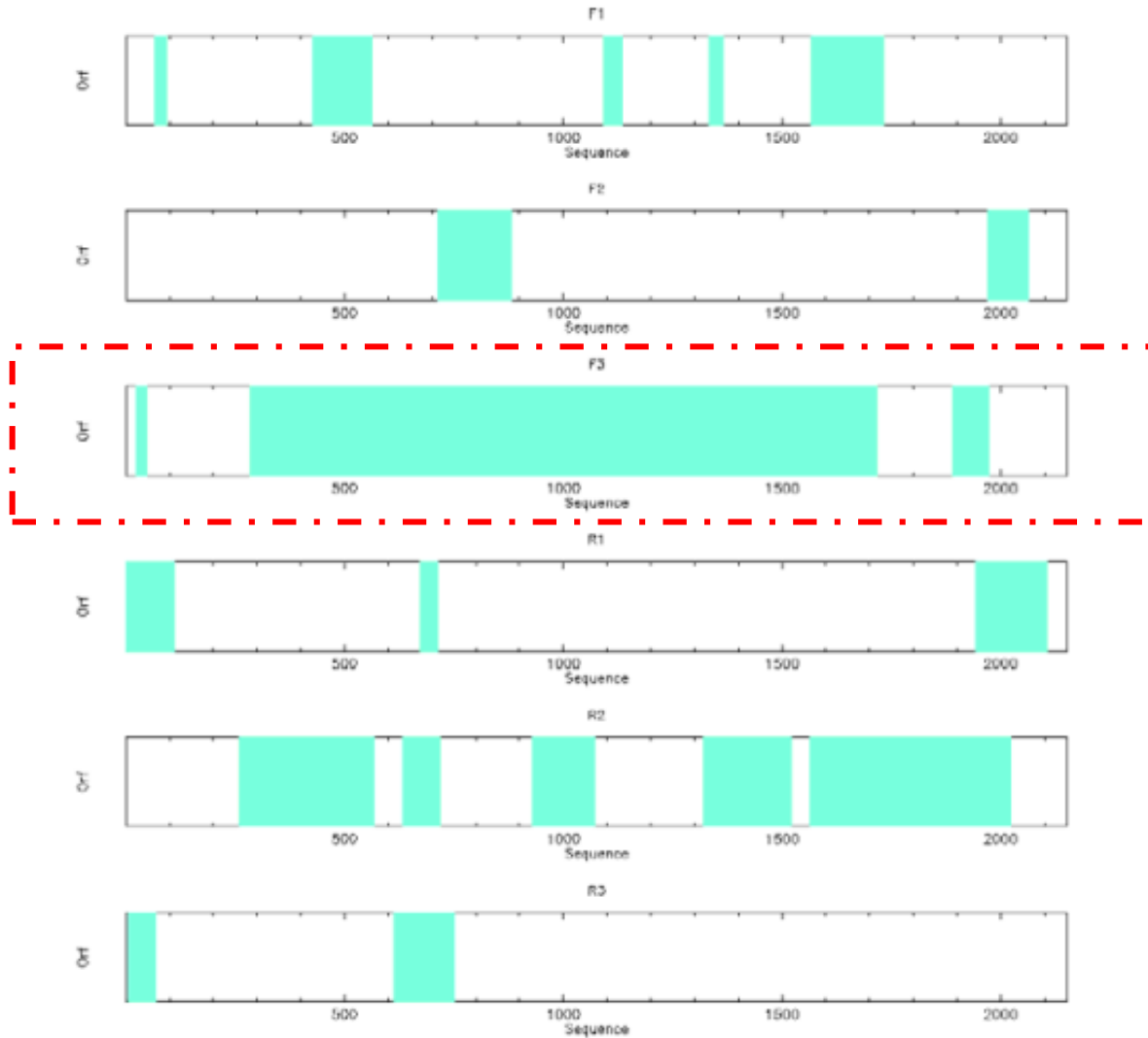


Dottup: fasta::855340:31214021-31225189 vs fasta::855340...
Thu 25 Jun 2015 01:32:28



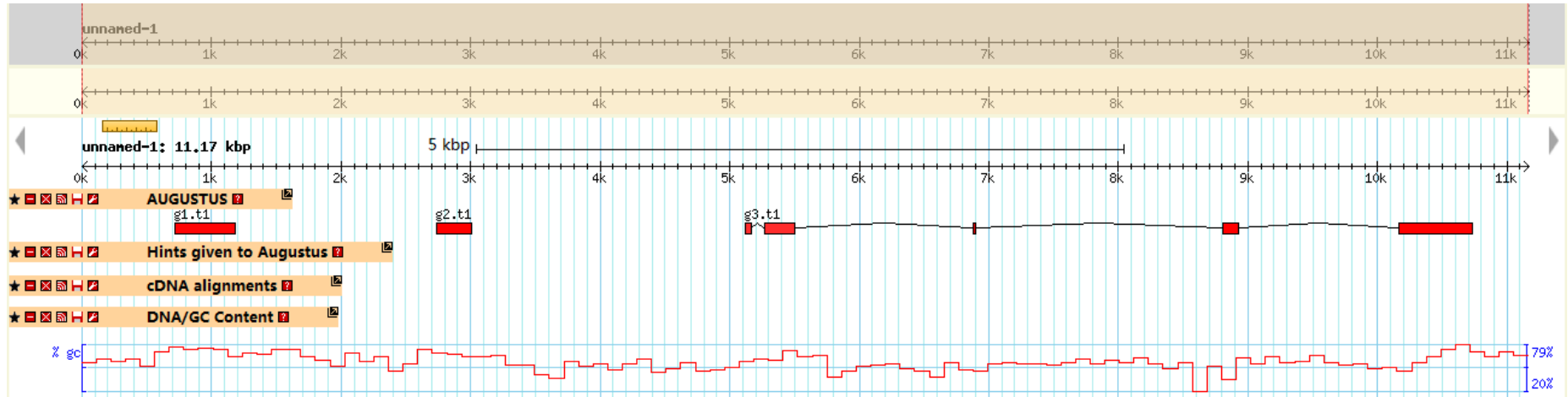
Dottup WebLab

TRIM72 GENE ANALYSIS



PlotORF WebLab

Gene identification



mRNA:

