Supplemental Information for:

Less-invasive age estimation using hair based on DNA methylation in brown bears

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Supplementary Table S_M1. List of hair samples used in this study.

Captive/Wild	Bear ID	Sex	Birth year	Sampling date	Age at sampling (year)
C	Nonno	F	2022	2023/12/11	1.9
C	Ikoro	M	2022	2024/2/29	2.1
C	Shouhei	M	2018	2020/7/19	2.5
C	Daichi	M	2017	2020/11/1	3.7
C	Kutta	M	2016	2020/6/3	4.3
C	Kai	M	2016	2020/6/3	4.3
C	Haru	F	2019	2023/11/29	4.8
C	Tocky	M	2018	2023/5/12	5.3
C	Ana	F	2015	2020/6/4	5.3
C	Fuku	M	2015	2020/6/23	5.4
C	Lucky	F	2015	2021/4/27	6.2
C	Kotetsu	M	2017	2023/5/19	6.3
C	Honoka	F	2015	2021/6/9	6.4
C	Sasuke	M	2015	2021/6/22	6.4
C	Yuzuru	M	2014	2020/7/19	6.5
C	Maron	F	2013	2020/6/4	7.3
C	Marin	F	2013	2021/3/22	8.1
C	Tsurara	M	2013	2021/5/7	8.3
C	Syosuke	M	2013	2021/5/20	8.3
C	Meruru	M	2013	2021/6/18	8.4
C	Ren	M	2011	2020/6/23	9.4
C	Rocky	M	2010	2020/6/25	10.4
C	Tokuto	M	2010	2020/6/25	10.4
C	Sumiri	F	2006	2020/12/24	14.9
C	Kanta	M	2006	2021/6/7	15.3
C	Mantarou	M	2006	2021/6/7	15.3
C	Bekky	F	2002	2019/4/23	17.2
C	Moriko	F	2001	2020/11/11	19.8
C	Momoko	F	1999	2019/4/24	20.2
C	Koron	F	1999	2019/4/24	20.2
C	Erika	F	1999	2019/12/6	20.8
C	Torue	F	1999	2023/12/8	24.8

C	Masako	F	1992	2021/5/4	29.3
C	Kiko	F	1990	2021/4/27	31.2
C	Raisa	F	1991	2024/3/21	33.1
W	Button	F	2018	2022/6/2	4.3
W	Lucky	F	2019	2024/6/3	5.3
W	Crayon	F	2015	2022/8/24	7.6
W	Betty	F	2014	2022/8/23	8.6
W	Ein	F	2014	2023/8/22	9.6
W	Guri	F	2010	2022/8/22	12.6
W	Hatch	F	2007	2021/6/24	14.4
W	Drum	F	2004	2019/7/5	15.4
W	Wacky	F	2002	2020/8/29	18.6
W	Rich	F	2002	2021/8/18	19.5
W	Kiriko	F	2001	2024/6/4	23.3
W	Linda	F	1996	2023/8/22	27.6

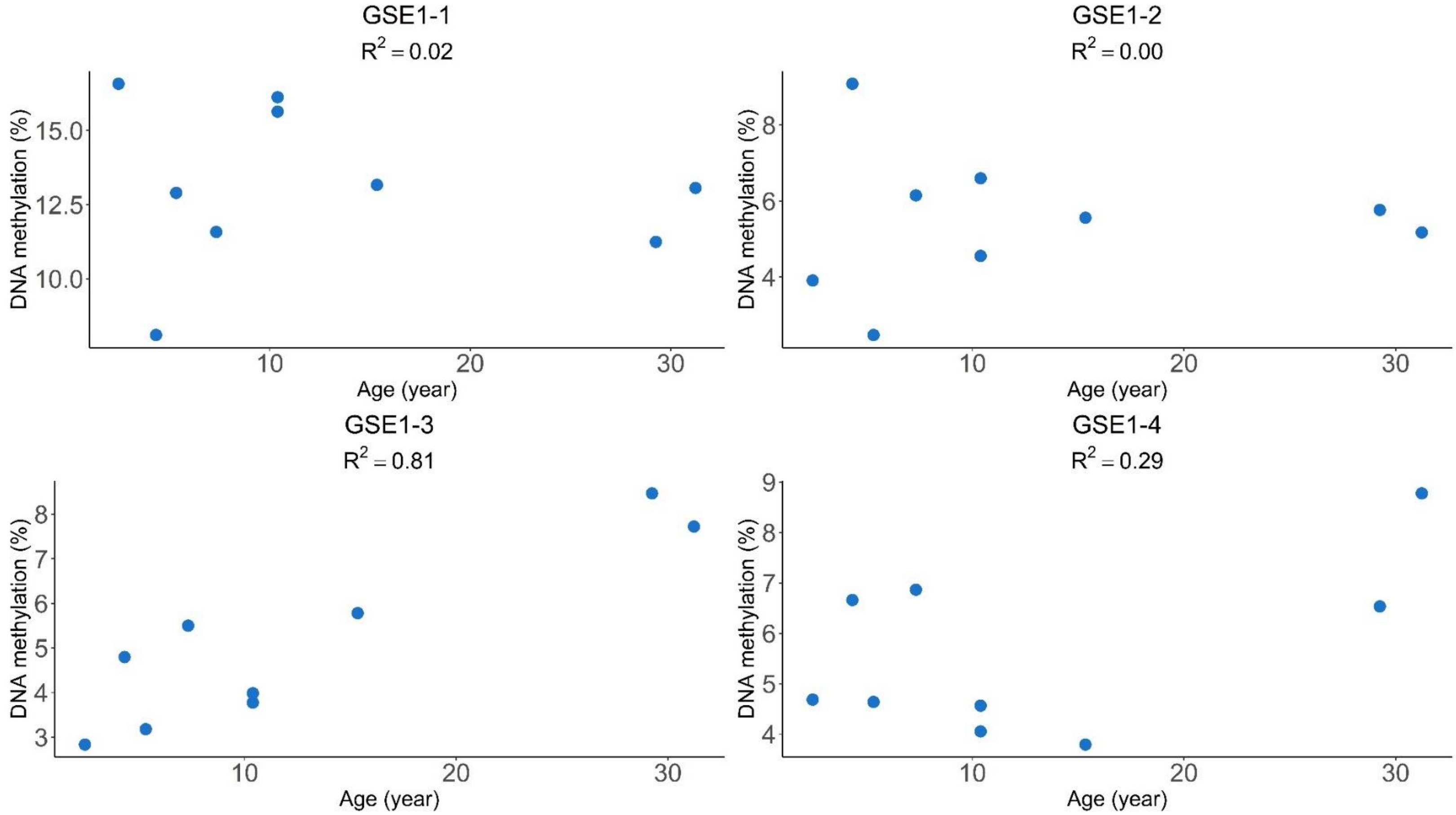
C: captive; W: wild; M: male; F: female

Supplementary Table S_R1. Correlation between DNA methylation level and chronological age. This table includes correlation coefficients (cor) and *p*-values for each CpG sites.

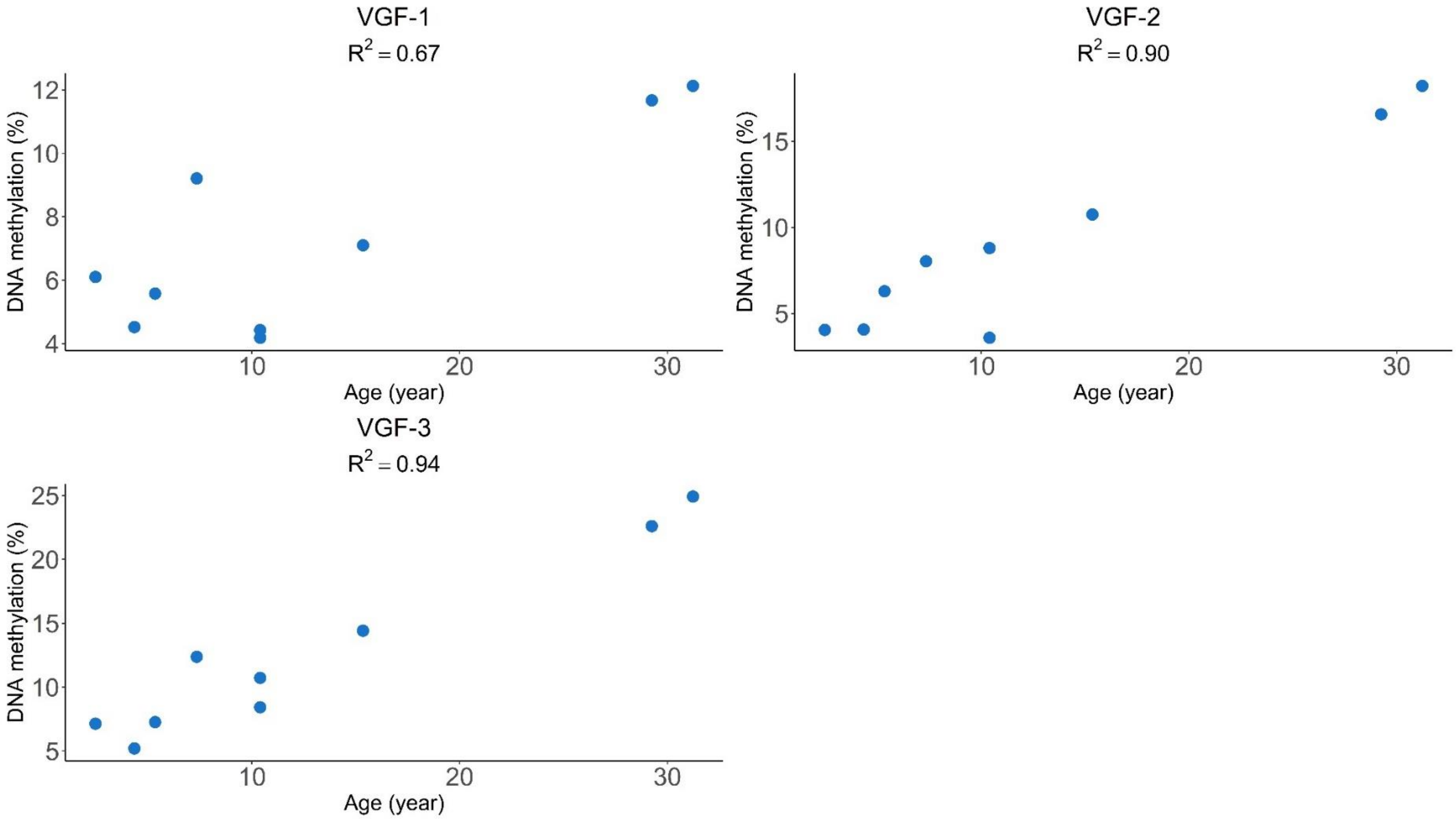
CpG site	**cor**	***p*-value**
VGF-1	0.682	＜0.0001
VGF-2	0.726	＜0.0001
VGF-3	0.784	＜0.0001
KCNK12-1	0.755	＜0.0001
KCNK12-2	0.706	＜0.0001
KCNK12-3	0.810	＜0.0001
ELOVL2-1	0.371	<0.05
ELOVL2-2	0.458	<0.01
ELOVL2-3	0.518	<0.001

Supplementary Table S_R2. Optimized parameters for the elastic net regression and the SVR models.

