Supplemental Information for:

Epigenetic age estimation for Hawaiian false killer whales (*Pseudorca crassidens*) in the absence of 'known age' individuals

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Table. S1. Primer design and optimization details for the 12 loci for which we designed primers. The first eight loci were used in this study, while the remaining four were eliminated following optimization. Start and end positions and sequence length refer to the total sequence extracted from the O. orca genome for use in primer design, while amplicon length is length of the product amplified by the primers we designed. The final column lists the number of mutations between the O. orca sequence and the false killer whale consensus sequence generated from our data (for loci retained in study) or optimization notes (for loci omitted).

Locus	Reference	O. orca chromosome	Start position	End position	Sequence length	Forward Tm	Reverse Tm	Final annealing temp	Amplicon length	# CpG sites	Substitutions relative to O. orca
TET2	Grönniger et al 2010	NW_004438445.1	6543392	6543750	358	55.9	58.4	57	192	10	0
GRIA2	Chakrabarti et al 2001, Koch et al 2011	NW_004438421.1	18635500	18635950	450	61.9	57.9	58	243	15	2
DIRAS3	Horvath 2013	NW_004438546.1	4692750	4693250	500	55.6	55.4	54	278	28	0
FLJ0945	Horvath 2013	NW_004438525.1	7905350	7905850	500	55.2	55.4	54	285	20	2-bp deletion in FKW
KCNC4	Horvath 2013	NW_004438417.1	25124695	25125250	555	60.7	57.8	58	325	40	0
VGF	Horvath 2013	NW_004438442.1	15154650	15155220	570	55.9	56.5	55	348	35	0
HMG20B	Horvath 2013	NW_004438719.1	477160	477777	617	58	57.6	57	265	19	1
PRDM12	Horvath 2013	NW_004438443.1	2072900	2073400	500	64.3	65	64	353	40	1

Loci not chosen:

Locus	Reference	O. orca chromosome	Start position	End position	Sequence length	Forward Tm	Reverse Tm		Amplicon length	# CpG sites	Notes
KHDRBS2	Horvath 2013	NW_004438437.1	19776130	19776679	549	57.6	56.1		324	9	Inconsistent amplification
CDKN	Koch et al 2011	NW_004438482.1	622300	622800	500	59.7	58.8		340	41	Inconsistent amplification
KLF14	Horvath 2013	NW_004438464.1	12005300	12005869	569	56.3	55.4		279	30	Inconsistent amplification
SCGN	Horvath 2013	NW_004438470.1	858233	858813	580	55.7	57.1		343	20	