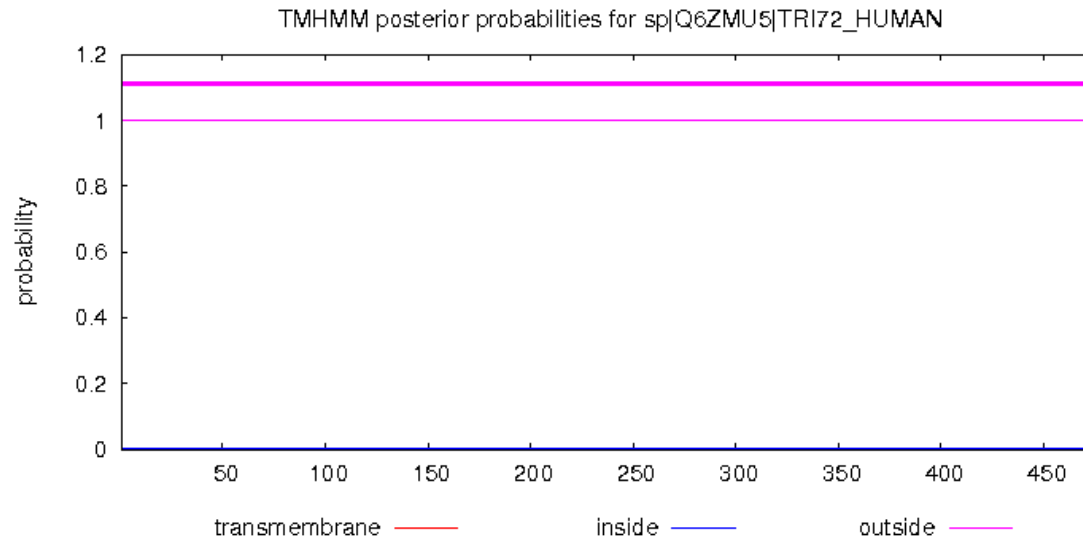
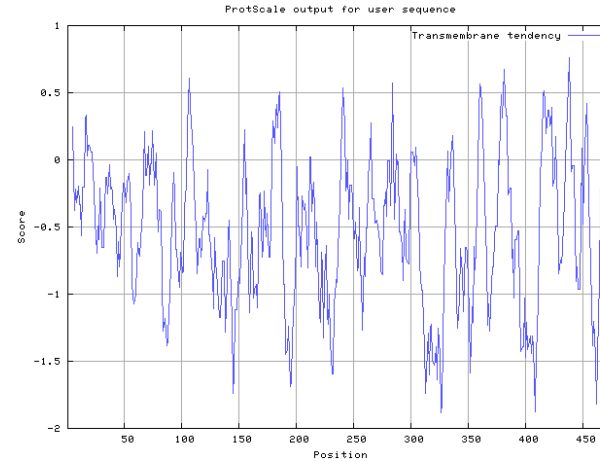
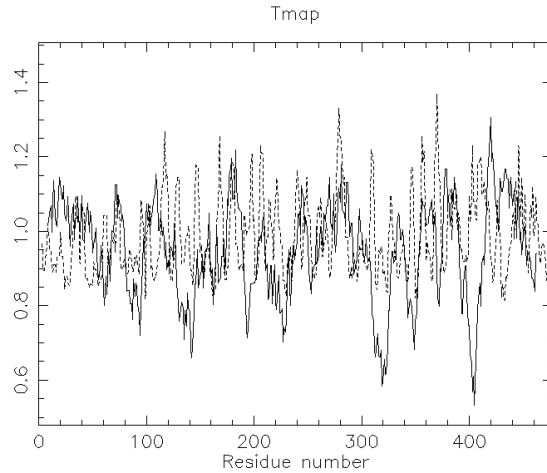
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
2152	6428.0	1002/2152 (46.6%)	1002/2152 (46.6%)	1150/2152 (53.4%)

Protein:

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
477	1714.0	333/477 (69.8%)	333/477 (69.8%)	144/477 (30.2%)

AUGUSTUS

Transmembrane Protein Prediction



Tmap WebLab
TMHMM
ProtScale ExPASy



人TRIM72_HUMAN 蛋白亚细胞定位预测

```
### targetp v1.1 prediction results #####  
Number of query sequences: 1  
Cleavage site predictions included.  
Using NON-PLANT networks.
```

Name	Len	mTP	SP	other	Loc	RC	TPlen
gi_270265876_ref_NP_	477	0.033	0.654	0.477	S	5	27
cutoff		0.000	0.000	0.000			

TargetP

BlastP



物种蛋白	相似度 (%)
人类TRIM72	100
家兔TRIM72	94
大鼠TRIM72	91
小鼠TRIM72	91
热带爪蟾TRIM72	59
非洲爪蟾TRIM72	58
小鼠营养不良相关fer-1相似蛋白	33



NCBI

PSI-blast



物种蛋白	相似度 (%)
人类TRIM72	100
家兔TRIM72	94
大鼠TRIM72	91
小鼠TRIM72	91
热带爪蟾TRIM72	59
非洲爪蟾TRIM72	58



