SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1: DNA sequences

Part	Sequence
His-Flag-Tag (HF-tag)	CACCATCACCATCACCATGGTAGCGGTGACTACAAAGACGATGACGACAAG
hCD4 extracellular domain	ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCA GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACC TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAG ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCT GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAG ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTG CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTG GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACT CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG GAATCCAACATCAAGGTTCTGCCCACATGGTCGACCCCGGTGCAGCCAATGGCCCTGATT GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC AGGTGCCGGCACTGA
mClover3	GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC GACGTAAACGGCCACAAGTTCAGCGTCCGCGGCGAGGGCGAGGGCGATGCCACCAACGGC AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC GTGACCACCTTCGGCTACGGCGTGGCCTGCTTCAGCCGCTACCCCGACCACATGAAGCAG CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCTTTC AAGGACGACGGTACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG CTGGAGTACAACTTCAACAGCCACTACGTCTATATCACGGCCGACAAGCAGAAGAACTGC ATCAAGGCTAACTTCAAGATCCGCCACAACGTTGAGGACGGCAGCGTGCAGCTCGCCGAC CACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCCATCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTG CTGGAGTTCGTGACCGCCGCCGGGATTACACATGGCATGGACGAGCTGTACAAG
mScarlet-I	GTGAGCAAGGGCGAGGCAGTGATCAAGGAGTTCATGCGGTTCAAGGTGCACATGGAGGGC TCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGC ACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCTCCTGGGACATC CTGTCCCCTCAGTTCATGTACGGCTCCAGGGCCTTCATCAAGCACCCCGCCGACATCCCC GACTACTATAAGCAGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG GACGGCGGCGCCGTGACCGTGACCCAGGACACCTCCCTGGAGGACGGCACCCTGATCTAC AAGGTGAAGCTCCGCGGCACCAACTTCCCTCCTGACGGCCCCGTAATGCAGAAGAAGACA ATGGGCTGGGAAGCGTCCACCGAGCGGTTGTACCCCGAGGACGGCGTGCTGAAGGGCGAC ATTAAGATGGCCCTGCGCCTGAAGGACGGCGGCCGCTACCTGGCGGACTTCAAGACCACC TACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTCGACCGCAAGTTGGAC ATCACCTCCCACAACGAGGACTACACCGTGGTGGAACAGTACGAACGCTCCGAGGGCCGC CACTCCACCGGCGGCATGGACGAGCTGTACAAG

CFP-P2A-BlaR	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGAC GGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC CTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAG CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTG GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCAC AAGCTGGAGTACAACGCCATCAGCGACAACGTCTATATCACCGCCGACAAGCAGAAGAAC GGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCC GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC TACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC CTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGAA TTCGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAAC CCTGGACCTCACGTGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCA ACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTC TCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGT GCAGAACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATC GTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTG CTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACG GCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAA
LoxP Site	ATAACTTCGTATAGCATACATTATACGAAGTTAT
FrtF Site	GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC
Frt3 Site	GAAGTTCCTATTCCGAAGTTCCTATTCTTCAAATAGTATAGGAACTTC
dClover2	GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC GACGTAAACGGCCACAAGTTCAGCGTCCGCGGCGAGGGCGAGGGCGATGCCACCATCGGC AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC GTGACCACCTTCGGCTACGGCGTGGCCTGCTTCAGCCGCTACCCCGACCACATGAAGCAG CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTACTTC AAGGACGACGGTACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG CTGGAGTACAACTTCAACAGCCACTACGTCTATATCACGGCCGACAAGCAGAACAACAGC ATCAAGGCTAACTTCACCATCCGCCACAACGTTGAGGACGGCAGCGTGCAGCTCGCCGAC CACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCCATCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTG CTGGAGTTCGTGACCGCCGCCGGGATTACACATGGCATGGACGAGCTGTACAAG