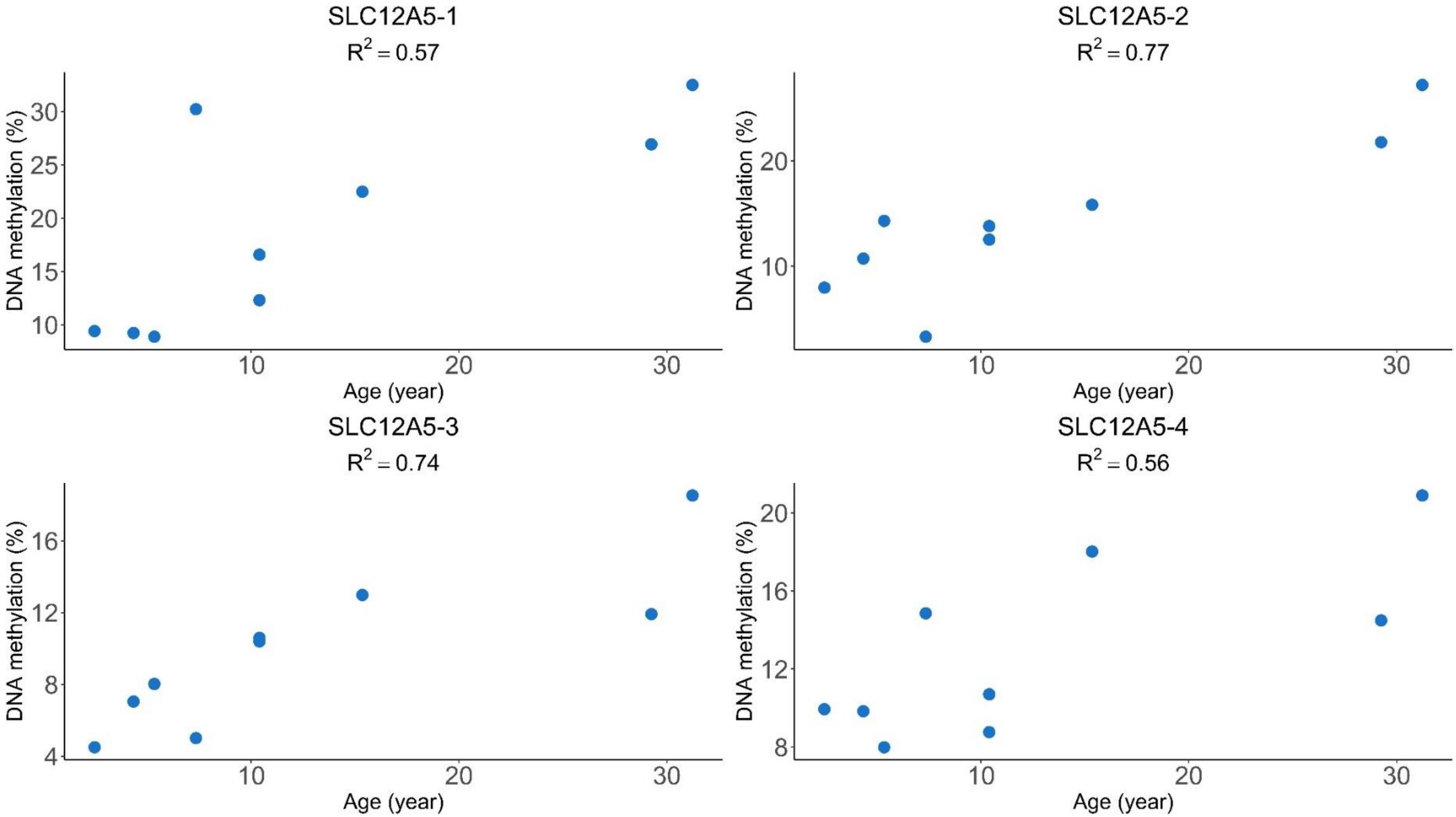
Model	Used CpGs	Alpha	Lambda	Cost	Gamma	Epsilon
Elastic net regression	All (9 CpGs)	0.04	0.4369317			
Support vector regression	all (9 CpGs)			3.981072	0.005011872	0.1
	VGF-1, -2, -3, KCNK12-1, -2, -3, ELOVL2 -2, -3			5.011872	0.003981072	0.1
	VGF-1, -2, -3, KCNK12-1, -2, -3, ELOVL2 -3			39.81072	0.05011872	0.1
	VGF-1, -3, KCNK12-1, -2, -3, ELOVL2 -3			1.258925	0.01258925	0.1
	VGF-1, -3, KCNK12-2, -3, ELOVL2 -3			1.258925	0.01584893	0.1
	VGF-1, -3, KCNK12-2, -3			1.995262	0.01584893	0.1
	VGF-1, -3, KCNK12-3			50.11872	0.006309573	0.1
	VGF-3, KCNK12-3			0.7943282	0.03162278	0.1
	VGF-1, -2, -3			79.43282	0.07943282	0.1
	KCNK12-1, -2, -3			1.258925	0.03162278	0.1



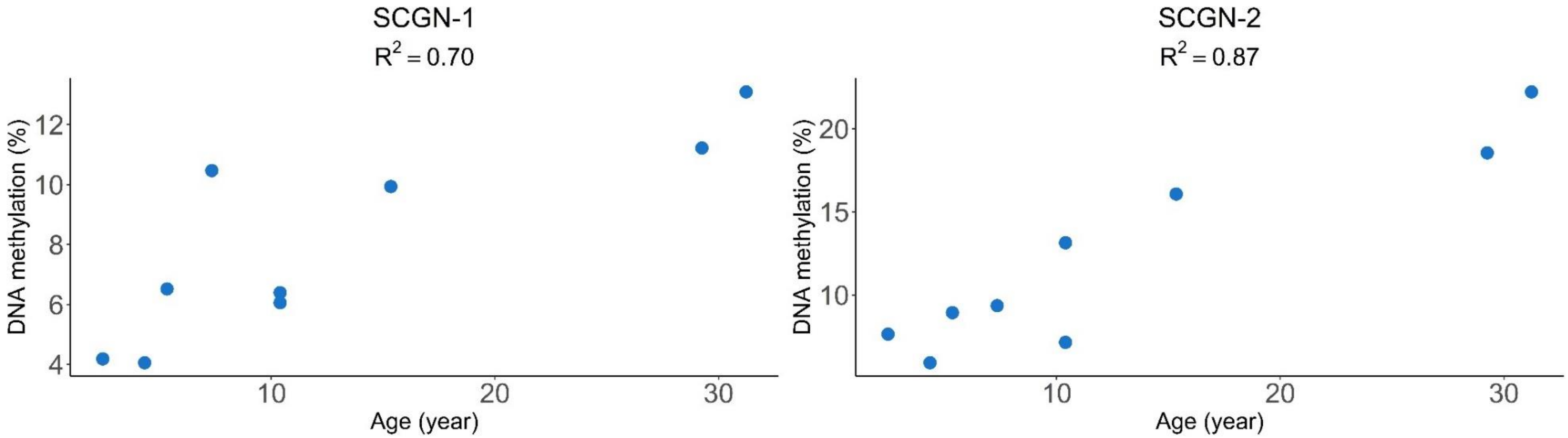
Supplementary Figure S_R1_1. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *GSE1* used for the screening process.



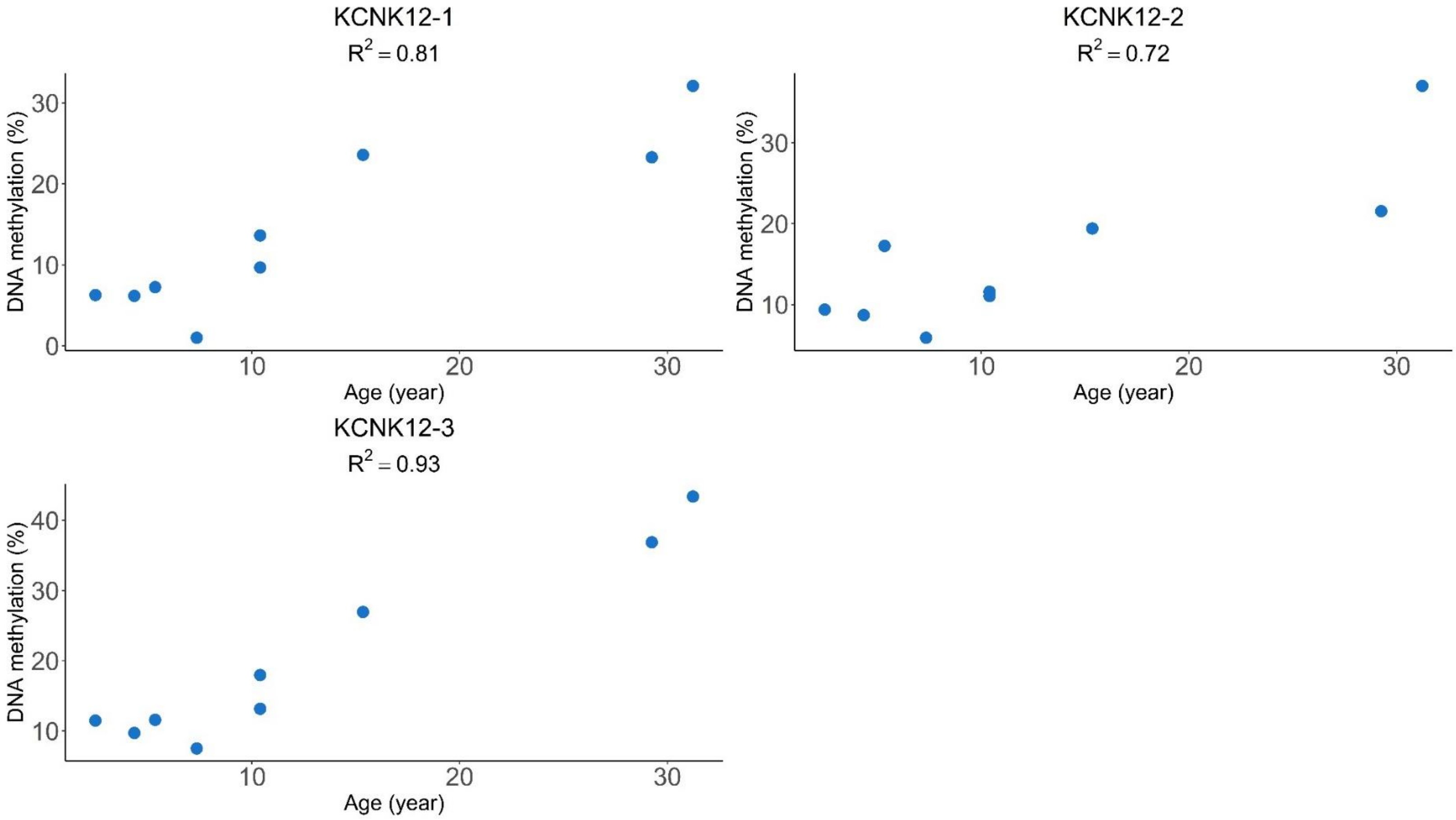
Supplementary Figure S_R1_2. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *VGF* used for the screening process.



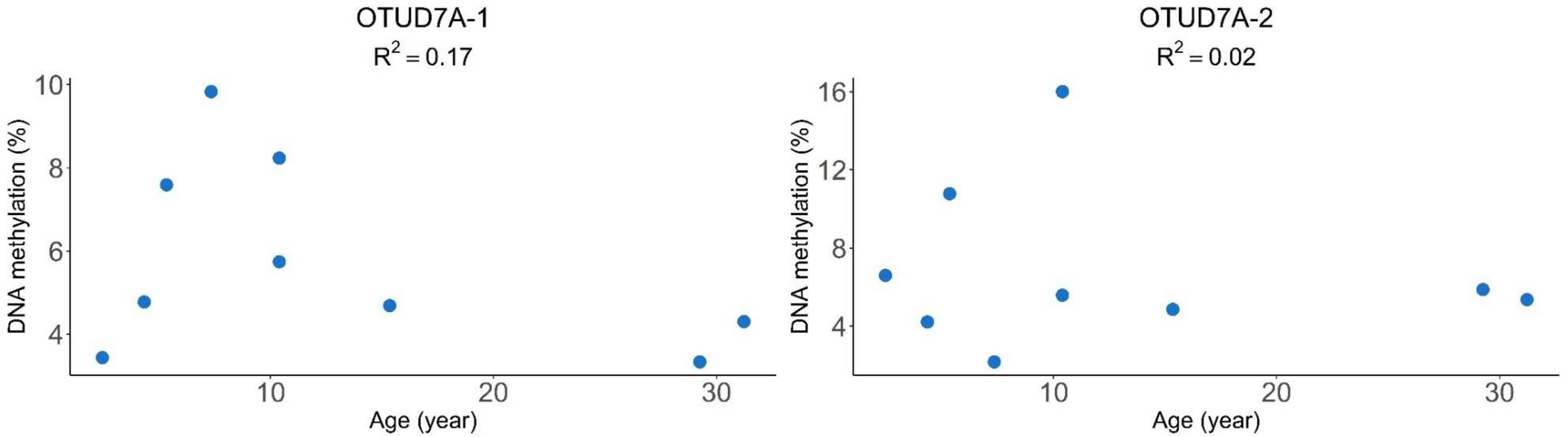
Supplementary Figure S_R1_3. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *SLC12A5* used for the screening process.



