

Mouse Embryo Fibroblast (MEF) Preparation. MEFs were prepared from individual embryos at embryonic day 14.5 (E14.5; 129/Sv) bearing E2F-1^{+/+}, E2F-1^{+/-}, and E2F-1^{-/-} genotypes. The head and internal organs were removed, and the torso was minced and dispersed in 0.1% trypsin (45BB60 min at 37E°C). Cells were grown for two population doublings (considered as one passage) and then viably frozen. These MEFs were used for all subsequent experiments. MEFs were maintained in DMEM containing 10% fetal bovine serum (FBS; GIBCO) and subcultured 1:4 upon reaching confluence. For serum starvation experiments, MEFs were plated in DMEM containing 0.1% FBS and then incubated at 37E°C for 72 hr, before stimulation with DMEM containing 10% FBS.

To obtain primary MEFs from 15-day-old chimaeric embryos, organs and head were removed and the remaining tissue was washed in PBS and minced. After a second PBS wash, the tissue was incubated with 100 µl trypsin/EDTA (Gibco) overnight on ice. The next morning, 100 µl trypsin/EDTA was added and the tissue was incubated at 37°C for 30 min. The tissue was then dissociated to near-homogeneity in complete medium and transferred to a 100-mm dish. MEFs were maintained in DMEM (Gibco) supplemented with 10% FBS (PAA Laboratories) and 0.1 mM - mercapto ethanol.