Table S2. Predictive accuracy of all models (n = 318). The MAE and mean residual are given for all samples, as well as for those with Age_{best} estimates between 0 and 9 years, 10 to 24 years, and 25 to 40 years. Corr is the Pearson's correlation coefficient between Age_{best} and predicted age. Models are sorted by overall MAE.
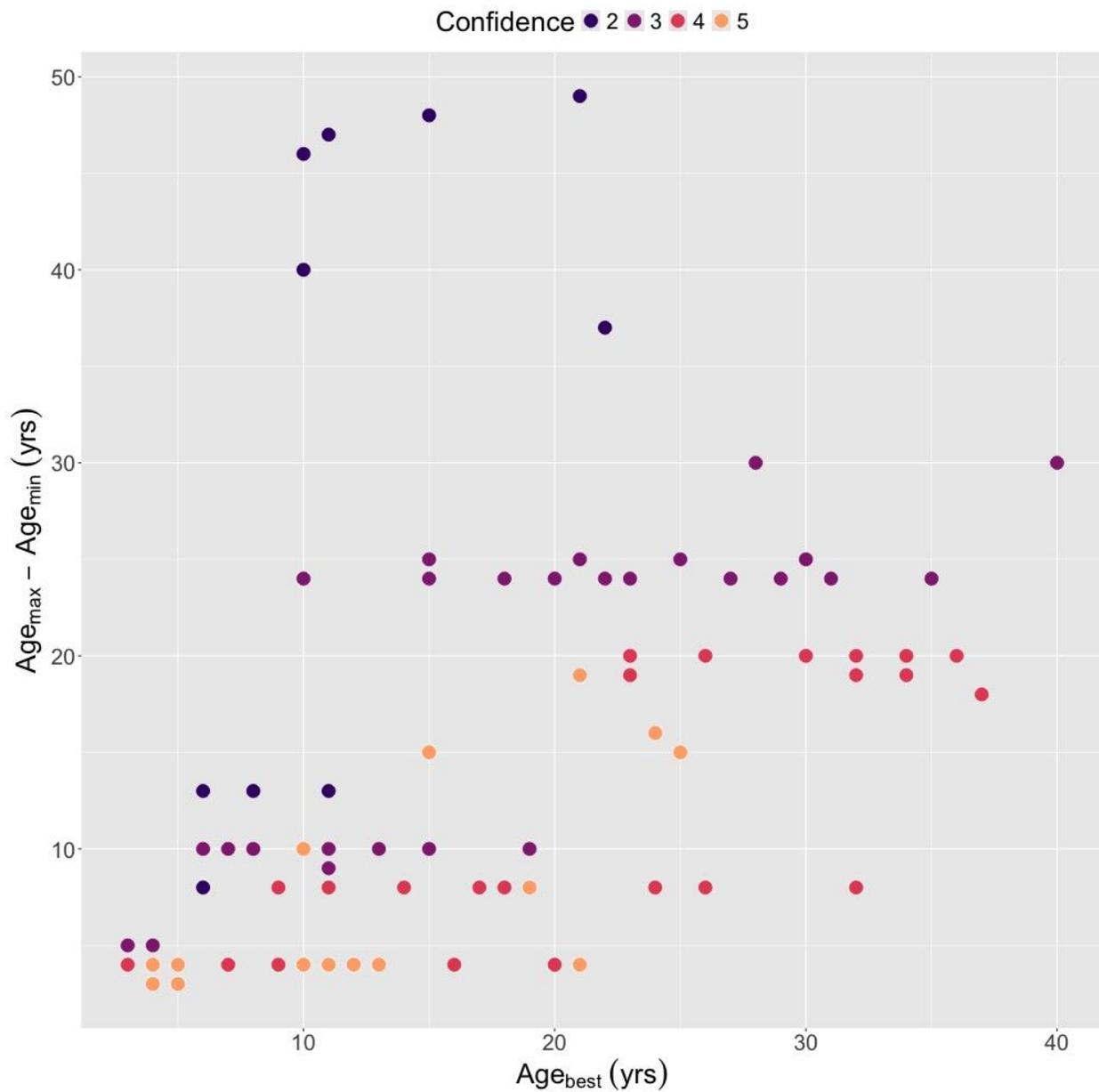
Table in separate Excel spreadsheet