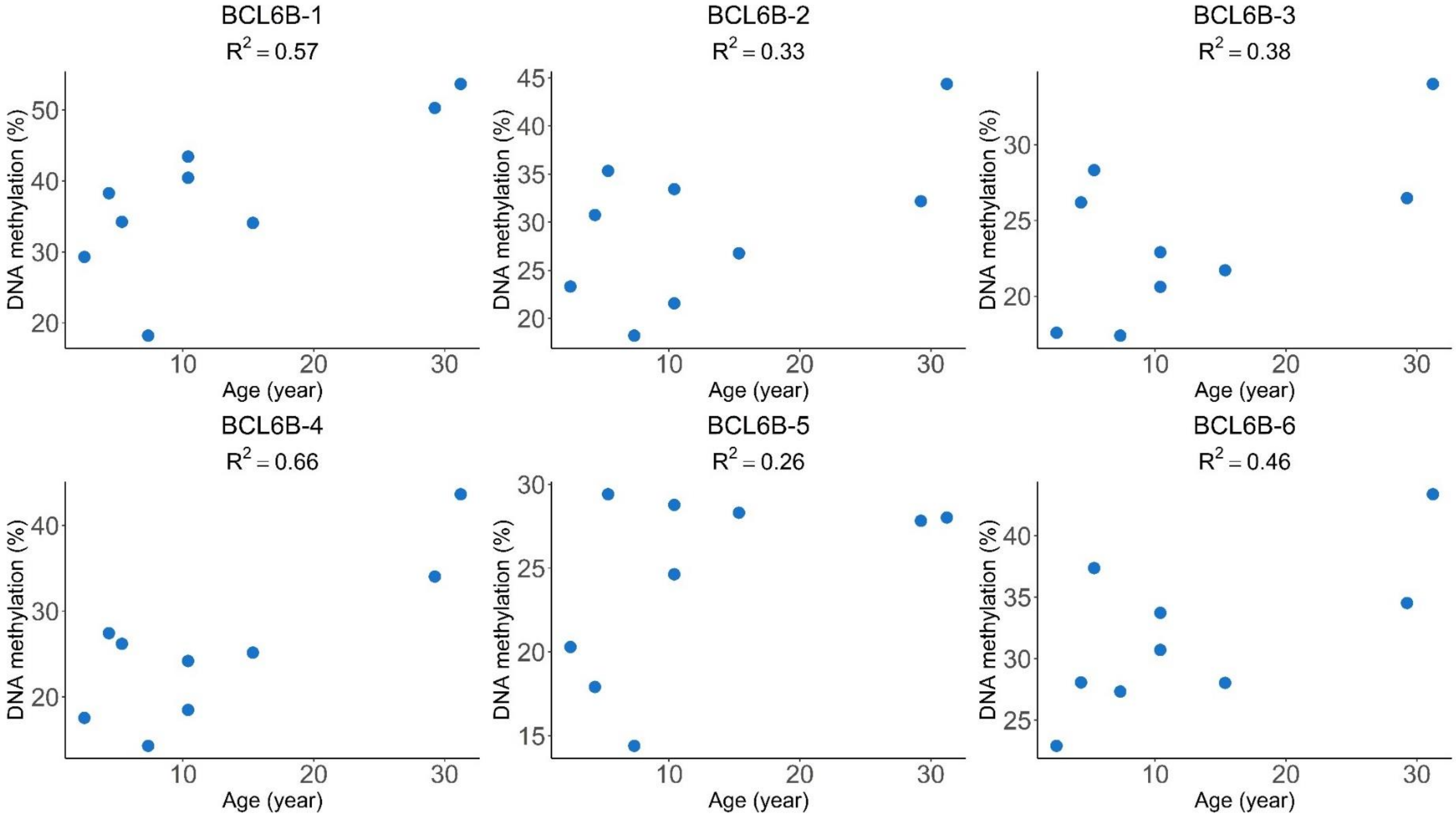
Supplementary Figure S_R1_4. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *SCGN* used for the screening process.



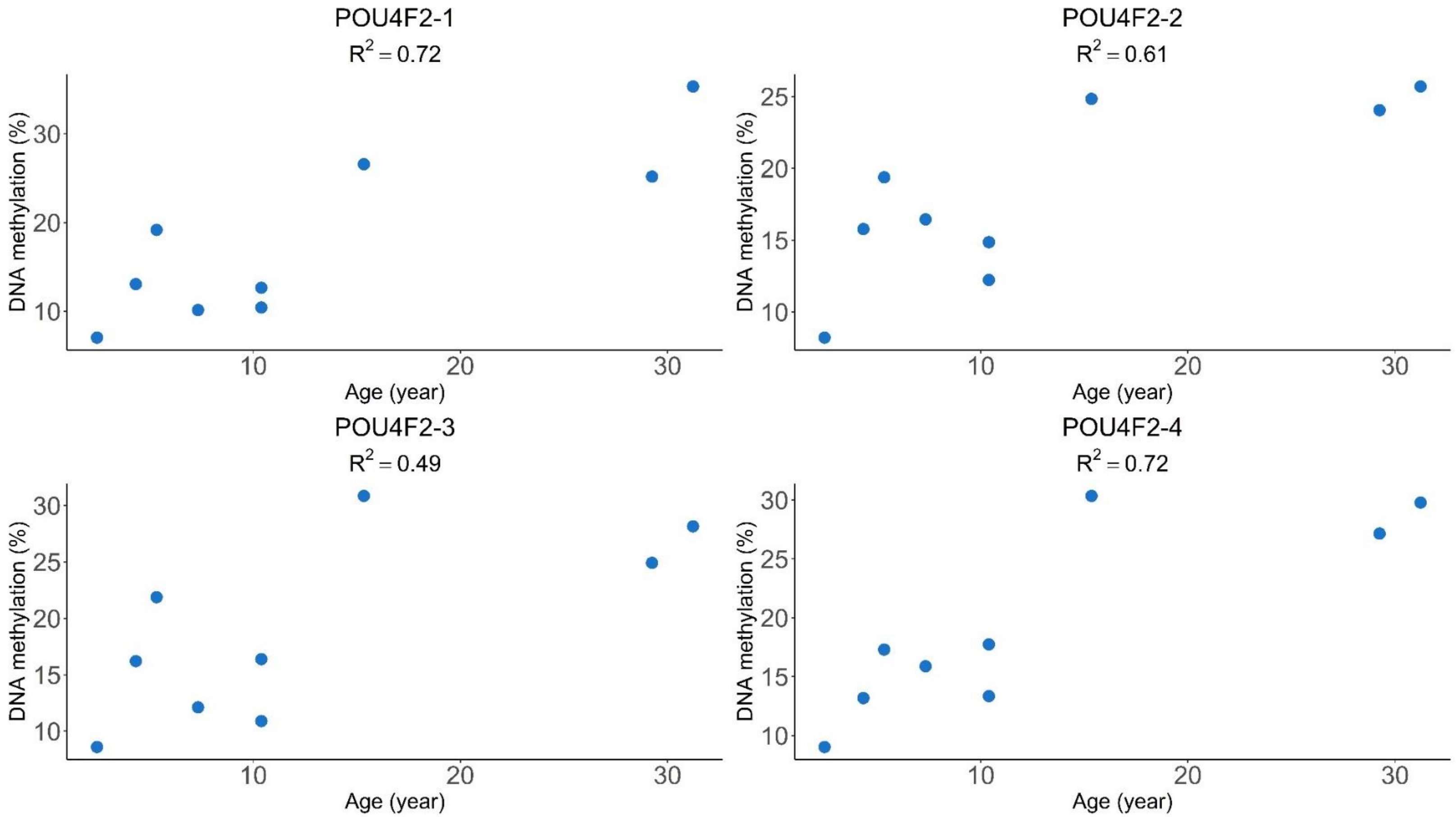
Supplementary Figure S_R1_5. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *KCNK12* used for the screening process.



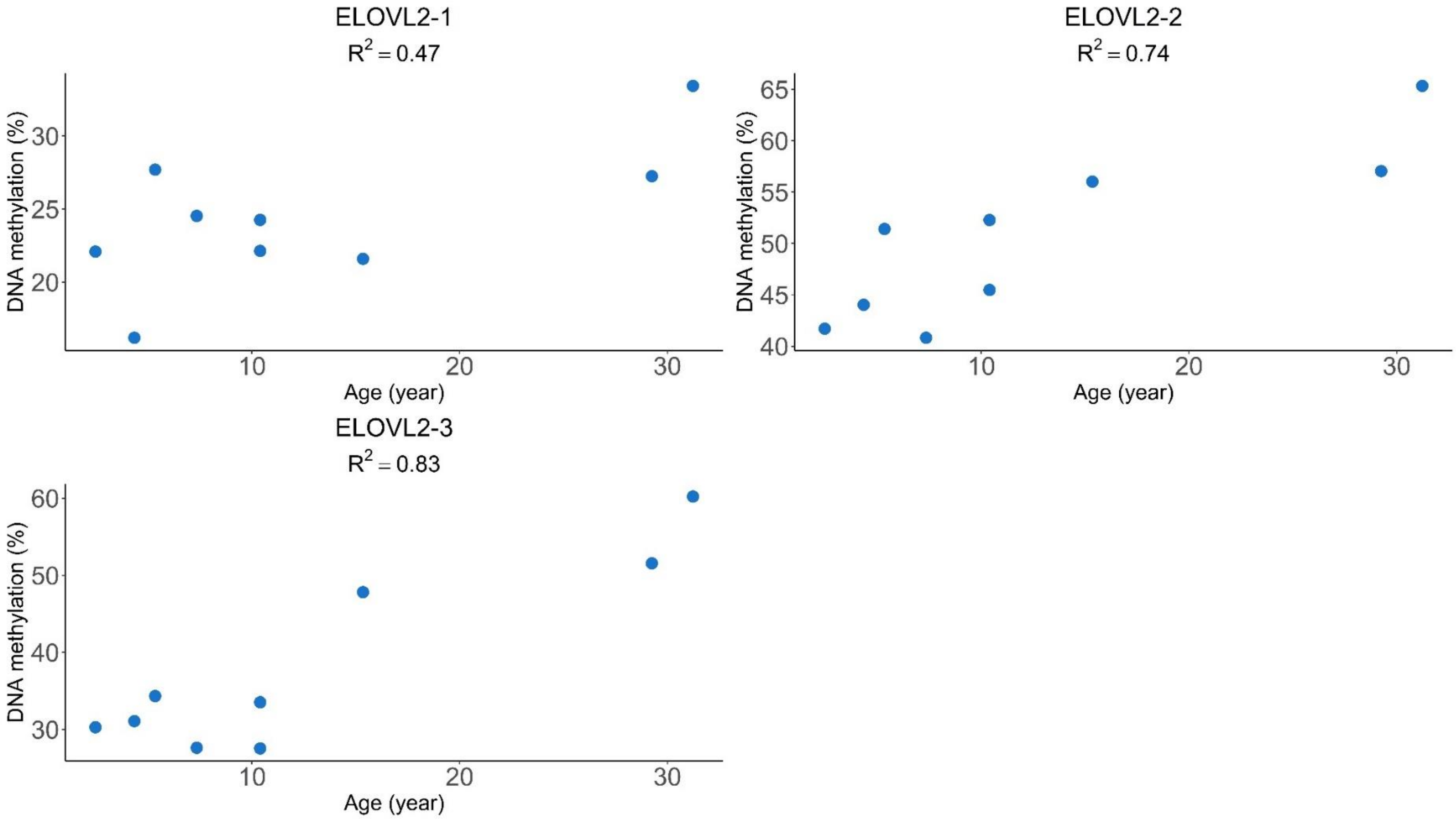
Supplementary Figure S_R1_6. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *OTUD7A* used for the screening process.



