ADVANCED HEALTHCARE MATERIALS

Supporting Information

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The Epitope Basis of Embryonic Stem Cell-Induced Antitumor Immunity against Bladder Cancer

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The epitope basis of embryonic stem cell-induced antitumor immunity against bladder cancer

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Supplementary data.

Methods:

1. Histopathology of explanted organs

At the time of sacrifice, tumors were explanted from vaccinated mice and processed for histopathology. The organs were fixed overnight in 4% paraformaldehyde and transferred to 70% ethanol for 24 h. Fixed samples were embedded in paraffin and cut into 5-mm sections, followed by staining with hematoxylin and eosin (H&E) for the histological analysis.

2. LEGENDplex multiplex cytokine assay

Serum was collected from vaccinated mice. The profiling of cytokines in serum was performed in a Flow Cytometer analyzer using fluorescence-encoded beads (LEGENDplex, BioLegend, San Diego, CA) in which the Mouse Th Cytokine Panel (category no. 741044, 12-plex for IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-22, IFN-γ, and TNF-α) were selected.

3.ESCs degradation detection

Stain ESCs with dissolved VivoTrack 680 (0.2mg of dye in 1g of PEG). Incubate for 15 min at room temperature, protected from light, washing by adding 15-20 mL PBS containing 1% FBS. Male C57BL/6 mice (n=3) were injected with 5□10^5 MB49 bladder cancer cells s.c. in the right flank, after one week, mice were immunized with stained ESCs (129), and ESCs were observed using in vivo imaging system spectrum 0 h, 2 h, 20 h after the injection.

4. Peptide synthesis

Epitope peptides were synthesized by GL Biochem (Shanghai, China). Briefly, weigh 0.3mmol (0.87g) of Fmoc-Gly-Wang Resin with a substitution degree of 0.344mmol/g. Put the weighed dry resin into the reaction column, and add solvent to swell. The Fmoc protection was removed with hexahydro pyridone. Weigh the second amino acid Fmoc-Cys(Trt)-OH at the C-terminal, put the condensing agent TBTU DIEA into the reaction column, and react with DMF as the solvent for 30min. Use a certain proportion of ninhydrin solution to detect whether the coupling is complete. Repeat these steps until the last amino acid is connected, then weigh Fmoc-Ala-OH and condensing agent TBTU, put them into the reaction column, react with DMF for 30 minutes, and remove Fmoc protection with hexahydro pyridone. Finally, wash 3 times with methanol, the resin shrinks, and drain. Use TFA cutting solution to cut the resin, and dilute the precipitate with ether to obtain the crude peptide. Purified peptide.

5. Tumor-infiltrating lymphocyte (TIL) isolation and $CD8^+$ T cell isolation

Briefly, tumors were initially divided into segments and then finely diced into RPMI1640 containing 20% FBS, 1mg/ml collagenase type 4 (Worthington Biochemical, Lakewood, NJ), 30 U/ml DNase (Roche Diagnostics, Indianapolis, IN, USA), and was incubated for 30min at 37 °C on a rocker. Digested tumor pieces were teased through a 70-µm sieve. Then, the sieve was irrigated with Dulbecco’s PBS, and the cells were collected into a 50-ml conical tube. Pelleted cells underwent different gradient percoll solutions and centrifuge for 30 min at 400g. Then collect lymphocytes at the middle stage. Wash cells with PBS and counted the cells for Elispot. After isolating the lymphocytes from tumors, then $CD8^+$ T cells were sorted by magnetic beads (Miltenyi Biotec, 130-104-075).

5. Evaluate the effects of adjuvant on tumor growth inhibition

Peptides were dissolved in DMSO or PBS. Vaccine formulations were prepared by mixing 100 μg of peptides with 1 μM CpG ODN 1826, or 5μg Poly IC (TLR3 agonist), or 1μM cGAMP (sting activator) in PBS at a final injection volume of 100 μL per mouse. Control vaccine formulations were prepared using PBS in place of the epitope peptides.

Legend:

Figure S1: (a) In the therapeutic mouse model, Vaccination of C57BL/6 mice with PBS, ESC (129)+ GM-CSF, CpG +GM-CSF, ESC (129) + CpG + GM-CSF resulted in a significant reduction of MB49 tumor sizes (n = 5), (b) In the therapeutic model, mice were injected with PBS, E(C57) vaccine or E(C57)+CpG, and the tumor growth was measured every two days (n=6). (c) In the therapeutic model, mice were injected with PBS, CpG, E(129), E(129)+C+G, E(C57) vaccine or E(C57)+CpG, and tumor growth was captured 2 weeks or 4 weeks after tumor inoculation (n=4). (d) ESC vaccination increases percentages of effector and memory $CD8^+$ T cells. 2 weeks after tumor inoculation in the prophylactic mouse model, ESC (129) + CpG vaccinated C57BL/6 mice showed a significant increase in the percentage of cytotoxic T cells ($CD8^+$granzyme-B^+), effector/memory $CD8^+$ T cells ($CD8^+CD44^+$) in the dLN (n=3). (Data represent mean ± SEM, ANOVA with Tukey's multiple comparison test; *$p< 0.05$, **$p < 0.001$, ***$p< 0.001$).

Figure S2 ESCs vaccination leads to a systemic immune profile in the low level

(a and b) LEGENDplex multiplex cytokine analysis of serum from the different treatment groups 4 weeks after tumor cell introduction reveals a significantly lower presence of systemic cytokines in the vaccination mice (ESC (129) + CpG/ESC (129) +CpG +GM-CSF) compared to PBS control mice (PBS) (n=3). (c) In the therapeutic model, ESCs vaccination leads to immune cell infiltration into tumors. C57BL/6 mice were vaccinated with ESC (129) + CpG + GM-CSF for four weeks, the tumor was stained with CD11c^{+} and CD4^{+} specific antibodies, respectively (n=3). (d, e) In the prophylactic mouse model, mice were injected with PBS, CpG, E(129), E(129)+C, E(C57) vaccine or E(C57)+CpG, and the infiltration of immune cells (CD3, CD4, CD8, and Treg) in tumors were tested after 4 weeks of tumor inoculation (n=3). (Data represent mean ± SEM, ANOVA with Tukey's multiple comparison test; *p< 0.05, **p < 0.001, ***p< 0.001).

Figure S3 (a) Further selection of genes highly expressed in tumor tissues across multiple cancer types. (b) Analysis of four ESCs-cancer signature gene mRNA expression in major human cancer types and normal tissues. (c) Prediction of selective epitopes.

Figure S4. (a) IFN-γ ELISPOT assay for ECT2-3, TOP2α-1, and TOP2α-2 peptides, Quantitative analysis of the ELISPOT assay for IFNγ secretion (n = 4). (b) In the therapeutic model, mice were injected with PBS, peptides+CpG, peptides+Poly IC, or peptides+cGAMP, the tumor volume was measured every two days, IFN-γ ELISPOT assay for Anln, Ect2-1 and Ect2-2 peptides was performed. Quantitative analysis of the ELISPOT assay for IFNγ secretion (n=5). (c) In the therapeutic model, mice treated with different peptides plus CpG and combined peptides + CpG were compared, and tumor volume was measured every two days (n=5). (d) IFN-γ ELISPOT assay for Melk was performed using TIL. Quantitative analysis of the ELISPOT assay for IFNγ secretion was shown (n=4). (e) NK, MDSC, and T cell groups were measured by flow cytometry (n=3) (Data represent mean ± SEM, ANOVA with Tukey's multiple comparison test; *p< 0.05, **p < 0.001, ***p< 0.001).

Figure S5. Irradiated ESCs were digested by immune cells. ESCs were stained with VivoTrack 680, and ESCs changes were observed using in vivo imaging system spectrum in 2 or 20 h after injection (n=3).

Figure S6 In the therapeutic model, flow cytometry data for PBMC samples (a, b) and dLN samples from the mice injected with PBS or E(129)+C +G (c, d). In the therapeutic model, flow cytometry data for dLN samples from the mice injected with PBS or E(C57) (e, f) (n=3).

Figure S1

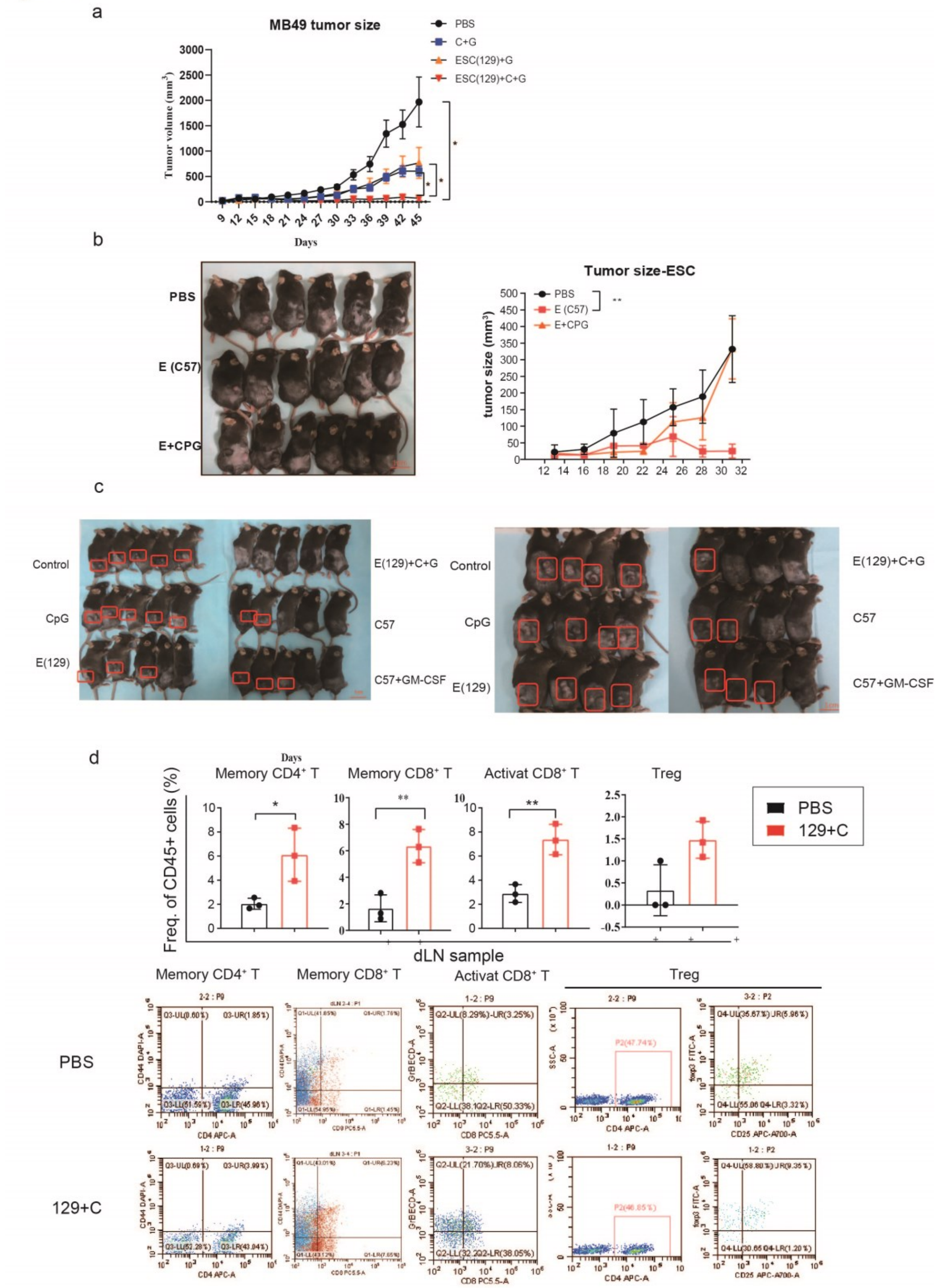

a
MB49 tumor size
PBS
C+G
ESC(129)+G
ESC(129)+C+G
Tumor volume (mm³)
Days
b
PBS
E (C57)
E+CPG
Tumor size-ESC
tumor size (mm³)
c
Control
CpG
E(129)
E(129)+C+G
C57
C57+GM-CSF
d
Memory CD4⁺ T
Memory CD8⁺ T
Activat CD8⁺ T
Treg
Freq. of CD45+ cells (%)
PBS
129+C
dLN sample