Figure S1. The difference between Age_{max} and Age_{min} as a function of Age_{best}. Points are color-coded based on their confidence rating. Note that, for a given age class, the range of plausible ages for high-confidence samples (confidence ≥4) is consistently smaller than for low-confidence samples.
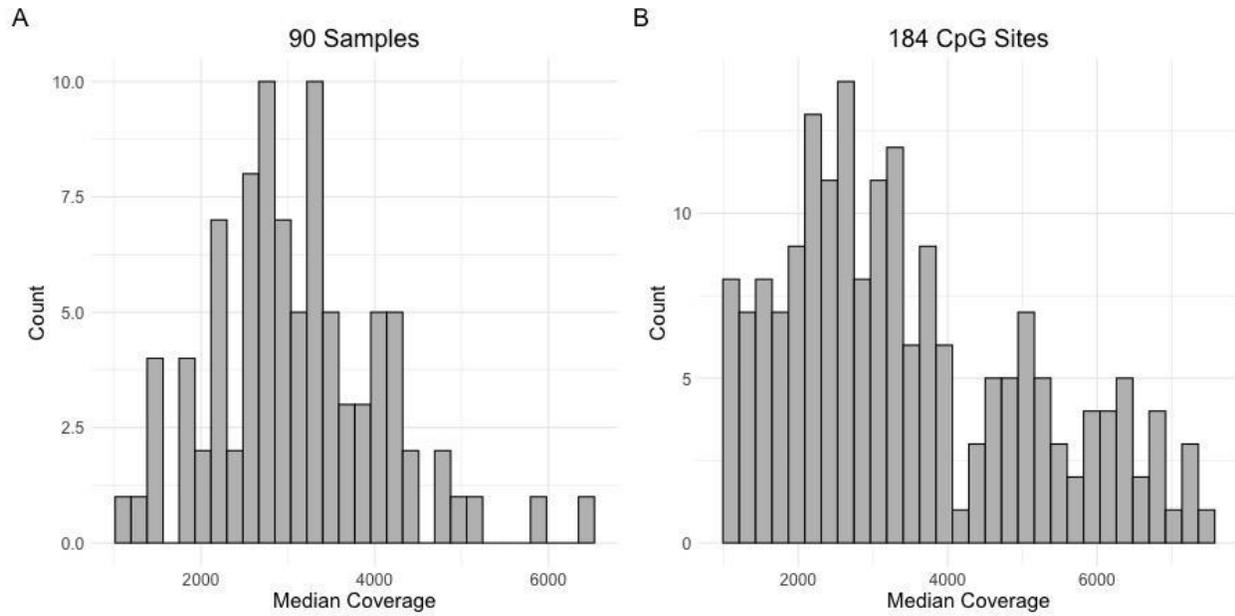
Figure S2. Median number of reads per sample (A) and per CpG site (B) in the final data set.
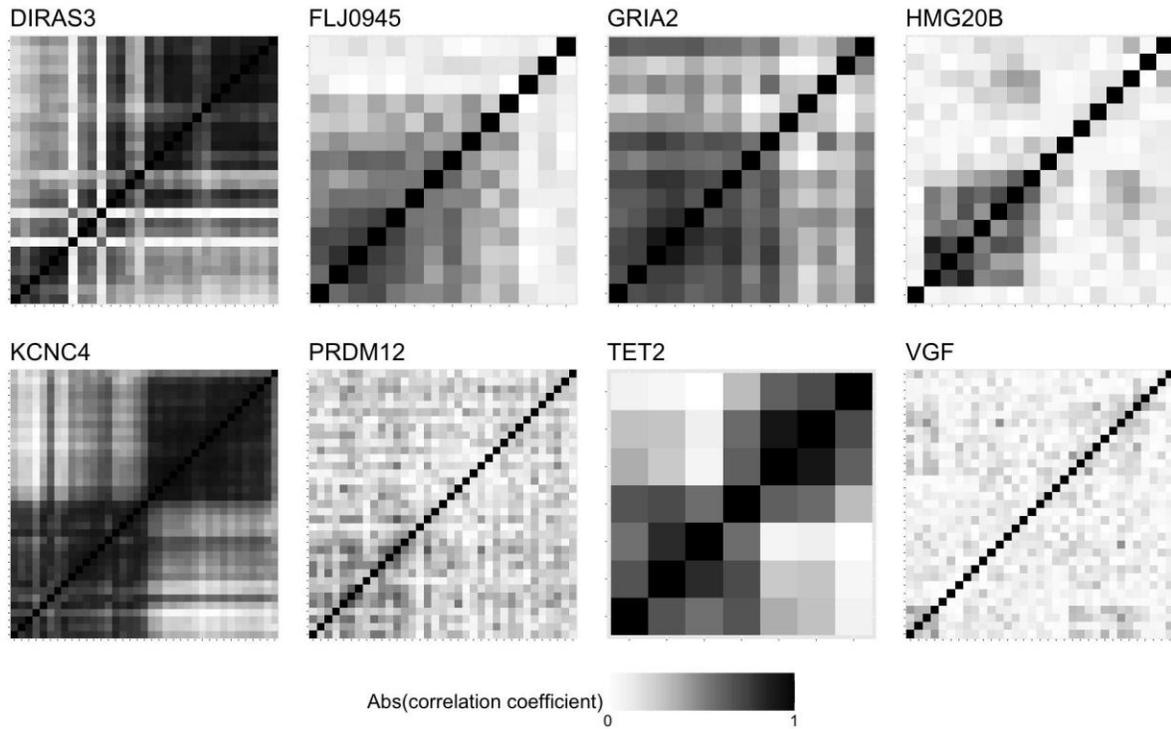