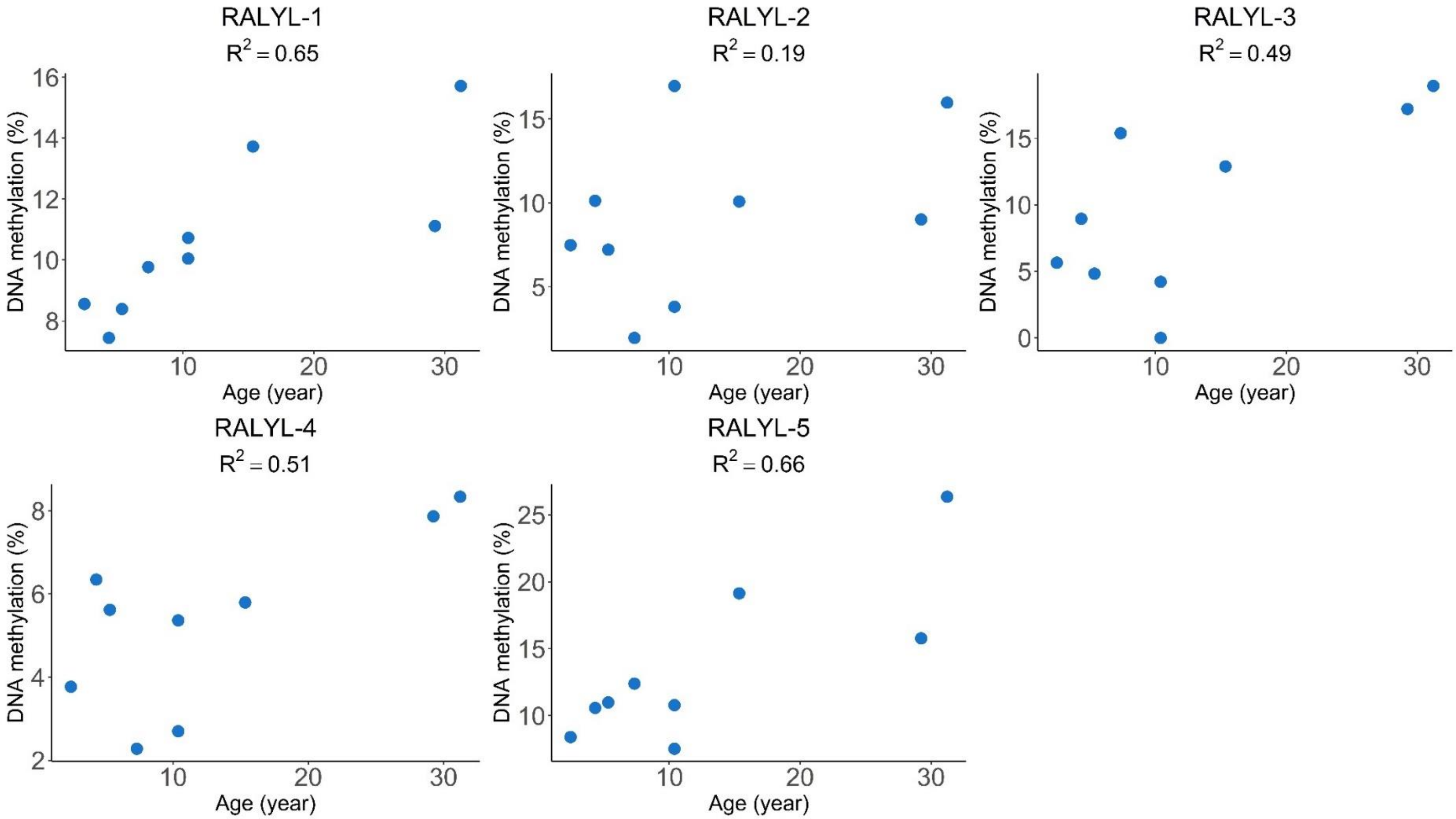
Supplementary Figure S_R1_7. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *BCL6B* used for the screening process.



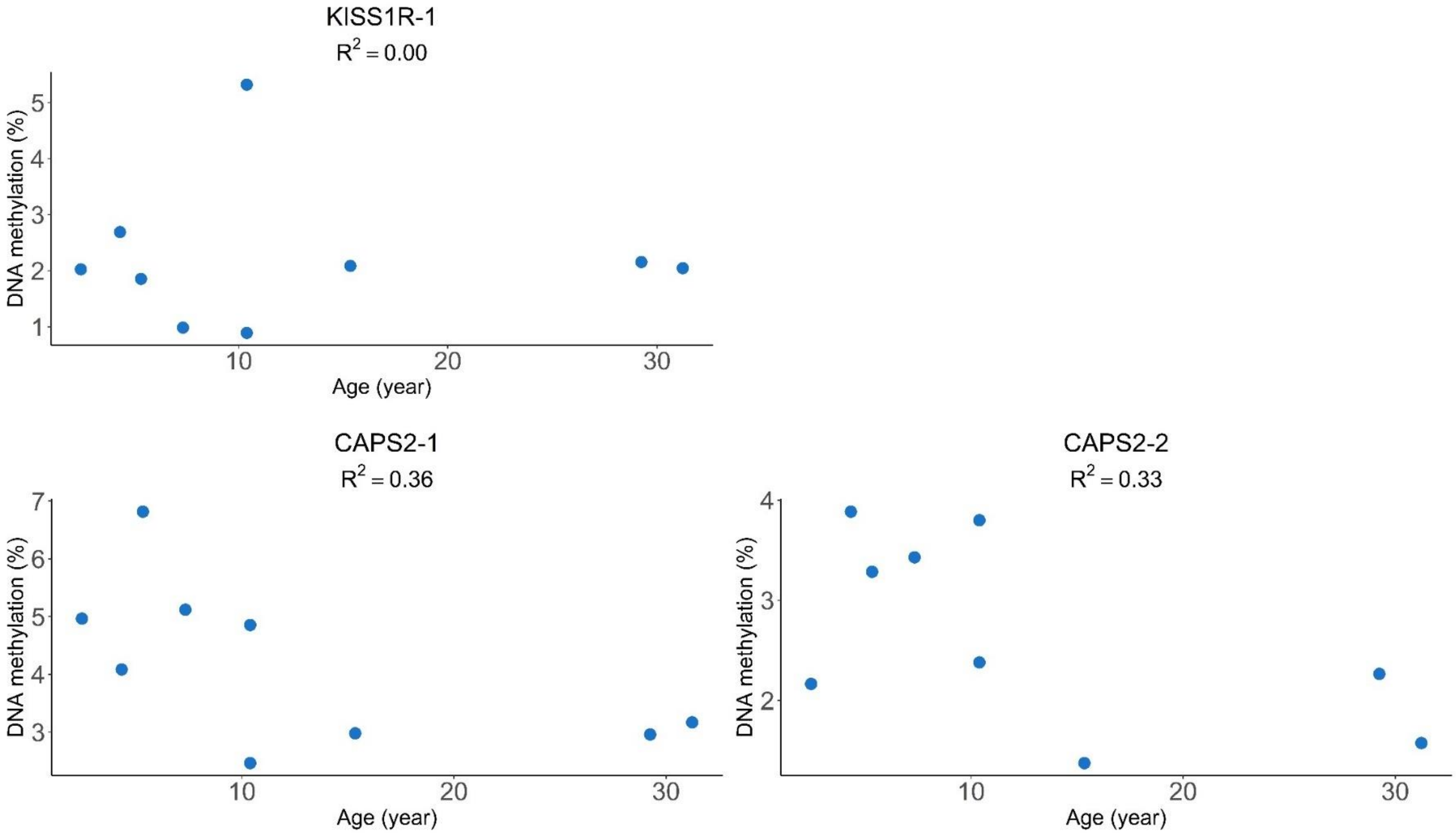
Supplementary Figure S_R1_8. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *POU4F2* used for the screening process.



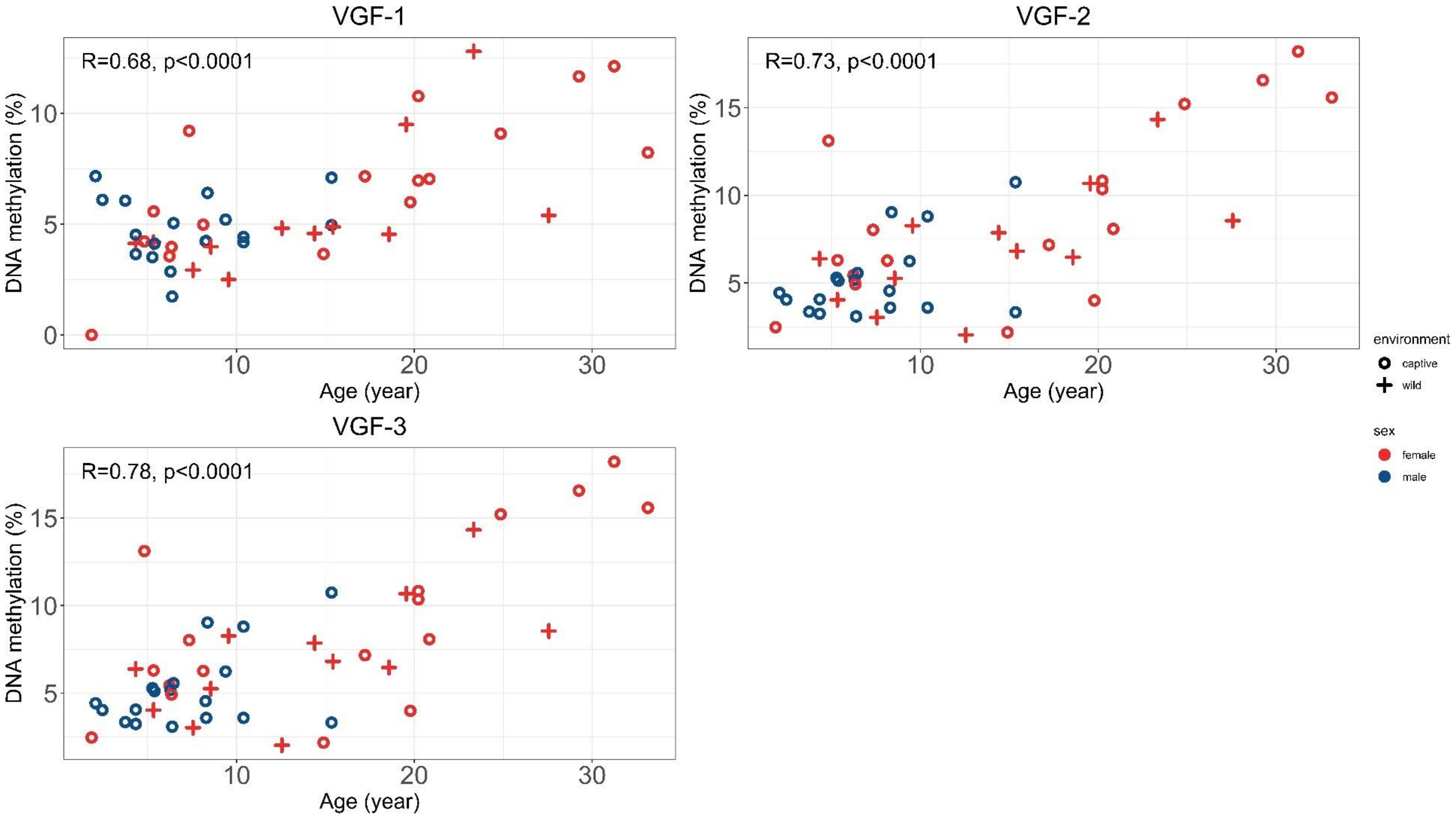
Supplementary Figure S_R1_9. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *ELOVL2* used for the screening process.



