Supplementary information

Molecular determinants of SR-B1-dependent *Plasmodium* sporozoite entry into hepatocytes.

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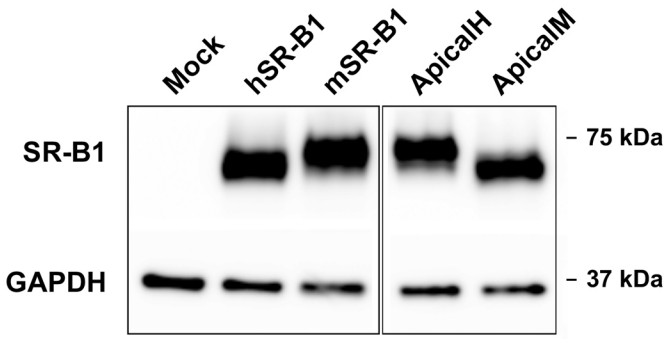
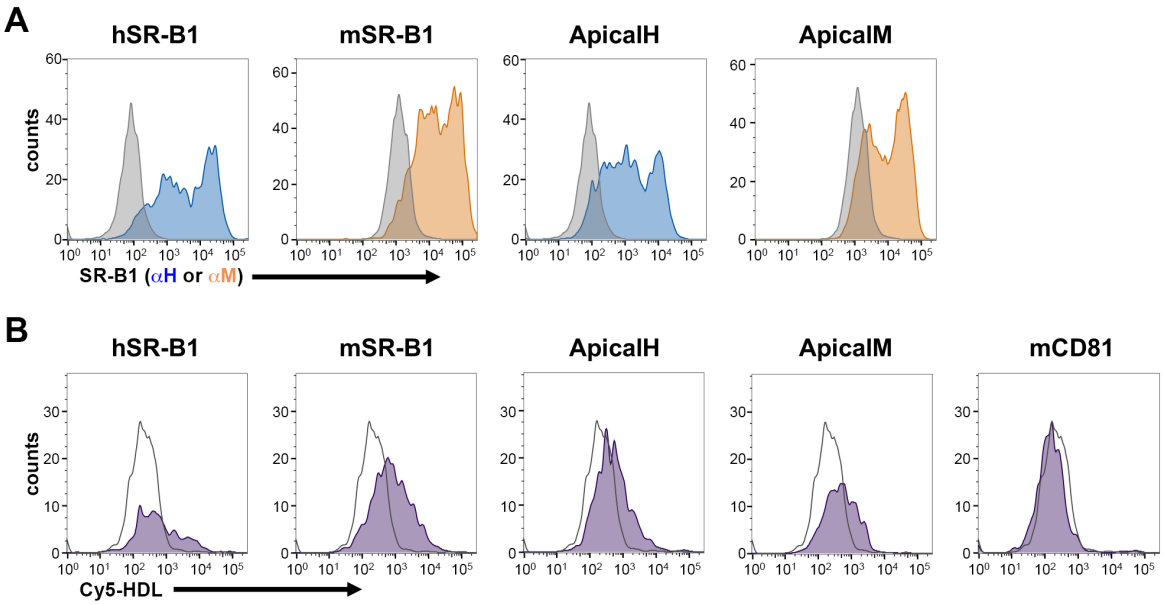
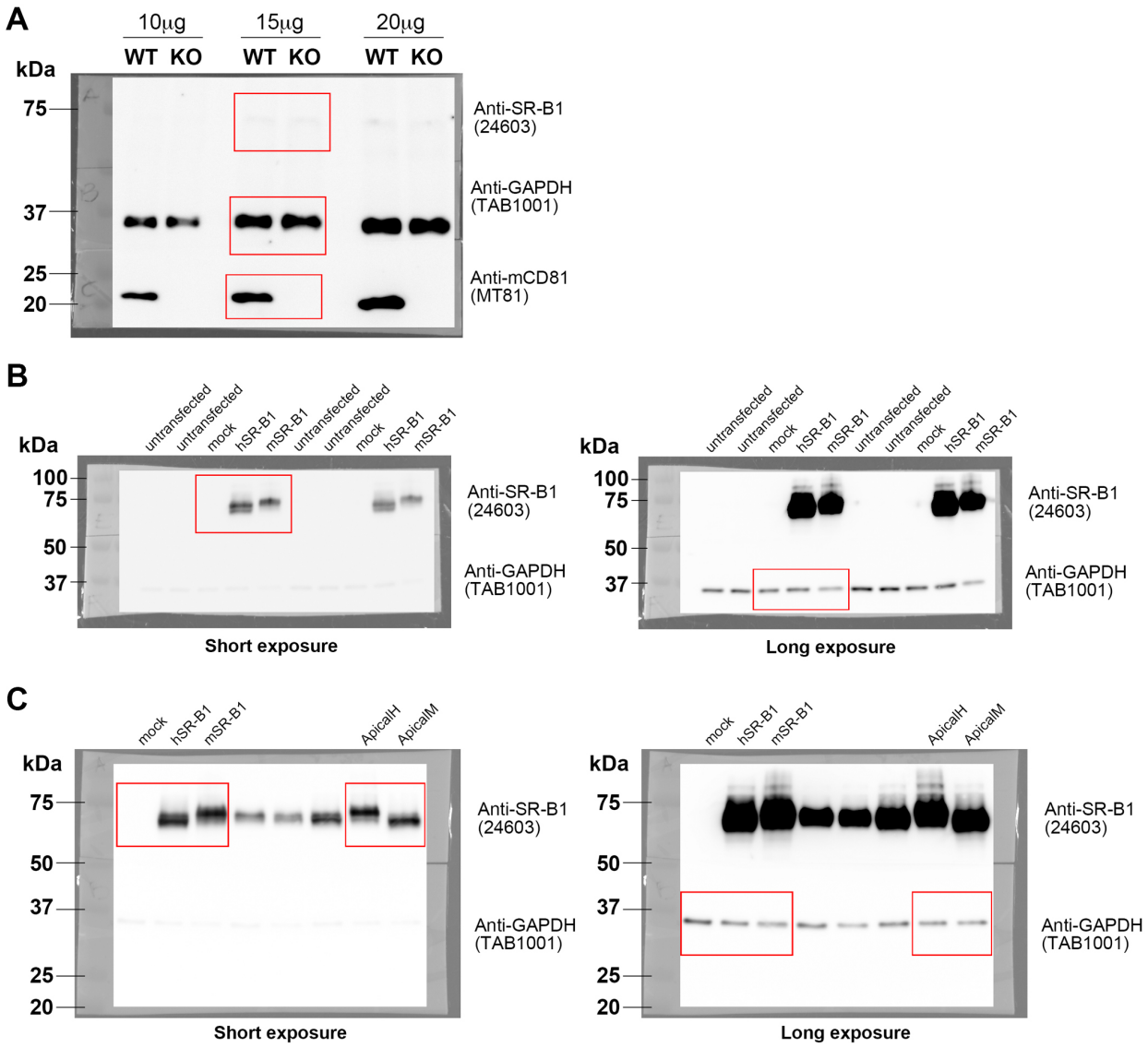