Figure S3. Heat maps showing absolute value of correlation coefficients of methylation among CpG sites within a locus.
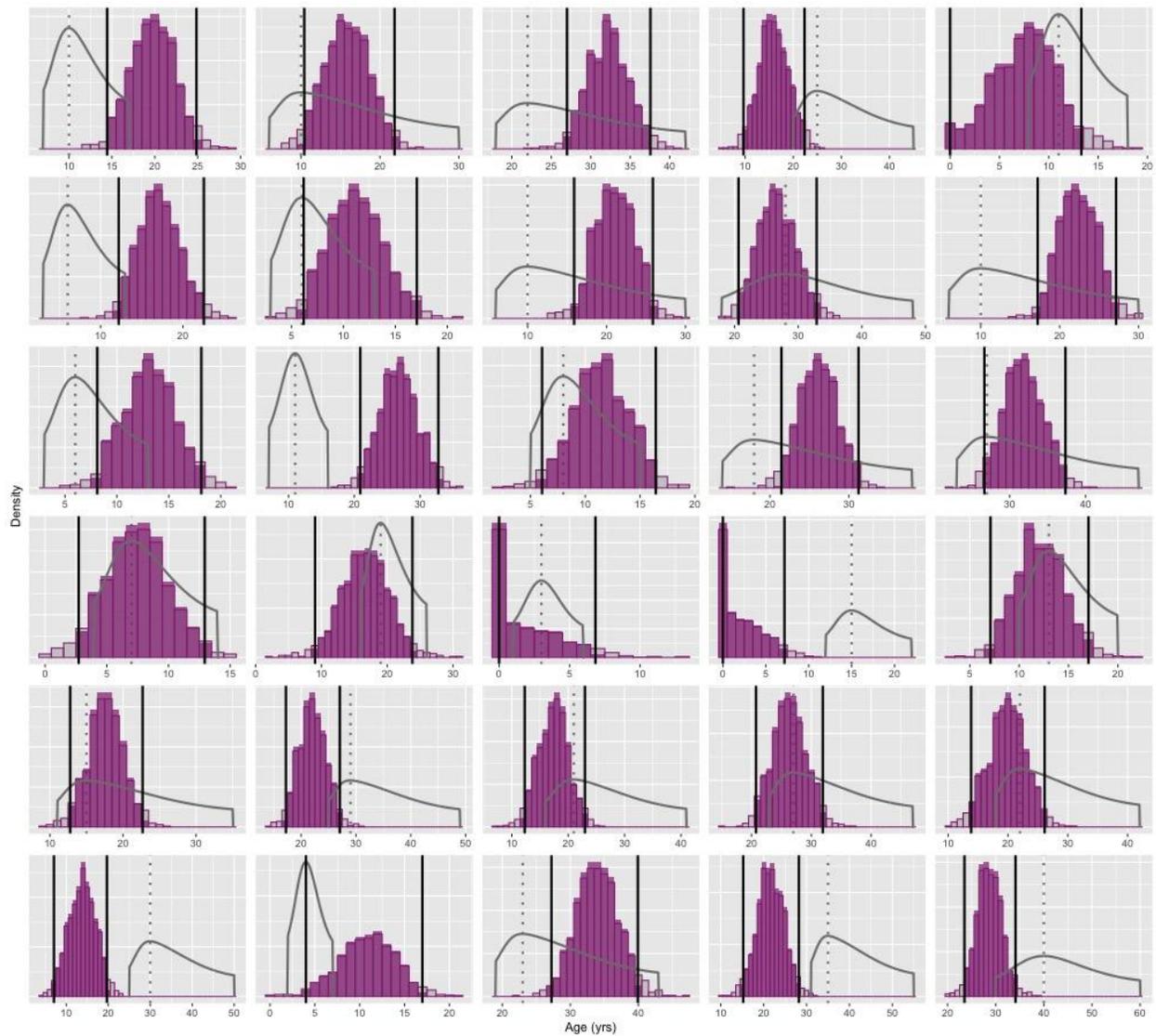
Figure S5. Predicted age probability distributions from the false killer whale age clock for samples with confidence ratings of 3. Each panel shows results for a different individual. The solid black lines show the lower and upper limits of the 95% high-density interval (HDI) of the distribution. Values outside of the HDI are shown in gray bars. The dotted gray line is at Age_{best}, while the solid gray lines show the Skew-Normal age probability distribution from Kratofil et al. (2026).