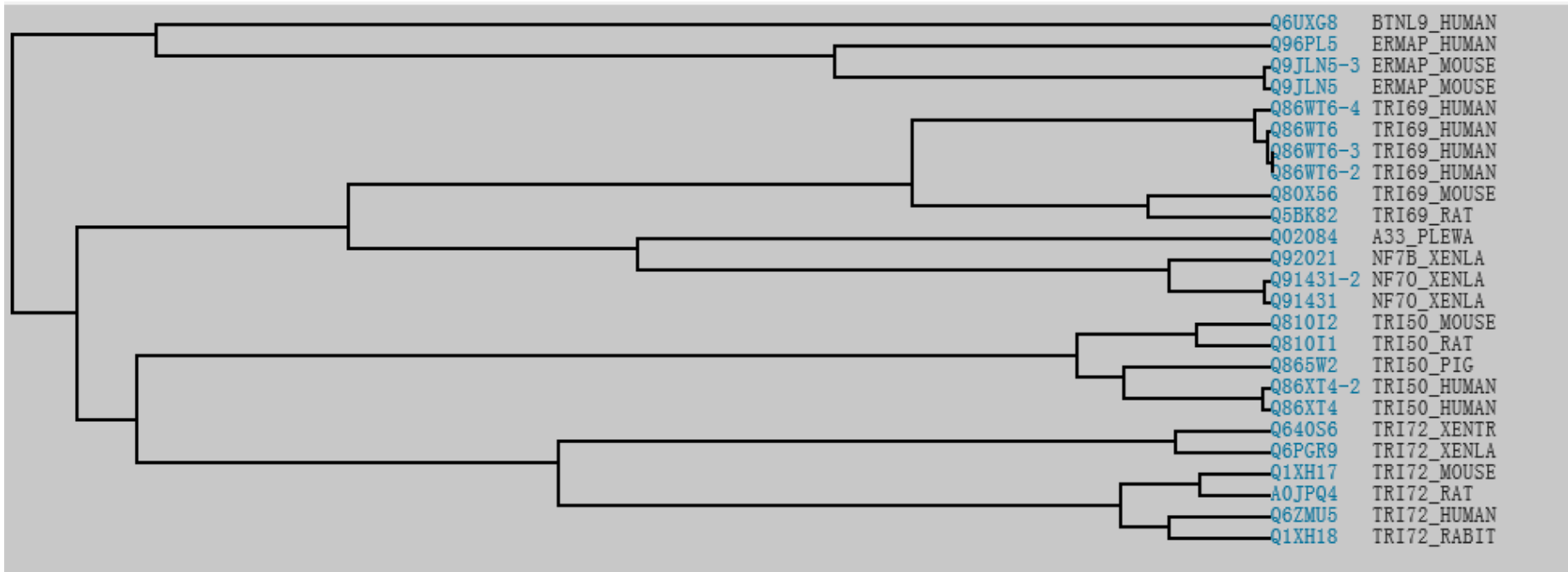
NCBI

分析对象: TRIM72_HUMAN的PRY-SPRY 结构域

目标数据库: swiss-prot

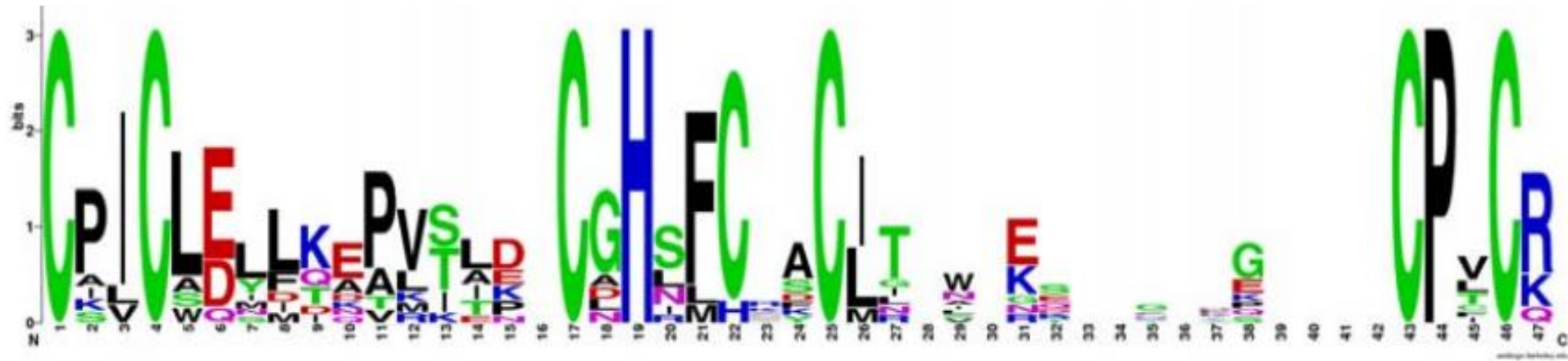
高分匹配分析:

1. 大部分为**TRIM**家族成员, 其它也多为**锌指结构蛋白**
2. 涉及Homo sapiens, Mus musculus, Rattus norvegicus, Pan troglodytes, Pongo abelii, Bos taurus, Sus scrofa, Xenopus, laevis等物种
3. Query coverage : 90%左右 (**整体结构较为保守**)
4. Identity: 除Trim家族外, 30%左右 (**序列本身保守性较差**)



RING finger 结构域分析

- RING finger 结构域是锌指结构的一种类型，其氨基酸序列通常为40 – 60 aa，包含保守的Cys3HisCys4 motif以螯合锌原子。具有该结构域的蛋白如TRIM家族多个成员通常都具有E3泛素连接酶活性。