Supplementary Figure 1. CD81 KO Hepa1-6 cells were transfected with either mSR-B1, hSR-B1, ApicalH or ApicalM construct plasmids, or no plasmid as a control (Mock). Total protein expression was analyzed by western blot using polyclonal anti-SR-B1 antibodies (Ab24603) and anti-GAPDH antibodies as a loading control. Full-length blots are shown in Supplementary Figure S3.



Supplementary Figure 2. **(A-B)** CD81KOH16 cells were transfected with hSR-B1, mSR-B1, ApicalH or ApicalM chimeric constructs, or with a plasmid encoding mCD81 (negative control). **(A)** Protein surface expression was analyzed using anti-hSR-B1 ("αH", blue histograms) and anti-mSR-B1 ("αM", orange histograms), 24 hours after transfection. The grey histogram represents untransfected cells stained with the corresponding antibody. **(B)** Cy5 fluorescent HDLs were added to transfected cells 24 hours after transfection to measure HDL binding (purple peak). HDL binding to non-transfected cells (negative control) is shown as a white peak. Cells transfected with a mouse CD81 construct did not bind HDLs, as expected.



Supplementary Figure 3. **A**. Full-length blot corresponding to Figure 2B. Protein extracts (10µg, 15 µg or 20 µg) were analyzed by SDS-PAGE. After transfer, the membrane was cut into three pieces, which were probed with anti-SR-B1, anti-GADPH and anti-CD81 antibodies, respectively. **B**. Full-length blot corresponding to Figure 3A. Protein extracts from mock, hSR-B1 or mSR-B1 transfected cells were analyzed by SDS-PAGE. After transfer, the membrane was cut into two pieces, which were probed with anti-SR-B1 and anti-GADPH antibodies, respectively. **C**. Full-length blot corresponding to Supplementary Figure S1. Protein extracts from mock, hSR-B1, mSR-B1, ApicalH or ApicalM transfected cells were analyzed by SDS-PAGE. After transfer, the membrane was cut into two pieces, which were probed with anti-SR-B1 and anti-GADPH antibodies, respectively. The red boxes highlight which parts of the full blots are reproduced in the corresponding figures.

Supplementary table 1. Sequences of oligonucleotides and synthetic genes used to assemble the chimeric constructs.