Supplementary Table S2: Sequences of single guide RNAs

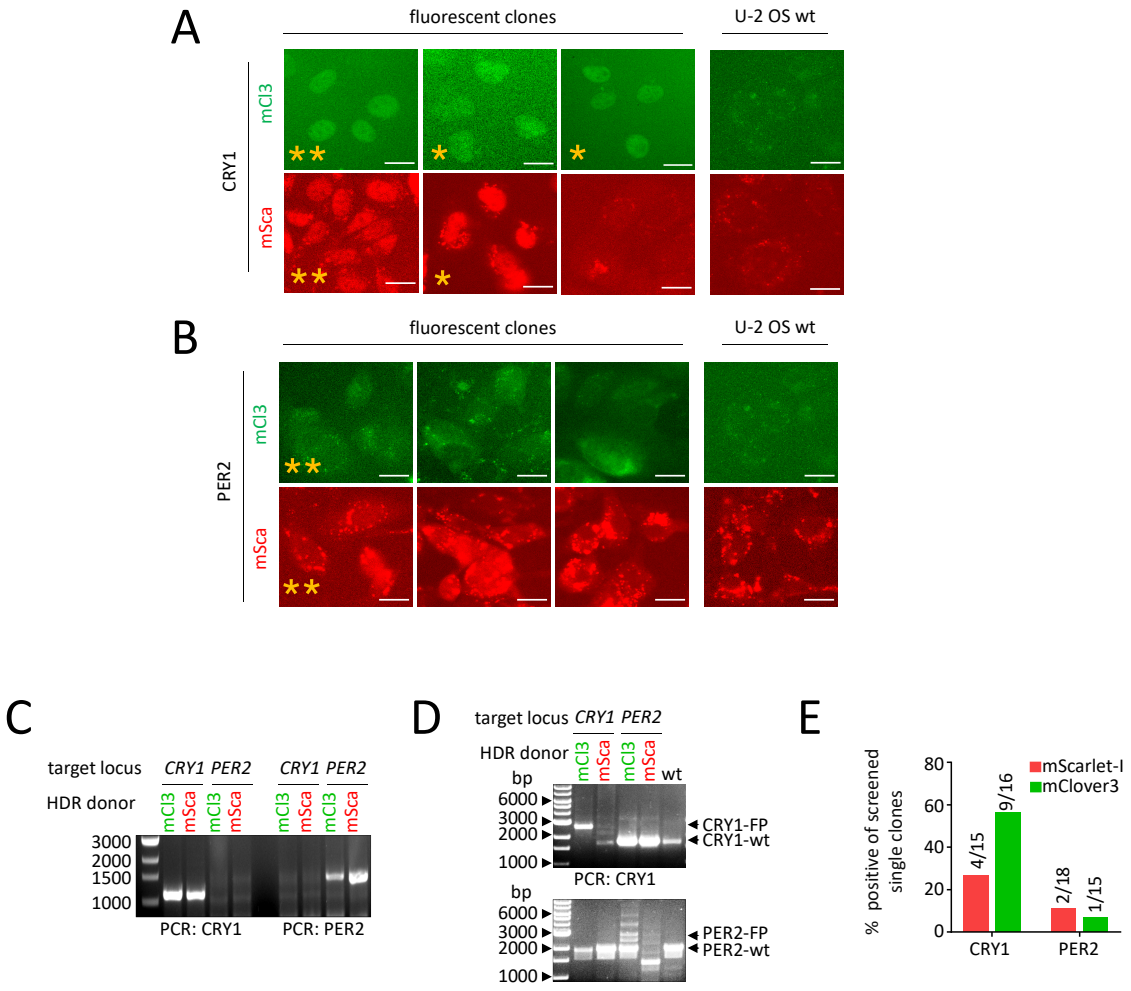
Target	Guide sequence	Target sequence fw strand (PAM underlined)
CRY1 (fw strand)	GGAAACGTCCTAGTCAGGAAG	GGAAACGTCCTAGTCAGGAAGAGG
PER2-1 (rv strand)	CACCACCTGGTGTACCTCGC	CCAGCGAGGTACACCAGGTGGTG
PER2-2 (fw strand)	ATGGATCCCCCTTGAATCAC	ATGGATCCCCCTTGAATCACAGG
PER3-3 (fw strand)	GGCAGCCAGCGAGGTACACC	GGCAGCCAGCGAGGTACACCAGG