Weblogo

RING finger 结构域分析

□ RING finger 结构域序列BlastP后序列比对:

4CFG_A	1	-----	--X[5]LAEELS	CSICL	EPFKEPV	TTP	CGHNFCGSCLNETWAVQ	-----GspyLCPQCR[383]	437
2YSL_A	1	GSSGSSGM[5]	VNK	LQEEVIC	CPICLDILQK	PVTID	CGHNFCLKCITQIGETS _{cg} FFK	-----CPLCK	64
4AYC_B	1	[29]EEKEKMQA[5]	LSH[4]	LENELQ	CIICSEYFIEAV	TLN	CAHSFCSYCIN _{EW} MKRKi	-----ECPICR	94
2ECJ_A	1	GSSGSSGA	LEN	LQVEASCSV	CLEYLKEPVI	IE	CGHNFCKACITRWWEDL _{er} DFP	-----CPVC-	58
4ORH_C	1	[40]EEKEKMQA[5]	LSH[4]	LENELQ	CIICSEYFIEAV	TLN	CAHSFCSYCIN _{EW} MKRKi	-----ECPICR	105
4AYC_A	1	[29]EEKEKMQA[5]	LSH[4]	LENELQ	CIICSEYFIEAV	TLN	CAHSFCSYCIN _{EW} MKRKi	-----ECPICR	94
1JM7_A	1	--MDLSAL[5]	QNV[3]	MQKILEC	PICLLELIKEP	VSTK	CDHIFCKFCMLKLLNQK	---KG-psQCPLCK	65
2ECV_A	1	GSSGSSGM[4]	LVN	VKEEVI	CPICLELLTQPL	SLD	CGHSFCQACLTANHKKS _{m1} DKG-esSCP	VCR	66
3VGO_A	1	[334]GST-----	FQL	-----	CKICAENDKDV	KIEP	CGHLMCTSCLTAWQESD	---GQG---CPFCR	380
3ZNI_A	1	[333]GST-----	FQL	-----	CKICAENDKDV	KIEP	CGHLMCTSCLTAWQESD	---GQG---CPFCR	379
4TXA_A	1	-----	MPV[4]	WIDFLS	CPICTQTFDET	IRKP[4]	CGHTVCKMCLNKLHRKA	-----CPFDQ[424]	478
2YSJ_A	1	GSSGSSGM[5]	VNK	LQEEVIC	CPICLDILQK	PVTID	CGHNFCLKCITQIGETS _{cg} FFK	-----CPLC-	63
2Y1M_A	1	[328]GST-----	FQL	-----	CKICAENDKDV	KIEP	CGHLMCTSCLTSWQESE	---GQG---CPFCR	374
4A4C_A	1	[330]GST-----	FQL	-----	CKICAENDKDV	KIEP	CGHLMCTSCLTSWQESE	---GQG---CPFCR	376
4A4B_A	1	[330]GST-----	FQL	-----	CKICAENDKDV	KIEP	CGHLMCTSCLTSWQESE	---GQG---CPFCR	376
1FBV_A	1	[328]GST-----	FQL	-----	CKICAENDKDV	KIEP	CGHLMCTSCLTSWQESE	---GQG---CPFCR	374
2EGP_A	1	GSSGSSG-	--N	VQEEVI	CPICLELLTEPL	SLD	CGHSLCRACITVSNKEA _{vt} SMGgksSCP	VCG	60
2ECW_A	1	GSSGSSGM[4]	LEM	IKEEVI	CPICLELLKEP	VSAD	CNHSFCRACITLNYESN _{rn} TDG-kgNCP	VCR	66
2ECI_A	1	GSSGSSGM[5]	YDV[5]	LESKYE	CPICLMALREAV	QTP	CGHRFCKACIIKSIRDA	---G---hKCPVDN	67
4QPL_A	1	-----	---[1]	SLTVPE	CAICLQTCVHP	VSLP	CKHVFCYLCV	-----KGasWLG---kRCALCR	46
4LAD_B	1	[3]NYLRVVG	N[5]	AVANDD	CAICWDSMQA	ARKLP	CGHLFHNSCLRSWLEQD _t	-----SPTCR	69
3HCT_A	1	-----	M[5]	YDV[5]	LESKYE	CPICLMALREAVQTP	CGHRFCKACIIKSIRDA	---G---hKCPVDN	60
2JMD_A	1	-----	---[2]	LGSKYE	CPICLMALREAV	QTP	CGHRFCKACIIKSIRDA	---G---hKCPVDN	48

RING finger 结构域分析

□ 人TRIM72的RING finger结构域

■ >hTRIM72

CPLCLQLFDAPVTAECGHSFCRACLGRVAGEP-AADGTVLCPCCCQ

□ 小鼠TRIM30的RING结构域 (2ECW)