	Oligonucleotide	**Sequence 5´ → 3´**
ApicalH construct	ApicalHfor	ACCGATCCAGCCTCCGCGGGCCCTGC
	ApicalHrev	GAGGCGCACCAAACCTGCAGGTGCTG
ApicalM construct	ApicalMfor	CGTGTCCTTCCTCGAGTACCGCACCTTCCAGTTCC
	ApicalMrev	GAGGTGGATCCTCGAGATGTTCTGGACCCCCGTG
D1 construct	mSRBIfor	ACCGATCCAGCCTCCGCGGGCCCTGC
	D1rev	CATGATGAGCTTCAGGGTCATGGGCTTATTCTCCATCAATATCGAGCCCCCCAG
	D1for	CTGAAGCTCATCATGACCTTGGCATTCACCACGATGGGCCAGCGTGCTTTTATG
	mSRBIrev	GAGGCGCACCAAACCTGCAGGTGCTG
D2 construct	mSRBIfor	ACCGATCCAGCCTCCGCGGGCCCTGC
	D2rev	GGGGAACATGCCTGGAAAGTACTTGTTGAGAAAATGCACGAAGGGATCGTC
	D2for	CCAGGCATGTTCCCCTTCAAGGACAAATTTGGCCTGTTTGTTGGGATGAAC
	mSRBIrev	GAGGCGCACCAAACCTGCAGGTGCTG
D3 construct	mSRBIfor	ACCGATCCAGCCTCCGCGGGCCCTGC
	D3rev	AAATAATCCGAACTTGTCCTTGAAGGGAAGCATGTCTGGGAGGTACGTG
	D3for	AAGTTCGGATTATTTGCTGAGCTCAACAACTCGAATTCTGGGGTCTTCACTGTC
	mSRBIrev	GAGGCGCACCAAACCTGCAGGTGCTG
ApicalH synthetic gene		accgatccagcctccgcgggccctgccaccatgggcggcagctccagggcgcgc tgggtggccttggggttgggcgccctggggctgctgtttgctgcgctcggcgtt gtcatgatcctcatggtgccctccctcatcaagcagcaggtgctcaagaatgtc cgcatagacccgagcagcctgtccttcgggatgtggaaggagatccccgtccct ttctacttgtctgtctacttcttcgaagtggtcaacccaaacgaggtcctcaac ggccagaagccagtagtccgggagcgtggaccctatgtctacagggagttcaga caaaaggtcaacatcaccttcaatgacaacgacaccgtgtccttcgtggagaac cgcagcctccatttccagcctgacaagtcgcatggctcagagagtgactacatt gtactg**CCCAACATCCTGGTCTTGGGTGCGGCGGTGATGATGGAGAATAAGCCC ATGACCCTGAAGCTCATCATGACCTTGGCATTCACCACCCTCGGCGAACGTGCC TTCATGAACCGCACTGTGGGTGAGATCATGTGGGGCTACAAGGACCCCCTTGTG AATCTCATCAACAAGTACTTTCCAGGCATGTTCCCCTTCAAGGACAAGTTCGGA TTATTTGCTGAGCTCAACAACTCG**aattctggggtcttcactgtcttcacgggc gtccagaatttcagcaggatccatctggtggacaaatggaacggactcagcaag atcgattattggcattcagagcagtgtaacatgatcaatgggacttccgggcag atgtgggcacccttcatgacacccgaatcctcgctggaattcttcagcccggag gcatgcaggtccatgaagctgacctacaacgaatcaagggtgtttgaaggcatt cccacgtatcgcttcacggcccccgatactctgtttgccaacgggtccgtctac ccacccaacgaaggcttctgcccatgccgagagtctggcattcagaatgtcagc acctgcaggtttggtgcgcctc
ApicalM synthetic gene		cgtgtccttcctcgagtaccgcaccttccagttccagccctccaagtcccacgg ctcggagagcgactacatcgtcatg**CCCAACATCCTGGTCCTGGGGGGCTCGAT ATTGATGGAGAGCAAGCCTGTGAGCCTGAAGCTGATGATGACCTTGGCGCTGGT CACCATGGGCCAGCGTGCTTTTATGAACCGCACAGTTGGTGAGATCCTGTGGGG CTATGACGATCCCTTCGTGCATTTTCTCAACACGTACCTCCCAGACATGCTTCC CATAAAGGGCAAATTTGGCCTGTTTGCTGAGCTCAACAACTCC**gactctgggct cttcacggtgttcacgggggtccagaacatctcgaggatccacctc

Supplementary Table 2. Protein sequences of hSR-B1, mSR-B1, and chimeras. Sequences of human and murine origin are in blue and orange, respectively. Conserved residues at the chimera boundaries between human and murine SR-B1 are indicated in black.

