

Supplementary information, Figure S1

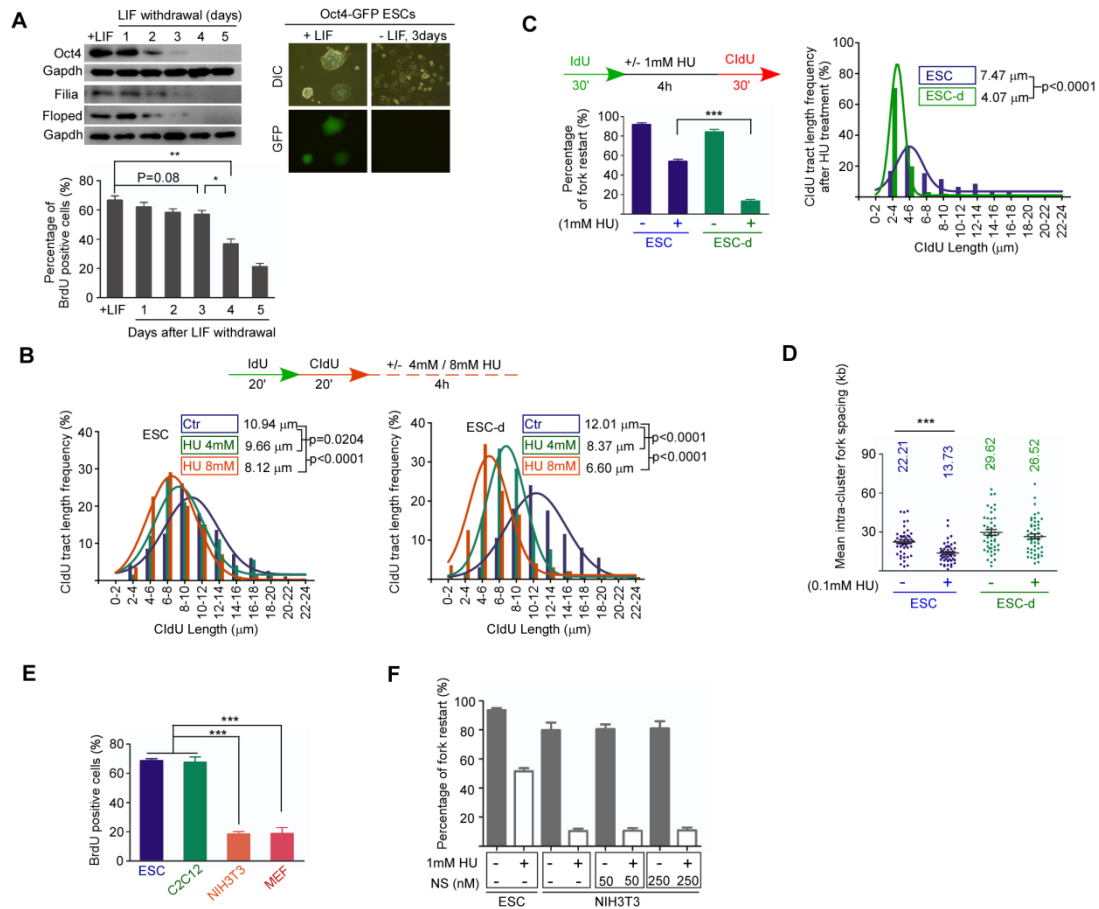


Figure S1 PSCs are superior at resolving DNA replication stress. **(A)** Spontaneous differentiation of mouse ESCs after LIF withdrawal. Left upper panel, immunoblotting confirmed the drastic downregulation of Oct4, Filia and Floped at day 3 after LIF withdrawal; left lower panel, bromodeoxyuridine (BrdU) proliferation assay following different days of LIF withdrawal; right panel, bright field and fluorescent images of mESCs with GFP expression driven by *Oct4* promoter and the isogenic differentiated cells. **(B)** CldU tract length frequency distribution was analyzed in ESCs (left panel) and the isogenic differentiated cells (ESC-d, right panel). Statistical significance was determined by the two-tailed Student's *t*-test, $n = 200$. **(C)**

After stalling by 1 mM HU for 4 h, ESCs were better than the isogenic differentiated cells at restarting the stalled replication forks (left panel). Moreover, the medium CldU tract length was longer in ESCs than in differentiated cells (right panel). **(D)** HU treatment induced significant decrease in the mean intra-cluster fork spacing in ESCs, indicating the active firing of the dormant replication origins. However, the mean intra-cluster fork spacing was similar before and after HU treatment in isogenic differentiated cells, reflecting that dormant replication origin firing was not active. Mean IdU track length was indicated. Mean spacing was calculated from 50 replication clusters. **(E)** Bromodeoxyuridine (BrdU) proliferation assay. **(F)** Rates of stalled replication fork restart in ESCs and NIH3T3 cells. The addition of 50 nM and 250 nM nucleosides (NS) had no impact on stalled fork restart in NIH3T3 cells. Data are represented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.