Supplementary Table S3: shRNA constructs

Target	Hairpin sequence (targeting sequence underlined)
PER2 (pGIPZ V2LHS_52938)	TGCTG TTGAC AGTGA GCGCG CATCC ATATT TCACT GTAAA TAGTG AAGCC ACAGA TGTAT TTACA GTGAA ATATG GATGC ATGCC TACTG CCTCG GA
CRY1 (pGIPZ V2LHS_172866)	TGCTG TTGAC AGTGA GCGCG CTGAG GCAAG CCGTT TGAAT TAGTG AAGCC ACAGA TGTAA TTCAA ACGGC TTGCC TCAGC ATGCC TACTG CCTCG GA

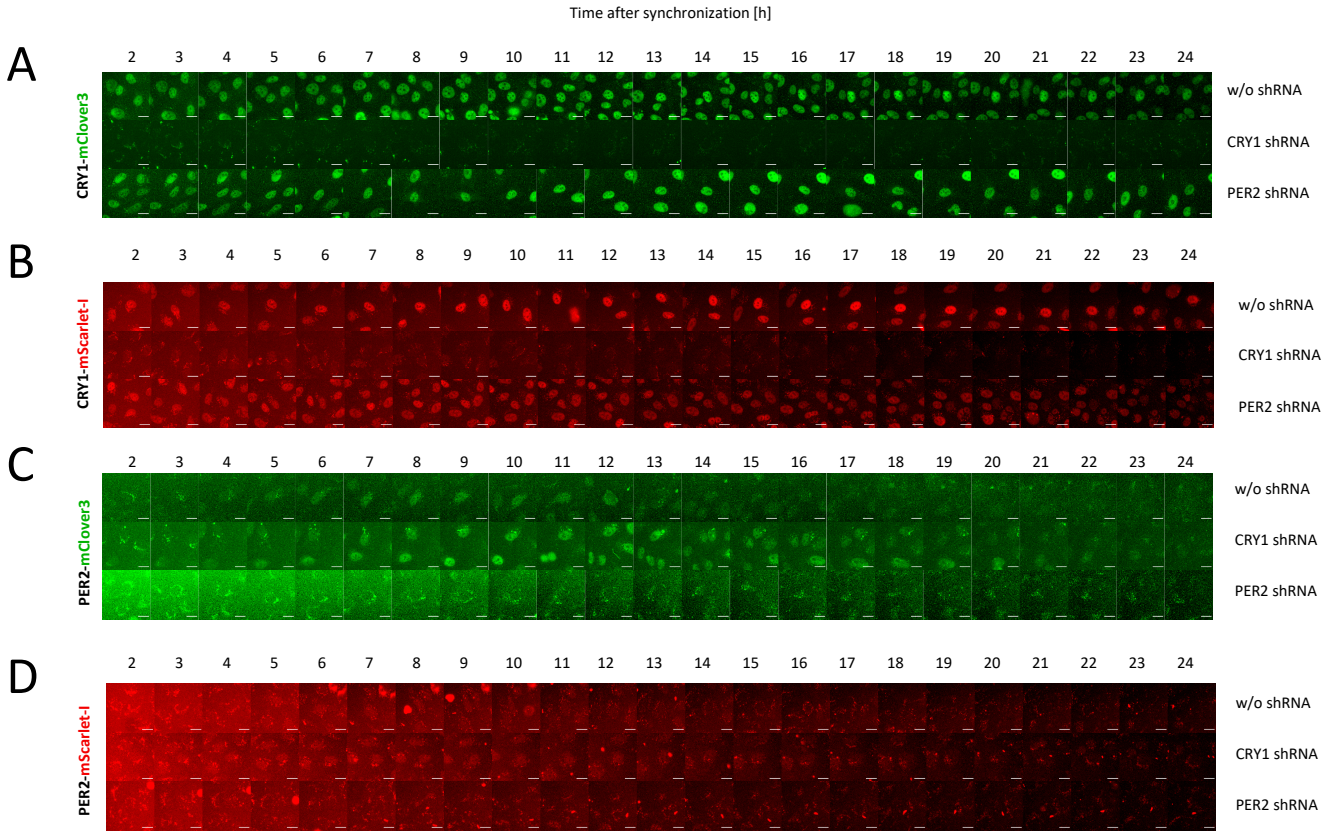
Supplementary Table S4: Sequences of PCR primers