	Protein sequence
hSR-B1	MGCSAKARWAAGALGVAGLLCAVLGAVMIVMVPSLIKQQVLKNVRIDPSSLSFNMWKEIPIPFYLSVYFFDVMN PSEILKGEKPQVRERGPYVYREFRHKSNITFNNNDTVSFLEYRTFQFQPSKSHGSESDYIVMPNILVLGAAVMM ENKPMTLKLIMTLAFTTLGERAFMNRTVGEIMWGYKDPLVNLINKYFPGMFPFKDKFGLFAELNNSDSGLFTVF TGVQNISRIHLVDKWNGLSKVDFWHSDQCNMINGTSGQMWPPFMTPESSLEFYSPEACRSMKLMYKESGVFEGI PTYRFVAPKTLFANGSIYPPNEGFCPCLESGIQNVSTCRFSAPLFLSHPHFLNADPVLAEAVTGLHPNQEAHSL FLDIHPVTGIPMNCSVKLQLSLYMKSVAGIGQTGKIEPVVLPLLWFAESGAMEGETLHTFYTQLVLMPKVMHYA QYVLLALGCVLLLVPVICQIRSQEKCYLFWSSSKKGSKDKEAIQAYSESLMTSAPKGSVLQEAKL
mSR-B1	MGGSSRARWVALGLGALGLLFAALGVVMILMVPSLIKQQVLKNVRIDPSSLSFGMWKEIPVPFYLSVYFFEVVN PNEVLNGQKPVVRERGPYVYREFRQKVNITFNDNDTVSFVENRSLHFQPDKSHGSESDYIVLPNILVLGGSILM ESKPVSLKLMMTLALVTMGQRAFMNRTVGEILWGYDDPFVHFLNTYLPDMLPIKGKFGLFVGMNNSNSGVFTVF TGVQNFSRIHLVDKWNGLSKIDYWHSEQCNMINGTSGQMWAPFMTPESSLEFFSPEACRSMKLTYNESRVFEGI PTYRFTAPDTLFANGSVYPPNEGFCPCRESGIQNVSTCRFGAPLFLSHPHFYNADPVLSEAVLGLNPNPKEHSL FLDIHPVTGIPMNCSVKMQLSLYIKSVKGIGQTGKIEPVVLPLLWFEQSGAMGGKPLSTFYTQLVLMPQVLHYA QYVLLGLGGLLLLVPIICQLRSQEKCFLFWSGSKKGSQDKEAIQAYSESLMSPAAKGTVLQEAKL
ApicalH	MGGSSRARWVALGLGALGLLFAALGVVMILMVPSLIKQQVLKNVRIDPSSLSFGMWKEIPVPFYLSVYFFEVVN PNEVLNGQKPVVRERGPYVYREFRQKVNITFNDNDTVSFVENRSLHFQPDKSHGSESDYIVLPNILVLGAAVMM ENKPMTLKLIMTLAFTTLGERAFMNRTVGEIMWGYKDPLVNLINKYFPGMFPFKDKFGLFAELNNSNSGVFTVF TGVQNFSRIHLVDKWNGLSKIDYWHSEQCNMINGTSGQMWAPFMTPESSLEFFSPEACRSMKLTYNESRVFEGI PTYRFTAPDTLFANGSVYPPNEGFCPCRESGIQNVSTCRFGAPLFLSHPHFYNADPVLSEAVLGLNPNPKEHSL FLDIHPVTGIPMNCSVKMQLSLYIKSVKGIGQTGKIEPVVLPLLWFEQSGAMGGKPLSTFYTQLVLMPQVLHYA QYVLLGLGGLLLLVPIICQLRSQEKCFLFWSGSKKGSQDKEAIQAYSESLMSPAAKGTVLQEAKL
ApicalM	MGCSAKARWAAGALGVAGLLCAVLGAVMIVMVPSLIKQQVLKNVRIDPSSLSFNMWKEIPIPFYLSVYFFDVMN PSEILKGEKPQVRERGPYVYREFRHKSNITFNNNDTVSFLEYRTFQFQPSKSHGSESDYIVMPNILVLGGSILM ESKPVSLKLMMTLALVTMGQRAFMNRTVGEILWGYDDPFVHFLNTYLPDMLPIKGKFGLFAELNNSDSGLFTVF TGVQNISRIHLVDKWNGLSKVDFWHSDQCNMINGTSGQMWPPFMTPESSLEFYSPEACRSMKLMYKESGVFEGI PTYRFVAPKTLFANGSIYPPNEGFCPCLESGIQNVSTCRFSAPLFLSHPHFLNADPVLAEAVTGLHPNQEAHSL FLDIHPVTGIPMNCSVKLQLSLYMKSVAGIGQTGKIEPVVLPLLWFAESGAMEGETLHTFYTQLVLMPKVMHYA QYVLLALGCVLLLVPVICQIRSQEKCYLFWSSSKKGSKDKEAIQAYSESLMTSAPKGSVLQEAKL
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