



Figure 2 - supplement 2. Establishment of Conditions for The Screen. Screen Set Up, Parameters and Correlation Between Biological Replicates. (A) Titration of the lentiviral library so that only ~15% of the E9.5 skin progenitors were infected, thereby ensuring a multiplicity of infection <1. (B) Screen coverage calculation. ~56 embryos are required per biological replicates to ensure that at least 100 E9.5 skin progenitors that will become HF progenitors are transduced with the same shRNA (100x coverage). (C) Calculations to ensure adequate amplification of genomic DNAs at E18.5. Shown are calculations for the HF fraction. The reader is referred to *Beronja et al.* (2013) for details on the epidermal fraction. (D) Flow cytometry analysis of dispase separated epidermal and HF fractions from *Krt14-Actin-GFP* mice. The separated dermal fraction contains a high proportion (80%) of HF cells at E18.5 (GFP+), which is used in our amplification calculation. Representation of n=3 embryos. (E) The sequencing library was prepared using custom primers containing adapter and sequencing features for the Illumina flow cells as well as a 4 nucleotide barcode for multiplex sequencing to amplify the shRNAs present in each population (Beronja et al., 2013). Shown is a representative example showing that the PCR reaction produces a clean PCR product after 31 cycles of amplification. (F-H) Correlation between biological replicates. Spearman correlation, R² and P values for each comparison are indicated on the corresponding graphs.