■ >hTRIM5

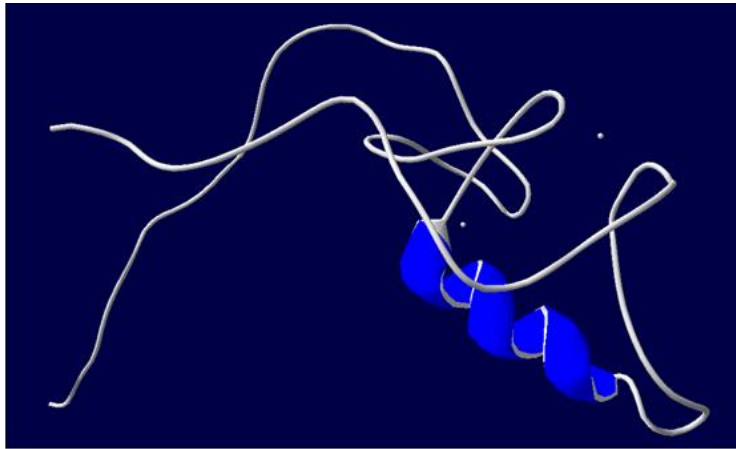
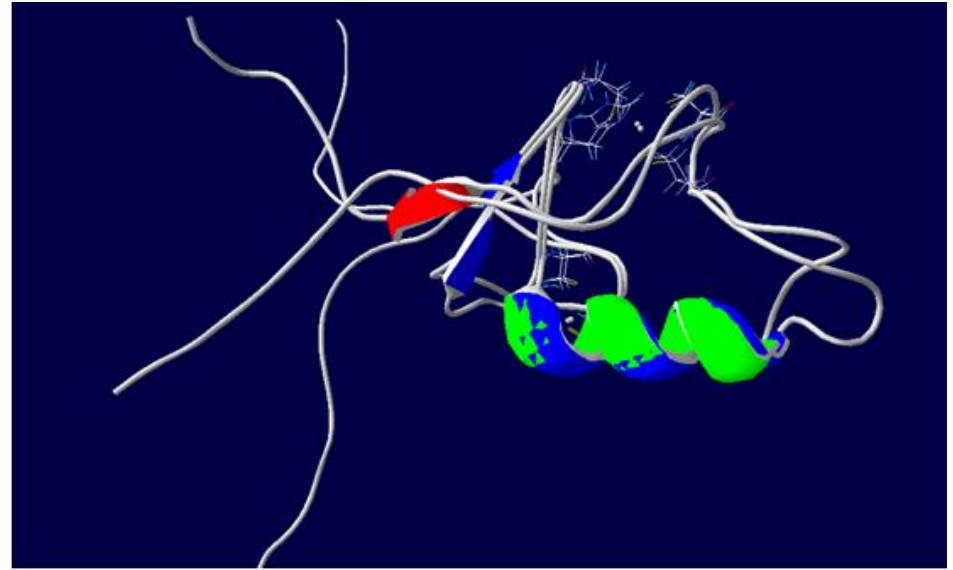
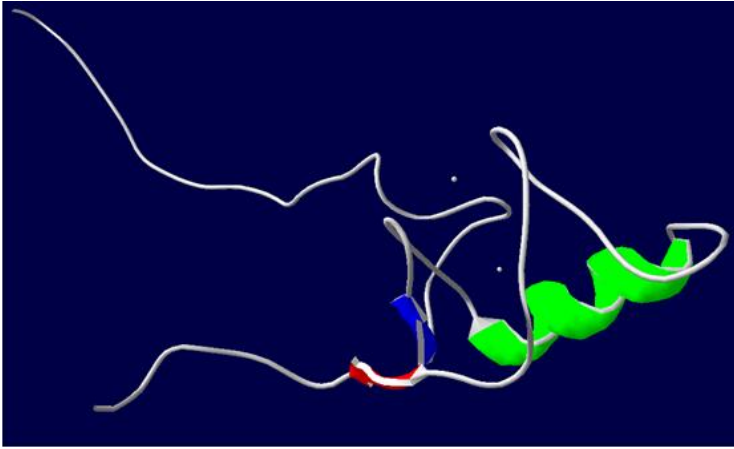
CPICLELLTQPLSLDCGHSFCQACL TAN HKKSMLDKGESS
CPVCR

□ 人TRIM5 的RING结构域 (2ECV)