Figure S2

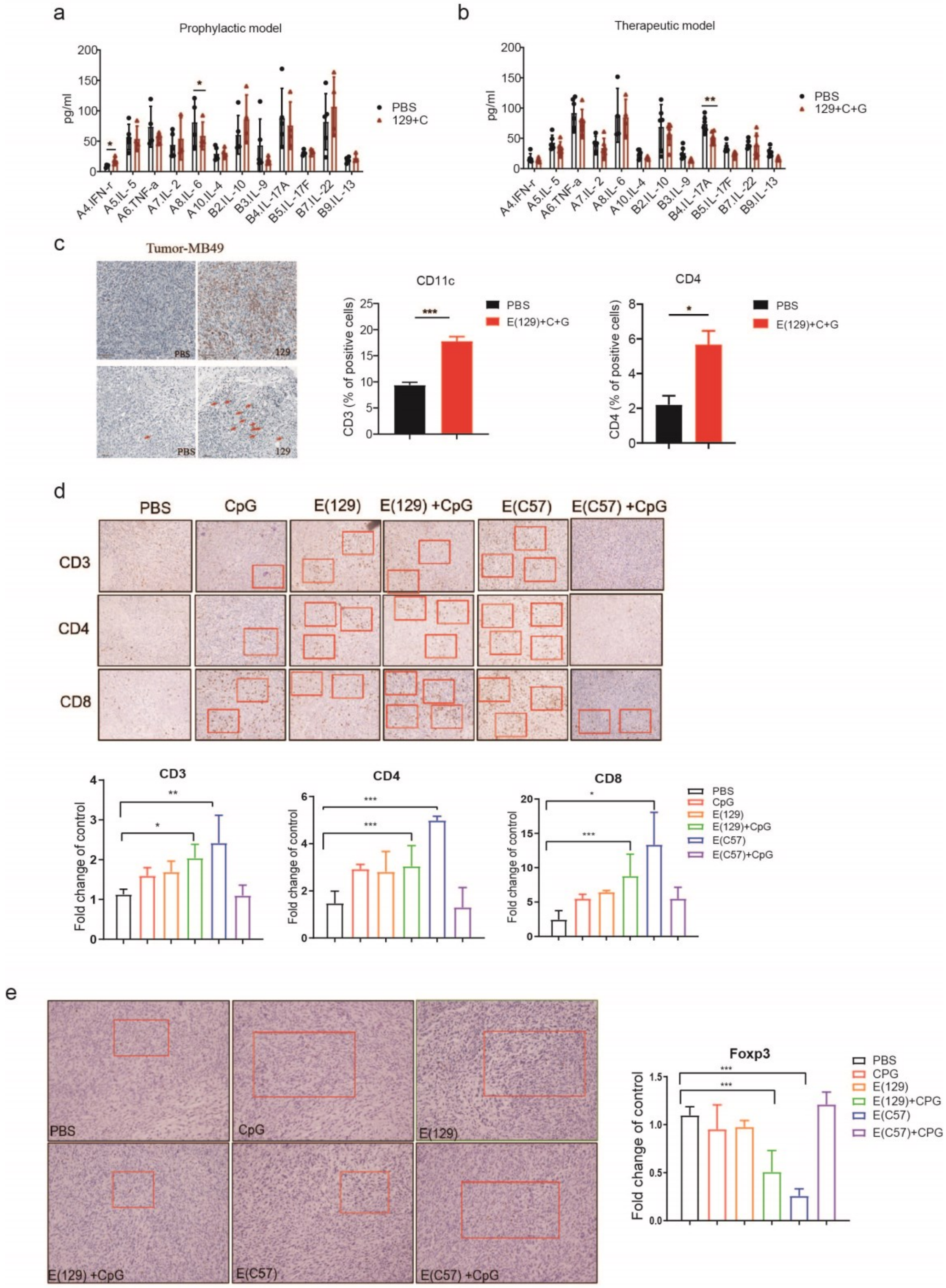
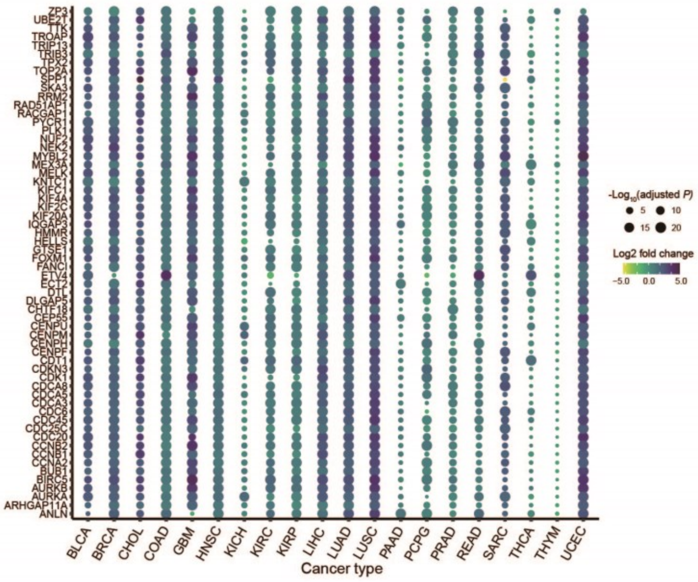
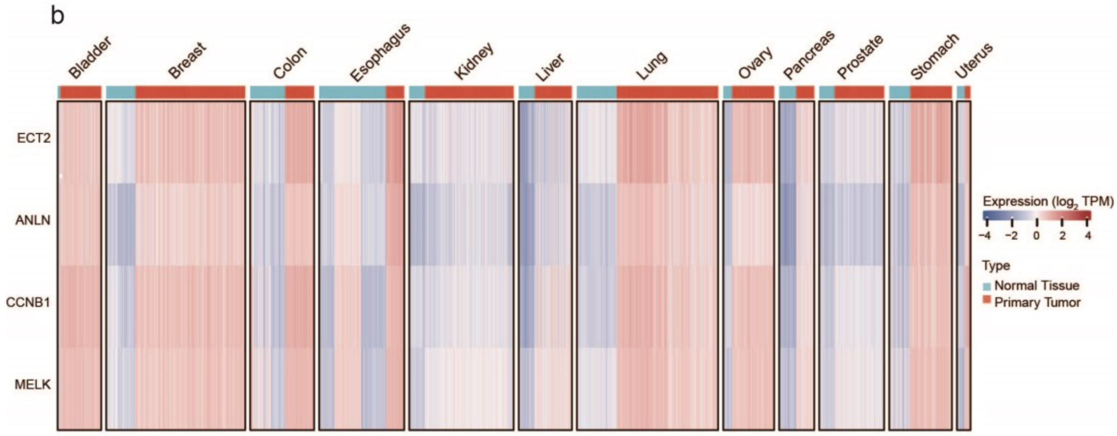

a
Prophylactic model
pg/ml
PBS
129+C
A4.IFN-r
A5.IL-5
A6.TNF-a
A7.IL-2
A8.IL-6
A10.IL-4
B2.IL-10
B3.IL-9
B4.IL-17A
B5.IL-17F
B7.IL-22
B9.IL-13
b
Therapeutic model
129+C+G
c
Tumor-MB49
PBS
129
CD11c
CD3 (% of positive cells)
E(129)+C+G
CD4
CD4 (% of positive cells)
d
PBS
CpG
E(129)
E(129) +CpG
E(C57)
E(C57) +CpG
CD3
CD4
CD8
Fold change of control
e
Foxp3
CPG
E(129)+CPG
E(C57)+CPG

Figure S3

a



b



c

Index	Gene	Epitope	Extend sequence	Strongest binding affinity to mouse (Allele)	Predicted effective population coverage
EP-1	CCNB1	YVKDIYAYL	ADPNLCSEYVKDIYAYLRQLEEEQA	193.2 nM (H2-Kb)	78.90%
EP-2	TOP2A-1	FIHHNWPSL	HIKGLLINFIHHNWPSLLRHRFLEE	555.7 nM (H2-Db)	60.12%
EP-3	TOP2A-2	SYHHGEMSL	AGSVAEMSSYHHGEMSLMMTIINLA	4302.5 nM (H2-Kb)	34.79%
EP-4	ECT2-3	FQDCILSFL	RNEFKVPPFQDCILSFLGFSDEEKT	2125.2nM (H-2-Db)	46.64%
EP-5	MELK	SSILLLQQM	DVPKWLSPSSILLLQQMLQVDPKKR	1198.4 nM (H2-Db)	14.87%
EP-6	ANLN	VIYQASQAL	NEINMQQTVIYQASQALNCCVDEEH	57.1 nM (H-2-Kb)	41.49%
EP-7	ECT2-1	VAVSLGTPI	CTQGEKFRVAVSLGTPIMKPEWIYK	264.3nM (H-2-Db)	31.83%
EP-8	ECT2-2	KTYPPFVNF	LKYSKDLVKTYPPFVNFFEMSKETI	90.1 nM (H-2-Kb)	44.26%
EP-9	CCNB1	LQLVGVTAM	NNCVPKKMLQLVGVTAMFIASKYEE	1433.1nM (H-2-Db)	36.01%
EP-10	TOP2A	FNYLLDMPL	SVTDSGPTFNYLLDMPLWYLTKEKK	86.8 nM (H-2-Kb)	12.65%
EP-11	TOP2A	VLFDHVGCL	TSLTCNSMVLFDHVGCLKKYDTVLD	178.1 nM (H-2-Kb)	25.43%
EP-12	MELK	VTMQFELEV	QTQSDFGKVTMQFELEVCQLQKPDV	192.1 nM (H-2-Kb)	13.08%
EP-13	ANLN	SSMSLLAPL	EQEDALNISSMSLLAPLAQTVGVVS	22.5 nM (H-2-Kb)	46.95%
EP-14	HMMR	VSFQKSQRF	TLEVLKGPVSFQKSQRFKQQKESKQ	91.9 nM (H-2-Kb)	13.91%
EP-15	IGF2BP1	IGHFYASQMAQRKIF	VIVKIIGHFYASQMAQRKIRDILAQ	46.0 nM (H-2-Kb)	89.33%
EP-16	IGF2BP1	AAMSLQSHL	REAYENDVAAMSLQSHLIPGLNLAA	226.5 nM (H-2-Kb)	19.10%
EP-17	IGF2BP1	WAMKAIETF	FVDCPDEHWAMKAIETFSGKVELQG	954.6 nM (H-2-Db)	55.56%
EP-18	IGF2BP1	ISYSGQFLV	EKVFAEHKISYSGQFLVKSGYAFVD	135.9 nM (H-2-Kb)	31.56%

