Supplemental material

Fig. S1.

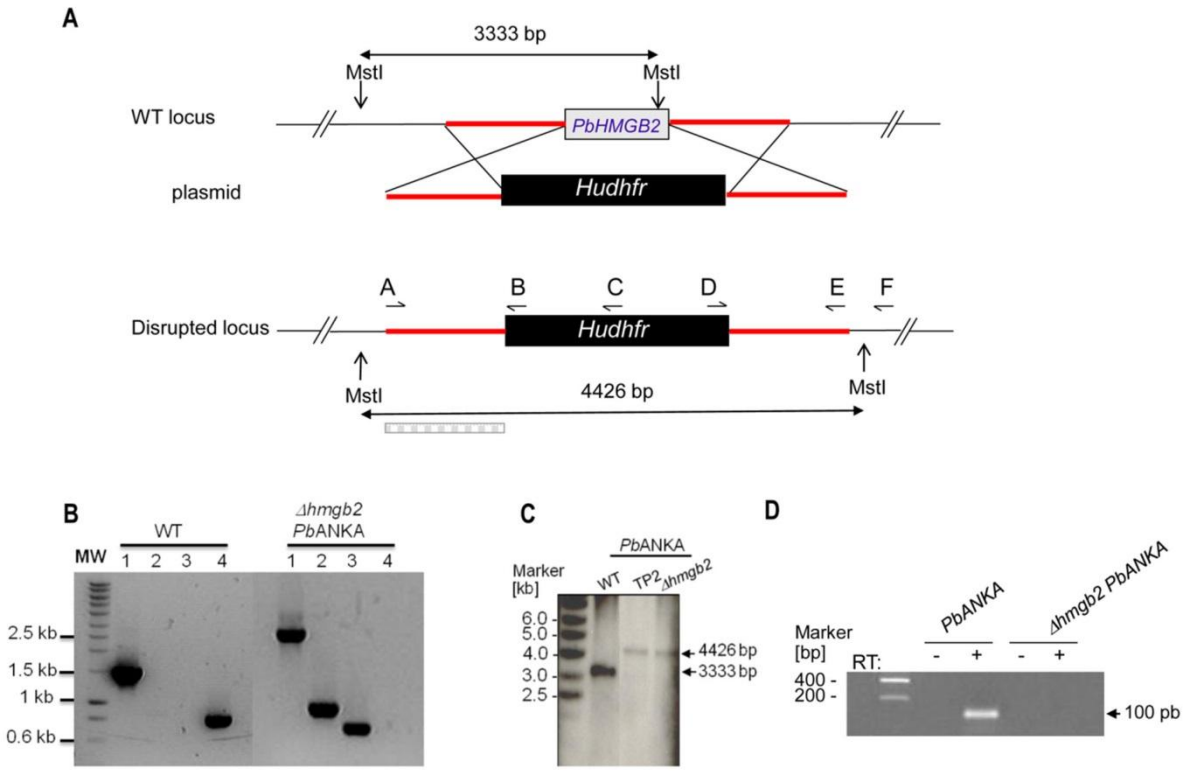

Fig. S1. Disruption of the *pbhmgb2* gene.

A. Schematic representation of *pbhmgb2* gene disruption by double cross-over homologous recombination. Sites of recombination are indicated by thick red lines. Genomic DNA from mutant and wild-type (WT) *Pb*ANKA and *Pb*NK65 parasites was digested by MstI for Southern blotting analysis. The position of the Southern probe is represented by the white bar (bottom of the figure). Predicted size of the fragments is indicated for each locus. Arrows show the position of primers used in the diagnostic PCR (see Table S1). **B.** Fifty ng of genomic DNA extracted from WT and *Δhmgb2 Pb*ANKA, were analysed by the primers listed in Supporting information Table T1. The combinations of primers used for amplification are the following: lane 1 (A+E), lane 2 (D+F), lane 3 (A+B), lane 4 (A+C). **C.** Southern blot analysis of MstI-digested genomic DNA for the WT, transfer populations (TP) and one of the *Δhmgb2* clones. The predicted size of the expected fragments (Fig. S1A) is indicated on the right side of the figure. **D.** RT-qPCR analysis of *pbhmgb2* expression in both WT and *Δhmgb2 Pb*ANKA parasites following a reverse transcription step or not.