Primer Sequences:
>TET2_F1
TGGGTTGATGTTATTATTTAATTATAAAGTTGG
>TET2_F2
TGGGTCGATGTTATTATTTAATTATAAAGTTGG
>GRIA2_F1
GAGTGTATGGGAGGGTGTTGAGTGTG
>DIRAS3_F1
TTATTTGTATTTGTTTGTTTTAGAGGAGG
>DIRAS3_F2
TTATTCGTATTTGTTTGTTTTAGAGGAGG
>FLJ0945_F1
TTGTTTAATTTAAATATGGAAATGTTTAGGTTAGG
>KCNC4_F1
GGTGTTGTTGGTAGGTTATTTAGTAGTAGGG
>KCNC4_F2
GGTGTTGTCGGTAGGTTATTTAGTAGTAGGG
>VGF_F1
TTAGTGGGTTGGGTTTAGTTGG
>HMG20B_F1
TTTTTTAGAGTTTTTTTTTGAGGGAGGATTTAGG
>PRDM12_F1
AGGGGTTGTAGGGAGGAGGGG
>PRDM12_F2
AGGGGTCGTAGGGAGGAGGGG
>TET2_R1
TTTTAAAAACTCAACAAATTAAAAATTTAACCCAAACC
>TET2_R2
TTTTAAAAACTCGACAAATTAAAAATTTAACCCAAACC
>GRIA2_R1
TTTCCAAAAAAAATAAAACATCCACAAAATAC
>DIRAS3_R1
TTTAAATTAACAAATCAACTCAAAAAAAAACTTC
>DIRAS3_R2
TTTAAATTAACGAATCAACTCAAAAAAAAACTTC
>FLJ0945_R1
ATACCAAAAAAAAATAAAACCCAAATACCC
>KCNC4_R1
CCTCCATCCAAAACATATCTAAAAAAAAAAATAAC
>KCNC4_R2
CCTCCGTCCAAAACATATCTAAAAAAAAAAATAAC

>VGF_R1
CATTCTACCATTCATTCATTCATCCTTTTC
>HMG20B_R1
AAACAAAACCTCAACATCTAAAAAAAAATCTAAAC
>HMG20B_R2
AAACGAAACCTCAACATCTAAAAAAAAATCTAAAC
>PRDM12_R1
TCCTCATCCTCCCCCATCACAACC
>PRDM12_R2
TCCTCGTCCTCCCCCATCACAACC

Killer whale locus sequences:
>TET2-Oorc-wPrimer
TGGGTCGATGTCATTACTTAATTATAAAGCTGGACATTTTTTCAAAATAGAGGCTCTTATGGGGGTAAATTTAAGCATTT
GAAAGTGCAGATTTATTTCTTACAACCGAATCCAGCGCGAAGTGGAGAGTCACTGGTGCGAAAGCCCTCTCGGATCTG
CGTGAATCCTTCTTCCCAGCCCACGTGGCCAAAGTAAACAGAAGGTGGGCCGGGGCGGGGAGAAGCGGGCCTGGGTC
AAATTCCTAATTTGTCGAGTCTTTAAAA
>GRIA2-Oorc-wPrimer
GAGTGCATGGGAGGGTGCTGAGTGTGCCGAGGCGCTGGGACCACAGCGGCAGCCTGCTGGAAGCTGCATCCAGCCA
GTCTCCGGACTTCGCGAGCGGGGACCGGGCGCAGGAAGGCAGCCACCCGCAGGACCTTGGAAATAGGGATTCCTCTG
CCACCACGTCGGGTTTCTAGCAGCTCGGTGCTACATTGCTGTCTCAAAATGCAGAGGATCTAATTTGCCGAGGAGAACG
GCCAAAGAAGGAAGAGGAGGAAAAGGGGGGAAAAAAAGGGTATTTTGTGGATGCTCTACTTTTCTTGGAAA
>DIRAS3-Oorc-wPrimer
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TTTGAGTTGATTCGTTAATTTAAA
>FLJ0945-Oorc-wPrimer
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GTGTTGTCTGTGCCTTTATGGGACTCAGGAGACAGGAGGCGGGGGAGGCCCCGCAGGCCGGCCGGGGGCTGGGGGG
CGGCCGCCGGGCATCTGGGCCTCACCCTCCCCTGGCAT
>KCNC4-Oorc-wPrimer
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GG

>VGF-Oorc-wPrimer
CCAGTGGGCTGGGCTCAGCTGGATCCGCGCGGCTCCGGGAGACTCGCTCGCTCAGGCTTCAGCACGCTGGACAGCGCC
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>HMG20B-Oorc-wPrimer
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>PRDM12-Oorc-wPrimer
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GGCCGGACGGCCGGGCCGAGGCGGGTATGTGGTGGGCGAGTGGGGAGCACAGGGGCGAGAGAAGAGGGCTGTGAT
GGGGGAGGACGAGGA

