Supporting Information

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SI Materials and Methods

Statistical Methods: Period. For each luciferase measurement, the period of oscillation was calculated by least-mean-squares fitting of dampened sine wave functions to the actual data. The period of the sine wave with the best least-squares fit to the data was assumed to be the true period of oscillation. Because the period length of the first day after synchronization varied according to the conditions of synchronization, it was not included in these calculations; rather, period was determined by analyzing only days 2–5. For each period measurement, at least three separate experimental measurements were done for each biopsy, using two biopsies from each individual. Period values are presented as mean ± SE.

Statistical Methods: Relative Amplitude. The amplitudes of the second and third cycles of circadian expression were obtained as the difference between the peak and nadir expression values of these cycles. These measurements then were normalized using the absolute raw-data magnitude of the first peak as an approximate measure of reporter virus infection efficiency in each culture.

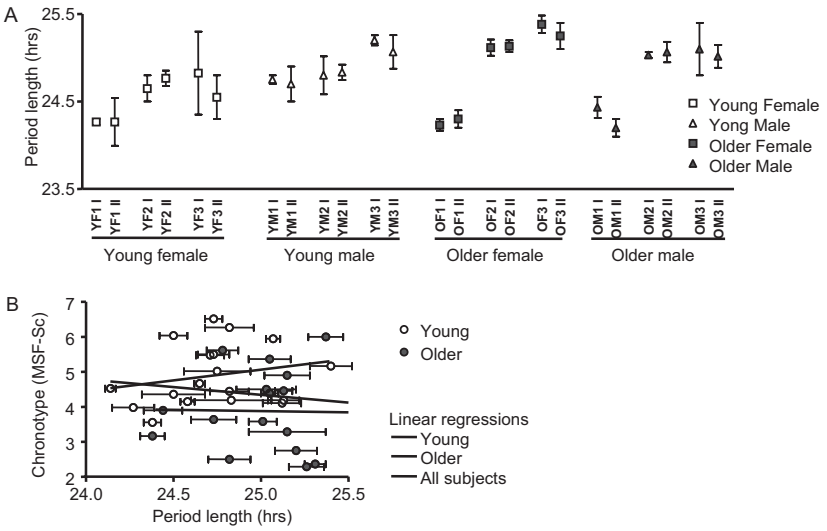

Fig. S1. (*A*) Period lengths obtained from two different biopsies taken from three young females (YF), three young males (YM), three older females (OF), and three older males (OM). Each bar represents the mean of three independent measurements per biopsy ± SEM. (*B*) Comparison between chronotype and in vitro circadian period length for the two groups of subjects ($P_{all\ subjects}$ = 0.3707; P_Y = 0.3785; P_O = 0.9229). The *x* axis shows period length in hours. Period length values are shown as the mean of six independent measurements ± SEM. The *y* axis shows subject mean sleep phase corrected for sex and cumulative sleep debt (MSF-Sc). This statistic is the output of the Munich Chronotype Questionnaire and is widely used as a reliable measure of human chronotype.

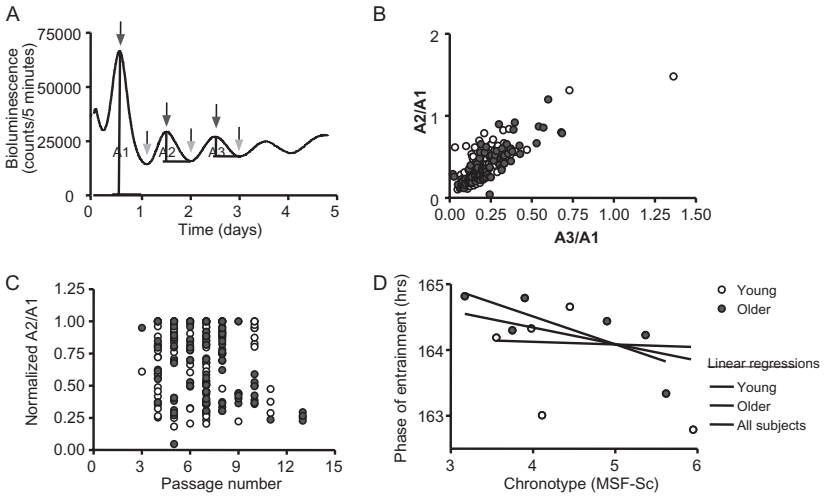

Fig. S2. Influence of age on amplitude and phase of entrainment. (*A*) Schematic representation of the amplitude measurement. Amplitude of the first three peaks (first peak = A1; second peak = A2; third peak = A3) was measured. Damping was approximated as the ratio A2/A1 or A3/A1, which produced equivalent results (*SI Materials and Methods*). (*B*) Diagram showing the correlation between A2/A1 and A3/A1 for young and old subjects. Age did not influence either of these ratios (unpaired *t* test: $P > 0.05$). (*C*) Graph reporting the influence of cellular senescence on the damping rate of peripheral oscillators. The *x* axis shows the passage number of the measured cells; the *y* axis shows the damping rate A2/A1, normalized to the highest value obtained from each subject. There was no difference in amplitude of fibroblasts between young and old subjects with increasing passage number (unpaired *t* test: $P > 0.05$). (*D*) Graph showing chronotype (MSF-Sc) vs. phase of entrainment of reporter expression for fibroblasts from five young and six older subjects. Statistical analyses revealed no significant correlation between phase of entrainment, chronotype, and aging ($P_{All\ subjects} = 0.3699$; $P_Y = 0.9462$; $P_O = 0.0698$).

