

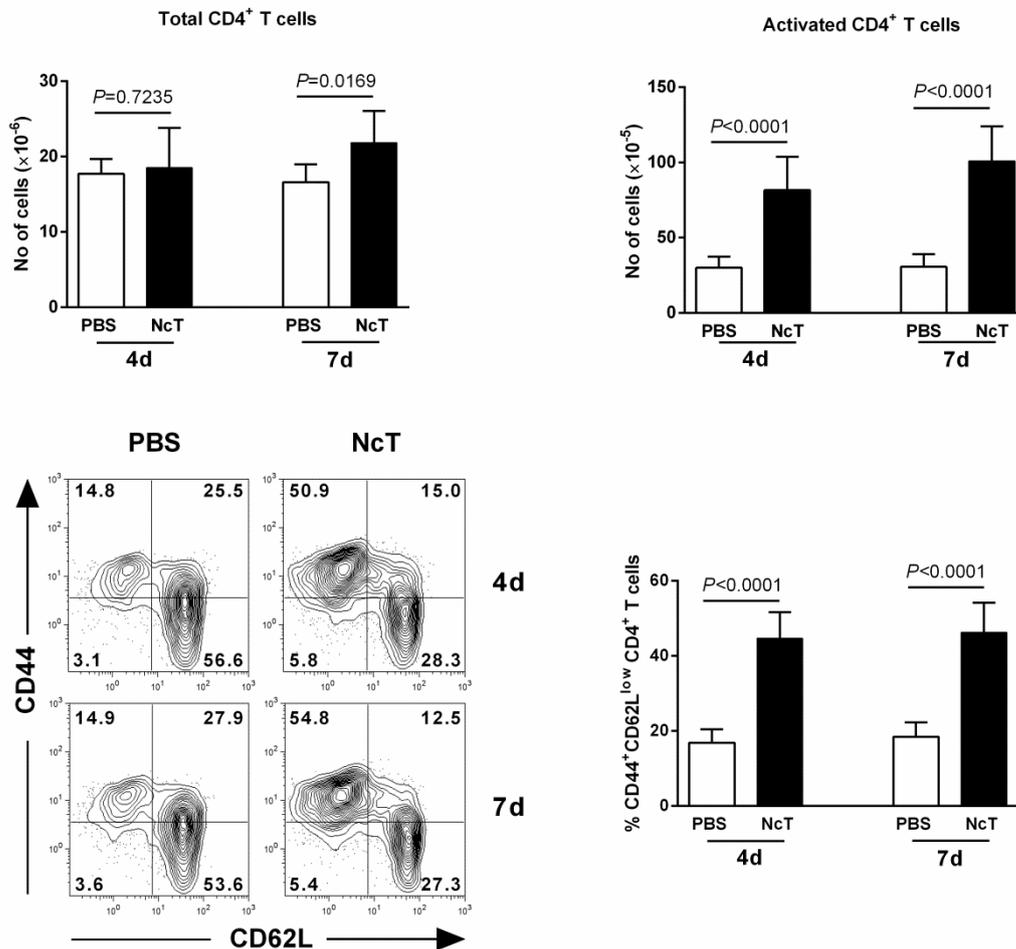
## **Predominant role of interferon- $\gamma$ in the host protective effect of CD8<sup>+</sup> T cells against *Neospora caninum* infection**

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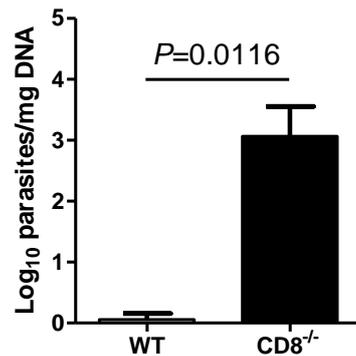
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**Supplementary Figure S1: CD4<sup>+</sup> T cells are activated and expand in the spleen of *N. caninum*-infected C57BL/6 WT mice**