Target	Sequence	Usage	Figure
CRY1 genomic locus (fw)	ACTGCCACTGATTGCCTGGGATTGAAGT	fw primer genomic PCR	Fig. S1D, Fig. S5C
CRY1 genomic locus (rv)	CAGCTGCAACAGTATTCCTCCTG	Rv primer Genomic PCR	Fig. S1D, Fig. S5C
PER2 genomic locus (fw)	ACCGGCTTCCAGGAGCCTCACTTGCA	fw primer genomic PCR	Fig. S1D, Fig. S5C
PER2 genomic locus (rv)	AAGCTGTCAGACTGAGTGGC	Rv primer genomic PCR	Fig. S1D, Fig. S5C
HF tag (rv)	TTGCTAGCCTTGTCGTCATC	RT primer	Fig. 1C Fig. S1C Fig. S5B
HF tag (rv)	ATCGTCTTTGTAGTCACCGCTACC	Rv primer RT-PCR	Fig. 1C Fig. S1C
CRY1 mRNA	TGCTGAGGCAAGCCGTTTGA	Fw primer RT-PCR	Fig. 1C Fig. S1C
PER2 mRNA	ACGCCCTTTCCACGTCAAGC	Fw primer RT-PCR	Fig. 1C Fig. S1C
mScarlet-I	GTCTTGAAGTCCGCCAGGTAGC	Rv primer RT-PCR	Fig. S5B
mClover3	ACGCTGAACTTGTGGCCGTTT	Rv primer RT-PCR	Fig. S5B



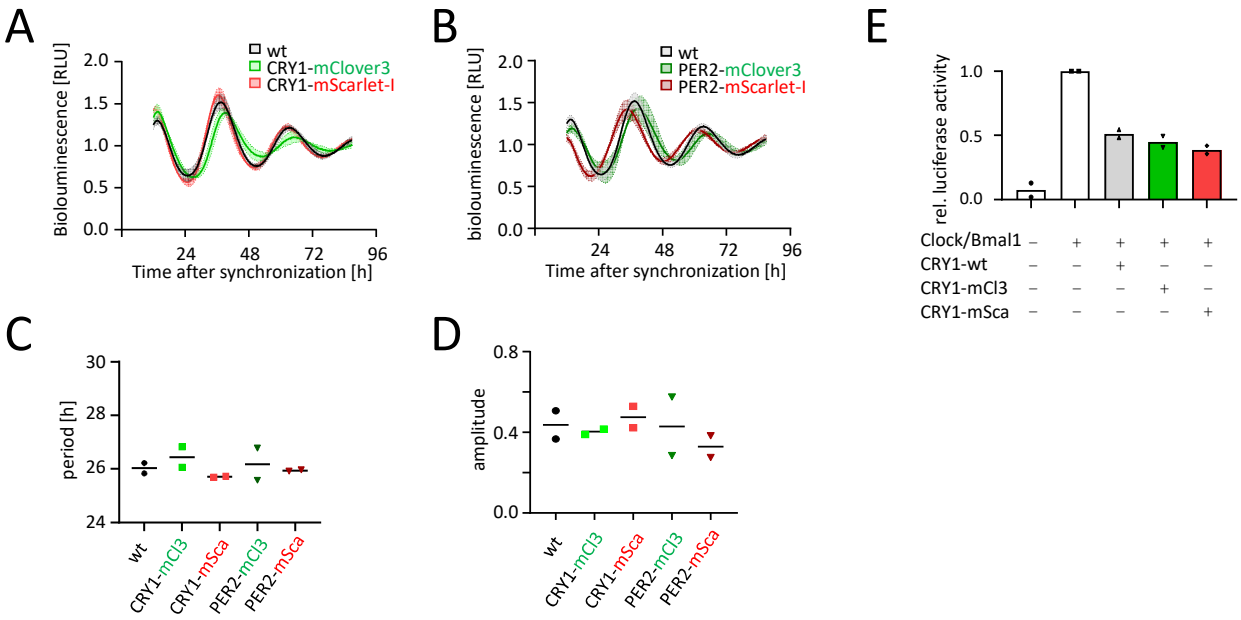
Supplementary Figure S1: Screening of potential single knock-in clones. (A) and **(B)**: Screening of potential CRY1 (A) and PER2 (B) knock-in clones by fluorescence microscopy using YFP and RFP channel. For each knock-in, 3 examples of fluorescent cells are shown along with wild-type cells that show autofluorescence only. Clones that were confirmed positive for correct knock-in by PCR afterwards are marked with *, clones used for all further analysis with **. **(C)** Chimeric mRNA was detected in single clones by RT-PCR (as in Fig. 1C). **(D)** Successful knock-in was confirmed by amplification of the targeted region of PER2 and CRY1 genomic loci by PCR, which shows either wild-type allele, the larger knock-in allele or both. Additional bands are probably heteroduplexes of wild-type and knock-in product. **(E)** Percentage of positive knock-in clones in relation to all screened clones for the 4 different knock-in experiments. Numbers indicate count of correct and initially screened clonal colonies, respectively.