Fig. S2.

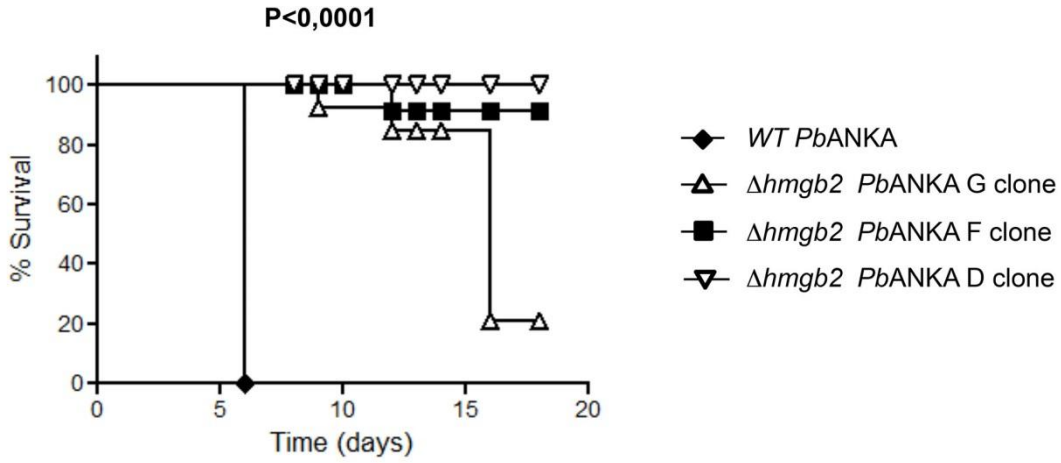

Fig. S2. Kaplan-Meier survival plots of C57BL/6 infected with either *Pb*ANKA WT (ECM reference) or three *⊿hmgb2 Pb*ANKA clones. C57BL/6 mice were infected by i.p. inoculation with 10^5 pRBCs WT and *⊿hmgb2 Pb*ANKA and monitored every day, starting on d5 p.i., for ECM symptoms. Significant differences in mortality/survival between WT and *⊿hmgb2 Pb*ANKA infected C57BL/6 were analysed by Log-rank test *(p*<0.0001, n=5).

Fig. S3.