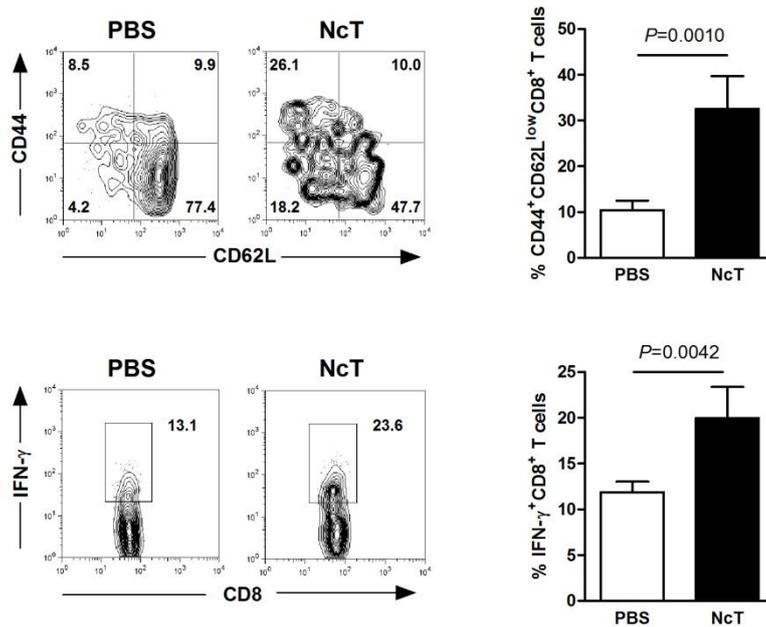
Numbers of total and activated (CD4<sup>+</sup>CD62L<sup>low</sup>) CD4<sup>+</sup> T cells, and percentage of activated CD4<sup>+</sup> T cells, as indicated, detected in the spleen of C57BL/6 mice infected i.p. with 1×10<sup>7</sup> *N. caninum* tachyzoites (NcT), or sham-infected injected i.p. with PBS alone (PBS), 4 and 7 days upon the parasitic challenge.. Bars represent means plus one SD of pooled data from two independent experiments (n=6 and n=9 for control and infected groups, respectively). Unpaired two-tailed t-test was used to compare parasite-inoculated vs respective control mouse groups. Statistical significance between infected mice and controls is indicated above bars. Contour plots correspond to a representative example of CD4-gated T cells of the analysed samples. Quadrants and regions were set according to isotype control-stained samples. Numbers within contour plots correspond to the percentage of cells in each quadrant.

**Supplementary Figure S2: CD8-deficient mice are more susceptible to long-term *N. caninum* infection than control WT mice**