Figure S4

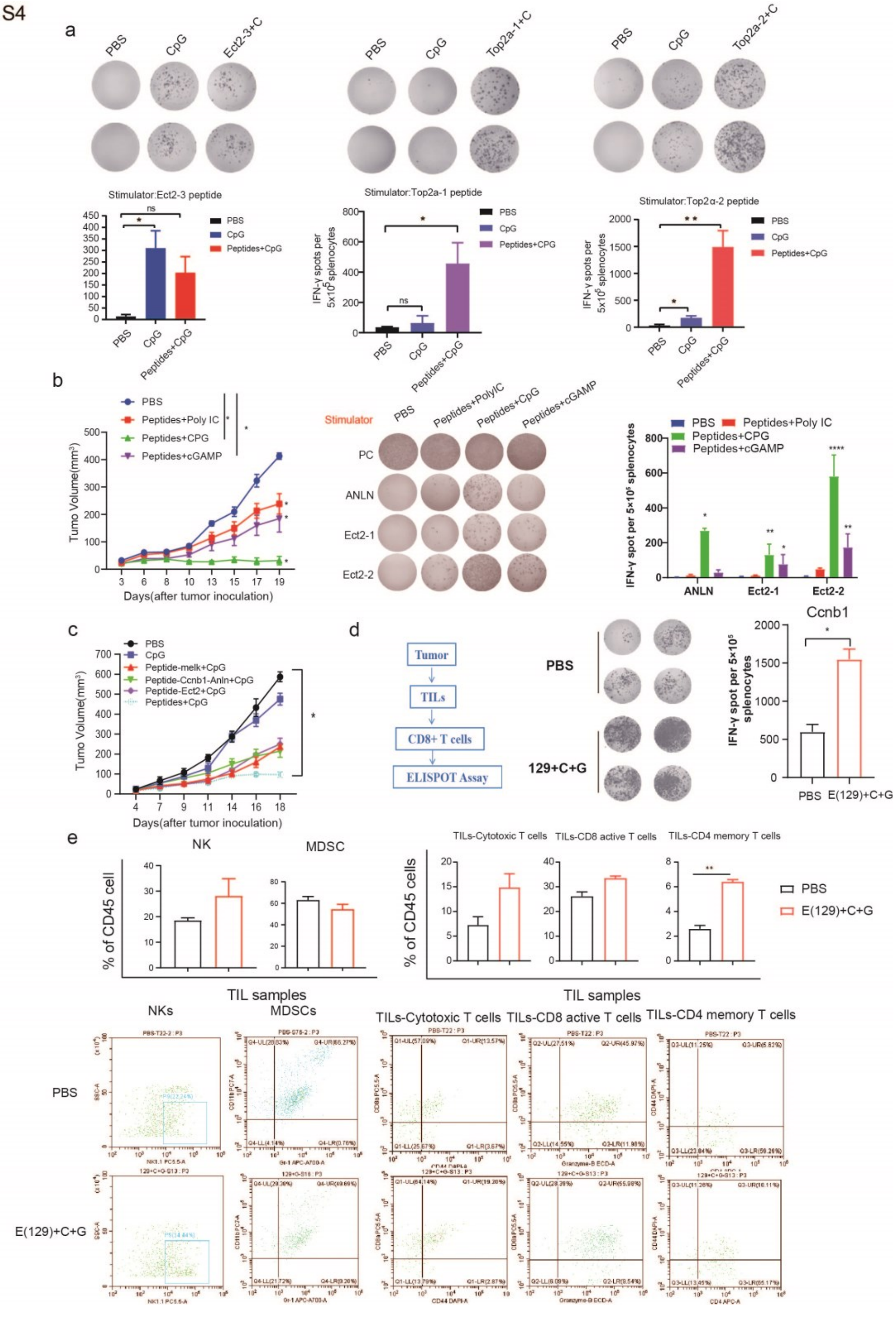

a
PBS
CpG
Ect2-3+C
Top2a-1+C
Top2a-2+C
Stimulator:Ect2-3 peptide
Stimulator:Top2a-1 peptide
Stimulator:Top2α-2 peptide
Peptides+CpG
Peptides+CPG
IFN-γ spots per 5x10⁵ splenocytes
b
PBS
Peptides+Poly IC
Peptides+CPG
Peptides+cGAMP
Tumo Volume(mm³)
Days(after tumor inoculation)
Stimulator
Peptides+PolyIC
PC
ANLN
Ect2-1
Ect2-2
IFN-γ spot per 5x10⁵ splenocytes
c
Peptide-melk+CpG
Peptide-Ccnb1-Anln+CpG
Peptide-Ect2+CpG
Peptides+CpG
d
Tumor
TILs
CD8+ T cells
ELISPOT Assay
129+C+G
Ccnb1
IFN-γ spot per 5x10⁵ splenocytes
E(129)+C+G
e
NK
MDSC
% of CD45 cell
TILs-Cytotoxic T cells
TILs-CD8 active T cells
TILs-CD4 memory T cells
% of CD45 cells
TIL samples
NKs
MDSCs

Figure S5

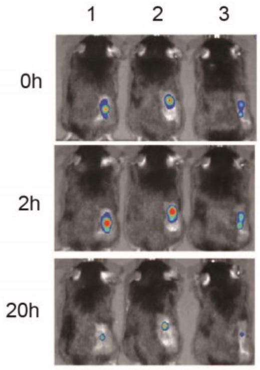
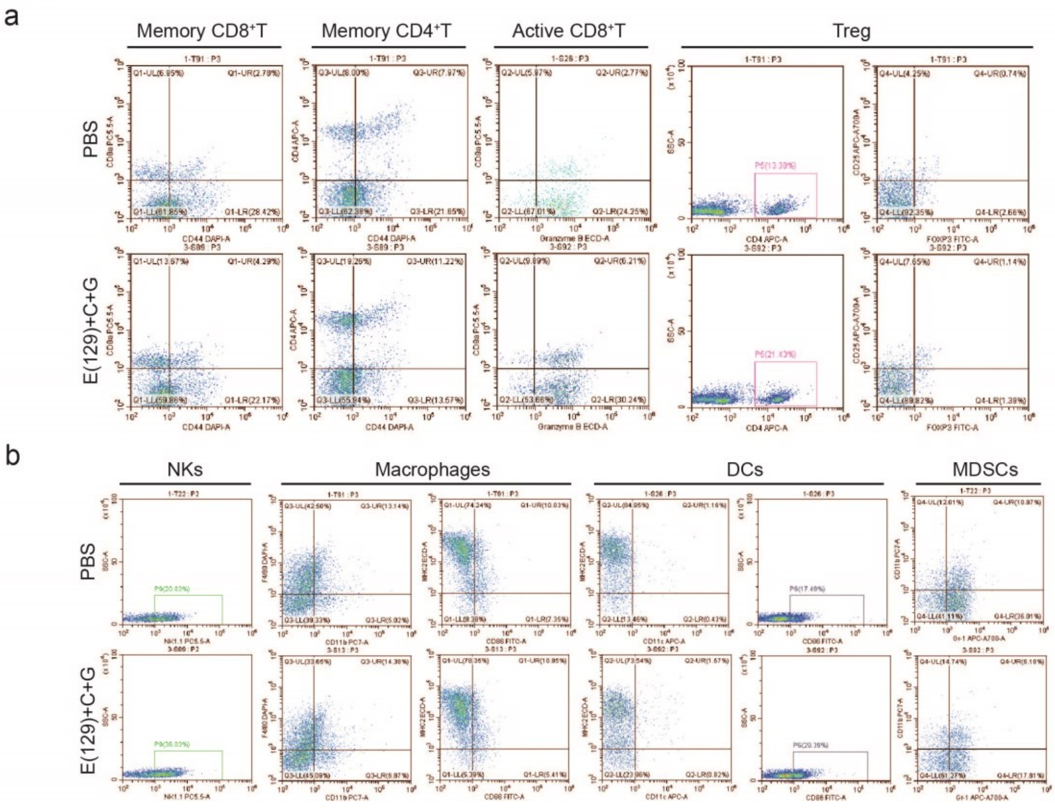
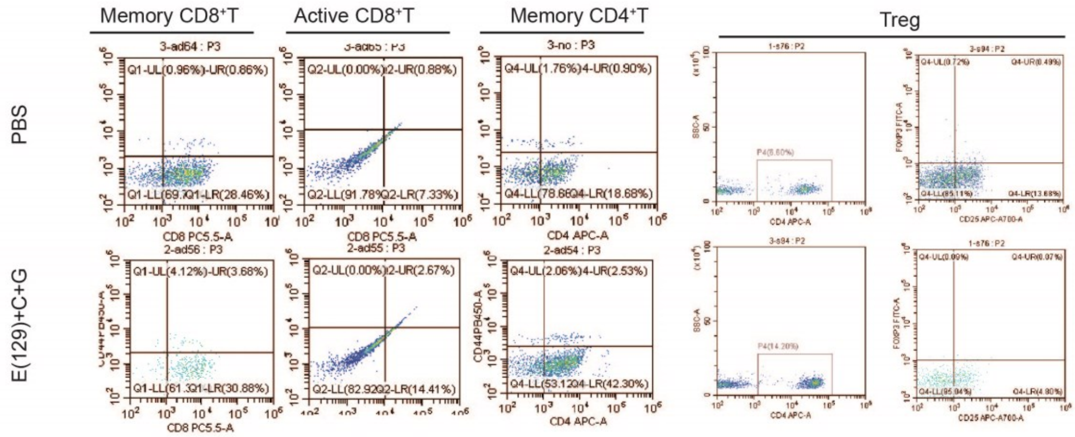