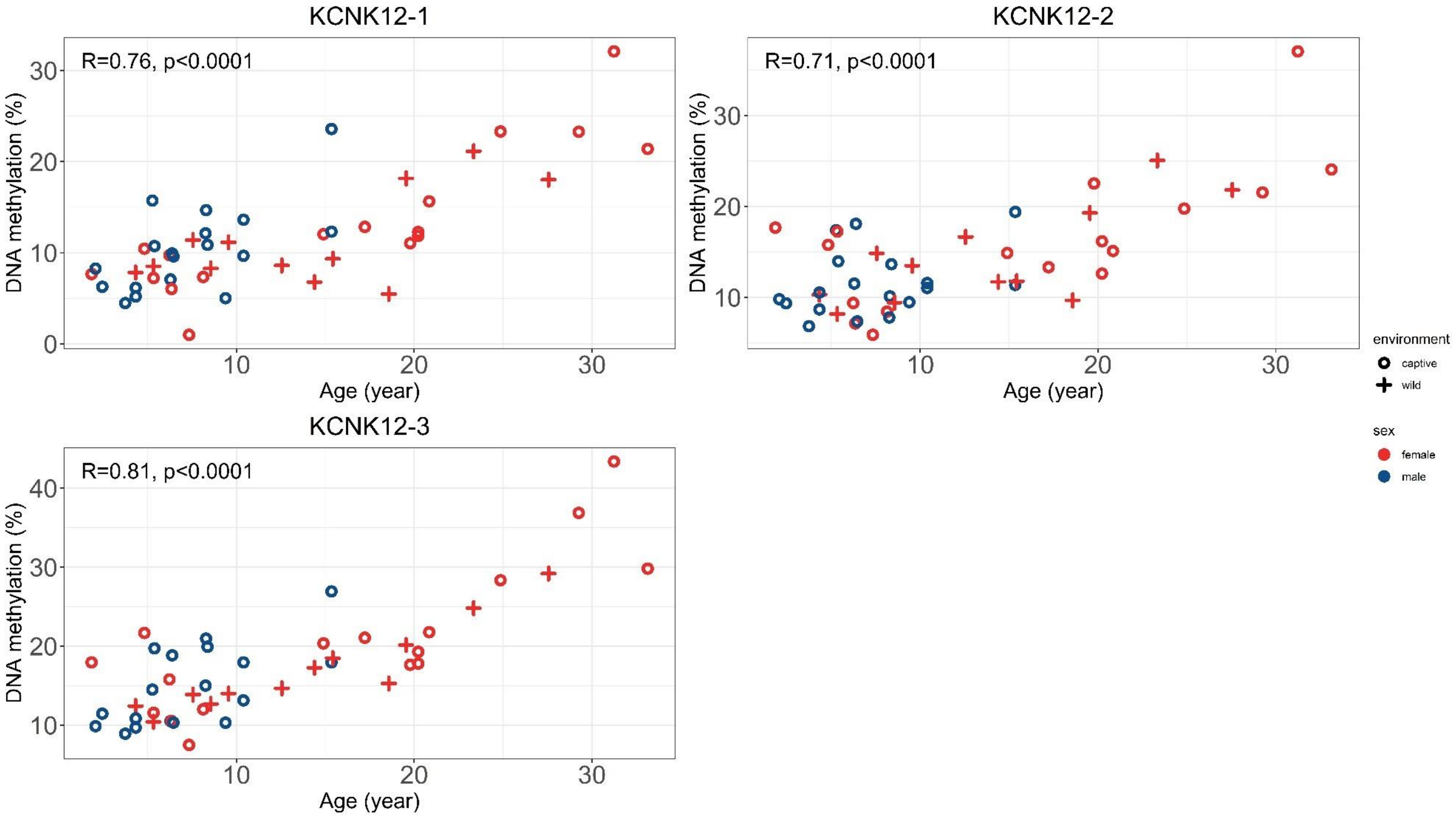
Supplementary Figure S_R1_10. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *RALYL* used for the screening process.



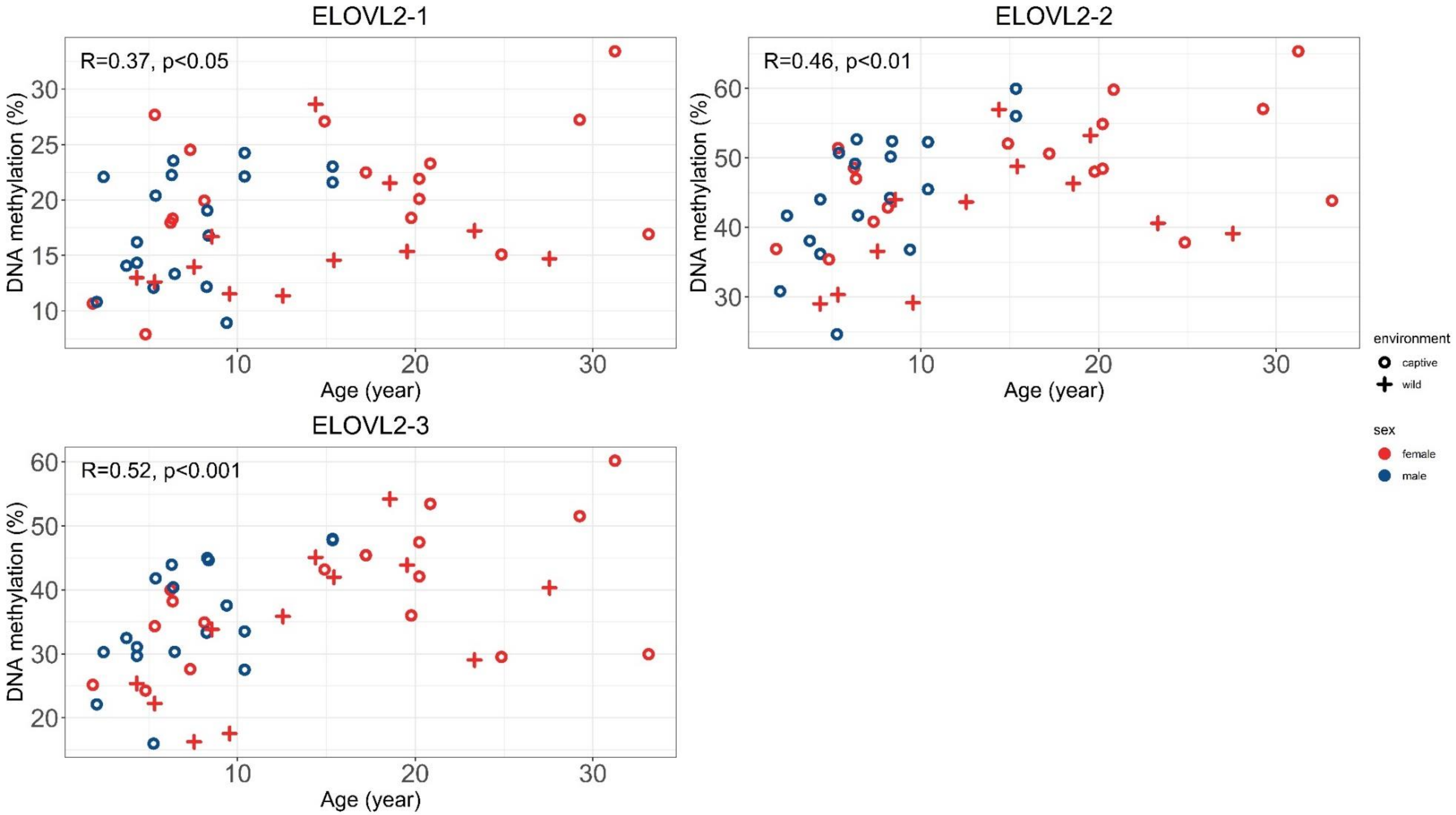
Supplementary Figure S_R1_11. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *KISS1R* and *CAPS2* used for the screening process.



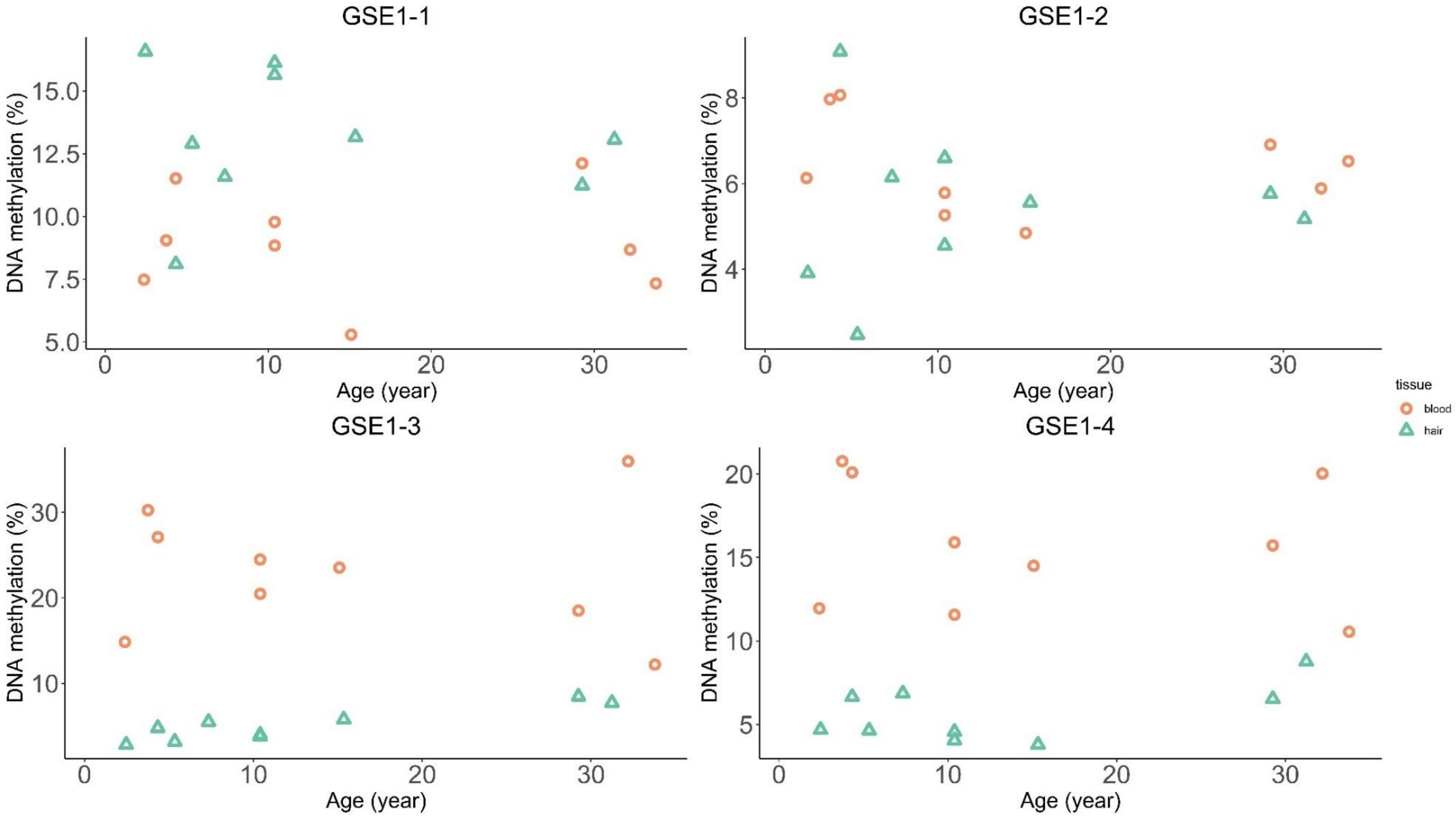
Supplementary Figure S_R2_1. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites. This figure shows the CpGs adjacent to *VGF*.