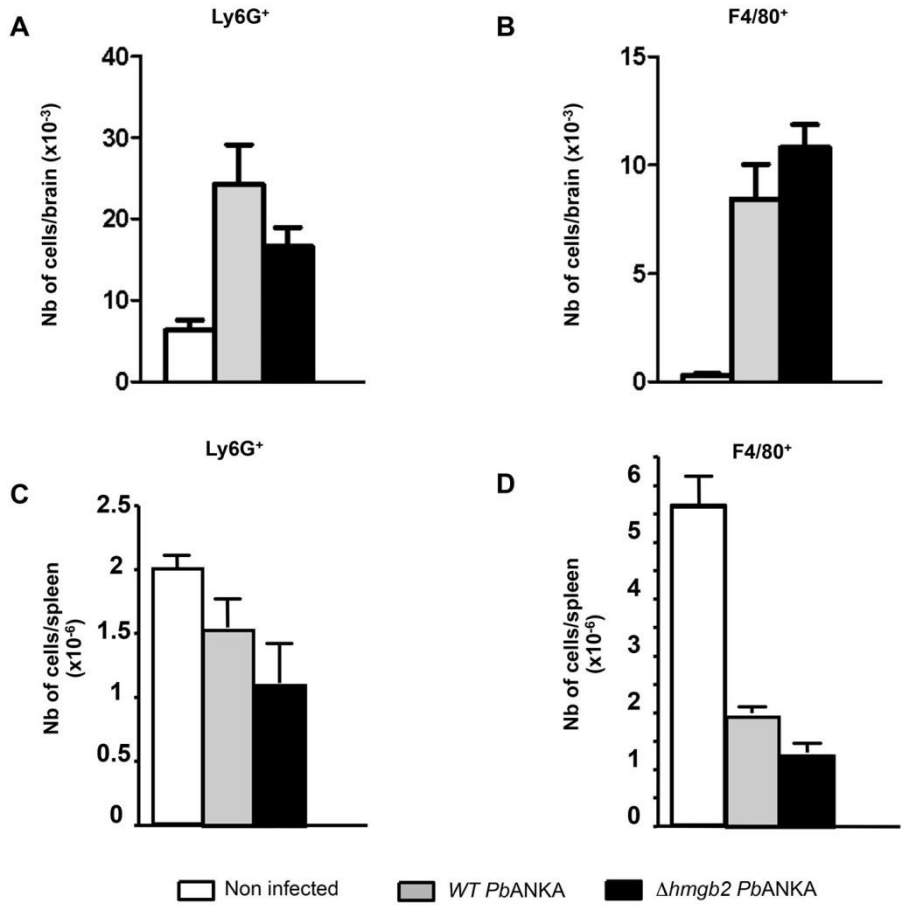

Fig. S3. Determination of the number of polymorphonuclear neutrophils and macrophages in the brain and spleen of mice infected with WT or *Δhmgb2 Pb*ANKA parasites. At the coma stage (day 6 p.i.), brains and spleens from perfused WT or *Δhmgb2 Pb*ANKA-infected C57BL/6 mice with 10^5 infected erythrocytes per mouse were taken and neutrophils and macrophages associated with cerebral and spleen tissue were analysed by FACS and expressed as absolute numbers per brain and spleen. Six mice per group were used. Experiment was reproduced twice.