■ >mTRIM30

CPICLELLKEPVSADCNHSFCRACITL NYESNRNTDG
KGNCPVCR

RING finger 结构域分析

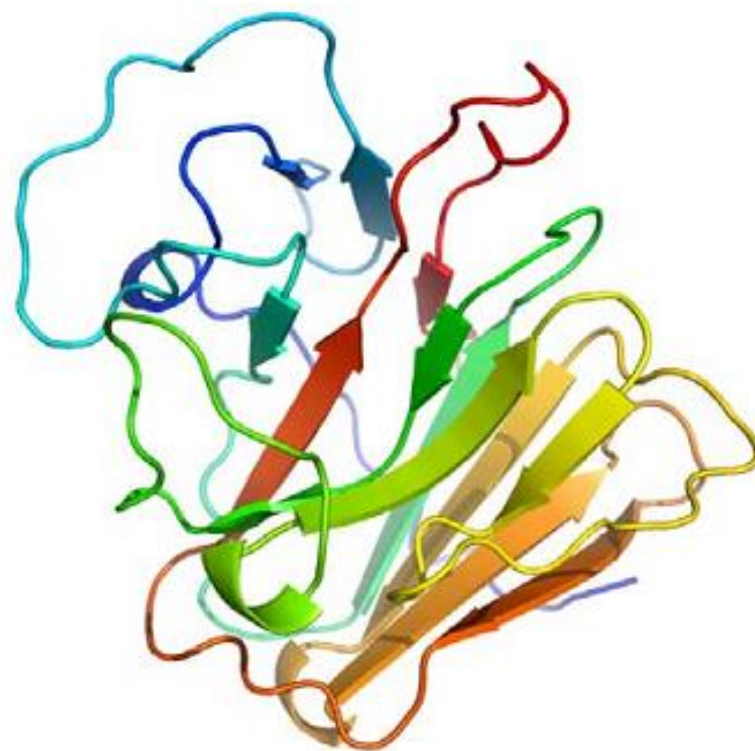


- ✓ 三维结构相似程度很高
- ✓ 锌原子的位置也很吻合
- ✓ RING结构域具有较为保守的序列和结构特征

PRY-SPRY结构域分析

PyMOL

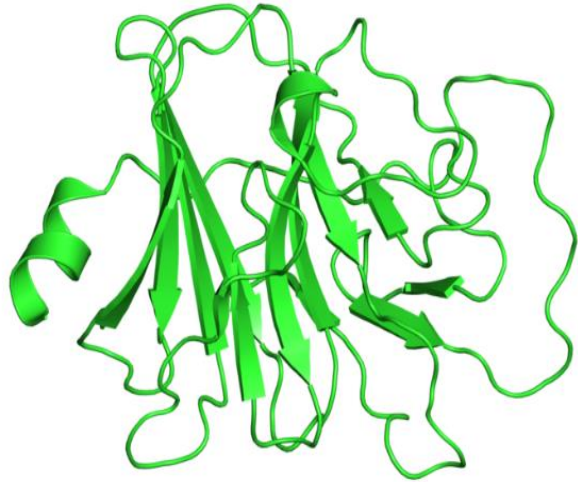
- 实验解析的PRY-SPRY 结构域：5个
- 亲缘关系最高：小鼠TRIM21分子的相应结构域



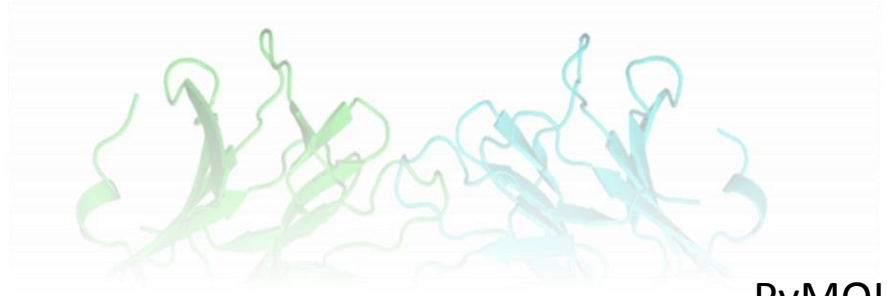
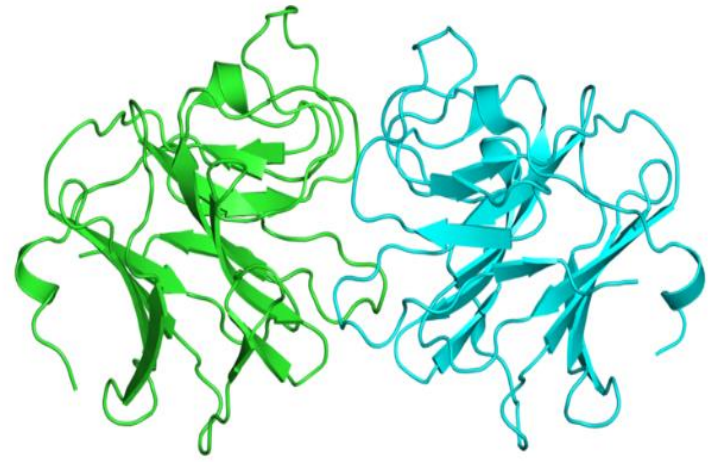
小鼠 TRIM21 的 PRY-SPRY 结构域（2VOK_a）的三维结构

PRY-SPRY 三维结构

TRIM72 PRY-SPRY结构域 (3KB5)

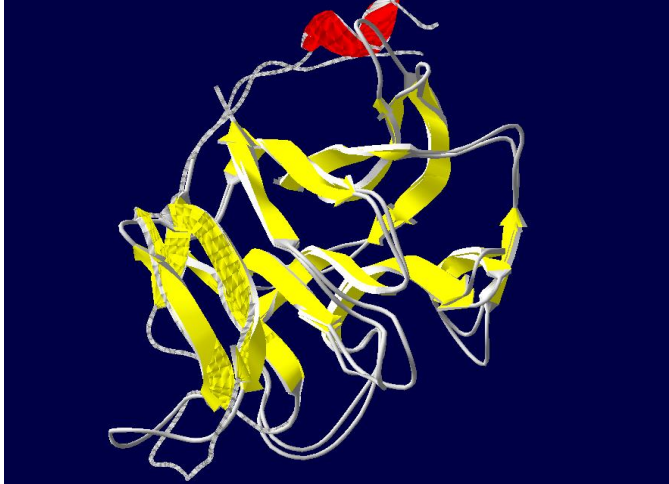


TRIM21 PRY-SPRY结构域 (2VOK)

