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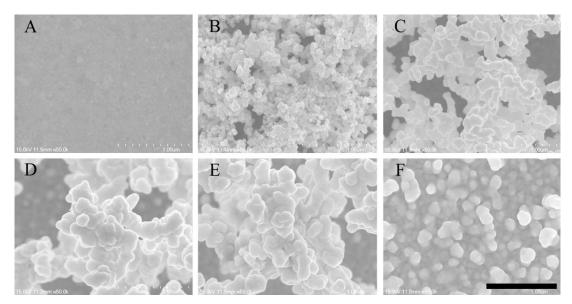
## **Supporting Information for**

## Maintaining the pluripotency of mouse embryonic stem cells on gold nanoparticle layers with nanoscale but not microscale surface roughness

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**Fig. 1** Top-view SEM images of the smooth Au and GNPLs with surface roughnesses from nanoscale to microscale. A: Au; B: GL-1; C: GL-2; D: GL-3; E: GL-4; F: GL-5. The three-dimensional structures of the GNPL became more densely aggregated with the increment of the gold plating solution which resulted in the GNPLs with rougher surface morphology. Bar,  $1 \mu m$ .

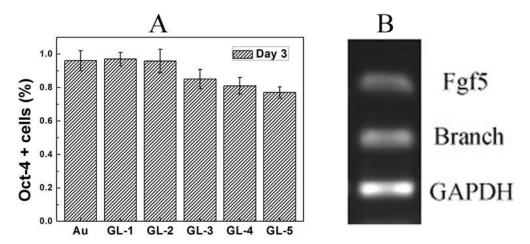


Fig. 2 A: Percentage of Oct-4 + mESCs on Au and GNPLs with various surface roughnesses after culture of 3 days; Data are the mean  $\pm$  SD (n = 3). B: RT-PCR analysis of the expression of specific germ layer maker genes of mESCs on GL-5 with microscale surface roughness (Rq of 1205 nm) after culture of 7 days. Both the expression of Branchyury (endoderm, middle panel) and Fgf5 (ectoderm, upper panel) were detected which indicated the spontaneous differentiation of the cells cultured on microrough GL-5 was undirectional.

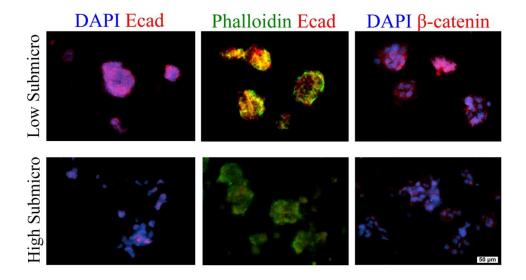


Fig. 3 Immunofluorescence images of mESCs on GNPLs with low sub-microscale and high sub-microscale surface roughnesses after culture of 3 days. A: The cells were costained for nuclei (DAPI; blue) and E-cadherin (red); B: The cells were costained for cytoskeleton (Phalloidin; green) and E-cadherin (red); C: The cells were costained for nuclei (DAPI; blue) and  $\beta$ -catenin (red).

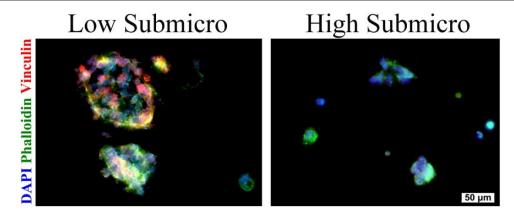


Fig. 4 Immunofluorescence images of mESCs on the smooth Au and GNPLs with nanoscale and microscale surface roughnesses after culture of 3 days. The cells were costained for nuclei (DAPI; blue), cytoskeleton (Phalloidin; green) and vinculin (red).