Fig. S4

A **Identity %.**

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DmHMG_D    36%      MSDKPKRPLSAYMLWLNSARESIKRENP--GIKVTEVAKRGGELWRAM--KDKSEWEAKAAKAKDDYDRAVKEFE
OsHMGB1    45%  AGKDPNKPKRAPSAFFVFMEEFRKEFKEKNPK-NKSVAAVGKAAGDRWKSLTEADKAPYVAKANKLKAEYNKAIAAYN
GmHMG1     41%  AAKDPNKPKRPPSAFFVFMEEFRKVFNKEHPE-NKAVSAVGKAAGAKWKTMSDAEKAPYVAKSEKRKVEYEKNMRAYN
BbNHP1     41%  AAKDPNKPKRPPSAFFVFMEEFRKVFNKEHPE-NKAVSAVGKAAGAKWKTMSDAEKAPYVAKSEKRKVEYEKNMRAYN
MmHMG1_B   42%   FKDPNAPKRPPSAFFLFCSEYRPKIKGEHP--GLSIGDVAKKLGEMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYR
HsHMG1_B   42%   FKDPNAPKRPPSAFFLFCSEYRPKIKGEHP--GLSIGDVAKKLGEMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYR
HsHMG2_B   40%   KKDPNAPKRPPSAFFLFCSEHRPKIKSEHP--GLSIGDTAKKLGEMWSEQSAKDKQPYEQKAAKLKEKYEKDIAAYR
ScNHP6A    54%   KKDPNAPKRALSAYMFFANENRDIVRSENPDIT--FGQVGKKLGEKWKALTPEEKQPYEAKAQADKKRYESEKELYN
PvHMGB1    64%  NKKDPHAPKRSLSAYMFFAKEKRAEIISRDPDLSKDVATVGKMIGEAWNKLDEREKAPYEKKAQEDKLRYEREKVEYA
PkHMGB1    66%  NKKDPHAPKRSLSAYMFFAKEKRAEIISRDPDLSKDVATVGKMIGEAWNKLDEREKAPYEKKAQEDKVRYEREKVEYA
PyHMGB1    65%  NXKDPHAPKRSLSAYMFFAKEKRAEIITRDPSLSKDVATVGKMIGEAWNKLDEREKAPYEKKAQEDKIRYEKEKMEYA
PbHMGB1    66%  NKKDPHAPKRSLSAYMFFAKEKRAEIITRDPSLSKDVATVGKMIGEAWNKLDEREKAPYEKKAQEDKIRYEKEKMEYA
PfHMGB1    66%  NKKDPHAPKRSLSAYMFFAKEKRAEIISKQPELSKDVATVGKMIGEAWNKLGEKEKAPFEKKAQEDKLRYEKEKAEYA
PfHMGB2    94%  KKKDPLAPKRALSAYMFYVKDKRLEIIKEKPELAKDVAQVGKLIGEAWGQLSPAQKAPYEKKAQLDKVRYSKEIEEYR
PbHMGB2   100%  KKKDPLAPKRALSAYMFYVKDKRLEIIQERPELAKEVAQVGKLIGEAWGQLTPAQKAPYEKKAELDKVRYSKEIEEYR
PyHMGB2   100%  KKKDPLAPKRALSAYMFYVKDKRLEIIQERPELAKEVAQVGKLIGEAWGQLTPAQKAPYEKKAELDKVRYSKEIEEYR
PkHMGB2    89%  KKKDPLAPKRALSAYMFYVKDKRLELIKEKPELARNVAQVGKLVGEAWGKLSAAQKTPYEKKAQLDKVRYSKEIEEYR
PvHMGB2    90%  KKKDPLAPKRALSAYMFYVKDKRLELIKERPELAKDVAQVGKLVGEAWGQLSAAQKTPYEKKAQLDKVRYSKEIEEYR
MmGMG1_A   30%   KGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYI
HsHMG1_A   30%   KGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYIk
ZmMNB1b    30%  AGKDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYI
HsHMG2_A   32%   KGDPNKPRGKMSSYAFFVQTCREEHKKKHPDSSVNFAEFSKKCSERWKTMSAKEKSKFEDMAKSDKARYDREMKNYV
Prim.cons.         DPNAPKRALSAYMFFVKEKR3EIIKEHPELSKDVAQVGKIGEAWK2LSAAEKAPYEKKAQKDKVRYEKEIEEYR
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B

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PfHMGB1     KKDPHAPKRSLSAYMFFAKE
PbHMGB1     KKDPHAPKRSLSAYMFFAKE
MuHMGB2-B   KKDPHAPKRSLSAYMFFAKE
PfHMGB2     KKDPLAPKRALSAYMFYVKD
PBHMGB2     KKDPLAPKRALSAYMFYVKD
MuHMGB1-B   FKDPNAPKRPPSAFFLFCSE
             *** ****. **:::: .:

PfHMGB2     KKDPLAPKRALSAYMFYVKD
PbHMGB2     KKDPLAPKRALSAYMFYVKD
PfHMGB1     KKDPHAPKRSLSAYMFFAKE
PbHMGB1     KKDPHAPKRSLSAYMFFAKE
MuHMGB1-A   KGDPKKPRGKMSSYAFFVQTC
MuHMGB2-A   KGDPNKPRGKMSSYAFFVQTC
             * **  *.  :*:* *:.:
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Identity	Homology
45 %	80 %
35 %	65 %

A. Multiple alignments of HMGB domains from several eukaryotic High Mobility Group B proteins including diverse *Plasmodium* proteins.
The various amino-acid (aa) sequences are taken from the previous article*. The alignment was achieved by NPS@: Network Protein Sequence AnalysisTIBS** programme as regards to the *Pb*HMGB2 protein sequence (highlighted in yellow) and the % of identity are included right part of the figure. Red stands for identical, blue and green for highly conserved aa. The TNFα activating domain is highlighted in grey i) for the both Hs HMGB proteins as the domain of HMGB1 box B was reported to bear *per se* the activating pro-inflammatory activity and ii) for the *Pb*HMGB2. Abbreviations are as follows: Bb, *Babesia bovis*; Dm, *Drosophila melanogaster*; Gm, *Glycine max*; Os, *Oryza sativa*; Pb, *Plasmodium berghei*; Pf, *Plasmodium falciparum*; Pk, *Plasmodium knowlesi*; Pv, *Plasmodium vivax*; Py, *Plasmodium yoelii*; Mu, *Mus musculus*; Sc, *Saccharomyces cerevisiae*; Zm, *Zea mays*. Bottom line corresponds to the consensus sequence.