False killer whale bisulfite-converted consensus sequences:
>TET2-Pcra-consensus
GTTGATGTTATTATTTAATTATAAAGTTGGATATTTTTTTAAAATAGAGGTTTTTATGGGGGTAAATTTAAGTATTTGAAA
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>GRIA2-Pcra-consensus
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>DIRA-Pcra-consensus
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>FLJ0945-Pcra-consensus
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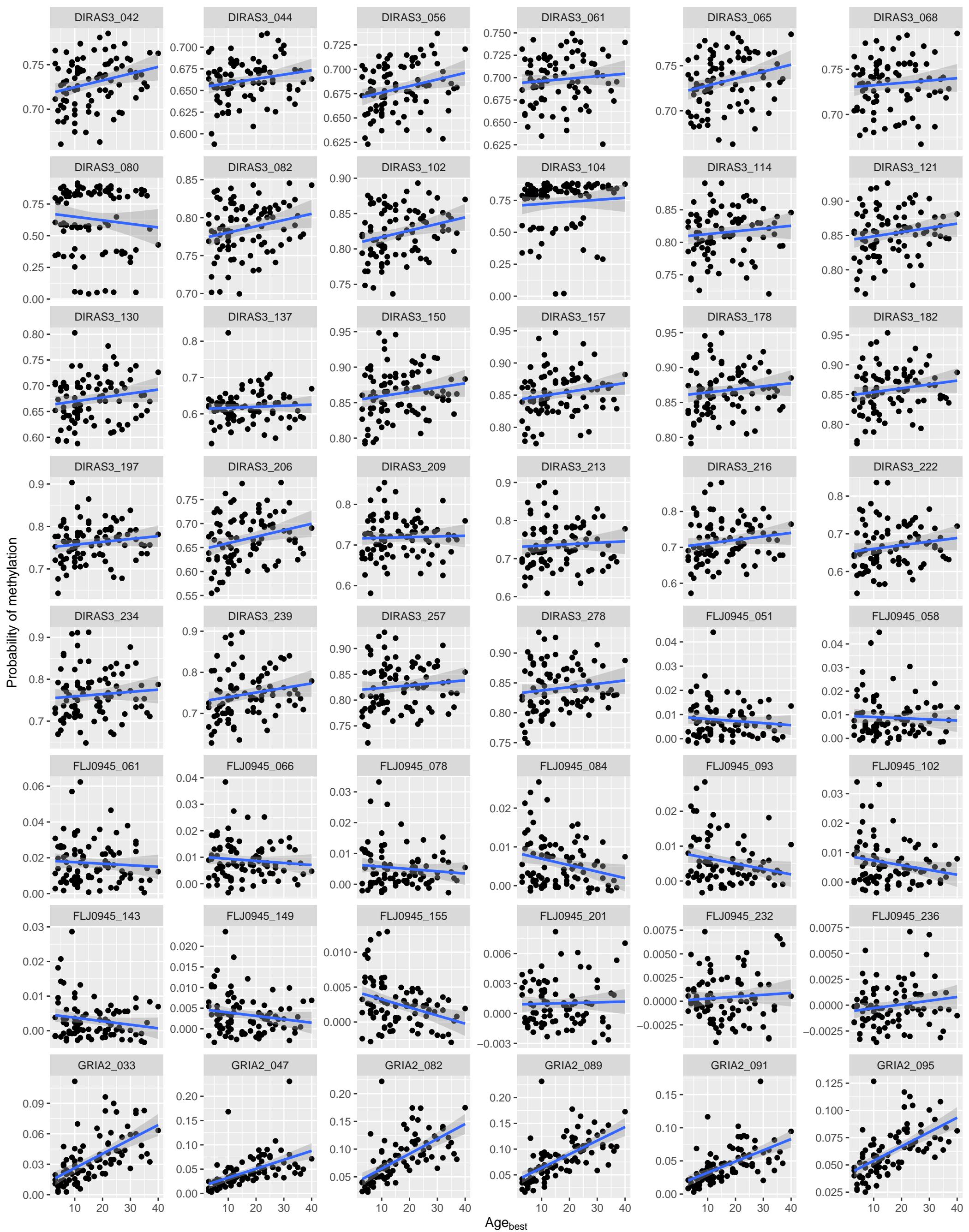