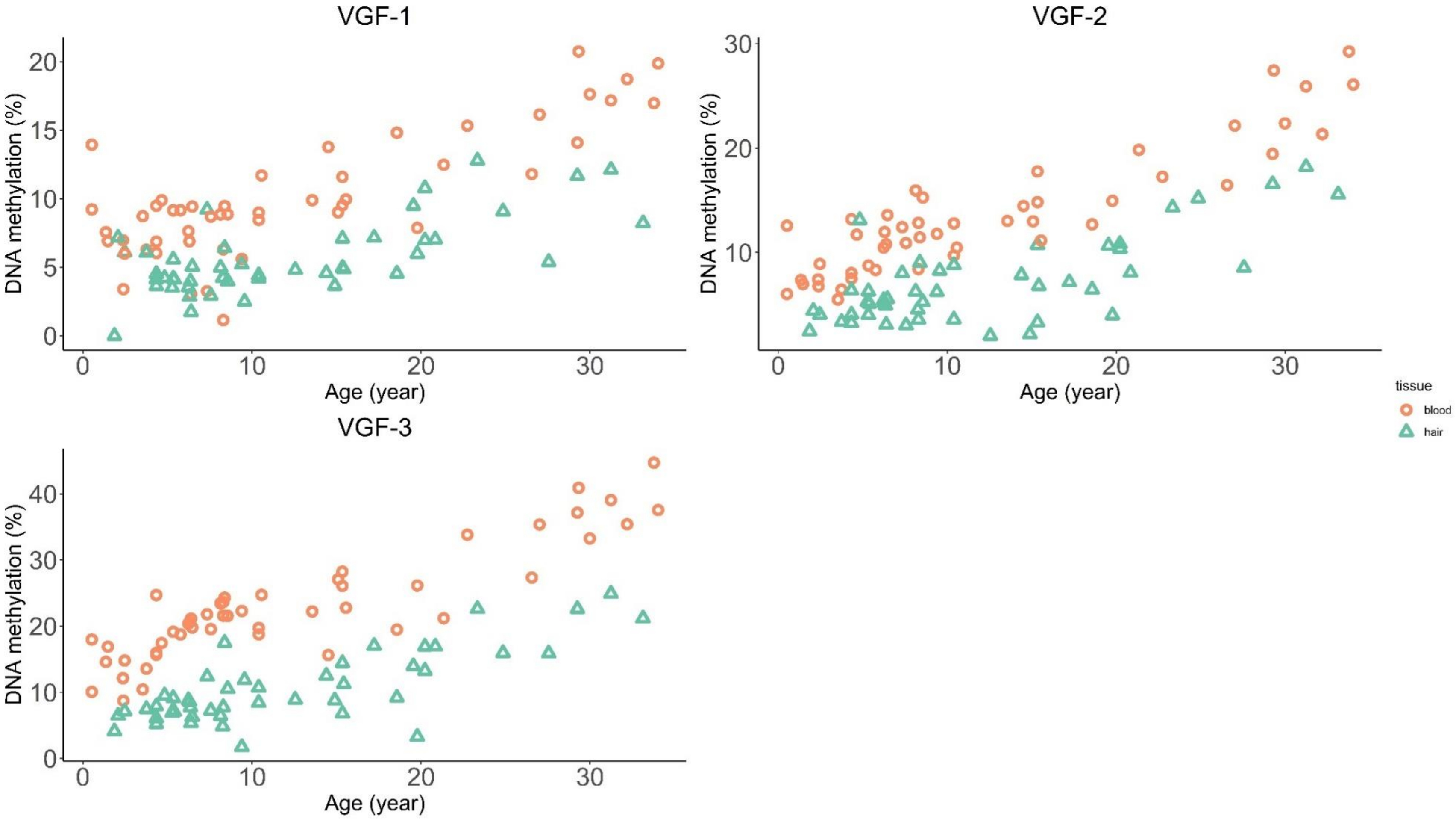
Supplementary Figure S_R2_2. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites. This figure shows the CpGs adjacent to *KCNK12*.



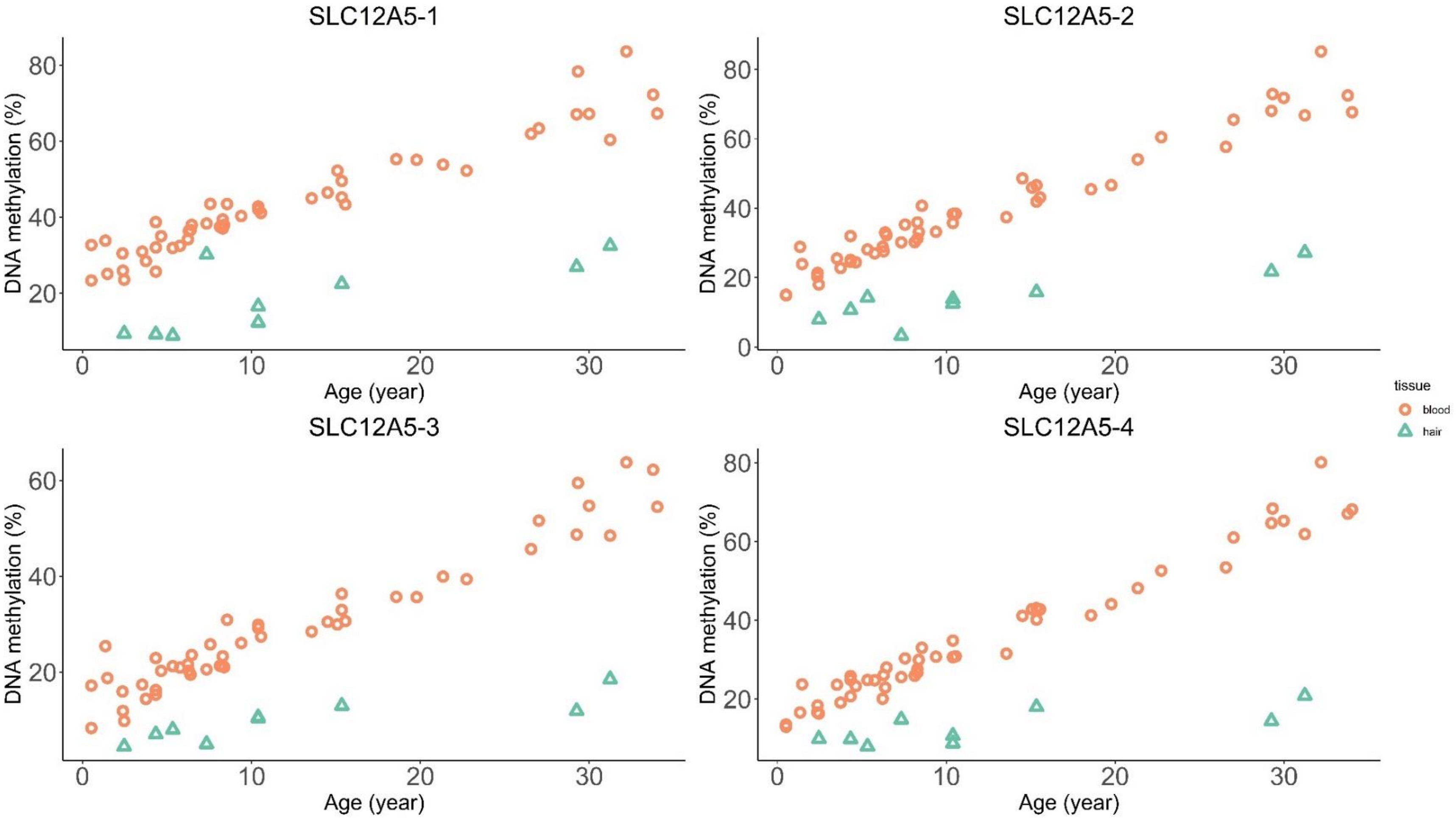
Supplementary Figure S_R2_3. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites. This figure shows the CpGs adjacent to *ELOVL2*.



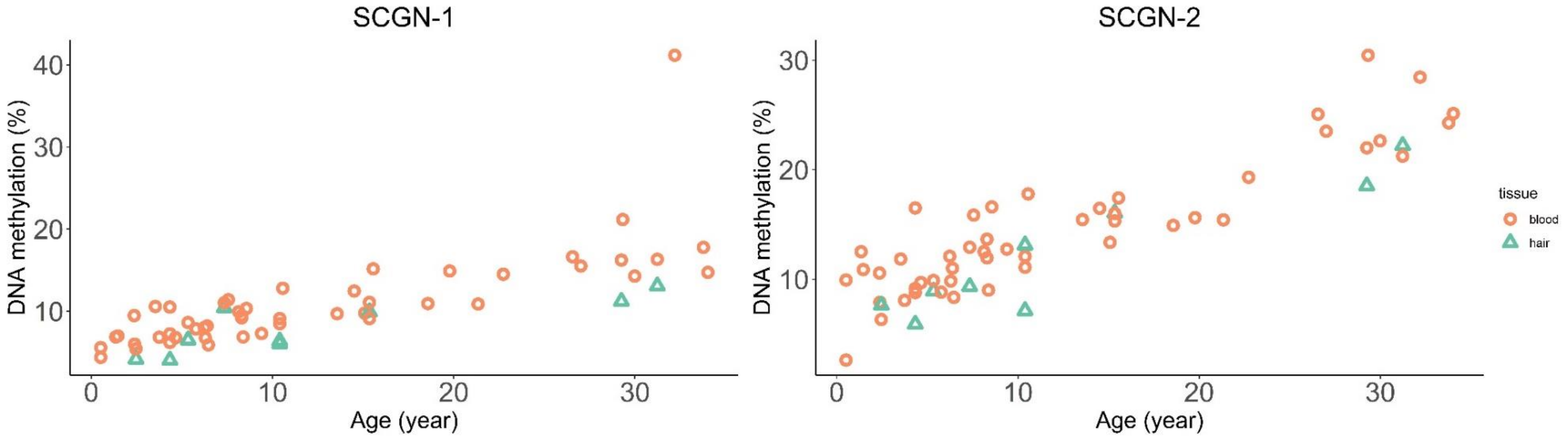
Supplementary Figure S_R3_1. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *GSE1*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



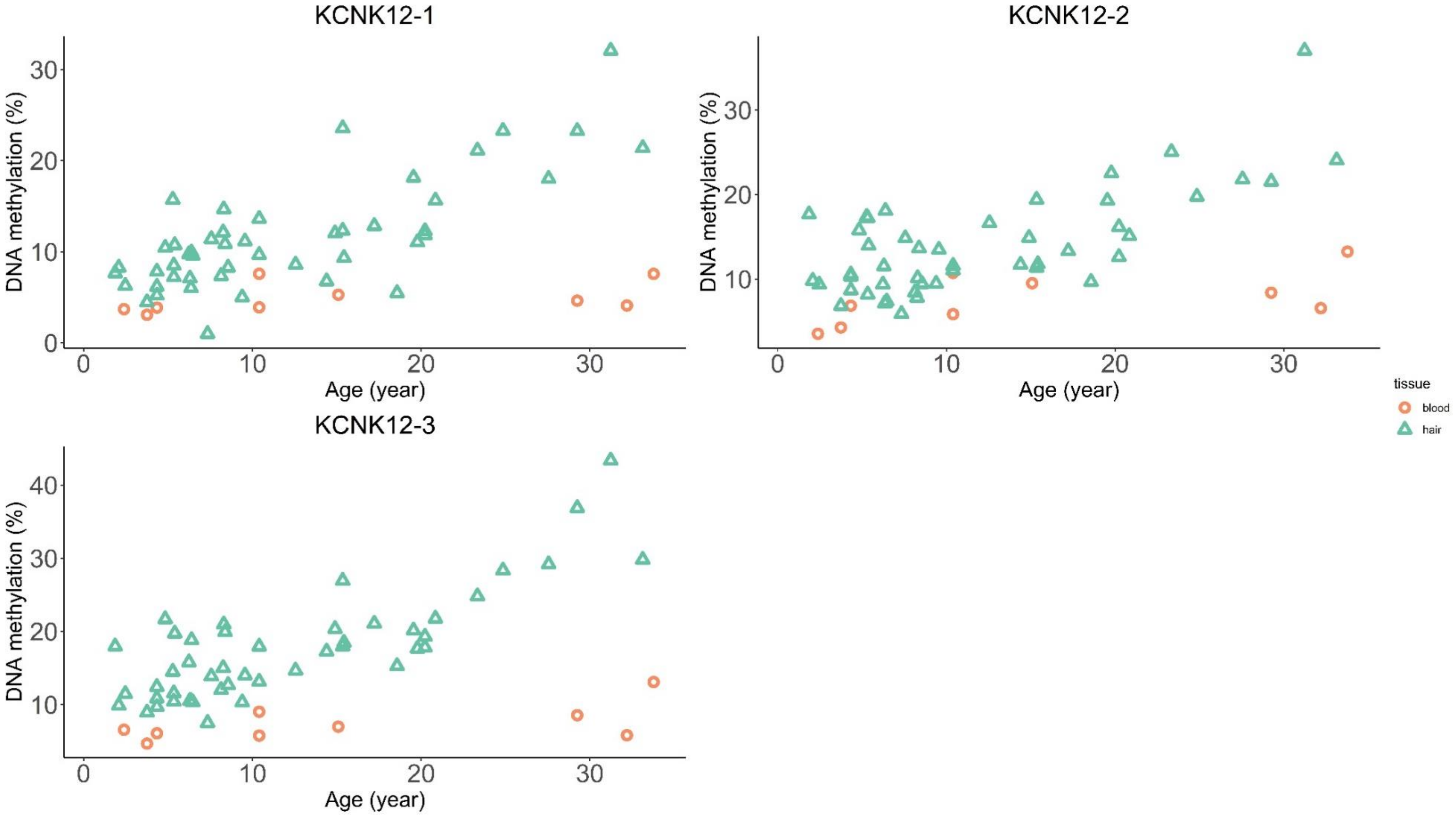
Supplementary Figure S_R3_2. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *VGF*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



