Figure S3

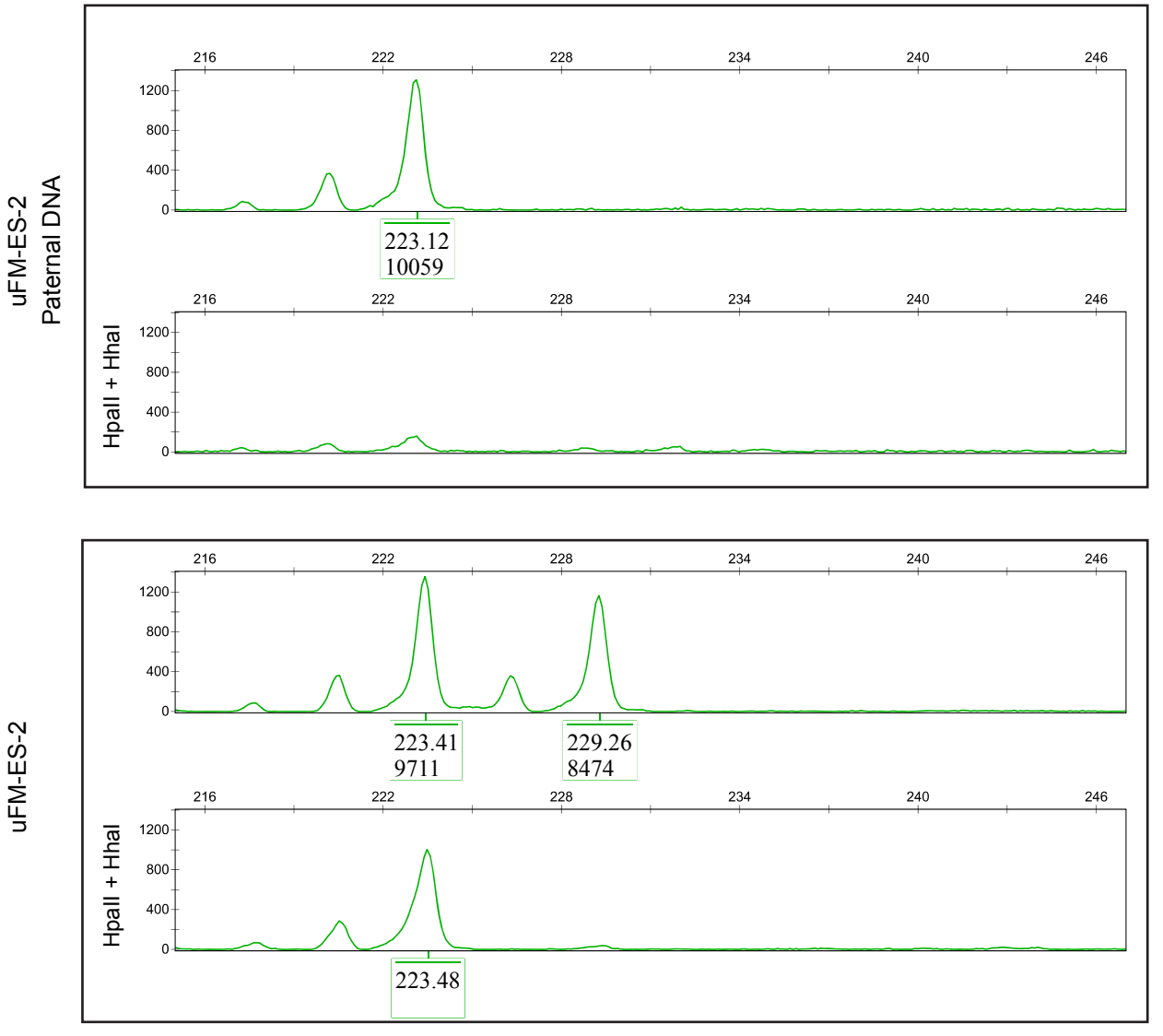

Figure S3. Skewed X-inactivation test in uFM-ES-2 hESCs. Skewed X-inactivation (Xi) was confirmed using an established methylation-sensitive quantitative assay, as described in Avitzour et al., 2014. The test is based on digestion with a methylation-sensitive restriction enzyme followed by PCR amplification of a short fragment within the X-linked ANDROGEN RECEPTOR gene. The fragment includes a highly polymorphic region (CAG repeat) and several sites that are liable to differential methylation by X-inactivation. Whereas the highly polymorphic CAG repeat is used to distinguish maternal from paternal inherited X-chromosomes, the methylation-sensitive sites allow selective amplification of alleles that are exclusively present on the inactive X chromosome, regardless of parental origin. Accordingly, by comparing the relative amount and fragment size of digested and undigested PCR products using capillary electrophoresis, a skewed bias from the expected 50:50 ratio between the inactive maternal or paternal X chromosomes can be readily identified. Paternal DNA was used to confirm full digestion and to distinguish the maternal (carrying the *FMR1* CGG expansion) from the paternal inherited X chromosome. According to this assay, complete X-inactivation of the maternal X chromosome is evident in uFM-ES-2 hESCs by the detection of a single PCR product of 223bp following digestion with a methylation-sensitive enzyme.