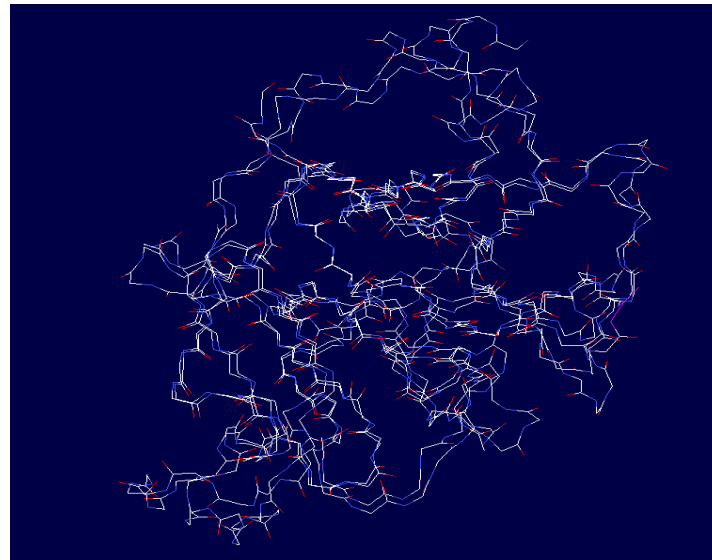
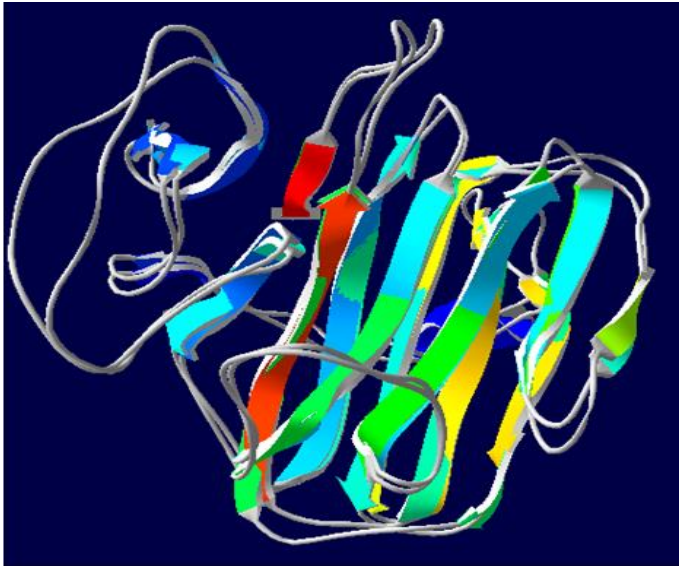