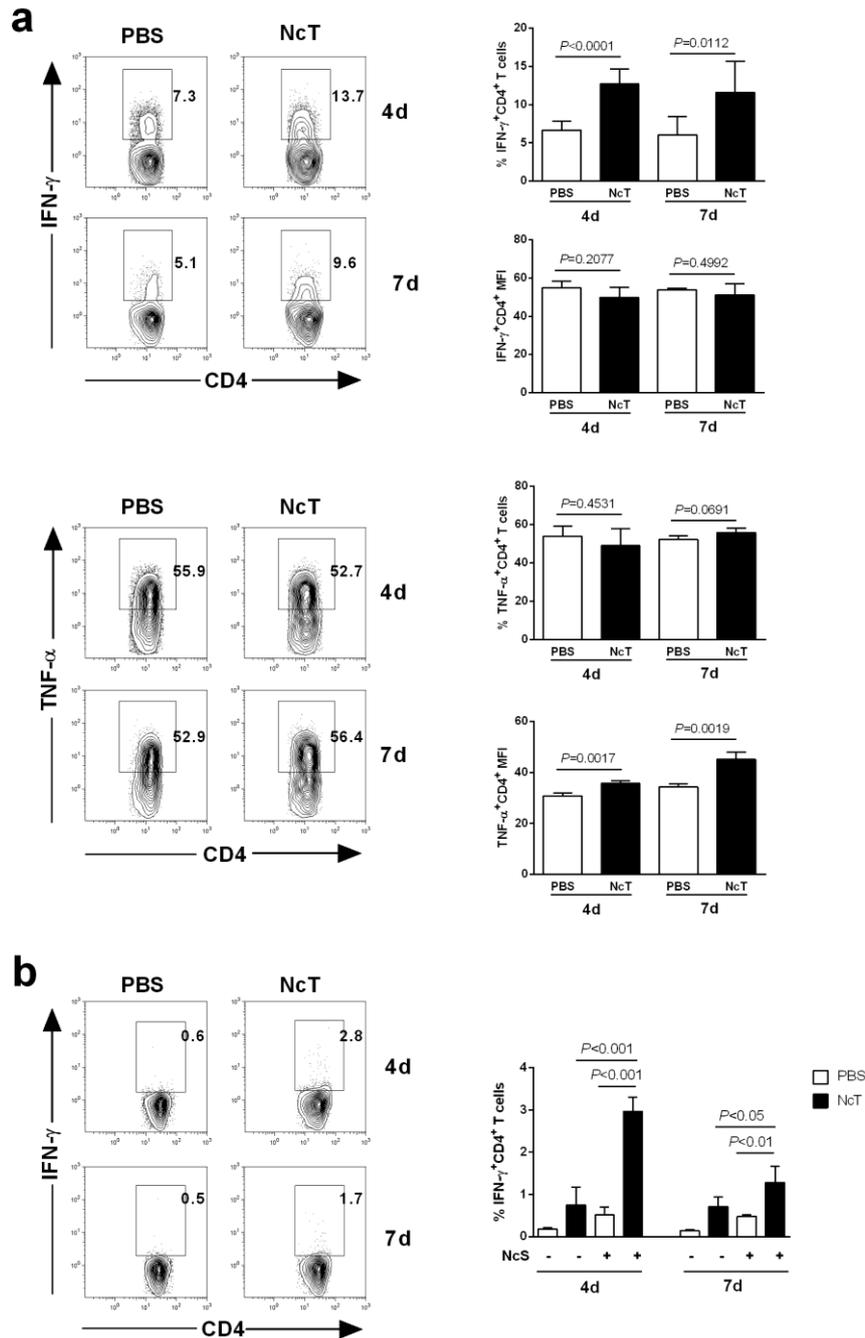
Parasitic load of brain tissue assessed by qPCR specific for *N. caninum* DNA in WT or *CD8a*<sup>-/-</sup> mice, as indicated, 40 days after i.p. inoculation of  $1 \times 10^7$  NcT. Bars represent means plus one SD of pooled data from two independent experiments (n=6 per group). Statistical significance between infected mice and controls is indicated above bars.

**Supplementary Figure S3: CD8<sup>+</sup> T cells are activated and produce IFN- $\gamma$  in *N. caninum*-infected C57BL/10 ScSn mice**



Proportions of activated (CD44<sup>+</sup>CD62L<sup>low</sup>) and of IFN- $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells, as indicated, detected in the spleen of ScSn mice 7 days upon infection i.p. with  $1 \times 10^7$  NcT (NcT) or sham-infection with PBS alone (PBS). Bars represent means plus one SD of four mice per group. Unpaired two-tailed t-test was used to compare parasite-inoculated vs respective control mouse groups. Statistical significance between infected mice and controls is indicated above bars. Contour plots correspond to a representative example of CD8-gated T cells of the analysed samples. Quadrants and regions inside contour plots were set according to isotype control-stained samples. Numbers within contour plots correspond to the percentage of cells in each quadrant or region.

**Supplementary Figure S4: IFN- $\gamma$  and TNF- $\alpha$  expression in *in vitro* stimulated splenic CD4<sup>+</sup> T cells of *N. caninum* infected mice and controls**

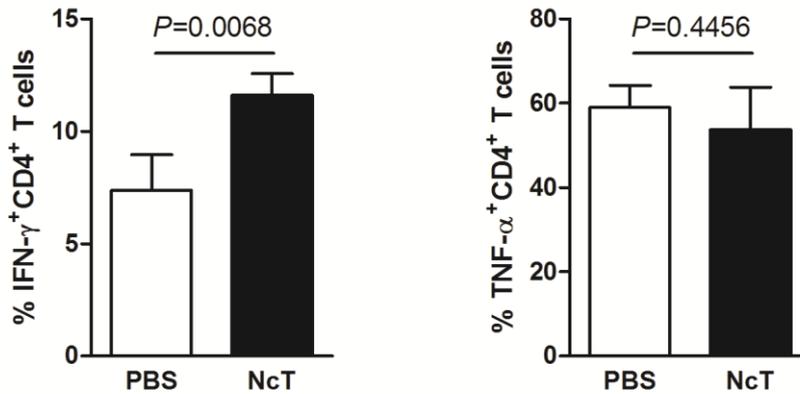


(a) Percentage of splenic CD4-gated T cells expressing IFN- $\gamma$  or TNF- $\alpha$  of  $1 \times 10^7$  NcT-infected mice (NcT) and sham-infected controls (PBS), detected by intracellular staining after stimulation with PMA/ionomycin. Mean fluorescence intensities (MFI) due to respective cytokine staining are also presented. Bars represent means plus one SD of pooled data from two independent