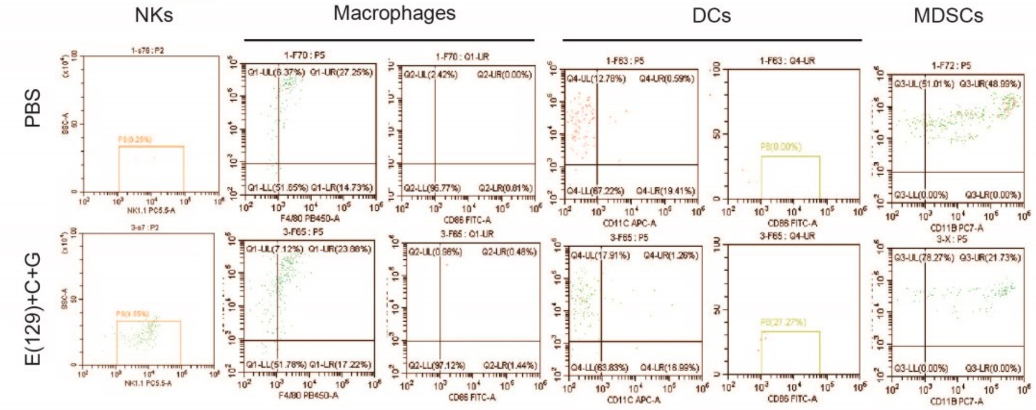
Figure S6



c


Memory CD8⁺T
Active CD8⁺T
Memory CD4⁺T
Treg
PBS
E(129)+C+G

d


NKs
Macrophages
DCs
MDSCs
PBS
E(129)+C+G

e

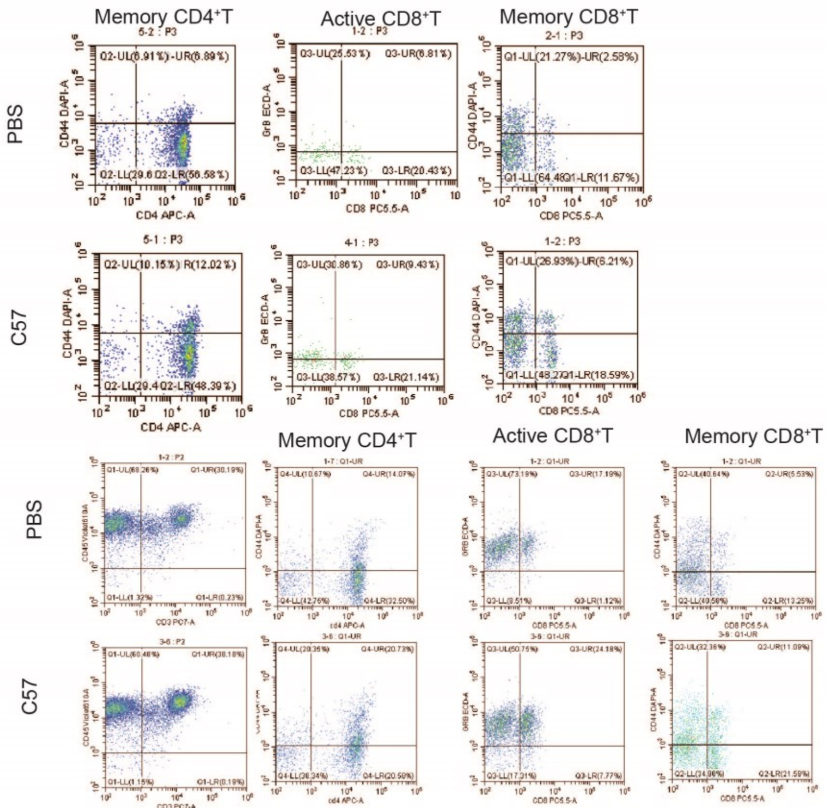

Memory CD4⁺T
Active CD8⁺T
Memory CD8⁺T
PBS
C57
Memory CD4⁺T
Active CD8⁺T
Memory CD8⁺T
PBS
C57

	Table S1:Reagent or resource	source	identifier
	antibody		
1	PE/Cy7 anti-mouse CD3	Biolegend	100220
2	APC anti-mouse CD4	Biolegend	100412
3	Alexa Fluor® 700 anti-mouse CD25	Biolegend	102024
4	PerCP/Cyanine5.5 anti-mouse CD8a	Biolegend	100733
5	Pacific Blue™ anti-mouse/human CD44	Biolegend	103020
6	Brilliant Violet 605™ anti-mouse CD45	Biolegend	103139
7	PE/Dazzle™ 594 anti-human/mouse Granzyme B Recombinant	Biolegend	372207
8	Alexa Fluor® 488 anti-mouse FOXP3	Biolegend	126405
9	PE anti-mouse H-2Dk	Biolegend	110307
10	Brilliant Violet 421™ anti-mouse F4/80	Biolegend	123131
11	PE/Dazzle™ 594 anti-mouse I-A/I-E	Biolegend	107647
12	FITC anti-mouse CD86	Biolegend	105005
13	PE/Cy7 anti-mouse/human CD11b	Biolegend	101215
14	APC anti-mouse CD11c	Biolegend	117309
15	PerCP/Cyanine5.5 anti-mouse CD49b (pan-NK cells)	Biolegend	108915
16	Alexa Fluor® 700 anti-mouse Ly-6G/Ly-6C (Gr-1)	Biolegend	108421
17	PE/Cy7 Rat IgG2b, κ Isotype Ctrl	Biolegend	400617
18	APC Rat IgG2b, κ Isotype Ctrl	Biolegend	400611
19	PerCP/Cyanine5.5 Rat IgG2a, κ Isotype Ctrl	Biolegend	400531
20	Pacific Blue™ Rat IgG2b, κ Isotype Ctrl	Biolegend	400627
21	Brilliant Violet 605™ Rat IgG2b, κ Isotype Ctrl	Biolegend	400657
22	Alexa Fluor® 488 Rat IgG2b, κ Isotype Ctrl	Biolegend	400625
23	PE Mouse IgG2a, κ Isotype Ctrl	Biolegend	400211
24	Brilliant Violet 421™ Rat IgG2a, κ Isotype Ctrl	Biolegend	400549
25	PE/Dazzle™ 594 Rat IgG2b, κ Isotype Ctrl	Biolegend	400659
26	FITC Rat IgG2a, κ Isotype Ctrl	Biolegend	400505
27	Alexa Fluor® 700 Rat IgG2b, κ Isotype Ctrl	Biolegend	400628
28	TruStain fcX™ (anti-mouse CD16/32)（达科为）	Biolegend	101320
29	True-Nuclear™ Transcription Factor Buffer Set	Biolegend	424401
30	Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	thermo fisher	A-11001

Chemicals, peptides and Recombinant Proteins

1	Penicillin-Streptomycin-Glutamine (100X)	Gibco	10378016
2	FBS	GIBCO	10099-141
3	KnockOut™ Serum Replacement - Multi-Species	Gibco	A3181502
4	Knockout DMEM	GIBCO	10829018
5	DMEM	GIBCO	10566-016

6	Opti-MEM	Gibco	31985070
7	Lipofectamine™ 3000 Transfection Reagent	Gibco	L3000015
8	TrypLE™ Express Enzyme (1X), no phenol red	Gibco	12604021
9	Trypsin-EDTA (0.25%), phenol red	Gibco	25200072
10	InVivoMab anti-mouse CD4	BioXcell	BE0003-1-5MG
11	InVivoMab anti-mouse CD8	BioXcell	BE0004-1-5MG
12	InVivoMab anti-mouse NK1.1	BioXcell	BE0036-5MG
13	100um cell strainer	Falcon	352360
14	70um cell strainer	Falcon	352350
15	Percoll	GE Health	17-0891-02
16	DPBS	Hyclone	SH30028.02
17	MEM-Non-essential AA(10mM),100mL	Stem cell	#07600
18	Gelatin,500mL	Stem cell	#07903
19	anti-SSEA-1(CD15)MicroBeads, m, h	Miltenyi Biotec	130-094-530
20	TransDetect PCR Mycoplasma Detection Kit	Transgene Biotec	FM311-01
21	Myco-Blue Mycoplasma Detector	Vazyme	D101-02
22	TLRL-1826 ODN	Invivogen	tlrl-1826-1
23	Mouse IFN-r precoated ELISPOT kit	Dakewe Biotech Co., Ltd.	2210006
24	Dimethyl sulfoxide （DMSO）	Sigma-Aldrich	D2650
25	Collagenase A	Roche	10103586001
26	Human LIF	Novaprotein	C017
27	HBSS	Servicebio	G4204-500
28	10XPBS	Servicebio	G4207-500
29	Human LIF	Novaprotein	C017
30	anti-SSEA-1(CD15)MicroBeads, m, h	Miltenyi Biotec	130-094-530
31	CLDN6 recombinant protein	Dima Biotech	PME100063
32	mouse IFN-gamma elispot plus(plus) HRP	MabTech	3321-4HST-2
33	VivoTrack 680 Sample Size	PerkinElmer	NEV12001
34	Bovine Serum Albumin	SIGMA	V900933-100G
35	CD8a+ MicroBeads, mouse	Miltenyi Biotec	130－104－075
36	mouse IFN-gamma elispot plus(plus) HRP	MabTech	3321-4HST-2
37	CD45 MicroBeads, mouse	Miltenyi Biotec	130-052-301
38	2’3’-cGAMP VacciGrade™	Invivogen	vac-nacga23
39	Poly(I:C) (HMW) VacciGrade™	Invivogen	vac-pic
40	Mouse IFN-gamma ELISpot PLUS (HRP), strips	MabTech	3321-4HST-10
41	EmbryoMax™ Nucleosides (100X)	merck	ES-008
42	Dead cell removal kit	Miltenyi Biotec	130-090-101
43	anti-SSEA-1(CD15)MicroBeads, m, h	Miltenyi	130-094-530

		Biotec	
44	Liberase™ TL 研究级	Roche	5401020001
45	PD0325901	MCE	HY-10254
46	CHIR99021	MCE	HY-10182
47	Mitomycin C	MCE	HY-13316
48	Pioglitazone	MCE	HY-13956
49	LEGENDplex MU Th Cytokine Panel (12-plex) w/ VbP V03	Biolegend	741044
50	mouse GM-CSF（Ecoli）	Novoprotein	CK02

Table S2

Protein local pairwise alignment between human and mouse gene, epitopes were highlighted in yellow. Canonical protein sequences were downloaded from uniprot, pairwise alignment were conducted by EMBOSS-Water with default parameter.