PyMOL

3kb5 与 2voka

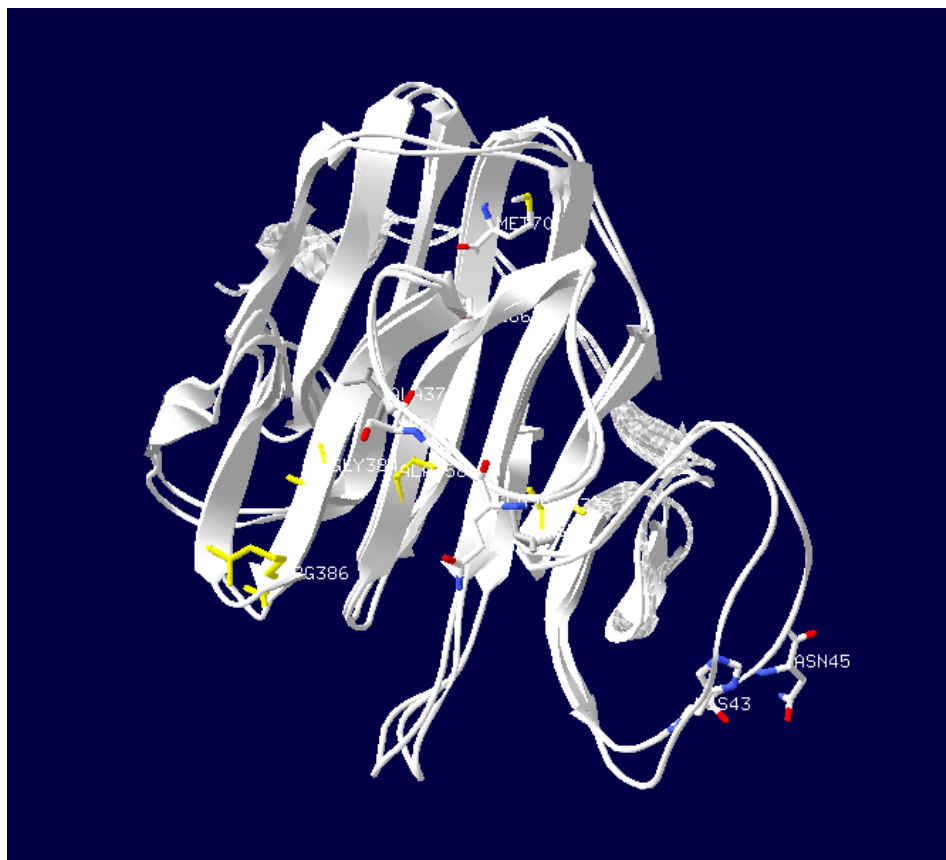


RMS:
1.00

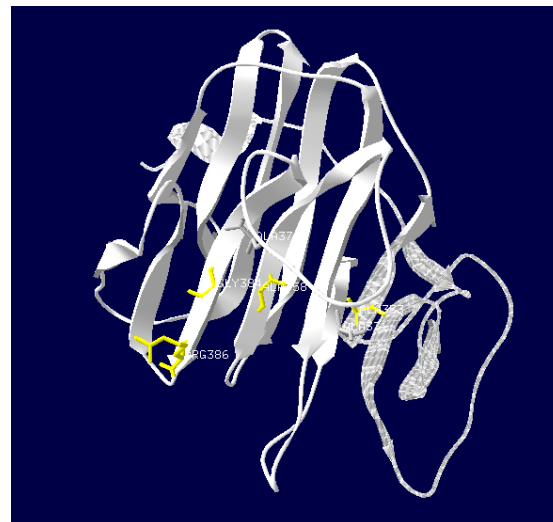


PDBviewer

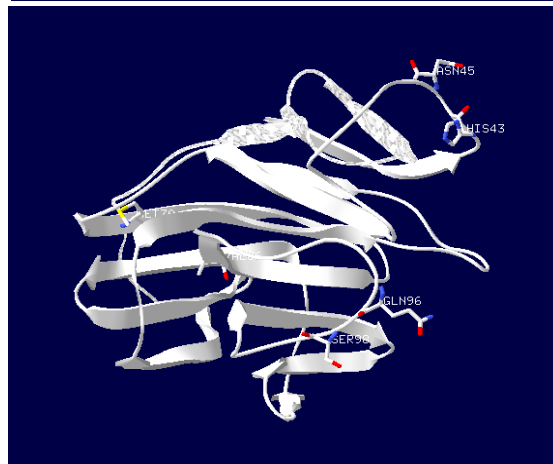
Loop区的几对疏水氨基酸残基



3kb5



2voka

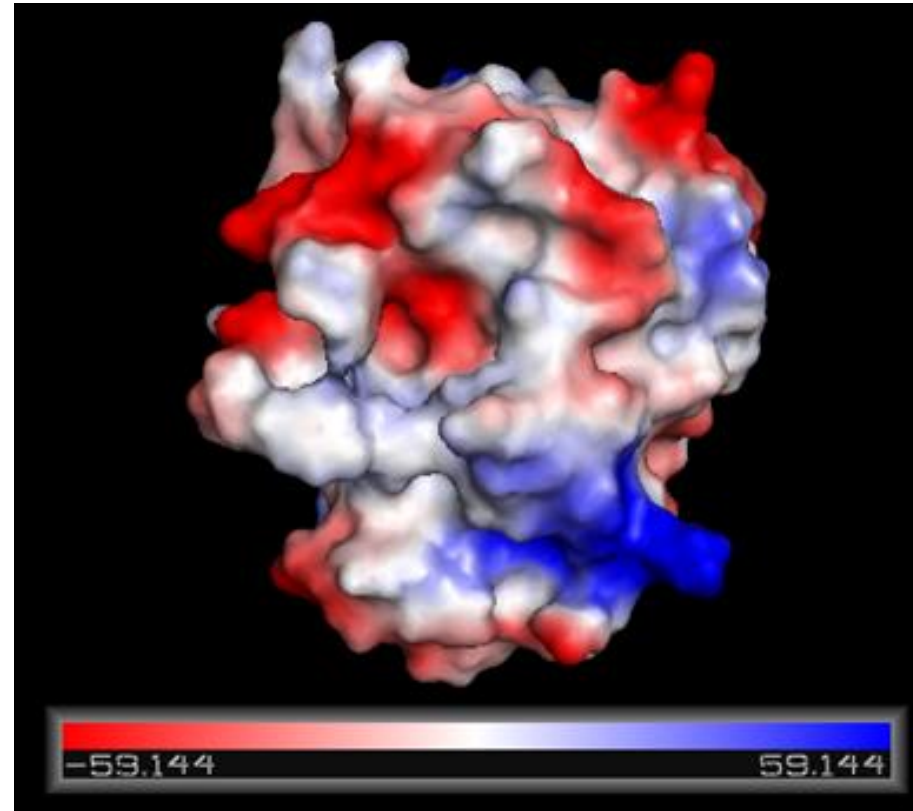
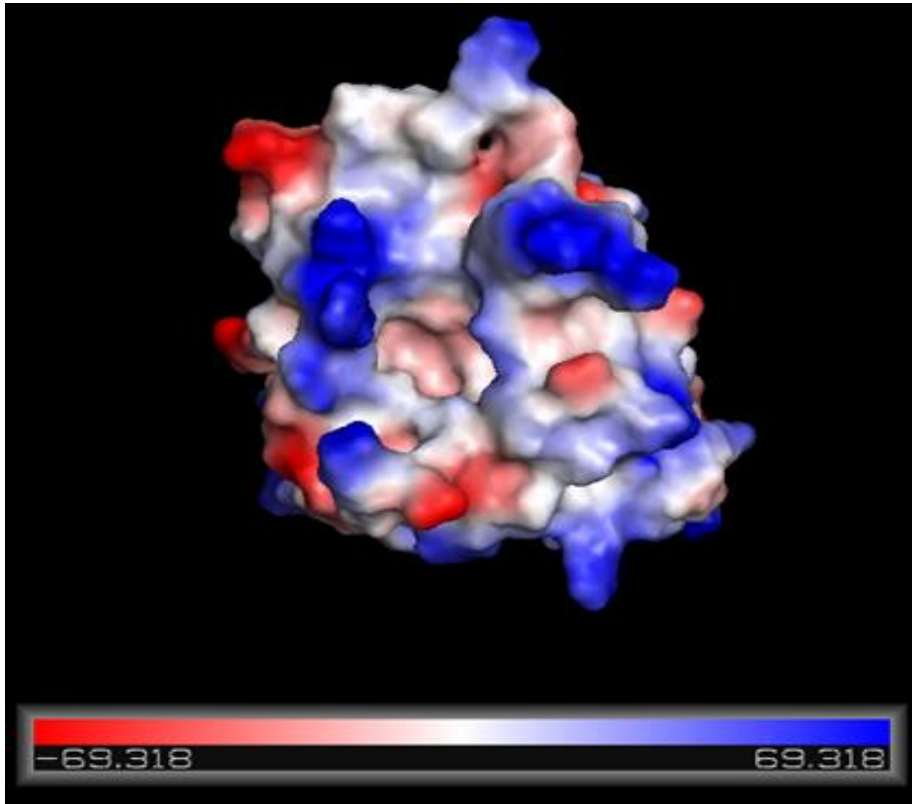


PDBviewer

Surface electrostatic charge comparison

□ hTrim72_PRYSPRY (3KB5):

□ mTrim21_PRYSPRY (2VOK_a):



对TRIM72_HUMAN的PRY-SPRY结构域进行同源模建





Acknowledgement

感谢罗老师!

感谢G08组的小伙伴!

谢谢大家!