B. Comparison of the TNFα activating domains of *P. falciparum* and *P. berghei* (in black) with that of the murine domain of Box A and Box B sequences (in red) of both HMGB1 and HMGB2 proteins. Identity and homology are indicated. ***** stands for identical, **:** and **.** for highly and less similar aa, respectively

* Briquet *et al.* 2006
** Combet C., Blanchet C., Geourjon C. and Deléage G, Trends Biochem Sci, 2000; 25, N° 3 [291]:147-150

Table S1. List of the oligonucleotides used for PCR of wild-type and recombinant parasites. Capital letters refer to the oligonucleotide positions in Fig. S1A.

ApaI-5'hmgb2-For **(A)**	CGATGCGGGCCCAAAAAGGTAAATATGAAAAAGAAAGGTT
SmaI-5'hmgb2-Rev **(B)**	CGATGCCCCGGGTTTGCAATGGAAACATATCAGTT
NotI-3'hmgb2-For **(D)**	CCAGTGAGTGCGGCCGCGGAGCCTTTTGTATGTGCTTTTTG
AscI-3'hmgb2-Rev **(E)**	AGCTGGCGCGCCAAGTATTGCAGTAGCATTTTCTTTAAAT
ORF-Rev **(C)**	CCCAAGCTTCTCCAATTAGTTTTCCAACTTGTGCAACTTC
Ana-For **(F)**	AAATGATTAGCTATAAAATAAGCGCAAAAATAATA
huDHFR-For	TGTTGTCTCTTCAATGATTCATAAATAGTTGG
huDHFR-Rev	TGCTTTGAGGGGTGAGCATTTAAAGC

Table S2. List of the oligonucleotides used for the transcript analyses by RT-qPCR.

pbhmgb2	101-For	AAAAGAGAGCAGAGATAATAACTCGAGATC
	201-Rev	CCTTTCGTCTAATTTATTCCATGCTT
mu hmgb1	415-For	AGTTCAAGGACCCCAATGCAC
	516-Rev	TGGATAAGCCAGGATGCTCG
mu hmgb2	14-For	GCGGAGAACTCTGCAAAACAA
	115-Rev	CTTCCCTCAGACCTCCGCA
mu tnfα	1032-For	GAAACACAAGATGCTGGGACAGT
	1133-Rev	GACATTCGAGGCTCCAGTGAAT
mu il-6	55-For	CTTCCATCCAGTTGCCTTCTTG
	155-Rev	TGGGAGTGGTATCCTCTGTGAAGT
mu il-10	113-For	TGACTGGCATGAGGATCAGC
	213-Rev	AGTCCGCAGCTCTAGGAGGCA
mu hprt	For	GTTGGATACAGGCCAGACTTTGTTG
	Rev	GATTCAACCTTGCGCTCATCTTAGGC
mu ifnγ	For	CACACTGCATCTTGGCTTTG
	Rev	TCTGGCTCTGCAGGATTTTC
mu icam-1	For	CGAAGGTGGTTCTTCTGAGC
	Rev	GTCTGCTGAGACCCCTCTTG
mu vcam-1	For	AGTCCGTTCTGACCATGGAG
	Rev	TGTCTGGAGCCAAACACTTG
mu hmox1	For	TCTCAGGGGGTCAGGTC
	Rev	GGAGCGGTGTCTGGGATG
pb 18S	1827-For	ATTAATCTTGAACGAGGAATGGCT
	1927-Rev	TCAATCGGTAGGAGCGACG