CCNB1

```
CCNB1_HUMAN        1 MALRVTRNSKINAENKAKINMAGAKRVPTAPAATSKPGLRPRTALGDIGN     50
                     ||||||||:|||||||||::||||||||....|.||||||||||||||||
CCNB1_MOUSE        1 MALRVTRNTKINAENKAKVSMAGAKRVPVTVTAASKPGLRPRTALGDIGN     50

CCNB1_HUMAN       51 KVSEQLQAKMPMKKEAKPSATGKVIDKKLPKPLEKVPMLVPVPVSEP---     97
                     ||||:|||::|:|:|||...|||...|.||||:||      |||.||
CCNB1_MOUSE       51 KVSEELQARVPLKREAKTLGTGKGTVKALPKPVEK------VPVCEPEVE     94

CCNB1_HUMAN       98 VPEPEPEPEPEPVKEEKLSPEPILVDTASPSPMETSGCAPAEEDLCQAFS    147
                     :.|||||||.|.|:||||||||||||..|||||||||||||||.||||||
CCNB1_MOUSE       95 LAEPEPEPELEHVREEKLSPEPILVDNPSPSPMETSGCAPAEEYLCQAFS    144

CCNB1_HUMAN      148 DVILAVNDVDAEDGADPNLCSEYVKDIYAYLRQLEEEQAVRPKYLLGREV    197
                     ||||||:||||:||||||||||||||||||||||||||:|||||.||||
CCNB1_MOUSE      145 DVILAVSDVDADDGADPNLCSEYVKDIYAYLRQLEEEQSVRPKYLQGREV    194

CCNB1_HUMAN      198 TGNMRAILIDWLVQVQMKFRLLQETMYMTVSIIDRFMQNNCVPKKMLQLV    247
                     ||||||||||||:||||||||||||||||||||||||||||:||||||||||
CCNB1_MOUSE      195 TGNMRAILIDWLIQVQMKFRLLQETMYMTVSIIDRFMQNSCVPKKMLQLV    244

CCNB1_HUMAN      248 GVTAMFIASKYEEMYPPEIGDFAFVTDNTYTKHQIRQMEMKILRALNFGL    297
                     ||||||||||||||||||||||||||:|||||||||||||||||.|||.|
CCNB1_MOUSE      245 GVTAMFIASKYEEMYPPEIGDFAFVTNNTYTKHQIRQMEMKILRVLNFSL    294

CCNB1_HUMAN      298 GRPLPLHFLRRASKIGEVDVEQHTLAKYLMELTMLDYDMVHFPPSQIAAG    347
                     ||||||||||||||:|||||||||||||||||:|||||||||.|||||||
CCNB1_MOUSE      295 GRPLPLHFLRRASKVGEVDVEQHTLAKYLMELSMLDYDMVHFAPSQIAAG    344

CCNB1_HUMAN      348 AFCLALKILDNGEWTPTLQHYLSYTEESLLPVMQHLAKNVVMVNQGLTKH    397
                     ||||||||||||||||||||||||:|:|||||||||||||||||.|||||
CCNB1_MOUSE      345 AFCLALKILDNGEWTPTLQHYLSYSEDSLLPVMQHLAKNVVMVNCGLTKH    394

CCNB1_HUMAN      398 MTVKNKYATSKHAKISTLPQLNSALVQDLAKAVAK    432
                     ||||||||.|||||||||.|||..|||:|:|||.|
CCNB1_MOUSE      395 MTVKNKYAASKHAKISTLAQLNCTLVQNLSKAVTK    429
```

TOP2A

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TOP2A_HUMAN        1 MEVSPLQPVNENMQVNKIKKNEDAKKRLSVERIYQKKTQLEHILLRPDTY     50
                     ||:||||||||||.:|| |||||.|||||:||||||||||||||||||||
TOP2A_MOUSE        1 MELSPLQPVNENMLMNK-KKNEDGKKRLSIERIYQKKTQLEHILLRPDTY     49

TOP2A_HUMAN       51 IGSVELVTQQMWVYDEDVGINYREVTFVPGLYKIFDEILVNAADNKQRDP    100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE       50 IGSVELVTQQMWVYDEDVGINYREVTFVPGLYKIFDEILVNAADNKQRDP     99
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TOP2A_HUMAN        101 KMSCIRVTIDPENNLISIWNNGKGIPVVEHKVEKMYVPALIFGQLLTSSN     150
                       ||||||||||||||:||||||||||||||||||||:|||||||||||||||
TOP2A_MOUSE        100 KMSCIRVTIDPENNVISIWNNGKGIPVVEHKVEKIYVPALIFGQLLTSSN     149

TOP2A_HUMAN        151 YDDDEKKVTGGRNGYGAKLCNIFSTKFTVETASREYKKMFKQTWMDNMGR     200
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        150 YDDDEKKVTGGRNGYGAKLCNIFSTKFTVETASREYKKMFKQTWMDNMGR     199

TOP2A_HUMAN        201 AGEMELKPFNGEDYTCITFQPDLSKFKMQSLDKDIVALMVRRAYDIAGST     250
                       ||:||||||:||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        200 AGDMELKPFSGEDYTCITFQPDLSKFKMQSLDKDIVALMVRRAYDIAGST     249

TOP2A_HUMAN        251 KDVKVFLNGNKLPVKGFRSYVDMYLKDKLDETGNSLKVIHEQVNHRWEVC     300
                       ||||||||||.|||||||||||:|||||:|||||||||||||||.|||||
TOP2A_MOUSE        250 KDVKVFLNGNSLPVKGFRSYVDLYLKDKVDETGNSLKVIHEQVNPRWEVC     299

TOP2A_HUMAN        301 LTMSEKGFQQISFVNSIATSKGGRHVDYVADQIVTKLVDVVKKKNKGGVA     350
                       |||||:||||||||||||||||||||||||||||:|||||||||||||||
TOP2A_MOUSE        300 LTMSERGFQQISFVNSIATSKGGRHVDYVADQIVSKLVDVVKKKNKGGVA     349

TOP2A_HUMAN        351 VKAHQVKNHMWIFVNALIENPTFDSQTKENMTLQPKSFGSTCQLSEKFIK     400
                       ||||||||||||||||||||||||||||||||||.|||||||||||||||
TOP2A_MOUSE        350 VKAHQVKNHMWIFVNALIENPTFDSQTKENMTLQAKSFGSTCQLSEKFIK     399

TOP2A_HUMAN        401 AAIGCGIVESILNWVKFKAQVQLNKKCSAVKHNRIKGIPKLDDANDAGGR     450
                       ||||||||||||||||||||:|||||||||||.:||||||||||||||.|
TOP2A_MOUSE        400 AAIGCGIVESILNWVKFKAQIQLNKKCSAVKHTKIKGIPKLDDANDAGSR     449

TOP2A_HUMAN        451 NSTECTLILTEGDSAKTLAVSGLGVVGRDKYGVFPLRGKILNVREASHKQ     500
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        450 NSTECTLILTEGDSAKTLAVSGLGVVGRDKYGVFPLRGKILNVREASHKQ     499

TOP2A_HUMAN        501 IMENAEINNIIKIVGLQYKKNYEDEDSLKTLRYGKIMIMTDQDQDGSHIK     550
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        500 IMENAEINNIIKIVGLQYKKNYEDEDSLKTLRYGKIMIMTDQDQDGSHIK     549

TOP2A_HUMAN        551 GLLINFIHHNWPSLLRHRFLEEFITPIVKVSKNKQEMAFYSLPEFEEWKS     600
                       ||||||||||||||||||||||||||||||||||||:|||||||||||||
TOP2A_MOUSE        550 GLLINFIHHNWPSLLRHRFLEEFITPIVKVSKNKQEIAFYSLPEFEEWKS     599

TOP2A_HUMAN        601 STPNHKKWKVKYYKGLGTSTSKEAKEYFADMKRHRIQFKYSGPEDDAAIS     650
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        600 STPNHKKWKVKYYKGLGTSTSKEAKEYFADMKRHRIQFKYSGPEDDAAIS     649

TOP2A_HUMAN        651 LAFSKKQIDDRKEWLTNFMEDRRQRKLLGLPEDYLYGQTTTYLTYNDFIN     700
                       |||||||:||||||||||||||||||||||||||||||:|:|||||||||
TOP2A_MOUSE        650 LAFSKKQVDDRKEWLTNFMEDRRQRKLLGLPEDYLYGQSTSYLTYNDFIN     699

TOP2A_HUMAN        701 KELILFSNSDNERSIPSMVDGLKPGQRKVLFTCFKRNDKREVKVAQLAGS     750
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        700 KELILFSNSDNERSIPSMVDGLKPGQRKVLFTCFKRNDKREVKVAQLAGS     749

TOP2A_HUMAN        751 VAEMSSYHHGEMSLMMTIINLAQNFVGSNNLNLLQPIGQFGTRLHGGKDS     800
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE        750 VAEMSSYHHGEMSLMMTIINLAQNFVGSNNLNLLQPIGQFGTRLHGGKDS     799

TOP2A_HUMAN        801 ASPRYIFTMLSSLARLLFPPKDDHTLKFLYDDNQRVEPEWYIPIIPMVLI     850
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                          |||||||||||.||||||||||||||:|||||||||||||||||||||||
TOP2A_MOUSE          800 ASPRYIFTMLSPLARLLFPPKDDHTLRFLYDDNQRVEPEWYIPIIPMVLI     849

TOP2A_HUMAN          851 NGAEGIGTGWSCKIPNFDVREIVNNIRRLMDGEEPLPMLPSYKNFKGTIE     900
                         |||||||||||||||||||||:|||||||:||||||||||||||||||||
TOP2A_MOUSE          850 NGAEGIGTGWSCKIPNFDVREVVNNIRRLLDGEEPLPMLPSYKNFKGTIE     899

TOP2A_HUMAN          901 ELAPNQYVISGEVAILNSTTIEISELPVRTWTQTYKEQVLEPMLNGTEKT     950
                         |||.|||||:||||||:||||||||||:||||||||||||||||||||||
TOP2A_MOUSE          900 ELASNQYVINGEVAILDSTTIEISELPIRTWTQTYKEQVLEPMLNGTEKT     949

TOP2A_HUMAN          951 PPLITDYREYHTDTTVKFVVKMTEEKLAEAERVGLHKVFKLQTSLTCNSM    1000
                         |.|||||||||||||||||:||||||||||||||||||||||:|||||||
TOP2A_MOUSE          950 PSLITDYREYHTDTTVKFVIKMTEEKLAEAERVGLHKVFKLQSSLTCNSM     999

TOP2A_HUMAN         1001 VLFDHVGCLKKYDTVLDILRDFFELRLKYYGLRKEWLLGMLGAESAKLNN    1050
                         |||||||||||||||||||||||||||||||||||||||||||||:||||
TOP2A_MOUSE         1000 VLFDHVGCLKKYDTVLDILRDFFELRLKYYGLRKEWLLGMLGAESSKLNN    1049

TOP2A_HUMAN         1051 QARFILEKIDGKIIIENKPKKELIKVLIQRGYDSDPVKAWKEAQQKVPDE    1100
                         |||||||||||||:||||||||||||||||||||||||||||||||||||
TOP2A_MOUSE         1050 QARFILEKIDGKIVIENKPKKELIKVLIQRGYDSDPVKAWKEAQQKVPDE    1099

TOP2A_HUMAN         1101 EENEESDNEKETEKSDSVTDSGPTFNYLLDMPLWYLTKEKKDELCRLRNE    1150
                         |||||||.  ||..|||..::||||||||||||||||||||||||:.|||
TOP2A_MOUSE         1100 EENEESDT--ETSTSDSAAEAGPTFNYLLDMPLWYLTKEKKDELCKQRNE    1147

TOP2A_HUMAN         1151 KEQELDTLKRKSPSDLWKEDLATFIEELEAVEAKEKQDEQVGLPGKGGKA    1200
                         |||||:|||:||||||||||||.||||||.||||||||||||||||.|||
TOP2A_MOUSE         1148 KEQELNTLKQKSPSDLWKEDLAVFIEELEVVEAKEKQDEQVGLPGKAGKA    1197