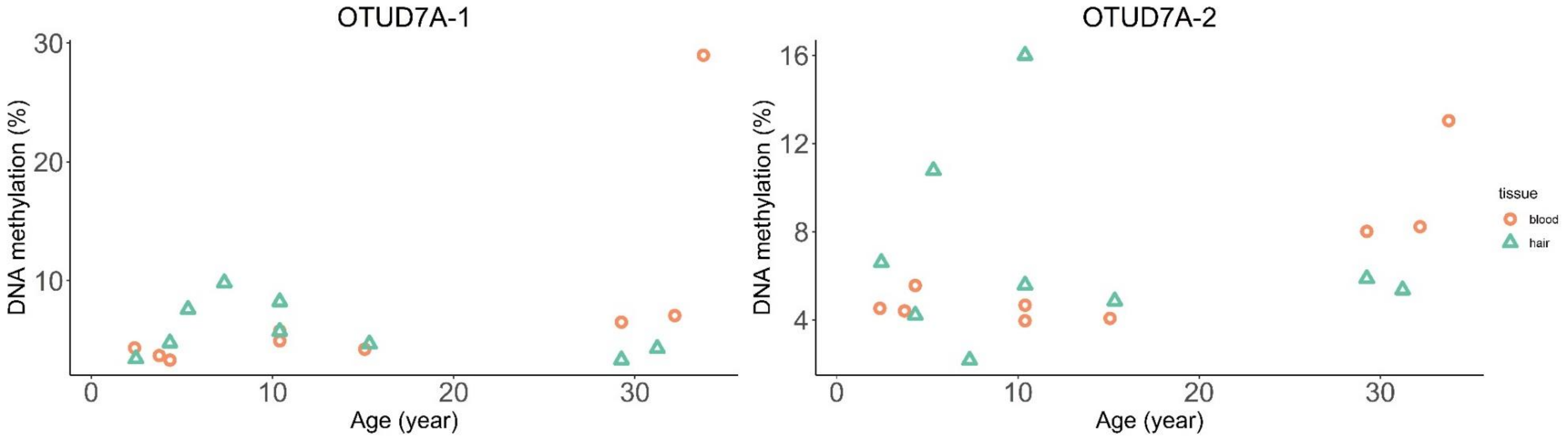
Supplementary Figure S_R3_3. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *SLC12A5*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



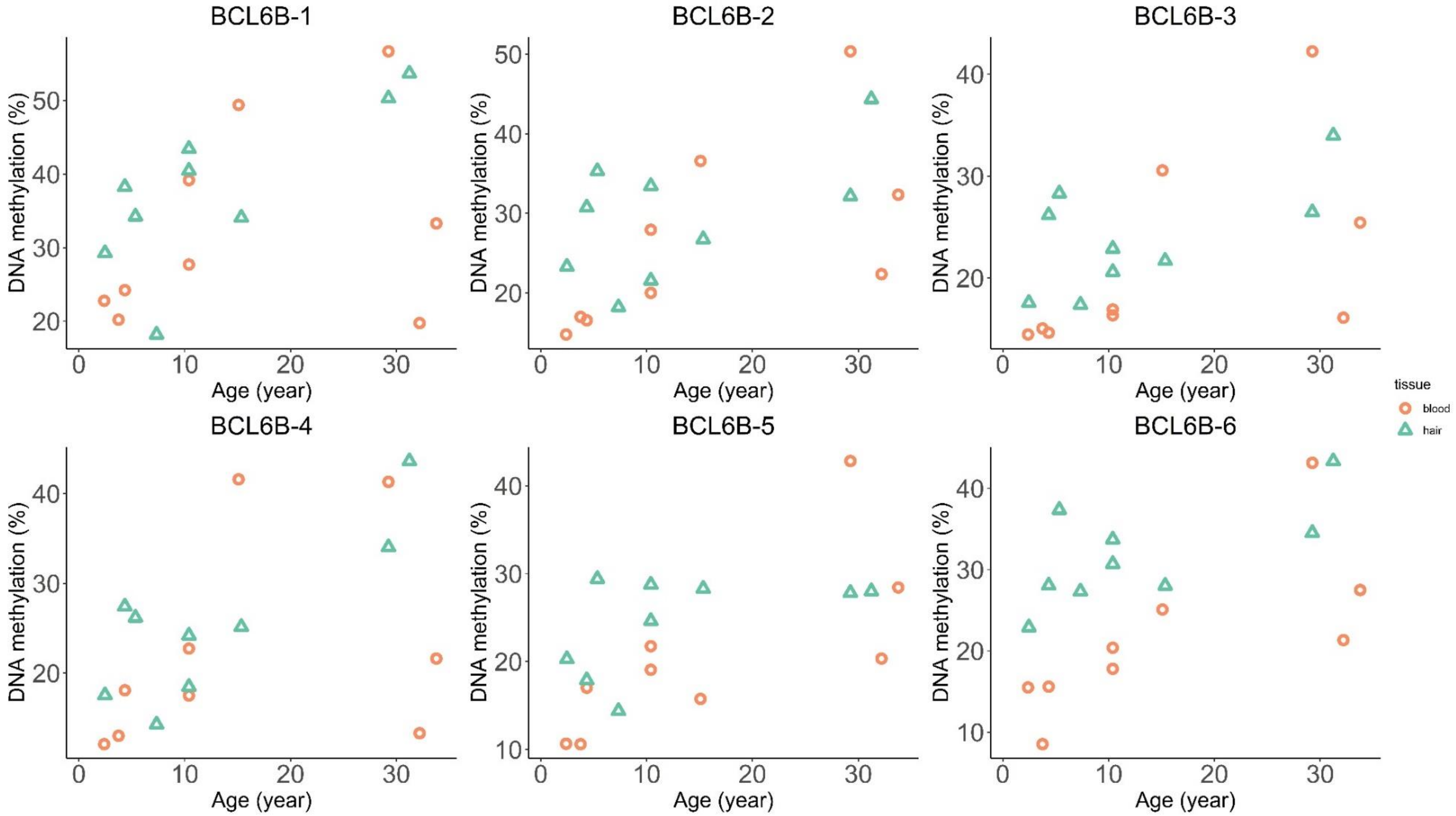
Supplementary Figure S_R3_4. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *SCGN*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



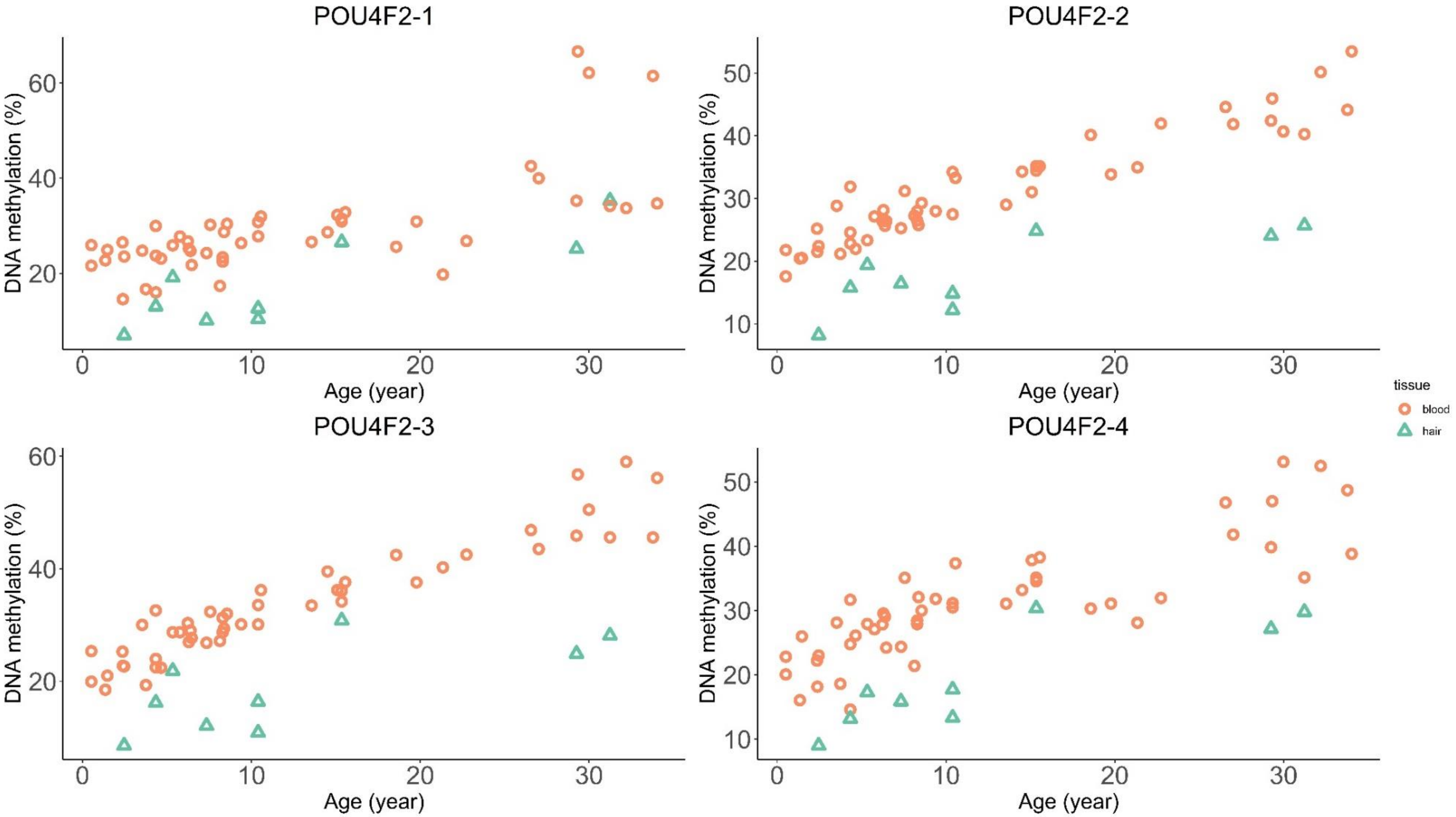
Supplementary Figure S_R3_5. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *KCNK12*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