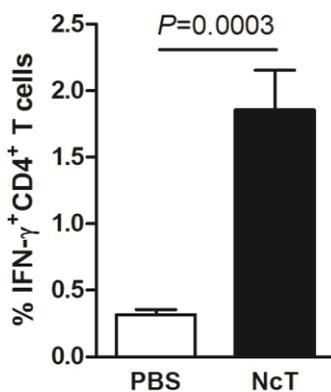
experiments (n=6 for controls and n=9 for infected mice). Statistical significance between infected mice and controls is indicated above bars. (b) Percentage of IFN- $\gamma$ <sup>+</sup> cells on total CD4<sup>+</sup> T cells of infected mice (NcT) and controls (PBS) detected in *in vitro* splenocytes cultures non stimulated (-) or stimulated for 16 h with *N. caninum* sonicates (+); n=5 and n=7 for non-stimulated and stimulated groups, respectively. Contour plots correspond to a representative example of stimulated CD4-gated T cells of the analysed samples. Analysis regions were set according to isotype control-stained samples. Numbers within contour plots correspond to the percentage of cells in the region shown.

**Supplementary Figure S5: Proportions of IFN- $\gamma$ -expressing CD4<sup>+</sup> T cells in *CD8a*<sup>-/-</sup> mice infected with *N. caninum*.**

**a**

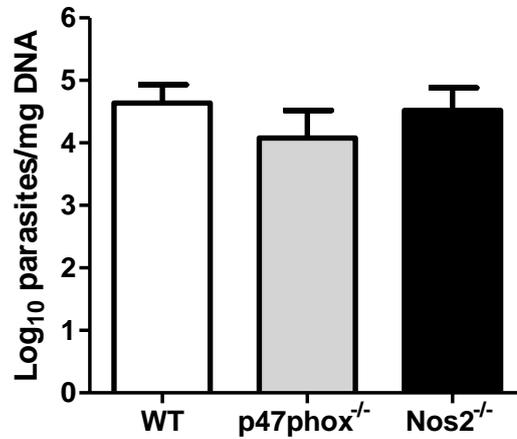


**b**



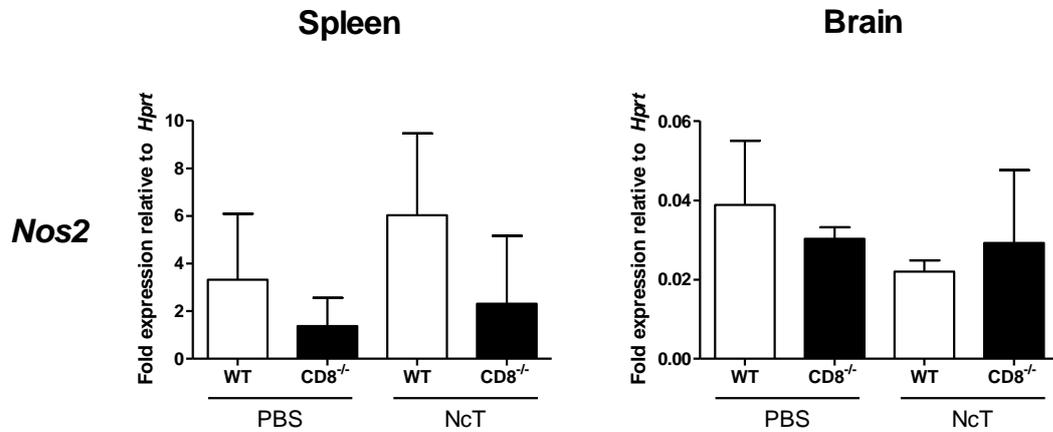
(a) Percentage of CD4-gated T cells expressing IFN- $\gamma$  or TNF- $\alpha$  in the spleen of *N. caninum* i.p. infected *CD8a*<sup>-/-</sup> mice (NcT) and sham-infected controls (PBS), detected by intracellular staining after stimulation with PMA/ionomycin 7 days upon the parasitic challenge. (b) Percentage of CD4-gated T cells expressing IFN- $\gamma$  detected in spleen cell cultures of 7-day infected *CD8a*<sup>-/-</sup> mice (NcT) and sham-infected controls (PBS), after 16 h *N. caninum* antigen-stimulation. Bars represent the mean plus one SD (n=3 for controls and n=4 for infected mice). Statistical significance between infected mice and controls is indicated above bars.