Supplementary Figure S1



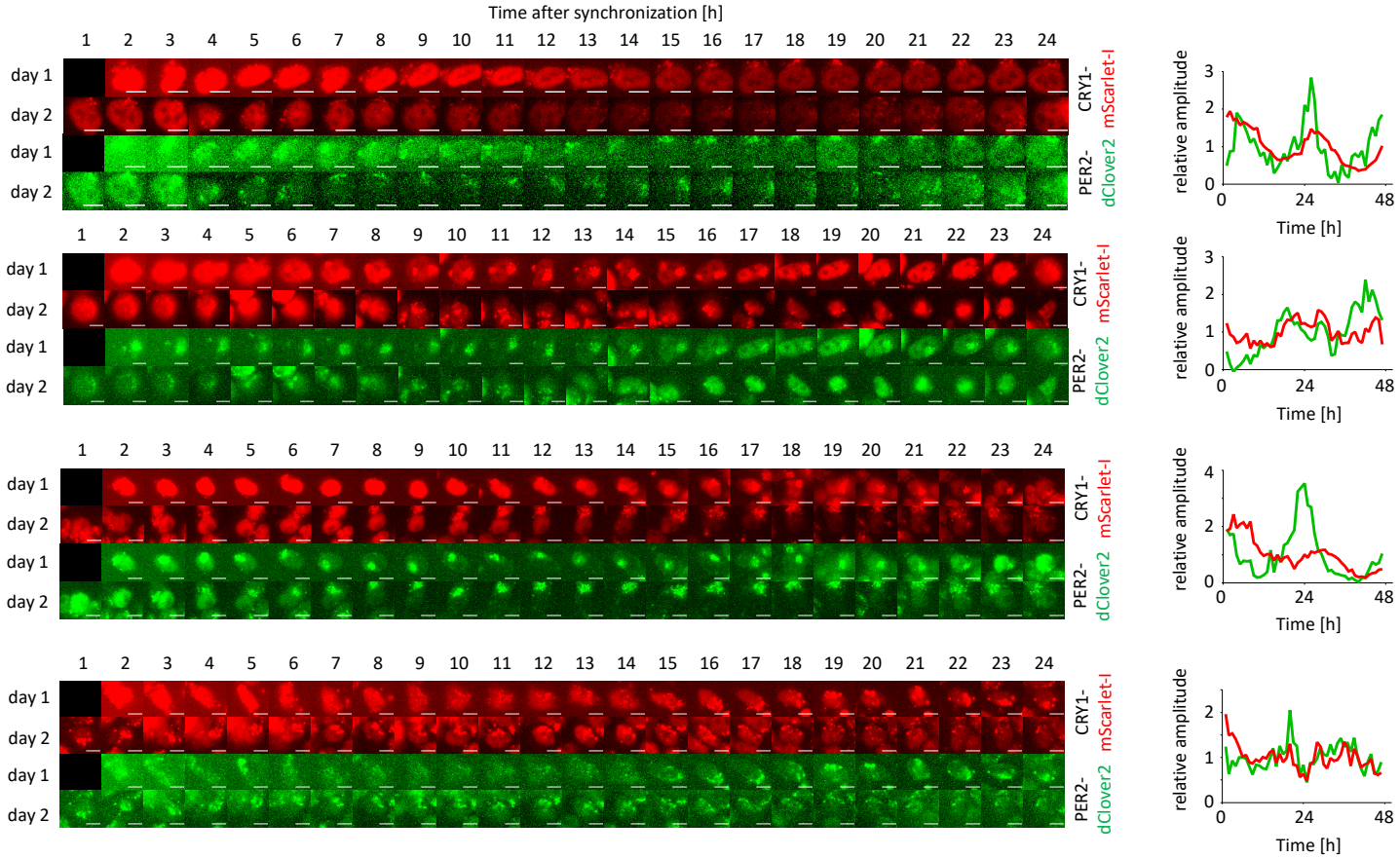
Supplementary Figure S2: **Complete time series of knock-down experiment (Fig. 1F).** U-2 OS knock-in cells expressing CRY1-mClover3 **(A)**, CRY1-mScarlet-I **(B)**, PER2-mClover3 **(C)** or PER2-mScarlet-I **(D)**, respectively, were either left untreated or transduced with shRNA targeting either CRY1 or PER2. After synchronization, fluorescence in the respective channel was recorded for 24 hours. Scale bar: 20 µm.