TOP2A_HUMAN         1201 KGKKTQM-AEVLPSPRGQRVIPRITIEMKAEAEKKNKKKIKNENTEGSPQ    1249
                         ||||.|| |:|||||||:||||::|:|||||||||.:||||:||.||:|.
TOP2A_MOUSE         1198 KGKKAQMCADVLPSPRGKRVIPQVTVEMKAEAEKKIRKKIKSENVEGTPA    1247

TOP2A_HUMAN         1250 EDGVELEGLKQRLEKKQKREPGTKTKKQTTLAFKPIKKGKKRNPWSDSES    1299
                         |||.|...|:||:|||||:|||  .||||||.|||:|||:|:||||||||
TOP2A_MOUSE         1248 EDGAEPGSLRQRIEKKQKKEPG--AKKQTTLPFKPVKKGRKKNPWSDSES    1295

TOP2A_HUMAN         1300 DRSSDESNFDVPPRETEPRRAATKTKFTMDLDSDEDFSDFDEKTDDEDFV    1349
                         |.||:|||.|||||:.|.|.||.|.|||:|||||||||..|||.:||||:
TOP2A_MOUSE         1296 DVSSNESNVDVPPRQKEQRSAAAKAKFTVDLDSDEDFSGLDEKDEDEDFL    1345

TOP2A_HUMAN         1350 PSDASPPKTKTSPKLSNKELKPQKSVVS--DLEADDVKGSVPLSSSPPAT    1397
                         |.||:|||.|..||.:.|.||.|.|.:|  |||: |||.|||.|...||.
TOP2A_MOUSE         1346 PLDATPPKAKIPPKNTKKALKTQGSSMSVVDLES-DVKDSVPASPGVPAA    1394

TOP2A_HUMAN         1398 HFPDETEITNPVPKKNVTVKKTAAKSQSSTSTTGAKKRAAPKGTKRDPAL    1447
                         .||.|||.:.| .||.|.|||||.|||||.||.|.||||||||||.|.||
TOP2A_MOUSE         1395 DFPAETEQSKP-SKKTVGVKKTATKSQSSVSTAGTKKRAAPKGTKSDSAL    1443

TOP2A_HUMAN         1448 NSGVSQKPDPAKTKNRRKRKPSTSDDSDSNFEKIVSKAVTSKKSKGESDD    1497
                         ::.||:||.|||.||.||||||:||.|||:||:.:||..||||:|||..|
TOP2A_MOUSE         1444 SARVSEKPAPAKAKNSRKRKPSSSDSSDSDFERAISKGATSKKAKGEEQD    1493

TOP2A_HUMAN         1498 FHMDFDSAVAPRAKSVRAKKPIKYLEESDEDD   1529
                         |.:|.:..:||||||.||:||||||||||:||
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TOP2A_MOUSE      1494 FPVDLEDTIAPRAKSDRARKPIKYLEESDDDD    1525
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ECT2

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ECT2_HUMAN           1 MAENSVLTSTTGRTSLADSSIFDSKVTEISKENLLIGSTSYVEEEMPQIE      50
                       ||::|||.|.:..|||||||:|||||.|:|||||.:.|||.|:|||||:|
ECT2_MOUSE           1 MADDSVLPSPSEITSLADSSVFDSKVAEMSKENLCLASTSNVDEEMPQVE      50

ECT2_HUMAN          51 TRVILVQEAGKQEELIKALKTIKIMEVPVIKIKESCPGKSDEKLIKSVIN     100
                       .|||:||:|||||||:||||||||||||||||||||||||:||||||:||
ECT2_MOUSE          51 ARVIMVQDAGKQEELLKALKTIKIMEVPVIKIKESCPGKSEEKLIKSIIN     100

ECT2_HUMAN         101 MDIKVGFVKMESVEEFEGLDSPEFENVFVVTDFQDSVFNDLYKADCRVIG     150
                       |::||..|||:|:||||.||||||||:|||||||:||||||||||||::|
ECT2_MOUSE         101 MEMKVPCVKMDSMEEFESLDSPEFENIFVVTDFQNSVFNDLYKADCRIVG     150

ECT2_HUMAN         151 PPVVLNCSQKGEPLPFSCRPLYCTSMMNLVLCFTGFRKKEELVRLVTLVH     200
                       |||:|||:|:||||||||||||||||:||||||||||||||||:||||||
ECT2_MOUSE         151 PPVILNCAQRGEPLPFSCRPLYCTSMLNLVLCFTGFRKKEELVKLVTLVH     200

ECT2_HUMAN         201 HMGGVIRKDFNSKVTHLVANCTQGEKFRVAVSLGTPIMKPEWIYKAWERR     250
                       ||||||||:.||||||||||||||||||||||||||||||||||||||||
ECT2_MOUSE         201 HMGGVIRKECNSKVTHLVANCTQGEKFRVAVSLGTPIMKPEWIYKAWERR     250

ECT2_HUMAN         251 NEQDFYAAVDDFRNEFKVPPFQDCILSFLGFSDEEKTNMEEMTEMQGGKY     300
                       |||.|.||||||||||||||||||||||||||||||.:||||||||||.|
ECT2_MOUSE         251 NEQCFCAAVDDFRNEFKVPPFQDCILSFLGFSDEEKHSMEEMTEMQGGSY     300

ECT2_HUMAN         301 LPLGDERCTHLVVEENIVKDLPFEPSKKLYVVKQEWFWGSIQMDARAGET     350
                       ||:||||||||:||||.||||||||||||:||||||||||||||||||||
ECT2_MOUSE         301 LPVGDERCTHLIVEENTVKDLPFEPSKKLFVVKQEWFWGSIQMDARAGET     350

ECT2_HUMAN         351 MYLYEKANTPELKKSVSMLSLNTPNSNRKRRRLKETLAQLSRETDVSPFP     400
                       |||||||||||||||||:|||:|||||||||||||||||||||||:||||
ECT2_MOUSE         351 MYLYEKANTPELKKSVSLLSLSTPNSNRKRRRLKETLAQLSRETDLSPFP     400

ECT2_HUMAN         401 PRKRPSAEHSLSIGSLLDISNTPESSINYGDTPKSCTKSSKSSTPVPSKQ     450
                       |||||||||||||||||||||||||||:||:|||||.|||:||||||.||
ECT2_MOUSE         401 PRKRPSAEHSLSIGSLLDISNTPESSIHYGETPKSCAKSSRSSTPVPPKQ     450

ECT2_HUMAN         451 SARWQVAKELYQTESNYVNILATIIQLFQVPLEEEGQRGGPILAPEEIKT     500
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
ECT2_MOUSE         451 SARWQVAKELYQTESNYVNILATIIQLFQVPLEEEGQRGGPILAPEEIKT     500

ECT2_HUMAN         501 IFGSIPDIFDVHTKIKDDLEDLIVNWDESKSIGDIFLKYSKDLVKTYPPF     550
                       ||||||||||||.||||||||||.|||||:|||||||||:||||||||||
ECT2_MOUSE         501 IFGSIPDIFDVHMKIKDDLEDLIANWDESRSIGDIFLKYAKDLVKTYPPF     550

ECT2_HUMAN         551 VNFFEMSKETIIKCEKQKPRFHAFLKINQAKPECGRQSLVELLIRPVQRL     600
                       |||||||||.||||||||||||||||||||||||||||||||||||||||
ECT2_MOUSE         551 VNFFEMSKEMIIKCEKQKPRFHAFLKINQAKPECGRQSLVELLIRPVQRL     600

ECT2_HUMAN         601 PSVALLLNDLKKHTADENPDKSTLEKAIGSLKEVMTHINEDKRKTEAQKQ     650
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
ECT2_MOUSE         601 PSVALLLNDLKKHTADENPDKSTLEKAIGSLKEVMTHINEDKRKTEAQKQ     650

ECT2_HUMAN         651 IFDVVYEVDGCPANLLSSHRSLVQRVETISLGEHPCDRGEQVTLFLFNDC     700
                       ||||||||||||||||||||||||||||:|||||||||||||||||||||
ECT2_MOUSE         651 IFDVVYEVDGCPANLLSSHRSLVQRVETVSLGEHPCDRGEQVTLFLFNDC     700
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ECT2_HUMAN        701 LEIARKRHKVIGTFRSPHGQTRPPASLKHIHLMPLSQIKKVLDIRETEDC    750
                      ||||||||||||||||||.:||||||||||||||||||||||||||||||
ECT2_MOUSE        701 LEIARKRHKVIGTFRSPHDRTRPPASLKHIHLMPLSQIKKVLDIRETEDC    750

ECT2_HUMAN        751 HNAFALLVRPPTEQANVLLSFQMTSDELPKENWLKMLCRHVANTICKADA    800
                      |||||||||||||||||||||||||:|||||:||||||||||||||||||
ECT2_MOUSE        751 HNAFALLVRPPTEQANVLLSFQMTSEELPKESWLKMLCRHVANTICKADA    800

ECT2_HUMAN        801 ENLIYTADPESFEVNTKDMDSTLSRASRAIKKTSKKVTRAFSFSKTPKRA    850
                      |||:|.||||||||||||||||||||||||||||||||||||||||||||
ECT2_MOUSE        801 ENLMYVADPESFEVNTKDMDSTLSRASRAIKKTSKKVTRAFSFSKTPKRA    850

ECT2_HUMAN        851 LRRALMTSHGSVEGRSPSSNDKHVMSRLSSTSSLAGIPSPSLVSLPSFFE    900
                      ||.||.:||.| |||||.|:.|..:|||||||||||||||||||||||||
ECT2_MOUSE        851 LRMALSSSHSS-EGRSPPSSGKLAVSRLSSTSSLAGIPSPSLVSLPSFFE    899

ECT2_HUMAN        901 RRSHTLSRSTTHLI    914
                      ||||||||||||||
ECT2_MOUSE        900 RRSHTLSRSTTHLI    913
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MELK

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MELK_HUMAN           1 MKDYDELLKYYELHETIGTGGFAKVKLACHILTGEMVAIKIMDKNTLGSD     50
                       |||||||||||||:||||||||||||||||:||||||||||||||.||||
MELK_MOUSE           1 MKDYDELLKYYELYETIGTGGFAKVKLACHVLTGEMVAIKIMDKNALGSD     50

MELK_HUMAN          51 LPRIKTEIEALKNLRHQHICQLYHVLETANKIFMVLEYCPGGELFDYIIS    100
                       |||:||||:|||:|||||||||||||||.|||||||||||||||||||||
MELK_MOUSE          51 LPRVKTEIDALKSLRHQHICQLYHVLETKNKIFMVLEYCPGGELFDYIIS    100

MELK_HUMAN         101 QDRLSEEETRVVFRQIVSAVAYVHSQGYAHRDLKPENLLFDEYHKLKLID    150
                       ||||||||||||||||:|||||||||||||||||||||||||.|||||||
MELK_MOUSE         101 QDRLSEEETRVVFRQILSAVAYVHSQGYAHRDLKPENLLFDENHKLKLID    150

MELK_HUMAN         151 FGLCAKPKGNKDYHLQTCCGSLAYAAPELIQGKSYLGSEADVWSMGILLY    200
                       ||||||||||||||||||||||||||||||||||||||||||||||||||
MELK_MOUSE         151 FGLCAKPKGNKDYHLQTCCGSLAYAAPELIQGKSYLGSEADVWSMGILLY    200

