**Supplementary Figure 5** Expression of rostral-caudal markers in differentiating ES cells. (a) Illustration of distributions of *Six3* and *Irx3* in early CNS tissues (neural plate) with respect to the relative position of the zona limitans (Zli). te, telencephalon; di, diencephalon; ms, mesencephalon; mt/my, metencephalon/myelencephalon; pcp, prechordal plate; nc, notochord; rp, roof plate; ap, alar plate; bp, basal plate; fp, floor plate. (b and c) Expression of *Six3* (marker of the CNS region rostral to the zona limitans) and *Irx3* (the CNS region caudal to the zona limitans) in SFEB-induced ES cell aggregates shown by *in situ* hybridization. (d) Percentages of *Six3*\(^+\) and *Irx3*\(^+\) aggregates/colonies in the SFEB and SDIA culture. Cells were analyzed on day 5. (e) RT-PCR analysis of rostral-caudal marker genes. SFEB cells were treated with 0.2 M (lane 2) or 2 M (lanes 3,5) RA during days 3–10 (lane 5) or 6–10 (lanes 2,3). (f,g) Immunostaining using Bf1 antibody with (g) or without (f) pre-immunoabsorption with 1 M antigen peptide.