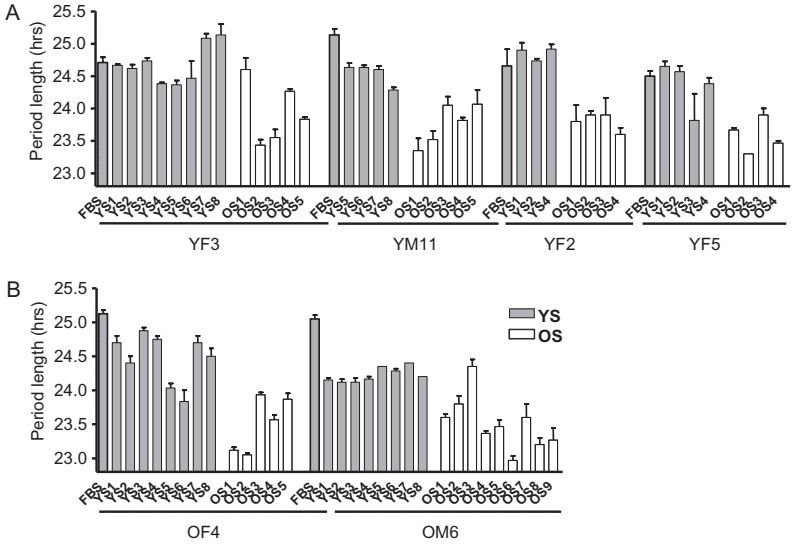

Fig. S3. Effects of individual sera upon individual cell lines from younger (*A*) and older (*B*) subjects used in our study. The length of the circadian period in each cell line used in our study is shown, as measured in the presence of each sample of human serum from young individuals (YS, gray) or from older individuals (OS, white). Bars represent the mean of three independent measurements ± SEM. Cell line designations refer to young (Y) or older (O) male (M) or female (F) donors. Donors are described in detail in Table S2.

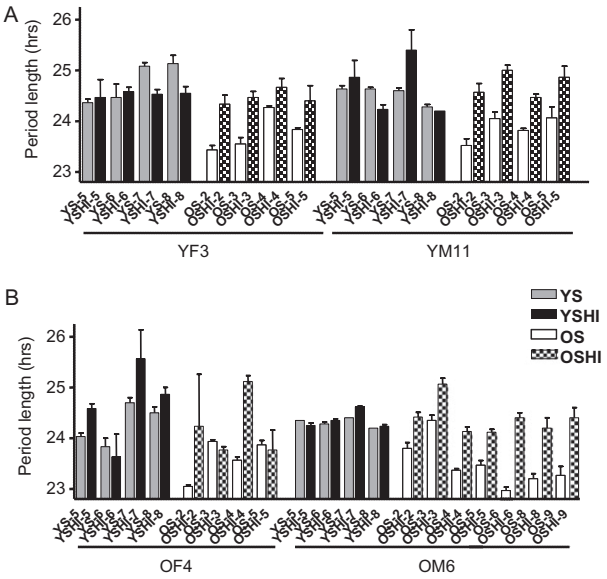

Fig. S4. Effects of individual normal and heat-inactivated sera on the individual cell lines used in our study. (For ease of analysis, data for normal serum are replotted from Fig. S3.) Circadian period lengths of cell lines from young male (YSM) or female (YSF) donors (*A*) and from older male (OSM) and older female (OSF) donors. (*B*) Cell lines, measured in media containing normal (OS, YS) or heat-inactivated (OSHI, YSHI) human serum from four young and four to eight older donors. Every bar shows the mean of three independent measurements ± SEM.