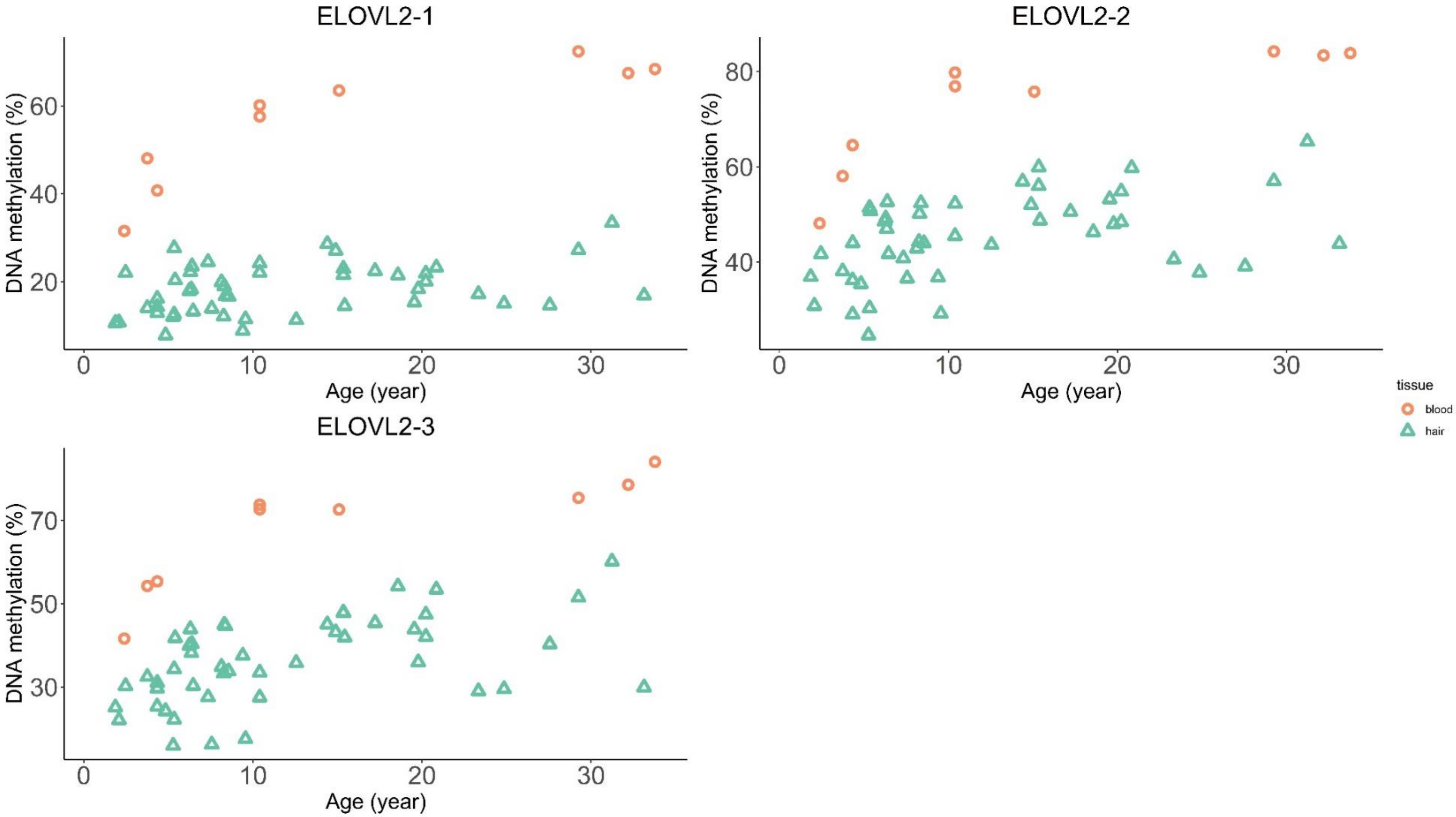
Supplementary Figure S_R3_6. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *OTUD7A*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



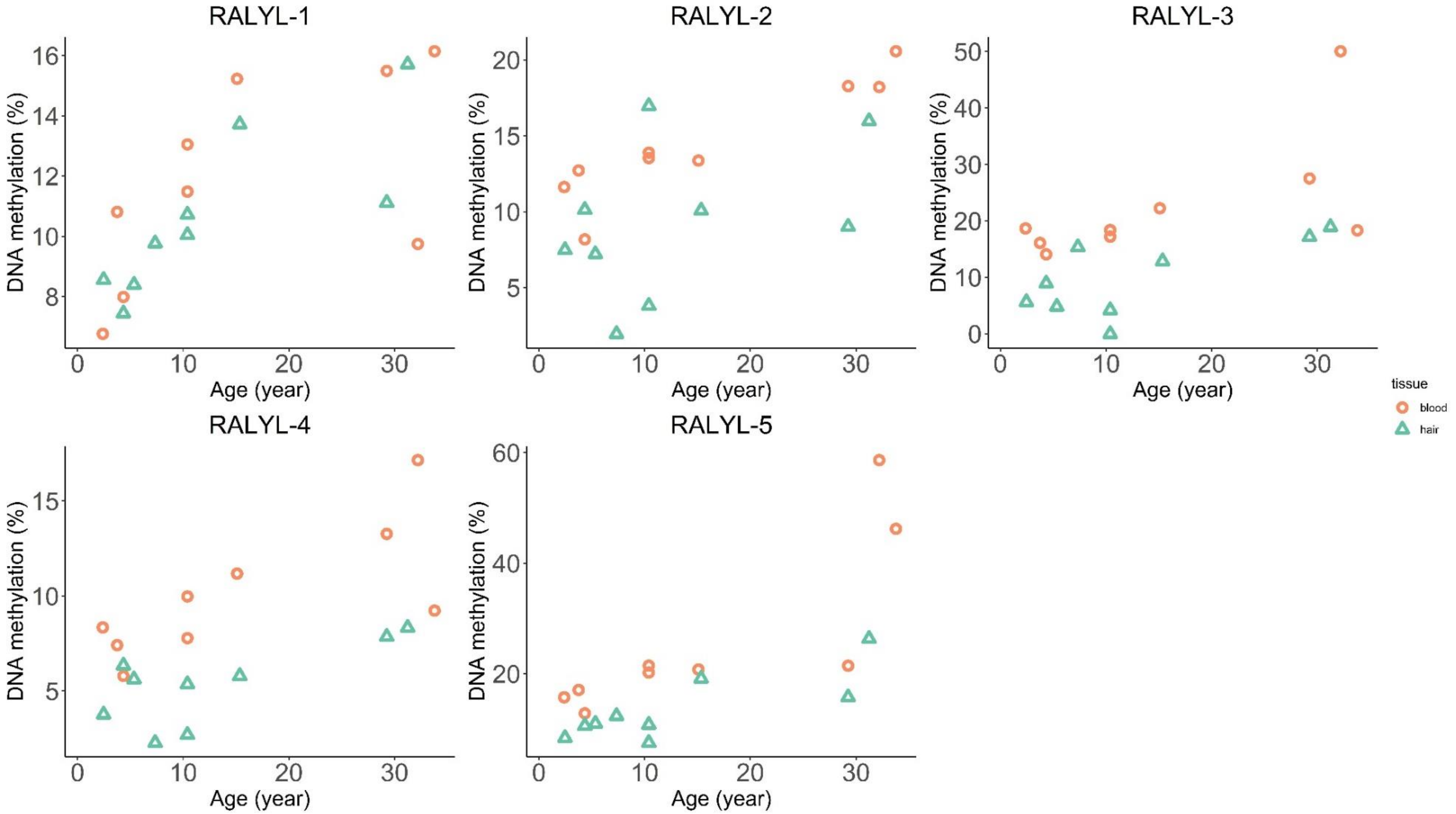
Supplementary Figure S_R3_7. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *BCL6B*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



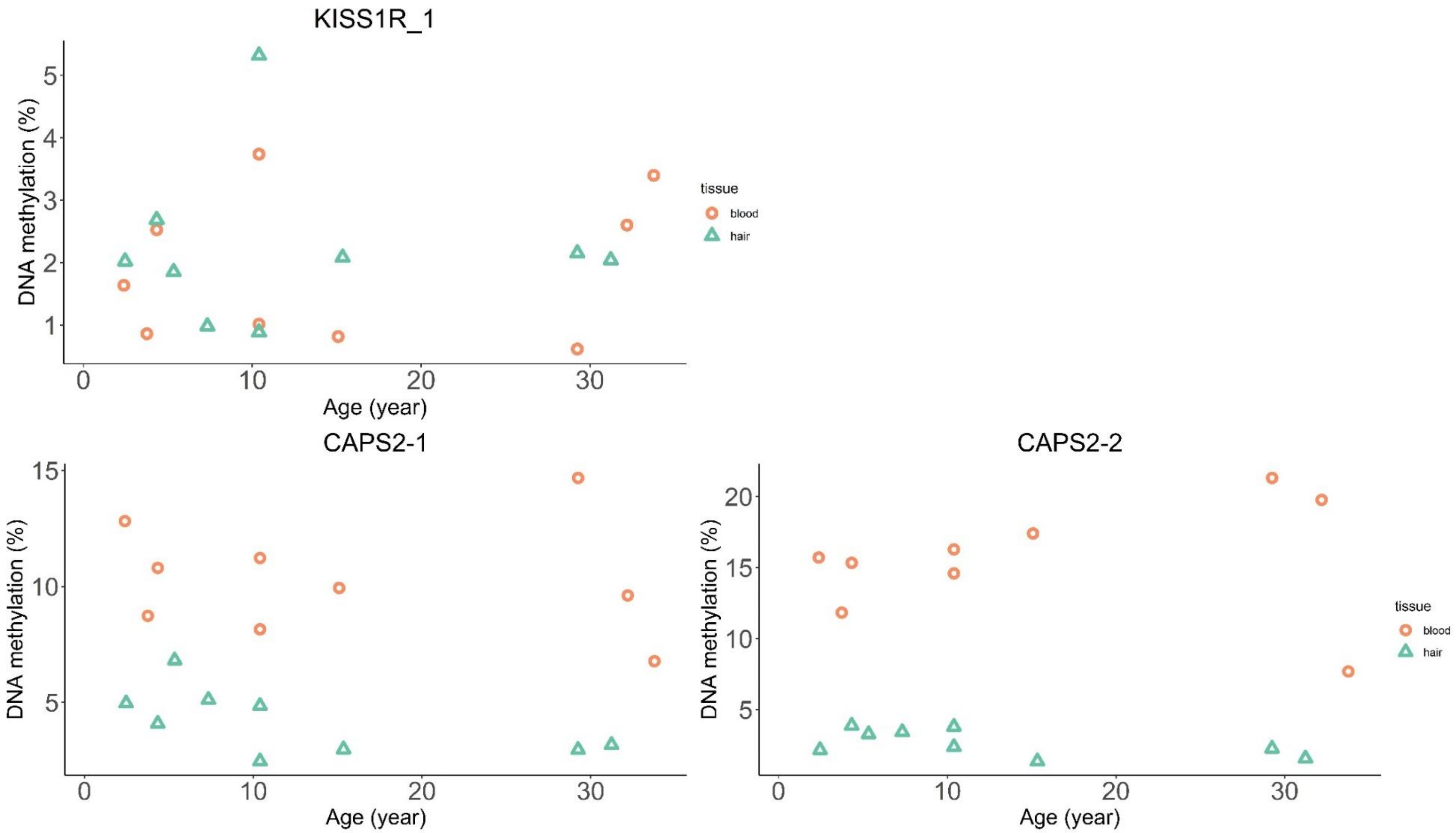
Supplementary Figure S_R3_8. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *POU4F2*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



Supplementary Figure S_R3_9. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *ELOVL2*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



Supplementary Figure S_R3_10. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *RALYL*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).



Supplementary Figure S_R3_11. Scatter plots of chronological age (year) versus DNA methylation level (%) at the CpG sites adjacent to *KISS1R* and *CAPS2*, combining the results of both this hair study and a previous blood study (Nakamura et al., 2023).