Gontan et al., http://www.jcb.org/cgi/content/full/jcb.200810106/DC1

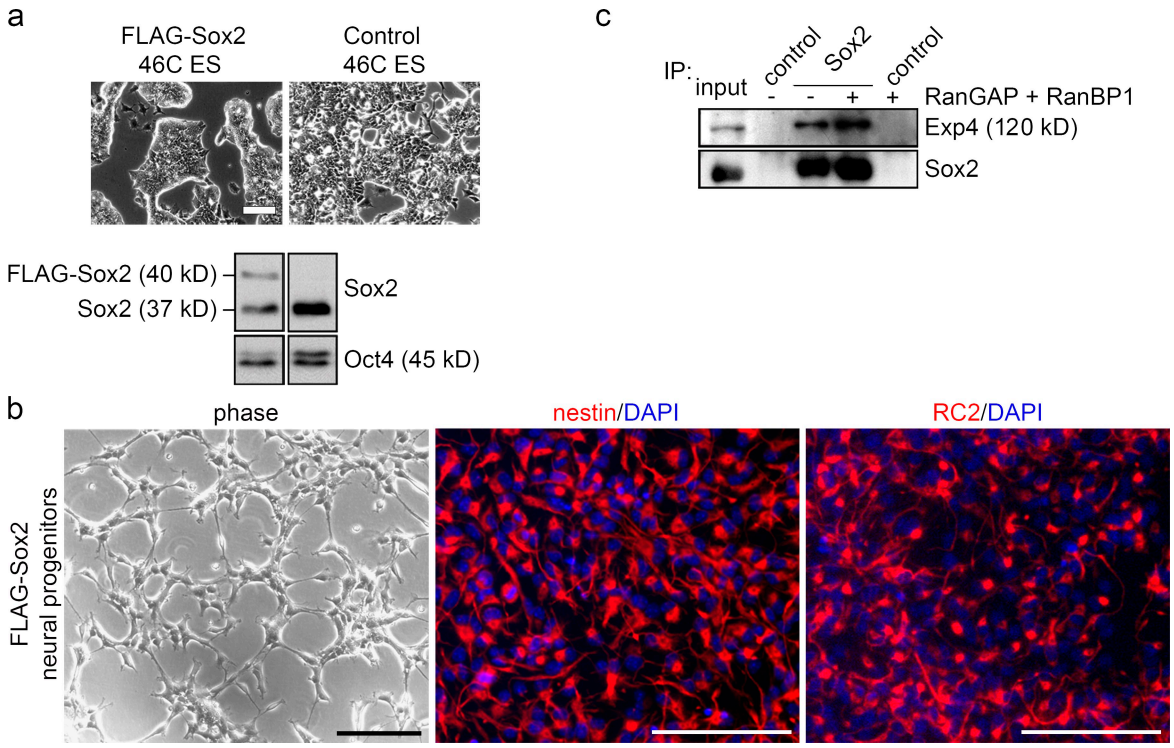

Figure S1. **Characterization of Flag-Sox2 ES cells and neural progenitor cells.** (a) Flag-Sox2 cells display normal ES cell growth behavior and express the ES cell marker Oct4. (top) Phase-contrast images of control 46C ES cells and 46C ES cells stably transfected with Flag-Sox2. (bottom) Anti-Sox2 and Oct4 Western blots using extracts from Flag-Sox2 ES cells (left) and control 46C ES cells (right). (b) Characterization of ES cell–derived Flag-Sox2 neural progenitors. The left panel shows a phase-contrast image of Flag-Sox2 neural progenitors. Immunostaining shows that the cells expressed the neural progenitor markers nestin (middle) and RC2 (right). (c) The Sox2–Exp4 complex is insensitive to RanGTPase activation by RanGTPase-activating protein (RanGAP) and RanBP1. Coimmunoprecipitation of Exp4 with endogenous Sox2 as in Fig. 1 b but with the indicated additions. IP, immunoprecipitation. Bars: (a) 200 µm; (b) 100 µm.