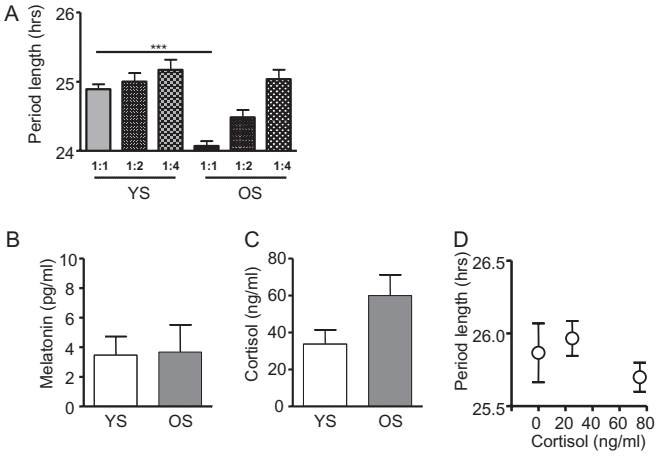

Fig. S5. (*A*) Period length measured in cell lines from two young subjects and from two older subjects in the presence of two sera from young donors (YS) and two sera from older donors (OS), serially diluted with FBS as indicated. A significantly shorter period is observed in the presence of OS (unpaired *t* test: $P < 0.001$); this effect gradually diminishes with increasing dilution. (*B*) Melatonin levels (pg/mL) in four YS and four OS. There was no statistical difference in melatonin concentration between the two groups of sera (unpaired *t* test: $P > 0.05$). (*C*) Cortisol levels (ng/mL) in four YS and four OS. There was no statistical difference in cortisol concentration between YS and OS (unpaired *t* test: $P > 0.05$). Because a (nonsignificant) trend was seen, the effects of cortisol on period length were studied in more detail, as shown in *D*. (*D*) Period lengths of one cell line in serum supplemented with 0 ng/mL, 25 ng/mL, or 75 ng/mL cortisol. Every point represents the mean of three independent measurements ± SEM. No significant differences were observed (unpaired *t* test: $P > 0.05$).

Table S1. Subject characteristics

Category	N	Age (y ± SD)	Chronotype (MSF-Sc ± SD)	Fibroblast period length (h ± SD)
Young	18	25.44 ± 3.58	4.98 ± 0.84	24.73 ± 0.32
Female	7	25.86 ± 2.48	4.86 ± 0.78	24.80 ± 0.38
Male	11	25.18 ± 4.24	5.06 ± 0.91	24.69 ± 0.29
Older	18	67.89 ± 7.32	3.88 ± 1.12	24.94 ± 0.37
Female	7	65.43 ± 4.12	4.01 ± 1.22	24.96 ± 0.35
Male	11	69.45 ± 8.61	3.79 ± 1.10	24.92 ± 0.40

MSF-Sc, mean sleep phase, corrected for sex and cumulative sleep debt. (Further details are given in the main text and in the legend for Fig. S1.)

Table S2. Detailed subject information

			Fibroblast period length		
Age (y)	Sex	Chronotype (MSF-Sc)	Time (h)	SD	*n*
28	F	3.98	24.27	0.30	6
23	F	6.04	24.50	0.21	7
27	F	5.48	24.71	0.20	6
29	F	5.02	24.75	0.45	6
27	F	4.19	24.83	0.51	5
23	F	4.11	25.12	0.27	6
24	F	5.17	25.40	0.27	5
27	M	4.53	24.14	0.08	6
21	M	3.56	24.38	0.12	6
31	M	4.36	24.50	0.39	5
21	M	4.15	24.58	0.11	7
21	M	4.67	24.65	0.06	6
31	M	5.50	24.73	0.17	4
22	M	6.52	24.73	0.13	6
24	M	6.27	24.82	0.34	6
31	M	4.45	24.82	0.26	6
26	M	5.95	25.07	0.11	6
22	M	4.19	25.13	0.23	6
65	F	3.17	24.27	0.14	6
61	F	3.64	24.73	0.31	6
73	F	3.58	25.01	0.20	6
62	F	4.46	25.13	0.13	6
63	F	4.90	25.15	0.30	5
68	F	2.29	25.26	0.24	6
66	F	6.00	25.20	0.17	6
64	M	3.90	23.99	0.27	6
65	M	5.62	24.32	0.21	6
70	M	2.50	24.82	0.24	4
70	M	4.50	25.03	0.40	7
60	M	5.37	25.05	0.27	6
65	M	4.39	25.05	0.13	5
70	M	3.29	25.15	0.55	6
83	M	2.75	25.20	0.30	6
66	M	2.37	25.18	0.20	6
63	M	3.27	25.29	0.08	6
88	M	3.75	25.01	0.19	7

Subjects highlighted in gray also were the source of biopsied fibroblasts used in characterization of blood sera.

Table S3. Blood donor characteristics

Blood donor	Sex	Age (y)	Medications
Y1	M	21	No
Y2	M	20	No
Y3	M	32	Citalopram-hormosan (120 mg/d)
Y4	M	21	No
Y5	M	26	No
Y6	M	27	No
Y7	M	31	No
Y8	M	26	No
O1	M	81	Daflon (500 mg/d)
O2	F	83	Aspirine Cardio (100 mg/d); metoprolol (50 mg/d; perindopril/indapamin (4 mg/d/1.25 mg/d)
O3	F	67	No
O4	M	80	Aspirine Cardio (100 mg/d); amlodipine maleate (2.5 mg/d); chlorthalidone (12,5 mg/d)
O5	M	61	Aspirine Cardio (100 mg/d); simvastatin (40 mg/d)
O6	F	61	No
O7	M	63	No
O8	F	56	No
O9	M	61	No

O, old; Y, young.