

Figure S9.

Characterisation of the H3R26me2 and H3R2me2 antibodies.

(a)Total cell extracts of Mouse Embryonic Fibroblasts from CARM1+/+ or CARM1-/homozygous mice or 500 ng of purified histone H3 (H3) were processed for Western Blot and probed with the indicated antibodies. Molecular weight markers (kDa) are indicated. (b-c) Mouse Embryonic Fibroblasts derived from homozygous CARM1-/- or CARM1+/+ mice (Yadav, N et al. 2003) were processed for immunostaining with the H3R26me2 (b) or the H3R2me2 (c) antibodies. Distribution of both H3R26me2 and H3R2me is euchromatic. To confirm the specificity of these antibodies, we performed peptide competition by incubating the antibodies in the absence or presence of specific peptides as indicated. Note that the signal detected by the two antibodies is nuclear and that the H3R26me2 staining is lost when challenged with an H3 peptide dimethylated in R26 but unchanged when challenged with a peptide dimethylated in R2. Similarly, incubation of the H3R2me2 antibody with the dimethylated R2 peptide, but not with the dimethylated R26 peptide, abolishes staining. Accordingly, histone H3 methylation of R2 and R26 are reduced in the CARM1-/- cells. All panels are shown at the same magnification. Scale bar is 30 µm.