**Supplementary Figure S6: Parasitic load in *N. caninum*-infected C57BL/6 WT, *p47phox*<sup>-/-</sup> and *Nos2*<sup>-/-</sup> mice**



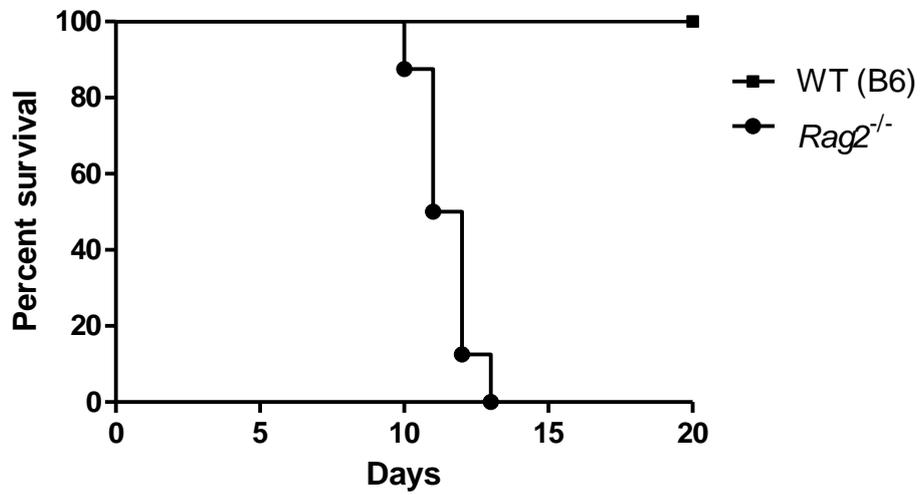
Parasitic load of brain tissue assessed by qPCR specific for *N. caninum* DNA in C57BL/6 WT (n=10), *p47phox*<sup>-/-</sup> (n=9) and *Nos2*<sup>-/-</sup> (n=8) mice, as indicated, 30 days after i.p. inoculation with  $1 \times 10^7$  *N. caninum* tachyzoites. Bars represent means plus one SD. No statistically significant differences were observed among the different infected mouse groups (One-way ANOVA and Tukey's *post-hoc* test).

**Supplementary Figure S7: Expression of inducible nitric oxide synthase (*Nos2*) mRNA in infected and control B6 WT and *CD8a*<sup>-/-</sup> mice**