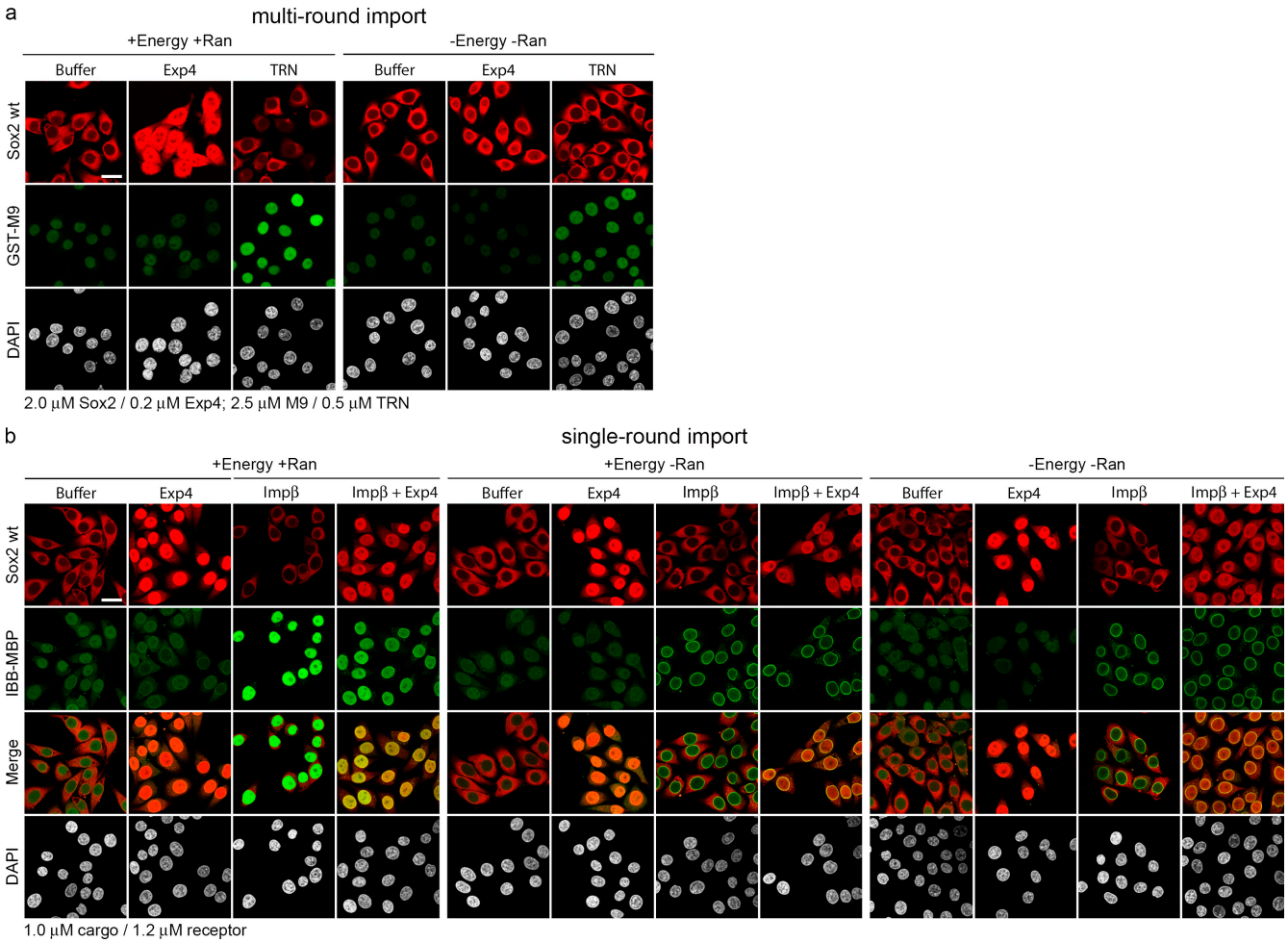

Figure S2. **Ran and energy dependence of Exp4-mediated nuclear import of Sox2.** (a) Efficient multiround nuclear import by Exp4 depends on Ran and metabolic energy. The import experiment was performed as in Fig. 2 a, but import substrates were in 10-fold (Sox2) or fivefold (M9) excess over the receptors, making efficient nuclear accumulation of cargoes dependent on receptor recycling. In the absence of Ran and energy, nuclear accumulation of both GST-M9 and GST-Sox2 was impaired. wt, wild type; TRN, transportin. (b) Single-round nuclear import of Exp4–Sox2 is independent of Ran and metabolic energy. The import experiment was performed as in Fig. 2 b, but transport receptors were in slight excess over the substrates to render nuclear accumulation of the cargoes independent of receptor recycling. Omission of Ran or of Ran and energy did not affect GST-Sox2 import by Exp4 but did prevent the nuclear accumulation of Imp-β–IBB-MBP complexes (which instead arrested at nuclear pores). This was also evident when both Imp-β and Exp4 had been added simultaneously (right). Bars, 25 μm.

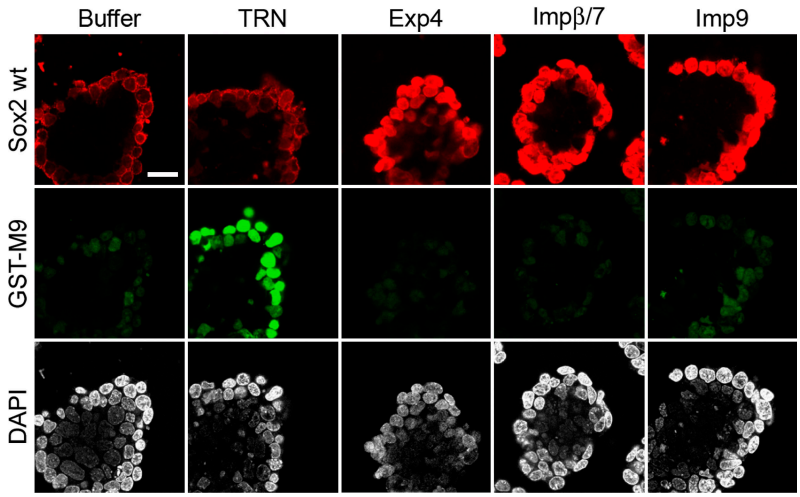

Figure S3. **Exp4, Imp-β/7, and Imp9 also mediate nuclear import of Sox2 in permeabilized ES cells.** NPC composition may vary among different cell types and change during development (Lupu, F., A. Alves, K. Anderson, V. Doye, and E. Lacy. 2008. *Dev. Cell.* 14:831–842). As it cannot be excluded that such changes regulate nuclear import, we tested the import activity of Exp4, Imp-β/7, and Imp9 with permeabilized 46C ES cells instead of HeLa cells. The import experiment was performed as in Fig. 2 a. The transportin (TRN) substrate GST-M9 was used as an internal specificity control. wt, wild type. Bar, 25 µm.