MELK_HUMAN         201 VLMCGFLPFDDDNVMALYKKIMRGKYDVPKWLSPSSILLLQQMLQVDPKK    250
                       ||||||||||||||||||||||||||:|||||||||||||||||||||||
MELK_MOUSE         201 VLMCGFLPFDDDNVMALYKKIMRGKYEVPKWLSPSSILLLQQMLQVDPKK    250

MELK_HUMAN         251 RISMKNLLNHPWIMQDYNYPVEWQSKNPFIHLDDDCVTELSVHHRNNRQT    300
                       ||||:|||||||:||||:.|||||||.|..|||:|||||||||||::|||
MELK_MOUSE         251 RISMRNLLNHPWVMQDYSCPVEWQSKTPLTHLDEDCVTELSVHHRSSRQT    300

MELK_HUMAN         301 MEDLISLWQYDHLTATYLLLLAKKARGKPVRLRLSSFSCGQASATPFTDI    350
                       ||||||.||||||||||||||||||||||.||:|.|||||.||.||
MELK_MOUSE         301 MEDLISSWQYDHLTATYLLLLAKKARGKPARLQLLSFSCGTASTTP----    346

MELK_HUMAN         351 KSNNWSLEDVTASDKNYVAGLIDYDWCEDDLSTGAATPRTSQFTKYWTES    400
                       ||.|.||||::.||.|.|||||||:.|||.|    ..|:|.|.||:..||
MELK_MOUSE         347 KSKNLSLEDMSTSDDNCVAGLIDYELCEDKL--- LAPKTPQVTKHLAES    392
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MELK_HUMAN       401 NGVESKSLTPALCRTPANKLKNKENVYTPKSAVKNEEYFMFPEPKTPVNK    450
                     |...|||..|.:.|..||||.:||||.||||:|||||.|:|.|||.||:|
MELK_MOUSE       393 NHAASKSPAPGVRRAVANKLMDKENVCTPKSSVKNEEQFVFSEPKIPVSK    442

MELK_HUMAN       451 NQHKREILTTPNRYTTPSKARNQCLKETPIKIPVNSTGTDKLMTGVISPE    500
                     ||:||||..:|.|:.||:|||.|||:|.|::.|.||.|.|.|.|||||||
MELK_MOUSE       443 NQYKREIPASPTRFPTPAKARAQCLREAPVRTPGNSAGADTLTTGVISPE    492

MELK_HUMAN       501 RRCRSVELDLNQAHMEETPKRKGAKVFGSLERGLDKVITVLTRSKRKGSA    550
                     |||||:::||||||||:|||:||..||||||||||||:|.|||:|:||||
MELK_MOUSE       493 RRCRSMDVDLNQAHMEDTPKKKGTNVFGSLERGLDKVLTALTRNKKKGSA    542

MELK_HUMAN       551 RDGPRRLKLHYNVTTTRLVNPDQLLNEIMSILPKKHVDFVQKGYTLKCQT    600
                     |||||:.||||||||||||||||||:|||:|||||:||||||||||||||
MELK_MOUSE       543 RDGPRKRKLHYNVTTTRLVNPDQLLSEIMAILPKKNVDFVQKGYTLKCQT    592

MELK_HUMAN       601 QSDFGKVTMQFELEVCQLQKPDVVGIRRQRLKGDAWVYKRLVEDILSSCK    650
                     |||||||||||||||||||:|||||||||||||||||||||||||||.||
MELK_MOUSE       593 QSDFGKVTMQFELEVCQLQRPDVVGIRRQRLKGDAWVYKRLVEDILSGCK    642

MELK_HUMAN       651 V    651
                     :
MELK_MOUSE       643 M    643
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ANLN

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ANLN_HUMAN         1 MDPFTEKLLERTRARRENLQRKMAERPTAAPRSMTHAKRARQPLSEASN-     49
                     |||||||||||||||||||||||||||||..||..||||.|:|||||||
ANLN_MOUSE         1 MDPFTEKLLERTRARRENLQRKMAERPTAVARSAPHAKRGREPLSEASNQ     50

ANLN_HUMAN        50 QQPLSGGEEKSCTKPSPSKKRCSDNTEVEVSNLENKQPVESTSAKSCSPS     99
                     ||||.|||||||||||||||||||..||...:|||.:|::  .||.|||.
ANLN_MOUSE        51 QQPLPGGEEKSCTKPSPSKKRCSDKIEVGAPDLENTEPID--VAKPCSPM     98

ANLN_HUMAN       100 PVSPQVQPQAADTISDSVAVPASLLGMRRGLNSRLEATAASSVKTRMQKL    149
                     |...|.:|.|...||:|||.||:||...|||||..||:|.||||||||:|
ANLN_MOUSE        99 PAPRQAKPPAPAAISESVAAPAALLSADRGLNSGSEASATSSVKTRMQRL    148

ANLN_HUMAN       150 AEQRRRWDNDDMTDDIPESSLFSPMPSEEKAASPPRPLLSNASATPVGRR    199
                     |||||.||: |:|||:.|||.|:|:|:|:|||||.:|.:|||||||||||
ANLN_MOUSE       149 AEQRRHWDS-DLTDDVSESSYFAPVPTEDKAASPSKPPISNASATPVGRR    197

ANLN_HUMAN       200 GRLANLAATICSWEDDVNHSFAKQNSVQEQPGTACLSKFSSASGASARIN    249
                     |||||||||||||||||:||.|||||||||||||||||.||||||||.||
ANLN_MOUSE       198 GRLANLAATICSWEDDVSHSSAKQNSVQEQPGTACLSKSSSASGASASIN    247

ANLN_HUMAN       250 SSSVKQEATFCSQRDGDASLNKALSSSADDASLVNASISSSVKATSPVKS    299
                     ||||:||||.||.|||:||:.|..||:|....|::||:||||||:|||.:
ANLN_MOUSE       248 SSSVQQEATCCSPRDGNASVRKDPSSNAAHGPLLSASVSSSVKASSPVTA    297

ANLN_HUMAN       300 TTSITDAKSCEGQNPELLPKTPISPLKTGVSKPIVKSTLSQTVPSKGELS    349
                     .|.||:.:  |.||||||.|| .|||||...||..|.||||....|.|.:
ANLN_MOUSE       298 ATFITENR--EAQNPELLHKT-ASPLKTEARKPCEKPTLSQGAQPKEEAN    344
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ANLN_HUMAN        350 REICLQSQSKDKSTTPGGTGIKPFLERFGERCQEHSKESPARSTPHRTPI    399
                      ||:|||||||||..||||.|||||||||||||||||||||:....|:||.
ANLN_MOUSE        345 REVCLQSQSKDKLATPGGRGIKPFLERFGERCQEHSKESPSYRASHKTPN    394

ANLN_HUMAN        400 ITPNTKAIQERLFKQDTSSSTTHLAQQLKQERQKELACLRGRFDKGNIWS    449
                      |||||||||||||||:|.||||||||||||||:|||||||||.||||:||
ANLN_MOUSE        395 ITPNTKAIQERLFKQNTCSSTTHLAQQLKQEREKELACLRGRLDKGNLWS    444

ANLN_HUMAN        450 AEKGGNSKSKQLETKQETHCQSTPLKKHQGVSKTQSLPVTEKVTENQIPA    499
                      |||...|:||.||||||.|||:|||||||.|:.|....||:||.||:...
ANLN_MOUSE        445 AEKNEKSRSKHLETKQEVHCQNTPLKKHQTVASTPLTSVTDKVAENEPAV    494

ANLN_HUMAN        500 KNSSTEPKGFTECEMTKSSPLKITLFLEEDKSLKVTSDPKVEQKIEVIRE    549
                      |.|||||.|.||.||||||||||||||||:|||||.||.:|||..|.:||
ANLN_MOUSE        495 KLSSTEPAGSTESEMTKSSPLKITLFLEEEKSLKVASDLEVEQNTEAVRE    544

ANLN_HUMAN        550 IEMSVDDDDINSSKVINDLFSDVLEEGELDMEKSQEEMDQALAESSEEQE    599
                      :||||||:|||||:||||:|||||||||||:|||||||||..||:|||||
ANLN_MOUSE        545 VEMSVDDEDINSSRVINDIFSDVLEEGELDVEKSQEEMDQVGAENSEEQE    594

ANLN_HUMAN        600 DALNISSMSLLAPLAQTVGVVSPESLVSTPRLELKDTSRSDESPKPGKFQ    649
                      ||||||||||||||||||||||.|:::|:|..||:|::.|..||||||||
ANLN_MOUSE        595 DALNISSMSLLAPLAQTVGVVSLENVISSPPSELRDSNLSAASPKPGKFQ    644

ANLN_HUMAN        650 RTRVPRAESGDSLGSEDRDLLYSIDAYRSQRFKETERPSIKQVIVRKEDV    699
                      |||||||||.||||||||||||||||||||||||||||||||||||||||
ANLN_MOUSE        645 RTRVPRAESADSLGSEDRDLLYSIDAYRSQRFKETERPSIKQVIVRKEDV    694

ANLN_HUMAN        700 TSKLDEKNNAFPCQVNIKQKMQELNNEINMQQTVIYQASQALNCCVDEEH    749
                      ||||.||.|.|..|||||||||||||:||:||||||||||||||||||||
ANLN_MOUSE        695 TSKLGEKKNVFSGQVNIKQKMQELNNDINLQQTVIYQASQALNCCVDEEH    744

ANLN_HUMAN        750 GKGSLEEAEAERLLLIATGKRTLLIDELNKLKNEGPQRKNKAS--PQSEF    797
                      ||||||||||||||||||.||.||||||||||:|||||:||.|  .||||
ANLN_MOUSE        745 GKGSLEEAEAERLLLIATEKRALLIDELNKLKSEGPQRRNKTSVISQSEF    794

ANLN_HUMAN        798 MPSKGSVTLSEIRLPLKADFVCSTVQKPDAANYYYLIILKAGAENMVATP    847
                      .|||||||||||.|||||||||||.||.||:||||||:||||||.|||||
ANLN_MOUSE        795 APSKGSVTLSEICLPLKADFVCSTAQKTDASNYYYLIMLKAGAEQMVATP    844

ANLN_HUMAN        848 LASTSNSLNGDALTFTTTFTLQDVSNDFEINIEVYSLVQKKDPSGLDKKK    897
                      ||||:|||:||||||.|||||.||||||||||||||||||||..|.||||
ANLN_MOUSE        845 LASTANSLSGDALTFPTTFTLHDVSNDFEINIEVYSLVQKKDSLGPDKKK    894

ANLN_HUMAN        898 KTSKSKAITPKRLLTSITTKSNIHSSVMASPGGLSAVRTSNFALVGSYTL    947
                      |.||||||||||||||||:||::|||||||||||.|||||||.||||:||
ANLN_MOUSE        895 KASKSKAITPKRLLTSITSKSSLHSSVMASPGGLGAVRTSNFTLVGSHTL    944