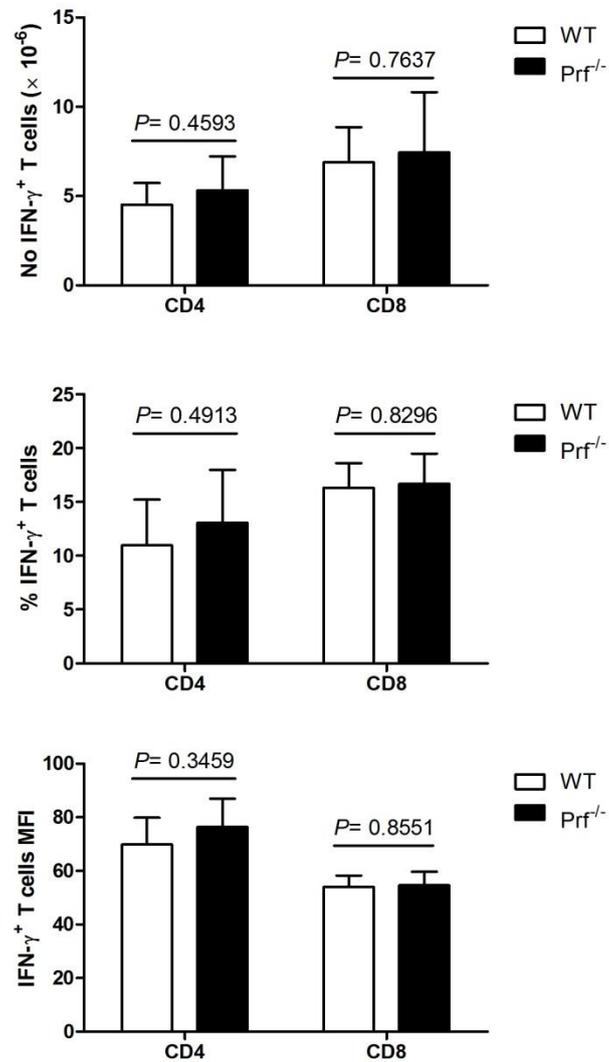
Relative levels of *Nos2* mRNA, normalized to hypoxanthine guanine phosphoribosyl transferase (*Hprt*) mRNA, detected by real-time PCR in the spleen and brain of WT and *CD8a*<sup>-/-</sup> mice, as indicated, 7 days after i.p. injection of  $1 \times 10^7$  *N. caninum* tachyzoites (NcT; n=4) or PBS (PBS; n=3). Bars represent mean values of the respective group plus one SD. No statistical significant differences were observed among mouse groups (one-way ANOVA and Tukey's post-hoc test).

**Supplementary Figure S8: *Rag2*<sup>-/-</sup> mice are lethally susceptible to *N. caninum* infection**



Survival of C57BL/6 WT (n=10) and *Rag2*<sup>-/-</sup> (n=10) mice infected i.p. with  $1 \times 10^7$  *N. caninum* tachyzoites. Statistical difference between mouse groups was calculated with the log-Rank test ( $P < 0.001$ ).

**Supplementary Figure S9: Expression of IFN- $\gamma$  by splenic T cells of *N. caninum* infected C57BL/6 WT and *Prf1*<sup>-/-</sup> mice**



Numbers, proportions and MFI due to IFN- $\gamma$  staining of IFN- $\gamma$ -producing CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as indicated, detected in the spleen of WT and *Prf1*<sup>-/-</sup> mice 7 days upon infection with  $1 \times 10^7$  *N. caninum* tachyzoites. Bars represent means plus one SD of five mice per group. Statistical significances between mouse groups are indicated above bars.

**Supplementary Table S1: Reconstitution of *Rag2*<sup>-/-</sup> mice**

Relative proportions and total numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells detected by flow cytometry in the spleen of 7-day *N. caninum* infected *Rag2*<sup>-/-</sup> mice reconstituted 28 days prior to infection with 1.5×10<sup>6</sup> WT CD4<sup>+</sup> T cells and equal numbers of either WT or *Ifng*<sup>-/-</sup> CD8<sup>+</sup> T cells, as indicated.

Individual mouse number	<i>IFNG</i> gene status in CD8 <sup>+</sup> T cells	CD4 <sup>+</sup> T cells		CD8 <sup>+</sup> T cells	
		%	total number (millions)	%	total number (millions)
#1	-/-	80,07	12,35	19,93	3,07
#2	-/-	86,46	19,82	13,54	3,10
#3	-/-	93,45	18,54	6,55	1,30
#4	-/-	83,38	18,23	16,62	3,63
#5	-/-	83,70	21,46	16,30	4,18
#6	-/-	78,68	33,30	21,32	9,02
#7	-/-	77,65	46,79	22,35	13,47
#8	-/-	90,13	24,53	9,87	2,69
#9	-/-	88,57	31,10	11,43	4,01
#10	-/-	78,93	13,31	21,07	3,55
#11	+/+	82,45	34,89	17,55	7,43
#12	+/+	60,98	38,04	39,02	24,34
#13	+/+	7,26	3,53	92,74	45,05
#14	+/+	74,22	37,33	25,78	12,97
#15	+/+	74,85	44,02	25,15	14,79
#16	+/+	80,69	43,86	19,31	10,49
#17	+/+	72,21	39,36	27,79	15,15
#18	+/+	62,35	48,19	37,65	29,10
#19	+/+	76,88	26,86	23,12	8,08
#20	+/+	85,22	10,48	14,78	1,82