Supplementary Figure S2



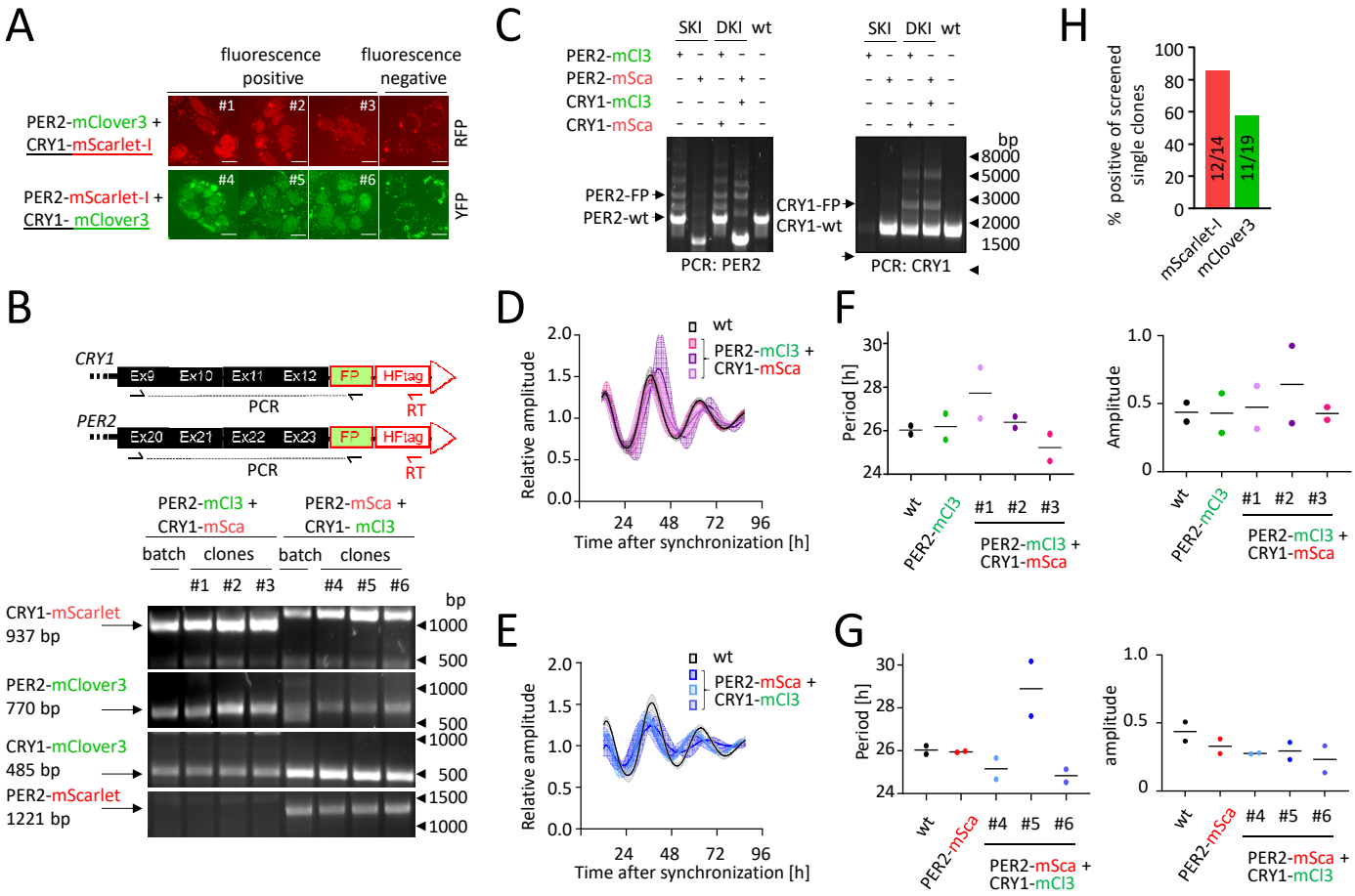
Supplementary Figure S3: Analysis of circadian rhythms in single knock-in cells. (A)-(D) Individual clones and wild-type cells were transduced with a *Bmal1*:Luc reporter and luminescence was recorded over four days. Depicted are mean + SD of four individual, detrended traces resulting from two independent experiments **(A)** and **(B)**, and mean calculated period lengths **(C)** and amplitude **(D)** for both experiments. **(E)** Ability of CRY fusion proteins to inhibit CLOCK/BMAL1 induced activation of an E-BOX reporter plasmid. HEK-293 cells were transfected with an 6xEBOX-Luciferase reporter plus the indicated constructs and reporter activity was measured (n=2).

Supplementary Figure S3



Supplementary Figure S4: Time series of HCT-116 double knock-in cells. Montages of bicolor fluorescence microscopy images of individual HCT-116 double-knock-in (PER2-dClover2/CRY1-mScarlet-I) cell's nuclei over the course of 2 days. Traces of 4 individual cells are shown. Mean nuclear fluorescence signals were quantified, backgrounds subtracted and signals normalized by dividing by mean signal of the time course. Scale bar: 10 µM.

Supplementary Figure S4



Supplementary Figure S5: Selection and characterization of double knock in clones. (A) Screening of clones with potential CRY1-knock in by fluorescence microscopy. For each knock-in, 3 example clones with the expected pattern are shown along with a negative clone. **(B)** Chimeric mRNA was detected in the three single clones from A by RT-PCR using RT-primer specific to the insertion, gene specific forward and fluorophore specific reverse primer. Arrows indicate the expected band for righteous insertion. **(C)** Successful knock-in was confirmed by amplification of the edited genomic locus using out-out PCR followed by Sanger sequencing. Results exemplarily shown for DKI clones #3 and #6. **(D-G)** Individual double knock-in clones, the corresponding parental clone and wt cells were transduced with a *Bmal1*:Luc reporter and luminescence was recorded over four days. Depicted are mean + SD of four individual, detrended traces resulting from two independent experiments **(D-E)**, and mean calculated period lengths and amplitude for both experiments **(F-G)**. Clone #6 was used for imaging analysis. **(H)** Percentage of positive knock-in clones in relation to all screened clones. Scale bar: 20 µm. mCl3 = mClover3, mSca = mScarlet-I, FP = fluorescent protein (mScarlet-I or mClover3).

Supplementary Figure S5