ANLN_HUMAN        948 SLSSVGNTKFVLDKVPFLSSLEGHIYLKIKCQVNSSVEERGFLTIFEDVS    997
                      ||||||:|||.||||||||.|||||.|||.|||||:|||:||||||||||
ANLN_MOUSE        945 SLSSVGDTKFALDKVPFLSPLEGHICLKISCQVNSAVEEKGFLTIFEDVS    994

ANLN_HUMAN        998 GFGAWHRRWCVLSGNCISYWTYPDDEKRKNPIGRINLANCTSRQIEPANR   1047
                      ||||||||||||||||||||||||||:|||||||||||||.|.|||||||
ANLN_MOUSE        995 GFGAWHRRWCVLSGNCISYWTYPDDERRKNPIGRINLANCISHQIEPANR   1044

ANLN_HUMAN       1048 EFCARRNTFELITVRPQREDDRETLVSQCRDTLCVTKNWLSADTKEERDL   1097
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                ||||||||.|||||||||||||||||||||||||||||||||||||||||
ANLN_MOUSE     1045 EFCARRNTLELITVRPQREDDRETLVSQCRDTLCVTKNWLSADTKEERDL
               1094

ANLN_HUMAN     1098 WMQKLNQVLVDIRLWQPDACYKPIGKP    1124
                    ||||||||:||||||||||||||:|||
ANLN_MOUSE     1095 WMQKLNQVIVDIRLWQPDACYKPVGKP    1121
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HMMR

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HMMR_HUMAN        1 MSFPKAPLKRFNDPSGCAPSPGAYDVKTLEVLKGPVSFQKSQRFKQQKES      50
                    ||||||||||||||||||||||||||||.|..|||||||||||||.|:||
HMMR_MOUSE        1 MSFPKAPLKRFNDPSGCAPSPGAYDVKTSEATKGPVSFQKSQRFKNQRES      50

HMMR_HUMAN       51 KQNLNVDKDTTLPASARKVKSSES-KESQKNDKDLKILEKEIRVLLQERG      99
                    :||||:||||||.|||:|.|.|.| |:|||||||:|.||||||.||||||
HMMR_MOUSE       51 QQNLNIDKDTTLLASAKKAKKSVSKKDSQKNDKDVKRLEKEIRALLQERG     100

HMMR_HUMAN      100 AQDRRIQDLETELEKMEARLNAALREKTSLSANNATLEKQLIELTRTNEL     149
                    .||:||||:|:||||.||:||||:||||||||:||:|||:|.||||.|||
HMMR_MOUSE      101 TQDKRIQDMESELEKTEAKLNAAVREKTSLSASNASLEKRLTELTRANEL     150

HMMR_HUMAN      150 LKSKFSENGNQKNLRILSLELMKLRNKRETKMRGMMAKQEGMEMKLQVTQ     199
                    ||:||||:|:|||:|.|||||||||||||||||.||.||||||:|||.||
HMMR_MOUSE      151 LKAKFSEDGHQKNMRALSLELMKLRNKRETKMRSMMVKQEGMELKLQATQ     200

HMMR_HUMAN      200 RSLEESQGKIAQLEGKLVSIEKEKIDEKSETEKLLEYIEEISCASDQVEK     249
                    :.|.||:|||.|||||||||||||||||.|||||||||:|||||||||||
HMMR_MOUSE      201 KDLTESKGKIVQLEGKLVSIEKEKIDEKCETEKLLEYIQEISCASDQVEK     250

HMMR_HUMAN      250 YKLDIAQLEENLKEKNDEILSLKQSLEENIVILSKQVEDLNVKCQLLEKE     299
                    .|:|||||||:||||:.|||||||||||||. .|||:|||.|||||||.|
HMMR_MOUSE      251 CKVDIAQLEEDLKEKDREILSLKQSLEENIT-FSKQIEDLTVKCQLLETE     299

HMMR_HUMAN      300 KEDHVNRNREHNENLNAEMQNLKQKFILEQQEREKLQQKELQIDSLLQQE     349
                    :::.|:::||..|.|:||||.|.::..||:||.|||||||||..||||||
HMMR_MOUSE      300 RDNLVSKDRERAETLSAEMQILTERLALERQEYEKLQQKELQSQSLLQQE     349

HMMR_HUMAN      350 KELSSSLHQKLCSFQEEMVKEKNLFEEELKQTLDELDKLQQKEEQAERLV     399
                    ||||:.|.|:||||||||..|||:|:|||||..|.|||.:||||||:||||
HMMR_MOUSE      350 KELSARLQQQLCSFQEEMTSEKNVFKEELKLALAELDAVQQKEEQSERLV     399

HMMR_HUMAN      400 KQLEEEAKSRAEELKLLEEKLKGKEAELEKSSAAHTQATLL---------     440
                    ||||||.||.||:|..|:..|:.||.||||..|||.||.|:
HMMR_MOUSE      400 KQLEEETKSTAEQLTRLDNLLREKEVELEKHIAAHQAILIAQEKYNDTA     449
                    --------------------------------------------------
HMMR_HUMAN      441                                                      440

HMMR_MOUSE      450 QSLRDVTAQLESVQEKYNDTAQSLRDVTAQLESEQEKYNDTAQSLRDVTA     499

HMMR_HUMAN      441 -------------------------LQEKYDSMVQSLEDVTAQFESYKAL     465
                                             :||||:...|||.|||||.||||:.
HMMR_MOUSE      500 QLESEQEKYNDTAQSLRDVTAQLESVQEKYNDTAQSLRDVTAQLESYKSS     549

HMMR_HUMAN      466 TASEIEDLKLENSSLQEKAAKAGKNAEDVQHQILATESSNQEYVRMLLDL     515
                    |..|||||||||.:||||.|.|.|:.||||.|||..||:||||.||:.||
HMMR_MOUSE      550 TLKEIEDLKLENLTLQEKVAMAEKSVEDVQQQILTAESTNQEYARMVQDL     599

HMMR_HUMAN      516 QTKSALKETEIKEITVSFLQKITDLQNQLKQQEEDFRKQLEDEEGRKAEK     565
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                       |.:|.|||.||||||.|||:|||||:|||:||:||||||||::..|.|||
HMMR_MOUSE        600  QNRSTLKEEEIKEITSSFLEKITDLKNQLRQQDEDFRKQLEEKGKRTAEK    649

HMMR_HUMAN        566  ENTTAELTEEINKWRLLYEELYNKTKPFQLQLDAFEVEKQALLNEHGAAQ    615
                       ||...|||.||||||||||||.||||||.||||||.|||||||||||.|
HMMR_MOUSE        650  ENVMTELTMEINKWRLLYEELYEKTKPFQQQLDAFEAEKQALLNEHGATQ    699

HMMR_HUMAN        616  EQLNKIRDSYAKLLGHQNLKQKIKHVVKLKDENSQLKSEVSKLRCQLAKK    665
                       |||||||||||:||||||||||||||||||||||||||||||||.||.|:
HMMR_MOUSE        700  EQLNKIRDSYAQLLGHQNLKQKIKHVVKLKDENSQLKSEVSKLRSQLVKR    749

HMMR_HUMAN        666  KQSETKLQEELNKVLGIKHFDPSKAFHHESKENFALKTPLKEGNTNC    712
                       ||:|.:||.||:|.|||:||||||||.|.|||||   |||||||.||
HMMR_MOUSE        750  KQNELRLQGELDKALGIRHFDPSKAFCHASKENF---TPLKEGNPNC    793
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IGF2BP1

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IF2B1_HUMAN        1  MNKLYIGNLNESVTPADLEKVFAEHKISYSGQFLVKSGYAFVDCPDEHWA     50
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE        1  MNKLYIGNLNESVTPADLEKVFAEHKISYSGQFLVKSGYAFVDCPDEHWA     50

IF2B1_HUMAN       51  MKAIETFSGKVELQGKRLEIEHSVPKKQRSRKIQIRNIPPQLRWEVLDSL    100
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE       51  MKAIETFSGKVELQGKRLEIEHSVPKKQRSRKIQIRNIPPQLRWEVLDSL    100

IF2B1_HUMAN      101  LAQYGTVENCEQVNTESETAVVNVTYSNREQTRQAIMKLNGHQLENHALK    150
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE      101  LAQYGTVENCEQVNTESETAVVNVTYSNREQTRQAIMKLNGHQLENHALK    150

IF2B1_HUMAN      151  VSYIPDEQIAQGPENGRRGGFGSRGQPRQGSPVAAGAPAKQQQVDIPLRL    200
                      |||||||||.||||||||||||||||||||||||||||||||.|||||||
IF2B1_MOUSE      151  VSYIPDEQITQGPENGRRGGFGSRGQPRQGSPVAAGAPAKQQPVDIPLRL    200

IF2B1_HUMAN      201  LVPTQYVGAIIGKEGATIRNITKQTQSKIDVHRKENAGAAEKAISVHSTP    250
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE      201  LVPTQYVGAIIGKEGATIRNITKQTQSKIDVHRKENAGAAEKAISVHSTP    250

IF2B1_HUMAN      251  EGCSSACKMILEIMHKEAKDTKTADEVPLKILAHNNFVGRLIGKEGRNLK    300
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE      251  EGCSSACKMILEIMHKEAKDTKTADEVPLKILAHNNFVGRLIGKEGRNLK    300

IF2B1_HUMAN      301  KVEQDTETKITISSLQDLTLYNPERTITVKGAIENCCRAEQEIMKKVREA    350
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE      301  KVEQDTETKITISSLQDLTLYNPERTITVKGAIENCCRAEQEIMKKVREA    350

IF2B1_HUMAN      351  YENDVAAMSLQSHLIPGLNLAAVGLFPASSSAVPPPPSSVTGAAPYSSFM    400
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE      351  YENDVAAMSLQSHLIPGLNLAAVGLFPASSSAVPPPPSSVTGAAPYSSFM    400

IF2B1_HUMAN      401  QAPEQEMVQVFIPAQAVGAIIGKKGQHIKQLSRFASASIKIAPPETPDSK    450
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE      401  QAPEQEMVQVFIPAQAVGAIIGKKGQHIKQLSRFASASIKIAPPETPDSK    450

IF2B1_HUMAN      451  VRMVIITGPPEAQFKAQGRIYGKLKEENFFGPKEEVKLETHIRVPASAAG    500
                      ||||:|||||||||||||||||||||||||||||||||||||||||||||
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IF2B1_MOUSE       451 VRMVVITGPPEAQFKAQGRIYGKLKEENFFGPKEEVKLETHIRVPASAAG     500

IF2B1_HUMAN       501 RVIGKGGKTVNELQNLTAAEVVVPRDQTPDENDQVIVKIIGHFYASQMAQ     550
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
IF2B1_MOUSE       501 RVIGKGGKTVNELQNLTAAEVVVPRDQTPDENDQVIVKIIGHFYASQMAQ     550

IF2B1_HUMAN       551 RKIRDILAQVKQQHQKGQSNQAQARRK     577
                      ||||||||||||||||||||.||||||
IF2B1_MOUSE       551 RKIRDILAQVKQQHQKGQSNLAQARRK     577
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