GAGGTGGGTGGGGTAGGGGGGTTATGAATGAATGAAGGAGGGATGAGGAGAAAAGGATGAATGAATGAATGGTAG
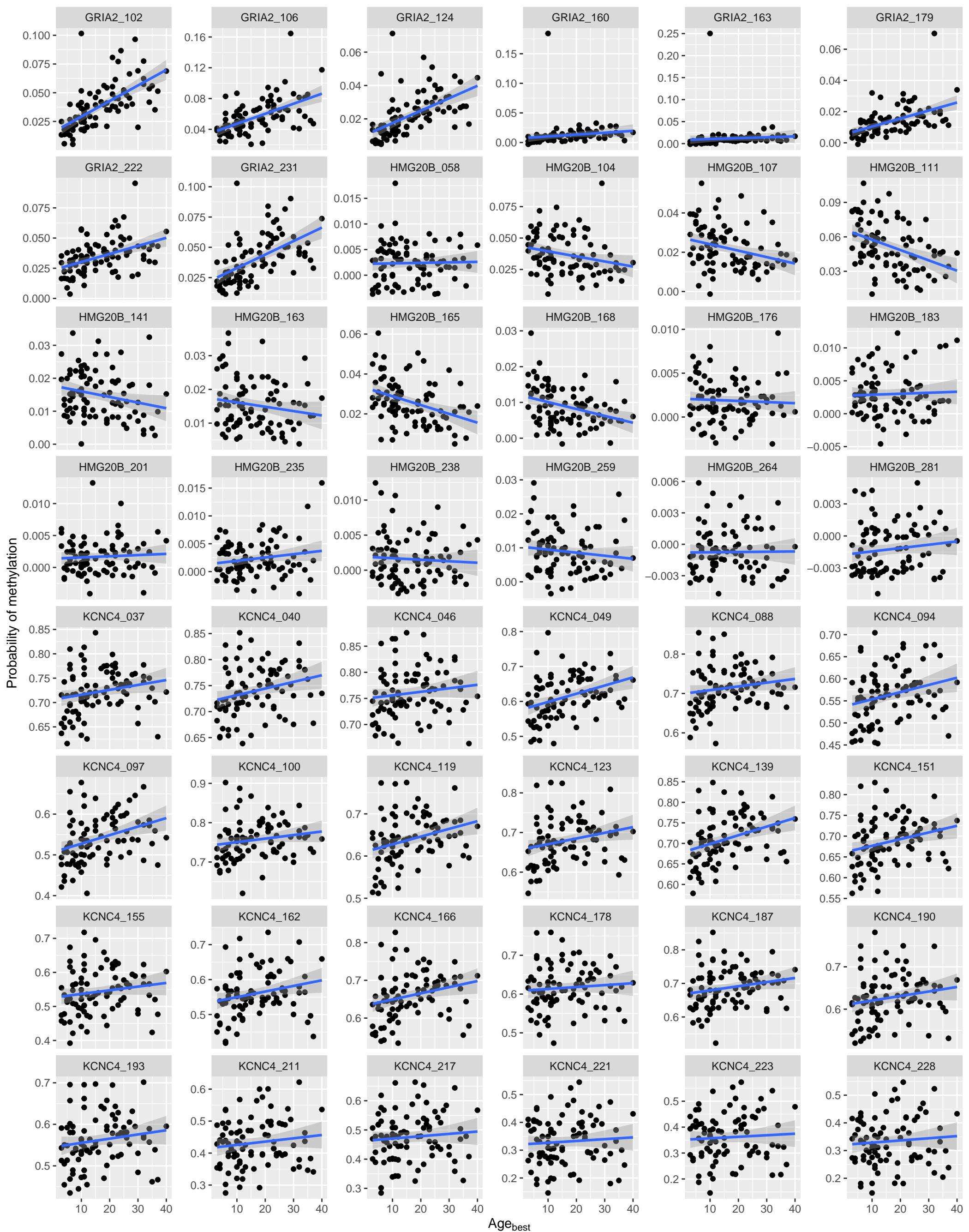>HMG20B-Pcra-consensus
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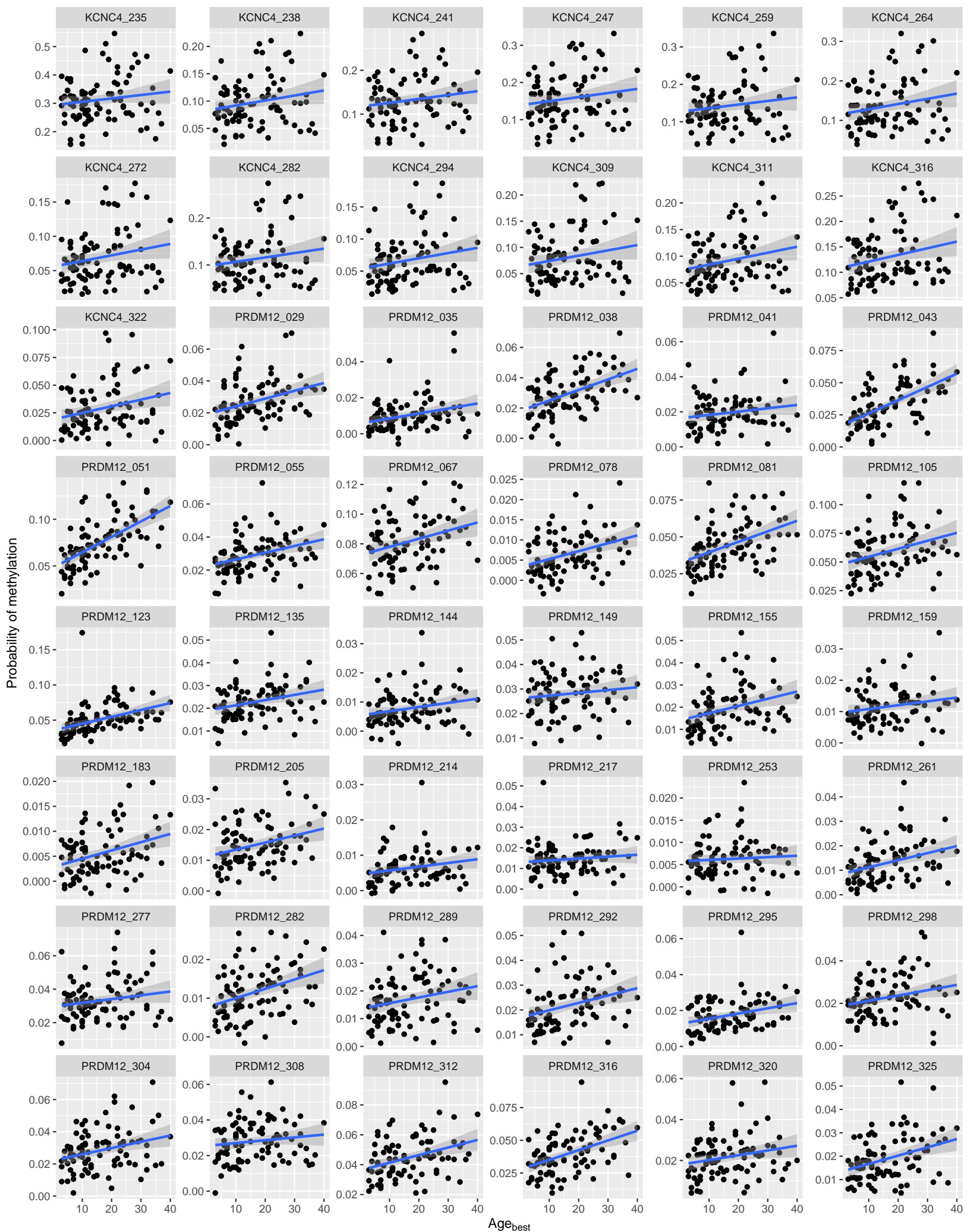TTAGTTTTGAAGTTTTGTTTTGAATTTAATTTGAATTTGTTGTGGTTTTGTTTATAGTTTAGATTTTTTTTTAGATGTTGAG
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AGGAAGTTGTGTAGGATGTTGGAGGTGATGATTTTTGTTAGTGTTAGTTTTGGTGTTTTTAGTTTTGTTTTGAGTATTAG
GGTTTTAGTTGGGAGTATGGAGTTTATTATGGGTGGTTTGGGGTTTGTTGGTGTTGGGGATGGATGGTTGGATGGTTG
GGTTGAGGTGGGTATGTGGTGGGTGGGTGGGGAGTATAGGGGTGAGAGAAGAGGGTTGTGATGGGGGAGGAT

Figure S4. Plots of Age_{best} versus the probability of methylation ($\widehat{P_m}$) for all 184 CpG sites included in the final data set. Each plot is labeled with [locus name]_[position]. The line of best fit, as calculated using the R function *geom_smooth(method = 'lm'), is shown in blue.*

Probability of methylation

Age_best